# A Molecular Investigation of the Aleocharinae (Coleoptera: Staphylinidae) Phylogeny

BY



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## Abstract

The subfamily Aleocharinae is a hyper-diverse group of staphylinid beetles that account for 40% of the diversity within the family Staphylinidae. These small to minute beetles are primarily dominant generalist predators in leaf litter and soil communities (Ashe, 1998). Several lineages of aleocharines have invaded and diversified in many unusual habitats such as mushroom habitats, seashore habitats, and tropical canopy habitats. These beetles are also known as the most successful group of inquilines in the nests of social insects (Seevers, 1978). At present, the aleocharine subfamily contains 52 tribes, over 1,000 genera, and about 12,000 species. Ashe (1984) considered the Aleocharinae to be "the most inadequately understood large group within the Coleoptera."

The subfamily was first shown to be monophyletic by Hammond (1975) and later by Ashe (1994) based on unique characteristics of the adeagus. In subsequent morphological phylogenetic analyses, Ashe and Newton (1993) and Ashe (2005) recovered a monophyletic 'higher' aleocharinae lineage based primarily on the presence of a tergal gland in both larvae and adults. 'Basal' lineages of aleocharine beetles do not possess this structure. Shortly there after, Haasl (unpublished thesis, 2005) recovered a monophyletic 'higher' aleocharine clade using molecular techniques.

Fragments of the 12s and 16s mithchondrial rDNA genes were sequenced and compared to investigate the phylogenetic relationships among selected tribes from within the 'higher' aleocharinae. The aim of this study is to investigate the usefulness of these two rDNA genes in resolving relationships at the tribal level, examine and improve upon some of the predominant morphological hypotheses that have been proposed for tribes in this subfamily, and further our understanding of the tribal-level relationships within this group.

Results from this study confirm that the 'higher' Aleocharinae form a monophyletic group. This study also supports the placement of the tribe Myllaenini within the 'higher' aleocharine clade. All datasets and analyses recovered a monophyletic Gyrophaenina lineage. The 12s and combined dataset hypothesized the paraphyly of the genus *Gyrophaena* with respect to *Phanerota*. There was also evidence that the genus *Tachyusa* and allied genera are closely related to members of the tribe Falagriini, as historically hypothesized by Bernhauer and Sheerpeltz (1926).

*For Michael Julian and Michael Ashley Leonard*

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# Chapter 1: An Introduction to the subfamily Aleocharinae

*General Characteristics of the subfamily Aleocharinae.*

Aleocharine beetles represent one of the most fascinating and diverse monophyletic radiations in the order Coleoptera. These minute beetles are mainly predatory generalists that dominate leaf litter and soil communities (Ashe, 1998). What makes them stand out among the staphylinid beetles is their ability to invade and diversify in a multitude of unique and specialized habitats. For instance, the larvae of the genus *Aleochara* are parasites of cyclorrhaphous diptera puparia (Maus, 2000). A multitude of aleocharine lineages, within numerous tribes (Homalotini, Oxypodini, Aleocharini, Athetini, Falagriini, Crematoxeninini), are known to be associated with mushrooms, and a few have become exclusively mycophagous. Several of the most well known mycophagous species are included in the present study. In terms of the numbers of lineages and species, aleocharines are by far the most successful group of inquilines in the nests of social insects, especially in ant and termite nests (Seevers 1957, 1965; Ashe, 1998). Another of the more extraordinary habitats hosting aleocharine beetles is the intertidal habitat. Several tribes of aleocharines (Phytosini, Aleocharinae, Falagriini, Athetini, Diglottini, Lipoarocephalini, and Myllaenini) boast genera that have invaded and diversified in seashore habitats (Ahn & Ashe, 2004). "The great described diversity of these beetles only hints at the true diversity of aleocharines, with many thousands of species, and numerous higher taxa, remaining to be described from throughout the

world, especially in tropical regions" (Ashe, 1998: http://tolweb.org/Aleocharinae). Naturally, the large majority of aleocharines studied thus far have evolved unique ecological histories making them stand out among the thousands of generalist ancestor species that exist in the subfamily. Currently, there are 52 tribes, over 1,000 described genera, and at least 12,000 described species. "Unfortunately, the seemingly endless diversity, the small size of most adults, and the virtual lack of illustrated keys and descriptions of aleocharines for most geographical regions make the Aleocharinae one of the most taxonomically difficult groups of beetles" (Ashe, 1998: http://tolweb.org/Aleocharinae). However, there is hope for this hyper-diverse radiation of life—subfamilial and tribal level phylogenetic studies are starting to shed light on the Aleocharinae.

*The Tachyporine Group.*

The family Staphylinidae has been broken up into 4 main lineages that are collectively known as informal 'groups'. A detailed overview of the Staphylinidae can be found on the The Tree of Life webpage: http://www.tolweb.org/Staphylinidae (Newton & Thayer, 1992). One of these 'informal' groups, known as the tachyporine group, includes the subfamily Aleocharinae. The remaining three lineages that comprise the staphylinid subfamily are: omaliine, oxyteline, and staphylinine groups.

The relationships among the tachyporine group subfamilies were first elucidated in Ashe and Newton's study of the larvae of *Trichophya* (Ashe & Newton, 1993). In 2005, Ashe published a phylogeny of the tachporine group subfamilies based on adult and larval characters, including a detailed look at the aleocharine

subfamily. Ashe's morphological phylogenetic study supported a monophyletic Aleocharinae, and showed that Trichophyinae + Habrocerinae form the sister group to the Aleocharinae. Haasl (unpublished thesis, 2005) completed a molecular analysis of the tachyporine group subfamilies based on DNA sequence analysis of the 18s nuclear rDNA, 12s and 16s mitochondrial rDNA and the nuclear gene 'wingless'. Haasl's study robustly supported Ashe's morphological investigation of the tachyporine group phylogeny. Most importantly, Haasl's molecular data recovered the same relationships: ((Trichophyinae + Habrocerinae) + Aleocharinae). Figure 1 presents the currently accepted tachyporine group phylogeny following Ashe, 2005.

*Aleocharinae as a monophyletic group.*

Hammond (1975) was the first investigator to provided evidence for a monophyletic Aleocharinae: presence of large lateral lobes on the aedagus, a unique synapomorphy shared by all aleocharines. Additionally, Ashe and Newton (1993) described two larval synapomorphies that further supported a monophyletic Aleocharinae: the presence of an enlarged molar area and a reduced number of stemmata relative to closely related staphylinid subfamilies. In Ashe's (2005) combined analysis of the tachyporine group subfamilies, the monophyly of the Aleocharinae is robustly supported by eleven morphological synapomorphies, seven of which are unique. Haasl's (2005) molecular study also strongly supported a monophyletic Aleocharinae.

*The 'Basal' Aleocharinae.*

Given the recent advances in the phylogenetic study of higher-level relationships among tachyporines, it is generally accepted that within the Aleocharinae, a 'basal' set of tribes (Gymnusini, Deinosini, Mesoporini, Trichophynini) gave rise to a staggeringly large and diverse group of aleocharines known as the 'higher' Aleocharinae. The relationships among the basal aleocharines are reasonably well supported. The Gymnusini (2 genera) + Deinopsini (3 or 4 genera) group was robustly supported as a basal, monophyletic lineage of Aleocharinae based on five unique morphological synapomorphies (Ashe and Newton, 1993; Ashe 2005). In his 2005 work, Ashe also showed that the subfamily Trichopseniinae is actually a member of the Aleocharinae and should be reduced in rank. The same study moderately supported a sister-group relationship between the Mesoporini and the newly demoted Trichopseniini. Ashe and Newton have both proposed, in several phylogenetic statements and analyses, that the 'basal' aleocharines represent a polyphyletic assemblage based on several pleisiomorphic characters (Ashe and Newton, 1993; Ashe, 1998, 2005). Haasl's (2005) molecular investigation of the 'basal' Aleocharinae recovered the monophyletic sister-group: Gymnusini + Deinopsini. The tribe Mesoporini was recovered as a monophyletic group as was the 'basal' Aleocharinae. Unfortunately, Haasl was unable to obtain specimens of Trichopseniini for his study, so molecular support for a monophyletic

'basal' Aleocharinae is still lacking. Figure 2 represents the currently accepted relationships among the four tribes of 'basal' aleocharines.

*The 'Higher' Aleocharinae.*

There is abundant morphological evidence in support of a monophyletic, informal group known as the 'higher' Aleocharinae. The higher aleocharines are characterized by one striking adult synapomorphy: the presence of a unique tergal gland at the base of tergum VII in the adults, or the apex of tergum VIII in the larvae (Ashe, 1994). None of the 'basal' aleocharines possess this structure. 'Higher' aleocharines also possess two larval synapomorphies that support the monophyly of this group-- fusion of the basal segment of the urogomphi to tergum IX and two pairs of distinct hooks on the pygopodium (Ashe and Newton, 1993). In addition to Ashe's morphological treatments, Haasl's (2005) molecular investigation of the Aleocharinae provided strong corroborating evidence for the monophyly of the 'higher' Aleocharinae. Beyond their monophyletic status, relationships among the tribes of 'higher' aleocharines are patchy and exceedingly artificial. "There have been no comprehensive studies, and the relatively few past studies of phylogeny in this large group have been addressed by examining phylogenetic structure in higher taxon subunits"(Ashe, 1998, http://tolweb.org/Aleocharinae). The logical next step was obvious: begin to examine the tribal-level relationships using a combination of morphological and molecular data.

*Tribal Level Relationships among 'higher' aleocharines.*

Some sister group relationships among various tribes have been proposed: Falagriini + Sceptobiini, Aleocharini + Hoplandriini. Seevers (1978) first proposed that the tribes Falagriini and Sceptobiini together formed a monophyletic group based on shared presence of a divided velum of the paramere. Danoff-Burg (1994) and Ahn and Ashe (1995) provided additional evidence for the monophyly of the Falagriini + Sceptobiini. Seevers (1978) noted that the members of the tribes Aleocharini and Hoplandriini shared presence of a pseudosegment on the maxillary palpi and an unusual reticulated array of sclerotized supports in the velum of the parameres. Based on these characters he proposed that they form a monophyletic group (Ashe, 1998). Jacobson and Kistner (1991) proposed a sister group relationship between Crematoxenini and Leptanillophilini. Steidle and Dettner (1993) suggested that the tribes Oxypodini, Athetini, Lomechusini (then called the "Myrmedoniini"), and Aleocharini form a monophyletic group based on shared presence of topically extremely effective hydrocarbons associated with a tergal gland at the base of tergum VIII. Although the biochemistry was compelling, the taxon sampling for this study was insufficient given that only eleven of the 52 tribes were represented. Steidle and Dettner's study supported Ashe's placement of the Myllaenini as a member of the 'higher' aleocharinae based on the presence of a small tergal gland at the base of tergum VII. In at least two of his phylogenetic treatments, Ashe concluded that the Myllaenini are, in fact, members of the 'higher' Aleocharinae, not closely allied with the Deinopsini as earlier work had suggested (Ashe, 1993, 2005). Results from this molecular investigation confirm the following morphological hypotheses: 1) the

'higher' Aleocharinae form a monophyletic group, and 2) the tribe Myllaenini is a member of the 'higher' Aleocharinae.

*An Introduction to the Tribes represented in this study.*

At least two representatives from eight of the 52 tribes were sampled to further investigate the tribal-level relationships within the aleocharine subfamily. Partial sequences of the mitochondrial genes, 12s and 16s, were used to infer these tribal level relationships. Three representatives from the basal tribe Deinopsini were selected as outgroup taxa. The Deinopsini are known to have a worldwide distribution. Two of the three genera included in this tribe occur in North America (Ashe, Chapter 22 Staphylinidae, Aleocharinae: p. 359). Deinopsini species are characteristic inhabitants of marshes, bogs, pond and stream edges and similar riparian habitats (Ashe & Chatzimanolis, 2003).

Among the 'higher' Aleocharinae, the Myllaenini and closely related Pronomaenini are each represented by one species, *Myllaena* sp. and *Pseudomniophila* sp., respectively. As a result of Ahn and Ashe's 2004 study of the Myllaenini, the tribe now contains nine genera, two of which occur in North America (Ahn & Ashe, 2004; Ashe, Chapter 22 Staphylinidae, Aleocharinae: p.364). Five of these genera—*Bryothinusa, Brachypronomaea, Rothium, Lautaea,* and *Polypea* occur exclusively in the intertidal region (Ahn and Ashe, 2004). The remaining four genera—*Myllaena, Amazonopora, Philomina,* and *Dimonomera*—are associated with

freshwater riparian habitats (Ahn and Ashe, 2004). According to this same study, the Pronomaeini was well recovered as a monophyletic group containing the following four genera: *Pronomaea, Pseudomniophila, Nopromaea,* and *Tomoxelia* (Ahn and Ashe, 2004). Both of these tribes form monophyletic lineages based on the unique, apomorphic morphological characters described in Ahn and Ashe's 2004 work. Species in these genera are known from terrestrial and riparian habitats (Ahn and Ashe, 2004). Together, these two tribes occur in most, if not all, zoogeographic regions (Seevers, 1978).

In Ahn and Ashe's 1995 revision of the North American Falagriini, they recognized 11 North American genera. In their study, the Falagriini are robustly supported as a monophyletic lineage. There are roughly 30 Falagriine genera known from throughout the world (Ashe, Chapter 22 Staphylinidae, Aleocharinae, p 372). Many of these species are known to have diversified in the intertidal zone, and many more are known from various seashore, and more general riparian habitats (Hoebeke, 1985). One of the two falagriines represented in this study, *Lissagria laeviscula,* is known only from California. The other, *Myrmecophalus cingulatus*, is widely distributed in eastern North America, from Nova Scotia and Ontario south to Louisiana and Alabama and west to eastern Kansas (Hoebeke, 1985). The genus *Myrmecocephalus* contains a cosmopolitan species, *Myrmecocephalus concinnus* (Hoebeke, 1985).

The Homalotini (= Bolitocharini) is another large tribe with 123 genera worldwide, 22 of which occur in North America (Ashe, Chapter 22 Staphylinidae,

Aleocharinae, p. 365). These species are known from typical leaf litter habitats much like those described for the Oxypodini. There are as many as 14 defined subtribes within the Homalotini, two of which are represented in this study: Bolitocharina and Gyrophaenina. Ashe provided extensive genus-level revisions for both of these subtribes (Ashe, 1984,1992). Both of these subtribes have a worldwide distribution. Larvae and adults in both of these subtribes are strictly mycophagous. The Bolitocharina and Gyrophaenina are most closely associated with Polyporaceae and lignicolous Agaricales (Ashe, Chapter 22 Staphylinidae, Aleocharinae, p.365). The Bolitocharina is represented here by one genus, *Strictalia*. A historically closely related genus, *Leptusa*, is also included in this study. The Gyrophaenina are represented by three genera (*Eumicrota, Gyrophaena, and Phanerota*) and 7 species in the present study. In his revision of the Bolitocharina, Ashe also provides morphological evidence (beyond 4,4,5 tarsal segmentation) for a monophyletic Homalotini (Ashe, 1992).

The tribe Aleocharini is represented by one species, *Aleochara valida*, in this study. This tribe contains some 15 genera worldwide, placed in three subtribes; only one genus occurs in North America (Ashe, Chapter 22 Staphylinidae, Aleocharinae, p. 360). Adult species in the subtribe Aleocharina are primarily predators of Diptera eggs, larvae, and pupae at dung, carrion, or rotting fungi, seaweed, or cacti (Ashe, Chapter 22 Staphylinidae, Aleocharinae, p 360). Aleocharine larvae are ectoparasitoids of cychlorrhaphous Diptera pupae near these habitats (American

Beetles, p. 360). The other two subtribes of Aleocharini are known to be termitophilous.

The last two tribes, Athetini, Oxypodini, included in this study are known to be large, paraphyletic assemblages each containing several monophyletic clades. According to Ashe, " The tribe Athetini is by far the most difficult tribe in the Aleocharinae. "The current classification of North American athetine taxa is completely inadequate, and many of the genus-level taxa currently recognized, and the subtribes proposed by Seevers (1978), cannot be clearly delimited or diagnosed…..this very large and poorly characterized tribe contains about 173 genera worldwide, 64 of which occur in North America" (Ashe, Chapter 22 Staphylinidae, Aleocharinae, p.368). According to Seevers (1978), "The tribe Athetini is the largest of the major groups of Aleocharinae, numbers thousands of species and occurs in every zoogeographic region." *Atheta* sp., one of the Athetini representatives, was once an extremely large complex of species (Bernhauer & Scheerpeltz, 1926), but is now much more restricted in size and geographic scope. *Atheta (sensu stricto)* species are known as boreal, north temperate species extending from Europe and Asia on into Alaska and western Canada (Seevers, 1978). *Leptonia* sp., the second of three Athetini genera represented in this study, possesses only one described species, *Leptonia picta* (Sharp), from Mexico. *Leptonia* was most recently treated as a subgenus of *Atheta* (Ashe, http://nhm.ku.edu/ksem/peet/catalogs/aleogen.htm) following a precedent set by Bernhauer and Scheerpeltz in 1926. The last athetine genus represented in this study is *Sableta.* This genus only contains one species,

*Sableta infulata*. This species was described by Casey (1906) based on a single specimen from Mississippi (Ashe & Gusarov, 2004). All three of the species mentioned above have been collected on, or associated with polypore mushrooms (Ashe, pers. comm.). "Dominant predators in many microhabitats, they occur in great abundance in decaying animal and vegetable materials—carcasses, dung, decaying fungi, rotting fruit, and vegetation. They may occupy such habitats as flowers, stream and pond shores, bird and mammal nests, and ant nests. Without a doubt, the athetines are among the most successful of small beetles" (Seevers, 1978).

The Oxypodini is another weakly delimited tribe containing 146 genera worldwide, with 40 of these genera occurring in North America (Ashe, Chapter 22 Staphylinidae, Aleocharinae, p.360-61). The diversity of habitats hosting oxypodine species does not seem to be as far reaching as the aforementioned Athetine habitats. Oxypodines are most frequently encountered in moist leaf litter habitats, rotting wood, moss, and a few species have been reported from ant nests, although this is probably a facultative condition (Seevers, 1978). Both of the oxypodine genera, *Oxypoda* sp. and *Tachyusa* sp., represented in this study deserve special mention. *Oxypoda* is a cosmopolitan genus with more than 350 recorded species, and *Tachyusa* is also, most likely, a cosmopolitan genus with a comparable number of recorded species (Seevers, 1978). Bernhauer and Scheerpeltz (1926) concluded that the *Tachyusa* and allied genera in the 'Tachyusae' subtribe were most closely allied with members of the tribe Falagriini. In fact, Bernhauer and Scheerpeltz did not separate *Tachyusa* (and allied genera) from the Falagriini, they included *Tachyusa* (and allied

genera) in the same subtribe with all of the Falagriini genera, calling this group the Falagriae, a subtribe of the Myrmedoniini. Seevers (1978) removed *Tachyusa* (and allied genera) from the Falagriae, created a new subtribe (Tachyusae), and placed this new subtribe within the tribe Oxypodini. Seevers then elevated the Falagriae, giving this group tribal status. Seevers used aedeagal characters, unique to the falagriine genera, to support his decision to elevate this subtribe to the tribal level. Defining morphological characters that are useful at the tribal level is not an easy task for a group of beetles that have proved to be so superficially similar.

Ashe (1998) provided the only published cladogram for the 52 tribes in the aleocharine subfamily: http://www.tolweb.org/Aleocharinae. This cladogram really showcases the need for a comprehensive, phylogenetic investigation of the tribal level relationships within the Aleocharinae. This type of revision is needed to stabilize the current proposed relationships within the 'higher' Aleocharinae, as well as to establish a functional tribal level classification system that truly reflects the evolutionary history of this hyper-diverse group of staphylinid beetles.

As a preliminary step towards that end, fragments of the 12s and 16s mithchondrial rDNA genes were sequenced and compared to investigate the phylogenetic relationships among selected tribes from within the 'higher' Aleocharinae. These genes seemed like the logical place to start considering Haasl's success using them for his phylogenetic study of the 'basal' Aleocharinae. In addition, several authors have used one or both of these genes to resolve subfamilial and tribal level relationships (Caterino et. al, 2005, Rasmussen & Cameron, 2007,

Yoshinzawa & Johnson, 2003). The goals of this study are as follows: 1) investigate the usefulness of these two rDNA genes in resolving relationships at the tribal level, 2) examine and improve upon some of the predominant morphological hypotheses that have been proposed for tribes in this subfamily, 3) further our understanding of the tribal level relationships within this group, and 4) examine several methods of phylogenetic analysis including parsimony, maximum likelihood, and Bayesian methods.

# Chapter 2: Materials and Methods

*Taxon Sampling.*

A total of 21 Aleocharinae species representing seven tribes and sixteen genera are preserved in 100% ethanol. Voucher specimens are deposited in the Snow Entomology Collection at the University of Kansas. Dr. James S. Ashe collected 13 specimens during his 2005 field season in the Great Smoky Mountains, Reed Lake (Illinois), and Calaveras California. Two specimens were collected in La Selva, Mexico in 1995. DNA extractions for the remaining six representatives in this study were obtained from Ryan Haasl (unpublished thesis, 2005). Table 2 includes detailed locality information for all the specimens in this study. 13 out of the 21 taxa are identified to the species level. The remaining taxa are only identified to the level of genus (Table 2). The Homalotini are represented by 9 species in 3 different subtribes. A subtribe of the Homalotini, Gyrophaenina, is represented by 7 of these 9 species. The following tribes are represented by at least two species: Falagriini, Athetini, Oxypodini, and Deinopsini. The last three tribes, Aleocharini, Myllaenini, and Pronomaenini, are only represented by 1 species each.

*DNA extractions*.

DNA extractions were obtained using a Guanidine Thiocyanate protocol developed for use in the KU-NHM Molecular lab. The general steps are as follows: cell lysis and RNAse treatment, a protein precipitation using Guanidine Thiocyanate, a DNA precipitation using 100% isopropanol and ethanol, and finally DNA hydration using a Tris-Cl solution. For each extraction, one or two complete specimens were ground up using sterile pestles and microcentrifuge tubes.

*PCR amplifications.*

PCR amplifications were carried out following a protocol published by Palumbi in 1996. For the 12s fragment, each 50ul PCR reaction contained 31.25uls water, 9 uls MgCl2, 5uls buffer, 0.5uls dNTP's, 1.0 ul of each a forward and a reverse primer, 0.25uls Taq, and 2uls of template. For the 16s fragment, each 25ul PCR reaction contained 14.6uls water, 4.5uls MgCl2, 2.5uls buffer, 0.25uls dNTP's, 0.5uls of each a forward and a reverse primer, 0.125uls Taq, and 2uls of template. For some specimens, PCR beads were also used to enhance the amplification of the 16s fragment. PCR products were visualized on a 8% agarose gel enfused with Ethidium Bromide. ExoSap-IT® was used to clean up the PCR reactions. 12s and 16s rDNA genes were amplified using 12Sai and 12Sbi, and 16Sar and 16Sbr, respectively. The primer sequences are listed in Table X. The following thermal cycle conditions were

used for 12s: 94 3min. (1x), 94ºC 1min, 50ºC 1min., 72ºC 1min. (35x). The thermal cycle conditions for 16s are as follows: 94ºC 1min., 93ºC 30sec., 48ºC 30sec., 72ºC 2 mins. (40x), 72ºC for 10mins. Before moving on to the sequencing reactions, PCR amplifications were cleaned up using Exo-SapIt (USB Corporation).

Table 1. Primer sequences used for PCR amplification in this study. Primer sequences are written in the 5' to 3' direction.

| *Primer* | *Sequence* |
|---|---|
| 12Sai | AAACTAGGATTAGATACCCTATTAT |
| 12Sbi | GAGAGCGACGGGCAATATGT |
| 16Sar | CGCCTGTTTAACAAAAACAT |
| 16Sbr | CCGGTCTGAACTCAGATCATGT |

*DNA Sequencing.*

Sequencing reactions were carried out following the sequencing protocol included with the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Each sequencing reaction included 0.5uls 3.1 Big Dye, 1.75uls 5x buffer, 0.5uls DMSO 0.2uls of one unidirection primer, 6.55uls water, and 0.5uls PCR product. The thermal cycle conditions are as follows: 95ºC 2mins. (1x), 95ºC 15sec., 50ºC 15sec, 60º 1:10mins. (45x). Sequencing reactions were cleaned up using magnetic beads (Agencourt Bioscience Corporation). Dr. Michael Grose (Ichthyology, University of Kansas Molecular Laboratory) performed the automated sequencing using 3100 ABI chemistry.

*Alignment.*

Sequencher 4.7 (Gene Code Corporation) was used to compile the contig files needed to perform an automated sequence alignment. Blast searches were conducted in GenBank to confirm the orthology of each of the DNA sequences used in this study. The Clustal X program was employed to create a preliminary alignment, but after comparing these alignments to Aleocharinae sequences available on Genbank, it became apparent that further, more in depth, alignment analysis was necessary. Published secondary structures for domains II-IV were used to manually align the 12s sequences (Ballard et. al. 1998). 16s alignments were also created manually using published secondary structures for domains IV and V of the 16s rDNA gene (Buckley et. al. 2000). A comparison of the Manual alignments and ClustalX generated alignments revealed a number of ambiguous gaps in the ClustalX alignments.

*Phylogenetic Analysis.*

Parsimony, Maximum Likelihood, and Bayesian analyses were performed on each of the three datasets: 12S, 16S, and combined datasets. In all analyses, gaps were treated as missing data. PAUP*4.0b10 (Swofford, 2002) was employed to perform all of the Parsimony analyses. Heuristic searches were performed on all three datasets with 1000 random addition replicates and TBR branch swapping. Bootstrap analyses (500 replicates, 10 random sequence additions per replicate) were conducted for all three datasets generating 50% Majority-Rule consensus trees for all three datasets.

Modeltest (Posada & Crandall, 1998) was used as a starting point for the Maximum Likelihood analyses of these three datasets. Using the Akaike Criterion,

model parameters were used in PAUP for each of the analyses. The most complex model, GTR+I+G was selected for all three datasets. Heuristic searches with random sequence addition and TBR branch swapping were used for the Maximum Likelihood analyses. Bootstrap analyses (500 replicates, 10 random addition sequences per replicate) produced 50% Majority-Rule concensus trees for all three datasets. Parsimony and Maximum Likelihood bootstrap values are generally discussed as being weakly supported (50-70%), moderately supported (71%-90%), or robustly supported (91%-100%).

Bayesian analyses were treated in a slightly more detailed fashion. In the Bayesian nexus files, DNA sequences were partitioned into stems and loops. Mr. Modeltest was then employed to delineate an appropriate evolutionary model for each of the stem and loop partitions in each of the datasets (12s, 16s, Combined). Under Akaike criterion, the most complex model of sequence evolution (GTR + I + G) was choosen for all but one of the stem and loop partitions, the 12s loop partition. The non-stem regions of the 12s gene fragment are AT rich regions that evolve under the HKY (Hasegawa) model of evolution. Mr. Bayes 3.0b4 (Huelsenbeck and Ronquist, 2001) program was used to conduct all Bayesian analyses for all three datasets. Each analysis consisted of four Markov chains (2 million generations each) sampled every 1000 generations, random starting trees, and default priors. The Tracer program (Rambaut & Drummond) was employed to determine a suitable burn-in percentage, and to calculate the mean –LnL score for each analysis. Only significant (95%) Posterior Probabilities are reported for Bayesian analyses.

# Chapter 3: Results

*The 12s dataset.*

The 'higher' Aleocharinae lineage was robustly supported to be a monophyletic lineage based on three separate analyses of the 12s dataset. All three analyses have shown that the subtribe, Gyrophaenina, is a monophyletic lineage. Parsimony and Maximum likelihood analyses supported this hypothesis with 67% and 77% confidence, respectively. Bayesian analysis produced a 99% Posterior Probability in support of a monophyletic Gyrophaenina clade. *Eumicrota* (represented by the type species *Eumicrota corruscula)*, the most basally derived genus in the Gyrophaenina clade, is hypothesized to be the sister group to the *Phanerota-Gyrophaena* lineage. In all three analyses, the genus *Gyrophaena* was shown to be paraphyletic with respect to *Phanerota.* Within the Gyrophaenina clade, the following sister-group relationships were moderately supported by all three analyses: (*Phanerota fasciata* + *Phanerota dissimilis), (Gyrophaena flavicornis* + *Gyrophaena vitirina*).

In the Maximum Likelihood and Bayesian trees (Figures 4 & 5), the myllaenine representative is hypothesized to be most closely related to the Gyrophaenina clade. This hypothesis did not receive any significant posterior probability or bootstrap support. In the Parsimony analysis, a sister-group relationship

was hypothesized between the myllaenine and pronomeanine tribes (Figure 3), although, this relationship did not receive any bootstrap support. This sister-group resolved well within the 'higher' aleocharine clade.

Monophyly of the athetine tribe was hypothesized by Parsimony and Bayesian methods (Figures 3 & 5). *Tachyusa* sp., an Oxypodine representative, was shown to be a basal ancestor of the Athetini clade. These relationships did not receive any significant posterior probability or bootstrap support. Within the Athetini clade, Parsimony analysis found weak bootstrap support for a sister-group relationship between *Sableta infulata* and *Leptonia* sp. Maximum Likelihood analysis did not resolve any significant relationship among the athetine representatives. The "*Oxypoda sp*" clade, hypothesized by Parsimony and Maximum Likelihood methods, did not receive any bootstrap support, and failed to resolve any discernable relationships among the tribes represented in this study.

*The 16s dataset.*

The 16s dataset did not support the 'higher' Aleocharinae as a monophyletic clade. All three analyses of this dataset recovered a monophyletic Gyrophaenina with moderate bootstrap support and significant posterior probabilities. Within this clade, Parsimony analysis hypothesized the genus *Eumicrota* to be a basal, sister lineage of the *Gyrophaena-Phanerota* lineage (Figure 6). Alternatively, Maximum likelihood analysis hypothesized this genus as the basal sister-group to all representatives of the genus *Gyrophaena* (Figure 7). In this analysis, the genus *Phanerota* was hypothesized to be the sister lineage to the *Eumicrota-Gyrophaena* clade. In the

Parsimony and Maximum Likelihood analyses, representatives of the genus *Gyrophaena* were hypothesized to be a monophyletic group with the following topology: (*G. flavicornis* + (*G. vitirina* + (*G. coniciventris* + *Gyrophaena*))). Parsimony bootstrap analysis strongly (97%) supported this monophyly, whereas, Maximum Likelihood bootstrap analysis failed to render any support for this clade. Bayesian analysis failed to resolve relationships among the genera in the Gyrophaenina clade (Figure 8). All three analyses strongly supported a sister-group relationship between the two *Phanerota* representatives. In the Maximum Likelihood analysis, the Myllaenini representative was hypothesized to be most closely related to the Gyrophaenina lineage. This hypothesis did not receive any bootstrap support, and Parsimony and Bayesian methods failed to corroborate this result.

A sister-group relationship between the two Deinopsini representatives, *Deinopsis erosa* and *Deinopsis illinoisensis,* was strongly supported by all three analyses. Maximum Likelihood and Bayesian methods robustly supported a sister-group relationship between the two Falagriini representatives, *Lissagria laeviscula* and *Myrmecophalus cingulatus.* Parsimony analysis failed to resolve this sister-group relationship. I was unable to obtain sequence data for the athetine representative, *Atheta* sp., and sequence data for *Leptonia* sp. was of poor quality. As a result, the 16s dataset only includes one athetine representative. None of the remaining clades represented in figures 7 and 8 were supported by significant bootstrap or posterior probability values, nor did they resolve any meaningful relationships.

*The Combined dataset.*

Pasimony analysis of the combined dataset weakly supported a monophyletic 'higher' Aleocharinae (Figure 9). Maximum Likelihood and Bayesian analyses of the combined dataset failed to recover a monophyletic 'higher' aleocharine clade (Figures 10&11). All three analyses recovered a monophyletic Gyrophaenina with, at least, 90% bootstrap support and a 95% posterior probability value. Within this clade, all three analyses hypothesized *Eumicrota* to be the basal sister lineage to the *Gyrophaena-Phanerota* clade. This hypothesis was strongly supported by bootstrap analysis and a significant (95%) posterior probability. In all three analyses, the genus *Gyrophaena* was recovered as a paraphyletic assemblage containing the genus *Phanerota*. The following sister-group relationships were strongly supported by all three analyses: (*Phanerota fasciata* + *Phanerota dissimilis), (Gyrophaena flavicornis* + *Gyrophaena vitirina*).

In the Parsimony analysis, the Myllaenini representative, *Myllaena* sp., is hypothesized to be a basal member of the 'higher' Aleocharinae, although this relationship was not supported in the bootstrap analysis. In the Maximum Likelihood analysis, representatives of Myllaenini and Pronomaenini were most closely related to the Gyrophaenina lineage, although bootstrap support for this result was lacking. In the Bayesian analysis, the Myllaenini representative was most closely related to the Gyrophaenina lineage, but without a significant posterior probability value. The Pronomeanini representative remained unresolved in the Bayesian analysis.

Maximum likelihood and Bayesian analyses supported a sister-group relationship between the two representatives of Falagriini with 100% confidence. The Parsimony analysis also resolved this relationship, but without bootstrap support. In the single combined parsimony tree and the Maximum Likelihood tree, the Oxypodini and Falagriini representatives form a monophyletic clade.

All three analyses also moderately supported a sister-group relationship between the two Athetini representatives. A sister-group relationship between the two representatives of Deinopsini was recovered by all three analyses with weak parsimony bootstrap support, strong Maximum Likelihood support, and a significant (96%) posterior probability value. In all three analyses, the clade containing *Strictalia* sp. did not receive any bootstrap support, and did not recover any disernable relationships among the tribes represented in this clade.

# Chapter 4: Conclusions

*The individual datasets.*

Monophyly of the 'higher' aleocharines was robustly supported by all three analyses of the 12s dataset. This result corroborates the morphological, phylogenetic studies that previously proposed this monophyletic group (Ashe & Newton, 1993; Ashe, 1994). Analysis of the 16s dataset failed to support a monophyletic 'higher' Aleocharinae. The subtribe, Gyrophaenina, was moderately supported as a monophyletic group by all three analyses of both, the 12s and 16s, datasets. The following topology (for the Gyrophaenina clade) was consistently recovered with moderate bootstrap and significant posterior probability values: (*Eumicrota* + (*Gyrophaena* + *Phanerota*)). These results fully support Ashe's (1984) morphological, phylogenetic revision of this subtribe. Within the Gyrophaenina lineage, Parsimony and Maximum Likelihood analysis of the 16s dataset recovered a monophyletic *Gyrophaena* clade. Bootstrap support for this clade was strong (97%). This clade was not resolved using Bayesian methods. None of the analyses of the 12s dataset recovered a monophyletic *Gyrophaena*. In all three analyses of the 12s dataset, the following sister-group relationships were robustly supported within the Gyrophaenina clade: (*Phanerota fasciata* + *Phanerota dissimilis), (Gyrophaena flavicornis* + *Gyrophaena vitirina*). All three analyses of the 16s dataset robustly supported a sister-group relationship between *Phanerota fasciata* and *Phanerota dissimilis*. Parsimony analysis of the 12s dataset also resolved a sister-group

relationship between Myllaenini and Pronomaenini, but without bootstrap support. This sister group was resolved well within the 'higher' aleocharine clade. The myllaenine tribe was hypothesized to be a basal ancestor of the gyrophaenina subtribe by Maximum Likelihood and Bayesian analyses of the 12s dataset, and Maximum Likelihood analysis of the 16s dataset. None of these hypotheses were supported by significant bootstrap or posterior probability values.

Analysis of the 12s dataset has shown that the representatives of the athetine clade form a monophyletic group, although support for this clade was lacking. The 16s dataset could not be used to hypothesize relationships among the Athetini representative because sequence data for two of the specimens was unattainable. A sister-group relationship between the representatives of Falagriini was strongly supported (100%) by Maximum Likelihood and Bayesian Analyses. All three analyses of the 16s dataset moderately supported a sister-group relationship between the two *Deinopsis* species.

*The Combined dataset.*

Pasimony analysis of the combined dataset weakly supported a monophyletic 'higher' Aleocharinae. Maximum Likelihood and Bayesian analyses of the combined dataset failed to recover a monophyletic 'higher' aleocharine clade. All three analyses robustly supported a monophyletic Gyrophaenina clade with the following topology: (*Eumicrota* + (*Gyrophaena* + *Phanerota*)). Within the Gyrophaenina clade, all three analyses strongly supported the following sister-group relationships: (*Phanerota fasciata* + *Phanerota dissimilis), (Gyrophaena flavicornis* + *Gyrophaena vitirina*).

Unlike the 16s dataset, none of the analyses of the combined dataset were able to recover a monophyletic *Gyrophaena* clade.

Maximum likelihood analysis resolved the closely related tribes, Myllaenini and Promnomaeini as basal ancestors of the Gyrophaenina clade. This result was not supported by significant bootstrap values. In the Bayesian analysis, the myllaenine representative was hypothesized to be a basal ancestor of the Gyrophaenina clade, although this relationship was not supported by a significant posterior probability value. Although bootstrap support was lacking, Parsimony analysis of the combined dataset resolved the myllaenine tribe as the basal member of the monophyletic 'higher' aleocharine clade. The results of this molecular investigation support the previous morphological hypothesis (Ashe, 2005) that the tribe, Myllaenini, is a member of the 'higher' aleocharine lineage.

All three analyses also moderately supported a sister-group relationship between the two Athetini representatives. All three analyses recovered a sister-group relationship between the two representatives of Deinopsini with weak parsimony bootstrap support, strong Maximum Likelihood bootstrap support, and a significant (96%) posterior probability values. A sister-group relationship between the to Falagriini representatives was robustly supported (100%) by Maximum Likelihood and Bayesian Analyses. Parsimony analysis also obtained this result, but without bootstrap support. Parsimony and Maximum likelihood analysis of the combined dataset suggest that oxypodine and falagriine tribes form a monophyletic clade. As outlined in the introduction, this result supports the historical hypothesis that

*Tachyusa* and allied oxypodine genera are closely related to the Falagriini (Bernhauer and Scheerpeltz, 1926).

*Discussion.*

Despite the paucity of informative characters, and the small size of the dataset, 12s and 16s rDNA genes did succeed in resolving relationships at the subfamilial and tribal level. Given the data presented here, it is not unreasonable to assume that the 16s rDNA gene is evolving at a slightly increased rate compared to the 12s rDNA gene. Namely, because 12s was able to consistently (in all three analyses) resolve the monophyly of the Aleocharinae, but was unable to resolve relationships among the genera of *Gyrophaena*. 16s, on the otherhand, could not resolve this deeper relationship (monophyly), but was able to obtain a monophyletic *Gyrophaena.*

Expanding the breath of gene coverage for this group would also greatly enhance our ability to derive a tribal level classification that hypothesizes the true evolutionary history for this seemingly endlessly diverse group of beetles. COI and COII were investigated during the early stages of this project. After experimenting with various primer pairs, it became apparent that COI/COII primers might have to be redesigned before moving forward with this particular gene. EF1alpha and 'wingless' (a nuclear gene) should also be investigated.

Beyond the limited gene coverage in this study, taxon sampling is an equally significant problem for this particular group of beetles, as well as other hyper-diverse insect lineages. Hillis et. al. (2002) reviewed several studies about the correlation between sparse taxon sampling and phylogenetic error. This review confirmed that, in

cases where the underlying evolutionary processes differ among sites, increased taxon sampling dramatically improves the accuracy of phylogenetic inference. It has been shown that the highly conserved stem and variable loop regions within the secondary structure of non-coding mitochondrial DNA (12s and 16s) molecules actually evolve under different models of evolution (Brandley et. al., 2005) Therefore, adequate taxon sampling is especially important for molecular phylogenetic studies that are based on the 12s and 16s mDNA genes. No matter what types of phylogenetic studies are being conducted on groups as large as the Aleocharinae, two representatives for tribes that contains hundreds of genera and thousands of species is, at best, immensely inadequate.

# Literature Cited

**Ahn, Kee-Jeong & Ashe, J. S. 1995**. Systematic Position of the Intertidal Genus Bryobiota Casey and a Revised Phlogeny of the Falagriine Genera of America North of Mexico (Coleoptera: Staphylinidae: Aleocharinae). Annals of the Entomological Society of America. Vol. 88, no. 2.

**Ashe, James S. 2001.** Chapter 22 Staphylinidae, Aleocharinae. American Beetles Volume 1 (Archostemata, Myxophaga, Adephaga, Polyphaga: Staphyliniformia. Copyright CRC Press.

**Ashe, James S. 1982.** Generic revision of the subtribe Gyrophaenina (Coleoptera: Staphylinidae: Aleocharinae) with a review of described subgenera and major features of evolution. PhD thesis. University of Alberta.

**Ashe, J.S. 1992**. Phylogeny and revision of genera if the subtribe Bolitocharina (Coleoptera: Staphylinidae: Aleocharinae). The University of Kansas Science Bulletin Vol. 54 (10): 335-406.

**Ashe, James S. and Newton, Al. 1993**. Larvae of *Trichophya* and phylogeny of the tachyporine group of subfamilies (Coleoptera: Staphylinidae) with a review, new species and characterization of the Trichophyinae. Systematic Entomology Vol. 18: 267-286.

**Ashe, J.S. 1994**. Evolution of aedeagal parameres of aleocharine staphylinids (Coleoptera: Staphylinidae: Aleocharinae). Canadian Entomologist, 126: 475-491.

**Ashe, James S. 1998.** Subfamily Aleocharinae. The Tree of Life: a distributed internet resource containing information about phylogeny and biodiversity. (http://phylogeny.arizona.edu/tree/eukaryotes/animals/arthropoda/hexapoda/coleopteran/polyphaga/staphylinoidea/staphylinidae/aleocharinae/aleocharinae.html).

**Ashe, James S. & Chatzimanolis, Stylianos. 2003**. Deinopsini. Version 06 November 2003. http://tolweb.org/Deinopsini/ 9815/ 2003.11.06 in The Tree of Life Web Project, http://tolweb.org/

**Ashe, J.S. and Gusarov, V. I. 2004.** Review of the genus *Sableta* Casey, 1910 (Coleoptera: Staphylinidae: Aleocharinae: Athetini). Zootaxa 745: 1-15.

**Ashe, James S. 2005**. Phylogeny of the tachyporine group subfamilies and 'basal' lineages of the Aleocharinae (Coleoptera: Staphylinidae) based on larval and adult characteristics. Systematic Entomology. Vol. 30. 3-37.

**Ballard ‚J. William O., Thayer, Margaret K. Newton, Alfred F. Jr., and Grismer, Elizabeth R. 1998.** Data Sets, Partitions, and Characters: Philosophies and Procedures for Analyzing Multiple Data Sets. Systematic Biology. Vol.47(3): 367-396.

**Bernhauer, M. and Scheerpeltz, O. 1926**. Coleopterorum catalogus, pars 82, Staphylinidae VI: 499-988. W. Junk ed., Berlin.

**Brandley, M.C., Schmitz, A., Reeder, T.D. 2005**. Partitioned Bayesian Analyses, Partition Choice, and the Phylogenetic Relationships of Scincid Lizards. Systematic Biology 54 (3): 373-390.

**T. R. Buckley, C. Simon, P. K. Flook, B. Misof. 2000**. Secondary structure and conserved motifs of the frequently sequenced domains IV and V of the insect mitochondrial large subunit rRNA gene. Insect Molecular Biology. Vol. 9(6), 565-580.

**Caterino, M.S., Hunt, T., Vogler, A.P. 2005**. On the constitution and phylogeny of Staphyliniformia (Insecta: Coleoptera). Molecular Phylogenetics and Evolution 34: 655-672.

**Danoff-Burg, J.A. 1994.** Evolving under myrmecophily: a cladistic revision of the symphilic beetle tribe Sceptobiini (Coleoptera: Staphylinidae: Aleocharinae). Systematic Entomology, 19: 25-45.

**Haasl, Ryan. 2005.** A Molecular Investigation of Tachyporine Group (Coleoptera: Staphylinidae) Phylogeny. Master's Thesis. University of Kansas.

**Hammond, P. M. 1975**. The phylogeny of a remarkable new genus and species of gymnusine staphylinid (Coleoptera) from the Auckland Islands. Journal of Entomology, (B) 44: 153-173.

**Hillis, D. M., Pollock, D. D., McGuire, J.A., Zwickl, D. J. 2003**. Is Sparse Taxon Sampling a Problem for Phylogenetic Inference? Systematic Biology. 52 (1): 124-126.

**Hoebeke, Richard E. 1985**. Revision of the Rove beetle tribe Falagriini of North America and Mexico (Coleoptera: Staphylinidae: Aleocharinae). Journal of the New York Entomological Society. 93 (2): 913-1018.

**Huelsenbeck, J. P. and F. Ronquist. 2001**. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

**Jacobson, H.R. & Kistner, D.H. 1991.** Cladistic study, taxonomic restructuring, and revision of a myrmecophilous tribe Leptanillophilini with comments on its evolution and host relationships (Coleoptera: Staphylinidae; Hymenoptera: Formicidae). Sociobiology 18: 1-150.

**Klimaszewski, J. 1982b**. A redefinition of the Myllaenini Ganglbauer and redescriptions of **Camacopalpus** Motschulsky and **Polypea** Fauvel (Coleoptera: Staphylinidae). Can. Entomol. 114: 411-429.

**Maus, C., Peschke, K., Dobler, S. 2001.** Phylogeny of the Genus Aleochara Inferred from Mitochondrial Cytochrome Oxidase Sequences (Coleoptera: Staphylinidae). Molecular Phylogenetics and Evolution Vol.18 (2): 202-216.

**Newton, A.F., Jr., and Thayer, M.K., 1992**. Current classification and family group names in Staphyliniformia (Coleoptera), Fieldiana (Zoology), New Series, 67: 1-92.

**Posada D and Crandall KA 1998**. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9): 817-818.

**Rambaut A, Drummond A, J. 2005.** Tracer: A program for analysing results from Bayesian MCMC programs such as BEAST & MrBayes. [http://evolve.zoo.ox.ac.uk/software.html?id=tracer].

**Rasmussen, C. & Cameron, S. A. 2007**. A molecular phylogeny of the Old World stingless bees (Hymenoptera: Apidae: Meliponini) and the non-monophyly of the large genus Trigona. Systematic Entomology 32: 26-39.

**Ronquist, F. and J. P. Huelsenbeck. 2003.** MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

**Seevers, C.H. 1957**. A monograph on the termitophilous Staphylinidae. Fieldiana: Zoology. 40: 1-334.

**Seevers, C. H. 1978**. A generic and tribal revision of the North American Aleocharinae (Coleoptera: Staphylinidae). Fieldiana: Zoology 71: vi - 275.

**Seevers, C.H. 1965**. The systematics, evolution, and zoogeography of staphylinid beetles associated with army ants. Fieldiana: Zoology, 47: 139-351.

**Steidle, J. L. M. and K. Dettner. 1993.** Chemistry and morphology of the tergal gland of freeliving Aleocharinae (Coleoptera: Staphylinidae). Syst. Entomol. 18: 149-168.

**Swofford, D.L. 2002.** PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

**Yoshizawa, Kazunori & Johnson, K. P. 2003**. Phylogenetic position of Phthiraptera (Insecta: Paraneoptera) and elevated rate of evolution in mitochondrial 12S and 16S rDNA. Molecular Phylogenetics and Evolution 29: 102-114.

**Table 2: Locality information for the taxa included in this analysis.**

| *Taxon* | *Tribe* | *Locality* |
|---|---|---|
| *Aleochara valida* | Aleocharini | Extraction from Smith Lab, 2004 Maricopa Co., AZ |
| *Atheta* sp. | Athetini | Extraction from Smith Lab, 2004. Douglas Co., KS |
| *Leptonia* | Athetini | C.R. La Selva June, 1995 |
| *Sableta infalata* | Athetini | GSMNP July 2005 |
| *Eumicrota corruscula* | Homalotini | GSMNP July 2005 |
| *Gyrophaena coniciventris* | Homalotini | GSMNP July 2005 |
| *Gyrophaena flavicornis* | Homalotini | GSMNP July 2005 |
| *Gyrophaena vitirina* | Homalotini | GSMNP July 2005 |
| *Gyrophaena* sp. | Homalotini | GSMNP July 2005 |
| *Leptusa opaca* | Homalotini | GSMNP July 2005 |
| *Phanerota dissimilis* | Homalotini | GSMNP July 2005 |
| *Phanerota fasciata* | Homalotini | GSMNP July 2005 |
| *Strictalia* sp. | Homalotini | CA., Calaveras Co., July, 2005 |
| *Tachyusa* sp. | Oxypodini | Illinois, Rend Lake, July 2005 |
| *Oxypoda* sp. | Oxypodini | Extraction, Smith Lab, 2004 |
| *Myllaena* sp. | Myllaenini | Illinois, Rend Lake, July 2005 |
| *Pseudomniophila* sp. | Pronomaeini | C.R. La Selva,June, 1995 |
| *Lissagria laeviscula* | Falagriini | CA., Calaveras Co., July,2005 |
| *Myrmecocephalus cingulatus* | Falagriini | GSMNP,July 2005 |
| *Deinopsis erosa* | Deinopsini | Extraction, Smith Lab, 2004. Limburg Prov., Holland |
| *Deinopsis illinoisensis* | Deinopsini | Extraction, Smith Lab, 2004. Douglas Co., KS |
| *Adinopsis* sp. | Deinopsini | Extraction, Smith lab, 2004. Heredia Prov., Costa Rica |

## Figure Captions

**Figure 1.** Currently accepted Tachyporine group phylogeny based on Ashe's recent treatment of these subfamilies.

**Figure 2.** Currently accepted phylogeny of the 'basal Aleocharinae' after Ashe, 2005 and Haasl, 2005.

**Figure 3.** One of 19 trees most parsimonious trees (tree length = 579, CI = 46, RI = 41) based on the 12s dataset. Bootstrap values are included for supported clades.

**Figure 4.** Maximum Likelihood ( –Ln likelihood score = 2287) tree based on the 12s dataset. Bootstrap values are included for supported clades.

**Figure 5.** 50% Majority-Rule consensus tree generated from 4 separate Bayesian Analyses with a mean –LnL score of 2275. All Posterior probability values are included for supported clades.

**Figure 6.** Bootstrap 50% Majority-Rule consensus tree (tree length 729, CI= 46, RI=41) for the 16s dataset.

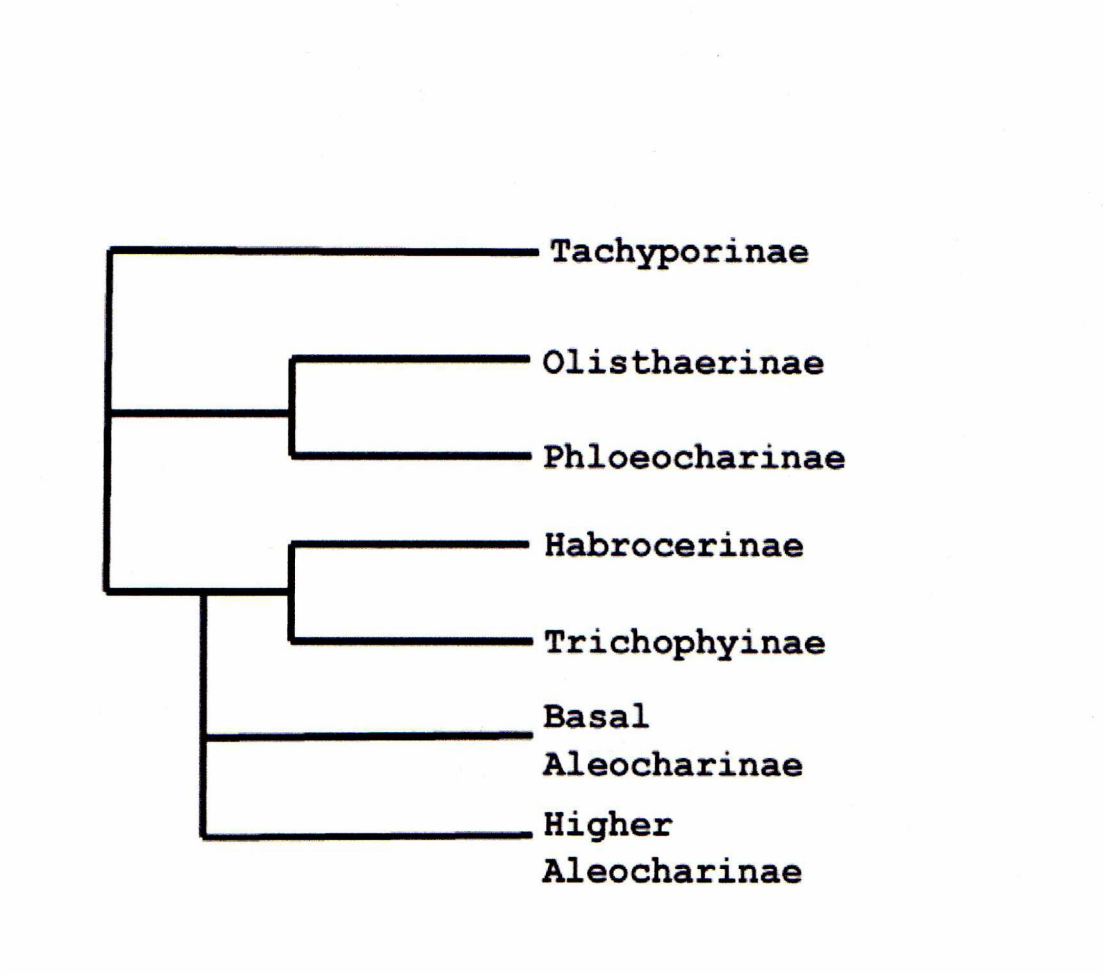
**Figure 7.** Maximum Likelihood tree for the 16s dataset with a –LnL score of 2439. Bootstrap values are included for supported clades.

**Figure 8.** 50% Majority-Rule consensus tree generated from 4 separate Bayesian analyses with a mean –LnL score of 3276. All Posterior probability values are included for supported clades.

**Figure 9.** Single most parsimonious tree (tree length = 1113, CI = 53, RI = 43) for the combined dataset. Bootstrap values are included for supported clades.

**Figure 10.** Maximum Likelihood analysis of the combined dataset produced one tree with a LnL score of 5391. Bootstrap values are included for supported clades.

**Figure 11.** 50% Majority-Rule consensus tree (–LnL of 5315) generated from 4 separate Bayesian Analyses of the combined dataset. All Posterior probability values are included for supported clades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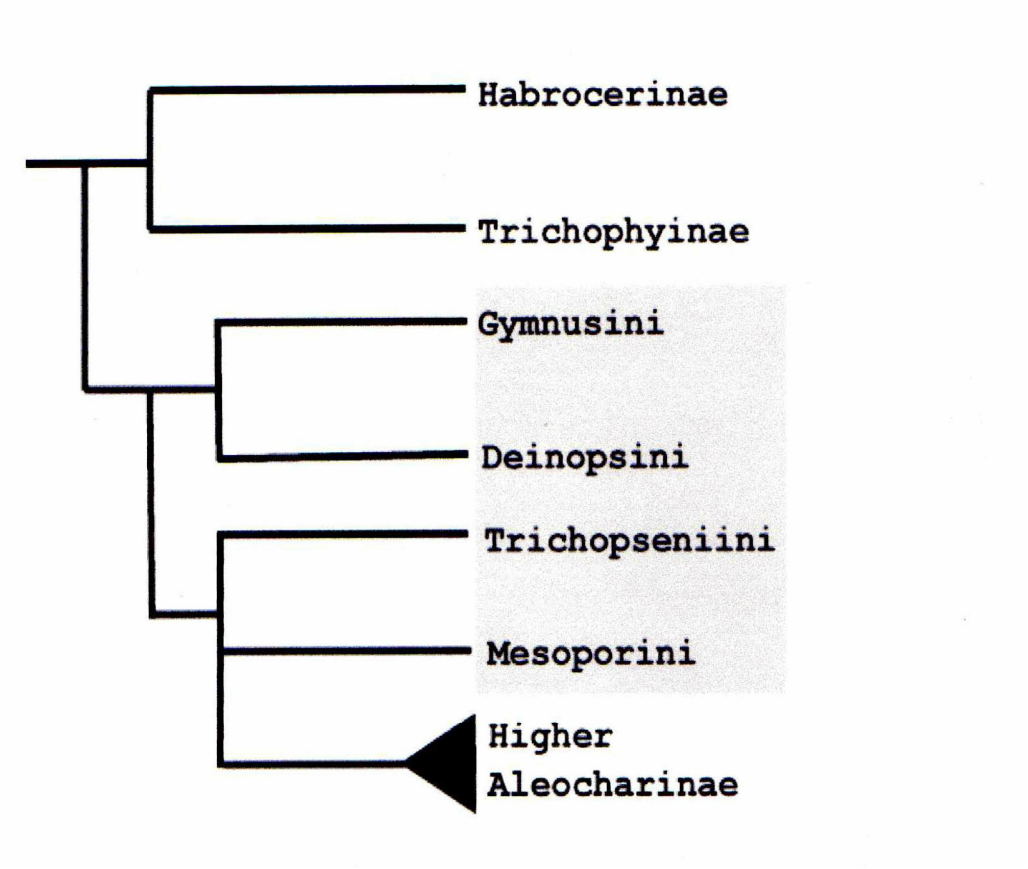
Olisthaerinae
Phloeocharinae
Habrocerinae
Trichophyinae
Basal
Aleocharinae
Higher
Aleocharinae

Figure 1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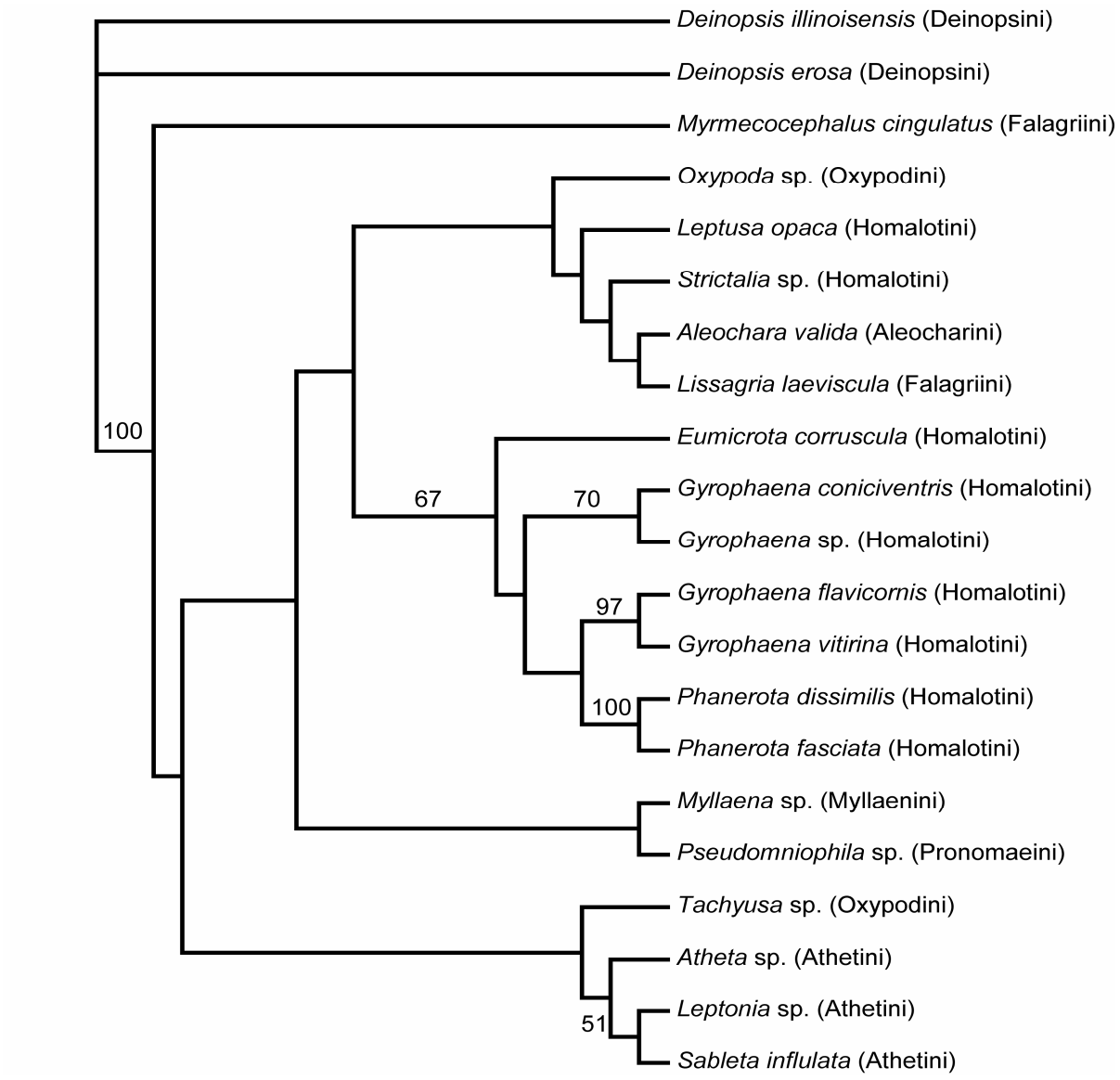
Trichopseniini
Mesoporini
Higher
Aleocharinae

Figure 2


Deinopsis illinoisensis (Deinopsini)
Deinopsis erosa (Deinopsini)
Myrmecocephalus cingulatus (Falagriini)
Oxypoda sp. (Oxypodini)
Leptusa opaca (Homalotini)
Strictalia sp. (Homalotini)
Aleochara valida (Aleocharini)
Lissagria laeviscula (Falagriini)
Eumicrota corruscula (Homalotini)
Gyrophaena coniciventris (Homalotini)
Gyrophaena sp. (Homalotini)
Gyrophaena flavicornis (Homalotini)
Gyrophaena vitirina (Homalotini)
Phanerota dissimilis (Homalotini)
Phanerota fasciata (Homalotini)
Myllaena sp. (Myllaenini)
Pseudomniophila sp. (Pronomaeini)
Tachyusa sp. (Oxypodini)
Atheta sp. (Athetini)
Leptonia sp. (Athetini)
Sableta influlata (Athetini)
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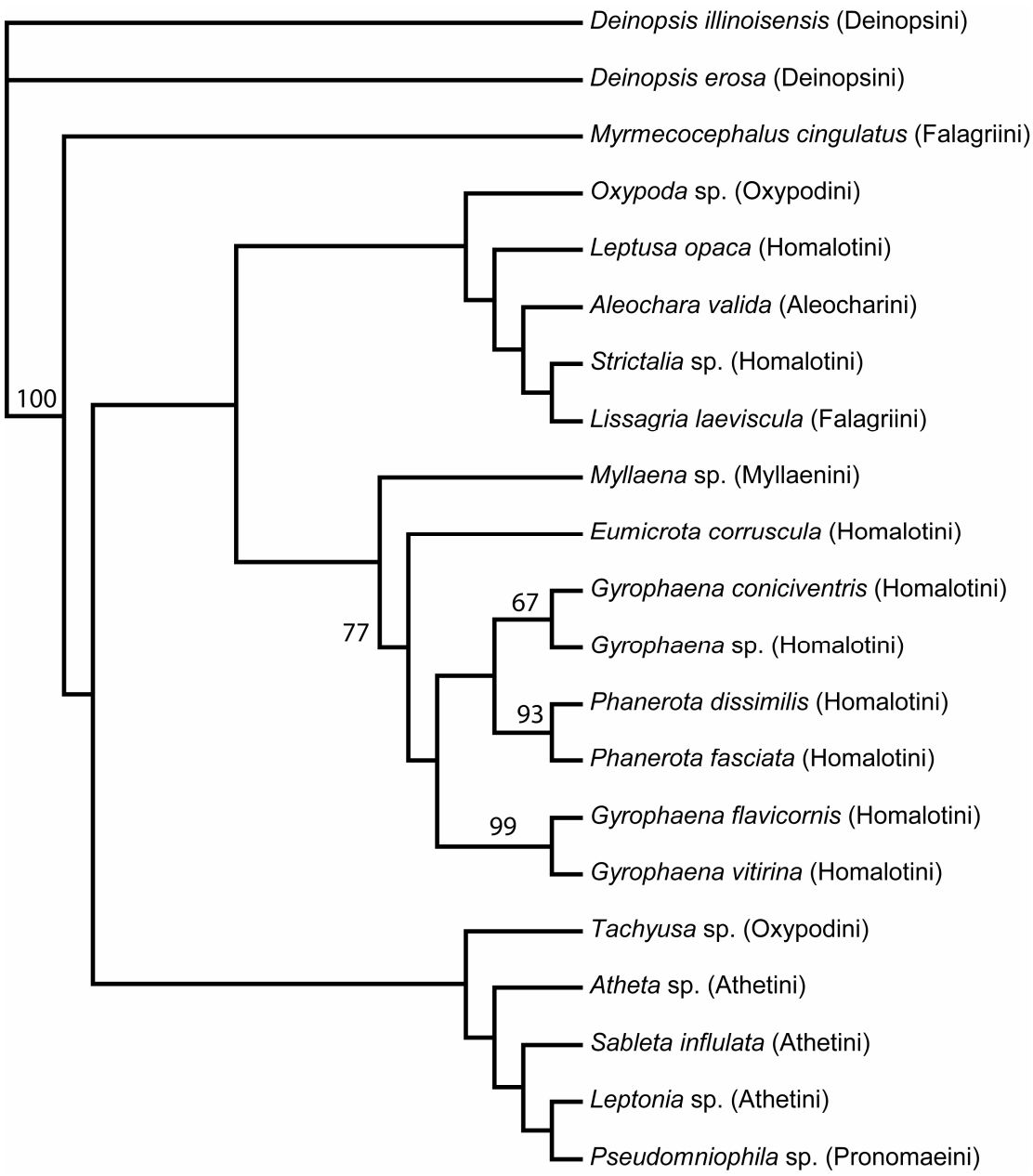
67
70
97
100
51

Figure 3


*Deinopsis illinoisensis* (Deinopsini)
*Deinopsis erosa* (Deinopsini)
*Myrmecocephalus cingulatus* (Falagriini)
*Oxypoda* sp. (Oxypodini)
*Leptusa opaca* (Homalotini)
*Aleochara valida* (Aleocharini)
*Strictalia* sp. (Homalotini)
*Lissagria laeviscula* (Falagriini)
*Myllaena* sp. (Myllaenini)
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*Phanerota fasciata* (Homalotini)
*Gyrophaena flavicornis* (Homalotini)
*Gyrophaena vitirina* (Homalotini)
*Tachyusa* sp. (Oxypodini)
*Atheta* sp. (Athetini)
*Sableta influlata* (Athetini)
*Leptonia* sp. (Athetini)
*Pseudomniophila* sp. (Pronomaeini)
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77
67
93
99

Figure 4

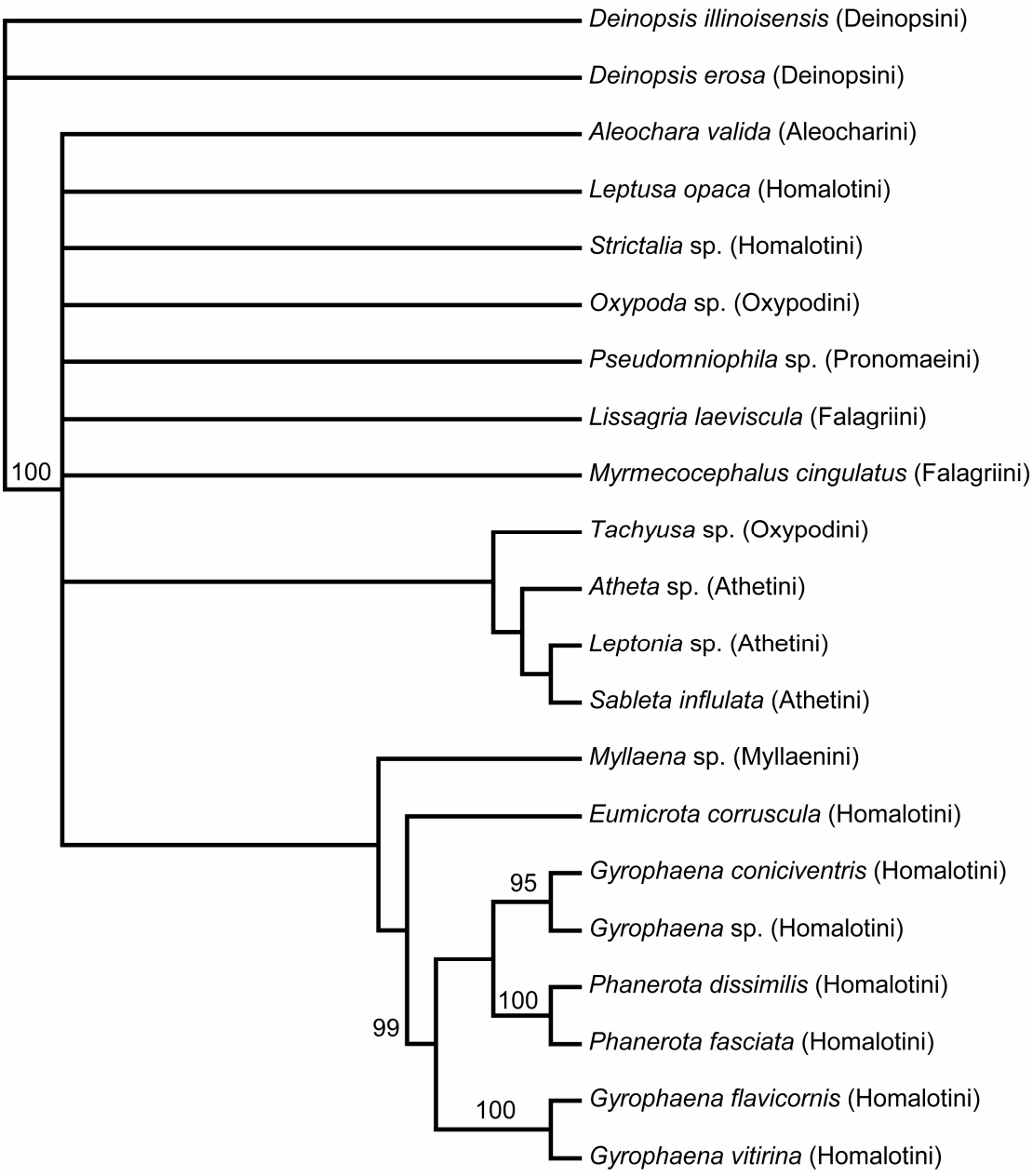
*Deinopsis illinoisensis* (Deinopsini)
*Deinopsis erosa* (Deinopsini)
*Aleochara valida* (Aleocharini)
*Leptusa opaca* (Homalotini)
*Strictalia* sp. (Homalotini)
*Oxypoda* sp. (Oxypodini)
*Pseudomniophila* sp. (Pronomaeini)
*Lissagria laeviscula* (Falagriini)
100
*Myrmecocephalus cingulatus* (Falagriini)
*Tachyusa* sp. (Oxypodini)
*Atheta* sp. (Athetini)
*Leptonia* sp. (Athetini)
*Sableta influlata* (Athetini)
*Myllaena* sp. (Myllaenini)
*Eumicrota corruscula* (Homalotini)
95
*Gyrophaena coniciventris* (Homalotini)
*Gyrophaena* sp. (Homalotini)
100
*Phanerota dissimilis* (Homalotini)
99
*Phanerota fasciata* (Homalotini)
100
*Gyrophaena flavicornis* (Homalotini)
*Gyrophaena vitirina* (Homalotini)

Figure 5

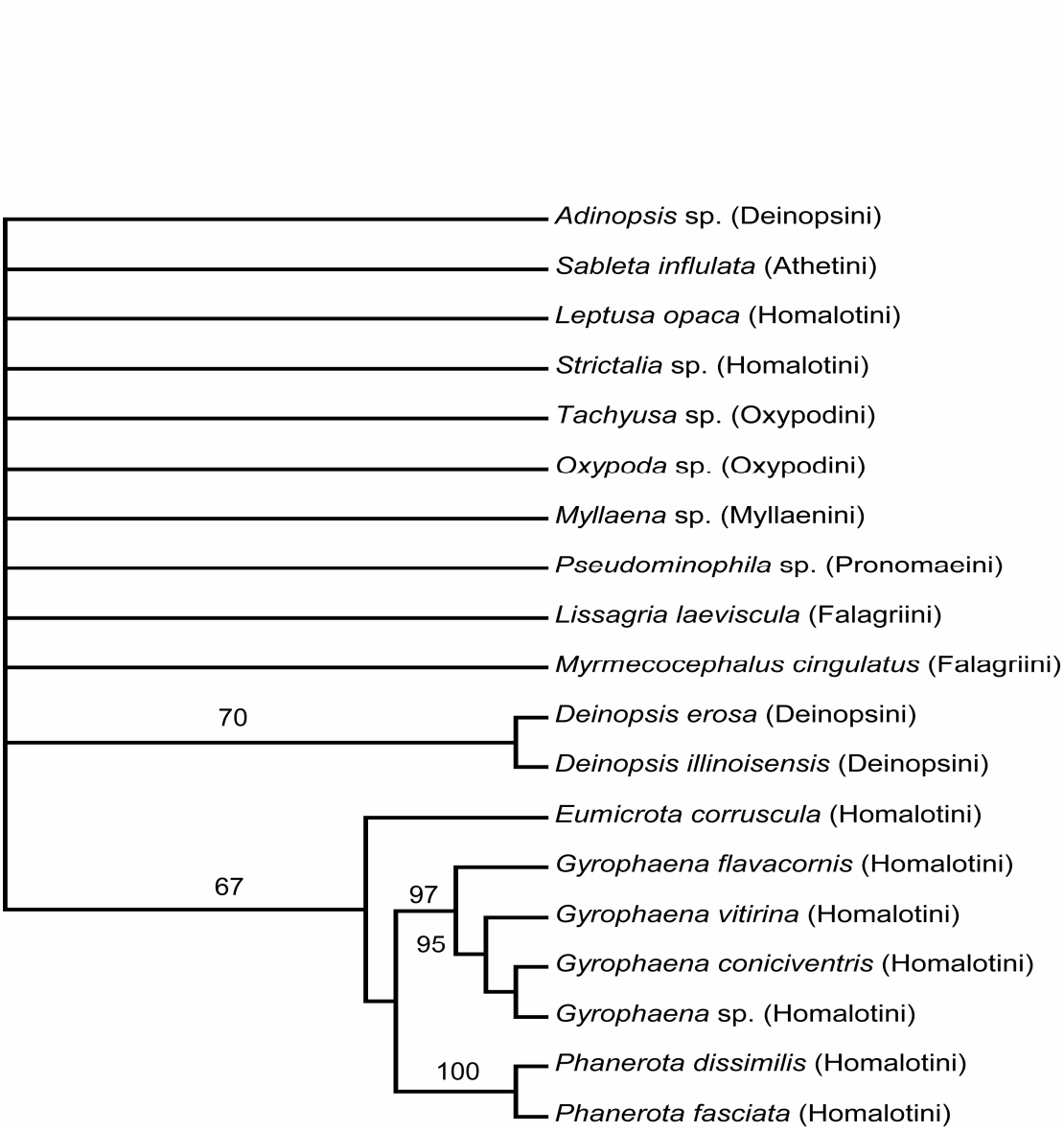
*Adinopsis* sp. (Deinopsini)
*Sableta influlata* (Athetini)
*Leptusa opaca* (Homalotini)
*Strictalia* sp. (Homalotini)
*Tachyusa* sp. (Oxypodini)
*Oxypoda* sp. (Oxypodini)
*Myllaena* sp. (Myllaenini)
*Pseudominophila* sp. (Pronomaeini)
*Lissagria laeviscula* (Falagriini)
*Myrmecocephalus cingulatus* (Falagriini)
70
*Deinopsis erosa* (Deinopsini)
*Deinopsis illinoisensis* (Deinopsini)
*Eumicrota corruscula* (Homalotini)
*Gyrophaena flavacornis* (Homalotini)
67
97
*Gyrophaena vitirina* (Homalotini)
95
*Gyrophaena coniciventris* (Homalotini)
*Gyrophaena* sp. (Homalotini)
100
*Phanerota dissimilis* (Homalotini)
*Phanerota fasciata* (Homalotini)

Figure 6

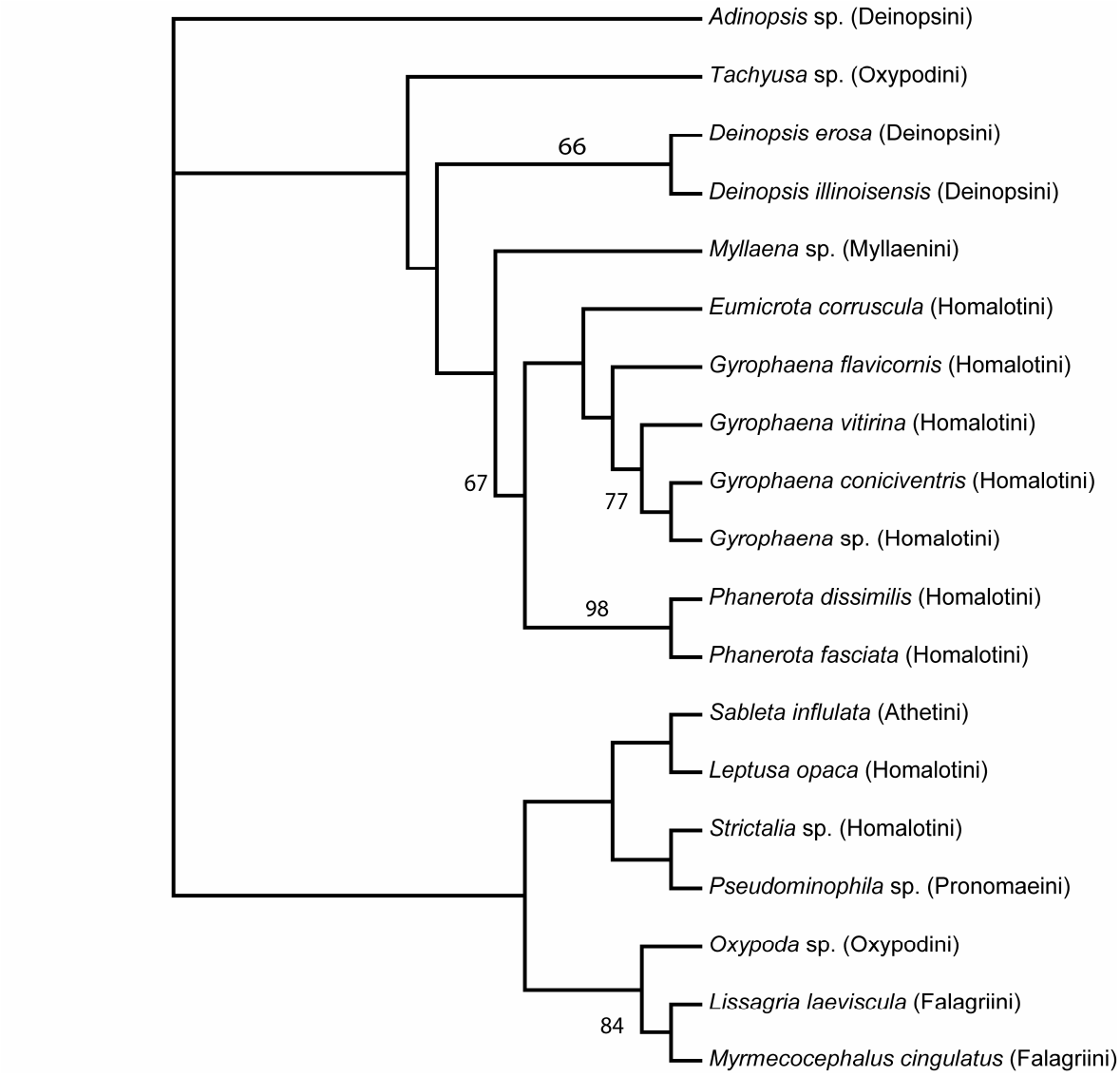
Adinopsis sp. (Deinopsini)
Tachyusa sp. (Oxypodini)
66
Deinopsis erosa (Deinopsini)
Deinopsis illinoisensis (Deinopsini)
Myllaena sp. (Myllaenini)
Eumicrota corruscula (Homalotini)
Gyrophaena flavicornis (Homalotini)
Gyrophaena vitirina (Homalotini)
67
77
Gyrophaena coniciventris (Homalotini)
Gyrophaena sp. (Homalotini)
98
Phanerota dissimilis (Homalotini)
Phanerota fasciata (Homalotini)
Sableta influlata (Athetini)
Leptusa opaca (Homalotini)
Strictalia sp. (Homalotini)
Pseudominophila sp. (Pronomaeini)
Oxypoda sp. (Oxypodini)
Lissagria laeviscula (Falagriini)
84
Myrmecocephalus cingulatus (Falagriini)

Figure 7

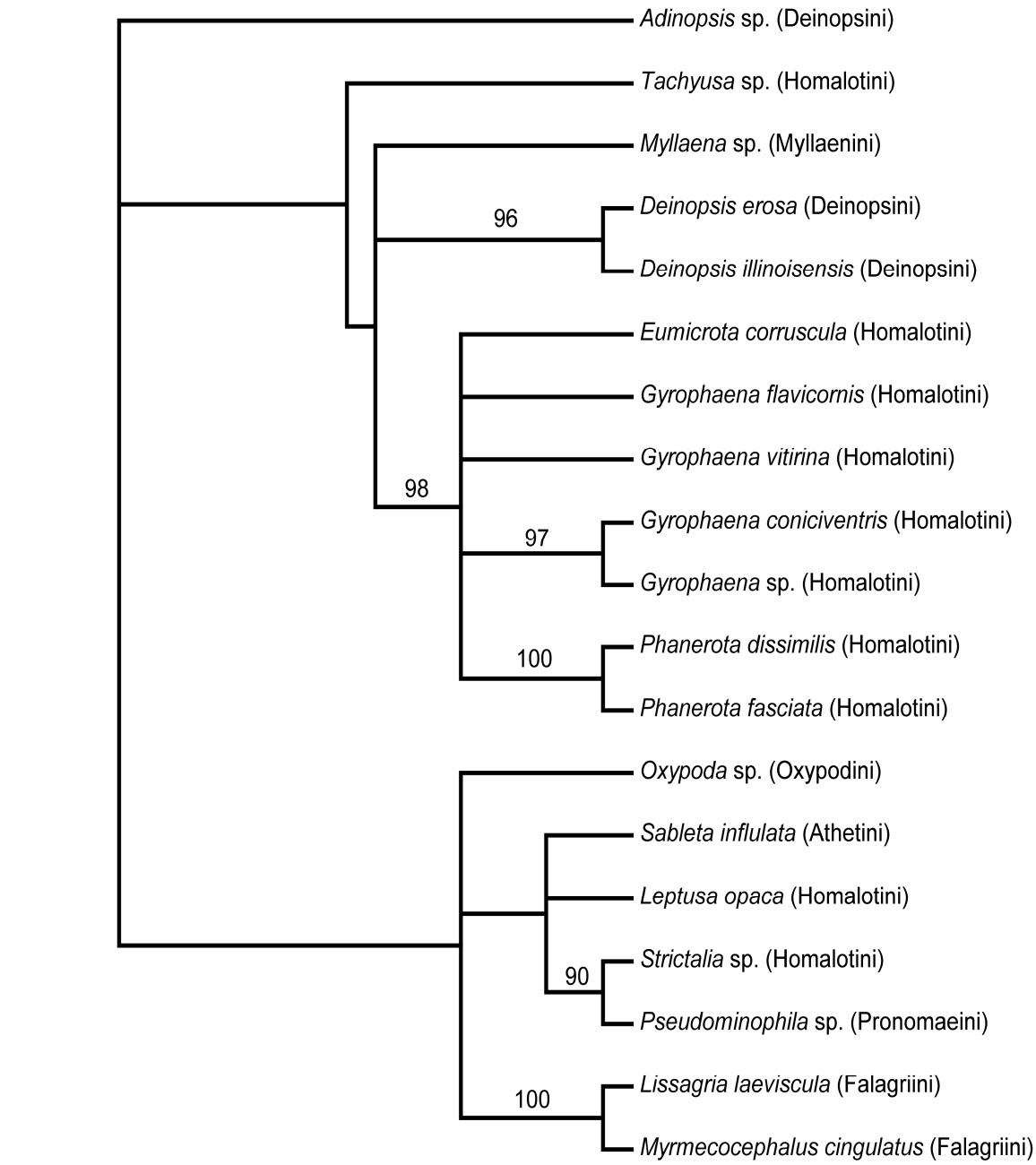
Adinopsis sp. (Deinopsini)
Tachyusa sp. (Homalotini)
Myllaena sp. (Myllaenini)
96
Deinopsis erosa (Deinopsini)
Deinopsis illinoisensis (Deinopsini)
Eumicrota corruscula (Homalotini)
Gyrophaena flavicornis (Homalotini)
Gyrophaena vitirina (Homalotini)
98
Gyrophaena coniciventris (Homalotini)
97
Gyrophaena sp. (Homalotini)
Phanerota dissimilis (Homalotini)
100
Phanerota fasciata (Homalotini)
Oxypoda sp. (Oxypodini)
Sableta influlata (Athetini)
Leptusa opaca (Homalotini)
Strictalia sp. (Homalotini)
90
Pseudominophila sp. (Pronomaeini)
Lissagria laeviscula (Falagriini)
100
Myrmecocephalus cingulatus (Falagriini)

Figure 8

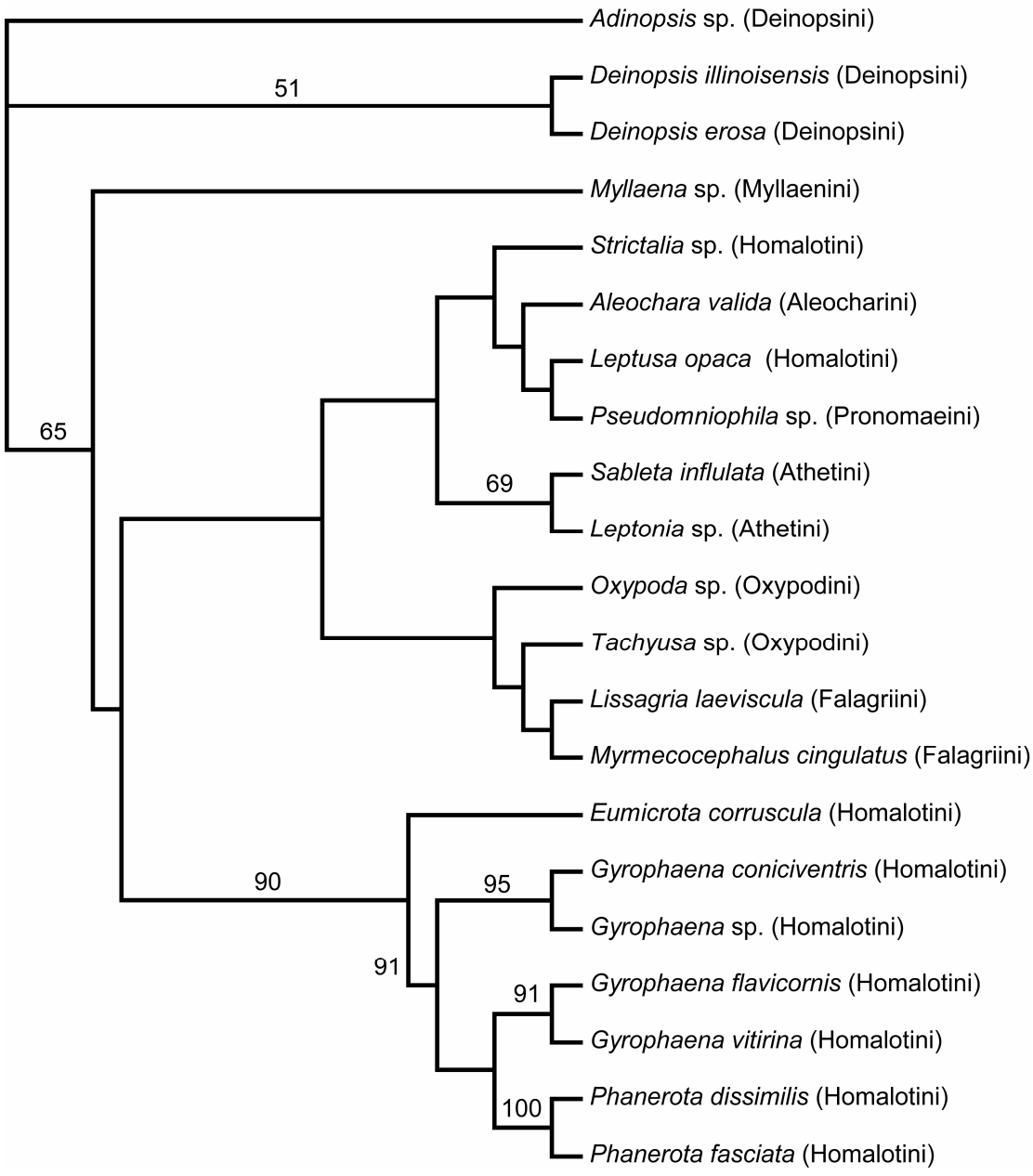
Adinopsis sp. (Deinopsini)
Deinopsis illinoisensis (Deinopsini)
Deinopsis erosa (Deinopsini)
Myllaena sp. (Myllaenini)
Strictalia sp. (Homalotini)
Aleochara valida (Aleocharini)
Leptusa opaca (Homalotini)
Pseudomniophila sp. (Pronomaeini)
Sableta influlata (Athetini)
Leptonia sp. (Athetini)
Oxypoda sp. (Oxypodini)
Tachyusa sp. (Oxypodini)
Lissagria laeviscula (Falagriini)
Myrmecocephalus cingulatus (Falagriini)
Eumicrota corruscula (Homalotini)
Gyrophaena coniciventris (Homalotini)
Gyrophaena sp. (Homalotini)
Gyrophaena flavicornis (Homalotini)
Gyrophaena vitirina (Homalotini)
Phanerota dissimilis (Homalotini)
Phanerota fasciata (Homalotini)
51
65
69
90
95
91
91
100

Figure 9

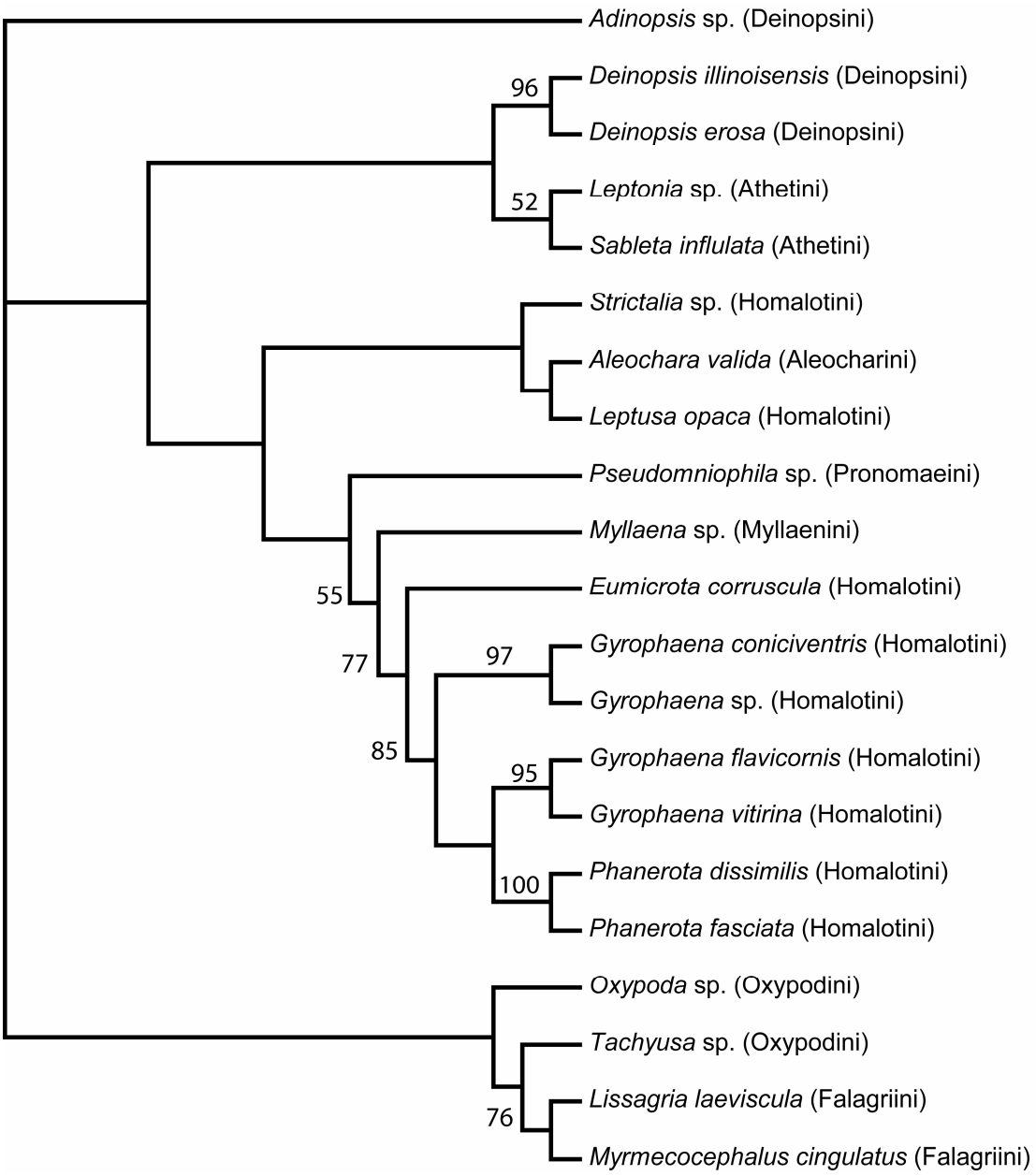
*Adinopsis* sp. (Deinopsini)
96
*Deinopsis illinoisensis* (Deinopsini)
*Deinopsis erosa* (Deinopsini)
52
*Leptonia* sp. (Athetini)
*Sableta influlata* (Athetini)
*Strictalia* sp. (Homalotini)
*Aleochara valida* (Aleocharini)
*Leptusa opaca* (Homalotini)
*Pseudomniophila* sp. (Pronomaeini)
*Myllaena* sp. (Myllaenini)
55
*Eumicrota corruscula* (Homalotini)
77
97
*Gyrophaena coniciventris* (Homalotini)
*Gyrophaena* sp. (Homalotini)
85
95
*Gyrophaena flavicornis* (Homalotini)
*Gyrophaena vitirina* (Homalotini)
100
*Phanerota dissimilis* (Homalotini)
*Phanerota fasciata* (Homalotini)
*Oxypoda* sp. (Oxypodini)
*Tachyusa* sp. (Oxypodini)
76
*Lissagria laeviscula* (Falagriini)
*Myrmecocephalus cingulatus* (Falagriini)

Figure 10

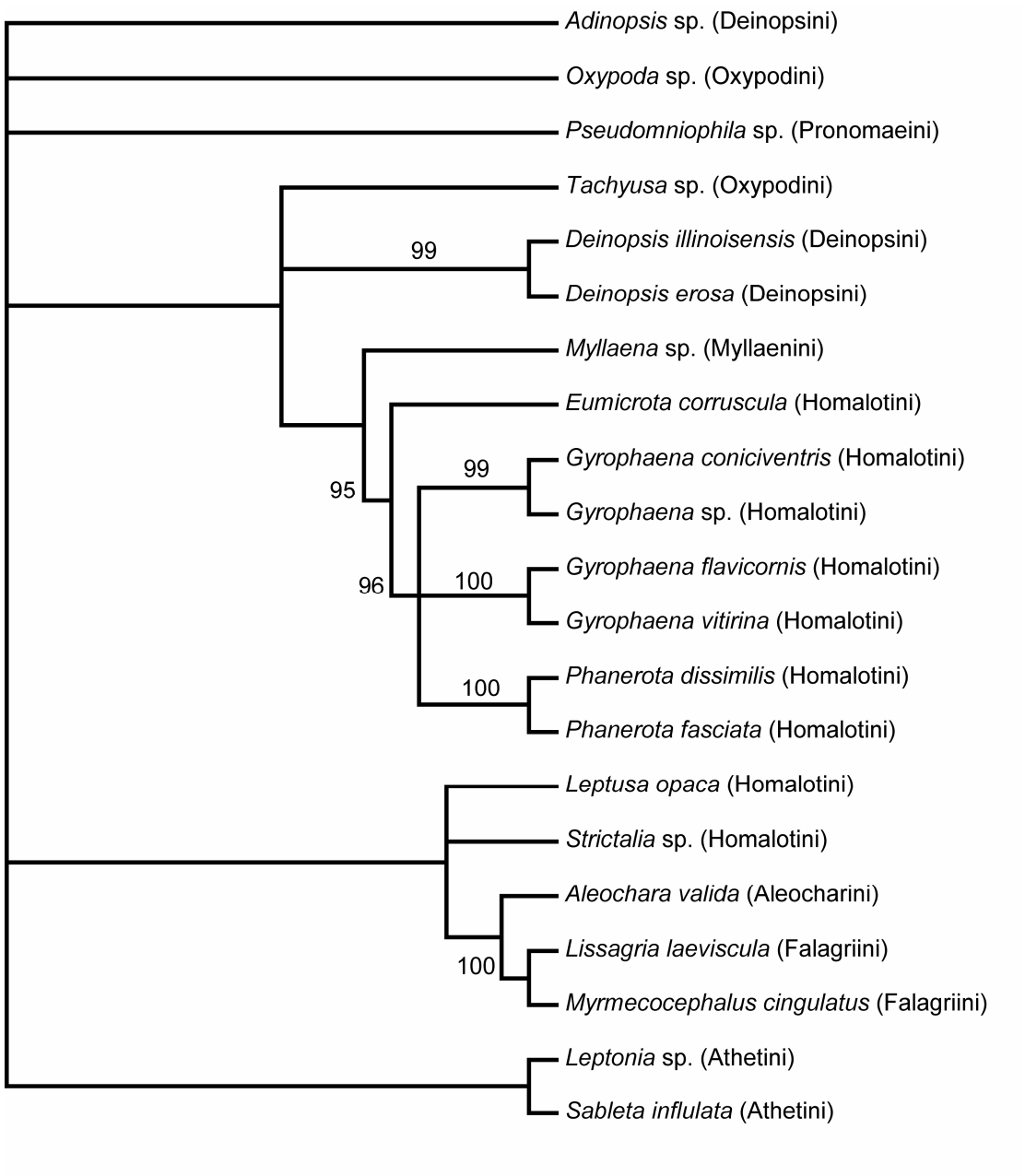
*Adinopsis* sp. (Deinopsini)
*Oxypoda* sp. (Oxypodini)
*Pseudomniophila* sp. (Pronomaeini)
*Tachyusa* sp. (Oxypodini)
99
*Deinopsis illinoisensis* (Deinopsini)
*Deinopsis erosa* (Deinopsini)
*Myllaena* sp. (Myllaenini)
*Eumicrota corruscula* (Homalotini)
99
95
*Gyrophaena coniciventris* (Homalotini)
*Gyrophaena* sp. (Homalotini)
96
100
*Gyrophaena flavicornis* (Homalotini)
*Gyrophaena vitirina* (Homalotini)
100
*Phanerota dissimilis* (Homalotini)
*Phanerota fasciata* (Homalotini)
*Leptusa opaca* (Homalotini)
*Strictalia* sp. (Homalotini)
*Aleochara valida* (Aleocharini)
100
*Lissagria laeviscula* (Falagriini)
*Myrmecocephalus cingulatus* (Falagriini)
*Leptonia* sp. (Athetini)
*Sableta influlata* (Athetini)

Figure 11

Table 3: Alignment of the 12s rDNA fragment of the 21 taxa used in this phylogenetic study.

```
                                 1111111111222222222233333333334444444444555555555566666666667777
Taxon/Node                       1234567890123456789012345678901234567890123456789012345678901234567890123456789012
-------------------------------------------------------------------------------------------------------------------
 Constant:                         *                     *  *** *****   *************** **     *******
    Uninf:                       ****** *    *    *     * *  *** *****   *************** **  ** *******
 Excluded:                       ********************************************
-------------------------------------------------------------------------------------------------------------------
Deinopsis illinoisensis          ??????????????AATTTATTTTTTGAAAKTTAAAGGTTTTGGCGGTATTTTAGTCTTTTTAGAGGAACT
Deinopsis erosa                  ???????GAGTAGTAATTTATTTTTTAGAAAGTTAAAGGTTTTGGCGGTATTTTAGTCTTTTTAGAGGAACT
Aleochara valida                 ???????????AATAGTTATAATCTATAAAATTAAAAAATTTGGCGGTATTTTAGTCTTTTTAGAGGAACC
Atheta sp.                       ????????????ATAGTTATATTCTTAAAAGTTAAAAATTTTGGCGGTATTTTAATCTACTCAGAGGAACC
Leptonia sp.                     ???????????????????TATAGTCTTTAAAGTTAAAAAATTTGGCGGTATTTTAATCTATCCAGAGGAACC
Sableta sp.                      ???AAATTATTAATATRTTATARTCTTTAAAGTTAAAAAATTTGGCGGTATTTTAATCTCTTCAGAGGAACC
Eumicrota corruscula             ???????????AATAGATAAAGTCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCCTTTCAGAGGAACC
Gyrophaena coniciventris         ???????????AATAGTTAAAGTCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCTTTTCAGAGGAACC
Gyrophaena flavicornis           ???????TAGGTAATAGTTAAAATCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCCTTTCAGAGGAACC
Gyrophaena vitirina              ??????????TGAATAGTTAAAATCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCTTTTCAGAGGAACC
Gyrophaena sp.                   ????????????????????AGAGTCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCTTTTCAGAGGAACC
Leptusa opaca                    ?????????????????????GGTCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCTATTCAGAGGAACC
Phanerota dissimilis             ???????TATTAATRGTTATAGTCTTATAAAATTAAAAAATTTGGCGGTATTTTAGTCTTTTCAGAGGAATC
Phanerota fasciata               ??????????????????????????????????AATTAARRAATTTGGSGGTATTTTAGTCTTTTCAGAGGAATC
Strictalia sp.                   ????????????????GAGTTATAGTCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCTTTTCAGAGGAACC
Tachyusa sp.                     ????AATTGAGTAATAGTTATAGTCTTAAAAGTTAAAAAATTTGGCGGTATTTTAATCTATTCAGAGGAACC
Oxypoda sp.                      ???TAAGWTWTWAAAAGTTATTGTCTAGAAAGTTAAAGATTTTGGCGGTATTTTAGTCTTTTCAGAGGAACC
Myllaena sp.                     TAAGGGRWTATTAATAGTTATGTTCTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCAATTCAGAGGAATC
Pseudomniophilas sp.             GKRWRRTKRAKWWAMGKWRTATTTCTCTAWWTTTAAAGATTTTGGCGGTATTTTATTCTATTTAGAGGAATC
Lissagria laeviscula             ????????TATTAATAGTTATATACTTTAAAATTAAAAAATTTGGCGGTATTTTAGTCTATTCAGAGGAACC
Myrmecocephalus cing.            ???????????AATAGTTATATTCTTAAAAGTTAAAAATTTTGGCGGTATTTTAGTCTATTCAGAGGAACT
```

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                                                                  1111111111111111111111111111111111111111111111111
                                 7777777888888888899999999990000000000111111111122222222233333333334444444
Taxon/Node                       3456789012345678901234567890123456789012345678901234567890123456789012345678901234
-------------------------------------------------------------------------------------------------------------------
 Constant:                       ***    **** **** ******** *     ****   **   * ***  ***** * *** * ********
    Uninf:                       ***    ********* ******** *     **** * ***  * ***  ******* *** * ********
-------------------------------------------------------------------------------------------------------------------
Deinopsis illinoisensis          TGTCCTATAATTGATATTCCACGATAAATTTTACTTTTATTAGAAGTTTACATATCGTCGTAGTTTGAATAT
Deinopsis erosa                  TGTCCTATAATTGATATTCCACGATAAATTTTACTTTTATTAGAAGTTTACATATCGTCGTAGTTTGAATAT
Aleochara valida                 TGTTCTGTAATTGATAATCCACGATTAGAAATACTTATTTTAAAAATTTATATATCGTTGTAGTT-GAATAT
Atheta sp.                       TGTCCTATAATTGATATTCCACGATTAAATTAACTTGTTTTAAAAATTTGTATATCGTCGTAGTT-GAATAT
Leptonia sp.                     TGTCCTGTAATTGATATTCCACGATTAAATTGACTTATATTAAAAATTTACATATCGTCGTAGTT-GAATAT
Sableta sp.                      TGTTCTATAATTGATATTCCACGATTAAATTAACTTATGTTAGAAATTTGCATATCGTCGTAATT-GAATAT
Eumicrota corruscula             TGTCCTATAATTGATAATCCACGATTAGAATTACTTTTGTTAAAAATTTGTATATCGTTGTAGTT-GAATAT
Gyrophaena coniciventris         TGTTCTATAATTGATAATCCACGATTAGAATTACTTGTATTAAAAATTTGTATATCGTTGTAGTT-GAATAT
Gyrophaena flavicornis           TGTCCTATAATTGATAGTCCACGATTAGTATTACTTATATTAAAAATTTGTATATCGTTGTAGTT-GAATAT
Gyrophaena vitirina              TGTCCTATAATTGATAGTCCACGATTAGTGATACTTATATTAATAATTTGTATATCGTTGTAGTT-GAATAT
Gyrophaena sp.                   TGTTCTGTAATTGATAATCCACGATTAGAATTACTTATATTCAAAATTTGTATATCGTTGTAGTT-GAATAT
Leptusa opaca                    TGTTCTATAATTGATAATCCACGATTAGTTTAACTTTATTTTAAAATTTGTATATCGTTGTAGTA-GAATAT
Phanerota dissimilis             TGTCCTGTAATTGATAATCCACGATTAGAATTACTTATTTTACAAATTTGTATATCGTTGTAGTT-GAATAT
Phanerota fasciata               TGTCCTGTAATCGATAATCCACGATTAGAATTACTTATTTTACAAATTTGTATATCGTTGTAGTT-GAATAT
Strictalia sp.                   TGTTCAGTAATTGATAATCCACGATTAGAATTACTTATGTTAATAATTTGTATATCATTGTAGTT-GAATAT
Tachyusa sp.                     TGTCCTGTAATTGATAATCCACGATTAAAGTTACTTATTTTAAAAATTTGTATATCGTTGTAGTT-GAATAT
Oxypoda sp.                      TGTTCTTTAATTGATAGTCCACGATTAGAATTACTTGTTTTAAAAATTTGTATATCGTTGTAGTT-GAATAT
Myllaena sp.                     TGTCTTATAATTGATAATCCACGATTAGTTGTACTTATATTATTAGTTTATATATCGTTGTAATA-GAATAT
Pseudomniophilas sp.             TGTCCTATAATTGATAATCCACGATTAGAATTACTTATGTTAAAAATTTATATATCGTTGTAGTT-GAATAT
Lissagria laeviscula             TGTTTATTAATTGATATTCCACGATTAAATTTACTTATTTTAATAATTTGTATATCGTTGTAGTT-GAATAT
Myrmecocephalus cing.            TGTCTTATAATTGATAATCCACGATTAGAATGACTTATTTTATTAATTTGTATATCGTCGTAGTA-GAATAT
```

Table 3 continued: Alignment of the 12s rDNA fragment of the 21 taxa used in this study.

```
                         1111111111111111111111111111111111111111111111111111111111222222222222222222
                         4444455555555556666666666777777777788888888889999999999000000000011111111
Taxon/Node               5678901234567890123456789012345678901234567890123456789012345678901234567890123456
-------------------------------------------------------------------------------------------------------------
 Constant:                *    * *       ** ***   *  *         *    ** *** ****** ** ** ****    *
    Uninf:               **    ****     *** ***   *  *   *     *    ** *** ****** ** ********   *
-------------------------------------------------------------------------------------------------------------
Deinopsis illinoisensis  TTTATTAGAAATTGA-ATATTCAAAATTTAAT-ATATTAATAAAATTCAGATCAAGGTGTAGTTTATATAAA
Deinopsis erosa          TTTATCAGAATTTAA-ATATTCAAAATTTATT-ATATTAATAAAATTCAGATCAAGGTGTAGTTTATATAAA
Aleochara valida         TTTATTAGAATTATA-ATATTCATAAATTTAA-ATTAGAAAATAAATCAAATCAAGGTGTAGTTTATAAATA
Atheta sp.               TTTAATAGAAAATAA-ATGTTCTAAATTTTTT-ATTTAAAAATAAATCAGATCAAGGTGCAGTTTATAGATA
Leptonia sp.             TTTAAAAGAAATTTA-ATATTCAAGATTTCTT-ATTAGAAAGTAAATCAGATCAAGGTGTAGTTTATATGTA
Sableta sp.              TTTTGAAGAATTTTA-ATATTCAAAATTTTTT-ATTAAAAAATAAATCAGATCAAGATGTAGTTTATATATA
Eumicrota corruscula     TTTTTAAGAAAATTGAATATTCATAATTTTAA-ATATAAAAATAAATCAAATCAAGGTGCAGTTTATATAAA
Gyrophaena coniciventris TTTTTAAGAATTTTA-ATATTCATTATATTTT-ATAAAATAGAAAATCAAATCAAGGTGCAGTTTATGTACA
Gyrophaena flavicornis   TTCTTAAGAATAAAA-ATGTTCATTATATTTAATAAAAATTTTAAATCAAATCAAGGTGCAGTTTATATATA
Gyrophaena vitirina      TTCTTTAGAATTAAA-ATGTTCGTAATATTTT-ATAAAATTATAAATCAAATCAAGGTGCAGTTTATATATA
Gyrophaena sp.           TTTTTAAGAATTTAA-ATATTCATTATATTAA-ATAAAATAAAAAATCAAATCAAGGTGCAGTTTATATATA
Leptusa opaca            TTTATAAGAATTAATAATTTTCAAAATATTAT-ATAAAATAATAAATCAAATCAAGGTGCAGTTTATAATAA
Phanerota dissimilis     TTTTTAAGAATTCA-AATATTCATAAAATTTA-ATTAAATAAAAAATCAAATCAAGGTGCAGTTTATAAATA
Phanerota fasciata       TTTTTAAAAATTAA-AATATTCATAAAATTTA-ATTAAATAGAAAATCAAATCAAGGTGCAGTTTATAAATA
Strictalia sp.           TTTAAAAGAATTTT-AATATTCAAAATTTTTT-ATTAAAAAATAAATCAGATCAAGATGCAGTTTATATATA
Tachyusa sp.             TTTAATAGAATTTA-AATATTCTAAATTTTTATTATAAAAAATAAATCAGATCAAGGTGCAGTTTATAAATA
Oxypoda sp.              TTTATAAGAATTTA-AATGTTCAAAATTTTTGATAAAAAAAGTAAATCAAATCAAGGTGCAGTTTATAAATA
Myllaena sp.             TTTTAAAGAATATATAATATTCAAGATTTTTT-TTAAAAAAATAAATCAAATCAAGATGTAGCTTATATATA
Pseudomniophilas sp.     GTTATTAGATTTTT-AATATTCAAAATTTTTT-AAAATAAAATAAATCAAATCAAGGTGTAGTTTATATGTA
Lissagria laeviscula     TTTAGAAGAATTAA-AATATTCATAATTTTTT-ATTAAAAAATAAATCAAATCAAGGTGCAGTTTATAAATA
Myrmecocephalus cing.    TTTATTAGAATTAT-CATGTTCAAAATGTTGA-ATTATAATATAAATCAGATCAAGGTGCAGTTTATAAATA


                         2222222222222222222222222222222222222222222222222222222222222222222222222222
                         1112222222222333333333344444444445555555555666666666677777777778888888888
Taxon/Node               7890123456789012345678901234567890123456789012345678901234567890123456789012345678
-------------------------------------------------------------------------------------------------------------
 Constant:               **  *   ***  ****   *            **          *                *  ** * **
    Uninf:               **  *   ***  *****  *            ***         **  ***       * *** *******
-------------------------------------------------------------------------------------------------------------
Deinopsis illinoisensis  AGAA-GAGATGAGTTACAATAAATTTATTTATAT-GGATTTTAAATTGTAAATTT-AAATGAAATTGGATTT
Deinopsis erosa          AGGA-GAGATGAGTTACAATAAATTTATTTAAAT-GGATTTAGAATTGTAAGTTTTTAT-GAAAGTGGATTT
Aleochara valida         AGAA-GAAATGGGTTACAATAAAAGTATTTTT---GGATAATTAATTTAAAATTAATTT-GAAATTGGATTT
Atheta sp.               AGAT-GAGATGGGTTACAATAAAGTTATTTTT---GGATTAATTT-TTAAAAAAAATTATGAAAATGGATTT
Leptonia sp.             AGGA-GAGATGGGTTACAAAAAAGTTAATTAT---GGATAATTTTTTTTAAAAAAAA-TTTAAAATTGGATTT
Sableta sp.              AGAA-GAAATGGGTTACAATATATTATTAATATTTGGAAA-ATTTTTTGAGAAAAGATTTGAAATTGGATTT
Eumicrota corruscula     AGAA-GAGATGGGTTACTATAAATTTATATTT---GGCTCTTAAATTTAAAATTTA-ATTAAAAATGGATTT
Gyrophaena coniciventris AGAA-GAGATGGGTTACAGTATTTCTATATGT---GGATTTTTAGTTTAAAATTAA-AATGAAAATGGATTT
Gyrophaena flavicornis   AGAA-GAGATGGGTTACAAAATTTATATATGT---GAATTTTTAATTTAAAATTAA-ATTGAAAATGGATTT
Gyrophaena vitirina      AGAA-GAGATGGGTTACAAAATTTTTAAATGT---GGATTTTAAATTTAAAATTAA-AATGAAAATGGATTT
Gyrophaena sp.           AGAA-GAGATGGGTTACAGTATTCCTATGTGT---GAATTTTTAATTTAAAATTAA-AATGAAAATGGATTT
Leptusa opaca            AGCAAGAAATGGGTTACATTAAATATATTTTT---GGAATTTTAATTTAAAATTAATA-TAAAATTGGATTT
Phanerota dissimilis     AGAA-GAGATGGATTACAATATAATAATATGT---GAATTTTTAATTTAAAATTAAA-ATGAAAATGGATTT
Phanerota fasciata       AGAA-GAGATGGATTACAATATATTAATATGT---GAATTTTTAATTTAAAATTAAA-ATGAAAATGGATTT
Strictalia sp.           AGAT-GAAATGGGTTACAATAAAGATATTTTT---GGAAATATAGTTTAGAATTATTTTTGAAATTGGATTT
Tachyusa sp.             AGAA-GTGATGGGTTACAATAATTTTGGTTTT---GGATTTAATTTTTAAAAAGTTA-ATGAAATTGGATTT
Oxypoda sp.              AGAA-GTAATGGGTTACAAAAAATTTATTTTT---TGATTTGTAGTTTAAAATTACA-ATGAAATTGGATTT
Myllaena sp.             AGTA-AAGATGGATTACAATATTGTTAAATTT---GAATTTATATTATAATAGTGTA-ATGAAATTGGATTT
Pseudomniophilas sp.     AGAA-GAGATGGATTACAAAAATATATATATTT--GGATTAGTTATTTAAAATAA---AATTATATGAAATT
Lissagria laeviscula     AGAT-AAAATGGGTTACAAAAATAATAATTAT---GGCTTATTAATTGAAAATTA-ATTTAAAATTGGATTT
Myrmecocephalus cing.    AGAT-TTGATGAGTTACAATAATTATAGTTATA--GATTTAATAATTTAAAATTTT-AATGAAATTGGATTT
```

Table 3 continued: Alignment of the 12s rDNA fragment of the 21 taxa used in this study.

```
                        2222222222233333333333333333333333
                        8999999999900000000001111111111122
Taxon/Node              9012345678901234567890123456789012
------------------------------------------------------------
 Constant:                   ***                     * **
    Uninf:                  ****                    *****
------------------------------------------------------------
Deinopsis illinoisensis  AAAAGTAATTTAATTTATTTTAATTAAATGATT
Deinopsis erosa          AAAAGTAATTTAATTGATTTTAA??????????
Aleochara valida         AAATGTAATTTATTTTATTTAGAATAAA?????
Atheta sp.               GATAGTAATTAGGTTAATATAGATTTA??????
Leptonia sp.             GGAAGTAATTTAGTTTATTTTG???????????
Sableta sp.              GATAGTAATTAATTTCTATT-GTT?????????
Eumicrota corruscula     GAAAGTAATTTTGTTAATTTAAATAAAATGATT
Gyrophaena coniciventris GAAAGTAATTTTTTTTTATTTA???????????
Gyrophaena flavicornis   GAAAGTAATTTTCTAAATTTATGGAAAATGATT
Gyrophaena vitirina      GAAAGTAATTTTTTAAATTTATAAAAAATG???
Gyrophaena sp.           GAAAGTAATTTTAA-AATTT????????????
Leptusa opaca            GATAGTAAAAAAAATTATTTAGAT?????????
Phanerota dissimilis     GAAAGTAATTTTTAAATTTTAWTAAAAT?????
Phanerota fasciata       GAAAGTAATTTTTAAATTTTAATAAMATGRTTT
Strictalia sp.           GAAAGTAATTTAGTTGATTTAAATTAAATGA??
Tachyusa sp.             GAAAGTAATTTAGTTAATATTAGTTAAATGWTT
Oxypoda sp.              GAATGTAATTAAGTTAATATTAATTAAAT????
Myllaena sp.             GAAGTAAATTTGTTTATAATGATAAA??????
Pseudomniophilas sp.     GGATTTAATTGTAAT-YTGATTTATTT??????
Lissagria laeviscula     GAATGTAATATAGTTATTTTTGATTATATG???
Myrmecocephalus cing.    GAATGTAATAATATTTATT--WAAATTAT????
```

Table 4: Alignment of the 16s rDNA fragment of the 19 taxa used in this phylogenetic study.

```
                                 1111111111222222222233333333334444444444555555555566666666667777
Taxon/Node                       1234567890123456789012345678901234567890123456789012345678901234567890123456789012
-------------------------------------------------------------------------------------------------------------------
 Constant:                                *           ** ****** ******* ******** * ************* * ***
    Uninf:                        *       *** **      ** ********************** *************** *****
-------------------------------------------------------------------------------------------------------------------
Adinopsis sp.                    ??GTCTAACCTGCCCAATGAGTAGTTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Deinopsis erosa                  ??GTCTAACCTGCCCTATGATTATTTAAATGGCTGCAGTATTTTGACTGTACAAAGGTAGCATAATAATTAG
Deinopsis illinoisensis          GTCTAATCTGCCCAATGAATTGATTTAAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATAATTAG
Sableta sp.                      AAGTCTAGCCTGCCCAATGAAGTTTTGAATGGCCGCAGTATTTTGACTGTGCGAAGGTAGCATAATCATTAG
Eumicrota corruscula             ????CTGACCTGCCCAATGAGTAATTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGMAWAATCATTAG
Gyrophaena coniciventris         ?AGTCTAGCCTGCCCAATGAGGATTTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Gyrophaena flavicornis           ??GTCTAGCCTGCCCAMTGAGTAATTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Gyrophaena vitirina              ?AGTCTAGCCTGCCCAATGAGTATTTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Gyrophaena sp.                   ???TCTAGCCTGCCCAATGAGGATTTGAATGGCCGCAGTATCTTGACTGTGCAAAGGTAGCAKAATCATTAG
Leptusa opaca                    ????????????????ATGAATTTTTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Phanerota dissimilis             ??GTCTAGCCTGCCCAATGAGGAATTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCMTTAG
Phanerota fasciata               ?GTCTACGCCTGCCCAATGAGGAATTGAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Strictalia sp.                   ?AGTCTGGCCTGCCCAATGAGTAATTTAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Tachyusa sp.                     ????????????CCAATGAGGAGTTGAATGGCCGCAKTWTATTGACTGTGCAAAGGTAGCATAATCATTAG
Oxypoda sp.                      ??GTCTGACCTGCCCAATGAGTAATTAAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Myllaena sp.                     ????????????????????????????????????????????GACTGTACTAAGGTAGCATAATCATTAG
Pseudominophilas sp.             ???????ACCTGCCCAATGATTTATTTAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCAATAG
Lissagria laeviscula             ????????????????TGAAATAATTTAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCATAATCATTAG
Myrmecocephalus cing.            AGTCTGACCTGCCCAATGATTTAATTTAATGGCCGCAGTATTTTGACTGTGCAAAGGTAGCAWAATCATTAG


                                                                  1111111111111111111111111111111111111111111111
                                 7777777888888888899999999990000000000111111111122222222233333333334444444
Taxon/Node                       3456789012345678901234567890123456789012345678901234567890123456789012345678901234
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 Constant:                       *** ******   * **** ***** * **  ** *   **   ** * *     * * *       ** **
    Uninf:                       *** ******  ** **** ***** ***** ** * *****  ********   * * **    * ** **
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Adinopsis sp.                    TTTCTTAATTAGTAACTGGAATGAAAGGTTGGACGAGAAAATAACTGTCTCTTTATAAATTATTTAGAATTT
Deinopsis erosa                  TTTTTTAATTGAAAGCTGGTATGAATGGTTGGACGAGAAAATAGCTGTCTCTATTTAAATTTAATAGAATTT
Deinopsis illinoisensis          TTTTTTAATTGAAAGCTGGAATGAATGATTGAACGAGAAAAATACTTTATTAAATTTAATTTAAATGAATTT
Sableta sp.                      TTTCTTAATTAGAAACTGGAATGAAGGGTTGAACGAGAAAATAACTGTCTCTTTGTAAATTAATTAGAAATT
Eumicrota corruscula             TTTTTTAATTTAAAGCTGGAATGAAAGGTTGGACAAAAAAAATAGCTGTCTCTTTATAATTTAAAAAAAAATT
Gyrophaena coniciventris         TTTTTTAATTGAAAGCTGGAATGAAAGGTTGGACAAGAAAATAGCTGTCTCTTTATAAATTAATTAGAATTT
Gyrophaena flavicornis           TTTTTTAATTGAAAGCTGGAATGAAAGGTTGGACAAGAAAATAGCTGTCTCTTTATAAATTAGTTAGAATTT
Gyrophaena vitirina              TTTTTTAATTGAAAGCTGGAATGAAAGGTTGGACAAGAAAATAGCTGTCTCTTTATAAATTTGTTAGAATTT
Gyrophaena sp.                   TTTTTTAATTGAAAGCTGGAATGAAAGGTTGGACAAGAAAATAGCTGTCTCTTTATAAATTAATTAGAATTT
Leptusa opaca                    TTTTTTAATTAAAAACTGGAATGAAAGGTTGAACGAGAAAATAACTGTCTCTGTATAAATTATTTATAATTT
Phanerota dissimilis             TTTTTTAATTGAAAGCTGGAATGAAAGGTTGGAMAAGAAAATAACTGTCTCTTTATAAATTTAATAGAATTT
Phanerota fasciata               TTTTTTAATTGAAAGCTGGAATGAAAGGTTGGACAAGAAAATAACTGTCTCTTTATAAATTTAATARAATTT
Strictalia sp.                   TTTCTTAATTAGAAACTGGAATGAAAGGTTAAACGAGAAAATAACTGTCTCTTATTAAATTATTTAGAATTT
Tachyusa sp.                     TTTTTTAATTGAAAACTGGTATGAAGGGTTGGACGAGAAAATAGCTGTCTCTTTATAAATTTTTTATAATTT
Oxypoda sp.                      TTTCTTAATTAGAAGCTGGAATGAAAGGTTGAACGAAAAAATAACTGTCTCTTTATAAATTTATTAGAATTT
Myllaena sp.                     TTTTTTAATTGAAAGCTGGTATGAATGGTTGGACAAAGTAATAGCTGTCTCTTTATAAATTATT--GAATTT
Pseudominophilas sp.             TTTCTTAATTAGGAGCTGGAATGAAAGGTTGAACGAGAAAATTTCTGTCTCTGTTTTATTAATTTAGAATTT
Lissagria laeviscula             TTTCTTAATTAGAAACTGGAATGAAGGGTTTTACGAGAAAATAACTGTCTCTTTATAATTTTTTTAAAATTT
Myrmecocephalus cing.            TTTCTTAATTAGAAACTGGAATGAAGGGTTGAACGAAAAAATATCTGTCTCTTTATTAATGATTTAGAATTT
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Table 4 continued: Alignment of the 16s rDNA fragment of the 19 taxa used in this study.

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                        4444455555555556666666666677777777778888888888999999999900000000001111111
Taxon/Node              5678901234567890123456789012345678901234567890123456789012345678901234567890123456
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 Constant:              **** ** *** ******** ****    *  ****************** ***** ** **
    Uninf:              ******* ************ ****   *** ****************** ******** ***
 Excluded:                                                                                        ********
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Adinopsis sp.           TATTTTTAAGTTAAAAAGCTTAAATTTATTTAAAAGACGAGAAGACCCTATAGAGTTTTATATAATTATATT
Deinopsis erosa         TATTTTTAAGTTAAAAAGCTTAAATAATTTTAAAAGACGAGAAGACCCTATARARTTTTATATTTTTTTTTT
Deinopsis illinoisensis TATTTTTGAGTTAAAAAGCTTAAATATAATTAAAAGACGAGAAGACCCTATAGAGTTTTATAAAAATGAAAA
Sableta sp.             TATTTTTAAGTTAAAAAGCTTAAATATTTTTAAAAGACGAGAAGACCCTATAGAGTTTTATACATGAAGATT
Eumicrota corruscula    TATTTTTAAGTTAAAAAGCTTAAATAATTTTAAAAGACGAGAAGACCCTATAGAGTTTTATATATTTTTTAG
Gyrophaena coniciventris TATTTTTAAGTTAAAAAGCTTAAATATATTTAAAAGACGAGAAGACCCTATAGAGTTTTATAAATTTTATAG
Gyrophaena flavicornis  TATTTTTAAGTTAAAAAGCTTAAATAAATTTAAAAGACGAGAAGACCCTATAGAGTTTTATAAATTTTATAA
Gyrophaena vitirina     TATTTTTAAGTTAAAAAGCTTAAATAAATTTAAAAGACGAGAAGACCCTATAGAGTTTTATAAAATTTGTTA
Gyrophaena sp.          TATTTTTAAGTTAAAAAGCTTAAATATATTTAAAAGACGAGAAGACCCTATAGAGTTTTATAAATTTAATAG
Leptusa opaca           TATTTTTAAGTTAAAAAGCTTAAATTTATTTGAAAGACGAGAAGACCCTATAGAGTTTTATAATAATGATAA
Phanerota dissimilis    TATTTTTAAGTTAAAAAGCTTAAATATATTTAAAAGACGAGAAGACCCTTTAGAGTTTTATAGATTTTATTA
Phanerota fasciata      TATTTTTAAGTTAAAAAGCTTAAATGTATTTAAAAGACGAGAAGACCCTTTAGAGTTTTATAAATTTTGTAA
Strictalia sp.          TATTTTTTAGTTAAAAAGCTAAAATAAATTTAAAAGACGAGAAGACCCTATAGAGTTTAATAATTTTTATTA
Tachyusa sp.            TATTTTTAAGTTAAAAAGCTTAAATATACTTAAAAGACGAGAAGACCCTATAGAGTTTTATATAATTATTTT
Oxypoda sp.             TATTTTTAAGTTAAAAAGCTTAAATAGTTTTAAAAGACGAGAAGACCCTATAGAGTTTTATATAATTTATTA
Myllaena sp.            TATTTTTAAGTGAAAAARCTTAAATTATTTTAAAAGACGAGAAGACCCTATAGAGCTTTATTATTAATTATT
Pseudominophilas sp.    TATTATTTAGTTAAAAAGCTAAAATGAATTTAAAAGACGAGAAGACCCTATAGAGTTTTATAAAATTGTTTT
Lissagria laeviscula    TATTTTTAAGTTAAAAAGCTTAAATATATTTAAAAGACGAGAAGACCCTATAGAGTTTAATAATTTTTTAAT
Myrmecocephalus cing.   TATTTTTAAGTAAAAAAGCTTAAATATTTTAGAAAGACGAGAAGACCCTATAGAGTTTTATATATTTTGTAT


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                        1112222222222233333333333444444444445555555555666666666677777777778888888888
Taxon/Node              7890123456789012345678901234567890123456789012345678901234567890123456789012345678
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 Constant:                                                          * ****** ***    **** *
    Uninf:                                                        * ** **********   *******
 Excluded:              ********************************************************
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Adinopsis sp.           TAATAATTTGAATTGAATTTTAAAAATTTTACTTAATATTATATTTGA--TTGGGGTGATTGAAAAATTTAA
Deinopsis erosa         GTAATAAATTTAATAAATTATTTTTTTATATTATGAAATATTTTAT-----TGGGGTGATAGTAAAATTTAA
Deinopsis illinoisensis ATTATTTTAATAATTTTTAAGTTAGTTTTTTCTTTTTATTTTAT-------TGGGGTGATAATTAAATTTAG
Sableta sp.             TTAATAATTAATTTATTATACTTATAATTTTATTATTTGTTGTATTTG-ATTGGGGTGATTAGAAAATTTGT
Eumicrota corruscula    TTAATTATTTTTAGTATTATTAATATTTAATTTTTAAATATATTTTAT---TGGGGTGATAGAAAAATTTAA
Gyrophaena coniciventris TTAATTTTTTTTAGTAATTGTTTAAGTGAATTATAAAATTTATTTTAT---TGGGGTGATAGAAAAATTTTA
Gyrophaena flavicornis  TTAATTTTTTTTTGTAATAATTTAAATAAATTATAAATTTTATTTTGT---TGGGGTGATGGAAAAATTTTA
Gyrophaena vitirina     TTATTTTTTTTTATTGTTTAAATTAATTATAAATTTTATTTTAT-------TGGGGTGATGGAAAAATTTTA
Gyrophaena sp.          TAAATTTTTTTTAGTAATTGTATAAATAGATTATTTAATTTATTTTAT---TGGGGTGATAGAAAAATTTTA
Leptusa opaca           TAAATATATTATTGAATCTGTAAATATTTATTTTAATAATTATTTGA---TTGGGGTGATTGAAAAATTTGT
Phanerota dissimilis    TTATTTTTTTTTTAGATTTTTAATAAATTAATTATAATATTTATTTT---GTTGGGGTGATGGAAAAATTTTA
Phanerota fasciata      TTATTTTTTTTTTAGATTTTTAATAAACTAATTATAGAATTTATTTT---GTTGGGGTGATGGGAAAATTTTA
Strictalia sp.          TAAATAATTATTTATTTATTATTTAATTTTTTTAATAAAATTTATTTT-ATTGGGGTGATAAAAAAATTTAT
Tachyusa sp.            ATAAATAATATTTTTAGGATATGATATATTTTTTAAAATATTATATTTAATTGGGGTGATTAAAAAATTTAA
Oxypoda sp.             TAATTAAATTATTTATAATTATAAAAATTTTATATTAAATTATATTT-AATTGGGGTGATTGAAAAATTTAA
Myllaena sp.            AATTTATTTAATAGAAATATTTTGTTAATTAAAAGTTAATAATTTAA---TTGGGGCGATTGAAAAATTTAG
Pseudominophilas sp.    TAAATTTGTTTTGGAAAATTTAATTTTTTAAGAATTTTTTTATTTT---GTTGGGGTGATAGAAAAATTTAT
Lissagria laeviscula    AAATAATAAAAATTGAATTTTTTTTTTATTTTTATAAGAAAAATTATTTAATTGGGGTGATTAAAAAATGTAA
Myrmecocephalus cing.   TATTCTAAATTTAGAATTTTTTTTTATTATTATAGTTTGCAATGTATTTAGTTGGGGTGATTAAAAAATTTAA
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Table 4 continued: Alignment of the 16s rDNA fragment of the 19 taxa used in this study.

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                        2222222222233333333333333333333333333333333333333333333333333333333333333333
                        8999999999900000000001111111111222222222233333333334444444444555555555556
Taxon/Node              9012345678901234567890123456789012345678901234567890123456789012345678901234567890
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 Constant:                ******             ****       ***      ****** *         *** ** **   *
    Uninf:                ******* **  *      ****   *  ****  *  ******* *       ******** **** **
-------------------------------------------------------------------------------------------------------------
Adinopsis sp.           TAAACTTTTTTTTAATAAAAA-CATTAATTTA-TGATTTATTGATCCATTATTTTA-T-GATTAAAA-GATT
Deinopsis erosa         TAAACTTTTATTTAAATTTAA-CATTAATTTA-TGATTAATTGATCCATTATAAT----GATTATAA-GATT
Deinopsis illinoisensis TAAACTTTAATATATAAAAA--CATGAATTAA-TGAGTTATTGATCCATTATAAT----GATTAAAA-GATT
Sableta sp.             TAAACTTTTTTTTTTATTTAAA-CATTAGTATA-TGATTTATTGATCCATATTTTAT---GATTAAAA-GATT
Eumicrota corruscula    TAAACTTTTTTTTTATAAAAA-CATAAATTTA-TGAATTATTGATCCTTAAATTATTAAGACTATAAGATTA
Gyrophaena coniciventris TAAACTTTTTTTTTAATTTTA-CATATATAAA-TGATTTATTGATCCTTAAATTAA---GATTAAAA-GATT
Gyrophaena flavicornis  TTAACTTTTTTTAAATAAAAA-CATATATTAA-TGATTTATTGATCCTTAAATTAA---GATTAAAA-GATT
Gyrophaena vitirina     TAAACTTTTTTTATATTTTTA-CATATTTAAA-TGATTTATTGATCCTTAGTTAAA---GATTATAA-GATT
Gyrophaena sp.          TAAACTTTTTTTTAAAATTTGACATATATAAA-TGGTTTATTGATCCTTATATTAA---GATTAAAA-GATT
Leptusa opaca           AAAACTTTTTTTTACTAAAAA-CATTAATTTA-TGAATATTTGATCCATATATTAT---GATTAAAAAGATT
Phanerota dissimilis    TAAACTTTTTTTTTATAAAAT-CATTTATTTA-TGAATTATTGATCCTTTAATAAA---GATTAAAA-GATT
Phanerota fasciata      TTAACTTTTTTTTTATAAAAT-CATTTATTTA-TGAATTATTGATCCTTTAATTAA---GATTAAAA-GATT
Strictalia sp.          ATAACTTTTTTTTAATAATAA-CATTAATATA-TGATTTTTTGATCCATAAATTAT---GATTAAAA-GATT
Tachyusa sp.            TGAACTTTTTTTTTATAAAAA-CATTAATTTA-TGAATTATTGATCCATTAAAATAAT-GATTATAA-GATT
Oxypoda sp.             TAAACTTTTTTTTTATAGAA--CATTTTATATATGATTAGTTGATCCATTATTTAT---GATTAAAA-GATT
Myllaena sp.            TAAACTTTTTTTTTATTTAA--CATTAATTTA-TGGTATAATGATCCATTTTTAT----GATTATAA-GATT
Pseudominophilas sp.    TTAACTTTTTTTTTATTAATAACATAAATTTA-TGATTTTTTGATCCATAGATAAT---GATTATAA-GTTT
Lissagria laeviscula    AAAACTTTTTTTTTATATAAA-CATTTATTTA-TGAATTATTGATCCATTTATTAT---GATTATAA-GATT
Myrmecocephalus cing.   AAAACTTTTTGTTAGTAAAT--CATTAATTTA-TGATTATTTGATCCATTATTTAT---GATTATAA-GATT


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                        6666666667777777777888888888899999999900000000001111111111222222222233333
Taxon/Node              1234567890123456789012345678901234567890123456789012345678901234567890123456789012
-------------------------------------------------------------------------------------------------------------
 Constant:              ** *  * *  **   *         ***   **  ***** *  **  * *    ****************
    Uninf:              ** ********************** **** ****  *******  ***** ***  ****************
-------------------------------------------------------------------------------------------------------------
Adinopsis sp.           AAATTACCTTAGGGATAACAGCGTAATTTCTTT-TTAGAGTTCTAATCGATAAAGAAGTTTGCGACCTCGAT
Deinopsis erosa         AAATTACCTTAGGGATAACAGCGTAATTTTTTT-GGAGAGTTCTAATCGATAAGAAAGTTTGCGACCTCGAT
Deinopsis illinoisensis AAATTACCTTAGGGATAACAGCGTTATTTTTCT--TAGAGTTCTTATTAAAAGAGAAGTTTGCGACCTCGAT
Sableta sp.             AAATTACCTTAGGGATAACAGCGTAATTTTTTT-TGAGAGTCCTAATCGATAAAAAAGTTTGCGACCTCGAT
Eumicrota corruscula    AATTACCTTAGGGATAACAGCGTAATTTTTTT--TGAGAGTTCTAATCGATAAAAAAGTTTGCGACCTCGAT
Gyrophaena coniciventris AAATTACCTTAGGGATAACAGCGTAATTTTTTT-GGAGAGTTCAAATCGATAAAAAAGTTTGCGACCTCGAT
Gyrophaena flavicornis  AAATTACCTTAGGGATAACAGCGTAATTTTTTT-GGAGAGTTCAAATCGATAAAAAAGTTTGCGACCTCGAT
Gyrophaena vitirina     AAATTACCTTAGGGATAACAGCGTAATTTTTTT-GGAGAGTTCAAATCGATAAAAAAGTTTGCGACCTCGAT
Gyrophaena sp.          AAATTACCTTAGGGATAACAGCGTAATTTTTTT-TAAGAGTTCTAATCGATAAAAAAGTTTGCGACCTCGAT
Leptusa opaca           AAATTACCTTAGGGATAACAGCGTAATTTTCTTTTTAGAGTTCTAATCGATAAAGAAGTTTGCGACCTCGAT
Phanerota dissimilis    AAATTACCTTAGGGATWACAGCGTAATTTTTTT-GAAGAGTTCTAATCGATAAAAAAASTTTKCGACCTCGAT
Phanerota fasciata      AAATTACCTTAGGGATAACAGCGTAATTTTTTT-GAAGAGTTCTAATCGATAAAAAAGTTTGCGACCTCGAT
Strictalia sp.          AAGTTACCTTAGGGATAACAGCGTAATTTTTTT-TAAGAGTTCAAATCGATAAAAGAGTTTGCGACCTCGAT
Tachyusa sp.            AAATTACCTTAGGGATAACAGCGTAATTTCTTT-GAAGAGTTCTAATCGATAAAGGAGTTTGCGACCTCGA?
Oxypoda sp.             AAATTACCTTAGGGATAACAGCGTAATTTCTTT-TTAGAGTTCAAATCGATAAAGAWGTTTGCGACCTCGAT
Myllaena sp.            AAGTTACCTTAGGGATAACAGCGTTATTTTTTT-TTAGAGTTCTTATCGAAAAAAAAGTTTGCGACCTCGAT
Pseudominophilas sp.    AAATTACCTTAGGGATAACAGCGTAATTTTTTT-GGAGAGTTCAAATCGATAAAAAAGTTTGCGACCTCGAT
Lissagria laeviscula    AAATTACCTTAGGGATAACAGCGTTATTTCTTT-TAAGAGTTCAAATCGATAAAGAAGTTTGCGACCTCGAT
Myrmecocephalus cing.   AAATTACCTTAGGGATAACAGCGTAATTTCTTT-GGAGAGTTCTAATCGATAAAGAAGTTTGCGACCTCGAT
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Table 4 continued: Alignment of the 16s rDNA fragment of the 19 taxa used in this study.

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                        4444444444444444444444444444444444444444444444444
                        3333333444444444455555555556666666666777777777
Taxon/Node              3456789012345678901234567890123456789012345678 9
-------------------------------------------------------------------------
 Constant:              ********** *     * **   ****    ** *    *  * *
    Uninf:              ********** *  *  ***** ******** ** *   **  ***
-------------------------------------------------------------------------
Adinopsis sp.           GTTGGATTAAAATTTAATTTT-GGTGTAGAAGCTAAAAAAATTA???
Deinopsis erosa         GTTGGATTAAATTTTATTTTT-GGTGTAGAAGCTAAAAAATTA????
Deinopsis illinoisensis GTTGGATTAAATTTTATATTT-GGTGTAGAGGCTAAATTATTA????
Sableta sp.             GTTGGATTAAAATTAATTTTT-GGTGTAGAAGCTAAAATAT??????
Eumicrota corruscula    ????????????????????????????????????????????
Gyrophaena coniciventris GTTGGATTAAAATTAAATTTT-GGTGTAGAAGCTAAAATAT??????
Gyrophaena flavicornis  GTTGGATTAAAATTAAATTTT-GGTGTAGAAGCTAAAATATTWRKS?
Gyrophaena vitirina     GTTGGATTAAAATTAAATTTT-GGTGTAGAAGCTAAAATATTTAGG?
Gyrophaena sp.          GTTGGATTAAAATTAAATTTT-GGTGTAGAAGCT???????????????
Leptusa opaca           GTTGGATTAAAATTTTATTTTTGGTGTAGAAGCTAAAATATTAGGT?
Phanerota dissimilis    GTTGGATTAAAATTAAATTTT-GGTGTAGAAGCTAGAATTTTTAG??
Phanerota fasciata      GTTGGATTAAAATTAAATTTT-GGTGTAGAAGCTAGAATTT??????
Strictalia sp.          GTYGGATTAAAATTTAATTTT-GGTGTAGAAGCTAAAATAT??????
Tachyusa sp.            ????????????????????????????????????????????
Oxypoda sp.             GTTGGATTAAAATATAATTTT-GGTGTAGAAGCTAGAATATTA????
Myllaena sp.            ????????????????????????????????????????????
Pseudominophilas sp.    GTTGGATTAAAATTAAAATAT-GGCGTAGAATCTATATGTT??????
Lissagria laeviscula    GTTGGATTAAAATATAGTTTT-GATGTAGCAGTTAAAATACTAG???
Myrmecocephalus cing.   GTTGGATTAAAATAAATTTTT-AATGTAGTAGTTAAAATA???????
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