Report

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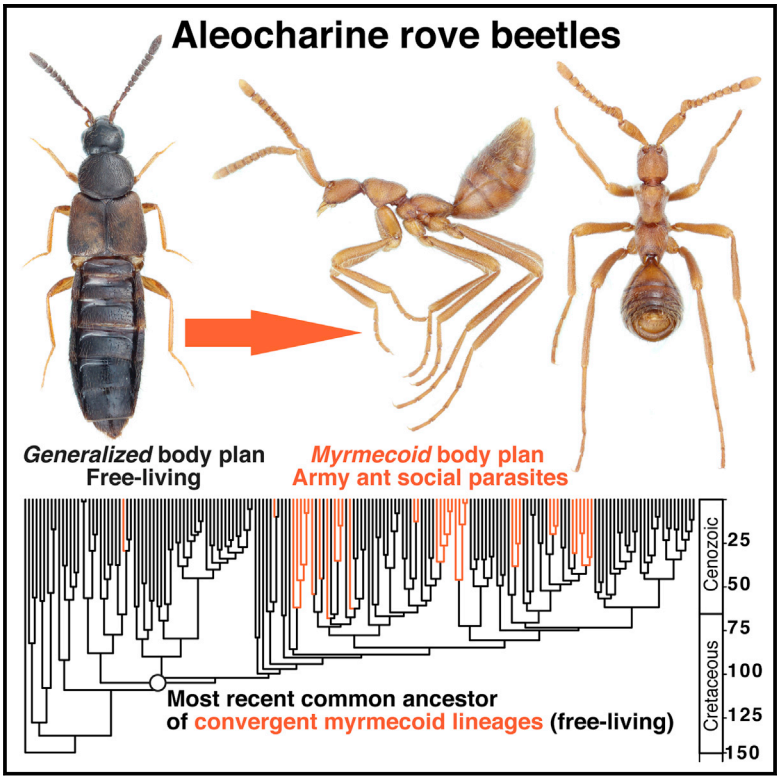
Deep-Time Convergence in Rove Beetle Symbionts of Army Ants

Graphical Abstract



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In Brief

Maruyama and Parker reveal that ant-mimicking rove beetles living symbiotically with army ants evolved at least 12 times. The convergent beetle lineages share a free-living common ancestor in the Early Cretaceous. Discovery of an ancient system of complex convergence challenges the notion of evolutionary contingency over deep timescales.

Highlights

- Army ant-parasitic rove beetles comprise an ancient system of complex convergence
- Beetles anatomically mimic and deceive host ants to exploit nest resources
- Convergent clades arose in the Cenozoic and share a Cretaceous common ancestor
- Convergence over deep time challenges the notion of evolutionary contingency

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Deep-Time Convergence in Rove Beetle Symbionts of Army Ants

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SUMMARY

Recent adaptive radiations provide striking examples of convergence [1–4], but the predictability of evolution over much deeper timescales is controversial, with a scarcity of ancient clades exhibiting repetitive patterns of phenotypic evolution [5, 6]. Army ants are ecologically dominant arthropod predators of the world's tropics, with large nomadic colonies housing diverse communities of socially parasitic myrmecophiles [7]. Remarkable among these are many species of rove beetle (Staphylinidae) that exhibit ant-mimicking "myrmecoid" body forms and are behaviorally accepted into their aggressive hosts' societies: emigrating with colonies and inhabiting temporary nest bivouacs, grooming and feeding with workers, but also consuming the brood [8–11]. Here, we demonstrate that myrmecoid rove beetles are strongly polyphyletic, with this adaptive morphological and behavioral syndrome having evolved at least 12 times during the evolution of a single staphylinid subfamily, Aleocharinae. Each independent myrmecoid clade is restricted to one zoogeographic region and highly host specific on a single army ant genus. Dating estimates reveal that myrmecoid clades are separated by substantial phylogenetic distances—as much as 105 million years. All such groups arose in parallel during the Cenozoic, when army ants diversified into modern genera [12] and rose to ecological dominance [13, 14]. This work uncovers a rare example of an ancient system of complex morphological and behavioral convergence, with replicate beetle lineages following a predictable phenotypic trajectory during their parasitic adaptation to host colonies.

RESULTS AND DISCUSSION

The degree to which biological evolution is idiosyncratic or predictable is a fundamental question in evolutionary biology. Convergence—the acquisition of similar traits in different taxa evolving under comparable selective regimes—provides a compelling argument for predictability in evolutionary change [15]. The most striking convergent systems are recent adaptive radiations, in which independent lineages have followed seemingly parallel evolutionary trajectories. Darwin's finches [1], Hawaiian *Tetragnatha* spiders [2], African lake cichlids [3], and three-spined sticklebacks [4] represent natural experiments, where exposure to similar selection pressures has led to analogous phenotypes in separate lineages. Although predictable evolution is manifestly demonstrated by these systems, the likelihood of convergence may nevertheless be enhanced by the young ages of these clades: the close genetic relatedness of lineages is expected to bias the production of genetic variation, enhancing the probability that similar traits will evolve repeatedly [16, 17]. Molecular studies of such recently descended convergent taxa support this notion, often revealing selection acting on the same loci or signaling pathways [18, 19]. With increasing phylogenetic divergence between taxa, however, the likelihood of such marked convergence has been shown to decrease markedly [6]. Ancient clades displaying equivalently conspicuous repeated evolution are rare, lending apparent credence to Gould's view that evolution is inherently contingent [5] and that adaptive responses to a given selection pressure are likely to be different in distantly related taxa.

Here, we report a novel example of predictable evolution of a highly complex phenotype that has occurred over a deep timescale. We explored the evolutionary origins of specialized rove beetles (Staphylinidae) that live symbiotically with army ants, uncovering an ancient system of marked convergence. Army ants are dominant eusocial predators of the tropics: their colonies are nomadic, with hundreds of thousands of workers that emigrate between temporary nest sites and engage in group foraging (raiding) to harvest invertebrate prey [20]. Although notoriously aggressive, army ant colonies represent major concentrations of resources, attracting numerous myrmecophiles that form obligate symbioses with their hosts [7]. Diverse taxa including mites, silverfish, flies, wasps, and beetles exploit this resource, employing either defensive morphologies, or behavioral and chemical strategies to evade worker hostility. A dramatic manifestation of this lifestyle occurs in numerous genera of the staphylinid subfamily Aleocharinae, where the beetles anatomically mimic their host ants and are recognized and accepted by them [8, 10, 11]. Such species live as behaviorally integrated social parasites—appearing at least partially assimilated into

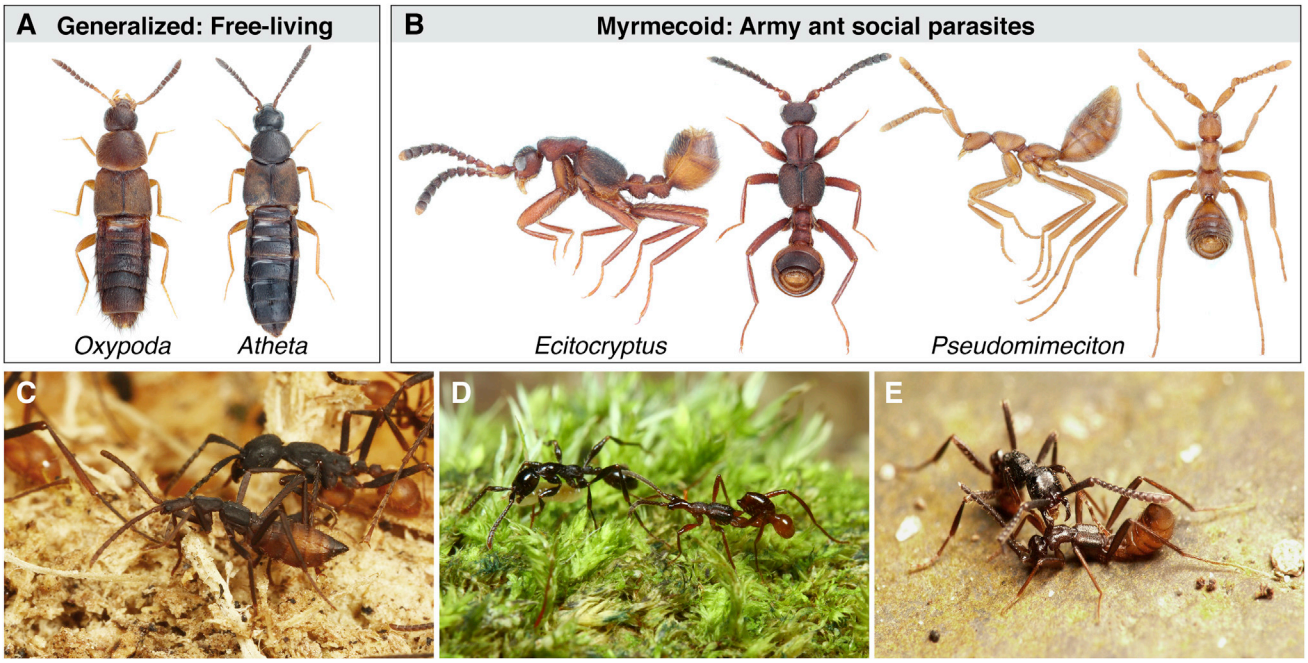


Figure 1. Myrmecoid Syndrome in Aleocharine Rove Beetles

(A) Examples of free-living Aleocharinae with generalized morphology, *Oxypoda* and *Atheta*.

(B) Examples of army ant social parasites with myrmecoid morphology, *Ecitocryptus* (associated with *Nomamyrmex*) and the eyeless, elytra-less *Pseudomimeciton* (associated with *Labidus*).

(C–E) Living myrmecoids with host ants: *Ecitophya* with *Eciton* host (Peru), *Rosciszewskia* with *Aenictus* host (Malaysia), *Beyeria* with *Neivamyrmex* host (Ecuador).

colony life but simultaneously feeding on the ants' brood and raided food. In contrast to the majority of the ~16,000 species of Aleocharinae, which are mainly free-living species with "generalized" staphylinid morphology and extremely similar in body form (Figure 1A), ant-like "myrmecoid" aleocharines are heavily modified (Figures 1B–1E), with a petiolate abdomen (a narrowed waist and expanded gaster), elongate appendages, geniculate (elbowed) antennae, and further similarities to host ant body size, thorax shape, and cuticle sculpturation. The myrmecoid ecomorph is thought to mediate tactile mimicry of nestmate recognition cues [10, 11, 21–23] and is accompanied by a suite of behaviors, including grooming and licking of workers [9], cohabitation of temporary nesting bivouacs, and synchronicity with the colony where the beetles emigrate with hosts and join them on raids, sometimes being carried by or phoretically attaching to workers [10, 24]. Where known, the beetle's cuticular hydrocarbons match those of the host [24], and novel glands on the beetles' cuticles are thought to facilitate chemical integration into the ant society [11].

The myrmecoid morphological and behavioral syndrome presents an evolutionary puzzle: because these beetles are so anatomically modified, their phylogenetic relationships to other aleocharines are obscure. Prominent aleocharine taxonomists have proposed conflicting evolutionary scenarios: Seevers [8] argued for a single principal origin of these beetles within Aleocharinae, forming the large tribe Dorylomimini, and posited an ancient association with army ants followed by codiversification with hosts throughout the tropics. In contrast, Kistner and Jacobson argued for multiple origins [22, 23, 25, 26], splitting Dorylomimini into numerous small tribes and invoking potentially widespread—and extraordinary—morphological and behavioral convergence. Neither scenario has been tested phylogenetically, and to date, the relationships of these beetles have been uncertain. A molecular approach is essential but has been enormously problematic due to difficulties in obtaining specimens. The beetles rank among the rarest and most challenging of insects to find in nature, with many known only from a small number of museum specimens. In this study, we present the outcome of efforts to collect these beetles and explore their evolutionary relationships. Over the course of a decade, we observed army ant colonies across the world's tropics, accumulating myrmecoid aleocharines. In reconstructing their evolutionary history, we uncovered evidence of conspicuous, repeated evolution over deep time that runs counter to the notion of evolutionary contingency and represents a new paradigm for understanding the origins of interspecies relationships.

Parallel Evolution of Myrmecoid Syndrome in Aleocharinae

Army ants include the New World genera *Eciton*, *Labidus*, *Neivamyrmex*, *Nomamyrmex*, and *Cheliomyrmex* and Old World *Aenictus*, *Aenictogiton*, and *Dorylus*. These "true" army ants exhibit classical nomadic biology [20] and are split into separate Old and New World clades within the subfamily Dorylinae [12]. We collected aleocharines associated with all genera except the poorly known *Cheliomyrmex* and *Aenictogiton*. Additionally, we collected beetles known to associate with two distantly related ants, *Carebara* (Myrmicinae) and *Liometopum* (Dolichoderinae),

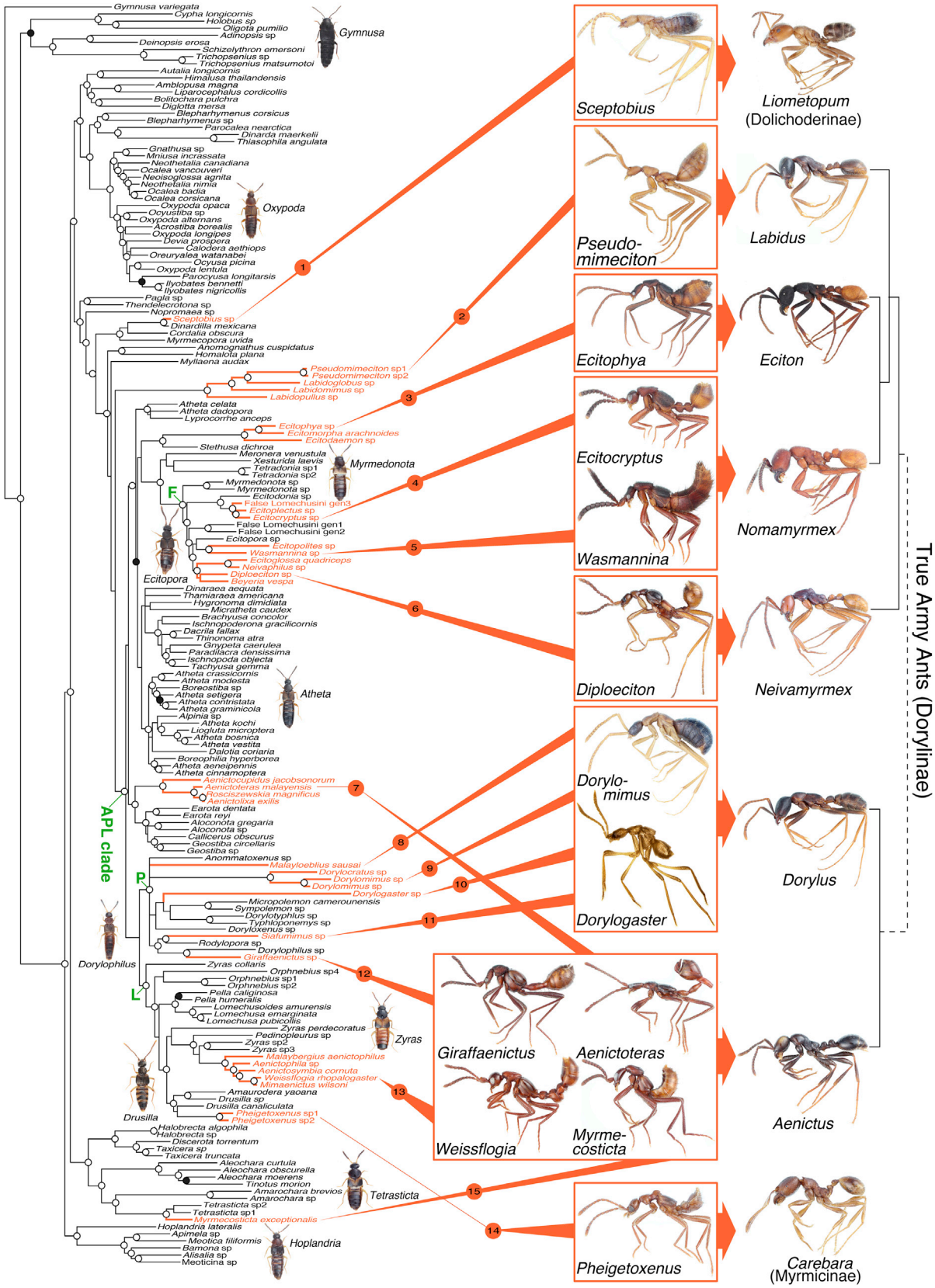
which display group-foraging behavior. Both generalized and myrmecoid aleocharines were collected, and to avoid subjectivity on our part, we defined myrmecoid species as those with petiolate abdomens and long legs that previous authors judged to be myrmecoid [8, 22, 23, 25–27] (see "Specimen collection and taxon sampling" in Supplemental Experimental Procedures). Most species collected were new to science, so DNA was extracted non-destructively [28] to permit taxonomic description ([29–31] and ongoing efforts). We sequenced five loci previously used in aleocharine phylogenetics: nuclear *28S rRNA*, *18S rRNA*, and *Topoisomerase 1*; mitochondrial *Cytochrome c oxidase subunit I* and *16S rRNA* [32, 33]. Army ant myrmecophile data were integrated with sequences from free-living, morphologically generalized taxa representing a broad taxonomic spectrum of Aleocharinae including all major tribes, and outgroups from the related subfamily Tachyporinae (see Data S1). We performed Bayesian phylogenetic inference on the resulting 181-taxon matrix (see Data S2). The topology produced by this analysis, along with exemplar beetle and host ant images, is shown in Figure 2.

The pattern of convergence is dramatic and clear to the eye. Generalized aleocharines form an ancestral backbone to the tree, from which numerous elaborate myrmecoid lineages have emerged in parallel (Figures 2, S2A, and S2B; beetles enlarged in Figures S1A–S1O). Each independent origin of the myrmecoid ecomorph is represented by a small clade or single taxon that is host specific on a single ant genus. All host ant genera have been targeted: each of the "true," doryline army ants have their own, dedicated symbiont clade(s) and so too do the group-foraging *Liometopum* and *Carebara*. We estimated the number of origins using parsimony optimization and Bayesian ancestral state reconstruction. For parsimony, we assumed Dollo-type irreversibility of myrmecoid syndrome [34], which may be a valid assumption in this system: the "tippy" distribution of myrmecoid lineages across the tree is consistent with it being a terminal phenotype, and an improbably large number of regains of primitive characters would be required to lose myrmecoid morphology and restore generalized morphology (together with reversion to ancestral behavior). Such a model of evolution produces the 15 origins depicted in Figure 2. However, for a more conservative estimate taking branch lengths and support values into account, and including the possibility of trait reversal, ancestral states were calculated over a Bayesian tree distribution, giving an estimate of 12 origins (Figure S2C). We think 12–15 origins is an underestimate: there remain numerous myrmecoid genera associated with both Old and New World army ants that we were unable to collect, some of which—given the polyphyletic evolution of this syndrome—likely represent additional origins. A detailed anatomical study of myrmecoid taxa and their inferred, non-myrmecoid relatives revealed characters supporting some of our molecular groupings (Figure S3 and "Systematics and Behavior of Myrmecoid Aleocharinae" in Supplemental Discussion, which also summarizes known behavior of each clade).

Importantly, we see no evidence in any of the myrmecoid clades of a lineage promiscuously switching to a different host genus, indicating that all these relationships are highly host specific. The converse of this relationship does not hold, however, with some ant genera—*Aenictus* and *Dorylus* in particular—playing host to multiple beetle clades. The stringency with which each beetle clade associates with its ant genus likely extends to species level, since individual beetle species have generally been recorded living with single ant species [8, 10]. From this evolutionary pattern we determine the following: (1) separate aleocharine lineages evolved to socially parasitize each army ant genus; (2) during subsequent adaptation of these lineages to ants, they specialized and became host specific; (3) most dramatically, their morphology and aspects of behavior followed a predictable evolutionary trajectory, leading to an overtly stereotyped symbiosis. Cumulatively the outcome is an extraordinary system of parallel evolution in the classical sense, where multiple ancestral taxa sharing a relatively conserved body plan have each evolved in the same direction [35]. This degree of conspicuous, repeated parallelism is rare in the natural world and is generally associated with young clades [6, 36]. In contrast, Aleocharinae are ancient, with crown-group fossils known from the mid-Cretaceous and a rich fauna of modern tribes and genera already diversified by the Eocene [37]. Substantial phylogenetic distances should therefore separate many myrmecoid lineages scattered across the tree.

To gauge the timescale over which this system emerged, we dated the tree using a Bayesian lognormal relaxed clock, calibrating nodes with Cretaceous Burmese and Middle Eocene Baltic amber fossils, and a compression from the Jurassic Talbragar Fish Bed (see Supplemental Experimental Procedures for details). Our analysis shows that virtually all myrmecoid clades arose in parallel during the Cenozoic (Figure 3; Figure S2D). This temporal window is consistent with when ants in general (including army ants) are thought to have risen to ecological dominance [13, 14], promoting the diversification of myrmecophiles [38]. Although army ant dating estimates are problematic due to limited fossils (only a single, Miocene Dominican amber *Neivamyrmex* is known [39]), recent dating estimates hypothesize that stem groups of doryline army ants date to the Upper Cretaceous (~80 mya,) radiating into crown-group genera ~35–20 mya [12]. This time frame is broadly consistent with the origins of myrmecoid clades inferred in this study (Figure 3; Figure S2D), although we see no clear relationship between the age of each ant genus and its corresponding myrmecoid clade(s) (data not shown).

Of foremost interest, however, is that we estimate the most recent common ancestor of all myrmecoid clades to have existed in the Early to mid-Cretaceous. In our focal analysis, this ancestor lived ~105 mya (Figure 3; Figure S2D). There is thus an ancient, inherent potential for Aleocharinae to evolve symbioses with army ants, which was realized by multiple lineages in parallel during the Cenozoic and which has led to the repeated evolution of symbionts with matching ecomorphologies and similar behaviors over an extraordinarily deep timescale. We note that origins of myrmecoid syndrome are unevenly distributed across the subfamily: 12 of the 15 myrmecoid lineages are clustered within a clade, "APL" (Figures 2 and 3), comprising the tribes Pygostenini, Lomechusini, and the vast, paraphyletic Athetini, where myrmecoid lineages occur among the "False Lomechusini" (clade F) [32], a group of New World genera formerly placed in Lomechusini. This bias probably represents the tropical dominance of APL tribes relative to other aleocharines, ecologically juxtaposing the beetles with army ants and hence elevating the likelihood of evolving this type of symbiosis. In contrast, no myrmecoid lineages emerge within the speciose



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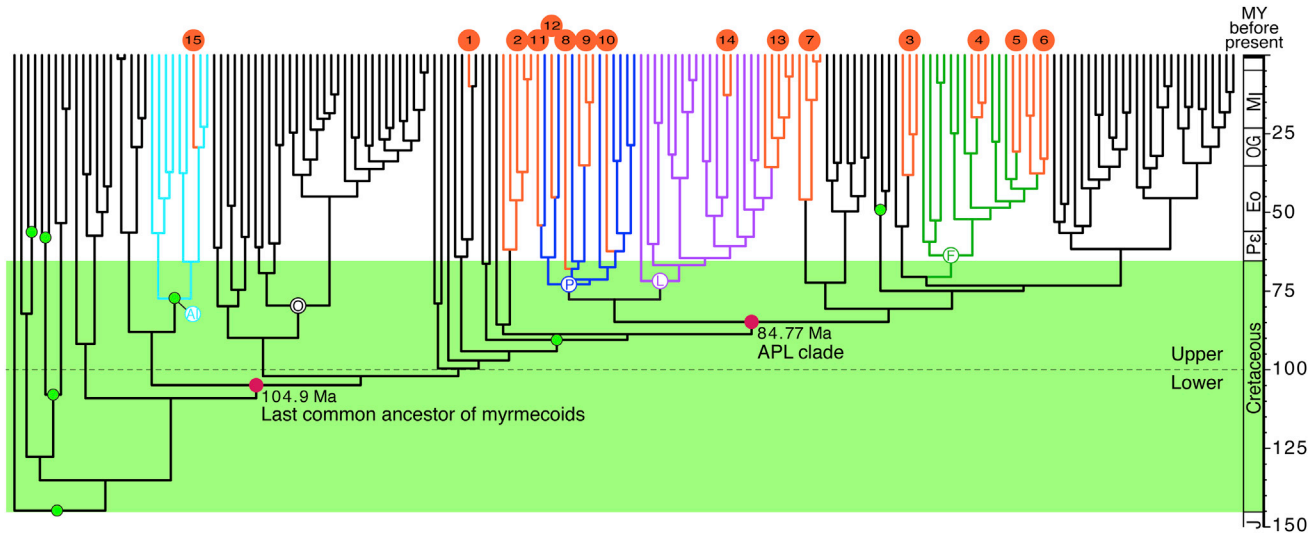

Figure 3. Dating the Evolution and Ancestry of Myrmecoid Clades

Dated phylogeny produced by BEAST2 and eight calibration points under a Bayesian lognormal relaxed clock. Outgroups belonging to Tachyporinae have been removed. Green circles indicate seven out of eight fossil calibration points; all eight calibration points, including the remaining one within Tachyporinae, are shown in Figures S2A and S2B. Myrmecoid clades are highlighted in orange, with clade numbers corresponding to those in Figure 2. The position and age of the APL clade as well as the positions of the P, L, and F subclades are indicated. The O (Oxypodini) and Al (Aleocharini) clades are also highlighted, and the age of the common ancestor of all myrmecoid lineages is indicated. See also Figures S2A, S2B, and S2D.

but largely temperate tribe Oxypodini (clade O; Figure 3). Despite this lineage clustering, the APL clade is itself still comparatively ancient (84.77 mega-annum [Ma]), and three additional origins outside the APL clade (clades 1, 2, and 15) confirm that the potential for evolving myrmecoid syndrome extends broadly across the subfamily (Figure 3).

What circumstances permitted this deep-time convergent system to arise? We deduce that historical selection pressures imposed by different army ant genera on separate aleocharine lineages were likely similar; so too were the adaptive responses of the beetles as they evolved with their hosts. This inherency in the outcome of selection begs the question of why myrmecoid syndrome has evolved repeatedly in Aleocharinae in particular, as opposed to all other groups of beetles, including 31 other staphylinid subfamilies numbering some 45,000 species—most of which have generalized staphylinid morphology similar to aleocharines. We previously argued that aleocharines' predatory habits, small body size, and major defensive capacity in the form of a dorsal abdominal tergal gland constitute a groundplan unique among Coleoptera [11]. This suite of characters predisposes aleocharines to successful entry and exploitation of ant colonies, providing the basis for why myrmecophily has evolved numerous times [10, 11], including repeated associations with army ants [8]. While many army ant associates are morphologically generalized (e.g., multiple APL-clade genera such as *Tetradonia* [40]), such species tend not to be socially accepted in nests. We propose that to gain the selective advantage of unlocking colony resources via social integration, many ancestrally generalized taxa experienced intense selection to conform to the myrmecoid shape, enabling the beetles to pass tactile assessment by workers [10, 11, 21–23]. Myrmecoid aleocharines are associated only with army ants and some other group foraging hosts that may employ such tactile cues to orchestrate collective behavior. If the narrow niche of social acceptance in such colonies demands an ant-like form, then the generalized aleocharine anatomy, comprising short elytra and an exposed, flexible abdomen, is conducive to such developmental remodeling [8, 11, 41]. Consequently, aleocharines are evolutionarily poised for myrmecophily and also for becoming myrmecoid as a major socially parasitic strategy when specializing on army ants. This near-clade-wide preadaptive groundplan may underlie the repeated evolution of myrmecoid syndrome in Aleocharinae.

Documented examples of deep-time convergence are mostly limited to the evolution of single traits with few instances of repeated evolution, and where a narrow range of alternative functional solutions are available. The independent origin of wings in birds, bats, and insects is an example. Similarly, although an expanding body of work has shown parallel genetic changes occurring in widely separated taxa [19, 42], such cases are typically functionally equivalent mutations in single, broadly conserved genes governing relatively simple traits, such as pigmentation [43, 44] or toxin resistance [45]. In contrast, we

Figure 2. Bayesian Consensus Tree of Aleocharinae

Myrmecoid clades are highlighted in orange, with representative taxa shown along with their respective host army ant genera. Clade numbers indicate independent origins of myrmecoid syndrome inferred from Dollo-type parsimony optimization. Anatomically generalized species that embody the ancestral morphology in Aleocharinae are also shown for comparison. Circles on nodes signify posterior probability (PP) values (open circles: PP > 0.95; closed circles: PP > 0.9). "APL" marks the "Athetini, Pygostenini, Lomechusini" clade; "F" labels the "False Lomechusini" clade. Outgroups belonging to Tachyporinae have been removed. The full topology, with PP values and taxonomic groupings indicated is shown in Figures S2A and S2B. See also Figures S2C and S3.

have found that a complex morphological and behavioral syndrome has evolved recurrently over >100 Ma, across a clade approximately equal in species richness to mammals and birds combined. To our knowledge, convergence at this frequency, timescale, and phenotypic complexity is without close precedent. The most comparable convergent system at roughly half the age may be the Caribbean anoles [46], where different ecomorphs share an Eocene common ancestor [47].

Our discovery challenges Gould's view that if the tape of life were replayed, an entirely different assemblage of life would exist [5]. On the contrary, the tape of life appears to be highly predictable whenever aleocharines ecologically coexist with army ants. We note that despite this overarching determinism, however, there is nevertheless an element of contingency: as Seevers appreciated [8], the segmental construction of the abdominal petiole differs among myrmecoid genera; some have unique specializations, such as the gland-associated abdominal lobes of *Aenictoteras*, or the complete loss of eyes and elytra in *Pseudomimeciton*; behavioral differences in how the beetles interact with ants also likely exist [9]. While this spectrum of variation could represent a continuum of specialization, we posit that at least some apparently idiosyncratic elements in this otherwise parallel system stem from clade-specific peculiarities: genetic and phenotypic disparities between ancestors of different myrmecoid lineages, discrepancies in selection pressures imposed by different host ants, as well as mutational and environmental stochasticity. Future studies on these beetles promise to reveal much about the nature of complex phenotypic change and the genetic and evolutionary forces shaping intricate symbioses in the animal kingdom.

ACCESSION NUMBERS

See Data S1 for a full list of NCBI accession numbers used in this study.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Discussion, Supplemental Experimental Procedures, three figures, and two datasets and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.02.030.

AUTHOR CONTRIBUTIONS

J.P. conceived and designed the project together with M.M. M.M. collected specimens with help from J.P. M.M. and J.P. sequenced specimens. M.M. photographed specimens and produced illustrations. J.P. performed analyses, made figures, and wrote the paper with input from M.M.

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Supplemental Information

Deep-Time Convergence in Rove Beetle Symbionts of Army Ants

Munetoshi Maruyama and Joseph Parker

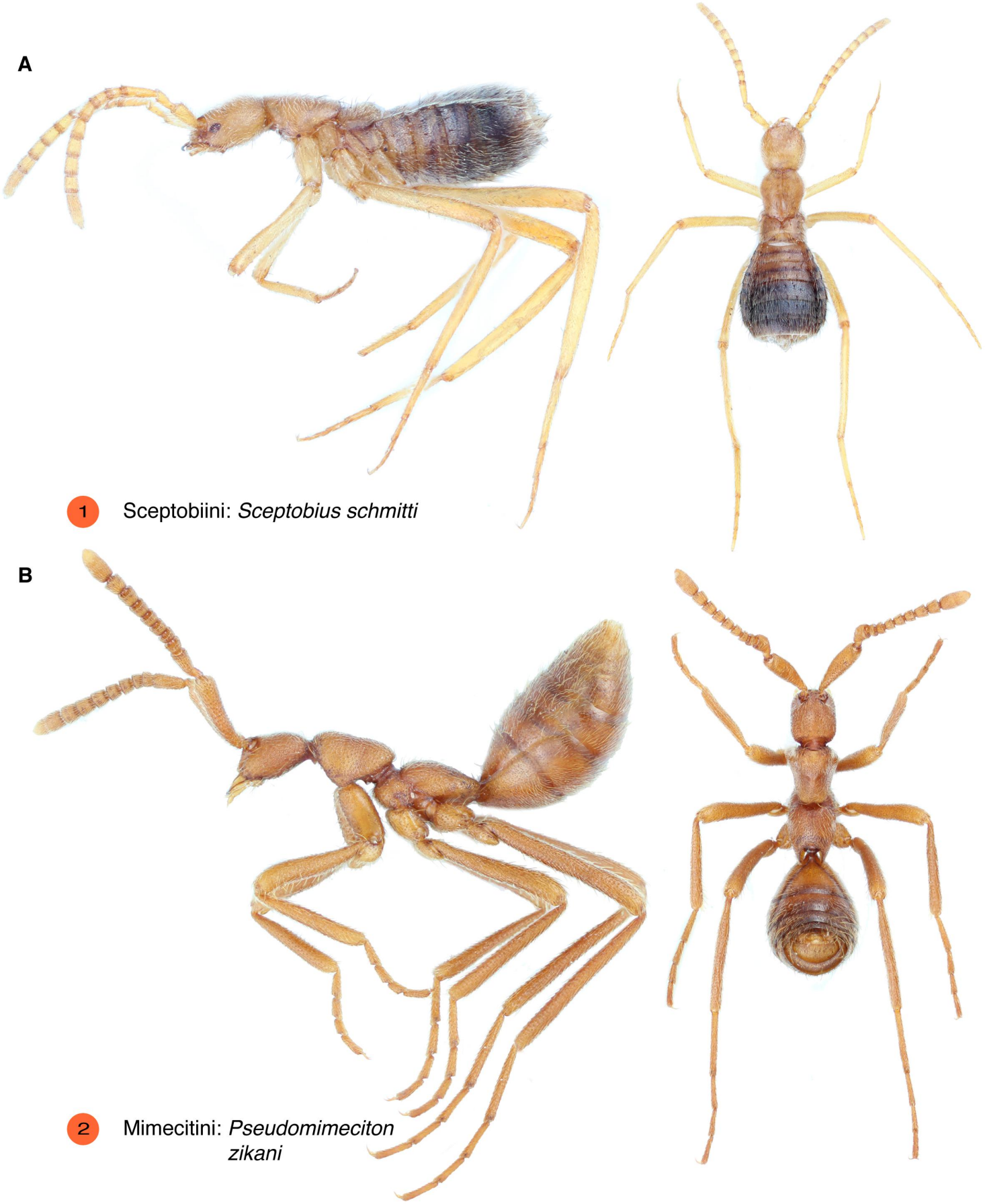

Figure S1 (continued on next page)

3 Ecitocharini: *Ecitophya simulans*

4 Athetini: *Ecitocryptus sulcatus*

Figure S1 (continued on next page)

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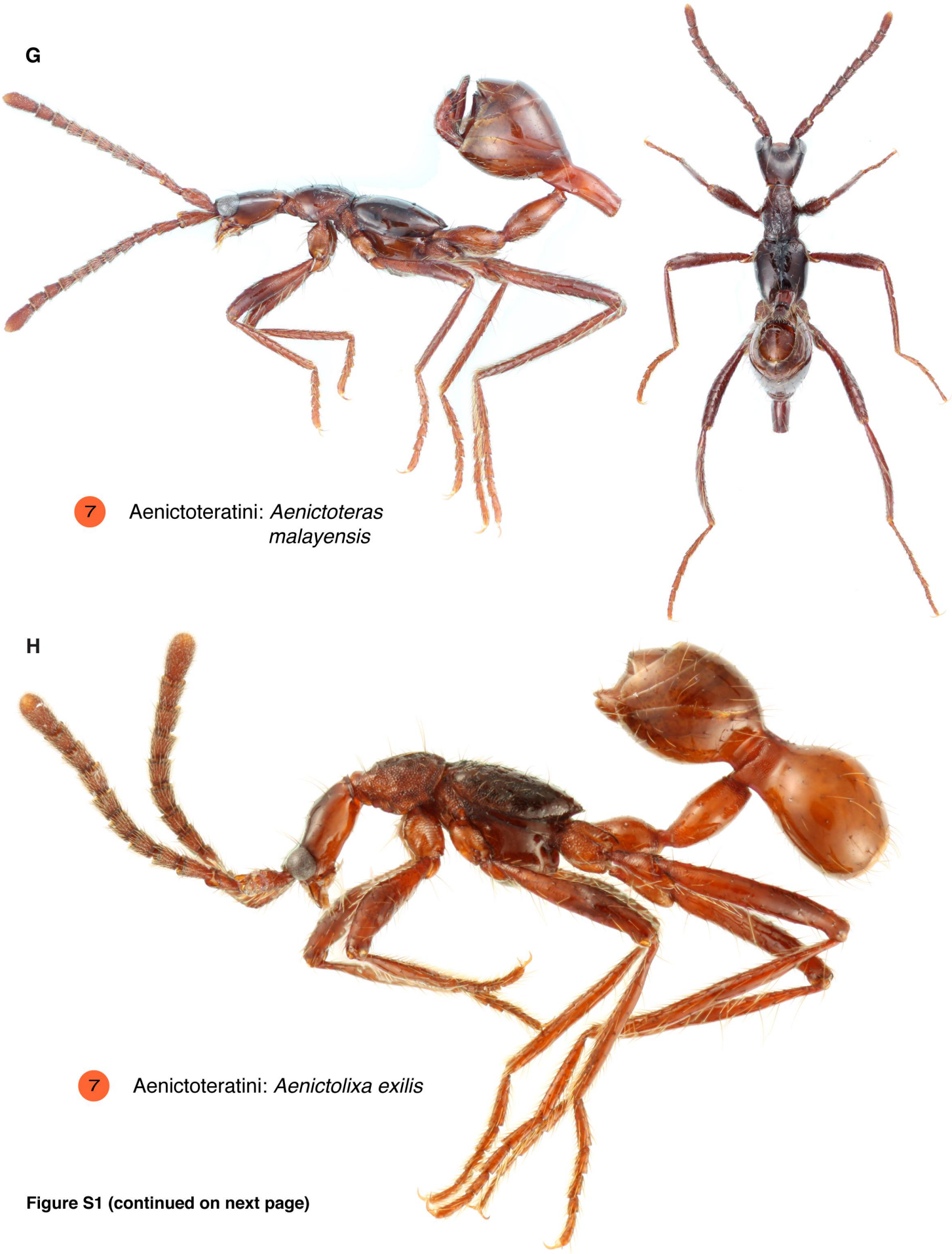

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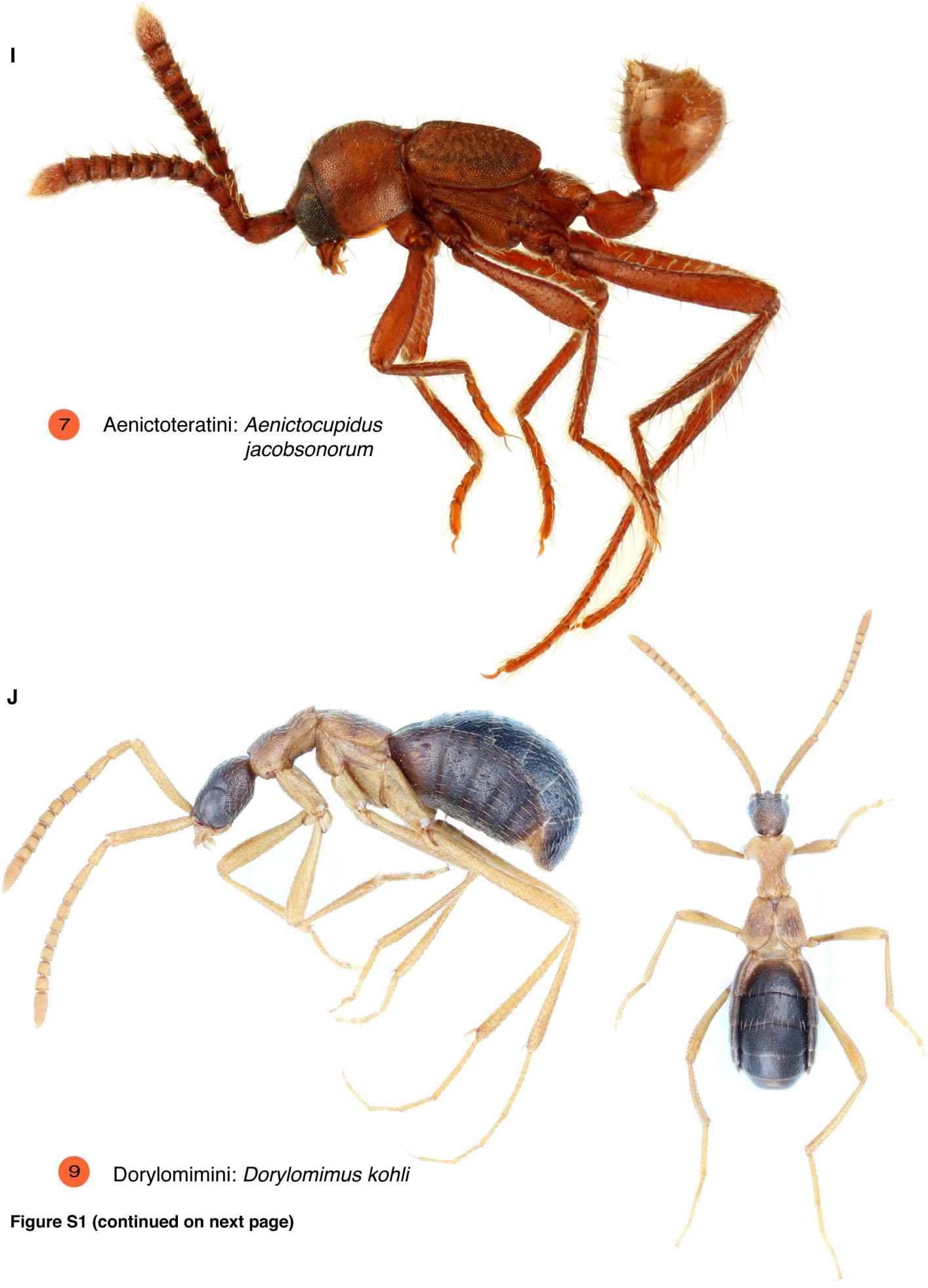

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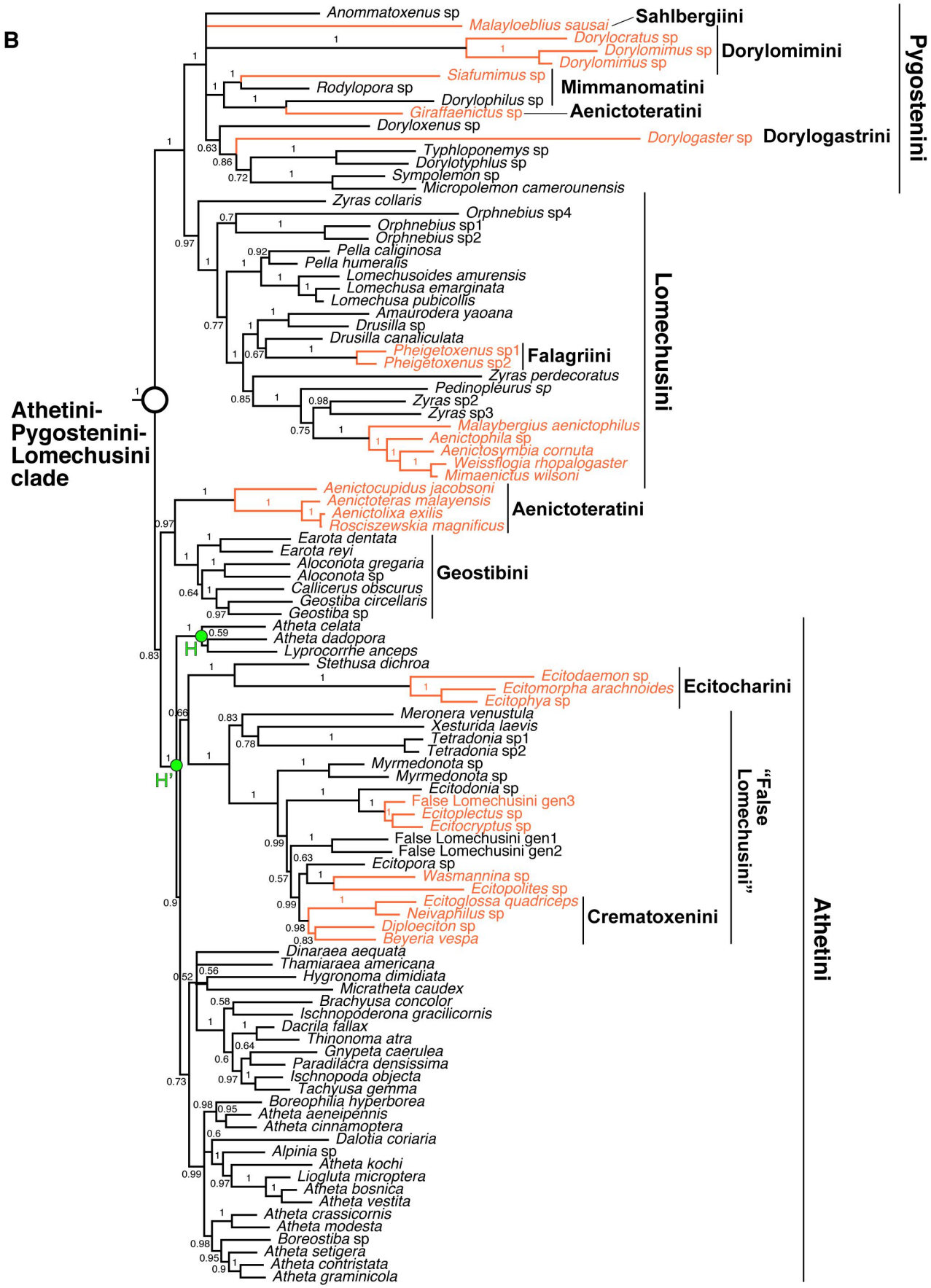
Figure S1. Habitus images of myrmecoid Aleocharinae (related to Figure 2). Numbers correspond to myrmecoid clades in Figure 2.

A

Figure S2 (continued on next page)



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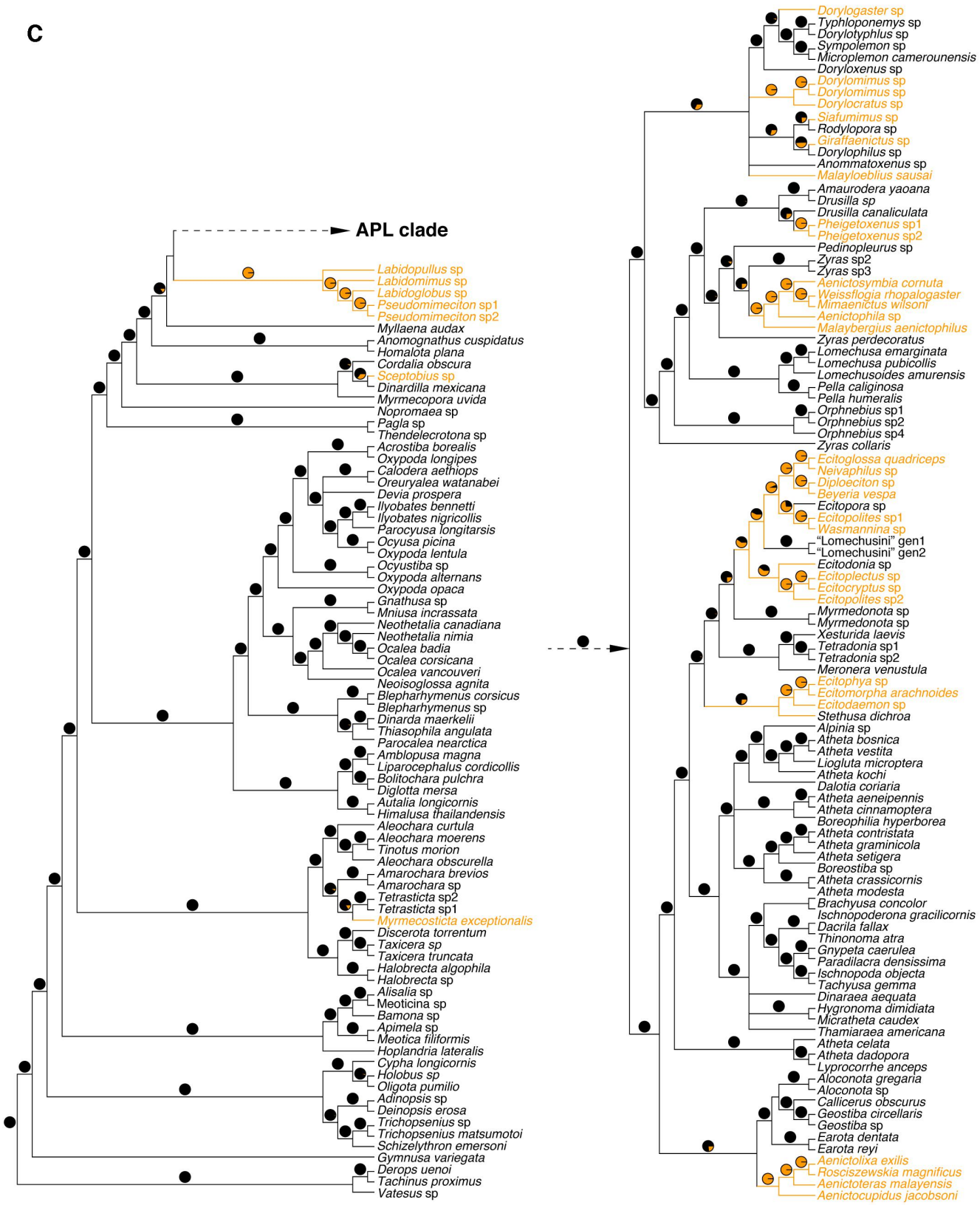

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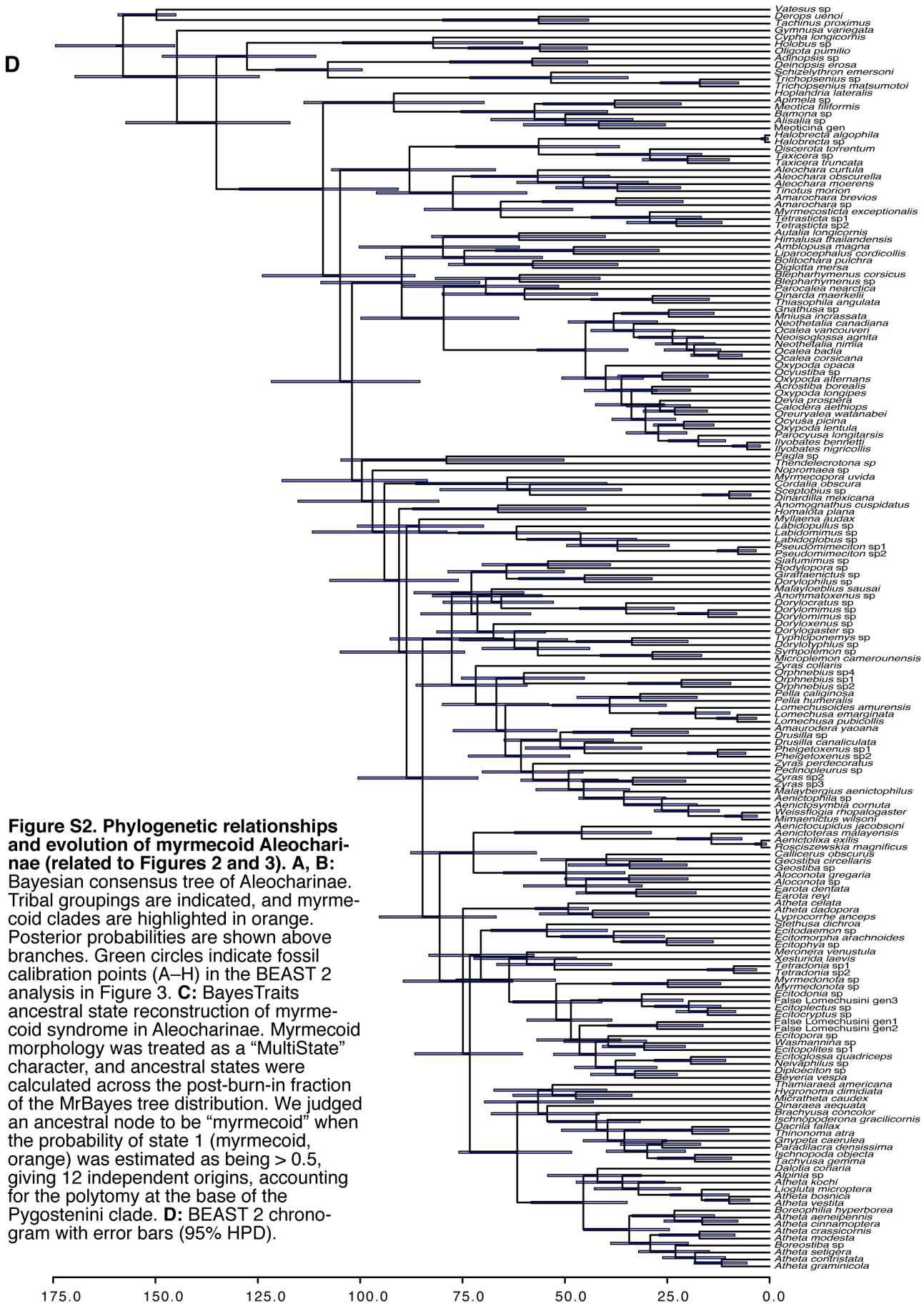

Figure S2. Phylogenetic relationships and evolution of myrmecoid Aleocharinae (related to Figures 2 and 3). A, B: Bayesian consensus tree of Aleocharinae. Tribal groupings are indicated, and myrmecoid clades are highlighted in orange. Posterior probabilities are shown above branches. Green circles indicate 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).

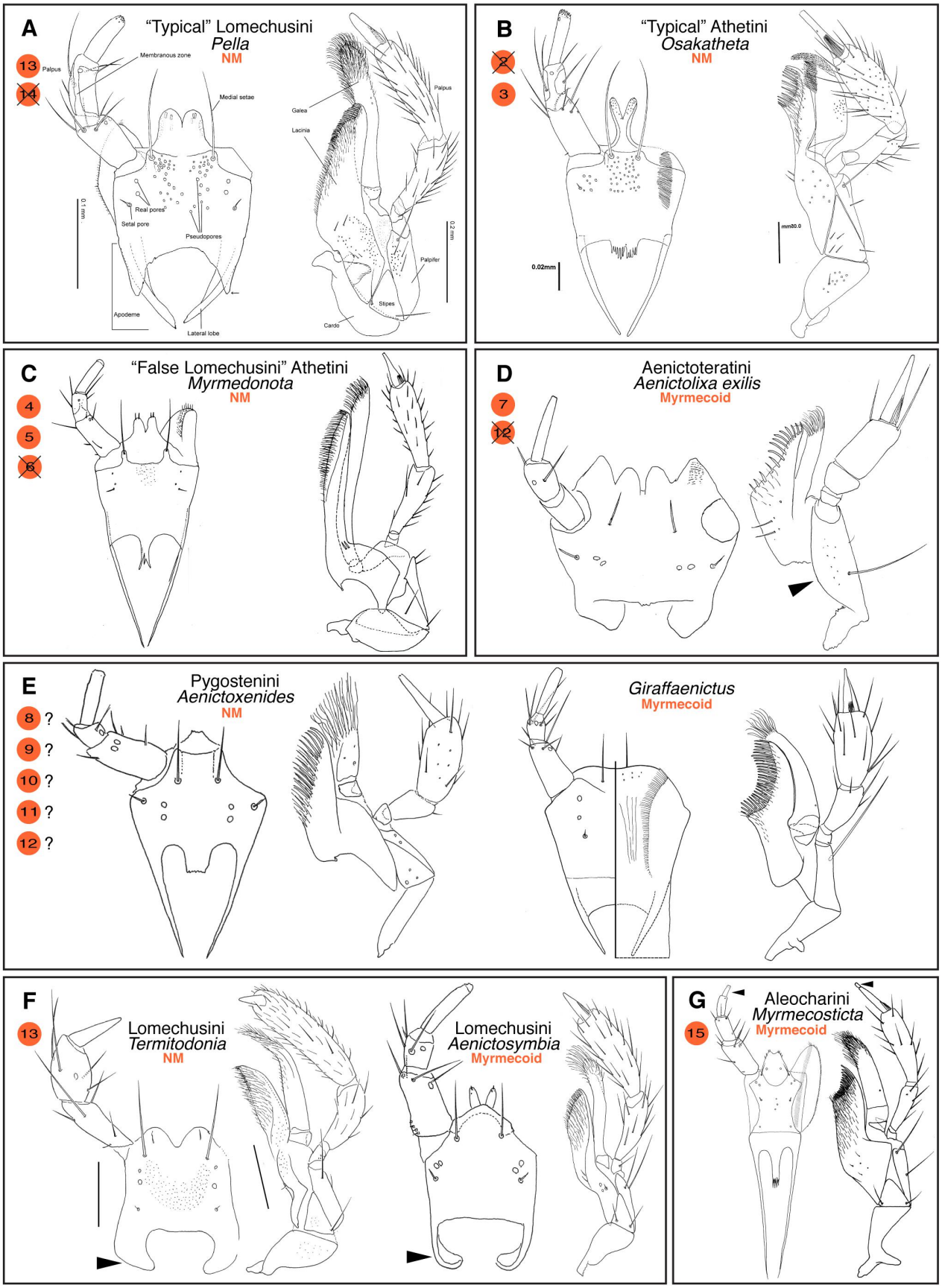

Figure S3. Mouthpart morphology (related to Figure 2). Labia and maxillae from relevant non-myrmecoid (NM) Aleocharinae are shown, together with structures from selected myrmecoid taxa. Below panel letters, myrmecoid clades related to and exhibiting putative synapomorphies with the figured NM genus are 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 *Dinardilla* 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 *Dinardilla*, 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 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 *Rosciszewskia magnificus*. The beetles followed *Aenictus* emigration columns and were not carried by worker ants. However, in subsequent observations, MM observed both *Aenictoteras malayensis* and *Rosciszewskia 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 *Rosciszewskia* 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 we 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, Procantonnetia* 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 *Procantonnetia* 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 × 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 accessions 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'-GACTCCTTGGTCCGTGTTTCAAG + **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 miniprepped 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) Trabalgar 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). *Baltioligota* 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 *Baltioligota* 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 10^{th} 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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