DELIMITING SPECIES: PHYLOGENY AND TAXONOMY OF THE FUNGUS-GROWING ANT GENUS *SERICOMYRMEX*

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INTRODUCTION

Sericomyrmex Mayr, 1865 is a poorly known genus of fungus-growing ants (Myrmicinae: Attini) that is closely related to the leaf-cutting genera *Atta* and *Acromyrmex*. *Sericomyrmex* includes 22 described species and subspecies, and it is distributed throughout most of South and Central America[1]. *Sericomyrmex* ants are commonly collected in leaf-litter samples in biodiversity studies, but are impossible to identify. Species are morphologically very similar and within-nest variation is substantial, confounding easy recognition of species boundaries. The most recent key to the species of *Sericomyrmex* was published in 1916 and most of the original species descriptions are vague, with almost no precise measurements[2]. The vague morphological species boundaries in *Sericomyrmex* are accompanied by very little variation in nuclear genes. Only mitochondrial COI shows some variation in 67 specimens of *Sericomyrmex*. Numerous studies of other organisms demonstrate the importance of a combination of data sources for delimiting species, especially in problematic taxa[3,4]. Here I summarize my ongoing efforts to find molecular variation in *Sericomyrmex*, discover useful morphological characters, and collect ecological data about nest architecture as an additional source of information.

MATERIALS & METHODS

Morphology

More than 15,000 *Sericomyrmex* specimens (~12 nests) were collected during extensive personal field work in Peru, Guyana, Brazil, Guatemala, and Mexico, and over 1,000 specimens were obtained from collections and colleagues. All pinned specimens were sorted into tentative morphospecies based mostly on the morphology of the head and pubescence.

Molecular work

Details of the processing of raw reads and data analyses can be found on the poster handout.

1) SANGER SEQUENCING: 77 specimens were extracted and amplified for mitochondrial COI and 7 nuclear gene fragments (Opsin, EF1αF1, Wingless, Abdominal A, Arginin Kinase and 28S).

2) TRANSCRIPTOMES: Colonies of 3 morphologically and molecularly distant species were chosen for transcriptomics. 10 workers per colony were used for each of the 3 samples for RNA extraction. Library preparation and sequencing (Ilumina HiSeq 1000) were carried out at IBBR at UMD. Except for the transcriptome of *Apterostigma megacephal*a, the transcriptomes of non-*Sericomyrmex* taxa are from Nygaard et al. (unpublished), courtesy of the authors, and from published genome data[5-10].

3) ULTRACONSERVED ELEMENTS (UCEs): 13 different *Sericomyrmex* specimens were chosen for a trial phylogenomic study utilizing UCE markers. This is a reduced genome representation method in which targeted enrichment for UCEs is combined with multiplexed Next Gen sequencing[11]. Extractions, library preparations and target enrichments were performed in the NMNH L.A.B. and sequenced on Illumina HiSeq at UCLA.

Nest architecture

Sericomyrmex nests were excavated in Guyana, Peru, Brazil, and Mexico. During each excavation detailed measurments were taken of: the nest entrance, and, for each chamber, chamber height, width, depth, and distance from the surface to the chamber roof.

RESULTS

Morphology

Based solely on morphology, all of the assembled specimens can be separated into 5 to 11 morphospecies depending on how conservative we set species-delimitation criteria. Combining the morphological data with a molecular phylogeny based on mtCOI (which included less taxa) 8 different species of *Sericomyrmex* can be identified. In both cases the total number of species is much less than the number described by previous authors (19 species).

Molecular data

1) SANGER SEQUENCING: In comparison with similar ant genera, including the closely related genus *Trachymyrmex*, I found surprisingly little variation in the nuclear genes that were sequenced for *Sericomyrmex*; significant variation was present only in mtCOI (Fig. 5.).

2) TRANSCRIPTOMES: For 3 *Sericomyrmex* taxa and *A. megacephala* 11,447 orthologs were identified. The complete matrix had 649,095 AA sites for all 13 taxa.The maximum likelihood phylogeny (Fig. 6.) confirmed the position of *Sericomyrmex* as the sister group to *T. zeteki* clade. The branches within *Sericomyrmex* are surprisingly short, but with high support values. More than 90% of orthologs present in all 3 *Sericomyrmex* taxa were 99% to 100% sequence-identical.

3) UCEs: From 642 to 707 UCE loci per taxa were sequenced for 7 *Sericomyrmex* taxa and 3 outgroups, varying in length from 142-1,852 bp. The complete, aligned data matrix contained 517,979 bp. The resulting phylogeny (Fig. 7.) has a topology that mostly matches the COI tree topology for the taxa that are present in both analyses, but the support for clades is much higher in the UCE phylogeny.

Nest architecture

A table with measurements for each of the nests can be found in the poster handout. A total of 12 nests were excavated. *Sericomyrmex* nests show great variation. Two different species collected in Tambopata, Peru, both excavated in same habitat, differ with regard to depth and chamber size (Fig. 2.).

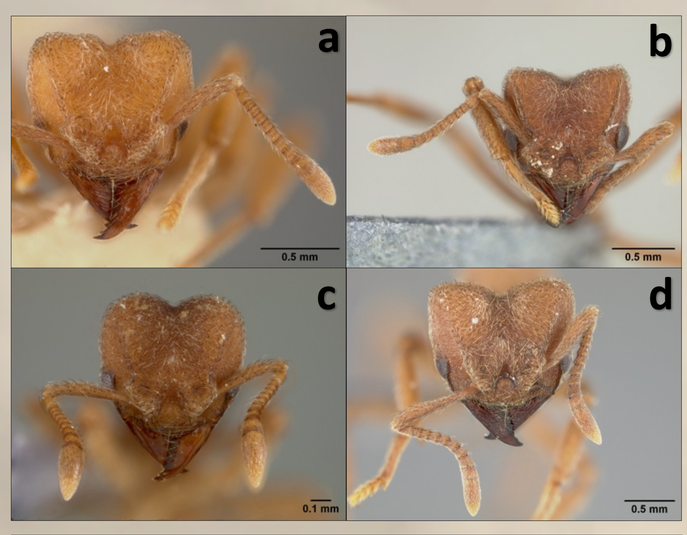

Fig. 1. Morphologically distinct *Sericomyrmex* species: a) *S. amabilis* (Panama), b) *S. scrobifer* (Brazil), c) *S. parvulus* (Brazil), d) *S. cf. luederwaldti* (Brazil).

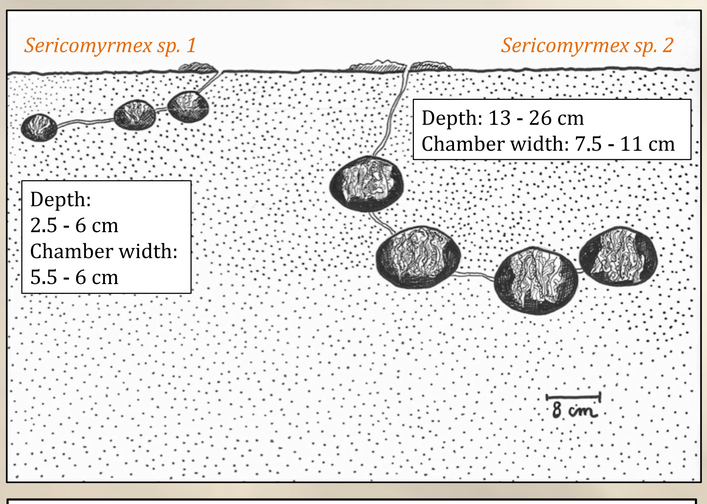

Fig. 2. DIFFERENT NEST MORPHOLOGIES: Sketch of of 2 different *Sericomyrmex* species nests from the same locality. Differences include the size of the chambers, depth, and the position of the fungus. Based on data from 4 nests from Peru.

Fig. 3. Locations visited by AJ, for collecting and/or visits to museum collections.

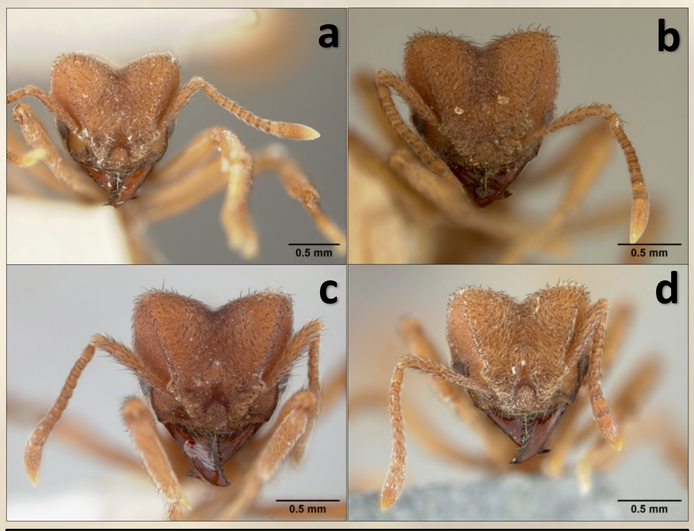

Fig. 4. ALL THE SAME?: Type specimens of a) *S. beniensis* (Bolivia), b) *S. impexus* (Guyana), and specimens of c) *S. bondari* (Brazil), and d) "Morphospecies 2" (Peru).

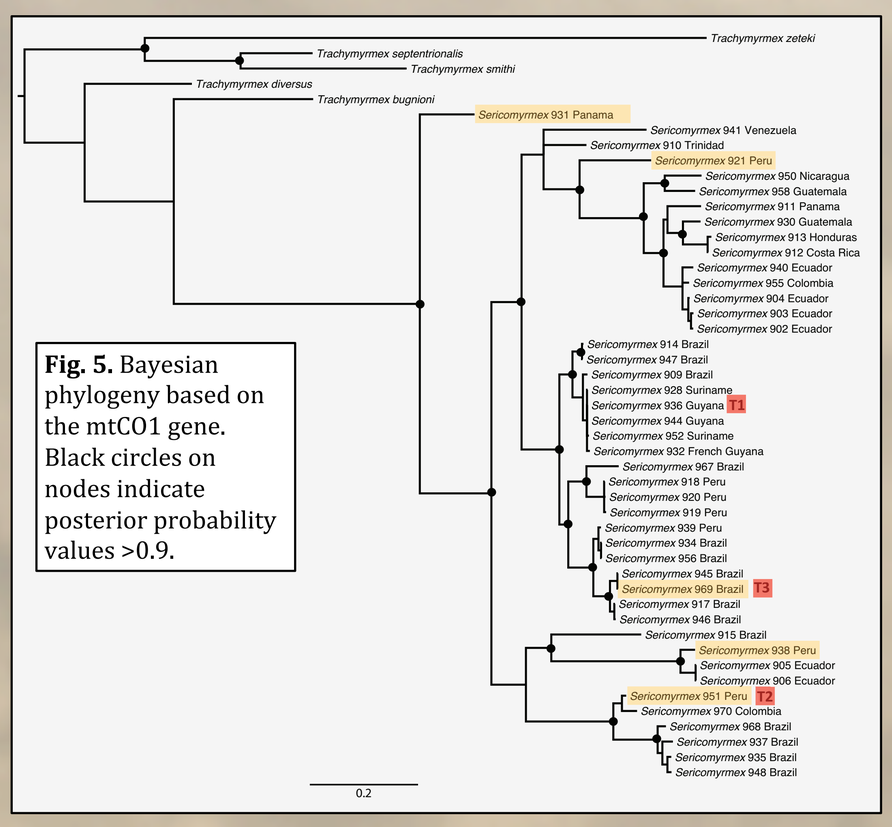

Fig. 5. Bayesian phylogeny based on the mtCO1 gene. Black circles on nodes indicate posterior probability values >0.9.

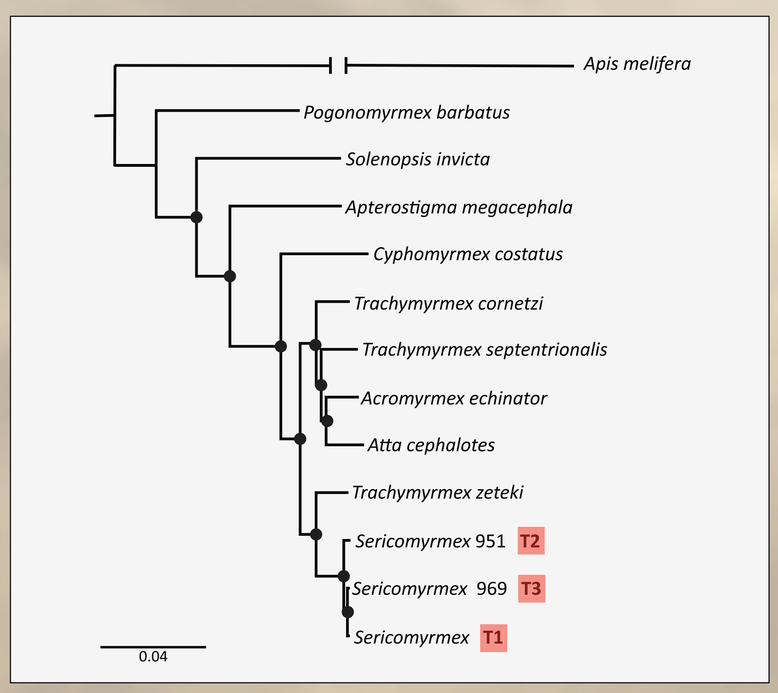

Fig. 6. Maximum likelihood phylogeny based on transcriptomic and genomic data: 1,317 orthologs and 649,095 AA sites present in all 13 taxa. Black circles on nodes indicate bootstrap values of 100.

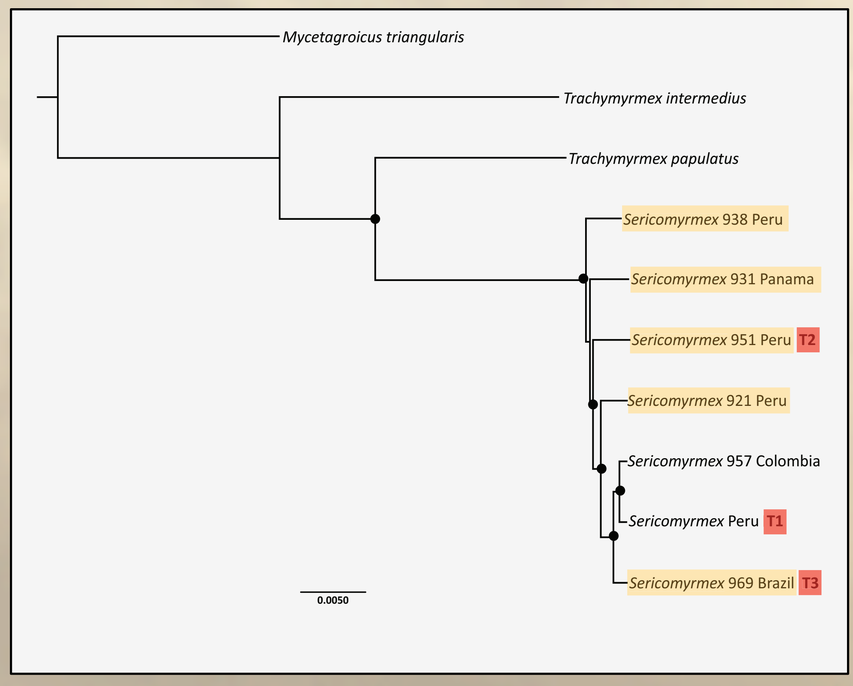

Fig 7. Maximum likelihood phylogeny based on UCE data: 642 to 707 UCE loci per taxa, each 147-1,852 bp in length, 517,979 bp total. Black circles on nodes indicate bootstrap values of 100.

DISCUSSION AND CONCLUSION

Morphological studies of worker variation, genetic data from 8 genes, transcriptome data from 3 specimens, and a preliminary UCE dataset, all indicate that there may be far less than the 19 species described by previous authors. The preliminary UCE data show particular promise for delimiting species and reconstructing phylogeny because almost all of the relationships are well supported and the topology corroborates that of the COI phylogeny. This suggests that UCE or similar reduced genome representation methods, which capture large amounts of sequence data from across the entire genome, may be the best way to obtain molecular variation sufficient for delimiting species and robustly reconstructing the phylogeny of *Sericomyrmex* species. This results suggests that *Sericomyrmex* has achieved a broad geographic and biotic distribution (in savannahs, cerrados, and rain forests from Argentina to Mexico) with only a small degree of accompanying speciation, in contrast to most other ant species, including those in its similarly distributed sister taxon *Trachymyrmex*. One possible explanation is that *Sericomyrmex* has recently and rapidly radiated geographically with minimal diversification. Additional investigations will focus on whether or not this radiation may have been driven by coevolution with a specialized clade of higher attine fungi, as has been suggested for *Atta*[12,13].

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