




ORIGINAL ARTICLE**Biogeography of mutualistic fungi cultivated by leafcutter ants**

Ulrich G. Mueller¹  | Heather D. Ishak¹ | Sofia M. Bruschi^{1,2} | Chad C. Smith¹ |
 Jacob J. Herman¹ | Scott E. Solomon^{1,2,3} | Alexander S. Mikheyev^{1,4}  |
 Christian Rabeling^{1,5} | Jarrod J. Scott¹ | Michael Cooper¹ | Andre Rodrigues² |
 Adriana Ortiz⁶ | Carlos R. F. Brandão⁷ | John E. Lattke⁸ | Fernando C. Pagnocca² |
 Stephen A. Rehner⁹ | Ted R. Schultz¹⁰ | Heraldo L. Vasconcelos¹¹ |
 Rachelle M. M. Adams¹² | Martin Bollazzi¹³ | Rebecca M. Clark¹⁴ | Anna G. Himler^{1,15} |
 John S. LaPolla^{10,16} | Inara R. Leal¹⁷ | Robert A. Johnson⁵ | Flavio Roces¹⁸ |
 Jeffrey Sosa-Calvo⁵ | Rainer Wirth¹⁹ | Maurício Bacci Jr² 

¹Department of Integrative Biology, University of Texas at Austin, Austin, TX, USA²Centro de Estudos de Insetos Sociais, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil³Department of Ecology & Evolutionary Biology, Rice University, Houston, TX, USA⁴Okinawa Institute of Science & Technology, Kunigami, Okinawa, Japan⁵School of Life Sciences, Arizona State University, Tempe, AZ, USA⁶Universidad Nacional de Colombia, Medellín, Colombia⁷Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil⁸Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil⁹Mycology and Nematology Genomic Diversity and Biology Laboratory, Beltsville, MD, USA¹⁰Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA¹¹Instituto de Biologia, Federal University of Uberlândia, Uberlândia, MG, Brazil¹²Department of Evolution, Ecology & Organismal Biology, Museum of Biological Diversity, Columbus, OH, USA¹³Section of Entomology, Universidad de la República, Montevideo, Uruguay¹⁴Integrative Biology, University of California-Berkeley, Berkeley, CA, USA¹⁵Department of Biology, College of Idaho, Caldwell, ID, USA¹⁶Department of Biological Sciences, Towson University, Towson, MD, USA¹⁷Departamento de Botânica, Universidade Federal de Pernambuco, Recife, PE, Brazil¹⁸Department of Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Würzburg, Germany¹⁹Department of Plant Ecology and Systematics, University of Kaiserslautern, Kaiserslautern, Germany**Correspondence**Ulrich G. Mueller, Integrative Biology,
University of Texas at Austin, Austin, TX,
USA.Email: umueller@austin.utexas.edu
andMaurício Bacci, Centro de Estudos de
Insetos Sociais, Universidade Estadual
Paulista, Rio Claro, São Paulo, Brazil.
Email: mbacci@rc.unesp.br**Funding information**Conselho Nacional de Desenvolvimento
Científico e Tecnológico, Grant/Award
Number: 302777/2003-2; Fundação de**Abstract**

Leafcutter ants propagate co-evolving fungi for food. The nearly 50 species of leafcutter ants (*Atta*, *Acromyrmex*) range from Argentina to the United States, with the greatest species diversity in southern South America. We elucidate the biogeography of fungi cultivated by leafcutter ants using DNA sequence and microsatellite-marker analyses of 474 cultivars collected across the leafcutter range. Fungal cultivars belong to two clades (Clade-A and Clade-B). The dominant and widespread Clade-A cultivars form three genotype clusters, with their relative prevalence corresponding to southern South America, northern South America, Central

Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 03/08112-0; CAPES Fellowships; National Science Foundation, Grant/Award Number: 9707209, 0110073, 998379, 0639879, 0919519, 0949689, 1354666, 0206372, 0407772, 07012333, 0508613, 0508698, 0808164, 1456964, 1654829

and North America. Admixture between Clade-A populations supports genetic exchange within a single species, *Leucocoprinus gongylophorus*. Some leafcutter species that cut grass as fungicultural substrate are specialized to cultivate Clade-B fungi, whereas leafcutters preferring dicot plants appear specialized on Clade-A fungi. Cultivar sharing between sympatric leafcutter species occurs frequently such that cultivars of *Atta* are not distinct from those of *Acromyrmex*. Leafcutters specialized on Clade-B fungi occur only in South America. Diversity of Clade-A fungi is greatest in South America, but minimal in Central and North America. Maximum cultivar diversity in South America is predicted by the Kusnezov–Fowler hypothesis that leafcutter ants originated in subtropical South America and only dicot-specialized leafcutter ants migrated out of South America, but the cultivar diversity becomes also compatible with a recently proposed hypothesis of a Central American origin by postulating that leafcutter ants acquired novel cultivars many times from other nonleafcutter fungus-growing ants during their migrations from Central America across South America. We evaluate these biogeographic hypotheses in the light of estimated dates for the origins of leafcutter ants and their cultivars.

KEYWORDS

Attamyces bromatificus, insect–fungus mutualism, *Leucoagaricus gongylophorus*, *Leucoagaricus weberi*, *Leucocoprinus gongylophorus*, symbiosis

1 | INTRODUCTION

Biogeographic distributions provide clues about evolutionary processes, such as ancient dispersal and vicariance events that shaped macroevolutionary patterns, or adaptation and gene flow influencing microevolutionary processes (Avice, 2009; Brown & Lomolino, 1998; Wallace, 1876). In mutualistic associations between two partners, similarities or differences in biogeographic distributions between codependent partners can facilitate inference of such evolutionary processes (Alvarez, McKey, Kjellberg, & Hossaert-McKey, 2010; Hembry & Althoff, 2016; Satler & Carstens, 2016, 2017; Thompson, 2005). Cobiogeographic patterns of mutualistic partners require cautious interpretation, however, particularly regarding congruence and incongruence of patterns, because evolutionary forces and demographies can differ markedly between partners (Alvarez et al., 2010; Chomicki, Janda, & Renner, 2017; Espindola, Carstens, & Alvarez, 2014; Herre, Knowlton, Mueller, & Rehner, 1999; Tian et al., 2015). For example, population sizes, migration rates, mutation rates and generation times can differ by orders of magnitude between a host and a symbiotic partner (Degnan, Lazarus, Brock, & Wernegreen, 2004; Lutzoni & Pagel, 1997; Moran & Wernegreen, 2000; Woolfit & Bromham, 2003), and dispersal barriers restricting gene flow for one partner (e.g., a pollinating bee) may not impede gene flow for the other partner (e.g., the pollinated plant). Such differences in evolutionary forces are particularly pronounced in mutualistic associations between macro-organisms and fast-evolving microbial symbionts, or microbial symbionts that do not comigrate with a host, disperse independently of the host and that are acquired by hosts

from local microbial populations (e.g., many plant–endophyte, mycorrhizal plant–fungus, lichen algal–fungus or host–microbe gut mutualisms) (Dal Grande, Widmer, Wagner, & Scheidegger, 2012; Kaltentpoth, Roeser-Mueller, Stubblefield, Seger, & Strohm, 2014; Palmer, Pringle, Stier, & Holt, 2015; Silverstein, Correa, & Baker, 2012; Weiblen & Treiber, 2015; Wornik & Grube, 2010).

In many mutualistic host–microbe associations, a greater dispersal ability of the microbial partners results in predictable differences in population-genetic and biogeographic patterns between hosts and microbial symbionts, for example lesser genetic differentiation between populations for the symbiont compared to the host (Hulcr & Stelinski, 2017; Kellner et al., 2013; Mueller, Mikheyev, Solomon, & Cooper, 2011; Nobre, Koné, Konaté, Linsenmair, & Aanen, 2011; Six, 2012), or greater potential for a single symbiont lineage to interact with different allopatric host species (Mueller & Gerardo, 2002; Palmer et al., 2015; Thompson, 2005; Weiblen & Treiber, 2015). In contrast, when symbiont dispersal is limited, populations of symbionts are predicted to differentiate across space, as, for example, in the symbiotic ectomycorrhizal fungus *Rhizopogon* where limited dispersal by vectoring mammals maintains population-genetic structure between proximate islands (Grubisha, Bergemann, & Bruns, 2007). As a general rule, however, widely dispersing symbionts are thought to be associated with a greater diversity of hosts than symbionts with limited dispersal (Herre et al., 1999; Roy et al., 2008). Biogeographic analyses of such microbial symbionts are often complicated by insufficient knowledge of species boundaries of microbial symbionts, requiring high-resolution genetic analyses to differentiate species and population boundaries (e.g., Douhan, Vincenot, Gryta, &

specialized to cut grass or dicot leaves, or utilized both types of leaves for fungiculture. *Ac. striatus* and *Ac. silvestrii* reportedly cut both grass and dicots, with foraging preferences possibly changing seasonally between grass and dicots (Bucher & Montenegro, 1974; Fowler & Claver, 1991; Fowler, Forti, Pereira-da-Silva, & Saes, 1986; Gonçalves, 1961).

5 | CONCLUSION

Most efforts to elucidate leafcutter ant–fungus associations focused so far on leafcutter ants in Central and North America (Table S6), but these leafcutter symbioses, all of them involving dicot-specialized leafcutter species, are not representative of the more complex leafcutter symbioses existing across South America (Figures 1 and S1). Leafcutter species specialized on cultivation of Clade-B fungi occur only in South America (ranging from Argentina to Colombia; Figure S1), the highest concentration of Clade-B-cultivating leafcutter nests found so far is in southern South America (Table S1), and Clade-A fungi of leafcutter ants are more diverse in South America than in Central and North America (Figure 1). This co-occurrence of the greatest leafcutter ant species diversity and greatest cultivar diversity in southern South America may not be a coincidence, yet the leafcutter ant–fungus associations in the grasslands of southern South America are far less understood than those in highly disturbed Central America forests dominated by weedy leafcutter ant species. If the Kusnezov–Fowler hypothesis for the origin of leafcutter ants in subtropical southern South America is correct and accounts for the concentrated diversity of leafcutter species there (Bacci et al., 2009; Borgmeier, 1959; Brandão et al., 2011; Delabie et al., 2011; Della Lucia, 2011; Farji-Brener & Ruggiero, 1994; Fowler, 1983; Gonçalves, 1961; Kusnezov, 1963; Mariconi, 1970; Mueller & Rabeling, 2008; Wild, 2007), a comprehensive cultivar survey in Argentina, Uruguay, Paraguay, Bolivia and sub-Amazonian Brazil is most likely to uncover unknown types of leafcutter fungi (i.e., “Clade-C” or “Clade-D” cultivars), which will inform hypotheses on the diversity of cultivars available for cultivation at the origin of leafcutter ants.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for comments on the manuscript. The work was supported by the Ministério da Educação do Brasil, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Estado de São Paulo of Brazil (awards CAPES/UT 05/02, CNPq 302777/2003-2 and FAPESP 03/08112-0 to MBJr; CAPES Fellowships to SMB & RA); the National Science Foundation (award 9707209 to UGM, TRS, SAR; 0110073 to UGM, TRS & HLV; 998379, 0639879, 0919519, 0949689 and 1354666 to UGM; 0206372 to AGH; 0407772 and 07012333 to SES; 0508613 to ASM; 0508698 to RMMA; 0808164 to CR; 1456964 and 1654829 to CR and TRS); and the W.M. Wheeler Endowment of the University of Texas at Austin.

DATA ACCESSIBILITY

DNA sequences: GenBank accessions GQ853919–GQ854367, GQ854817–GQ855186, HQ391561–HQ391895.

Sample information, microsatellite genotypes, analyses: Tables S1–S10 in Supporting Information.

AUTHOR CONTRIBUTIONS

Project coordination: M.Ba. Jr, U.G.M. Coordination of regional collections: M.Ba. Jr, M.Bo., C.R.F.B., A.G.H., J.E.L., I.R.L., A.S.M., U.G.M., A.O., F.C.P., C.R., F.R., S.A.R., S.E.S., T.R.S., R.W., H.L.V. Field work, sample vouchering: R.M.A., M.Ba. Jr, M.Bo., R.M.C., A.G.H., J.E.L., J.S.L., I.R.L., R.A.J., A.S.M., U.G.M., A.O., F.R., C.R., S.A.R., A.R., T.R.S., J.J.S., S.E.S., J.S.-C., H.L.V., R.W. Fungal isolations: A.O., A.R., U.G.M., S.A.R. Molecular analyses: S.M.B., H.D.I., M.C., A.S.M., J.J.S. Microsatellite marker development: J.J.S., M.C., H.D.I. Microsatellite marker scoring, phylogenetic analyses: H.D.I. Population genetic analyses: C.C.S., J.J.H. Writing of manuscript: U.G.M., H.D.I., T.R.S., C.R., S.A.R., F.R. All authors edited, commented on, and approved submission of the manuscript.

ORCID

Ulrich G. Mueller  <http://orcid.org/0000-0003-2677-8323>

Alexander S. Mikheyev  <http://orcid.org/0000-0003-4369-1019>

Maurício Bacci Jr  <http://orcid.org/0000-0002-5619-1411>

REFERENCES

- Adams, R. M. M., Mueller, U. G., Holloway, A. K., Green, A. M., & Narozniak, J. (2000). Garden sharing and garden stealing in fungus-growing ants. *Naturwissenschaften*, *87*, 491–493. <https://doi.org/10.1007/s001140050765>
- Alvarez, N., McKey, D., Kjellberg, F., & Hossaert-McKey, M. (2010). Phylogeography and historical biogeography of obligate specific mutualisms. In S. Morand & B. R. Krasnov (Eds.), *The biogeography of host–parasite interactions* (pp. 31–39). Oxford, UK: Oxford University Press.
- Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography*, *36*, 3–15. <https://doi.org/10.1111/j.1365-2699.2008.02032.x>
- Bacci, Jr, M., Solomon, S. E., Mueller, U. G., Martins, V. G., Carvalho, A. O. R., Vieira, L. G. E., & Silva-Pinhati, A. C. O. (2009). Phylogeny of leafcutter ants in the genus *Atta* Fabricius (Formicidae: Attini) based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution*, *51*, 427–437. <https://doi.org/10.1016/j.ympev.2008.11.005>
- Bich, G. A., Castrillo, M. L., Villalba, L. L., & Zapata, P. D. (2016). Isolation of the symbiotic fungus of *Acromyrmex pubescens* and phylogeny of *Leucoagaricus gongylophorus* from leaf-cutting ants. *Saudi Journal of Biological Sciences*, *24*, 851–856.
- Borgmeier, T. (1959). Revision der Gattung *Atta* Fabricius (Hymenoptera, Formicidae). *Studia Entomologica*, *22*, 321–390.
- Bot, A. N., Rehner, S. A., & Boomsma, J. J. (2001). Partial incompatibility between ants and symbiotic fungi in two sympatric species of *Acromyrmex* leaf-cutting ants. *Evolution*, *55*, 1980–1991. <https://doi.org/10.1111/j.0014-3820.2001.tb01315.x>