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Symbiont fidelity and the origin of species in fungus-growing ants

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A major problem in evolutionary biology is explaining the success of mutualism. Solving this problem requires understanding the level of fidelity between interacting partners. Recent studies have proposed that fungus-growing ants and their fungal cultivars are the products of 'diffuse' coevolution, in which single ant and fungal species are not exclusive to one another. Here we show for ants and associated fungi in the *Cyphomyrmex wheeleri* species group that each ant species has been exclusively associated with a single fungal cultivar 'species' for millions of years, even though alternative cultivars are readily available, and that rare shifts to new cultivars are associated with ant speciation. Such long-term partner fidelity may have facilitated 'tight' ant-fungus coevolution, and shifts to new fungal cultivars may have had a role in the origin of new ant species.

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Mutualisms occur at all biological levels—from mitochondria within eukaryotic cells to a myriad of species interactions—and they contribute significantly towards increasing complexity in the evolution of life^{1–5}. The long-term stability and success of mutualisms present a challenge for evolutionary theory, for how can such cooperative associations persist, much less proliferate, when selection favours selfishness and conflict^{4,6,7}?

Answering this fundamental question requires understanding of the level of fidelity between interacting partners. Mutualists can be tightly integrated, resulting in coadaptation and cospeciation (that is, speciation in one partner results in simultaneous speciation in the other), or ‘diffusely’ associated because of frequent host switching. Diffuse coevolution has been proposed to occur in a variety of mutualisms, such as those between figs and fig-wasp pollinators^{4,8,9}, squids and their bioluminescent bacterial symbionts¹⁰, and fungus-growing insects and their cultivated fungi^{11–17}.

All of the 235 described species of fungus-growing ants (tribe Attini) obligately depend on the cultivation of fungus for food¹⁸. In return, the ants nourish, protect and disperse their fungal cultivars¹⁸. Although all attine ants are obligate symbionts, the cultivated fungi vary from facultative to obligate in their associations with ants. In ‘lower’ attine fungiculture, the ancestral fungicultural system for attine ants^{12,19,20}, fungal cultivars belong to two distinct, separate lineages (‘Clade 1’ and ‘Clade 2’) within the tribe Leucocoprineae. Lower-attine fungi are thought to facultatively associate with ants because the fungi can also live outside the symbiosis^{12,21}. In ‘higher’ attine fungiculture, however, a highly derived group of fungi descended from a lower-attine fungal ancestor is obligately associated with ants and, as far as is known, cannot live outside the symbiosis^{13,14,16,17,22,23}.

Because cultivar clones are typically propagated vertically from parent to offspring nests via transport by foundress queens, it had been long assumed that the two mutualistic partners evolve by strict coevolution (that is, a fungal species evolves in close association with its host ant species)^{19,24}. However, empirical work over the past two decades has shown that cultivars are frequently transmitted horizontally between different ant colonies (sometimes between colonies of different ant species) and that lower-attine cultivars are closely related to, and in some cases identical to, free-living fungal species, suggesting frequent import of fungi into the symbiosis from free-living sources^{12–14,17,21,23,25–29}. This apparent absence of one-to-one host fidelity (that is, long-term association of ant and fungal genotypes), combined with the recent discovery that the lower-attine fungal Clade 2 (Fig. 1) may be evolutionarily much younger than its ant hosts¹⁶, has led to a general expectation of low partner fidelity (that is, frequent reassociation of ant and fungal genotypes) in lower-attine fungiculture.

Because of the difficulty of obtaining large samples of both ants and fungi from the same nests across the full geographic range of a species, most previous work on the fidelity of association of attine ants and their fungi involved reconstructing phylogenies of fungi but not of their ant hosts, or of ants and fungi drawn from limited localities^{12,14,21,26}. A complete understanding of the shared evolutionary history of attine ants and their associated fungi, however, requires detailed understanding of the phylogenies of both interacting partners across their geographic ranges³⁰, preferably ant–fungus pairs sampled from the same nests.

It has long been known that two lower attine species in Panama, *Cyphomyrmex longiscapus* and *C. muelleri*, cultivate phylogenetically distantly related fungi from Clades 1 and 2, respectively (Fig. 1), even though nests of the two species occur in sympatry and, indeed, may be separated by only a few centimetres in some localities^{12,26,31–33}. Based on evidence from morphology and biogeography, it was previously hypothesized that *C. muelleri* may be derived from an ancestor resembling *C. longiscapus* s.l. and that the origin of *C. muelleri* may have coincided with, or even have been driven by, a switch from the

Clade 1 to Clade 2 cultivar³¹. The younger evolutionary age of Clade 2 relative to Clade 1¹⁶ is consistent with this scenario, as is experimental work demonstrating that Clade 2-cultivating Panamanian *C. muelleri* ants accept the cultivar of *C. longiscapus*, whereas *C. longiscapus* ants do not readily accept the cultivar of *C. muelleri*^{33,34}, reflecting an asymmetric ancestral/derived host-tolerance pattern also known from insect–plant coevolution³⁵.

These prior observations on the *C. longiscapus* and *C. muelleri* symbioses prompted the present investigation of the co-phylogenetic patterns between ants and fungi of these species and their close relatives in the *Cyphomyrmex wheeleri* group. Here, we reconstruct the relationships of 138 individual ants and 405 fungus-cultivar samples, including ants and fungi taken from the same 88 nests of members of the ‘lower’ attine *C. wheeleri* species group (currently consisting of the four species *C. wheeleri*, *C. costatus*, *C. longiscapus* and *C. muelleri*) drawn from their known ranges in North, Central and South America. Contrary to expectation, we found that each ant species has been exclusively associated with a single fungal cultivar ‘species’ for millions of years—even though alternative cultivars are readily available—and that rare shifts to new cultivars are associated with ant speciation. These results strongly support a pattern of ant fidelity to particular fungi in this clade of fungus-growing ants.

Results

Global phylogeny of the fungal tribe Leucocoprineae. Consistent with previous studies^{12,21}, our ITS-based phylogeny of the fungal tribe Leucocoprineae (Fig. 1) indicates that associations of fungi with ants have arisen repeatedly, that particular leucocoprineaceous species may be both ant-associated and free-living, and that ant-associated fungi are non-randomly distributed in the phylogeny and concentrated within the ‘Clade 1’ and ‘Clade 2’ fungus groups (T-PTP test: $n_{\text{global}} = 271$, $n_{\text{Clade 1}} = 138$, $n_{\text{Clade 2}} = 69$; $P < 0.001$).

Clade 1 and Clade 2 fungal cultivar phylogenies. Within ‘Clade 1’ and ‘Clade 2’, cultivar associations with *C. wheeleri*-group ants are concentrated within particular, narrowly defined lineages (Fig. 2a and b). For example, although cultivar clade B (Fig. 2a) includes fungi associated with seven observed fungus-growing ant species, a derived lineage within this clade (darker coloured box) accounts for all associations with *Cyphomyrmex cf. longiscapus* n. sp. 2 and, within this derived lineage, a single genotype (that is, a single ITS sequence-identical strain) (red arrow), occurring in Ecuador, Panama and Costa Rica, accounts for 64 of 70 such associations. Likewise, although cultivar clade D (Fig. 2a) includes fungi associated with three observed ant species, a derived lineage within that clade (darker coloured box) accounts for 18 of a total of 20 observed associations with *C. longiscapus*. Finally, cultivar clade G (Fig. 2b) is associated with five observed ant species, but a derived lineage within that clade (darker coloured box) accounts for all observed associations with just two species, *C. muelleri* and *C. cf. costatus* sp. 2; within that derived lineage, a single genotype (red arrow), occurring in Ecuador, Panama and Costa Rica, accounts for 49 out of 82 such associations. These patterns indicate that each ant species appears to be exclusively associated with a single species of fungus independent of geography.

***C. wheeleri* group ant phylogeny.** The ant phylogeny (Fig. 3) indicates that, in addition to four known and two previously hypothesized cryptic species (*C. cf. longiscapus* n. sp. 1 and 2³¹), currently circumscribed ant species consist of additional, previously undetected, sibling species. *C. wheeleri* and *C. muelleri* are each divided into two distinct geographic species, Texas versus California (K2P-corrected distance = 0.11) and Ecuador versus Panama (K2P-corrected distance = 0.06), respectively. The geographic species pairs in both *C. wheeleri* and *C. muelleri* are old, diverging approximately 5.3 and 5.8 mya, respectively (Fig. 3). *C. costatus* is also divided into

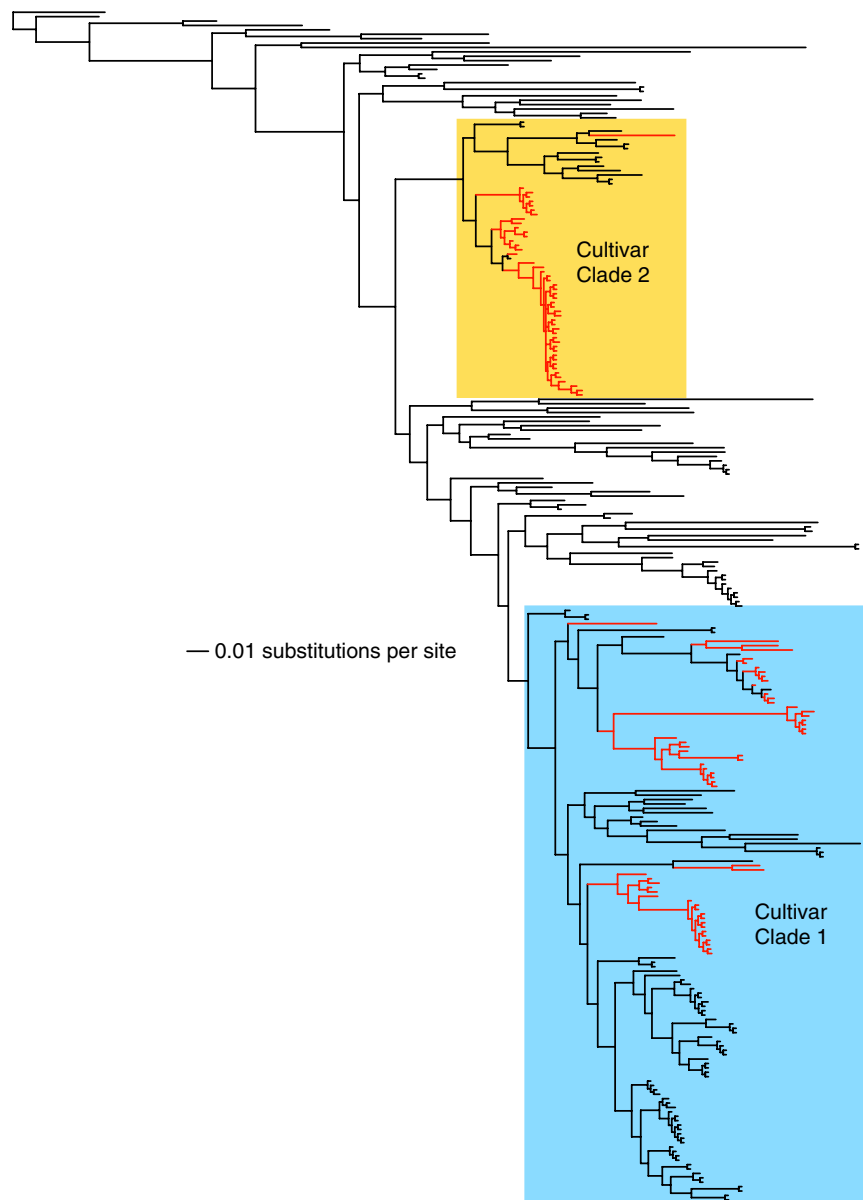


Figure 1 | Global phylogeny of the fungal tribe Leucocoprineae. The phylogeny was generated by Bayesian analyses of ITS sequence for 405 taxa (271 unique genotypes). Free-living fungi are indicated by black branches. Ant-associated fungi (red branches) have arisen multiple times and are concentrated in cultivar Clade 1 (shown in blue) and Clade 2 (shown in yellow).

two distinct species-level lineages (K2P-corrected distance = 0.10) that diverged approximately 7 mya and that co-occur in Ecuador. *C. cf. longiscapus* n. sp. 1, *C. cf. longiscapus* n. sp. 2, and *C. longiscapus* co-occur in Panama.

Discussion

Contrary to expectation, since their origins, all *C. wheeleri* group ant species have been tightly associated with narrowly defined fungal groups (Fig. 3) (BayesTraits ancestral-state posterior probabilities [PP] ≥ 0.98). *C. cf. costatus* sp. 1, occurring in Ecuador, is entirely associated with cultivar clade B, whereas *C. cf. costatus* sp. 2, occurring in Ecuador, Costa Rica and Panama, is entirely associated with cultivar clade G. *C. cf. longiscapus* n. sp. 1 is associated with cultivar clade D, *C. cf. longiscapus* n. sp. 2 is associated with fungal cultivar clade B, *C. longiscapus* is associated with cultivar clade D and both *C. wheeleri* sp. 1 and sp. 2 are associated with cultivar clade A. With a single exception (*C. muelleri* sp. 2 AGH020526-03), both *C. muelleri* sp.

1 and sp. 2 are associated with cultivar clade G. Data on the exception, *C. muelleri* sp. 2 AGH020526-03, were included in BayesTraits ancestral-state analyses and in ParaFit analyses of coevolution and did not alter the respective conclusions that the ancestor of *C. muelleri* s.l. cultivated a clade G fungus (PP = 1.0) and that significant ant–fungus coevolution has occurred between clade G fungi and their associated ants ($P < 0.05$). In short, although not all observed ant speciation events are associated with shifts in cultivar association, every observed shift in cultivar association is associated with an ant speciation event.

Taken together, these results strongly support a pattern of ant fidelity to particular fungi that is independent of geography and stable over evolutionary time spans of several millions of years. For example, *C. wheeleri* s.l. consists of two distinct geographic species that have both remained associated with cultivar clade A for approximately 5 million years. Likewise, *C. muelleri* s.l. includes two distinct cryptic sibling species that have both been associated with

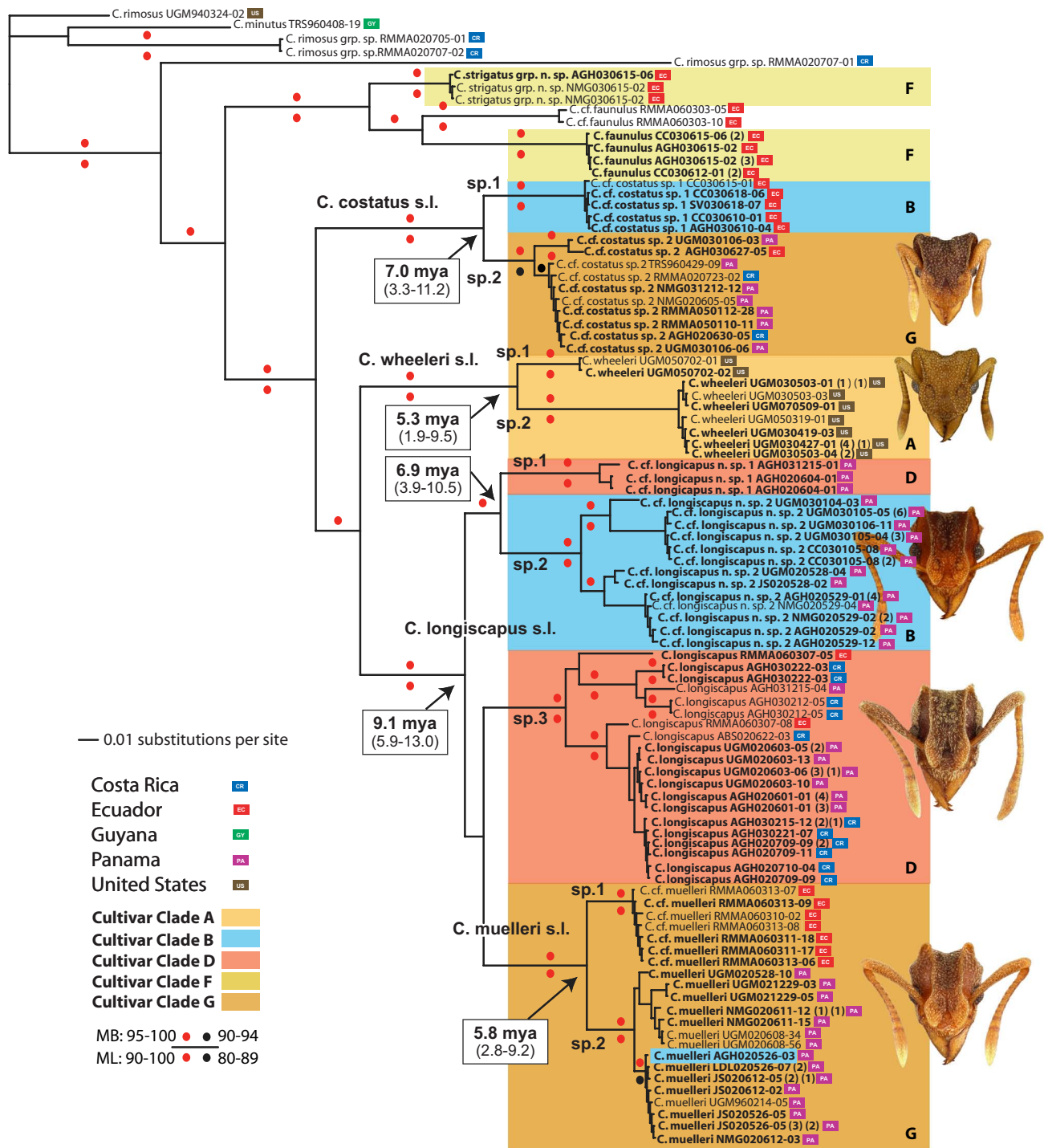


Figure 3 | Phylogeny of the *C. wheeleri* group of fungus-growing ants and outgroup species. The phylogeny was generated by Bayesian (shown) and maximum-likelihood analyses of 1,043 bp of mtCOI and 634 bp of nuclear wingless genes from 138 taxa (96 unique genotypes). Large coloured boxes indicate ancestral associations of ants with the cultivar clades shown in Fig. 2, as reconstructed in BayesTraits (all posterior probabilities ≥ 0.98). Although geographic speciation events within *C. wheeleri* s.l. and *C. muelleri* s.l. were not associated with shifts in cultivar association, five observed shifts in cultivar association were consistently associated with ant speciation events, for example, in *C. costatus* s.l. (sp. 1 versus sp. 2), *C. longiscapus* s.l. (sp. 1 and sp. 3 versus sp. 2) and at the origin of *C. muelleri* (*C. muelleri* s.l. versus *C. longiscapus* s.l.). Divergence-date estimates from Bayesian relaxed-clock analyses are indicated for major lineages. Coloured circles indicate Bayesian posterior probabilities (above branch) and maximum likelihood bootstrap proportions (below branch); small coloured labels beside taxon names indicate localities; numbers in parentheses refer to the number of samples belonging to a given ant genotype; and names in non-bold type indicate ant species for which fungal cultivars were not available. Ant heads from top to bottom: *C. cf. costatus* sp. 2, *C. wheeleri* sp. 2, *C. cf. longiscapus* sp. 2, *C. longiscapus* sp. 3 (syntype specimen) and *C. muelleri* sp. 2.

prior studies^{12,21} to construct a global phylogeny of 405 leucocoprineaceous fungi (reduced to 271 when all but one exemplar of each group of redundant genotypes were excluded; Supplementary Table S2). These samples were in most cases

preserved in ethanol in the field; some fungi were cultured from gardens of laboratory nests and their mycelium was lyophilized and preserved at -80°C for later study. Ant vouchers and fungal samples are deposited in the USNM Department

of Entomology collections. Information detailing extraction, PCR and sequencing methods can be found in the Supplementary Methods.

Alignments and data matrices. Ants: For this study we generated sequence data for 138 individual ants consisting of a 1,043-bp COI protein-coding fragment, two wg protein-coding fragments totalling 316 bp and a variable-length wg intron consisting of 220–318 bp. Sequences were edited in Sequencher (Lasergene version 7.0; DNASTAR, Madison, WI) and aligned using ClustalW³⁶ and by eye. After eliminating all but one representative of each unique genotype and one taxon judged to contain a COI pseudogene, and after excluding 44 bp of autapomorphic indel regions occurring in the wg intron, our ant data set consisted of 96 ant taxa and 1720 nucleotide sites. Because 32 taxa contained one or more missing fragments, for each treatment two phylogenetic analyses were conducted, one with the full 96-taxon data set and one with the 64-taxon data set in which there were no missing fragments. To check for possible gene tree/species tree discordance problems, single-gene analyses were also conducted utilizing data matrices consisting of, for COI, 96 taxa and 1,043 nucleotide characters and, for wg, 68 taxa and 633 nucleotide characters. Fungi: For this study we generated ITS sequence data for 193 ant garden fungi for a variable-length fragment (~700–750 bp) of the nuclear ITS region. After eliminating all but one representative of each unique genotype, our fungal data set consisted of 59 exemplar taxa, which we analysed in three separate alignments: global, Clade 1 and Clade 2. Global analyses utilized a data matrix in which the 59 new sequences were combined with 212 leucocoprineaceous sequences generated for previous studies, producing a data matrix of 271 fungal taxa and 721 nucleotide sites. Clade 1 analyses utilized a data matrix of 138 taxa (out of a total of 183 Clade 1 taxa), including all Clade 1 fungi known to be ant-associated, and 838 nucleotide sites. Clade 2 analyses utilized a data matrix of 69 taxa and 950 nucleotide sites. All alignments were produced in the program MAFFT v6.814b³⁷ using the E-INS-I algorithm, a 200PAM/k = 2 scoring matrix, a gap opening penalty of 1.53, and an offset value of zero.

Data partitioning. Ants: Based on genes and on the variability of codon-position sites within each gene, and based on the results of Ward *et al.*³⁸, we partitioned the ant data set into six partitions: COI first positions, COI second positions, COI third positions, wg first and second positions, wg third positions and the wg intron. Fungi: In global analyses, the fungal ITS region was analysed as a single unpartitioned unit. For Clade 1 and Clade 2 analyses, we partitioned the fungal ITS region into two partitions, a 'rapidly evolving' partition and a 'conserved' partition. In all cases, best-fit models of sequence evolution were selected for each partition under the Akaike information criterion as calculated in ModelTest v3.7³⁹.

Bayesian phylogenetic inference. All ant and fungal data sets were analysed under Bayesian criteria as implemented in MrBayes v3.1.2⁴⁰ with nucmodel = 4by4 and samplefreq = 500 (ants) or 100 (fungi). All parameters, including branch-length rate multipliers, were unlinked across partitions except branch lengths and topology. All analyses were carried out using parallel processing (one chain per CPU) with 8 chains per run and 2 runs per analysis (nrns = 2) and consisted of 10 million generations.

Maximum likelihood analyses. All ant data sets were analysed under maximum likelihood criteria as implemented in Garli 0.97.r737^{41,42} using parallel processing. ML bootstrap analyses consisted of 1000 pseudoreplicates, deviating from default settings as follows: genthreshfortopterm = 5000; scorethreshforterm = 0.10; starptprec = 0.5; minoptprec = 0.1; numberofpreductions = 1; treerejectionthreshold = 20.0; topweight = 0.01; brlenweight = 0.002. ML 'best tree' analyses consisted of 100 searches, deviating from the default settings as follows: topweight = 0.01; brlenweight = 0.002.

T-PTP tests. The correlation with phylogeny of ant-associated vs. free-living fungi was tested for the global ($n = 271$), Clade 1 ($n = 138$), and Clade 2 ($n = 69$) fungal phylogenies using the T-PTP test⁴³ with 1000 iterations as implemented in PAUP* v4.0a118⁴⁴. In each case, the significance of the parsimony-based length difference between the observed tree and the tree in which ant-associated fungi were constrained to be monophyletic was assessed by comparing it to the distribution of such length differences calculated from 1,000 replicates in which ant associations were randomized across fungal taxa. For all three data sets, the observed length difference was consistently less than the length differences for the permuted data, that is, the observed length difference was significant at $P < 0.001$.

Test for coevolution. The permutation-based ParaFit test for significant global coevolution between 'parasites' (attine ants) and 'hosts' (leucocoprineaceous fungi)⁴⁵ was applied to a reduced data set of 99 ants and 90 fungi, that is, to the largest overlapping subset of ants and fungi for which we had sequence data and host associations and, for ants, for which we had sufficient sequence data to calculate pairwise distances. Distances were calculated for both ants and fungi in PAUP* v4.0a118⁴⁴ under the GTR + I + G model using maximum-likelihood values for the proportion of sites invariant and gamma alpha calculated in PAUP on maximum-likelihood topologies identified by GARLI-PART 0.97.r737⁴¹. Distances were converted into principal coordinate matrices⁴⁶ in DistPCoA⁴⁷ with negative eigenvalues removed.

The principal-coordinate-transformed distance data were analysed in ParaFit using 999 permutations, resulting in significant support for a hypothesis of global coevolution between *Cyphomyrmex wheeleri*-group ants and their fungal cultivars ($P = 0.001$), as well as for ant-fungus coevolution in fungal clades A, B, D, F and G ($P < 0.05$) (Figs 2 and 3).

Ancestral state reconstruction. We inferred the ancestral fungal lineage for each ant species using the Bayesian method⁴⁸ implemented by BayesMultiState in the computer package BayesTraits (www.evolution.rdg.ac.uk). We coded each fungal lineage within the *Cyphomyrmex wheeleri* group (cultivars A, B, D, F, G) as a separate state; in cases where the fungal association was unknown for a particular ant specimen, the state was coded as unknown. We estimated Bayesian posterior distributions for each state using MCMC on a set of 1,000 trees selected randomly without replacement from the total set of post-burnin trees obtained from the MrBayes analyses. As suggested in the program manual, exploratory analyses were conducted to determine the appropriate range for hyperpriors and to select a ratedev value that produced an acceptance rate between 20 and 40%. We used hpall (0, 10) and ratedev = 20 for the results reported below; alternative distributions and values produced virtually identical results. The MCMC chain was run for 100 million generations with a burnin of 10 million and sampled every 10,000 generations. The following fungal cultivar lineages were reconstructed at the crown-group node for each ant species: *C. strigatus* group n. sp., cultivar F with PP = 0.99; *C. faunulus*, cultivar F with PP = 1.0; *C. costatus* sp.1, cultivar B with PP = 1.0; *C. costatus* sp.2, cultivar G with PP = 1.0; *C. wheeleri*, cultivar A with PP = 0.98; *C. longiscapus* sp.1, cultivar D with PP = 1.0; *C. longiscapus* sp.2, cultivar B with PP = 1.0; *C. longiscapus* sp.3, cultivar D with PP = 1.0; *C. muelleri*, cultivar G with PP = 1.0.

Divergence dating. Divergence times for ant lineages within the *Cyphomyrmex wheeleri* group were inferred under a Bayesian framework using BEAST v1.6.1 by employing an uncorrelated lognormal-relaxed clock model^{49,50}. We used the same nucleotide-partitioning scheme and substitution models as detailed above. Substitution models were unlinked and clock and tree models linked among partitions. A Yule speciation process with a random starting tree was used for the tree prior. We assigned a priori age distributions to two nodes. The root node was assigned a lognormal prior of log(mean) = 1.6, log(s.d.) = 1.0, offset = 15 (equivalent to a minimum bound of 15 Ma, median value of 20 Ma and a 95% quantile of 40 Ma) based on the presence of *Cyphomyrmex maya* and *C. taino*, both members of the *C. rimosus* group, in Dominican amber^{20,51}. The crown group of the *C. wheeleri* group was also assigned a normal prior of mean = 20, s.d. = 5 (equivalent to a median of 20 Ma and a 95% confidence interval of 1.8–18.2 Ma) based on prior divergence dates from a largely independent data set²⁰. Elimination of this second prior, which is not directly linked to the fossil record, altered resulting ages by less than 1 Ma. MCMC searches were conducted for 100,000,000 generations with the first 20,000,000 generations discarded as burn-in. Trees and other parameter values were saved every 10,000 generations. Convergence and stationarity were assessed with Tracer v1.5⁵² using ESS scores, log likelihood values and the consistency of results between multiple runs. The results from two independent runs were combined and visualized using FIGTREE v1.3.1⁵³.

References

- Wallin, I. E. *Symbioticism and the Origin of Species* (Waverly Press, 1927).
- Thompson, J. N. *The Coevolutionary Process* (Univ. of Chicago Press, 1994).
- Maynard Smith, J. & Szathmáry, E. *The Major Transitions in Evolution* (Oxford Univ. Press, 1995).
- Herre, E. A., Knowlton, N., Mueller, U. G. & Rehner, S. A. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol. Evol.* **14**, 49–53 (1999).
- Bourke, A. F. G. *Principles of Social Evolution* (Oxford Univ. Press, 2011).
- Sachs, J. L., Mueller, U. G., Wilcox, T. P. & Bull, J. J. Evolution of cooperation. *Q. Rev. Biol.* **79**, 135–160 (2004).
- Foster, K. R. & Wenseleers, T. A general model for the evolution of mutualisms. *J. Evol. Biol.* **19**, 1283–1293 (2006).
- Machado, C. A., Robbins, N., Gilbert, M. T. P. & Herre, E. A. Critical review of host specificity and its coevolutionary implications in the fig/wasp mutualism. *Proc. Natl Acad. Sci. USA* **102**, 6558–6565 (2005).
- Jackson, A. P., Machado, C. A., Robbins, N. & Herre, E. A. Multi-locus phylogenetic analysis of neotropical figs does not support co-speciation with the pollinators: the importance of systematic scale in fig/wasp cophylogenetic studies. *Symbiosis* **45**, 57 (2008).
- Dunlap, P. V. *et al.* Phylogenetic analysis of host symbiont specificity and codivergence in bioluminescent symbioses. *Cladistics* **23**, 507 (2007).
- Nobre, T., Fernandes, C., Boomsma, J. J., Korb, J. & Aanen, D. K. Farming termites determine the genetic population structure of *Termitomyces* fungal symbionts. *Mol. Ecol.* **20**, 2023–2033 (2011).
- Mueller, U. G., Rehner, S. A. & Schultz, T. R. The evolution of agriculture in ants. *Science* **281**, 2034–2038 (1998).
- Mikheyev, A. S., Mueller, U. G. & Abbott, P. Cryptic sex and many-to-one coevolution in the fungus-growing ant symbiosis. *Proc. Natl Acad. Sci. USA* **103**, 10702–10706 (2006).

14. Mikheyev, A. S., Mueller, U. G. & Boomsma, J. J. Population genetic signatures of diffuse coevolution between Panamanian leaf-cutter ants and their cultivar fungi. *Mol. Ecol.* **16**, 209–216 (2007).
15. Mikheyev, A. S., Vo, T. & Mueller, U. G. Phylogeography of post-Pleistocene population expansion in a fungus-gardening ant and its microbial mutualists. *Mol. Ecol.* **17**, 4480 (2008).
16. Mikheyev, A. S., Mueller, U. G. & Abbott, P. Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals coevolutionary synchrony and discord. *Am. Nat.* **175**, E126–E133 (2010).
17. Mueller, U. G., Mikheyev, A. S., Solomon, S. E. & Cooper, M. Frontier mutualism: Co-evolutionary patterns at the northern range limit of the leafcutter ant-fungus symbiosis. *Proc. R. Soc. Lond. B* **278**, 3050–3059 (2011).
18. Mehdiabadi, N. J. & Schultz, T. R. Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myr. News* **13**, 37–55 (2010).
19. Chapela, I. H., Rehner, S. A., Schultz, T. R. & Mueller, U. G. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* **266**, 1691–1694 (1994).
20. Schultz, T. R. & Brady, S. G. Major evolutionary transitions in ant agriculture. *Proc. Natl Acad. Sci. USA* **105**, 5435–5440 (2008).
21. Vo, T. L., Mikheyev, A. S. & Mueller, U. G. Free-living fungal symbionts (Lepiotaceae) of fungus-growing ants (Attini: Formicidae). *Mycologia* **101**, 206–210 (2009).
22. Schultz, T. R., Mueller, U. G., Currie, C. R. & Rehner, S. A. in *Ecological and Evolutionary Advances in Insect-Fungal Associations* (eds Vega, F. & Blackwell, M.) Ch. 7, pp. 149–190 (Oxford Univ. Press, 2005).
23. Silva-Pinhati, A. C. O. *et al.* Low variation in ribosomal DNA and internal transcribed spacers of the symbiotic fungi of leaf-cutting ants (Attini: Formicidae). *Braz. J. Med. Biol. Res.* **37**, 1463–1472 (2004).
24. Hinkle, G., Wetterer, J. K., Schultz, T. R. & Sogin, M. L. Phylogeny of the attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. *Science* **266**, 1695–1697 (1994).
25. Bot, A. N. M., Rehner, S. A. & Boomsma, J. J. Partial incompatibility between ants and symbiotic fungi in two sympatric species of *Acromyrmex* leaf-cutting ants. *Evolution* **55**, 1980–1991 (2001).
26. Green, A. M., Mueller, U. G. & Adams, R. M. M. Extensive exchange of fungal cultivars between sympatric species of fungus-growing ants. *Mol. Ecol.* **11**, 191–195 (2002).
27. Mueller, U. G., Schultz, T. R., Currie, C. R., Adams, R. M. M. & Malloch, D. The origin of the attine ant-fungus mutualism. *Q. Rev. Biol.* **76**, 169–197 (2001).
28. Munkacsy, A. B. *et al.* Convergent coevolution in the domestication of coral mushrooms by fungus-growing ants. *Proc. R. Soc. Lond. B* **271**, 1777–1782 (2004).
29. Dentinger, B. T. M., Lodge, D. J., Munkacsy, A. B., Desjardins, D. E. & McLaughlin, D. J. Phylogenetic placement of an unusual coral mushroom challenges the classic hypothesis of strict coevolution in the *Apterostigma pilosum* group ant-fungus mutualism. *Evolution* **63**, 2172–2178 (2009).
30. Thompson, J. N. *The Geographic Mosaic of Coevolution* (Univ. of Chicago Press, 2005).
31. Schultz, T. R. *et al.* Cryptic speciation in the fungus-growing ants *Cyphomyrmex longiscapus* Weber and *Cyphomyrmex muelleri* Schultz and Solomon, new species (Formicidae: Attini). *Insectes Sociaux* **49**, 331–343 (2002).
32. Mueller, U. G. & Wcislo, W. T. Nesting biology of the fungus-growing ant *Cyphomyrmex longiscapus* Weber (Attini, Formicidae). *Insectes Sociaux* **45**, 181–189 (1998).
33. Mehdiabadi, N. J., Hughes, B. & Mueller, U. G. Cooperation, conflict, and coevolution in the attine ant-fungus symbiosis. *Behav. Ecol.* **17**, 291–296 (2006).
34. Mueller, U. G., Poulin, J. & Adams, R. M. M. Symbiont choice in a fungus-growing ant (Attini, Formicidae). *Behav. Ecol.* **15**, 357–364 (2004).
35. Winkler, I. S. & Mitter, C. in *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed Tilmon, K. J.) Ch. 18, 240–263 (Univ. California Press, 2008).
36. Thompson, J. D., Higgins, D. G. & Gibson, T. J. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680 (1994).
37. Katoh, K., Misawa, K., Kuma, K. & Miyata, T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059–3066 (2002).
38. Ward, P. S., Brady, S. G., Fisher, B. L. & Schultz, T. R. Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Syst. Biol.* **59**, 342–362 (2010).
39. Posada, D. & Crandall, K. A. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818 (1998).
40. Ronquist, F. & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574 (2003).
41. Zwickl, D. J. *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion* Ph.D. thesis, The University of Texas at Austin (2006).
42. Zwickl, D. J. GARLI-part v0.97, <http://garli.googlecode.com> (2010).
43. Faith, D. P. Cladistic permutation tests for monophyly and nonmonophyly. *Syst. Zool.* **40**, 366–375 (1991).
44. Swofford, D. L. *PAUP*: Phylogenetic Analysis using Parsimony (*and other methods)* Version 4, (Sinauer Associates, Sunderland, MA, 2002).
45. Legendre, P., Desdés, Y. & Bazin, E. A Statistical test for host-parasite coevolution. *Syst. Biol.* **51**, 217–234 (2002).
46. Gower, J. C. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**, 325–338 (1966).
47. Legendre, P. & Anderson, M. J. *Program DistPCoA* (Département de sciences biologiques, Université de Montréal, Canada, 1998).
48. Pagel, M., Meade, A. & Barker, D. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* **53**, 673–684 (2004).
49. Drummond, A. J., Ho, S. Y. W., Phillips, M. J. & Rambaut, A. Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**, e88 (2006).
50. Drummond, A. J. & Rambaut, A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214 (2007).
51. de Andrade, M. L. First descriptions of two new amber species of *Cyphomyrmex* from Mexico and the Dominican Republic (Hymenoptera: Formicidae). *Beiträge zur Entomologie* **53**, 131–139 (2003).
52. Rambaut, A. & Drummond, A. J. Tracer v1.5, <http://tree.bio.ed.ac.uk/software/tracer/> (2009).
53. Rambaut, A. Figtree v1.3.1, <http://tree.bio.ed.ac.uk/software/figtree/> (2010).

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Author contributions

N.J.M., U.G.M., and T.R.S. designed experiments; U.G.M., A.G.H., and T.R.S. collected samples; T.R.S. identified ant specimens; N.J.M. performed experiments; N.J.M., S.G.B., and T.R.S. analysed the data; N.J.M. and T.R.S. wrote the manuscript; U.G.M., S.G.B., and A.G.H. commented on the manuscript.

Additional information

Accession codes: The sequence data have been deposited in GenBank NCBI database under accession codes JQ617314 to JQ617746 listed in Supplementary Tables S1 and S2.

Supplementary Information accompanies this paper at <http://www.nature.com/naturecommunications>

Competing financial interests: The authors declare no competing financial interests.

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Supplementary Information:

Symbiont fidelity and the origin of species in fungus-growing ants

Natasha J. Mehdiabadi, Ulrich G. Mueller, Seán G. Brady,

Anna G. Himler, and Ted R. Schultz

This Supplementary Information includes:

Supplementary Table S1

Supplementary Table S2

Supplementary Methods

Supplementary References

Supplementary Table S1. Collecting code, species name, collection locality, collector, and Genbank accessions for all ants used in data matrices.

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
AGH020529.01	NACYPHO103	JQ617456	JQ617432	1	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	A.G. Himler
SP020529.03	NACYPHO173	JQ617525	JQ617434	1	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	S.L. Price
UGM020528.06	NACYPHO175	JQ617461	JQ617388	1	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM020528.12	NACYPHO177	JQ617489	JQ617362	1	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
CC030105.08	NACYPHO125	JQ617475	JQ617423	2	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	C.R. Currie
UGM030105.14	NACYPHO198	JQ617440	JQ617411	2	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
NMG020529.02	NACYPHO138	JQ617454	JQ617363	3	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	N.M. Gerardo
NMG020529.05	NACYPHO140	JQ617448	JQ617379	3	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	N.M. Gerardo
UGM030105.04	NACYPHO192	JQ617439	JQ617358	4	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030106.09	NACYPHO202	JQ617482	JQ617415	4	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030106.12	NACYPHO204	JQ617503	JQ617418	4	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030105.05	NACYPHO193	JQ617534	JQ617384	5	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030105.06	NACYPHO194	JQ617526	JQ617371	5	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030105.07	NACYPHO195	JQ617458	JQ617393	5	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030105.12	NACYPHO196	JQ617452	JQ617346	5	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030105.13	NACYPHO197	JQ617518	JQ617414	5	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030105.16	NACYPHO199	JQ617496	JQ617378	5	Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
JS020526.05	NACYPHO129	JQ617479	JQ617359	6	Cyphomyrmex muelleri	Panama	J.J. Scott
JS020526.07	NACYPHO130	JQ617486	JQ617389	6	Cyphomyrmex muelleri	Panama	J.J. Scott

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
JS020612.01	NACYPHO132	JQ617512	JQ617429	6	Cyphomyrmex muelleri	Panama	J.J. Scott
NMG020526.08	NACYPHO137	JQ617519	JQ617400	6	Cyphomyrmex muelleri	Panama	N.M. Gerardo
NMG020612.04	NACYPHO145	JQ617531	JQ617403	6	Cyphomyrmex muelleri	Panama	N.M. Gerardo
AGH020612.08	NACYPHO108	JQ617556	JQ617404	7	Cyphomyrmex muelleri	Panama	A.G. Himler
JS020612.05	NACYPHO134	JQ617449	JQ617433	7	Cyphomyrmex muelleri	Panama	J.J. Scott
NMG020526.03	NACYPHO136	JQ617480	JQ617355	7	Cyphomyrmex muelleri	Panama	N.M. Gerardo
LDL020526.07	NACYPHO135	JQ617477	JQ617413	8	Cyphomyrmex muelleri	Panama	L.D. Loudermilk
NMG020612.05	NACYPHO146	JQ617517	JQ617398	8	Cyphomyrmex muelleri	Panama	N.M. Gerardo
NMG020611.12	NACYPHO141	JQ617516	JQ617380	9	Cyphomyrmex muelleri	Panama	N.M. Gerardo
NMG020611.18	NACYPHO143	JQ617550	JQ617344	9	Cyphomyrmex muelleri	Panama	N.M. Gerardo
AGH020601.01	NACYPHO105	JQ617332	JQ617377	10	Cyphomyrmex longiscapus	Panama	A.G. Himler
AGH020602.01	NACYPHO4	JQ617318		10	Cyphomyrmex longiscapus	Panama	A.G. Himler
UGM020601.01	NACYPHO179	JQ617473	JQ617385	10	Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020602.04	NACYPHO180	JQ617325	JQ617348	10	Cyphomyrmex longiscapus	Panama	U.G. Mueller
AGH020601.01	NACYPHO3	JQ617322		11	Cyphomyrmex longiscapus	Panama	A.G. Himler
AGH020602.01	NACYPHO106	JQ617323	JQ617436	11	Cyphomyrmex longiscapus	Panama	A.G. Himler
UGM020602.04	NACYPHO8	JQ617315		11	Cyphomyrmex longiscapus	Panama	U.G. Mueller
AGH020709.09	NACYPHO110	JQ617328	JQ617354	12	Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH020710.01	NACYPHO112	JQ617521	JQ617417	12	Cyphomyrmex longiscapus	Costa Rica	A.G. Himler

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
AGH030215.12	NACYPHO115	JQ617507	JQ617399	13	Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030218.05	NACYPHO116	JQ617488	JQ617386	13	Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030222.07	NACYPHO119	JQ617555	JQ617372	13	Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030615.02	NACYPHO13	JQ617470		14	Cyphomyrmex faunulus	Ecuador	A.G. Himler
CC030612.01	NACYPHO126	JQ617331	JQ617351	14	Cyphomyrmex faunulus	Ecuador	C.R. Currie
CC030615.08	NACYPHO128	JQ617326	JQ617410	14	Cyphomyrmex faunulus	Ecuador	C.R. Currie
CC030612.01	NACYPHO14	JQ617324		15	Cyphomyrmex faunulus	Ecuador	C.R. Currie
CC030615.08	NACYPHO16	JQ617330		15	Cyphomyrmex faunulus	Ecuador	C.R. Currie
CC030615.06	NACYPHO127	JQ617520		16	Cyphomyrmex faunulus	Ecuador	C.R. Currie
CC030615.06	NACYPHO15	JQ617515		16	Cyphomyrmex faunulus	Ecuador	C.R. Currie
UGM020603.05	NACYPHO181	JQ617509	JQ617341	17	Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020603.13	NACYPHO186	JQ617317	JQ617353	17	Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020603.06	NACYPHO182	JQ617468	JQ617426	18	Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020603.07	NACYPHO183	JQ617522	JQ617342	18	Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020603.10	NACYPHO184	JQ617474	JQ617408	18	Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020603.12	NACYPHO185	JQ617491	JQ617396	18	Cyphomyrmex longiscapus	Panama	U.G. Mueller
AR061001.02	NACYPHO124	JQ617485	JQ617394	19	Cyphomyrmex wheeleri	USA	A. Rodrigues
UGM030427.01	NACYPHO206	JQ617446	JQ617402	19	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM051215.05	NACYPHO214	JQ617441	JQ617376	19	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
UGM060406.03	NACYPHO215	JQ617466	JQ617350	19	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM061001.02	NACYPHO217	JQ617462	JQ617343	19	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM030503.01	NACYPHO207	JQ617540	JQ617395	20	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM030503.02	NACYPHO208	JQ617467	JQ617337	20	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM030503.04	NACYPHO210	JQ617513	JQ617401	21	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM061001.01	NACYPHO216	JQ617460	JQ617366	21	Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
ABS020622.03	NACYPHO101	JQ617447	JQ617424		Cyphomyrmex longiscapus	Costa Rica	A.B. Smith
AGH020526.03	NACYPHO102	JQ617545	JQ617370		Cyphomyrmex muelleri	Panama	A.G. Himler
AGH020529.02	NACYPHO104	JQ617544	JQ617334		Cyphomyrmex cf. longiscapus n. sp. 2	Panama	A.G. Himler
AGH020529.12	NACYPHO178	JQ617450	JQ617416		Cyphomyrmex cf. longiscapus n. sp. 2	Panama	A.G. Himler
AGH020604.01	NACYPHO107	JQ617487	JQ617360		Cyphomyrmex cf. longiscapus n. sp. 1	Panama	A.G. Himler
AGH020604.01	NACYPHO5	JQ617541			Cyphomyrmex cf. longiscapus n. sp. 1	Panama	A.G. Himler
AGH020630.05	NACYPHO109	JQ617510	JQ617352		Cyphomyrmex cf. costatus sp. 2	Costa Rica	A.G. Himler
AGH020709.09	NACYPHO6	JQ617319			Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH020709.11	NACYPHO111	JQ617442	JQ617409		Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH020710.04	NACYPHO113	JQ617459			Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030212.05	NACYPHO114	JQ617327	JQ617438		Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030212.05	NACYPHO7	JQ617333			Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030221.07	NACYPHO117	JQ617549	JQ617345		Cyphomyrmex longiscapus	Costa Rica	A.G. Himler

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
AGH030222.03	NACYPHO118	JQ617320	JQ617437		Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030222.03	NACYPHO12	JQ617314			Cyphomyrmex longiscapus	Costa Rica	A.G. Himler
AGH030610.04	NACYPHO222	JQ617547	JQ617387		Cyphomyrmex cf. costatus sp. 1	Ecuador	A.G. Himler
AGH030615.02	NACYPHO120	JQ617471			Cyphomyrmex faunulus	Ecuador	A.G. Himler
AGH030615.06	NACYPHO121	JQ617530	JQ617405		Cyphomyrmex strigatus grp. n. sp	Ecuador	A.G. Himler
AGH030627.05	NACYPHO223	JQ617527	JQ617357		Cyphomyrmex cf. costatus sp. 2	Ecuador	A.G. Himler
AGH031215.01	NACYPHO122	JQ617481	JQ617367		Cyphomyrmex cf. longiscapus n. sp. 1	Panama	A.G. Himler
AGH031215.04	NACYPHO123	JQ617504	JQ617430		Cyphomyrmex longiscapus	Panama	A.G. Himler
CC030105.08	NACYPHO1	JQ617553			Cyphomyrmex cf. longiscapus n. sp. 2	Panama	C.R. Currie
CC030610.01	NACYPHO221	JQ617499	JQ617368		Cyphomyrmex cf. costatus sp. 1	Ecuador	C.R. Currie
CC030615.01	A416	JQ617464			Cyphomyrmex cf. costatus sp. 1	Ecuador	C.R. Currie
CC030618.06	NACYPHO220	JQ617552	JQ617383		Cyphomyrmex cf. costatus sp. 1	Ecuador	C.R. Currie
JS020526.05	NACYPHO2	JQ617444			Cyphomyrmex muelleri	Panama	J.J. Scott
JS020528.02	NACYPHO131	JQ617536	JQ617349		Cyphomyrmex cf. longiscapus n. sp. 2	Panama	J.J. Scott
JS020612.02	NACYPHO133	JQ617508	JQ617369		Cyphomyrmex muelleri	Panama	J.J. Scott
NMG020529.04	NACYPHO139	JQ617483			Cyphomyrmex cf. longiscapus n. sp. 2	Panama	N.M. Gerardo
NMG020605.05	NACYPHO219	JQ617537	JQ617335		Cyphomyrmex cf. costatus sp. 2	Panama	N.M. Gerardo
NMG020611.15	NACYPHO142	JQ617548	JQ617391		Cyphomyrmex muelleri	Panama	N.M. Gerardo
NMG020612.03	NACYPHO144	JQ617505	JQ617338		Cyphomyrmex muelleri	Panama	N.M. Gerardo

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
NMG030615.02	NACYPHO147	JQ617329	JQ617419		Cyphomyrmex strigatus grp. n. sp	Ecuador	N.M. Gerardo
NMG030615.02	NACYPHO17	JQ617316			Cyphomyrmex strigatus grp. n. sp	Ecuador	N.M. Gerardo
NMG031212.12	NACYPHO149	JQ617532	JQ617364		Cyphomyrmex cf. costatus sp. 2	Panama	N.M. Gerardo
RMMA020705.01	NACYPHO150	JQ617469	JQ617406		Cyphomyrmex rimosus grp. sp.	Costa Rica	R.M.M. Adams
RMMA020707.01	NACYPHO151	JQ617500	JQ617428		Cyphomyrmex rimosus grp. sp.	Costa Rica	R.M.M. Adams
RMMA020707.02	NACYPHO152	JQ617501			Cyphomyrmex rimosus grp. sp.	Costa Rica	R.M.M. Adams
RMMA020723.02	NACYPHO156	JQ617490	JQ617374		Cyphomyrmex cf. costatus sp. 2	Costa Rica	R.M.M. Adams
RMMA050110.11	NACYPHO159	JQ617502	JQ617420		Cyphomyrmex cf. costatus sp. 2	Panama	R.M.M. Adams
RMMA050112.28	NACYPHO160	JQ617554	JQ617339		Cyphomyrmex cf. costatus sp. 2	Panama	R.M.M. Adams
RMMA060303.05	NACYPHO162	JQ617451			Cyphomyrmex cf. faunulus	Ecuador	R.M.M. Adams
RMMA060303.10	NACYPHO163	JQ617497			Cyphomyrmex cf. faunulus	Ecuador	R.M.M. Adams
RMMA060307.05	NACYPHO164	JQ617463			Cyphomyrmex longiscapus	Ecuador	R.M.M. Adams
RMMA060307.08	NACYPHO165	JQ617524	JQ617390		Cyphomyrmex longiscapus	Ecuador	R.M.M. Adams
RMMA060310.02	NACYPHO166	JQ617538			Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams
RMMA060311.17	NACYPHO167	JQ617546	JQ617375		Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams
RMMA060311.18	NACYPHO168	JQ617529	JQ617373		Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams
RMMA060313.06	NACYPHO169	JQ617476			Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams
RMMA060313.07	NACYPHO170	JQ617494	JQ617397		Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams
RMMA060313.08	NACYPHO171	JQ617465			Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg ¹	Genotype Number ²	Species ID	Locality (Country)	Collector
RMMA060313.09	NACYPHO172	JQ617478	JQ617340		Cyphomyrmex cf. muelleri	Ecuador	R.M.M. Adams
SV030618.07	NACYPHO224	JQ617514	JQ617361		Cyphomyrmex cf. costatus sp. 1	Ecuador	S. Villamarin
TRS960408.19	A318	JQ617495			Cyphomyrmex minutus	Guyana	T.R. Schultz
TRS960429.09	Cypho48	JQ617542			Cyphomyrmex cf. costatus sp. 2	Panama	T.R. Schultz
UGM020528.04	NACYPHO174	JQ617533	JQ617336		Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM020528.10	NACYPHO176	JQ617455	JQ617407		Cyphomyrmex muelleri	Panama	U.G. Mueller
UGM020603.10	NACYPHO10	JQ617443			Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020603.13	NACYPHO11	JQ617321			Cyphomyrmex longiscapus	Panama	U.G. Mueller
UGM020608.34	NACYPHO187	JQ617511	JQ617347		Cyphomyrmex muelleri	Panama	U.G. Mueller
UGM020608.56	NACYPHO188	JQ617445	JQ617422		Cyphomyrmex muelleri	Panama	U.G. Mueller
UGM021229.03	NACYPHO189	JQ617523	JQ617425		Cyphomyrmex muelleri	Panama	U.G. Mueller
UGM021229.05	NACYPHO190	JQ617528	JQ617381		Cyphomyrmex muelleri	Panama	U.G. Mueller
UGM030104.03	NACYPHO191	JQ617472	JQ617392		Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030106.03	NACYPHO200	JQ617457	JQ617365		Cyphomyrmex cf. costatus sp. 2	Panama	U.G. Mueller
UGM030106.06	NACYPHO201	JQ617535	JQ617427		Cyphomyrmex cf. costatus sp. 2	Panama	U.G. Mueller
UGM030106.11	NACYPHO203	JQ617453	JQ617412		Cyphomyrmex cf. longiscapus n. sp. 2	Panama	U.G. Mueller
UGM030419.03	NACYPHO205	JQ617543	JQ617356		Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM030503.03	NACYPHO209	JQ617551	JQ617431		Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM050319.01	NACYPHO211	JQ617539			Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller

Collection ID	DNA Extraction Number	GenBank Accession Number - CO1	GenBank Accession Number - Wg¹	Genotype Number²	Species ID	Locality (Country)	Collector
UGM050702.01	NACYPHO212	JQ617484	JQ617421		Cyphomyrmex wheeleri	USA: California	U.G. Mueller
UGM050702.02	NACYPHO213	JQ617493	JQ617435		Cyphomyrmex wheeleri	USA: California	U.G. Mueller
UGM070509.01	NACYPHO218	JQ617498	JQ617382		Cyphomyrmex wheeleri	USA: Texas	U.G. Mueller
UGM940324.02	Cypho4	JQ617506			Cyphomyrmex rimosus	USA	U.G. Mueller
UGM960214.05	A423	JQ617492			Cyphomyrmex muelleri	Panama	U.G. Mueller
¹ Shaded cells represent samples that did not produce Wg sequence.							
² Genotype numbers only assigned for those with more than one exemplar.							

Supplementary Table S2. Collecting code, species name, collection locality, collector, and Genbank accessions for all fungi used in data matrices.

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
AGH020709.11	C176	JQ617583	1	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH020710.01	C175	JQ617630	1	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH020710.04	C186	JQ617601	1	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH020710.04	C177	JQ617615	1	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
UGM020603.06	C112	JQ617692	1	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
AGH030222.03	C109	JQ617634	2	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
UGM020602.04	C113	JQ617651	2	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
CC020529.04	C92	JQ617610	3	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
NMG020529.05	C167	JQ617707	3	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	N.M. Gerardo
AGH020526.03	C155	JQ617700	4	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020529.01	C166	JQ617715	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020529.01	C26	JQ617729	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020529.02	C168	JQ617636	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020529.07	C71	JQ617575	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020529.08	C9	JQ617668	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020529.12	C149	JQ617672	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	A.G. Himler
AGH030610.04	C103	JQ617563	4	Cyphomyrmex cf. costatus sp. 1 symbiont	Ant-cultivated	Ecuador	A.G. Himler
CC011108.03	C90	JQ617593	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011114.02	C54	JQ617705	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011114.03	C29	JQ617560	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
CC011114.03	C3	JQ617746	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011114.04	C61	JQ617650	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011114.04	C60	JQ617662	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011209.04	C18	JQ617592	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011209.04	C28	JQ617674	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011211.08	C91	JQ617732	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011213.01	C40	JQ617695	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011213.09	C51	JQ617661	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011213.10	C27	JQ617654	4	Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	C.R. Currie
CC011213.18	C2	JQ617730	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC020529.07	C93	JQ617743	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC020529.10	C94	JQ617648	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC020531.10	C45	JQ617703	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC030104.05	C33	JQ617713	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	C.R. Currie
CC030105.08	C119	JQ617604	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	C.R. Currie
CC030105.08	C111	JQ617643	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	C.R. Currie
CC030610.01	C105	JQ617683	4	Cyphomyrmex cf. costatus sp. 1 symbiont	Ant-cultivated	Ecuador	C.R. Currie
CC030618.06	C107	JQ617606	4	Cyphomyrmex cf. costatus sp. 1 symbiont	Ant-cultivated	Ecuador	C.R. Currie
LDL020526.03	C36	JQ617626	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	L.D. Loudermilk
LDL020526.05	C95	JQ617658	4	Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	L.D. Loudermilk

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
NMG011101.07	C1	JQ617698	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011101.12	C67	JQ617736	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011108.08	C19	JQ617708	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011114.03	C10	JQ617659	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011114.05	C64	JQ617689	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020529.01	C148	JQ617675	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020529.02	C156	JQ617618	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020531.04	C52	JQ617623	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
RMMA010317.03	C58	JQ617742	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010321.11	C79	JQ617721	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA030818.04	C76	JQ617633	4	Cyphomyrmex sp. symbiont	Ant-cultivated	Costa Rica	R.M.M. Adams
SP020529.03	C53	JQ617619	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	S.L. Price
SP020529.03	C162	JQ617642	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	S.L. Price
SP020531.03	C80	JQ617685	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	S.L. Price
SP020531.05	C17	JQ617681	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	S.L. Price
SV030618.07	C89	JQ617669	4	Cyphomyrmex cf. costatus sp. 1 symbiont	Ant-cultivated	Ecuador	S. Villamarin
TV020531.02	C63	JQ617586	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	T. Vo
UGM020528.06	C163	JQ617744	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020528.12	C165	JQ617607	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030104.03	C132	JQ617670	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
UGM030104.04	C110	JQ617735	4	Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.04	C133	JQ617677	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.05	C134	JQ617680	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.06	C135	JQ617622	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.07	C136	JQ617679	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.12	C137	JQ617614	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.13	C138	JQ617620	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.14	C139	JQ617564	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030105.16	C140	JQ617640	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030106.09	C143	JQ617577	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030106.11	C144	JQ617676	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030106.12	C145	JQ617590	4	longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
Unknown01	C88		4	Cyphomyrmex sp. symbiont	Ant-cultivated	Unknown	Unknown
AR061001.02	C201	JQ617580	5	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	A. Rodrigues
UGM030419.03	C191	JQ617720	5	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
UGM030427.01	C200	JQ617646	5	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
UGM061001.01	C197	JQ617684	5	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
UGM061001.02	C198	JQ617596	5	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
UGM070509.01	C206	JQ617602	5	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
UGM030503.04	C195	JQ617701	6	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
UGM060406.03	C207	JQ617737	6	Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
CC030615.06	C123	JQ617629	7	Cyphomyrmex faunulus symbiont	Ant-cultivated	Ecuador	C.R. Currie
CC030615.08	C122	JQ617716	7	Cyphomyrmex faunulus symbiont	Ant-cultivated	Ecuador	C.R. Currie
AGH020522.03	C47	JQ617682	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020524.01	C59	JQ617726	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020525.01	C66	JQ617588	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020526.10	C35	JQ617666	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020612.08	C153	JQ617686	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020630.05	C152	JQ617613	8	Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030627.05	C108	JQ617600	8	Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Ecuador	A.G. Himler
AGH031218.04	C102a_small	JQ617706	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	A.G. Himler
AGH031218.04	C102b_large	JQ617731	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	A.G. Himler
AS020605.01	C48	JQ617611	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	A. Smith
CC011024.01	C74	JQ617616	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011124.06	C23	JQ617725	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
CC011129.05	C56	JQ617608	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
CC020602.04	C99	JQ617663	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
CC030104.02	C87	JQ617664	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
JS020524.01	C50	JQ617625	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	J.J. Scott
JS020526.05	C118	JQ617702	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	J.J. Scott

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
JS020612.05	C170	JQ617573	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	J.J. Scott
NMG010811.01	C22	JQ617635	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG010815.04	C65	JQ617639	8	Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011101.06	C14	JQ617687	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011101.06	C82	JQ617722	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011105.02	C75	JQ617711	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011110.02	C83	JQ617594	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011114.02	C11	JQ617632	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020529.06	C96	JQ617558	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020531.07	C62	JQ617591	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020611.12	C172	JQ617628	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020612.03	C173	JQ617570	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020612.03	C174	JQ617581	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020612.05	C150	JQ617734	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG031216.04	C106	JQ617562	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	N.M. Gerardo
RMMA010302.01	C85	JQ617641	8	Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010311.01	C41	JQ617598	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010311.04	C7	JQ617699	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010311.04	C84	JQ617738	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010311.05	C25	JQ617667	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
RMMA010311.06	C42	JQ617655	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010320.06	C73	JQ617585	8	Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA050110.11	C203	JQ617631	8	Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Panama	R.M.M. Adams
SP011105.01	C15	JQ617557	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	S.L. Price
SP011108.06	C8	JQ617740	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	S.L. Price
SP020605.01	C5	JQ617665	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	S.L. Price
TV020524.01	C98	JQ617728	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	T. Vo
UGM011209.04	C101	JQ617719	8	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020528.10	C160	JQ617688	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM021229.03	C146	JQ617567	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM021229.05	C147	JQ617637	8	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	U.G. Mueller
Unknown02	pos1 ITS5_4		8	Cyphomyrmex sp. symbiont	Ant-cultivated	Unknown	Unknown
JS020612.01	C171	JQ617710	9	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	J.J. Scott
JS020612.02	C151	JQ617727	9	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	J.J. Scott
CC011025.02	C16	JQ617691	10	Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
NMG011101.11	C68	JQ617704	10	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020612.04	C154	JQ617724	10	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
RMMA010313.05	C32	JQ617723	10	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
SP011108.01	C57	JQ617714	10	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	S.L. Price
NMG011110.04	C34b_large	JQ617574	11	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
NMG011110.04	C34a_small	JQ617621	11	Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
AGH020601.01	C117	JQ617572		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020602.01	C116	JQ617673		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020604.01	C115	JQ617561		longiscapus n. sp. 1 symbiont	Ant-cultivated	Panama	A.G. Himler
AGH020709.09	C114	JQ617566		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH020710.03	C55	JQ617645		Cyphomyrmex sp. symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030215.12	C183	JQ617741		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030218.05	C185	JQ617694		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030221.07	C182a_small	JQ617617		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030221.07	C182b_large	JQ617644		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030222.03	C78	JQ617712		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Costa Rica	A.G. Himler
AGH030615.02	C125	JQ617565		Cyphomyrmex faunulus symbiont	Ant-cultivated	Ecuador	A.G. Himler
AGH030615.06	C130	JQ617612		Cyphomyrmex strigatus grp. n. sp. symbiont	Ant-cultivated	Ecuador	A.G. Himler
AGH031215.01	C180	JQ617582		longiscapus n. sp. 1 symbiont	Ant-cultivated	Panama	A.G. Himler
CC011110.04	C46	JQ617579		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	C.R. Currie
CC020605.06	C104	JQ617571		Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	C.R. Currie
CC030612.01	C124	JQ617717		Cyphomyrmex faunulus symbiont	Ant-cultivated	Ecuador	C.R. Currie
HLV020524.03	C12	JQ617584		Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	H.L. Vasconcelos
JS020528.02	C164	JQ617739		longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	J.J. Scott
KER031215.01	C77	JQ617603		Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	K.E. Richardson

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
LDL020526.07	C158	JQ617745		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	L.D. Loudermilk
NMG010812.04	C38	JQ617690		Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG010816.04	C37	JQ617587		Cyphomyrmex sp. symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011102.02	C4	JQ617559		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG011110.05	C49	JQ617595		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020526.01	C43	JQ617693		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG020611.15	C169	JQ617597		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	N.M. Gerardo
NMG031212.12	C202	JQ617568		Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Panama	N.M. Gerardo
RMMA010311.03	C24	JQ617647		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010317.07	C20	JQ617569		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA010317.08	C86	JQ617657		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA050112.28	C205	JQ617718		Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Panama	R.M.M. Adams
RMMA060307.05	RMMA060307.05	JQ617733		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Ecuador	R.M.M. Adams
RMMA060311.17	RMMA060311.17	JQ617653		Cyphomyrmex cf. muelleri symbiont	Ant-cultivated	Ecuador	R.M.M. Adams
RMMA060311.18	RMMA060311.18	JQ617656		Cyphomyrmex cf. muelleri symbiont	Ant-cultivated	Ecuador	R.M.M. Adams
RMMA060313.06	RMMA060313.06	JQ617589		Cyphomyrmex cf. muelleri symbiont	Ant-cultivated	Ecuador	R.M.M. Adams
RMMA060313.09	RMMA060313.09	JQ617627		Cyphomyrmex cf. muelleri symbiont	Ant-cultivated	Ecuador	R.M.M. Adams
SP011108.02	C21	JQ617578		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	S.L. Price
SP011108.03	C13	JQ617652		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	S.L. Price
SP011108.04	C72	JQ617660		Cyphomyrmex muelleri symbiont	Ant-cultivated	Panama	S.L. Price

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
TV020531.04	C44	JQ617697		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	T. Vo
UGM020528.04	C159	JQ617599		longiscapus n. sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020528.05	C81	JQ617605		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020601.01	C131	JQ617576		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020603.05	C179	JQ617678		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020603.07	C178	JQ617696		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020603.10	C128	JQ617671		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM020603.13	C127	JQ617638		Cyphomyrmex longiscapus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030106.03	C141	JQ617649		Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030106.06	C142	JQ617609		Cyphomyrmex cf. costatus sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM030503.01	C192	JQ617624		Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: Texas	U.G. Mueller
UGM050702.02	C199	JQ617709		Cyphomyrmex wheeleri symbiont	Ant-cultivated	USA: California	U.G. Mueller
Unknown03	C70			Cyphomyrmex sp. symbiont	Ant-cultivated	Unknown	Unknown
Unknown04	C100			Cyphomyrmex sp. symbiont	Ant-cultivated	Unknown	Unknown
UGM960619-01		AF079676		Cyphomyrmex costatus symbiont	Ant-cultivated	Panama	U.G. Mueller
920823-01		AF079678		Cyphomyrmex faunulus symbiont	Ant-cultivated	Brazil	T.R. Schultz
UGM960208-31		AF079679		Cyphomyrmex longiscapus slow symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM951227-05		AF079680		Cyphomyrmex longiscapus fast symbiont	Ant-cultivated	Panama	U.G. Mueller
X95010602		AF079681		Cyphomyrmex minutus symbiont	Ant-cultivated	Trinidad	U.G. Mueller
X95010603		AF079682		Cyphomyrmex minutus symbiont	Ant-cultivated	Trinidad	U.G. Mueller

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
X95011208		AF079683		Cyphomyrmex minutus symbiont	Ant-cultivated	Trinidad	U.G. Mueller
X95011309		AF079684		Cyphomyrmex minutus symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM930327-01		AF079685		Cyphomyrmex minutus symbiont	Ant-cultivated	Florida	U.G. Mueller
UGM930317-05		AF079686		Cyphomyrmex minutus symbiont	Ant-cultivated	Florida	U.G. Mueller
UGM930801-01		AF079687		Cyphomyrmex minutus symbiont	Ant-cultivated	Florida	U.G. Mueller
UGM960408-19		AF079688		Cyphomyrmex minutus symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM951216-02		AF079689		Cyphomyrmex minutus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM960408-14		AF079692		Cyphomyrmex rimosus symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM960408-18		AF079693		Cyphomyrmex rimosus symbiont	Ant-cultivated	Guyana	U.G. Mueller
921106-02		AF079694		Cyphomyrmex rimosus symbiont	Ant-cultivated	Trinidad and Tobago	T.R. Schultz
UGM960104-08		AF079696		Cyphomyrmex salvini symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM960415-12		AF079697		Mycocepurus goeldii symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM960116-01		AF079699		Mycocepurus smithii symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM951229-01		AF079701		Mycocepurus smithii slow symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM950110-02		AF079703		Mycocepurus smithii symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM950109-01		AF079704		Mycocepurus smithii symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM950110-09		AF079705		Mycocepurus smithii symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM950118-03		AF079707		Mycocepurus smithii symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM960110-03		AF079708		Mycocepurus curvispinosus symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM950612-03		AF079709		Mycocepurus curvispinosus symbiont	Ant-cultivated	Costa Rica	U.G. Mueller

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UGM960412-14		AF079711		Myrmicocrypta cf. buenzlii symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM960415-11		AF079712		Myrmicocrypta cf. buenzlii symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM960416-05		AF079713		Myrmicocrypta cf. buenzlii symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM951222-01		AF079715		Myrmicocrypta ednaella symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM960410-14		AF079716		Myrmicocrypta infusata symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM951227-01		AF079718		Myrmicocrypta sp. 2 symbiont	Ant-cultivated	Panama	U.G. Mueller
UGM950118-01		AF079719		Myrmicocrypta urichi symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM950107-02		AF079720		Myrmicocrypta urichi symbiont	Ant-cultivated	Trinidad	U.G. Mueller
UGM960421-02		AF079721		Mycetophylax conformis; type 2/3 symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM960421-06		AF079722		Mycetophylax conformis; type 2/3 symbiont	Ant-cultivated	Guyana	U.G. Mueller
UGM960404-10		AF079724		Mycetophylax emeryi symbiont	Ant-cultivated	Guyana	U.G. Mueller
240892-01		AF079725		Mycetoarotes paralleleus symbiont	Ant-cultivated	Brazil	T.R. Schultz
070892-11		AF079726		Mycetarotes senticosus symbiont	Ant-cultivated	Brazil	T.R. Schultz
3527-d		AF079727		Mycetosoritis hartmanni symbiont	Ant-cultivated	USA: Texas	S.P. Cover
UGM950613-02		AF079728		Mycetosoritis hartmanni symbiont	Ant-cultivated	Costa Rica	U.G. Mueller
DA373		AF079754		Trachymyrmex papulatus symbiont	Ant-cultivated	Argentina	D. Agosti
Leucoagaricus sp Vellinga		AY243641		Leucoagaricus sp Vellinga	Free-living	USA	
PA147		AF079729		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA148		AF079730		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA152		AF079731		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
PA156		AF079732		Lepiota cf. abruptibulba	Free-living	Panama	U.G. Mueller
PA165		AF079733		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA170		AF079734		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA185		AF079735		Lepiota cf. subclypeolaria	Free-living	Panama	U.G. Mueller
PA205		AF079736		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA234		AF079737		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA250		AF079738		Leucocoprinus cf. fragillissimus	Free-living	Panama	U.G. Mueller
PA252		AF079739		Leucocoprinus cf. phaeosticta	Free-living	Panama	U.G. Mueller
PA272		AF079740		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA280		AF079741		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA288		AF079742		Leucocoprinus cf. brebissonii	Free-living	Panama	U.G. Mueller
PA294		AF079743		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA300		AF079744		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA302		AF079745		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA309		AF079746		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA341		AF079747		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA351		AF079748		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA356		AF079749		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA363		AF079750		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA375		AF079751		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller

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PA409		AF079752		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA415		AF079753		Leucocoprinus cf. zamurensis	Free-living	Panama	U.G. Mueller
Leucoagaricusbresadolae		AF295929		Leucoagaricus bresadolae	Free-living		
Leucoagaricusleucothites		AF482865		Leucoagaricus leucothites	Free-living	Netherlands	
Leucoagaricusmeleagris		AF482867		Leucoagaricus meleagris	Free-living	Netherlands	
Leucoagaricusnympharum		AF482868		Leucoagaricus nympharum	Free-living	Germany	
Leucoagaricusamericanus		AY176407		Leucoagaricus americanus	Free-living	USA: Michigan	
Leucoagaricuscinerascens		AY176408		Leucoagaricus cinerascens	Free-living	USA: Hawaii	
Leucoagaricusmelanotrichus		AY176417		Leucoagaricus melanotrichus	Free-living	Netherlands	
Leucoagaricustener		AY176444		Leucoagaricus tener	Free-living	Netherlands	
Leucoagaricuscretaceus		AY176447		Leucocoprinus cretaceus	Free-living	Malaysia	
Leucoagaricussp Huijser		AY243643		Leucoagaricus sp Huijser	Free-living	Netherlands	
ASM1		EF527280		Lepiotaceae sp.	Free-living	Guadeloupe: Basse Terre	A.S. Mikheyev
ASM2		EF527281		Lepiotaceae sp.	Free-living	Guadeloupe: Basse Terre	A.S. Mikheyev
BR001		EF527282		Lepiotaceae sp.	Free-living	Brazil	A.S. Mikheyev
BR002		EF527283		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR003		EF527284		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR004		EF527285		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR005		EF527286		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR006		EF527287		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev

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BR007		EF527288		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR008		EF527289		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR009		EF527290		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR010		EF527291		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR011		EF527292		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR013		EF527293		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR014		EF527294		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR015		EF527295		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR016		EF527296		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR017		EF527297		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR018		EF527298		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR019		EF527299		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR020		EF527300		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR021		EF527301		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR023		EF527302		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR024		EF527303		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR025		EF527304		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR026		EF527305		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR027		EF527306		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR028		EF527307		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev

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BR029		EF527308		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR030		EF527309		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR031		EF527310		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR032		EF527311		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR033		EF527312		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR034		EF527313		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR037		EF527314		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR038		EF527315		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR039		EF527316		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR040		EF527317		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR041		EF527318		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR042		EF527319		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR043		EF527320		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR044		EF527321		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
BR045		EF527322		Lepiotaceae sp	Free-living	Brazil	A.S. Mikheyev
PA298		EF527323		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA303		EF527324		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA393		EF527325		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA451A		EF527326		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA455		EF527327		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller

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PA465		EF527328		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA476		EF527329		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA477		EF527330		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA480		EF527331		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA481		EF527332		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA491		EF527333		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA493		EF527334		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA501		EF527335		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA511		EF527336		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA519		EF527337		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA528		EF527338		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA530		EF527339		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA531		EF527340		Lepiotaceae sp.	Free-living	Panama	U.G. Mueller
PA600		EF527341		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA603		EF527342		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA606		EF527343		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA607		EF527344		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA608		EF527345		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA609		EF527346		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA610AandB		EF527347		Lepiotaceae sp.	Free-living	Panama	T. Vo

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
PA611		EF527348		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA614		EF527349		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA615		EF527350		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA616		EF527351		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA617		EF527352		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA618		EF527353		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA619		EF527354		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA620		EF527355		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA621		EF527356		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA623		EF527357		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA624		EF527358		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA625		EF527359		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA628		EF527360		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA629		EF527361		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA630AandB		EF527362		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA631		EF527363		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA633		EF527364		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA634		EF527365		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA635		EF527366		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA636		EF527367		Lepiotaceae sp.	Free-living	Panama	T. Vo

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
PA639		EF527368		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA640		EF527369		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA641		EF527370		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA643		EF527371		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA644		EF527372		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA645		EF527373		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA646		EF527374		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA647		EF527375		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA648		EF527376		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA650AandB		EF527377		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA652		EF527378		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA653		EF527379		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA654		EF527380		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA655		EF527381		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA657		EF527382		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA659		EF527383		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA660		EF527384		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA663		EF527385		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA664		EF527386		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA665		EF527387		Lepiotaceae sp.	Free-living	Panama	T. Vo

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number ¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
PA667		EF527388		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA669		EF527389		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA671		EF527390		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA672		EF527391		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA673		EF527392		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA675		EF527393		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA676		EF527394		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA677		EF527395		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA678		EF527396		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA680		EF527397		Lepiotaceae sp.	Free-living	Panama	T. Vo
PA681		EF527398		Lepiotaceae sp.	Free-living	Panama	T. Vo
TX006		EF527399		Lepiotaceae sp.	Free-living	USA: Texas	U.G. Mueller
TX008AandB		EF527400		Lepiotaceae sp.	Free-living	USA: Texas	U.G. Mueller
Leucoagaricus sp		LSU85320		Leucoagaricus sp	Free-living	Costa Rica	
Lagarnaucin		U85315		Leucoagaricus naucinus	Free-living	USA: Virginia	
Lagarrubrot		U85316		Leucoagaricus rubrotinctus	Free-living	Costa Rica	
Lagarameric		U85317		Leucoagaricus americanus	Free-living	USA: New York	
Lagarhorten		U85318		Leucoagaricus hortensis	Free-living	Colombia	
LagarcepaesCol		U85321		Leucoagaricus cepaestipes	Free-living	Colombia	
Lcoprlongis		U85322		Leucocoprinus longistriatus	Free-living	USA: North Carolina	

Collection ID	DNA Extraction Number	GenBank Accession Number - ITS	Genotype Number¹	Ant Host or Free-living Fungus ID	Free-living/ Ant-cultivated	Locality (Country)	Collector
Lagarbirnba		U85323		Leucoagaricus birnbaumii	Free-living	United Kingdom	
Lepioclypeo		U85326		Lepiota clypeolaria	Free-living	South Korea	
Lepiocrista		U85327		Lepiota cristata	Free-living	USA: Montana	
Lepiofelin		U85330		Lepiota felina	Free-living	USA: Montana	
Lepioflamme		U85331		Lepiota flammeotincta	Free-living	Costa Rica	
¹ Genotype numbers only assigned for those with more than one exemplar.							

Supplementary Methods

DNA extraction and sequencing. We extracted DNA from ants by grinding tissue of a single ant (if multiple ants were collected per colony) or a leg (if only one ant was collected from a colony) in liquid nitrogen and then followed the standard protocol of a Qiagen DNEasy extraction kit. For fungi, we extracted DNA by placing lyophilized or alcohol-preserved tissue in approximately 200 μ L of 10% Bio-Rad Chelex solution and heated the samples in a programmable thermal controller (step 1: 60°C for 1.5 h; step 2: 99°C for 15 min). We amplified 1,043 bp of mitochondrial COI and an approximately 600 bp fragment of wingless for the ants in 25 μ L polymerase chain reactions (PCR; 1.125 μ L MgCl₂, 0.2 μ L dNTPs, 1 μ L forward primer, 1 μ L reverse primer, 1 μ L 10X Buffer, 0.1 μ L Platinum Taq DNA Polymerase (Invitrogen), 4.575 μ L water, and 1 μ L DNA) using the following protocol (step1: 94.0°C for 2min; step2: 94.0°C for 30 sec; step 3: 65.0°C decreasing 1.0°C every 30 sec cycle; step 4: 72.0°C for 1 min; step 5: 15 cycles to step 2; step 6: 94.0°C for 30 sec; step 7: 50.0°C for 30 sec; step 8: 72.0°C for 1 min; step 9: 25 times to step 6; step 10: 72.0°C for 15 min). Primer pairs used to amplify the mtCOI were 5' -CAACATTTATTTTGATTTTTTGG - 3'; 5' - GGTCAACAAATCATAAAGATATTGG - 3' (forward primers) and 5' - CATGATACMTATTAYGTWGTWGC - 3'; 5' - TGATTTTTTGGTCACCCTGAAGTTTA - 3' (reverse primers) and wingless were 5' - GCWGTRACTCACAGYATCGC - 3' (forward primer) and 5' - CGRTCCTTBAGRTRTCGCC - 3' (reverse primer). For the fungal cultivars, we amplified an approximately 700-750 bp fragment of ITS using the same PCR cocktail as for the ants. Primer pairs used to amplify ITS were 5'-

GGAAGTAAAAGTCGTAACAAGG - 3' (forward primer) and 5' -
GCATATCAATAAGCGGAGGA - 3' (reverse primer).

After amplification of PCR products, we purified the DNA with exonuclease I and shrimp alkaline phosphatase (EXO-SAP IT, USB Corporation, Cleveland, OH) to remove unincorporated nucleotides and primers. One microliter of the cleaned PCR product provided template for sequencing. Sequencing reactions were prepared using the ABI Prism BigDye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA), then cleaned with Sephadex columns, and run on an ABI 3100 automated sequencer (Applied Biosystems) for analysis. Sequences have been deposited in GenBank under accession numbers JQ617314-JQ617746.

Data partitioning. Listed below are the models employed for each data partition based on results obtained from ModelTest v3.7³⁹. For models that are unavailable in MrBayes, the next most complex available model was used.

Partition	Model
Ants	
COI position 1	GTR+I+G
COI position 2	HKY+I+G
COI position 3	GTR+G
wg positions 1&2	HKY+I
wg position 3	HKY
wg intron	HKY+I

Fungi: Clade 1

Conserved TVM+I+G

Rapidly evolving HKY+I+G

Fungi: Clade 2

Conserved: TVM+G

Rapidly evolving TrNef

Bayesian phylogenetic inference. To address known problems with branch-length estimation in MrBayes^{e.g., 38,54-57}, we reduced the branch-length priors by setting `brlenspr=unconstrained:Exp(100)` and we used the *props* command to increase the proposal rate from 1,000 to 10,000 and to decrease the Dirichlet alpha parameter from 500 to 250 for the rate multipliers (proposal mechanism 26 in MrBayes).

Burn-in, convergence, and stationarity were assessed using Tracer v1.5⁵² by examining PSRF values in the MrBayes.stat output files, and by using Bayes factor comparisons of marginal likelihoods of pairs of runs in Tracer, which employs the weighted likelihood bootstrap estimator of Newton and Raftery⁵⁸ as modified by Suchard et al.⁵⁹, with standard error estimated using 1,000 bootstrap pseudoreplicates.

Maximum likelihood analyses. In all analyses the value for modweight was calculated as $0.0005 \times (\#\text{subsets} + 1)$ (Zwickl, pers. comm.). Most ML analyses were carried out on the University of Maryland distributed-computing Lattice Project (<http://boinc.umiacs.umd.edu/about.php>⁶⁰⁻⁶²).

Supplementary References

54. Brown, J. M., Hedtke, S. M., Lemmon, A. R. & Lemmon, E. M. When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates.

Syst. Biol. **59**, 145-161 (2010).

55. Marshall, D. C., Simon, C. & Buckley, T.R. Accurate branch length estimation in partitioned Bayesian analyses requires accommodation of among-partition rate variation and attention to branch length priors. *Syst. Biol.* **55**, 993-1003 (2006).

56. Marshall, D. C. Cryptic failure of partitioned Bayesian phylogenetic analyses: lost in the land of long trees. *Syst. Biol.* **59**, 108-117 (2010).

57. Spinks, P. Q. & Shaffer, H. B. Conflicting mitochondrial and nuclear phylogenies for the widely disjunct *Emys* (Testudines: Emydidae) species complex, and what they tell us about biogeography and hybridization. *Syst. Biol.* **58**, 1-20 (2009).

58. Newton, M. A. & Raftery, A. E. Approximate Bayesian inference by the weighted likelihood bootstrap. *J. R. Stat. Soc. Ser. B* **56**, 3-48 (1994).

59. Suchard, M. A., Weiss, R. E. & Sinsheimer, J. S. Bayesian selection of continuous-time Markov chain evolutionary models. *Mol. Biol. Evol.* **18**, 1001-1013 (2001).

60. Bazinet, A. L., Myers, D. S., Fuetsch, J. & Cummings, M. P. Grid Services Base Library: a high-level, procedural application program interface for writing Globus-based Grid services. *Future Generation Computer Systems* **23**, 517-522 (2007).

61. Bazinet, A. L. & Cummings, M. P. in *Distributed and Grid Computing - Science Made Transparent for Everyone. Principles, Applications and Supporting Communities* (ed Weber, M. H. W.) 2-13 (Rechenkraft.net, 2009).

62. Myers, D. S., Bazinet, A. L. & Cummings, M. P. in *Grids for Bioinformatics and Computational Biology* (eds Talbi, E.-G. & Zomaya, A.) Ch. 4, 71-85 (Wiley, 2008).