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Phylogenomic reconstruction reveals new insights into the evolution and biogeography of *Atta* leaf-cutting ants (Hymenoptera: Formicidae)

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Abstract. *Atta* Fabricius is an ecologically dominant leaf-cutting ant genus, the major herbivore of the Neotropics, and an agricultural pest of great economic importance. Phylogenetic relationships within *Atta* have until now remained uncertain, and the delimitation and identification of a subset of *Atta* species are problematic. To address these phylogenetic uncertainties, we reconstruct the most comprehensive phylogenetic estimate to date of *Atta* by employing ultraconserved elements (UCEs). We recovered 2340 UCE loci from 224 *Atta* specimens, which include 14 out of the 15 identifiable species from across their geographic distributions, and 49 outgroup specimens. Our results strongly support the monophyly of *Atta* and of the four clades that coincide with the previously recognized subgenera *Archeatta* Gonçalves, *Atta* s.s. Emery, *Epiatta* Borgmeier, and *Neoatta* Gonçalves. The Archeatta clade contains three species occurring in North and Central America and the Caribbean and is the sister group of the remainder of all other *Atta* species. The Atta s.s. clade is composed of two species occupying North, Central, and South America. The Epiatta clade contains seven entirely South American species and the two species of the Neoatta clade occur in Central and South America. Divergence-dating analyses identify a series of major events in the Miocene, such as the divergence of *Acromyrmex* Mayr and *Atta* 16.7 million years ago (Ma) and the crown-group origin of *Atta* around 8.5 Ma. Extant *Atta* species evolved very recently, originating in the early Pleistocene, approximately 1.8–0.3 Ma (crown-group ages). We provide the first evidence that *Atta goiana* Gonçalves belongs to the Epiatta clade and that *Atta robusta* Borgmeier is the species with the youngest crown-group age of 0.3 Ma. The very young ages of *Atta* and its component species indicate a recent, rapid radiation. Biogeographic analyses suggest that the range of the most recent common ancestor of *Atta* consisted of the combined North/Central America and NW South America bioregions and that one daughter lineage subsequently dispersed into South America, rapidly diversifying in the newly formed Cerrado biome and Chaco, and further dispersing into the Atlantic Forest, Caatinga, and Pampas bioregions.

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Introduction

The fungus-growing ants are perhaps the most conspicuous and charismatic ants in the Neotropics. Their association with fungal cultivars originated approximately 55–65 million years ago (Ma) and has become one of the most successful symbiotic systems in nature (Weber, 1972; Hölldobler & Wilson, 1990; Mueller *et al.*, 1998; Schultz & Brady, 2008; Nygaard *et al.*, 2016; Branstetter *et al.*, 2017a). Leaf-cutting ants, a monophyletic group composed of four valid genera (*Atta* Fabricius, *Acromyrmex* Mayr, *Amoimyrmex* Cristiano *et al.*, and *Pseudoatta* Gallardo, a workerless social parasite of multiple *Acromyrmex* species), constitute what is arguably the most highly specialized subset (∼15%) of the larger group of fungus-growing ants (tribe Attini, subtribe Attina; the *Atta* genus-group *sensu* Ward *et al.*, 2015, hereafter referred to as 'attine' ants). Leaf-cutting ants are thought to have originated around 15–19 Ma (Nygaard *et al.*, 2016; Ješovnik *et al.*, 2016; Branstetter *et al.*, 2017a; Li *et al.*, 2018) and belong to the 'higher-attine' ants, which also include five genera of non-leaf-cutting ants (Schultz & Brady, 2008; Mueller *et al.*, 2017, 2018; Solomon *et al.*, 2019). Compared with fungi cultivated by the ancestral attine ant and still cultivated by the so-called 'lower-attine' ants, which are facultative symbionts, fungi cultivated by leaf-cutting ants are obligate mutualists that may have originated more recently, around 22–30 Ma, coincident with or subsequent to the origin of the higher-attine ants (Schultz & Brady, 2008; Mikheyev *et al.*, 2010; Nygaard *et al.*, 2016; Branstetter *et al.*, 2017a; Mueller *et al.*, 2017). Further derived within higher-attine agriculture, the mutualistic symbiotic system of leaf-cutting ants is extremely specialized, consisting of approximately 50 species that mostly cultivate a single species of fungus, *Leucoagaricus gongylophorus* Möller (also referred to as 'Clade-A' or *Attamyces* fungi) (Silva-Pinhati *et al.*, 2004; Mikheyev *et al.*, 2006; Mueller *et al.*, 2017, 2018); although a few leaf-cutting ant species were recently shown to cultivate fungal species arising in the sister clade of *L. gongylophorus*, sometimes referred to as 'Clade-B' (Mueller *et al.*, 2018; Solomon *et al.*, 2019), which are also cultivated by non-leaf-cutting higher-attine ants. In addition to being obligate symbionts, higher-attine fungi, including *L. gongylophorus*, are polyploid, consistently produce nutritious swollen hyphal tips (gongylidia) and express significantly elevated enzyme profiles mostly associated with the degradation of fresh plant material (Schultz & Brady, 2008; De Fine Licht *et al.*, 2013; Masiulionis *et al.*, 2014; Mueller *et al.*, 2018).

Like the other fungus-growing ants, leaf-cutting ants depend on their fungus for food (Weber, 1982; Siqueira *et al.*, 1998). They differ from other fungus-growing ants, however, by cutting and using fresh plant material as the substrate on which they grow their gardens (Weber, 1966; Hölldobler & Wilson, 2011). Because of this behaviour and the large quantities of vegetal material that they harvest, several species have become pests of human agriculture, with significant economic impact (Moser, 1986; Fowler *et al.*, 1989; Cherrett, 1989; Hölldobler & Wilson, 1990; Wirth *et al.*, 2003; Della Lucia, 2003, 2011; Leal *et al.*, 2014; Fischer, 2015; Schowalter & Ring, 2017). Many species have large underground colonies, particularly those of *Atta* with up to eight million workers (Fernández *et al.*, 2015), and have been characterized as ecosystem engineers due to their high capacity to modify the environment (Jones *et al.*, 1994; Leal *et al.*, 2014). The population densities of some leaf-cutting species are enhanced by anthropic activities (Fowler *et al.*, 1986) such as deforestation and agricultural land use (Forti *et al.*, 2006; Urbas *et al.*, 2007; Wirth *et al.*, 2007). In addition, leaf-cutting ants are characterized by colony longevity (queens of *Atta* can live 15 or more years; Fernández & Serna, 2019), multiple interactions with other organisms, multiple queen mating frequencies, and, in *Atta*, claustral nest founding (Mehdiabadi & Schultz, 2010; Hölldobler & Wilson, 1990, 2011).

In addition to their biological and ecological importance, the long-lived colonies of *Atta* leaf-cutting ants represent a consistently dependable nutritional resource for diverse organisms, and several Native Amerindian groups used them as food. Their gigantic subterranean nests significantly contribute to nutrients in the soil (Weber, 1966; Dufour, 1987). Leaf-cutting nests host many underground microorganisms and insects, which derive nutrition from the large quantity of organic matter provided by the ants (Bacci *et al.*, 1995; Mountinho *et al.*, 2003; Rodrigues *et al.*, 2005). Fifteen species are currently recognized in the genus *Atta* (Bacci *et al.*, 2009; Hölldobler & Wilson, 2011; Fernández *et al.*, 2015; Bolton, 2021), and their biogeographic distributions extend from the southern United States in the north, to north-eastern Argentina and western Uruguay in South America (Borgmeier, 1959; Kempf, 1972; Hölldobler & Wilson, 1990, 2011; Fernández & Sendoya, 2004; Janicki *et al.*, 2016), and to some islands in the Caribbean (Cuba, Trinidad & Tobago, and the Lesser Antilles; Weber, 1968; Wilson, 1986; Fontenla Rizo, 1995). Due to its high ecological and economic importance, the biology, behaviour, and control of the genus *Atta* are relatively well studied compared with that of most other tropical insects (Hölldobler & Wilson, 2011; Della Lucia, 2003, 2011; Mueller & Rabeling, 2008). However, the delimitation of species and their taxonomic identification remain problematic (Borgmeier, 1959; Schultz & Brady, 2008; Bacci *et al.*, 2009; Solomon *et al.*, 2019).

Early studies on the phylogeny and systematics of the genus *Atta* were largely based on morphological characters. Emery (1913, 1922) was the first entomologist to differentiate species using characters of the male genitalia. These characters of the males and those of major workers were combined with biological information and nest architecture to produce the first complete revision of the genus, including the grouping of species into subgenera (Gonçalves, 1942). Later, Borgmeier (1950, 1959) added information to Gonçalves' (1942) study, analysing 14 of the species recognized at that time and constructing the identification key still currently in use. These studies require updating for a number of reasons, including the use of subspecific names, a category disfavoured by modern ant taxonomists (Wilson & Brown Jr, 1953; Bolton *et al.*, 2006), as well as taxonomic changes that have occurred in the last decades (e.g., Fontenla Rizo, 1995; Delabie, 1998) but have not yet been included in a general taxonomic review.

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Studies of morphology, nesting behaviour, ecology, biogeography, and pest control, among other topics, can provide information about the classification, taxonomy, and identification of *Atta* species as well as of leaf-cutting ants in general (Hölldobler & Wilson, 2011; Fernández *et al.*, 2015). However, the sole use of morphological characters for species identification is problematic because large-sized workers have a high level of within-species polymorphism (Borgmeier, 1950, 1959) and small-sized workers from different species are very similar to each other. Male genitalic morphology is informative for taxonomy (Gonçalves, 1942, 1986), but males only emerge once each year during the mating season and are therefore underrepresented in collections. Very few collections have complete series representing all sexes and female castes from the same nest for all or most of the species (S.E. Solomon, personal communication; C.A. Barrera, personal observation), providing fragmented information for systematic studies. In addition, to date, there have been only a few studies, mainly molecular phylogenetic, that have focused on leaf-cutting ants in general or, in particular, on individual leaf-cutting ant genera (Sumner *et al.*, 2004; Bacci *et al.*, 2009; Cristiano *et al.*, 2013, 2020; Pereira *et al.*, 2018; Rabeling *et al.*, 2018; Sánchez-Restrepo *et al.*, 2019). These challenges, particularly in *Atta* taxonomy and phylogenetics, motivated this present study, which aims to improve our understanding of the evolutionary history and phylogenetic relationships of the leaf-cutting ants and which will serve as a reference for future taxonomic studies of the genus *Atta*.

During the past four decades, phylogenetic systematics has progressed rapidly due to the use of molecular genetic markers. Most recently, during the past decade, progress in understanding evolutionary relationships in ants has accelerated due to techniques employing massive sequencing of genomic data (Nygaard *et al.*, 2016; Ward *et al.*, 2016; Ješovnik *et al.*, 2016; Branstetter *et al.*, 2017b). Techniques involving selective enrichment and next-generation sequencing have allowed the efficient processing of large numbers of taxa for a large number of independent loci in a short time frame (Faircloth *et al.*, 2012, 2015; Lemmon & Lemmon, 2013; Do Amaral *et al.*, 2015). In particular, ant phylogenetics has benefited most from the sequencing of so-called ultraconserved elements (UCEs). UCEs are genomic regions that have been highly conserved in genomic evolution (Bejerano *et al.*, 2004) and, together with more variable flanking regions, provide valuable data for phylogenetic studies at a broad range of phylogenetic levels (Crawford *et al.*, 2012; Faircloth *et al.*, 2012; Smith *et al.*, 2014; Blaimer *et al.*, 2015).

Technological advances have also greatly improved the ability to reconstruct the evolutionary history of fungus-growing ants. Molecular data have provided critical information for reconstructing evolutionary relationships in the tribe Attini, the larger group that includes the fungus-growing ants (Ward *et al.*, 2015), thereby revealing the major transitions in ant-fungus co-evolution (Schultz & Brady, 2008; Mehdiabadi *et al.*, 2012; Nygaard *et al.*, 2016; Sosa-Calvo *et al.*, 2017; Branstetter *et al.*, 2017a), the history of ant-Actinobacteria co-evolutionary associations (Currie *et al.*, 2003; Meirelles *et al.*, 2015; Li *et al.*, 2018), and the evolution of asexual reproduction and social parasitism (Sumner *et al.*, 2004; Rabeling *et al.*, 2011, 2014; Schrader *et al.*, 2021), as well as the revision, recognition, and description of new genera and species (Ješovnik *et al.*, 2017; Rabeling *et al.*, 2015, 2019; Sosa-Calvo *et al.*, 2013, 2018; Solomon *et al.*, 2019; Cristiano *et al.*, 2013, 2020). Molecular data have likewise proven useful in studies of systematics and, in particular, the phylogenetic relationships of leaf-cutting ants. Such previous studies (Nygaard *et al.*, 2016; Branstetter *et al.*, 2017a; Li *et al.*, 2018; Rabeling *et al.*, 2018; Sánchez-Restrepo *et al.*, 2019; Cristiano *et al.*, 2013, 2020) revealed that *Atta* is the sister genus to *Acromyrmex*, and that both are sister to *Amoimyrmex*. They also indicate that the genus *Atta* is remarkably young.

Prior studies of *Atta* utilizing mitochondrial and nuclear DNA sequence data produced important discoveries about the ecology and evolution of the genus. For example, Solomon *et al.* (2008) examined the phylogeography of a subset of *Atta* species with broad distributions in order to test three hypotheses about the causes underlying those distributions. A subsequent phylogenetic study produced the first nearly complete phylogeny of the genus based on molecular data (Bacci *et al.*, 2009), supporting the monophyly of the genus and placing, with strong support, the 13 studied species into four clades representing the four previously recognized but subsequently synonymized (Bolton *et al.*, 2006) subgenera *Archeatta* Gonçalves, *Atta* s.s. Emery, *Epiatta* Borgmeier, and *Neoatta* Gonçalves. Bacci *et al.* (2009) cautioned, however, that their data were unable to resolve a number of important phylogenetic relationships, which were reconstructed as polytomies. More recently, a phylogeny of six *Atta* species was reconstructed based on mitogenomes (Barbosa *et al.*, 2019).

Here, we provide the results of a phylogenomic analysis of the leaf-cutting ant genus *Atta* based on UCE data and comprehensive taxon sampling. By reconstructing a robust phylogeny, we aimed to infer: (i) the phylogenetic relationships of the genus *Atta* to other higher-attine ants; (ii) the phylogenetic relationships of species and species groups within *Atta*; (iii) the evolutionary history and divergence times separating *Atta* from other higher-attine ant groups and separating species within the genus *Atta*; and (iv) the historical biogeography and the current biogeographic distributions of *Atta* species. It is our hope that the reconstruction of *Atta* internal relationships, divergence times, and biogeography will inspire and inform further evolutionary and taxonomic studies of leaf-cutting and other attine ants.

Materials and methods

Taxon sampling

For this study, we assembled a total of 865 samples (i.e., sets of specimens from the same nest for 865 colonies) from 19 countries and from 25 of 26 Brazilian states. Our samples include 14 of the 15 recognized, extant species currently assigned to the genus *Atta*. We do not include the species *Atta cubana* Fontenla Rizo due to the unavailability of specimens. Two additional North American taxa, *Atta pilosa* Buckley and *Atta tardigrada* Buckley, are *incertae sedis* in the genus (Bolton, 2021),

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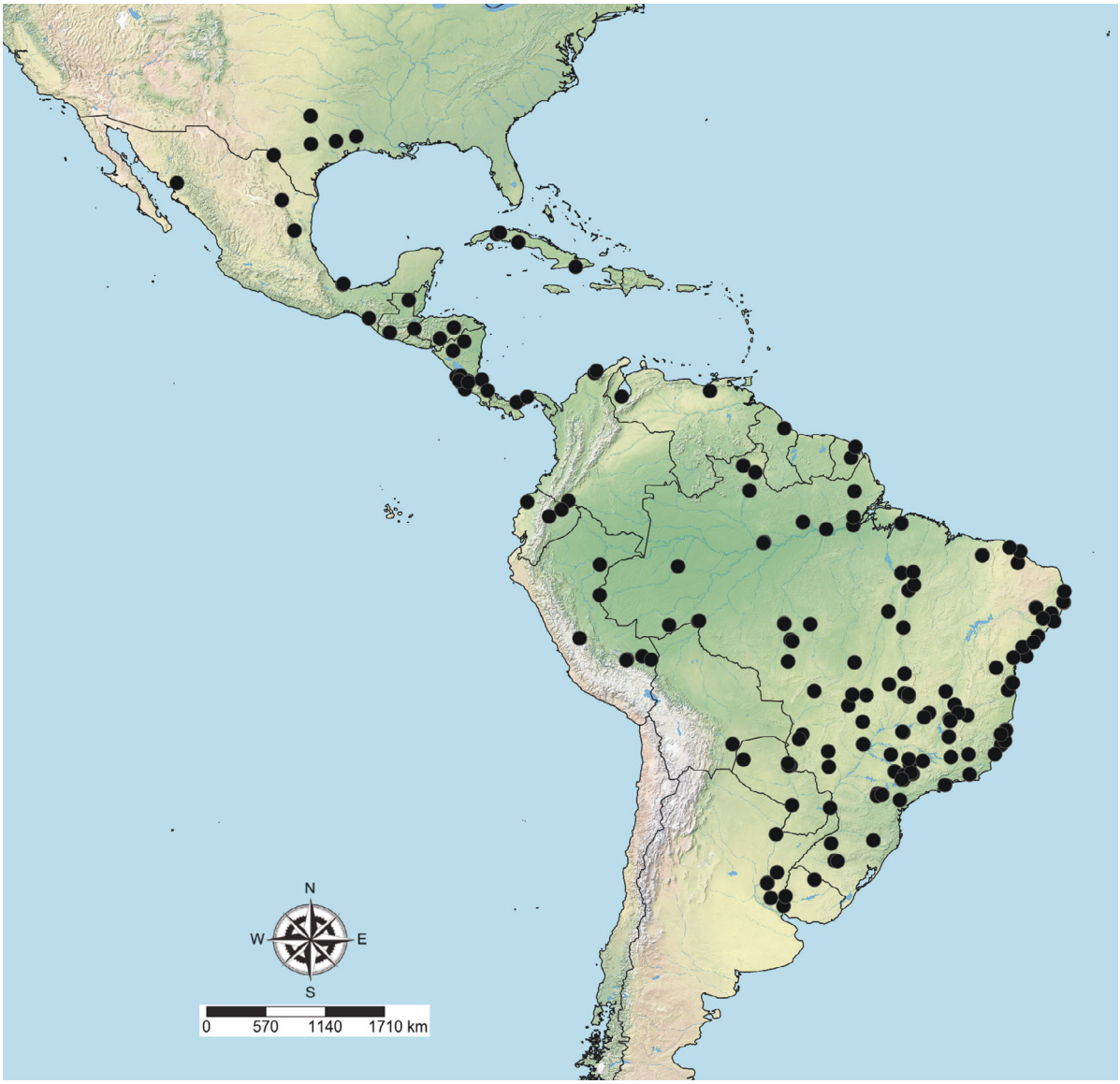

Fig. 1. Map of collection sites sampled for specimens of *Atta* used in the phylogenetic analyses.

i.e., they were inadequately described in the 19th century without reference to voucher or type specimens and, whatever the true identities of the two species, they were certainly not species of *Atta* as the genus is currently understood. We identified *Atta* specimens using the keys and descriptions in the currently available literature (Borgmeier, 1959; Gonçalves, 1963, 1967, 1971; Kusnezov, 1978). Wherever possible, we densely sampled specimens of *Atta* to represent the entire geographic distributions of the species (Figs 1, 2a). Most of the samples used in this study belong to the Bacci (*Universidade Estadual Paulista*, UNESP) and Rabeling (Arizona State University, ASU) collections. We complemented our sampling with specimens either donated or loaned by colleagues, borrowed from major entomological collections in Brazil and the United States (Table 1), and collected during various field trips. We thoroughly sampled the known widely distributed species to account for potential cryptic species (Fig. 1 and Table S1).

During field work, at each collection site, we collected at least ten individuals per nest from at least three nests, including workers of different sizes, preferentially large workers. We collected the individuals into Eppendorf® vials and Falcon centrifuge tubes filled with 95–100% ethanol. We stored these collections in a −20°C freezer in the *Laboratório de Evolução Molecular* (LEM) at the *Centro de Estudos de Insetos Sociais* (CEIS) at UNESP in Rio Claro, SP, Brazil. Borrowed specimen series were both point-mounted and preserved in ethanol. The list of all taxa used in this study is presented in Table S1, and includes sampling information, DNA extraction code, voucher specimen code, and Sequence Read Archive (SRA) accession numbers. The voucher specimens are deposited in the Social

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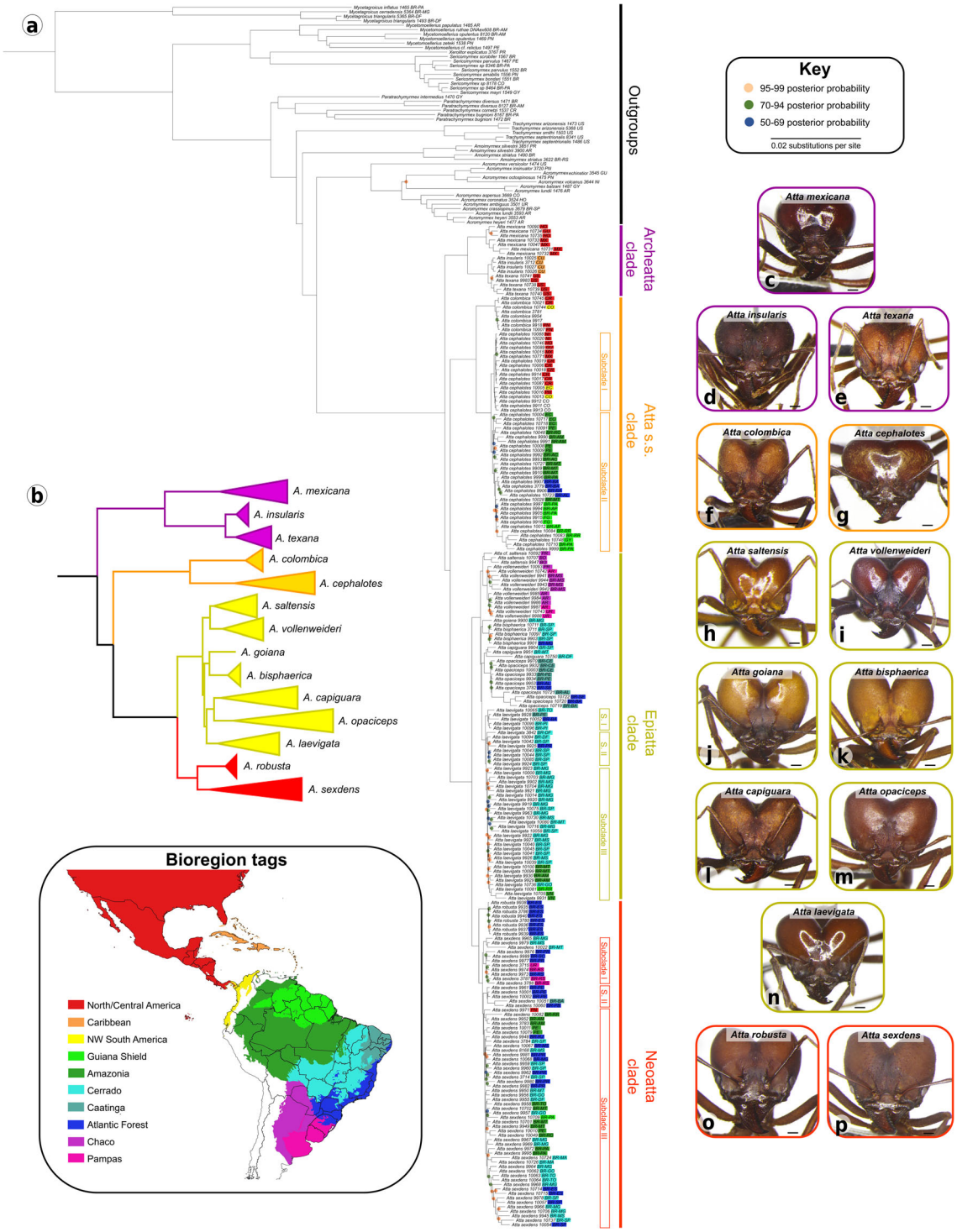

Fig. 2. Legend on next page.

Insect Biodiversity Repository (SIBR) in the School of Life Sciences at ASU in Tempe, AZ, U.S.A. Replicate vouchers (individuals from the same nest) are deposited to the *Museu de Zoologia da Universidade de São Paulo* (MZSP) in São Paulo, SP, Brazil. Borrowed voucher specimens are deposited in the corresponding insect collections listed in Table 1 (for more details, see Table S1). We plotted our samples on a map using SimpleMappr (Shorthouse, 2010) (Fig. 1) and the map for bioregions using QGIS v2.18.14 software (Esri, Redlands, CA, U.S.A.) (Figs 2, 4).

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Fig. 2. Ultraconserved element (UCE) phylogeny of *Atta* and outgroups. (a) The maximum-likelihood phylogeny produced in IQ-TREE from the 70% complete SWSC-EN partitioned UCE matrix. Coloured circles at nodes represent less than 100% support and the colour indicates the maximum-likelihood bootstrap support (see figure key). Roman numerals I, II, and III represent within-species subclades. Samples are listed by species name and DNA extraction number. Locality tags (small coloured boxes) to the right of taxon names indicate the bioregion (by colour, see figure key) and the collection country (including state for Brazil) as follows. AR, Argentina; BR, Brazil (AC, Acre, AL, Alagoas, AP, Amapá, AM, Amazonas, BA, Bahia, CE, Ceará, ES, Espírito Santo, DF, Distrito Federal, GO, Goiás, MA, Maranhão, MG, Minas Gerais, MS, Mato Grosso do Sul, MT, Mato Grosso, PA, Pará, PB, Paraíba, PR, Paraná, PE, Pernambuco, PI, Piauí, RJ, Rio de Janeiro, RO, Rondônia, RR, Roraima, RS, Rio Grande do Sul, SC, Santa Catarina, SP, São Paulo, SE, Sergipe, TO, Tocantins); BO, Bolivia; CO, Colombia; CR, Costa Rica; CU, Cuba; EC, Ecuador, FG, French Guiana; GU, Guatemala; GY, Guyana; HO, Honduras; MX, Mexico; NI, Nicaragua; PN, Panama; PR, Paraguay; PE, Peru; UR, Uruguay; US, United States of America; VN, Venezuela. (b) Phylogram of collapsed clades Archeatta (purple branches), Atta s.s. (orange branches), Epiatta (yellow branches), and Neoatta (red branches) and species of *Atta*. (c-p) Images of *Atta* worker voucher specimens (individuals from the same nest as those sampled for DNA sequencing). Unique specimen identifiers of the extracted voucher and collection repository follow the taxon name. (c) *Atta mexicana*, ASU-SIBR00000689, *Museu de Zoologia da Universidade de São Paulo* (MZSP). (d) *Atta insularis*, ASU-SIBR00000676, *Instituto de Ciências Biológicas e da Saúde* (ICBS) (e) *Atta texana*, ASU-SIBR00000690, MZSP. (f) *Atta colombica*, ASU-SIBR00000691, MZSP. (g) *Atta cephalotes*, ASU-SIBR00000692, MZSP. (h) *Atta saltensis*, ASU-SIBR00000693, MZSP. (i) *Atta vollenweideri*, ASU-SIBR00000694, MZSP. (j) *Atta goiana*, ASU-SIBR00000695, MZSP. (k) *Atta bisphaerica*, ASU-SIBR00000696, MZSP. (l) *Atta capiguara*, ASU-SIBR00000697, MZSP. (m) *Atta opaciceps*, ASU-SIBR00000699, MZSP. (n) *Atta laevigata*, ASU-SIBR00000701, MZSP. (o) *Atta robusta*, ASU-SIBR00000702, MZSP. (p) *Atta sexdens*, ASU-SIBR00000703, MZSP. Scale bars represent 0.6 mm. Ant photos were taken and edited by Corina Barrera.

UCE data collection

We carried out the UCE laboratory methods at ASU, Tempe, AZ, U.S.A. following protocols based on Blaimer *et al.* (2015) and Borowiec (2019a), and as described below.

Taxon selection and specimen conditioning. We selected 224 specimens (165 minor workers, 50 major workers, five males, and four queens) for DNA extraction and UCE sequencing to represent as fully as possible both the morphospace and geographic distributions of the species within *Atta* (Fig. 1). We also chose 49 outgroup specimens that we had already sequenced, which included species in the leaf-cutting genera *Amoimyrmex* and *Acromyrmex*, other higher attines in the genera *Mycetomoellerius* Solomon *et al.*, *Paratrachymyrmex* Solomon *et al.*, *Sericomyrmex* Mayr, *Trachymyrmex* Forel, and *Xerolitor* Sosa-Calvo *et al.*, and a more distantly related lower-attine genus, *Mycetagroicus* Brandão & Mayhé-Nunes (for more detailed information on sample numbers per genus and species, see Table S1). This selection was based on results from previously published phylogenetic analyses employing multi-locus and genomic data (Schultz & Brady, 2008; Cristiano *et al.*, 2013, 2020; Schultz *et al.*, 2015; Branstetter *et al.*, 2017a; Li *et al.*, 2018; Ješovnik *et al.*, 2017; Rabeling *et al.*, 2018; Sosa-Calvo *et al.*, 2017, 2018; Solomon *et al.*, 2019).

We extracted DNA from specimens with minimum morphological damage (non-destructive extraction) or with partial damage (removing and pulverizing three legs per individual). For point-mounted specimens, we removed the individuals from their paper triangles and placed them in 95% ethanol to decrease cuticle stiffness and to prevent excessive damage. For non-destructive extractions, we poked small holes with a sterilized entomological pin on the right side of the pronotum, propodeum and metasoma to facilitate the lysis process. For

Table 1. Entomological collections, listing institutions from which specimens were donated or borrowed, where specimens were studied during visits, and/or where vouchers are deposited.

CEIS	Centro de Estudos de Insetos Sociais, Universidade Estadual Paulista (UNESP), Laboratório de Ecologia e Sistemática de Fungos, Rio Claro, SP, Brazil
CPDC	Centro de Pesquisas do Cacau, Comissão Executiva do Plano de Lavoura Cacaueira (CEPLAC), Jacques Delabie, Itabuna, BA, Brazil
CU	Cornell University, Corrie Moreau, personal collection, Ithaca, NY, U.S.A.
DZUP	Coleção Entomológica 'Padre Jesus Santiago Moure', Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil
EMBRAPA	Embrapa Florestas, Laboratório de Entomologia, Colombo, PR, Brazil
ICBS	Instituto de Ciências Biológicas e da Saúde, Coleção Entomológica Ângelo Moreira da Costa Lima, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil
INPA	Instituto Nacional de Pesquisas da Amazônia, Coleção Zoológica de Invertebrados, Manaus, AM, Brazil
JTLC	John T. Longino Collection, University of Utah, Salt Lake City, UT, U.S.A.
MPEG	Museu Paraense 'Emílio Goeldi', Coleção de Entomologia, Belém, PA, Brazil
MZSP	Museu de Zoologia, Universidade de São Paulo, São Paulo, SP, Brazil
SIBR	Social Insect Biodiversity Repository, School of Life Sciences, Arizona State University, Tempe, AZ, U.S.A.
UCDC	Bohart Museum of Entomology, University of California, Davis, CA, U.S.A.
UFU	Universidade Federal de Uberlândia, Coleção Zoológica do Museu de Biodiversidade do Cerrado, Uberlândia, MG, Brazil
UFV	Universidade Federal de Viçosa, Coleção Entomológica do Laboratório de Coleoptera, Viçosa, MG, Brazil
USNM	National Museum of Natural History, Smithsonian Institution, Washington, DC, U.S.A.

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partially destructive extractions, we removed the right three legs of large specimens (major workers, queens, and males), placed them in a 2.0 mL tube with a previously sterilized glass bead, and then disrupted the sample by using a vortexer. After extraction, we retained all specimens (voucher collection, see Table 1).

DNA extraction and library preparation. We extracted DNA using either the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA. U.S.A.) for samples that were recently collected (<30 years old), or the QIAmp DNA Micro Kit (Qiagen, Valencia, CA, U.S.A.) for samples that were older than 30 years or for which we suspected DNA had degraded due to poor preservation conditions. For both kits, we followed the recommendations of the manufacturer, with the following modifications to the protocols: overnight cell lysis with 20 μL of Proteinase-K in a dry bath shaker at 56°C and at 500 rpm and eluting the extracted DNA from the spin-collection columns in two steps, each using 65 μL of nuclease-free water (manufacturer's protocol calls for 1–8 h cell lysis and 200 μL of AE buffer for DNA elution). After extraction, we cleaned the specimen (voucher) with ethyl acetate (5–10 min), rinsed it and preserved it in 95–100% ethanol. Later, we point-mounted the vouchers, labelled them, and deposited them in the insect collections mentioned above for future taxonomic study.

We quantified each DNA extraction by using 2 μL of DNA template with a Qubit 3.0 Fluorometer employing the High Sensitivity Kit (Thermo Fisher Scientific, Inc.). Prior to library preparation, we sheared <5–50 ng of DNA template to an average fragment length of 300–600 bp using a Qsonica Q800R3 Sonicator (Qsonica LLC, Newton, CT, U.S.A.) generally for 60 s. However, we adjusted the shearing time in relation to the preservation of the sample and the concentration of the DNA extracted, varying the shearing time to 30 or 15 s. Library preparations were carried out on 96-well plates using the KAPA Hyper Prep Library Kit (Kapa Biosystems, Inc., Wilmington, MA, U.S.A.) as described in Faircloth *et al.* (2015) with the iTru Adapters protocol. We implemented all magnetic bead clean-up steps (Fisher *et al.*, 2011) using an SPRI substitute (Rohland & Reich, 2012), also as described in Faircloth *et al.* (2015). We used dual-indexing TruSeq adapters (Faircloth & Glenn, 2012; Glenn *et al.*, 2019) for ligation. We followed the ligation step by PCR amplification of 15 μL of the library using the KAPA HiFi Ready Mix (Kapa Biosystems, Inc., Wilmington, MA, U.S.A.) with 25 μL of HiFi mix, 2.5 μL of each of Illumina TruSeq i5 and i7 primers, and 5 μL nuclease-free ddH_2O. For the PCR, we employed the following thermal cycler program: 98°C for 45 s; 14 cycles of 98°C for 15 s, 60°C for 30 s, 72°C for 60 s; and final extension at 72°C for 5 m. Following PCR, we purified DNA products using 1.2× speedbeads and rehydrated the purified product in 22 μL of Elution Buffer (EB, pH = 8). We quantified individual libraries using 2 μL of library products in a Qubit 3.0 Fluorometer using the Broad Range Kit (Thermo Fisher Scientific, Inc.).

Sample pooling and target enrichment of libraries. We pooled post-PCR libraries (eight to 12 libraries together) at equimolar concentrations into 22 pools. We adjusted pool concentrations to 147 ng/μL by drying samples in a vacuum centrifuge for 45–60 m (or until all liquid was evaporated) at 60°C, and then resuspending them in nuclease-free water at volumes estimated for each pool. We used 2 μL of the resuspended product to measure each pool concentration in a Qubit 3.0 Fluorometer with the Broad Range Kit. The final concentrations of pre-enrichment pools were 37.8–149 ng/μL.

We enriched each pool using 9446 baits (myBaits®; Arbor BioSciences) targeting 2524 conserved loci in Hymenoptera (Branstetter *et al.*, 2017b) for 24 h at an incubation temperature of 65°C. Enrichment, bead-cleaning, and PCR reaction procedures followed Borowiec (2019a). The resulting reactions were purified using 1.0× speedbeads and the enriched pools were then rehydrated in 22 μL EB. Following this step, we quantified 2 μL of each enriched pool using the Qubit 3.0 Fluorometer and the Broad Range Kit. To obtain reliable estimates of DNA concentration for each enriched pool, we performed a quantitative qPCR on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems™, Thermo Fisher Scientific, Inc.) using the KAPA Library Quantification Kit (Kapa Biosystems, Inc.) with the KAPA SYBR® FAST qPCR Master Mix, universal Illumina primers, and dilutions of 1:1 000 000 and 1:2 000 000 for each pool. We then used the resulting concentration estimates to pool the libraries at equimolar concentrations in a single final pool, with a final volume of 200 μL. We sent two final enriched pools, containing libraries for 128 and 120 samples, respectively, to the University of Utah High Throughput Genomics Core Facility for quality control and sequencing of two full lanes of a HiSeq 2500 (125 Cycle Paired-End Sequencing v4) run. Raw sequences generated as part of this study are deposited at the NCBI SRA (BioProject ID PRJNA742376).

Processing of UCE sequence data

The raw sequence data were demultiplexed and converted to FASTQ format at the University of Utah High Throughput Genomics Core Facility. We cleaned, assembled, and aligned the output from the demultiplexed FASTQ data using the PHYLUCE package v1.6.7 (Faircloth, 2016) and following the recommendations in Branstetter *et al.* (2017b). Within the PHYLUCE package, we took advantage of wrapper scripts designed to facilitate batch processing of large amounts of data. We trimmed raw reads for adapter contamination and low-quality bases using ILLUMIPROCESSOR v.2.0.6 (Faircloth, 2013), which includes TRIMMOMATIC v0.39 (Bolger *et al.*, 2014). We used TRINITY v.r2013_2_25 (Grabherr *et al.*, 2011) for assembly of reads into contigs and LASTZ v1.0 (Harris, 2007) to match the assembled contigs to the input probe set (ant-specific-hym-v2 bait file) with the script *phyluce_assembly_match_contigs_to_probes* with default settings.

Following UCE contig extraction, we aligned each UCE locus using MAFFT v7.407 (Katoh & Standley, 2013) with the L-INS-i option. We trimmed the resulting alignments with GBLOCKS v.091b (Talavera & Castresana, 2007) using relaxed settings (−b1 = 0.5 −b2 = 0.5 −b3 = 12 −b4 = 7). We concatenated the trimmed single-locus UCE alignments using

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the scripts *phyluce_align_get_only_loci_with_min_taxa* and *phyluce_align_format_nexus_files_for_raxml* to generate the datasets or alignments. Most of the ingroup taxa involved in our study had over 2000 UCE loci. We identified long branches associated with sequences from older specimens or from specimens with low-quality DNA and less than 1000 loci, probably caused by problems in the alignment of sequence fragments with large length differences. If we determined that such long-branched sequences could negatively affect the analyses, we removed them using manual cut-off. We did not use the program SPRUCEUP (Borowiec, 2019b) to address these sequences, because it would have removed even more characters from those taxa leading to the problem of large amounts of missing data, a problem that has been shown to interfere with the accuracy of phylogenetic estimation (Wiens & Morrill, 2011). We ultimately generated three final alignments, a dataset including loci in which 70% of the taxa were present (Atta273t_70per), a more 'complete' dataset that included loci in which 90% of the taxa were present (Atta273t_90per), and a reduced dataset for the divergence-dating analysis (Atta73t_div). We calculated summary statistics for the final data matrices using the script *phyluce_align_get_align_summary_data* in the PHYLUCE package. For the construction and analysis of the phylogeny presented in our results (Fig. 2a), we used the dataset from Atta273t_70per matrix, which resulted in a final dataset of 2340 UCE loci and 2 308 679 bp of sequence data; and for the divergence-dating analysis, we used the reduced dataset Atta73t_div matrix (Table 2; for more details on alignment matrices statistics, see Table S2).

UCE phylogenetic analysis

We ran preliminary, unpartitioned analyses using IQ-TREE multicore v.1.6.12 (Nguyen *et al.*, 2015). Branch support was calculated with 1000 ultrafast bootstraps (Hoang *et al.*, 2018), using -m GTR + G, and estimating a neighbour-joining tree (−t BioNJ) for the three datasets (Chernomor *et al.*, 2016). We then partitioned the datasets using the sliding-window site characteristics algorithm based on entropy (SWSC-EN, Tagliacollo & Lanfear, 2018), which uses a sliding window method to partition UCE loci into three regions (the core and its right and left flanking regions). This method takes advantage of the general pattern in which UCE core regions are highly conserved, whereas the variability of the flanking regions increases with their distance from the core (Faircloth *et al.*, 2012). The resulting data subsets were then analysed using MODELFINDER (Kalyaanamoorthy *et al.*, 2017) as implemented in IQ-TREE multicore v.1.6.12 (Nguyen *et al.*, 2015), setting the selection criterion as corrected Akaike information criterion (AICc), and the search algorithm as rclusterf (Lanfear *et al.*, 2017). We also partitioned the alignments by each locus. Each dataset was analysed as non-partitioned, SWSC-EN partitioned, and partitioned by locus.

We performed maximum-likelihood (ML) analyses on the three generated datasets with their respective partitioning schemes using IQ-TREE multicore v.1.6.12 (Nguyen *et al.*, 2015), estimating branch support with ultrafast bootstrapping set at 1000 replicates (Hoang *et al.*, 2018), and other settings kept at the default conditions.

Divergence time inference

To estimate species divergence times, we analysed a reduced dataset (Atta73t_div) in which, for each species, we included specimens with the largest number of UCE loci and with consideration of their locations on the tree. We used the reduced alignment and topology as input for the program MCMCTREE in the PAML v4.9j package (Yang, 2007), implementing the approximate-likelihood approach. To calibrate our analysis, we used a Dominican amber fossil [*Mycetomoellerius primaevus* (Baroni Urbani); see Baroni Urbani, 1980]. This fossil species, which was previously used to calibrate analyses (Schultz & Brady, 2008; Branstetter *et al.*, 2017a; Li *et al.*, 2018), was recently placed in the newly created genus *Mycetomoellerius* in a recent study in which the formerly paraphyletic higher-attine genus *Trachymyrmex* s.l. was divided into three monophyletic genera (Solomon *et al.*, 2019). However, this placement is associated with a high degree of uncertainty (T.R. Schultz and J. Sosa-Calvo, personal observation). Due to this uncertainty, we conservatively chose to use the fossil to calibrate the ancestral crown node of all the higher-attine ants corresponding, in the present study, to the most recent common ancestor (MRCA) of *Mycetomoellerius* and *Atta*, rather than to calibrate the stem node of the genus *Mycetomoellerius*. The fossil calibration was specified as a truncated Cauchy probability distribution indicated by L(*tL*, *P*, *c*), where *tL* = minimum-age bound (set as 15 Ma), *P* = offset value (default value of 0.1) and *c* = the scale parameter value (default value of 1.0) representing a heavy-tailed density (Inoue *et al.*, 2010). Since the dating of Dominican amber is ambiguous, ranging from 15 to 20 Ma (Iturralde-Vinent & MacPhee, 1996; Grimaldi & Agosti, 2000), we chose a conservative minimum age of 15 Ma and employed a relatively flat (c = 1.0) prior probability distribution to

Table 2. Datasets consisting of partitioned alignments (SWSC-EN) used in the phylogenetic and divergence-dating analyses, with summary statistics for the aligned and concatenated matrices.

Matrix	Data matrix	Number of taxa	UCE loci	Alignment length (pb)	Partitions	Distinct alignment patterns	UCE mean loci length	% missing data
Atta273t_70per	70%	273	2340	2 308 679	1520	1 386 996	986.61	4.54
Atta273t_90per	90%	273	1038	1 064 781	774	648 340	1025.8	2.45
Atta73t_div	70%	73	2274	2 168 673	1039	848 122	953.68	7.09

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accommodate a wide range of prior dates. Because of the lack of a fossil with which to directly calibrate the root node, we employed a secondary calibration based on the inferred age of the corresponding internal node from Branstetter *et al.* (2017a), Ješovnik *et al.* (2017), and Li *et al.* (2018). The root node was calibrated with a uniform distribution of B(0.259, 0.372) to incorporate the 95% highest posterior density (HPD) value range estimated in the studies listed above (25.9–37.2 Ma).

We performed the MCMCTree dating analyses using an unpartitioned concatenated alignment, the independent-rates clock model, and the HKY85 + G model of sequence evolution. For each analysis, we ran two independent chains for 50 million generations at a sampling frequency of 500 and a burn-in of 20%. We assessed run convergence and performance by examining the mcmc.txt files in TRACER v1.7.1 (Rambaut *et al.*, 2018) and convergence plots in Excel.

Biogeographical analysis

To reconstruct the biogeographic history of *Atta*, we used the BIOGEOBEARS R package (Matzke, 2013, 2015) and a modified version of the BIOGEOBEARS original R scripts (A.F Sánchez-Restrepo, personal communication; Magalhaes, 2021). As input, we submitted the chronogram obtained from the MCMCTree Bayesian divergence-dating analysis (Fig. 3); a geography data matrix coding the presence/absence of each sample, including *Atta* species and outgroup taxa; an areas-allowed matrix specifying pairs of states allowed and disallowed in area ranges; and a dispersal multiplier matrix quantifying the relative probabilities of dispersal between adjacent areas vs. between non-adjacent areas across 10 biogeographic regions (see Table S3). Biogeographic regions were adapted from Morrone's (2006) classification of Neotropical entomofauna and were defined as: A = North/Central America; B = Caribbean; C = NW South America; D = Guiana Shield; E = Amazonia; F = Cerrado; G = Caatinga; H = Atlantic Forest; I = Chaco; J = Pampas (see maps in Figs 2, 4). A maximum limit of three was placed on the number of areas allowed in ancestral ranges. Taxon sampling was necessarily limited to the specimens in the dating analysis (Fig. 3). As per the instructions of the author of BIOGEOBEARS (Matzke, 2021), we did not include every available specimen in the analysis; rather, we included one representative of each species or, in the cases of the widespread species *Atta cephalotes* (Linnaeus), *Atta laevigata* (Smith), and *Atta sexdens* (Linnaeus), one representative, as available, of every geographic subclade, which may be geographic populations or cryptic species.

By dividing the three widespread *Atta* species into geographic subclades, it was possible to limit the range of each taxon to no more than three areas (see Tables S3 and S4). A three-area range limit was necessary for making analysis run times computationally tractable and to be able to assign a maximum number of three areas to the ancestral ranges. To achieve the three-area limit and to avoid introducing non-adjacent area combinations (e.g., Guiana Shield + Atlantic Forest) in one of the subclades of the three widespread species, the Guiana Shield was eliminated from the coding because in these species the Guiana Shield is clearly a derived rather than an ancestral area (Fig. 2a; see Discussion: Historical biogeography and evolution). To code non-*Atta* outgroups, in which our sampling was mostly limited to a single specimen per species and for which species boundaries and distributions are uncertain, we always included the area in which the sampled specimen was collected and only conservatively coded for additional areas based largely on our own background knowledge as well as on sceptical consideration of distributional data in AntWeb (http://antweb.org), AntMaps (http://antmaps.org) (Janicki *et al.*, 2016; Guénard *et al.*, 2017), and the literature (e.g., Gonçalves, 1961; Weber, 1966; Kempf, 1972; Fowler, 1985, 1988; Wilson, 1986; Brandão, 1991; Farji-Brener & Ruggiero, 1994; Fernández & Sendoya, 2004; Rando & Forti, 2005; Fernández & Serna, 2019). Although this strategy undoubtedly led to underreporting the distributions of a subset of widespread outgroup species, we judge it preferable to extrapolating distributions (i.e., assigning dubious areas to a taxon) based on possibly erroneous presumptions about species identification, species boundaries, and species distributions.

Using BIOGEOBEARS with the above-described parameter constraints, we tested and compared six commonly implemented models: the likelihood-based dispersal–extinction cladogenesis model (DEC; Ree *et al.*, 2005; Ree & Smith, 2008), the likelihood-based version of dispersal–vicariance analysis model (DIVA-like, DVL; Ronquist, 1997; Matzke, 2013), the Bayesian binary model (BayArea-like, BAL; Landis *et al.*, 2013; Matzke, 2013), and all three models with the additional parameter of founder-event speciation (+J) (Matzke, 2014), as recommended in BIOGEOBEARS, but see Ree & Sanmartín (2018) for a criticism of DEC + J. We used the AICc score to compare the fit of the models to our data.

Because the Central/South American land connection has been variously open and closed to terrestrial dispersal over the past 46 million years (Bacon *et al.*, 2015), we considered implementing the time-stratification option of BIOGEOBEARS, which allows different area adjacency and dispersal multiplier values in different time strata. Under such a scheme, Central America could be adjacent to NW South America in time strata during which the land bridge was connected, but non-adjacent in time strata during which the land bridge was not connected. Arguing against this scheme, however, are the considerable confidence intervals on Bacon *et al.*'s (2015) reconstructions and, most importantly, the considerable confidence intervals on the nodes in our dating analysis (Fig. 3). Because node ages are treated as point values rather than as confidence intervals in BIOGEOBEARS, minor imprecision in node dating could lead to a critical node being placed within the wrong time stratum. To avoid this problem, we chose to address the varying adjacencies of the two bioregions over time by assigning a dispersal multiplier value intermediate between those assigned to adjacent areas and those assigned to non-adjacent areas (Table S3). The time periods during which the land bridge was connected and disconnected, as reported by Bacon *et al.* (2015), are depicted graphically in Fig. 4.

Analyses were conducted on the Smithsonian Institution high performance computing cluster (n.d.), Smithsonian Institution, and the Agave Computing Cluster at ASU.

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Clade	Mean	95% HPD
Higher-attine ants crown	23.2	16.4–30.6
MRCA *Trachymyrmex* + *Atta*	18.9	12.9–24.9
MRCA *Amoimyrmex* + *Atta*	18.2	12.4–24.1
Acromyrmex + *Atta*	16.7	11.3–22.3
Atta crown	8.5	4.9–12.5
Archeatta crown	4.8	2.3–7.8
Atta s.s. + Epiatta + Neoatta crown	7.5	4.2–11.2
Atta s.s. crown	3.3	1.5–5.5
Epiatta + Neoatta crown	3.7	2.0–5.7
Epiatta crown	2.2	1.3–3.3
Neoatta crown	2.2	1.1–3.5

HPD, highest posterior density. Ages in millions of years ago (Ma)

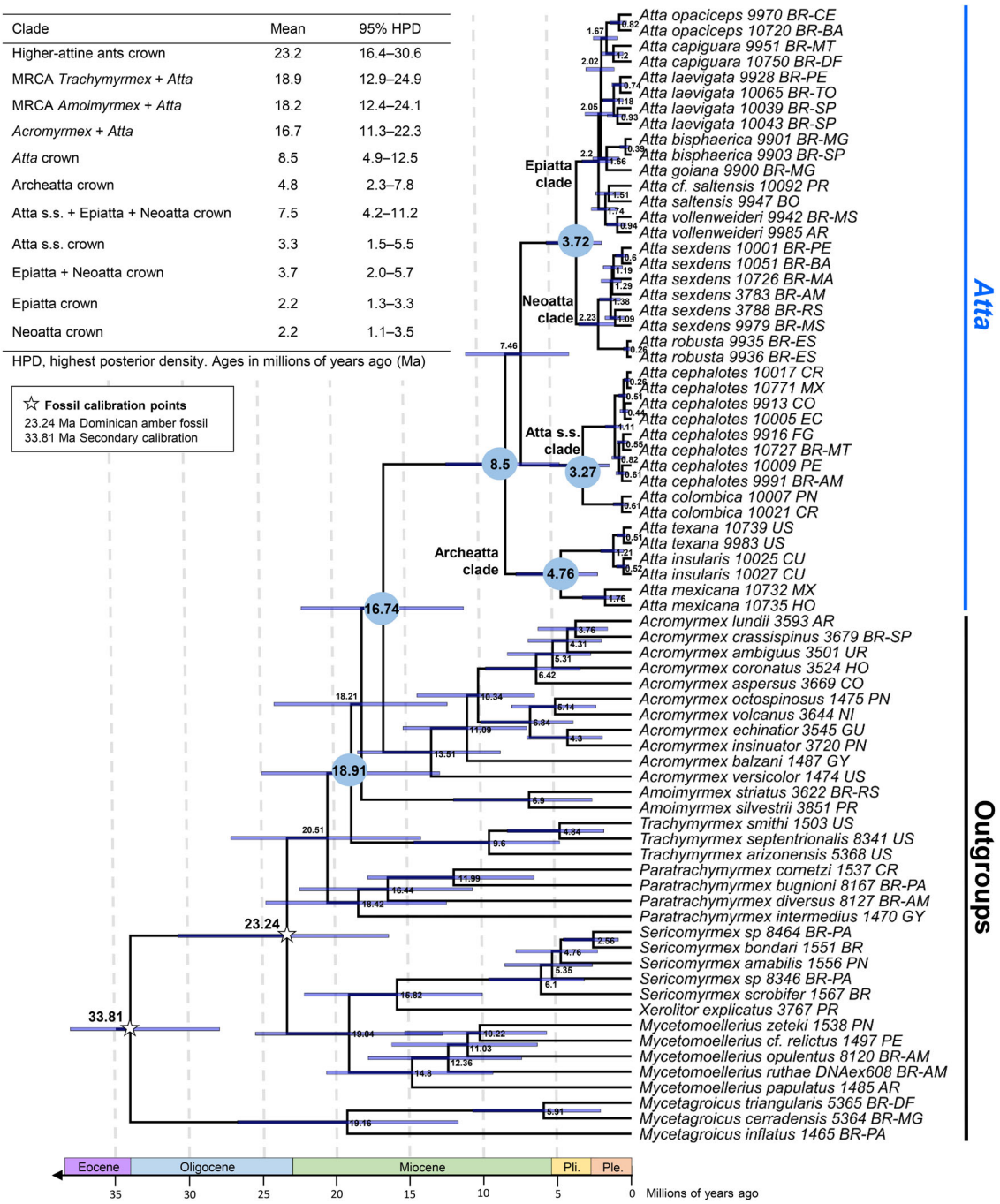

Fig. 3. Divergence-date estimates based on Bayesian analyses conducted in MCMCTree. Numbers at nodes indicate the mean age and blue bars represent 95% highest posterior density (HPD) values. Ages are in millions of years ago (Ma). The table on the upper left of the figure shows the estimated divergence dates for major clades and subclades of genus *Atta*; for more detailed estimates by clade, see Table S6.

Results

UCE sequencing

The mean DNA post-extraction concentration of the 246 taxa extracted for this study was 3.01 ng/μL (range: 0.06–31.3 ng/μL) and 24.21 ng/μL (range: 4.36–53 ng/μL) for the post-PCR libraries. The mean number of raw reads sequenced was 2 083 262 (range: 78 145–7 307 553) and the mean number of UCE loci captured was 2307 (range: 1088–2447) (Table 3). Full UCE data and sequencing statistics for each taxon are presented in Table S5.

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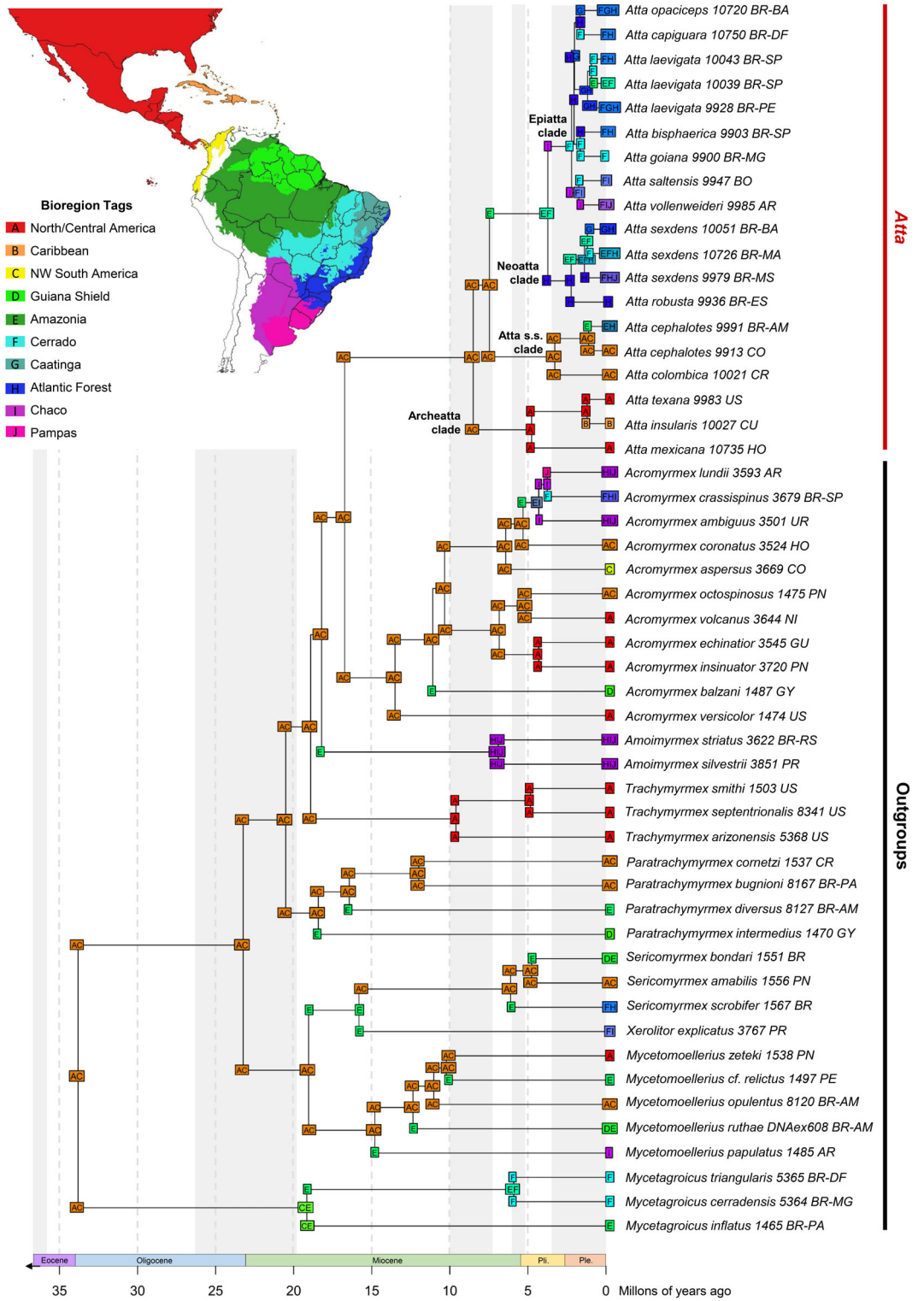

Fig. 4. Chronogram of historical biogeography constructed using the program BIOGEOBEARS under the Bayesian binary biogeographic model with founder-event speciation and incorporating the dispersal multiplier parameter (BAL + J_ArAlw_Disp). The letters at nodes represent the most probable ancestral geographic ranges and inherited ranges, respectively (by letter for the single bioregions, see figure key). Grey columns indicate the approximate time periods during which the Panamanian land bridge connecting Central and NW South America was present (Bacon *et al.*, 2015). Ages are in millions of years ago (Ma).

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Table 3. Summary of ultraconserved element (UCE) processing and sequencing statistics for all taxa extracted in this study. For more detailed statistics per taxon, see Table S5.

	DNA conc. extract (ng/μL)	DNA conc. post-PCR (ng/μL)	Raw reads	Contigs	UCE loci
Mean	3.01	24.21	2 083 262.2	38 133.73	2307.5
Min	0.06	4.36	78 145	1276	1088
Max	31.3	53	7 307 553	236 430	2447
Standard deviation	4.4	9	984 256.78	32 721.06	164.38

Phylogenetic reconstruction and divergence dating

Here, we provide the most comprehensive phylogenetic estimate to date for the leaf-cutting ant genus *Atta* and its associated outgroups based on the sequence capture of UCEs (Fig. 2a). The results of the SWSC-EN partitioned phylogeny recovered the leaf-cutting ants as a monophyletic group that consists of three reciprocally monophyletic genera: *Atta*, *Acromyrmex*, and *Amoimyrmex*. Our phylogeny also indicates that the monophyly of our target group, the genus *Atta*, is maximally supported and recovered the 14 studied species and separated them into four clades, each supported by maximum bootstrap frequency (Fig. 2a). These clades correspond to previously recovered species groups (Bacci *et al.*, 2009) and to previously recognized subgenera of *Atta*, including *Archeatta*, *Atta* s.s., *Epiatta*, and *Neoatta* (Fig. 2a, b). Hereafter we use these subgenus names, currently considered synonyms of the genus *Atta* (Bolton *et al.*, 2006), to refer to specific clades within *Atta* as informal species-group names.

All *Atta* species studied are monophyletic with maximal support (Fig. 2a). The Archeatta clade includes the North and Central American as well as the Caribbean species, is composed of *Atta mexicana* (Smith) (Fig. 2c), *Atta insularis* Guérin (Fig. 2d), and *Atta texana* (Buckley) (Fig. 2e), and is the sister clade to all the remaining *Atta* species, which occupy North, Central, and South America. The species of the Atta s.s. clade, *Atta colombica* Guérin (Fig. 2f) and *A. cephalotes* (Fig. 2g), are from North, Central, and South America. Our results indicate that Epiatta is the most species-rich clade and includes species with biogeographic distributions restricted to South America: *Atta saltensis* Forel (Fig. 2h), *Atta vollenweideri* Forel (Fig. 2i), *Atta goiana* Gonçalves (Fig. 2j), *Atta bisphaerica* Forel (Fig. 2k), *Atta capiguara* Gonçalves (Fig. 2l), *Atta opaciceps* Borgmeier (Fig. 2m), and *A. laevigata* (Fig. 2n). Within the Epiatta clade, *A. saltensis* and *A. vollenweideri* form the sister clade to the remaining species. Our phylogeny provides the first evidence that *A. goiana* belongs to the Epiatta clade. In the case of the Neoatta clade, we recovered two species, *Atta robusta* Borgmeier (Fig. 2o), one of the most biogeographically restricted species in the genus *Atta*, and *A. sexdens* (Fig. 2p), from Central and South America and one of the most widely distributed species in the genus (for more details on the distribution of each species, see Table S4).

Our results indicate that the higher-attine ants originated around 23.2 Ma (crown age; 95% HPD: 16.4–30.6 Ma, Fig. 3). The leaf-cutting ants subsequently arose around 18.9 Ma (stem age; 95% HPD: 12.9–24.9 Ma) and radiated relatively rapidly (crown age; 18.2, 95% HPD: 12.4–24.1 Ma). The stem and crown estimated divergence times of the genus *Atta* are 16.7 Ma (95% HPD: 11.3–22.3 Ma) and 8.5 Ma (95% HPD: 4.9–12.5 Ma), respectively, indicating that a period of approximately 8.2 million years separates the stem age of *Atta* from the MRCA of all extant species (i.e., the crown-group age), suggesting either a relatively long delay until the first speciation event or subsequent extinction of early-diverging lineages (Fig. 3; for more details on divergence-date estimates, see Table S6).

The Archeatta clade (crown age 4.8; 95% HPD: 2.3–7.8 Ma) is the sister to all remaining *Atta* species and consists of notably fewer species (3 spp.) than its sister group (11 spp.), suggesting either a lower rate of speciation or a higher rate of extinction within this North American and Caribbean clade. Another three clades, Atta s.s., Epiatta, and Neoatta, share a common ancestor around 7.5 Ma (95% HPD: 4.2–11.2 Ma). Interestingly, the species *A. cephalotes* is divided into two distinct clades that diverged around 1.1 Ma (95% HPD: 0.6–1.7 Ma). This divergence is correlated with biogeography, with one clade inhabiting North, Central, and NW South America north of the Andes and contiguous with Central America, and with a second clade inhabiting northern South America, including the Guiana Shield and Amazonia, and the Atlantic Forest of north-eastern Brazil (Fig. 2a). The common ancestor of the species in the Epiatta and Neoatta clades arose approximately 3.7 Ma (95% HPD: 2.0–5.7 Ma), and both groups subsequently diversified roughly simultaneously during the Pleistocene, 2.2 Ma (95% HPD: 1.3–3.3 Ma) and 2.2 Ma (95% HPD: 1.1–3.5 Ma), respectively.

Historical biogeography

The AICc, likelihood, and other values of the BIOGEOBEARS analyses under 12 different models and incorporating the areas-allowed parameter are reported in Table S7. The best-fitting model that incorporates the dispersal multiplier parameter is BayArea-like with founder-event speciation (hereafter BAL + J_ArAlw_Disp) (Fig. 4 and Table S7). Although six other models were found to be better-fitting based on both likelihood and AICc scores, none of those incorporated the dispersal multiplier parameter. In spite of the differences in likelihood and AICc scores, however, the results of all the models evaluated in BIOGEOBEARS (Table S7) are generally very similar (Figs 4, S6–S16), particularly with regard to a North/Central American and/or northern South American origin of leaf-cutting ants in general and of *Atta* in particular. We note

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that the reconstruction of the two-area 'backbone' ancestral range of AC (North/Central America + NW South America) for leaf-cutting ants, for *Atta* + *Acromyrmex*, and for the genus *Atta*, is not an artefact of the maximum limit we placed on ancestral ranges, which was three areas. In fact, in the results of all 12 models in the BIOGEOBEARS analyses (Table S7 and Figs S6–S16), the ranges of these ancestors consistently contain only one area (A or C) or two areas (AC or CE).

Based on the BAL + J + Disp results, which incorporate the dispersal multiplier parameter, the combined North/Central America + NW South America range played a critical role in the evolutionary history of the higher-attine ants. It was the ancestral range of the MRCA of the higher-attine ants at the beginning of the Oligocene (33.8 Ma), it was the ancestral range of the MRCA of leaf-cutting ants in the early Miocene (18.9 Ma), and it was the ancestral range of *Atta* + *Acromyrmex*, also in the early Miocene (16.7 Ma) (Figs 3, 4). The stem (16.7 Ma) and crown (8.5 Ma) ancestors of the genus *Atta* also occupied this range during the early and late Miocene, respectively. It also was the ancestral range of the crown ancestor (7.5 Ma) of the Atta s.s., Epiatta, and Neoatta clades, whereas the range of the crown ancestor of the Archeatta clade was restricted only to North/Central America around 4.8 Ma (Figs 3, 4). The crown ancestor of Atta s.s. + Epiatta + Neoatta spawned two daughter clades, Atta s.s. and Epiatta + Neoatta. The range of the crown ancestor of Atta s.s. (3.3 Ma) was, again, North/Central America + NW South America, whereas the range of the stem ancestor of Epiatta + Neoatta (7.5 Ma) was Amazonia, from which it subsequently expanded into the Cerrado, producing the combined Amazonia + Cerrado range of the crown ancestor (3.7 Ma). Atlantic Forest is the reconstructed range of the stem ancestor (3.7 Ma) of Neoatta and Chaco is the reconstructed range of the stem ancestor (3.7 Ma) of Epiatta.

Discussion

Reconstruction of Atta *phylogeny and divergence dating*

In this study, we inferred a comprehensive molecular phylogeny of the leaf-cutting ant genus *Atta* utilizing phylogenomic markers and sampling a large number of taxa to capture diversity and population variation across the biogeographic range of this widely distributed ant genus. Our well-resolved and well-supported phylogeny clarifies relationships between species and species groups and reveals previously unknown historical divergences that allow us to test hypotheses about the origin of the genus *Atta*, the divergences of its species, and their historical dispersal into North, Central, and South America as well as the Caribbean. Our study adds to a growing body of phylogenetic research that utilizes UCEs to infer comprehensive, well-resolved, and statistically well-supported phylogenies in order to study insect evolution, as well as to test evolutionary and biogeographic hypotheses (Faircloth *et al.*, 2012, 2015; Blaimer *et al.*, 2016a; Branstetter *et al.*, 2017c, 2021). Lending support to the conclusions of multiple prior studies, our results indicate that UCEs are extremely effective for reconstructing divergences in higher-attine and other groups of ants at a wide range of levels, from ancient (>30 Ma) to very recent, including the delimitation of species and populations, even in very recent, short-branched evolutionary radiations (Blaimer *et al.*, 2016b; Ješovnik *et al.*, 2017; Branstetter *et al.*, 2017a; Borowiec *et al.*, 2020; Prebus, 2020; Williams *et al.*, 2020; van Elst *et al.*, 2021; Rabeling *et al.* in prep.).

Our phylogeny supports the monophyly of the leaf-cutting ants and of the genus *Atta*, in agreement with previous phylogenetic studies (Schultz & Brady, 2008; Bacci *et al.*, 2009; Cristiano *et al.*, 2013, 2020; Branstetter *et al.*, 2017a; Rabeling *et al.*, 2018), and our estimated dates for both the stem and crown-group ages of leaf-cutting ants and for the stem age of *Atta* are also similar to those found in previous studies (Ješovnik *et al.*, 2016; Nygaard *et al.*, 2016; Branstetter *et al.*, 2017a). Some of the divergence times inferred here, however, are slightly older, such as the root of our phylogeny and the *Atta* crown-group age (see table in Fig. 3 and Table S6). Our divergence-dating analyses additionally provide new insights into the timing of the origin and subsequent diversification of leaf-cutting ant species in the genus *Atta*. We find that *Atta* is remarkably young, originating between 16.7 (stem) and 8.5 (crown) Ma at the Miocene. Leaf-cutting agriculture, originating ~19 Ma, is young as well, although some previous studies have suggested that the origins of leaf-cutting ants and their fungal cultivars may not have occurred simultaneously, whether judged by stem or crown-group ages (Mikheyev *et al.*, 2010; Nygaard *et al.*, 2016; Mueller *et al.*, 2017). Unlike the diversification pattern in *Acromyrmex*, our data indicate a long delay separating the stem from the crown node of *Atta*, followed by a burst of diversification in the early Pliocene, producing extant *Atta* species, that occurred during the past 4–5 million years, i.e., following the origins of the clades within the genus. A similar pattern was observed in the fungus-growing ant genus *Sericomyrmex*, which also diversified during the past 4 million years (Ješovnik *et al.*, 2017). In fact, Ješovnik *et al.* (2017) compared *Sericomyrmex* with *Atta* when they explained the possible evolutionary processes underlying the recent and rapid radiations of both genera; our results support this prior conjecture. *Atta* species have conquered a wide diversity of habitats spanning a vast geographical range, differing in climatic conditions and in phytophysiognomies (e.g., Amazon forest, Cerrado, restinga, and Caatinga, among others). This radiation has been accompanied by few phenotypic changes, particularly in the morphology of smaller workers. Like *Sericomyrmex*, *Atta* is the product of a large amount of initial phenotypic evolution, making it immediately distinguishable from other leaf-cutting genera as well as from any other fungus-growing ant species. Our data and those of Ješovnik *et al.* (2017) suggest that the radiations of both *Atta* and *Sericomyrmex* are correlated with the rise and great expansion of Cerrado habitats in South America during the past 4 million years (Prado & Gibbs, 1993; Silva, 2011; Meyer *et al.*, 2014).

Our results indicate that *Atta* comprises four well-defined clades, which correspond to the clades recovered in a previous phylogeny of the genus (Bacci *et al.*, 2009) and to the subgenera that were previously defined on the basis of morphological

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characters (Gonçalves, 1942; Borgmeier, 1950). The topology of our phylogeny differs from the mitochondrial phylogeny inferred by Bacci *et al.* (2009), which recovered Archeatta and Atta s.s. as sister clades, and it agrees with the topologies inferred from nuclear genes and from alignments that combined both mitochondrial and nuclear DNA, which, like ours, placed Archeatta as the sister group to the remaining species of the genus (Bacci *et al.* 2009). *Atta* species were originally grouped into subgenera based primarily on the morphology of males (Gonçalves, 1942, 1986; Borgmeier, 1950, 1959), but Bolton *et al.* (2006) synonymized the subgenera under the genus *Atta*. Although our results support the reciprocal monophyly of the *Atta* clades (former subgenera) and conform to clear biological and biogeographical patterns, revisionary taxonomy is beyond the scope of the present study, including any changes to the current status of the synonymized subgenera (for more details on the taxonomic history of the subgenera, see Table S8).

Phylogenetic relationships within the genus Atta

Our study indicates, like phylogenetic studies using nuclear genes (Bacci *et al.*, 2009; Cristiano *et al.*, 2013), that Archeatta is the sister clade to all other species of *Atta* and its species retain the most plesiomorphic traits within the genus. This result contradicts Gonçalves' (1986) theory that *A. colombica* (belonging to Atta s.s. clade) occupies an early-diverging position in the genus. The only *Atta* species that is not included in our study, *A. cubana*, likely also belongs to the Archeatta group. Known only from its original description (Fontenla Rizo, 1995), this species has never been included in a phylogenetic analysis. Based on the species description, and assuming that it is a valid species (rather than conspecific with *A. insularis*, in which case *A. cubana* is a junior synonym of *A. insularis*), *A. cubana* is likely the sister species of *A. insularis*. If so, the two species, which occur sympatrically, very likely diverged on the island of Cuba, as is suggested by their differing habitat preferences. Whereas *A. cubana* generally occupies coastal regions and sandy soils, *A. insularis* prefers forest and grassland habitats (Fontenla Rizo, 1995; J.L. Fontenla Rizo, personal communication).

Our UCE analysis reconstructed the Atta s.s. clade as the sister clade to the remaining Central and South American species in the Epiatta and Neoatta clades and the transitional group between those two clades and Archeatta, consistent with the results inferred by a previous phylogeny (Bacci *et al.*, 2009), as well as with inferences in early morphological studies (Borgmeier, 1950, 1959; Gonçalves, 1986). The phylogenetic position of the Atta s.s. clade suggests that the ancestral species of this clade diverged during *Atta* evolution and that its daughter species occupied the northernmost regions of South America. *Atta colombica* is a forest-dwelling species that lives mainly in lower-elevation regions of Central America from Guatemala to Colombia in north-western South America. In contrast, *A. cephalotes*, which is sympatric with *A. colombica* in Central America, can inhabit higher-elevation regions and is distributed from Mexico in the north to South America as far south as the coast of eastern Brazil, making it one of the most widely distributed species in the genus (Weber, 1969; Fernández & Sendoya, 2004; Fernández *et al.*, 2015; Forti *et al.*, 2020). However, the northernmost extension of the Andes seems to be a biogeographic barrier potentially restricting gene flow between *A. cephalotes* populations (Muñoz-Valencia *et al.*, 2021), as suggested by the clear separation of the northern and southern clades of *A. cephalotes* in our phylogeny (Fig. 2a; see also Fig. S17). In fact, these two well-supported, reciprocally monophyletic sister groups of *A. cephalotes* may be separate species (Lovato, 2006), a hypothesis that needs to be tested by future taxonomic and phylogenetic analyses. With regard to a similar phylogenetic split in the southern clade, the absence of *A. cephalotes* in the Cerrado regions separating the Amazonian and Atlantic Forests is consistent with the hypothesis that the Amazonian and Atlantic Forest populations of *A. cephalotes* arose in allopatry after they were separated by a climatic barrier, because *A. cephalotes* is restricted to the wet forest (Solomon *et al.*, 2008) and absent from the semi-arid Cerrado (Gonçalves, 1960).

Containing seven species, the Epiatta clade represents the largest rapid radiation in the genus (see Section 4.3). Although closely related, the species of Epiatta have different biological and ecological characteristics. *Atta saltensis*, *A. vollenweideri*, *A. goiana*, *A. bisphaerica*, *A. capiguara*, and *A. opaciceps* occur in semi-arid and dry regions of South America and have restricted geographic distributions. By contrast, *A. laevigata* is widely distributed (Fig. 2a) (Daguerre, 1945; Gonçalves, 1942, 1960; Borgmeier, 1959; Fernández & Sendoya, 2004; Solomon *et al.*, 2008; Sabattini *et al.*, 2017; Forti *et al.*, 2020). The geographic distributions are correlated with the degree of specialized foraging: *A. vollenweideri*, *A. goiana*, *A. bisphaerica*, and *A. capiguara* forage mainly for monocotyledonous plants, whereas *A. saltensis* and *A. opaciceps* forage exclusively for dicotyledonous plants, and, in contrast to both other groups, *A. laevigata* is a generalist that forages for both types of plants (Fowler *et al.*, 1989; Gonçalves, 1971; Mueller *et al.*, 2017). This generalist foraging behaviour likely allowed *A. laevigata* to occupy novel ecological niches and expand its geographic range, and it is consistent with its ability to live in both arid and humid environments (Solomon *et al.*, 2008). This unspecialized foraging behaviour also made *A. laevigata* an important pest of human agriculture in some regions (Della Lucia, 2003).

Our analyses inferred the reciprocal monophyly and sister-group relationship of *A. capiguara* and *A. opaciceps*, differing from the topology reconstructed by Bacci *et al.* (2009). Previous studies placed these two species in the Neoatta group (see Table S8) because of morphological similarities to the other species that belonged to this group (e.g., *A. sexdens*, *A. vollenweideri*, and *A. laevigata*), mainly relying on characters of the male genitalia (Gonçalves 1942, 1944, 1986; Borgmeier, 1959). By contrast, our results indicate that *A. capiguara* and *A. opaciceps* are members of the Epiatta clade, which is consistent with the molecular phylogenetic results of Bacci *et al.* (2009). *Atta capiguara* inhabits secondary, open forests and grassland habitats, mainly in the Brazilian Cerrado, and it was originally known only from São Paulo State. It was

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subsequently found to occur in Mato Grosso, Mato Grosso do Sul, Goiás, Minas Gerais, and Paraná (Della Lucia *et al.*, 1993; Forti & Boaretto, 1997; Forti *et al.*, 2020). *Atta opaciceps*, the only leaf-cutting ant species endemic to the Caatinga, a dry, desert-like forest biome, is restricted to the north-eastern region of Brazil (Brandão, 1995; Ulysséa & Brandão, 2013; Siqueira *et al.*, 2018). Adaptations to high temperatures and low humidity in the dry forest are traits shared by these two species.

We originally included two specimens of *A. goiana* (10023 BR-MT and 9900 BR-MG) in our analyses, which formed a strongly supported clade. However, the taxon 10023 BR-MT arose on an extremely long branch, and we excluded the sample from subsequent analyses, as we did other anomalously long-branched taxa (see Materials and methods: Processing of UCE sequence data). The sister-group relationship of *A. goiana* and *A. bisphaerica* is consistent with the prior results of Gonçalves (1986) based on morphological characters that indicate species- rather than population-level differences. Major-worker specimens of 10023 BR-MT and 9900 BR-MG were positively identified as *A. goiana* based on the morphological characters described by the author of the species (Gonçalves, 1971), including, when compared with *A. bisphaerica*: larger size, shinier head and gaster, reduced sculpture on the head and gaster, shallower occipital groove, coarser sculpture on the mesosoma, and more developed anterior mesonotal spines (see Fig. 2j, k). This is the first time that *A. goiana* was included in a molecular phylogenetic analysis. Although our study indicates that *A. goiana* is distinct from the five included specimens of *A. bisphaerica*, future analyses will need to evaluate this result by including specimens from different populations and locations as well as more comprehensive taxonomic study. *Atta goiana* was previously included in the no longer recognized subgenus *Palaeatta* (see Table S8), consisting of *A. bisphaerica*, *A. saltensis*, and *A. goiana* (Borgmeier, 1950, 1959; Gonçalves, 1986). As in the previous analyses of Bacci *et al.* (2009), our analyses contradict the monophyly of a clade equivalent to *Palaeatta* because, although *A. bisphaerica* and *A. goiana* are sister species, they are not closely related to *A. saltensis*.

The clear monophyly of *A. sexdens* in our study resolves the (weakly supported) polytomy of *A. sexdens* species with respect to *A. robusta* recovered in the previous study of Bacci *et al.* (2009). The well-supported subclades of *A. sexdens* in our study could be interpreted as different species or as geographically correlated, divergent populations (Figs 2a, S19). Our results are consistent with those of Martins (2011), who demonstrated that mitochondrial alleles of *A. sexdens* are divided into three distinct clades. The lineages in our phylogeny and the taxonomic identification of these taxa, however, are not strictly correlated with previously recognized subspecies of *A. sexdens*, including *A. sexdens sexdens* (Linnaeus), *A. sexdens rubropilosa* Forel, and *A. sexdens piriventris* Santschi (Gonçalves, 1963). The subspecies of *A. sexdens* were synonymized by Borgmeier (1959), and this synonymization was subsequently upheld by Bolton (1995, 2021). Because Bolton (2021) does not accept the proposals of Gonçalves (1963) and Mayhé-Nunes (2002) to revive the subspecies of *A. sexdens*, these infraspecific names are currently considered junior synonyms of the nominal species.

The species of the Neoatta clade exhibit an evolutionary pattern that is similar to that discussed previously for the Epiatta clade, in which closely related species have distinctly different ecologies. *Atta robusta* is endemic to the restingas in the States of Rio de Janeiro and Espírito Santo in Brazil (Fowler, 1995; Teixeira *et al.*, 2003; Dáttilo *et al.*, 2012). *Atta robusta* is very well adapted to the semiarid climate of this region, building its nests in sandy soil, producing medium-sized adult colonies, and specializing on dicotyledonous plants (Gonçalves, 1945; Teixeira & Schoereder, 2003; Teixeira *et al.*, 2008). Because it is restricted to such a narrow ecological environment, *A. robusta* has been registered in the National List of Endangered Species of Brazilian Fauna since 2003 (Machado *et al.*, 2008; ICMBio, 2018). In contrast, its sister species *A. sexdens* is distributed from Central America to north-eastern Argentina and western Uruguay (i.e., the southernmost limit of the distribution of the genus) (Weber, 1966; Gonçalves, 1963; Fernández & Sendoya, 2004), and even inhabits the Brazilian coastal regions, but unlike *A. robusta*, it is more frequent in urban and/or disturbed areas (Fowler, 1995). *Atta sexdens* is adapted to a wide variety of environments across a larger climate range (Solomon *et al.*, 2008) and, for this reason, it is a widely distributed pest of human agriculture (Della Lucia, 2003). This species also builds extremely large and populous nests and forages on a wide range of plants (Gonçalves, 1945; Fowler, 1985; Mueller *et al.*, 2017). For example, in the south, central, and north-western parts of its distribution, *A. sexdens* forages mainly on dicotyledonous plants, but less frequently on monocotyledonous plants, whereas in south-eastern Brazil, it exclusively forages on dicotyledonous plants (Gonçalves, 1967, 1971; Fowler, 1985; Farji-Brener & Ruggiero, 1994).

Historical biogeography and evolution

Our phylogeny strongly supports the monophyly of all 14 species of *Atta*. However, for the three most widely distributed species, *A. cephalotes*, *A. laevigata*, and *A. sexdens*, it also strongly supports intraspecific subgroups (Fig. 2a). *Atta cephalotes* is divided into well-supported northern and southern subclades (Fig. S17), as described above, and our data support a scenario in which one daughter lineage of this species secondarily dispersed from the north across the Colombian Andes, initially into Amazonia, and then, secondarily, into the Guiana Shield and Atlantic Forest bioregions (Figs 2, 4). This contradicts the conclusions of a study based on mitochondrial DNA sequencing of *A. cephalotes*, which also identified two geographic daughter lineages, but which concluded that their MRCA originated in the south and secondarily dispersed into the north (Muñoz-Valencia *et al.*, 2021). *Atta laevigata* is divided into three subclades, including a clade located in north-eastern Brazil and two clades with overlapping distributions in central and south-eastern Brazil (Fig. S18). Finally, *A. sexdens* is divided into three subclades that are also correlated with geography. The first

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subclade occupies Uruguay and southern Brazil, the second subclade is restricted to north-eastern Brazil and the third subclade occupies a large area encompassing south-eastern, central western and northern South America (Fig. S19). The northern and southern subclades appear to be divided by the Tropic of Capricorn (or the South Tropic) at Latitude 23°26′, so sampling in this transition zone would allow testing for the presence or absence of gene flow and thus for reproductive isolation and thereby determine the species-level status of these subclades. In addition, both *A. laevigata* and *A. sexdens* include subclades composed of taxa from north-eastern Brazil, suggesting the presence of some environmental and/or geographic barrier that restricts gene flow from other populations into this region (Carnaval & Moritz, 2008).

Our results indicate estimated crown-group ages for extant *Atta* species between 0.3 and 1.8 Ma (95% HPD: 0.1–3.3 Ma) (see Fig. 3 and Table S6). Interestingly, within each clade, the species with the broadest distribution conforms to a common pattern with regard to its age of divergence. The crown-group age of each widespread species is somewhat older (between 1.0 and 2.0 Ma) than the crown-group age of less widespread species, which may be correlated with the longer time required to colonize multiple regions. It is also possible that it is due, at least in part, to sampling bias favouring the widespread species. Most of the geographically restricted species were younger (less than 1.0 Ma), with the exception of two Epiatta species: (i) *Atta saltensis*, which could be restricted in distribution due to environmental barriers, such as climatic differences between the dry Chaco and the wet Chaco of Paraguay to the north (Willig *et al.*, 2000; Wild, 2007) and the decrease in temperature in the extreme south (central-southern region of Argentina) (Farji-Brener & Ruggiero, 1994; Sánchez-Restrepo *et al.*, 2019); and (ii) *A. capiguara*, which presents an unusual case because its currently recognized distribution may be due to poor sampling. Recently, it was found that the increase of human-disturbed areas, in which native vegetation was replaced with grassland, facilitated the geographic expansion of this species (Forti *et al.*, 2020). Even if *A. capiguara* speciated simultaneously with *A. laevigata*, perhaps it has not achieved a similar geographic range due to its specialization in cutting monocotyledonous plants.

The results of the biogeographic analysis employing the best-fitting model that incorporates the dispersal multiplier parameter, BAL + J_ArAlw_Disp (Table S7), indicate that the MRCA of all leaf-cutting ants originated in the combined North/Central America + NW South America bioregions and that the MRCA of *Atta* + *Acromyrmex* likewise originated in this combined region (Fig. 4). The best-fitting model overall, which does not incorporate the dispersal multiplier parameter (BAL + J_ArAlw), indicates instead that the leaf-cutter ancestor originated in North/Central America (Fig. S6). Interestingly, regardless of model, the results of all of the BIOGEOBEARS analyses (Figs 4, S6–S16) agree on a northern origin of the leaf-cutting ants, contradicting most previous hypotheses, which favour a southern Cerrado origin (Kusnezov, 1963; Fowler, 1983; Mueller *et al.*, 2017; Cristiano *et al.*, 2020), but agreeing with the hypotheses of Gonçalves (1986) and Branstetter *et al.* (2017a), the latter, like this study, based on a quantitative biogeographic analysis. However, the question of the biogeographic origin of the leaf-cutting ants is still unsettled and requires, primarily, expanded taxon sampling for the genus *Acromyrmex*.

The reconstruction of a combined area consisting of North/Central America + NW South America throughout higher-attine-ant evolution might seem to be contradicted by the presumed absence of a Panamanian land-bridge connection prior to 3.5 Ma, but, based on distribution data for diverse animal groups, Bacon *et al.* (2015) concluded that during the past 45 million years there have been at least four Isthmus of Panama land connections in addition to the current connection (Fig. 4, grey columns). One of these occurred 41.1 Ma (with a confidence interval of 35.9–46.2 Ma), another occurred 23.7 Ma (with a confidence interval of 19.9–26.2 Ma), and three of them, including the current connection, occurred during the past 10 million years. In addition to the land-bridge connection, it is also possible that the oceanic barrier, when present, may have at times been sufficiently narrow to allow dispersal by flying attine-ant queens.

The crown ancestor of the species of the Archeatta clade originated in North/Central America and the distribution of one species, *A. insularis*, is the result of a presumably rare dispersal to the Caribbean island of Cuba (Fig. 4). Because paleogeographic evidence indicates that the island of Cuba separated from the continent more than 34 Ma (Iturralde-Vinent, 2006), the dispersal of the ancestor of (*A. insularis* + *A. cubana*) must have occurred across an oceanic barrier (Bacci *et al.*, 2009; Mueller *et al.*, 2017), most likely from the Florida or Yucatan peninsula.

Because the BAL + J_ArAlw model ignores the dispersal multiplier parameter, within the biogeographic history of *Atta* it reconstructs two improbable dispersals: (i) from North/Central America to Atlantic Forest in the ancestor of Epiatta + Neoatta and (ii) from North/Central America to Atlantic Forest within *A. cephalotes*, but other within-*Atta* dispersals take place between adjacent areas and appear reasonable (Fig. S6). All dispersals within the genus *Atta* as reconstructed by the BAL + J_ArAlw_Disp model, which incorporates the dispersal multiplier parameter, occur between adjacent areas and generally appear reasonable (Fig. 4). Because we were unable to include the Guiana Shield in our coding for the geographic population represented by *Atta cephalotes* 9991, it is helpful to examine the geographic distributions in *A. cephalotes* and *A. colombica* in the more comprehensively sampled phylogeny in Fig. 2a. The relationships of *A. cephalotes* to *A. colombica* and the relationships of geographic subclades (populations or, possibly, cryptic species) within *A. cephalotes* are consistent with the BIOGEOBEARS-reconstructed range of Central/North America and NW South America for the crown ancestor of the Atta s.s. clade and with the subsequent dispersal of one of two sister lineages within *A. cephalotes* across the Andes into the south. Dispersal into the Atlantic Forest from Amazonia around 1.1 Ma corresponds with the existence of gallery forest corridors connecting the two bioregions at that time (Oliveira *et al.*, 1999; Auler *et al.*, 2004; Wang *et al.*, 2004; Batalha-Filho *et al.*, 2013), discussed further below.

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The biogeographic reconstruction in Fig. 4 indicates that the common crown ancestor of the Atta s.s., Epiatta, and Neoatta clades occupied the combined area of North/Central America + NW South America approximately 7.5 Ma. This ancestor diverged into two sister clades, the Atta s.s. clade, discussed above, and the stem ancestor of Epiatta + Neoatta, reconstructed as occupying the Amazonia bioregion. As this stem ancestor evolved into the crown ancestor between 7.5 and 2.2 Ma, the reconstruction indicates that it dispersed into the Cerrado, then dispersed into the Chaco in the stem ancestor of Epiatta and into the Atlantic Forest in the stem ancestor of Neoatta. In both Epiatta and Neoatta, beginning around 2.2 Ma, there were subsequent dispersals between Cerrado, Atlantic Forest, Chaco, and Pampas. Although the modern Amazonia and Atlantic Forest bioregions are non-adjacent, they have been connected in the past, most recently through gallery forests within the dry diagonal of South America in north-eastern Brazil during a period of wetter conditions in the Quaternary that began 2.5 Ma. The existence of this past connection, spanning what is present-day Cerrado and Caatinga, likely explains the reconstructed Amazonia-Cerrado-Atlantic Forest dispersal pattern in Atta s.s., Epiatta, and Neoatta in our biogeographic reconstruction. The beginning of these dispersal events around 2.2 Ma corresponds remarkably well with the timing of the Quaternary forest corridors (Oliveira *et al.*, 1999; Auler *et al.*, 2004; Wang *et al.*, 2004; Batalha-Filho *et al.*, 2013).

The evolution and geographic radiation of South American *Atta* species in the Epiatta and Neoatta clades was likely associated with adaptations to dry habitats and the expansion of the Cerrado around 4–8 Ma. This rapid radiation is correlated with environmental and climatic changes, as well as with the increase of forest clearings and edges during the Pleistocene due to the increasing interpolation of grasslands, Cerrado, and forest, which likely created conditions that favoured the diversification of species distributed in a South-to-North direction (Prado & Gibbs, 1993; Silva, 2011; Meyer *et al.*, 2014). If correct, this would explain why the current distributions of species such as *A. laevigata* and *A. sexdens* can include both Cerrado and rainforest habitats. Our results suggest that a mosaic of dry and moist habitats was critical to the diversification of most South American *Atta* species, driving a burst of phenotypic diversification, speciation, and range expansion into other bioregions.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Tables S1–S8: Supplementary tables.

Appendix S2. Figs S1–S5: Supplementary phylogenetic trees.

Appendix S3. Figs S6–S16: Supplementary BioGeoBEARS results.

Appendix S4. Figs S17–S19: Supplementary maps.

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Data availability statement

All new raw Illumina reads of UCE loci generated for this study are deposited in the NCBI Sequence Read Archive (SRA), BioProject# PRJNA742376. The Illumina reads of some samples, including two *Atta* specimens (*Atta cephalotes* 3779 and *Atta insularis* 3712) and several outgroup taxa (see Supplementary Table S5b), were generated for previous studies and can be found in the NCBI SRA, BioProjects# PRJNA454746, PRJNA298486, and PRJNA379607. BioGeoBEARS scripts used in this study were provided by A.F. Sánchez-Restrepo and I.L.F. Magalhaes and are available on GitHub: https://github.com/ivanlfm/BGB_model_selection. Additional data, including TRINITY assembly contigs, tree files, and supplementary tables, are deposited in the Dryad repository (https://doi.org/10.5061/dryad.547d7wm86).

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References

Auler, A.S., Wang, X., Edwards, R.L., Cheng, H., Cristalli, P.S., Smart, P.L. & Richards, D.A. (2004) Quaternary ecological and geomorphic changes associated with rainfall events in presently semi-arid northeastern Brazil. *Journal of Quaternary Science*, **19**, 693–701. https://doi.org/10.1002/jqs.876.

Bacci Jr., M., Ribeiro, S.B., Casarotto, M.E.F. & Pagnocca, F.C. (1995) Biopolymer-degrading bacteria from nests of the leaf-cutting ant *Atta sexdens rubropilosa*. *Brazilian Journal of Medical and Biological Research*, **28**, 79–82.

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.

Bacon, C.D., Silvestro, D., Jaramillo, C., Smith, B.T., Chakrabarty, P. & Antonelli, A. (2015) Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences*, **112**, 6110–6115. https://doi.org/10.1073/pnas.1423853112.

Barbosa, J.T.V., Barbosa, M.S., Morais, S., Santana, A.E.G. & Almeida, C. (2019) Mitochondrial genomes of genus *Atta* (Formicidae: Myrmicinae) reveal high gene organization and giant intergenic spacers. *Genetics and Molecular Biology*, **42**, e20180055. https://doi.org/10.1590/1678-4685-gmb-2018-0055.

Baroni Urbani, C. (1980) First description of fossil gardening ants (Amber collection Stuttgart and Natural History Museum Basel; Hymenoptera: Formicidae. I: Attini.). *Stuttgarter Beiträge zur Naturkunde Serie B*, **54**, 1–13.

Batalha-Filho, H., Fjeldså, J., Fabre, P.H. & Miyaki, C.Y. (2013) Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. *Journal of Ornithology*, **154**, 41–50.

Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W.J., Mattick, J.S. & Haussler, D. (2004) Ultraconserved elements in the human genome. *Science*, **304**, 1321–1325. https://doi.org/10.1126/science.1098119.

Blaimer, B.B., Brady, S.G., Schultz, T.R., Lloyd, M.W., Fisher, B.L. & Ward, P.S. (2015) Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: a case study of formicine ants. *BMC Evolutionary Biology*, **15**, 271. https://doi.org/10.1186/s12862-015-0552-5.

Blaimer, B.B., LaPolla, J.S., Branstetter, M.G., Lloyd, M.W. & Brady, S.G. (2016b) Phylogenomics, biogeography and diversification of obligate mealybug-tending ants in the genus *Acropyga*. *Molecular Phylogenetics and Evolution*, **102**, 20–29. https://doi.org/10.1016/j.ympev.2016.05.030.

Blaimer, B.B., Lloyd, M.W., Guillory, W.X. & Brady, S.G. (2016a) Sequence capture and phylogenetic utility of genomic ultraconserved elements obtained from pinned insect specimens. *PLoS One*, **11**, 161531. https://doi.org/10.1371/journal.pone.0161531.

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, **30**, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bolton, B. (1995) *A New General Catalogue of the Ants of the World*. Harvard University Press, Cambridge, Massachusetts.

Bolton, B. (2021) *An Online Catalog of the Ants of the World* [WWW document]. URL https://antcat.org/ [accessed on 10 October 2020].

Bolton, B., Alpert, G., Ward, P.S. & Naskrecki, P. (2006) *Bolton's Catalogue of Ants of the World: 1758–2005*. Harvard University Press, Cambridge, Massachusetts.

Borgmeier, T. (1950) Estudos sobre *Atta* (Hym., Formicidae). *Memórias do Instituto Oswaldo Cruz*, **48**, 239–263.

Borgmeier, T. (1959) Revision der Gattung *Atta* Fabricius (Hymenoptera, Formicidae). *Studia Entomologica*, **2**, 321–390.

Borowiec, M.L. (2019a) Convergent evolution of the army ant syndrome and congruence in big-data phylogenetics. *Systematic Biology*, **68**, 642–656. https://doi.org/10.1093/sysbio/syy088.

Borowiec, M.L. (2019b) Spruceup: fast and flexible identification, visualization, and removal of outliers from large multiple sequence alignments. *Journal of Open Source Software*, **4**, 1635. https://doi.org/10.21105/joss.01635.

Borowiec, M.L., Cover, S.P. & Rabeling, C. (2020) The evolution of social parasitism in *Formica* ants revealed by a global phylogeny. *bioRxiv*. https://doi.org/10.1101/2020.12.17.423324.

Brandão, C.R.F. (1991) Adendos ao catálogo abreviado das formigas da região Neotropical (Hymenoptera: Formicidae). *Revista Brasileira de Biologia*, **35**, 319–412.

Brandão, C.R.F. (1995) *Formigas dos Cerrados e Caatingas*. PhD Thesis, Instituto de Biociências da Universidade de São Paulo. Departamento de Zoologia, São Paulo.

Branstetter, M.G., Danforth, B.N., Pitts, J.P. *et al.* (2017c) Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Current Biology*, **27**, 1019–1025. https://doi.org/10.1016/j.cub.2017.03.027.

Branstetter, M.G., Ješovnik, A., Sosa-Calvo, J., Lloyd, M.W., Faircloth, B.C., Brady, S.G. & Schultz, T.R. (2017a) Dry habitats were crucibles of domestication in the evolution of agriculture in ants. *Proceedings of the Royal Society B: Biological Sciences*, **284**, 20170095. https://doi.org/10.1098/rspb.2017.0095.

Branstetter, M.G., Longino, J.T., Ward, P.S. & Faircloth, B.C. (2017b) Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other hymenoptera. *Methods in Ecology and Evolution*, **8**, 768–776. https://doi.org/10.1111/2041-210X.12742.

Branstetter, M.G., Müller, A., Griswold, T.L., Orr, M.C. & Zhu, C.D. (2021) Ultraconserved element phylogenomics and biogeography of the agriculturally important mason bee subgenus *Osmia* (*Osmia*). *Systematic Entomology*, **46**, 453–472. https://doi.org/10.1111/syen.12470.

Carnaval, A.C. & Moritz, C. (2008) Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *Journal of Biogeography*, **35**, 1187–1201. https://doi.org/10.1111/j.1365-2699.2007.01870.x.

Chernomor, O., von Haeseler, A. & Minh, B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, **65**, 997–1008. https://doi.org/10.1093/sysbio/syw037.

Cherrett, J.M. (1989) Biology, pest status and control of leaf-cutting ants. *Biology and Population Dynamics of Invertebrate Crop Pests* (ed. by G.E. Russell), pp. 1–38. Intercept, Andover, Minnesota.

Crawford, N.G., Faircloth, B.C., McCormack, J.E., Brumfield, R.T., Winker, K. & Glenn, T.C. (2012) More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. *Biology Letters*, **8**, 783–786. https://doi.org/10.1098/rsbl.2012.0331.

Cristiano, M.P., Cardoso, D.C. & Fernandes-Salomão, T.M. (2013) Cytogenetic and molecular analyses reveal a divergence between *Acromyrmex striatus* (Roger, 1863) and other congeneric species: taxonomic implications. *PLoS One*, **8**, e59784. https://doi.org/10.1371/journal.pone.0059784.

Cristiano, M.P., Cardoso, D.C., Sandoval-Gómez, V.E. & Simões-Gomes, F.C. (2020) *Amoimyrmex* Cristiano, Cardoso & Sandoval, gen. nov. (Hymenoptera: Formicidae): a new genus of leaf-cutting ants revealed by multilocus molecular phylogenetic and morphological analyses. *Austral Entomology*, **59**, 643–676. https://doi.org/10.1111/aen.12493.

 doi: 10.1111/syen.12513

Currie, C.R., Wong, B., Stuart, A.E. *et al.* (2003) Ancient tripartite coevolution in the attine ant-microbe symbiosis. *Science*, **299**, 386–388. https://doi.org/10.1126/science.1078155.

Daguerre, J.B. (1945) Hormigas del género *Atta* Fabricius de la Argentina (Hymenop. Formicidae). *Revista de la Sociedad Entomológica Argentina*, **12**, 438–460.

Dáttilo, W., Falcão, J.C. & Teixeira, M.C. (2012) Predictive model of distribution of *Atta robusta* Borgmeier 1939 (Hymenoptera: Formicidae): subsidies for conservation of a Brazilian leaf-cutting ant endangered species. *Studies on Neotropical Fauna and Environment*, **47**, 193–201. https://doi.org/10.1080/01650521.2012.700791.

De Fine Licht, H.H., Schiøtt, M., Rogowska-Wrzesinska, A., Nygaard, S., Roepstorff, P. & Boomsma, J.J. (2013) Laccase detoxification mediates the nutritional alliance between leaf-cutting ants and fungus-garden symbionts. *Proceedings of the National Academy of Sciences*, **110**, 583–587. https://doi.org/10.1073/pnas.1212709110.

Delabie, J.C.H. (1998) *Atta silvai* Gonçalves, sinônimo júnior de *Atta laevigata* (Fred Smith) (Hymenoptera, Formicidae, Attini). *Revista Brasileira de Entomologia*, **2**, 339–341.

Della Lucia, T.M.C. (2003) Hormigas de importancia económica en la región Neotropical. *Introducción a las Hormigas de la Región Neotropical* (ed. by F. Fernández), pp. 337–349. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá.

Della Lucia, T.M.C. (2011) *Formigas-Cortadeiras: da Bioecologia ao Manejo*. Editora Universidade Federal de Viçosa, Viçosa.

Della Lucia, T.M.C., Fowler, H.G. & Moreira, D.D.O. (1993) Espécies de formigas cortadeiras no Brasil. *As Formigas Cortadeiras* (ed. by T.M.C. Della Lucia), pp. 26–32. Folha de Viçosa, Viçosa.

Do Amaral, F.R., Neves, L.G., Resende, M.F. Jr, Mobili, F., Miyaki, C.Y., Pellegrino, K.C.M. & Biondo, C. (2015) Ultraconserved elements sequencing as a low-cost source of complete mitochondrial genomes and microsatellite markers in nonmodel amniotes. *PLoS One*, **10**, e0138446. https://doi.org/10.1371/journal.pone.0138446.

Dufour, D.L. (1987) Insects as food: a case study from the Northwest Amazon. *American Anthropologist*, **89**, 383–397.

Emery (1913) Éstudes sur les Myrmicinae: V–VII. *Annales de la Société Entomologique de Belgique*, **57**, 250–262.

Emery, C. (1922) Hymenoptera. *Fam. Formicidae. Subfam. Myrmicinae. Genera Insectorum*, Vol. **174** (ed. by P. Wytsman), pp. 207–397. Verteneuil and Desmet, Brussels.

Faircloth, B. C. (2013) *Illumiprocessor: A Trimmomatic Wrapper for Parallel Adapter and Quality Trimming* [WWW document] https://doi.org/10.6079/J9ILL [accessed on 18 July 2019].

Faircloth, B.C. (2016) PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics*, **32**, 786–788. https://doi.org/10.1093/bioinformatics/btv646.

Faircloth, B.C., Branstetter, M.G., White, N.D. & Brady, S.G. (2015) Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Molecular Ecology Resources*, **15**, 489–501. https://doi.org/10.1111/1755-0998.12328.

Faircloth, B.C. & Glenn, T.C. (2012) Not all sequence tags are created equal: designing and validating sequence identification tags robust to indels. *PLoS One*, **7**, e42543. https://doi.org/10.1371/journal.pone.0042543.

Faircloth, B.C., Mccormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T. & Glenn, T.C. (2012) Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, **61**, 717–726. https://doi.org/10.1093/sysbio/sys004.

Farji-Brener, A.G. & Ruggiero, A. (1994) Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range sizes. *Journal of Biogeography*, **21**, 391–399. https://doi.org/10.2307/2845757.

Fernández, F., Castro-Huertas, A.V. & Serna, F. (2015) *Hormigas Cortadoras de Hojas de Colombia: Acromyrmex & Atta (Hymenoptera: Formicidae). Fauna de Colombia, Monografia No. 5*. Universidad Nacional de Colombia, Facultad de Ciencias, Instituto de Ciencias Naturales, Bogotá.

Fernández, F. & Sendoya, S. (2004) Synonymic list of Neotropical ants (Hymenoptera: Formicidae). *Biota Colombiana*, **5**, 3–93.

Fernández, F. & Serna, F.J. (2019) Subfamilia Myrmicinae. *Hormigas de Colombia* (ed. by F. Fernández, R.J. Guerrero and T. Delsinne), pp. 791–888. Universidad Nacional de Colombia, Facultad de Ciencias, Instituto de Ciencias Naturales, Bogotá.

Fischer, M. (2015) *Forest health: leaf-cutting ant. Texas A&M Forest Service*. [WWW document] URL http://texasforestservice.tamu.edu/LeafCuttingAnt/ [accessed on 17 February 2020].

Fisher, S., Barry, A., Abreu, J. *et al.* (2011) A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. *Genome Biology*, **12**, 1–5. https://doi.org/10.1186/gb-2011-12-1-r1.

Fontenla Rizo, J.L. (1995) Nueva especie de *Atta* (Hymenoptera: Formicidae) del archipiélago cubano. *Avicennia*, **3**, 77–86.

Forti, L.C. & Boaretto, M.A.C. (1997) *Formigas Cortadeiras: Biologia, Ecologia, Danos e Controle*. Departamento de Defesa Fitossanitária, Universidade Estadual Paulista, Botucatu.

Forti, L.C., De Andrade, M.L., Andrade, A.P.P., Lopes, J.F.S. & Ramos, V.M. (2006) Bionomics and identification of *Acromyrmex* (Hymenoptera: Formicidae) through an illustrated key. *Sociobiology*, **48**, 1–18.

Forti, L.C., Rando, J.S., da Silva Camargo, R. *et al.* (2020) Occurrence of leaf-cutting and grass-cutting ants of the genus *Atta* (Hymenoptera: Formicidae) in geographic regions of Brazil. *Sociobiology*, **67**, 514–525. https://doi.org/10.13102/sociobiology.v67i4.5741.

Fowler, H.G. (1983) Latitudinal gradients and diversity of the leaf-cutting ants (*Atta* and *Acromyrmex*) (Hymenoptera: Formicidae). *Revista de Biología Tropical*, **31**, 213–216.

Fowler, H.G. (1985) Leaf-cuttings ants of the genera *Atta* and *Acromyrmex* of Paraguay (Hymenoptera Formicidae). *Deutsche Entomologische Zeitschrift*, **32**, 19–34. https://doi.org/10.1002/mmnd.19850320105.

Fowler, H.G. (1988) Taxa of the neotropical grass-cutting ants, *Acromyrmex* (*Moellerius*) (Hymenoptera: Formicidae: Attini). *Científica*, **16**, 281–295.

Fowler, H.G. (1995) The population status of the endangered Brazilian endemic leaf-cutting ant *Atta robusta* (Hymenoptera: Formicidae). *Biological Conservation*, **74**, 147–150. https://doi.org/10.1016/0006-3207(95)00009-S.

Fowler, H.G., Forti, L.C., Pereira-da-Silva, V. & Saes, N.B. (1986) Economics of grass-cutting ants. *Fire Ants and Leaf-Cutting Ants* (ed. by C.S. Lofgren and R.K. Vandermeer), pp. 18–35. Westiview, Boulder.

Fowler, H.G., Pagani, M.I., Da Silva, O.A., Forti, L.C., Da Silva, V.P. & De Vasconcelos, H.L. (1989) A pest is a pest is a pest? The dilemma of neotropical leaf-cutting ants: keystone taxa of natural ecosystems. *Environmental Management*, **13**, 671–675.

Glenn, T.C., Nielsen, R.A., Kieran, T.J. *et al.* (2019) Adapterama I: universal stubs and primers for 384 unique dual-indexed or 147, 456 combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ*, **7**, e7755. https://doi.org/10.7717/peerj.7755.

Gonçalves, C.R. (1942) Contribuição para o conhecimento do gênero *Atta* Fabr., das formigas saúvas. *Boletim da Sociedade Brasileira de Agronomia*, **5**, 333–358.

Gonçalves, C.R. (1944) Descrição de uma nova saúva brasileira (Hym., Form.). *Revista Brasiliera de Biologia*, **4**, 233–238.

Gonçalves, C.R. (1945) Saúvas do sul e centro do Brasil. *Boletim Fitossanitário*, **2**, 183–218.

 doi: 10.1111/syen.12513

Gonçalves, C.R. (1960) Distribuição, biologia e ecologia das saúvas. *Divulgação Agronômica*, **1**, 2–10.

Gonçalves, C.R. (1961) O gênero *Acromyrmex* no Brasil (Hym. Formicidae). *Studia Entomologica*, **4**, 113–180.

Gonçalves, C.R. (1963) Nota sobre a sistemática de *Atta sexdens* (L, 1758) e de suas sub-espécies (Hym., Formicidae). *Boletim Fitossanitário*, **9**, 1–3.

Gonçalves, C.R. (1967) As formigas cortadeiras da Amazônia, dos generos "*Atta"* Fabr. e "*Acromyrmex"* Mayr (Hym., Formicidae). *Atas do Simpósio sôbre a Biota Amazônica*, **5**, 181–202.

Gonçalves, C.R. (1971) *As saúvas de Mato Grosso, Brasil (Hymenoptera, Formicidae)*, Vol. **54**, pp. 249–253. *Arquivos do Museu Nacional*, Rio de Janeiro.

Gonçalves, C.R. (1986) *Filogenia do gênero Atta Fabricius (Hymenoptera, Formicidae). Anais do VI Congresso Brasileiro de Zoologia*, Vol. **65**, pp. 13–17. *Publicações Avulsas do Museu Nacional*, Rio de Janeiro.

Grabherr, M.G., Haas, B.J., Yassour, M. *et al.* (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, **29**, 644–652. https://doi.org/10.1038/nbt.1883.

Grimaldi, D. & Agosti, D. (2000) A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. *Proceedings of the National Academy of Sciences*, **97**, 13678–13683. https://doi.org/10.1073/pnas.240452097.

Guénard, B., Weiser, M., Gomez, K., Narula, N. & Economo, E.P. (2017) The global ant biodiversity informatics (GABI) database: synthesizing data on the geographic distribution of ant species (Hymenoptera: Formicidae). *Myrmecological News*, **24**, 83–89.

Harris, R. (2007) *Improved Pairwise Alignment of Genomic DNA*. PhD Thesis, Pennsylvania State University, University Park, Pennsylvania.

Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, **35**, 518–522. https://doi.org/10.1093/molbev/msx281.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Harvard University Press, Cambridge, Massachusetts.

Hölldobler, B. & Wilson, E.O. (2011) *The Leafcutter Ants: Civilization by Instinct*. WW Norton & Company, New York.

Inoue, J., Donoghue, P.C. & Yang, Z. (2010) The impact of the representation of fossil calibrations on Bayesian estimation of species divergence times. *Systematic Biology*, **59**, 74–89. https://doi.org/10.1093/sysbio/syp078.

Instituto Chico Mendes de Conservação da Biodiversidade, ICMBio. (2018) Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume VII - Invertebrados. *Instituto Chico Mendes de Conservação da Biodiversidade* (org.). Livro Vermelho da Fauna Brasileira Ameaçada de Extinção, Brasília.

Iturralde-Vinent, M.A. (2006) Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review*, **48**, 791–827. https://doi.org/10.2747/0020-6814.48.9.791.

Iturralde-Vinent, M.A. & MacPhee, R.D.E. (1996) Age and paleogeographical origin of Dominican amber. *Science*, **273**, 1850–1852 URL https://www.jstor.org/stable/2891099.

Janicki, J., Narula, N., Ziegler, M., Guénard, B. & Economo, E.P. (2016) Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: the design and implementation of antmaps.org. *Ecological Informatics*, **32**, 185–193.

Ješovnik, A., González, V.L. & Schultz, T.R. (2016) Phylogenomics and divergence dating of fungus-farming ants (Hymenoptera: Formicidae) of the genera *Sericomyrmex* and *Apterostigma*. *PLoS One*, **11**, e0151059. https://doi.org/10.1371/journal.pone.0151059.

Ješovnik, A., Sosa-Calvo, J., Lloyd, M.W., Branstetter, M.G., Fernández, F. & Schultz, T.R. (2017) Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera: Formicidae): ultraconserved elements (UCEs) resolve a recent radiation. *Systematic Entomology*, **42**, 523–542. https://doi.org/10.1111/syen.12228.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994) Organisms as ecosystem engineers. *Ecosystem Management* (ed. by F.B. Samson and F.L. Knopf), pp. 130–147. Springer, New York.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, **14**, 587–589. https://doi.org/10.1038/nmeth.4285.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780. https://doi.org/10.1093/molbev/mst010.

Kempf, W.W. (1972) Catálogo abreviado das formigas da região Neotropical. *Studia Entomologica*, **15**, 3–344.

Kusnezov, N. (1963) Zoogeografía de las hormigas en Sudamérica. *Acta Zoologica Lilloana*, **19**, 25–186.

Kusnezov, N. (1978) *Hormigas Argentinas: claves para su identificación*. Miscelánea 61, Ministerio de Cultura y Educación, Instituto Miguel Lillo, Tucumán.

Landis, M.J., Matzke, N.J., Moore, B.R. & Huelsenbeck, J.P. (2013) Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology*, **62**, 789–804. https://doi.org/10.1093/sysbio/syt040.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**, 772–773. https://doi.org/10.1093/molbev/msw260.

Leal, I.R., Wirth, R. & Tabarelli, M. (2014) The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified Neotropical forests. *Biotropica*, **46**, 516–528. https://doi.org/10.1111/btp.12126.

Lemmon, E.M. & Lemmon, A.R. (2013) High-throughput genomic data in systematics and phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 99–121. https://doi.org/10.1146/annurev-ecolsys-110512-135822.

Li, H., Sosa-Calvo, J., Horn, H.A., Pupo, M.T., Clardy, J., Rabeling, C. & Currie, C.R. (2018) Convergent evolution of complex structures for ant–bacterial defensive symbiosis in fungus-farming ants. *Proceedings of the National Academy of Sciences*, **115**, 10720–10725. https://doi.org/10.1073/pnas.1809332115.

Lovato, L. (2006) *Estudos morfológicos e análises de sequências do gene mitocondrial citocromo oxidase I (COI) em populações de Atta cephalotes (L., 1758)*. PhD Thesis, Universidade Federal do Paraná, Curitiba.

Machado, A.B.M., Drummond, G.M. & Paglia, A.P. (2008) *Livro vermelho da fauna brasileira ameaçada de extinção - Ministério do Meio Ambiente*, Vol. **1**. Fundação Biodiversitas, Brasília, DF.

Magalhaes, I.L.F. (2021) *BGB_Model_Selection*. *Script to Perform Model Selection Using BioGeoBEARS* [WWW document]. URL https://github.com/ivanlfm/BGB_model_selection [accessed on 2021].

Martins, J.J. (2011) *Filogenia Molecular de Atta sexdens (Myrmicinae: Attini) e investigação de pseudogenes em formigas da tribo Attini*. PhD Thesis, Universidade Estadual Paulista, Instituto de Biociências, Rio Claro.

Masiulionis, V.E., Rabeling, C., Henrik, H., Schultz, T., Bacci Jr., M., Bezerra, C.M.S. & Pagnocca, F.C. (2014) A Brazilian population of the asexual fungus-growing ant *Mycocepurus smithii* (Formicidae,

Myrmicinae, Attini) cultivates fungal symbionts with gongylidia-like structures. *PLoS One*, **9**, e103800. https://doi.org/10.1371/journal.pone.0103800.

Matzke, N.J. (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography*, **5**, 242–248. https://doi.org/10.21425/F5FBG19694.

Matzke, N.J. (2014) Model selection in historical biogeography reveals that founder-event speciation is a crucial process in Island clades. *Systematic Biology*, **63**, 951–970. https://doi.org/10.1093/sysbio/syu056.

Matzke N.J. (2015) *Script: Example that Should Run out of the Box: Hawaiian* Psychotria [WWW document] URL http://phylo.wikidot.com/biogeobears#script [accessed on 2020].

Matzke, N.J. (2021) *PhyloWiki: Biogeobears Mistakes to Avoid: OTUs of the Phylogeny Should Be Species/Populations*, *Not Specimens* [WWW document] URL http://phylo.wikidot.com/biogeobears-mistakes-to-avoid#no_specimen_trees [accessed on 02 July 2021].

Mayhé-Nunes, A.J. (2002) A controvérsia sobre as subespécies de *Atta sexdens* (L., 1758) das formigas saúvas (Hymenoptera: Formicidae: Myrmicinae: Attini). *Folha Florestal*, **101**, 12.

Mehdiabadi, N.J., Mueller, U.G., Brady, S.G., Himler, A.G. & Schultz, T.R. (2012) Symbiont fidelity and the origin of species in fungus-growing ants. *Nature Communications*, **3**, 1–7. https://doi.org/10.1038/ncomms1844.

Mehdiabadi, N.J. & Schultz, T.R. (2010) Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecological News*, **13**, 37–55.

Meirelles, L.A., Solomon, S.E., Bacci Jr., M., Wright, A.M., Mueller, U.G. & Rodrigues, A. (2015) Shared *Escovopsis* parasites between leaf-cutting and non-leaf-cutting ants in the higher attine fungus-growing ant symbiosis. *Royal Society Open Science*, **2**, 150257. https://doi.org/10.1098/rsos.150257.

Meyer, K.E.B., Cassino, R.F., Lorente, F.L., Raczka, M. & Parizzi, M.G. (2014) Paleoclima e paleoambiente do Cerrado durante o Quaternário com base em análises palinológicas. *Paleontologia: Cenários da Vida*, **5**, 403–420.

Mikheyev, A.S., Mueller, U.G. & Abbot, P. (2006) Cryptic sex and many-to-one coevolution in the fungus-growing ant symbiosis. *Proceedings of the National Academy of Sciences*, **103**, 10702–10706. https://doi.org/10.1073/pnas.0601441103.

Mikheyev, A.S., Mueller, U.G. & Abbot, P. (2010) Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals coevolutionary synchrony and discord. *The American Naturalist*, **175**, E126–E133. https://doi.org/10.1086/652472.

Morrone, J.J. (2006) Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. *Annual Review Entomology*, **51**, 467–494. https://doi.org/10.1146/annurev.ento.50.071803.130447.

Moser, J.C. (1986) Estimating timber losses from a town ant colony with aerial photographs. *Southern Journal of Applied Forestry*, **10**, 45–47. https://doi.org/10.1093/sjaf/10.1.45.

Mountinho, P., Nepstad, D.C. & Davidson, E.A. (2003) Influence of leaf-cutting ant nests on secondary forest growth and soil properties in Amazonia. *Ecology*, **84**, 1265–1276. https://doi.org/10.1890/0012-9658(2003)084[1265:IOLANO]2.0.CO;2.

Mueller, U.G., Ishak, H.D., Bruschi, S.M. *et al.* (2017) Biogeography of mutualistic fungi cultivated by leafcutter ants. *Molecular Ecology*, **26**, 6921–6937. https://doi.org/10.1111/mec.14431.

Mueller, U.G., Kardish, M.R., Ishak, H.D. *et al.* (2018) Phylogenetic patterns of ant-fungus associations indicate that farming strategies, not only a superior fungal cultivar, explain the ecological success of leafcutter ants. *Molecular Ecology*, **27**, 2414–2434. https://doi.org/10.1111/mec.14588.

Mueller, U.G. & Rabeling, C. (2008) A breakthrough innovation in animal evolution. *Proceedings of the National Academy of Sciences*, **105**, 5287–5288. https://doi.org/10.1073/pnas.0801464105.

Mueller, U.G., Rehner, S.A. & Schultz, T.R. (1998) The evolution of agriculture in ants. *Science*, **281**, 2034–2038. https://doi.org/10.1126/science.281.5385.2034.

Muñoz-Valencia, V., Vélez-Matínez, G.A., Montoya-Lerma, J. & Diaz, F. (2021) The role of the Andean uplift as an asymmetrical barrier to gene flow in the Neotropical leaf-cutting ant *Atta cephalotes*. *bioRxiv*. https://doi.org/10.1101/2021.03.24.436865.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**, 268–274. https://doi.org/10.1093/molbev/msu300.

Nygaard, S., Hu, H., Li, C. *et al.* (2016) Reciprocal genomic evolution in the ant-fungus agricultural symbiosis. *Nature Communications*, **7**, 1–9. https://doi.org/10.1038/ncomms12233.

Oliveira, P.E., Barreto, A.M.F. & Suguio, K. (1999) Late Pleistocene/Holocene climatic and vegetational history of the Brazilian caatinga: the fossil dunes of the middle São Francisco River. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **152**, 319–337. https://doi.org/10.1016/S0031-0182(99)00061-9.

Pereira, T.T.P., dos Reis, A.C.C.C., Cardoso, D.C. & Cristiano, M.P. (2018) Molecular phylogenetic reconstruction and localization of the (TTAGG) n telomeric repeats in the chromosomes of *Acromyrmex striatus* (Roger, 1863) suggests a lower ancestral karyotype for leaf-cutter ants (Hymenoptera). *Comparative Cytogenetics*, **12**, 13–21. https://doi.org/10.3897/CompCytogen.v12i1.21799.

Prado, D.E. & Gibbs, P.E. (1993) Patterns of species distributions in the dry seasonal forests of South America. *Annals of the Missouri Botanical Garden*, **80**, 902–927. https://doi.org/10.2307/2399937.

Prebus, M.M. (2020) Phylogenomic species delimitation in the ants of the *Temnothorax salvini* group (Hymenoptera: Formicidae): an integrative approach. *Systematic Entomology*, **46**, 307–326. https://doi.org/10.1111/syen.12463.

Rabeling, C., Schultz, T.R., Bacci Jr., M. & Bollazzi, M. (2015) *Acromyrmex charruanus*: a new inquiline social parasite species of leaf-cutting ants. *Insectes Sociaux*, **62**, 335–349. https://doi.org/10.1007/s00040-015-0406-6.

Rabeling, C., Bollazzi, M., Delabie, J.H.C., Schultz, T.R. & Bacci Jr., M. (2018) Reconstructing the evolutionary history of leaf-cutting ants reveal multiple origins of inquiline social parasitism via sympatric speciation. *XVIII International Conference of the Union for the Study of Social Insects*. Guaruja, SP, Brazil.

Rabeling, C., Gonzales, O., Schultz, T.R. *et al.* (2011) Cryptic sexual populations account for genetic diversity and ecological success in a widely distributed, asexual fungus-growing ant. *Proceedings of the National Academy of Sciences*, **108**, 12366–12371. https://doi.org/10.1073/pnas.1105467108.

Rabeling, C., Messer, S., Lacau, S. & do Nascimento, I.C., Bacci Jr., M. & Delabie, J.H.C. (2019) *Acromyrmex fowleri*: a new inquiline social parasite species of leaf-cutting ants from South America, with a discussion of social parasite biogeography in the Neotropical region. *Insectes Sociaux*, **66**, 435–451. https://doi.org/10.1007/s00040-019-00705-z.

Rabeling, C., Schultz, T.R., Pierce, N.E. & Bacci Jr., M. (2014) A social parasite evolved reproductive isolation from its fungus-growing ant host in sympatry. *Current Biology*, **24**, 2047–2052. https://doi.org/10.1016/j.cub.2014.07.048.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using

 doi: 10.1111/syen.12513

Tracer 1.7. *Systematic Biology*, **67**, 901–904. https://doi.org/10.1093/sysbio/syy032.

Rando, J.S.S. & Forti, L.C. (2005) Ocorrência de formigas *Acromyrmex* Mayr, 1865, em alguns municípios do Brasil. Acta Scientiarum. *Biological Sciences*, **27**, 129–133. https://doi.org/10.4025/actascibiolsci.v27i2.1322.

Ree, R.H., Moore, B.R., Webb, C.O. & Donoghue, M.J. (2005) A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, **59**, 2299–2311. https://doi.org/10.1111/j.0014-3820.2005.tb00940.x.

Ree, R.H. & Sanmartín, I. (2018) Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography*, **45**, 741–749. https://doi.org/10.1111/jbi.13173.

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4–14. https://doi.org/10.1080/10635150701883881.

Rodrigues, A., Pagnocca, F.C., Bacci Jr., M., Hebling, M.J.A., Bueno, O.C. & Pfenning, L.H. (2005) Variability of non-mutualistic filamentous fungi associated with *Atta sexdens rubropilosa* nests. *Folia Microbiologica*, **50**, 421–425.

Rohland, N. & Reich, D. (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research*, **22**, 939–946. https://doi.org/10.1101/gr.128124.111.

Ronquist, F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, **46**, 195–203. https://doi.org/10.1093/sysbio/46.1.195.

Sabattini, J.A., Zerda, H.R., Sabattini, R.A. & Savino, C. (2017) Distribución geográfica potencial de *Atta vollenweideri* Forel en la provincia de Entre Ríos (Argentina). *Ambiência*, **13**, 31–46.

Sánchez-Restrepo, A.F., Jiménez, N.L., Confalonieri, V.A. & Calcaterra, L.A. (2019) Distribution and diversity of leaf-cutting ants in northeastern Argentina: species most associated with forest plantations. *International Journal of Pest Management*, **65**, 244–257. https://doi.org/10.1080/09670874.2018.1555343.

Schowalter, T.D. & Ring, D.R. (2017) Biology and management of the Texas leafcutting ant (Hymenoptera: Formicidae). *Journal of Integrated Pest Management*, **8**, 16. https://doi.org/10.1093/jipm/pmx013.

Schrader, L., Pan, H., Bollazzi, M. *et al.* (2021) Relaxed selection underlies genome erosion in socially parasitic ant species. *Nature Communications*, **12**, 1–13. https://doi.org/10.1038/s41467-021-23178-w.

Schultz, T.R. & Brady, S.G. (2008) Major evolutionary transitions in ant agriculture. *Proceedings of the National Academy of Sciences*, **105**, 5435–5440. https://doi.org/10.1073/pnas.0711024105.

Schultz, T.R., Sosa-Calvo, J., Brady, S.G., Lopes, C.T., Mueller, U.G., Bacci Jr., M. & Vasconcelos, H.L. (2015) The most relictual fungus-farming ant species cultivates the most recently evolved and highly domesticated fungal symbiont species. *The American Naturalist*, **185**, 693–703. https://doi.org/10.1086/680501.

Shorthouse, D.P. (2010) *SimpleMappr*, *an Online Tool to Produce Publication Quality Point Maps* [WWW document] URL http://www.simplemappr.net [accessed on 2020].

Silva, M.L. (2011) A dinâmica de expansão e retração de Cerrados e Caatingas no período Quaternário: uma análise segundo a perspectiva da teoria dos refúgios e redutos florestais. *Revista Brasileira de Geografia Física*, **4**, 57–73.

Silva-Pinhati, A.C.O., Bacci Jr., M., Hinkle, G. *et al.* (2004) Low variation in ribosomal DNA and internal transcribed spacers of the symbiotic fungi of leaf-cutting ants (Attini: Formicidae). *Brazilian Journal of Medical and Biological Research*, **37**, 1463–1472. https://doi.org/10.1590/S0100-879X2004001000004.

Siqueira, C.G., Bacci Jr., M., Pagnocca, F.C., Bueno, O.A. & Hebling, M.J.A. (1998) Metabolism of plant polysaccharides by *Leucoagaricus gongylophorus*, the symbiotic fungus of the ant *Atta sexdens* L. *Applied and Environmental Microbiology*, **64**, 4820–4822. https://doi.org/10.1128/AEM.64.12.4820-4822.1998.

Siqueira, F.F.S., Ribeiro-Neto, J.D., Tabarelli, M., Andersen, A.N., Wirth, R. & Leal, I.R. (2018) Human disturbance promotes herbivory by leaf-cutting ants in the Caatinga dry forest. *Biotropica*, **50**, 779–788. https://doi.org/10.1111/btp.12599.

Smith, B.T., Harvey, M.G., Faircloth, B.C., Glenn, T.C. & Brumfield, R.T. (2014) Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. *Systematic Biology*, **63**, 83–95. https://doi.org/10.1093/sysbio/syt061.

Smithsonian Institution. (2021) Smithsonian institution high performance computing cluster. https://doi.org/10.25572/SIHPC [accessed on 2021].

Solomon, S.E., Bacci Jr., M., Martins, J. Jr, Vinha, G.G. & Mueller, U.G. (2008) Paleodistributions and comparative molecular phylogeography of leafcutter ants (*Atta* spp.) provide new insight into the origins of Amazonian diversity. *PLoS One*, **3**, e2738. https://doi.org/10.1371/journal.pone.0002738.

Solomon, S.E., Rabeling, C., Sosa-Calvo, J. *et al.* (2019) The molecular phylogenetics of *Trachymyrmex* ants and their fungal cultivars provide insights into the origin and co-evolutionary history of "higher-attine" ant agriculture. *Systematic Entomology*, **44**, 939–956. https://doi.org/10.1111/syen.12370.

Sosa-Calvo, J., Ješovnik, A., Vasconcelos, H.L., Bacci Jr., M. & Schultz, T.R. (2017) Rediscovery of the enigmatic fungus-farming ant "*Mycetosoritis*" asper Mayr (Hymenoptera: Formicidae): implications for taxonomy, phylogeny, and the evolution of agriculture in ants. *PLoS One*, **12**, e0176498. https://doi.org/10.1371/journal.pone.0176498.

Sosa-Calvo, J., Schultz, T.R., Brandão, C.R. *et al.* (2013) *Cyatta abscondita*: taxonomy, evolution, and natural history of a new fungus-farming ant genus from Brazil. *PLoS One*, **8**, e80498. https://doi.org/10.1371/journal.pone.0080498.

Sosa-Calvo, J., Schultz, T.R., Ješovnik, A., Dahan, R.A. & Rabeling, C. (2018) Evolution, systematics, and natural history of a new genus of cryptobiotic fungus-growing ants. *Systematic Entomology*, **43**, 549–567. https://doi.org/10.1111/syen.12289.

Sumner, S., Aanen, D.K., Delabie, J. & Boomsma, J.J. (2004) The evolution of social parasitism in *Acromyrmex* leaf-cutting ants: a test of Emery's rule. *Insectes Sociaux*, **51**, 37–42. https://doi.org/10.1007/s00040-003-0723-z.

Tagliacollo, V.A. & Lanfear, R. (2018) Estimating improved partitioning schemes for ultraconserved elements. *Molecular Biology and Evolution*, **35**, 1798–1811. https://doi.org/10.1093/molbev/msy069.

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, **56**, 564–577. https://doi.org/10.1080/10635150701472164.

Teixeira, M. & Schoereder, J.H. (2003) The effect of plant cover on *Atta robusta* (Hymenoptera: Formicidae) distribution in restinga vegetation. *Sociobiology*, **41**, 615–623.

Teixeira, M.C., Schoereder, J.H. & Andrade, I. (2008) *Atta robusta* (Hymenoptera: Formicidae): endemismo, extinção ou ausência de estudos. *Insetos Sociais: da Biologia à Aplicação* (ed. by E. Villela, J.H. Schoereder and I. Andrade), pp. 359–367. Universidade Federal de Viçosa, Viçosa.

Teixeira, M.C., Schoereder, J.H. & Mayhé-Nunes, A.J. (2003) Geographic distribution of *Atta robusta* Borgmeier (Hymenoptera: Formicidae). *Neotropical Entomology*, **32**, 719–721. https://doi.org/10.1590/S1519-566X2003000400026.

 doi: 10.1111/syen.12513

Ulysséa, M.A. & Brandão, C.R. (2013) Ant species (Hymenoptera, Formicidae) from the seasonally dry tropical forest of northeastern Brazil: a compilation from field surveys in Bahia and literature records. *Revista Brasileira de Entomologia*, **57**, 217–224. https://doi.org/10.1590/S0085-56262013005000002.

Urbas, P., Araújo, M.V. Jr, Leal, I.R. & Wirth, R. (2007) Cutting more from cut forests: edge effects on foraging and herbivory of leaf-cutting ants in Brazil. *Biotropica*, **39**, 489–495. https://doi.org/10.1111/j.1744-7429.2007.00285.x.

van Elst, T., Eriksson, T.H., Gadau, J., Johnson, R.A., Rabeling, C., Taylor, J.E. & Borowiec, M.L. (2021) Comprehensive phylogeny of *Myrmecocystus* honey ants highlights cryptic diversity and infers evolution during aridification of the American Southwest. *Molecular Phylogenetics and Evolution*, **155**, 107036. https://doi.org/10.1016/j.ympev.2020.107036.

Wang, X., Auler, A.S., Edwards, R.L. *et al.* (2004) Wet periods in northeastern Brazil over the past 210 kyr linked to distant climate anomalies. *Nature*, **432**, 740–743. https://doi.org/10.1038/nature03067.

Ward, P.S., Blaimer, B.B. & Fisher, B.L. (2016) A revised phylogenetic classification of the ant subfamily Formicinae (Hymenoptera: Formicidae), with resurrection of the genera *Colobopsis* and *Dinomyrmex*. *Zootaxa*, **4072**, 343–357. http://doi.org/10.11646/zootaxa.4072.3.4.

Ward, P.S., Brady, S.G., Fisher, B.L. & Schultz, T.R. (2015) The evolution of Myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology*, **40**, 61–81. https://doi.org/10.1111/syen.12090.

Weber, N.A. (1966) Fungus-growing ants. *Science*, **153**, 587–604. https://doi.org/10.1126/science.153.3736.587.

Weber, N.A. (1968) Tobago Island fungus-growing ants (Hymenoptera: Formicidae). *Entomological News*, **74**, 141–145.

Weber, N.A. (1969) Ecological relations of three *Atta* species in Panama. *Ecology*, **50**, 141–147. https://doi.org/10.2307/1934676.

Weber, N.A. (1972) *Gardening Ants, the Attines*. The American Philosophical Society, Philadelphia, Pennsylvania.

Weber, N.A. (1982) Fungus Ants. *Social Insects* (ed. by H.R. Hermann), pp. 255–363. Academic Press, New York.

Wiens, J.J. & Morrill, M.C. (2011) Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. *Systematic Biology*, **60**, 719–731. https://doi.org/10.1093/sysbio/syr025.

Wild, A.L. (2007) A catalogue of the ants of Paraguay (Hymenoptera: Formicidae). *Zootaxa*, **1622**, 1–55. https://doi.org/10.11646/zootaxa.1622.1.1.

Williams, J.L., Zhang, Y.M., Lloyd, M.W., LaPolla, J.S., Schultz, T.R. & Lucky, A. (2020) Global domination by crazy ants: phylogenomics reveals biogeographical history and invasive species relationships in the genus *Nylanderia* (Hymenoptera: Formicidae). *Systematic Entomology*, **45**, 730–744. https://doi.org/10.1111/syen.12423.

Willig, M.R., Presley, S.J., Owen, R.D. & López-González, C. (2000) Composition and structure of bat assemblages in Paraguay: a subtropical-temperate interface. *Journal of Mammalogy*, **81**, 386–401. https://doi.org/10.1644/1545-1542(2000)081<0386:CASOBA>2.0.CO;2.

Wilson, E.O. (1986) The defining traits of fire ants and leaf-cutting ants. *Fire Ants and Leaf-Cutting Ants* (ed. by C.S. Lofgren and R.K. Vandermeer), pp. 1–9. Westiview, Boulder.

Wilson, E.O. & Brown, W.L. Jr (1953) The subspecies concept and its taxonomic application. *Systematic Zoology*, **2**, 97–111. https://doi.org/10.2307/2411818.

Wirth, R., Herz, H., Ryel, R.J., Beyschlag, W. & Hölldobler, B. (2003) *Herbivory of Leaf-Cutting Ants: A Case Study on Atta Colombica in the Tropical Rainforest of Panama*. Springer-Verlag, Berlin Heidelberg, New York.

Wirth, R., Meyer, S.T., Almeida, W.R., Araújo, M.V. Jr, Barbosa, V.S. & Leal, I.R. (2007) Increasing densities of leaf-cutting ants (*Atta* spp.) with proximity to the edge in a Brazilian Atlantic forest. *Journal of Tropical Ecology*, **23**, 501–505. https://doi.org/10.1017/S0266467407004221.

Yang, Z. (2007) PAML 4: phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, **24**, 1586–1591. https://doi.org/10.1093/molbev/msm088.

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