

A



1 Sceptobiini: *Sceptobius schmitti*

B



2 Mimecitini: *Pseudomimeciton zikani*

Figure S1 (continued on next page)

C



3 Ecitocharini: *Ecitophya simulans*

D



4 Athetini: *Ecitocryptus sulcatus*

Figure S1 (continued on next page)

E



6 Crematoxenini: *Diploeciton nevermanni*

F



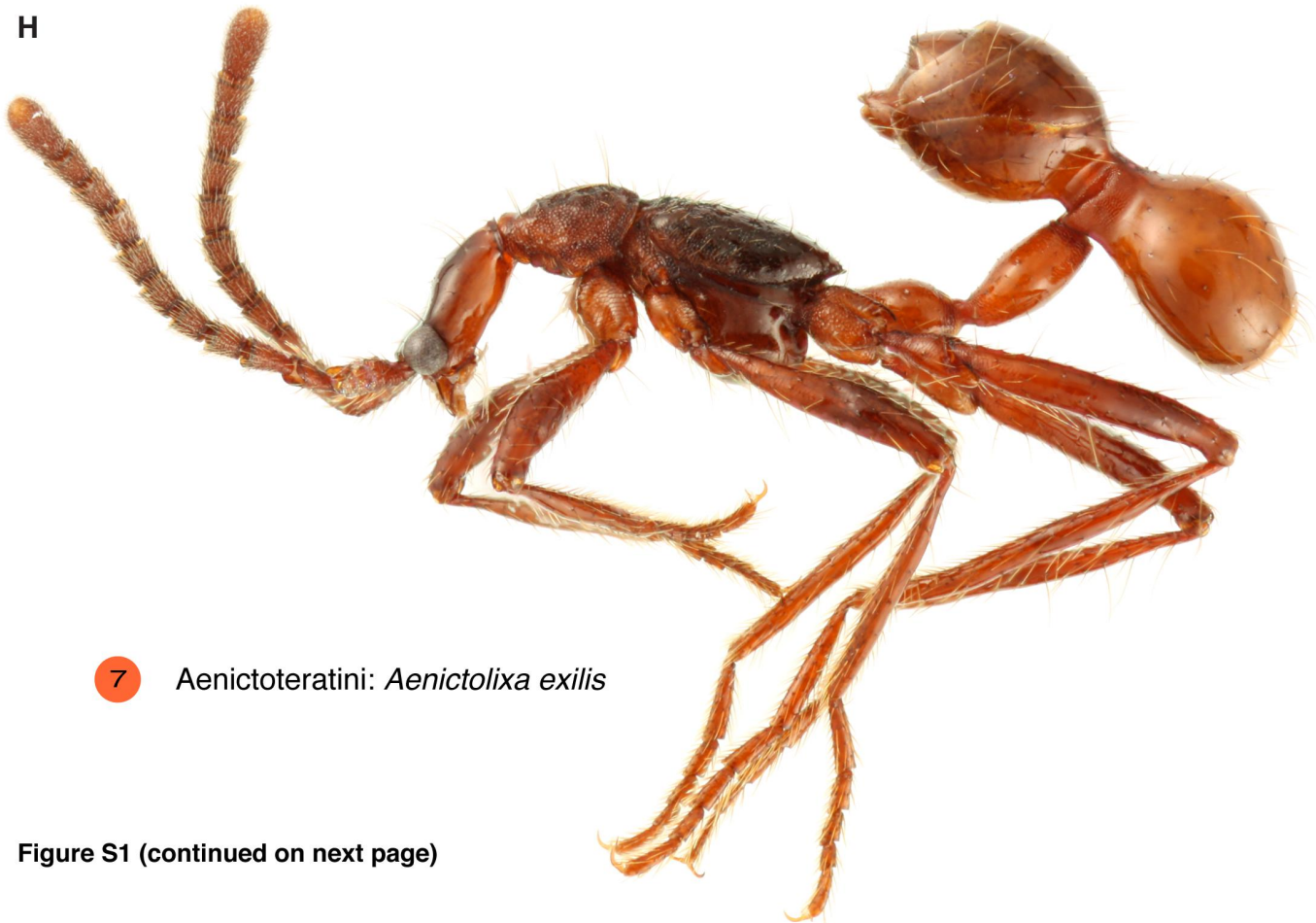
6 Crematoxenini: *Ecitoglossa quadriceps*

G



7 Aenictoteratini: *Aenictoteras malayensis*

H



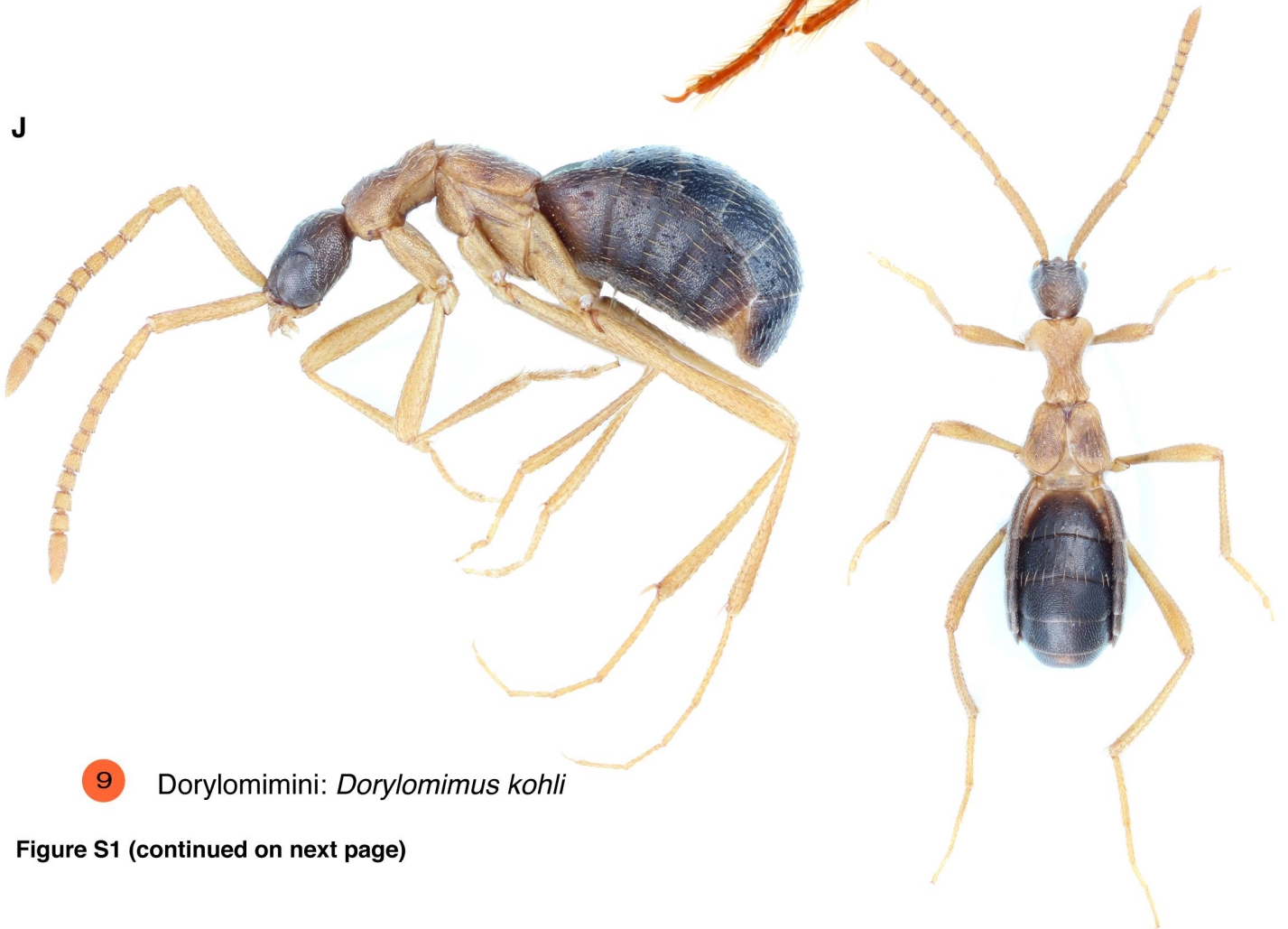
7 Aenictoteratini: *Aenictolixa exilis*

I



7 Aenictoteratini: *Aenictocupidus jacobsonorum*

J



9 Dorylomimini: *Dorylomimus kohli*

K



10 Dorylogastrini:
Dorylogaster sp

L



12 Aenictoteratini: *Giraffaenictus* sp

Figure S1 (continued on next page)

M



13 Lomechusini: *Weisfloggia pubescens*

N



13 Lomechusini: *Aenictosymbia cornuta*

O



14 Falagriini: *Pheigetoxenus* sp

P



15 Aleocharini: *Myrmecosticta exceptionalis*

Figure S1. Habitus images of myrmecoid Aleocharinae (related to Figure 2). Numbers correspond to myrmecoid clades in Figure 2.



Figure S2 (continued on next page)

B

Athetini-
Pygostenini-
Lomechusini
clade

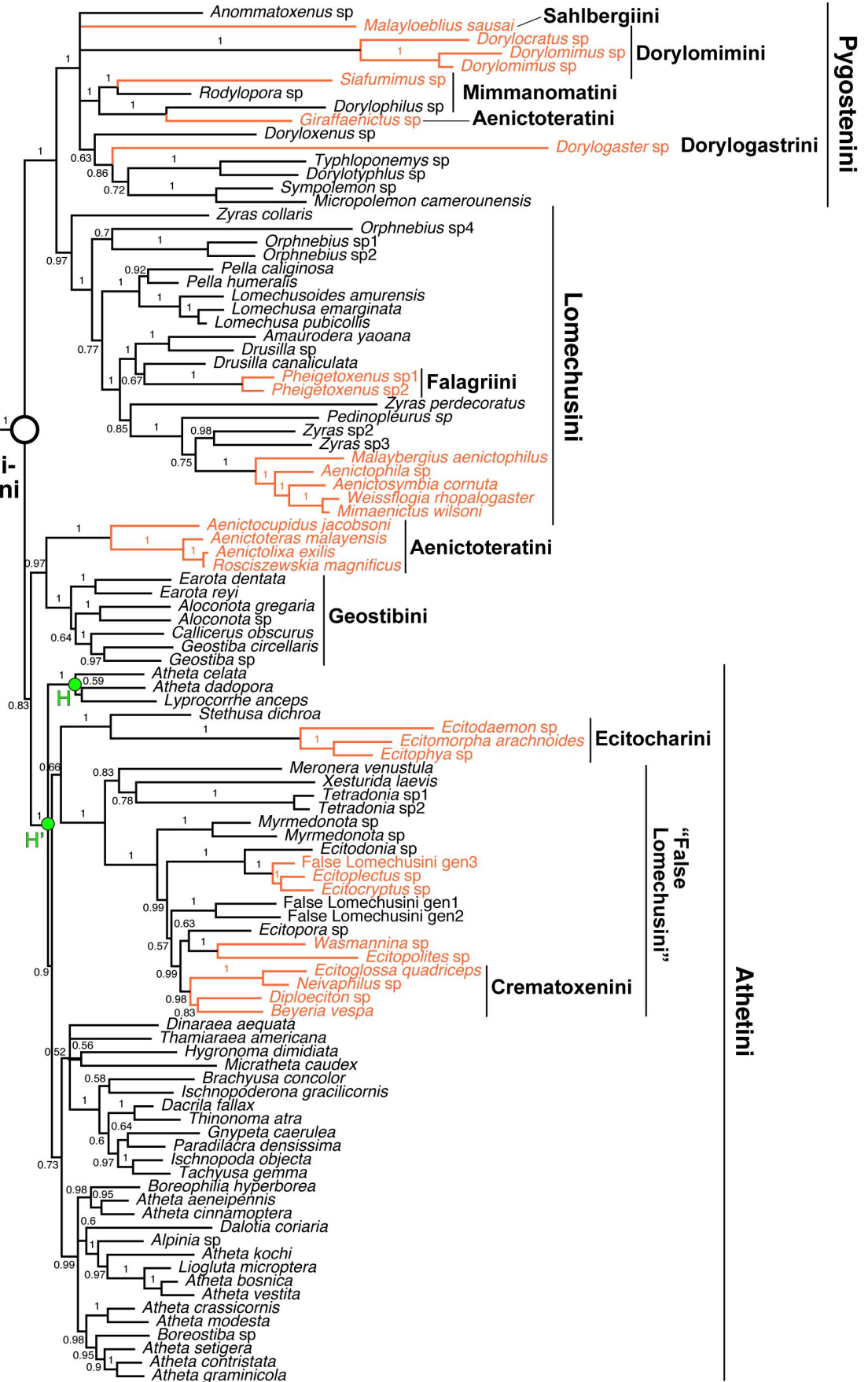


Figure S2 (continued on next page)

C

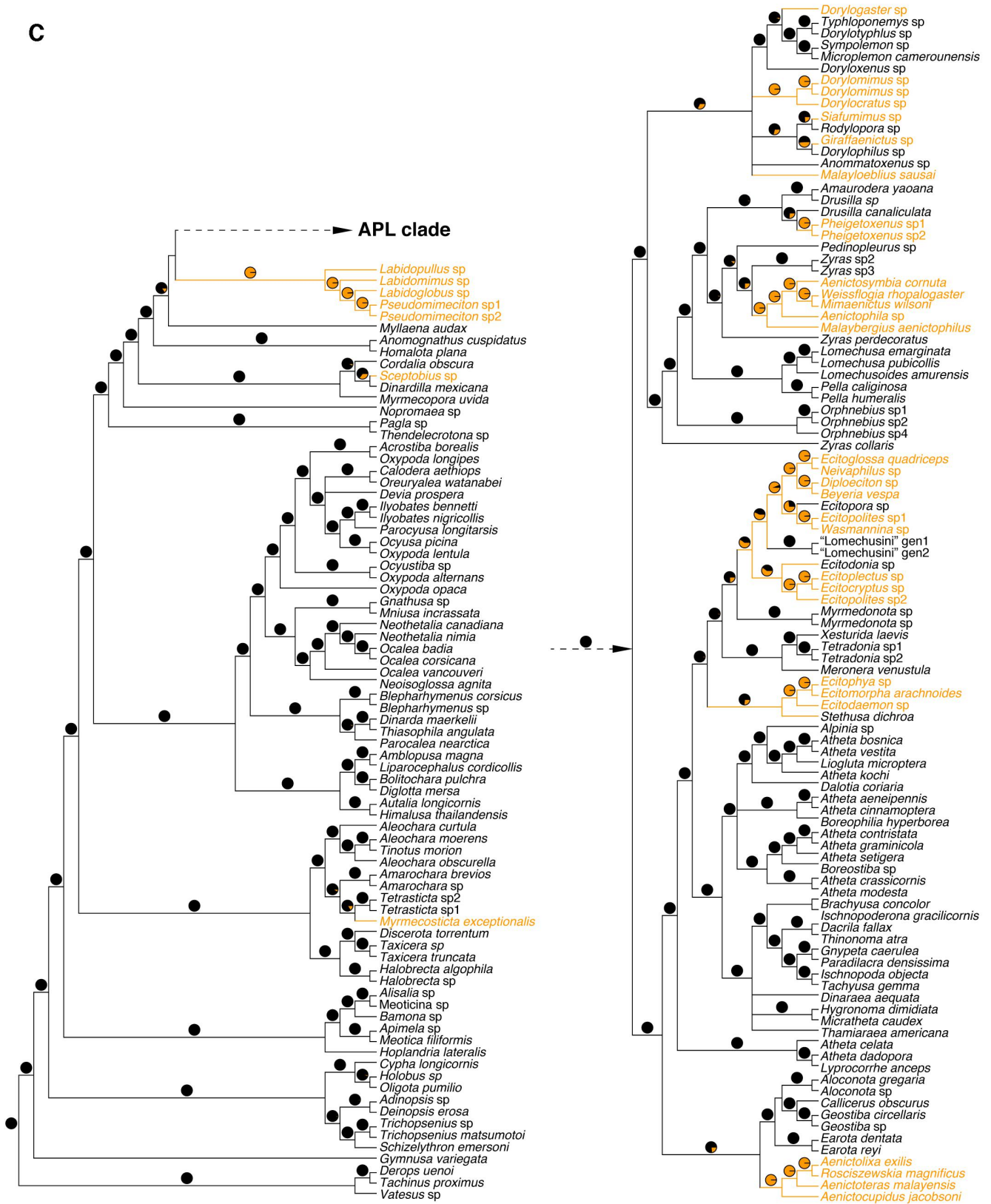


Figure S2 (continued on next page)

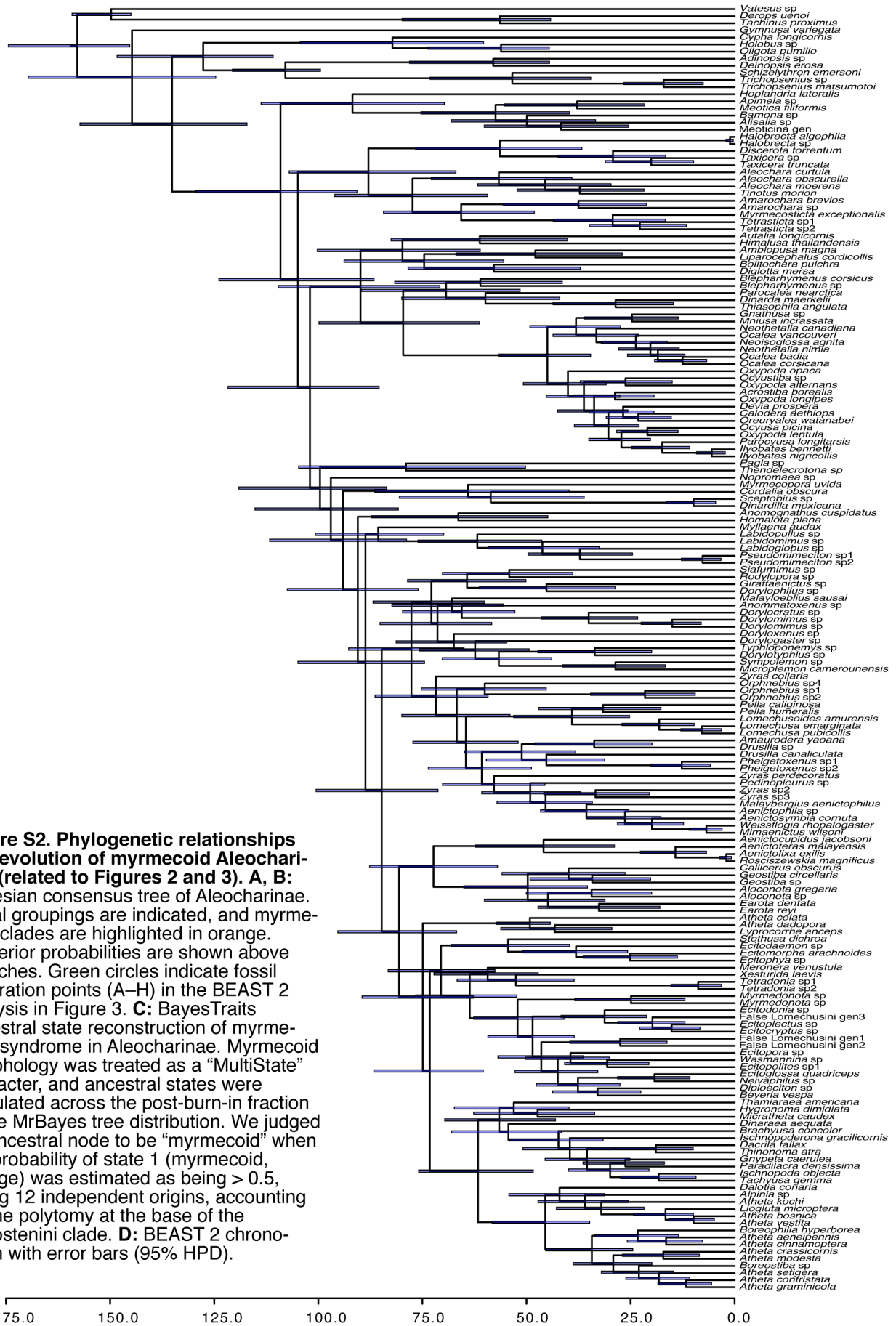
D

Figure S2. Phylogenetic relationships and evolution of myrmecoid Aleocharinae (related to Figures 2 and 3). A, B: Bayesian consensus tree of Aleocharinae. Tribal groupings are indicated, and myrmecoid clades are highlighted in orange. Posterior probabilities are shown above branches. Green circles indicate fossil calibration points (A–H) in the BEAST 2 analysis in Figure 3. **C:** BayesTraits ancestral state reconstruction of myrmecoid syndrome in Aleocharinae. Myrmecoid morphology was treated as a “MultiState” character, and ancestral states were calculated across the post-burn-in fraction of the MrBayes tree distribution. We judged an ancestral node to be “myrmecoid” when the probability of state 1 (myrmecoid, orange) was estimated as being > 0.5, giving 12 independent origins, accounting for the polytomy at the base of the Pygostenini clade. **D:** BEAST 2 chronogram with error bars (95% HPD).

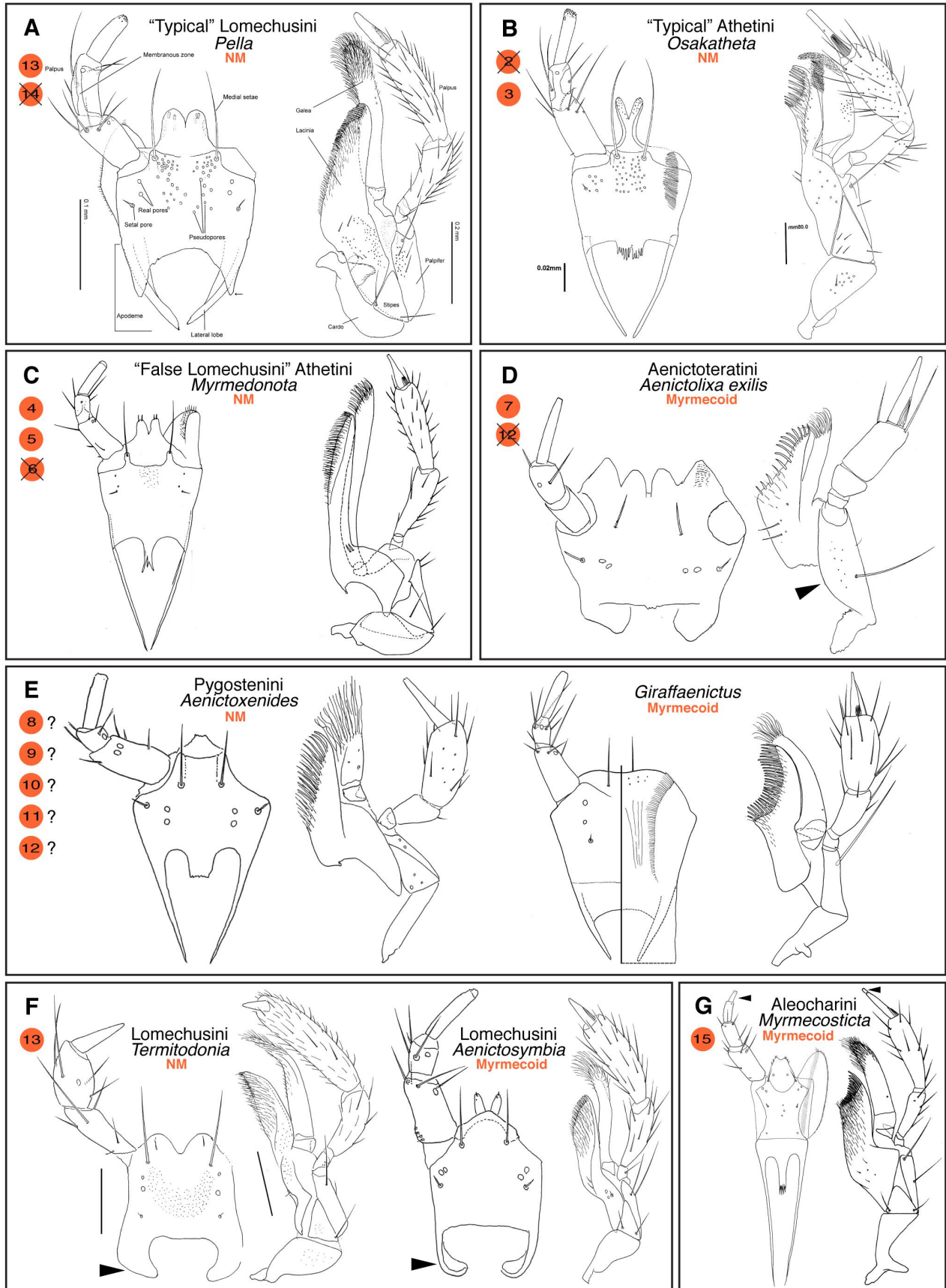


Figure S3. Mouthpart morphology (related to Figure 2). Labia and maxillae from relevant non-myrmecoid (NM) Aleocharinae are shown, together with structures from selected myrmecoid taxa. Below panel letters, myrmecoid clades related to and exhibiting putative synapomorphies with the figured NM genus are listed; if the clade number is crossed through, mouthpart morphology differs between the NM and myrmecoid clade; question marks indicate ambiguity over mouthpart homologies at this time. Typical labia and maxillae of Lomechusini and Athetini are shown in **A** and **B**, respectively, with key morphological structures labeled in **A**. Myrmecoid clade numbers correspond to Fig 2.

Supplemental Discussion

Systematics and Behavior of Myrmecoid Aleocharinae

What follows is a discussion of the relationships between the fifteen myrmecoid lineages in the phylogeny in Figure 2 and their non-myrmecoid relatives. Morphological features supporting or conflicting with these relationships are mentioned, and important mouthpart characters are illustrated in Figure S3. A summary of observed behavioral interactions with ants is also included for each lineage or group of lineages. Historically, most of these fifteen lineages were grouped together by Seevers, into the large, monophyletic tribe Dorylomimini [S1], which contained the vast majority of anatomically modified, myrmecoid aleocharines. Seevers recognized eight principal divisions within the Dorylomimini based on morphological characters such as the form of the abdominal petiole. The Dorylomimini was later dismantled by Kistner and Jacobson: in a series of revisions, they split the tribe into 8 smaller tribes, largely along the divisions recognized by Seevers [S2-5]. However, the monophyly of some these tribes, and their relationships with each other and with the remaining Aleocharinae were unclear. Several of these tribes correspond to distinct myrmecoid lineages in the phylogeny in Figure 2, and these are noted below.

Clade 1: Sceptobiini (Fig S1A)

Relationships: Sceptobiini includes two genera, *Sceptobius* and *Dinardilla*. All the species are associated with dolichoderine ants of the genus *Liometopum* in the southern Nearctic region [S6]. Seevers [S7] speculated that this tribe is closely related to the tribe Falagriini based on the shared presence of a divided velum of the paramere, and in our analysis both genera form part of the Falagriini clade. Further morphological support for this grouping can be found in Danoff-Burg [S6] and Ahn and Ashe [S8].

Behavior: The body shapes of *Sceptobius* and *Dinardiella* are contrasting: *Sceptobius* species are myrmecoid, but *Dinardiella* species have a more “limuloid” (teardrop-shaped) defensive morphology. The beetles are found in foraging columns of host ants. They mount and groom the ants, and the ants also groom the beetles [S9]. Consistent with its myrmecoid morphology, *Sceptobius* appears to be more socially integrated into colonies, and unlike *Dinardiella*, is not treated aggressively by workers.

Clade 2: Mimecitini (Fig S1B)

Relationships: Mimecitini is one of the tribes erected by Kistner and Jacobson that was formerly included in Seevers’ broader concept of Dorylomimini [S5]. The tribe includes 14 genera in four subtribes from the New World tropics. All members are associated with army ants of the genera *Labidus*, *Neivamyrmex* or *Nomamyrmex* of Ecitonini. Of four subtribes, three are associated only with *Labidus* while members of the remaining one, Leptanillophillina, which we were unable to sample, is associated with *Neivamyrmex* or *Nomamyrmex* although this subtribe’s membership of Mimecitini remains to be verified. All members of Mimecitini are extremely morphologically modified and show reductions of various characters, including eyes, wings, elytra and genitalia; the genera *Pseudomimecton* and *Labidoglobus* are eyeless, wingless and elytraless and rank among the most heavily modified army ant myrmecophiles known. Nearly all morphological characters that could be used to help define the relationships of Mimecitini to other aleocharines have been secondarily lost or are difficult to distinguish. A morphology-based phylogenetic position of this tribe has therefore been impossible to establish [S5]. In our analysis, the relationships of Mimecitini are still unclear since no free-living sister group was detected in the present tree; instead, the tribe nested as the sister to the vast Athetini-Pygostenini-Lomechusini (APL) clade. This may represent the tribe’s true position, but it is also possible that with further taxon sampling of aleocharine tribes, a more closely related free-living sister group will be recovered. Mimecitini lack the “athetine bridge” of the male aedeagus, a putative synapomorphy of the APL clade, and their labium and maxilla are not clearly of the general athetine type (Fig S3B; [S5]).

Behavior: Mimecitines are generally observed in emigration columns of the host ants [S5], but we also observed them in raiding columns (Maruyama, personal observation). Thus far, limited interactions between the beetles and ants have been observed in most species, but *Mimonilla ecitonis* has been seen being carried and groomed by a worker ant [S5]. This species also followed trails of its host ant in experimental conditions [S10].

Clade 3: Ecitocharini (Fig S1C)

Relationships: Ecitocharini is a former “dorylomimine” tribe, sensu Seevers [S1], that was erected by Kistner and Jacobson [S2] and is composed of 10 genera from the New World, all of which are associated with army ants of the genus *Eciton*. Prior to the present study, Ecitocharini was the only myrmecoid group with molecular data: Elven et al [S11] resolved them as sister to the genus *Stethusa* (Athetini) which are Nearctic, leaf-litter dwellers. Although this tribe is morphologically not clearly defined, they are similar to each other in possessing a rather long head (with a “neck”), prominent eyes, a more or less myrmecoid body shape, and characteristic sculpturation of the body surface. The mouthparts and genitalia are rather variable in shape but their general structures appear to approximately match those of Athetini (Fig S3B), including the presence of an athetine bridge of the aedeagus [S2].

Behavior: The behavior of *Ecitomorpha* and *Ecitophya* beetles was reviewed by Kistner & Jacobson [S2]. They are observed in both emigration and raiding columns; beetles and ants groom each other and no aggression by ants toward the beetles was observed [S12]. No behavioral records have been published for the other members of the tribe. However, MM observed *Ecitodaemon* sitting on ant cocoons that were being carried by *Eciton vagans* ants during their emigration, and also recorded an *Ecitochara connexa* beetle on an ant larva being carried by *Eciton burchellii* ants (Maruyama, personal observation). Ecitocharine species associated with day-raiding *Eciton* species show mimicry of host body color, which may be performing a role in Batesian mimicry to protect against vertebrate predators [S13].

Clades 4–6: Athetini “False-Lomechusini” clade including Crematoxenini (Fig S1D–F)

Relationships: The clade 'false-Lomechusini' was first recovered by Elven et al [S11] as a group of generalized aleocharines that included several New World genera that were formerly classified into Lomechusini. These genera share an elongate galea and lacinia of the maxilla, which were previously considered important character states for defining Lomechusini (e.g., [S7]) (Compare Figure S3C to S3A) but these New World genera are evidently phylogenetically distant from “true Lomechusini”, including the type genus *Lomechusa* [S11], which are predominantly an Old World tribe. In our study (Fig 2), members of the myrmecoid tribe Crematoxenini (clade 6, including *Diploeciton* and *Ecitoglossa*; this tribe is another one erected by Kistner and co-workers) which are associated with *Neivamyrmex*, as well as several myrmecoid genera associated with *Nomamyrmex* (clades 4 and 5, containing *Ecitocryptus* and *Wasmannina*), emerge from within this clade so are also evidently false lomechusines. These beetles are highly modified and some genera mark an extreme in the myrmecoid body shape (e.g., *Diploeciton* and *Ecitocryptus*). The sister group genus of each myrmecoid clade in the false Lomechusini is a non-myrmecoid, morphologically generalized myrmecophile genus of ecitonine army ants, potentially representing the ancestral condition of the symbiotic association with army ants from which the myrmecoid clades have evolved. Notably, members of Crematoxenini do not share the elongate galea and lacinia of false lomechusines (Fig S3C), but mouthpart morphology, in addition to body shape, is highly diverse in this tribe, and we are unable to satisfactorily define the group morphologically at present.

Behavior: The behavior of Crematoxenini species was reviewed by Jacobson & Kistner [S4]. Some species are known to be highly integrated into ant societies, licking and grooming the ants, which groom the beetles in return. The beetles were also observed eating prey booty that had been raided by the ants [S12,14]. Behavior of myrmecoid “false-Lomechusini” species associated with *Nomamyrmex* has not been reported, probably due to their rarity. MM observed the behavior of *Ecitocryptus*, *Wasmannina*, *Ecitopolites* and *Ecitoplectus* species in the field in Peru (Maruyama, personal observation). They followed raiding columns of *Nomamyrmex* ants, but no grooming or licking between the ants and beetles was seen during the period of observation, although the ants were never aggressive towards the beetles. The beetles ate dead cockroaches that were hunted by the ants in a raiding column.

Clade 7: Aenictoteratini (Fig S1G–I)

Relationships: Aenictoteratini, another tribe created by Kistner that was formerly a branch within Dorylomimini [S3], is composed of six genera from tropical Asia. All species are associated with army ants of the genus *Aenictus*. In Figure 2, Aenictoteratini emerged as the sister group of Geostibini. Members of

Geostibini are leaf-litter dwellers and distinctive due to a long, apically truncate mesoventral process. Although mouthparts and almost all other body structures of Aenictoteratini are strongly modified, the state of the mesoventrite appears to match that of Geostibini (not shown). We found two putative mouthpart autapomorphies of Aenictoteratini (Fig S3D, *Aenictolixa* is shown): the lateral apodeme of the labium is rounded and shortened, although this condition is also approached in some true Lomechusini; the palpifer of the maxilla is extremely large and conceals the stipes underneath it. Geostibini lack these character states, and so too does *Giraffaenictus*, a genus currently placed in Aenictoteratini [S15], but which emerges from the “Pygostenini” clade in our tree (Fig 2) and has seemingly more generalized athetine-type mouthparts (Fig S3E).

Behavior: Maruyama et al [S16] reported the behavior of *Aenictoteras malayensis* and *Rosciszewska magnificus*. The beetles followed *Aenictus* emigration columns and were not carried by worker ants. However, in subsequent observations, MM observed both *Aenictoteras malayensis* and *Rosciszewska magnificus* being carried by workers in an emigration column in Malaysia (Maruyama, personal observation). On steep and slippery surfaces, the ants grasped the beetles between the eyes in area that is excavated to hold the ants’ mandibles, and carried the beetles to the next bivouac. In the laboratory, both *Aenictoteras* and *Rosciszewska* were palpated by host workers. Adult beetles of both genera showed similar cuticular hydrocarbon profiles to their host colonies.

Clades 8–12: “Pygostenini” clade including Dorylomimini, Dorylogastrini, Sahlbergiini, Mimanommatini and *Giraffaenictus* (Fig S1J–L)

Relationships: Five Old World tribes, Dorylomimini (e.g., *Dorylomimus*, *Dorylocratus*), Dorylogastrini (*Dorylogaster*), Sahlbergiini (*Malaybergius*), Mimanommatini (e.g., *Siafumimus*) and Pygostenini (e.g., *Anommatoxenus* and *Sympolemon*) formed a monophyletic group in our tree (Fig 2, clade “P”). All except the latter tribe were erected or revised by Kistner [S3], and were formerly included in the broader concept of Dorylomimini by Seevers [S1]. Although this clade was maximally supported (PP = 1), interrelationships between many of the descendent lineages are unclear and weakly supported, and Mimanommatini and Pygostenini became paraphyletic. All the species belonging to this clade are associated with *Dorylus* army ants in Africa and Asia, except *Giraffaenictus*, which is associated with *Aenictus* ants. The various myrmecophile groups within this clade are morphologically extremely diverse including limuloid (all Pygostenini), myrmecoid (all Dorylomimini, Dorylogastrini and Sahlbergiini, some Mimanommatini and *Giraffaenictus*), and rather generalized species (some Mimanommatini). The myrmecoid genus *Giraffaenictus* was formerly classified into Aenictoteratini [S15] but clearly does not belong in this tribe (see Discussion under Aenictoteratini, above), and instead emerges from the Mimanommatini clade with strong support. The general mouthpart and aedeagal morphology of all members of this heterogeneous assemblage of tribes more or less correspond to those of Athetini (Fig S3E, a “typical” pygostenine genus, *Aenictoxenides*, and *Giraffaenictus* are shown), but due to the large species richness and exceptional morphological diversity of this assemblage of tribes, we have thus far been unable to find clear morphological character states to define the clade as a whole.

Behavior: Behavior of myrmecoid species belonging to Dorylomimini, Dorylogastrini, Mimanommatini was reviewed or described for the first time by Kistner [S3]. *Dorylomimus kohli* (Dorylomimini) beetles are highly integrated, never attacked or captured by *Dorylus* ants in their raiding columns, and were palpated by the ants as if they were workers [S17]. Behavior of *Dorylonannus* sp. (Dorylomimini) is similar to that of *Dorylomimus kohli*. *Jeanneliusa alzadae* and *Dorylocratus* spp. (Dorylomimini) beetles were observed in emigration and/or raiding columns of *Dorylus* ants. They are also integrated into the ant society: the ants licked their physogastric abdomens and thoraces. *Dorylogaster* (Dorylogastrini) beetles were observed mainly in the central parts of raiding and emigration columns. The ants palpated the beetles with their antennae. When ant activity was intense and the density of ants became high, the beetles were found riding on the thoraces of workers (phoresy). *Mimanomma* and *Siafumimus* (Mimanommatini) beetles are also probably both integrated into the ant society: *Mimanomma spectrum* was observed in the central parts of raiding and emigration columns and was frequently palpated by the ants. *Siafumimus alzadae* was collected only once, but it was found at the center of an active raiding column and was not treated aggressively by the ants. *Giraffaenictus* sp. is associated with *Aenictus binghami* ants in the Indochinese Peninsula of tropical Asia. Unlike other Mimanommatini, including the myrmecoid *Mimanomma* and *Siafumimus*,

which are very ant-like but have relatively short legs, *Giraffaenictus* has exceptionally long legs. *Giraffaenictus* is found in emigration columns and is also sometimes palpated by the ants. There are presently no published behavioral records for Sahlbergini species. However, MM observed *Malayloeblius sausai* running among ants in a raiding column. No aggression from the ants was observed, and the ants palpated the abdomen of the beetle with their antennae (Maruyama, personal observation).

Clades 13, 14: Lomechusini (Fig S1M–O)

Relationships: The tribe Lomechusini is composed mostly of myrmecophilous and termitophilous species that predominantly occur in the Old World [S18]. The members of this tribe are well characterized by a combination of an elongate galea and lacinia of the maxilla and a long, apically truncate metaventral process. In our phylogeny (Fig 2), myrmecoid syndrome appears to have arisen twice in Lomechusini, in the Indomalayan clades of *Mimaenictus* and its related genera (Clade 13) and separately, the genus *Pheigetoxenus* (Clade 14). *Mimaenictus* and its related genera have the classical elongate lomechusine galea and lacinia of the maxilla (a representative of this myrmecoid clade, *Aenictosymbia*, is shown in Fig S3F). Further, this clade is nested together with *Zyras* (sensu lato) spp. and *Pedinopleurus*; in support of this grouping, a putative synapomorphy that these genera share is the presence of a pair of sclerites in the internal sac of the aedeagus, which in other lomechusine genera are usually exposed from the apex of the median lobe (structure not illustrated here). We note further a possible synapomorphy in the form of the base of labial apodeme, which is rounded in genera in this myrmecoid clade (arrowheads in Fig S3F), similar to genera allied to *Pedinopleurus* such as *Termitodonia*. In contrast, *Pheigetoxenus* emerged from a *Drusilla* + *Amaurodera* clade. *Pheigetoxenus* was previously classified into the tribe Falagriini [S19], and it does not share the elongate galea and lacinia of the lomechusine maxilla (Fig S3F). This appears to represent a secondary loss of these lomechusine character states. However, excluding these maxilla states, the head and pronotal structures of *Pheigetoxenus*, as well as the morphology of the metasternal process, are similar to some Lomechusine genera such as *Drusilla*. The myrmecophagous (ant-hunting) behavior of *Pheigetoxenus* also matches that of *Drusilla*. We therefore think it plausible that *Pheigetoxenus* evolved from a *Drusilla*-like ancestor.

Behavior: Kistner and Jacobson [S20] and Maruyama et al. [S16] reported the behavior of *Mimaenictus*, *Procantonetia* and *Weissflogia* beetles. They are highly integrated into the ant society and are found in the center of bivouacs, where they are palpated by the ants in the same manner that the ants palpate other workers. In emigration columns, *Mimaenictus* and *Procantonetia* beetles were carried by the ants, which grasp the bases of the antennae to pick the beetles up. No feeding behavior was observed. Kistner [S19] reported *Pheigetoxenus* spp. beetles in raiding columns of *Pheidologeton* (now a synonym of *Carebara*), a non-doryline ant that exhibits army ant-like behavior. MM observed that *Pheigetoxenus* hunt worker ants on the raiding columns (Maruyama, personal observation). The beetles bite at the base of the ant head, killing the ant, which is then dragged 10–20 cm away from the column where it is consumed. Another non-doryline ant genus with army ant-like behavior, *Leptogenys*, also plays host to a myrmecoid lomechusine, *Leptogenopapus* [S21]

Clade 15: Aleocharini (Fig S1P)

Relationships: *Myrmecosticta exceptionalis* is the only myrmecoid species known from Aleocharini, a tribe in which most species are generalized in body shape or limuloid (some termitophiles). *Myrmecosticta* shares with other Aleocharini the pseudosegments on the labial and maxillary palpi (Fig S3G, arrowheads). This species is associated with *Aenictus sonchaengi* and found in Borneo; as discussed by Maruyama et al. [S22], two genera of Aleocharini are also associated with *Aenictus* ants, but are generalized in body shape. We think it probable that *Myrmecosticta* and these genera share a recent common ancestor.

Behavior: No behavioral observations have been made on *Myrmecosticta exceptionalis*.

Supplemental Experimental Procedures

Specimen collection and taxon sampling

Myrmecoid aleocharines are rarely collected. They require targeted sampling of army ant colonies and often live at what appear to be very low abundances in nature [S13]. Numerous species and genera are known from only single or small numbers of specimens. We set out to obtain fresh, DNA-grade material of myrmecoid aleocharines throughout the world's tropics, and over the course of multiple expeditions spanning a decade, collected beetle species associated with doryline army ants of the genera *Eciton*, *Labidus*, *Neivamyrmex* and *Nomamyrmex* in the Neotropics, and *Dorylus* and *Aenictus* in the Afrotropics and Indomalaya. Only the rarely encountered army ant genera *Cheliomyrmex* and *Aenictogiton* were not sampled from. Our targeted search, assisted by several other myrmecophile enthusiasts, totaled hundreds of man-hours spent observing emigrating and swarm-raiding army ant columns. We accumulated a taxon sample that spans the Dorylomimini sensu Seevers [S1] including all of the smaller tribes into which Dorylomimini was split by Kistner and Jacobson in their series of revisions [S2-5]. Many new species and several new genera were collected, and we also sampled myrmecoid species from the group-foraging ants *Liometopum* and *Carebara diversa*.

We employed a definition of “myrmecoid” based on the historical views of the morphology of such taxa by previous authors [S1-5,13,19]. Myrmecoid body shape is very distinctive, but difficult to define quantitatively or qualitatively with a blanket rule that fits all taxa. However, in general, myrmecoid taxa can be defined as those species that i) have an abdominal constriction (petiole) with the first few abdomen segments clearly narrower and more dorsoventrally constricted than posterior segments (so the petiole is usually less than 3/4 maximal abdomen width and depth), and ii) legs that are elongate, with the combined hind femur + tibia length greater than or equal to $1.5 \times$ abdomen length. This criterion appears to be a working approximation that reconciles the views of previous authors with consistent features of myrmecoid beetles. We integrated these sequences with data from non-myrmecoid aleocharines from across the Aleocharinae phylogeny [S11,23]. As our phylogeny took shape, we slightly expanded taxon sampling of non-myrmecoid species by sequencing some early diverging lineages to help with dating analysis, and also to increase taxon sampling density in areas where multiple myrmecoid lineages appeared to have emerged. These additional taxa belong to the tribes Deinopsini, Trichopseniini, Hypocyphtini, Sceptobiini, Athetini (including False Lomechusini), Pygostenini and Mimmanomatini. Our taxon inventory, including Genbank accession numbers, is provided in Data S1.

DNA extraction and sequencing.

Ethanol-preserved specimens were vacuum dried and incubated without damaging them in DNA extraction buffer [S24] for 2 days at 55°C. DNA was phenol-chloroform extracted using the protocol in reference [S25]. DNA was resuspended in Tris-EDTA and clontech Advantage 2 polymerase was used to amplify gene fragments with an annealing temperature of 51°C in almost all PCR reactions. Expanding on previous molecular work on Aleocharine [S11,23], the following loci and primer combinations were used (asterisks indicate primers designed for this study):

18s rRNA: **18Sai** 5'-CCTGAGAAACGGCTACCACATC / **18Sbi** 5'-
GAGTCTCGTTCGTTATCGGA

Or in two sections: **18Sai** 5'-CCTGAGAAACGGCTACCACATC / **18sMID_R*** 5'-
GTGTTGAGTCAAATTRAGCCGC + **18sMID_F*** 5'-
GGGCAAGTCTGGTGCCAGC / **18Sbi** 5'-GAGTCTCGTTCGTTATCGGA

28s rRNA: **28sC1-FWD** 5'-ACCCGCTGAATTTAAGCAT / **28S-1118r** 5'-
GTATAGTTCACCATCTTTCGGG

Or in two sections: **28sC1-FWD** 5'-ACCCGCTGAATTTAAGCAT / **28sR-01** 5'-
GACTCCTGGTCCGTGTTTCAAG + **28s-751f** 5'-
GTAGGACGTCGCGACCCGTTGGGTGTCGGTCT / **28S-1118r** 5'-
GTATAGTTCACCATCTTTCGGG

Topoisomerase I: Nested two step PCR:

Reaction 1: 30 cycles, 55°C (TP643F 5'-GACGTTGGAARTCNAARGARATG / TP932R 5'-GGWCCDGCATCDATDGCCCA).

Reaction 2: 1 µl from reaction 1, 30 cycles 55°C (TP675F 5'-GAGGACCAAGCNGAYACNGTDGGTTGTTG / TP932R 5'-GGWCCDGCATCDATDGCCCA)

16s rRNA: 16saR 5'-CGCCTGTTTATCAAAAACAT / 16sb 5'-CTCCGGTTTGAACTCAGATCA or 16sb_3 5'-TTAATCCAACATCGAGGTCG

COI: TL2-N-3014PAT 5'-TCCAATGCACTAATCTGCCATATTA / C1-J-2183JERRY 5'-CAACATTTATTTTGATTTTTTGG or Jerry2nd 5'-GATTTTTTGGWCAYCCWGAAG)

Bands were cut from gels, purified, and ligated into pCR4-TOPO (Life Technologies), and transformed into DH5a cells. Colonies were minipreped and test digested and plasmids containing the correct inserts were sequenced with T7 and M13R primers using Macrogen Corp. (NY, USA).

Phylogenetic analysis

Sequences were aligned in MAFFT v. 7 [S26], and concatenated in SequenceMatrix [S27]. PartitionFinder [S28] was used to simultaneously identify the optimal partitioning scheme and select a substitution model for each partition. Nine partitions were identified under the Bayesian information criterion using the “greedy” algorithm in PartitionFinder: 16s rRNA, 18s rRNA, 28s rRNA and three partitions each for COI and TOPO corresponding to first, second and third codon positions. Partitions and models were as follows: 16s rRNA (GTR+I+G), 18s rRNA (SYM+I+G), 28s rRNA (SYM+I+G), COI 1st positions (HKY+I+G), COI 2nd positions (GTR+I+G), COI 3rd positions (GTR+I+G), TOPO 1st positions (SYM+I+G), TOPO 2nd positions (GTR+I+G), TOPO 3rd positions (GTR+I+G). We performed Bayesian inference on the 9-partition data set using MrBayes 3.2 [S29], available online through the Cipres Science Gateway [S30]. Search consisted of two runs of 8 chains, with a temperature set at 0.03, which yielded chain swap statistics between 0.4–0.5. We sampled every 5000 generations, and runs were judged to have converged at 100 million generations, when the standard deviation of split frequencies of the two runs was 0.003, and all ESS values were above 200 in Tracer [S31]. The first 25% of samples were discarded as burn-in. This analysis was repeated in triplicate and in each case gave largely indistinguishable consensus topologies, branch lengths and posterior probabilities. We also repeated the analysis with ribosomal RNA sequences aligned using SINA 1.2.11 [S32] and found this to also have a negligible effect on the outcome. The nexus file for our focal MrBayes analysis that generated the tree in Figure 2 is available online as Data S2.

Molecular dating

To date the diversification of myrmecoid aleocharines, we employed a Bayesian uncorrelated lognormal relaxed clock model [S33] using Beast 2.3.2 [S34]. To create a starting tree, a rooted and fully resolved maximum clade credibility tree from the MrBayes analysis was made by combining log files in TreeAnnotator [S29]. The tree was made ultrametric and scaled to conform to dating priors in TreeEdit [S35]. This starting topology was fixed during the BEAST analysis. We used the same 9 partitions that were used in the MrBayes analysis with separate nuclear and mitochondrial clocks [S36], and used the bModelTest plug-in in BEAST 2 [S37] to infer site models during the analysis. The models selected by bModelTest in our focal analysis (Fig 3) are presented below as the 95% HPD of models. This is smallest set of models that cover 95% of the posterior: the first column represents the posterior covered by a model,

the second the cumulative probability (the posterior covered by a given model and models above it), and third column is the model itself:

BEAST RUN 1

substmodel.16s
used cumulative model
73.35% 73.35% 123456
26.38% 99.73% 123451

substmodel.18s
used cumulative model
89.34% 89.34% 123451
10.64% 99.99% 123456

substmodel.28s
used cumulative model
90.28% 90.28% 123456
9.37% 99.65% 123145

substmodel.CO1_1
used cumulative model
36.88% 36.88% 121121
12.54% 49.43% 121321
11.41% 60.83% 121131
7.94% 68.78% 121123
7.42% 76.20% 121323
3.71% 79.91% 121341
2.61% 82.52% 121324
2.26% 84.78% 121134
2.04% 86.82% 121343
1.98% 88.80% 123321
1.69% 90.49% 123121
1.61% 92.10% 123123
1.52% 93.62% 123323
0.73% 94.35% 121345
0.72% 95.07% 123341

substmodel.CO1_2
used cumulative model
57.44% 57.44% 123451
42.53% 99.97% 123456

substmodel.CO1_3
used cumulative model
41.22% 41.22% 123324
13.26% 54.48% 123345
11.83% 66.31% 121123
11.42% 77.74% 121324
10.59% 88.33% 123425
3.08% 91.40% 121134
3.03% 94.43% 123456
2.91% 97.34% 121345

substmodel.TOPO_1

used cumulative model
43.55% 43.55% 123453
33.35% 76.90% 123345
16.87% 93.77% 123456
5.47% 99.24% 123343

substmodel.TOPO_3
used cumulative model
33.12% 33.12% 121321
29.49% 62.60% 123321
17.59% 80.19% 123421
3.73% 83.93% 123423
3.36% 87.28% 123341
3.26% 90.54% 121341
2.52% 93.07% 123324
2.31% 95.38% 121324

substmodel.TOPO_2
used cumulative model
62.37% 62.37% 123456
24.56% 86.93% 121345
11.98% 98.91% 123453

BEAST RUN 2

substmodel.16s
used cumulative model
73.78% 73.78% 123456
25.88% 99.66% 123451

substmodel.18s
used cumulative model
89.41% 89.41% 123451
10.57% 99.98% 123456

substmodel.28s
used cumulative model
90.30% 90.30% 123456
9.40% 99.70% 123145

substmodel.CO1_1
used cumulative model
36.76% 36.76% 121121
13.34% 50.11% 121321
11.43% 61.54% 121131
7.55% 69.08% 121123
7.11% 76.19% 121323
3.98% 80.17% 121341
2.65% 82.82% 121324
2.10% 84.92% 121343
2.08% 87.00% 121134
1.80% 88.80% 123121
1.77% 90.57% 123321
1.70% 92.27% 123323
1.65% 93.91% 123123
0.77% 94.69% 121345

0.62% 95.31% 123423

substmodel.CO1_2

used cumulative model

57.32% 57.32% 123451

42.66% 99.98% 123456

substmodel.CO1_3

used cumulative model

41.58% 41.58% 123324

13.14% 54.72% 123345

12.00% 66.72% 121123

11.28% 78.00% 121324

10.12% 88.12% 123425

3.23% 91.35% 123456

3.07% 94.43% 121134

2.85% 97.28% 121345

substmodel.TOPO_1

used cumulative model

43.47% 43.47% 123453

33.07% 76.55% 123345

17.36% 93.90% 123456

5.23% 99.13% 123343

substmodel.TOPO_3

used cumulative model

32.76% 32.76% 121321

30.27% 63.03% 123321

17.39% 80.41% 123421

3.67% 84.08% 123423

3.37% 87.45% 123341

3.12% 90.58% 121341

2.50% 93.08% 123324

2.41% 95.49% 121324

substmodel.TOPO_2

used cumulative model

62.51% 62.51% 123456

24.32% 86.82% 121345

12.04% 98.86% 123453

In our focal analysis (Analysis #1), two BEAST runs of 200 million generations each were combined, giving convergence based on high ESS values (>200) following removal of a 10% burn in fraction. We used fossils to calibrate eight nodes, A–H, which are indicated on the phylogeny in Figure S2A, B. The dating priors used to calibrate these nodes are listed below. In parentheses are given the prior distribution class (exponential or lognormal), followed by the hard minimum age (offset), the mean (in real space) and standard deviation (if lognormal):

A) Tachyporinae-Aleocharinae split (exponential, 145, 20). Presence of Tachyporinae in the Late Jurassic (Kimmeridgian) Tralgar Fish bed [S38], indicates a split from Aleocharinae in the Late Jurassic at the latest. This is our deepest calibration point, and also the deepest node in our tree.

B) *Tachinus* (lognormal, 44, 10, 1.0). *Tachinus* in Baltic amber [S39]. *Tachinus* specimens are common in Baltic amber, although none have been formally described [S40].

C) *Oligota* (lognormal, 44, 10, 1.0). *Baltiologota* in Baltic amber [S41]. This genus appears to be very close to *Oligota* so in our focal analysis was placed at the node joining *Oligota* and *Holobus*. In analysis #2 we placed *Baltiologota* at a more conservative position, one node deeper in the tree, at the common ancestor of the Hypocyphtini clade (position C' in Fig S2A).

D) *Adinopsis* (lognormal, 44, 10, 1.0). *Adinopsis* in Baltic amber [S42].

E) *Deinopsini* (lognormal, 99, 20, 1.0). *Cretodeinopsis* in Burmese amber [S43].

F) *Aleochara* including *Tinotus* (lognormal, 44, 10, 1.0). *Aleochara* in Baltic amber [S44].

G) *Homalotini* (lognormal, 44, 10, 1.0). *Leptusa* in Rovno amber [S45] and *Phymatura* in Baltic amber [S44].

H) *Atheta celata* (lognormal, 44, 10, 1.0). *Atheta jantarica* in Baltic amber is thought to be a member of the subgenus *Datomicra*, close to *Atheta celata* [S41]. *Atheta* species are notoriously difficult to identify, so in analysis #2 we placed *Atheta jantarica* one node deeper in the tree, at the common ancestor of the Athetini clade (including Crematoxenini, Ecitocharini) (position H' in Fig S2B).

In addition to Analysis #1, we performed Analysis #2 where fossils C and H were placed at more conservative positions on the tree (see Figure S2A, B). The same overall pattern and timescale of diversification of Aleocharinae was observed to that produced by Analysis #1, with myrmecoid clades arising in parallel in the Cenozoic with similar date estimates, and all such lineages sharing a common ancestor deep in the Cretaceous. Because overly-strong dating priors can override signal from molecular data, a precautionary analysis was also run without any molecular data. [S46,47]. Sampling from the prior alone led to obvious dating discrepancies with our focal analysis, confirming that our dating priors were not constraining the outcome.

Ancestral State Reconstruction

For ancestral state reconstruction of myrmecoid syndrome across the Aleocharinae phylogeny, we scored taxa as 0 (non-myrmecoid) or 1 (myrmecoid) based on the criterion in “Specimen collecting and taxon sampling” above. For Dollo-type parsimony optimization, we modelled “myrmecoid” as an “irreversible” character in Macclade 4.08a [S48], optimizing it onto the fully resolved maximum clade credibility tree produced by the MrBayes analysis. For Bayesian reconstruction of ancestral states, BAYESTRAITS V.2 [S49] was used. A MultiState analysis was conducted using a distribution of the 10,000 trees from the MrBayes analysis that was pruned to every 10th tree of the post-burn-in 75% of trees, giving 750 trees. TreeGraph 2 [S50] was used to create an AddMRCA command file to estimate states at all nodes in the phylogeny. The BAYESTRAITS analysis was run for 1010000 generations, sampling every 1000 generations, with the first 10000 generations discarded as burn-in. Ancestral state probabilities were mapped onto the MrBayes consensus tree in TreeGraph 2 (Fig S3).

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