Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus Sericomymrex Mayr (Hymenoptera: Formicidae): ultraconserved elements (UCEs) resolve a recent radiation

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Abstract. Ants in the Neotropical genus Sericomymrex Mayr cultivate fungi for food. Both ants and fungi are obligate, coevolved symbionts. The taxonomy of Sericomymrex is problematic because the morphology of the worker caste is generally homogeneous across all of the species within the genus, species limits are vague, and the relationships between them are unknown. We used ultraconserved elements (UCEs) as genome-scale markers to reconstruct evolutionary history and to infer species boundaries in Sericomymrex. We recovered an average of ~990 UCE loci for 88 Sericomymrex samples from across the geographical range of the genus as well as for five outgroup taxa. Using maximum likelihood and species-tree approaches, we recovered nearly identical topologies across datasets with 50–95% matrix completeness. We identify nine species-level lineages in Sericomymrex, including two new species. This is less than the previously described 19 species, even accounting for two species for which we had no UCE samples, which brings the total number of Sericomymrex species to 11. Divergence-dating analyses recovered 4.3 Ma as the crown-group age estimates for Sericomymrex, indicating a recent, rapid radiation. We also sequenced mitochondrial cytochrome oxidase subunit I (COI) for 125 specimens. Resolution and support for clades in our COI phylogeny are weak, indicating that COI is not an appropriate species-delimitation tool. However, taxa within species consistently cluster together, suggesting that COI is useful as a species identification (‘DNA barcoding’) tool. We also sequenced internal transcribed spacer (ITS) and large subunit (LSU) for 32 Sericomymrex fungal cultivars. The fungal phylogeny confirms that Sericomymrex fungi are generalized higher-attine cultivars, interspersed with Trachymyrmex-associated fungal species, indicating cultivar sharing and horizontal transfer between these two genera. Our results indicate that UCEs offer immense potential for delimiting and resolving relationships of problematic, recently diverged species.

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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 approach to taxonomy advocates the combination of evidence from several different sources: molecular, ecological, chemical and/or behavioural, 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 when other approaches fail.

The genus Sericomyrmex Mayr is one such problematic ant taxon (Wheeler, 1925; Ješovník et al., 2016). It belongs to the fungus-farming ants (Formicidae: Myrmicinae: Attini: Atta genus group, hereafter referred to as 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 (Mehlisbadi & 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 three 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, and from sea level to over 1000 m in elevation (Mayhe-Nunes & Jaffe, 1998; Fernández & Sendoya, 2004; Bolton, 2014). Surprisingly, this species-level diversity and geographic coverage are 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, brown and covered with a dense layer of hairs, giving them a velvety appearance. Differences between most species are subtle and are obscured by sometimes marked intraspecific and intracolonial 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 transcripts found strong evidence that Sericomyrmex species are probably the product of a very recent radiation and that they are separated from their nearest relatives, the Trachymyrmex iheringi clade, by a long branch (Ješovník et al., 2016). However, a more recent molecular phylogeny of the attine ants reveals that the sister of Sericomyrmex is Mycetosoritis explicantus Kempf (M.G. Branstetter et al., unpublished data), an enigmatic species considered by some authors to be a lower attine (Forel, 1912; Kempf, 1968; Weber, 1972), and by others as a higher attine (Sosa-Calvo et al., 2009).

Because variations in both morphology and nuclear gene sequence data (Ješovník et al., 2016) are very low across Sericomyrmex species – probably 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, 2016a). Here we use UCEs to reconstruct the evolutionary history of Sericomyrmex and estimate its divergence times, with five outgroup and 88 ingroup samples from across the geographical range of the genus (Fig. 1). Among other purposes, this phylogeny serves as the foundation for a species-level taxonomic revision of Sericomyrmex (A. Ješovník & T.R. Schultz, unpublished data). In addition to UCEs, we sequenced two fragments of the mitochondrial cytochrome oxidase subunit I gene (COI) for most UCE samples plus an additional 38 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 expensive relative to Sanger sequencing techniques. This is especially true for researchers in developing countries where Sericomyrmex occurs, including those conducting ecological and conservation studies. Despite its well-known shortcomings (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 (Ma) (Schultz & Brady, 2008; Ward Published 2017. This article is a U.S. Government work and is in the public domain in the USA. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 42, 523–542
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Fig. 1. Collection localities. Localities from which Sericomymex ants (white circles) and fungi (black triangles) were collected. [Colour figure can be viewed at wileyonlinelibrary.com].

et al., 2015, M.G. Branstetter et al., unpublished data), 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 the attine symbiosis. The cultivation of facultatively symbiotic fungi, practised 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; Mehdiahabi & 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 Sericomymex 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 Sericomymex species spanning the geographical range of the genus, as is done here. Our investigation of Sericomymex fungi was guided by two aims. First, we wanted to determine whether the radiation of Sericomymex ants 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 Sericomymex 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).

Materials and methods

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 1 illustrates collecting localities for all Sericomyrmex ant and fungal specimens used in this study. Table S1 lists all ant specimens used in this study, including collecting information, voucher numbers and GenBank accession numbers. Voucher specimens are deposited in 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) and on AntWeb (http://www.antweb.org). Table S2 contains the same information for fungal specimens. Methods for locating and excavating Sericomyrmex nests in the field follow 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 Fig. 1 was prepared with ARCGIS v10.3.1 software (Esri, Redlands, CA) using a basemap from the U.S. National Park Service.

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 (M.G. Branstetter et al., unpublished data). These included the newly identified sister species of Sericomyrmex, M. expilcatus, and four species from the Trachymyrmex iheringi clade, the large sister clade of Sericomyrmex + M. expilcatus. 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.

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., U.S.A.

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, Valencia, CA, U.S.A.) 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 bp) and Ben-Jerry (~380 bp), was attempted for all specimens with PCR Master Mix (Go Taq and Hot Start kits; Promega, Madison, WI). We ran 15 µL PCR reactions with 1 µL of DNA extract with several different protocols (Table S3), but mostly with a 48°C annealing temperature. Out of 135 extractions performed, ten failed to amplify or sequence due to DNA degradation, and thus the COI dataset 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 min 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, Bartlesville, OK, U.S.A.). We submerged the tubes in liquid nitrogen for 3 min, and then put them into the TissueLyser (Qiagen) for 1 min to ensure tissue disruption. After tissue disruption we extracted DNA from each sample manually using the Plant DNeasy kit (Qiagen) following the standard manufacturer protocol. For the final elutions we used 100 µL of elution buffer. We quantified DNA concentrations for all extracts with a Qubit 2.0 fluorometer high sensitivity kit (Life Technologies, Rockville, MD, U.S.A.). 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 µL of the DNA extract with 15 µL of ddH2O (1:1 dilution); each subsequent dilution was prepared by diluting 15 µL of the previous dilution with another 15 µL of ddH2O. This was repeated three 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 and 0.1 ng/µL. For PCR reactions we used a PCR Master Mix (Hot Start kit). For samples that repeatedly did not amplify, we ran 10 µL of DNA extract on a low-concentration agarose gel (60 min, 60 V) 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 a generic SPRI substitute Sera-Mag Magnetic SpeedBeads (GE Healthcare, Chicago, IL, U.S.A.), hereafter ‘speedbeads’ (Fisher et al., 2011; Rohland & Reich, 2012), to remove possible PCR inhibitors (see File S1 for detailed protocol).

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 DNA. 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 S3).

We succeeded in amplifying fungal DNA from six out of 64 ant extractions. For two of those six 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.

### Laboratory methods for UCEs

#### Extraction and library preparation.
We destructively extracted ants as described earlier, 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 cations. For the final DNA elution step, we used 130 extracted ants as described earlier, except for a few modifications.

Laboratory methods for UCEs

We used a Kapa Hyper Prep Library Kit (Kapa Biosystems, Wilmington, MA, U.S.A.) for the remaining 66 Sericomyrmex samples, sequenced subsequently, we used a Kapa Hyper Prep Library Kit (Kapa Biosystems, Wilmington, MA, U.S.A.). All ‘with-bead’ clean-up steps were performed using a generic SPRI substitute (Fisher et al., 2011), ‘speedbeads’ (Fisher 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 (Kapa Biosystems, Wilmington, MA, U.S.A.), 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 s, 10–13 cycles of 98°C for 15 s, 65°C for 30 s, 72°C for 60 s, and 72°C for 5 min.

#### Enrichment verification and size selection.
We tested our enrichment by amplifying seven UCE loci with relative quantitative PCR (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 (Kapa 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, U.S.A.). Size-selected library pools were sent to either the UCLA Neuroscience Genomics Core or Cornell’s Institute of Biotechnology and sequenced as three partial lanes (each of the lanes included other samples) on an Illumina HiSeq 2500 (2 × 150 rapid run).

### Phylogenomic 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

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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 125 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 58 ITS and 25 LSU sequences downloaded from GenBank, consisting of both Sericomyrmex and non-Sericomyrmex fungal cultivars (Table S4). Numbers of taxa and other information for each dataset are summarized in Table S1. Alignments were generated with MAFFT v1.3.5 (Katoh & 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 dataset we used PARTITIONFINDER v1.1.1 (Lanfear et al., 2012) and for all three fungal datasets we used PARTITIONFINDER 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 datasets, 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 dataset 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 dataset and for each analysis can be found in supplemental material (File S2). We conducted simultaneous best-tree and rapid bootstrapping (1000 pseudoreplicates; --f 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 datasets using MRBAYES v3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For each dataset we performed two runs (four chains per run) of 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., 2014). The number of generations and other computational details for each mrbayes run are listed in supplemental material (File S2). 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Following trimming, the data were processed through a series of scripts available in the phy Luca 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 (assemble_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 datasets (summarized in Table 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% dataset, 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 dataset (get_align_summary_data.py).

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) which 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, personal communication), most probably 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 hecluster search algorithm on the 90% complete dataset. We conducted phylogenetic analyses of all UCE datasets under the 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 v8.0.3 (also using the simultaneous best-tree search and rapid bootstrapping analysis): of an unpartitioned dataset (100 bootstrap replicates), of a dataset partitioned with UCE loci defined as data blocks (100 bootstrap replicates), and of a kmeans-partitioned dataset (500 bootstrap replicates).

Datasets and some of the analysis details are summarized in Table 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 v8.0.3 (simultaneous best-tree and rapid bootstrap search, 200 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., 2014b), 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 earlier for the non-binned ASTRAL analysis.

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

To explore the effect of taxa with long branches, especially as some of them had lower coverages and read counts, we also created two reduced datasets, one (Ex1) from which Sericomyrmex opacus 1614 was excluded, and one (Ex2) from which Sericomyrmex opacus 1614, Sericomyrmex mayri 1555 and Sericomyrmex amabilis VE 1559 were excluded. We created these datasets 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 datasets we used only the 90% level of taxon filtering.

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 datasets, each with the same reduced numbers of taxa and UCE loci. In order to estimate species divergence dates using the Yule prior, in all datasets we reduced the number of taxa to a single representative per species, so that the dataset included 15 taxa (10 ingroup and five 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 dataset consisting only of the 100 UCE loci with the highest bootstrap support (dataset bs_best_15T) from our 90% complete dataset. We used a reduced number of loci because BEAST analyses are
computationally demanding and larger datasets take intractably long periods of time to converge. To ensure that this subsampling of loci did not bias our results, we created three additional datasets consisting of 100 UCE loci (datasets 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 (M.G. Branstetter et al., unpublished data) because no fossils exist for Sericomymrex or the outgroups included in this study, whereas M.G. Branstetter et al. (unpublished data) 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 (HPDs) of M.G. Branstetter et al. (unpublished data) (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 datasets using unperturbed data because of the much longer analysis times required for partitioned datasets 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 dataset 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 (ulcl.mean: alpha = 0.001, beta = 1000), with other settings at default, in all of our BEAST v2.4.1 analyses. For each dataset 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) 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 two independent runs for each of the four datasets consisting of 1 billion generations with log and tree sampling frequencies of 5000 generations. We also set up two independent runs for the partitioned bs_best dataset, with 500 million generations and with log and tree sampling frequencies of 1000 generations. For each dataset, two independent log files were combined in LOGCOMBINE v.2.4.1 (Bouckaert et al., 2014). Examination of the combined log files in TRACER v1.6 (Rambaut et al., 2014) 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 dataset 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 Sericomymrex 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 $t_0(lnN_t - lnN_0)$, where $t_0$ is the time to a single common ancestor in millions of years, $N_t$ is a number of lineages, and $N_0$ the number of original species, which is one in monophyletic lineages (Coyne & Orr, 2004).

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 datasets excluding outgroups and a phylogeny of the ant UCE loci, including only those ants that had associated fungi in the fungal phylogeny (dataset Ex3; Table 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 Sericomymrex-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 dataset with only 30 taxa, excluding the two associations with non-matching localities. Lists of taxa in both datasets can be found in Table S6. Analyses consisted of 999 permutations with the cailliez correction (Legendre & Legendre, 1998; Legendre et al., 2002).

Results

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 (497–1054). Table 2 summarizes statistics for sequencing and pipeline processing (full statistics for each taxon are presented in Table S7). 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 datasets ranged from 100 to 1062. The most complete dataset, the 95% complete concatenated matrix, included 530 UCE loci.
and another new species, distinct populations or as fully-fledged species. For morphological species-level clades could be interpreted as geographically gated with respect to geography and morphology. For geographical species-level clade in the UCE-based phylogeny was investigated to see if they were unable to include in the phylogeny, S. lutzi 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 datasets (Fig. 2, Figures S1 and 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, S. mayri 1555 TT from Trinidad and Tobago, which occupies differing positions in phylogenies produced by different datasets 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 dataset 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 artefact 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 earlier, the UCE-based phylogenetic results are inconclusive. The phylogenetic position and species-level status of S. mayri in Trinidad certainly deserves further research; we were able to include only a single nest series in this study.

Table 2. Ultraconserved element (UCE) sequencing statistics.

|                 | DNA concentration of extract (ng/µL) | DNA concentration post-PCR library (ng/µL) | Raw read count | Contig number after trimming | Contigs coverage | Contig mean length | UCE loci coverage | Sequence coverage | Mean UCI loci length (bp) |
|----------------|-------------------------------------|--------------------------------------------|---------------|------------------------------|------------------|-------------------|-------------------|-------------------|-------------------------|
| Average        | 3.87                                | 79                                         | 1 423 497.91  | 15 352.15                    | 19.94            | 398.4             | 989.23            | 72.78             | 923.15                  |
| Minimum        | 0.619                               | 17                                         | 299 485       | 4201                         | 9.2              | 273.2             | 497               | 9.04              | 284.8                   |
| Maximum        | 50                                  | 136                                        | 3 500 409     | 71 228                       | 31.7             | 768.4             | 1054              | 191.83            | 1218.4                  |
| Standard deviation | 5.3                             | 23.98                                      | 594 466.6     | 11 971.71                    | 3.64             | 78.31             | 59.75             | 29.62             | 133.25                  |

Mean, standard deviation, minimum and maximum sequencing and assembly statistics averaged for all 93 taxa in the ultraconserved element (UCE) dataset. For more detailed statistics per taxon, see supplemental material Table S6.
Fig. 2. Ultraconserved element (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 (numbers following species names) and country codes as follows: BR, Brazil; CO, Colombia; CR, Costa Rica; HN, Honduras; EC, Ecuador; 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) coloured boxes.

In addition to *S. mayri* 1555 TT, we identified two other taxa as potentially problematic. *Sericomymex opacus* 1614 CO has very low sequence quality (Table S7) and occupies a relatively long branch. *S. 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 analysed two data subsets with these taxa excluded, Ex1 and Ex2 (Table 1). ML analyses of datasets 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. 1), with similarly high support values, indicating that the inclusion of these taxa does not have a negative effect on tree topology.

Divergence dating

All 10 BEAST v2.4.1 analyses, two for each of four different datasets and two for the partitioned dataset, recovered similar divergence dates (Table 3, Fig. 3). The dates recovered by the partitioned and unpartitioned analyses of the bs_best dataset were nearly identical (Table 3). The bs_best and random1 datasets recovered the same topology as the larger UCE datasets with the full number of taxa and the greater number of loci. The other two random datasets, random2 and random3, recovered topologies in which relationships within the amabilis clade were different, and were also different from each other (Figure S2). 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 datasets 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 datasets (Table 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) Ma, respectively (Fig. 4). These dates, especially the stem-date estimate, are younger than mean estimates in a number of studies (all four datasets). The mean stem-date estimate, are younger than mean estimates in a number of studies (all four datasets).

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CI ant phylogeny

We generated COI sequence data for 125 Sericomyrmex specimens in order to: (i) inform taxon selection for the UCE dataset; (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 dataset consisted of 150 taxa and 885 bp of aligned sequence data (Table 1). Bayesian and ML 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 S3). The exceptions are S. mayri

### Table 3. Divergence date estimates.

| Node                          | Average (all four datasets) | bs best 15T partitioned | random 1 | random 2 | random 3 |
|-------------------------------|-----------------------------|-------------------------|----------|----------|----------|
| Sericomyrmex 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) |
| Sericomyrmex 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) |
| Sericomyrmex amabilis         | 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) |
| bondari 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) |
| mayri + bondari 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) |
| parvulus crown                | 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)  |
| opacus 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           |
| S. nsp 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) |
| scrobifer + nsp 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) |

Divergence dates estimated in BEAST analyses.
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 S3). The uniqueness of the COI sequence corroborates our morphology-based conclusion that it represents a new species. Even though this sequence does not contain any stop codons, it is also possible that, due to degraded DNA, we sequenced a nuclear pseudogene of mitochondrial origin (numt) instead of the targeted fragment of COI (Kronauer et al., 2007; Martins et al., 2007; Cristiano et al., 2014). In either case, we currently lack the molecular data for confidently estimating the phylogenetic position of S. n. sp. 3.

**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 four from ant DNA extracts) associated with six different Sericomyrmex ant species and combined them with 58 ITS and 25 LSU sequences downloaded from GenBank (Table S4). Fungal phylogenies resulting from analyses of three different datasets (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 dataset resulted in higher support values than those based on LSU-only datasets, 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 dataset is probably 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) (Fig. 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).
Fig. 4. Fungal phylogeny. The maximum-likelihood phylogeny of the ITS+LSU data set. Black circles at 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 Lepiotaecae. Names of sequences generated for this study follow the color scheme of Fig. 2 and have a fungal extraction number of the form “fun123” following the taxon name, whereas sequences downloaded from GenBank are listed in black and 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.

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 (Fig. 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

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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–fungus association dataset (32 ant–fungus associations) (Table 56). However, when the dataset was reduced to 30 ant–fungus 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).

**Discussion**

**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 taxonomic structure due to the availability key's being outdated and incomplete, and, with regard to morphological variation, most species look similar. 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 (A. Ješovník & T.R. Schultz, unpublished data). 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 S. 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 their 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, this is a large number of species and a high rate of speciation given the very short time since the origin of the genus. The crown-group age estimate for the entire genus Sericomyrmex is recovered as 4.3 Ma. For comparison, sister-species pairs in the Cyphomyrmex wheeleri group are estimated to have diverged 5.3–7.0 Ma ( Mehdiabadi et al., 2012) and the crown-age estimate for the species pair Mycetophylax simplex and M. conformis is 6.6 Ma (Cardoso et al., 2014). So in the time in which comparable groups gave rise to two species, Sericomyrmex gave rise to 11 or 12. The crown-age estimate for the lower-attine species Mycocepurus goeldi and its social parasite M. castrator (2.04 Ma) (Rabeling et al., 2014) is similar to those of within-Sericomyrmex species divergences (2.3–4 Ma). However, in the case of Mycocepurus, this recent divergence is attributed to its social-parasite biology because social parasites are known to have faster evolutionary rates than non-parasite ant species (Bromham & Leys, 2005; Jansen et al., 2010; Rabeling et al., 2014). The 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 Ma; Kambysellis et al., 1995) 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 the common ancestor shared with M. explicatus from the most recent common ancestor of extant Sericomymnex species (Fig. 3), but that, following the origin of the novel Sericomymnex habitus, morphological evolution slowed down dramatically. One possible explanation for this rapid burst of phenotypic evolution at the genus origin is genome duplication, because Sericomymnex 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 suggest that chromosome evolution is correlated with genus and species diversification in ants (Lorite & Palomeque, 2010; Cardoso et al., 2014).

Another distinguishing trait of Sericomymnex 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 Sericomymnex certainly had such bacterial films (e.g. they are present in M. 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).

Diffuse coevolution with generalized higher-attine fungi

Sericomymnex 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 Masuilions et al. (2014) for gongylidia-like structures in a lower-attine ant]. Our results indicate that Sericomymnex-associated fungi are typical higher-attine ant fungi, a clade of multiple fungal species cultivated interchangeably between species of Sericomymnex and Trachymyrmex. We find no evidence of tight (i.e. species-to-species) coevolution between Sericomymnex ants and their fungi. Rather, our fungal phylogeny indicates the horizontal exchange of cultivars between Sericomymnex and Trachymyrmex ant species (Fig. 4, Figure S4). However, associations between ants and their fungal cultivars do not appear to be entirely random. Most Sericomymnex and some Trachymyrmex species and populations in our study grow a single fungal species, which we refer to as the amabilis-mayri fungus species. This species may 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 Sericomymnex and Trachymyrmex that are most ecologically successful are consistently associated with the same, presumably more productive, fungal species.

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 Sericomymnex. 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 nine Sericomymnex species with strong support. In comparison, the COI phylogeny recovered six of those species, but failed to recover S. bondari, S. mayri and S. opacus. The obvious advantage of COI compared with phylogenomic markers is its affordability, but the disadvantage is that, judging by its success rate in Sericomymnex, it is inaccurate a significant proportion of the time, probably 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 for delimiting species, 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

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that COI performs well, i.e. with the exception of S. n. sp. 3, COI-based species identifications referencing our archived COI sequence data (KY202274–KY202398) are expected to be accurate (Figure S3).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12228

Figure S1. Additional UCE phylogenies. Phylogenies from all datasets: 50, 70, 80, 90—unpartitioned, 90%–UCE—partitioned, 95%, bs_best_all, ASTRAL analysis of individual UCE loci and of binned UCE loci.

Figure S2. BEAST phylogenies for each dataset (bs_best, bs_best partitioned, random 1, 2, and 3).

Figure S3. COI ant phylogeny. Maximum likelihood tree from RAxML and MrBayes analyses.

Figure S4. Associated ant and fungal phylogenies. The maximum likelihood phylogenies of the reduced ant UCE dataset, including only ants for which associated fungi were available, and of the fungal ITS + LSU dataset, including only Sericomyrmex fungi.

Table S1. List of ant specimens. Collecting information, extraction codes, voucher specimen numbers, and GenBank and SRA accession numbers.

Table S2. List of fungal specimens. Collecting information, extraction codes, voucher specimen numbers, and GenBank accession numbers.

Table S3. Primers and PCR protocols. Primers and protocols used for Sanger sequencing of ant COI and fungal ITS and LSU.

Table S4. List of sequences downloaded from GenBank. Ant COI and fungal ITS and LSU, with locality information when available.

Table S5. Calibrations for ant divergence dating in BEAST.

Table S6. Ant–fungus association table with global and individual PARAFIT results, per taxon, for both PARAFIT runs. Grey-highlighted cells indicate a significant result at $P < 0.05$.

Table S7. UCE sequencing statistics for all taxa.

File S1. Speedbeads cleaning protocol.

File S2. Single-gene analyses details.

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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), also listed in Table S1.

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