

# **A tree of sticks: dispersal and speciation of Australian Phasmatodea**

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**Thesis submitted to fulfil requirements for the  
degree of Doctor of Philosophy**

**2025**

## Authorship Attribution Statement

All chapters of this doctoral thesis comprise stand-alone manuscripts that have been submitted or prepared for submission to peer-reviewed journals. All chapters are tied to the central theme of this thesis, which explores the evolution and taxonomy of Australian phasmids. Accordingly, there is inevitable repetition between chapters, particularly in the methods and taxonomic sections of Chapters 2-4

Within this thesis, I am the sole author of Chapters 1 and 5. All other chapters are co-authored and therefore make use of the plural “we”. Detailed author contributions are presented at the start of each chapter. Unless explicitly specified, I am the main contributor for data collection and analysis, as well as the conception and writing of all chapters.

Appendix 1 shows a paper I co-authored during my PhD candidature, which describes numerous new species of *Candovia* and *Paracandovia*, with *Paracandovia* representing a new genus. Since I did not lead this work, I did not include it as a research chapter. My role in this paper involved specimen collection, imaging and figure creation, and writing and editing species descriptions.



## **Statement of Originality**

I certify that, to the best of my knowledge, the content of this thesis is my own work, unless otherwise acknowledged. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual contents of this thesis are the product of my own work, and that all assistance received in preparing this thesis have been appropriately acknowledged.

## Acknowledgements

From my very first moment glimpsing the most wonderful phasmids at the CSIRO Discovery Centre in Canberra back in 2005, my most loving mother, Joranda Jones, made sure to support my fascination with these amazing creatures. She recognized my love at first sight for these insects and arranged for CSIRO to graciously give me some eggs to take home. To whoever gave me those eggs, I am sorry that you may never know how much of an impact you had on such a young boy. To this day, I continue to breed the same family line of *Eurycnema goliath* stick insects. My mum has been there every step of my life, continuously supporting my passions and dreams, and always compromising to allow me to keep insects inside her home. You are the reason I am who I am today, submitting this thesis.

To my father, Robert Jones: ever since I first started collecting leaves to feed my phasmids, you have been there helping me reach those high branches I could not. When I was away, you never hesitated to change their leaves for me. You are one of the most generous people I know, always willing to drop everything you are doing to drive me anywhere. You even dropped me off at Mount Isa, a 4910 km round trip from Sydney, just so I could look for stick insects with a friend. To both my parents, I love you so much and could not possibly hope to have more loving and supportive people in my life.

My sister, Imogen Jones, you have always been my best friend, and I have loved making you listen to all my amazing phasmid facts that you refuse to remember. Thank you for loving me and occasionally showing me how much you appreciate my passion. I will always love you. Thank you to my grandparents, Anne-Marie van Heeswyk and Michael van Heeswyk, for always showing interest and providing loving support for whatever I do. I love you both very much!

To my amazing and supportive partner, Jennifer Le: you have been such a tremendous help in getting me over the line, always offering to read over all parts of my thesis. Your tireless support right up until the end means more to me than words can express. I would not be where I am today without all your effort. I love you so very much!

I would like to thank my three supervisors, Professor Nathan Lo, Professor Simon Ho, and Dr. David Yeates, for their amazing efforts. To my primary supervisor, Nathan, you have been there for me the whole time, supporting everything I do so earnestly. Whenever I had questions, you were more than happy to hear me out. I hope I have not added too many extra grey hairs. There is no way I would have made it to the end without your continuous support. Thank you so much! Simon, I am forever grateful for our conversations over lunch or in the office. Your advice has been such a blessing that I never knew I needed. David, your entomological knowledge and expertise have blown me away and have been incredibly valuable in developing my taxonomic writing style. I am sure your input will last my entire academic career.

To all the members of the Molecular Ecology, Evolution, and Phylogenetics (MEEP) lab: each of you has provided me with advice or helped me prepare presentations over the years. I would especially like to thank Toby Kovacs, Perry Beasley-Hall, and Maxim Adams, who have received way too many questions from me. You three never complained and always took the time to explain phylogenetics to me. This research reported in this thesis was supported by the award of a Research Training Program scholarship to the PhD Candidate. Additional support was also provided by the award of a CSIRO Postgraduate Scholarship - National Research Collections of Australia 62947.

The endless mentorship and generous help provided by Paul Brock have profoundly impacted my academic journey. Paul, you have helped me develop my skills and deepen my

love for phasmids. No matter the questions I come up with, you have always guided me towards greater knowledge and passion for these insects. I cannot express how grateful I am for your guidance. Thank you for your continuous support and communication!

To all the people who have helped me collect phasmids for this thesis, particularly Noelene Tweed, my mum, and my dad: your amazing generosity of time and support has left me with some of the fondest memories of my life.

All the amazing friends who have helped keep me sane throughout this long journey: you are all special to me, particularly Justin McNab, Joanne Lim, Marcus Chung, Henry Bell, Nathan Ricardo, Stephanie Bagala, James Listberger, and many more. Thank you everyone!

Finally, to the most amazing insects I could ever imagine studying: I would not be here without your existence. Unravelling the complexities of your behaviour and evolution brings me so much joy. I know my passion for you will never end and I hope you continue to show me the amazing world you live in, which is still so totally unexplored!

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# CHAPTER 1

## General introduction

### 1.1 Phasmatodean biodiversity and systematics

Phasmatodea, encompassing stick insects, leaf insects, and walking sticks, are large, herbivorous, nocturnal, insects famous for their remarkable mimicry and camouflage towards sticks and leaves (Fig. 1.1a, b, c, d, e, f, g, h). The order contains ~3500 species that mostly inhabit tropical and subtropical regions, but are less abundant in temperate areas. Phasmids are herbivores, with their diets consisting of variations between monophagous (consumption of one plant) to polyphagous (generalist feeders capable of surviving on a variety of host plant species) (Bedford 1978).

Systematically, the phasmid order is a close sister taxon of Embioptera (web-spinners; Misof et al. 2014). Among Phasmatodea, there are 20 distinct clades; Timematidae represents the first divergence and is sister to all other stick insects, the Euphasmatodeans. Within Euphasmatodea, the family Aschiphasmatidae is the first to diverge from the remaining phasmids, the Neophasmatodea. The Neophasmatodea is divided into Occidophasmata (Agathemeridae + Pseudophasmatidae + Heteronemiinae + Diapheromerinae) and Oriophasmata (Heteropterygidae + Gratidiidae + Bacillinae + Clitumninae + African and Malagasy clade (Achriopteridae + Anisacanthidae + Antongiliidae + Damasippoididae + Xylicinae) + Phylliidae + Pharnaciinae + Prosentoria + Palophidae + Cladomorphinae + Xenophasmina + Stephanacridini + Lanceocercata + Lonchodinae + Necrosciinae).

Molecular studies are working to address all 20 clades, but many species are yet to be included in phylogenetic studies (Robertson et al. 2018; Simon et al. 2019; Bank and Bradler 2022; Boisseau and Woods 2024).

In the most recent studies, the deeper relationships within the Phasmatodean phylogeny are all strongly supported, with >95% support and congruence across studies (Simon et al. 2019; Bank and Bradler 2022; Boisseau and Woods 2024). The strongly supported groupings include the splits between Timematidae and Euphasmatodea, Aschiphasmata and Neophasmatodea, and Occidophasmata and Oriophasmata. While deeper relationships are strongly supported, more recent divergences remain uncertain. For example, the position of the Phylliidae clade differs between the most recently published trees (Robertson et al. 2018; Simon et al. 2019; Bank and Bradler 2022; Boisseau and Woods 2024). Although numerous phylogenetic studies have been conducted, some taxonomic groups remain incompletely sampled, meaning further research is needed for a comprehensive analysis of Phasmatodea.

Within Australia, four major clades exist: Phylliidae, Lonchodidae (Lonchodinae and Necrosiinae), *Pachymorpha* (nested within Clitumninae) within and Lanceocercata. The Phylliidae clade represents ‘true’ leaf insects, which display impressive mimicry of leaves. These species mimic leaf shape, colour, and patterning. Leaf insects are exclusively found in tropical habitats, found between Southeast Asia and Far North Queensland, Australia. The Lonchodidae clade consists of the subfamilies Lonchodinae and Necrosiinae (Fig. 1.1f). These insects are well-known stick and grass mimics. The two groups can be morphologically distinguished by size and wing state. Lonchodinae species are generally larger, more robust and lacking wings. Necrosiinae species are small, delicate and often have wings. Lonchodinae can be found in tropical and subtropical regions across the world.

Necrosciinae are found in tropical and temperate regions, across Asia, Madagascar and Australia.

The *Pachymorpha* are stocky, robust stick insects, mimicking dead leaves and sticks. These insects are found in tropical and subtropical regions, between South Africa, Asia, Australia, and New Zealand (Brock et al. 2024). The Lanceocercata clade (representing Eurycanthinae + Pachymorphinae + Phasmatinae + Platycraninae + Tropicoderinae + Xeroderinae) is the most morphologically diverse group among Australian stick insects (Fig. 1.1a, b, c, d, e, g, h; Bradler and Buckley 2018). They mostly consist of large, robust insects. Mimicry in Lanceocercata is varied, mimicking different types of plant material, including dead and alive leaves, sticks, bark, and lichen (Brock and Hasenpusch 2009).

The evolutionary adaptations of phasmids make them a fascinating model to study a wide range of phenomena, including mimicry, wing and egg evolution, dispersal and reproductive behaviours, particularly parthenogenesis (where females reproduce asexually). Through their array of unique characters, phasmids display a large diversity of ecological roles and life histories.



**Figure 1.1:** Photos of various Australian phasmid species. (a) Female *Extatosoma tiaratum*. (b) Two female *Dryococelus australis* showing social behaviour. (c) Pair of mating *Tropidoderus gracilifemur*. (d) Female *Onchestus rentzi* showing deimatic display. (e) Female *Megacrania batesii*. (f) Pair of mating *Candovia annulate*, in situ. (g) Female *Tropidoderus* sp. (h) Female *Cigarrophasma tessellatum*. Photos by Braxton Jones.

## 1.2 Phasmatodean morphology

The typical body form envisioned when stick insects are mentioned is a simple looking ‘stick’. However, phasmids possess diverse sets of morphological features to help them remain hidden and defend against predators. Phasmatodea range in adult size between 2 cm (*Timema cristinae*) and 64 cm (*Phryganistria ‘chinensis’*, undescribed sp.). They can also reach a maximum weight of 65 g (*Heteropteryx dilatate*). Compared to other insect groups, phasmids are elongated, large insects. Their abdomens are flexible and, in some species, capable of being curled (Fig. 1.1a).

Phasmatodeans are masters of disguise: they can completely blend into their environment, whilst remaining physically exposed (Fig. 1.1h). It is common for species to have spines and flange-like appendages all over their bodies, used for defence and camouflage respectively. One of the most well-known species worldwide, *Extatosoma tiaratum*, is a perfect example of stick insects adapting to different environmental habitats while still maintaining crypsis. They show both brown and lichen colour forms, which mimic dead leaves and lichen respectively (Brock and Büscher 2022; Fig. 1.1a). *Trychopeplus laciniatus* species provide one of the most impressive examples of flange use, perfectly mimicking the moss in which they inhabit (Fig. 1.2a; Conle et al. 2011; Brock and Büscher 2022).

The stereotypical ‘brown stick’ imagery associated with stick insects is far removed from the impressive displays of colour exhibited by more visually striking phasmids. It is common for species to display greens and browns, but species are capable of displaying any colour, including many species which showcase multiple bright contrasting colours. For

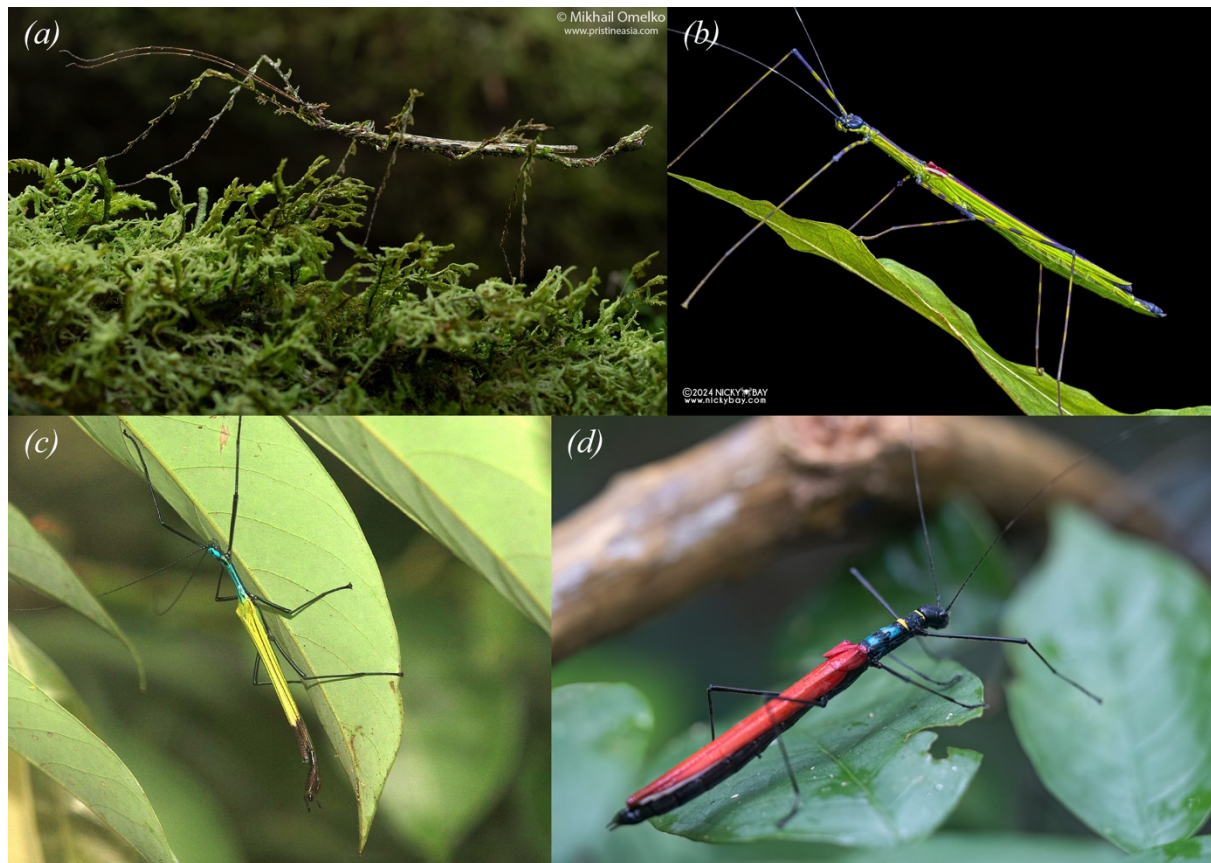


instance, *Orthonicroscia keatsooni* females have blue, purple, green, yellow and white pigmentations across their bodies (Fig. 1.2b). *Euryneicroscia nigrofasciata* males have a blue head, blue to dark green pigmented thorax, bright yellow wings, red-tipped wings, and black legs (Fig. 1.2c; Brock and Büscher 2022). *Calvisia kneubuehleri* females have highly contrasting colouration over their primarily black bodies, flaunting yellow bands and bright blue patches upon their thoraxes and bright red wings (Fig. 1.2d; Brock and Büscher 2022). However, perhaps the most visually astonishing phasmid species are those found in the genus *Achrioptera* (Glaw et al. 2019). *Achrioptera punctipes* males are purple, blue, black, yellow, red, orange and green (Brock and Büscher 2022). Phasmid species also display colour phenotypic plasticity. For example, the genus *Tropidoderus* often has orange or pink colour variants, contrasting with their typical green colour (Brock and Hasenpusch 2009; Brock and Büscher 2022; Fig. 1.1c, g).

Perhaps one of the most diverse morphological traits within Phasmatodea are their wings, or lack thereof. Wing states include the presence and absence of wings, and variation in wing size. However, the presence of wings often does not confer the capacity of flight (Whiting et al. 2003; Trueman et al. 2004). Across virtually all phasmatodeans, females are larger than males, and often brachypterous or apterous (Bank and Bradler 2022). Phasmids use their wings in deimatic displays, and rely on their morphological traits to engage in some of the most striking camouflage strategies of crypsis or masquerading adaptations (Ioannou and Krause 2009; Whiting et al. 2003; Trueman et al. 2004; Endler 1978; Merilaita and Lind 2005; Fig. 1.1c, d, g, h).

Despite flight being one of the foremost examples of diversification in phasmids, certain lineages have undergone wing size reductions (Wagner and Liebherr 1992; Roff 1990; Roff 1994; Wipfler et al. 2015). Phasmatodea show widespread wing loss, with most

species presenting with brachypterous or apterous wing states (Bank and Bradler 2022). Regardless, numerous species still maintain the ability to fly, with functional flight more common in males (Bank and Bradler 2022).



**Figure 1.2.** All images have been taken by other people and uploaded to iNaturalist. (a) *Trychopeplus laciniatus*, photo by Mikhail Omelko. (b) *Orthonectroscia keatsooni*, photo by Nicky Bay. (c) *Eurynectroscia nigrofasciata*, photo by Les Day. (d) *Calvisia kneubuehleri*, photo by James Jolokia.

### 1.3 Phasmatodea eggs

The egg stage often represents a large period of an organism's life cycle, thus behavioural and ecological research on eggs across the animal kingdom has been broad (Kilner 2006; Altig and McDiarmid 2007; Cherry and Gosler 2010; Guerra-Grenier 2019; Hallman and Griebler 2015). Due to the innate vulnerability of eggs, many vertebrate animals provide protective parental care, such as choice of laying site or defending eggs from predators (Montgomerie and Weatherhead 1988; Refsneider and Janzen 2010; Mainwaring et al. 2014; Katsis et al. 2018; Meats and Pando 2002). Contrastingly, parental protection of invertebrate eggs is not common (Wong et al. 2013; Tallamy 2000; Royle et al. 2012). Instead, insect eggs must deal with predation and environmental challenges by themselves (Church et al. 2019). Therefore, invertebrates experience strong selection pressures in this life stage.

Intense selection pressures acting on eggs have driven diverse evolutionary adaptations. For example, ladybirds use chemicals to avoid predator consumption of their eggs (Agarwala and Yasuda 2001). The egg stage can also be used for dispersal, for example wind-borne crustacean eggs (Brendonck and Riddoch 1999; Pincel et al. 2016). Among insects, morphological variation seems to be more correlated to their ecological niche than the traits of adults (Church et al. 2019). Accordingly, the ecological interactions of eggs with their environment likely drive the vast diversity of egg behaviour and morphology.

Behavioural and morphological adaptations pertaining to phasmatodean eggs are multifarious. Complexity in phasmid egg morphology can extend to taxonomy, in that identification is often possible to the species level (Clark 1976; Sellick 1988, 1997a, b; Mazzini et al. 1993; Jintsu et al. 2010; Fig. 1.3). Egg morphological variation is similarly linked to even more diverse ovipositional strategies, and the two have likely evolved together (Robertson et al. 2018; Boisseau and Woods 2024). However, even with ongoing interest in

egg morphology, the functional significance of associated ovipositional behaviours are comparatively lacking (Goldberg et al. 2015; Robertson et al. 2018; Büscher et al. 2023; Boisseau and Woods 2024).

Phasmids are known to follow two overarching egg-laying strategies: eggs placed without specific intention (dropping or flicking eggs to the ground), and eggs placed with specific intention (buried or adhered to substrates) (Carlberg 1983). Eggs can be laid individually or in a clutch. The ancestral phasmatodean egg laying strategy has been inferred to be dropping eggs to the ground (Robertson et al. 2018). The tough outer shell encasing phasmid eggs is thought to facilitate this strategy, such that eggs are not damaged when dropped from trees. The ancestral phasmid is thus inferred to have dwelled in trees, like most extant taxa (Robertson et al. 2018; Boisseau and Woods 2024). Eggs that are dropped are typically round. The majority of eggs which are dropped also exhibit a capitulum, which serves to attract ants (Compton and Ware 1991; Hughes and Westoby 1992; Windsor et al. 1996; Stanton et al. 2015). The substances present in the capitulum strongly resemble the well-known fatty acid-rich elaiosome present in plant seeds, which attracts ants that mediate dispersal (Giladi 2006; Brew et al. 1989; Fischer et al. 2008). This egg dropping strategy requires the least amount of parental care in Phasmatodea.

Compared to the dropping of eggs, adult Phasmatodea also use strategies whereby they specifically place an egg or eggs. This behaviour may encourage more morphological variation, due to the wide variety of habitats and substrates available to phasmid species. The most simple examples involve adult females that bury or wedge eggs into substrates by manoeuvring their ovipositor (Carlberg 1989). For example, *Sipyloidea* spp. are known to place their eggs in the crevices of bark (Bedford 1978; Sellick 1988). An example of a more complicated placement of eggs comes from *Anisomorpha buprestoides*, which manoeuvre

their abdomen over the top of their body and in front of their head, where they will drop eggs into a hole they have dug (Hetrick 1949). *Epidares nolimetangere* have been reported to flick their egg over their bodies, and catch them with their antennae and forelimbs, before again placing the eggs into holes they have dug (Abercrombie 1992). *Epidares nolimetangere* eggs have hooked hairs which facilitate this action.

Gluing eggs is also routinely used amongst phasmids. In Australia, the genus *Denhama* is one of the more well-known gluers, but *Spinosityloidea doddi* are also observed securing eggs to host plant leaves (Brock and Hasenpusch 2009). However, perhaps the most famous example is from the genus *Korinninae*, whose individuals lay eggs in ootheca-like clutches, showing convergent evolution to praying mantises (Goldberg et al. 2015).

The mimicry phasmid eggs display towards plant seeds is outstanding. Eggs resemble seed morphology, through similar size, ovoid shape, dark colour, and hard outer shell (Stockard 1908). This mimicry is further evidenced through the consistent relocation by ants of both phasmid eggs and seeds to their nests (Compton and Ware 1991; Hughes and Westoby 1992; Windsor et al. 1996; Stanton et al. 2015), and can be considered a form of local dispersal. These similarities could be mere coincidence (Severin 1910) or could have been selected for protective mimicry to avoid predators and parasites (Stockard 1908). However, comparative studies between seeds and eggs are limited and inconclusive. Thus, the functional significance behind egg morphology and oviposition strategy is still an outstanding question (Goldberg et al. 2015; Robertson et al. 2018). With research on phasmids suggesting their convergence with plants, there is value in exploring their egg laying, and its associated evolutionary history.





**Figure 1.3:** Various eggs representing the Australian Phasmatodea. Photos by Braxton Jones.

## 1.4 Dispersal in Phasmatodea

### *1.4.1 Dispersal*

Dispersal describes the movement of organisms or their gametes from one area to another in the interests of growth and reproduction. Dispersal contributes to gene flow, reduces parental and sibling competition, and promotes genetic diversity (Bohonak 1999). For motile organisms (those who can walk, fly, or swim) dispersal is relatively uncomplicated. However, motile organisms still need to efficiently locate suitable habitats and avoid predators along the way.

Sessile organisms rely on random dispersal through biotic and abiotic means. For example, marine corals (Anthozoa) disperse during the pelagic stage after mass spawning events, before settling on the seafloor (Jackson 1986). In sessile organisms, the embryonic stage (when organisms are smaller and can move more easily) is optimal for dispersal (Wang and Smith 2002; Panov and Caceres 2007). This is commonly seen amongst sessile marine species, where juveniles are capable of moving through water. However, in terrestrial environments, embryonic dispersal is less common. Examples are seen in marine and freshwater crustaceans that use wind to disperse eggs (Brendonck and Riddoch 1999; Pincel et al. 2016).

Embryonic dispersal is particularly well understood in plants. Because plants are unable to move, the seed stage is the only viable period for plants to relocate to a different growing environment from that of the parent plant. For local seed dispersal, plants utilise gravity or ballistic methods, and for long distance dispersal, seeds are moved with abiotic means, such as water and wind, or biotic means, though animals (Murray 1986; Burns 2012).

One of the more striking dispersal adaptations found in seeds is the presence of morphological appendages (known as ‘wings’) to facilitate wind dispersal (Horn et al. 2001). Seeds are often associated with colourful fruits that attract animals to consume them. This mechanism is complicated by the prerequisite to survive digestion (Traveset et al. 2007). This is known as endozoochory. Seeds can also grow hooks or barbs for adhesion to animals (Kulbaba et al. 2009), known as epizoochory. Finally, as mentioned above, seeds can be locally dispersed by ants into their nests, known as myrmecochory. Ant dispersal can provide protection from fire and predation, and provides an optimal, nutrient-rich environment for germination (Giladi 2006). Whilst dispersal mechanisms used by sessile terrestrial plants are well studied, there are comparatively little studies focusing on sessile invertebrates.

#### *1.4.2 Flight, rafting and egg based dispersal in phasmids*

Within a widespread population of phasmids, individuals may need to transverse large distances to locate a mate. In this case, selection may drive the maintenance of wings. In geographically restricted species, wings may be less important due to the relatively short distances required to locate a mate. This idea is supported by studies of *Asceles tanarata*, whereby wings are reduced with increased altitude. Again, it is often the case that males are the only ones to retain flight capabilities (Zeng et al. 2020), which would maintain continuous intraspecific gene flow across widespread populations.

Despite the prevalence of males maintaining functional wings in many phasmid species (while females are brachypterous or apterous), it is still common for other species to be brachypterous or apterous in both sexes. For entirely flightless species to disperse, other means are required. Considering the clear resemblance phasmid eggs have to plant seeds, it is



possible that phasmatodeans have convergently evolved similar dispersal mechanisms to seeds.

The distributions of some phasmids are indicative of oceanic egg dispersal. Considering the infeasibility of live stick insects to survive in sea water, oceanic dispersal seems only viable during the egg stage. Whilst not confirmed with molecular data, it is thought that the morphological features of *Mauritiophasma cliquennois* (from the Mascarene Islands near Madagascar) are most similar to species from Australia (Cliquennois and Brock 2004; Bradler et al. 2015). Regardless of which species is more similar to *M. cliquennois*, the Mascarene Islands are entirely volcanic in nature, meaning the pioneering species would have most likely rafted across the ocean (Cliquennois and Brock 2004). The likelihood of this happening is conceivable in species which adhere their eggs to bark or leaves (Bedford 1978; Sellick 1988). It is likely that *Dryococelus australis* (Lord Howe Island Stick-insect) also dispersed across the ocean, considering they are found on Ball's Pyramid, a volcanic island isolated from mainland Australia.

More recently, the genus *Megacrania* has been proposed to use ocean dispersal. This idea is driven by the ongoing viability of eggs after exposure to saltwater for extended periods (Kobayashi et al. 2014), combined with the distributions of both the host plants (*Pandanus*) (Gallaher et al. 2015) and insects (Ushirokita 1998). Ocean dispersal through currents is a mechanism frequently used by coastal plants (Nathan et al. 2008). Some of the more famous examples include coconuts and mangrove seeds (Murray 1986; Smith 1994). These plants remain buoyant in ocean water because of internal air pockets, which enable them to float through ocean currents. The same happens in the genus *Pandanus* (Gallaher et al. 2015), which are the exclusive food plant for *Megacrania* (Brock and Hasenpusch 2009). The shared habitats and morphological adaptations (suggesting ocean dispersal) of *Pandanus*

and *Megacrania*, bolster the plausibility of these two species sharing convergent evolution. *Megacrania* species appear to have coevolved this strategy to implement the same dispersal strategy as their host plants, allowing them to effectively relocate to habitats which support *Pandanus*.

#### *1.4.3 Factors driving egg laying strategies*

The vast diversity in phasmid egg laying strategies raises questions concerning the selection pressures that drive such diverse evolution. Numerous studies have set out to compare adult physiology and oviposition strategy (Robertson et al. 2018; Boisseau and Woods 2024), but functional explanations behind these strategies are yet to be sufficiently addressed. However, it has been proposed that some strategies are driven by predator and parasite protection, and host plant availability (Giladi 2006; Krombein 1956; Baker 2016; Hughes and Westoby 1992).

Many phasmids are limited to individual host food plants, and without them they are unable to survive. Accordingly, it is vital these insects do not stray far from their host plant (Cernak and Hasenpusch 2000; Bragg 2001). Given this scenario, it makes sense that individuals, particularly those of more robust species, would secure eggs to, or nearby, host plants (Goldberg et al. 2015). However, how this influences insect dispersal remains untested. In contrast, these strategies might also provide protection from predators, such as gluing eggs to disguise them as part of a plant or burying eggs to avoid detection altogether.

Parasitoid wasps, from the Chrysididae subfamilies (Amiseginae and Lobosceliidinae), are well-known to predate on phasmid eggs (Krombein 1956; Baker 2016; Riek et al. 1974 p. 928). Whilst there is evidence to support predator avoidance strategies in

*Didymuria violescens* eggs (Hughes and Westoby 1992), additional studies are still needed to prove the impact on egg evolution.

## **1.5 Historical Biogeography of Australia**

Biogeography is the study of life on earth to better understand how species distributions are shaped by geological and biological processes (Parenti and Ebach 2013). By understanding these processes, science can explore diversification and speciation, the interaction of organisms in their environments, and discern historical events that have shaped present day biodiversity (Sanmartín 2012).

Historical biogeography is where geographical distribution patterns are combined with molecular data to investigate the evolution and relationship of species across time and space. As this field of study is relatively young, it is still being improved. For instance, the introduction of phylogeography occurred quite recently (Avice 2000). This concept uses phylogenetic data combined with molecular data (associated with a geographical location) to describe the patterns of evolution and biological diversity. Techniques being used to reconstruct ancestral ranges, and describe ecological niches, are continuously improving (Rode and Lieberman 2005; Yu et al. 2013).

For hundreds of years, observations of geographically distinct fauna have been made. For instance, Alexander von Humboldt (1805) observed similarities among different species across the same climactic habitats. These observations eventually incited different hypotheses for how speciation influenced their present-day geographical distribution. It is now thought speciation can occur through: 1) allopatric speciation, populations becoming geographically isolated, 2) sympatric speciation, species diverging from the same population through

specialisation of ecological or behavioural differences, 3) parapatric speciation, two different populations diverging and still maintaining gene flow within population boundaries, 4) peripatric speciation, a small part of the larger population being isolated, typically at the edge of the species boundary. In allopatric speciation, two processes drive species separation, dispersal and vicariance. Vicariance occurs when a natural barrier physically divides up a population.

Most Australian ecosystems have their origins in rainforest habitats. Ancient climate change and a general drying of the Australian landscape led to these rainforests contracting and becoming fragmented. This change first began during the Cretaceous period (~145 Ma), and is still continuing (Sluiter and Kershaw 1982; Bryant and Krosch 2016; Truswell 1993; Byrne et al. 2011; Martin 2006; Hill 2004). During the Cenozoic period (~65 Ma), the northward movement of the Australian continent altered ocean currents, causing decreased rainfall in Australia (Byrne et al. 2011; Wilford and Brown 1994; Cook and Crisp 2005; Hill 2004). This initiated aridification in Australia. Aridification continued until ecosystems moved into grasslands and open sclerophyll woodlands during the Miocene to Pliocene (~23-2.6 Ma) (Martin 1998; Byrne et al. 2011, 2008; VanDerWal et al. 2009; Bryant and Krosch 2016).

Along the Australian eastern coast, eight distinct geographical barriers were established by the fragmentation of rainforest ecosystems (Bryant and Krosch 2016). These geographical barriers are drier and hotter regions, and are open woodland and savanna landscapes, which prevent gene flow between adjacent inhabitable rainforest areas. Although the beginning of aridification in Australia is not recent (starting ~20 Ma), the current arid zones (characteristic of the sandy deserts in central Australia) only appeared within the last ~5-2.6 Ma. Fragmented rainforest habitats and arid sandy deserts are a result of climate

change in Australia, and represent opportune systems to explore species diversification and dispersal across geographically isolated habitats.

## **1.6 Phylogenetics and integrative systematics**

The discipline of taxonomy investigates organisms in an attempt to discover new species. When first introduced, taxonomy suffered from various methodological problems, which often lead to errors and inconsistency. In 1753, Carl Linnaeus established what became the basis for traditional taxonomy (Linnaeus 1753). Although species are continually being described, the vast majority of the estimated 1-6 billion species on earth remain undiscovered (Larsen et al. 2017). With the emergence of cheaper and faster molecular techniques, it is becoming common practice for taxonomy to now rely on a combination of molecular, morphological and biogeographical methods, known as integrative systematics (Dayrat 2005; Yeates et al. 2010). Systematics combines taxonomy and phylogenetics to better describe species evolution and relatedness, and offers the basis of evolutionary research (Yang and Rannala 2012). Given the current extinction rate (Barnosky et al. 2011), employing systematic techniques is vital for species delimitation and management of biodiversity (May 2002; Mace 2004). Of particular importance is the identification of wide-ranging species that could potentially reveal cryptic species complexes, in which morphologically similar species are indistinguishable using traditional taxonomic techniques alone (Gill and Kemp 2002).

Traditional taxonomy has been based on morphological data only. However, morphological data are often inconclusive due to the existence of cryptic species, homoplasy, intraspecific variation in morphology, and phenotypic plasticity (Givnish and Sytsma 1997; Nadler 1995; Schlick-Steiner et al. 2010; San Jose et al. 2018; Wendel and Doyle 1998).

Accordingly, it has become best practice to perform taxonomic descriptions by utilising both morphological and molecular data (Wiley and Lieberman 2011; Dayrat 2005; Will et al. 2005; Baker and Gatesy 2002). With the rise of systematics, similar advancements have been made with phylogenetic trees inferred from molecular data (Yang and Rannala 2012). The two most used statistical methods are maximum likelihood (Felsenstein 1981) and Bayesian inference (Yang and Rannala 1997). Given the differences between the two models, it is optimal to apply both methods to a molecular dataset (Yang and Rannala 2012).

The different methods used to infer phylogenetic trees determine the robustness of the estimated relationships. Maximum likelihood analyses use molecular datasets to search across tree space and locate the phylogenetic tree with the highest likelihood (Felsenstein 1981). Bayesian inference analyses use posterior probabilities to determine the probability of a tree based on prior information and dataset signal (Yang and Rannala 1997; Huelsenbeck et al. 2001). Maximum likelihood analyses offer a more conservative approach, which thoroughly tests the data (Felsenstein 1985; Alfaro et al. 2003), whilst Bayesian inference uses more parameter-rich models (Drummond et al. 2006).

The use of molecular data to build phylogenetic trees and assist in the inference of species is vital to integrative systematics. Consideration of how many specimens to sample, which genes to sequence, DNA sequence quality, and choice of the best model for phylogenetic analyses are required. The mitochondrial genome remains the most used marker for use in integrative systematics (Zink and Barrowclough 2008; Herbert et al. 2003; Luo et al. 2010). However, mitochondrial genes can sometimes be insufficient for delineating species due to phenomena such as incomplete lineage sorting or hybridisation (Rubinoff et al. 2006). Therefore, it is best practice to combine mitochondrial data with morphological

characters, as well as nuclear markers, where feasible, to increase confidence when inferring species relationships (Degnan and Rosenberg 2009).

By combining multiple lines of evidence (from morphological and molecular, and in some cases behavioural and biogeographical data), integrative systematics provides the most comprehensive estimate of evolutionary history (Padial et al. 2010; Schlick-Steiner et al. 2010; Valdecasas et al. 2008; Dayrat 2005; Will et al. 2005). In essence, modern practice includes these methods to unravel cryptic taxa, which are typically missed by traditional methods (Levasseur and Lapointe 2001).

#### *1.6.1 Molecular dating*

Molecular dating is a method that involves examining genetic sequences and quantifying them into evolutionary timescales (Bromham and Penny 2003). When such information is available, the understanding of the context in which evolutionary processes occur is greatly enhanced. Molecular dating is inferred through the use of molecular clocks, which were originally based on the assumption that the evolutionary rate of change is constant between different lineages (Zuckerkandl and Pauling 1962). Whilst first proposed for amino acid sequences, continual advancement has led to its wider use, including analysis with nucleotide sequence data (Ho 2014). The molecular clock is continually improving, but its advancement is complex (Kumar 2005). One key development was the discovery of varied evolutionary rates (Lanfear et al. 2010). The relaxed molecular clock model was developed to effectively model rate heterogeneity, and has become the dominant paradigm for estimation of evolutionary timescales (Welch and Bromham 2005; Ho and Duchêne 2014).

In order to use a molecular clock, a calibration is needed. Using fossil calibrations to place ages on particular nodes is the most commonly adopted method here, and generally the larger the number of fossil calibrations used, the higher the levels of confidence in the estimates (Paradis 2013; Duchêne et al. 2014). However, this method can only be used to provide a minimum age, and therefore cannot provide a definitive date for when the particular node divergence occurred. In the absence of fossil data, it is possible to use other forms of calibrations, including biogeographical events (for example, the formation of an isthmus or river) to constrain a particular node (Ho et al. 2015). This method, of course, relies on the confidence surrounding the timing of the biogeographical isolation event.

Molecular clock models are complex, and it is important that appropriate methods are applied to provide confidence in the obtained estimates. For instance, when looking at recent events, if a fossil from an ancient group is used, the evolutionary timescale may be overestimated (Ho and Larson 2006). When suitable fossils are not available, an estimated substitution rate of a closely related taxon may be used (Ho et al. 2011). Molecular clock methods continue to evolve, and there is ongoing debate surrounding insect evolutionary timescales (Misof et al. 2014; Tong et al. 2014; Montagna et al. 2019; Klopstein 2021).

## **1.7 Thesis outline**

To explore the evolution of Australian phasmids, this thesis has used a variety of methods. Morphological and molecular data were used to delimit species, and the latter to examine phylogenetic relationships. Molecular clock analyses were used to determine the age of divergence times. The results were then looked at in a historical biogeographical context to hypothesise likely mechanisms influencing divergence times and distributions of Australian



phasmid taxa. Finally, where possible, results were considered in the context of established theories of dispersal and oviposition strategies.

The selection of genera in this thesis was based on the presence or absence of wings and their distribution in rainforest and arid environments. The first two chapters focus on two genera found in tropical coastal regions along the Queensland coast. Chapter 2 examines species without wings, while Chapter 3 focuses on species with wings. Chapter 4 investigates differences in distribution patterns between rainforest species with and without wings, as well as desert species without wings.

Chapter 2 focuses on the genus *Austrocarausius*. These phasmids are exclusively apterous and are found in highly endemic rainforest populations. Initially, these phasmids represented two species, but preliminary surveys of taxa suggested otherwise. Morphological and molecular analyses were conducted, including species delimitation analyses, to revise the systematics of *Austrocarausius*. The results yielded nine new species and showed this genus was highly endemic to fragmented rainforest habitats.

Chapter 3 investigated the genus *Anchiale*. *Anchiale* and *Austrocarausius* species are found in similar habitats, but *Anchiale* shows lower levels of endemism. Both sexes of the genus *Anchiale* have wings, with males being capable of flight. This study aimed to revise the systematics of *Anchiale*. This study described two new species, removed one from synonym status, and transferred one to another genus. The distributions for known taxa were also updated, showing they are widespread across all habitats along the Queensland coast.

Chapter 4 investigates relationships among members of the Australian Lonchodini, including comprehensive sampling of the genera *Denhama* and *Hyrtacus*, and comparisons with data from *Austrocarausius* (Chapter 2). These Lonchodini species are all apterous, and with the exception of *Austrocarausius* and *Leprocaulinus insularis*, are presumed to either

glue or bury their eggs. These ‘grass’ stick insects are distributed across large areas of the continent, particularly in arid habitats. Whole mitochondrial genome and nuclear ribosomal datasets were used for analyses of *Denhama* and *Hyrtacus*. Combining morphological and molecular data, there is evidence for four new morphogroups within the Australian Lonchodini, the reinstatement of one genus, at least five new species, and reinstatement of two species. There remains some uncertainty concerning boundaries between genera, with missing egg morphology and previously examined overseas samples that are likely to have been misidentified. Therefore, probable new genera are only hypothesised (rather than formally proposed) in this chapter, whilst new species (which are more certain) are proposed.

Chapter 5 of the thesis is a general discussion of the results in the thesis. This chapter details the progress each chapter has made in understanding the evolutionary diversification and dispersal of Australian Phasmatodea across the landscape. It also provides future directions needing further research

## CHAPTER 2

### **Integrative taxonomy of the stick insect genus *Austrocarausius* Brock, 2000 (Phasmatodea: Lonchodidae) reveals cryptic species in remnant Queensland rainforests**

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and Nathan Lo

Disclaimer: This chapter is published in volume 36 of *Invertebrate Systematics*. For consistency with this thesis, the published paper has been reformatted. To view the published version, refer to Jones et al. 2022 in the references.

**Authors' contributions.** B.R.J. and N.L. devised the study. B.R.J. conducted the analyses and wrote the manuscript with input from all authors. All authors approved of the final version.

## 2.1 Abstract

*Austrocarausius* (Brock 2000) is a stick insect (Phasmatodea: Lonchodidae) genus containing two species restricted to the tropical rainforests of northern Queensland. Recent specimen collections between the two species' type localities, Lizard Island and Rockhampton, have suggested that *Austrocarausius* might represent more than the two nominal species. Here, we apply morphological and molecular analyses to revise the taxonomy of this genus. Using both field-collected and historic museum samples, we developed morphological species hypotheses and descriptions. Genetic sequencing of mitochondrial *COI* and *16S* were undertaken for species delimitation and phylogenetic analysis, including an estimate of the evolutionary timescale of the genus. Based on these results, we propose nine new *Austrocarausius* species, increasing the number of species in the genus to eleven: *A. nigropunctatus* (Kirby, 1896), *A. mercurius* (Stål, 1877), *A. coronatus* sp. nov., *A. decorus* sp. nov., *A. eirmosus* sp. nov., *A. gasterbulla* sp. nov., *A. tuberosus* sp. nov., *A. macropunctatus* sp. nov., *A. truncatus* sp. nov. *A. waiben* sp. nov. and *A. walkeri* sp. nov. Our results suggest *Austrocarausius* species diversified over the last c. 25-70 Ma, resulting in the now endemic distributions in the tropical rainforests of the central and northern Queensland coasts. This is the first integrative systematic study of an Australian phasmid genus, combining morphological, molecular and biogeographical methods. Additional species of *Austrocarausius* likely remain undescribed as can be inferred from methodical sampling of rainforest patches along the Queensland coast.

## 2.2 Introduction

The Lonchodidae is the largest family of Phasmatodea (stick insects), comprising two subfamilies, four tribes and approximately 1200 species that represent 35% of known phasmids (P. D. Brock, T. Büscher and E. Baker, Phasmida Species File Online, ver. 5.0/5.0, <http://phasmlida.speciesfile.org/HomePage/Phasmida/HomePage.aspx>). These insects are typically slender and have long antennae, and are comparatively small to medium in size, with bodies ranging from 5 to 15 cm long (Robertson et al. 2018). Lonchodids can largely be found throughout Asia and Australasia. The family Lonchodidae has been proposed to accommodate the two subfamilies Lonchodinae, all the members of which are wingless and the often-winged Necroschiinae (Robertson et al. 2018). Lonchodinae is found mainly in Asia, Australasia and the Seychelles (Cliquennois 2020).

The tribe Lonchodini comprises 39 genera of which three are endemic to Australia: *Austrocarausius* Brock, 2000; *Denhama* Werner, 1912; and *Hyrtacus* Stål, 1875. *Austrocarausius* was erected by Brock (2000) to accommodate Australian species from *Carausius* Stål, 1875 and *Lonchodes* Gray, 1835 that were *Carausius*-like but more elongate. The genus comprises wingless phasmids that have simple ‘stick-like’ body forms (Fig. 1.1a, b). Two species, *A. mercurius* (Stål, 1877) and *A. nigropunctatus* (Kirby, 1896), are currently known from the genus. These have very similar morphologies, differing only in some minor head and genitalic structures. These insects have been observed feeding on *Eucalyptus*, *Areaceae*, *Dendrocnide moroides* and *Acacia* spp. as host plants (Brock and Hasenpusch 2009; P. D. Brock, pers. obs.). *Austrocarausius mercurius* and *A. nigropunctatus* are found in two very different regions of Queensland: the Rockhampton to Peak Downs area and the Lizard Island to Cooktown area respectively (Brock 2000; Brock et al., <http://phasmlida.speciesfile.org/HomePage/Phasmida/HomePage.aspx>). Several samples

collected along the coast that separates these two regions (spanning ~1000 km) have been assigned to this genus, although species assignment has been difficult due to a lack of defining morphological characters (Brock and Hasenpusch 2009).

As these are all wingless, *Austrocarausius* species are expected to have poor dispersal ability. The distribution across an ~1000-km stretch of north-eastern Australia in patchy rainforest areas raises the possibility that this genus is more species-rich than currently considered. We focused this systematic study on *Austrocarausius* because of the likelihood of additional undescribed species occupying separate rainforest patches and the fact that many more specimens of the genus have accumulated in collections.



**Figure 2.1.** (a) *Austrocarausius mercurius* in copula from Byfield, note diagnostic whitish knees (photo by Paul D. Brock). (b) *Austrocarausius nigropunctatus* from Home Rule Camping, Rossville in copula (photo by Beth Ripper).

Integrative taxonomy is a process in which species are delimited and described using a combination of morphological, biogeographical and molecular data (Dayrat 2005; Yeates et al. 2010). More robust, biologically meaningful taxa can be delimited by employing multiple lines of evidence (Will et al. 2005; Yeates et al. 2010). In this study, we integrate morphological and evolutionary species concepts to assess the species diversity of *Austrocarausius* before revising the taxonomy of the genus.

We have undertaken detailed anatomical analysis of numerous specimens collected during extensive fieldwork and where possible, generated relevant molecular sequence data. Here, we also estimate the evolutionary timescale of *Austrocarausius* and use quantitative species delimitation methods to delimit putative undescribed species. In this study we identify and describe nine new species and provide egg descriptions, where available, to assist with species identification. Species keys are also provided to identify all species, focusing on diagnostic morphological characters.

## **2.3 Methods**

### *2.3.1 Specimen collection*

Specimens were collected from the field by Paul Brock, Braxton Jones, Jack Hasenpusch, Jerome Constant and Linda Semeraro or borrowed from museums (Supplementary Tables S2.1, S2.2). Specimens collected in Queensland by B. R. Jones were obtained under the permit number WITK18701717 and other ABRS collecting and export permits for PB. Specimens donated by J. Constant and L. Semeraro were financially supported under the



Leopold III Funds project ‘Fulgoridae et Eurybrachidae d’Australie: (I) Taxa de la côte SE du Queensland’.

Eggs were collected from live insects and specimens mounted, with the right midleg stored in 100% ethanol for downstream molecular analysis. Specimens used in this paper can be found in the Queensland Museum, Brisbane, Australia under registration numbers T250900-T250927 and T258567-T258572 (Supplementary Table S2.2).

### *2.3.2 Abbreviations used*

Australian National Insect Collection, Canberra, Australia (ANIC); Natural History Museum, London, UK (NHMUK); Naturhistorisches Museum Wien, Vienna, Austria (NHMW); Naturhistoriska Riksmuseet, Stockholm, Sweden (NHRM); Queensland Museum, Brisbane, Australia (QM); Zoologisches Institut und Zoologisches Museum, Universität von Hamburg, Hamburg, Germany (ZMUH).

### *2.3.3 Morphological analysis and imaging*

Fifty specimens were examined, of which 25 were used for morphological measurements (Supplementary Table S2.1). Morphological analysis was performed by B. R. Jones. Images were taken with a Canon 7D mark I with a 100- mm macro lens. Images were processed in Adobe Photoshop 2020 (ver. 21.2.0) and, when needed, photos were stacked either in Adobe Photoshop 2020 (ver. 23.3, Adobe, <https://www.adobe.com/au/>) or ZereneStacker (ver. 1.04, Zerene Systems, <http://zerenesystems.com/cms/home>). Species measurements and diagnoses were performed prior to molecular analysis to avoid bias when inferring morphological groupings.

#### 2.3.4 Molecular and species delimitation analysis

For all molecular and species delimitation analyses, DNA sequence data were obtained from 31 representatives from eight of the eleven species described in this study (ranging from one to seven individuals per species; Supplementary Table S2.1). DNA extraction and PCR were attempted on the remaining three species, however, the museum specimens were too degraded to allow successful PCR. Specimens used in molecular analyses were collected in the field by P. D. Brock, B. R. Jones, J. Constant and L. Semeraro. Among all sampled species, pairwise distance data were obtained for cytochrome oxidase subunit *I* (*COI*) (Supplementary Table S2.3).

DNA was extracted using a High Pure Roche Kit, following the manufacturer's instructions. The primers LCO1490 (5' GTCAACAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA 3') (Folmer et al. 1994) were used to amplify *COI*. For PCR of *16S* ribosomal RNA we used LR-J-12961 (5' TTACGCTGTTATCCTAA 3') and LR-N-13398 (16SAR)F (5' CACCTGTTTAACAAAAACAT 3') (Simon et al. 1994). PCR amplification was performed in a BIO RAD MyCycler Thermal Cycler as follows (as per Velonà et al. 2015): initial denaturation at 94°C for 1 min, five cycles of 94°C for 30 s, annealing at 45°C for 40 s and extension at 72°C for 1 min, followed by 30 cycles of 94°C for 30 s, 51°C for 40 s and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were sent to Macrogen (South Korea) for Sanger sequencing. Sequences were edited in Genious Prime (ver. 2020.2.2, [https:// www.geneious.com/](https://www.geneious.com/); Kearse et al. 2012) by aligning the raw forward and reverse reads. These were trimmed and reviewed for nucleotide ambiguities. Sequences were aligned with Muscle (ver. 3.2, [http://www.drive5. com/muscle/](http://www.drive5.com/muscle/); Edgar 2004) and checked for

correct reading frame in Seqotron (ver. 1.0.1, <https://github.com/4ment/seqotron>; Fourment and Holmes 2016).

Mitochondrial *COI* and *I6S* were sampled from a total of 30 and 26 *Austrocarausius* individuals respectively. A total of 23 outgroup taxa were used to estimate the *Austrocarausius* timescale (*COI* from 12 individuals, *I6S* from 22 individuals; Supplementary Table S2.4). Outgroup taxa were downloaded from GenBank where sequence data were available.

Phylogenetic trees were constructed using Bayesian inference and maximum likelihood, and partitioned by *I6S* and the 1st, 2nd and 3rd codon positions for *COI*. Bayesian inference analyses were performed in BEAST (ver. 2.6.3, <https://www.beast2.org/>; Bouckaert et al. 2014). The bModelTest package (ver. 1.3.0, <https://github.com/BEAST2-Dev/bModelTest>; Bouckaert and Drummond 2017) was used in BEAST to estimate substitution models, invariant sites and rate heterogeneity with default settings. Maximum likelihood analyses were performed using the IQ-TREE web server (ver. 1.6.12, <http://www.iqtree.org/>; Trifinopoulos et al. 2016) with 1000 bootstrap replicates. The inbuilt ModelFinder (ver. 1, <http://www.iqtree.org/ModelFinder/>) was used to specify models; *I6S* GTR+F+G4, *COI* 1st codon TIM2+F+G4, *COI* 2nd codon HKY+F+I, *COI* 3rd codon TN+F+G4. Output for both trees were visualised in Figtree (ver. 1.4.4, <http://tree.bio.ed.ac.uk/software/figtree/>, accessed 13 May 2021).

To infer the evolutionary timescale using Bayesian inference, crown groups were assigned representing five fossils, *Eophyllum messelense* at a minimum age of 47 Ma (Wedmann et al. 2007), *Clonistria* sp. at 20 Ma (Poinar 2011), *Eophasma* spp. at 44 Ma (Poinar 2011), *Anisomorphini* at 44 Ma (Sellick 1994) and *Malacomorpha* sp. at 20 Ma (Poinar 2011) (Supplementary Table S2.5). Fossil calibration nodes, including soft upper

bounds of 110 Ma for each fossil were selected based on recent studies of Phasmatodea (Robertson et al. 2018; Simon et al. 2019). A lognormal relaxed clock model was selected for our analysis and we selected a birth-death tree beforehand. A lognormal prior distribution was selected for each fossil calibration. The resulting tree was checked for adequate sampling and convergence in TRACER (ver. 1.7, <https://github.com/beast-dev/tracer/releases/tag/v1.7>; Rambaut et al. 2018). The maximum clade credibility tree was produced in TreeAnnotator (ver. 2.6.3, <https://www.beast2.org/treeannotator/>; Bouckaert et al. 2014) with 10% burn-in.

Species delimitation was performed on the browser-based implementation of the Automatic Barcode Gap Discovery (ABGD) program (ver. 2.1, <https://bioinfo.mnhn.fr/abi/public/abgd/>; Puillandre et al. 2012). Our alignment of *COI* was submitted under the JC69 model. The following settings were used: Pmin 0.001, Pmax 0.1, steps 10 and Nb bins 20. Generalised Mixed Yule Coalescent (GMYC) (Kapli et al. 2017) analyses were performed in RStudio (ver. 1.1.463, RStudio, Inc., Boston, MA, USA, <http://www.rstudio.com/>) using the BEAST output tree file with the ‘splits’ R package (ver. 1.2, T. Ezard, T. Fujisawa and T. G. Barraclough, <https://rdr.io/rforge/splits/>) downloaded from R-Forge.

### 2.3.5 Data accessibility

Molecular data for this project can be found in GenBank under the following accession numbers. For *COI*: HM425710.1, HM425711.1, HM425716.1, HM425725.1-HM425728.1, KJ201995.1, KJ202015.1, KJ202026.1, KJ202027.1, ON077343-ON077348, ON101990-ON102002; and for *16S*: OM992332-OM992357 (Supplementary Table S2.2).

### 2.3.6 Ethics and permits

All material was collected under permits with approved ethics, under the ABRS collecting and export permits for P. D. Brock, and in Queensland protected areas under the Entomological Society of Queensland Scientific Purpose Permit for National Parks and CYPAL WITK18701717-3 and State Forests WITF18701717 with Authority Holder Christine Lambkin for B. R. Jones.

## 2.4 Results

### 2.4.1 Phylogenetic analyses

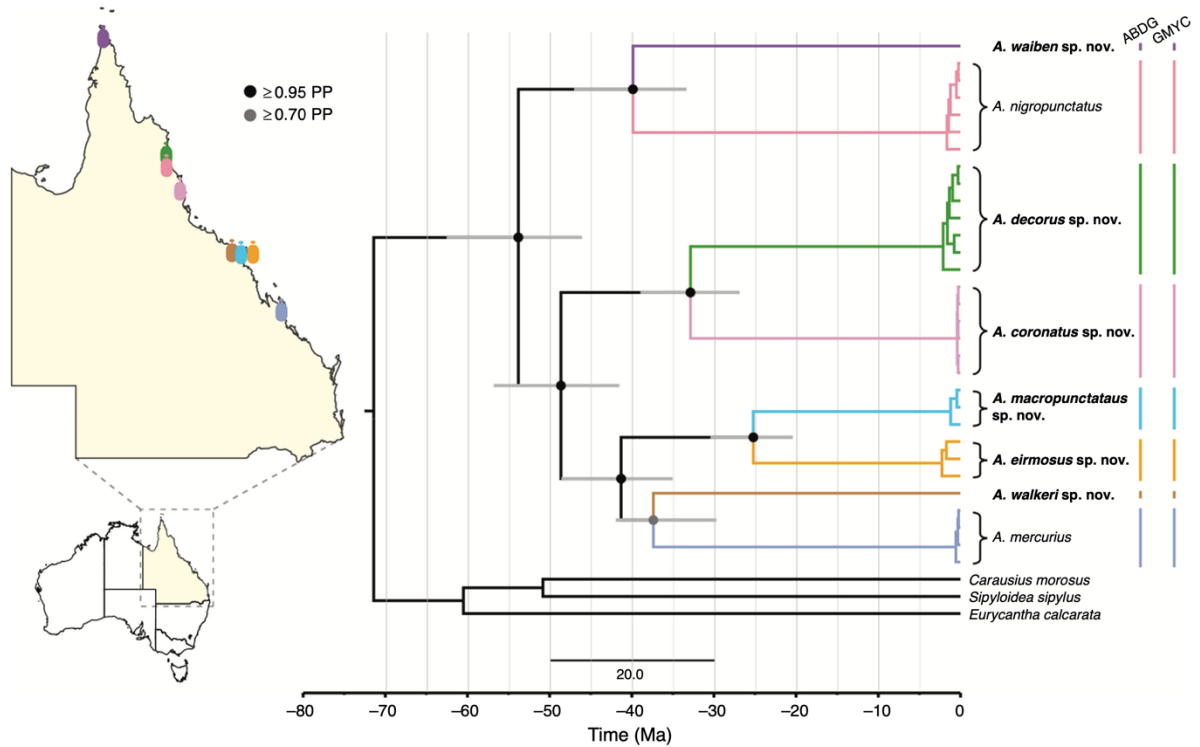
Phylogenetic relationships among *Austrocarausius* spp. were congruent between our Bayesian inference and maximum likelihood trees (Fig. 2.2, Supplementary Figure S2.1, S2.3). GMYC and ABGD analyses both divided the taxa examined into eight putative species (Fig. 2.2), in agreement with our morphological examinations (see Taxonomy section below).

Individuals from each putative species were typically found in a restricted area and displayed high endemism. In some cases, two sympatric species were found in one area, e.g. *A. decorus* and *A. nigropunctatus* from the Daintree rainforest, and *A. macropunctatus* and *A. walkeri* from the Whitsundays region.

The earliest divergence in the genus, occurring at c. 53.8 Ma (95% HPD 46.12-62.49 Ma), separates the group comprising *A. waiben* and *A. nigropunctatus* from the rest of the genus (Fig. 2.2); *A. waiben* and *A. nigropunctatus* diverged at c. 39.9 Ma (95% HPD 33.37-46.99 Ma). A second major divergence occurred at c. 48.6 Ma (95% HPD 41.56-56.77 Ma), separating *A. decorus* and *A. coronatus* from *A. macropunctatus*, *A. eirmosus*, *A. walkeri* and *A. mercurius*; *A. decorus* and *A. coronatus* diverged at c. 32.9 Ma (95% HPD 26.91-38.91

Ma). The latter represent the most southerly distributed species. *Austrocarausius macropunctatus* and *A. eirmosus* diverged from *A. walkeri* and *A. mercurius* at c. 41.3 Ma (95% HPD 35.05-48.53 Ma); *A. macropunctatus* and *A. eirmosus* diverged at c. 25.2 Ma (95% HPD 20.42-30.39 Ma), and *A. walkeri* and *A. mercurius* diverged at c. 35.5 Ma (95% HPD 29.72-41.94 Ma).

In our Bayesian analyses, all node support values had >0.95 posterior probability support (Fig. 2.2), except for the node uniting *A. walkeri* and *A. mercurius* (0.70 posterior probability). In our maximum likelihood analyses (Supplementary Figure S2.2), the divergence between *A. waiben*, *A. nigropunctatus*, *A. decorus*, and *A. coronatus* (northern clade) and *A. macropunctatus*, *A. eirmosus*, *A. walkeri* and *A. mercurius* (southern clade) had >95% bootstrap and >95 ESS values.



**Figure 2.** Fossil calibrated Bayesian inference tree for eight *Austrocarausius* species and outgroups inferred from mitochondrial *COI* and *16S*. Distant outgroups used for time calibrations are not shown here, see Supplementary Figure S2.1 for the full tree. The tree is colour-coded by species. Species distributions are likewise colour-coded by egg markers on map. Bold text indicates new species. ABGD and GMYC analyses are mapped next to species names on the tree corresponding to each species. Grey bars at each node represent 95% highest posterior density (HPD) divergence times. Node support values are shown for Bayesian inference, as PP, posterior probability. Scale bar represents substitutions per million years.

## 2.5 Taxonomy

Family **LONCHODIDAE** Brunner, 1893

Subfamily **LONCHODINAE** Brunner, 1893

Tribe **LONCHODINI** Brunner, 1893

Genus *Austrocarausius* Brock

*Austrocarausius* Brock, 2000, p. 51. Type species: *Lonchodes nigropunctatus* Kirby, 1896, p. 453, by original designation.

*Austrocarausius* Brock – Otte & Brock, 2005, p. 57; Brock & Hasenpusch, 2007, p. 69; Hennemann & Conle, 2008, p. 76; Brock & Hasenpusch, 2009, p. 160.

### Description

Described in Brock (2000, p. 52).

### Species included

*Austrocarausius coronatus* sp. nov. (northern Qld: Garradunga; ZooBank number: CC454399-B83A-4648-BA43-DE5B9311F5C5).

*Austrocarausius decorus* sp. nov. (far northern Qld: Cow Bay to Cape Tribulation; ZooBank number: 6E563988-FC74-4B69-BA2B-FB3FD14AC9CB).



*Austrocarausius eirmosus* sp. nov. (central Qld: Whitsunday Islands National Park; ZooBank number: 94FDB207-785C-46A1-BFBC-C0620D194111).

*Austrocarausius gasterbulla* sp. nov. (central Qld: Mt Macartney; ZooBank number: DA8F455B-9606-4861-8670-068DE9BEE5C6).

*Austrocarausius macropunctatus* sp. nov. (central Qld: Shute Harbour; ZooBank number: 4B75CBB7-DB6F-4C5F-BF60-6686008066FD).

*Austrocarausius mercurius* (Stål, 1877) (central Qld: Rockhampton to Byfield; ZooBank number: 7DE90F0E-3B91-4205-9598-5D19B55E3312).

*Austrocarausius nigropunctatus* (Kirby, 1896) (northern Qld: Cow Bay to Cooktown and Lizard Island; ZooBank number: FDBFAAF1-B21D-462A-A636-63A3387AD7C8).

*Austrocarausius truncatus* sp. nov. (far northern Qld: Iron Range; ZooBank number: CD533028-371A-4158-BDD2-8D2BA5F97D3C).

*Austrocarausius tuberosus* sp. nov. (far northern Qld: Mount Hemmant; ZooBank number: 09B01C1E-F81A-4D5D-9F7A-9EF99872F210).

*Austrocarausius waiben* sp. nov. (far northern Qld: Thursday Island; ZooBank number: B90F0632-863E-446A-9993-691E339555E6).

*Austrocarausius walkeri* sp. nov. (central Qld: Mount Dryander; ZooBank number: D8EB61F2-AD3A-4EB5-B997-DAAAC82D64E9).

## Key to adult *Austrocarausius* females

Unknown females: *A. truncatus*, *A. tuberosus* and *A. walkeri*.

**1** No disjointed black ventral thorax stripe ..... **2**

- Disjointed black ventral stripe on prosternum, mesosternum and metasternum, pair of spines between eyes, dark coloured tubercles over entire body, short tumescence protruding at end of abdominal segments 1-6, white bands at end of each femora (Fig. 2.3f, 2.4f, 2.5f, Supplementary Figure S2.3i, S2.4f)..... ***A. eirmosus* sp. nov.**

**2** Lacks two large black dots surrounding tubercles on mesonotum ..... **3**

- Large black dots surrounding tubercles on mesonotum, black- and brown-tipped tubercles over dorsal and lateral body surface, two black spines between eyes, white banding present at ends of all femora (Fig. 2.3e, 2.4e, 2.5e, Supplementary Figure S2.3h, S2.4e) ..... ***A. macropunctatus* sp. nov.**

**3** No lateral black stripe on head behind eyes..... **4**

- Lateral black stripe on head behind eyes, dorsal and lateral black-tipped tubercles across body, pair of spines between eyes (Fig. 2.3a, 2.4a, 2.5a, Supplementary Figure S2.3b, S2.4a) ..... ***A. waiben* sp. nov.**

4 Preopercular organ extends into abdominal segment 8 .....5

- Preopercular organ truncate, not extending into segment 8, uniform dorsal and lateral body tubercles, some black-tipped, medium- sized head protuberances between eyes, 6th abdominal segment swollen, most segments blotched with black markings, mostly on ventral surface of metanotum and abdomen, short tumescence protruding at end of each abdominal segment (Fig. 2.3d, 2.4d, 2.5d, Supplementary Figure S2.3g, S2.4d) ....*A. gasterbulla* sp. nov.

5 Pair of flange-like protuberances between eyes.....6

- Pair of spines between eyes, black granulations across dorsal and ventral body surface .....*A. nigropunctatus*

6 Fore tibiae or tarsi without dorsal lobe protrusions .....7

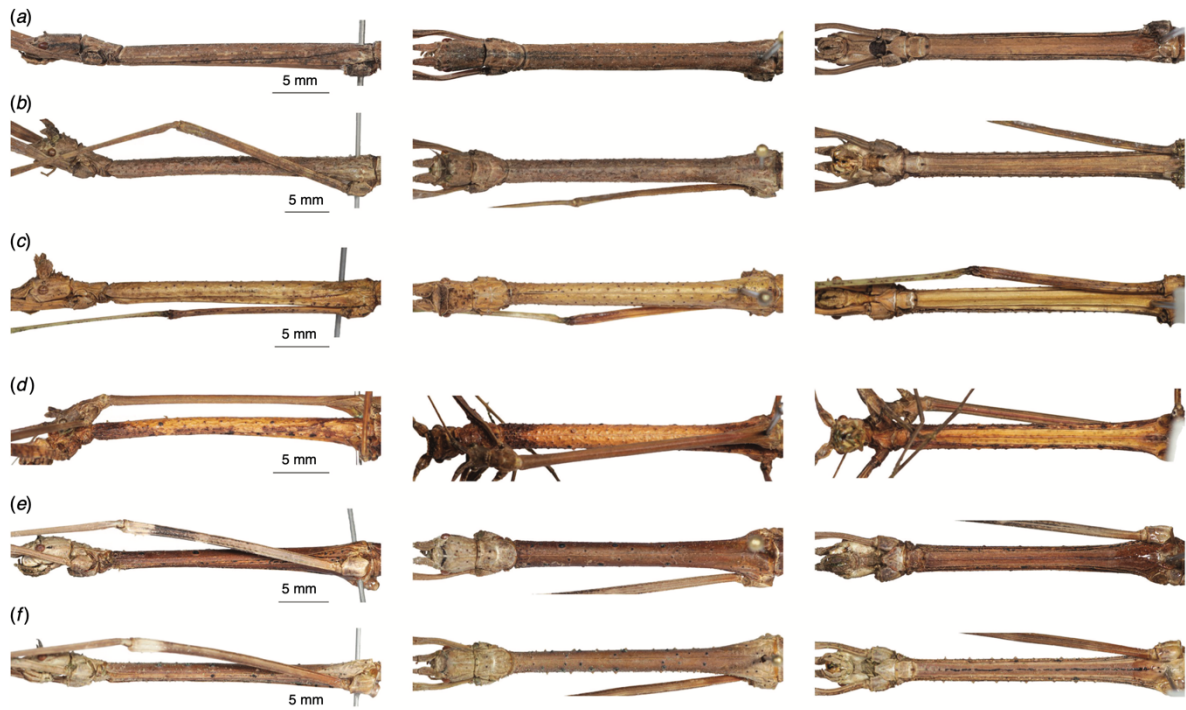
- Fore tibiae or tarsi with dorsal lobe protrusions, pair of large flange- like protuberances between eyes, three pairs of small spines behind these protuberances, each decreasing in size, black-tipped tubercles dorsally and laterally across body, ventral tubercles only on metanotum, dorsal bumps at the end of each abdominal segment (Fig. 2.3b, 2.4b, 2.5b, Supplementary Figure S2.3e, S2.4b) ..... *A. decorus* sp. nov.

7 Abdominal segment 6 swollen, dorsal and lateral black-tipped tubercles across body, flange-like protuberance between eyes..... *A. mercurius*

- Abdominal segment 6 not swollen, black-tipped tubercles dorsally and laterally across body, ventral surface of metanotum has black- tipped tubercles, large flange-like protuberance between eyes, preopercular organ spinelike with two pairs of appendage-like structures, dorsal bumps at the end of each abdominal segment (Fig. 2.3c, 2.4c, 2.5c, Supplementary Figure S2.3f, S2.4c) .....***A. coronatus* sp. nov.**



**Figure 2.3.** Female head: lateral, dorsal and anterior views. (a) *A. waiben*. (b) *A. decorus*. (c) *A. coronatus*. (d) *A. gasterbulla*. (e) *A. macropunctatus*. (f) *A. eirmosus*.



**Figure 2.4.** Female thorax: lateral, dorsal and ventral views. (a) *A. waiben*. (b) *A. decorus*. (c) *A. coronatus*. (d) *A. gasterbulla*. (e) *A. macropunctatus*. (f) *A. eirmosus*.



**Figure 2.5.** Female, end of abdomen: lateral, dorsal and ventral views. (a) *A. waiben*. (b) *A. decorus*. (c) *A. coronatus*. (d) *A. gasterbulla*. (e) *A. macropunctatus*. (f) *A. eirmosus*.

### Key to adult *Austrocarausius* males

- |                                                                                           |   |
|-------------------------------------------------------------------------------------------|---|
| 1 Genital claspers not truncate .....                                                     | 2 |
| - Genital claspers truncate, approximately equal in length to 9th abdominal segment ..... | 3 |
| 2 Dorsal bumps at the end of some abdominal segments .....                                | 4 |
| - No dorsal bumps at the end of any abdominal segments .....                              | 5 |

- 3** Body smooth throughout, no ornamentation, genital claspers truncate, appearing severed (Fig. 2.6c, 2.7c, 2.8c, Supplementary Figure S2.3) ..... *A. truncatus* sp. nov.
- Black-tipped tubercles on mesonotum and metanotum, sparser on metanotum, two spines between eyes, genital claspers rounded (Fig. 2.6d, 2.7d, 2.8d, Supplementary Figure S2.3) . *A. tuberosus* sp. nov.
- 4** Two large protuberances between eyes, two tubercles behind these protuberances, fore tarsi flanged, short tumescence protruding at end of abdominal segments 1-6 (Fig. 2.7j, 2.9e, 2.10e, Supplementary Figure S2.3j) ..... *A. walkeri* sp. nov.
- Two spines between eyes, sparse lateral tubercles on mesonotum, short tumescence protruding at end of abdominal segments 1-7 (Fig. 2.7i, 2.9d, 2.10d, Supplementary Figure S2.3i)..... *A. eirmosus* sp. nov.
- 5** No dark lateral dots or black-tipped tubercles on thorax..... **6**
- Dark lateral dots on thorax, two spines between eyes (Fig. 2.7h, 2.9c, 2.10c, Supplementary Figure S2.3h)..... *A. macropunctatus* sp. nov.
- 6** No protuberances between eyes..... **7**
- Two large protuberances between eyes, body smooth throughout, no ornamentation (Fig. 2.7g, 2.9b, 2.10d, Supplementary Figure S2.3g) ..... *A. gasterbulla* sp. nov.



|                                                                                                                                                            |                              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| 7 Pair of spines between eyes .....                                                                                                                        | 8                            |
| - No spines between eyes, genital claspers distinctly straight and sharply pointed at end (Fig. 2.6b, 2.7b, 2.8b, Supplementary Figure S2.3b) .....        |                              |
|                                                                                                                                                            | <i>A. waiben</i> sp. nov.    |
| 8 Genital claspers pointed apically .....                                                                                                                  | 9                            |
| - Genital claspers rounded apically .....                                                                                                                  |                              |
|                                                                                                                                                            | 10                           |
| 9 No white bands at ends of femora, pair of small spines between eyes .....                                                                                | <i>A. nigropunctatus</i>     |
| - White bands on apices of all femora, pair of spines between eyes (Fig. 2.6a, 2.7a, 2.8a, Supplementary Figure S2.3a).....                                |                              |
|                                                                                                                                                            | <i>A. mercurius</i>          |
| 10 Genital claspers duck bill-shaped at end, two spines between eyes, fore tarsi dorsally flanged (Fig. 2.6e, 2.7e, 2.8e, Supplementary Figure S2.3e)..... | <i>A. decorus</i> sp. nov.   |
| - Genital claspers rounded but not duck bill-shaped, two spines between eyes (Fig. 2.7f, 2.9a, 2.10a, Supplementary Figure S2.3f) .....                    |                              |
|                                                                                                                                                            | <i>A. coronatus</i> sp. nov. |



**Figure 2.6.** Male head: lateral, dorsal and anterior views. (a) *A. mercurius*. (b) *A. waiben*. (c) *A. truncatus*. (d) *A. tuberosus*. (e) *A. decorus*.

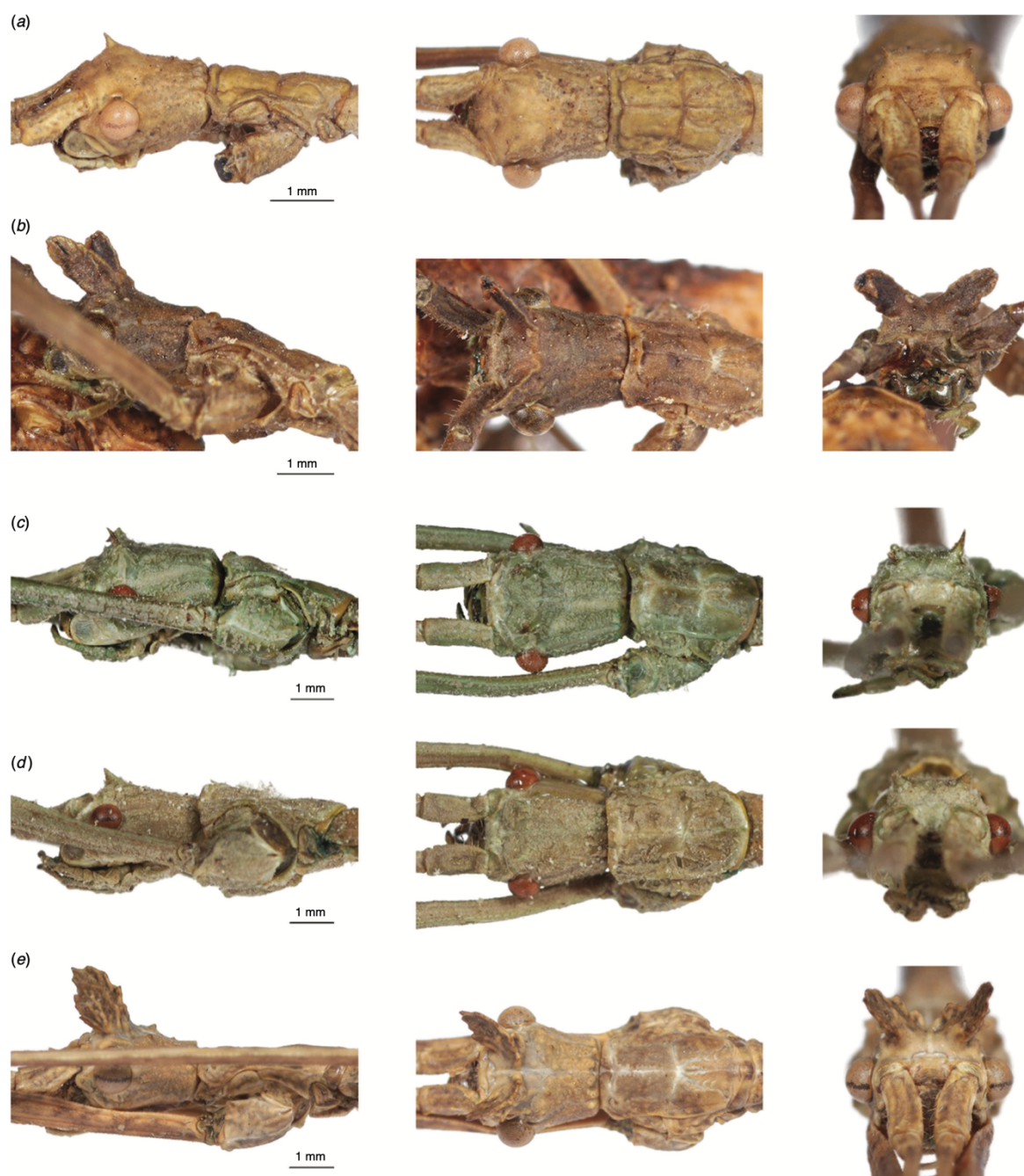


**Figure 2.7.** Male thorax: lateral, dorsal and ventral views. (a) *A. mercurius*. (b) *A. waiben*. (c) *A. truncatus*. (d) *A. tuberosus*. (e) *A. decorus*. (f) *A. coronatus*. (g) *A. gasterbulla*. (h) *A. macropunctatus*. (i) *A. eirmosus*. (j) *A. walkeri*.



**Figure 2.8.** Male, end of abdomen: lateral, dorsal and ventral views. (a) *A. mercurius*. (b) *A. waiben*. (c) *A. truncatus*. (d) *A. tuberosus*. (e) *A. decorus*.





**Figure 2.9.** Male head: lateral, dorsal and anterior views. (a) *A. coronatus*. (b) *A. gasterbulla*. (c) *A. macropunctatus*. (d) *A. eirmosus*. (e) *A. walkeri*.



**Figure 2.10.** Male, end of abdomen: lateral, dorsal and ventral views. (a) *A. coronatus*. (b) *A. gasterbulla*. (c) *A. macropunctatus*. (d) *A. eirmosus*. (e) *A. walkeri*.

### Key to eggs of *Austrocarausius*

- 1 Egg with two large lateral depressions .....2
- Egg without lateral depressions .....3

**2** Egg oval and thicker than other *Austrocarausius* eggs, lateral bulge above micropylar plate, two large circular lateral depressions, capitulum stem thin (Fig. 2.11a). *A. nigropunctatus* sp. nov.

- Egg angular and elongate, lateral pointed bulge above micropylar plate, two large oval lateral depressions, capitulum stem thin (Fig. 2.11b) ..... *A. waiben* sp. nov.

**3** Uniform surface patterns on egg ..... **4**

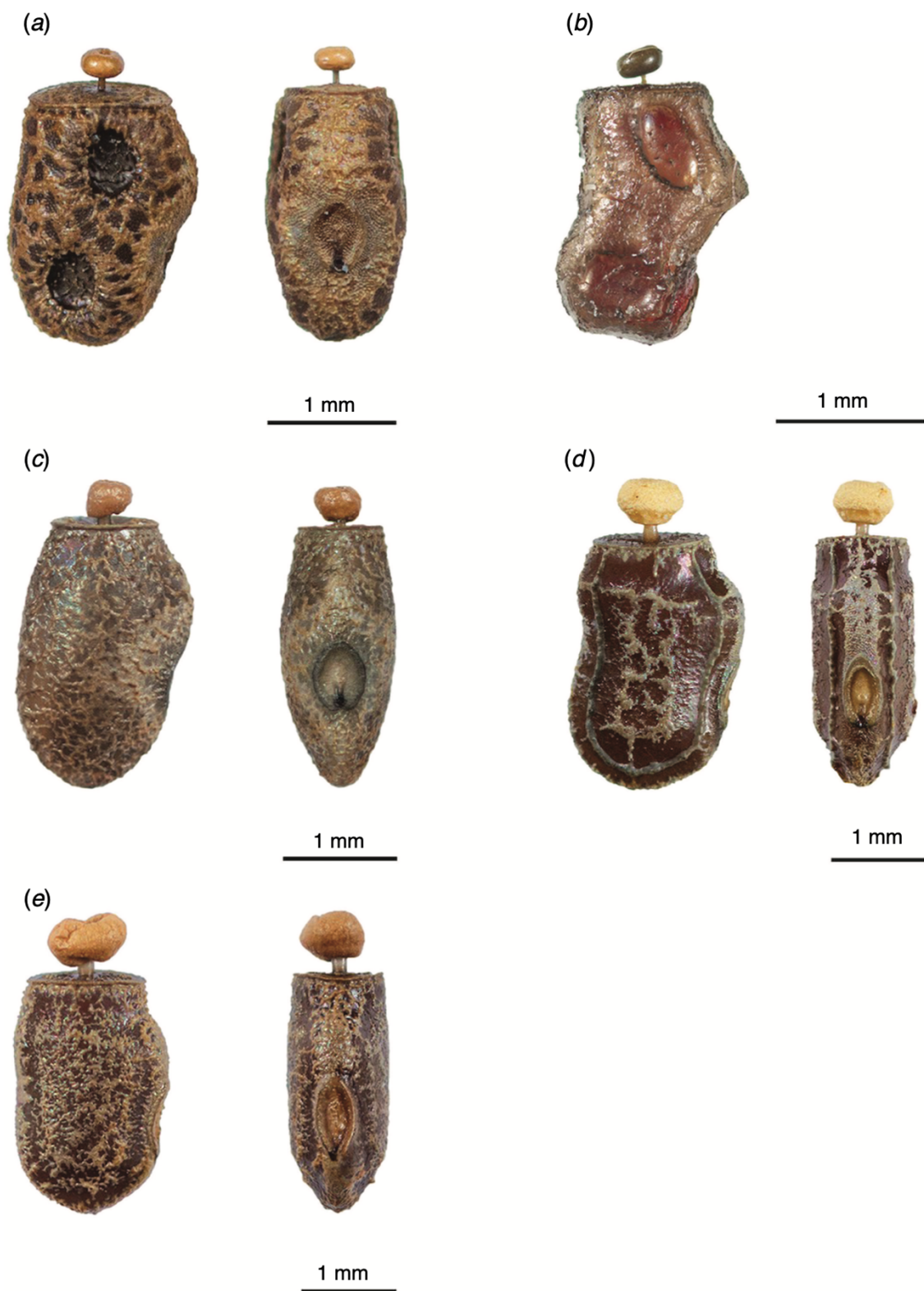
- Egg surface is smooth with network of granulation in the centre, singular ridge running along the outside of the egg on the lateral side, starting and ending at the operculum in a 'U'-like shape (Fig. 2.11d) ..... *A. macropunctatus*

**4** Micropylar plate more oval in shape ..... **5**

- Egg oval, lateral bulge above micropylar plate, surface uniformly rough, micropylar plate more round than oval (Fig. 2.11c) ..... *A. decorus* sp. nov.

**5** Egg oval, reduced lateral bulge above micropylar plate, not protruding further out than the top of the micropylar plate, surface rough, micropylar plate ovular (Fig. 2.11e).... *A. eirmosus* sp. nov.

- Egg oval, lateral bulge slightly pointed, protruding further out than the top of the micropylar plate, micropylar plate ovular ..... *A. mercurius*



**Figure 2.11.** *Austrocarausius* eggs, lateral and dorsal views. (a) *A. nigropunctatus*. (b) *A. waiben*. (c) *A. decorus*. (d) *A. macropunctatus*. (e) *A. eirmosus*.



*Austrocarausius nigropunctatus* (Kirby, 1896)

(Black-spotted Stick-insect)

*Lonchodes nigropunctatus* Kirby, 1896, p. 453. Holotype: ♀ Australia: Qld, Lizard Island (NHMUK).

*Lonchodes nigropunctatus* Kirby – Rainbow, 1897, p. 38; Tepper, 1903, p. 278.

*Staelonchodes nigropunctatus* (Kirby) – Kirby, 1904, p. 317; Vickery, 1983, p. 10; Balderson et al. 1998, p. 374.

*Austrocarausius nigropunctatus* (Kirby) – Brock, 2000, p. 52; Otte & Brock, 2005, p. 57; Brock & Hasenpusch, 2007, p. 69; Brock & Hasenpusch, 2009, pp. 80, 160.

*Carausius macerrimus* Brunner von Wattenwyl, 1907, p. 275. Holotype: ♂ Australia: Qld, Cooktown (NHMW), synonymised by Brock and Hasenpusch (2007, p. 70).

*Carausius macerrimus* Brunner – Vickery, 1983, p. 4; Balderson et al. 1998, p. 364; Brock, 2000, p. 83; Otte & Brock, 2005, p. 85.

## **Description**

### **Female**

Described in Brock (2000, p. 52) (NHMUK).

## **Male**

Described in Brunner von Wattenwyl (1907, p. 275) (as the synonym *Carausius macerrimus*).

## **Egg**

Described in Brock (2000, p. 52) (NHMUK) (Fig. 2.11a).

## **Specimens examined**

♂ Quandong Road, Cow Bay, Qld, 16°13'51"S, 145°27'15"E, 30.i.2019, B. Jones and N. Tweed, BJ-240 (QM; Registration number: T258567; GenBank numbers: ON101990, OM992332); ♂ same data, BJ-242 (QM; Registration number: T258569; GenBank numbers ON101991, OM992334); ♂ same data, BJ-241 (QM; Registration number: T258568; GenBank number: OM992333); ♂ same data, BJ-243 (QM; Registration number: T258570; GenBank numbers: ON101992, OM992335); ♀ same data, BJ-245 (QM; Registration number: T258571; GenBank numbers: ON077343, OM992337); ♀ same data, BJ-246 (QM; Registration number: T258572; GenBank numbers: ON101994, OM992338).

*Austrocarausius mercurius* (Stål, 1877)

(Plain Stick-insect)

*Carausius mercurius* Stål, 1877, p. 62. Holotype: ♀ Australia: Qld, Rockhampton, Mus. Godeffroy (NHRS).

*Carausius mercurius* Stål – Kirby, 1904, p. 320; Balderson et al., 1998, p. 364.

*Austrocarausius mercurius* (Stål) – Brock, 2000, p. 52; Otte & Brock, 2005, p. 57; Brock and Hasenpusch, 2007, p. 69; observations are noted from NSW, but physical specimens do not exist for this observation, which is likely incorrect (Brock and Hasenpusch, 2009, pp. 79, 160).

*Carausius australicus* Brunner von Wattenwyl, 1907, p. 273. Lectotype: ♀ Australia: Qld, Rockhampton, leg. Thorey (NHMW) designated by Brock (2000, p. 52). Paralectotype series: ♀ Australia: Qld, Rockhampton, leg. Thorey (NHMW); 2 ♀♀ Australia: Qld, Rockhampton, leg. Thorey (ZMUH); ♀ Australia: Qld, Peak Downs (ZMUH), synonymised by Brock (2000, p. 52).

*Carausius australicus* Brunner – Vickery, 1983, p. 4; Balderson et al., 1998, p. 364.

## Diagnosis

Pair of short spines between eyes. Body smooth, with no flanges (Fig. 2.6a, 2.7a, 2.8a, Supplementary Figure S2.3a, Supplementary Table S2.3).

## **Description**

### **Female**

Described in Brock and Hasenpusch (2007) (NHRS).

### **Male**

Uniform brown with reddish tinge on the thorax. White bands present on apices of all femora.

**Head.** Elongate, width  $0.6\times$  length. Antennae long, 62 segments, extending past forelegs,  $\sim 2.5\times$  length of fore femora. Pair of spines present between eyes (Fig. 2.6a).

**Thorax.** Pronotum slightly shorter than head, mesonotum  $8\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum (Fig. 2.7a).

**Abdomen.** Segments 2-5 are equal in length,  $2\times$  length of median segment. Segments 6 and 7 equal in length, slightly shorter than segments 2-5. Segments 8 and 9 equal in length,  $\sim 0.5\times$  length of segment 10. Cerci concealed,  $0.2\times$  length of 10th abdominal segment (Fig. 2.8a).  
Genital claspers pointed.

**Legs.** Without ornamentation (Supplementary Figure S2.3a).

## **Egg**

Described in Brock (2000, p. 52) (NHMUK).

## **Measurements (mm)**

For 2 males. Body length: 82.4-92. Head: 2.1-2.8. Antennae: 62 segments. Pronotum: 2.3-2.6. Mesonotum: 19.9-22.1. Metanotum: 11.0-12.4. Median segment: 3.2-3.5. Femora: fore, mid, hind: 22.4-24.4, 17.1, 19.7-21.4. Tibiae: fore, mid, hind: 24.8-26.9, 18.3, 24.1-27.1.

## **Variation among allotypes**

Females uniform dark brown or heavily mottled with grey and dark brown. Dorsal surface of head behind eyes sometimes light brown. Thick, white, dorsal longitudinal stripe on last two abdominal segments.

## **Specimens examined**

Allotypes: ♂ Australia: Qld, Byfield 19.xi.2011 P. Brock and N. Tweed, PB-0078 (QM; Registration number: T250901; GenBank number: KJ202015.1); ♂ same data, PB-0079 (QM; Registration number: T250900; GenBank numbers: KJ202027.1, OM992352).

Specimens used in DNA only: PB-0081 (GenBank numbers: KJ201995.1, OM992353); PB-0080 (GenBank number: KJ202026.1).

*Austrocarausius waiben* sp. nov.

(Waiben Stick-insect)

**Diagnosis**

Females with black-tipped tubercles sparsely scattered across head, thorax and first three segments of abdomen (Fig. 2.3a, 2.4, 2.5a, Supplementary Figure S2.3b, S2.4a, Supplementary Table S2.3). Two small spines between eyes. Males uniformly smooth (Fig. 2.6b, 2.7b, 2.8b, Supplementary Figure S2.3b). Genital claspers distinctly straight and apically pointed.

**Description**

**Female**

Body uniform brown. Lateral black stripe on head behind eyes.

**Head.** Small pair of spines between eyes (Fig. 2.3a). Head smooth, with sparse black-tipped tubercles. Head slightly longer than wide. Antennae reaching end of forelegs, 54 segments, ~2× length of fore femora.

**Thorax.** Pronotum slightly shorter than head, mesonotum  $6\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum. Metanotum sparsely covered in dorsal only black-tipped tubercles (Fig. 2.4a).

**Abdomen.** First three segments sparsely tuberculate. Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of 2-6. Segments 9 and 10 equal in length and  $0.6\times$  length of segment 8. Tip of operculum rounded, sub-acute (Fig. 2.5a). End of supra-anal plate forming a rounded bump. End of operculum and supra-anal plate level. Supra-anal plate  $\sim 0.3\times$  length of anal segment. Cerci mostly concealed, approximately equal in length to the supra-anal plate. End of preopercular organ consists of two outer lobes either side of a spine, almost reaching end of segment 8 (Fig. 2.5a).

**Legs.** Without ornamentation (Supplementary Figure S2.3b).

## **Male**

Uniform green.

**Head.** Longer than wide (Fig. 2.6b). Antennae long, reaching fore tarsi, 74 segments,  $2\times$  length of fore femora.

**Thorax.** Smooth (Fig. 2.7b). Pronotum  $0.75\times$  head length, mesonotum  $7\times$  length of pronotum length. Metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7 is slightly shorter than segments 2-6. Segments 8 and 9 equal in length and  $\sim 0.5\times$  length of segment 10. Cerci visible,  $0.3\times$  length of 10th abdominal segment (Fig. 2.8b). Genital claspers very straight and apically pointed.

**Legs.** Without ornamentation (Supplementary Figure S2.3b).

## **Egg**

Small, flat, somewhat angular and elongate with lateral bulge above micropylar plate that comes to a sharp point (Fig. 2.11b). Egg capitate, connected by a thin stem. Egg surface is uniformly coarse. Two large oval lateral depressions. Micropylar plate and dorsal view too damaged to make any observations.

## **Measurements (mm)**

For 3 females and 1 male. Body length: female 79-92, male 66. Head: female 4.2-4.5, male 3.1. Antennae: female 54 segments, male 74 segments. Pronotum: female 3.1-3.7, male 2.4. Mesonotum: female 18.6-22.2, male 16.4. Metanotum: female 8.5-9.8, male 7.8. Median segment: female 3.2-4.5, male 3.0. Femora: fore, mid, hind: female 21.1-22.3, 16.5-17.6,



19.6-20.6, male 19.5, 14.6, 16.9. Tibiae: fore, mid, hind: female 20.3-22.2, 15.9-17.6, 20.0-21.6, male 21.2, 16.1, 21.1. Eggs: capsule length 2.49, height 1.44, width too damaged for accurate measurements.

### **Variation among paratypes**

Females sometimes reddish over dorsal and ventral surfaces of thorax and median segment. May also have black dorsal stripe running either head to thorax or entire length of body.

### **Specimens examined**

Holotype: ♀ Qld, Thursday Island, 28 Sep 2020, Dominic Funnell, BJ-324 (QM; Registration number: T250904). Paratypes: ♂ same data, BJ-323 (QM; Registration number: T250903); ♀ same data, BJ-327 (QM; Registration number: T250906); ♀ same data, BJ-326 (QM; Registration number: T250905). Specimens used in DNA only: ♀ Qld, Thursday Island, 28 Sep 2020, Dominic Funnell, BJ-322 (QM; Registration number: T250902; GenBank numbers: ON102002, OM992351).

### **Etymology**

Waiben is the local indigenous name for Thursday Island, the type locality of the species. To be treated as a noun in apposition.

*Austrocarausius truncatus* sp. nov.

(Truncate Stick-insect)

**Diagnosis**

Males notably shorter than in other species (Fig. 2.6c, 2.7c, 2.8c, Supplementary Figure S2.3c). Without ornamentation. Apices of male genital claspers truncate, distinct from other species.

**Description**

**Female**

Not known.

**Male**

Uniform brown.

**Head.** Elongate, width  $0.6\times$  length (Fig. 2.6c). Antennae long, 68 segments, extend past forelegs,  $\sim 2.5\times$  length of fore femora.

**Thorax.** Pronotum  $0.6\times$  head length, mesonotum  $8\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum (Fig. 2.7c).

**Abdomen.** Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of 2-6. Segments 8-10 equal in length. Cerci concealed,  $0.25\times$  length of 10th abdominal segment (Fig. 2.8c). Apices of genital claspers truncate.

**Legs.** Without ornamentation (Supplementary Figure S2.3c).

## **Egg**

Not known.

## **Measurements (mm)**

For 1 male. Body length: 71. Head: 3.2. Antennae: 68 segments. Pronotum: 2.5. Mesonotum: 19.3. Metanotum: 8.9. Median segment: 2.8. Femora: fore, mid, hind: 21.8, 15.3, 20.2. Tibiae: fore, mid, hind: 25.6, 18.4, 26.5.

## **Specimens examined**

Holotype; ♂ Gordon's Mine Area, Iron Range, N. Qld. 12-19.ii.1996, G. B. Monteith, Rainforest (QM; Registration number: T250907).

## **Etymology**

The species name is derived from the shape of the genital claspers.

*Austrocarausius tuberosus* sp. nov.

(Tuber Stick-insect)

## **Diagnosis**

Males lack foreleg flanges and have small spines between eyes (Fig. 2.6d, 2.7d, 2.8d, Supplementary Figure S2.3d). Black tipped dorsal and lateral tubercles on mesonotum and metanotum, sparser on metanotum.

## **Description**

### **Female**

Not known.

### **Male**

Uniform brown.

**Head.** Slightly longer than wide (Fig. 2.6d). Antennae long, 35 segments, reaching beyond end of forelegs,  $\sim 2.25\times$  length of fore femora.

**Thorax.** Pronotum and head equal in length, mesonotum  $7\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum (Fig. 2.7d).

**Abdomen.** Short tumescence protruding at end of abdominal segments 1-3. Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of segments 2-6. Segments 8 and 9 equal in length and  $\sim 0.6\times$  length of segment 10. Cerci short,  $0.3\times$  length of segment 10 (Fig. 2.8d). Genital claspers short and rounded.

**Legs.** Without ornamentation (Supplementary Figure S2.3d).

## **Egg**

Not known.

## Measurements (mm)

For 1 male. Body length: 84. Head: 3. Antennae: 35 segments. Pronotum: 3.3. Mesonotum: 22.5. Metanotum: 10.2. Median segment: 3.8. Femora: fore, mid, hind: 23.3, 16.6, 19.1. Tibiae: fore, mid, hind: 26.3, 18.0, 24.0.

## Specimens examined

Holotype: ♂ north-eastern Qld: 16°07'S, 145°25'E, Mt Hemmant, 1050 m, 25-27.xi.1993, Monteith, Cook, Janetzki and Roberts (QM; Registration number: T250908).

## Etymology

The species name is derived from the black tipped protuberances on the thorax.

*Austrocarausius decorus* sp. nov.

(Flanged Stick-insect)

## Diagnosis

Females with black tipped tubercles over body, ventral tubercles only on metanotum (Fig. 2.3b, 2.4b, 2.5b, Supplementary Figure S2.3e, S2.4b, Supplementary Table S2.3). Pair of large flange-like protuberances between eyes, with three smaller pairs caudally, each reducing in size. Fore tibiae and tarsi have dorsal flange like ridge, fore tibiae with two dorsal

lobes and tarsi with one. Short tumescence protruding at end of each abdominal segment. Males smooth, with two small spines between eyes (Fig. 2.6e, 2.7e, 2.8e, Supplementary Figure S2.3e). Fore tarsi dorsally flanged. Thorax with few black dots sparsely covering dorsal surface.

## **Description**

### **Female**

Uniform brown, fore tibiae flanges green.

**Head.** Pair of large flange-like protuberances between eyes (Fig. 2.3b). Two pairs of blunt spines and rounded tubercles behind horns. Head slightly longer than wide. Antennae long, 71 segments, extending past forelegs,  $\sim 2\times$  length of fore femora.

**Thorax.** Black tipped tubercles are present dorsally and laterally, ventral tubercles only on metanotum (Fig. 2.4b). Pronotum slightly shorter than head, mesonotum  $8\times$  length of pronotum. Metanotum  $\sim 0.5\times$  length of mesonotum.

**Abdomen.** Covered in dorsal and ventral black tipped tubercles. Short tumescence protruding at end of each abdominal segment. Operculum surpassing last abdominal segment and in line with the supra-anal plate (Fig. 2.5b). Operculum terminating ventrally in a point. End of operculum and supra-anal plate truncate. Cerci short, reaching end of last abdominal

segment. Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7 slightly shorter than segment 2-6. Segments 9 and 10 equal in length and  $0.2\times$  shorter than segment 8. Preopercular organ extending to  $0.5\times$  length of segment 8, from midpoint abruptly becoming constricted and spinelike (Fig. 2.5b).

**Legs.** Fore tibiae and tarsi highly flanged, with two dorsal lobes on tibiae and one on tarsi; flange like ridge present between lobes (Supplementary Figure S2.3e).

## **Male**

Dark brown.

**Head.** Pair of spines between eyes, with two smaller pairs caudally (Fig. 2.6e). Head slightly longer than wide. Antennae reach beyond forelegs, 76 segments,  $\sim 2.5\times$  length of fore femora.

**Thorax.** Smooth with few black dots sparsely covering dorsal surface (Fig. 2.7e). Pronotum and head equal in length, mesonotum  $9\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Segments 2-5 equal in length,  $2\times$  length of median segment. Segment 6 slightly shorter than segments 2-5, segment 7 slightly shorter than segment 6. Segments 8 and 9 equal



in length and  $\sim 0.5\times$  length of segment 10. Cerci concealed,  $0.2\times$  length of 10th abdominal segment (Fig. 2.8e). Genital claspers rounded, bend outwards.

**Legs.** Fore tarsi dorsally flanged (Supplementary Figure S2.3e).

### **Egg**

Small, flat, oval in shape with a lateral bulge above the micropylar plate (Fig. 2.11c). Egg capitulate, connected by enlarged stem. Egg surface rough throughout. Micropylar plate low and round.

### **Measurements (mm)**

For 3 females and 3 males. Body length: female 85-130, male 76-94. Female can occur in lichen form at a significantly reduced body size. Head. Female 2.8-4.4, male 2-2.4. Antennae: female 71 segments, male 76 segments. Pronotum: female 2.4-3.9, male 2.2-2.9. Mesonotum: female 18.5-30.1, male 18.1-23.0. Metanotum: female 13.5-18.1, male 11.1-15.4. Median segment: female 3.5-4.6, male 2.7-3.5. Femora: fore, mid, hind: female 19.3-29.9, 13.4-21.4, 15.1-23.0, male 21.6-24.1, 13.6-17.6, 16.2-20.3. Tibiae: fore, mid, hind: female 19.0-32.3, 14.1-21.2, 18.1-27.0, male 21.6-29.5, 15.4-19.9, 21.8-27.1. Eggs: capsule length 2.25, height 1.58, width 1.29.

## Variation among paratypes

Females may have some mottling of darker brown over body. Males can be uniform green.

Females may have reduced dorsal head protuberances and swollen 6th abdominal segments.

Male tarsi can be found without dorsal flange.

## Specimens examined

Holotype: ♀ Cape Tribulation Road, Thornton Beach, Qld, 16°10'24"S, 145°26'27"E, 3.ii.2018. B. Jones and N. Tweed, BJ-294 (QM; Registration number: T250911; GenBank numbers: ON101995, OM992340). Paratypes: ♂, Quandong Road, Cow Bay, Qld, 16°13'51"S, 145°27'15"E, 30J.i.2019, B. Jones and N. Tweed, BJ-244 (QM; Registration number: T250909; GenBank numbers: ON101993, OM992336). ♀ Cape Tribulation Rd, Cape Tribulation, Qld, 16°05'25"S, 145°27'52"E, 19.v.2019, B. Jones BJ-295 (QM; Registration number: T250912; GenBank numbers: ON077345, OM992341). ♀ Cape Tribulation Rd, Cape Tribulation, Qld, 16°05'25"S, 145°27'52"E, 19.v.2019, B. Jones BJ-298 (QM; Registration number: T250915; GenBank numbers: ON077347, OM992344). ♂ Cape Tribulation Rd, Cape Tribulation, Qld, 16°05'25"S, 145°27'52"E, 19.v.2019, B. Jones BJ-296 (QM; Registration number: T250913; GenBank numbers: ON101996, OM992342). ♂ Cape Tribulation Rd, Cape Tribulation, Qld, 16°05'25"S, 145°27'52"E, 19.v.2019, B. Jones BJ-297 (QM; Registration number: T250914; GenBank numbers: ON077346, OM992343).

## Etymology

The species is named after the Latin *decorus*, meaning beautiful or ornamented, in reference to the flanged fore legs and head protuberance of this species.

## Notes

Females may have unflanged tarsi and tibiae. Males and females observed to feign death when disturbed. Female lichen form was observed to hang vertically holding branch with only the two midleg tarsi, whereas all legs were straightened lengthwise along body.

*Austrocarausius coronatus* sp. nov.

(Crowned Stick-insect)

*Austrocarausius mercurius* ♀ Brock & Hasenpusch, 2009, p. 79.

*Austrocarausius mercurius* ♂ Brock & Hasenpusch, 2009, p. 79.

*Austrocarausius mercurius* egg Brock & Hasenpusch, 2009, p. 79.

## Diagnosis

Females have tubercles across body, black-tipped on thorax (Fig. 2.3c, 2.4c, 2.5c, Supplementary Figure S2.3f, S2.4c, Supplementary Table S2.3). Pair of large flange-like

protuberances between eyes, notably larger than any other species. Dorsal flanges found on fore tarsi and tibiae. This species has black-tipped tubercles over body. Short tumescence protruding at end of each abdominal segment. Male body without ornamentation, two spines between eyes (Fig. 2.7f, 2.9a, 2.10a, Supplementary Figure S2.3f).

## **Description**

### **Female**

Mottled light and dark brown with blotches of dark spots, mostly on ventral side.

**Head.** Pair of large flange-like protuberances between eyes (Fig. 2.3c). Behind this protuberance are two pairs of spines each smaller than the other. Other tubercles and smaller spines can be found on the dorsal side of the head. Head slightly longer than wide. Antennae long, 58 segments, extending past forelegs,  $\sim 1.5\times$  length of fore femora.

**Thorax.** Black-tipped tubercles cover the dorsal and lateral surfaces of whole thorax, only metanotum has ventral black-tipped tubercles (Fig. 2.4c). Pronotum and head equal in length, mesonotum  $8\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Short tumescence protruding at end of each abdominal segment. Abdomen speckled with black-tipped tubercles throughout the dorsal and ventral surface. Segments 2-5 equal in length,  $2\times$  length of median segment. Segment 6 slightly shorter than segments 2-5.

Segment 7 slightly shorter than segment 6. Segments 8 and 9 equal in length and  $\sim 0.6\times$  length of segment 10. Segments 9 and 10 equal in length and 0.3 shorter than segment 8. Operculum shape is angular but extends to end of supra-anal plate (Fig. 2.5c). End of operculum is round but supra-anal plate is emarginate. Cerci short, extending  $0.5\times$  length of the supra-anal plate. Preopercular organ extends to  $0.5\times$  length of segment 8, spine-like in shape, consisting of two pairs of elongated appendages on either side of the preopercular organ (Fig. 2.5c).

**Legs.** Fore tibiae and tarsi have dorsal flange like ridge (Supplementary Figure S2.3f).

## **Male**

Uniform brown, thorax has a dorsal reddish tinge.

**Head.** Pair of small spines between eyes, dorsal surface heavily tuberculate (Fig. 2.9a). Antennae reach to the end of the fore tarsi, 69 segments. Head slightly longer than wide. Antennae extends past forelegs,  $\sim 2.5\times$  length of fore femora.

**Thorax.** Thorax smooth (Fig. 2.7f). Pronotum and head equal in length, mesonotum  $9\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Segments 2-5 equal in length,  $2\times$  length of median segment. Segment 6 slightly shorter than segments 2-5. Segment 7 slightly shorter than segment 6. Segments 8 and 9 equal in length and  $\sim 0.6\times$  length of segment 10. End of genital claspers curved (Fig. 2.10a). Genital claspers parallel and partly conceal cerci. Cerci short, extending  $0.2\times$  length of 10th abdominal segment.

**Legs.** Without ornamentation (Supplementary Figure S2.3f).

## **Egg**

Not known.

## **Measurements (mm)**

For 1 female and 1 male. Body length: female 110, male 83. Head: female 3.8, male 2.2.

Antennae: female 58 segments, male 69 segments. Pronotum: female 3.2, male 2.3.

Mesonotum: female 24.9, male 20.2. Metanotum: female 14.5, male 11.5. Median segment: female 4.5, male 3. Femora: fore, mid, hind: female 23.3, 17.1, 18.8, male 21.0, 15.7, 18.6.

Tibiae: fore, mid, hind: female 22.4, 17.3, 22.7, male 26.0, 16.9, 23.3.

## **Specimens examined**

Holotype: ♀ Polly Ck, Garradunga, N. Qld, 27.xi.2009, J. Hasenpusch, JH-0055 (QM;

Registration number: T250917; GenBank numbers: HM425711.1, OM992354). Paratype: ♂

Polly Ck, Garradunga, N. Qld, 27.xi.2009, J. Hasenpusch, JH-0054 (QM; Registration number: T250918; GenBank numbers: HM425710.1, OM992356). Specimens used in DNA only: PB-0028 (GenBank number: HM425728.1), PB-0026 (GenBank number: HM425727.1), PB-0025 (GenBank numbers: HM425726.1, OM992357), PB-0024 (GenBank numbers: HM425725.1, OM992355).

### **Etymology**

Derived from the Latin corona meaning crown, based on the distinct crown-like head structure of the female. The species was also discovered during the corona-virus pandemic in 2020.

*Austrocarausius gasterbulla* sp. nov.

(Gaster Stick-insect)

### **Diagnosis**

Females have uniform tubercles across body, black-tipped on thorax (Fig. 2.3d, 2.4d, 2.5d, Supplementary Figure S2.3g, S2.4d). Pair of moderately sized protuberances between eyes. Fore tibiae and tarsi are dorsally flanged. Short tumescence protruding at end of each abdominal segment. Sixth abdominal segment swollen. Females have black spots sparsely covering body. Male body without ornamentation (Fig. 2.7g, 2.9b, 2.10b, Supplementary Figure S2.3g), pair of horns between eyes.

## **Description**

### **Female**

Blotched with black spots sparsely over body, mostly on ventral side. Female mottled light and dark brown.

**Head.** Pair of moderately sized flange-like protuberances between eyes (Fig. 2.3d). Heavily covered in dorsal and lateral tubercles. Head slightly longer than wide. Antennae do not extend past forelegs, 48 segments,  $\sim 1.5\times$  length of fore femora.

**Thorax.** Thorax dorsally and laterally tuberculate throughout (Fig. 2.4d). Pronotum and head equal in length, mesonotum  $6\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Dorsally and laterally tuberculate across abdomen. Abdominal segment 6 swollen and has a rough tuberculate surface (Supplementary Figure S2.4d). Short tumescence protruding at end of each abdominal segment. Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of segments 2-6. Segments 9 and 10  $0.5\times$  length of segment 8. Tip of operculum and supra-anal plate rounded (Fig. 2.5d). Operculum extends past the supra-anal plate by approximately  $0.3\times$  length of the supra-anal plate. Cerci are not visible. Preopercular organ rounded and short, not extending into segment 8 (Fig. 2.5d).



**Legs.** Fore tibiae and tarsi have dorsal flange-like ridge (Supplementary Figure S2.3g).

## **Male**

Uniform brown, thorax has a dorsal reddish tinge.

**Head.** Slightly longer than wide (Fig. 2.9b). Antennae extend past forelegs, 59 segments,  $\sim 2.5\times$  length of fore femora. Large pair of horns between eyes.

**Thorax.** Thorax smooth (Fig. 2.7g). Pronotum and head equal in length, mesonotum  $6\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Segments 2-5 equal in length,  $2\times$  length of median segment. Segment 6 slightly shorter than segments 2-5. Segment 7 slightly shorter than segment 6. Segment 9  $0.6\times$  length of segment 8 and  $\sim 0.5\times$  length of segment 10. Cerci concealed,  $0.2\times$  length of 10th abdominal segment (Fig. 2.10b). Genital claspers rounded.

**Legs.** Without ornamentation (Supplementary Figure S2.3g).

## **Egg**

Not known.

## **Measurements (mm)**

For 1 female and 1 male. Body length: female 112, male 92. Head: female 4, male 2.6.

Antennae: female 48 segments, male 59 segments. Pronotum: female 4, male 2.7.

Mesonotum: female 25.5, male 23.6. Metanotum: female 13.9, male 12.5. Median segment:

female 4.7, male 3.6. Femora: fore, mid, hind: female 24.0, 16.6, 17.6, male 25.4, 17.0, 20.2.

Tibiae: fore, mid, hind: female 22.7, 16.8, 20.3, male 29.8, 20.2, 27.1.

## **Specimens examined**

Holotype; ♀ Mt Macartney, 600-850 m, Cathu State Forest, C. Qld, 20-21.iv.1979 G. B.

Monteith (QM; Registration number: T250919). Paratype: ♂ Mt Macartney, 600-850 m,

Cathu State Forest, C. Qld, 20-21.iv.1979 G. B. Monteith (QM; Registration number:

T250920).

## **Etymology**

The new species is a combination of the Latin gaster, meaning belly and bulla, meaning bubble, in reference to the swollen and bulbous 6th abdominal segment of the female. To be treated as a noun in apposition.

*Austrocarausius macropunctatus* sp. nov.

(Black-dotted Stick-insect)

**Diagnosis**

Females are covered in black- and brown-tipped tubercles (Fig. 2.3e, 2.4e, 2.5e, Supplementary Figure S2.3h, S2.4e, Supplementary Table S2.3). Pair of black spines between eyes. Insects have large black dots surrounding tubercles on mesonotum. Male body without ornamentation (Fig. 2.7h, 2.9c, 2.10c, Supplementary Figure S2.3h). Pair of spines between eyes. Dark dots present on thorax of both sexes.

**Description**

**Female**

Uniform brown, white banding present on all femora.

**Head.** Two large black spines between eyes, longer than wide, with several smaller black-tipped spines caudally (Fig. 2.3e). Antennae extend past forelegs, 104 segments,  $\sim 2.5\times$  length of fore femora.

**Thorax.** Dorsal and lateral thorax sparsely covered in black-tipped and brown tubercles, on mesonotum black-tipped tubercles are surrounded by black (Fig. 2.4e). Pronotum and head equal in length, mesonotum  $7\times$  length of pronotum, metanotum  $0.5\times$  length of mesonotum.

**Abdomen.** Smooth with black-tipped tubercles. Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of segments 2-6. Segments 9 and 10 equal in length,  $0.6\times$  length of segment 8. End of supra-anal plate is mucronate (Fig. 2.5e). Overall shape of operculum curves to the supra-anal plate. The end of the operculum is rounded. Preopercular organ square and short, not extending into segment 8 (Fig. 2.5e).

**Legs.** Without ornamentation (Supplementary Figure S2.3h).

## **Male**

Light brown with green legs.

**Head.** Pair of large elongate spines between eyes (Fig. 2.9c). Head otherwise without tubercles. Head slightly longer than wide. Antennae extend past forelegs, 130 segments,  $2.5\times$  length of fore femora.

**Thorax.** Smooth thorax with few darker dots covering parts of the dorsal and lateral surface (Fig. 2.7h). Pronotum slightly longer than head, mesonotum  $8\times$  length of pronotum, metanotum slightly more than  $0.5\times$  length of mesonotum.

**Abdomen.** Abdominal segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of segments 2-6. Segment 9  $0.6\times$  length of segment 8 and  $\sim 0.5\times$  length of segment 10. Cerci short,  $0.2\times$  length of 10th abdominal segment (Fig. 2.10c). Genital claspers pointed at end, dorsal bump at half the length.

**Legs.** Without ornamentation (Supplementary Figure S2.3h).

## **Egg**

Small, flat, roughly oval in shape, lateral bulge above the micropylar plate (Fig. 2.11d). Egg capitula connected by enlarged stem. Underlying egg surface smooth with network of granulation in the centre, on both lateral and dorsal sides. Singular ridge running along the outside of the egg on the lateral side, starting and ending at the operculum in a 'U'-like shape. Micropylar plate is low and ovular.

## **Measurements (mm)**

For 1 female and 2 males. Body length: female 121, male 98-103. Head: female 4.6, male 2.8-3.3. Antennae: female 104 segments, male 130 segments. Pronotum: female 3.7, male

2.8-3. Mesonotum: female 26.2, male 23.7-24.9. Metanotum: female 15.3, male 14.7-15.3. Median segment: female 4.3, male 2.9-3.3. Femora: fore, mid, hind: female 31.7, 22.7, 25.3, male 30.8-34.1, 23.7-26.6, 27.1-30.7. Tibiae: fore, mid, hind: female 33.9, 25.4, 31.4, male 38.3-40.9, 30.2-32.5, 38.5-42.5. Eggs: capsule length 2.79, height 1.93, width 1.32.

### **Specimens examined**

Holotype; ♀ Shute Harbour, Qld, 22.iv.2020, Leg J. Constant and L. Semeraro, Leopold III Funds Expedition – Qld, BJ-302 (QM; Registration number: T250921; GenBank numbers: ON102000, OM992348). Paratype: ♂ same data, BJ-304 (QM; Registration number: T250922; GenBank numbers: ON102001, OM992350); ♂ same data, BJ-303 (QM; Registration number: T250923; GenBank numbers: ON077348, OM992349).

### **Etymology**

The new species is a combination of macro meaning large and Latin punctum meaning dot and spot, in reference to the large black dots surrounding the lateral tubercles on the mesonotum.

*Austrocarausius eirmosus* sp. nov.

(Black-striped Stick-insect)

**Diagnosis**

Females have a pair of spines between eyes and dark tubercles over body (Fig. 2.3f, 2.4f, 2.5f, Supplementary Figure S2.3i, S2.4f, Supplementary Table S2.3). Disjointed black ventral stripe on mesonotum and metanotum, only in this species. Short tumescence protruding at end of abdominal segments 1-6. Males have a smooth body with few lateral tubercles on thorax (Fig. 2.7i, 2.9d, 2.10d, Supplementary Figure S2.3i). Pair of spines between eyes. Some lateral tubercles on thorax. Short tumescence protruding at end of abdominal segments 1-6.

**Description**

**Female**

Uniform brown, legs lightly mottled brown with white bands on apices of all femora.

**Head.** Slightly longer than wide (Fig. 2.3f). Two black spines between eyes, longer than wide, with several smaller brown spines caudally. Antennae reach end of fore tarsi, 103 segments, 2× length of fore femora.

**Thorax.** Large black-tipped lateral tubercles on thorax (Fig. 2.4f). Disjointed black ventral stripe on mesonotum and metanotum. Pronotum and head equal in length, mesonotum  $7\times$  length of pronotum, metanotum  $0.6\times$  length of mesonotum.

**Abdomen.** Short tumescence protruding at end of abdominal segments 1-6. Segments 2-6 equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of segments 2-6. Segments 9 and 10  $0.5\times$  length of segment 8. Cerci extends to tip of ovipositor (Fig. 2.5f). Supra-anal plate emarginate. Ovipositor curved and extending to end of supra-anal plate, apically pointed. Preopercular organ cordate, approximately  $0.3\times$  length of segment 8 (Fig. 2.5f).

**Legs.** Without ornamentation (Supplementary Figure S2.3i).

## **Male**

Light brown with green legs.

**Head.** Pair of spines between eyes, otherwise without ornamentation (Fig. 2.9d). Head slightly longer than wide. Antennae extend past forelegs, 11 segments,  $2.5\times$  length of fore femora.



**Thorax.** Smooth, with few lateral tubercles (Fig. 2.7i). Pronotum and head equal length, mesonotum  $8\times$  length of pronotum, metanotum just over  $0.5\times$  length of mesonotum.

**Abdomen.** Short tumescence protruding at end of abdominal segments 1-6. Abdominal segments 2-6 are equal in length,  $2\times$  length of median segment. Segment 7  $0.6\times$  length of segments 2-6. Segment 9  $0.6\times$  length of segment 8, and  $0.5\times$  length of segment 10. Cerci short,  $0.2\times$  length of 10th abdominal segment (Fig. 2.10d). Genital claspers pointed, at half the length reduce  $0.5\times$  in height.

**Legs.** Without ornamentation (Supplementary Figure S2.3i).

## **Egg**

Small, flat, roughly oval in shape with a slight lateral bulge above the micropylar plate (Fig. 2.11e). Egg capitula connected by enlarged stem. Egg surface rough throughout. Micropylar plate low and ovular.

## **Measurements (mm)**

For 2 females and 1 male. Body length: female 135-137, male 111. Head: female 4.4-4.5, male 3. Antennae: female 103 segments, male 111 segments. Pronotum: female 4.1-4.5, male 3.3. Mesonotum: female 30.6-31.2, male 27. Metanotum: female 18.1, male 16.4. Median segment: female 4.4-4.6, male 3.3. Femora: fore, mid, hind: female 33.6-36.0, 24.1-26.0,

27.8-30.0, male 32.6, 24.8, 28.1. Tibiae: fore, mid, hind: female 35.8-40.1, 27.5-29.7, 35.7-36.8, male 28.0, 29.5, 37.0. Eggs: capsule length 3.08, height 1.72, width 1.25.

### **Specimens examined**

Holotype: ♀ Whitsunday Islands National Park, Qld, 21-22.iv.2020, Leg J. Constant and L. Semeraro, Leopold III Funds Expedition – Qld, BJ-300 (QM; Registration number: T250924; GenBank numbers: ON101998, OM992346). Paratype: ♂ same data, BJ-301 (QM; Registration number: T250925; GenBank numbers: ON101999, OM992347); ♀ same data, BJ- 299 (QM; Registration number: T250926; GenBank numbers: ON101997, OM992345).

### **Etymology**

Derived from the Latin eirmos meaning series and train, in reference to the disjointed black ventral stripe on the mesonotum and metanotum of the female.

*Austrocarausius walkeri* sp. nov.

(Walker's *Austrocarausius*)

**Diagnosis**

This species has the largest male dorsal head protuberance in the genus (Fig. 2.7j, 2.9e, 2.10e, Supplementary Figure S2.3j, Supplementary Table S2.3). The body is smooth. Fore tarsi are flanged. Short tumescence protruding at end of abdominal segments 1-6.

**Description**

**Female**

Not known.

**Male**

Very elongated, brown with mottled body and particularly legs, the latter with whitish markings on femora and tibiae. Upper half of metanotum with reddish flush; ventral surface of mesothorax and upper half of metathorax reddish.

**Head.** As long as wide, pair of large flange-like protuberances between eyes, with two pairs of smaller spines caudally, decreasing in size and lateral distance from each other (Fig. 2.9e). Antennae extend to end of forelegs, 68 segments,  $2.5\times$  length of fore femora.

**Thorax.** Smooth (Fig. 2.7j). Pronotum slightly longer than head, mesonotum  $8\times$  length of pronotum, metanotum slightly over  $0.5\times$  length of mesonotum.

**Abdomen.** Short tumescence protruding at end of abdominal segments 1-6. Segment 2 slightly over  $2\times$  length of median segment. Segments 2-5 approximately equal in length. Segment 6 slightly shorter than segments 2-5. Segment 7 shorter than segment 6. Segments 8-9 combined are approximately the same as segment 7. Segment 10  $0.6\times$  longer than segment 9. Genital claspers lobe-like and splayed outwards, rounded apically (Fig. 2.10e). Cerci short. Poculum tapered to tip, reaching end of 9th abdominal segment.

**Legs.** Fore tarsi flanged (Supplementary Figure S2.3j).

## **Egg**

Not known.

## **Measurements (mm)**

Body length: 111. Head: 2.8. Antennae: 68 segments. Pronotum: 3.4. Mesonotum: 27.5.

Metanotum: 15.2. Median segment: 3.8. Femora: fore, mid, hind: 31.5, 21.5, 24.0. Tibiae: fore, mid, hind: 39.0, 26.3, 33.2.

## **Specimens examined**

Holotype: ♂ Qld, Mt Dryander, 21.11.2009, P. D. Brock and I. O. Walker, PB-0014 (QM; Registration number: T250927; GenBank number: HM425716.1).

## **Etymology**

Named after Ian O. Walker, a keen naturalist from Bowen with a vast knowledge of that area and Mt Dryander, who greatly assisted P. D. Brock with his trip to the area in 2009.

## **2.6 Discussion**

Using a combination of molecular and morphological data, we have found evidence that *Austrocarausius* represents multiple undescribed species and has diversified at the species level within the tropical rainforests of Queensland over the last c.25-70 Ma.

### *2.6.1 Species delimitation*

Both ABGD and GMYC species delimitation methods suggest eight undescribed species among the individuals examined (Fig. 2.2). These species delimitation results also corroborate the species hypotheses presented in our morphological analyses. We note that for some specimens, we were unable to obtain molecular data. Among these specimens, we were able to confidently describe three new species on the basis of morphological data (4.

*truncatus*, *A. tuberosus* and *A. gasterbulla*). Future molecular studies are required to determine the phylogenetic position among the other eight *Austrocarausius* species.

### 2.6.2 Morphological analysis

Close inspection reveals that this genus has some of the most ornate and unusual features seen in Australian phasmids, including dorsal head structures in the form of a flange-like protuberance, horn or spine (Fig. 2.3, 2.6, 2.9), and preopercular organs located ventrally on the 7th abdominal segment in females (Fig. 2.5). Although head structures can be variable within each *Austrocarausius* species (Fig. 2.3, 2.6, 2.9), the overall form (protuberance, horn, spine or absent) is useful for species identification. The variation in preopercular organ shape and additional appendage structures observed only in this genus, make for some of the most morphologically unique phasmid species. No intraspecific variation was observed in this morphological structure. The preopercular organ is known to be used for reproduction (Bragg 2001), however, further behavioural investigation into these organs and structures is needed to fully understand the precise function. The eggs of *Austrocarausius* are capitulate, like other closely related taxa such as *Carausius*.

### 2.6.3 Phylogenetic placement of *Austrocarausius* among other lonchodids

In agreement with other studies (Robertson et al. 2018, Simon et al. 2019), our Bayesian analysis places *Eurycantha* (Lonchodidae: Lonchodinae: Eurycanthini), *Carausius* (Lonchodidae: Lonchodinae: Lonchodini), and *Sipyloidea* (Lonchodidae: Necrosiinae) as closely related taxa to *Austrocarausius* (Supplementary Figure S2.1). Previous studies have placed *Carausius* as sister to *Eurycantha* (Robertson et al. 2018; Simon et al. 2019).

However, our maximum likelihood analysis did not recover this relationship (Supplementary Figure S2.1) and our Bayesian analysis places *Carausius* as sister to *Sipyloidea* instead of *Eurycantha*. The support values for these relationships are notably not high among outgroup taxa. Our results regarding the closest relative of *Austrocarausius* should therefore be considered preliminary. Compared to our study, both Robertson et al. (2018) and Simon et al. (2019) used a significantly larger number of taxa and more molecular evidence. These authors found that *Eurycantha* and *Carausius* are consistently placed in the subfamily Lonchodinae and *Sipyloidea* in Necrosciinae. The lack of resolution in our phylogenetic trees is likely due to limited genetic and taxon sampling.

*Austrocarausius* has only been included in one previous molecular analysis (Velonà et al. 2015). In that study, *Austrocarausius* was grouped with *Denhama* Werner, 1912 (Lonchodidae: Lonchodinae: Lonchodini). Further studies including the two genera will help determine the phylogenetic position with more confidence.

#### 2.6.4 Historical biogeography of *Austrocarausius*

The drivers of ancient speciation events within *Austrocarausius* are difficult to determine, since these occurred at a time when rainforests were considered to have dominated the Australian landscape (Greenwood and Christophel 2005; Byrne et al. 2008; Byrne et al. 2011). However, the formation and maintenance of ancient biogeographic barriers from the Cretaceous to late Oligocene may offer one possible explanation for speciation in *Austrocarausius*. For example, the Black Mountain Corridor, where *A. nigropunctatus*, *A. decorus* and *A. coronatus* are found, has been suggested as a geographical barrier for earthworms between the Eocene and Cretaceous periods (Moreau et al. 2015), this being a similar time period for the divergence of *A. waiben* and *A. nigropunctatus* (c. 53.8 Ma (95%

HPD 46.12-62.49 Ma)), and *A. decorus* and *A. coronatus* (c. 48.6 Ma (95% HPD 41.56-56.77 Ma)). The St Lawrence Gap is a geographical barrier for assassin spiders appearing during the Eocene (Rix and Harvey 2012) and between the distributions of *A. mercurius* and *A. macropunctatus*, *A. eirmosus* and *A. walkeri*. Although these geographical barriers may play a role in the ancient speciation events of *Austrocarausius*, our study is only based on two mitochondrial genes and our biogeographic inferences should be regarded as preliminary.

*Austrocarausius* is highly endemic to specific patches of remnant rainforest, unlike other phasmids that can be found in a variety of vegetation communities. For example, *Extatosoma tiaratum* and *Tropidoderus rhodomus* can be found along most of the eastern coast of Australian between Cape York and southern New South Wales (~2500 km) (Brock and Hasenpusch 2009; Atlas of Living Australia, <http://www.ala.org.au>, accessed 12 April 2021). These species have winged males and are generalist feeders (Brock and Hasenpusch 2009), and this may explain the widespread distribution. In contrast, *Austrocarausius* species are wingless with limited dispersal capabilities, and are restricted to tropical rainforests. Loss of these already fragmented rainforests could put such endemic species at risk of extinction. *Austrocarausius nigropunctatus* was given an IUCN Red List conservation status of 'Data Deficient' in 2017 (Rudolf and Brock 2017).

A previous study of the rainforest phasmid genus *Parapodacanthus* showed that this comprises only two species with restricted distributions (one in the border ranges near Brisbane and the other in the Daintree rainforest) (Brock and Monteith 2018). Whether members of *Parapodacanthus* were previously present in the patchy rainforest areas inhabited by *Austrocarausius* but later went extinct, or whether the current distribution is the result of long-distance dispersal, is unclear. Our study indicates that *Austrocarausius* has



been able to survive in rainforests over tens of millions of years, during which time these vegetation communities disappeared from large parts of Queensland.

Our analyses show that morphologically different species of *Austrocarausius* can coexist in sympatry within the same habitats (Fig. 2.2). For example, *A. nigropunctatus* and *A. decorus* both live within identical habitats in the Daintree rainforest and have similar distributions. *Austrocarausius walkeri* and *A. macropunctatus* are found in similar rainforest habitat close together (Shute Harbour), although the distributions are separated by an area devoid of rainforest.

Although this study analyses specimens from different museum collections along with the addition of new material, there is still a great deal to learn. For example, no DNA was recovered from specimens of *A. nigropunctatus* from the type locality of Lizard Island, as no specimens were available. Additionally, *A. coronatus* might also live in Kuranda and Paluma (Paul Brock, pers. comm., 2022). Given the reported distributions of *A. mercurius* along the Queensland coast (Brock and Hasenpusch 2009), it is likely that these records represent different species. Further research on these species will benefit from field surveys in central and northern Queensland and to discover the currently undescribed females of *A. truncatus*, *A. tuberosus* and *A. walkeri*, how far the distributions of each species spread, and exactly how many other species there might be in *Austrocarausius*.

## Chapter 3

### **A revision of Australian *Anchiale* (Stål, 1875) (Phasmida: Phasmatini), with the description of two new species.**

**Braxton R. Jones**, Paul D. Brock, Ross M. Coupland, Angus J. Emmott and Nathan Lo

**Authors' contributions.** B.R.J., P.D.B. and R.M.C. devised the study. B.M., A.L. and S.C extracted genetic material. B.R.J. conducted the analyses and wrote the manuscript with input from all authors.

### 3.1 Abstract

The examination of Australian species within the genus *Anchiale* uncovered two new species: *Anchiale mabiensis* Coupland and Emmott, sp. nov., and *Anchiale robusta* Brock and Jones, sp. nov., both of which are described and depicted. Comparative keys, supported by genetic analysis where feasible, are included to facilitate differentiation of species. The taxonomic status of *A. albopunctata* (Kirby, 1896) stat. rev. is revised, removing it from synonymy with *A. briareus* (Gray 1834) and a lectotype has been designated. A lectotype is also designated for *A. austrotessulata* (Brock and Hasenpusch, 2007; initially described as *Ctenomorpha tessulata* in Gray, 1835), now proposed as a new synonym of *A. briareus* (Gray, 1834). Another lectotype is designated for *Ctenomorpha nigrovaria* (Stål, 1875) stat. rev., previously considered synonymous with *A. briareus* (Gray, 1834), now suggested as a new synonym of *A. albopunctata* (Kirby, 1896). *Anchiale spinicollis* (Gray, 1833) is revised and placed in *Acrophylla* (Gray, 1833) stat. rev. Additional Australian locations for these taxa are documented and illustrated keys for adults and eggs are provided.

## 3.2 Introduction

The Phasmatidae (Leach, 1815) is a family of phasmids found in several biogeographic areas worldwide, but with a concentration of taxa in Australia and surrounding islands. This family contains 10 subfamilies, 21 tribes, 133 genera, and 703 species (Brock et al. 2024). One of these subfamilies includes Phasmatinae, which contains the tribe Phasmatini (Leach, 1815). The Phasmatini tribe consists of 13 genera and 48 species (Brock et al. 2024), which are known for their large body size, and large, colourful wings (Hennemann et al. 2015).

The genus *Anchiale* (Stål, 1875) (Fig. 3.1) currently comprises a total of 16 species living across most of the Papuan and Australian regions, such as the Maluku Islands, Peleng Island, Kei Islands, Aru Islands, Bismarck Archipelago, New Guinea, Solomon Islands, and Australia, with the tentative inclusion of French Polynesia (Hennemann et al. 2015). This geographic range is atypical for Australian phasmids, which commonly exhibit endemism across many genera.

While catalogued in Australian (Brock and Hasenpusch 2007; Brock and Hasenpusch 2009) and global phasmid compilations (Otte and Brock 2005; Brock and Büscher 2022), the Australian *Anchiale* species have not undergone a comprehensive revision, despite being the most frequently observed. One of these species, *A. austrotessulata* Brock and Hasenpusch, 2007, has been documented as a pest species (previously known as *Ctenomorphodes tessulatus* (Gray, 1835; Baker 2015)). This species undergoes outbreaks in various colour forms, a phenomenon termed kentromorphism by Key (1957). Extensive fieldwork in New South Wales and Queensland has revealed the common occurrence of *Anchiale* representatives on the upper sections of *Eucalyptus* spp. saplings or branches during the day and night (Brock and Hasenpusch 2009). Citizen science data for *Anchiale* was collected (~1100) from iNaturalist and the Atlas of Living Australia (ALA) (iNaturalist 2024; Atlas of

Living Australia 2024). A review of relevant literature, examination of all type material, and scrutinising non-type specimens in major Australian collections (with a focus on AMS, ANIC, and QM) and private collections, has revealed additional records of *Anchiale* (Paul D. Brock, pers. obs.). Genetic material for numerous specimens has also been analysed (Velonà et al. 2015).

The substantial interest in *Anchiale* justifies a re-evaluation of the status of certain species and the introduction of two newly described species: *Anchiale mabiensis* Coupland and Emmott sp. nov., and *Anchiale robusta* Brock and Jones sp. nov. Morphological and molecular analyses were performed across museum and field collected specimens to investigate the status of all Australian *Anchiale* species and describe new *Anchiale* species.



**Figure 3.1.** A representative of *Anchiale*: *A. mabiensis* sp. nov. female, in situ. Photo AJE (Angus J. Emmott).

### 3.3 Methods

#### 3.3.1 Specimen collection

Specimens were either obtained from museum collections or collected in the field by Jack Hasenpusch, Graham Milledge, David C. F. Rentz, Paul D. Brock, and Ross M. Coupland. No permits were required to obtain specimens. Eggs were collected from some live females. Specimens in this study can be found in QM under registration numbers T258381-T258409, T259412, T259413.

### 3.3.2 Abbreviations used

The following abbreviations have been used for depositories: Australian Museum, Sydney, Australia (AMS); Australian National Insect Collection, Canberra, Australia (ANIC); Linnean Society, London, United Kingdom (LSUK); Museum Victoria, Melbourne Museum, Australia (MVMA); Natural History Museum, London, United Kingdom (NHMUK); Naturhistoriska Riksmuseet, Stockholm, Sweden (NHRM); Oxford University Museum, Hope Entomological Collections, United Kingdom (OUMNH); P.D. Brock collection, United Kingdom (PDB); Queensland Museum, Brisbane, Australia (QM).

### 3.3.3 Morphological analysis and imaging

Morphological measurements for a total of 35 specimens were taken by P. D. Brock. Images of specimens were taken by A. Emmott, R. M. Coupland, P. D. Brock, and B. R. Jones, using a Canon 7D Mark I with a 100-mm macro lens. Images, where applicable, were further processed by B. R. Jones in Adobe photoshop 2024 (ver. 25.1, <https://www.adobe.com/au/>). Any additional image stacking was performed in ZereneStacker (ver. 1.04, Zerene Systems, <http://zerenesystems.com/cms/home>).

### 3.3.4 Molecular analysis

DNA extraction was performed by Barbara Mantovani, Andrea Luchetti, and Simona Corneti and molecular analyses were performed by Braxton Jones on a total of 33 *Anchiale* specimens, with an additional 22 non-*Anchiale* specimens included as outgroup taxa (Supplementary Table S3.1). Unfortunately, it was not possible to examine *A. mabiensis* specimens due to specimen availability. For all *Anchiale* specimens, and some outgroup

specimens, sequences encoding cytochrome oxidase subunit 1 (*COXI*) were obtained as per Velonà et al. (2015) or processed by iBOL staff. Sequences for other outgroup specimens were downloaded from Genbank. Specimens previously submitted to Genbank and BOLD under different names have been updated (Supplementary Table S3.1).

To obtain *COXI* sequences, DNA was isolated using NucleoSpin DNA Insect, Mini kit for insect DNA (MACHERAY/NAGEL). PCR amplification was performed using the DreamTaq DNA Polymerase (Thermo Scientific), with the standard thermal cycling conditions specified by manufacturer, with the following amendments: amplification following program: 1 initial denaturation cycle at 95°C for 2 min; 30 cycles of denaturation, annealing and extension: denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extending at 72°C for 1 min; and a final extension at 72°C for 10 min. The following primers were used: LCO1490 (GTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994).

Raw sequences were trimmed in Geneious and aligned with Genbank sequences using Muscle (ver. 3.2, <http://www.drive5.com/muscle/>; Edgar 2004) in Seqotron (ver. 1.0.1, <https://github.com/4ment/seqotron>; Fourment and Holmes 2016).

Phylogenetic trees were inferred using both Bayesian inference and maximum likelihood approaches. Substitution models were applied to both analyses, with no data partitioning. Bayesian inference analyses were performed in MrBayes (ver. 3.2.7a, <https://nbisweden.github.io/MrBayes/>; Ronquist and Huelsenbeck 2003). Modelgenerator (Keane et al. 2006) was used to estimate the best substitution model, with GTR+G being optimal. The Effective Sample Size was checked in Tracer (ver. 1.7.2 <https://github.com/beast-dev/tracer/releases/tag/v1.7.2>; Bouckaert et al. 2014) and the tree files were combined in LogCombiner (ver. 2.6.3, <https://www.beast2.org>; Bouckaert et al.



2014). TreeAnnotator (ver. 2.6.3, <https://www.beast2.org>; Bouckaert et al. 2014) was used to create a maximum clade credibility tree. Maximum likelihood analyses were performed in IQTREE (ver. 2.1.3, iqtree2; Minh et al. 2020) with 1000 Ultrafast bootstrap replicates. ModelFinder (ver. 1, <http://www.iqtree.org/ModelFinder/>; Kalyaanamoorthy et al. 2017) was used to select the best fitting model according to the Bayesian information criterion, selecting TIM2+F+I+G4. Output for both trees were visualised in Figtree (ver. 1.4.4, <http://tree.bio.ed.ac.uk/software/figtree/>, accessed 13 May 2021).

### 3.3.5 Data accessibility

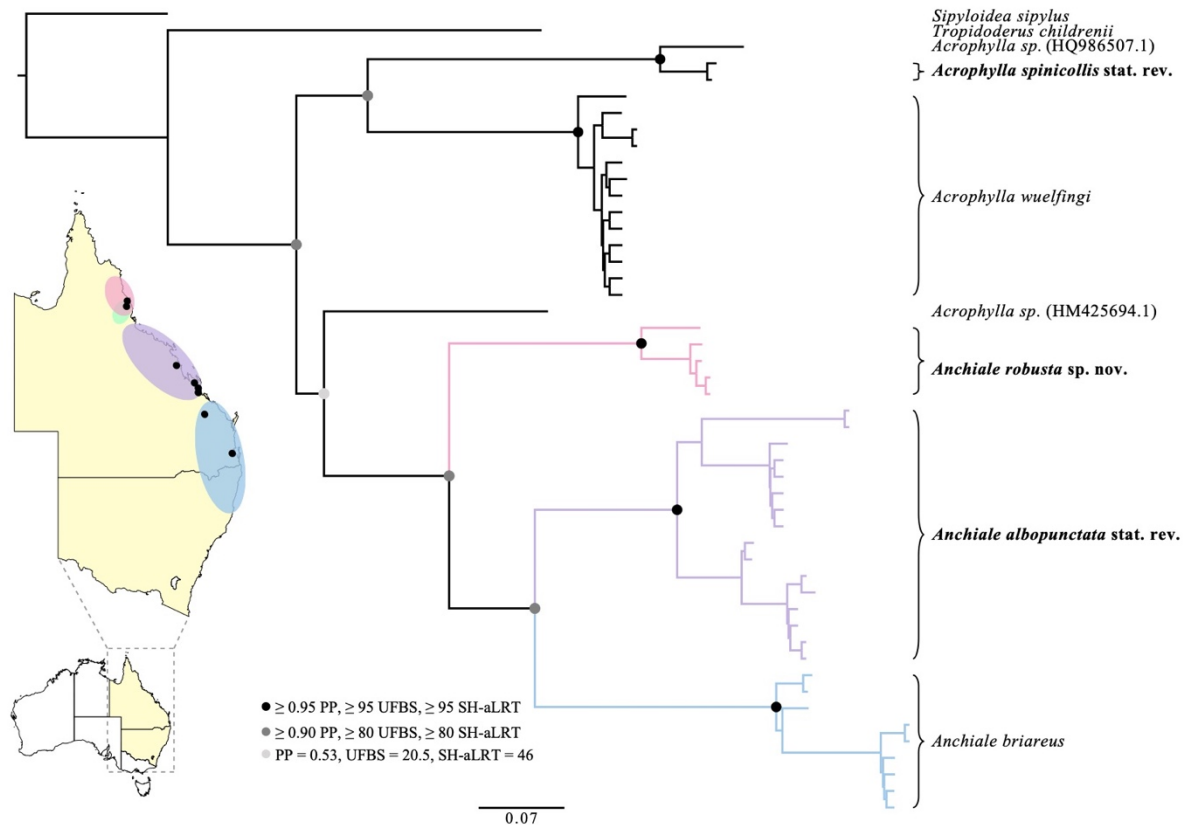
Molecular data used in this study is available on GenBank under the following accession numbers HM425670.1-HM425674.1, HM425694.1, HM425701.1-HM425705.1, HM425712.1-HM425715.1, HM425717.1, HM425718.1, HM425723.1, HM425732.1-HM425736.1, KJ201976.1, KJ201977.1, KJ201982.1, KJ201983.1, KJ201992.1, KJ201993.1, KJ202000.1, KJ202001.1, KJ202003.1, KJ202004.1, KJ202009.1, KJ202010.1, KJ202014.1, KJ202020.1, KJ202021.1, KJ202028.1, KJ202041.1, KJ202046.1, KJ202050.1, KJ202056.1, PP373352, PP373354 (Supplementary Table S3.1).

## 3.4 Results

### 3.4.1 Phylogenetic analyses

Phylogenetic relationships among Australian *Anchiale* were congruent in Bayesian inference and maximum likelihood analyses (Fig. 3.2; Supplementary Figure S3.1, S3.2). Australian *Anchiale* were found to form a monophyletic group (0.1 PP, 94.2 BS, 94 SH-aLRT) that was the sister group of *Acrophylla* sp. (HM425694.1) from Montville, Qld, Australia. Without

any genetic material from non-Australian *Anchiale*, it remains inconclusive whether they are monophyletic when exotic species are included. The *Acrophylla* sp. (HM425694.1) groups with *Anchiale* species, instead of with the other *Acrophylla* species (0.53 PP, 20.5 BS, 46 SH-aLRT). The first divergence in the genus represents a split between *A. robusta* and *A. albopunctata* + *A. briareus*. The two species *A. albopunctata* and *A. briareus* are sister species (1 PP, 91.3 BS, 95 SH-aLRT).



**Figure 3.2.** Bayesian inference tree for three Australian *Anchiale* species and outgroups inferred from mitochondrial *COX1*. Species distributions on the map correspond to the colours used in tree branches, except for the green colour representing the distribution of *A. mabiensis*, for which no genetic material could be examined. Black point markers on the distribution map indicate specific collection localities. Bold text indicates new and revised

species. Node support values are shown for Bayesian inference as posterior probability (PP) and maximum likelihood as ultrafast bootstrap (UFBS) and Shimodaira-Hasegawa Approximate Likelihood Ratio Test (SH-aLRT). Scale bar represents substitutions per site.

### 3.5 Taxonomy

Family **PHASMATIDAE** Leach, 1815

Subfamily **PHASMATINAE** Leach, 1815

Tribe **PHASMATINI** Leach, 1815

Genus *Anchiale* Stål, 1875

**Type species:** *Mantis maculata* (Olivier, 1797: 636), Indonesia, Maluku, Ambon Island, by original monotypy. *Anchiale* (Stål 1875: 36, 84; Kirby 1904: 392; Redtenbacher 1908: 460; Vickery 1983: 3; Balderson et al. 1998: 361; Otte and Brock 2005: 44; Brock and Hasenpusch 2007: 72; Brock and Hasenpusch 2009: 201; Hennemann et al. 2015: 10; Brock and Büscher 2022: 558; Brock et al. 2023).

*Ctenomorphodes* (Karny, 1923: 240). Synonymised by Brock and Hasenpusch (2007: 72). Zompro (2009:42) reinstatement of the genus ‘because of the striking differences in the structure of eggs’, following rearing of an unidentified species of *Ctenomorphodes* from New Guinea, here regarded as *Anchiale*.

**Type species:** *Phasma (Diura) briareus* (Gray, 1834: 45), by original designation.

**Comment:** *Anchiale briareus* (Gray), has variably been regarded as belonging to *Acrophylla*, *Ctenomorpha*, and *Ctenomorphodes* since Karny (1923) and *Anchiale* since Brock and Hasenpusch (2007).

### **Generic characteristics**

Medium to large size (115-170 mm in females, 75-120 mm in males), elongate or robust insects. Thoracic tubercles vary in size and abundance. Wings and operculum vary in proportional size within species. Males smaller and less elongate than females. Head rounded, slightly longer than wide, often with lateral black marking on front part of eyes. Eyes large, three ocelli in both sexes, less noticeable in some females. Antennae for females shorter than fore femora and longer in males. Metanotum just longer than mesonotum, can be smooth, granulated, or with tubercles. Mesonotum 3-4× length of pronotum; with granulated or cone-shaped tubercles. Forewings long, leaf-like, c.0.5× length of hind wings in females and less than 0.5× length in males, variation possible within species. Hindwings with black and white tessellation or uniform black, length variation between species, males consistently longer. Operculum boat-like, just exceeding end of abdomen. Male poculum just reaching end of abdomen. Cerci ovate to oblong, ranging from shorter than anal segment (segment 10) to 2× length. Mid and hind legs, uniform spines, sometimes with reduction of spines in fore femora and tibiae. Closely related to the genus *Acrophylla* (Gray 1835), both being in the tribe Phasmatini. Female *Acrophylla* are distinguished by more robust, spinier bodies with longer wings, reaching end of abdominal segment 6, in *Anchiale* wings just extend abdominal segment 4. The operculum of *Acrophylla* extends beyond end of abdomen, forming a chute. The shiny, more ovate eggs of *Anchiale* differ from *Acrophylla* which have more circular and less shiny, punctured eggs. The egg operculum of *Anchiale* species has a raised central cone,

with a white to yellow capitulum; the micropylar plate has a raised rim extending most of the capsule. The capsule is smooth to heavily sculptured, over time lightening in colour and losing its glossiness. The capsule in the type species (*Acrophylla maculata*) has keels and a strongly sloping operculum. In Australian *Anchiale* species the opercular angle is slight, a maximum of c.-10°.

### Species included:

*Anchiale albopunctata* stat. rev. (Kirby, 1896) (Rockhampton to Paluma, Qld) (Fig. 3.3a, 3.4)

*Anchiale briareus* (Gray, 1834) (NSW to SE Qld) (Fig. 3.3b, 3.5)

*Anchiale mabiensis* sp. nov. (Atherton tablelands, Qld) (Fig. 3.3c, 3.6-3.8)

*Anchiale robusta* sp. nov. (Tully to Cooktown, Qld) (Fig. 3.3d, 3.9-3.11)

### Keys to adult females of Australian *Anchiale*

- 1 tip of hindwings closer to posterior end of third abdominal segment than fourth.....2
- tip of hindwings closer to posterior end of fourth abdominal segment than third. Hindwings medium-sized (average 50 mm long), c.2.3× length of forewings. Body length 122-162 mm (Fig. 3.3d, 3.9, 3.11b, c).....***A. robusta* sp. nov.**

2 white spots absent at base of mesonotum tubercles.....3

- white spots present at base of mesonotum tubercles. Forewings sometimes with obvious white spot. Hindwings short (average 23 mm long), less than 2× length of forewings.

Mesonotum with sparse tubercles, some black-tipped and ringed with white (where this is the case, the dorsal anterior corners of abdominal segments 1-5 have a large white streak). Cerci oblong, about length of anal segment. Body length 115-140 mm (Fig. 3.3a, 3.4a, b, c).....*A.*

*albopunctata* stat. rev.

3 Cerci longer than anal segment. Dorsal tubercles absent on pronotum. Mesonotum with sparse, usually black-tipped, small, pointed tubercles. Hind part of abdominal segment 6 not swollen or lobed. Forewings plain, hindwings short (each one on average 27 mm long), c.2× length of forewings. Fore femora unarmed or slightly spiny. Body length 123-170 mm (Holotype 146 mm) (Fig. 3.3b, 3.5a, b, d)..... *A. briareus*

- Cerci shorter than anal segment. Hindwings short (27 mm long), c.2× length of forewings. Mesonotum with stout spines, mostly light brown (some black-tipped). Cerci short, c.0.6× length of anal segment. Body length 115-122 mm (Fig. 3.3c, 3.6, 3.8a) .*A. mabiensis* sp. nov.

### Keys to adult males of Australian *Anchiale*

1 white dorsal spot present on forewings.....2

- white dorsal spot missing on forewings. Spines on mesonotum black. Mesonotum with sparse, usually black-tipped, small, pointed tubercles. Body length 80-102 mm (Lectotype of synonym *Ctenophora tessulata* (Gray, 1835) syn. n., 89 mm) (Fig. 3.5a, e) ..... *A. briareus*

2 mesonotum with dorsal spines .....4

- mesonotum lacking dorsal spines. Forewings with less distinct white spot. Body length 93-104 mm (Fig. 3.10, 3.11a).....*A. robusta* sp. nov.

3 mesonotum spines uniformly black. Forewings with less distinctive white spot. Cerci short, c.0.6× length of anal segment. Body length 82 mm (Fig. 3.7, 3.8b) .....*A. mabiensis* sp. nov.

- mesonotum spines black tipped only. Forewings with obvious white spot. Mesonotum with sparse tubercles, usually with few small, black spines ringed with white. Cerci oblong, about length of anal segment. Body length 75-90 mm (Lectotype 87 mm) (Fig. 3.4a, b) .....*A. albopunctata* stat. rev.

#### Keys to eggs of Australian *Anchiale*

1 no inner rings on operculum. Capsule fairly smooth to moderately sculptured. Capitulum present, whitish or yellow .....2

- inner rings on operculum. Capsule heavily sculptured. Capitulum absent, instead operculum with a black cone (Fig. 3.3c).....*A. mabiensis* sp. nov.

2 egg elliptical or rounded .....3

- egg oblong, capsule moderately sculptured, with narrow micropylar plate. Capitulum large (Fig. 3.3d).....*A. robusta* sp. nov.

3 capsule fairly smooth (although microscopic examination shows it to be uneven) and, with narrow micropylar plate, with raised black ridge at base. Capitulum ragged (Fig. 3.3b).....*A. briareus*

- capsule moderately sculptured, black at base. Capitulum variable (Fig. 3.3a) .....*A. albopunctata* stat. rev.





**Figure 3.3.** *Anchiale* eggs, dorsal and lateral views. (a) *Anchiale albopunctata* stat. rev., photo PDB (Paul D. Brock). (b) *Anchiale briareus*, photo PDB. (c) *Anchiale mabiensis* sp. nov. also showing operculum view, photo BRJ (Braxton R. Jones). (d) *Anchiale robusta* sp. nov. also showing faded egg, photo PDB.

*Anchiale albopunctata* (Kirby, 1896) stat. rev.

(White-spotted *Anchiale*)

*Ctenomorpha albopunctatum* (Kirby, 1896: 472) (Fig. 3.4).

*Ctenomorpha albopunctatum* (Tepper, 1903: 281; Kirby 1904: 389; Redtenbacher 1908: 459), synonymised with *Ctenomorpha briareus*. Vickery (1983: 5) and Balderson et al. (1998: 366) both as synonym of *Ctenomorphodes briareus*. Otte and Brock (2005: 113) Brock and Hasenpusch (2007: 72) Hennemann et al. (2015: 11) Brock et al. (2016: 159) synonym of *Anchiale briareus*. **Comment:** Kirby specified ‘Queensland’ as the type locality, but as recorded in Brock et al. 2016) the register entry in NHMUK provided additional collecting information. Kirby continued, ‘I suspect that *Diura briareus* Gray’, is the female of this species, in which case the name *briareus* must be retained for it.’ Redtenbacher (1908) synonymised this species with *A. briareus*. Here *Anchiale albopunctata* is removed from synonymy with *Anchiale briareus*, confirmed by the examination of many specimens including genetic material for additional specimens in and near the type locality, Rockhampton.

## Examined material

**Lectotype** ♂, AUSTRALIA: Queensland, Rockhampton, (18)72/18, G. L. Pilcher, Purch[ased]. E. W. Janson, BMNH(E) #845023 (NHMUK) (here designated to change the status of this species, by selecting the specimen mounted with outstretched wings);

**Paralectotype** ♂, AUSTRALIA: Queensland, Rockhampton, register (18)72/18, G. L. Pilcher, Purch[ased]. E. W. Janson, BMNH(E) #845024 (NHMUK) (specimen mounted with closed wings). The first specimens used for genetic analysis, originally identified as *Anchiale briareus*, include: 2 ♂♂, AUSTRALIA: Queensland, Waste ground by L'amor Apts, Lammermore Beach, nr. Rockhampton, 23.1575S, 150.7669 E, 14.xi.2009, P.D. Brock, DNA project PB0001, PB0017 (QM); ♂, 4 ♀♀, AUSTRALIA: Queensland, Nr. Middle Creek Dam surrounds, Nr. Sarina, 21.475S, 149.0983E, 17.xi.2009, P.D. Brock, DNA project

PB0012, PB0015, PB0022, JH0076-JH0077 (QM); ♂, AUSTRALIA: Queensland, Old Byfield Road Reserve, Byfield, 22.8453S, 150.65E, 16.xi.2009, P.D. Brock, DNA project PB0013 (QM); 2 ♀♀, same data except 15.xi.2011, DNA project JH0078-JH0079 (QM); 3 ♂♂, 2 ♀♀, AUSTRALIA: Queensland, Gladstone, 23.8787S, 151.2343E, 29.xi.2011, N. Tweed, DNA project PB0046-PB0049, PB0083 (QM); ♀, same data, PB-0097 (NHMUK).

## **Description**

Light green to light brown, males sometimes darker brown, medium-sized to large insects. Light brown insects can have darker brown markings on some body segments. Males have pinkish-red lateral lines along length of mesonotum. Forewings sometimes with obvious white spot in females, always present in males. Hindwings long in males, short in females, less than 2× length of forewings. Mesonotum with sparse tubercles, some black-tipped and ringed with white (where this is the case, the dorsal anterior corners of abdominal segments 1-5 have a large white streak). Cerci oblong, about length of anal segment.

## **Egg**

Capsule glossy in appearance, dark brown with grey around micropylar plate, some eggs mostly grey (Fig. 3.3a). Shape, oval, moderately sculptured with slightly raised lines and bumps. Operculum black, almost flat, outer border grey. Capitulum yellowish and uneven. Micropylar plate slight enlargement at base, raised black rim, extending most of capsule length, not reaching operculum. Measurements (mm): capsule length 2.6, height 2, width 1.6.

### **Habitat and foodplants**

Forests, parks and gardens, coastal and inland, commonly feeding on different *Eucalyptus* spp. (Myrtaceae).

### **Season**

Adults have been found between October and June (iNaturalist 2024; Atlas of Living Australia 2024).

### **Distribution**

Central and northern Queensland from Gladstone to Paluma, (~900 km; Fig. 3.2), some sites checked by genetic material. Whilst not confirmed, distributions may extend beyond this region as specimen records exist on the ALA. For example, records include Childers, ~200 km south of Gladstone, and isolated populations near Brisbane, given correct localities submitted to ALA.



**Figure 3.4.** *Anchiale albopunctata* in situ. (a) Mating pair, Gladstone, Qld, 15Nov2011, photo PDB. (b) Mating pair, Airlie Beach, Qld, 14Feb2016, photo Steve and Alison Pearson. (c) Female, common colour form, missing forelegs, Nr Middle Creek Dam, Sarina, Qld, 17Nov2009, DNA reference number PB0015 (Supplementary Table S3.1), photo PDB.

*Anchiale briareus* (Gray, 1834)

(Tessellated Stick-insect)

*Phasma (Diura) briareus* (Gray, 1834: 45) (Fig. 3.3b, 3.5). **Holotype** ♀, AUSTRALIA

(MVMA, T13886). **Comment:** Gray provided a description of the ♀ ‘...from Mrs

Dunn’s’ collection, who received it with *Ph. Goliath*’, now recognised as

*Clemacantha goliath* (Gray, 1834: 45). The description of *Phasma (Diura) goliath* indicates Mrs Dunn ‘is uncertain from which part of the country it was obtained’. However, as *C. goliath* is mostly found in New South Wales and southeast Queensland, it most likely originated in the distribution range now known for *A. briareus*. Specimens used for genetic analysis, originally identified as *Anchiale austrotessulata*: 3 ♂♂, AUSTRALIA: Queensland, Brisbane, Alexandra Hills 27.5238S, 153.2289E, 12.xi.2011, P.D. Brock and N. Tweed, DNA project PB0059, PB0075, PB0076 (QM); 3 ♂♂, 3 ♀♀, AUSTRALIA: Queensland, Cania Gorge, 24.7986S, 151.0293E, 24.xi.2011, P.D. Brock and N. Tweed, DNA project PB0087-PB0092 (QM)

*Acrophylla briareus* (Gray, 1835: 40; Westwood 1859: 114; Rainbow 1897: 40; Tepper 1903: 280).

*Ctenomorpha briareus* (Kirby, 1904: 389; Redtenbacher 1908: 459, pl. 22: 4).

*Ctenomorphodes briareus* (Karny, 1923: 240; Key 1960: 236; China and Riley 1962: 19; Vickery 1983: 5; Clark 1976: 99, egg description included with uncertainty due to the larger size and longer wings in the culture stock (this is the egg of *Anchiale robusta* sp. nov.); Balderson et al. 1998: 366; Otte and Brock 2005: 113).

*Anchiale briareus* (Brock and Hasenpusch, 2007: 72; Brock and Hasenpusch 2009: 2, 16, 20, 97, 165 (in part); Velonà et al. 2015: 458 (in part); Hennemann et al. 2015: 11; Brock and Büscher 2022: 454, 558 (in part); additional checklist and other references in Brock et al. (2023)).

*Anchiale austrotessulata* syn. nov. (Brock and Hasenpusch, 2007: 72, 96; Hennemann et al. 2015: 11; Brock et al. 2015: 15), new name for *Ctenomorpha tessulata* (Gray, 1835).

*Anchiale austrotessalata* (Baker, 2015: 12), subsequent misspelling.

*Ctenomorpha tessulata* (Gray, 1835: 44), primary homonym, not *Mantis tessulata* of Goeze (1778), later moved to *Anchiale* by Kirby (1904). **Lectotype** ♂, AUSTRALIA (MVMA, T13881), here designated to fix the status of this species, by selecting a specimen that unquestionably belongs to the original type series, i.e. ‘in coll. D. Curtis.’, if there is more than one ♂. Kirby (1904: 389) marked the species as ‘\*\*’ indicating existing type material in NHMUK. **Paralectotypes** ♂, AUSTRALIA (NHMUK), ♂, AUSTRALIA (OUMNH), renamed by Brock and Hasenpusch (2007: 72).

*Ctenomorpha tessellate* (Westwood, 1859: 115, pl. 35:1-2, unjustified emendation; Kirby 1904: 389; Redtenbacher 1908: 458).

*Acrophylla tessalata* (of Curtiss) Rainbow (1897: 41) subsequent misspelling.

*Acrophylla tesselata* (Tepper, 1903: 281).

*Ctenomorphodes tessulata* (Karny, 1923: 240; Key 1957: 247; Vickery 1983: 5; Balderson, et al. 1998: 366; Otte and Brock 2005: 113).

*Ctenomorphodes tessulatus* (Key, 1960: 237), species name ends -us, as the gender of *Ctenomorphodes* is masculine.

*Ctenomorpha macleayi* (Gray, 1835: 41) **Syntypes** ♂, ♀, AUSTRALIA (LSUK, lost), (Otte and Brock 2005: 112), synonymised with *Anchiale briareus* by Hasenpusch and Brock (2006: 4).

*Acrophylla macleaii* (Westwood, 1859: 115) subsequent misspelling. **Comment:**

*Ctenomorpha tessulata* (Gray, 1835) later renamed *Anchiale austrotessulata* Brock

and Hasenpusch (2007), is here changed to a new synonym of *A. briareus*, supported through examination of museum specimens and genetic material, including related species. The elongate, slender *A. briareus* form has resulted in uncertain identification of this taxon in the literature by authors, for example, Redtenbacher (1908: 459) who stated *briareus* to have spiny fore femora in both sexes (unarmed or partly unarmed in *A. tessulata*), spinier mid and hind femora, and the posterior end of abdominal segment 6 swollen (not the case in *A. tessulata*). Apart from shorter wings, this description could belong to the species reared by the Phasmid Study Group as PSG 15, around 1960s or 1970s, originating from Australia. More recently reared specimens were collected in Cairns and Garradunga, near Innisfail, north Queensland, as described in this paper as *A. robusta* sp. nov. The holotype of *A. briareus* is missing both fore and hind pairs of legs, and earlier literature would not have been able to help Redtenbacher (1908) distinguish these features. Therefore, Redtenbacher used specimens labelled as *Ctenomorpha briareus* in NHMW, drawer 10-17 (6 ♂♂, 6 ♀♀) to make assumptions, including *A. albopunctata*, and compared these to *C. macleayi* (now recognized as *A. austrotessulata*); this can be verified through the recording of white spots on ♂ forewings, which are absent in true *A. briareus*. Although damaged, the *A. briareus* holotype ♀ matches *A. austrotessulata*, and its name has age priority over *A. austrotessulata*. Westwood (1859: 114) stated ‘I have not seen this species.’ The type material of *C. macleayi* (Gray, 1835) is lost, possibly the 97.2 mm syntype ♀ may refer to a nymph. The ♂ is 80.4 mm., but the brief description is inconclusive. *Ctenomorpha nigrovaria* (Stål, 1875) requires further examination, incorporating genetic material, as this morphologically similar individual could represent a new species.



*Ctenomorpha nigrovaria* (Stål, 1875: 83, as *nigro-varia*) syn. N., stat. rev. Lectotype ♀, AUSTRALIA, Cape York (NHRS) (here designated to amend the status of this species); Paralectotype ♂, AUSTRALIA, Cape York (NHRS), synonymised with *Anchiale briareus* by Redtenbacher (1908: 459).

*Ctenomorpha nigro-varia* (Sjöstedt, 1933: 6), type data reference only ♀, but both sexes in NHRS.

### **Description**

Insects mottled light brown to dark brown, medium-sized to large insects. Male mesonotum without mottling and with light pink lateral line along length. Forewings without white dorsal spot, long in males and short in females, c.2× length of forewings. Mesonotum spines black-tipped in females black in males. In females dorsal anterior corners of abdominal segments 1-5 have may have a large white streak. Cerci longer than anal segment.

### **Egg**

Capsule glossy, black with dark brown around micropylar plate (Fig. 3.3b). Capsule fairly smooth, slightly uneven under microscope. Operculum black and flat. Capitulum yellow and ragged. Micropylar plate narrow, with raised black ridge at base. Measurements (mm): capsule length 2.4, height 1.7, width 1.4.

## **Habitat and foodplants**

Drier to wet sclerophyll forests, parks and gardens, coastal and inland sites in all altitudes except the highest, feeds on various *Eucalyptus* spp. (Myrtaceae), *Acacia*, including *A. floribunda* (Fabaceae), *Syncarpia glomulifera* (Myrtaceae), *Allocasuarina torulosa* (Casuarinaceae), and many other plants (Fig. 3.5c). Sometimes occurs in plague proportions, which leads to trees being stripped of leaves. The species can reproduce parthenogenetically, including producing eggs of both sexes (Campbell and Hadlington 1967). Key (1957) noted kentromorphic phases, relating to three Australian phasmid species, due to overcrowded captive raised populations, all in isolated specimens. Wild observations infer occurrence of high numbers, with nymphs and adults showcasing black and yellow markings instead of plain green or brown (Baker 2015; Brock 2012, Brock 2019; Brock et al. 2015). A thorough ecology and parasites paper for this species exists (Hadlington and Hoschke 1959).

## **Season**

Adults have been observed between November and March (iNaturalist 2024; Atlas of Living Australia 2024).

## **Distribution**

New South Wales and southeast Queensland, up to 610 metres a.s.l. (Fig. 3.2).

## Notes

Females can be plain green or brown, or green with brown fore and pre-anal part of hindwings (Fig. 3.5a).



**Figure 3.5.** *Anchiale briareus* in situ. (a) Mating pair, Broken Head Nature Reserve, NSW, 8Feb2017, photo PDB. (b) Female, Beerwah, Qld, 29Nov2011, startle defence display and elaborate body pattern (kentromorphic pest phase), photo PDB. (c) Habitat for panel b, Beerwah, Qld, 29Nov2011 photo PDB. (d) Thorax shot of female from panel b, Beerwah, Qld, 29Nov2011, photo PDB. (e) Male, showing typical tubercles on mesonotum, ranging from 8-13, and absence of forewing white spot, Cania, Qld, 23Nov2011, photo PDB.

*Anchiale mabiensis* (Coupland and Emmott) sp. nov.

(Mabi Anchiale)

**Holotype** ♀, AUSTRALIA, Queensland, Malanda, -17.355S 145.5861E, 02.ii.2023, R.M. Coupland, 730m a.s.l. (QM; Registration number: T259412).

**Paratype** ♂, same data as Holotype (QM; Registration number: T259413).

**Description**

Small, somewhat robust *Anchiale* species, mostly leaf green in ♀ with shades of brown markings on some body segments including head, coxae, trochanters and laterally on abdominal segments. Forewings and pre-anal part of hindwings are mottled with different shades of brown, more reddish-brown in ♂. Hindwings short in ♀, long in ♂ with strong tessellation. Conspicuous, numerous, small spines on mesonotum, light brown in ♀, some black-tipped, black in ♂. Black-tipped serrations on all femora, mid and hind tibiae more brown. Serrations shorter on fore femora and mid and hind tibiae. Abdominal segment 6 laterally swollen at end of segment in ♀ (Fig. 3.6-3.8).

*Anchiale mabiensis* is smaller and more slender than *A. robusta* sp. nov., which occurs sympatrically on the Atherton Tablelands, albeit *A. robusta* sp. nov. is observed less frequently than at other localities, including the mountain village of Kuranda and at lower altitudes around Cairns and Innisfail. A key morphological feature for *A. robusta* sp. nov. is

the hindwing reaching just beyond abdominal segment 4. *Anchiale briareus* although longer and more slender than *A. mabiensis* sp. nov., occurs much further south in Queensland and New South Wales. *Anchiale briareus* also lacks the swollen abdominal segment 6. In northern Queensland, short-winged species include *A. albopunctata* and *A. mabiensis*, with only *A. mabiensis* recorded from Atherton Tablelands. *Anchiale mabiensis* can be distinguished from *A. albopunctata* and other species by its short cerci and unique eggs. Members of the genus *Acrophylla* (Gray, 1835) are much larger and have more spines, but likely to be a close sister genus, *Acrophylla wuelfingi* (Redtenbacher, 1908) and *Acrophylla thoon* (Stål, 1877) are known to occur on the Atherton Tablelands.

## **Female**

Head rounded, slightly longer than wide, with light to dark brown longitudinal lines (Fig. 3.6b, c). Eyes light brown with two longitudinal, dark brown bands. Antennae shorter than fore femora, 25 segments, basal segment longer and wider than segment 2. Pronotum slightly shorter and narrower than head, leaf green colour with two brown longitudinal lines, in rough rectangular patterns. Dark brown dorsal marking at end of pronotum. Mesonotum leaf green, 4× length of pronotum, with c.35 brown dull-tipped spines on dorsal and lateral surfaces, anterior tubercles darker in colour (Fig. 3.6b, c). Ventral mesothorax surface smooth, with four small posterior tubercles. Length of metathorax and median segment shorter than mesothorax. Abdomen smooth, leaf green, more yellow dorsally (Fig. 3.6a). Faint mid-dorsal longitudinal line on abdomen, with mottling more laterally. Dorsal dark brown spot at end of each abdominal segment. Segment 6 with brown lateral lobe. Segments 7-9 shorter and narrower than segments 1-6. Segment 9 and anal segment equal in length. One to two dark brown spots on ventral surface of most abdominal segments, otherwise smooth and uniform

leaf green colour. Segments 7-10 sparsely covered in tiny, dark hairs. Operculum tapered to rounded tip, reaching end of anal segment (Fig. 3.6d, e, f). Anal segment and supra-anal plate obcordate with, supra-anal plate short. Cerci ovate, coming to a point. Forewings less than 0.5× length of hindwings (Fig. 3.6a). Hindwings mottled black and white; pre-anal part of hindwing light brown with dark brown mottling and dark-pinkish inner margin. Uniform tessellated venations on forewings and pre-anal parts of hindwings. Black-tipped serrations on all femora, shorter on fore femora and mid and hind tibiae (Fig. 3.8a).



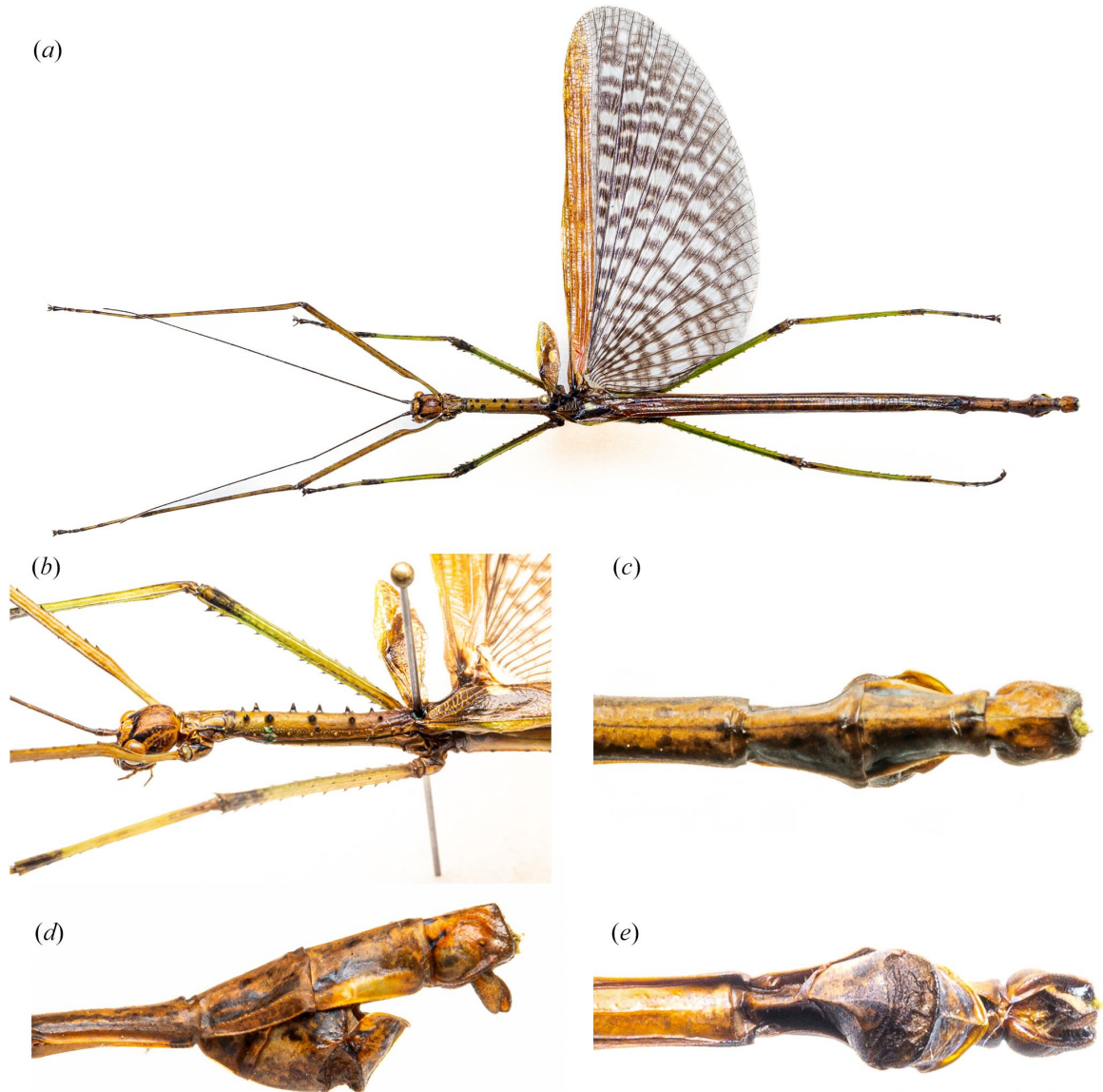
**Figure 3.6.** *Anchiale mabiensis* sp. nov. female holotype. (a) Full dorsal view. (b) Thorax, dorsal view. (c) Thorax, lateral view. (d) End of abdomen, dorsal view. (e) End of abdomen, lateral view. (f) End of abdomen, ventral view. All photos AJE

### Male

Small and slender compared to female. More brown than green (Fig. 3.7, 3.8b). Three distinct ocelli (Fig. 3.7b). Mesonotum with 11 randomly spaced, black-tipped tubercles, smaller lateral tubercles present. Posterior abdominal segments lighter in colour, patterned with



symmetrical dark markings (Fig. 3.7a). Segments 8 and 9 have bulbous appearance due to swollen poculum beneath (Fig. 3.7c, d, e). Poculum reaching over 0.5× length of segment 9. Segment 8 broadened at end, c.0.5× length of segment 7. Segment 9 tapers to tip, slightly longer than segment 8. Anal segment, shorter than segment 9. Anal segment swollen. Cerci short. Segment 6 swollen. Forewings with lighter lateral margin, white central spot present (Fig. 3.7a). Hindwings almost reaching end of abdominal segment 6. Antennae almost reaching end of tibiae, 25 segments.



**Figure 3.7.** *Anchiale mabiensis* sp. nov. male paratype. (a) Full dorsal view. (b) Thorax, lateral view. (c) End of abdomen, dorsal view. (d) End of abdomen, lateral view. (e) End of abdomen, ventral view. All photos AJE

### Egg

Capsule black, oval and glossy, heavily sculptured with raised ridges (Fig. 3.3c). Operculum almost flat except for raised central cone. Without capitulum. Two to three inner rings on

operculum, absent in other *Anchiale* species. Micropylar plate with raised ridges, slender with enlarged base, extending most of capsule length, not reaching operculum.

### Measurements (mm)

Holotype: ♀ Body Length 115.4, head 5.2, antennae 15.6, pronotum 4.6, mesonotum 17.2, metanotum and median segment about equal, combined 13.3. forewing 11.5, hindwing 25.9, femora: fore 20, mid 13.8, hind 18, tibiae: fore 19.8, mid 12.4, hind 18.5, tarsi: fore 4.4, mid 1.4, hind 3.6, cerci 3.15. Paratype: ♂ Body Length 82, head 3.8, antennae 39.2, pronotum 3, mesonotum 10.9, metanotum and median segment about equal, combined 9.5, forewing 7.9, hindwing 46.8, femora: fore 20.9, mid 14.1, hind 18.4, tibiae: fore 21.1, mid 11.4, hind 18, tarsi: fore 5.4, mid 3.4, hind 4.8, cerci 1.5. Eggs: capsule length 2.7, height 2.1, width 1.8.

### Etymology

Mabi is the locally indigenous Dyirbal and Yidiny language name for the Lumholtz's tree-kangaroo, *Dendrolagus lumholtzi*. This threatened species has significant populations in this critically endangered ecological community. As such, the forest type is named after it, 'Mabi forest'.

### Habitat and foodplants

The species is predominantly found in the Mabi forest which is a complex mesophyll vine forest (CMVF), with high rainfall, and cloudy uplands on basalt (Fig. 3.8c). Also known from

lowland, riparian tropical rainforest. Feeds on Grey Sassafras *Doryphora aromatica* (Atherospermataceae).

### **Season**

So far only observed between February and April (the latter, a 122 mm specimen, was found on 7Apr2023, Noelene Tweed, pers. comm.), but likely to be present for several months.

### **Distribution**

Known only from the type locality on the Atherton tablelands, Queensland, Australia and Babinda, Queensland, Australia (Fig. 3.2).



**Figure 3.8.** *Anchiale mabiensis* sp. nov. in situ. (a) Female holotype, photo RMC (Ross M. Coupland). (b) Male paratype, photo RMC. (c) Montane rainforest habitat, photo AJE.

***Anchiale robusta*** (Brock and Jones) sp. nov.

(Robust Anchiale)

**Holotype** ♀, AUSTRALIA: Queensland, Bramston Beach, 17.3606S, 146.0289E, 29.ii.2009, J. Hasenpusch, DNA project JH0001 (QM; Registration number: T258381)

**Paratypes** ♂, 3 ♀, AUSTRALIA: Queensland, Bramston Beach, 17.3606S, 146.0289E, 29.ii.2009, J. Hasenpusch, DNA project JH0002, JH0004-JH0005 (QM; Registration numbers: T258382-T258385) (although JH0003 is in QM, it is not included in the type series due to poor sequencing. The DNA project material was provisionally identified as *Anchiale briareus* (Supplementary File 1)); ♂, ♀ in copula, AUSTRALIA: N. Queensland, Wongaling Beach, 6.xi.1989, G. Milledge (PDB); ♂, ♀ in copula, AUSTRALIA: N. Queensland, 2.5 km. S. of Bald Hills Stn, 15.19S, 145.01E, 4.iv.1991, G. Milledge (PDB); 2 ♂♂, AUSTRALIA: N. Queensland, Garradunga, 17.ii.1993, J. Hasenpusch (PDB); 3 ♀♀, AUSTRALIA: N. Queensland, Garradunga, xii.1995, J. Hasenpusch (PDB); ♂, AUSTRALIA: N. Queensland, Kuranda, 18.v.1992, P.D. Brock (PDB); ♂, AUSTRALIA: N. Queensland, Kuranda, 335m., top of the Range, 19 Butler Dr, 16.48S, 145.38E, 15-28.ii.2005, D.C.F. Rentz (PDB); 5 ♂♂, 1 ♀, AUSTRALIA: N. Queensland, Garradunga, x.1992-i.1993, reared by P.D. Brock (PDB); ♀, AUSTRALIA: N. Queensland, Ella Bay, 14.ii.2006, J. Hasenpusch and P.D. Brock (PDB); ♂, ♀, AUSTRALIA: N. Queensland, Harvey Creek, N. of Babinda, 22.ii.2006, J. Hasenpusch and P.D. Brock (PDB). Although not part of the type series, eggs have also been examined and deposited in collections mentioned.

## Description

Light to dark brown, sometimes greenish-brown, medium-sized to large insects. Live specimens with distinct black marks on head and pronotum, more subtle on hind part of mesonotum, metanotum, abdomen and legs. Forewings and pre-anal part of hindwings uniform reddish brown. Hindwings in females vary from slight to strong mottling, with strong mottling being observed in the more robust individuals. In slight mottled individuals, which are often dark brown, forewings usually have white mark. Hindwings of robust mottled individuals are larger than other Australian species, mostly black, and get whiter on outer part (Fig. 3.11c inset). Ventral part of pre-anal hindwings more reddish than dorsal, likely to be visible to potential predators during deimatic displays. Mesonotum with variable number and size of tubercles, more so in ♀ where they are often orange or brown; rarely black (Fig. 3.11c).

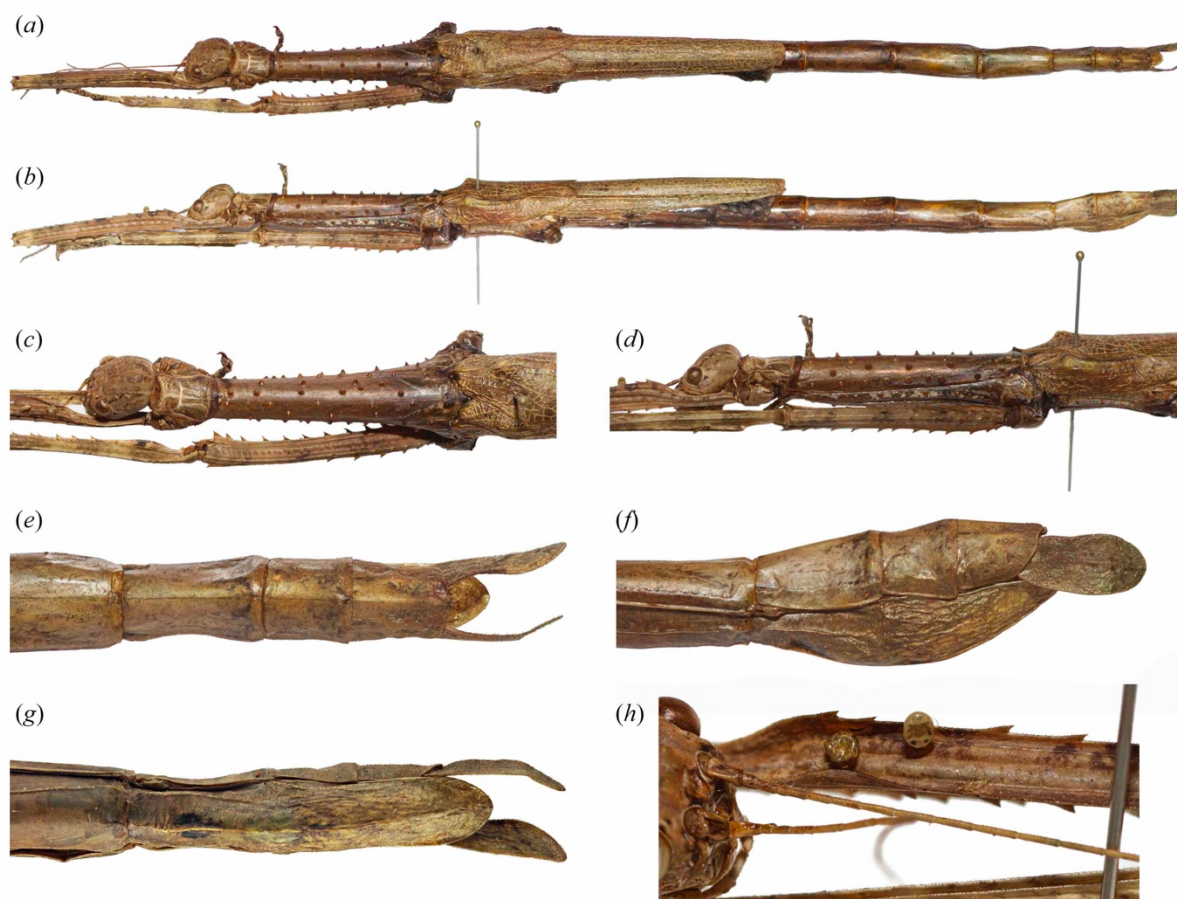
The general form of *A. robusta* is larger, more robust than other *Anchiale* species. It is the only Australian species where females have mid-length hindwing, reaching just beyond abdominal segment 4, *A. mabiensis* with the second longest wings, reaching beyond segment 3. Both *A. mabiensis* sp. nov. and *A. robusta* sp. nov., occur in low numbers on the Atherton Tablelands. Members of the genus *Acrophylla* (Gray, 1835), likely to be closely related sister genus, are larger and have more spines. *Acrophylla wuelfingi* (Redtenbacher, 1908) is particularly common in northern Queensland.

## Female

Head with up to eight broad, black longitudinal bands from back of eyes to hind margin and other smaller black marks. Slightly rounded, longer than wide. Eyes large, with up to eight

longitudinal stripes (Fig. 3.9c, d). Three indistinct ocelli present. Antennae shorter than fore femora, 25 segments. Basal segment broader than other segments, c.2.5× length of segment 2. Pronotum slightly shorter than head, with central impression and distinctive, variable black marks. Mesonotum broadened towards base. Captive raised specimens have low, sparse dorsal tubercles on mesonotum, from c.12 to c.26, about the same number laterally (Fig. 3.9c, d). Tubercles are large irregular cone-like, and orange or brown in colour (Fig. 3.11c). Mesonotum 3.4× length of pronotum. Ventral surface of mesothorax and metathorax with few sparse tubercles. Length of metathorax and median segment shorter than mesothorax. Forewings leaf-like, oval. Hindwings medium-sized, heavily tessellated, reaching end of abdominal segment 4 to just beyond (Fig. 3.9a, b, 3.11b, c, 3.11c inset). Abdomen elongate and robust-looking, segment 6 often swollen (Fig. 3.9a, b). Operculum tapered to tip, reaching just beyond end of anal segment (Fig. 3.9e, f, g). Cerci broad, elliptical in shape, almost 2× length of anal segment. Hindlegs reaching about end of segment 6 or 7. Femora with orange or brown spines, shorter in fore femora, typical for the genus, and shorter still in tibiae. Tarsi hairy.





**Figure 3.9.** *Anchiale robusta* sp. nov. female holotype. (a) Full dorsal view. (b) Full lateral view. (c) Thorax, dorsal view. (d) Thorax, lateral view. (e) End of abdomen, dorsal view. (f) End of abdomen, lateral view. (g) End of abdomen, ventral view. (h) Parasitic mites on left fore femora. All photos BRJ.

## Male

Small and slender compared to female, mostly dark brown (Fig. 3.10a, b, 3.11a). Head slightly rounded, usually with black rings around eyes, three distinct ocelli, black ‘U’ or ‘V’-shaped mark beneath ocelli (Fig. 3.10c, d). Black longitudinal bands from back of eyes to hind margin. Dorsal black band either side of central longitudinal line. Head slightly longer than wide, large eyes, with two pale longitudinal stripes. Antennae shorter than forelegs, 28

segments. Basal segment broader than other segments, c.2.5× length of segment 2; segment 3 onwards are hairy. Pronotum slightly shorter than head, with central impression and distinctive, but variable black marks, lines or bands. Mesonotum often with sparse, indistinct tubercles, rarely black, and more noticeable in some specimens (Fig. 3.10c, d). Mesonotum 4× length of pronotum. Few sparse tubercles on ventral surface of mesothorax and metathorax. Length of Metathorax and median segment shorter than mesothorax. Forewings leaf-like, oval, with a cream lateral margin (Fig. 3.10a, b, 3.11a). Hindwings long, not reaching end of abdominal segment 7. Heavily tessellated. Abdomen elongate, segments 8 and 9 have bulbous appearance due to swollen poculum beneath (Fig. 3.10a, b). Poculum almost reaching end of segment 9 (Fig. 3.10e, f, g). Segment 8 broadened at end, 0.5× length of segment 7. Segment 9 tapers to tip, equal length of segment 8. Anal segment, shorter than segment 9. Anal segment swollen at sides, modified to form claspers, which bear spines. Cerci oblong, 1.5× length of anal segment. Legs elongate, hindlegs reaching or just exceeding end of abdomen (Fig. 3.10a, b). All femora with serrations, shorter in fore femora, shorter still in mid and hind tibiae. Tarsi, hairy (Fig. 3.10e, f, g).



**Figure 3.10.** *Anchiale robusta* sp. nov. male paratype. (a) Full dorsal view. (b) Full lateral view. (c) Thorax, dorsal view. (d) Thorax, lateral view. (e) End of abdomen, dorsal view. (f) End of abdomen, lateral view. (g) End of abdomen, ventral view. All photos BRJ.

## Egg

Capsule originally described by Clark (1976), as *Ctenomorphodes briareus* due to misidentification. Black or dark brown, oval, sculptured with low grey ridges. Glossy when laid, over time fades to become paler (Fig. 3.3d). Operculum almost flat except for raised central cone. Capitulum detachable, uneven, whitish or yellowish. Opercular angle c.-5°. Micropylar plate slender with raised rim, extending most of capsule length, not reaching operculum.

## Measurements (mm)

Holotype: ♀ Body Length 160, head 9.5, antennae 28, pronotum 7, mesonotum 27, metanotum 19, median segment 6, forewing 23.7, hindwing 52.5, femora: fore 36.2, mid 26.8, tibiae: fore 36.7, mid 23, tarsi: fore 16, mid 12, cerci 6.8. Paratype: ♀♀ Body Length 122-162, head 6-9, antennae 17-30 (often broken), pronotum 5-7, mesonotum 20-27, metanotum 8-10, median segment 8-10, forewing 17-23, hindwing 42-55, femora: fore 27-39, mid 18-25, hind 26-35, tibiae: fore 28-40, mid 17-23, hind 23-32, tarsi: fore 12-17, mid 10-12, hind 11-15, cerci 4-8. Paratype: ♂♂ Body Length 93-104, head 4-5, antennae 47-50, pronotum 3.5-4, mesonotum 14-15, metanotum 6-8.9, median segment 3.7-6.5, forewing 11-12.8, hindwing 56-57.2, femora: fore 28-30, mid 20-22, hind 26-29, tibiae: fore 30.5-33, mid 19-20, hind 26-28, tarsi: fore 13.7-15, mid 10, hind 11, cerci 2.5-3.1. Eggs: capsule length 2.7-2.9, width 1.6-1.7, height 2-2.1.

## Etymology

Named after the robust appearance of this species.

## Habitat and foodplants

Mainly coastal rainforest, feeding on a wide variety of vegetation, often on *Acacia*, but also including *A. melanoxylon* (Fabaceae), *Eucalyptus* species (Myrtaceae), *Corymbia intermedia* and *Syzygium* species (Myrtaceae), *Buckinghamia celcissima* (Protaceae) and *Litsea leefeana* (Lauraceae) (Brock and Hasenpusch 2009) (Fig. 3.11d, e).

### **Season**

So far only observed between October and May, likely to occur in low numbers outside this season (iNaturalist 2024; Atlas of Living Australia 2024).

### **Distribution**

Northern Queensland, from Tully to north of Cooktown, probably a range of at least 450 km (Fig. 3.2). An overlap with other *Anchiale* species occurs in the Atherton Tablelands, with *A. mabiensis* sp. nov., and a possibility of *A. albopunctata*. ALA records include Fitzroy Island.

### **Notes**

Female holotype preserved with parasitic mites on left fore femora (Fig. 3.9h).





**Figure 3.11.** *Anchiale robusta* sp. nov. in situ. (a) Male. (b) Female. (c) Female defence display, inset: wings of preserved female paratype. (d) Coastal habitat, Bramston Beach, Qld. (e) Garden habitat, Kuranda, Qld. All photos PDB.

*Acrophylla spinicollis* (Gray, 1833) comb. nov.

(Spiny Acrophylla Stick-insect)

*Phasma* (*Ctenomorpha*) *spinicolle* Gray (1833: 16, 27, pl. 1: 1) (Fig. 3.12), page 16 refers to *Ctenomorpha spinicollis*, but on page 15, Gray makes it clear all listed genera are subgenera of *Phasma*. **Holotype** ♂, AUSTRALIA, Northern Territory, Melville Island (OUMNH).

*Ctenomorpha spinicollis* (Gray, 1835: 41; Burmeister 1840: 38; Westwood 1859: 114, as synonym of *Acrophylla japetus* (Gray, 1833); Rainbow 1897: 41, as synonym of *Acrophylla japetus*; Tepper 1903: 280, as synonym of *Acrophylla osiris* (Gray, 1834, now recognised as *Eurycnema osiris*); Redtenbacher 1908: 459, as synonym of *Ctenomorpha japetus*.

*Anchiale spinicollis* (Kirby, 1904: 393, as '?' in *Anchiale*; Vickery 1983: 3; Balderson et al. 1998: 362; Otte and Brock 2005: 45; Brock and Hasenpusch 2007: 72; Brock and Hasenpusch 2009: 98, 165; Hennemann et al. 2015: 13; Brock and Büscher 2022: 558; additional checklist and other references in Brock et al. 2023).

*Phasma* (*Diura*) *japetus* (Gray, 1833: 20, 26, pl. 5: 1). **Holotype** ♀, AUSTRALIA, Northern Territory, Melville Island (OUMNH); synonymised by Burmeister (1840: 38) who was first reviser. **Comment:** *Anchiale spinicollis* is here removed from *Anchiale* and provisionally placed in *Acrophylla* as *Acrophylla spinicollis*. This is supported by molecular and morphological work (Fig. 3.2). ♂, has the following morphological features to better place it in *Acrophylla*; reduced cerci, green colouring on fore wings and pre-anal part of hindwings, and mostly clear hindwings (Fig. 3.12b). ♀, has the

following morphological features to better place it in *Acrophylla*; elongate and pointed cerci (unlike the more ovate, oblong, or reduced in length cerci of other Australian *Anchirole*), more robust body, round egg (*Anchirole* eggs being more oval) with large capitulum (Fig. 3.12a, 3.12a inset).

## **Description**

Insects light green to light brown, medium-sized to large. Pronotum with several tubercles. Male forewings with lighter lateral margin. Hindwings tessellated in females, males only lightly tessellated towards outer margin.

## **Egg**

Capsule glossy, smooth and round (Fig. 3.12a inset). Pigmented black, micropylar plate dark brown. Operculum raised towards central cone. Capitulum white to pale yellow. Micropylar plate narrow, swollen at base. Measurements (mm): capsule length 2.8, height 2.4, width 2.

## **Habitat and foodplants**

Not known, but most likely tropical eucalypt forests. In a study of eucalypt forests from the type locality, Melville Island (Fensham 1990), phasmids were described as comprising only a ‘miniscule proportion of the total collection’.

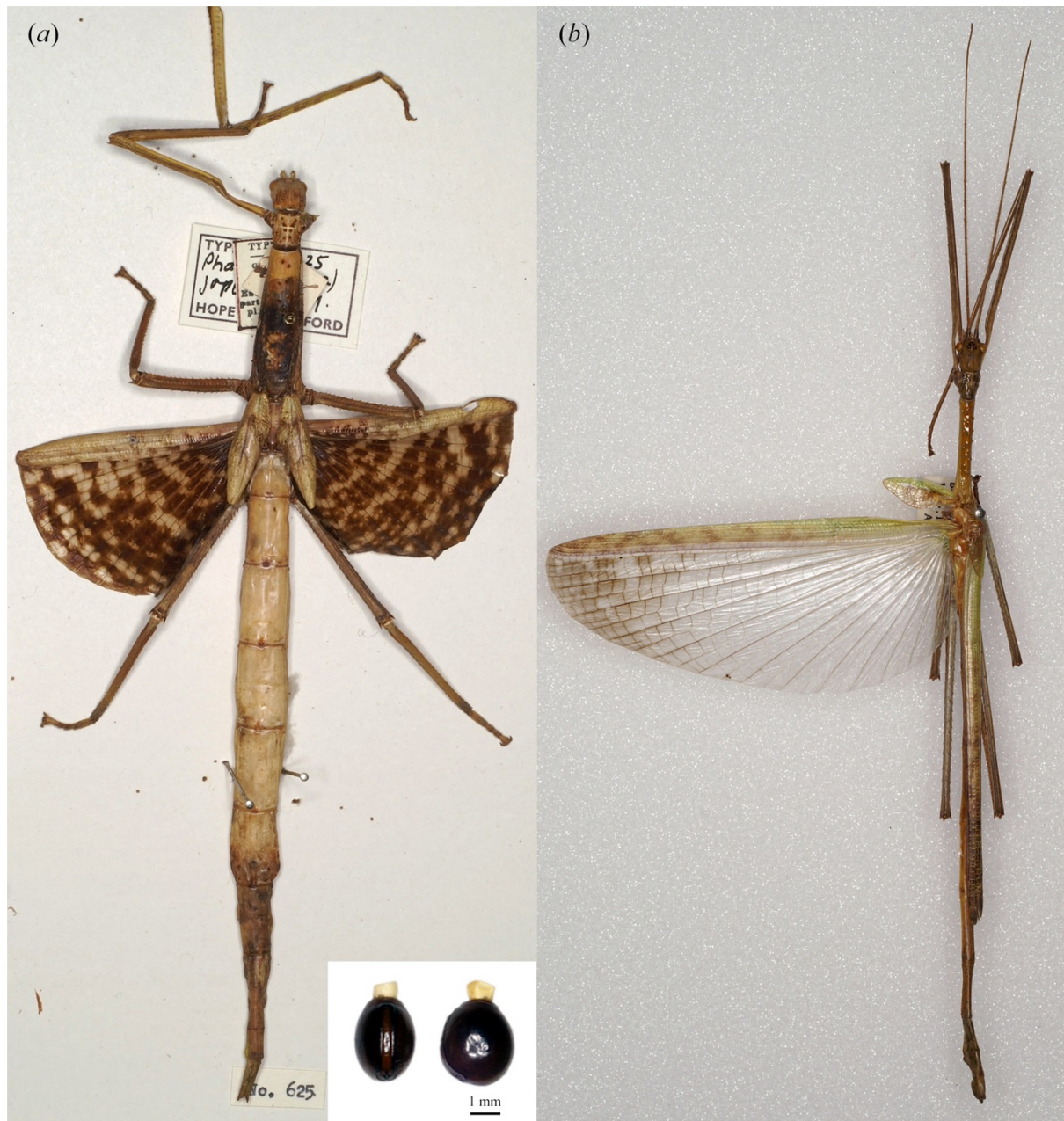


**Season**

So far only observed between September and March (iNaturalist 2024; Atlas of Living Australia 2024).

**Distribution**

Northern Territory, Northern Queensland, Western Australia, assuming records do belong to *A. spinicollis*. Tubercles on thorax vary in number between habitat location. ♂ cerci length varies, short in holotype.



**Figure 3.12.** *Acrophylla spinicollis* comb. nov. (a) Female (holotype of synonym *Phasma (Diura) japetus*), Melville Island, NT (OUMNH), inset: eggs of non type *A. spinicollis*, dorsal and lateral views. (b) Male, Berrimah, NT, 19Feb1972, B.C. Abbey (ANIC). All photos PDB.

## 3.6 Discussion

Based on molecular results and examination of morphological characters, a redefinition of the genus *Anchiale* is proposed. Two new species are described, one removed from synonymy, and one transferred to another genus.

### 3.6.1 Morphological analysis

Australian *Anchiale* possess similarities in wing patterning, cerci shape and egg morphology. Although differences exist in hindwing mottling between species, all Australian *Anchiale* species have dark brown to black patterns (Fig. 3.5b, 3.6a, 3.7a, 3.11c, 3.11c inset). Wings in ♀ *Anchiale* are also reduced in size compared to the closely related sister genus *Acrophylla*, reaching to only abdominal segment 4 (Fig. 3.4, 3.5a, b, 3.6a, 3.8a, 3.9a, b, 3.11b, c). Some species have a distinct white spot on the fore wings (Fig. 3.4, 3.7a, 3.8b, 3.10a, 3.11a). ♀ *Anchiale* also exhibit distinct cerci shapes, typically being large and ovate or oblong (Fig. 3.4, 3.5b, 3.9e, f, g), except for *A. mabiensis*, whose cerci are reduced in size (Fig. 3.6d, e, f). A similar pattern (where *A. mabiensis* is an outlier) is also seen among *Anchiale* eggs. Eggs belonging to *A. albopunctata*, *A. briareus*, and *A. robusta* are all small, shiny when laid, black to dark brown in colour, oval and contain a small capitulum (Fig. 3.3a, b, d) differing to the round *Acrophylla* eggs with a larger capitulum. However, eggs laid by *A. mabiensis* are not shiny (because of sculpturing on their surfaces), brown, less elongate than other Australia *Anchiale* and exhibit inner rings on operculum (Fig. 3.3c). This paper also marks the first time inner rings on the operculum are recorded in Phasmatodea. The distribution of *A. mabiensis* also differs with other *Anchiale* species, as it seems to be endemic to a small geographic region. Members of the genus *Austrocarausius* show similarly small ranges in Qld rainforest habitats, having diversified in these habitats over the last ~40 Ma (Jones et al.

2022; Bryant and Krosch 2016). Considering the outlying morphology of *A. mabiensis*, and lack of any genetic material, it seems like an interesting topic to explore its placement within the genus in subsequent studies.

It is hoped that the photos and keys provided in this paper will clarify the complicated morphological variation to assist with species identification. When looking at morphology within the genus, delimitation of each species is somewhat challenging due to colour variation and size differences within species. Colour variation could possibly stem from differing foodplants or camouflage, as well as kentromorphism. Across Australia, *Anchiale* species are the most sighted phasmids (iNaturalist 2024; Atlas of Living Australia 2024). One species, *A. briareus*, displays kentromorphism and is known to undergo outbreaks: the “low-density phase” is uniform and green, while the “high-density phase” can be mottled black, yellow and white (Key 1957). Different colour forms in *A. albopunctata* also occur among the same populations, but so far this trait is unlinked to kentromorphism.

### 3.6.2 Phylogenetic placement of *Anchiale*

Within the different species of Australian *Anchiale*, species clades are well supported in both Bayesian inference and maximum likelihood (Fig. 3.2; Supplementary Figure S3.1, S3.2).

The movement of *A. spinicollis* from *Anchiale* to *Acrophylla* is supported by both morphology and molecular data; *A. spinicollis* is placed as the sister taxon to *A. wuelfingi* (Fig. 3.2). *Acrophylla spinicollis* is known to occur across the entire northern region of Australia. However, it is unclear whether there is continuous gene flow across its entire distribution, or if the species is comprised of fragmented, genetically diverse populations or cryptic species. As seen across two other Australian Phasmatodea subfamilies (Necrosiinae: *Candovia*, Lonchodinae: *Austrocarausius*), cryptic taxa often harbour multiple species (Jones

et al. 2022; Forni et al. 2023). The same question arises for *A. albopunctata* where two clades within the genus are observed (Fig. 3.2). The two *A. albopunctata* clades are on average 4.8% divergent, which supports splitting up the species, considering that the COI sequence divergence between *Megacrania tsudai* and *M. batesii* is 3.1% (Wu et al. 2020). However, without supporting morphological and specimen data, the two *A. albopunctata* clades should remain as one species. Further sampling and systematic study of these species will help to address these questions.

The monophyly of Australian *Anchiale* species are well supported in molecular analyses, although the position of *A. mabiensis* was not examined. The relationships between *Anchiale* and close sister taxa are not conclusive (Fig. 3.2; Supplementary Figure S3.1, S3.2). *Acrophylla* (Gray, 1835) was previously considered the sister genus of *Anchiale* (Hennemann et al. 2015). However, *Phasma* (Lichtenstein, 1796) was placed as sister to *Anchiale* in a recent study (Robertson et al. 2018). In this study, the grouping of species within *Acrophylla* is not well supported. The genus *Acrophylla* is paraphyletic, with *Acrophylla* sp. (HM425694.1) (most likely *A. cookorum* (Brock and Coupland, 2022)) grouping with *Anchiale* (0.53 PP, 20.5 BS, 46 SH-aLRT). It is possible that this sample has not been identified correctly or that it represents a new species within *Anchiale*. Similarly, *Acrophylla thoon* does not group with other *Acrophylla* (Robertson et al. 2018). Further extensive study of *Acrophylla* in combination with study of *Anchiale* across all the isolated islands around Australia will help to better define the systematics of these genera.

Collection of non-Australian *Anchiale*, particularly from the Papuan region, is also required to better understand the evolution and systematics of the genus. Eggs of Australian *Anchiale* are known to differ from those of the type species, *A. maculata* (Olivier, 1797) (Zompro 2009). However, without molecular data it is not possible to determine

biogeographical origins of the genus. A similar question exists in the genus *Megacrania*, where it is suspected *M. batesii* from Maluku and Kai Islands differ to those found in Australia and could be separate species (Cermak and Hasenpusch 2000; O’Hanlon et al. 2020). In the absence of genetic data this question is also unresolved.

## CHAPTER 4

### **Phylogenetic analysis of Australian Lonchodini stick insects, with reinstatement of the genus *Marcenia* Sjöstedt, 1918 and description of 5 new species.**

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Hartley, Stephen Bent and Nathan Lo

**Authors' contributions.** B.R.J. and N.L. devised the study with assistance from D.K.Y. and  
A.Z. Genetic sequence extraction was performed by H.Y., J.N., D.H., and S.B. B.R.J.  
conducted the analyses and wrote the manuscript with input from N.L.

## 4.1 Abstract

The Australian tribe Lonchodini (Brunner von Wattenwyl, 1893) consists of the genera *Austrocarausius*, *Denhama*, and *Hyrtacus*. The genus *Austrocarausius* contains numerous cryptic species, and species diagnoses within *Denhama* and *Hyrtacus* are unclear due to morphological similarities. *Denhama* and *Hyrtacus* are apterous, small to medium sized species that inhabit arid environments Australia wide. Recent examination of live and preserved specimens suggests there may be more species and genera which do not reside within the six described *Denhama* and four described *Hyrtacus* species. This study uses morphological and molecular data from field-collected and historic museum samples from *Denhama* and *Hyrtacus* specimens to perform an integrative taxonomic investigation and describe new species and reinstate one genus. Genetic sequences from whole mitochondrial genomes and two nuclear genes are used to perform phylogenetic analysis, and estimate the evolutionary timescale of species and genera, to uncover the arid Lonchodini diversification across Australia. The results suggest the reinstatement of *Marcenia* (Sjöstedt, 1918), the description of five new *Marcenia* species, *M. cylindrix* sp. nov., *M. gojira* sp. nov., *M. spinosa* sp. nov., *M. sticcosa* sp. nov., and *M. apheles* sp. nov., and reinstating *M. cunctatrix* (Sjöstedt, 1918; removing from synonymy with *Hyrtacus caurus* (Tepper, 1905)) and *M. imitans* (Brunner von Wattenwyl, 1907; removing from synonymy with *Denhama longiceps* (Brunner von Wattenwyl, 1907)). The molecular dating analyses indicate that most *Marcenia* species diversified between ~8.2-15.8 Ma, corresponding to aridification and increasing grassland habitats across the Australian landscape. This investigation of arid zone Lonchodini is the most comprehensive review of species and genera to date, and the first to examine their evolution with integrative systematics. It is likely that many other species exist across the whole of Australia, and this study highlights gaps in the taxonomy for all Australian Lonchodini species and genera.



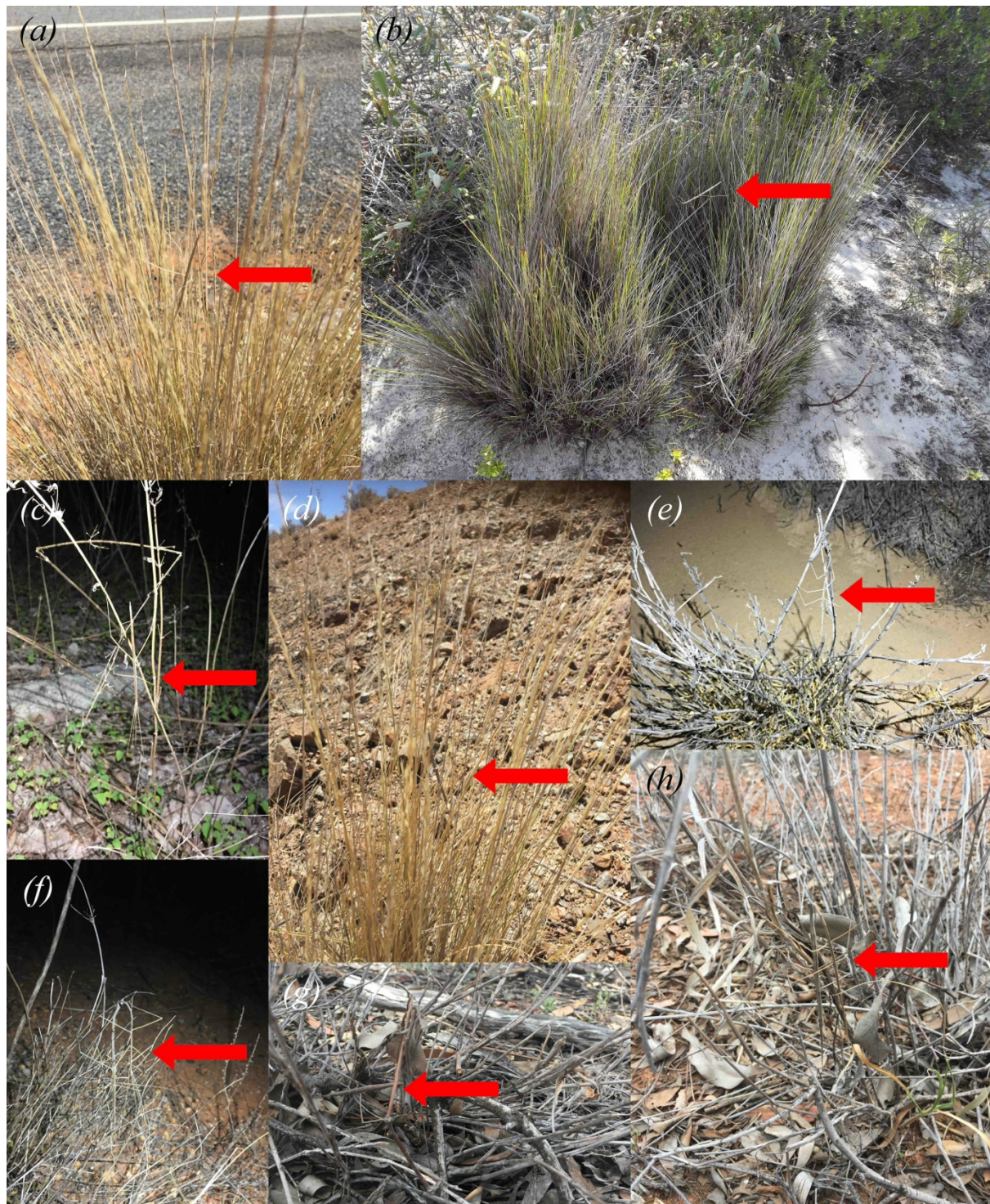
## 4.2 Introduction

The majority of described Australian Phasmatodea can be found on the eastern coast, with many of these species endemic and restricted to mesic environments. However, there are many arid-adapted stick insect species found in the central areas of the Australian continent, which are often under-studied. One of the families found in Australia is the Lonchodidae (Brunner von Wattenwyl, 1893), which consists of three genera from the tribe Lonchodini (Brunner von Wattenwyl, 1893). This family comprises stick insects found in both coastal and central Australian environments.

Lonchodini comprises 39 genera and 295 species distributed across the Oriental, Australasian, Palearctic, Afrotropical, and Nearctic regions. This group of insects consists of many medium- to small-sized species resembling sticks and grasses, which often lack wings. The three Australian Lonchodini genera are apterous in both sexes and consist of *Austrocarausius*, *Denhama*, and *Hyrtacus*. Difficulties in species diagnoses have been persistent in these genera due to morphological similarities between cryptic species. However, through the use of integrative approaches that incorporate both morphological and genetic data, progress towards delineating species within these groups has recently been made (Jones et al. 2022).

*Austrocarausius* is entirely endemic to isolated Australian rainforest patches. In contrast, the *Denhama* (six species) and *Hyrtacus* (four species) groups are found across most Australian states and territories, except for the ACT and Tasmania. *Denhama* and *Hyrtacus* are Australian endemics except for two *Hyrtacus* species from New Guinea, *H. procerus* (Brunner von Wattenwyl, 1907) and *H. semoni* (Brunner von Wattenwyl, 1907). The habitats of *Denhama* and *Hyrtacus* are unique for Australian taxa, as they are ground dwelling and mostly found in arid grassland habitats (Brock and Hasenpusch 2009; Fig. 4.1a, b, c, d, e, f,

g, h). Species from these two genera feed on *Acacia* spp. and *Triodia microstachya*, and *Denhama* are documented to glue their eggs to host plants (Brock and Hasenpusch 2009). *Denhama* have elongated bodies and legs and imitate grasses, while *Hyrtacus* are more physically robust and imitate sticks (Brock and Hasenpusch 2009). Recently, the number of *Austrocarausius* species from the Queensland coast was expanded from two to eleven using an integrative taxonomic approach (Jones et al. 2022). This study aims to expand this work to investigate whether *Hyrtacus* and *Denhama* also exhibit cryptic diversity, through an integrative taxonomic approach.



**Figure 4.1.** In situ photos of arid Australian Lonchodini species. All photos are of undescribed species belonging to Lonchodini. Arrows point out individuals within each image. (a) Female during the day, by the side of a highway on live grass. (b) Female during the day, in a coastal environment on live grass. (c) Female at nighttime, on dead grass. (d)



Female during the day, on live grass. (e) Female at nighttime, on dead grass. (f) Male and female in copula, at nighttime on dead grass. (g) Female during the day, on dead grass. (h) In copula male and female during the day, on dead grass.

Observations of specimens in museums and field collections (pers. obs.) have revealed numerous individuals from *Denhama* and *Hyrtacus* that do not fit into any key and do not match any existing species descriptions. From these observations, the currently synonymised *M. cunctatrix* (Sjöstedt, 1918), known as *H. caurus* (Tepper, 1905), potentially represents its own valid species and genus. Accordingly, this study aimed to: 1) perform a comprehensive molecular phylogenetic analysis of Australian *Denhama* and *Hyrtacus* specimens across their distributions, to test the monophyly of genera, 2) investigate the status of *M. cunctatrix* using both molecular and morphological approaches to clarify potential reinstatement of *Marcenia*, 3) describe any new species within *Marcenia*, 4) investigate the diversification of the Lochodini across Australia, and 5) examine how dispersal is maintained within species across large population distributions.

## 4.3 Methods

### 4.3.1 Abbreviations used

Australian National Insect Collection, Canberra, Australia (ANIC); Queensland Museum, Brisbane, Australia (QM); Natural History Museum, London, UK (NHMUK); Naturhistoriska Riksmuseet, Stockholm, Sweden (NHRS); Naturhistorisches Museum Wien, Vienna, Austria (NHMW); Zoologisches Institut und Zoologisches Museum, Universität von Hamburg, Hamburg, Germany (ZMUH).

#### 4.3.2 Specimen collection

Specimens used in this paper were either collected live or sampled from museum specimens stored at ANIC. Specimens were collected from the field by Braxton Jones, under permit number WITK18701717. The permit was applicable to Queensland only, and no other permits were required. All samples from ANIC were reorganised into either *Denhama* or *Hyrtacus* groups. Similarly, all specimens collected in the field represented species most closely related to those two genera. Each specimen was observed for overall body morphology, and the shapes and sizes of either the anal segment or the sub-anal plate. These two attributes were compared to those of *Denhama* and *Hyrtacus*, and all other Australian genera. Before mounting collected specimens, the right midleg was cut off and stored in 100% ethanol until DNA extraction for molecular analysis. All specimens for this paper are found in ANIC, QM, or SAM.

#### 4.3.3 Morphological analysis and imaging

A total of 269 specimens across the collections of ANIC and Braxton Jones were morphologically examined. Initial morphological analysis used overall body morphology and the shape and size of either the anal segment or sub-anal plate, and revealed a number of taxa, including one species previously described as *Marcenia cunctratix*, which shared a synapomorphic character. This character was the absence of the sub-anal plate, which is present and elongated in *Denhama* and *Hyrtacus*. Accordingly, these taxa were grouped into a putative genus *Marcenia* prior to subsequent analyses.

Although numerous new species are likely to be present among the examined specimens, only a subset of specimens here within the candidate genus *Marcenia* were examined in this study (Supplementary Table 4.1). This subset was chosen based on the *Marcenia* clades which were consistently grouped with the type species (*M. cunctatrix*) clade in phylogenetic analyses. Examination of additional specimens will be completed in future studies, after collection of more adult specimens and their eggs, to increase accuracy of species diagnoses. Similarly, new genera are not described until complete morphological data can be generated. Male specimens were lacking across the examined genera, due either to possible parthenogenesis in some species, or males simply being harder to find. Consequently, only female specimens were used, leading to a more consistent foundation against which new genera and species could be proposed. From initial analyses, females appeared to have more useful characters for species delineation than males, including characters associated with the anal segment or supra-anal plate, and egg morphology (Fig. 4.2).



**Figure 4.2.** Eggs from different Australian Lonchodini species and genera to illustrate the morphological egg variation. Refer to Supplementary Figure S4.1 for annotated figure.

Photos by Braxton Jones.

Images were taken using a Canon 7D Mark I camera body fitted with a 100 mm Canon macro lens. Processing and stacking of images were performed in Adobe Photoshop 2022 (ver. 23.1.0, Adobe, <https://www.adobe.com/au/>) and in ZereneStacker (ver. 1.04, Zerene Systems, <http://zerenesystems.com/cms/home>). Morphological analyses were performed before molecular analyses to avoid biases inferred from molecular analyses. When results from morphological and molecular analyses were incongruent, conservativeness was prioritised, with new species not inferred.

#### 4.3.4 Molecular analysis

DNA extraction was attempted for 203 specimens selected from the 269 previously examined *Denhama*, *Hyrtacus*, and various undescribed taxa which displayed similarities to these genera. Extraction was successful in 186 out of 203 of these samples. DNA extraction and sequencing was performed by ANIC, who have expertise in extracting fragmented DNA from historical, dried specimens. A proteinase K digestion with a silica filter-based method was used to extract 5 ng of DNA to build ligation-based whole-genome shotgun sequence libraries. This was performed using an acoustic liquid handler (Echo 525; Beckman Coulter, California, USA), to increase the reaction efficiency. DNA libraries for different samples were pooled equimolar to be sequenced by an Illumina NovaSeq 6000 platform, with S1 flow cell and 300-cycle kit, at the Biomolecular Resource Facility at Australian National University. Results yielded paired-end reads of 150 bp in length, which were then demultiplexed (further details on methodology are provided in Jin et al. 2020 and Zwick and Zwick 2023). This method led to the successful assembly of the whole mitochondrial genome, except for the control region, and nuclear genes *18S*, *ITS1*, *5.8S*, *ITS2*, and *28S*.

Once raw reads were obtained, mitochondrial genome and nuclear contigs were assembled with SPAdes v.3.12.0 (Bankevich et al. 2012), using default settings (*k* 33, 55, 77, 91, and 121). To extract contigs, the SPAdes output was mapped to an *Extatosoma tiaratum* mitochondrial reference genome and *Carausius morosus* 18S and 28S nuclear genes, downloaded from GenBank, in Geneious Prime (v.2023.2.1, <https://www.geneious.com>). In some cases, full mitochondrial genome and nuclear gene contigs could not be assembled, so partial contigs were accepted. Additionally, samples without any full or partial contigs were processed with a separate pipeline whereby the raw reads were filtered against reference



genomes using Burrows-Wheeler Aligner (Li and Durbin 2009). Reference genomes included all available phasmid genomes from GenBank, in addition to the newly assembled genomes from the SPAdes output. The filtered output was mapped in Geneious with low sensitivity to one of the ingroup reference genomes extracted from the SPAdes output. The same process was also applied to the assembled nuclear genes. The consensus sequences were extracted under Highest Quality 60% (sites below 60% were coded as N's) and when coverage was  $\geq 10$ .

All samples were then aligned to the annotated *Extatosoma tiaratum* mitochondrial reference genome, to allow extraction of each individual gene. Sequences were then checked for alignment with the correct reading frame, where applicable, in Seqotron (ver. 1.0.1, <https://github.com/4ment/seqotron>; Fourment and Holmes 2016). They were also aligned in Seqotron using Muscle (ver. 3.2, <http://www.drive5.com/muscle/>; Edgar 2004). The resulting alignment consisted of 274 samples, including 186 new specimens extracted in this study and 34 Lonchodini and 54 outgroup taxa from GenBank (Supplementary Table 4.2).

Phylogenetic relationships were inferred using maximum likelihood and Bayesian inference. A total of 17 genes were included across analyses (*COXI*, *COXII*, *COXIII*, *ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5*, *ND6*, *CYTB*, *ATP8*, *ATP6*, *16S*, *12S* (15 mitochondrial genes), *18S*, *28S* (2 nuclear genes)). For outgroup taxa, molecular data were sourced from GenBank for up to five genes per sample: *COXII*, *16S*, *12S*, *18S*, and *28S* (Supplementary Table 4.2). The best partitioning scheme and substitution models was selected using ModelFinder (-m MFP+MERGE) in IQTREE (ver. 2.1.3, <http://www.iqtree.org/>; Trifinopoulos et al. 2016), which considered each gene separately, with protein coding genes further divided by codon position (Chernomor et al. 2016). The resulting best partition scheme consisted of 13

partitions (Supplementary Table. 4.3). This same partitioning scheme was also used in Bayesian analyses.

To infer phylogenetic relationships among Australian Lonchodini, a total of ten different analyses were performed (Table 4.1). Two taxon datasets were used in the analyses: the first, contained all 274 samples, while the second comprised 124 samples (representing the *Marcenia* clade, selected Lonchodini genera, and outgroups). The latter (smaller) dataset was used to save computational time. For the two taxon datasets, separate analyses were performed on only nuclear genes, only mitochondrial genes, and a combined genetic dataset with both nuclear and mitochondrial genes (Table 4.1). Analyses on all datasets were performed using maximum likelihood and Bayesian inference methods. Evolutionary timescales were only estimated for the smaller dataset. Topologies from all analyses were compared to examine the consistency of taxon groupings at both the genus and species levels.

**Table 4.1.** Graphical depiction of the 10 analyses used, showing different gene alignments, phylogenetic software programs, and sample datasets.

|                | <b>Combined: mtDNA<br/>+ Nuclear</b> | <b>Mitochondrial</b> | <b>Nuclear</b>  |
|----------------|--------------------------------------|----------------------|-----------------|
| <b>IQTREE</b>  | Full dataset                         | Full dataset         | Full dataset    |
|                | reduced dataset                      | reduced dataset      | reduced dataset |
| <b>MrBayes</b> | reduced dataset                      | reduced dataset      | reduced dataset |
| <b>BEAST</b>   | reduced dataset                      |                      |                 |

Maximum likelihood analyses were performed in IQTREE (ver. 2.1.3, <http://www.iqtree.org/>; Trifinopoulos et al. 2016). Node support values were estimated by ultrafast bootstrapping 1000 replicates and performing SH-like approximate likelihood-ratio test. Final trees were visualised in FigTree (ver. 1.4.4, <http://tree.bio.ed.ac.uk/software/figtree/>, accessed 13 May 2021).

Bayesian inference analyses were executed in MrBayes (ver. 3.2.7a; Ronquist et al. 2012) and BEAST (ver. 2.6.3, <https://www.beast2.org/>; Bouckaert et al. 2014). Each of the 13 partitions had a separate GTR+I+G substitution model applied, the closest to the models estimated in ModelFinder. MrBayes analyses were conducted using Markov chain Monte Carlo sampling with two independent chains. Each analysis was run for  $2 \times 10^7$  generations and checked for convergence in TRACER (ver. 1.7.2, <https://github.com/beast-dev/tracer/releases/tag/v1.7>; Rambaut et al. 2018), making sure the effective sample size for each or most parameters was reached  $\geq 200$ .

For the estimation of evolutionary timescales in BEAST the substitution model selection was done using the bModelTest package (ver. 1.3.0, <https://github.com/BEAST2-Dev/bModelTest>; Bouckaert and Drummond 2017). Default settings were applied to invariant sites and rate heterogeneity. An uncorrelated lognormal relaxed clock was used to model among-lineage rate variation (Drummond et al. 2006), with a birth-death tree prior. To infer evolutionary timescale, crown groups were assigned representing three fossils, *Eophyllium messelense* (minimum age of 47 Ma; Wedmann et al. 2007), *Eophasma* spp. (minimum age of 44 Ma; Poinar 2011), and *Clonistria* sp. (minimum age 20 Ma; Poinar 2011) (Supplementary Table 4.4). Soft upper bounds of 110 Ma for each fossil were used based on the soft upper bounds of previous studies (Misof et al. 2014; Tong et al. 2014; Montagna et

al. 2019; Robertson et al. 2018; Simon et al. 2019). The outgroup topology of the BEAST tree analysis was constrained based on relationships found previously (Robertson et al. 2018; Simon et al. 2019). An exponential prior distribution was applied to fossil calibrations. Each analysis was run for  $2 \times 10^7$  generations and checked for convergence in TRACER (ver. 1.7.2, <https://github.com/beast-dev/tracer/releases/tag/v1.7>; Rambaut et al. 2018), making sure the effective sample size for each or most parameters was reached  $\geq 200$ . The maximum clade credibility trees were produced in TreeAnnotator (ver. 2.6.3, <https://www.beast2.org/treeannotator/>; Bouckaert et al. 2014) with 10% burn-in, and the final trees were visualised in FigTree (ver. 1.4.4, <http://tree.bio.ed.ac.uk/software/figtree/>, accessed 13 May 2021).

## 4.4 Results

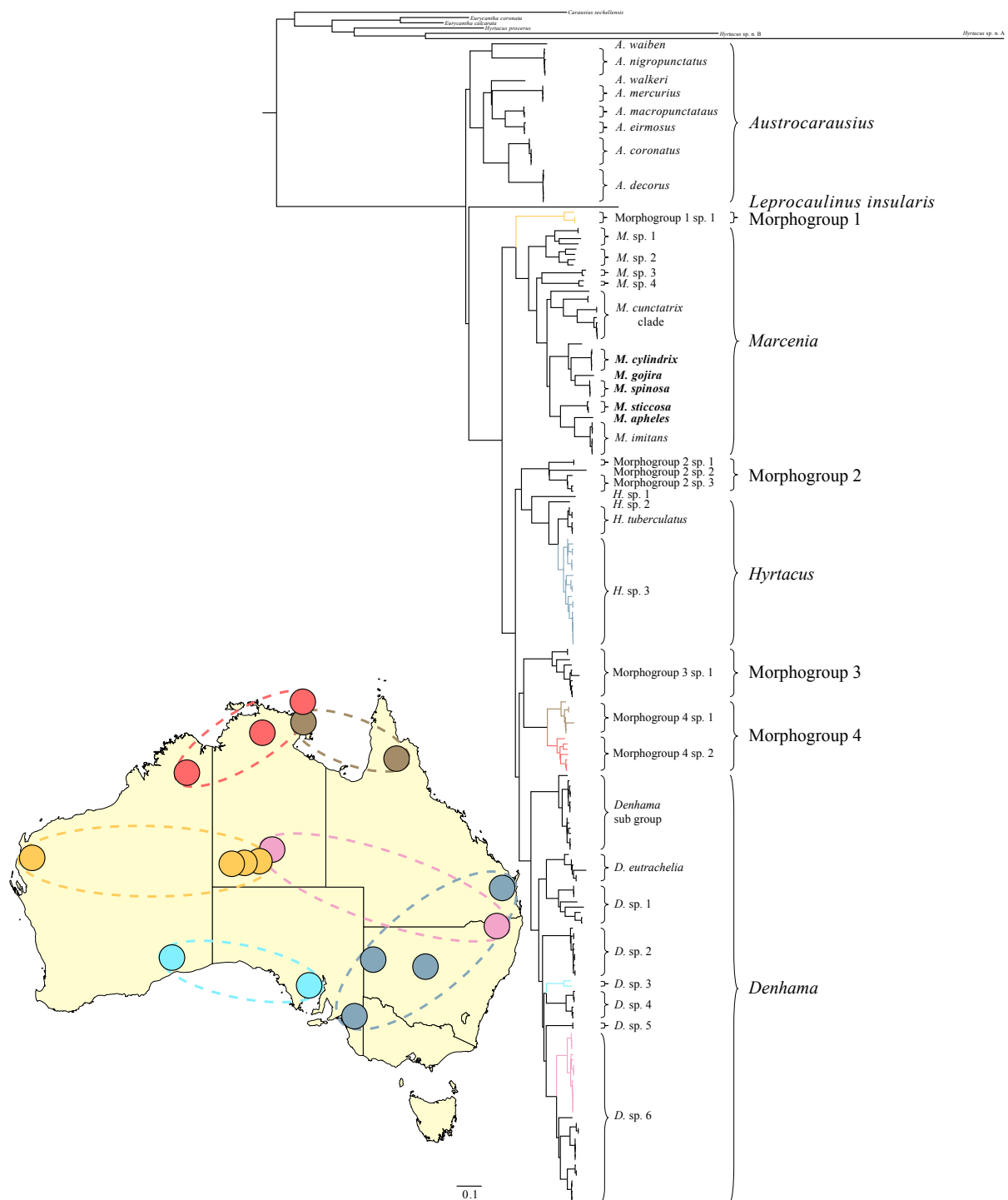
### 4.4.1 Phylogenetic relationships among all examined taxa

Based on the combined mitochondrial + nuclear and mitochondrial full datasets (Table 4.1), the two earliest diverging lineages among the examined Australian Lonchodini were *Austrocarausius* and *Leprocaulinus insularis*, which are sister taxa to all other arid Lonchodini samples (Fig. 4.3; Supplementary Figure S4.2-3). Among the clade of arid taxa, a clade comprising morphogroup 1 + *Marcenia* was the earliest diverging lineage, followed by morphogroup 2 + *Hyrtacus*, and finally morphogroup 3 + morphogroup 4 + *Denhama*. The topology inferred from mitochondrial data (Supplementary Figure S4.3) was the same as that from the combined dataset, except for the mitochondrial data inferring the earliest diverging genus *L. insularis* diverged before *Austrocarausius*. The topology inferred from the nuclear dataset (Supplementary Figure S4.4) was the least well-resolved, and differs the most from the trees inferred from the combined and mitochondrial datasets. The earliest branching

lineage in this tree was the *Denhama* subgroup, followed by the two remaining clades comprising morphogroup 3 + morphogroup 4 + *Denhama*, and *Hyrtacus* + *L. insularis* + morphogroup 2 + morphogroup 1 + *Marcenia*.

In combined mitochondrial + nuclear and mitochondrial full dataset trees (Fig. 4.3; Supplementary Figure S4.3; Table 4.1), all seven proposed genera were recovered as monophyletic. In the analysis of nuclear sequences (Supplementary Figure S4.4), only morphogroup 1, morphogroup 2, morphogroup 3 and morphogroup 4 were recovered as monophyletic. In the combined nuclear full dataset tree, the specimens for both *H. tuberculatus* and *D. eutrachelia* were split up within each respective genus. In all three trees, *H. procerus*, *Hyrtacus*. sp. nov. A, and *Hyrtacus*. sp. nov. B cluster with *Eurycantha* and *Carausius*. All these taxa can be found outside Australia, in either New Guinea or Mahé, Seychelles (for *Carausius sechellensis* only).

The phylogenetic positions of two individuals, ANIC\_086 and ANIC\_123, varied across all trees and therefore remain uncertain. Differing positions for sample ANIC\_086 occur across combined and nuclear data sets, where this sample is placed in morphogroup 2 and *Marcenia* respectively. Mitochondrial data were not available for ANIC\_086. ANIC\_123 was placed within *Hyrtacus* across both combined and mitochondrial trees, but nested within *Marcenia* in the nuclear tree.



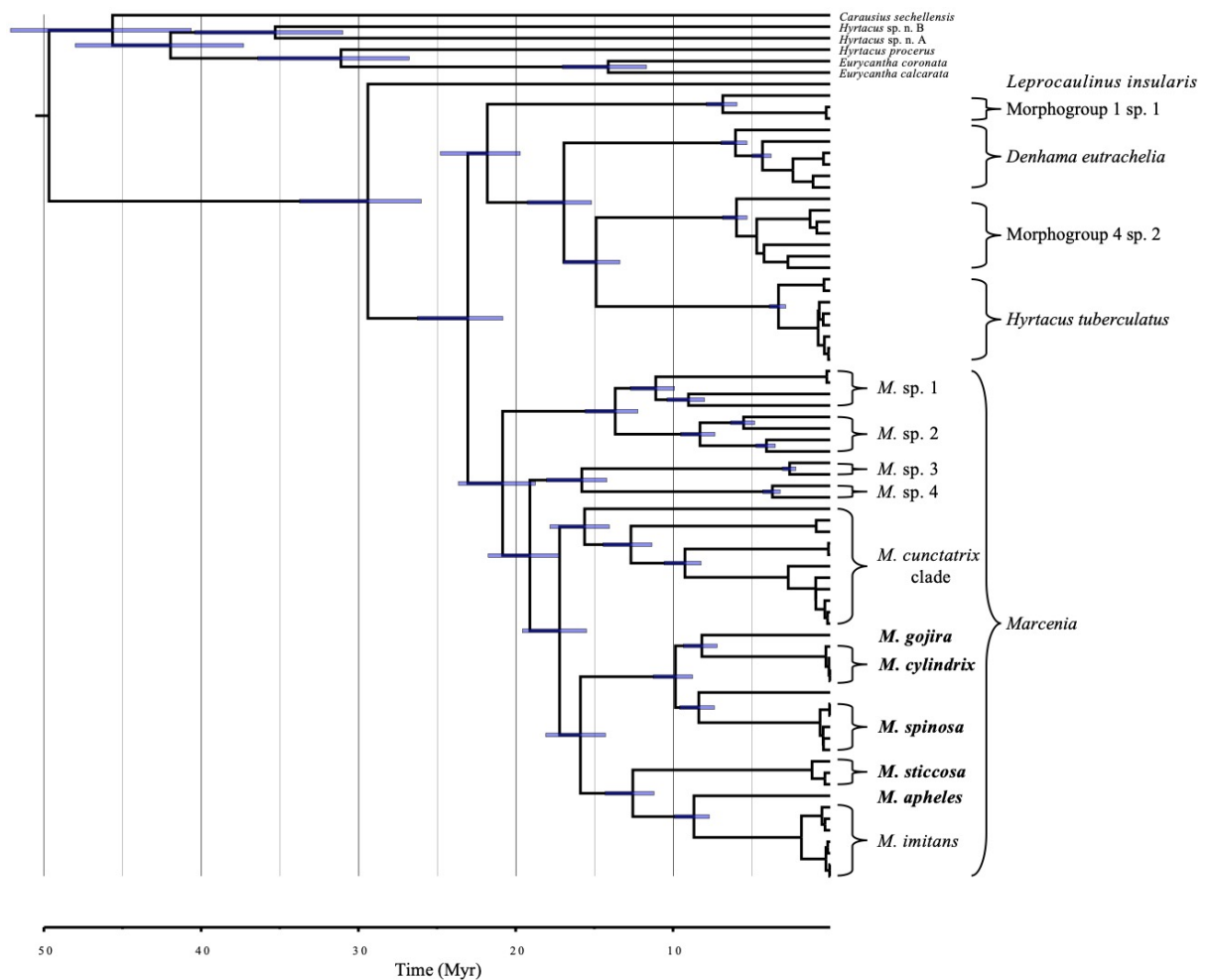
**Figure 4.3.** Maximum likelihood tree for all Lonchodini taxa and outgroups, inferred from 17 mitochondrial and nuclear genes. Species distributions are colour-coded, correspond to coloured branches, and represent single species with widespread distributions. Bold text indicates new species. Scale bar represents branch length substitutions per site. Refer to Supplementary Figure S4.2 for tree showing outgroups and node support values.

#### 4.4.2 Phylogenetic relationships inferred from the reduced dataset and *Marcenia*

Across all seven reduced dataset analyses (Table 4.1), except nuclear analyses, *L. insularis* was recovered as a sister to all Australian Lonchodini (Fig. 4.4; Supplementary Figure S4.5-11). In all reduced dataset analyses, the seven *Marcenia* species (i.e. those reinstated, and those newly described here) group together. Except for in the nuclear reduced datasets (performed in both maximum likelihood, and Bayesian inference analyses), the seven species separate into three clades: 1) the putative *M. cunctatrix* clade, 2) *M. gojira* + *M. spinosa* + *M. sp. BJ\_069* + *M. cylindrix*, and 3) *M. sticcosa* + *M. apheles* + *M. imitans*. In one analysis (Bayesian inference, nuclear only), the ANIC\_141 sample was displaced from the putative *M. cunctatrix* clade, and was instead grouped with *L. insularis*, as a sister to all other Lonchodini. In this same analysis, the *M. sp. BJ\_069* sample was repositioned into the *M. cylindrix* species (Supplementary Figure S4.11). There are no nuclear sequences available for *M. apheles*.

Included in the time-calibrated phylogenetic tree are *L. insularis* + morphogroup 1 + *Denhama* + morphogroup 4 + *Hyrtacus* + *Marcenia* (Fig. 4.4). *Leprocaulinus insularis* diverged first from all other Australian Lonchodini at 29.4 Ma (95% HPD 26-33.75 Ma). Then *Marcenia* diverged from morphogroup 1 + *Denhama* + morphogroup 4 + *Hyrtacus* at 23 Ma (95% HPD 20.81-26.26 Ma). Between morphogroup 1 + *Denhama* + morphogroup 4 + *Hyrtacus*, morphogroup 1 diverged first at 21.9 Ma (95% HPD 19.71-24.79 Ma), then *Denhama* at 16.9 Ma (95% HPD 15.18-19.25 Ma), and morphogroup 4 and *Hyrtacus* diverged at 14.9 Ma (95% HPD 13.38-16.99 Ma). Within the genus *Marcenia*, the first clade diverged at 20.8 Ma (95% HPD 18.75-23.65 Ma), followed by a second divergence at 19.1 Ma (95% HPD 17.24-21.75 Ma). The remaining three clades in *Marcenia* include the *M.*

*cunctatrix* clade, which diverged at 17.2 Ma (95% HPD 15.5-19.57 Ma), and the two remaining clades (*M. gojira* + *M. spinosa* + *M. sp. BJ\_069* + *M. cylindrix* and *M. sticcosa* + *M. apheles* + *M. imitans*) which diverged at 15.9 Ma (95% HPD 14.29-18.09 Ma). For all *Marcenia*, the species level divergences occurred between 8.2-15.8 Ma.



**Figure 4.4.** Fossil calibrated Bayesian inference tree for reduced Lonchodini dataset and outgroups, inferred from 17 mitochondrial and nuclear genes. Bold text indicates new species. Blue bars at each node represent 95% highest posterior density (HPD) divergence times. Scale bar represents substitutions per million years. Refer to Supplementary Figure S4.5 for tree showing outgroups, node support values and Fossil node points.



## 4.5 Taxonomy

Family **LONCHODIDAE** Brunner, 1893

Subfamily **LONCHODINAE** Brunner, 1893

Tribe **LONCHODINI** Brunner, 1893

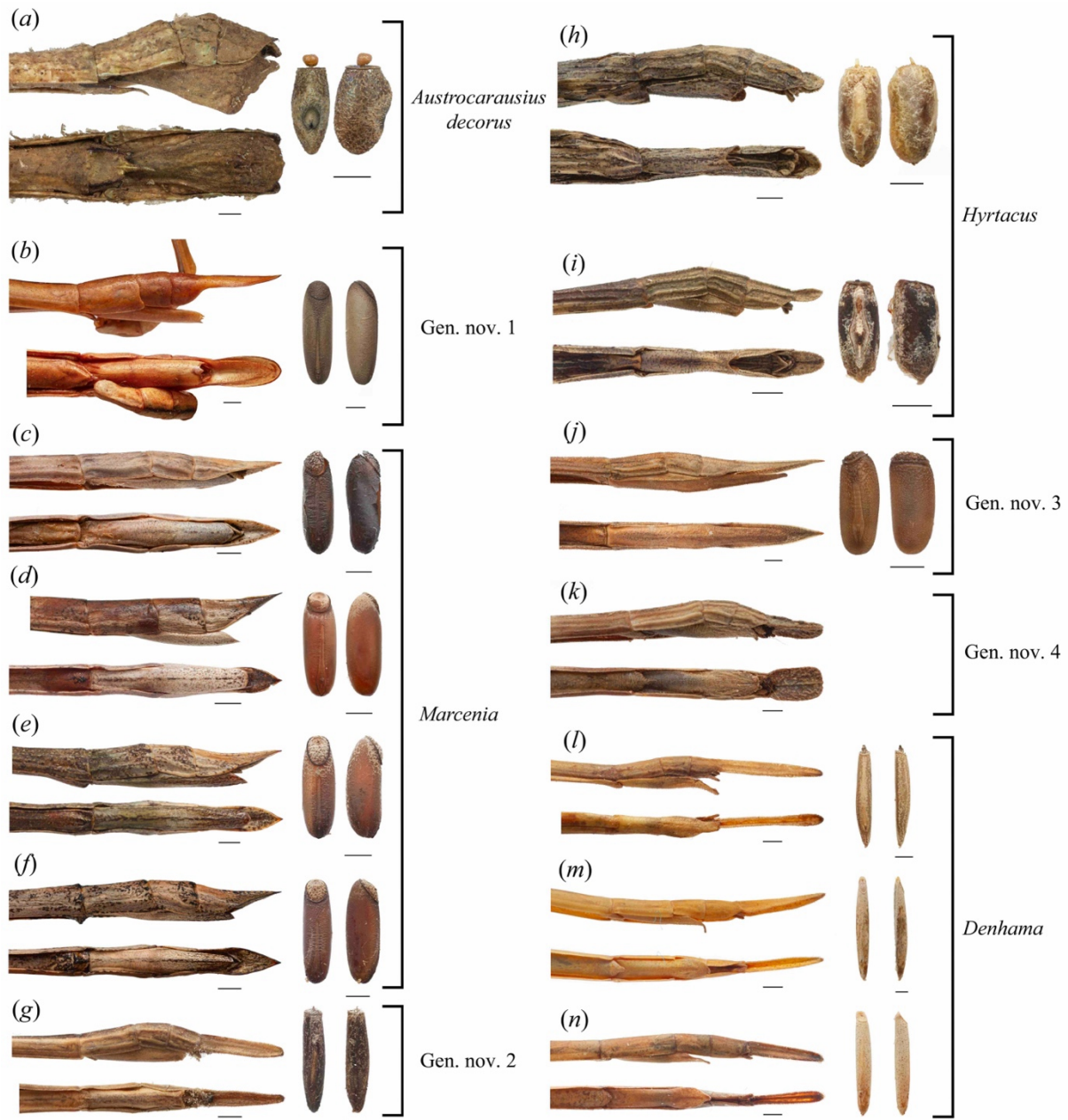
### Diagnosis

Body is elongate to very elongate, the mesothorax is significantly longer than head and prothorax combined. Body surface mostly covered in granulation, sometimes spines are present. Antennae long. Ocelli and wings apterus in both sexes. Females can have a distinct preopercular organ on abdominal segment 7. The subgenital plate of females is boat-shaped but not elongated or lanceolate, never noticeably extending past the anal segment. In some genera the female supraanal plate is elongated. In males vomer is strongly reduced or lacking.

### *Key to females of endemic Australian Lonchodini genera*

- 1 supra-anal plate not present .....2
- supra-anal plate present .....4

|                                                                                                        |                        |
|--------------------------------------------------------------------------------------------------------|------------------------|
| 2 anal segment rounded (Fig. 4.5b, 4.6b) .....                                                         | <b>Morphogroup 1</b>   |
| - anal segment pointed .....                                                                           | <b>3</b>               |
| 3 operculum not extending to end of anal segment (Fig. 4.5c, d, e, f, 4.6c) <i>Marcenia</i> stat. rev. |                        |
| - operculum almost reaching end of anal segment (Fig. 4.5j) .....                                      | <b>Morphogroup 3</b>   |
| 4 supra-anal plate not extending past operculum (Fig. 4.5a) .....                                      | <i>Austrocarausius</i> |
| - supra-anal plate extending past operculum .....                                                      | <b>5</b>               |
| 5 supra-anal plate truncate (Fig. 4.5k) .....                                                          | <b>Morphogroup 4</b>   |
| - supra-anal plate not truncate .....                                                                  | <b>6</b>               |
| 6 segments 8, 9 and 10 flat, not hump-like (Fig. 4.5l, m, n, 4.6a, d, e, f) .....                      | <i>Denhama</i>         |
| - segments 8, 9 and 10 raised and hump-like .....                                                      | <b>7</b>               |
| 7 strong dorsal black stripe (Fig. 4.5g) .....                                                         | <b>Morphogroup 2</b>   |
| - no strong dorsal black stripe (Fig. 4.5h, i) .....                                                   | <i>Hyrtacus</i>        |



**Figure 4.5.** End of abdomen and egg photos of all endemic Australian Lonchodini genera, lateral and ventral genital and dorsal and lateral egg views. (a) GenBank sample, BJ\_294. (b) ANIC\_140. (c) BJ\_284. (d) BJ\_120. (e) *M. cylindrix*, BJ\_017. (f) *M. imitans*, BJ\_088. (g) BJ\_167. (h) BJ\_165. (i) BJ\_060. (j) BJ\_099. (k) ANIC\_139. (l) BJ\_248 (m) BJ\_020. (n) BJ\_172. Scale bars represent 1 mm.

*Key to eggs of Australian Lonchodini genera*

- 1 capitulum present (Fig. 4.5a) ..... ***Austrocarausius***
- capitulum not present..... **2**
  
- 2 with Anterior protuberance (Fig. 4.5h, i)..... ***Hyrtacus***
- without Anterior protuberance..... **3**
  
- 3 egg elongate ..... **4**
- egg oblong ..... **5**
  
- 4 operculum flat or almost flat (Fig. 4.5g)..... **Morphogroup 2**
- operculum angled, c.45° or steeper (Fig. 4.5l, m, n, 4.6a, d, e, f) ..... ***Denhama***
  
- 5 operculum with central crater (Fig. 4.5c, d, e, f, 4.6c)..... ***Marcenia* stat. rev.**
- operculum without central crater ..... **6**
  
- 6 operculum flat or almost flat (Fig. 4.5b, 4.6b)..... **Morphogroup 1**
- operculum angled, c.45° (Fig. 4.5j)..... **Morphogroup 3**



**Figure 4.6.** egg traits showcased for arid Australian Lonchodini species. All photos were taken in captivity. (a) Improperly laid eggs of *Denhama* sp., adhered both together and on supra-anal plate. (b) Newly hatched morphogroup 1 nymphs, eggs mixed in the frass under nymphs. (c) Buried *Marcenia* sp. egg. (d) Glued *Denhama* sp. egg. (e) *Denhama* sp. BJ\_149 with eggs glued to body by a different *Denhama* sp. (f) Glued *Denhama* sp. egg, with closed and open operculum.

## Description of morphogroups and genera

Differences are observed between the seven arid genera and morphogroups: morphogroup 1, *Marcenia*, morphogroup 2, *Hyrtacus*, morphogroup 3, morphogroup 4 and *Denhama* (Fig. 4.5a, b, c, d, e, f, g, h, i, j, k, l, m, n). Morphogroup 1, *Marcenia*, and morphogroup 3 can be differentiated from morphogroup 2, *Hyrtacus*, morphogroup 4, and *Denhama*, based on the presence of a sclerotized anal segment and the absence of any supra-anal plate. Within morphogroup 1, *Marcenia*, and morphogroup 3, morphogroup 1 differs in its rounded anal segment, which the others do not possess. Morphogroup 3 differs in its long ovipositor, which again, the others do not possess. In the remaining clades, which possess an unsclerotised supra-anal segment, morphogroup 2 and *Hyrtacus* can be differentiated from morphogroup 4 and *Denhama* by their raised and hump-like shape across abdominal segments 8-10. Morphogroup 2 can be differentiated from *Hyrtacus* by its strong dorsal black stripe. Morphogroup 4 can be differentiated from *Denhama* by its truncate supra-anal plate.

Eggs of *Hyrtacus* can easily be distinguished by their curved shape and presence of an anterior appendage; it is not known how they deposit their eggs. Morphogroup 2 and *Denhama*, which glue their eggs, can be differentiated from other clades by their very elongated shape, and then differentiated from each other by the angle of the operculum, with that of morphogroup 2 being relatively flat. *Marcenia*, which bury their eggs (Fig. 4.5a, b, c, d, e, f, g, h, i, j, l, m, n, 4.6a, b, c, d, e, f), can be differentiated from morphogroup 1 and morphogroup 3 (which also bury their eggs) by the central crater located in the operculum. Morphogroup 1 and morphogroup 3 can be differentiated by the angle of the operculum, with that of morphogroup 1 being angled (c. -45°) and morphogroup 3 being relatively flat.

## **Morphogroup 1**

Anal segment sclerotized and extended c.3× length of segment 9 (Fig. 4.5b). Anal segment rounded in the shape of a duck bill. Females robust, medium size. A strong dorsal black stripe spans from the head to the anal segment. Uniform light brown colour. Egg capsule oblong, slightly raised sculpturing near operculum and micropylar plate and on operculum (Fig. 4.5b). Pigmented brown to grey, darker around and on operculum. Operculum raised towards central cone, angle c. 45°. No capitulum. Micropylar plate very narrow, lanceolate. Micropylar plate swollen at base, spoon-like shape, c. 0.75× length of capsule. Measurement (mm): capsule length 5.7.

## ***Marcenia***

Anal segment sclerotized and extended c.2× length of segment 9 (Fig. 4.5c, d, e, f). Anal segment pointed, often acute. Females moderately robust, and small to medium size. Females often have thorax spines and abdominal protuberances. Brown to dark brown colour. Egg capsule oblong, raised sculpturing near operculum and micropylar plate and on operculum, sculpturing mostly pigmented white (Fig. 4.5c, d, e, f). Pigmented light brown, darker around and on operculum. Operculum raised towards central cone which is recessed and crater-like, angle c. 45°-65°. Operculum sculpturing pigmented white. No capitulum. Micropylar plate oblong and narrow with raised rim. Micropylar plate swollen at base, spoon-like shape, c. 0.75× length of capsule. Measurement (mm): capsule length 3.9-4.6. Some specimens were found walking between grasses at nighttime (Fig. 4.7a, b, c, d, e, f, g).

## Morphogroup 2

Supra-anal segment not sclerotized and extended, c.2× length of segment 9 (Fig. 4.5g).

Females small. A strong dorsal black stripe spans from the head to the anal segment. A strong dorsal black stripe spans from the head to the anal segment. Uniform grey, brown, or light brown colour. Abdominal segments 8, 9 and 10 raised and hump-like. Egg capsule elongate and sculptured (Fig. 4.5g). Capsule pigmented dark brown, sculpturing pigmented white. Operculum almost flat except for raised central cone which is pointed. Operculum pigmented white. No capitulum. Micropylar plate rhomboid and elongated. Pigmented light brown, c. 0.6× length of capsule. Measurement (mm): capsule length 5.4.

## *Hyrtacus*

Supra-anal segment not sclerotized and extended, sometimes short sometimes long c. 0.25-1× length of segment 9 (Fig. 4.5h, i). Females moderately robust, small to medium size. Females often have thorax spines and abdominal protuberances. Brown to dark brown colour. Abdominal segments 8, 9 and 10 raised and hump-like. Egg capsule oval, sometimes curved, and raised sculpturing (Fig. 4. 5h, i). Capsule pigmented light to dark brown, sculpturing pigmented white. Anterior protuberance present on capsule. Operculum flat with raised central bump. No capitulum. Micropylar plate oblong and swollen in the middle, extending almost length of capitulum. Measurement (mm): capsule length 2.7-3.



### **Morphogroup 3**

Anal segment sclerotized and extended c.2× length of segment 9 (Fig. 4.5j). Ovipositor sclerotized and extended, almost reaching end of anal segment. Females robust, small. Brown to dark brown colour. Egg capsule oblong, raised sculpturing near and on operculum (Fig. 4.5j). Pigmented light brown. Operculum raised towards central cone. No capitulum. Micropylar plate oblong and swollen at base, spoon-like shape, c. 0.75× length of capsule. Measurement (mm): capsule length 3.1. Some specimens were found walking between grasses at nighttime (Fig. 4.7a, b, c, d, e, f, g).

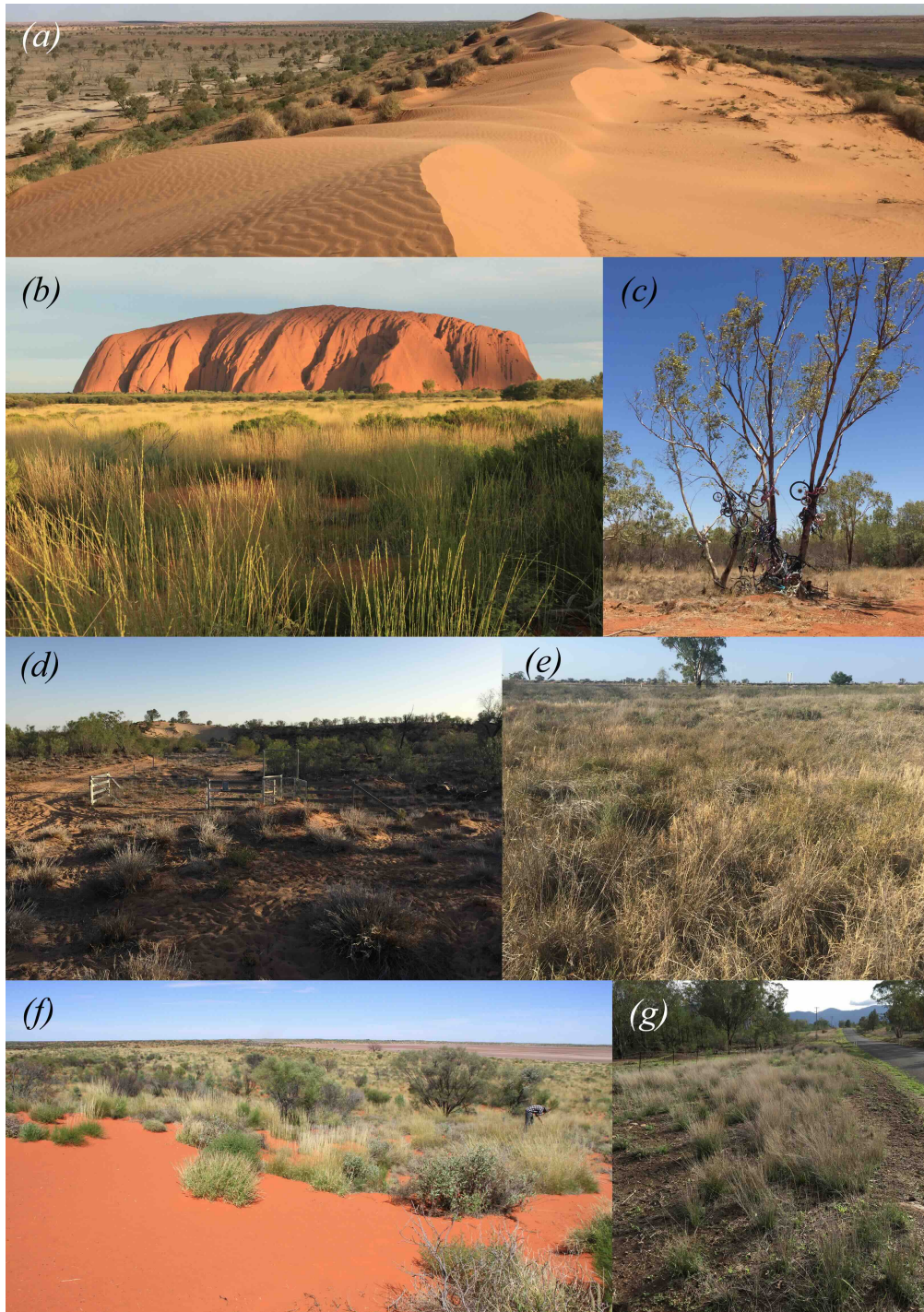
### **Morphogroup 4**

Supra-anal segment not sclerotized and extended, c.1-2× length of segment 9 (Fig. 4.5k). Supra-anal plate often truncate. Females moderately robust, small to medium size. Brown to light brown colour. Egg not known.

### ***Denhama***

Supra-anal segment not sclerotized and often greatly extended, c.1-6× length of segment 9 (Fig. 4.5l, m, n). Females elongate, small to medium size. Often uniform light brown colour. Abdominal segments 8, 9 and 10 flat, not hump-like. Egg capsule elongate, pigmented white to light brown to dark brown (Fig. 4.5l, m, n). Operculum flat, only *Denhama* sub group with capitulum-like protuberance, angle c. 30°-70°. No capitulum. Micropylar plate lanceolate, can be elongated or short and more swollen at base, c. 0.25-0.75× length of capsule.

Measurement (mm): capsule length 6.1-9. Some specimens were found walking between grasses at nighttime (Fig. 4.7a, b, c, d, e, f, g).



**Figure 4.7.** Typical habitats for arid Australian *Lonchodini*. (a) Birdsville, Qld. (b) Uluru, NT. (c) Dajarra Bike Tree, nr. Mount Isa, Qld. (d) Cunnamulla, Qld. (e) Boolooroo Rest Area, nr. Moree, NSW. (f) Alice Springs, NT. (g) Cobar, NSW.

Genus *Marcenia* Sjöstedt, 1918 stat. rev.

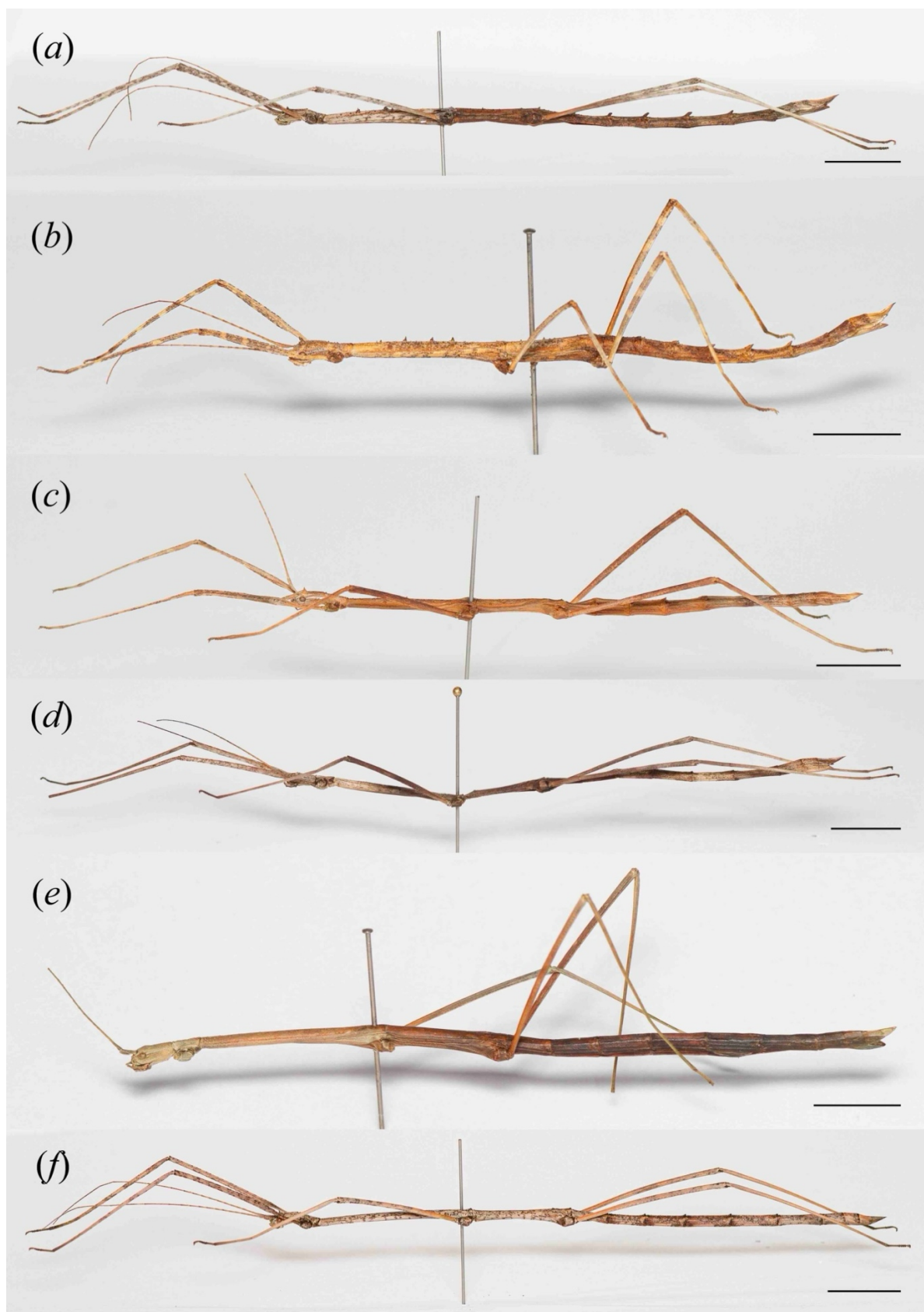
Type species: *Marcenia cunctatrix* Sjöstedt, 1918

*Marcenia* Sjöstedt, 1918, p. 37. Type species *Marcenia cunctatrix* Sjöstedt, 1918, p. 37, by original designation. Synonymised as *Hyrtacus* (Brock and Hasenpusch, 2007), but here reinstated.

### Diagnosis

Females elongate, wingless (Fig. 4.8a, b, c, d, e, f). Body often granulated, can be black tipped in some species. All insects are pigmented grey, dark brown to light brown, or yellow and may be mottled. Faint black dorsal stripe sometimes running length of body. Lateral reddish line along length of mesonotum and metanotum, on both sides. Head often has pair of spines or flange-like protuberances between the eyes, can be reduced in size within the species (Fig. 4.9a, b, c, d, e, f). Without ocelli. Antennae reaching past fore femora, almost reaching tarsus. Thorax often covered in dorsal granulation, tubercles and/or spines (Fig. 4.9a, b, c, d, e, f). Slight dorsal protuberance often present at end of pronotum, mesonotum, and metanotum. Mesonotum c.6-8× length of pronotum. Metanotum c.0.5× length of mesonotum. Legs slender and unarmed. Dorsal protuberance often present at end of abdominal segments 1-6, when larger, fin-like in shape (Fig. 4.8a, b, c, d, e, f). Anal segment sclerotized and pointed (Fig. 4.10a, b, c, d, e, f). Anal segment 2× length of segment 9. End of operculum rounded. Operculum c.0.6× length of anal segment. Without supra-anal plate.

Cerci very short. Preopercular organ ventrally protrudes. Eggs oblong, surface can be sculptured and/or smooth. Operculum angled, c.45°, with central crater. Micropylar plate elongated, enlarged at base, c.0.6× length.



**Figure 4.8.** full body: lateral view. (a) *M. cylindrix*. (b) *M. gojira*. (c) *M. spinosa*. (d) *M. sticcosa*. (e) *M. apheles*. (f) *M. imitans*. Scale bars represent 10 mm.

## Species included

*Marcenia cunctatrix* (Sjöstedt, 1918) stat. rev., (northern WA: Kimberley District)

*Marcenia cylindrix* sp. nov. BJ\_017 (central NSW: Cobar)

*Marcenia gojira* sp. nov. ANIC\_155 (south western Qld: Noccundra)

*Marcenia spinosa* sp. nov. BJ\_087 (western NSW: Broken Hill)

*Marcenia sticcosa* sp. nov. BJ\_119 (central Qld: Clermont)

*Marcenia apheles* sp. nov. (northern Qld: Georgetown)

*Marcenia imitans* (Brunner von Wattenwyl, 1907) stat. rev., (central Qld: Peak Downs to Cape River)

## Key to adult females of *Marcenia*

1 no dorsal abdominal protuberances ..... *Marcenia apheles* sp. nov. (Fig. 4.8e, 4.9e, 4.10e)

- dorsal abdominal protuberances ..... 2

2 pair of blunted spine protuberances between eyes..... 3

- pair of pointed spine protuberances between eyes ..... 4

**3** one or more cylindrical mesonotum spines ..... *Marcenia cylindrix* **sp. nov.** (Fig. 4.8a, 4.9a, 4.10a)

- no cylindrical mesonotum spines ..... *Marcenia gojira* **sp. nov.** (Fig. 4.8b, 4.9b, 4.10b)

**4** dorsal black stripe from head to anal segment..... *Marcenia cunctatrix*

- dorsal black stripe from head to anal segment not present..... **5**

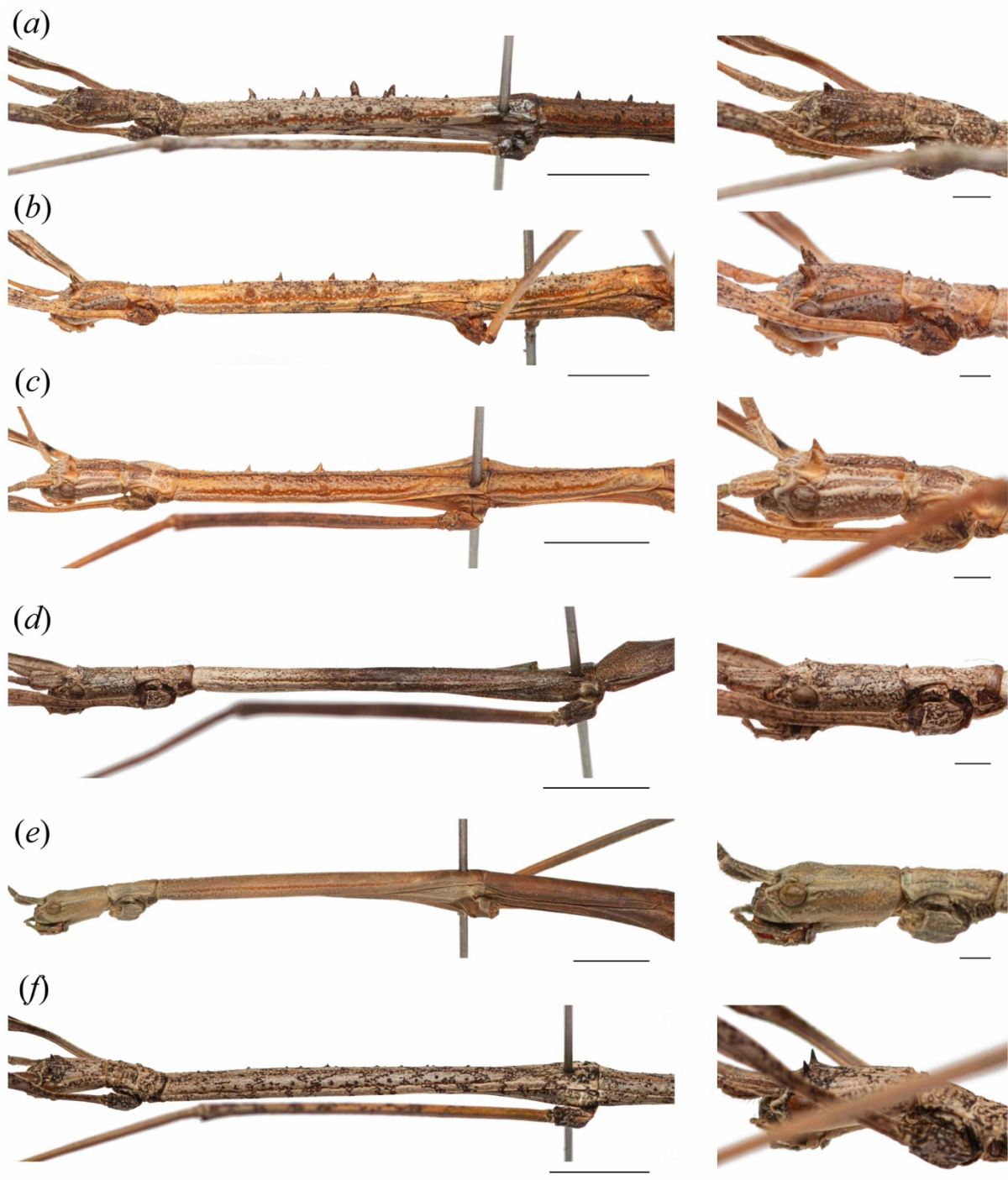
**5** no thorax spines ..... *Marcenia sticcosa* **sp. nov.** (Fig. 4.8d, 4.9d, 4.10d)

- thorax spines ..... **6**

**6** abdominal protuberances fin-like ..... *Marcenia spinosa* **sp. nov.** (Fig. 4.8c, 4.9c, 4.10c)

- abdominal protuberances not fin-like ..... *Marcenia imitans* (Fig. 4.8f, 4.9f, 4.10f)





**Figure 4.9.** Thorax and head: lateral view. (a) *M. cylindrix*. (b) *M. gojira*. (c) *M. spinosa*. (d) *M. sticcosa*. (e) *M. apheles*. (f) *M. imitans*. Scale bars represent 5 mm for thorax and 1 mm for head views.





**Figure 4.10.** End of abdomen: dorsal, lateral, and ventral views. (a) *M. cylindrix*. (b) *M. gojira*. (c) *M. spinosa*. (d) *M. sticcosa*. (e) *M. apheles*. (f) *M. imitans*. Scale bars represent 1 mm.

***Marcenia cunctatrix* Sjöstedt, 1918 stat. rev.**

*Marcenia cunctatrix* Sjöstedt, 1918 stat. rev., Kimberley District, WA, Australia. This male is removed from synonym with *H. caurus* (Tepper, 1905).

*Hyrtacus nigrogranulosus* (Sjöstedt, 1918) syn. nov., Kimberley District, WA, Australia.

This female is removed from synonym with *H. caurus* (Tepper, 1905). It was collected along with *M. cunctatrix* male type and matches species diagnosis, so it is here transferred to synonymy with *M. cunctatrix*.

*Bacteria frenchi* (Wood-Mason, 1877) – was first transferred to *Hyrtacus frenchi* (Kirby, 1904), then transferred to *Marcenia frenchi* Vickery, 1983, and later synonymised as *H. tuberculatus* (Brock and Hasenpusch, 2007). It is here changed to a new synonym of *M. cunctatrix*. The *B. frenchi* type is lost.

*Hyrtacus caurus* (Brock and Hasenpusch, 2007) – Brock and Hasenpusch 2007, p.70.

## **Description**

Females light brown. Faint black dorsal stripe length of the body. Dorsal protuberances at end of most body segments missing. No protuberances between eyes. Thorax with sparse granulation. No dorsal spines on thorax. No dorsal granulation on abdomen. Anal segment pointed.

## **Specimens examined**

Syntypes: ♀ Kimberley District, WA, Australia, 1918, Sjöstedt, (NHRS). ♀ same data (NHRS).

***Marcenia cylindrix* (Jones and Lo) sp. nov.**

(Cylinder Stick-insect)

**Diagnosis**

Females mottled light and dark brown (Fig. 4.8a). Dorsal protuberances at end of most body segments, becoming fin-like on abdomen. Pair of spine protuberances between eyes (Fig. 4.9a). Thorax with dense tubercles, some black tipped. Dorsal spines on mesonotum and metanotum, some cylindrical. Dorsal granulation on abdomen. Anal segment acute (Fig. 4.10a).

**Description**

**Female**

Mottled light and dark brown across whole body, mesothorax and legs lighter colour. Lateral reddish line along length of mesonotum and metanotum, on both sides.

**Head.** Pair of blunted spine protuberances between eyes (Fig. 4.9a). Head surface smooth.

Head longer than wide, length almost 2× width. Antennae long, 32 segments, almost reaching fore tarsus, c.2× length of fore femora.

**Thorax.** Pronotum, mesonotum, and metanotum covered in tubercles, more dense on mesonotum, and metanotum, more pointed on mesonotum (Fig. 4.9a). Tubercle size varies on mesonotum, and metanotum. Tubercles on mesonotum, and metanotum are the same colour as thorax surface while spines are dark brown. Some tubercles and spines are black tipped. Pronotum with pair of small anterior tubercles, one tubercle shorter. Mesonotum and metanotum covered in dorsal spines of various size and shape, mesonotum with the largest number and size c.17 spines, metanotum c.5 spines. Most mesonotum spines are sharply pointed and angular, longest 2 spines are cylindrical. Slight dorsal protuberance at end of pronotum, mesonotum, and metanotum, increasing in size and becoming more pointed. Pronotum slightly shorter than head, mesonotum c.7× length of pronotum. Metanotum c.0.5× length of mesonotum.

**Abdomen.** Rounded dorsal protuberance at end of abdominal segments 1-6, increasing in size, until segment 5, and becoming more fin-like (Fig. 4.8a). Protuberances with pronounced lateral lobes each side of structure, more prominent when larger. Segment 6 protuberance 0.5× size of segment 5. Pair of large dorsal tubercles at start of segments 2-8. Several dorsal tubercles anteriorly located on segments 2-7, slightly decreasing in size and number from segment 3. Abdominal segments 1-7 covered in mostly dorsal granulation, becoming more sparse towards segment 7. Median segment 0.5× length of abdominal segment 2. Segments 2-4 equal in length. Segments 5 and 6 equal in length, slightly longer than segment 4. Segment 7 slightly shorter than segment 6. Segment 8 0.5× length of segment 6. Segment 9 0.6× length of segment 8. Anal segment 2× length of segment 9. Operculum 0.6× length of anal segment. Operculum rounded (Fig. 4.10a). Anal segment acute. Cerci short, not quite reaching end of operculum. Preopercular organ ventrally protrudes.

## Measurements (mm)

For 1 female. *Body length*: 75.1. *Head*: 3.3. *Antennae*: 32 segments. *Pronotum*: 2.6. *Mesonotum*: 17.5. *Metanotum*: 9.6. *Median segment*: 2.1. *Femora*: *fore, mid, hind*: 18.8, 17.5, 21.3. *Tibia*: *fore, mid, hind*: 19.9, 18.6, 24.3.

## Variation among types

Females can have faint black dorsal stripe length of the body. Without head protuberances. Thorax spines can be reduced or not present. Abdominal protuberances can be significantly reduced, not fin-like.

## Specimens examined

Holotype: ♀ -31.503836S, 145.812242E, Cobar, NSW, Australia 8 Jan 2018, Braxton Jones BJ\_017 (ANIC). Paratypes: ♀ -31.510607S, 145.724141E, Cobar, NSW, Australia 19 Jan 2018, Braxton Jones BJ\_074 (ANIC). ♀ same data, BJ\_075 (ANIC). ♀ same data, BJ\_076 (ANIC).

## Etymology

The epithet “*cylindrix*” is a Latin word for “cylinder” and refers to the cylindrical spines on thorax.

## Notes

The majority of type specimens were found in grasses growing no more than 1 meter from highway.

*Marcenia gojira* (Jones and Lo) sp. nov.

(Godzilla Stick-insect)

## Diagnosis

Females mottled brown and yellow (Fig. 4.8b). Dorsal protuberances at end of most body segments, becoming fin-like on abdomen. Pair of blunted protuberances between eyes (Fig. 4.9b). Thorax with dense tubercles. Dorsal spines on mesonotum and metanotum, larger spines with lateral black tipped tubercles. Dorsal granulation on abdomen. Anal segment acute (Fig. 4.10b).

## Description

### Female

Mottled light shades of brown to yellow, mesonotum lightest colour, even lighter in colour on anterior end, abdomen darkest. Lateral pale orange line along length of mesonotum and metanotum, on both sides.

**Head.** Pair of protuberances between eyes, more flange-like than spine (Fig. 4.9b). Head smooth. Head longer than wide, length almost  $2\times$  width. Antennae long, 26 segments, almost reaching fore tarsus,  $c.2\times$  length of fore femora.

**Thorax.** Pronotum, mesonotum, and metanotum covered in uniformly rounded tubercles, more dense on mesonotum, and metanotum (Fig. 4.9b). Pronotum with several pairs of large tubercles, including anterior pair. Mesonotum and metanotum covered in dorsal spines of various size and shape, mesonotum with the largest number and size,  $c.12$  spines, metanotum  $c.4$  spines. Largest 5 mesonotum spines with lateral tubercles, black tipped when well defined. Slight dorsal protuberance at end of pronotum, mesonotum, and metanotum, increasing in size and becoming more pointed. Pronotum slightly shorter than head, mesonotum  $c.7\times$  length of pronotum. Metanotum  $c.0.5\times$  length of mesonotum.

**Abdomen.** Pointed dorsal protuberance at end of abdominal segments 1-6, increasing in size, until segment 5, and becoming more fin-like (Fig. 4.8b). Protuberances with pronounced lateral lobes each side of structure, more prominent when larger. Segment 6 protuberance  $0.5\times$  size of segment 5. Pair of large dorsal tubercles at start of segments 2-8. Several dorsal tubercles anteriorly located on segments 2-8, slightly decreasing in size and number from segment 3. Abdominal segments 1-6 covered in mostly dorsal granulation, becoming more sparse. Median segment  $0.5\times$  length of abdominal segment 2. Segments 2-4 equal in length. Segments 5-7 equal in length, slightly longer than segment 4. Segment 8  $0.5\times$  length of segment 6. Segment 9  $0.6\times$  length of segment 8. Anal segment  $2\times$  length of segment 9.

Operculum 0.6× length of anal segment. Operculum rounded (Fig. 4.10b). Anal segment acute. Cerci short, just extending end of operculum. Preopercular organ ventrally protrudes.

### Measurements (mm)

For 1 female. *Body length*: 70.1. *Head*: 3.7. *Antennae*: 26 segments. *Pronotum*: 1.2. *Mesonotum*: 1.3. *Metanotum*: 9.7. *Median segment*: 2.5. *Femora*: *fore, mid, hind*: 15.4, 14.9, 18.2. *Tibia*: *fore, mid, hind*: 16, 17.4, 20.9.

### Specimens examined

Holotype: ♀ -27.696306S, 142.750577E, 3 miles SW. of Nockatunga H.S., Qld, Australia, 28 Jan 1965. L. J. Chinnick, ANIC\_155 (ANIC).

### Etymology

The species is named after the fictional Godzilla, where the multi-tipped thorax spines on *M. gojira* resemble Godzilla's back spines. To be treated as a noun in apposition.



*Marcenia spinosa* (Jones and Lo) sp. nov.

(Thorned Stick-insect)

**Diagnosis**

Females mottled light brown (Fig. 4.8c). Faint black dorsal stripe length of the body. Dorsal protuberances at end of most body segments, becoming fin-like on abdomen. Pair of spine protuberances between eyes (Fig. 4.9c). Thorax with dense tubercles. Dorsal spines on mesonotum and metanotum. Dorsal granulation on abdomen. Anal segment acute (Fig. 4.10c).

**Description**

**Female**

Mottled light brown. Lateral reddish light brown line along length of mesonotum and metanotum, on both sides. Faint black dorsal stripe length of the body.

**Head.** Pair of spines between eyes (Fig. 4.9c). Head uneven, longer than wide, length almost 2× width. Antennae long, 34 segments, almost reaching fore tarsus, c.2× length of fore femora; antennae broken in Holotype, measurements based of paratype BJ\_086.

**Thorax.** Pronotum, mesonotum, and metanotum covered in pointed and rounded tubercles, more dense on mesonotum, and metanotum (Fig. 4.9c). Pronotum with several pairs of large tubercles, including anterior pair. Mesonotum c.7 spines, 4 larger spines, metanotum c.2. Slight dorsal protuberance at end of pronotum, mesonotum, and metanotum. Pronotum slightly shorter than head, mesonotum c.6× length of pronotum. Metanotum c.0.5× length of mesonotum.

### **Abdomen.**

Pointed dorsal protuberance at end of abdominal segments 1-6, increasing in size, until segment 5, and becoming more fin-like (Fig. 4.8c). Protuberances with pronounced lateral lobes each side of structure, more prominent when larger. Segment 6 protuberance 0.5× size of segment 5. Several dorsal tubercles anteriorly located on segments 2-8, slightly decreasing in size and number from segment 3. Abdominal segments 1-8 with some dorsal granulation, becoming more sparse. Median segment 0.5× length of abdominal segment 2. Segments 2-4 equal in length. Segments 5-7 equal in length, slightly longer than segment 4. Segment 8 0.5× length of segment 6. Segment 9 0.6× length of segment 8. Anal segment 2× length of segment 9. Operculum 0.6× length of anal segment. Operculum rounded (Fig. 4.10c). Anal segment acute. Cerci short, just extending end of operculum. Preopercular organ ventrally protrudes.

### Measurements (mm)

For 1 female. *Body length*: 66.3. *Head*: 3.7. *Antennae*: 35 segments. *Pronotum*: 2.6.

*Mesonotum*: 14.8. *Metanotum*: 8.5. *Median segment*: 1.9. *Femora*: *fore, mid, hind*: 15.5, 14.5, 17.3. *Tibia*: *fore, mid, hind*: 16.3, 15.6, 19.1.

### Variation among types

Thorax spines can vary in number c.8 in paratype.

### Specimens examined

Holotype: ♀ -31.9081S, 141.6489E, Barrier Highway, Broken Hill, NSW, Australia 8 Jan 2018, Braxton Jones BJ\_087 (ANIC). Paratypes: ♀ same data, BJ\_086 (ANIC).

### Etymology

The epithet “*spinosa*” is a Latin word for “spine” and refers to the thorn-like spines on the thorax and head.

*Marcenia sticcosa* (Jones and Lo) sp. nov.

(Stick Stick-insect)

**Diagnosis**

Females mottled grey and dark brown (Fig. 4.8d). Small dorsal protuberances at end of most body segments. Pair of small spine protuberances between eyes (Fig. 4.9d). Thorax with sparse granulation. No dorsal spines on thorax. No dorsal granulation on abdomen. Anal segment acute (Fig. 4.10d).

**Description**

**Female**

Mottled grey and dark brown across whole body, more grey on head, prothorax and anterior mesothorax, fore femora and all tibiae, and abdominal segments 6, 8-10. Lateral reddish line, becoming lighter in colour, along length of mesonotum and metanotum, on both sides.

**Head.** Pair of bumps apexed with small spines between eyes (Fig. 4.9d). Head surface smooth. Head longer than wide, length almost 2× width. Antennae broken.

**Thorax.** Pronotum, mesonotum, and metanotum sparsely covered in granules, almost no granulation on metanotum, some larger black tipped granules on mesonotum (Fig. 4.9d).

Mesonotum granules mostly anterior positioned. Pronotum with pair of anterior tubercles, black tipped. Slight dorsal protuberance at end of mesonotum, and metanotum. Pair of dorsal white tipped granules and end of mesonotum and metanotum. Pronotum slightly shorter than head, mesonotum c.6× length of pronotum. Metanotum c.0.5× length of mesonotum.

**Abdomen.** Rounded dorsal protuberance at end of abdominal segments 1-6, increasing in size, until segment 5 (Fig. 4.8d). Protuberances on segments 3-5 with slight lateral lobes each side of structure. Segment 6 protuberance 0.5× size of segment 5. Pair of dorsal white tipped granules and end of median segment. Pair of dorsal tubercles at start of segments 2-7. Several dorsal tubercles located on segments 2-6, decreasing in size and number from segment 3, becoming less dense at end of segments. Median segment 0.5× length of abdominal segment 2. Segments 2-4 equal in length. Segments 5 and 6 equal in length, slightly longer than segment 4. Segment 7 slightly longer than segment 6. Segment 8 0.5× length of segment 6. Segment 9 0.6× length of segment 8. Anal segment 2× length of segment 9. Operculum 0.5× length of anal segment. Operculum mucronate (Fig. 4.10d). Anal segment acute. Cerci short, just extending end of operculum. Preopercular organ ventrally protrudes.

### **Measurements (mm)**

For 1 female. *Body length:* 81.2. *Head:* 3.8. *Pronotum:* 3. *Mesonotum:* 19.1. *Metanotum:* 10. *Median segment:* 2.1. *Femora: fore, mid, hind:* 20, 17.7, 21.5. *Tibia: fore, mid, hind:* 20.2, 19.8, 26.4.

### **Specimens examined**

Holotype ♀ -22.8031S, 147.6402E, Clermont, Qld, Australia, 28 Jan 2018, Braxton Jones  
BJ\_119 (ANIC).

### **Etymology**

The epithet “*sticcosa*” is a Latin word for “stick” and refers to the species’ resemblance to dead grass and sticks.

***Marcenia apheles*** (Jones and Lo) sp. nov.

(Simple Stick-insect)

### **Diagnosis**

Females light and dark brown (Fig. 4.8e). Faint black dorsal stripe length of the body. Dorsal protuberances at end of most body segments missing. No protuberances between eyes (Fig. 4.9e). Thorax with sparse granulation. No dorsal spines on thorax. No dorsal granulation on abdomen. Anal segment obtuse (Fig. 4.10e).

## Description

### Female

Light and dark brown across whole body, head, pronotum, mesothorax and legs light brown colour. Lateral reddish line along length of mesonotum and metanotum, on both sides. Faint black dorsal stripe length of the body.

**Head.** Swollen bumps between the eyes, where pair of spines would be (Fig. 4.9e). Head surface smooth. Head longer than wide, length almost  $2\times$  width. Antennae broken.

**Thorax.** Pronotum and mesonotum, with sparse granules, more dense at the start of mesonotum (Fig. 4.9e). Pronotum with pair of small anterior tubercles. Slight dorsal protuberance at end of metanotum. Pronotum slightly shorter than head, mesonotum  $c.6\times$  length of pronotum. Metanotum  $c.0.5\times$  length of mesonotum.

**Abdomen.** No dorsal protuberance at end of any abdominal segments (Fig. 4.8e). Pair of small dorsal granules at start of segments 3-7. Segments 6 and 5 with one additional dorsal granule, anteriorly located. Abdomen otherwise smooth. Median segment  $0.5\times$  length of abdominal segment 2. Segments 2 and 3 equal in length. Segments 4-7 equal in length, slightly longer than segment 3. Segment 8  $0.5\times$  length of segment 6. Segment 9  $0.7\times$  length of segment 8. Anal segment  $2\times$  length of segment 9. Operculum  $0.6\times$  length of anal segment. Operculum rounded (Fig. 4.10e). Anal segment obtuse. Cerci short, reaching end of operculum. Preopercular organ ventrally protrudes.

### Measurements (mm)

For 1 female. *Body length*: 89.2. *Head*: 4.5. *Pronotum*: 3.4. *Mesonotum*: 21.7. *Metanotum*: 11.8. *Median segment*: 2.2. *Femora: fore, mid, hind*: 22.2, 19.8, 26.1. *Tibia: fore, mid, hind*: 24.4, 21.3, 28.6.

### Specimens examined

Holotype ♀ -18.289842S, 143.549595E, 8 miles W. of Georgetown, Qld, Australia, 7 Apr 1962, K.H.L. Key and E.L. Corby, ANIC\_143 (ANIC).

### Etymology

The epithet “*apheles*” is a Latin word for “smooth” and refers to the species’ smooth and simple body.

*Marcenia imitans* (Brunner von Wattenwyl, 1907) comb. nov.

*Marcenia imitans* (Brunner von Wattenwyl, 1907) stat. rev., Peak Downs, Qld, Australia.

Removed from synonymy with *D. longiceps* (Brunner von Wattenwyl, 1907).



*Hyrtacus imitans* (Brunner von Wattenwyl, 1907) – Brunner von Wattenwyl 1907, p.302; Weidner 1966, p.230; Vickery 1983, p.7; Balderson et al. 1998, 370; Zompro 2002, p.189.

*Hyrtacus longiceps* (Otte and Brock, 2005) – Otte and Brock 2005, p.163; Brock and Hasenpusch 2007, p.6.

### **Diagnosis**

Females light and dark brown (Fig. 4.8f). Faint black dorsal stripe length of the body. Dorsal protuberances at end of most body segments. Pair of small spine protuberances between eyes (Fig. 4.9f). Thorax with granulation. Spine-like tubercles on mesonotum. Anal segment acute (Fig. 4.10f).

### **Description**

#### **Female**

Light and dark brown across whole body, head and abdomen darker colour. Lateral reddish line along length of mesonotum and metanotum, on both sides. Faint black dorsal stripe length of the body.

**Head.** Pair of bumps apexed with small spines between eyes (Fig. 4.9f). Head surface smooth. Head longer than wide, length almost 2× width. Antennae long, almost reaching fore tarsus, c.1.5× length of fore femora.

**Thorax.** Mesonotum, and metanotum with granules and tubercles, more dense and pointed on mesonotum (Fig. 4.9f). Some mesonotum tubercles larger becoming spines c.5. Pronotum slightly shorter than head, mesonotum c.8× length of pronotum. Metanotum c.0.5× length of mesonotum.

**Abdomen.** Dorsal protuberance present at end of some abdominal segments, increasing in size, until segment 5 (Fig. 4.8f). Pair of dorsal tubercles at start of most segments. Several dorsal tubercles anteriorly located, slightly decreasing in size and number from segment 3. Median segment 0.5× length of abdominal segment 2. Segments 2-4 equal in length. Segments 5 and 6 equal in length, slightly longer than segment 4. Segment 7 slightly shorter than segment 6. Segment 8 0.5× length of segment 6. Segment 9 0.6× length of segment 8. Anal segment 2× length of segment 9. Operculum 0.5× length of anal segment. Operculum rounded (Fig. 4.10f). Anal segment acute. Cerci short, not quite reaching end of operculum. Preopercular organ ventrally protrudes.

### **Measurements (mm)**

For 1 female. *Body length:* 86.1. *Head:* 4.4. *Pronotum:* 2.8. *Mesonotum:* 21.9. *Metanotum:* 11.9. *Median segment:* 2.3. *Femora: fore, mid, hind:* 21.6, 18.9, 23.8. *Tibia: fore, mid, hind:* 19, 18.2, 20.2.

## Variation among types

Females can be without faint black dorsal stripe length of the body. Small dorsal bump between eyes. Thorax granules very sparse and no tubercles. Abdominal protuberances can be significantly reduced, more sparse dorsal tubercles.

## Specimens examined

Holotype ♀ -22.255073S, 148.184099E, Peak Downs, Queensland, Australia, 1907, Brunner von Wattenwyl (ZMUH). Additional material: ♀ -20.9875S, 146.4195E, Cape River, 111km S of Charters Towers, Qld, Australia, 15 Dec 2017, Braxton Jones BJ\_088 (ANIC). ♀ same data, BJ\_106 (ANIC). ♀ same data, BJ\_114 (ANIC). ♀ same data, BJ\_115 (ANIC).

## 4.6 Discussion

Based on the morphological and molecular analyses, there is evidence for the reinstatement of the genus *Marcenia*, and description of several new species in the genus *Marcenia*. This study describes five new species of *Marcenia*, and reinstates *M. cunctatrix* (Sjöstedt, 1918) from synonymy with *Hyrtacus caurus* (Tepper, 1905), and reinstates *M. imitans* (Brunner von Wattenwyl, 1907) from synonymy with *Denhama longiceps* (Brunner von Wattenwyl, 1907). The remaining undescribed species from other genera or clades (i.e. *Denhama*, *Hyrtacus*, morphogroup 1, 2, 3, and 4) will be the focus of future work.

#### 4.6.1 Morphological analysis

The *Marcenia* type species, *M. cunctatrix*, which was previously synonymised into the genus *Hyrtacus*, differs morphologically from both *Hyrtacus* and *Denhama* in that it lacks the supra-anal segment, and the anal segment is sclerotized. This sclerotized anal segment character is also observed in all species that cluster with *M. cunctatrix* in molecular analyses. For this reason, *Marcenia* should be reinstated as a valid genus (Fig. 4.5, 4.10; Supplementary Figure S4.12). The genetic variation within *Marcenia* is generally higher than that found within other Australian Lochodini genera, but the morphology of this genus is relatively conserved.

Morphological oviposition characters and egg morphology are linked to the type of egg laying strategy. For example, oviposition strategies that favour burying or gluing are more likely to have elongated egg morphologies (Boisseau and Woods 2024). Burying of eggs is also accompanied by morphological changes to the body (including in robust ground-dwelling species with modified ovipositors), which facilitates the deposition of eggs into substrate (Robertson et al. 2019). As such, the morphology of oviposition characters (including the ovipositor, anal segment and supra-anal plate) can indicate oviposition strategy.

The subfamily Lonchodinae are well documented egg buriers (Robertson et al. 2019; Boisseau and Woods 2024; Jolma 2020), but until now, the egg gluing genus *Denhama* has been excluded from any phylogenetic trees. This study therefore represents the first inclusion of the egg gluing trait for Lonchodinae. All confirmed arid Australian Lonchodini species either bury (morphogroup 1 + *Marcenia* + morphogroup 3) or glue (morphogroup 2 + *Denhama*) their eggs. However, the egg laying strategies are not known for all species and genera; none are confirmed in morphogroup 4 and *Hyrtacus*. In those that bury eggs, all

clades and species lack the supra-anal plate, instead having an elongated, sclerotized anal segment. In those that glue eggs, all clades and species have an elongated, unsclerotised supra-anal plate, instead of an elongated anal segment. This supra-anal plate is also present in morphogroup 4 and *Hyrtacus*, suggesting that they too could glue eggs. Given the widespread occurrence of egg burying across all Lonchodinae species, and also in other families (Robertson et al. 2019; Boisseau and Woods 2024), it is most likely that egg burying is the precursor to egg gluing. Likewise, the elongated and sclerotized anal segment appears to be the ancestral trait for Lonchodini.

All seven *Marcenia* species are diagnosed with a combination of different protuberances across the head, thorax and abdomen, and the presence or absence of a dorsal black stripe. *Marcenia apheles* is easily distinguished by a lack of any abdominal protuberances, while all other *Marcenia* species have these protuberances. Most noticeably, *M. cylindrix*, *M. gojira* and *M. spinosa* all have larger protuberances that are more fin-like in appearance. Another useful character for species level differences is the shape and number of mesothorax spines. Again, *M. cylindrix*, *M. gojira* and *M. spinosa* showcase enlarged thorax spines. However, the shape of these spines differ between individuals, i.e. *M. cylindrix* with cylindrical spines, *M. gojira* with spines having lateral tubercles, and *M. spinosa* with a typical spine shape.

Like *Austrocarausius* (Jones et al. 2022), all other Australian Lonchodini appear to have pronounced preopercular organs. But unlike *Austrocarausius* species, *Marcenia* species cannot be diagnosed according to differences of this organ, as all *Marcenia* species lack morphological variation of this organ. However, the preopercular organ remains useful for delineating genera and clades. Based on available data, it is unclear if this character can be

used for species level differences among the other six genera and clades. Further examination of this character for remaining Lonchodini genera is needed.

Specimens from the *M. cunctatrix* type locality were not available. Therefore, no genetic material could be gathered from specimens matching the exact type locality. Additionally, no eggs were collected from the female type specimen. Due to the cryptic nature of *Marcenia* species, it is difficult to discern which of the examined species best matches the type, but it is likely closely related to members of the *M. cunctatrix* clade (Fig. 4.3). Until specimens from the exact type locality can be sampled, with egg collection and DNA extraction, any further diagnosis of available individuals cannot be completed.

Whilst *M. sp. BJ\_069* is significantly divergent from *M. cylindrix* + *M. gojira* + *M. spinosa*, based on molecular analyses, only one individual is available for examination, and no eggs were collected. Until additional, closely related individuals can be collected and examined, this individual is not proposed as a new species.

#### 4.6.2 Incorrectly labelled *Hyrtacus* specimens in GenBank

*Hyrtacus procerus*, *Hyrtacus. sp. nov. A* and *Hyrtacus. sp. nov. B* (which are from Papua New Guinea) clearly do not group with *Hyrtacus* or any other Australian Lonchodini in any molecular analyses (Fig. 4.3, 4.4; Supplementary Figure S4.2-11). Although no physical specimens were examined, photos of the *H. procerus* and *H. semoni* types strongly suggest they do not belong within *Hyrtacus*. The anal segment for *H. semoni* is sclerotized, and the ovipositor extends to reach the end of anal segment, characters not shared with known *Hyrtacus*. The *H. procerus* type specimen is damaged, so it is missing the anal segment. However, the ovipositor on this specimen extends beyond the typical length of those of

*Hyrtacus* species. These findings are further supported by previous studies (Robertson et al. 2019; Boisseau and Woods 2024), where samples claimed as belonging to *Hyrtacus*, are found in three distinct clades, likely representing three different genera. Without proper examination of other non-Australian Lonchodini, placement of these specimens into other genera is not possible. The *H. tuberculatus* sample (data downloaded from GenBank) represent a species from morphogroup 3. Likewise, Lonchodinae sp. 1 and sp. 2 samples (data also from GenBank) reside in morphogroup 4.

#### 4.6.3 Diversification across an arid environment

The reduction of rainforests and increased occurrence of arid habitats across Australia created new and vast environmental niches, likely driving evolution of the Australian Lonchodini ancestors. How species evolved in these environments is known to be complex and poorly understood (Rix et al. 2017). The central parts of the Australian continent experienced aridification, with forests opening into grasslands during the Miocene to Pliocene (~23-2.6 Ma) (Martin 1998; Byrne et al. 2011, 2008; Bryant and Krosch 2016). The divergence times between arid Lonchodini species (~8.2-15.8; Fig. 4.4) overlap with increased expansion of grassland habitats.

Diversification of the genus *Marcenia* has occurred throughout Australia and is likely influenced by the aridification of Australian landscapes. Findings suggest the diversification of *Marcenia* may have started in Central/Western Australia and spread to Eastern Australia: the two earliest diverging *Marcenia* clades (~20.8 Ma and ~19.1 Ma; Fig. 4.3, 4.4) span across Western and Central Australia (WA, NT, and SA), the *M. cunctatrix* clade (~17.2 Ma) can be found across Northern Australia (WA, NT, Qld), and the remaining two clades (~15.9 Ma; *M. gojira* + *M. spinosa* + *M. sp. BJ\_069* + *M. cylindrix* and *M. sticcosa* + *M. apheles* +

*M. imitans*) are found in Eastern Australia (NSW, Qld). Interestingly, the diversification of *Marcenia* species across Australia may have coincided with the more recent aridification of rainforests, across the northern and eastern parts of Australia (Byrne et al. 2011). A potential mechanism facilitating arid diversification and dispersal throughout Australia could have been parthenogenesis.

It has been suggested that parthenogenesis might drive diversification across arid environments (Kearney 2005). The ability for asexual populations to break off from the original population and diversify into new environments has been demonstrated in arid Australian taxa including the phasmid genus *Sipyloidea* (Kearney and Moussalli 2003; Kearney 2003), grasshoppers (Hoffmann et al. 2023; Kearney and Moussalli 2003; Kearney et al. 2006; Kearney et al. 2022) as well as some lizard species (Kearney et al. 2003). It appears some *Denhama* species lack male specimens, but further sampling is needed to confirm whether populations are truly asexual or not.

When considering mechanisms underlying dispersal and speciation of arid stick insects that inhabit grasslands, parallels in other taxa found in these habitats might offer insight. *Astrebla* (Mitchell grass) is found across large parts of northern Australia and extends into central northern NSW (Orr 1975). These grasses are known to occur across large areas during flood periods; therefore, floods might offer a means of dispersal through increased habitat connectivity for any invertebrates that feed on grasses. Additionally, the native *Themeda triandra* grass has recently dispersed across the Australian continent and outcompeted other species (Dunning et al. 2017). It is possible this species of grass has played a role in increasing habitat availability for herbivores. It is also likely that the *Spinifex* grass consumed by *Denhama* has influenced their habitat availability (Brock and Hasenpusch 2009). *Spinifex* and many other grassland plants started to become more widespread in the



early Pliocene (~5-2.6 Ma; Morton et al. 2011), when the forests were replaced by savannah woodlands (Fujioka and Chappell 2010). Although the spread of *Spinifex* appears to have occurred following the diversification of arid Australian Lonchodini, it may have facilitated intraspecific gene flow in arid Lonchodini species.

Despite the presumed lack of dispersal in apterous insects that bury and glue their eggs (O’Hanlon et al. 2020), the arid Australian Lonchodini are very widespread. Three different species, each with high genetic similarity (one from each of morphogroup 1, *Hyrtacus*, and *Denhama*), have geographical ranges of ~1300-2000 km (Fig. 4.3). Given the genetic similarity of geographically disparate populations within these species, it is clear that gene flow has been maintained across the entire population, despite species burying and gluing their eggs, and not having wings. It is unclear how these species disperse, but potentially, these insects might maintain gene flow by walking, which is achieved over several generations.

Contrary to studies highlighting relatively small geographic ranges of flightless insects (Yeates et al. 2002; Hoffmann et al. 2023), findings from this study indicate some Lonchodini species have widespread distributions across Australia (Fig. 4.3). Increased aridification within the last ~5-2.6 Ma (Byrne et al. 2011) might have enforced population boundaries, but it appears that these were subsequently relaxed in some cases. Given the widespread distributions of all other arid Australian Lonchodini, additional sampling across nearby grass habitats will likely expand species distributions.

## Chapter 5

### General discussion

#### 5.1 Thesis summary

All studies in this thesis used integrative taxonomy to deduce evolution and diversification of Australian Phasmatodea species, with a focus on the tribe Lonchodini. The first chapter introduced the morphology and behaviour of Phasmatodea species and their eggs, which are likely to have contributed diversification and dispersal. This chapter also introduced the integrative taxonomic methods used in subsequent chapters.

Chapter 2 revised the genus *Austrocarausius* (Brock, 2000). Morphological and molecular data were used to perform species delimitation, phylogenetic analysis, and an estimation of the evolutionary timescale. Results revealed nine new *Austrocarausius* species, increasing the genus from two species to eleven (*A. nigropunctatus* (Kirby, 1896), *A. mercurius* (Stål, 1877), *A. coronatus* sp. nov., *A. decorus* sp. nov., *A. eirmosus* sp. nov., *A. gasterbulla* sp. nov., *A. tuberosus* sp. nov., *A. macropunctatus* sp. nov., *A. truncatus* sp. nov. *A. waiben* sp. nov., and *A. walkeri* sp. nov.). These *Austrocarausius* species were shown to have diversified over the last c.25-70 Ma. Their diversification can be attributed to the reduction of tropical rainforest habitats, which restricted their dispersal across more arid biogeographical barriers. The present day *Austrocarausius* species are highly endemic, found only in small, isolated patches of rainforest across the Queensland coast in Australia. More cryptic species are expected to occur in other fragmented rainforest habitats not sampled in this study. This

exploration of cryptic species demonstrated that many species remained undiscovered. Therefore, future sampling across all rainforests in Queensland should be encouraged.

Chapter 3 examined morphological and molecular data from Australian members of the genus *Anchiale*. *Anchiale* species are large, with males capable of flight. *Anchiale* is one of the most observed Australian stick insects and are found in coastal areas of Queensland. This chapter described two new species (*Anchiale mabiensis* (Coupland and Emmott) sp. nov. and *Anchiale robusta* (Brock and Jones) sp. nov.), removed one from synonymy (*A. albopunctata* (Kirby, 1896)), and transferred one to another genus (*Anchiale spinicollis* (Gray 1833) revised to *Acrophylla spinicollis* (Gray, 1833)). No genetic data exist for the two type species (*Acrophylla maculata* (Olivier, 1797)) or *Anchiale mabiensis*. Further DNA sampling from these two species will facilitate more accurate placement of the Australian *Anchiale* species amongst members of the genus globally. Phylogenetic data indicated that one *Acrophylla* sp. (most likely *A. cookorum* (Brock and Coupland, 2022)) was grouped with *Anchiale* species. These results suggest the relationships between *Acrophylla* species are not well resolved. A follow-up study on the closely related genus *Acrophylla* would be beneficial.

Chapter 4 involved an extensive mitogenome and nuclear ribosomal DNA investigation of Australian representatives of *Denhama* and *Hyrtacus* (Brunner von Wattenwyl, 1893), along with morphological investigations of selected taxa. These two grass-mimicking genera are small to medium in size and live across all arid Australian environments. Initial field and morphological studies indicated many cryptic species might exist within these genera. Molecular dating analyses were also used to describe diversification of selected Lonchodini across Australia. The collective results suggest the existence of four new morphogroup, and the reinstatement of the genus *Marcenia* (Sjöstedt,

1918). This chapter described five new *Marcenia* species, and reinstated *M. cunctatrix* (Sjöstedt, 1918) and *M. imitans* (Brunner von Wattenwyl, 1907). The relatively recent divergence of many *Marcenia* species (~8.2-15.8 Ma) suggests that the aridification of Australia and the subsequent increase in grassland habitats drove distributions of arid Australian Lonchodini.

## 5.2 Future directions

The costs of DNA sequencing continue to decrease, and technologies in recent decades have arisen that facilitate long read sequencing (Jain et al. 2016; Eid et al. 2009; McCormack et al. 2013; Reuter et al. 2015; Goodwin et al. 2016). It is therefore becoming more feasible and common to include integrative taxonomic techniques when describing new species.

Molecular dating methods provide key insights into species diversification. These methods allow us to piece together the evolution of species and clades when combined with historical biogeography. Applying such methods to taxonomic studies provides a greater understanding of the evolution of behavioural and morphological traits. Future studies should focus on including both morphological and molecular evidence to infer species relationships.

There has been an increase in the use of molecular data to investigate deeper family-level relationships between phasmatodean clades (Robertson et al. 2018; Simon et al. 2019; Bank and Bradler 2022; Boisseau and Woods 2024). However, relationships between deeper clades differ in these studies when using different genetic techniques. Most phasmid species remain unsequenced, and therefore many taxon groups are missing from previous studies. Until now, the Australian Lonchodini had not been represented. Accordingly, gaps remain for

all phasmid species worldwide, and many Australian genera and species are still missing genetic representation, including in species investigated in this thesis.

Investigation of Australian Phasmatodea in each chapter has highlighted knowledge gaps requiring further study. These have been discussed in detail at the end of each chapter, and are summarised more briefly in this discussion. Whilst Chapters 2, 3 and 4 extensively explored species within the *Austrocarausius*, *Anchiale*, and *Marcenia* genera, these studies also highlight the need for further investigations within these genera, and increased sampling across habitats.

The camouflage and life history traits exhibited by Phasmatodea species make the acquisition of their samples difficult. For instance, when *Ctenomorpha gargantua* (Australia's longest insect at 56.5 cm) was described in 2006, no female specimens could be collected despite photographic evidence of their existence (Hasenpusch and Brock 2006). The first *Gibbernecroscia kooymani* specimen was only found in 2007. This is significant considering its relatively widespread occurrence in northern New South Wales and southeastern Queensland rainforests (Brock et al. 2019). Further, this area includes Mt Wollumbin (Mt Warning), known as a biodiverse hotspot to entomologists (Brock and Coupland 2022). The challenges of finding phasmid species, combined with Australia's vast size, means thorough exploration of every habitat is necessary to document all Australian Phasmatodea species.

The aridification and fragmentation of habitats within Australia has influenced diversification of phasmid species. For instance, the *Austrocarausius* (from Chapter 2) have been restricted to the tropical rainforests of northern Queensland. These species seem to be endemic and unable to disperse across large geographical ranges. This fragmentation of species is common for tropical rainforest taxa. The two described *Parapodocanthus* species

can only be found in what is considered remnant Gondwanan rainforests in far northern Queensland, and Springbrook in southern Queensland (Kitching et al. 2010; Brock and Monteith 2018). The *Megacrania batesii* species is restricted to small isolated populations in coastal environments (iNaturalist 2024). If aridification due to the changing climate continues, these species are likely to become threatened or extinct.

Although rainforest species commonly showcase high endemism, arid genera do not necessarily, despite the fact they are unable to fly. The *Anchiale* from Chapter 3 and arid Lonchodini from Chapter 4 inhabit large geographical distributions, up to 2000 km (morphogroup 1 from Chapter 4). It is known that the main habitat and food plant for the arid Lonchodini genera are grasses. Since the reduction of rainforests in central Australia, these rainforest habitats transitioned into open sclerophyll woodlands and grasslands (~23-2.6 Ma; Byrne et al. 2011, 2008; Bryant and Krosch 2016). Accordingly, physically continuous grassland habitats have likely enabled large geographical distributions of phasmid species. With increasing conversion of grassland to agricultural land, it is unclear the extent to which grazing animals have influenced habitat availability and connectivity of the “grass” stick insects. Additionally, several species from Chapter 4 were collected on roadsides. Regular trimming and maintenance of vegetation by the governing authorities is likely putting strain on population boundaries. Better exploration of grassland habitats is needed in the future to assess the vulnerability of arid insect populations.

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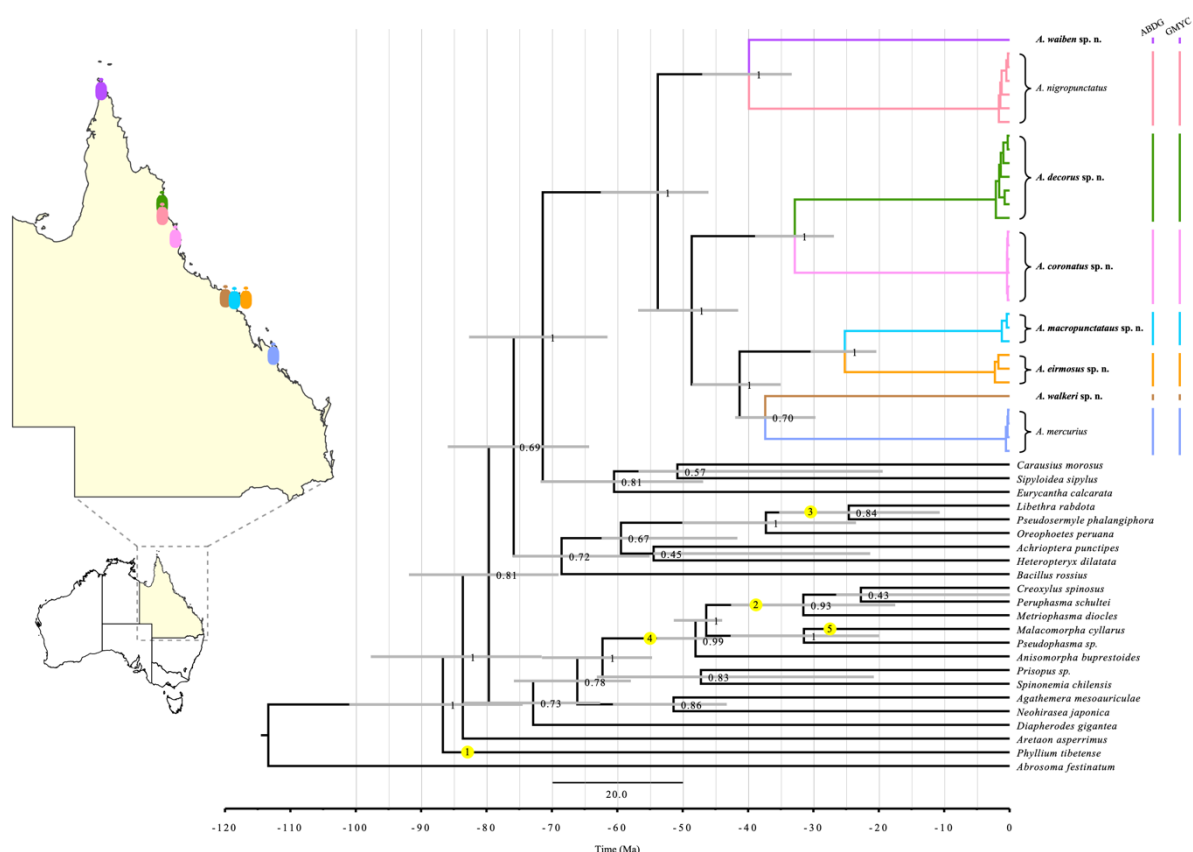
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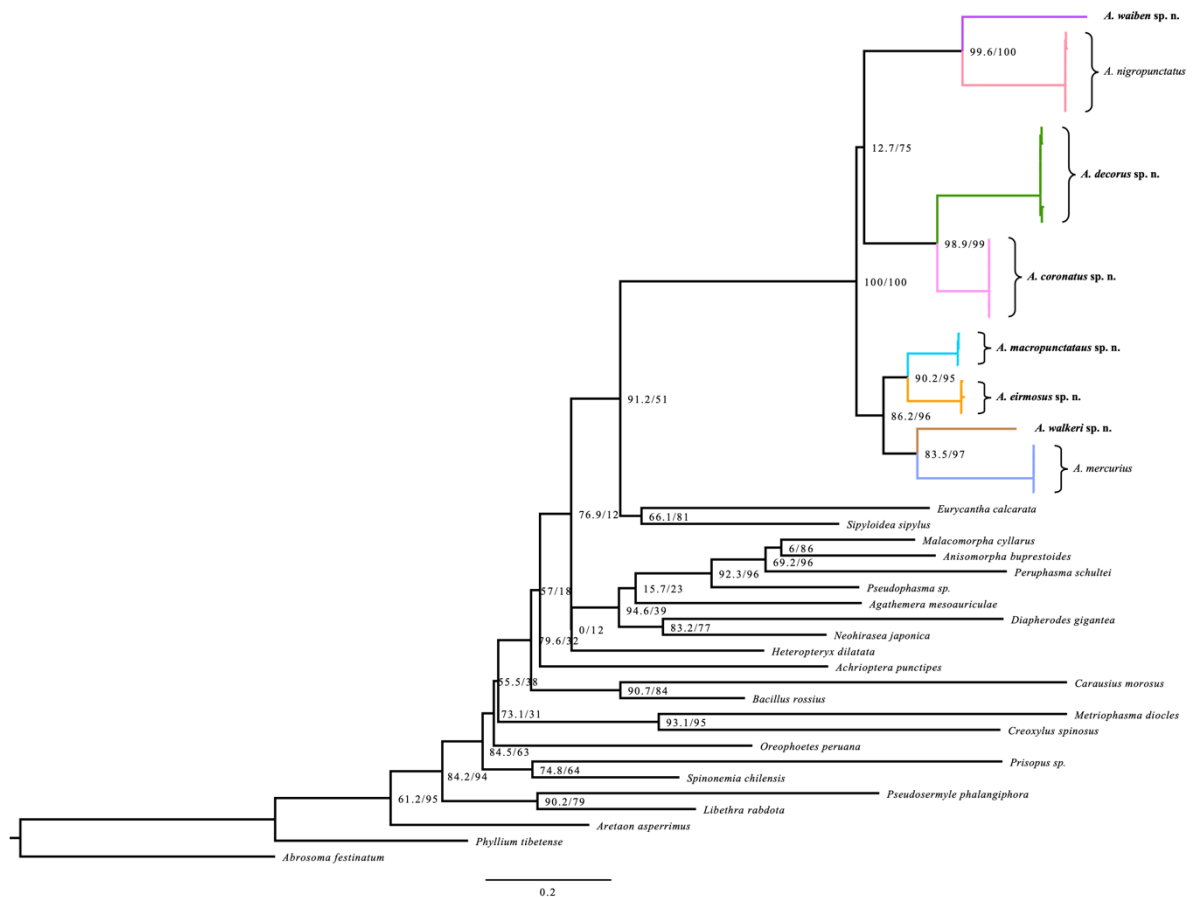


## **Appendix**

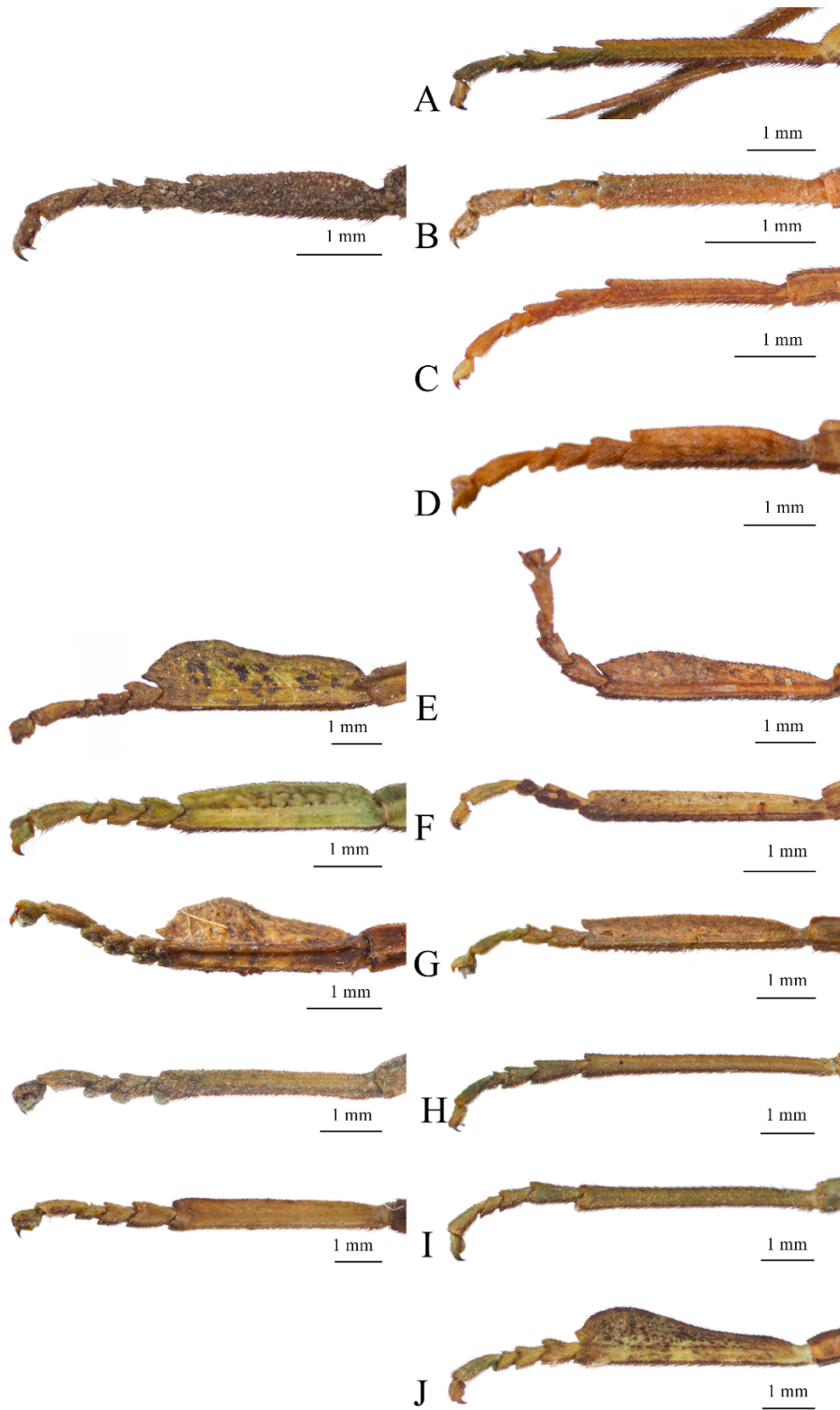
## Supplementary material for Chapter 2



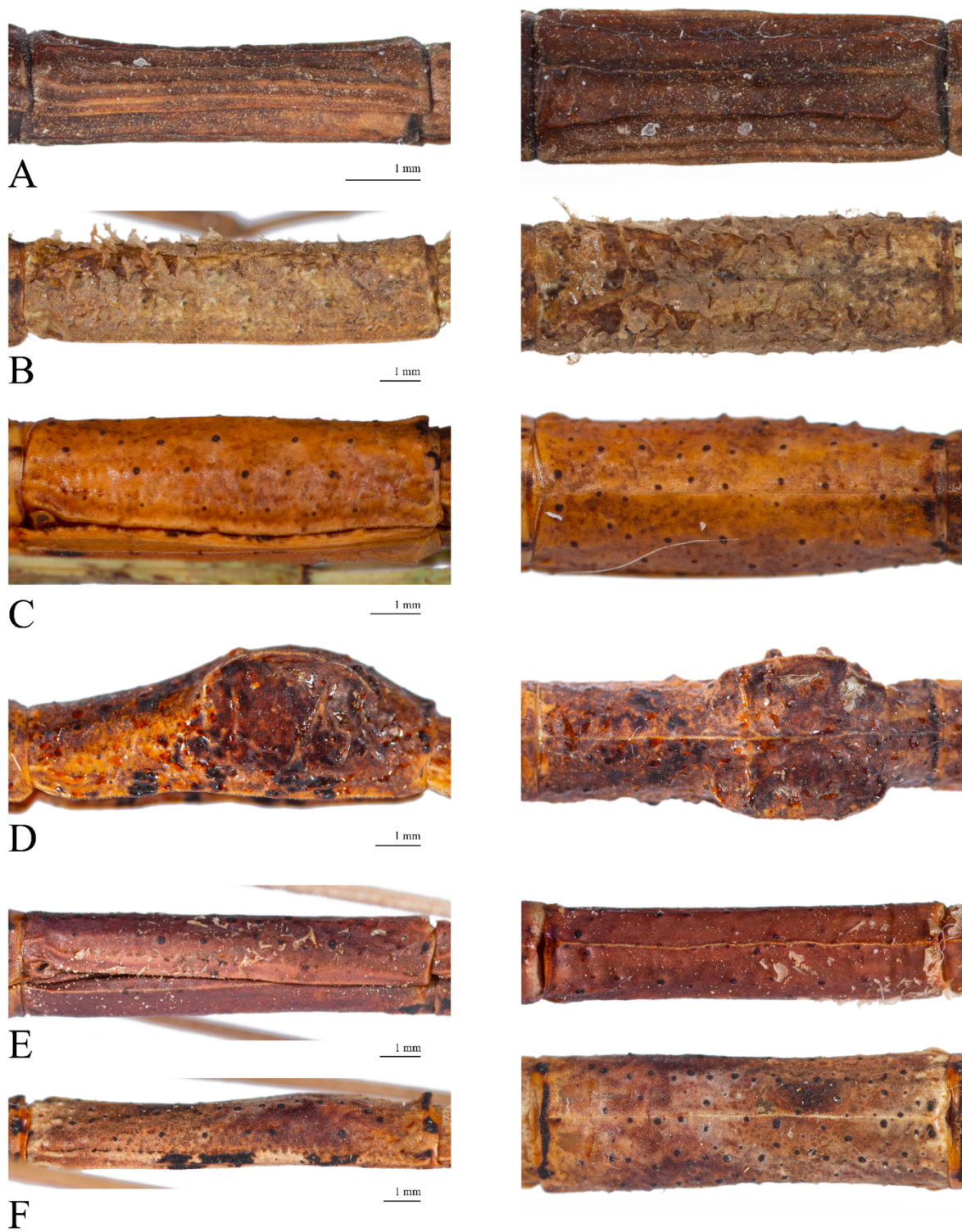
**Figure S2.1.** Fossil calibrated Bayesian inference tree for eight *Austrocarausius* species and outgroups inferred from mitochondrial *COI* and *16S*. The tree is colour coded by species. Species distributions are likewise colour coded by egg markers on map. Bold text indicates new species. Grey bars at each node represent 95% highest posterior density (HPD) divergence times. Node support values are shown for Bayesian inference, as posterior probability. Yellow markers on tree correspond to fossils used in time calibration (Supplementary Table S2.5). Scale bar represents substitutions per million years.



**Figure S2.2.** Maximum likelihood tree for eight *Austrocarausius* species and outgroups inferred from *COI* and *16S* genes. Tree is colour coded by species. Bold text indicates new species. Node support values represent bootstrap and ESS values. Scale bar represents substitution rate.



**Figure S2.3.** Lateral views for tarsi of females (left) and males (right). Each row is a single species. (A) *A. mercurius*; (B) *A. waiben*; (C) *A. truncatus*; (D) *A. tuberosus*; (E) *A. decorus*; (F) *A. coronatus*; (G) *A. gasterbulla*; (H) *A. macropunctatus*; (I) *A. eirimosus*; (J) *A. walkeri*.



**Figure S2.4.** Lateral and dorsal views for the 6th abdominal segment of females. Each row is a single species. (A) *A. waiben*; (B) *A. decorus*; (C) *A. coronatus*; (D) *A. gasterbulla*; (E) *A. macropunctatus*; (F) *A. eirmosus*.

**Table S2.1.** Morphological measurements for specimens examined in species diagnosis.

| individual   | body region | character                                 | measurment 1 (mm)      | measurment 2 | measurment 3 | average   |
|--------------|-------------|-------------------------------------------|------------------------|--------------|--------------|-----------|
| male PB-0079 | general:    | whole insect                              | small tubercels on hea |              |              |           |
|              |             | colouration                               | uniform brown, reddis  |              |              |           |
|              |             | Body length                               | 82.5                   | 82.3         | 82.3         | 82.366667 |
|              | head:       | head length                               | 2.6                    | 2.8          | 3            | 2.8       |
|              |             | head width                                | 1.9                    | 1.9          | 1.9          | 1.9       |
|              |             | antennae length                           | 52.4                   | 53           | 52.5         | 52.633333 |
|              |             | femur length                              | 21.8                   | 21.7         | 21.7         | 21.733333 |
|              |             | antennae segment no.                      |                        |              |              | 62        |
|              | Thorax:     | pronotum length                           | 2.3                    | 2.3          | 2.4          | 2.333333  |
|              |             | mesonotum length                          | 19.9                   | 19.9         | 19.8         | 19.866667 |
|              |             | metanotum length                          | 11                     | 11.08        | 10.89        | 10.99     |
|              | Abdomen:    | median segment                            | 3.1                    | 3.22         | 3.41         | 3.243333  |
|              |             | abdominal segment 2 length                | 7                      | 6.9          | 6.9          | 6.933333  |
|              |             | abdominal segment 3 length                | 5.9                    | 6.1          | 6.1          | 6.033333  |
|              |             | abdominal segment 4 length                | 5.9                    | 6            | 6.1          | 6         |
|              |             | abdominal segment 5 length                | 5.9                    | 5.8          | 5.8          | 5.833333  |
|              |             | abdominal segment 6 length                | 5.4                    | 5.4          | 5.4          | 5.4       |
|              |             | abdominal segment 7 length                | 4.6                    | 4.4          | 4.4          | 4.466667  |
|              |             | abdominal segment 8 length                | 2.6                    | 2.5          | 2.5          | 2.533333  |
|              |             | abdominal segment 9 length                | 1.9                    | 2            | 2            | 1.966667  |
|              |             | abdominal segment 10 length               | 3.7                    | 3.5          | 3.3          | 3.5       |
|              |             | cerci                                     | N/A                    |              |              |           |
|              |             | supra anal plate length                   | N/A                    |              |              |           |
|              |             | operculum tip covering how much supra tip | N/A                    |              |              |           |
|              | Wings:      | wings                                     | apterus                |              |              |           |
|              | Legs:       | leg spines                                | none                   |              |              |           |
|              |             | fore femur                                | 22.18                  | 22.6         | 22.34        | 22.373333 |
|              |             | mid femur                                 | 17.6                   | 16.56        | 16.78        | 16.98     |
|              |             | hind femur                                | 19.84                  | 19.71        | 19.66        | 19.736667 |
|              |             | fore tibia                                | 24.69                  | 24.82        | 24.9         | 24.803333 |
|              |             | mid tibia                                 | 18.53                  | 18.61        | 17.71        | 18.283333 |
|              |             | hind tibia                                | 24.4                   | 24.03        | 23.78        | 24.07     |
|              |             | length of basitarsus hind leg             | 2.6                    | 2.5          | 2.7          | 2.6       |
|              |             | length of tarsomears hind leg             | 2.1                    | 2.2          | 2.2          | 2.166667  |
|              |             | length of basitarsus fore leg             | 3.3                    | 3.3          | 3.4          | 3.333333  |
|              |             | length of tarsomears fore leg             | 2.4                    | 2.3          | 2.2          | 2.3       |
| male PB-0078 | general:    | whole insect                              | mostly same as type, l |              |              |           |
|              |             | colouration                               | same as type           |              |              |           |
|              |             | Body length                               | 92.2                   | 92.3         | 91.6         | 92.033333 |
|              | head:       | head length                               | 2.9                    | 3.2          | 3            | 3.033333  |
|              |             | head width                                | 2.1                    | 2.1          | 2.1          | 2.1       |
|              |             | antennae length                           | 55                     | 55.8         | 55.1         | 55.3      |
|              |             | femur length                              | 23.6                   | 24.3         | 24           | 23.966667 |
|              |             | antennae segment no.                      |                        |              |              |           |
|              | Thorax:     | pronotum length                           | 2.6                    | 2.7          | 2.6          | 2.633333  |
|              |             | mesonotum length                          | 22                     | 22.1         | 22.3         | 22.133333 |
|              |             | metanotum length                          | 12.75                  | 12.23        | 12.31        | 12.43     |
|              | Abdomen:    | median segment                            | 3.15                   | 3.67         | 3.59         | 3.47      |
|              |             | abdominal segment 2 length                | 7.2                    | 7.1          | 7.3          | 7.2       |
|              |             | abdominal segment 3 length                | 6.6                    | 6.6          | 6.5          | 6.566667  |
|              |             | abdominal segment 4 length                | 6.4                    | 6.5          | 6.5          | 6.466667  |
|              |             | abdominal segment 5 length                | 6.6                    | 6.7          | 6.7          | 6.666667  |
|              |             | abdominal segment 6 length                | 6                      | 5.9          | 5.9          | 5.933333  |
|              |             | abdominal segment 7 length                | 4.9                    | 4.8          | 4.5          | 4.733333  |
|              |             | abdominal segment 8 length                | 3                      | 3            | 2.8          | 2.933333  |
|              |             | abdominal segment 9 length                | 1.7                    | 1.8          | 1.7          | 1.733333  |
|              |             | abdominal segment 10 length               | 4                      | 4.2          | 4.2          | 4.133333  |
|              |             | cerci                                     | N/A                    |              |              |           |
|              |             | supra anal plate length                   | N/A                    |              |              |           |
|              |             | operculum tip covering how much supra tip | N/A                    |              |              |           |
|              | Wings:      | wings                                     | apterus                |              |              |           |
|              | Legs:       | leg spines                                | none                   |              |              |           |
|              |             | fore fenur                                | 24.18                  | 24.84        | 24.23        | 24.416667 |
|              |             | mid femur                                 | missing                |              |              | 0         |
|              |             | hind femur                                | 21.51                  | 21.01        | 21.82        | 21.446667 |
|              |             | fore tibia                                | 26.92                  | 26.66        | 27.07        | 26.883333 |
|              |             | mid tibia                                 | missing                |              |              | 0         |
|              |             | hind tibia                                | 27.13                  | 27.11        | 27.19        | 27.143333 |
|              |             | length of basitarsus hind leg             | missing                |              |              |           |
|              |             | length of tarsomears hind leg             | missing                |              |              |           |
|              |             | length of basitarsus fore leg             | 3.4                    | 3.6          | 3.7          | 3.566667  |
|              |             | length of tarsomears fore leg             | 2.6                    | 2.6          | 2.4          | 2.533333  |

| individual    | body region | character                                 | measurment 1 (mm) | measurment 2 | measurment 3 | average     |
|---------------|-------------|-------------------------------------------|-------------------|--------------|--------------|-------------|
| female bj-324 | general:    | whole insect                              |                   |              |              |             |
|               |             | colouration                               |                   |              |              |             |
|               |             | Body length                               | 92.17             | 92.15        | 91.92        | 92.08       |
|               | head:       | head length                               | 3.93              | 4.17         | 4.82         | 4.30666667  |
|               |             | head width                                | 2.47              | 2.62         | 2.59         | 2.56        |
|               |             | antennae length                           | 37.58             | 38.06        | 40.51        | 38.71666667 |
|               |             | femur length                              |                   |              |              |             |
|               |             | antennae segment no.                      |                   |              |              | 54          |
|               | Thorax:     | pronotum length                           | 3.68              | 3.91         | 3.43         | 3.67333333  |
|               |             | mesonotum length                          | 22.33             | 22.11        | 22.08        | 22.17333333 |
|               |             | metanotum length                          | 9.75              | 9.77         | 9.81         | 9.77666667  |
|               | Abdomen:    | median segment                            | 4.77              | 4.36         | 4.39         | 4.50666667  |
|               |             | abdominal segment 2 length                | 7.19              | 6.9          | 7.29         | 7.12666667  |
|               |             | abdominal segment 3 length                | 6.49              | 6.6          | 6.71         | 6.6         |
|               |             | abdominal segment 4 length                | 7.71              | 7.17         | 7.57         | 7.48333333  |
|               |             | abdominal segment 5 length                | 7.67              | 7.89         | 7.46         | 7.67333333  |
|               |             | abdominal segment 6 length                | 7.16              | 7.63         | 7.25         | 7.34666667  |
|               |             | abdominal segment 7 length                | 5.61              | 5.39         | 5.54         | 5.51333333  |
|               |             | abdominal segment 8 length                | 2.55              | 2.97         | 2.65         | 2.72333333  |
|               |             | abdominal segment 9 length                | 1.81              | 2.1          | 2.13         | 2.01333333  |
|               |             | abdominal segment 10 length               | 2.35              | 2.43         | 2.14         | 2.30666667  |
|               |             | cerci                                     | N/A               |              |              |             |
|               |             | supra anal plate length                   | N/A               |              |              |             |
|               |             | operculum tip covering how much supra tip | N/A               |              |              |             |
|               | Wings:      | wings                                     |                   |              |              |             |
|               | Legs:       | leg spines                                |                   |              |              |             |
|               |             | fore fenur                                | 21.36             | 21.93        | 21.46        | 21.58333333 |
|               |             | mid femur                                 | 16.58             | 16.58        | 16.49        | 16.55       |
|               |             | hind femur                                | 20.71             | 19.87        | 20.08        | 20.22       |
|               |             | fore tibia                                | 21.97             | 22.36        | 22.14        | 22.15666667 |
|               |             | mid tibia                                 | 16.72             | 17.15        | 16.42        | 16.76333333 |
|               |             | hind tibia                                | 21.07             | 21.36        | 21.09        | 21.17333333 |
|               |             | length of basitarsus hind leg             | 2.11              | 2.34         | 2.17         | 2.20666667  |
|               |             | length of tarsomears hind leg             | 2.35              | 2.77         | 2.46         | 2.52666667  |
|               |             | length of basitarsus fore leg             | 2.25              | 2.59         | 2.25         | 2.36333333  |
|               |             | length of tarsomears fore leg             | 2.03              | 2.1          | 2.1          | 2.07666667  |
| male bj-323   | general:    | whole insect                              |                   |              |              |             |
|               |             | colouration                               |                   |              |              |             |
|               |             | Body length                               | 65.62             | 66.23        | 65.86        | 65.90333333 |
|               | head:       | head length                               | 3.05              | 3.05         | 3.3          | 3.13333333  |
|               |             | head width                                | 1.6               | 1.5          | 1.47         | 1.52333333  |
|               |             | antennae length                           | 41.34             | 40.61        | 40.92        | 40.95666667 |
|               |             | femur length                              |                   |              |              |             |
|               |             | antennae segment no.                      |                   |              |              | 74          |
|               | Thorax:     | pronotum length                           | 2.13              | 2.21         | 2.73         | 2.35666667  |
|               |             | mesonotum length                          | 16.3              | 16.35        | 16.6         | 16.41666667 |
|               |             | metanotum length                          | 7.67              | 7.7          | 8.06         | 7.81        |
|               | Abdomen:    | median segment                            | 3.23              | 3.02         | 2.84         | 3.03        |
|               |             | abdominal segment 2 length                | 5.03              | 5.48         | 5.1          | 5.20333333  |
|               |             | abdominal segment 3 length                | 4.29              | 4.45         | 4.33         | 4.35666667  |
|               |             | abdominal segment 4 length                | 4.27              | 4.28         | 4.26         | 4.27        |
|               |             | abdominal segment 5 length                | 4.24              | 4.51         | 4.49         | 4.41333333  |
|               |             | abdominal segment 6 length                | 4.02              | 4.22         | 4.14         | 4.12666667  |
|               |             | abdominal segment 7 length                | 3.27              | 3.18         | 3.17         | 3.20666667  |
|               |             | abdominal segment 8 length                | 1.91              | 2            | 1.99         | 1.96666667  |
|               |             | abdominal segment 9 length                | 1.8               | 2.21         | 1.97         | 1.99333333  |
|               |             | abdominal segment 10 length               | 3.7               | 4.02         | 3.93         | 3.88333333  |
|               |             | cerci                                     | N/A               |              |              |             |
|               |             | supra anal plate length                   | N/A               |              |              |             |
|               |             | operculum tip covering how much supra tip | N/A               |              |              |             |
|               | Wings:      | wings                                     |                   |              |              |             |
|               | Legs:       | leg spines                                |                   |              |              |             |
|               |             | fore fenur                                | 19.48             | 19.53        | 19.48        | 19.49666667 |
|               |             | mid femur                                 | 14.52             | 14.73        | 14.53        | 14.59333333 |
|               |             | hind femur                                | 16.24             | 17.06        | 17.47        | 16.92333333 |
|               |             | fore tibia                                | 20.94             | 21.48        | 21.28        | 21.23333333 |
|               |             | mid tibia                                 | 16.17             | 16.26        | 15.89        | 16.10666667 |
|               |             | hind tibia                                | 20.5              | 21.96        | 20.92        | 21.12666667 |
|               |             | length of basitarsus hind leg             | 2.11              | 2.64         | 2.56         | 2.43666667  |
|               |             | length of tarsomears hind leg             | 2.12              | 2.2          | 2.24         | 2.18666667  |
|               |             | length of basitarsus fore leg             | 2.19              | 1.88         | 2.16         | 2.07666667  |
|               |             | length of tarsomears fore leg             | 1.43              | 1.37         | 1.81         | 1.53666667  |

|               |          |                                           |         |       |       |            |
|---------------|----------|-------------------------------------------|---------|-------|-------|------------|
| female bj-327 | general: | whole insect                              |         |       |       |            |
|               |          | colouration                               |         |       |       |            |
|               |          | Body length                               | 79.5    | 79.34 | 79.02 | 79.2866667 |
|               | head:    | head length                               | 4.44    | 4.51  | 4.4   | 4.45       |
|               |          | head width                                | 2.64    | 2.63  | 2.7   | 2.6566667  |
|               |          | antennae length                           | 39.53   | 38.83 | 40.03 | 39.4633333 |
|               |          | femur length                              |         |       |       |            |
|               |          | antennae segment no.                      |         |       |       |            |
|               | Thorax:  | pronotum length                           | 3.13    | 3.43  | 2.81  | 3.12333333 |
|               |          | mesonotum length                          | 18.42   | 18.59 | 18.73 | 18.58      |
|               |          | metanotum length                          | 8.33    | 8.59  | 8.71  | 8.54333333 |
|               | Abdomen: | median segment                            | 3.2     | 3.24  | 3.04  | 3.16       |
|               |          | abdominal segment 2 length                | 6.03    | 6.32  | 5.89  | 6.08       |
|               |          | abdominal segment 3 length                | 5.69    | 5.91  | 5.59  | 5.73       |
|               |          | abdominal segment 4 length                | 5.99    | 6.06  | 6.21  | 6.0866667  |
|               |          | abdominal segment 5 length                | 6.42    | 6.73  | 6.66  | 6.60333333 |
|               |          | abdominal segment 6 length                | 6.28    | 6.5   | 6.67  | 6.48333333 |
|               |          | abdominal segment 7 length                | 4.86    | 4.84  | 4.94  | 4.88       |
|               |          | abdominal segment 8 length                | 2.66    | 2.55  | 2.38  | 2.53       |
|               |          | abdominal segment 9 length                | 1.6     | 1.73  | 1.58  | 1.6366667  |
|               |          | abdominal segment 10 length               | 1.67    | 1.87  | 1.74  | 1.76       |
|               |          | cerci                                     | N/A     |       |       |            |
|               |          | supra anal plate length                   | N/A     |       |       |            |
|               |          | operculum tip covering how much supra tip | N/A     |       |       |            |
|               | Wings:   | wings                                     | apterus |       |       |            |
|               | Legs:    | leg spines                                | none    |       |       |            |
|               |          | fore femur                                | 20.93   | 20.8  | 21.51 | 21.08      |
|               |          | mid femur                                 | 16.35   | 16.55 | 16.54 | 16.48      |
|               |          | hind femur                                | 19.43   | 19.55 | 19.69 | 19.5566667 |
|               |          | fore tibia                                | 20.49   | 20.23 | 20.11 | 20.2766667 |
|               |          | mid tibia                                 | 15.72   | 16    | 16    | 15.9066667 |
|               |          | hind tibia                                | 20.04   | 20    | 20    | 20.0133333 |
|               |          | length of basitarsus hind leg             | 2.11    | 2.16  | 2.39  | 2.22       |
|               |          | length of tarsomeres hind leg             | 2.66    | 2.79  | 2.87  | 2.77333333 |
|               |          | length of basitarsus fore leg             | 2.47    | 2.44  | 1.92  | 2.2766667  |
|               |          | length of tarsomeres fore leg             | 2.55    | 2.16  | 2.62  | 2.44333333 |
| female bj-326 | general: | whole insect                              |         |       |       |            |
|               |          | colouration                               |         |       |       |            |
|               |          | Body length                               | 90.32   | 91.25 | 90.53 | 90.7       |
|               | head:    | head length                               | 4.12    | 4.29  | 4.21  | 4.2066667  |
|               |          | head width                                | 2.72    | 2.77  | 2.72  | 2.7366667  |
|               |          | antennae length                           | broken  |       |       |            |
|               |          | femur length                              |         |       |       |            |
|               |          | antennae segment no.                      | broken  |       |       |            |
|               | Thorax:  | pronotum length                           | 3.35    | 3.49  | 2.75  | 3.1966667  |
|               |          | mesonotum length                          | 21.01   | 21.04 | 20.86 | 20.97      |
|               |          | metanotum length                          | 9.01    | 9.7   | 9.4   | 9.37       |
|               | Abdomen: | median segment                            | 4.6     | 3.77  | 4.32  | 4.23       |
|               |          | abdominal segment 2 length                | 7.06    | 6.74  | 6.57  | 6.79       |
|               |          | abdominal segment 3 length                | 6.58    | 6.46  | 6.45  | 6.4966667  |
|               |          | abdominal segment 4 length                | 6.54    | 6.44  | 6.46  | 6.48       |
|               |          | abdominal segment 5 length                | 7.21    | 7.67  | 7.52  | 7.4666667  |
|               |          | abdominal segment 6 length                | 7.05    | 7.17  | 7.37  | 7.1966667  |
|               |          | abdominal segment 7 length                | 5.68    | 5.6   | 6.69  | 5.99       |
|               |          | abdominal segment 8 length                | 3.01    | 2.96  | 3.01  | 2.99333333 |
|               |          | abdominal segment 9 length                | 1.83    | 1.82  | 1.79  | 1.81333333 |
|               |          | abdominal segment 10 length               | 2.01    | 2.28  | 1.13  | 1.8066667  |
|               |          | cerci                                     | N/A     |       |       |            |
|               |          | supra anal plate length                   | N/A     |       |       |            |
|               |          | operculum tip covering how much supra tip | N/A     |       |       |            |
|               | Wings:   | wings                                     |         |       |       |            |
|               | Legs:    | leg spines                                |         |       |       |            |
|               |          | fore femur                                | 22.13   | 22.25 | 22.6  | 22.3266667 |
|               |          | mid femur                                 | 17.75   | 17.46 | 17.54 | 17.5833333 |
|               |          | hind femur                                | 20.92   | 20.2  | 20.82 | 20.6466667 |
|               |          | fore tibia                                | 21.7    | 21.87 | 21.9  | 21.8233333 |
|               |          | mid tibia                                 | 17.44   | 17.6  | 17.83 | 17.6233333 |
|               |          | hind tibia                                | 21.73   | 21.54 | 21.67 | 21.6466667 |
|               |          | length of basitarsus hind leg             | 2.52    | 2.21  | 2.51  | 2.41333333 |
|               |          | length of tarsomeres hind leg             | 2.31    | 2.58  | 2.66  | 2.5166667  |
|               |          | length of basitarsus fore leg             | 1.91    | 2.06  | 2.1   | 2.02333333 |
|               |          | length of tarsomeres fore leg             | 2.62    | 2.56  | 2.5   | 2.56       |



| individual | body region | character                                 | measurment 1 (mm)      | measurment 2 | measurment 3 | average     |
|------------|-------------|-------------------------------------------|------------------------|--------------|--------------|-------------|
| T250907    | general:    | whole insect                              | plain, no head spines, |              |              |             |
|            |             | colouration                               | brown                  |              |              |             |
|            |             | Body length                               | 71                     | 71           | 71           | 71          |
|            | head:       | head length                               | 3.4                    | 3.2          | 3.1          | 3.23333333  |
|            |             | head width                                | 2.2                    | 2            | 2.3          | 2.16666667  |
|            |             | antennae length                           | 52.2                   | 50.2         | 51.8         | 51.4        |
|            |             | femur length                              | 22.3                   | 22.3         | 21.9         | 22.16666667 |
|            |             | antennae segment no.                      |                        |              |              | 68          |
|            | Thorax:     | pronotum length                           | 2.5                    | 2.3          | 2.6          | 2.46666667  |
|            |             | mesonotum length                          | 19.3                   | 19.9         | 18.8         | 19.33333333 |
|            |             | metanotum length                          | 8.71                   | 8.9          | 9.1          | 8.90333333  |
|            | Abdomen:    | median segment                            | 2.59                   | 2.8          | 2.9          | 2.76333333  |
|            |             | abdominal segment 2 length                | 5.7                    | 5.7          | 5.8          | 5.73333333  |
|            |             | abdominal segment 3 length                | 5.3                    | 5.3          | 5.5          | 5.36666667  |
|            |             | abdominal segment 4 length                | 5.4                    | 5.4          | 5.1          | 5.3         |
|            |             | abdominal segment 5 length                | 5.4                    | 5.3          | 5.6          | 5.43333333  |
|            |             | abdominal segment 6 length                | 4.9                    | 5.1          | 5.1          | 5.03333333  |
|            |             | abdominal segment 7 length                | 3.8                    | 3.6          | 3.6          | 3.66666667  |
|            |             | abdominal segment 8 length                | 2.1                    | 1.9          | 2.1          | 2.03333333  |
|            |             | abdominal segment 9 length                | 1.7                    | 1.7          | 1.8          | 1.73333333  |
|            |             | abdominal segment 10 length               | 2                      | 2.1          | 2.1          | 2.06666667  |
|            |             | cerci                                     | N/A                    |              |              |             |
|            |             | supra anal plate length                   | N/A                    |              |              |             |
|            |             | operculum tip covering how much supra tip | N/A                    |              |              |             |
|            | Wings:      | wings                                     | apterus                |              |              |             |
|            | Legs:       | leg spines                                | none                   |              |              |             |
|            |             | fore fenur                                | 21.94                  | 21.75        | 21.6         | 21.76333333 |
|            |             | mid femur                                 | 15.4                   | 15.3         | 15.19        | 15.29666667 |
|            |             | hind femur                                | 20.06                  | 20.51        | 20.16        | 20.24333333 |
|            |             | fore tibia                                | 25.7                   | 25.54        | 25.58        | 25.60666667 |
|            |             | mid tibia                                 | 18.42                  | 18           | 18.65        | 18.35666667 |
|            |             | hind tibia                                | 26.63                  | 26.04        | 26.7         | 26.45666667 |
|            |             | length of basitarsus hind leg             | 2.2                    | 2.4          | 2.8          | 2.46666667  |
|            |             | length of tarsomeres hind leg             | 2                      | 2.2          | 2.7          | 2.3         |
|            |             | length of basitarsus fore leg             | 2.2                    | 2.4          | 2.4          | 2.33333333  |
|            |             | length of tarsomeres fore leg             | 2.4                    | 2.7          | 2.5          | 2.53333333  |

| individual | body region | character                                 | measurment 1 (mm)      | measurment 2 | measurment 3 | average    |
|------------|-------------|-------------------------------------------|------------------------|--------------|--------------|------------|
| T250908    | general:    | whole insect                              | mostly plain, tubercel |              |              |            |
|            |             | colouration                               | uniform brown          |              |              |            |
|            |             | Body length                               | 84.1                   | 83.1         | 83.8         | 83.6666667 |
|            | head:       | head length                               | 3.3                    | 3            | 2.8          | 3.03333333 |
|            |             | head width                                | 2.8                    | 2.5          | 2.4          | 2.56666667 |
|            |             | antennae length                           | 51.29                  | 54.27        | 51.3         | 52.2866667 |
|            |             | femur length                              | 23.3                   | 23.4         | 23.3         | 23.3333333 |
|            |             | antennae segment no.                      |                        |              |              | 35         |
|            | Thorax:     | pronotum length                           | 3.3                    | 3.4          | 3.1          | 3.26666667 |
|            |             | mesonotum length                          | 22.4                   | 22.4         | 22.6         | 22.4666667 |
|            |             | metanotum length                          | 9.96                   | 10.17        | 10.41        | 10.18      |
|            | Abdomen:    | median segment                            | 3.74                   | 3.93         | 3.59         | 3.75333333 |
|            |             | abdominal segment 2 length                | 6.8                    | 6.7          | 6.4          | 6.63333333 |
|            |             | abdominal segment 3 length                | 6.3                    | 6.2          | 6.4          | 6.3        |
|            |             | abdominal segment 4 length                | 6.1                    | 6            | 6.4          | 6.16666667 |
|            |             | abdominal segment 5 length                | 6.1                    | 6            | 6.1          | 6.06666667 |
|            |             | abdominal segment 6 length                | 5.5                    | 5.6          | 5.4          | 5.5        |
|            |             | abdominal segment 7 length                | 4.4                    | 4.3          | 4.3          | 4.33333333 |
|            |             | abdominal segment 8 length                | 2.6                    | 2.7          | 2.7          | 2.66666667 |
|            |             | abdominal segment 9 length                | 2.3                    | 2.1          | 2.1          | 2.16666667 |
|            |             | abdominal segment 10 length               | 2.4                    | 2.2          | 2.5          | 2.36666667 |
|            |             | cerci                                     | N/A                    |              |              |            |
|            |             | supra anal plate length                   | N/A                    |              |              |            |
|            |             | operculum tip covering how much supra tip | N/A                    |              |              |            |
|            | Wings:      | wings                                     | apterus                |              |              |            |
|            | Legs:       | leg spines                                | none                   |              |              |            |
|            |             | fore fenur                                | 23.11                  | 23.41        | 23.31        | 23.2766667 |
|            |             | mid femur                                 | 16.67                  | 16.52        | 16.56        | 16.5833333 |
|            |             | hind femur                                | 18.78                  | 19.64        | 18.81        | 19.0766667 |
|            |             | fore tibia                                | 25.8                   | 26.75        | 26.48        | 26.3433333 |
|            |             | mid tibia                                 | 17.91                  | 17.85        | 18.1         | 17.9533333 |
|            |             | hind tibia                                | 23.84                  | 24.27        | 24           | 24.0366667 |
|            |             | length of basitarsus hind leg             | 2                      | 2.3          | 2.1          | 2.13333333 |
|            |             | length of tarsomears hind leg             | 2.6                    | 2.9          | 2.8          | 2.76666667 |
|            |             | length of basitarsus fore leg             | 2.6                    | 2.4          | 2.4          | 2.46666667 |
|            |             | length of tarsomears fore leg             | 2.5                    | 2.9          | 2.9          | 2.76666667 |

| individual    | body region | character                                 | measurment 1 (mm)       | measurment 2 | measurment 3 | average     |
|---------------|-------------|-------------------------------------------|-------------------------|--------------|--------------|-------------|
| female BJ-294 | general:    | whole insect                              | tubercels over entire l |              |              |             |
|               |             | colouration                               | uniform dowl brown, f   |              |              |             |
|               |             | Body length                               | 129.9                   | 129.9        | 129.7        | 129.833333  |
|               | head:       | head length                               | 4.6                     | 4.3          | 4.2          | 4.36666667  |
|               |             | head width                                | 3.7                     | 3.7          | 3.7          | 3.7         |
|               |             | antennae length                           | 60.9                    | 59.7         | 61.2         | 60.6        |
|               |             | femur length                              | 29.9                    | 29.6         | 29.7         | 29.7333333  |
|               |             | antennae segment no.                      |                         |              |              | 71          |
|               | Thorax:     | pronotum length                           | 4.1                     | 3.9          | 3.8          | 3.93333333  |
|               |             | mesonotum length                          | 30.1                    | 30.1         | 30.2         | 30.1333333  |
|               |             | metanotum length                          | 18                      | 18.14        | 18.04        | 18.06       |
|               | Abdomen:    | median segment                            | 4.7                     | 4.46         | 4.56         | 4.57333333  |
|               |             | abdominal segment 2 length                | 10.1                    | 10.2         | 10.3         | 10.2        |
|               |             | abdominal segment 3 length                | 10                      | 9.7          | 9.9          | 9.86666667  |
|               |             | abdominal segment 4 length                | 10.4                    | 10.5         | 10.4         | 10.4333333  |
|               |             | abdominal segment 5 length                | 10.8                    | 10.7         | 10.7         | 10.7333333  |
|               |             | abdominal segment 6 length                | 9.8                     | 9.8          | 9.7          | 9.76666667  |
|               |             | abdominal segment 7 length                | 8                       | 8            | 7.7          | 7.9         |
|               |             | abdominal segment 8 length                | 3.8                     | 3.5          | 3.7          | 3.66666667  |
|               |             | abdominal segment 9 length                | 2.2                     | 2.2          | 2.3          | 2.23333333  |
|               |             | abdominal segment 10 length               | 2.5                     | 2.5          | 2.5          | 2.5         |
|               |             | cerci                                     | N/A                     |              |              |             |
|               |             | supra anal plate length                   | N/A                     |              |              |             |
|               |             | operculum tip covering how much supra tip | N/A                     |              |              |             |
|               | Wings:      | wings                                     | apterus                 |              |              |             |
|               | Legs:       | leg spines                                | none                    |              |              |             |
|               |             | fore fenur                                | 29.4                    | 30.63        | 29.7         | 29.91       |
|               |             | mid femur                                 | 21.46                   | 21.39        | 21.34        | 21.39666667 |
|               |             | hind femur                                | 22.71                   | 23.06        | 23.32        | 23.03       |
|               |             | fore tibia                                | 31.9                    | 32.36        | 32.68        | 32.3133333  |
|               |             | mid tibia                                 | 21.4                    | 21.47        | 20.83        | 21.2333333  |
|               |             | hind tibia                                | 26.54                   | 27.22        | 27.13        | 26.9633333  |
|               |             | length of basitarsus hind leg             | 2.8                     | 2.6          | 2.5          | 2.63333333  |
|               |             | length of tarsomears hind leg             | 3.4                     | 3.8          | 3.7          | 3.63333333  |
|               |             | length of basitarsus fore leg             | 4.5                     | 4.5          | 4.8          | 4.6         |
|               |             | length of tarsomears fore leg             | 2.7                     | 2.1          | 2.5          | 2.43333333  |
| male BJ-244   | general:    | whole insect                              | small spines on head, f |              |              |             |
|               |             | colouration                               | dark brown              |              |              |             |
|               |             | Body length                               | 94.4                    | 94.1         | 94.3         | 94.2666667  |
|               | head:       | head length                               | 2.4                     | 2.4          | 2.4          | 2.4         |
|               |             | head width                                | 2.1                     | 2.1          | 2.1          | 2.1         |
|               |             | antennae length                           | 55.6                    | 56.3         | 56.5         | 56.1333333  |
|               |             | femur length                              | 23.7                    | 23.6         | 23.5         | 23.6        |
|               |             | antennae segment no.                      |                         |              |              | 76          |
|               | Thorax:     | pronotum length                           | 2.4                     | 2.4          | 2.7          | 2.5         |
|               |             | mesonotum length                          | 23                      | 23           | 22.9         | 22.9666667  |
|               |             | metanotum length                          | 13.07                   | 13.25        | 13.14        | 13.1533333  |
|               | Abdomen:    | median segment                            | 3.53                    | 3.46         | 3.56         | 3.51666667  |
|               |             | abdominal segment 2 length                | 7.8                     | 7.8          | 7.9          | 7.83333333  |
|               |             | abdominal segment 3 length                | 7.1                     | 7.1          | 6.8          | 7           |
|               |             | abdominal segment 4 length                | 6.6                     | 7.1          | 6.9          | 6.86666667  |
|               |             | abdominal segment 5 length                | 7.2                     | 7.2          | 7            | 7.13333333  |
|               |             | abdominal segment 6 length                | 6.5                     | 6.6          | 6.7          | 6.6         |
|               |             | abdominal segment 7 length                | 5.1                     | 5.1          | 5.2          | 5.13333333  |
|               |             | abdominal segment 8 length                | 2.2                     | 2.2          | 2.3          | 2.23333333  |
|               |             | abdominal segment 9 length                | 1.8                     | 1.9          | 1.7          | 1.8         |
|               |             | abdominal segment 10 length               | 3.6                     | 3.5          | 3.9          | 3.66666667  |
|               |             | cerci                                     | N/A                     |              |              |             |
|               |             | supra anal plate length                   | N/A                     |              |              |             |
|               |             | operculum tip covering how much supra tip | N/A                     |              |              |             |
|               | Wings:      | wings                                     | apterus                 |              |              |             |
|               | Legs:       | leg spines                                | none                    |              |              |             |
|               |             | fore fenur                                | 24.33                   | 24.05        | 23.8         | 24.06       |
|               |             | mid femur                                 | 17.36                   | 16.9         | 17           | 17.0866667  |
|               |             | hind femur                                | 20.62                   | 20.17        | 20.24        | 20.3433333  |
|               |             | fore tibia                                | 28.96                   | 29.98        | 29.5         | 29.48       |
|               |             | mid tibia                                 | 19.63                   | 19.85        | 20.29        | 19.9233333  |
|               |             | hind tibia                                | 27.31                   | 27.16        | 26.88        | 27.1166667  |
|               |             | length of basitarsus hind leg             | 2.8                     | 2.9          | 2.8          | 2.83333333  |
|               |             | length of tarsomears hind leg             | 3                       | 3            | 2.9          | 2.96666667  |
|               |             | length of basitarsus fore leg             | 3.9                     | 4            | 4            | 3.96666667  |
|               |             | length of tarsomears fore leg             | 3.3                     | 3.3          | 3.4          | 3.33333333  |

|               |          |                                           |                        |       |       |             |
|---------------|----------|-------------------------------------------|------------------------|-------|-------|-------------|
| female BJ-295 | general: | whole insect                              | small crown, black tip |       |       |             |
|               |          | colouration                               | uniform brown, maybe   |       |       |             |
|               |          | Body length                               | 107.6                  | 107.6 | 107.5 | 107.566667  |
|               | head:    | head length                               | 3.8                    | 3.8   | 3.9   | 3.83333333  |
|               |          | head width                                | 3.2                    | 3.2   | 3.2   | 3.2         |
|               |          | antennae length                           | 53.5                   | 53.4  | 52.7  | 53.2        |
|               |          | femur length                              | 23.8                   | 24.5  | 23.9  | 24.066667   |
|               |          | antennae segment no.                      |                        |       |       |             |
|               | Thorax:  | pronotum length                           | 3.8                    | 3.8   | 3.6   | 3.73333333  |
|               |          | mesonotum length                          | 24.2                   | 24.1  | 24.2  | 24.166667   |
|               |          | metanotum length                          | 13.45                  | 13.66 | 13.51 | 13.54       |
|               | Abdomen: | median segment                            | 4.45                   | 4.34  | 4.49  | 4.426667    |
|               |          | abdominal segment 2 length                | 9                      | 9     | 8.7   | 8.9         |
|               |          | abdominal segment 3 length                | 8.1                    | 8.2   | 8.2   | 8.166667    |
|               |          | abdominal segment 4 length                | 8.3                    | 8.3   | 8.2   | 8.266667    |
|               |          | abdominal segment 5 length                | 9.2                    | 9.1   | 8.8   | 9.03333333  |
|               |          | abdominal segment 6 length                | 8.4                    | 8.5   | 8.3   | 8.4         |
|               |          | abdominal segment 7 length                | 6.5                    | 6.6   | 6.7   | 6.6         |
|               |          | abdominal segment 8 length                | 2.8                    | 3     | 3.2   | 3           |
|               |          | abdominal segment 9 length                | 1.9                    | 1.7   | 1.8   | 1.8         |
|               |          | abdominal segment 10 length               | 1.9                    | 2     | 2.1   | 2           |
|               |          | cerci                                     | N/A                    |       |       |             |
|               |          | supra anal plate length                   | N/A                    |       |       |             |
|               |          | operculum tip covering how much supra tip | N/A                    |       |       |             |
|               | Wings:   | wings                                     | apterus                |       |       |             |
|               | Legs:    | leg spines                                | none                   |       |       |             |
|               |          | fore femur                                | 24.26                  | 25.14 | 24.78 | 24.726667   |
|               |          | mid femur                                 | 17.5                   | 17.83 | 18.18 | 17.836667   |
|               |          | hind femur                                | 19.36                  | 18.99 | 19.49 | 19.28       |
|               |          | fore tibia                                | 25.64                  | 25.69 | 25.26 | 25.53       |
|               |          | mid tibia                                 | 17.94                  | 17.86 | 17.54 | 17.78       |
|               |          | hind tibia                                | 24.11                  | 23.9  | 23.78 | 23.93       |
|               |          | length of basitarsus hind leg             | 2.4                    | 2.7   | 2.7   | 2.6         |
|               |          | length of tarsomeres hind leg             | 3.1                    | 3.2   | 3.3   | 3.2         |
|               |          | length of basitarsus fore leg             | 3.4                    | 3.3   | 3.4   | 3.366667    |
|               |          | length of tarsomeres fore leg             | 2.9                    | 2.8   | 2.8   | 2.83333333  |
| female BJ-298 | general: | whole insect                              | spine at end of abdom  |       |       |             |
|               |          | colouration                               | lichen form            |       |       |             |
|               |          | Body length                               | 85.1                   | 84.9  | 84.9  | 84.966667   |
|               | head:    | head length                               | 2.9                    | 2.7   | 3     | 2.866667    |
|               |          | head width                                | 2.5                    | 2.5   | 2.5   | 2.5         |
|               |          | antennae length                           | 37.6                   | 38.2  | 37.5  | 37.766667   |
|               |          | femur length                              | 17.4                   | 18.5  | 17.4  | 17.766667   |
|               |          | antennae segment no.                      |                        |       |       |             |
|               | Thorax:  | pronotum length                           | 2.4                    | 2.4   | 2.5   | 2.43333333  |
|               |          | mesonotum length                          | 18.2                   | 18.5  | 18.7  | 18.466667   |
|               |          | metanotum length                          | 14.86                  | 14.92 | 15.01 | 14.93       |
|               | Abdomen: | median segment                            | 3.34                   | 3.58  | 3.69  | 3.536667    |
|               |          | abdominal segment 2 length                | 7.1                    | 6.9   | 7.1   | 7.03333333  |
|               |          | abdominal segment 3 length                | 6.6                    | 6.6   | 6.5   | 6.566667    |
|               |          | abdominal segment 4 length                | 6.7                    | 6.7   | 6.7   | 6.7         |
|               |          | abdominal segment 5 length                | 7.1                    | 7.3   | 6.8   | 7.066667    |
|               |          | abdominal segment 6 length                | 6                      | 6.2   | 6.3   | 6.166667    |
|               |          | abdominal segment 7 length                | 5.1                    | 5.3   | 5.3   | 5.23333333  |
|               |          | abdominal segment 8 length                | 2.2                    | 2.2   | 2.2   | 2.2         |
|               |          | abdominal segment 9 length                | 1.3                    | 1.5   | 1.3   | 1.366667    |
|               |          | abdominal segment 10 length               | 1.9                    | 1.8   | 1.8   | 1.83333333  |
|               |          | cerci                                     | N/A                    |       |       |             |
|               |          | supra anal plate length                   | N/A                    |       |       |             |
|               |          | operculum tip covering how much supra tip | N/A                    |       |       |             |
|               | Wings:   | wings                                     | apterus                |       |       |             |
|               | Legs:    | leg spines                                | none                   |       |       |             |
|               |          | fore femur                                | 19.14                  | 18.98 | 19.63 | 19.25       |
|               |          | mid femur                                 | 13.34                  | 13.37 | 13.49 | 13.4        |
|               |          | hind femur                                | 15.28                  | 15.33 | 14.75 | 15.12       |
|               |          | fore tibia                                | 19.33                  | 18.76 | 18.99 | 19.026667   |
|               |          | mid tibia                                 | 14.21                  | 13.95 | 14.17 | 14.11       |
|               |          | hind tibia                                | 18.23                  | 18.12 | 17.99 | 18.11333333 |
|               |          | length of basitarsus hind leg             | 1.6                    | 1.7   | 1.6   | 1.63333333  |
|               |          | length of tarsomeres hind leg             | 2.7                    | 2.6   | 2.7   | 2.666667    |
|               |          | length of basitarsus fore leg             | 2.9                    | 2.8   | 3.1   | 2.93333333  |
|               |          | length of tarsomeres fore leg             | 2.1                    | 2.4   | 2.3   | 2.266667    |

|             |          |                                           |                         |       |       |             |
|-------------|----------|-------------------------------------------|-------------------------|-------|-------|-------------|
| male BJ-296 | general: | whole insect                              | medium spines, tarsi fl |       |       |             |
|             |          | colouration                               | brown over body exce    |       |       |             |
|             |          | Body length                               | 83.8                    | 83.7  | 83.5  | 83.6666667  |
|             | head:    | head length                               | 2.4                     | 2.5   | 2.4   | 2.43333333  |
|             |          | head width                                | 2.1                     | 2.1   | 2.1   | 2.1         |
|             |          | antennae length                           | 60.2                    | 60.5  | 60.3  | 60.33333333 |
|             |          | femur length                              | 23.6                    | 23.6  | 23.6  | 23.6        |
|             |          | antennae segment no.                      |                         |       |       |             |
|             | Thorax:  | pronotum length                           | 2.7                     | 2.7   | 3.2   | 2.86666667  |
|             |          | mesonotum length                          | 19.4                    | 19.4  | 19.3  | 19.3666667  |
|             |          | metanotum length                          | 11.58                   | 10.95 | 10.74 | 11.09       |
|             | Abdomen: | median segment                            | 3.12                    | 3.35  | 3.46  | 3.31        |
|             |          | abdominal segment 2 length                | 6.7                     | 6.7   | 6.9   | 6.76666667  |
|             |          | abdominal segment 3 length                | 6.1                     | 6.3   | 6.4   | 6.26666667  |
|             |          | abdominal segment 4 length                | 6.2                     | 6.3   | 6.1   | 6.2         |
|             |          | abdominal segment 5 length                | 6                       | 6.1   | 6.2   | 6.1         |
|             |          | abdominal segment 6 length                | 6.2                     | 6     | 6     | 6.06666667  |
|             |          | abdominal segment 7 length                | 4.9                     | 5     | 4.8   | 4.9         |
|             |          | abdominal segment 8 length                | 2.2                     | 2.4   | 2.4   | 2.33333333  |
|             |          | abdominal segment 9 length                | 1.6                     | 1.7   | 1.5   | 1.6         |
|             |          | abdominal segment 10 length               | 3.8                     | 3.6   | 3.4   | 3.6         |
|             |          | cerci                                     | N/A                     |       |       |             |
|             |          | supra anal plate length                   | N/A                     |       |       |             |
|             |          | operculum tip covering how much supra tip | N/A                     |       |       |             |
|             | Wings:   | wings                                     | apterus                 |       |       |             |
|             | Legs:    | leg spines                                | none                    |       |       |             |
|             |          | fore femur                                | 24.04                   | 23.94 | 23.97 | 23.98333333 |
|             |          | mid femur                                 | 17.53                   | 17.48 | 17.69 | 17.5666667  |
|             |          | hind femur                                | 19.82                   | 20.04 | 19.77 | 19.8766667  |
|             |          | fore tibia                                | 27.49                   | 27.52 | 27.25 | 27.42       |
|             |          | mid tibia                                 | 19.3                    | 19.58 | 19.71 | 19.53       |
|             |          | hind tibia                                | 25.1                    | 24.95 | 25.53 | 25.19333333 |
|             |          | length of basitarsus hind leg             | 2.2                     | 2.2   | 2.1   | 2.16666667  |
|             |          | length of tarsomeres hind leg             | 3.2                     | 3     | 2.9   | 3.03333333  |
|             |          | length of basitarsus fore leg             | 3.6                     | 3.6   | 3.5   | 3.56666667  |
|             |          | length of tarsomeres fore leg             | 2.7                     | 2.7   | 2.9   | 2.76666667  |
| male BJ-297 | general: | whole insect                              | small dorsal head spin  |       |       |             |
|             |          | colouration                               | brown over body exce    |       |       |             |
|             |          | Body length                               | 75.7                    | 75.8  | 75.4  | 75.63333333 |
|             | head:    | head length                               | 2.1                     | 2.1   | 2     | 2.06666667  |
|             |          | head width                                | 2.1                     | 2.1   | 2.1   | 2.1         |
|             |          | antennae length                           | 47.3                    | 46.7  | 48.4  | 47.4666667  |
|             |          | femur length                              | 20.5                    | 20.4  | 20.6  | 20.5        |
|             |          | antennae segment no.                      |                         |       |       |             |
|             | Thorax:  | pronotum length                           | 2.2                     | 2.2   | 2.1   | 2.16666667  |
|             |          | mesonotum length                          | 18.1                    | 18.1  | 18.1  | 18.1        |
|             |          | metanotum length                          | 15.43                   | 15.34 | 15.53 | 15.43333333 |
|             | Abdomen: | median segment                            | 2.67                    | 2.76  | 2.57  | 2.66666667  |
|             |          | abdominal segment 2 length                | 6.6..2                  |       | 6.2   | 6.1         |
|             |          | abdominal segment 3 length                | 5.7                     | 5.6   | 5.6   | 5.63333333  |
|             |          | abdominal segment 4 length                | 5.3                     | 5.5   | 5.2   | 5.33333333  |
|             |          | abdominal segment 5 length                | 5.5                     | 5.4   | 5.6   | 5.5         |
|             |          | abdominal segment 6 length                | 4.7                     | 5.1   | 5.1   | 4.96666667  |
|             |          | abdominal segment 7 length                | 3.9                     | 4.3   | 4.2   | 4.13333333  |
|             |          | abdominal segment 8 length                | 2                       | 1.8   | 1.8   | 1.86666667  |
|             |          | abdominal segment 9 length                | 1.1                     | 1.2   | 1.3   | 1.2         |
|             |          | abdominal segment 10 length               | 3.2                     | 3     | 3.3   | 3.16666667  |
|             |          | cerci                                     | N/A                     |       |       |             |
|             |          | supra anal plate length                   | N/A                     |       |       |             |
|             |          | operculum tip covering how much supra tip | N/A                     |       |       |             |
|             | Wings:   | wings                                     | apterus                 |       |       |             |
|             | Legs:    | leg spines                                | none                    |       |       |             |
|             |          | fore femur                                | 19.89                   | 24.7  | 20.13 | 21.57333333 |
|             |          | mid femur                                 | 14.21                   | 13.53 | 13.1  | 13.61333333 |
|             |          | hind femur                                | 15.92                   | 16.18 | 16.44 | 16.18       |
|             |          | fore tibia                                | 21.6                    | 21.64 | 21.59 | 21.61       |
|             |          | mid tibia                                 | 15.54                   | 15.71 | 14.97 | 15.4066667  |
|             |          | hind tibia                                | 21.98                   | 21.75 | 21.68 | 21.80333333 |
|             |          | length of basitarsus hind leg             | 2.2                     | 2.2   | 2     | 2.13333333  |
|             |          | length of tarsomeres hind leg             | 2.4                     | 2.4   | 2.2   | 2.33333333  |
|             |          | length of basitarsus fore leg             | 3                       | 3.2   | 3.3   | 3.16666667  |
|             |          | length of tarsomeres fore leg             | 2.5                     | 2.4   | 2.4   | 2.43333333  |

| individual     | body region | character                                 | measurment 1 (mm)        | measurment 2 | measurment 3 | average    |
|----------------|-------------|-------------------------------------------|--------------------------|--------------|--------------|------------|
| female JH-0055 | general:    | whole insect                              | black and white tipped   |              |              |            |
|                |             | colouration                               | light brown over body    |              |              |            |
|                |             | Body length                               | 109.6                    | 109.4        | 109.6        | 109.533333 |
|                | head:       | head length                               | 3.9                      | 3.5          | 4            | 3.8        |
|                |             | head width                                | 3.1                      | 3.1          | 3.1          | 3.1        |
|                |             | antennae length                           | 50.1                     | 50           | 49.5         | 49.8666667 |
|                |             | femur length                              | 22.7                     | 23.3         | 22.9         | 22.9666667 |
|                |             | antennae segment no.                      |                          |              |              | 58         |
|                | Thorax:     | pronotum length                           | 3.3                      | 3.1          | 3.3          | 3.23333333 |
|                |             | mesonotum length                          | 25                       | 24.9         | 24.9         | 24.9333333 |
|                |             | metanotum length                          | 15.07                    | 14.13        | 14.36        | 14.52      |
|                | Abdomen:    | median segment                            | 4.03                     | 4.87         | 4.64         | 4.51333333 |
|                |             | abdominal segment 2 length                | 9.3                      | 9.4          | 9.5          | 9.4        |
|                |             | abdominal segment 3 length                | 8.6                      | 8.5          | 8.6          | 8.56666667 |
|                |             | abdominal segment 4 length                | 8.7                      | 8.9          | 8.9          | 8.83333333 |
|                |             | abdominal segment 5 length                | 9                        | 9            | 8.9          | 8.96666667 |
|                |             | abdominal segment 6 length                | 7.8                      | 7.6          | 7.9          | 7.76666667 |
|                |             | abdominal segment 7 length                | 6.3                      | 6.4          | 6.3          | 6.33333333 |
|                |             | abdominal segment 8 length                | 3.3                      | 3.4          | 3.4          | 3.36666667 |
|                |             | abdominal segment 9 length                | 1.6                      | 1.8          | 1.7          | 1.7        |
|                |             | abdominal segment 10 length               | 2                        | 2.1          | 2.1          | 2.06666667 |
|                |             | cerci                                     | N/A                      |              |              |            |
|                |             | supra anal plate length                   | N/A                      |              |              |            |
|                |             | operculum tip covering how much supra tip | N/A                      |              |              |            |
|                | Wings:      | wings                                     | apterus                  |              |              |            |
|                | Legs:       | leg spines                                | none                     |              |              |            |
|                |             | fore fenur                                | 23.1                     | 23.52        | 23.55        | 23.39      |
|                |             | mid femur                                 | 17.06                    | 17.1         | 17.02        | 17.06      |
|                |             | hind femur                                | 19.06                    | 18.68        | 18.68        | 18.8066667 |
|                |             | fore tibia                                | 22.23                    | 22.53        | 22.39        | 22.3833333 |
|                |             | mid tibia                                 | 17.35                    | 17.15        | 17.3         | 17.2666667 |
|                |             | hind tibia                                | 22.88                    | 22.7         | 22.65        | 22.7433333 |
|                |             | length of basitarsus hind leg             | 2.2                      | 2.3          | 2.3          | 2.26666667 |
|                |             | length of tarsomears hind leg             | 3.2                      | 3.1          | 2.9          | 3.06666667 |
|                |             | length of basitarsus fore leg             | 3.3                      | 3.4          | 3.4          | 3.36666667 |
|                |             | length of tarsomears fore leg             | 2.7                      | 2.9          | 2.9          | 2.83333333 |
| male JH-0054   | general:    | whole insect                              | .plain, no fore tarsi or |              |              |            |
|                |             | colouration                               | uniform brown except     |              |              |            |
|                |             | Body length                               | 83.3                     | 83.4         | 83.5         | 83.4       |
|                | head:       | head length                               | 2.3                      | 2.2          | 2.2          | 2.23333333 |
|                |             | head width                                | 1.9                      | 1.9          | 1.9          | 1.9        |
|                |             | antennae length                           | 54.2                     | 53.6         | 53.4         | 53.7333333 |
|                |             | femur length                              | 20.5                     | 20.6         | 20.8         | 20.6333333 |
|                |             | antennae segment no.                      |                          |              |              | 69         |
|                | Thorax:     | pronotum length                           | 2.2                      | 2.3          | 2.3          | 2.26666667 |
|                |             | mesonotum length                          | 20.1                     | 20.3         | 20.3         | 20.2333333 |
|                |             | metanotum length                          | 11.41                    | 11.57        | 11.48        | 11.4866667 |
|                | Abdomen:    | median segment                            | 2.99                     | 3.03         | 3.02         | 3.01333333 |
|                |             | abdominal segment 2 length                | 7                        | 7            | 6.9          | 6.96666667 |
|                |             | abdominal segment 3 length                | 6.5                      | 6.5          | 6            | 6.33333333 |
|                |             | abdominal segment 4 length                | 6.2                      | 6.4          | 6.5          | 6.36666667 |
|                |             | abdominal segment 5 length                | 6.4                      | 6.4          | 6.4          | 6.4        |
|                |             | abdominal segment 6 length                | 5.7                      | 5.7          | 5.8          | 5.73333333 |
|                |             | abdominal segment 7 length                | 4.5                      | 4.6          | 4.5          | 4.53333333 |
|                |             | abdominal segment 8 length                | 2.3                      | 2.2          | 2            | 2.16666667 |
|                |             | abdominal segment 9 length                | 1.5                      | 1.5          | 1.6          | 1.53333333 |
|                |             | abdominal segment 10 length               | 3.1                      | 3            | 2.9          | 3          |
|                |             | cerci                                     | N/A                      |              |              |            |
|                |             | supra anal plate length                   | N/A                      |              |              |            |
|                |             | operculum tip covering how much supra tip | N/A                      |              |              |            |
|                | Wings:      | wings                                     | apterus                  |              |              |            |
|                | Legs:       | leg spines                                | none                     |              |              |            |
|                |             | fore fenur                                | 20.91                    | 20.75        | 21.22        | 20.96      |
|                |             | mid femur                                 | 16.26                    | 15.89        | 15.28        | 15.81      |
|                |             | hind femur                                | 18.77                    | 18.54        | 18.62        | 18.6433333 |
|                |             | fore tibia                                | 25.97                    | 26.05        | 25.92        | 25.98      |
|                |             | mid tibia                                 | 17.29                    | 16.8         | 16.82        | 16.97      |
|                |             | hind tibia                                | 23.24                    | 23.44        | 23.17        | 23.2833333 |
|                |             | length of basitarsus hind leg             | 2.2                      | 2.2          | 2.5          | 2.3        |
|                |             | length of tarsomears hind leg             | 2.9                      | 2.7          | 2.8          | 2.8        |
|                |             | length of basitarsus fore leg             | 3.3                      | 3.5          | 3.4          | 3.4        |
|                |             | length of tarsomears fore leg             | 2.3                      | 2.2          | 2.1          | 2.2        |

| individual | body region | character                                 | measurment 1 (mm)         | measurment 2 | measurment 3 | average     |
|------------|-------------|-------------------------------------------|---------------------------|--------------|--------------|-------------|
| T250919    | general:    | whole insect                              | fore leg and tarsi flange |              |              |             |
|            |             | colouration                               | mottled light and dark    |              |              |             |
|            |             | Body length                               | 112                       | 112          | 112          | 112         |
|            | head:       | head length                               | 3.8                       | 4            | 4.1          | 3.96666667  |
|            |             | head width                                | 3.3                       | 3.1          | 3            | 3.13333333  |
|            |             | antennae length                           | 43.8                      | 44.2         | 43.9         | 43.96666667 |
|            |             | femur length                              | 25.1                      | 24.9         | 24.3         | 24.76666667 |
|            |             | antennae segment no.                      |                           |              |              | 48          |
|            | Thorax:     | pronotum length                           | 4.1                       | 3.8          | 4            | 3.96666667  |
|            |             | mesonotum length                          | 25.4                      | 25.3         | 25.9         | 25.53333333 |
|            |             | metanotum length                          | 13.72                     | 13.75        | 14.37        | 13.94666667 |
|            | Abdomen:    | median segment                            | 4.88                      | 4.65         | 4.63         | 4.72        |
|            |             | abdominal segment 2 length                | 9.6                       | 10           | 10           | 9.86666667  |
|            |             | abdominal segment 3 length                | 9.4                       | 9.5          | 9.7          | 9.53333333  |
|            |             | abdominal segment 4 length                | 9.2                       | 9.3          | 9.3          | 9.26666667  |
|            |             | abdominal segment 5 length                | 9.5                       | 9.4          | 9.4          | 9.43333333  |
|            |             | abdominal segment 6 length                | 9                         | 9            | 8.9          | 8.96666667  |
|            |             | abdominal segment 7 length                | 7                         | 6.7          | 6.8          | 6.83333333  |
|            |             | abdominal segment 8 length                | 3.7                       | 3.5          | 3.8          | 3.66666667  |
|            |             | abdominal segment 9 length                | 1.9                       | 1.8          | 1.7          | 1.8         |
|            |             | abdominal segment 10 length               | 1.9                       | 1.9          | 1.8          | 1.86666667  |
|            |             | cerci                                     | N/A                       |              |              |             |
|            |             | supra anal plate length                   | N/A                       |              |              |             |
|            |             | operculum tip covering how much supra tip | N/A                       |              |              |             |
|            | Wings:      | wings                                     | apterus                   |              |              |             |
|            | Legs:       | leg spines                                | none                      |              |              |             |
|            |             | fore fenur                                | 24.01                     | 24.2         | 23.71        | 23.97333333 |
|            |             | mid femur                                 | 16.74                     | 16.49        | 16.61        | 16.61333333 |
|            |             | hind femur                                | 17.44                     | 17.67        | 17.65        | 17.58666667 |
|            |             | fore tibia                                | 22.51                     | 22.3         | 23.37        | 22.72666667 |
|            |             | mid tibia                                 | 16.66                     | 16.7         | 16.96        | 16.77333333 |
|            |             | hind tibia                                | 20.5                      | 20.23        | 20.27        | 20.33333333 |
|            |             | length of basitarsus hind leg             | 2.7                       | 2.7          | 2.8          | 2.73333333  |
|            |             | length of tarsomears hind leg             | 2.8                       | 3.2          | 3.5          | 3.16666667  |
|            |             | length of basitarsus fore leg             | 3.1                       | 3.1          | 3            | 3.06666667  |
|            |             | length of tarsomears fore leg             | 2.5                       | 2.6          | 2.6          | 2.56666667  |
| T250920    | general:    | whole insect                              | crown present on head     |              |              |             |
|            |             | colouration                               | body a uniform brown      |              |              |             |
|            |             | Body length                               | 92                        | 92           | 92           | 92          |
|            | head:       | head length                               | 2.4                       | 2.7          | 2.7          | 2.6         |
|            |             | head width                                | 2.2                       | 2.2          | 2.2          | 2.2         |
|            |             | antennae length                           | 61                        | 61.9         | 60           | 60.96666667 |
|            |             | femur length                              | 25.9                      | 25.5         | 26.1         | 25.83333333 |
|            |             | antennae segment no.                      |                           |              |              | 59          |
|            | Thorax:     | pronotum length                           | 2.8                       | 2.8          | 2.5          | 2.7         |
|            |             | mesonotum length                          | 23.4                      | 23.8         | 23.5         | 23.56666667 |
|            |             | metanotum length                          | 12.52                     | 12.56        | 12.29        | 12.45666667 |
|            | Abdomen:    | median segment                            | 3.58                      | 3.54         | 3.61         | 3.57666667  |
|            |             | abdominal segment 2 length                | 8.2                       | 8.4          | 8.2          | 8.26666667  |
|            |             | abdominal segment 3 length                | 7.7                       | 7.9          | 7.9          | 7.83333333  |
|            |             | abdominal segment 4 length                | 7.5                       | 7.4          | 7.3          | 7.4         |
|            |             | abdominal segment 5 length                | 7.2                       | 7.4          | 7.5          | 7.36666667  |
|            |             | abdominal segment 6 length                | 6.5                       | 6.2          | 6.2          | 6.3         |
|            |             | abdominal segment 7 length                | 5.2                       | 5            | 5            | 5.06666667  |
|            |             | abdominal segment 8 length                | 2.8                       | 3.1          | 3.2          | 3.03333333  |
|            |             | abdominal segment 9 length                | 1.9                       | 1.7          | 1.9          | 1.83333333  |
|            |             | abdominal segment 10 length               | 3.6                       | 3.5          | 3.9          | 3.66666667  |
|            |             | cerci                                     | N/A                       |              |              |             |
|            |             | supra anal plate length                   | N/A                       |              |              |             |
|            |             | operculum tip covering how much supra tip | N/A                       |              |              |             |
|            | Wings:      | wings                                     | apterus                   |              |              |             |
|            | Legs:       | leg spines                                | none                      |              |              |             |
|            |             | fore fenur                                | 25.69                     | 25.11        | 25.46        | 25.42       |
|            |             | mid femur                                 | 17.23                     | 16.69        | 16.93        | 16.95       |
|            |             | hind femur                                | 20.2                      | 20.1         | 20.24        | 20.18       |
|            |             | fore tibia                                | 29.92                     | 29.79        | 29.56        | 29.75666667 |
|            |             | mid tibia                                 | 20.45                     | 19.86        | 20.24        | 20.18333333 |
|            |             | hind tibia                                | 27.3                      | 27.17        | 26.75        | 27.07333333 |
|            |             | length of basitarsus hind leg             | 2.7                       | 2.5          | 2.6          | 2.6         |
|            |             | length of tarsomears hind leg             | 2.6                       | 2.3          | 2.3          | 2.4         |
|            |             | length of basitarsus fore leg             | 3.7                       | 4.2          | 3.8          | 3.9         |
|            |             | length of tarsomears fore leg             | 2.4                       | 2.3          | 2.1          | 2.26666667  |

| individual    | body region | character                                 | measurment 1 (mm)      | measurment 2 | measurment 3 | average     |
|---------------|-------------|-------------------------------------------|------------------------|--------------|--------------|-------------|
| female BJ-302 | general:    | whole insect                              | black and brown tube   |              |              |             |
|               |             | colouration                               | uniform brown, white   |              |              |             |
|               |             | Body length                               | 121.8                  | 120.6        | 120.3        | 120.9       |
|               | head:       | head length                               | 4.6                    | 4.6          | 4.5          | 4.56666667  |
|               |             | head width                                | 3.8                    | 3.9          | 4            | 3.9         |
|               |             | antennae length                           | 72.9                   | 72.7         | 73.5         | 73.03333333 |
|               |             | femur length                              | 31.1                   | 31.1         | 31.5         | 31.23333333 |
|               |             | antennae segment no.                      |                        |              |              | 104         |
|               | Thorax:     | pronotum length                           | 3.6                    | 3.8          | 3.7          | 3.7         |
|               |             | mesonotum length                          | 25.9                   | 26.3         | 26.4         | 26.2        |
|               |             | metanotum length                          | 15.35                  | 15.26        | 15.23        | 15.28       |
|               | Abdomen:    | median segment                            | 4.35                   | 4.24         | 4.27         | 4.28666667  |
|               |             | abdominal segment 2 length                | 9.2                    | 8.8          | 8.6          | 8.86666667  |
|               |             | abdominal segment 3 length                | 9.8                    | 9.9          | 10           | 9.9         |
|               |             | abdominal segment 4 length                | 10                     | 10.2         | 10.3         | 10.16666667 |
|               |             | abdominal segment 5 length                | 10.5                   | 10.5         | 10.2         | 10.4        |
|               |             | abdominal segment 6 length                | 10                     | 9.9          | 9.9          | 9.933333333 |
|               |             | abdominal segment 7 length                | 7.3                    | 7.4          | 7.4          | 7.36666667  |
|               |             | abdominal segment 8 length                | 3.6                    | 3.7          | 3.6          | 3.633333333 |
|               |             | abdominal segment 9 length                | 2.2                    | 2.2          | 2.3          | 2.233333333 |
|               |             | abdominal segment 10 length               | 2.2                    | 2.4          | 2.5          | 2.36666667  |
|               |             | cerci                                     | N/A                    |              |              |             |
|               |             | supra anal plate length                   | N/A                    |              |              |             |
|               |             | operculum tip covering how much supra tip | N/A                    |              |              |             |
|               | Wings:      | wings                                     | apterus                |              |              |             |
|               | Legs:       | leg spines                                | none                   |              |              |             |
|               |             | fore femur                                | 31.52                  | 31.69        | 31.83        | 31.68       |
|               |             | mid femur                                 | 22.81                  | 22.47        | 22.7         | 22.66       |
|               |             | hind femur                                | 25.34                  | 25.28        | 25.17        | 25.26333333 |
|               |             | fore tibia                                | 34.12                  | 33.92        | 33.67        | 33.90333333 |
|               |             | mid tibia                                 | 25.29                  | 25.45        | 25.41        | 25.38333333 |
|               |             | hind tibia                                | 31.2                   | 31.39        | 31.74        | 31.44333333 |
|               |             | length of basitarsus hind leg             | 3.7                    | 3.7          | 3.7          | 3.7         |
|               |             | length of tarsomeres hind leg             | 3.7                    | 3.6          | 3.5          | 3.6         |
|               |             | length of basitarsus fore leg             | 3.5                    | 3.6          | 3.6          | 3.56666667  |
|               |             | length of tarsomeres fore leg             | 2.8                    | 2.9          | 2.9          | 2.86666667  |
| male BJ-304   | general:    | whole insect                              | .plain medium spines c |              |              |             |
|               |             | colouration                               | brown body, green leg  |              |              |             |
|               |             | Body length                               | 102.8                  | 103.2        | 102.7        | 102.9       |
|               | head:       | head length                               | 3.3                    | 3.3          | 3.4          | 3.333333333 |
|               |             | head width                                | 2.5                    | 2.5          | 2.5          | 2.5         |
|               |             | antennae length                           | 86.7                   | 86.3         | 86           | 86.33333333 |
|               |             | femur length                              | 33.3                   | 33.4         | 33.5         | 33.4        |
|               |             | antennae segment no.                      |                        |              |              | 130         |
|               | Thorax:     | pronotum length                           | 3.1                    | 3            | 2.9          | 3           |
|               |             | mesonotum length                          | 25                     | 24.9         | 24.9         | 24.93333333 |
|               |             | metanotum length                          | 15.34                  | 15.25        | 15.2         | 15.26333333 |
|               | Abdomen:    | median segment                            | 3.26                   | 3.35         | 3.2          | 3.27        |
|               |             | abdominal segment 2 length                | 8.1                    | 8.1          | 8.2          | 8.133333333 |
|               |             | abdominal segment 3 length                | 7.7                    | 7.6          | 7.6          | 7.633333333 |
|               |             | abdominal segment 4 length                | 7.3                    | 7.5          | 7.6          | 7.46666667  |
|               |             | abdominal segment 5 length                | 7.3                    | 7.3          | 7.6          | 7.4         |
|               |             | abdominal segment 6 length                | 8.2                    | 8.2          | 8            | 8.133333333 |
|               |             | abdominal segment 7 length                | 5.6                    | 5.6          | 5.7          | 5.633333333 |
|               |             | abdominal segment 8 length                | 2.8                    | 2.5          | 2.9          | 2.733333333 |
|               |             | abdominal segment 9 length                | 1.7                    | 1.5          | 1.6          | 1.6         |
|               |             | abdominal segment 10 length               | 3.8                    | 4            | 3.7          | 3.833333333 |
|               |             | cerci                                     | N/A                    |              |              |             |
|               |             | supra anal plate length                   | N/A                    |              |              |             |
|               |             | operculum tip covering how much supra tip | N/A                    |              |              |             |
|               | Wings:      | wings                                     | apterus                |              |              |             |
|               | Legs:       | leg spines                                | none                   |              |              |             |
|               |             | fore femur                                | 34.34                  | 33.95        | 34           | 34.09666667 |
|               |             | mid femur                                 | 26.57                  | 26.68        | 26.51        | 26.58666667 |
|               |             | hind femur                                | 30.83                  | 30.61        | 30.68        | 30.70666667 |
|               |             | fore tibia                                | 40.93                  | 40.85        | 40.98        | 40.92       |
|               |             | mid tibia                                 | 32.73                  | 32.5         | 32.27        | 32.5        |
|               |             | hind tibia                                | 42.24                  | 42.71        | 42.68        | 42.54333333 |
|               |             | length of basitarsus hind leg             | 4                      | 4.1          | 4.1          | 4.06666667  |
|               |             | length of tarsomeres hind leg             | 3.3                    | 3.1          | 3.3          | 3.233333333 |
|               |             | length of basitarsus fore leg             | 4.9                    | 4.8          | 4.8          | 4.833333333 |
|               |             | length of tarsomeres fore leg             | 3.5                    | 3.5          | 3.4          | 3.46666667  |



|             |          |                                           |                        |       |       |            |
|-------------|----------|-------------------------------------------|------------------------|-------|-------|------------|
| male BJ-303 | general: | whole insect                              | .plain medium spines c |       |       |            |
|             |          | colouration                               | brown body, green leg  |       |       |            |
|             |          | Body length                               | 98                     | 97.9  | 97.6  | 97.8333333 |
|             | head:    | head length                               | 2.9                    | 2.9   | 2.8   | 2.86666667 |
|             |          | head width                                | 2.4                    | 2.3   | 2.3   | 2.33333333 |
|             |          | antennae length                           | 85.6                   | 84.8  | 85.6  | 85.3333333 |
|             |          | femur length                              | 30.1                   | 30.2  | 30    | 30.1       |
|             |          | antennae segment no.                      |                        |       |       |            |
|             | Thorax:  | pronotum length                           | 2.8                    | 2.8   | 2.9   | 2.83333333 |
|             |          | mesonotum length                          | 23.7                   | 23.7  | 23.7  | 23.7       |
|             |          | metanotum length                          | 14.93                  | 14.4  | 14.71 | 14.68      |
|             | Abdomen: | median segment                            | 2.77                   | 2.9   | 2.89  | 2.85333333 |
|             |          | abdominal segment 2 length                | 7.6                    | 7.5   | 7.4   | 7.5        |
|             |          | abdominal segment 3 length                | 7.1                    | 7.1   | 7.1   | 7.1        |
|             |          | abdominal segment 4 length                | 7.2                    | 7     | 7.1   | 7.1        |
|             |          | abdominal segment 5 length                | 7.4                    | 7.6   | 7.5   | 7.5        |
|             |          | abdominal segment 6 length                | 7                      | 7     | 6.9   | 6.96666667 |
|             |          | abdominal segment 7 length                | 5.3                    | 5.4   | 5.3   | 5.33333333 |
|             |          | abdominal segment 8 length                | 2.8                    | 2.9   | 2.7   | 2.8        |
|             |          | abdominal segment 9 length                | 1.9                    | 1.8   | 2     | 1.9        |
|             |          | abdominal segment 10 length               | 3.5                    | 3.6   | 3.6   | 3.56666667 |
|             |          | cerci                                     | N/A                    |       |       |            |
|             |          | supra anal plate length                   | N/A                    |       |       |            |
|             |          | operculum tip covering how much supra tip | N/A                    |       |       |            |
|             | Wings:   | wings                                     | apterus                |       |       |            |
|             | Legs:    | leg spines                                | none                   |       |       |            |
|             |          | fore fenur                                | 30.83                  | 30.84 | 30.62 | 30.7633333 |
|             |          | mid femur                                 | 23.87                  | 23.91 | 23.38 | 23.72      |
|             |          | hind femur                                | 26.92                  | 27.14 | 27.26 | 27.1066667 |
|             |          | fore tibia                                | 38.28                  | 38.02 | 38.72 | 38.34      |
|             |          | mid tibia                                 | 30.08                  | 30.45 | 29.94 | 30.1566667 |
|             |          | hind tibia                                | 38.6                   | 38.45 | 38.5  | 38.5166667 |
|             |          | length of basitarsus hind leg             | 3.6                    | 3.8   | 4     | 3.8        |
|             |          | length of tarsomears hind leg             | 3                      | 3     | 2.9   | 2.96666667 |
|             |          | length of basitarsus fore leg             | 5                      | 5.1   | 4.9   | 5          |
|             |          | length of tarsomears fore leg             | 3.3                    | 3.2   | 3.3   | 3.26666667 |

| individual    | body region | character                                 | measurment 1 (mm)        | measurment 2 | measurment 3 | average    |
|---------------|-------------|-------------------------------------------|--------------------------|--------------|--------------|------------|
| female BJ-300 | general:    | whole insect                              | large dorsal head spin   |              |              |            |
|               |             | colouration                               | uniform brown, femur:    |              |              |            |
|               |             | Body length                               | 137                      | 137.1        | 136.5        | 136.866667 |
|               | head:       | head length                               | 4.5                      | 4.5          | 4.5          | 4.5        |
|               |             | head width                                | 3.8                      | 3.8          | 3.8          | 3.8        |
|               |             | antennae length                           | 81.8                     | 80.8         | 80.5         | 81.0333333 |
|               |             | femur length                              | 35.5                     | 35.9         | 35.4         | 35.6       |
|               |             | antennae segment no.                      |                          |              |              | 103        |
|               | Thorax:     | pronotum length                           | 3.9                      | 4.2          | 4.2          | 4.1        |
|               |             | mesonotum length                          | 30.6                     | 30.5         | 30.8         | 30.6333333 |
|               |             | metanotum length                          | 18.07                    | 17.93        | 18.44        | 18.1466667 |
|               | Abdomen:    | median segment                            | 4.63                     | 4.77         | 4.26         | 4.55333333 |
|               |             | abdominal segment 2 length                | 10.8                     | 10.5         | 10.6         | 10.6333333 |
|               |             | abdominal segment 3 length                | 10.3                     | 10.5         | 10.2         | 10.3333333 |
|               |             | abdominal segment 4 length                | 11.2                     | 11.3         | 11.1         | 11.2       |
|               |             | abdominal segment 5 length                | 11.3                     | 11.3         | 11.4         | 11.3333333 |
|               |             | abdominal segment 6 length                | 11                       | 11.1         | 11.1         | 11.0666667 |
|               |             | abdominal segment 7 length                | 8.5                      | 8.4          | 8.5          | 8.4666667  |
|               |             | abdominal segment 8 length                | 4.6                      | 4.5          | 4.5          | 4.53333333 |
|               |             | abdominal segment 9 length                | 2.2                      | 2.2          | 2.3          | 2.23333333 |
|               |             | abdominal segment 10 length               | 2.8                      | 2.5          | 2.5          | 2.6        |
|               |             | cerci                                     | N/A                      |              |              |            |
|               |             | supra anal plate length                   | N/A                      |              |              |            |
|               |             | operculum tip covering how much supra tip | N/A                      |              |              |            |
|               | Wings:      | wings                                     | apterus                  |              |              |            |
|               | Legs:       | leg spines                                | none                     |              |              |            |
|               |             | fore fenur                                | 35.95                    | 36.01        | 35.99        | 35.9833333 |
|               |             | mid femur                                 | 25.84                    | 26.1         | 26.18        | 26.04      |
|               |             | hind femur                                | 29.97                    | 30.19        | 29.73        | 29.9633333 |
|               |             | fore tibia                                | 40.02                    | 40.19        | 40.11        | 40.1066667 |
|               |             | mid tibia                                 | 29.87                    | 29.73        | 29.57        | 29.7233333 |
|               |             | hind tibia                                | 36.62                    | 36.94        | 36.8         | 36.7866667 |
|               |             | length of basitarsus hind leg             | 3.8                      | 3.8          | 3.7          | 3.7666667  |
|               |             | length of tarsomears hind leg             | 3.8                      | 3.6          | 3.7          | 3.7        |
|               |             | length of basitarsus fore leg             | 4.8                      | 4.7          | 4.7          | 4.73333333 |
|               |             | length of tarsomears fore leg             | 3.7                      | 3.4          | 3.2          | 3.43333333 |
| male BJ-301   | general:    | whole insect                              | .plain, small spines loc |              |              |            |
|               |             | colouration                               | body brown legs green    |              |              |            |
|               |             | Body length                               | 110.5                    | 110.7        | 110.3        | 110.5      |
|               | head:       | head length                               | 3                        | 2.9          | 3.2          | 3.03333333 |
|               |             | head width                                | 2.6                      | 2.6          | 2.3          | 2.5        |
|               |             | antennae length                           | 85.5                     | 84.1         | 85.3         | 84.9666667 |
|               |             | femur length                              | 32.3                     | 32.5         | 32.6         | 32.4666667 |
|               |             | antennae segment no.                      |                          |              |              | 111        |
|               | Thorax:     | pronotum length                           | 3.4                      | 3.2          | 3.4          | 3.33333333 |
|               |             | mesonotum length                          | 27                       | 27           | 27.1         | 27.0333333 |
|               |             | metanotum length                          | 16.35                    | 16.4         | 16.52        | 16.4233333 |
|               | Abdomen:    | median segment                            | 3.35                     | 3.3          | 3.18         | 3.2766667  |
|               |             | abdominal segment 2 length                | 8.4                      | 8.7          | 8.6          | 8.5666667  |
|               |             | abdominal segment 3 length                | 8                        | 8            | 7.8          | 7.93333333 |
|               |             | abdominal segment 4 length                | 7.9                      | 7.7          | 7.8          | 7.8        |
|               |             | abdominal segment 5 length                | 8.2                      | 8.3          | 8.4          | 8.3        |
|               |             | abdominal segment 6 length                | 7.9                      | 7.7          | 7.8          | 7.8        |
|               |             | abdominal segment 7 length                | 6.3                      | 6.3          | 6.4          | 6.33333333 |
|               |             | abdominal segment 8 length                | 3.2                      | 3.3          | 3            | 3.1666667  |
|               |             | abdominal segment 9 length                | 1.9                      | 2            | 2            | 1.9666667  |
|               |             | abdominal segment 10 length               | 4.5                      | 4.3          | 4.3          | 4.3666667  |
|               |             | cerci                                     | N/A                      |              |              |            |
|               |             | supra anal plate length                   | N/A                      |              |              |            |
|               |             | operculum tip covering how much supra tip | N/A                      |              |              |            |
|               | Wings:      | wings                                     | apterus                  |              |              |            |
|               | Legs:       | leg spines                                | none                     |              |              |            |
|               |             | fore fenur                                | 32.27                    | 32.86        | 32.65        | 32.5933333 |
|               |             | mid femur                                 | 24.81                    | 24.7         | 24.8         | 24.77      |
|               |             | hind femur                                | 27.83                    | 28.41        | 28.03        | 28.09      |
|               |             | fore tibia                                | 27.54                    | 28.1         | 28.38        | 28.0066667 |
|               |             | mid tibia                                 | 29.15                    | 29.68        | 29.52        | 29.45      |
|               |             | hind tibia                                | 37.07                    | 37.01        | 36.9         | 36.9933333 |
|               |             | length of basitarsus hind leg             | 3.7                      | 3.5          | 3.5          | 3.5666667  |
|               |             | length of tarsomears hind leg             | 3.4                      | 3.2          | 3.4          | 3.33333333 |
|               |             | length of basitarsus fore leg             | 4.5                      | 4.7          | 4.7          | 4.63333333 |
|               |             | length of tarsomears fore leg             | 3.4                      | 3.5          | 3.3          | 3.4        |

|               |          |                                           |                        |       |       |            |
|---------------|----------|-------------------------------------------|------------------------|-------|-------|------------|
| female BJ-299 | general: | whole insect                              | black tubercels, large |       |       |            |
|               |          | colouration                               | uniform brown, mottle  |       |       |            |
|               |          | Body length                               | 134.7                  | 135.5 | 134.5 | 134.9      |
|               | head:    | head length                               | 4.3                    | 4.5   | 4.5   | 4.43333333 |
|               |          | head width                                | 3.7                    | 3.7   | 3.7   | 3.7        |
|               |          | antennae length                           | 77                     | 76.6  | 77    | 76.8666667 |
|               |          | femur length                              | 33.9                   | 33.9  | 33.9  | 33.9       |
|               |          | antennae segment no.                      |                        |       |       |            |
|               | Thorax:  | pronotum length                           | 4.4                    | 4.5   | 4.6   | 4.5        |
|               |          | mesonotum length                          | 31.2                   | 31.2  | 31.2  | 31.2       |
|               |          | metanotum length                          | 18.02                  | 17.93 | 18.32 | 18.09      |
|               | Abdomen: | median segment                            | 4.48                   | 4.57  | 4.08  | 4.37666667 |
|               |          | abdominal segment 2 length                | 9.7                    | 9.7   | 10    | 9.8        |
|               |          | abdominal segment 3 length                | 9.8                    | 10.2  | 10    | 10         |
|               |          | abdominal segment 4 length                | 11.1                   | 11.1  | 11.1  | 11.1       |
|               |          | abdominal segment 5 length                | 11.1                   | 11    | 11.2  | 11.1       |
|               |          | abdominal segment 6 length                | 11                     | 10.8  | 11    | 10.9333333 |
|               |          | abdominal segment 7 length                | 7.9                    | 8     | 8.2   | 8.03333333 |
|               |          | abdominal segment 8 length                | 4.2                    | 4.2   | 4.2   | 4.2        |
|               |          | abdominal segment 9 length                | 2.4                    | 2.2   | 2.2   | 2.26666667 |
|               |          | abdominal segment 10 length               | 2.6                    | 2.7   | 2.7   | 2.66666667 |
|               |          | cerci                                     | N/A                    |       |       |            |
|               |          | supra anal plate length                   | N/A                    |       |       |            |
|               |          | operculum tip covering how much supra tip | N/A                    |       |       |            |
|               | Wings:   | wings                                     | apterus                |       |       |            |
|               | Legs:    | leg spines                                | none                   |       |       |            |
|               |          | fore fenur                                | 33.34                  | 33.91 | 33.49 | 33.58      |
|               |          | mid femur                                 | 24.15                  | 24.25 | 23.82 | 24.0733333 |
|               |          | hind femur                                | 27.85                  | 27.75 | 27.8  | 27.8       |
|               |          | fore tibia                                | 35.88                  | 35.77 | 35.85 | 35.8333333 |
|               |          | mid tibia                                 | 27.48                  | 27.69 | 27.36 | 27.51      |
|               |          | hind tibia                                | 35.37                  | 35.84 | 35.74 | 35.65      |
|               |          | length of basitarsus hind leg             | 3.5                    | 3.5   | 3.6   | 3.53333333 |
|               |          | length of tarsomears hind leg             | 3.9                    | 3.8   | 3.9   | 3.86666667 |
|               |          | length of basitarsus fore leg             | 4.5                    | 4.5   | 4.2   | 4.4        |
|               |          | length of tarsomears fore leg             | 3.8                    | 4.1   | 4.1   | 4          |

| individual   | body region | character                                 | measurment 1 (mm)     | measurment 2 | measurment 3 | average   |
|--------------|-------------|-------------------------------------------|-----------------------|--------------|--------------|-----------|
| male PB-0014 | general:    | whole insect                              | large crown on dorsal |              |              |           |
|              |             | colouration                               | uniform brown, mott   |              |              |           |
|              |             | Body length                               | 110.2                 | 110.5        | 111.5        | 110.73333 |
|              | head:       | head length                               | 2.7                   | 2.9          | 2.7          | 2.7666667 |
|              |             | head width                                | 2.7                   | 2.7          | 2.9          | 2.7666667 |
|              |             | antennae length                           | 75.6                  | 75.3         | 74.9         | 75.266667 |
|              |             | femur length                              | 28.1                  | 28.1         | 28.5         | 28.233333 |
|              |             | antennae segment no.                      | 68                    |              |              | 68        |
|              | Thorax:     | pronotum length                           | 3.3                   | 3.5          | 3.3          | 3.3666667 |
|              |             | mesonotum length                          | 27.5                  | 27.5         | 27.4         | 27.466667 |
|              |             | metanotum length                          | 15.22                 | 15.22        | 15.22        | 15.22     |
|              | Abdomen:    | median segment                            | 3.81                  | 3.68         | 3.88         | 3.79      |
|              |             | abdominal segment 2 length                | 9.9                   | 9.9          | 10.2         | 10        |
|              |             | abdominal segment 3 length                | 8.5                   | 8.7          | 8.9          | 8.7       |
|              |             | abdominal segment 4 length                | 7.9                   | 7.9          | 8.5          | 8.1       |
|              |             | abdominal segment 5 length                | 8.6                   | 8.4          | 8.6          | 8.5333333 |
|              |             | abdominal segment 6 length                | 7.6                   | 7.7          | 7.8          | 7.7       |
|              |             | abdominal segment 7 length                | 5.9                   | 5.9          | 6            | 5.9333333 |
|              |             | abdominal segment 8 length                | 3.2                   | 3.2          | 3.1          | 3.1666667 |
|              |             | abdominal segment 9 length                | 2.4                   | 2.3          | 2.3          | 2.3333333 |
|              |             | abdominal segment 10 length               | 3.8                   | 3.7          | 3.9          | 3.8       |
|              |             | cerci                                     | N/A                   |              |              |           |
|              |             | supra anal plate length                   | N/A                   |              |              |           |
|              |             | operculum tip covering how much supra tip | N/A                   |              |              |           |
|              | Wings:      | wings                                     | apterus               |              |              |           |
|              | Legs:       | leg spines                                | none                  |              |              |           |
|              |             | fore femur                                | 31.41                 | 31.13        | 31.87        | 31.47     |
|              |             | mid femur                                 | 20.87                 | 21.3         | 22.19        | 21.453333 |
|              |             | hind femur                                | 24.13                 | 23.98        | 23.85        | 23.986667 |
|              |             | fore tibia                                | 39.2                  | 39.02        | 38.72        | 38.98     |
|              |             | mid tibia                                 | 26.2                  | 26.13        | 26.45        | 26.26     |
|              |             | hind tibia                                | 33.16                 | 33.17        | 33.18        | 33.17     |
|              |             | length of basitarsus hind leg             | 2.7                   | 2.7          | 2.6          | 2.6666667 |
|              |             | length of tarsomeres hind leg             | 3.1                   | 3.3          | 3.2          | 3.2       |
|              |             | length of basitarsus fore leg             | 4.5                   | 4.4          | 4.6          | 4.5       |
|              |             | length of tarsomeres fore leg             | 2.8                   | 2.6          | 2.7          | 2.7       |

**Table S2.2. Metadata for all specimens used in paper.**

| Family      | Subfamily   | Tribe      | Genus                  | Species               | Type classification | DNA Number Code | Specimen deposition | Registration Number | Genbank Accession Number (COI) | Accession Number (16S) | Location and date of collection                               | Collector                         | Sex          |
|-------------|-------------|------------|------------------------|-----------------------|---------------------|-----------------|---------------------|---------------------|--------------------------------|------------------------|---------------------------------------------------------------|-----------------------------------|--------------|
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>mercurius</i>      | Paratype            | PB-0079         | QM                  | T250900             | KJ202027.1                     | OM1992352              | Byfield, QLD, Australia, 19Nov2011                            | Paul Brock, Noelene Tweed         | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>mercurius</i>      | Paratype            | PB-0078         | QM                  | T250901             | KJ202015.1                     | not sampled            | Byfield, QLD, Australia, 19Nov2011                            | Paul Brock, Noelene Tweed         | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>mercurius</i>      | missing specimen    | PB-0080         | NHMLUK              | NA                  | KJ202026.1                     | not sampled            | not examined                                                  | Paul Brock, Noelene Tweed         | not examined |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>mercurius</i>      | missing specimen    | PB-0081         | NHMLUK              | NA                  | KJ201995.1                     | OM1992353              | not examined                                                  | Paul Brock, Noelene Tweed         | not examined |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>waibeni</i>        | Paratype            | BI-322          | QM                  | T250902             | ON102002                       | OM1992351              | Thursday Island, QLD, Australia, 15sep2021                    | Dominic Funnell                   | female nymph |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>waibeni</i>        | Paratype            | BI-323          | QM                  | T250903             | not sampled                    | not sampled            | Thursday Island, QLD, Australia, 15sep2021                    | Dominic Funnell                   | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>waibeni</i>        | Holotype            | BI-324          | QM                  | T250904             | not sampled                    | not sampled            | Thursday Island, QLD, Australia, 15sep2021                    | Dominic Funnell                   | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>waibeni</i>        | Paratype            | BI-326          | QM                  | T250905             | not sampled                    | not sampled            | Thursday Island, QLD, Australia, 15sep2021                    | Dominic Funnell                   | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>waibeni</i>        | Paratype            | BI-327          | QM                  | T250906             | not sampled                    | not sampled            | Thursday Island, QLD, Australia, 15sep2021                    | Dominic Funnell                   | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>truncatus</i>      | Holotype            | NA              | QM                  | T250907             | not sampled                    | not sampled            | Gordon's Mine Area, Iron Range, N. Qld, 12-19Feb1976          | G. B. Monteith                    | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>tuberosus</i>      | Holotype            | NA              | QM                  | T250908             | not sampled                    | not sampled            | Mt Hemmant, North East QLD, Australia, 25-27Nov1993           | Monteith, Cook, Janetzki, Roberts | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | BI-244          | QM                  | T250909             | ON101993                       | OM1992336              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | BI-293          | QM                  | T250910             | ON077344                       | OM1992339              | Cape Tribulation road, Cape Tribulation, QLD, 16.0883333333   | Braxton Jones                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Holotype            | BI-294          | QM                  | T250911             | ON101995                       | OM1992340              | Cape Tribulation Rd, Thornton Beach, QLD, 16.173888895        | Braxton Jones                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | BI-295          | QM                  | T250912             | ON077345                       | OM1992341              | Cape Tribulation Rd, Cape Tribulation, QLD, 16.090555565      | Braxton Jones                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | BI-296          | QM                  | T250913             | ON101996                       | OM1992342              | Cape Tribulation Rd, Cape Tribulation, QLD, 16.090555565      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | BI-297          | QM                  | T250914             | ON077346                       | OM1992343              | Cape Tribulation Rd, Cape Tribulation, QLD, 16.090555565      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | BI-298          | QM                  | T250915             | ON077347                       | OM1992344              | Cape Tribulation Rd, Cape Tribulation, QLD, 16.090555565      | Braxton Jones                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>deconus</i>        | Paratype            | NA              | QM                  | T250916             | not sampled                    | not sampled            | Cape Tribulation Rd, Cape Tribulation, QLD, 16.090555565      | Braxton Jones                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>coronatus</i>      | Holotype            | JH-0055         | QM                  | T250917             | HM425711.1                     | OM1992354              | Polly Creek, Garradunga, N.Qld, Australia, 27Nov2009          | J. Hasenpusch                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>coronatus</i>      | Paratype            | JH-0054         | QM                  | T250918             | HM425710.1                     | OM1992356              | Polly Creek, Garradunga, N.Qld, Australia, 27Nov2009          | J. Hasenpusch                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>coronatus</i>      | missing specimen    | PB-0028         | NHMLUK              | NA                  | HM425728.1                     | not sampled            | not examined                                                  | Paul Brock, Noelene Tweed         | not examined |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>coronatus</i>      | missing specimen    | PB-0026         | NHMLUK              | NA                  | HM425727.1                     | not sampled            | not examined                                                  | Paul Brock, Noelene Tweed         | not examined |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>coronatus</i>      | missing specimen    | PB-0025         | NHMLUK              | NA                  | HM425726.1                     | OM1992357              | not examined                                                  | Paul Brock, Noelene Tweed         | not examined |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>coronatus</i>      | missing specimen    | PB-0024         | NHMLUK              | NA                  | HM425725.1                     | OM1992355              | not examined                                                  | Paul Brock, Noelene Tweed         | not examined |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>gasterbulla</i>    | Holotype            | NA              | QM                  | T250919             | not sampled                    | not sampled            | Mt Macartney, Cathu State Forest, C.Qld                       | G.B. Monteith                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>gasterbulla</i>    | Paratype            | NA              | QM                  | T250920             | not sampled                    | not sampled            | Mt Macartney, Cathu State Forest, C.Qld                       | G.B. Monteith                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>macropunctatus</i> | Holotype            | BI-302          | QM                  | T250921             | ON102000                       | OM1992348              | Shute Harbour, QLD, Australia, 22Mar2020                      | Leg. J. Constant, L. Semeraro     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>macropunctatus</i> | Paratype            | BI-304          | QM                  | T250922             | ON102001                       | OM1992350              | Shute Harbour, QLD, Australia, 22Mar2020                      | Leg. J. Constant, L. Semeraro     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>macropunctatus</i> | Paratype            | BI-303          | QM                  | T250923             | ON077348                       | OM1992349              | Shute Harbour, QLD, Australia, 22Mar2020                      | Leg. J. Constant, L. Semeraro     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>elimosus</i>       | Holotype            | BI-300          | QM                  | T250924             | ON101998                       | OM1992346              | Whitsunday Island National Park, QLD, Australia, 21-22Mar2020 | Leg. J. Constant, L. Semeraro     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>elimosus</i>       | Paratype            | BI-301          | QM                  | T250925             | ON101999                       | OM1992347              | Whitsunday Island National Park, QLD, Australia, 21-22Mar2020 | Leg. J. Constant, L. Semeraro     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>elimosus</i>       | Paratype            | BI-299          | QM                  | T250926             | ON101997                       | OM1992345              | Whitsunday Island National Park, QLD, Australia, 21-22Mar2020 | Leg. J. Constant, L. Semeraro     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>walkeri</i>        | Holotype            | PB-0014         | QM                  | T250927             | HM425716.1                     | not sampled            | Mt Dryander, 21Nov2009                                        | Paul Brock                        | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>nigropunctatus</i> | NA                  | BI-240          | QM                  | T258567             | ON101990                       | OM1992332              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>nigropunctatus</i> | NA                  | BI-241          | QM                  | T258568             | not sampled                    | OM1992333              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>nigropunctatus</i> | NA                  | BI-242          | QM                  | T258569             | ON101991                       | OM1992334              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>nigropunctatus</i> | NA                  | BI-243          | QM                  | T258570             | ON101992                       | OM1992335              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | male         |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>nigropunctatus</i> | NA                  | BI-245          | QM                  | T258571             | ON077343                       | OM1992337              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | female       |
| Lonchodidae | Lonchodinae | Lonchodini | <i>Austrocarausius</i> | <i>nigropunctatus</i> | NA                  | BI-246          | QM                  | T258572             | ON101994                       | OM1992338              | 100, Rosewood Rd, Cow Bay, QLD, Australia, 16.2300000005      | Braxton Jones                     | female       |

**Table S2.3.** DNA divergence data for COI, where individual scores represent similarity to one another. The holotype for each species was used.

|                          | <i>A. mercurius</i> | <i>A. waiben</i> | <i>A. decorus</i> | <i>A. coronatus</i> | <i>A. macropunctatus</i> | <i>A. eirmosus</i> | <i>A. walkeri</i> | <i>A. nigropunctatus</i> |
|--------------------------|---------------------|------------------|-------------------|---------------------|--------------------------|--------------------|-------------------|--------------------------|
| <i>A. mercurius</i>      | NA                  | 83.40%           | 84.30%            | 87.10%              | 86.60%                   | 86.50%             | 87.70%            | 85.10%                   |
| <i>A. waiben</i>         | 83.40%              | NA               | 86%               | 87.10%              | 85.10%                   | 86.20%             | 86.30%            | 87.00%                   |
| <i>A. decorus</i>        | 84.30%              | 86%              | NA                | 90.20%              | 87.90%                   | 88.10%             | 87.50%            | 85.50%                   |
| <i>A. coronatus</i>      | 87.10%              | 87.10%           | 90.20%            | NA                  | 88.50%                   | 89.20%             | 88.00%            | 86.90%                   |
| <i>A. macropunctatus</i> | 86.60%              | 85.10%           | 87.90%            | 88.50%              | NA                       | 91.70%             | 89.20%            | 87.50%                   |
| <i>A. eirmosus</i>       | 86.50%              | 86.20%           | 88.10%            | 89.20%              | 91.70%                   | NA                 | 90.30%            | 85.70%                   |
| <i>A. walkeri</i>        | 87.70%              | 86.30%           | 87.50%            | 88.00%              | 89.20%                   | 90.30%             | NA                | 85.60%                   |
| <i>A. nigropunctatus</i> | 85.10%              | 87.00%           | 85.50%            | 86.90%              | 87.50%                   | 85.70%             | 85.60%            | NA                       |

**Table S2.4.** Accession numbers for outgroup taxa used in phylogenetic trees.

| Family            | Genus                | Species              | Gene        | Accession Number |
|-------------------|----------------------|----------------------|-------------|------------------|
| Agathemeridae     | <i>Agathemera</i>    | <i>mesoauriculae</i> | <i>16S</i>  | JN228857.1       |
| Agathemeridae     | <i>Agathemera</i>    | <i>mesoauriculae</i> | <i>COXI</i> | JN228874.1       |
| Aschiphasmataidae | <i>Abrosoma</i>      | <i>festinatum</i>    | <i>16S</i>  | MK291730.1       |
| Aschiphasmataidae | <i>Abrosoma</i>      | <i>festinatum</i>    | <i>COXI</i> | FJ474257.1       |
| Bacillidae        | <i>Bacillus</i>      | <i>rossius</i>       | <i>COXI</i> | MH324774.1       |
| Diapheromeridae   | <i>Libethra</i>      | <i>rabdota</i>       | <i>16S</i>  | KX857211.1       |
| Diapheromeridae   | <i>Libethra</i>      | <i>rabdota</i>       | <i>COXI</i> | KX857191.1       |
| Diapheromeridae   | <i>Oreophoetes</i>   | <i>peruana</i>       | <i>16S</i>  | KJ024428.1       |
| Diapheromeridae   | <i>Oreophoetes</i>   | <i>peruana</i>       | <i>COXI</i> | KT426643.1       |
| Diapheromeridae   | <i>Pseudosermyle</i> | <i>phalangiphora</i> | <i>16S</i>  | MK291744.1       |
| Diapheromeridae   | <i>Pseudosermyle</i> | <i>phalangiphora</i> | <i>COXI</i> | FJ474318.1       |
| Diapheromerinae   | <i>Pseudophasma</i>  | sp.                  | <i>16S</i>  | MK291722.1       |
| Heteronemiidae    | <i>Spinonemia</i>    | <i>chilensis</i>     | <i>16S</i>  | JN228840.1       |
| Heteronemiidae    | <i>Spinonemia</i>    | <i>chilensis</i>     | <i>COXI</i> | JN228858.1       |
| Heteropterygidae  | <i>Aretaon</i>       | <i>asperrimus</i>    | <i>16S</i>  | KJ024430.1       |
| Heteropterygidae  | <i>Aretaon</i>       | <i>asperrimus</i>    | <i>COXI</i> | KP300911.1       |
| Heteropterygidae  | <i>Heteropteryx</i>  | <i>dilatata</i>      | <i>16S</i>  | KJ024429.1       |
| Heteropterygidae  | <i>Heteropteryx</i>  | <i>dilatata</i>      | <i>COXI</i> | MK548353.1       |
| Lonchodidae       | <i>Carausius</i>     | <i>morosus</i>       | <i>16S</i>  | KJ024434.1       |
| Lonchodidae       | <i>Carausius</i>     | <i>morosus</i>       | <i>COXI</i> | FJ474268.1       |
| Lonchodidae       | <i>Eurycantha</i>    | <i>calcarata</i>     | <i>16S</i>  | MN449612.1       |
| Lonchodidae       | <i>Eurycantha</i>    | <i>calcarata</i>     | <i>COXI</i> | MN449923.1       |
| Lonchodidae       | <i>Neohirasea</i>    | <i>japonica</i>      | <i>COXI</i> | AB477469.1       |
| Lonchodidae       | <i>Sipyloidea</i>    | <i>sipylus</i>       | <i>16S</i>  | MN449601.1       |
| Lonchodidae       | <i>Sipyloidea</i>    | <i>sipylus</i>       | <i>COXI</i> | MN449958.1       |
| Phasmataidae      | <i>Achrioptera</i>   | <i>punctipes</i>     | <i>16S</i>  | MK291712.1       |
| Phasmataidae      | <i>Achrioptera</i>   | <i>punctipes</i>     | <i>COXI</i> | FJ474262.1       |
| Phasmataidae      | <i>Diapherodes</i>   | <i>gigantea</i>      | <i>16S</i>  | MN449572.1       |
| Phasmataidae      | <i>Diapherodes</i>   | <i>gigantea</i>      | <i>COXI</i> | MN449806.1       |
| Phylliidae        | <i>Phyllium</i>      | <i>tibetense</i>     | <i>COXI</i> | KY124332.1       |
| Prisopodidae      | <i>Prisopus</i>      | sp.                  | <i>16S</i>  | MK291749.1       |
| Pseudophasmatidae | <i>Anisomorpha</i>   | <i>buprestoides</i>  | <i>16S</i>  | JN228842.1       |
| Pseudophasmatidae | <i>Anisomorpha</i>   | <i>buprestoides</i>  | <i>COXI</i> | JN228860.1       |
| Pseudophasmatidae | <i>Creoxylus</i>     | <i>spinosus</i>      | <i>16S</i>  | MK291689.1       |
| Pseudophasmatidae | <i>Malacomorpha</i>  | <i>cyllarus</i>      | <i>16S</i>  | MK291677.1       |
| Pseudophasmatidae | <i>Metriophasma</i>  | <i>diocles</i>       | <i>16S</i>  | MK291683.1       |
| Pseudophasmatidae | <i>Peruphasma</i>    | <i>schultei</i>      | <i>COXI</i> | FJ474309.1       |

**Table S2.5.** Fossils used in time calibrated Bayesian inference tree (Supplementary Figure S2.1).

| Fossil                      | Life stage | Reference                  | Minimum age (Ma) | Soft upper bound (Ma) | Mean | Calibration node                 |
|-----------------------------|------------|----------------------------|------------------|-----------------------|------|----------------------------------|
| <i>Eophyllum messelense</i> | Adult      | Wedmann <i>et al.</i> 2007 | 47               | 110                   | 17   | Phylliinae (node 1)              |
| <i>Eophasma</i> sp.         | Egg        | Poinar 2011                | 44               | 110                   | 18   | Pseudophasmatinae (node 2)       |
| <i>Clonistria</i> sp.       | Egg        | Poinar 2011                | 20               | 110                   | 24.3 | Diapheromerinae (node 3)         |
| <i>Anisomorpha</i>          | Egg        | Sellick 1994               | 44               | 110                   | 18   | Pseudophasmatinae (node 4)       |
| <i>Malacomorpha</i>         | Egg        | Poinar 2011                | 20               | 110                   | 24.3 | Malacomorpha and sister (node 5) |

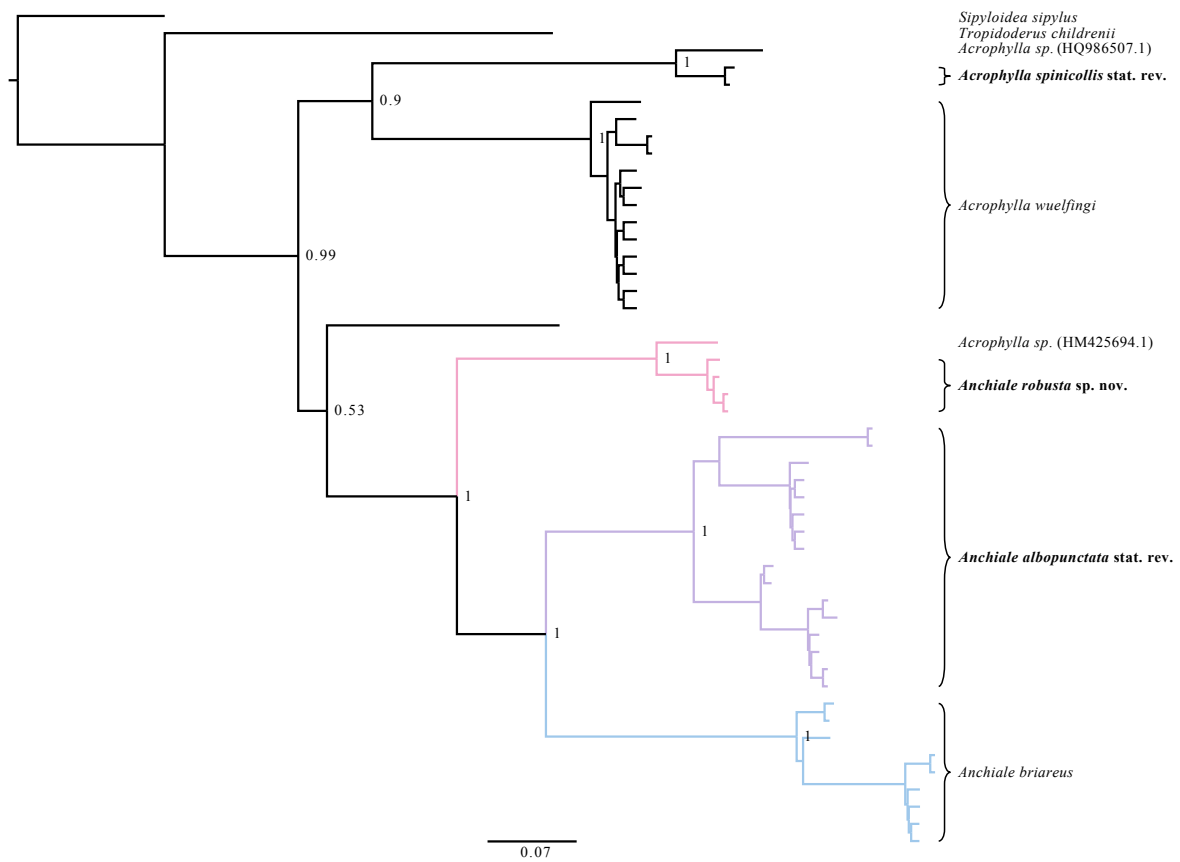
| Fossil node                        | 1                                 | 2                     | 3                           | 4                        | 5                     |
|------------------------------------|-----------------------------------|-----------------------|-----------------------------|--------------------------|-----------------------|
| <b>Included taxa (with sister)</b> | Achrioptera_punctipes             | Creoxylus_spinosus    | Libethra_rabdota            | Anisomorpha_buprestoides | Malacomorpha_cyllarus |
|                                    | Agathemera_mesoauriculae          | Malacomorpha_cyllarus | Oreophoetes_peruana         | Creoxylus_spinosus       | Pseudophasma_sp       |
|                                    | Anisomorpha_buprestoides          | Metriophasma_diocles  | Pseudosermyle_phalangiphora | Malacomorpha_cyllarus    |                       |
|                                    | Aretaon_asperrimus                | Peruphasma_schultei   |                             | Metriophasma_diocles     |                       |
|                                    | Austrocarausius_mercurius_JH_0054 | Pseudophasma_sp       |                             | Peruphasma_schultei      |                       |
|                                    | Austrocarausius_mercurius_JH_0055 |                       |                             | Prisopus_sp              |                       |
|                                    | Austrocarausius_mercurius_PB_0024 |                       |                             | Pseudophasma_sp          |                       |
|                                    | Austrocarausius_mercurius_PB_0025 |                       |                             | Spinonemia_chilensis     |                       |
|                                    | Austrocarausius_mercurius_PB_0026 |                       |                             |                          |                       |
|                                    | Austrocarausius_mercurius_PB_0028 |                       |                             |                          |                       |
|                                    | Austrocarausius_mercurius_PB_0078 |                       |                             |                          |                       |
|                                    | Austrocarausius_mercurius_PB_0079 |                       |                             |                          |                       |
|                                    | Austrocarausius_mercurius_PB_0080 |                       |                             |                          |                       |
|                                    | Austrocarausius_mercurius_PB_0081 |                       |                             |                          |                       |
|                                    | Austrocarausius_sp_PB_0014        |                       |                             |                          |                       |
|                                    | BJ_240                            |                       |                             |                          |                       |
|                                    | BJ_241                            |                       |                             |                          |                       |
|                                    | BJ_242                            |                       |                             |                          |                       |

| Fossil node | 1                           | 2 | 3 | 4 | 5 |
|-------------|-----------------------------|---|---|---|---|
|             | BJ_243                      |   |   |   |   |
|             | BJ_244                      |   |   |   |   |
|             | BJ_245                      |   |   |   |   |
|             | BJ_246                      |   |   |   |   |
|             | BJ_293                      |   |   |   |   |
|             | BJ_294                      |   |   |   |   |
|             | BJ_295                      |   |   |   |   |
|             | BJ_296                      |   |   |   |   |
|             | BJ_297                      |   |   |   |   |
|             | BJ_298                      |   |   |   |   |
|             | BJ_299                      |   |   |   |   |
|             | BJ_300                      |   |   |   |   |
|             | BJ_301                      |   |   |   |   |
|             | BJ_302                      |   |   |   |   |
|             | BJ_303                      |   |   |   |   |
|             | BJ_304                      |   |   |   |   |
|             | BJ_322                      |   |   |   |   |
|             | Bacillus_rossius            |   |   |   |   |
|             | Carausius_morosus           |   |   |   |   |
|             | Creoxylus_spinosus          |   |   |   |   |
|             | Diapherodes_gigantea        |   |   |   |   |
|             | Eurycantha_calcarata        |   |   |   |   |
|             | Heteropteryx_dilatata       |   |   |   |   |
|             | Libethra_rabdota            |   |   |   |   |
|             | Malacomorpha_cyllarus       |   |   |   |   |
|             | Metriophasma_diocles        |   |   |   |   |
|             | Neohirasea_japonica         |   |   |   |   |
|             | Oreophoetes_peruana         |   |   |   |   |
|             | Peruphasma_schultei         |   |   |   |   |
|             | Phyllium_tibetense          |   |   |   |   |
|             | Prisopus_sp                 |   |   |   |   |
|             | Pseudophasma_sp             |   |   |   |   |
|             | Pseudosermyle_phalangiphora |   |   |   |   |
|             | Sipyloidea_sipylus          |   |   |   |   |
|             | Spinonemia_chilensis        |   |   |   |   |

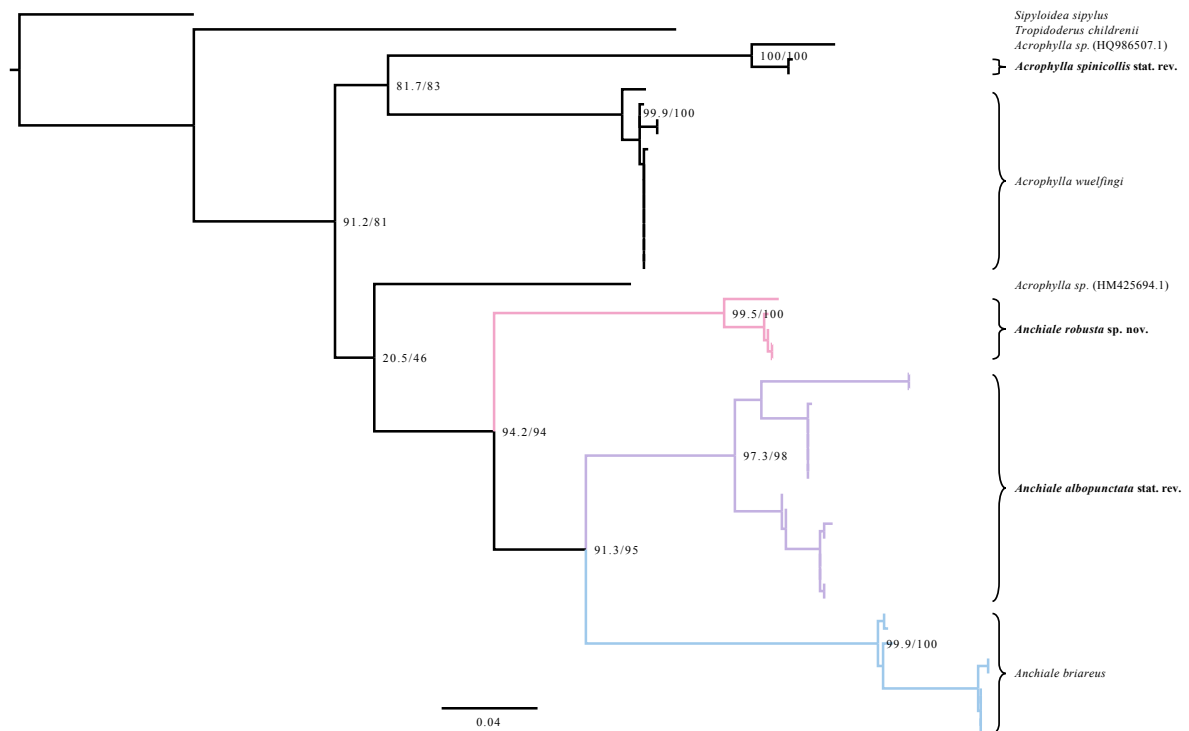
[illegible]

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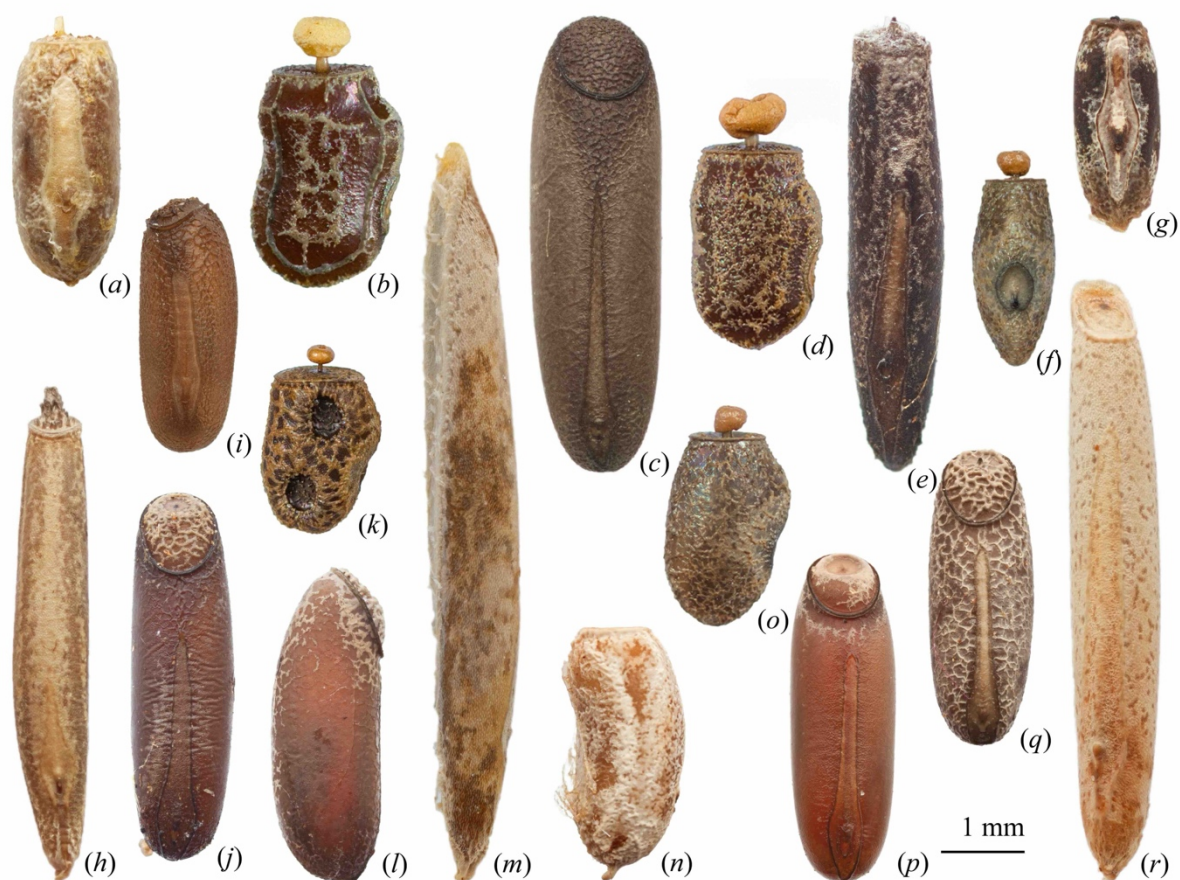


**Figure S3.1.** Bayesian inference tree for three Australian *Anchiale* species and outgroups inferred from mitochondrial *COX1*. Colours used on tree branches corresponds to species distributions used in Figure 3.2. Bold text indicates new and revised species. Node support values are shown as posterior probability. Scale bar represents substitutions per site.

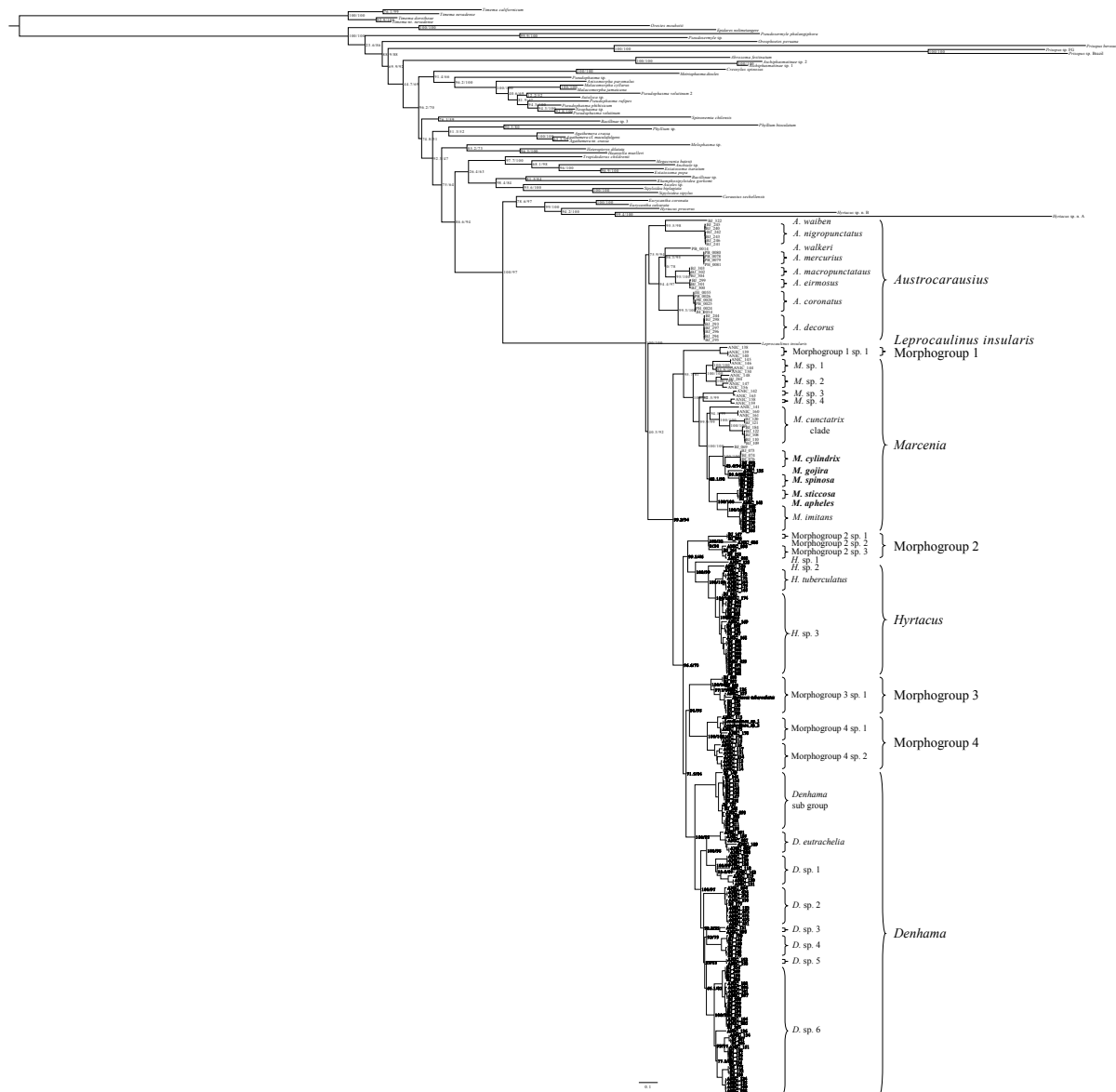


**Figure S3.2.** Maximum likelihood tree for three Australian *Anchiale* species and outgroups inferred from mitochondrial *COX1*. Colours used on tree branches corresponds to species distributions used in Figure 3.2. Bold text indicates new and revised species. Node support values are shown as ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents substitutions per site.

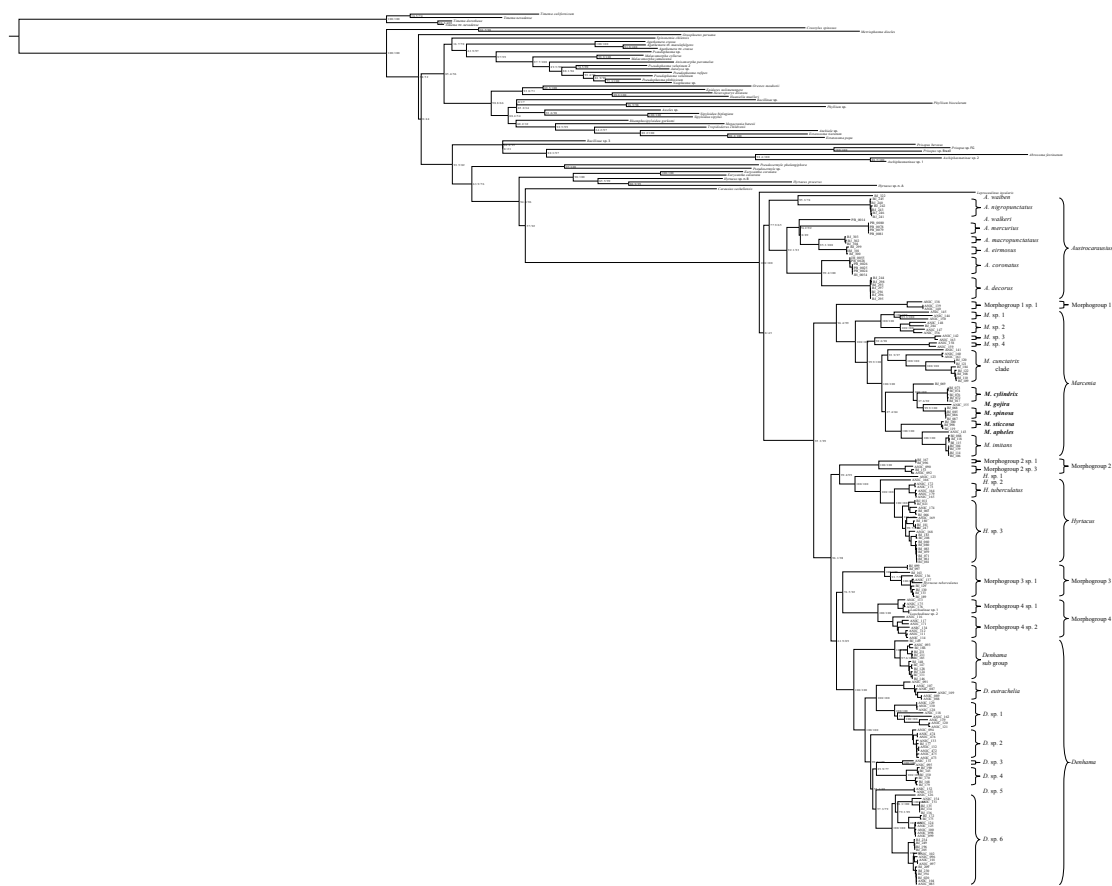
## Supplementary material for Chapter 4



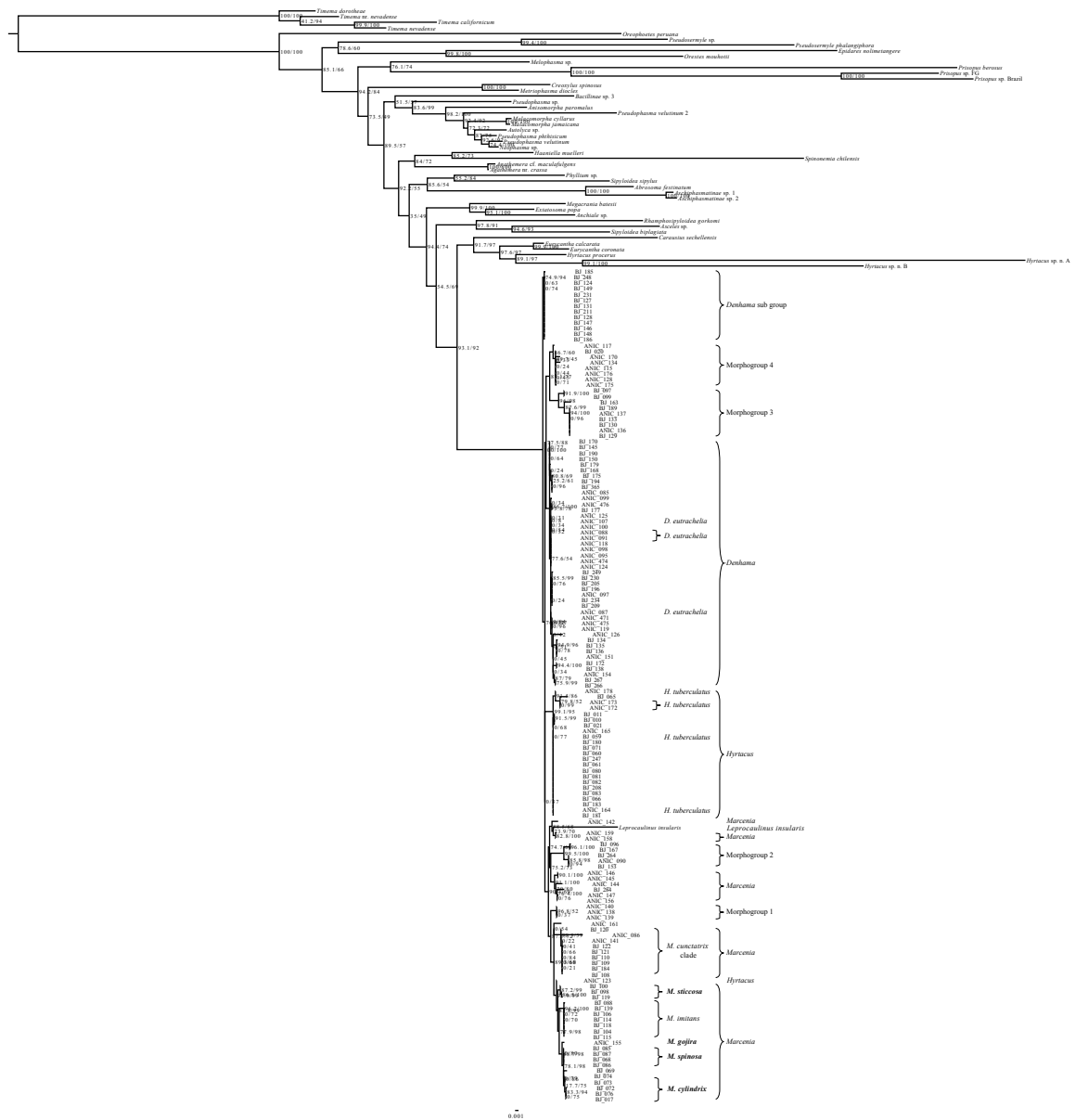
**Figure S4.1.** Eggs from different Australian Lonchodini species and genera to illustrate the morphological egg variation. (a) *Marcenia* sp.. (b) *Austrocarausius macropunctatus*. (c) morphogroup 1, ANIC\_140. (d) *A. eirmosus*. (e) morphogroup 2, BJ\_167. (f) *A. decorus*. (g) Lonchodini sp.. (h) *Denhama* sp., BJ\_248. (i) morphogroup 3, BJ\_099. (j) *M. imitans*, BJ\_088. (k) *A. nigropunctatus*. (l) *M. cylindrix*, BJ\_017. (m) *Denhama* sp. BJ\_020. (n) *Marcenia* sp.. (o) *A. decorus*. (p) *Marcenia* sp. BJ\_120. (q) *Marcenia* sp.. (r) *Denhama* sp. BJ\_172. Photos by Braxton Jones.



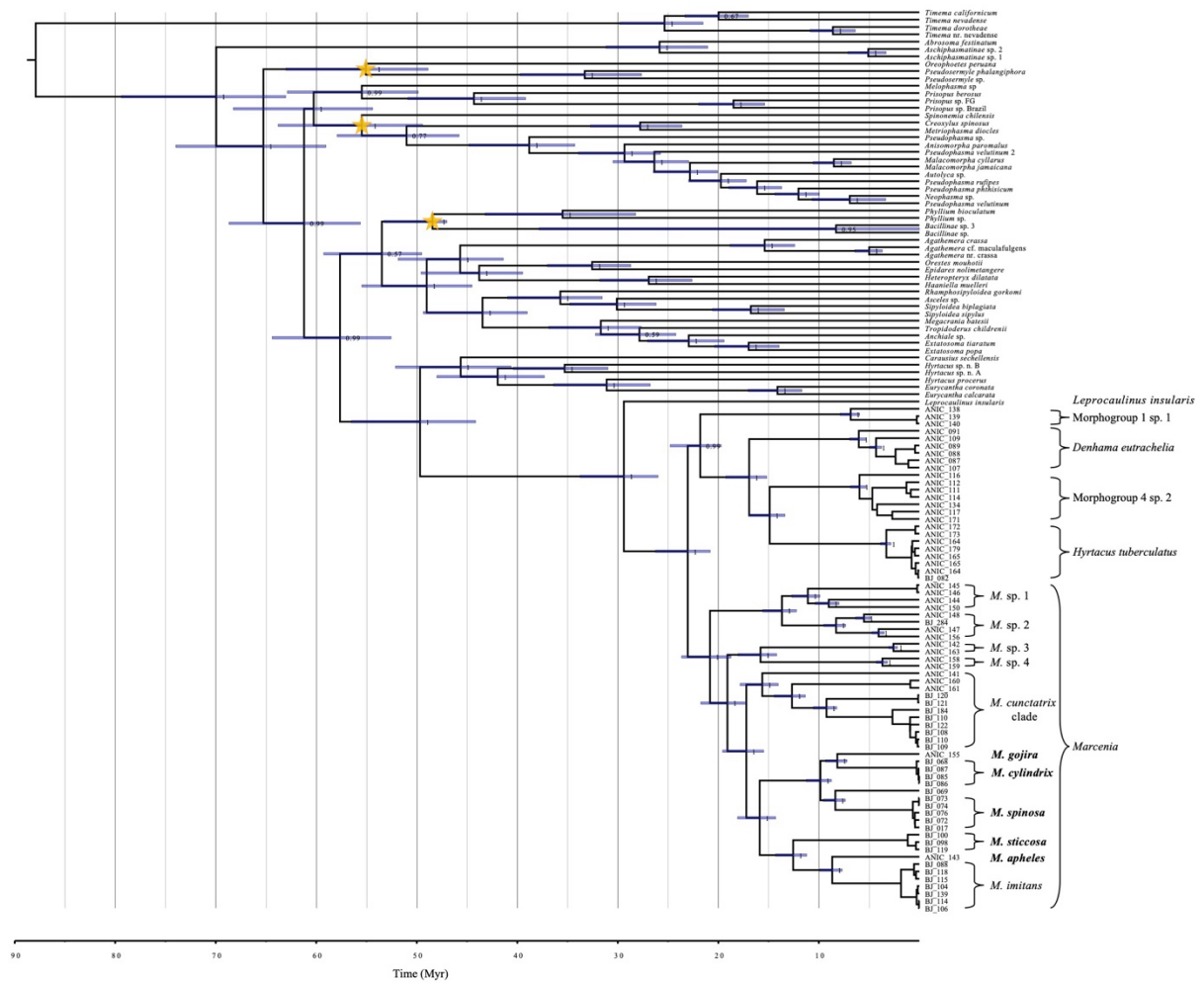
**Figure S4.2.** Maximum likelihood tree for all Lonchodini taxa and outgroups, inferred from 17 mitochondrial and nuclear genes. Bold text indicates new species. Node support values are shown for ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents branch length substitutions per site.



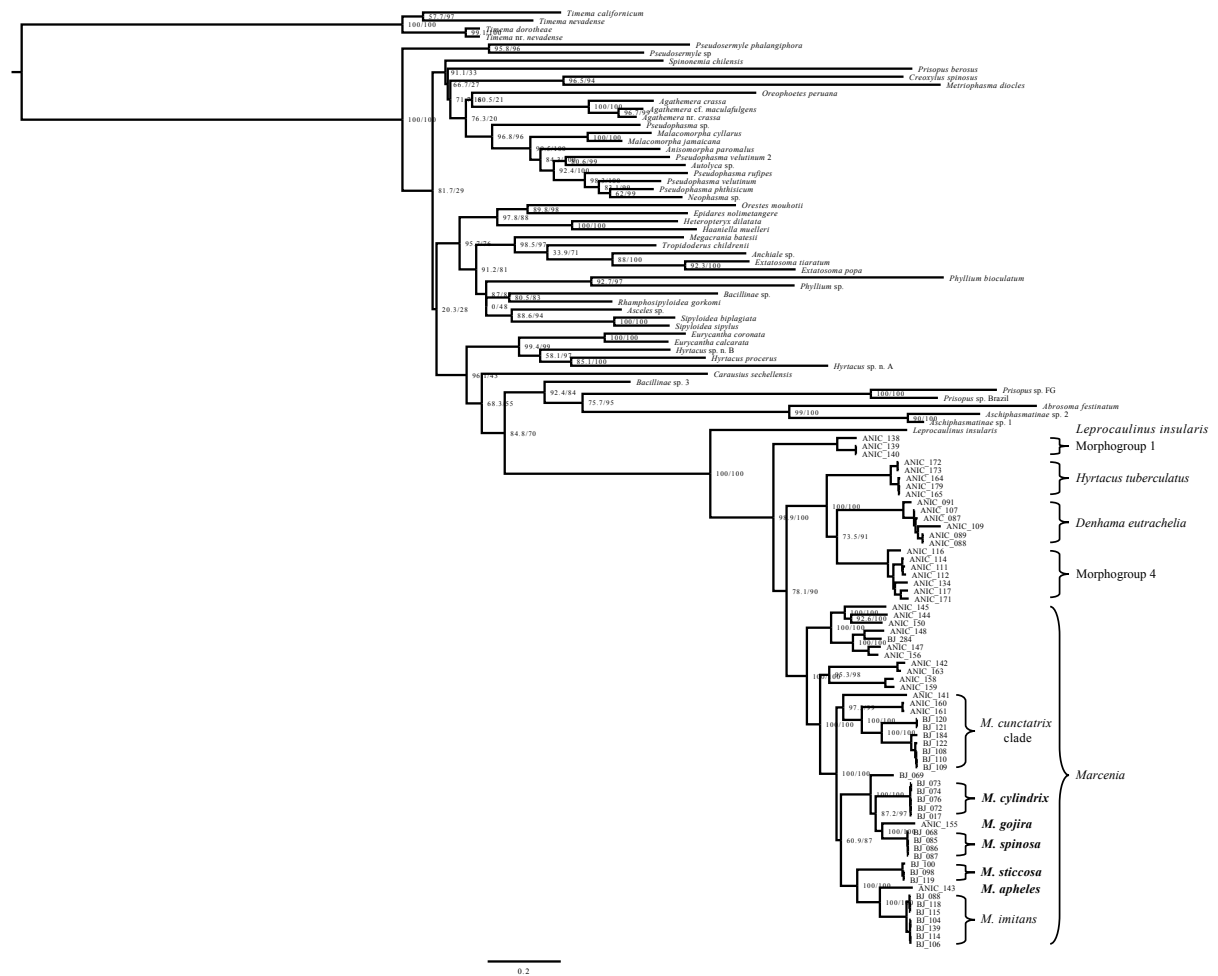
**Figure S4.3.** Maximum likelihood tree for all Lonchodini taxa and outgroups, inferred from 15 mitochondrial genes. Bold text indicates new species. Node support values are shown for ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents branch length substitutions per site.



**Figure S4.4.** Maximum likelihood tree for all Lonchodini taxa and outgroups, inferred from 2 nuclear genes. Bold text indicates new species. Node support values are shown for ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents branch length substitutions per site.

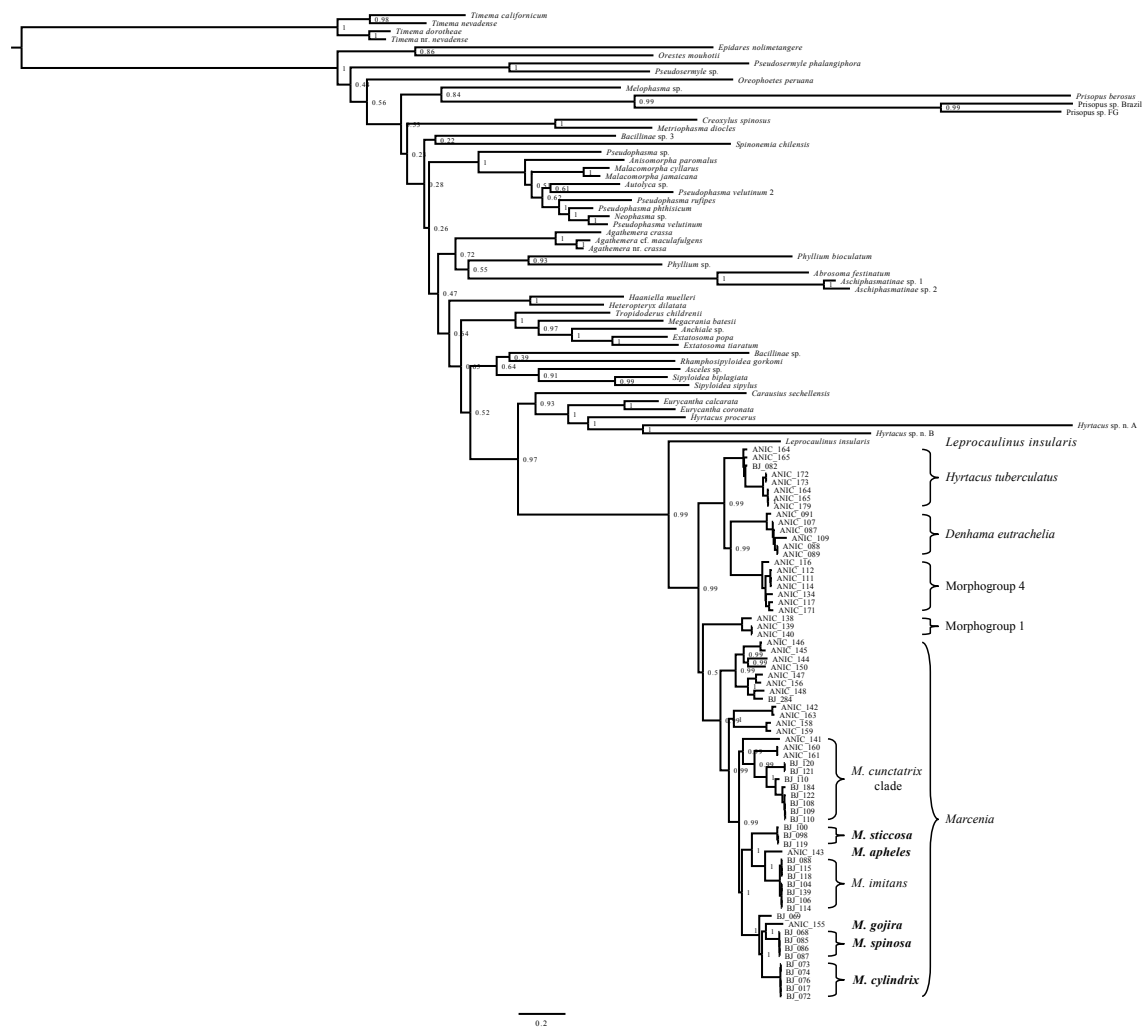


**Figure S4.5.** Fossil calibrated Bayesian inference tree for reduced Lonchodini dataset and outgroups, inferred from 17 mitochondrial and nuclear genes. Fossil node points are marked by an yellow star. Bold text indicates new species. Blue bars at each node represent 95% highest posterior density (HPD) divergence times. Node support values are shown for Bayesian inference, as posterior probability. Scale bar represents substitutions per million years.

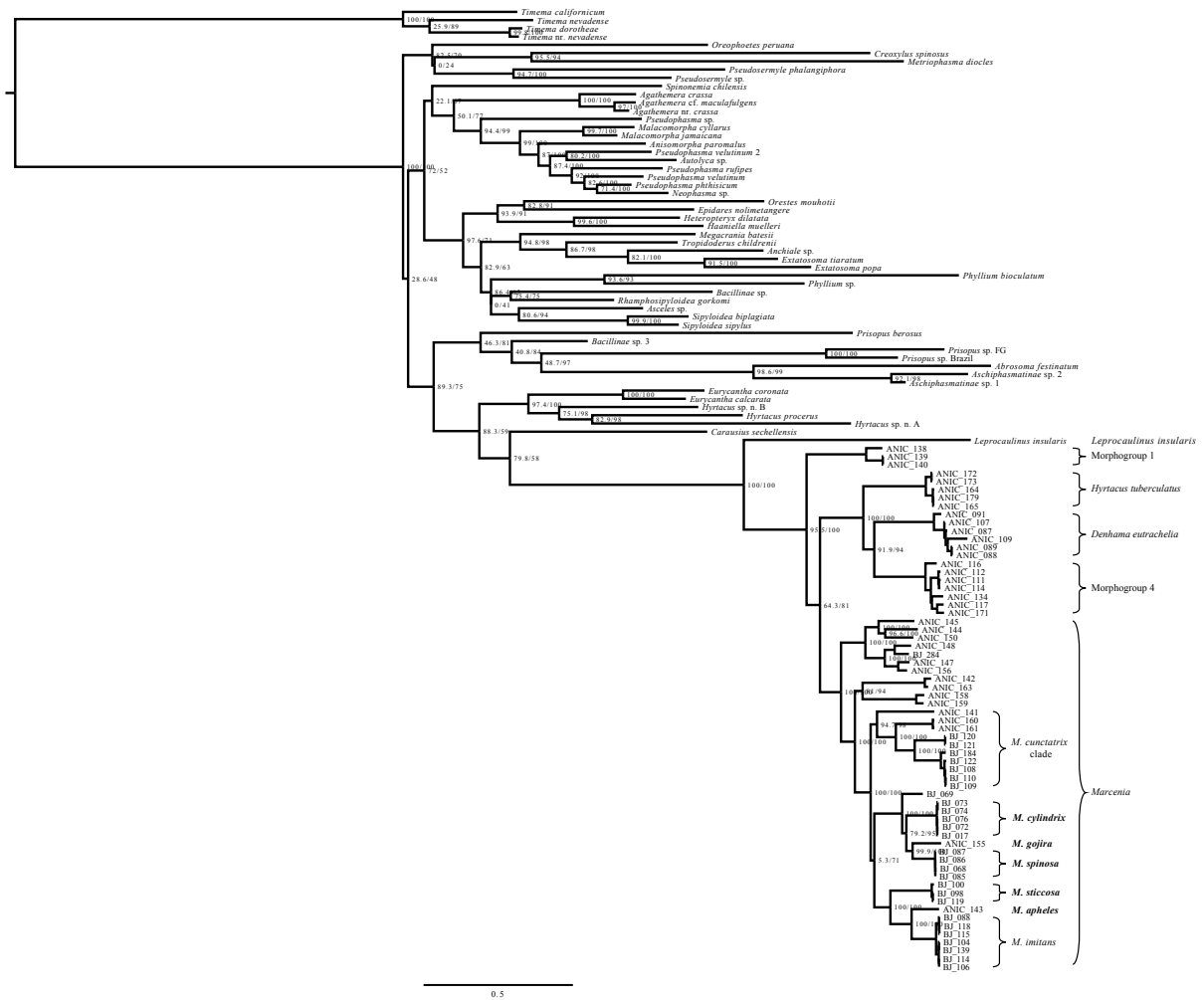


**Figure S4.6.** Maximum likelihood tree for reduced Lonchodini dataset and outgroups, inferred from 17 mitochondrial and nuclear genes. Bold text indicates new species. Node support values are shown for ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents branch length substitutions per site.

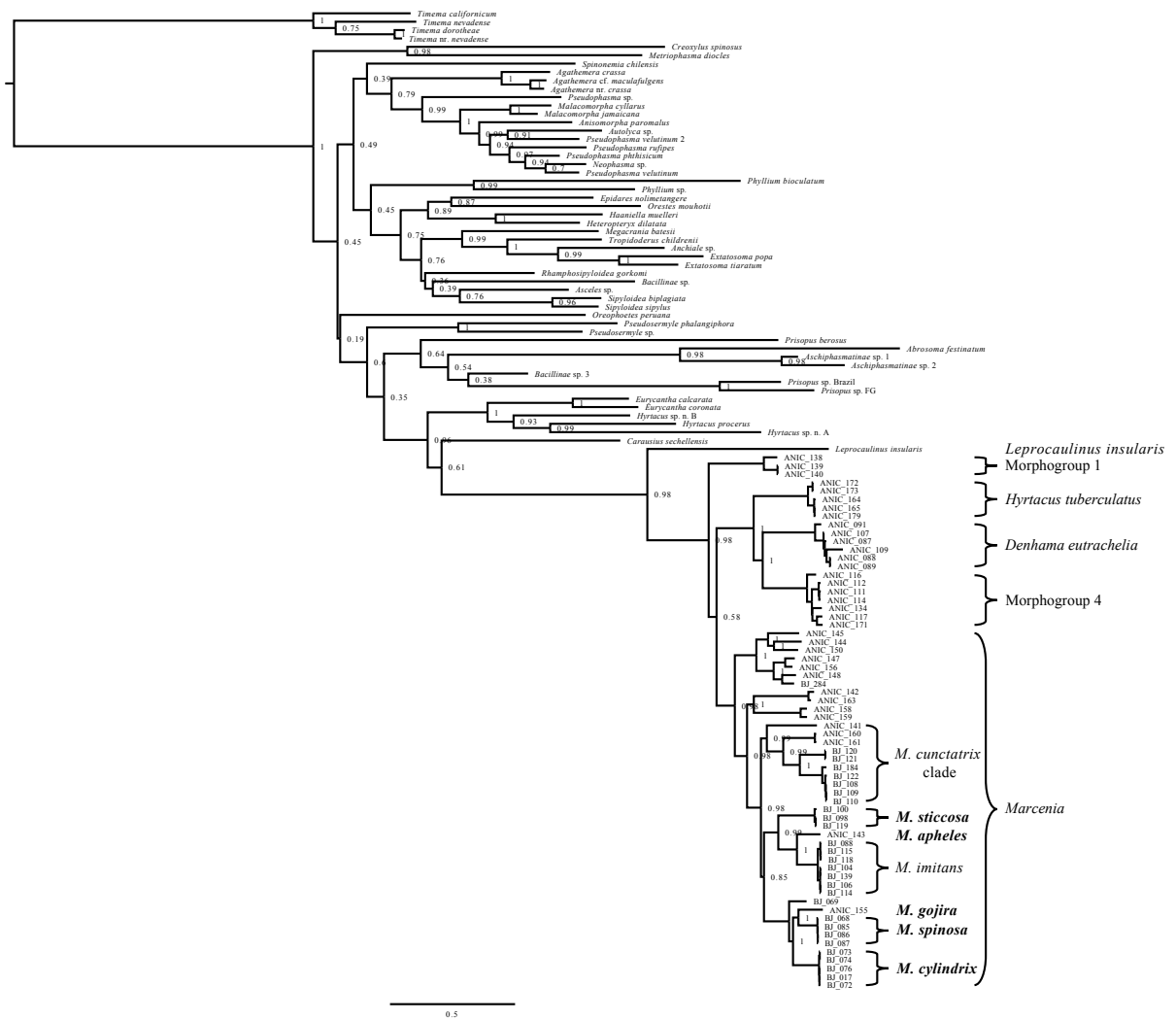




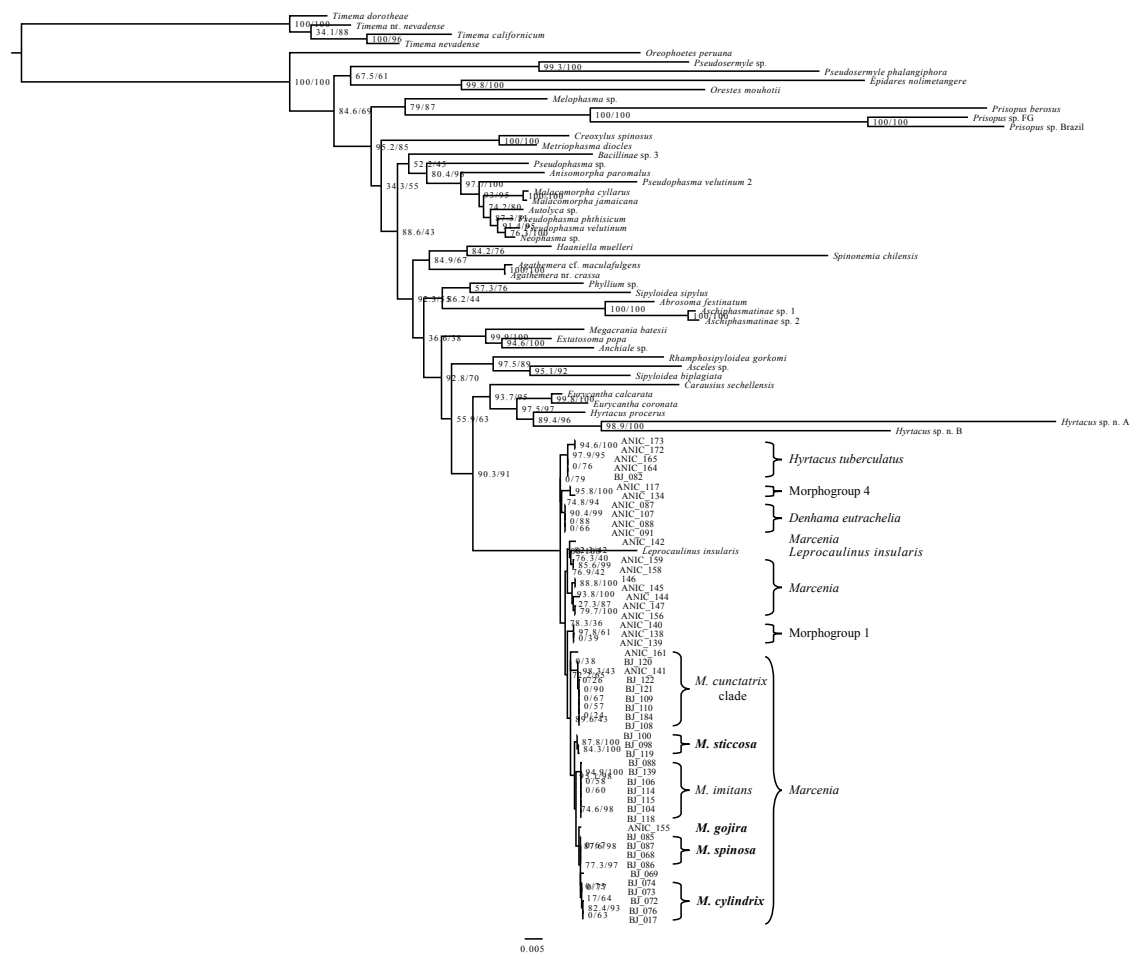
**Figure S4.7.** Bayesian inference tree for reduced Lonchodini dataset and outgroups, inferred from 17 mitochondrial and nuclear genes. Bold text indicates new species. Node support values are shown for Bayesian inference, as posterior probability. Scale bar represents substitutions per site.



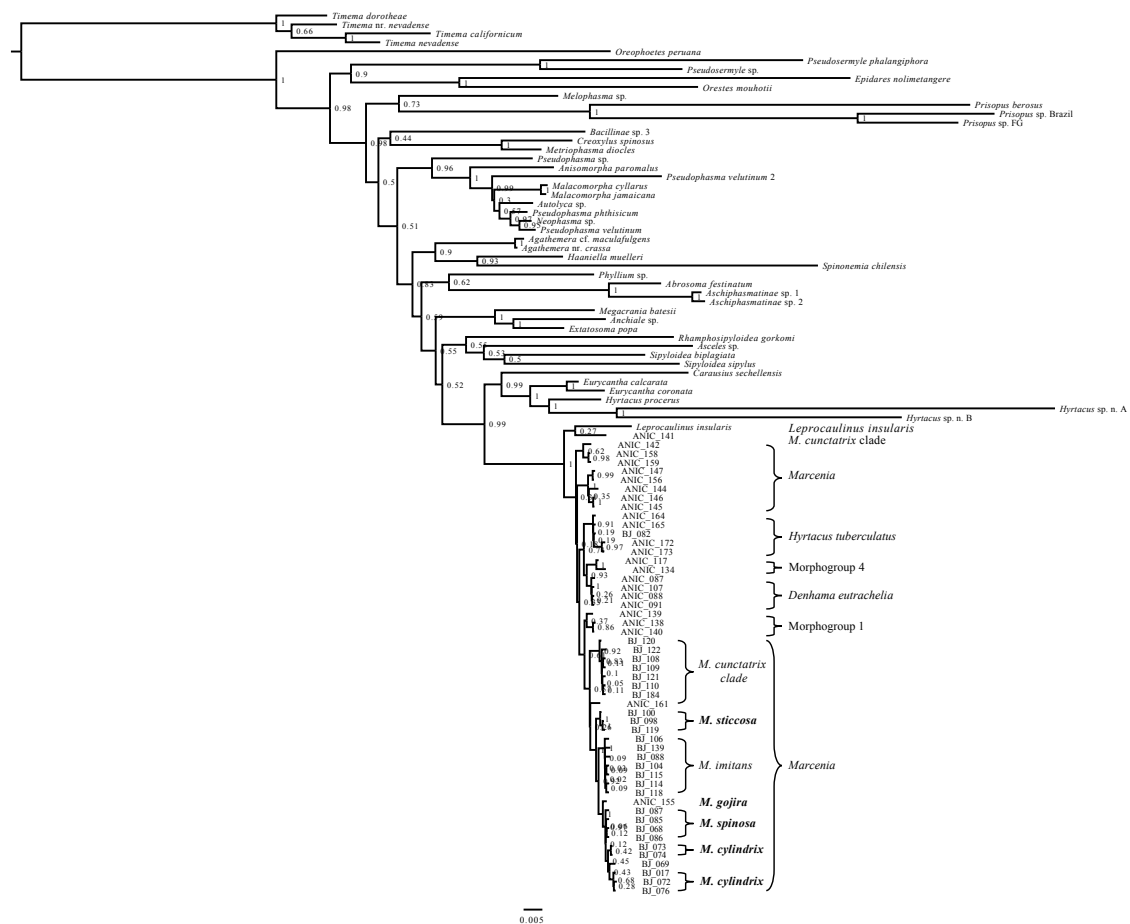
**Figure S4.8.** Maximum likelihood tree for reduced Lonchodini dataset and outgroups, inferred from 15 mitochondrial genes. Bold text indicates new species. Node support values are shown for ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents branch length substitutions per site.



**Figure S4.9.** Bayesian inference tree for reduced Lonchodini dataset and outgroups, inferred from 15 mitochondrial genes. Bold text indicates new species. Node support values are shown for Bayesian inference, as posterior probability. Scale bar represents substitutions per site.



**Figure S4.10.** Maximum likelihood tree for reduced Lonchodini dataset and outgroups, inferred from 2 nuclear genes. Bold text indicates new species. Node support values are shown for ultrafast bootstrap and Shimodaira-Hasegawa Approximate Likelihood Ratio Test. Scale bar represents branch length substitutions per site.



**Figure S4.11.** Bayesian inference tree for reduced Lonchodini dataset and outgroups, inferred from 2 nuclear genes. Bold text indicates new species. Node support values are shown for Bayesian inference, as posterior probability. Scale bar represents substitutions per site.



**Figure S4.12.** Photos of representatives across *Marcenia* species depicting minimal variation in preopercular organ.

**Table S4.1.** Morphological measurements for specimens examined in species diagnosis.

| individual                   | body region | character                                     | measurment 1 (mm) |
|------------------------------|-------------|-----------------------------------------------|-------------------|
| <i>M. cylindrix</i> (BJ_017) | general:    | whole insect                                  |                   |
|                              |             | colouration                                   |                   |
|                              |             | Body length                                   | 75.1              |
|                              | head:       | head length                                   | 3.3               |
|                              |             | head width                                    | 1.7               |
|                              |             | antennae length                               | 31.3              |
|                              |             | antennae segment no.                          | 32                |
|                              | Thorax:     | pronotum length                               | 2.6               |
|                              |             | mesonotum length                              | 17.5              |
|                              |             | metanotum length                              | 9.6               |
|                              | Abdomen:    | median segment                                | 2.1               |
|                              |             | abdominal segment 2 length                    | 4.7               |
|                              |             | abdominal segment 3 length                    | 4.8               |
|                              |             | abdominal segment 4 length                    | 4.8               |
|                              |             | abdominal segment 5 length                    | 6.1               |
|                              |             | abdominal segment 6 length                    | 6.2               |
|                              |             | abdominal segment 7 length                    | 5.6               |
|                              |             | abdominal segment 8 length                    | 3                 |
|                              |             | abdominal segment 9 length                    | 1.9               |
|                              |             | abdominal segment 10 length                   | 4.4               |
|                              |             | cerci                                         |                   |
|                              |             | length of operculum starting from anal segmen | 2.6               |
|                              | Legs:       | leg spines                                    |                   |
|                              |             | fore femur                                    | 18.8              |
|                              |             | mid femur                                     | 17.5              |
|                              |             | hind femur                                    | 21.3              |
|                              |             | fore tibia                                    | 19.9              |
|                              |             | mid tibia                                     | 18.6              |
|                              |             | hind tibia                                    | 24.3              |

| individual                  | body region | character                                     | measurment 1 (mm)    |
|-----------------------------|-------------|-----------------------------------------------|----------------------|
| <i>M. gojira</i> (ANIC_155) | general:    | whole insect                                  |                      |
|                             |             | colouration                                   | brown and mottled, t |
|                             |             | Body length                                   | 70.1                 |
|                             | head:       | head length                                   | 3.7                  |
|                             |             | head width                                    | 2.4                  |
|                             |             | antennae length                               | 25.3                 |
|                             |             | antennae segment no.                          | 26                   |
|                             | Thorax:     | pronotum length                               | 3.2                  |
|                             |             | mesonotum length                              | 18.6                 |
|                             |             | metanotum length                              | 9.7                  |
|                             | Abdomen:    | median segment                                | 2.5                  |
|                             |             | abdominal segment 2 length                    | 4.5                  |
|                             |             | abdominal segment 3 length                    | 4.1                  |
|                             |             | abdominal segment 4 length                    | 4.5                  |
|                             |             | abdominal segment 5 length                    | 5.1                  |
|                             |             | abdominal segment 6 length                    | 5.2                  |
|                             |             | abdominal segment 7 length                    | 5                    |
|                             |             | abdominal segment 8 length                    | 3.3                  |
|                             |             | abdominal segment 9 length                    | 2.2                  |
|                             |             | abdominal segment 10 length                   | 3.9                  |
|                             |             | cerci                                         |                      |
|                             |             | length of operculum starting from anal segmen | 2                    |
|                             | Legs:       | leg spines                                    |                      |
|                             |             | fore femur                                    | 15.4                 |
|                             |             | mid femur                                     | 14.9                 |
|                             |             | hind femur                                    | 18.2                 |
|                             |             | fore tibia                                    | 16                   |
|                             |             | mid tibia                                     | 17.4                 |
|                             |             | hind tibia                                    | 20.9                 |



| individual                 | body region | character                                     | measurment 1 (mm) | Notes                    |
|----------------------------|-------------|-----------------------------------------------|-------------------|--------------------------|
| <i>M. spinsoa</i> (BJ_087) | general:    | whole insect                                  |                   |                          |
|                            |             | colouration                                   |                   |                          |
|                            |             | Body length                                   | 66.3              | paratype (BJ-086) = 70.2 |
|                            | head:       | head length                                   | 3.7               |                          |
|                            |             | head width                                    | 1.9               |                          |
|                            |             | antennae length                               | na                | paratype (BJ-086) = 25.4 |
|                            |             | antennae segment no.                          | na                |                          |
|                            | Thorax:     | pronotum length                               | 2.6               |                          |
|                            |             | mesonotum length                              | 14.8              |                          |
|                            |             | metanotum length                              | 8.5               |                          |
|                            | Abdomen:    | median segment                                | 1.9               |                          |
|                            |             | abdominal segment 2 length                    | 3.7               |                          |
|                            |             | abdominal segment 3 length                    | 3.9               |                          |
|                            |             | abdominal segment 4 length                    | 4.2               |                          |
|                            |             | abdominal segment 5 length                    | 4.9               |                          |
|                            |             | abdominal segment 6 length                    | 5                 |                          |
|                            |             | abdominal segment 7 length                    | 4.9               |                          |
|                            |             | abdominal segment 8 length                    | 3.3               |                          |
|                            |             | abdominal segment 9 length                    | 1.9               |                          |
|                            |             | abdominal segment 10 length                   | 4                 |                          |
|                            |             | cerci                                         |                   |                          |
|                            |             | length of operculum starting from anal segmen | 2.1               |                          |
|                            | Legs:       | leg spines                                    |                   |                          |
|                            |             | fore femur                                    | 15.5              | paratype (BJ-086) = 15.3 |
|                            |             | mid femur                                     | 14.5              |                          |
|                            |             | hind femur                                    | 17.3              |                          |
|                            |             | fore tibia                                    | 16.3              |                          |
|                            |             | mid tibia                                     | 15.6              |                          |
|                            |             | hind tibia                                    | 19.1              |                          |

| individual                  | body region | character                                     | measurment 1 (mm) |
|-----------------------------|-------------|-----------------------------------------------|-------------------|
| <i>M. sticcosa</i> (BJ_119) | general:    | whole insect                                  |                   |
|                             |             | colouration                                   |                   |
|                             |             | Body length                                   | 81.2              |
|                             | head:       | head length                                   | 3.8               |
|                             |             | head width                                    | 1.9               |
|                             |             | antennae length                               | na                |
|                             |             | antennae segment no.                          | na                |
|                             | Thorax:     | pronotum length                               | 3                 |
|                             |             | mesonotum length                              | 19.1              |
|                             |             | metanotum length                              | 10                |
|                             | Abdomen:    | median segment                                | 2.1               |
|                             |             | abdominal segment 2 length                    | 5.4               |
|                             |             | abdominal segment 3 length                    | 5.2               |
|                             |             | abdominal segment 4 length                    | 5.3               |
|                             |             | abdominal segment 5 length                    | 6                 |
|                             |             | abdominal segment 6 length                    | 6.1               |
|                             |             | abdominal segment 7 length                    | 6.6               |
|                             |             | abdominal segment 8 length                    | 3.7               |
|                             |             | abdominal segment 9 length                    | 2.2               |
|                             |             | abdominal segment 10 length                   | 4.2               |
|                             |             | cerci                                         |                   |
|                             |             | length of operculum starting from anal segmen | 2.2               |
|                             | Legs:       | leg spines                                    |                   |
|                             |             | fore femur                                    | 20                |
|                             |             | mid femur                                     | 17.7              |
|                             |             | hind femur                                    | 21.5              |
|                             |             | fore tibia                                    | 20.2              |
|                             |             | mid tibia                                     | 19.8              |
|                             |             | hind tibia                                    | 26.4              |

| individual                   | body region | character                                    | measurment 1 (mm) |
|------------------------------|-------------|----------------------------------------------|-------------------|
| <i>M. apheles</i> (ANIC_143) | general:    | whole insect                                 |                   |
|                              |             | colouration                                  |                   |
|                              |             | Body length                                  | 89.2              |
|                              | head:       | head length                                  | 4.5               |
|                              |             | head width                                   | 2.5               |
|                              |             | antennae length                              | na                |
|                              |             | antennae segment no.                         | na                |
|                              | Thorax:     | pronotum length                              | 3.4               |
|                              |             | mesonotum length                             | 21.7              |
|                              |             | metanotum length                             | 11.8              |
|                              | Abdomen:    | median segment                               | 2.2               |
|                              |             | abdominal segment 2 length                   | 5.4               |
|                              |             | abdominal segment 3 length                   | 5.5               |
|                              |             | abdominal segment 4 length                   | 6.2               |
|                              |             | abdominal segment 5 length                   | 6.5               |
|                              |             | abdominal segment 6 length                   | 6.2               |
|                              |             | abdominal segment 7 length                   | 6.2               |
|                              |             | abdominal segment 8 length                   | 3.3               |
|                              |             | abdominal segment 9 length                   | 2.4               |
|                              |             | abdominal segment 10 length                  | 3.9               |
|                              |             | cerci                                        |                   |
|                              |             | length of operculum starting from anal segme | 2.2               |
|                              | Legs:       | leg spines                                   |                   |
|                              |             | fore femur                                   | 22.2              |
|                              |             | mid femur                                    | 19.8              |
|                              |             | hind femur                                   | 26.1              |
|                              |             | fore tibia                                   | 24.4              |
|                              |             | mid tibia                                    | 21.3              |
|                              |             | hind tibia                                   | 28.6              |

| individual                 | body region | character                                     | measurment 1 (mm) |
|----------------------------|-------------|-----------------------------------------------|-------------------|
| <i>M. imitans</i> (BJ_088) | general:    | whole insect                                  |                   |
|                            |             | colouration                                   |                   |
|                            |             | Body length                                   | 86.1              |
|                            | head:       | head length                                   | 4.4               |
|                            |             | head width                                    | 2                 |
|                            |             | antennae length                               | 29.9              |
|                            |             | antennae segment no.                          | na                |
|                            | Thorax:     | pronotum length                               | 2.8               |
|                            |             | mesonotum length                              | 21.9              |
|                            |             | metanotum length                              | 11.9              |
|                            | Abdomen:    | median segment                                | 2.3               |
|                            |             | abdominal segment 2 length                    | 5.5               |
|                            |             | abdominal segment 3 length                    | 5.5               |
|                            |             | abdominal segment 4 length                    | 5.7               |
|                            |             | abdominal segment 5 length                    | 6.3               |
|                            |             | abdominal segment 6 length                    | 6.1               |
|                            |             | abdominal segment 7 length                    | 6                 |
|                            |             | abdominal segment 8 length                    | 3.2               |
|                            |             | abdominal segment 9 length                    | 1.9               |
|                            |             | abdominal segment 10 length                   | 3.8               |
|                            |             | cerci                                         |                   |
|                            |             | length of operculum starting from anal segmen | 2                 |
|                            | Legs:       | leg spines                                    |                   |
|                            |             | fore femur                                    | 21.6              |
|                            |             | mid femur                                     | 18.9              |
|                            |             | hind femur                                    | 23.8              |
|                            |             | fore tibia                                    | 19                |
|                            |             | mid tibia                                     | 18.2              |
|                            |             | hind tibia                                    | 20.2              |

**Table S4.2.** Metadata for all specimens used in paper.

| Species                                    | COXII      | 16S      | 12S      | 18S      | 28S      |
|--------------------------------------------|------------|----------|----------|----------|----------|
| <i>A. waiben</i> BJ-322                    | ON102002   | OM992351 | -        | -        | -        |
| <i>A. nigropunctatus</i> BJ-240            | ON101990   | OM992332 | -        | -        | -        |
| <i>A. nigropunctatus</i> BJ-241            | -          | OM992333 | -        | -        | -        |
| <i>A. nigropunctatus</i> BJ-242            | ON101991   | OM992334 | -        | -        | -        |
| <i>A. nigropunctatus</i> BJ-243            | ON101992   | OM992335 | -        | -        | -        |
| <i>A. nigropunctatus</i> BJ-245            | ON077343   | OM992337 | -        | -        | -        |
| <i>A. nigropunctatus</i> BJ-246            | ON101994   | OM992338 | -        | -        | -        |
| <i>A. walkeri</i> PB-0014                  | HM425716.1 | -        | -        | -        | -        |
| <i>A. mercurius</i> PB-0079                | KJ202027.1 | OM992352 | -        | -        | -        |
| <i>A. mercurius</i> PB-0078                | KJ202015.1 | -        | -        | -        | -        |
| <i>A. mercurius</i> PB-0080                | KJ202026.1 | -        | -        | -        | -        |
| <i>A. mercurius</i> PB-0081                | KJ201995.1 | OM992353 | -        | -        | -        |
| <i>A. macropunctataus</i> BJ-302           | ON102000   | OM992348 | -        | -        | -        |
| <i>A. macropunctataus</i> BJ-304           | ON102001   | OM992350 | -        | -        | -        |
| <i>A. macropunctataus</i> BJ-303           | ON077348   | OM992349 | -        | -        | -        |
| <i>A. eirmosus</i> BJ-300                  | ON101998   | OM992346 | -        | -        | -        |
| <i>A. eirmosus</i> BJ-301                  | ON101999   | OM992347 | -        | -        | -        |
| <i>A. eirmosus</i> BJ-299                  | ON101997   | OM992345 | -        | -        | -        |
| <i>A. coronatus</i> JH-0055                | HM425711.1 | OM992354 | -        | -        | -        |
| <i>A. coronatus</i> JH-0054                | HM425710.1 | OM992356 | -        | -        | -        |
| <i>A. coronatus</i> PB-0028                | HM425728.1 | -        | -        | -        | -        |
| <i>A. coronatus</i> PB-0026                | HM425727.1 | -        | -        | -        | -        |
| <i>A. coronatus</i> PB-0025                | HM425726.1 | OM992357 | -        | -        | -        |
| <i>A. coronatus</i> PB-0024                | HM425725.1 | OM992355 | -        | -        | -        |
| <i>A. decorus</i> BJ-244                   | ON101993   | OM992336 | -        | -        | -        |
| <i>A. decorus</i> BJ-293                   | ON077344   | OM992339 | -        | -        | -        |
| <i>A. decorus</i> BJ-294                   | ON101995   | OM992340 | -        | -        | -        |
| <i>A. decorus</i> BJ-295                   | ON077345   | OM992341 | -        | -        | -        |
| <i>A. decorus</i> BJ-296                   | ON101996   | OM992342 | -        | -        | -        |
| <i>A. decorus</i> BJ-297                   | ON077346   | OM992343 | -        | -        | -        |
| <i>A. decorus</i> BJ-298                   | ON077347   | OM992344 | -        | -        | -        |
| <i>Abrosoma festinatum</i>                 | -          | MK291730 | MK291654 | MK291809 | MK291884 |
| <i>Timema nevadense</i>                    | MK297263   | MK291711 | MK291635 | MK291788 | MK291863 |
| <i>Timema californicum</i>                 | MK297264   | -        | MK291636 | MK291789 | MK291864 |
| <i>Timema dorotheae</i>                    | MK297262   | MK291710 | MK291634 | MK291787 | MK291862 |
| <i>Timema</i> nr. <i>nevadense</i>         | MK297288   | MK291746 | MK291666 | MK291824 | MK291901 |
| <i>Aschiphasmatinae</i> sp. 1              | -          | -        | MK291671 | -        | MK291908 |
| <i>Aschiphasmatinae</i> sp. 2              | MK297291   | -        | MK291672 | -        | MK291909 |
| <i>Oreophoetes peruana</i>                 | KJ024540   | KJ024428 | -        | AY121152 | AY125292 |
| <i>Pseudosermyle</i> sp.                   | -          | MK291723 | MK291648 | MK291801 | MK291876 |
| <i>Pseudosermyle phalangiphora</i>         | MK297286   | MK291744 | MK291665 | -        | MK291899 |
| <i>Agathemera</i> cf. <i>maculafulgens</i> | MK297289   | MK291747 | MK291667 | MK291825 | MK291902 |
| <i>Agathemera crassa</i>                   | KJ024537   | KJ024421 | KJ024472 | -        | -        |

|                                     |          |          |          |          |          |
|-------------------------------------|----------|----------|----------|----------|----------|
| <i>Agathemera</i> nr. <i>crassa</i> | MK297290 | MK291748 | MK291668 | MK291826 | MK291903 |
| <i>Phyllium bioculatum</i>          | KJ024570 | KJ024463 | -        | -        | -        |
| <i>Phyllium</i> sp.                 | -        | KJ024464 | KJ024508 | KJ024418 | KJ024397 |
| <i>Bacillinae</i> sp.               | -        | MK291752 | -        | -        | MK291910 |
| <i>Bacillinae</i> sp. 3             | MW686187 | -        | -        | -        | MW703233 |
| <i>Spinonemia chilensis</i>         | FJ474365 | JN228840 | -        | -        | FJ474134 |
| <i>Prisopus</i> sp.                 | -        | MK291750 | -        | MK291828 | MK291905 |
| <i>Prisopus</i> sp. Brazil          | -        | MK291749 | MK291669 | MK291827 | MK291904 |
| <i>Melophasma</i> sp.               | -        | -        | -        | -        | MK291914 |
| <i>Prisopus berosus</i>             | -        | ON888631 | -        | ON898258 | MK291913 |
| <i>Creoxylus spinosus</i>           | MK297247 | MK291689 | MK291613 | MK291766 | MK291841 |
| <i>Metriophasma diocles</i>         | -        | MK291683 | MK291608 | MK291760 | MK291835 |
| <i>Pseudophasma</i> sp.             | MK297272 | MK291722 | MK291647 | MK291800 | MK291875 |
| <i>Anisomorpha paromalus</i>        | OK314799 | OK333530 | OK333675 | OK334005 | OK333846 |
| <i>Autolyca</i> sp.                 | MK297246 | MK291688 | MK291612 | MK291765 | MK291840 |
| <i>Pseudophasma velutinum</i>       | -        | MW138619 | -        | MW138543 | MW138694 |
| <i>Pseudophasma velutinum</i> 2     | FJ474388 | -        | -        | -        | FJ474163 |
| <i>Malacomorpha jamaicana</i>       | MK297280 | MK291732 | MK291656 | MK291811 | MK291886 |
| <i>Malacomorpha cyllarus</i>        | MK297240 | MK291677 | MK291603 | MK291754 | MK291829 |
| <i>Pseudophasma phthisicum</i>      | MK297256 | MK291700 | MK291624 | MK291777 | MK291852 |
| <i>Neophasma</i> sp.                | MK297294 | -        | MK291675 | ON898266 | MK291922 |
| <i>Pseudophasma rufipes</i>         | KJ024573 | KJ024467 | KJ024510 | -        | -        |
| <i>Orestes mouhotii</i>             | MK297275 | MK291726 | MK291650 | MK291805 | MK291880 |
| <i>Epidares nolimetangere</i>       | MK297243 | MK291680 | MK291606 | MK291757 | MK291832 |
| <i>Heteropteryx dilatata</i>        | KJ024542 | KJ024429 | KJ024476 | -        | -        |
| <i>Haaniella muelleri</i>           | MK297244 | MK291681 | MK291607 | MK291758 | MK291833 |
| <i>Sipyloidea sipylus</i>           | MN449877 | MN449601 | MN449683 | -        | MN449501 |
| <i>Asceles</i> sp.                  | KJ024557 | KJ024447 | KJ024493 | KJ024411 | KJ024388 |
| <i>Rhamphosipyloidea gorkomi</i>    | -        | KJ024436 | KJ024482 | KJ024401 | KJ024378 |
| <i>Sipyloidea biplagiata</i>        | MK297296 | -        | MK291676 | ON898300 | MK291924 |
| <i>Carausius sechellensis</i>       | -        | MK291714 | MK291639 | MK291792 | MK291867 |
| <i>Leprocaulinus insularis</i>      | KJ024552 | KJ024441 | KJ024487 | KJ024405 | KJ024382 |
| <i>Hyrtacus procerus</i>            | MK297250 | MK291693 | MK291617 | MK291770 | MK291845 |
| <i>Hyrtacus</i> sp. A               | -        | MK291704 | MK291628 | MK291781 | MK291856 |
| <i>Hyrtacus</i> sp. B               | MK297251 | MK291694 | MK291618 | MK291771 | MK291846 |
| <i>Hyrtacus tuberculatus</i>        | -        | MK291742 | MK291664 | MK291821 | MK291897 |
| <i>Lonchodinae</i> _sp_1            | MK297283 | MK291737 | MK291661 | MK291817 | MK291892 |
| <i>Lonchodinae</i> _sp_2            | MK297284 | MK291662 | MK291738 | MK291818 | MK291893 |
| <i>Eurycantha calcarata</i>         | MK297258 | MK291705 | MK291629 | MK291782 | MK291857 |
| <i>Eurycantha coronata</i>          | MK297248 | MK291690 | MK291614 | MK291767 | MK291842 |
| <i>Tropidoderus childrenii</i>      | KJ024575 | KJ024469 | KJ024512 | -        | -        |
| <i>Anchiale</i> sp.                 | -        | MK291725 | -        | MK291804 | MK291879 |
| <i>Megacrania batesii</i>           | KJ024572 | KJ024465 | KJ024509 | KJ024419 | KJ024398 |
| <i>Extatosoma popa</i>              | MK297249 | MK291691 | MK291615 | MK291768 | MK291843 |

*Extatosoma tiaratum*

NC\_017748 NC\_017748 NC\_017748 -

-

**Table S4.3.** Partitions schemes and models used in molecular analyses.

| Partition                                                      | Iqtree<br>Combined | Iqtree<br>Mito  | Iqtree<br>Nuclear | MrBayes<br>Combined                    | MrBayes Mito                           | MrBayes<br>Nuclear                     |
|----------------------------------------------------------------|--------------------|-----------------|-------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| COX1_1_COX2_1                                                  | TIM2+F+I+<br>G4    | TIM2+F<br>+I+G4 |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| COX1_2_COXII_2_CO<br>X3_2_CYTB_2                               | TPM3+F+R<br>4      | TPM3+<br>F+R4   |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| COXI_3_COXIII_3_ND<br>2_3_ND3_3_ND6_3_CY<br>TB_3_ATP8_3_ATP6_3 | TVM+F+R6           | TVM+F<br>+R6    |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| COXII_3                                                        | TN+F+R4            | TN+F+<br>R4     |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| COXIII_1_ND3_1_CYT<br>B_1_ATP6_1                               | GTR+F+I+<br>G4     | GTR+F<br>+I+G4  |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| ND1_1_ND4_1_ND4L_1<br>_ND5_1                                   | GTR+F+I+<br>G4     | GTR+F<br>+I+G4  |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| ND1_2_ND4_2_ND4L_2<br>_ND5_2                                   | GTR+F+I+<br>G4     | GTR+F<br>+I+G4  |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| ND1_3_ND4_3_ND4L_3<br>_ND5_3                                   | TPM3+F+R<br>6      | TPM3+<br>F+R6   |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| ND2_1_ND6_1_ATP8_1<br>_ATP8_2                                  | TIM2+F+I+<br>G4    | TIM2+F<br>+I+G4 |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| ND2_2_ND3_2_ND6_2_<br>ATP6_2                                   | TVM+F+I+<br>G4     | TVM+F<br>+I+G4  |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| 16S_12S                                                        | GTR+F+R1<br>0      | GTR+F<br>+R10   |                   | nst=6<br>rates=invgamma<br>ngammacat=6 | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        |
| 18S.nex                                                        | TNe+I+G4           |                 | TNe+I+<br>G4      | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        | nst=6<br>rates=invgamma<br>ngammacat=6 |
| 28S.nex                                                        | GTR+F+I+<br>G4     |                 | GTR+F<br>+I+G4    | nst=6<br>rates=invgamma<br>ngammacat=6 |                                        | nst=6<br>rates=invgamma<br>ngammacat=6 |



**Table S4.4.** Fossils used in time calibrated Bayesian inference tree (Supplementary Figure S4.11).

| <b>Fossil</b>               | <b>Life stage</b> | <b>Reference</b>           | <b>Minimum age (mya)</b> | <b>Soft upper bound (mya)</b> | <b>Mean</b> | <b>Calibration node</b>    |
|-----------------------------|-------------------|----------------------------|--------------------------|-------------------------------|-------------|----------------------------|
| <i>Eophyllum messelense</i> | Adult             | Wedmann <i>et al.</i> 2007 | 47                       | 110                           | 17          | Phylliinae (node 1)        |
| <i>Eophasma</i> sp.         | Egg               | Poinar 2011                | 44                       | 110                           | 18          | Pseudophasmatinae (node 2) |
| <i>Clonistria</i> sp.       | Egg               | Poinar 2011                | 20                       | 110                           | 24.3        | Diapheromerinae (node 3)   |

## Taxonomic revision of the Australian stick insect genus *Candovia* (Phasmida: Necrosiinae): insight from molecular systematics and species-delimitation approaches

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Received 18 March 2022; revised 4 July 2022; accepted for publication 8 August 2022

The Phasmida genus *Candovia* comprises nine traditionally recognized species, all endemic to Australia. In this study, *Candovia* diversity is explored through molecular species-delimitation analyses using the *COI<sub>mt</sub>* gene fragment and phylogenetic inferences leveraging seven additional mitochondrial and nuclear loci. Molecular results were integrated with morphological observations, leading us to confirm the already described species and to the delineation of several new taxa and of the new genus *Paracandovia*. New *Candovia* species from various parts of Queensland and New South Wales are described and illustrated (*C. alata* sp. nov., *C. byfieldensis* sp. nov., *C. dalglishae* sp. nov., *C. eungellensis* sp. nov., *C. karasi* sp. nov., *C. koensi* sp. nov. and *C. wollumbinensis* sp. nov.). New combinations are proposed and species removed from synonymy with the erection of the new genus *Paracandovia* (*P. cercata* stat. rev., comb. nov., *P. longipes* stat. rev., comb. nov., *P. pallida* comb. nov., *P. peridromes* comb. nov., *P. tenera* stat. rev., comb. nov.). Phylogenetic analyses suggest that the egg capitulum may have independently evolved multiple times throughout the evolutionary history of these insects. Furthermore, two newly described species represent the first taxa with fully developed wings in this previously considered apterous clade.

ADDITIONAL KEYWORDS: Australian fauna – capitulum – molecular phylogenetics – *Paracandovia* – phasmids – wings.

### INTRODUCTION

The order Phasmida Leach, 1815 (Hexapoda: Insecta) consists of c. 3400 valid species (Brock *et al.*, 2022), mostly with a tropical or subtropical distribution. It contains insects that are well known for their remarkable mimicry, from crypsis and background matching to disruptive coloration and masquerade (Merilaita & Lind, 2005; Skelhorn, 2015). Their

morphology presents instances of interspecific convergent evolution, possibly combined with intraspecific phenotypic plasticity (Gutiérrez-Valencia *et al.*, 2017). Therefore, it is important to rely on the integration of different approaches to unravel their diversity, such as the integration of molecular data and morphological observations. Despite several shortcomings about the usage of a single mitochondrial gene locus for this purpose having been highlighted (Moritz & Cicero 2004), this approach is widespread as a quick and efficient exploratory strategy for the delimitation and validation of species boundaries. Yet, many empirical studies have shown that some species-delimitation methods can lead to under- or over-splitting of species, therefore the outcomes

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[Version of record, published online 10 October 2022; <http://zoobank.org/> urn:lsid:zoobank.org:pub:D2036A60-F34D-409A-A0A2-A84D5ACB0004]

of molecular species-delimitation should be better interpreted along with complementary information of species morphology and/or ecology (Dellicour & Flot, 2018; Jacobs *et al.*, 2018; Luo *et al.*, 2018).

Molecular species delimitation has been successfully used in phasmids in several instances (Glaw *et al.*, 2019; Bank *et al.*, 2021; Cumming *et al.*, 2021). Velonà *et al.* (2015) first applied DNA barcoding on 16 Australian stick insect taxa, and retrieved a high differentiation among three putative morphospecies of the genus *Candovia* Stål, 1875: *Candovia* spp. A, B and C, and found their divergence from *C. annulata* (Brunner, 1907). Traditionally, the genus *Candovia* (Lonchodidae: Necrosiinae: Necrosiini) consisted of nine recognized species, all endemic to Australia, feeding on a wide range of trees, shrubs and ferns. The species *C. evoneobertii* Zompro & Adis, 2001, usually listed as the tenth taxon, is endemic to South America, sharing only a superficial similarity to *Candovia* (Brock & Hasenpusch, 2009); it has recently been synonymized with *Arumatia dubia* (Caudell, 1904) (Ghirotto *et al.*, 2022). *Candovia* are small to medium-sized, stick-like, Australian phasmids known from few records, with the apparent scarcity of specimens relating to limited interest in phasmids and to their nocturnal lifestyle. Thus their species richness and true distribution may be much more extensive than recorded. Although enthusiasts rear them from time to time, there is no published information on the biology of *Candovia* species, with the exception of the publication by Brock & Hasenpusch (2009). Furthermore, older phasmid descriptions are often listed from just a single location and rely on damaged material of one sex only: the type species of *Candovia*, originally described as *Phasma* (Bacteria) *coenosum* Gray, 1833, is a fitting example, consisting of a damaged holotype female.

A recent sampling effort revealed morphologically diverse *Candovia* specimens, not recognizable as known species. Therefore, this study aims to unravel the *Candovia* species richness, to support and validate formal descriptions of putative new taxa and to understand their phylogenetic relationships.

As a result, the new genus *Paracandovia* (described below) is erected alongside the traditional *Candovia*, while we also validate already known species and describe several new ones.

#### TAXONOMIC HISTORY OF CANDOVIA

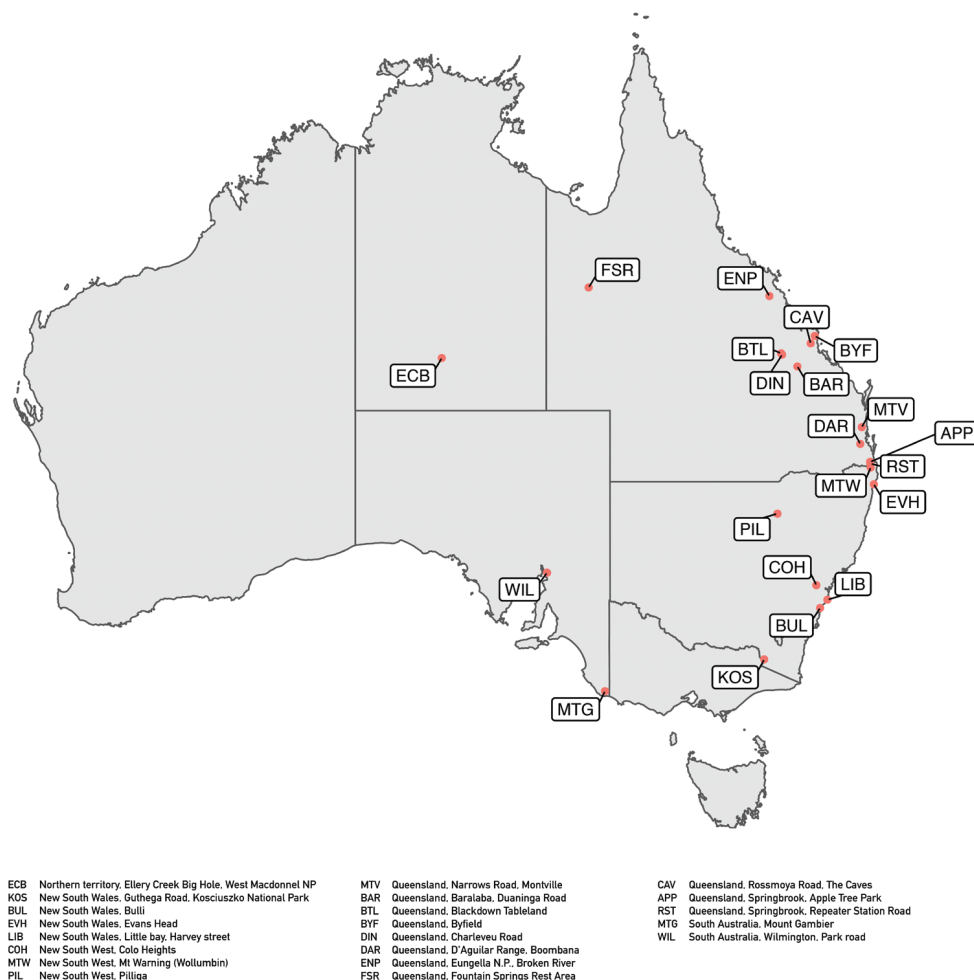
Since *Candovia* was described (Stål, 1875) to accommodate *Phasma* (Bacteria) *coenosum* Gray, 1833, the genus has been little mentioned and was often confused by subsequent authors. Stål believed Gray's holotype to be a male, but Westwood (1859) clarified this, and also described and figured the supposed male and synonymized *Phasma* (Bacteria) *fragilis*

Gray, 1833. In a checklist of phasmids (Kirby, 1904), *Candovia* was synonymized with *Hyrtacus* Stål, 1875. Brock & Hasenpusch (2007) redescribed *Candovia* and removed the genus from synonymy with *Hyrtacus* (the latter is also in need of revision, as the type species has spine-like tubercles on the head, thorax and abdomen, which are lacking in other taxa, including undescribed species; in females, the end of abdomen is beak-shaped and pointed at tip). They also transferred several species to *Candovia* that had been originally listed as *Parasipyloidea* Redtenbacher, 1908 in checklists of Australian phasmids by Vickery (1983) and Balderson *et al.* (1998). Vickery (1983) was the first to place several wingless Australian species in *Parasipyloidea* (an Asian genus of several small, wingless species), presumably thinking they were allied to *C. strumosa* (Redtenbacher, 1908), originally described in *Parasipyloidea*. *Candovia* species were otherwise originally described as belonging to *Bacillus* Berthold, 1827, *Bacunculus* Burmeister, 1838, *Dyme* Stål, 1875, *Hyrtacus*, *Marcenia* Sjöstedt, 1918 or *Menexenus* Stål, 1875, due to vague similarity; that is, in most cases elongate, wingless taxa, originating from different geographical regions, with various distinguishing morphological characters. To add to confusion, later Zompro & Adis (2001) incorrectly linked a South American species as 'Australian' and placed it in the Australian genus *Echetlus* Stål, 1875 (which have short antennae, not reaching the end of fore femora), instead of the more appropriate *Candovia* (with long antennae), if it had really been an Australian species [transferred to *Candovia* in Brock & Hasenpusch (2007)]. As with many phasmids, the several genera mentioned above have not been studied in any detail by researchers and are in need of revision.

## MATERIAL AND METHODS

### TAXON SAMPLING

Adult specimens were collected between 2009 and 2017 across 21 locations in southern and eastern Australia (Fig. 1; Table 1) by Paul Brock (PDB), Braxton Jones (BRJ) and Noelene Tweed, or were examined in museums. All type material of existing species was examined by visits to various collections, with photographs and type data included on the Phasmida Species File (Brock *et al.*, 2021). Specimens of new taxa collected in Queensland by BRJ were obtained under the permit #WITK18701717; others associated with PDB via Australian Biological Resources Study permits over several years. In a few cases, Jack Hasenpusch (JH) reared specimens on PDB's behalf. Sampling sites were visualized geographically using the R package



**Figure 1.** Geographical distribution of the 21 Australian sampling sites.

ozmaps (<https://cran.r-project.org/package=ozmaps>). Eggs from live insects were collected where possible (with females kept in suitable plastic containers with ventilation and a supply of foodplant leaves) and specimens were mounted, with the right midleg stored in 100% ethanol for molecular analysis. Voucher specimens were deposited at the Queensland Museum, Brisbane (QM), Australian National Insect Museum, Canberra (ANIC) and the Natural History Museum, London (NHMUK). We referred to new specimens which could not be reliably assigned to any known

species as morphospecies D to L, therefore adding to the three putative morphospecies A–C previously proposed (Supporting Information, Table S1; Velonà *et al.*, 2015). Sixty-seven individuals (Table 1) were selected for molecular analyses; their leg tissues were preserved in ethanol and stored at 4 °C until use. For phylogenetic analyses, 18 publicly available sequences of Necrosciinae species were drawn from GenBank and *Extatosoma tiaratum* (Macleay, 1826) (Phasmatidae, Extatosomatinae) was used as outgroup (Supporting Information, Table S1a).

**Table 1.** Sampling information about analysed *Candovia* samples. Map IDs refer to [Figure 1](#). Sampling site abbreviations are: QLD = Queensland, NSW = New South Wales, NT = Northern Territory, SA = South Australia. Sample IDs refer to [Figure 2](#).

| Map ID | Sampling site                                             | Initial species/<br>morphospecies | N | Latitude | Longitude | Sample ID                                 |
|--------|-----------------------------------------------------------|-----------------------------------|---|----------|-----------|-------------------------------------------|
| APP    | Springbrook, Apple Tree Park (QLD)                        | <i>C. annulata</i>                | 5 | -28.166  | 153.259   | PB-(0207-0211)                            |
| APP    | Springbrook Apple Tree Park(QLD)                          | <i>C. strumosa</i>                | 5 | -28.166  | 153.259   | PB-(0214-0218)                            |
| BAR    | Baralaba, Duaninga Road (QLD)                             | morphospecies G                   | 1 | -24.136  | 149.830   | PB-0295                                   |
| MTV    | Montville, Narrows Road(QLD)                              | <i>C. annulata</i>                | 9 | -26.698  | 152.866   | JH-00(37,39-42);<br>PB-00(66,67,77,98)    |
| MTV    | Montville,Narrows Road (QLD)                              | morphospecies A                   | 9 | -26.698  | 152.866   | JH-00(26-<br>31,43,44); PB-0060           |
| MTV    | Montville, Baroon Pocket Dam (QLD)                        | morphospecies C                   | 5 | -26.698  | 152.866   | JH-00(66-70)                              |
| MTV    | Montville, Baroon Pocket Dam (QLD)                        | morphospecies A                   | 1 | -26.698  | 152.866   | JH-0027                                   |
| BTL    | Blackdown Tableland (QLD)                                 | morphospecies D                   | 4 | -23.582  | 149.063   | PB-0(188-191)                             |
| BTL    | Blackdown Tableland(QLD)                                  | morphospecies E                   | 4 | -23.582  | 149.063   | PB-0(184-187)                             |
| BUL    | Bulli (NSW)                                               | <i>C. robinsoni</i>               | 7 | -34.330  | 150.901   | PB-<br>0(107,108,112,115,<br>118,119,121) |
| BYF    | Byfield (QLD)                                             | morphospecies F                   | 3 | -22.847  | 150.650   | PB-0(325, 327, 328)                       |
| CAV    | Rossmoya Road, The Caves (QLD)                            | morphospecies F                   | 4 | -23.159  | 150.457   | PB-<br>0(258,261,271,272)                 |
| COH    | Colo Heights (NSW)                                        | <i>C. coenosa</i>                 | 1 | -33.369  | 150.722   | PB-0164                                   |
| DAR    | D'Aguilar Range, Boombana (QLD)                           | morphospecies A                   | 1 | -27.404  | 152.794   | PB-0058                                   |
| DIN    | Charlevue Road, 18km West of Dingo (QLD)                  | morphospecies I                   | 4 | -23.638  | 149.110   | BJ-00(91-94)                              |
| ECB    | Ellery Creek Big Hole, West Macdonnell National Park (NT) | <i>C. pallida</i>                 | 4 | -23.778  | 133.073   | BJ-00(30,31,32,34)                        |
| ENP    | Eungella National Park, Broken River (QLD)                | morphospecies B                   | 4 | -21.162  | 148.512   | PB-00(18-21)                              |
| EVH    | Evans Head (NSW)                                          | <i>C. aberrata</i>                | 5 | -29.108  | 153.431   | PB-0(157-161)                             |
| FSR    | Fountain Springs Rest Area, 60km East of Mount Isa (QLD)  | morphospecies L                   | 1 | -20.800  | 139.996   | BJ-0095                                   |
| KOS    | Kosciuszko National Park, Guthega Road (NSW)              | <i>C. spurcata</i>                | 2 | -36.500  | 148.266   | PB-00(155, 156)                           |
| LIB    | Little bay, Harvey Street (NSW)                           | <i>C. coenosa</i>                 | 2 | -33.983  | 151.244   | BJ-00(41-42)                              |
| MTG    | Mount Gambier (SA)                                        | <i>C. peridromes</i>              | 3 | -37.843  | 140.765   | PB-0(178-180)                             |
| MTW    | Mt. Warning, Wollumbin (NSW)                              | <i>Candovia</i> sp. H             | 2 | -28.400  | 153.282   | PB-0(173, 174)                            |
| PIL    | Pilliga (NSW)                                             | <i>C. robinsoni</i>               | 1 | -30.350  | 148.890   | PB-0162                                   |
| RST    | Springbrook, Repeater Station Rd (QLD)                    | <i>C. granulosa</i>               | 4 | -28.234  | 153.267   | PB-0(220-223)                             |
| WIL    | Wilmington, Park road (SA)                                | <i>C. peridromes</i>              | 5 | -32.840  | 138.036   | BJ-0(12-16)                               |

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING  
Genomic DNA was isolated for the 67 newly collected specimens using the kit Smarter Nucleic Acid Preparation (Stratagene) following the manufacturer's standard protocol. The Folmer region of cytochrome *c* oxidase subunit I (*COI*) for DNA barcoding (Folmer *et al.*, 1994; henceforth referred to as *COI<sub>Fol</sub>*) was polymerase chain reaction (PCR) amplified from all samples using primers and the condition is given in the [Supporting Information, Table S2](#). On the basis of the species delimitation results, we selected 39 specimens

for which, in addition to the *COI<sub>Fol</sub>*, we amplified seven further loci: four mitochondrial [a *COI* fragment downstream of the *COI<sub>Fol</sub>* region, and indicated as *COI*; cytochrome oxidase *c* subunit II (*COII*); 12S and 16S rDNAs] and three nuclear markers [histone subunit 3 (*H3*); 18S and 28S rDNAs]. Amplified fragment length, primers and thermal cycling conditions can be found in the [Supporting Information, Table S2](#). All PCR products were visualized by 1% agarose gel electrophoresis, subsequently purified using ExoSAP-IT PCR Product Cleanup Reagent (ThermoFisher) and sequenced by

MacroGen Inc. Chromatograms were inspected using SEQTRACE v.0.9.0 (Stucky, 2012) and the resulting sequences were manually checked using ALIVIEW v.1.26 (Larsson, 2014). We then compared the obtained sequences with the NCBI GenBank database using BLAST (with *blastn* algorithm: Altschul *et al.*, 1990) to identify potential contaminants. New sequences were submitted to GenBank under the accession numbers MT077516–MT077845 (Supporting Information, Table S1b for the *COI<sub>fol</sub>* sequences used for species delimitation and the Supporting Information, Table S1c for the seven additional gene fragments used for phylogenetic analyses).

#### MOLECULAR SPECIES DELIMITATION

*COI<sub>fol</sub>* fragments have been aligned as amino acids using MAFFT v.7 with *--auto* parameter setting (Katoh & Standley, 2013) and then retrotranslated to nucleotides. The dataset was partitioned in the three-codon positions and analysed by both maximum likelihood (ML) and Bayesian inference (BI) methods. All phylogenetic analyses were carried out with XSEDE (eXtreme Science and Engineering Discovery Environment, <https://www.xsede.org>) through CIPRES Science Gateway ([www.phylo.org](http://www.phylo.org); Miller, Pfeiffer & Schwartz, 2012). ML tree was obtained with IQ-TREE v.1.6.12 (Nguyen *et al.*, 2014). The best-fit models of nucleotide substitution were identified using ModelFinder (Kalyaanamoorthy *et al.*, 2017; Supporting Information, Table S3a); then, ten ML searches were run with 1000 ultrafast bootstrap replicates and the run with the best likelihood was selected as the most reliable. For the BI, we determined the best-fit nucleotide substitution model using PARTITIONFINDER2, based on the corrected Bayesian Information Criterion using the edge-linked parameter and the greedy strategy (Lanfear *et al.*, 2016; Supporting Information, Table S3a). But the partition scheme and the substitution model selected by PARTITIONFINDER led to poor mixing, most likely due to over-parametrization. We thus modified the partition scheme to use the less parametrized model HKY.

The *COI<sub>fol</sub>* dataset was analysed using several species-delimitation approaches to explore the species diversity of the genus and to explore the species hypotheses. For this purpose, we used both distance and evolutionary model-based methods, without a priori species hypothesis: (1) automatic barcode gap discovery (ABGD; Puillandre *et al.*, 2012) analysis was performed using Jukes–Cantor distances, a relative gap width of 1 and 10 bins; (2) the different Poisson tree processes approaches (PTP and mPTP; Zhang *et al.*, 2013; Kapli *et al.*, 2017) were carried out using the

IQ-TREE best ML tree as input: two runs of 1 000 000 generations with sampling every 100 and a burn-in of 20% as parameters were carried out and compared for convergence; (3) the general mixed Yule coalescent (GMYC; Pons *et al.*, 2006) was run on ultrametric trees, using the single threshold parameter. The concatenated sequence alignment (see below) was analysed with BEAST v.2.5 (Bouckaert *et al.*, 2019) to generate ultrametric trees for the GMYC species-delimitation method. We used four combinations of different clock and speciation model priors: strict or relaxed clock model and Yule or coalescent process. Each analysis was run with trees and parameter values sampled every 5000 steps over a total of 60 million generations. A burn-in of 20% steps was discarded and adequate sample sizes (ESS > 200) were checked using TRACER v.1.6 (Rambaut *et al.*, 2018). Trees were summarized in the maximum clade credibility tree from the posterior distribution in TREEANNOTATOR v.1.4.7 (Drummond *et al.*, 2012). GMYC and ABGD were used with their online implementation (available at <https://species.hits.org/gmyc/> and <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>, respectively).

#### MOLECULAR PHYLOGENETIC ANALYSES

Each gene was aligned separately using MAFFT v.7 with the option *--auto* for protein-coding genes (PCGs) and with the option *--X-INS-i* for the rDNA genes (Katoh & Standley, 2013). We then manually inspected alignments using AliView (Larsson, 2014) to select the correct reading frame and to check for stop codons. Ambiguously aligned regions were removed from the single-gene alignments with GBLOCKS (Talavera & Castresana, 2007) with options for a less stringent selection. We then concatenated gene sequences to form a single-character matrix using PHYUTILITY (Smith & Dunn, 2008).

Model selection, ML and BI analyses were carried out with XSEDE (eXtreme Science and Engineering Discovery Environment, <https://www.xsede.org>) through the CIPRES Science Gateway ([www.phylo.org](http://www.phylo.org); Miller *et al.*, 2012). For the ML approach, the best-fit partitioning scheme and models of nucleotide substitution were identified using IQ-TREE v.1.6.12 ModelFinder (Kalyaanamoorthy *et al.*, 2017) (Supporting Information, Table S3b) using the edge-proportional parameter. We then inferred ten trees using IQ-TREE v.1.6.12 (Nguyen *et al.*, 2014) with 1000 ultrafast bootstraps, and the run with the best likelihood was selected. For the BI, we determined the best-fit nucleotide substitution model and the optimal partitioning scheme using PARTITIONFINDER2, based on the corrected Bayesian information criterion, using the edge-linked parameter and the greedy strategy



(Lanfear *et al.*, 2016), with each rDNA and each codon position of the four PCGs as separate initial partition (Supporting Information, Table S3b). The MCMC analysis was run with the tree and parameter values sampled every 5000 steps over a total of 50 million generations. A burn-in of 10% steps was discarded, and adequate sample sizes (ESS > 200) were checked using TRACER v.1.7.7 (Rambaut *et al.*, 2018).

#### MORPHOLOGICAL EXAMINATION

Many specimens were examined as part of this study, including the type and molecular material detailed in this paper. Images were taken with a Nikon D5500 with a Nikon 105 mm macro lens and macro flash, often in nature at night. Images were processed in ADOBE PHOTOSHOP with slight changes, if any. When measuring specimens, the body length of examined specimens was measured to the nearest 0.5 mm and eggs to the nearest 0.1 mm. Microscopes used included available equipment during visits to ANIC and QM, also a specially made GX research microscope (GT Vision, UK), with a flat base suitable for phasmids by PDB.

### RESULTS

#### MOLECULAR SPECIES DELIMITATION

The *COI<sub>pol</sub>* alignment was 666 bp long, and correctly translated for 222 amino acids sequences without any stop codon. BLAST searches against NCBI GenBank database consistently resulted in congeneric species best-hits. The final dataset consisted in 67 newly generated sequences and 29 previously published sequences, bringing the total number of operational taxonomic units (OTUs) to 96. The obtained ML and BI trees showed fully compatible topologies (Fig. 2; Supporting Information, Fig. S1). Using the different species-delimitation approaches, the number of hypothetical species ranged from 19 to 24 for PTP, which proved to be the most conservative approach, while GMYC was the method identifying the highest number of hypothetical species (24 spp.; Fig. 2).

A full agreement of molecular-delimitation approaches with most known taxa can be observed, such as for *C. aberrata* (Brunner, 1907), *C. granulosa* (Brunner, 1907), *C. pallida* (Sjöstedt, 1918), *C. robinsoni* Brock & Hasenpusch, 2007 and *C. strumosa*. This holds also for the previously recognized putative morphospecies B and C. In other instances, morphospecies are split in different taxa or merged in a single entity. *Candovia peridromes* (Westwood, 1859) splits in all approaches in two groups, according to a geographical pattern, while this occurs for *C. annulata* in the GMYC analyses only. The specimen PB-0178,

identified as *C. peridromes*, is further found as a separate taxon by sPTP and the two GMYC approaches using the strict molecular clock, therefore calling for particular attention on this entity. *Candovia coenosa* (Gray, 1833) is consistently recognized as composed by two putative species, separating the PB-0164 specimen from the other two congeneric samples. On the other hand, the mPTP method merges all *C. coenosa* specimens with those of *C. spurcata* (Brunner, 1907) in a single hypothetical species. Finally, the specimen JH-0027 of morphospecies A is recognized as divergent by two GMYC analyses (Fig. 2). Regarding the newly collected specimens, in both ML and BI trees they have been partitioned into well-defined clusters identified by the species-delimitation analysis as new putative taxonomic entities (henceforth named spp. D to L; Fig. 2). Although, for sp. F, two specimens were recognized as a separate taxon by two GMYC analyses (Fig. 2).

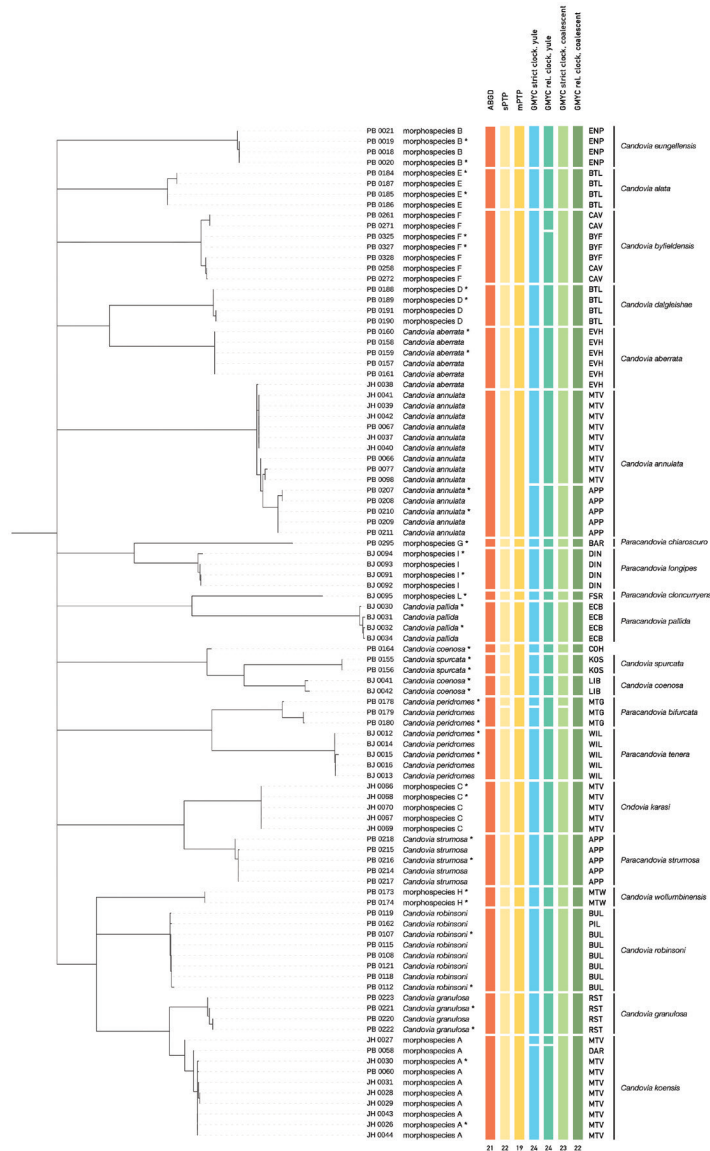
#### MOLECULAR PHYLOGENETIC ANALYSES

The final dataset used for phylogenetic analyses, a concatenation of the eight analysed mitochondrial and nuclear markers, consisted of 4540 sites and 58 specimens representing 30 species. The ML and BI analyses provided identical tree topologies, with most nodes more strongly supported in the BI tree than in the ML tree. Both phylogenetic analyses identify *Candovia* as monophyletic with respect to the other Necrosiinae taxa included in the present analysis (Fig. 3; Supporting Information, Fig. S2). Within the clade, the observed clustering pattern appeared more resolved, in terms of phyletic relationships and nodal supports, than in the analysis of *COI<sub>pol</sub>* dataset alone.

The first split within the clade identified a cluster including *C. peridromes*, *C. pallida* and five putative species (*Candovia* spp. G, I and L) in a sister-relationship to all remaining *Candovia* species (consisting in the newly erected *Paracandovia* genus). Samples of *C. spurcata* and *C. coenosa* (the type species of *Candovia*) form a monophyletic clade, with the latter taxon being paraphyletic. Further well-defined clusters are given by: *C. annulata* + spp. B and E; *C. strumosa* + spp. C and F; *C. aberrata* + sp. D; *C. robinsoni* and *C. granulosa* + spp. A and H (Fig. 3). The pattern observed in the species-delimitation analysis for *C. coenosa* appears also here, with the sample PB-164 more related to the *C. spurcata* clade than to the putatively conspecific specimens.

#### MORPHOLOGICAL ANALYSES

Thanks to a detailed taxonomic analysis supported by molecular data, we present the description and illustration of seven new *Candovia* species from various parts of Queensland and New South Wales, including



**Figure 2.** Species delimitations summarised on the maximum likelihood tree with a bootstrap cut off of 80%. The names at the tips of the  $COI_{Pst}$  phylogenetic tree represent the initial species identifications and hypotheses; coloured bars represent hypothetical species identified by the different approaches; the names on the right represent the final species identified. Acronyms on the right are relative to geographic collection points, as reported in Figure 1.





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eggs, where available (named below as *C. alata* Brock & Jones sp. nov., *C. byfieldensis* Brock & Jones sp. nov., *C. dalglishae* Brock & Jones sp. nov., *C. eungellensis* Brock & Jones sp. nov., *C. karasi* Brock & Jones sp. nov., *C. koensi* Brock & Jones sp. nov. and *C. wollumbinensis* Brock & Jones sp. nov.). These are placed in species groups also in agreement with molecular phylogenetic evidence. Finally, several new *Candovia* species combinations are proposed and removed from synonymy. To partially account for molecular suggestions, but also given clear-cut morphological characters, a new genus *Paracandovia* is erected: *P. cercata* (Redtenbacher, 1908) stat. rev., comb. nov., *P. longipes* (Brunner, 1907) stat. rev., comb. nov., *P. pallida* (Sjöstedt, 1918) comb. nov., *P. peridromes* (Westwood, 1859) comb. nov. and *P. tenera* (Brunner, 1907) stat. rev., comb. nov. Three taxa were removed from synonymy with *C. peridromes* (Westwood, 1859). Detailed images of type specimens will be available upon publication of this paper via the Phasmida Species File on-line <http://phasmida.speciesfile.org> (see Taxonomic account for details). A key to *Candovia* groups and a guide to *Candovia* and *Paracandovia* species are given in [Supporting Information, File S1](#).

## TAXONOMIC ACCOUNT

*CANDOVIA* Stål, 1875: 12, 70.

Type species: *Phasma* (Bacteria) *coenosum* Gray, 1833: 28, pl. 2: 2 [= *Candovia coenosa*], by original monotypy.

Remarks: Brock & Hasenpusch, 2007: 7, 70 (removed from synonymy with *Hyrtacus* Stål, 1875, in which it had been placed by Kirby, 1904: 331). Brunner, 1907: 301 [listed the type species as a synonym of *Hyrtacus eutrachelia* (Westwood, 1859)], Vickery, 1983: 7 (reinstated the type species as valid, as *Hyrtacus coenosa*). For other references, see Brock *et al.* (2021), but it is notable that Westwood (1859: 33) regarded the figure in Gray (1833) as being 'too large and robust' but he rightly points out that the female body has 'shrunk in various parts' and this is an accurate assessment. He illustrated a potential male on pl. 27: 2. To further add to confusion, several species currently in *Candovia* were regarded as belonging to *Parasipyoidea* Redtenbacher, 1908 by Vickery (1983) and Balderson *et al.* (1998); hence Brock & Hasenpusch, 2007 placed all Australian *Parasipyoidea* species and *Candovia coenosa* in *Candovia*, as a temporary step, in the knowledge that this needed splitting and that several other undescribed wingless, and even winged, species appeared to belong to this genus.

Description: Small to medium-sized (40–89 mm), plain or mottled, in nature brown or green, sometimes (as in type species and some others) with central black marks at end of most body segments, usually wingless (if

winged, can be short-winged in female), elongate (one species group with plumper females), moderately long to long-legged phasmids, with the body smooth, granulated or with sparse tubercles, the latter particularly conspicuous in females. Cerci short in both sexes.

Head at least as long as, or slightly longer than, wide. Head, pronotum and mesonotum smooth, sparsely or extensively granulate with some tubercles possibly present on mesonotum and metanotum, including laterally. Antennae exceeding length of forelegs, with numerous segments (up to 132); two basal segments broadened. Pronotum slightly shorter or equal in length to head, mesonotum long, 4.9–6.4 × length of pronotum; mesonotum 1.5–2.4 × combined length of metanotum and the shorter median segment. Wingless, except wings known in two species so far, which are short in the female of one species (forewings short, hindwings tiny) and hindwings in male reach up to just beyond the end of the fifth abdominal segment. Anal segment in female rounded at tip, may be slightly incised in centre, with supra-anal plate just visible beneath. Operculum not broad, rounded at tip, almost reaching end of ninth abdominal segment to beyond it. Anal segment tip in male slightly triangular emarginated, poculum a modest size with rounded tip, reaching just over half length of ninth abdominal segment. Cerci short, slender but sometimes stout and hairy. Legs long, smooth; hindlegs reaching in excess of elongate abdomen (midlegs longer than many phasmids, only slightly shorter than hindlegs, except for some species in *C. strumosa* group, which have midlegs short, only just reaching beyond end of hind femora when set near body). Egg: oval or nearly so, or almost rectangular, some species with low raised, broad mound-like structure on operculum ([Supporting Information, Fig. S3](#)). Micropylar plate elliptical or spear-shaped. Capsule rugged appearance, with various sculpturing and sometimes pitted.

Distribution: Australia.

## Species included:

*C. aberrata* group

*Candovia aberrata* (Brunner, 1907) [aberrant stick insect]: New South Wales, Queensland.

*Candovia dalglishae* [Dalglish's stick insect] [= *Candovia* sp. D]: Queensland.

*C. annulata* group

*Candovia alata* [winged candovia] [= *Candovia* sp. E]: Queensland.

*Candovia annulata* (Brunner, 1907) [banded-legged stick insect]: New South Wales, Queensland.

*Candovia eungellensis* [Eungella stick insect] [= *Candovia* sp. B]: Queensland.

*C. coenosa* group

*Candovia coenosa* (Gray, 1833) [muddy stick insect]: New South Wales.

*Candovia spurcata* (Brunner, 1907) [Blue Mountain stick insect]: New South Wales

*C. robinsoni* group

*Candovia granulosa* (Brunner, 1907) [granulated stick insect]: New South Wales, Queensland.

*Candovia koensi* [Koens's stick insect] [= *Candovia* sp. A]: Queensland.

*Candovia robinsoni* [Robinson's stick insect] (Brock & Hasenpusch, 2007): New South Wales, Queensland.

*Candovia wollumbinensis* [Wollumbin stick insect] [= *Candovia* sp. H]: New South Wales.

*C. strumosa* group

*Candovia byfieldensis* [Byfield stick insect] [= *Candovia* sp. F]: Queensland.

*Candovia karasi* [Karas's stick insect] [= *Candovia* sp. C]: Queensland.

*Candovia strumosa* (Redtenbacher, 1908) [Richmond River stick insect]: New South Wales, Queensland.

*Removed from the Australian list: Candovia evoneoberti* (Zompro & Adis, 2001) [Evoneoberti's stick insect], a species with long cerci thought to be 'introduced' to Brazil and Paraguay but misidentified as *Candovia*. Brock & Hasenpusch (2009) stated that this species belongs near *Paracalynda* Zompro, 2001. The species is now a synonym of *Arumatia dubia* (Caudell, 1904) (Ghirotto et al., 2022).

**CANDOVIA ALATA BROCK & JONES SP. NOV.**  
(SUPPORTING INFORMATION, FIG. S4A FOR FEMALE  
AND S4B FOR MALE)

[winged candovia]

*Zoobank registration*: urn:lsid:zoobank.org:act:099FF0EA-9365-4786-94E6-8B5519ADB4EA.

*Type material*: *Holotype*: ♀, QUEENSLAND, Blackdown Tableland National Park, 23.582°S, 149.063°E, 14.iii.2016, N. Tweed, DNA project PB-0184 (QM). *Paratypes*: QUEENSLAND: 1 ♂, same data, DNA project PB-0185 (QM); 2 ♂♂, same data, DNA project PB-0186 to PB-0187 (NHMUK). (Note, all DNA project data was provisionally labelled *Candovia* sp. E). Eggs also deposited in NHMUK (not paratypes).

*Overview of both sexes*: Elongate body brown in both sexes, with lateral tubercles on thorax. Black line on

head between eyes and also black, longitudinal line running length of body. Hindwings dusky brown, modest length. Legs indistinctly mottled.

*Female* (Supporting Information, Fig. S4A): Damaged. Head: longer than wide. Antennae long, with numerous indistinct segments; basal segment and shorter segment 2 broader than remaining segments. Thorax: mesonotum with granules and several tubercles laterally. Pronotum slightly shorter than head, with central impression. Mesonotum  $4.3 \times$  length of pronotum. Mesonotum  $1.5 \times$  combined length of metanotum and median segment, the latter about same length as metanotum. Winged, hindwings of modest length. Abdomen: eighth to tenth segment similar in length. Operculum tapered to pointed tip, reaching end of anal segment; the anal segment has tapered sides towards tip, which is incised in centre. Cerci shorter than anal segment. Legs: long.

*Male* (Supporting Information, Fig. S4B): Much slenderer, but similar in general appearance, with more black marks than female. Wings reach up to just beyond up of fifth abdominal segment. Anal segment tip slightly incised in centre; cerci shorter than segment. Poculum not reaching end of ninth abdominal segment.

*Egg* (Supporting Information, Fig. S3A): Brown, dark and heavily sculptured in paler brown; broad, almost oval capsule. Micropylar plate pear-shaped, dark brown, surrounded by paler mottled area; whilst central, higher up than eggs of related species. Operculum dome-like and heavily sculptured.

*Measurements (mm)*. *Length of body*: Female 84, male 61–64. *Head*: female 4, male 2. *Antennae*: female 50 (tip broken), male 57–64. *Pronotum*: female 3.5, male 2. *Mesonotum*: female 15, male 10–11. *Metanotum*: female 5, male 3.5–4.0. *Median segment*: female 5, male 3.5–4.0. *Forewings*: female 5, male 3. *Hindwings*: female 36, male 28–30. *Femora, fore, mid, hind*: female 25, 19, 22, male 21–22, 16, 21–22. *Tibiae, fore, mid, hind*: female 24, 20, 27, male 25, 17–18, 25–26. *Cerci*: female 2.3, male 1.2–1.3. *Eggs*: capsule length 2, width 1.6, height 1.6.

*Distribution* (Fig. 2): This species is so far only known from the Blackdown Tableland National Park.

*Habitat and foodplants*: The species is found in forest edge, foodplants not recorded.

*Etymology*: Named after the presence of wings in this species.

***CANDOVIA BYFIELDENSIS* BROCK & JONES SP. NOV.**  
(SUPPORTING INFORMATION, FIG. S5A, B)

[Byfield stick insect]

*Zoobank registration:* urn:lsid:zoobank.org:act:C7C16BF6-C376-4D18-AB0B-B3770398D2CE.

*Type material:* *Holotype:* ♀, QUEENSLAND, Byfield, 22.847°S, 150.650°E, 13.i.2013, N. Tweed, DNA project PB-0327 (QM). *Paratypes:* QUEENSLAND: 1 ♂, same data, except 09.i.2013, DNA project PB-0324 (QM); 1 ♂, same data, 09.i.2013, DNA project PB-0325 (NHMUK); 1 ♂, 1 ♀, same data, 18.i.2013, DNA project PB-0330 to PB-0331 (NHMUK); 1 ♀, same data, 15.i.2013, DNA project PB-0328; (NHMUK); 1 ♂, 1 ♀, Rossmoya Road, The Caves, 23.159°S, 150.457°E, 15.i.2012, DNA project PB-0271 to PB-0272 (NHMUK); 1 ♀, same data, 22.i.2012, DNA project PB-0258 (NHMUK); 1 ♀, same data, 04.ii.2012, DNA project PB-0261 (NHMUK); 1 ♀, Pistol Gap, 10 km SSE of Byfield, 10.i.1970, Britton, Holloway & Misco (ANIC). (Note, all DNA project data was provisionally labelled *Candovia* sp. F.) Eggs also deposited in NHMUK (not paratypes).

*Overview of both sexes:* Female body fairly elongate, plain brownish green, head yellowish, mesonotum with sparse whitish tubercles, mainly laterally. Head with suffused blackish band from eyes to back of head. Antennae dark brown. The male has a dark greenish mesonotum and metanotum with pale yellowish brown head, pronotum and abdomen, both with black lines and streaks, including broad, black, median line on whole body, which may be broken in places. Antennae black. Both sexes have eyes with two horizontal, dark mauve to black lines.

*Female* (Supporting Information, Fig. S5A): Head: longer than wide. Antennae longer than forelegs, with numerous indistinct segments; basal segment broad and much longer than segment 2. Thorax: pronotum slightly shorter than head, with central impression. Mesonotum about 6 × length of pronotum. Mesonotum 1.7 to 2.0 × combined length of metanotum and short median segment. Wingless. Abdomen: elongate eighth segment longer than ninth; the latter and tenth (anal) segment are of similar size. Operculum tapered to pointed tip, reaching up to half length of segment, which has rounded tip. Cerci short. Legs: moderately long, hindlegs sometimes exceeding tip of abdomen.

*Male* (Supporting Information, Fig. S5B): Apart from being much slenderer, similar in general appearance, except distinctive dark colour. Anal segment subtruncate at tip, cerci short, curved. Poculum

reaching about half length of ninth abdominal segment.

*Egg* (Supporting Information, Fig. S3C): Dark brown, with a modest amount of pale brown sculpturing, almost oval capsule. Micropylar plate central, broad and pointed at tip, slightly lighter shade of brown than capsule ground colour, surrounded by broad pale whitish brown area, as on opercular rim. Operculum with sculptured paler inner circle.

*Measurements (mm).* *Length of body:* 74–88 (holotype 76), male 59–68. *Head:* female 4–4.5, male 2.5–2.7. *Antennae:* female 60–70, male 55–65. *Pronotum:* female 3, male 2.3. *Mesonotum:* female 18–20, male 14–16. *Metanotum:* female 5.5–6.0, male 4–5. *Median segment:* female 3.5–4.0, male 2.7–3.0. *Femora, fore, mid, hind:* female 21–23, 16–18, 20–24, male 18–22, 18–20, 20–22. *Tibiae, fore, mid, hind:* female 24–26, 16–18, 24–27, male 20–28, 15–21, 22–29. *Cerci:* female 0.5, male 0.5. *Eggs:* capsule length 1.8, width 1.2, height 1.4.

*Distribution* (Fig. 2): This species is fairly widespread in the Byfield area and also recorded 84 km away at The Caves.

*Habitat and foodplants:* The species is found in rainforest edge and more open areas with saplings, foodplants include *Acacia* Mill. and *Eucalyptus* L'Hér. species.

*Etymology:* Named after the main type locality, Byfield, which has some superb rainforest habitat, in gardens as well as national parks.

***CANDOVIA DALGLEISHAE* BROCK & JONES SP. NOV.**  
(SUPPORTING INFORMATION, FIG. S6A, B)

[Dalglish's stick insect]

*Zoobank registration:* urn:lsid:zoobank.org:act:93A1D5CA-92ED-43A1-947D-503BD20B6009.

*Type material:* *Holotype:* ♀, QUEENSLAND, Blackdown Tableland National Park, 23.582°S, 149.063°E, ii.2017, reared by B. Jones, DNA project PB-0168 (QM). *Paratypes:* QUEENSLAND: 3 ♂♂, same data, DNA project PB-0165 to PB-0167 (QM); 1 ♀, 3 ♂♂, same data, i.2017, N. Tweed, DNA project PB-0188 to PB-0191 (NHMUK). (Note, this DNA project data was provisionally labelled *Candovia* sp. D.)

*Overview of both sexes:* Elongate, pale greenish female, each side of head with a narrow, part blackish longitudinal band, running from eyes to back of head. Upper part of pronotum with two black lines well

separated from narrow black median line, which runs length of body. The male has a dark green thorax (except black pronotum, hind part of mesonotum and metanotum) with pale yellowish brown head and abdomen, both with black streaks. Antennae black. Both sexes have hind part of thoracic and abdominal segments with broad orange band (preceded by indistinct darkish marks and more distinct marks beneath), also brown legs. Eyes paler than head with horizontal black line.

**Female** (*Supporting Information, Fig. S6A*): Head: longer than wide. Antennae long and pale, not as long as forelegs, with numerous indistinct segments, darkened at tip; basal segment and much shorter segment 2 broader than remaining segments. Thorax: smooth, pronotum slightly shorter than head, with central impression. Mesonotum just under  $6 \times$  length of pronotum. Mesonotum  $1.5 \times$  combined length of metanotum and short median segment. Wingless. Abdomen: elongate. Eighth segment much longer than ninth and tenth (anal), which are of similar size. Operculum tapered to tip, not reaching end of anal segment, which has rounded tip. Cerci short. Legs: elongate.

**Male** (*Supporting Information, Fig. S6B*): Apart from being much slenderer, similar in general appearance, except distinctive dark colour. Anal segment rounded at tip; cerci short. Poculum reaching about half length of ninth abdominal segment.

**Egg** (*Supporting Information, Fig. S3E*): Brown, dark with pale brown sculpturing, almost oval capsule. Micropylar plate central, broad and pointed at tip, lighter shade of brown than capsule ground colour, surrounded by broad pale whitish brown area.

**Measurements (mm).** *Length of body:* female 89, male 60–66. *Head:* female 4, male 2.5. *Antennae:* female 25, male 53 (tips broken off in both sexes). *Pronotum:* female 3.5, male 2.5. *Mesonotum:* female 20, male 14. *Metanotum:* female 9, male 6.5. *Median segment:* female 4, male 3. *Femora, fore, mid, hind:* female 27, 21, 26, male 22, 22, 24. *Tibiae, fore, mid, hind:* female 30, 24, 31, male 30, 25, 33. *Cerci:* female 0.4, male -. *Eggs:* Capsule length 2.2, width 1.1, height 1.2.

**Distribution** (*Fig. 2*): This species is widespread, at least in the Blackdown Tableland National Park (Beth Ripper, Noelene Tweed, pers. comm.).

**Habitat and foodplants:** The species is found in forest and often seen paired, where they feed on *Acacia* and *Eucalyptus* spp., also accepted in captivity.

**Etymology:** Named after Kirsten Dalgleish, a keen phasid rearer who travelled a considerable distance to hand PDB samples of live *Candovia* spp. for molecular work.

#### **CANDOVIA EUNGELLENSIS BROCK & JONES SP. NOV.**

(*SUPPORTING INFORMATION, FIG. S7A, B*)

[Eungella stick insect]

*Zoobank registration:* urn:lsid:zoobank.org:act:27778BA0-C336-440F-82D1-C0978110A3F1.

**Type material:** *Holotype:* ♂, QUEENSLAND, Broken River, Eungella National Park, 21.1681°S, 148.5044°E, 19.xi.2009, P.D. Brock, DNA project PB-0018 (QM). *Paratypes:* QUEENSLAND: 1 ♂, 2 ♀♀, same data, DNA project PB-0019 to PB-0021 (QM). (Note, this DNA project data was provisionally labelled *Candovia* sp. B), 2 ♂♂, 1 ♀, QUEENSLAND, Eungella National Park, late xii.1992 (the ♀ labels state 'laid eggs' the ♂♂ state reared from eggs laid in laboratory xii.1992). Hatched 15.ii.1993 and 15.xii.1993 respectively (ANIC).

**Overview of both sexes:** Elongate green or brown female, possibly with darker marks and indistinctly banded legs. Male slenderer, brown with black marks laterally on pronotum, hind part of mesonotum, metanotum (more indistinct or absent in female), including specks on abdomen, apices of femora and tibiae. Whitish marks may also be present laterally. Head and pronotum with sometimes indistinct darker marks. Both sexes with black lines from eyes to back of head, orange mouthparts and eyes brown or orange with two horizontal black lines. Thorax granules and tubercles sometimes whitish. Dull-orange bands may be present on hind part of all thoracic and abdominal segments, with small black central mark, but this seems occasional and mainly in some females.

**Female** (*Supporting Information, Fig. S7A*): Head: slightly longer than wide. Antennae long, with numerous indistinct segments; basal segment and shorter segment 2 broader than remaining segments. Thorax: with granules and tubercles, the latter more on mesonotum. Pronotum about same length as head, with central impression. Mesonotum up to  $4.4 \times$  length of pronotum. Mesonotum  $2.4 \times$  combined length of metanotum and median segment, the latter slightly longer than metanotum. Wingless. Abdomen: ridged, with granules. Eighth segment longer than ninth which is longer than tenth (anal) segment. Operculum tapered to tip, just exceeding end of segment 9; the anal segment has tapered sides towards tip. Cerci short. Legs: moderately long.



**Male** (*Supporting Information, Fig. S7B*): Slenderer, but similar in general appearance, except for distinctive body patterning. Anal segment rounded at tip; cerci short. Poculum about reaching end of ninth abdominal segment.

**Egg** (*Supporting Information, Fig. S3F*): Brown, dark with paler brown mottled sculpturing, broad almost oval capsule. Micropylar plate central, broad and pointed at tip, lighter shade of brown than capsule, surrounded by paler mottled area. Operculum dome-like and heavily sculptured.

**Measurements (mm).** *Length of body*: female 71–75, male 53–55. *Head*: female 4–5, male 2.5. *Antennae*: female 54–60, male 60. *Pronotum*: female 4–5, male 2.3. *Mesonotum*: female 17–18, male 10. *Metanotum*: female 3.3–4.0, male 3.5. *Median segment*: female 3.7, male 3.5–4.8. *Femora, fore, mid, hind*: female 22, 16, 20, male 18, 12, 17. *Tibiae, fore, mid, hind*: female 22, 14, 21, male 18, 11, 17. *Cerci*: female 1, male 0.8. *Eggs*: Capsule length 2.2, width 1.6, height 1.8.

**Distribution** (*Fig. 2*): This species is so far only known from Eungella National Park.

**Habitat and foodplants**: The species is found in forest edge, feeding on *Eucalyptus* spp. and others.

**Etymology**: Named after the type locality, Eungella National Park, a superb area of subtropical rainforest, well known for its platypuses.

***CANDOVIA KARASI* BROCK & JONES SP. NOV.**  
(*SUPPORTING INFORMATION, FIG. S8A, B*)

[Karas's stick insect]

*Zoobank registration*: urn:lsid:zoobank.org:act:EB26E337-EC01-401E-8F84-E01AE5B878DB.

**Type material**: *Holotype*: ♂, QUEENSLAND, Narrows Road, nr Baroon Pocket Dam, Montville, 26.6983°S, 152.8656°E, 10.xii.2009, A. Karas, DNA project JH-0070 (QM). *Paratypes*: QUEENSLAND: 1 ♀, same data, DNA project, JH-0068 (QM); 1 ♂, 2 ♀♀, same data, DNA project JH-0069, JH-0066, JH-0067 (NHMUK). (Note, this DNA project data was provisionally labelled *Candovia* sp. C.)

**Overview of both sexes**: Female body greenish brown, with black marks on head and pronotum, also remainder of thorax and abdomen. Male more colourful, greenish brown with green mesonotum and black marks on head, pronotum and abdomen. Mesonotum and metanotum with sparse granules/

occasional tubercles. Hind part of female abdominal segment 6 slightly swollen. Forewings and pre-anal part of hindwings dark brown, net-like with pale veins, hindwings dusky. Female short-winged with tiny hindwings, male long-winged, hindwings reaching beyond end of abdominal segment 5. In both sexes eyes are green and bases of antennal segments indistinctly pale banded.

**Female** (*Supporting Information, Fig. S8A*): Head: slightly longer than wide. Antennae longer than forelegs, with numerous indistinct paler bands at base of segments; basal segment and much shorter segment 2 broader than remaining segments. Thorax: pronotum slightly shorter than head, with central impression. Mesonotum almost 5 × length of pronotum. Mesonotum 1.6 × combined length of metanotum and short median segment. Mesonotum and metanotum with sparse granules and few well-spaced tubercles on mesonotum. Forewings short, hindwings tiny. Abdomen: elongate. Eighth segment much longer than ninth and tenth (anal), which are of similar size. Operculum rounded at tip, reaching about half length of anal segment, which has almost truncate tip. Cerci short. Legs: all of modest length, hindlegs not reaching end of abdomen.

**Male** (*Supporting Information, Fig. S8B*): Apart from being much slenderer, similar in general appearance, except wings. The forewings are truncate, hindwings reaching beyond end of abdominal segment 5. Anal segment rounded at tip; cerci short. Poculum reaching about end of ninth abdominal segment.

**Eggs**: Unknown.

**Measurements (mm).** *Length of body*: female 70–75, male 54–55. *Head*: female 3.5–3.6, male 2.4. *Antennae*: female 34–36, male 45. *Pronotum*: female 3.2, male 2. *Mesonotum*: female 15.0–15.5, male 9.8. *Metanotum*: female 4.2, male 2.8. *Median segment*: female 5.0–5.2, male 3.3. *Forewings*: female 2.0–2.3, male 2.8. *Hindwings*: female 1.8–2.0, male 27. *Femora, fore, mid, hind*: female 13–14, 9–10, 14, male 13, 8, 14. *Tibiae, fore, mid, hind*: female 12–13, 8–9, 15, male 14–15, 8, 16. *Cerci*: female 0.5, male 0.7.

**Distribution** (*Fig. 2*): This species is so far only recorded from Montville.

**Habitat and foodplants**: The species is found in forest, foodplants include *Eucalyptus* spp.

**Etymology**: Named after Alexander Karas who supplied the research stock, via Jack Hasenpusch who kindly processed it before a visit by PDB.

**CANDOVIA KOENSI** BROCK & JONES SP. NOV.

(SUPPORTING INFORMATION, FIG. S9A, B)

[Koens's stick insect]

*Zoobank registration:* urn:lsid:zoobank.org:act:5BCC0EB0-6241-4B45-A400-6133BBDE78F3.

*Type material:* *Holotype:* ♀, QUEENSLAND, Narrows Road, nr Baroon Pocket Dam, Montville, 26.6983°S, 152.8656°E, 15.xi.2011, A. Karas, P.D. Brock & J. Koens, DNA project PB-0060 (QM). *Paratypes:* QUEENSLAND: 4 ♀♀, 2 ♂♂, same data, 04.ix.2009, J. Koens, DNA project, JH-0026 to JH-0031 (QM); 2 ♀♀, same data, xi.2009, A. Karas, DNA project, JH-0043 to JH-0044 (QM); 1 ♀, D'Aguilar Range, Boombana, 27.4045°S, 152.794°E, 13.xi.2011, P.D. Brock & N. Tweed, DNA project PB-0058 (QM). (Note, this DNA project data was provisionally labelled *Candovia* sp. A.)

*Overview of both sexes:* Stout, brown female, with darker marks and indistinctly mottled legs, but can vary and include light and dark areas and whitish marks. Thorax heavily granulated and abdomen ridged. Small, orange marks may be present on thorax and thorax, also part of mouthparts. Male slenderer, brown with broad, black line laterally on body from back of eyes (orange line above this on mesonotum and metanotum). Darker on head, pronotum and towards hind part of thoracic and abdominal segments. Thorax with less distinct granules. Both sexes with eyes whitish with two horizontal black lines.

*Female* (Supporting Information, Fig. S9A): Head: longer than wide, sparse granules present. Antennae long, with numerous indistinct segments; basal segment broader than remaining segments. Thorax: heavily granulated dorsally and ventrally. Pronotum slightly shorter than head, with central impression. Mesonotum up to  $4.3 \times$  length of pronotum. Mesonotum  $1.7 \times$  combined length of metanotum and median segment, the latter shorter than metanotum. Wingless. Abdomen: heavily ridged dorsally and ventrally, with granules, less numerous than on thorax. Eighth segment longer than ninth, which is about the same length as tenth (anal) segment. Operculum reaching end of segment 9; anal segment tip tapered, with tip incised in centre. Cerci broad but short. Legs: long.

*Male* (Supporting Information, Fig. S9B): Much slenderer, but similar in general appearance. Anal segment subtruncate at tip, cerci short. Poculum not reaching half length of ninth abdominal segment.

*Eggs:* Unknown.

*Measurements (mm).* Length of body: female 71.6–82.0, male 59–60. Head: female 4.2–5.0, male 3.2. Antennae: female 48–62, male 48. Pronotum: female 3.4–4.4, male 2.9. Mesonotum: female 14–19, male 13. Metanotum: female 5–6, male 4.4. Median segment: female 4–5, male 3.2. Femora, fore, mid, hind: female 17–20, 13–16, 15–20, male 21, 15, 20. Tibiae, fore, mid, hind: female 16–23, 11–16, 17–25, male 25, 16, 23.5. Cerci: female 0.6, male 0.5.

*Distribution* (Fig. 2): This species is so far only known from Montville and Boombana.

*Habitat and foodplants:* The species seen by forest tracks, feeding on various rainforest plants; often observed resting on ferns.

*Etymology:* Named after John Koens who helped PDB research this species in the wild.

**CANDOVIA WOLLUMBINENSIS** BROCK & JONES SP. NOV.

(SUPPORTING INFORMATION, FIG. 10A, B)

[Wollumbin stick insect]

*Zoobank registration:* urn:lsid:zoobank.org:act:91D10862-1E32-4A7A-8ADF-91E5FF99AC34.

*Type material:* *Holotype:* ♀, NEW SOUTH WALES, Mt. Warning (Wollumbin), 28.4°S, 153.270278°E, 14.ii.2017, B. Jones, DNA project PB-0174 (QM). *Paratypes:* NEW SOUTH WALES: 1 ♂, same data, DNA project, PB-0173 (QM). [Note, this DNA project data was provisionally labelled *Candovia* sp. H (initially thought to be *C. robinsoni*).]

*Overview of both sexes:* Stout female, slenderer male, green with cream lateral stripe (in male running length of body, narrower stripe in female and mainly from thorax, low down); eyes whitish with two horizontal black lines. Narrow, black stripe running from eyes to back of head, but indistinct or partial in female. Mouthparts orange, also trochanters. Cerci pink.

*Female* (Supporting Information, Fig. S10A): Head: longer than wide. Antennae long and hairy, with numerous indistinct segments; basal segment broader than remaining segments. Thorax: sparsely granulated and ridged. Pronotum shorter than head, with central impression. Mesonotum about  $3.5 \times$  length of pronotum. Mesonotum  $1.8 \times$  combined length of metanotum and median segment, the latter shorter than metanotum. Wingless. Abdomen: heavily ridged, with sparse granules. Eighth segment longer than ninth, which is longer than tenth (anal)

segment. Operculum reaching end of anal segment, of which tip tapered at sides and incised in centre. Cerci short and slender. Legs: long, tibiae and tarsi hairy.

**Male** (*Supporting Information, Fig. S10B*): Much slenderer, but similar in general appearance. Anal segment slightly triangular incised at tip, cerci short and slender, slightly incurved. Poculum reaching end of ninth abdominal segment.

**Egg** (*Supporting Information, Fig. S3K*): Almost rectangular, heavily sculptured and rugged capsule, with keel; micropylar plate with surrounding area pale.

**Measurements (mm):** *Length of body*: female 52.8, male 47.5. *Head*: female 3.6, male 2.8. *Antennae*: female 37, male 38. *Pronotum*: female 3.1, male 2.5. *Mesonotum*: female 10.7, male 10.8. *Metanotum*: female 3.6, male 3.3. *Median segment*: female 2.4, male 2.6. *Femora, fore, mid, hind*: female 13, 9.5, 13, male 15, 12, 16. *Tibiae, fore, mid, hind*: female 17, 11, 14, male 18, 10, 19. *Cerci*: female 1.4, male 1. *Eggs*: Capsule length 2.54, width 1.71, height 1.94.

**Distribution** (*Fig. 2*): This species is so far only known from high altitude ~900–1000m in Mt. Warning (Wollumbin).

**Habitat and foodplants**: The species occurs at the top of a mountain, foodplant(s) uncertain, but accepts *Eucalyptus* spp. in captivity.

**Etymology**: Named after Mt. Warning (Wollumbin), given its English name by Captain Cook in 1770.

#### **PARACANDOVIA FORNI ET AL. GEN. NOV.**

**Zoobank registration**: urn:lsid:zoobank.org:act:59B2EB61-EF82-4457-8421-B4CC58BF99B2.

**Type species**: *Paracandovia chiaroscuro*, here designated.

This group has confused entomologists, with five described species (including three with revised status) being initially linked to five different mainly non-related genera, then later often transferred to other genera. Notably *Echetlus* Stål, 1875 was used by Zompro & Adis (2001), which is similar in general appearance, but has short, not long antennae, in addition to tiny forewings in known females, also long cerci in both sexes (only females in *Paracandovia*). *Candovia evoneobertii* (Zompro & Adis, 2001) was erroneously described in *Echetlus*, and this South American species is removed from the Australian

phasmid fauna in this paper. As no existing generic name is appropriate for this particular group, it is necessary to erect a new genus. It became apparent when studying adults and eggs in detail, also considering molecular analyses, that samples originally identified as *P. peridromes* (PB178–PB180 and BJ13–BJ16) were not a single species, hence three other synonymized species (*cercata*, *longipes* and *tenera*) are reinstated as valid. The only one with distribution not known with certainty, nor egg, is *P. peridromes*. Other species from Western Australia and Three Hummock Island (near Tasmania) await formal description once further research has been undertaken. Molecular work indicates that this genus has a sister relationship to all *Candovia* species.

**Description**: Small to medium-sized (45–118 mm), plain, in nature brown or green (sometimes with lines by head), wingless, elongate, long-legged, fragile-looking phasmids, with the body smooth, granulated or with tubercles, the latter particularly conspicuous in females. Abdomen with carina or ridges distinct or indistinct. Cerci long in females, short in male. Head much longer than wide (typically about  $1.8 \times$  longer). Head, pronotum and mesonotum often smooth, or sparsely granulose in some species. Mesonotum more heavily granulose in one species and tubercles noted in one species. Antennae exceeding length of forelegs, with numerous segments (up to 89); two basal segments slightly broadened. Pronotum equal in length or shorter than head, mesonotum long,  $4.3\text{--}6.0 \times$  length of pronotum; mesonotum  $1.6\text{--}1.9 \times$  longer than combined length of metanotum and the shorter median segment. Wingless. Anal segment in female rounded at tip, usually tapered at sides and may be triangular incised in centre, with supra-anal plate visible in some species. Operculum not broad, rounded at tip, reaching beyond end of ninth abdominal segment, up to half length of anal segment. Anal segment tip in male (where known) with rounded tip, poculum a modest size with rounded tip, not reaching end of ninth abdominal segment. Anal segment tips are splayed. Cerci long in female,  $2.0\text{--}2.4 \times$  length of anal segment; much shorter in male, no more than same length as anal segment. Legs long, smooth; hindlegs reaching in excess of elongate abdomen. Egg: more elongate, almost rectangular, possibly with indented micropylar plate. Micropylar oval or round. Capsule rugged appearance, with various sculpturing. Distribution: Australia.

**Etymology**: Named after the close resemblance and affinity to *Candovia*.



*Species included:* The first five here transferred from *Candovia* Stål, 1875:

*Paracandovia cercata* (Redtenbacher, 1908) [Sydney stick insect] stat. rev., comb. nov.: New South Wales (known from culture stock), Victoria [removed from synonymy with *Candovia peridromes* (Westwood, 1859), which was originally described in the genus *Bacillus*]. No molecular data were available for this taxon, but is supported by its different-shaped egg.

*Paracandovia longipes* (Brunner, 1907) [Bowen Stick insect] stat. rev., comb. nov.: Queensland [lectotype locality Bowen = sample *Candovia* sp. I from Dingol]. The paralectotype from Western Australia is a misidentified female of *Echetlus peristhenes* (Westwood, 1859) [removed from synonymy with *Candovia peridromes* (Westwood, 1859)].

*Paracandovia pallida* (Sjöstedt, 1918) [pale stick insect] comb. nov.: Northern Territory, Western Australia.

*Paracandovia peridromes* (Westwood, 1859) [peridrome stick insect] comb. nov.: 'Australia' localities as yet uncertain, possibly Western Australia. No morphological nor molecular data were available for this species.

*Paracandovia tenera* (Brunner, 1907) [Adelaide stick insect] stat. rev., comb. nov.: South Australia [type locality Adelaide, = sample of 'peridromes' from Wilmington, SA] [removed from synonymy with *Candovia peridromes* (Westwood, 1859)].

*Paracandovia bifurcata* sp. nov. [bilobed stick insect] [= 'peridromes']: South Australia.

*Paracandovia chiaroscuro* sp. nov. [Chiaroscuro stick insect] [= *Candovia* sp. G]: Queensland.

*Paracandovia cloncurriensis* sp. nov. [Cloncurry stick insect] [= *Candovia* sp. L]: Queensland.

**PARACANDOVIA BIFURCATA BROCK & JONES  
SP. NOV.**

(SUPPORTING INFORMATION, FIG. S11A, B)

[bilobed stick insect]

*Zoobank registration:* urn:lsid:zoobank.org:act:CC72FDC8-078B-4808-A538-90569087852F.

*Type material:* Holotype: ♀, SOUTH AUSTRALIA, Mount Gambier, 37.842931°S, 140.764820°E, ii.2017, B. Jones, DNA project PB-0180 (QM). Paratypes: 1 ♂, same data, DNA project PB-0178 (QM), 1 ♂, same data,

DNA project PB-0179 (NHMUK). (Note, this DNA project data was provisionally labelled *Candovia peridromes*).

*Description:* The female specimen is based on the only one found so far. Elongate, greenish brown, each side of head with a narrow, brown longitudinal band, running from eyes to back of head. The male is brown with reddish beneath mesothorax and metathorax.

*Female* (Supporting Information, Fig. S11B): Head: twice as long than wide. Antennae long and pale, not as long as forelegs, with numerous indistinct segments; basal segment broader than remaining segments. Thorax: pronotum slightly shorter than head, with central impression. Mesonotum  $4.6 \times$  length of pronotum, with 18 tubercles laterally up to final third. Mesonotum  $1.7 \times$  combined length of metanotum and short median segment. Wingless. Abdomen: elongate, with two indistinct ridges each side. Length of eighth, ninth and tenth (anal) segments similar, although anal segment is a little shorter. End of anal segment with bilobed tip with supra-anal plate visible beneath. Operculum tapered to tip, reaching half length of anal segment. Cerci elongate,  $2 \times$  length of anal segment, tapered to tip. Legs: elongate.

*Male* (Supporting Information, Fig. S11B): Apart from being much slenderer, similar in general appearance, except colour. Mesonotum  $1.9 \times$  combined length of metanotum and short median segment. Cerci appear short, less than length of anal segment, which is slightly triangular incised at tip. Poculum tapered towards rounded tip, not reaching end of ninth abdominal segment.

*Egg* (Supporting Information, Fig. S3L): Brown, rough, heavily sculptured, almost oval capsule with darker markings. Micropylar plate darker brown, central and almost oval.

*Measurements* (mm): Two males and 1 female. *Length of body:* female 62, male 45–51. *Head:* female 3.5, male 2.5–3.0. *Antennae:* female 33, male 29. *Pronotum:* female 3, male 2.0–2.4. *Mesonotum:* female 13.6, male 9–12. *Metanotum:* female 5.8, male 7.5. *Median segment:* female 2.4, male 1.4–1.5. *Femora, fore, mid, hind:* female 21, 16, 24, male 17, 14, 18. *Tibiae, fore, mid, hind:* female 22, 17, 25, male 17, 15, 19. *Cerci:* female 6, male 1. *Eggs:* Capsule length 1.8, width 1.3, height 1.4.

*Distribution* (Fig. 2): The type series is from Mount Gambier and is expected to be found elsewhere in at least South Australia.

*Habitat and foodplants:* The species was taken in forest.

*Etymology:* Named after the bilobed anal segment.

**PARACANDOVIA CHIAROSCURO** BROCK & JONES SP. NOV.

(SUPPORTING INFORMATION, FIG. S12A, B)

[Chiaroscuro stick insect]

*Zoobank registration*: urn:lsid:zoobank.org:act:26AC230F-4E1B-4D21-B279-4ADF9983A59A.

*Type material*: *Holotype*: ♀, QUEENSLAND, Baralaba, Duaninga Road, 24.136°S, 149.830°E, 09.i.2013, N. Tweed, DNA project PB-0295 (QM). *Paratypes*: QUEENSLAND: 1 ♂, same data, except on 19.i.2013, DNA project PB-0305 (QM); 7 ♀♀, same data, except for dates as shown, DNA project PB-0290 on 20.xii.2012, PB-0293 and PB-0294 both on 31.xi.2012, PB-0310, PB-0311, PB-0312, PB-0313 all on 22.ii.2012 (NHMUK). (Note, all DNA project data was provisionally labelled *Candovia* sp. G). Eggs also deposited in NHMUK (not paratypes).

*Overview of both sexes*: Elongate, green female with whitish sides, tip of abdomen, cerci and antennae (underside paler) basal area of legs brownish. Sometimes brown and may have a black, central line running length of the body, which can have black flecks; they can change shade in captivity. The male is invariably brown, darker on the dorsal surface, with a broad brown longitudinal band from eye to back of head and a black longitudinal lateral line running length of much of the body (second black sublateral line on mesonotum), also a broader whitish lateral stripe, reaching to about end of second abdominal segment. There is also an orange patch of the underside of the thorax. Eyes in both sexes with narrow brown stripe in centre.

*Female* (Supporting Information, Fig. S12A): Head: twice as long than wide. Antennae long and slightly hairy, with numerous segments (c. 45, but indistinctly segmented and difficult to determine under a microscope), basal segment broader than remaining segments. Thorax: smooth, pronotum slightly shorter than head, with central impression. Mesonotum  $4.8 \times$  length of pronotum. Mesonotum also  $1.6 \times$  combined length of metanotum and short median segment. Wingless. Abdomen: elongate, with two indistinct ridges each side. Length of eighth, ninth and tenth (anal) segments similar, although anal segment is a little shorter. End of anal segment with rounded tip, supra-anal plate visible. Operculum tapered to tip, reaching half the length of anal segment. Cerci elongate,  $2 \times$  length of anal segment, slightly hairy and tapered to tip. Legs: elongate.

*Male* (Supporting Information, Fig. S12B): Apart from being much slenderer, similar in general appearance, except colour. Mesonotum  $5.8 \times$  length

of pronotum, mesonotum  $1.5 \times$  combined length of metanotum and short median segment. Cerci appear short, but are the same length as anal segment, which is triangular incised at tip. Poculum tapered towards rounded tip, not reaching end of ninth abdominal segment.

*Egg* (Supporting Information, Fig. S3N): Small, brown, rough, uneven and pitted but almost rectangular capsule with wide indentations, also raised ridge before operculum. Micropylar plate indented, surrounded by darker brown area.

*Measurements* (mm): One male and 8 females. *Length of body*: female 66–76, male 56. *Head*: female 4–5, male 2.5. *Antennae*: female 37–44, male 54. *Pronotum*: female 3.6–4.5, male 2.3. *Mesonotum*: female 14–18, male 13. *Metanotum*: female 7.5–9.0, male 7.5. *Median segment*: female 2.5–3.2, male 1.5. *Femora, fore, mid, hind*: female 20–22, 18–20, 24–26, male 23, 20, 23. *Tibiae, fore, mid, hind*: female 19–21, 18–20, 24–28, male 22, 18, 25. *Cerci*: female 3–5, male 1. *Eggs*: Capsule length 1.9, width 1.2, height 1.3.

*Note*: As is usual in phasmids, there can be variation in colour within a species. Detailed photographs will be uploaded to <http://phasmida.speciesfile.org>.

*Distribution* (Fig. 2): The type series is from Baralaba (95 km NW of Biloela) but is likely to be found elsewhere in Queensland.

*Habitat and foodplants*: The species was taken in forest with a dense understorey of grasses and low-growing plants feeding on a mixture of the latter. Grasses are sometimes dead, which may influence female body colour. In captivity they also accept *Eucalyptus* spp. and *Acacia* spp. It has been noted as adults so far at least between December and February.

*Etymology*: Named after *chiaroscuro* (from the Italian chiaro, 'light,' and scuro, 'dark') due to the contrast of green dorsal and whitish lateral and ventral surface, similar to strong contrasts of light and dark (a technique used in the visual arts).

**PARACANDOVIA CLONCURRIENSIS** BROCK & JONES SP. NOV.

(SUPPORTING INFORMATION, FIG. S13A, B)

[Cloncurry stick insect]

*Zoobank registration*: urn:lsid:zoobank.org:act:535570ED-0C5E-4AE6-AFBE-26F3519719E0.

**Holotype:** ♀, QUEENSLAND, Fountain Springs Rest Area, Cloncurry, 60 km E of Mount Isa, 20.800°S, 139.996°E, 30.i.2018, B. Jones, DNA project BJ-0095 (QM). (Note, this DNA project data was provisionally labelled *Candovia* sp. L.)

**Overview of female:** A single female only found so far (in poor condition, with only three legs intact). Elongate, greenish brown, each side of head with a broad, dark-brown longitudinal band, running from eyes to back of head, followed by broad lateral whitish band. Lower part of pronotum dark brown, narrow longitudinal sublateral dark line either side of thorax.

**Female** (Supporting Information, Fig. S13A): Head: twice as long than wide. Antennae long and pale, tips broken, but presumed longer than forelegs, with numerous indistinct segments, basal segment broader than remaining segments. Thorax: smooth, pronotum slightly shorter than head, with central impression. Mesonotum  $6 \times$  length of pronotum. Mesonotum  $1.9 \times$  combined length of metanotum and short median segment. Wingless. Abdomen: elongate. Length of eighth, ninth and tenth (anal) segments similar, although ninth segment is a little shorter. End of anal segment with tapered tip. Operculum tapered to tip, reaching almost end of anal segment. Cerci elongate,  $2 \times$  length of anal segment, tapered to tip. Legs: elongate.

**Male:** Unknown.

**Egg** (Supporting Information, Fig. S30): Greyish, rough, almost oval capsule with large black spots and markings. Micropylar plate black, low down, surrounded by lighter area.

**Measurements (mm):** One female. Length of body: 84. Head: 4.3 Antennae: 50 + but tips broken off. Pronotum: 3.7. Mesonotum: 22. Metanotum: 8. Median segment: 2. Femora, fore, mid, hind: 22, 20, 25. Tibiae, fore, mid, hind: 22, ? damaged, 29. Cerci: 6. Eggs: mislaid, not available for measurement.

**Distribution** (Fig. 2): The type series is from Cloncurry and is likely to be found elsewhere in the vicinity, in Queensland.

**Habitat and foodplants:** The species was taken in forest with understorey near the permanent waterhole of Fountain Springs, on spinifex.

**Etymology:** Named after the type locality, Cloncurry.

## DISCUSSION

A consensus on which is the more robust method for molecular species delimitation is lacking, and different methods can outperform, depending on the specific characteristics of a dataset (Luo *et al.*, 2018). Therefore, the combination of different species-delimitation approaches and phylogenetic reconstruction methods (Tang *et al.*, 2014) gave us more confidence in exploring the diversity of this phasmid clade. Although all methods, whether distance (ABGD) or tree-based (PTP and GMYC), were able to detect most interspecific boundaries, the use of a single approach could have been misleading in some instances. Generally, the different species-delimitation analyses consistently recognized previously described species and identified new putative species (spp. A–L; Fig. 2). For some lineages, the GMYC method highlighted some degree of intraspecific divergence, for example specimen JH-0027 of sp. A, specimens PB-0261 and PB-0271 of sp. F and five specimens of *C. annulata*. This could be due to either general properties of our dataset, including unbalanced geographical range sampling, skewed species abundances and the availability for analyses of a single specimen (Talavera *et al.*, 2013; Ahrens *et al.*, 2016), or because of paraphyly/polyphyly of some clades (Hendrich *et al.*, 2010; Scicchitano *et al.*, 2018). Interestingly, the intraspecific differentiation observed in *C. annulata* corresponds to samplings at different geographic areas (Montville and Apple Tree Park – Springbrook). Therefore, the geographical ground of the observed pattern suggests the presence of structured populations that may represent different subspecific entities. A peculiar situation is seen with specimen PB-164 of the *C. coenosa* species, recognized as a different entity by all species-delimitation methods except the mPTP method. Although this could be due to the paraphyly of *C. coenosa*, which is also confirmed by phylogenetic analyses with high nodal support (see below), it is possible that specimen PB-164 may represent a more differentiated taxon. Despite a substantial proportion of species-level diversity being represented by species described on a single specimen (Lim *et al.*, 2012), in our opinion, only a wider sampling of specimens from different geographical locations will allow to properly describe the aforementioned lineage. Overall, data observed here do not seem to suggest significant deviations from known or provisional taxonomy, especially considering that inferences drawn from species-delimitation approaches should rely on a conservative consensus (Carstens *et al.*, 2013). The results of the detailed analyses on body and egg morphology well match molecular species delimitation; formal descriptions of novel taxa are presented in the Taxonomic account in the Results with keys to *Candovia* groups/species,

also including egg morphology, which in some lineages appear to be a more reliable character than traditional body morphology (Scali *et al.*, 1987; Sellick, 1988; Cubillos & Vera, 2020 and references therein).

The phyletic relationships in the clade appear well defined, despite a general decrease of nodal support at the deeper nodes (Fig. 3), and agree with morphological characters (such as leg length and egg morphology) allowing species identification of the *Candovia* groups (see Taxonomic account and Supporting Information, File S1 for details).

While in the present analysis the nodal support of the clade monophyly is high (BS:90; PP:1.0), it is to be noted that the dataset for comparison is fairly limited, as no other Australian Necrosiinae species were available for comparison. Moreover, two recent studies considering all Phasmida provided contrasting evidence, showing this clade either monophyletic (Forni *et al.*, 2022) or paraphyletic (Bank & Bradler, 2022). Therefore, the possible monophyly of the clade should be reconsidered after a more extensive taxon sampling. On the other hand, body and egg morphological characters are clear-cut in defining an intraclade divergence of generic level, so that a split of the genus *Candovia* into two genera (*Candovia* and *Paracandovia*) is below reported (see Taxonomic account for details), which coincides with differences in morphological characters, although this genus has up to now been largely neglected by researchers and poorly understood, hence previous errors in synonymy. At variance with some intraspecific divergences retrieved in the species-delimitation analysis, no clear geographical pattern emerges from the phylogenetic analysis. Most *Candovia* taxa are distributed along the eastern Australian coasts, with overlapping ranges. Yet, the newly erected *Paracandovia* genus appears to have a broader geographic distribution, with *P. pallida* found in central-western Australia, *P. cloncurriensis* collected from central Australia and *P. longipes* and *P. chiaroscuro* from the east coast.

As for *Candovia coenosa*–*spurcata* relationships, also the sample morphologically identified as *Candovia peridromes*, they have revealed a situation that will require a more extensive sampling to be clarified. When considering morphological and molecular analyses, the samples initially identified as *C. peridromes* (PB178–PB180 and BJ13–BJ16) were retrieved as multiple species, and morphologically differentiated from *C. peridromes*, thus reinstating as valid its synonyms *P. cercata*, *P. longipes*, *P. tenera* (all now placed in the new genus *Paracandovia*). Further analyses are required for *P. peridromes* itself, along with: (1) *P. cercata*, which is reinstated as valid on the basis of eggs morphology observations (Supporting Information, Fig. S3); (2) *P. tenera*, which is reinstated as valid in molecular analyses (Figs 2, 3); and (3) *P. longipes*, which is also reinstated as valid on molecular ground. For the

two latter species, a formal description is in preparation, but additional samples are necessary.

The phylogenetic analysis presented here provides a framework to correctly identify the morphological characters that can be used for clade taxonomy, but also allows a better understanding of taxa evolution. Phasmida taxonomy often relies on egg morphological character, like the capitulum (Clark, 1976; O'Hanlon *et al.*, 2020): the function of this lipid-rich extension of the operculum was not understood until recently, when it was shown to serve as a reward to promote ant-mediated dispersal of the egg (myrmecochory; Compton & Ware, 1991). This adaptation represents one of the most extraordinary examples of convergent evolution across different kingdoms of life, where the capitulum in phasmid eggs is analogous to elaiosomes of angiosperm seeds (Stanton *et al.*, 2015). Both structures are used to exploit ant behaviour: this mutualistic relationship allows for both plant seeds and phasmid eggs to be buried in the soil, protected from environmental changes, predators and parasites, also greatly enhancing the dispersal abilities of the species (Hughes & Westoby, 1992). A study on an African phasmid species also suggests the possibility to escape recurrent bushfire when ants drag the egg into their nest (Compton & Ware, 1991). *Candovia aberrata*, *C. coenosa* and *C. spurcata* lay eggs with a capitulum, (Supporting Information, Fig. S3) and mapping this character on to our phylogenetic tree (Fig. 3) suggests the non-monophyly of this feature. At the order level, it has been shown that capitula probably evolved independently in several lineages across the phasmid tree of life (Robertson *et al.*, 2018 and references therein), but to our knowledge this has never been proposed among congeneric species. It is interesting to note that, although belonging to different phylogenetic clusters, the three *Candovia* species that present the capitulum, have strictly overlapping distribution in the southern part of the Australian east coast. Further efforts should investigate whether the presence of a capitulum can be linked to local adaptations, such as the presence of a particular ant species performing myrmecochory. Recently, a strong interest has been renewed towards the patterns of wing evolution in Phasmida (Bank & Bradler, 2022; Forni *et al.*, 2022). Among the newly described species, two species (*C. alata* and *C. karasi*) present males with fully developed hindwings and females with reduced hindwings. The latter represents the first winged species in this previously considered apterous clade. As such, the observed pattern may support the phenomenon initially proposed by Whiting *et al.* (2003): wings were absent in extant phasmid ancestors, to which they subsequently reverted back independently in several instances throughout their evolutionary history.

## CONCLUSION

Molecular analyses represent a reliable framework to refine taxonomy and systematics, and to reconsider diagnostic characters for species description and identification in this Australian phasmid clade. While this work represents a substantial step forward, further species collection and rearing is needed – including similar specimens from different geographical areas, which may prove to be valid, cryptic species. Museum collections could also represent useful resources, with a large amount of material available in Australia clearly suggesting the presence of additional undescribed taxa, not considered in this study. Furthermore, molecular data for *P. peridromes* and *P. cercata* are still lacking, as their distribution remains uncertain, and no samples were available for molecular analyses. Nonetheless, our effort represents a way forward to better understand the phenomena underlying the evolution and diversification of this overlooked, yet remarkable, clade.

## ACKNOWLEDGEMENTS

The authors are grateful to all the people who kindly helped in obtaining material, sometimes with PDB and/or BRJ. In particular, the authors want to thank Noelene Tweed, Steve Cross, Kirsten Dalgleish, Alexander Karas, Aila Keto, John Koens and Andreas Urban. Geoff Monteith and Helen Brock assisted in the Springbrook area and Geoff organised permits. Ross Coupland, Brian Cox and Beth Ripper supplied photographs of species in parts of Queensland. Oskar Conle and Frank Hennemann are also thanked for their assistance. Export and collecting permits were otherwise obtained thanks to the Australian Biological Resources Study (ABRS). Jack Hasenpusch helped in rearing some stock sent by collectors. Curators of museum collections have assisted us over the years. This work has been supported by Canziani Funding to BM.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY

The sequence data underlying this article are available in the NCBI nucleotide database at <https://www.ncbi.nlm.nih.gov/nucleotide>, and can be accessed with the accession numbers presented in [Supporting Information, Table S1](#).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** Maximum likelihood  $COI_{\text{pol}}$  tree used for the species delimitation, presented as (A) cladogram with bootstrap proportion and (B) with branch lengths.

**Figure S2.** Trees obtained using (A) maximum likelihood inference and (B) Bayesian inference on the phylogenetic dataset.

**Figure S3.** Eggs from the species: A, *Candovia aberrata*; B, *C. alata*; C, *C. annulata*; D, *C. byfieldensis*; E, *C. coenosa*; F, *C. dalgleishae*; G, *C. eungellensis* (Beth Ripper; Ripper 2017); H, *C. granulosa*; I, *C. robinsoni*; J, *C. spurcata*; K, *C. strumosa*; L, *C. wollumbinensis*; M, *Paracandovia bifurcata*; N, *P. cercata* (Brian Cox); O, *P. chiaroscuro*; P, *P. cloncurriensis*; Q, *P. pallida*; R, *P. tenera*.

**Figure S4.** *C. alata* female (A) and male (B) specimens.

**Figure S5.** *C. byfieldensis* female (A) and male (B) specimens.

**Figure S6.** *C. dalgleishae* female (A) and male (B) specimens.

**Figure S7.** *C. eungellensis* female (A) and male (B) (Beth Ripper; Ripper 2017) specimens.

**Figure S8.** *C. karasi* female (Ross Coupland) (A) and male (B) specimens.

**Figure S9.** *C. koensi* female (A) and male (B) specimens.

**Figure S10.** *C. wollumbinensis* female (A) and male (B) specimens.

**Figure S11.** *P. bifurcata* female (A) and male (B) specimens.

**Figure S12.** *P. chiaroscuro* female (A) and male (B) specimens (both Noelene Tweed).

**Figure S13.** *P. cloncurriensis* female specimen.

**Table S1.** NCBI GenBank accession numbers of all the sequences included in the species delimitation and phylogenetic datasets.

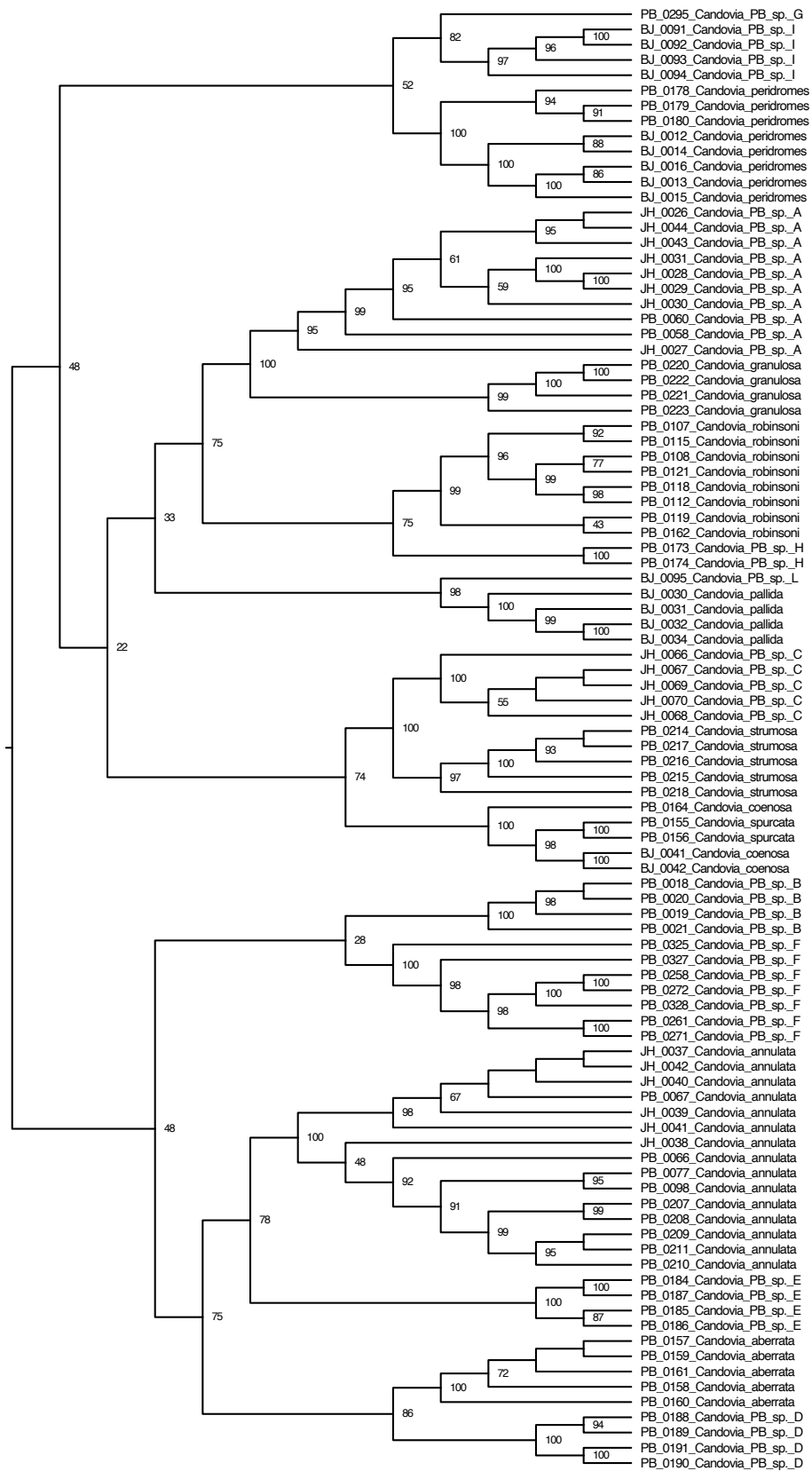
**Table S2.** Gene target, sequence, GC%, amplicon size (bp), temperature and time of annealing of the primers used in the present study.

**Table S3.** Results of the model selection performed with ModelFinder and PARTITIONFINDER2 on (1) the species-delimitation dataset (2) the phylogenetics dataset.

**File S1.** Key to *Candovia* groups and guides to *Candovia* and *Paracandovia* species.

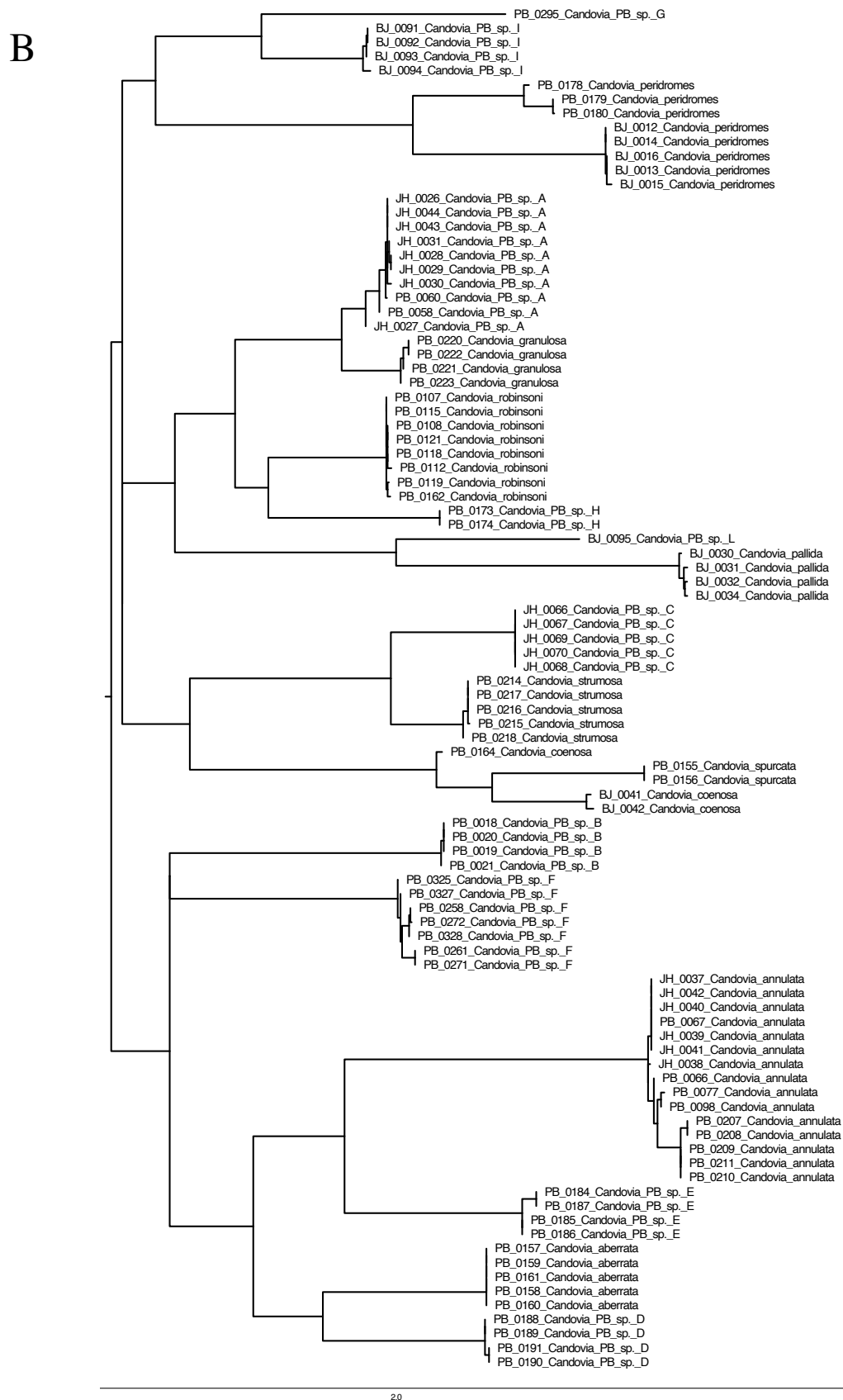
# Supplementary material for Publication

A



2.0

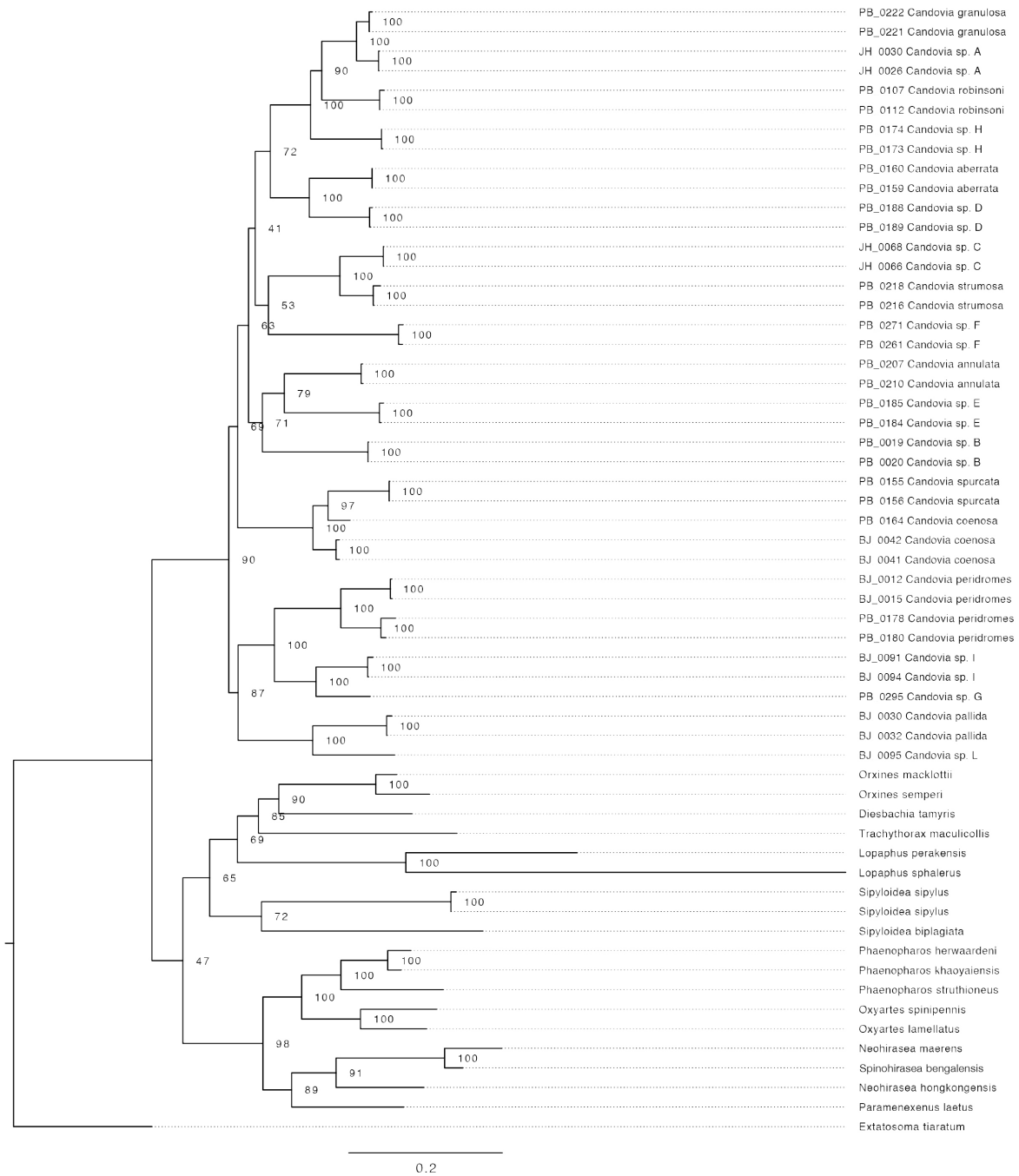




20

**Figure S1.**

A



B

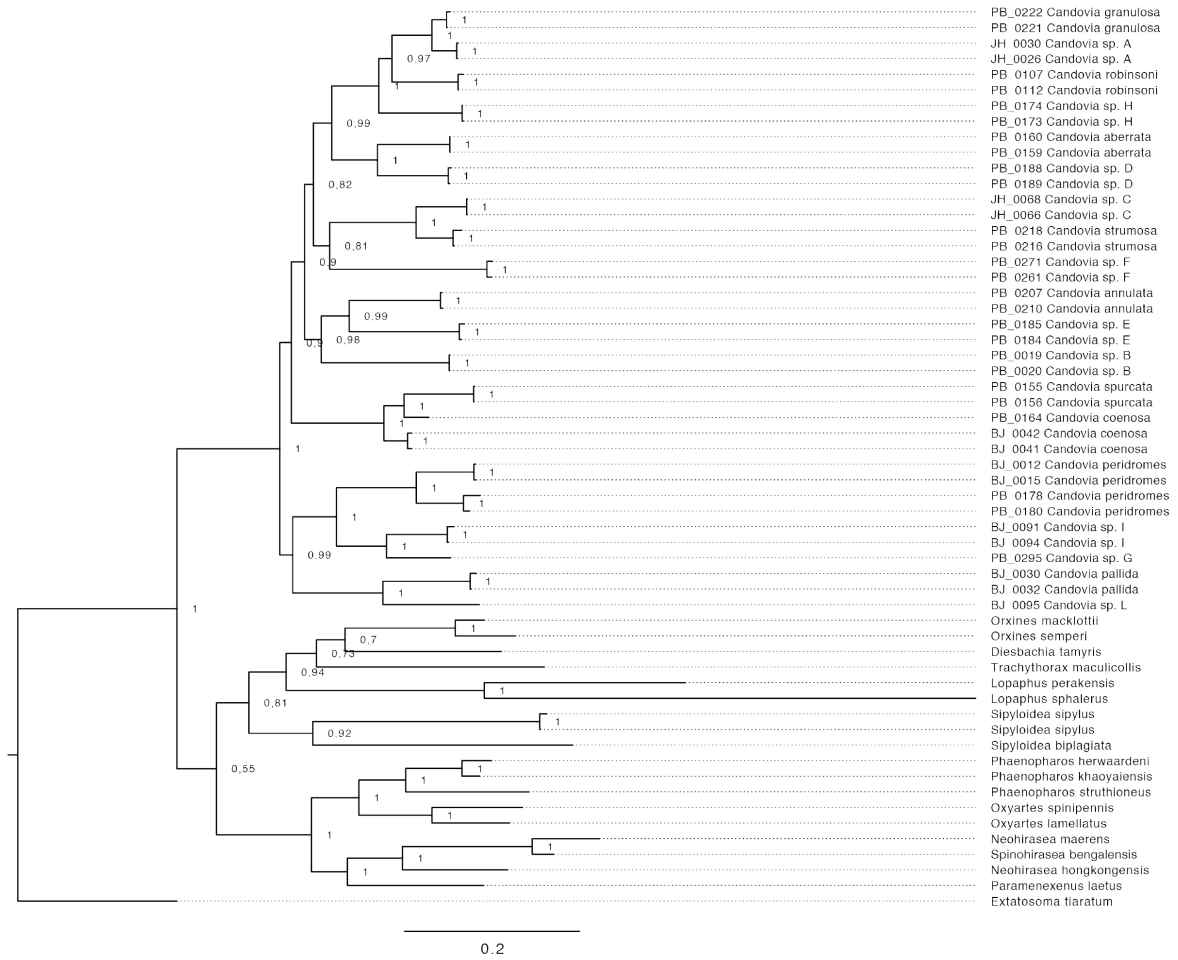


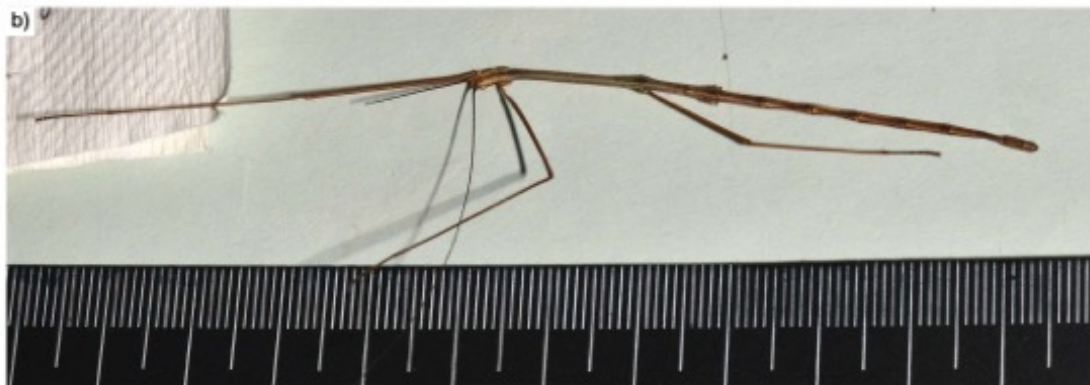
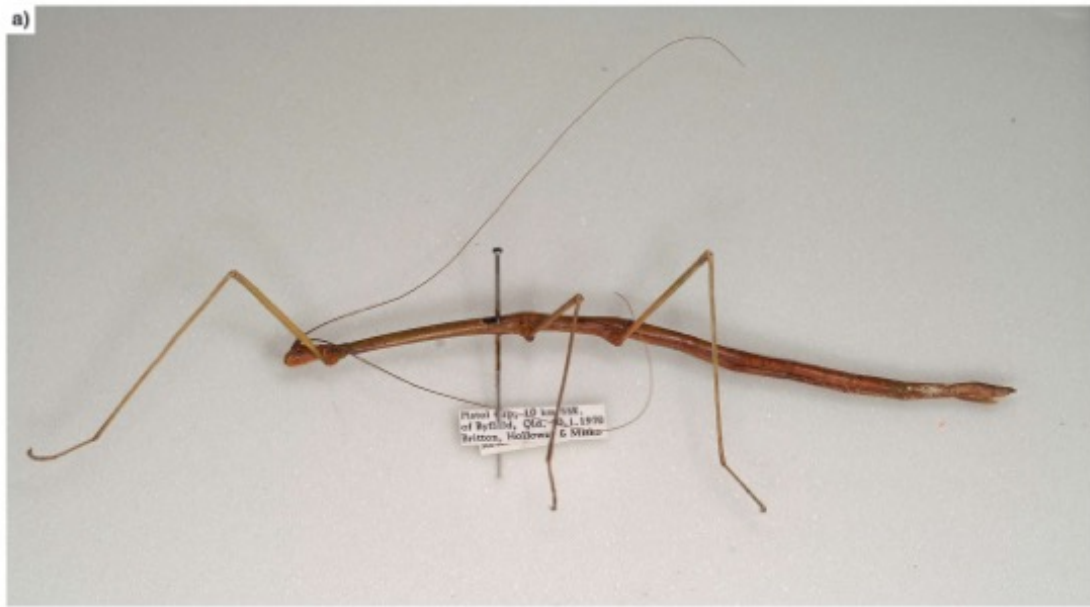
Figure S2.



**Figure S3.**



**Figure S4.**



**Figure S5.**





**Figure S6.**



**Figure S7.**





**Figure S8.**



**Figure S9.**





Figure S10.

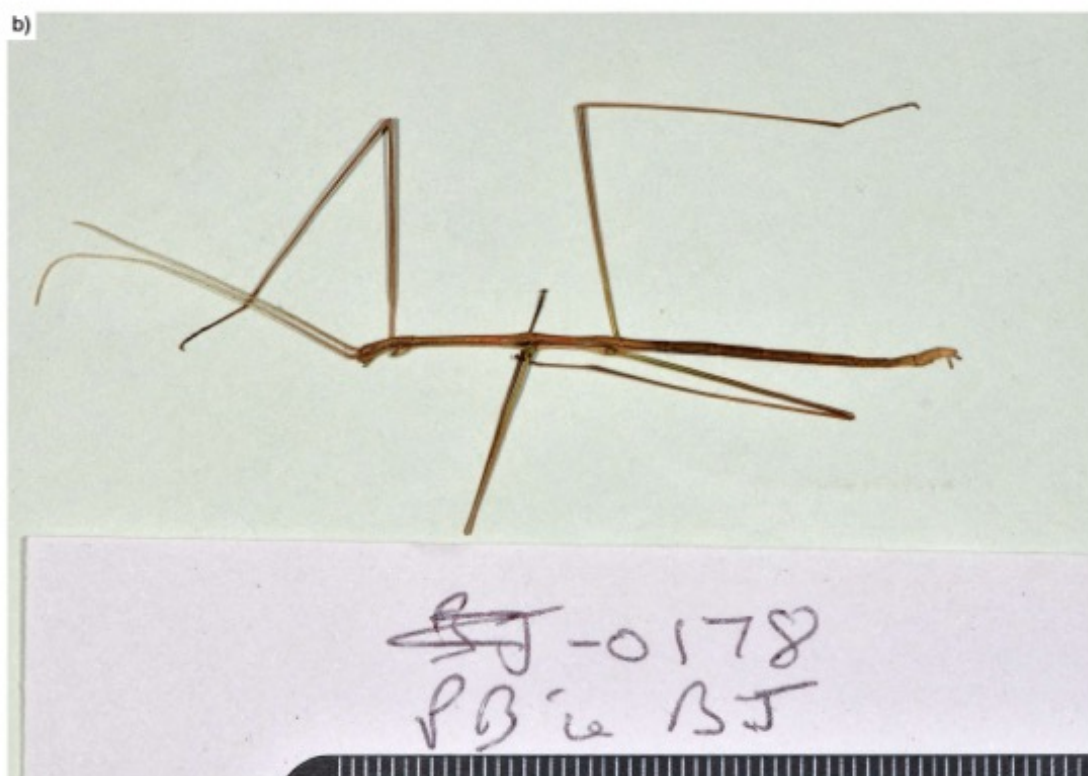
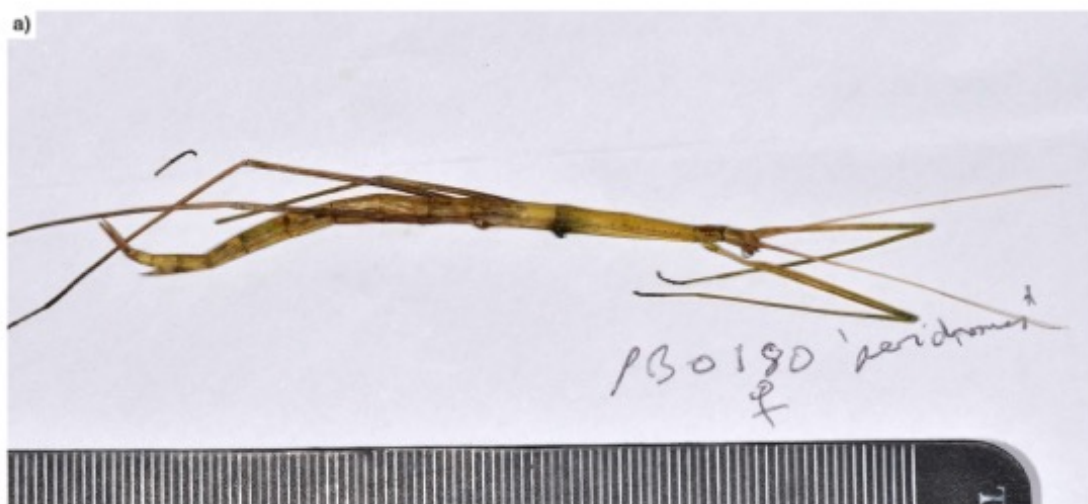


Figure S12.



**Figure S13.**

**Table S1.**

**Table S1a.** Accession number of the sequences of the species used as outgroups.

|                                  | 12S        | 16S        | 18S        | 28S        | cox1       | cox2       | H3         |
|----------------------------------|------------|------------|------------|------------|------------|------------|------------|
| Lonchodidae                      |            |            |            |            |            |            |            |
| Necrosiinae                      |            |            |            |            |            |            |            |
| <i>Diesbachia tamyris</i>        | KJ024494.1 | -          | -          | KJ024389.1 | -          | KJ024558.1 | KJ024526.1 |
| <i>Lopaphus perakensis</i>       | KJ024485.1 | KJ024439.1 | AY121171.1 | AY125311.1 | -          | -          | AY125254.1 |
| <i>Lopaphus sphalerus</i>        | -          | -          | AY121182.1 | AY125322.1 | -          | -          | AY125265.1 |
| <i>Neohirasea hongkongensis</i>  | KJ024484.1 | -          | KJ024403.1 | KJ024380.1 | -          | -          | KJ024517.1 |
| <i>Neohirasea maerens</i>        | KJ024480.1 | KJ024432.1 | AY121168.1 | AY125308.1 | -          | KJ024546.1 | AY125251.1 |
| <i>Orxines macklottii</i>        | -          | KJ024438.1 | AY121153.1 | AY125293.1 | -          | -          | AY125237.1 |
| <i>Orxines semperi</i>           | -          | -          | -          | FJ474150.1 | FJ474306.1 | -          | FJ474228.1 |
| <i>Oxyartes lamellatus</i>       | -          | -          | -          | FJ474151.1 | FJ474307.1 | FJ474380.1 | FJ474229.1 |
| <i>Oxyartes spinipennis</i>      | KJ024489.1 | KJ024443.1 | KJ024407.1 | KJ024384.1 | -          | -          | KJ024521.1 |
| <i>Paramenexenus laetus</i>      | KJ024490.1 | KJ024444.1 | KJ024408.1 | KJ024385.1 | -          | KJ024554.1 | KJ024522.1 |
| <i>Phaenopharos herwardeni</i>   | KJ024498.1 | KJ024451.1 | KJ024414.1 | KJ024393.1 | -          | -          | KJ024530.1 |
| <i>Phaenopharos khaoyaiensis</i> | -          | -          | -          | FJ474157.1 | FJ474313.1 | FJ474383.1 | FJ474235.1 |
| <i>Phaenopharos struthioneus</i> | KJ024486.1 | KJ024440.1 | KJ024404.1 | KJ024381.1 | -          | KJ024551.1 | KJ024518.1 |
| <i>Sipylodea biplagiata</i>      | MK291676.1 | -          | -          | MK291676.1 | -          | -          | -          |
| <i>Sipylodea sipylus</i>         | -          | -          | -          | FJ474168.1 | -          | KJ024550.1 | AY125264.1 |
| <i>Sipylodea sipylus</i>         | -          | -          | -          | FJ474168.1 | FJ474324.1 | FJ474393.1 | FJ474246.1 |
| <i>Trachythorax maculicollis</i> | KJ024495.1 | J024448.1  | -          | KJ024448.1 | -          | KJ024559.1 | KJ024527.1 |
| <i>Spinohirasea bengalensis</i>  | -          | -          | -          | FJ474169.1 | FJ474325.1 | FJ474394.1 | FJ474247.1 |
| Phasmatidae                      |            |            |            |            |            |            |            |
| Extatosomatinae                  |            |            |            |            |            |            |            |
| <i>Extatosoma tiaratum</i>       | KJ024511.1 | KJ024468.1 | -          | -          | FJ474284.1 | KJ024574.1 | -          |

**Table S1b.** Information about newly analyzed specimens; map IDs refer to Figure 2. QLD = Queensland, NSW = New South Wales, NT = Northern Territory, SA = South Australia.

| Map ID | Sampling site | Initial species / | N | Latitude | Longitude | Sample ID | NCBI accession numbers |
|--------|---------------|-------------------|---|----------|-----------|-----------|------------------------|
|--------|---------------|-------------------|---|----------|-----------|-----------|------------------------|

|     |                                    | morphospecies      |   |          |         |                                     |                                                                                                                                          |
|-----|------------------------------------|--------------------|---|----------|---------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| APP | Springbrook, Apple Tree Park (QLD) | <i>C. annulata</i> | 5 | - 28.166 | 153.259 | PB-(0207-0211)                      | MT077791.1<br>MT077792.1<br>MT077793.1<br>MT077794.1<br>MT077795.1                                                                       |
| APP | Springbrook Apple Tree Park (QLD)  | <i>C. strumosa</i> | 5 | - 28.166 | 153.259 | PB-(0214-0218)                      | MT077827.1<br>MT077830.1<br>MT077828.1<br>MT077829.1<br>MT077831.1                                                                       |
| BAR | Baralaba, Duaninga Road (QLD)      | morphospecies G    | 1 | - 24.136 | 149.830 | PB-0295                             | MT077816.1                                                                                                                               |
| MTV | Montville, Narrows Road (QLD)      | <i>C. annulata</i> | 9 | - 26.698 | 152.866 | JH-00(37,39-42); PB-00(66,67,77,98) | HM425695.1<br>HM425697.1<br>HM425698.1<br>HM425700.1<br>HM425700.1<br>KJ202029.1<br>MT077786.1<br>KJ202030.1<br>MT077790.1               |
| MTV | Montville, Narrows Road (QLD)      | morphospecies A    | 9 | - 26.698 | 152.866 | JH-00(26-31,43,44); PB-0060         | MT077751.1<br>HM425690.1<br>MT077755.1<br>MT077756.1<br>MT077757.1<br>KJ202019.1<br>KJ202049.1<br>KJ202047.1<br>KJ202002.1<br>KJ201984.1 |
| MTV | Montville, Baroon Pocket Dam (QLD) | morphospecies C    | 5 | - 26.69  | 152.866 | JH-00(66-70)                        | KJ202033.1<br>KJ202011.1                                                                                                                 |

|     |                                    |                     |            |         |                                    |                                                                                                |
|-----|------------------------------------|---------------------|------------|---------|------------------------------------|------------------------------------------------------------------------------------------------|
|     |                                    |                     | 8          |         |                                    | MT077824.1<br>MT077825.1<br>MT077826.1                                                         |
| MTV | Montville, Baroon Pocket Dam (QLD) | morphospecies A     | 1 - 26.698 | 152.866 | JH-0027                            | HM425690.1                                                                                     |
| BTL | Blackdown Tableland (QLD)          | morphospecies D     | 4 - 23.582 | 149.063 | PB-0(188-191)                      | MT077805.1<br>MT077808.1<br>MT077807.1<br>MT077806.1                                           |
| BTL | Blackdown Tableland (QLD)          | morphospecies E     | 4 - 23.582 | 149.063 | PB-0(184-187)                      | MT077796.1<br>MT077798.1<br>MT077799.1<br>MT077797.1                                           |
| BUL | Bulli (NSW)                        | <i>C. robinsoni</i> | 7 - 34.330 | 150.901 | PB-0(107,108,112,115, 118,119,121) | MT077767.1<br>MT077769.1<br>MT077772.1<br>MT077768.1<br>MT077770.1<br>MT077773.1<br>MT077771.1 |
| BYF | Byfield (QLD)                      | morphospecies F     | 3 - 22.847 | 150.650 | PB-0(325, 327, 328)                | MT077812.1<br>MT077813.1<br>MT077811.1                                                         |
| CAV | Rossmoya Road, The Caves (QLD)     | morphospecies F     | 4 - 23.159 | 150.457 | PB-0(258,261,271,272)              | MT077809.1<br>MT077814.1<br>MT077815.1<br>MT077810.1                                           |
| COH | Colo Heights (NSW)                 | <i>C. coenosa</i>   | 1 - 33.369 | 150.722 | PB-0164                            | MT077763.1                                                                                     |
| DAR | D'Aguilar Range, Boombana (QLD)    | morphospecies A     | 1 - 27.404 | 152.794 | PB-0058                            | MT077759.1                                                                                     |
| DIN | Charlevue Road, 18km West of       | morphospecies I     | 4 - 23.63  | 149.110 | BJ-00(91-94)                       | MT077818.1<br>MT077819.1                                                                       |

|     |                                                           |                       |            |         |                    |                                                                    |
|-----|-----------------------------------------------------------|-----------------------|------------|---------|--------------------|--------------------------------------------------------------------|
|     | Dingo (QLD)                                               |                       | 8          |         |                    | MT077820.1<br>MT077821.1                                           |
| ECB | Ellery Creek Big Hole, West Macdonnell National Park (NT) | <i>C. pallida</i>     | 4 - 23.778 | 133.073 | BJ-00(30,31,32,34) | MT077843.1<br>MT077844.1<br>MT077608.1<br>MT077845.1               |
| ENP | Eungella National Park, Broken River (QLD)                | morphospecies B       | 4 - 21.162 | 148.512 | PB-00(18-21)       | HM425719.1<br>HM425720.1<br>HM425721.1<br>HM425722.1               |
| EVH | Evans Head (NSW)                                          | <i>C. aberrata</i>    | 5 - 29.108 | 153.431 | PB-0(157-161)      | MT077800.1<br>MT077801.1<br>MT077802.1<br>MT077803.1<br>MT077804.1 |
| FSR | Fountain Springs Rest Area, 60km East of Mount Isa (QLD)  | morphospecies L       | 1 - 20.800 | 139.996 | BJ-0095            | MT077817.1                                                         |
| KOS | Kosciuszko National Park, Guthega Road (NSW)              | <i>C. spurcata</i>    | 2 - 36.500 | 148.266 | PB-00(155, 156)    | MT077832.1<br>MT077833.1                                           |
| LIB | Little bay, Harvey Street (NSW)                           | <i>C. coenosa</i>     | 2 - 33.983 | 151.244 | BJ-00(41-42)       | MT077784.1<br>MT077762.1                                           |
| MTG | Mount Gambier (SA)                                        | <i>C. peridromes</i>  | 3 - 37.843 | 140.765 | PB-0(178-180)      | MT077834.1<br>MT077835.1<br>MT077836.1                             |
| MTW | Mt Warning, Wollumbin (NSW)                               | <i>Candovia</i> sp. H | 2 - 28.400 | 153.282 | PB-0(173, 174)     | MT077775.1<br>MT077776.1                                           |
| PIL | Pilliga (NSW)                                             | <i>C. robinsoni</i>   | 1 - 30.350 | 148.890 | PB-0162            | MT077774.1                                                         |
| RST | Springbrook, Repeater Station Rd (QLD)                    | <i>C. granulosa</i>   | 4 - 28.234 | 153.267 | PB-0(220-223)      | MT077761.1<br>MT077765.1<br>MT077764.1                             |

|     |                            |                      |   |                 |         |             |                                                                                  |
|-----|----------------------------|----------------------|---|-----------------|---------|-------------|----------------------------------------------------------------------------------|
| WIL | Wilmington, Park road (SA) | <i>C. peridromes</i> | 5 | -<br>32.84<br>0 | 138.036 | BJ-0(12-16) | MT077766.1<br>MT077837.1<br>MT077839.1<br>MT077841.1<br>MT077840.1<br>MT077568.1 |
|-----|----------------------------|----------------------|---|-----------------|---------|-------------|----------------------------------------------------------------------------------|

**Table S1c.** Accession number of newly generated sequences used for phylogenetics analyses.

|                                |         | 12S        | 16S        | 18S        | 28S        | cox1       | cox2       | H3         |
|--------------------------------|---------|------------|------------|------------|------------|------------|------------|------------|
| <i>Candovia granulosa</i>      | PB-0222 | MT077518.1 | MT077553.1 | MT077590.1 | MT077629.1 | MT077719.1 | -          | MT077693.1 |
| <i>Candovia granulosa</i>      | PB-0221 | MT077519.1 |            | MT077587.1 | MT077619.1 | MT077720.1 | MT077657.1 | MT077692.1 |
| <i>Candovia koensi</i>         | JH-0030 | MT077516.1 | MT077551.1 | MT077589.1 | MT077618.1 | MT077721.1 | MT077655.1 | MT077688.1 |
| <i>Candovia koensi</i>         | JH-0026 | MT077517.1 | MT077552.1 | MT077586.1 | MT077628.1 | KJ201989.1 | MT077656.1 | MT077700.1 |
| <i>Candovia robinsoni</i>      | PB-0107 | MT077520.1 | MT077556.1 | MT077612.1 | -          | -          | MT077658.1 | -          |
| <i>Candovia robinsoni</i>      | PB-0112 | MT077521.1 | MT077557.1 | MT077588.1 | MT077617.1 | MT077722.1 | MT077659.1 | MT077694.1 |
| <i>Candovia wollumbinensis</i> | PB-0174 | MT077522.1 | MT077555.1 | MT077592.1 | MT077623.1 | MT077728.1 | MT077660.1 | MT077696.1 |
| <i>Candovia wollumbinensis</i> | PB-0173 | -          | MT077554.1 | MT077591.1 | MT077622.1 | MT077727.1 | MT077661.1 | MT077695.1 |
| <i>Candovia aberrata</i>       | PB-0160 | MT077526.1 | MT077561.1 | MT077583.1 | MT077649.1 | MT077723.1 | MT077663.1 | MT077710.1 |
| <i>Candovia aberrata</i>       | PB-0159 | MT077527.1 | MT077560.1 | MT077582.1 | MT077650.1 | MT077724.1 | MT077664.1 | MT077709.1 |
| <i>Candovia dalgleishae</i>    | PB-0188 | MT077528.1 | MT077565.1 | MT077585.1 | MT077626.1 | MT077726.1 | MT077662.1 | MT077711.1 |
| <i>Candovia dalgleishae</i>    | PB-0189 | MT077529.1 | MT077562.1 | MT077584.1 | MT077627.1 | MT077725.1 | -          | MT077712.1 |

|                              |         |            |            |            |            |            |            |            |
|------------------------------|---------|------------|------------|------------|------------|------------|------------|------------|
| <i>Candovia karasi</i>       | JH-0068 | MT077523.1 | MT077572.1 | MT077594.1 | MT077635.1 | MT077731.1 | MT077665.1 | MT077689.1 |
| <i>Candovia karasi</i>       | JH-0066 | MT077524.1 | MT077573.1 | MT077593.1 | MT077634.1 | MT077732.1 | MT077666.1 | MT077690.1 |
| <i>Candovi strumosa</i>      | PB-0216 | -          | -          | -          | MT077636.1 | MT077730.1 | MT077668.1 | MT077691.1 |
| <i>Candovi strumosa</i>      | PB-0218 | MT077525.1 | -          | -          | MT077652.1 | MT077729.1 | MT077667.1 | -          |
| <i>Candovia byfieldensis</i> | PB-0271 | MT077535.1 | MT077558.1 | MT077616.1 | MT077654.1 | -          | MT077675.1 | MT077702.1 |
| <i>Candovia byfieldensis</i> | PB-0261 | -          | MT077559.1 | MT077615.1 | MT077653.1 | MT077735.1 | MT077674.1 | MT077701.1 |
| <i>Candovia anulata</i>      | PB-0207 | MT077530.1 | MT077574.1 | MT077611.1 | MT077630.1 | MT077733.1 | MT077669.1 | MT077699.1 |
| <i>Candovia anulata</i>      | PB-0210 | MT077531.1 | -          | MT077613.1 | MT077631.1 | MT077734.1 | MT077670.1 | -          |
| <i>Candovia alata</i>        | PB-0185 | MT077548.1 | MT077578.1 | MT077609.1 | MT077632.1 | -          | -          | MT077697.1 |
| <i>Candovia alata</i>        | PB-0184 | MT077549.1 | MT077577.1 | MT077595.1 | MT077633.1 | -          | -          | MT077698.1 |
| <i>Candovia eungellensis</i> | PB-0019 | MT077546.1 | MT077575.1 | MT077597.1 | MT077625.1 | MT077736.1 | MT077679.1 | -          |
| <i>Candovia eungellensis</i> | PB-0020 | MT077547.1 | MT077576.1 | MT077596.1 | MT077624.1 | -          | MT077680.1 | -          |
| <i>Candovia spurcata</i>     | PB-0155 | MT077543.1 | -          | -          | MT077639.1 | -          | -          | -          |
| <i>Candovia spurcata</i>     | PB-0156 | MT077544.1 | -          | -          | MT077638.1 | MT077739.1 | MT077678.1 | -          |
| <i>Candovia coenosa</i>      | PB-0164 | MT077541.1 | -          | MT077604.1 | MT077637.1 | MT077737.1 | MT077676.1 | MT077704.1 |
| <i>Candovia coenosa</i>      | BJ-0042 | MT077542.1 | MT077563.1 | MT077599.1 | MT077620.1 | -          | MT077677.1 | MT077703.1 |
| <i>Candovia coenosa</i>      | BJ-0041 | MT077545.1 | MT077621.1 | MT077606.1 | MT077564.1 | MT077738.1 | -          | -          |
| <i>Paracandovia tenera</i>   | BJ-     | MT077536.1 | MT077566.1 | MT077610.1 | MT077643.1 | MT077738.1 | MT077683.1 | MT077705.1 |



|                                    |         |            |            |            |            |            |            |            |
|------------------------------------|---------|------------|------------|------------|------------|------------|------------|------------|
|                                    | 0012    |            |            |            |            |            |            |            |
| <i>Paracandovia tenera</i>         | BJ-0015 | MT077537.1 | MT077568.1 | MT077647.1 | MT077568.1 | MT077743.1 | MT077684.1 | MT077706.1 |
| <i>Paracandovia bifurcata</i>      | PB-0178 | MT077538.1 | -          | MT077601.1 | MT077642.1 | MT077741.1 | MT077682.1 | MT077707.1 |
| <i>Paracandovia bifurcata</i>      | PB-0180 | -          | MT077567.1 | MT077602.1 | MT077644.1 | MT077740.1 | MT077681.1 | MT077708.1 |
| <i>Paracandovia longipes</i>       | BJ-0091 | MT077533.1 | MT077569.1 | MT077605.1 | MT077648.1 | MT077745.1 | MT077687.1 | MT077713.1 |
| <i>Paracandovia longipes</i>       | PB-0094 | MT077534.1 | MT077570.1 | MT077598.1 | MT077646.1 | MT077746.1 | MT077686.1 | MT077714.1 |
| <i>Paracandovia chiaroscuro</i>    | PB-0295 | MT077532.1 | MT077571.1 | MT077603.1 | MT077645.1 | MT077744.1 | MT077685.1 | MT077715.1 |
| <i>Paracandovia pallida</i>        | BJ-0030 | MT077539.1 | MT077579.1 | MT077607.1 | MT077651.1 | MT077747.1 | MT077671.1 | MT077716.1 |
| <i>Paracandovia pallida</i>        | BJ-0032 | MT077540.1 | MT077580.1 | MT077608.1 | MT077640.1 | MT077748.1 | MT077672.1 | MT077717.1 |
| <i>Paracandovia cloncurryensis</i> | BJ-0095 | MT077550.1 | MT077581.1 | MT077614.1 | MT077641.1 | MT077749.1 | MT077673.1 | MT077718.1 |

**Table S2.**

**Table S2.** Gene target, sequence, GC%, amplicon size (bp), temperature and time of annealing of the primers used in the present study.

| GENE               |          | SEQUENCE                       | %G<br>C | AMPLICON<br>SIZE (bp) | ANNEALING TIME AND<br>TEMPERATURE |
|--------------------|----------|--------------------------------|---------|-----------------------|-----------------------------------|
| <b>12S</b>         | <i>F</i> | CTGCACCTTGACYT<br>GAAATA       | 42,5    | 399                   | 56°C, 30s                         |
|                    | <i>R</i> | CAGCATACGCGGT<br>TATACAA       | 45,0    |                       |                                   |
| <b>16S</b>         | <i>F</i> | CGCCTGTTTATCAA<br>AAACAT       | 35,0    | 548                   | 56°C, 30s                         |
|                    | <i>R</i> | CTCCGGTTTGAAC<br>CAGATCA       | 47,6    |                       |                                   |
| <b>COX1</b>        | <i>F</i> | TTGATTTTTTGGTC<br>ATCCAGAAGT   | 33,3    | 819                   | 61°C, 60s                         |
|                    | <i>R</i> | TCCAATGCACTAAT<br>CTGCCATATTA  | 36,0    |                       |                                   |
| <b>COX2</b>        | <i>F</i> | CAGATAAGTGCATT<br>GGATTT       | 35,0    | 1000                  | 50°C, 60s                         |
|                    | <i>R</i> | GTTTAAGAGACCA<br>GTAATTG       | 40,0    |                       |                                   |
| <b>COX1</b><br>FoL | <i>F</i> | GTCAACAAATCATA<br>AAGATATTGG   | 55,0    | 708                   | 55°C, 45s                         |
|                    | <i>R</i> | TAAACTTCAGGGTG<br>ACCAAAAAATCA | 41,3    |                       |                                   |
| <b>18S</b>         | <i>F</i> | CCTGAGAAACGGC<br>TACCACATC     | 54,5    | 800                   | 57°C, 60S                         |
|                    | <i>R</i> | GAGTCTCGTTCGTT<br>ATCGGA       | 50,0    |                       |                                   |
| <b>28S</b>         | <i>F</i> | TCAAGACGGGTCG<br>GGAGA         | 61,0    | 564                   | 58°C, 30s                         |
|                    | <i>R</i> | AGAACTTTGAAGAG<br>AGAGTTCAAGA  | 36,0    |                       |                                   |
| <b>H3</b>          | <i>F</i> | ATGGCTCGTACCA<br>AGCAGAC       | 55,0    | 500                   | 58°C, 30s                         |
|                    | <i>R</i> | ATATCCTTRGGCAT<br>RATRGTGAC    | 41,3    |                       |                                   |

**Table S3.**

**Table S3.** Results of the model selection performed with ModelFinder and PartitionFinder2 on (1) the species delimitation dataset (2) the phylogenetics dataset.

**a) Cox1<sub>Fol</sub> dataset (species delimitation analyses)**

*IQTree model selection with ModelFinder*

| PARTITION NAMES | ID | MODEL     | LogL       | AIC       | w-AIC   | AICc      | w-AICc  | BIC       | w-BIC   |
|-----------------|----|-----------|------------|-----------|---------|-----------|---------|-----------|---------|
| FOLst           | 1  | TN+F+I+G4 | -1033.5600 | 2083.1200 | +0.0000 | 2083.7960 | +0.0000 | 2110.3414 | +0.0000 |
| FOLnd           | 2  | F81+F+I   | -421.4696  | 852.9393  | +0.0000 | 853.2170  | +0.0000 | 869.9526  | +0.0000 |
| FOLrd           | 3  | TIM2+F+G4 | -3602.3608 | 7222.7216 | +0.0000 | 7223.5706 | +0.0000 | 7187.6429 | +0.0000 |

*BEAST 2.5 model selection with PartitionFinder2*

| BEST MODEL | # SITES | PARTITION NAMES |
|------------|---------|-----------------|
| GTR+G+X    | 222     | FOLst           |
| HKY+I+X    | 222     | FOLnd           |
| GTR+G+X    | 222     | FOLrd           |

**b) Species phylogenetic analyses**

*IQTree model selection with ModelFinder*

| PARTITION NAME                                          | BEST MODEL | Log L | AIC         | w-AIC | AICc        | w-AICc | BIC         | w-BIC |
|---------------------------------------------------------|------------|-------|-------------|-------|-------------|--------|-------------|-------|
| 12S                                                     | GTR+F+G4   |       | 4373.988529 | 0     | 4374.909032 | 0      | 4409.203139 | 0     |
| 16S                                                     | GTR+F+I+G4 |       | 7614.369048 | 0     | 7614.86343  | 0      | 7661.697857 | 0     |
| 18S+H03 <sup>st</sup> +H03 <sup>nd</sup>                | TNe+I      |       | 5105.018321 | 0     | 5105.183762 | 0      | 5130.650312 | 0     |
| 28S                                                     | TIM2e+G4   |       | 8950.841332 | 0     | 8951.33479  | 0      | 8992.206915 | 0     |
| H03 <sup>rd</sup>                                       | TVM+F+G4   |       | 3386.588787 | 0     | 3388.444457 | 0      | 3410.644247 | 0     |
| CO1 <sup>st</sup> +CO2 <sup>st</sup> +FOL <sup>st</sup> | TIM2+F+G4  |       | 7247.319122 | 0     | 7247.656029 | 0      | 7285.468161 | 0     |
| CO1 <sup>nd</sup> +CO2 <sup>nd</sup> +FOL <sup>nd</sup> | HKY+F+R2   |       | 3635.414644 | 0     | 3635.751551 | 0      | 3671.506054 | 0     |
| CO1 <sup>rd</sup> +CO2 <sup>rd</sup> +F                 | GTR+F+R    |       | 22471.54    | 0     | 22472.10    | 0      | 22530.01    | 0     |

|                  |   |     |     |     |
|------------------|---|-----|-----|-----|
| OL <sup>rd</sup> | 3 | 156 | 156 | 922 |
|------------------|---|-----|-----|-----|

*MrBayes model selection with PartitionFinder2.*

| BEST MODEL | # SITES | PARTITION NAMES                                           |
|------------|---------|-----------------------------------------------------------|
| GTR+G      | 250     | 12S                                                       |
| GTR+I+G    | 546     | 16S                                                       |
| SYM+I      | 1098    | H03 <sup>st</sup> , H03 <sup>nd</sup> , 18S               |
| GTR+G      | 547     | 28S                                                       |
| GTR+G      | 664     | CO1 <sup>st</sup> , CO2 <sup>st</sup> , FOL <sup>st</sup> |
| HKY+I+G    | 664     | FOL <sup>nd</sup> , CO2 <sup>nd</sup> , CO1 <sup>nd</sup> |
| GTR+G      | 664     | CO2 <sup>rd</sup> , CO1 <sup>rd</sup> , FOL <sup>rd</sup> |
| GTR+G      | 107     | 28S <sup>rd</sup>                                         |

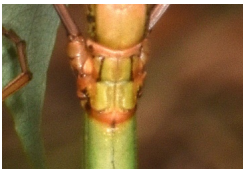
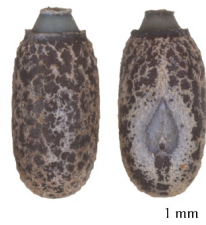
# File S1.

## Key to *Candovia* groups (mainly wingless)

|   |                                                                                                                                                                                                                                                                                                                                                |   |
|---|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| 1 | Body elongated or stout when egg-laying. Wingless or winged (if winged, females with tiny or moderately long hindwings). Species with short cerci in both sexes. Eggs of various shape, none with indented micropylar plate<br><b><i>Candovia</i> Stål</b><br><i>See 2 onwards to distinguish between species groups</i>                       | 2 |
| - | Body elongated. Wingless. Species with long cerci in female (not male), 2-2.4 x length of anal segment. Eggs more elongate, almost rectangular, possibly with indented micropylar plate<br><b><i>Paracandovia</i> gen. n.</b>                                                                                                                  |   |
| 2 | Body elongated and drab. Legs easily exceeding end of abdomen. Eggs oval or nearly so, with low raised, broad mound-like structure on operculum<br><b><i>C. coenosa</i> group</b>                                                                                                                                                              |   |
| - | Body elongated or stout when egg-laying, drab to brightly coloured species. Legs shorter than, about length of, or exceeding end of abdomen. Eggs with or lacking taller raised mound on operculum                                                                                                                                             | 3 |
| 3 | Body elongated, legs reaching almost to or beyond end of abdomen. Eggs oval with large, raised mound present or absent                                                                                                                                                                                                                         | 4 |
| - | Body stout when egg-laying. Legs reaching beyond end of abdomen. Eggs rectangular, rugged appearance, with bumps; lacking mound on operculum<br><b><i>C. robinsoni</i> group</b>                                                                                                                                                               |   |
| 4 | Body elongated with brightly coloured species with various marks, at least in male. Female thorax may have granules. Wingless. Legs just reaching end of abdomen. Eggs oval with tall, broad mound on operculum.<br><b><i>C. aberrata</i> group</b>                                                                                            |   |
| - | Body elongated, female thorax with conspicuous tubercles. Wingless or winged. Eggs rounded, lacking mound on operculum                                                                                                                                                                                                                         | 5 |
| 5 | Legs reaching beyond end of abdomen of which no segments are swollen. Head and/or thorax in female usually lacking black marks, often plain. Wingless or winged (female with moderately long wings). Eggs stout and almost rounded, heavily sculptured<br><b><i>C. annulata</i> group</b>                                                      |   |
| - | Legs of modest length, hindlegs usually not reaching end of abdomen in female, of which segment 6 may be swollen. Head and/or thorax in female with various black marks, even in plain specimens. Wingless or winged (female with tiny wings). Eggs rounded, micropylar plate surrounded by broad pale band<br><b><i>C. strumosa</i> group</b> |   |

**Guide to *Candovia* species**

***C. aberrata* group**

| Species                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Thorax                                                                                                                                                                                                                                                                                                                                             | Genitalia                                                                                                              | Egg                                                                                                                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b><i>aberrata</i></b><br/> BL ♂ 55-61 mm., ♀ 65-83 mm.<br/> Body brown in female (thorax green in male), mesonotum and metanotum may have blackish and white lateral marks (also on head and abdomen in male). Hind part of thorax and abdominal segments reddish with black central mark. In male, hind part of mesothorax and metathorax is also black. Both sexes with legs plain or indistinctly mottled and antennae segment tips indistinctly paler</p>                                                                                                                    | <p>Mesonotum and metanotum with sparse granules including laterally (more conspicuous in female). Pronotum with characteristic black mark on pronotum (less broad in female), shown below in male, of which head has an inverted black 'V' between its eyes:</p>  | <p>Operculum reaching just beyond end of 9th abdominal segment. Poculum also reaching end of 9th abdominal segment</p> | <p>Almost oval, slightly raised mound on operculum, micropylar plate in basal half of capsule (Queensland: Springbrook N.P.)</p>                                        |
| <p><b><i>dalgleishae</i> sp. n.</b><br/> BL ♂ 60-66 mm., ♀ 89 mm.<br/> Body pale greenish in female, each side of head with a narrow, part blackish longitudinal band, running from eyes to back of head. Black median line runs length of body. The male has a dark green thorax (except black pronotum, hind part of mesonotum and metanotum) with pale yellowish-brown head and abdomen, both with black streaks. Antennae black. Both sexes have hind part of thoracic and abdominal segments with broad orange band (preceded by indistinct darkish marks), also brown legs</p> | <p>Mesonotum and metanotum smooth. Pronotum in male black, in female upper part with two black lines well separated from narrow black median line, lower marks not black, as shown below:</p>                                                                   |                                                                                                                        | <p>Almost oval, slightly raised mound on operculum, micropylar plate central, surrounded by broad pale whitish-brown area (Queensland: Blackdown Tableland N.P.)</p>  |

***C. annulata* group**

| Species                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Thorax                                                 | Wings   | Egg                                                                                                                                                                                                                                                                                                                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|---------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b><i>alata</i> sp. n.</b><br/>           BL ♂ 61-64 mm., ♀ 84 mm.<br/>           Body brown in both sexes, with lateral tubercles on thorax. Black line on head between eyes and also black longitudinal line running length of body. Legs indistinctly mottled</p>                                                                                                                                                                                                                                                                                                                                                             | Smooth                                                 | Present | <p>Oval, heavily sculptured, broad capsule. Micropylar plate pear-shaped, surrounded by paler area; whilst central, higher up than eggs of related species. Operculum slightly raised<br/>           (Queensland: Blackdown Tableland N.P.)</p>  |
| <p><b><i>annulata</i></b><br/>           BL ♂ 53-60 mm., ♀ 76-86 mm.<br/>           Body green or brown in female, with several white tubercles on thorax, as in slenderer male. However, male body often dark greenish-brown on head and thorax. Black line on head between eyes bordered by narrow whitish bands; whitish streaks also present laterally on pronotum and towards end of mesonotum and metanotum. Legs with distinctive subapical white band on all femora. In dark forests, specimens are darker, but the female may have a greenish head, purple thorax, orange abdomen with brown marks and green underside</p> | Mesonotum and metanotum with several lateral tubercles | Absent  | <p>Oval, heavily sculptured, micropylar plate same colour as capsule, large, broad and almost pear-shaped. Operculum slightly raised (Queensland: Springbrook N.P.)</p>                                                                        |
| <p><b><i>eungellensis</i> sp. n.</b><br/>           BL ♂ 53-55 mm., ♀</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Thorax granules and tubercles sometimes                | Absent  | <p>Oval capsule with mottled sculpturing. Micropylar</p>                                                                                                                                                                                                                                                                           |



|                                                                                                                                                                                                                                                                                                                                                                                         |                                       |  |                                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 71-75 mm.<br>Elongate green or brown female, possibly with darker marks and indistinctly banded legs. Male slenderer, brown with black marks laterally on thorax, abdomen, apices of femora and tibiae. Whitish marks may also be present laterally. Both sexes with black lines from eyes to back of head, orange mouthparts and eyes brown or orange with two horizontal black lines. | whitish; the latter more on mesonotum |  | plate central, broad and pointed at tip, surrounded by paler mottled area. Operculum dome-like (Queensland: Eungella N.P.)<br> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

#### ***C. coenosa* group**

| Species                                                                                                                                                                                                                                                                                                     | Thorax                                                                            | Genitalia                                                                                                  | Egg                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><i>coenosa</i></b><br>BL ♂ 43-60 mm., ♀ 68-83 mm.<br>Body brown, colour variation not known but antennae black-ringed for up to basal half                                                                                                                                                               | Mesonotum and metanotum with two rows of granules/tubercles, laterally and nearby | Operculum reaching well beyond end of 9th abdominal segment [male uncertain]                               | Almost oval, slightly raised mound on operculum, micropylar plate in basal half of capsule (New South Wales: Colo Heights*)<br> |
| <b><i>spurcata</i></b><br>BL ♂ 43-44 mm., ♀ 68 mm.<br>Male with dark brown band from eye to back of head, colour variation not known but from specimens seen, both sexes brown and rather mottled (including black marks on most segments, with black ring between antennal segments, sometimes indistinct) | Mesonotum and metanotum with sparse granules in both sexes                        | Operculum almost reaching end of 9th abdominal segment. Poculum reaching ½ length of 9th abdominal segment | Oval, slightly raised mound on operculum, micropylar plate central (New South Wales: Kosciuszko N.P.)<br>                       |

\*Further research needed, due to species of very similar appearance – we have two similar egg forms and in the one illustrated (which the authors believe to be this species), the male has not yet been observed. It is

likely further species will be identified in this group, which look similar and lay eggs similar in appearance, with a slightly raised mound on the operculum.

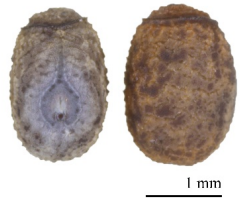
***C. robinsoni* group**

| Species                                                                                                                                                                                 | Thorax                                               | Genitalia                                                                                                            | Egg                                                                                                                                                                                                                                                                   |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><i>granulosa</i></b><br>BL ♂ 43-50 mm., ♀ 58-70 mm.<br>Body brown, occasionally green with some brown marks, abdomen heavily ridged. Mouthparts brown                                | Heavily granulated in female, only sparsely in male  | Operculum not reaching $\frac{1}{2}$ length of anal segment. Poculum not reaching end of 9 <sup>th</sup> segment.    | Almost rectangular, rugged with various pits, some large, including opercular rim and surrounding micropylar plate, but some population differences (New South Wales: Binna Burra)  |
| <b><i>koensi</i> sp. n.</b><br>BL ♂ 59-60 mm., ♀ 71.6-82 mm.<br>Body brown, male with black lateral line, abdomen heavily ridged. Mouthparts brown or in part red                       | Heavily granulated in female, only sparsely in male  | Operculum reaching end of 9th abdominal segment, poculum not reaching $\frac{1}{2}$ length of anal segment           | Undescribed                                                                                                                                                                                                                                                           |
| <b><i>robinsoni</i></b><br>BL ♂ 40-42 mm., ♀ 52-62 mm.<br>Body green or brown in female with some darker marks, brown or greenish-brown male, abdomen heavily ridged. Mouthparts orange | Mesonotum and metanotum with few sparse granulations | Operculum reaching beyond end of 9th abdominal segment, poculum over $\frac{1}{2}$ length of 9 <sup>th</sup> segment | Almost rectangular, heavily sculptured and rugged capsule, broad micropylar plate in basal half of capsule, with surrounding area swollen (Queensland: Springbrook N.P.)          |
| <b><i>wollumbinensis</i> sp. n.</b><br>BL ♂ 47.5 mm., ♀ 52.8 mm.<br>Body green, male with cream lateral stripe, abdomen heavily ridged.                                                 | Sparsely granulated                                  | Operculum reaching end of anal segment, poculum end of 9 <sup>th</sup> segment                                       | Almost rectangular, heavily sculptured and rugged capsule, with keel; micropylar plate with surrounding area pale (New South Wales: Mt Warning (Wollumbin))                                                                                                           |

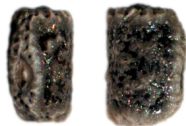
|                   |  |  |                                                                                    |
|-------------------|--|--|------------------------------------------------------------------------------------|
| Mouthparts orange |  |  |  |
|-------------------|--|--|------------------------------------------------------------------------------------|

***C. strumosa* group**

| Species                                                                                                                                                                                                                                                                                                                                                                       | Thorax & abdomen                                                                                                                                                | Wings                                                                                                           | Egg                                                                                                                                                                                                                                                                                                          |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><i>byfieldensis</i> sp. n.</b><br>BL ♂ 59-68 mm., ♀ 74-88 mm.<br>Female plain brownish-green, head yellowish, body more elongate                                                                                                                                                                                                                                           | More elongate than other species in the group, mesonotum with sparse whitish granules, mainly laterally.<br>Hind part of female abdominal segment 6 not swollen | Absent                                                                                                          | Oval, rough capsule, heavily sculptured, micropylar plate central, surrounded by broad pale band, remainder of capsule dark with lighter brown marks. Operculum brown with inner area partly paler (Queensland: Byfield)  |
| <b><i>karasi</i> sp. n.</b><br>BL ♂ 54-55 mm., ♀ 70-75 mm.<br>Female body greenish-brown, with black marks on head and pronotum, also remainder of thorax and abdomen. Male more colourful, greenish-brown with green mesonotum and black marks on head, pronotum and abdomen. Forewings and pre-anal part of hindwings dark brown, net-like with pale veins, hindwings dusky | Mesonotum and metanotum with sparse granules/occasional tubercles.<br>Hind part of female abdominal segment 6 slightly swollen                                  | Female short-winged with tiny hindwings, male long-winged, hindwings reaching beyond end of abdominal segment 5 | Undescribed [misaid, unable to figure]                                                                                                                                                                                                                                                                       |
| <b><i>strumosa</i></b><br>BL ♂ 50 mm., ♀ 59-61 mm.<br>Female body various shades of                                                                                                                                                                                                                                                                                           | Mesonotum with several sparse tubercles, often whitish.                                                                                                         | Absent                                                                                                          | Oval, rough capsule, heavily sculptured, micropylar plate broad and central with spear-shaped point almost reaching                                                                                                                                                                                          |

|                                                                                                                                                                                                                                                      |                                                                             |  |                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>brown or green, grey or black, some specimens heavily mottled, marked with black or whitish; even plain specimens have black marks on head and/or thorax and abdomen. Male colourful, mostly green with black and orange markings on segments</p> | <p>Hind part of female abdominal segment 6 usually considerably swollen</p> |  | <p>top of capsule; plate surrounded by broad pale band (Queensland: Kroombit Tops N.P.)</p>  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Guide to *Paracandovia* species**

| Species                                                                                                                                 | Thorax                                                  | Abdomen                        | Egg                                                                                                                                                                                                                                                          |
|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><i>bifurcata</i> sp. n.</b><br>BL ♂ 45-51 mm., ♀ 62 mm.<br>Type locality – South Australia: Mt Gambier                               | Mesonotum with 18 tubercles laterally up to final third | 2 indistinct ridges each side  | Rough, heavily sculptured (South Australia: Mt Gambier)<br><br>1 mm                                                                                                        |
| <b><i>cercata</i></b><br>BL ♂ 45 mm., ♀ 60 mm.<br>Type locality - New South Wales: Sydney                                               | Heavily granulated                                      | Several (c.6) ridges each side | Almost rectangular capsule (New South Wales: Gibraltar N.P.)<br><br>1 mm                                                                                                   |
| <b><i>chiaroscuro</i> sp. n.</b><br>BL ♂ 56 mm., ♀ 66-76 mm.<br>Type locality - Queensland: Baralaba<br><i>Body with pale underside</i> | Smooth                                                  | 2 indistinct ridges each side  | Rough, uneven, pitted but almost rectangular capsule with indentations, also raised ridge before operculum. Micropylar plate indented (Queensland: Baralaba)<br><br>1 mm |
| <b><i>cloncurriensis</i> sp. n.</b><br>BL ♂ ? mm., ♀ 83 mm.<br>Type locality – Queensland, Mt Isa/Cloncurry                             | Smooth                                                  | Indistinct ridges              | Not quite oval, heavily marked with black blotches, micropylar plate low down (Queensland: Mt Isa)<br>                                                                   |
| <b><i>longipes</i></b><br>BL ♂ 47 mm., ♀ 65 mm.                                                                                         | Sparsely granulated                                     | 3 uneven ridges each side      | Undescribed                                                                                                                                                                                                                                                  |

|                                                                                                                                                               |                                                                         |                                                        |                                                                                                                                                                                                                     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Type locality -<br>Queensland: Bowen                                                                                                                          |                                                                         |                                                        |                                                                                                                                                                                                                     |
| <b><i>pallida</i></b><br>BL ♂ 60-79 mm., ♀<br>76-118 mm.<br>Type locality -<br>Western Australia                                                              | Smooth                                                                  | Indistinct ridges                                      | Heavily sculptured,<br>indistinct micropylar plate<br>(Northern Territory: Ellery<br>Creek Big Hole, West<br>Macdonnell N.P.)<br> |
| <b><i>peridromes</i></b><br>BL ♂ 50 mm., ♀ 67-<br>72 mm.<br>Type locality –<br>Australia (possibly<br>Western Australia)                                      | Mesonotum with 3<br>or 4 very fine<br>granules either side<br>near base | Lateral and<br>sublateral slender<br>ridge either side | Undescribed                                                                                                                                                                                                         |
| <b><i>tener</i></b><br>BL ♂ ?, ♀ 58 mm.<br>Type locality – South<br>Australia: Adelaide<br><i>Type has central<br/>black longitudinal<br/>line along body</i> | Heavily granulated                                                      | 3 ridges each side                                     | Capsule pitted. Micropylar<br>plate indented (South<br>Australia: Wilmington)<br>                                               |