Taxonomy of the *Elymus scaber* complex (Triticeae: Poaceae)

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Declaration

I hereby declare that this thesis contains the results of my own work, except where otherwise indicated, and has not been previously presented for a higher degree in this or any other university.

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Abstract

Elymus L., following Löve's (1984) genomically defined system, consists of about 150 species which are widely distributed throughout the world. Eight species: *E. apricus, E. falcis, E. enysii, E. multiflorus, E. rectisetus, E. sacandros, E. solandri* and *E. tenuis*, are currently recognised in New Zealand, and three taxa, *E. scaber* var. *scaber*, *E. scaber* var. *plurinervis* and *E. multiflorus*, were recorded from Australia. *Elymus rectisetus* was occasionally also considered to be present in Australia. There were taxonomical problems about the number and the circumscription of Australian taxa.

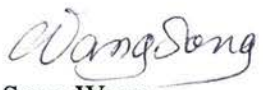
The morphology of 102 samples representing the full range of Australian taxa was examined. Thirty-seven morphological characters were subjected to a phenetic analysis using cluster analysis and Semi- Strong- Hybrid multidimensional scaling (SSH). RAPD analysis was carried out on a sub-set of Australian samples. Characters dealing with the rachilla, callus and palea apices were revealed to be taxonomically informative. These characters were subsequently used to augment a large character set in order to investigate Australian and New Zealand taxa.

Five taxa from Australia are formally recognised: *E. fertilis* sp. nov., *E. multiflorus*, *E. plurinervis* comb. nov., *E. scaber* subsp. *scaber* and *E. scaber* subsp. *rectisetus* comb. nov. Furthermore, *E. rectisetus sensu* Löve et Connor (1982) from New Zealand is confirmed to be identical to *E. scaber* subsp. *scaber,* and *E. enysii* is shown not to belong to *Elymus.* Both agreements and disagreements were found between the results obtained from morphological and RAPD data sets. The final taxonomic conclusions were attained based on comprehensive application of all information.

Nomenclatural Disclaimer

I do not consider the new names and new combinations used in this thesis to be effectively published based on the International Code of Botanical Nomenclature (Art. 29.1; Greuter *et al.*, 1994). Latin diagnoses of new taxa have been deliberately omitted for this reason.

It is my intention to validly publish the new names and new combinations contained within this thesis in journals later on.



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List of Abbreviations

This list includes technical terms. Herbaria in this thesis are abbreviated following Holmgren *et al.* (1990).

AGE	Agarose Gel Electrophoresis.
bp	Base pair.
CTAB	Hexadecyltrimethylammonium bromide.
ICBN	International Code of Botanical Nomenclature
OTU, OTUs	Operational Taxonomic Unit(s).
PCR	Polymerase Chain Reaction.
RAPD	Random Amplified Polymorphic DNA.
SEM	Scanning Electron Microscopy.

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Chapter 1.

Introduction

1.1. Classificatory history

This section includes a brief review of the taxonomic history of the genus *Elymus* L. to place the more detailed review of the *Elymus scaber* complex into its broader context.

1.1.1. Classificatory history of the genus *Elymus* L.

Taxonomic problems encountered within *Elymus* reflect those of the tribe Triticeae as a whole. Barkworth (1992) pointed out that only five of the genera currently recognised within Triticeae have had more than 50% of their species being treated as members of the same genus, and considerable disagreement over the taxonomic treatment also exists at the specific level.

Arguments relating to the circumscription of *Elymus* have existed since Linnaeus (1753) established this genus based on six species, namely: *E. arenarius, E. sibiricus* (lectotype), *E. canadensis*, *E. viriginicus*, *E. caput-medusae* and *E. hystrix*. Two of these species are excluded from the current concept of *Elymus* L. and are transferred to other genera such as *Leymus* Hochst. (*L. arenarius* (L.) Hochst.) and *Taeniatherum* Nevski. (*T. caput-medusae* (L.) Nevski). Table 1-1 shows selected taxonomic treatments compared with Löve's (1984) currently accepted sectional concepts.

The earlier classifications relied almost entirely on the characteristics of the inflorescence and the number of spikelets at each node of the rachis. Bentham and Hooker (1883) separated species with a single spikelet at each node (his Triticeae) from

Table 1-1. The sections of *Elymus sensu* Löve (1984) in alternative taxonomic treatments by different authors. Only genera or sections with one or more species assigned to *Elymus* by Löve (1984) are listed. Superscripts following genera indicate either partial or total agreement with one of Löve's sections.

Löve (1984)	Linnaeus (1753)	Bentham (1883)	Nevski (1933)	Hitchcock (1951)
Elymus	*Elymus*[1, 3, 5]	*Agropyrum*	*Terrella*[3]	*Agropyron*[4,10]
1.Elymus		sect. *Agropyrum*[7,8]		
2.Turczabubivua	*Triticum*[4]		*Clinelymus*[2,3]	*Elymus*[1,3]
3.Macrolepis		*Elymus*		
4.Goulardia		sect. *Sitanion*[6]	*Asperella*[5]	*Hystrix*[5]
5.Hystrix		sect. *Clinelyma*[2,3]		
6.Sitanion			*Roegneria*[4,7,8]	*Sitanion*[6]
7.Clinelymopsis		*Asprella*[5]		
8.Anthosachne			*Anthosachne*[8]	
9.Stenostachys				
10.Dasystachyae			*Sitanion*[6]	
11.Hyalolepis				
			Elymus[*]	

Table 1-1. Continued

Tzvelev (1976, 1989)	Melderis (1978, 1980)	Clayton & Renvoize (1986)	Kuo *et al.* (1987)	Watson & Dallwitz (1992)
Elymus	*Elymus*	*Elymus*[1, 3, 4, 7, 8]	*Elymus*[1,2,3,10]	*Cockaynea*[9]
sect. *Turczaninovia*[2]	sect. *Elymus*[1,2,3,7]			
sect. *Goulardia*[4]	sect. *Goulardia*[4]	*Hystrix*[5, 9]	*Roegneria*[1,4,8]	*Elymus*[1-5, 7-9]
sect. *Clinelymopsis*[7]				
sect. *Anthosachne*[8]		*Sitanion*[6]	*Hystrix*[5]	*Hystrix*[5]
sect. *Clinelymus*[1]				
				Sitanion[6]
Elytrigia[11]				
Hystrix[5]				
Sitanion[6]				
Cockaynea[9]				

* *Elymus sensu* Nevski only contained species of *Leymus sensu* Löve.

those with multiple spikelets at each node (his Elymeae). *Elymus* by Bentham and Hooker's definition included only about 20 species whereas the species with a single spikelet at each node were placed in *Agropyron* J. Gaertn. Similarly, Nevski (1933) applied solitary *vs.* multiple spikelets at each node, but he also used other characters — such as glume shape and anther dimensions — to split the large traditional genera *Elymus* and *Agropyron* into smaller genera. Nevski subdivided *Elymus* into eight genera: *Elymus, Aneurolepidium* Nevski, *Malacurus* Nevski, *Clinelymus* (Griseb.) Nevski, *Terrella* Lunell, *Taeniatherum, Asperella* Humb. and *Sitanion* Rafin.. *Agropyron* was further subdivided into five genera: *Agropyron*, *Elytrigia* Desv., *Anthosachne* Steud., *Roegneria* C. Koch and *Eremopyrum* (Ledeb.) Jaub. & Spach. However, Nevski's concept of *Elymus* did not contain any species of *Elymus* currently recognised by Löve, but rather it included the species of *Leymus* as currently circumscribed by Löve (spikelets in pairs at nodes, and long anthers). Nevski's system influenced at least those authors working on regional floras. For example, the genus *Roegneria* was accepted by Chinese botanists (Keng *et al.*, 1959; Kuo *et al.*, 1987) but was never taken up in North America (Dewey, 1982). The principal character of a solitary spikelet at each node or not, was also employed by Hitchcock (1951) in his classification of wheat grasses in North America. In Hitchcock's treatment, *Elymus* and its related genera *Hystrix* and *Sitanion* encompassed only species with multiple spikelets at each node, whereas those species with a single spikelet at each node were placed in *Agropyron*.

Runemark and Heneen (1968) expressed concern that convergent evolution might lead to the misplacement of unrelated species within the same genus if characters of external morphology were emphasised. Thus, the traditional subdivision into two genera: *Agropyron* and *Elymus* distinguished solely by the number of spikelets at the nodes, was subsequently recognised to be artificial. Similarly, the splitting of two genera into 13 small genera by Nevski (1933) was also considered inappropriate because the characters used for generic definitions were found to be less sound. Based on a comprehensive investigation using morphological, anatomical and cytological data, Runemark and Heneen (1968) suggested the union of the traditional genera *Agropyron* and *Elymus* under the name *Elymus* L. with the exception that some annual species of *Agropyron*

were morphologically and cytologically defensible as a separate genus (*Eremopyrum*), and some annual species of the original *Elymus* were distinct enough, by their morphology of the palea, to be accorded generic status (*Taeniatherum*).
When dealing with the Grasses of the Soviet Union, Tzvelev (1976) distinguished *Agropyron* by keeled glumes and lemmas from *Elymus*. Therefore, the former was restricted to a group of only 15 species with a solitary spikelet at each node, whereas the latter encompassed about 100 species mostly with a single spikelet at each node and rarely with multiple spikelets at each node. He also accepted a number of small genera such as *Elytrigia* and *Hystrix* Moench. In his later, worldwide grass taxonomic system (1989) two more sections of *Elymus sensu* Löve were elevated to generic status - *Cockaynea* Zotov and *Sitanion*. Tzvelev's system was endorsed, with a few exceptions, by Dewey (1982; 1983) for he considered that "it closely reflects biological and phylogenetic relationships."

In accordance with Runemark and Heneen, Melderis (1978), when discussing the classification adopted for the treatment of the tribe Triticeae in Flora Europaea, pointed out the limited taxonomic value of separating genera by the number of spikelets at each node. He agreed with Tzvelev to include *Clinelymus* and *Roegneria* in the genus *Elymus* due to the similar characters of morphology, leaf-anatomy, ecology and cytology. Melderis thought that there was not sufficient evidence (including morphological, anatomical, cytological and genomic data) for keeping *Elytrigia* and *Elymus* apart, and, therefore, he united them in his contribution of the European *Elymus* species.

Löve (1982) pointed out that in the traditional system, the morphologically defined genera may unite taxa that show little signs of genetical relationship but divide those that are closely related. Therefore, he emphasised the importance of genome analysis, a method for the study of polyploids and their ancestry, as crucial for studies of the evolution and definition of the basic taxonomical categories. According to Löve, genomically homogeneous groups could be also morphologically distinct. Therefore, "a genomically homogeneous taxon ... based either on a single haplome with or without a polyploid series or on a distinct combination of two or more haplome, would be most

reasonably classified as a genus". In his exclusively genomic classification of the Triticeae (Löve, 1982; Löve, 1984), *Elymus* is the largest and geographically most widespread genus comprising about 150 species. The genus was subsequently subdivided into 11 sections (Table 1-1) based on the shape of the spike, the number of spikelets at each node, the dimensions and number of nerves of glumes, the length and curvature of lemma awns and the length of anthers. He asserted that all sections have the haplomes H and S with some variations, and the genomic constitutions include allotetraploid SH, autoallohexaploid HHS or SSH, and autoallooctoploid SSHH.

Dewey endorsed Löve's genomic classification system. He (1982) admitted that morphological classification may approximately represent genetic and phylogenetic relationships. However, he stated that taxonomic disagreements arise whenever morphological and biological concepts of generic boundaries fail to coincide. He insisted that genome analysis indubitably contributes to a better understanding of evolution, phylogeny, and systematics of plant species, even though the procedure has obvious limitations. He enumerated a series of possible shortcomings of the genomic system such as the existence of genes for asynapsis or desynapsis, the *Ph* gene which can selectively suppress pairing between partly homologous chromosomes, and the difficulties of interpreting pairing relationships if the parent species are high polyploids. He made the species alignments of North American wheat grasses based solely on genome relationships, such as 'S' taxa, 'H' taxa, 'SH' taxa, 'JX' taxa and 'SHJX' taxa, theoretically similar to Löve's system. He used *Elymus* as the generic name for SH taxa and stated that the type species (*E. sibiricus*) of *Elymus* is an SSHH species, therefore, other species with the S and H genomes should become part of *Elymus*. Consequently *Hystrix, Sitanion* and some members of *Agropyron* would be combined into *Elymus*.

The principle involved in circumscribing a genus on genomic grounds was clarified in Dewey's later publication (Dewey, 1984). Three steps were involved 1) determine the genomic constitution of the type species of the genus, 2) bring all taxa with the same basic genome or combination of genomes into that genus, and 3) exclude from that genus all taxa that do not have the same basic genome or combination of genomes as the type species. The genus *Elymus sensu* Dewey (1984) contains approximately 150

species with the combinations of genomes SH, SY and SHY. Dewey's treatment of *Elymus* was similar to that of Löve but with some modifications. For example, besides genome S and H designated by Löve, Dewey recognised genome Y in some species. Dewey also admitted that "the genome structure and constitution of many New Zealand – Australian species of *Elymus* is still uncertain. After the genomic relationships are fully understood in *Elymus*, several new genera may need to be constructed."

Löve and Dewey's genomic classification system was considered to reflect biological relationships and phylogeny, and to be more useful, even though less convenient, than a system based on morphology (Dewey, 1982).

The problems of generic circumscription within the tribe Triticeae did not, however, end with the genomic system. On the contrary, it aroused an extensive debate. Questions raised included the ability of chromosome pairing to indicate phylogenetic relationships, when to recognise a new genome, and the nature of genomes as characters (Seberg, 1989; Jarvie and Barkworth, 1992).

Seberg (1989) pointed out that the precise relationship between the theoretical and operational definitions of chromosome homology is shown to be inconclusive owing to the uncertainty of the mechanism controlling chromosome pairing. Furthermore, Seberg (1989) asserted that from a phylogenetic point of view, pairing ability is a plesiomorphic character (or character state) which is uninformative with respect to an analysis of the phylogeny of the whole group. Therefore, a phylogeny inferred from data obtained by genome analysis alone will not be any better supported than a phylogeny inferred from phenetic similarity, and genera defined by genome analysis may be either monophyletic or non-monophyletic. Jarvie and Barkworth (1992) concluded, based on the phenetic analysis using morphological data, that supraspecific groups suggested by morphological variation do reflect their genomic constitution. However, they also noted that the similarities between the morphological groups are not in complete agreement with genomically based classifications. Therefore, they suggested the use of a broad range of data for informative and predictive treatments of the Triticeae.

Baum *et al.* (1987) strongly disagreed with Löve and Dewey based on the results obtained from cladistic analyses of the genomically defined genera. The arguments they presented were: the results of genome analysis are not more conclusive than those of other techniques and approaches, genome analysis is also more or less subjective, a genomic system is unstable as the discovery of new genomes or genome combinations will require the taxonomic realignment of already well-defined and well-marked taxa. Baum *et al.* (1987) further advocated that as many characters as possible, rather than only a single character like a genome, should be included in the study of classification. Nevertheless, Baum *et al.*'s view that genome analysis can only supplement other criteria in assessing phylogeny was considered as inaccurate by later authors (Jauhar and Crane, 1989). Jauhar and Crane (1989) admitted that, like other techniques, genome analysis does have its disadvantages, however this technique is still one of the most useful criteria for revealing phylogenetic relatedness especially in consideration of other techniques of genome analysis, such as karyotype analysis and *in situ* hybridisation, have also been employed successfully. Furthermore, Jauhar and Crane (1989) believed that it is not appropriate to adopt cladistic analysis of the Triticeae because reticulate evolution is obvious due to allopolyploidy and homoploid recombinational speciation.

Kellogg (1989) pointed out one of the problems created by using genomes to indicate similarity. If genomes are not discrete genetic units but a continuum, as Dewey (1984) admitted, taxonomists will differ on the extent of similarity as with any continuous character (Kellogg, 1989). This may be one of the reasons why scientists disagree about the genomic constitution of some taxa such as the *Elymus scaber* complex, which would result in generic ambiguity if Löve and Dewey's principle to delimit the genera is strictly followed. With respect to the cladistic point of view, there is an assumption that classification should reflect evolutionary history, that is, that all taxa should be strictly monophyletic, including any and all the descendants of a given ancestor. However, Kellogg (1989) argued that in Löve's genomic system one third of the total genera are heterogenomic, thus they are not monophyletic. Based on a phylogeny of the Triticeae using monogenomic groups, Kellogg endorsed Stebbins' (1956) notion that the entire tribe should be included in one genus. The polyphyly of some heterogenomic genera, such as *Elymus sensu* Löve, has been confirmed by Linde-Laursen *et al.* (1994) from

giemsa C-banding and N-banding data, and by Svitashev *et al.* (1996) based on an analysis of molecular hybridisation.

Since the initial work by Löve and Dewey, much new cytological information obtained from genomic analysis has questioned their generic delimitation as well as the circumscription of the sections within *Elymus*. To date, five basic genomes in different combinations have been found in *Elymus*: SH (Dewey, 1974), SY (Dewey, 1980b), SHY (Dewey, 1980a), SYP (Jensen *et al.*, 1986; Jensen, 1990a; 1990b; Yen and Yang, 1990b), and SYW (Torabinejad *et al.*, 1987; Torabinejad and Mueller, 1993a). The donors of each genome, except Y which is of unknown origin, were assumed to be *Pseudoroegneria* (S), *Hordeum* (or *Critesion sensu* Löve; H), *Agropyron* (P) and *Australopyrum* (W). The S genome is the most consistent component of the genus which is represented in all *Elymus* species.

Torabinejad *et al.* (1987; 1989) and Torabinejad and Mueller (1993a) confirmed the formula for Australian *Elymus* species as SSYYWW, and suggested further investigation of the distribution of the W genome in the *Elymus* complex from Australia and New Zealand. Connor (1994) partly accepted the result that the Australian hexaploids possess a unique genomic formula as defined by Torabinejad *et al.*, but claimed that this explanation can only accommodate the hexaploids but not the tetraploid (*E. enysii*) nor the octoploid (*E. tenuis*) endemic to New Zealand. Another group of workers (Svitashev *et al.*, 1996) revealed, through molecular hybridisation, that in *E. enysii* the genomic constitution is H with an unknown element (not S). This cast doubt on the status of *E. enysii* in the genus *Elymus*.

Further studies by many authors (Jensen, 1990a; Salomon and Lu, 1992; Lu, 1993) revealed the absence of congruence between genome constitution of the species and current sectional delimitation. It was recommended by Lu (1993) that one particular genomic combination should delimit a section, a principle similar to Löve's for delimitation of genera within Triticeae.

Based on new cytological information, as well as the fact that the morphological discontinuity between the taxa is absent, Assadi and Runemark (1995) supported the delimitation of *Elymus sensu* Melderis (1980) in Flora of Europaea, that is to combine *Pseudoroegneria* (S), *Elymus* (SH, SY, sometimes with additional genomes), *Elytrigia* (SSH, SSHX), and *Thinopyrum* (SJ, SJJ, J) into a single genus, *Elymus.* On the contrary, splitting *Elymus* into smaller genera has also been presented by other authors. The examples are: the separation from *Elymus* of a large number of taxa into *Roegneria* based on the genome constitution (SY) and morphology (a single spikelet at each node and palea structure) (Yen and Yang, 1990a; Baum *et al.*, 1991), and a subsequent separation of other species into *Kengyilia* which has SYP genomes and intermediate morphological characters between *Agropyron* and *Roegneria* (Yen and Yang, 1990b; Baum *et al.*, 1995), the remainder is *Elymus sensu stricto.* Moreover, Connor (1994) reinstated the section *Stenostachys* of *Elymus* at generic level mainly because of its spikelet shape.

Australian *Elymus* species were involved in the separation of *Roegneria* from *Elymus.* Being insistent on weighting genome and other characteristics equally, Baum *et al.* (1991) documented that *Roegneria* is worth recognising as a separate genus alongside *Elymus, Elytrigia* and *Agropyron.* In Baum *et al.*'s (1991) treatment, about 127 species were transferred to *Roegneria* from *Elymus* and only 20-30 species were considered valid in *Elymus.* The key character used by these authors to separate *Roegneria* from *Elymus* was whether the spikelets were solitary (*Roegneria*) or double (*Elymus*) at each node of the rachis. Some other characters were also compared between these two genera, and they are listed in Table 1-2. Baum *et al.* (1991) also intended to link the genus *Roegneria* with the genomic information. They believed that some generic characters may be correlated with particular genomes, thus the characters of *Roegneria* may result from the existence of the Y genome and the genomic formulae for this genus are possibly SSYY and SSHHYY. However, this hypothesis seemed not to be supported by later genomic information (Lu, 1993). According to Baum *et al.*'s treatment, some elements of *Elymus* from southern hemisphere will fall into the genus *Roegneria* (*E. scaber* was the only representative mentioned by the authors).

Connor (1994) declared the retention of *Elymus* and the rejection of *Roegneria* for indigenous species from New Zealand. Meanwhile, he discussed the possibility that a new genus be erected for Australian hexaploids if Löve's (1984) principle was followed, thus *Anthosachne* would be the name for the new genus.

Table 1-2. Summary of characters used for differentiating *Elymus sensu* Baum *et al.* and *Roegneria sensu* Baum *et al.* (1991).

Characters	*Roegneria*	*Elymus*
Spikelets / node	one	two
Glumes	wide, 3-9 nerves	narrow, 1-5 nerves
Palea length	shorter than, or equal to, the lemma	equal to, or slightly longer than, the lemma
Palea tip	obtuse, truncate or retuse	acute

In view of the discussion above, it is clear that the arguments focusing on the generic delimitation within the tribe Triticeae, as well as the genome constitution and its employment in distinguishing *Elymus* from its related genera have been the subject of controversy over the years, and will continue to be the subject of taxonomic disagreement. Numerous systems have been presented by different authors, but none, however, were universally accepted. In this research, the nomenclature follows Löve's treatment of Triticeae (1984). In this treatment, Australian and New Zealand taxa are included in *Elymus* sect. *Anthosachne* (Steud.) Tzvelev.

1.1.2. General description of *Elymus* L. as currently defined

The latest complete description of *Elymus* was provided by Löve (1984) and Lu (1993). According to these authors, *Elymus* is distinguished by its perennial and mostly caespitose habit, a single spike with spikelets solitary or in groups of two or more at each rachis node, narrowly lanceolate glumes, muticous to long-awned lemmas, and short anthers (1-3.5 mm). All species are polyploid including tetraploid, hexaploid and

octoploid. To date, the following patterns of haplome and genome combinations have been reported: SH, SY, SHH, SSH, SHY, SSY, SYP and SYW.

Elymus L., as it is currently recognised, consists of about 150 species (Dewey, 1984; Löve, 1984) in an exclusively genomically defined system. It is by far the largest and most geographically widespread genus in the tribe Triticeae of the grass family (Gramineae or Poaceae).

Asia, particularly the central Asiatic mountain region, was considered to be a likely centre for the origin and diversity of *Elymus* (Lu, 1993). About 100 *Elymus* species (based on the taxonomic revisions by Lu, 1993) are distributed in Asia, a small number of species are found in Europe, North and South America, and about 10 species have been reported from Australia and New Zealand.

Many species of this genus are hay and pasture-fodder plants of varying quality (Hitchcock, 1951; Tzvelev, 1976; Clayton and Renvoize, 1986), and the species on dunes are useful sand binders (Tzvelev, 1976; Clayton and Renvoize, 1986). Disease resistance, wide adaptation, and apomixis are characteristics that make *Elymus* a genus of interest to breeders of annual Triticeae cereal crops (Dewey, 1984).

Elymus L. was subsequently divided into eleven sections by Löve (1984) based on several morphological characters. The *Elymus scaber* complex falls into the section *Anthosachne* (Steud.) Tzvelev. According to Löve (1984), section *Anthosachne* is delimited by the following characters: Spike enlarged at the top, usually nodding; rachis tough; spikelets 3-7 in two rows, solitary, short-pedicelled to sessile. Glumes unequal in size, 4-12 mm long, 3-5 veined, lanceolate to linear-lanceolate. Lemmas awned; awns 15-50 mm long, canaliculate and tapering. Anthers long. The type species is *Elymus rectisetus* (Nees in Lehm.) A. Löve and Connor based on *Vulpia rectiseta* Nees in Lehm.(1846). This section includes about 20 species distributed in Africa (1 species), eastern and south central Asia to Australia and New Zealand.

1.1.3. Classificatory history of the *Elymus scaber* complex in Australia

Elymus scaber was first described by Labillardière (1805) as *Festuca scabra* based on a specimen collected from Hobart, Tasmania. The defining characters used by Labillardière included the lemma being terminated by a long awn and the palea apex being emarginate. However, this name was previously and validly published by Vahl (1791) based on a different type, and, as such, Labillardière's name was illegitimate according to the ICBN (Art. 53; Greuter *et al.*, 1994). The epithet "*scabrum*" was legitimately published by Brown (1810) as *Triticum scabrum* based on Labillardière's *Festuca scabra*. Subsequently, Beauvois (1812) transferred this species to the genus *Agropyron* as *A. scabrum*, and was taken up by many authors working on the Australian flora (Bentham, 1878; Bailey, 1902; Ewart, 1930; Black, 1948; Vickery, 1951; Willis, 1970; Simon, 1978; Wheeler *et al.*, 1984). Not until recently, has the species been referred to as *Elymus scaber* following Löve's (1984) treatment (Jessop, 1986; Simon, 1986; Stanley and Ross, 1989; Jacobs and Hastings, 1993; Walsh, 1994).

The morphological diversity (e.g. awn length and curvature) of *E. scaber* has given rise to what has been termed the *E. scaber* complex. Numerous names have been applied to the more distinct elements of the complex (Table 1-3). Nees in Lehm. (1846) described *Vulpia rectiseta* based on the specimen collected from Western Australia (Preiss. No. 1819 in MEL). Meanwhile, he also listed two other species, namely, *V. browniana* based on *Triticum scabrum* R. Br. and *V. scabra* based on *Festuca scabra* Labill. The species *V. rectiseta* was accepted by some later authors (Walpers, 1849; Steudel, 1854; Löve and Connor, 1982; Löve, 1984; Connor, 1994), however, not by Australian workers (Table 1-3).

Steudel (1854) accepted *V. browniana*, *V. rectiseta* and *V. scabra* as separate taxa but transferred them to the genus *Festuca* and renamed *F. scabra* Labill. as *F. billardieri*. He apparently realised that *Festuca scabra* had been previously published by Vahl based on another type, therefore he cited Labillardière's name as a synonym of *F. billardieri*. Furthermore, he established a new taxon, *Anthosachne australasica,* based on

Table 1-3. Summary of classifications of the *Elymus scaber* complex from Australia*

Epithet	Localities of types	Labillardière (1805)	R. Brown (1810)	Beauvois (1812)	Nees in Lehm. (1846)	Walp. (1849)	Hook. f. (1853)	Steud. (1854)
scaber	Hobart, Tasmania, Labillardière; holo: FI.	***Festuca scabra*** *(nom. illeg.)*	***Triticum scabrum***	***Agropyron scabrum***	***Vulpia browniana*** [*Triticum scabrum* R. Br.] ***Vulpia scabra*** [*Festuca scabra* Labill.]	***Festuca browniana*** ***Festuca scabra***	***Triticum scabrum***	***F. browniana*** ***F. billardieri*** [*V. scabra; F. scabra*]
rectisetus	[1]WA, Preiss 1819; holo: MEL. [2]WA, Drummond 382-384; syn: K.				[1]***Vulpia rectiseta***	***Festuca rectiseta***		***Festuca rectiseta*** [2]***Anthosachne australasica***
multiflorus	NZ, Banks & Solander, lecto: BM.						***Triticum multiflorus***	
plurinervis	NSW, Thomas; holo: NSW (No. 8245)							

Table 1-3. continued

Epithet	Nevski (1934)	Tzvelev (1973)	Bentham (1878)	Bailey (1902)	Ewart (1930)	Black (1948)	Vickery (1951)	Willis (1970)
scaber	***Anthosachne scabra***		***Agropyron scabrum***	***Agropyron scabrum***	***Agropyron scabrum***	***Agropyron scabrum***	***Agropyron scabrum***	***Agropyron scabrum***
rectisetus	***Anthosachne australasica***	***Elymus australasicus*** [*Anthosachne australasica*]						
multiflorus	As a syn. of *Anthosachne scabra*							
plurinervis							***Agropyron scabrum* var. *plurinerve***	

Table 1-3. continued

Epithet	Simon (1978)	Wheeler *et al.*(1982)	Simon (1986)	Jessop (1986)	Stanley & Ross (1989)	Simon (1990, 1993)	Jacobs and Hastings (1993)	Walsh (1994)
scaber	***Agropyron scabrum***	***A. scabrum***	***E. scaber***	***E. scaber***	***E. scaber***	***E. scaber***	***E. scaber***	***E. scaber***
rectisetus								
multiflorus					***E. multiflorus***	***E. multiflorus***		***E. multiflorus***
plurinervis	***A. scabrum* var. *plurinerve***	***A. scabrum* var. *plurinerve***	***E. scaber* var. *plurinervis***		***E. scaber* var. *plurinervis***	***E. scaber* var. *plurinervis***	***E. scaber* var. *plurinervis***	

*: The names in [ ] were cited as synonyms by the authors. 1, 2 : Different names were based on different types.

specimens from Western Australia (Drummond 382-384, syn: K) which was only used by Russian authors (e.g. Nevski, 1933). It was reduced to sectional rank in *Agropyron* by Melderis (1970) or *Elymus* by Tzvelev (1973).

For a long time only one taxon, *Agropyron scabrum,* had been recognised in Australia and the other names including *Vulpia* (or *Festuca*) *rectiseta* and *Anthosachne australasica* were all treated as the synonyms of *A. scabrum* (Bentham, 1878; Bailey, 1902; Vickery, 1951). Vickery (1951) increased the number of entities within the complex by distinguishing *Agropyron scabrum* var. *plurinerve*, characterised by wider and longer glumes (10-15 mm long) with 6-8 nerves, and lemmas apex narrowing quickly into the awn. This variety was widely accepted by other authors (Table 1-3). As to the remainder of the complex, Vickery (1951) concluded that: "the combination of characters is so variable that attempts to recognise taxonomic units within the species (*Agropyron scabrum*) have not so far been successful." Jacobs and Hastings (1993) recognised two taxa (e.g. the two varieties of *E. scaber*) but pointed out the possibility of more taxa.

Rees (1972) suggested the existence of two distinct forms in *E. scaber* var. *plurinervis*, namely form A and form B. Two forms were differentiated on the basis of morphology, ecology and their roles in epidemiology of rust (*Puccinia graminis*) though they were sympatric (Rees, 1972). According to the descriptions presented by Rees, it is obvious that form A is typical *Elymus scaber* var. *plurinervis*, whereas form B is the short-awned form treated as either *E. scaber* var. scaber or *E. multiflorus* by later authors (see the subsequent discussion).

A third taxon recently recognised in Australia is *Elymus multiflorus*. It was first described by Hooker *f.* (1853) as *Triticum multiflorus* based on Banks and Solander's collection from New Zealand in 1769. Since then the taxon has been taken up by other workers working on the New Zealand flora under several different names (*Agropyron mutiflorum* (Hook. f.) Cheeseman (1906), *Agropyron kirkii* Zotov (1943, nom. superfl.) and *Elymus multiflorus* (Hook. f.) Löve and Connor (1982)). The key character used to distinguish it from other related taxa is the short and straight awn approximately equal

in length with the lemma (Connor, 1994). This taxon, nevertheless, did not gain recognition in Australia until recently when it was recorded in some regional floras (Table 1-3). The diagnostic character used by different Australian authors (Stanley and Ross, 1989; Simon, 1993; Walsh, 1994) to separate *E. multiflorus* from *E. scaber* is similarly the former's shorter lemma awn length.

Torabinejad *et al.* (1987) distinguished three taxa: *E. scaber* var. *plurinervis*, *E. multiflorus* and *E. rectisetus*, on the basis of glume and lemma characteristics. According to Torabinejad *et al.*, *Elymus scaber* var. *plurinervis* is characterised by large glumes with many nerves and limited to the dark basaltic heavy clays of Queensland and northern New South Wales. The second taxon, a short-awned form, which has smaller glumes and is sympatric with *E. scaber* var. *plurinervis* but with a much broader geographic distribution, resembles *E. multiflorus* from New Zealand. However, further study is still needed to confirm their taxonomic synonymization. The last taxon, *E. rectisetus*, is apomictic, and has small glumes and long awns, and occurs widely throughout Australia except the Northern Territory.

Carman and Wang (1992) also accepted three Australian taxa in *Elymus*. Their definitions of *E. rectisetus* and *E. scaber* var. *plurinervis* agreed with those of Torabinejad *et al.* (1987), however, they equated the short-awned type with Labillardière's type-specimen of *E. scaber* instead of with *E. multiflorus* (*sensu* Torabinejad *et al.*) of New Zealand. Furthermore, they pointed out the necessity of revising the species complex by formally synonimizing the Australian apomicts with *E. rectisetus* apomicts of New Zealand, and by elevating *E. scaber* var. *plurinervis* to species status, as its hybrids with *E. scaber* are sterile.

Some authors foreshadowed the presence of more taxa in the *Elymus scaber* complex. Crane and Carman (1987) argued that at least four taxa could be distinguished morphologically in the species complex. The definitions of *E. rectisetus* and two varieties of *E. scaber* by Crane and Carman (1987) concurred with other authors (Carman and Wang, 1992). However, Crane and Carman (1987) described a fourth taxon consisting of intermediate to mostly long-awned individuals, which are

characterised by highly fertile seeds and distributed in montane forests from the Australian Capital Territory to south-central Victoria. According to Crane and Carman, the fourth taxon corresponds somewhat to montane forms of *E. rectisetus* in New Zealand.

A recent informal classification of the *Elymus scaber* complex was presented by Murphy and Jones (1999) based on the phenetic analysis of 19 specimens from the New England Tablelands of New South Wales. Four major entities were identified: short-awned forms of *E. scaber* (= *E. scaber sensu* Labillardière); long-awned apomictic forms of *E. scaber* (= *E. rectisetus*); entities with large glumes, often bifid lemmas and a high palea to caryopsis ratio (= *E. scaber* var. *plurinervis*); and very short-awned entities (= *E. multiflorus*).

Apart from the four entities listed, Murphy and Jones (1999) mentioned an ambiguous form characterised by some intermediate characters, such as medium-awn length and similar percentage seed set and floret shape to the short-awned form. This form was found in montane areas of NSW and south-central Vic. The only specimen of this form was clustered together with the short-awned form of *E. scaber* in their phenetic analysis, but together with the long-awned specimen in some analyses of the robustness test. It is apparent that this form is the fourth taxon described by Crane and Carman (1987). Murphy and Jones (1999) concluded that the current *E. scaber* var. *scaber* may represent one large species complex that includes *E. rectisetus*, *E. scaber* and a mass of hybrid and/or aberrant sexual forms.

In addition, Murphy and Jones (1999) noted the difficulties when separating the long-awned specimens from the short- and medium-awned specimens of *E. scaber*, and the distinction of *E. multiflorus* and *E. scaber* var. *plurinervis* from one another and from all other forms. They admitted that although *E. multiflorus* was separated from the short- and medium-awned specimens of *E. scaber*, their hierarchical relationship had not been clarified. They also suggested further work on the *Elymus scaber* complex: to confirm obligate sexuality in the short-awned forms, and to determine the breeding system of the intermediate forms and their relationships to the long-awned forms.

Finally, they suggested the separation of *E. scaber* var. *plurinervis* from *E. scaber* at the specific level, which concurred with Carman (Carman in Carman and Wang, 1992) and Connor (pers. comm.).

The viewpoint of four taxa existing in Australia was also articulated by Löve (1982) and Connor (1994). Connor (1994) insisted that there are several hexaploid taxa in Australia: *E. multiflorus* and *E. rectisetus* are found in both New Zealand and Australia, but the latter was naturalised from Australia, and *E. scaber* and *E. scaber* var. *plurinervis* are endemic to Australia.

1.1.4. Classificatory history of *Elymus scaber* complex in New Zealand

Connor and his colleagues have undertaken a comprehensive study on the related species of *Elymus* from New Zealand during past decades. When they started this research (Connor, 1954), only four species were recognised: *Agropyron enysii* (= *Elymus enysii*), *A. multiflorum* (= *E. multiflorus*), *A. scabrum* (= *E. scaber*), and *A. tenue* (= *E. tenuis*). *Agropyron enysii* is apparently different from others and was recognised earlier by Kirk (Kirk, 1895). *Agropyron multiflorum* was first described by J. D. Hooker (*Triticum multiflorum* Hook. f.; Hooker, 1853) and transferred to *Agropyron* by Cheeseman (1906). *Agropyron scabrum* was found to be widely distributed in New Zealand and Australia. *Agropyron tenue* (=*E. tenuis*) was originally described as a variety of *A. scabrum* by Buchanan (1880) and was separated from *Agropyron scabrum* and accorded species level by Connor (1954) based on morphological and cytological grounds, such as extravaginal branching, bifid lemma apex, laxly tufted and trailing habit of culm, more slender spikelets, and 56 chromosomes (8x). In the latest publication by Connor (1994), however, eight species were listed in *Elymus* from New Zealand: *E. enysii*, *E. apricus*, *E. sacandros*, *E. solandri*, *E. falcis*, *E. tenuis*, *E. multiflorus* and *E. rectisetus*. Except for *E. enysii*, *E. multiflorus* and *E. tenuis*, the others were split gradually from the aggregate species *Agropyron scabrum.*

Connor (1954) noted that the collections of *Agropyron scabrum* from different localities were morphologically variable, and that the transplants of these morphological variants

retained their particular characters in experimental gardens. Based on their morphological characters, reproductive peculiarities and geographic distribution, Connor (1954) grouped them into three major series comprising twelve groups. Connor indicated that he considered that some formal, taxonomic ranks could possibly be recognised from these infraspecific groups.

New taxonomic results were presented by Löve and Connor (1982). It was claimed, based on the hybridisation studies, that one of the groups (Series three) was genetically and morphologically distinct enough to be recognised as a new species, *E. apricus*. However, the remaining groups were not considered to be worthy of formal taxonomic recognition. They were included in one species, *E. rectisetus*, a new name selected for New Zealand elements by the authors based on the assumption that *Agropyron scabrum* based on Labillardière specimens is a short-awned form and should only occur in Australia.

The most recent revision of the *Elymus scaber* complex from New Zealand was made by Connor (1994) when two new species and a new combination of *Elymus* were described. *Elymus falcis* (a sexual group in Connor's Series two) and *E. sacandros* (one population from an apomictic group in Series one) were described as new and differentiated from others by involute leaf-blades and minute or absent auricles. The former was distinct from the latter by its peculiar leaf-blades (rather short, variously falcate, sinuous or straight, abaxially glaucous usually with scattered erect long hairs) and prostrate to ascending culms. *Elymus solandri* (the remainder of sexual Series two) was revived from the synonymy of *Triticum solandri* and separated from *E. rectisetus* (the remainder of apomictic Series one) on account of its usually glaucous and abaxially glabrous leaf blades, its recurved awns, and its pointed, bifid paleas. Furthermore, both *E. rectisetus* and *E. solandri* were widely distributed throughout New Zealand.

It should be noted that the taxonomic conclusions summarised above were reached not only on the basis of morphological variation but also on thorough biosystematic studies and ecological evidence (Connor, 1954; 1956; Löve and Connor, 1982).

1.1.5. Related studies on the *Elymus scaber* complex

Reproductive biology

Elymus rectisetus sensu Löve and Connor is the only apomict so far known in the tribe Triticeae (Dewey, 1984; Carman and Wang, 1992). Its relatedness to wheat, one of the most important crops, makes it potentially useful for breeding programs. That is to say, if the gene controlling apomixis can be transferred to wheat then elite lines of wheat can be preserved which would facilitate commercial production of our major food. Bashaw and Hanna (1990) believed that producing partially fertile interspecific hybrids and backcrosses which can maintain a high level of apomictic reproduction would make progress in transference of genes controlling apomixis.

Much work has been done to demonstrate the apomictic mechanism (Hair, 1956; Crane and Carman, 1987; Carman *et al.*, 1991; Peel *et al.*, 1997). Many attempts have been made to transfer apomixis from wild species to domestic Triticeae (Carman and Wang, 1992) and some of the earlier efforts failed (Zenkteler and Nitzsche, 1984; Torabinejad *et al.*, 1987). However, more recent work has been successful to some extent (Ahmad and Comeau, 1991; Torabinejad and Mueller, 1993b; Liu *et al.*, 1994). Obviously much work needs to be done, but it is believed that the transfer of apomixis to cereal species of Triticeae is possible (Torabinejad and Muller, 1993b).

Apart from the apomictic groups taxonomically recognised as *E. rectisetus*, all *Elymus species* in New Zealand were found to be apparently obligately sexual and self-compatible (Connor, 1954, 1994; Löve and Connor, 1982). *Elymus rectisetus* was believed to be of Australian origin, having been introduced into New Zealand about 130-140 years ago (Löve and Connor, 1982; Connor, 1994). Nevertheless, apomixis had not been investigated in Australian collections of *Elymus* species until Crane and Carman (1987) examined the megasporogenesis in cleared ovaries of *Elymus*, and confirmed that the intermediate- to long-awned forms from Australia corresponded to the apomictic *E. rectisetus* found in New Zealand. This result suggested that apomictic forms were widespread in Australia. They were scattered across southern Australia but were most common along the inland side of the Dividing Range from southwestern

Victoria to northeastern New South Wales (Crane and Carman, 1987). Crane and Carman (1987) also identified that individuals of *E. scaber* var. *scaber* and *E. scaber* var. *plurinervis* from Queensland were obligately sexual. This finding was confirmed by Murphy and Jones (1999) who indicated that the long-awned forms of *E. scaber* var. *scaber* in Australia were characterised by low seed set, a reputed feature indicating apomixis. Furthermore, Murphy (pers. comm.) suggested that long awns and low seed set indicated apomixis in Australian *Elymus*, but that short awns and average to high seed set did not exclusively indicate obligate sexuality, especially where polymorphism within a population was evident. Therefore, Murphy (pers. comm.) considered the possibility that the intermediate awn length indicated a stronger influence from the apomictic genes.

Cytology and genome analyses

Extensive inter- and intra-generic hybridisations with the *Elymus scaber* complex from Australia and New Zealand laid the foundation for the discussion of the inter- and intra-specific relationships of this species complex, and inter-generic relationships of *Elymus* and some related genera. The genome constitutions were suggested as SH for tetraploid (*E. enysii*), SSH for hexaploids (*E. rectisetus*, *E. multiflorus* and *E. apricus*), and SSHH for octoploid (*E. tenuis*) based on the results obtained from the hybridisation between this group and *Elytrigia*, *Pseudoroegneria*, *Critesion, Leymus, Lophopyrum, Thynopyrum, Hordeum, Aegilops,* and some *Elymus* species (e.g. *E. longearistatus* from India, and *E. trachycaulus* from Canada (Löve and Connor, 1982)). Torabinejad *et al.* (1987) and Torabinejad and Mueller (1993a) hybridised this group with *Elymus* species of Asian and American origin, as well as with *Australopyrum*, *Psathyrostachys* and *Thynopyrum*. Their results revealed that the genome formula for *E. scaber* obtained from Australia should be written as SSYYWW. Connor (1994) accepted the result, but considered that the formula could only accommodate the hexaploids, and that further studies are needed for the tetraploid *E. enysii* and the octoploid *E. tenuis*.

Biosystematic investigations within the species complex

The fertility of hybrids is considered to be one of the most important indicators of biosystematic relationships, for it may be a measure of the ability of the species to

exchange genes (Connor, 1956). The current taxonomic conclusions about the *Elymus scaber* complex, especially those from New Zealand, were achieved largely based on biosystematic investigations (Connor, 1954; 1956; 1962a; 1962b; Löve and Connor, 1982). Connor (1956; 1962b) reported that gene exchange took place between *Agropyron multiflorum* (= *Elymus multiflorus*) and some sexual strains of *A. scabrum* in their hybridisation experiments, therefore hybrids and introgression between these two were likely in nature if they were sympatric. It was generalised by Löve and Connor (1982) that, although artificial hybrids of all combinations of New Zealand *Elymus* species have been produced, the natural hybridisation between these native species is rare. This may result from the agamospermy in *E. rectisetus* and predominantly autogamy in others except *E. multiflorus* which is allogamous.

Similar work has seldom been done on Australian species with the exception that Carman and Wang (1992) reported sterile hybrids between the two varieties of *E. scaber*, and the male sterile hybrids between *E. scaber* and *E. rectisetus*.

Molecular analysis

The *Nor* and *5SDna* loci of the Triticeae have been well studied over the past 20 years and have provided useful examples of the application of DNA analytic information to problems such as assessing the relationships between species (Appels and Baum, 1992). A study by Appels and Baum (1992) indicated that certain *Nor* loci from some *Elymus* species are more closely related to the *Australopyrum retrofractum* (W genome) probe. This result further supported Torabinejad and Mueller's (1993a) suggestion that the W genome should be included in the genome formula for *E. scaber*. The study also suggested a relatively close relationship between *Australopyrum* and *Elymus*/*Pseudoroegeneria*, which raised the possibility that *Australopyrum* is not a Gondwanan relic but found its way into Australia in relatively recent times, possibly via an Asian route. Further investigation of the Triticeae species in Australia and Asia may present interesting information about the further calibration of sequence changes in DNA in time (Scoles *et al.*, 1988).

Wei and Wang (1995) developed genome- and species-specific markers based on the Random Amplified Polymorphic DNA (RAPD) technique and claimed that these markers were useful in studies of genome evolution, analysis of genome composition, and genome identification. Their results showed that genetic similarity was greater amongst species having the same basic genome than amongst species having different basic genomes. Furthermore, the W genome had the largest number of unique RAPD bands indicating that this genome has diverged from other genomes early in the evolution of genome. Four genome-specific markers were found to be connected to the S genome and five to the W genome respectively. However, no genome-specific markers were developed for the Y genome.

Recently Svitashev *et al.* (1996) employed four repetitive DNA sequences cloned from the barley (*Hordeum vulgare*) genome for a molecular study using the Southern blot hybridisation technique. Their aims were to study phylogenetic relationships within 28 *Elymus* species including *E. scaber* and *E. enysii* (both were obtained from New Zealand), to test whether this approach was useful for discriminating between *Elymus* species with different genomes, and to elucidate the genomic constitution of species with uncertain or unknown genomes. Their results showed that the W genome, unlike genomes H and S, generally gave rather poor hybridisation signals and no specific bands different from the other genomes were revealed. Similarly, a weak hybridization signal and no specific hybridization patterns were observed on the Y genome. Furthermore, the results confirmed the presence of the genome H but rejected the genome S in *E. enysii* which posed a question of the status of this species in the genus *Elymus* and also implied the necessity for further study including other Australasian taxa and a broader set of probes.

1.2. Species problems of Australian *Elymus*

The number of taxa

Currently, the number of taxa constituting the *Elymus scaber* complex in Australia is controversial. *Elymus scaber* and *E. rectisetus* were listed in a recent world wide summary of the tribe Triticeae (Löve, 1984) regardless of the fact that the name *E.*

rectisetus (= *Vulpia rectiseta* Nees in Lehm.) has never been accepted by Australian taxonomists. According to Löve's work, the *Elymus scaber* complex from Australia should contain at least four taxa, namely *E. rectisetus*, *E. scaber* var. *scaber*, *E. scaber* var. *plurinervis* and *E. multiflorus*. This was recommended strongly by Connor (1994), and was also supported by Crane and Carman (1987) and Murphy and Jones (1999) based on their independent investigation of Australian material.

The circumscription of taxa

No matter how many taxa are preferred by different workers, the names, *E. rectisetus*, *E. scaber*, *E. scaber* var. *plurinervis*, and *E. multiflorus* have, at one time or another, been used in some regional floras of Australia and related studies. A careful examination revealed that there has been confusion about the circumscriptions of these taxa used by different workers. Examples are shown in Table 1-4.

It is clear that confusion exists in all taxa except *E. scaber* var. *plurinervis* for its glume characters and restricted geographic area render it easily distinguishable from the other species. The taxonomic treatments by Australian workers (Stanley and Ross, 1989; Jacobs and Hastings, 1993; Walsh, 1994) are all similar in including *E. rectisetus* within *E. scaber* var. *scaber*. Other workers (Crane and Carman, 1987; Torabinejad *et al.*, 1987; Murphy and Jones, 1999), who accepted *E. rectisetus* as a separate taxon, agreed with each other that this taxon is characterised by long awns, apomixis, widespread distribution in Australia and that it is synonymous with the New Zealand *E. rectisetus*.

However, disagreements occurred when these authors defined *E. scaber* var. *scaber* and *E. multiflorus* (Table 1-4). The short-awned specimens found from southeastern Queensland to Victoria and probably South Australia were treated as either *E. multiflorus* (Torabinejad *et al.*, 1987; Stanley and Ross, 1989; Walsh, 1994) or *E. scaber* var. *scaber* (Crane and Carman, 1987; Murphy and Jones, 1999), and part of the form (very short-awned) was probably defined as *E. multiflorus* by Murphy and Jones (1999). Torabinejad *et al.* (1987), however, seemed not so confident of the synonymization of the short-awned Australian *E. scaber* type with New Zealand *E. multiflorus*, therefore, they still used the name *E. scaber* for the material (Plant

Table 1-4. Circumscription elements of the *Elymus scaber* complex from Australia interpreted by different workers

Taxa	Crane & Carman (1987)	Torabinejad *et al.* (1987)	Stanley & Ross (1989)	Walsh (1994)	Jacobs & Hastings (1993)	Murphy & Jones (1999)
E. rectisetus	intermediate- to long-awned, seed sterile; widespread	small glumes, long-awned, apomictic, low seed set; wide distributed				long-awned, apomictic; widespread
E. scaber. var. *scaber*	smaller glumes, shorter awns; Qld, SW NSW, north-central Vic. and along the coast of E Vic.		awns > 15 mm; throughout the region on sandy soils	awns 15 mm or more; wide distributed including New Zealand	glumes 3-4- nerved, awns 6-15 mm; widespread	short-awned, sexual; widespread from Qld to Vic.
E. scaber var. *plurinervis*	large glumes; SE Qld and NE NSW	large glumes with many nerves, sexual; N NSW and SE Qld.	large glumes with 6-8 nerves; Darling Downs		glumes 6-8- nerved; widespread	large glumes, bifid lemmas and high palea:caryopsis ratio; Qld & NSW
E. multiflorus		short-awned, smaller glumes, 3-5-nerved, sexual; wide distributed from Qld. to Vic. and SA.	awns < 15 mm; throughout the region	awns < 15 mm; coastal sands and alluvial loams in Vic, and Qld, NSW		very short-awned
Others	intermediate to mostly long-awned, highly seed-fertile; in montane forests from ACT to south central Vic.					medium-awned forms

Identification No. D-2888) in their experiment and suggested hybridisation and taxonomic studies between Australian material and New Zealand *E. multiflorus*. Connor (1994) seemed to concur with Torabinejad *et al.* (1987) on the definition of *E. multiflorus* and interpreted the material which Torabinejad *et al.* (1987) used in their experiments as *E. multiflorus*. Interestingly, Stanley and Ross (1989) described both *E. scaber* var. *scaber* and *E. multiflorus* from southeastern Queensland. The former was recorded throughout the region generally on sandy soils, whereas the latter was found throughout the region on black soils. However, the illustrations (Fig. 21 D1-2; see Stanley and Ross, 1989) for *E. scaber* var. *scaber* indicated that it is the short-awned form sympatric with *E. scaber* var. *plurinervis*. No illustrations for *E. multiflorus* were given by the authors, therefore, it is not clear what is *E. multiflorus* sensu Stanley and Ross.

The montane form, described by Crane and Carman (1987) and Murphy and Jones (1999), is another problem. Crane and Carman described it as highly seed-fertile, distributed in montane forests from the Australian Capital Territory to south-central Victoria, and that it corresponded somewhat to montane forms of *E. rectisetus* in New Zealand. Murphy portrayed it as "a continuum of intermediate forms" or "a mass of hybrid and/or aberrant sexual forms". No taxonomic conclusions were put forward by either authors.

Diagnostic characters used by previous classifications

All past classifications have employed numerical characters as the basis for taxonomic circumscription. Lemma awn length longer than 15 mm was used to distinguish *E. scaber* var. *scaber* from *E. multiflorus* (Stanley and Ross, 1989; Simon, 1993; Walsh, 1994). The ratio of lemma length to awn length was used to separate *E. rectisetus* from *E. scaber* var. *scaber* (Murphy and Jones, 1999; the reproductive pattern was also used). The size and number of veins of glumes were used to define *E. scaber* var. *plurinervis*. However, Vickery (1951) noticed that the awn lengths "often vary considerably even on the one specimen". An initial observation by the present author indicated that, owing to extensive morphological overlap, it is difficult to use numerical characters to distinguish taxa within this species complex. Furthermore, the lemma of some *Elymus* species

narrows gradually and becomes canaliculate before its central nerve is clearly defined as an awn (Connor, 1994). Due to the variable nature in which awns arise a degree of ambiguity between treatments would be introduced depending on how awn length has been defined.

1.3. Principles and concepts

The taxonomic problems within the *Elymus scaber* complex have been elaborated in the previous sections. It is obvious that the problems centre on the delimitation of species. If one admits, as Templeton (1989) pointed out, that speciation is a process and is still occurring, and that incomplete speciation will produce "bad species", then one should not be surprised by the condition existing in the taxonomy of *Elymus*, especially in consideration of both apomixis and self-fertilisation being in this group. Moreover, disagreements apparently resulted not only from the diversity of natural individuals but also from different criteria employed by different taxonomists. In order to deal with the taxonomic problems encountered in the present study, it is necessary to have a general view of some basic principles and concepts, and to make decisions on what criteria are to be applied and what techniques for analysis are applicable in this research.

1.3.1. Reproductive patterns

The genus *Elymus* was described as comprising species of small-anthered and self-pollinating individuals (Löve and Connor, 1982; Dewey, 1984; Lu, 1993), and the only report of apomixis in the Triticeae comes from *Elymus scaber* (Hair, 1956). This necessitates a brief discussion about these phenomena, as well as the effect on variability in natural populations in the following paragraphs.

Self-pollination

It is possible that self-fertilisation derived from cross fertilising ancestors (Stebbins, 1957). Not only cleistogamy but also chasmogamy were exhibited in self-pollinating species (Connor, 1979). In the first case, pollination occurs within the same floret of grasses, which should be easy to be examined. In the latter case, however, self-fertilisation may be conducted through the passage of pollen from one floret to another

of the same plant (Connor, 1952; Stebbins, 1957), which could be experimentally demonstrated in the form of seed setting when inflorescences are isolated from one another (Connor, 1957). Self-fertilization was found to be widespread among the grasses and the number of genera is about one hundred (Connor, 1979).

Stebbins (1957) summarised the variation pattern and reproductive behaviour within species of this type. The natural population consists of several morphological types that can be recognised by distinctive morphological characteristics. In addition to these dominant morphological types, there may exist also several other recognisable types that are represented by only one or two individuals. Each successful biotype maintains itself as a constant, genetically homozygous pure line and isolated by self fertilisation from other biotypes. New lines may result from the occasionally accidental crossing between biotypes. When different morphological types belonging to the same or adjacent populations are intercrossed, the F_1 hybrids may be completely fertile or more or less sterile (Stebbins, 1957). The latter example will result in species problem.

Apomixis

Similar to sexual plants, pollination and fertilisation may take place in apomixis, but the male and female sex cells do not unite to form a zygote. Instead, the embryo of the seed develops from an unreduced vegetative cell in the ovule of the female and receives no genetic material from the male and, therefore, the offspring of an apomict are identical to each other and to their parent (Bashaw and Hanna, 1990). There are two classes of apomict: obligate apomicts (no sexual reproduction) and facultative apomicts (with some degree of sexual reproduction). It was noted that within a facultative apomictic species some plants and some strains may be completely sexual whereas others are obligate apomicts; apomixis is known to be heritable; and the environment affects the method of reproduction in these plants (Knox and Heslop-Harrison, 1963; Bashaw and Hanna, 1990). According to Hanna and Bashaw (1987), apomixis can be recognised directly or indirectly by a set of indicators. The roles of apomixis in evolution were generalised by Bashaw and Hanna (1990) as the production of true-breeding hybrids with permanent heterosis, assuring fertility in derivatives of wide crosses that might not otherwise survive,

providing a method for preservation of genotypes over aeons of time, and providing an effective method for polyploid build-up.

Over 300 species from Rosaceae and Poaceae are known apomicts (Hanna and Bashaw, 1987) and 125 species representing most tribes of Poaceae have been reported as apomicts (Bashaw and Hanna, 1990). Most apomictic grasses are polyploid, highly heterozygous, generally of hybrid origin and frequently behave like derivatives of wide crosses (Bashaw and Hanna, 1990). *Elymus rectisetus sensu* Löve et Connor is the only apomict documented in the tribe Triticeae. The transference of apomixis from this species to wheat has been considered economically important and much work has been done worldwide (1.1.5).

The classification of apomicts has long been a problem for taxonomists. As explained by Löve (1960), apomictic populations fulfil the classical requirements of a species by being morphologically distinguishable, having geographical or ecological requirements of their own, and do not regularly hybridise. However, Löve (1960) stated that the lack of hybridisation is not only between populations but also between individuals, so that if the classical requirements of a species is stressed, every individual plant must be regarded as a biological species. In consideration of this, Löve (1960) suggested that the criterion based on hybridisation capabilities within species does not apply to the apomictic groups, and "in some cases even the geographical criterion may break down".

Löve (1960) believed that the variation within a population of obligate apomicts, and also between ecologically distinct populations, probably resulted from cytological mechanisms or some other unknown processes, but mostly from facultative sexuality that allowed occasional hybridisation in the past. He suggested that the variants of apomicts should be recognised as good species if they were not only morphologically and physiologically distinct but also had a characteristic habitat and area. Otherwise they could be treated as subspecies if they were major geographical races.

1.3.2. Species and species concepts

It has been universally recognised that species is a fundamental category of the taxonomic hierarchy, and that populations are the basic unit of the evolutionary process (Jacobs, pers. comm.; Nelson, 1989). Owing to its primary role in biological classification, various papers have been written on this topic and many workers have contributed to the development of the species concepts. Several current species concepts will be briefly discussed below based on the notion that the species have reality, and that species are classes instead of individuals.

Morphological species concept

The most frequently employed concept is the morphological species concept, of which several synonyms have been involved such as phenetic species concept (Ridley, 1993), Linnaean or classical species concept (Burger, 1975), and the typological species concept (Mayr, 1963). In essence, scientists define species by the morphological similarities of the organisms under consideration. It is apparent that if one group of organisms consistently differs from other organisms, it will be defined as a separate species (Ridley, 1993).

One example of the application of this concept comes from the phenetic school. The species level is that at which distinct phenetic clusters can be observed (Sneath, 1976). The phenetic clusters could result from different data, such as cytology, chemistry, and anatomy. Nevertheless, in practice, morphology would be mainly relied upon (Stuessy, 1990). Criticisms of this species concept are acute and centre on its perceived lack of a sound philosophic basis (Ridley, 1993), and that it is highly misleading when dealing with species with polymorphic diversity or two or more species that are morphologically extremely similar (Mayr, 1963).

Nevertheless, many taxonomists have still stressed the importance of recognising species on a morphological basis alone. The reason has probably been that, in practice, morphological characters are usually much easier collected than other characters, that morphologically defined species are more readily recognised, and that even if

intermediates and hybridisation occur, meaningful taxa can still be identified by morphological characters (Burger, 1975).

Evolutionary species concept

Darwin's (1859) *Origin of Species* marked the beginning of the evolutionary species concept which is still in use today with some modifications. A strictly evolutionary criterion for this species concept was presented by Simpson (1951) as "A phyletic lineage (ancestral-descendant sequence of interbreeding populations) evolving independently of others, with its own separate and unitary evolutionary role and tendencies, is a basic unit in evolution". It is apparent that, in this case, a species is defined and maintained by the ancestral-descendant sequence and interbreeding, whereas the asexual and self-fertilising organisms were not taken into account even though it was believed that this theory "may even resolve the theoretical difficulty of defining species in asexually reproducing groups" (Simpson, 1951).

A modified version of evolutionary species concept was provided by Van Valen (1976) as "a lineage (or a closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range". In contrast with that presented by Simpson (1951), this version extends its consideration of species to asexual organisms and "emphasises the fact that a species unit can be held together not only through gene flow but also through developmental, genetic, and ecological constraints" (Templeton, 1989).

Biological species concept

The biological species concept was formulated by Mayr (1963) as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". Reproductive isolation of organisms as a standard for defining species has been clearly stressed here. Isolation mechanisms could occur before mating, after mating but either before zygotic formation or after zygotic formation (Templeton, 1989). As soon as reproductive isolation is confirmed, a species is defined.

This species concept has been applied extensively in taxonomic research on *Elymus* as well as *Triticeae* (see 1.1) — especially, the current taxonomic conclusions of *Elymus* species in New Zealand (1.1.4).

The practical difficulties encountered by the biological species concept have been widely discussed by many authors. For example, difficulties occurred when dealing with species in a multidimensional system, sibling species, and those species in which the acquisition of isolating mechanisms lags far behind morphological divergence (Mayr, 1963). Difficulties also occurred in collecting accurate data of interbreeding within and between populations due to the tremendous work load involved (Stuessy, 1990). The interpretation of the crossing results, as Stuessy further argued, could also be a problem because of known genetic control of chromosomal pairing in some groups and other cytogenetic events. Moreover, the common occurrence of interspecific hybridisation between species of flowering plants caused the biological species concept problems as well (Stuessy, 1990). An extreme objection was raised by Ehrlich (1961) who stressed the difficulties of determining reproductive barriers and suggested that the genetic definition of species, rarely employed in practice, be discarded as an ideal, and relationships at the lower levels of the taxonomic hierarchy should be expressed numerically.

Besides all of these problems, "asexuality poses the biological species concept with the most formidable and most fundamental obstacle" (Mayr, 1963). Asexual organisms, such as apomicts found in the *Elymus scaber* complex, are actually isolated even from one individual to another, therefore, the criterion of interbreeding is considered to be inapplicable to them . In this case, the interbreeding criterion as a standard for defining species of self-fertilising organisms is also pointless because these organisms exist in nature as several morphological types and are isolated by self fertilisation from other biotypes of the same species (Stebbins, 1957).

Another idea that was considered to be closely related to the biological species concept is the genetic species concept (Stuessy, 1990). According to this concept, a species could be defined by measuring the genetic differences among populations or among

groups of populations. The measurement has been based on the data obtained from different molecular analyses. The genetic species concept has gained wide application and rapid development in recent years.

Cohesion species concept

Templeton (1989) claimed that this concept was formed by borrowing parts of other species concepts (e.g. evolutionary and biological species concept) and avoiding their serious defects. He defined the cohesion concept species as "the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms". The intrinsic cohesion mechanisms include all factors that cause speciation. These cohesion mechanisms fall into two categories: genetic exchangeability (the factors act through gene flow) and demographic exchangeability (the factors act through gene drift and natural selection) (Templeton, 1989). In consideration that reproductive continuum exist among organisms, the relative importance of the two categories is different: for asexual organisms only demographic exchangeability acts, whereas for outbreeding organisms the genetic exchangeability is more important. In other words, two cohesion mechanisms can change their effect over the whole reproductive continuum (Templeton, 1989). It seems that the cohesion species concept incorporated most extensive considerations about taxonomic problems, it is presumably more applicable in taxonomic practice.

Cladistic species concept

The cladistic species (or phylogenetic species) concept differentiates itself from all other species concepts with exception of evolutionary species concept in so much as it is "vertical" or, in other words, it takes evolutionary time into account. This is because the cladistic concept recognises species by branch points. That is, if branch points are recognised, the set of organisms in a lineage between two branch points, or between one branch point and an extinction event or a modern population will be defined as cladistic species (Ridley, 1993). The cladistic species are considered to be monophyletic (all individuals within the species are the descendants of a single ancestor), and the recognition of synapomorphies (modified characters shared by a group of descendants) is the strategy of cladistic analysis (Crisp and Weston, 1986).

One of the problems encountered by the cladistic species concept is that it may contradict the current nomenclatural system — a more-than-one-type system may result from the taxa defined by the cladistic concept (Lidén *et al.*, 1997). Lidén *et al.* (1997) further pointed out that even an ancestor could not be recognised from its descendants if the latter did not change from the former after the 'branch point', whereas, they would be treated as two separate species if the cladistic concept was adhered to. All these as stated above will cause confusion and problems with taxonomy in practice.

Other species concepts

Some other types of species concepts were found in the literature with regard to the research of *Elymus* species complex e.g. ecospecies, coenospecies (Connor, 1956; 1962a). Cain (1953) defined coenospecies as two distinct forms that cannot be made to produce fertile hybrids artificially; ecospecies within the same coenospecies as two closely related, but ecologically distinct forms that can produce fertile hybrids occasionally when they meet in nature. However, Cain (1953) equated both coenospecies and ecospecies with the biological species concept.

1.3.3. Infraspecific taxa

When dealing with taxa in which complex patterns of variation occur, taxonomists usually need to circumscribe infraspecific taxa. Up to now, only one infraspecific rank, variety, has been applied to Australian *Elymus* species (*E. scaber* var. *plurinervis*), and all New Zealand taxa are accorded species rank (1.1.4). It is necessary, then, to understand the definitions for these infraspecific taxa and their practical application.

Though five infraspecific ranks are recognised by the ICBN (Greuter *et al.*, 1994), a recent survey conducted by Hamilton and Reichard (1992) indicated that the most commonly used infraspecific taxa include subspecies and variety, and that most authors used geographical, ecological and phylogenetic coherence as the standard to define infraspecific taxa. However, different source of coherences was possibly emphasised by different authors, and many authors regarded these two categories as equivalent (Hamilton and Reichard, 1992).

Stuessy (1990) recommended some criteria for determining infraspecific ranks. Under the criteria, morphological differences among populations within a species are the essential factors needed to be considered. If no morphological differences occur then no formal designations should be provided. Geography is another important factor. Morphological variants that geographically overlap are suggested to be treated as good species, whereas those that are morphologically distinct and geographically allopatric probably represent subspecies.

Stebbins (1957) suggested a possible treatment of self-fertilising plants. Different biotypes of self-fertilising plants that represent extreme morphological and ecological variants occurring sympatrically over wide areas of geographic distribution are justifiably recognised as different subspecies because of a low incidence of crossing, in spite of their potential interfertility. Accordingly, Löve (1960) considered that the major geographical races of apomictic species could be treated as subspecies.

1.3.4. Species concept applied in the present study

The cohesion species concept (Templeton , 1989) is more applicable for taxonomic operation because it incorporates most extensive considerations about taxonomic problems to date. Therefore, the principles of this species concept were adopted for the present study. Accordingly, investigation of morphological variations within the *Elymus scaber* complex were carried out using phenetic analysis (see the following section) in order to display the phenotypic cohesion (Templeton, 1989). Subsequently, the available evidence of ecology, geography, cytology and genetics (cohesion mechanisms; Templeton, 1989) were taken into account to taxonomically interpret the morphological variation patterns revealed.

1.3.5. Classification: definition and techniques

Classification dates back to Theophrastus in the third century B. C. and refers to the practice of ordering organisms into groups based on similarities and/or differences, a meaning retained to the present time. The term has been used in two general senses,

namely, the process of classification and the results of the process (Stuessy, 1990). Both clustering and ordination division are considered the more recent and more formal (to some extent, automated) processes of classification. The roles of classification summarised by Stuessy (1990) include devices for information storage and retrieval, identification services to be performed, reflecting evolutionary relationships in some fashion, and most significantly, devices of summarisation and prediction.

Since Darwin's time, three major approaches i.e. phyletics, phenetics, and cladistics, have been advocated. Each has its own rationale and process. Phyletics was so called because it emphasises primitive vs. derived character states and draws lines of descent between and amongst taxa (Stuessy, 1990). The search for more objectivity and repeatability resulted in the other two approaches. Cladistics can be defined as the concepts and methods for the determination of branching patterns of evolution (Stuessy, 1990). Based on the consideration of morphological species concept, it is the second approach, phenetics, which will be attempted in the present study and explained in detail subsequently.

Phenetics investigates overall similarity of a group of samples that is selected for analysis. This relies on the analysis of numerous, precisely delimited, characters of equal weight (Stuessy, 1990). Being different from the other two approaches, phenetics does not take evolution into account. Five steps are usually involved in the process of phenetic analysis, namely, selection of taxa or individuals (Operational Taxonomic Units, OTUs), selection of characters, description and/or measurement of character states, determining overall similarity (phenetic relationship) between each pair of OTUs and the taxonomic structure by comparison of character states and, finally, ranking of all OTUs into the categories of the taxonomic hierarchy. Some points need to be made on the process of analysis:

1. Theoretically, only individuals should be treated as OTUs but it is not always workable in practice. Instead, attempts should be made to select OTUs adequately reflecting the diversity at a lower level in the hierarchy of the group being treated.

2. Both the appropriate number and the particular set of OTUs are important for accurate representation of homogeneous phenetic clusters. Therefore, one needs to take as many characters as is feasible from as widely as possible over the various parts of the plant, life history stages, tissues, and levels of organisation of the organisms. As for the number of OTUs, it was suggested that at least three individuals being selected for each known homogeneous cluster (usually species) (Sneath, 1976).

3. Effort is needed to establish unit characters (those that cannot be subdivided logically within the particular study group).

4. Minimise the within-OTU variable characters. Determination of phenetic structure follows the calculation of similarity and two basic approaches are involved i.e. clustering and ordination. Both clustering (hierarchical, agglomerative) and ordination will be used in the present study and the algorithms concerned will be detailed in chapter 2.

The contributions of phenetics to taxonomy were summarised by Stuessy (1990) as follows: the stress on having sound philosophical underpinning to what we are doing in classification activities; the stress on characters (stimulating the insights on what is a taxonomic character, what kinds of characters to use, how many, the difference between character and character state, and the importance of examining as many characters as practicable); and the ability to handle large amounts of data especially those encompassing complex patterns of variation. Defects of this approach have also been recognised. For example, subjectivity, though less than phyletics, exists in the analysis process (e.g. selecting samples, selecting and measuring characters and character states, and determining phenetic structure); and unlike the other two approaches, it has not been based on evolutionary thinking (Stuessy, 1990). Nevertheless, phenetics has been applied extensively to solving taxonomic problems particularly at the level of species.

1.4. Aims of this study

In view of the above, it was apparent that confusion occurs in the number and the circumscription of taxa within the *Elymus scaber* complex from Australia. Therefore, it was believed that a detailed analysis of the internal relationships of the species complex from Australia was desirable.

This research was aimed firstly at quantifying the morphological variation within the complex by employing phenetic methods of analysis. Taxonomic problems resulting from the use of some numerical characters, such as lemma awn length, the dimensions of glumes and the number of nerves of glumes, demanded critical reassessment of the circumscription of taxa based on analysis of a large number of samples. Subsequently, more informative characters would be investigated by using SEM and anatomical techniques. Further analysis would be conducted using combined characters (traditional characters and those developed in this study if they were available).

In order to confirm the synonymisation of some taxa shared by Australia and New Zealand (e.g. *E. rectisetus* and *E. multiflorus*), a phenetic analysis carried out on a combined data set including Australian and New Zealand samples was necessary.

Considering that some morphological characters, especially those being used for diagnostic purposes (e.g. lemma awn length in this study) may be modified by environment, molecular analysis (RAPD) would be applied to investigate the genetic distinctness based on the results obtained from phenetic analyses. It was also aimed to confirm the genomic constitution of Australian taxa using genome-specific RAPD markers developed in other labs (Wei and Wang 1995).

In summary it was hoped to answer the following questions:

- Is the variation within the complex continuous or discontinuous?
- How many groups exist in Australia?
- Are any of the groups worthy of formal, taxonomic recognition, and if so, at what level?
- Which Australian groups are synonymised with *E. rectisetus* and *E. multiflorus* from New Zealand?

Chapter 2.

General Methods

This chapter deals only with general methods and especially with computer programs utilised in this study. Other methods specific to a particular analysis will be presented in the appropriate chapters.

2.1. Specimen sampling and preparation

Three hundred and eighty herbarium specimens were obtained from AD, BRI, HO, MEL, SYD and CHR covering the known geographic range of the *Elymus scaber* complex. Herbarium specimens were augmented by new collections made by the author.

The following types were obtained: *Elymus multiflorus* (= *Triticum multiflorum* Hook. f.; lecto: BM), *E. rectisetus* (= *Vulpia rectiseta* Nees in Lehm.; holo: MEL), *Anthosachne australasica* Steud. (syn: K), and a photograph of the type of *E. scaber* var. *scaber* (= *Triticum scabrum* R. Br. based on Labillardière's specimen; holo: FI). The type of *E. scaber* var. *plurinervis* (= *Agropyron scabrum* var. *plurinerve* Vickery; holo: NSW) was not seen in the National Herbarium of New South Wales. However, another specimen from the same locality of the type (Inverell, NSW) was examined (see Chapter 7).

The Australian specimens were grouped into 10 broadly defined morphological forms (named as 'A' to 'J') based on a preliminary examination. Subsequently, 102 Operational Taxonomic Units (OTUs) were sampled to cover the observed range of variation (Appendix I). A sub-sample (30 OTUs, see Appendix I) was selected to carry out further investigation on more informative characters by scanning electron

microscope and leaf blade anatomy. The material preparations especially for SEM and anatomical investigation will be discussed in Chapter 3.

A loan of 109 herbarium specimens obtained from CHR, New Zealand were examined. Subsequently, 24 OTUs (three samples of each species) were sampled (Appendix I). Many specimens were immature and consequently some characters could not be obtained (e.g. those of fruit). Nevertheless, the size of sample fulfilled the requirements for phenetic analysis suggested by Sneath (1976).

Specimen sampling and preparation, DNA isolation, primer selection, optimisation of DNA reaction conditions and agrose gel electrophoresis for molecular analysis will be discussed in Chapter 6.

2.2. Transplantation

Live individuals representing different morphological forms, populations and geographic regions were collected from the field and established in an outdoor garden or in a greenhouse. All field-collected material is supported by voucher specimens lodged at SYD. Seed of some mature individuals was also collected in the field and established as above. This part of the work permitted regular observation of the habit and adaptability of this species complex. Furthermore, it also facilitated the selection of reliable characters, namely, characters not influenced by environmental factors for the purpose of analysis. Fresh material for molecular analysis was collected directly from transplanted individuals or from the seedlings germinated from the seeds.

Individuals from Western Australia and Tasmania did not survive under the conditions available in Sydney and most of them died. The survivors did not flower normally. Otherwise, the new generation growing up from the seeds of Tasmanian collections grew well in the vegetative growing stage and even flowered abundantly. Nevertheless, no mature seeds were obtained. The new generation of Western Australian plants also grew healthily until anthesis, then some (e.g. S95001) produced mature seeds, and others died in the first year (the parent of one such sample, S. Wang 95041, was

observed to be annual). In contrast, transplantations and new plants grown from seed from Queensland flourished under cultivation. They grew well and produced a large number of mature seeds. The short-awned form was especially vigorous. Individuals of this form flowered throughout the year and germinated readily. It was observed that some collections of *Elymus multiflorus* obtained from Broulee Beach, New South Wales were prone to infection by an unidentified rust.

2.3. Computer programs

The computer programs used in the present study were important tools; some of them enabled data coding and manipulation, whereas others were used to conduct similarity or dissimilarity calculations, and some were employed to generate various plots for illustration purposes.

The DELTA system (Dallwitz *et al.*, 1993; Dallwitz *et al.*, 1996) was used to code the data and to deal with data maintenance and format-conversion when natural language descriptions or data formats were required by several other programs.

In order to investigate the presence of 'pattern' in the data, both cluster and ordination analyses were conducted. To detect if the results had been altered markedly when conducting cluster analyses by using re-coded data (which will be discussed later), the clustering algorithms from both PATN (Belbin, 1995) and DELTA were selected.

A number of computer programs have been developed to analyse DNA markers. The RAPDistance Programs (Armstrong *et al.*, 1994) were used to analyse DNA fragment data generated by RAPD assay in this research. A brief introduction to RAPDistance Programs will be presented in Chapter 6.

A wide variety of options is available in these computer programs, such as the measure of association, and different algorithms of clustering and ordination. These will be presented under the relevant program systems as follows.

2.3.1. DELTA system

DELTA is a flexible system for gathering, organising and storing taxonomic data and maximising its use (Van den Borre, 1994). Its manifold functions facilitated this research in many aspects which are revealed through the following related sections.

2.3.1.1. CONFOR

CONFOR is a program for translating DELTA-format data into various formats, including natural language. The execution of the program is controlled by the contents of the directives, and by the order in which they appear (Dallwitz *et al.* 1993). Therefore, in order to run CONFOR a directive file and some basic DELTA-format files are necessary. The examples of directive files are CHECK, TODIS, TOKEY and TONAT, which contain directives cause the data to be checked, translated into DIST or KEY format, or into natural languages, respectively. SPECS, CHARS, and ITEMS are three essential DELTA-format files which will be discussed in detail later on.

When entering the original data at the first stage of this study, CONFOR CHECK was run regularly to detect and correct any possible mistakes. A cluster analysis can only be carried out based on the similarity or dissimilarity calculations through the program DIST. Before implementing the distance measurements in DIST, CONFOR TODIS was carried out to transform DELTA-format data into the format required.

2.3.1.2. DELFOR

This program has been designed for reformatting DELTA data and directives. Extensive additions or alterations were often carried out to the data when preparing basic files. In this case, running DELFOR (followed by a directive file) will keep SPECS, CHARS, and ITEMS matched with each other. For example, when the character list in the file 'Chars' was reordered, the operation of DELFOR REORDER simultaneously changed SPECS, ITEMS and other directive files to make them all consistent with the new order.

2.3.1.3. DIST

Running the program DIST can generate a distance matrix. Distances are calculated using a modified version of Gower's (1971) similarity coefficient (Dallwitz, *et al.* 1993).

Different types of characters coded in the DELTA format were treated in different ways. For unordered multistate characters, a directive, MATCH OVERLAP specifies the contribution of a character to the distance between two OTUs (items). That is to say that the contribution is 0 if any values of the character are shared by the two OTUs. Therefore, if the directive MATCH OVERLAP is not used, given P_{isk} is the probability of OTU i having state s of character k, P_{jsk} is the probability of OTU j having state s of character k, and n is the number of states of character k, the contribution D_{ijk} of character k to the distance between OTUs i and j is

$$D_{ijk} = 0.5 \left(| P_{i1k} - P_{j1k} | \ldots + | P_{isk} - P_{jsk} | \ldots + | P_{ink} - P_{jnk} | \right)$$

If the number of characters is m, then

$$D_{ij} = \sum_{k=1}^{m} D_{ijk}$$

If the character has more than one value in an OTU, the probability is divided equally between the values present. Whereas the calculation of the distance is different if MATCH OVERLAP is used, then D_{ijk} is 0 if i and j have any state values in common, and 1 otherwise.

For ordered multistate and numeric characters, the contribution D_{ijk} becomes

$$D_{ijk} = | X_{ik} - X_{jk} | \,/\, R_k$$

where X_{ik} is calculated for OTU *i* and character *k* during the translation from DELTA format, and R_k is range of the X_{ik} for all included OTUs *i*. And the total distance between OTU *i* and OTU *j* is

$$D_{ij} = \sum_{k=1}^{m} D_{ijk}$$

Unknown and inapplicable characters make no contribution to the total distance.

2.3.1.4. ISS-flexible clustering method

ISS or SS (Increment in Sum of Squares; Burr, 1970) is an agglomerative, hierarchic clustering strategy. The formula (Burr, 1970) is provided below:

$$D_{(i,j)k} = [(n_i + n_k)\, D_{ik} + (n_j + n_k)\, D_{jk} - n_k D_{ij}] \,/\, (n_i + n_j + n_k)$$

where $D_{(i,j)k}$ stands for the distance between the previously formed cluster (including clusters or only OTUs *i* and *j*) and a new cluster or only an OTU *k*; n represents the size or the number of clusters or only OTUs .

A small modification (Dallwitz, in Van den Borre and Watson, 1994) was made to the formula as follows:

$$D_{(i,j)k} = [(n_i + \sigma\, n_k)\, D_{ik} + (n_j + \sigma\, n_k)\, D_{jk} - \sigma\, n_k D_{ij}] \,/\, (n_i + n_j + \sigma n_k)$$

There were few differences between these two formulae except the intensity parameter σ which made the strategy more flexible. By varying the σ value, some groups can change position in a way that would not be expected and thus give more information about their stability or lack of it in the dendrogram. UPGMA and ISS are the extreme cases of the formula: when $\sigma = 0$, the formula is the equivalent of UPGMA (Sneath and Sokal, 1973; Belbin, 1995); when $\sigma = 1$, the formula is the equivalent of ISS (Burr, 1970). In this research, $\sigma = 0$, 0.25, 0.50, and 1.0 were used because the comparison of their influence on the analyses would be conducted, and the UPGMA result was needed

to compare with the result obtained from analysing re-coded data in the PATN system. When $\sigma = 0.25$, the results produced were taxonomically meaningful, and the values 0.5 and 1.0 could also generate dendrograms comparable with those when $\sigma = 0.25$. However, the results from strict UPGMA were somewhat different from other strategies (see Chapter 4). This indicates that the intensity parameter σ does not obviously influence the structure of dendrogram within some range of values (e.g. > o.25), but may result in different pattern at extreme value (e.g. $\sigma = 0$).

2.3.1.5. Data files

All applications of the DELTA system are based on information contained in three files: the character list (CHARS), the coded taxon list (ITEMS) and the specifications (SPECS). The last file contains directives that give CONFOR information about the characters and items such as the number and types of characters, the number of states of the characters, the dependency of the characters and implicit values.

All three basic data files, together with a supplementary file CNOTES, which contains additional notes about the character list, were developed in this study. The file CHARS encompasses 251 characters including essential information of the taxa (e.g. nomenclature), habit, vegetative morphology, reproductive morphology, anatomy, distribution and additional information (flowering period, citation of specimens and so on). The majority of these characters were based on original observations during this study, only a few, such as the genomic information, were cited from the literature. The character list was constructed for general use including the identification and description of the taxa. The selection of characters useful for cluster and ordination analysis was conducted in another related program INTKEY (2.3.1.6). The details and interpretation of these characters will be given in Chapter 3. Another important data file, ITEMS, contains all information obtained about the 126 specimens or OTUs. The final analysis resulted in the recognition of five taxa. Information about the five taxa was obtained through the 'summarise' routine in the program INTKEY. Subsequently, the five taxa were added as new items into the file ITEMS.

2.3.1.6. INTKEY

Dallwitz *et al.* (1996) stated that INTKEY is an interactive program for identifying a specimen by comparing it with stored descriptions. Furthermore, the program can be used to interrogate the stored data by carrying out operations specified by relevant INTKEY commands. For example, a request for the summary or description on a set of characters of a set of taxa or OTUs can be readily specified by the selection of 'summarise' or 'describe' commands from the 'Descriptions' menu. The commands in the 'File' menu (e.g. 'output' and 'summary') can be used to withdraw data coded in DELTA system, which could be useful for other computer programs (e.g. PATN). The program INTKEY, indeed, facilitated this study in many ways as stated above. An Interactive Identification Key to Australian *Elymus* species, generated from the database, is appended to this thesis on a CD-ROM (see Appendix III for installation procedure).

2.3.2. PATN system

In recent years, the program PATN has been one of the most popular computer packages used to analyse different types of biological data from different disciplines. PATN procedures are objective in the sense of bringing no memory to bear on the problem and being confined to the boundaries of the set of data under examination. PATN often highlights new features or relationships that were not seen by the original investigator (Belbin, 1995). Over 50 separate programs covering a wide range of multivariate data analysis are collected in PATN. According to their function they can be grouped into three types, those for data preparation, for data analysis including cluster and ordination analysis and for post-processing. Some of these programs considered important and used in this research are briefly explained below.

2.3.2.1 Data preparation

DATN

DATN is used for data input and output. Data were prepared as rectangular forms (OTUs by characters) in FORTRAN free format (values separated by commas) or FORTRAN floating point (F) format which were accepted by DATN.

In my data file, most multistate and binary characters coded in the DELTA system could be classified as a nominal scale. In this case, the value assigned to each state is artificially consistent. For example, plant colour is described as:

#9. <plant colour>/
1. glaucous/
2. not glaucous <implicit>/

It is important that 'not glaucous' (scored as '2') is not bigger than 'glaucous' (scored as '1'). However, PATN will treat them as if they were ratio scaled ('2' > '1') rather than accept them as nominal scale characters as they stand (Belbin, 1995). This makes it necessary to re-code all nominal characters as ratio characters in DATN. The re-coded character of a 'glaucous' sample will be presented as '10' and a 'not glaucous' sample will be '01'. In order to avoid confusion, the re-coded characters will be referred to as 'binary expression of multistate and binary characters'.

All characters coded as "unknown" and "inapplicable" in the DELTA system were treated as a missing data value (-99.00 in this study) which was inserted in every relevant position in the preparation of the input files. However, all states of "inapplicable" characters were given value "0" for binary expression of multistate and binary characters to differentiate "inapplicable" from "unknown" characters in the final analysis (4.2.1). The results were not influenced dramatically by the different modes of scoring.

2.3.2.2. Association measures

The measure of association is defined by using the ASO routine in PATN. ASO is one of the most important modules in PATN, being the main way of generating association measures between all pairs of rows in the datafile (Belbin, 1995). To avoid apparent impact on overall association, attention was paid to the number of missing values contained in rows or OTUs, because ASO will skip a comparison of two rows on a single attribute if either has a missing value. Of nine algorithms, Bray and Curtis and Gower Metric were used in the generation of association measures. The latter, Gower Metric, has consistently performed well in this research - it sorted OTUs into several groups which were considered to be taxonomically meaningful. This algorithm, therefore, was selected to conduct most analyses.

Belbin (1995) recommends Bray and Curtis for data that requires some type of ratio responses and as such, is the default measure. By ratio responses, it means that the values of association are affected by comparisons at different points in the character range. For presence/absence data, if

		Row_j	
		1	0
Row_i	1	*A*	*B*
	0	*C*	*D*

then the formula is

$$D_{ij} = 1 - 2A \ (2A + B + C)$$

where A, B, C, and D represent the ratio of 1-1, 1-0, 0-1, and 0-0 matches between *i* and *j* respectively, of which 0-0 matches are excluded which means that they will not be considered as adding to the overall similarity between the two OTUs. While for continuous data, the measure of Bray and Curtis is formulated as

$$D_{ij} = \sum |D_{ik} - D_{jk}| \ / \ \sum (D_{ik} + D_{jk})$$

where D_{ik} is the data value of the *i*th OTU row and *k*th character.

As suggested by Belbin (1995), Gower Metric is probably one of the better algorithms to use when dealing with continuous data (which is the case in this study). For numeric characters, the formulation is:

$$D_{ij} = (1/M) * \sum | D_{ik} - D_{jk} | / R_k$$

while for presence/absence data, it is:

$$D_{ij} = 1 - (A+D) / (A+B+C+D)$$

where 0-0 matches are included and will be considered as adding to the overall similarity between the two OTUs.

Both algorithms generate a measure of dissimilarity in which smaller values indicate an increased similarity.

2.3.2.3. Clustering methods

Clustering aims to connect OTUs to each other by using algorithms based on the association measure obtained. In this way, the variation pattern present in the OTUs will be graphically displayed as dendrograms, so that taxonomic conclusions can be attained. OTUs which are sorted into one group by clustering algorithms may be considered to be more similar to each other than to those OTUs from other groups. However, a taxonomic conclusion should be reached with care because these clusters are not necessary representative of true or natural taxa (Belbin, 1995). Anyhow, clustering is one of the most common approaches at the present time and is employed widely for different subjects.

FUSE

Given the size of the dataset (102 OTUs), FUSE (Agglomerative Hierarchical Fusion) is an appropriate choice. Unweighted pair-group method using arithmetic averages (UPGMA) is the sub-choice for, according to Belbin (1995), it has been "well trialed over many years on a wide variety of data".

UPGMA algorithm calculates dissimilarities between two OTUs, or an OTU and a cluster, or two clusters by the following formula

$$D_{(i,j)k} = (n_i D_{ik} + n_j D_{jk}) \; / \; (n_i + n_j)$$

where $D_{(i,j)k}$ is the dissimilarity coefficient between the candidate OTU or cluster k and the cluster comprising OTUs (or clusters) i and j, D_{ik} is the distance between i and k, D_{jk} is the distance between j and k, and n_i and n_j represent the size of clusters i and j separately. Following each calculation, the smallest term of D will be located and two clusters (or OTUs, or an OTU and a cluster) with the smallest D will be fused. New D will be computed and new fusion will be conducted following steps above. The cycles of fusion will be repeated until all OTUs are connected to the dendrogram.

2.3.2.4. Ordination methods

As an alternative to clustering methods, ordination is the preferred method of analysis by most taxonomists. Sneath and Sokal (1973) suggested that far better representations of the data can be obtained by ordination rather than by the hierarchic classifications, because the latter often perform poorly when presenting the actual phenetic relationships. However, ordination also has its disadvantages. As Sneath and Sokal (1973) pointed out, in the case of many OTUs and clusters, the ordination may give no simple result in low-dimensions (e.g. 2- or 3-dimensional space) though the result is quite good in hyperspace. In addition, if large numbers of characters and OTUs are dealt with, the ordination is possibly impracticable (Sneath and Sokal, 1973).

The basic strategy of any ordination method is to produce a summary of significant information contained in all OTUs by condensing them into two or three new OTUs with minimal information loss. Following this, the distance or similarity of OTUs can be graphically displayed. Unlike cluster analysis, no classification of OTUs is performed here. The method will provide an indication of whether true or 'natural' clusters exist.

SSH

Semi-Strong-Hybrid multidimensional scaling (SSH) is considered to be superior to other ordination methods such as principal components analysis (PCA), principal coordinates analysis (PCoA), correspondence analysis (CA) or reciprocal averaging (RA). It permits combinations of ordinal (monotonic), interval or ratio scaling.

SSH asks users to select the number of dimensions and assumes an initial configuration of OTUs in those dimensions. The inter-object association matrix is then produced by measuring from this initial configuration between all pairs of OTUs. Furthermore, regression is used to try and predict the input association measures from the configuration (output) associations. To estimate the degree of match between input and output associations, stress is used as the indicator which ranges from 0 to 1. The larger the disparity, the larger the stress. Some calculating strategies are used to find a minimum value of stress. The number of dimensions selected by researchers is yet another factor to influence the value of stress. Generally speaking, the more dimensions a user selects, the easier it will be for SSH to position the OTUs and the lower the stress (Belbin 1995). However, too many dimensions will result in too many degrees of freedom on the OTUs, which will add superfluous noise to the analysis. Two and three dimensions were selected in this research, and both produced interpretable results with low stress values.

PCC

PCC (Principal axis correlation) is a program designed to see how well a set of characters can be fitted to an ordination space. It takes each attribute and finds the location of the best fitted vector in the ordination space. By running the program, a

table containing the original ordination followed by OTUs fitted with PCC will be produced. These fitted OTUs represent the co-ordinates of the head of the vector in the ordination space. The correlation between OTUs and the fitted attribute in this direction is presented as an additional column in the table.

2.3.3. Computer programs for miscellaneous illustration purposes

Several computer programs were employed as tools to generate miscellaneous plots or maps which helped to graphically reveal the results. Some examples are: SYSTAT (Wilkinson, 1990) - producing graphical representations of all ordination analysis results; MapInfo Professional (1992-1995 MapInfo Corporation) - mapping the geographical distribution of the taxa; PowerPoint 5.0 and Pagemaker 4.0 - facilitating the refinement of pictures and graphs. Many manuals and user's guides of these programs are available for instructions, therefore, no details will be discussed here.

Chapter 3.

Characters of the *Elymus scaber* complex

3.1. Introduction

The variation in the *Elymus scaber* complex can be observed over a wide range of characteristics including the habit, vegetative morphology, reproductive structures, habitat and geographic distribution, and leaf anatomy. The recognisable and definable variation amongst the individuals are termed 'characters' which are represented by numeric values or ranges, or mutually exclusive states (Watson and Dallwitz, 1992). Therefore, these observed features within *Elymus* are translated into recorded characters and states (Appendix II) for the purposes of phenetic analysis and general descriptions. This chapter summarises the important variation within the species complex based on the examination of a comprehensive set of samples. In order to facilitate the interpretation of these characters, some photographs are included at the end of this chapter. The taxon names used in illustrations follow the taxonomic treatment by other authors (see Chapter 1) except that *E. rectisetus* is used to represent one group of Australian samples defined in this study rather than the New Zealand *E. rectisetus sensu* Löve et Connor (see Chapter 4 and 7). For the final treatment of these taxa, refer to Chapter 7.

3.2. Material and methods

Morphological characters, except those of SEM, were obtained from 126 OTUs selected from Australian and New Zealand specimens (Chapter 2). Further investigation using SEM and anatomical techniques was conducted on 54 OTUs (including Australian and New Zealand material; see Appendix I). Material preparation and observation methods in the latter case are presented as follows.

3.2.1. Scanning electron microscopy

At least two florets together with rachillas were obtained from the mid-segments of the spikelet at the lower half of the inflorescence of each sample. The florets were mounted on aluminium stubs using double sided tape and coated with gold to a thickness of 20 nm. The material was viewed and photographed using a Philip 505 SEM , using secondary electron image detection. The operating voltage was 10 kV.

3.2.2. Anatomical preparation

Abaxial leaf epidermis scrapes and hand cut transverse sections were made from the mid-point of leaf blades (preferably from the second leaf down from the inflorescence). The technique followed Van den Borre (1994) with some modifications. The leaf blades were boiled in 2% NaOH until the tissue softened. Abaxial leaf blade epidermal preparations were obtained by scraping flattened blade mid-zones with a scalpel, thus removing adaxial epidermis and mesophyll. For transverse sections, the leaf blades were boiled in water with a few drops of Teepol (detergent) for about 10 to 30 minutes, to soften the tissues properly. Transverse sections were handcut from the blade midzones. For precision, both these procedures were executed under a stereo microscope. Both transverse sections and abaxial epidermal preparations were stained in Phenolic Bismarck Brown solution (Bismarck Brown 1g + phenol crystals 1g + distilled water 100 ml) for 10-20 minutes, rinsed in distilled water for 5 minutes, transferred to 50% glycerol/distilled water for 5 minutes. Permanent slides were made by sealing cover slips with nail varnish.

The slides of leaf epidermises and transverse sections were observed under a compound light microscope.

3.3. Characters of the *Elymus scaber* complex

3.3.1. Habit

Elymus species were described as perennial in the literature. Only two specimens from arid areas of Western Australia (e.g. near Perenjori, 29°26'S, 116°17'E; NW of Bungalbin Hill, 30°20'S, 119°31'E) were observed to be annual (without remains of old sheaths or culms, and no underground part either).

Differences in growth habit (erect, geniculate, ascending, drooping and decumbent) were observed within *Elymus*. The short-awned forms and *E. scaber* var. *plurinervis* from Queensland were more often erect, whereas a combination of different habits were observed in other taxa. Reliable information about plant habit should be obtained from living material. Moreover, it was observed that an individual's habit could change according to habitat. For example, some specimens of *Elymus multiflorus* (S. Wang 95163 and S. Wang 95164) appeared to be erect in the original habitat (sandy soil near the beach), whereas the new shoots of the transplants in cultivation acquired an ascending habit. Connor (1994) used this character: whether culms prostrate to ascending (e.g. *E. falcis*) or erect (e.g. *E. sacandros*), for diagnostic purposes. However, the character is possibly not taxonomically valuable for Australian taxa based on the above observations.

Only five OTUs (all from *E. scaber* var. *scaber*) of Australian individuals and one New Zealand taxon (*E. apricus*; Connor, 1994) were shortly rhizomatous. Five New Zealand taxa were described as stoloniferous (Connor, 1994) but this character seemed not to be present in Australian individuals. Most specimens were caespitose.

Plant glaucescence was often described for New Zealand material and was even used, together with other characters, for diagnostic purposes (e.g. *E. solandri* is glaucous whereas *E. rectisetus sensu* Löve et Connor is not; Connor 1994). This character, however, was shown to vary within Australian taxa. Both glaucous and non glaucous individuals were growing together at some localities (e.g. OTUs 86 and 87 from Gara

River, NSW). Nevertheless, most individuals from the open areas of Western Australia (e.g. Kalgoorlie, Stirling Range, Ravensthorpe, and Bungalbin Hill) demonstrated this feature. Some *Elymus* plants under cultivated conditions appeared green when young and became glaucous when they began to flower.

3.3.2. Vegetative morphology

Most Australian individuals have long and clasping or minute auricles, a character useful for distinguishing *Elymus* species in the field. The auricles of two species from New Zealand (*E. falcis* and *E. sacandros*) were described as minute or absent and, therefore, distinguishable from other species (Connor, 1994). However, minute or no auricles were observed to be common in most New Zealand specimens sampled (e.g. 10 out of 15 specimens have minute auricles, and 14 out of 15 specimens were coded as non auriculate). For the variation of auricles see Plate 3.1.

Observed variation of leaf blades included whether the leaf blades were flat or not, their dimensions and their indumentum. Leaf blade shape in transverse section indicates its shape in nature. The leaf blades of some specimens looked involute, however, this probably resulted from improper treatment when collecting in the field (e.g. having not been pressed promptly as soon as they were collected). Such leaf blades reshaped after boiling in Teepol solution. All except five individuals possessed flat leaf blades. Some taxa from New Zealand (*E. falcis* and *E. sacandros*) have folded or involute leaf blades which are falcate or strict in outline (Connor, 1994). The anatomy of folded or involute leaf blades was distinct from the flat ones (3.3.11). Accordingly, these characters (#32 and #33, Appendix II) were selected for phenetic analysis.

A wide range of dimensional variation of leaf blades was observed within *Elymus*. The length of the leaf blade ranged from 28 to 400 mm and the width from 1.3 to 6.9 mm. These characters apparently were influenced by habitat and possibly also by geographic location. For example, some individuals from sandy soil of the coastal areas had linear-lanceolate leaf blades whereas the leaf blades from Tasmanian individuals under cultivated conditions were linear. Moreover, the width of the leaf blades was not a

stable character — it changed markedly in cultivation (Table 4-1). Therefore, these characters are possibly not reliable for phenetic analysis. For indumentum of leaf blades, *E. multiflorus*, *E. scaber* var. *plurinervis* and the short-awned samples from Queensland were mostly coded as glabrous and/or scabrous, whereas, the remainder were mostly coded as hairy. Connor (1994) used indumentum of leaf blades to distinguish some New Zealand species e.g. *E. solandri* has abaxially glabrous leaf blades, whereas *E. rectisetus* has coarsely hirsute leaves. Murphy (pers. comm.) insisted that leaf indumentum was useful to distinguish the short-awned individuals (glabrous) in Queensland from *E. scaber* var. *scaber* (scabrous or coarsely hirsute) if they are sympatric. It is considered by the current author, however, that the character may, in some cases, be helpful in some populations but it may not be reliable for phenetic analysis of a comprehensive geographic sample. This was confirmed by an analysis using only vegetative characters, such as the dimensions and indumentum of leaf blades. All groups completely mixed up and formed several taxonomically meaningless groups compared with the results from all other data sets (result not shown).

3.3.3. Inflorescence and spikelets

Some inflorescence and spikelet characters were shown to be taxonomically valuable for the *Elymus scaber* complex.

According to Connor (1994), two species from New Zealand were defined mainly by the position of the inflorescence and the position of spikelets on the rachis. *Elymus tenuis* is characterised by a long trailing inflorescence due to the obvious elongation of the uppermost internode of the culms after anthesis. Another species, *E. apricus*, was separated from others by its erect and stiff inflorescences, as well as by its widely divergent spikelets. The position of spikelets on the rachis, however, can only be scored from living material in the field or from cultivated material. Similarly, the character, whether the upmost internode elongated or not after anthesis, can be only obtained from living material. This obviously caused some problems when these characters were coded, and limited their use. Some Tasmanian individuals (e.g. OTU 78) were characterised by the inflorescence trailing to the ground when cultivated.

Nevertheless, this possibly did not result from the elongation of the uppermost internodes of the culms after anthesis. Australian samples were not seen to have erect and stiff inflorescences with spikelets widely divergent from the rachis.

Remarkable differentiation of the peduncle length between individuals was observed: some were very short even at the end of anthesis (e. g. some individuals of Australian *E. rectisetus* and *E. scaber* var. *scaber*) and some quickly extended at the beginning of anthesis (e. g. the short-awned individuals from southeastern Queensland). It is presumably useful for taxonomic purposes, although its application is limited because the data obtained from herbarium specimens were not reliable due to these individuals being collected at different developmental stages in the field.

The length of inflorescence internodes was apparently different within *Elymus* species, therefore, a character (#53) was developed to measure the average length of the lowest 2–3 internodes of the rachis. Another character (#62) was used to record the ratio of spikelet length to the average length of the lowest 2–3 internodes of the rachis. The lowest value of character 53 occurred in *E. rectisetus* (average = 17 mm), whereas the highest value was measured from the short-awned individuals in Queensland (average = 32 mm). For character 62, the contrary results were revealed: *E. rectisetus* has the highest value (average = 4.0), whereas the short-awned individuals has the lowest one (average = 1.0). Both characters were included in the phenetic analysis and exhibited high correlation coefficients (Chapter 4 and 5) which indicated their taxonomic value.

Australian *Elymus* species were informally separated as long-, intermediate-, and short-awned forms by some authors (see Chapter 1), therefore spikelet length was measured in this study. The results indicated that spikelet length forms a cline from *E. rectisetus* and *E. scaber* var. *scaber* to *E. multiflorus*, and the short-awned individuals from Queensland and New South Wales fall in between. The average values of spikelet lengths were used for phenetic analysis and gained high correlation coefficients (see Chapter 4 and 5). However, the range of spikelet length obtained from different groups extensively overlapped one another (Figure 4-8), therefore, the utility of this character is limited in practice.

Elymus species in the section *Anthosachne sensu* Löve were described to have a solitary spikelet at each node (Löve, 1984). This was observed in most individuals sampled in this study with the exception that paired spikelets occasionally occurred at the base of inflorescence of *E. multiflorus* (Connor, 1994; also Australian specimens).

3.3.4. Glumes

A set of characters was developed to investigate the polymorphism of glumes within the *Elymus scaber* complex. These included the dimensions, the number of nerves, the apex structure (awned or not awned), and features of margins. Several of these characters were informative for taxonomic purposes.

When Vickery (1951) established *E. scaber* var. *plurinervis*, she used the dimensions and the number of nerves of the glumes to distinguish this variety from others. Traditionally, this taxon is characterised by glumes 10–15 mm long with 6–8 veins. In this study, it is confirmed that *E. scaber* var. *plurinervis* is distinguishable by a set of glume characters including the longer and wider (*in situ*) glumes (Figure 4-9. a-d), and apparently wider membranous margins of glumes than other taxa (Plate 3.2. e). In addition, the distinction of glumes (e.g. the size and number of nerves) was also observed between the short-awned forms (including *E. multiflorus* and Queensland individuals) and the long-awned forms (including *E. rectisetus* and *E. scaber* var. *scaber*). The former has narrowly ovate and 5–6 veined glumes (Plate 3.2. d), whereas the latter has subulate and 3–5 veined glumes (Plate 3.2. b and c). Furthermore, the New Zealand taxon, *E. tenuis*, is characterised by long-awned glumes (Connor, 1994; Plate 3.2. a).

The number of nerves of glumes was observed to be less valuable for diagnostic purposes than it was traditionally thought. The number of nerves of lower glumes coded for *E. scaber* var. *plurinervis* (5-6) overlapped with that of *E. multiflorus* (4-6). Nevertheless, the number of nerves of upper glumes is still informative although overlap also occurred between *E. scaber* var. *plurinervis* and a few individuals of other taxa (Figure 4-9. e). In any case, it is important to examine a set of characters (e.g.

characters of glumes, lemmas and the ratio of fruit to palea length for *E. scaber* var. *plurinervis*) rather than to examine only one character in taxonomic practice.

For *Elymus* species, the glume apices were characterised by tapering into longer or shorter points or awns. Therefore, it was difficult to determine whether they were awned or not awned, and, if so, how long the awn was. In order to standardise the measurement, the 'awn' was measured from the point at which glume apex was about 0.5 mm wide.

3.3.5. Rachilla and callus

Twenty characters were developed to describe rachillas and calluses of the *Elymus scaber* complex. Fourteen of these characters were formulated based on observation by scanning electron microscope. It was demonstrated that these characters are informative and can be used for taxonomic purposes within this species complex. Moreover, *E. enysii* was observed to be distinct from all other *Elymus* taxa from Australia and New Zealand by characters of rachilla and callus. This raised a possibility that *E. enysii* does not belong to *Elymus*, which is supported by molecular evidence (see Chapter 1).

The variation of rachillas within the *Elymus scaber* complex was expressed in the indumentum and the shape of the top. Variation in calluses (abaxial and adaxial) include the shape and the indumentum.

Hairy rachillas were observed in all individuals. However, there was differentiation of the length and density of hairs between taxa. *Elymus multiflorus, E. enysii, E. tenuis, E. falcis* and the individuals from Queensland (e.g. the short-awned forms and *E. scaber* var. *plurinervis*) were characterised by short-hairy rachillas (Plate 3.3. c, d and e), whereas other species or forms e.g. *E. scaber* var. *scaber, E. solandri, E. apricus, E. sacandros* (except one sample) and New Zealand *E. rectisetus sensu* Löve and Connor, usually had long-hairy rachillas (Plate 3.3. b). Australian *E. rectisetus* was distinguishable from all other species or forms by hairs densely distributed on the upper and central portion of the rachilla, and, therefore, covering the base of callus (Plate 3.3.

a; callus was removed), rather than hairs more or less evenly distributed on the whole surface of the rachilla and not covering the base of the callus (Plate 3.3. b). The rachillas of one sample of *E. sacandros* were densely and very long-hairy (Plate 3.3. f), thereby differing from all other samples.

Rachilla apex, the point at which the rachilla is connected to the callus of the upper floret, was shown to be facing inwards in most species (Plate 3.3. a, b, c, d and f) with the exception that the rachilla apex of *E. enysii* was nearly horizontal and sharply expanded (Plate 3.3. e). This character obviously distinguishes *E. enysii* from all other *Elymus* species from Australia and New Zealand. The shapes of rachilla apices of other species were also observed to be informative: Australian *E. rectisetus* was distinct by an oblong or elliptical rachilla apex (Plate 3.3. a), *E. scaber* var. *plurinervis* was distinguishable by semi-circular to transverse-circular rachilla apex (Plate 3.3. c), and the remainder were shown to be more various having ovate, obovate to circular rachilla apices.

Some taxonomically useful characters were developed from the callus. The variation of the abaxial view of calluses involve the shape, the indumentum and the tip structure (thickened or not thickened). The calluses of *Elymus enysii* were very short and glabrescent (Plate 3.4. e), and distinct from all other species. Australian *E. rectisetus* was characterised by the narrowly triangular and dorsally raised calluses with hairs restricted to the margins (Plate 3.4. a), whereas *E. multiflorus*, *E. scaber* var. *plurinervis* and the short-awned forms from Queensland differed from others by their widely triangular and glabrous or glabrescent calluses (Plate 3.4. c and d). However, these characters were more variable in the remainder of *Elymus* species. Typical *E. scaber* var. *scaber* was distinguishable by triangular and hairy callus with hairs distributed on the surface and the margins, and with thickened tips (Plate 3.4. b). *Elymus apricus*, *E. sacandros*, *E. falcis*, and New Zealand *E. rectisetus sensu* Löve et Connor were observed to be similar to *E. scaber* var. *scaber* by those characters of callus discussed above, with the exception that the callus tips of the former three species were not thickened. One sample of *E. sacandros* was revealed to have very dense and long hairs on its callus surface (Plate 3.4. f). *Elymus solandri* had somewhat intermediate

characters of callus — the dorsally raised surface and marginally distributed hairs were similar to Australian *E. rectisetus*, whereas the hairy areas were wider than those of *E. rectisetus*, and the shape of callus was triangular to widely triangular which was similar to *E. scaber* var. *scaber* (Plate 3.4. g).

The adaxial view was not so informative as the abaxial view of the callus. However, some variation was also observed. Again, *E. enysii* was separable from all other species by its distinctly raised callus (Plate 3.5. d). The marginal thickening of the callus of other species was revealed to be either restricted to below the mid-point (Plate 3.5. a, e and f) or extended approximately to the mid-point of the callus (Plate 3.5. b and c). The former character seemed to be related to the narrowly triangular to triangular and hairy calluses, whereas the latter related to the widely triangular and glabrous or glabrescent calluses.

The traditionally-defined *Elymus* species from Australia and New Zealand can be grouped into three categories based on their rachilla and callus characters. The first category contains only *E. enysii* which is characterised by sharply expanded rachilla apices, very short (abaxial) and distinctly raised (adaxial) calluses. These characters indicate that *E. enysii* should not be included in *Elymus*. The second category consists of *E. scaber* var. *plurinervis*, whose semi-circular to transverse-circular rachilla apex and widely triangular and glabrous calluses indicate its distant relationship to other species. The remainder form the third category whose members are closer to one another than to the first two categories. Although Australian *E. rectisetus*, *E. multiflorus* and the short-awned form from Queensland are distinct from one another and from other species in the third category (see discussion above), some individuals with intermediate characters blur the distinctions between these species based on characters of rachilla and callus. For example, some individuals of *E. scaber* var. *scaber* were characterised by widely triangular and glabrous calluses, similar to *E. multiflorus*, and a few specimens were found to have flat to sunken calluses but with hairs mostly restricted to the margins showing their intermediate positions between *E. scaber* var. *scaber* and *E. rectisetus*. Moreover, the thickened tip of the callus (abaxial) is considered to be valuable for distinguishing *E. scaber* var. *scaber*, however, it is not

so typical in some individuals especially in those with widely triangular and glabrous calluses.

3.3.6. Lemmas

Thirty-three characters were developed to describe the variation of lemmas (Appendix II). These include the texture, shape and dimensions, indumentum, margins, apical features and awns. The analysis indicates that some lemma characters are useful for taxonomic purposes.

The length of lemmas was obtained from the proximal lemma of median spikelets (to avoid the proximal or apical spikelets within an inflorescence because their dimensions were usually markedly different from the others). The lemma apices of the *E. scaber* complex were mostly characterised by tapering into awns (Plate 3.6), and, especially in *E. rectisetus*, lemma apices were much wider than in other species (Plate 3.6. 1 and 2). In order to measure the length of either lemmas or awns, the same standard as that for measuring the length of glume awns was used (3.3.4). The exceptions were the samples of *E. scaber* var. *plurinervis* whose awns were considered to originate from the point where two lateral veins of lemmas end (Plate 3.7. h). The lemmas of *E. enysii, E. tenuis* and *E. multiflorus* were measured from their bases to their bifid apices (Plate 3.9). The way in which the length of lemmas and awns were measured resulted in much higher values of lemma length for some species — especially the range of values from Australian *E. rectisetus* (10–40 mm; average = 20.43 mm). For the texture of lemmas, *Elymus enysii* was characterised by thinner (chartaceous) lemmas than all other species which have coriaceous lemmas.

Under the light microscope, *Elymus scaber* var. *plurinervis* was observed to be more scabrous on the dorsal surface of its lemmas. This was confirmed by observations with the SEM. The SEM revealed that there are more papillae and short cells on the dorsal surface of this taxon than the other taxa (Plate 3.8).

The width of the membranous margins of lemmas ranged from 0.05 to 0.5 mm and the average was 0.07 mm. It seemed that *E. scaber* var. *plurinervis* possessed distinctly wider margins of different texture than others with the range of values from 0.1 to 0.5 mm and the average of 0.14 mm (Plate 3.7. f).

The lemma apex of Australian *E. rectisetus* was nearly lorate and long tapering into the awn, and markedly canaliculate (Plate 3.6. 1 and 2), being distinguishable from other taxa in the complex that have acuminate and flat lemma apices (Plate 3.6. 3-6). Lemma apex characters had a strong influence on the phenetic analysis (see Chapter 4). Folded lemma apices were found in *E. enysii* (Plate 3.9. c) and sometimes also in *E. scaber* var. *plurinervis*.

Bifid lemma apices were observed in the individuals of *Elymus multiflorus*, *E. apricus*, *E. enysii* and *E. tenuis* (Plate 3.9. a-d). The bifid lemma apex of *E. tenuis* was different from others in as much as it formed two lacerate membranous blades (Plate 3.9. d). In addition, the lemma apex of *E. scaber* var. *plurinervis* became lacerate with maturity. Bifid lemma apices were more common in *E. multiflorus* than in the short-awned forms from Queensland.

All *Elymus* species in Australia and New Zealand were characterised by awned lemmas with the exception that mucronate or muticous lemmas were scored for some individuals of *E. multiflorus* (Plate 3.9. a), and occasionally also scored for some individuals of *E. scaber* var. *scaber*. As discussed in Chapter 1, Australian *Elymus* species were informally separated as long-awned, intermediate-awned and short-awned forms by some authors (Crane and Carman, 1987; Torabinejad *et al.*, 1987; Murphy and Jones, 1999). Furthermore, formal taxonomic treatments based on these forms suggested that *E. rectisetus* was equated with the long-awned form (Crane and Carman, 1987; Torabinejad *et al.*, 1987; Murphy and Jones, 1999), *E. scaber* var. *scaber* with short-awned form (Crane and Carman, 1987; Murphy and Jones, 1999), and the very short-awned form with *E. multiflorus* (Murphy and Jones, 1999). In order to investigate the variation of lemma awn length between and within *Elymus* taxa, the average values of lemma awn length (excluding proximal and apical 1–2 lemmas; #152) were used for

phenetic analysis, and the result indicated that they were useful to distinguish *E. multiflorus* and the short-awned form from Queensland from the remainder (Figure 4-8. d). However, the range of values of lemma awn length overlap extensively (Table 4-4), which would cause ambiguity in taxonomic practice. The awn length of the proximal lemmas was measured separately, and its values were distinct in *E. multiflorus* and in the short-awned form from Queensland by the phenetic analysis (Figure 4-8. e). The ratio of average awn length to proximal lemma length was calculated and shown to be different in *E. multiflorus* (Figure 4-8. f). Connor (1994) also used 'long-awned' or 'short-awned' as diagnostic characters for New Zealand species, and, *E. multiflorus* and *E. enysii* were grouped into the 'short-awned' category, whereas the remainder fell into the 'long-awned' category.

The curvature of lemma awns has created problems with scoring of samples. For Australian material, the short-awned individuals from Queensland and *E. multiflorus* are characterised by straight awns when young and mature no matter whether the environment is dry or wet and, therefore, these samples were scored confidently from herbarium specimens. Nevertheless, it was difficult to score other specimens because the curvature of lemma awns was observed to be somewhat connected with the maturity and the environment from which the specimens were collected. Based on the observation of the cultivated individuals, *Elymus scaber* var. *plurinervis* and Australian *E. rectisetus* are characterised by lemma awns incurved when young and regularly recurved when mature. The individuals of *Elymus scaber* var. *scaber* have straight awns when young and straight to variously recurved awns when dry. Therefore, it is possible that some herbarium specimens were incorrectly scored. Connor (1994) used this character to distinguish several taxa. For example, *E. multiflorus* and *E. enysii* were distinguished from other taxa by short and straight awns, and *E. solandri* was described as being different from *E. rectisetus sensu* Löve et Connor on account of its recurved awns. Generally, this character may be used for taxonomic purposes based on the observations of cultivated material.

The character of one vein entering the lemma awn (Plate 3.7. h) was valuable for defining *E. scaber* var. *plurinervis*, whereas, all other taxa were characterised by three veins entering the awn (Plate 3.7. g).

3.3.7. Paleas

Salomon and Lu (1992) claimed that two features of the palea of *Elymus* species were correlated with genome constitution, namely the apex shape and size of the cilia. Connor (1994) has also used palea apex shape for distinguishing the *E. scaber* complex in New Zealand. Coincidently, it was revealed in the present study that some features of the palea apex were useful for classification of the *Elymus scaber* complex. Several new characters discussed below were developed and used for phenetic analysis, some of which received high correlation values (see characters 171, 175 and 176 Table 4-4).

Palea apices were narrower than or wider than the lemma apices (Plate 3.7. a-d). In *E. rectisetus* and *E. scaber* var. *plurinervis*, palea apices were commonly narrower than lemma apices whereas, in the short-awned individuals from Queensland, palea apices were usually equal to or wider than lemma apices.

Cilia of markedly different sizes exist on keels of some samples, especially those of *E. rectisetus*. In such cases, both "scabrous" and "ciliolate" were scored (Plate 3.11. g). The length of cilia ranged from 0.2 – 0.4 mm for *E. rectisetus* and *E. scaber* var. *scaber*, and from 0.1 – 0.3 mm for the remainder.

In Connor's (1994) description, all taxa from New Zealand have pointed palea apices against the truncate or obtuse apex for Australian taxa. However, pointed palea apices were also observed in some Australian elements (e. g. OTUs 9, 19, 21 and 46; Plate 3.11. f & g) and truncate or obtuse palea apices were discovered in some New Zealand samples (e. g. *E. apricus* [OTU 103], *E. falcis* [OTU 107] and *E. solandri* [OTU 117]). Furthermore, there was occasionally uncertainty between obtuse and pointed palea apices. The incision of the palea apex is another obvious character, and three states: entire, retuse and bifid, were established to describe the variation. The meaning of

"retuse" used here was expanded: it refers to all states exclusive of "entire" and "bifid" (e.g. emarginate, Plate 3.11. c; retuse, Plate 3.11. d; and erose, Plate 3.11. e), to avoid redundancy and confusion. Different combinations were observed within a single specimen (Plate 3.11. g). Although overlap occurred, pointed and bifid palea apices played important roles in separating several New Zealand taxa from Australian taxa (Figure 5-2).

Palea flanks (abaxial view) ending at different positions against the apices were first revealed by SEM. This character was then applied to the examination of samples under the stereo-microscope. Individuals of *E. rectisetus* were distinct with flanks ending at lower points than other taxa and, therefore, the tips above the flanks were obviously longer (Plate 3.12. b) than in the other taxa (Plate 3.12. a, c and d). A high correlation coefficient (0.8785) for this character was obtained by phenetic analysis (see Chapter 4 and Table 4-4).

3.3.8. Lodicules and stamens

Lodicules were uninformative in this study. Only 13 characters were included and none of them was used for analysis owing to either uniformity or polymorphism observed between individuals. Polymorphisms occur in the shape of lodicules (Plate 3.13). Eight states were established to describe the various shapes of lodicules.

The variation in anthers exist in the length and the colour. These characters were used by Connor (1994) to distinguish *E. tenuis* from *E. apricus*. For Australian material, anther length ranged from 0.5 – 4.4 mm with the average 2.0 mm. The mean values of each sample were used in the phenetic analysis, but a correlation analysis indicated that its contribution to the ordination space was poor (data not shown). Therefore, this character was discarded from the final analysis.

Yellow anthers were observed in most samples, whereas both yellow and purple anthers were observed in a few specimens. Based on observations of cultivated material, it was noticed that anther colour was not a stable feature within this species complex (see

Table 4-2). Two individual plants (OTUs 12 and 33), growing five meters apart in the field and bearing either yellow or purple anthers, were included in the RAPD analysis (Table 6-1). The cluster analysis indicated no genetic distance between them (Figure 5-2). Thus this character is presumably not informative for classifications.

3.3.9. Fruit

Low seed set was considered to be an important feature of apomictic species of *Elymus*, namely *E. rectisetus* (Carman and Wang, 1992). The character of seed set was expected to provide useful data on their reproductive pattern. However, only less than half of the samples were scored because some of the samples were at anthesis or had some mature seeds already shed. Furthermore, identifying apomicts should depend on more explicit techniques (Hanna and Bashaw, 1987) rather than inferred from herbarium specimens.

The characters of fruit were obtained from the mature ones and the pinched fruit were avoided as far as possible. *Elymus scaber* var. *plurinervis* was distinct from other Australian taxa by several characters of fruit. It had narrowly obovoid rather than narrowly elliptical shaped fruit (Plate 3.14. a), a higher ratio of fruit width to fruit length (0.22-0.32, average = 0.27), and a lower ratio of fruit length to palea length (0.47-0.55, average = 0.51). *Elymus rectisetus* was also characterised by a rather low ratio of fruit length to palea length (0.52-0.68, average = 0.59) and was apparently distinct from *E. scaber* var. *scaber* (0.63-0.81, average = 0.72), *E. multiflorus* (0.61-0.71, average = 0.64) and the short-awed form from Queensland (0.61-0.79, average = 0.71). The fruit of New Zealand taxa were more variable (Plate 3.14. b), and no conclusions were obtained owing to the small sample size for each taxon. However, it was noted that New Zealand *E. rectisetus sensu* Löve and Connor was more similar to Australian *E. scaber* var. *scaber* than to Australian *E. rectisetus* on account of its high ratio of fruit length to palea length (average = 0.77).

In order to observe the transverse section, the fruit were hand sectioned from the mid-segments. The structure of the transverse section generally appeared to be crescent shaped and 'U' shaped (Plate 3.15). However, only *E. scaber* var. *plurinervis* was

consistently characterised by crescent-shaped fruit in t.s. (Plate 3.15. a), and other taxa were variable. This character is obviously connected with the maturity of fruit.

3.3.10. Abaxial leaf blade epidermis

For descriptive purposes, Metcalfe's (1960) convention that the epidermis is viewed as though the leaf was arranged horizontally across the slide is followed. That is, with the veins running across the field of view. 'Horizontal' thus indicates 'parallel to the long axis of the leaf and its main veins'. It should be noted that the "epidermis near the leaf margin is sometimes rather markedly dissimilar from that elsewhere" (Clifford and Watson, 1977), such as the number of rows of long-cells, the distribution of stomata, and so forth. Therefore, descriptions from this region were avoided.

The variation in the epidermis of the leaf blades was described as the differences in the costal and the intercostal zones, the number of long-cell rows on the intercostal region, the feature of long-cell walls and short-cells, papillae, stomata and prickles.

The epidermises of most individuals were characterised by conspicuous costal and intercostal zones (Plate 3.16. a), i.e. the cells (e.g. long-cells, short-cells, papillae, prickles and macrohairs) were distributed differently between, and over, the veins. However, in some material, there were no differences between costal and intercostal zones, except that stomata occurred only on the intercostal region (Plate 3.16. b).

The number of rows of long-cells on the intercostal region ranged from 4 to 27. However, 5–12 rows occurred more often (about 86 %), which indicated that apparent differences occurred across the width of the intercostal region. The length of long-cells was markedly different from one sample to another (Plate 3.16. c, d and e). The wall thickness of long-cells seemed to be related to their length. Generally, shorter cells had tessellate or sinuous walls (Plate 3.16. c; Plate 3.17. c), whereas longer cells were characterised by sinusoid or straight walls (Plate 3.16. d and e). It appeared that some of the characters of long-cells discussed above were connected with folded or involute leaves (e.g. those of *E. falcis*, *E. apricus* and *E. sacandros*). Therefore, the epidermis of

folded or involute leaves was usually characterised by rather short long-cells with sinuous to tessellate walls.

Stomata in the abaxial epidermis were observed in most material (about 80%) except a few specimens from New Zealand (e.g. *E. enysii* [OTU 106], *E. sacandros* [OTU 115], and *E. solandri* [OTUs 116 and 117]). Stomata were distributed on the intercostal region irregularly in *Elymus* species, and the number of rows ranged from 0 to 3 per intercostal region. Three rows seldom occurred (about 1%). Interrupted stomatal rows were observed often. Parallel-sided subsidiary cells (Plate 3.16. b and d) were encountered often in the *Elymus scaber* complex. In some individuals with folded leaves, the subsidiary cells seemed wider than those in flat leaves (Plate 3.17. a). Moreover, low, dome-shaped subsidiary cells (Plate 3.16. f) were also observed in several individuals.

Short-cells were seen in all samples even though the density and shape of them differed between individuals. Short-cells were usually distributed on the intercostal regions and over veins, and arranged in different patterns, such as individually (Plate 3.16. c), in pairs (Plate 3.16. d) or in horizontal rows (Plate 3.16. e). The paired short-cells occurred commonly both on the intercostal regions and over the veins, whereas, single short-cells were observed more often on the intercostal regions than over the veins. Short-cells in horizontal rows existed more frequently over the veins than on the intercostal regions.

Clifford and Watson (1977) and Metcalfe (1960) described many kinds of silica bodies. Based on their descriptions, silica bodies were categorised in *Elymus* species: tall and narrow (Plate 3.16. c), rounded, cuboidal (Plate 3.17. b), and horizontally long and sinuous or smooth silica bodies (Plate 3.16. e) were seen often, whereas crescent, acutely angled (Plate 3.16. e; Plate 3.17. b) and cross-shaped silica bodies were rarely observed.

Papillae are variously shaped protrusions from the outer walls of epidermal cells (Metcalfe, 1960) and are referred to as 'crown cells' by some workers (Runemark and

Heneen, 1968; Macfarlane, 1979). This structure occurred in most samples examined (78%) but was absent in others. Of ten samples which lacked papillae, eight were from New Zealand (namely, *E. apricus*, *E. enysii*, *E. tenuis* and *E. multiflorus*). Nevertheless, papillae were observed on two samples of *E. multiflorus* from Australia, which indicated the differentiation within species. Mostly, papillae occurred only over the veins (Plate 3.17. a), whereas, in some individuals, papillae were observed both over the veins and on the intercostal regions (Plate 3.17. c).

Prickles and macrohairs occurred in some individuals. Metcalfe (1960) described a prickle as a structure that is robust, sharply but shortly pointed with the point directed towards the apex of the leaf, and its base is swollen. He also noted that ambiguity sometimes occurred when distinguishing prickles from macrohairs, especially when the former have long barbs or the latter are short, rigid and thick-walled. Therefore, he suggested that prickles are homologous to macrohairs. The same problem was encountered in this study. For descriptive purposes, protrusions with barbs of more than average length were treated as macrohairs, otherwise, as prickles.

Prickles were observed in about 60% of samples. They were mostly distributed only over the veins (Plate 3.16. a), and rarely distributed both over the veins and on the intercostal regions (Plate 3.17. d). Basically, two kinds of prickles were observed: big prickles (Plate 3.17. e) which had elliptical bases and thick barbs, and were observed over the veins; small prickles (Plate 3.16. d) which were much smaller than the former, had circular bases, and occurred in the intercostal regions and were also over the veins.

Macrohairs (Plate 3.17. f) were seen on the abaxial epidermis of only 24% samples.

3.3.11. Leaf blade transverse sections

In transverse section, most samples were characterised by distinct, prominent adaxial ribs (Plate 3.18. a). Nevertheless, some individuals (15%) had both adaxial and abaxial ribs (Plate 3.18. b), and this was called 'nodular structure' (Clifford and Watson, 1977). The variation described above could sometimes be observed within a single taxon. For

example, the nodular structure was observed in two samples of *E. multiflorus* from New Zealand but not in Australian *E. multiflorus*.

Adaxial ribs of different sizes (excluding the midrib; Plate 3.18. c) occurred in most samples, but more or less consistent ribs were also observed in some individuals (Plate 3.18. a). Bigger ribs differed from smaller ribs not only by their size, but also by the amount and structure of sclerenchyma within them. Bigger ribs usually consisted of more sclerenchyma which had a 'T'-shaped or 'I'-shaped profile (Plate 3.18. c and d), whereas smaller ribs usually comprised less sclerenchyma which sometimes only showed a strand-like structure (Plate 3.18. d) or no sclerenchyma. In folded or involute leaves, the adaxial ribs were apparently truncate at the tops (Plate 3.18. c and e) rather than obtuse at tops as in flat leaves (Plate 3.18. d).

Species of Triticeae are described as having a Non-Kranz leaf anatomy (Brown, 1975; 1977) which consists of irregular chlorenchyma, more than four cells between adjacent sheaths and two bundle sheaths (Clayton and Renvoize, 1986). All these structures were observed in this study (Plate 3.18. d) with the exception that the outer sheaths were not obvious in some individuals.

Abaxial-hypodermal sclerenchyma (Plate 3.18. e), although not obvious, occurred in a few involute or folded leaves of some samples (e.g. *E. falcis* and *E. sacandros*) but not in all such leaves. This kind of structure was also reported from *Thinopyrum junceum* (L.) A. Löve (= *Triticum junceum* L.) and was used as a taxonomic character in some grasses such as *Festuca* and *Avena* (Runemark and Heneen, 1968). However, experiments showed that this character is mutable in response to the prevailing ecological conditions (e.g. humidity), and, therefore, is possibly of little taxonomic value (Runemark and Heneen, 1968).

Plate 3.1.

Auricles and leaf sheaths.

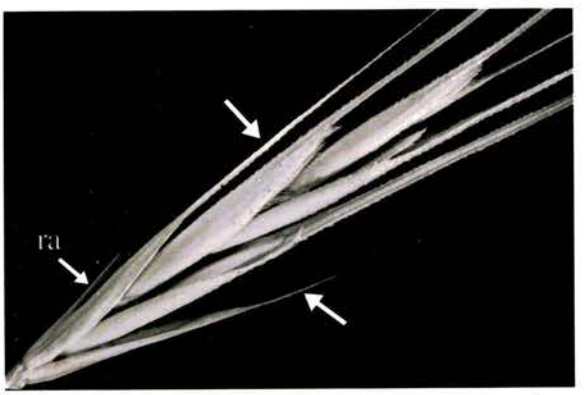
a. ***Elymus multiflorus*** (S. Wang 95176). Auricles long, unequal in size, pungent, glabrous; leaf sheaths glabrous and margins membranous and smooth (arrow). **b.** ***Elymus multiflorus*** (S. Wang 95174). Auricles minute, nearly equal in size, acute, glabrous. **c.** ***Elymus rectisetus*** (S. Wang 95115). Auricles long, equal in size (one of which not seen in photo, arrow), pungent, hairy; leaf sheaths with retrorse hairs. **d.** ***Elymus solandri*** (CHR260318). Auricles absent. **Bar** = 1 mm.


a
c
b
d

Plate 3.2.

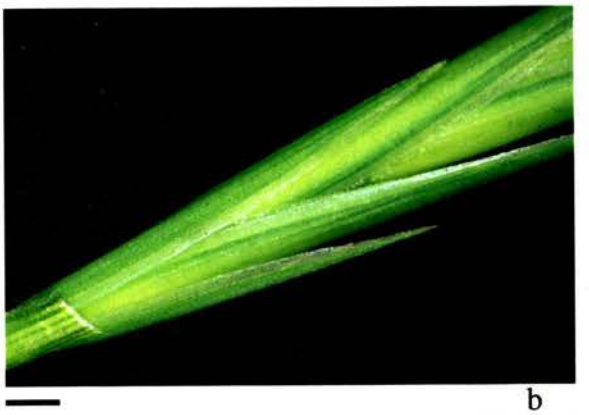
Polymorphism of glumes.

a. ***Elymus tenuis*** (CHR249122). Glumes with long awns (arrows; ra: rachilla). **b.** ***Elymus scaber*** var. ***scaber*** (S. Wang 95104). Glumes subequal in length, subulate (shape *in situ* of half glume); margins membranous, narrow. **c.** ***Elymus scaber*** var. ***scaber*** (S. Wang 95104). Lower glume subulate, 3-veined. **d.** ***Elymus*** sp. (S. Wang 95140, the short-awned from Queensland). Lower glume narrowly ovate (shape *in situ* of half glume), 5-veined; margins membranous, narrow (arrow). **e.** ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95157). Glumes subequal in length; margins membranous, wide (arrow). **f.** ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95157). Upper glume 7-8-veined. **Bar** = 1 mm.



a

d



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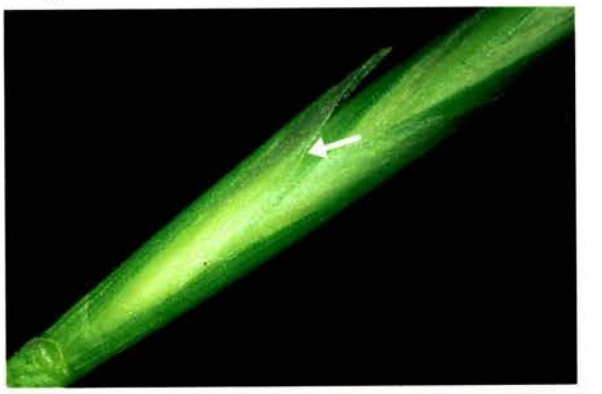
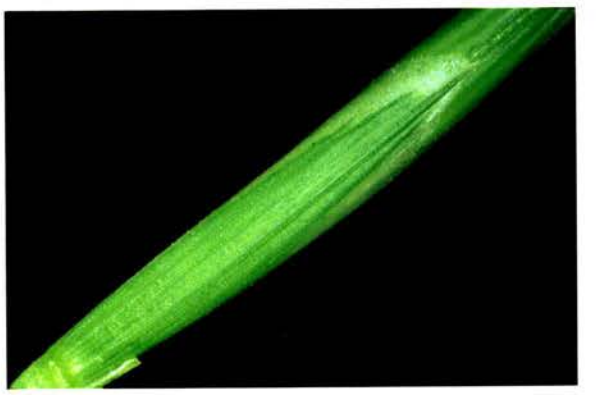


e

c

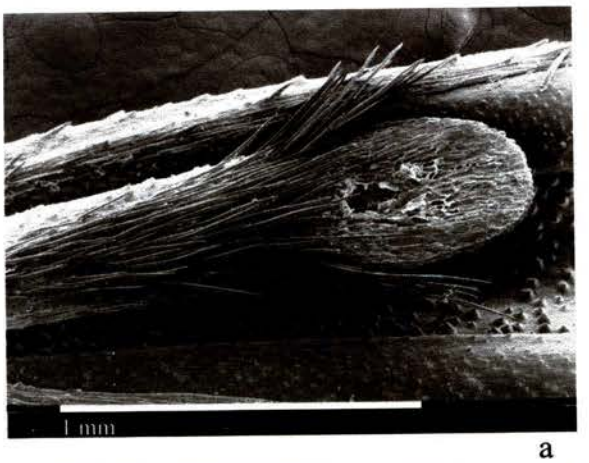


f

Plate 3.3.

Scanning electron micrographs illustrating polymorphism of the rachillas.

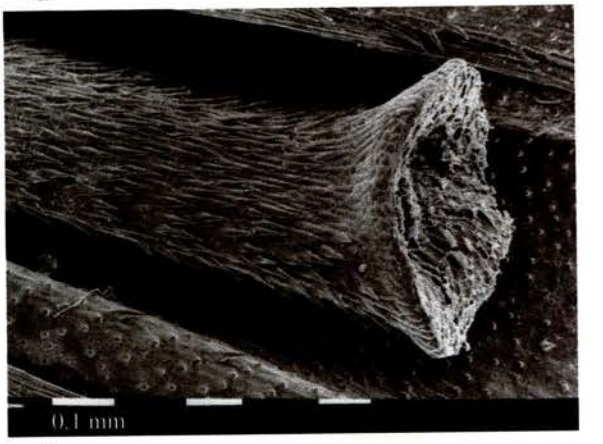
a. ***Elymus rectisetus*** (S. Wang 95087). Rachilla hairs long and dense, covering callus (removed) base; rachilla apex elliptical. **b.** ***Elymus scaber*** var. ***scaber*** (S. Wang 95135). Rachilla hairs rather long, not covering callus (removed) base; rachilla apex obovate. **c.** ***Elymus scaber*** var. ***plurinervis*** (L.S. Smith & S.L. Everist 811A). Rachilla hairs short; rachilla apex transverse-circular. **d.** ***Elymus multiflorus*** (CHR1594). Rachilla hairs short; rachilla apex obovate. **e.** ***Elymus enysii*** (CHR471937). Rachilla hairs short; rachilla apex nearly horizontal, sharply expanded. **f.** ***Elymus sacandros*** (CHR387017). Rachilla hairs very long and dense, covering callus (removed) base; rachilla apex ovate.



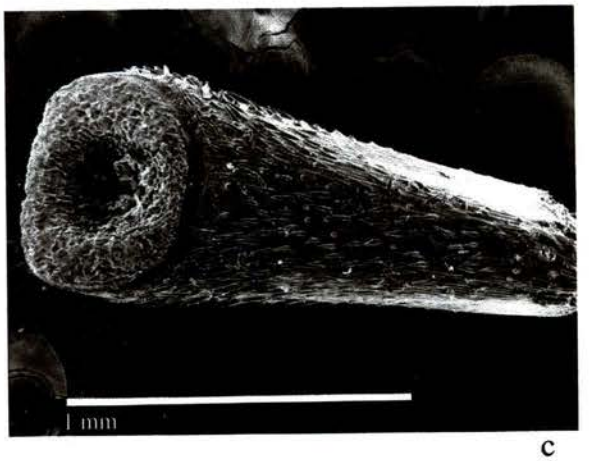
a

d

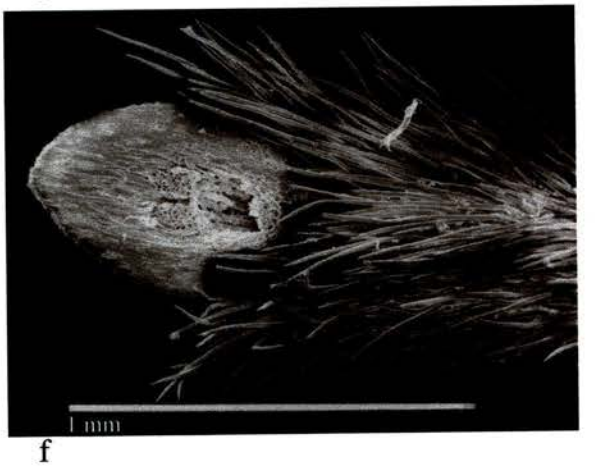
b



e



c

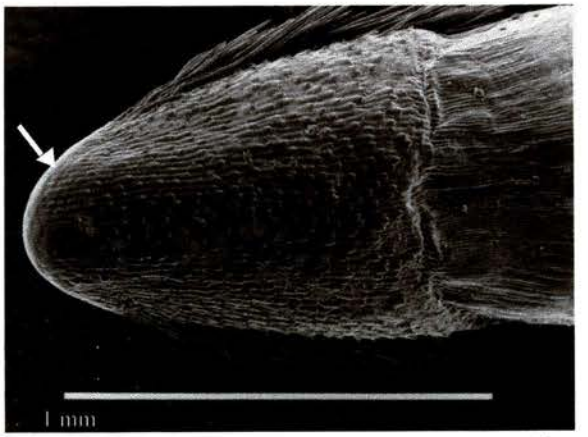


f

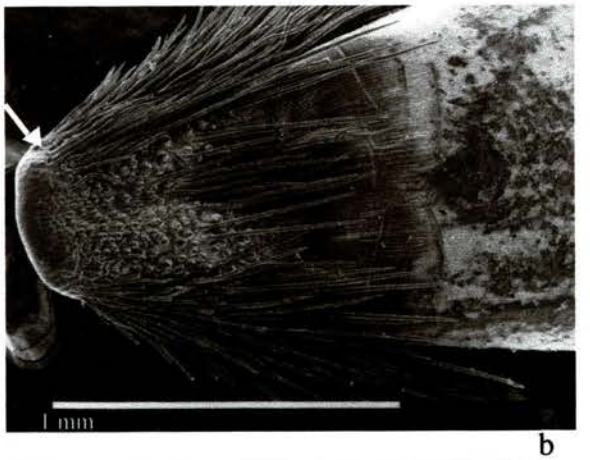
Plate 3.4.

Scanning electron micrographs illustrating polymorphism of the calluses (abaxial).

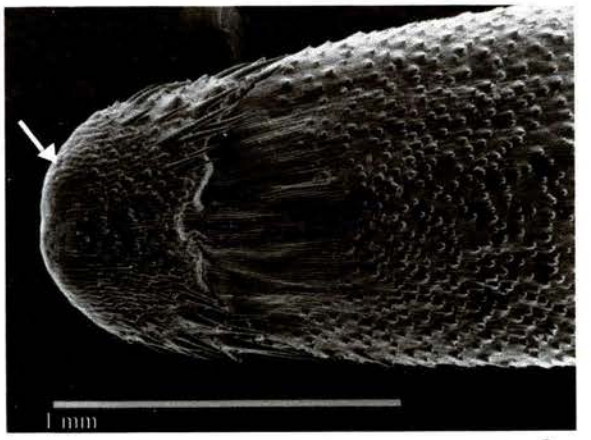
a. ***Elymus rectisetus*** (S. Wang 95001). Callus (abaxial) narrowly triangular; dorsal surface rounded; hairs restricted to margins; tip not thickened (arrow). **b.** ***Elymus scaber*** var. ***scaber*** (MEL1560495). Callus (abaxial) triangular; dorsal surface sunken; hairs on margins and surface; tip thickened (arrow). **c.** ***Elymus scaber*** var. ***plurinervis*** (R.J. Fensham 1728). Callus (abaxial) broadly triangular; dorsal surface flat, glabrescent; tip not thickened (arrow). **d.** ***Elymus multiflorus*** (CHR1594). Callus (abaxial) broadly triangular; dorsal surface flat, glabrescent; tip not thickened (arrow). **e.** ***Elymus enysii*** (CHR127516). Callus (abaxial) latitudinally much wider (> triple wider than longer). **f.** ***Elymus sacandros*** (CHR387017). Callus (abaxial) triangular, dorsal surface flat; hairs long and dense, distributed on margins and surface; tip not thickened (arrow). **g.** ***Elymus solandri*** (CHR260318). Callus (abaxial) widely triangular, dorsal surface raised; hairs long and dense, mostly distributed on margins.



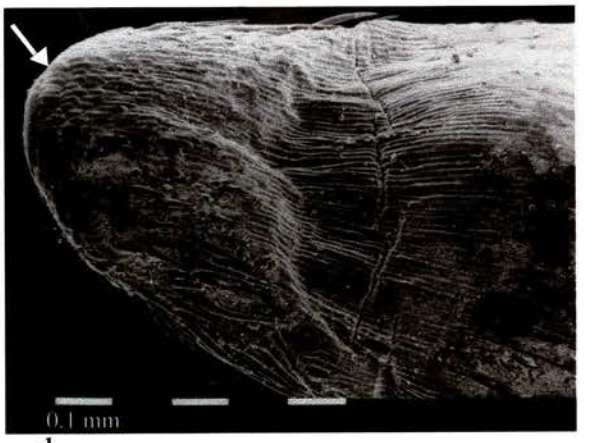
a



b

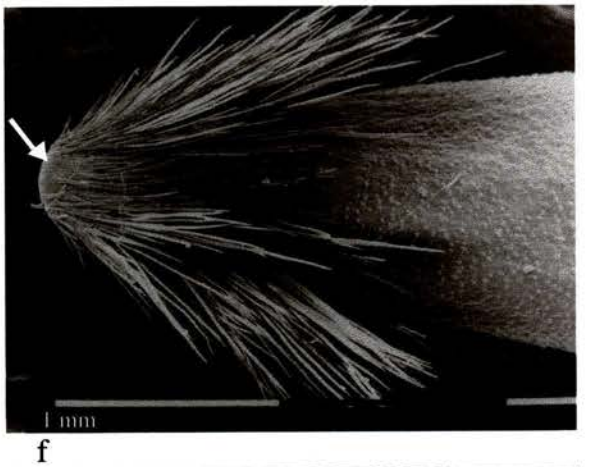


c

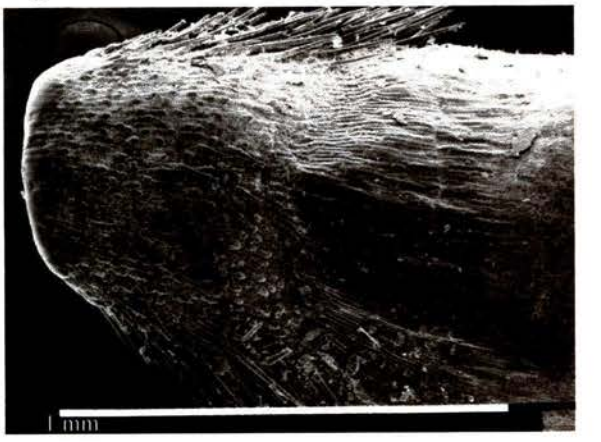


d

e



f

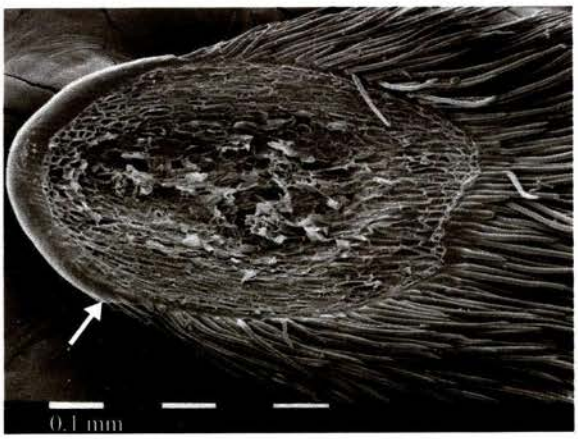


g

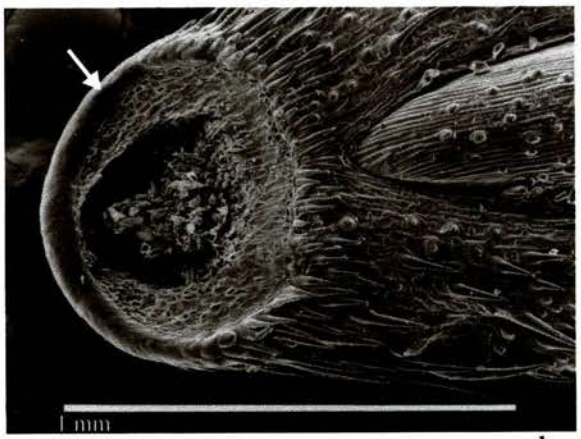
Plate 3.5.

Scanning electron micrographs illustrating polymorphism of the calluses (adaxial).

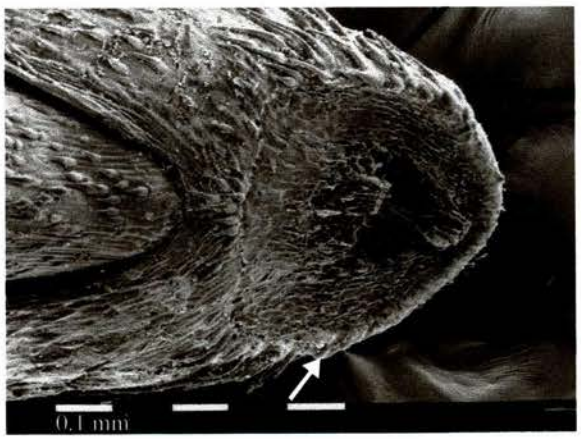
a. ***Elymus rectisetus*** (S. Wang 95001). Callus flat; marginal thickening distinct (arrow), restricted to below the mid-point; hairy. **b.** ***Elymus scaber*** var. ***plurinervis*** (R.J. Fensham 1343). Callus sunken; marginal thickening distinct (arrow), extending approximately to the mid-point; glabrescent. **c.** ***Elymus multiflorus*** (CHR1594). Callus sunken; marginal thickening distinct (arrow), extending approximately to the mid-point; glabrous. **d.** ***Elymus enysii*** (A.P. DRUCE 1345). Callus raised, with remnants of rachilla (arrow); marginal thickening not distinct; hairy. **e.** ***Elymus sacandros*** (CHR387017). Callus sunken; marginal thickening distinct (arrow), restricted to below the mid-point; hairy. **f.** ***Elymus apricus*** (P.N. JOHNSON 1220). Callus sunken, marginal thickening distinct (arrow), restricted to below the mid-point; hairy.



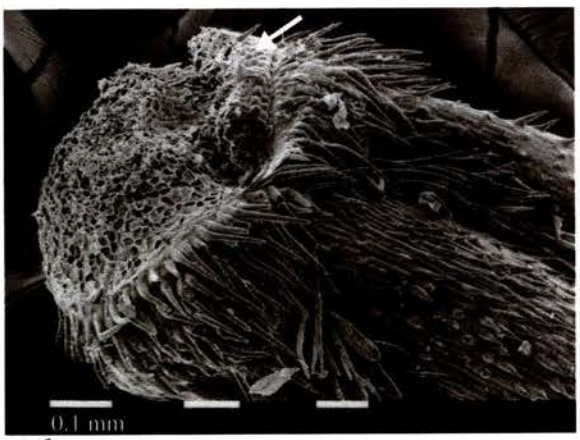
a



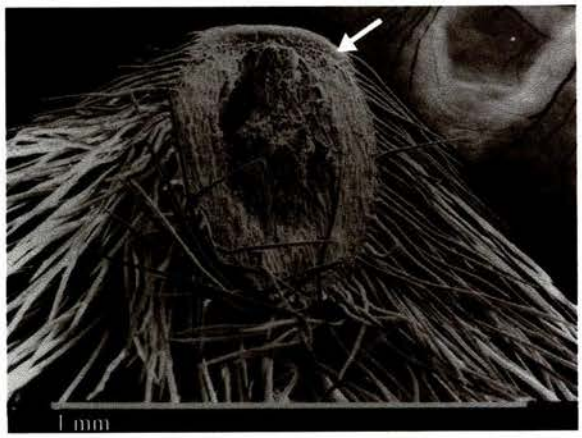
b



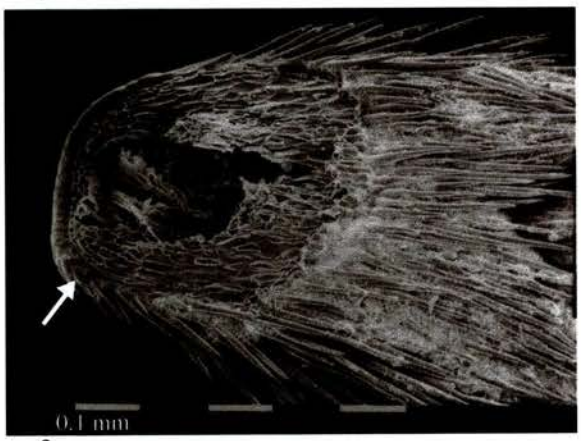
c



d



e



f

Plate 3.6.

Polymorphism of lemma apices.

a. Lemma apices (adaxial). 1 & 2: ***Elymus rectisetus*** (N.C. Beadle s.n.; S. Wang 95001). Lemma apex nearly lorate (shape of half lemma, *in situ*), canaliculate (shape in t.s.). 3. ***Elymus scaber*** var. ***scaber*** (J.B. Muir 353). Lemma apex acuminate, nearly flat. 4. ***Elymus*** sp (S. Wang 95141, the short-awned from Queensland). Lemma apex acuminate, flat. 5. ***Elymus multiflorus*** (S. Wang 96163). Lemma apex acuminate, nearly flat. 6. ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95153). Lemma apex acuminate, nearly flat. **b**. Lemma apices (abaxial). The taxa are the same as those in **a**. Lemma apex nearly lorate: 1-2; Lemma apex acuminate: 3-6. Lemma apically keeled (arrows). **Bar** = 1 mm.

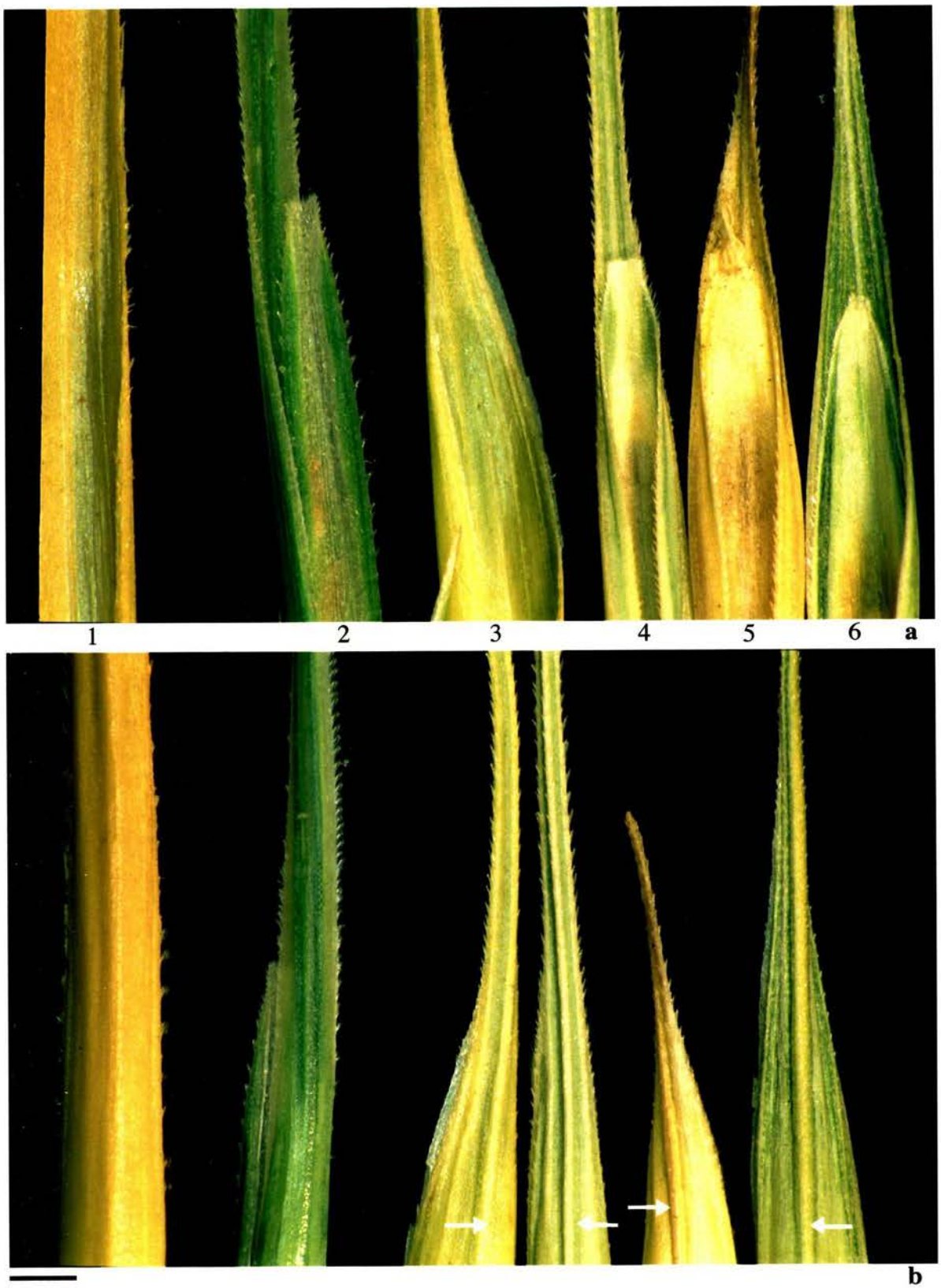

1
2
3
4
5
6
a
b

Plate 3.7.

The relationships between lemma and palea; lemma margins and the number of nerves entering the awns.

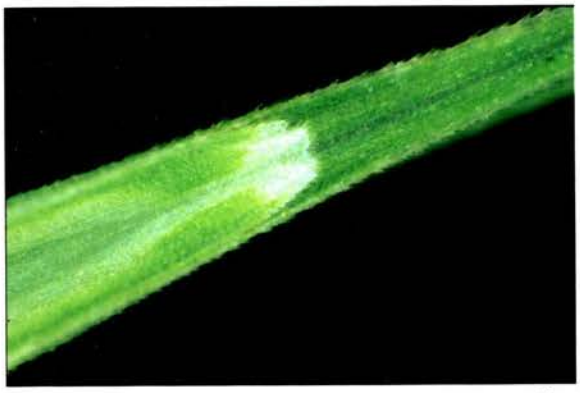
a. ***Elymus rectisetus*** (S. Wang 95001). Palea apex narrower than lemma apex. **b**. ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95157). Palea apex narrower than lemma apex. **c**. ***Elymus*** sp. (S. Wang 95140). Palea apex nearly equal to lemma apex. **d**. ***Elymus*** sp. (S. Wang 95141). Palea apex wider than lemma apex. **e**. ***Elymus rectisetus*** S. Wang 95001). Lemma margins hyaline (arrow), narrow, smooth. **f**. ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95153). Lemma margins membranous (arrow), wide, smooth. **g**. ***Elymus*** sp. (S. Wang 95141). Lemma awn entered by three veins (arrow). **h**. ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95157). Lemma awn entered by one vein (arrow). **Bar** = 1 mm.

a

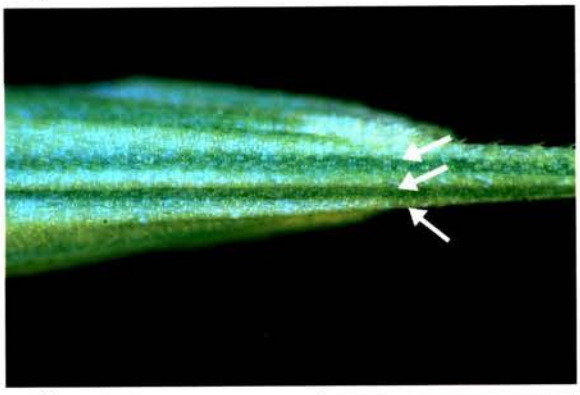
e

b

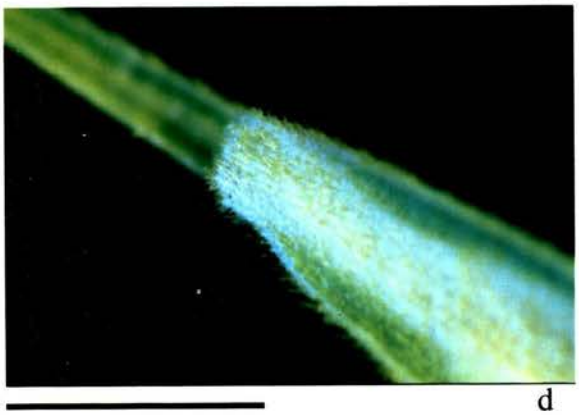
f



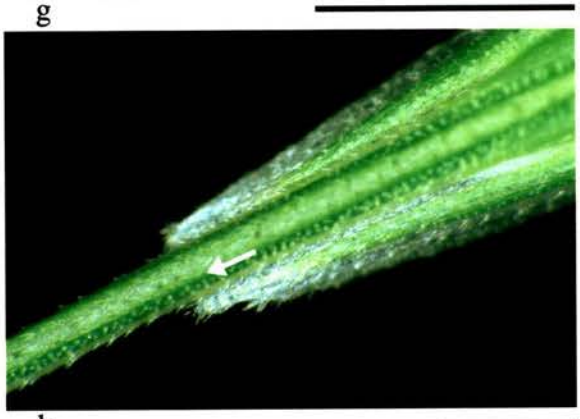
c



g



d

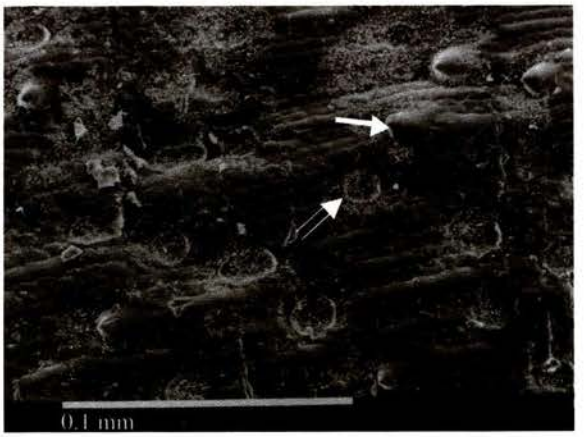


h

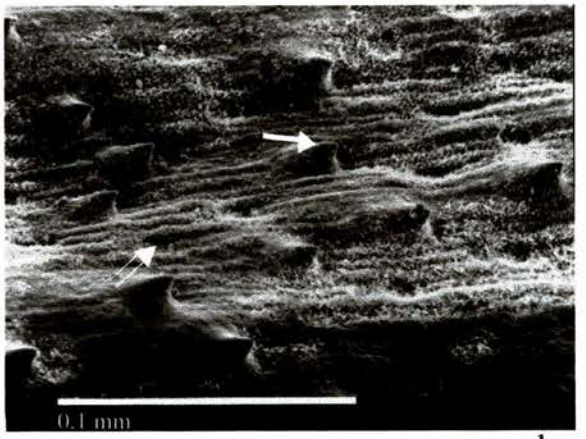
Plate 3.8.

Scanning electron micrographs illustrating the lemma surface (abaxial, lower part), papillae (closed arrows) and short cells (open arrows).

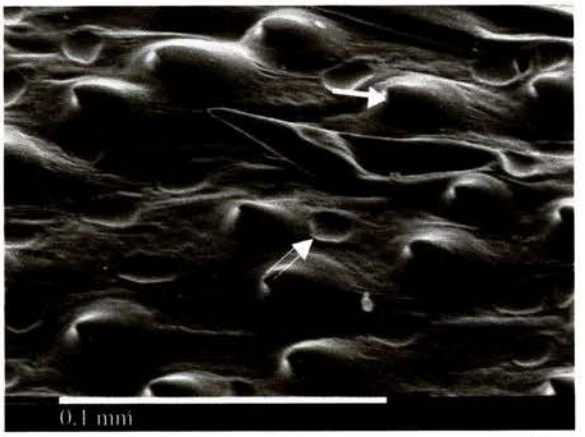
a. ***Elymus rectisetus*** (B.J. Lepschi 2027). **b.** ***Elymus scaber*** var. ***scaber*** (S. Wang 95104). **c.** ***Elymus scaber*** var. ***plurinervis*** (L.S. Smith & S.L. Everist 811A). **d.** ***Elymus multiflorus*** (CHR1594). **e.** ***Elymus tenuis*** (CHR145548). Glaucous. **f.** ***Elymus solandri*** (CHR223898).



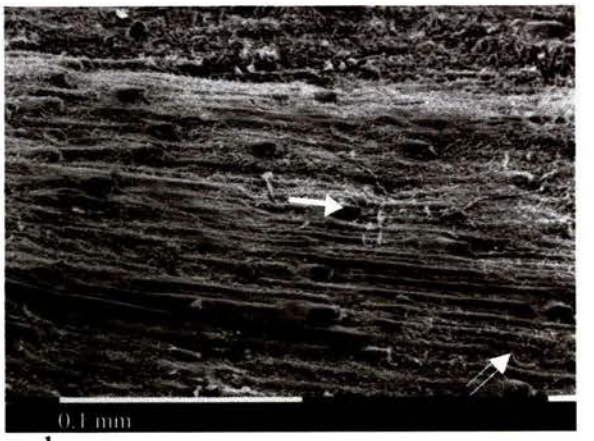
a



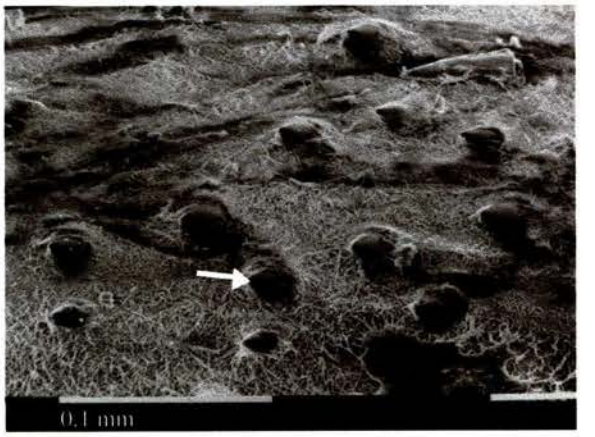
b



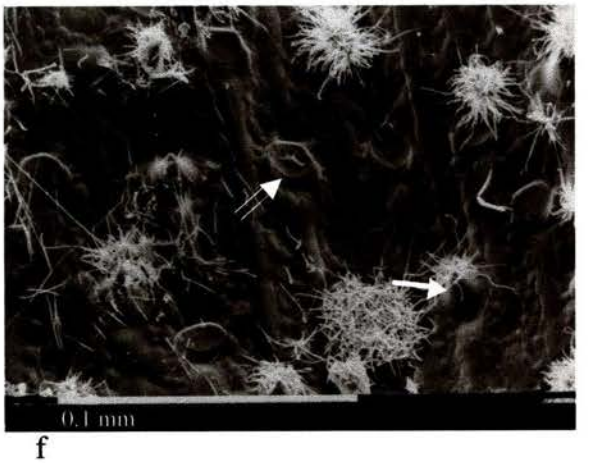
c



d



e



f

Plate 3.9.

Lemma apices, incisions.

a. ***Elymus multiflorus*** (CHR1595). Lemma apex bifid (arrow), mucronate (with awn-like point). **b**. ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95153). Lemma apex folded, bifid (when dry; arrow), awned. **c**. ***Elymus enysii*** (CHR127516). Lemma apex folded, bifid (arrow), awned. **d**. ***Elymus tenuis*** (CHR249122). Lemma apex bifid (arrow), awned. **Bar** = 1mm.

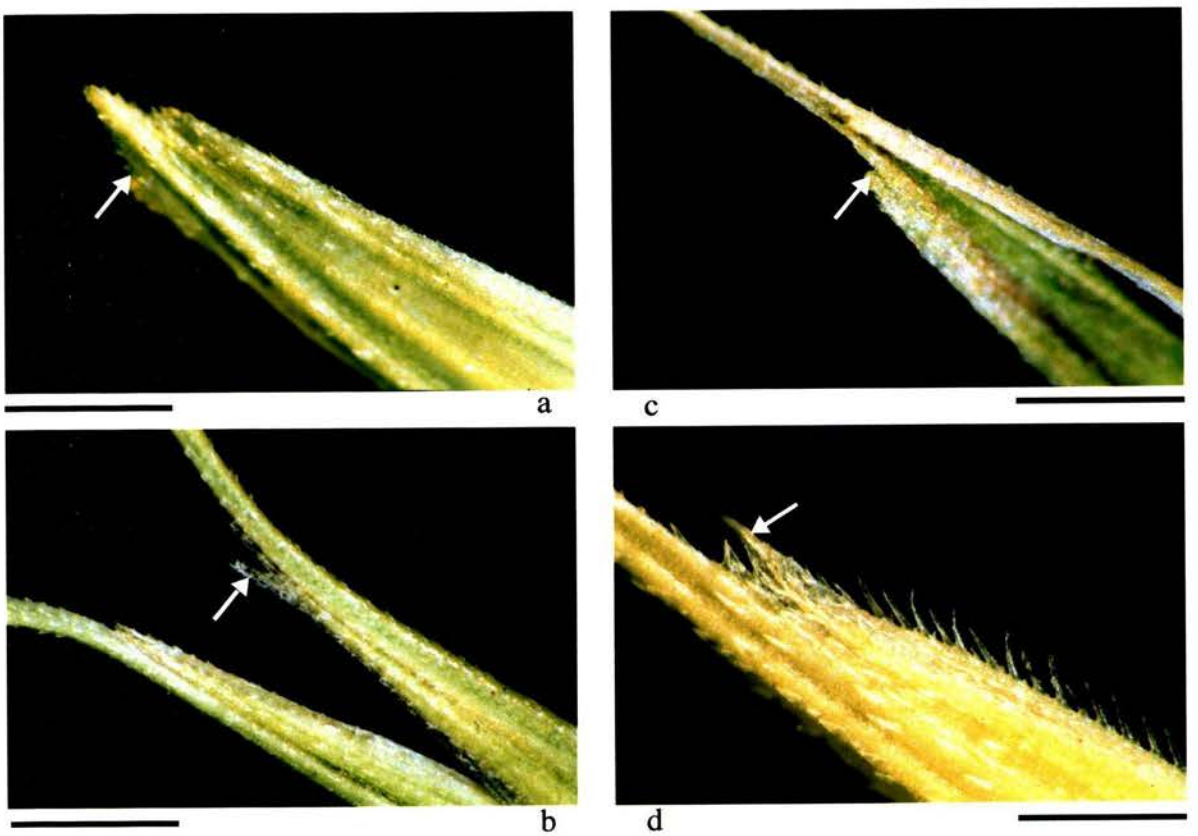
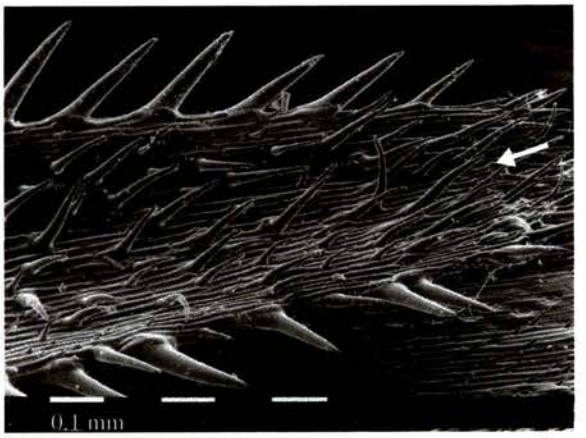

a
c
b
d

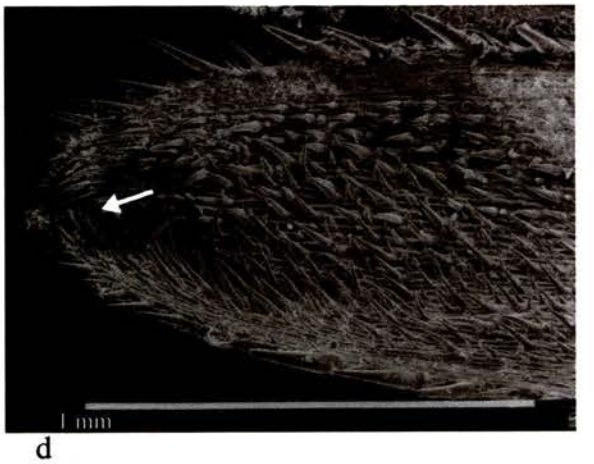
Plate 3.10.

Scanning electron micrographs illustrating hairiness in intercostal regions of the paleas, and the hairiness of lemmas (ventrally).

a. ***Elymus rectisetus*** (S. Wang 95087). Palea apex truncate, retuse (arrow); intercostal region hairy. **b**. ***Elymus scaber*** **var**. ***scaber*** (J.S. Whinray 709). Palea apex truncate, entire (arrow); intercostal region hairy. **c**. ***Elymus sacandros*** (CHR279258). Palea apex pointed, bifid (arrow); intercostal region hairy. **d**. ***Elymus multiflorus*** (CHR473347). Palea apex obtuse, retuse (not so clear in the photo; arrow); intercostal region hairy. **e**. ***Elymus scaber*** **var.** ***plurinervis*** (L.S. Smith & S.L. Everist 811A). Palea apex truncate, entire (arrow); intercostal region scabrous (or glabrous under the light microscope). **f**. ***Elymus tenuis*** (CHR145548). Palea apex pointed, bifid (arrow); intercostal region hairy. Lemma ventrally pubescent: **b, c** & **e** (open arrows).

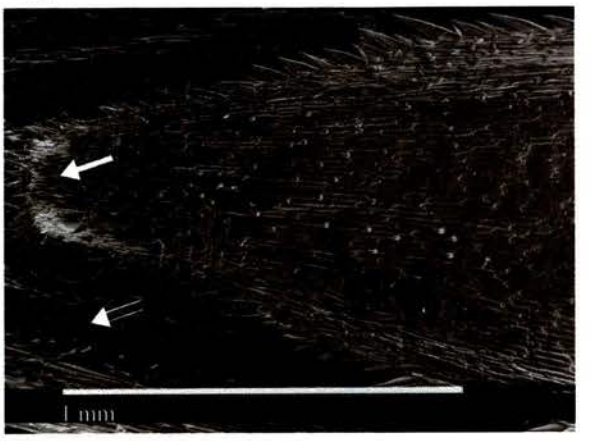


a

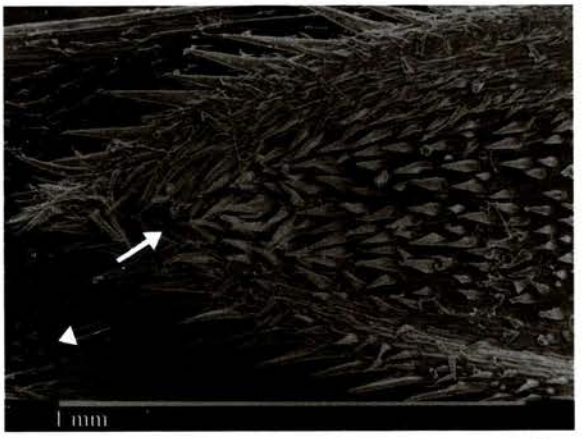


d

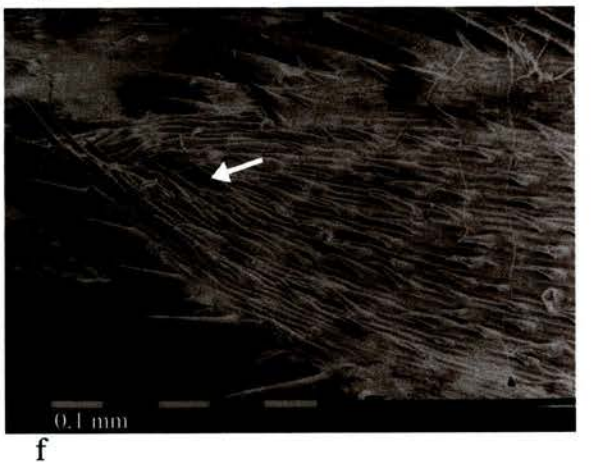
b



e



c



f

Plate 3.11.

Palea apices, incisions; Palea keels, hairiness; Palea margins, hairiness.

a. ***Elymus scaber*** var. ***scaber*** (S. Wang 95104). Palea apex truncate, entire, ciliolate. **b**. ***Elymus rectisetus*** (A.D.J. Piesse 143). Palea apex truncate, entire, ciliolate. **c**. ***Elymus rectisetus*** (N.C. Beadle s.n.). Palea apex truncate, emarginate, ciliate; **d**. ***Elymus multiflorus*** (S. Wang 95163). Palea apex obtuse, retuse, ciliolate. **e**. ***Elymus rectisetus*** MEL1560436). Palea apex truncate, erose, ciliate; keels ciliate. **f**. ***Elymus scaber*** var. ***scaber*** (A. Moscal 1577). Palea apex pointed, bifid; margins smooth (arrow). **g**. ***Elymus rectisetus*** (A. C. Beauglehole 29323). Different combinations of palea apex structure were observed within a single specimen: palea apex pointed and deeply bifid from a mature inflorescence, and palea apex truncate and emarginate from a basal young inflorescence; keels ciliolate and scabrous (arrow). **h**. ***Elymus enysii*** (A.P. Druce 1345). Palea apex pointed and bifid (arrow). **Bar** = 1 mm.

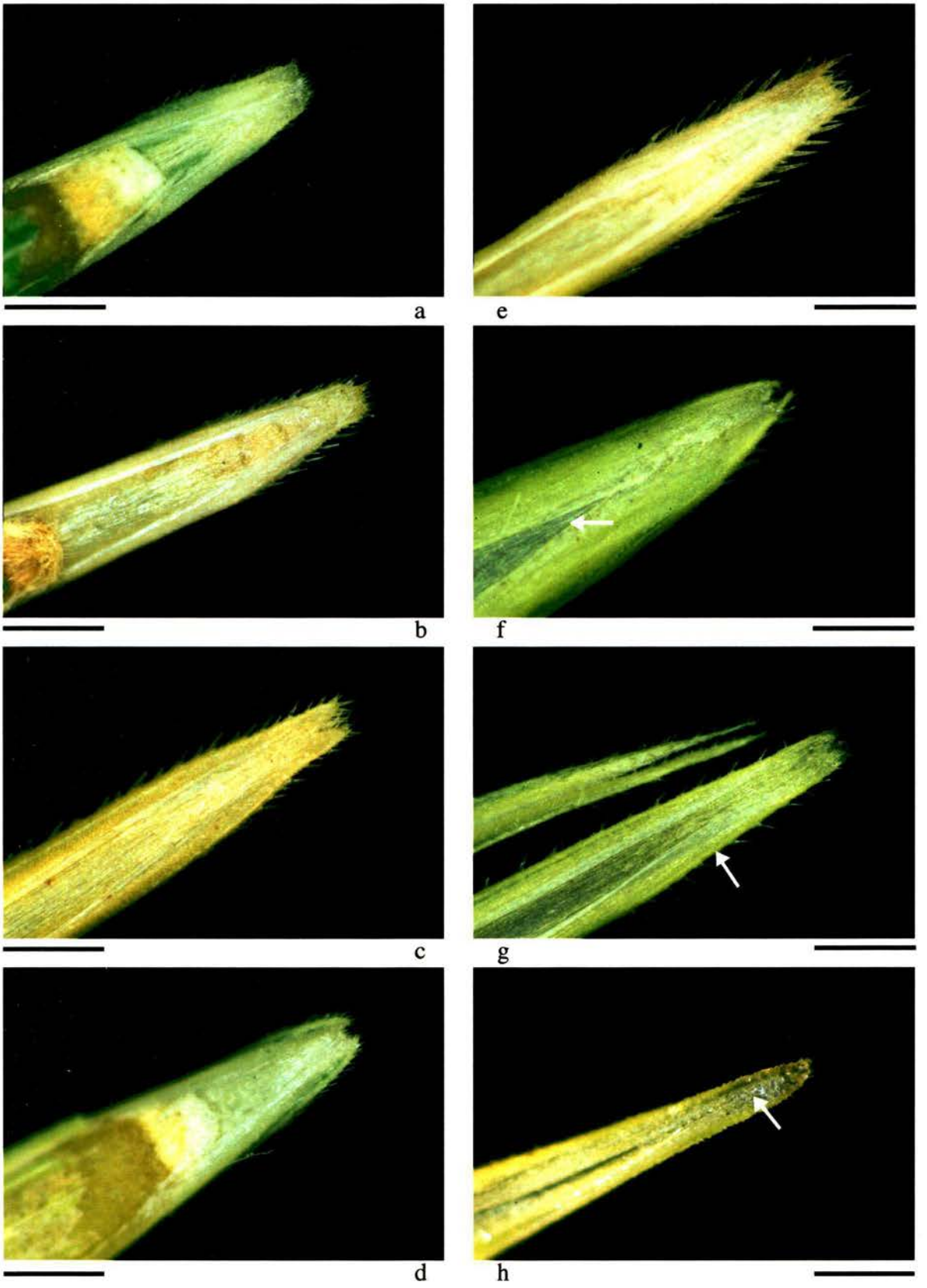
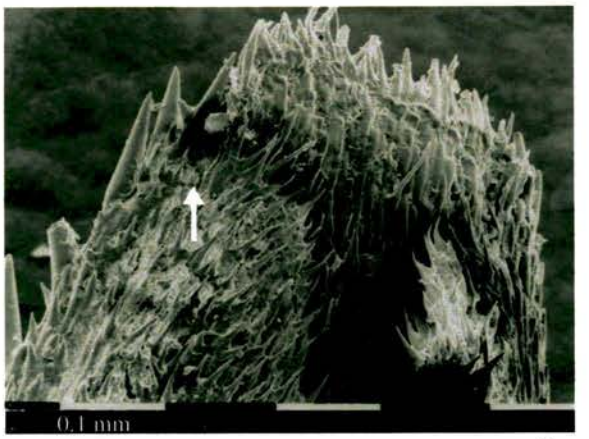

a
e
b
f
c
g
d
h

Plate 3.12.

Palea flanks (abaxial), ending positions.

a. ***Elymus scaber*** var. ***scaber*** (S. Wang 95129). Palea flanks ending under the top (arrow). **b**. ***Elymus rectisetus*** (S. Wang 95041). Palea flanks ending far away under the top (arrow). **c**. ***Elymus multiflorus*** (CHR1594). Palea flanks ending at the top (arrow). **d**. ***Elymus scaber*** var. ***plurinervis*** (L.S. Smith & S.L. Everist 811A). Palea flanks ending under the top (large closed arrow); palea tip retroflex (open arrow); and keels winged (small arrow). **Bar** = 0.5 mm.



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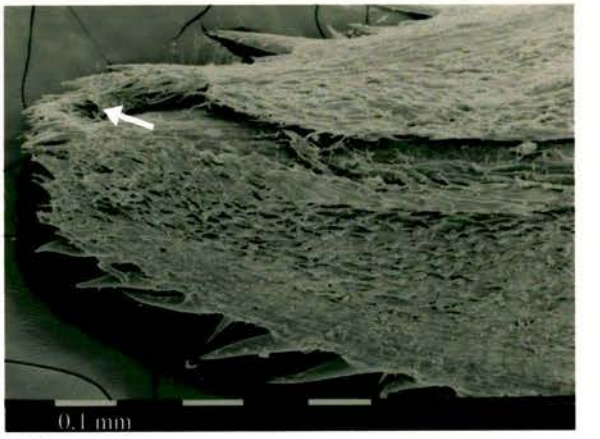

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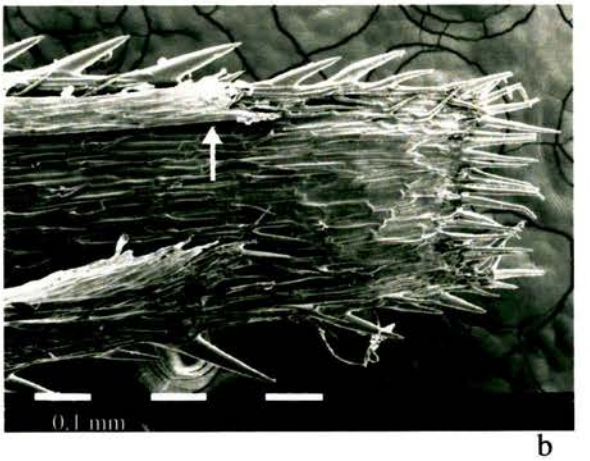

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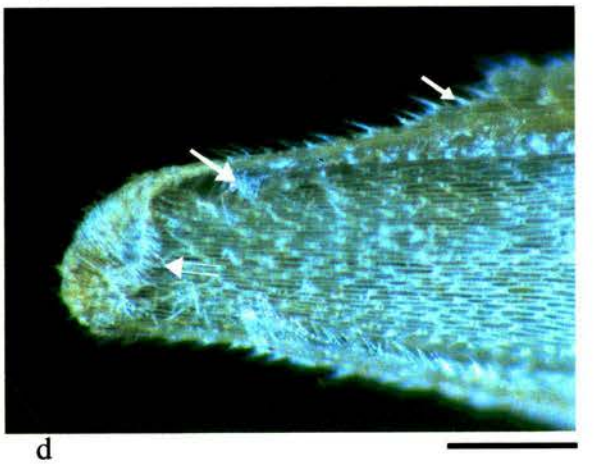


d

Plate 3.13.

Lodicule shapes.

a-e: ***Elymus scaber*** var. ***scaber*** (S. Wang 95104, S. Wang 95132, HO28260, O.D. Evans, A. Moscal 1577); **f**: ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95153). **a**. Lodicules irregular, glabrous, lobed; divisions lateral (arrow). **b**. Lodicules sickle-shaped, entire, keeled (arrow). **c**. Lodicules oblong, entire, glabrous. **d**. Lodicules cuneate, entire, keeled (arrow). **e**. Lodicules obovate, lobed; divisions terminal (arrow); margins ciliolate. **f**. Lodicules ovate, entire. **Bar** = 0.5 mm.

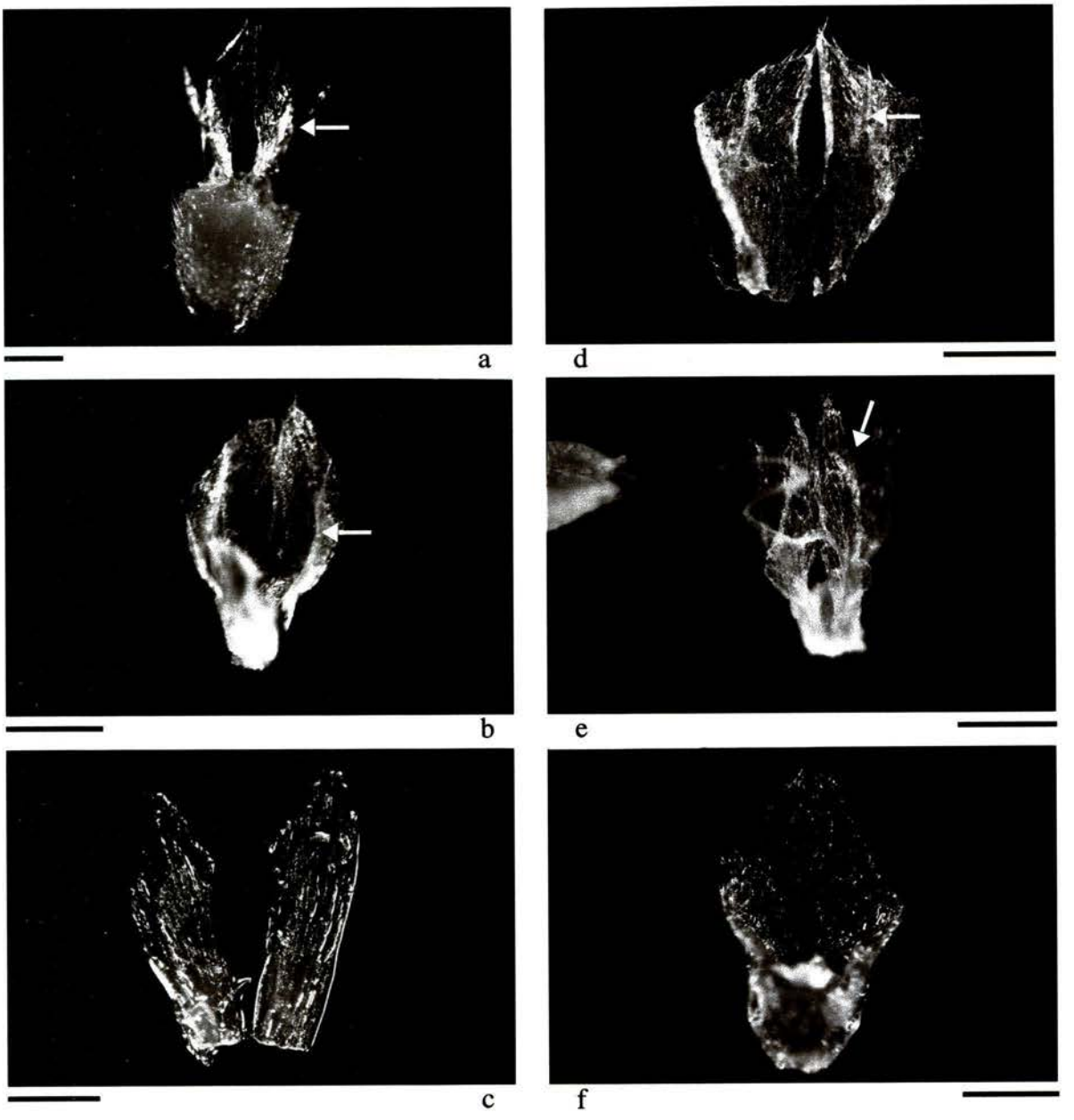
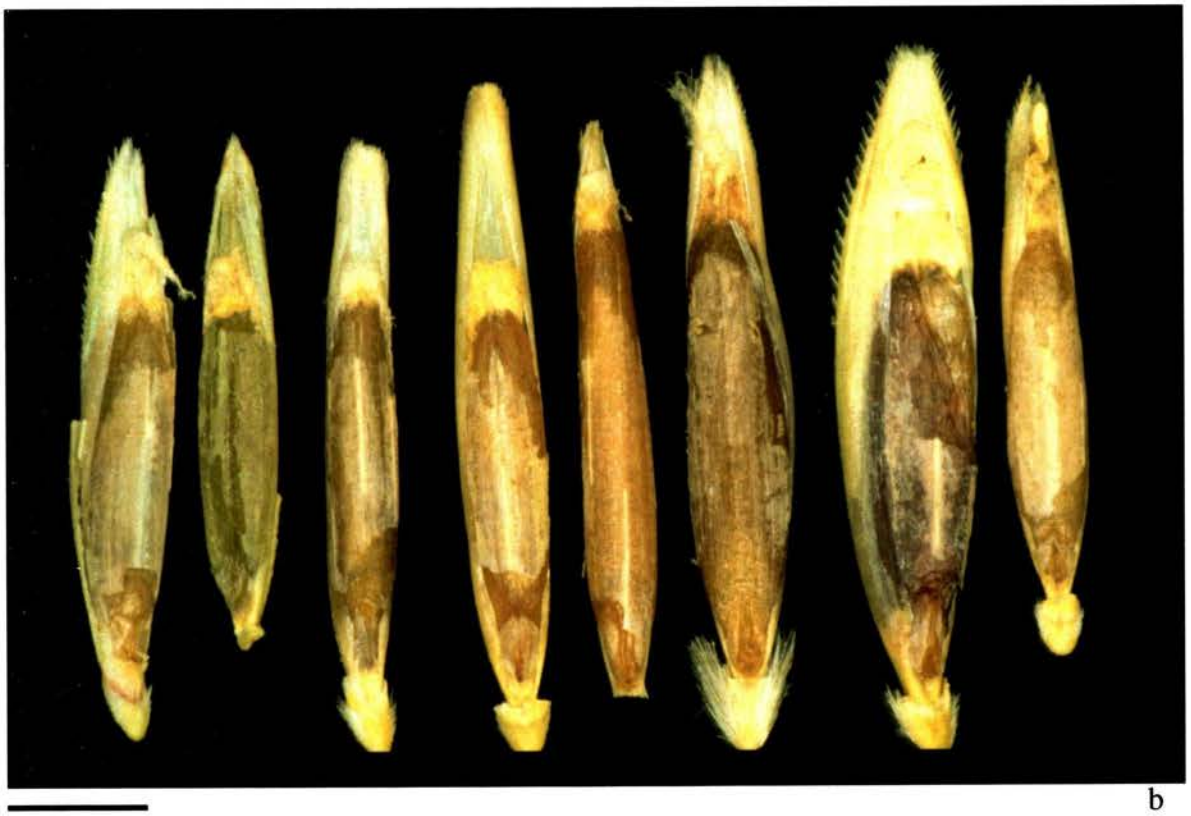

a
d
b
e
c
f

Plate 3.14.

Fruit shapes and the relationship between fruit and palea.

a. Australian *Elymus* species. From left to right: ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95153), ***Elymus rectisetus*** (S. Wang 95001), ***Elymus scaber*** var. ***scaber*** (S. Wang 95128), ***Elymus multiflorus*** (S95163), and ***Elymus sp*** (S. Wang 95141, the short-awned from Queensland). **b.** New Zealand *Elymus* species. From left to right: ***Elymus apricus*** (CHR494196), ***Elymus enysii*** (CHR127516), ***Elymus falcis*** (CHR143004), ***Elymus multiflorus*** (CHR1594), ***Elymus rectisetus*** *sensu* Löve et Connor (CHR387016), ***Elymus sacandros*** (CHR387017), ***Elymus solandri*** (CHR223898), and ***Elymus tenuis*** (R. Mason 10575). **Bar** = 2 mm.

a

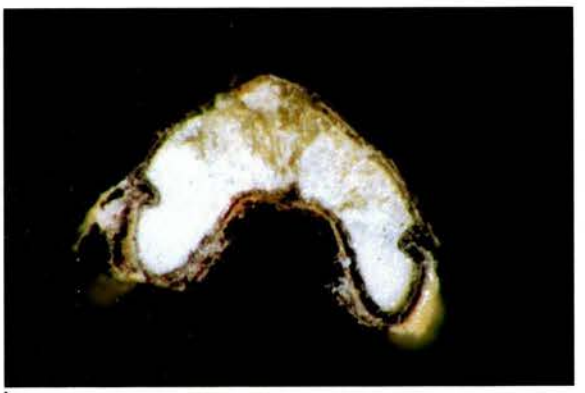


b

Plate 3.15.

Fruit shapes in transverse section.

a. ***Elymus scaber*** var. ***plurinervis*** (S. Wang 95153). Fruit crescent-shaped. **b-e**: Fruit "U"-shaped. **b**. ***Elymus rectisetus*** (S. Wang 95041). c. ***Elymus scaber*** var. ***scaber*** (S. Wang 95104). **d**. ***Elymus multiflorus*** (S. Wang 95163). **e**. ***Elymus*** sp. (S. Wang 95158). **Bar** = 0.5 mm.



a

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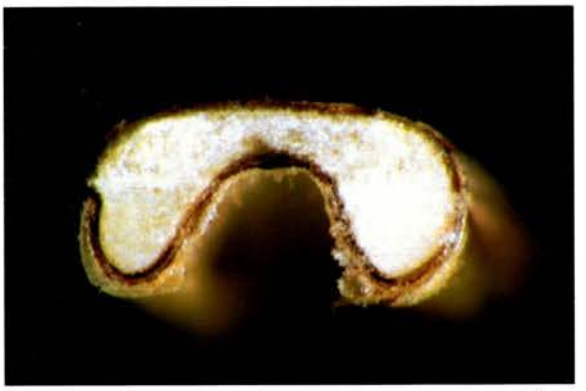

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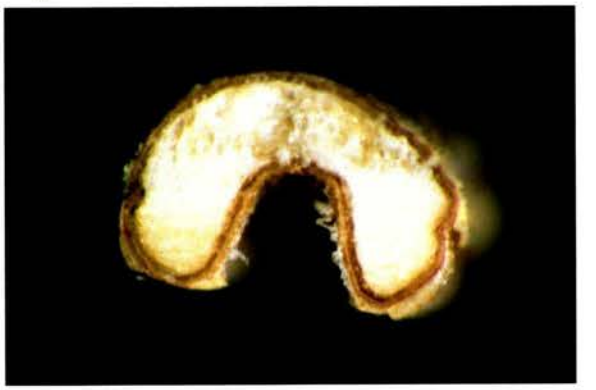


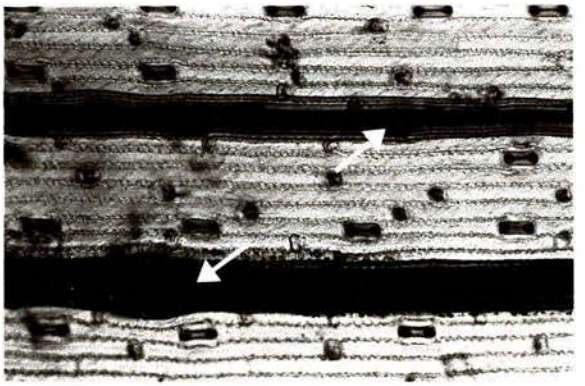
e

c

Plate 3.16.

Abaxial leaf blade epidermis.

a. ***Elymus rectisetus*** (S. Wang 95087). The difference between costal and intercostal zones conspicuous; prickles present over veins (arrow). **b.** ***Elymus apricus*** (P.N. Jonson 1220). The difference between costal and intercostal zones inconspicuous. **c.** ***Elymus sacandros*** (CHR279258). Long-cell walls tessellate; short-cells individual, silica bodies tall and narrow (arrow). **d.** ***Elymus apricus*** (P.N. Jonson 1220). Long-cell walls sinusoid; short-cells in pairs, subsidiary cells parallel-sided (arrow). **e.** ***Elymus multiflorus*** (CHR1595). Long-cell walls straight; short-cells in horizontal row, silica bodies horizontally long and sinuous (closed arrow) or acutely angled (open arrow). **f.** ***Elymus rectisetus*** (B.J. Lepschi 2027). Long-cell walls straight; subsidiary cells low dome-shaped (arrow). **Bar** = 0.1 mm.



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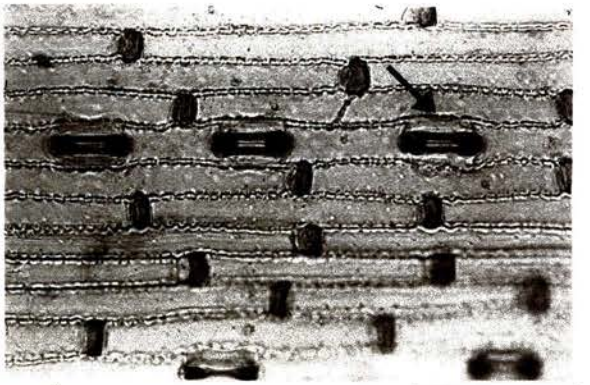

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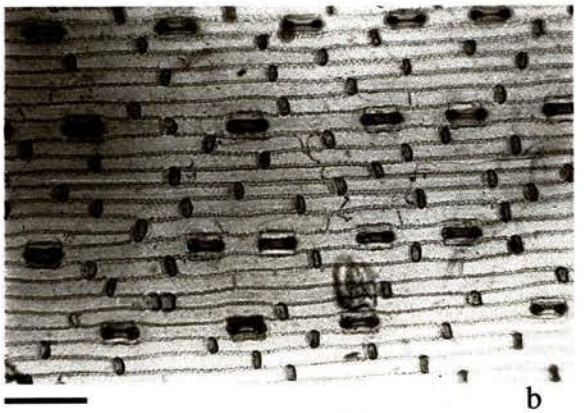

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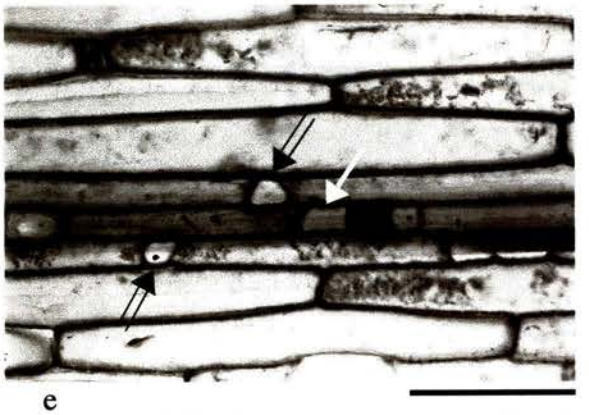

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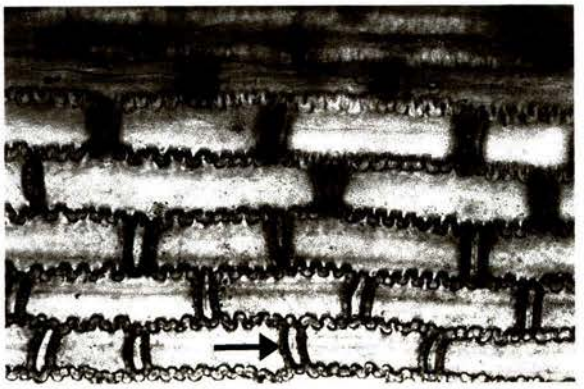

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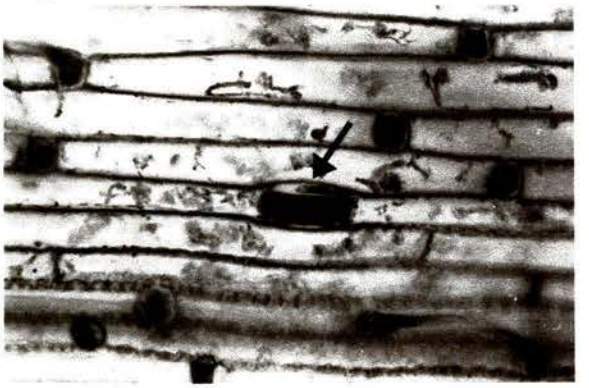
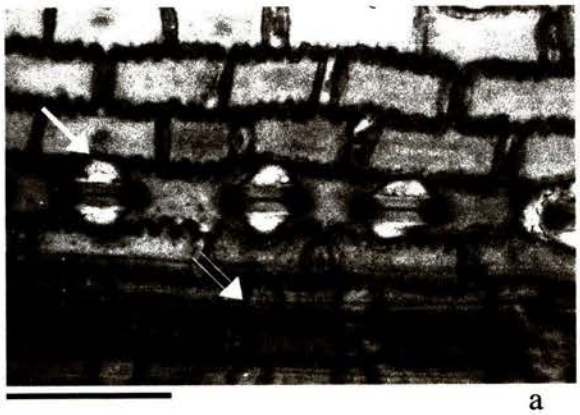


f

Plate 3.17.

Abaxial leaf blade epidermis (continued).

a. ***Elymus falcis*** (CHR402714). Long-cell walls tessellate; subsidiary cells parallel-sided but wider than some other material (closed arrow); papillae present only over veins (open arrow) . **b.** ***Elymus multiflorus*** (CHR1595). Silica body cuboidal (closed arrow), acutely angled (open arrow). **c.** ***Elymus scaber*** var. ***scaber*** (S. Wang 95132). The difference between costal and intercostal zones conspicuous; long-cell walls sinuous; papillae present over veins and on the intercostal region (arrow). **d.** ***Elymus rectisetus*** (S. Wang 95087). Small prickles present over veins and on the intercostal region (arrow). **e.** ***Elymus rectisetus*** (S. Wang 95087). Big prickle present over vein. **f.** ***Elymus rectisetus*** (N.C. Beadle s.n.). Macrohairs present over veins and on the intercostal region (arrow). **Bar** = 0.1 mm.



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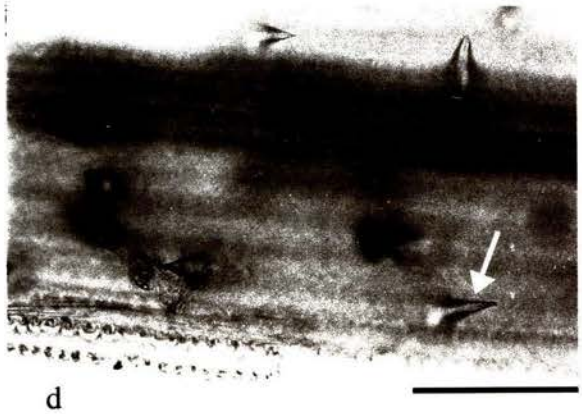

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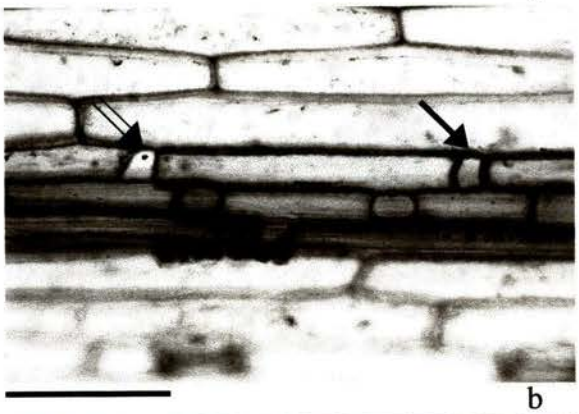

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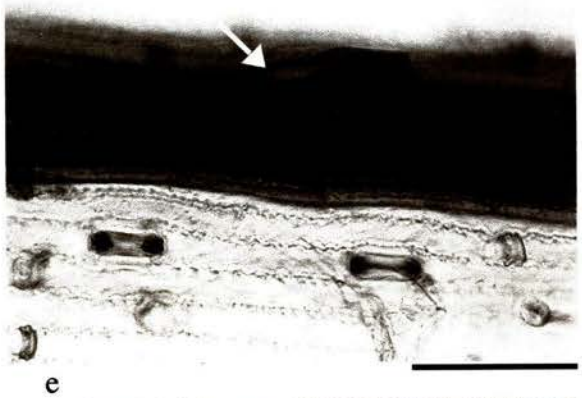

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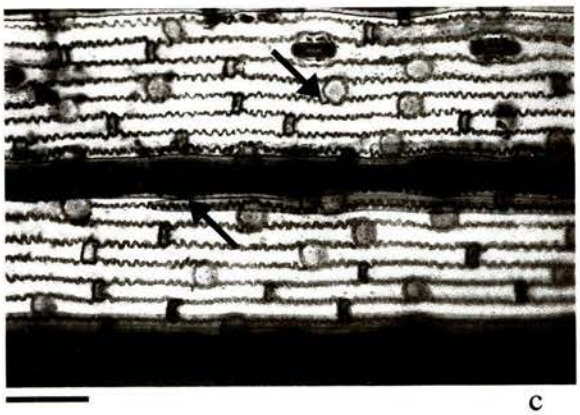

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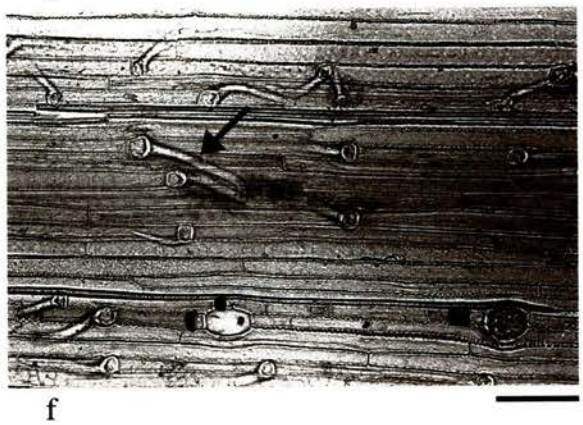


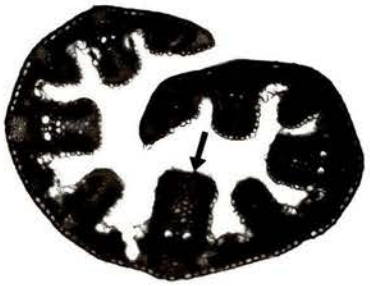
f

Plate 3.18.

Leaf blade transverse sections.

a. ***Elymus scaber*** **var.** ***plurinervis*** (R.J. Fensham 1343). Leaf blade flat, having distinct, prominent adaxial ribs only; adaxial ribs (midrib, arrowed) more or less constant in size. **b.** ***Elymus*** **sp.** (S. Wang 95140, the short-awned from Queensland). Leaf blade having nodular structure. **c.** ***Elymus apricus*** (CHR95426). Leaf blade involute; adaxial ribs very irregular in size, rib tops truncate (arrow); sclerenchyma forming a 'T'. **d.** ***Elymus scaber*** **var.** ***plurinervis*** (R.J. Fensham 1343). Adaxial ribs obtuse; Non-Kranz pattern: irregular chlorenchyma, more than four cells between adjacent sheaths and two bundle sheaths (closed arrow); bulliform cells occuring in simple fan-shaped groups (open arrow). **e.** ***Elymus apricus*** (CHR95426). Abaxial-hypodermal sclerenchyma present (arrow). **Bar** = 0.1 mm.

a



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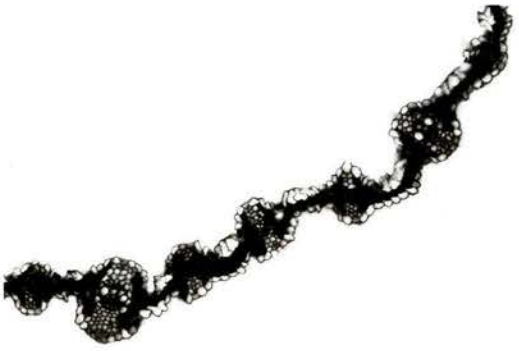

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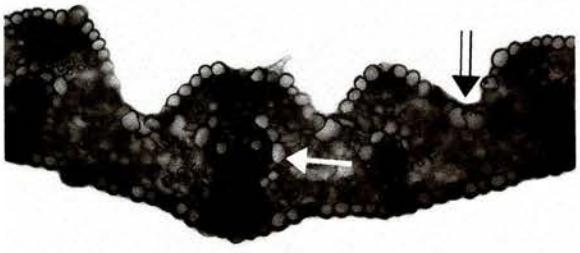

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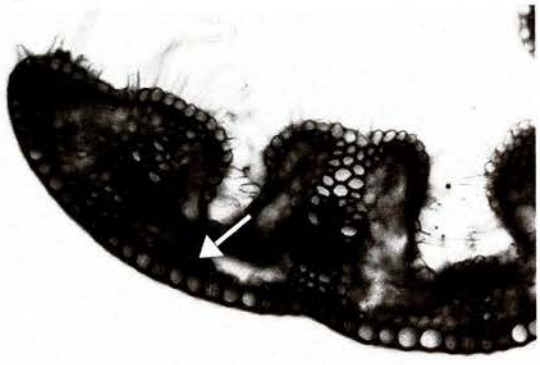


e

Chapter 4.

Classification of the *Elymus scaber* complex from Australia

4.1. Introduction

The morphological diversity encountered in the *Elymus scaber* (= *Agropyron scabrum* (R. Br.) P. Beauv.) species complex has posed a problem to taxonomists for over 100 years (see Chapter 1). Currently three taxa: *E. scaber* var. *scaber*, *E. scaber* var. *plurinervis* and *E. multiflorus* are recognised in Australia (Jacobs and Hastings, 1993; Simon, 1993; Walsh, 1994). However, some workers (Löve and Connor, 1982; Connor, 1994) have suggested that at least four taxa, *E. rectisetus*, *E. scaber* var. *scaber*, *E. scaber* var. *plurinervis*, and *E. multiflorus*, exist in Australia. In accordance with Löve and Connor's view, Murphy and Jones (1999) suggested four major entities based on the phenetic analysis of a small group of samples. Similar names, but probably with different taxonomic implications, have been applied in publications related to this species complex (Chapter 1).

The confusion concerns the number of taxa and the circumscription of each. The creation of a separate species for *E. scaber* var. *plurinervis* has been suggested by Connor (pers. comm.), Murphy and Jones (1999), and Carman and Wang (1992). This view seems to be supported by a crossing experiment (Carman and Wang, 1992; unpublished data).

All past classifications have employed the relative dimensions of glumes and lemmas as the basis for taxonomic circumscription. The character, lemma awn length longer than 15 mm, was used to distinguish *E. scaber* var. *scaber* from *E. multiflorus*; the size and number of glume veins were used to define *E. scaber* var. *plurinervis* (Simon, 1993;

Walsh, 1994). My initial observations indicated, owing to extensive morphological overlap, that it is difficult to use traditional numerical characters such as awn length and glume size to distinguish taxa within this species complex. Given this, a detailed analysis of the morphological affinities within *E. scaber* and its allies seemed desirable.

4.2. Material and methods

Specimen sampling refers to chapter 2. A hundred and two OTUs were selected to conduct phenetic analysis using traditional morphological characters (4.3.1). Subsequently, a sub-sample including 32 OTUs from Australia was chosen from the groups defined in the first analysis to carry out further investigation on more informative characters by scanning electron microscope (3.2.1) and leaf blade anatomy (3.2.2). Phenetic analysis was further conducted on sub-sample by using combined characters (i.e. traditional morphological and those of SEM and anatomy; 4.3.2).

4.2.1. Character selection

All samples were coded according to the Character List (Appendix II), but not all data obtained were suitable for computer analyses. Characters were selected for analyses as follows:

1. Reliable characters — that is, the states of those characters that could be decided unambiguously by only examining the herbarium sheets. For example, character 5 (Plant habit) was observed to be variable within the complex, and was used to distinguish some taxa of this species complex (Connor, 1994; Murphy and Jones, 1999). However, it could not be reliably coded from dried material and therefore was discarded. Some characters (e.g. the width of leaf blades) were possibly influenced by environmental factors such as water availability or sunlight, and were excluded from the analyses. Table 4-1 shows the comparison of leaf width between field-collected and cultivated individuals. Inconsistent characters were also considered not suitable for analyses. For example, anther colour (character 193) was found to be different even

within a inflorescence or within a spikelet, or from time to time (Table 4-2). Auricle hairiness (character 24) was observed on young plants but rarely on mature plants. All these characters were not included in the analyses.

Table 4-1. Comparison of leaf width between field and cultivated material

Sample	Field material		Cultivated material		Average rate of increment	
	Culm leaf (mm)	Flag (mm)	Culm leaf (mm)	Flag (mm)	Culm leaf (%)	Flag (%)
S95038	4.0	2.3	4.5	c. 4.0	12.5	73.9
S95103	1.8	1.5	4.5	3.5	150	133
S95109	2.3	2.5	4.1	3.5	78.3	40
S95115	4.3	3.0	6.1	4.1	41.9	36.7
S95135	c.5.0	nd	5.5	4.5	10	nd
S95139	3.7	3.3	4.2	3.5	13.5	6.1
S95140	3.6	4.2	4.3	4.7	19.4	11.9
S95144	nd	2.3	3.3	2.8	nd	21.7
S95158	3.0	4.5	4.0	4.2	33.3	- 6.7
S95159	2.5	2.2	4.0	2.8	60	18.2

Average rate of increment = Cult. – Field / Field • 100%. nd: no data.

Table 4-2. Anther colour of *Elymus scaber* complex

Sample	S95038	S95103	S95109	S95139	S95140	S95144	S95157	S95159
field	yellow & purple*	yellow & purple	yellow	purple	yellow	yellow	yellow	yellow
cultivated	yellow with purple stripes	yellow & purple	purple	purple	yellow (March) purple (Oct.)	purple & yellow	purple & yellow	yellow

*Yellow and purple anthers were observed within a inflorescence or a spikelet.

2. Characters showing polymorphism — it is worthless to use characters monomorphic among different forms. Examples of these are: the number of lodicules and stamens (character 179 and 191), of which all specimens have the same value; spikelet grouping, (character 58), of which only a few specimens (e.g. *E. multiflorus*) were found to have paired spikelets at the base of inflorescence.

3. Characters without many missing data — for numerical characters (including real and integer numeric), both 'inapplicable' and 'unknown' characters are treated as 'missing data (-99.00)' by the PATN computer programs even though they are basically different. For binary expression of multistate characters, all states of 'inapplicable' characters were scored as '0', whereas 'unknown' characters were scored as 'missing data', thereby expressing the difference between them. As stated above, missing data could influence the results of the analyses, therefore those characters with over 1/3 missing data (especially unknown characters) were deleted from the following analyses.

Characters selected for analyses are listed in Appendix IV.

4.2.2. Data analysis

In the DELTA system, coded data were used directly to get a distance matrix by running DIST. However, the DELTA-formatted data were processed in several ways before running computer programs in PATN system.

- they were transformed into the form of a two dimensional matrix in ASCII format (operating in EXCEL).
- numerical characters and multistate characters were separated into different files so that they could be dealt with in different ways.
- the calculation of the average values on numerical characters (real and integer numeric) was carried out because range values were not accepted by the PATN system, such that for one DELTA-formatted numerical character three new characters were generated: minimum, mean and maximum. Average values were used for all analyses in PATN system.

- multistate characters were re-coded into binary form for the reason stated in Chapter 2 (2.3.2.1.).

A much expanded new data matrix, which had to be kept in three separate files (-.xls), was generated after the initial processing procedure. Each file was separately submitted to PATN and then merged if necessary by running MERG.

Analyses were run on a Pentium PC 486. The steps followed are summarised in Figure 4-1 and Figure 4-2.

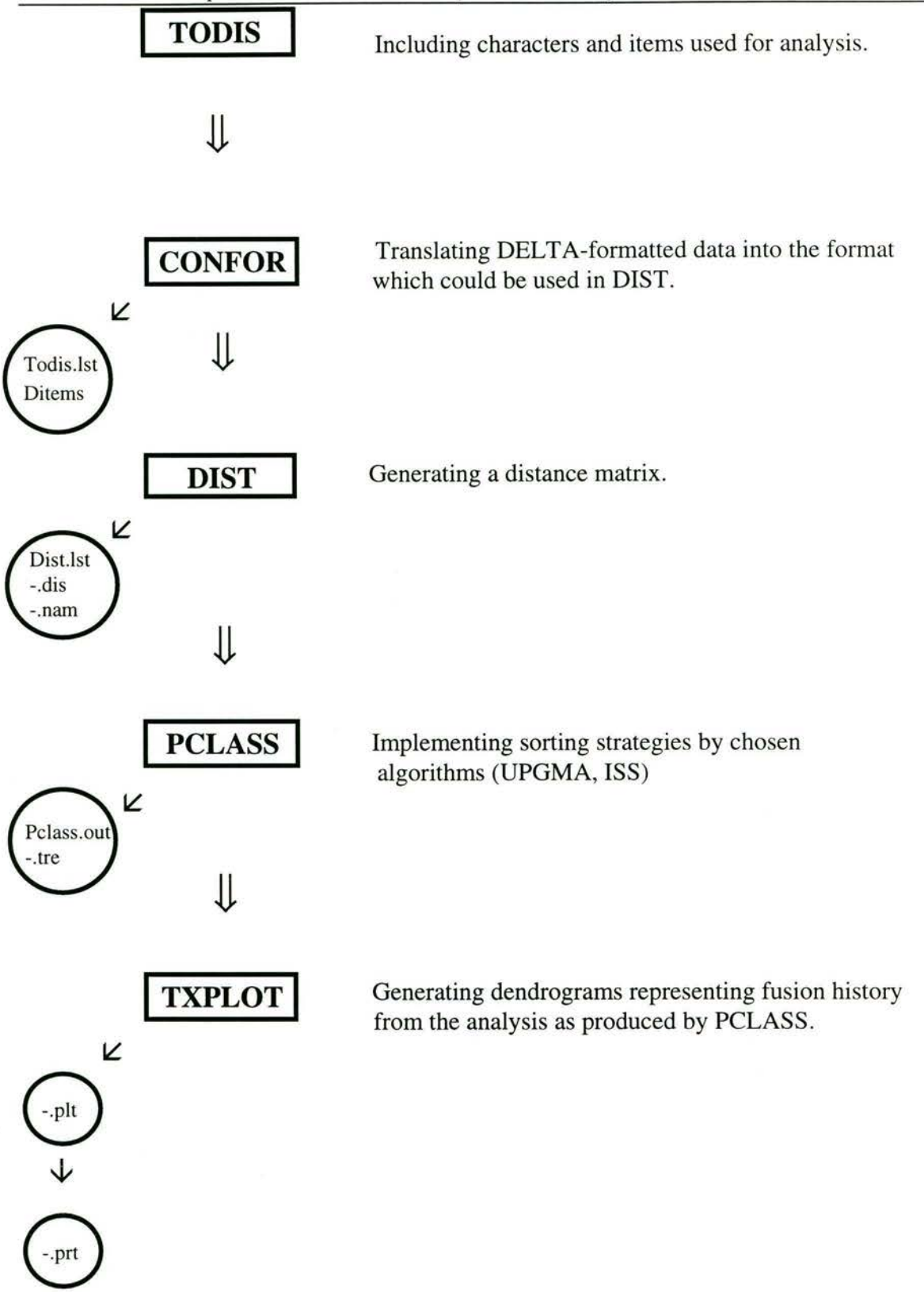


Figure 4-1. Flow diagram illustrating the steps followed in the analyses using the DELTA system. Rectangles denote programs used; circles denote files created.

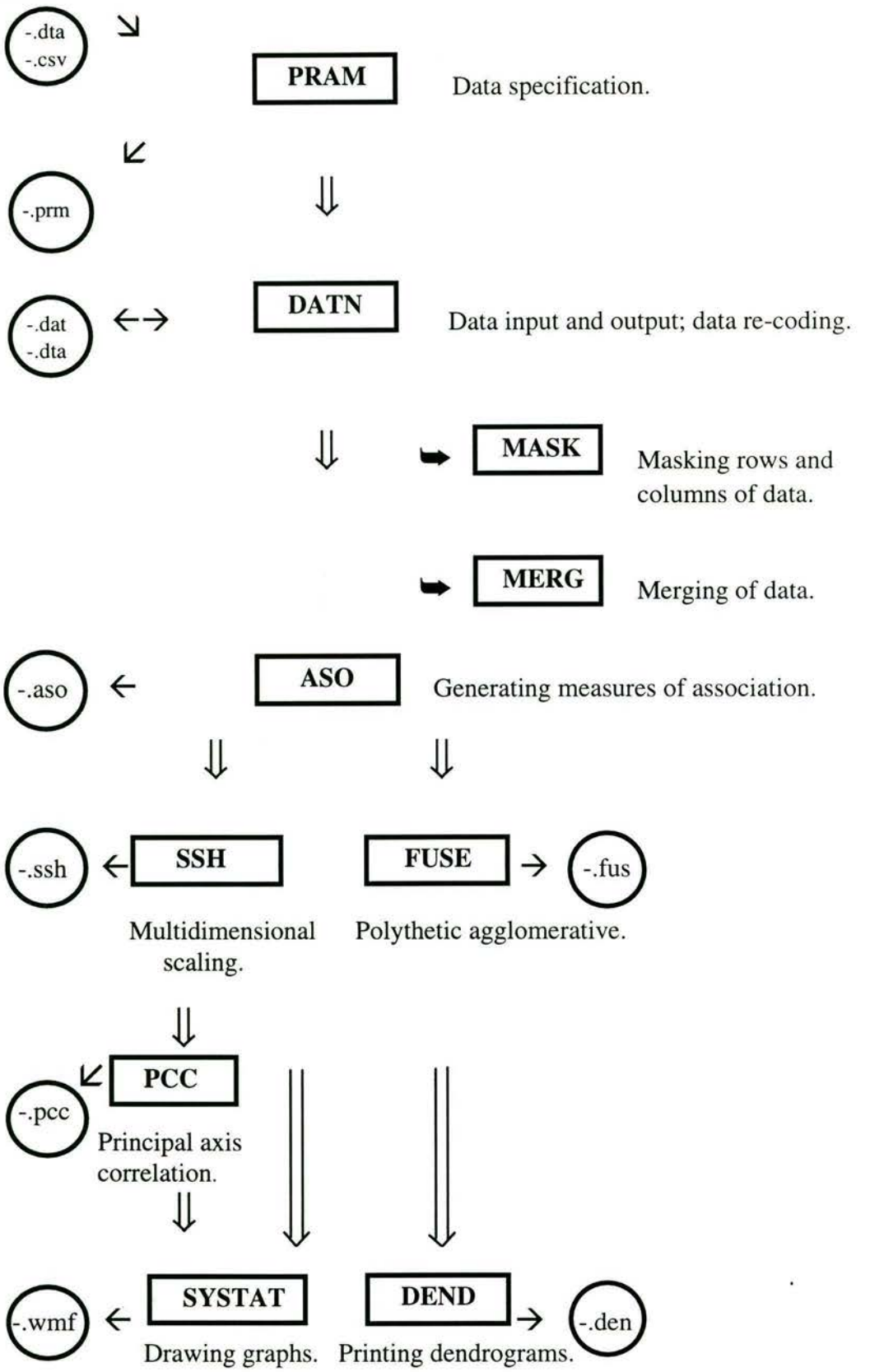


Figure 4-2. Flow diagram illustrating the steps followed in the analysis using the PATN system. Rectangles denote PATN modules; circles denote files created.

4.3. Results

4.3.1. Analysis of 102 OTUs

Twenty eight traditional morphological characters were used in this analysis (Appendix IV). These included multistate characters such as the structure of lemma apices and palea apices, and numerical characters such as the average length of the internodes, spikelet length, the dimensions and nerve number of the glumes, the length of the proximal lemmas, the average length of lemma awns and the length of anthers.

Cluster analysis was generated in both the DELTA and PATN systems, and Semi-Strong-Hybrid multidimensional scaling (SSH) was conducted in the PATN system.

4.3.1.1. DELTA system

Distances were calculated using a modified version of Gower's (1971) similarity coefficient available in the DIST program of the DELTA system (2.3.1.3.).

In order to compare the different results influenced by the different intensities, $\sigma = 0.00$ (UPGMA), $\sigma = 0.25$, $\sigma = 0.50$, and $\sigma = 1.00$ (ISS) were used to produce the dendrograms respectively. In general, four main groups were generated in each performance except $\sigma = 0.00$, in which five groups formed at dissimilarity of 0.21 (a few individuals were detached from Group IV and comprised a small cluster) and Group III was pulled away from others (result not shown). All other dendrograms had very similar constituents to each other with only a few OTUs being placed differently. Figure 4-3 shows the result generated from $\sigma = 0.25$. It indicates that:

1. Four groups comprise the *Elymus scaber* species complex at about 0.5 Gower dissimilarities.

2. Group I consisted of most members of Form C, a few members from Form D (OTU 25, or together with OTU 95 when different σ values were used), and one individual in Form F.

3. Group II encompassed most individuals of Forms B, D and E. Some individuals from other forms also fell into this group. These included OTUs 70 (Form A), 18 and 49 (Form C), 9, 35, 36 and 37 (Form I). This group was subdivided into two small clusters. However, they combined at 0.3 Gower dissimilarities indicating their morphological resemblances. Re-examination of the individuals of the two small clusters showed that they were not worthy to be treated as separate groups based on a comprehensive comparison of characters used in this analysis.

4. All members of Form J, except OTU 4, were included in Group III.

5. Group IV included most individuals of Form H and I, whereas OTUs 9, 35, 36 and 37 were consistently placed together with Group II indicating their dissimilarity to other members of these two short-awned forms. Unexpectedly, some individuals from other forms such as Form B (OTU 88), D (OTU 20), E (OTUs 61 and 66), G (OTUs 32 and 45), and J (OTU 4) were also clustered into this group.

6. Group I was morphologically similar to Group II, whereas Group III and IV were morphologically close to one another.

The individuals that were not associated with the majority of individuals of the same form (e.g. OTUs 9, 35, 36 and 37 away from Group IV, OTU 4 from Group III, and OTUs 61 and 66 from Group II) had intermediate characters. For example, OTU 61 had rather long awns similar to the members of Group II. However, it also had some characters similar to Group IV, such as unequal awn length within a spikelet and short awn of the proximal lemma. These individuals blur the differences between the groups.

4.3.1.2. PATN system

Distance matrices were produced by both Bray and Curtis and Gower Metric routines. The latter, Gower Metric, was finally selected to conduct the whole analysis, because the results generated by this algorithm were considered to be taxonomically meaningful. It confirmed Belbin's suggestion (1995) that Gower Metric is the better choice when the data set contains continuous elements. Both cluster analysis and Semi-Strong-Hybrid multidimensional scaling (SSH) analysis were conducted.

Based on the results obtained from the DELTA system, the same combination of characters (Appendix IV) was used in the present analysis. The difference was that both numerical and multistate characters were not completely the same as those in the DELTA system, but were modified to fulfil the requirement of the PATN system (2.3.2.1).

Cluster analysis

Four groups were found at about 0.40 Gower dissimilarities in this analysis. Each group comprised similar elements as those in the DELTA system (Fig. 4-4) with a few exceptions such as OTU 29 which was clustered into Group II rather than Group IV. This result demonstrated that modified characters did not overly influence the results and that the two results were comparable with each other.

SSH analysis

Figure 4-5 is a scatter plot of the SSH in three dimensions, of which vector 1 vs. vector 2 is illustrated. The stress (0.14) is fair according to Belbin (1995). Generally, Group III is well separated from other groups. Most individuals from Group I are reasonably well defined. The main clusters of Group II and IV are fairly distinct from one another, but are linked by some individuals with intermediate characters (labelled as 'x' in Fig. 4-5). Table 4-3 lists the correlation coefficients, including only those over 0.5, produced by the PCC program.

In general, the results generated from both the DELTA and PATN systems, from both the cluster and SSH analyses, agreed with each other. They indicated that at least four taxa exist in this species complex. However, most of the characters used for the analyses were numerical ones (Table 4-3), and were not suitable for diagnostic purposes, even though some of them showed high correlation values (> 0.5). In considering these characters, such as spikelet length and lemma awn length, all groups except Group III (which was shown to be distinguishable by characters of glumes and lemma apex) could be interpreted as a species with clinal variation. A further investigation was, therefore, carried out using SEM and anatomical techniques.

Table 4-3. Correlation coefficients between OTUs and the fitted characters (102 samples)

Character	Correlation	Character	Correlation	Character	Correlation
53	0.5370	**130**	0.7646	150-2	0.8414
61	0.8337	135-1	0.6121	**152**	0.8222
62	0.6665	135-2	0.5821	**158**	0.7729
72	0.6904	146-1	0.7774	156	0.6700
73	0.5753	146-2	0.7144	157-1	0.5329
86	0.5575	147-1	0.6413	**169**	0.5258
88	0.6833	147-3	0.6239	171-1	0.5667
90	0.7064	148-2	0.5395	171-2	0.7264
91	0.6659	149-2	0.6656	171-3	0.5563
106	0.6182	150-1	0.8424	173-1	0.7046

Numerical characters are in boldface.

4.3.2. Sub-sample

A sub-sample of OTUs was selected from those with mature fruit in each of the groups discerned from cluster analysis and SSH analysis. Two OTUs (29 and 61), which were found to have intermediate characters or were sorted into different groups in the previous analyses, were selected. Two specimens of New Zealand *E. multiflorus* (OTUs 109 and 110) were also included in this analysis. The sampled individuals are listed in Appendix I. Investigations of new characters were conducted on the sub-sample using scanning electron microscope and anatomical techniques (3.2.1 and 3.2.2).

When selecting the characters for this analysis, attention was concentrated on the micro-structures of rachilla, callus and palea inspected by scanning electron microscope, on the ratio of the length and width of fruit, the ratio of fruit length to palea length and also on the anatomical characters of leaf blades. Some of the new characters obtained by SEM were found to be informative and these were used, together with those with high correlation values in previous analyses, to generate a new analysis. However, examination of anatomical preparations of leaf blades indicated that variation occurs between individuals (e.g. the number of long-cell rows on the intercostal regions, the structure of long-cell walls, the existence of papillae and prickles), or that monomorphism was observed (e.g. the structure of the transverse sections; see Chapter 3). Therefore, none of the anatomical characters were used for this analysis. Most traditional characters of which high correlation coefficients were found in the analyses of 102 OTUs (4.3.1) were also included. A total of 37 characters were selected for this analysis (Appendix IV).

Both cluster analysis and SSH multidimensional scaling were generated in PATN system.

Cluster analysis

Figure 4-6 shows the result generated by flexible UPGMA in the PATN system. Five distinct clusters formed (the orders of the groups followed those in Fig. 4-3 and 4-4).

Similar to the analyses of 102 OTUs, all members of Group I and III defined in the previous analyses were placed together respectively. Most members of Group II obtained from the analyses of 102 OTUs were clustered together, whereas two specimens (OTUs 85 and 87) were pulled out. Group IV was split up such that three specimens (OTUs 3, 30 and 45) were detached from the main cluster, and grouped together with the two individuals of *Elymus multiflorus* from New Zealand and formed a new cluster — Group V. The current Group IV contained the remainder and OTUs 85 and 87 from Group II. As to the two OTUs with somewhat intermediate characters, OTU 29 was sorted with Group IV, whereas OTU 61 was clustered together with Group II. Group I and II were closer to one another than to other groups, and they combined at 0.4 of Gower similarity. Group III seemed more similar to Group IV by merging with it at the association value of 0.53 and both were closer to Group V than to Group I and II.

SSH analysis

The clustering of specimens was more evident in the scatter plot in the two dimensions produced from the Gower Metric association measure with a rather low stress (0.11) (Figure 4-7). All members of Group I and III were completely separated from the remainder in the scatter plot. Most elements of Group II were clustered together and formed a distinct cluster close to Group I but distant from Group IV. The specimens from Group V formed a somewhat looser cluster which was fairly well separated from Group III but touched the margin of Group IV showing morphological similarities to it.

Most characters showed high correlation values in this analysis. Those with correlation values more than 0.7 are listed in Table 4-4. Some of them are illustrated in the vector plot superimposed above the scatter plot showing direction of maximum linear correlation between the ordination space and the fitted characters (Figure 4-7).

Table 4-4. Correlation coefficients between OTUs and the characters (sub-sample)

Characters	Correlation	Characters	Correlation	Characters	Correlation
61	0.8699	121	0.9655	156	0.5218
62	0.7404	125	0.9046	157-1	0.6225
72	0.7411	126	0.9232	157-2	0.5623
73	0.7876	**130**	0.7417	**158**	0.7972
86	0.5586	146-1	0.8805	**159**	0.5877
88	0.8051	146-2	0.8126	**169**	0.5876
90	0.7604	147-2	0.5448	171-2	0.6073
91	0.8148	148-2	0.6646	175-1	0.7033
106	0.7982	149-1	0.5623	**176**	0.8785
111	0.8451	149-2	0.6646	**205**	0.7428
114-1	0.7980	149-3	0.6225	**206**	0.8056
114-2	0.8581	150-1	0.5759	comb-1	0.8451
114-3	0.7468	150-2	0.7444	comb-2	0.7669
120	0.9387	**152**	0.8899	comb-3	0.9387

Boldface: Numerical characters.
comb-1-3: Indicate a combined character including characters 117, 119 and 122 in the Character List. comb-1: callus narrowly triangular, rounded at back, tip not thickened; comb-2: callus triangular, dorsal surface flat or sunken, tip thickened; comb-3: callus broadly triangular, dorsal surface flat or sunken, tip not thickened. When dealing with natural language descriptions, this character was split into three characters.

4.4. Discussion

4.4.1. Summaries of five groups

The analysis of 102 OTUs using morphological data demonstrated that at least four groups exist in the *Elymus scaber* complex. Furthermore, the analysis of a sub-sample of OTUs using extra characters obtained by SEM revealed five distinct groups. The introduction of two OTUs of *E. multiflorus* from New Zealand to the analysis and the inclusion of the characters of SEM may help the separation of Group IV and V. Those characters with high correlation values (Table 4-4) show their diagnostic power and

contribute to distinguishing these groups. Subsequently, these characters were summarised for each group using INTKEY. The summaries are shown in Table 4-5.

It was noticed that both numerical and multistate characters played important roles in differentiating all the groups. The multistate characters reflect the polymorphism of rachillas (characters 111 and 114), calluses (characters 117, 119-122, 125-126), lemma awns (characters 149 and 150) and lemma apices (146-148) within the *Elymus scaber* complex. The important numerical characters include the length of spikelets and lemma awns (characters 61, 62, 152, and 158), the dimensions of glumes (characters 72, 73, 90, and 91), the number of glume nerves (characters 88 and 106), the structure of palea apices (character 176) and fruit (characters 205 and 206). Some numerical characters are graphically presented in Figures 4-8 and 4-9 which display clearly the differences between the groups.

These characters as indicated above were used to re-examine those OTUs which were not included in the sub-sample. For most OTUs (about 80%), the results obtained from the first analysis using traditional morphological characters (4.3.1) were confirmed, with the exception that individuals of the previous Group IV (4.3.1) were sorted into the current Group IV and V (4.3.2) based on their morphological characters and geographical distribution. The remainder had somewhat intermediate characters and will be discussed in the following section (4.4.2). Based on the above summaries, five groups are generally described as follows.

Group I and II are distinct from Group IV and V by long spikelets (Figure 4-8. a) and long- or intermediate-awned lemmas (Figure 4-8. d). Although these characters overlap with those of Group III, the latter is distinguishable from Group I and II by a set of characters of glumes and lemma apices (see the following discussion). The individuals of Group I and II are possibly the representatives of the so called long-awned apomicts defined by other authors (see Chapter 1). They are separated, however, as two distinct groups in all analyses in this study.

The characters which are diagnostic for Group I are: long-hairy rachilla, elliptical apices of rachilla, narrowly triangular callus (abaxial) with hairs restricted to the margins, usually lorate and canaliculate apices of lemmas. Whereas Group II has characters of triangular callus (abaxial) with hairs on margins and dorsal surface, thickened tip of callus (abaxial), and usually acuminate and flat apices of lemmas. Several numerical characters are also distinct between the two groups. For example, Group I has longer palea tip (above flanks; Figure 4-8. c) and lower ratio of fruit length to palea length (Figure 4-9. f) than Group II.

Examination of the type of *Elymus rectisetus* (*Vulpia rectiseta* Nees in Lehm., W.A., L. Preiss 1819; holo: MEL) indicted that it possesses the same characters of callus, rachilla, lemma awn and palea as those of Group I. Therefore, Group I should be named as *Elymus rectisetus*.

Only a photograph of the type of *E. scaber* var. *scaber* (= *Triticum scabrum* R. Br. based on Labillardière's specimen; holo: FI) was obtained and examined because the loan of types is not allowed by FI. Moreover, the request for photographs of rachilla and callus was also rejected. The type specimen of *E. scaber* var. *scaber*, by the photograph, was shown to have rather short awns, and the ratio of awn length to lemma length was probably less than 2:1 (the exact value cannot be obtained owing to the way in which this character was determined in this study; see Chapter 3). This was also noticed by Connor (pers. comm.) and Murphy and Jones (1999). Nevertheless, the decision that Group II should be designated by the name of *E. scaber* var. *scaber* has been made based on the following consideration:

- The type was collected from Hobart, Tasmania (Labillardière, 1793).
- Tasmanian specimens including those from Hobart (OTUs 75 and 76) examined mostly fell into Group II, only a few from coastal areas were grouped into *E. multiflorus* (OTU 40).
- Specimens with rather short awns were observed in some individuals (e.g. OTUs 9, 72,73, 75 and 93), and a wide variation of lemma awn length was revealed in Group II (Figure 4-8. d).

Group III was consistently shown to be markedly distinct from others. The characters which are important for distinguishing this group include: semi-circular to transverse-circular apices of rachilla, lemma awn entered by one vein, widely triangular and glabrous callus (abaxial). In addition, a set of numerical characters are also diagnostic for this group, such as the dimensions of glumes as traditionally described (Figure 4-9. a, b, c and d), wide membranous margins of lemmas and low ratio of fruit length to palea length (Figure 4-9. f). As for the number of veins of glumes which was used for diagnostic purposes by Vickery (1951) and later authors, the number of veins of the upper glumes (up to eight) are confirmed to be informative (Figure 4-9. e) though it overlaps somewhat with Group V, whereas that of the lower glumes are completely overlapped by the individuals of Group V. The members of Group III have long been treated as a variety of *Elymus scaber*. However, the results obtained in this study indicated that this taxon is probably worthy of recognition at species rank, concurred with other authors (Connor, pers. comm.; Murphy and Jones, 1999; Carman and Wang., 1992).

Group IV and Group V represent the so called "short-awned" *Elymus* species in Australia. Both are distinct from other groups by short spikelets and lemma awns (Figure 4-8. a, d and e). Moreover, the characters of shortly hairy rachillas, broadly triangular and glabrous callus (abaxial) are also useful for distinguishing the members of the two groups from the individuals of Group I and II. According to some workers, all members of these two groups should be interpreted as *Elymus multiflorus* by the lemma awn length, namely awn < 15 mm long (Simon, 1990, 1993) or awn length approximately equal to lemma length (Connor, 1994). In contrast to the previous taxonomic concepts, nevertheless, the results of this study indicated that morphological distinctions can be seen between Group IV and V. For example, the members of Group V have shorter spikelets and lemma awns (Figure 4-8. a and d), lower ratio of awn length to lemma length (Figure 4-8. f) than those of Group IV, and usually have bifid lemma apices whereas Group IV often has entire lemma apices. From an ecological point of view, individuals of Group V are usually found in the sandy soil of coastal areas, whereas the specimens of Group IV are restricted to the black heavy soil of southeastern areas of Queensland or to northern New South Wales (Figure 4-7).

Observation on the transplants revealed that the elements of Group V could not grow well under the cultivated condition in Sydney area — they changed their habit (from erect to ascending), and some individuals could not set seed normally. However, the members of Group IV, especially those from southeastern Queensland, grew well and set abundant seed. Based on the above observation and the current analyses, they are preferably to be treated as separate taxa.

In consideration of the similarities to New Zealand *E. multiflorus* (OTUs 109 and 110), Group V should be accorded the name *Elymus multiflorus*. This was confirmed by examining the type (*Elymus multiflorus*, = *Triticum multiflorum* Hook. f.; lecto: BM). However, Group IV is an as yet un-named taxon.

4.4.2. Uncertain OTUs

There are some OTUs with more or less intermediate characters. Their taxonomic status is uncertain. The examples are given as follows:

1. Specimens similar to both *E. rectisetus* and *E. scaber* var. *scaber*. OTUs 79 and 82 have characters typical of *E. rectisetus* such as hairy callus and the hairs restricted to margins, hairy rachilla and hairs dense and long and covering callus base. However, their palea tips are characteristic of *E. scaber* var. *scaber* — the length of the palea tip above the flanks is only 0-0.3 mm long which falls into the range of *E. scaber* var. *scaber*.

2. Specimens resembling *E. scaber* var. *scaber* and the short-awned entities. There are several characters useful for distinguishing *E. scaber* var. *scaber* from the short-awned entities, namely *E. multiflorus* and the new entity (see Table 4-5). However, some OTUs exhibit ambiguity between these two categories.

OTU 8 (Shepparton area of Victoria) has short spikelets (24–30 mm) and short lemma awns (3–10.5 mm). It was grouped into Form I and consistently clustered into Group IV (Figure 4-3 and 4-4). However, its calluses are more or less hairy on the surface and its

appearance is more like *E. scaber* var. *scaber* instead of the short-awned entities. Walsh (1994) claimed that *E. multiflorus* was distributed on the alluvial loams in the Shepparton - Numurkah area of Victoria. This specimen is probably one of these representatives.

OTUs 20, 22, 23, 25, 32, 66, 85, 87, 88 and 90 are from either Victoria or New South Wales. Considering the dimensions of their spikelets (24-65 mm) and lemma awns (11-29 mm), they should be placed together with the remainder of *E. scaber* var. *scaber*. However, their rachillas are short-hairy and calluses are glabrous to glabrescent, which suggests that they are morphologically similar to the short-awned entities. These specimens presumably contribute to the polymorphism of the widely distributed and extremely diverse taxon *E. scaber* var. *scaber*.

3. Specimens placed between *E. scaber* var. *scaber* and var. *plurinervis*. Two OTUs (4 and 45) were revealed being similar either to *E. scaber* var. *scaber* or to another variety of this species. The lower and upper glumes of both individuals are more than 8 mm long and 7-8 veined, which are distinct characters of *E. scaber* var. *plurinervis*. However, their lemma awns are entered by three veins instead of one vein, which suggests their differentiation from this taxon. Nevertheless, OTU 4 was consistently pulled away from the main cluster of *E. scaber* var. *plurinervis* but placed in Group IV (Figure 4-3 and 4-4) and OTU 45 was clustered into Group IV (Figure 4-3 and 4-4) or into Group V (Figure 4-6). This probably resulted from their possession of some characters such as the unequal length of lemma awns, the bifid proximal lemma apex and the short proximal lemma awns (0–2.5 mm).

4. OTUs 10 and 11 were constantly placed in Group IV (4.3.1). However, their peculiar appearances cast doubt upon the positions related to the short-awned entities, suggesting that they are probably anomalous members of *E. scaber* var. *scaber*.

4.5. Conclusions

Based on the current analyses of morphological characters, preliminary conclusions about the taxonomy of the *Elymus scaber* complex are presented as follows. Five taxa are morphologically distinguishable from one another: *E. rectisetus* (Group I), *E. scaber* var. *scaber* (Group II), *E. scaber* var. *plurinervis* (Group III), *E. multiflorus* (Group V), and a new entity comprising the short-awned form from Queensland, New South Wales and possibly Victoria. Moreover, *Elymus scaber* var. *plurinervis* is worthy of being raised to species level. There are some individuals that have intermediate characters as discussed above. These individuals, especially those resembling both *E. scaber* var. *scaber* and the short-awned entities (Group IV and Group V), blur the distinction between the taxa.

A further investigation using molecular techniques (RAPD), therefore, was undertaken to test the above results obtained by using morphological characters. Moreover, it was hoped to resolve the taxonomic position of some individuals with more or less intermediate morphological characters.

A final taxonomic conclusion will be given in Chapter 7.

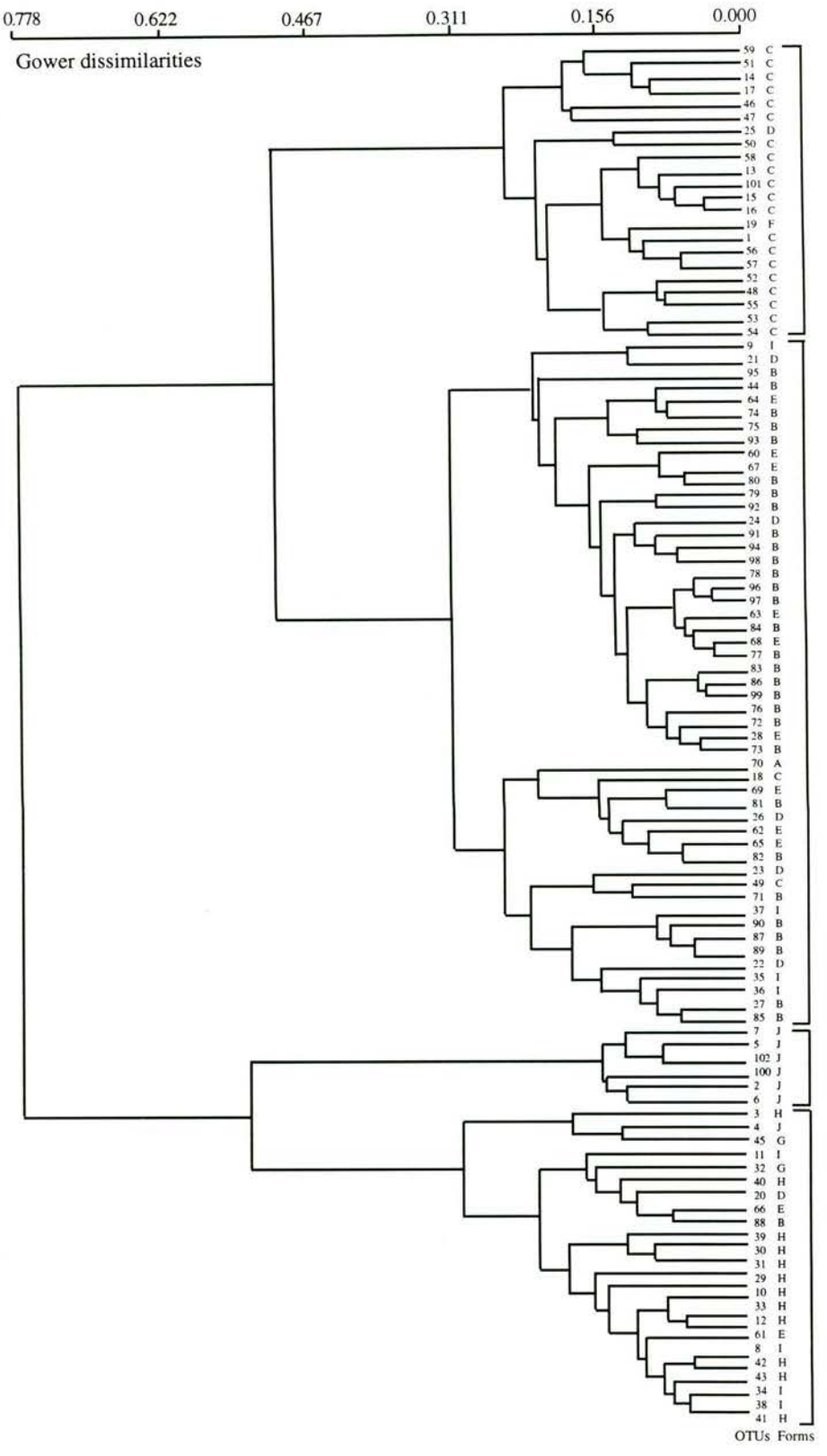


Figure 4-3. ISS-flexible analysis ($\sigma = 0.25$) of the 102 OTUs, using 38 selected classification characters (Appendix IV). To facilitate discussion, the main groups are referred to as I, II, III and IV.

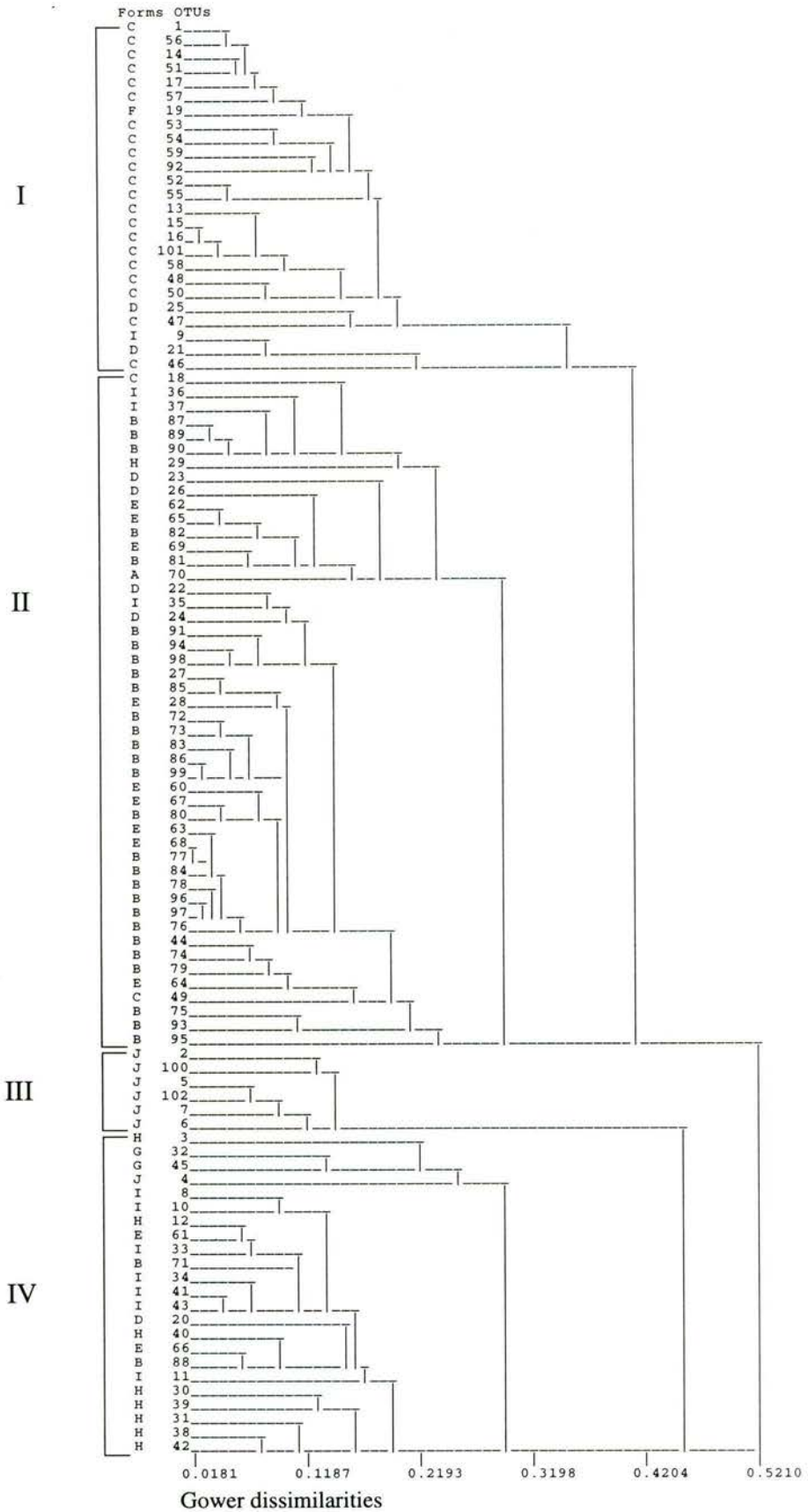


Figure 4-4. Dendrogram of 102 OTUs produced from UPGMA cluster analysis using Gower Metric association measure.

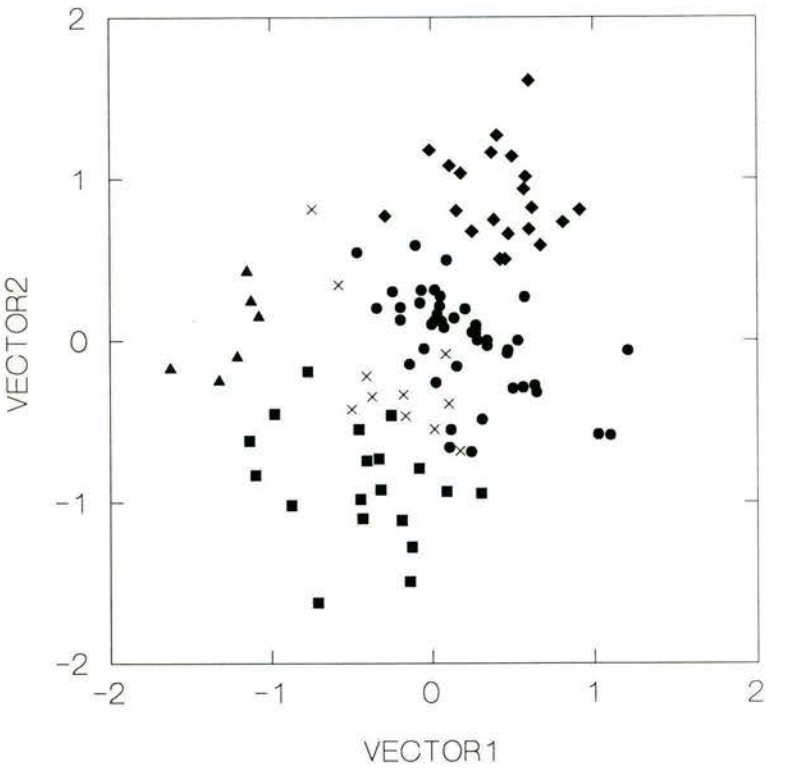


Figure 4-5. Scatter plot of SSH of 102 OTUs in three dimensions: vector 1 vs. vector 2. Stress = 0.14. Group I (◆); Group II (●); Group III (▲); Group IV (■); Individuals with intermediate characters (×).

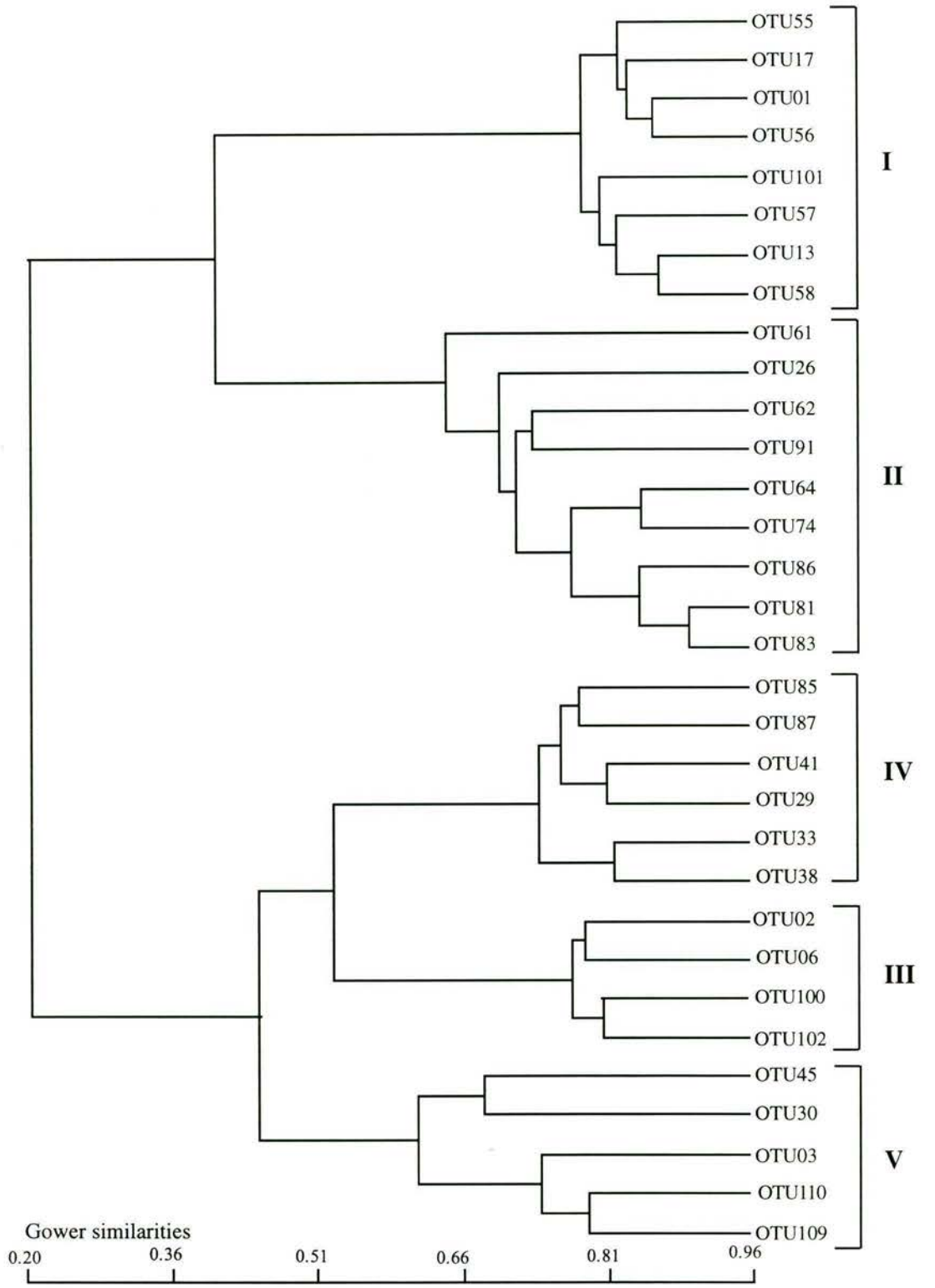


Figure 4-6. Dendrogram of sub-sample produced from UPGMA cluster analysis using Gower Metric association measure.

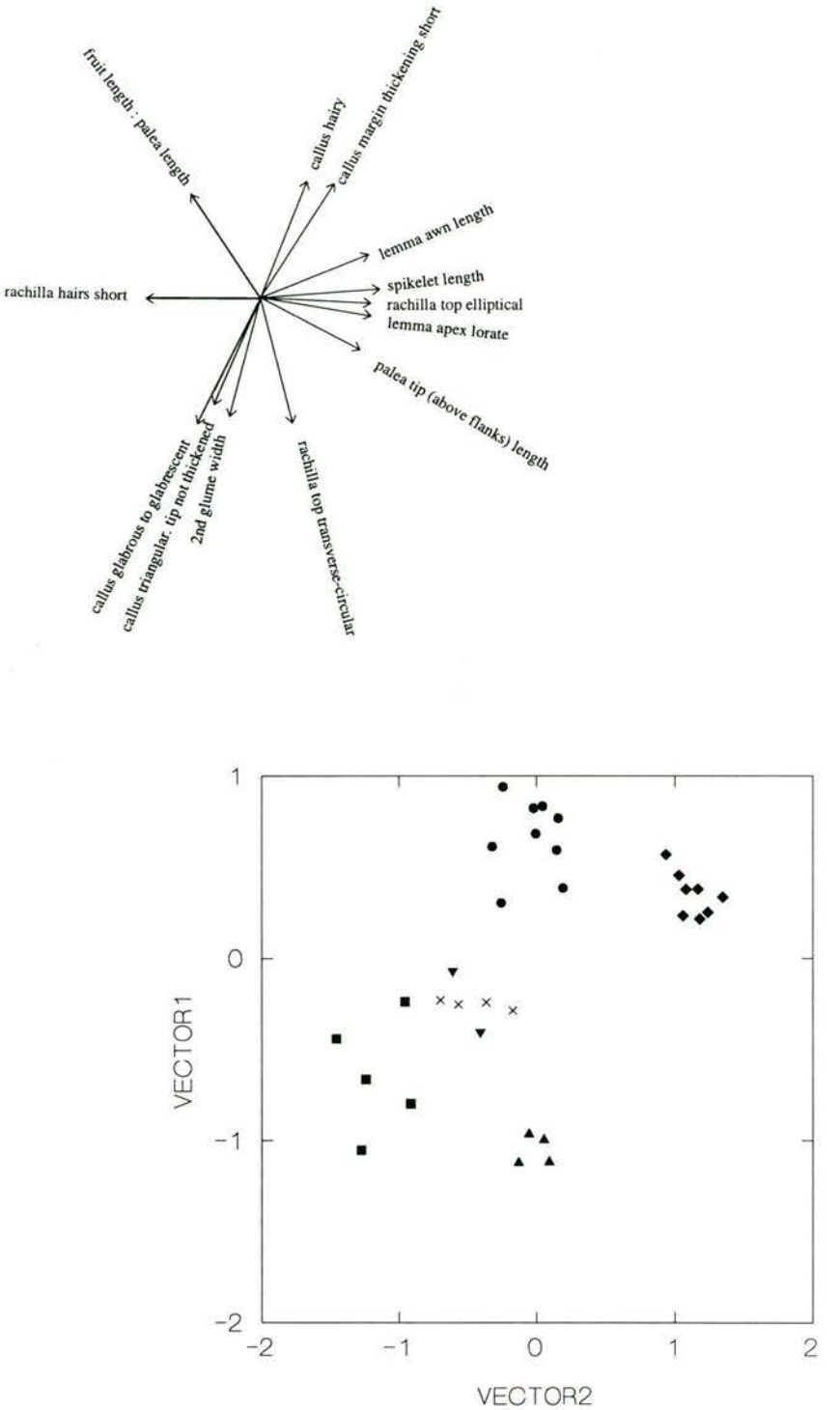


Figure 4-7. Scatter plot of SSH (sub-sample) in two dimensions using Gower Metric association measure. Stress = 0.11. Group I (◆); Group II (●); Group III (▲); Group IV (▼: from SE Queensland; ×: from New South Wales); Group V (■). A vector plot showing direction of maximum linear correlation (values > 0.7) between the ordination space and the fitted characters is superimposed above the plot.

Table 4-5. Summary of characters of groups*

Numerical characters

Character	Group I		Group II		Group III		Group IV		Group V	
	mean	range	mean	range	mean	range	mean	range	mean	range
61	**61.77**	38-101	44.12	28-56	37.12	25-47	**30**	18-36	**21.37**	10-29
62	**3.8**	2.72-5.6	2.55	1.3-4.4	**1.55**	1.3-2.09	**1.19**	0.5-2.8	**1.23**	1.09-1.43
72	3.9	1.8-6.1	4.11	1.9-7	**9.85**	**8-11**	4.87	4.1-6.1	4.96	3.5-7
73	0.44	0.3-0.5	0.71	0.4-1	**1.48**	**1.3-1.7**	**0.97**	**0.9-1.1**	**0.92**	**0.5-1.23**
86	0.26	0.1-0.4	0.33	0.1-0.5	**0.74**	**0.4-0.9**	0.38	0.3-0.5	0.31	0.23-0.4
88	2.75	2-3(-4)	3.5	3-4	**5.4**	**5-6**	4.67	4(-7)	**5.13**	**4-6(-7)**
90	5.14	3.6-6.8	5.16	2-7.6	**11.52**	**10.6-12.5**	5.78	4.7-7	5.91	4-8
91	0.5	0.4-0.6	0.73	0.5-1	**1.3**	**1-1.5**	0.88	0.8-1	**1.02**	**0.64-1.2**
106	3.3	(2-)3(-5)	4.38	(3-) 4(-6)	**6.5**	**5-8**	**5.83**	**5-7**	**5.75**	**5(-7)**
130	20.43	10-40	12.36	8.7-17	13.25	12-14.5	**8.73**	**7-9.6**	**8.31**	**6-11**
152	32.25	26-40	25.31	15-36.5	21.5	17-26.5	**12.3**	**5-22**	**1.68**	**0-7**
158	23.81	11-31	21.81	2-33	15.93	8.5-21	**8.2**	**1.3-18**	**0.25**	**0-1**
159	1.63	0.9-2.6	2.19	1.5-3.6	1.72	1.5-2.0	1.41	0.9-2.4	**0.21**	**0-0.6**
169	0.26	0.2-0.3	0.25	0.2-0.4	**0.15**	**0.1-0.3**	**0.19**	**0.1-0.3**	0.22	0-0.55
176	**0.45**	(0-)1	0.07	0-0.36	0.3	0.1-0.64	0.15	0-0.36	0.05	0-0.18
205	0.20	0.15-0.24	0.19	0.15-0.26	**0.27**	**0.22-0.32**	0.19	0.14-0.29	0.23	0.2-0.28
206	**0.59**	**0.52-0.68**	0.72	0.63-0.81	**0.51**	**0.47-0.55**	0.71	0.61-0.79	0.64	0.61-0.71

Multistate characters

Character	Group I	Group II	Group III	Group IV	Group V
111	**1**	2	2	2	2
114	**1**(7), 2(1)	**2**	**3**	**2**	**2**
120	**2**	**2**	**1**	**1**	**1**
121	**1**	**2**	-	-	-
125	**1**	**1**	**2**	**2**	**2**
126	**1**	**1**	**2**	**2**	**2**
146	**1**(7),2	2	2	2	2
147	1(5), 2(4)	2	2/3	2	1,2(1)
148	1	1	1	1	**1/2**
149	1	1	1	1	**1-2(-3)**
150	**1**	**1**	**1/2**	**1/2/3**	**2**
156	1	1	**2**	1	1
157	3	3	3	3	**1/2/3**
171	1	1/2	1/2	1/2/3	1/2/3
175	1(1),2	1/2	2	1/2	1/2
comb.	**1**	**2**	**3**	**3**	**3**

* Boldface indicates marked differences between groups.

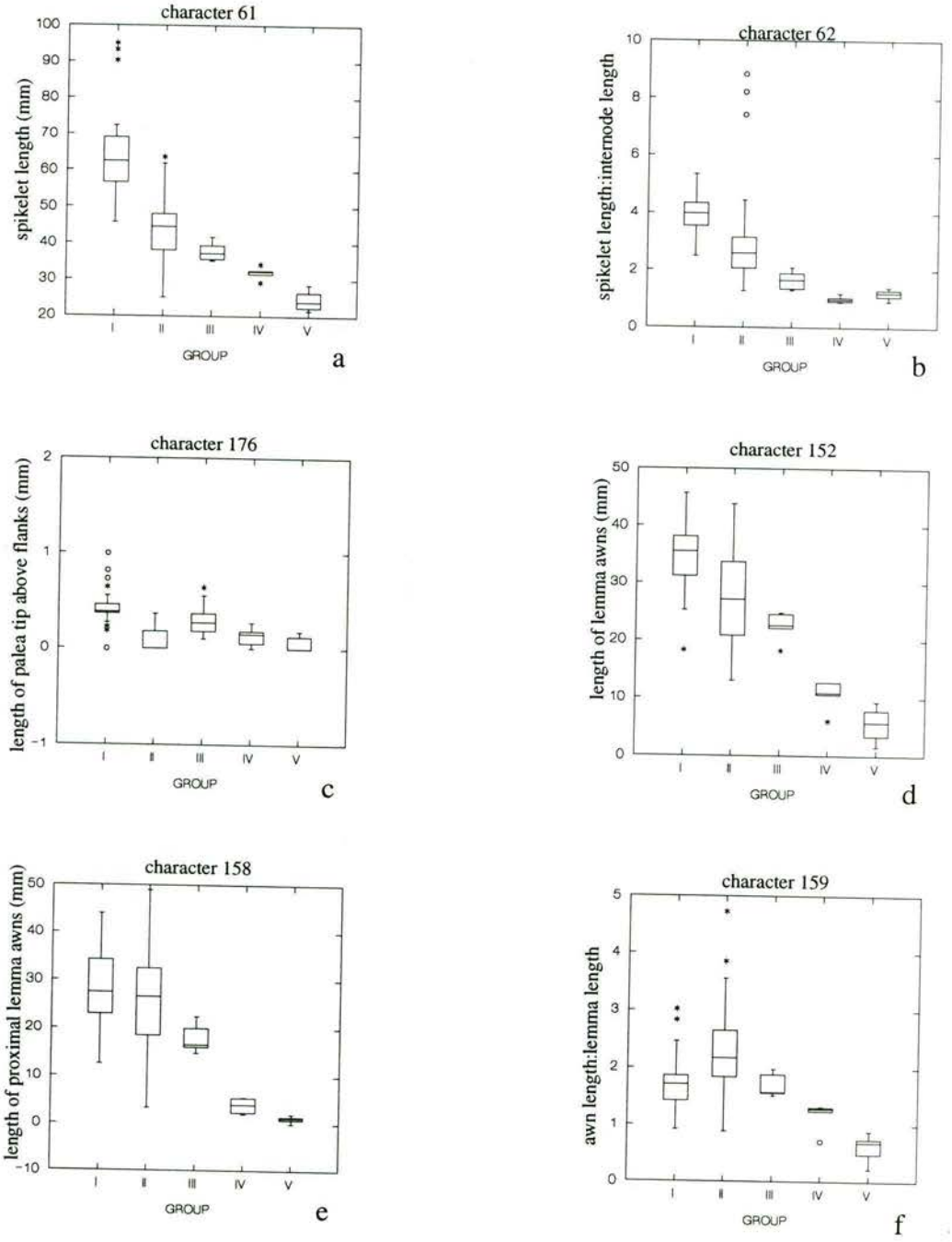


Figure 4-8. Boxplots of numerical characters. The samples of each group are represented as a box which is divided at the median. The top and bottom of the box are drawn at the upper and lower quartiles. The whiskers indicate the range between the largest and the smallest observation within 1.5 interquartile ranges. Values outside the whiskers are plotted as '*' (< 3 interquartile ranges) or 'o' (> 3 interquartile ranges) (Sokal & Rohlf, 1995).

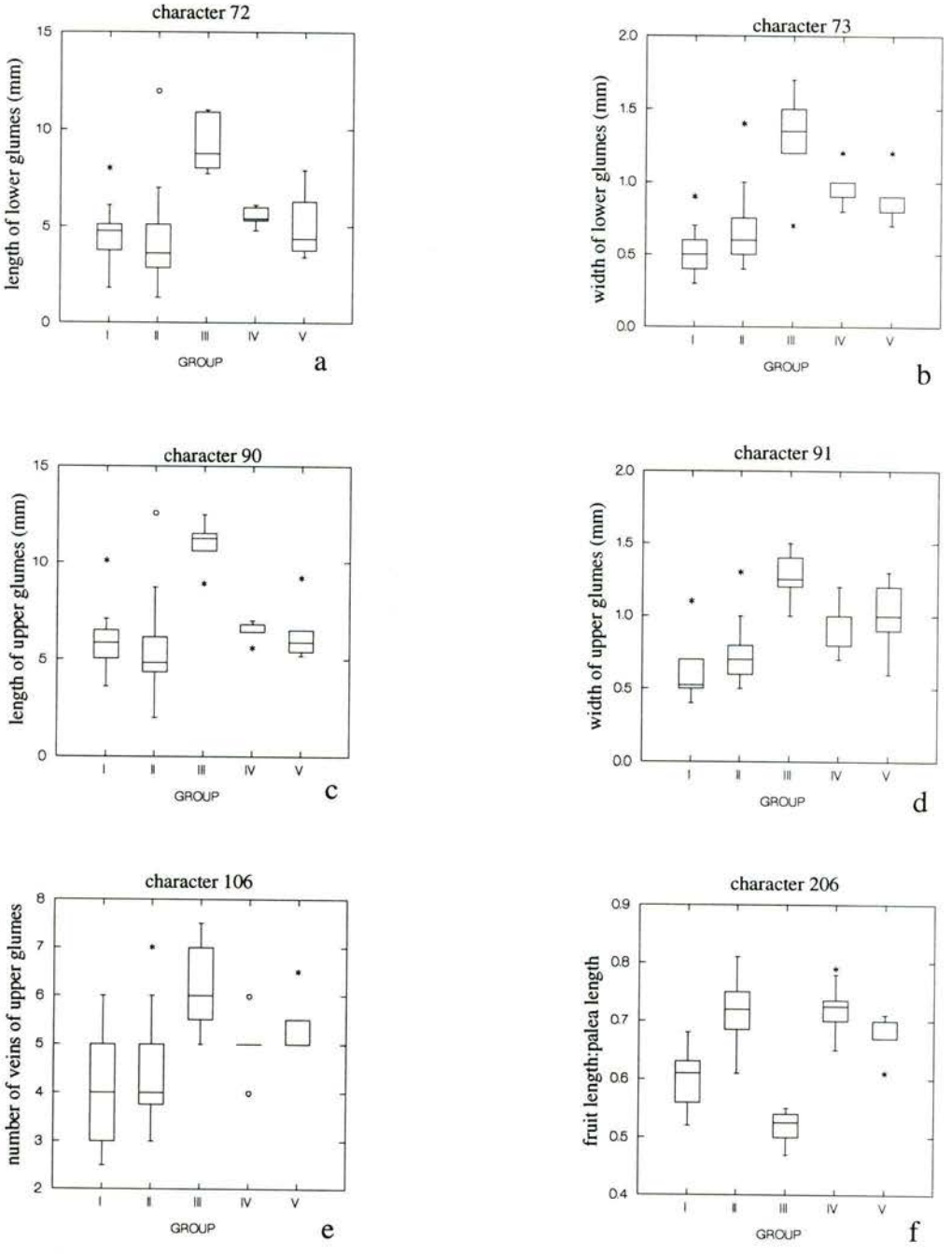


Figure 4-9. Boxplots of numerical characters. For interpretation of the plots refer to Figure 4-8.

Chapter 5.

Research on the relationships between Australian and New Zealand *Elymus* species

5.1. Introduction

Löve and Connor (1982) pointed out, based on their biosystematic investigation of the *Elymus* (or *Agropyron*) species in New Zealand, that "the name *Agropyron scabrum* has been used by New Zealand botanists for over 100 years for widespread and well known plants, but *Agropyron scabrum* (R. Br.) P. Beauv. based on the Labillardière specimens, does not occur in New Zealand. It is an Australian taxon, and is undoubtedly part of the agamospermous complex found there. The web of nomenclature surrounding it is not relevant to New Zealand plants." Eight taxa of *Elymus* are currently recognised in New Zealand (Connor, 1994). The long-recognised species *A. enysii* (= *E. enysii*), *A. tenue* (= *E. tenuis*), and *A. multiflorum* (= *E. multiflorus*) are found to be cytogenetically sound (Löve and Connor, 1982); four taxa, namely *E. apricus*, *E. falcis*, *E. sacandros* and *E. solandri* were split from the collective hexaploid taxon *Agropyron scabrum* (Chapter 1), and the remainder was called *E. rectisetus*. All taxa except *E. rectisetus* were considered indigenous elements, whereas *E. rectisetus* was thought to be an introduction from Australia — probably only 130–140 years ago (Löve and Connor, 1982). In addition, the synonymisation of Australian *E. multiflorus* with New Zealand material is uncertain. In order to confirm the synonymisation of Australian *E. rectisetus* and *E. multiflorus* with New Zealand material, and to inspect if the new characters developed in this study were also valuable for New Zealand taxa, a phenetic analysis was conduct on a mixed sample including both Australian and New Zealand specimens using the same data set (sub-sample) as that for Australian *Elymus* species.

5.2. Material and methods

Twenty four OTUs were selected from 109 herbarium specimens (Chapter 2). Material for the investigation of SEM and leaf-blade anatomy were prepared in the same way as presented in Chapter 3.

5.2.1. Character selection

Character selection was based on the data set (sub-sample) used for analysis of Australian material (see Chapter 4). Some morphological characters are restricted to New Zealand elements or were used for diagnostic purposes (Connor, 1994). The examples are: auricles size (long, minute or absent), the shape and hairiness of leaf-blades (flat or involute; glabrous or coarsely hirsute), inflorescence position (erect, nodding, prostrate or trailing to the ground), spikelet position to rachis (appressed to or widely divergent from the rachis), awn length of glumes, rachilla apex (horizontal and expanded or not), callus (adaxially raised or not), lemma texture and palea apex (truncate or pointed; entire, retuse or bifid). Most of these characters were selected for analysis. However, the hairiness of leaf-blades, which was considered to be variable even within a group and to be possibly influenced by environmental factors, was not included. Moreover, Connor (1994) used the dimensions and the colours of anthers to distinguish some of New Zealand species. Nevertheless, the dimensions of the anthers showed very low correlation coefficients in the analyses of Australian samples (data not shown), and the colours of anthers were found to differ even within a spikelet. These characters were not employed in the analysis.

A total of 51 characters (20 numerical plus 31 binary and multistate characters) were used in this analysis and they are given in Appendix IV.

5.2.2. Data analysis

The PATN computer program package was used to conduct both cluster analysis and ordination analysis. Similar to the previous analyses, average values of numerical

characters were calculated, and binary and multistate characters were coded directly as binary form. The Gower metric association measure was selected to calculate similarities, and followed by the Flexible UPGMA routine for constructing dendrograms. Multidimensional scaling was conducted by the SSH program and a 2-dimensional scatter plot was produced. In order to understand the contributions of characters to the ordination space the program Principal Axis Correlation (PCC) was also conducted.

5.3. Results

Cluster analysis

Figure 5-1 shows the clustering result generated by the flexible UPGMA. Seven groups were formed at the Gower dissimilarity value of 0.30. Group I included all members of Australian *E. rectisetus.* Group II encompassed all Australian *E. scaber* var. *scaber* plus New Zealand '*E. rectisetus*'. Group III was a mixed cluster comprising several small clusters, such as *E. solandri, E. apricus* (two OTUs) and *E. tenuis* (two OTUs together with an element of *E. apricus*). A mixed cluster consisting of one of the New Zealand *E. multiflorus* (OTU 111), Australian *E. multiflorus* and other short-awned samples formed Group IV. *Elymus scaber* var. *plurinervis*, two elements of New Zealand *E. multiflorus* and *E. enysii* were separated into Group V, VI and VII respectively.

Elymus enysii was morphologically distinct from all other *Elymus* species by connecting with others at the dissimilarity of 0.70. Being dissimilar to the cluster analysis of Australian material (Figure 4-6), *E. scaber* var. *scaber* was here revealed to be closer to *E. multiflorus*, *E. scaber* var. *plurinervis* and the short-awned forms than to Australian *E. rectisetus* by the combination of the first four clusters occurring before the incorporation of *E. rectisetus*. Five New Zealand species: *E. apricus*, *E. falcis*, *E. sacandros*, *E. solandri* and *E. tenuis*, formed a mixed cluster and joined *E. scaber* var. *scaber* at the dissimilarity level of 0.36, indicating that the complex of these five species was morphologically similar to Australian *E. scaber* var. *scaber*.

SSH analysis

Figure 5-2 is a scatter plot of SSH in two dimensions using the Gower Metric association measure. A stress of 0.15 was reached. *Elymus enysii* was placed far away from the others. *Elymus rectisetus* and *E. scaber* var. *plurinervis* were also well defined. Individuals of *E. multiflorus* from both Australia and New Zealand with one exception (OTU 111, labelled as mn) formed a loose cluster and touched the mixed cluster of the short-awned samples, indicating their morphological similarities. Australian *E. scaber* var. *scaber* together with New Zealand '*E. rectisetus*' formed a reasonably well defined cluster which was placed between the Australian short-awned samples and the remainder of New Zealand samples. The latter comprised *E. apricus*, *E. falcis, E. sacandros*, *E. solandri* and *E. tenuis*, forming a loose cluster.

The vector plot superimposed above the scatter plot indicates that the characters (correlation coefficients > 0.7) contributing to the division of *E. enysii* from others included raised adaxial callus, non-thickening callus margin, horizontal and sharply expanded rachilla apex, and chartaceous lemma. The important characters pulling most New Zealand samples (except *E. multiflorus* and "*E. rectisetus*") away from Australian ones were the pointed and bifid palea apices and the absence of auricles. It was obvious that *E. plurinervis* and the short-awned entities (e.g. *E. multiflorus*, *E.* sp. nov. and the short-awned form) have characters of glabrous or glabrescent callus, short spikelets and lemma awns, wider glumes, and long marginal thickening of callus (adaxial), which were distinct from the remainder. Characters with correlation coefficients more than 0.7 are shown in Table 5-1.

Table 5-1. Correlation coefficients between OTUs and the characters

Characters	Correlation	Characters	Correlation	Characters	Correlation
20	0.7903	118-2	0.7183	126-2	0.7912
61	0.7966	123-1	0.7183	128-1	0.7183
73	0.7785	123-2	0.7183	128-2	0.7183
88	0.7075	124-1	0.7183	**152**	0.7900
91	0.7182	124-2	0.7183	156-1	0.7102
112-1	0.7183	125-1	0.8361	172-2	0.8177
112-2	0.7183	125-2	0.8597	173-3	0.8177
113-1	0.7183	126-1	0.7912	**176**	0.7188

Boldface = numerical characters.

5.4. Discussion

The classificatory history of New Zealand *Elymus* species was presented in Chapter 1. Eight species of *Elymus* from New Zealand are currently recognised (Connor, 1994). It should be noted that no infraspecific categories were used and that all taxa of *Elymus* in New Zealand were accorded species rank.

It was claimed that the taxonomic treatment of these species was related to their biosystematic status (Connor, 1956). All these species were considered morphologically distinguishable. Some of them have their own ecological requirements (e.g. *E. multiflorus* is a predominantly coastal and lowland species, *E. tenuis* is largely restricted to damp or very shaded sites, and *E. enysii* is confined to high altitudes in inland localities in Canterbury province). Hybridisation was rare in nature even though spontaneous hybrids were not uncommon under cultivated conditions. Sympatric distribution occurred among most of these species e.g. both '*E. rectisetus*' and *E. solandri* were widespread and sympatric in New Zealand, the geographic distribution of

both species overlaps with *E. falcis*, *E. apricus*, *E. tenuis*, *E. enysii* and *E. multiflorus*, and *Elymus tenuis* was found together with *E. enysii* in some localities (see Connor, 1954). The mechanism keeping these species morphologically distinct was explained as an intrinsic barrier for apomicts, and predominant autogamy or, in some cases, the differences of flowering time for the sexual species (Connor, 1954; Löve and Connor, 1982).

The distinctness of *E. enysii* was upheld in the current analysis. It was placed away from all other species in both cluster and SSH analyses. Besides some characters, such as small inflorescence and few-flowered shining spikelets (Plate 5.1. a) emphasised by Connor, a group of features were revealed specific to this taxon: sharply expanded rachilla apex (Plate 5.1. e), much shorter callus than others (Plate 5.1. f), and adaxially raised callus (Plate 5.1. d). New information concerning the genome constitution obtained from molecular studies (Svitashev *et al.* , 1996) confirmed the presence of the H genome but rejected the S genome in *E. enysii.* All this casts doubt over the inclusion of this species in *Elymus.*

Elymus scaber var. *plurinervis* was separated completely from all other species which confirmed its endemic status in Australia. The distinctive characters of this taxon were discussed in detail in Chapter 4.

Elymus multiflorus is another sound New Zealand species which, together with two Australian elements from coastal areas, was fairly well defined in the scatter plot. However, one member of this species from New Zealand (OTU 111) was placed together with Australian short-awned forms (Group IV) probably due to its entire lemma apex and longer lemma awns (14.5–20 mm compared with 5–22 mm of Group IV) than other two New Zealand OTUs (0–0.95 mm). This specimen may represent *E. multiflorus* var. *longisetus* (a variety established by Hackel in Cheeseman, 1906) which was reduced to synonymy with *E. multiflorus* by Connor (1994). The reason why Connor did so was that Hackel's variety with awns 5–6 mm long is encompassed by the lectotype (Connor, 1994). Furthermore, it was found that both short-awned and conspicuously long-awned plants co-existed, and that the plants raised from seed had

shorter awns than the parents, which indicated that the character may not be directly heritable (Connor, 1994). The arguments may stand for New Zealand material but may not for Australian short-awned forms from inland areas. In consideration of morphological and ecological differences known to date between *E. multiflorus* and the members of Group IV from Australia (4.4.1), it is preferred to keep them as separate taxa. Moreover, evidence from RAPD data (Chapter 6) also support this treatment.

The cluster of Australian *E. rectisetus* was apparent in both dendrogram and scatter plot. The Gower dissimilarity at which *E. rectisetus* connected with others was relatively high (0.59), which indicated its morphological distinctness from all other species. The characters that contribute to this result are: relatively longer spikelets and lemma awn, long and densely hairy rachilla, narrowly triangular callus with hairs restricted to margins, lorate and canaliculate lemma apices, distinct structure of palea apices and rather high ratio of palea length to fruit length.

Three OTUs of '*E. rectisetus*' from New Zealand were grouped with Australian *E. scaber* var. *scaber* instead of *E. rectisetus*. This indicated that the so called *E. rectisetus*, widely distributed in New Zealand, actually equates to *E. scaber* var. *scaber* found in Australia mostly from New South Wales down to Tasmania and rarely to Western Australia. Some arguments might be raised here. For example, the currently defined Australian *E. scaber* var. *scaber* may be named as *E. rectisetus* by some authors (Connor, pers. comm.), because its awn is quite long (average value 25.31 mm) and the ratio of awn to lemma is rather high (1.5–3.6, average 2.19), whereas the awn of the type specimen of *E. scaber* var. *scaber* is rather short. If this argument is tenable, then the currently defined Australian *E. rectisetus* should be included too, because it has even longer awns (average 32.25 mm) than *E. scaber* var. *scaber*. This deduction agrees with some current authors that the long- to intermediate-awned samples be treated as *E. rectisetus* (see Chapter 1). In this case, nothing could be designated by the name of *E. scaber* var. *scaber*. Though some authors might take the members of Group IV as *E. scaber* var. *scaber* (Table 1-5), this is rejected based on the following consideration: no specimens from Tasmania were found to be similar to the short-awned forms from New South Wales and Queensland, whereas Connor (1994) would interpret the Queensland

specimens to be *E. multiflorus*. Moreover, this research revealed that awn length should not be used alone for diagnostic purpose in this species complex (see Chapter 4), which concurred with other workers (Connor, 1954). Finally, in the present study, a set of characters including those obtained by SEM (see Chapter 3 and 4) separated the notorious mixture of long-awned to intermediate-awned forms into two distinct groups: Group I - *E. rectisetus*, and Group II - *E. scaber* var. *scaber*. The consideration for such treatments has been detailed in Chapter 4 (4.4.1). This result has also been supported by the molecular data (see Chapter 6).

Given this, Connor (1954) correctly accepted *Anthosachne scabra* (= *Agropyron scabrum*; *Elymus scaber*) for New Zealand specimens, but did not explicitly reject *Anthosachne australasica*. Nevertheless, Connor (1954) indicated the impossibility to divide these two species by Nevski's (1934) characters (awn length and curvature), and by this, he hinted at the synonymy of *A. australasica* with *A. scabra*. The former was reduced to synonymy with *E. rectisetus* (Löve and Connor, 1982; Löve, 1984).

The remainder of the New Zealand *Elymus* species: *E. apricus*, *E. falcis*, *E. sacandros*, *E. solandri* and *E. tenuis,* were not resolved in this analysis. Although some small clusters can be seen (e.g. *E. apricus* and *E. solandri* in Figure 5-1; *E. tenuis* in Figure 5-2), they form a single heterogeneous cluster away from, but touching, the margin of *E. scaber* var. *scaber* cluster. This result probably indicates the morphological similarities within these New Zealand species and their closer relationships to Australian *E. scaber* var. *scaber*. The characters of triangular and hairy callus, hairy rachilla, acuminate lemma apices, and rather long awns shared by Australian *E. scaber* var. *scaber* and these New Zealand species, confirmed their relationships. Nevertheless, differences between New Zealand material and Australian specimens were also observed and examples are: pointed and bifid palea apices were not uncommon in New Zealand specimens but were rare in Australian ones (though truncate and retuse palea apices were also found in some New Zealand individuals e.g. *E. apricus* (CHR95426), *E. falcis* (CHR143004), and *E. solandri* (CHR223898)). The hairiness of calluses of New Zealand elements was more variable than that of Australian ones (Chapter 3, Plate 3.4. f), and auricles were observed to be mostly minute (10 out of 15 specimens) or absent

(14 out of 15 specimens) even though only *E. falcis* and *E. sacandros* were described with minute or absent auricles (Connor, 1994). Based on the assumption that the New Zealand wheatgrasses originated from boreal ancestors by their dispersal from Asia via Australia (Löve and Connor, 1982), it is possible that these morphological differences arose after their arrival in New Zealand, and some morphotypes also evolved in the new environments. However, these morphotypes may not be distinct enough to be revealed by phenetic analysis at the present time. Moreover, if autogamy is predominant among these morphotypes (Connor, 1954; Löve and Connor, 1982), they can maintain themselves as a constant pure line by self-fertilisation (Stebbins, 1957).

There is, of course, an alternative interpretation of this result. The inconsistency between biosystematic and phenetic data exhibited in this study may also indicate some of the weaknesses of phenetic analyses. For example, some characters are important for diagnostic purpose but are hard to score, therefore their taxonomic value could be reduced even to nil. It is noted that *E. tenuis* is a rather distinct taxon on account of its greatly elongating flowering culms, awned glumes and different genome constitution (8x) from all other taxa. However, the first character cannot be scored by only examining herbarium specimens and was not scored in this research. In addition, genome constitution was not included in the phenetic analysis. Some combination of these might be responsible for the lack of resolution of *E. tenuis*.

Elymus species from New Zealand were reported (Connor, 1994) as hexaploid (*E. apricus*, *E. solandri*, *E. falcis*, *E. multiflorus*, "*E. rectisetus*"), tetraploid (*E. enysii*) and octoploid (*E. tenuis*). However, the genomic constitution of *E. sacandros* was not mentioned by Connor (1994). Genetically, *E. enysii* and *E. tenuis* are distantly related to the others. Löve and Connor (1982) believed that although the austral elements of *Elymus* have similar genome constitutions to boreal ones (SH, SSH and SSHH), they have also gained significant chromosomal differences, and that the distinctness probably arose after their arrival in the regions, or the tetraploid and hexaploid ancestors obtained variations before their migration southward. Nevertheless, if the new genome formula SYW (instead of SSH) put forward by Torabinejad *et al.* (1987) and Torabinejad and Mueller (1993a) is accepted, the origin of Australian and New Zealand wheatgrasses

would be different from the above hypothesis. The hypotheses raised by Crane (unpublished in Ryan, 1987) could provide alternative possibilities: *Roegneria* (with SY genomic composition according to Baum *et al.*, 1991) and ancestral *Australopyrum* entered Australia and there formed amphiploids to yield hexaploid *Elymus* species, or at least three diploid species were introduced into Australia, with assembly there of the remaining 4x and 6x species. However, this explanation can only accommodate the Australian and New Zealand hexaploids, but cannot account for the tetraploid *E. enysii* and the octoploid *E. tenuis* (Connor, 1994). The octoploid *E. tenuis* was considered to be a young endemic that evolved in New Zealand from the tetraploid *A. enysii* by simple autoalloploidy (Löve and Connor, 1982), whereas, if the status of *E. enysii* in the genus *Elymus* is questionable, the origin of *E. tenuis* needs to be re-considered.

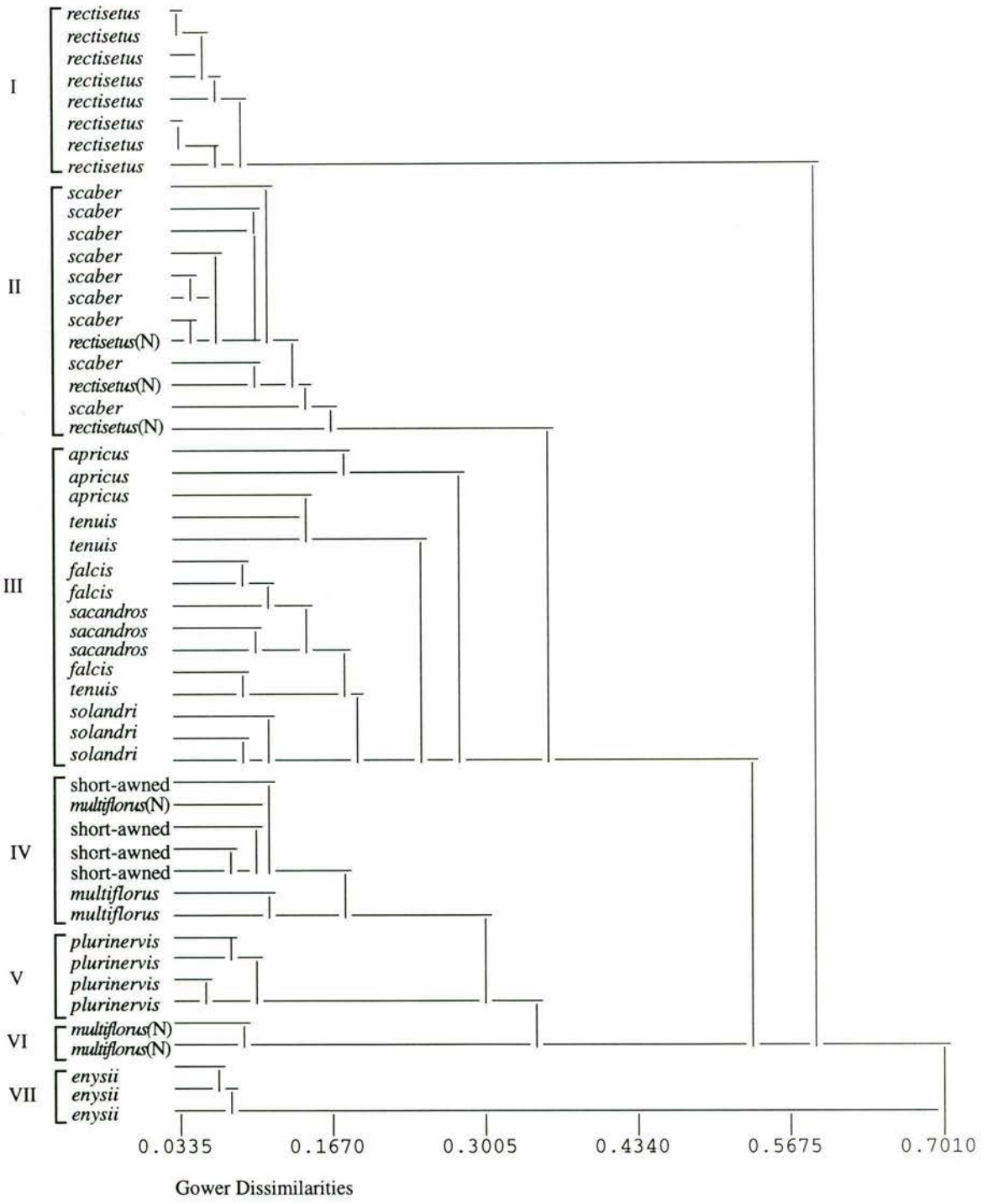


Figure 5-1. Dendrogram of Australian and New Zealand samples obtained from UPGMA cluster analysis using Gower Metric association measure. N = New Zealand samples.

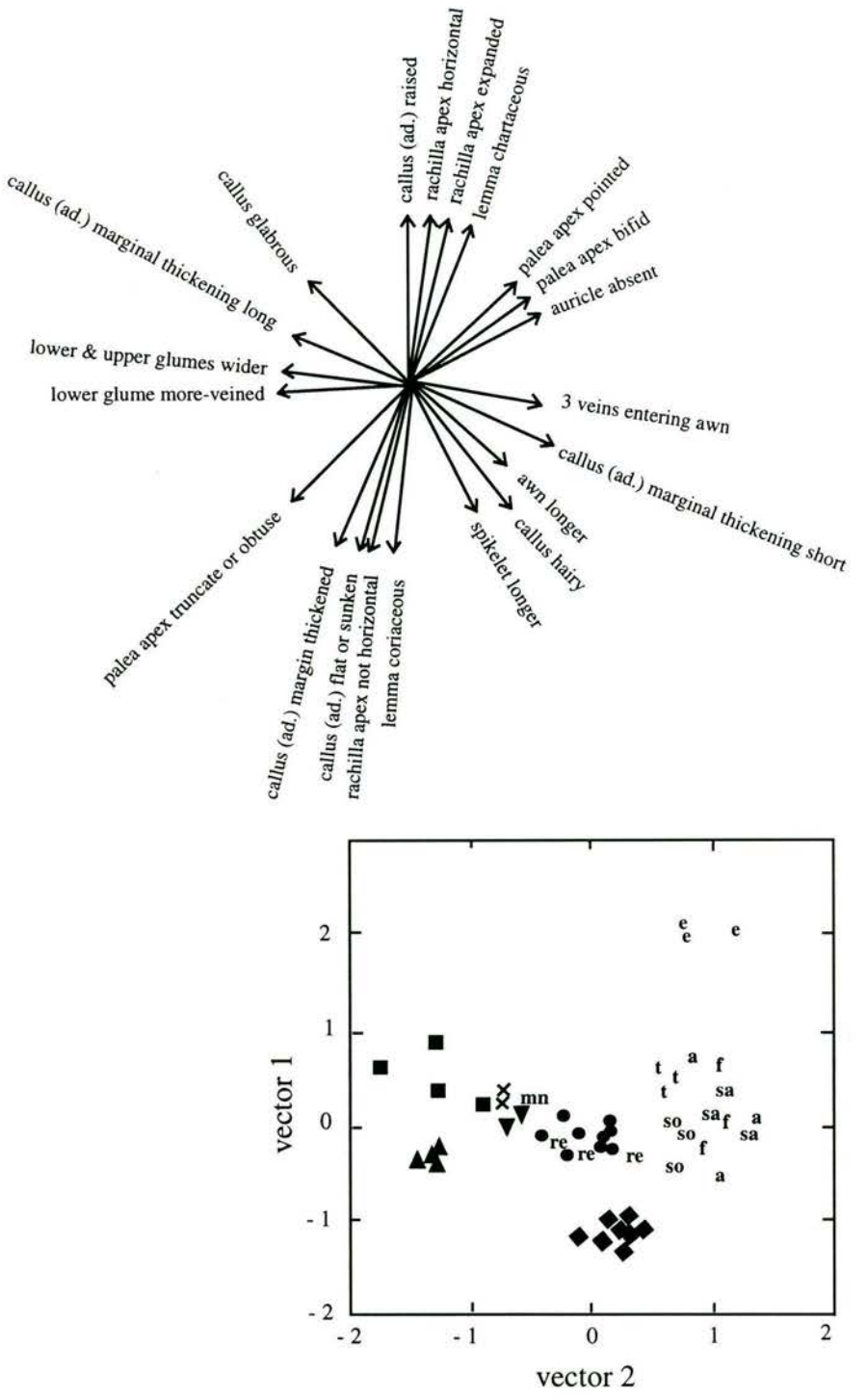
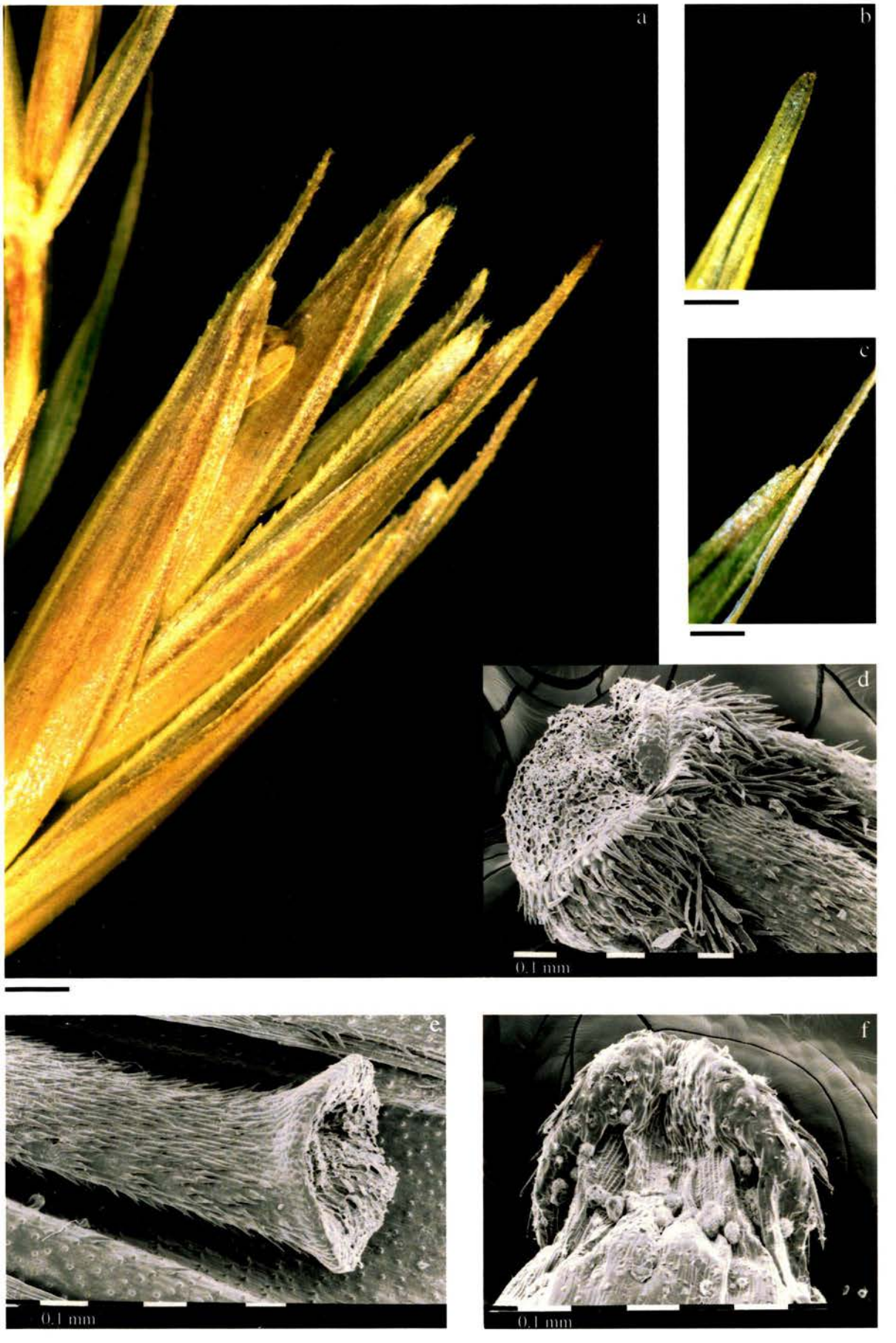


Figure 5-2. Scatter plot of SSH in two dimensions using Gower Metric association measure. Stress = 0.15. *E. rectisetus* (◆); *E. scaber* var. *scaber* (●); *E. scaber* var. *plurinervis* (▲); *E. multiflorus* (■, mn); short-awned from NSW (×); short-awned from Qld (▼); *E. apricus* (a); *E. enysii* (e); *E. falcis* (f); *E. rectisetus* (re, NZ); *E. sacandros* (sa); *E. solandri* (so); *E. tenuis* (t). A vector plot showing direction of maximum linear correlation (values > 0.7) between the ordination space and the fitted characters is superimposed above the plot.

Plate 5.1.

***Elymus enysii*, an alien of the genus *Elymus*.**

a. Spikelet small, consisting of several densely arranged florets. **b**. Palea apex pointed and bifid. **c**. Lemma apex folded and bifid; awn short and straight. **d**. Callus (adaxial) raised. **e**. Rachilla apex horizontal and sharply expanded. **f**. Callus (abaxial) very short. **Bar** = 1 mm (except where indicated).


a
b
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0.1 mm
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Chapter 6.

Molecular research on the *Elymus scaber* complex

6.1. Introduction

This chapter describes the analysis and results for genomic DNA polymorphism of the *Elymus scaber* complex from Australia using the random amplified polymorphic DNA (RAPD) technique. The same analysis was not carried out on New Zealand material due to time limitation.

The PCR-based RAPD technique was developed independently by two groups (Welsh and McClelland, 1990; Williams *et al.*, 1990). Both assays detect DNA polymorphisms based upon the amplification of genomic DNA segments with single primers of arbitrary nucleotide sequence. Williams *et al.* suggested that "these polymorphism be called RAPD markers, after Random Amplified Polymorphic DNA"; whereas Welsh *et al.* (1990) called the method AP-PCR, representing Arbitrarily Primed PCR. DNA Amplification Fingerprinting (DAF) is another related technique developed by Caetano-Anollés *et al.* (1991). Of all the acronyms, RAPD has become widely used as a generic term.

The RAPD method uses a decamer oligonucleotide (primer) to search for variations in DNA (Hedrick, 1992). It is assumed that when the primer is short (e.g. 10-mer), there is a high probability that the genome will contain several priming sites close to one another that are in an inverted orientation, and, therefore, DNA segments of variable length can be amplified from the sequences between them (Hadrys *et al.*, 1992; Tingey and del Tufo, 1993). Base substitutions within either priming site, or deletions and insertions between the priming sites, will affect the efficiency of amplification, changing the profile of segments produced by a given primer (Williams *et al.*, 1990; Caetano-

Anollés and Bassam, 1993; Kazan *et al.*, 1993). RAPD primers have been found to be an efficacious size, and a G + C content of the primer similar to the G + C content of the analysed genome will maximise the frequency of binding sites and hence amplification products (Hadrys *et al.*, 1992). The amplification products are resolved by gel electrophoresis and polymorphisms serve as dominant genetic markers, which are inherited in a Mendelian fashion (Williams *et al.*, 1990; Carlson *et al.*, 1991; Hadrys *et al.*, 1992; Newbury and Ford-Lloyd, 1993).

The advantages of RAPD over other DNA fingerprinting techniques are: it requires little knowledge of the biochemistry or molecular biology of the species, simplified technology, does not require specific primers, requires only traces of DNA (as little as nanograms) (Welsh and McClelland, 1990; Williams *et al.*, 1990; Hadrys *et al.*, 1992; Hedrick, 1992; Newbury and Ford-Lloyd, 1993; Tingey and del Tufo, 1993; Williams *et al.*, 1993), reduced time (Welsh and McClelland, 1990; Newbury and Ford-Lloyd, 1993; Tingey and del Tufo, 1993) and even expense (Hadrys *et al.*, 1992), has comparatively higher frequency with which variation is detected than RFLP (Newbury and Ford-Lloyd, 1993) and is nonradioactive (Tingey and del Tufo, 1993; Williams *et al.*, 1993).

Some of the limitations and difficulties of the RAPD technique have also been described. RAPD is extremely sensitive to reaction conditions such as the temperature profile, type of polymerase and Mg^{2+} concentration. Slight changes in the conditions may affect the reproducibility, therefore only strictly standardised reaction conditions will guarantee reproducible amplification products (Welsh and McClelland, 1990; Williams *et al.*, 1990; Hadrys *et al.*, 1992). Some RAPD fragments may be ambiguous and difficult to score. These fragments may derive from non-specific priming, or from heteroduplex formation between related amplification products or other secondary structure artefacts (Wenger and Nielsen, 1991; Hadrys *et al.*, 1992). Therefore, all RAPD amplification should be repeated two or more times, to ensure that the majority of markers are clearly reproducible and scorable (Hadrys *et al.*, 1992). There is also the chance that two amplified fragments, from different individuals, which have the same size as monitored by gel electrophoresis, may not actually have the same sequence (Newbury and Ford-Lloyd, 1993). In addition, RAPD markers are the least informative

of all known DNA markers since they are dominant and heterozygosity is normally not detectable (Hadrys *et al.*, 1992; Newbury and Ford-Lloyd, 1993; Peakall, 1997) .

RAPD technology has quickly gained widespread acceptance and application because it has provided a tool for genetic analysis in biological systems that have not previously benefited from the use of molecular markers (Tingey and del Tufo, 1993). RAPDs have been used in the construction of genetic maps (Williams *et al.*, 1990; Carlson *et al.*, 1991; Newbury and Ford-Lloyd, 1993). It has been demonstrated that RAPD is useful for taxonomic studies at levels ranging from population (Huff *et al.*, 1993; Castagnone-Sereno *et al.*, 1994; Peakall *et al.*, 1995), strains or cultivars (Welsh and McClelland, 1990; Demeke *et al.*, 1992; Mailer *et al.*, 1994; Multani and Lyon, 1995; Ho *et al.*, 1997), varieties or subspecies (Novy *et al.*, 1994), species (Demeke *et al.*, 1992; Kazan *et al.*, 1993; Castagnone-Sereno *et al.*, 1994; Transue *et al.*, 1994), and perhaps genera (Demeke *et al.*, 1992). Owing to its speed and ease, RAPD can enhance the assessment of genetic diversity on a large scale in natural populations, ecosystems, nature reserves or *in situ* conservation (Newbury and Ford-Lloyd, 1993). The identification of diagnostic RAPD markers linked with genes of interest allows for the use of the RAPD technique in breeding programs, saving considerable time in the production of new varieties (Newbury and Ford-Lloyd, 1993; Wight *et al.*, 1994). Another area where the RAPD technique is being increasingly applied is the development of chromosome-, population-, species-, and genome-specific markers (Wei and Wang, 1995).

Recently, RAPD technology has been applied within the tribe Triticeae and the genus *Elymus*. Wei and Wang (1995) claimed that the genome relationships of Triticeae generated from RAPD markers agree with the conclusions from studies of chromosome pairing, isozyme and DNA sequence analyses. Some RAPD fragments are considered to be genome- and species-specific markers which are useful in studies of genome evolution, analysis of genome composition, and genome identification. Furthermore, these genome-specific bands were cloned and demonstrated to be mainly low-copy sequences present in various Triticeae species (Svitashev *et al.*, 1998). Considering that the genomic constitution of approximately 40% of the species within *Elymus* is still unknown and there are many *Elymus* species whose genomic constitution could be

questioned (Svitashev *et al.*, 1998), the RAPD technique provides a very powerful tool for study on this genus. Analysis of RAPD data from some *Elymus* species concurs with the morphological classifications and also supports the genomic classification showing that *Elymus* is not a monophyletic genus (Sun *et al.*, 1997; Svitashev *et al.*, 1998). Some species have already been shown not to belong to *Elymus* based on RAPD markers, RFLP markers and cytological evidence (Jensen and Wang, 1997; Svitashev *et al.*, 1998).

Based on the morphological data collected from the *Elymus scaber* complex in Australia, five groups have been identified (Chapter 4). The results indicated that morphological traits, such as callus, rachilla, palea tip, together with some numerical characters, namely the length of spikelet and lemma awn, the ratio of fruit : palea and the size of glume, are extremely useful. However, these characters can sometimes be influenced by environmental conditions, and it was noted that the range of lemma awn length of some transplants changed under cultivated conditions. In contrast, DNA-based markers clearly allow the direct comparison of the genetic material of two individual plants avoiding any environmental influences on gene expression, and provide ideal tools for assessing genetic variation, identifying taxa, and defining genetic relationships (Newbury and Ford-Lloyd, 1993; Peakall, 1997).

When considering the genome structure and constitution of wheat grasses in Australia, it seems that the genomic formula SSYYWW instead of SSSSHH has been universally accepted (Torabinejad *et al.*, 1987; Carman and Wang, 1992; Torabinejad and Mueller 1993; Connor, 1994; Liu *et al.*, 1994). However, confusion concerning the taxa used in analyses exists. In other words, different workers may designate the experimental material as different taxa (Chapter 1).

The aim of the present study is to find out if RAPD markers will confirm the differences between all the taxa indicated by the previous phenetic analysis. In particular, to find out the relationships between the individuals with somewhat intermediate characters and the morphologically defined taxa. It is also expected that

the genomic constitution of Australian taxa can be confirmed using some genome-specific RAPD markers developed by Wei *et al.* (1995).

6.2. Material and methods

6.2.1. Specimen sampling and preparation

Thirty-five samples were selected for RAPD fingerprinting (Appendix VI). The samples were obtained mostly from seedlings or young shoots, but one was from a herbarium specimen (collected in 1968) used to test if dry plant material can be used to extract DNA suitable for RAPD analysis.

6.2.2. DNA isolation

Healthy leaves were collected from one- to six-month old greenhouse-grown plants germinated from seeds, or from young culms transplanted from the field. Three protocols of DNA extraction were followed and compared (Table 6-1).

In the modified DNA extraction protocol using hexadecyltrimethylammonium bromide (CTAB) (Porebski *et al.*, 1997), approximately 0.5 g of fresh leaves were ground in a mortar and pestle with liquid nitrogen. The frozen powdered leaf tissue was transferred to a 15-ml polypropylene centrifuge tube and 5 ml of extraction buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% CTAB, 0.3% 2-mercaptoethanol) pre-heated to 60°C and 50 mg PVP were added. The mixture was incubated at 60°C for 25 - 60 minutes, and 6 ml chloroform : Isoamylalcohol (IAA) (24 : 1) was added followed by centrifugation at 3000 rpm. Thereafter, 1/2 volume 5 M NaCl and 2 volumes 100% ethanol (-20°C) were added to the aqueous layer, and the DNA was precipitated by centrifugation at 3000 rpm. The DNA was washed with 70% ethanol and air dried. The

DNA was then dissolved in 700 µl TE (10 mM tris-HCl, 1 mM EDTA, pH 8.4) containing 3 µl of 10 mg/ml RNase A and incubated at 37 °C overnight. Subsequently, 3 µl of 1 mg/ml proteinase K was added to the mixture and it was incubated at 37 °C for 30 minutes. To remove the proteins, 1/2 volume phenol and 1/2 volume chloroform : IAA were added and the mixture was spun at 14000 rpm, followed by another extraction with 1 volume chloroform : IAA and spinning at 14000 rpm. The DNA was precipitated from the aqueous layer by the addition of 1 /10 volume of 2 M sodium acetate and 2 volumes 100% ethanol. The DNA was spun down at 14000 rpm and the pellet was washed with 70% ethanol, air dried and dissolved in 300 µl TE.

The second DNA extraction protocol was modified from the method described by Wu *et al.* (1995). Fresh leaf tissue (0.2 - 0.5 g) was ground to a fine powder with liquid nitrogen and then 500 µl DNA extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 500 mM NaCl) and 30 µl 10% SDS were added to the mixture and it was incubated at 65 °C for 20 minutes. Thereafter, 150 µl 5 M potassium acetate was added and the mixture was left at 4 °C for 20 minutes. This was followed by one extraction with phenol and chloroform. The supernatant was transferred to 400 µl isopropanol and spun at 13000 rpm to obtain the DNA pellet. The DNA was air dried and dissolved in 100 µl MQ water. After treatment with 1 µl of 10 mg/ml RNase A for 0.5 to 1 hour, one extraction with phenol/chloroform was conducted. The supernatant was transferred to a fresh tube and 20 µl 3 M sodium acetate pH 5.5 and 400 µl 100% ethanol were added and the DNA was spun down. The DNA was washed with 70% ethanol, air dried and dissolved in 50 - 100 µl MQ water.

Bahl and Pfenninger (1996) developed a rapid method of DNA isolation using commercial biological laundry detergent concentrate as a detergent/buffer-system. As this method does not need special preparation of the detergent/buffer-system and can be used to rapidly extract DNA from bulk plant material in two days, this protocol was followed to isolate most of the DNA samples in this study. About 0.2 - 0.3 g fresh leaf tissue was ground with liquid nitrogen, then 300 µl 6% laundry detergent (OMO, *Lever Rexona*®) was added and the mixture was incubated at 37 °C overnight. After incubation, 250 µl potassium acetate was added, and the homogenate was extracted once

with 250 µl phenol and 250 µl chloroform-IAA. The supernatant was transferred to 500 µl isopropanol and centrifuged to precipitate the DNA. The DNA was washed in 70% ethanol, air dried and dissolved in 300 µl TE. This was followed by RNase treatment for 1 hour at 37 °C by the addition of 5 µl of 10 mg/ml RNase A, and by one extraction with 400 µl phenol and 400 µl chloroform-IAA. The DNA was precipitated by the addition of 80 µl 3 M sodium acetate pH 5.5, 800 µl 100% ethanol and centrifugation at 13000 rpm for 5 minutes. The DNA pellet was washed with 500 µl 70% ethanol, air dried for 30 minutes and dissolved in 50 - 100 µl MQ water.

The DNA concentration was determined by spectroscopy using a QuantaGene DNA/RNA calculator (Pharmacia Biotech), taking readings at 260 nm wavelength. The accurate quantitation of DNA was not possible probably due to contamination with protein or phenol. The DNA quality was determined by gel electrophoresis of sample DNA on a 1% agarose gel containing 0.5 µg/ml ethidium bromide (cf. 6.2.5.).

Table 6-1. Comparison of the three DNA extraction methods

Protocol (modified)	Detergent/buffer system	Time required to obtain DNA	Protein concentration (mg/ml)	DNA Yield (µg DNA/g leaf tissue)
Porebski *et al.* (1997)	100 mM Tris, 1.4 M NaCl, 20 mM EDTA pH 8.0, 2% CTAB, 0.3% 2-mercantoethanol, plus 50 mg PVP / 0.5 g leaf tissue.	> 72 hours	1.7 - 4.2 Average: 2.3	300 - 900 Average: 497[1]
Wu *et al.* (1995)	100 mM Tris-HCl, 50 mM EDTA pH8.0, 500 mM NaCl	> 8 hours	4 - 5 Average: 4.9	216 - 280 Average: 248[2]
Bahl & Pfenninger (1996)	Commercial biological laundry detergent concentrate	> 24 hours	0.5 - 2.7 Average: 1.8	107 - 466 Average: 243[3]

1: Average value was calculated from six samples; *2*: Average value was calculated from two samples; *3*: Average value was calculated from 27 samples.

6.2.3. Primer selection

Demeke *et al.* (1992) suggested that at least ten primers, which would generate approximately 100 total bands, are required to adequately portray taxonomic relationships. Sixty-four different decamer primers, purchased from Operon Technologies Inc. (Alameda, USA), were used in an initial screening and 11 primers (Table 6-2), which generated informative amplification products, were chosen for the whole experiment. The other primers, which generally produced extremely complex banding patterns or monomorphism, were subsequently discarded from the study (data not shown). Available information on genomic constitution of this species complex (Löve and Connor, 1982; Löve, 1984; Torabinejad *et al.*, 1987; 1989; Torabinejad and Mueller, 1993a; Connor, 1994) was considered, and those primers which were reported to generate genome-specific markers (Wei and Wang, 1995) were taken into account (Table 6-2).

Table 6-2. Decamer primers used in this study, the number of markers obtained, and genome-specific markers developed in other laboratories

Primer	Sequence (5' to 3')	Genome & markers (Wei & Wang, 1995) *	Total markers	Polymorphic markers
OPC-04	CCGCATCTAC		16	15
OPD-14	CTTCCCCAAG	St: D14-640	12	11
OPE-02	GGTGCGGGAA		13	11
OPE-09	CTTCACCCGA		14	12
OPF-15	CCAGTACTCC	W: F15-581	10	10
OPL-06	GAGGGAAGAG		14	11
OPL-17	AGCCTGAGCC		16	14
OPR-05	GACCTAGTGG	St: R5-860	14	13
OPR-16	CTCTGCGCGT	W: R16-570	18	18
OPW-05	GGCGGATAAG	H: W5-700	19	19
OPW-19	CAAAGCGCTC	W: W19-402	12	12
Total	-	-	158	146 (92%)

* H, St, and W are genomes. The genome designations follow the rules recently proposed (Wang *et al.*, 1994).

6.2.4. Optimisation of DNA amplification conditions

It is best to begin the RAPD protocol with the standard conditions (Williams *et al.*, 1993), because amplification parameters tailor fingerprints in their complexity and polymorphic DNA content (Caetano-Anollés and Bassam, 1993). Some crucial parameters suggested by Williams *et al.* (1993) were considered for modification in the current study: DNA concentration, primer concentration, dNTP concentration, DNA polymerase, magnesium ion concentration and annealing temperature.

According to Williams *et al.* (1993), higher concentrations of DNA may result in smears or in poorly defined bands, whereas lower concentrations may give unreproducible patterns. Lower concentrations of primer may generate too little product, but higher concentrations may result in evident smearing. For dNTP, the intensity of stained bands in the gel may become progressively weaker at lower concentrations (less than 100 μM for each of the four bases). DNA polymerase from different sources may influence the patterns of amplified bands. Finally, both magnesium ion concentration and annealing temperature may distinctively affect the relative amounts of amplified bands.

DNA amplification was performed using a PTC-100-60 thermal cycler (MJ Research, USA). Optimisation experiments were carried out in which the concentrations of magnesium chloride (1.0 - 3.0 mM), dNTPs (0.1 - 0.24 mM), primer (0.4 - 1.3 mM), genomic DNA (25 - 60 ng), and *Taq* DNA polymerase (Advanced Biotechnologies, UK, or Gibco BRL, USA; 1 - 2 units per reaction) were varied. Initially the PCR cycling conditions and times followed the standard conditions of Wei and Wang (1995), but the results were unsatisfactory (obvious smearing instead of bands). Therefore, adjustments were made by referring to another PCR cycling program used extensively in the laboratory for cotton DNA amplification (Multani and Lyon, 1995). The two programs are compared in Table 6-3.

Table 6-3. Comparison of PCR profiles used for RAPD analysis

Wei & Wang (1995)	denaturation	94 °C	1 min	40 cycles
	annealing	36 °C	1 min	
	extension	72 °C	1 min	
Multani & Lyon (1995)	denaturation	94°C	1 min	41 cycles
	denaturation	93 °C	45 sec	
	annealing	35 °C	1 min	
	extension	74 °C	3 min	
	extension	74 °C	10 min	

It was observed that the concentration of primer, magnesium chloride and dNTP determined the RAPD banding pattern. Lower concentrations clearly reduced the background smearing. Different sources of *Taq* also influenced the banding pattern. *Taq* polymerase obtained from Advanced Biotechnologies was found to generate a greater number of clearly defined bands and was selected for all RAPD amplifications. The concentration of genomic DNA did not appear to affect the RAPD amplification in the range of 25 - 60 ng. Increasing annealing temperature (36 - 38 °C) and extension time (1 - 3 min) generated more discrete amplification products.

The parameters that were finally selected gave the highest yield of specific RAPD bands with the least amount of background smear due to non-specific amplification. The optimised PCR conditions were as follows: 25 µl total reaction volume containing 1 unit *Taq* DNA polymerase (Advanced Biotechnologies), 1 x buffer (20 mM $[NH_4]_2SO_4$, 75 mM Tris-HCl, pH 8.8, 0.01% [v/v] Tween®20), 1 mM magnesium chloride, 0.4 mM primer, 0.1 mM dNTP, and about 40 ng template DNA. The cycling program comprised an initial denaturation step at 94 °C for 1 minute followed by 40 cycles of 1 min at 94 °C, 1 min at 38 °C, and 3 min at 72 °C. There was a final extension of 10 min at 72 °C and a hold step at 4 °C.

The reproducibility of DNA banding patterns is one of the most important factors determining the usefulness and reliability of the RAPD technique for the identification of genetic variation (Ho *et al.*, 1997). Band reproducibility was checked by repeating amplifications on each template DNA.

6.2.5. Agarose gel electrophoresis (AGE)

Amplified DNA fragments were resolved by gel electrophoresis in 1.6 - 2.0 % agarose gels containing ethidium bromide (0.5 µg/ml) in 0.5 x TBE (90 mM Tris, 90 mM Boric acid, 2 mM EDTA pH 8). Prior to electrophoresis, 1/5 volume of loading dye (30% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol) was added to each tube containing the reaction products. The electrophoresis was carried out for 2 hours at 100 - 120 V using an HE99X max submarine unit (Hoefer, USA) and then visualised and photographed under UV light.

6.2.6. Data analysis

Photographs were used to score the data for RAPD analysis. The reproducible bands (both bright and faint) were scored as 1 for their presence and 0 for absence. DNA fragment sizes were determined by comparison with the following DNA size markers run on each gel: pBluescript plasmid cut with *Hpa*II whose sizes are 710, 489, 404, 328, 242, 190, 157, 147, 110, 67, and 57 bp, and Lambda DNA cut with *Eco*RI and *Hin*dIII with sizes of 21226, 5148, 4973, 4268, 3530, 2027, 1904, 1584, 1375, 947, 831, and 561 bp. The RAPDistance Program was used to calculate similarities. The similarities were then used to construct dendrograms using the UPGMA routine in the computer program package, Australian National Genomic Information Service (ANGIS), and to generate a scatter plot of SSH in the PATN program. The RAPDistance program is discussed in the following section.

The RAPDistance programs were designed to help record and analyse the RAPD fragment data (Armstrong *et al.*,). The primary data can be coded (presence/absence of bands) and edited with the programs, or recorded using a spreadsheet program (such as

EXCEL as in this study), and transformed into the format used by the RAPDistance programs. Pairwise distances can then be calculated using any of the 18 metrics provided by the program. The algorithm selected to calculate similarity for RAPD data in this study was that of Dice (1945). The formulation is simply

$$2n11 / (2n11 + n01 + n10)$$

where n11 is the number of bands shared by two objects, n01 and n10 the number of bands that only exist in one of the objects. The 00 matches representing absence of the bands in both objects are not taken into account here.

The calculation resulted in a distance matrix, the output file -.DIS, which was, subsequently, used to construct dendrograms and to generate scatter plots of SSH as discussed above.

6.3. Results

6.3.1. RAPD amplification products

A total of 158 amplification bands were obtained (Table 6-2), of which 146 (92%) were polymorphic and these were used to conduct similarity calculations. The average number of bands amplified was 14 per primer. DNA fragment sizes ranged from 240 - 2200 bp.

Table 6-4 shows the distribution of markers unique to different groups within the species complex and the corresponding random primers. Analysis of these polymorphic bands indicated that 13 (9%) were unique to *E. scaber* var. *plurinervis,* another 13 were unique to *E. multiflorus* and 11 (8%) were unique to the short-awned form from Queensland. In these three taxa, distinct band patterns were obtained with almost all primers, but only 4 (3%) markers were fixed in *E. rectisetus* and no markers were found to be unique to *E. scaber* var. *scaber* from New South Wales. The genetic differentiation between short-awned individuals from Queensland and from New South

Wales was obvious because no markers were shared exclusively between them. The distinction of Tasmanian individuals was indicated by the presence of two unique markers and the absence of two bands (OPD14-450 and OPR5-850) which were present in all other individuals sampled.

Examination of the eleven primers used showed that OPF-15 resulted in the most informative band pattern (Figure 6-1). It demonstrated that *E. rectisetus*, *E. scaber* var. *scaber*, *E. scaber* var. *plurinervis*, *E. multiflorus*, and the short-awned samples from Queensland are genetically distinct from one another. It also indicated that the short-awned individuals from New South Wales are genetically similar to *E. scaber* var. *scaber* instead of being similar to their morphologically resemblant samples from Queensland. Unique markers were obtained using all primers selected in this research. OPW-05 gave the most (10) while OPD-14 the least (1; Table 6-4).

Table 6-4. Specific markers generated from RAPD amplification with 11 primers

Primer	C4	D14	E2	E9	F15	L6	L17	R5	R16	W5	W19	Total markers
E. rectisetus					1	1	1	1				4
E. scaber var. *scaber* (NSW)												
E. scaber var. *scaber* (Tas.)										2		2
E. scaber var. *plurinervis*	2	1	1	1	3		1		1	2	1	13
E. multiflorus			2		2	1	4	1		2	1	13
Short-awned (Qld)	2			2	1			1	1	2	2	11
Short-awned (NSW)										2		2

Reproducibility of the majority of polymorphisms was revealed in the present study. Nearly all bands except a few faint ones were reproducible under standardised conditions. Figure 6-2 shows two of the band patterns, generated on separate occasions, with primer OPL-06 using replicate genomic DNA samples from *Elymus rectisetus*, *E.*

scaber var. *plurinervis*, and the short-awned samples. More details will be discussed in the related section (6.4.1).

One sample of *E. scaber* var. *scaber* (s_1, S95059; cf. Appendix VI) was found to have the same band pattern as the short-awned form from Queensland. It appears that the short-awned sample from Queensland was erroneously introduced into the *E. scaber* var. *scaber* batch. DNA extracted from the herbarium specimen (m_6, HO116080; cf. Appendix VI) was shown by agarose gel electrophoresis to be degraded. Four faint bands were obtained from this template but none of them was informative for analysis. Both samples, s_1 and m_6, were deleted from the data analysis.

6.3.2. Genome-specific markers

None of the genome-specific markers developed by Wei and Wang (1995; Table 6-2) were observed in this research, but six markers (E2-580, E9-860, E9-1750, L6-1600, L17-1000, and R5-370) were present in all samples, and two (D14-450 and R5-850) were found in all samples except the Tasmanian individuals.

6.3.3. Cluster analysis

Figure 6-3 shows the results of the cluster analyses. All members of *E. rectisetus*, *E. scaber* var. *plurinervis* and *E. multiflorus* were divided into distinct clusters. On the basis of morphology, it was not expected that the short-awned samples from southeastern Queensland would be grouped together with the members of *E. scaber* var. *scaber*. Three sub-clusters can be seen in *E. scaber* var. *scaber*: long-awned samples which are close to the short-awned samples from Queensland and New South Wales, and the Tasmanian samples together with two New South Wales samples make up the third sub-cluster.

Elymus rectisetus was more closely related to the mixed *E. scaber* var. *scaber* than to *E. scaber* var. *plurinervis* and *E. multiflorus*. The first two clusters fused at 0.23 of Dice similarity, and connected with *E. scaber* var. *plurinervis* at 0.22. *Elymus multiflorus*

demonstrated that it is distantly related to others by combining with them at 0.1 of Dice similarity.

6.3.4. Ordination

Figure 6-4 is a scatter plot of SSH in two dimensions with the stress = 0.19. The results were similar to those of cluster analysis which showed that *E. multiflorus* and *E. scaber* var. *plurinervis* were well defined and that *E. rectisetus* was well separated from, but fairly close to, *E. scaber* var. *scaber*. In contrast with the dendrogram, short-awned specimens from Queensland were detached from the main cluster of *E. scaber* var. *scaber*. *Elymus scaber* var. *scaber* formed a loose cluster with three specimens from Tasmania lay on one side and the short-awned individuals from New South Wales on another side.

6.4. Discussion

6.4.1. Problems with RAPD amplification

Reproducibility of polymorphisms was reported as the main problem encountered with the RAPD technique (Devos and Gale, 1992; Johnston, 1997). This was confirmed by the fact that genome-specific markers developed in other laboratories cannot be reproduced in the current study (cf. 6.4.2.). However, under the standardised conditions used in this research, only a few faint bands were not reproducible (Figure 6-2) and these were ignored for scoring purposes.

One of the other problems encountered in this research was that agarose gel electrophoresis was readily affected by some physical variables. It was found that higher voltages (i. e. 100 V or over with small gel casting kit [15 x 10 cm] and 110 V or over with big gel casting kit [15 x 20 cm]) could result in some close bands not being clearly resolved, or thick bands migrating unevenly. This result agrees with that of Dowling *et al.* (1996) who suggested that fragments are best resolved using low voltages. In addition, the ionic buffer and support matrix affected electrophoresis and

"gel smile" (bands moving at different speeds) resulted from unknown problems with the buffer or support matrix. The mobility of RAPD markers was also found to be different from time to time and even from the top half to the bottom half of the same gel. The differences ranged from 20 - 100 bp (same gel) or 20 - 190 bp (different gels). This might not be a problem with scoring if the band spectrum is regular, but it made the data scoring of *E. scaber* var. *scaber* more difficult due to the rather diverse band patterns. One way to resolve the problem was to load samples in a different order along the gel for comparable results.

DNA purity noticeably affected RAPD amplification, which was contrary to other workers' results (Caetano-Anollés and Bassam, 1993). One sample of *E. rectisetus* (r_5, S95103; cf. Appendix VI) was firstly obtained using the second protocol (see 6.2.2), its protein concentration was recorded as approx. 5 mg/ml, and no degradation of DNA was observed (data not shown). The band patterns for this sample were consistently found to be different from others in this group. For instance, in the RAPD amplification results obtained with primer OPL-06 (Figure 6-2), r_5 was seen to be lacking two markers of 1278 and 900 bp. A new DNA extraction of this sample using the third protocol (see 6.2.2) contained only 2.7 mg/ml of protein, and its RAPD amplification products were similar to other members of this group. This suggests that the techniques of DNA extraction would influence DNA quality and then affect the reproducibility of polymorphisms exhibited by the RAPD technique.

6.4.2. Genome-specific markers

Wei and Wang (1995) claimed that they developed 29 genome-specific markers and 11 species-specific markers, and that these RAPD markers are useful in studies of genome evolution, analysis of genome composition and genome identification.

It was hoped to use some of those markers to confirm the genomic constitutions of the *E. scaber* complex from Australia. This aim has not been achieved due to problems with poor reproducibility of characterised polymorphic markers between laboratories. No genome-specific markers for St, H or W genomes (Table 6-2) were observed in this

research presumably due to the sensitivity of the RAPD technique to slight variations in amplification conditions (Johnston, 1997). The most important differences of reaction conditions between this research and that of Wei and Wang (1995) are probably due to different sources of polymerase, different thermal cycling profiles, and different PCR machines (Table 6-5). This exhibited one of the limitations of the RAPD technique.

Meanwhile, eight markers were present in all, or nearly all, samples with primers D-14, E-02, E-09, L-06, L-17, and R-05 under our reaction conditions (cf. 6.3.2.). No conclusions can be drawn about whether they are genome-specific markers since only a small range of samples from Australia were analysed.

Nevertheless, 83 polymorphic markers (57%) were obtained from these six primers which generated genome-specific markers in other laboratories, and the most informative band pattern which can be used to distinguish the five groups was produced by RAPD amplification with OPF-15.

Table 6-5. Comparisons of important RAPD amplification conditions between Wei & Wang and this research

	Source of primers	Source of polymerase	Temperature profile	PCR machine
This research	Operon Technologies	Advanced Biotechnologies	94°C for 1 min; followed by 40 cycles of 1 min at 94 °C, 1 min at 38 °C, and 3 min at 72 °C; finally 10 min at 72 °C.	PTC-100-60 thermal cycler (MJ Research, USA)
Wei and Wang (1995)	Operon Technologies	Perkin Elmer	40 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C.	Gene Amp PCR System 9600

6.4.3. Taxonomic interpretation

Generally speaking, the results of RAPD fingerprinting on *Elymus scaber* complex from Australia concur with those of the morphological analyses.

Elymus rectisetus was genetically distinct from *E. scaber* var. *scaber* which mostly comprises long-awned individuals. This indicates that the characters of callus, rachilla, and palea tip are informative for taxonomic purposes within this species complex.

Elymus scaber var. *plurinervis* was more distantly related to the main cluster of *E. scaber* var. *scaber*, which is in accordance with the previous morphological analysis. The 13 RAPD markers unique to *E. scaber* var. *plurinervis,* and the specific band profiles seen in all the agarose gels support the raising of this taxon to specific rank.

Elymus multiflorus lay distantly from other short-awned elements owing to its distinguishable band patterns and 13 specific markers. This result confirmed that this taxon, which matched the New Zealand entity morphologically, should only refer to those specimens distributed along the coastal area in Australia. Specimens from riverine areas of Victoria (e.g. MEL1554652 from west of Mildura) were morphologically similar to this taxon, and, therefore, may also belong to this species.

Unlike the morphological results, short-awned samples were placed close to the main cluster of *E. scaber* var. *scaber* rather than to *E. multiflorus*. The relationship between short-awned samples from southeastern Queensland and New South Wales was also resolved. They are genetically different from one another because they always demonstrated different band patterns in RAPD assays. Queensland individuals deserve to be treated as a new taxon which has special band patterns in all RAPD amplifications and 11 specific markers fixed to it. Whereas, the short-awned form from New South Wales represents a group of individuals with more or less intermediate morphological characters, and are possibly widespread in the Great Dividing Range. They are revealed to be the members of *E. scaber* var. *scaber*, contribute morphological polymorphism to this diverse entity.

A wide range of genetic variation was observed within the remainder of *E. scaber* var. *scaber*. There were no unique markers observed within this group and individuals from Tasmania were separated from the main cluster. This resulted in a very loose cluster, widely scattered in the ordination space. This result may indicate that remarkably local genetic differentiation has occurred within this taxon (Heywood, 1991). In order to confirm this assumption, further research on populations and regions is needed.

Final taxonomic decisions about the *Elymus scaber* complex will be made based on a synthesis of morphological and molecular data. This will be discussed in detail in Chapter 7.

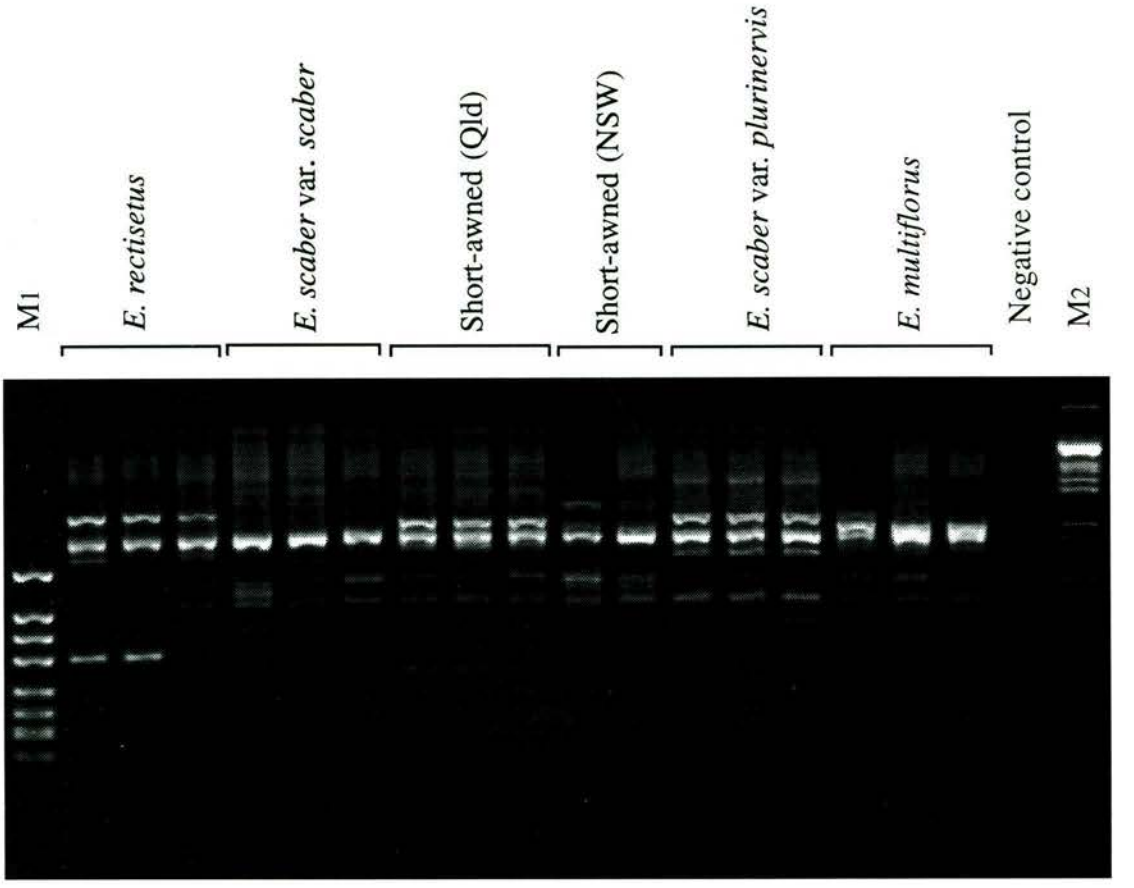
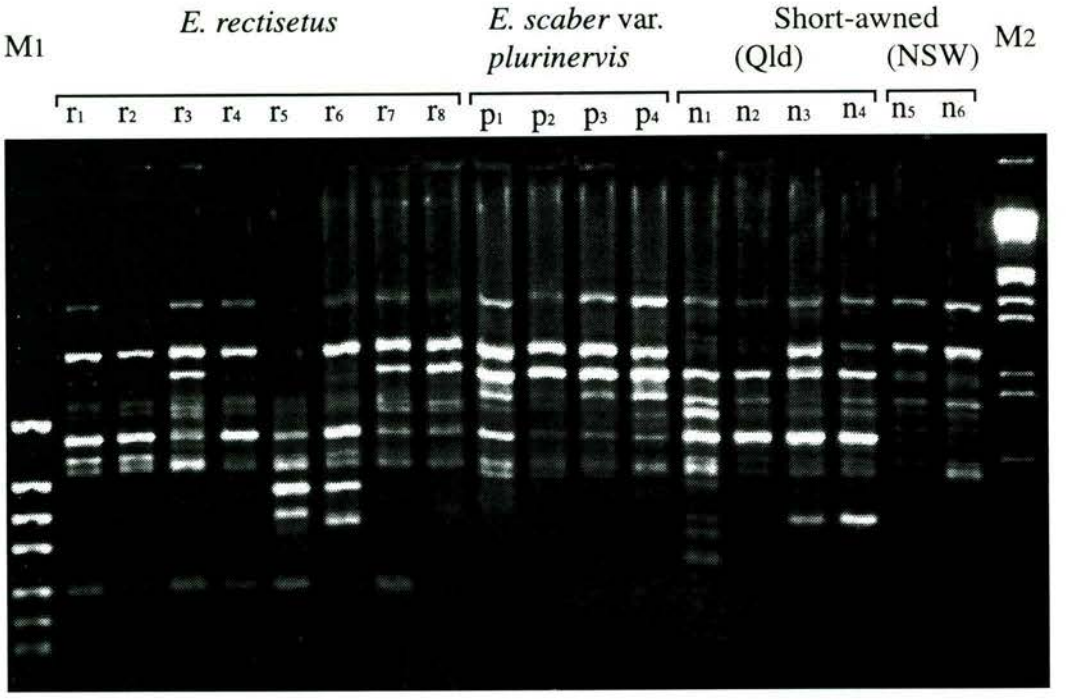
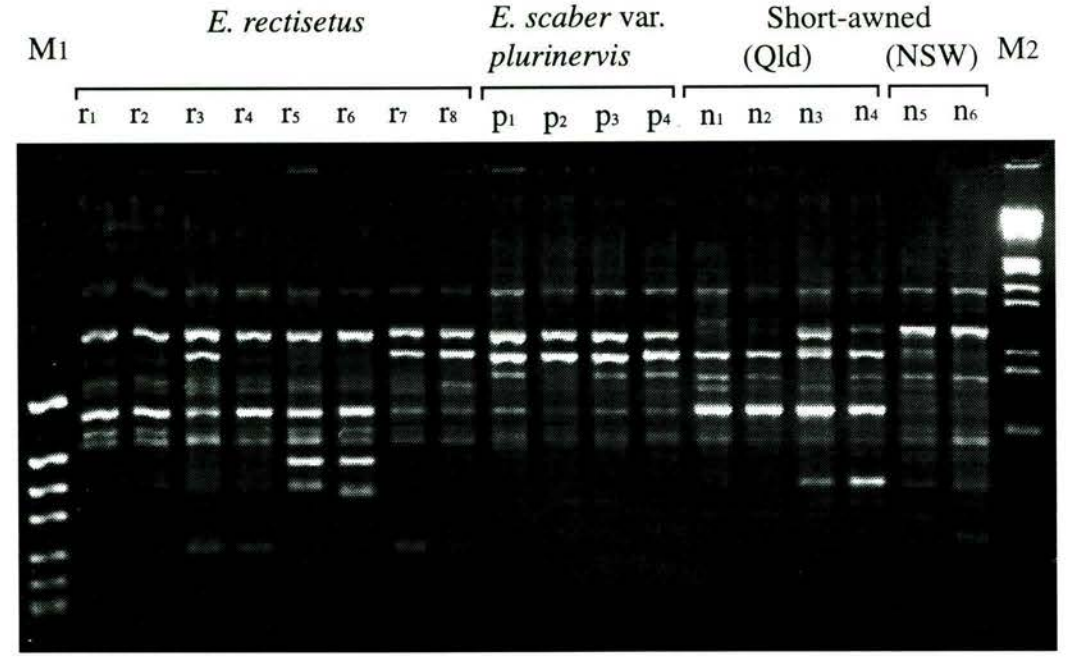


Figure 6-1. RAPD results from genomic DNA of the *Elymus scaber* complex generated with primer OPF-15 showing polymorphic patterns of each taxon. Negative control contains no DNA. M1: pBluescript DNA digested with *Hpa*II; M2: Lambda DNA digested with *Hin*dIII / *Eco*RI.



a



b

Figure 6-2. Duplicated RAPD results generated with primer OPL-06 using genomic DNA from *Elymus rectisetus*, *E. scaber* var. *plurinervis*, and the short-awned samples. Most bands are reproducible. The differences for r5 between the two gels (a and b) demonstrates that DNA quality may have adversely affected the band pattern in (a) (see 6.4.1). Protein concentration of r5 in gel (a) 5 mg/ml compared to gel (b) 2.7 mg/ml. M1: pBluescript DNA digested with *Hpa*II; M2: Lambda DNA digested with *Hin*dIII / *Eco*RI.

Figure 6-3. Dendrogram produced from UPGMA cluster analysis using Dice association measure in RAPDistance programs.

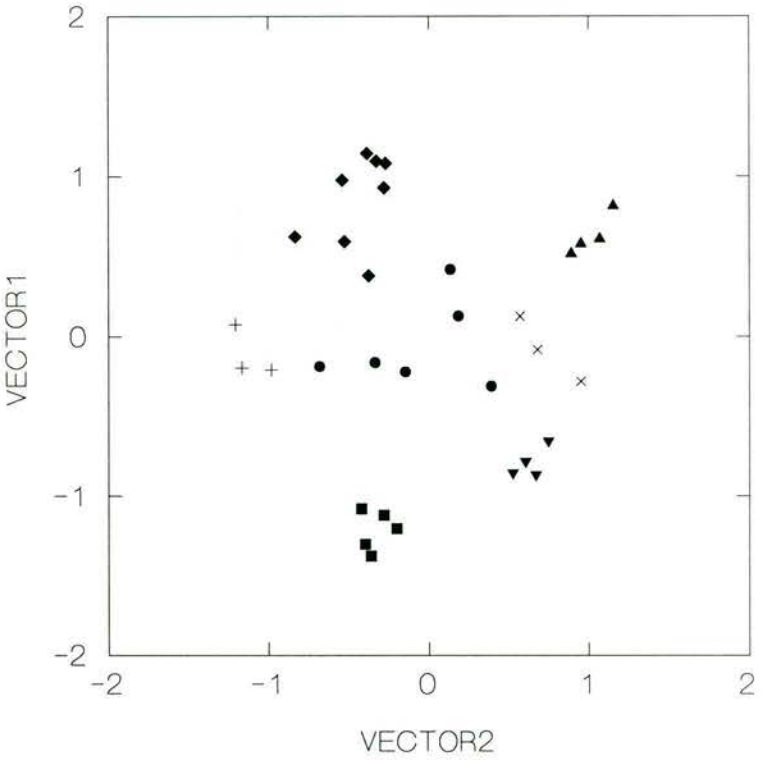


Figure 6-4. Scatter plot of SSH in two dimensions using Dice association measure generated from RAPD data. Stress = 0.19. *E. rectisetus* (◆); *E. scaber* var. *scaber* (NSW: ●; Tas.: +); *E. scaber* var. *plurinervis* (▲); *E. multiflorus* (■); Short-awned samples (Qld.: ▼; NSW: ×).

Chapter 7.

General discussion and conclusions

7.1. General discussion

7.1.1. Summaries and comparisons, what the analyses demonstrated

In order to investigate the variation within the *Elymus scaber* complex, four analyses were conducted using different data sets (Chapter 4–6).

The first analysis aimed to find out the variation pattern within Australian taxa using traditional morphological characters. To do this, 102 OTUs from Australia representing the full range of variation observed were selected and both cluster and SSH analysis were conducted (4.3.1). The results obtained from the analysis of 102 OTUs enabled a further investigation of a sub-sample for more informative characters using SEM and anatomical techniques. In the second analysis, a combined data set including new characters (e.g. those of rachillas, calluses, paleas and fruit) and those used in the first analysis with high correlation coefficients were analysed using the same programs (clustering and SSH; see 4.3.2). Generally, the results obtained from the two data sets concurred with each other with the exception that Group IV defined in the first analysis was split into two groups. The reason for the splitting was possibly the introduction of new OTUs and new characters (4.4.1). The clusters of Group I (*E. rectisetus*) and Group III (*E. scaber* var. *plurinervis*) appeared rather stable in both analyses, whereas the remainder did not (e.g. OTUs 29, 85, 87 were placed into Group II in the first analysis or into Group IV in the second, and Group IV was split into two groups in the second analysis; see Fig. 4-4 and 4-5). Two points can be deduced here:

1. There is a variation pattern with reference to the traditionally used morphological characters (mostly numerical ones such as spikelet length and awn length) if the average values of these characters were referred to (as it has been done in this analysis). However, the range values of some of these characters (e.g. spikelet length and lemma length) between groups extensively overlap (Figure 4-8) which caused taxonomical problems as discussed in Chapter 1.

2. The new characters (e.g. those of rachillas, calluses, paleas and fruit) are somewhat related to the traditional characters—long-hairy rachillas and calluses related to intermediate- to long-awned lemmas except those of individuals of Group III, transverse-circular rachilla apices and low ratio of fruit length to palea length related to large dimensions of glumes, and broadly triangular and glabrous to glabrescent calluses as well as short-hairy rachillas related to short-awned lemmas (except those of individuals of Group III).

The new characters are more apparent and more convenient to distinguish the taxa than the traditional numerical characters. Some of them (those of rachilla, callus and palea) were used to re-examine those OTUs which were not included in the second analysis (due to the impossibility to obtain all new characters, such as those of fruit, from all OTUs). Consequently, a total of 80% OTUs were confidently resolved — they were sorted with the five groups defined in the second analysis, and the remainder were uncertain (4.4). Furthermore, the same character set was used to examine all other specimens obtained in this study (Chapter 2) and most of them were confidently grouped (Appendix VIII). The practice indicated that the characters of rachilla, callus, palea and fruit are informative in the taxonomy of the *Elymus scaber* complex.

The third analysis aimed to confirm the occurrence of taxa (e.g. *E. rectisetus* and *E. multiflorus*) in both Australia and New Zealand, and to inspect the taxonomic values of new characters to New Zealand material. The characters used in this analysis were augmented by characters unique to New Zealand taxa and determined from the data set of the second analysis (5.2.1). Consequently, it was confirmed that *E. multiflorus* occurs in both countries, whereas *E. rectisetus sensu* Löve et Connor (1982) from New

Zealand was found to be equal to *E. scaber* var. *scaber* from Australia (5.4). In the later case, their similarity were clearly revealed by the characters of rachilla, callus, palea and fruit, which repeatedly indicated that these characters are taxonomically valuable. The taxonomic value of rachilla and callus was further highlighted by the separation of *E. enysii* from all other *Elymus* species — the former has distinct characters of rachilla and callus from the latter (Chapter 5). In addition, Australian *E. rectisetus*, *E. scaber* var. *scaber* and *E. scaber* var. *plurinervis* still formed distinct clusters, *E. multiflorus* and the new entity (Group IV) were closely related to each other, as it appeared in the second analysis. The results obtained from the latter two analyses indicated that the variation pattern revealed by SSH in this study (Figure 4-7 and 5-2) was not influenced by the introduction of new data (characters and OTUs) and, therefore, they are comparable. Whereas, the dendrograms appeared somewhat unstable — individuals separated into two groups (Group IV and V; Figure 4-6) in the second analysis were combined in the third analysis (Figure 5-1, short-awned: individuals of Group IV; multiflorus: individuals of Group V), and both appeared more closely related to *E. scaber* var. *plurinervis* than to the other two members of *E. multiflorus* from New Zealand. Considering that "ordination has the advantage of making few assumptions about the nature of relationships in the data" (Crisp, 1991), it is here preferred to interpret the variation patterns based on the results of SSH.

Finally, RAPD analysis was carried out on Australian material. The aim of this analysis, as previously stated (Chapter 1 and 6), was to confirm the results obtained from morphological data sets, to clarify the relationships blurred by individuals with intermediate characters (especially those resembling both *E. scaber* var. *scaber* and the short-awned entities; see 4.4.2), and to confirm the genomic constitution of Australian taxa (SYW or SSH). The first two were achieved, whereas the last one was not for reasons presented in Chapter 6. Although results obtained from RAPD and morphological data generally agreed, disagreements were also found and, therefore, the two data sets were not completely comparable. Morphologically similar taxa are not necessarily genetically similar (e.g. *E. multiflorus* is morphologically similar to but genetically distinct from the new entity). In addition, genetic diversity seems to be related to the distance of geographic distribution (e.g. Tasmanian individuals were

genetically distinct from other individuals of *E. scaber* var. *scaber*). All these demand that a final taxonomic conclusion should be attained based on comprehensive application of all information (see below).

7.1.2. Taxonomic conclusions

The taxonomic confusion concerning the *Elymus scaber* complex has long been recognised and species problems centred on the delimitation of taxa (see Chapter 1). Speciation is still occurring and is incomplete in the sense of Templeton (1989), they are really nebulous species. The cohesion species concept (Templeton, 1989) was considered to be more applicable for this taxonomic study and, therefore, its principles were followed. Accordingly, four analyses were conducted using different data sets as summarised above. A final taxonomic conclusion, however, cannot be reached by using phenetic or genetic distinctness as a sole criterion, but relies on comprehensive consideration of all available evidence e.g. ecology, geography, cytology and genetics (cohesion mechanisms; Templeton, 1989).

The results generated from different data sets and from different algorithms (cluster and SSH) were displayed and discussed in the preceding chapters, and were summarised and compared as above. In order to facilitate the following discussion, some of the results are selected and shown in Figure 7-1 and 7-2. Based on these results, five taxa: *E. rectisetus*, *E. scaber*, *E. plurinervis*, *E. multiflorus* and a new taxon exist in Australia. However, the final taxonomic rank of these taxa will be discussed in the subsequent sections. With regard to New Zealand taxa, two species, *E. multiflorus* and *E. enysii* are upheld. *Elymus rectisetus* is found to be a misidentification of *E. scaber*. *Elymus tenuis* is considered to be rather distinct, as discussed in Chapter 5, though the small cluster of this species was not detached from other four species in the phenetic analysis (Figure 7-2. b). The remainder, *E. apricus*, *E. falcis*, *E. sacandros* and *E. solandri* cannot be resolved by the current phenetic analysis as previously presented (Chapter 5).

Comparing the results generated from different data sets (Figure 7-1 and 7-2), both agreements and disagreements were found. Agreements: *E. rectisetus*, *E. plurinervis*,

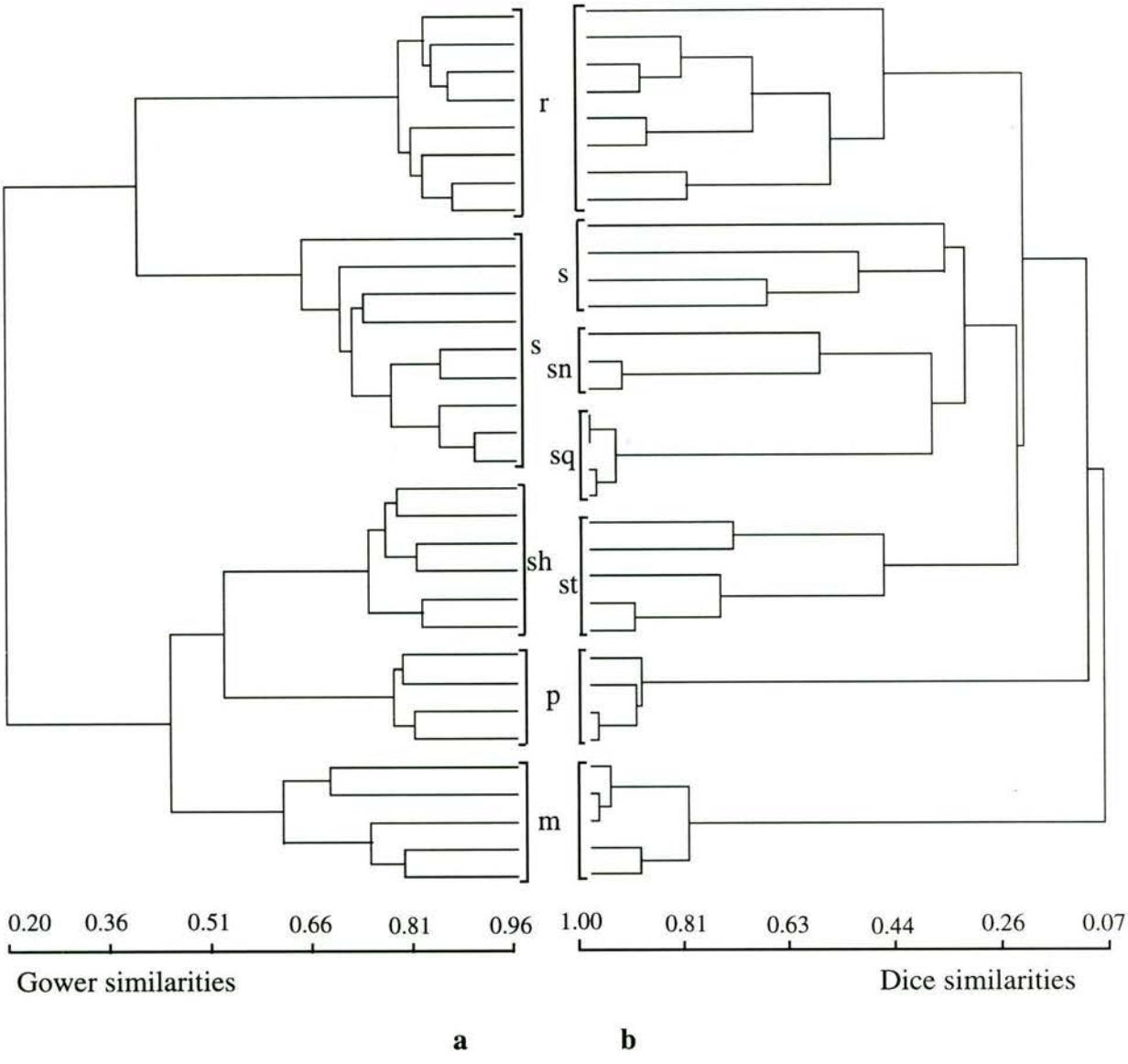


Figure 7-1. Dendrograms produced from UPGMA cluster analyses using **a**: Gower Metric for morphological data, and **b**: Dice association measure for RAPD data. r: *Elymus rectisetus*; s: *E. scaber*; sn: short-awned samples from NSW; sq: *E.* sp. nov.; sh: *E.* sp. nov. and short-awned samples from NSW; st: Tasmanian samples of *E. scaber*; p: *E. plurinervis*; m: *E. multiflorus*.

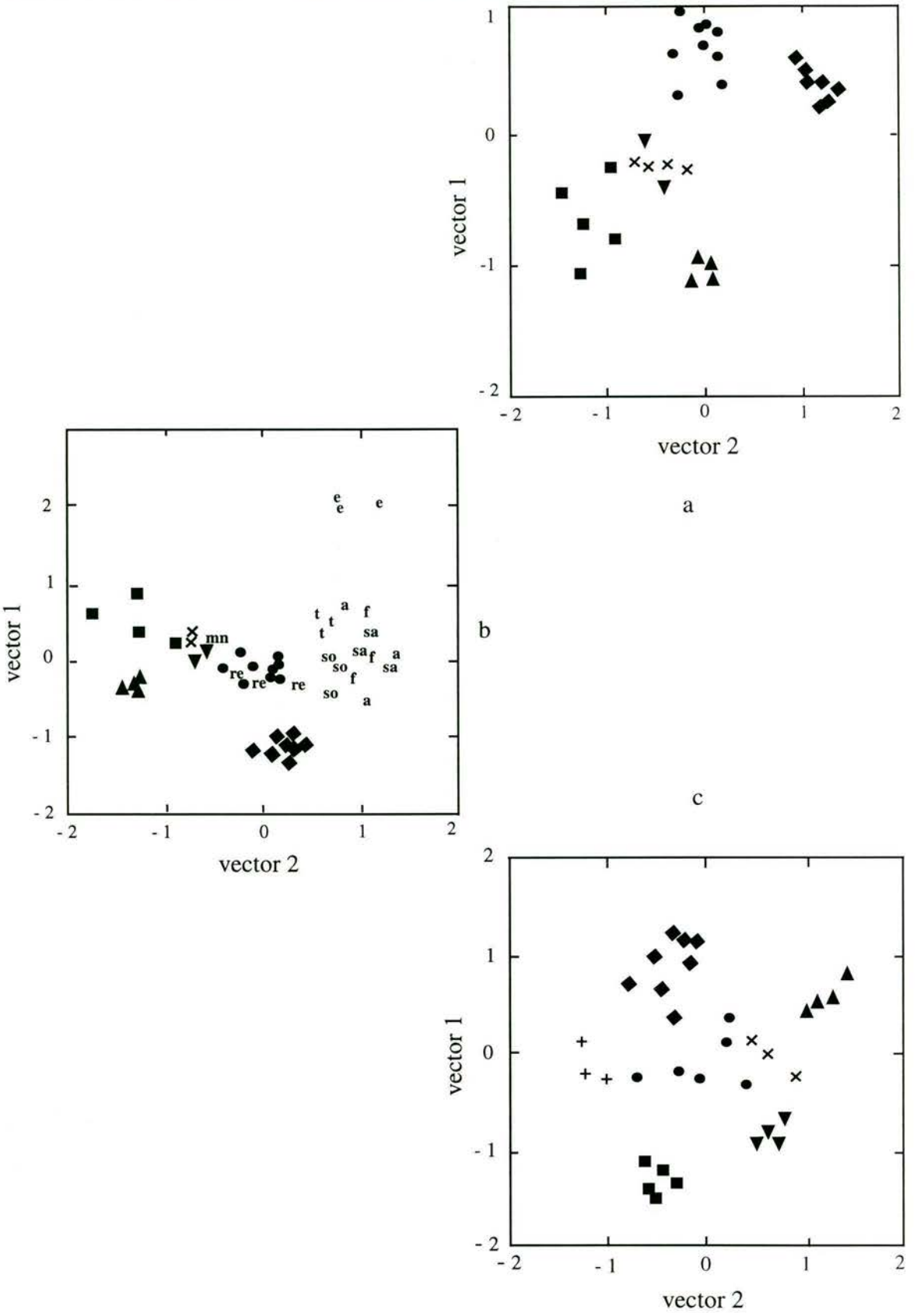


Figure 7-2. Scatter plots of SSH in two dimensions using **a** & **b**: Gower Metric for morphological data, and **c**: Dice association measure for RAPD data. **a**: Australian samples; **b**: Austalian & New Zealand samples; *E. rectisetus* (◆); *E. scaber* (●); *E. plurinervis* (▲); *E.* sp. nov. (▼); *E. multiflorus* (■); short-awned samples from NSW (×); Tasmanian *E. scaber* (+); *E. apricus* (a); *E. falcis* (f); *E. enysii* (e); *E. sacandros* (sa); *E. solandri* (so); *E. tenuis* (t); New Zealand *E. rectisetus* (re); a member of *E. multiflorus* from New Zealand (mn).

and *E. multiflorus* are well defined. *Elymus rectisetus* is morphologically and molecularly close to *E. scaber*. There is an unrecognised taxon, *E.* sp. nov. Disagreements: *E. scaber* is morphologically distinct but is genetically more varied. The short-awned samples from New South Wales are morphologically similar to *E.* sp. nov. (Figure 7-2. a & b) whereas they genetically more closely resemble *E. scaber* (Figure 7-2. c). Owing to these disagreements, problems arise when attempting to reach a final taxonomic conclusion. The problems are:

- How many taxa should be recognised for Australian *Elymus*, five (morphologically: Figure 7-1. a; Figure 7-2. a) or six (genetically: Figure 7-2. c)?
- Should the short-awned samples from areas other than Queensland be grouped with *E.* sp. nov. or with *E. scaber* var. *scaber*?
- Is *E.* sp. nov. worthy of a separate species or not (Figure 7-1.b)?
- Finally, is it defensible to accord *E. rectisetus* and *E. scaber* specific ranks if other information, such as reproductive pattern, is taken into account?

If taxonomy is to be based on morphological distinctness alone, then five clusters (figure 7-1. a) may be interpreted as five species, and the short-awned samples from areas other than Queensland should undoubtedly be part of *E.* sp. nov. However, these conclusions will not only ignore the genetic information but also contradict some other available evidence, such as ecology, geography, cytology and genetics. Following the principle of the cohesion species concept, reasonable conclusions about the *Elymus* complex should be attained based on comprehensive and consistent application of all information. This consideration leads to the detailed discussion presented in the following paragraphs.

Elymus plurinervis is a good species in the *Elymus scaber* complex for the following reasons: its distinctness is revealed by morphological data (large glumes with broad membranous margins, upper glumes with numerous veins, glabrous and broadly triangular callus, semi-circular to transverse-circular rachilla top, awn entered by one vein, high ratio of palea : fruit length), and molecular data (13 specific markers are fixed to it). In practice, it is easily distinguished in the field and from herbarium specimens.

Geographically, it has a characteristic habitat and geographical distribution — it is found on dark brown basaltic soils of southeastern Queensland and northern New South Wales. In addition, presumably no gene flow occurs between *E. plurinervis* and the sympatric *E.* sp. nov. because their hybrids are sterile (Carman and Wang, 1992).

Elymus multiflorus is distinguished by its morphological characters (very short and straight awns, glabrous and broadly triangular callus), genetic markers (13 specific markers are fixed to it), and ecological preferences (coastal or riverine areas). It is not difficult to identify it from *E.* sp. nov. even from herbarium material, and its morphological characters and coastal or riverine habitat help to distinguish it from the short-awned forms of *E. scaber* found in New South Wales and Victoria. Specimens collected from Australian coastal and riverine areas are synonymous with New Zealand *E. multiflorus*.

The reasons for recognising *E.* sp. nov. are: firstly, it is morphologically distinct (erect habit, rather short and straight awns, glabrous and broadly triangular callus), and the differences between this taxon and the closely related *E. multiflorus* are also obvious (e.g. *E.* sp. nov. has longer spikelets and lemma awns, higher ratio of awn length to lemma length than those of *E. multiflorus*, and the latter has more often bifid lemma apices than *E.* sp. nov; see Chapter 4). Secondly, its genetic differentiation from other taxa is revealed by molecular data (11 specific markers are fixed to it). Thirdly, it is restricted to dark brown basaltic soils of southeastern Queensland and northern New South Wales, sympatric with *E. plurinervis*. Finally, it is obligately sexual (Crane and Carman, 1987; Murphy (pers. comm.), which makes it distinguishable from the apomictic *E. scaber* (see the following discussion) though it was placed close to the latter in the molecular analysis (Figure 7-2. c). In addition, emphasis is placed on the heterogeneity of the short-awned form from other areas with *E.* sp. nov., and the former is now grouped into *E. scaber* (see below).

Elymus enysii is markedly different from all *Elymus* species from Australia and New Zealand. Evidence includes morphological data (short and compact inflorescence, distinctive callus and rachilla characters; see Plate 5.1), as well as genetic data

(tetraploid, having the H genome but no S genome; Svitashev *et al.* , 1996). The revision of its generic status is probably unavoidable. This taxon is endemic to New Zealand.

Elymus tenuis is a rather distinct species restricted to New Zealand as previously indicated (Chapter 5). The morphological and genetic distinctness of *E. tenuis* are: greatly elongating flowering culms, long-awned glumes (confirmed in this study) and octoploidy (Connor, 1994).

Elymus rectisetus was established based on Western Australian material, and *E. scaber* on Tasmanian material. Their morphological distinctness revealed in this research includes rachilla hairiness, callus shape and hairiness, lemma apex shape, palea apex structure, and the ratio of fruit length to palea length (see Chapter 4). Molecular data also indicate their genetic differences: four specific markers were fixed to *E. rectisetus* (see Chapter 6). This evidence supports the two taxa being accorded specific rank. However, the following consideration casts doubt on this treatment.

Most members of these taxa may be included in the so called long-awned category where apomixis was reported (Crane and Carman, 1987; Murphy and Jones, 1999). Therefore, if the two taxa were treated as separate species, both would probably be found to be apomicts. This deduction has been partly confirmed by the embryological work conducted by Murphy (pers. comm.) on two specimens of *E. scaber* (OTUs 62 and 78). Both are confirmed to be apomictic. However, sexual reproduction probably also occurs in the two taxa (Murphy, 1998). In other words, they are possibly both facultative apomicts. Their morphological and genetic distinctness may be preserved for a long time by the reproductive pattern even though they are sympatric. Moreover, the remarkable level of local genetic differentiation (e.g. Tasmanian individuals) within *E. scaber* revealed in this study can be also interpreted as the result of isolation by distance and reproduction, a phenomenon occurring in apomictic or selfing species (Heywood, 1991).

Considering their apomictic reproductive pattern, one might simply lump them together as a polymorphic taxon as was traditionally done. However, a suggestion raised by Löve (1960) is followed here. That is to treat them as two subspecies of *E. scaber*: subsp. *rectisetus* and subsp. *scaber*. This decision is based upon their morphological distinctness and geographical distribution pattern: each has its own distribution area (subsp. *rectisetus* in Western Australian, and subsp. *scaber* in Tasmania), and both are sympatric in the Great Dividing Range to South Australia.

Elymus scaber subsp. *rectisetus* is characterised by narrowly triangular calluses with hairs restricted to the margins, long and densely hairy rachillas with hairs covering the base of callus, typically lorate and canaliculate lemma apices, long palea tips above the flanks, and a rather high ratio of palea : fruit length. *Elymus scaber* subsp. *scaber* is characterised by triangular and hairy calluses with hairs distributed on the surface, thickened tips of calluses (abaxial), hairy rachillas but hairs not covering the callus base, acuminate lemma apices, and short to nil palea tip above the flanks. In addition, New Zealand *E. rectisetus* is considered to be a mis-identification and should be identified as *E. scaber* subsp. *scaber*.

The short-awned form from New South Wales (excluding those from dark brown basaltic soils of northern areas) represents a group of individuals occurring along the Great Dividing Range. They have somewhat intermediate characters, such as short to rather long awns, triangular to broadly triangular and glabrous calluses. These characters pull them closer to *E.* sp. nov. and *E. multiflorus* (Figure 7-2. a & b). However, molecular data separated them from *E.* sp. nov. and *E. multiflorus*, and placed them together with the main cluster of *E. scaber* subsp. *scaber*. The result obtained from molecular data is supported by the geographical and ecological data. For example, some individuals (e.g. OTUs 86 and 87) are separated into two clusters (*E. scaber* subsp. *scaber* vs. *E.* sp. nov.) but are found from the same localities. The molecular data are judged here to be more indicative of the situation. This conclusion is further upheld by embryological evidence (Murphy, pers. comm.) which indicated that the short-awned forms from NSW are facultatively apomictic, whereas *E.* sp. nov. and *E. multiflorus* are obligately sexual. Individuals characterised by short-awned

lemmas and/or broadly triangular and glabrous calluses are considered to contribute to the polymorphism of the currently defined *E. scaber* subsp. *scaber*. The variation may result from facultative apomixis observed in *E. scaber* subsp. *scaber* which allows occasional hybridisation to occur when it is sympatric with *E.* sp. nov. in some localities. The alternative explanation is probably that *E.* sp. nov. evolved from the short awned form by gene drift and subsequent natural selection.

A formal taxonomic conclusion about the remainder of New Zealand *Elymus* species, namely, *E. apricus*, *E. falcis*, *E. sacandros* and *E. solandri*, cannot be attained here due to the unresolved morphological variation patterns obtained in this study (see Chapter 5). Presumably they represent morphological types and maintain themselves as pure lines by self-fertilisation.

The final taxonomic conclusions are given as follows:

Elymus scaber
subsp. *scaber* (Aust. and NZ)
subsp. *rectisetus comb. et stat. nov.* (Aust.)
Elymus plurinervis comb. et stat. nov. (Aust.)
Elymus multiflorus (Aust. and NZ)
Elymus fertilis sp. nov. (Aust.)
Elymus tenuis (NZ)
Elymus solandri (NZ)
Elymus apricus (NZ)
Elymus falcis (NZ)
Elymus sacandros (NZ)

Elymus enysii (NZ)

7.1.3. Important morphological characters

The traditional characters used for diagnostic purposes within Australian *Elymus* species were the dimensions of glumes, the number of nerves of glumes, and the length of the lemma awns. The present study indicates that the first two characters are informative (with the restriction that only the number of nerves of the upper glumes is informative) for distinguishing *E. plurinervis*, whereas awn length longer than 15 mm or not, is revealed as being less informative than previously considered (see Chapter 4). Nevertheless, characters of the callus, rachilla and palea tip developed in this research are diagnostically valuable for identification of *Elymus* species, and for the separation of the alien element (*E. enysii*) from *Elymus*. These characters may be correlated somewhat to the reproductive patterns found in this species complex. For example, the hairy callus and densely hairy rachilla are correlated with apomixis, and the broadly triangular and glabrous callus appear to be indicators of sexuality.

7.1.4. Consideration of the phenetic school

Subjectivity is one of the defects of the phenetic school (Stuessy, 1990). This has been experienced in this research. For example, though some characters were continuous (e.g. rachilla hairiness, callus shape), they were scored as several states. Some characters are related to growing period (e. g. awn curvature) and could not be confidently scored from herbarium specimens, whereas other characters (e.g. culm elongating after anthesis or not) could be only obtained by observing the living material for at least one growing season. Such characters could not be included in these analyses though their potential taxonomic value was apparent.

Theoretically, characters used in phenetic classification should include all aspects, even molecular aspects, of the whole specimen examined (see Chapter 1). Practically, this is hard to be fulfilled. On one hand, not any taxonomists can examine all characters — collaboration with other workers may be needed; on the other hand, some characters may be discarded before analysis because preconceived notions by taxonomists are unavoidable (Barkworth, 1992). For the present study, 251 characters were developed

whereas only small part of them were used in the analyses (28 were used for the first analysis, 37 for the second, and 51 for the third). Selection was based on the author's observation of the characters (see Chapter 3). There is also limitation for analysis using combined data sets (e.g. combination of all four data sets in this study). One reason was that not all characters were available from all OTUs as discussed above. Another obvious reason was that the samples from which molecular data and morphological data were collected were partly different — morphological data were mainly obtained from herbarium specimens, whereas molecular data were obtained from living material. It is considered, nevertheless, that a final taxonomic interpretation can be made by comprehensive consideration of different data sets without a further analysis using combined data set.

Mayer (1963) criticised the morphological species concept as being highly misleading when dealing with polymorphic species or species that are morphologically extremely similar. This has been the case in the taxonomic history of the *Elymus scaber* complex. As for the currently defined taxa, the morphological distinction between the short-awned entities (*E.* sp. nov. and *E. multiflorus*) and *E. scaber* subsp. *scaber* is blurred by the short-awned form of the latter taxon. In order to distinguish them, ecological, geographical and genetic data, as well as reproductive pattern, are needed to be referred to.

Although there are some defects, phenetic analysis is still a useful method to find the morphological pattern amongst individuals. However, one should realise the possible incorrectness introduced by an unspecified level of subjectivity in the analytic process. Furthermore, it seems unnecessary to equate a cluster with a taxon, or to distinguish the taxa based on the same variation level. For example, the morphological distinction between the two subspecies of *E. scaber* is comparable with that between *E. plurinervis* and *E. multiflorus* (Figure 7-1. a), but they are only granted infraspecific rank. It is here preferred to treat the variation patterns revealed by phenetic analysis as a general guide, and to subsequently bring as much information as possible together to attain a reasonable taxonomic conclusion. In this case, the principle of the cohesion species concept is actually followed.

7.2. Formal taxonomic treatment of the Australian *Elymus*

7.2.1. Key to the Australian species of *Elymus*

Notes on the Key

- The apices of glumes and lemmas of *Elymus* species, except *E. plurinervis*, characteristically taper into points or awns, therefore the lengths of awns are obtained following a standard used in this research: 'awns' are measured from the point at which glume or lemma apex is about 0.5 mm wide.
- Average awn length of a spikelet does not include the proximal and apical 1–2 lemmas.

Key to the Australian species of *Elymus*

1 Rachilla hairs very short (< 0.2 mm); callus broadly triangular, glabrous to glabrescent.

2 Glumes usually < 7 mm long; lemma awn straight when dry, entered by 3 veins; the ratio of fruit : palea length ≥ 0.61.

3 length of lemma awns 4.4–15 mm; length of proximal lemma awns 2–7.5 mm; average awn length : lemma length 0.7–1.3; restricted to dark brown basaltic soils of southeastern Qld and northern NSW ..... 1. *E. fertilis*

3 length of lemma awns 0–11 mm; length of proximal lemma awns 0–3 mm; average awn length : lemma length 0.2–0.8; coastal or riverine plants ..... 2. *E. multiflorus*

2 Glumes ≥ 7.7 mm long; lemma awn regularly curved when dry, entered by 1 vein; the ratio of fruit : palea length < 0.55; Qld and NSW commonly on dark brown basaltic soils..... 3. *E. plurinervis*

1 Rachilla hairs usually rather long (0.2–1.0 mm); callus usually triangular to narrowly triangular, usually distinctly hairy (if rachilla shortly hairy and callus glabrous to glabrescent, then lemma awn usually curved when dry) ..... 4. *E. scaber*

7.2.2. Description of taxa

The characters of morphology, anatomy and some related information of the Australian *Elymus* species are briefly described as follows. Five specimens are listed for each taxon and more specimens are given in Appendix VIII.

1. ***Elymus fertilis*** S. Wang *et* M. J. Henwood, sp. nov.

T: Bank of Brisbane River, Ascot, Brisbane, Qld., 13 Aug. 1930, C. E. Hubbard 3654; holo: MEL.

Illustration: Plates 7.1 & 7.2.

Habit, vegetative morphology. Caespitose, perennial. Culms erect or drooping, to c. 55 cm tall, 0.7–1.7 mm diameter, glaucous, 3–6 noded. Basal leaf sheaths glabrous or hairy; margins hyaline or membranous, smooth. Auricles long (clasping) or minute or absent, 0–1.9 mm long, glabrous. Ligule 0.4–0.5 mm long, membranous, truncate. Collar glabrous or hairy. Leaf blade flat, to c. 20 cm long, 1.8–4.7 mm wide, adaxially glabrous or scabrous or hairy, abaxially glabrous or scabrous.

Inflorescence. Inflorescence axis 210–685 mm long. Peduncles glabrous or scabrous. Rachis 115–245 mm long, average length of internode 20.3–38 mm, glabrous or scabrous.

Spikelets, florets, fruit. Spikelets 5–10 on the rachis, solitary at each node, 26–39 mm long, 4–9-flowered, average spikelet length : internode length of rachis 0.9–1.19. Glumes subequal. Lower glume 4.8–6.1 mm long, 0.8–1.2 mm wide, glabrous or scabrous, 4–5-veined; apex awned, awn 1–1.8 mm long; margin membranous, 0.3–0.6 mm wide. Upper glume 5.6–7 mm long, 0.7–1.2 mm wide, glabrous or scabrous, 4–6-veined; apex awned, awn 1–1.5 mm long; margin hyaline or membranous, 0.3–0.5 mm wide. Rachilla segments 1.8–2.5 mm long, hairy; hairs short; apex ovate, obovate to circular. Callus 0.6–0.9 mm long, broadly triangular; dorsal surface flat or sunken,

glabrous to glabrescent; tip not thickened; adaxial margin thickening extending approximately to the mid-point. Lemma 8.5–10 mm long, dorsally glabrous or scabrous, not pruinose; 5-veined; margin hyaline or membranous, 0.1–0.3 mm wide; apex acuminate, flat, entire or bifid, awned; awns 4.4–14.5 mm long, remaining straight when dry, scabrous, entered by 3 veins. Proximal lemma awned, awn 2–7.5 mm long. Average awn length : proximal lemma length 0.7–1.3. Palea 8–9.1 mm long, 1–1.4 mm wide, 2-keeled; keels ciliolate, hairs 0.1–0.18 mm long; intercostal region scabrous or hairy; apex narrower to wider than lemma apex, truncate or obtuse, entire or retuse; flanks (abaxial) ending at or under the top; tip above flanks 0–0.27 mm long. Lodicules 0.9–1.4 mm long; margin lobed or entire, divisions lateral. Stamens 3; anthers 1.6–2.6 mm long, yellow or purple. Fruit narrowly elliptical or narrowly oblong, 5.2–6.7 mm long, 1–1.3 mm wide; ratio of width : length 0.18–0.22; ratio of fruit length : palea length 0.67–0.73; longitudinally grooved or deeply furrowed.

Abaxial leaf blade epidermis. Long-cells 8–12 rows on the intercostal region. Long-cell wall straight or nearly straight, or sinusoid. Stomata 2 rows on intercostal region; subsidiary cells parallel-sided. Short-cells present or absent on intercostal region, individually or in pairs; present over veins, individually or in pairs. Silica bodies tall and narrow, or cuboidal, or horizontally long and sinuous or smooth. Papillae present, only over veins. Prickles present, over veins or on intercostal region, different in shape if present over veins and on intercostal region. Macrohairs absent.

Transverse sections of the leaf blade. Leaf having distinct, prominent adaxial ribs only, or having nodular structure; adaxial ribs very irregular in size, top obtuse; midrib inconspicuous. Bulliform cells in simple fan-shaped groups, or no bulliform cells in simple fan-shaped groups. Vascular bundles accompanied by sclerenchyma, forming an "I".

Genomic information. Genomic polyploidy: 2n = 42. Genomic constitution: SYW (Torabinejad *et al.* 1987; Torabinejad and Mueller, 1993).

Distribution. Found in southeastern Qld. and northern NSW, usually from dark brown basaltic soils in open areas, along roads. Sympatric with *E. plurinervis* (Figure 7-3).
Flowering. August to March.

Etymology. The epithet '*fertilis*' refers to the high fertility of the species.

Selected specimens. Qld: Wyreema, 17 Mar. 1931, C. E. Hubbard 5879 (MEL); Between Laidley and Forest Hill, 28 Nov. 1930, C. E. Hubbard, 5311 (MEL); Cherribah, 14 Jan. 1996, S. Wang 95141 (RAPD, SYD); Toowoomba, 15 Jan. 1996, S. Wang 95158 (RAPD, SYD). NSW: Moree, 7 May 1997, M. Murphy 431 (NE).

Comments. This species is similar to *E. multiflorus* on account of its short-awned spikelets, and was synonymised with the latter by Connor (1994), or with *E. scaber* subsp. *scaber* by others (Crane and Carman, 1987; Murphy and Jones, 1999). It is here treated as a new species. The awn length of *E. fertilis* somewhat overlaps with *E. multiflorus*. However, *E. fertilis* is restricted to the dark brown basaltic soils of southeastern Queensland and northern New South Wales, whereas *E. multiflorus* is usually found in coastal and riverine areas. Moreover, *E. fertilis* is genetically different from *E. multiflorus* as revealed by molecular analysis. *Elymus fertilis* is further distinguished by its high fertility observed both in the field and under cultivation. Carman *et al.* (1991) and Murphy and Jones (1999) confirmed that this species is obligately sexual. It is probable that this species was used by Torabinejad *et al.* (1987) and Torabinejad and Mueller (1993a) to determine the current genomic constitution of all Australian *Elymus* species.

2. ***Elymus multiflorus*** (Banks *et* Sol. ex Hook. f.) A. Löve *et* Connor, New Zeal. J. Bot. 20: 183 (1982)

Triticum multiflorum Banks *et* Sol. ex Hook. f., Fl. New Zeal. 1: 311(1853); *Agropyron multiflorum* (Banks. *et* Sol. ex Hook. f.) Cheeseman, Man. New Zeal. Fl. 921 (1906); *Agropyron kirkii* Zotov T. R. S. N. Z. 73: 233 (1943). T: Mercury Bay, New Zeal., Nov. 1769, J. Banks and D. Solander; lecto: BM.

Illustration: Plates 7.3 & 7.4.

Habit, vegetative morphology. Caespitose, perennial. Culms erect or geniculate or ascending or drooping, to c. 55 cm tall, 0.9–3 mm diameter, glaucous or not glaucous, 3–7 noded. Basal leaf sheaths glabrous, or hairy; margins hyaline or membranous, smooth. Auricles long (clasping) or minute, 0–1.7 mm long, glabrous or hairy. Ligule 0.5–0.6 mm long, membranous or chartaceous, truncate. Collar glabrous. Leaf blade flat, to c. 30 cm long, 1.8–6.8 mm wide, adaxially glabrous or scabrous, abaxially glabrous or scabrous or hairy.

Inflorescence. Inflorescence axis 125–470 mm long. Peduncles glabrous. Rachis 58–240 mm long, average length of internode 10.7–33.3 mm, glabrous or scabrous.

Spikelets, florets, fruit. Spikelets 6–18 on the rachis, solitary at each node or paired at base (rare), 16–35 mm long, 4–10-flowered, average spikelet length : internode length of rachis 0.6–1.6. Glumes subequal. Lower glume 3.4–7.9 mm long, 0.7–1.2 mm wide, glabrous, 4–6-veined; apex muticous or awned, awn 0.4–1.3 mm long; margin hyaline or membranous, 0.1–0.4 mm wide. Upper glume 5.2–9.2 mm long, 0.6–1.3 mm wide, glabrous, 5–7-veined; apex muticous or awned, awn 0.3–1.2 mm long; margin hyaline or membranous, 0.1–0.5 mm wide. Rachilla segments 1.1–2.3 mm long, hairy; hairs short; apex ovate, obovate to circular. Callus 0.8–1 mm long, broadly triangular; dorsal surface sunken, glabrous to glabrescent; tip not thickened; adaxial margin thickening extending approximately to the mid-point. Lemma 6.9–12.5 mm long, dorsally glabrous or scabrous, not pruinose; 5-veined; margin hyaline or membranous or chartaceous, 0.1 mm wide; apex acuminate, canaliculate or flat, entire or bifid, awned or mucronate or muticous; awns 0–11.5 mm long, remaining straight when dry, scabrous, entered by 3 veins. Proximal lemma muticous or mucronate or awned, awn 0–3 mm long. Average awn length : proximal lemma length 0.2–0.8. Palea 7.1–9.8 mm long, 0.9–1.2 mm wide, 2-keeled; keels ciliolate, hairs 0.14–0.3 mm long; intercostal region scabrous or hairy; apex narrower to wider than lemma apex, truncate or obtuse, entire or retuse; flanks (abaxial) ending at or under the top; tip above flanks 0–0.18 mm long. Lodicules 0.9–1.3 mm long; margin lobed or entire, divisions lateral. Stamens 3; anthers 1.6–3.8

mm long, yellow or purple or yellow and purple. Fruit narrowly elliptical or narrowly ovoid or narrowly oblong, 5.8–6.6 mm long, 1.1–1.7 mm wide; ratio of width : length 0.22–0.28; ratio of fruit length : palea length 0.61–0.71; longitudinally grooved or deeply furrowed.

Abaxial leaf blade epidermis. Long-cells 5–8 rows on the intercostal region. Long-cell wall sinuous or tessellate. Stomata 0–2 rows on intercostal region; subsidiary cells parallel-sided, or low dome-shaped. Short-cells present on intercostal region, individually or in pairs; present over veins, individually or in pairs or in horizontal rows. Silica bodies tall and narrow, or rounded. Papillae present, only over veins. Prickles present or absent, over veins, different in shape. Macrohairs absent.

Transverse sections of the leaf blade. Leaf having distinct, prominent adaxial ribs only; adaxial ribs very irregular in size, top truncate or nearly truncate or obtuse; midrib inconspicuous. Bulliform cells occurring in simple fan-shaped groups, or no bulliform cells in simple fan-shaped groups. Vascular bundles accompanied by sclerenchyma, forming a "T" or forming an "I".

Genomic information. Genomic polyploidy: 2n = 42. Genomic constitution: SSH? or SYW? The genomic constitution of this species was first reported by Löve and Connor (1982) as SSH. However, it was possibly not used by Torabinejad *et al.* (1987) in their experiment. Therefore, further investigation is suggested in order to confirm its genomic constitution.

Distribution. Found in eastern NSW, Vic., SA and Tas., usually from sand dunes of foreshore, river bank, or some inland areas. Also in New Zealand (Figure 7-5).

Flowering. October to March (June?).

Selected specimens. NSW: Broulee Beach, 6 June 1981, K. R. Thiele 249 (MEL); Boat Harbour, 23 Oct 1969, S. Jacobs (SYD). Vic.: Captain Cook NP., 11 Dec. 1969, A. C.

Beauglehole & E. W. Finck 32316 (MEL); West of Mildura, Nov. 1986, D. Cheal (MEL). Tas.: Maria Island, 24 Nov. 1968, J. E. S. Townrow (HO).

Comments. This species is widespread but not common in Australian coastal or riverine areas. In some localities (e.g. Broulee, NSW), individuals occurring on the open beach seem to be shorter-awned than those under the forest. The latter are somewhat morphologically similar to *E. fertilis*. However, the RAPD data indicated that individuals from the beach and under the forest were genetically similar to each other but both are distinct from *E. fertilis*. The transplants from coastal areas markedly changed their habit during cultivation. They shifted from an erect to an ascending habit, and some of them did not produce normal inflorescences. In addition, they are probably prone to rusts. Some New Zealand specimens have no awns or much shorter awns than Australian material, or some have more or less longer awns similar to Australian individuals under the forest. Connor (1994) discussed the variations of the awn length within New Zealand *E. multiflorus*, and he (Connor, 1982) reduced var. *longisetus*, erected by Hackel in Cheeseman (1906), to synonymy with *E. multiflorus*. Presumably Connor's circumscription of *E. multiflorus* included only the Australian coastal and riverine material but not the short-awned individuals currently defined as *E. fertilis*.

3. ***Elymus plurinervis*** (Vickery) S. Wang *et* M. J. Henwood, comb. et stat. nov.

Agropyron scabrum (R. Br.) P. Beauv. var. *plurinerve* Vickery, Contr. New South Wales Nat. Herb. 1: 342 (1951). *Elymus scaber* var. *plurinervis* (Vickery) B. Simon, Austrobaileya 2(3): 242 (1986). T: Inverell, N.S.W., Dec. 1912, E. O. Thomas; holo: NSW, *n.v., fide* J. W. Vickery, Contr. New South Wales Nat. Herb. 1: 342 (1951). I have based my interpretation of this taxon on: Inverell, NSW, Nov. 1912, E. O. Thomas (NSW 8246).

Illustration: Plates 7.5 & 7.6.

Habit, vegetative morphology. Caespitose, perennial. Culms erect or geniculate, to c. 55 cm tall, 1–2.1 mm diameter, not glaucous, 2–4 noded. Basal leaf sheaths glabrous,

or hairy; margins hyaline or membranous, smooth. Auricles long (clasping) or minute, 0.05–1.6 mm long, glabrous. Ligule 0.3–0.8 mm long, hyaline or membranous, obtuse or truncate. Collar glabrous. Leaf blade flat, to c. 30 cm long, 1.8–4.1 mm wide, adaxially glabrous or scabrous, abaxially glabrous.

Inflorescence. Inflorescence axis 210–700 mm long. Peduncles glabrous. Rachis 80–250 mm long, average length of internode 12.7–35.3 mm, glabrous or hairy.

Spikelets, florets, fruit. Spikelets 6–13 on the rachis, solitary at each node, 25–48 mm long, 4–8 -flowered, average spikelet length : internode length of rachis 1.3–2.09. Glumes equal or subequal. Lower glume 7.7–11 mm long, 0.7–1.7 mm wide, glabrous, 5–6-veined; apex muticous or apiculate or awned, awn 0.5–0.7 mm long; margin hyaline or membranous, 0.4–0.9 mm wide. Upper glume 8.9–12.5 mm long, 1–1.5 mm wide, glabrous, 5–8-veined; apex muticous or apiculate or mucronate or awned, awn 0.5 mm long; margin hyaline or membranous, 0.4–0.8 mm wide. Rachilla segments 1.3–2.1 mm long, hairy; hairs short; apex semi-circular to transverse-circular. Callus 0.7–1.2 mm long, broadly triangular; dorsal surface flat, glabrous to glabrescent; tip not thickened; adaxial margin thickening extending approximately to the mid-point. Lemma 12–14.5 mm long, dorsally glabrous or scabrous or pubescent, not pruinose; 5–7-veined; margin hyaline or membranous, 0.1–0.5 mm wide; apex acuminate, flat or folded, entire or bifid, awned; awns 17–27 mm long, uniformly recurved when dry, scabrous, entered by 1 vein. Proximal lemma awned, awn 8.5–22.5 mm long. Average awn length : proximal lemma length 1.5–2. Palea 8.1–11 mm long, 1.4–1.8 mm wide, 2-keeled; keels scabrous or ciliolate, hairs 0.1–0.3 mm long; intercostal region glabrous; apex narrower than lemma apex or equal to lemma apex, truncate or obtuse, entire or retuse; flanks (abaxial) ending under the top; tip above flanks 0.1–0.64 mm long. Lodicules 0.9–1.4 mm long; margin lobed or entire, divisions lateral. Stamens 3; anthers 2.3–3 mm long, yellow or purple. Fruit narrowly obovoid, 4.1–5.8 mm long, 1.1–1.7 mm wide; ratio of width : length 0.22–0.32; ratio of fruit length : palea length 0.47–0.55; longitudinally grooved.

Abaxial leaf blade epidermis. Long-cells 5–10 rows on the intercostal region. Long-cell wall straight or nearly straight, or sinusoid. Stomata 0–2 rows on intercostal region; subsidiary cells parallel-sided. Short-cells present on intercostal region, individually or in pairs; present over veins, in pairs or in horizontal rows. Silica bodies tall and narrow, or rounded, or cuboidal, or horizontally long and sinuous or smooth. Papillae present, over veins and on intercostal region. Prickles present or absent, over veins or on intercostal region, different or similar in shape. Macrohairs absent.

Transverse sections of the leaf blade. Leaf having distinct, prominent adaxial ribs only; adaxial ribs more or less constant in size or very irregular in size, top truncate or nearly truncate or obtuse; midrib inconspicuous. Bulliform cells occurring in simple fan-shaped groups, or no bulliform cells in simple fan-shaped groups. Vascular bundles accompanied by sclerenchyma, forming a "T" or forming an "I".

Genomic information. Genomic polyploidy: 2n = 42. Genomic constitution: SYW.

Distribution. Found in southeastern Qld, also in northern NSW, usually occurs in dark brown basaltic soils of open area, along road and fence lines (Figure 7-4).

Flowering. October to April.

Selected specimens. Qld: 4 km E. of Maryvale, 15 Jan. 1996, S. Wang 95153 (SYD); 7 km SW of Toowoomba, 10 Apr. 1994, R. J. Fensham 1728 (BRI); Oakey, 10 Apr. 1994, R. J. Fensham 1343 (BRI); 18 km ESE of Cecil Plains, 13 Apr. 1994, R. J. Fensham 1307 (BRI). NSW: 58 km N of Moree on Newell Hwy, 14 Oct. 1988, N. Lloyd 735 (NSW).

Comments. This species is distinct from all other *Elymus* species in the Southern Hemisphere. In the field, it is characterised by the robust nature of its glumes, its regularly curved awns when mature, and its somewhat elliptical spikelets when young. The characters of one nerve entering the awn, wide membranous margins of glumes and lemmas, and high ratio of fruit to palea length indicate its distant relationship to other

Elymus species. A few specimens of *E. scaber* from islands (e.g. MEL1560479) were observed to have larger glumes. However, the callus characters (e.g. callus hairy and tip thickened) and the awns entered by three veins helped to group them with *E. scaber* instead of with *E. plurinervis*. There are a few specimens (e.g. MEL1560511) with somewhat intermediate characters: their larger glumes and characteristic spikelet shape are similar to *E. plurinervis*, but three nerves entering awn is similar to *E. scaber*. These specimen may be of hybrid again.

4. ***Elymus scaber*** (R. Br.) A. Löve, Feddes Repert. 95: 468 (1984)

Festuca scabra Labill., Nov. Holl. Pl. Sp. 1: 22, t. 26 (1804) nom. illeg. non Vahl; *Triticum scabrum* R. Br., Prodr. Fl. Nov. Holl.: 178 (1810); *Agropyron scabrum* (R. Br.) P. Beauv., Ess. Agrostogr. 102 (1812); *Vulpia scabra* (R. Br.) Nees in Lehm., Pl. Preiss. 2: 108 (1846); *Vulpia Browniana* Nees in Lehm., Pl. Preiss. 2: 107 (1846); *Festuca Browniana* (Nees in Lehm.) Steud., Syn. Pl. Glum., I: 304 (1854); *Festuca Billardieri* Steud., Syn. Pl. Glum. I: 304 (1854); *Anthosachne scabra* (R. Br.) Nevski, Tr. Sredneaz. Univ., ser. 8B, 17: 65 (1934). T: in capite Van-Dieman (Tas.), 1793, J. J. H. de Labillardière; holo: FI.

Habit, vegetative morphology. Rhizomatous (rare) or caespitose, annual (rare) or perennial. Culms erect or geniculate or ascending or drooping, to c. 1 m tall, 0.6–2.3 mm diameter, glaucous or not glaucous, 2–5 noded. Basal leaf sheaths glabrous, or hairy; margins hyaline or membranous or chartaceous, smooth. Auricles long (clasping) or minute or absent, 0–2.4 mm long, glabrous or hairy. Ligule 0.2–1.2 mm long, membranous or chartaceous, obtuse or truncate. Collar glabrous, or hairy. Leaf blade flat, to c. 30 cm long, 1.6–6.9 mm wide, adaxially glabrous or scabrous or hairy, abaxially glabrous or scabrous or hairy.

Inflorescence. Inflorescence axis 67–1255 mm long. Peduncles glabrous, or scabrous, or pubescent. Rachis (0–)15–325 mm long, average length of internode (0–)8–45.3 mm, glabrous or scabrous or hairy.

Spikelets, florets, fruit. Spikelets (1–)3–12(–19) on the rachis, solitary at each node, 18–101 mm long, 2–12 -flowered, average spikelet length : internode length of rachis 0.5–5.8. Glumes equal or subequal. Lower glume 1.3–12 mm long, 0.3–1.4 mm wide, glabrous or scabrous, 2–5-veined; apex muticous or awned, awn 0–5.3 mm long; margin hyaline or membranous or chartaceous, 0.1–0.9 mm wide. Upper glume 2–12.6 mm long, 0.4–1.3 mm wide, glabrous or scabrous, 3–6 (7)-veined; apex muticous or awned, awn 0–7.6 mm long; margin hyaline or membranous, 0.1–0.7 mm wide. Rachilla segments 1–3.9 mm long, hairy; hairs long and dense, covering callus base, or short to rather long, not covering callus base; apex oblong or elliptical, or ovate, obovate to circular. Callus 0.5–2.5 mm long, narrowly triangular to broadly triangular; dorsal surface rounded or flat or sunken, glabrous to glabrescent or densely hairy; hairs restricted to margins, or on margins and surface; tip thickened or not thickened; adaxial margin thickening restricted to below the mid-point, or extending approximately to the mid-point. Lemma 6–40 mm long, dorsally glabrous or scabrous, pruinose or not pruinose; 5 (–7)-veined; margin hyaline or membranous, 0.05–0.2 mm wide; apex nearly lorate or acuminate, canaliculate or flat, entire, awned or mucronate; awns (3–)15–49 mm long, straight to variously recurved when dry, scabrous, entered by 3 veins. Proximal lemma awned, awn (0.5–)2–49 mm long. Average awn length : proximal lemma length 0.9–5.8. Palea 5.2–12.7 mm long, 0.7–1.7 mm wide, 2-keeled; keels scabrous or ciliolate, hairs 0.1–0.5 mm long; intercostal region scabrous, or hairy; apex narrower to wider than lemma apex, truncate or obtuse or pointed, entire or retuse or bifid; flanks (abaxial) ending at or under the top; tip above flanks 0–1 mm long. Lodicules 0.7–1.9 mm long; margin lobed or entire, divisions lateral or terminal. Stamens 3; anthers 0.5–3.9 mm long, yellow or purple. Fruit narrowly elliptical or narrowly ovoid or narrowly oblong, 4.2–7.4 mm long, 0.8–1.7 mm wide; ratio of width : length 0.14–0.29; ratio of fruit length : palea length 0.52–0.81; longitudinally grooved, or deeply furrowed.

Abaxial leaf blade epidermis. Long-cells 5–27 rows on the intercostal region. Long-cell wall straight or nearly straight, or sinusoid, or sinuous, or tessellate. Stomata 0–2(–3) rows on intercostal region; subsidiary cells parallel-sided, or low dome-shaped. Short-cells present or absent on intercostal region, individually or in pairs or in

horizontal rows; present over veins, individually or in pairs or in horizontal rows. Silica bodies tall and narrow, or crescent, or rounded, or cuboidal, or horizontally long and sinuous or smooth, or cross-shaped. Papillae present or absent, only over veins or over veins and on intercostal region. Prickles present or absent, over veins or on intercostal region, different in shape or similar in shape. Macrohairs present, or absent.

Transverse sections of the leaf blade. Leaf having distinct, prominent adaxial ribs only, or having nodular structure; adaxial ribs more or less constant or very irregular in size, top truncate or nearly truncate or obtuse; midrib inconspicuous. Bulliform cells occurring in simple fan-shaped groups, or no bulliform cells in simple fan-shaped groups. Vascular bundles accompanied by sclerenchyma or unaccompanied by sclerenchyma, forming a "T" or forming an "I".

Genomic information. Genomic polyploidy: 2n = 42. Genomic constitution: SYW? Further investigation is needed to confirm its genomic constitution.

Distribution. Found throughout Australia except N.T. Also in New Zealand.

Comments. An extremely variable species. Two subspecies are recognised.

Key to subspecies

1 Rachilla hairs usually not covering callus base; callus usually triangular or more various, dorsal surface flat to sunken; callus hairy, the hairs on margins and surface, or sometimes glabrous to glabrescent; callus tip usually thickened ..... subsp. *scaber*

1 Rachilla hairs covering callus base; callus usually narrowly triangular, dorsal surface rounded; callus distinctly hairy, hairs restricted to margins; callus tip not thickened ..... subsp. *rectisetus*

Elymus scaber (R. Br.) A. Löve subsp. ***scaber***

Illustration: Plates 7.7 & 7.8.

Spikelets 18–75 mm long, spikelet length : internode length of rachis (0.5–)1.3–4.4 (average: 2.4). Lemma apex usually acuminate and flat. Palea tip above flanks 0–0.36 (average: 0.1) mm long. Ratio of fruit length : palea length 0.61–0.81 (average: 0.72).

Distribution. Found in NSW, Vic., SA, WA, and Tas.. Occurs in various habitats, from islands to mountains (up to 1650 m), in shallow sandy soil, in dry sclerophyll forests, in open woodland areas, or in swampy areas with rocks. Also in New Zealand (Figure 7-6).

Flowering. September to March.

Selected specimens. NSW: Gara River, 13 Jan. 1996, S. Wang 95128 (SYD). Vic.: Central Gippsland, Hazelwood, 1 Dec. 1965, R. N. Auchterlonie (MEL); Mt. Buffalo NP., 18, Feb. 1963, J. H. Willis (MEL). Tas.: Penstock Lagoon, c. 1 km E. of Crisps Point, 24 Jan. 1980, J. J. Yates (HO). WA: Round Island, Recherche Archipelago, 18 Nov. 1950, J. H. Willis (MEL). New Zealand: Hodder V., Inland Karthoura Range, Feb. 1981, A. P. Druce (CHR).

Comments. This taxon is variable in awn length and curvature, callus shape and indumentum. Individuals of this subspecies with typical characters (e.g. triangular callus markedly hairy on margins and surface, callus tip thickened) are found in Victoria and Tasmania. However, specimens with somewhat intermediate characters, such as short to rather long awns, glabrous to glabrescent callus and non-thickened callus tip, are not uncommon in the Great Dividing Range. Molecular and embryological data indicate their relatedness to typical *E. scaber* subsp. *scaber*. These specimens blur the distinction between the present subspecies and *E. fertilis* or *E. multiflorus*. Example specimens are cited as follows: NSW: Near Duri, 12 Jan. 1996, S. Wang 100 (SYD); E of Glen Innes, 14 Jan. 1996, S. Wang 95137 (SYD). Vic.: Shepparton, 4 Nov. 1980, G.

K. Patterson 577 (MEL); Splitters Range Forest Block, 8 Dec. 1984, G. W. Carr 10246 (MEL).

From callus characters (e.g. hairs distributed on the margins and surface, tip thickened), New Zealand individuals should be included in this subspecies instead of subsp. *rectisetus*.

Elymus scaber subsp. ***rectisetus*** (Nees in Lehm.) S. Wang *et* M. J. Henwood, comb. et stat. nov.

Vulpia rectiseta Nees in Lehm. Pl. Preiss.2: 107 (1846); *Festuca rectiseta* (Nees in Lehm.) Walp., Ann. Bot. 1: 943 (1849); *Festuca rectiseta* (Nees in Lehm.) Steud., Syn. Pl. Glum. I: 304 (1854); *Elymus rectisetus* (Nees in Lehm.) A. Löve *et* Connor, New Zeal. J. Bot. 20: 183 (1982). T: WA, L. Preiss 1819; holo: MEL.

Anthosachne australasica Steud. Syn. Pl. Glum. I: 237 (1854); *Elymus australasicus* (Steud.) Tzvel. Nov. Sist. Vyssch. Rast. 10: 25 (1973). T: W.A., Drummond 382, 383, 384; syn: K.

Illustration: Plate 7.9 & 7.10.

Spikelets 35–101 mm long, spikelet length : internode length of rachis 2.49–5.6 (average: 3.8). Lemma apex usually nearly lorate and canaliculate. Palea tip above flanks (0–) 0.18–1 (average: 0.45) mm long. Ratio of fruit length : palea length 0.52–0.68 (average: 0.59).

Distribution. Found in NSW, Vic., SA, and WA. Usually grows in open area along road, on grassy hillside and burnt ground, or on flood plain of rivers (Figure 7-7).

Flowering. August to April.

Selected specimens. WA: c. 12 km NW of Bungalbin Hill, western extremity of Helena and Aurora Range, 26 Sep. 1995, B. J. Lepschi 2027 (PERTH). Vic.: 31 km NW from Wangaratta, 9 Nov. 1985, A. D. J. Piesse 247 (MEL); Wyperfeld NP., Lost Lake, 10 Oct. 1968, A. C. Beauglehole & E. W. Finck 29232 (MEL). NSW: 5 miles W of Ootha, 10 Oct. 1945, N. C. Beadle (SYD). SA: Alligator Gorge NP., 1988, R. Bates 15856 (BRI).

Comments. A few specimens from arid areas of WA (e.g. S. Wang 95041, B. J. Lepschi 2027) appear to be annual. This subspecies usually has longer lemma awns than other *Elymus* taxa in Australia. The informal term, "long-awned form", used by some workers may refer to this taxon together with some individuals of subsp. *scaber*.

With regard to the characters of rachilla, callus, palea tip and the ratio of fruit to palea length, subsp. *rectisetus* is rather uniform, and is distinct from subsp. *scaber*. The genetic distinction between these two subspecies was also revealed. However, considering that both are probably apomicts, and that their differentiation may result from reproductive isolation and geographic distance, the treatment of two subspecies is considered here to be more acceptable than that of two separate species.

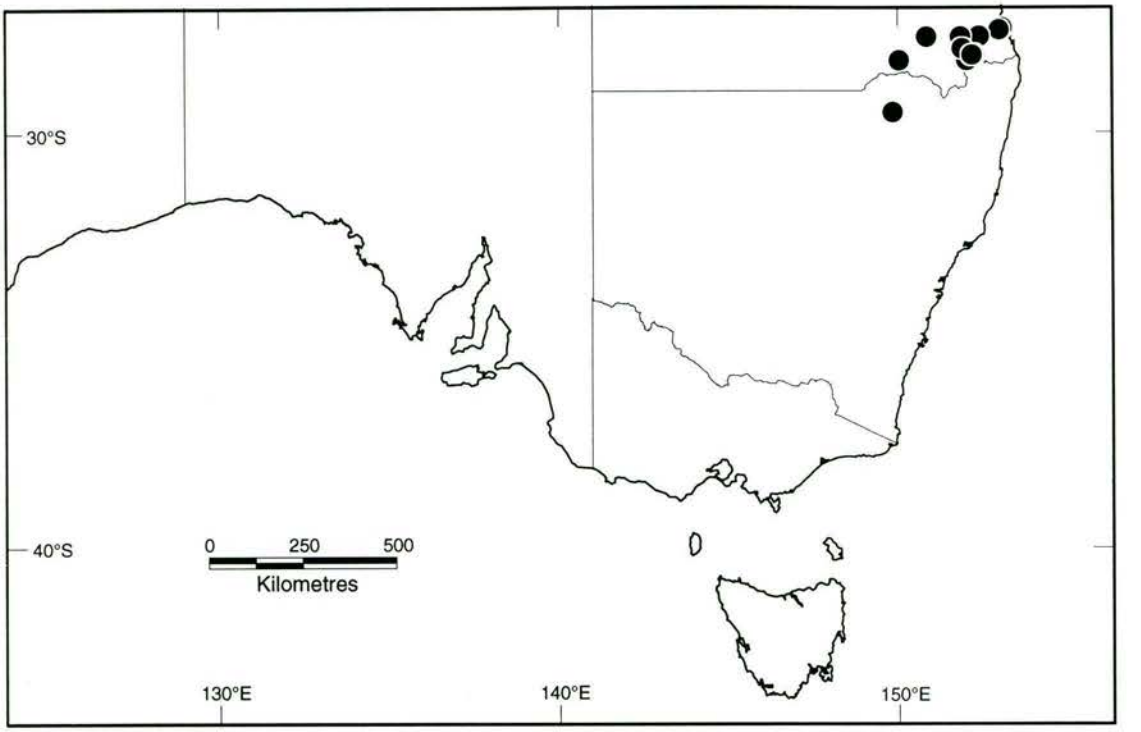


Figure 7-3. Distribution of *Elymus fertilis*.

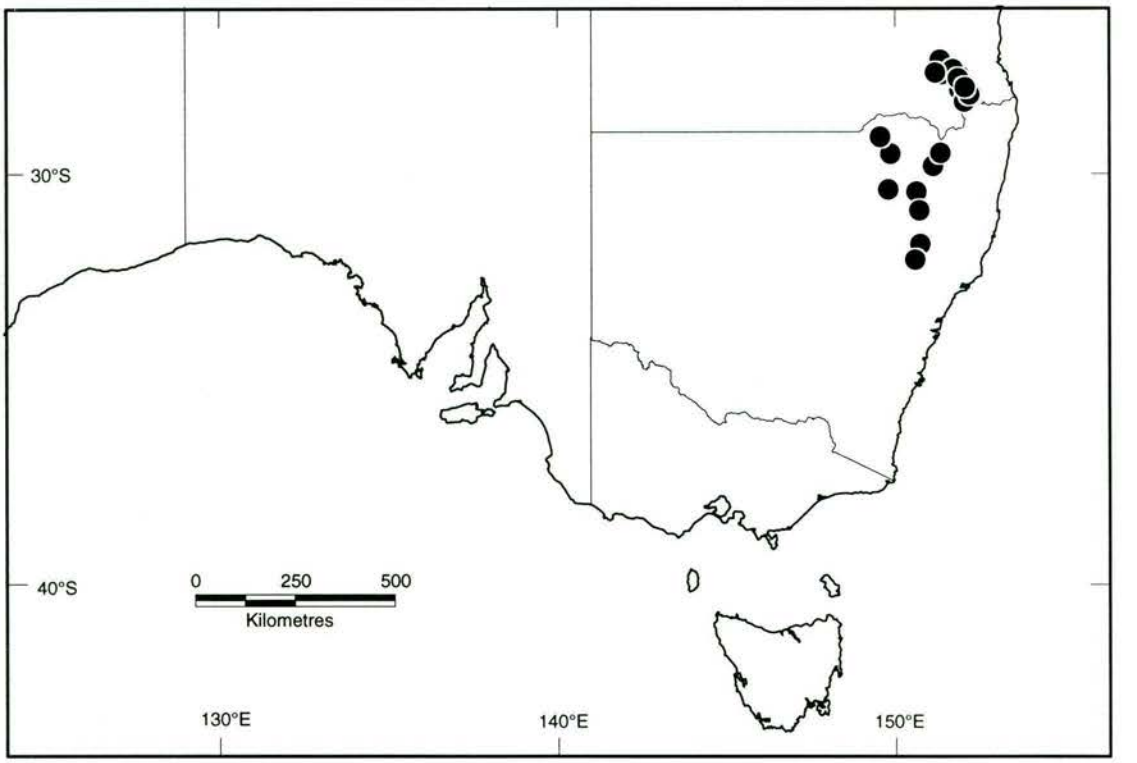


Figure 7-4. Distribution of *Elymus plurinervis*.

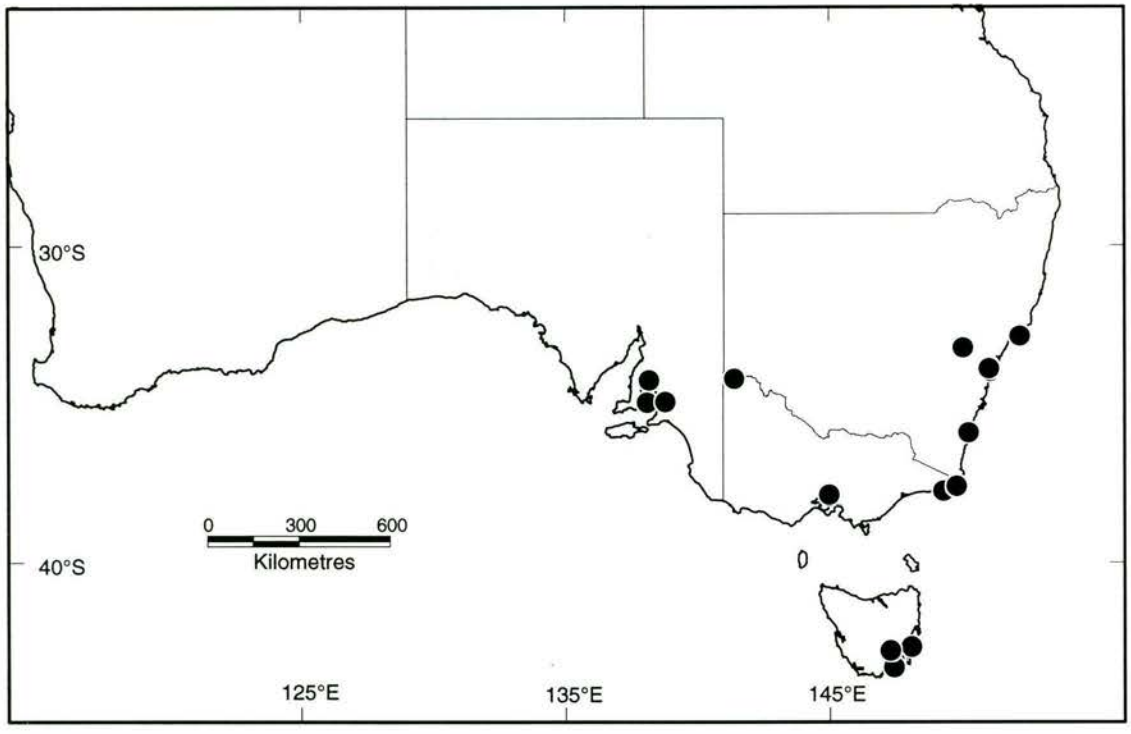


Figure 7-5. Distribution of *Elymus multiflorus*.

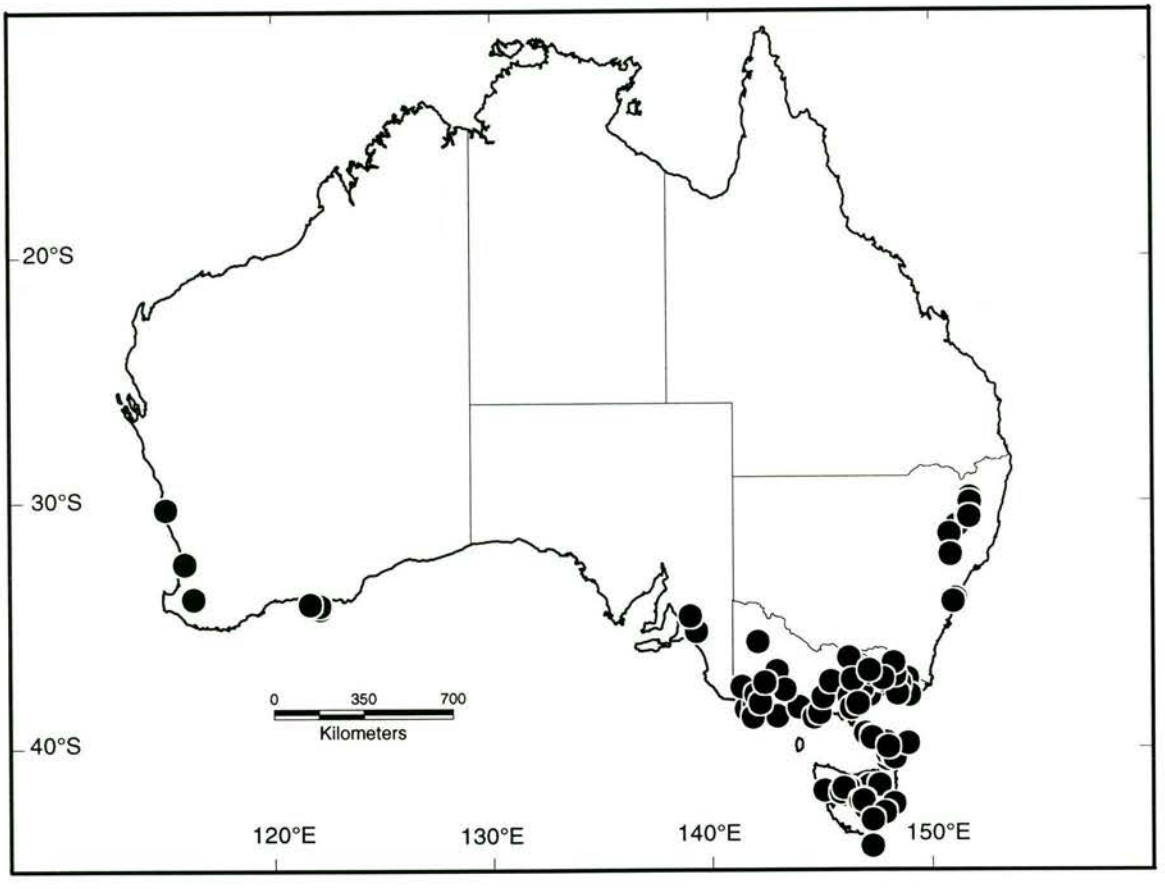


Figure 7-6. Distribution of *Elymus scaber* subsp. *scaber*.

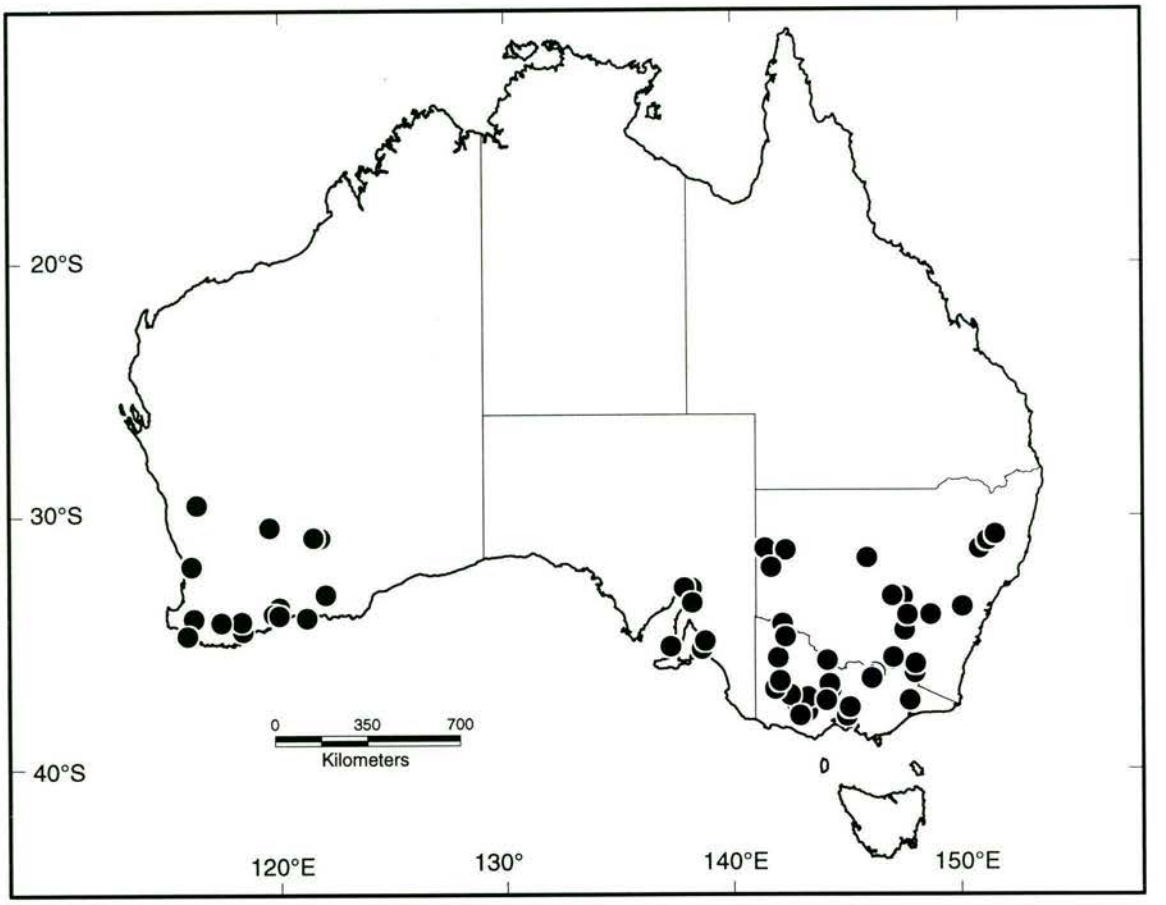
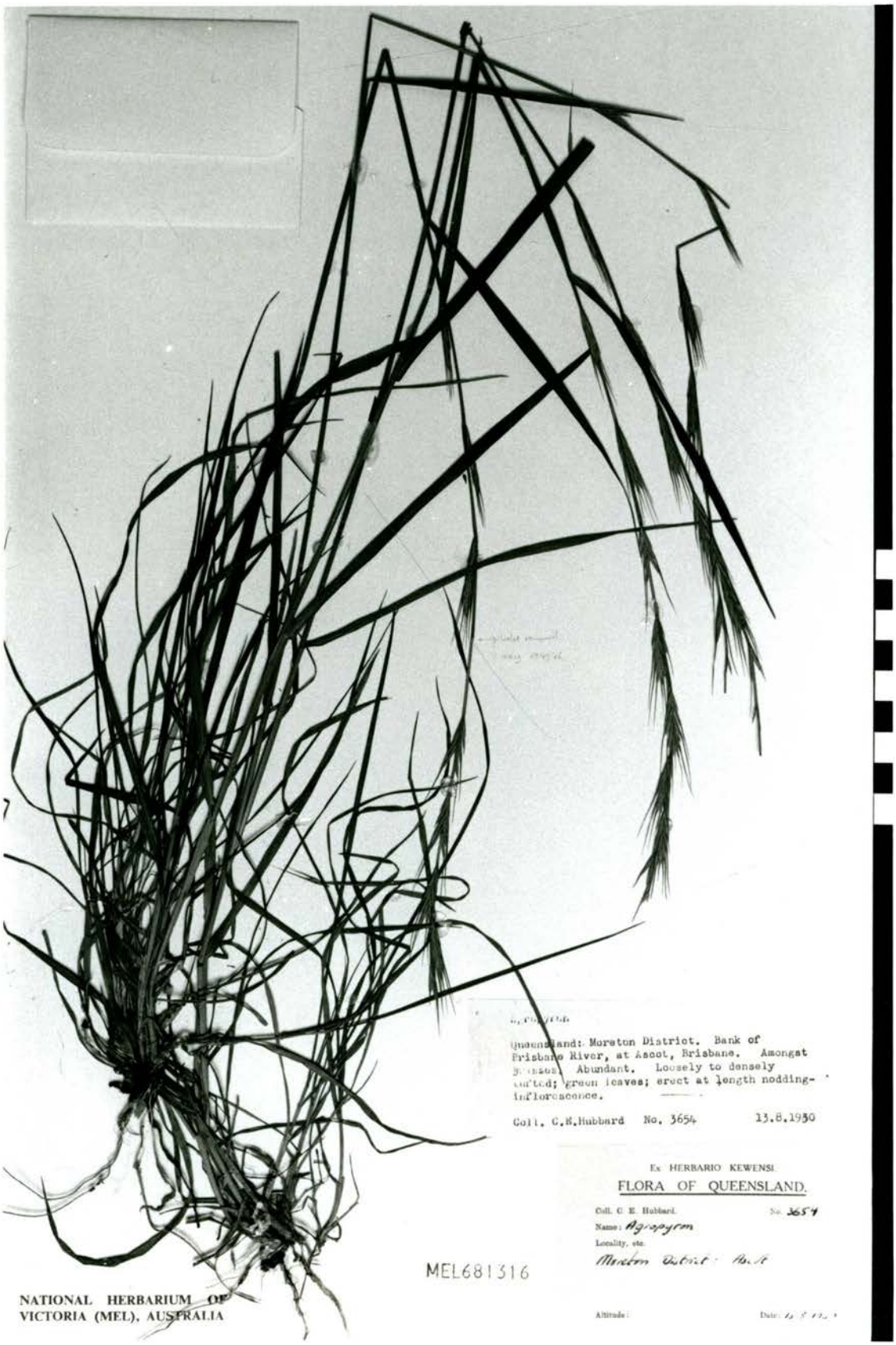


Figure 7-7. Distribution of *Elymus scaber* subsp. *rectisetus*.

Plate 7.1.

Elymus fertilis S. Wang et M. J. Henwood (C. E. Hubbard 3654, holo: MEL).

Bar = 1 cm.



Queensland: Moreton District. Bank of Brisbane River, at Ascot, Brisbane. Amongst [illegible] Abundant. Loosely to densely tufted; green leaves; erect at length nodding-inflorescence.

Coll. C.E.Hubbard No. 3654 13.8.1930

Ex HERBARIO KEWENSI

FLORA OF QUEENSLAND.

Coll. C. E. Hubbard. No. 3654

Name: Agropyron

Locality, etc.

Moreton District: Ascot

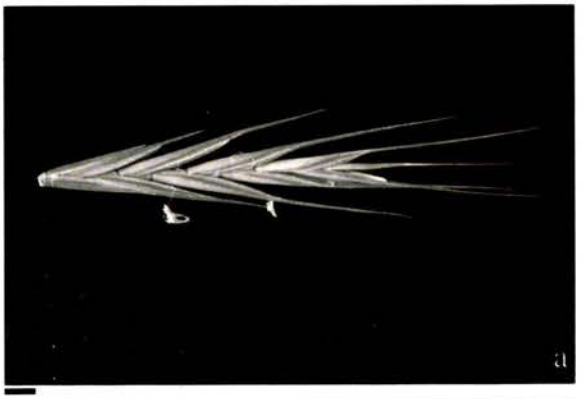
Altitude: Date:

MEL681316

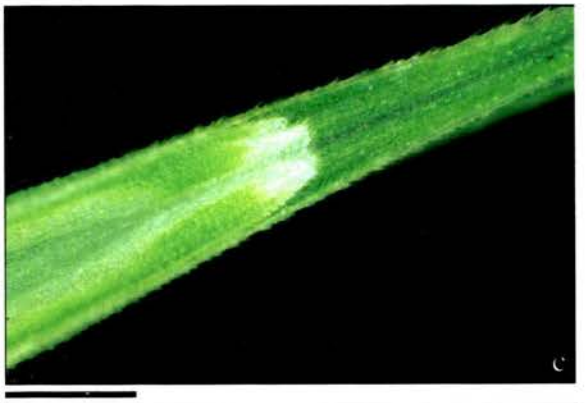
Plate 7.2.

Elymus fertilis (continued).

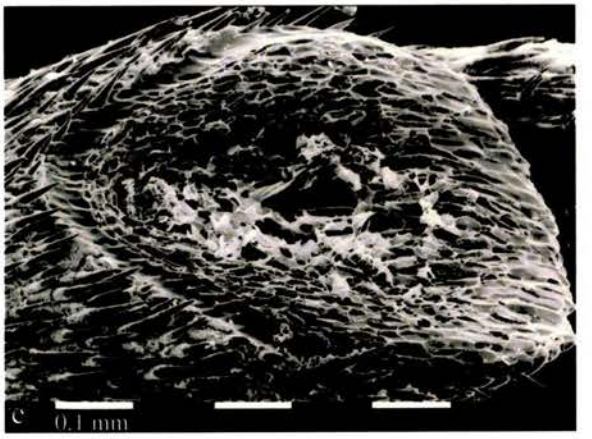
a. Spikelet (S. Wang 95140; bar = 2 mm). **b**. Lower glume (S. Wang 95140; bar = 1 mm). **c**. Palea apex (S. Wang 95140; bar = 1 mm). **d**. Fruit (S. Wang 95141; bar = 1mm). **e**. Rachilla apex and hairs (S. Wang 95158). **f**. Callus (abaxial; S. Wang 95140). **g**. Callus (adaxial; S. Wang 95158).

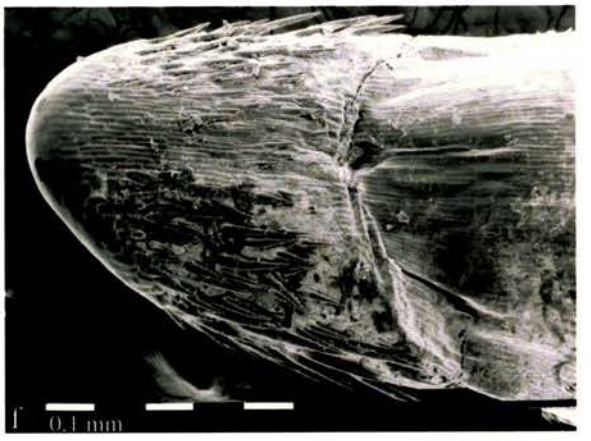

a


b


c


d


e
0.1 mm


f
0.1 mm

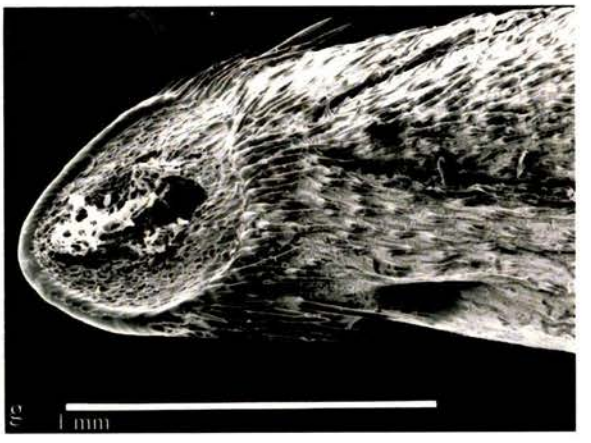

g
1 mm

Plate 7.3.

Elymus multiflorus (Banks et Sol. ex Hook.f.) A. Löve et Connor (K. R. Thiele 249).

Bar = 1 cm.

MEL667857

HERBARIUM OF K.R.THIELE

Flora of Australia

Agropyron junceum

Loc.: NSW : N end of Broulee Beach, 18 km S of Batemans Bay

Lat. 35°51'S Long. 150°11'E Date: 6 June 1981 Alt.:

Coll.: K.R.Thiele 249

Notes: Sheltered dune hollows up to the landward side of the first dune

NATIONAL HERBARIUM OF VICTORIA (MEL), AUSTRALIA

Seen for *Flora of Australia* M.I.H. Brownsey 9.3.1989

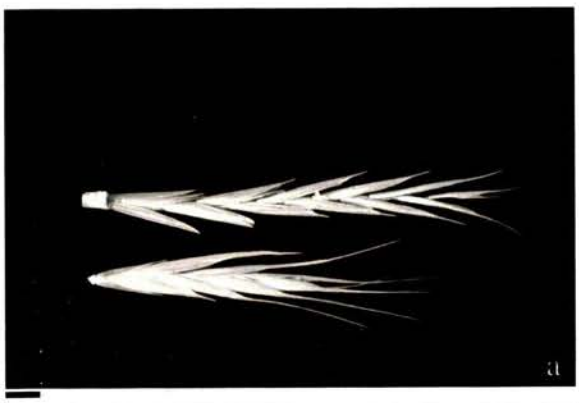
Det.: K.R.T

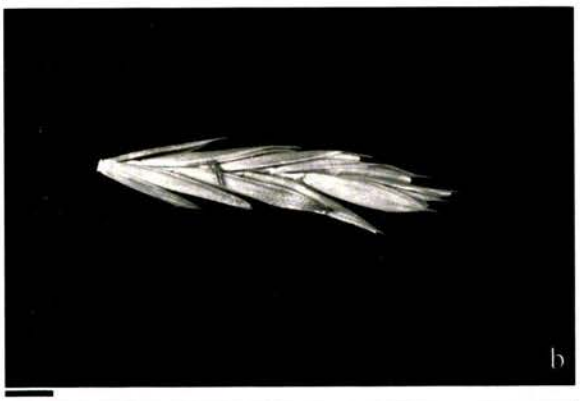
Dup.:

Plate 7.4.

***Elymus multiflorus* (continued).**

a. Spikelets (S. Wang 95163; bar = 3 mm). Upper: under cultivated condition; lower: from the field. **b**. Spikelet (CHR 1594; bar = 3 mm). **c**. Lemma apex (CHR 1595; bar = 1mm). **d.** Fruit (S. Wang 95163; bar = 1mm). **e**. Rachilla hairs and apex (CHR 1594). **f**. Callus (abaxial; CHR 1594). **g**. Callus (adaxial; CHR1594).

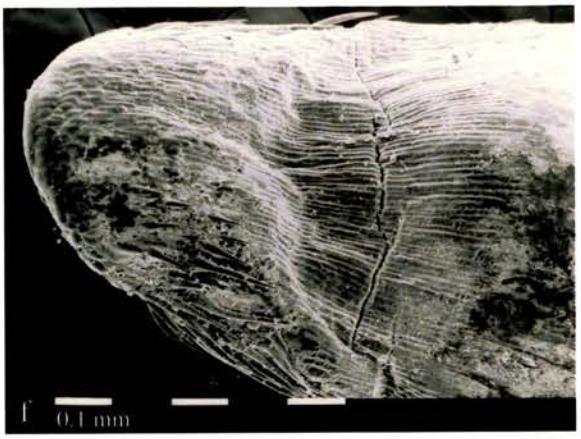

a


b


c


d


e
1 mm


f
0,1 mm

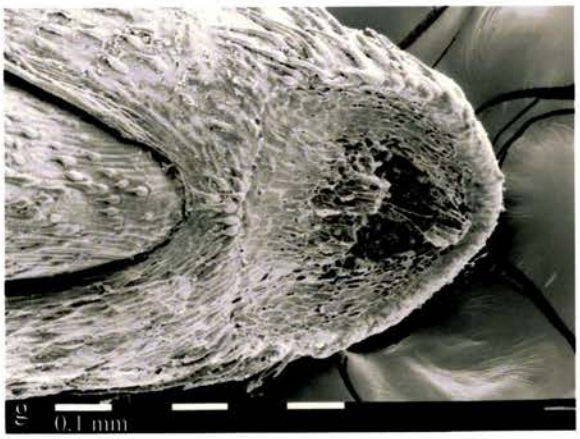

g
0,1 mm

Plate 7.5.

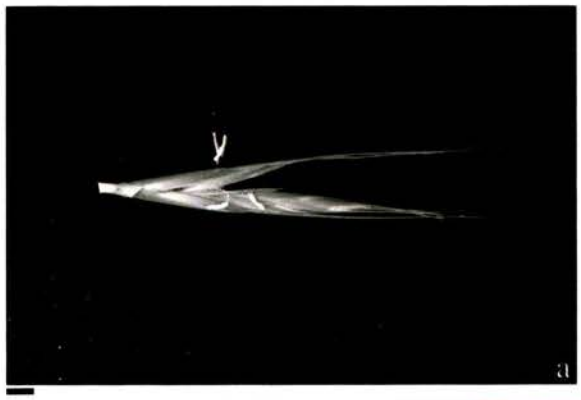
Elymus plurinervis (Vickery) S. Wang et M. J. Henwood (S. Wang 95153).

Bar = 1 cm.

Plate 7.6.

Elymus plurinervis (continued).

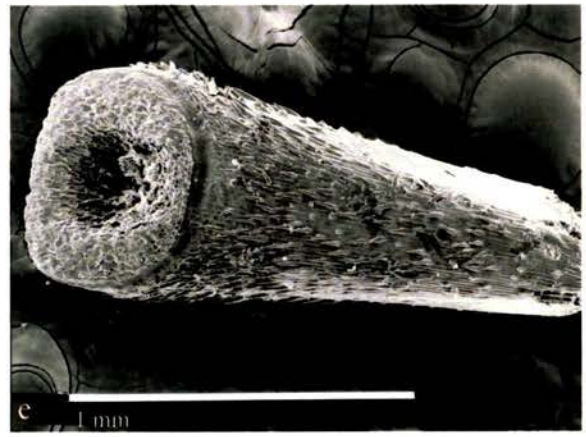
a. Young spikelet (S. Wang 95153; bar = 3 mm), awns incurved. **b**. Glume with wide membranous margins (S. Wang 95157; bar = 1mm). **c**. Upper glume 7-8 veined (S. Wang 95157; bar = 1mm). **d**. Lemma awn entered by one vein (S. Wang 95157; bar = 1mm). **e**. Rachilla hairs and apex (L. S. Smith & S. L. Everist 811 A). **f**. Callus (abaxial; R. J. Fensham 1728). **g**. Callus (adaxial; R. J. Fensham 1343). **h**. Fruit (S. Wang 95153; bar = 1mm).

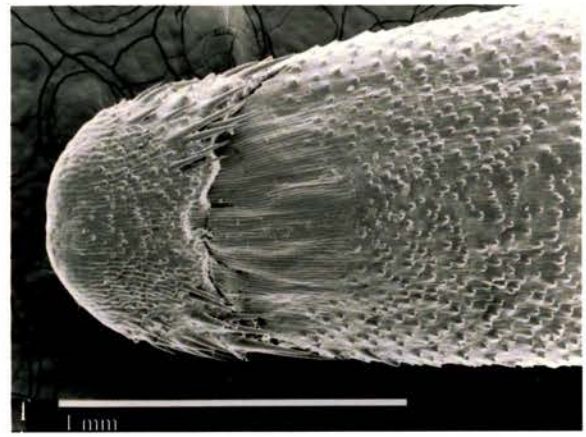

y
a

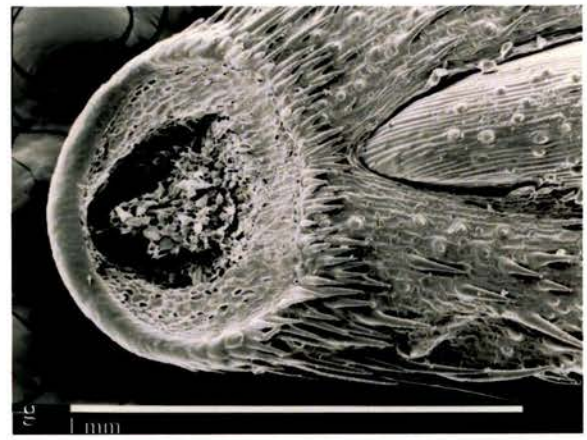

b


c


d


e
1 mm

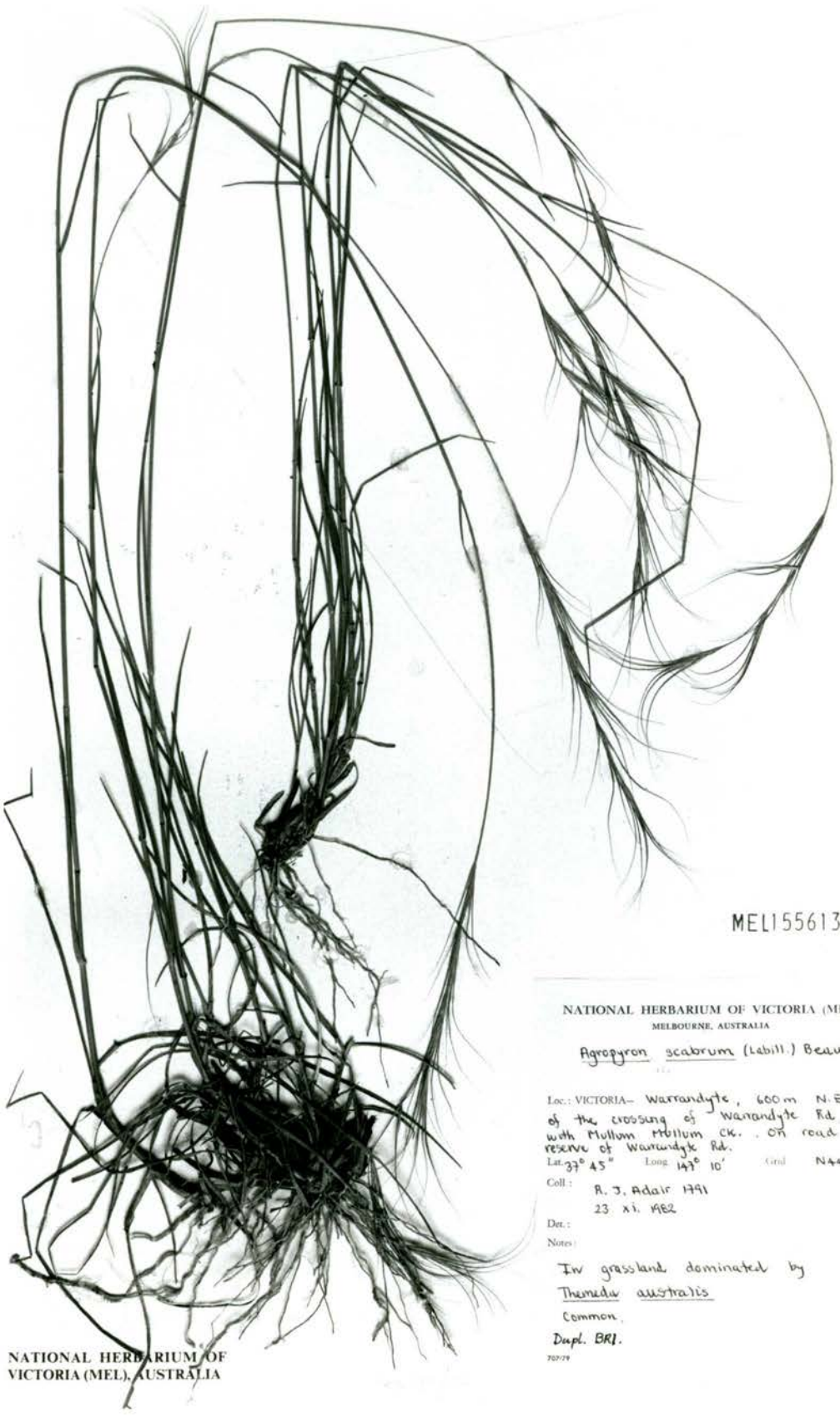

f
1 mm


g
1 mm


h

Plate 7.7.

Elymus scaber (R. Br.) A. Löve subsp. ***scaber*** (R. J. Adair 1791).



MEL1556138

NATIONAL HERBARIUM OF VICTORIA (MEL)
MELBOURNE, AUSTRALIA

Agropyron scabrum (Labill.) Beauv.

Loc.: VICTORIA– Warrandyte, 600 m N.E. of the crossing of Warrandyte Rd. with Mullum Mullum Ck., on road reserve of Warrandyte Rd.
Lat. 37° 45" Long. 145° 10' Grid N44

Coll.: R. J. Adair 1791
23 xi. 1982

Det.:

Notes:

In grassland dominated by Themeda australis
Common.

Dupl. BRI.

707/79

NATIONAL HERBARIUM OF
VICTORIA (MEL), AUSTRALIA

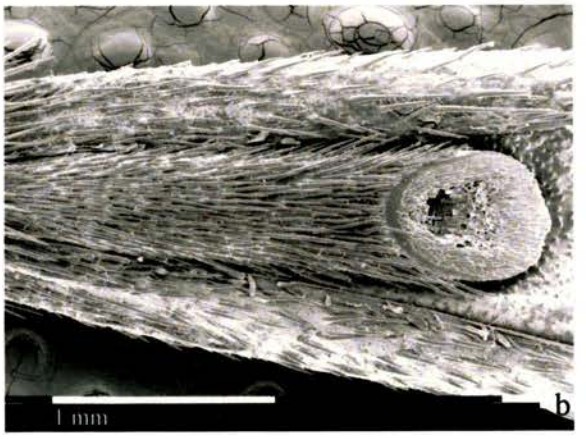
Plate 7.8.

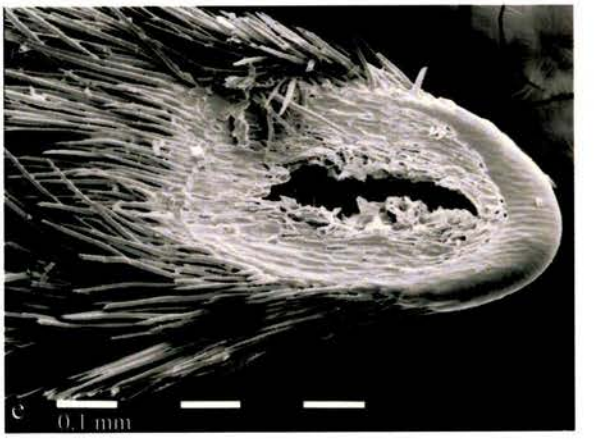
Elymus scaber subsp. ***scaber*** (continued).

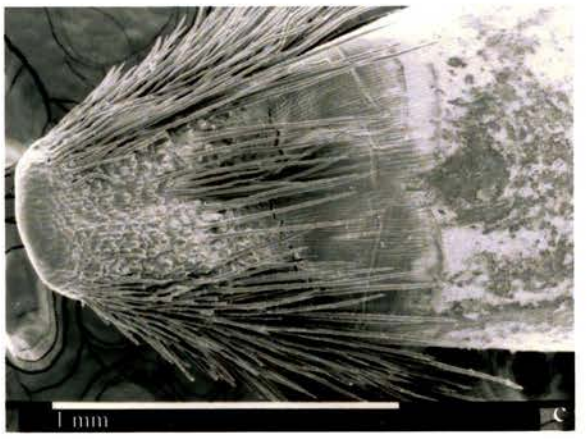
a. Spikelets (upper: S. Wang 96104; lower: A. C. Beauglehole 71547. Bar = 3 mm). **b**. Rachilla hairs and apex (S. Wang 95135). **c**. Callus (abaxial; MEL 1560495). **d**. Fruit (S. Wang 95129, bar = 1 mm). **e**. Callus (adaxial; HO 91358). **f**. Palea apex (S. Wang 95129).


a


d


1 mm
b


e
0.1 mm


1 mm
c

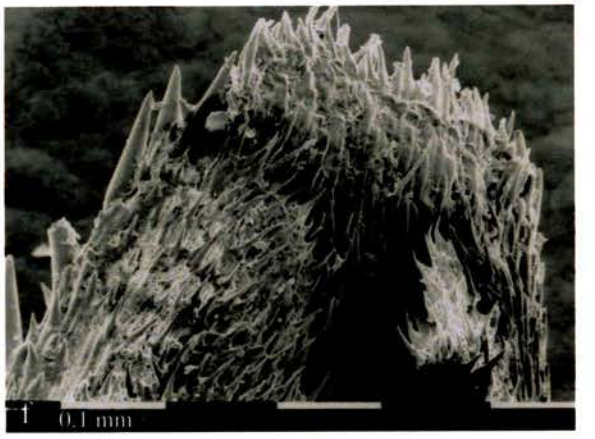

f
0.1 mm

Plate 7.9.

Elymus scaber subsp. ***rectisetus*** (Nees in Lehm.) S. Wang et M. J. Henwood (A. C. Beauglehole 29368). **Bar** = 1 cm.

ROYAL BOTANIC GARDENS
AND
NATIONAL HERBARIUM, MELBOURNE
VICTORIA, AUSTRALIA

Agropyron scabrum

Loc.: VICTORIA– B 34
Wyperfeld National Park
1 3½ m. S.W. of Wonga Hut
Notes: E. side of road to Entrance
– open areas amongst
Eucalyptus largiflorens

Coll.: A.C. Beauglehole 15-10-1968
AEB 29368

Det.: A.C.B.

Dup.: N.P.A.

MEL531316

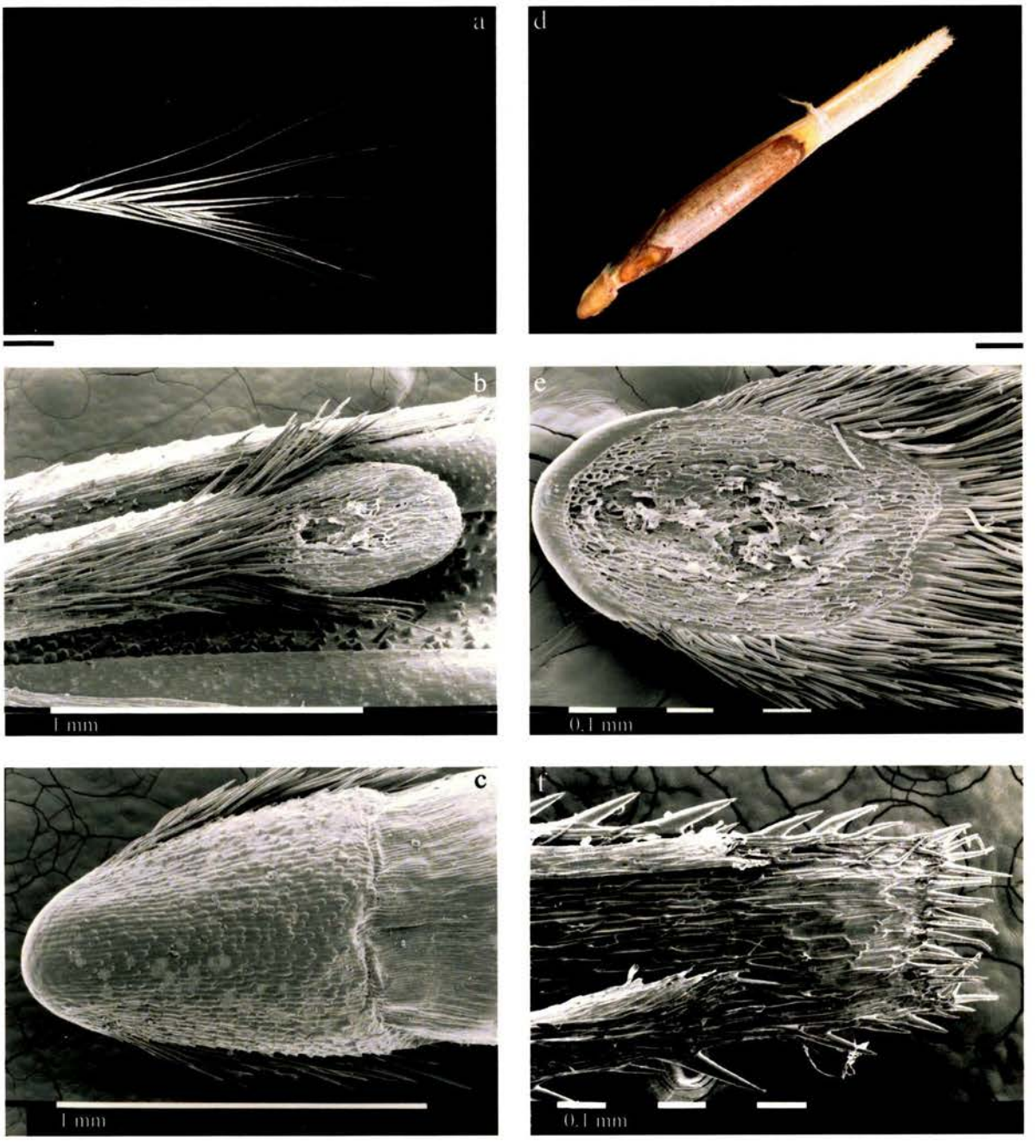
CONFIRMAVIT
RMcM
12/4/83 Herb. MEL

ROYAL BOTANIC GARDENS
& NATIONAL HERBARIUM
MELBOURNE, AUSTRALIA

Plate 7.10.

Elymus scaber subsp. ***rectisetus*** (continued).

a. Spikelet (Lindseys Exp. 138; bar = 1 cm). **b**. Rachilla hairs and apex (S. Wang 95087). **c**. Callus (abaxial; S. Wang 95001). **d**. Fruit (S. Wang 95001; bar = 1 mm). **e**. Callus (adaxial; S. Wang 95001). **f**. Palea apex (S. Wang 95041).


a
d
b
1 mm
e
0.1 mm
c
1 mm
f
0.1 mm

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Appendix I
Specimens sampled for morphological and anatomical study

Australian samples

OTUs	Names used on sheets	Form	Collector	Sheet No
1*	*Elymus scaber*	C	R. Bates 15856	BRI 467208
2*	*Elymus scaber* var. *plurinervis*	J	S. Wang 95153	SYD
3*	*Agropyron junceum* *Elymus elongatum*	H	K. R. Thiele 249	MEL 667857
4	*Agropyron scabrum* var. *plurinerve*	J	K. H. Bennett	MEL 1560505
5	*Elymus scaber* var. *scaber*	J	R. J. Fensham 1307	BRI 631026
6*	*Agropyron scabrum*	J	L. S. Smith & S. L. Everist 811A	MEL 1560507
7		J	S. Wang 95154	SYD
8	*Agropyron scabrum*	I	G. K. Patterson 577	MEL 619680
9	*Agropyron scabrum*	I	A. Moscal 1577	HO 64456
10	*Agropyron scabrum*	I	Johnson	MEL 1560418
11	*Festuca billardieri*	I		MEL 1560493
12		H	S. Wang 95139	SYD
13*		C	S. Wang 95103	SYD
14	*Agropyron scabrum*	C	A. C. Beauglehole 42443	MEL 1507858
15	*Agropyron scabrum*	C	J. B. Muir 2389	MEL 1560322
16	*Agropyron scabrum*	C	A. C. Beauglehole 29368	MEL 531316
17*	*Agropyron scabrum*	C	N. C. Beadle	SYD
18		C	S. Wang 95116	SYD
19	*Agropyron scabrum*	F	A. C. Beauglehole 29323	MEL 531309
20	*Agropyron scabrum*	D	G. W. Carr 10246	MEL 678632
21	*Agropyron scabrum*	D	J. B. Muir 1081	MEL 1560496
22	*Agropyron scabrum*	D	A. C. Beauglehole 32114	MEL 531312
23		D	?, 248	MEL 1560415
24	*Festuca billardieri*	D	Dr Story	MEL 1560401
25	*Agropyron scabrum*	D	A. C. Beauglehole 33131	MEL 531317
26*	*Agropyron scabrum*	D	J. H. Willis	MEL 1560495
27	*Agropyron scabrum*	B	D. E. Albrecht 346	MEL 1526153
28	*Agropyron scabrum*	E	J. B. Muir 353	MEL1560499
29*		H	S. Wang 95137	SYD
30*	*Elymus multiflorus*	H	A. C. Beauglehole 32316	MEL 531303
31	*Agropyron scabrum* *Vulpia browniana* *Festuca billardieri*	H		MEL 1560356
32		G	William Banerlen	MEL 1560417
33*		I	S. Wang 95140	SYD
34	*Agropyron*	I	C. E. Hubbard 3654	MEL 681316
35	*Agropyron scabrum*	I	O. D. Evans	SYD
36		I	S. Wang 95124	SYD
37		I	S. Wang 95133	SYD

38*		I	S. Wang 95158	SYD
39	*Elymus multiflorus*	H	A. C. Beauglehole 32282	MEL 531305
40	*Agropyron scabrum*	H	J. E. S. Townrow	HO 116080
41*		H	S. Wang 95132	SYD
42	*Festuca billardieri*	H		MEL 1560339
43		H	S. Wang 95162	SYD
44	*Agropyron scabrum*	B	W. M. Curtis	HO 27735
45*		G	Miss A. Bell	MEL 1560416
46	*Agropyron scabrum*	C	A. C. Beauglehole 29323	MEL 531315
47		C		MEL 1560436
48		C	S. Wang 95105 - a	SYD
49		C	S. Wang 95105 - b	SYD
50	*Vulpia browniana*	C		MEL 1560345
51	*Vulpia browniana*	C		MEL 1560340
52	*Festuca ?*	C	D. Sullivan	MEL 1560457
53	*Agropyron scabrum*	C		MEL 1560472
54	*Agropyron scabrum*	C	A.D.J. Piesse	MEL 1554171
55*	*Agropyron scabrum*	C	A. D. J. Piesse 247	MEL 689066
56*		C	S. Wang 95087	SYD
57*		C	S. Wang 95001	SYD
58*		C	S. Wang 95041	SYD
59	*Agropyron scabrum*	C	B. A. Rowe	HO 28260
60	*Agropyron scabrum*	E	A. C. Beauglehole 15740	MEL1507853
61*		E	S. Wang 95104	SYD
62*		E	S. Wang 95135	SYD
63		E	S. Wang 95046	SYD
64*	*Agropyron scabrum*	E	J. H. Willis	MEL 521279
65		E	AVDB 313	SYD
66	*Agropyron scabrum*	E	J. B. Muir 1955	MEL 1560500
67	*Agropyron scabrum*	E	J. B. Muir 3009	MEL 1560319
68	*Agropyron scabrum*	E	S. J. Forbes 1890	MEL 667927
69	*Agropyron scabrum*	E	M. G. Corrick 7973	MEL 602667
70	*Agropyron scabrum*	A	J. H. Willis	MEL 1560479
71	*Agropyron scabrum*	B	R. Story 7075	MEL 1560498
72	*Agropyron scabrum*	B	J. S. Whinray 959	MEL 532206
73	*Agropyron scabrum*	B	J. S. Whinray 2013	MEL 576573
74*	*Agropyron scabrum*	B	J. S. Whinray 709	MEL 594669
75	*Agropyron scabrum*	B	J. H. Hemsley	HO 91358
76	*Elymus scabrus* var. *scabrus*	B	S. T. Blake 18271	HO 110935
77	*Agropyron scabrum*	B	J. J. Yates	HO 32695
78		B	AVDB 334	SYD
79	*Agropyron scabrum*	B	A. D. J. Piesse 103	MEL 1543130
80	*Agropyron scabrum*	B	R. N. Auchterlonie	MEL 1560321
81*	*Agropyson scabrum*	B	Austin Brown 14	MEL 690519
82	*Agropyron scabrum*	B	S. J. Forbes 1928	MEL 667941
83*	*Agropyron scabrum*	B	J. H. Hemsley	HO 91348
84		B	AVDB 309	SYD
85*		B	S. Wang 95100	SYD
86*		B	S. Wang 95128	SYD
87*		B	S. Wang 95129	SYD

88		B	S. Wang 95102	SYD
89		B	S. Wang 95134	SYD
90		B	S. Wang 95123	SYD
91*		B	S. Wang 95136	SYD
92	*Agropyron scabrum*	B	A. C. Beauglehole 21488	MEL 1507856
93	*Agropyron scabrum*	B	S. J. Forbes 269	MEL 595510
94	*Agropyron scabrum*	B	J. B. Muir 2951	MEL 1560324
95	*Agropyron scabrum*	B	J. B. Muir 3541	MEL 1560323
96	*Agropyron scabrum*	B	S. Rennick 73	MEL 687191
97		B	S. Wang 95038	SYD
98	*Agropyrum scabrum*	B	W. M Curtis	MEL 647906
99	*Agropyron scabrum*	B	J. S. Whinray	HO 26150
100*	*Elymus scaber* var. *scaber*	J	R. J. Fensham 1728	BRI 630488
101*	*Elymus sp.*	C	B. J. Lepschi 2027	PERTH 04195280
102*	*Elymus scaber* var. *scaber*	J	R. J. Fensham 1343	BRI 635876

New Zealand samples

103*	*Elymus apricus*		V. Zotov (?)	CHR95426
104*	*Elymus apricus*		P. N. Johnson 1220	CHR494196
105*	*Elymus enysii*		M. J. A. Lempson 3442	CHR127516
106*	*Elymus enysii*		A. P. Druce 1345	CHR471937
107*	*Elymus falcis*		Melville 6021	CHR143004
108*	*Elymus falcis*		H. E. Connor	CHR402714
109*	*Elymus multiflorus*		D. Petrie	CHR1594
110*	*Elymus multiflorus*		D. Petrie	CHR1595
111*	*Elymus multiflorus*		P. J. de Lange 832	CHR473347
112*	*Elymus rectisetus*		A. P. Druce	CHR387016
113*	*Elymus rectisetus*		A. P. Druce	CHR209684
114*	*Elymus sacandros*		A. P. Druce	CHR387017
115*	*Elymus sacandros*		A. P. Druce	CHR279258
116*	*Elymus solandri*		H. E. Connor	CHR260318
117*	*Elymus solandri*		P. Wardle (?)	CHR223898
118*	*Elymus solandri*		B.P.J.Molloy & AD.Campbell	CHR212978
119*	*Elymus tenuis*		R. Mason 10575	CHR145548
120*	*Elymus tenuis*		A. P. Druce	CHR402401
121*	*Elymus tenuis*		A. P. Druce	CHR249122
122*	*Elymus apricus*		V. D. Zotov	CHR19722
123*	*Elymus enysii*		A. E. Esler 3117	CHR207335
124*	*Elymus rectisetus*		S. Aiken 2830	CHR354371
125*	*Elymus sacandros*		A. P. Druce	CHR279243
126*	*Elymus falcis*		H. H. Allan	CHR9514

A – J: morphological forms based on preliminary examination (Chapter 2); *:Specimens sampled for anatomical and SEM investigations.

Appendix II
DELTA Character List

For illustrations of some characters refer to the plates in Chapter 3 as indicated. Notes concerning the definition of character states are included when appropriate.

Nomenclature and references

#1. <Reference to the place of valid publication for the current names>/

First valid publication of the current name.

#2. <Nomenclature: Type and synonyms>/

Information of type and synonyms of the current name.

#3. Illustration:/

Habit, vegetative morphology

#4. <Plants - proliferation>/
1. <conspicuously long> rhizomatous (rare)/
2. <conspicuously long> stoloniferous (rare)/
3. caespitose <not long rhizomatous nor long stoloniferous, implicit>/

#5. <Longevity of plants>/
1. annual <without remains of old sheaths or culms>/
2. perennial <with remains of old sheaths and/or culms, implicit>/

#5. <Plants, habit> culms/
1. erect/
2. geniculate/
3. ascending/
4. drooping/
5. decumbent/

#7. <Mature> culms <height, range>/
cm tall/

Mature culm height: the height (or length) under the uppermost node.

#8. <Mature> culms <diameter, range>/
mm diameter/

Mature culm diameter: from the lower 2nd or 3rd internode.

#9. <Plants, colour>/
1. glaucous/
2. not glaucous <implicit>/

#10. <Plants> branching/
1. intravaginal/
2. extravaginal/

#11. <Culms, number of nodes (on the aerial part of the culm, below the inflorescence), range>/
noded/

#12. <Culm> nodes <whether hairy or glabrous>/
1. hairy/
2. glabrous <implicit>/

#13. <Culm> nodes <whether pigmented>/
1. pigmented/
2. not pigmented <implicit>/

#14. <Aerial> mid-culm internodes <whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. pubescent/
4. pilose/
5. hispid/

Reference: Hewson, H. J. 1988. Plant indumentum.

#15. Basal leaf sheaths <colour>/
1. the same colour as the lamina/
2. purple/
3. paler than the lamina <eg. straw - coloured>/

#16. Basal leaf sheaths <whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. hairy/

#17. <Basal leaf sheaths> hairs <orientation>/
1. antrorse/
2. retrorse/
3. erect <implicit>/
4. variable/

#18. <Basal leaf sheaths> margins <texture in free sheaths, if differing from dorsal portion>/
1. hyaline/
2. membranous/
3. chartaceous/

#19. <Margins of basal leaf sheaths, indumentum>/
1. smooth/
2. ciliate/

#20. <Leaf> auricles <whether present or absent>/
1. long (clasping) <Plate 3.1. a & c>/
2. minute <Plate 3.1. b>/
3. absent <Plate 3.1. d>/

#21. <Auricles, size>/
1. nearly equal in size <Plate 3.1. b & c>/
2. unequal in size <Plate 3.1. a>/

#22. <Auricles, shape>/
1. obtuse/
2. acute <Plate 3.1. b>/
3. pungent <= subulate; Plate 3.1. a & c>/

#23. Auricles <length, range>/
mm long/

#24. Auricles <hairiness>/
1. glabrous <Plate 3.1. a & b>/
2. hairy <Plate 3.1. c>/

#25. <Adaxial> ligule <length at middle. Length of trichomes included for ciliolate ligules>/
mm long/

#26. <Adaxial ligule, texture>/
1. hyaline/
2. membranous/
3. chartaceous/

#27. <Adaxial ligule, in situ (unflattened) shape of apex>/
1. obtuse/
2. truncate <implicit>/

#28. <Adaxial ligule> margins <whether divided>/
1. entire/
2. erose/
3. dentate/

#29. <Adaxial ligule margin, hairiness - avoid seedlings>/
1. smooth/
2. ciliolate/
3. ciliate/

#30. <Adaxial ligule> abaxially <whether glabrous or hairy>/
1. glabrous <implicit>/
2. hairy/

#31. Collar <whether glabrous or hairy, either abaxially or adaxially>/
1. glabrous/
2. hairy/

#32. Leaf blade <shape in t.s.>/
1. flat <implicit>/
2. folded/
3. involute <Plate 3.18. c>/

Leaf blade shape in t.s. indicates its shape in nature. Some involute leaf blades probably resulted from improper treatment when collected in the field, but usually reshape after boiling in water.

#33. <Leaf blade, whether falcate or not>/
1. falcate <curved like a sickle>/
2. strict/

Reference: Connor, 1994: Indigenous New Zealand Triticeae: Gramineae.

#34. <Leaf blades, length of cauline leaves, range>/
mm long/

#35. <Leaf blades, mid-width, flat, range>/
mm wide/

#36. <Leaf blades, rigidity>/
1. stiff/
2. not stiff <implicit>/

#37. <Leaf blades> adaxially <whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. hairy/

#38. <Adaxial leaf blades> hairs <orientation>/
1. antrorse/
2. retrorse/
3. erect <implicit>/
4. variable/

#39. <Leaf blades> abaxially <whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. hairy/

#40. <Abaxial leaf blades> hairs <orientation>/
1. antrorse/
2. retrorse/
3. erect <implicit>/
4. variable/

#41. <Leaf blades> margins <indumentum>/
1. smooth/
2. scabrous/
3. hairy/

#42. Flag leaves <length, range>/
mm long/

#43. Flag leaves <mid-width, flat, range>/
mm wide/

Inflorescence

#44. Inflorescence <when exserted: overall form>/
1. a single spike <implicit>/
2. a spike-like raceme/

A single spike: including spikes with sessile or subsessile spikelets (refer to character 64). A spike-like raceme: spikelets pedicellate.

#45. Inflorescence axis <length: taken from the last culm node to the base of the terminal spikelet, range>/
mm long/

#46. <Inflorescence, position>/
1. erect, stiff/
2. usually nodding or drooping to the ground <implicit>/

This character can only be scored from living material in the field or from the cultivated material. For New Zealand samples, data were obtained from Connor 1954: Studies in New Zealand Agropyron. Part I and II; and Connor 1994: Indigenous New Zealand Triticeae: Gramineae.

#47. <Inflorescence, colour at maturity>/
1. purple/
2. not purple <implicit>/

#48. Peduncles <length: taken from the last node to the first floral branch or spikelet (in racemes & spikes), range>/
mm long/

#49. <Inflorescence> peduncles <whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. pubescent/

#50. <Peduncle> hairs <orientation>/
1. antrorse/
2. retrorse/
3. erect <implicit>/
4. variable/

#51. Rachis prolongation <whether present or absent>/
1. absent <implicit>/
2. present/

#52. Rachis <length from the first inflorescence node to the last, range>/
mm long/

#53. <Rachis> average length of internode <from the lowest 2-3 internodes>/
mm/

#54. <Rachis, whether dorsally glabrous or hairy: of strap-like, subterete and 3-cornered rachis, record as the entire surface of terete rachis>/
1. glabrous/
2. scabrous/
3. hairy/

#55. <Rachis> hairs <orientation>/
1. antrorse/
2. retrorse/
3. erect <implicit>/

#56. <Rachis> angles <whether glabrous or hairy: applies mainly to strap-like and subterete rachis, may apply to strongly ridged terete rachis>/
1. glabrous/
2. scabrulous/
3. scabrous/
4. pubescent/

Spikelets

#57. Spikelets <the number of spikelets on a rachis>/
on the rachis/

#58. <Spikelets, grouping>/
1. solitary at each node <implicit>/
2. paired at base <occasionally>/

#59. <Spikelets, position to rachis>/
1. flatwise on rachis <implicit>/
2. edgewise on rachis/

#60. <Spikelets, whether appressed to the rachis of inflorescence>/
1. not divaricate <implicit>/
2. widely divaricate <from the rachis>/

This character can only be scored from living material. Reference: Connor, 1954 and 1994.

#61. <Spikelet length, including awns, range>/
mm long/

#62. <Spikelet> average spikelet length : internode length <the lowest 2-3 internodes> of rachis/

#63. <The number of florets per spikelet>/
-flowered/

Counting usually did not include the uppermost one or two undeveloped florets.

#64. <Spikelets, whether pedicellate>/
1. sessile <implicit>/
2. subsessile <length less than diameter>/
3. pedicellate <length greater than diameter>/

#65. <Spikelets> pedicels <length>/
mm long/

#66. <Pedicels of spikelets, hairiness>/
1. glabrous/
2. scabrous/
3. pubescent/

Glumes

#67. Glumes <of spikelets, whether divergent>/
1. diverging from the spikelets/
2. not diverging from the spikelets <implicit>/

#68. <Glumes of spikelets, whether markedly dissimilar in form, texture or size difference>/
1. similar <implicit>/
2. <very> dissimilar <lower glume : upper glume less than 2/3s>/

#69. Glumes <of spikelets, relative length in situ>/
1. equal <in situ, 1:1>/
2. subequal <in situ, less than 1.5:1>/
3. <very> unequal <in situ, equal to or more than 1.5:1>/

#70. Lower <glume approximate ratio of upper glume length>/
1. c. 1/2 the length of the upper glume/
2. c. 2/3s the length of the upper glume/
3. approximately equal to the upper glume <more than 2/3s, less than or equal to 1>/

#71. Lower glume <shape in situ of half glume>/
1. subulate <awl-like; Plate 3.2. b & c>/
2. narrowly oblong <= linear (12:1)>/
3. narrowly ovate <6:1-3:1; Plate 3.2. d>/
4. narrowly obovate/

For glumes with eccentric keels, data were obtained from the wider portion.

#72. Lower glume <length, excluding awn>/
mm long/

#73. <Lower glume width, in situ of half glume at widest point>/
mm wide/

#74. <Lower glume texture>/
1. chartaceous/
2. coriaceous/

#75. <Lower glume, hairiness>/
1. glabrous/
2. scabrous/

#76. <Lower glume, whether carinate>/
1. keeled <at least in part>/
2. dorsally rounded <not keeled>/

#77. <Lower glume> keels <whether centric or eccentric>/
1. centric/
2. eccentric <implicit>/

#78. <Lower glume keels, hairiness>/
1. glabrous/
2. scaberulous/
3. scabrous/

#79. <Lower glume> apex <shape of half glume excluding mucro or awn>/
1. acuminate/
2. acute/
3. obtuse/
4. subulate/

#80. <Lower glume apex, whether incised>/
1. entire <implicit>/
2. emarginate/
3. erose/
4. bifid/

#81. <Lower glume> apex <apical extension>/
1. muticous/
2. apiculate/
3. mucronate/
4. awned <Plate 3.2. a>/

Awned: The lower glume apices of most samples were characterised by tapering into a longer or shorter point, thus a standard for 'awned' was needed. See character 82.

#82. <Lower glume> awn <length>/
mm long/

The 'awn' was measured from the point at which glume apex was about 0.5 mm wide.

#83. <Lower glume awn, whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. puberulous/

#84. <Lower glume> margin <texture, if differing from dorsal portion>/
1. hyaline/
2. membranous <Plate 3.2. e>/
3. chartaceous/

#85. <Lower glume> widest part <of membranous margin, position>/
1. basipetal/
2. in the middle/
3. acropetal/
4. almost equal/

#86. <Lower glume margin, width of different texture at widest point>/
mm wide/

#87. <Lower glume margin, indumentum>/
1. smooth <implicit>/
2. scabrous/
3. ciliolate/
4. ciliate/

#88. <Lower glume, number of conspicuous veins>/
veined/

#89. Upper glume <shape in situ of half glume>/
1. subulate <awl-like>/
2. narrowly oblong <= linear (12:1)>/
3. narrowly ovate <6:1-3:1>/
4. narrowly elliptic/
5. narrowly obovate/

For glumes with eccentric keels, data were obtained from wider portion.

#90. Upper glume <length, excluding awn >/
mm long/

#91. <Upper glume width, in situ of half glume at widest point>/
mm wide/

#92. Upper glume <texture>/
1. chartaceous/
2. coriaceous/
3. cartilaginous/

#93. <Upper glume, hairiness>/
1. glabrous/
2. scabrous/

#94. <Upper glume, whether carinate>/
1. keeled <at least in part>/
2. dorsally rounded <not keeled>/

#95. <Upper glume> keels <whether centric or eccentric>/
1. centric/
2. eccentric <implicit>/

#96. <Upper glume keels, hairiness>/
1. glabrous/
2. scaberulous/
3. scabrous/

#97. <Upper glume> apex <shape of half glume excluding mucro or awn>/
1. acuminate/
2. acute/
3. obtuse/
4. truncate/
5. subulate/

#98. <Upper glume apex, whether incised>/
1. entire <implicit>/
2. bifid/

#99. <Upper glume> apex <apical extension>/
1. muticous/
2. apiculate/
3. mucronate/
4. awned <Plate 3.2. a>/

#100. <Upper glume> awn <length>/
mm long/

#101. <Upper glume awn, whether glabrous or hairy>/
1. glabrous/
2. scabrous/
3. puberulous/

#102. <Upper glume> margin <texture, if differing from dorsal portion>/
1. hyaline/
2. membranous/
3. chartaceous/

#103. <Upper glume margin> widest part <of different texture, position>/
1. basipetal/
2. in the middle/
3. acropetal/
4. almost equal/

#104. <Upper glume margin, width of different texture at widest point>/
mm wide/

#105. <Upper glume margin, indumentum>/
1. smooth <implicit>/
2. scabrous/
3. ciliolate/
4. ciliate/

#106. Upper glume <number of conspicuous veins>/
veined/

Rachilla and callus

#107. Rachilla <location of disarticulation positions>/
1. <readily> disarticulating above the glumes <when mature, implicit>/
2. disarticulating below the glumes/

#108. <Rachilla, whether disarticulates between the florets of spikelets>/
1. not disarticulating between the florets/
2. and <disarticulating> between the florets <implicit>/

#109. Rachilla segments <length, between first and second lemmas>/
mm long/

#110. <Rachilla, whether glabrous or hairy>/
1. glabrous/
2. hairy <implicit>/

#111. <Rachilla> hairs <distribution>/
1. long and dense, covering callus base <Plate 3.3. a & f>/
2. short to rather long, not covering callus base <Plate 3.3. b-e>/

State 1 indicates that long and dense hairs are apparently distributed on the central and upper portion of rachilla, therefore, cover the base of calluses. State 2 describes that short to rather long hairs are evenly distributed on the whole surface of rachillas, and do not cover the base of calluses.

#112. <Rachilla> apex <the face direction>/
1. nearly horizontal <Plate 3.3. e>/
2. not horizontal <implicit; Plate 3.3. a-d, f>/

#113. <Rachilla apex, shape>/
1. sharply expanded <Plate 3.3. e>/
2. <not sharply expanded, implicit; Plate 3.3. a-d, f>/

Rachilla apex was observed to be sharply expanded in Elymus enysii.

#114. Rachilla apex <shape of interface>/
1. oblong or elliptical <Plate 3.3. a>/
2. ovate, obovate to circular <Plate 3.3. b, d, f>/
3. semi-circular to transverse-circular <Plate 3.3. c>/

#115. Callus <length>/
mm long/

#116. <Callus, whether pigmented at maturity>/
1. discolourous/
2. concolourous <implicit>/

#117. Callus <abaxial; shape when mature>/
1. narrowly triangular <longer than wide; distinct; Plate 3.4. a>/
2. triangular <equally wide as long; Plate 3.4. b>/
3. broadly triangular <wider than longer; distinct; Plate 3.4. c-e>/

#118. <Callus, abaxial shape>/
1. <latitudinally> less than 3 times wider than long <implicit; Plate 3.4. c & d>/
2. <latitudinally> more than 3 times wider than long <Plate 3.4. e>/

#119. <Callus> dorsal surface <shape>/
1. rounded <Plate 3.4. a>/
2. flat <Plate 3.4. c & d>/
3. sunken <Plate 3.4. b>/

#120. <Callus, abaxial surface, hairiness >/
1. glabrous to glabrescent <Plate 3.4. c & d>/
2. densely hairy <Plate 3.4. a, b & f>/

#121. <Callus abaxially; hair distribution> hairs/
1. restricted to margins <Plate 3.4. a>/
2. on margins and <abaxial> surface <Plate 3.4. b & f>/

#122. <Callus> tip <in abaxial view; whether thickened>/
1. thickened <Plate 3.4. b>/
2. not thickened <Plate 3.4. a, c, d & g>/

#123. Adaxial <callus, whether sunken>/
1. flat or sunken <implicit; Plate 3.5. a-c, e-f>/
2. raised <Plate 3.5. d>/

#124. < Callus, adaxial, whether marginal thickening distinct>/
1. distinctly thickened <implicit; Plate 3.5>/
2. not distinctly thickened <Plate 3.5. d>/

#125. <Callus> adaxial margin thickening <extent of thickening>/
1. restricted to below the mid-point <distinct, Plate 3.5. a, e, f>/
2. extending approximately to the mid-point <Plate 3.5. b & c>/

#126. Callus <adaxial surface; hairiness>/
1. distinctly hairy <hairs long and dense; Pate 3.5. a, e, f>/
2. glabrous to glabrescent <hairs very short and sparse; Plate 3.5. b & c>/

Lemmas

#127. Lemma <firmness, relative to the glumes>/
1. less firm than the glumes/
2. similar in firmness to the glumes < implicit>/
3. <decidedly> firmer than the glumes/

#128. Lemma <texture when mature>/
1. chartaceous/
2. coriaceous/
3. cartilaginous/

#129. Lemma <shape in situ of half lemma>/
1. narrowly oblong <= linear (12:1)>/
2. narrowly ovate <6:1-3:1>/
3. narrowly elliptic/
4. narrowly obovate/
5. oblong <2:1-3:2>/

#130. Lemma <length, excluding awn>/
mm long/

A similar standard was established for defining lemma and awn: awn originates from the point at which lemma apex is about 0.5 mm wide.

#131. <In situ width at widest point of half> lemma <of proximal floret>/
mm wide/

#132. <Lemma, whether dorsally glabrous or hairy> dorsally/
1. glabrous/
2. scabrous/
3. pubescent/

#133. Hairs <distribution>/
1. over the entire dorsal surface/
2. only superior/
3. only inferior/
4. only to the margin/

#134. <Lemma, whether ventrally glabrous or hairy> ventrally/
1. glabrous/
2. scabrous/
3. pubescent/
4. hirsute/

#135. <Lemma, whether pruinose or not>/
1. pruinose/
2. not pruinose <implicit>/

#136. <Lemma, presence of keel>/
1. rounded/
2. apically keeled <i. e. above the mid-point; Plate 3.6. b>/
3. keeled <i. e. keel runs the entire length of the lemma>/

#137. <Lemma, hairiness of keel> keel/
1. glabrous/
2. scaberulous/
3. scabrous/
4. hirsute/

#138. <Lemma number of veins>/
veined/

#139. <Lemma> veins <confluence>/
1. confluent towards the apex <implicit>/
2. not confluent apically/

#140. <Lemma veins, prominence, applies only to laterals when lemma keeled>/
1. obscure <not raised>/
2. prominent acropetally/

#141. <Lateral veins of lemma, whether dorsally glabrous or hairy>/
1. glabrous/
2. scabrous/

#142. <Lemma> margin <texture if different to dorsal surface>/
1. hyaline/
2. membranous/
3. chartaceous/

Hyaline margins were easily seen in fresh and young material. When getting mature, this structure became cracked or disappeared.

#143. <Lemma margin> widest part <of different texture, position>/
1. basipetal/
2. in the middle/
3. acropetal/
4. nearly equal/

#144. <Lemma margin, width of different texture at widest point>/
mm wide/

#145. <Lemma margin, indumentum>/
1. smooth <implicit>/
2. scabrous/
3. ciliolate/
4. ciliate/

#146. <Lemma> apex <shape of half lemma, in situ>/
1. nearly lorate <Plate 3.6>/
2. acuminate <implicit; Plate 3.6.>/

#147. <Lemma apex, shape in t.s., just above the palea apex, when mature>/
1. canaliculate <Plate 3.6>/
2. flat <or nearly flat; Plate 3.6>/
3. folded <Plate 3.9. c>/

#148. <Lemma apex, incision>/
1. entire <implicit>/
2. bifid <Plate 3.9>/

#149. <Lemma, whether with typical awn or not>/
1. awned/
2. mucronate <Plate 3.9. a>/
3. muticous/

#150. <Mature lemma> awn length <within a single spikelet, whether equal or unequal>/
1. almost equal within a single spikelet/
2. very unequal <within a single spikelet>/
3. variable/

Almost equal: the proximal 1-2 awns usually shorter but not much shorter than the median lemma awns. Very unequal: the proximal lemma is awned but typically shorter than the median lemma awns, or mucronate or muticous. Variable: different states were observed within a single specimen.

#151. Longest awn <of lemma within a spikelet, position>/
1. acropetal/
2. medial/
3. basipetal/
4. variable/

The position should only be applicable to the median lemmas (excluding the proximal and the apical undeveloped lemmas).

#152. <Average length of> awns <range, excluding proximal and apical 1-2 lemmas>/ mm long/

The proximal and apical lemmas usually have shorter awns.

#153. <Lemma awns of young spikelets, whether straight or incurved>/
1. straight when young <implicit>/
2. incurved when young/

#154. <Lemma awns of mature spikelet, whether straight or recurved, when dry>/
1. remaining straight when dry/
2. uniformly recurved when dry/
3. straight to variously recurved when dry <implicit>/

It seemed that the curvature of awns is relative to the environment under which the specimen was collected. State 3 indicates that different states could be seen within the same specimen, or the awns were curved irregularly.

#155. <Awns, whether glabrous or hairy>/
1. glabrous/
2. scabrous <implicit>/

#156. <Lemma, number of veins entering awn>/
1. entered by 3 veins <implicit; Plate 3.7. g>/
2. entered by 1 vein <Plate 3.7. h>/

#157. Proximal lemma <whether awned or not>/
1. muticous/
2. mucronate/
3. awned <implicit>/

#158. Awn <of proximal lemma, length>/ mm long/

#159. Average awn length : proximal lemma length/

Paleas

#160. <Proximal> palea <length, relative to lemma>/
1. minute <less than half the length of the lemma>/
2. 1/2 the length of the lemma/
3. 2/3s the length of the lemma/
4. slightly shorter than the lemma/
5. equalling the lemma/
6. shortly exceeding the lemma/

#161. <Proximal> palea <shape in situ of region between the keels>/
1. narrowly oblong <= linear, 12:1>/
2. narrowly ovate <6:1-3:1>/
3. narrowly elliptic/
4. oblong/
5. oblanceolate/

#162. <Proximal> palea <length, range>/ mm long/

#163. <Proximal> palea <width in situ of region between the keels>/ mm wide/

#164. <Proximal> palea <texture relative to the lemma>/
1. thinner than the lemma <implicit>/
2. similar in texture to the lemma/

#165. <Proximal> palea <excluding keels, texture>/
1. membranous/
2. chartaceous/

#166. <Proximal> palea <whether dorsally 2-keeled>/
1. 2-keeled <implicit>/
2. keel-less/

#167. <Proximal palea> keels <whether winged>/
1. winged <rare; Plate 3.12. d>/
2. wingless <implicit>/

#168. <Proximal palea> keels <hairiness>/
1. scabrous/
2. ciliolate/

#169. <Proximal palea keel> hairs <length>/ mm long/

#170. Intercostal region <of proximal palea, hairiness>/
1. glabrous/
2. scabrous <Plate 3.10. e>/
3. hairy <implicit; Plate 3.10>/

#171. <Palea> apex <whether wider or narrower than lemma apex>/
1. narrower than lemma apex <Plate 3.7. a & b>/
2. equal to lemma apex <Plate 3.7. c>/
3. wider than lemma apex <protruding laterally; Plate 3.7. d>/

#172. <Palea apex shape, dorsal view in situ>/
1. truncate or obtuse <implicit; Plate 3.10. a, b & e; Plate 3.11. a-e>/
2. pointed <Plate 3.10. c & f; Plate 3.11. f-h>/

Reference: Connor, 1994: Indigenous New Zealand Triticeae: Gramineae.

#173. <Palea apex, whether incised>/
1. entire <Plate 3.11. a & b>/
2. retuse <Plate 3.11. c-e>/
3. bifid <Plate 3.10. c & f; Plate 3.11. f-h>/

#174. <Palea apex, hairiness>/
1. smooth/
2. ciliolate <implicit; Plate 3.11. a & d>/
3. ciliate <Plate 3.11. c & e>/

#175. <Palea> flanks (abaxial) <whether end at/under the top; abaxial view in situ>/
1. ended at the top <Plate 3.12. c>/
2. <ended> ended under the top <distinct; Plate 3.12. a, b & d>/

#176. <Palea> tip above flanks <length>/
mm long/

#177. <Palea> margin <abaxial, hairiness>/
1. smooth/
2. ciliolate/
3. ciliate/

Lodicules

#178. Lodicules <presence in florets>/
1. present <implicit>/
2. absent/

#179. <Lodicules, number in a floret>/

#180. <Lodicules, whether joined>/
1. free <implicit>/
2. joined <at least basally>/

#181. Lodicules <texture>/
1. hyaline <implicit>/
2. basally fleshy/
3. fleshy/

#182. <Lodicules, colour>/
1. white <implicit>/
2. brown or brownish/

#183. <Lodicules, shape>/
1. ovate <Plate 3.13. f>/
2. cuneate <Plate 3.13. d>/
3. oblong <Plate 3.13. c>/
4. elliptic/
5. oblanceolate/
6. obovate <Plate 3.13. e>/
7. sickle-shaped <Plate 3.13. b>/
8. irregular <those cannot be sorted into states 1-7; Plate 3.13. a>/

#184. <Lodicules, whether keeled or not>/
1. keeled on two lodicules <Plate 3.13. b, d & e>/
2. keeled on one lodicule/
3. not keeled <implicit>/

#185. <Lodicules, length>/
mm long/

#186. Lodicules <hairiness>/
1. glabrous/
2. pilose/

#187. <Lodicules> apex <shape of main division>/
1. acuminate/
2. acute/
3. obtuse/
4. truncate/
5. pungent/

#188. <Lodicules> margin <type of margin divisions>/
1. lobed/
2. entire/

#189. <Lodicule margin> divisions <position>/
1. lateral/
2. terminal/

#190. <Lodicules> margin <hairiness>/
1. smooth/
2. ciliolate/

Stamens

#191. Stamens <number per floret>/

#192. Anthers <length, range>/
mm long/

#193. <Anthers, colour>/
1. yellow/
2. purple/

It is common that two colours of anthers could be found within an individual plant, or even within a single spikelet.

Ovary

#194. Ovary <shape>/
1. oblong/
2. elliptic/
3. obovate <implicit>/

#195. Ovary <whether keeled or not>/
1. keeled <e. g. MEL 667857>/
2. not keeled <implicit>/

#196. Ovary <hairiness>/
1. glabrous/
2. pubescent/
3. pilose <implicit>/
4. hispid/

#197. Hairs <distribution>/
1. over the entire ovary/
2. only at the base/
3. only at the apex <implicit>/

#198. Styles <number>/

#199. Styles <position>/
1. apical <implicit>/
2. subterminal/

#200. Styles <whether fused or not>/
1. fused/
2. free to their bases <implicit>/

Fruit

#201. Plant <average seed set per spikelet, based on Sarah Ryan, unpublished data>/
1. sterile <1 seed/spikelet>/
2. low fertility <1-2 seed/spikelet>/
3. moderate fertility <3 seed/spikelet>/
4. fertile <>3 seed/spikelet>/
5. variable/

#202. Fruit <shape 3D>/
1. narrowly elliptical <implicit>/
2. narrowly ovoid/
3. narrowly obovoid/
4. narrowly oblong/

#203. Fruit <length when mature>/
mm long/

#204. <Fruit, width>/
mm wide/

#205. <Fruit> ratio of width : length/

#206. <Fruit> ratio of fruit length : palea length/

#207. <Fruit, whether grooved in transverse section>/
1. longitudinally grooved/
2. deeply furrowed/

Grooved: canaliculate, wider and shallow compared with state 2.

#208. <Fruit shape, in t.s.> forming/
1. a "U" in t. s. <Plate 3.15. b, c and e>/
2. crescent shape in t. s. <Plate 3.15. a>/

This is complement of character 207. Typical 'U' shape in t.s. is described as that the middle part of the fruit is thinner than the marginal parts, and the typical crescent shape is characterised by thicker parts in the middle instead of on the margins.

#209. <Fruit, whether glabrous or hairy>/
1. glabrous <implicit>/
2. hairy/

#210. <Fruit hairs, distribution> hairs/
1. confined to a terminal tuft/
2. covering most of the body/

#211. Fleshy apex <presence, i. e. connate base of styles>/
1. present/
2. absent <implicit>/

#212. Embryo <length>/
mm long/

Abaxial leaf blade epidermis

#213. The difference between costal and intercostal zones/
1. conspicuous <Plate 3.16. a>/
2. inconspicuous <Plate 3.16. b>/

Conspicuous: There are different cell components, except stomata, distributed between, and over, the veins, or at least there are more short-cells over, than between, the veins. Inconspicuous: There are no differences as stated above, except stomata, between costal and intercostal zones.

#214. Long-cells <number of rows on the intercostal region>/
rows on the intercostal region/

Long-cells are cells markedly elongated in the horizontal direction (Clifford and Watson, 1977).

#215. <Long-cells> <number of cells, horizontal counting of the medium line of intercostal region per field of view>/

Counting was carried out under 10 x 10 magnification. This character shows the relative length of long-cells, e.g. the higher the number, the shorter the long-cells and vice versa.

#216. Long-cell wall <thickness>/
1. straight or nearly straight <Plate 3.16. e & f>/
2. sinusoid <Plate 3.16. d>/
3. sinuous <Plate 3.17.c>/
4. tessellate <Plate 3.16. c>/

Reference: An Van den Borre, 1994: Taxonomy of the Chloridoideae (Poaceae), with special reference to the genus *Eragrostis* (thesis).

#217. Stomata <whether present on intercostal region>/
1. present/
2. absent/

#218. Stomata <number of rows on intercostal region>/
rows on intercostal region/

#219. Subsidiary cells <of stomata, shape>/
1. parallel-sided <Plate 3.16. b & d; Plate 3.17. a>/
2. low dome-shaped <Plate 3.16. f>/

#220. Short-cells <whether present on intercostal region>/
1. present on intercostal region/
2. absent on intercostal region/

Short-cells are approximately isodimensional, but sometimes they are somewhat longer horizontally than they are vertically (e.g. those containing horizontally long silica bodies).

#221. Short-cells <on intercostal region, arrangement>/
1. single <Plate 3.16. c>/
2. in pairs <Plate 3.16.d>/
3. in horizontal rows/

#222. Short-cells <whether present over veins>/
1. present over veins/
2. absent over veins/

#223. <Short cells over veins, arrangement>/
1. single/
2. in pairs/
3. in horizontal rows <Plate 3.16. e>/

#224. Silica bodies <shape>/
1. tall and narrow <Plate 3.16. c>/
2. crescent/
3. rounded/
4. cuboidal <Plate 3.17. b>/
5. acutely angled <Plate 3.17. b>/
6. horizontally long and sinuous or smooth <Plate 3.16. e>/
7. cross-shaped/

Reference: Metcalfe, 1960: Anatomy of the Monocotyledons, Vol. I. Gramineae; Clifford & Watson, 1977: Identifying Grasses: Data, Methods and Illustrations.

#225. Papillae <whether present/absent>/
1. present/
2. absent/

#226. Papillae <distribution>/
1. only over veins <Plate 3.17. a>/
2. over veins and on intercostal region <Plate 3.17. c>/

#227. Prickles <whether present/absent; ignore that on margin>/
1. present/
2. absent/

#228. Prickles <distribution>/
1. over veins <Plate 3.16. a>/
2. on intercostal region <Plate 3.17. d>/

#229. Prickles <whether different in shape, especially between those over veins and those on intercostal region; ignore that on margin>/
1. different in shape/
2. similar in shape/

#230. Macrohairs <whether present/absent>/
1. present <Plate 3.17.f>/
2. absent/

Leaf blade transverse sections

#231. Leaf <whether having distinct, prominent ribs in t.s.>/
1. having distinct, prominent adaxial ribs only <Plate 3.18.a & c>/
2. having nodular structure <Plate 3.18. b>/

#232. Adaxial ribs <whether more or less constant in size>/
1. more or less constant in size <Plate 3.18. a>/
2. very irregular in size <i. e., of two or more orders of size; ignore midrib; Plate 3.18. c>/

#233. <Adaxial rib> top <shape>/
1. truncate or nearly truncate <at least the bigger ribs; Plate 3.18. c>/
2. obtuse <Plate 3.18. d>/

'Nodular structure' involves the presence of quite well-defined adaxial ribs and, opposite them, abaxial ribs of comparable dimensions (Clifford and Watson, 1977).

#234. Midrib <whether conspicuous in the t.s.>/
1. conspicuous/
2. inconspicuous <Plate 3.18. a>/

#235. <Whether bulliform cells occurring in simple fan-shaped groups>/
1. bulliform cells in simple fan-shaped groups <Plate 3.18. d>/
2. no bulliform cells in simple fan-shaped groups/

#236. <Whether colourless cells penetrating deeply into the mesophyll>/
1. colourless cells penetrating deeply into the mesophyll/
2. no colourless cells penetrating deeply into the mesophyll/

#237. <All or nearly all> vascular bundles <whether accompanied by sclerenchyma>/
1. accompanied by sclerenchyma/
2. unaccompanied by sclerenchyma/

#238. Sclerenchyma <existing as girders or strands>/
1. existing as adaxial & abaxial girders/
2. existing as either abaxial or adaxial girder/
3. existing as strands on one or both sides of vascular bundles/

#239. <Combined adaxial & abaxial "girders" of sclerenchyma, whether forming an "I" or "T" in one or more bundles>/
1. forming a "T" <Plate 3.18. c>/
2. forming an "I" <Plate 3.18. d>/

#240. Bundle sheath <whether both two sheaths distinct or not>/
1. with two distinct layers <Plate 3.18. d>/
2. with only one distinct layer/

#241. Chlorenchyma <whether radiate round the bundles>/
1. not radiate round the bundles <Plate 3.18. d>/
2. radiate round the bundles/

#242. Abaxial-hypodermal sclerenchyma <whether present or not, except that associated with the veins>/
1. present <Plate 3.18. e>/
2. absent/

Genomic information

#243. Genomic polyploidy: <chromosome number>/

References: Löve, A. and Connor, H. E. (1982). Relationship and taxonomy of New Zealand wheatgrasses. New Zealand Journal of Botany 20, 169-186. Löve (1984). Conspectus of the Triticeae. Feddes Repert. 95, 425-521.

#244. Genomic constitution:/

References: Löve, A. and Connor, H. E. (1982). Relationship and taxonomy of New Zealand wheatgrasses. New Zealand Journal of Botany 20, 169-186. Torabinejad, J., Carman, J. G. and Crane, C. F. (1987). Morphology and genome analyses of interspecific hybrids of *Elymus scabrus*. Genome 29, 150-155. Torabinejad, J. and Mueller, R. J. (1993a). Genome constitution of the Australian hexaploid grass *Elymus scabrus* (Poaceae: Triticeae). Genome 36, 147-151.

Distribution

#245. Distribution in Australia: <by state, according to notes on the sheets>/
1. QLD <Queensland>/
2. NSW <New South Wales>/
3. VIC <Victoria>/
4. SA <South Australia>/
5. WA <Western Australia>/
6. TAS <Tasmania>/
7. NT <Northern Territory>/

#246. <Distribution, format following Flora of Australia>/

#247. <Whether distributed in New Zealand; data from Connor, 1994>/
1. also in New Zealand/
2. <not distributed in New Zealand>/

Additional information

#248. <Flowering period>/

#249. <Citation of specimens>/

Cite 5 specimens representative of each taxon, including: location, date, collector, number (herbarium/ia), e.g. HO, PERTH, BRI, SYD, NSW, MEL, etc.)

#250. <Comments for Flora of Australia>/

Comments on variation of characters, relationship to other taxa, questions, etc.

#251. <Comments of taxon>/

Appendix III
Interactive Identification Key

An interactive key to the species of *Elymus* from Australia treated in this study is included on a CD-ROM which can be found in a pocket inside the back cover of this thesis.

The data set has been compiled for use with the DELTA program INTKEY. INTKEY Version 4 requires Microsoft Windows V3.1, 95, 98, or NT. INTKEY Version 5 requires Windows 95, 98 or NT. The program files for both versions have been included on the CD-ROM either for running directly from the CD-ROM, or by first installing the appropriate program on a computer's hard drive.

This data set is designed to run on the above versions and has been tested on windows 3.1 with INTKEY v4 and on Windows 95 with INTKEY v5. Previous versions of the program had three modes of operation instead of two and thus may not behave correctly.

Installation and starting the program

Running directly from the CD-ROM

In windows 95/98/NT, INTKEY v5 may run automatically from the CD-ROM when inserted in the drive, and will load the *Elymus* data set (this may take about ten seconds — if this is disliked, holding the shift key as the disk is loaded by the computer will over-ride the autorun). If this does not occur, a 'shortcut to INTKEY' can be found in the main (root) directory of the CD-ROM. The short-cut assumes the CD-ROM is in drive D:\. Locate this file, either in Windows Explorer, or by double clicking 'My Computer' on the Desktop followed by the CD-ROM drive icon, and double click on the shortcut to run INTKEY. If the CD-ROM drive is not D:\, locate and run INTKEY5.EXE in the directory INTK32. When the program begins, a 'Select data set'

box will be presented. Click on the 'Browse' button at the bottom of the box, locate the file INTKEY.INI in the ELYMUS directory, highlight it and click the 'Open' button. In Windows 3.1, place the CD-ROM in the drive, select Run... from the File menu of the Program Manager, and type 'D:\INTK16\INTKEY4.EXE (replacing D:\ with the actual drive letter if necessary) and press enter. When the program begins, a 'Select data set' box will be presented. Click on the 'Browse' button at the bottom of the box, locate the file INTKEY.INI in the ELYMUS directory, highlight it and click the 'Open' button.

Installing to a hard drive

Windows 95/98/NT: Copy INTK32.EXE from the INSTALL subdirectory of the CD-ROM to a temporary directory on the hard drive. Double click on this copy to run the installation program and follow the on screen instructions. Once installation is complete, run INTKEY from the icon in the Start Menu. When the program begins, a 'Select data set' box will be presented. Click on the 'Browse' button at the bottom of the box, locate the file INTKEY.INI in the ELYMUS directory, highlight it and click the 'Open' button.

Windows 3.1: Copy INTK16.EXE from the INSTALL subdirectory of the CD-ROM to a temporary directory on the hard drive. Double click on this copy to run the installation program and follow the on screen instructions. Once installation is complete, run INTKEY from the icon in the Program Manager. When the program begins, a 'Select data set' box will be presented. Click on the 'Browse' button at the bottom of the box, locate the file INTKEY.INI in the ELYMUS directory, highlight it and click the 'Open' button.

Making an identification

There are two modes of operation in INTKEY, normal and advanced. In normal mode, identifications are made using the toolbar buttons at the top of the screen and all available images are displayed automatically. In advanced mode, both the toolbar

(which has some additional buttons) and menu options are available, but images will not be displayed unless invoked manually. Normal mode is recommended unless the user is familiar with operation of the program.

To identify a specimen:

Press the toolbar button labelled 'Start a new identification' (traffic light) and then the button 'Continue identification using 'best' characters' (blue star). The program displays a list of characters, with the 'best' ones (those with the greatest separation power for the remaining taxa) near the top. Choose a character and click on it, and press 'OK'.

The program displays an image with labelled character states, or a list of character states, or a box for entering values such as lengths. Some of the character states have images which are also included in the thesis (Chapter 3) and the reference numbers are given after the states. Click on the state, or enter the value that applies to the specimen, and press 'OK'. If you are not sure of the correct state or value, do not guess. Instead, select more than one state, enter a range of values, or press 'Cancel' and try another character. Notes for some characters, containing more detailed information, can be accessed using the 'Notes' button when present. Repeat this process until only one taxon remains or you have run out of available information with which to make an identification.

When only one taxon remains, the program should automatically cease returning to the 'best characters' box and will list the name of the taxon identified. In Normal Mode the first image is displayed automatically and in Advance Mode images can be accessed by pressing the toolbar button 'display illustrations of the remaining taxa' (the eye). Other images may be viewed by holding down the control key and using the up and down arrows, or via the Subject or Control menus of the open image.

If, at any stage, you think you have made a mistake and want to correct it, press 'Cancel' to close any dialog boxes that are open. Press the toolbar button labelled 'Change previously entered information about the specimen being identified' (dark blue arrow).

Click on the character you think is wrong, press 'Change', and make the correction. To recommence the identification using the 'best' characters as before, press the toolbar button 'continue identification using best characters' (blue star).

More detailed instructions can be found in the help menu or in the 'User's Guide to INTKEY' (Dallwitz *et al.* 1996) which has been included in the files manual.rtf and command.txt which can be found in the main directory of the CD-ROM.

Conditions of Use

This is not a licensed data set. Use of the program beyond a test period of one month is prohibited unless you have registered. Details of the conditions of use and registration fees are contained in the files DELTAUSE.TXT and DELTAREG.TXT, which can be found in the main directory on the CD-ROM.

The DELTA programs (including INTKEY), documentation and additional data sets are available on the internet at http://biodiversity.uno.edu/delta/

Appendix IV.
Characters used in the analyses*

Characters	102 OTUs	Sub-sample	Aust. & NZ. samples
20			+
32			+
33			+
46			+
53	+	+	+
60			+
61	+	+	+
62	+	+	+
72	+	+	+
73	+	+	+
82			+
86	+	+	+
88	+	+	+
90	+	+	+
91	+	+	+
100			+
104	+		
106	+	+	+
111		+	+
112			+
113			+
114		+	+
117		+	+
118			+
119		+	+
120		+	+
121		+	+

Characters	102 OTUs	Sub-sample	Aust. & NZ samples
122		+	+
123			+
124			+
125		+	+
126		+	+
128			+
130	+	+	+
135	+		
146	+	+	+
147	+	+	+
148	+	+	+
149	+	+	+
150	+	+	+
152	+	+	+
156	+	+	+
157	+	+	+
158	+	+	+
159	+	+	+
169	+	+	+
171	+	+	+
172	+		+
173	+	+	+
175		+	+
176		+	+
192	+		
205		+	+
206		+	+

*The orders of characters follow the Character List (Appendix II). +: Characters selected for analyses.

Appendix V
Phenetic Analysis Data

Morphological data matrix used in the analysis of the *Elymus scaber* complex from Australia and New Zealand (Chapter 4). For character order refer to Appendix II. Multistate characters

OTUs	#20	#32	#33	#46	#60	#111	#112	#113	#114	#117	#118	#119	#120	#121	#122	#123	#124	#125	#126	#128	#146	#147	#148	#149	#150	#156	#157	#171	#172	#173	#175
OTU001	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	10	10	10	10	10	00	10	10	01	01
OTU013	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU017	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU055	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU056	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU057	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU058	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU101	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU026	111	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU061	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU062	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU064	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU074	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU081	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU083	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU086	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU091	111	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU041	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU002	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU006	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU100	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU102	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU003	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU029	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU030	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU033	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU038	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU103	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU104	001	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU105	111	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU106	111	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU107	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU108	010	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU109	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU110	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU111	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU112	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU113	100	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU114	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU115	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU116	001	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU117	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU118	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU119	001	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU120	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU121	110	10	00	01	10	10	01	01	10	10	00	10	01	10	01	10	10	10	10	01	10	01	10	10	10	10	00	10	10	01	01
OTU122	001	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU123	011	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU124	011	10	00	01	10	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU125	001	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10
OTU126	011	01	00	10	01	01	10	10	01	01	00	01	10	01	10	01	01	01	01	10	01	10	01	01	01	01	00	01	01	10	10

Appendix V
Phenetic Analysis Data

(Continued) Numerical characters

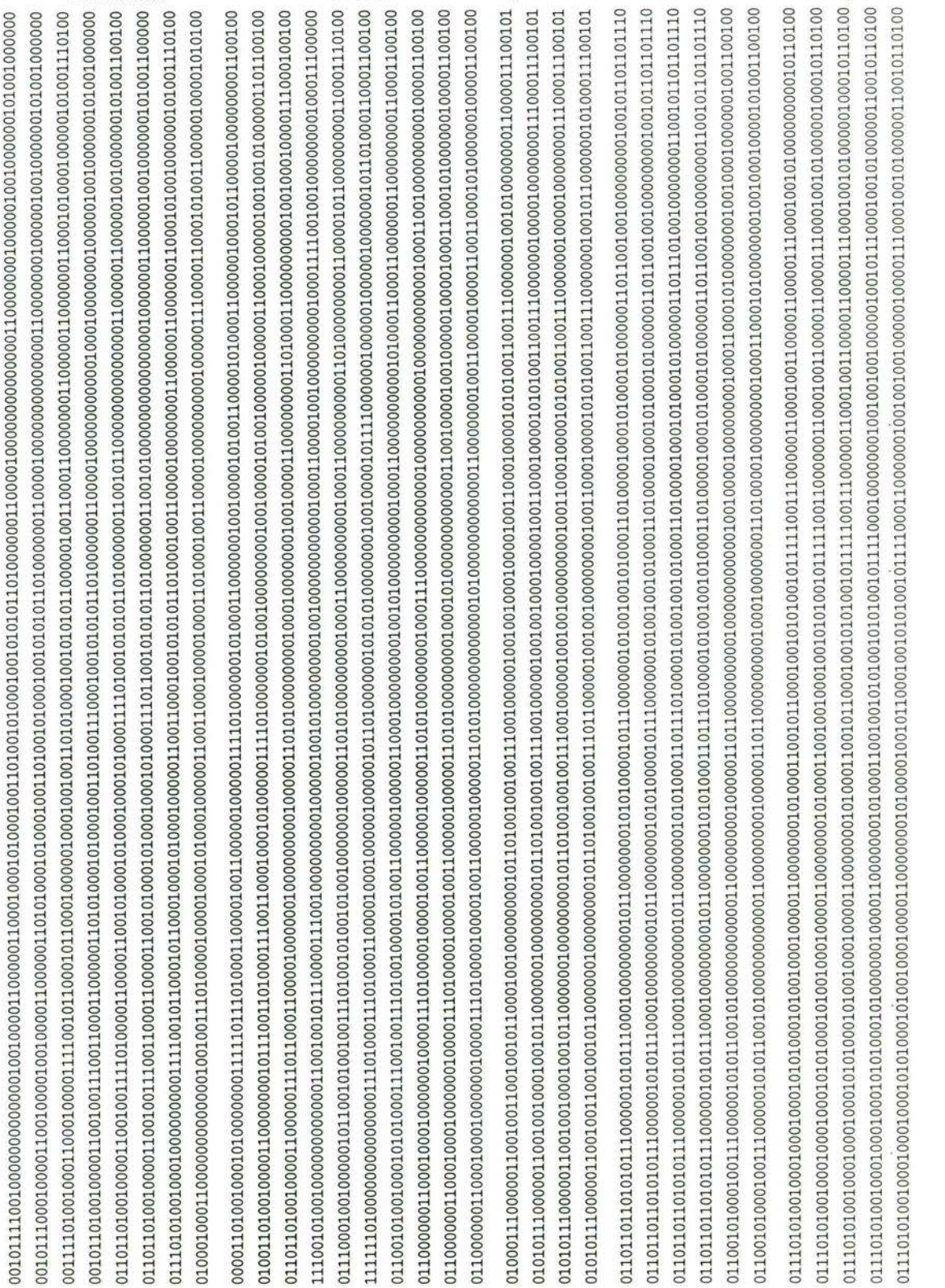
OTUs	#53	#61	#62	#72	#73	#82	#86	#88	#90	#91	#100	#106	#130	#152	#158	#159	#169	#176	#205	#206
OTU001	14.57	66.70	4.57	6.00	0.50	0.00	0.40	2.00	5.50	0.60	0.00	2.50	18.00	34.75	24.00	1.93	0.20	0.39	0.18	0.62
OTU013	13.96	56.17	4.02	5.10	0.50	2.40	0.30	3.00	4.70	0.50	3.30	3.00	11.50	32.00	13.50	2.83	0.30	0.61	0.18	0.64
OTU017	21.90	95.00	4.34	6.10	0.30	2.30	0.10	3.00	6.80	0.40	2.40	4.00	40.00	36.60	28.00	0.92	0.30	0.37	????	????
OTU055	17.85	62.60	3.51	1.80	0.40	1.90	0.20	4.00	3.60	0.60	1.10	5.00	18.50	31.88	22.00	1.72	0.30	0.42	0.18	0.66
OTU056	14.26	48.57	3.41	3.20	0.50	1.20	0.30	3.00	5.20	0.50	2.00	3.00	20.00	30.57	29.50	1.54	0.20	0.33	0.22	0.56
OTU057	21.00	57.22	2.72	3.40	0.40	1.30	0.30	3.00	5.10	0.50	1.70	3.00	21.00	30.20	27.00	1.44	0.25	0.46	0.21	0.59
OTU058	15.66	58.00	3.70	3.70	0.50	2.40	0.30	2.00	6.60	0.40	2.00	3.00	17.50	?????	20.50	????	0.25	0.42	0.21	0.61
OTU101	13.44	57.63	4.29	1.90	0.40	3.60	0.20	2.00	3.60	0.50	3.70	3.00	17.00	30.33	26.00	1.78	0.30	0.40	0.22	0.54
OTU026	14.00	44.33	3.17	7.00	0.80	1.80	0.50	3.00	7.60	0.90	1.30	5.00	14.00	21.50	18.00	1.54	0.30	0.00	0.18	0.69
OTU061	30.58	40.50	1.32	4.90	0.80	1.70	0.40	4.00	6.10	0.50	1.30	4.00	11.50	18.50	3.25	1.61	0.20	0.17	0.20	0.69
OTU062	25.30	52.67	2.08	5.60	1.00	1.70	0.50	4.00	6.40	1.00	1.10	6.00	17.00	26.17	28.00	1.54	0.33	0.22	0.18	0.69
OTU064	18.93	37.64	1.99	2.90	0.50	1.80	0.10	3.00	3.40	0.60	1.20	3.00	8.70	20.75	22.30	2.39	0.25	0.08	0.21	0.77
OTU074	4.50	33.50	7.44	1.30	0.40	1.20	0.20	3.00	3.50	0.50	1.10	4.00	9.00	19.00	20.00	2.11	0.25	0.18	0.18	0.71
OTU081	16.60	47.67	2.87	3.50	0.70	1.90	0.30	4.00	4.90	0.80	1.90	4.00	13.70	32.50	29.00	2.37	0.20	0.00	0.17	0.79
OTU083	15.08	44.67	2.96	1.90	0.50	1.80	0.20	3.00	2.00	0.60	1.50	4.00	13.00	26.50	25.00	2.04	0.20	0.00	0.19	0.69
OTU086	17.56	43.38	2.47	2.50	0.40	1.90	0.20	3.00	4.80	0.60	1.60	4.00	11.00	24.90	16.00	2.26	0.20	0.03	0.21	0.73
OTU091	21.57	46.50	2.16	4.60	1.00	4.30	0.40	4.00	6.10	0.80	2.40	5.00	10.00	35.50	33.00	3.55	0.30	0.03	0.18	0.71
OTU041	31.13	33.00	1.06	4.70	1.10	1.00	0.50	4.00	6.20	1.00	0.60	7.00	9.00	10.20	4.65	1.13	0.25	0.12	0.19	0.72
OTU002	29.00	37.75	1.30	10.9	1.30	0.00	0.40	5.50	12.5	1.40	0.00	7.50	14.50	22.60	16.00	1.56	0.10	0.18	0.25	0.52
OTU006	26.00	35.00	1.35	11.0	1.50	0.00	0.90	6.00	11.5	1.50	0.00	5.50	12.00	18.17	16.00	1.51	0.10	0.50	0.26	0.49
OTU100	17.00	35.57	2.09	8.00	1.70	0.70	0.90	5.00	10.6	1.00	0.00	7.00	12.50	24.75	14.75	1.98	0.10	0.23	0.28	0.51
OTU102	27.15	39.33	1.45	9.50	1.40	0.50	0.75	5.00	11.5	1.30	0.50	6.00	14.00	?????	17.00	????	0.30	0.29	0.28	0.53
OTU003	22.70	24.67	1.09	6.30	1.20	0.00	0.40	6.00	6.50	1.20	0.00	6.50	7.50	1.64	0.00	0.22	0.20	0.10	????	????
OTU029	39.52	26.09	0.66	4.30	0.90	0.40	0.30	4.00	5.60	1.00	0.30	6.00	8.25	9.40	5.65	1.17	0.20	0.16	0.16	0.74
OTU030	17.85	23.40	1.31	3.80	0.90	0.40	0.30	5.00	5.40	1.00	0.70	5.00	8.50	5.33	1.00	0.63	0.30	0.05	0.25	0.67
OTU033	31.23	32.50	1.04	6.10	1.00	1.70	0.30	5.00	7.00	0.80	1.20	5.00	9.60	12.70	5.40	1.32	0.10	0.15	0.21	0.72
OTU038	34.85	31.50	0.90	4.80	1.00	1.30	0.40	4.00	5.60	0.80	1.40	5.00	8.50	10.57	5.50	1.24	0.16	0.06	0.19	0.69
OTU103	22.08	47.71	2.16	3.83	0.44	2.40	0.29	3.67	6.67	0.51	3.50	5.33	10.50	27.18	24.25	2.59	0.15	0.09	0.16	0.67
OTU104	17.17	34.57	2.01	3.50	0.43	5.90	0.39	3.00	5.25	0.62	6.90	3.00	8.25	22.50	21.00	2.73	0.18	0.00	0.24	0.65
OTU105	8.35	12.36	1.48	3.00	0.46	3.70	0.00	2.50	3.50	0.52	2.80	2.75	9.00	2.00	2.38	0.22	0.13	0.00	0.26	0.59
OTU106	10.00	17.00	1.70	0.00	0.00	7.00	0.00	1.00	0.00	0.00	8.00	1.00	9.13	7.79	7.63	0.85	0.11	0.00	????	????
OTU107	12.34	41.20	3.34	3.50	0.50	0.70	0.27	3.00	6.60	0.45	1.80	4.00	10.00	27.50	22.50	2.75	0.23	0.00	0.20	0.71
OTU108	7.61	40.67	5.34	2.67	0.42	0.80	0.23	3.00	3.67	0.48	1.00	4.00	8.75	29.57	20.50	3.38	0.21	0.00	0.27	0.56
OTU109	7.44	14.10	1.90	3.67	0.62	0.80	0.26	4.00	5.00	0.85	0.80	5.33	7.20	0.00	0.00	0.00	0.12	0.00	0.20	0.62
OTU110	18.11	25.33	1.40	6.00	0.93	1.30	0.28	5.50	6.67	1.06	1.20	6.00	9.88	0.39	0.00	0.04	0.17	0.00	????	????
OTU111	26.56	33.00	1.24	4.33	0.71	1.50	0.43	4.33	5.67	0.59	1.10	4.00	9.00	17.44	13.00	1.94	0.15	0.00	????	????
OTU112	19.54	58.00	2.97	4.67	0.59	1.80	0.32	3.67	6.83	0.62	2.70	5.67	12.00	39.14	39.67	3.26	0.18	0.12	0.21	0.78
OTU113	42.34	57.09	1.35	5.33	0.65	2.10	0.27	3.33	7.00	0.64	2.50	4.33	13.33	32.30	26.00	2.42	0.20	0.06	0.17	0.76
OTU114	16.34	52.50	3.21	3.00	0.36	1.70	0.23	2.00	3.67	0.56	1.80	3.67	10.67	44.31	43.00	4.15	0.20	0.00	0.21	0.78
OTU115	17.50	73.67	4.21	4.50	0.57	2.70	0.25	4.50	5.75	0.77	2.30	5.50	13.00	52.43	54.00	4.03	0.15	0.00	????	????
OTU116	22.44	90.00	4.01	5.00	0.74	1.20	0.51	4.00	6.33	1.11	1.00	6.67	16.17	44.00	58.50	2.72	0.20	0.19	0.28	0.68
OTU117	13.45	47.40	3.52	4.67	0.66	1.50	0.29	4.67	5.83	0.86	1.20	5.33	15.00	28.50	28.33	1.90	0.28	0.13	0.28	0.67
OTU118	18.42	48.33	2.62	4.00	0.59	1.40	0.23	3.50	6.50	0.73	1.30	5.50	11.00	27.56	32.50	2.51	0.13	0.00	0.24	0.63
OTU119	23.11	43.86	1.90	5.25	0.73	6.00	0.41	3.25	6.38	0.72	8.00	4.00	9.00	29.88	30.00	3.32	0.24	0.03	0.23	0.63
OTU120	18.22	29.67	1.63	3.00	0.43	2.70	0.19	3.00	3.75	0.50	4.30	3.00	7.00	16.40	19.25	2.34	0.12	0.00	0.20	0.68
OTU121	23.70	45.40	1.90	5.70	0.65	5.30	0.13	3.00	7.00	0.65	10.3	3.00	8.50	28.70	31.80	3.40	0.28	0.00	????	????
OTU122	12.00	50.00	4.17	4.50	0.52	1.00	0.33	4.00	5.30	0.71	1.30	4.00	11.30	27.00	24.00	2.40	0.26	0.18	0.16	0.64
OTU123	9.20	13.40	1.46	3.00	0.43	4.60	0.00	1.30	3.00	0.46	4.80	1.70	9.10	3.00	3.40	0.33	0.12	0.00	????	????
OTU124	22.60	59.30	2.62	4.70	0.53	2.60	0.18	3.00	5.30	0.62	3.00	4.20	11.10	41.60	31.00	3.70	0.23	0.00	0.15	0.77
OTU125	12.90	49.00	3.80	3.50	0.57	2.50	0.25	3.30	5.20	0.60	2.20	4.00	11.30	37.60	34.70	3.30	0.17	0.00	????	????
OTU126	16.10	43.30	2.69	4.20	0.56	0.20	0.33	4.00	5.60	0.68	1.30	4.70	10.60	27.40	22.00	2.58	0.17	0.00	????	????

Appendix VI.
Accessions of *Elymus scaber* complex used in RAPD assay

Accessions	Abbr.	Species	Localities	Sources
S95001 (OTU57)	r_1	*E. rectisetus*	Hampton Hills Station, WA	Seedlings.
S95041	r_2	*E. rectisetus*	Near Perenjori, WA	Seedlings.
S95070	r_3	*E. rectisetus*	Stirling Range, WA	Young leaves.
S95087	r_4	*E. rectisetus*	North of Ravensthorpe, WA	Seedlings.
S95103 (OTU13)	r_5	*E. rectisetus*	Near Duri, NSW	seedlings.
S59109	r_6	*E. rectisetus*	Duri, NSW	seedlings.
S95115	r_7	*E. rectisetus* (?)	Near Walcha, NSW	Seedlings.
S95122	r_8	*E. rectisetus* (?)	Near Uralla, NSW	Seedlings.
S95059	s_1	*E. scaber* (?)	Manjimup, WA	Young leaves.
S95128 (OTU86)	s_2	*E. scaber*	Gara River, NSW	Seedlings.
S95104 (OTU61)	s_3	*E. scaber*	Duri, NSW	Young leaves.
S95129 (OTU87)	s_4	*E. scaber* (?)	Gara River, NSW	Seedlings.
S95108	s_5	*E. scaber* (?)	Duri, NSW	Seedlings.
S95125	s_6	*E. scaber*	Armidale, NSW	Seedlings.
S95142	s_7	*E. scaber*	Near Cherribah, Qld	Seedlings.
S95130	s_8	*E. scaber*	Near Uralla, NSW	Seedlings.
AVDB309	s_9	*E. scaber*	Bothwell, Tas.	seedlings.
AVDB333	s_{10}	*E. scaber*	Hobart, Tas.	seedlings.
AVDB334 (OTU78)	s_{11}	*E. scaber*	Mt. Nelson, Tas.	Young leaves.
S95163	m_1	*E. multiflorus*	Broulee Beach, NSW.	Young leaves.
S95170	m_2	*E. multiflorus*	Broulee Beach, NSW.	Young leaves.
S96168	m_3	*E. multiflorus*	Broulee Beach, NSW.	Young leaves.
S95177	m_4	*E. multiflorus*	4 km S. of the intersection of Broulee Rd. to Garlandtown, NSW.	Young leaves.
S95174	m_5	*E. multiflorus*	20 m away from S95177.	Young leaves.
HO116080	m_6	*E. multiflorus*	Maria Is., Tas., collected in 1968.	Dry material.
S95139 (OTU12)	n_1	Short-awned	Near Warwick, Qld.	Seedlings.
S95140 (OTU33)	n_2	Short-awned	Near Warwick, Qld.	Seedlings.
S95141	n_3	Short-awned	Near Cherribah, Qld.	Seedlings.
S95158 (OTU38)	n_4	Short-awned	Near Toowoomba, Qld.	Seedlings.
S95137 (OTU29)	n_5	Short-awned	Near Glen Innes, NSW	Young leaves.
S95132 (OTU41)	n_6	Short-awned	Armidale, NSW	Seedlings
S95153 (OTU2)	p_1	*E. plurinervis*	Near Maryvale, Qld.	Young leaves.
S95154	p_2	*E. plurinervis*	Near Cliffton, Qld.	Seedlings.
S95157	p_3	*E. plurinervis*	Near Drayton, Qld.	Seedlings.
S95159	p_4	*E. plurinervis*	Cecil Plains, Qld.	Seedlings.

Appendix VII
RAPD Analysis Data

RAPD data matrix used in the analysis of the *Elymus scaber* complex from Australia (Chapter 6).



Appendix VIII
Specimen Citation of Taxa (Including Geographic Information)

Elymus fertilis

No	Collectors	Sheet No	Locality	Latitude	Longitude
1	S. Wang 95139	SYD	12 km SW of Warwick, on New England HWY. Qld.	28°13'S	152°02'E
2	S. Wang 95140	SYD	12 km SW of Warwick, on New England HWY. Qld.	28°13'S	150°02E
3	S. Wang 95141	SYD	11.7 km E. of New England HWY on the Rd to Cherribah. Qld.		
4	C. E. Hubbard 3654	MEL 681316 PERTH00296546	Moreton District, Bank of Brisbane R., at Ascot, Brisbane. Qld.	27°26'S	153°04'E
5	S. Wang 95158	SYD	Turnoff to Wyreema from Cliffton to Toowoomba Rd. via Drayton. Qld.	27°39'S	150°51'E
6	S. Wang 95162	SYD	5.3 km from Warrego HWY on Brookvale Park Rd. Qld.		
7	C. E. Hubbard 5311	MEL595107	Morton District; between Laidley and Forest Hill. Qld.	27°38'30"S (Laidley)	152°24'30"E
8	C. E. Hubbard 1310	MEL681310	Morton District; banks of Brisbane River, Toowong. Qld.	27°29'S	152°59'E
9	C. E. Hubbard 5879	MEL594850	Darling Downs District; Wyreema. Qld.	27°39'S	151°51'E
10	S. Wang 95155	SYD	2.1 km from New England HWY to Cliffton. Qld.	27°56'00"S	151°54'00"E
11	S. Wang 95145	SYD	Maryvale Qld.	28°04'00"S	152°14'00"E
12	S. Wang 95143	SYD	1 km E of Gladfield, on Cunnigham Rd.	28°05'00"S	152°11'00"E
13	M. Murphy 431	NE	Moree, NSW.	29°28'S	149°50'
14	E. C. Hubbard 5879	MEL594850	Wyreema, Qld.	27°39'S	151°51'E
15	C. E. Hubbard 4811	MEL681310	Toowong, Qld.	27°29'S	153°E
16	N. Lloyd 759	NSW220373	Urban area, Moree	29°28'S	149°51'E
Specimens no detailed notes: MEL1560399 (Morton Bay, Qld.), MEL1560492 (Morton Bay, Qld.), MEL1560488 (Qld.), MEL1560489 (Qld.), MEL1560487 (T. M. Bailey, 1874), MEL1560496 (R. Brown; Qld.)					

Elymus multiflorus

1	K. R. Thiele 249	MEL 667857	N end of Broulee Beach, 18 km S of Batemans Bay. NSW.	35°51' S	150°11'E
2	A. C. Beauglehole 32316	MEL 531303	Captain Cook National Park, W of Thrra R., NNE of C Everard. Vic.	37°42'S	149°15'E
3		MEL 1560356	Pine forest, St. Vincent Gulf. SA.	34°33' - 35°13'S	137°55' - 138°15'E
4	A. C. Beauglehole 32282	MEL 531305	Captain Cook National Park, lighthouse track, c. 1.5m NE of Point Hicks. Vic.	37°42'S	149°15'E
5	J. E. S. Townrow	HO 116080	Darlington jetty, Maria Is. Tas.	42°34'S	148°03'E
6		MEL 1560339	Torrens R. SA.	34°52'S	138°46'E

7	W.M. Curtis	HO27735	South Arm. Tas.	43°02'S	147°25'E
8	S. Wang 95163	SYD	Near the beach, Broulee, NSW.	35°51'S	150°12'E
9	S. Wang 95168	SYD	Near the beach, Broulee, NSW.	35°51'S	150°12'E
10	S. Wang 95170	SYD	Near the beach, Broulee, NSW.	35°51'S	150°12'E
11	S. Wang 95174	SYD	4 km south of the intersection of Broulee Rd to Garlandtown.	35°51'S	150°12'E
12	S. Wang 95177	SYD	4 km south of the intersection of Broulee Rd to Garlandtown.	35°51'S	150°12'E
13	D. Cheal	MEL1554652	W. of Mildura. 4.7 km N of Lindsay Point Rd. on track to Potterwalkagee Creek.	34°08'40"S	141°23'45"E
14	S. Jacobs	SYD	Boat Harbour, NSW. Semi-stabilized sand dunes.	32°47'S	152°06'30''E
15	A. M. Buchanan 8350	HO96144	Variety Bay, North Bruny Island	43°12'S	147°24'E
16	I.C. Clarke 1354	MEL625872	Kew (Melbourne), Willsmere Park. Vic.	37°49'9"S	144°58'27"E
17		MEL1560343	St. Vincent Gulf, SA.	34°11'00"S	138°09'00"E
18	A. C. Beauglehole 31129	MEL531302	Mallacoota - Wingan coast, Vic.	37°33'40"S	149°45'4"E
19		MEL1560335	On the bank of the Little River, Vic.		
20	W. W. Spicer 10	MEL1560407	Pontville, Tas.	42°41'S	147°16'
21	C. Gibson s.n.	NSW369350	Morion St., Condell Park, Sydney.	33°54'30"S	150°59'30"E
22	G. Errington 109	NSW267217	100 m SW of Pontia St. Bridge on high bank, W side of creek, Toongabbie, NSW.	33°47'20"S	150°56'25"E
23	P. Kravchenko G9	NSW269503	Greystanes Creek, Toongabbie, NSW.	33°49'S	150°57'E
24	W. Semple OR226	NSW260429	'Goolloinboine', between Capertee and Glen Davis, NSW.	33°09'S	149°59'E
25		MEL1560430	Blue Mountains, NSW.		
Specimens no detailed notes: MEL1560410 (NSW), MEL1560327.					

Elymus plurinervis

1	S. Wang 95153	SYD	4 km E of Maryvale. Qld.	28°04'S	152°14'E
2	R. J. Fensham 1307	BRI 631026	18 km ESE of Cecil Plain. Grassy rail reserve. Qld.	27°33'S	151°22'E
3	L. S. Smith & S. L. Everist 811A	MEL 1560507	Darling Downs, about 3 miles SE of Blaxland. Qld.	27°12'S	151°19'E
4	S. Wang 95154	SYD	2.1 km from New England HWY to Cliffton. Qld.	27°56'S	151°54'E
5	R. J. Fensham 1728	BRI 630488	7 km SW of Toowoomba, grassy remnant, on basalt, roadside. Qld.	27°36'S	151°54'E
6	R. J. Fensham 1343	BRI 635876	Oakey, grassy roadside. Qld.	27°26' S	151°42'E

7	S. Wang 95144	SYD	2nd turn off to Maryvale	28°04' S	152°14'E
8	S. Wang 95159	SYD	18 km out Cecil Plains on the Cecil Plain RD	27°31' S	151°11' E
9	S. Wang 95157	SYD	Turn off to Wyreema from Clifton to Toowoomba Rd. via Drayton	27°39' S	151°51' E
10		MEL1560509	Warwick	28°13' S	152°02'E
11		MEL1560514	Warwick	28°13' S	152°02'E
12	J. Morrow 98	NSW99999	15 miles from Ashford on road to Emmaville, NSW.	c. 29°27'S	c.151°20'E
13	H. M. R. Rupp s.n.	NSW8248	Barraba, NSW.	30°23'S	150°37'E
14	F. Hely s.n.	NSW10703	Between Somerton and Manilla, NSW.	c. 30°50'S	c.150°42'E
15	D. Curry s.n.	NSW150564	Willow Tree District, NSW.	31°39'S	150°44'E
16		NSW8252	Narrabri, NSW.	30°20'S	149°47'E
17	F. W. Cutting s.n.	NSW57512	Moree, NSW.	29°28'S	149°51'E
18	E. O. Thomas 06	NSW8246	Inverell, NSW.	29°46'S	151°07'E
19	N Lloyd 735	NSW221730	58 km N of Moree on Newell HYW, NSW.	29°04'S	149°33'E
20	J. Carmen 1020	NSW	1 km E of Gladfield, Qld.	28°03'S	152°11'E
21	B. K. Simon & J. R. Harlan 2488	NSW	Pilton, grassy area outside planted pasture, Qld.	27°52'S	152°03'E
22	R. Story 6943	NSW	1 mile E of Bunnan, Hunter Valley, NSW.	32°02'S	150°35'E
Specimens no detailed notes: MEL1560398 (Qld.), MEL15060412, MEL1560501 (Qld.), MEL1560502 (Qld.), MEL1560503, MEL1560504, MEL1560508, MEL1560510, MEL1560512, MEL1560513					

Elymus scaber subsp. *scaber*

1	A. Moscal 1577	HO 64456	Lake Ayr, Tas.	41°49'S	146°04'E
2	S. Wang 95116	SYD	3.1 km E of New England HWY, on Oxley HWY. NSW.	c. 30°53'S	c. 151°10'E
3	J. B. Muir 1081	MEL 1560496	Upper slopes of Mt. Delusion, 17 m SW of Omeo. Vic.	37°19'S	147°32'E
4	Dr Story	MEL 1560401	Swanport. SA.	35°09'S	139°19'E
5	J. H. Willis	MEL 1560495	Mt. Buffalo NP. NE Alps. Vic.	c. 36°40'S	146°40'E
6	D. E. Albrecht 346	MEL 1526153	Plum Creek Flora Reserve, 20km along Dawsons Track from its intersection with Glenmore Rd. Vic.	37°23'S	148°10'30"E
7	J. B. Muir 353	MEL1560499	Mt. Buller. Vicinity of Whittaker Lodge. Vic.	37°09'S	146°26'E
8	S. Wang 95137	SYD	Near intersection of Lynnwood Rd. & Red Range Road, E of Glen Innes. NSW.	29°44'S	151°44'E
9	O. D. Evans	SYD	Concord West. NSW.	33°50'30"S	151°05'00"E
10	S. Wang 95124	SYD	Northern Hill at UNE, Armidale. NSW.	30°28'54"S	151°38'30"E
11	S. Wang 95133	SYD	Turnoff of Glenshiel R. on New Eangland HWY, 5 km N. of Guyra. NSW.	30°13'S	151°40'E

12	S. Wang 95132	SYD	Northern Hill at UNE, Armidale. NSW.	30°28'54"S	151°38'30"E
13	B. A. Rowe	HO28260	Life Sience Building. University. Sandy Bay. Tas.	42°54'S	147°18'E
14	A. C. Beauglehole 15740	MEL1507853	Bogong High Plains, Buckety Plain. Vic.	36°56'S	147°21'E
15	S. Wang 95104	SYD	Duri, Kelsos Lane, 300 m W of railway. NSW.	31°13'S	150°49'E
16	S. Wang 95135	SYD	0.5 km S of William Ck., N. of Glencoe. NSW.	29°56'S	151°44'E
17	S. Wang 95046	SYD	Serpentine Falls NP. across river. WA	32°22'S	115°58'E
18	J. H. Willis	MEL 521279	Hill behind Treasure Is. Caravan Park, Glen Dhu St., Launceston. Tas.	41°27'S	147°10'E
19	A.V.D. Borre 313	SYD	Penstock Lagoon (near Shannon), N side where road ends. Tas.	42°06'S	146°46'E
20	J. B. Muir 3009	MEL 1560319	SE of Tali Kargn, near the Sentinels. Vic.	37°33'S(Tali kargn Lake)	146°47'E
21	M. G. Corrick 7973	MEL 602667	N-central Highlands, Mt. Stirling, S slopes near Howqua Gap. Vic.	37°09'S	146°29'30"E
22	J. H. Willis	MEL 1560479	Round Is. (SW of Mondrain Is.),, Recherche Archipelago, WA	34°12'S	122°07'E
23	R. Story 7075	MEL 1560498	11 miles SW of Scone, NSW.	32°03'S	150°52'E
24	J. S. Whinray 959	MEL 532206	Long Is., Furneaux Group. Tas.	40°22'S	148°00'E
25	J. S. Whinray 2013	MEL 576573	Hogan Is., Hogan Group, Big Bay. Tas.	39°19'S	147°00'E
26	J. S. Whinray 709	MEL 594669	Erith Is., Kents Group. Tas.	39°27'S	147°17'E
27	J. H. Hemsley	HO 91358	Mt. Nelson, Hobart. Tas.	42°54'S	147°19'E
28	S. T. Blake 18271	HO 110935	Hobart, the Domain. Tas.	42°53'S	147°19'E
29	J. J. Yates	HO 32695	Penstock Lagoon, ca. 1 km E. of Crisps Point. Tas.	42°06'S	146°46'E
30	A.V.D. Borre 334	SYD	Mt. Nelson, Tas.	42°54'S	147°19'E
31	R. N. Auchterlonie	MEL 1560321	Central Gippsland, Hazelwood. Vic.	38°17'S	146°22'E
32	Austin Brown 14	MEL 690519	Dargo High Plains. Vic.	37°05'S	147°08'E
33	J. H. Hemsley	HO 91348	Mt. Nelson. Tas.	42°54'S	147°19'E
34	A.V.D. Borre 309	SYD	Bothwell, 1 -2 km out of town on the Melton Mowbray Rd. Tas.	42°23'S	147°00'E
35	S. Wang 95128	SYD	Gara R. TSR no. 58. NSW.	30°32'24"S	151°43'49"E
36	S. Wang 95134	SYD	0.5 km S of William Ck., N. of Glencoe. NSW.	29°56'S	151°44'E
37	S. Wang 95136	SYD	0.5 km S of William Ck., N. of Glencoe. NSW.	29°56'S	151°44'E
38	A. C. Beauglehole 21488	MEL 1507856	Port Cambell National Park, bw. Crown of Thorns & the Gotto. Vic.	38°37'S	143°00'E
39	S. J. Forbes 269	MEL 595510	Mt. Dawsons Track,, Buchan R. crossing, 7km NE of Mt. Johnson. Vic.	37°26'40"S	148°09'35"E
40	J. B. Muir 2951	MEL 1560324	Gippsland. Nightingale Ck., just above Tali Karng. Vic.	37°33'S	146°47'E
41	J. B. Muir 3541	MEL 1560323	Yea District, 5 miles S of Highlands, on the Yea Rd. Vic.	37°13'S	145°26'E
42	S. Rennick 73	MEL 687191	Nepean State Park Arthurs Seat, Dromana. Vic.	38°21'S	144°56'E

43	S. Wang 95038	SYD	Cockleshell Gully, on the Cockleshell Gully Rd. WA	30°09'S	115°07'E
44	W. M Curtis	MEL 647906	The Domain, Hobart. Tas.	42°51'S	147°19'E
45	J. S. Whinray	HO 26150	Furneaux Group, Flinders Is. Tas.	40°08'S	148°00'E
46	J. H. Willis	MEL1560470	Crest of Bolangum Range, c. 2 miles N of Kanya. Vic.	36°47'09"S	142°59'45"E
47	A. C. Beauglehole 1945	MEL1507844	Portland, Gorae West. Vic.	38°20'30"S	141°35'59"E
48		MEL1560409	Murrumbidgee. NSW.	34°31'36"-36°7'S	143°19' - 149°8'E
49	A. C. Beauglehole 9733	MEL531310	Wyperfeld NP. Wonga Hut area. Vic.	35°34'01"S	142°07'00"E
50	Dr. Behr.	MEL1560350	Road towards Angaston. SA.	34°31'S	139°3'E
51	R. C. Gunn	HO91356	Penquite. Tas.	41°27'S	147°12'E
52	A. C. Beauglehole 63652	MEL547406	Lake Dubban Wildlife Reserve, 7 km ENE of Winchelsea P.O. Vic.	38°15'S	143 59'E
53	J. H. Willis	MEL1560408	Pasco Is. Rechercche Archipelago. WA	34°4'S	122°6'E
54	J. H. Willis	MEL1560480	Boxer Is. Recherche Archipelago. WA	34°S	121°40'E
55	C. A. Gardener 6625	PERTH008861 65	Jeyes, near Boyup Brook. WA	33°47'36"S	116°21'12"E
56	L. Rodway	HO91355	Domain, Hobart. Tas.	42°51'S	147°19'E
57	N. A. Wakefield 3353	MEL1507849	East Gippsland, Cann River. Vic.	38°40'S	144°40'E
58	A. C. Beauglehole 34738	MEL531314	East Gippsland, Mt. Delegate. Vic.	37°06'50"S	148°53'47"E
59	R. J. Adair 1857	MEL 113110	Toongabbie. Vic.	38°6'S	146°40'E
60	S. J. Forbes 194	MEL574816	Mt Seldom Seen. Vic.	37°6'5"S	148°11'30"E
61	R. W. Lawrence 249	HO99346	Probably Formosa. Tas.		
62	J. Whinray 5		Bass Strait. West Sister Is. Tas.	39°41'57"S	147°55'38"E
63	W. D. Jackson 243	HO54109	Dunes N of the mouth of Piema n River. Tas.	41°39'S	145°8'E
64	L. Rodway	HO91361	Hobart. Tas.	42°51'S	147°19'E
65	O. Rodway	HO91360	Blackmans Bay. Tas.	43°S	147°19'E
66	L. Rodway	HO91352	Queens Domain, Hobart. Tas.	42°51' S	147°19'E
67	J. Ireson	HO43939	234 Mt. Nelson Road	42°54'S	147°19'E
68	M. G. Temple - Smith	HO 43952	The Domain, near old Tip. Hobart. Tas.	42°51's	147°19'E
69	M. Gillham	MEL1515075	Great Dog Is. Tas.	40°15'07"S	148°15'08"E
70	S. T. Blake 18271	HO91362	The Domain, Hobart. Tas.	42°51'S	147°19'E
71	L Rodway	HO91353	Queens Domain, Hobart. Tas.	42°51'S	147°19'E
72	J. S. Whinray 21	HO26186	Furneaux Group; W Sister Is. Tas.	39°43'S	148°55'E
73	A. Moscal 1978	HO62106	Mt Inglis. Tas.	41°44'S	145°53'E

74	A. Moscal 8436	HO99957	Pittwater Bluff. Tas.	42°49'S	147°30'E
75	R. C. Gunn	HO91359	Penquite, near Norwood, Launceston. Tas.	41°27'S	147°12'E
76	D. I. Morris	HO74912	Great Lake, SW corner of Swan Bay. Tas.	41°59'S	146°40'E
77	A. C. Beauglehole 19060	MEL1507854	Bessiebelle, near Eumeralla River	38°09'35"S	141°57'32"E
78	R. J. Adair 1791	MEL1556138	Warrandyte. Vic.	37°45'S	147°10'E
79	J. S. Wihnray 1878	MEL1503068	Deal Is. Kents Group. Tas.	43°56'31"S	147°18'59"E
80	D. E. Albrecht 1425	MEL674989	Alpine Study Area. Nunniong Rd. directly S of the Tambo Valley Golf Club. Vic.	37°10'20" S	147°46'40"E
81	J. B. Muir 3820	MEL1560325	Gippsland. On headwaters of Moroka River, 1 mile N of Mt Wellinton. Vic.	37°30'34"S	146°50'42"E
82	M. G. Corrick 8469	MEL657759	Warrock Rd. c. 14 km SSW Chetwynd. Vic.	37°25'S	141°23'E
83	A. C. Beauglehole 71547	MEL677588	Cape Schanck Coastal Park. Vic.	38°29'26"S	144°55'04"E
84	A. D. J. Piesse 198	MEL1545490	NE Middle Creek, 43 km S from Wangaratta. Vic.	36°14'S	146°15'E
85	M. G. Noble 29102	HO79296	Ben Nevis - in E. archeri forest. Tas.	41°25'S	147°37'E
86	J. S. Whinray 197	HO25894	Kent Group; Deal Is. Tas.	39°30'S	147°17'E
87	A. Moscal 2403	MEL652633	Croesus Cave State Reserve. Tas.	41°34'S	146°13'E
88	A. M. Buchanan 735	HO50285	Mouth of Lagunta Creek. Tas.	42°11'S	148°17'E
89	J. B. Muir 345	MEL1560462	Mt. Buller. N slope of Baldy, alt. 5650ft. Vic.	37°08'48"S	146°25'29"E
90	A. C. Beauglehole 21432	MEL1507855	Port Campbell NP, the Arch area. Vic.	38°39'05"S	141°53'47"E
91	R. Melville 3261	MEL524866	Mt. Buller. Vic.	37°08'48"S	146°25'29"E
92	J. B Muir 342	MEL1560464	Mt. Buller, North slope of Baldy, alt. 5650ft. Vic.	37°08'48"S	146°25'29"E
93	A. C. Beauglehole 17965	MEL531308	East Gippsland, Sydenham Inlet. Vic.	37°45'57"S	148°58'32"E
94	A. Moscal 6507	HO95454	Jacks Creek. Tas.	42°4'S	146°54'E
95	A. Moscal 2403	HO64941	Croesus Cave State Reserve. Tas.	41°34'S	146°13'E
96	E. A. Chesterfield	MEL626566	East Gippsland, upper Rodger River. Vic.	37°18'5"S	148°32'E
97	F. Swindley 1461	MEL1294	Lake Goldsmith. Vic.	37°32'11"S	143°20'55"E
98	S. Harris	HO112950	Pelican Is. Off NE corner of Cape Barren Is. Tas.	40 18'S	148°19'E
99	J. S. Whinray 2087	MEL576572	Flinders Is, Furneaux Group. Tas.	39°52'17"S	148°01'02"E
100	A. Moscal 6526	HO96898	1.5 km W of Norths Hill. Tas.	42°3'S	146°54'E
101	W. M. Curtis	HO55990	The Domain, Hobart. Tas.	42°51'S	147°19'E
102	T. B. Muir 5627	MEL1510202	Ashburto, SE suburb of Melbourne. Vic.	37°51'S	145°5'E
103	A. C. Beauglehole 17962	MEL531307	East Gippsland, Orbost. Vic.	37°42'15"S	148°27'28"E

104	S. Wang 95126	SYD	Just cross Commissioners Water, 7 km E of Armidale. NSW.	30°32'23"S	151°43'50"E
105	L. Rodway	HO91351	Middlesex Plain. Tas.	41°33'S	146°E
106	A. C. Beauglehole 22550	MEL1507847	Bogong High Plains, E of Mt. Jim. Vic.	36°53'43"S	147°16'18"E
107	N. A. Wakefield 3352	MEL1507848	East Gippsland, Suggan Buggan. Vic.	36°57'11"S	148°19'31"E
108	O. D. Evans	SYD	Casula, near Liverpool. NSW.	33°57'00"S	150°59'00"E
109	J. M^{c}L. & A.H.K.P.	SYD	Kosciusko. NSW.	36 27'S	148°16'E
110	A. C. Beauglehole 22578	MEL1507852	Bogong High Plains, Basalt Hill. Vic.	36°53'43"S	147°16'18"E
111	J. H. Willis	MEL1560320	Mrytleford. Vic.	36°33'32"S	146°43'26"E
112	G. W. Carr 10256	MEL678290	Alpine Study Area. Slitters Range Forest Block.Vic.	37°5'55"S	147°46'35"E
113	A. C. Beauglehole 72580	MEL677587	Baw Baw NP. Vic.	37°50'46"S	146°16'25"E
114	P. R. eMilne 348	MEL575493	Hamilton, on Ararat railway line, c. 7 km NE of Hamilton G.P.O. Vic.	37°44'25"S	142°01'17"E
115	R. Turner	HO97526	Earham, Orford. Tas.	42°33'S	147°52 E
116	J. B. Muir 326	MEL1560463	Mt. Buller. Top of House Hill, alt. 4860 ft. Vic.	37°08'48"S	146°25'29"E
117	H. B. Williamson	MEL570569	Hawkesdale West? Vic.	38°05'58"S	142°14'15"E
118	A. C. Beauglehole 16405	MEL531318	Grampians c. 5.5 m S of Halls Gap. Glenbower Creek. Vic.	37°15'46"S	142°26'37"E
119	M. J. Henwood 382	SYD	c. 1 km from Jenolin Caves towards Hartley? NSW.		
120	L. Rodway	HO91354	Hobart. Tas.	42°51'S	147°19'E
121	A. C. Beauglehole 22396	MEL1507850	Clover Dam area, between Mt Beauty & Falls Creek. Vic.	36°45'01"S	147°10'53"E
122	G. W. Anderson	MEL651339	South east shore of Question Lake, Hattah Lakes National Park. Vic.		
123	D. I. Morris 8234	MEL652591	Tasman HWY, c. 3.5 km N of Cranbrook. Tas.	41°59'S	148°02'E
124	D. I. Morris 8234	HO59242	Tasman HWY, c. 3.5 km N of Cranbrook. Tas.	41°59'S	148°02'E
125	W. M. Curtis	HO27733	The Domain, Hobart. Tas.	42°50'S	147°19'E
126	D. Martin	SYD	Hobart. Tas.	42°51'S	147°19'E
127	J. L. Boorman	SYD	Awaba. NSW.	33°01'S	151°33'E
128	H. S. Mekee 8851	NSW	Canberra. A. C. T.	35°17'S	149°08'E
129	J. Carmen 1023	NSW	23 km from Warwick on Stanthorpe Rd. Qld.	28°22'S	151°55'E
Specimens no detailed notes: MEL1560446, MEL1560453, MEL1560347, MEL1560341 (Torrens, SA?)					

Elymus scaber subsp. *rectisetus*

1	R. Bates 15856	BRI 467208	Alligator Gorge NP. Flinders Ranges. SA.	32°46'S	138°10'E
2	S. Wang 95103	SYD	4.15 km E. of Werris Ck - Tamworth Rd. on the Duri - Dungowan Rd. NSW.	31°13'S	150°49'E
3	A. C. Beauglehole 42443	MEL 1507858	Bendigo, Mystery Paddock. Vic.	36°46'S	144°17'E
4	J. B. Muir 2389	MEL 1560322	Jattans Lookout, on the Murray Valley HWY, 3 miles N of Towong. Vic.	36°08'S	147°59'E
5	A. C. Beauglehole 29368	MEL 531316	Wyperfeld NP, c. 3.5 miles SW of Wonga Hut, E side of road to Entrance. Vic.	35°32'S	141°58'E
6	N. C. Beadle	SYD	5 miles W of Ootha. NSW.	33°07'S	147°27'E
7	A. C. Beauglehole 29323	MEL 531309	N. side of Wyperfeld NP. Vic.	35°32'S	141°58'E
8	A. C. Beauglehole 29232	MEL 531315	Wyperfeld NP., Lost Lake, mid-N end of park. Vic.	35°32'S	141°58'E
9		MEL 1560436	Near Dimboola. Vic.	36°37'S	142°02'E
10	S. Wang 95105a	SYD	Kelsos Lane, 430 m W of railway, Duri. NSW.	31°13'S	150°49'E
11	S. Wang 95105b	SYD	Kelsos Lane, 430 m W of railway, Duri. NSW.	31°13'S	150°49'E
12		MEL 1560345	Crystal Brook. SA.	33°21'S	138°12'E
13		MEL 1560340	Near Torrence R. SA.	34°52'S	138°46'E
14	D. Sullivan	MEL 1560457	Moyston. Vic.	37°18'S	142°46'E
15		MEL 1560472	Between Esperance Bay & Frasers Range (?). WA	c. 33°S	c. 122°E
16	A.D.J. Piesse 143	MEL 1554171	Murrey Valley - Loves Bend (?) Flora Reserve, 30 km N from Wangaratta. Vic.	36°08'S	146°14'E
17	A. D. J. Piesse 247	MEL 689066	Murray Valley, Boweya Flora Reserve, 31 km NW from Wangaratta. Vic.	36°15'S	146°06'E
18	S. Wang 95087	SYD	Waricul Private Property, driveway to 13.3 km N of Ravensthorpe of Ravensthorpe - Lake King Rd. WA	33°30'S	119°57'E
19	S. Wang 95001	SYD	23.6 km E of Kalgoorlie, Hampton Hills Station. WA	30°45'S	121°46'E
20	S. Wang 95041	SYD	600 m S of Munckton Rd on Fallon Rd. near Perenjori. WA	29°26'35"S	116°17'E
21	S. J. Forbes 1890	MEL667927	Broken Ck. c. 1.5 km NNE Mooramong H.S., Horseshoe Paddock c. 11 km NW Skipton. Vic.	37°38'50"S	143°15 E
22	B. J. Lepschi 2027	PERTH 04195280	c. 12 km NW of Bungalbin Hill, W extremity of Helena & Aurora Range. WA	30°20'S	119°31'E
23	S. Wang 95070	SYD	Stirling Range Caravan Park. WA.	c. 34°25'S	c. 118°15'E
24	S. Wang 95109	SYD	500 m W of railway, Kelsos Lane, Duri. NSW.	31°13'S	150°49'E
25	S. Wang 95115	SYD	3.1 km E of New England HWY on Oxley HWY. NSW.	30°53'S	151°10'E
26	S. Wang 95122	SYD	Right side of the Walcha-Uralla Rd, 5.1 km S of railway. NSW	30°39'00"S	151°30'00"E
27	J. B. Muir 1746	MEL1560318	7 miles SW of Thoona, near the Yarrawonga-Benalla Rd. Vic.	36°21'S	146°5' E

28		MEL1560413	Barrier Range. NSW.	31°11'41"S	141°24'34"E
29		MEL1560358	Yorke Peninsula, SA.	35°5'S	137°15'E
30		MEL1560357	Near upper end of Spencers Gulf. SA.	32°45'S	137°50'E
31	G. H. Robinson	MEL1560332	Burnley, Royal Horticultural Gardens. Vic.	37°49'34"S	145°00'38"E
32	Leiut. Dittruch. Lindseys Exp. 138	MEL1560346	S-central Australia. SA.		
33	S. Mathews 1889	MEL1560451	Mildura. Vic.	34°11'S	142°10'E
34	A. C. Beauglehole 21710	MEL1507857	Ararat, McDonald Park. Vic.	37°17'S	142°56'E
35	J. Vickery 10189	MEL532205	Barham. NSW.	35°38'S	144°8'E
36	K. R. Thiele 128	MEL667858	4 km due west of Cowra. NSW.	33°50'S	148°41'E
37	E. J. McB.	SYD	Henty. NSW.	35°31'S	147°2'E
38	K. Newbey 11058	PERTH00832685	35 km SW of Renvensthorpe. WA	33°46'11"S	119°42'15"E
39	C. A. Gardener 6592a	PERTH00886092	E from Warrungup,Stirling Range. WA	34°17'59"S	118°8'E
40	R. D. Royce 9148	PERTH00886149	Fitzgerald River NP. WA	33°49'47"S	119°56'23"E
41	B. Jones (per A. C. Linto)	PERTH00886084	Hampton Hills Station, Kalgoorlie. WA	30°44'23"S	121°28'6"E
42	R. D. Royce 4702	PERTH00886130	Swanview, NE of Perth. WA	31°52'59"S	116°2'59"E
43	A. S. Weston 9006	PERTH00296538	Peak Charles. WA	33°55'S	121°10'E
44	S. Wang 95071	SYD	Stirling Range Caravan Park. WA	c. 34°25'S	118°15'E
45		MEL1560344	Crystal Brook. SA.	33°21'S	138°12'E
46	J. P. Echert	MEL1560443	Wimmera. Vic.	c. 37°S	c.142°30'E
47	G. J. Keighery 5712	PERTH00322482	Southern margin Quarderwardup Lake, eastern Stirling Ranges. WA	34°27'54"S	118°19'53"E
48	E. J. MacB. 906	SYD	Holbrook? NSW.		
49	G. H. Robinson (1894)	MEL1560449	Goulburn River. Vic.	37°02'09"S	145°07'41"E
50	G. A. Robinson	MEL1560471	Pyrenees. Vic.	37°5'S	143°16'E
51	A. C. Beauglehole 22005	MEL1507851	Stawell, Three Jacks Reserve. Vic.	37°12'S	147°45'E
52	A. W. Bell 59	MEL527423	Southern Lofty Region. SA.	35°11'S	138°37'E
53	C. A. Gardener 6566	PERTH00886181	Mourcowrup, S of Borden. WA	34°4'30"S	118°16'5"E
54	M. Koch 1134	PERTH00886122			
55	A. C. Beauglehole 17963	MEL531306	East Gippsland, Mc Killop Bridge. Vic.		
56	E. J. McB	SYD	Tumbarumba. NSW.	35°46'S	148°1'E
57	R. H. Kennedy	MEL1560414	Mt. Lyell, Darling district? NSW.	33°31'00"S	150°04'00"E

58	R. D. Royce 8040	PERTH008861 06	Tunny, S. of Kojonup. WA	34°6'54"S	117°21'47"E
59	J. P. Echert	MEL1560455	Wimmera. Vic.	c. 37°S	c. 142°30'E
60	G. H. Robinson	MEL1560450	Goulbourn River. Vic.	37°02'09"S	145°07'41"E
61	A. C. Beauglehole 30867	MEL1507845	Grampians Mt. Arapiles, SW side. Vic.	36°45'14"S	141°50'01"E
62	A. C. Beauglehole 935	MEL1507846	Far NW Hattah Lakes NP. Vic.	34°42'15"S	142°17'18"E
63	W. Perry	MEL531174	Near Neilborough. Vic.	36°34'S	144°14'E
64	S. J. Forbes 1915	MEL667937	Nerrin Nerrin - Woorndpp Rd. 4 km WSW of Mt. Hamilton, near Pagel's Lane. Vic.	37°48'S	142°56'30"E
65	W. M. Carne	PERTH008866 02	Bridgetown. WA	33°57'33"S	116°08'22"E
66	M. M^{c}C. Webster	PERTH 00886599	Warren, at road junction 1/4 W of Denmark. WA	34°38'00"S	115°51'04"E
67	J. Edwards 22	MEL1539560	McKenzie Flora Reserve, jus toutside Alexandra township. Vic.	37°12'S	145°43'E
68		MEL1560437	Near Dimboola. Vic.	36°27'S	142°3'E
69	N. Lloyd 1444	NSW295843	7 km NW of Temora on the Wyalong road, NSW.	34°27'S	147°32'E
70	N. Lloyd 1027	NSW256284	11 km E of Condobolin on the Parkes road, NSW.	c. 33°05'S	c. 147°
71	N. Lloyd 1128	NSW	11 km E of Caragabal on the Mid-Western HWY, NSW.	c. 33°51'S	c. 147°40'E
72	P. L. Milthorpe & G. M. Cunningham 2808	NSW	10 km E of Cobar, NSW.	31°30'S	145°55'E
73	C. H. Miller 537	NSW331175	11 km S of highway turnoff near Cobar, NSW.	31°35'S	145°53'E
74	I. Crawford 1045	CBG	Mootwingee NP., 112 km NE of Broken Hill, NSW.	31°16'S	142°18'E
75	L. Richley 1436	NSW	Donsandel (Broken Hill), NSW.	31°57'S	141°40'E
76	Drummond 384 ?	MEL1560481	WA		
77	Drummond 384 ?	MEL1560482	WA		
78	Pidgeon	SYD	Currabubula, NSW.		