

ABSTRACT

Title of Dissertation: TAXONOMIC REVISION, PHYLOGENOMIC ANALYSES, AND NATURAL HISTORY OF THE FUNGUS-FARMING ANT GENUS *SERICOMYRMEX* (HYMENOPTERA: FORMICIDAE)

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Fungus-farming ("attine") ants, a New World clade of nearly 300 species in 16 genera, are model systems for studies of symbiosis, coevolution, and advanced eusociality. All attine ants cultivate fungal symbionts for food. "Lower" attine ants cultivate facultatively symbiotic fungi, but in "higher" attine ants both ants and fungi are obligate, highly coevolved symbionts. *Sericomyrmex* is a poorly studied higher-attine genus known for its problematic taxonomy. This work represents the first comprehensive study of *Sericomyrmex* species and their fungal cultivars.

For Chapter One I sequenced, assembled, and analyzed transcriptomes of three species of *Sericomyrmex* and a lower-attine-ant species, *Apterostigma megacephala*. I characterize the transcriptomes, conduct phylogenetic analyses, and search for genes of interest, most importantly arginine biosynthesis pathway genes, the absence of which in *A. megacephala* strongly suggests that attine ants became dependent on their fungi early in their evolution.

In Chapter Two I address the phylogenomics of *Sericomyrmex*, sequencing ~990 ultra-conserved-element loci for 88 *Sericomyrmex* samples. Maximum-likelihood and species-tree phylogenetic methods recover nearly identical topologies across data sets, identifying nine species-level lineages. Divergence-dating analyses indicate that *Sericomyrmex* is the product of a recent, rapid radiation, with a crown-group age estimate of 4.3 million years. I sequence two nuclear ribosomal regions for 32 *Sericomyrmex* fungi. The fungal phylogeny indicates that *Sericomyrmex* cultivars are generalized higher-attine cultivars, which, rather than forming a separate clade, are interspersed with *Trachymyrmex*-associated fungi, indicating cultivar sharing and horizontal transfer between those genera.

In Chapter Three, guided by my phylogenomic results, I study the morphology of *Sericomyrmex* workers, males, queens, and larvae and conduct a comprehensive taxonomic revision, including a key to the worker caste and species distribution maps. *Sericomyrmex* comprises 11 species, including three new species. The number of recognized species is lower than the previously recognized 19 species and three subspecies.

In Chapter Four I review the literature of and report newly acquired data on the natural history of *Sericomyrmex*, with a focus on nesting biology, presenting data for 19 nests of seven *Sericomyrmex* species.

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HISTORY OF THE FUNGUS-FARMING ANT GENUS *SERICOMYRMEX*
(HYMENOPTERA: FORMICIDAE)

by

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Dedication

To Marko.

Acknowledgements

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fungus extraction number of the form "fun123" following the taxon name, whereas sequences downloaded from GenBank have the GenBank accession number following the taxon name. All sequences for which the collection locality is known have a two-letter country code following the taxon name as follows: BR–Brazil, CR–Costa Rica, EC–Ecuador, GY–Guyana, GF– French Guiana, MX–Mexico, PA–Panama, PE–Peru, TT– Trinidad and Tobago, US–United States, VE– Venezuela.

Chapter 3

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Figure 3.7. Worker morphology and terminology.

a Head; **b** postpetiole lateral view; **c** gaster lateral view. Posterior cephalic corner shapes: **d** acute posterior cephalic corner; **e** rectangular posterior cephalic corner; **f** rounded posterior cephalic corner. Frontal lobe shapes: **g** trapeziform frontal lobes; **h** rectangular frontal lobes; **i** triangular frontal lobes. Posterior cephalic border shapes: **j** posterior cephalic border, gradual emargination; **k** posterior cephalic border, abrupt emargination. Mesosoma morphology: **l** mesosoma lateral view; **m** mesosoma dorsal view.

PCC posterior cephalic corner, **PCE** posterior cephalic emargination, **Tu** tumuli, **V** vertexal impression, **FrC** frontal carinae, **FrL** frontal lobe, **post** posterior frontal lobes margin, **lat** lateral frontal lobes margin, **med** medial frontal lobe margin, **vs** postpetiolar ventral sternite, **LC** lateral gastral carina, **AMC** anteromedian gastral carina, **IFC** incomplete frontal carina, **CFC** complete frontal carina, **Fe** frontal lobe fenestra, **PMT** pronotal median tumulus, **PLS** pronotal lateral spine, **MS** mesonotal spine, **MT** mesonotal tubercle.

Figure 3.9. *Sericomyrmex* male genitalia. *S. amabilis* (**a–d**), *S. mayri* (**e–h**), *S. opacus* (**i–l**), *S. saussurei* (**m–p**). Abdominal sternum IX, ectal view (**a, e, i, m**); genital capsule, ventral view (**b, f, j, n**); volsella (**c, g, k, o**) and penisvalve (**d, h, l, p**).

Bs basimere; **Te** telomere; **D** digitus; **C** cuspis; **DBP** distal basivolsellar process; **PBP** proximal basivolsellar process; **Vr** valvura; **Vc** valviceps.

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Figure 3.37. *S. mayri* worker variation; head, full-face view. **a** *mayri* worker from Brazil, Amazonas (USNMENT00444066); **b** *mayri* worker from Brazil, Bahia (USNMENT01125172); **c** *mayri* worker from Colombia, Meta (USNMENT01125151).

Figure 3.38. *S. mayri* worker (USNMENT01126229), SEM images. **a** Head, full-face view; **b** mandibles; **c** mesosoma and gaster, lateral view; **d** eye.

Figure 3.39. *S. mayri* queen and male; head, lateral profile, and dorsal view. Queen (USNMENT01126007): **a, c, e**. Male (USNMENT01126022): **b, d, f**.

Figure 40. *S. mayri* larva (USNMENT01126227: **a, f**; USNMENT01126230: **b, c, d, e**), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

Figure 3.41. Distribution map of *S. mayri*.

Figure 3.42. Distribution map of *S. mayri* populations. This map is based on the subset of *S. mayri* samples for which molecular data (either UCE or COI) were obtained. Detailed specimen locality data are included in Table S1.

Figure 3.43. *S. opacus* worker; head, lateral profile, and dorsal view. Variation in the eyes: with the white layer (USNMENT01125124): **a, c, e**; without the white layer (USNMENT01125118): **b, d, f**.

Figure 3.44. *S. opacus* worker (USNMENT01125331), SEM images. **a** Head, full-face view; **b** mandibles; **c** mesosoma and gaster, lateral view; **d** eye.

Figure 3.45. *S. opacus* queen and male; head, lateral profile, and dorsal view. Queen (USNMENT00305214): **a, c, e**. Male (USNMENT01125314): **b, d, f**.

Figure 3.46. *S. opacus* larva (USNMENT01125317), SEM. **a** Lateral view; **b** ventral view; **c** head, frontolateral view; **d** head and thorax, frontolateral view; **e** mouthparts; **f** anal setae.

Figure 3.47. Distribution map of *S. opacus* and *S. parvulus*.

Figure 3.48. *S. parvulus* worker and queen; head, lateral profile, and dorsal views. Worker (USNMENT00446157): **a, c, e**. Queen (USNMENT01125594): **b, d, f**.

Figure 3.49. *S. parvulus* worker, SEM (USNMENT01125593). **a** Head, full-face view; **b** mandibles; **c** mesosoma and gaster, lateral view; **d** eye.

Figure 3.50. *S. parvulus* larva (USNMENT01125592), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

Figure 3.51. *S. n. sp. 3* worker (USNMENT00924059). **a** Head; **b** lateral profile; **c** dorsal view; and **d** frontodorsal view of mesonotal spines.

Figure 3.52. *S. n. sp. 3* worker (USNMENT00924061), SEM image. **a** Head, full-face view; **b** mandibles; **c** eye; **d** mesosoma, lateral view; **e** mesosoma, lateral view; **f** propodeum, petiole, postpetiole, and gaster, anterolateral view.

Figure 3.53. *S. n. sp. 2* worker (USNMENT00924064) and queen (USNMENT00924065); head, profile and dorsal views. Worker: **a, c, e**. Queen: **b, d, f**.

Figure 3.54. *S. n. sp. 2* worker (USNMENT01125262), SEM images. **a** Head, full-face view; **b** mandibles; **c** gaster, lateral view; **d** eye.

Figure 3.55. *S. n. sp. 2* larva (USNMENT01125266), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

Figure 3.56. *S. saussurei* worker; head, profile, and dorsal view. Striate-mandibled form (USNMENT01125217): **a, c, e**. Smooth-mandibled form (USNMENT01125221): **b, d, f**.

Figure 3.57. *S. saussurei* worker (USNMENT01126237), SEM images. **a** Head, full-face view; **b** mandibles; **c** mesosoma, lateral view; **d** gaster, lateral view; **e** eyes completely covered with white layer, individual ommatidia not visible; **f** eyes with thick white layer, ommatidia visible through narrow holes in the layer.

Figure 3.58. *S. saussurei* queen and male; head, lateral profile, and dorsal view. Queen (USNMENT01125514): **a, c, e** (USNMENT01125515). Male: **b, d, f**.

Figure 3.59. *S. saussurei* larva (USNMENT01126236), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontolateral view; **d** head, dorsal view; **e** mouthparts; **f** anal setae.

Figure 3.60. Distribution map of *S. saussurei* populations: striate-mandibled and smooth-mandibled (SM) *S. saussurei*.

Figure 3.61. *S. scrobifer* worker (USNMENT01125115). **a** Head; **b** head, close-up view (USNMENT01125290); **c** lateral profile; and **d** dorsal view.

Figure 3.62. *S. scrobifer* worker (USNMENT01125273), SEM images. **a** Head, full-face view; **b** eye; **c** mesosoma, lateral view; **d** gaster, dorsolateral view.

Chapter 4

Figure 4.1. Localities from which *Sericomyrmex* nests (yellow circles) were collected.

Figure 4.2. Nest measurements. **a)** Cutaway view of a fungus-farming ant nest indicating standardized measurements: E = nest entrance; T = tunnel; CH = chamber; F = fungus garden; d = chamber depth; w = chamber width; h = chamber height; ed = offset distance from the entrance; **b)** Schematic representation of a single chamber, with measurements indicated: w = chamber width; w2 = chamber width 2, h = chamber height (adapted from Sosa-Calvo *et al.*, 2015).

Figure 4.3. Nest entrances of *Sericomyrmex*. **a)** *S. amabilis* nest mound (AJ131005-01), Mexico; **b)** *S. mayri* (JSC111119-04) nest mound, Guyana; **c)** *S. bondari* (AJ141001-05) nest entrance, Brazil; **d)** *S. bondari* (AJ141001-05) nest entrance after rain, Brazil; **e)** *S. bondari* (AJ120926-02) nest entrance, Brazil; **f)** *S. parvulus* (JCMC001) nest entrances, Brazil; **g)** *S. parvulus* (AJ120728-09) nest entrance, Peru; **h)** *S. parvulus* (AJ120728-09) nest entrance with bait (Cream of Rice) accumulated around it, Peru. White arrow points to the nest entrance hole.

Figure 4.4. Subterranean *Sericomyrmex* nests. **a)** *S. bondari* (AJ120926-02) underground chamber after the fungus garden and ants have been removed; **b)** *S. bondari* (AJ120926-02) chamber immediately after opening, with the fungus garden visible; **c)** chambers of *S. mayri* (JSC111119-04) after garden removal; **d)** *S. mayri* (JSC111119-04) chamber immediately after opening, with the fungus garden visible; **e)** *S. parvulus* (AJ120728-09) carrying bait (Cream of Rice) into its nest (arrow indicates nest entrance); **f)** plastic nest box with part of a *S. amabilis* (AJ131005-01) colony one day after excavation: the fungus

garden has been re-assembled and soil particles introduced during excavation have been removed by the ants.

Figure 4.5. Nest drawings 1. **a)** *S. amabilis* (AJ131005-01), Mexico; **b)** *S. mayri* (JSC111122-13), Guyana; **c)** *S. bondari* (AJ141001-05), Brazil; **d)** *S. bondari* (AJ120926-02), Brazil; **e)** *S. parvulus* (AJ120728-10), Peru; **f)** *S. parvulus* (AJ120803-03), Peru; **g)** *S. parvulus* (JCMC001), Brazil; **h)** *S. n. sp. 2* (AJ120729-03), Peru. White, unfilled chambers on the drawing indicate chambers that are behind other chambers.

Figure 4.6. Nest drawings 2. **a)** *S. saussurei* (JCMC002), Brazil; **b)** *S. mayri* (AJ111125-08), Guyana; **c)** *S. mayri* (AJ120801-03), Peru; **d)** *S. mayri* (AJ120726-03), Peru. White, unfilled chambers on the drawing indicate chambers that are behind the other chambers.

Chapter 1: Phylogenomics and Divergence Dating of Fungus-Farming Ants (Hymenoptera: Formicidae) of the Genera *Sericomyrmex* and *Apterostigma*

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1.1. Introduction

Fungus-farming (hereafter “attine”) ants are a monophyletic group in which all known species grow fungus for food. The most conspicuous attine ants, the leaf-cutting genera *Atta* and *Acromyrmex*, are dominant herbivores in Neotropical ecosystems (Wirth *et al.*, 2003) and have become model organisms for studies of symbiosis, higher eusociality, and coevolution (Schultz & Brady, 2008; Mehdiabadi & Schultz, 2010). The attine ants have been divided into two informal groups: the lower and the higher attine ants, the former paraphyletic with respect to the latter. Higher attine ants include, in addition to the leaf-cutters, the non-leaf-cutting genera *Trachymyrmex* and *Sericomyrmex*. All of the higher attine ants grow highly derived, obligately symbiotic fungal cultivars, in contrast to the lower attine ants, which grow facultatively symbiotic cultivars capable of living outside of the symbiosis (Schultz & Brady, 2008).

The higher attine ant genus *Sericomyrmex* contains 19 nominal species and 3 nominal subspecies and has a broad Neotropical distribution (Mayhe-Nunes & Jaffe, 1998; Bolton, 2014). *Sericomyrmex* ants are commonly collected in leaf-litter samples in biodiversity studies in South and Central America, but are hard to identify to the species

level. Species are morphologically highly similar and within-nest variation is substantial, confounding easy recognition of species boundaries (Wheeler, 1925). In addition, preliminary multiple-gene studies have shown surprisingly low molecular divergence, which makes this genus a compelling group in which to study speciation. The phylogenetic position of *Sericomyrmex* within the higher attine ant clade, and its similarities with *Atta* leaf-cutter ants (Weber, 1976), makes understanding the evolutionary history and biology of *Sericomyrmex* species important for reconstructing the origin and evolution of higher attine agriculture and for explaining the ecological success of the leaf-cutting genera *Atta* and *Acromyrmex*.

The lower attine ant genus *Apterostigma*, with 45 described species (Latke, 1997), is remarkable for its symbiotic plasticity. Like all other genera of lower attine ants, one clade of *Apterostigma* species grows fungi in the tribe *Leucocoprineae*, whereas, unlike any other lower or higher attine ant, another clade of *Apterostigma* species cultivates coral fungi in the distantly related family *Pterulaceae* (Schultz & Brady, 2008). Most remarkably, a recent study revealed the only known case of a lower attine ant cultivating a higher attine fungus: *A. megacephala* grows *Leucoagaricus gongylophorus*, the most highly derived and recently evolved leucocoprineaceous fungal species, an obligate symbiont otherwise known to be cultivated only by leaf-cutting ants (Schultz *et al.*, 2015).

Attine ant genomic studies (Nygaard *et al.*, 2011, 2016; Suen *et al.*, 2011) have significantly advanced our understanding of the evolution of fungus-farming in ants. The goal of this study was to sequence, *de novo* assemble, and characterize transcriptomes for species in the genera *Sericomyrmex* and *Apterostigma*, in order to better understand the

evolution of fungus-farming behavior and species boundaries within the genus *Sericomyrmex*. Here we report the first genomic data for both genera. For *Sericomyrmex* we sequenced the transcriptomes of three different morphospecies. These three morphospecies were chosen to be the most morphologically and molecularly diverged of the samples assembled for a taxonomic revision of the genus that were properly preserved for RNA extraction. We combined the data produced in this study with published ant and honey bee genomes (Munoz-Torres *et al.*, 2011; Nygaard *et al.*, 2011, 2016; Smith *et al.*, 2011; Suen *et al.*, 2011; Wurm *et al.*, 2011; Elsik *et al.*, 2014) in order to confirm the phylogenetic position of the genus *Sericomyrmex* and to infer divergence dates for *Sericomyrmex*. The transcriptome of *A. megacephala* is the first genomic data generated for any species in the Paleoattini, one of the two sister clades produced by the basal-most divergence in the fungus-farming ant phylogeny. The other sister clade, the Neoattini, includes the higher attines and all previously sequenced species, so genetic data for a paleoattine species significantly improves our ability to date the loss of arginine biosynthesis.

1.2. Materials and Methods

1.2.1. Sample preparation and sequencing

No animal ethics approvals are required to conduct research on ants. Research in Brazil was covered by Brazilian Council of Research and Scientific Development permit Processo CNPq 001884/2012-3 and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) collecting permit 14789-6. Research in Peru was covered by Ministerio de Agricultura Instituto Nacional de Recursos Naturales INRENA

Autorización No. 034-2004-INRENA-IFFS-DCB; Modificación a la Autorización No. 034-2004-INRENA-IFFS-DCB; Ministerio de Agricultura Instituto Nacional de Recursos Naturales INRENA Autorización No. 12 C/C-2004-INRENA-IANP; Carta No. 553-2014-MINAGRI-DGFFS/DGEFFS; Ministerio de Agricultura Instituto Nacional de Recursos Naturales INRENA Autorización No. 088-2005-INRENA-IFFS-DCB; CARTA No. 0217-2012-SERNANP-JRNTAMB; Resolución del Jefe de la Reserva Nacional Tambopata No. 020-2012-SERNANP-JEF; Permiso para Fauna y Flora Silvestre No. 004905-AG-INRENA; Permiso para Fauna y Flora Silvestre No. 006771-AG-INRENA; and Permiso para Fauna y Flora Silvestre No. 009154-AG-DGFFS.

Specimens of three *Sericomyrmex* species were collected from live colonies in the field and preserved in RNAlater. Specimens of *A. megacephala* were collected from a live laboratory nest and also preserved in RNAlater. Voucher specimens for each of the species sequenced are deposited in the Department of Entomology, National Museum of Natural History, Washington, DC, USA (Table 1.1), as recorded in the NMNH K-EMu database (<http://collections.nmnh.si.edu/search/ento/>). In order to ensure sufficient quantities of RNA, we included ten workers per sample for each *Sericomyrmex* species and five workers for the larger species, *A. megacephala*, crushing them with sterilized wooden sticks to enable the RNAlater to rapidly penetrate the integument. All specimens were stored at -20°C until RNA extraction. We extracted RNA using the Promega SV Total RNA Isolation System, following the standard protocol. The Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland prepared libraries using the standard Illumina TruSeq RNA Sample Preparation protocol (all

libraries were normalized and redundant rRNA was removed), and performed 100bp pair-end sequencing on Illumina HiSeq.

	<i>Apterostigma megacephala</i>	<i>Sericomyrmex</i> cf. <i>mayri</i>	<i>Sericomyrmex</i> cf. <i>parvulus</i>	<i>Sericomyrmex</i> cf. <i>luederwaldti</i>
Collection code	JSC110911-16	AJ120726-03	AJ120728-10	AJ121019-03
Collection date	11 September 2011	26 July 2012	28 July 2012	19 October 2012
Preservation method	RNAlater	RNAlater	RNAlater	RNAlater
Country	Brazil	Peru	Peru	Brazil
Locality	Para, Carajas	Madre de Dios, Tambopata NP	Madre de Dios, Tambopata NP	Minas Gerais, Panga Field Station
Habitat	Disturbed rain forest	Primary rain forest	Primary rain forest	Cerrado <i>sensu stricto</i>
Coordinates	-6.0632, -50.0577	-12.8186, -69.3636	-12.8500, -69.3721	-19.1831, -48.4014
Elevation	676 m	224 m	196 m	813 m
Voucher Specimen	USNMENT01124335	USNMENT01124333	USNMENT01124334	USNMENT01124336

Table 1.1. Specimen data.

1.2.2. Data cleaning and assembly

We concatenated the raw data and performed a quality check with FastQC (Andrews, 2010) before and after trimming (Figure 1.1). Raw reads were cleaned and trimmed with Trimmomatic (Bolger *et al.*, 2014) and the resultant cleaned reads were assembled with Trinity (Grabherr *et al.*, 2011; Haas *et al.*, 2013) using the default parameters. Basic assembly statistics (number of transcripts, average transcript length, and N50) were obtained from Trinity. We processed the resulting assemblies in TransDecoder (Haas *et al.*, 2013) to identify candidate coding regions within the transcripts. Predicted peptides were then filtered with CD-HIT-EST (98% global similarity) (Fu *et al.*, 2012) to remove redundant sequences. After removal of duplicates, peptides were further filtered in order to select only one peptide per putative transcript by

choosing the longest ORF (Open Reading Frame) per Trinity subcomponent with a custom-made Python script (Python Software Foundation). This step ensured that we had removed variation in the coding regions of our assemblies due to alternative splicing, closely related paralogs, and allelic diversity. Peptide sequences for the seven sampled ant genomes were filtered at 98% similarity in CD-HIT-EST. To calculate coverage we multiplied the number of raw reads with the expected length (100 bp), and divided that by total number of bases in the assembly (Riesgo *et al.*, 2012).

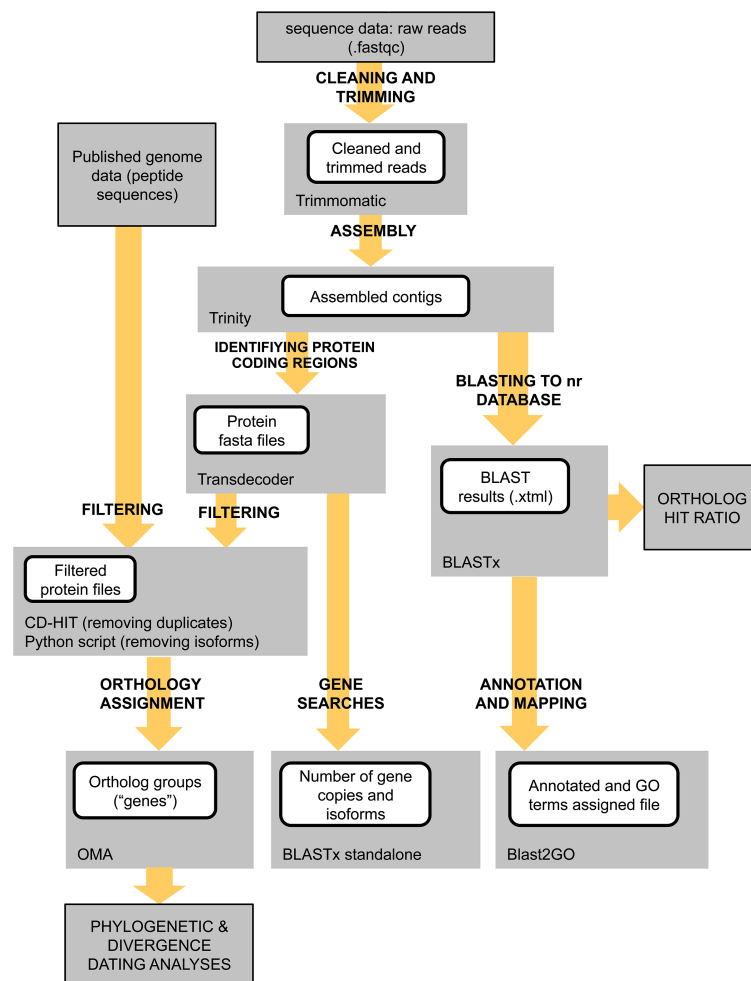


Figure 1.1. Workflow summary.

1.2.3. Orthology Assignment and Alignment

We identified orthologs using OMA stand-alone v.0.99w software (Roth *et al.*, 2008; Altenhoff *et al.*, 2011) with default settings on 100 cores on the Smithsonian Lattice high-performance computing cluster (Linux-based with AMD processors). We constructed an amino acid supermatrix by concatenating the set of OMA groups containing all taxa (1,317). Each putative ortholog group (from now on “gene”) was aligned individually using MAFFT (Kato & Standley, 2013). Aligned genes were then trimmed with Gblocks (Castresana, 2000) to cull regions of dubious alignment. We separately ran an orthology assignment in OMA for a data subset containing only the *Sericomyrmex* transcriptomes. For this we used TransDecoder to identify the nucleotide sequences for the candidate protein-coding regions and performed the same filtering as described above with the larger dataset on the resultant nucleotide sequence data. Individual gene alignments were trimmed with Gblocks (Castresana, 2000) and analyzed in Geneious v.8 (Kearse *et al.*, 2012).

1.2.4. Phylogenetic analyses

Maximum-likelihood inference was conducted with PhyML-PCMA (Guindon *et al.*, 2005; Zoller & Schneider, 2013). We selected 10 PCs (principal components) in the PhyML-PCMA analyses and used empirical amino-acid frequencies. PhyML-PCMA estimates a model through the use of a principal component (PC) analysis; in this case using 10 PCs. Bootstraps were calculated in PhyML-PCMA for 100 replicates. Concomitantly, tree searches were conducted in PhyloBayes MPI 1.4e (Lartillot *et al.*, 2013) using the site-heterogeneous CAT + GTR model of evolution. Three independent

chains were run for 1295–1445 cycles, and the initial cycles discarded as *burn-in* were determined for each analyses using the “*tracecomp*” executable, with convergence assessed using the maximum bipartition discrepancies across chains ($\text{maxdiff} < 0.1$).

1.2.5. Divergence dating

Divergence dates were estimated using the Bayesian relaxed molecular clock approach as implemented in PhyloBayes v.3.3f (Lartillot *et al.*, 2013) applying an auto-correlated model of clock relaxation (Lepage *et al.*, 2007; Rehm *et al.*, 2011). Three calibration constraints (Table S1.1), based on a recent study of myrmicine ants (Ward *et al.*, 2015), were used with soft bounds (Yang & Rannala, 2006) under a birth-death prior in PhyloBayes. PhyloBayes was run for 32,664 cycles, sampling posterior rates and dates every 5 cycles. The initial 5000 cycles were discarded as burn-in. We excluded the outgroup taxon *Apis mellifera* from final analyses because we considered it to be an inappropriate outgroup for the purposes of divergence dating. All other taxa are members of a single subfamily, the Myrmicinae, which occupies a highly derived position within the ant family Formicidae, whereas *A. mellifera*, the honey bee, is a highly derived member of a highly derived family within the superfamily Apoidea. The most recent common ancestor of the lineages containing the Apoidea and the Formicidae dates to at least 140 mya, whereas the ancestor of the Myrmicinae, the focus of our dating analyses, dates to ~99 mya (Brady *et al.*, 2006; Johnson *et al.*, 2013; Ward *et al.*, 2015).

1.2.6. Annotation and Ortholog Hit Ratio

We used BLASTx (Altschul *et al.*, 1990) (cut-off E-value $1e^{-5}$) to compare the unfiltered, assembled transcripts (Trinity output) against the non-redundant (nr) protein database of NCBI. Resulting xml files were used as input for CLC Workbench (CLC Inc., Aarhus, Denmark). We performed functional annotation and mapping to GO terms, using Blast2GO with default settings, in order to summarize functional categories of the genes, annotate our data set, and determine the quality of our transcriptomes. We also used the resultant xml output to run Orthology Hit Ratio calculations following previously described methods and scripts (Ewen-Campen *et al.*, 2011). This analysis estimates the degree to which a transcriptome is fully sequenced and assembled by comparing the length of the contigs that recovered BLAST hits with the length of their top BLAST hits (O'Neil *et al.*, 2010; O'Neil & Emrich, 2013).

1.2.7. Gene search

Based on previous studies of attine ant genomes and ant transcriptomes (Nygaard *et al.*, 2011, 2016; Suen *et al.*, 2011; Badouin *et al.*, 2013) we considered the following genes or gene families of particular interest: genes in the arginine metabolism pathway (*arginase*, *nitric oxide synthase*, *argininosuccinate synthase*, and *argininosuccinate lyase*), detoxification genes (*cytochrome P450 monooxygenase*), hexamerins (*hex 70a*, *hex 70b*, *hex 70c* and *hex 110*), desaturase, RYamide, and chitinases. The nucleotide or protein sequence for each of these genes was downloaded from NCBI GenBank or, in the case of the desaturase gene, obtained from the supplemental material of a previous study (Badouin *et al.*, 2013). We used sequences from the closely related species *Atta*

cephalotes and *Acromyrmex echinator* for searches for *arginase*, *nitric oxide synthase*, and *cytochrome P450*. For chitinase searches we assembled protein and nucleotide sequences that were used in similar analyses in a study of the *Atta cephalotes* genome (Suen *et al.*, 2011), for a total of ten different chitinase-like proteins from *Acromyrmex echinator*, *Nasonia vitripennis*, and *A. mellifera* (Table S1.2). For *argininosuccinate synthase* and *argininosuccinate lyase*, which are lost in leaf-cutter ants, we used copies from the closely related myrmicine ants *Solenopsis invicta*, *Pogonomyrmex barbatus*, and *Wasmannia auropunctata*. For hexamerin searches we used *Apis mellifera* sequences in order to repeat the methods of previous studies (Suen *et al.*, 2011) and because some of the attine ant hexamerin sequences were unavailable. For searches for ryamide, which is absent in ants, we used a *Drosophila melanogaster* sequence. We used BLAST+ standalone (Camacho *et al.*, 2009) to manually create a BLAST database for each of the transcriptomes separately (using unfiltered, assembled transcripts) and searched the created databases for each of the query sequences with the cut-off E-value $1e^{-5}$. Returned hits were then BLASTed against NCBI to confirm that they had returned the same protein.

1. 3. Results and Discussion

1.3.1. Sequencing

We sequenced the transcriptomes of the lower attine ant *Apterostigma megacephala* and three morphospecies of the higher attine ant genus *Sericomyrmex* (Figure 1.1, Table 1.1). The total number of raw sequences was 329.6 Mb, varying from 72 to more than 91 million reads per sample. After trimming and cleaning, between

92.2% and 93.8% of the raw reads were used for assembly. The number of assembled contigs varied from 71,391 for *S. cf. luederwaldti* to 95,242 for *A. megacephala*. The N50, contig length, and other statistics for each of the sequenced taxa can be found in Table 1.2. The number of raw reads and assembled contigs were highest for *A. megacephala*, which can be attributed to the high quality of preservation of the extracted RNA for this sample.

Coverage for the transcriptomes varied from 53.8 to 169.7 reads per base (Table 1.2). Ortholog Hit Ratios (OHR) were calculated in order to estimate transcriptome assembly quality and completeness (O’Neil *et al.*, 2010). An OHR value close to 1 suggests complete transcript assembly, with a value of 1 being an identical match. On average, our transcriptomes had high OHR values, with 47.7 to 53.5 % of total contigs having an OHR greater than 0.8, and 63.7 to 69.5 % of contigs having an OHR higher than 0.5 (Table 1.2, Figure S1.1). A number of contigs recovered values over 1, which is not uncommon (O’Neil & Emrich, 2013), and represents possible sequence expansions. Additionally, we calculated annotation metrics to estimate transcriptome quality (e.g., number of contigs that recovers at least one BLAST hit). Based on these metrics (Figures 1.2 & 1.3, Table 1.2, S1.3 & S1.4), all four transcriptomes are of high quality with values that are either within or slightly higher than those reported in similar studies (Ewen-Campen *et al.*, 2011; Riesgo *et al.*, 2012; Badouin *et al.*, 2013).

	<i>Apterostigma megacephala</i>	<i>Sericomyrmex cf. mayri</i>	<i>Sericomyrmex cf. parvulus</i>	<i>Sericomyrmex cf. luederwaldti</i>	Stats. source
Number of raw reads after sequencing	91,666,468	85,655,146	72,383,100	80,111,038	1
Percentage of reads after trimming	93.87%	92.29%	92.85%	92.56%	2
Number of transcripts (contigs) after assembly	95,242	65,983	83,935	71,391	3
Assembled contigs length: min-max (average, st dev)	201 - 30,835 (1,670.9)	201 - 20,168 (1,358.4)	201 - 28,459 (1,601.9)	201 - 28,605 (1,405.2)	4
N50	3,466	2,755	3,168	2,739	3
Number of potential protein-coding regions	38,113	33,585	40,801	37,563	5
Number of contigs without BLAST hits (%)	44,986 (49.11%)	31,597 (48.28%)	38,205 (46.60%)	31,133 (44.11%)	6 (7)
Number of contigs with BLAST results (%)	17,558 (19.16%)	10,659 (16.28%)	15,165 (18.49%)	14,277 (20.22%)	6 (7)
Number of contigs with mapping results	3,212 (3.5%)	1,630 (2.49%)	1,796 (2.19%)	1,239 (1.75%)	6 (7)
Number of annotated aontigs (%)	25,843 (28.21%)	21,555 (32.93%)	26,808 (32.70%)	23,925 (33.90%)	6 (7)
Total number of sequences annotated with BP (Biological Processes)	50,366	46,495	52,420	47,920	6
Total number of sequences annotated with MF (Molecular Function)	32,999	27,495	34,303	30,926	6
Total number of sequences annotated with CC (Cellular Component)	20,098	18,641	21,954	18,944	6
Species with highest number of BLAST top-hits	<i>Acromyrmex echinator</i>	<i>Acromyrmex echinator</i>	<i>Acromyrmex echinator</i>	<i>Acromyrmex echinator</i>	6
Species with highest number of BLAST hits	<i>Cerapachys biroi</i>	<i>Acromyrmex echinator</i>	<i>Acromyrmex echinator</i>	<i>Acromyrmex echinator</i>	6
Number of contigs with OHR >0.5	72.24%	64.60%	69.50%	63.70%	8
Number of contigs with OHR >0.8	55.15%	49.70%	53.50%	47.80%	8
COVERAGE	57.6	169.7	53.8	79.8	7
NCBI SRA BioSample Accession Number	SAMN04166284	SAMN04166285	SAMN04166286	SAMN04166287	

Table 1.2. Transcriptome sequencing, assembly, and analysis statistics. Sources of statistics: 1: FastQC, 2: Trimmomatic, 3: Trinitystats.pl, 4: Geneious, 5: Transdecoder, 6: Blast2GO, 7: calculated from other statistics, 8: Ortholog Hit Ratio.

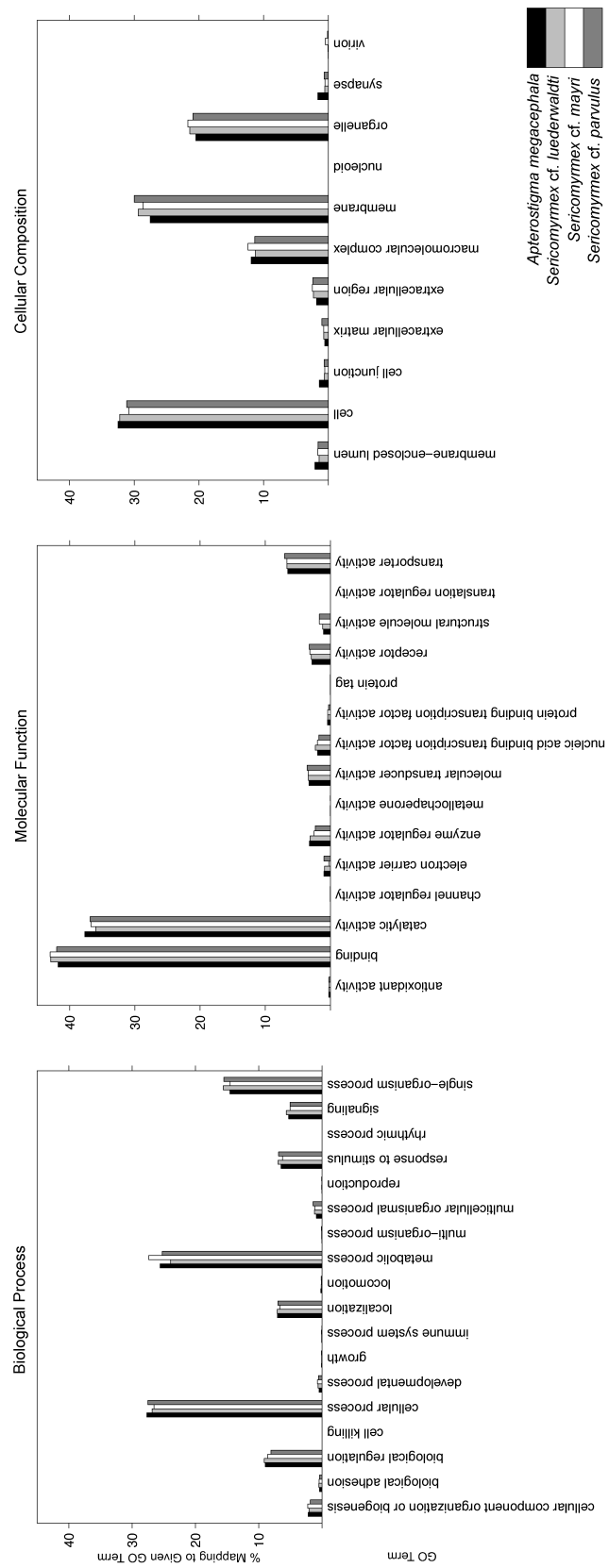


Figure 1.2. Gene Ontology (GO) distributions by level, by species.

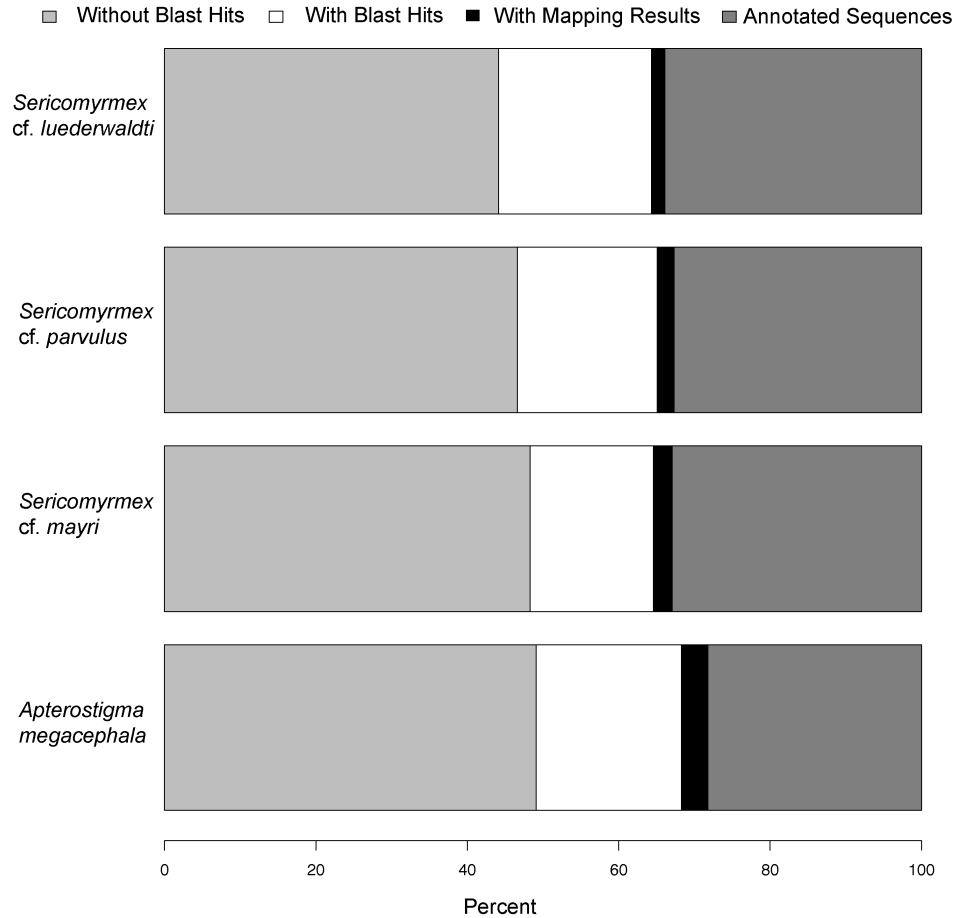


Figure 1.3. Functional annotation. Percentages of BLAST-hits, annotated sequences, mapped sequences, and no-BLAST hits for each species.

1.3.2. Orthology assignment

We used OMA standalone software (Altenhoff *et al.*, 2011) to place predicted genes into orthologous groups. OMA's advantage over standard bidirectional best-hit approaches is that OMA's algorithm uses evolutionary distances instead of scores, considers distance inference uncertainty, includes many-to-many orthologous relations, and accounts for differential gene losses (Altenhoff *et al.*, 2011; Dalquen *et al.*, 2013). The number of orthologs obtained for the complete dataset (ten ingroup and three outgroup taxa) was 1,317 and the number of phylogenetically informative sites ($n > 4$) was

11,447. The final concatenated matrix contains a total of 649,095 amino-acid sites present in all taxa.

1.3.3. Functional annotation

The percentage of reads that had at least one BLAST hit varied between 50% and 55.8% of total transcripts (Table 1.2), which is slightly higher than in similar transcriptome studies (Ewen-Campen *et al.*, 2011; Badouin *et al.*, 2013). Final number of annotated sequences varied between 21,555 and 26,808 sequences. Our data were annotated with a wide range of functional categories represented on all levels of the Gene Ontology database and were comparable to other similar studies, with no biases toward any particular category (Figure 1.2, Table 1.2). For all four transcriptomes the top BLAST hit species was recovered as *Acromyrmex echinator*, which is a higher-attine leaf-cutting species, a very close relative of *Sericomyrmex*, and a close relative of *Apterostigma*. This was also the species with the largest number of total BLAST hits for the three *Sericomyrmex* species. For *A. megacephala* the top BLAST hit species was the non fungus-farming ant *Cerapachys biroi*.

1.3.4. Phylogeny and dating

Results from phylogenetic analyses of this dataset (Figure 1.4) are congruent with existing phylogenies, including a monophyletic *Sericomyrmex* clade that is the sister of *Trachymyrmex zeteki*, and with *A. megacephala* as the sister taxon to all other attine ant taxa included in this study. Considering the large number of characters in this dataset, the branches subtending the three *Sericomyrmex* species are reconstructed as remarkably

short in both analyses. This result indicates a very low genetic divergence between *Sericomyrmex* species. To investigate this further we ran a separate orthology search including only the three *Sericomyrmex* species, which revealed 4,217 orthologous genes present in all three samples, varying in length from 303 to 10,530 bp. Of those 4,217 loci, 92.71% (3,910 sequences) are 99% to 100% pairwise identical, as defined by Geneious (Kearse *et al.*, 2012). Of the remaining orthologs, 290 are between 90% and 99% pairwise identical. Combining these results, 99.59% of all orthologs shared by these three *Sericomyrmex* morphospecies are at least 90% pairwise identical. This result is unexpected given that we selected the most molecularly and morphologically divergent *Sericomyrmex* samples available for RNA extraction. Based on morphology and multiple gene sequences, of the samples chosen for this study *S. cf. luederwaldti* and *S. cf. mayri* were considered *a priori* to be more similar, so the high similarity of their transcriptome sequences is less surprising. However, *S. cf. parvulus* is morphologically distinctly different from the other two species and is considered to be a member of one of the most basally diverging lineages of *Sericomyrmex* species. Even though phylogenetic analyses of the transcriptome data agreed with prior analyses regarding these relationships, i.e., that *S. cf. luederwaldti* and *S. cf. mayri* are more closely related to each other than either is to *S. cf. parvulus*, the degree of divergence separating *S. cf. parvulus* from those two species is much lower than expected based on divergences separating similarly related taxa, e.g., some leaf-cutter species. In general, the transcriptomes indicate that even morphologically divergent *Sericomyrmex* species are separated by remarkably low genetic distances, which means that accurately recovering species boundaries may require more variable data from non-coding regions. Importantly, this pattern, also

supported by our divergence-dating analyses (see next paragraph) indicates that *Sericomyrmex* has rapidly radiated into multiple, morphologically distinguishable species that occupy a broad geographic distribution (Mayhe-Nunes & Jaffe, 1998; Sánchez-Peña, 2010) with only a small degree of accompanying genetic divergence, in contrast to most other comparable ant groups, including its sister taxon, the *Trachymyrmex iheringi* species group. This conclusion must obviously be investigated further, given that transcriptomes from only three *Sericomyrmex* species were included in this study. It will be particularly important to test whether this rapid radiation may have been driven by coevolution with a specialized clade of higher attine fungi, as has been suggested for *Atta* (Schultz & Brady, 2008; Mueller *et al.*, 2010), especially since recent research suggests the possibility of high symbiont fidelity between *Sericomyrmex* species and a single species of fungal cultivar (De Fine Licht & Boomsma, 2014). Another potentially important factor in this radiation could be major changes in genetic architecture such as chromosome duplications and rearrangements, because data from two species indicate that, compared to most other attine ants, *Sericomyrmex* has an unusually high number of chromosomes and an unusually large genome (Tsutsui *et al.*, 2008; Cardoso *et al.*, 2014).

The divergence dating analysis, which included ant species only (the outgroup *A. mellifera* was excluded in the dating analysis), recovered the stem-group age (i.e., the earliest possible origin) of fungus-farming ants as 66.7 million years ago (mya) and the crown-group age (i.e., the latest possible origin) as 53.6 mya. The crown-group age estimate is consistent with previous estimates of 50-60 mya (Schultz & Brady, 2008; Rabeling *et al.*, 2011; Cardoso *et al.*, 2014; Schultz *et al.*, 2015; Ward *et al.*, 2015), whereas the stem-group age estimate is slightly younger than a stem-group estimate from

a recent study (74.6 mya) of a large genomic data set (Nygaard *et al.*, 2016). However, these results are in some cases imprecise and are not directly comparable because neither included taxa from the closest non-fungus-farming relatives (the sister group) of the attine ants (Ward *et al.*, 2015) and only this study included representatives of both clades resulting from the basal-most divergence at the origin of the fungus-farming ants. Our age estimate (33.3 -53.6 mya) of this divergence between the Paleoattini (including the genera *Myrmicocrypta*, *Apterostigma*, and *Mycocepurus*) and the Neoattini (including all other attine ant genera) should be interpreted with caution because our taxon sampling is arguably inadequate for the task. Our taxon sampling for the higher attine ants is better and represents the first genomic data set that includes *Sericomyrmex*. We estimate the crown-group age of the higher attine ants to be 29.2 (31.3-27.4) mya; the crown-group age of the *Sericomyrmex*+*Trachymyrmex zeteki* clade (i.e., the *Trachymyrmex iheringi* group) as 22.6 (25.3-20.4) mya; and the crown-group age of the leaf-cutting ants (genera *Atta* and *Acromyrmex*) to be 17.9 (20.4 – 15.6) mya (Table 1.3). For comparison, Schultz and Brady (Schultz & Brady, 2008) recovered ~16 mya, ~10.5, and ~8 mya for those same nodes, respectively, and Nygaard and colleagues estimate ~17 mya for the leaf-cutter origin (Nygaard *et al.*, 2016). Our dating analyses, based on transcriptome data, are most likely overestimating divergence times due to the small taxon sample. This possible overestimation notwithstanding, the crown-group age of *Sericomyrmex* is reconstructed as very recent, estimated at 4.8 mya for the age of the ancestor of all extant species.

For comparison, the stem- and crown-group ages of the clade containing the attine host/parasite species pair *Mycocepurus goeldii* and *M. castrator* are estimated to be 2.04 and 3.31 million years, respectively (Rabeling *et al.*, 2014), and crown-group divergences

between sister species pairs in the *Cyphomyrmex wheeleri* group range from 5.3 to 7.0 mya (Mehdiabadi *et al.*, 2012). The branch separating *Sericomyrmex* from the most recent common ancestor it shares with the *Trachymyrmex iheringi* group (represented here by *T. zeteki*) is very long (Figure 1.4), which might be explained by taxon undersampling, especially if there are more extant *Sericomyrmex* species that could split that long branch if they had been included. We consider this unlikely, however, because the three sampled *Sericomyrmex* species were chosen to represent the full range of diversity in the genus both morphologically and molecularly and because other phylogenomic studies currently in preparation, employing much denser taxon sampling, corroborate that the genus *Sericomyrmex* is the sister group of the entire *T. iheringi* clade, i.e., that the branch subtending *Sericomyrmex* is unoccupied by other *Trachymyrmex* species.

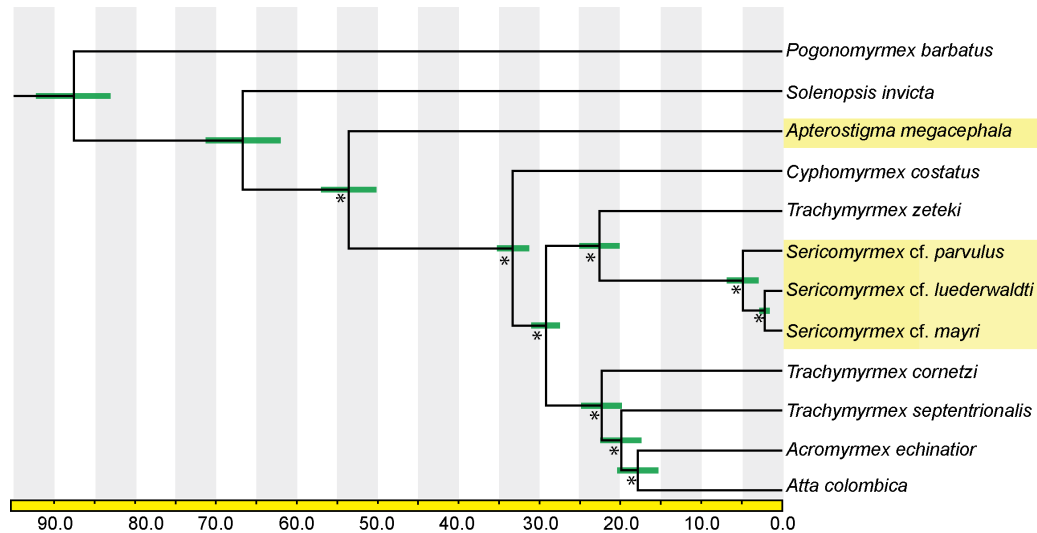


Figure 1.4. Time-calibrated phylogeny of attine ants. Highlighted taxa are novel transcriptomes obtained for this study. This topology was recovered in both ML and Bayesian analysis. Asterisks (*) at nodes indicate bootstrap values of 100 and posterior probabilities of 1.0. Green error bars at nodes indicate minimum and maximum age estimates; the time scale at the bottom is in million of years.

Clade		This study	Schultz & Brady 2008	Nygaard <i>et al.</i> 2016
All attine ants				
	Crown	53.6 (56.7-49.2)*	50 (56-44)	
	Stem	66.7 (70.7-61.6)*	51(57-45)	74.63 (84.28- 65.24)
Neoattini				
	Crown	33.3 (35.1-31.3)*		
	Stem	53.6 (56.9-49.2)*		
Higher attine ants				
	Crown	29.2 (31.3-27.4)*	16 (19-13)	22.87 (17.17, 28.57)
	Stem	33.3 (35.1-31.3)*	20 (24-17)	26.60 (19.60, 33.80)
<i>Trachymyrmex</i> + <i>Sericomyrmex</i>				
	Crown	22.6 (25.3-20.4)*	10.58	
	Stem	29.2 (31.3-27.4)*	16	
Genus <i>Sericomyrmex</i>				
	Crown	4.9 (7.1-3.4)*		
	Stem	22.6 (25.3-20.4)*		
<i>S. cf. mayri</i> + <i>S cf. luederwaldti</i>				
	Crown	2.1 (3.4-1.4)*		
	Stem	4.9 (7.1-3.4)*		
<i>Trachymyrmex cornetzi</i> + leafcutter ants				
	Crown	22.3 (25.1-20.1)*		19.18 (23.57- 14.69)
	Stem	29.2 (31.2-27.4) *		22.87 (17.17, 28.57)
<i>Trachymyrmex septentrionalis</i> + leafcutter ants				
	Crown	19.9 (22.5-17.7)*		17.77(21.72- 13.70)
	Stem	22.3 (25.1-20.1)*		19.18 (23.57- 14.69)
Leaf-cutter ants				
	Crown	17.9 (20.4-15.6)*	8 (10-6)	7.05 (8.44-5.60)
	Stem	19.9 (22.5-17.7)*	9 (7-11)	16.21 (19.74- 12.57)

Table 1.3. Crown-group and stem-group age estimates. In units of million years ago, with standard error given in parentheses. An asterisk (*) indicates a posterior probability of 1.

1.3.5. Gene family searches

In order to further investigate the sequenced transcriptomes, we chose several genes of interest based on previous ant genome and ant transcriptome studies (Nygaard *et al.*, 2011; Suen *et al.*, 2011; Badouin *et al.*, 2013) and performed separate searches for those genes. In general, the similarity of the results of our gene BLAST searches to those of other attine ant genomic studies serves as a confirmation of the completeness of our transcriptomes.

Arginine metabolic pathway genes

The first genomic studies of the higher-attine leaf-cutting ant genera *Atta* and *Acromyrmex* demonstrated losses of two genes in the arginine anabolic pathway, whereas all other ants for which data are available, including species in the same subfamily (Myrmicinae) as the attine ants, have functional copies of those genes (Nygaard *et al.*, 2011; Suen *et al.*, 2011). It was suggested that the leaf-cutting ants, or even all higher attine ants, had become obligately dependent on their fungi for arginine. Such a metabolic division of labor is perhaps not surprising in the highly derived higher attine ants, given that their fungi are also obligate symbionts, and similar examples are known from other mutually obligate symbioses (Graf & Ruby, 1998; Klasson, 2004; Feldhaar *et al.*, 2007). However, a more recent study, which included one lower attine ant species that is relatively closely related to the higher attines, indicates that those two genes, *argininosuccinate synthase* and *argininosuccinate lyase*, were lost prior to the origin of the higher attines (Nygaard *et al.*, 2016), raising the question of at what point in attine

evolution the ants became obligately dependent on their fungal cultivars for the amino acid arginine.

Our BLAST searches for *argininosuccinate synthase* and *argininosuccinate lyase* in the three *Sericomyrmex* species did not retrieve any hits, which, because *Sericomyrmex* species are higher attines, was to be expected. However, BLAST searches also failed to find these genes in *A. megacephala*. *A. megacephala* is a member of the Paleoattini, one of two sister clades formed by the basal-most divergence in the fungus-farming ant phylogeny. The other sister clade, the Neoattini, contains *Sericomyrmex* and all previously studied attine ant species, so the absence of these two genes in a paleoattine indicates that the dependence of attine ants on their fungal cultivars for arginine arose in the common ancestor of all fungus-farming ants. This result is unlikely to be due to inadequate transcriptome data for two reasons. First, our Ortholog Hit Ratios are high, and, second, searches for two other enzymes in the arginine metabolic pathway, the catabolic genes *arginase* and *nitric oxide synthase*, produced hits in all of our transcriptomes (Table 1.4). Functional copies of these two genes are likewise present in the genomes of *Atta* and *Acromyrmex* (Nygaard *et al.*, 2011; Suen *et al.*, 2011).

Hexamerins

Hexamerins are another protein family that has been associated with the specialized diet of attine ants (Suen *et al.*, 2011). Four copies of hexamerins are commonly found in insects, *hex 70a*, *hex 70b*, *hex 70c*, and *hex 110*, serving as storage proteins during development as well as in the adult stage (Martins *et al.*, 2010). In the genome study of the leaf-cutting ant *Atta cephalotes*, the gene *hex 70c* was not found

(Suen *et al.*, 2011). BLAST searches with *Apis mellifera* hexamerins recovered two to four different hexamerins per ant transcriptome (Table 1.4), but unfortunately we were not able to distinguish between the different copies. In the transcriptome of *S. cf. luederwaldti* our search recovered only two copies.

	<i>Apterostigma megacephala</i>		<i>Sericomyrmex</i> cf. <i>mayri</i>		<i>Sericomyrmex</i> cf. <i>parvulus</i>		<i>Sericomyrmex</i> cf. <i>luederwaldti</i>	
	# of copies	# of isoforms	# of copies	# of isoforms	# of copies	# of isoforms	# of copies	# of isoforms
Hexamerins	3	0	4	0	4	0	2	0
Desaturase	5	9	8	11	8	12	8	15
CytP450	31	106	42	118	38	117	38	97
Arginase	4	0	3	0	4	0	9	0
Nitric Oxide Synthase	7	0	26	0	10	0	10	0
Chitinases	9	0	10	0	10	0	10	0

Table 1.4. Gene searches. Number of gene copies and isoforms per species.

RYamide

We found no gene sequences for the RYamide protein in the *Sericomyrmex* and *Apterostigma* transcriptomes. RYamide proteins are recently discovered and have been found in all insects for which genomes are available (Hauser *et al.*, 2010) except for ants. The functional roles of RYamides are poorly known, but it has been suggested that in ants the absence of RYamide is connected with the differentiation between reproductive and non-reproductive castes (Nygaard *et al.*, 2011).

Desaturases

The desaturase gene family plays an important role in the synthesis of cuticular hydrocarbons (CHC), which are one of the key elements of nestmate recognition in social insects. Using the *desat1* gene of the ant *Formica exsecta* (Badouin *et al.*, 2013) as a query sequence, we found five copies of desaturase in *A. megacephala* and eight copies in each of the three *Sericomyrmex* species, all with various numbers of isoforms (Table 1.4). When the found copies were BLASTed back to the NCBI protein database, the highest similarity was found with delta(Δ)11 desaturase genes of other ants. This finding is comparable to that of a similar study of the *Atta cephalotes* genome, in which six out of eleven identified desaturase-like genes matched Δ 11 desaturase (Suen *et al.*, 2011).

Cytochrome P450

Cytochrome P450 (*CytP450*) is a large protein family, members of which are found in enzymatic pathways central to the metabolism of toxic compounds as well as to development and reproduction (Feyereisen, 1999). We found 31 copies of *CytP450* in *A. megacephala*, and between 38 and 42 copies in the three *Sericomyrmex* morphospecies (Table 1.4). These numbers are surprisingly small. The 54 copies of *CytP450* identified in *Atta cephalotes* is regarded as a reduced number in comparison to other ants (136 in *Camponotus floridanus* and 107 in *Harpegnathos saltator*) (Bonasio *et al.*, 2010). Like fungus-farming ants, both of the two other insects with known low numbers of *CytP450*, the honey bee *Apis mellifera* (with 62 copies) and the body louse *Pediculus humanus* (with 39 copies) (Claudianos *et al.*, 2006; Kirkness *et al.*, 2010) have specialized diets

and it has been suggested that insects with predictable diets may have a reduced need to metabolize toxins.

Chitinases

Chitinases, enzymes with chitinolytic activity, play important roles in digestion and moulting in insects. The number of chitinases in Hymenoptera is considered reduced in comparison to that in *Drosophila melanogaster* (Suen *et al.*, 2011). In studies of attine ants, however, chitinases have been shown to have experienced positive selection, presumably because of their importance in the digestion of the chitinous cell walls of their fungal cultivars (Nygaard *et al.*, 2016). We found 10 chitinase-like genes, in different copy numbers, in all three *Sericomyrmex* species. In *A. megacephala* we found 9 of them, with *CG8460*, chitinase-like protein 1, absent. Interestingly, chitinase gene *K06A9.b*, which is found in *A. mellifera* and *A. cephalotes* but is not known in *D. melanogaster*, was found in all of our transcriptomes. It was beyond the scope of this paper to test for positive selection in the chitinase genes, but we found 13-23 copies of *Chitinase 3*, for which positive selection has been detected in other attine ants (Nygaard *et al.*, 2016). Details on copy numbers for each of the chitinases for each of the species can be found in Table S6.

Our transcriptomes were constructed from worker-caste ants only, and some of the identified genes of interest, including the arginine metabolic pathway and *CytP450* genes, are expressed at higher levels in the larval stage (Feyereisen, 1999; Suen *et al.*, 2011). Their absence in our assemblies could therefore be attributed to insufficient sequencing depth due to low levels of expression. This seems unlikely, however, because,

as noted above, our Ortholog Hit Ratios are high and we detected the other genes in the arginine metabolic pathway, *arginase* and *nitric oxide synthase*. Rather, our results suggest that the reduction in *CytP450* genes and the losses of *argininosuccinate synthase* and *argininosuccinate lyase* likely occurred at the origin of fungus-farming ants. An alternative hypothesis, at least for the arginine metabolic pathway genes, is that they were lost twice, once in *A. megacephala* and once in the ancestor of the higher attine ants. This appears initially plausible because *A. megacephala* is the only lower attine known to cultivate a higher-attine fungal cultivar and it is clear that *A. megacephala* secondarily acquired its cultivar during the course of its evolution (Schultz *et al.*, 2015). Hence, if arginine dependence is specifically associated with higher attine fungal cultivars, then it could have occurred in parallel in the higher attine ants and in *A. megacephala*. On closer inspection, we consider this hypothesis unlikely because the two arginine anabolic genes are also absent in the lower attine ant *Cyphomyrmex costatus*, which cultivates a lower-attine fungus. Clearly, genomic data from other species of paleoattine and basally diverging neoattine lineages are needed to better understand the history of genomic evolution in the fungus-farming ants.

1.4. Conclusions

Results from our phylogenetic and dating analyses suggest that the genus *Sericomyrmex* has undergone a recent, rapid diversification, reflected by short branch lengths and recent divergence dates. Most surprisingly, the overall genetic similarity between the three *Sericomyrmex* morphospecies is unexpectedly high. It is our hope that these results will inspire further investigation into the genetic mechanisms underlying

rapid radiation with little genetic change in *Sericomyrmex*. In addition to this phenomenon and its implications for speciation, recent research on social parasites and agro-predators makes this genus an exciting model system for studying behavioral ecology, coevolution, and chemical ecology (Adams *et al.*, 2012, 2013; Bruner *et al.*, 2013). The transcriptomes sequenced in this study could provide the foundation for future research in gene discovery, phylogenomics, population genomics, and conservation genetic studies (Peters *et al.*, 2014; Romiguier *et al.*, 2014). In our analyses of gene families (arginine metabolic pathway, *Cytochrome P450*, hexamerins, RYamide, desaturase, and chitinase) we failed to find two arginine anabolic enzymes in the paleoattine ant *A. megacephala*, suggesting that the loss of these enzymes, confirmed previously only in the clade Neoattini, was likely lost at the origin of the fungus-farming ants. We recovered a surprisingly small number of *CytP450* genes, which are associated with detoxification and unspecialized diets. Additional research is required to confirm these gene losses, since our data do not include larval transcripts, and therefore cannot be interpreted as conclusive. Finally, we hope the transcriptome of *A. megacephala* will prove a useful tool for future research on the underlying genetics that makes this species the only lower attine ant known to cultivate a higher attine fungus.

1.5. Supplemental Information

Node	Lower and upper soft bound prior
<i>Pogonomyrmex barbatus</i> , <i>Solenopsis invicta</i>	106 - 85.2
<i>Apterostigma megacephala</i> , <i>Cyphomyrmex costatus</i>	63.5 - 47.6
<i>Cyphomyrmex costatus</i> , <i>Trachymyrmex zeteki</i>	33.8 - 19.6

Table S1.1. Calibration priors used in PhyloBayes divergence dating analysis. Unit: Million of years ago.

Gene Name*	Query Sequence: Protein (annotation in database)	Query Sequence: Organism	Query Sequence: GenBank accession no.	No. of copies <i>A. megacephala</i>	No. of copies <i>S. cf. mayri</i>	No. of copies <i>S. cf. parvulus</i>	No. of copies <i>S. cf. luederwaldti</i>
CG8460	chitinase domain-containing protein 1	<i>Acromyrmex echinator</i>	XM_011065531.1	0	3	4	2
Chitinase 2	probable chitinase 2	<i>Acromyrmex echinator</i>	XM_011063909.1	18	14	13	18
Chitinase 3	probable chitinase 3	<i>Acromyrmex echinator</i>	XM_011063337.1	23	13	15	20
Chitinase 5	similar to chitinase 5	<i>Nasonia vitripennis</i>	XP_001602780.1	23	13	15	20
Chitinase 6	similar to brain chitinase and chia	<i>Nasonia vitripennis</i>	XP_001601999.1	25	23	25	23
Chitinase 7	similar to chitinase 7	<i>Nasonia vitripennis</i>	XP_001604515.1	23	14	15	20
Chitinase 11	acidic mammalian chitinase	<i>Nasonia vitripennis</i>	XP_001604954.1	19	13	13	19
Idgf4	chitinase-like protein Idgf4	<i>Acromyrmex echinator</i>	XM_011068662.1	19	13	13	16
K06A9.1b	similar to K06A9.1b	<i>Apis mellifera</i>	XP_391993.2	10	16	10	13
CG5613	hypothetical protein	<i>Nasonia vitripennis</i>	XP_001606984.1	7	2	1	2

Table S1.2. Chitinase gene copies in transcriptomes, for each gene searched, for each species. * Following Suen *et al.*, 2011, S18.

		<i>Apterostigma megacephala</i>	<i>Sericomyrmex cf. mayri</i>	<i>Sericomyrmex cf. parvulus</i>	<i>Sericomyrmex cf. luederwaldti</i>
GO-id	GO-term	#Seqs	#Seqs	#Seqs	#Seqs
	Biological processes	50366	46495	52420	47920
GO:0009987	cellular process	13940	12345	14420	12865
GO:0008152	metabolic process	12887	12730	13230	11462
GO:0044699	single-organism process	7323	6747	8143	7475
GO:0065007	biological regulation	4520	3995	4243	4376
GO:0051179	localization	3548	3093	3660	3387
GO:0050896	response to stimulus	3261	2908	3601	3327
GO:0023052	signaling	2665	2355	2653	2702
GO:0071840	cellular component organization or biogenesis	1115	1054	976	993
GO:0032501	multicellular organismal process	448	521	749	581
GO:0032502	developmental process	236	350	293	305
GO:0022610	biological adhesion	196	216	221	237
GO:0040011	locomotion	97	53	72	69
GO:0040007	growth	45	31	52	34
GO:0002376	immune system process	31	41	26	28
GO:0000003	reproduction	27	25	31	40
GO:0051704	multi-organism process	24	28	45	37
GO:0001906	cell killing	2	1	2	0
GO:0048511	rhythmic process	1	2	3	2
	Molecular Function	32999	27495	34303	30926
GO:0005488	binding	13788	11820	14406	13271
GO:0003824	catalytic activity	12436	10104	12645	11131
GO:0005215	transporter activity	2158	1830	2412	2060
GO:0060089	molecular transducer activity	1084	934	1223	1041
GO:0030234	enzyme regulator activity	1056	686	796	962
GO:0004872	receptor activity	935	868	1119	899
GO:0001071	nucleic acid binding transcription factor activity	645	545	603	712
GO:0005198	structural molecule activity	343	462	577	362
GO:0009055	electron carrier activity	325	68	332	274
GO:0000988	protein binding transcription factor activity	144	113	96	126
GO:0016209	antioxidant activity	78	58	81	75
GO:0016530	metallochaperone activity	4	0	4	8
GO:0031386	protein tag	3	4	4	3
GO:0016247	channel regulator activity	0	2	4	2
GO:0045182	translation regulator activity	0	1	1	0
	Celular Component	20098	18641	21954	18944
GO:0005623	cell	6529	5743	6842	6108
GO:0016020	membrane	5531	5335	6586	5566
GO:0043226	organelle	4115	4049	4594	4055
GO:0032991	macromolecular complex	2400	2316	2500	2137
GO:0031974	membrane-enclosed lumen	421	305	353	272
GO:0005576	extracellular region	369	465	530	438
GO:0045202	synapse	330	101	146	106
GO:0030054	cell junction	281	106	146	116
GO:0031012	extracellular matrix	113	133	223	134
GO:0019012	virion	8	88	34	12
GO:0009295	nucleoid	1	0	0	0

Table S1.3. GO distributions by level, by species, with GO-ID numbers, the output of BLASTtoGO

Annotation analyses.

Classes	<i>Apterostigma megacephala</i>	<i>Sericomyrmex cf. mayri</i>	<i>Sericomyrmex cf. parvulus</i>	<i>Sericomyrmex cf. luederwaldti</i>
1.- Oxidoreductases	768	617	761	668
2.- Transferases	3161	2135	2921	2423
3.- Hydrolases	2662	1946	2655	2485
4.- Lyases	241	141	201	221
5.- Isomerases	160	209	339	211
6.- Ligases	643	479	872	635
Total	7635	5527	7749	6643

Table S1.4. Enzyme code distribution. Number of sequences annotated with different Enzyme code per species, the output of BLASTtoGO Annotation analyses.

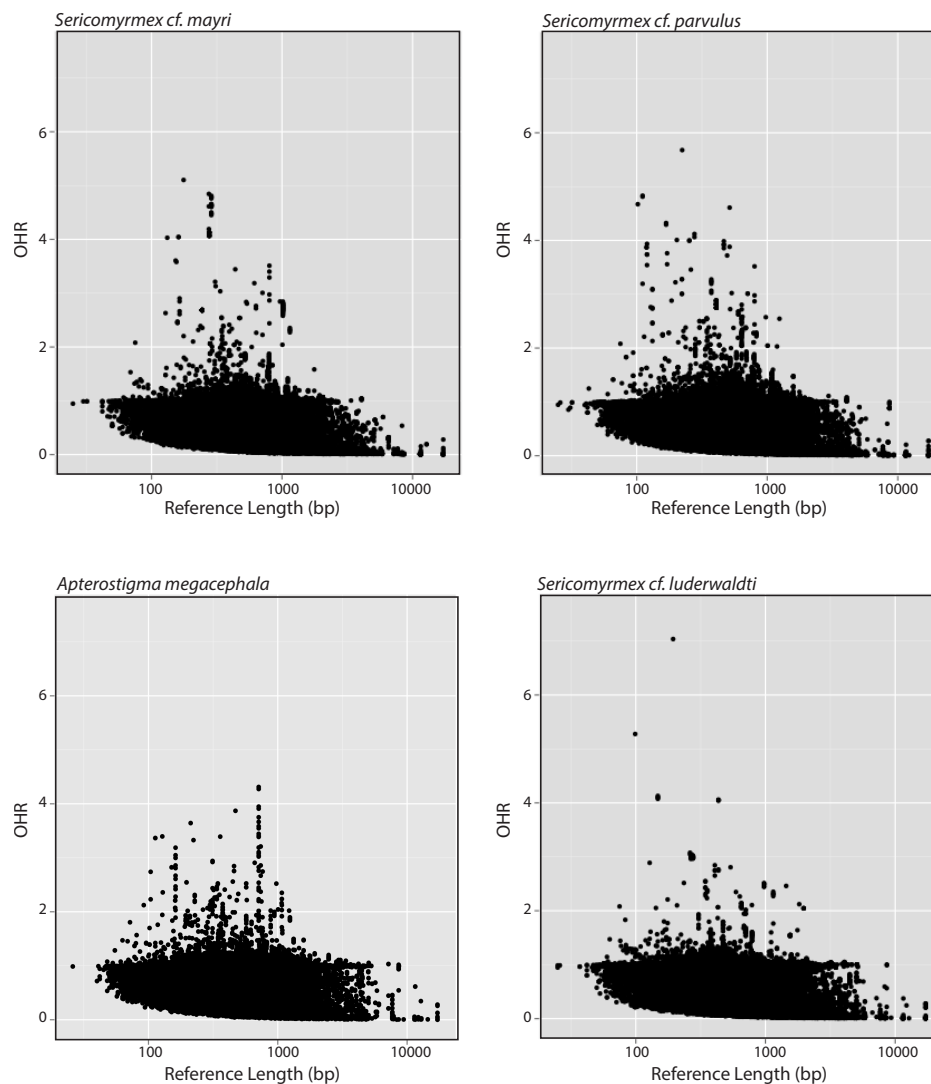


Figure S1.1. Ortholog Hit Ratio values for each of the species sequenced.

Chapter 2: Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera: Formicidae): Ultraconserved elements (UCEs) resolve a recent radiation

2.1. Introduction

The practice of alpha taxonomy, defining and delimiting species, has evolved considerably over the last two decades due to the rise of multidisciplinary species delimitation (Sites & Marshall, 2003; Schlick-Steiner *et al.*, 2010; Yeates *et al.*, 2011). This "integrative taxonomy" approach advocates the combination of evidence from several different sources: molecular, ecological, chemical, and/or behavioral, in addition to traditional morphological characters, especially for what are considered difficult groups (Martin *et al.*, 2008; Ross *et al.*, 2010; Seppa *et al.*, 2011; Ward, 2011; Ronque *et al.*, 2016). In ants, integrative taxonomy is especially useful in cases where various processes obscure species boundaries, e.g., hybridization (Helms Cahan & Vinson, 2003; Anderson *et al.*, 2006; Steiner *et al.*, 2011; Kulmuni & Pamilo, 2014), morphologically cryptic species (Schlick-Steiner *et al.*, 2006), high interspecific variability (Blaimer & Fisher, 2013; Branstetter, 2013), rapid or recent divergences (Goropashnaya *et al.*, 2004), and clonal reproduction (Pearcy *et al.*, 2004; Foucaud *et al.*, 2010; Rabeling *et al.*, 2011).

Most recently, advances in DNA sequencing technology have made genomic data affordable, effecting major changes in our ability to reconstruct deeper evolutionary divergences, such as relationships between families or genera (Johnson *et al.*, 2013; Blaimer *et al.*, 2015), and to resolve species boundaries (Wang *et al.*, 2011; Crawford *et al.*, 2015; Manthey *et al.*, 2016), especially where other approaches failed.

The genus *Sericomyrmex* is one such problematic ant taxon (Wheeler, 1925; Ješovnik *et al.*, 2016). It belongs to the fungus-farming ants (Formicidae: Myrmicinae: Attini: *Atta* genus group, hereafter “attine ants”), a New World clade comprising over 240 extant species that depend on the fungi they farm for food (Schultz & Brady, 2008), and whose most well-known representatives, leaf-cutting ants, are major herbivores in the Neotropics (Wirth & Leal, 2007). Attine ants, and leaf-cutters in particular, have become model organisms for the study of symbiosis and coevolution, and due to years of research attine ants are better understood than many other comparable ant groups (Mehdiabadi & Schultz, 2010; Nygaard *et al.*, 2016). Fungus-farming ants in the genus *Sericomyrmex*, however, have received little attention, and our knowledge of their evolution, taxonomy, natural history, and fungal cultivars remains poor. With 19 described species and 3 subspecies, *Sericomyrmex* has radiated into a diverse biota across a large geographic area, occupying wet and dry forests, pastures, cerrado, and urban and agricultural habitats from southern Brazil to northern Mexico, from sea level to over 1000 meters in elevation (Mayhe-Nunes & Jaffe, 1998; Fernández & Sendoya, 2004; Bolton, 2014). Surprisingly, this species-level diversity and geographic coverage is accompanied by remarkably little morphological variation. The genus itself is highly distinctive and unmistakably diagnosable from other attine genera, but species-level variation is subtle and complex.

Sericomyrmex ants are small- to medium-sized, ferrugineous brown, and covered with a dense layer of hairs, giving them a silky, velvety appearance. Differences between most species are indistinct and are obscured by sometimes significant within-species and within-colony variation, as some species are mildly polymorphic (Wheeler, 1925; Weber, 1972). A comprehensive taxonomic revision has never been attempted, and our knowledge of species boundaries still relies on the literature from the beginning of the 20th century (Wheeler, 1916). A recent genomic study that included three *Sericomyrmex* transcriptomes found strong evidence that *Sericomyrmex* species are likely the product of a very recent radiation and that they are separated from their nearest relative, the *Trachymyrmex iheringi* clade, by a long branch (Ješovnik *et al.*, 2016). However, a more recent molecular phylogeny of the attine ants reveals that the sister of *Sericomyrmex* is *Mycetosoritis explicatus* (Branstetter *et al.*, in prep.), an enigmatic species considered by some authors to be a lower attine (Forel, 1912; Kempf, 1968; Weber, 1972) but by others to be a higher attine (Sosa-Calvo *et al.*, 2009).

Because variation in both morphology and in nuclear gene sequence data (Ješovnik *et al.*, 2016) is very low across *Sericomyrmex* species – likely the result of a recent, rapid radiation – we consider *Sericomyrmex* to be a good model for judging the utility of a phylogenomic approach for delimiting closely related, recently diverged species. We chose ultraconserved elements (UCEs) as our phylogenomic markers. UCE loci are characterized by having a conserved core and increasingly variable flanking regions (Bejerano *et al.*, 2004). This gradient of variation makes UCEs useful for recovering phylogenetic relationships ranging from deep to shallow scales (McCormack *et al.*, 2012; Smith *et al.*, 2014), including population-level studies and studies of rapid

evolutionary radiations (Lim & Braun, 2015; Meiklejohn *et al.*, 2016). Recently developed UCE probes for Hymenoptera (Faircloth *et al.*, 2015) have been used successfully in multiple phylogenetic studies of ants (Blaimer *et al.*, 2015, 2016). Here we use UCEs to reconstruct the evolutionary history of *Sericomyrmex* and estimate its divergence times, with 5 outgroup and 88 ingroup samples from across the geographical range of the genus (Figure 2.1). Among other purposes, this phylogeny serves as the foundation for a species-level taxonomic revision of *Sericomyrmex* (Ješovnik & Schultz, in prep.). In addition to UCEs, we sequenced two fragments of the mitochondrial cytochrome oxidase subunit I gene (COI) for most UCE samples plus an additional 48 *Sericomyrmex* specimens, and we generated a COI phylogeny, which we compare with the results of analyses of the UCE data. Although UCEs and similar methods are rapidly becoming easier and cheaper to use, they are still not as inexpensive or, consequently, as commonly employed as single-gene markers. This is especially true for researchers in developing countries (*i.e.*, the places where *Sericomyrmex* occurs), including those conducting ecological and conservation studies. Despite its well-known shortcomings, such as reduced effective population size and maternal inheritance (Brower, 2006; Rubinoff *et al.*, 2006), COI has proven useful as a tool for species identification in some ant groups (Smith *et al.*, 2005; Ng'endo *et al.*, 2013). By comparing our UCE and COI results, we can judge the usefulness of COI for species identifications in *Sericomyrmex*.

Finally, to examine host-symbiont coevolution in *Sericomyrmex*, we obtained DNA sequences for the fungal cultivars of *Sericomyrmex* ant species. Attine ants have been obligately dependent on their fungi since soon after their origin around 55-60 million years ago (mya) (Schultz & Brady, 2008; Ward *et al.*, 2015), whereas, so far as is

known, most (but not all) attine fungal species (phylum Basidiomycota, order Agaricales) are facultative symbionts (Ješovnik *et al.*, 2016; Nygaard *et al.*, 2016) capable of living freely outside of the attine symbiosis. The cultivation of facultatively symbiotic fungi, practiced by the majority of attine genera, is known as “lower” attine agriculture (Chapela *et al.*, 1994; Hinkle *et al.*, 1994; Mueller *et al.*, 1998; Vo *et al.*, 2009). In contrast, the so-called “higher” attine fungi, which originated from a lower-attine fungal ancestor, form a clade cultivated by so-called “higher” attine ants (with one notable exception; see Schultz *et al.*, 2015). Higher attine fungi are obligate symbionts, incapable of living apart from the ants, and they have evolved various adaptations for life with ants (Schultz & Brady, 2008; Mehdiabadi & Schultz, 2010; Masiulionis *et al.*, 2014; Nygaard *et al.*, 2016).

Sericomyrmex cultivars are higher attine fungi. Previous phylogenetic studies of attine fungi have included a few *Sericomyrmex* fungal cultivars (De Fine Licht *et al.*, 2010; De Fine Licht & Boomsma, 2014; Kooij *et al.*, 2015), but no prior phylogeny has included fungi from multiple *Sericomyrmex* species spanning the geographical range of the genus, as it is done here. Our investigation of *Sericomyrmex* fungi was guided by two aims. First, we wanted to determine whether the radiation of *Sericomyrmex* ant species was driven by coevolution with a specialized clade of higher attine fungi, as has been suggested for *Atta* (Schultz & Brady, 2008; Mueller *et al.*, 2010). Second, we wanted to quantify the degree to which *Sericomyrmex* ants and fungi have coevolved. In particular, we wanted to investigate the degree of ant-fungus symbiont fidelity across the genus or in any subset of species pairs. Even though general patterns of attine ant–fungus coevolution have been described as “diffuse coevolution” (Mikheyev *et al.*, 2007), it is

also the case that phylogenies of attine ants and their fungal cultivars are strongly congruent at deeper phylogenetic levels (Schultz *et al.*, 2015), and that ant and fungal associations in the *Cyphomyrmex wheeleri* species group conform to a pattern of strong symbiont fidelity (Mehdiabadi *et al.*, 2012).

2.2. Materials and methods

2.2.1. Field Work

Field work was conducted in Guyana, Peru, Mexico, Guatemala, and in multiple localities in Brazil. In addition to those collected in the field, samples were obtained from colleagues and museum collections. Figure 2.1 illustrates collecting localities for all *Sericomyrmex* ant and fungal specimens used in this study. Table S2.1 lists all ant specimens used in this study, including collecting information, voucher numbers, and GenBank accession numbers. Voucher specimens are deposited at the Smithsonian Institution's National Museum of Natural History in Washington (NMNH), DC, with all data publicly available online in the Museum's database (<http://collections.nmnh.si.edu/search/ento/>). Table S2.2 contains the same information for fungal specimens. Methods for locating and excavating *Sericomyrmex* nests in the field follow Sosa-Calvo *et al.* (Sosa-Calvo *et al.*, 2015). After opening a nest chamber in the field, we used flame-sterilized soft metal forceps to immediately transfer fungus from the fungus garden into vials of 95% ethanol. Field-collected ant samples were likewise preserved in 95% ethanol. Alcohol-preserved material borrowed from museums and colleagues was preserved in ethanol of various concentrations. The map for Figure 2.1.

was prepared with ArcGIS v10.3.1 software (Esri, Redlands, CA) using a basemap from the U.S. National Park Service.

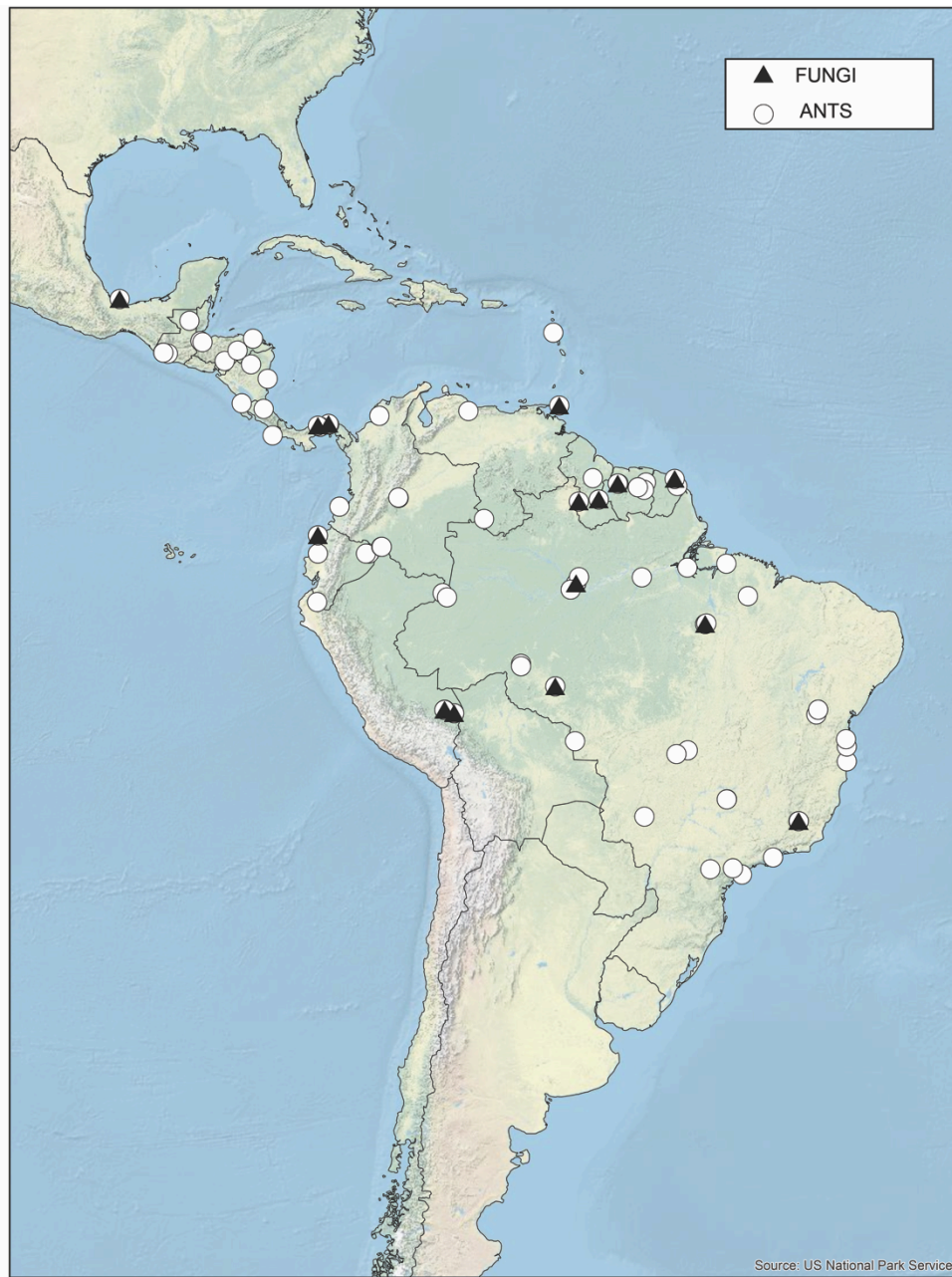


Figure 2.1. Localities from which *Sericomyrmex* ants (white circles) and fungi (black triangles) were collected.

2.2.2. Taxon selection

Specimens for UCE sequencing were selected to encompass the full range of morphological and molecular variation, as well as geographical distribution, of *Sericomyrmex*. Molecular variation was preliminarily assessed based on two fragments of mitochondrial COI (885 base pairs) sequenced for 125 specimens. Outgroup taxa were chosen based on a recent attine phylogenomic study (Branstetter *et al.*, in prep.). These included the newly identified sister species of *Sericomyrmex*, *Mycetosoritis explicatus*, and four species from the *Trachymyrmex iheringi* clade, the large sister clade of *Sericomyrmex* + *M. explicatus*. Two *Sericomyrmex* species were available only as old, point-mounted, museum specimens: *Sericomyrmex lutzi*, for which, due to the small number of specimens, we did not attempt any DNA extractions, and *Sericomyrmex* n. sp. 3 from Venezuela, for which we only sequenced COI.

2.2.3. Laboratory methods for Sanger sequencing

We conducted all laboratory work in the Laboratories of Analytical Biology (LAB) at the National Museum of Natural History (NMNH), Smithsonian Institution, in Washington, D.C., USA.

Ants. For the COI dataset we assembled 135 specimens of *Sericomyrmex*. We extracted ants destructively or non-destructively (for rare specimens) using the DNeasy Blood and Tissue Kit (Qiagen, Inc.) and following the standard protocol described by the manufacturer, except that we prolonged the tissue lysis step (incubation with Proteinase K) to overnight. For the final DNA elution step we used two washes of 50 µL double-distilled water (ddH₂O) each, ending with 100 µL of genomic DNA. Amplification for

two fragments of COI, defined by primer pairs CI13-CI14 (~ 560 base pairs) and Ben-Jerry (~380 base pairs), was attempted for all specimens with PCR Master Mix (Go Taq and Hot Start kits, Promega, Madison, WI). We ran 15 μ L PCR reactions with 1 μ L of DNA extract with several different protocols (Table S2.3), but mostly with a 48° C annealing temperature. Out of 135 extractions performed ten failed to amplify or sequence due to DNA degradation, thus the COI data set contained 125 newly sequenced taxa.

Fungi. We assembled 38 samples of *Sericomyrmex* fungal cultivars, each collected from a different *Sericomyrmex* nest, and attempted to sequence two nuclear ribosomal regions: internal transcribed spacer (ITS) and large subunit (LSU). For each sample we took a small amount of ethanol-preserved fungal tissue, dried it for 1–3 minutes on tissue paper, and placed it in a 2.0 mL tube that was previously partly filled with 2.3 mm zirconia/silica beads and 0.5 mm glass beads (BioSpec Products). We submerged the tubes in liquid nitrogen for 3 minutes, and then put them into the TissueLyser (Qiagen, Inc.) for 1 minute to ensure tissue disruption. After tissue disruption we extracted DNA from each sample manually using the Plant DNeasy kit (Qiagen, Inc.) following the standard manufacturer protocol. For the final elutions we used 100 μ L of elution buffer. We quantified DNA concentrations for all extracts with a Qubit 2.0 fluorometer high sensitivity kit (Life Technologies, Inc.). Because fungal samples with high DNA concentrations can be problematic to amplify, we made a series of dilutions for all the samples, resulting in increased amplification success. We prepared the first dilution by mixing 15 μ L of the DNA extract with 15 μ L of ddH₂O (1:1 dilution); each subsequent dilution was prepared by diluting 15 μ L of the previous

dilution with another 15 μ L of ddH₂O. This was repeated 3 times for a final dilution of 12.5% of the original extraction. Based on our PCR results, the optimal DNA concentration for PCR amplification was between 0.01 to 0.1 ng/ μ L. For PCR reactions we used a PCR Master Mix (Hot Start kit, Promega, Madison, WI). For samples that repeatedly did not amplify we ran 10 μ L of DNA extract on a low-concentration agarose gel (60 min, 60V) to check for DNA fragmentation. Four of the fungal samples repeatedly failed to PCR-amplify due to degraded DNA. If the DNA did not seem severely fragmented, we purified it with generic Sera-Mag Magnetic Speed-beads (Rohland & Reich, 2012), hereafter “speedbeads” (Fisher Scientific, Inc.), to remove possible PCR inhibitors.

In addition to fungal DNA samples, we tried to amplify fungal DNA from ant DNA extractions, a method that was partially successful in a previous study of lower attine ant genera (Sosa-Calvo, 2015). We ran PCR reactions with fungal primers on 64 samples of destructively extracted ant extracts. We tried the following primer combinations for both fungal and ant extracts: Long ITS (ITS1F-LR1, ITS5-ITS4), ITS Fragment 1 (ITS1F-ITS2, ITS1-ITS2, ITS1-5.8S), ITS Fragment 2 (ITS3-ITS4, ITS4-LR1), LSU (ITS3-LR16, LR3R-LR7, LROR-LR3-1) and SSU (BMB-BR-ITS2). (Table S2.3).

We succeeded in amplifying fungal DNA from 6 out of 64 ant extractions. For 2 of those 6 ants we had also sampled and sequenced the fungus garden, which enabled us to confirm that the fungus sequenced from the ant DNA extract was identical to the fungus grown in the nest. We sequenced a total of 32 different fungi, from ants and fungi combined.

2.2.4. Laboratory methods for UCEs

Extraction and library preparation. We destructively extracted ants as described above, except for a few modifications. For the final DNA elution step we used 130 μL of ddH₂O, to ensure that we had enough extract for all of the quality checks and library preparation. We quantified the DNA extracts with the Qubit 2.0 fluorometer broad range kit (Life Technologies, Inc.) and ran 10 μL of each extract on an agarose gel to check for possible DNA fragmentation. We sheared 61-417 ng of DNA extract to an average fragment length of 500-600 bp with a Qsonica Q800r sonicator (Qsonica LLC, Newton, CT) and then used the sheared DNA for library preparation. Library preparations were carried out in 1.5 mL tubes. For 22 *Sericomyrmex* and all 5 outgroup samples we used the standard Kapa library preparation kit (Faircloth *et al.*, 2015). For the remaining 66 *Sericomyrmex* samples, sequenced subsequently, we used a Kapa Hyper Prep Library Kit (Kapa Biosystems, Wilmington, MA). All SPRI bead cleanup steps were performed using speedbeads (Fisher *et al.*, 2011; Faircloth *et al.*, 2015). During the ligation step of the library preparations we added either custom, single-indexing TruSeq-style barcode adapters (Faircloth *et al.*, 2012, 2015) or custom, dual-indexing TruSeq-style adapters (iTru; i5 and i7 primers) (Faircloth & Glenn, 2012). After ligation we PCR-amplified 50% of the resulting library volume (15 μL) with the following reaction mix: 25 μL HiFi HotStart polymerase (Kappa Biosystems), either 5 μL of Illumina TruSeq primer mix (5 μM) or 2.5 μL of each of Illumina TruSeq-style i5 and i7 primers, and 5 μL ddH₂O, with the following thermal protocol: 98 °C for 45 sec, 10-13 cycles of 98 °C for 15 sec, 65 °C for 30 sec, 72 °C for 60 sec, and 72 °C for 5 min.

Pooling. Following PCR, we purified the product using 1.0X speedbeads and we eluted the purified DNA with 23 μ L using a pH 8 elution buffer (EB). We checked the quality of the libraries by measuring DNA concentration with a Qubit 2.0 fluorometer broad range kit and running 2 μ L of library on an agarose gel. Following the quality checks we pooled 6-10 libraries together at equimolar concentrations into enrichment pools and we adjusted pool concentrations to ~147 ng/ μ L using a vacuum centrifuge. Final concentrations of enrichment pools were 140-162 ng/ μ L.

Enrichment. We enriched each pool with 2,749 custom-designed probes (MYcroarray, Ann Arbor, MI), targeting 1510 UCE loci in Hymenoptera (Faircloth *et al.*, 2015). We followed the manufacturer's protocol for the MYcroarray MYBaits kit (Blumenstiel *et al.*, 2010), except that we used a 1:5 dilution of the standard MYBaits concentration, and we added 0.7 μ L of 500 μ M custom blocking oligos (designed for our custom sequence tags). Enrichment incubation was performed at 65 °C for 24 hours, after which we bound all pools to streptavidin beads (MyOne C1; Life Technologies) and purified enriched libraries according to a standard target enrichment protocol (Blumenstiel *et al.*, 2010). We PCR-enriched libraries using the 'with-bead' approach (Faircloth *et al.*, 2015). We combined 15 μ L of the streptavidin bead-bound enriched library with 25 μ L of HiFi HotStart Taq (Kappa Biosystems), 5 μ L of Illumina TruSeq primer mix (5 μ M each), and 5 μ L of ddH₂O, and ran it at 98 °C for 45 sec, 18 cycles of 98 °C for 15 sec, 60 °C for 30 sec, 72 °C for 60 sec, and 72 °C for 5 min. We purified the resulting reactions using 1.0X speedbeads and rehydrated the enriched pools in 22 μ L EB. We quantified 2 μ L of each enriched pool using a Qubit 2.0 fluorometer broad range kit (Life Technologies, Inc.).

Enrichment verification and size selection. We tested our enrichment by amplifying seven UCE loci with relative qPCR. For each of the seven loci we compared amplification profiles between enriched and unenriched pools (adjusted to the same concentration). We used a SYBR® FAST qPCR kit (Kappa Biosystems) to perform qPCR on a ViiA™ 7 Real-Time PCR System (Life Technologies). Following enrichment verification we quantified the DNA concentration of each pool using qPCR. We used the resulting concentrations to pool libraries at equimolar concentrations, creating a pool-of-pools. This final pool-of-pools was then size-selected to a fragment range of 250–800 bp using the BluePippin size-selection instrument (SageScience, Beverly, MA). Size-selected library pools were sent to either the UCLA Neuroscience Genomics Core or Cornell’s Institute of Biotechnology and sequenced as 3 partial lanes (each of the lanes included other samples) on an Illumina HiSeq 2500 (2x150 rapid run).

2.2.5. Phylogenetic analyses of single-gene data (ants and fungi)

We edited, aligned, and concatenated the ant and the fungal sequences in Geneious v9 (Kearse *et al.*, 2012) and compared our results with NCBI GenBank sequences using BLASTn (Altschul *et al.*, 1990; Johnson *et al.*, 2008) to check for possible contamination. We combined our newly generated sequences, ant COI and fungal ITS and LSU data, with previously published sequences downloaded from NCBI GenBank. The COI data included 135 specimens sequenced in this study, with the addition of 12 *Sericomyrmex* and 12 non-*Sericomyrmex* attine ant GenBank sequences, the latter serving as outgroups. The fungal data included 32 *Sericomyrmex* fungal sequences generated in this study and 60 ITS and 4 LSU sequences downloaded from

GenBank, consisting of both *Sericomyrmex* and non-*Sericomyrmex* fungal cultivars. Numbers of taxa and other information for each data set are summarized in Table 2.1. Alignments were generated with MAFFT v1.3.5 (Kato & Standley, 2013) using default settings, as implemented in Geneious v9 (Kearse *et al.*, 2012), and further refined manually. Three matrices were generated for the fungal data: one for ITS, one for LSU, and one with both alignments combined (ITS+LSU). To select the best model of sequence evolution and data partitioning schemes for the COI data set we used PartitionFinder v1.1.1 (Lanfear *et al.*, 2012) and for all three fungal data sets we used Partition Finder v2.0 (Frandsen *et al.*, 2015). For COI we defined data blocks by gene and codon position and used the "search = all" algorithm, whereas for the fungal data sets, which are from non-protein-coding regions, we used the "search = kmeans" algorithm, which does not require predefined data blocks (Frandsen *et al.*, 2015). For each data set we ran two PartitionFinder analyses using "AICc" model selection criteria, one with "models" set to "raxml" and one with "models" set to "mrBayes". The number of partitions and their models for each data set and for each analysis can be found in supplemental material (Text S2.1). We conducted simultaneous best-tree and rapid bootstrapping (1000 pseudoreplicates; -f a option) maximum-likelihood (ML) analyses in RAxML v8.2.8 (Stamatakis, 2014). The resulting phylogenies were examined in FigTree v1.4.2 (Rambaut, 2009). We also conducted Bayesian analyses of all data sets using MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For each data set we performed two runs (four chains per run) with the chain length set to 30–50 million generations. We confirmed that our runs reached stationarity and converged by making sure that the average standard deviation of split frequencies was below 0.01,

by examining the estimated sample size (ESS) values for all parameters, and by comparing the two independent runs in Tracer v1.6 (Rambaut *et al.*, 2016). The number of generations and other computational details for each MrBayes run are listed in supplemental material (Text S1). The resulting consensus trees were examined in FigTree v1.4.2 (Rambaut, 2009). All phylogeny figures were prepared using FigTree v1.4.2 and Adobe Illustrator CC.

2.2.6. Processing of UCE data

The raw sequence data were converted to FASTQ format and demultiplexed by the sequencing centers. Using the FASTQ files we cleaned and trimmed reads using Illumiprocessor (Faircloth, 2013), which incorporates Trimmomatic (Bolger *et al.*, 2014). Following trimming, the data were processed through a series of scripts available in the PHYLUCE package v1.4 (Faircloth, 2015), with parallel processing on a 12-core Intel-processor Apple computer. We computed summary statistics for the trimmed FASTQ files (`get_fastq_stats.py`), assembled the trimmed reads with a script wrapper (`assemblo_trinity.py`) in the program Trinity (version `trinityrnaseq_r20140717`) (Grabherr *et al.*, 2011), and calculated the coverage of all assembled contigs (`get_trinity_coverage.py`). After assembly we mapped Trinity contigs to UCE loci (`match_contigs_to_probes.py`, with settings: `min_coverage=50`, `min_identity=80`), and calculated coverage statistics for UCE contigs (`get_trinity_coverage_for_uce_loci.py`). We generated a list of UCE loci shared across all taxa (`get_match_counts.py`) and used this list to create a monolithic FASTA file containing all samples and sequence data (`get_fastas_from_match_counts.py`). We separated data by locus and aligned each locus

using a wrapper script around MAFFT (Katoh & Standley, 2013). The resulting alignments were trimmed with Gblocks (Castresana, 2000) (get_gblocks_trimmed_alignment_from_untrimmed.py, settings: b1=0.5, b2=0.5, b3=12, b4=7).

After trimming, we created multiple data sets (summarized in Table 2.1) based on filtering UCE loci for differing levels of taxon occupancy (95%, 90%, 80%, 70%, and 50%) using the get_only_loci_with_min_taxa.py script,. The 70% data set, for example, contained only those UCE loci that included sequences for at least 70% of the taxa. We then added missing data designators (“?”) for taxa missing from each alignment of a given locus (add_missing_data_designators.py) and generated matrix statistics for each data set (get_align_summary_data.py).

Sequencing	Organism	Data matrix	Taxa number	UCE loci or genes	Description	Alignment length (bp)	Number of partitions	UCE mean loci length
nextgen	ant	50%	93	1,062	50 complete matrix	873,481	29	873.481
nextgen	ant	70%	93	1016	70 complete matrix	850,814	16	837.42
nextgen	ant	80%	93	956	80 complete matrix	814,997	29	852.51
nextgen	ant	90%	93	799	90 complete matrix	702,574	13 , 799*	879.32
nextgen	ant	95%	93	530	95 complete matrix	499,504	17	942.46
nextgen	ant	ex1	92	816	one taxon excluded	716,032	31	877.49
nextgen	ant	ex2	90	788	3 taxa excluded	695,579	33	882.71
nextgen	ant	ex3	32	676	only 32 taxa for paraFit included	625,196	25	814.56
nextgen	ant	bs best all	93	100	100 UCE loci with highest bs values, all taxa	83,533	13	835.33
nextgen	ant	bs best 15T	15	100	100 UCE loci with highest bs values, 15 taxa	88,157	11	881.157
nextgen	ant	random 1	15	100	100 random loci, for 15 taxa	90,771	12	907.71
nextgen	ant	random 2	15	100	100 random loci, for 15 taxa	94,740	13	947.4
nextgen	ant	random 3	15	100	100 random loci, for 15 taxa	90,760	13	907.6
Sanger	ant	CO1	150	1	CO1 alignment	885	3	NA
Sanger	funga	ITS	81	1	ITS alignment	953	5	NA
Sanger	funga	ITS+LSU	93	2	ITS + LSU alignments	2329	6	NA
Sanger	funga	LSU	54	1	LSU alignment	1412	3	NA

Table 2.1. Data sets. Summary statistics for all data sets used in analyses. * The two numbers listed under "Number of partitions" for the 90% matrix indicate the results of PartitionFinder analyses using the kmeans and greedy algorithms (the latter with UCE loci as data blocks), respectively. (bp – base pairs.)

2.2.7. UCE Phylogenetic analyses

For data partitioning and model estimation of the UCE data, we used the kmeans algorithm in PartitionFinder 2.0 (Frandsen et al., 2015; Lanfear et al., in prep.) that is designed to infer partitions and models directly, rather than through predefined data blocks (e.g., UCE loci), which is particularly useful for genome-scale data. Because of a concern, based on simulated data, that the kmeans search algorithm can produce odd results (P. Frandsen, pers. comm.), most likely associated with a large subset of invariant sites, we also ran a PartitionFinder analysis in which UCE loci were defined as data blocks. We ran this analysis with the “hcluster” search algorithm on the 90% complete data set. We conducted phylogenetic analyses of all UCE data sets under the maximum likelihood (ML) criterion in RAxML v8.0.3 (Stamatakis, 2014) using a simultaneous best-tree search and rapid bootstrapping analysis (-f a option) with 100 bootstrap replicates. To investigate the effects of partitioning and number of bootstrap replicates on the results we performed additional analyses on the 90% complete matrix in RAxML v.8.0.3 (also using the simultaneous best-tree search and rapid bootstrapping analysis): of an unpartitioned data set (100 bootstrap replicates), of a data set partitioned with UCE loci defined as data blocks (100 bootstrap replicates), and of a kmeans-partitioned data set (500 bootstrap replicates). Data sets and some of the analysis details are summarized in Table 2.1.

In addition to concatenated analyses, we partitioned each UCE locus separately using PartitionFinder 2.0 (using kmeans), and inferred partitioned gene trees with RAxML v.8.0.3 (simultaneous best tree and rapid bootstrap search, 100 pseudoreplicates). We then carried out a species tree analysis using the resulting gene

trees as input into the program ASTRAL v4.7.6 (Mirarab *et al.*, 2014), a species-tree method that uses a multi-species coalescent model to estimate the true species tree from unrooted gene trees. We ran the program with 100 multi-locus bootstrap replicates. ASTRAL has been shown to outperform similar methods in cases of rapid evolutionary radiations (Meiklejohn *et al.*, 2016).

To further explore the effects of possible gene-tree disagreement on our species-tree results, we performed statistical binning (Mirarab *et al.*, 2014). This method sorts UCE loci that agree topologically into bins, taking into account only well-supported bipartitions as determined by bootstrap proportions (Mirarab *et al.*, 2014a; Bayzid *et al.*, 2015), producing, for our data, 433 bins. We concatenated the loci from each bin into supergene alignments and used these alignments in gene-tree species-tree analyses using PartitionFinder, RAxML, and ASTRAL, as described above for the non-binned ASTRAL analysis.

We also used the gene trees as an input for the script `gene_stats.R` (Borowiec *et al.*, 2015) to calculate the average bootstrap support for each locus in R v3.1.2 (R Development Core Team, 2014) and created a data set consisting of the 100 UCE loci with the highest bootstrap support (data set `bs_best_all`) for all taxa.

To explore the effect of taxa with long branches, especially since some of them had lower coverages and read counts, we also created two "reduced" data sets, one (Ex1) from which *Sericomyrmex opacus* 1614 was excluded, and one (Ex2) from which *Sericomyrmex opacus* 1614, *Sericomyrmex mayri* 1555, and *Sericomyrmex amabilis* 1559 were excluded. We created these data sets by running the PHYLUCE pipeline from

the `get_match_counts.py` step, with those taxa that we wished to exclude absent from the configuration taxon list. For these data sets we used only the 90% level of taxon filtering.

2.2.8. UCE Divergence Dating Analyses

For divergence-dating analyses in BEAST v2.4.1 (Drummond *et al.*, 2012; Bouckaert *et al.*, 2014) we created four separate data sets, each with the same reduced numbers of taxa and UCE loci. In order to estimate species divergence dates using the Yule prior, in all data sets we reduced the number of taxa to a single representative per species, so that the data set included 15 taxa (10 ingroup and 5 outgroup species). Using the script `gene_stats.R` (Borowiec *et al.*, 2015), we calculated the average bootstrap support for each locus in R v3.1.2 (R Development Core Team, 2014) and used the results to create a data set consisting only of the 100 UCE loci with the highest bootstrap support (data set `bs_best_15T`) from our 90% complete data set. We used a reduced number of loci because BEAST analyses are computationally demanding and larger data sets take intractably long periods of time to converge. To ensure that this subsampling of loci did not bias our results, we created three additional data sets consisting of 100 UCE loci (data sets `random 1`, `random 2`, and `random 3`), randomly subsampled using the function `sample` in R v 3.1.2 (R Development Core Team, 2014).

We calibrated two nodes (Table S5) using secondary calibrations taken from the most recent phylogeny of attine ants (Branstetter *et al.*, unpublished data) because no fossils exist for *Sericomyrmex* or the outgroups included in this study, whereas Branstetter *et al.* (unpublished) is calibrated with nine fossils and includes a much larger taxon sample from across the tribe Attini s.l. We used very broad normal distributions in

our prior calibrations (Ho & Phillips, 2009) that encompassed more than the 95% highest posterior densities (HPD) of Branstetter *et al.* (unpublished) (Table S5) in order to account for the use of secondary calibrations, which are known to cause unrealistically narrow confidence intervals (Drummond & Bouckaert, 2015).

We conducted BEAST v2.4.1 analyses of all four data sets using unpartitioned data because of the much longer analysis times required for partitioned data sets and because of concerns that partitioned analyses do not produce consistent divergence dates (Dos Reis *et al.*, 2014; Zhu *et al.*, 2014; Ward & Fisher, 2016). However, we also performed one partitioned analysis, on the bs_best data set only, to test whether partitioning had any effect on the results. We used the relaxed lognormal molecular clock, Yule tree prior, and diffuse gamma distribution on the mean branch lengths (uclد.mean; alpha=0.001, beta= 1000), with other settings at default, in all of our BEAST v2.4.1 analyses. For each data set and for each analysis we started two independent runs using the same xml file. In the first set of analyses we used the GTR model of evolution with base frequencies set to “estimated” for 600 million generations and with log and tree sampling frequencies set at 5000 generations. The resulting log files had low (<100) estimated sample size (ESS) values for some of the statistics (“prior” and “posterior”) when examined in Tracer v1.6.0, even though the ESS for other parameters (e.g., likelihood of the tree) was high, and even though our runs converged on the same dates. Since overparameterization can cause low ESS values in BEAST v2.4.1 runs (Drummond & Bouckaert, 2015), and a complex model of evolution is one of the causes of this behaviour, we conducted a second set of analyses with a simpler model of evolution, HKY with base frequencies set to “empirical”. We set up 2 independent runs for each of

the 4 data sets consisting of 1 billion generations with log and tree sampling frequencies of 5000 generations. We also set up 2 independent runs for the partitioned bs_best data set, with 500 million generations and with log and tree sampling frequencies of 1000 generations. For each data set two independent log files were combined in LogCombiner v.2.4.1 (Bouckaert *et al.*, 2014). Examination of the combined log files in Tracer v1.6 (Rambaut *et al.*, 2016) indicated that ESS values were high for all parameters (>200) and that stationarity had been achieved by 100 million generations (burn-in of 10%). Because of computational limitations, we summarized the resulting tree files using a burn-in of 50% with Tree Annotator v2.4.1 (Bouckaert *et al.*, 2014) and examined them in FigTree v1.4.0. For each data set we also combined two tree files from two independent runs, using Log Combiner, and summarized these combined trees with Tree Annotator v2.4.1.

To compare *Sericomyrmex* with other examples of recent radiations, we calculated the net diversification index (NDI), a time elapsed between the origin of a new lineage and the next branching of that lineage, assuming no extinction. NDI is defined as $t / (\ln Nt - \ln N_0)$, where t is the time to a single common ancestor in millions of years, Nt is a number of lineages, and N_0 the number of original species, which is one in monophyletic lineages (Coyne & Orr, 2004).

2.2.9. Analyses of coevolution

We tested for evidence of ant-fungus coevolution using ParaFit, a permutation-based test for significant coevolution (Legendre *et al.*, 2002), as implemented in the R software package ape v.3.4 (Paradis *et al.*, 2004). For ParaFit analyses we used the phylogeny resulting from the analysis of the combined fungal ITS+LSU data sets

excluding outgroups and a phylogeny of the ant UCE loci including only those ants that had associated fungi in the fungal phylogeny (data set Ex3; Table 2.1). We exported the patristic distance matrices of both phylogenies from Geneious v.9 and created a matrix of 32 associated ants and fungi. Six *Sericomyrmex*-associated fungi did not have an exact ant match because the fungus originated from a nest that was not represented in the UCE ant phylogeny. For four of those cases we associated the fungus with an ant of the same species from the same locality, and for the remaining two samples we associated the fungus with the same ant species but from a different locality. To determine whether the latter substitution biased our results, we created a second data set with only 30 taxa, excluding the two associations with non-matching localities. Lists of taxa in both data sets can be found in Table S2.5. Analyses consisted of 999 permutations with the “cailliez” correction (Legendre & Legendre, 1998; Legendre *et al.*, 2002).

2.3. Results

2.3.1. UCE sequencing

The mean DNA sample concentration for our 93 taxa was 3.32 ng/μL (0.619–50 ng/μL) post-extraction, and 59.25 ng/μL (22.7–136 ng/μL) post-PCR libraries. The mean number of raw reads sequenced per sample was 1,447,502 (229,485–3,500,409) and the mean number of UCE loci captured per taxon was 994.23 (497–1,054). Table 2.2 summarizes statistics for sequencing and pipeline processing. These statistics are similar to those in previous UCE studies using the same Hymenoptera probes (Blaimer *et al.*, 2015; Faircloth *et al.*, 2015). The total number of UCE loci in our concatenated data sets

ranged from 100 to 1,062. The most complete data set, the 95% complete concatenated matrix, included 530 UCE loci.

	DNA conc. extraction (ng/μL)	DNA conc. post PCR Library (ng/μL)	Raw read count	Contigs coverage	Contig mean length	UCI Loci coverage	Sequence coverage	Mean UCI loci length (bp)
Average	3.87	79	1,423,497.91	19.94	398.4	989.23	72.78	923.15
Minimum	0.619	17	299,485	9.2	273.2	497	9.04	284.8
Maximum	50	136	3,500,409	31.7	768.4	1054	191.83	1,218.4
Standard deviation	5.30	23.98	594,466.6	3.64	78.31	59.75	29.62	133.25

Table 2.2. UCE sequencing statistics. Mean, standard deviation, minimum, and maximum sequencing and assembly statistics averaged for all 93 taxa in the UCE data set.

2.3.2. UCE phylogeny and species delimitation

Most data sets and analyses produced exactly the same topology and had bootstrap frequencies of 99–100 at all nodes, except one, for divergences at the species level and deeper (Fig. 2, Figure S1). The results indicate that the genus *Sericomyrmex* consists of two main groups, which we refer to as the *scrobifer* clade and the *amabilis* clade. This is the first such division to be discovered in *Sericomyrmex*, for which no species groups have been previously proposed. The *scrobifer* clade comprises 3 species: *S. scrobifer* and two new species, which we refer to as *S. n. sp. 1* and *S. n. sp. 2*. All *scrobifer*-clade species occupy relatively long branches supported by bootstrap frequencies of 100 in all analyses. The larger *amabilis* clade includes 5 species: *S. opacus*, *S. parvulus*, *S. saussurei*, *S. amabilis*, *S. bondari*, and *S. mayri*. It is highly likely, based on morphology (Ješovnik & Schultz, unpublished data), that the two *Sericomyrmex* species that are not represented in our UCE-based phylogeny, *S. lutzi* and *S. n. sp. 3*, also

belong to the *amabilis* clade, with *S. lutzii* a close relative to *S. mayri*, and *S. n. sp. 3* a close relative to *S. bondari*.

In an iterative process of species delimitation, each putative species-level clade in the UCE-based phylogeny was investigated with respect to geography and morphology. For geography, we studied distribution data to determine whether putative species-level clades could be interpreted as geographically distinct populations or as full-fledged species. For morphology, we studied >1500 specimens, including type specimens, to determine whether putative species-level clades were characterized by discrete morphological character combinations. We have chosen not to recognize putative species-level clades as distinct species if (i) they can alternatively be interpreted to be allopatric populations of a single widespread species and (ii) they are morphologically indistinguishable from other populations of that widespread species. Based on these criteria, there are nine species-level clades present in our UCE-based phylogeny, including two new species. This number is lower than the currently recognized 19 species, even accounting for the two species that we were unable to include in the phylogeny, *S. lutzii* and another new species, *S. n. sp. 3*, which increase to 11 the total number of *Sericomyrmex* species.

A single specimen of *S. amabilis* from Venezuela occupies a long branch in the UCE phylogeny as the sister to the combined *saussurei* and *amabilis*, a position with maximum support in all analyses of all data sets (Fig. 2, Figure S1, S3). This position, as well as intervening branch lengths, indicates that this specimen is molecularly distinct from the rest of the *amabilis* clade and could represent a new species. It is, however, morphologically indistinguishable from *S. amabilis* and is represented in our study by

only three specimens, one destructively sampled for DNA extraction and two pinned. For these reasons, and because it is possible for one of two sister species to be paraphyletic with respect to the other (Coyne & Orr, 2004), we are for now treating *amabilis* VE as an allopatric population of *amabilis* that renders *amabilis* paraphyletic with respect to *saussurei*.

The only species-level clade supported by a low bootstrap frequency is *S. mayri* (68-100, depending on the analysis, Figure S1) and this is caused by the inclusion of a single rogue specimen, *Sericomyrmex mayri* 1555 TT from Trinidad and Tobago, which occupies differing positions in phylogenies produced by different data sets or analytical conditions. In phylogenies resulting from RAxML v8.0.3 analyses of the 50%, 70%, 80%, and 90% complete concatenated matrices, *S. mayri* 1555 TT consistently occupies the position of the most basally diverging lineage in the *S. mayri* species-level clade (Fig. 2), although supported by low bootstrap frequencies (68-87). It likewise occupies this position in analyses of the 100_best_all data set with a bootstrap frequency of 100. In ML analyses of the 95% matrix, however, *S. mayri* 1555 TT occupies the position of sister to *S. bondari*, while in both ASTRAL v4.7.6 species-tree analyses (of individual UCE loci and of binned UCE loci) it is recovered as the sister of the combined *S. bondari* and *S. mayri* clades, in both cases with a bootstrap frequency of 100 (Figure S1). The number of UCE loci sequenced for this taxon is average; however, some of the sequencing statistics are very low, including the number of raw reads and UCE locus length (Table S7), so its unstable position in some analyses could be an artifact of poor sequence quality and missing data. Considering all the evidence, we have chosen not to recognize *S. mayri* 1555 TT as a new species. Morphologically, it is indistinguishable from other members

of *S. mayri* and, as summarized above, the UCE-based phylogenetic results are inconclusive. The phylogenetic position and species-level status of *Sericomyrmex mayri* in Trinidad certainly deserves further research; we were able to include only a single nest series in this study.

In addition to *S. mayri* 1555 TT, we identified two other taxa as potentially problematic. *Sericomyrmex opacus* 1614 CO has very low sequence quality (Table S2.7) and occupies a relatively long branch. *Sericomyrmex amabilis* 1559 VE is likewise placed on a relatively long branch, outside of the *amabilis*+*saussurei* clade, even though it is morphologically identical to *S. amabilis*. We analyzed two data subsets with these taxa excluded, Ex1 and Ex2 (Table 2.1). ML analyses of data sets with one (*S. mayri* 1555 TT) or all three of these taxa excluded resulted in phylogenies with the same topology as those obtained in most analyses (e.g., the topology in Fig. 2.2), with similarly high support values, indicating that the inclusion of these taxa does not have a negative effect on tree topology.

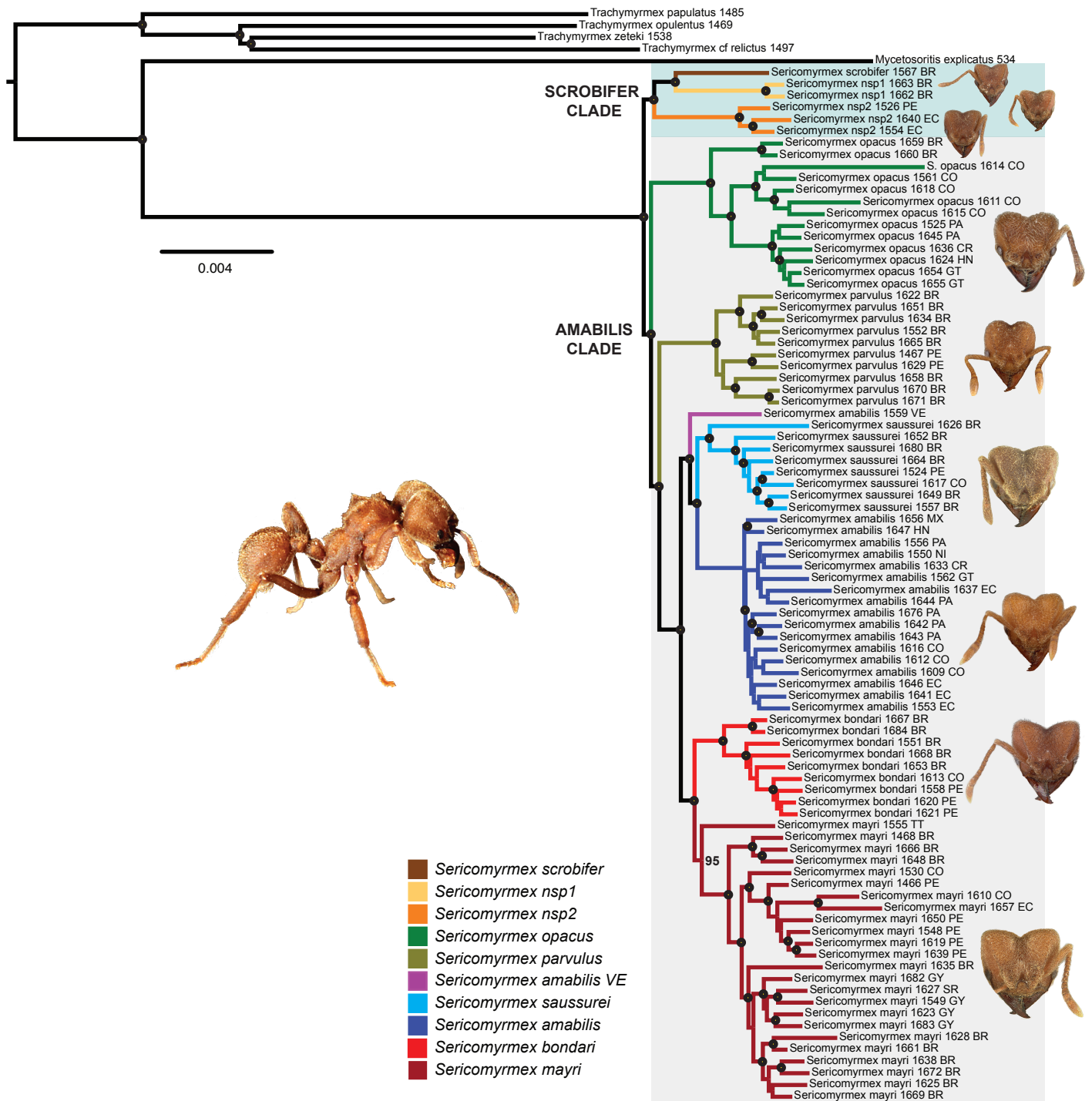


Figure 2.2. UCE phylogeny. The maximum-likelihood phylogeny of the 90% complete concatenated matrix containing 799 UCE loci (702,574 bp). Black circles at nodes indicate 98–100 bootstrap frequencies (N=500). Taxon names include DNA extraction codes and country codes as follows: BR–Brazil, CO–Colombia, CR–Costa Rica, HN–Honduras, EC–Ecuador, GY–Guyana, GF– French Guiana, GT–Guatemala, MX–Mexico, NI–Nicaragua, PA–Panama, PE–Peru, SR–Surinam, TT– Trinidad and Tobago, VE– Venezuela. The two major *Sericomymex* clades are indicated with green (scrobifer clade) and grey (amabilis clade) colored boxes.

2.3.3. Divergence dating

All 10 BEAST v2.4.1 analyses, two for each of four different data sets and two for the partitioned data set, recovered similar divergence dates (Table 2.3, Fig. 2.3). The dates recovered by the partitioned and unpartitioned analyses of the *bs_best* data set were nearly identical (Table 2.3). The *bs_best* and *random1* data sets recovered the same topology as the larger UCE data sets with the full number of taxa and the greater number of loci. The other two random data sets, *random2* and *random3*, recovered topologies in which relationships within the *amabilis* clade were different, and were also different from each other (Figure S2.2). This is not surprising considering the small number of loci used in the BEAST analysis (100 loci versus 799 in 90% complete matrix). We used a reduced number of loci because BEAST analyses are computationally demanding and larger data sets take intractably long periods of time to converge. However, the nodes that we were most interested in dating, the stem and crown nodes of the genus *Sericomyrmex*, the stem and crown nodes of the two main clades, *amabilis* and *scrobifer*, and major internal nodes within the *scrobifer* clade, were dated similarly in BEAST analyses of all the data sets (Table 2.3).

The mean estimates for *Sericomyrmex* stem and crown divergence times are 15.13 (95% HPD: 8.56–21.85) and 4.32 (95% HPD: 2.26–6.86) mya, respectively (Fig. 2.4). These dates, especially the stem-date estimate, are younger than mean estimates in a recent transcriptome and genome data study, which were 22.6 and 4.9 mya (Ješovnik *et al.*, 2016) for the same nodes. This discrepancy can be explained by taxon sampling; including the sister taxon *M. explicatus* in this study breaks the branch connecting *Trachymyrmex* and *Sericomyrmex*. The similarity between the dates in the current study

and those in Branstetter *et al.*, which are just slightly older, 16.18 and 4.71 mya (Branstetter *et al.*, unpublished data), are no doubt partly due to the overlapping UCE data in the two studies and to the use of dates from Branstetter *et al.* as secondary calibration priors in the current study. However, a large number of taxa, both attine ants and outgroups, included in Branstetter *et al.* were not included in this study, and, similarly, most of the *Sericomyrmex* species included in this study were not included in Branstetter *et al.*, which included only five *Sericomyrmex* species. Within the *amabilis* clade, the most recent divergences occur between the sister species *amabilis* and *saussurei* (2.33 mya) and *mayri* and *bondari* (2.37 mya). Within the *scrobifer* clade species divergences are somewhat older: *scrobifer* and *S. n. sp. 1* share a most recent common ancestor at 3.4 mya, and together they share a most recent common ancestor with *S. n. sp. 2* at 4.19 mya.

Node	Average (all 4 data sets)	bs best 15T	bs best 15T partitioned	random 1	random 2	random 3
<i>Sericomyrmex</i> stem	15.13 (8.56– 21.85)	15.63 (8.8– 22.37)	15.63 (8.87– 22.44)	15.41 (8.75– 21.99)	14.73 (8.15– 21.14)	15.29 (8.53– 21.92)
<i>Sericomyrmex</i> crown	4.32 (2.26– 6.86)	4.95 (2.47– 7.54)	4.93 (2.49– 7.54)	3.7 (1.92– 5.5)	4.48 (2.29– 6.9)	4.78 (2.36– 7.49)
<i>S. amabilis</i> + <i>saussurei</i> crown	2.33 (1.01– 3.41)	1.86 (0.87– 2.93)	1.85 (0.88– 2.94)	2.01 (0.99– 3.08)	NA	2.64 (1.16– 4.21)
<i>S. amabilis</i> VE crown	2.77 (1.26– 3.98)	2.15 (1.05– 3.35)	2.14 (1.03– 3.33)	2.46 (1.27– 3.72)	NA	3.1 (1.45– 4.86)
<i>mayri</i> + <i>bondari</i> crown	2.37 (1.04– 3.36)	1.72 (0.81– 2.73)	1.72 (0.81– 2.73)	2.28 (1.16– 3.47)	NA	2.45 (1.15– 3.88)
<i>mayri</i> + <i>bondari</i> stem	3.09 (1.36– 4.46)	2.42 (1.16– 3.7)	2.42 (1.2– 3.75)	2.77 (1.44– 4.16)	NA	3.42 (1.46– 5.5)
<i>parvulus</i> crown	3.35 (1.67– 4.98)	3.21 (1.59– 4.93)	3.2 (1.6–4.94)	3.35 (1.75– 5.023)	NA	NA
<i>opacus</i> crown	3.59 (2– 5.96)	4.29 (2.12– 6.55)	4.26 (2.12– 6.52)	3.59 (1.18– 5.37)	NA	NA
<i>S. nsp</i> 2 crown	4.03 (2.1– 6.45)	4.68 (3.27– 7.2)	4.66 (2.35– 7.18)	3.41 (1.77– 5.1)	4.13 (2.06– 6.35)	4.56 (2.2– 7.11)
<i>scrobifer</i> + <i>nsp</i> 1 crown	3.22 (1.63– 5.26)	3.92 (1.85– 6.06)	3.91 (1.88– 6.1)	2.84 (1.43– 4.31)	3.42 (1.63– 5.34)	3.42 (1.64– 5.33)

Table 2.3. Divergence dates estimated in BEAST analyses of four data sets (2 runs per data set, 1 billion generations per run).

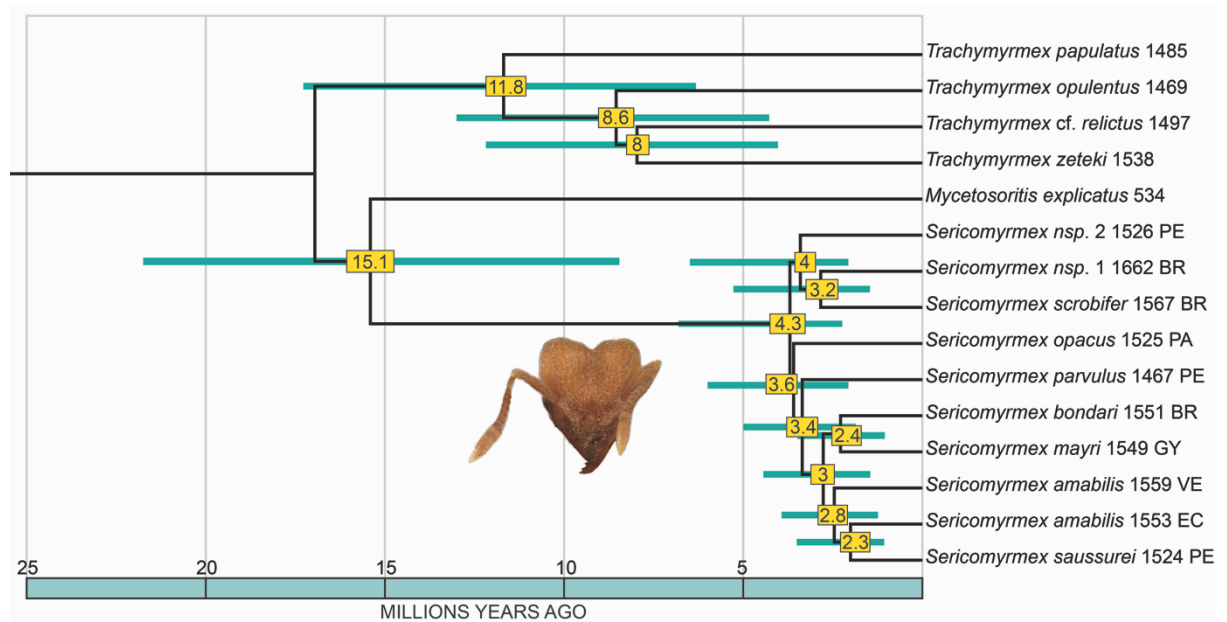


Figure 2.3. Chronogram. The phylogeny resulting from the BEAST analysis of the UCE data, with mean dates indicated in yellow boxes on nodes, calculated from all 8 runs for all 4 data sets (Table 2.3). Green bars on nodes represent 95% posterior densities.

2.3.4. COI ant phylogeny

We generated COI sequence data for 125 *Sericomymex* specimens in order to (i) inform taxon selection for the UCE data set, (ii) provide additional information for a taxonomic revision and for understanding geographic distributions, and (iii) assess the usefulness of COI for species identification. Combined with the sequences downloaded from GenBank, the COI data set consisted of 150 taxa and 885 base pairs of aligned sequence data (Table 2.1). Bayesian and maximum-likelihood analyses recovered similar phylogenies. Unsurprisingly, relationships between species were not congruent with those in the UCE phylogeny, and support values were very low across the entire COI

tree. However, the two phylogenies generally agreed at the species level, i.e., in most cases terminal taxa grouped together into the same species-level clusters in both phylogenies (Figure S2.2). The exceptions are *S. mayri* and *S. bondari*, which were not recovered as monophyletic in the COI-based phylogeny but instead overlapped with each other with regard to some taxa, and *S. opacus*, which was recovered as paraphyletic.

We were particularly interested in the phylogenetic position of *Sericomyrmex* n. sp. 3 VE, a sample from Rio Negro, Venezuela, because of its distinct morphology and because we did not obtain UCEs for this specimen. We had only nine, dry-preserved, museum specimens greater than 30 years old, which, based on morphology, certainly represent a new species. Recent work has demonstrated that acceptable UCE sequences can be obtained from old, pinned, museum specimens (Blaimer *et al.*, 2016b), but, unfortunately, this information was published subsequent to the molecular phase of our study. In the COI phylogeny, *Sericomyrmex* n. sp. 3 is the sole occupant of a very long branch and, depending on the analysis, it groups with different clades, always with very low support (Figure S2.2). The uniqueness of the COI sequence corroborates our morphology-based conclusion that it represents a new species, however, we currently lack the molecular data for confidently estimating the phylogenetic position of *S.* n. sp. 3.

2.3.5. Fungal phylogeny and coevolution

We successfully sequenced one or both fragments of the ITS and LSU ribosomal nuclear gene regions for 32 fungi (28 from fungus garden samples and 4 from ant DNA extracts) associated with six different *Sericomyrmex* ant species and combined them with 58 ITS and 25 LSU sequences downloaded from GenBank. Fungal phylogenies resulting

from analyses of three different data sets (ITS, LSU, ITS+LSU) and two different analytical methods (ML and Bayesian) have similar topologies.

The phylogeny produced by the ITS+LSU and ITS-only data set resulted in higher support values than those based on LSU-only data sets, a pattern observed in analyses of other fungal taxa (Schoch *et al.*, 2012), although LSU seems to perform better in analyses of fungi associated with lower attine ants (Schultz *et al.*, 2015). The poorer performance of the LSU-only data set is likely due to the low levels of divergence in LSU among higher-attine fungi.

The fungal phylogeny indicates that *Sericomyrmex* fungi are higher attine fungi and that they belong to the same clade as all the *Trachymyrmex* fungi included in our analysis, in agreement with previous studies (Chapela *et al.*, 1994; Schultz & Brady, 2008) (Figure 2.4). *Sericomyrmex* cultivars are not monophyletic, but instead are interspersed with fungi cultivated by *Trachymyrmex* species. The most derived *Sericomyrmex* cultivar species, which we call the *amabilis-mayri* fungus, is cultivated by the majority of *Sericomyrmex* species, including most *S. amabilis* from Central America, all of *S. mayri* from across its range, all *S. parvulus*, and some *S. bondari*. This fungus is also grown by three *Trachymyrmex* species included in our phylogeny. A separate, small fungal clade, most closely related to the *amabilis-mayri* fungus, is cultivated by *S. amabilis* in Ecuador and a single *Sericomyrmex* from GenBank from an unknown locality (GU202430).

The three most basally diverging, less inclusive lineages within the *Sericomyrmex*-*Trachymyrmex* fungal clade are fungi cultivated by *S. bondari* and *S. saussurei*, as well as a fungus cultivated by *Trachymyrmex opulentus* (="wheeleri"),

joined by long branches (Figure 2.4) and grown at multiple localities across Brazil and Peru. Both *saussurei* and *bondari* also cultivate the more derived *amabilis-mayri* fungal species, sometimes at the same localities where they cultivate the aforementioned basally diverging fungal lineages. A single fungal cultivar (*Sericomyrmex* n. sp. 2 fun284 PE) collected from a nest of *Sericomyrmex* n. sp. 2 in Peru occupies a long branch that assumes different positions in different analyses, albeit with low support values. Although this fungus is clearly a member of the higher attine fungi, it does not cluster closely with any of the sequences produced for this study or with any sequences from GenBank. This is interesting because it is the only representative of the fungi associated with *scrobifer*-clade ant species, and it prompts the question of whether *scrobifer*-clade ant species may cultivate a clade of fungal species distinct from those cultivated by species in their larger and more diverse sister clade.

Evidence for global coevolution was found to be significant at the 0.05 level, ($p = 0.029$) in a ParaFit analysis of the full ant-fungal association data set (32 ant-fungal associations) (Table S2.5). However, when the data set was reduced to 30 ant-fungal associations, coevolution was not found to be significant at the level of 0.05 ($p = 0.235$). This result is in agreement with a previously observed pattern of “diffuse coevolution” in which higher attine ants are strongly associated with higher-attine fungi (Schultz & Brady, 2008), but in which ant-fungus associations within these groups are labile, i.e., in which fungi are apparently horizontally transmitted across closely related (Green *et al.*, 2002; Mikheyev *et al.*, 2007, 2008) and distantly related ants (Schultz *et al.*, 2015).

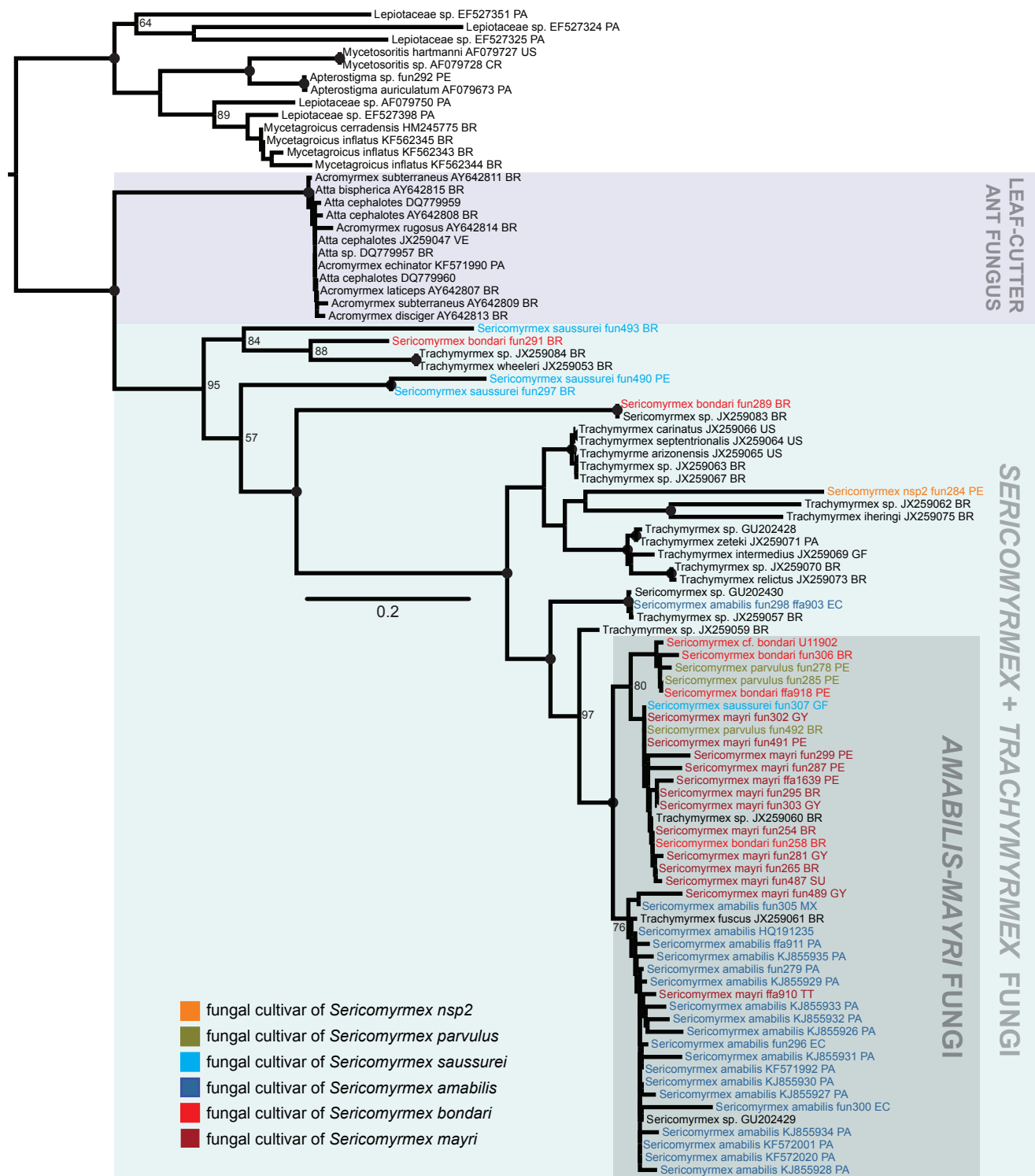


Figure 2.4. Fungal phylogeny. The maximum-likelihood phylogeny of the ITS+LSU data set. Black circles at the nodes indicate 98–100 bootstrap frequencies. Only values of > 50% bootstrap frequency are indicated. Terminal taxa are named by their ant host species or genera except for free-living Lepiotaceae. Sequences generated for this study have the fungal extraction number of the form "fun123" following the taxon name, whereas sequences downloaded from GenBank have the GenBank accession number following the taxon name. All sequences for which the collection locality is known have a two-letter country code following the taxon name as follows: BR–Brazil, CR–Costa Rica, EC–Ecuador, GY–Guyana, GF– French Guiana, MX–Mexico, PA–Panama, PE–Peru, TT– Trinidad and Tobago, US–United States, VE– Venezuela.

2.4. Discussion

2.4.1. Evolution of the genus *Sericomyrmex*: a recent radiation with a high speciation rate

The evolution of the genus *Sericomyrmex* was, until recently, poorly studied. The genus is known for its problematic taxonomy, mostly because species descriptions are old and confusing, the available key (Wheeler, 1916) is outdated and incomplete, and, with regard to morphological variation, most species look alike. Our analyses of the UCE data resulted in a fully resolved and well-supported phylogeny, which we used to define two species-groups within the genus (*scrobifer* and *amabilis*), and which guided our efforts to delimit species. The criteria we use to define species of *Sericomyrmex* are (i) they form well-supported lineages in the UCE phylogeny and (ii) they are morphologically distinct from other putative species (Ješovnik & Schultz, in prep.). The number of species-level lineages in *Sericomyrmex* (11) was found to be lower than previously described (19), even after accounting for the discovery of three new species. It is possible that we failed to detect morphologically indistinguishable, cryptic species, especially given our incomplete knowledge of the full morphological character and character-state space. One possible candidate is *Sericomyrmex amabilis* VE, representing the distinct sister lineage of the *amabilis* + *saussurei* clade. The two available specimens of *S. amabilis* VE are morphologically identical to the specimens assigned to *amabilis*. Because of the small number of specimens, the lack of distinguishing morphological character states, and its allopatric distribution, and because of the biological reality that a species may be paraphyletic with respect to its sister species, especially during the early stages of speciation (Coyne & Orr, 2004), we are, for now recognizing *S. amabilis* VE as an

allopatric population of *amabilis* that renders *amabilis* paraphyletic. The collection of additional specimens may improve our ability to determine whether or not this population is a separate species.

Two species that we recognize, *S. opacus* and *S. mayri*, contain particularly well-supported subspecific clades, which could be alternatively interpreted as distinct species or as genetically distinct populations. Because these clades are morphologically indistinguishable and because their distributions are correlated with geography, we do not recognize them as separate species. It is likely, however, that they represent cases of incipient species. There is little doubt that improved sampling, especially in regions where our sampling was poor (e.g., Venezuela, Bolivia, Ecuador, Amazonian Colombia and Brazil) would result in the discovery of populations or species that would clarify *Sericomyrmex* species boundaries and relationships.

Our results indicate that the current distribution of *Sericomyrmex* is the product of a recent and rapid radiation. *Sericomyrmex* ants inhabit a wide variety of habitats from northern Mexico to southern Brazil, including wet and dry forests, riverbanks, cerrado, and urban and agricultural habitats. Even though the number of species (11) is lower than previously described, given the very short time since the origin of the genus, this is a large number of species and a high rate of speciation. The crown-group age estimate for the entire genus *Sericomyrmex* is recovered as 4.3 mya. For comparison, sister-species pairs in the *Cyphomyrmex wheeleri* group are estimated to be diverged 5.3 to 7.0 mya (Mehdiabadi *et al.*, 2012) and the crown-age estimate for the species pair *Mycetophylax simplex* and *M. conformis* is 6.6 mya (Cardoso *et al.*, 2014). So in the time in which comparable groups gave rise to two species, *Sericomyrmex* gave rise to twelve. Similar to

within-*Sericomyrmex* species divergences (2.3–4 mya) is a crown-age estimate for the lower attine species *Mycocepurus goeldi* and its social parasite *M. castrator* (2.04 mya) (Rabeling *et al.*, 2014). However, in the case of *Mycocepurus* this recent divergence is attributed to its social-parasite biology; social parasites are known to have faster evolutionary rate than non-parasite ant species (Bromham & Leys, 2005; Jansen *et al.*, 2010; Rabeling *et al.*, 2014). The net diversification rate (NDI) of *Sericomyrmex* is 1.8 (0.9–2.8) million years, which is comparable to that of cichlid fishes in Lake Tanganyika (NDI=1.6–2.2 Ma) (Turner *et al.*, 2001). Known examples of higher speciation rates (lower NDI) than that of *Sericomyrmex* are rapid radiations associated with island biogeography (radiation of the genus *Drosophila* in the Pacific ocean: NDI=0.88 My, Kambysellis *et al.*, 1995; and Hawaiian crickets, Mendelson & Shaw, 2005) or with geographic isolation and selection pressures generated by a host symbiont, as in the remarkable ant-nest beetle genus *Paussus* on Madagascar (NDI=0.36) (Moore & Robertson, 2014).

Perhaps more remarkable than the number of species, however, is that the rapid radiation of *Sericomyrmex* into diverse habitats across a large swathe of Central and South America was accompanied by remarkably little molecular and morphological change. As a group, *Sericomyrmex* ants are morphologically distinct, instantly recognizable at the genus level, and quite different from any other ant, whether attine or non-attine. This indicates that a large amount of phenotypic evolution took place initially, possibly generating now-extinct species, on the very long branch separating the stem node from the crown node, i.e., the branch separating *M. explicatus* from *Sericomyrmex* (Figure 2.3), but that, following the origin of the novel *Sericomyrmex* habitus,

morphological evolution slowed down dramatically. One possible explanation for this rapid burst of phenotypic evolution at the genus origin is genome duplication, because known *Sericomyrmex* chromosome numbers and genome sizes are very high in comparison to those in other fungus-growing and non-fungus-growing ants (Murakami *et al.*, 1998; Tsutsui *et al.*, 2008; Cardoso *et al.*, 2014), and a number of cytogenetic studies suggests that chromosome evolution is correlated with genus and species diversification in ants (Lorite & Palomeque, 2010; Cardoso *et al.*, 2014).

Another distinguishing trait of *Sericomyrmex* ants is that they do not nourish and cultivate actinomycete bacteria (*Pseudonocardia*) on their integuments (Fernández-Marín *et al.*, 2009). *Pseudonocardia* bacterial films are associated with all other attine ants, with the notable exception of those in the leaf-cutting genus *Atta*, also the product of a recent burst of phenotypic evolution. Attine-associated actinomycetes secrete narrow-spectrum antibiotics that are thought to be employed by the ants to protect their fungus gardens from infection (Currie *et al.*, 1999, 2003, 2006; Poulsen *et al.*, 2003), among other purposes (Mattoso *et al.*, 2012). The ancestors of both *Atta* and *Sericomyrmex* certainly had such bacterial films (e.g., they are present in *Mycetosoritis explicatus*), so it is possible that the rapid bursts of phenotypic evolution that accompanied the origins of both genera have been correlated in some way with the secondary loss of the integumental microbial symbionts present in all other attine ants. In response to garden infections, both genera compensate for the lack of *Pseudonocardia* by increased use of metapleural gland secretions, which are known to contain wide-spectrum antibiotics (Fernández-Marín *et al.*, 2006, 2009).

2.4.2. Diffuse coevolution with generalized higher attine fungi

Sericomyrmex fungi belong to the clade of higher attine fungi, distinct from lower attine fungi by their polyploidy, their obligate rather than facultative symbiont status (Nygaard *et al.*, 2016), and their consistent production of gongylidia, swollen hyphal tips harvested by the ants for food (but see (Masiulionis *et al.*, 2014), for gongylidia-like structures in a lower attine ants). Our results indicate that *Sericomyrmex*-associated fungi are typical higher attine ant fungi, a clade of multiple fungal species cultivated interchangeably between species of *Sericomyrmex* and *Trachymyrmex*. We find no evidence of tight (i.e., species-to-species) coevolution between *Sericomyrmex* ants and their fungi. Rather, our fungal phylogeny indicates the horizontal exchange of cultivars between *Sericomyrmex* and *Trachymyrmex* ant species (Figures 2.4 & S2.3). However, associations between ants and their fungal cultivars do not appear to be entirely random. Most *Sericomyrmex* and some *Trachymyrmex* species and populations in our study grow a single fungal species, which we refer to as the *amabilis-mayri* fungus species. Based on branch lengths, this species appears to have arisen relatively recently, but it has a large geographic distribution (Mexico to southern Peru and Brazil) and it is grown by all populations of *S. amabilis*, *S. mayri*, and *S. parvulus* species included in our study. As with the leaf-cutting ant fungus species *Leucoagaricus gongylophorus*, which is also recently diverged and widely distributed (Aylward *et al.*, 2013), this pattern prompts the question of whether this particular fungus species is somehow better adapted for life with ants than other higher-attine fungus species, for example whether it is nutritionally superior, and, if so, why it has not replaced all the others. We know that nutritionally superior attine fungi promote, at least in the short term, the evolution of larger,

ecologically dominant colonies (Mueller *et al.*, 2008, 2011; Nygaard *et al.*, 2016; Shik *et al.*, 2016) (with *Apterostigma megacephala* as a notable exception, Schultz *et al.*, 2015). In the long term, however, poorly understood ant-fungus incompatibilities can reduce the fitness of some ant-fungus species pairs (Seal & Mueller, 2014), possibly explaining why some fungi may replace resident fungi and spread to dominance across some species, but not others. Along these lines, it would be interesting to test whether the species of *Sericomyrmex* and *Trachymyrmex* that are most ecologically successful are consistently associated with the same, presumably more productive, fungal species.

2.4.3. UCE loci as a tool for species delimitation

Our results indicate that UCEs encompass variation sufficient for capturing recent species- and population-level divergences in *Sericomyrmex*. We conclude that UCEs represent an efficient choice for multi-level questions in insect phylogenomics, including species delimitation in recent and rapid radiations. A distinct advantage of UCEs as phylogenomic markers is the wide range of variation they encompass, ranging from the slowly evolving sites adjacent to the invariant core to increasingly variable sites in the flanking regions. This range of variability facilitates the incorporation of data across multiple phylogenetic studies carried out at multiple phylogenetic levels, obviating the need to resequence taxa for different markers.

The UCE-based phylogeny recovered all *Sericomyrmex* species with strong support. In comparison, the COI phylogeny recovered eight of those species, but failed to recover *S. bondari*, *S. mayri*, and *S. opacus*. The obvious advantage of COI compared to phylogenomic markers is its affordability, but the disadvantage is that, judging by its

success rate in *Sericomyrmex*, it is inaccurate a significant proportion of the time, likely due to incomplete lineage sorting, hybridization, and other known problems (Brower, 2006; Rubinoff *et al.*, 2006). For large-scale projects with many specimens that are not concerned with correctly recovering supraspecific relationships, COI may be useful, but our results indicate that it should be used with caution and only in combination with other lines of evidence, such as morphology and geography. As a species identification ("DNA barcoding") tool, however, our results indicate that COI should perform well. COI-based species identifications referencing our archived COI sequence data (KY202274–KY202398), with the exception of *S. n. sp. 3*, are expected to be accurate (Figure S2.2).

Data availability

Quality-trimmed sequence reads generated in this study are available from the NCBI Sequence Read Archive under the Bioproject ID PRJNA354064, sample numbers SAMN0604441-SAMN0604533 (Table S1) . Single gene sequences are available on GenBank under accession numbers KY173361-KY173382 (ITS), KY176772-KY176799 (LSU), KY202274-KY202398 (COI)

2.5. Supporting information

Genus	Species	DNA extraction number UCE	DNA extraction number COI	Voucher specimen number	Country
<i>Sericomyrmex</i>	<i>amabilis</i>	1550	950	USNMENT01125190	Nicaragua
<i>Sericomyrmex</i>	<i>amabilis</i>	1553	902	USNMENT00419919	Ecuador
<i>Sericomyrmex</i>	<i>amabilis</i>	1556	911	USNMENT00307446	Panama
<i>Sericomyrmex</i>	<i>amabilis</i>	1559	941	USNMENT00924110	Venezuela
<i>Sericomyrmex</i>	<i>amabilis</i>	1562	1562	USNMENT01125184	Guatemala
<i>Sericomyrmex</i>	<i>amabilis</i>	1609	1609	USNMENT01125196	Colombia
<i>Sericomyrmex</i>	<i>amabilis</i>	1612	1612	USNMENT01125194	Colombia
<i>Sericomyrmex</i>	<i>amabilis</i>	1616	1616	USNMENT01125193	Colombia
<i>Sericomyrmex</i>	<i>amabilis</i>	1633	912	USNMENT00305937	Costa Rica
<i>Sericomyrmex</i>	<i>amabilis</i>	1637	925	USNMENT00419901	Ecuador
<i>Sericomyrmex</i>	<i>amabilis</i>	1641	904	USNMENT00419900	Ecuador
<i>Sericomyrmex</i>	<i>amabilis</i>	1642	1642	USNMENT01125177	Panama
<i>Sericomyrmex</i>	<i>amabilis</i>	1643	1643	USNMENT01125179	Panama
<i>Sericomyrmex</i>	<i>amabilis</i>	1644	1644	USNMENT01125176	Panama
<i>Sericomyrmex</i>	<i>amabilis</i>	1646	940	USNMENT01125181	Ecuador
<i>Sericomyrmex</i>	<i>amabilis</i>	1647	913	USNMENT01125189	Honduras
<i>Sericomyrmex</i>	<i>amabilis</i>	1656	1656	USNMENT01125192	Mexico
<i>Sericomyrmex</i>	<i>amabilis</i>	1676	/	USNMENT01125178	Panama
<i>Sericomyrmex</i>	<i>amabilis</i>		956	USNMENT01125182	Honduras
<i>Sericomyrmex</i>	<i>amabilis</i>		929	USNMENT01125180	Costa Rica
<i>Sericomyrmex</i>	<i>amabilis</i>		958	USNMENT01125186	Guatemala
<i>Sericomyrmex</i>	<i>amabilis</i>		954	USNMENT01125185	Guatemala
<i>Sericomyrmex</i>	<i>amabilis</i>		942	USNMENT01125183	Guatemala
<i>Sericomyrmex</i>	<i>amabilis</i>		962	USNMENT01125188	Guatemala
<i>Sericomyrmex</i>	<i>amabilis</i>		930	USNMENT01125187	Guatemala
<i>Sericomyrmex</i>	<i>amabilis</i>		901	USNMENT00419913	Ecuador
<i>Sericomyrmex</i>	<i>amabilis</i>		903	USNMENT00419883	Ecuador
<i>Sericomyrmex</i>	<i>amabilis</i>		955	USNMENT01125195	Colombia
<i>Sericomyrmex</i>	<i>amabilis</i>		977	USNMENT01125191	Nicaragua
<i>Sericomyrmex</i>	<i>bondari</i>	1551	967	USNMENT01125209	Brazil
<i>Sericomyrmex</i>	<i>bondari</i>	1558	918	USNMENT01125197	Peru
<i>Sericomyrmex</i>	<i>bondari</i>	1613	1613	USNMENT01125203	Colombia
<i>Sericomyrmex</i>	<i>bondari</i>	1620	920	USNMENT01125199	Peru
<i>Sericomyrmex</i>	<i>bondari</i>	1621	919	USNMENT01125198	Peru
<i>Sericomyrmex</i>	<i>bondari</i>	1653	1653	USNMENT01125205	Brazil
<i>Sericomyrmex</i>	<i>bondari</i>	1667	1667	USNMENT01125206	Brazil
<i>Sericomyrmex</i>	<i>bondari</i>	1668	1668	USNMENT01125207	Brazil
<i>Sericomyrmex</i>	<i>bondari</i>	1684	1684	USNMENT01125208	Brazil
<i>Sericomyrmex</i>	<i>bondari</i>		1782	USNMENT01125202	Suriname
<i>Sericomyrmex</i>	<i>bondari</i>		926, 1675	USNMENT01124297	Suriname
<i>Sericomyrmex</i>	<i>bondari</i>		927	USNMENT01125201	Suriname
<i>Sericomyrmex</i>	<i>bondari</i>		1797	USNMENT01125210	Brazil
<i>Sericomyrmex</i>	<i>bondari</i>		1799	USNMENT01125204	Colombia
<i>Sericomyrmex</i>	<i>bondari</i>		961	USNMENT01125243	Peru
<i>Sericomyrmex</i>	<i>bondari</i>		1679	USNMENT01125200	Peru
<i>Sericomyrmex</i>	<i>bondari</i>		1716	USNMENT01125242	Peru
<i>Mycetosoritis</i>	<i>explicata</i>	534	/	USNMENT01125245	Paraguay
<i>Sericomyrmex</i>	<i>mayri</i>	1466	1466	USNMENT01125146	Peru
<i>Sericomyrmex</i>	<i>mayri</i>	1468	969	USNMENT01125170	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1530	957	USNMENT01125151	Colombia
<i>Sericomyrmex</i>	<i>mayri</i>	1548	939	USNMENT01125147	Peru
<i>Sericomyrmex</i>	<i>mayri</i>	1549	944	USNMENT01125169	Guyana
<i>Sericomyrmex</i>	<i>mayri</i>	1555	910	USNMENT00443135	Trinidad
<i>Sericomyrmex</i>	<i>mayri</i>	1610	1610	USNMENT01125237	Colombia
<i>Sericomyrmex</i>	<i>mayri</i>	1619	923	USNMENT01125145	Peru
<i>Sericomyrmex</i>	<i>mayri</i>	1623	936	USNMENT00535745	Guyana
<i>Sericomyrmex</i>	<i>mayri</i>	1625	914	USNMENT01125160	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1627	952	USNMENT01125164	Suriname

<i>Sericomyrmex</i>	<i>mayri</i>	1628	975	USNMENT01125162	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1635	909	USNMENT00444061	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1638	1638	USNMENT01125159	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1639	1639	USNMENT01125236	Peru
<i>Sericomyrmex</i>	<i>mayri</i>	1648	917	USNMENT01125171	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1650	939	USNMENT01125148	Peru
<i>Sericomyrmex</i>	<i>mayri</i>	1657	/	USNMENT01125150	Ecuador
<i>Sericomyrmex</i>	<i>mayri</i>	1661	1661	USNMENT01125158	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1666	1666	USNMENT01125172	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1669	1669	USNMENT01125157	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1672	1672	USNMENT01125155	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>	1682	1682	USNMENT01125167	Guyana
<i>Sericomyrmex</i>	<i>mayri</i>	1683	1683	USNMENT01125168	Guyana
<i>Sericomyrmex</i>	<i>mayri</i>		1677, 1786	USNMENT01125165	Suriname
<i>Sericomyrmex</i>	<i>mayri</i>		1785	USNMENT01125166	Suriname
<i>Sericomyrmex</i>	<i>mayri</i>		1789	USNMENT01125239	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		933	USNMENT01125152	Colombia
<i>Sericomyrmex</i>	<i>mayri</i>		1784	USNMENT00538352	Guyana
<i>Sericomyrmex</i>	<i>mayri</i>		932	USNMENT01125238	French Guiana
<i>Sericomyrmex</i>	<i>mayri</i>		928	USNMENT00445613	Suriname
<i>Sericomyrmex</i>	<i>mayri</i>		946	USNMENT01125174	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		947	USNMENT01125161	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		949	USNMENT01125175	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		934	USNMENT01125153	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		965	USNMENT01125154	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		963	USNMENT01125163	Brazil
<i>Sericomyrmex</i>	<i>mayri</i>		1678, 1740	USNMENT01125149	Peru
<i>Sericomyrmex</i>	<i>mayri</i>		1748	USNMENT01125235	Peru
<i>Sericomyrmex</i>	<i>mayri</i>		1783	USNMENT00419810	Ecuador
<i>Sericomyrmex</i>	<i>mayri</i>		945	USNMENT01125173	Brazil
<i>Sericomyrmex</i>	n. sp. 1	1662	1662	USNMENT00924095	Brazil
<i>Sericomyrmex</i>	n. sp. 1	1663	1663	USNMENT01125225	Brazil
<i>Sericomyrmex</i>	n. sp. 1		1793	USNMENT01125234	Brazil
<i>Sericomyrmex</i>	n. sp. 2	1526	938	USNMENT00924080	Peru
<i>Sericomyrmex</i>	n. sp. 2	1554	905	USNMENT00419538	Ecuador
<i>Sericomyrmex</i>	n. sp. 2	1640	906	USNMENT00419555	Ecuador
<i>Sericomyrmex</i>	n. sp. 2		1794	USNMENT01125117	Colombia
<i>Sericomyrmex</i>	n. sp. 3		1798	USNMENT00924063	Venezuela
<i>Sericomyrmex</i>	<i>opacus</i>	1525	931	USNMENT01125118	Panama
<i>Sericomyrmex</i>	<i>opacus</i>	1561	/	USNMENT01125127	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>	1611	1611	USNMENT01125123	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>	1614	1614	USNMENT01125124	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>	1615	1615	USNMENT01125125	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>	1618	1618	USNMENT01125126	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>	1624	943	USNMENT01125122	Honduras
<i>Sericomyrmex</i>	<i>opacus</i>	1636	1636	USNMENT00443109	Costa Rica
<i>Sericomyrmex</i>	<i>opacus</i>	1645	1645	USNMENT01125119	Panama
<i>Sericomyrmex</i>	<i>opacus</i>	1654	1654	USNMENT01125120	Guatemala
<i>Sericomyrmex</i>	<i>opacus</i>	1655	1655	USNMENT01125121	Guatemala
<i>Sericomyrmex</i>	<i>opacus</i>	1659	1659	USNMENT01125128	Brazil
<i>Sericomyrmex</i>	<i>opacus</i>	1660	1660	USNMENT01125129	Brazil
<i>Sericomyrmex</i>	<i>opacus</i>		1796	USNMENT01125131	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>		1804	USNMENT01125130	Colombia
<i>Sericomyrmex</i>	<i>opacus</i>		1801	USNMENT01125132	Guadeloupe
<i>Trachymyrmex</i>	<i>opulentus</i>	1469	/	USNMENT00443295	Panama
<i>Trachymyrmex</i>	<i>papulatus</i>	1485	/	USNMENT00444749	Argentina
<i>Sericomyrmex</i>	<i>parvulus</i>	1467	951	USNMENT01125138	Peru
<i>Sericomyrmex</i>	<i>parvulus</i>	1552	968	USNMENT01125137	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>	1622	937	USNMENT00755930	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>	1629	1629	USNMENT01125140	Peru
<i>Sericomyrmex</i>	<i>parvulus</i>	1634	935	USNMENT00756784	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>	1651	1651	USNMENT00756697	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>	1658	1658	USNMENT01125134	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>	1665	1665	USNMENT01125139	Brazil

<i>Sericomyrmex</i>	<i>parvulus</i>	1670	1670	USNMENT01125135	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>	1671	1671	USNMENT01125136	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>		976	USNMENT01125143	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>		948	USNMENT01125141	Brazil
<i>Sericomyrmex</i>	<i>parvulus</i>		924	USNMENT01124296	Suriname
<i>Sericomyrmex</i>	<i>parvulus</i>		1803	USNMENT01125133	Colombia
<i>Sericomyrmex</i>	<i>parvulus</i>		970	USNMENT01125142	Colombia
<i>Sericomyrmex</i>	<i>parvulus</i>		1791	USNMENT01125144	Brazil
<i>Trachymyrmex</i>	<i>relictus</i>	1497	/	USNMENT01125244	Peru
<i>Sericomyrmex</i>	<i>saussurei</i>	1524	921	USNMENT01125215	Peru
<i>Sericomyrmex</i>	<i>saussurei</i>	1557	916	USNMENT01125216	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>	1617	1617	USNMENT01125213	Colombia
<i>Sericomyrmex</i>	<i>saussurei</i>	1626	974	USNMENT01125224	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>	1649	922	USNMENT01125217	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>	1652	1652	USNMENT01125211	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>	1664	1664	USNMENT01125214	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>	1680	1680	USNMENT01125212	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>		1787	USNMENT01125218	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>		971	USNMENT01125222	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>		960	USNMENT00446292	Suriname
<i>Sericomyrmex</i>	<i>saussurei</i>		1795	USNMENT01125219	Colombia
<i>Sericomyrmex</i>	<i>saussurei</i>		1724	USNMENT01125241	Peru
<i>Sericomyrmex</i>	<i>saussurei</i>		1732	USNMENT01125240	Peru
<i>Sericomyrmex</i>	<i>saussurei</i>		1792	USNMENT01125221	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>		1790	USNMENT01125220	Brazil
<i>Sericomyrmex</i>	<i>saussurei</i>		1781	USNMENT01125223	French Guiana
<i>Sericomyrmex</i>	<i>scrobifer</i>	1567	915	USNMENT01125115	Brazil
<i>Sericomyrmex</i>	<i>scrobifer</i>		1802	USNMENT01125116	Brazil
<i>Trachymyrmex</i>	<i>zeteki</i>	1538	/	USNMENT01125246	Panama

Table S2.1. List of ant specimens. Collecting information, extraction codes, and voucher specimen numbers.

Fungus	Ant host: genus	Ant host: species	Fungal extraction number	Fungus from ant extraction number	Collection code	Country
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>amabilis</i>	/	ffa911	TRS960426-11	Panama
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>amabilis</i>	fun279	/	JB130	Panama
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>amabilis</i>	fun305	/	AJ131005-01	Mexico
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>amabilis</i>	fun296	/	AGH030627-10	Ecuador
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>amabilis</i>	fun298	ffa903	AGH030626-02	Ecuador
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>amabilis</i>	fun300	/	AGH030627-11	Ecuador
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>bondari</i>	/	ffa918	TRS041004-02	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>bondari</i>	fun258	/	AJ141001-05	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>bondari</i>	fun289	/	AJ120926-02	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>bondari</i>	fun291	/	TRS141003-03	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>bondari</i>	fun306	/	TRS041008-02	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	/	ffa1639	JSC051123-14	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	/	ffa910	UGM950108-03	Trinidad
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun254	/	AJ141002-01	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun265	/	AJ141004-01	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun302	/	AJ111125-08	Guyana
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun281	/	JSC111119-04	Guyana
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun303	ffa944	JSC111122-13	Guyana
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun491	/	AJ120801-03	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun287	/	AJ120726-03	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun295	/	TRS920809-15	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun299	/	JSC051123-07	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun487	/	JSC060311-07	Suriname
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>mayri</i>	fun489	/	TRS960405-13	Guyana
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	n. sp. 2	fun284	/	AJ120729-03	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>parvulus</i>	fun278	/	AJ120728-10	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>parvulus</i>	fun285	/	AJ120803-03	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>parvulus</i>	fun492	/	JCMC#001	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>saussurei</i>	fun297	/	TRS081007-05	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>saussurei</i>	fun490	/	JSC051122-06	Peru
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>saussurei</i>	fun493	/	JCMC#002	Brazil
<i>Leucocoprinus sp.</i>	<i>Sericomyrmex</i>	<i>saussurei</i>	fun307	/	MF141218	Fr. Guiana

Table S2.2. List of fungal specimens. Collecting information and extraction codes.

Primer name	Primer sequence (5'-->3')	Organism
5.8SR	TCGATGAAGAACGCAGCG	fungus
LROR-1	ACC CGC GAA CTT AAG C	fungus
LR7	TACTACCACCAAGATCT	fungus
LR16	TTCCACCCAAACACTCG	fungus
LR3R	GTCTTGAAACACGGACC	fungus
LR3-1	CCGTGTTTCAAGACGGG	fungus
BMB-BR	CTTAAAGGAATTGACGGAA	fungus
LR1	GGTTGGTTTCTTTTCCT	fungus
ITS1	TCCGTAGGTGAACCTGCGG	fungus
ITS2	GCTGCGTTCTTCATCGATGC	fungus
ITS3	GCATCGATGAAGAACGCAGC	fungus
ITS4	TCCTCCGCTTATTGATATGC	fungus
ITS5	GGAAGTAAAAGTCGTAACAAGG	fungus
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	fungus
CI13	ATAATTTTTTTTATAGTTATACC	ants
CI14	GTTTCTTTTTTCTCTTTC	ants
Jerry	CAACATTTATTTGATTTTGG	ants
Ben	GCWACWACRTAATAKGTATCATG	ants

PCR name	Protocol	Organism
PCR 50	95°C 3 min, 35x [94°C 45sec, 50°C 45sec, 72°C 1min], 72°C 5 min	fungus
PCR 55	95°C 3 min, 35x [94°C 45sec, 55°C 45sec, 72°C 1min], 72°C 5 min	fungus
PCR 50	94°C 2 min, 34x [94°C 1min, 50°C 1min, 72°C 1min], 72°C 5 min	ants
PCR 48	94°C 2 min, 34x [94°C 1min, 48°C 1min, 72°C 1min], 72°C 5 min	ants

Table S2.3. Primers and PCR protocols used for Sanger sequencing of ant COI and fungal ITS and LSU.

Node	Branstetter <i>et al.</i>	Calibration: prior distribution	Calibration: mean	Calibration: sigma
<i>M. explicatus</i> + all <i>Sericomyrmex</i> (<i>Sericomyrmex</i> stem)	16.18 (10.44-21.79)	Normal	16.18	4
<i>Trachymyrmex papulatus</i> + all other <i>Trachymyrmex</i> (<i>T. iheringi</i> group crown)	12.29 (7.29-18.67)	Normal	12.29	4

Table S2.4. Calibrations for ant divergence dating in BEAST.

Fungi (ant host name and fungal dna extraction number)	Ant host species	Run 1 (32 taxa)	Run 2 (30 taxa)
		p (individual host-parasite links)	p (individual host-parasite links)
Sericomyrmex nsp2 fun284 PE	<i>Sericomyrmex nsp. 2</i>	0.048	0.082
Sericomyrmex parvulus fun492 BR	<i>Sericomyrmex parvulus</i>	0.085	0.074
Sericomyrmex parvulus fun278 PE	<i>Sericomyrmex parvulus</i>	0.011	0.076
Sericomyrmex parvulus fun285 PE	<i>Sericomyrmex parvulus</i>	0.022	0.043
Sericomyrmex amabilis fun296 EC	<i>Sericomyrmex amabilis</i>	0.009	0.024
Sericomyrmex amabilis fun300 EC	<i>Sericomyrmex amabilis</i>	0.005	0.027
Sericomyrmex amabilis fun298 EC	<i>Sericomyrmex amabilis</i>	0.011	0.2
Sericomyrmex amabilis fun279 PA	<i>Sericomyrmex amabilis</i>	0.008	0.019
Sericomyrmex amabilis ffa911 PA	<i>Sericomyrmex amabilis</i>	0.01	0.013
Sericomyrmex amabilis fun305 MX	<i>Sericomyrmex amabilis</i>	0.011	0.163
Sericomyrmex saussurei fun307 GF	<i>Sericomyrmex saussurei</i>	0.214	NA
Sericomyrmex saussurei fun297 BR	<i>Sericomyrmex saussurei</i>	0.038	0.2
Sericomyrmex saussurei fun490 PE	<i>Sericomyrmex saussurei</i>	0.063	0.1
Sericomyrmex saussurei fun493 BR	<i>Sericomyrmex saussurei</i>	0.056	0.689
Sericomyrmex mayri fun299 PE	<i>Sericomyrmex mayri</i>	0.942	0.726
Sericomyrmex mayri ffa1639 PE	<i>Sericomyrmex mayri</i>	0.092	0.321
Sericomyrmex mayri fun287 PE	<i>Sericomyrmex mayri</i>	0.94	0.772
Sericomyrmex mayri fun491 PE	<i>Sericomyrmex mayri</i>	0.948	0.644
Sericomyrmex mayri fun265 BR	<i>Sericomyrmex mayri</i>	0.066	0.446
Sericomyrmex mayri fun254 BR	<i>Sericomyrmex mayri</i>	0.112	0.267
Sericomyrmex mayri fun295 BR	<i>Sericomyrmex mayri</i>	0.026	0.124
Sericomyrmex mayri fun489 GY	<i>Sericomyrmex mayri</i>	0.98	NA
Sericomyrmex mayri fun302 GY	<i>Sericomyrmex mayri</i>	0.964	0.95
Sericomyrmex mayri fun487 SU	<i>Sericomyrmex mayri</i>	0.99	0.947
Sericomyrmex mayri fun303 GY	<i>Sericomyrmex mayri</i>	0.221	0.429
Sericomyrmex mayri fun281 GY	<i>Sericomyrmex mayri</i>	0.091	0.243
Sericomyrmex mayri ffa910 TT	<i>Sericomyrmex mayri</i>	0.53	0.971
Sericomyrmex bondari fun258 BR	<i>Sericomyrmex bondari</i>	0.672	0.27
Sericomyrmex bondari ffa918 PE	<i>Sericomyrmex bondari</i>	0.327	0.964
Sericomyrmex bondari fun306 BR	<i>Sericomyrmex bondari</i>	0.576	0.961
Sericomyrmex bondari fun289 BR	<i>Sericomyrmex bondari</i>	0.584	934
Sericomyrmex bondari fun291 BR	<i>Sericomyrmex bondari</i>	0.546	0.04
p (ParaFit global coevolution)		0.029	0.235

Table S2.5. Ant-fungus association with global and individual ParaFit results, per taxon, for both ParaFit runs. Gray-highlighted cells indicate a significant result at $p < 0.05$.

Text S1. Single-gene analyses details.

CO1 data set (ants) matrix: 150 taxa, 885 sites

Partition Finder (v1.1.1) for raxml

Settings used:

models : raxml

model_selection : aic

search : all

Partition Finder (v1.1.1) for mr bayes

Settings used:

models : mrbayes

model_selection : aic

search : all

Best partitioning scheme:

Subset	Best Model	Subset Partitions	Subset Sites
1	GTR+I+G	CO1_pos1	1-883\3
2	GTR+I+G	CO1_pos2	2-884\3
3	GTR+I+G	CO1_pos3	3-885\3

Best partitioning scheme:

Subset	Best Model	Subset Partitions	Subset Sites
1	GTR+I+G	CO1_pos1	1-883\3
2	HKY+I+G	CO1_pos2	2-884\3
3	GTR+I+G	CO1_pos3	3-885\3

RAxML (v8.2.8) analysis

Alignment has 544 distinct alignment patterns. Proportion of gaps and completely undetermined characters in this alignment: 18.03%.

RAxML was called as follows: raxmlHPC -m GTRGAMMA -f a -p 100298 -x 12345 -s

SERICO_150T_CO1.phy -q col_partitions.txt -N 1000 -n serico_150T_CO1

Mr Bayes (v3.2.6) analysis

mcmc = 50 million generations, 2 runs, each with 4 chains, 3 hot and one cold chain (default settings)

After 50 million generations: Average standard deviation of split frequencies: 0.009599.

PSRF = Potential Scale Reduction Factor was 1.0 for all parameters.

ESS off all paramaters > 200 (run1.p and run2.p files examined in Tracer v1.6.0).

Tree files summarizzed and the consensus tree created using the command sumt with the default burn-in of 0.25%.

ITS (fungus) matrix: 81 taxa, 953 sites

Partition Finder (v2) for raxml

models : raxml

model_selection : aicc

search : kmeans

Subset | Best Model | # sites

1	GTR	76
2	GTR	145
3	GTR+I+G	339
4	GTR+G	124
5	GTR+G	269

Partition Finder (v2) for mr bayes

models : mrbayes

model_selection : aicc

search : kmeans

Subset | Best Model | # sites

1	K80	76
2	SYM	75
3	F81+I	339
4	K80	70
5	HKY	77
6	GTR+G	269
7	K80+G	47

RAxML (v8.2.8) analysis

Alignment has 792 distinct alignment patterns. Proportion of gaps and completely undetermined characters in this alignment: 35.63%.

RAxML was called as follows: raxmlHPC -m GTRGAMMA -f a -p 100298 -x 12345 -s ITS_R1r_81T.phy -q its_partitions.txt -N 1000 -n ITS_part

Mr Bayes (v3.2.6) analysis

mcmc=50 mil generations, 2 runs, each with 4 chains, 3 hot and one cold chain (default settings)

After 50 mil generations: Average standard deviation of split frequencies: 0.001566.

ESS off all paramaters > 200 (run1.p and run2.p files examined in Tracer v1.6.0). Tree files summarizzed and the consensus tree created using the command sumt with the default burn-in of 0.25%.

ITS+LSU (fungus) matrix: 93 taxa, 2329 sites

Partition Finder (v1.1.1) for raxml

models : raxml
model_selection : aicc
search : kmeans

Best partitioning scheme

Subset	Best Model	# sites
1	GTR	448
2	GTR+I+G	1220
3	GTR	130
4	GTR+G	150
5	GTR+G	327
6	GTR	54

Partition Finder (v1.1.1) for mr bayes

models : mrbayes
model_selection : aicc
search : kmeans

Best partitioning scheme

Subset	Best Model	# sites
1	TVMEF	254
2	F81+I	1549
3	K80	55
4	TRN+G	144
5	TVM+G	327

RAxML (v8.2.8) analysis

Alignment has 1079 distinct alignment patterns. Proportion of gaps and completely undetermined characters in this alignment: 65.11%.

RAxML was called as follows: raxmlHPC -m GTRCAT -f a -p 100298 -V -x 12345 -s

ITS_LSU_FINAL.phy -q its_lsu_partitions.txt -N 1000 -n ITS_LSU_part_cat

Mr Bayes (v3.2.6) analysis

mcmc=50 milion, 2 runs, each with 4 chains, 3 hot and one cold chain (default settings)

After 50 milion generations: average standard deviation of split frequencies: 0.004659.

ESS off all paramaters > 200 (run1.p and run2.p files examined in Tracer v1.6.0). Tree files summarizzed and the consensus tree created using the command sumt with the default burn-in of 0.25%.

LSU (fungus) matrix: 54 taxa, 1421 sites

Partition Finder (v1.1.1) for raxml

models : raxml
model_selection : aicc
search : kmeans

Best partitioning scheme:

Subset	Best Model	# sites
1	GTR	1187
2	GTR	130
3	GTR+G	104

Partition Finder (v1.1.1) for mr bayes

models : mrbayes
model_selection : aicc
search : kmeans

Best partitioning scheme:

Subset	Best Model	# sites
1	F81+I	1187
2	K80	130
3	HKY+G	104

RAxML (v8.2.8) analysis

Alignment has 351 distinct alignment patterns. Proportion of gaps and completely undetermined characters in this alignment: 45.93%.

RAxML was called as follows: raxmlHPC -m GTRCAT -f a -p 100298 -V -x 12345 -s LSU_R1r.phy -q

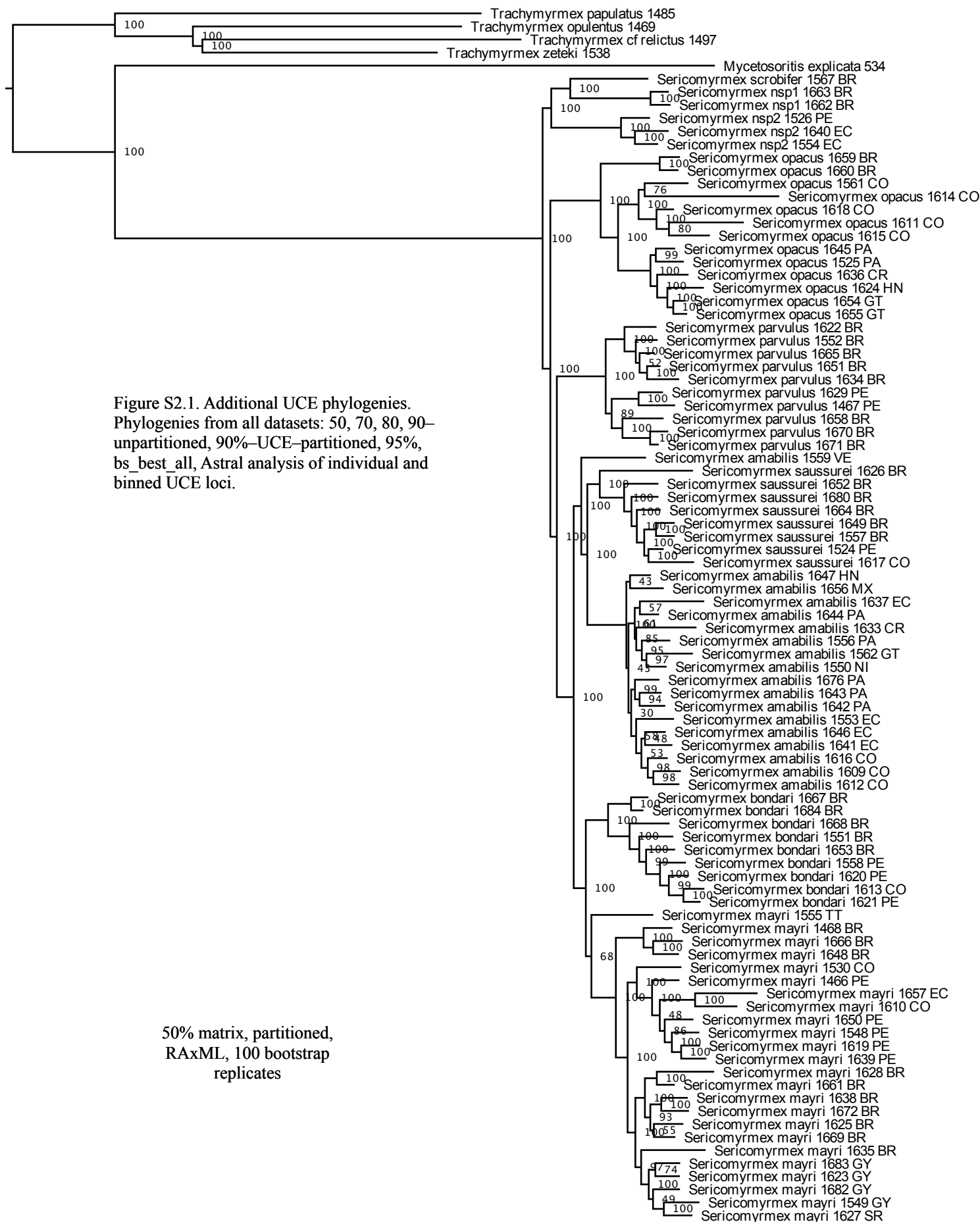
lsu_partitions.txt -N 1000 -n LSU_R1r_part_cat

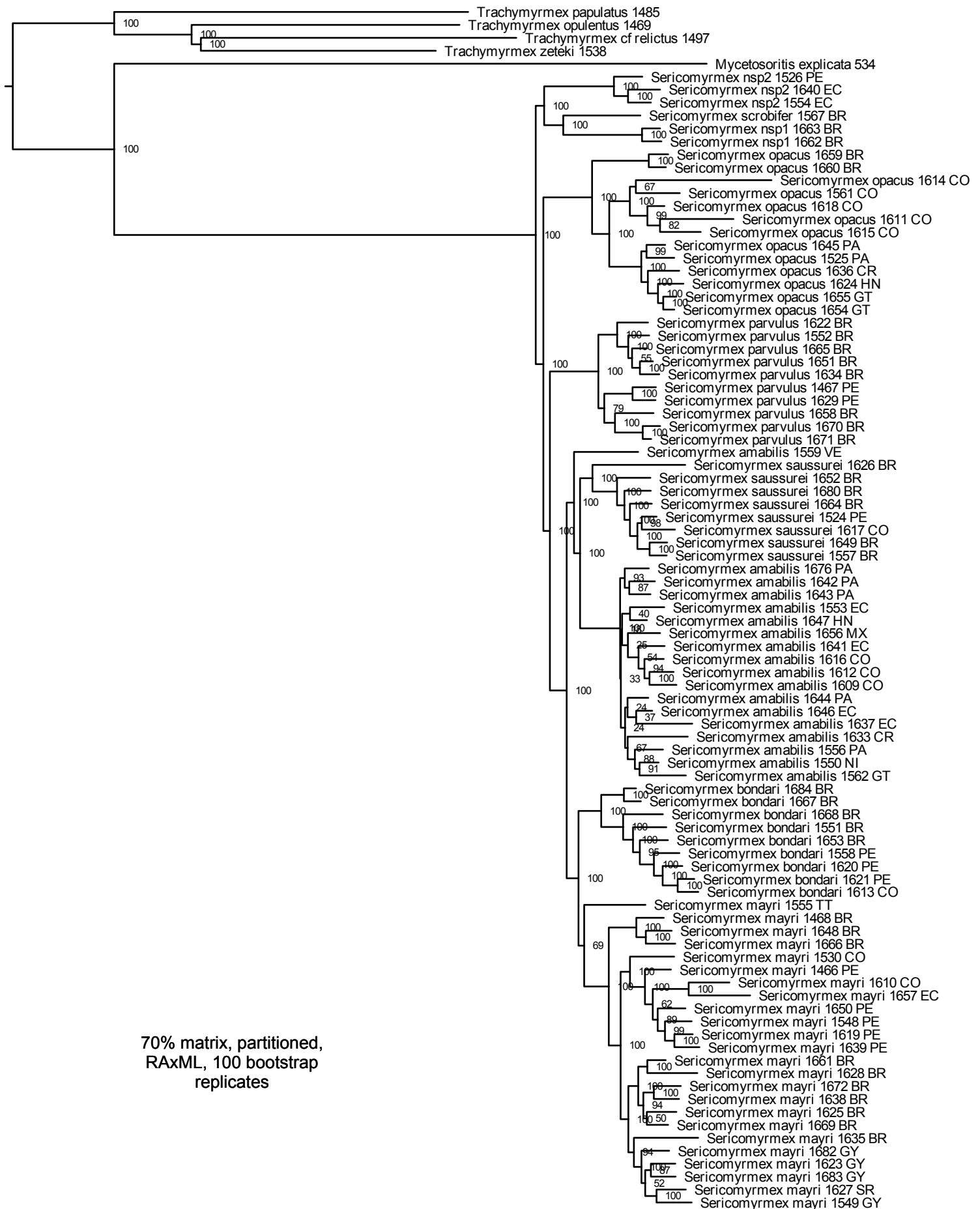
Mr Bayes (v3.2.6) analysis

mcmc= 30 milion, 2 runs, each with 4 chains, 3 hot and one cold chain (default settings)

After 30 milion generations: average standard deviation of split frequencies: 0.003566.

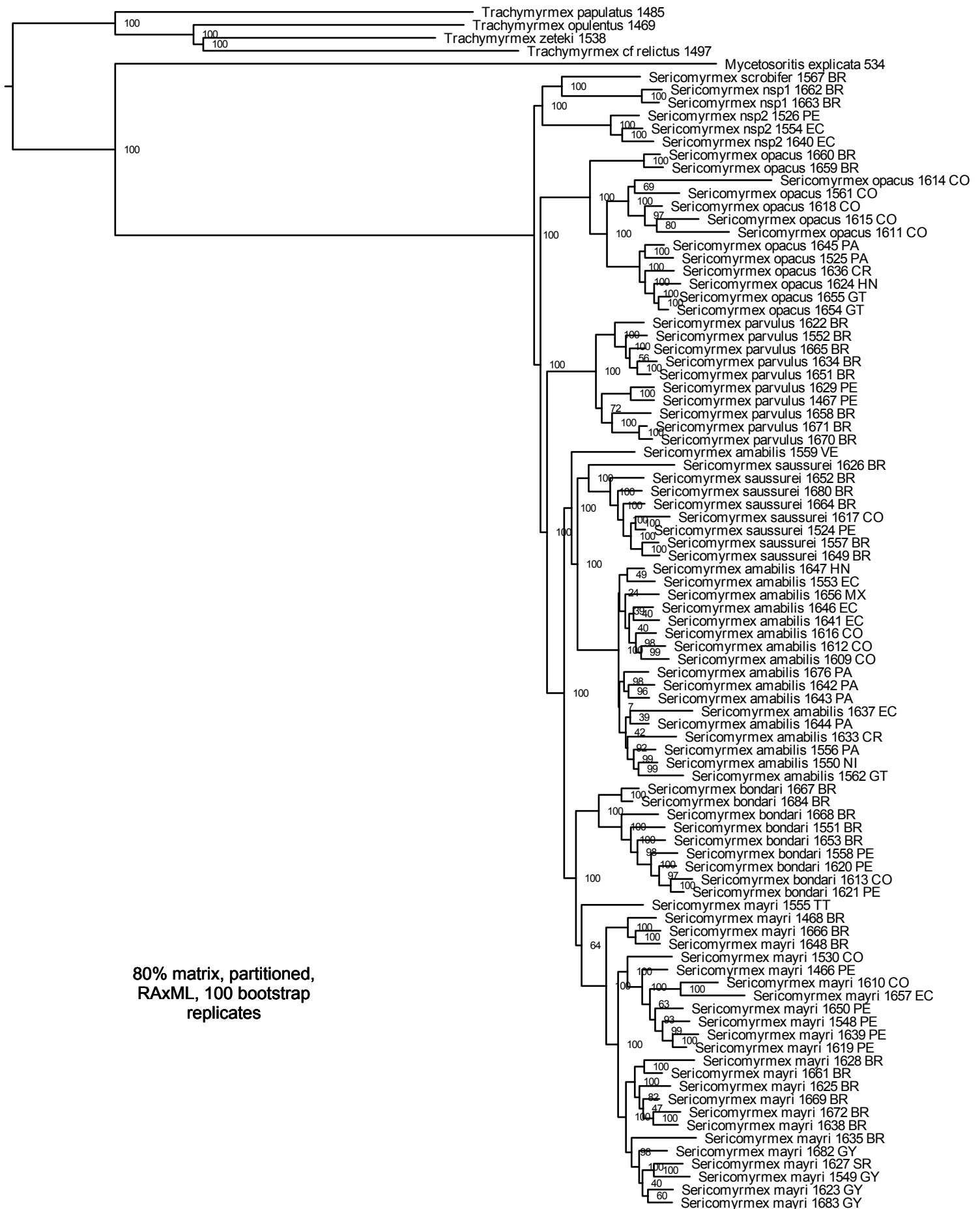
ESS off all paramaters > 200 (run1.p and run2.p files examined and combined in Tracer v1.6.0). Tree files summarizzed and the consensus tree created using the command sumt with the default burn-in of 0.25%.





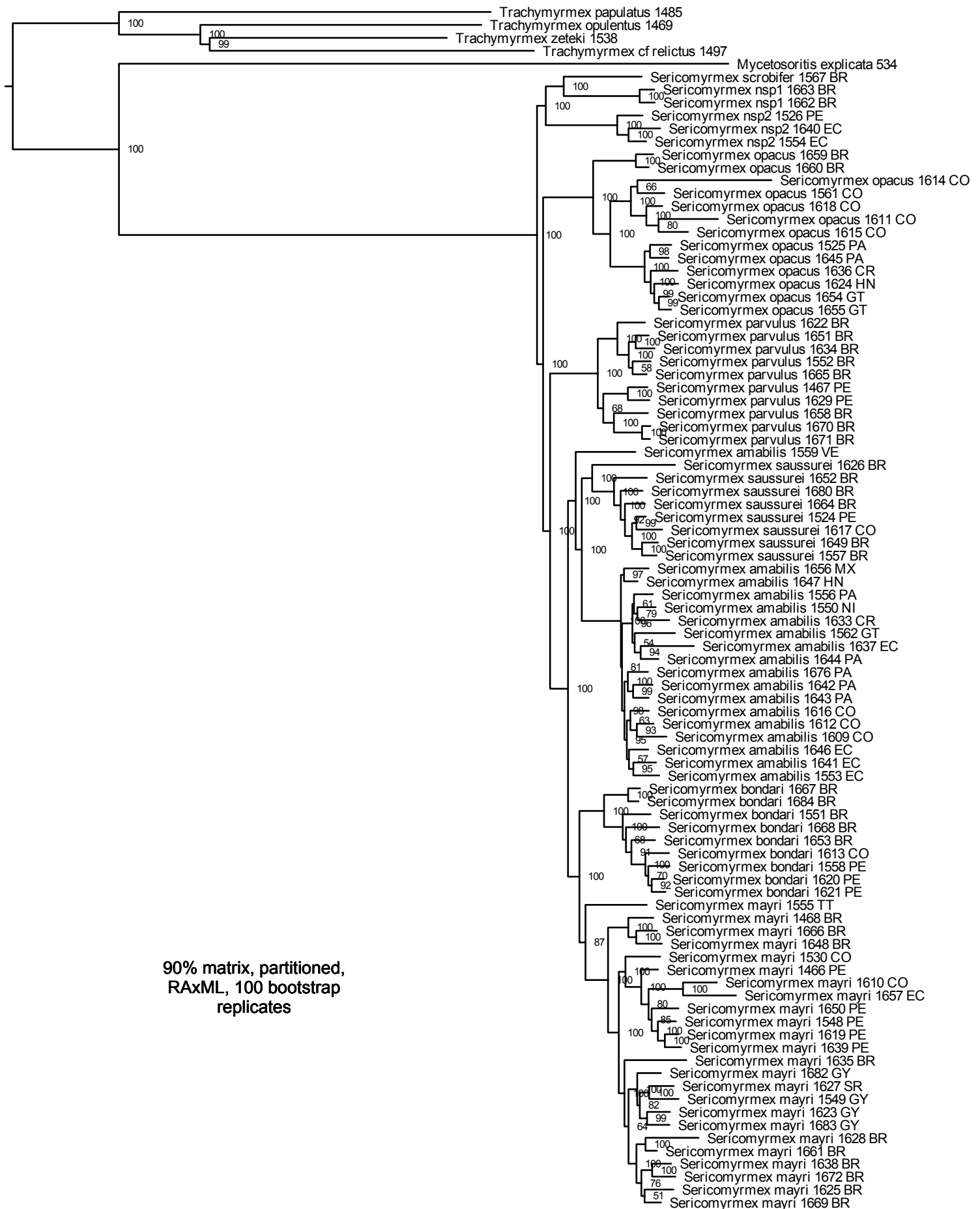
70% matrix, partitioned,
RAxML, 100 bootstrap
replicates

0.006



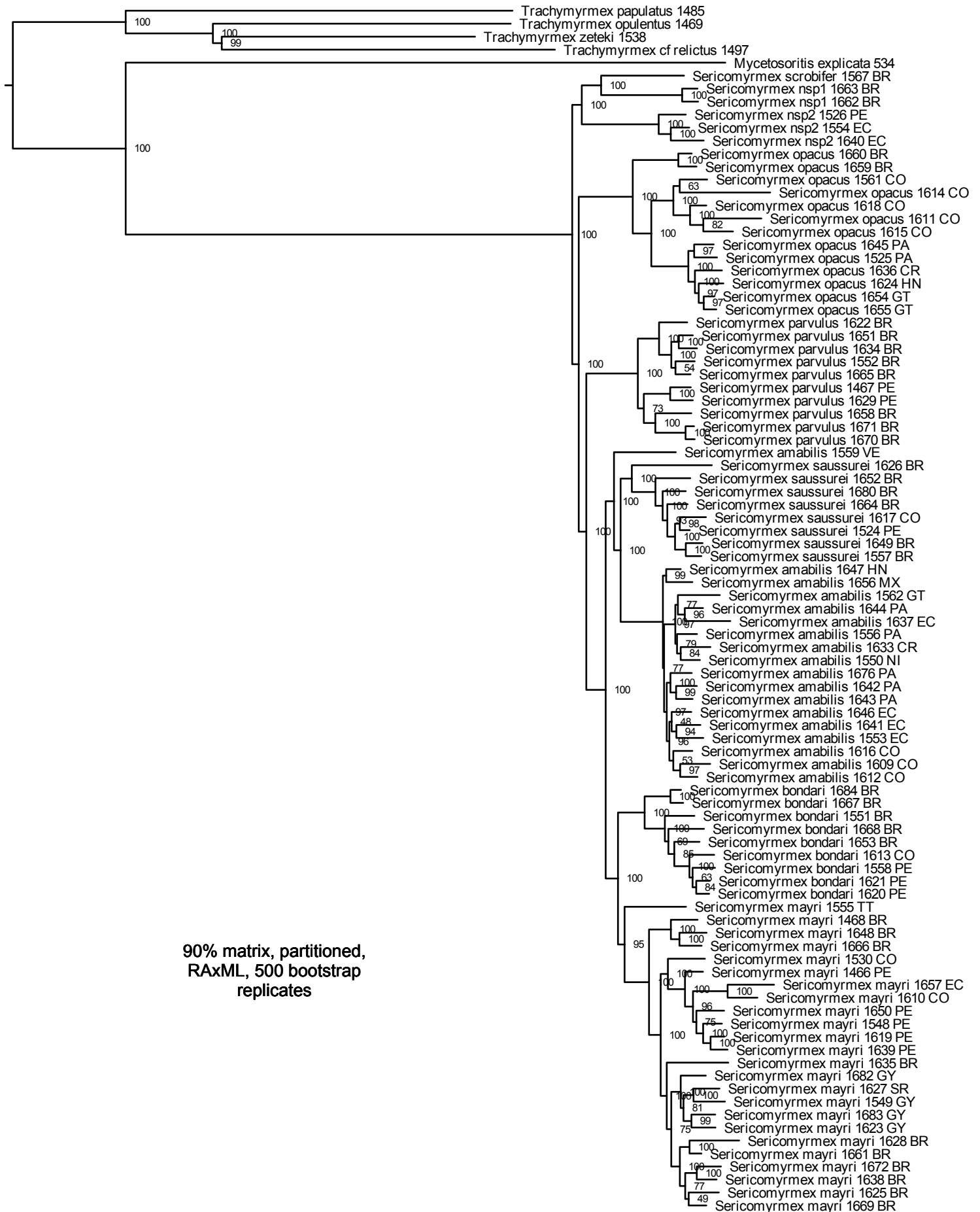
80% matrix, partitioned,
RAxML, 100 bootstrap
replicates

0.006



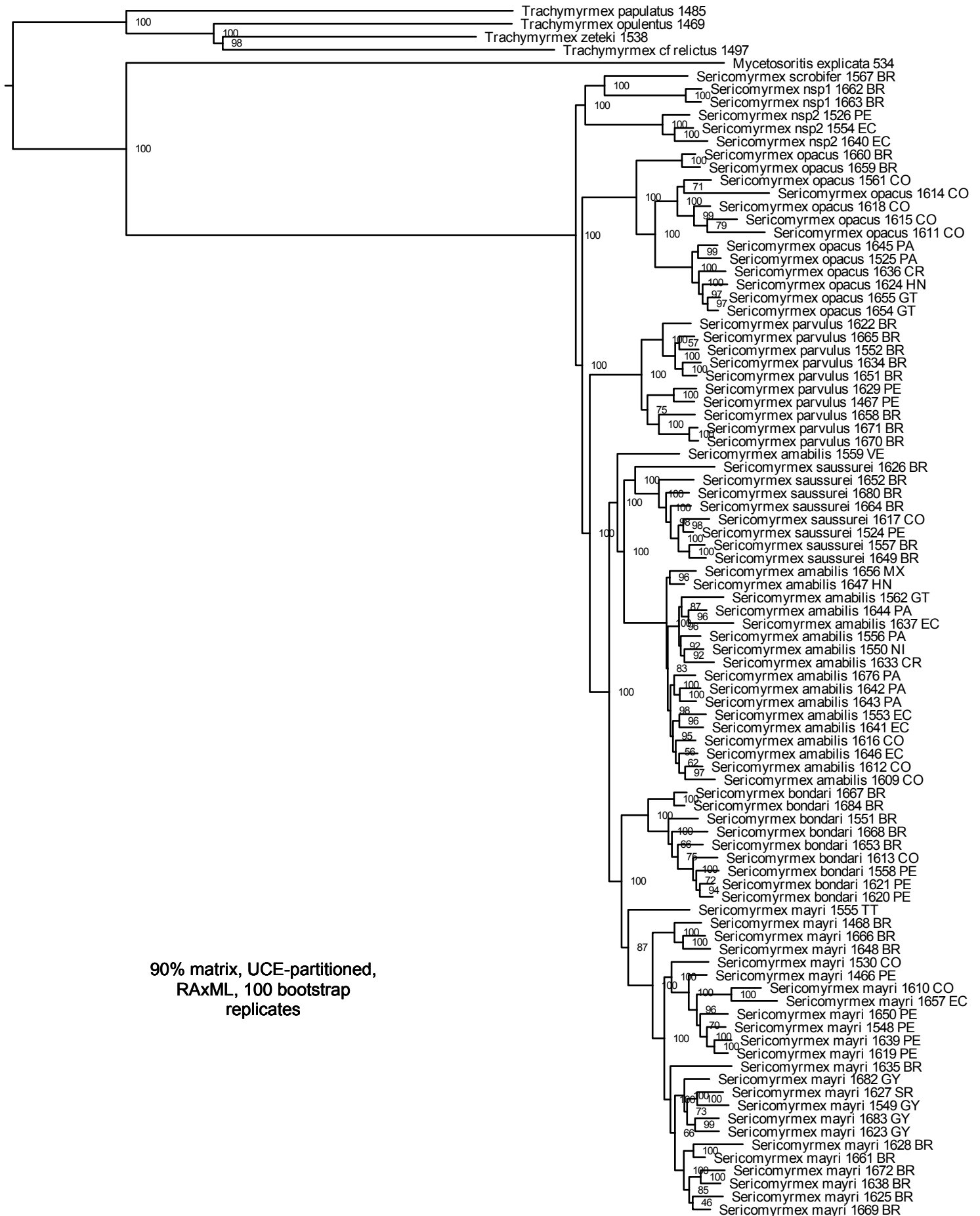
90% matrix, partitioned,
RAxML, 100 bootstrap
replicates

0.005



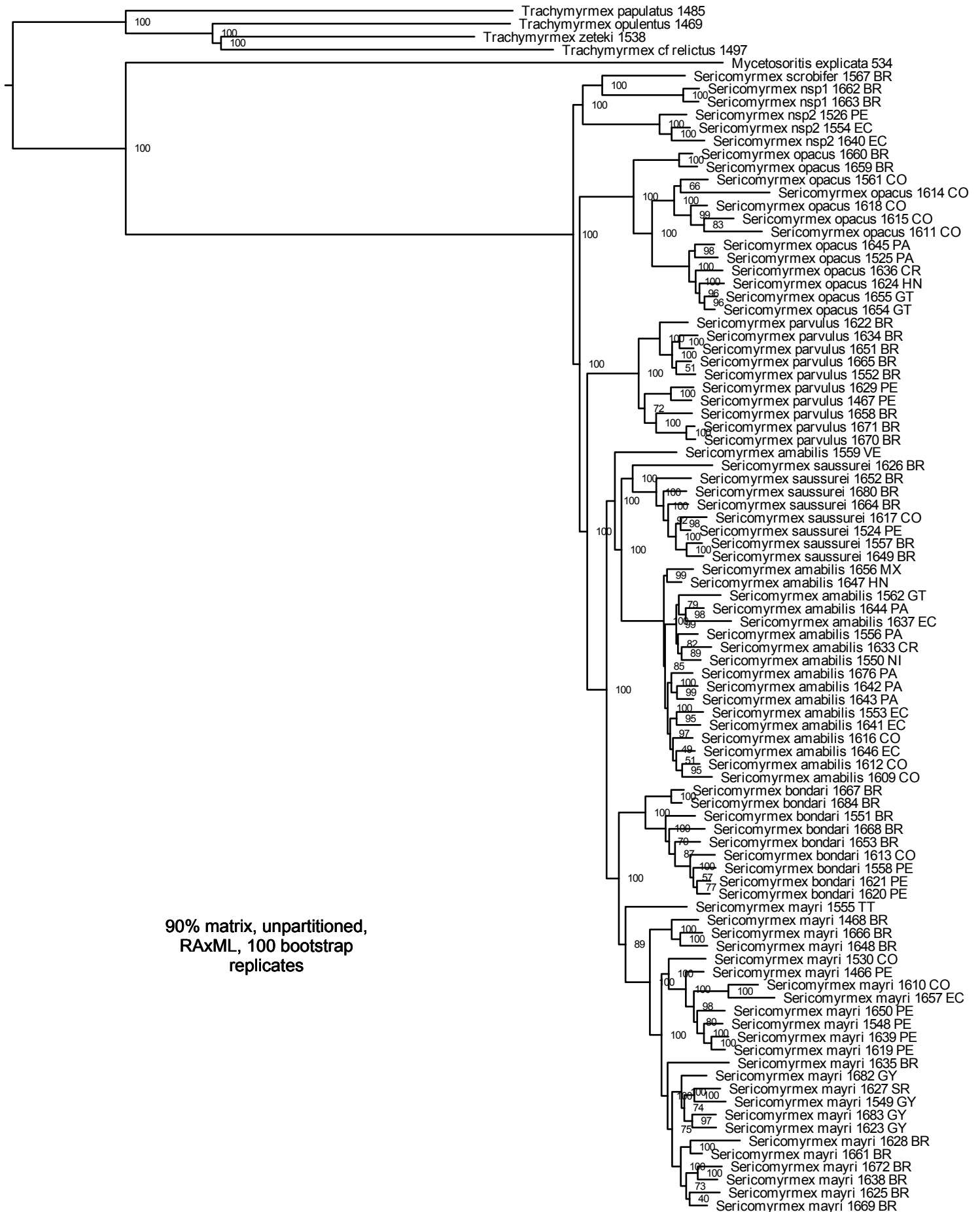
90% matrix, partitioned,
RAxML, 500 bootstrap
replicates

0.005



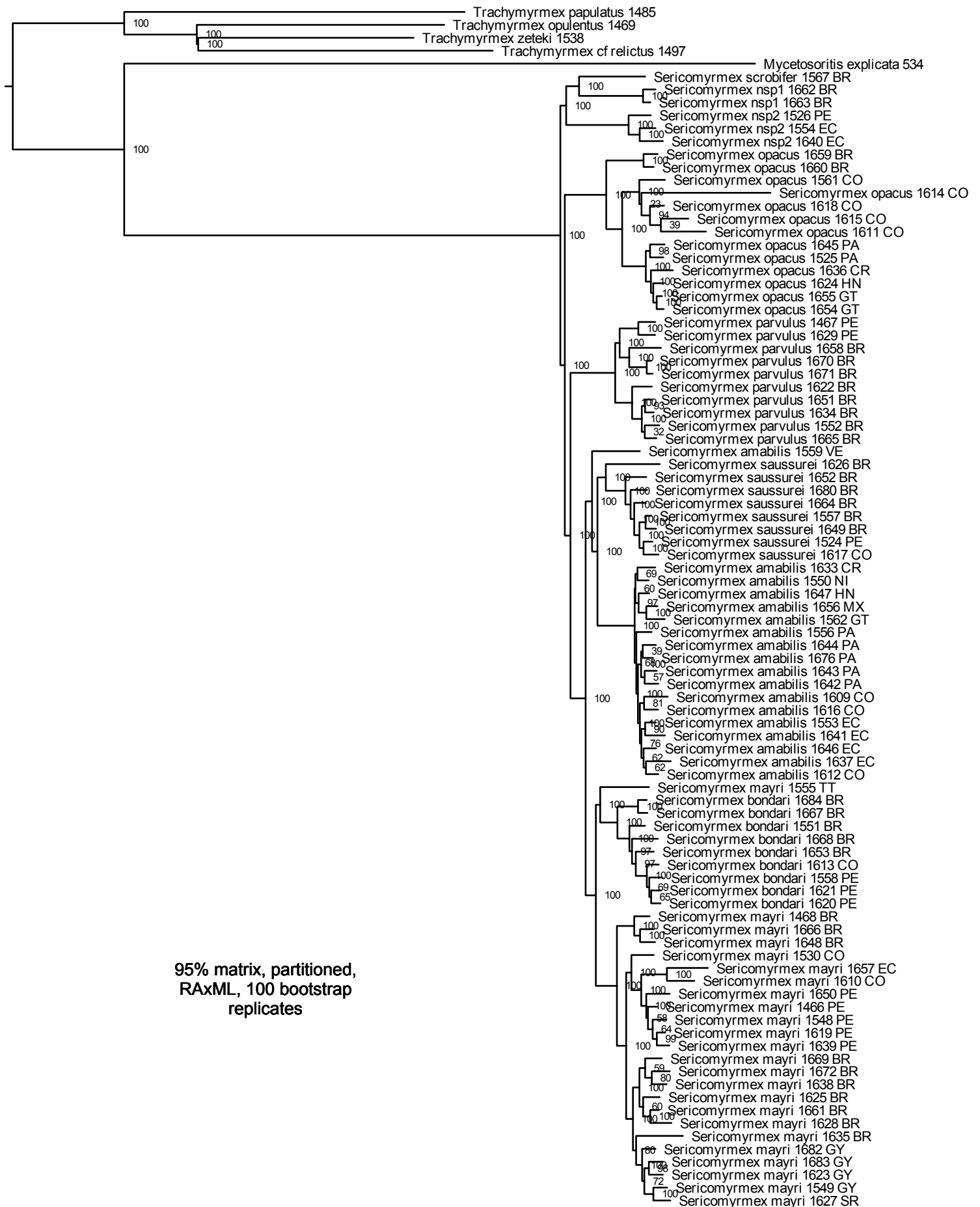
90% matrix, UCE-partitioned,
RAxML, 100 bootstrap
replicates

0.006

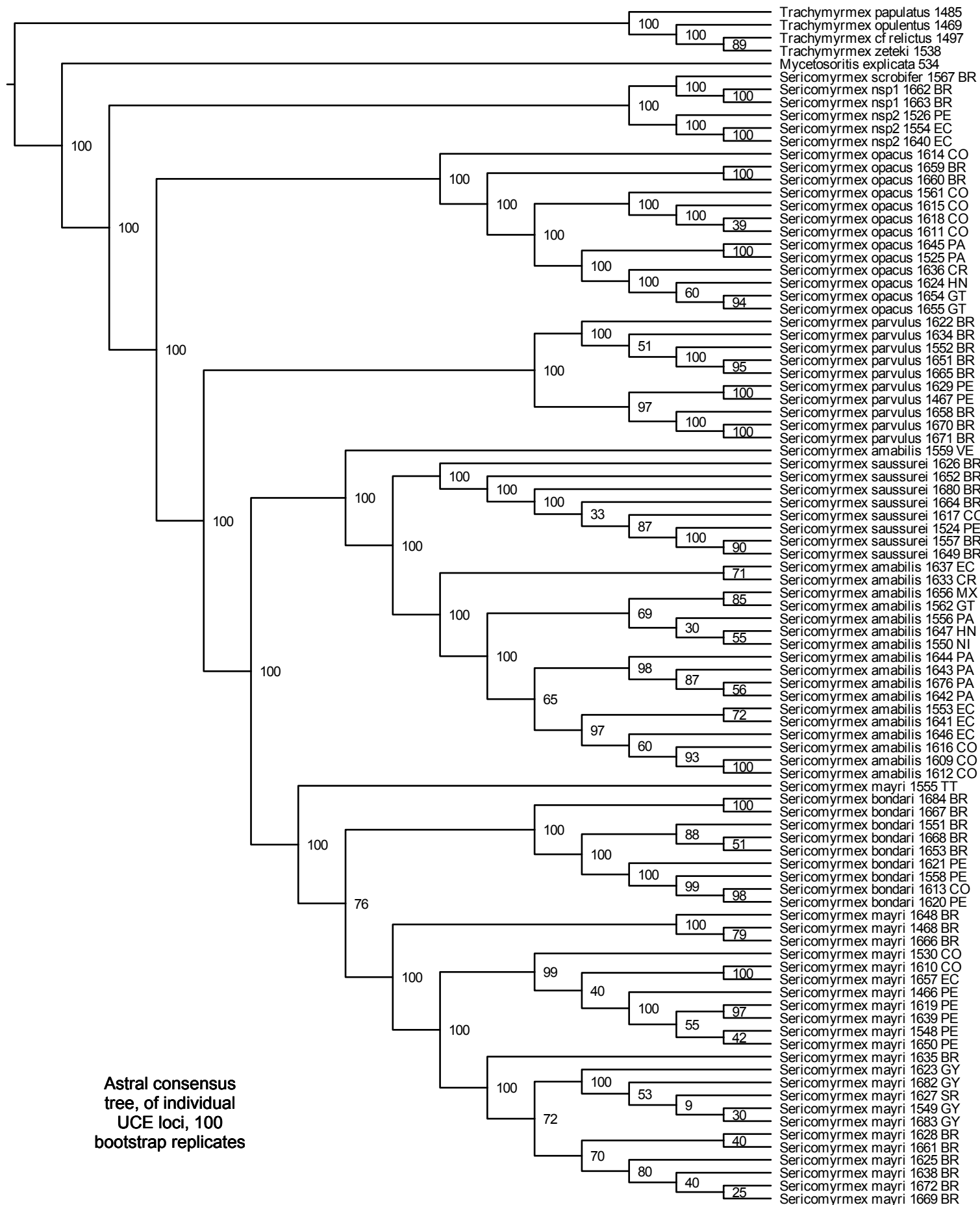


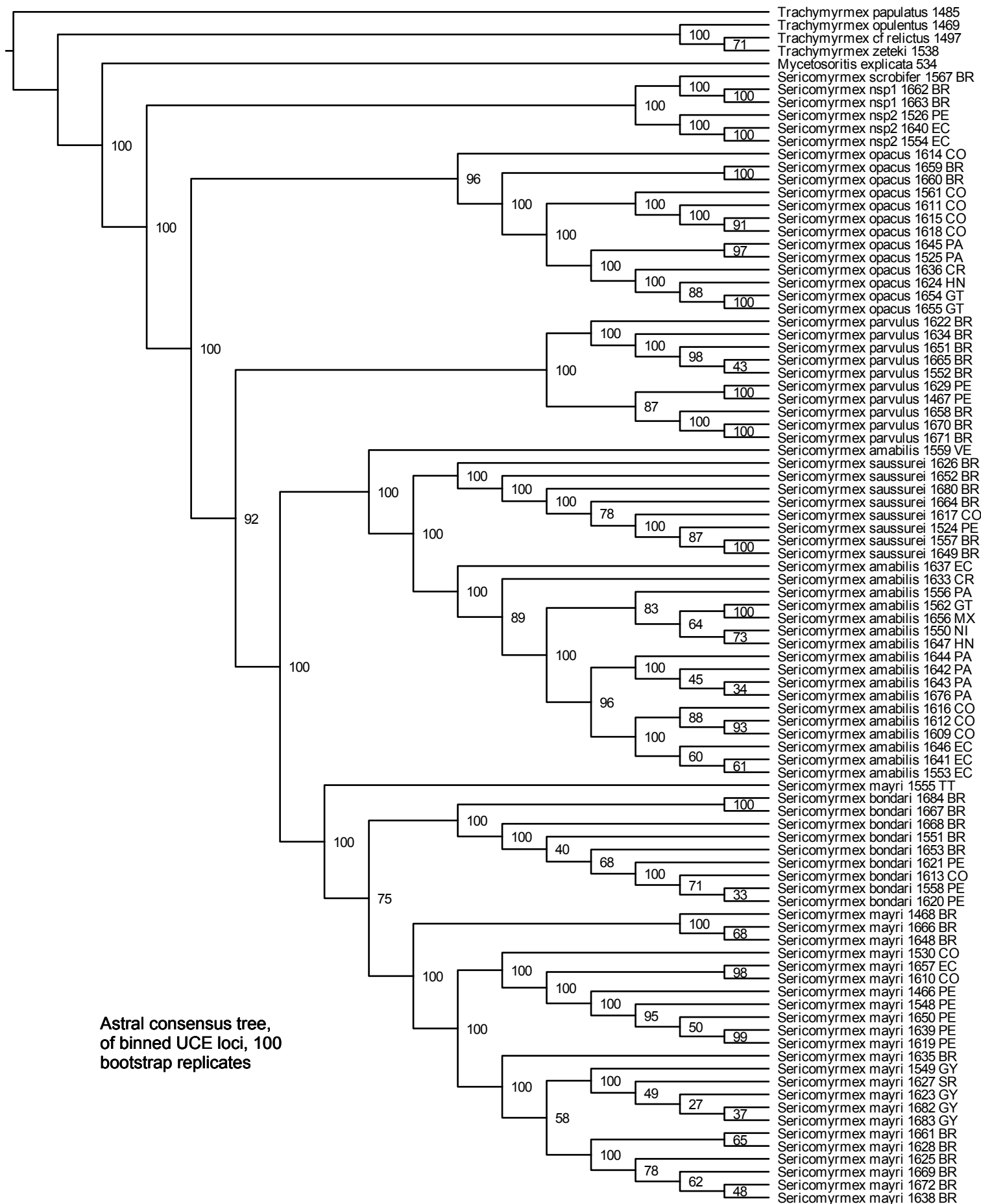
90% matrix, unpartitioned,
RAxML, 100 bootstrap
replicates

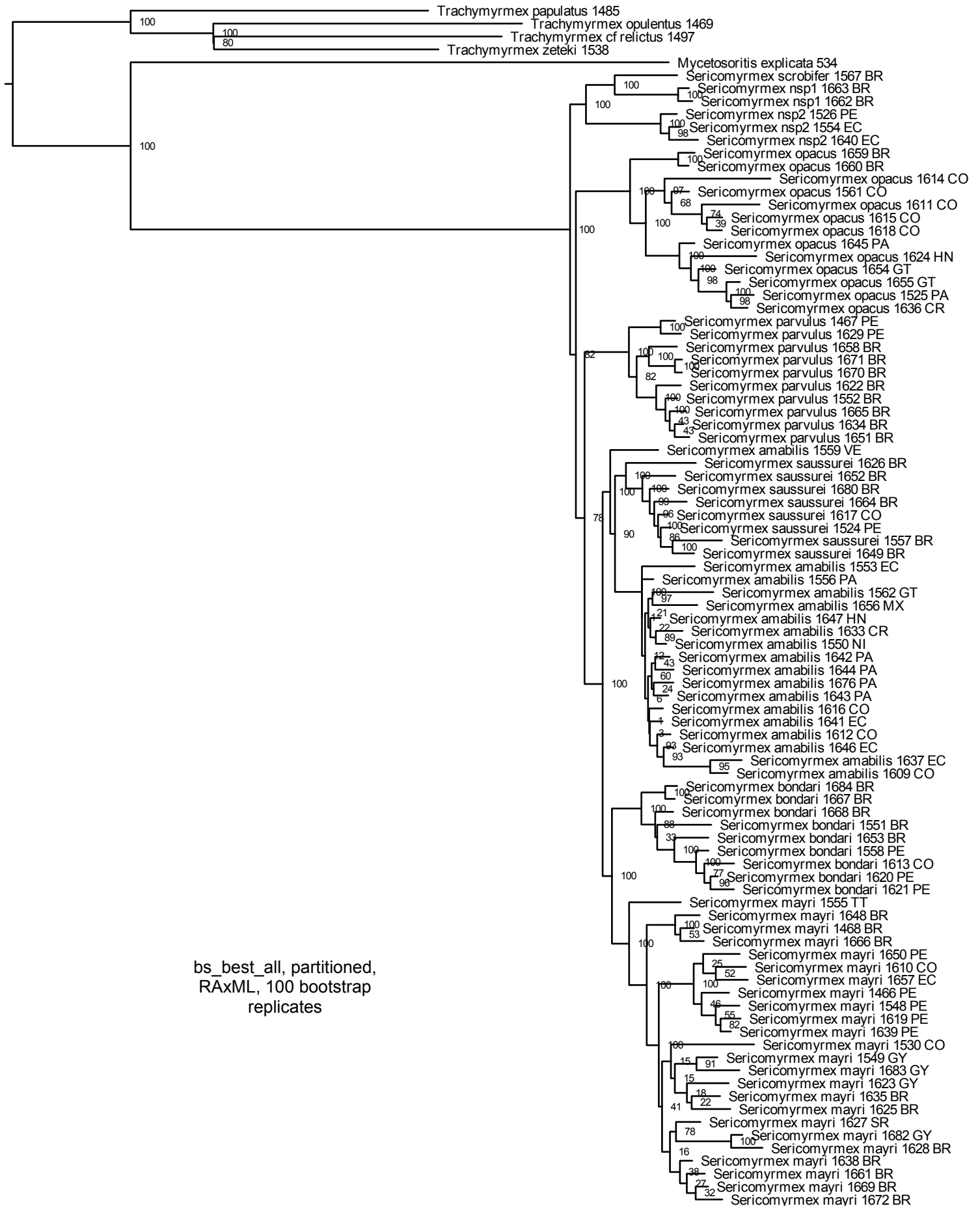
0.005



95% matrix, partitioned,
RAxML, 100 bootstrap
replicates







bs_best_all, partitioned,
RAxML, 100 bootstrap
replicates

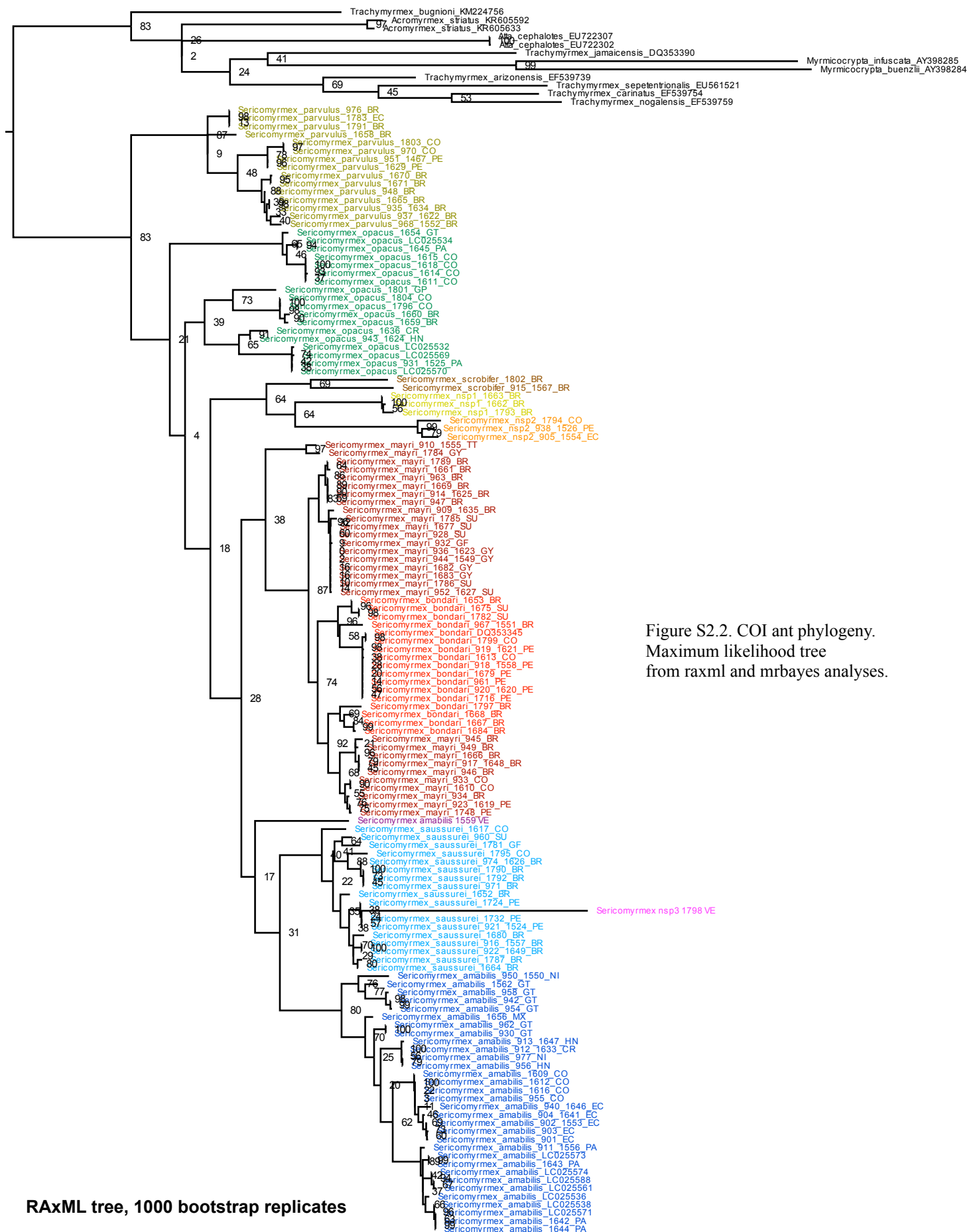


Figure S2.2. COI ant phylogeny.
 Maximum likelihood tree
 from raxml and mrbayes analyses.

RAXML tree, 1000 bootstrap replicates

0.3

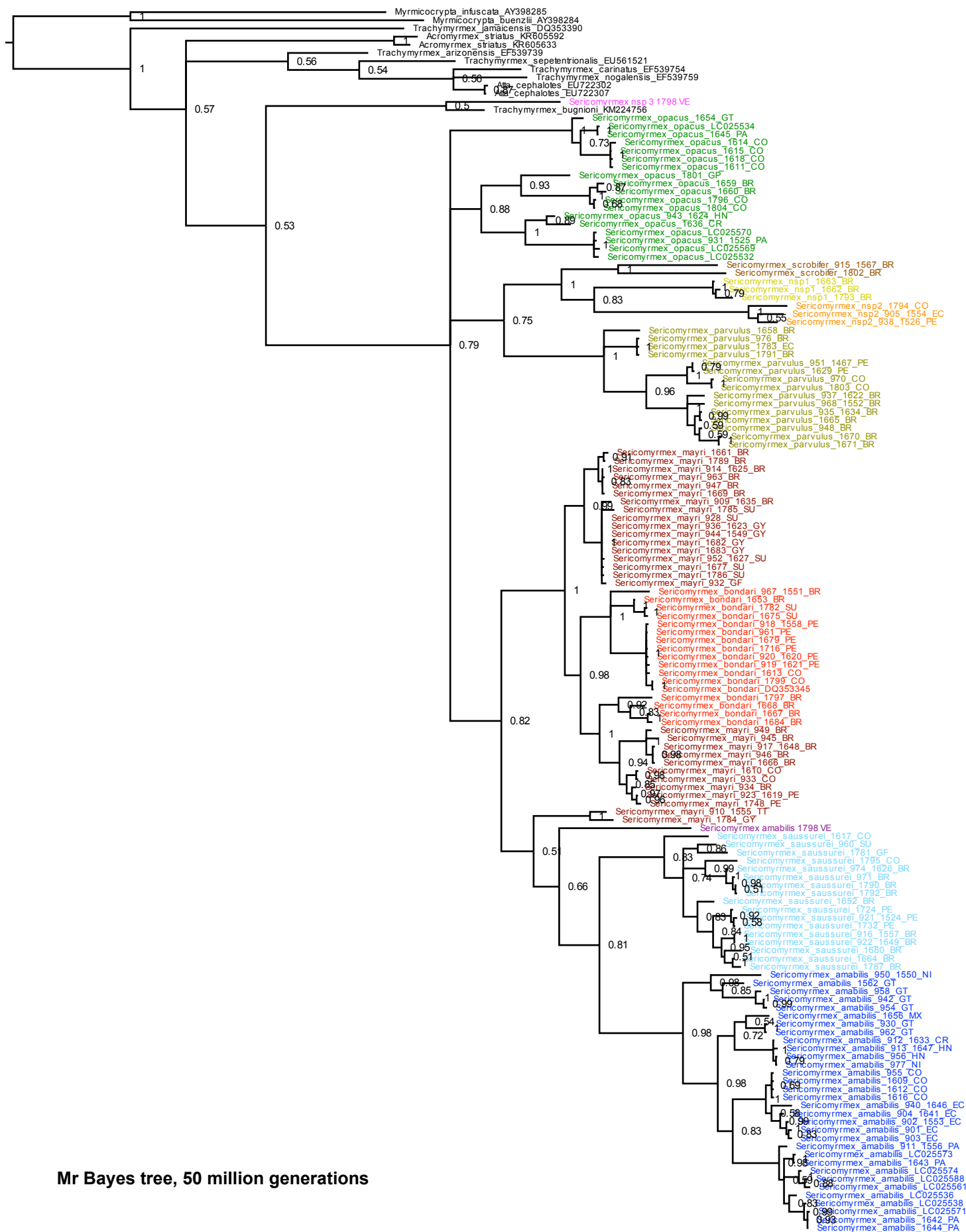
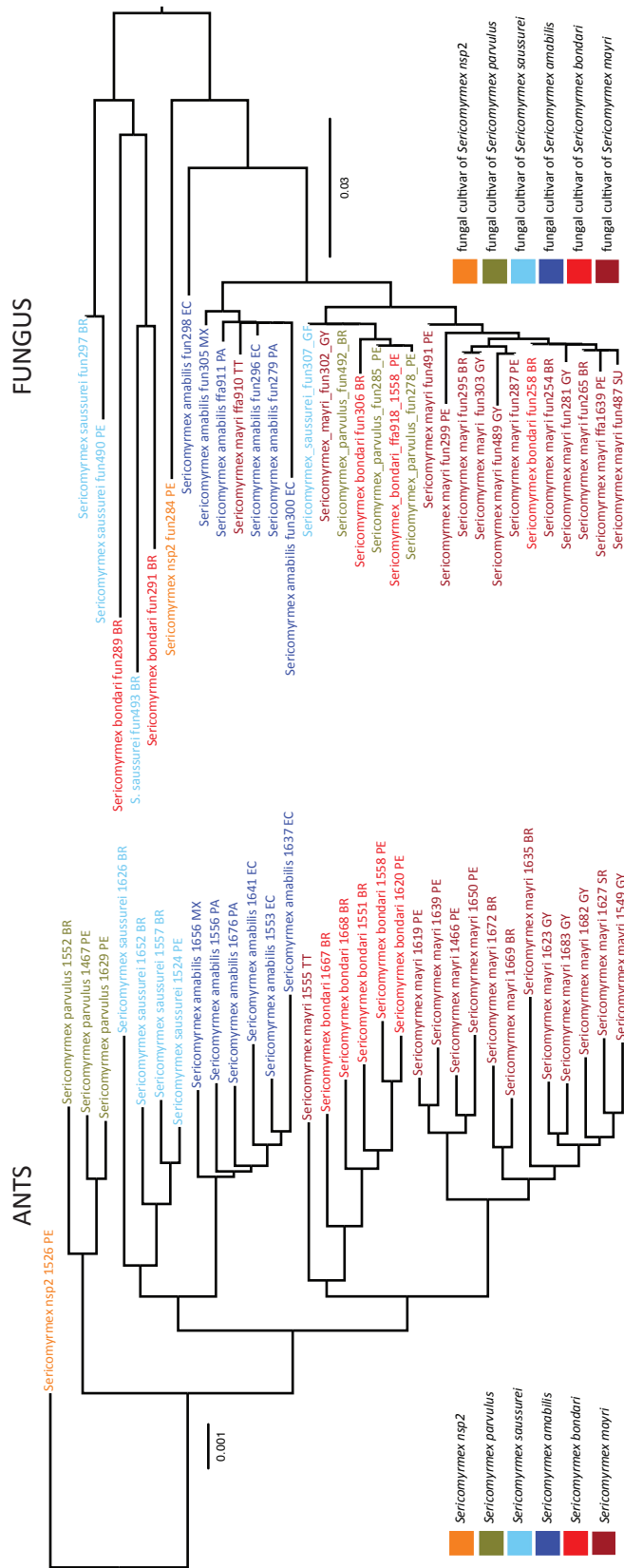


Figure S2.3. Associated ant and fungal phylogenies. The maximum-likelihood phylogenies of the reduced ant UCE data set, including only ants for which associated fungi were available, and of the fungal ITS+LSU data set, including only *Sericomyrmex* fungi.



Chapter 3: Taxonomic revision of the fungus-farming ant genus *Sericomyrmex* (Hymenoptera: Formicidae)

3.1. Introduction

The ant genus *Sericomyrmex* belongs to the fungus-farming (hereafter: “attine”) ants (Formicidae: Myrmicinae: Attini: subtribe Attina), a New World clade of over 250 species (Bolton, 2014), all of which cultivate fungus gardens for food (Mehdiabadi & Schultz, 2010). The ants provide the fungus with the substrate on which it grows, either organic detritus or fresh vegetation, thus effectively practicing agriculture (Schultz *et al.*, 2005). *Sericomyrmex* belongs to the so-called “higher” attine ants, a clade of ants that farms a corresponding clade of highly specialized fungal cultivars. These higher attine fungal cultivars are derived from a “lower” attine cultivar, but unlike lower attine cultivars, which are facultative symbionts capable of living freely apart from attine ants, higher attine cultivars have become obligate symbionts of a corresponding clade of higher attine ants, and evolved specialized adaptations for life with ants (Schultz & Brady, 2008). The higher attine ants include three other genera: the paraphyletic *Trachymyrmex* and the leaf-cutting *Atta* and *Acromyrmex*. The position of *Sericomyrmex* in the attine ant phylogeny and its close relatedness to leaf-cutting ants makes the study of *Sericomyrmex* species important for understanding the evolutionary origins of higher

attine ant agriculture and the factors that influenced the immense ecological success of leaf-cutter ants.

Like the majority of attine ant genera, *Sericomyrmex* has a wide Neotropical distribution (Figure 3.1), ranging from northern Mexico (Sánchez-Peña, 2010) southward to Bolivia, Paraguay, and Paraná, Brazil (Mayhe-Nunes & Jaffe, 1998; Fernández & Sendoya, 2004). *Sericomyrmex* species can be found in a variety of habitats, from dry savanna to tropical wet forest, as well as in disturbed, open, and urban habitats (Mehdiabadi & Schultz, 2010). Mayr described the genus *Sericomyrmex* and its first species, *Sericomyrmex opacus*, in 1865, based on specimens collected by American naturalist E. Norton in Cordoba, Mexico (Mayr, 1865). Since then, a total of 19 species and 3 subspecies have been described, mostly during the early 20th century. The most recent description, of *S. beniensis* from Bolivia (Weber, 1938), was published in 1938. Forel published the first key to *Sericomyrmex* in 1912 (Forel, 1912), but it contained only the nine species known at that time. Wheeler modified that same key a few years later (Wheeler, 1916), adding the newly described *S. lutzii*, and since then no updated keys have been attempted. The genus *Sericomyrmex* is considered taxonomically difficult mostly because of its substantial morphological homogeneity, further exacerbated by considerable variation within species. The differences between some species (e.g., between *S. parvulus* and *S. opacus*) can be very subtle, lacking discrete character states, and not much greater than the differences sometimes observed between two workers of the same species. The original species descriptions tend to be short and are often based on just few collected foragers instead of on complete nest series taken from several geographical locations, both common practices at the time in which they were written. As

a consequence, some of the characters cited in those descriptions are not useful for distinguishing between *Sericomyrmex* species, since they are now known to vary within species. Thus, even though *Sericomyrmex* ants are commonly collected by standard arthropod collecting methods (e.g., Winkler sifting and pitfall traps), most present-day biologists cannot identify them to species level, and even sorting to morphospecies can be difficult. The old authors were in fact aware of this problem, e.g., Wheeler, in his 1925 paper, writes: "...it now appears that there are several forms (...) which are so closely related that they may be merely geographical races, or subspecies of one or few highly variable species" (Wheeler, 1925). An intermediate degree of within-colony polymorphism, as well as other natural history traits, are shared by *Sericomyrmex* and *Trachymyrmex* species, and differentiates them from both the lower attine ants, which typically have smaller colonies, collect insect frass and organic debris, and are monomorphic, and from the leaf-cutters, which have enormous colonies, forage for fresh vegetation, and have highly polymorphic worker castes (Leal *et al.*, 2011).

3.1.1. Short review of natural history

Sericomyrmex ants are light yellow to deep ferrugineous brown and densely covered with long, flexuous hairs, which gives them a silky, velvety appearance to the naked eye, and which earned them their name: "*sericeus*" means "silky" in Latin. When encountered in nature they move slowly, and when disturbed they react like most other attine ants: they stop moving, curl into a ball, and feign death. Because of their slow movements and opaque integuments, they are difficult to notice on the forest floor. The nest entrances of some species can be recognized by their raised cylindrical craters

consisting of excavated soil particles, but those of other species often consist of just a simple hole in the ground, hard to notice on the forest floor (Urich, 1895; Forel, 1912; Wheeler, 1925). *Sericomyrmex* species nest underground, and the nest consists of a minimum of one but usually of at least a few subspherical to subelliptical chambers connected by narrow tunnels. Each chamber is filled with a fungus garden, a spongy, yellow-brown or yellow-grey mass containing eggs, brood, workers, and, in one chamber, the queen. The fungus garden can be sessile on the chamber floor (Leal *et al.*, 2011), but is more often suspended from the roof of the chamber by small rootlets (Weber, 1972; Fernández-Marín *et al.*, 2004; Mehdiabadi & Schultz, 2010). Colony sizes of *Sericomyrmex* species vary from medium (several hundred workers) to large (~5000 workers), but never come close to the sizes of gigantic *Atta* leaf-cutter ant colonies, which can contain millions of workers (Weber, 1972).

Sericomyrmex workers mostly forage alone, but in species with larger colonies (e.g., *S. mayri* and *S. bondari*), they sometimes form short, dense foraging columns in the immediate area of the nest entrance. They collect organic material, mostly freshly fallen leaflets, sometimes moss, grass, leaves, and fruits (Leal & Oliveira, 1998; De Fine Licht & Boomsma, 2010). They can also feed on seeds, but will usually not bring them back to the nest (Feldmann *et al.*, 2000). They will cut leaves on some occasions (Weber, 1967, 1972), but they do not climb vegetation in order to cut leaves as leaf-cutter ants do (Leal *et al.*, 2011).

Local occurrences of *Sericomyrmex* are often patchy, with colonies abundant in some areas but absent or sparse in adjacent areas. In areas where they are abundant they can be very dense, with nest entrances very close to each other. For example, during an

attine ant study in Trinidad, 67 *Sericomyrmex* nest entrances were observed in an 18 x 77 meters area in one year (Weber, 1972). The species *Sericomyrmex amabilis* is the host to a socially parasitic ant guest, *Megalomyrmex symmetochus*, which specializes on *Sericomyrmex* species (Wheeler, 1925; Adams *et al.*, 2013) and which provides protection to the host ant colony by defending it from specialized agro-predator *Gnamptogenys* ants (Adams *et al.*, 2013).

Here we present the first comprehensive taxonomic revision of the ant genus *Sericomyrmex*, recognizing 11 species. Eleven species and two subspecies are synonymized and three new species are described. In addition to morphological characters of workers, queen, males, and larvae, this revision was strongly informed by molecular data. A well-supported phylogeny of 88 *Sericomyrmex* individuals and 5 outgroup taxa (*Trachymyrmex* and *Mycetosoritis*), based on genomic data from ultraconserved elements (UCE), informed decisions about the relationships of species and species groups, and the combination of the UCE-based phylogeny and a phylogeny based on mitochondrial DNA cytochrome oxidase 1 subunit (COI) sequences from 136 individuals informed decisions about species delimitation (Chapter 2).

3.2. Materials and Methods

3.2.1. Morphological analyses

The majority of specimens examined for this study were collected during extensive field work in South and Central America by members of the Smithsonian Ant Lab, mainly Ted R. Schultz, Jeffrey Sosa-Calvo, and Ana Ješovnik. A total of ~19,000 specimens from 17 countries were obtained for this study, which included 14 complete

Sericomyrmex nests, that accounted for >17,550 individuals (even though each of those individuals was counted, not all were examined). The total number of pinned specimens examined was ~1,500. The collecting localities for all of the specimens examined are indicated on Figure 3.1.

Adult ant specimens were examined and measured using a MZ16 Leica stereomicroscope with an ocular micrometer. Measurements were taken to the nearest 0.001 mm, but are conservatively reported to an accuracy of the second decimal place. A minimum of 25 workers were measured for each species, except for *S. lutzii* and *S. n. sp.* 3, for which only 8 and 9 specimens were available, respectively. Measured workers were chosen to represent the geographic range of the species and to estimate within-colony variation, with up to 12 workers from the same nest measured when nest series were available.

Workers, queens, and males were photographed using a JVC KY-F70B video camera mounted on a M420 Leica stereomicroscope. The images were assembled using Automontage Pro version 5.03.0018 software. Queen and male wings were prepared on microscope slides with Euparal mounting medium. The larvae were dehydrated in 100% absolute ethanol, critical-point dried in liquid CO₂ in Balzers CPD-030, and coated with gold and palladium alloy in a Cressington Scientific 108 Auto sputter coater to a thickness of 20–25 nm. Coated larval specimens were then imaged with a Philips XL–30 ESEM Scanning Electron Microscope (SEM) in the NMNH SEM Lab. Adult worker, queen, and male specimens were air-dried for a few minutes, then coated with gold to a thickness of 50–70 nm using a Cressington Scientific 108 Auto sputter coater in the USNM Lab at NMNH. SEM images of the worker, queen, and males were taken with the

Hitachi SEM. All Automontage and SEM images were edited in Adobe Photoshop, following the image editing instructions of the AntWeb (<http://www.antweb.org>).

All latitudes and longitudes are provided in decimal degrees, and elevations in metric units. For specimens for which GPS coordinates were unavailable, the longitude and latitude were estimated based on locality data using AntWeb, GEONet Names Server (<http://geonames.nga.mil/gns/html/index.html>), Google Earth (<http://www.google.com/earth/index.html>), and/or Google Maps (<https://www.google.com/maps>). Estimated coordinates are indicated by square brackets. Date of collection is in the day/month/year format, with the month spelled with the first three letters to avoid uncertainty. Data for type material follows the format: [Country], [First administrative district], [Locality], [GPS coordinates], [Elevation], [Collection code], [Collector], [Collection date], [Habitat], and for each of the type specimens examined: (Repository: number and form of specimens, specimen code). In cases where no holotype exists, we designate one of the specimens as the lectotype and the rest of the syntype specimens as paralectotypes. When there is more than one ant on the pin, the position on the pin of the lectotype is indicated (e.g., “topmost specimen on the pin”). Data for the material examined is not an exhaustive list of every specimen seen, but instead provides an overview of the geographic range of the examined specimens. The data for material examined is organized alphabetically by country, first administrative district, and then in the following format: [Locality], [GPS coordinates], [Elevation], [Collection date], [Collector].

The collections visited and from which material was borrowed and/or into which material was deposited are referred to by the following acronyms:

AMNH	American Museum of Natural History, New York, New York, USA.
BMNH	The Natural History Museum, London, U.K.
DZUP	Museu de Entomologia Pe. Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.
CASC	California Academy of Sciences, San Francisco, California, USA.
CEPEC	Laboratório de Mirmecologia Itabuna, Bahia, Brazil.
IAVH	Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Colombia.
ICN	Insect Collection, Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá D.C., Colombia.
INPA	Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.
MBC–UFU	Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.
MHNG	Museum d'Histoire Naturelle, Genève, Switzerland.
MHNL	Museo de Historia Natural, Lima, Peru.
MPEG	Museu Paraense Emilio Goeldii, Belem, Brazil.
MPUJ	Museo Javeriano de Historia Natural “Lorezo Uribe”, S.J. Pontificia Universidad Javeriana, Bogotá D.C., Colombia.
MSNG	Museo Civico de Storia Naturale “Giacomo Doria”, Genoa, Italy.
MZSP	Museu de Zoologia de Universidade de São Paulo, Brazil.
NHMB	Naturhistorisches Museum, Basel, Switzerland.

NHMW	Naturhistorisches Museum Wien, Vienna, Austria.
PSWC	Philip S. Ward Collection, UC Davis, California.
UFVB	Museu de Entomologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.
USNM	National Museum of Natural History, Washington, DC, USA.
UVGC	Colección de Artrópodos, Universidad del Valle de Guatemala, Guatemala City, Guatemala.

Measurements and indices

Morphological terminology and measurement indices follow Snodgrass (1910), Tulloch (1929), Bolton (1994), Schultz & Meier (1995), Sosa-Calvo & Schultz (2010), and Ješovnik *et al.* (2013). Male genitalia terminology follows Boudinot (2013).

Morphological measurements, index abbreviations, and definitions are as follows:

Female (worker and queen)

HWe Head width: in full-face view, the maximum width of the head including eyes.

HW Head width: in full-face view, the maximum width of the head just above the eyes.

HW1 Head width at the top of head: in full-face view, the maximum width between the points where the frontal carinae meet the posterior cephalic margin.

HW2 Head width posterior: in full-face view, the maximum width of the posterior part of the head.

HW3 Anterior head width most: in full-face view the maximum width of most anterior end of head capsule, measured between the points where lateral most edge of the clypeus connects with the head capsule.

HL1 Head length 1: in full-face view, the maximum length of the head from a line tangential to the posteriormost margins of the head to the line tangential to anteriormost margin of clypeal apron (since both posterior cephalic border and clypeus are medially notched, but see Figure 3.2 a).

HL2 Head length 2: HL1 minus the posterior cephalic notch.

SL Scape length: maximum length of the scape in full face-view, measured from the middle of the frontal lobe fenestra to the distal end of the scape.

IFW1 Interfrontal width 1: in full face-view, the maximum distance between the lateralmost points of the frontal lobes, which, in all *Sericomyrmex*, is the point where the lateral and posterior margins of the frontal lobes meet.

IFW2 Interfrontal width 2: in full face-view, the maximum distance between the interior margins of the frontal lobes, or the distance between the points where the lateral and medial margins of each frontal lobe meet.

EL Eye length: in lateral view, the maximum diameter of the eye.

EW Eye width: in full-face view, the maximum width of the eye. Measured only in queens and males, not in workers.

Om Ommatidia count: number of ommatidia visible in the maximum diameter of the eye, mostly in lateral view.

WL Weber length: in lateral view, length of the mesosoma from the anteriormost point of the pronotum to the posteriormost ventral angle of the propodeum.

HFL Hind femur length: length of the hind femur in the most appropriate view, which most often is the dorsal view.

PL Petiole length: in lateral view, the maximum length of the petiole measured from the posteriormost end of the mesonotal gland bulla.

PPL Postpetiole length: in lateral view, the maximum length of the postpetiole.

GL Gaster length: in lateral view, the distance from the anteriormost point of the tergo-sternal gaster articulation to the posterior tip.

PW Pronotal width: in dorsal-anterior view, the maximum length of pronotum,

FWg Front wing length (queen only).

HWg Hind wing length (queen only).

CI Cephalic index: $(HW/HL) \times 100$.

FLI Frontal lobe index: $(IFW1/HW) \times 100$.

SI Scape index: $(SL/HW) \times 100$.

EI Eye index: $(EL/HW) \times 100$.

CEI Cephalic Emargination Index: $((HL1-HL2)/HW) \times 100$.

Male (In addition to all worker measurements except HL2, HW1, HW2 and HW3, and all indices except CEI)

IOD Interocular distance: in the male, maximum width of the head in frontal view measured at the midpoint of the internal margin of the eyes (Figure 3.2 d).

SL Scape length (measured differently in males because the base of the scape is visible): in full face-view, maximum length of the scape, excluding the constriction that occurs just distal of the condylar bulb (Figure 3.2 d).

FWg Front wing length.

HWg Hind wing length.

CI Cephalic index: $(\text{IOD}/\text{HL}) \times 100$.

FLI Frontal lobe index: $(\text{IFW}/\text{IOD}) \times 100$.

SI Scape index: $(\text{SL}/\text{IOD}) \times 100$.



Figure 3.1. Distribution map of *Sericomymex*. Collecting localities of all specimens examined for this study.

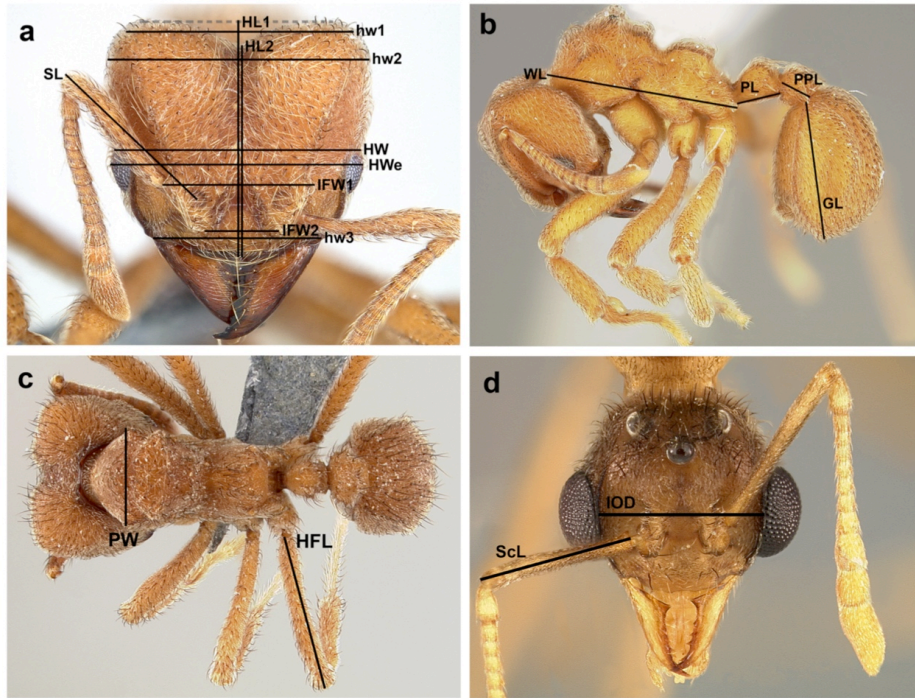


Figure 3.2. Measurements. Measurements of worker (**a**, **b**, **c**) and male (**d**) indicated.

HWWe Head width including eyes; **HW** head width just above the eyes; **HW1** head width at the top of head, between the points where the frontal carinae meet the posterior cephalic margin; **HW2** posterior head width, the maximum width of the posterior part of the head, **HW3** anterior head width, measured between the points where lateral most edge of the clypeus connects with the head capsule; **HL1** head length 1; **HL2** head length 2, HL1 minus the posterior cephalic notch; **SL** scape length **IFW1** interfrontal width 1; **IFW2** interfrontal width 2; **WL** Weber length; **PL** petiole length, **PPL** postpetiole length; **GL** gaster length; **PW** Pronotal width; **HFL** hind femur length; **IOD** Interocular distance.

3.2.2. Morphometric analysis

The quantitative morphological data set consists of 525 measured worker individuals and 21 variables (18 measurements and 5 indices). Measurements that were not possible to take (e.g., due to a damaged specimen or a specimen badly positioned on a pin) were scored as “NA” and treated as missing values in the analyses, except for the Principal Component Analysis (PCA), from which individuals with missing values were

excluded. All analyses were performed in R (R Development Core Team, 2014) using the MorphoTools functions set (Koutecký, 2015) and R packages *vegan* and *ade4*.

We ran basic descriptive statistics to examine the data visually and to detect possible errors and outliers. We manually rechecked all outliers for all species by remeasuring them. Not all size-related measurements were repeated, however, in cases in which the remeasuring of one variable was sufficient to confirm an outlying large- or small-sized individual.

Based on Shapiro-Wilk normality test run on the entire data set only two variables, CI (cephalic index) and SI (scape index), had normal distributions, so the data set was log-transformed for PCA analyses. To check for correlations between different variables we calculated Spearman's correlation coefficient. After removing the correlated parameters (HW, HL1, HL2, WL, HFL) the data set for PCA contained 17 variables. We calculated the PCA of individuals using the functions *pca.cor*, *pca.eigen*, and *pca.scpres*, and the correlations of characters (character loadings) with the function *pca.cor*. The character loadings indicate to what extent each character contributes to each of the components, and therefore indicate what characters are responsible for most of the variation.

In addition to the full data set, containing all the species, we created reduced data matrices, with only certain taxa or populations included, to further explore morphological separation in just those groups. The species-level reduced data sets included

1) *S. opacus* and *S. amabilis*, 2) *S. opacus* and *parvulus*, and 3) *S. scrobifer* and n. sp. 1.

The population-level reduced data sets included: 1) populations of striate-mandible vs. smooth-mandible *S. amabilis* 2) populations 1, 2, and 3 of *S. opacus*, 3) populations of

typical *S. bondari* vs. reduced-hair *S. bondari*, 4) populations of striate-mandible vs. smooth-mandible *S. saussurei*, and 5) populations of *S. mayri*. Also, we ran basic descriptive statistical analyses on data sets of queen-only (47 individuals) and male-only specimens (34 individuals).

3.3. Results

3.3.1. Morphometric analysis

Table 3.1 summarizes the statistics for each species, with mean and standard deviation values (SD) for chosen variables. Figure 3.3 contains box plots for chosen variables. The plot a in Figure 3.4 is based on the first two principal components identified by the principal components analysis, which together are responsible for 71.8% of the observed variance. The first principal component is strongly correlated with head width and other measurements of the head (Table 3.2), and it accounts for 60.8% of the observed variance. The second axis, principal component two, accounts for 11.2% of the observed variance. It is influenced mostly by the eye index (EI) and the cephalic emargination index (CEI), and somewhat less by the cephalic index (CI) and the frontal lobe index (FLI). We observe that along the y axis (PCA2), individuals are separated mainly by the relative sizes of their eyes and by the relative depths of their posterior cephalic emarginations, but also to a lesser extent by head shape and relative frontal lobe width. In general PC1 represents size and PC2 represents shape, the latter represented by ratios of certain characters. The third principal component did not add more resolution when plotted in three dimensions. It was similar to PC2, most correlated with CEI and EI, and it accounted for 6.971% of variance. The fourth component (PC4), which

accounts for 5.018 % of variance, is interesting because it is highly correlated with SI, the scape index, with all other variables having much lower variation (Table 3.2).

In general, the results of the PCA analyses (Figure 3.4) are congruent with genetic data for *Sericomyrmex*, which indicate that all species are closely related and that speciation in this genus occurred recently (Chapter 2). Species clusters greatly overlap, with a subset of species separated mostly due to size (axis x). PCA analyses of the reduced data sets are easier to interpret (Figure 3.5). These results are discussed in more detail in the notes section of the corresponding species.

Species (N)	<i>amabilis</i> (70)		<i>bondari</i> (59)		<i>lutzi</i> (6)		n. sp. 1 (30)	
	mean	SD	mean	SD	mean	sd	mean	SD
HWe	1.06	0.07	1.15	0.09	1.22	0.09	0.92	0.04
IFW1	0.72	0.06	0.76	0.07	0.78	0.07	0.62	0.05
HL1	1.02	0.07	1.10	0.07	1.25	0.08	0.88	0.05
ScL	0.75	0.05	0.81	0.05	0.86	0.05	0.66	0.04
WL	1.37	0.10	1.44	0.11	1.52	0.13	1.18	0.06
HFL	1.15	0.09	1.27	0.09	1.29	0.07	0.99	0.06
CI	104.32	2.83	104.06	3.45	102.53	2.48	104.16	2.86
FLI	67.68	2.46	66.52	2.48	64.28	1.89	67.53	3.31
SI	70.83	2.86	70.64	3.36	71.07	2.28	72.26	2.87
CEI	10.33	1.84	13.02	1.96	15.35	1.42	10.42	2.28

Species (N)	<i>mayri</i> (103)		<i>opacus</i> (68)		<i>parvulus</i> (55)		n. sp. 3 (9)	
	mean	SD	mean	SD	mean	SD	mean	SD
HWe	1.35	0.13	0.90	0.05	0.81	0.06	1.03	0.02
IFW1	0.85	0.08	0.64	0.05	0.56	0.05	0.63	0.02
HL1	1.25	0.12	0.90	0.04	0.80	0.06	1.02	0.03
ScL	0.91	0.07	0.64	0.07	0.58	0.05	0.80	0.02
WL	1.71	0.18	1.15	0.08	1.04	0.10	1.39	0.03
HFL	1.48	0.13	0.92	0.06	0.82	0.08	1.24	0.04
CI	107.83	3.46	100.39	2.64	101.61	2.70	100.78	1.66
FLI	63.16	2.54	70.35	2.90	69.06	3.13	61.09	1.22
SI	67.66	3.12	70.60	2.53	71.44	3.04	77.44	2.13
CEI	9.96	2.11	9.47	3.13	8.55	1.73	13.80	1.72

Species (N)	n. sp. 2 (25)		<i>saussurei</i> (68)		<i>scrobifer</i> (31)	
	mean	SD	mean	SD	mean	SD
HWe	1.01	0.06	1.02	0.08	1.00	0.05
IFW1	0.68	0.06	0.70	0.07	0.76	0.04
HL1	0.98	0.07	0.99	0.08	0.97	0.05
ScL	0.72	0.04	0.73	0.06	0.70	0.04
WL	1.28	0.08	1.33	0.11	1.29	0.07
HFL	1.10	0.07	1.12	0.12	1.11	0.08
CI	102.77	3.04	103.74	3.19	103.47	3.32
FLI	67.71	3.87	68.61	2.34	76.39	2.70
SI	72.07	2.78	71.19	2.81	69.88	3.51
CEI	9.56	1.53	9.56	2.07	11.51	1.41

Table 3.1. Morphological measurements. Mean and standard deviation (SD) values for chosen morphological measurements and indices, for species: *S. amabilis*, *S. bondari*, *S. n. sp. 1*, *S. mayri*, *S. opacus*, *S. parvulus*, *S. n. sp. 3*, *S. saussurei*, and *S. scrobifer*. All values are in millimeters. N represents the number of individuals measured for each species.

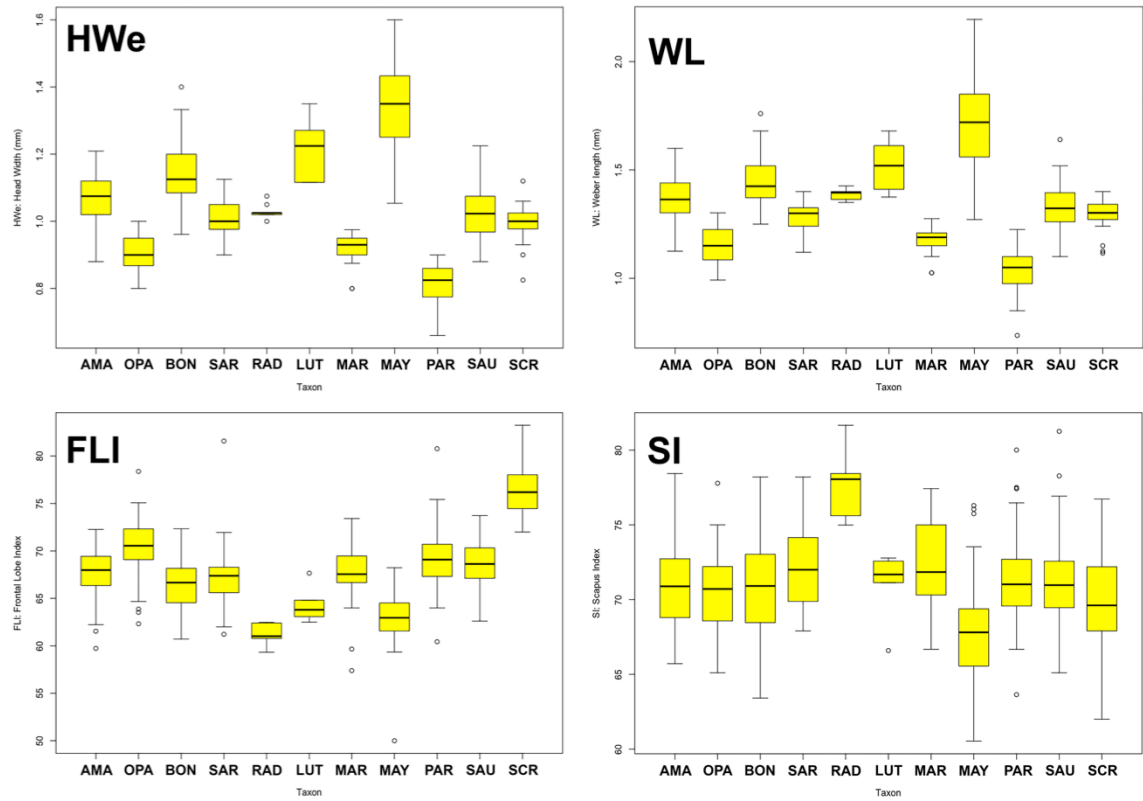


Figure 3.3. Box plots for morphological variables. **HWe**–head width including the eyes, **FLI**–frontal lobe index, **SI**–scapus index, **WL**–Weber length. Species name abbreviations: AMA–*amabilis*, OPA–*opacus*, BON–*bondari*, SAR–n. sp. 2, LUT–*lutzi*, MAR–n. sp. 1, MAY–*mayri*, PAR–*parvulus*, RAD– n. sp. 3, SAU–*saussurei*, SCR–*scrobifer*.

Character	PC1	PC2	PC3	PC4	PC5	PC6
HWe	0.98	-0.14	0.03	0.04	0.00	-0.04
IFW1	0.96	0.07	-0.07	-0.11	0.19	-0.11
IFW2	0.87	-0.06	-0.02	0.08	0.18	0.02
ScL	0.95	-0.05	0.11	0.25	0.08	-0.11
EL	0.80	0.37	-0.37	0.16	-0.21	0.04
Om	0.77	0.30	-0.26	0.10	-0.18	-0.14
PL	0.79	0.04	-0.03	0.00	0.18	0.39
PPL	0.80	0.13	0.00	0.07	0.17	0.26
GL	0.94	-0.06	-0.05	0.03	0.07	-0.02
PrW	0.96	-0.06	-0.01	0.02	0.05	0.01
hw3	0.95	-0.13	0.02	0.08	0.03	0.01
CI	0.54	-0.54	-0.29	-0.06	-0.31	-0.26
FLI	-0.38	0.58	-0.27	-0.40	0.49	-0.17
SI	-0.45	0.35	0.23	0.68	0.29	-0.22
EI	-0.33	0.69	-0.54	0.16	-0.27	0.11
FLD	0.88	0.15	-0.10	-0.24	0.16	-0.18
CEI	0.37	0.62	0.57	-0.20	-0.30	-0.03

Table 3.2. Principal component analysis. Character loadings of the first six principal components.

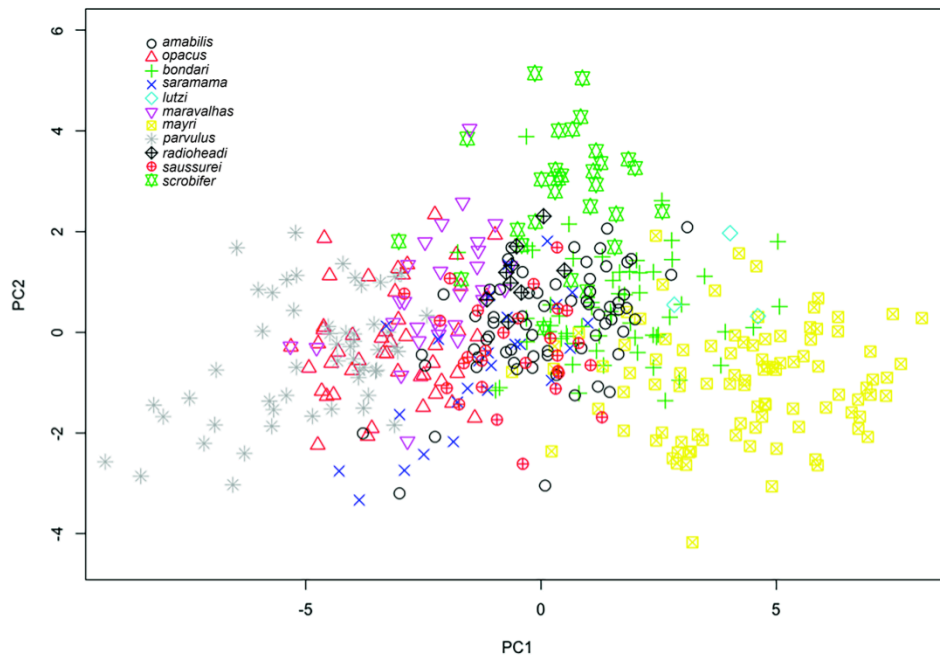


Figure 3.4. Principal components analysis for all species, scatter plot. Species name abbreviations: AMA–*amabilis*, OPA–*opacus*, BON–*bondari*, SAR–n. sp. 2, LUT–*lutzi*, MAR–n. sp. 1, MAY–*mayri*, PAR–*parvulus*, RAD– n. sp. 3, SAU–*saussurei*, SCR–*scrobifer*.

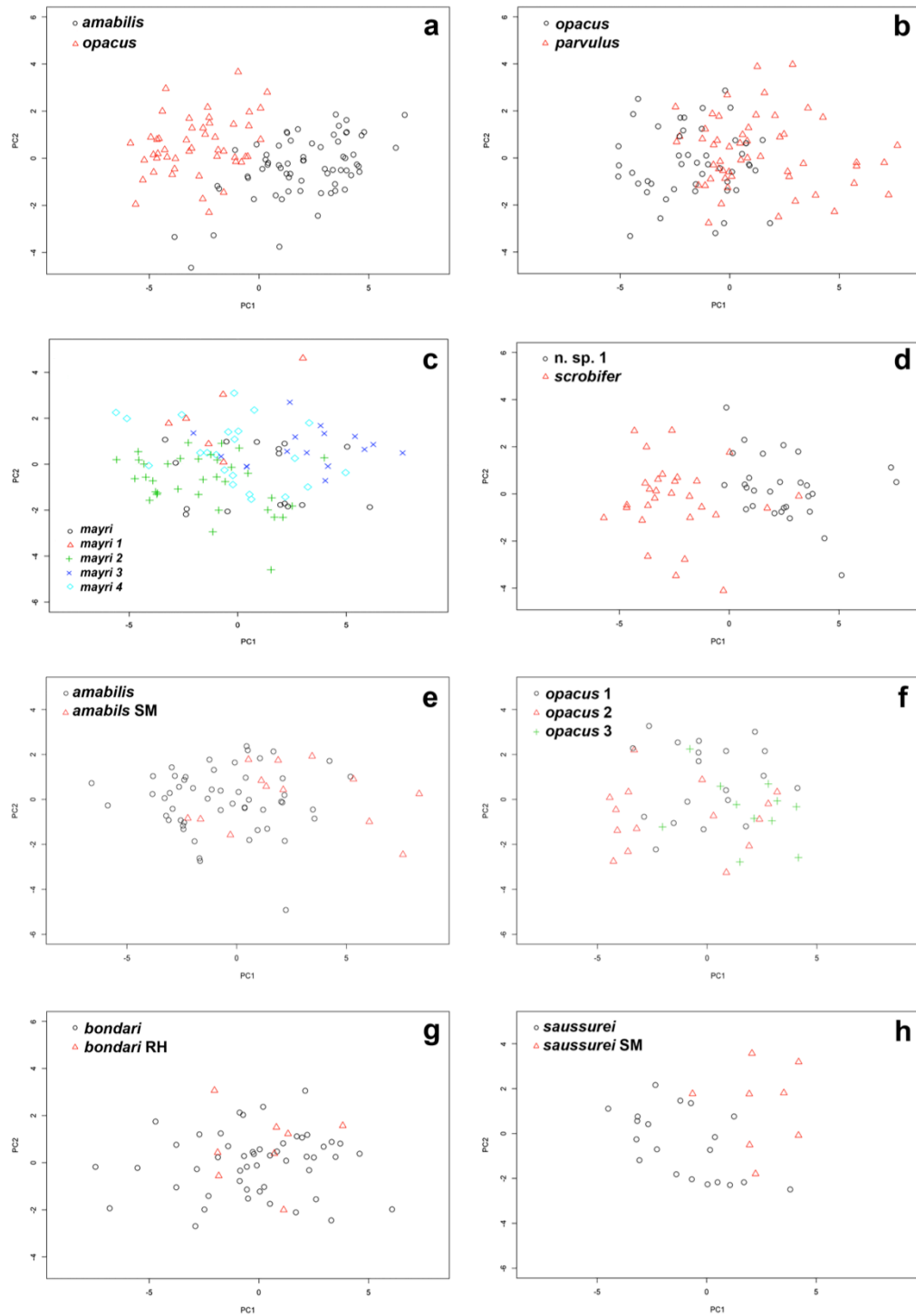


Figure 3.5. Principal component analysis of reduced data sets, scatter plots.

a *S. amabilis* and *S. opacus*; **b** *S. opacus* and *S. parvulus*; **c** populations of *S. mayri*; **d** *S. n. sp. 1* and *S. scrobifer*; **e** populations of striate-mandibled *S. amabilis* vs. smooth-mandibled (SM) *S. amabilis*; **f**

populations of *S. opacus*; **g** populations of typical *S. bondari* vs. reduced-hair (RH) *S. bondari*; **h** populations striate-mandibled *S. saussurei* vs. smooth-mandibled (SM) *S. saussurei*.

3.3.2. Species delimitation

In delimiting species, we adopted the modified biological species concept (BSC) (Mayr, 1942) in the sense of Coyne and Orr, which defines species as groups of interbreeding natural populations that are characterized by substantial but not necessarily complete reproductive isolation, thus not excluding some degree of introgression after evolutionary divergence (Coyne & Orr, 2004). Unlike phylogenetic species concepts (De Queiroz & Donoghue, 1988; Cracraft, 1989; Baum & Donoghue, 1995), which require sister species to be reciprocally monophyletic, the BSC allows for the reality that phylogenies of genes in sister species may be paraphyletic or polyphyletic with regard to species boundaries (Brown *et al.*, 1994; Funk *et al.*, 1995; Harrison, 1998; Coyne & Orr, 2004). Given the relatively recent origins of *Sericomyrmex* species (Chapters 1&2), such an allowance is critical. In fact, we encountered one such possible case of a species that was paraphyletic with respect to its sister species (see *S. amabilis* notes section for details).

Our practical criteria for recognizing species were (i) a well-supported sister-group relationship (not necessarily reciprocally monophyletic, see above) on our molecular phylogeny and (ii) a unique combination of morphological character states, including characters of workers, queens, males, and larvae, as well as measurement characters based on our morphometric analyses of workers. In recognizing species we also took into account the geographic distributions of populations. E.g., we did not describe two molecularly distinct (i.e., reciprocally monophyletic) allopatric populations

as separate species if they were morphologically indistinguishable, whereas we described co-occurring, genetically and/or morphologically distinct, sympatric populations as two separate species.

3.3.2. Genus *Sericomyrmex* description

Following are genus-level descriptions and diagnoses of *Sericomyrmex* workers, queens, males, and larvae, describing character states that are shared by all species within the genus. In the “Character Discussion” we discuss characters with states that are variable across the genus but fixed within species, and therefore important for distinguishing between *Sericomyrmex* species.

***Sericomyrmex* worker**

Small to medium-sized ants (mean WL: *parvulus*=1.04 mm, *mayri*=1.71 mm), body color evenly light yellow or yellowish-brown to deep, ferrugineous-brown. Some individuals with darker areas on frontal lobe fenestrae and along the frontal carinae. Integument dull and opaque, in adult workers covered with what appears to be a waxy cuticular layer (Figure 3.6 a, b, c.). This layer is absent in callow workers and males (Figure 3.6 d, e, f). It is also completely or partially absent in some workers of *S. n. sp. 1* (see *S. n. sp. 1* notes for details).

Entire body covered with dense pubescence: short, thin, appressed to decumbent, light yellow hairs. In addition to pubescent hairs entire body covered with thicker (Figure 3.6 b, c.), longer, often flexuous hairs, which are yellow to gray or black, often darker in color at the base, appressed to erect. We refer to these thicker, non-pubescent hairs

simply as “hairs”, whereas, when referring to pubescence, we use the terms “pubescence” or “pubescent hairs”. The hairs vary in length and density: dorsum of head, mesosoma, and gaster usually with denser and longer hairs than the rest of the body. The only areas of the body with the integument glossy instead of opaque and mostly devoid of hairs are the mandibles and the metapleural gland bulla.

Head cordate, tapering anteriorly, lateral borders of head slightly convex, shape of posterior cephalic corners acute to round, depending on the species, but never with occipital tubercles or spines as in most *Trachymyrmex* species. Posterior cephalic margin medially emarginate, the depth and shape of the emargination varying between and, to a lesser extent, within species. Vertex medially impressed in frontal view and with two small, low tumuli (Figure 3.7 a: Tu) present laterad of vertexal impression, one on each side, sometimes distinct and sometimes barely visible.

Anterior clypeal border broadly convex and medially notched. The notch shallow but almost always visible under higher magnifications. Unpaired median clypeal seta arising from the middle of the clypeal apron, somewhat posterior from the anterior edge (Figure 3.6 g: MCS.). Mandibles triangular, glossy, variably striate or smooth. Masticatory margin of mandible with 7 to 9 teeth, varying within species, no consistent teeth differences to separate species observed, sometimes in a single specimen left and right mandible with different number of teeth. Lateral margin of mandible straight in basal two-thirds in frontal view, curving at apex. Edge of masticatory margin with variable 2–6 short, decumbent hairs, directed medially. Slender, light-colored, short hairs evenly but sparsely distributed over entire mandibular surface, directed anteriorly (Figure 3.6 g, h, i). Palp formula 4, 2.

Preocular carinae present, directed posterad but fading at the tops of the eyes, never reaching the posterior cephalic corners to join with the frontal carinae to form complete antennal scrobes. Eyes distinct, placed laterally on anterior half of head, variable in size and shape, from almost flat to convex and protruding laterally, in some species covered with a silvery-white layer of an unknown, waxy substance, hereafter referred to as a "white layer". Number of ommatidia across the largest eye diameter 7–14, depending on the species. Frontal lobes always present, completely covering the antennal condylar bulb, with three distinct margins: posterior, lateral and medial (Figure 3.7 a). Frontal lobe size and shape vary between species. Frontal lobe with a dorsal fenestra, i.e., a thinner, sometimes almost translucent area just above the condylar bulb, circular in shape and darker in color on the edges (Figure 3.7 g: Fe). Frontal carinae straight to slightly curved laterally, diverging toward the posterior cephalic corners, in some species fading before reaching the cephalic corners, in others complete. Area laterad of frontal carinae, the incomplete antennal scrobe, with less hair than rest of head, mostly appressed pubescence. Antenna 11-segmented, lacking a distinct antennal club. Antennal scape relatively short, never extending beyond posterior cephalic margin, slightly narrower at the base than distally, angled in the first third, slightly curved distally. Antennal pedicel longer than funicular segments two and three combined.

Mesosoma (Figure 3.7 l, m). Inferior pronotal corner forming an obtuse angle, sometimes bearing a small denticle pointing anterad, best seen in lateral view, variable within species. Pronotum anteriorly with a single, low median tumulus, and two larger, lateral pronotal spines, both best seen in postero-dorsal view. Mesonotum with two pairs of spines or tubercles: the anterior mesonotal spines, the most prominent feature of the

mesosoma, and the posterior mesonotal tubercles, smaller and lower (Figure 3.7 l, m.). Metanotal groove distinct, best seen in lateral view, with less hairs than the rest of the mesosomal dorsum. Propodeum dorsally with two longitudinal, low, posteriorly diverging carinae, sometimes weakly serrate, each often ending in a small and blunt denticle. Propodeal spiracular carina absent, propodeal spiracle opening oval, directed posterad, mounted on a small tubercle. Metapleural gland bulla transparent and glossy, devoid of hairs. Mid and hind tibial spurs absent. Arolium present.

Metasoma. Petiole with a short peduncle, in lateral view longer than postpetiole, lacking a subpetiolar process. Nodes of petiole and postpetiole each with a pair of low, short, serrate, longitudinal carinae, which in the petiole are sometimes reduced to two low denticles, best seen in dorsolateral view. Postpetiole in dorsal view broader than long, and broader than petiole. Anterior edge of ventral sternite of postpetiole protruding anteriorly, so that in lateral view it sometimes looks like an anteriorly directed ventral postpetiolar process (Figure 3.7 b). First gastral tergite (A4) larger and longer than first sternite, dorsally overhanging the remaining segments (A5–A8). First gastral tergite laterally impressed on both sides, and with a pair of lateral longitudinal carinae in the anterior two-thirds, weakly to strongly developed, curved ventrally, in some species a pair of anteromedian dorsal carinae also present, weakly to strongly developed (Figure 3.7 c).

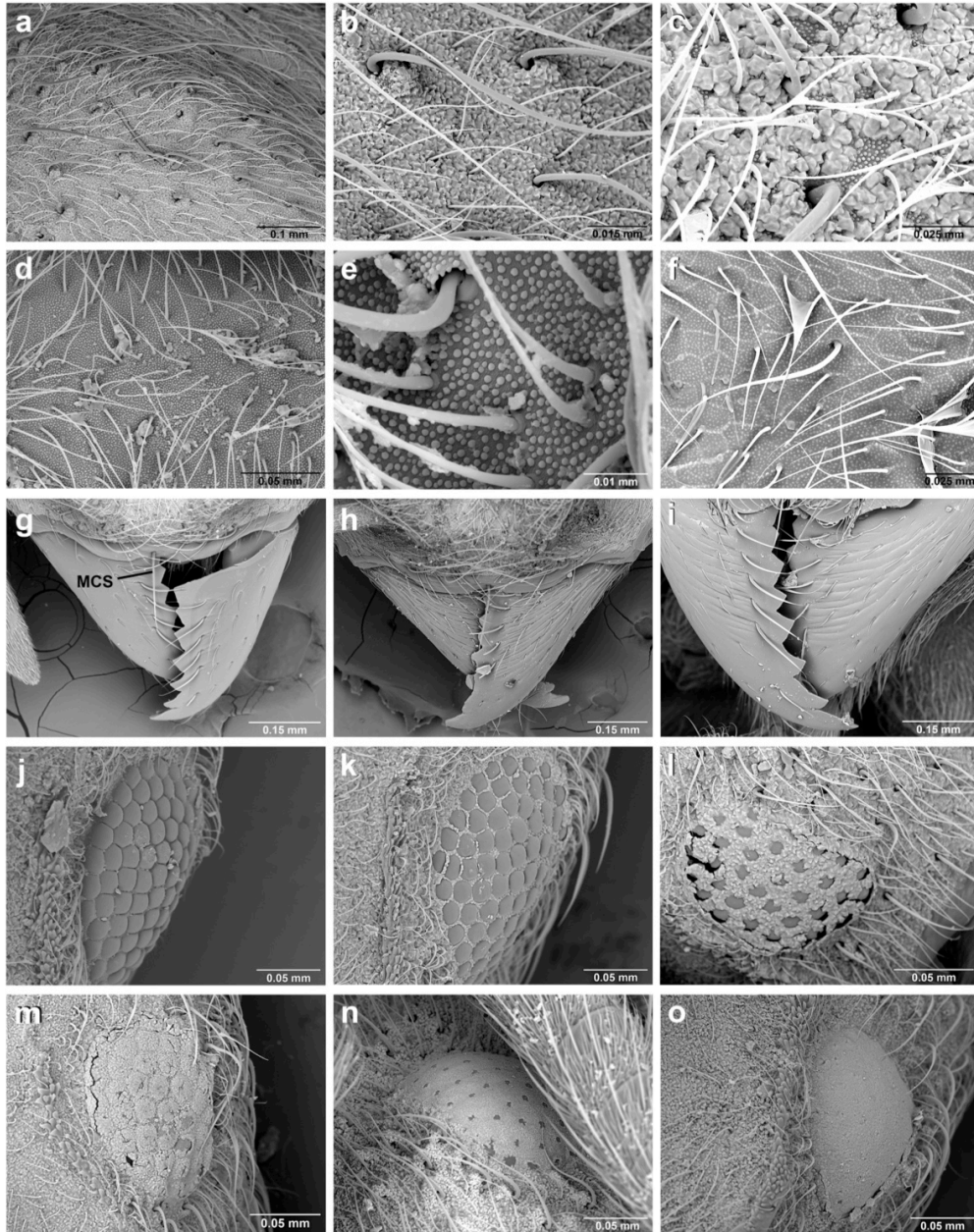


Figure 3.6. Morphology (SEM images): integument, mandibles, and eyes.

Integument: **a, b, c** adult worker integument; **d, e** callow worker integument; **f** male integument.

Mandibles: **g** smooth (*S. parvulus* worker), MCS—Median clypeal seta; **h** striate (*S. saussurei* worker); **i**

faintly striate (callow worker of *S. mayri*). Eyes: **j, k** eyes without the white layer; **i** partially covered eyes

(*S. parvulus*); **m** completely covered eyes, but individual ommatidia still discernible (*S. opacus*); **n** eyes

with thick white layer, ommatidia visible through narrow holes in the layer (*S. saussurei*); **o** eyes

completely covered with white layer, individual ommatidia not visible (*S. saussurei*).

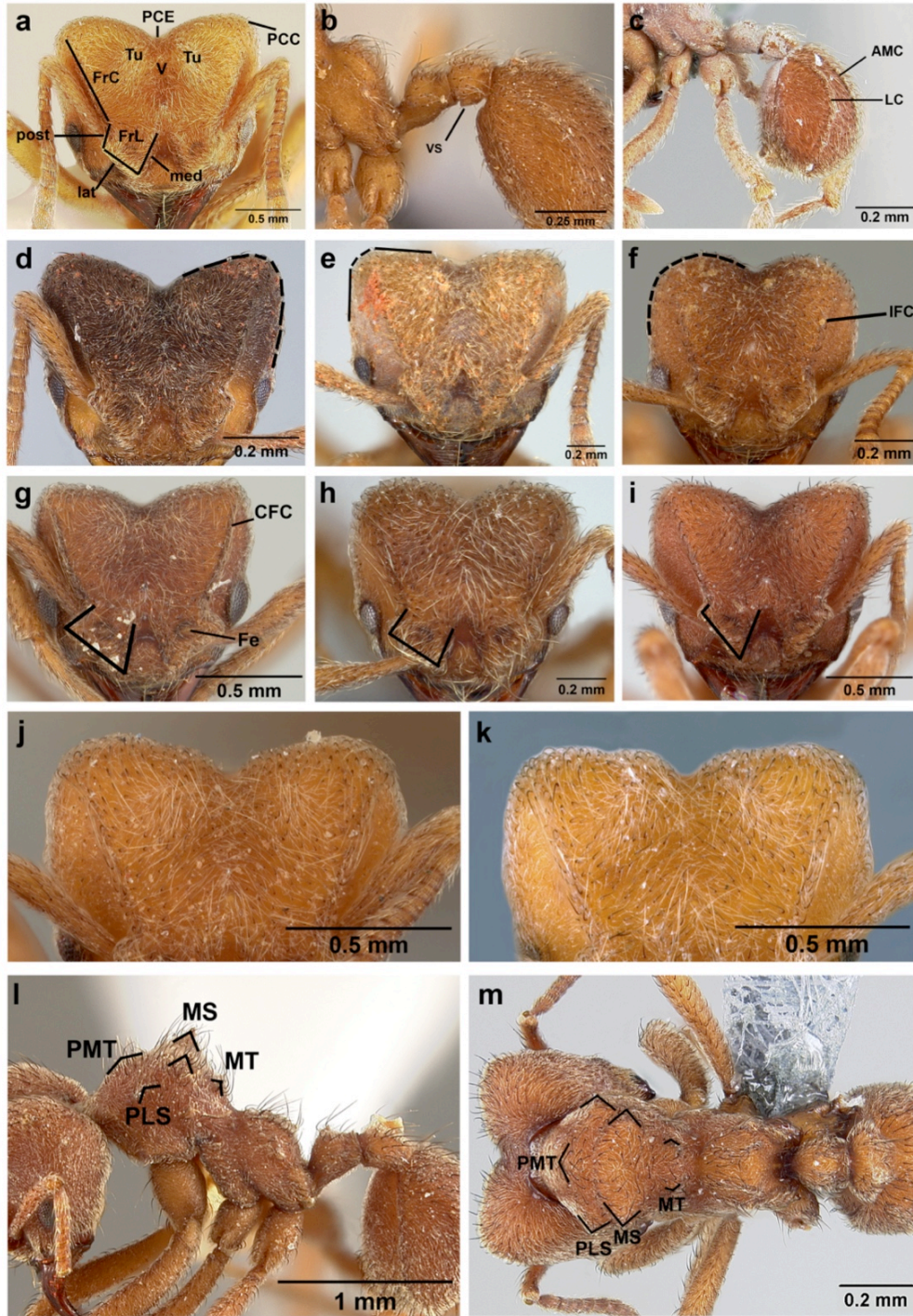


Figure 3.7. Worker morphology and terminology.

a Head; **b** postpetiole lateral view; **c** gaster lateral view. Posterior cephalic corner shapes: **d** acute posterior cephalic corner; **e** rectangular posterior cephalic corner; **f** rounded posterior cephalic corner. Frontal lobes shapes: **g** trapeziform frontal lobes; **h** rectangular frontal lobes; **i** triangular frontal lobes. Posterior cephalic

border shapes: **j** posterior cephalic border, gradual emargination; **k** posterior cephalic border, abrupt emargination. Mesosoma morphology: **l** mesosoma lateral view; **m** mesosoma dorsal view.

PCC–posterior cephalic corner, **PCE**–posterior cephalic emargination, **Tu**–tumuli, **V**–vertexal impression, **FrC**–frontal carinae, **FrL**–frontal lobe, **post**–posterior frontal lobes margin, **lat**–lateral frontal lobes margin, **med**–medial frontal lobes margin, **vs**–postpetiolar ventral sternite, **LC**–lateral gastral carinae, **AMC**–anteromedian gastral carinae, **IFC**–incomplete frontal carinae, **CFC**–complete frontal carinae, **Fe**–frontal lobe fenestra, **PMT**–pronotal median tumulus, **PLS**–pronotal lateral spine, **MS**–mesonotal spine, **MT**–mesonotal tubercle.

***Sericomyrmex* queen**

(Genus level description of *Sericomyrmex* queen is based on *S. amabilis*, *S. bondari*, *S. lutzi*, *S. n. sp. 1*, *S. mayri*, *S. opacus*, *S. parvulus*, *S. n. sp. 2*, and *S. saussurei*. Queens of *S. n. sp. 3* and *S. scrobifer* unknown.

Larger than worker (e.g., *amabilis* worker and queen, mean values: HW=1.06 w, 1.32 q; WL=1.37 w, 2.05 q; GL=0.96 w, 1.77 q), body color ferrugineous-brown, often darker than worker, integument dull, opaque, pilosity same as in worker.

Head. Head cordate, with cephalic emargination always distinct. Vertexal tumuli more pronounced than in worker, bearing ocelli, integument surrounding ocelli sometimes darker in color than elsewhere. Glossy, light yellow or white to dark grey ocelli present, half embedded in integument and covered with hair so in frontal view usually only the median anterior ocellus is visible. Preocular carinae present and, in some species, fading shortly beyond the tops of the eyes, just as in the worker. However, in other species, preocular carinae extending beyond the eyes, almost converging with the frontal carinae to form complete scrobes; in these species, however, the preocular carinae are much thinner and weaker posterad of the eye (Figure 3.8 a.). In a third state, the

preocular carinae fade posterad of the eye, but, posterior to the eye, are replaced by one or more short, weak carinae that never reach the posterior cephalic corners (Figure 3.8 b, c). Eyes larger and more convex than in worker, almost never covered with a white layer (except in the *S. saussurei* queen, in which a thin white layer occurs at the borders of the eyes). Frontal lobes similar to worker, but sometimes more robust.

Mesosoma (Figure 3.8 d, e). Lateral pronotal tubercles present, conical, very short and blunt, best seen in dorsal view. Anapleural suture present (=median episternal groove); wide, shallow, and curved in lateral view. Anepisternum inflated. Mesoscutum in dorsal view with reduced notauli in the form of faint, shallow impressions, sometimes entirely absent. Median mesoscutal line sometimes present, often reduced, and visible only in its anterior portion, this anterior portion sometimes developed into a weak costa, and its posterior end with shallow longitudinal impressions on each side. Parapsidal lines present, faint, slightly curved. Axillae in dorsal view entirely separated from one another, laterally rounded, narrowing medially. In dorsal view a transverse shallow groove separating axillae from the scutellum, this groove sometimes transversely costate. Scutellum slightly convex in lateral view, in dorsal view narrowing posteriorly, posterior margin with wide, shallow, median V-shaped notch, this notch sometimes continuing into a median impression that appears to divide the scutellum into two lateral parts. Propodeum with two low carinae, posteriorly diverging in dorsal view, often reduced to laterally flattened, obtuse denticles.

Wings (Figure 3.8 f, h) Light to dark brown, opaque, covered with minute pilosity, veins dark brown. Forewing (Length: 4.85–8.03) with 5 closed cells (terminology follows Goulet and Huber, 1993): costal (C), radial (R), cubital (Cu), first radial 1 (1R1), and first

radial 2 (2R1). Pterostigma not visible. Cubitus and media fading towards the wing margin. In a few specimens of *S. mayri* cubitus with 1–2 short spur veins distally. Cubital cell rounded distally, anal vein not extending distad beyond cell as a free vein or spur. Hindwing (Length: 3.6–5.28) with reduced venation, just two enclosed cells: radial and cubital, cubital much smaller than radial. Cubitus and distal part of radius faded. In a few specimens of *S. mayri* media with 1–3 short spur veins distally. Anterior margin of hindwing with 7–9 hamuli, varying within species.

Metasoma. Petiole compact, without a subpetiolar process, petiolar peduncle short. Node of petiole with two denticles dorsally, often acute, and with two smaller lateral denticles, best seen in frontodorsal view. Dorsal petiolar denticles more prominent than in the worker. Postpetiole broader than long, and broader than petiole in dorsal view, with four short longitudinal carinae, two dorsal and two lateral, which are sometimes reduced to low denticles, best seen in frontodorsal view. The size and sharpness of the petiolar denticles is usually correlated with size, so bigger species have larger and sharper denticles. First gastral tergite (A4) larger and longer than first sternite, dorsally overhanging the remaining segments (A5–A8). First gastral tergite laterally impressed on both sides, and with a pair of usually strongly developed lateral longitudinal carinae. First gastral tergite dorsum with shallow longitudinal anteromedian groove (Figure 3.8 d), anteromedian dorsal carinae on each side of median groove vestigial or absent.

***Sericomyrmex* male**

(Genus level description of *Sericomyrmex* male is based on *S. amabilis*, *S. lutzi*, *S. mayri*, *S. opacus*, and *S. saussurei*. Males of *S. bondari*, *S. n. sp. 1*, *S. parvulus*, *S. n. sp. 3*, *S. n. sp. 2*, and *S. scrobifer* unknown.)

Body color pale yellow-brown to dark brown. Head darker, antennae and legs lighter than the rest of the body. Mesosoma in dorsal view sometimes differently colored along mesoscutal lines, i.e., the notauli, parapsidal lines, and median mesoscutal sulcus may be darker or sometimes lighter than the surrounding areas. A combination of fine pubescence and thicker hairs covering the body, but sparser than in worker and queen. The hairs distributed unevenly: area around ocelli and dorsum of petiole and postpetiole with more hair; gaster and thorax, dorsally and laterally, with less hair. Integument relatively dull, with reticulate sculpture, but appearing shinier than the very opaque cuticles of worker and queen. This difference is especially striking in SEM images, which reveal that males do not have what appears to be a cuticular wax layer that is present in workers and queens (Figure 3.6 f).

Head. Obovate to subquadrate in frontal view, from equally long and wide to longer than wide (CI=100–133), posterior cephalic margin straight, without posterior cephalic median emargination. Lateral margins of the head straight or slightly convex. Vertex with three large, white to grey ocelli, mounted on sides of small tumuli. Clypeus in frontal view broadly convex, medially with shallow notch. Unpaired median clypeal seta arising from the middle of clypeal apron, somewhat posterior from the anterior edge. Mandible triangular, with lateral margin straight, except curving at apex. Dorsal surface of mandible finely reticulated near the basal angle, and with thin, light yellow, sparsely

distributed hairs, directed anterad. Masticatory margin of mandibles with 5–7 teeth, usually 4–5 teeth in the distal two-thirds of the mandible, a single basal tooth separated from the rest by a gap (Figure 3.18 i); however, sometimes this gap absent and teeth distributed more or less evenly along the mandible (Figure 3.50 b). Palp formula: 4, 2. Preocular carina present, varying in length, directed posterad, slightly curved medially before fading. Eyes convex, large, protruding laterally, 20–28 ommatidia across the largest diameter. Frontal lobes very small and narrow, not completely concealing condylar bulbs, and without clearly defined medial, lateral, and posterior margins. Frontal carinae absent. Antennal scape long, straight, extending well beyond the posterior cephalic border (SI=74–95). Antenna 12-segmented, a departure from usual 13 segments in male attine ants, without distinct club. Pedicel thicker and longer than funicular segments two or three.

Mesosoma (Figure 3.8 e). Inferior pronotal corner obtuse, never denticulate. Lateral pronotal tubercle absent. Mesopleuron with shallow anapleural suture. Katepisternum inflated and rounded ventrally, best seen in lateral view. Mesoscutum in dorsal view with notauli, most visible anteriorly, not converging posteriorly. Median mesoscutal line present, best visible in antero-dorsal view, fading posteriorly. Parapsidal lines present. Notauli, mesoscutal, and parapsidal lines sometimes lighter than the surrounding integument and sometimes darker; this variation is seen within males from the same nest, and is not useful in separating species. Axillae rounded laterally, entirely separated from one another, from scutum anteriorly, and from scutellum posteriorly by a shallow sulcus. This sulcus can be smooth but often has transverse short costae, sometimes just one or two medially, sometimes more. Scutellum inflated, convex in

lateral view, in dorsal view narrowing posteriorly, posterior margin straight or with very shallow medial notch. Propodeum dorsally completely smooth or with two very small, low, short propodeal carinae. Propodeal spiracle directed posterad, mounted on a small tubercle, best seen in dorsal view, propodeal spiracular carina absent.

Wings (Figure 3.8 g, i). Venation and cells same as in queen, but wings smaller and lighter in color. Forewing length: 3.48–6.25, hindwing: 2.20–4.29 mm. Anterior margin of hindwing with 7–10 hamuli.

Metasoma Petiole with short peduncle, without subpetiolar process, longer than postpetiole in lateral view. Petiole with a pair of small denticles laterally, and sometimes also dorsally. Postpetiole in dorsal view broader than long, and broader than petiole. Postpetiole laterally with a pair of very reduced denticles, dorsally smooth. Gaster in dorsal view elliptical, without any carinae. First gastral tergite and sternite equal in length and size, gastral tergites and sternites 2–5 (i.e. A5 to A8) visible in dorsal view. Rows of long, decumbent to suberect hairs present on posterior borders of gastral segments 2–5 (A5–A8), the hairs on the dorsal side slightly longer than the ventral ones.

Genitalia. (Figure 3.9) Parameres simple, with basimere smooth to longitudinally striate towards its posterior margins, telomeres narrow, elongated, medially curved and apically rounded, with sparse simple hairs. Volsellae each with a strongly medially curved, clubbed digitus, cuspis present as a rounded lobe, sometimes with 1–2 short teeth, covered with thin, sparse hairs. Volsellae basally with two additional processes, best seen in ventral view, here named the proximal basivolsellar and distal basivolsellar process. These processes represent previously undescribed, novel structures of male ant genitalia. Valviceps of penisvalve with 10–12 denticles ventrally, posteriorly notched.

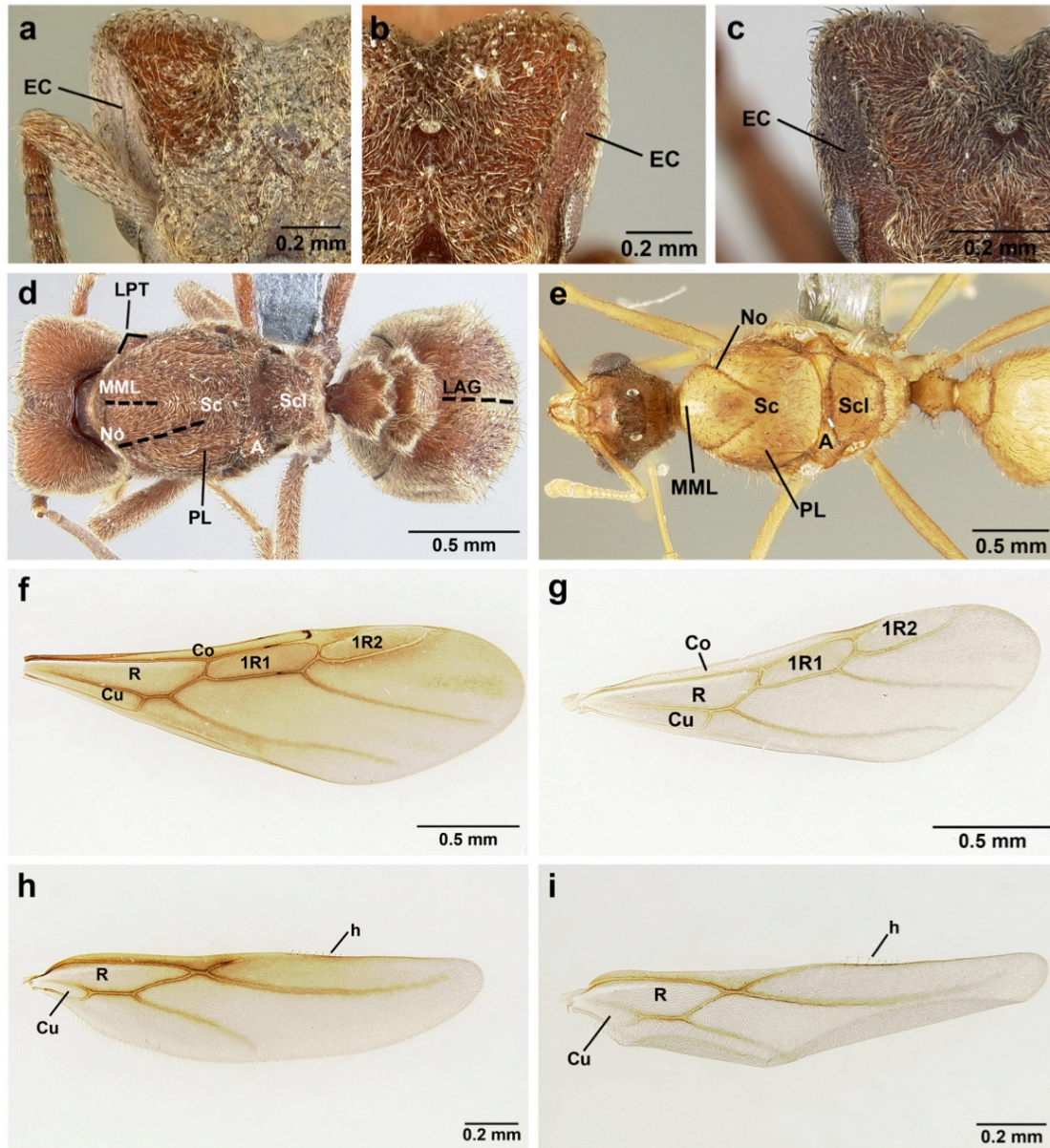


Figure 3.8. Queen and male morphology. **a** *S. n. sp. 1* queen head, frontal view, eye carina; **b** *S. parvulus* queen head, frontal view, eye carina; **c** *S. bondari* queen head, frontal view, eye carina; **d** queen dorsal view; **e** male dorsal view; **f** queen front wing; **g** queen hind wing; **h** male front wing; **i** male hind wing. **EC**—preocular carina; **MML**—median mesoscutal line; **No**—notauli; **LPT**—lateral pronotal tubercle; **PL**—parapsidal line; **A**—axilla; **Sc**—scutum; **Scl**—scutellum; **LAG**—longitudinal anteromedian groove; **Co**—costal cell; **R**—radial cell, **Cu**—cubital cell; **1R1**—First radial 1 cell; **1R2**—First radial 2 cell.

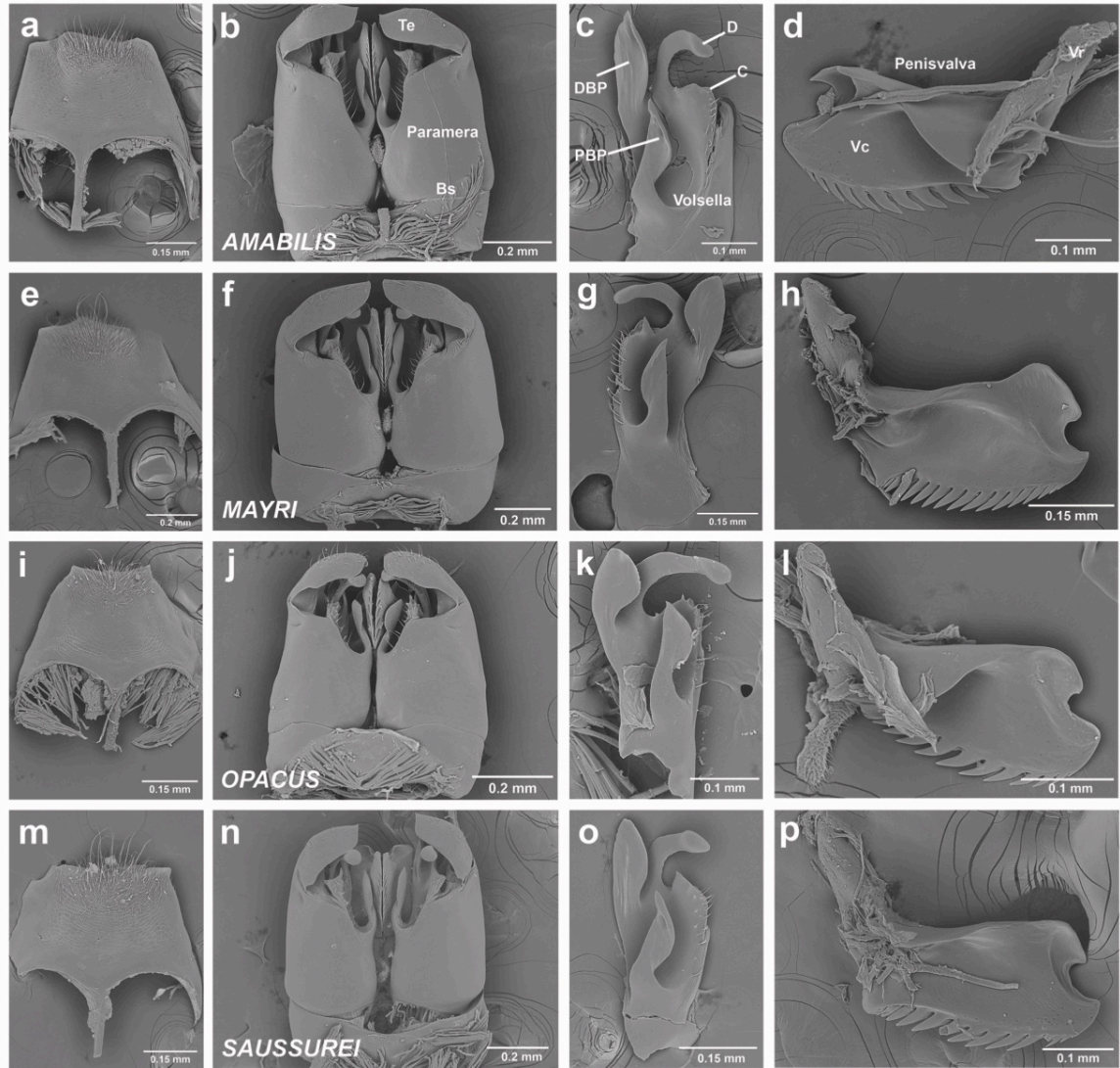


Figure 3.9. *Sericomymex* male genitalia. *S. amabilis* (a–d), *S. mayri* (e–h), *S. opacus* (i–l), *S. saussurei* (m–p). Abdominal sternum IX, ectal view (a, e, i, m); genital capsule, ventral view (b, f, j, n); volsella (c, g, k, o) and penisvalve (d, h, l, p). Bs basimere; Te telomere; D digitus; C cuspid; DBP distal basivolsellar process; PBP proximal basivolsellar process; Vr valvura; Vc valviceps.

Sericomymex larvae

Description based on SEM study of 23 prepupae specimens from 9 different *Sericomymex* nests of the larvae of *Sericomymex amabilis*, *S. bondari*, *S. mayri*, *S. opacus*, *S. parvulus*, *S. n. sp. 2*, and *S. saussurei*. No larvae were available for *S. lutzi*, *S.*

n. sp. 3, *S. scrobifer*, and *S. n. sp. 1*. Terminology follows Schultz and Meier (Schultz & Meier, 1995), including the usage of the terms “seta” (a structure with a visible basal setal socket) and “hair” (a structure without a visible socket). We only use the term seta in our description, since the socket is always visible. When the setal base is visible but the seta is either absent or very short, we refer to it as a sensilliform or papilliform seta, respectively.

General body. Body profile “attoid” *sensu* Wheeler & Wheeler (1948), i.e., longitudinally curved, bean-shaped, with ventral profile shorter than dorsal profile (Figure 3.10). As in all other attine ants, thoracic-abdominal articulation absent, thoracic intersegmental constrictions superficial, deep lateral depressions associated with abdominal spiracles absent, and leg vestiges present and visible as open slits ventrally on the thorax. Setae on dorsal and lateral body surfaces vary, from bare (*S. parvulus*) to more than 15 setae on each side (*S. amabilis*). All setae on the body are simple, sometimes long and flexuous. Some setae (e.g., on clypeus and labrum) very reduced, papilliform, with only the setal socket and a very short bristle visible (Figure 3.10 f: PfS). *Head.* Genal lobes present (Figure 3.10 c, d, e: Gn). Presence of supra-antennal setae (setae above the level of antennae, Figure 3.10 c: SaS) variable: one long seta present over each antenna (“*Sericomyrmex* condition” *sensu* Schultz & Meier 1995) in some species (*S. amabilis*, *S. bondari*, *S. mayri*), absent in others (*S. opacus*, *S. parvulus*, *S. n. sp. 2*, *S. saussurei*).

No setae between the antennae, and four setae present on each gena, except in *S. mayri*, which has 6 genal setae on each side. Two supraclypeal setae present in all species, reduced to papillae, except in *S. bondari*, which has two long supraclypeal setae

(Figure 3.10 d: ClyS). Spinules on head present, but restricted to clypeus, genae, and vertex; in *S. opacus* almost completely absent, restricted to a few on the clypeus.

Mouthparts. Labrum monolobate, narrow, and inflated, with anterior setae either entirely absent or reduced to sensilla or papillae. Mandibles fleshy and subconical; a synapomorphy for attine ants. Spinules on mandibles covering entire surface, not just the base. Subapical mandibular tooth absent, mandibular apical tooth distinct, divided in some species (Figure 3.10 g), undivided in others. Mandibular gnathobases absent. Basal portion of maxilla fused with the head capsule and the maxillary palp widely removed laterad from galea, as in other neoattine ants. Maxillary palp digitiform, maxillary accessory palpal sensillum present (Figure 3.10 e: PlpS). Area between maxillary galea and palp with 2 reduced setae. As in most attine ants, labium short, only feebly protruding, lateral sericteral protuberances absent, labial palps reduced to sensilla. Spinules on labium either completely absent or sparse, present anterior to sericteries. Ventral part of labium (posterior to sericteries) usually not visible. This state is shared with *M. explicatus*, which also has sparse labial spinules and ventral surface of labium hidden. Hypopharyngeal spinules densely distributed and predominantly multidentate.

Thorax and abdomen. Thoracic segment one (T1) ventrally with transverse rows of sparsely distributed multidentate spinules (see *S. saussurei* description for an exception). Thoracic segments two and three (T2 and T3) without multidentate spinules, except in *S. bondari*, which has sparse spinules on T2. Number of setae on ventral thoracic segments varies from 0–5 on each side of each segment, and in general T1 with more setae than T2 or T3. Total number of setae on T1 is less than 10 in most species. An exception is *S. mayri*, which has 10–14 setae on T1, the number of setae considered

sufficient to form, in combination with the genal setae (6 in *S. mayri*), the “feeding basket” of attine ant larvae (Schultz & Meier 1995). Abdominal segments lacking any ventromedian protuberances, and with variable numbers of relatively long and simple setae, except in *S. parvulus*, which has no ventral abdominal setae. Anal setal pattern conforming to typical “*Sericomyrmex* pattern” (Schultz, & Meier, 1995), with a single pair of long to papilliform setae anterior to the anal opening (Figure 3.10 h, i). An additional pair of setae sometimes present ventrolaterally on segment 9 (e.g., in *S. bondari*), but widely removed (Figure 3.10 j). Ventral anal lip absent.

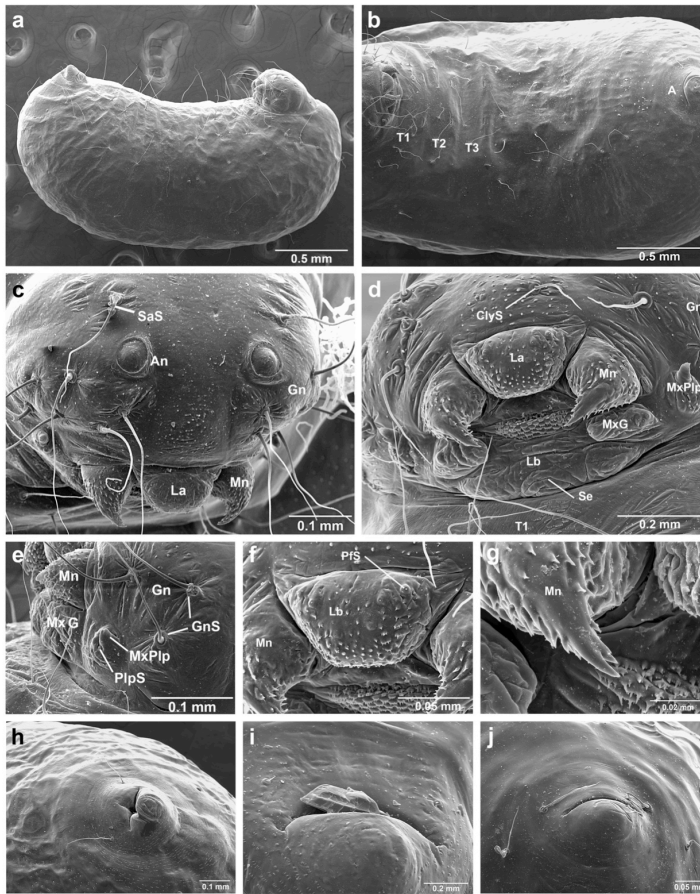


Figure 3.10. Larval morphology (SEM images). **a)** Lateral view; **b)** ventral view; **c)** head dorso–frontal view; **d)** mouthparts, frontal view; **e)** head, lateral view; **f)** labrum detail; **g)** mandible detail; **h, i, j)** anal setae. **T1, T2, T3**–thoracic segments 1, 2 and 3; **A**–anus; **SaS**–supra antennal seta; **An**–antenna; **Gn**–gena,

La–labrum; **Mn**–mandible; **MxG**–maxilla: galea; **Lb**–labium; **Se**–sericteries; **MxPlp**–Maxillary palp; **ClyS**–clypeal setae; **GnS**–genal setae; **PlpS**–maxillary palp sensillum; **PfS**–papilliform seta.

3.3.3. Genus *Sericomyrmex* diagnosis

Ants of the genus *Sericomyrmex* are small- to medium-sized, larger than ants of most of the lower attine ant genera (e.g., *Cyphomyrmex*, most *Apterostigma*), and similar in size to most *Trachymyrmex* species and to the minor workers of leaf-cutter ants. They are monomorphic to slightly polymorphic, and can easily be separated from other attine ant genera by the combination of the following characters: a silky, woolly appearance due to dense pilosity; cordate head shape; well-developed frontal lobes; robust and short, moderately tuberculate mesosoma; and smooth gaster, without obvious tubercles. Members of the *Trachymyrmex iheringi* clade are similar to *Sericomyrmex* in size, general appearance, and presence of long, flexuous hairs (in some species, e.g., *T. opulentus* and *T. dichrous*), but *Sericomyrmex* can be distinguished from them by the following: posterior cephalic corners in frontal view smooth (most *Trachymyrmex* with multiple small to large denticles, tubercles, or spines, with exception of *T. dichrous*), antennal scape short, in frontal view almost reaching cephalic margin but never surpassing it (scape longer in *Trachymyrmex*, always surpassing cephalic margin), frontal lobes wide, concealing the base of scape, with 3 distinct margins (in *Trachymyrmex* often rounded or with just two margins, smaller and narrower, often not concealing the base of the scape), tubercles on mesosoma smooth, simple, without minute tubercles on them (“tubercles on tubercles” is very common in *Trachymyrmex*, giving the mesosoma a spiny appearance), gaster smooth, unsculptured, if mildly tuberculate this is visible only under SEM (in *Trachymyrmex* gaster often visibly tuberculate). Because of the absence

of denticles and spines on the posterior cephalic corners, dense hair, and tubercles on the mesosoma without additional, minute tubercles, *T. dichrous* can be mistaken for a *Sericomyrmex* species. It is easily identified and separated from *Sericomyrmex* by its bicolored integument (head dark brown, rest of body light ferruginous brown), narrow and rounded frontal lobes, and strongly convex eyes protruding from the sides of the head.

Ants of the genus *Apterostigma* can be mistaken for *Sericomyrmex* because of their long, silky hairs. *Apterostigma* can be separated from *Sericomyrmex*, even with a field hand lens, as follows: head long, narrowing posterad of the eyes (except for *A. megacephala*); posterior cephalic border evenly rounded, never medially emarginate (median emargination always present in *Sericomyrmex*); frontal lobes frequently rounded (rectangular or triangular with 3 distinct margins in *Sericomyrmex*); mesosoma and general appearance slender, similar to some non-attine ant Myrmicinae genera (e.g., *Aphaenogaster*) (*Sericomyrmex* habitus more robust).

Males of *Sericomyrmex* can be separated from most other attine males by the presence of 12 antennal segments, deviating from the usual 13 segments in the males of most attine species. However, 12 antennal segments occur in parallel in males of the following attine species: *Cyphomyrmex* (*C. faunulus* and *C. auritus*), *Trachymyrmex opulentus*, *Mycetagriocus inflatus*, and in a number of social parasites in the genus *Acromyrmex* (*A. charruanus*, *A. ameliae*, *A. insinator*). Also, males of the socially parasitic *Mycocepurus castrator* and *Pseudoatta argentina* have further reduced, 11-segmented antennae.

Males of *Sericomyrmex* can be separated from the other 12-segmented attine ants by the following: presence of relatively dense, long, flexuous hairs on most of the body (no hair

or very short and sparse hair in *M. inflatus*, *Cyphomyrmex*, and *Trachymyrmex*); dull, light brown integument, faintly reticulate and not strongly sclerotized (strongly sclerotized and dark brown to black in *A. charruanus* and *C. faunulus*, light brown but costate on head and mesosoma in *Trachymyrmex*), scutellum with flat to slightly notched posterior margin, but never with sharp and long posterior processes (as in *Trachymyrmex*, *C. faunulus*), smooth posterior cephalic corners (with a small sharp denticle in *C. faunulus*), and lateral pronotal spine or teeth absent (present in *Acromyrmex charruanus*). The larvae of *Sericomyrmex* can easily be recognized as those of attine ants because of the following synapomorphies: short, narrow, monolobate labrum; fleshy subconical mandibles; leg vestiges present as the open slits in the integument; and a reduced number of supra-antennal setae (one to no setae in *Sericomyrmex*). The larvae of *Sericomyrmex* can be separated from those of closely related *Trachymyrmex* species by a single pair of hairs anterior to the anus (5–8 extremely short to sensilliform setae in the *Trachymyrmex iheringi* group) and the presence of setae on the lateral and dorsal surfaces of the body in most species (setae absent in *Trachymyrmex*).

There are some characters that *Sericomyrmex* larvae share with those of the closely related *M. explicatus*, in particular labial spinules that are present only on the anterior surface dorsal to sericteries (ventral side of labium hidden) and the absence of supra-antennal setae (in some species of *Sericomyrmex* one seta present). The larva of *M. explicatus* differs from *Sericomyrmex* larvae by the anal setae pattern: *M. explicatus* with ~8 extremely short setae anterad and 0–2 setae dorsad of anus, all *Sericomyrmex* species we examined with 2 setae anterad, and 0–2 dorsad; and by the number of genal setae: 3 in *M. explicatus*, 4–6 in *Sericomyrmex*.

3.3.4. Character discussion and terminology definitions

Worker

General appearance. Worker color varies within species and even within single colonies, and can be altered by collecting and preservation methods, as well as by age, especially in dried, pinned specimens. The body color of an individual *Sericomyrmex* worker is homogeneous except in some *S. bondari* individuals from Carajas, Brazil (Figure 3.23 a, b, c), and in a few workers of *S. saussurei* in which some, but not all, nestmate workers have lighter-colored areas on their heads. The density of pubescence may vary both between and within species. Hair length, color, and degree of curvature (appressed to nearly erect) vary within species. Curvature in particular can be altered by collecting and preservation methods, for example, the hair of specimens collected by pitfall trapping may differ from that of workers from same nest collected directly into ethanol and subsequently point-mounted. Hair is, however, a useful character for recognizing the species *S. bondari*, which possesses uniquely thicker, much darker hairs (Figure 3.22).

Body size. Body size in *Sericomyrmex* is a useful character for separating some species. Because it varies to some extent within species and within colonies, it should be used with caution and in combination with other characters. We use head width including the eyes (HWe), Weber's length (WL), and hind femur length (HFL) in combination as an approximation for body size to group *Sericomyrmex* species into three size categories. The mean values for the size categories are as follows: small (HW<0.92, WL<1.18,

HFL<0.99); medium (HW=1.0–1.06, WL=1.28–1.39, HFL=1.1–1.24); and large (HW>1.15, WL>1.44, HFL>1.27).

Head. Head shape in *Sericomyrmex* is characteristically cordate, but the shape of the posterior corners and the size and shape of the median posterior cephalic emargination vary between species. Here we distinguish between three main categories of posterior corner shape: 1) *acute*, in which (in dorsal, i.e., full-face view) the lateral and posterior cephalic margins meet at an angle of less than 90 degrees (Figure 3.7 d); 2) *angular*, in which these margins meet at an angle approaching 90 degrees and in which the lateral and posterior cephalic margins are more or less straight (Figure 3.7 e); and 3) *rounded*, in which the transition between the lateral and posterior borders is smoothly rounded and in which the lateral and posterior borders are usually convex (Figure 3.7 f). Even though intermediate states exist, these three character states are very useful in describing the variation across *Sericomyrmex* species and, in combination with other characters, are useful in distinguishing species.

The shape of the median cephalic emargination varies in a manner that can be broadly captured by two states. The posterior cephalic margin between the cephalic corner and the median emargination can be straight to convex and it can merge with the emargination gradually (Figure 3.7 j) or it can be more or less straight and abruptly merge with the emargination (Figure 3.7 k). Intermediate states also exist, and in some species, particularly *S. amabilis* and *S. saussurei*, both states are often present within the same colony. However, the second (straight and abruptly merging) state predominates in the species *S. mayri*, whereas the gradual state occurs almost exclusively in the species *S.*

bondari, *S. lutzi*, *S. n. sp. 1*, *S. opacus*, *S. parvulus*, *S. n. sp. 3*, *S. n. sp. 2*, and *S. scrobifer*.

Mandibles. In some species (*S. parvulus*, *S. scrobifer*, *S. n. sp. 2*, *S. opacus*, *S. bondari*) the dorsal surface of the mandibles is glossy and smooth, lacking any striae except for very short and faint ones restricted to the masticatory margin. In *S. amabilis*, *S. saussurei*, and *S. mayri*, however, the mandibles are usually striate across the entire dorsal surface (Figure 3.6 g, h). Exceptions occur in each species: smooth mandibles occur in some populations of *S. amabilis* in Costa Rica, Ecuador, and Colombia, in populations of *S. saussurei* mostly in southeast Brazil, and in populations of *S. mayri* in Trinidad and Suriname. In callow workers of all species, dorsal striation is not very distinct (Figure 3.6 i). An intermediate stage exists in all three species, with mandibles just partly or faintly striate. Because the presence of smooth mandibles may indicate either that a specimen belongs to one of the smooth-mandibled species or that it belongs to a rare population of an otherwise striate-mandibled species, additional characters obviously need to be examined for species diagnosis. The presence of striate mandibles, in contrast, always indicates that a specimen belongs to one of the striate-mandibled species.

Eyes. Relative eye size, reflected in the eye index (EI), varies moderately within most species (EI 13.8–16), but it is noticeably larger in two species, *S. scrobifer* and *S. n. sp. 1* (EI 18–19). For describing eye size, we use three categories based on EI value: “small” eyes for species with EI <14, “medium-sized” eyes for species with EI between 14–16 (the majority of species), and “large” eyes for species with EI 18–19. Eyes can be flat to strongly convex, the latter protruding from the lateral borders of the head. Most

species have flat to mildly convex eyes, but *S. scrobifer* and *S. saussurei* eyes are consistently moderately (*S. saussurei*) or strongly (*S. scrobifer*) convex, so eye shape is useful for recognizing those species.

Another important character of the eyes is the presence or absence of what appears to be a white, waxy layer. This layer may be absent (Figure 3.6 j, k); it may only partially cover the eyes (in *S. parvulus*, Figure 3.6 l), or it may completely cover them. When completely covering the eye it can be relatively thin, so that the individual ommatidia are still discernible (as in *S. opacus*, Figure 3.6 m), or it can be so thick that individual ommatidia are hard to distinguish (as in *S. saussurei*, Figure 3.6), even though in some individuals the centers of individual ommatidia can be seen showing through the white layer in rare adult individuals (as in some *S. saussurei*, Figure 3.6 n). Below we refer to this layer simply as a “white layer”; its origin, whether as a cuticular secretion or due to some other cause, remains the subject of future investigation.

Frontal lobes. Frontal lobe size and shape are useful characters for *Sericomyrmex*, and here we distinguish three main states: 1) *trapeziform* (Figure 3.7 g), in which the posterior margin of the frontal lobe is subequal in length to the medial margin and in which the angles between the lateral and the other two margins are subequal and often acute; 2) *rectangular* (Figure 3.7 h), similar to trapeziform, but in which the medial and posterior margins are parallel and in which the angles between the lateral and the other two margins approximate 90 degrees; and 3) *triangular* (Figure 3.7 i), in which the posterior margin is at least half the length of the medial margin, sometimes much less than half; in which the angle between the lateral and posterior margins is obtuse; and in which the angle between the lateral and medial margins is acute, sometimes giving the

lobes the appearance of downward-pointing arrows. Although variants and intermediate states occur, and some degree of variation frequently occurs between individuals in the same nest, frontal lobe shape remains a very informative character. The frontal lobes of *S. scrobifer*, for example, can vary from trapeziform to rectangular, but are never triangular; and the frontal lobes of *S. bondari* are always triangular and narrow, with long lateral margins. Among these three states, triangular is the one with the most variation.

Frontal carinae. The length, shape, and robustness of the frontal carinae vary between species, but also to some extent within species. Frontal carinae can be complete and robust or, at the opposite extreme, weak and incomplete, fading before reaching the posterior corners. Complete and robust frontal carinae (Figure 3.7 g: CFC) are a good diagnostic criterion for some species (*S. scrobifer*, *S. n. sp. 1*), but the alternate state of reduced frontal carinae can be fairly variable within species, e.g., *S. parvulus* and *S. mayri* mostly have reduced frontal carinae (Figure 3.7 f: IFC), but in some individuals or populations this is not the case, so this character must be examined across nest series and employed in conjunction with other characters.

Scape length. Scape index is a useful character only in combination with other characters, and is diagnostic only for *S. n. sp. 3*, which has an unusually long scape (Figure 3.3 a: SI).

Mesosoma and metasoma. The tubercles and spines of the mesosoma, including the propodeal carinae and denticles, are in general sharper and more prominent in some species and less sharp and shorter in others. However, because within-species and even within-colony variation are common, this character must be used with caution. The same holds for the petiolar and postpetiolar denticles and carinae, which, again, can be more or

less pronounced within workers of the same colony. The presence or absence of a pair of anteromedian dorsal carinae on the first gastral tergite (Figure 3.7 c) is a more consistent character that in general holds well for *S. scrobifer* and *S. n. sp. 1*, in which it is strongly present; and *S. parvulus* and *S. opacus*, in which it is faint or completely absent. In *S. amabilis* and *S. mayri* it is variable (usually present, but faint in some specimens).

Queen

Characters that are useful for separating workers of different species are also useful for separating queens, especially striation of the mandibles and shape of the frontal lobes. A queen-specific character that is useful for separating queens of *S. n. sp. 1* from queens of similarly sized species is the presence of a full preocular carina (Figure 3.8 a). Queens of other species either have the preocular carinae ending at the top of the eye or have a few short, faint carinae that do not reach the posterior cephalic corners. The notauli, median mesoscutal line, and parapsidal lines can vary from prominent to very faint, and the depth of the scutellar notch can also vary, but these variations seem to be as common within species as between species, and are thus not useful for separating species.

Male

Apart from size, we found no morphological characters of males useful for separating *Sericomyrmex* species, but this might be due to the small sample sizes and to the fact that we examined males for only 5 out of 11 species. The presence of 2 short teeth on the cuspis of the volsella in *S. mayri* is unique within the male genitalia examined in this study (Figure 3.9: *mayri*); however, males were not available for its

sister species, *S. bondari*, so we were unable to determine whether this character state is species-specific.

Larvae

Based on examination of the larvae of seven species, larval characters useful for separating *Sericomyrmex* species are number of dorsal and lateral setae (from absent, in *S. n. sp. 2* and *S. parvulus*, to ~30 in *S. amabilis*), supra-antennal setae (presence/absence), mandibular teeth (divided/undivided), number of genal setae (6 in *S. mayri*, 4 in all others), additional pair of anal setae (*S. bondari* only), and number of setae on T1.

3.3.5. Taxonomic synopsis

Previously described forms in squared brackets, newly described and re-described forms in parentheses; w–worker, m–male, q–queen, l–larvae.

Sericomyrmex amabilis Wheeler 1925, Barro Colorado, Panama [w, q, m] (w, q, m, l)

=*Sericomyrmex bierigi* Santschi 1931, Panama [w], junior synonym of *amabilis* by Weber, 1958.

Sericomyrmex bondari Borgmeier 1937, Aqua Preta, Bahia, Brazil [w]

=*Sericomyrmex beniensis* Weber 1938, Huachi Beni, Bolivia [w]. **New junior synonym.**

Sericomyrmex lutzi Wheeler 1916, Roraima, Guyana [w, q, m] (w, q).

Sericomyrmex mayri Forel 1912, Rio de Janeiro, Brazil [w, q] (w, q, m, l)

=*Sericomyrmex luederwaldti* Santschi 1925 Minas Gerais, Brazil [w]. **New junior synonym.**

=*Sericomyrmex moreirai* Santschi 1925, Rio de Janeiro, Brazil [w, f, m]. **New junior synonym.**

=*Sericomyrmex harekulli* Weber 1937, Oronoque river, Guyana [w]. **New junior synonym.**

=*Sericomyrmex harekulli arawakensis* Weber 1937, Mazaruni River, Guyana [w, f]. **New junior synonym.**

=*Sericomyrmex urichi* Forel 1912, Trinidad [w]. **New junior synonym.**

***Sericomyrmex* n. sp. 1** Mato Grosso, Brazil (w, q). **New species.**

***Sericomyrmex* n. sp. 3** Venezuela (w) **New species.**

***Sericomyrmex* n. sp. 2** Colombia, Ecuador & Peru (w, q, l) **New species.**

Sericomyrmex opacus Mayr 1865, (Brazil)* Mexico [w] (w, q, m, l)

=*Sericomyrmex aztecus*, Forel 1885, Orizaba, Mexico [w]. **New junior synonym.**

=*Sericomyrmex diego* Forel 1912, Sierra Nevada de Santa Marta, Colombia [w, m]. **New junior synonym.**

=*Sericomyrmex zacapanus* Wheeler, 1924, Zacapa, Guatemala [w]. **New junior synonym.**

Sericomyrmex parvulus Forel 1912, Para, Brazil [w] (w, q, m, l).

=*Sericomyrmex myersi* Weber, 1937, Upper Courantyne River, Surinam [w]. **New junior synonym.**

Sericomyrmex saussurei Emery 1894, Mato Grosso, Brazil [w] (w, q, m, l).

=*Sericomyrmex burchelli* Forel 1905, Goiás, Brazil [q, m]. **New junior synonym.**

=*Sericomyrmex impexus* Wheeler 1925 Kartabo, Guyana [w]. **New junior synonym.**

=*S. urichi maracas* Weber, 1937, Trinidad [w, q, m]. **New junior synonym.**

Sericomyrmex scrobifer Forel 1911, Ypiranga, São Paulo, Brazil [w] (w).

3.3.6. Key to the worker caste of *Sericomyrmex*

When we refer to sizes or shapes we provide mean values, indicated with bold font, followed by value ranges. Because of frequent overlap of minimum values of one species with maximum values of another, we advise users of this key to examine several individuals from the same nest/collection event whenever possible and to consult images and distribution maps when indicated. Body-size categories are as follows: small (HW<0.92, WL<1.18, HFL<0.99); medium (HW=1.0–1.06, WL=1.28–1.39, HFL=1.1–1.24) and large (HW>1.15, WL>1.44, HFL>1.27).

- 1–Mandibles smooth except for fine transverse striae along the masticatory margin (Figure 3.6 g, Figure 3.11 a).....**2**
- Mandibles striate across the entire or most of the dorsal surface (Figure 3.6 h, Figure 3.11 b).....**12**
- 2**
- Entire body covered with dark, long, thick hair (Figure 3.11 c). Large species (HWe=**1.15**, 0.96–1.4; WL=**1.44**, 1.25–1.76; HFL=**1.27**, 1.12–1.52) with deep posterior cephalic emargination and narrow, triangular frontal lobes (Figure 3.22 a).....*bondari*

–Body not covered with thick, dark hair (Figure 3.11 d); small to large species; posterior cephalic margin from shallow to deep; frontal lobes variable.....3

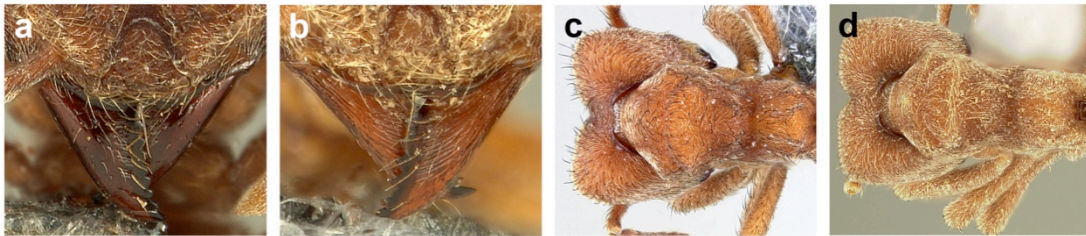


Figure 3.11. a) smooth mandibles (*S. scrobifer*), b) striate mandibles (*S. saussurei*); c) dorsal view of *S. bondari*: thick, dark hairs; d) dorsal view of *S. amabilis*, light yellow hairs.

3

–Mesosoma with very long and sharp mesonotal spines (Figure 3.12 c, Figure 3.44 d); scape long for *Sericomyrmex* (SI=77.44, 74.98–81.66), reaching the posterior cephalic corner in frontal view (Figure 3.12 a); deep posterior cephalic emargination (CEI=13.80, 11.76–16.67); narrow, triangular frontal lobes; medium-sized species.....n. sp. 3

–Mesosomal spines from low and obtuse to more developed, but never as long or as sharp as in n. sp. 3 (Figure 3.12 d); scape short to almost reaching the posterior cephalic corner in frontal view (Figure 3.12 b), cephalic emargination shallow to deep, size small to large, frontal lobes variable.....4

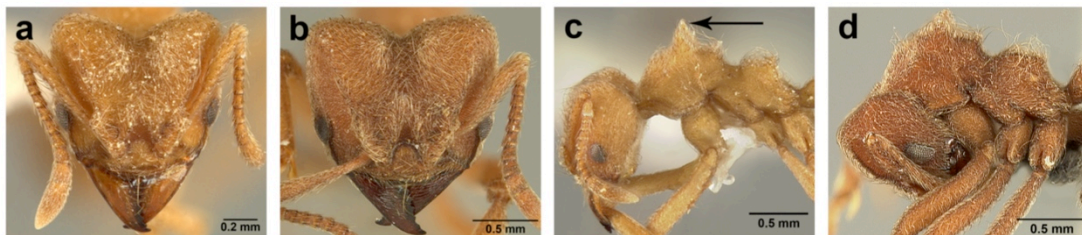


Figure 3.12. a) *S. n. sp. 3* head, frontal view; b) *S. mayri* head, frontal view; c) *S. n. sp. 3* head and mesosoma, lateral view (arrow indicates mesonotal spine); d) *S. mayri* head and mesosoma, lateral view.

4

– Cephalic emargination very deep (CEI=**15.35**, 13.78–16–54) (Figure 3.13 a); mesosomal spines low and obtuse; body size large (HWe=**1.22**, 1.12–1.35; WL=**1.52**, 1.38–1.68; HFL=**1.29**, 1.2–1.4); probably endemic to Mt. Roraima and surrounding tepuis in Guyana, Brazil, and Venezuela (Figure 3.30)*lutzi*

–Either body size small to medium (HWe<1.06, WL<1.39, HFL<1.24) and cephalic emargination shallow to moderately deep (CEI=8.55–11.51), or body size large (HWe=**1.35**, 1.05–1.6; WL=**1.71**, 1.27–2.20; HFL=**1.48**, 1.15–1.70) and cephalic emargination usually shallow (Figure 3.13 b) (CEI=9.96, 4.70–18.75)5

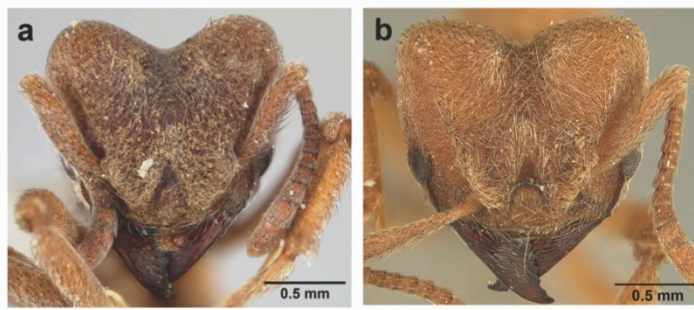


Figure 3.13. a) *S. lutzi* head, frontal view; **b)** *S. mayri* head, frontal view.

5

–Large eyes (EI>18), frontal carinae complete, usually robust (Figure 3.14 a); body size small or medium; gaster with 4 strong longitudinal carinae: 2 lateral and 2 anteromedian (Figure 3.14 c)6

–Eyes medium-sized (EI 14–16), frontal carinae complete to incomplete, but not robust (Figure 3.14 b), gaster with lateral carinae weakly to strongly developed, anteromedian carinae either absent or faint (Figure 3.14 d)7

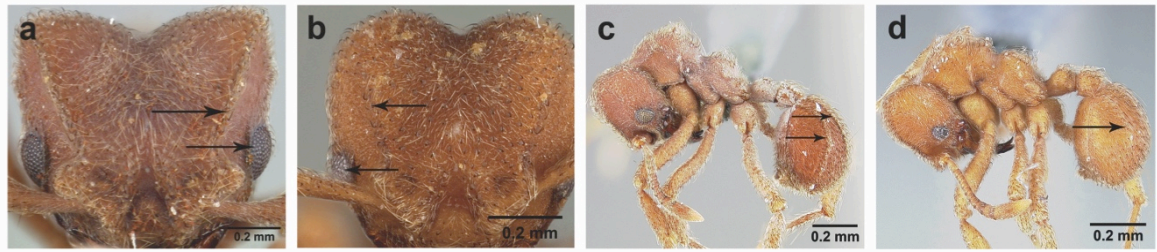


Figure 3.14. **a)** *S. scrobifer* head, frontal view, arrows indicate complete frontal carina and large, convex eye; **b)** *S. parvulus* head, frontal view, arrows indicate incomplete frontal carina and small, almost flat eyes; **c)** *S. n. sp. 1*, lateral view, arrows indicate lateral and anteromedian dorsal carinae on gaster; **d)** *S. parvulus*, lateral view, arrow indicating the lateral carina on gaster, anteromedian dorsal carinae absent.

6

–Wide, trapeziform frontal lobes (Figure 3.61 a) (FLI=**76.39**, 72–83.25), eyes large, strongly convex, and strongly protruding from the sides of the head, medium-sized species (HWe=**1.0**, 0.84–1.12; WL=**1.29**, 1.12–1.4; HFL=**1.11**, 0.93–1.26)***scrobifer***

– Triangular frontal lobes (Figure 3.39 a), narrower than in *scrobifer* (FLI=**68.53**, 64.0–73.41), eyes large and moderately protruding, less than in *scrobifer*, small-sized species (HWe=**0.92**, 0.8–0.98; WL=**1.18**, 1.03–1.28; HFL=**0.99**, 0.82–1.08)**n. sp. 1**

7

–Small to medium-sized species (mean WL=1.04–1.28), with narrower head (mean CI=100–102), frontal lobes either: small and reduced, rectangular, or triangular (Figure 3.15 a, b, c)

.....**8**

–Larger species (mean WL=1.33–1.71), head wider (mean CI=103.7–107), frontal lobes either triangular and diverging, or triangular and very narrow, pointing down (Figure 3.15 d, e, f)**10**

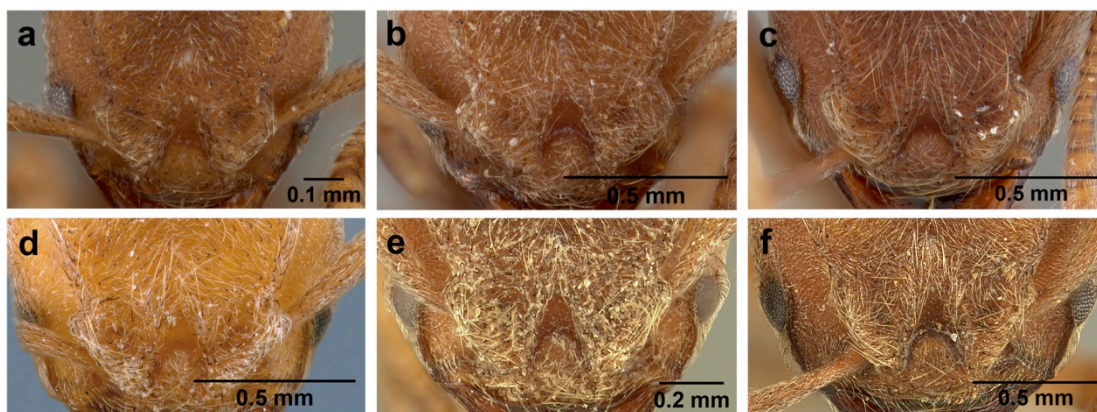


Figure 3.15. Frontal lobes. **a)** *S. parvulus*; **b)** *S. opacus*; **c)** *S. n. sp. 2*; **d)** *S. amabilis*; **e)** *S. saussurei*; **f)** *S. mayri*.

8

–Very small species (WL=**1.04**, 0.74–1.23; HWe=**0.82**, 0.66–0.90; HFL=**0.82**, 0.65–0.99); frontal carinae incomplete to complete, faint; frontal lobes small and triangular; eyes small and flat, often covered with thin white layer (Figure 3.14 b, d; Figure 3.53)

.....*parvulus*

– Small to medium-sized species (mean WL =1.15–1.28, mean HWe=0.9–1.01); frontal carinae incomplete or complete; frontal lobes rectangular to triangular; eyes larger and without white layer, or smaller in size and with white layer (as in *parvulus*), but then frontal lobes rectangular

9

9

–Small-sized species (WL=**1.16**, 0.99–1.30; HWe=**0.91**, 0.8–1.0; HFL=**0.92**, 0.8–1.02); frontal lobes rectangular (Figure 3.15 b); head corners smoothly rounded; eyes often with transparent white layer; frontal carinae incomplete to complete

opacus

–Medium-sized species (WL=**1.28**, 1.12–1.40; HWe=**1.01**, 0.90–1.13; HFL=**1.10**, 0.95–1.20); frontal lobes triangular (Figure 3.15 c); eyes without white layer

n. sp. 2

10

–Eyes distinctly convex, mildly protruding, covered with thick white layer that makes it hard to discern individual ommatidia (Figure 3.15 e, Figure 3.16 a); medium-sized speciesthe smooth mandibled variant of *saussurei*

–Eyes flat to mildly convex, without white layer, ommatidia visible; medium-sized to large (mean WL=1.37–1.71; mean HWe=1.06–1.35)11

11

–Large species (WL=**1.71**, 1.27–2.20; HWe=**1.35**, 1.05–1.60; HFL=**1.48**, 1.15–1.70); head broad (CI=**107.9**, 100.93–115.08); posterior cephalic corners acute to rectangular; frontal lobes narrow (FLI=**63.16**, 59.35–68.24), pointing anterad; frontal carinae often incomplete (Figure 3.16 c, Figure 3.33); distribution: South America (Figure 3.37)

.....smooth-mandibled variant of *mayri*

–Medium-sized species (WL=**1.37**, 1.13–1.60; HWe=**1.06**, 0.88–1.21; HFL=**1.15**, 0.93–1.38); head narrower than *mayri* (CI=**104.32**, 96.88–110.80); frontal lobes relatively wide (FLI=**67.68**, 59.73–72.26), directed laterally; frontal carinae complete (Figure 3.16 b); distribution: Central America, Colombia, Venezuela, Ecuador (Figure 3.21)

.....smooth mandibled-variant of *amabilis*

12

— Eyes distinctly convex, mildly protruding, covered with thick white layer that makes it hard to discern individual ommatidia (Figure 3.15 e, Figure 3.16 a); medium-sized species*saussurei*

– Eyes flat to mildly convex, without white layer, ommatidia visible; medium-sized to large species (mean WL=1.37–1.71; mean HWe=1.06–1.35)13

– Large species (WL=**1.71**, 1.27–2.20; HWe=**1.35**, 1.05–1.60; HFL=**1.48**, 1.15–1.70); head broad (CI=**107.9**, 100.93–115.08); posterior cephalic corners acute to rectangular; frontal lobes narrow (FLI=**63.16**, 59.35–68.24), pointing anterad; frontal carinae often incomplete (Figure 3.16 c, Figure 3.33); distribution: South America (Figure 3.37)

.....*mayri*

– Medium-sized species (WL=**1.37**, 1.13–1.60; HWe=**1.06**, 0.88–1.21; HFL=**1.15**, 0.93–1.38); head narrower than *mayri* (CI=**104.32**, 96.88–110.80); frontal lobes relatively wide (FLI=**67.68**, 59.73–72.26), directed laterally; frontal carinae complete (Figure 3.16 b); distribution: Central America, Colombia, Venezuela, Ecuador (Figure 3.21)*amabilis*



Figure 3.16. **a** *S. saussurei* head, frontal view; **b** *S. amabilis*, head, frontal view; **c** *S. mayri*, head, frontal view.

3.3.6. Species accounts

Sericomyrmex AMABILIS Wheeler 1925

Worker: Figures 3.17, 3.18; **Queen and male:** Figures 3.18, 3.19; **Larvae:** Figure 3.20;
Map: Figure 3.21.

Sericomyrmex amabilis, Wheeler, 1925: 166. Lectotype worker (here designated):

PANAMA, Panamá, Barro Colorado Island, [9.1543, -79.8461], 3 Aug 1924, W. M.

Wheeler, WMW838 (NMNH: 3w, USNMENT00920034, topmost specimen on the pin).

Paralectotypes: Same data as lectotype (NMNH: 2w, USNMENT00920034, lower two specimens on the pin) (MCZ: 1pin, 2w, 1q, MCZ 40-42 21197), same data as lectotype but the date is 21 July 1924 (NMNH: 1pin, 3w, USNMENT00528938).

=*Sericomyrmex bierigi* Santschi, 1931: 279.

Type material: PANAMA, Chiriquí, La Concepción, 16 Jul 1930, Bierigi, (NHMB: 1w, CASENT0912515) [image examined]. Weber, 1958: 263: junior synonym of *amabilis*.

S. amabilis worker description

Measurements in mm (range): HWe 0.88–1.21 IFW1 0.56–0.86 IFW2 0.19–0.35 HL1 0.82–1.2 HL2 0.76–1.04 ScL 0.65–0.87 EL 0.11–0.2 Om 7–11 WL 1.13–1.6 PL 0.22–0.4 PPL 0.16–0.29 GL 0.78–1.12 HFL 0.93–1.38 PrW 0.58–0.92 hw1 0.82–1.18 hw2 0.92–1.36 hw3 0.58–0.9 CI 96.88–110.8 FLI 59.73–72.26 SI 65.71–78.43 EI 12–19.12 CEI 4.88–14.81 [n=70]

Color uniformly yellowish-brown to deep ferrugineous-brown. Pubescence dense, often lighter than the integument, appressed to decumbent. Hairs curved, darker in color at the base, yellow to gray, appressed to suberect, but mostly decumbent.

Head. In frontal view slightly broader than long ($CI=104.32 \pm 2.83$, mean $\pm$ SD), posterior corners rounded to angular, posterior cephalic emargination distinct ($CEI=0.33 \pm 1.84$). The posterior cephalic margin between the cephalic corner and the median emargination can be straight to convex and it can merge with the emargination gradually (Figure 3.7 j) or it can be more or less straight and abruptly merge with the emargination (Figure 3.7 k). Vertexal impression and frontal tumuli usually distinct. Mandibles glossy and striate across the whole dorsal surface, except in some populations (see notes section). Masticatory margin of mandible with 7 to 9 teeth. Eyes medium-sized ($EI=15.79 \pm 1.39$), flat to slightly convex, 8 to 11 ommatidia across largest diameter. Frontal lobes triangular, with posterior margin shorter than medial, diverging laterally, relatively wide ($FLI=67.68 \pm 2.46$). Frontal carinae complete, reaching posterior cephalic corners. Antennal scape relatively short, never reaching posterior cephalic margin ($SI=70.83 \pm 2.86$).

Mesosoma. Lateral pronotal and mesonotal spines from low and obtuse to well developed. Propodeal carinae low, serrate, ending in small and blunt denticles.

Metasoma. Nodes of petiole and postpetiole dorsally each with a pair of low, short, serrate, longitudinal carinae. On the petiole the carinae are sometimes reduced to two low denticles, best seen in dorsolateral view. First gastral tergite with lateral longitudinal carinae strongly developed, one on each side, and anteromedian dorsal carinae at least faintly present on most specimens, in some populations strongly developed (e.g., population from Gorgona, Colombia).

***S. amabilis* worker diagnosis**

Medium-sized, with mandibles usually striate, complete frontal carinae, triangular frontal lobes, merging of posterior cephalic margin with emargination abrupt to gradual. Mesosomal spines from low and obtuse to well developed, but not as sharp as in *S. scrobifer* and *S. bondari* or as sharp and long as in *S. n. sp. 3*. In some specimens first gastral tergite with anteromedian dorsal carinae visible, but weaker than the lateral longitudinal carinae. Eyes without white layer, ommatidia clearly visible.

Differs from sympatric *S. opacus* by larger size, mandibles usually striate (in *opacus* always smooth), triangular frontal lobes (rectangular in *opacus*), the shape of posterior cephalic corners (in *opacus* smoothly rounded) and the presence of anteromedian dorsal carinae on gaster. *S. amabilis* can be separated from its sister species *S. saussurei* by flatter, uncoated eyes (convex, with conspicuous, thick white layer in *saussurei*), and usually by geography (Figure 3.21). It can be separated from *S. mayri* by smaller size, narrower head, complete frontal carinae, and wider frontal lobes.

***S. amabilis* queen description**

Measurements in mm (range): HWe 1.27–1.40 HW 0.34–1.45 IFW1 0.90–1.00 IFW2 0.27–0.37 HL1 1.27–1.45 HL2 1.15–1.25 ScL 0.81–0.99 EL 0.23–0.29 Om 15–22 EW 0.06–0.1 WL 1.95–2.2 PL 0.45–0.58 PPL 0.28–0.4 GL 1.68–1.87 HFL 1.16–1.58 PrW 1.05–1.52 hw1 1.30–1.52 hw2 1.40–1.52 hw3 0.87–1.04 FWg 5.85–6.93 HWg 3.79–4.41 CI 93–104.25 FLI 70–76.80 SI 62–74.42 EI 17.55–22.15 [n=10]

Head. Masticatory margin of mandible with 8–9 teeth. Mandibles striate across entire dorsal surface. Preocular carina present, usually fading shortly after reaching the top of the eye. Eyes large and nearly flat (EI=19.55), 15 to 22 ommatidia across largest

diameter. Frontal lobes similar to worker (FLI=72.52), antennal scape not reaching posterior cephalic margin (SI=68.27).

Mesosoma. Mesoscutum in dorsal view with weak notauli and reduced median mesoscutal sulcus. In dorsal view a transverse shallow groove separating axillae from the scutellum, this groove sometimes weakly transversely costate. Scutellum slightly convex in profile view, in dorsal view narrowing posteriorly, posterior margin medially with wide and shallow V-shaped notch, this notch sometimes continuing into median impression that appears to separate scutellum into two lateral halves. Propodeum in dorsal view with two posteriorly diverging carinae that end in denticles.

Metasoma. Longitudinal lateral carinae on first gastral tergite strongly developed, anteromedian dorsal carinae absent, shallow longitudinal anteromedian groove present.

***S. amabilis* queen diagnosis**

The *S. amabilis* queen can be separated from that of the sympatric *S. opacus* by its larger size, striate mandibles (smooth in *opacus* queen), and, although the strength of notauli and median mesoscutal line can vary in both *amabilis* and *opacus*, they are often pronounced in *amabilis* and faint or absent in *opacus*.

***S. amabilis* male description**

Measurements in mm (range): HWe 0.61–0.98 HW 0.6–0.78 IFW1 0.24–0.48 IFW2 0.17–0.25 HL1 0.61–0.78 ScL 0.59–0.76 EL 0.24 0.35 Om 20–26 EW 0.1–0.18 WL 1.43–2.02 PL 0.25–0.43 PPL 0.18–0.25 GL 1.08–1.75 HFL 1.48–1.95 PrW 0.58–0.90

IOD 0.54–0.63 FWg 4.28–5.77 HWg 2.68–3.68 CI 100–129.03 FLI 32.82–59.55 SI
74.29–98.68 EI 33.96–39.97

Head in frontal view longer than broad (CI=120.19 $\pm$ 7.47). Eyes large (EI=37.54 $\pm$ 2.04), ~23 ommatidia across the largest diameter. Preocular carinae fading after reaching the top of the eyes, slightly curved medially before fading. Notauli and mesoscutal line well developed, the groove between axillae with up to four transverse costae. Propodeal carinae short and faint. Petiole with lateral and dorsal denticles, postpetiole only with very reduced lateral denticle.

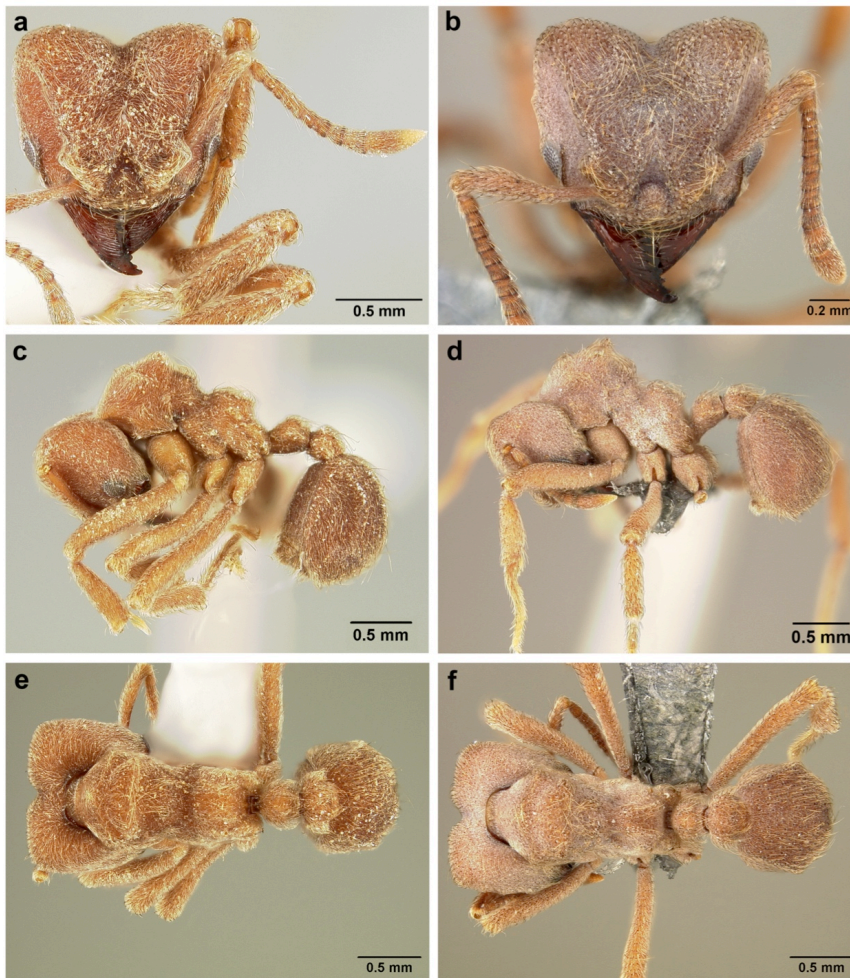


Figure 3.17. *S. amabilis* worker; head, profile, and dorsal view.

Striate-mandibled form (USNMENT00308236): **a, c, e**. Smooth-mandibled form (USNMENT01125194): **b, d, f**.

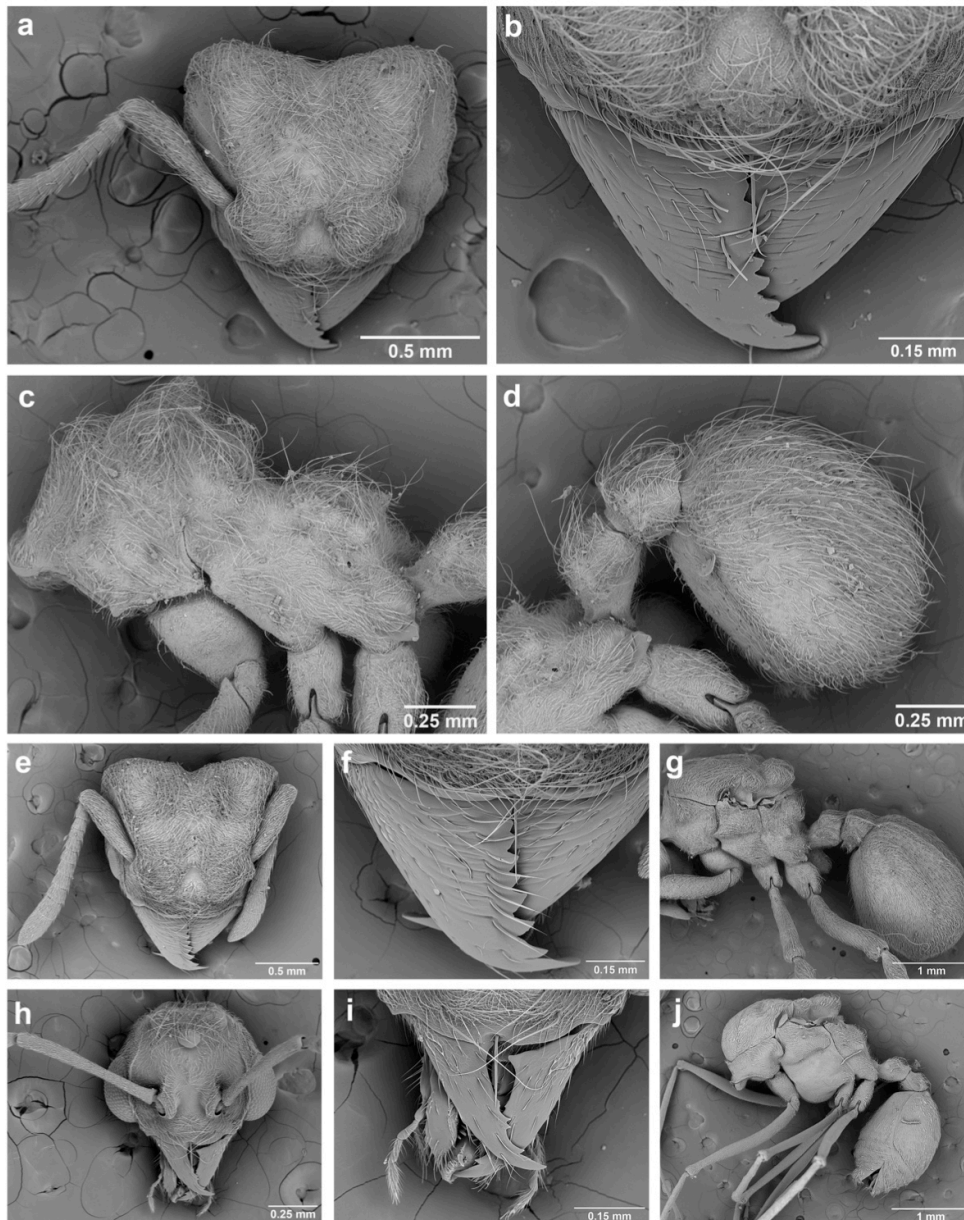


Figure 3.18. *S. amabilis* worker, queen, and male (SEM images).

Worker (USNMENT01125862): **a** head, full-face view; **b** mandibles; **c** mesosoma, lateral view; **d** gaster, lateral view. Queen (USNMENT01125864): **e** head, full-face view; **f**

mandibles; **g** mesosoma and gaster, lateral view. Male (USNMENT01125863): **h** head, full-face view; **i** mandibles; **j** mesosoma and gaster, lateral view.

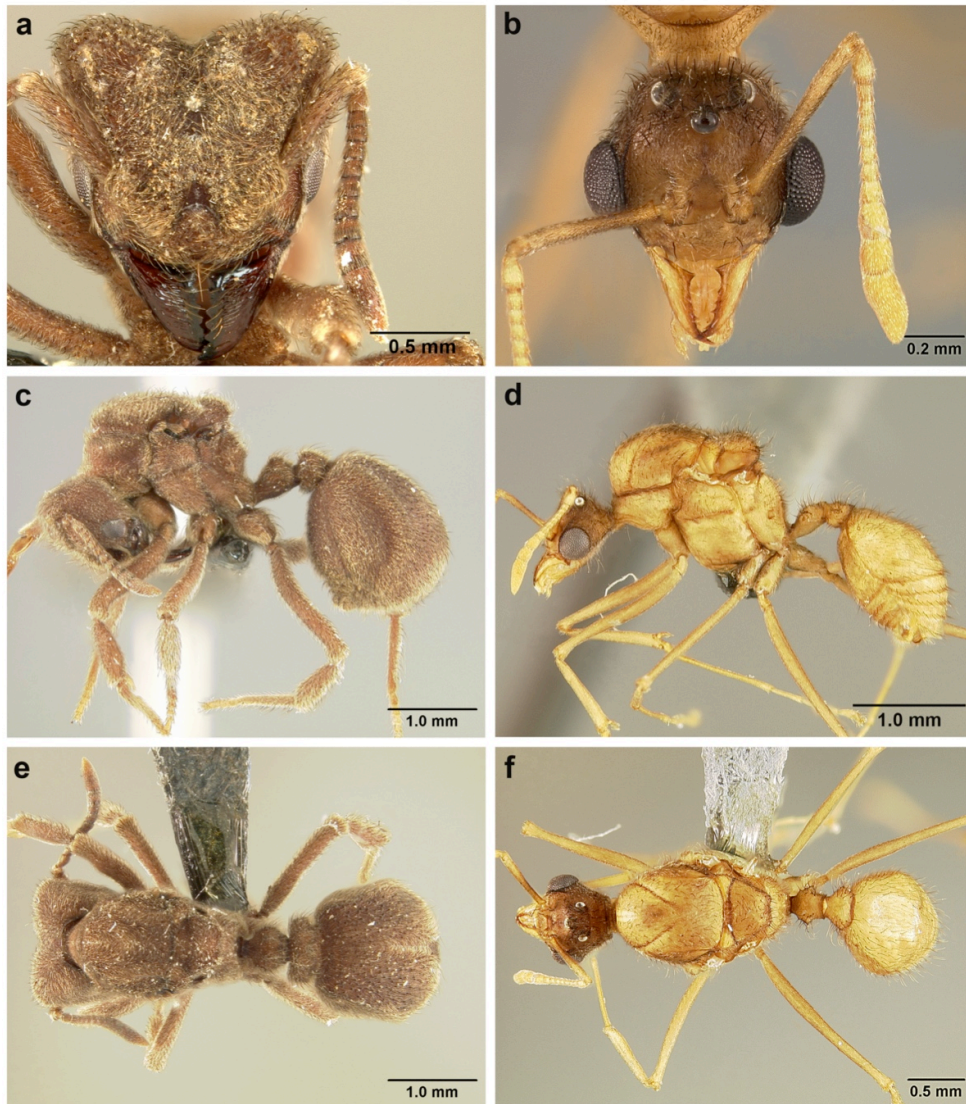


Figure 3.19. *S. amabilis* queen and male; head, profile, and dorsal view.

Queen (USNMENT01125871): **a, c, e**. Male (USNMENT01125846): **b, d, f**.

***S. amabilis* larvae description**

About 15 setae on each side of dorsal and lateral body surfaces (i.e., total ~30).

Supra-antennal setae present, one over each antennae. Four genal setae on each side.

Mandibular apical teeth divided. Labial denticles present anterior to sericteries, sparse.

First thoracic segment ventrally with multiple multidentate spinules, arranged in transverse rows. Numbers of ventral setae: six on T1, four on T2, four on T3, and around 10 setae on abdomen. Single pair of setae anterior to anal opening.

***S. amabilis* geographic range.** Mexico to Ecuador, Colombia, and Venezuela. Map:

Figure 3.21.

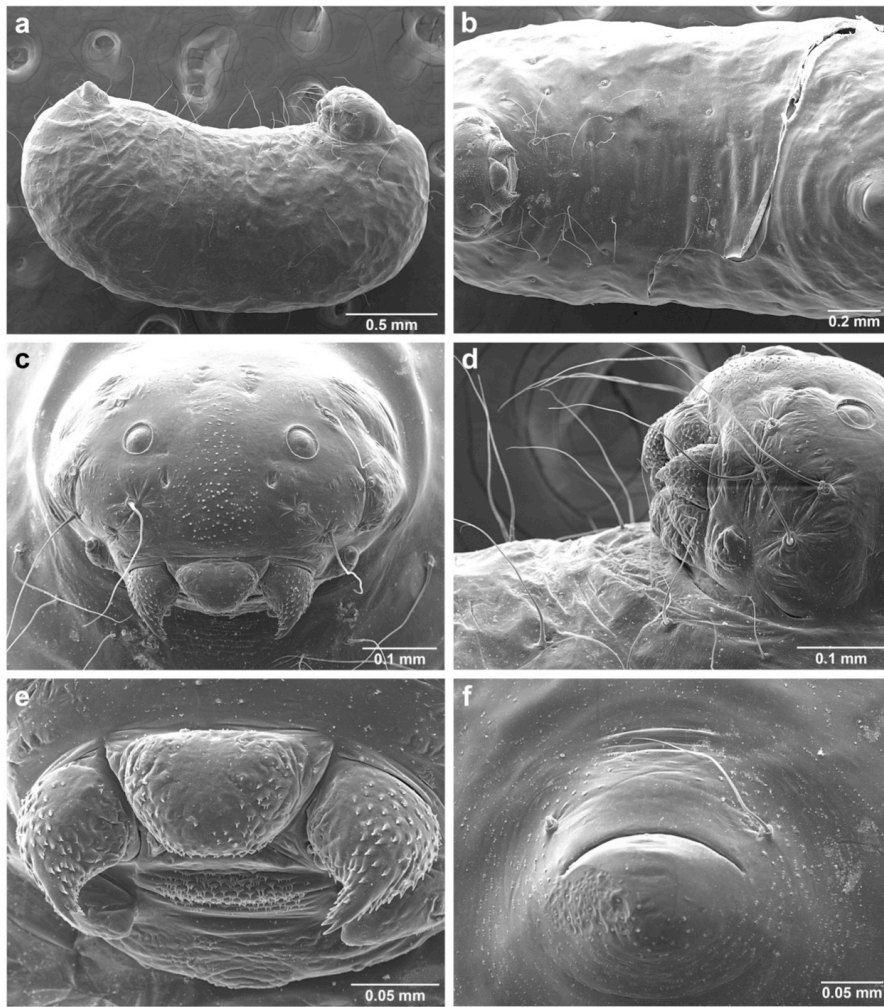


Figure 3.20. *S. amabilis* larva (USNMENT01126216) SEM images.

a Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

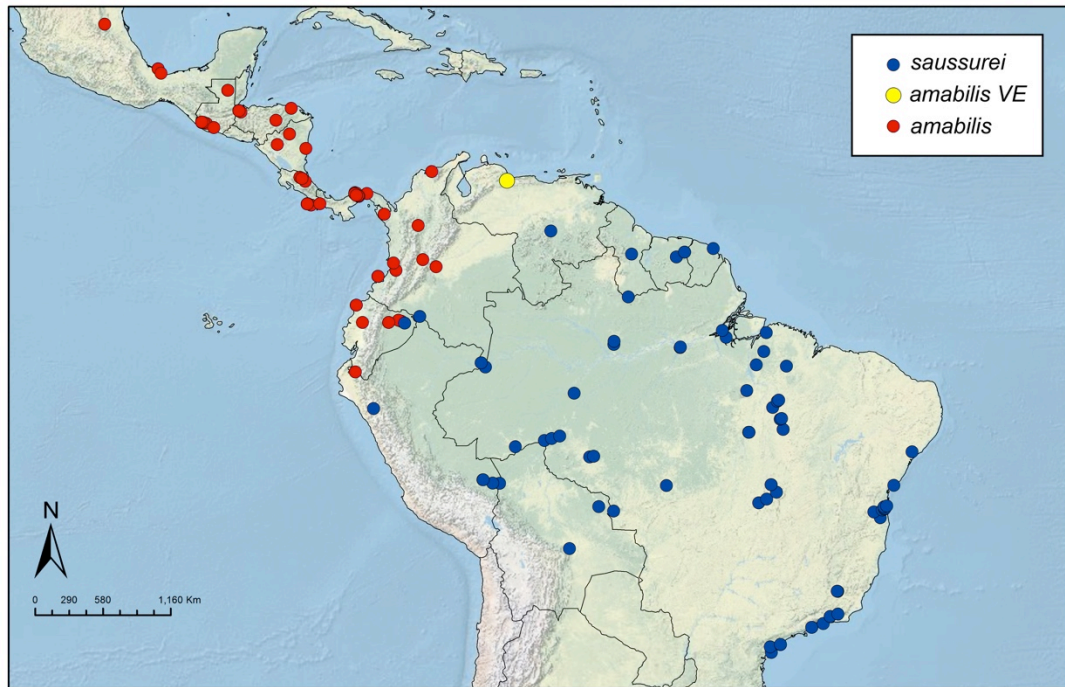


Figure 3.21. Distribution map of *S. amabilis* and *S. saussurei*.

***S. amabilis* notes**

Within *S. amabilis*, there is striking variation in the character of smooth versus striate mandibles. In the majority of specimens examined for this revision (including the type specimens) the mandibles are striate across the entire dorsal surface (Figure 3.17 a) and in most populations of *amabilis* the mandibles are consistently striate. In some populations, however, both forms occur: some of the specimens from a given locality have striate mandibles, and some have completely smooth mandibles (Figure 3.17 b). In those populations intermediate forms are also present, with faint striations. In the intermediate state of faint striation, the striations are most obvious at the posterior lateral edge of the mandibles, in dorsal view, where the mandibles connect with the head capsule.

The population of *amabilis* in Costa Rica, Heredia, La Selva Biological Station, exemplifies the polymorphic state in which both smooth and striate mandibles occur, whereas the population from Costa Rica, Puntarenas, Osa Peninsula, contains only the striate state. A few specimens from Nicaragua and Ecuador also have smooth or faintly striate mandibles. Specimens of *amabilis* from Gorgona National Park (an island 35 km off the Pacific coast, in the department of Cauca, Colombia) show the complete range of variation, from fully striate through intermediate to completely smooth mandibles. The smooth-mandibled populations do not form distinct clusters in molecular phylogenies of *amabilis*; rather, smooth-mandibled specimens group with striate-mandibled ones and vice versa. Likewise, statistical analyses of morphological measurements do not identify any distinct clusters correlated with mandibular sculpture (Figure 3.5 e).

Members of the Gorgona population of *amabilis* have longer and sharper anterior mesonotal spines and more robust anteromedian dorsal carinae on the gaster compared to Central American populations. Some specimens from Nicaragua have a thin, translucent white layer covering part of the eye, similar to the condition observed in *S. opacus*. Other variable characters in *amabilis* are: frontal carinae (usually complete, but less developed in some), head shape (from angular to more rounded posterior head corners), and the size of the frontal lobes.

A single sequenced specimen from the population of *S. amabilis* from Venezuela (indicated with a yellow circle in the Figure 3.21) is molecularly distinct from the rest of the *amabilis* clade, as indicated by its position in the molecular phylogeny of *Sericomyrmex* (Chapter 2, Figure 2.2). This specimen, to which we refer as *S. amabilis* VE, is the sister of a clade that contains two reciprocally monophyletic subclades: (i) the

monophyletic *S. saussurei* and (ii) all *S. amabilis* except *S. amabilis* VE. The molecular phylogeny, based on UCEs, reconstructs *S. amabilis* VE as a separate, third species (Chapter 2, Figure 2.2). The two available specimens, however, are morphologically identical to the specimens assigned to *amabilis*. Because of the small number of specimens and the lack of distinguishing morphological data, and because the molecular data are consistent with a scenario in which *S. saussurei* arises from within a paraphyletic *S. amabilis*, we are for now treating *S. amabilis* VZ as an allopatric population of *amabilis* that renders *amabilis* paraphyletic. We would like to encourage further study of this population and more thorough sampling in Venezuela in general, which will hopefully bring more insight into the species status of this population.

***S. amabilis* material examined**

COLOMBIA: Antioquia: Amalfi Canon del Porce, El Caiman, 6.8572, -75.0958, 970m, 19 Dec 1999, E. Vergara, F. Serna; **Magdalena:** 4 km N San Pedro, 10.95, -74.05, 220m, 14 Aug 1985, P. S. Ward; **Meta:** San Martín Caduceo, [3.6970, -73.6982], 400m, 4 May 2006, J. Ordóñez; **Valle del Cauca:** PNN Farallones de Cali, Anchicaya, 3.4333, -76.8, 730m, 1 Jul 2000, S. Sarria; **Cauca:** PNN Gorgona Alto El Mirador, 2.9666, -78.1833, 180m, 21 Oct 2000, R. Duque; PNN Gorgona Antigua Laguna, 2.9666, -78.1833, 70m, 20 Dec 2000, R. Duque; PNN Gorgona El Helechal, 2.966, -78.1833, 30m, 17 Mar 2002, R. Duque; PNN Gorgona El Roble, 2.9666, -78.1833, 120m, 17 Jun 2001, R. Duque; PNN Gorgona El Saman, 2.9666, -78.1833, 5m, 14 Sep 2001, H. Torres ; PNN Gorgona Mancora, 2.9666, -78.1833, 60m, 20 Dec 2000, R. Duque; **Cudinamarca:** Melgar to Girardot, [4.249, -74.726], 28 Mar 1967, R. B.

Root, W. L. Brown; **Valle:** Buenaventura, Bajo Calima, Villa Clara, [3.996, -76.974
 ±2000m], 30m, 18 Mar 1967, R. B. Root, W. L. Brown; **COSTA RICA: Heredia:** La
 Selva Biol. Station, 10.43, -84.017, 50-150m, 1 Aug 2004, J. Sosa-Calvo; **Limón:** Puerto
 Viejo, [10.51, -84.18], 18 Jun 1979, J. Paich; R. Toro Amarillo Vic., Guapiles, 10.2166, -
 83.7667, 25 Feb 1966, W. L. Brown; **Puntarenas:** 15 km SSW Pto. Jimenez, [8.4083, -
 83.3278 ±30m], 170m, 7 Mar 2010, J. T. Longino; Osa Peninsula, Corcovado, Sirena
 Station, 8.50, -83.6166, 31 May 1992, T. R. Schultz; **ECUADOR: Esmeraldas:** Las
 Vegas, 0.7547, -79.8490, 26 Jun 2003, A. G. Himler; **Loja:** Reserva Jorupe, Macará, -
 4.372, -79.906, 600m, 15 Sep 2011, T. Delsinne; Los Ríos Rio Palenque, 2km SSE
 Patricia Pilar, -0.5833, -79.3666, 160m, 1 Sep 1991, M. J. Stern; **Napo:** Limoncocha, -
 0.4, -76.6, 250m, 18 Jun 1976, S., J. Peck; Tiputini, La Selva, Chorong trail, -0.4975, -
 76.3747, 12 Jun 2003, A. Little; **Pichincha:** 47 km S Sto. Domingo, Rio Palenque
 Research Station, [-0.583, -77.36], 28 May 1905, S., J. Peck; **GUATEMALA:**
Escuintla: Escuintla, [14.2445, -90.7995], 22 Sep 2008, W. M. Mann; **Izabal:** 16 km
 ESE Morales, 15.4111, -88.7118 ±58m, 440m, 19 May 2009, J. T. Longino; 5 km NW
 Morales, 15.5097, -88.8627 ±36m, 160m, 18 May 2009, J. T. Longino; **Petén:** Cerro
 Cahui, 17.0023, -89.7194, 210m, 3 Feb 2009, Llama team; **Retalhuleu:** El Asintal,
 14.6555, -91.7344, 720m, 19 Feb 2013, K. Delgado; Nuevo San Carlos, 110 km NW
 Retalhuleu, 14.6258, -91.7234, 440m, 27 Feb 2013, K. Delgado; San Felipe, 14.6309, -
 91.581, 745m, 8 Feb 2013, K. Delgado; **HONDURAS: Gracias a Dios:** Las Marias,
 15.6817, -84.8352 ±20m, 80m, Llama team; **Olancho:** 14 km WSW Catacamas, 14.7997,
 -86.0141 ±210m, 600m, 13 May 2009, J. T. Longino; **MEXICO: San Luis Potosí:** Rio
 Santa Maria, Tamul, -21.8025, -99.1803, 200m, 2 Jul 1992, S. Sanchez-Pena; **Veracruz:**

Catemaco, Tuxtla, 18.5865, -95.0779, 184m, 3 Oct 2013, A. Ješovnik; Ocotal Chico, 18.2588, -94.8619, G. N. Ross; **NICARAGUA: Matagalpa:** Pancasan, nr. Rio Guapotal, 12.92, -85.55, 450m, 17 Jun 1992, T. R. Schultz, J. C. Gomez; Selva Negra, ca. 12 km N Matagalpa, Reserva Natural Cerro El Arenal, 12.9812, -85.9136, 1009m, 28 Dec 2007, C. Rabeling; **Región Autónoma del Atlántico Norte:** PN Cerro Saslaya, 13.7705, -84.9789 ±20m, 290m, 7 May 2011, M. G. Branstetter; **Región Autónoma del Atlántico Sur:** RN Kahka Creek, 12.6851, -83.7136, 50m, 8 Jun 2011, M. G. Branstetter; **PANAMA:** **Colón:** Fort Sherman, 9.36, -79.95, 28 Apr 1996, T. R. Schultz, U. G. Mueller, S. Rehner; Gamboa, PN Soberanía, Pipeline Rd. ca. 2 km past Rio Frijoles, 9.1205, -79.7067, 54m, 25 May 2002, C. J. Marshall; Gatún, Punta da los Chivos, 3 km SW Gatún, [9.26, -79.91], 1 Jul 1979, W. L. Brown; Mt. Hope, nr. Colón, 9.2833, -79.9667, 24 Jul 1924, W. M. Wheeler; San Lorenzo Forest, 9.2833, -79.9666, 25 May 2004, R. K. Didham; **Darién:** Cana, 7.7166, -77.7, 800m, 23 Aug 1987, D. M. Olsen; **Panamá:** Barro Colorado Island, 9.15, -79.84, 1 Aug 1946, J. Zetek; Gamboa, Pipeline Rd, ca. 2 km past Rio Frijoles, 9.1205, -79.7066, 54m, 25 May 2002, C. J. Marshall; Nusagandi Biol. Stn, Markisgandi trail, [9.2848, -79.0280], 26 Apr 1996, T. R. Schultz; PN Soberanía, Plantation Rd., 9.08, -79.66, 4 May 2011, R. M. M. Adams; **VENEZUELA: Carabobo:** Mocundo, ca. Aguirre, [10.2533, -68.2683], 750m, 27 Dec 2010, J. Lattke.

***Sericomyrmex BONDARI* Borgmeier 1937**

Worker: Figures 3.22, 3.23, 3.24; **Queen:** Figure 3.25; **Larva:** Figure 3.26; **Map:** Figure 3.27.

Sericomyrmex bondari Borgmeier, 1937: 248. Lectotype worker (here designated): BRAZIL, Bahia, Sul da Bahia, Aqua Preta, [-8.8333, -66.1667], 1864, G. Bondar (MZSP: 3w; Borgmeier coll. Nr. 5779, topmost specimen on the pin). Paralectotypes: same data as lectotype (MZSP: 2w; Borgmeier coll. Nr. 5779, lower two specimens on the pin) =*Sericomyrmex beniensis* Weber, 1938: 182. **New junior synonym.**

Type material examined: BOLIVIA, Huachi Beni, 31 Dec 1921, W. M. Mann, WMM327. (MCZ: 5pin, 15w, two pins with small "327" label, one pin with "*S. beniensis* Weber" label)

***S. bondari* worker description**

Measurements in millimeters (range): HWe 0.96–1.40 HW 0.98–1.40 IFW1 0.65–1.00 IFW2 0.22–0.38 HL1 0.99–1.30 HL2 0.87–1.12 ScL 0.68–0.96 EL 0.14–0.20 Om 8.00–12.00 WL 1.25–1.76 PL 0.24–0.40 PPL 0.18–0.30 GL 0.81–1.24 HFL 1.12–1.52 PrW 0.66–1.02 hw1 0.93–1.40 hw2 1.00–1.56 hw3 0.65–0.90 CI 92.59–109.97 FLI 60.71–72.34 SI 63.41–78.20 EI 11.84–17.50 FLD 0.40–0.62 CEI 5.90–17.24 [n=59]

Hairs black and thick, the most distinguishing characteristic of this species, relatively dense, often curved, appressed to erect, mostly suberect. Hairs generally longer and denser on dorsal than on lateral surfaces, e.g., mesosoma laterally with barely any hairs, just pubescence.

Head. In frontal view slightly broader than long ($CI=102.93 \pm 3.70$), posterior corners rounded to acute, posterior cephalic emargination distinct and deep ($CEI=13.00 \pm 2.19$). Cephalic margin between cephalic corner and median emargination straight to slightly convex in most specimens and gradually merging into the emargination. Vertexal impression often distinct and deep, frontal tumuli usually faint. Mandibles smooth and glossy, finely transversely striate only along masticatory margin, this fine striation in some specimens difficult to see so that the mandibles look entirely smooth. Masticatory margin of mandible with 7–9 teeth. Eyes medium-sized ($EI=14.65 \pm 1.26$), mildly convex, lacking white layer, 8 to 12 ommatidia across largest diameter. Frontal lobes triangular, long but narrow ($FLI=66.52 \pm 2.48$), with posterior margin shorter than medial, and with a long lateral margin, giving the lobes the appearance of being directed downward (i.e., anterad). Frontal carinae complete, reaching posterior cephalic corners. Antennal scape almost reaching posterior cephalic corners ($SI=70.64 \pm 3.36$). Antennal scape with black thick hairs, antennal flagellum lacking thick black hairs, but with pubescence and long, light yellow, thin hairs.

Mesosoma. Lateral pronotal spine and anterior mesonotal spine in dorsal view subequal in length, low and conical to more developed, larger and sharp; this varies between specimens within the species and within colonies. Propodeal carinae low and diverging, usually ending in denticles.

Metasoma. Node of petiole with two low denticles, and node of postpetiole with four low, short, longitudinal carinae, two anteromedian dorsal and two lateral, the lateral pair sometimes very faint. Gaster with longitudinal lateral carinae on first tergite distinct, anteromedian dorsal carinae usually absent, sometimes present but very faint. Decumbent

to suberect hairs on gastral dorsum curved at the base and sometimes hooked at the tip, the margins of gastral segments 2–4 with curved suberect to erect hairs.

***S. bondari* worker diagnosis:** Thick, dark hairs are the most distinguishing feature of *S. bondari*, in combination with a deep posterior cephalic emargination, acute posterior cephalic corners, smooth mandibles, narrow and long triangular frontal lobes, sharp anterior mesonotal spines, large size, and no anteromedian dorsal carinae on the gaster. Hard to mistake for any other species, except when individuals with reduced hairs are encountered (see notes section for more details, and Figure 3.23). In such cases the other remaining character states, with the exception of dark hairs, are enough to identify *bondari*. The widespread, sympatric sister species, *S. mayri*, has striate mandibles, a wider head (CI *mayri*=107.83, CI *bondari*=104.6), narrower frontal lobes, and shallow posterior cephalic emargination. *S. n. sp. 3* is smaller (medium-sized species, while *bondari* is large); completely lacks dark hairs; and has a longer antennal scape (SI=77.44), reduced lateral pronotal spines, and unusually sharp, long anterior mesonotal spines.



Figure 3.22. *S. bondari* worker (USNMENT01125207). **a** Head; **b** dorsal view; and **c** lateral profile.

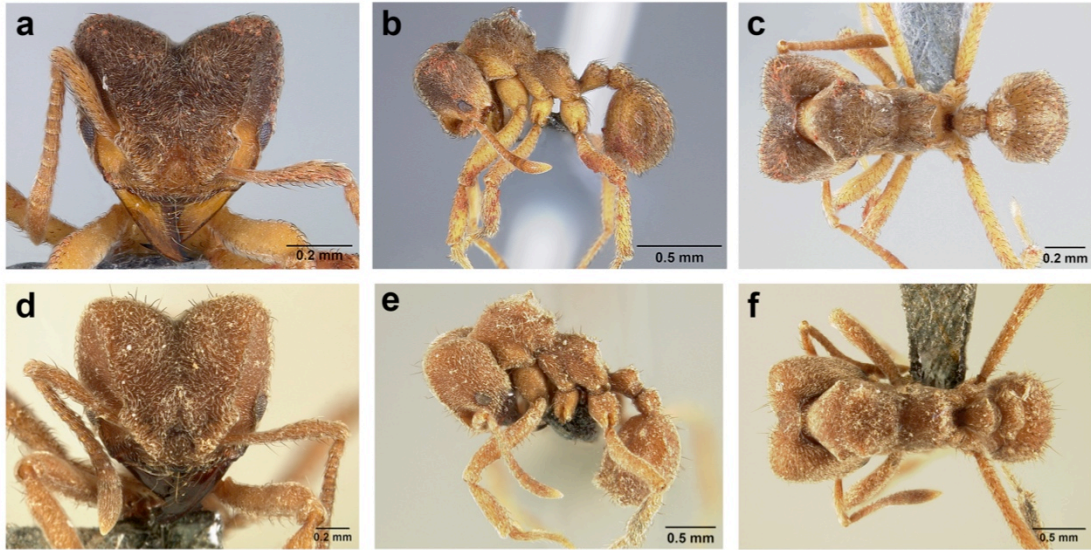


Figure 3.23. *S. bondari* worker variation. Worker with bicolored integument (USNMENT01125207): **a, b, c**. Worker with reduced hairs (USNMENT01125823): **d, e, f**.

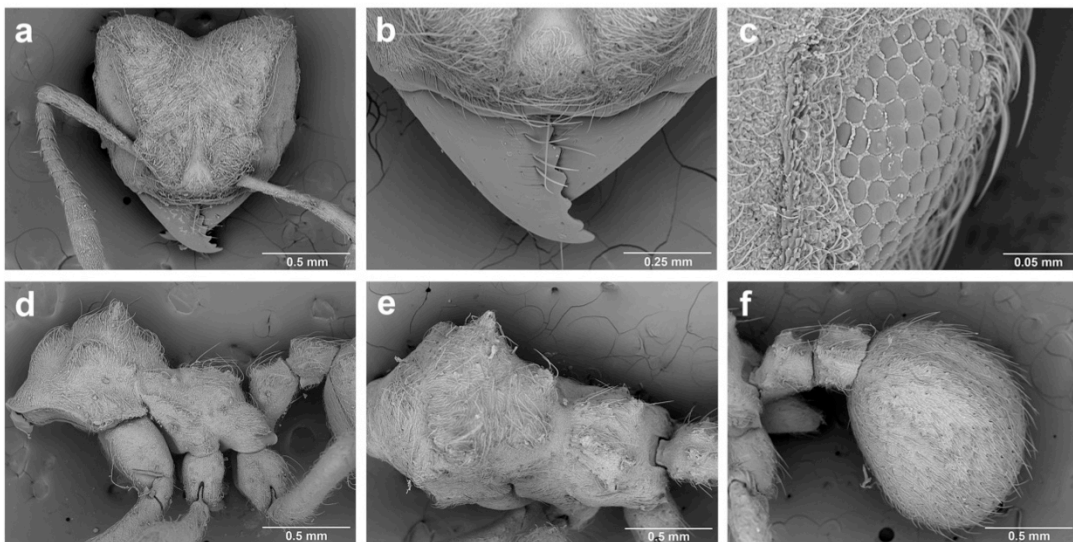


Figure 3.24. *S. bondari* worker (USNMENT01125813) SEM images. **a** Head, full-face view; **b** mandibles; **c** eye; **d** mesosoma, lateral view; **e** mesosoma, dorsal view; **f** gaster, dorsolateral view.

***S. bondari* queen description**

Measurements in mm (range): HWe 1.33–1.48 HW 1.35–1.48 IFW1 0.99–1.02 IFW2 0.34–0.40 HL1 1.32–1.44 HL2 1.12–1.24 ScL 0.92–1.00 EL 0.27–0.29 Om 17.00–21.00

EW 0.08–0.08 WL 1.96–2.25 PL 0.40–0.45 PPL 0.24–0.28 GL 1.95–2.05 HFL 1.60–1.64
PrW 1.15–1.28 hw1 1.40–1.46 hw2 1.15–1.58 hw3 0.93–1.09 FWg 6.71–6.71 HWg
4.66–4.66 CI 98.01–102.78 FLI 69.12–75.02 SI 67.57–71.11 EI 19.46–21.61 [n=3]

As in the worker, the body of the *S. bondari* queen is covered with thick black hairs.

Head. Masticatory margin of mandible with 7–8 teeth. Mandibles glossy and smooth across dorsal surface, finely transversely striate only along masticatory margin, this fine striation in some specimens hard to see so mandibles look entirely smooth. Preocular carina fading shortly after reaching the top of the eye, but several short and very thin posterior carinae present, never reaching the posterior cephalic corner (Figure 3.8 c: EC). Eyes large and convex (EI=19.80), 17 to 21 ommatidia across largest diameter. Frontal lobes wider than in worker (FLI=72.54, worker FLI=66.52), antennal scape relatively long, almost reaching posterior cephalic margin (SI=69.23).

Mesosoma. Lateral pronotal tubercle low and obtuse, but slightly more pronounced than in the other species. Mesoscutum in dorsal view with reduced notauli in the form of faint, shallow impressions, sometimes entirely absent. Median mesoscutal line present, its anterior portion sometimes developed into a weak costa, its posterior end with shallow longitudinal impressions on each side. Parapsidal lines present, faint, slightly curved. Scutellum inflated, short in dorsal view, narrowing posteriorly, medially often with a faint longitudinal impression, posterior notch V-shaped, relatively deep. Propodeum in dorsal view with low carinae, sometimes ending in low denticles, sometimes the carinae reduced and only the denticles visible.

Metasoma. Node of petiole in frontodorsal view with four distinct denticles: two dorsal, which are long and narrow, and a two smaller, lateral denticles. Node of postpetiole with 2 short and low carinae dorsally and two low denticles laterally. First gastral tergite with lateral longitudinal carinae strongly developed, one on each side, with anteromedian carinae absent or faint, and longitudinal anteromedian groove visible in dorsal view.

***S. bondari* diagnosis queen**

The large size, smooth mandibles, and presence of dark thick hairs are key characters for separating *S. bondari* from other queens. In populations of *S. bondari* with reduced hairs the combination of large size and smooth mandibles will distinguish it from the relatively similar *S. mayri* (which has striate mandibles) and *S. saussurei* (which is smaller in size).

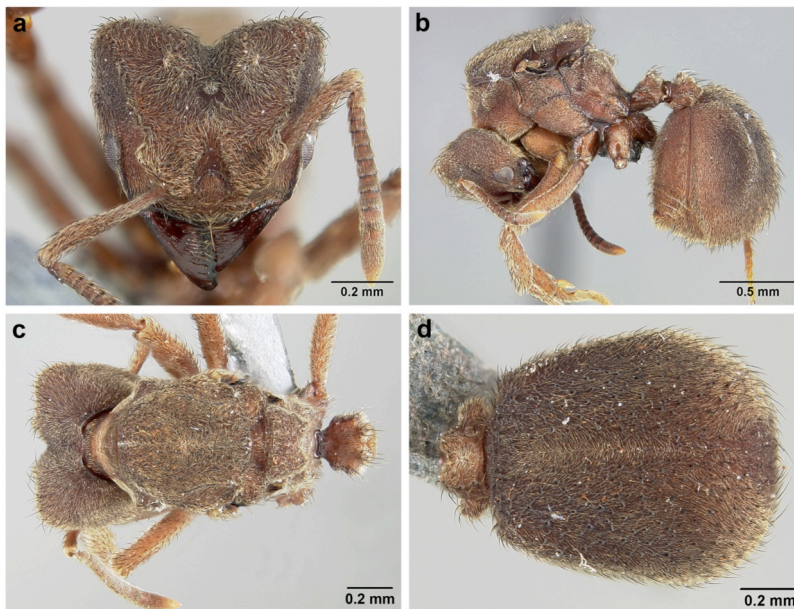


Figure 3.25. *S. bondari* queen (USNMENT01125803). **a** Head; **b** lateral profile; **c** mesosoma and **d** gaster, dorsal views.

***S. bondari* male** Unknown.

***S. bondari* larvae description**

Two to four setae on dorsal and lateral body surfaces on each side. Supra-antennal setae present, one over each antennae. Four genal setae on each side. Mandibular apical teeth undivided. Just a few labial denticles present anterior to sericteries. First and second thoracic segments ventrally with multiple multidentate spinules, arranged in transverse rows. Numbers of ventral setae: six on T1 and T3, four on T2, around six on abdomen. One pair of setae directly anterior to anal opening, and another pair on abdominal segment 9 positioned laterad, on each side of the anal opening.

***S. bondari* geographic range** Colombia, Ecuador, Peru, Guyana, Fr. Guiana, Surinam, and Brazil. Map: Figure 3.27.

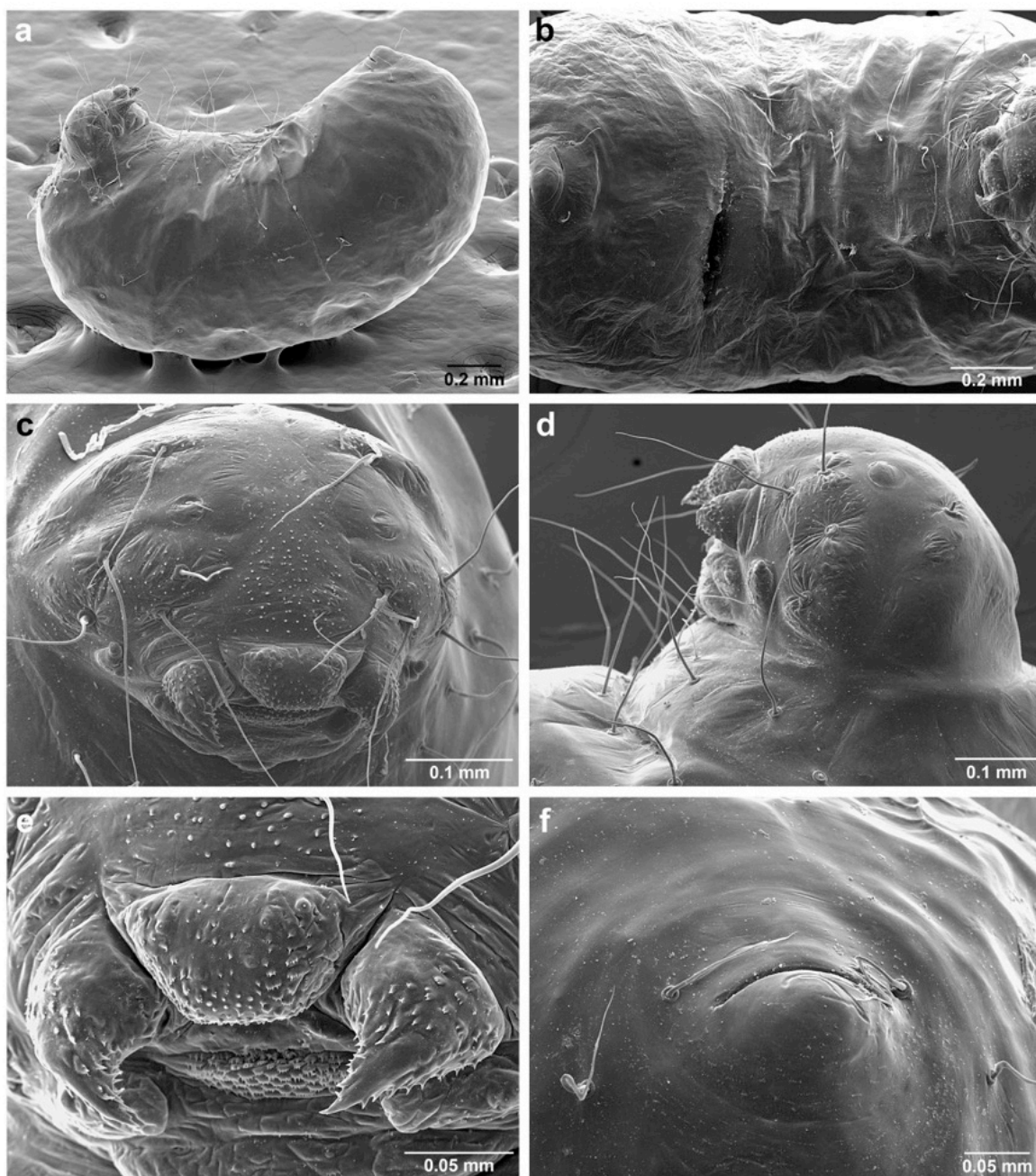


Figure 3.26. *S. bondari* larva (USNMENT01125807) SEM images.

a Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

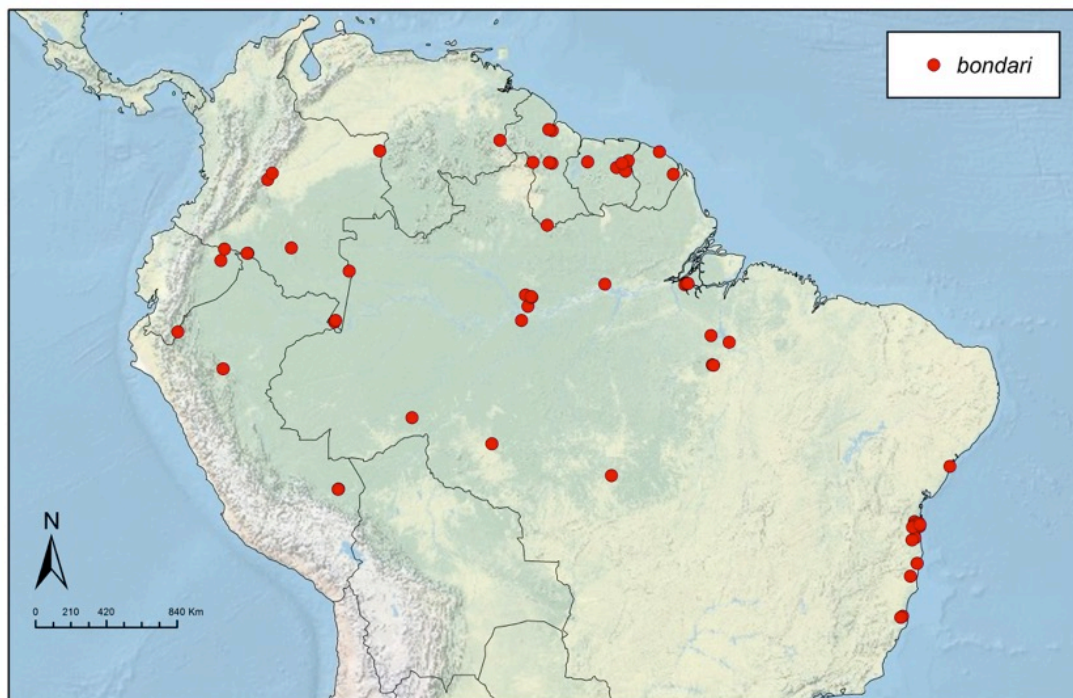


Figure 3.27. Distribution map of *S. bondari*.

***S. bondari* notes**

Some specimens of *S. bondari* have slightly to very reduced hairs, including five workers from Brazil (Melgaço, Para), one from Ecuador (Cuyabeno), and three from Venezuela (Bolívar) of a total of ~200 dry-mounted specimens examined. Aside from reduced hairs they have the typical *bondari* morphology and measurements (Figure 3.5 g). Molecular data (COI only) for the reduced-hair *bondari* specimens from Melgaço groups them with specimens originating in the geographically adjacent Carajas. The Carajas individuals have dense, dark, but slightly shorter hairs.

Individuals with an intermediate state (hairs slightly reduced) exist, some from Melgaço (Para) and some from Espírito Santo, Brazil. The existence of intermediate forms, the molecular phylogeny, and PCA analysis of morphological measurement data

all indicate that populations with reduced hairs are rare variants of *bondari* rather than separate species. Another variation within *bondari*, also occurring in the Carajas specimens, is a slightly bicolored (rather than uniformly colored) integument (Figure 3.23), but this state occurs only in a minority of workers from the same nest, the rest of which are homogeneously colored.

***S. bondari* material examined**

BRAZIL: Amazonas: Manaquiri, Br 319, km101, [-3.68, -60.31], 10 Oct 2010, F. Baccaro; Manaus, Camp 41, -2.4494, -59.7634, 118m, 10 Jan 2009, J. Sosa-Calvo; Manaus, Reserva Ducke, -2.9324, -59.9721, 95m, 26 Sep 2012, A. Ješovnik; Manaus, BDFFP Camp Gaviao, -2.4219, -59.8469, 29 Feb 2000, T. R. Schultz; Manaus, BDFFP Dimona Camp, 100-ha. Frag., -2.3388, -60.1026, 16 Aug 2000, R. M. M. Adams; ZF3-Km41, -2.4166, -59.8, 20 Sep 1996, A. C. Macedo; **Bahia:** Canavieiras, Oiticica, -14.4094, -30.0166, 30 Mar 1998, J. S. C. Carmo; Ilheus, Pimenteira mata W-A17, -14.5352, -39.4275, 6 Oct 1997, J. R. M. Santos, J. S. C. Carmo; Ilheus, Ponta do Ramo, -14.4977, -39.0405, 11 Feb 1997, J. R. M. Santos; Itacare, -14.3177, -39.0719, 3 Aug 1998, J. R. M. Santos; Itamaraju, [-17.0438, -39.5300], 29 Mar 2004, J. H. C. Delabie; Jussari, Pratos, -15.1955, -39.4452, 18 Jul 1997, J. R. M. Santos; Marau-tramanbe Mata WA4, -14.4022, -39.3233, 7 May 1997, J. R. M. Santos; Porto Seguro, E.E. pau brasil, -16.3925, -39.1694, 16 Jun 2000, J. R. M. Santos, S. M. Soares; Ubaitaba, -14.2502, -39.3213, 9 Apr 1998, J. R. M. Santos; Unacau A43, -15.0891, -39.295, 11 Feb 2000, J. R. M. Santos; Urucuca, Mata A19, -14.5125, -39.2002, 24 Oct 2002, J. R. M. Santos; **Espírito Santo:** Guriri, -18.7167, -39.75, 1 Mar 2005, M. C. Teixeira; Parque Sooretama,

Linhares, -19.0725, -39.9491, 17 Oct 1962, F. S. Pereira; **Mato Grosso:** Sinop, [-11.8581, -55.5056], 1 Oct 1974, Alv., Roppa; **Pará:** Melgaço, Caxiuanã, ECFPn V Transecto 3-700) Winkler #2, -1.7248, -51.4230, 26 Apr 2004, A. Y. Harada; Nova Ipixuna, Fazenda Bom Retiro, Parcela 04, -4.8412, -49.2180, 12 Apr 2012, M. Tavares, A. Palmeira; Novo Repartimento, Faz. Aratau, [-4.49, -50.19], 17 Jun 2002, A. M. Elizabeth; Oriximina, Alega Reloiado, -1.76, -55.85, 8 Oct 1982, A. Y. Harada; Parauapebas, FL Nacional de Carajás, Parque Zoobotanico, -6.0629, -50.0571, 626m, 1 Oct 2014, A. Ješovnik; **Rondônia:** Ouro Preto do Oeste, Res do INPA No 0158, [-10.2, -61.9], 27 Mar 1985, W. Franca; **Sergipe:** Sta. Luiza do Itanhy, Crasto, -11.3775, -37.4187, 21 Jun 2001, R. R. Silva, R. M. Feitosa, C. R. F Brandão; **COLOMBIA:** **Amazonas:** PNN Amacayacu Matamata, -3.6833, -70.25, 150m, 1 Oct 2001, D. Chota; PNN Amacayacu, San Martín, -3.7666, -70.3, 150m, 7 Nov 2001, D. Chota; **Caquetá:** Puerto Solano, PNN Chiribiquete, Serranía de Chiribiquete, Rio Sararamano, 0.167, -72.6097, 250m, 1 Apr 2000, E. Gonzales; **Meta:** PNN Sumpaz, Canana las Mirias, 3.8, -73.8666, 779m, 1 Oct 2003, H. Vargas; Villavicencio, La Vanguardia, Sector Pozo Azul, [4.1451, -73.6269], 375m, 16 Apr 2005; **Putumayo:** PNN La Paya Cabaña Chagra, -0.1166, -74.9333, 320m, 15 Oct 2001, R. Cobete; **Vaupés:** Est. Biol. Mosiro-Itajura (Caparu) Centro Ambiental, -1.0666, -69.5166, 60m, 1 Feb 2003, M. Sharkey, D. Arias; **Vichada:** Municipio Cumaribo Cgto. Santa Rita PNN El Tuparro, 5.3316, -67.8908, 135m, 8 Feb 2004, I. Quintero, E. Gonzales; **ECUADOR: Morona-Santiago:** Los Tayos, [-4.3, -78.67], 3 Aug 1976, Tjitte de Vries; **Napo:** Reserva Faunistico Cuyabeno, 0.1167, -76.1833, 1 Nov 1994, J. P. Caldwell; **Sucumbíos:** La Selva Lodge, Mandi Cocha, [-0.4973, -76.3747], 11 Jun 2003, S. Villamarin; **FRENCH GUIANA: Cayenne:**

10 km south Sinnamary, Paracou forest, 5.2808, -52.9465, 2006-2009; Nouragues Field Station, 4.09, -52.2, 1 Oct 2009; **GUYANA: Cuyuni-Mazaruni:** Mazaruni River, Forest Settlement, 6.3973, -58.6781, 1 Aug 1935, N. A. Weber; Oko R., Cuyuni trib., [6.4638, -58.8538], 22 Jun 1936, N. A. Weber; **Potaro-Siparuni:** Iwokrama For. Res. Whitewater Camp, 4.7168, -58.8333, 60m, 6 Nov 2002, J. S. LaPolla; Iwokrama, Kurapakari base Camp, [4.6698, -58.6854], 60m, 6 Apr 1996, T. R. Schultz; Paramakatoi, PK-Yawong Trail, [4.7167, -59.7], 704m, 16 Apr 1996, T. R. Schultz; **Upper Takutu-Upper Essequibo:** Acarai Mts, nr Romeo's camp, 1.3833, -58.9333, 735m, 16 Oct 2006, T. R. Schultz, C. J. Marshall; **PERU: Madre de Dios:** Puerto Maldonado, Los Amigos Biol. Station, -12.5617, -70.0924, 276m, 20 Nov 2005, J. Sosa-Calvo; San Martín Davidcillo, 30km NNE Tarapoto, -6.25, -76.25, 220m, 21 Aug 1986, P. S. Ward; **SURINAME: Brokopondo:** Maripaheuvel, near Dam on Sara creek, [4.67, -54.95], 1 Sep 1959, I. v. d. Drift; Poeroe man Kemisa, [4.67, -54.95], 1 Sep 1959, I. v. d. Drift; **Sipaliwini:** Bakhuis Mountains, 4.7451, -56.7832, 5 m, 11 Mar 2006, J. Sosa-Calvo; Lely Mountains, 4.2529, -54.7561, 619m, 26 Oct 2005, J. Sosa-Calvo; Nassau Mountains, 4.8172, -54.6067, 514m, 3 Nov 2005, J. Sosa-Calvo; **VENEZUELA: Bolivar:** Bolivar, via El Dorado-Sta. Elena Km. 80, [5.89, -61.46], 300m, 26 Jun 1984, J. Lattke.

***Sericomyrmex LUTZI* Wheeler 1916**

Worker: Figures 3.28, 3.29; **Queen & Male:** Figure 3.30; **Map:** Figure 3.31.

Sericomyrmex lutzi Wheeler, 1916: 9. Lectotype (here designated): GUYANA, Roraima, Kauwa creek [5.223, -60.73], 13 Aug 1911, A. Crampton, AC3097 (MCZ: 2w, 1 aq, MCZ 9-11 21139, topmost worker on the pin). Paralectotypes: same data as lectotype (MCZ: 2w, 1 aq, MCZ 9-11 21139, lower two specimens on the pin) (MCZ: 1pin, 3w, 12-14 21139).

***S. lutzi* worker description**

Measurements in mm (range): HWe 1.12–1.35 HW 1.12–1.35 IFW1 0.70–0.88 IFW2 0.27–0.33 HL1 1.16–1.30 HL2 0.96–1.12 ScL 0.80–0.93 EL 0.14–0.19 Om 9.00–11.00 WL 1.38–1.68 PL 0.30–0.44 PPL 0.22–0.26 GL 0.90–1.18 HFL 1.20–1.40 PrW 0.73–0.94 hw1 1.08–1.27 hw2 1.21–1.46 hw3 0.86–0.90 CI 99.69–104.22 FLI 62.50–67.66 SI 66.59–72.78 EI 12.10–15.05 FLD 0.40–0.55 CEI 13.78–16.54 [n=7]

Body color yellowish-brown to ferrugineous-brown. Pubescence dense, lighter than the integument, appressed to decumbent. Hairs curved, darker in color at the base, yellow to gray, appressed to suberect, but mostly decumbent.

Head. Head in frontal view evenly long and broad to slightly broader than long (CI=102.53 ± 2.02), posterior corners acute, lateral margins of the head convex, posterior cephalic emargination distinct, very deep (CEI=15.35 ± 1.16). The posterior cephalic margin between the cephalic corner and the median emargination straight, gradually merging with the emargination. Vertexal impression relatively deep, pronounced, frontal tumuli present. Mandibles smooth and glossy, finely transversely striate only along

masticatory margin. Masticatory margin of mandible with 7–9 teeth. Frontal carinae straight to slightly curved laterally, complete. Eyes medium-sized ($EI = 13.87 \pm 0.98$), weakly convex, lacking the white layer, 9 to 11 ommatidia across largest diameter. Frontal lobes triangular, relatively narrow ($FLI = 64.28 \pm 1.72$), posterior margin shorter than medial. Antennal scape not reaching posterior cephalic margin ($SI = 71.08 \pm 2.08$).

Mesosoma. Lateral pronotal and anterior mesonotal spines low and obtuse. Propodeal carinae low, reduced or ending in small denticle.

Metasoma. Nodes of petiole and postpetiole dorsally each with a pair of low, short, serrate, longitudinal carina. On the petiole the carinae are sometimes reduced to two low denticles, best seen in dorsolateral view. First gastral tergite with longitudinal lateral carinae, one on each side, anteromedian dorsal carinae absent.

***S. lutzii* worker diagnosis**

The most distinct character of *S. lutzii* is a very deep posterior cephalic emargination, in combination with the large size, smooth mandibles, triangular frontal lobes, and absence of anteromedian dorsal carinae on the gaster. It is most similar to *S. mayri*, but *mayri* has striate mandibles; a much shallower posterior cephalic emargination; narrower, directed sharply anterad frontal lobes; and often at least faint anteromedian dorsal carinae.

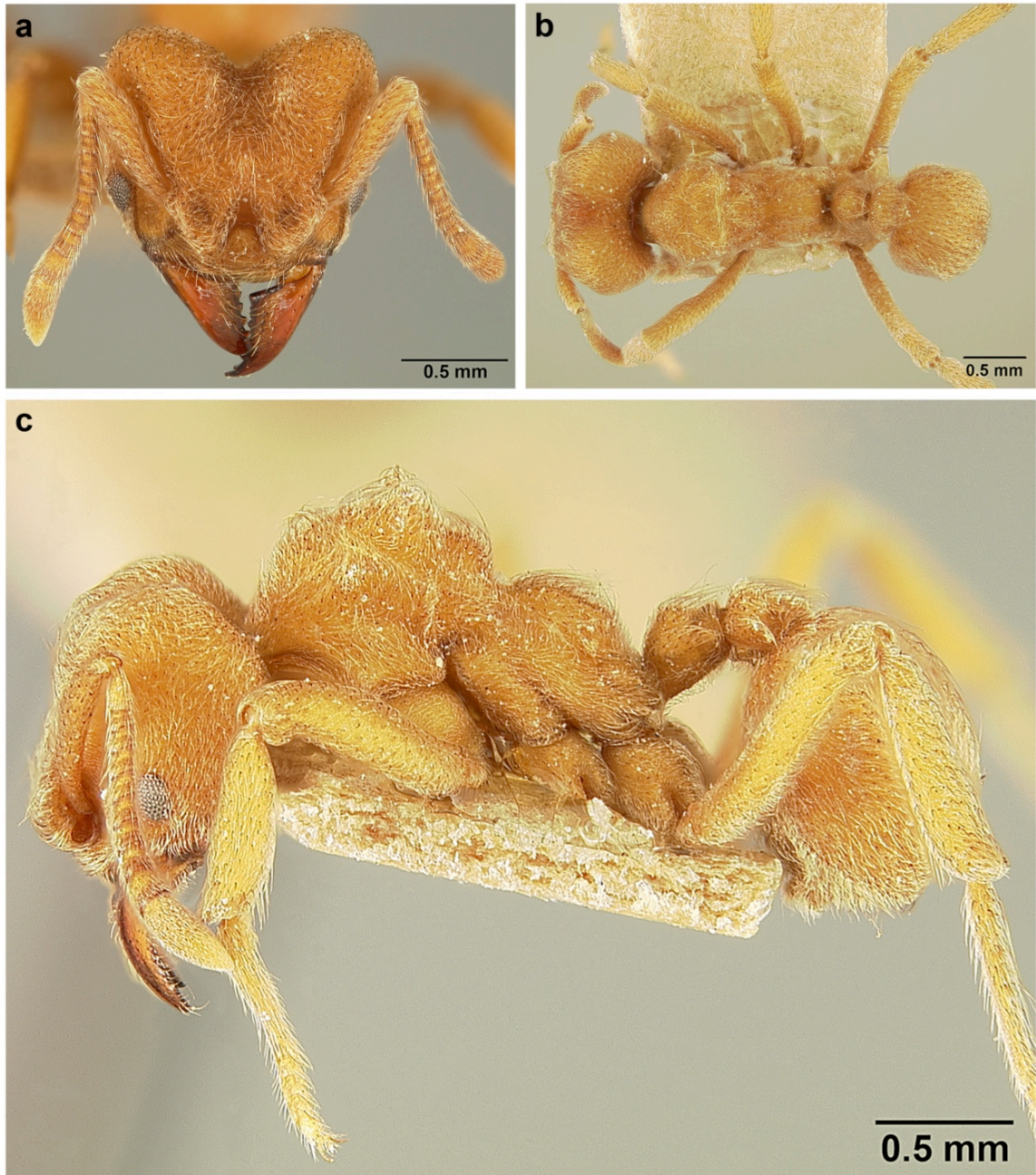


Figure 3.28. *S. lutzii* worker (MCZ 12-14 21139). **a** Head; **b** dorsal view; and **c** lateral profile.

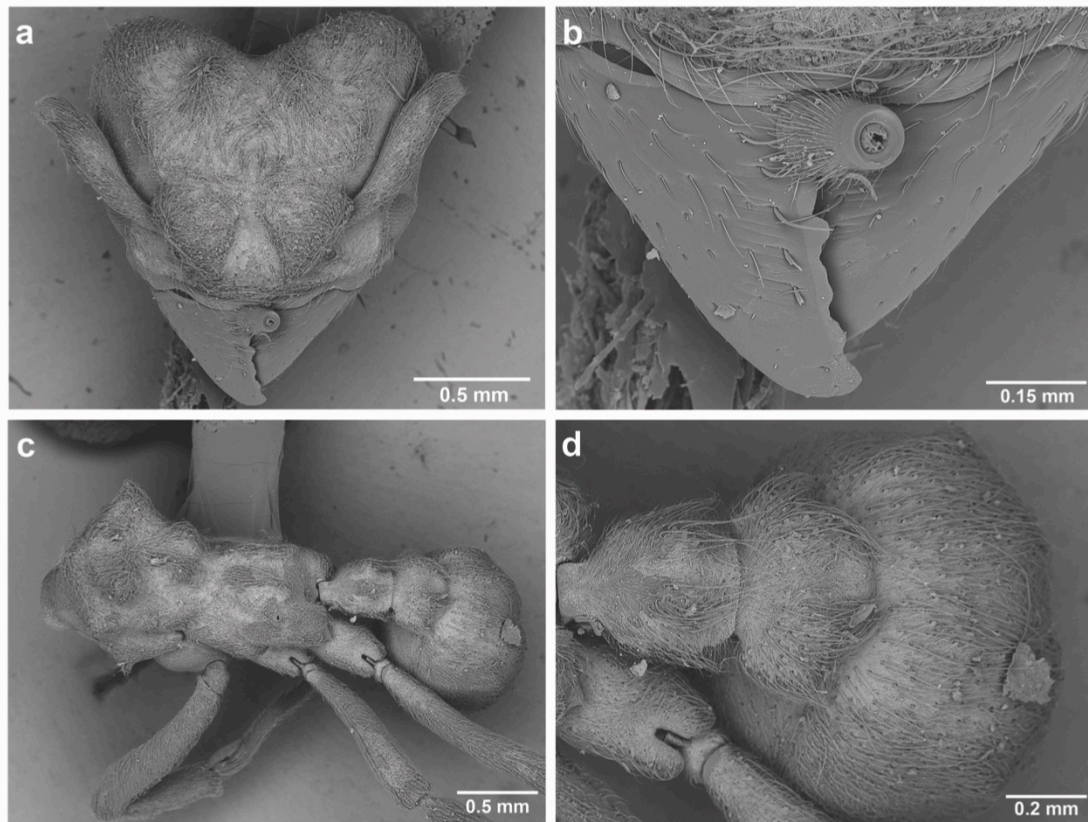


Figure 3.29. *S. lutzi* worker (USNMENT00445053) SEM images.

a Head, full-face view; **b** mandibles; **c** mesosoma and gaster, dorsolateral view; **d** petiole, postpetiole, and gaster, dorsal view.

***S. lutzi* queen description**

Measurements in mm: HWe 1.4 HW 1.4 IFW1 0.9 IFW2 0.34 HL1 1.36 HL2 1.16 ScL 0.92 EL 0.25 Om 18 EW 0.1 WL 2.08 PL 0.44 PPL 0.28 GL 1.72 HFL 1.56 PrW 1.18 hw1 1.32 hw2 1.52 hw3 1 FWg 6.82 HWg 4.6 CI 102.94 FLI 64.29 SI 65.71 EI 18 [n=1]

Head. Ocelli glossy, dark grey, half-embedded in the cuticle and surrounded with dense hairs; in full-face view only median ocellus visible. Masticatory margin of mandible with 9 teeth. Mandibles glossy and smooth across dorsal surface, finely transversely striate only along masticatory margin. Preocular carinae fading above the top

of the eye. Eyes large and convex (EI=18), number of ommatidia across largest diameter =18. Frontal lobes similar to worker in size and shape (FLI=74.80), antennal scape not reaching posterior cephalic margin (SI=64.29).

Mesosoma. Mesoscutum in dorsal view with reduced notauli and median mesoscutal line sometimes present, most distinct anteriorly. Parapsidal lines present, faint, slightly curved. Axillae in dorsal view entirely separated from one another, laterally rounded, narrowing medially. In dorsal view a transverse shallow groove separating axillae from the scutellum. Scutellum in dorsal view narrowing posteriorly, posterior notch shallow. Propodeum with two low, reduced denticles.

Metasoma. First tergite of gaster with strongly developed lateral longitudinal carinae, anteromedian dorsal carinae absent, shallow longitudinal anteromedian groove visible in dorsal view.

***S. lutzi* queen diagnosis**

Most similar in size to *S. mayri*, but with smooth mandibles and a deeper cephalic emargination. Also similar in size to *S. bondari* and *S. saussurei* populations with smooth mandibles, but larger and with a deeper cephalic emargination (than in both *bondari* and *saussurei*), and lacking thick dark hairs (as in *bondari*).

***S. lutzi* male**

Measurements in mm: HWe 0.9 HW 0.74 IFW1 0.27 IFW2 0.17 HL1 0.7 ScL 0.74 EL 0.29 Om 30 EW 0.13 WL 1.74 PL 0.4 PPL 0.22 GL 1.4 HFL 1.67 PrW 0.80 IOD 0.58 FWg 5.21 HWg 3.55 CI 128 FLI 30.13 SI 82.59 EI 32.14 [n=1]

Head in frontal view longer than broad (CI=128). Eyes large (EI=32.14), 30 ommatidia across the largest diameter. Preocular carinae extended posteriorly to the level of the median ocellus, curved medially before fading. Notauli and mesoscutal line well developed, integument around them slightly lighter in color, with dark-brown reticulate pattern, the groove between axillae with one transverse, short costa medially. Propodeal carinae and denticles absent, the only protuberances on the propodeum are the small tubercles on which the propodeal spiracles are mounted. Petiole with weak lateral carinae; postpetiole simple, smooth, without denticles or carinae.

***S. lutz* geographic range.** Guyana. Map: Figure 3.30.

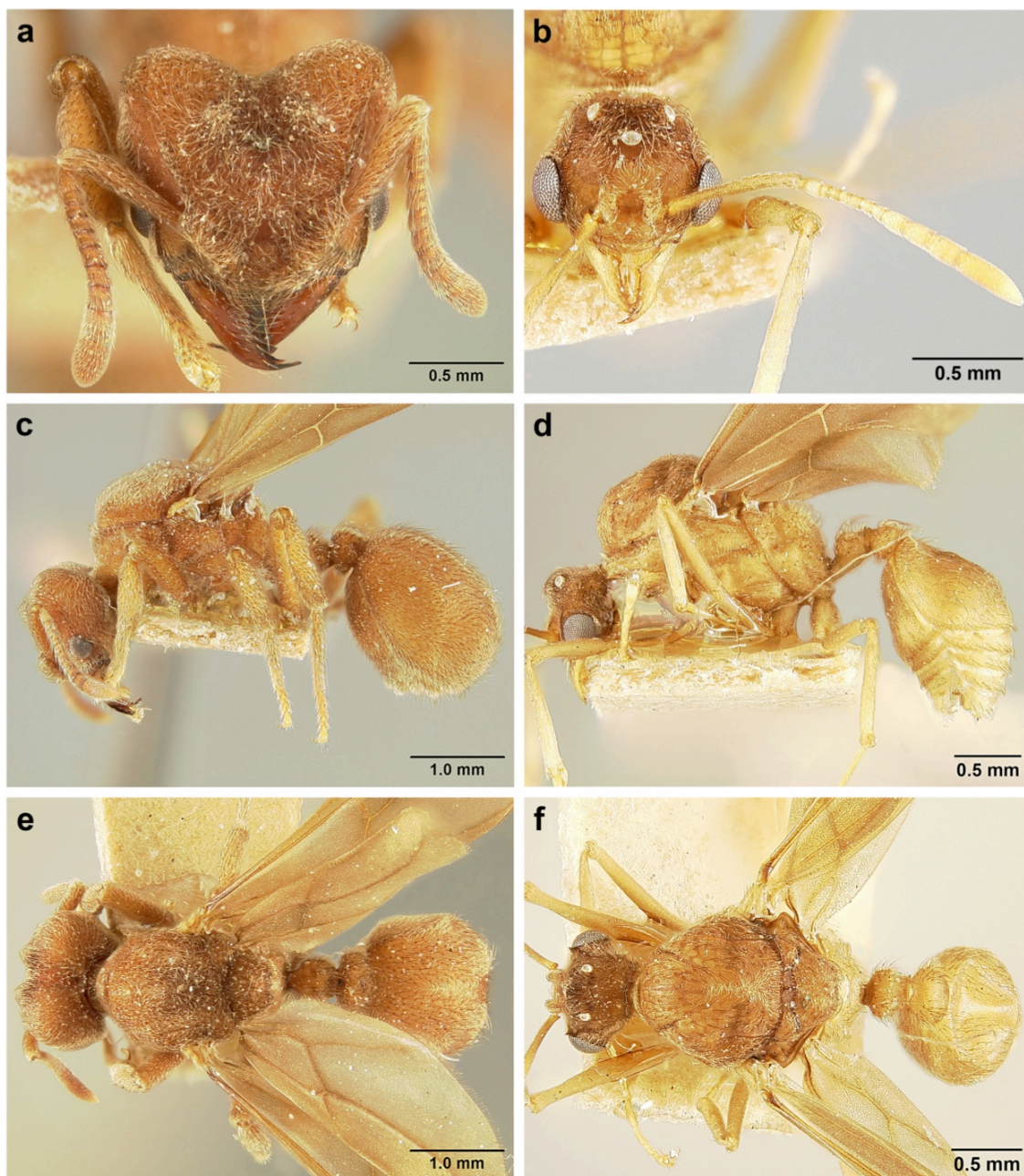


Figure 3.30. *S. lutzi* queen and male; head, profile, and dorsal view. Queen (MCZ 9-11 21139): **a, c, e.**
Male (USNMENT01126226): **b, d, f.**



Figure 3.31. Distribution map of *S. lutzi* and *S. n. sp. 3*.

***S. lutzi* notes**

Based on morphology this species seems to be most closely related to *S. mayri*. It has a restricted distribution in the mountainous region of western Guyana, near the borders of Venezuela and Brazil. It was described from a handful of specimens collected on Mt. Roraima, a mountain plateau (tepui) on the border of Guyana, Venezuela, and Brazil. The only other specimens of this species were collected on the slopes of the eastern-most tepui in Guyana, Mt. Ayanganna. It is very likely that this species has a restricted distribution and is endemic to the tepuis of Guiana Highlands. This area is known for its endemic flora and fauna (Brown, 1975; Steyermark, 1987; Berry *et al.*, 1995; Lapolla *et al.*, 2007).

***S. lutzii* material examined**

GUYANA: Cuyuni-Mazaruni: Mt. Ayanganna base camp, 5.3344, -59.9248, 732 m, 8 Oct 2002, T. R. Schultz.

***Sericomyrmex MAYRI* Forel 1912**

Worker: Figures 3.32, 3.33, 3.34; **Queen:** Figure 3.35; **Male:** Figure 3.35; **Larva:** Figure 3.36; **Map:** Figures 3.37, 3.38.

Sericomyrmex mayri Forel, 1912: 194. Lectotype (here designated): BRAZIL, Rio de Janeiro, Niterói, [-22.8751, -43.2775] A. Forel, ANTC31816 (MHNG: 1w, CASENT0909370).

=*Sericomyrmex luederwaldti* Santschi 1925: 15. **New junior synonym.**

Type material examined: BRAZIL, Minas Gerais, Pirapora, [-17.355, -44.9447], E. Garbe, ANTC35978, ANTC25817 (NHMB: 5w, CASENT0912516) (MSNG: 1w, CASENT0904989).

=*Sericomyrmex moreirai* Santschi, 1925: 16. **New junior synonym.**

Type material examined: BRAZIL, Rio de Janeiro, [-22.8751, -43.2775], Moreira, ANTC35979 (MCZ: 1pin, 2w, 1-2 21140) (NHMB: 3pin, 5w, 2q, CASENT0912517).

=*Sericomyrmex harekulli* Weber, 1937: 398. **New junior synonym.**

Type material examined: GUYANA, East Berbice-Corentyne, Oronoque river, [2.75, -57.4167], 27 Jul 1936, N. A. Weber, NAW 598 (NMNH: 1w, USNMENT00529483) (MCZ: 2w, USNMENT00924104; 2w, USNMENT00924105)

=*Sericomyrmex harekulli arawakensis* Weber, 1937: 399. **New junior synonym.**

Type material examined: GUYANA, Cuyuni-Mazaruni, Mazaruni River, Forest Settlement, [6.39733, -58.6781], 10 m, 15 Aug 1935, N. A. Weber, NAW 277 (MCZ: 2w, MCZ 23051; 2w, 1q, USNMENT00924106)

=*Sericomyrmex urichi* Forel 1912: 193. **New junior synonym.**

Type material examined: TRINIDAD, F. W. Urich, ANTC31818 (MHNG: 1pin, 3w, CASENT0909372).

***S. mayri* worker description**

Measurements in mm (range): HWe 1.05–1.60 HW 1.05–1.64 IFW1 0.66–1.00 IFW2 0.24–0.40 HL1 1.02–1.52 HL2 0.93–1.36 ScL 0.74–1.08 EL 0.15–0.35 Om 10.00–13.00 WL 1.27–2.20 PL 0.24–0.47 PPL 0.18–0.35 GL 0.92–1.42 HFL 1.15–1.70 PrW 0.68–1.20 hw1 1.02–1.68 hw2 1.12–1.80 hw3 0.74–1.12 CI 95.74–115.08 FLI 50.00–68.24 SI 60.53–76.29 EI 11.25–25.81 FLD 0.40–0.68 CEI 4.70–18.75 [n=103]

Body color yellowish-brown to ferrugineous-brown. Pubescence dense, often lighter than the integument, appressed to decumbent. Hairs straight to curved, darker in color at the base, yellow to gray, appressed to suberect, but mostly decumbent.

Head. Head in frontal view broader than long ($CI=107.88 \pm 3.50$), posterior corners angular to acute, lateral margins of the head mostly straight, sometimes slightly convex, posterior cephalic emargination distinct but usually relatively shallow. The posterior cephalic margin between the cephalic corner and the median emargination can be straight to convex and it can merge with the emargination gradually or, more common for *mayri*, it can be more or less straight and abruptly merge with the emargination. This character varies within the species and within colonies. Vertexal impression relatively

deep, pronounced, frontal tumuli often distinct. Mandibles glossy, usually striate across the whole dorsal surface. Masticatory margin of mandible with 7 to 8 teeth. Frontal carinae straight to slightly curved laterally, usually fading before reaching posterior cephalic corners, but sometimes complete. Eyes medium-sized ($EI=14.38 \pm 1.40$), flat to slightly convex, lacking the white layer, 9 to 12 ommatidia across largest diameter. Frontal lobes triangular, narrow ($FLI=63.14 \pm 2.08$), posterior margin much shorter than medial, and lateral margin long, giving the lobes the appearance of being directed downward (anteriorly). Antennal scape relatively short, never reaching posterior cephalic margin ($SI=68.11 \pm 3.14$).

Mesosoma. Mesosomal spines and tubercles low and obtuse. Propodeal carinae low, without denticle posteriorly or denticle low and hard to see.

Metasoma. Nodes of petiole and postpetiole each with a pair of two low, short, serrate, longitudinal carina, which in the petiole are sometimes reduced to a two low denticles, best seen in dorsolateral view. First gastral tergite with strong lateral longitudinal carinae, anteromedian dorsal carinae absent or faint.

***S. mayri* worker diagnosis**

The largest *Sericomyrmex* species. Head wide; narrow, frontal lobes directed strongly anterad; mandibles striate; frontal carinae often reduced, fading well before reaching posterior cephalic corners; eyes dark, clear, without white layer, flat to mildly convex. Posterior margin of head often appears flattened and joins abruptly with the posterior cephalic emargination (Figure 3.7 k); posterior cephalic corners angled in most specimens. Anterior mesonotal spine low and obtuse, anteromedian dorsal carinae usually

absent. In some specimens striation of dorsal mandibular surface is reduced, appearing smooth or just faintly striate. When faint striation is present, it is most obvious at the posterolateral edges of the mandibles, in dorsal view, where the mandibles connect with the head capsule.

Most similar to *S. amabilis* and *S. saussurei*, both with striate mandibles. Both *saussurei* and *amabilis* have wider frontal lobes, anteromedian dorsal carinae on the gaster, and are smaller in size than *mayri*. In addition, *saussurei* has eyes covered with a thick white layer, which is never the case with *mayri*. Similar in size to *S. lutzi*, but *lutzi* has a characteristic, much deeper cephalic emargination and smooth mandibles.

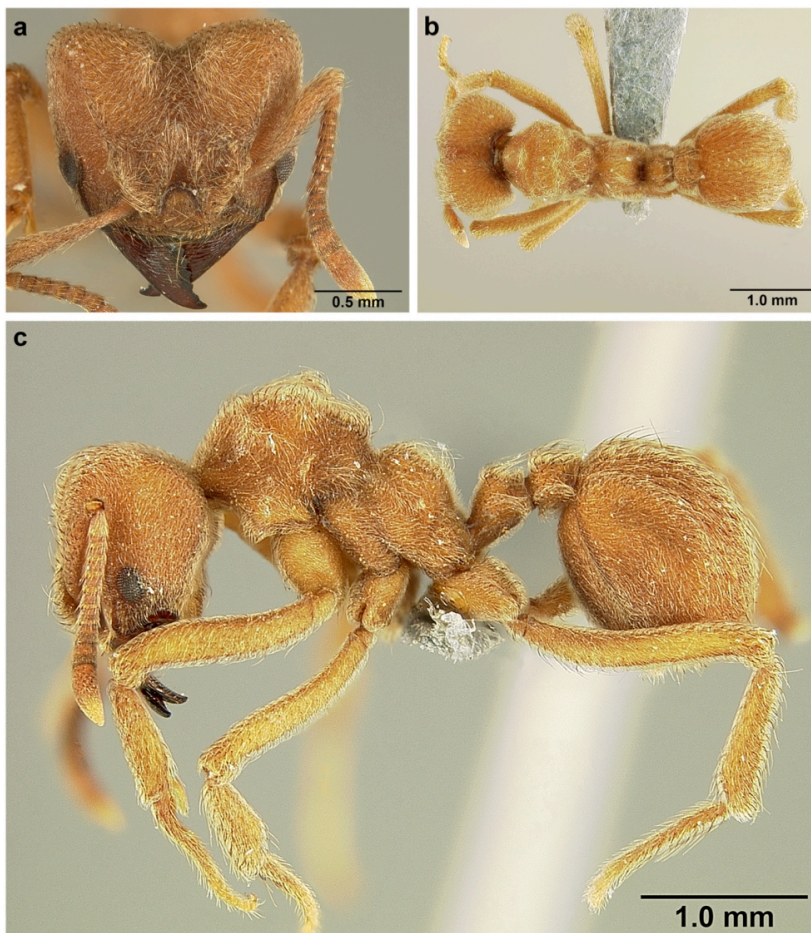


Figure 3.32. *S. mayri* worker (USNMENT01125171). **a** Head; **b** dorsal view; and **c** lateral profile.



Figure 3.33. *S. mayri* worker variation; head, full-face view. **a** *mayri* worker from Brazil, Amazonas (USNMENT00444066); **b** *mayri* worker from Brazil, Bahia (USNMENT01125172); **c** *mayri* worker from Colombia, Meta (USNMENT01125151).

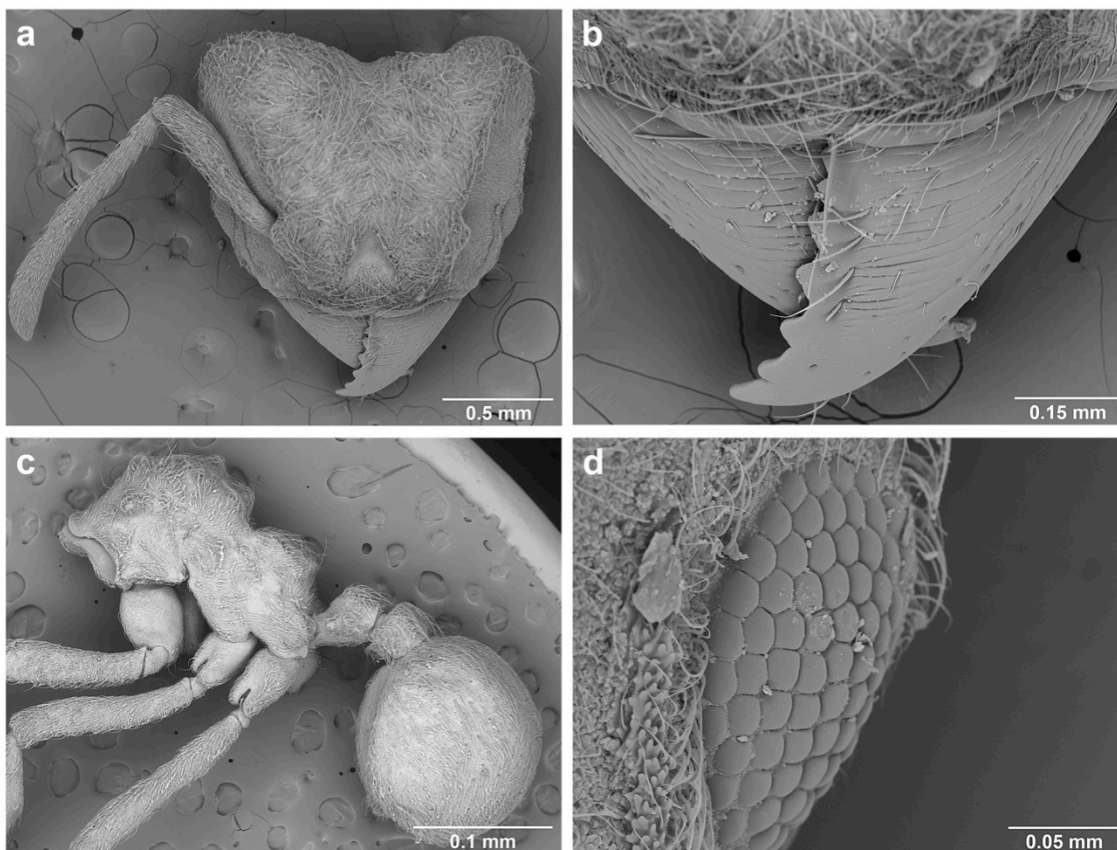


Figure 3.34 *S. mayri* worker (USNMENT01126229), SEM images. **a** Head, full-face view; **b** mandibles; **c** mesosoma and gaster, lateral view; **d** eye.

***S. mayri* queen description**

Measurements in mm (range): HWe 1.44–1.64 HW 1.48–1.68 IFW1 0.98–1.13 IFW2 0.36–0.45 HL1 1.4–1.56 HL2 1.24–1.4 ScL 0.96–1.09 EL 0.24–0.3 Om 16–21 EW 0.08–0.13 WL 2.12–2.5 PL 0.45–0.65 PPL 0.25–0.4 GL 1.76–2.21 HFL 1.5–1.85 PrW 1.24–1.46 hw1 0.52–1.76 hw2 1.64–1.84 hw3 1.03–1.2 FWg 6.56–8.03 HWg 4.29–5.28 CI 100–108.96 FLI 63.94–72.42 SI 62.38–70 EI 15.75–19.49 [n=15]

Head. Masticatory margin of mandible with 8–9 teeth. Mandibles striate across entire dorsal surface. Preocular carina fading above the eye, but rarely (in one queen from Ecuador) a few weak supraocular carinae are present, never reaching the posterior cephalic corner. Eyes large and convex ($EI=17.70 \pm 1.12$), sometimes mildly notched posteriorly. Number of ommatidia across largest diameter 16–21. Frontal lobes similar to worker ($FLI=67.57 \pm 2.41$), antennal scape not reaching posterior cephalic margin ($SI=66.39 \pm 2.43$)

Mesosoma. Lateral pronotal tubercle present, low and obtuse. Mesoscutum in dorsal view with reduced notauli, and faint, slightly curved parapsidal lines. Median mesoscutal line present, reduced, its anterior portion sometimes developed into a weak keel, posteriorly with a faint longitudinal impression on each side. In dorsal view scutellum short, narrowing posteriorly, medially often with a faint longitudinal impression, posterior notch shallow. Propodeal denticle reduced, low.

Metasoma. Node of petiole in frontodorsal view with four distinct denticles: two dorsal and two smaller, lateral denticles. Postpetiole with four short and low longitudinal carinae (sometimes also reduced to low denticles), two dorsal and two lateral. Lateral longitudinal carinae on first gastral tergite strongly developed. In dorsal view first gastral

tergite with longitudinal anteromedian groove and two anteromedian dorsal carinae, one on each side of the groove.

***S. mayri* queen diagnosis**

The largest *Sericomyrmex* species, separated from the sympatric, similarly sized *S. bondari* by the absence of dark, thick hairs, and by the striate mandibles (smooth in *S. bondari*). The sympatric *S. saussurei* also has striate mandibles, but *S. mayri* is larger, and has a wider head and more pronounced petiolar denticles.

***S. mayri* male description**

Measurements in mm (range): HWe 0.84–1.02 HW 0.71–0.84 IFW1 0.32–0.38 IFW2 0.15–0.27 HL1 0.72–0.8 ScL 0.77–0.85 EL 0.31–0.36 Om 24–32 EW 0.13–0.16 WL 1.88–2.05 PL 0.35–0.56 PPL 0.24–0.33 GL 1.32–1.8 HFL 1.9–2.2 PrW 0.87–1.12 IOD 0.65–0.74 FWg 5.4–6.25 HWg 3.79–4.29 CI 104.63–130.77 FLI 33.75–42.05 SI 78.43–95.58 EI 31.38–41.82 (n=8)

Head in frontal view longer than broad (CI=124.33 $\pm$ 8.37). Eyes large (EI=36.24 $\pm$ 3.17), ommatidia across the largest diameter 24–32 . Preocular carinae long, extended posteriorly beyond the lateral ocellus, slightly curved medially before fading. Notauli and mesoscutal line well developed, integument around them usually lighter in color, often with a reticulate pattern, the groove between the axillae with up to 4 transverse keels, but often with just one strong, short keel medially. Propodeum smooth, without propodeal carinae or denticles, the only protuberances the small tubercles on

which the propodeal spiracles are mounted. Petiole with lateral and dorsal serrate carinae, postpetiole only with very reduced lateral denticles.

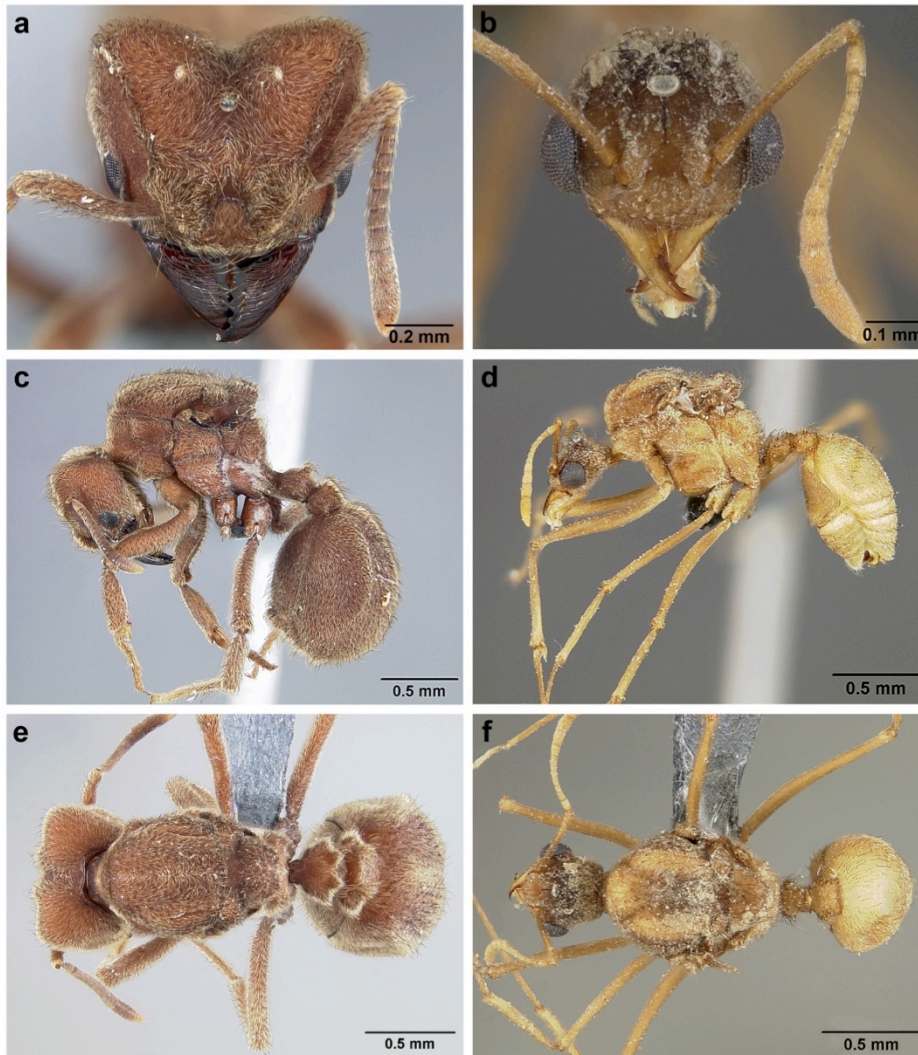


Figure 3.35. *S. mayri* queen and male; head, lateral profile, and dorsal view. Queen (USNMENT01126007): **a, c, e**. Male (USNMENT01126022): **b, d, f**.

***S. mayri* larvae description**

Two to four setae on each side of the lateral body surfaces, none dorsally. Supra-antennal setae present, one over each antennae. Six genal setae on each side. Mandibular apical teeth divided. Labial denticles absent. First thoracic segment ventrally with

multiple multidentate spinules, arranged in transverse rows. Numbers of ventral hairs: ten to fourteen on T1, six on T2, and four to six and T3, two to eight on the abdomen (not including anal setae). A single pair of setae anterior to anal opening, no additional setae laterally.

***S. mayri* geographic range:** Colombia, Peru, Ecuador, Guyana, Surinam, Fr. Guiana, Trinidad and Tobago, Venezuela, Brazil, Bolivia, and Paraguay. Map: Figure 3.37.

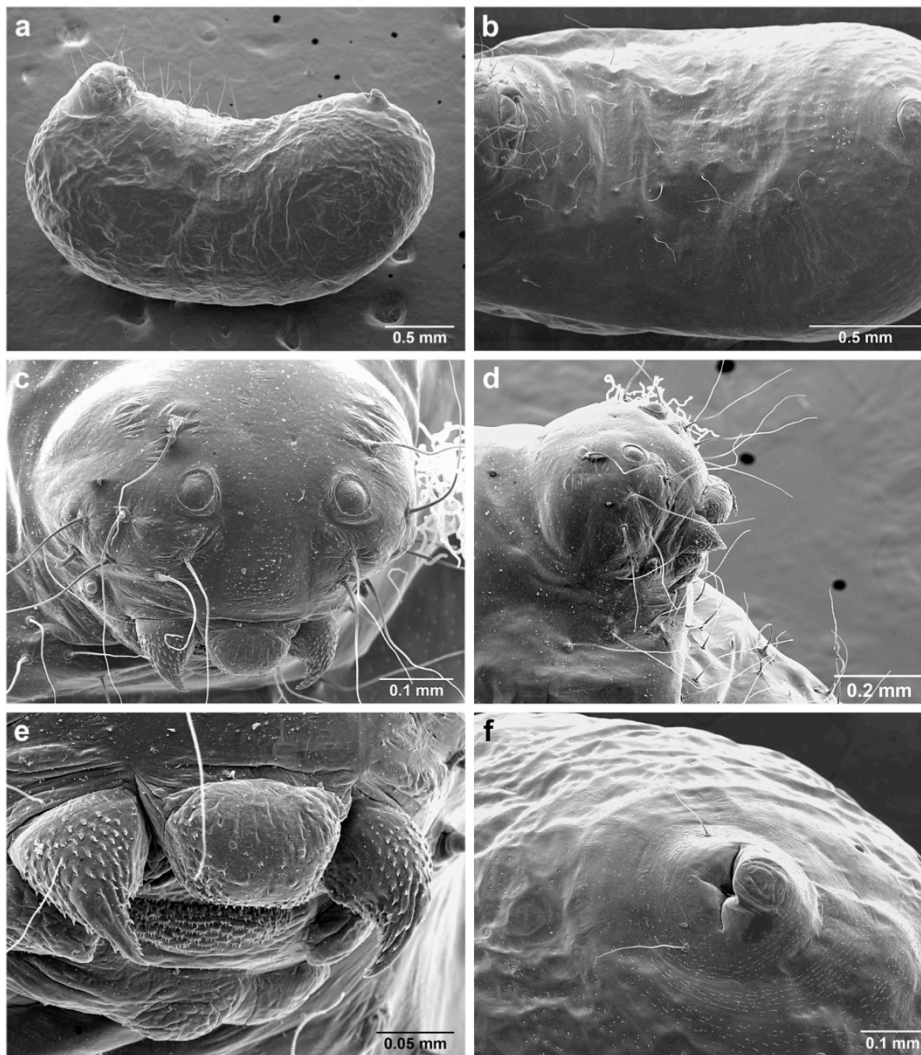


Figure 3.36. *S. mayri* larva (USNMENT01126227: **a, f**; USNMENT01126230: **b, c, d, e**), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

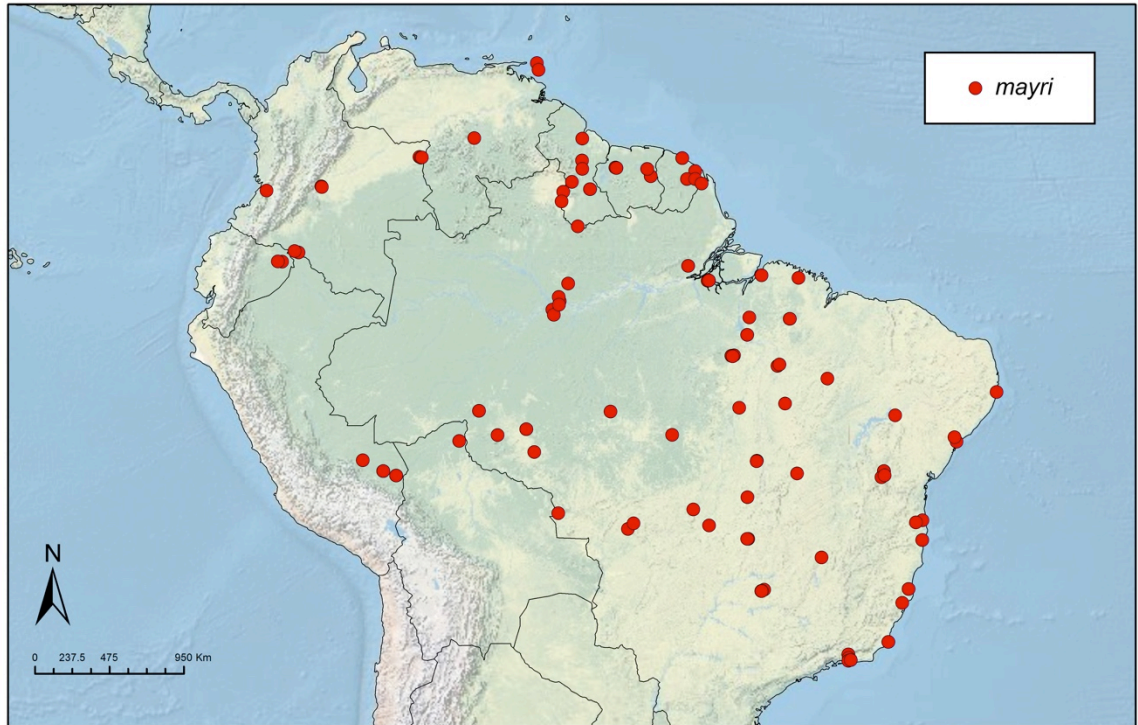


Figure 3.37. Distribution map of *S. mayri*.

***S. mayri* notes**

Variation within *mayri* includes the length of the scape, shape of the head, and striation of the mandibles (Figure 3.33). As with *S. amabilis* and *saussurei*, two other species with striate mandibles, some individuals or populations of *S. mayri* have smooth or faintly striate mandibles. These alternative states are encountered less commonly in *mayri* than in the other two species, mostly in populations from Trinidad (Figure 3.33 a). Intermediate-state workers (i.e., those with faint mandibular striation) can be collected at the same locality and, indeed, are found within the same nest as workers with typically striate mandibles. A single callow worker of *mayri* studied with SEM had smoother, faintly striate mandibles (Figure 3.6 i), so some observations of smooth mandibles may be due to sampling of recently enclosed workers, but is unlikely to explain smooth-

mandibled foragers. The presence/absence of the anteromedian dorsal gastral carinae and robustness of the lateral gastral carinae are also variable in *mayri*. Typically, both carinae are present, but in some specimens the anteromedian carinae are very faint or absent, while lateral carinae can be weak to robust, but always present. The posterior cephalic emargination can be very shallow, so that the posterior margin of the head appears almost straight (e.g., specimens from Minas Gerais, Uberlandia, Brazil, and the type series of *S. moreirai*). The shape of the head varies to some extent, from distinctly broad in some specimens to more narrow in others (the CI range is wide: 95.74–115.08).

We studied variation in the morphology of *S. mayri* with reference to the molecular phylogeny, which has high support values for subspecific clades within *S. mayri*, in order to investigate whether any of these separate, well-supported subclades could represent a separate species. Geographical separation can explain those clades to some extent (Figure 3.37), although the distribution ranges of two of the populations overlap in Brazil. Further, one specimen collected in Lençios, Brazil, has a COI sequence that differs from those of the other samples collected in that same locality, instead grouping with the other Brazilian *S. mayri* population. This could indicate the presence of two genetically distinct, sympatric populations, which would be consistent with a hypothesis of two separate Brazilian species or of introgression between two incipient species. However, we do not have UCE data for this sample and previous comparative study of UCE and COI data for *Sericomyrmex* (Chapter 2) indicates that COI is inadequate for delimiting species, especially in the *mayri* and *bondari* clades. Further, these two populations are morphologically indistinguishable (Figure 3.5c). Therefore, based on our morphological studies and inconclusive molecular evidence, we currently

regard *S. mayri* as a single, widespread species with geographically distinct populations. We find *S. mayri* interesting in terms of population structure, and we report these inconclusive data to encourage further research. Population study of *S. mayri*, with finer geographic sampling, could be important for understanding speciation and population genetics in attine ants.

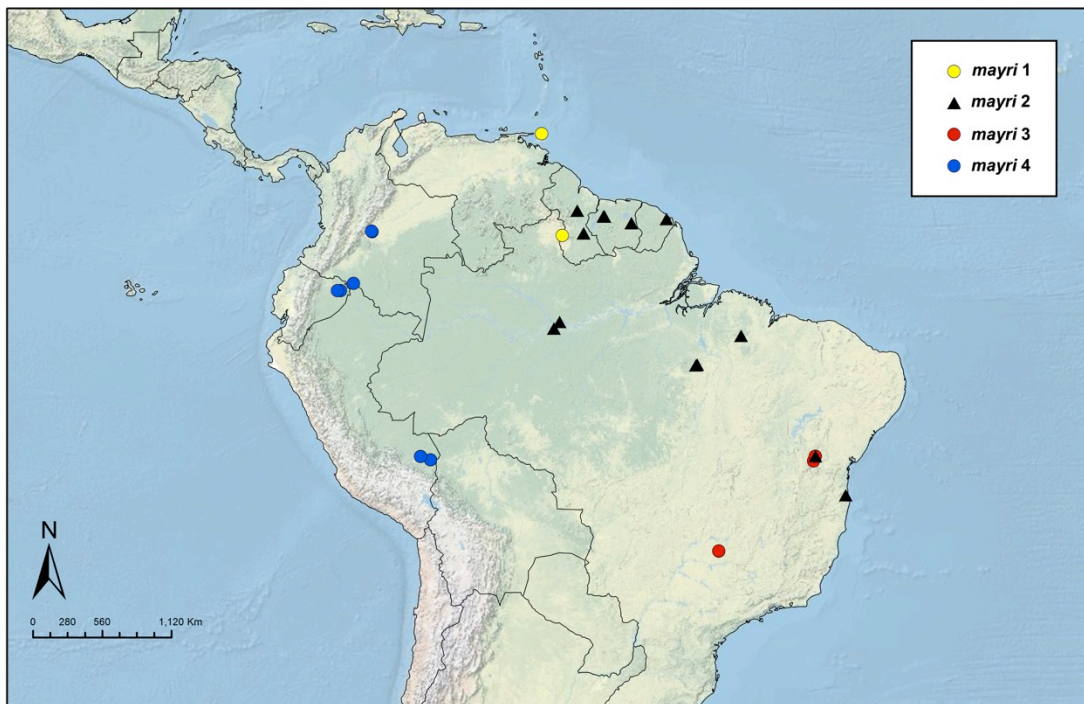


Figure 3.38. Distribution map of *S. mayri* populations. This map is based on the subset of *S. mayri* samples for which molecular data (either UCE or COI) were obtained.

Material examined

BOLIVIA: Beni: Vaca Diez, nr. Reserva Ecological El Tigre, [-10.8667, -65.75], 172m, 1 Jul 1999, R. Dunn; **BRAZIL: Amapá:** Oiapoque, [3.8333, -51.8333], 1 May 1979, W. L. Overal; **Amazonas:** Manaus, Rs2303, [-3.1133, -60.0253], 30 Sep 1993, A. B. Casimiro; Manaquiri, Br 319, km100, [-3.4, -60.4], 10 Oct 2010, F. Baccaro; Manaus,

Reserva Ducke, [-2.917, -59.983], 9 Aug 1992, T. R. Schultz; Pres. Figueredo, I. Pe
 Inchado, -1.8971, -59.4865, 23 Aug 1993, Queiroz; Reserva Campina, Km 44 EEST, [-
 2.67, -60.03], 18 Aug 1992, T. R. Schultz; **Bahia:** Andaraí, Mata Carrasco (castanha), [-
 12.8055, -41.3312], 13 Dec 1990, C. R. F Brandão, Diniz, Oliveira; NP Chapada
 Diamantina - Mucugê, -12.9053, -41.5005, 1032m, 6 Sep 2009, E. Borges; Lençóis, nr.
 NP Chapada Diamantina, -12.5598, -41.3708 ±5m, 530m, 9 Nov 2008, J. Sosa-Calvo;
 Una, Fazenda Ararauna, -15.3071, -39.1626, 80m, 9 May 2014, I. O. Fernandes; **Espírito**
Santo: Aracruz, [-19.8156, -40.3244], 10 Dec 1980, E. Campinos, D. R. Smith; Parque
 Sooretama, Linhares, -19.0725, -39.9491, 31 Mar 2004, J. H. C. Delabie; **Goiás:** Campo
 Limpo, Faz. Conceição, -16.3308, -49.1636, 20 Jan 2005, R. R. Silva, R. M. Feitosa;
 Colinas do Sul, Serra da Mesa, -14.0166, -49.2, 2 Dec 1995, B. H. Dietz, Campaner;
 Fazenda Pau Brasil, Reserva 19, -15.5813, -51.3987, 310m, 8 Apr 2008, S. E. Solomon;
 Ouro Verde, Faz Boa Vista, -16.3308, -49.2118, 1 Jul 2005, R. R. Silva, R. M. Feitosa;
Maranhão: Bom Jardim, REBIO Gurupi Parcela 01 08, -3.9258, -46.7712, 20 Sep 2014,
 A. Y. Harada; Estreito, Fazenda Itaueiras, -6.5317, -47.3711, 1 Jun 2005, R. R. Silva, R.
 M. Feitosa; **Mato Grosso:** Chapada dos Guimaraes, Cechoeira Pedra Purada, [-15.4671, -
 55.7363], 15 Feb 1985, J. C. Trager; Hwy to Sto. Antonio de Leverger, 10km S Cuiaba,
 [-15.780, -56.0638], 16 Feb 1985, J. C. Trager; Mata São João, Reserva Saporanga, -
 12.5581, -33.0431, 21 Jun 2001, R. R. Silva, R. M. Feitosa, C. R. F Brandão; Xingu, [-
 10.5233, -53.5264], 1 Nov 1961, Alvarenga & Werner; **Minas Geiras:** Cachoeira da
 Fumaça (district of Novo São Joaquim), -14.697, -52.312, 288m, 5 Nov 2011; Panga,
 Uberlândia, -19.1831, -48.4014, 813m, 19 Oct 2012, A. Ješovnik; Serra de Ricardo
 Franco State Park, -14.9076, -60.0646, 200m, 11 Feb 2014, J. Maravilhas; Unai, Faz.

Sto. Antonio, -16.7544, -46.4825, 1 Feb 2014, L. N. Paolucci; **Pará:** Baixo Amazonas, [-1.4256, -48.3906], 1 Feb 1949, C. R. Gonçalves; Goianesia, Faz. Rio Capim, [-3.8384, -49.0986], 1 Jun 2003, A. M. Elizabeth; Melgaço, Caxiuanã, -1.7248, -51.4230, 10 Oct 2006, A. Y. Harada; Nova Ipixuna, Fazenda Bom Retiro, -4.8412, -49.218, 12 Jun 2012, M. Tavares, A. Palmeira; Parauapebas, FL Nacional de Carajás, Parque Zoobotanico, -6.0629, -50.0571, 626m, 2 Oct 2014, A. Ješovnik; Viseu São Jose do Gurupi, Parcela 3, -1.5718, -46.2672, 10 Aug 2014, A. Y. Harada; **Pernambuco:** Recife, [-8.096, -34.904], 1 Jan 1988, L. Lima Castro; Tapera, [-9.4272, -40.7218], B. Pickal; **Piauí:** Rio Uruçuí Preto, [-7.3431, -44.6168], 20 Feb 1976, R. Negrett; **Rio de Janeiro:** Belford Roxo, [-22.7631, -43.3991], 15 Jun 1936, C. R. Gonçalves; Rio de Janeiro DF, [-22.8751, -43.2775], 1 Mar 1940, C. R. Gonçalves; Nova Iguacu, ReBlo Tingua, -22.5705, -43.4141, 2 Feb 2002, A. Mayhe, S. Veiga-Ferreira; Represa Rio Grande Guanabara, [-22.9167, -43.4167], F. M. Oliveira; S. Bento, [-21.9167, -41.1167], 25 Apr 1946, A. Silva; **Rondônia:** Fazenda São Sebastião, [-10.5336, -63.5457], 7 Oct 2008, S. E. Solomon; Ilha Pedras, km1, subparcela 150, -9.1744, -64.61222, 86m, 25 Oct 2013, I. O. Fernandes; Ouro Preto do Oeste, Res do INPA No 0078, [-10.2, -61.9], 26 Mar 1985, F. F. Ramos; **Tocantins:** Aguiarnópolis, -6.6137, -47.4814, 1 Jun 2005, R. R. Silva, R. M. Feitosa; Araguacema, -8.9888, -49.6780, 16 Nov 2005, R. R. Silva, R. M. Feitosa; Aurora do Tocantins, -12.6985, -46.3604, 9 Oct 2004, R. R. Silva, R. M. Feitosa; Gurupi, -12.0111, -48.6783, 30 Sep 2001, R.R. Silva, N.L. Albuquerque; Peixes, Fazenda Galileia, Transecto 1, -11.9788, -48.6591, 30 Sep 2001, R.R. Silva, N.L. Albuquerque; Recursolandia, Mata Ciliar Rio Mateiros, -8.7579, -47.0388, 9 May 2005, R. R. Silva, B. H. Dietz; **COLOMBIA: Meta:** El Caduceo Reserve, nr. San Martín, 3.6694, -73.6585,

374m, 30 Sep 2007, T. R. Schultz; San Martín, Finca El Caduceo, 3.6292, -73.6256, 364m, 1 Oct 2007, J. Sosa-Calvo; **Putumayo:** PNN La Paya Cabaña La Paya, -0.0333, -75.2, 330m, 15 Dec 2001, E. Lozano; PNN La Paya Cabaña Vivano Cocha, -0.1166, -74.9666, 320m, 30 Nov 2001, R. Cobete; **Valle del Cauca:** Valle del Cauca, PNN Farallones de Cali, Anchicaya, [3.4333, -76.8], 730m, 20 Jul 200, S. Sarria; **Vichada:** Cumaribo, Cgto. Santa Rita PNN El Tuparro, 5.3555, -68.0244, 135m, 1 Feb 2004, I. Quintero, E. Gonzales; Municipio Cumaribo Cgto. Santa Rita PNN El Tuparro, 5.3316, -67.8908, 135m; **ECUADOR: Napo:** Tiputini, La Selva, Chorongó trail, -0.6382, -76.1493, 16 Jun 2003, A. Little; **Orellana:** Estación Chiruisla-Petrobras, Río Huiririma, -0.6438, -75.9124, 18m, 10 Sep 2005, D. Donosov; **FRENCH GUIANA: Cayenne:** Kaw Mt., Amazon Nature Lodge, 4.55, -52.2, 950m, 20 Jul 2005, T. R. Schultz; Nouragues Field Station, XII trail, 4.09, -52.6768, 75m, 27 Jul 2005, T. R. Schultz; Paracou Experimental forest, 5.2808, -52.9465, 10 Jul 1999, S. Durou; **Régina:** Nouragues Field Station, XII trail, 4.09, -52.6768, 75m, 27 Jul 2005, T. R. Schultz; **GUYANA: Cuyuni-Mazaruni:** Mabura Hill, camp at the end of Rd. to Lethem, 5.1552, -58.6997, 64m, 30 Oct 2002, J. S. La Polla; Mazaruni River, Forest Settlement, 6.3973, -58.6781, 1 Aug 1935, N. A. Weber; **Potaro-Siparuni:** Iwokrama, Kurapakari base Camp, [4.6698, -58.6854], 60m, 7 Apr 1996, T. R. Schultz, U. G. Mueller; **Upper Takutu-Upper Essequibo:** Annai-Georgetown Rd., nr. Essequibo Riv., [3.93, -59.27], 9 Apr 1996, T. R. Schultz, U. G. Mueller; Karanambo, [3.36, -59.78], 5 Apr 1996, T. R. Schultz, U. G. Mueller; Kusad Mountains, 2.8120, -59.8668, 135m, 26 Oct 2013, J. A. Helms; Upper Essequibo, CI concesion, BBC camp, 3.5059, -58.2334, 11m, 21 Nov 2011, A. Ješovnik; Acarai Montains, nr. Romeo's camp, 1.3833, -58.9333, 282m, 7 Oct 2010, T. R. Schultz;

PERU: Madre de Dios: E. Pakitza- Manu, [-11.95, -71.2833], 13 Feb 1992, R. Cambra, D. Quintero; Puerto Maldonado, Los Amigos Biol. Station, -12.569, -70.1005, 272m, 23 Nov 2005, J. Sosa-Calvo; Tambopata Reserve, -12.8187, -69.3636, 224m, 1 Aug 2012, A. Ješovnik; **SURINAME: Brokopondo:** Maripaheuvel, near Dam on Sara creek, [4.67, -54.95], 1 Sep 1959, I. v. d. Drift; Poeroe man Kemisa, [4.67, -54.95], 1 Sep 1959, I. v. d. Drift; **Sipaliwini:** Bakhuis Mountains, 4.7208, -56.726, 249m, 5 Mar 2006, J. Sosa-Calvo; Lely Mts., 4.2529, -54.7561, 619m, 26 Oct 2005, J. Sosa-Calvo; Nassau Mountains, 4.2529, -54.7561, 619m, 26 Oct 2005, J. Sosa-Calvo; **TRINIDAD: Tunapuna-Piarco:** Simla Biol. Stn., [10.6836, -61.2833], 240m, 8 Jan 1995, U. G. Mueller; **VENEZUELA: Bolivar:** Rio Tawadu, Nichare Field Stn., 6.4333, -64.8833, 200m, 9 Feb 1966, D. M. Olsen.

***Sericomyrmex* n. sp. 1 new species**

Worker: Figures 3.39, 3.40; **Queen:** Figure 3.41; **Map:** Figure 3.42.

Type material. *Holotype worker:* BRAZIL, Minas Gerais, Serra Azul State Park, Barra do Garças, -15.8571, -52.2617, 539m, 5 Jun 2011, H. Vasconcelos (NMNH: 1w, USNMENT00924081) Paratypes: same data as holotype (NMNH: 1q, USNMENT00924082) (13w, USNMENT00924083, USNMENT00924084, USNMENT00924085, USNMENT00924086, USNMENT00924087, USNMENT00924088, USNMENT00924089, USNMENT00924090, USNMENT00924091, USNMENT00924092, USNMENT00924093, USNMENT00924094, USNMENT00924095)

S. n. sp. 1 worker description

Measurements in mm (range): HWe 0.80–0.98 HW 0.78–1.00 IFW1 0.51–0.68 IFW2 0.19–0.30 HL1 0.77–0.95 HL2 0.68–0.83 ScL 0.58–0.73 EL 0.13–0.20 Om 8.00–11.00 WL 1.03–1.28 PL 0.21–0.32 PPL 0.13–0.25 GL 0.72–0.95 HFL 0.82–1.08 PrW 0.52–0.71 hw1 0.75–1.20 hw2 0.82–1.08 hw3 0.54–0.75 CI 96.67–110.00 FLI 57.39–73.41 SI 66.67–77.42 EI 15.34–20.63 FLD 0.28–0.44 CEI 6.67–17.24 [n=30]

Body color yellowish-brown to ferrugineous-brown. Pubescence dense, lighter than the integument, appressed to decumbent. Hairs curved, darker in color at the base, appressed to suberect, but mostly decumbent.

Head. Head in frontal view slightly broader than long ($CI=105.71 \pm 3.28$), posterior corners angular to acute, lateral margins of the head slightly convex, posterior cephalic emargination distinct ($CEI=10.42 \pm 2.42$). The posterior cephalic margin between the cephalic corner and the median emargination straight to convex, merging gradually with the emargination. Vertexal impression present, frontal tumuli faint but visible. Mandibles triangular, dorsally smooth and shiny, finely transversely striate along masticatory margin. Masticatory margin of mandible with 7 to 8 teeth. Eyes large ($EI=18.06 \pm 1.41$), moderately convex, without white layer, 9 to 11 ommatidia across largest diameter. Frontal lobes relatively wide ($FLI=68.1 \pm 2.23$), triangular, with posterior margin shorter than medial. In some specimens the lateral margin of the frontal lobe mildly concave. Frontal carinae well developed, complete, reaching posterior cephalic corners. Antennal scape either slightly shorter or just reaching the posterior cephalic margin ($SI=72.26 \pm 2.87$).

Mesosoma. Lateral pronotal and anterior mesonotal spines low and obtuse to moderately pronounced, in dorsal view similar in length. Propodeal carinae low, sometimes serrate, ending in small denticles.

Metasoma. Nodes of petiole and postpetiole dorsally each with a pair of low, short, serrate, longitudinal carina. On the petiole the carinae are sometimes reduced to two low denticles, best seen in dorsolateral view. First gastral tergite with four strongly developed longitudinal carinae: two lateral and two anteromedian.

***S. n. sp. 1* worker diagnosis**

Small size; frontal lobes triangular; gaster with four sharp longitudinal carinae: two lateral and two anteromedian dorsal carinae. Frontal carinae robust, complete; eyes without white layer, convex, sometimes laterally protruding in frontal view; mandibles glossy and smooth. Differing from its sister species *S. scrobifer* in its smaller size; narrower, triangular instead of trapeziform frontal lobes; less robust frontal carinae; and eyes somewhat smaller and flatter (Figure 3.5 d). The closely related *S. n. sp. 2* is similar in size but it differs from *S. n. sp. 1* in the absence of anteromedian dorsal gastral carinae, differently shaped frontal lobes, and smaller eyes. *S. opacus*, which is similar in size and has smooth mandibles, can be easily distinguished from *S. n. sp. 1* by the absence of anteromedian dorsal gastral carinae; smaller, flat eyes, often with a white layer; weaker, incomplete frontal carinae; and *opacus*-typical rectangular frontal lobes.

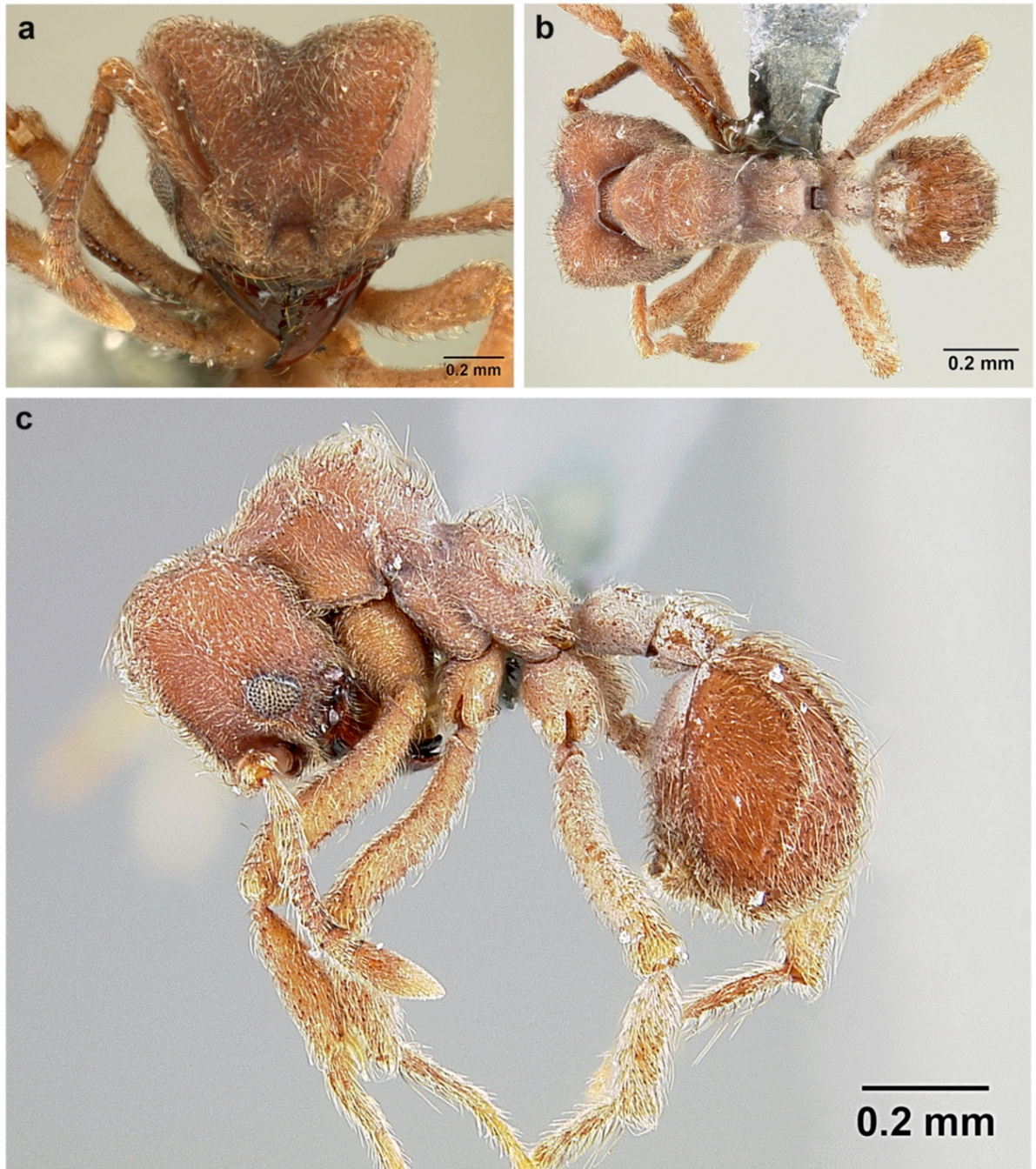


Figure 3.39. *S. n.* sp. 1 worker (USNMENT00924081). **a** Head; **b** dorsal view; and **c** lateral profile.

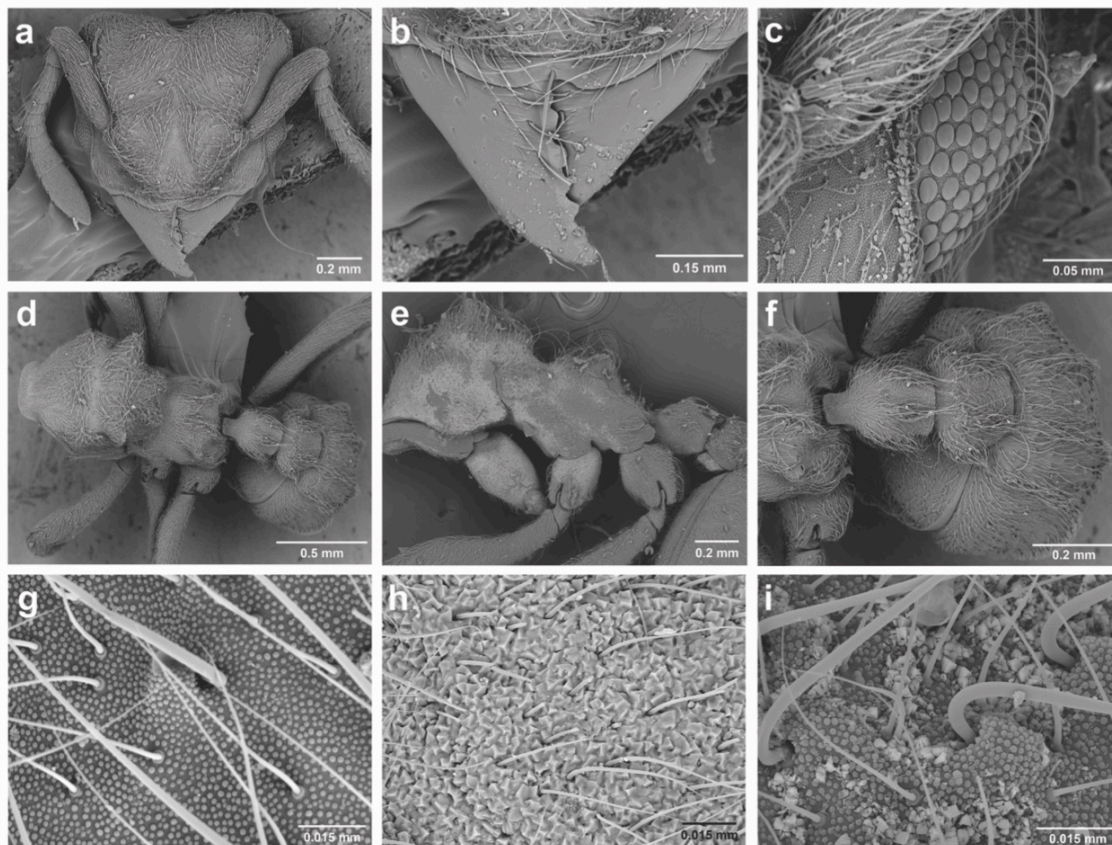


Figure 3.40. *S. n. sp. 1* worker (USNMENT00924090: **a, b, c, d, f, g**; USNMENT01126226: **e, h, i**) SEM images. **a** Head, full-face view; **b** mandibles; **c** eye; **d** mesosoma and gaster, dorsal view; **e** mesosoma, lateral view; **f** petiole, postpetiole, and gaster, dorsal view; **g** head, papillate integument; **h** thorax, integument with dense crystal-like layer; **i** thorax, papillate integument with sparse crystal-like layer.

***S. n. sp. 1* queen description**

Measurements in mm: HWe 1.12 HW 1.12 IFW1 0.84 IFW2 0.4 HL1 1.12 HL2 0.99 ScL 0.75 EL 0.24 Om 20 EW 0.08 WL 1.72 PL 0.33 PPL 0.3 GL 1.6 HFL 1.25 PrW 1 hw1 1.24 hw2 1.36 hw3 0.84 CI 100.36 FLI 75 SI 66.96 EI 21.7 [n=1]

Head. Masticatory margin of mandible with 8 teeth. Mandibles glossy and smooth across dorsal surface, finely transversely striate only along masticatory margin. Preocular carina extends beyond the eye, almost converging posteriorly with the frontal carina to

form a complete scrobe, but much thinner and weaker beyond the top of the eye, best seen in lateral view. Eyes large, convex, protruding from the sides of head in frontal view, number of ommatidia across largest diameter 20. Frontal lobes similar to worker, antennal scape not reaching posterior cephalic margin (SI=66.96).

Mesosoma. Lateral pronotal tubercle present, low and obtuse. Mesoscutum in dorsal view with reduced notauli in the form of faint, shallow impressions and with the median mesoscutal line visible only anteriorly. Parapsidal lines present, faint, slightly curved. A transverse shallow groove separating the axillae from the scutellum, transversely costate. Scutellum inflated, short in dorsal view, narrowing posteriorly, with relatively deep V-shaped posterior notch. Propodeum with two posteriorly laterally flattened, obtuse, diverging denticles, large in comparison to other *Sericomyrmex* queens.

Metasoma. Lateral longitudinal carinae on first tergite of gaster strongly developed. In dorsal view first gastral tergite with longitudinal anteromedian groove; faint anteromedian dorsal carinae present, one on each side of groove. *Wings*. Unknown.

***S. n. sp. 1* queen diagnosis**

The most diagnostic character of the *S. n. sp. 1* queen is the presence of fully developed preocular carinae that almost join with the frontal carinae posteriorly to form complete scrobes. However, because this description is based on a single specimen, we do not know if this character varies within the species. Other useful characters are the small size and smooth mandibles, which separate this species from *S. mayri*, *S. amabilis*, *S. n. sp. 2* (all of which have striate mandibles), and *S. bondari* (which is much larger than *S. n. sp. 1*). *S. n. sp. 1* can be distinguished from the similarly sized *S. parvulus* and

S. opacus by the presence of complete preocular carinae and a relatively deep notch in the posterior margin of the scutellum.

***S. n. sp. 1* male.** Unknown.

***S. n. sp. 1* geographic range.** Interior of Brazil, cerrado habitats. Map: Figure 3.42.

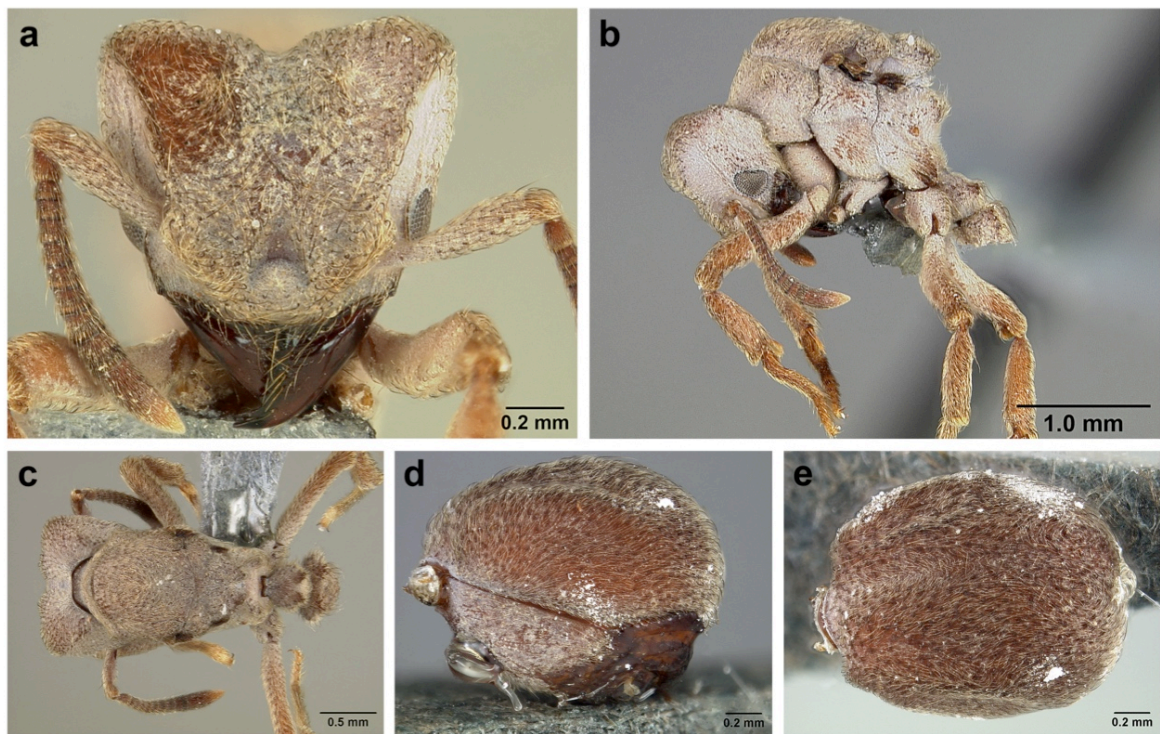


Figure 3.41. *S. n. sp. 1* queen (USNMENT00924082) **a** Head; **b** lateral profile; **c** mesosoma, dorsal view; and gaster, **d** lateral and **e** dorsal views.

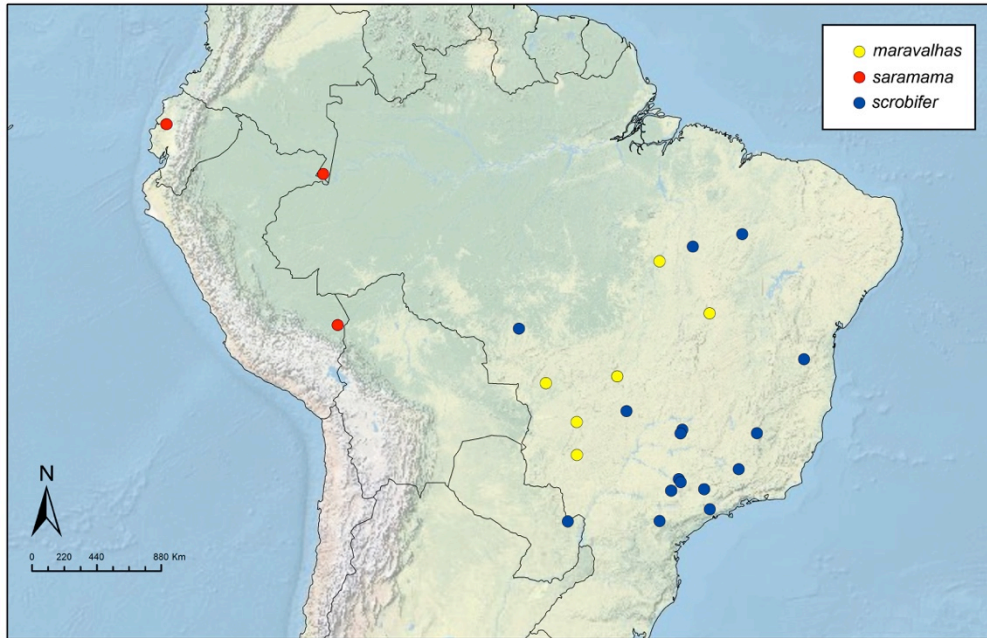


Figure 3.42. Distribution map of *S. n. sp. 1*, *S. n. sp. 2*, and *S. scrobifer*.

***S. n. sp. 1* notes**

Based on molecular data and examination of morphological characters, *S. n. sp. 1* is the sister species of *S. scrobifer*. Their distributions overlap, possibly substantially, but distributional data for both species are clearly incomplete (Figure 3.42). Consistent morphological and molecular differences in sympatry reinforce our decision to recognize *S. n. sp. 1* and *S. scrobifer* as distinct species. It remains entirely possible, however, that, due to undersampling, we may be unaware of morphological and molecular forms that are intermediate between the two species, which, if they exist, might compel us to reevaluate them to be a single species. Currently, however, based on all of the material examined, the results of the morphological measurement analyses (Figures 3.3 FLI, 3.5 d), and the branch lengths separating the two species in the molecular phylogeny (Chapter 2, Figure 2.2), we have chosen to recognize *n. sp. 1* as distinct from *scrobifer*.

Material examined

BRAZIL: Mato Grosso: Coxim, Rio Taquari, [-18.5264, -54.7465], 1 Dec 1963, V. C. Andzada; Near Pocone, Transpantaneira Km115, [-16.2597, -56.6269], 28 Nov 1984, J. C. Trager; **Mato Grosso do Sul:** Campo Grande, -20.4261, -54.7275, 532m, 7 Oct 2012; **Minas Gerais:** Serra Azul State Park, Barra do Garças, -15.8571, -52.2617, 539m, 5 Jun 2011, H. Vasconcelos; **Tocantins:** Araguacema, Rio Tiririca, -8.9886, -49.6675, 16 Nov 2005, R. R. Silva, R. M. Feitosa; Ponte Alta do Bom Jesus, -12.1212, -46.6176, 7 Oct 2004, R. R. Silva, B. H. Dietz.

Sericomyrmex n. sp. 2 new species

Worker: Figures 3.43, 3.44; **Queen:** Figure 3.43; **Larvae:** Figure 3.45; **Map:** Figure 3.42.

Type material. *Holotype:* PERU: Madre de Dios, Tambopata Reserve, -12.8187, -69.3636, 224m, A. Ješovnik, AJ120729-03, primary forest, nest on forest trail. (NMNH, 1w, USNMENT00924064). *Paratypes:* same data as holotype (NMNH: 1q, USNMENT00924065) (15w: USNMENT00924066, USNMENT00924067, USNMENT00924068, USNMENT00924069, USNMENT00924070, USNMENT00924071, USNMENT00924072, USNMENT00924073, USNMENT00924074, USNMENT00924075, USNMENT00924076, USNMENT00924077, USNMENT00924078, USNMENT00924079, USNMENT00924080)

S. n. sp. 2 worker description

Measurements in mm: HWe 0.90–1.13 HW 0.90–1.13 IFW1 0.58–0.78 IFW2 0.22–0.35
HL1 0.84–1.10 HL2 0.78–0.98 ScL 0.65–0.80 EL 0.11–0.18 Om 7.00–9.00 WL 1.12–
1.40 PL 0.22–0.38 PPL 0.19–0.30 GL 0.78–0.98 HFL 0.95–1.20 PrW 0.54–0.74 hw1
0.60–1.05 hw2 0.90–1.16 hw3 0.53–0.76 CI 96.84–108.57 FLI 61.22–81.58 SI 67.90–
78.20 EI 12.00–16.60 FLD 0.31–0.56 CEI 6.67–12.12 [n=25]

Body color uniformly yellowish-brown to ferrugineous-brown. Pubescence dense, lighter than the integument, appressed to decumbent. Hairs curved, darker in color at the base, appressed to suberect, but mostly decumbent.

Head in frontal view slightly broader than long ($CI=102.77 \pm 3.04$), posterior corners smoothly rounded to acute, lateral margins of the head slightly convex, posterior cephalic emargination distinct ($CEI=9.56 \pm 1.53$). Posterior cephalic margin between cephalic corner and median emargination straight to convex, merging gradually with emargination. Vertexal impression in most specimens distinct, frontal tumuli faint. Mandibles dorsally smooth and glossy, finely transversely striate only along masticatory margin. Masticatory margin of mandible with 7–8 teeth. Eyes medium-sized ($EI=14.54 \pm 1.23$), slightly convex, lacking the white layer, 7–9 ommatidia across their largest diameters. Frontal lobes moderately wide ($FLI=67.08 \pm 2.59$), triangular, weakly directed anterad, with the posterior margin shorter than the medial. Frontal carinae usually complete, reaching posterior cephalic corners, but not very robust. Antennal scape either slightly shorter or just reaching the posterior cephalic margin ($SI=72.07 \pm 2.78$).

Mesosoma. Mesosomal spines and tubercles low and obtuse. Propodeal carinae low, rarely ending in denticles.

Metasoma. Node of petiole with two low, reduced denticles and node of postpetiole with two faint, short, longitudinal carinae, both best seen in dorsolateral view. In dorsal view first gastral tergite with relatively weak lateral longitudinal carina, one on each side, anteromedian dorsal carinae absent.

***S. n. sp. 2* worker diagnosis**

Small size, mandibles smooth, frontal lobes triangular, with posterior margin short. Similar to other small, smooth-mandibled *Sericomyrmex*, but possible to separate from them by the following: *S. n. sp. 2* has weaker frontal carinae and lower anterior mesonotal spines; lacks the anteromedian dorsal gastral carinae found in *S. n. sp. 1* and *S. scrobifer*; and lacks the trapeziform, robust frontal lobes of *scrobifer*. *S. n. sp. 2* is larger than *S. parvulus*, has complete frontal carinae (in *parvulus* usually incomplete), a longer scape, and larger eyes that lack a white layer (which is common in *parvulus*). It can be separated from similar, sympatric *S. opacus* by lack of rectangular frontal lobes and the white layer on the eyes (triangular frontal lobes and uncoated eyes in *S. n. sp. 2*), and the posterior corners of the head that are not as smoothly rounded as in *opacus*.

***S. n. sp. 2* queen description**

Measurements in mm: HWe 1.35 HW 1.4 IFW1 0.93 IFW2 0.38 HL1 1.32 HL2 1.16 ScL 0.88 EL 0.22 Om 15 EW 0.11 WL 1.92 PL 0.48 PPL 0.36 GL 1.82 HFL 1.48 PrW 1.08 hw1 1.4 hw2 1.5 hw3 0.95 CI 102.27 FLI 68.89 SI 64.81 EI 16 [n=1]

Head. Masticatory margin of mandible with 8 teeth. Mandibles striate across dorsal surface, unlike workers. Preocular carina fading at the top of the eye. Eyes large

and slightly convex, 15 ommatidia across largest diameter. Frontal lobes slightly larger and wider than in the worker, more rectangular than triangular.

Mesosoma. Mesoscutum in dorsal view with very reduced notauli, median mesoscutal line absent. Parapsidal lines present, faint, slightly curved. Axillae in dorsal view entirely separated from one another, laterally rounded, narrowing medially. Scutellum in dorsal view narrowing posteriorly, posterior margin with a shallow notch. Propodeal teeth present, blunt and low, directed posterolaterad.

Metasoma. Lateral longitudinal carinae and anteromedian dorsal impression present on gaster, anteromedian carinae absent.

***S. n. sp. 2* queen diagnosis**

The queen of *S. n. sp. 2* is similar in size to *S. opacus* and *S. parvulus* queens, but it can be separated from them by its striate mandibles (smooth in *parvulus* and *opacus*). It is smaller in size than *S. mayri* and *S. amabilis* and lacks the dark hairs typical of the queen of *S. bondari*.

***S. n. sp. 2* male** Unknown.

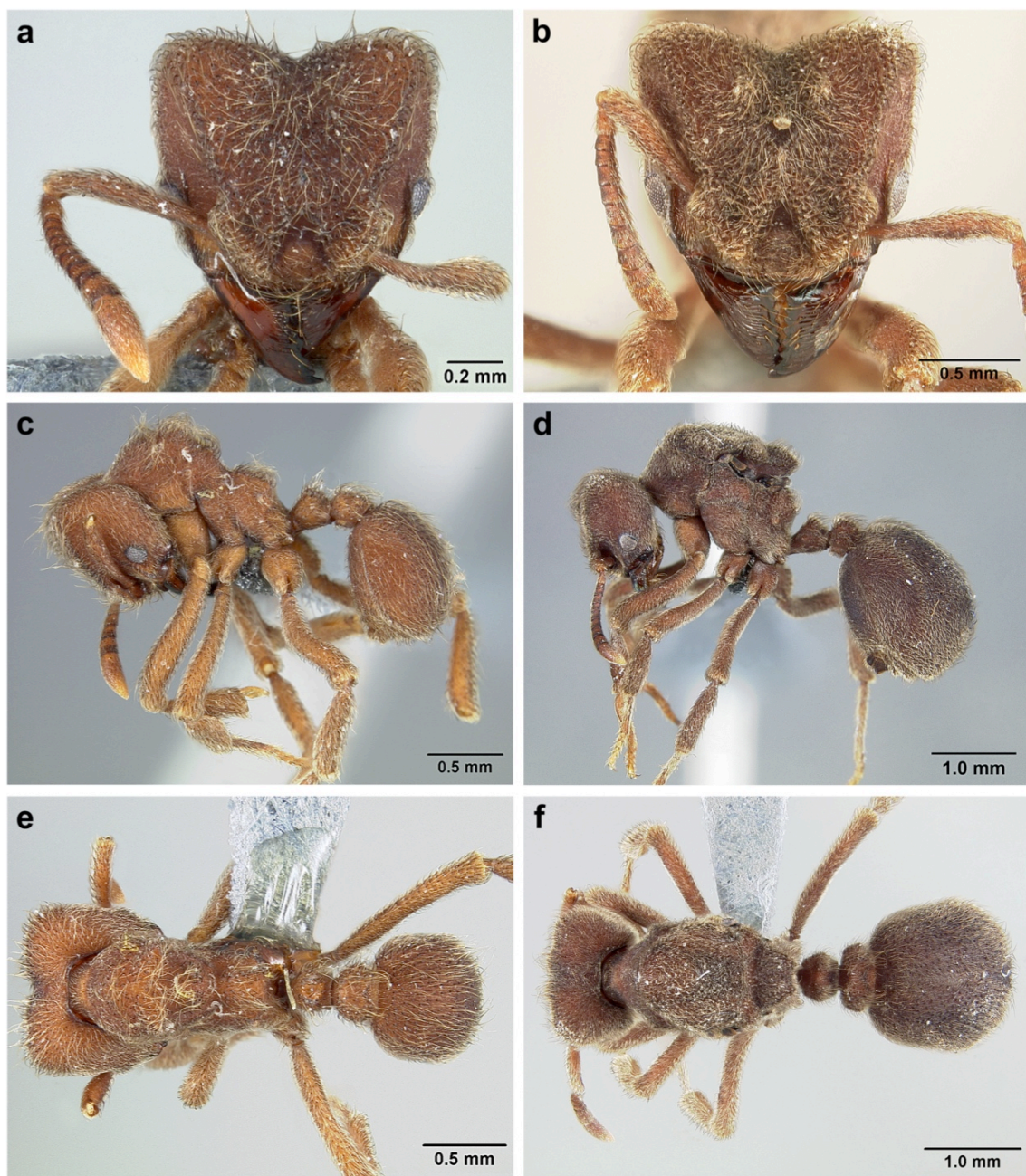


Figure 3.43. *S. n. sp. 2* worker (USNMENT00924064) and queen (USNMENT00924065); head, profile and dorsal views. Worker: **a, c, e**. Queen: **b, d, f**.

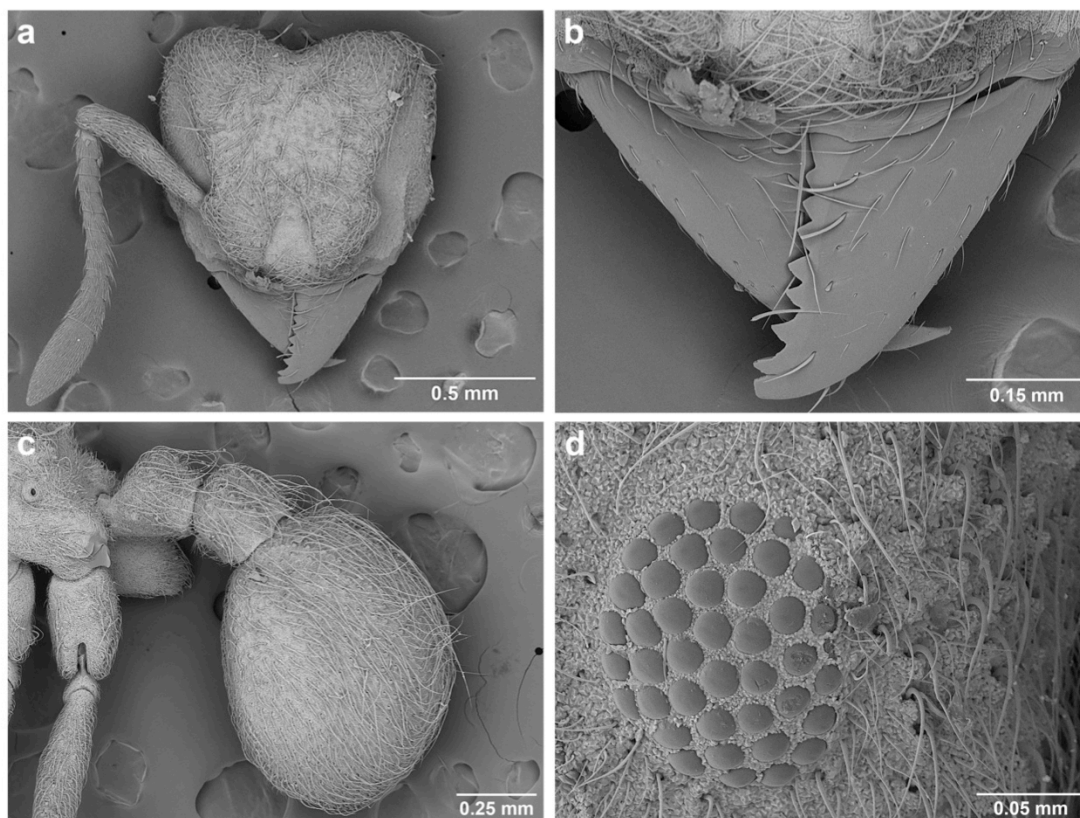


Figure 3.44. *S. n. sp. 2* worker (USNMENT01125262), SEM images. **a** Head, full-face view; **b** mandibles; **c** gaster, lateral view; **d** eye.

***S. n. sp. 2* larvae description**

Setae on dorsal and lateral body surfaces entirely absent. Supra-antennal setae absent. Four genal setae on each side. Mandibular apical teeth divided. Labial denticles absent. First thoracic segment ventrally with multiple multidentate spinules, arranged in transverse rows. Numbers of ventral setae: six on each thoracic segment, two on the abdomen (not including anal setae). A single pair of papilliform setae present anterior to anal opening.

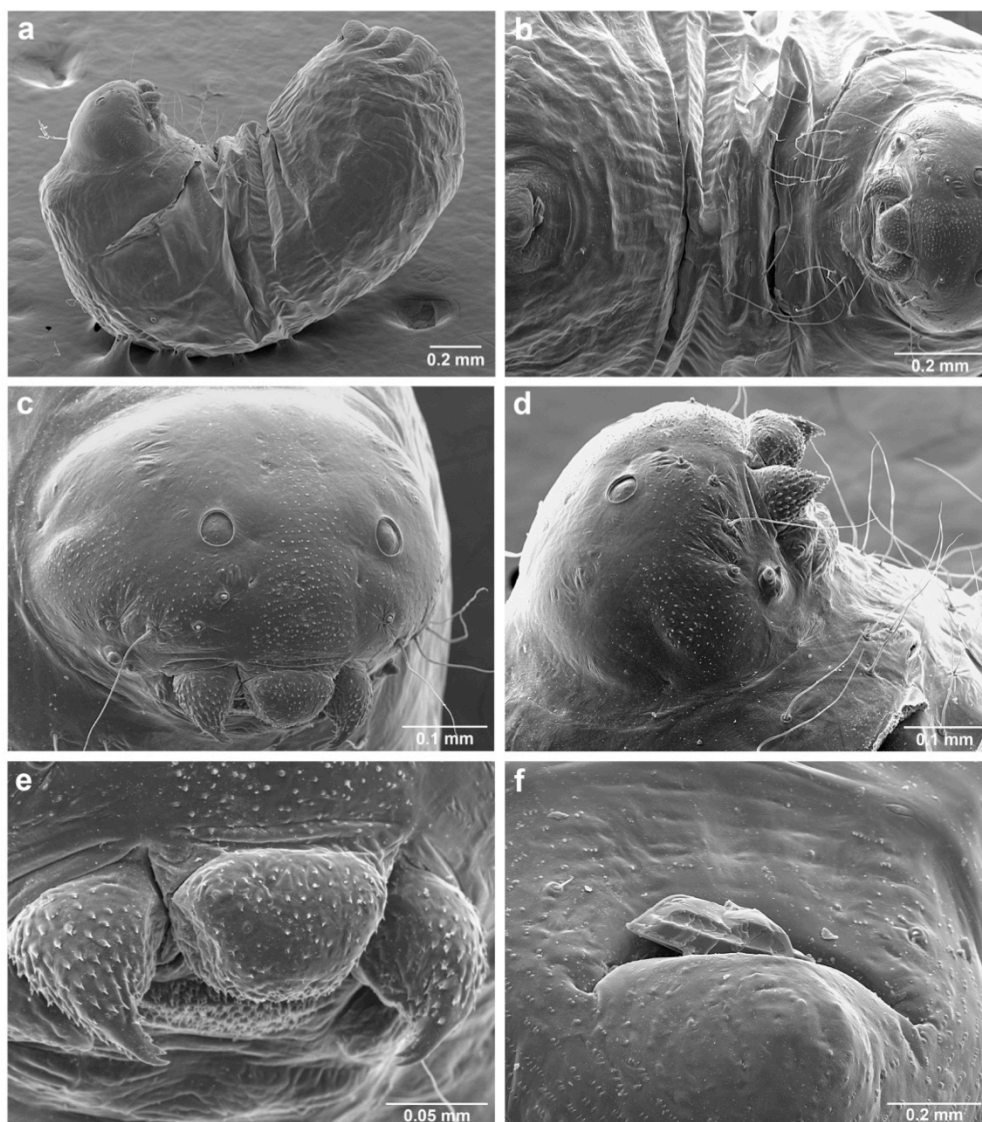


Figure 3.45. *S. n. sp. 2* larva (USNMENT01125266), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

***S. n. sp. 2* geographic range.** Colombia, Ecuador, Peru. Map: Figure 3.42.

***S. n. sp. 2* notes**

The anteromedian gastral carinae are robust in the Peru population, but hardly visible in the Ecuador population. Head shape and frontal lobe shape are consistent across

its geographic range. *S. n. sp. 2* occurs in forested habitats in Peru, Colombia, and Ecuador (Figure 3.42), but has never been collected in Amazonian Brazil. No other *Sericomyrmex* species has a similar distribution, although the known distribution could easily be an artifact of undersampling because *S. n. sp. 2* is rarely collected. It is the sister species of a clade containing *S. n. sp. 1* + *scrobifer*, Brazilian cerrado species that are also rarely collected (Figure 3.42). Together, *S. n. sp. 2* + (*S. n. sp. 1* + *S. scrobifer*) form a clade that is the sister to all remaining *Sericomyrmex* species (Chapter 2, Figure 2.2). Morphologically *S. n. sp. 2* resembles *opacus* more than its sister species, but it can be separated from *opacus* by its larger size and differently shaped frontal lobes. The fungal cultivar associated with this species is, unsurprisingly, a member of the diverse clade of generalized higher attine fungi grown by species of *Sericomyrmex* and *Trachymyrmex*, but it occupies a long, separate branch from all other higher attine cultivars (Chapter 2, Figure 2.4). *S. n. sp. 2* grows this cultivar species even when other cultivars are readily available; it was collected at the same locality in which two *mayri* and two *parvulus* nests were growing a different fungal species (Chapter 2).

Material examined

COLOMBIA: Amazonas: PNN Amacayacu, Matamata, -3.6833, -70.25, 150m, 1 Oct 2001, D. Chota; **ECUADOR: Napo:** Tiputini, Chorong trail, -0.6382, -79.8490, 15 Jun 2003, N. M. Gerardo; **PERU: Madre de Dios:** Tambopata Reserve, -12.81867, -69.36364, 224m, 29 Jul 2012, A. Ješovnik.

***Sericomyrmex* n. sp. 3 new species**

Worker: Figure 3.46, 3.47; **Map:** Figure 3.31.

Type material. *Holotype:* VENEZUELA, Amazonas, 10km N of San Carlos de Rio Negro, [2.0164, -67.0599] Jul-Aug 1978, K. Clark (MCZ: 1w, USNMENT00924059)
Paratypes: same data as holotype (NMNH: 4 pins, 8 w: USNMENT00924060, USNMENT00924061, USNMENT00924062, USNMENT00924063).

***S. n. sp. 3* worker description**

Measurements in mm (range): HWe 1.00–1.08 HW 1.00–1.08 IFW1 0.61–0.66 IFW2 0.24–0.27 HL1 1.00–1.08 HL2 0.84–0.90 ScL 0.75–0.84 EL 0.15–0.18 Om 9.00–12.00 WL 1.35–1.43 PL 0.24–0.34 PPL 0.20–0.25 GL 0.92–1.02 HFL 1.18–1.30 PrW 0.64–0.75 hw1 0.93–1.00 hw2 1.08–1.15 hw3 0.64–0.73 CI 97.67–102.50 FLI 59.32–62.48 SI 74.98–81.66 EI 15.00–17.95 FLD 0.35–0.38 CEI 11.76–16.67 [n=9]

Body color evenly light yellow; but, because only dry, pinned specimens were available for this species, the pale color is likely due to age. Pubescence dense, appressed to decumbent, light yellow. Hairs curved, darker in color at the base, yellow to gray, appressed to suberect, but mostly decumbent.

Head. In frontal view head evenly broad and long ($CI=100.78 \pm 1.57$), posterior corners acute, posterior cephalic emargination distinct and deep ($CEI=13.80 \pm 1.62$). The posterior cephalic margin between the cephalic corner and the median emargination straight to convex and merging with the emargination gradually. Vertexal impression distinct, frontal tumuli barely visible. Mandibles smooth and glossy, finely transversely striate only along masticatory margin, this fine striation in some specimens hard to see so

that mandibles can appear entirely smooth. Masticatory margin of mandible with 7–8 teeth. Eyes medium-sized to large ($EI = 16.46 \pm 0.86$), mildly convex, dark, without white layer, 9 to 12 ommatidia across largest diameter. Frontal lobes triangular, relatively long but narrow ($FLI = 61.09 \pm 1.15$), with posterior margin shorter than medial, lateral margin long and sometimes mildly convex. Frontal carinae straight to slightly curved laterally, complete, reaching posterior cephalic corners. Antennal scape slender and long ($SI = 77.44 \pm 2.13$), reaching posterior cephalic corners, the longest scape of all known *Sericomyrmex* species (Figure 3.3 SI).

Mesosoma. Anterior pronotal tumulus low; lateral pronotal spine short in comparison to mesonotal spine in dorsal view; mesonotal spine very long and sharp relative to other *Sericomyrmex* species (Figure 3.46 d, 3.47 e); posterior mesonotal tubercle low, blunt. Propodeal carinae very faint or absent, propodeal denticle present, thin, relatively large and obtuse.

Metasoma. Node of petiole with two small and low denticles and node of postpetiole with four low, short, longitudinal carina, two dorsal and two lateral, the lateral pair faint. Postpetiole in dorsal view broader than long. First gastral tergite with longitudinal lateral carinae distinct, anteromedian dorsal carinae absent or faint.

***S. n. sp. 3* worker diagnosis**

Mandibles smooth; antennal scape long and slender; posterior cephalic emargination deep; very distinct, sharp, and long anterior mesonotal spines; lateral pronotal spines very reduced; gastral anteromedian dorsal carinae faint or absent. Similar head shape, smooth mandibles, and sharp mesonotal spines are also present in *S. bondari*,

but *bondari* has at least some thick black hairs, a shorter scape, and the lateral pronotal spines more developed, while the mesonotal spines are shorter and blunter than in *S. n.* sp. 3.

***S. n.* sp. 3 queen, male, and larvae.** Unknown.

***S. n.* sp. 3 geographic range.** Amazonian Venezuela. Map: Figure 3.31.

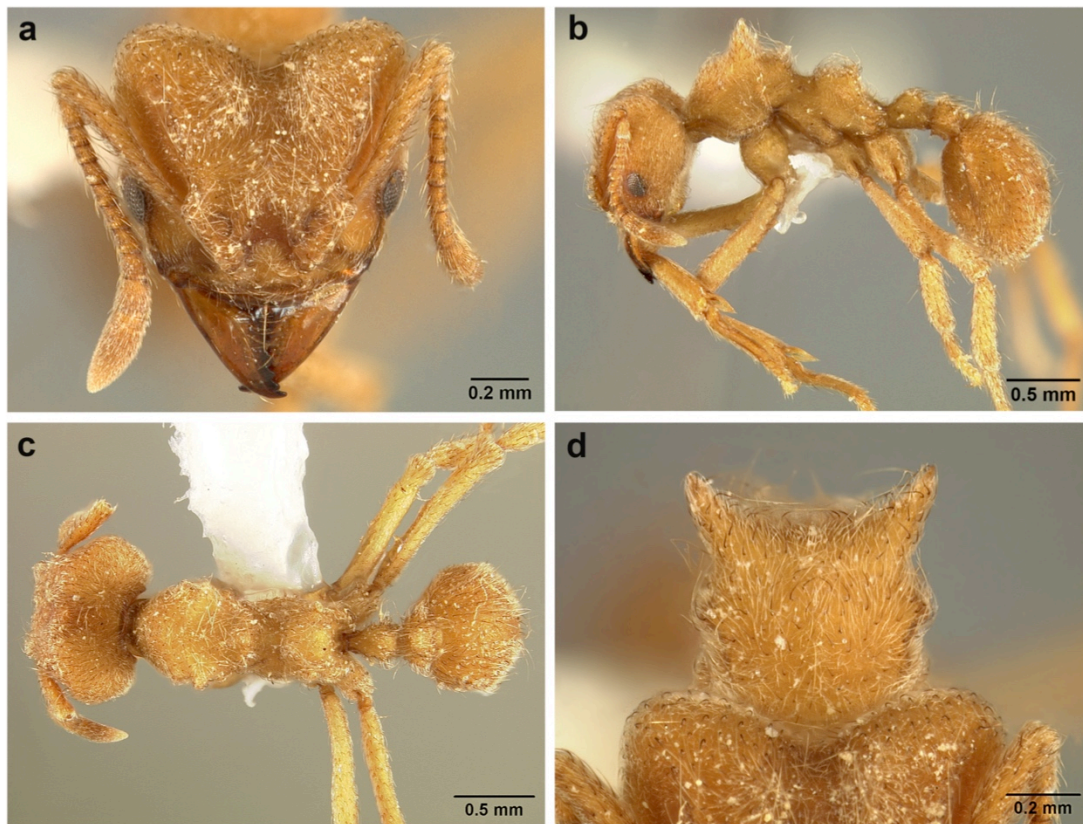


Figure 3.46. *S. n.* sp. 3 worker (USNMENT00924059). **a** Head; **b** lateral profile; **c** dorsal view; and **d** frontodorsal view of mesonotal spines.

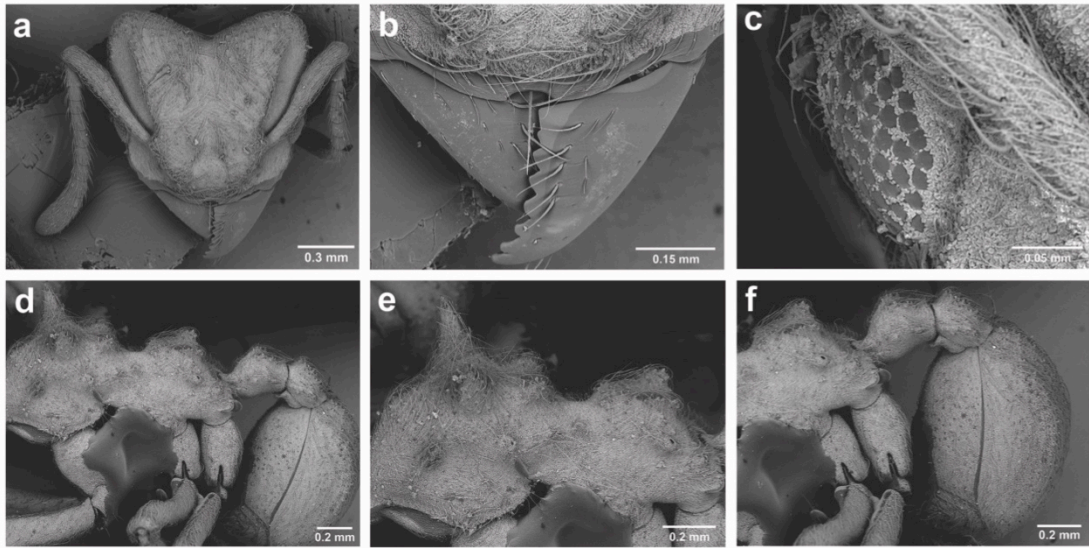


Figure 3.47. *S. n. sp. 3* worker (USNMENT00924061), SEM image. **a** Head, full-face view; **b** mandibles; **c** eye; **d** mesosoma, lateral view; **e** mesosoma, lateral view; **f** propodeum, petiole, postpetiole, and gaster, anterolateral view.

***S. n. sp. 3* notes**

Morphology and measurement data (Figure 3.5 c) indicate that *S. n. sp. 3* is the sister species to *S. bondari*. Like *n. sp. 3*, a few *bondari* populations have reduced hair. In addition to the absence of dark hairs, *n. sp. 3* also has a long antennal scape and distinctly longer and sharper anterior mesosomal spines than any other *Sericomyrmex* species (Figure 3.46 d, 3.47 e).

***Sericomyrmex opacus* Forel 1885**

Worker: Figures 3.48, 3.49; **Queen and male:** Figure 3.50; **Larva:** Figure 3.51; **Map:** Figure 3.52.

Sericomyrmex opacus Mayr 1865: 84. Lectotype worker (here designated):

(Brazil)* MEXICO, Veracruz, Córdoba, [18.8808, -96.9272], E. Norton (NHMW: 1w, CASENT0915956) Paralectotypes: Same data as lectotype: (NHMW: 1w, CASENT0915955) (NMNH: 1w, USNM00924096).

=*Sericomyrmex aztecus* Forel, 1885: 363. **New junior synonym.**

Type material examined. MEXICO, Veracruz, Orizaba, [18.85, -97.08], A. Forel (MSNG: 1w, CASENT0904987) (MHNG: 1w, CASENT0909368) (NHMW: 1w, USNMENT00924097).

=*Sericomyrmex diego* Forel 1912: 192. **New junior synonym.**

Type material examined: COLOMBIA, Magdalena, Don Diego, [11.23, -73.7], A. Forel (NMNH: 1w, USNMENT00529165; 2w, USNMENT00921744) (BMNH: 2w, CASENT0901678) (MSNG: 2w, CASENT0904988; 1m, USNMENT00924098) [MHNG: 3w, CASENT0909369).

=*Sericomyrmex zacapanus* Wheeler, 1925: 54. **New junior synonym.**

Type material examined: GUATEMALA, Zacapa, [14.9722, -89.5306], 15 Dec 1911, W. M. Wheeler, (MCZ: 3w, USNMENT00924099; 2w, USNMENT00924100; 3W, USNMENT00924101)

*See Supplementary file Text S1 *opacus* for discussion on locality of the type

***S. opacus* worker description**

Measurements in mm (range): HWe 0.80–1.00 HW 0.80–1.00 IFW1 0.54–0.73 IFW2 0.16–0.28 HL1 0.82–1.00 HL2 0.60–0.90 ScL 0.58–1.08 EL 0.12–0.18 Om 6.00–10.00 WL 0.99–1.30 PL 0.20–0.33 PPL 0.15–0.25 GL 0.78–1.00 HFL 0.68–1.02 PrW 0.50–0.72 hw1 0.78–1.00 hw2 0.88–1.13 hw3 0.54–0.74 CI 94.63–105.95 FLI 62.32–78.38 SI 65.10–77.78 EI 12.60–19.36 FLD 0.32–0.50 CEI 5.21–27.91 [n=68]

Body color uniformly yellowish-brown to ferrugineous-brown. Pubescence dense, often lighter than the integument, appressed to decumbent. Hairs curved, darker in color at the base, yellow to gray, appressed to suberect, but mostly decumbent.

Head. Head in frontal view almost equally broad and long ($CI=100.19 \pm 2.74$), posterior corners smoothly rounded, posterior cephalic emargination distinct but relatively shallow ($CEI=9.71 \pm 3.75$). The posterior cephalic margin between the cephalic corner and the median emargination convex, merging gradually with the emargination. Vertexal impression and frontal tumuli visible, but usually faint. Mandibles smooth and glossy, finely transversely striate only along masticatory margin. Masticatory margin of mandible with 7 to 8 teeth. Eyes medium-sized ($EI=15.53 \pm 1.27$), flat to slightly convex, often covered with thin white layer (Figure 3.49 d, Figure 4.48 a), but some individuals or populations lacking the white layer (Figure 3.48 b, and see notes section for details). Seven to 9 ommatidia across largest diameter. Frontal lobes rectangular to trapeziform in most specimens, wide ($FLI=71.27 \pm 2.57$), with posterior margin as long, or almost as long, as medial. In some populations frontal lobes more triangular and narrower (see notes section). Frontal carinae usually incomplete, fading before reaching

posterior cephalic corners in some specimens. Antennal scape relatively short, never reaching posterior cephalic margin ($SI=70.66 \pm 2.48$).

Mesosoma. Mesosomal spines and tubercles low and obtuse. Propodeal carinae sometimes absent, with just denticles marking their posterior extent, otherwise low and feeble, posteriorly diverging, sometimes serrate.

Metasoma. Node of petiole with two low, reduced denticles and node of postpetiole with two faint, short, longitudinal carina, both best seen in dorsolateral view. First gastral tergite usually with weak longitudinal lateral carinae, one on each side, anteromedian dorsal carinae absent.

***S. opacus* worker diagnosis**

Small size; mandibles smooth; posterior cephalic corners smoothly rounded; wide, rectangular frontal lobes; eyes often at least partially covered with white layer; anteromedian dorsal carinae on gaster absent. The most similar species are *S. parvulus*, *S. n. sp. 2*, and, in Central America, smooth-mandibled populations of *S. amabilis*. Typical *amabilis* can be distinguished from *opacus* by completely striate mandibles, triangular instead of rectangular frontal lobes, posterior cephalic corners not smoothly rounded, and larger size. The distinction between smooth-mandibled *amabilis* and *opacus* is less obvious, but the frontal lobes, head shape and size are still good indicators. An ameliorating factor for this difficulty is that, when sympatric, *amabilis* and *opacus* are very distinct; we have not encountered the smooth-mandibled *amabilis* variant sympatric with *opacus*, which might indicate character displacement.

Sericomyrmex opacus is in size and general appearance very similar to *S. n. sp. 2*; the main characters separating them are the shape of the frontal lobes (triangular in *S. n. sp. 2*) and the absence of a white layer over the eyes in *S. n. sp. 2*. *S. parvulus* can be distinguished from the typical *opacus* by having smaller, narrower, triangular frontal lobes; smaller overall size; and shorter frontal carinae, often fading well before reaching the posterior cephalic corners. Separating non-typical representatives of *opacus*, which may also have reduced frontal lobes, from *parvulus* is difficult (see notes for details).

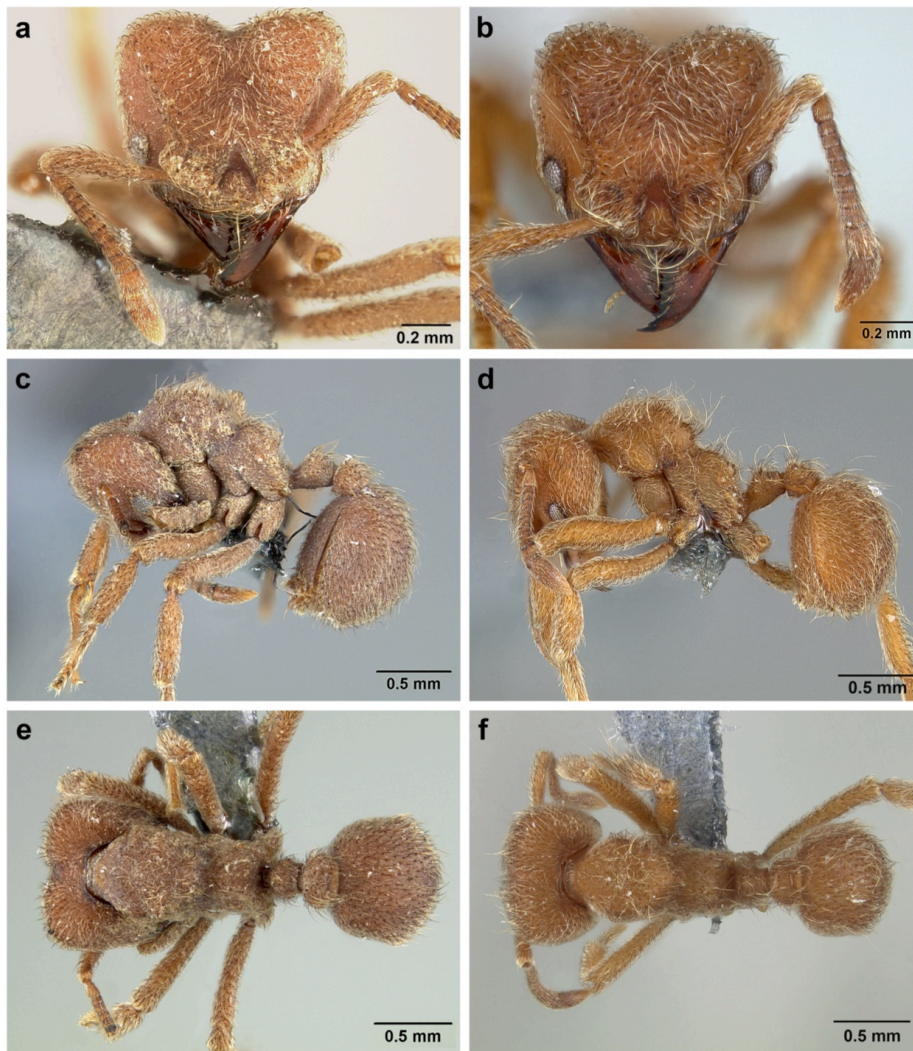


Figure 3.48. *S. opacus* worker; head, lateral profile, and dorsal view. Variation in the eyes: with the white layer (USNMENT01125124): **a, c, e**; without the white layer (USNMENT01125118): **b, d, f**.

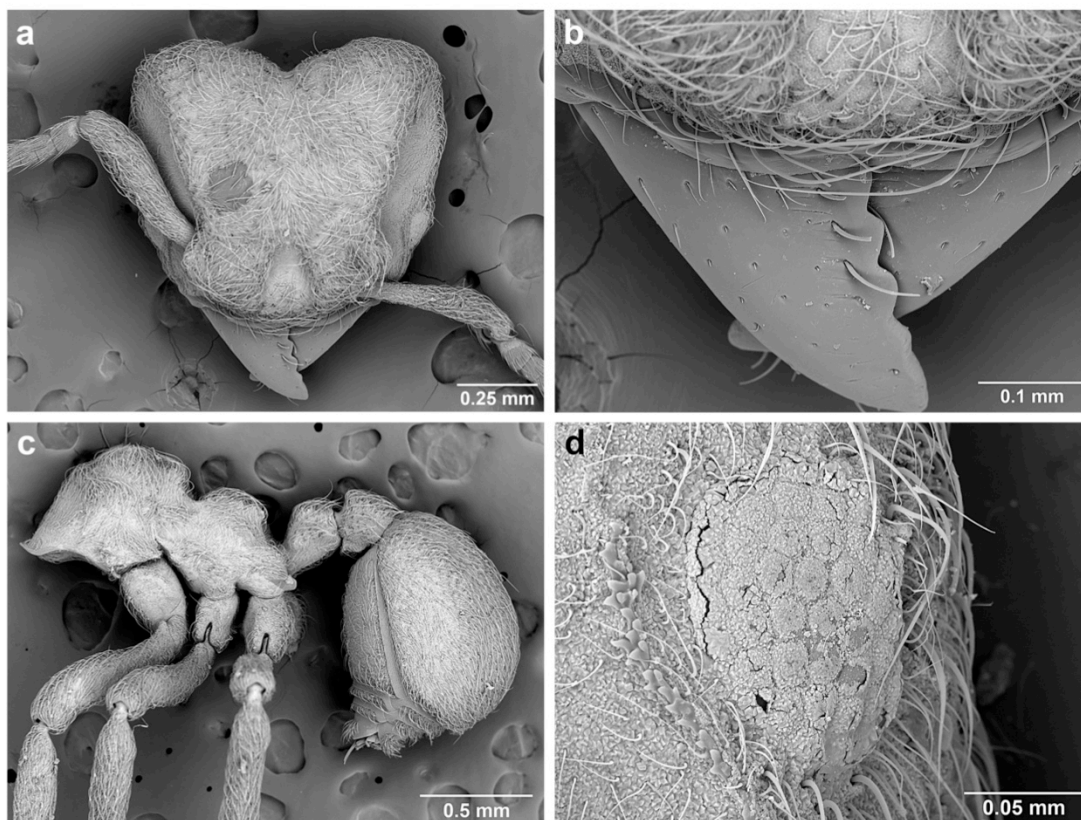


Figure 3.49. *S. opacus* worker (USNMENT01125331), SEM images. **a** Head, full-face view; **b** mandibles; **c** mesosoma and gaster, lateral view; **d** eye.

***S. opacus* queen description**

Measurements in mm (range): HWe 1.02–1.16 HW 1.05–1.20 IFW1 0.76–0.85 IFW2 0.24–0.33 HL1 1.09–1.12 HL2 0.98–1.00 ScL 0.74–0.78 EL 0.20–0.24 Om 15.00–17.00 EW 0.08–0.10 WL 1.58–1.80 PL 0.34–0.48 PPL 0.24–0.28 GL 1.50–1.64 HFL 0.99–1.28 PrW 0.90–0.96 hw1 1.10–1.25 hw2 1.20–1.30 hw3 0.72–0.85 FWg 4.85–5.17 HWg 3.60–3.64 CI 93.00–105.99 FLI 71.11–76.16 SI 64.14–73.31 EI 18.40–21.90 [n=6]

Color and pilosity as in the worker.

Head. Masticatory margin of mandible with 7–8 teeth. Mandible glossy and smooth across dorsal surface, finely transversely striate only along masticatory margin.

Preocular carina fading shortly after reaching the top of the eye, but more than one posterior carinae are sometimes present, short, thin, and never reaching the posterior cephalic corner. Eyes large and convex (EI=20.18), 15 to 22 ommatidia across the largest diameter. Frontal lobes similar to worker (FLI=73.37), antennal scape not reaching posterior cephalic margin (SI=68.51).

Mesosoma. Lateral pronotal tubercle present, low and obtuse. Mesoscutum in dorsal view with very reduced notauli and median mesoscutal line, parapsidal lines faint. Scutellum small and short, in some specimens the posterior notch continuing into the median impression that separates the scutellum in two lateral halves, but sometimes the notch is very shallow, almost absent. Propodeum in dorsal view with two low posteriorly diverging carinae that end in denticles.

Metasoma. Node of petiole in frontodorsal view with four low and obtuse denticles: two dorsal and two lateral, the lateral smaller than the dorsal. Node of postpetiole with two short and low longitudinal carinae dorsally and two low denticles laterally. Lateral carinae on first gastral tergite well developed, one on each side, anteromedian dorsal carinae absent, longitudinal anteromedian groove present.

Diagnosis queen

The queen of *S. opacus* can easily be separated from that of the sympatric *S. amabilis* by its smaller size, smooth mandibles (striate in *amabilis*), and usually less conspicuous notauli on the mesoscutum. It is similar in size to queens of *S. parvulus* and *S. n. sp. 2*. It is very difficult to separate queens of *opacus* and *parvulus*. The *parvulus* queen is slightly smaller and apparently lacks the faint carinae posterior to the eye,

visible in lateral view; however, these eye carinae are absent in some *opacus* queens as well. The region of overlap of the known distributions of these two species is limited, so geographic origin can aid in species identification (Figure 3.52). The *S. n. sp. 2* queen is similar to *opacus*, but the former can easily be separated from the latter by its striate mandibles (smooth in *opacus*).

***S. opacus* male description**

Measurements in mm (range): HWe 0.66–0.74 HW 0.46–0.58 IFW1 0.20–0.27 IFW2 0.13–0.18 HL1 0.53–0.61 ScL 0.49–0.62 EL 0.22–0.28 Om 19.00–28.00 EW 0.09–0.14 WL 1.20–1.44 PL 0.27–0.38 PPL 0.16–0.22 GL 0.96–1.12 HFL 1.20–1.50 PrW 0.50–0.80 IOD 0.36–0.50 FWg 3.48–4.10 HWg 2.20–2.84 CI 114.58–125.00 FLI 30.18–37.66 SI 70.54–87.52 EI 29.19–39.13 [n=7]

Head in frontal view longer than broad (CI=120.66). Eyes large (EI=33.47), 19 to 28 ommatidia across the largest diameter. Preocular carinae fading shortly before reaching the top of the eye, curved slightly mesad before fading. Notauli and mesoscutal line faint, the groove between the axillae usually smooth but sometimes weakly transversely costate. Propodeum without denticles or carinae. Petiole and postpetiole with very reduced lateral denticles, dorsal denticles absent.

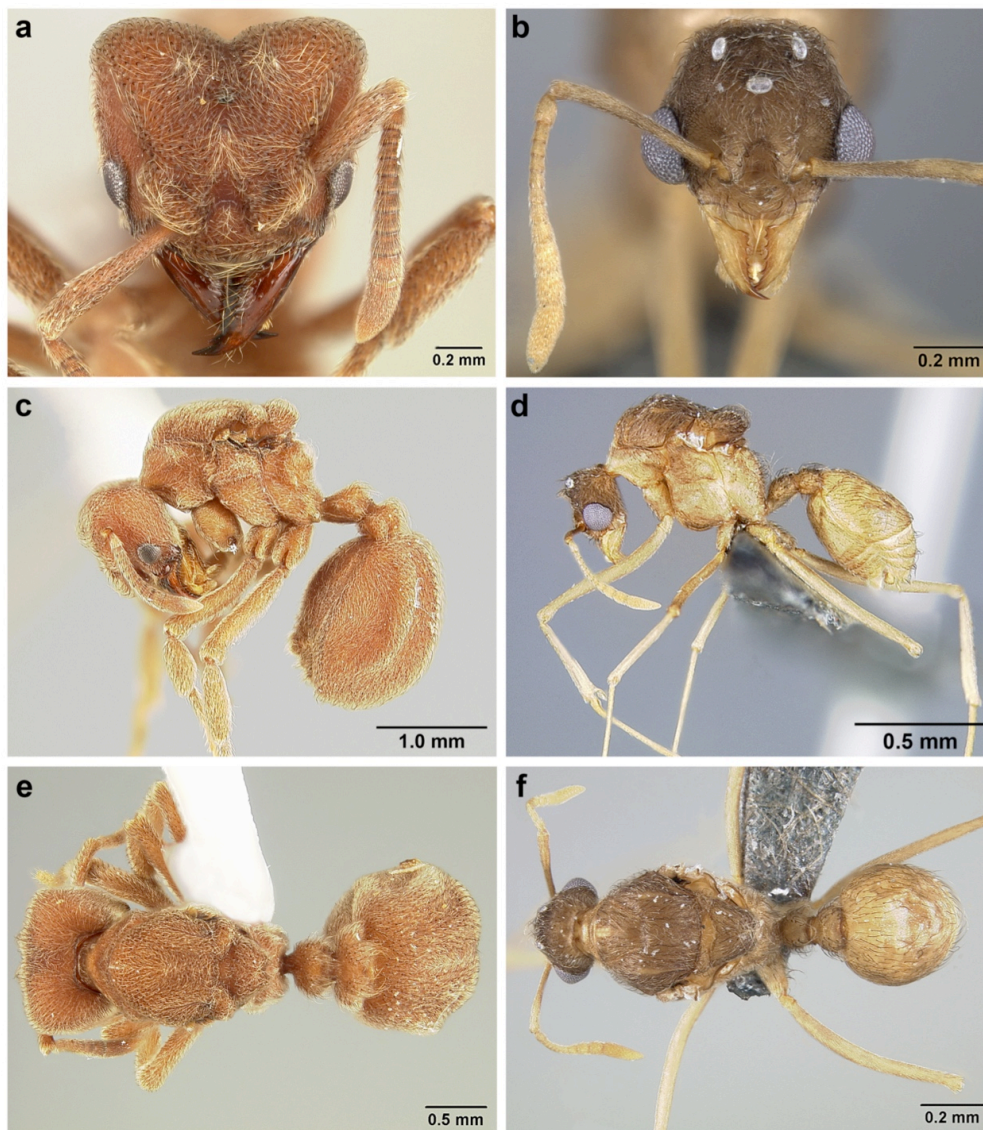


Figure 3.50. *S. opacus* queen and male; head, lateral profile, and dorsal view. Queen (USNMENT00305214): **a, c, e**. Male (USNMENT01125314): **b, d, f**.

***S. opacus* larvae description**

Approximately 8 setae on each side of dorsal and lateral body surfaces (i.e., approximately 16 total). Supra-antennal setae absent. Four genal setae on each side. Mandibular apical teeth undivided. Labial denticles not visible in the only specimen available. First thoracic segment ventrally with multiple multidentate spinules, arranged

in transverse rows. Numbers of ventral hairs: six on each thoracic segment, eight on the abdomen (not including anal setae). Single pair of sensilliform setae present anterior to anal opening.

***S. opacus* geographic range** Mexico to Ecuador and NW Brazil (Rondônia). Map:

Figure 3.52.

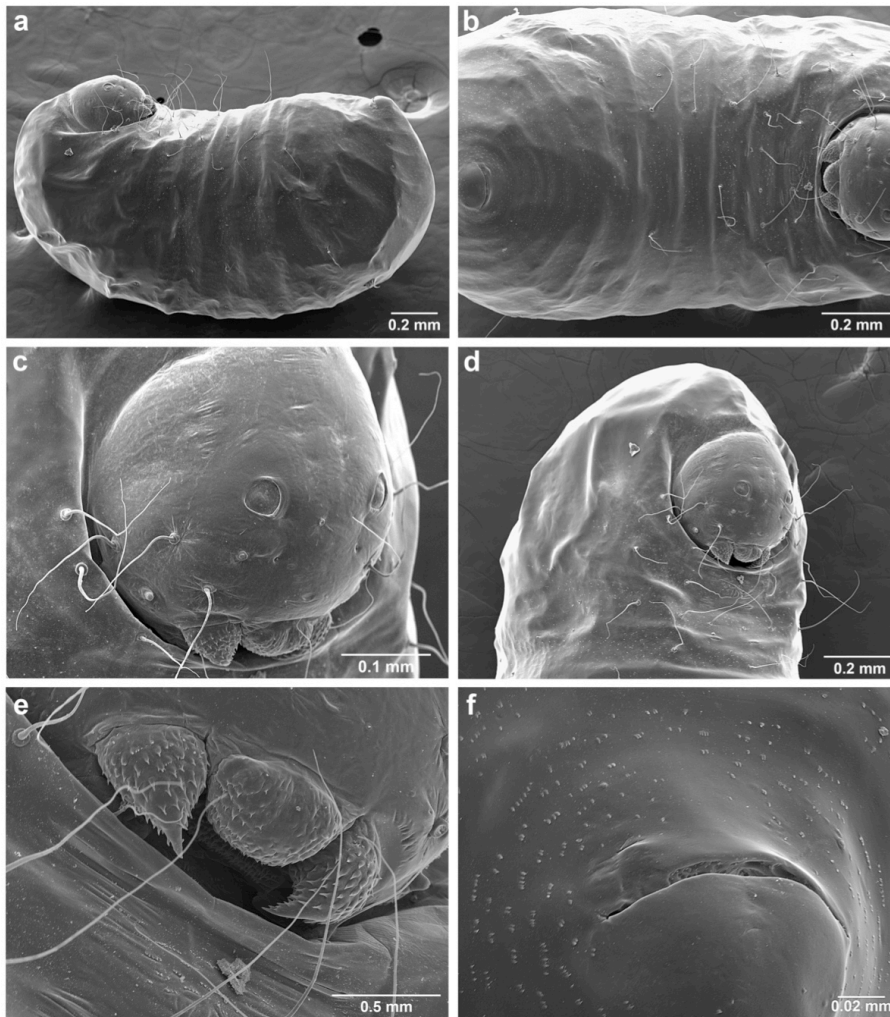


Figure 3.51. *S. opacus* larva (USNMENT01125317), SEM. **a** Lateral view; **b** ventral view; **c** head, frontolateral view; **d** head and thorax, frontolateral view; **e** mouthparts; **f** anal setae.

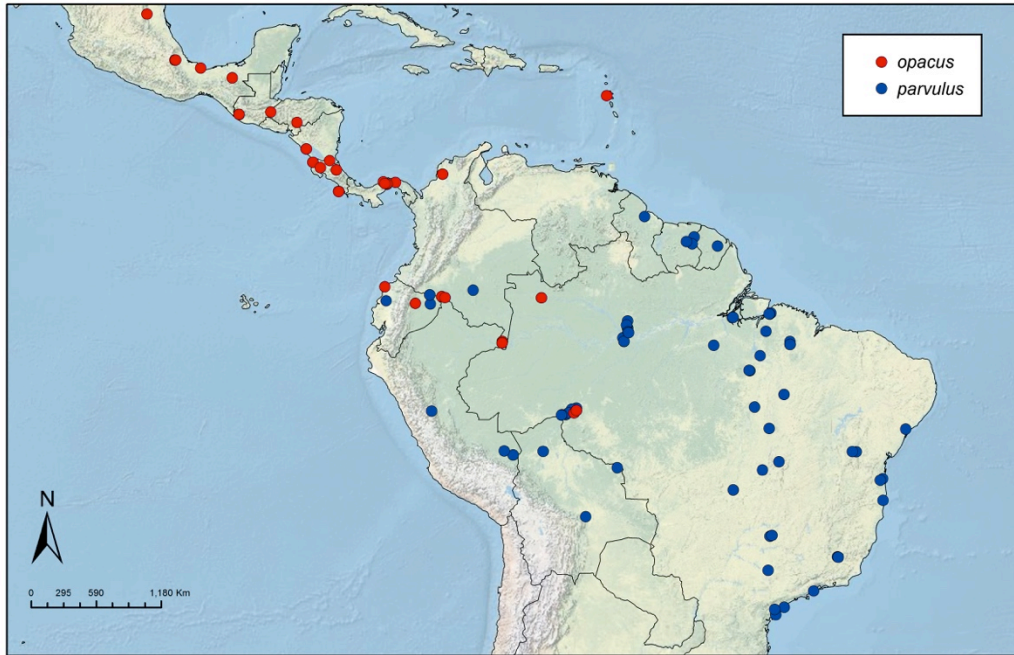


Figure 3.52. Distribution map of *S. opacus* and *S. parvulus*.

***S. opacus* notes**

S. opacus is sometimes hard to identify because it is morphologically variable across its geographic range and because it is similar to the sometimes sympatric *S. parvulus*. This variation is correlated with patterns in the molecular phylogeny as well as with geography (Chapter 2, Figure 2.2). The three main subspecific geographical and molecular subclades, all reciprocally monophyletic, are: Central American (population *opacus1*), North Colombian (population *opacus2*), and South Colombian & West Brazilian (population *opacus3*). The most pronounced variation within *opacus* occurs in the shape of the frontal lobes and in the eyes. The typical *S. opacus* has rectangular frontal lobes and the eyes covered with a thin white layer. In populations of *opacus1* there are occasional, rare individuals with smaller, almost triangular lobes and eyes lacking the white layer. These rare, odd specimens are also smaller in size, so these

atypical character states could be correlated with size (e.g., in nanitic workers). The specimens from *opacus2* populations, from Northern Colombia, are fairly uniform, typical representatives of the species. This uniformity, however, may be an artifact of the small sample size (20 individuals). Most of *opacus2* specimens were collected in pitfall traps, so no nest series were available for evaluating actual, within-nest variation. The specimens from the *opacus3* population all appear to have eyes lacking the white layer and the frontal lobes are often (but not always!) more triangular than rectangular. The number of samples available for this population is also low (19 specimens examined, in comparison to 97 for *opacus1*), and there are a few specimens from this population that are morphologically typical, including rectangular frontal lobes. The principal-component analysis of the morphological data for just these three *opacus* populations (Figure 3.5 f) shows no separation of the three populations along the two main axes. With regard to the high degree of morphological variation in *S. opacus*, one possibility well worth considering is that *opacus* and *parvulus* (which are similar in size and which overlap in distribution in the area where *opacus3* occurs) can hybridize (Figure 3.52). If so, then the specimens that are difficult to identify as either *parvulus* or *opacus* could be *opacus-parvulus* hybrids. Separate PCA analysis of only *parvulus* and *opacus* indicates a large amount of overlap (Figure 3.5 b) along the two main axes. The data currently available are insufficient for recognizing the three *opacus* subclades as species and we believe that the observed pattern (especially in *opacus3*) is likely an artifact of our very low sample sizes. It is entirely possible that further investigation and more sampling, especially of whole-nest series, might reveal that *S. opacus* actually consists of multiple species and/or that *opacus* hybridizes with *parvulus*.

S. opacus type locality. The original description of *S. opacus* by G. L. Mayr in 1865, which is also the original description of the genus *Sericomyrmex*, lists “Brasilien” as the type locality. Based on the route of the Novara-Expedition, which is the expedition from which Mayr's specimens supposedly originated, the Brazilian locality is most likely Rio de Janeiro (Mayr, 1865). However, the locality label of the type specimens (CASENT0915955, CASENT0915956, USNMMENT00924096) of *Sericomyrmex opacus* that we studied indicate “Mexico, Cordoba”, and “Norton” as the collector. The label looks original when compared to the labels of other Mayr types, both in terms of handwriting and in resemblance to the labels of other specimens collected by Norton. Indeed, Mayr described other species based on specimens collected by E. Norton in Mexico, so he clearly had access to Norton's Mexican material.

Importantly, the type specimen of *S. opacus* is identical to the type specimen of *S. aztecus*, a Mexican species described by Forel (1885), which in turn is identical to numerous specimens collected in Mexico and elsewhere in Central America. *S. aztecus* is the name most commonly applied to such specimens. Given these facts, the most likely explanation is that the locality of the type specimen of *S. opacus* (“Brasilien”) given in the original description is incorrect. The alternative explanation is that the published locality is instead correct, the specimen label is incorrect, and that the type specimen of *S. opacus* originated in Brazil. We judge this alternative to be highly unlikely, because *S. opacus* (under which we have synonymized *S. aztecus*, *S. diego*, and *S. zacapanus*) does not occur anywhere near Rio de Janeiro (Figure 3.52).

***S. opacus* material examined**

BRAZIL: Amazonas: São Gabriel de Cachoeira, [-0.1237, -67.0476], 120m, 23 Aug 1992, T. R. Schultz; **Rondônia:** Ilha do Bufalo, km 0.5, -9.2656, -64.2125, 90m, 19 Jan 2014, I. O. Fernandes; Jaci MD, km 3, -9.2656, -64.2125, 94m, 22 Jan 2014, I. O. Fernandes; Jaci-Paraná, km 2, -9.2656, -64.2125, 94m, 6 Jun 2012, I. O. Fernandes; Novo Modulo Jaci, km4, -9.4630, -64.3911, 122m, 24 Jan 2014, I. O. Fernandes; **COLOMBIA: Amazonas:** PNN Amacayacu Matamata, -3.6833, -70.25, 150m, 20 May 2000, A. Parente; PNN Amacayacu, -3.8103, -70.2662, 88m, 7 Oct 2007, J. Sosa-Calvo, J. Rodriguez; **Bolivar:** PNN Los Colorados, Villa Roca, 9.9, -75.1166, 180m, 26 May 2001, E. Deulufeut; PNN Los Colorados, La Yaya, 9.9, -75.1166, 280m, 21 Jul 2001, E. Deulufeut; PNN Los Colorados, Alto el Mirador, 9.9, -75.1166, 400m, 11 Apr 2001, E. Deulufeut; PNN Los Colorados, Venado, 9.9, -75.1166, 320m, 1 Jan 2001, E. Deulufeut; **Putumayo:** PNN La Paya Cabaña Chagra, -0.1166, -74.9333, 320m, 1 May 2002, R. Cobete; PNN La Paya Cabaña La Paya Chagra, -0.0333, -75.2, 330m, 26 Feb 2002, R. Cobete; PNN La Paya Cabaña La Paya, -0.0333, -75.2, 330m, 2 Jul 2002, R. Cobete; PNN La Paya Cabaña Viviano, -0.1166, -74.9333, 320m, 26 May 2002, A. Morales; PNN La Paya Rio Caucaya, -0.1166, -74.9333, 330m, 15 Oct 2001, R. Cobete; **COSTA RICA: Guanacaste:** Canas, Finca Pacifica, [10.42, -85.10], 16 Jul 1986, S. B. Peck; Hacienda La Pacifica, nr. Canas, 10.24, -83.80, 50m, 1 May 1979, P. S. Ward; PN Santa Rosa, [10.8378, -85.7051], 14 Jun 1995, U. G. Mueller; **Puntarenas:** Osa Peninsula, Corcovado, Sirena Station, Pavo trail, [8.51, -83.60], 2 Jun 1992, T. R. Schultz; **ECUADOR: Esmeraldas:** 10 km S Atacamen, [0.7755, -79.8462], 205m, 7 Nov 1987, C.R.F. Brandão, C.D. Bastidas; **Pichincha:** Rio Palenque Research Station, [-0.583, -

77.36], 20 Dec 1980, S. Sandoval; **GUATEMALA: Retalhuleu:** El Asintal, 14.6524, -91.73901, 670m, 30 Jul 2013, K. Delgado; Nuevo San Carlos, 14.6388, -91.72193, 585m, 9 Nov 2008, K. Delgado; **HONDURAS: Copán:** Copán Ruinas (archeological site), 14.8379, -89.1428, 629m, 4 Jan 2008, C. Rabeling; **Francisco Morazán:** Esc. Zamorano, 14.0134, -87.0076 $\pm$ 25m, 800m, 19 May 2009, J. T. Longino; **MEXICO: Chiapas:** 8km SE Salto de Agua, 17.5143, -92.2949 $\pm$ 200m, 70m, 16 Jun 2008, M. G. Branstetter; **San Luis Potosí:** Cuesta de los Cedros, 36 km E of Ciudad del Maíz, [22.3889, -99.2515], 685m, 12 Jun 1962; **Veracruz:** Ocotal Chico, [18.2588, -94.8619], 579m, 26 Jun 1963, G. N. Ross; **NICARAGUA: Masaya:** Masatepe, vic. San Marcos, Cafetal San Jose del Llano, 11.917, -86.25, 485m, 23 Jun 1992, T. R. Schultz; **Río San Juan:** Río San Juan, Isla de Diamante, [10.9794, -84.3415], 9 Oct 1994, J. P. Caldwell; **PANAMA: Colón:** Gamboa, PN Soberanía, Pipeline Rd. Km 6, 9.08, -79.66, 24 Apr 1996, T. R. Schultz, U. G. Mueller; San Lorenzo Forest R1, 9.2833, -79.9666, 30 Dec 2004, A. Dejean, J. Orivel, B. Corbara, H. Aberlenc, M. Leponce; **Panamá:** Barro Colorado Island, Canal Zone, 9.15, -79.84, 1 Nov 1941, J. Zetek; El Llano-Carti Suitupo Rd, ca. 6km ex El Llano, [9.22, -78.97], 27 Apr 1996, T. R. Schultz, U. G. Mueller, S. Rehner.

***Sericomyrmex parvulus* Forel 1912**

Worker: Figures 3.53, 3.54; **Queen:** Figure 3.53; **Larva:** Figure 3.55; **Map:** Figure 3.52.

Sericomyrmex parvulus Forel, 1912: 193. Lectotype (here designated): BRAZIL, Pará, [-4, -53] C. Emery (MHNB: 1w, USNM00445579, bottom specimen). Paralectotype: same data as lectotype (MHNB: 1w, USNM00445579, top specimen).

=*Sericomyrmex myersi* Weber, 1937: 400. **New junior synonym.**

Type material examined: SURINAM, Nickerie, Upper Courantyne River, 29 Dec 1935, J. G. Mayers, JGM 5931 (MCZ: 1w, USNMENT00924107)

***S. parvulus* worker description**

Measurements in mm (range): HWe 0.66–0.90 HW 0.66–0.93 IFW1 0.42–0.65 IFW2 0.15–0.28 HL1 0.62–0.90 HL2 0.58–0.82 ScL 0.48–0.72 EL 0.11–0.15 Om 6.00–9.00 WL 0.74–1.23 PL 0.16–0.34 PPL 0.13–0.24 GL 0.60–0.90 HFL 0.65–0.99 PrW 0.46–0.64 hw1 0.60–0.93 hw2 0.68–1.03 hw3 0.48–0.80 CI 93.75–106.45 FLI 60.43–80.77 SI 63.64–80.00 EI 13.30–18.67 FLD 0.26–0.42 CEI 5.00–12.20 [n=55]

Body color yellow-brown to ferrugineous-brown. Pubescence dense, lighter than the integument, appressed to decumbent. Hairs not very thick, often curved, yellow to gray, appressed to suberect, and mostly (but not always) sparser, thinner and shorter than in other *Sericomyrmex* species.

Head. In frontal view, head evenly broad and long ($CI=100.69 \pm 2.99$), posterior corners smoothly rounded, posterior cephalic emargination distinct, but shallow ($CEI=9.71 \pm 3.75$). The posterior cephalic margin between the cephalic corner and the median emargination convex, merging gradually with the emargination. Vertexal

impression very faint, frontal tumuli barely visible. Mandibles smooth and glossy, finely transversely striate only along masticatory margin. Masticatory margin of mandible with 7–8 teeth. Eyes medium-sized ($EI=16.35 \pm 1.37$), flat to slightly convex, either without white layer or partially covered with white layer, often with each ommatidium slightly protruding from that layer (Figure 3.6 I), 7 to 9 ommatidia across largest diameter. Frontal lobes triangular, relatively small and narrow ($FLI=69.03 \pm 2.77$), with posterior margin shorter than medial. Frontal carinae straight to slightly curved laterally, incomplete, in most specimen fading before reaching posterior cephalic corners. Antennal scape short, not reaching posterior cephalic margin ($SI=71.25 \pm 3.01$).

Mesosoma. Mesosomal spines and tubercles low and obtuse. Propodeal carinae low, ending in denticles, denticles sometimes small and hard to see.

Metasoma. Nodes of petiole and postpetiole each with a pair of two low, reduced denticles, best seen in dorsolateral view. Node of postpetiole with two low, short, serrate, longitudinal carinae, which are sometimes also reduced to low denticles. First gastral tergite with longitudinal lateral carinae present, one on each side, weakly developed in comparison with other *Sericomyrmex* species; anteromedian dorsal carinae absent, in very few specimens faintly present.

***S. parvulus* worker diagnosis**

Small size, cephalic lobes smoothly rounded, frontal lobes small and narrow, frontal carinae faint, fading well before posterior corners, gaster smooth, with only weak longitudinal lateral carinae, anteromedian dorsal carinae absent. Less hairy than other *Sericomyrmex* species. In the regions where their distributions overlap (Figure 3.52)

parvulus is most easily mistaken for *S. opacus*. In general *opacus* is larger, with wider, rectangular frontal lobes, but some smaller *opacus* workers can have atypical frontal lobes, more triangular than rectangular, that can be very similar to those in *parvulus*. *S. opacus* is easy to separate from the sympatric *S. amabilis* by its smaller size, smooth mandibles (striate in *amabilis*), and rectangular frontal lobes (triangular in *amabilis*).

***S. parvulus* queen description**

Measurements in mm (range): HWe 0.98–1.05 HW 1–1.08 IFW1 0.7–0.78 IFW2 0.24–0.28 HL1 0.95–1.08 HL2 0.88–0.95 ScL 0.64–0.7 EL 0.21–0.24 Om 14–15 EW 0.08–0.08 WL 1.56–1.65 PL 0.34–0.48 PPL 0.2–0.25 GL 1.4–1.58 HFL 1–1.18 PrW 0.82–0.92 hw1 1–1.13 hw2 1.08–1.22 hw3 0.74–0.8 CI 95.24–102.63 FLI 71.79–76 SI 65.64–70 [n=4]

Head. Masticatory margin of mandible with 7–8 teeth. Mandibles glossy and smooth across dorsal surface, finely transversely striate only along masticatory margin. Preocular carinae fading shortly after reaching the top of the eye. Eyes large and mildly convex ($EI=21.50 \pm 1.1$). Number of ommatidia across largest diameter ~14. Frontal lobes more robust than in worker ($FLI=73.98 \pm 1.88$), antennal scape not reaching posterior cephalic margin ($SI=67.40 \pm 1.86$).

Mesosoma. Lateral pronotal tubercle present but very low. Mesoscutum in dorsal view with notauli and median mesoscutal line absent or very faint. In dorsal view a transverse shallow groove separating axillae from the scutellum. Scutellum short in dorsal view, narrowing posteriorly, posterior notch V-shaped, in some specimens the

posterior notch seems to separate the scutellum into two halves. Propodeal denticle obtuse, laterally flattened, diverging posteriorly in dorsal view.

Metasoma. Lateral longitudinal carinae on first tergite of gaster present. In dorsal view first gastral tergite with shallow longitudinal anteromedian groove, anteromedian dorsal carinae either absent or weak.

***S. parvulus* queen diagnosis**

Small size, mandibles smooth, eyes flat, mesoscutum with very faint notauli and median scutal line. Difficult to separate from the queen of *S. opacus*. The *S. opacus* queen is slightly larger and in some specimens there are weak posterior eye carina (absent in *parvulus*), but this character is variable within *S. opacus*, the posterior eye carinae are absent in some queens. The *S. n. sp. 2* queen is similar in size but can be separated by its striate mandibles (smooth in *parvulus*).

***S. parvulus* male** Unknown.

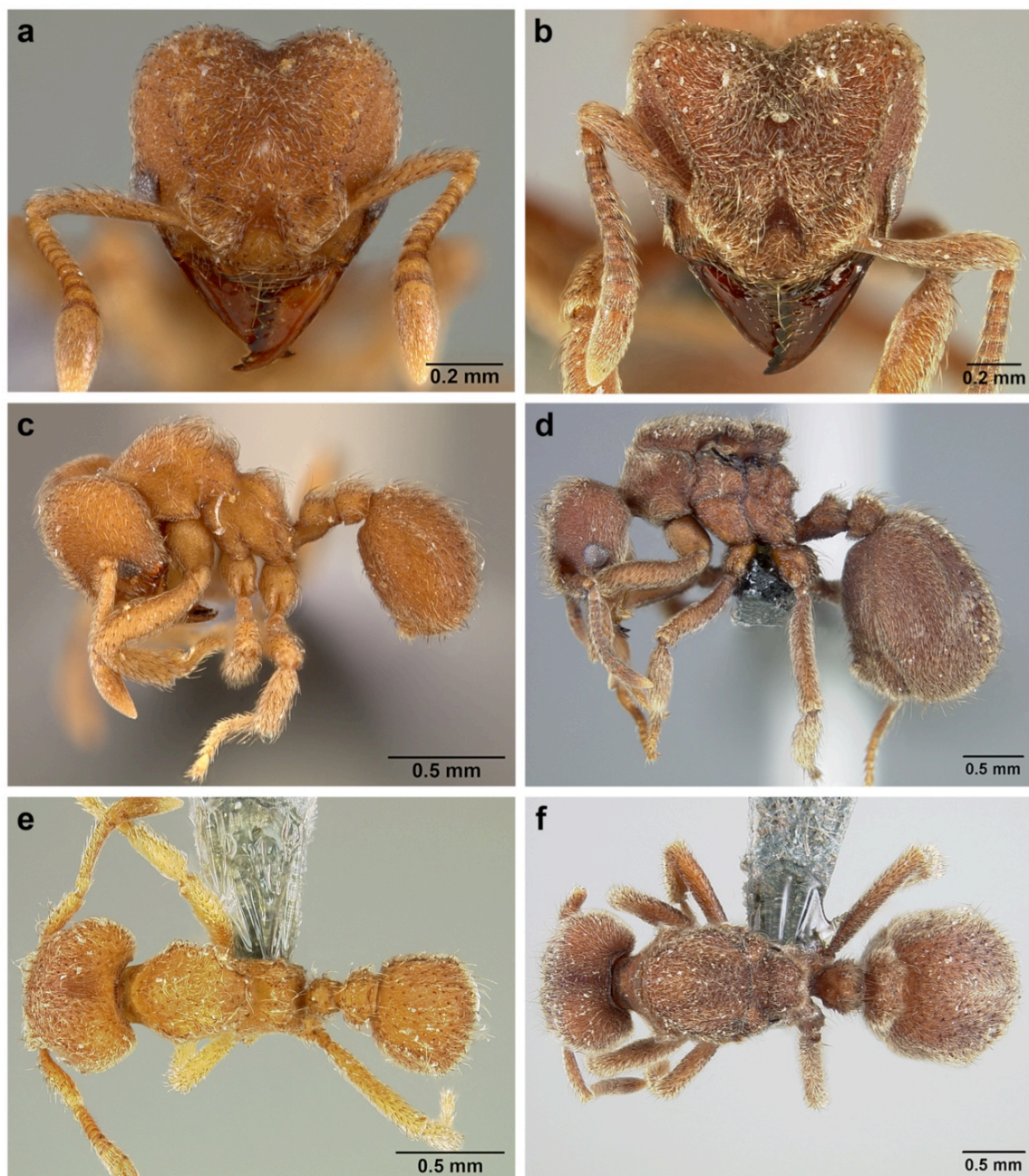


Figure 3.53. *S. parvulus* worker and queen; head, lateral profile, and dorsal views. Worker (USNMENT00446157): **a, c, e**. Queen (USNMENT01125594): **b, d, f**.

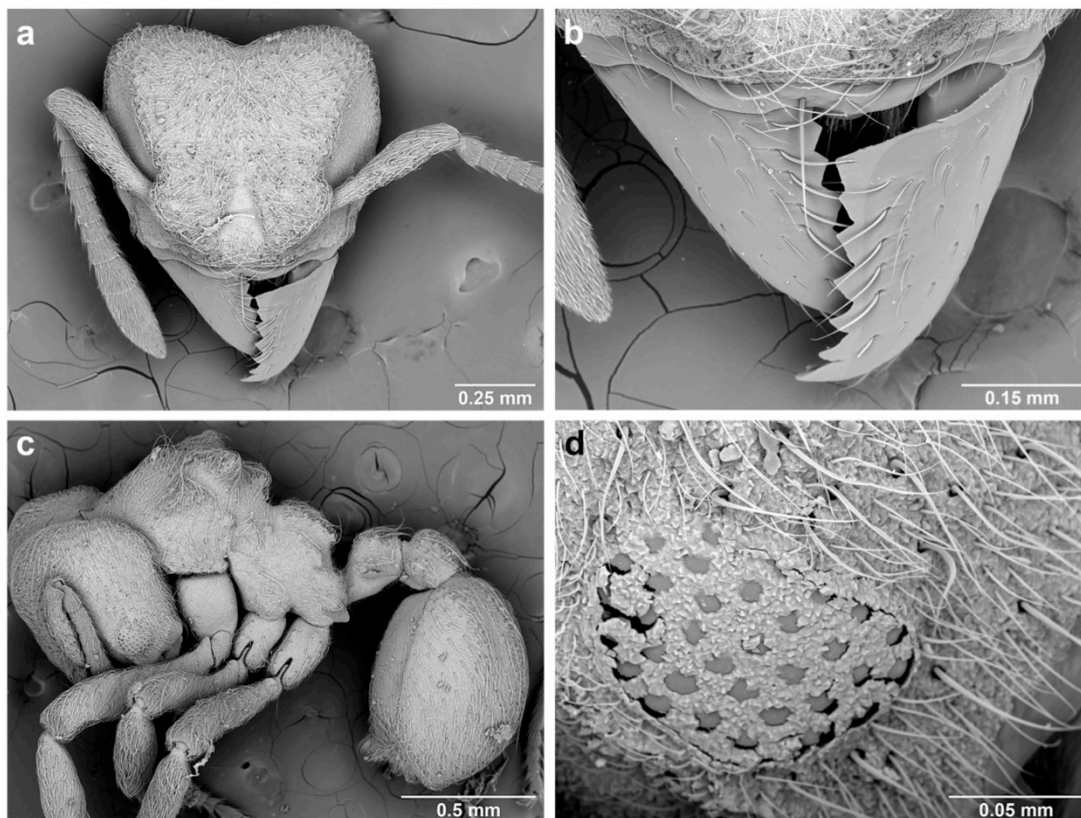


Figure 3.54. *S. parvulus* worker, SEM (USNMENT01125593). **a** Head, full-face view; **b** mandibles; **c** mesosoma and gaster, lateral view; **d** eye.

***S. parvulus* larvae**

Lateral and dorsal surfaces without any setae. Supra-antennal setae absent. Four genal setae on each side. Mandibular apical teeth undivided. Labial denticles either absent or just a few present, anterior to sericteries. Thoracic segment 1 (T1) ventrally with multidentate spinules. Number of ventral setae: T1, T2 and T3 with two setae each, abdomen without setae. Anterior to anal opening a single pair of reduced, sensilliform setae (only the setal sockets and very short setae visible).

***S. parvulus* geographic range.** Colombia, Ecuador, Peru, Bolivia, Fr. Guyana, Guyana, Suriname, Brazil. Map: Figure 3.52.

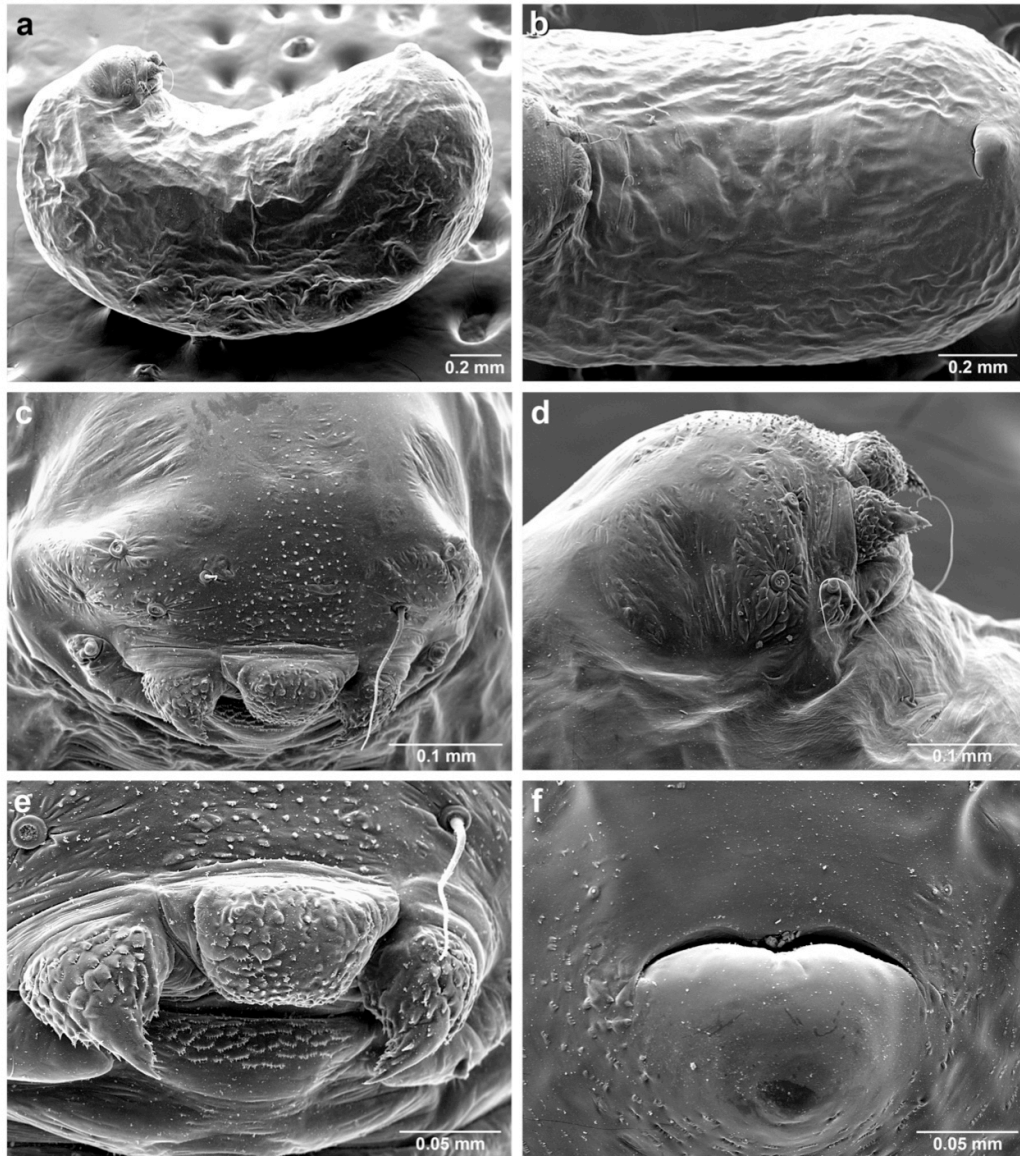


Figure 3.55. *S. parvulus* larva (USNMENT01125592), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontodorsal view; **d** head, lateral view; **e** mouthparts; **f** anal setae.

***S. parvulus* notes**

The aptly named *S. parvulus* is the smallest *Sericomyrmex* species. Within-species morphological variation includes the frontal carinae (typically incomplete and faint, but complete and stronger in some populations), eyes (sometimes covered with a white layer,

but sometimes not), and the general robustness of denticles and tubercles on the mesosoma and metasoma.

In the populations of *S. opacus* from southern Colombia and northwestern Brazil (clade *opacus*3) some of the workers have small, triangular frontal lobes, so that they can easily be mistaken for *parvulus*. They are usually larger than the typical *parvulus* worker, but given the overlap in size between the two species, they could be mistaken for larger *parvulus* workers. It is possible that these intermediate individuals are hybrids and that the molecular and morphological divergence between the Colombian and Brazilian populations of *opacus* is a consequence of hybridization and introgression with *parvulus* (see also discussion in notes for *S. opacus*).

***S. parvulus* material examined:**

BOLIVIA: Beni: Cavinass, [-12.5311, -66.9146], 30 Oct 1921, W. M. Mann;
Santa Cruz: 10km NW Terevinto, -17.6667, -63.45, 380m, 9 Dec 1993, P. S. Ward; 35 km SSE Flor de Oro, -13.8333, -60.8667, 450m, 27 Nov 1993, P. S. Ward; **BRAZIL:**
Amazonas: EMBRAPA Amazonia Ocidental, 30km N Manaus, -2.89824, -59.9903, 4 Sep 2006, C. Rabeling; Manaquiri, Br 319, km100, [-3.6829, -60.32], 10 Oct 2010, F. Baccaro; Manaus, Br 174 Km 46-EEST INPA, [-2.58, -60.03], 83m, 21 Feb 1991, A. Y. Harada; Manaus, Dimona, INPA, 100 ha plot, -2.3833, -60.1, 80m, 6 Jan 2009, J. Sosa-Calvo; Manaus, Rs2303, [-2.96, -59.93], 29 Sep 1993, A. B. Casimiro; Pres. Figueredo, I. Pe Inchado, [-2.02, -60.02], 27 Jan 1994, Queiroz; São Gabriel de Cachoeira, [-0.1237, -67.0476], 120m, 23 Aug 1992, T. R. Schultz; **Bahia:** Ilheus, Itabuna, CEPEC area Zoolog, km 22, [-14.7, -39.2], 1 Oct 1986, J. H. C. Delabie; Itabuna, Ferradas A27, -

14.8258, -39.4044, 21 Sep 2000, J. R. M. Santos; Lençóis, -12.56151, -41.36942 $\pm$ 5m, 487m, 10 Nov 2008, J. Sosa-Calvo; Lençóis, trevo, [-12.55, -41.6833], 30 Mar 2001, J. R. M. Santos; Porto Seguro, -16.3925, -39.1694; **Goiás:** Colinas do Sul, Serra da Mesa, -14.0166, -49.02, 2 Dec 1995, B. H. Dietz, Campaner; Faz. Pau, nr. Jussara, -15.5835, -51.3966 $\pm$ 6m, 305m, 30 Sep 2008, J. Sosa-Calvo, T. R. Schultz; **Maranhão:** Bom Jardim, REBIO Gurupi Parcela 01 08, -3.9258, -46.7712, 20 Sep 2014, A. Y. Harada; Centro Novo Maranhao, REBIO Gurupi, Parcela 02, -3.682111, -46.7798, 18 Jul 2014, A. Y. Harada; **Minas Gerais:** Uberlândia, Panga, -19.18314, -48.40141, 813m, 18 Oct 2012, A. Ješovnik; Uberlândia, Panga, -19.10557, -48.23849, 810m, 22 Sep 2008, J. Sosa-Calvo; Uberlândia, Panga, -19.1666, -48.3833, 790m, 22 Sep 2008, T. R. Schultz; Viçosa, -20.7833, -42.8333, 30 Oct 2014, R. Jesus, J. Chaul; **Pará:** Belem, IPEAN APEG, [-1.4373, -48.4706], 19 Jul 1971, I. B. de Almeida; Belem, Parque R.A., [-1.4585, -48.4372], R.C.G.; Belem, Utinga Forest Preserve, -1.4174, -48.4288 $\pm$ 5m, 45m, 3 Feb 2009, J. Sosa-Calvo; Marituba, CEPLAC Station, [-1.3666, -48.3333], 16 Oct 2004, J. R. M. Santos; Melgaço, Caxiuanã, ECFPn, -1.7248, -51.4230, 20 Oct 2007, A. Y. Harada; Nova Ipixuna, Fazenda Bom Retiro, Parcela 02, -4.8412, -49.218, 12 Apr 2012, M. Tavares, A. Palmeira; Parauapebas, FL Nacional de Carajás, Parque Zoobotanico, -6.06292, -50.05712, 626m, 3 Oct 2014, A. Ješovnik; Tailandia, Faz St Marta Empresa Jurua Florestal, [-3.0167, -4.2667], 28 May 2002; **Rondônia:** Ilha do Bufalo, km 0.5, subparcela 250, -9.0818, -64.2125, 90m, 26 Oct 2013, I. O. Fernandes; Ilha Pedras, km 4, subparcela 100, -9.1512, -64.578, 86m, 25 Oct 2013, I. O. Fernandes; Jaci-Paraná, km 4, subparcela 150, -9.2656, -64.2125, 94m, 26 Nov 2011, I. O. Fernandes; Rio Jamari, São Pedro, [-10.2, -63.25], 11 Jun 1960, OP Fora Hini ; **São Paulo:** Cananeia, Ilha do

Cardoso, -28.0968, -47.9298, 24 Dec 2002, R. R. Silva, C. R. F. Brandão, C. Scott; Faz. Itaquere, Nova Europa, [-21.7838, -48.5578], 2 Dec 1963, K. Lenko; Jacupiranga, [-24.7055, -48.0167], 1 Nov 1963, F. Plaumann; Jureia-Itantins, -24.5442, -47.235;

Sergipe: Areia Branca E.E. da Serra de Itabaiana, -10.765, -37.3326, 19 May 2003, R. R. Silva, B. H. Dietz, L. S. Ferreira; **Tocantins:** Araguacema, -8.9886, -49.6675, 16 Nov 2005, R. R. Silva, R. M. Feitosa; Goiatins, -7.9793, -47.2507, 3 May 2005, R. R. Silva, B. H. Dietz; Paraná, Serra da Contenda, -13.3576, -47.6756, 13 Oct 2004, R. R. Silva, B. H. Dietz; Porto Nacional, Fazenda Alto Paraíso, Transecto 1, -10.7089, -48.4680, 30 Sep 2001, R. R. Silva, N. L. Albuquerque; **COLOMBIA: Amazonas:** PNN Amacayacu Matamata, -3.6833, -70.25, 150m, 17 Jan 2001, A. Alvarado; **Caquetá:** Puerto Solano, PNN Chiribiquete, Serranía de Chiribiquete, Rio Cunare, 0.5, -72.631, 250m, 1 Nov 2000, E. Gonzales; **ECUADOR: Manabi:** 73km NE Chone, B364, [-0.363, -79.739 ±10000m], 300m, 12 Jun 1976, S., J. Peck; **Napo:** Reserva Faunístico Cuyabeno, 0.1167, -76.1833, 1 Nov 1994, J. P. Caldwell; **Orellana:** Tiputini Biodiversity Station, -0.6333, -76.1333, 10 Feb 2003, K. T. Ryder Wilkie; **FRENCH GUIANA: Cayenne:** Régina, Nouragues Field Station, intersect. trails 16&L, 4.0849, -52.6771, 95m, 4 Aug 2005, T. R. Schultz; **GUYANA: Cuyuni-Mazaruni:** Calm Water Creek, along Essequibo River, nr. Bartica, 6.466667, -58.65, 26 Sep 2002, J. S. LaPolla; **PERU: Huánuco:** Monson Valley, Tingo Maria, -9.3167, -76.0167, 600m, 10 Nov 1954, E. I. Schlinger, E. S. Ross; **Madre de Dios:** Tambopata Reserve, -12.8187, -69.3636, 224m, 1 Aug 2012, A. Ješovnik; **SURINAME: Sipaliwini:** Lely Mountains, 4.2529, -54.7561, 619m, 26 Oct 2005, J. Sosa-Calvo, R. Badal; Nassau Mountains, 4.8172, -54.6067, 514m, 3 Nov 2005, J. Sosa-Calvo.

***Sericomyrmex SAUSSUREI* Emery 1894**

Worker: Figures 3.56, 3.57; **Queen** and **Male:** Figure 3.58; **Larva:** Figure 3.59; **Map:** Figures 3.21, 3.60.

Sericomyrmex saussurei Emery, 1894: 223. *Lectotype* (here designated): BRAZIL, Mato Grosso, [-13, -56], 1886, P. Germain, ANTC25804 (MSNG: 1w, USNM00445513).

=*Sericomyrmex burchelli* Forel, 1905: 183. **New junior synonym.**

Type material examined: BRAZIL, Goiás [-15.9, -50.3], W. J. Burchell (MHNG: 1q, USNM00445563; 1m, USNM00445564).

=*Sericomyrmex impexus* Wheeler, 1925: 54. **New junior synonym.**

Type material examined: GUYANA, Cuyuni-Mazaruni, Kartabo [6.3551, -58.6944], Jul-Aug 1920, W. M. M. Wheeler (MCZ: 2w, MCZ-ENT00021138).

=*S. urichi maracas* Weber, 1937: 395. **New junior synonym.**

Type material examined: TRINIDAD, San Juan-Laventille, Maracas Valley [10.75, -61.43], 1 Oct 1935, N. A. Weber, NAW373-1 (MCZ: 2w, MCZ23048).

***S. saussurei* worker description**

Measurements in mm (range): HWe 0.88–1.23 HW 0.88–1.23 IFW1 0.59–0.88 IFW2 0.19–0.36 HL1 0.84–1.20 HL2 0.76–1.08 ScL 0.62–0.86 EL 0.12–0.24 Om 7.00–11.00 WL 1.10–1.64 PL 0.21–0.38 PPL 0.15–0.30 GL 0.70–1.13 HFL 0.75–1.38 PrW 0.61–0.85 hw1 0.82–1.32 hw2 0.92–1.56 hw3 0.55–0.84 CI 94.48–112.20 FLI 62.61–73.73 SI 65.10–81.25 EI 12.44–23.06 FLD 0.34–0.53 CEI 4.44–14.15 [n=68]

Body color yellowish-brown to ferruginous brown. Pubescence dense, often lighter than the integument, appressed to decumbent. Hairs are often curved, darker in color at the base, yellow to gray, appressed to suberect, but mostly decumbent.

Head. In frontal view, head slightly broader than long ($CI=103.86 \pm 2.33$), posterior corners rounded to angular, posterior cephalic emargination distinct ($CEI=10.18 \pm 1.79$). The posterior cephalic margin between the cephalic corner and the median emargination can be straight to convex and merge with the emargination gradually (Figure 3.7 j) or it can be more or less straight and abruptly merge with the emargination (Figure 3.7 k). Vertexal impression and frontal tumuli distinct. Mandibles glossy, striate across the whole dorsal surface except in some populations that have smooth mandibles (Figure 3.53 b, see notes for more detail). Masticatory margin of mandible with 7 to 8 teeth. Eyes medium-sized for *Sericomyrmex* ($EI=15.45 \pm 1.25$), but conspicuously convex, protruding slightly from sides of the head in frontal view, and always covered with a thick, white layer (Figure 3.6 n, o), which makes it hard to discern individual ommatidia in most specimens; 7 to 9 ommatidia across largest diameter. Frontal lobes relatively wide ($FLI=68.54 \pm 2.27$), triangular, slightly laterally expanded, with posterior margin shorter than medial. Frontal carinae straight to slightly curved laterally, complete, reaching posterior cephalic corners. Antennal scape relatively short, never reaching posterior cephalic margin ($SI=70.51 \pm 2.12$).

Mesosoma. Anterior pronotal tumulus low and faint, lateral pronotal and mesonotal spines low and obtuse to moderately pronounced, but never tall and sharp (as in, e.g., *S. bondari* or *S. n. sp. 3*). Propodeal carinae low, reduced or ending in small and blunt denticles.

Metasoma. Nodes of both petiole and postpetiole with two low, short, serrate, longitudinal carinae, which in the petiole are sometimes reduced to low denticles, best seen in dorsolateral view. First gastral tergite with strongly developed longitudinal lateral carinae, anteromedian dorsal carinae present, weak to well developed.

***S. saussurei* worker diagnosis**

The most distinguishing and consistent characters are the convex, slightly protruding eyes covered with thick white layer, striate mandibles (but see notes for exceptions), and triangular frontal lobes. The species most similar to *S. saussurei* is its sister species *S. amabilis*, but *amabilis* can be easily distinguished by its more or less flat eyes lacking the white layer, and usually by geography (Figure 3.21). *S. mayri*, also with striate mandibles, is larger, has a wider head, and flat, eyes without a white layer. The smaller species *S. parvulus* and *S. opacus* may have a similar white layer over the eyes, but their eyes are small and flat, and the white layer is thinner than in *saussurei*, sometimes incomplete, with individual ommatidia still distinguishable (Figure 3.6 l, m). Also, both *parvulus* and *opacus* have smooth mandibles.

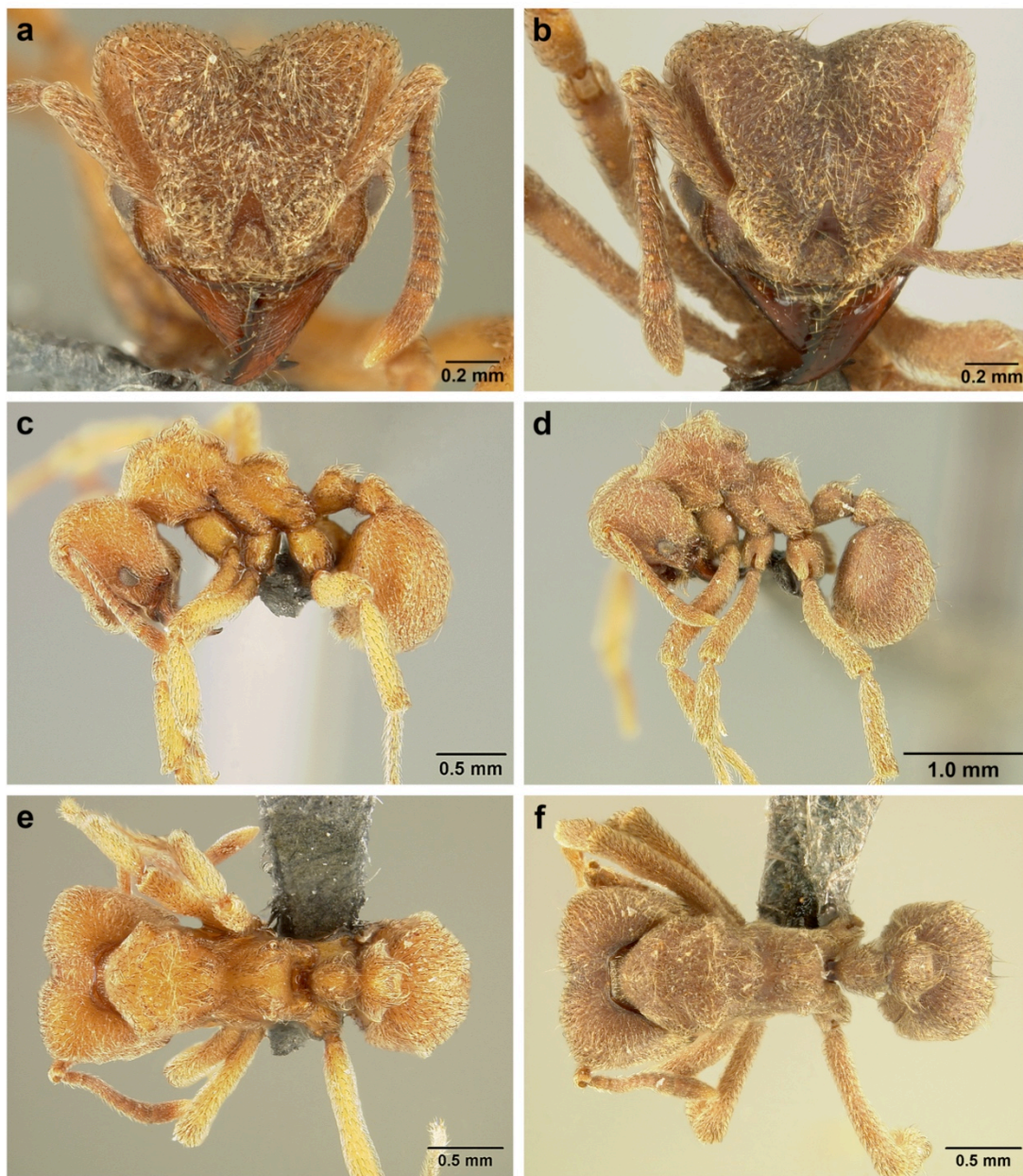


Figure 3.56. *S. saussurei* worker; head, profile, and dorsal view. Striate-mandibled form (USNMENT01125217): **a, c, e**. Smooth-mandibled form (USNMENT01125221): **b, d, f**.

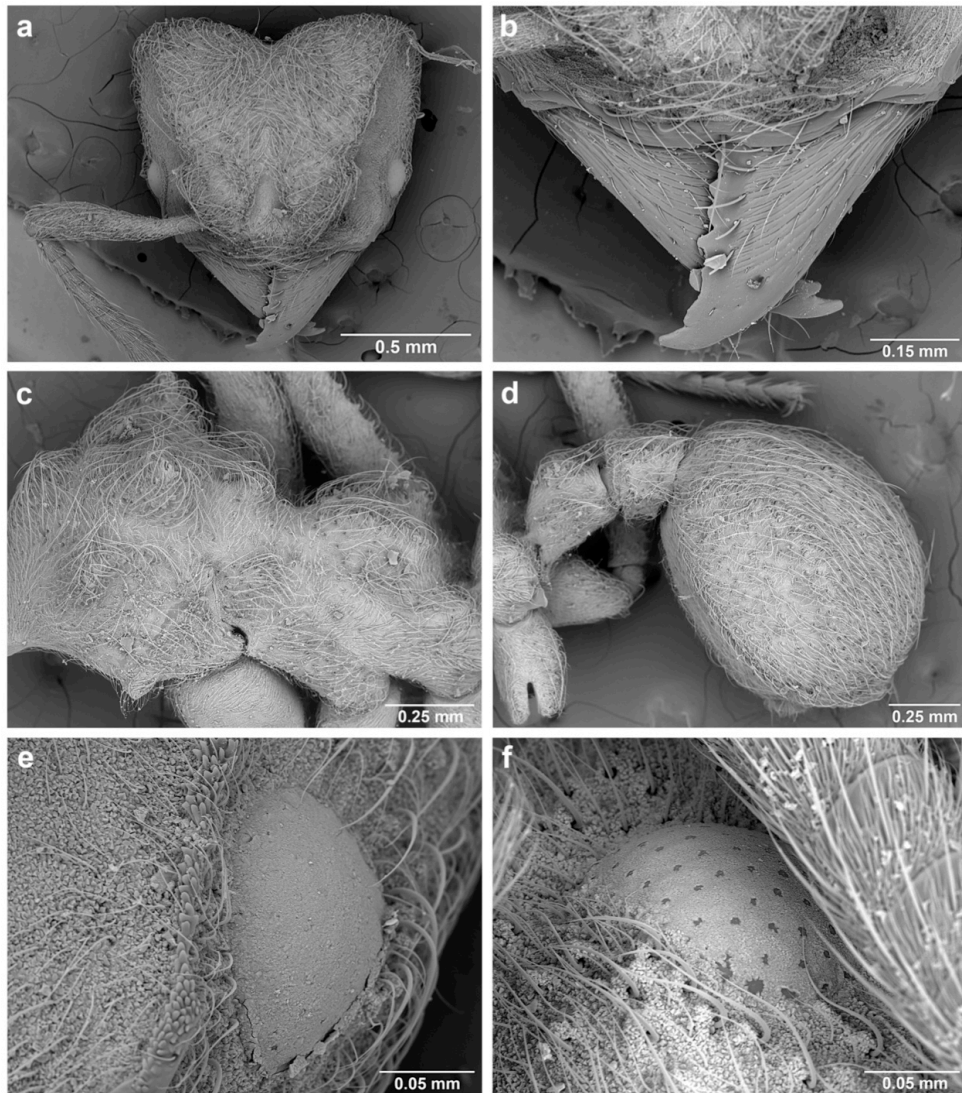


Figure 3.57. *S. saussurei* worker (USNMENT01126237), SEM images. **a** Head, full-face view; **b** mandibles; **c** mesosoma, lateral view; **d** gaster, lateral view; **e** eyes completely covered with white layer, individual ommatidia not visible; **f** eyes with thick white layer, ommatidia visible through narrow holes in the layer.

***S. saussurei* queen description**

Measurements in mm (range): HWe 1.3–1.38 HW 1.27–1.4 IFW1 0.95–1 IFW2 0.35–0.4
 HL1 1.27–1.33 HL2 1.18–1.25 ScL 0.9–0.96 EL 0.23–0.29 Om 20–24 EW 0.08–0.1 WL
 2–2.16 PL 0.43–0.56 PPL 0.25–0.3 GL 1.83–1.95 HFL 1.22–1.6 PrW 1.15–1.2 hw1 1.4–

1.46 hw2 1.49–1.56 hw3 0.9–0.93 FWg 7.04–7.37 HWg 4.73–4.73 CI 97.88–105.61 FLI 69.85–75.02 SI 65.38–72.09 EI 17.02–21.18 [n=6]

Head. Masticatory margin of mandible with 8–9 teeth. Mandibles glossy and smooth, finely transversely striate only along masticatory margin. Preocular carinae fading shortly after reaching the top of the eyes. Eyes large ($EI=19.31 \pm 1.57$), convex, and only partially covered with the white layer thinner than in workers, ~21 ommatidia across largest diameter. Frontal lobes similar to worker ($FLI=72.77 \pm 1.81$). Antennal scape not reaching posterior cephalic margin ($SI=67.87 \pm 3.04$).

Mesosoma. Mesoscutum in dorsal view without notauli or notauli very faint, median mesoscutal line visible only anteriorly. Parapsidal lines present, slightly curved. In dorsal view a transverse shallow groove separating axillae from the scutellum, this groove weakly transversely costate. Scutellum in dorsal view narrowing posteriorly, posterior margin medially with a wide and shallow V-shaped notch. Propodeum with two low carinae that end in denticles, posteriorly diverging in dorsal view.

Metasoma. Lateral longitudinal carinae on first tergite of gaster strongly developed. In dorsal view first gastral tergite with shallow longitudinal anteromedian groove, anteromedian dorsal carinae present, one on each side of impression.

***S. saussurei* queen diagnosis**

The typical white eye layer, which is distinct in the worker, is not as distinct in the queen. The *S. saussurei* queen can be separated from that of the sympatric *S. mayri* by its smooth mandibles and smaller size (the *mayri* queen is larger and has striate mandibles), and from that of the sympatric *S. bondari* by the absence of dark, thick hairs, which are

typical of *bondari*. The *S. saussurei* queen is larger than the queen of *S. parvulus* and it has wider frontal lobes. This diagnosis is based on the queen of the smooth-mandibled *saussurei* population, the only one for which we had samples, so it remains possible that queens from other *saussurei* populations could have striate mandibles.

***S. saussurei* male description**

Measurements in mm (range): HWe 0.74–0.9 HW 0.62–0.7 IFW1 0.3–0.32 IFW2 0.16–0.19 HL1 0.66–0.68 ScL 0.65–0.74 EL 0.25–0.3 Om 23–26 EW 0.13–0.14 WL 1.6–1.72 PL 0.28–0.38 PPL 0.18–0.22 GL 1.18–1.4 HFL 1.52–1.78 PrW 0.74–0.88 IOD 0.56–0.61 FWg 4.73–5.23 HWg 3.15–3.4 CI 112.2–133.33 FLI 34–41.3 SI 78.79–91.3 EI 32.14–36.36 [n=6]

Head longer than broad ($CI=124.89 \pm 7.08$). Eyes large ($EI=34.28 \pm 1.47$), ommatidia across the largest diameter ~25. Preocular carinae long, extending posteriorly almost to the level of the lateral ocelli, slightly curved medially before fading. Notauli and mesoscutal line well developed, integument around parapsidal lines darker in color on some specimens, the groove between axillae sometimes with one short costa. Propodeum smooth, without propodeal carinae or denticles, the only protuberance the small tubercle on which the propodeal spiracle is mounted. Petiole with lateral and dorsal serrate carinae, postpetiole with only very reduced lateral denticles.

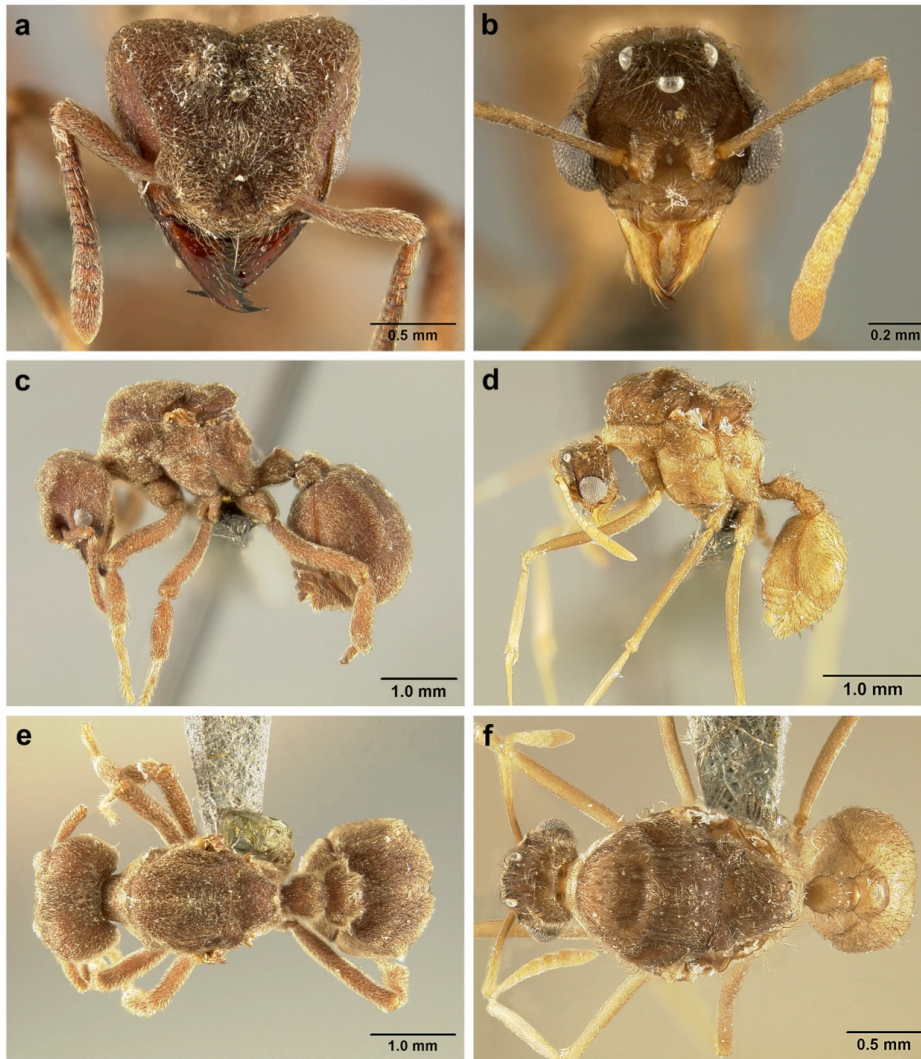


Figure 3.58. *S. saussurei* queen and male; head, lateral profile, and dorsal view. Queen (USNMENT01125514): **a, c, e** (USNMENT01125515). Male: **b, d, f**.

***S. saussurei* larvae**

Around 8 setae on each side of the lateral and dorsal body surfaces (i.e., ~16 total). Supra-antennal setae absent. Four genal setae on each side. Mandibular apical teeth divided. Labial denticles present anterior to sericteries. First thoracic segment without multidentate spinules. Numbers of ventral setae: two on T1, two on T2, three on T3, and around 10 on abdomen. Single pair of setae anterior to anal opening.

***S. saussurei* geographic range:** Brazil, Bolivia, Peru, Guyana, Suriname, French Guiana, Trinidad and Tobago, Venezuela, Ecuador. Map: Figure 3.21.

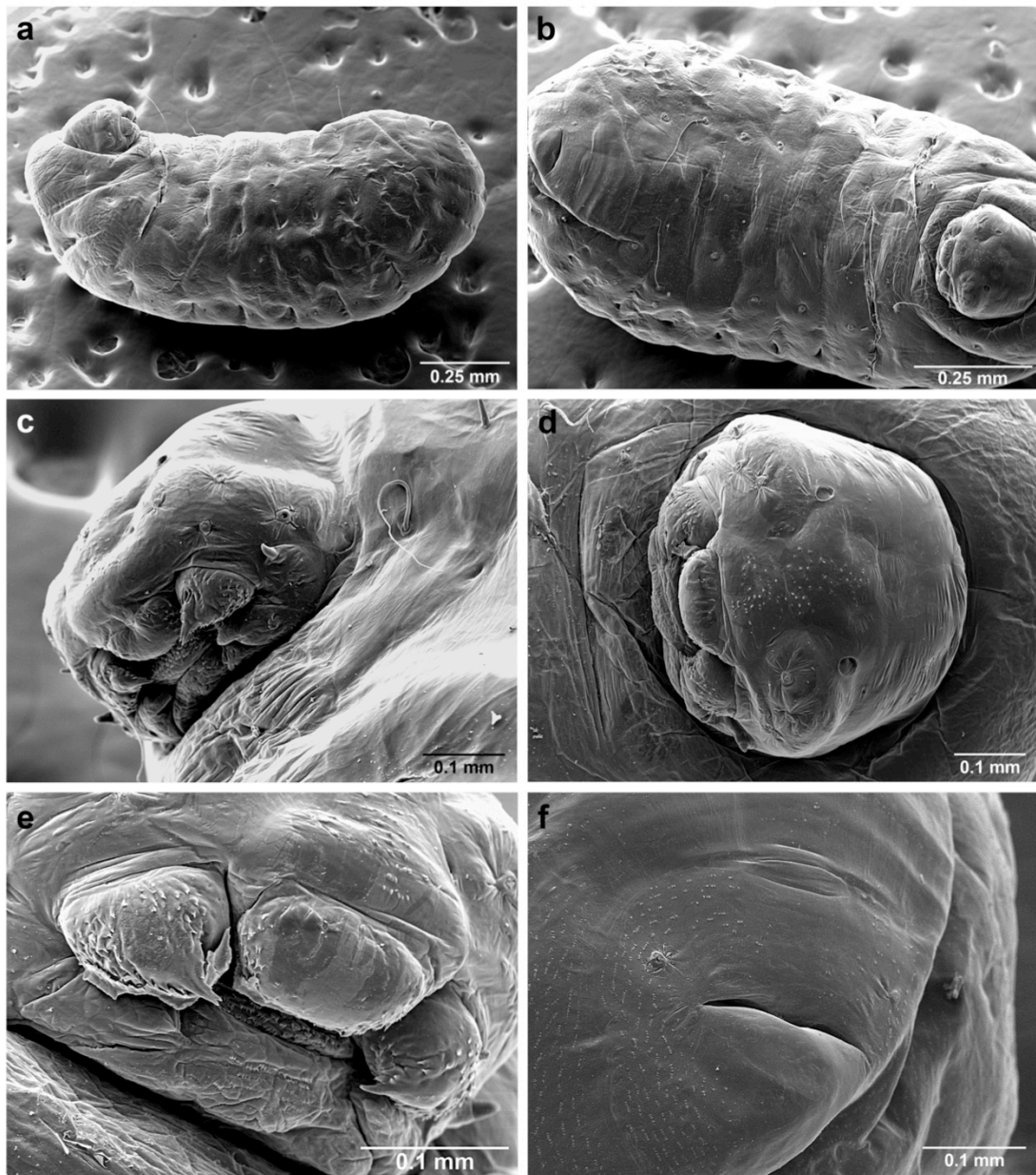


Figure 3.59. *S. saussurei* larva (USNMENT01126236), SEM images. **a** Lateral view; **b** ventral view; **c** head, frontolateral view; **d** head, dorsal view; **e** mouthparts; **f** anal setae.

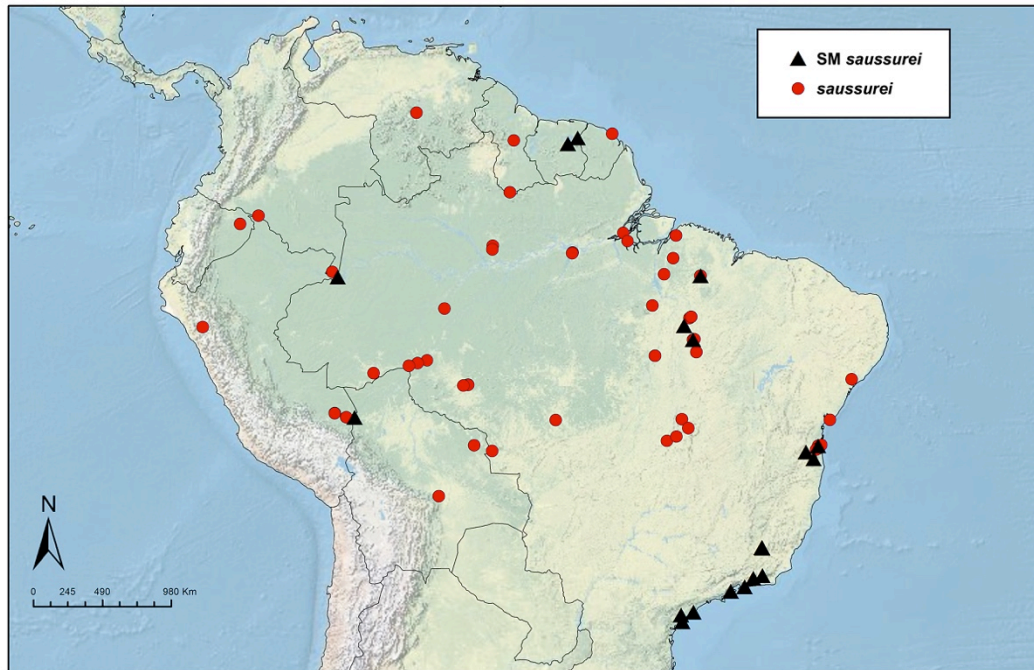


Figure 3.60. Distribution map of *S. saussurei* populations: striate-mandibled and smooth-mandibled (SM) *S. saussurei*.

***S. saussurei* notes**

Although the typical *S. saussurei* has striate mandibles, variation in this character occurs in several populations in which some individuals have faintly striate or completely smooth mandibles. The atypical smooth-mandibled state is in at least one case consistent within an entire nest; all individuals in a colony collected in Viçosa (Brazil) have smooth mandibles. Individuals with intermediate states are sometimes encountered, with mandibles entirely but faintly striate, or only partly striate. Smooth-mandibled *saussurei* are distributed more or less across the entire range of the species, but are more concentrated in eastern Brazil (Figure 3.60). In the molecular phylogeny the two forms cluster into two imperfect subclades in which some striate-mandibled forms mix with predominantly smooth-mandibled forms and vice versa, and a similar pattern is observed

in principle-component analyses of the morphological measurement data (Figure 3.5 h). More data are clearly needed to determine if the smooth-mandibled variant represents a new species. Here we choose to include both forms in a single species because they are morphologically very similar, the molecular evidence is indecisive, and this character is generally plastic (e.g., both *amabilis* and *mayri*, which typically have striate mandibles, likewise have a few populations with smooth or smoother mandibles). It remains possible, however, that within *Sericomyrmex*, this species may represent the most convincing case of a consistent correlation between mandibular sculpture and species boundaries because of the observation of an entire nest series with the fixed state of completely smooth mandibles.

S. saussurei is the sister species of *S. amabilis* based both on molecular data (UCE phylogeny) and morphology. It can be clearly separated from *amabilis* by its larger, convex eyes, covered with a thick white layer, and by larval characters. Interestingly, this white layer on the eyes of *saussurei* is the most constant species character within *Sericomyrmex*, and is exceptionally consistent. All other characters, including the white layer on the eyes, are more variable and less reliable in other *Sericomyrmex* species. In all specimens of *S. saussurei* examined, from across a large geographic range (Figure 3.21), the eyes are convex and covered with a thick white layer. Similar white layers are also seen in *parvulus* and *opacus*. In those species, however, the layer itself is thinner and often incomplete, and the eyes are generally smaller and flat, creating a distinctly different appearance (Figure 3.6 l, m). Also, in both *parvulus* and *opacus* the layer is completely absent in some individuals or populations. In the remaining species of *Sericomyrmex* the eyes are uncoated, without a white layer. It would be interesting to

determine the biological significance of this layer and to analyze its chemical properties. Based on our SEM images it seems to be an extension of the waxy cuticular layer found on the integuments of workers and queens in all *Sericomyrmex* species (Figure 3.6 b,c), but which is absent in males and in callow workers (Figure 3.6 d, e, f) as well as in some individuals of *S. n. sp. 1*. Why this layer extends to and completely covers the eyes in some species but not others remains unknown.

Material examined

BOLIVIA: Santa Cruz: 10 km NW Terevinto, -17.6667, -63.45, 380m, 9 Dec 1993, P. S. Ward; Aserradero Moira, -14.5667, -61.2, 180m, 27 Nov 1993, P. S. Ward;

BRAZIL: Amazonas: Floresta de Tapaua, km 4, -6.01, -63.1, 69m, 13 Oct 2013, I. O. Fernandes; Manaus, Br 174, Km.70, [-2.26, -60.04], 95m, 30 Aug 1995, H. Vasconcelos; Pres. Figueredo, I. Pe Inchado, [-2.02, -60.02], 26 Aug 1994, Queiroz; **Bahia:** CEPLAC, arboreto, -14.7535, -39.2313, 11 Oct 2013, J. H. C. Delabie; Ilheus, Ponta do Ramo, Cacau 02, -14.5294, -39.0619, 28 Aug 1998, J. R. Maia; Itabuna, Ferradas A27, -14.8258, -39.4044, 21 Sep 2000, J. R. M. Santos; Itororo, -14.9744, -40.0502, 11 Aug 2000, J. R. M. Santos; Marau, Faz. Agua Boa, -14.5847, -39.2672, 1 Jul 1997, J. R. M. Santos; Salvador, [-12.9833, -38.5167], 1 Oct 2012, T. S. Melo; Urucuca, -14.5847, -39.2672, 16 Dec 1997, J. R. M. Santos; **Goiás:** Calvacante, Serra da Contenda, -13.4951, -47.5504, 15 Oct 2004, R. R. Silva, B. H. Dietz; Niquelandia, -14.0166, -48.3, 24 Sep 1995, R. Silvestre, B. H. Dietz, C. R. F. Brandão; **Maranhão:** Bom Jardim, REBIO Gurupi Parcela 01 A2, -3.9258, -46.7712, 19 Sep 2014, A. Y. Harada; Estreito, Fazenda Itaueiras, -6.5317, -47.3711, 1 Jun 2005, R. R. Silva, R. M. Feitosa; Estreito, João

Lisboa, -6.5317, -47.3711, 1 Jun 2005, R. R. Silva, R. M. Feitosa; **Minas Gerais:** Camacan, Serra Bonita, -15.3907, -39.5634 $\pm$ 6m, 789m, 20 Mar 2009, J. Sosa-Calvo; Serra de Ricardo Franco State Park, -14.9076, -60.0646, 200m, 7 Feb 2014, J. Maravilhas; Viçosa, Mata do Seu Nico, -20.7833, -42.8333, 8 May 2013, R. Jesus, J. Chaul; Viçosa, UFV Mata da Biologia, -20.7578, -42.8636, 10 Oct 2015, J. Chaul, S. Epifânio; **Pará:** Alter do Chao, -2.4607, -54.926 $\pm$ 6m, 39m, 27 Jan 2009, J. Sosa-Calvo; Goianesia, Faz. Rio Capim, [-3.8384, -49.0986], 16 Jun 2003, A. M. Elizabeth; Gurupa, [-1.197, -51.7], 18 Oct 2003, J. M. S. Vilhena; Marituba, Cacao, [-1.3666, -48.3333], 16 Oct 2004, J. R. M. Santos; Melgaço, Caxiuanã, ECFpn IV Transecto 9-100) Winkler #5, -1.7248, -51.4230, 27 Jun 2003, A. Y. Harada; Paranapuebas Palmares, Lote BPR Mensa Ponto:35583, -5.8072, -49.8325, 9 Apr 2008, M. Martins; Tailandia, Fazenda Marupiara, Parcela 05, -2.8121, -48.5122, 25 Apr 2013, M. Tavares, A. Palmeira; **Rio de Janeiro:** Nova Iguaçu, ReBio Tingua, -22.5705, -43.4141, 2 Feb 2002, A. Mayhe, S. Veiga-Ferreira; Restinga da Marambaia, [-23.0685, -43.9531], P. S. Meneguete; Teresopolis, PN Serra dos Órgãos, -6.5317, -47.3711, 23 Nov 1999, Racha, B. H. Dietz, Rosa; **Rondônia:** Jaci-Paraná, km 0, subparcela 100, -9.2656, -64.2125, 94m, 27 Jan 2013, I. O. Fernandes; Ji-Paraná, -10.7997, -61.5947, 273m, 7 Oct 2008, T. R. Schultz; **São Paulo:** Cananeia, Ilha do Cardoso, -28.0968, -47.9298, 24 Dec 2002, R. R. Silva, C. R. F. Brandão, C. Scott; Jacupiranga, [-24.7055, -48.0167], 1 Nov 1963, F. Plaumann, C. Kempf; Jureia-Itantins, -24.54416, -47.235; Picinguaba, P.E. Serra do Mar, -23.3361, -44.8375, 1 Apr 2001, C. R. F. Brandão; **Tocantins:** Aguiarnópolis, -6.6137, -47.4814, 14 Jan 2005, R. R. Silva, R. Silvestre; Araguacema, Rio Tiririca, -8.9886, -49.6675, 16 Nov 2005, R. R. Silva, R. M. Feitosa; Babaculândia, -7.0878, -47.8286, 10 Dec 2001, R.

R. Silva, N. L. Albuquerque; Goiatins, -7.9413, -47.1586, 3 May 2005, R. R. Silva, B. H. Dietz; Paraná, -12.9343, -47.9618, 13 Oct 2004, R. R. Silva, B. H. Dietz; Recursolandia, -8.7579, -47.0388, 9 May 2005, R. R. Silva, B. H. Dietz; **COLOMBIA: Amazonas:** Leticia, Reserva Forestal Del Rio Calderon, Estac. Biol. El Zafire, -4.0058, -69.9125, 146m, 1 Dec 2007, L.E. Franco, S. Florez; PNN Amacayacu Matamata, -3.6833, -70.25, 150m, 15 Oct 2000, A. Parente; **Putumayo:** PNN La Paya Cabaña Viviano Cocha Montana, -0.1166, -74.9666, 350m, 1 Jun 2003, R. Cobete; **ECUADOR: Orellana:** Tiputini Biodiversity Station, -0.6333, -76.1333, 220m, 14 Feb 2002, K. T. Ryder Wilkie; **FRENCH GUIANA: Cayenne:** Kourou, 5.096, -52.404, 18 Dec 2014, M. Fichaux, Orivel; **GUYANA: Potaro-Siparuni:** Iwokrama, Kurapakari base camp, [4.6698, -58.6854], 60m, 9 Apr 1996, T. R. Schultz, U. G. Mueller; **Upper Takutu-Upper Essequibo:** Acari Mountains, nr Romeo's camp, 1.3833, -58.9333, 282m, 10 Oct 2006, J. Sosa-Calvo; **PERU: Cajamarca:** Cajamarca, 32 km W Jaen, -7.1667, -78.5167, 600m, 19 Jan 1955, E. I. Schlinger, E.S.Ross; **Madre de Dios:** PN Pampas de Heath, Rio Heath, -12.8333, -68.8333, 26 Jul 1991, B. L. Fischer; Puerto Maldonado, Los Amigos Biol. Station, trail 3, Huangana, -12.569, -70.1008, 277m, 9 Oct 2004, T. R. Schultz, J. Sosa-Calvo; Tambopata Reserve, -12.8187, -69.3636, 224m, 1 Aug 2012, A. Ješovnik; **SURINAME: Sipaliwini:** Lely Mountains, 4.4507, -55.2302, 550 m, 29 Oct 2005, J. Sosa-Calvo, R. Badal; Nassau Mountains, 4.8172, -54.6067, 514m, 3 Nov 2005, J. Sosa-Calvo; **VENEZUELA: Bolivar:** Rio Tawadu, Nichare Field St., 6.4333, -64.8833, 200m, 9 Feb 1966, D. M. Olsen.

***Sericomyrmex SCROBIFER* Forel 1911**

Worker: Figure 3.61, Figure 3.62; **Map:** Figure 3.42.

Sericomyrmex scrobifer Forel, 1911: 296. *Lectotype* (here designated): BRAZIL, São Paulo, Ypiranga, [-23.5, -46.6], Luederwaldt, ANTC35980 (NHMB, 1w, CASENT0912518).

***S. scrobifer* worker description**

Measurements in mm (range): HWe 0.83–1.12 HW 0.78–1.05 IFW1 0.66–0.84 IFW2 0.22–0.32 HL1 0.80–1.10 HL2 0.70–0.98 ScL 0.62–0.78 EL 0.15–0.24 Om 10.00–14.00 WL 1.12–1.40 PL 0.24–0.38 PPL 0.20–0.28 GL 0.74–1.68 HFL 0.93–1.26 PrW 0.63–0.80 hw1 0.83–1.13 hw2 0.88–1.20 hw3 0.55–0.85 CI 96.78–111.23 FLI 72.00–83.25 SI 62.00–76.73 EI 15.80–23.33 FLD 0.41–0.56 CEI 7.65–15.12 [n=31]

Body color uniformly ferrugineous yellow-brown to brown. Pubescence dense, often lighter than the integument, appressed to decumbent. Setae are often curved, darker in color at the base, appressed to suberect, but mostly decumbent.

Head. In frontal view, head slightly broader than long ($CI=103.48 \pm 2.58$), posterior corners angular to acute. Lateral margins of the head straight to slightly convex, posterior cephalic emargination distinct and relatively deep ($CEI=11.61 \pm 1.12$). Posterior cephalic margin between cephalic corner and median emargination can be straight to convex, and merges with the emargination gradually. Vertexal impression in most specimens distinct, frontal tumuli faint but usually visible. Mandibles dorsally smooth and shiny, finely transversely striate only along masticatory margin. Masticatory margin of mandible with 7 to 8 teeth. Eyes large for the genus ($EI=19.82 \pm 2.17$) and distinctly

convex, protruding from sides of the head in frontal view, without white layer, 10–14 ommatidia across largest diameter. Frontal lobes wide ($FLI=74.34 \pm 3.99$), laterally expanded, trapeziform to rectangular, with posterior margin as long as medial, or just slightly shorter in some specimens. In some specimens the lateral margin of the frontal lobe is mildly concave and serrate (Figure 3.61 b). Frontal carinae robust, complete, straight to slightly curved laterally. Antennal scape relatively long for *Sericomyrmex*, almost reaching the posterior corners ($SI=69.88 \pm 3.51$).

Mesosoma. Mesonotal spine well developed, acute, sometimes weakly tuberculate at the top. Propodeal carinae low, sometimes serrate, ending in small and blunt denticles, densely covered with hairs.

Metasoma. Node of petiole with two low, reduced denticles, and node of postpetiole with two faint, short, longitudinal carinae, both best seen in dorsolateral view. Postpetiole in dorsal view sometimes slightly posteriorly emarginate. First gastral tergite with four strongly developed longitudinal carinae: two lateral and two anteromedian.

***S. scrobifer* worker diagnosis**

Small size; frontal lobes large, wide, trapeziform to rectangular; frontal carinae diverging, robust, complete; eyes large, dark, convex, protruding laterally in frontal view. Sharp spines on mesosoma, anterior mesonotal spine most prominent, gastral tergum with two pairs of sharp longitudinal carinae. Mandibles always glossy and smooth, except for faint striae along the masticatory border. Most similar to its sister species *S. n. sp. 1*, from which *scrobifer* can be separated by its larger size; much wider, trapeziform frontal lobes; larger, more protruding eyes; and stronger frontal carinae. The combination of large eyes,

trapeziform frontal lobes, and 4 carinae on gaster will separate it from all other *Sericomyrmex* species.

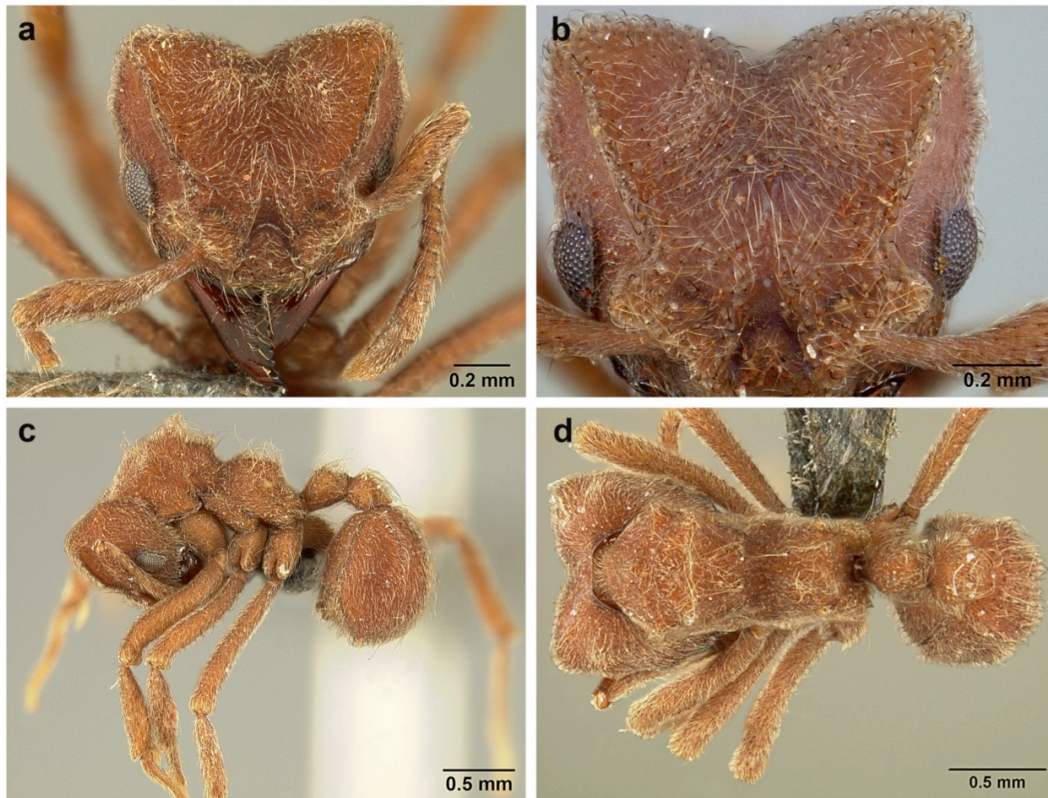


Figure 3.61. *S. scrobifer* worker (USNMENT01125115). **a** Head; **b** head, close-up view (USNMENT01125290); **c** lateral profile; and **d** dorsal view.

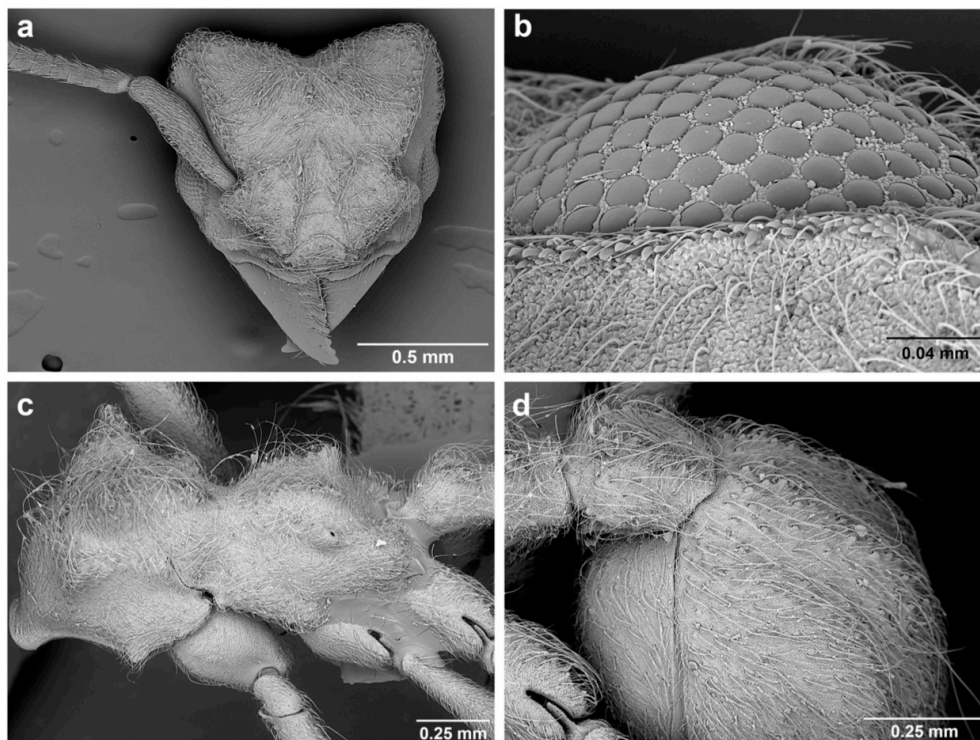


Figure 3.62. *S. scrobifer* worker (USNMENT01125273), SEM images. **a** Head, full-face view; **b** eye; **c** mesosoma, lateral view; **d** gaster, dorsolateral view.

***S. scrobifer* queen, male or larva.** Unknown

***S. scrobifer* geographic range.** Cerrado regions of Brazil, Paraguay. Map: Figure 3.42.

***S. scrobifer* notes**

The smaller individuals can have less pronounced mesonotal spines and weaker dorsal and lateral gastral carinae.

***S. scrobifer* material examined**

BRAZIL: Bahia: Vitoria de Conquista, -14.84, -40.84, 27 Jan 1997, J. H. C. Delabie; **Goiás:** Faz. Cachoeirinha, Jatai, [-17.89, -51.70], 28 Oct 1962, Exp. Dep. Zool.; **Mato Grosso:** Utiariti, Rio Papagaio, [-13.03, -58.28], 23 Oct 1966, Lenko, Pereira;

Minas Gerais: Santana do Riacho, -19.17, -43.72, 19 Feb 2001, S. M. Soares;
Uberlândia, clube Caça, Pesca, [-18.97, -48.28], 14 Sep 2007, R. M. Feitosa; Uberlândia,
Panga, -19.1667, -48.3833, 816m, 2 Sep 2008, T. R. Schultz; **Paraná:** Jaguariaíva, -
24.168, -49.667, 804m, 15 Jan 2015; **Piauí:** Rio Uruçuí Preto, [-7.3431, -44.6168], 20
Feb 1976, R. Negrett; **São Paulo:** São Paulo, [-21.8, -48.5], 26 May 1905, N. A. Weber;
Agudos, [-22.46, -48.97], 27 Apr 1952, W. W. Kempf; Faz. Itaquere, Boa Esperança do
Sul, [-21.9802, -48.3881], 25 Jan 1964, K. Lenko; Mogi Guaçu, -22.37, -46.94, 570m, 6
Feb 1997, I. R. Leal; **Tocantins:** Cartucho e Goiatins, [-8.10, -47.64], 9 Nov 1998, C. R.
F. Brandão, C. I. Yamamoto. **PARAGUAY:** Canindeyú: Res. Mbaracayú, Aguara Ñu, -
24.1833, -55.2833, 240m, 16 Nov 2002, A. L. Wild.

Chapter 4: Nest architecture and natural history of the fungus-farming genus *Sericomyrmex* (Hymenoptera: Formicidae)

4.1. Introduction

The nest of an ant, or of any animal, is considered to be part of its extended phenotype (Turner, 2002), and nest architecture and nest-building behavior have been studied in an evolutionary framework in many organisms, especially social insects (Schmidt, 1955; Wenzel, 1998; Zyskowski & Prum, 1999; Rasmussen & Camargo, 2008). After brood care and foraging for food, nest construction constitutes the largest energy investment for the colonies of many ant species (Sudd & Franks, 1987). Most subterranean ant nests consist of two basic nest modules: tunnels or shafts, which connect different parts of the nest, and chambers, where ants live, raise brood, and store food. The variation in size, shape, number, and arrangement of these basic nest modules is species-typical in many ants, and it is preserved as the colony grows (Cerquera & Tschinkel, 2010; Tschinkel, 2010). Many factors influence the structure of the ant nest, both biotic and abiotic. The volume of a nest, for example, is correlated with individual ant size and with colony size (Tschinkel, 2014), whereas the vertical distribution of brood and workers within the nest is influenced by the temperature of the soil (Tschinkel, 1999). The depth of the nest is correlated with colony size as well (Clémencet & Doums, 2007; Ramos-Lacau *et al.*, 2012), but it is also constrained by environmental factors such as the water table (Lapointe *et al.*, 1998; Mikheyev & Tschinkel, 2004; Clémencet & Doums, 2007).

In the fungus-farming ants (Formicidae: Myrmicinae: Attini: *Atta* genus group; hereafter “attine” ants), a New World clade that contains over 250 species that are all obligately symbiotic with fungi that they farm for food, underground nests need to accommodate the sometimes substantial mass of the fungus gardens. This requirement influences nest architecture: the nest chambers of most fungus-farming ants are subspherical, subovate, or subelliptical, rather than horizontally flattened as in many other ant species with subterranean nests, which only need to house brood and food (Tschinkel, 2003, 2015). Further, in addition to an optimal environment for brood development, fungus-farming ant nests need to optimize temperature, humidity, and CO₂ concentrations for the growth of the fungi as well (Roces & Kleineidam, 2000; Bollazzi & Roces, 2002; Bollazzi & Roces, 2007, Pielstorm & Roces, 2014). The importance of nesting biology for fungus-farming ants has been recognized by many researchers and nest architecture data have been recorded at least partially for the majority of the genera (Camargo et al., 2011; Diehl-Fleig and Diehl, 2007; Ješovnik et al., 2013; Jonkman, 1980; Klingenberg et al., 2007; Klingenberg and Brandão, 2009; Mayhé-nunes et al., 2006; Mehdiabadi and Schultz, 2010; Moreira et al., 2004; Mueller and Wcislo, 1998; Pielstorm and Roces, 2014; Rabeling et al., 2007; Schultz et al., 2002; Solomon et al., 2011; Verza et al., 2007; Weber, 1967; Weber, 1969; Weber, 1976; Wheeler, 1925).

Sericomyrmex, an ant genus with a broad Neotropical distribution, belongs to the so-called “higher” attine ants, a clade of ant species that grow a clade of highly coevolved, obligately symbiotic fungal species (Schultz & Brady, 2008). The closest relatives of *Sericomyrmex*, the other higher attine ants, include genus *Trachymyrmex*, the leaf-cutting genera *Atta* and *Acromyrmex*, and the rare and enigmatic species *Mycetosoritis explicatus*, the sister species of *Sericomyrmex* (Branstetter et al., in prep). *Sericomyrmex* consists of 11 recently

revised species (Chapter 3) that diverged from a most-recent common ancestor only 4.3 million years ago (Chapter 2), making *Sericomyrmex* the youngest attine ant genus.

A few *Sericomyrmex* natural-history studies have been published (Urich, 1895; Weber, 1967, 1976; Feldmann *et al.*, 2000), as well as a number of ecological or behavioral studies that included one or more *Sericomyrmex* species (Kaspari, 1996; Fernández-Marín *et al.*, 2004, 2006; Vasconcelos *et al.*, 2008; Leal *et al.*, 2011; Bruner *et al.*, 2014). Until recently, however, reliable species identifications for *Sericomyrmex* were impossible. The last key was published 100 years ago and is obsolete and incomplete (Wheeler, 1916), so most studies including *Sericomyrmex* species did not identify or wrongly identified the species. For these reasons our knowledge of the natural history of *Sericomyrmex* species is incomplete, lacking basic sociometric data such as colony size, queen number, and timing of mating flights.

Here we briefly review the natural history of *Sericomyrmex*, with a special focus on nest architecture. We organize and summarize previous work by species for cases in which we could determine species identity. We report results of our own work, based on nest excavations following the method of Sosa-Calvo *et al.* (Sosa-Calvo *et al.*, 2015), in which we excavated 19 *Sericomyrmex* nests belonging to 7 different species from across the geographic range of the genus. We summarize nest architecture and other natural history observations, including the first record for the genus of an external waste midden. We report colony demographics for each of the colonies collected and, based on 904 database records, we summarize *Sericomyrmex* species distributions and habitat occurrences.

4.2. Methods and materials

We conducted field work in Mexico, Costa Rica, Guyana, Peru, and three localities in Brazil (Figure 4.1). Localities and collecting data are summarized in Table 4.1. In addition to our collections, we acquired >1500 *Sericomyrmex* specimens from the entire geographic range of the genus from museum collections and colleagues. We constructed a data set containing the collecting information (locality, habitat, elevation, collection date) of those specimens. When analyzing these data to estimate habitat occurrences, we included only one data point for single specimens of the same species collected at the same locality in order to avoid collecting bias. If, for example, multiple individuals of the same species occurred in 20 leaf-litter samples taken at the same locality and in the same habitat, we recorded it as one occurrence of that species for that locality and habitat.

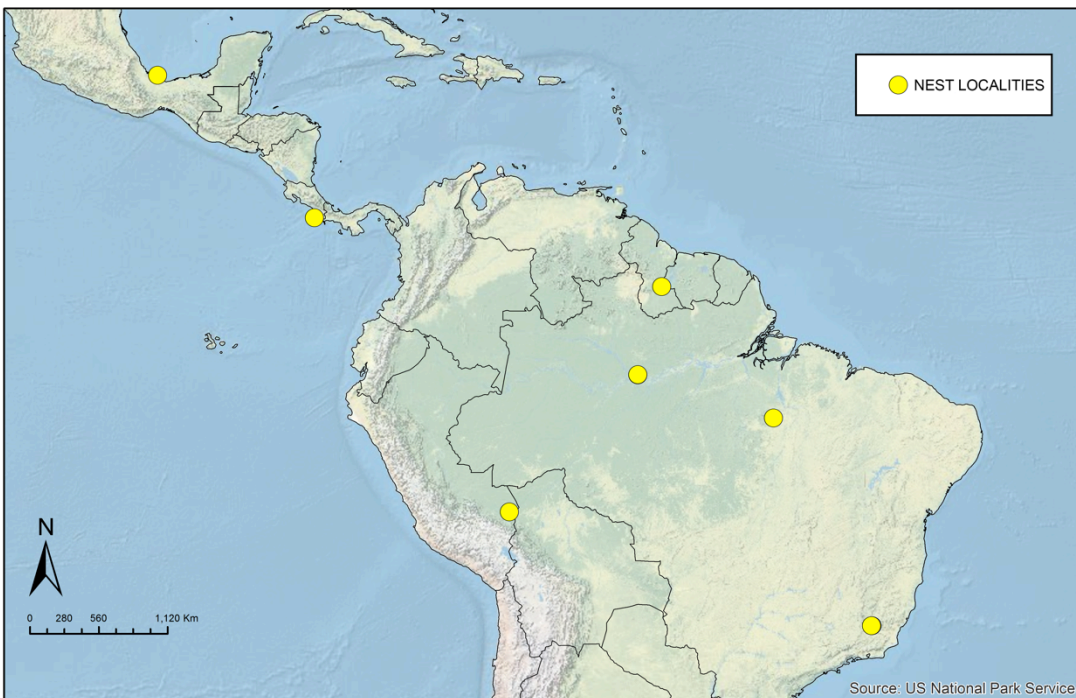


Figure 4.1. Localities from which *Sericomyrmex* nests were collected.

Country	Locality	Habitat	Coordinates	Collection codes	Collectors	Collection date	Species collected
Brasil	Amazonas, Manaus, Reserva Ducke	primary rainforest	-2.932 - 59.972	AJ120926-02	A. Jesovnik M. Rajkovic	26 Oct 2012	<i>bondari</i>
Brasil	Amazonas, Manaus, Reserva Ducke	primary rainforest	-2.917 - 59.983	TRS920809-15	T. R. Schultz	9 Aug 1992	<i>mayri</i>
Brasil	Pará, Parauapebas, FL Nacional de Carajás	secondary rainforest edge	-6.063 - 50.057	AJ141001-05 AJ141002-01 AJ141004-01	A. Jesovnik T. R. Schultz	Oct 2014	<i>mayri</i> <i>bondari</i>
Brasil	Minas Gerais, Viçosa, UFV, Mata de Biologia	yard in urban area	-20.758 - 42.864	JCMC002	J. Chaul S. Epifânio	10 Oct 2015	<i>saussurei</i>
Brasil	Minas Gerais, Viçosa	yard in urban area	-20.755 - 42.887	JCMC001	J. Chaul	27 Sep 2015	<i>parvulus</i>
Costa Rica	Puntarenas, Osa Peninsula, Corcovado, Sirena Station	primary rainforest	8.50 -83.617	TRS920531-03 TRS920602-08 TRS920606-05	T.R. Schultz	May–Jun 1992	<i>amabilis</i> <i>opacus</i>
Guyana	Upper Takutu-Upper Essequibo, CI concesion, base camp	primary rainforest	3.513 - 58.235	JSC111119-04 AJ111125-08 JSC111122-13	A. Jesovnik J. Sosa-Calvo	Nov 2011	<i>mayri</i>
Mexico	Vera Cruz, Catemaco, Tuxtla	rainforest	18.587 - 95.078	AJ131005-01	A. Jesovnik M. Rajkovic	5 Oct 2013	<i>amabilis</i>
Peru	Madre de Dios, Tambopata Reserve	primary rainforest	-12.819 - 69.364	AJ120729-03 AJ120726-03 AJ120801-03 AJ120803-03 AJ120728-10	A. Jesovnik	Jul–Aug 2012	<i>mayri</i> <i>n. sp. 2</i> <i>parvulus</i>

Table 4.1. Collection localities.

In the field we located nests of *Sericomyrmex* by visually searching for nest entrances and by baiting. For bait we used Cream of Rice cereal, which is loved by all non-leaf-cutter attine ants, and which, due to its white color, is easy to track as it is carried by foragers across the dark forest floor. After locating a nest we photographed the nest entrance and measured the height and the diameter of the mound, if present. When possible, we cleared an area with a radius of about 2 meters around the nest entrance of any debris and interfering vegetation in order to detect the presence of any additional nest entrances or external waste middens. For the nest in which a waste midden was found, we measured the distance from the midden to the nest and observed the

behavior of the workers transporting debris to the midden. Following these observations, we collected the midden, including associated arthropods, into 95% ethanol. We started nest excavations 0.5 to 1 meter away from the entrance on the side that was easiest for digging. Nest excavations followed Sosa-Calvo *et al.* (2015). In short, we attempted to excavate a trench deep enough to facilitate exposing the nest chambers from the side rather than from above, enabling careful collection of the chamber contents and accurate measurements of chamber dimensions. For each chamber we recorded the distance from the surface, the horizontal distance from the nest entrance (if applicable), height, and two measures of width (width and “chamber depth” sensu Jesovnik *et al.*, 2013; Sosa-Calvo *et al.*, 2015), as indicated in Figure 4.2. Whenever possible we collected the fungus garden and workers from different chambers separately in order to estimate ant density variation across the nest. We stopped the digging after we found the queen and/or after we had been digging for approximately half a meter without finding any new chambers. Also, when possible, we left the digging pit open until the following day. If there were no new tunnel openings or ant activity the following day, we refilled the pit. If we found new ant activity, we continued excavations. After returning to the lab, we counted all of the ants and the brood to obtain a colony census. Two colonies were maintained alive in the Smithsonian Ant Lab in Washington, D.C. (for live lab nest methods see Sosa-Calvo *et al.*, 2015). We calculated the volumes of chambers following the formula for the volume of an ellipsoid shape (Sun & Liu, 2003), which is $V = \pi/6 \times a \times b \times h$, where a, b, and h are height (h), width (w), and width 2 (w2) of the chamber (Figure 4.2 b: h, w, w2). To calculate correlations between the variables we created a data set with total nest volume, individual chamber volume, nest census (number of individual ants in the nest), and ant size (using mean head width of the species) for all collected nests. All of the statistical analyses were carried out in R (R Development Core Team, 2014).

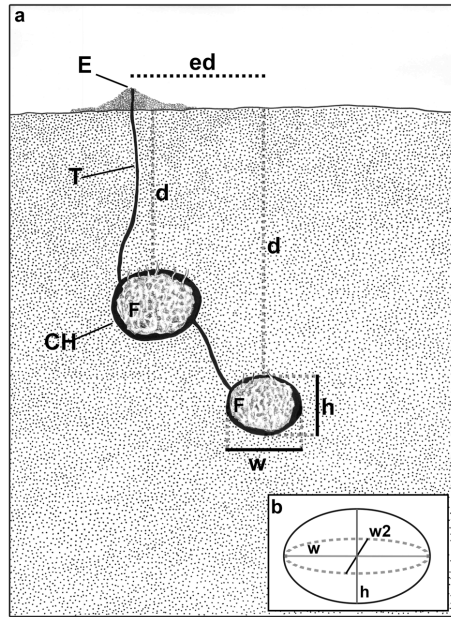


Figure 4.2. Nest measurements. **a)** Cutaway view of a fungus-farming ant nest indicating standardized measurements: E = nest entrance; T = tunnel; CH = chamber; F = fungus garden; d = chamber depth; w = chamber width; h = chamber height; ed = offset distance from the entrance; **b)** Schematic representation of a single chamber, with measurements indicated: w = chamber width; w2= chamber width 2, h = chamber height (adapted from Sosa-Calvo *et al.*, 2015).

4.3. Results

4.3.1. Natural history of *Sericomyrmex*

Beginning at the end of the 19th century, several researchers published observations of *Sericomyrmex* natural history, most notably W. M. Wheeler (Wheeler, 1916, 1925) and N. A. Weber (Weber, 1937, 1967, 1969a, 1969b, 1972a), the latter reporting the first demographic study based on a colony of *Sericomyrmex* maintained alive in the lab for 10 years (Weber, 1976). Early naturalists often at least partially excavated ant nests, noting the depths of the nests and the sizes of the chambers, and describing the fungus gardens (Urich, 1895; Forel, 1912; Weber, 1969a, 1972b). More recently, different aspects of attine ant nesting biology were studied in

Panama (Fernández-Marín *et al.*, 2004; Bruner *et al.*, 2014) and in Brazil (Leal & Oliveira, 1998, 2000; Vasconcelos *et al.*, 2008; Leal *et al.*, 2011), providing data on the nesting biology of *Sericomyrmex* as well as other attine genera. All of those data, including locality, nest measurements, and colony demography, are summarized in Table 4.2, organized by species, together with new data acquired in this study. Nest architecture data is summarized in Table 4.3 and nest demography data in Table 4.4.

Species	Distribution	Habitat	Elev. (m)	Nest entrance	Nest V	Nest depth	Ch. #	Ch. width	w #	Q #
<i>amabilis</i>	CR, HN, GT, MX, NI, PA, CO, EC	1° and 2° rainforest, dry forest, xeric open habitat, coffee plantation, disturbed habitat, forest edge (N=53)	333 (5–1009) (N=36)	soil mound (16 × 30 cm), entrance opening 2.5–3 mm	214.11 (194–234)	11.75 (2–25)	1–5	6.125 (3.5–9)	482 (45–2569)	1–2
<i>bondari</i>	BR, PE, CO, EC, GY	1° and 2° rainforest, mountain forest, restinga forest, forest edge, forest to semi open, cacao plantation (N=48)	325 (5–779) (N=21)	soil mound (23 × 18 cm), nest entrance opening 0.5–1.5 cm	866.4 (857–875)	17.65 (10–23)	2–3	9.625 (8–12)	845 (826–864)	1–3
<i>lutzi</i>	GY	1° rainforest (N=2)	732 (N=1)							
<i>maravalhas</i>	BR	Cerrado: cerrado, gallery forest & cerrado sensu stricto (N=6)	535 (532–539) (N=2)							
<i>mayri</i>	CO, EC, BR, PE, GY, SU, GF, TT,	1° and 2° rainforest, dry, Atlantic and riparian forest, cerrado, cerrado, agricultural habitat, pasture (N=75)	341 (5–1033) (N=31)	soil mound, about 2.5 cm in height	2706.8 (402–6609)	18.6 (10–32)	1–18	10.88 (5.5–18)	2102 (817–5165)*	1–3
<i>opacus</i>	CR, HN, GT, GP, MX, NI, PA, CO, EC, BR	1° and 2° rainforest, mountain forest, pasture, shrub vegetation, coffee plantation (N=29)	338 (50–800) (N=19)	excavated soil, no mound, 3–7.6 cm in diameter	103.6 (94–113)	12.3 (5–20)	1	6	173 (105–231)	1
<i>parvulus</i>	CO, PE, BR, GY, TT, SU	1° and 2° rainforest, Atlantic forest, gallery forest, cerrado, cerrado, cacao plantation, urban habitat (N=59)	325 (45–813) (N=26)	small hole in the ground	484.9 (222–904.5)	6.6 (2.5–13)	2–5	7.1 (4–8.5)	258 (190–312)	1–2
<i>radioheadi</i>	VE	1° rainforest (N=1)								
<i>saramama</i>	PE, EC, CO	1° rainforest (N=3)	187 (150–224) (N=2)	small hole in the ground	465.00	10.5	1	9–11	51	1
<i>saussurei</i>	GY, SU, GF, BR	1° and 2° rainforest, Atlantic forest, gallery forest, cerrado, cacao plantation, open habitats (N=60)	282 (39–789) (N=19)	soil mound	7179.22	31–35	5	13.9 (6–27)	1249	4
<i>scrobifer</i>	BR, PY	Cerrado: campo cerrado, cerrado sensu stricto, cerrado (N=9)	666 (240–910)	soil mound	2918	61–79	1–2		573	2

Table 4.2. Species summary. Distribution, habitat, elevation, total nest volume (Nest V), number of chambers (Ch #), chamber width (Ch width), number of workers (w #), and number of queens (Q #), summarized by species. Country codes: BR–Brazil, CR–Costa Rica, HN–Honduras, EC–Ecuador, GY–Guyana, GF– French Guiana, GP–Guadeloupe, GT–Guatemala, MX–Mexico, NI–Nicaragua, PA–Panama, PE–Peru, SR–Surinam, TT– Trinidad and Tobago, VE– Venezuela. *Two live nests not included.

Species	Coll. code	Ch. #	Nest V	Ch. V	d	h	w	w2
<i>amabilis</i>	TRS920531-03	1	234.45	234.45	3	~7	8	8
<i>amabilis</i>	AJ131005-01	2	193.77	96.89	6 (2–10)	4.5 (3.5–5.5)	6 (6–6)	6.25 (3.5–9)
<i>bondari</i>	AJ120926-02	3	857.3	285.78	18.3 (10–23)	6.3 (5–8.5)	8.83 (8.5–9)	9.67 (8–11)
<i>bondari</i>	AJ141001-05	2	875.5	437.75	17 (14–20)	8.5 (8–9)	11 (10–12)	9
<i>saramama</i>	AJ120729-03	1	466	466	10.5	9	11	9
<i>mayri</i>	TRS920809-15*	2	1041	520.5	10.5 (10–11)	9.5 (9–10)	10 (9–11)	10 (9–11)
<i>mayri</i>	JSC111119-04	18	6608.7	367.2	22.25 (15–29)	6.94 (5–8)	8.5 (5.5–14)	11.39 (6–18)
<i>mayri</i>	AJ111125-08	10	3249.3	324.9	22.9 (16–32)	7.4 (6–8)	7.7 (7–9)	10.7 (8–13)
<i>mayri</i>	JSC111122-13	5	1592.8	318.6	14.7 (10–18)	7 (6–7.5)	8.22 (7–10)	10.5 (9–12.5)
<i>mayri</i>	AJ120726-03	4	2231.4	557.8	18.63 (10.5–25)	9.825 (9–11)	9.63 (8–11)	11 (8–13)
<i>mayri</i>	AJ120801-03	4	2547.3	626.8	21.75 (13–26)	9.25 (8–10)	9.75 (7.5–11)	12.875 (10.5–17)
<i>mayri</i>	AJ141002-01*	2	2316.4	1158.2	20 (13–27)	10 (7–13)	16.5 (16–17)	11.5 (6–17)
<i>mayri</i>	AJ141004-01*	1	401.7	401.7	10	6	16	8
<i>opacus</i>	TRS920602-08	1	113	113	5	6	6	6
<i>opacus</i>	TRS920606-05	1	94.2	94.2	12	5	6	6
<i>parvulus</i>	AJ120728-10	5	904.5	180.9	3.9 (2.5–6)	6.1 (3.5–9)	6.9 (5.5–8.5)	7.8 (7.5–8.5)
<i>parvulus</i>	AJ120803-03	3	328.3	109.4	4.17 (2.5–6)	4.67 (4.5–5)	5.83 (5.5–6)	7.67 (7–8.5)
<i>parvulus</i>	JCMC 001	2	221.8	135.3	11.67 (9–13)	5.17 (4.5–5.5)	6.67 (4–8)	NA
<i>saussurei</i>	JCMC 002	5	7179.2	1435.9	33.8 (31–35)	10 (8–15)	13 (8–20)	14.8 (6–27)

Table 4.3. Nest architecture. Number of chambers (Ch #), total nest volume (Nest V), mean chamber volume (Ch V.), nest depth (d), chamber height (h), and chamber widths (w and w2) for all of the collected *Sericomyrmex* nests. For d, h, w and w2 mean and range values are reported. Asterisks* indicate incomplete excavations.

species	Collection code	workers	larvae	pupae	males	queen	al. queen	total	V
<i>amabilis</i>	AJ131005-01	455	42	37	0	1	0	535	234.45
<i>amabilis</i>	TRS920531-03	487	40	37	0	1	0	565	193.77
<i>bondari</i>	AJ120926-02	826	5	85	0	1	0	917	857.3
<i>bondari</i>	AJ141001-05	864	28	74	0	1	2	969	875.5
<i>saramama</i>	AJ120729-03	51	2	5	0	1	0	59	466
<i>mayri</i>	TRS920809-15*	540	16	1	0	0	32	589	1041
<i>mayri</i>	JSC111119-04	5165	532	869	74	2	0	6642	6608.7
<i>mayri</i>	AJ111125-08	2266	416	445	1	1	0	3129	3249.3
<i>mayri</i>	JSC111122-13	1202	155	207	0	1	0	1565	1592.8
<i>mayri</i>	AJ120726-03	817	124	125	0	1	0	1067	2231.4
<i>mayri</i>	AJ120801-03	1062	203	303	0	3	0	1571	2547.3
<i>mayri</i>	AJ141002-01*	181	25	27	0	1	0	234	2316.4
<i>mayri</i>	AJ141004-01*	1409	107	129	0	3	0	1648	401.7
<i>opacus</i>	TRS920602-08	105	3	4	0	0	0	112	113
<i>opacus</i>	TRS920606-05	231	0	0	8	1	3	243	94.2
<i>parvulus</i>	AJ120728-10	312	30	60	0	1	0	403	904.5
<i>parvulus</i>	AJ120803-03	271	31	65	0	1	0	368	328.3
<i>parvulus</i>	JCMC 001	190	60	0	0	2	0	252	221.8
<i>saussurei</i>	JCMC 002	1249	3	0	235	1	81	1565	7179.2

Table 4.4. Nest demography. Number of workers, larvae, pupae, males, dealate queens, alate queens, and nest volume (V) for each nest. Asterisks * indicate incomplete excavations.

4.3.2. Distribution and habitat

The northernmost record for *Sericomyrmex* is *Sericomyrmex "aztecus"* (either *S. opacus* or *S. amabilis*) in Cuesta de los Cedros, 36 km E of Ciudad del Maíz, in the state of San Luis Potosi in northern Mexico [21.8025, -99.1803] (Sánchez-Peña, 2010). Southernmost records include records of *S. parvulus* and *S. saussurei* in Cananeia, Ilha do Cardoso, southern Sao Paulo state, Brazil [-25.0968, -47.9298] and, further west, *S. scrobifer* in Reserva Mbaracayú, Aguara Ñu, in Canindeyú, southeast Paraguay [-24.1833, -55.2833]. *Sericomyrmex opacus* has been collected on two Caribbean islands, Dominica [15.4857, -61.424] and Guadeloupe [16.11735, -61.74291]. Based on our field observations, local distributions of *Sericomyrmex* are often patchy, with colonies densely distributed and abundant in some areas but absent or sparse in adjacent areas, even though the habitat appears the same. Where abundant, nest entrances may be very close to each other. For example, during a one-year attine-ant study in Trinidad, 67 *Sericomyrmex* nest entrances were observed in an 18 x 77 meter area (Weber, 1972b). An attine ant nest study in cerrado, a central Brazilian savanna biome, reported 17 to 26 nests per 160 m² for *S. cf. scrobifer* (Vasconcelos *et al.*, 2008). Differences in abundance in adjacent localities may be caused by microhabitat differences that cannot be detect with the naked eye, as has been shown for other attine ants (Lapointe *et al.*, 1998).

When redundant records are removed, our habitat data set consists of 904 habitat records. *Sericomyrmex* species can be found in wide variety of habitats, including dry open areas, dry forest, cerrado, open urban grassy areas, agricultural land (e.g., cacao plantations), and primary and secondary rainforest (Table 4.2). Nine out of 11 species are either exclusively or at least most commonly found in forested habitats. The species that are mostly, but not exclusively, found in forest are *S. amabilis* (82.7% occurrences are in forest habitats), *S. bondari* (91.7%), *S.*

mayri (80%), *S. parvulus* (88.13%), *S. saussurei* (88.9%), and *S. opacus* (75,8%). The forest habitats where these species are collected are diverse: primary and secondary wet tropical rainforest, Atlantic forest, dry forest, riparian, and gallery forest. These species are also found in open and semi-open habitats such as cacao, banana, and coffee plantations; savanna; pastures; orchards; and disturbed urban habitats such as yards and parks. Of the species that are most commonly found in forest habitats, *S. bondari* is apparently the most specialized; it is found in forested habitats 91.7 % of the time and it was never collected in cerrado (Chapter 3, Figure 3.27). This is interesting because *S. bondari* has a broad South American distribution and is found in forest habitats bordering cerrado areas, and because species with similar distributions, *S. mayri*, *S. parvulus*, and *S. saussurei*, have all been collected in cerrado habitats. It is possible, but not likely, that this is caused by undersampling. *S. bondari* is a large and easy to notice and our sampling of cerrado habitats is relatively thorough.

Three species that occur only in forest, *S. lutzi*, *S. radioheadi*, and *S. saramama*, have all been collected in primary rainforest only. However, these species are rarely collected in general, so this could be an effect of undersampling. Two species, *Sericomyrmex scrobifer* and *S. maravalhas*, occur almost exclusively in the cerrado, but within the cerrado they have been more often collected in forested cerrado than in open areas (Vasconcelos *et al.*, 2008).

About 400 records in our data set contained elevation data. The mean and range of elevation records for each species can be found in Table 4.2. In general, most species were found at mean elevations of 300 to 400 meters (*S. amabilis*, *S. bondari*, *S. mayri*, *S. parvulus*, *S. saussurei*, and *S. opacus*), with wide ranges (widest range in *S. mayri*, 5-1033 m). Two cerrado-specialist species had a narrower range and higher mean, 240–900 m (mean 666.2) for *S. scrobifer* and 532–539 m (mean 535) for *S. maravalhas*. The only elevation record for *S. lutzi* is

732 m. This species is known from the mountain plateaus (tepui) of the Pakaraima Mountains on the border of Guyana, Venezuela, and Brazil (Chapter 3), and is most likely endemic to that region.

4.3.3. Nest architecture and colony size

Nest entrances (Figure 4.3). Three early researchers reported that the nest entrances of *S. mayri*, *S. amabilis*, and *S. opacus* have raised cylindrical craters, up to 2.5 cm high and 7.5 cm wide, made of excavated soil particles (Urich, 1895; Wheeler, 1925; Weber, 1972b). For *S. opacus* (described at the same time as *S. zacapanus*) in Zacapa, Guatemala, W. M. Wheeler additionally noted that, apart from the soil particles, craters sometimes contain particles of exhausted fungus (Wheeler, 1925). In a study of the nesting biology of cerrado attine ants, Leal and colleagues report that nest mound volume in *S. cf. scrobifer* is a good predictor of colony size (Leal *et al.*, 2011), similar to what is reported for nest rings of harvester ants (Tschinkel, 2014). This correlation may, however, depend on the season because during the rainy season the amount of soil outside the nest entrance varies from day to day (Figure 4.3 c, d).

We studied the nests of seven different species: *S. amabilis* (2 nests), *S. bondari* (3 nests), *S. saramama* (1 nest), *S. mayri* (8 nests), *S. opacus* (2 nests), *S. parvulus* (3 nests), and *S. saussurei* (1 nest). Nest entrance morphology varied between two main forms, with and without the soil mound. *S. parvulus*, *S. opacus*, and *S. saramama* nest entrances consisted of either a simple, small hole in the ground (*S. parvulus*, *S. saramama*, Figure 4.3 g) or two such holes (*S. parvulus*, JCMC 002, Figure 4.2 f), with a small amount of excavated soil around it/them, but without an accompanying soil mound. The nests of *S. amabilis*, *S. bondari*, *S. mayri*, and *S. saussurei* all had irregular soil mounds around the entrance (Figure 4.3 a, b). The size of the

entrance varied from 0.5 cm to 1.5 centimeters. The largest soil mound we recorded was for the *S. bondari* nest in Brazil (AJ141001-05): 33×18 cm wide and 2–3 cm tall.

In four nests, belonging to the larger species *bondari* and *mayri*, we observed one to two, clearly defined, short foraging paths in the immediate vicinities of the nest entrances (Figure 4.3 c), usually radiating out from the entrance. These short paths were from 0.5 to 1 cm wide and were slightly impressed into the surrounding forest floor, and were most easily visible when the nest was surrounded with a mound of freshly excavated soil. There was a large number of openings in the soil mound of one *S. bondari* nest (AJ141001-05) immediately after an afternoon rain (Figure 4.3 d), probably consisting of additional temporary nest openings that were used by workers for faster removal of soil that was washed into the nest during the rain. In previous days, when no strong rains occurred, this nest had a single opening (Figure 4.3 c).



Figure 4.3. Nest entrances of *Sericomymex*. **a)** *S. amabilis* nest mound (AJ131005-01), Mexico; **b)** *S. mayri* (JSC111119-04) nest mound, Guyana; **c)** *S. bondari* (AJ141001-05) nest entrance, Brazil; **d)** *S. bondari* (AJ141001-05) nest entrance after rain, Brazil; **e)** *S. bondari* (AJ120926-02) nest entrance, Brazil; **f)** *S. parvulus* (JCMC001) nest entrances, Brazil; **g)** *S. parvulus* (AJ120728-09) nest entrance, Peru; **h)** *S. parvulus* (AJ120728-09) nest entrance with bait (Cream of Rice) accumulated around it, Peru. White arrow points to the nest entrance hole.

Nest architecture (Figure 4.4, 4.5, & 4.6). Several authors have noted the presence of a vertical, straight tunnel connecting the nest entrance to the uppermost chamber, and Ulrich reports a small antechamber, common in other attine ants as well (Ulrich, 1895; Fernández-Marín *et al.*, 2004; Sosa-Calvo *et al.*, 2015). Historically the shallowest reported nest depth records were 8 cm for both *S. mayri* in Guyana (Weber, 1937) and *S. saussurei* (described at the time as *S. urichi maracas*) in Trinidad and Tobago (Weber, 1937), and the deepest reported nest depth records were 79 cm for *S. cf. scrobifer* in the cerrado of Minas Gerais, Brazil (Leal *et al.*, 2011). The number of nest chambers recorded in the literature for *Sericomyrmex* species varies from 1 to 7 (Table 4.2), including the single species *S. mayri*, reported to encompass the full range of this variation (Ulrich, 1895; Weber, 1969a). The only nest chamber dimensions recorded in the literature are those of *S. mayri*, in which the recorded width varies from 5 to 15 cm and the height from 3 to 12 cm (Ulrich, 1895; Weber, 1937, 1969a), and for *S. saussurei*, for which chambers were recorded as 6 cm wide (Weber, 1937). The only living laboratory nest of *Sericomyrmex* reported in the literature was a single *S. mayri* nest, which produced more than 55,000 individuals during its nearly 11-year life span, including ~2,500 alate females, ~16,300 males, and the rest workers. This colony contained ~1600 individuals when it was collected in the field (Weber, 1967).

In 18 out of 19 nests excavated for this study, the tunnel leading from the nest entrance to the uppermost chamber was either vertical or deviated from vertical by a small angle. The tunnel was sometimes observed while digging (Figure 4.4 e), but more often its position and orientation were inferred based on the location of the nest entrance and the uppermost chamber because the majority of the nests had chambers either more or less directly beneath the nest entrance, or offset by up to 10 cm. In contrast to this general rule, however, in a nest of *S. bondari*

(AJ120926-02) collected in Reserva Ducke (Amazonas, Brazil), one of two nest entrances was horizontally displaced by more than 2 meters from the nest chambers (Figure 4.5 d), whereas a second nest entrance was positioned more or less immediately above the uppermost chamber. In our excavations, an antechamber was found only in one nest of *S. mayri* (AJ141002-01); it was smaller in size than other chambers and did not contain any fungus garden. This was a relatively mature nest, with a large fungus chamber beneath the antechamber, so the antechamber was probably a remnant of the first chamber created by the queen when founding the nest (Fernández-Marín *et al.*, 2004).

Nests of *S. opacus* and *S. saramama* had a single chamber each, nests of *S. amabilis*, *saussurei* and *parvulus* up to 5 chambers, and *S. bondari* nests had 2 to 3 chambers. The number of chambers in *S. mayri* nests varied the most, from 1 to 18, with two large nests collected in Guyana with 10 and 18 chambers.

Nest volume is usually related to body size, so that small species have smaller nests than larger species, even when the number of individuals in a colony is the same (Tschinkel, 2015). Another factor that influences nest volume is the number of workers in the colony; when the body size of an ant is the same, the number of individuals in the colony (nest census) is a good predictor of nest volume (Tschinkel, 2015). Our findings agree with these rules in general. Nest volumes varied from ~200 to ~7000 cm³ (Table 4.3), and the colony census varied from 59 to 6642 individual ants (Table 4.4). We found correlations between colony census and nest volume ($r=0.68$, $p=0.0012$) and between ant size and nest volume ($r=0.61$, $p=0.0051$). Individual chamber size was also correlated with ant size ($r=0.64$, $p=0.0033$) and strongly correlated with total nest size ($r=0.79$, $p=5.362e-05$). The largest species, *S. mayri*, had some of the largest nests, while the small species *S. parvulus* and *opacus* had some of the smallest nests (Table 4.3).

However, exceptions exist; *S. saussurei* had the largest nest volume of all the species collected, even though it didn't have the highest colony census (1565 individuals, in comparison to large *mayri* nests with ~3000 and ~6000 individuals) and even though it is a medium-sized ant (smaller than both *S. bondari* and *S. mayri*). Another exception is *S. amabilis*, which is almost identical in size to *S. saussurei*, and in which colony census is relatively high (550 ants) given its surprisingly small nest volumes (165.7 cm³) (Table 4.3). For example, *S. saramama*, a smaller species with a lower colony census (59 ants), has a larger nest volume (466 cm³). Before drawing any conclusions, however, it is important to note that our sample sizes are generally very low (19 nests) and particularly so for the species *S. saramama*, *S. saussurei* (a single nest for each species), and *S. amabilis* (two nests). Also, for most *Sericomyrmex* species we lack data that span different habitats, which are essential for understanding the environmental factors that influence nest characteristics.

The spacing of underground nest chambers varied within a single nest: neighboring chambers could be very closely separated (1–5 cm) (Figure 4.5 c, e, f; Figure 4.6 a), whereas other neighboring chambers could be separated by 10–30 cm (Figure 4.5 b, g). In some nests, both conditions applied, with clusters of closely spaced chambers separated from other such clusters by 10–20 cm (Figure 4.5 b). This pattern is at least partly explained by heterogeneity of the soil and the presence of large roots. We noticed that if a large tree root was present, chambers were often adjacent to it, incorporating the root to form at least part of the chamber wall (Figure 4.4 c)

In addition to the 19 successful nest excavations reported above, we attempted to excavate an additional seven nests for which we located the nest entrances and followed the tunnel for 1-3 days but ultimately did not locate the chambers. These unsuccessful attempts also

offer some insights. The uppermost chamber of the deepest *Sericomyrmex* nest we excavated was 35 cm below the surface (*S. saussurei* in Minas Gerais, Brazil), and the deepest record known from the literature, for *S. cf. scrobifer*, was 79 cm from the surface (Leal & Oliveira, 2011). During the 3-day excavation of an *S. parvulus* nest in the cerrado habitat of the Panga Ecological Reserve in Minas Gerais, Brazil, we created a pit more than 180 cm deep but still did not reach the chambers. The chambers were certainly deeper rather than displaced laterally because ants were observed emerging from a tunnel opening at the bottom of the pit. Similarly, during two attempted nest excavations of *S. parvulus* in Carajas (Para, Brazil), we excavated to greater than 1 meter in depth without encountering chambers, but observed the entrance tunnel continuing straight down.

Aside from depth, a separate cause of unsuccessful excavations was horizontal displacement of chambers from the nest entrance. In these cases, rather than descending vertically, the tunnel diverged from the nest entrance slightly below and almost parallel to the surface of the ground. We observed such horizontal entrance tunnels in two nests of *S. mayri* in Peru, one nest of *S. amabilis* in Mexico, and one nest of *S. mayri* in Minas Gerais, Brazil. We were invariably able to determine the direction of the chambers (directly below or horizontally displaced) by following the tunnel, or, when the tunnel was temporarily lost, by waiting for the ants to re-excavate the tunnel opening.



Figure 4.4. Subterranean *Sericomymex* nests. **a)** *S. bondari* (AJ120926-02) underground chamber after the fungus garden and ants have been removed; **b)** *S. bondari* (AJ120926-02) chamber immediately after opening, with the fungus garden visible; **c)** chambers of *S. mayri* (JSC111119-04) after garden removal; **d)** *S. mayri* (JSC111119-04) chamber immediately after opening, with the fungus garden visible; **e)** *S. parvulus* (AJ120728-09) carrying bait (Cream of Rice) into its nest (arrow indicates nest entrance); **f)** plastic nest box with part of a *S. amabilis* (AJ131005-01) colony one day after excavation: the fungus garden has been re-assembled and soil particles introduced during excavation have been removed by the ants.

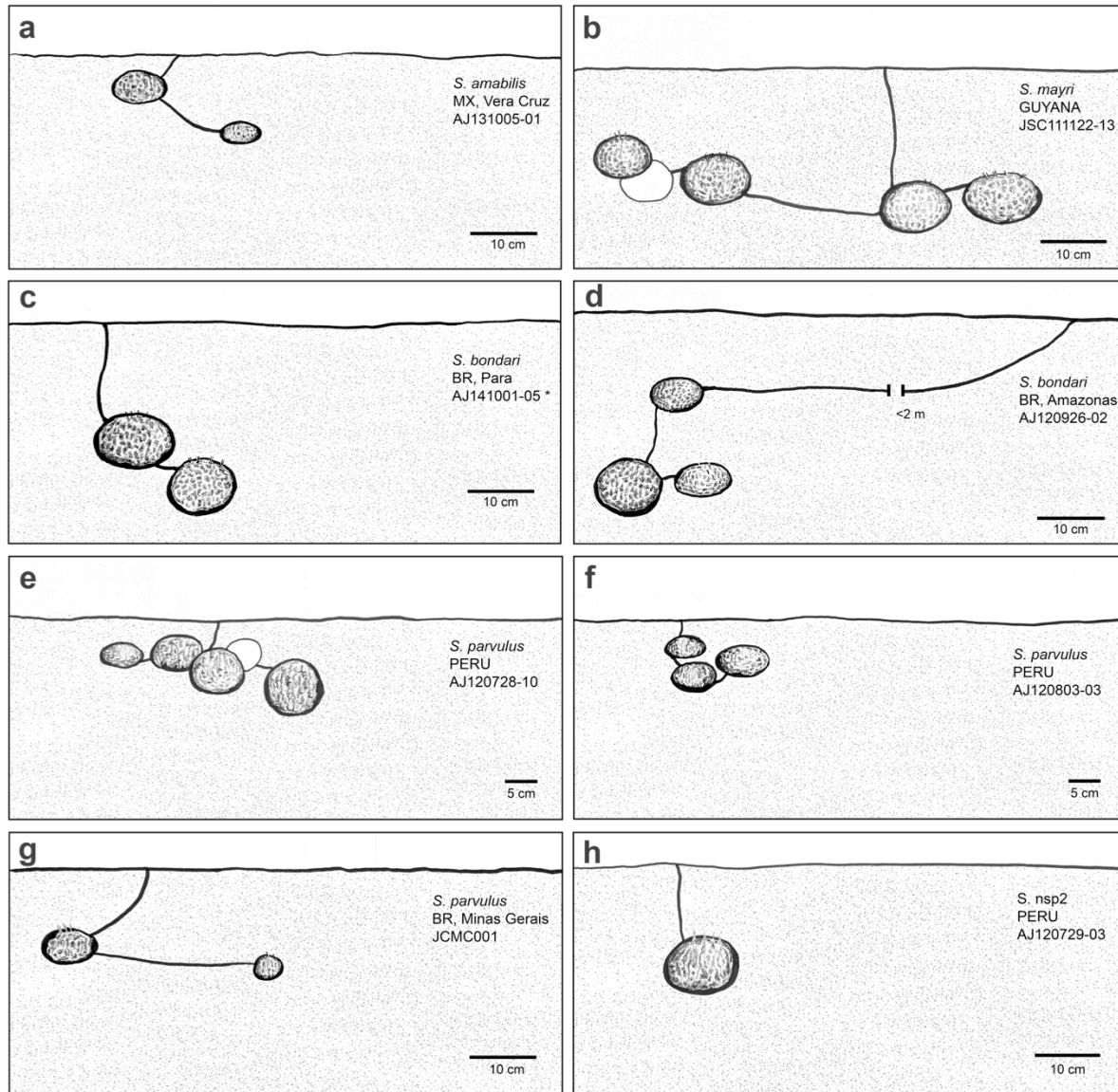


Figure 4.5. Nest drawings 1. **a)** *S. amabilis* (AJ131005-01), Mexico; **b)** *S. mayri* (JSC111122-13), Guyana; **c)** *S. bondari* (AJ141001-05), Brazil; **d)** *S. bondari* (AJ120926-02), Brazil; **e)** *S. parvulus* (AJ120728-10), Peru; **f)** *S. parvulus* (AJ120803-03), Peru; **g)** *S. parvulus* (JCMC001), Brazil; **h)** *S. saramama* (AJ120729-03), Peru. White, unfilled chambers on the drawing indicate chambers that are behind other chambers.

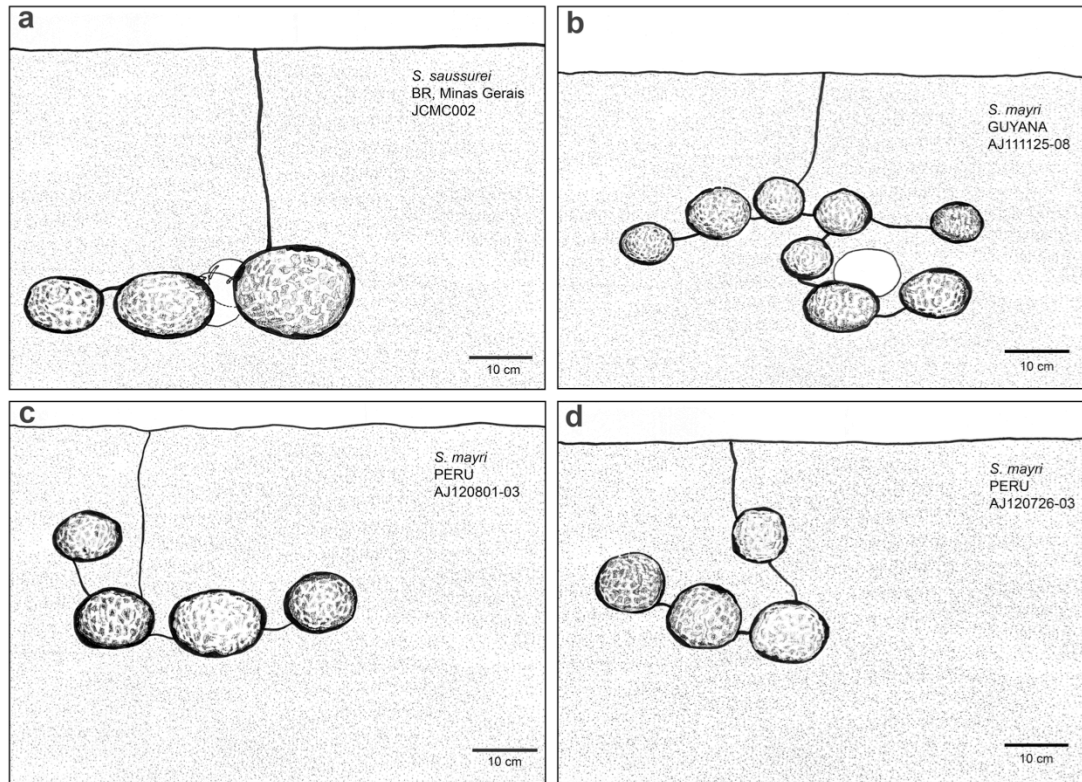


Figure 4.6. Nest drawings 2. **a)** *S. saussurei* (JCMC002), Brazil; **b)** *S. mayri* (AJ111125-08), Guyana; **c)** *S. mayri* (AJ120801-03), Peru; **d)** *S. mayri* (AJ120726-03), Peru. White, unfilled chambers on the drawing indicate chambers that are behind the other chambers.

4.3.4. Fungus gardens

Sericomyrmex fungi belong to the generalized higher attine fungi, a derived clade of obligately symbiotic species in the family Agaricaceae cultivated by *Trachymyrmex* and *Sericomyrmex* species. The majority of *Sericomyrmex* species grows a single, very recently diverged, higher-attine fungal species, the “*amabilis-mayri*” fungus, across the entire geographic range of the genus (Chapter 2, Figure 2.4). In general, *Sericomyrmex* gardens are large, almost always completely filling the chamber (Figure 4.4 b, d). Garden color depends on the substrate collected by ants; in the field the garden is yellow-brown to grey. Most gardens are suspended

from the roof of the chamber by small rootlets, but less frequently they can also be found sessile on the chamber floor (Wheeler, 1925; Leal *et al.*, 2011). If rootlets are not present the fungus can be suspended from rocks in the chamber ceiling (Fernández-Marín *et al.*, 2004). *Sericomyrmex* fungus gardens have a firm texture and in the lab can reach a size of 3 liters (Weber, 1976). Following field collection, fungus gardens are usually broken into fragments and embedded with particles of soil, but once stabilized in a nest container the ants clean and rebuild the garden within a single day (Figure 4.4 f).

4.3.5. Arthropods associated with an external waste midden

We observed an external waste midden outside of a nest of *S. mayri* in primary forest habitat in the Upper Essequibo region of Guyana. Located 50 cm from the nest entrance, the midden consisted mostly of exhausted fungus garden. During 30 minutes of observation, workers visited the midden every one to two minutes. Workers invariably approached the midden from the direction of the nest entrance carrying a fragment of what looked like exhausted fungus substrate in their mandibles, which they deposited on the midden. In addition to exhausted garden, the midden contained two dead *Sericomyrmex* ants as well as many living organisms. A total of 57 organisms were collected from the midden: 41 live Diptera larvae, 12 live Coleoptera larvae, 2 live mites, and 2 dead *Sericomyrmex* workers. The presence of developing larvae in the waste midden indicates that it is a relatively stable feature of the nest and is consistent with previous studies of attine waste midden commensals (Autuori, 1942; Moser, 1963; Navarrete-Heredia, 2001; Sanchez-Pena *et al.*, 2003; Steiner, 2004). Waste disposal is important for colony hygiene (Hart *et al.*, 1998; Bot *et al.*, 2001; Ballari *et al.*, 2007) because exhausted fungus garden becomes contaminated by numerous microorganisms and parasites that can threaten the health of

the growing garden (Bot *et al.*, 2001). Some leaf-cutter ants dispose of waste in specialized underground chambers, whereas others use external refuse dumps, a difference apparently driven by environmental factors (Farji-brener *et al.*, 2016). Prior to this study the only mention of *Sericomyrmex* waste management was made by Weber (1976), who observed *Sericomyrmex mayri* workers to carry their dead, as well as fragments of exhausted fungus garden, to one corner of a captive colony (Weber, 1976). Weber did not, however, report observations of waste middens in nature. External waste has been described in *Trachymyrmex arizonensis* (LaPolla *et al.*, 2002) and *Trachymyrmex sp.* (Hart & Ratnieks, 2001).

4.3.6. Foraging material and behavior

As substrate for the fungus garden, *Sericomyrmex* ants collect mostly fresh plant material consisting of fallen flowers and leaves, less frequently, depending on the season, moss, grass, seeds, seed husks, dry leaves, insect frass, and fruit pulp (Leal & Oliveira, 1998; De Fine Licht & Boomsma, 2010). Cerrado species of *Sericomyrmex* prefer pieces of fallen flowers and fruit during the wet season, and turn to vegetative parts of plants and moss during the dry season, depending on availability (Leal & Oliveira, 2000). *Sericomyrmex* are known to cut leaves and fruit in a manner similar to leaf-cutter ants. They do not climb vegetation like leaf-cutters and they cut more slowly and produce smaller fragments, up to 2 mm in diameter (Weber, 1967, 1969b, 1976; Leal & Oliveira, 2000). When presented with milled barley in a tropical forest in Costa Rica, *Sericomyrmex* workers (either *S. amabilis* or *S. opacus*) removed more than a half of the total bait, suggesting that they were taking it back to the nest as a fungus substrate (Kaspari, 1996). In a study of cerrado attine ants, *Sericomyrmex* visited plant, fruit, and seed baits less frequently than other attine ants in the study, and most of the baits were consumed on the spot

instead of taken to the nest (Leal & Oliveira, 1998). Similarly, in a seed dispersal study of the plant *Parkia panurensis* in Peru, Fieldman and colleagues observed *Parkia* seed predation by *Sericomyrmex* workers, which appeared in groups of 2-6 individuals, chewed on seeds slowly, and did not attempt to transport the seeds (Feldmann *et al.*, 2000).

In most of our field observations *Sericomyrmex* foraged on our bait, Cream of Rice cereal. We observed workers of *S. mayri* and *S. bondari* carrying small flowers, small pieces of fresh leaves, and dry leaves. We did not observe them cutting vegetation. *Sericomyrmex* workers move relatively slowly, and when disturbed they react like most other attine ants: they stop moving, curl into a ball, and feign death. They recruit nestmates when bait (Cream of Rice, fruit) is encountered. They do not form heavily populated foraging columns as in leaf-cutter ants, but *S. mayri* and *S. bondari* form short foraging columns close to the nest entrance. When large amounts of bait were present, we observed *S. parvulus* workers to deposit it around the nest entrance (Figure 4.4 h), probably so that other workers could take it into the nest. Foragers of *Sericomyrmex* cf. *scrobifer* have been recorded up to 7.2 meters away from the nest (Leal & Oliveira, 1998) and they are active during day and night, but peak activity is in afternoon and in the morning there is usually no activity at all. We observed crepuscular and nocturnal activity in *S. mayri*.

In a study of attine trail pheromones that investigated whether attine ants follow the trail pheromones of other attine and non-attine species and genera, it was found that *Sericomyrmex* foragers will not follow artificial trails prepared from glandular extracts of other genera, including the non-fungus-farming *Daceton armigerum* or fungus-farming *Trachymyrmex* and *Atta* species, indicating that their trail pheromones differ from those of the other ants (Blum &

Portocarrero, 1966). In the same study, the *Atta* and *Trachymyrmex* species followed each other's trails, as well as the trails of *Daceton armigerum*, but not those of *Sericomyrmex*.

4.3.7. Association with other ants

At least some *Sericomyrmex* and *Trachymyrmex* species, including *S. amabilis*, can be attacked by the agropredator ant species *Gnamptogenys hartmani* (Dijkstra & Boomsma, 2003), which forces the host ants to retreat from the nest, eats the fungus garden, and, once the garden is consumed, moves on to raid a new nest. The species *Sericomyrmex amabilis* is also the host of the *Megalomyrmex symmetochus*, which has long been known as a social parasite of *Sericomyrmex* (Wheeler, 1925). Interestingly recent studies have shown that *Megalomyrmex* protects *Sericomyrmex* from *Gnamptogenys* ants (Adams *et al.*, 2013). *Megalomyrmex* ants repel and kill *Gnamptogenys* much more effectively than *Sericomyrmex*, because they possess alkaloid venom. Also, *Gnamptogenys* foragers recognize the odor of *Sericomyrmex* colonies that are hosting *Megalomyrmex* and avoid them. This renders *M. symmetochus* colonies a functional soldier caste of a *Sericomyrmex* colony. A *M. symmetochus* colony spends its entire life in a single *Sericomyrmex* colony, so protection of that colony is a shared mutual interest (Adams *et al.*, 2013). Additional details can be found in Wheeler, 1925; Adams *et al.*, 2012, 2013; Bruner *et al.*, 2014; Liberti *et al.*, 2015. In the colonies we collected we did not encounter any of the *Sericomyrmex* ant associates.

4.3.8. Queen numbers

Villesen *et al.* (2002) studied two *Sericomyrmex* species that occur in Panama, *S. amabilis* and *opacus*, and overturned the conclusion of multiple mating (polyandry) previously

proposed by Murakami *et al.* (2000). Instead, they found evidence for single mating with occasional polygyny (Villesen *et al.*, 2002). Single-queen colonies appear to be the general rule in *Sericomyrmex* species, but multiple queens are occasionally encountered. A study of nest founding in attine ants found that 40 out of 44 nests of *S. amabilis* had a single foundress queen, and that 4 nests had more than one queen (Fernández-Marín *et al.*, 2004), while a study of Brazilian cerrado species reports 1-2 queens for *S. scrobifer* (Leal *et al.*, 2011). In 4 out of the 19 colonies we excavated there were 2-4 queens (Table 4.4), while the remainder had a single queen.

4.4. Discussion

We report the nest architecture data for seven species of attine genus *Sericomyrmex*, based on 19 excavated nests and literature data. This is one of the larger data sets on non-leafcutter attine ant nest architecture in terms of number of different species represented, with the *Trachymyrmex* as the only attine ant genus for which nest data exists for more species.

Our data on nesting architecture, together with the data culled from the literature, is in agreement with previous studies with regard to the relationship of nest and colony size (Tschinkel, 2015). *Sericomyrmex* nest volume is correlated both with ant size and colony census, meaning that smaller-bodied species have smaller nest volumes and larger-bodied species have larger nest volumes. Exceptions exist, and not all variations in chamber size and number we observed can be explained by body or nest size, and are likely also influenced by additional ecological and phylogenetic factors, which we are unable to further explore because of the small size of our data set.

The presence of very deep nests, e.g., in *S. parvulus* at two different localities, is intriguing. Nest depth could vary by season, since it is known that at least some species of fungus-farming ants move their nests deeper during the dry season (Weber, 1957; Navarro & Jaffe, 1985; Lapointe *et al.*, 1998). However, in Carajas we excavated three other nests, of *S. mayri* and *S. bondari*, at the same place and at the same time as an *S. parvulus* nest, and they occurred at much shallower depths (14-27 cm). Why did *S. parvulus* have such a deep nest in the same habitat and locality and during the same season in which other *Sericomyrmex* species had shallower nests, especially considering that at other localities we located and excavated *S. parvulus* nests at only 5-20 cm depth? Growing a different species of fungus, that has different ecological requirements, might be a possible explanation, but needs to be tested.

A nest of *S. bondari* had long horizontal tunnels connecting one nest entrance with the uppermost nest chamber almost two meters away, as well as a second nest entrance directly above the chamber. Similarly, leaf-cutter ant species *Atta laevigata* and *A. vollenweideri* have long underground tunnels leading from the nest to openings several meters away, possibly to protect foragers from predators or the sun (Mehdiabadi & Schultz, 2010). Protection from the sun is likely not important in the case of *S. bondari*, since this is a forest-dwelling species, but protection from predators is one possible explanation. If a predator were to attack the main nest entrance, an additional nest entrance some distance removed from the attacked entrance could offer an alternative escape route. Predator-avoidance behavior is well documented as a response to above-ground and underground army ant attacks in many ants, including attine ants, and it usually involves nest evacuation and frequent nest moving (Fowler, 1977; Topoff *et al.*, 1980; LaPolla *et al.*, 2002; Smith & Haight, 2008; Souza & Moura, 2008). Also, army ant could potentially explain uneven local distribution of *Sericomyrmex* nests we observed, and which is

also known from literature (Weber, 1972b). If *Sericomyrmex* queens do not display any preference of one site over other and found colonies relatively evenly across the area, localized army ant predation could eradicate some nests, thus creating the patchy distribution we observed in seemingly ecologically homogeneous habitats.

Interestingly, in most cases in which we encountered nests with two entrances, such as Amazon *S. bondari* nest, we also observed and in some cases excavated nests of the same species at that locality, sometimes only 10-20 meters away, with a single typical entrance located more or less directly above the uppermost chamber. This polymorphism in nest architecture within the same species could be caused by microhabitat differences that we were not able to detect. It is also possible that the presence of a second tunnel and a second entrance is more common than our data suggest, but that we did not always notice the second entrance. Such features as chamber depths and the presence or absence of horizontal entrance tunnels may be influenced by multiple environmental factors. Future studies should collect ecological data, include data for multiple nests of the same species from different habitats, and collect nests during both dry and wet seasons.

Our nesting biology results indicate *Sericomyrmex* is a good candidate for a more detailed study of nest architecture in an evolutionary ecology framework. *Sericomyrmex* is a moderately speciose ant genus, containing 11 closely related species, with broad distributions across multiple habitats. Nests differ in depth, position with regard to the nest entrance, and morphology, and colony size varies from several hundred to almost 7,000 ants. Having a resolved taxonomy and phylogeny means that comparative nest architecture study across different environments, would allow for the differentiation of environmental and genetic factors. We hope that this study will encourage further research on *Sericomyrmex* nest architecture.

Appendix

The Chapter One of this thesis was published in Plos One, the Chapter Two in Systematic Entomology, and the Chapter Three has been submitted to ZooKeys. Chapter Four is in preparation, but will also be submitted to a scientific journal.

References for the first three chapters:

(Ch. 1) Ješovnik A, Sosa-Calvo J, Lloyd M, Branstetter M, Fernandez F & Schultz TR (2016): Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera: Formicidae): Ultraconserved elements (UCEs) resolve a recent radiation. *Systematic Entomology*. doi: 10.1111/syen.12228

(Ch. 2) Ješovnik A, González VL & Schultz TR (2016): Phylogenomics and divergence dating of fungus-farming ants (Hymenoptera: Formicidae) of the genera *Sericomyrmex* and *Apterostigma*. *PLoS One* 11(7): e0151059.

(Ch. 3) Ješovnik A & Schultz TR: Revision of the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera, Formicidae, Myrmicinae). *ZooKeys*. Submitted.

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