

Evolution, systematics, and natural history of a new genus of cryptobiotic fungus-growing ants

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Abstract. *Xerolitor*, a new, monotypic genus of fungus-growing ants, is described to accommodate the phylogenetically isolated, relict species *Mycetosoritis explicatus* Kempf. We also diagnose the male and the larva of *Xerolitor explicatus* (Kempf) **comb.n.** and report ecological observations for the species, including nest architecture and foraging behaviour. *Xerolitor explicatus* **comb.n.** inhabits the dry habitats of the Brazilian Cerrado and the Bolivian and Paraguayan Gran Chaco. Bayesian multilocus phylogenetic analyses indicate that *X. explicatus* **comb.n.** is, contrary to some prior hypotheses, a member of the ‘higher’ fungus-growing ants and the sister taxon of the genus *Sericomyrmex* Mayr. Results from phylogenetic analyses of the fungal cultivar grown by *X. explicatus* **comb.n.** in Paraguay, as well as the presence of gongylidia, indicate that the fungal mutualist is a member of the clade of higher fungal cultivar species and that it is probably the same species cultivated by some *Trachymyrmex* Forel and *Sericomyrmex* species.

Introduction

The biology of the fungus-growing ants (Myrmicinae: Attini: Attina) (sensu Ward *et al.*, 2015) has inspired biologists for more than 150 years (Hölldobler & Wilson, 1990, 2010; Della Lucia, 2011). Historically, the leaf-cutting ants in the genera *Atta* Fabricius and *Acromyrmex* Mayr attracted the attention of naturalists and farmers alike, because they are the most conspicuous of the fungus-growing ants, they are ecologically dominant herbivores, and their leaf-cutting behaviour causes considerable damage in crop plantations across Latin America (Cherrett, 1986; Wirth *et al.*, 2003; Leal *et al.*, 2014). Despite the longstanding interest in leaf-cutting ant biology due to their adverse economic impact, the purpose of the leaf-cutting behaviour remained unknown until the latter half of the 19th century. Famously, Henry Walter Bates (1863: 26) reported in his travelogue *The Naturalist on the River Amazons* that the ‘leaf-carrying ants’ use the leaves to ‘thatch their domes which cover the entrances to their subterranean dwellings, thereby protecting from the deluging rains the young broods in the

nests beneath’. A decade later, both Belt (1874) and Fritz Müller (1874) arrived independently at the conclusion that the leaf-cutting ants in fact ‘feed upon the fungus growing on the leaves, that they carry into their nests’ (Müller, 1874: 102). Building on his uncle’s observations, Alfred Möller conducted the first detailed comparative study of the fungus gardens of several fungus-growing ant species, and his experiments established the mutualistic nature of the ant–fungus symbiosis (Möller, 1893).

Simultaneous to, and potentially inspired by, the fundamental biological discoveries of Bates and his contemporaries, late 19th-century entomologists described and classified many new species of fungus-growing ants. Fundamental treatises of the 19th and early 20th centuries include taxonomic monographs and evolutionary hypotheses authored by foundational ‘attinologists’ such as Auguste Forel (1892, 1893a,b), Carlo Emery (1905, 1912, 1913), and William Morton Wheeler (1907, 1910), laying the basis for subsequent work by Borgmeier (1959), Gonçalves (1961), Kempf (1963, 1964, 1966, 1972), Kusnezov (1963), Wilson (1971), Brandão & Mayhé-Nunes (2001, 2008), Mayhé-Nunes & Brandão (2002, 2005, 2006, 2007), Rabeling *et al.* (2007a, 2014), Klingenberg & Brandão (2009), Sosa-Calvo & Schultz (2010), and Ješovnik & Schultz (2017). Accompanying the dramatic increase in the number of described species due to these and other authors, a number of 20th-century

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researchers documented fungus-growing ant natural history, surveying the group's high morphological, behavioural, and ecological diversity (see, for example, Wheeler, 1907; Autuori, 1941, 1942; Wilson, 1971; Weber, 1972a,b,c; Hölldobler & Wilson, 1990, 2010). The advent of phylogenetic systematics and molecular phylogenetics provided the opportunity to focus on reconstructing the evolutionary history of the fungus-growing ants and their symbionts (e.g. Chapela *et al.*, 1994; Hinkle *et al.*, 1994; Schultz & Meier, 1995; Mueller *et al.*, 1998; Wetterer *et al.*, 1998). The combination of morphological, experimental, and molecular genetic techniques led to a number of important discoveries, such as additional key fungal and microbial symbionts, some of which parasitize and others of which assist the ants in defending their fungus gardens against harmful diseases (Currie *et al.*, 1999a,b, 2003, 2006; Little & Currie, 2007, 2008). Schultz & Brady (2008) provided a comprehensive molecular phylogeny of the fungus-growing ants that included the major ant evolutionary lineages and allowed for inferral of the evolution of fungiculture across the attine tree of life (Mueller & Rabeling, 2008). For the past decade, Schultz and Brady's attine phylogeny provided the phylogenetic backbone for evolutionary, behavioural-ecological, and systematic/taxonomic studies alike, addressing questions such as ant–fungus co-evolution (De Fine Licht *et al.*, 2010, 2014; Mehdiabadi *et al.*, 2012; Schultz *et al.*, 2015; Sosa-Calvo *et al.*, 2017a,b), fungus–parasite co-evolution (Gerardo & Caldera, 2007; Meirelles *et al.*, 2015), evolution of mating systems (Villessen *et al.*, 1999), the evolution of asexual reproduction (Rabeling *et al.*, 2009; Rabeling *et al.*, 2011), and the evolution of social parasitism (Sumner *et al.*, 2004; Adams *et al.*, 2013; Rabeling *et al.*, 2014) in a phylogenetic context.

Adding new and rarely collected species to the attine tree of life is essential for systematically revising the taxonomy of the attine ants to reflect their evolutionary relationships. Most importantly, due to their phylogenetic positions, certain key taxa are essential for correctly reconstructing phylogeny as well as for correctly reconstructing genetic distances and divergence dates (Ward *et al.*, 2010). New and rare fungus-growing ant species continue to be discovered. In 2001, for example, a new genus of fungus-growing ant, *Mycetagroicus* Brandão and Mayhé-Nunes, was described from Brazil (Brandão & Mayhé-Nunes, 2001, 2008). In 2013, and in efforts to synthesize taxonomy, field research, and molecular phylogenetics, the new fungus-growing ant genus *Cyatta* Sosa-Calvo *et al.* was described from Brazil (Sosa-Calvo *et al.*, 2013).

Recent studies (Branstetter *et al.*, 2017; Ješovnik *et al.*, 2017; Sosa-Calvo *et al.*, 2017b) provided new insights regarding the systematics and taxonomy of the genus *Mycetosoritis* Wheeler, which has troubled taxonomists for decades. Wheeler (1907) described *Mycetosoritis* as a subgenus of *Atta* with *M. hartmanni* Wheeler as the type species and transferred *Cyphomyrmex asper* (Mayr), which was previously described by Mayr (1887), to *Mycetosoritis*. In 1949, Kusnezov described *Cyphomyrmex (Mycetosoritis) clorindae* (Kusnezov) from Argentina (Kusnezov, 1949). In its early taxonomic history *Mycetosoritis* was first transferred as a subgenus from *Atta* to *Cyphomyrmex* Mayr (Emery, 1913) and then from *Cyphomyrmex* to

Trachymyrmex (Wheeler, 1922). Creighton (1950) stabilized the rather volatile status of *Mycetosoritis* by elevating the rank to genus. Subsequently, two additional species of *Mycetosoritis* were described: *M. explicatus* from Brazil (Kempf, 1968) and *M. vinsoni* MacKay from Costa Rica (MacKay, 1998). As Kempf rightfully noted (1968: 403), 'the striking features of this species [*M. explicatus*] warrant immediate recognition. But the generic placement offers some difficulties'. According to Kempf, the antennal scrobe morphology resembled *Cyphomyrmex*, whereas the presence of erect and suberect hairs reminded him of *Mycetosoritis*, to which he assigned the species. Kempf further noted that the genus, as defined by Emery (1922), was highly heterogeneous and that the South American species were quite distinct from the North American *M. hartmanni*. Recent molecular phylogenetic studies provide evidence that the currently known species of *Mycetosoritis* are polyphyletic and have at least three independent evolutionary origins (Branstetter *et al.*, 2017; Ješovnik *et al.*, 2017). In a continuing effort to stabilize the taxonomy of the fungus-growing ants by aligning it with the well-supported results of molecular phylogenetic studies, Sosa-Calvo *et al.* (2017b) transferred the South American species *M. asper* and *M. clorindae* to the genus *Mycetophylax* Emery. They also clarified the etymology of the genus name *Mycetosoritis* and explained why its gender is masculine rather than feminine as had been traditionally assumed.

Among the three species that currently remain in *Mycetosoritis*, *M. explicatus* is certainly the most poorly known. The species is known from a few specimens that were collected in two principal localities (Fig. 1A): (i) the Brazilian Cerrado; and (ii) the Paraguayan and Bolivian Chaco. From the Brazilian Cerrado, Kempf (1968) collected two workers (the holotype and paratype) near Anápolis in the state of Goiás; J.M.L. Diniz collected a single worker in Brasília; and Sosa-Calvo *et al.* (2009) described the queen of *M. explicatus* from a single alate queen that was also collected in the Distrito Federal of Brazil. Delsinne *et al.* (2007, 2010) and P.S. Ward (personal communication) collected a few specimens in the Paraguayan and Bolivian Chaco, respectively. To learn more about the general biology, fungal associations, taxonomy, evolutionary history, and phylogenetic position of *M. explicatus*, starting in 1992 we repeatedly searched for populations of this species at the type locality near Brasília, but our attempts were unsuccessful. Fortunately, in leaf-litter sampling studies, Thibaut Delsinne and his collaborators discovered a population of *M. explicatus* at the Parque Nacional Teniente Agripino Enciso in the Department of Boquerón in Paraguay (Delsinne *et al.*, 2007; Delsinne *et al.*, 2010), where we were able to study *M. explicatus* in more detail. Here we: (i) reconstruct the evolutionary history of *M. explicatus*; (ii) describe the male and larva of *M. explicatus*; (iii) document nest architecture and foraging behaviour based on four colonies collected; and (iv) identify the fungal cultivar grown by *M. explicatus* based on molecular genetic analyses. In light of our morphological and molecular phylogenetic data, and in order to establish an evolutionary classification in which genera are monophyletic, we describe the new genus *Xerolitor* gen.n. to accommodate *M. explicatus*.

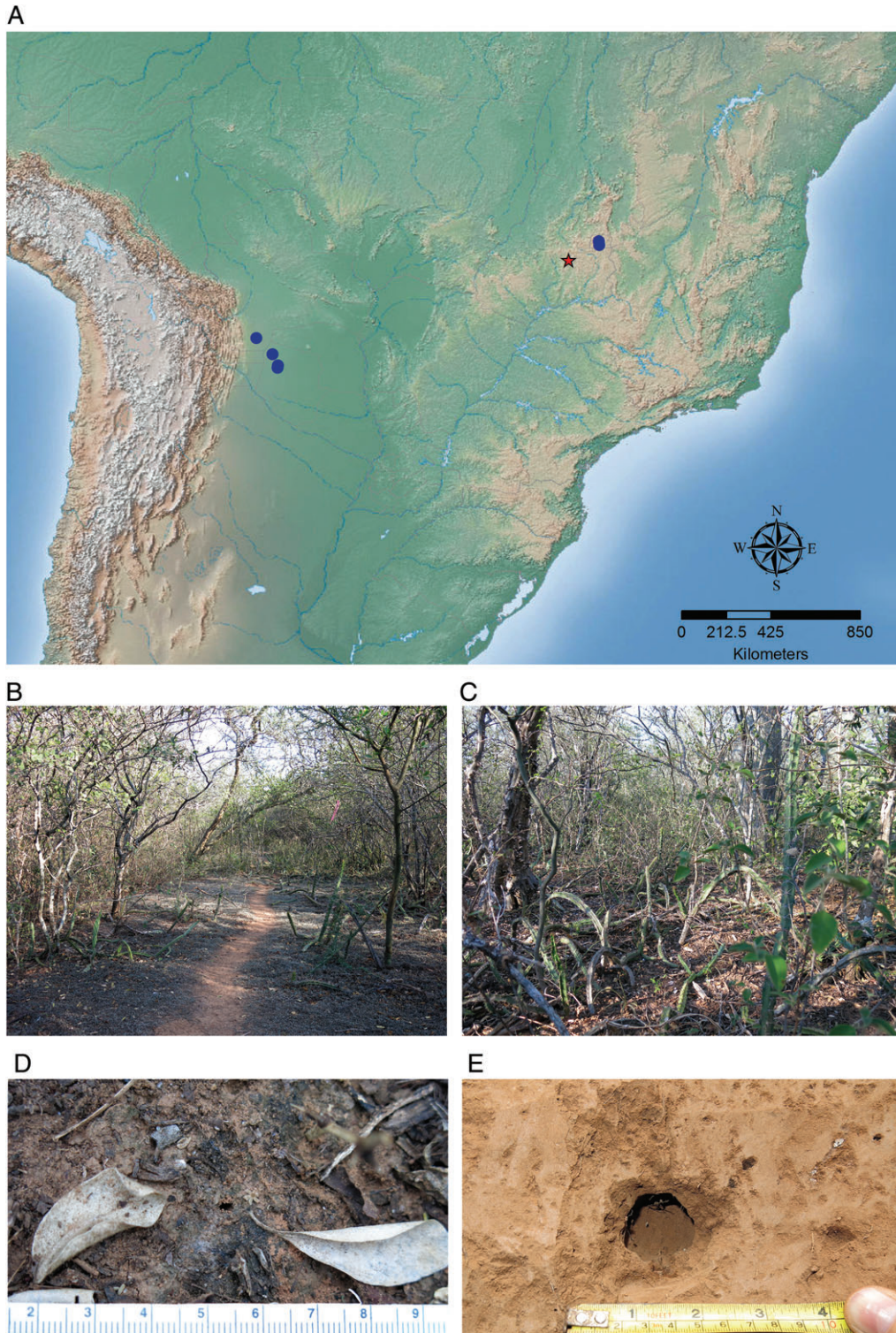


Fig. 1. Geographic distribution, habitat at the Parque Nacional Teniente Agripino Enciso in Paraguay, and nest architecture of *Xerolitor explicatus*. (A) The geographic distribution of *X. explicatus* – the type locality is indicated by a red star; (B) xeromorphic forest habitat; (C) ground vegetation, including cactus thicket, where nests were located; (D) nest entrance; and (E) spherical nest chamber of *X. explicatus*.

Materials and methods

Field site

Field research was conducted 27–31 October 2015 in the Parque Nacional Teniente Agripino Enciso, Department of Boquerón, Paraguay (GPS coordinates: S21.20595°, W061.65749°, elevation 257 meters above sea level). The park has an area of ~40 000 ha and is located in the Semi-Arid Chaco biome of Paraguay with mean annual rainfalls of 573 mm and mean annual temperatures of 23.8 °C (Delsinne *et al.*, 2010). The area is dominated by xerophytic thorn scrub forest with understorey vegetation consisting of arborescent cacti and terrestrial bromeliads (Fig. 1B, C). We searched for foraging workers of *X. explicatus* during day- and night-time hours (04.00–11.00 hours and 17.00–22.00 hours, respectively). To locate nests of *X. explicatus*, foraging ants were baited with dry, coarsely ground rice cereal, which they carried to their nest entrances (Fig. 1D). Nests were marked and subsequently excavated (Fig. 1E) following methods described in Schultz (1993), Rabeling *et al.* (2007b), and Sosa-Calvo *et al.* (2015).

Vouchers and material examined

Ant vouchers and previously collected specimens of *X. explicatus* were deposited in entomological collections at the following institutions:

DZUP	Coleção Entomológica 'Padre Jesús Santiago Moure', Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil
MBC-UFU	Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, MA, U.S.A.
MNHNP	Museo Nacional de Historia Natural del Paraguay, Asuncion, Paraguay
MZSP	Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil
SIBR	Social Insect Biodiversity Repository, Arizona State University, Tempe, AZ, U.S.A.
UCDC	The Bohart Museum of Entomology, University of California, Davis, CA, U.S.A.
USNM	United States National Museum of Natural History, Washington, DC, U.S.A.

Morphological examination and specimen preparation

Morphological measurements were recorded to the nearest 0.01 mm and are reported here in millimetres. Composite images were generated using a Leica DFC450 digital camera mounted on a Leica M205 C stereomicroscope (Buffalo Grove, IL, U.S.A.). Composite images were assembled using the LEICA APPLICATION SUITE (version 4.5) and the HELICON FOCUS (version 6.2.2) software packages. Male wings were removed from the left side of the specimen, placed on microscope slides with Euparal, and covered with a circular cover glass. The larvae were dehydrated sequentially through a series of ethanol concentrations to 100% absolute and then critical point-dried in a Balzers CPD–030 (Bal-Tec AG, Liechtenstein) using liquid CO₂ at the Scanning Electron Microscopy Laboratory in the

Smithsonian Institution–National Museum of Natural History, and mounted on aluminium stubs. In addition to the prepared larvae, one worker, one queen, and one male were sputter-coated with 60:40 wt% gold:palladium alloy on a Cressington Scientific 108 auto/SE sputter coater (Netherlands) to a thickness of 20–25 nm. Scanning electron micrographs of coated specimens were generated using a Philips XL-30 ESEM (Netherlands) with lanthanum hexaboride (LaB₆) source and with a backscatter detector. The male genital capsules from two males were removed and dissected in a Petri dish with ethanol under a Leica MZ16 stereomicroscope. The dissected genitalia were air-dried for a few seconds, mounted on a metal stub, and then coated with gold to a thickness of 30–70 nm using a Cressington Scientific 108 auto/SE sputter coater. Micrographs were taken with a Hitachi TM3030 plus tabletop microscope (Tarrytown, NY, U.S.A.). All images were edited using Adobe PHOTOSHOP CC 2017.0.1 (version 20161130.r.29 x64) (Adobe Inc.). We employed morphological terminology, measurements, and indices used in recent taxonomic studies of fungus-growing ants (Rabeling *et al.*, 2007a, 2015; Boudinot, 2013; Sosa-Calvo *et al.*, 2013, 2017b; Ješovnik & Schultz, 2017).

Abbreviations for measurements and indices are as follows:

EL	Eye length: in full-face view, the maximum diameter of the eye measured from the dorsal margin to the ventral margin.
FLD	Frontal lobe distance: in full-face view, the maximum horizontal distance between the outer borders of the frontal lobes.
GL	Gaster length: in profile, the length of the gaster from the anteriormost point of the first gastral segment (fourth abdominal segment) to the posteriormost point of the last segment.
HL	Head length: in full-face view, the maximum vertical distance from the posteriormost margin of the head to the mid-point of the anterior clypeal margin (clypeal apron), excluding the mandibles.
HFL	Hind femur length: in most appropriate view, the maximum length of the hind femur.
HTL	Hind tarsomere I length: in most appropriate view, the maximum length of the hind tarsomere I.
HW	Head width: in full-face view, the maximum horizontal width of the cephalic capsule excluding the eyes.
ML	Mandible length: in full-face view, the maximum diagonal-line distance from the base of the external mandibular insertion to the apical tooth.
MSL	Median clypeal seta length: in full-face view, the maximum length of the unpaired median clypeal seta from its point of origin on the clypeal apron to the tip (apex) of the seta.
PL	Petiole length: in lateral view, the straight-line distance from the posteriormost margin of the petiole to the posteriormost margin of the metapleural lobe.
PPL	Postpetiole length: in lateral view, the maximum length of the postpetiole.
PPW	Postpetiole width: in dorsal view, the maximum horizontal width of the postpetiole.
SL	Scape length: in full-face view, the maximum length of the scape, excluding the basal condyle.
TL	Total length: HL + ML + WL + PL + PPL + GL.
WL	Weber's length: in lateral view, the diagonal length of the mesosoma as measured from the anteriormost dorsal extent of the pronotum to the posteriormost ventral angle of the propodeum.

CI	Cephalic index: (HW/HL) × 100.
FLI	Frontal lobe index: (FLD/HW) × 100.
MI	Mandibular index: (ML/HL) × 100.
MSI	Median seta index: (MSL/HL) × 100.
OI	Ocular index: (EL/HW) × 100.
PPI	Postpetiole index: (PPW/PPL) × 100.
FLDI	Relative frontal lobe distance index: (FLD/HL) × 100.
SI	Scape index: (SL/HW) × 100.

Molecular phylogenetic analyses

Genomic DNA was extracted using a Qiagen DNEasy Blood and Tissue kit and a Qiagen DNEasy Plant kit for the ants and fungi, respectively (Qiagen, Germantown, MD, U.S.A.). For the ants, five nuclear protein-coding genes (*EF1 α -F1*, *EF1 α -F2*, *wg*, *LWRh* and *TOP1*) were amplified and sequenced following methodologies outlined in previous studies (Schultz & Brady, 2008; Ward *et al.*, 2015). For the fungal cultivar, two ribosomal gene fragments, internal transcribed spacer (ITS) and large subunit (LSU), were amplified and sequenced following previously described methods (Mueller *et al.*, 1998; Solomon *et al.*, 2011; Mehdiabadi *et al.*, 2012). New sequences generated for this study are deposited in GenBank under accession numbers MG607360 for the fungal cultivar and MG642983–MG642987 for the ant.

Ant DNA sequences, consisting of ~3.3 kb, were added to the aligned dataset of Sosa-Calvo *et al.* (2017b) and aligned using MAFFT v7.017 (Katoh *et al.*, 2002; Katoh *et al.*, 2005; Katoh & Standley, 2013) as implemented in GENEIOUS R9 v8.1.8 (Kearse *et al.*, 2012) and inspected and refined by eye in MESQUITE (Maddison & Maddison, 2015). DNA sequence data were partitioned and modelled using the program PARTITIONFINDER v1.1.0 (Lanfear *et al.*, 2012) under the Bayesian information criterion with 15 data blocks consisting of the first, second and third codon positions of each of the five gene fragments and with a user tree resulting from an unpartitioned maximum likelihood best-tree analysis conducted in RAXML v8.2 (Stamatakis, 2014). The eight partitions and models identified by PARTITIONFINDER were employed in Bayesian analyses with parameter conditions identical to those described in Sosa-Calvo *et al.* (2017b).

Internal transcribed spacer and LSU sequences for the fungal cultivar of *X. explicatus* were generated, but only those of ITS were added to a pre-existing dataset of agaricaceous (Basidiomycota, Agaricales, Agaricaceae, Leucocoprineae) ant-associated and free-living fungi and aligned in MAFFT, producing a matrix consisting of 62 taxa and 976 characters, including indels. Data were partitioned and modelled using PARTITIONFINDER v2.1.1 under the 'all' algorithm (Frandsen *et al.*, 2015; Baca *et al.*, 2017) and the corrected Akaike information criterion. Maximum likelihood phylogeny was inferred using simultaneous best-tree and rapid bootstrapping (100 pseudoreplicates; -f a option) maximum likelihood analyses in RAXML v8.2.8 (Stamatakis, 2014). The resulting phylogenies were examined in FIGTREE v1.4.2 (Rambaut, 2009), and the phylogeny figure was prepared using FIGTREE v1.4.2 and Adobe ILLUSTRATOR CC.

Fungus culture and voucher deposition

Fragments of fungus garden were isolated using sterilized forceps and were cultured on potato dextrose agar (PDA) medium with penicillin G, streptomycin sulphate, and chloramphenicol antibiotics (Sosa-Calvo *et al.*, 2017b). After several subcultures, pure mycelia were transferred from agarose to PDA liquid broth medium without antibiotics. The mycelia were then cultured at 30 °C under constant agitation using a New Brunswick Scientific Series 25 Incubator Shaker (Edison, NY, U.S.A.) for several weeks. Following incubation, the tissues were filtered, lyophilized, and placed into cryostorage for later DNA extraction. Vouchers of the lyophilized fungus garden are deposited at the USNM.

Morphological analysis of the fungal cultivar

The fungal cultivars of *X. explicatus* were found to bear gongylidia, which are hyphal-tip swellings that occur rarely in the fungus gardens of lower *Attina* (Masiulionis *et al.*, 2014) but consistently in the fungus gardens of higher *Attina*. To test whether the gongylidia morphology of *X. explicatus* cultivars differs from that of fungi cultivated by other higher-attine species, we measured the diameter of 40 gongylidia from *X. explicatus* fungi and compared the measurements with the data from four higher-attine species and one lower-attine species that were reported in Masiulionis *et al.* (2014). We performed an ANOVA test in R (R Development Core Team 2014) to determine whether ant species identity is associated with a difference in gongylidia diameter and a Tukey's honest significant difference for post hoc pairwise comparisons between species. Both tests were performed at the same significance level ($\alpha = 0.01$), which is consistent with the tests carried out by Masiulionis *et al.* (2014). Box plots were generated in STATISTICA v.13 (Tibco Software Inc., Palo Alto, CA, U.S.A.). Assumptions of normality, linearity and homogeneity of variance were confirmed by visual inspection of residual plots (Zuur *et al.*, 2009).

Results

Molecular phylogenies

Phylogeny of fungus-growing ants

Our fungus-growing ant phylogeny (Fig. 2), which is based on five nuclear protein-coding genes, indicates that the subtribe *Attina*, i.e. the fungus-growing ants, consists of two sister clades, the Paleoattina (the so-called 'lower-attine' genera *Apterostigma* Mayr, *Myrmicocrypta* Fr. Smith, and *Mycocarpus* Forel) and the Neoattina, consisting of the remaining genera (the lower-attine genera *Kalathomyrmex* Klingenberg and Brandão, *Cyatta*, *Mycetosoritis*, *Mycetarotes* Emery, *Mycetophylax*, *Cyphomyrmex*, *Mycetagroicus*, and the so-called 'higher-attine' genera). Contrary to the hypothesis of Kempf (1968), the molecular phylogeny (Fig. 2) indicates that, rather than a close relationship with a lower-attine genus such as *Cyphomyrmex* or *Mycetosoritis*, *Xerolitor explicatus* is a member of the higher-attine ants, a clade that includes *Sericomyrmex*, *Trachymyrmex*, and the leaf-cutting ant genera *Acromyrmex* and *Atta*. The phylogeny further indicates that,

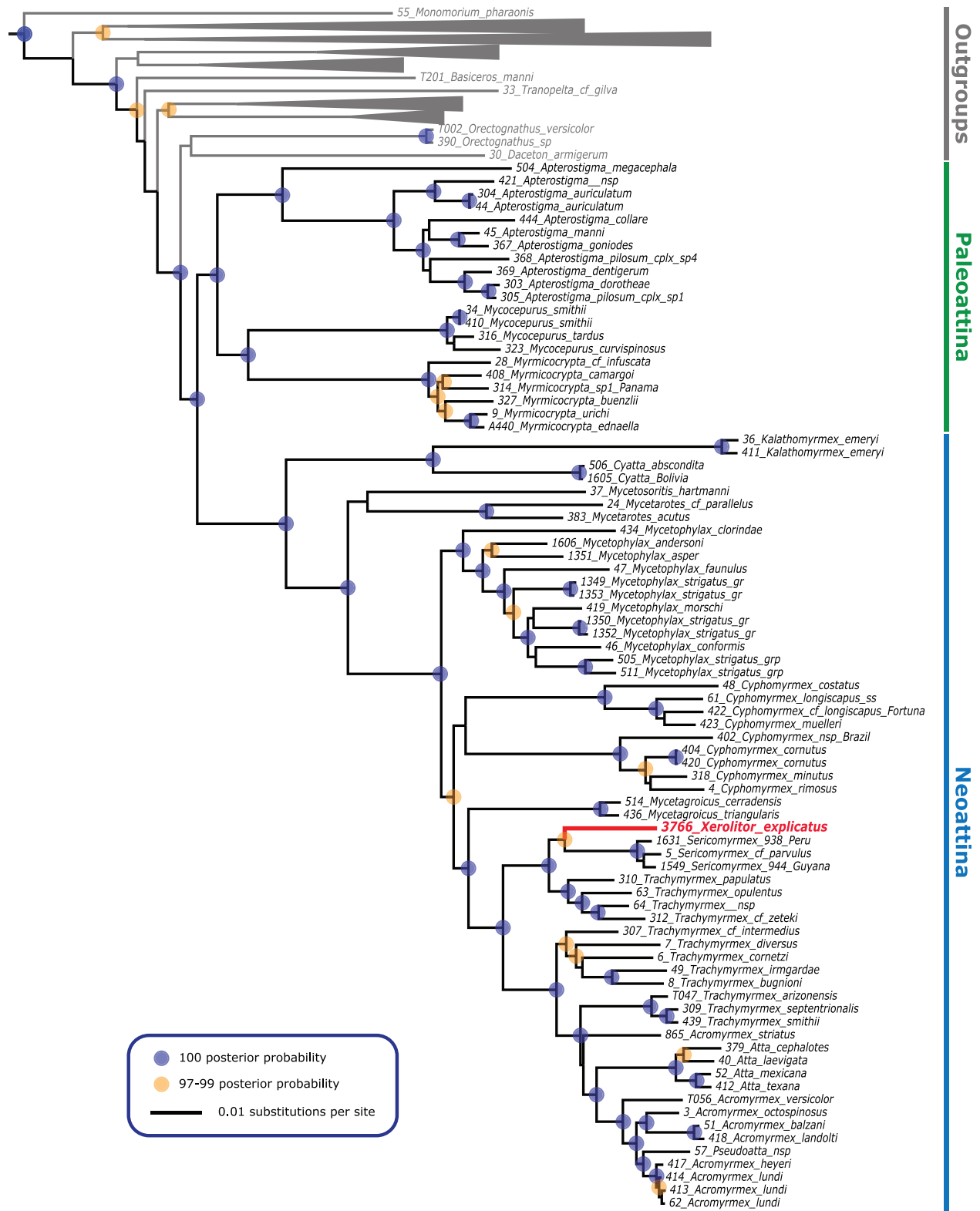


Fig. 2. Multilocus molecular phylogeny of the fungus-growing ants based on Bayesian analyses. *Xerolitor explicatus* is indicated in red.

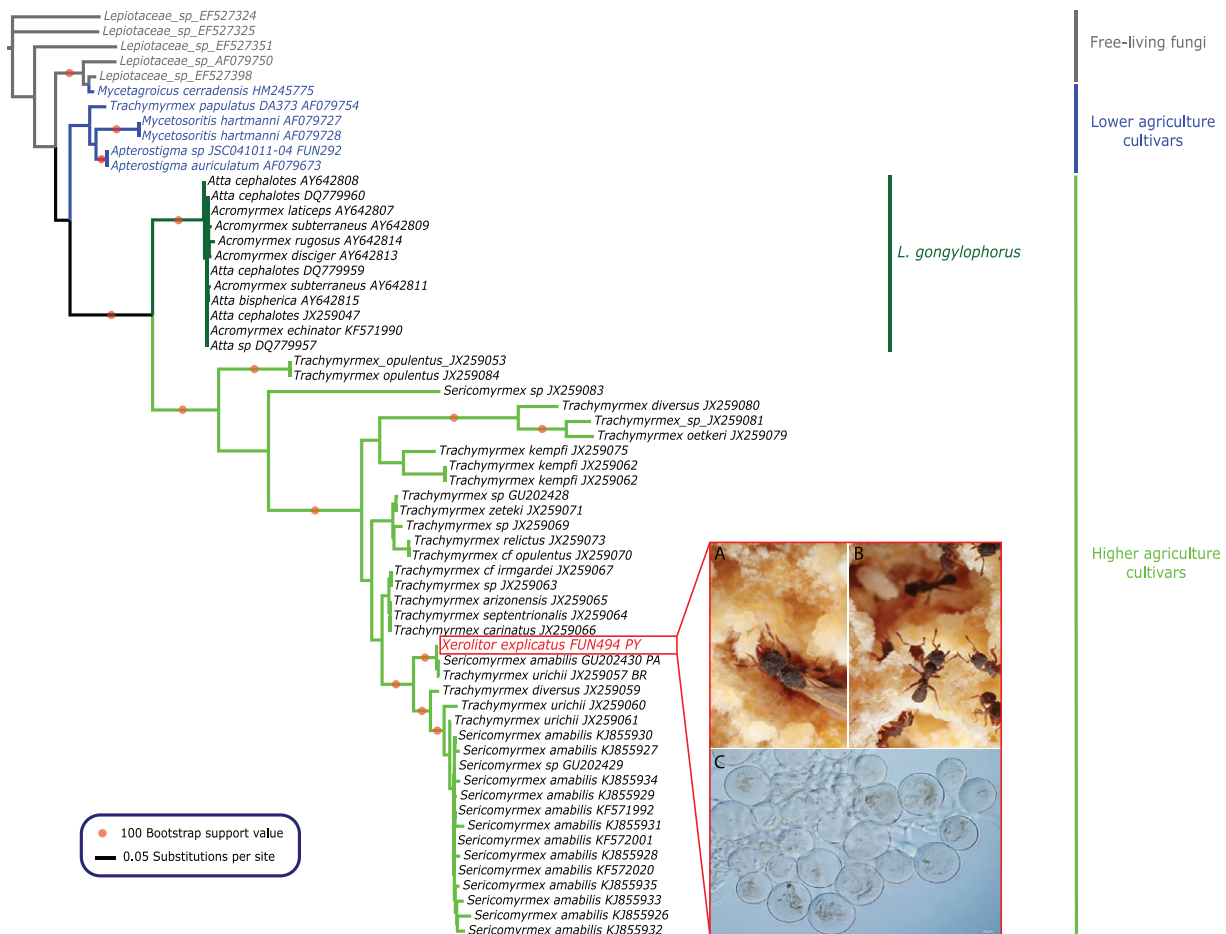


Fig. 3. Molecular phylogeny of internal transcribed spacer fungal cultivar sequences based on Bayesian analyses. Photograph insets: (A) alate queen on the fungus garden; (B) workers tending the cultivar; (C) gongylidia of mutualistic fungus cultivated by *Xerolitor explicatus*.

within the higher attines, *X. explicatus* is the sole representative of a depauperate evolutionary lineage that descended from a common ancestor, shared with the genus *Sericomyrmex*, that lived ~15 Ma (Ješovnik *et al.*, 2017). Our results are consistent with those of earlier studies (Branstetter *et al.*, 2017; Ješovnik *et al.*, 2017). Considering the cryptic biology of *X. explicatus*, it is possible that additional species of the currently monotypic genus *Xerolitor* remain to be discovered.

Phylogeny of mutualistic fungi

The molecular phylogeny of attine ant-associated fungi (Fig. 3), which is based on the ribosomal ITS gene that is commonly employed for fungal phylogenetics, indicates that the higher-attine fungi, which are, with a single known exception (Schultz *et al.*, 2015), exclusively associated with higher-attine ants, are a derived, monophyletic group (Fig. 3, green) closely related to the lower-attine fungi (Fig. 3, blue). With regard to relationships of ant-cultivated fungi, these results are consistent with those of earlier studies (Mueller *et al.*, 1998; Ješovnik *et al.*, 2017) and, in regard to the relationships of ant-cultivated and free-living fungi (Fig. 3, black), these results are consistent

with those of prior studies that have shown that, with the exception of pterulaceous fungi cultivated by ants in the *Apterostigma pilosum* Mayr group (Villesen *et al.* 2004; Schultz *et al.*, 2015), all lower- and higher-attine fungi belong to a group of genera in the fungal family Agaricaceae currently assigned to the tribe Leucocoprineae, including *Lepiota* (Pers.) Gray, *Leucoagaricus* Locq. ex. Singer, and *Leucocoprinus* Pat. (Vellinga, 2004; Schultz *et al.*, 2015). The fungal phylogeny (Fig. 3) further indicates that the fungal cultivar of *X. explicatus* in the Paraguayan Chaco is probably conspecific with fungi cultivated by *Sericomyrmex amabilis* Wheeler in Panama and *Trachymyrmex urichii* (Forel) in Brazil, as well as by *S. amabilis* in Ecuador (Ješovnik *et al.*, 2017).

Taxonomy

Xerolitor new genus

<http://zoobank.org/urn:lsid:zoobank.org:act:13A0F17E-67D3-4D4A-BDCE-334806098261> (Figs 4–7)

Type species. Xerolitor explicatus (Kempf, 1968), by monotypy.

Diagnosis. Dorsum of promesonotum forming a shield, carinate on all sides, separated from lateral portions of promesonotum by abrupt right angles, posteriorly, overhanging and elevated above propodeum; mandible with eight teeth (five to six in males); clypeal apron convex, interrupted by concavity medially; antennal scrobe complete; frontal lobe broadly expanded. Antenna of male with 12 segments.

Worker (Figs 4A, C, E; 6A, D, G, J). **Head:** Mandible triangular, masticatory margin with eight teeth, which increase gradually in size towards apex. Clypeal apron present, convex, medially concave. Median clypeal seta long (~1/5 of mandible length), originating on anterior margin of clypeal apron emargination; lateral portion of clypeus with cluster of four to five clypeal hairs each. Preocular carina raised and extending posterad joining frontal carina at posterior cephalic margin forming complete antennal scrobe. Frontal lobe greatly expanded, covering antennal insertion, and jagged. Leading (anterior) edge of antennal scape denticulate, covered with subdecumbent long hairs that project toward apex; posterior edge of antennal scape lacking denticles and bearing appressed hairs. **Mesosoma:** Dorsum of pronotum flat and with some very low tubercles that bear some decumbent or subdecumbent hairs. Lateral margins of pronotum with denticulate carina. Lateral pronotal spine triangular, large. Lateral mesonotal tubercles large, triangular and keeled, with broad base. Posterior mesonotal lobes carinate. Dorsum of promesonotum forming shield, carinate on all sides, separated from lateral portions of promesonotum by abrupt right angles, and, posteriorly, overhanging and elevated above propodeum. Anterolateral face of propodeum with carinules ending in small tubercles. **Metasoma:** Petiolar node approximately as long as broad. Postpetiole wider than long; side bearing several denticles of similar length; posterior margin vestigially emarginated. First gastral tergite ovate, with pair of lateral longitudinal carinae along anterior two-thirds; dorsum with small, pimple-like, piligerous tubercles connected to each other by weak but distinct rugae, forming an areolate surface sculpture. In lateral view, first gastral tergite longer than sternite, dorsally overhanging remaining segments.

Queen (Figs 4B, D, F; 6B, E, H, K). **Head:** In frontal view, clypeal apron broadly convex, convexity interrupted medially by conspicuous emarginate notch. Lateral margin of frontal lobe semicircular and greatly expanded, attaining width of head anterad of eye. Border of frontal lobe with irregular serrated appearance. Frontal carina produced into denticulate lamella. Supraocular tubercle absent. Frontal carina extending to posterolateral cephalic margin, there joining subocular carinae to form complete antennal scrobe. Antennal scape short, not exceeding length of scrobe. **Mesosoma:** Pronotum with pair of short lateral tubercles connected by conspicuous posterior pronotal carina, most easily seen in frontodorsal view. Anteroventral corner of pronotum forming obtuse angle, lacking tooth or spine. Scutellar process with pair of posterior rounded teeth. Propodeal tooth short and obtuse. Propodeal spiracle small, directed posterad. Outer surface of tibia with row of

denticles, inner margin devoid of denticles. **Metasoma:** In dorsal view, petiolar node approximately as long as broad, with two posterodorsal tubercles and several lateral denticles. Body dark reddish-brown. Sculpture scabrous and areolate, particularly on scape, legs, and gaster due to presence of scattered, pointed, piligerous pimples connected by irregular rugae. Hairs long, flexuous, and mostly strongly recurved, especially on clypeus, scape, and gaster.

Male (Figs 5A-E; 6C, F, J, L, M-R). **Head:** In frontal view, preocular carina extending posterad close to posterior cephalic margin and weakly developed when meeting frontal carina, forming feebly impressed antennal scrobe; scrobe areolate. Mandible triangular, masticatory margin with five to six teeth, increasing in size from base to apex. Clypeal apron narrow, shining, notched medially and dorsally rugulose with lateral portions of apron striate. Frontal lobe small, slightly convex, failing to cover antennal insertions. Antennal scape long, longer than length of funicular segments I–III combined. Antenna 12-segmented. Eye large, convex. Hypostomal teeth absent. Palpus formula 4,2. **Mesosoma:** Pronotum lacking humeral tubercles; lateral tubercles of pronotum reduced to carinae. Propodeal tooth short and blunt. **Metasoma:** Petiole sessile, lacking node. Dorsum of gastral segment I weakly sculpted, lustrous.

Etymology. *Xerolitor* is a neologism composed of the Greek word *xeros*, meaning dry, and the Latin *olitor*, meaning gardener or vegetable-grower. The genus name refers to the seasonally dry Cerrado and Chaco ecoregions that *X. explicatus* is known to inhabit and to its fungus-cultivating behaviour.

Comments. *Xerolitor explicatus* is the only known species in the genus. The molecular phylogeny indicates that *Sericomyrmex* and *X. explicatus* are sister taxa, sharing a common ancestor that lived ~15 Ma (Branstetter *et al.*, 2017; Ješovnik *et al.*, 2017). All species in the genus *Sericomyrmex* are morphologically homogenous and, as such, easily recognizable. As *X. explicatus* possesses none of the synapomorphic morphological characters that define *Sericomyrmex*, and because it diverged from that genus some ~15 Ma, we erect the new genus *Xerolitor* to accommodate *X. explicatus* to that genus. Specifically, *X. explicatus* can be easily distinguished from *Sericomyrmex* by its laterally expanded frontal lobes, its complete and deep antennal scrobes, and its uniquely shaped mesonotum (depicted in Figs 4A–F, 6A, B, G). Kempf (1968) recognized that *X. explicatus* is clearly distinct from all other species assigned to *Mycetosoritis* at that time, but suggested that the erect and suberect hairs constitute a shared characteristic uniting them. Our results indicate that erect and suberect hairs evolved independently in *X. explicatus* as well as in the sister-species pair *M. hartmanni* and *M. vinsoni*, which are clearly closely related. Despite the convergent hair morphology, *X. explicatus* can be clearly distinguished from *M. hartmanni* and *M. vinsoni* by the same morphological characters that distinguish it from *Sericomyrmex*.

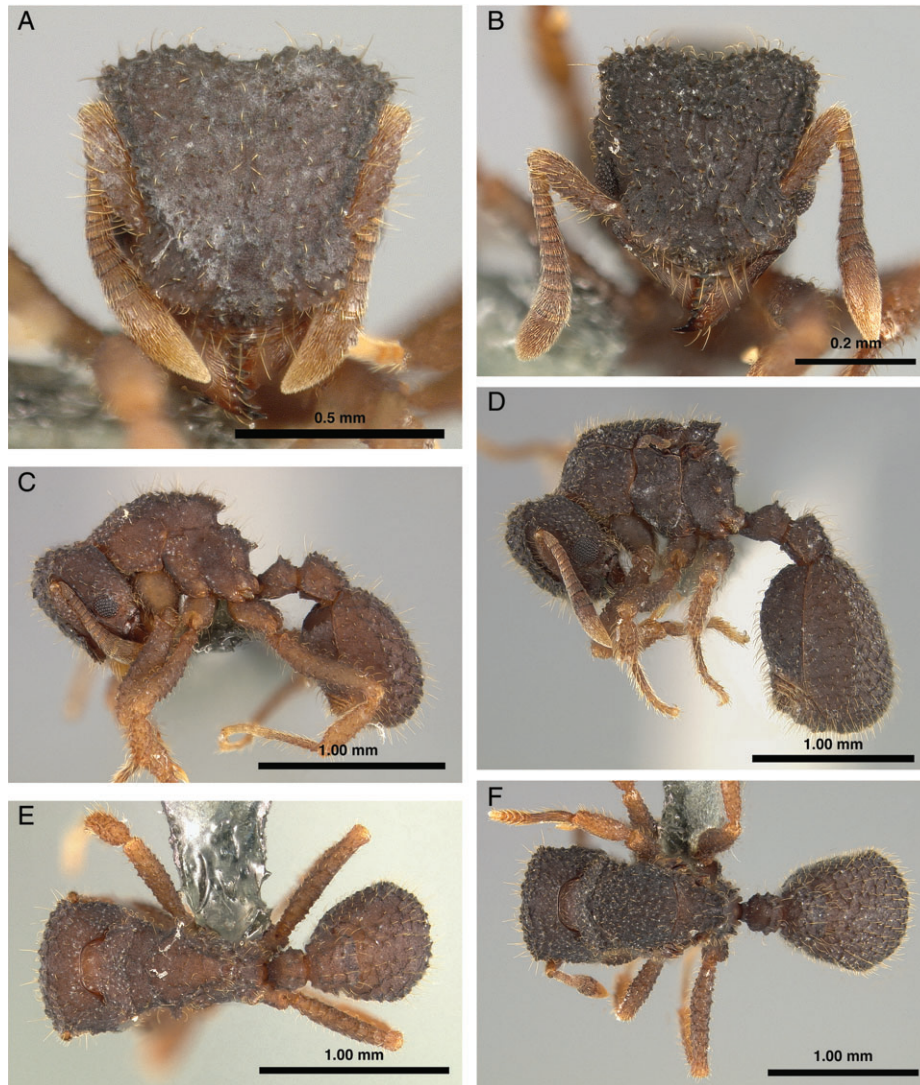


Fig. 4. The worker (A, C, E) and queen (B, D, F) of *Xerolitor explicatus*. (A–B) Full-face view; (C–D) lateral view; (E–F) dorsal view.

***Xerolitor explicatus* (Kempf, 1968) new combination (Figs 4–7)**

Mycetosoritis explicata Kempf, 1968: 401, figs. 14–16. Holotype and paratype workers, Anápolis, Goiás, Brazil. (MZSP) [paratype examined]. Sosa-Calvo *et al.*, 2009: 114–116, figs. 1–7, 9, 10. Description of gyne. (IBGE) [examined].

Description, male. Morphometric measurements: EL, 0.21–0.24; FLD, 0.31–0.32; GL, 1.02–1.18; HL, 0.58–0.61; HFL, 0.92–1.02; HTL, 0.68–0.75; HW, 0.58–0.60; ML, 0.31–0.34; MSL, 0.10–0.11; PL, 0.26–0.39; PPL, 0.21–0.31; PPW, 0.34–0.37; PW, 0.50–0.57; SL, 0.43–0.45; TL, 3.82–4.09; WL, 1.18–1.25; CI, 98–100; MI, 54–55; MSI, 17–19; OI, 37–47; PPI, 110–176; FLDI, 51–54; SI, 74–75 ($n = 3$).

Head. Approximately as long as wide (CI, 98–100) in full-face view, posterior cephalic margin convex; preocular

carina extending posterad close to posterior cephalic margin and weakly meeting frontal carina, forming feebly demarcated antennal scrobe; antennal scrobe areolate (Figs 5A, 6C) contrasting with torulose sculpture on dorsum of head (frons) and frontal lobes (Fig. 6C), some rugae on frons (Figs 5A, 6C); median carina present (Fig. 6C); frontal triangle present, crenulate (Fig. 6C). Mandible triangular, masticatory margin with five to six teeth, increasing in size from base to apex (Figs 5A, 6C, F); dorsum of mandibles mostly areolate-rugulose to smooth towards masticatory margin, and covered with appressed simple hairs (Fig. 6C, F). Clypeal apron narrow, shining, medially emarginate, and dorsally rugulose (Fig. 6C, F); long median seta (MSL, 0.10–0.11; MSI, 17–19) originating at, or very close to, anterior margin of clypeal apron (Figs 5A, 6C, F). Discal area of clypeus somewhat raised, sculptured as frons; in lateral view, clypeus in lateral view medially broadly convex; posterior margin of clypeus (base of frontal triangle)

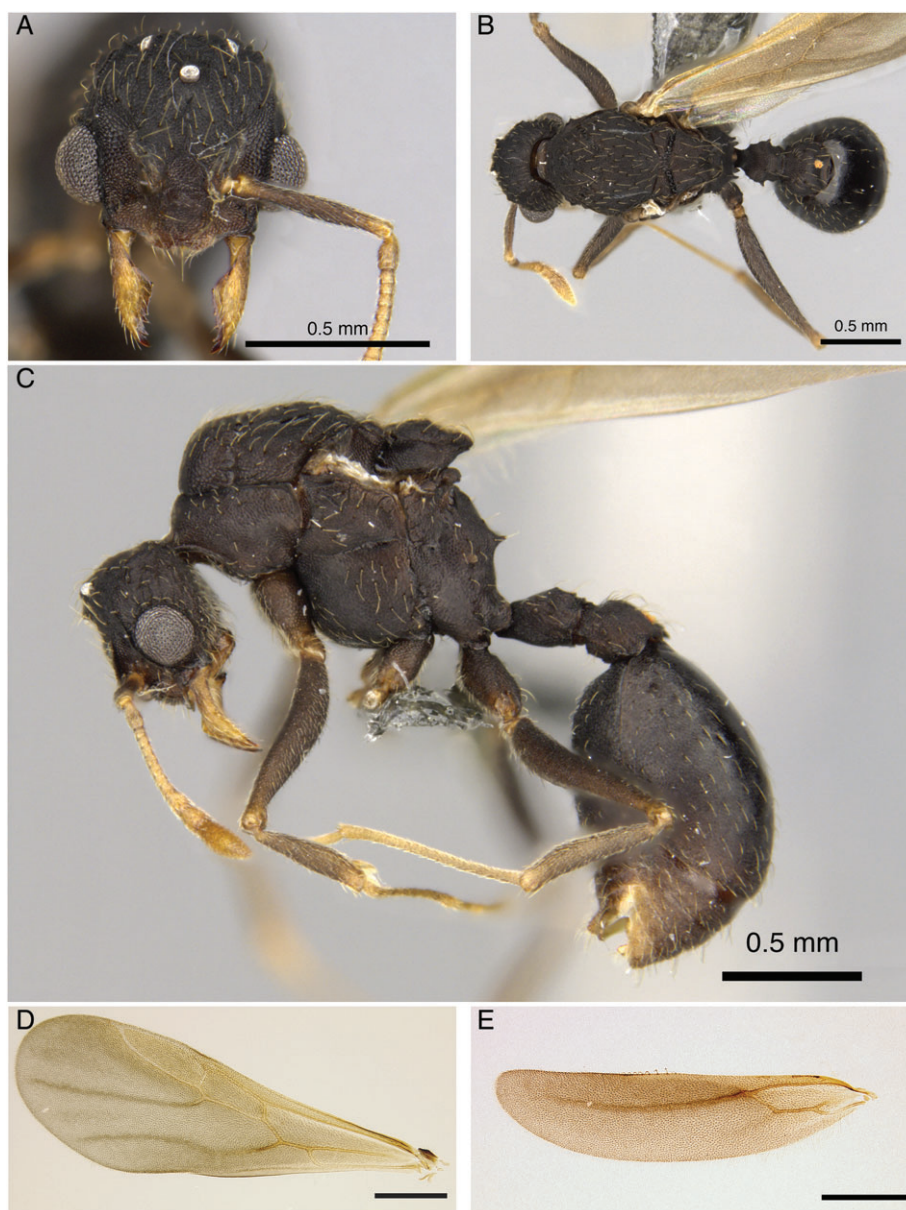


Fig. 5. The male of *Xerolitor explicatus*. (A) Full-face view; (B) dorsal view; (C) lateral view. (D, E) Forewing (D) and hindwing (E) of the male. The scale bar represents 0.5 mm.

extends as deep groove between frontal lobes, sculpture torulose, clearly contrasting with sculpture of clypeus and frontal triangle (Fig. 6C). In lateral view, frontal lobe somewhat projecting forward; in full-face view, frontal lobes small slightly convex (FLD, 0.31–0.32; FLDI, 51–54), failing to cover antennal insertions. Antennal scape long (SL, 0.43–0.45; SI, 73–75), longer than length of funicular segments I–III combined (Fig. 5A, C), and surpassing cephalic corner by $\sim 1/3$ of its length; scape thin with maximum width at midportion of its length; dorsum of scape covered with appressed simple hair; sculpturing of scape as in frons; antennae 12-segmented, shared with males of *Sericomyrmex* but departing from the plesiomorphic number of

antennal segments (13) for ant males (including, plesiomorphically, fungus-growing ant males). Eye large (EL, 0.21–0.24; OI, 36–40), convex. In ventral view, hypostomal teeth absent. Palp formula 4,2.

Mesosoma. Pronotum lacking humeral tubercles; lateral tubercles of pronotum reduced to carinae (Fig. 6I), in dorsal view, appearing as small triangular projections (Fig. 5B); antero-inferior margin of pronotum rounded, lacking tubercle or tooth (Figs 5C, 6I). Mesoscutum with shallow median sulcus, extending close to point of intersection of notauli (Fig. 6L); notauli present, deep, and with scattered, short ridges (Figs 5B, 6L); parapsidal lines present (Figs 5B, 6L); in dorsal view,

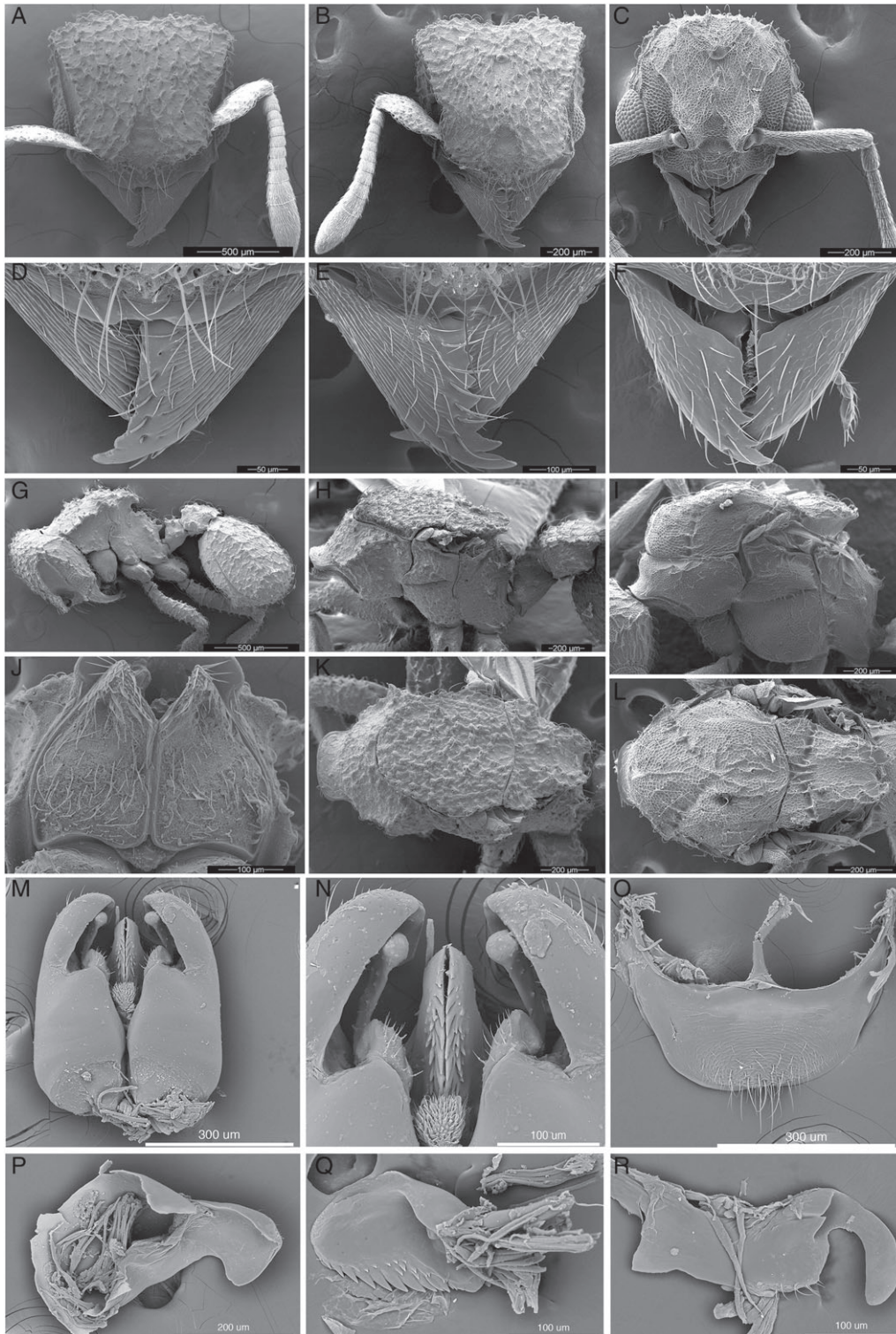


Fig. 6. Scanning electron micrographs of the worker (A, D, G, J), the queen (B, E, H, K), the male (C, F, I, L), and the male genitalia (M–R) of *Xerolitor explicatus*. (A–C) full-face views; (D–F) close-up of mandibles in full-face views; (G–I) lateral views; (J) close-up of propleural plates; (K, L) dorsal views; (M, N) genital capsule, ventral view; (O) abdominal sternum IX, ectal view; (P) basimera, mesal view; (Q) penisvalve, mesal view; (R) volsella, mesal view.

mesoscutum rugulose in addition to areolate with toruli within each areola (Fig. 6L). Oblique mesopleural sulcus (= anapleural sulcus) minutely scrobiculate, dividing mesopleuron into anepisternum and katepisternum (Fig. 6I); subalar sulcus (area above the anepisternum and below wing insertion) transversely costate (Figs 5B, 6I). Mesoscutellum with deep, transversely costate scutoscutellar sulcus (Figs 5B, 6L); mesoscutellar disc with transverse groove dividing mesoscutellar disc into axillae (Figs 5B, 6L); posterior margin of mesoscutellum bidentate (Figs 5B, 6L). Propodeal teeth short and blunt (Figs 5C, 6I).

Metasoma. Petiole in lateral view sessile, lacking node (Fig. 5B, C). Lateral margin of petiole with at least one pair of teeth (Fig. 5B). In dorsal view Postpetiole wider than long (PPL, 0.21–0.31; PPW, 0.34–0.37; PPI, 110–176), lateral margins with small tubercles, posterior margin broadly concave. Dorsum of abdominal segment IV weakly sculpted, lustrous (Fig. 5B); lateral portion of abdominal segment IV areolate. Anterior portion of gastral sternum I feebly emarginate.

Colour. Scapes, head, meso- and metasoma, coxae, and femora dark brown to black; funiculi, mandibles, tibiae, and tarsomeres yellowish to light brown.

Wings. Yellowish-brown, forewing with five closed cells (Fig. 5D). Hindwing with one closed cell (Fig. 5E).

Genitalia. Abdominal sternum IX broader than long, with thin and long spiculum, lateral margins extending anteriorly about length of spiculum. Posterior margin of abdominal sternum IX weakly rounded (almost flat), weakly reticulate, without apical triangular lobe, and medially with simple hairs (Fig. 6O). In ventral view, parameres with basimere longer than broad, simple, glabrous. Telomeres short, medially curved, with bluntly rounded apex, wider apically than basally, and with sparse simple hairs apically (Fig. 6M, N, P). Volsellae (Fig. 6R) with cuspis ventrally produced into simple angled lobe, lacking teeth or processes, and with thin, sparse, medially pointed hairs. Digitus strongly medially curved, simple, weakly clubbed, with additional structure at its base that might be homologous (it appears in the same position) to the distal basivolsellar process found in all known *Sericomyrmex* males, but less produced than in *Sericomyrmex*. The proximal basivolsellar process, which is present in *Sericomyrmex*, is absent in *X. explicatus*. Valviceps of penisvalve distally rounded, ventral edge with nine to 11 long, pointed penisvalvar teeth with minute denticles at base (Fig. 6N, Q).

Comments. The abdominal sternum IX in all known *Sericomyrmex* males is longer, with the lateral margin straight instead of concave, the meeting posterior margin at an angle (rounded in *X. explicatus*). The posterior margin of abdominal sternum IX simple and rounded in *X. explicatus*, whereas posterior margin completely flat in *S. opacus* Mayr and *S. saussurei* Emery, or with reduced apical triangular lobe in *S. amabilis* and *S. mayri* Forel. In *Sericomyrmex*, parameres similar to those in *X. explicatus*; however, telomeres narrower and more medially curved. In *Sericomyrmex*, volsellae with digitus longer and more distinctly clubbed, and cuspis with one larger or few smaller teeth. All known *Sericomyrmex* have a large leaf-shaped structure, termed distal and proximal basivolsellar process, whereas *X. explicatus* has a much shorter structure at base of digitus that could be, based on its position, homologous

to distal basivolsellar process. Penisvalve with broadly notched distal margin in *Sericomyrmex*, rounded in *X. explicatus*. Penisvalvar teeth similar in the two genera, long and pointed, but larger in *Sericomyrmex*, and lacking minute denticles at base.

Description of larva. Two last-instar worker prepupae were examined. Profile ‘atoid’ sensu Wheeler (1948) and Wheeler & Wheeler (1974), i.e. with a moderately curved, ventrally shortened profile. As in all other *Attina*, thoracic-abdominal articulation absent, thoracic intersegmental constrictions superficial, deep lateral depressions associated with abdominal spiracles absent, and leg vestiges present as open slits. As in most other *Attina* (Schultz & Meier, 1995), dorsal and lateral body surfaces devoid of setae. Setae on venter and head simple.

Ventral thoracic segments I and II medially bearing sparsely distributed rows of multidentate spinules; segment III with single feeble row of spinules (Fig. 7A); median lobes or protuberances absent. Thoracic segment I (T1) ventrally with two to three hairs laterally on each side; T2 with three hairs on each side; T3 with two hairs on each side (Fig. 7A); abdominal segment I (A1) ventrally with total of four evenly spaced hairs; A2 and A3 with two hairs; A3 to A8 with no hairs; A9 with four hairs (Fig. 7D–G).

Head devoid of setae except on gena, which bears three long setae, and on clypeus, which bears two short, setiform setae (Fig. 7A).

As in most *Neoattina*, genal lobe present. Labrum monolobate, narrow, bulging, synapomorphy for the *Attina*; four labral setae apparently present, at least some setiform (Fig. 7B, C). Mandible typically attine, i.e. short, fleshy, and subconical. Distinct, undivided apical mandibular tooth and no subapical teeth; spinules evenly distributed on all mandibular surfaces. Mandibular gnathobase absent. Basal portions of maxilla fused with head capsule. As in all other *Neoattina*, maxillary palp widely removed laterad from galea. Galea reduced, present as two sensilla surmounting low welt; shallow pit distal and immediately adjacent to galea. Maxillary palp digitiform, maxillary accessory palpal sensillum present. Two reduced/papilliform setae on maxilla between galea and palp. As in most attines, labium feebly protruding and lateral sericteral protuberances absent; labial palp papilliform. Labial spinules multidentate, sparse, present dorsal to sericteries, ventral surface hidden. This state is shared with most *Sericomyrmex* larvae examined, in which labial denticles are sparse or absent dorsal to sericteries and in which the ventral surface is hidden. Hypopharyngeal spinules multidentate, densely distributed (Fig. 7A).

Anal setal pattern conforms to ‘*Trachymyrmex opulentus* (Mann) pattern’ of Schultz & Meier (1995), with two sensilliform setae (only one asymmetrically positioned seta actually observed in one individual) dorsad of anus and six sensilliform setae arranged in linear row ventral to anus (Fig. 7D, F). Four setae present ventrally on abdominal segment 9, lateral pair long and setiform, median pair sensilliform, in this regard differing from all examined *Sericomyrmex* and closely related *Trachymyrmex* species, in which ventral setae are absent on A9.

Comments. As mentioned earlier, larval morphology (e.g. genal lobes, form of the maxilla) clearly places *M. explicatus*

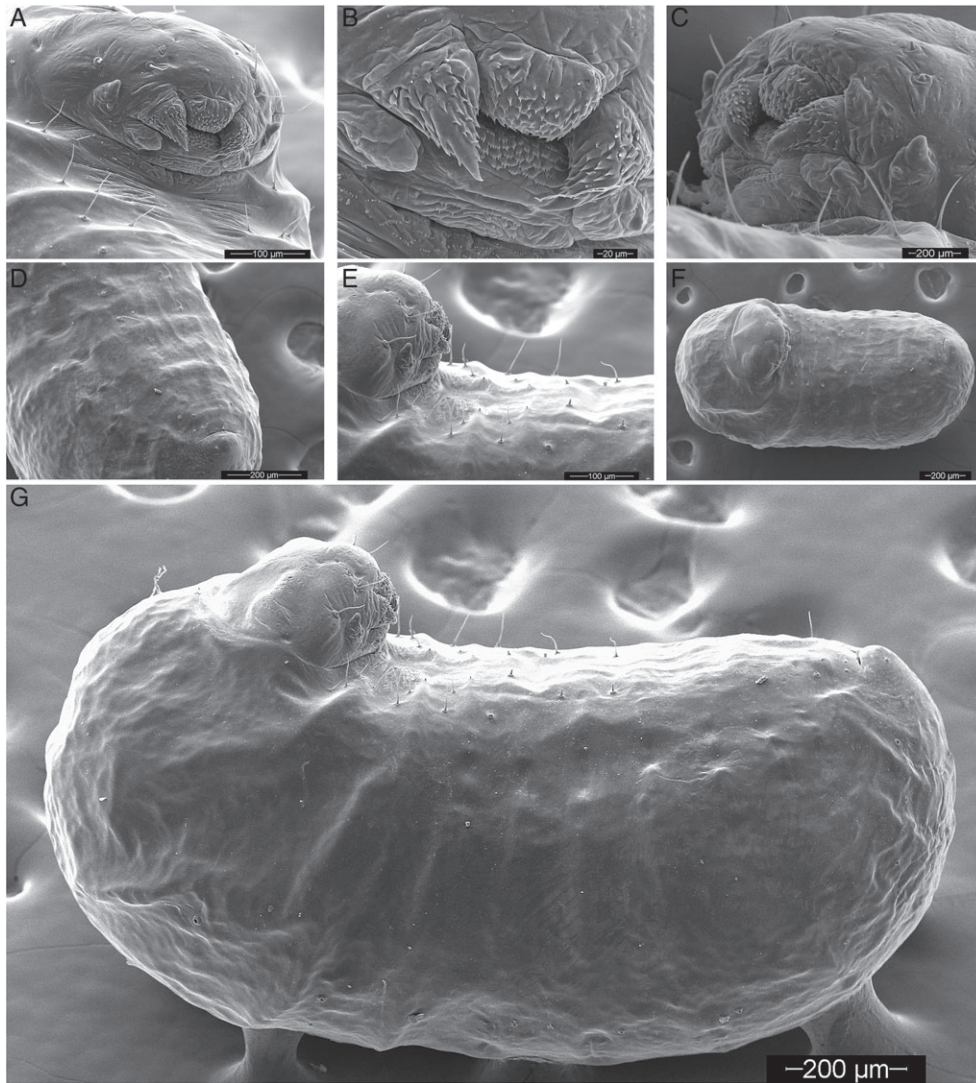


Fig. 7. Scanning electron micrographs of the prepupal larva of *Xerolitor explicatus*. (A) Head, frontolateral view; (B) mouthparts, frontolateral view; (C) mouthparts, including maxilla, frontolateral view; (D) abdomen, lateroventral view; (E) mouthparts and thorax, lateral view; (F) habitus, ventral view; (G) habitus, lateral view.

within the Neoattina. Larval morphology further places *X. explicatus* within the clade containing *Sericomyrmex* and the *Trachymyrmex iheringi* (Emery) clade. Notably, *X. explicatus* shares with members of the *T. iheringi* clade a distinctive pattern of setae on abdominal segment X (all examined *Sericomyrmex* species share a different pattern, in which two extremely long and flexuous setae arise ventrally below the lateral limits of the anal opening); the absence of hairs on the dorsal and lateral body surfaces (hairs present in some *Sericomyrmex* species); less than four genal setae (shared with all examined *T. iheringi* clade species and with some but not all *Sericomyrmex* species); and labial denticles present but sparsely distributed dorsal to the sericteries (the ventral surface hidden), shared with all examined *Sericomyrmex* species, in which labial denticles are either present and sparsely distributed or absent dorsal to the sericteries, and in which the ventral surface is likewise hidden.

Material examined. Brazil: Goiás, Anápolis, 15-iii-1968, *WW Kempf*, 4858 (1w, MZSP, Paratype); Distrito Federal, Brasília, Res. Ecol. IBGE, Km 0 BR 251-DF, 26-ix to 03-x-1980, 3A-47-1 m (1q, IBGE); Distrito Federal, Brasília, Universidade de Brasília Campus, 28-viii-1976, in Cerrado habitat, *JLM Diniz* (JLMD 1102) (1w, J. Diniz personal collection); **Bolivia:** Santa Cruz, Perforación 68 Km ESE Charagua, 11-xii-1993, 470 m, 19°55' S 62°34' W, ground forager, tropical dry forest, *PS Ward* (PSW 12335-5) (1w, MCZC); **Paraguay:** Boquerón, Enciso, 3-6-xi-2001, 21°12' S 61°40' W, dry Chaco, sifted litter, *M Leponce* and *T Delsinne* (No. 4075-41) (1w, A. Wild personal collection); Enciso, 4-5-xi-2001, 21°20' S 61°66' W, 400–590 m trail, Winkler 24 h, *M Leponce* (No. 4057) (3w, 1q, USNM); Enciso, 1-2-x-2002, 21°21' S 61°66' W, Winkler 24 h, *T Delsinne* (No. 11539) (1w, USNM); Enciso, 4-5-xii-2001, 21°20' S 61°66'

Table 1. Nest architecture and nest chamber measurements of four *Xerolitor explicatus* nests that were excavated at Parque Nacional Teniente Agrupino Enciso, Department of Boquerón, Paraguay. Asterisks (*) indicate missing measurements.

Nest	Collection code	Date	Depth (cm)	Chamber dimensions (cm)			Field notes
				Height	Width	Depth	
1	AJ151030-01	30 October 2015	32	4.4	3.5	3.5	Garden, several workers. Queen was not located.
2	CR151031-07	31 October 2015	56	3	3	3	One dealate queen and ~93 workers; fungus garden filled out the entire chamber; garden suspended from tiny rootlets hanging from the chamber ceiling; tunnel leading down from chamber, nest excavation continued to 120 cm depth but no further chambers were encountered. Colony is being maintained alive in laboratory as of September 2017.
3	TRS151031-01	31 October 2015	21	2.5	2	2	Empty
	TRS151031-01	31 October 2015	29.5	2.5	2	1.8	Empty
	TRS151031-01	31 October 2015	43	2.5	2	1	Garden, ~58 workers, and one queen. Brood present, including sexual brood. Small colony.
4	TRS151031-02	31 October 2015	16	2.3	2.2	2.0	Garden, workers, tunnel leading away from the side of the chamber.
	TRS151031-02	31 October 2015	27	*	*	*	Small empty chamber.
	TRS151031-02	31 October 2015	45	2.2	2	1.5	No garden, just workers.
	TRS151031-02	31 October 2015	66	2.5	3.5	3.5	Garden, ~190 workers, one queen, larvae, and pupae.
	TRS151031-02	31 October 2015	72.5	1.8	1.8	0.5	Small empty chamber connected with previous chamber with a tunnel.

W, 800–990 m trail, Winkler 24 h, *M Leponce* (No. 22851) (1w, USNM); Nueva Asunción, 1–2-xi-2001, 20°70' S 61°93' W, 0–190 m dunes, Winkler 24 h, *M Leponce* (No. 22959) (1w, USNM); PN Teniente Enciso, 31-x-2015, 244 m, 21.20599° S 61.65752° W, dry Chaco, nest series, nesting in ground, TR Schultz, J Sosa-Calvo, C Rabeling, A Ješovnik, R Dahan, TRS151031-01 (nest series, USNM, SIBR); same information as previous entry but TRS151031-02 (nest series, USNM, SIBR); same information as previous entry but CR151031-07 (nest series, USNM, SIBR); same information as previous entry but AJ151030-01 (nest series, USNM, SIBR).

Geographic distribution. *Xerolitor explicatus* is known to inhabit the tropical dry habitats of the Brazilian Cerrado and the Bolivian and Paraguayan Dry Chaco. The species has been recorded at elevations of between 250 and 1100 m above sea level. The original description of this species was based on two workers (the holotype and paratype) collected in 1968 along the highway that connects the cities of Goiânia and Anápolis in the state of Goiás in Brazil. Subsequently, a worker and a queen (the latter collected in a Malaise trap) were collected on the campus of the Universidade Federal de Brasília and at the Instituto Brasileiro de Geographia e Estatística (IBGE) in Brasília, respectively. This area is dominated by the Cerrado biome that is estimated to cover 22% of Brazil's surface area and is characterized by diverse phytophysiognomies (Oliveira-Filho & Ratter, 2002; Gottsberger & Silberbauer-Gottsberger, 2006).

The specimens from Bolivia and Paraguay were collected in the Gran Chaco ecoregion, which constitutes a large and continuous dry forest habitat covering a total area of more than 1 million km² and includes parts of Argentina, Bolivia, and

Paraguay (Bucher, 1982; Marinero *et al.*, 2015). The Chaco is considered a highly threatened biome due to its intensive conversion into agricultural land, for both crops (soybeans) and cattle grazing (Gasparri *et al.*, 2013, 2015; Hansen *et al.*, 2013; Caldas *et al.*, 2015; Baumann *et al.*, 2016). In the Chaco, prior to this study, a single worker of *X. explicatus* was collected by hand in Bolivia and the remainder, including a single dealate queen, was collected by sampling the leaf litter with Winkler electors in Paraguay (Delsinne *et al.*, 2007, 2010; Sosa-Calvo *et al.*, 2009).

Natural history, behaviour, and evolution

Microhabitat

Four nests of *X. explicatus* were excavated at PN Teniente Enciso (Table 1). The park, like most of the Gran Chaco habitat, is dominated by 'Quebrachal' forest (Spichiger & Ramella, 1989; Delsinne *et al.*, 2010; Baumann *et al.*, 2016), a xeromorphic forest habitat composed of large trees (*Aspidosperma quebradacho-blanco* Schltr. and *Ceiba insignis* (Kunth) P.E. Gibbs and Semir), tree-like cacti (*Stetsonia coryne* (Salm-Dyck) Britton and Rose and *Cereus stenogonus* K.Schum), and shrubs (*Ruprechtia triflora* Griseb, *Capparis* spp., and *Ziziphus mistol* Griseb). The undergrowth includes bromeliads (*Bromelia* spp. and *Dyckia* spp.) and cacti (*Opuntia* spp., *Cereus* spp., *Cleistocactus baumannii* (Lem.) Lem).

Foraging behaviour

Workers of *X. explicatus* were difficult to locate at our field site. The few observed foraging individuals were active during the early morning hours between 04.00 and 06.00 hours. After

sunrise (~ 06.15 hours) foraging activity ceased and apparently did not resume during the evening and early night hours (18.00–22.00 hours), presumably because of the already high surface temperatures in late October, which marks the end of the dry season. Although we only observed nocturnal/crepuscular foraging, it is possible that workers of *X. explicatus* forage diurnally during the rainy season and/or the cooler winter months. Kempf (1968) collected two foragers during the rainy season (15 March 1968) in Brazil and he did not report the collecting time. Therefore, we assume that Kempf collected the individuals during daylight. T. Delsinne et al. (personal communication) recovered specimens of *X. explicatus* from Winkler samples but the time of day when the foraging workers were trapped is unknown.

Nest architecture

The entrances of *X. explicatus* nests consisted of a single, inconspicuous hole in the ground approximately 3 mm in diameter lacking any visible turret or mound (Fig. 1B). Chambers containing fungus gardens were found in all four nests that we excavated (Table 1). Two of the four excavated nests contained a single, small, rounded garden chamber (Fig. 1E; Table 1). Two nests contained three to five chambers, of which only one or two chambers contained a fungus garden. Chambers varied in size from 1.8 to 4.4 cm high and 1.8 to 3.5 cm wide (Fig. 1E). Nest chambers were located between 16 and 72.5 cm below the soil surface. In each case, the fungus garden was attached to rootlets that were hanging from the chamber ceiling (Fig. 1E).

Demography

In three of the four excavated colonies, we were able to locate a single queen, suggesting that *X. explicatus* is monogynous. For the fourth colony, we failed to locate the queen. For three of the four colonies, we counted the number of workers and found 58–190 individuals (Table 1).

Gongylidia morphology of mutualistic fungus

The one-way ANOVA revealed that species was a significant predictor of gongylidia diameter (one-way ANOVA, $F = 211.4$, $P < 0.0001$). Furthermore, post hoc comparisons revealed that the diameters of gongylidia were significantly different for each species pair ($P < 0.001$). The diameters of the *X. explicatus* gongylidia varied between 31.42 and 57.12 μm ($n = 40$; mean = 45.77 μm ; SD = 6.59) and were significantly different from those of the lower-attine species *Mycocepurus smithii* (Forel) ($n = 40$; mean = 20.25 μm ; SD = 2.5), the higher-attine species *T. urichii* ($n = 40$; mean = 54.6 μm ; SD = 5.93) and the leaf-cutting ant species *Atta laevigata* (Smith) ($n = 40$; mean = 39.67 μm ; SD = 6.17), *Atta sexdens* (Linnaeus) ($n = 40$; mean = 31.72 μm ; SD = 4.02), and *Acromyrmex disciger* (Mayr) ($n = 40$; mean = 41.5 μm ; SD = 4.47). The only comparison that was not statistically significantly different was between *A. disciger* and *A. laevigata* ($P = 0.6$). The gongylidia occurring in the lower-attine *M. smithii* are significantly smaller than the gongylidia occurring in the fungus gardens of *X. explicatus*. Therefore, the average diameter of the gongylidia present in cultivars of *X. explicatus* (45.77 μm) places it within

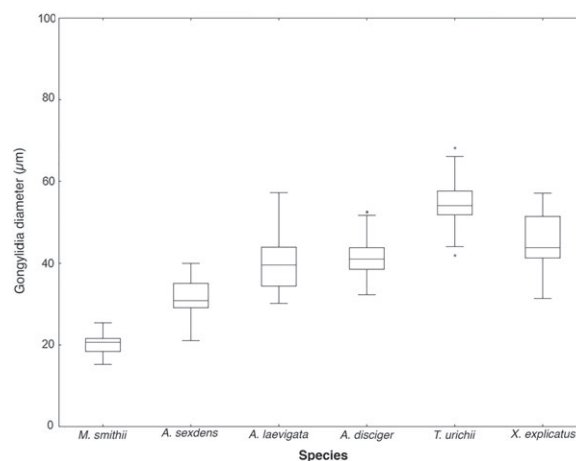


Fig. 8. Comparison of gongylidia diameter from fungi cultivated by a single species of lower-attine ant (*Mycocepurus smithii*), a single species of nonleaf-cutting, higher-attine ant (*Trachymyrmex urichii*), three species of leaf-cutting ants (*Atta sexdens*, *Atta laevigata*, and *Acromyrmex disciger*), and of *Xerolitor explicatus*. Boxes represent 25–75% quartiles and the bar inside each box represents the median.

the higher-attine (39.67–56.4 μm) rather than the lower-attine (20.25 μm) size range (Fig. 8).

Discussion

Our fungus-growing ant phylogeny (Fig. 2) indicates that, soon after their origin 55–60 Ma (Nygaard et al., 2016; Branstetter et al., 2017), the fungus-growing ants diverged into two sister clades, the Paleoattina (Fig. 2 right, green) and the Neoattina (Fig. 2 right, blue). The so-called ‘lower-attine’ ants (the paleoattine genera and the neoattine genera *Kalathomyrmex*, *Cyatta*, *Mycetosoritis*, *Mycetarotes*, *Mycetophylax*, *Cyphomyrmex*, and *Mycetagroicus*) are paraphyletic with respect to the so-called ‘higher-attine’ ant genera *Sericomyrmex*, *Trachymyrmex*, *Acromyrmex*, and *Atta*. The higher- versus lower-attine classification was not intended to reflect fungus-growing ant phylogeny; rather, it was invented to distinguish between morphologically highly modified fungi, i.e. those cultivated by the higher-attine ants, and unmodified fungi, i.e. those cultivated by lower-attine ants (Mueller et al., 1998). However, the derived status of the higher-attine ants is paralleled by the derived status of their fungal cultivars, which, as indicated by the fungal phylogeny (Fig. 3), constitute a derived clade (Fig. 3, green) within the larger group of Leucoprineaceous fungi (Agaricales: Agaricaceae: *Lepiota*, *Leucocoprinus*, and *Leucoagaricus*) which also contains the lower-attine fungi, except for the coral fungi cultivated by ants in the *A. pilosum* group. Although they are closely related, higher-attine fungi are distinct from lower-attine fungi in a number of morphological and life-history characters, including: (i) the consistent production of gongylidia, swollen hyphal tips produced by the fungus and preferentially harvested by the ants for food (Masiulionis et al., 2014; Mueller et al., 2017; Sosa-Calvo et al., 2017a); (ii) major shifts in decompositional enzyme profiles (De Fine Licht et al.,

2014); (iii) polyploidy, in contrast to the lower-attine fungi, which are, so far as is known, consistently diploid (Kooij *et al.*, 2015); and (iv) obligately symbiotic relationships with their ant hosts, i.e. an inability to live freely apart from fungus-growing ants, in contrast to the lower-attine fungi, which, so far as is known, are all capable of a free-living existence (Mueller *et al.*, 1998, 2017; Mueller, 2002; Vo *et al.* 2009; Mehdiabadi and Schultz 2010).

Most importantly for this study, the ant phylogeny indicates that *X. explicatus*, formerly assigned to the lower-attine genus *Mycetosoritis*, is in fact a higher-attine ant. The phylogeny also indicates that *X. explicatus* is the distant sister species of the derived higher-attine ant genus *Sericomyrmex*. We further know from prior studies (Branstetter *et al.*, 2017; Ješovnik *et al.*, 2017) that *Xerolitor* and *Sericomyrmex* share a most recent common ancestor that lived approximately 15 Ma. The phylogenetic position of *X. explicatus* is consistent with the phylogenetic position of its fungal cultivar (Fig. 3) among the higher-attine fungi as well as with our observation that the fungus cultivated by *X. explicatus* in the Paraguayan Chaco possesses gongyliidia (Fig. 3) that were organized into staphylae. The fungal phylogeny (Fig. 3) further indicates that the fungal cultivar of *X. explicatus* is very likely conspecific with fungal cultivars collected from the nests of *S. amabilis* in Panama and of *T. urichii* in Brazil as well as the cultivar of *S. amabilis* in Ecuador (Ješovnik *et al.*, 2017). Although it seems initially surprising that this obligately ant-associated fungal species is found across such a broad geographic range and in both wet and dry habitats, similar patterns have been observed in other ant-cultivated fungi and may in fact be the norm (e.g. Rabeling, 2004; Ješovnik *et al.*, 2013; Sosa-Calvo *et al.*, 2017b). It is possibly significant that the fungal species associated with *X. explicatus* is the sister group (along with another, more closely related species) to the derived ‘*amabilis-mayri*’ fungal cultivar group, which may comprise a single fungal species grown by a disproportionately large number of *Trachymyrmex* and *Sericomyrmex* species across a wide geographic range (Ješovnik *et al.*, 2017).

The origin and maintenance of biological diversity comprises a dynamic evolutionary process in which speciation generates and extinction eradicates species diversity. In this process, the presence of relictual, species-poor lineages is theoretically highly unlikely but, notwithstanding, these lineages persist throughout the ant tree of life (Pie & Feitosa, 2016), are often phylogenetically informative and help to resolve the evolutionary relationships among major ant clades (Rabeling *et al.*, 2008; Ward *et al.*, 2010; Borowiec *et al.*, 2017). The genus *Xerolitor* is one example of these relictual and phylogenetically informative lineages in the fungus-growing ants. Additional species-poor, phylogenetically isolated lineages remain in the Paleoattina, such as *Apterostigma megacephala* (Schultz *et al.*, 2015; Sosa-Calvo *et al.*, 2017a), and in the Neoattina. In the Neoattina, a series of historical, sequential cladogenetic events has repeatedly produced depauperate, phylogenetically isolated lineages such as the species-poor (*Cyatta* + *Kalathomyrmex*), (*Mycetosoritis hartmanni* + *Mycetarotes*), and *Mycetagroicus* (Schultz & Brady, 2008; Sosa-Calvo *et al.*, 2013; Nygaard *et al.*, 2016). Because of their distinct morphology, the species in these

isolated and species-poor taxa have historically been difficult to classify and the convergent evolution of similar morphological characters has repeatedly misled attine-ant taxonomists. The genus *Mycetosoritis* arguably epitomizes the pitfalls of convergent phenotypic evolution in the fungus-growing ants, because the five known species of *Mycetosoritis* belong to at least three independently evolved lineages. As part of a continuing effort to revise the taxonomy of the fungus-growing ants so that it accurately reflects the evolutionary relationships revealed by molecular phylogenetics, we erect the new genus *Xerolitor*, and we are currently revising a number of attine ant taxa, including the genera *Cyphomyrmex*, *Mycetophylax*, *Trachymyrmex*, *Acromyrmex*, as well as others.

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