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THE FUNGUS-GROWING ANT GENUS *APTEROSTIGMA* IN DOMINICAN AMBER

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ABSTRACT

The first fossil species of the fungus-growing ant genus *Apterostigma* (Myrmicinae: Attini) are described from Dominican amber. Two new species are described, and the likely placement of each within the genus is discussed. The phylogenetic position of *Apterostigma* within the Attini is summarized, as is the evolutionary history of the transition from the cultivation of leucocoprineaceous fungi (the ancestral condition) to the cultivation of pterulaceous fungi (the derived condition) by different lineages of *Apterostigma* species. I conclude by speculating on the possible implications of the fossil species for understanding this transition, which is unique within the fungus-growing ants.

Key words: Hymenoptera, Formicidae, Attini, *Apterostigma*, fungus-growing ants, fossils, Dominican amber.

INTRODUCTION

Fungus-growing ants in the tribe Attini are one of only a few animal groups that practice true agriculture. Because, so far as is known, this fungicultural life-history strategy is entirely unique in ants and, because it parallels human agriculture in many remarkable ways (Schultz *et al.*, 2005), the origin and evolution of the fungus-growing behavior has been the subject of recent intensive study (e.g., Chapela *et al.*, 1994; Hinkle *et al.*, 1994; Schultz & Meier, 1995; Wetterer *et al.*, 1998; Mueller *et al.*, 1998; Mueller *et al.*, 2001). Various lines of evidence suggest that the fungus-growing behavior arose a single time in an ancestral attine ant sometime between 45–65 million years ago on the South American continent (Mueller *et al.*, 2001). Perhaps the most compelling pattern supporting this hypothesis is the geographic distribution, extant and historical, of the Attini: fungus-growing ants occur only in North, Central, and South America, and on some Caribbean islands (Kempf, 1972; Wilson, 1988; Brandão, 1991). They also occur as fossils in Dominican amber (Oligo-Miocene, ~20 mya), but are unknown in Old World fossils, including Baltic amber (Eocene, ~45 mya). To date, three attine ant species have been described from Dominican amber: *Trachymyrmex primaevus* (Baroni Urbani, 1980), *Cyphomyrmex maya*, and *Cyphomyrmex taino* (de Andrade, 2003). In addition, Brown (1973) refers to possible *Mycetosoritis* males in Chiapas amber (Oligo-Miocene, ~20 mya), Wilson (1985) refers to *Cyphomyrmex* in Dominican amber, and Baroni Urbani (1995) refers to *Apterostigma* and *Cyphomyrmex* in Dominican amber.

Here I describe two fossil species of the fungus-growing ant genus *Apterostigma* preserved in Dominican amber.

MATERIALS AND METHODS

Amber specimens were studied and measured using a Leica Wild M10 stereomicroscope. Illustrations were executed by USNM scientific illustrator V. Malikul, who directly examined the specimens using a Nikon SMZ 1500 stereomicroscope. Specimens were photographed using a JVC KY-F70B video camera mounted on a Leica M420 microscope and attached to an IBM Intellistation M Pro computer, on which composite images were assembled using Auto-Montage Version 3.04 software (Synoptics Ltd.). The following are abbreviated in the text: American Museum of Natural History (AMNH), National Museum of Natural History (USNM), and the Smithsonian Institution Ant Database (SIANT).

SPECIES DESCRIPTIONS

Apterostigma electropilosum, new species

(Figures 1 and 2)

HOLOTYPE (worker): AMBER: Oligo-Miocene; Dominican Republic; AMNH no. DR-14-984; no other locality data. USNM SIANT database specimen reference number 00443127. Deposited in AMNH.

Measurements: head length = 1.06 mm; head width (not including eyes) = 0.68 mm; scape length = 0.99 mm; Weber's length = 1.37 mm; metafemur length = 1.30 mm.

DESCRIPTION: In full-face view, head elongate, evenly rounded at the posterolateral corners and slightly concave at the median posterior border. As in all *Apterostigma* species, occiput drawn out posteriorly into a "neck" or "collar"; in this species, the collar short, not expanded posteriorly, and lacking integumental rugosities. Frontal carinae strongly produced, extending posterad to well past the level of the eyes. Vertexal carinae not visible, possibly

obscured by the amber matrix and by the unusual texture of the integument, discussed below. Vestigial preocular carinae extending posterad to top of eye. Frontal lobes evenly rounded. Antennae with the typical attine number of eleven segments, the apical antennal segment $2.5\times$ as long as the subapical segment, the subapical segment subequal in length to the pedicel. Clypeus clearly with a smooth, shining anterior border, produced medially into a broad, V-shaped angle. Mandibles with eleven teeth, all unusually sharp and showing no wear, teeth decreasing evenly in size toward the mandibular base. Lateral corners of the hypostoma produced into blunt, rounded “hypostomal teeth.” Eyes with six ommatidia across at narrowest circumference (subparallel to the transverse plane) and ten ommatidia across at greatest circumference (subparallel to the longitudinal axis). Eyes prominent and hemispherical, each hemisphere interrupted from behind (i.e., ventrally) by a straight line of occluding integument.

Promesonotum with a pair of longitudinal, uniformly low carinae. Anterolateral mesonotal carinae apparently absent and humeral prominences vestigial. Posterior mesonotum with a pair of reduced, very short, almost vestigial carinae (“arista metanotal” of Lattke, 1997), best described as vestigial tubercles. Ventral mesopleural carinae present and complete, but not lamellate. Meso- and metacoxae of typical form, without lamellate or keel-like carinae. Propodeum carinate on the basal and declivous faces, the carinae essentially interrupted extensions of the promesonotal pair. Propodeal spines or tubercles absent, propodeal shoulder evenly rounded.

Petiole short and thick, entirely lacking a node; anterior ventral tooth apparently absent. Viewed dorsally, postpetiole roughly as long as broad, and about half as broad anteriorly as posteriorly. First gastral (fourth abdominal) tergite strongly laterally carinate for at least three-fourths of its length.

Body covered with long, fine, simple, erect setae with a maximum length of 0.15 mm. Integument with a mottled, coarsely granulate texture that interferes with the study of microsculpture. On close inspection, this texture apparently caused by shining bubbles, possibly of an oily secretion, that arose from punctures in the integument while the amber was in a semiliquid state. A similar phenomenon was observed in a modern *Apterostigma* specimen embedded in Canada balsam by N. A. Weber.

The shining clypeal border places this species in the *pilosum* group (*sensu* Lattke, 1997), one of two informal subgeneric groupings. The specimen keys out to *A. wasmanni* (southern Brazil) in Lattke’s (1997) key, but differs from that species in being less strongly sculptured and in having the median clypeal angle more pronounced. In habitus it is similar to many small cryptic species that key out to Lattke’s unrevised “*pilosum* complex” of species, but is distinct among them in combining evenly rounded frontal lobes, eleven mandibular teeth, and a medially angulate clypeus.

ETYMOLOGY: The species name indicates that this is a species of the *pilosum* group, and probably of the *pilosum* complex, in amber (*electrum*).

***Apterostigma eowilsoni*, new species**

(Figures 3, 4, and 5)

HOLOTYPE (worker): AMBER: Oligo-Miocene; Dominican Republic; AMNH no. DR-16-292; no other locality data. USNM SIANT database specimen reference number 00443150. Deposited in AMNH.

Measurements: head length = 0.99 mm; head width (not including eyes) = 0.68 mm; scape length = 0.99 mm; Weber’s length = 1.44 mm; metafemur length = 1.37 mm.

DESCRIPTION: In full-face view, head bluntly angled at posterolateral corners and again at vertexal carinae, and slightly indented medially. Occipital “collar” short and not expanded

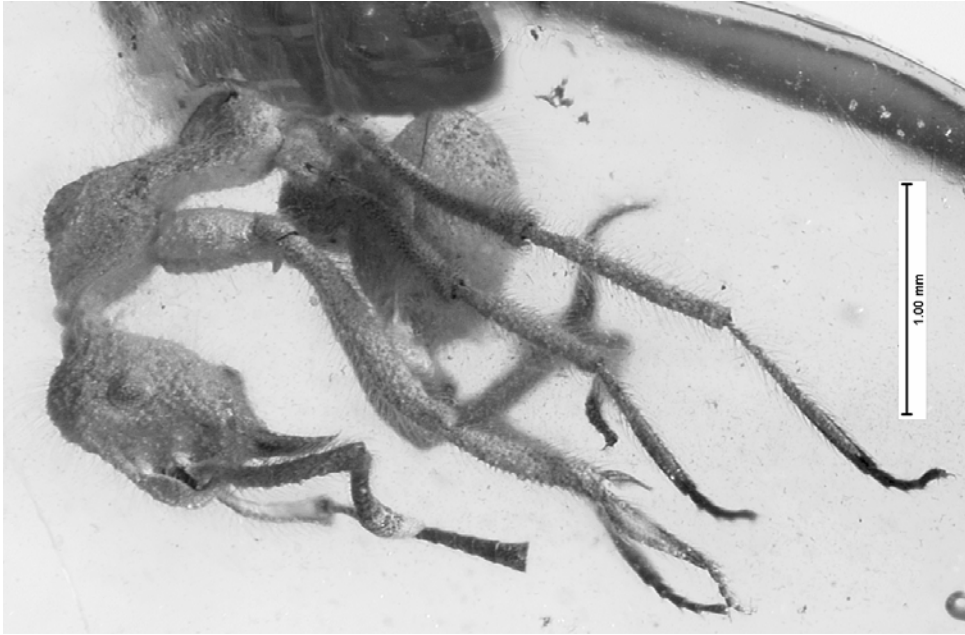


Fig. 1b. *Apterostigma electropilosum*, lateral view.

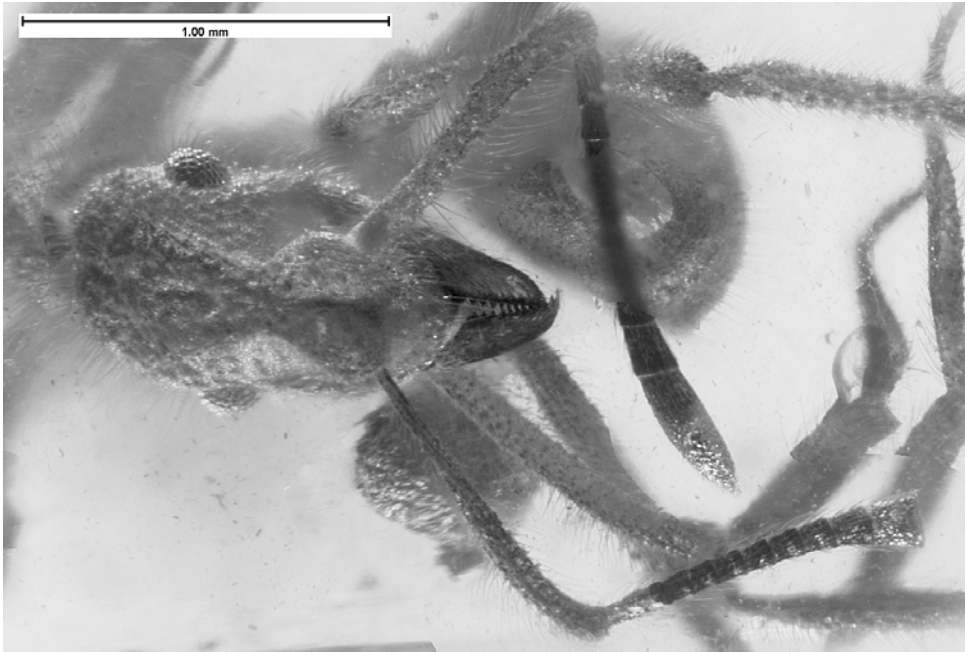


Fig. 1a. *Apterostigma electropilosum*, full-face view.

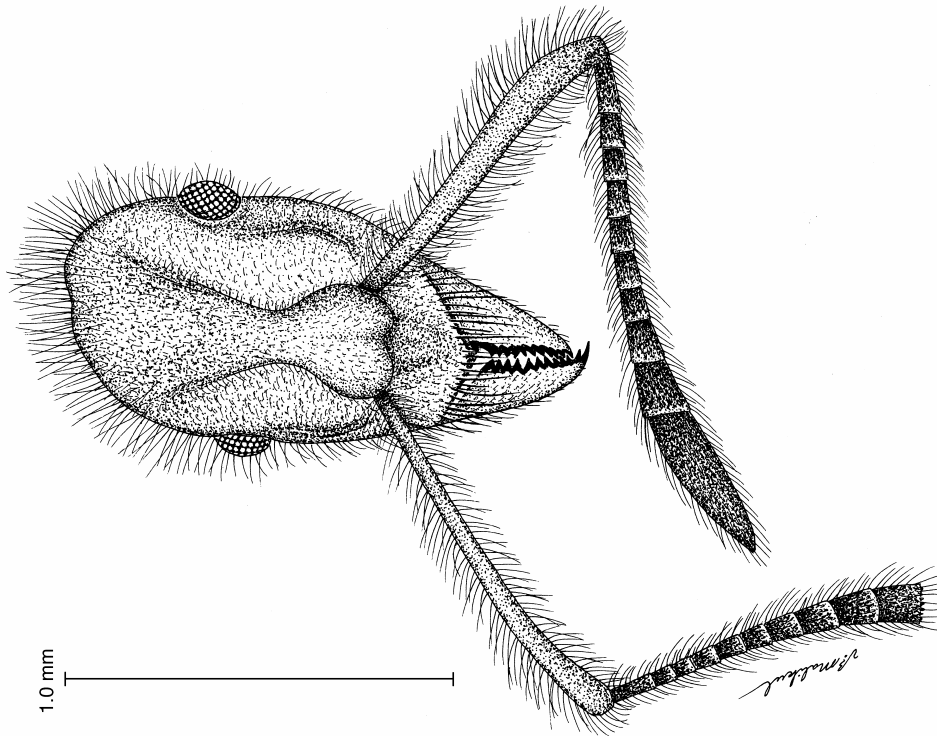


Fig. 2a. *Apterostigma electropilosum*, full-face view.

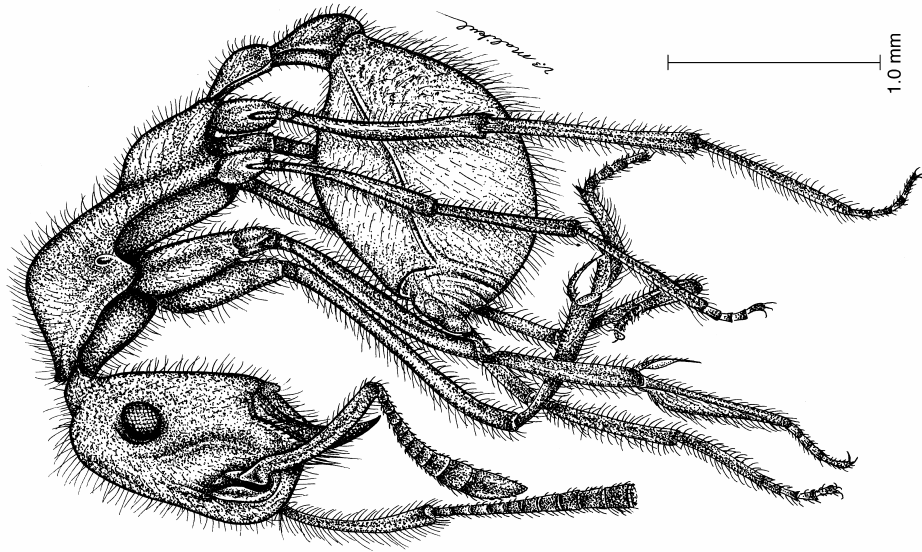


Fig. 2b. *Apterostigma electropilosum*, lateral view.



Fig. 3a. *Apterostigma eowilsoni*, lateral view.



Fig. 3b. *Apterostigma eowilsoni*, full-face view.

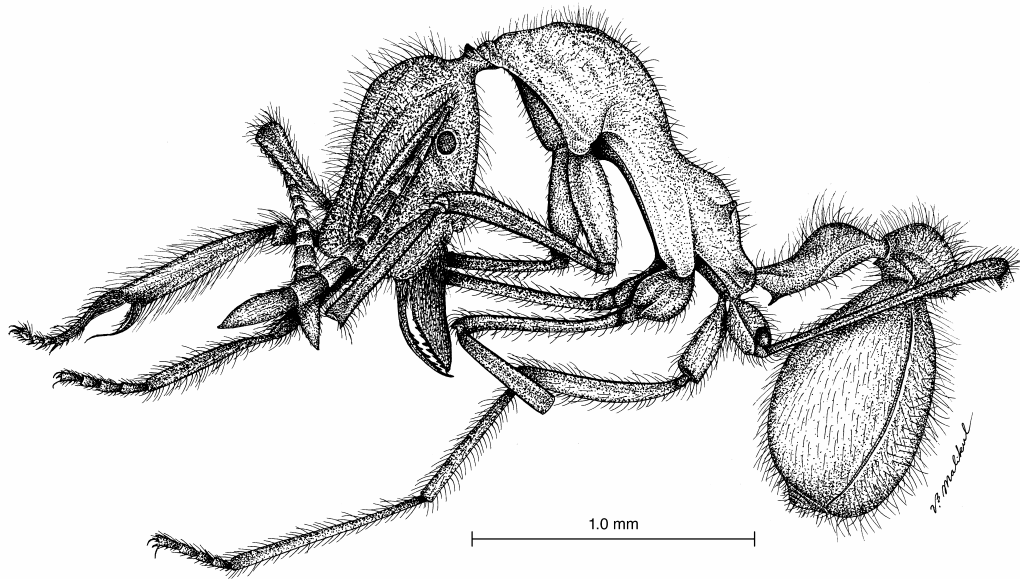


Fig. 4a. *Apterostigma eowilsoni*, lateral view.

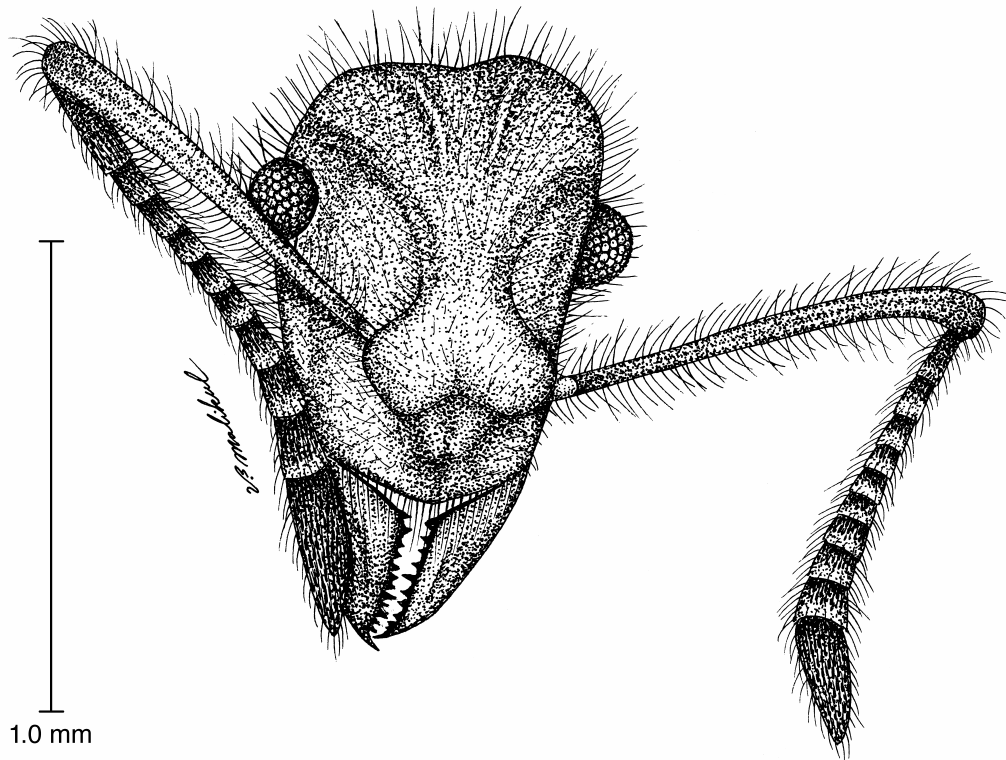


Fig. 4b. *Apterostigma eowilsoni*, full-face view.

posteriorly; collar integument with a series of longitudinal rugae. Frontal carinae strongly produced, extending posterad past the level of the eyes. Vertexal carinae strong, preocular carinae weak. Frontal lobes evenly rounded. Antennae with the typical attine number of eleven segments, the apical segment $2.25\times$ as long as the subapical segment, the subapical segment two-thirds the length of the pedicel. Clypeal border broadly convex, and, as far as can be seen, with only an exceedingly thin strip of cuticle, forming the anterior edge of the clypeus, smooth and of a darker color than the rest of the clypeal integument, the rest identical to the integument of the rest of the head. Mandibles apparently with eleven sharp teeth, the teeth arranged in a curious pattern heretofore unencountered in the genus: reckoning from the apex, the sixth and eighth teeth on the left mandible and the fourth and seventh teeth on the right mandible distinctly larger than the rest; the smaller teeth in between of various sizes, not decreasing in size toward the mandibular base. Lateral corners of the hypostoma produced into blunt, rounded hypostomal teeth. In frontal view the eyes typical, in lateral view the eyes half-hemispheres truncated from behind by integument, but in dorsal view the eyes forward-directed subconical hemispheres mounted on lobes or tubercles that project, perpendicular and earlike, from the sides of the head. As far as can be determined given the complications of observing through the amber matrix, eye width approximately nine ommatidia across the transverse circumference and approximately twelve ommatidia across the longitudinal circumference.

Promesonotum with a pair of strong longitudinal carinae, in lateral view the carinae ending anteriorly in an abrupt vertical wall above the promesonotal junction. Anterolateral mesonotal carinae strong and humeral prominences apparently absent. Posterior mesonotum ("metanoto" of Lattke, 1997) without sculpture. Ventral mesopleural carina present and complete, but not lamellate. Meso- and metacoxae of typical form, without lamellate or keel-like carinae. Propodeum carinate on the basal face, weakly carinate on the declivous face, and lacking propodeal spines. Propodeal shoulder evenly rounded, the propodeal spiracles directed posterad and mounted on tubercles. Ventral surfaces of the mesopleura covered with six small spherical objects, apparently clusters of minute bubbles.

Petiole with an elongate peduncle bearing an anterior ventral tooth and a low but distinct node. What appears to be a second, posterior ventral tooth is actually debris in the amber matrix. Viewed dorsally, the postpetiole approximately $1.3\times$ broader than long, subtriangular in shape, and about half as broad anteriorly than posteriorly. Entire length of the first gastral (fourth abdominal) tergite strongly laterally carinate. Body covered with long, fine, simple, erect setae with a maximum length of 0.15 mm.

By Lattke's (1997) primary criterion of the presence of a smooth and shining clypeal border, *A. eowilsoni* belongs to the *pilosum* group. The anterior clypeal border in this species is, however, extremely reduced and thus represents a credible intermediate in the morphocline spanning the typical (and presumably plesiomorphic; Lattke, 1999) state in the *pilosum* group and the derived state (clypeal border absent) in the *auriculatum* group. Of the species known to me, the reduced clypeal border in *A. eowilsoni* most resembles that of an undescribed Costa Rican species that keys out to the unrevised "*pilosum* complex" of species in Lattke's (1997) key. Certainly the most striking character of *A. eowilsoni* is the remarkably protruding eyes, which are similar in form to—but far more developed than—the eyes found in the *auriculatum*-group species *A. pariense* (Venezuela and Bolivia; specimens examined) and *A. reburum* (Colombia; specimens not seen), which also have their eyes mounted on markedly protruding tubercles. *Apterostigma eowilsoni* clearly possessed excellent stereoscopic forward vision, limited lateral vision, and no rearward vision.

ETYMOLOGY: It gives me great pleasure to name this striking and possibly phylogenetically important fossil fungus-growing ant after E.O. Wilson, in celebration of his long career of myrmecological discovery.

EVOLUTIONARY IMPLICATIONS

Current evidence suggests that the tribe Attini is divided into two major clades, the result of an ancient, phylogenetically basal divergence: (i) Kusnezov's (1963) "Paleoattini," consisting of the three genera *Myrmicocrypta*, *Mycocepurus*, and *Apterostigma*, and (ii) the "Neoattini," consisting of all other attine genera (Kusnezov, 1963; Schultz & Meier, 1995; Schultz, unpublished). The Paleoattini are characterized by a number of compelling synapomorphies, including: (i) short antennal pedicel in the male caste; (ii) the presence of a unique "fenestra" (clear spot) in the wings of gynes (Emery, 1913); (iii) paired hypostomal teeth in workers and gynes (secondarily lost in some species of *Apterostigma* and *Mycocepurus*); and (iv) the absence of an inferior pronotal angle or tooth. (The monophyly of the Paleoattini does not preclude the possibility that some attine species, known or as yet unknown and probably fitting our current concept of *Myrmicocrypta*, may belong to earlier diverging lineages.) Within the Paleoattini, ants of the genus *Apterostigma* are by far the most evolutionarily derived. This genus is characterized by a number of synapomorphies, including: (i) a reduced palpal formula of 3, 2 (Kusnezov, 1953), in contrast to the formula of 4, 2 present in all other Attini except the Argentinean social parasite *Pseudoatta argentina*; (ii) unusual wing venation, including loss of the *rsf1* vein; (iii) the wings of gynes with a distinctively positioned, reflective fenestrum, and of both sexes with a smoky coloration; (iv) pilosity of fine, simple, erect setae, in some species short and in most species long and flexuous; and (v) cytochrome oxidase mtDNA sequence with a unique amino acid insertion.

Most remarkably, with regard to symbiont association a subset of *Apterostigma* species are the most highly derived of all attine ants. All other attine ants, including the leaf-cutters in the genera *Atta* and *Acromyrmex*, cultivate "parasol mushrooms" in the single tribe Leucocoprineae (family Lepiotaceae), consisting of *Leucocoprinus* and *Leucoagaricus*, as well as a few species currently assigned to *Lepiota* (Mueller et al. 1998; Johnson 1999). *Apterostigma auriculatum* and, presumably, other species in the *auriculatum* group retain this ancestral attine association with the Leucocoprineae. In contrast, all *Apterostigma pilosum*-group species (*sensu* Lattke, 1997) studied to date cultivate "coral fungi" closely related to the genera *Pterula* and *Deflexula* (Pterulaceae), which are quite distantly related to the Leucocoprineae (Munkacsi and McLaughlin 2001; Munkacsi et al., 2004). A molecular phylogeny for *Apterostigma* (Villesen et al., 2004; summarized in Figure 6) indicates that the earliest *Apterostigma* species cultivated leucocoprineaceous fungi. Sometime after the origin of *Apterostigma*, in an unparalleled event in the history of the Attini, an *Apterostigma* species host-switched to a radically different fungal cultivar in the Pterulaceae, which all of its descendants (the majority of extant *Apterostigma* species, all derived) have been cultivating ever since. Moreover, it appears that the first Pterulaceae-cultivating *Apterostigma* species cultivated the distinctive "G4" fungal clade, whereas later in evolutionary time a *pilosum*-group *Apterostigma* species host-switched to the "G2" fungal clade, a behavior inherited by all of its descendant species. Unlike G4-cultivating species, G2-cultivating *Apterostigma* ants (including *A. dentigerum*, *A. dorotheae*, *A. collare*, and a subset of Lattke's [1997] "*pilosum* complex") weave the elongate aerial hyphae of their fungal cultivars into protective tent-like veils that entirely surround their gardens.



Fig. 5. *Apterostigma eowilsoni*, posterior view of head showing protruding eyes.

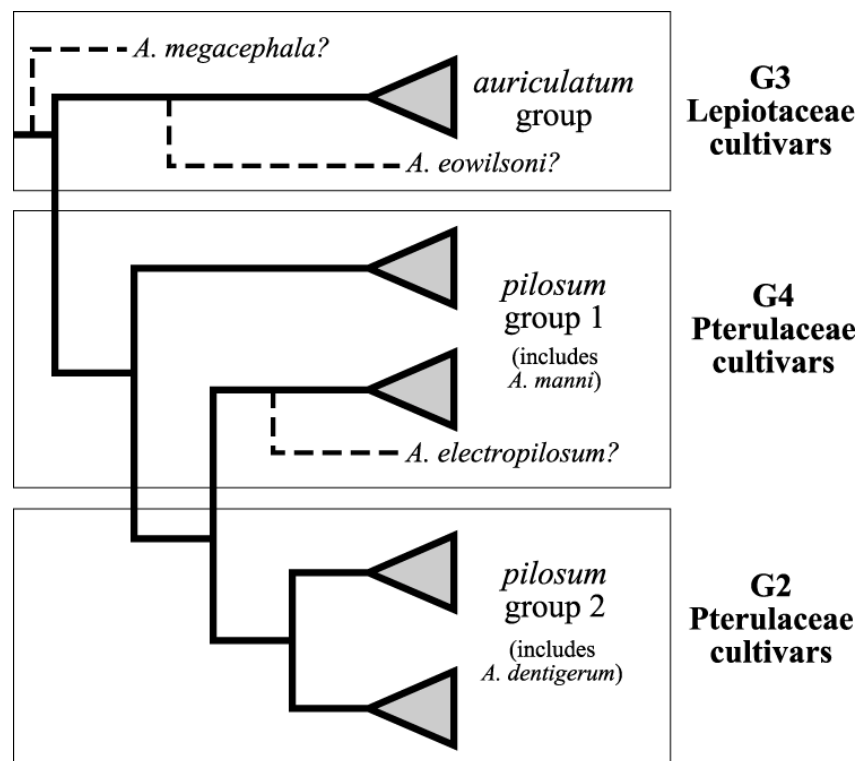


Fig. 6. A phylogeny of *Apterostigma* based on Villesen *et al.* (2004) indicating known cultivar associations and the speculative positions of *A. megacephala*, *A. eowilsoni*, and *A. electropilosum*.

Recently, Lattke (1999) described *Apterostigma megacephala*, a bizarre species that combines the smooth and shining clypeal border that is the primary character of the *pilosum* group with the strongly sculptured integument that is usually associated with the *auriculatum* group. The first *Apterostigma* very probably also combined these traits. A phylogeny based on morphological character data, incorporating *A. megacephala* as well as the two fossil species recorded here, is necessary for accurately reconstructing the phylogenetic positions of the fossil species, speculatively reconstructed in Figure 6. This information, combined with an expanded molecular phylogeny, will permit the assignment of minimum dates to particular nodes, which will in turn provide information critical for estimating the dates of origin of (i) the genus *Apterostigma*; (ii) the *auriculatum* group; (iii) the shift from lepiotaceous to pterulaceous cultivars; and (iv) the shift from “G4” to “G2” pterulaceous cultivars.

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Most of all, I am grateful to E.O. Wilson for inspiring me to become a myrmecologist. In particular, my life was transformed by his 1971 book *The Insect Societies*, in which appears the following passage: “So long as the evolutionary origin of the Attini remains a mystery, we cannot hope to gain any convincing insight into the evolutionary beginnings of fungus gardening.”

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