PHYLOGENY OF NORTH AMERICAN *APHAENOGASTER* SPECIES (HYMENOPTERA: FORMICIDAE) RECONSTRUCTED WITH MORPHOLOGICAL AND DNA DATA

By

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ABSTRACT

PHYLOGENY OF NORTH AMERICAN *APHAENOGASTER* SPECIES (HYMENOPTERA: FORMICIDAE) RECONSTRUCTED WITH MORPHOLOGICAL AND DNA DATA

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The ant genus *Aphaenogaster* Mayr is an ecologically diverse group that is common throughout much of North America. *Aphaenogaster* has a complicated taxonomic history due to variability of taxonomic characters. *Novomessor* Emery was previously synonymized with *Aphaenogaster,* which was justified by the partial mesonotal suture observed in *A. ensifera* Forel. Previous studies using Bayesian phylogenies with molecular data suggest *Aphaenogaster* is polyphyletic. Convergent evolution and retention of ancestral similarities are two major factors contributing to non-monophyly of *Aphaenogaster*. Based on 42 multi-state morphological characters and five genes, we found *Novomessor* more closely related to *Veromessor* Forel and that this clade is sister to *Aphaenogaster*. Our results confirm the validity of *Novomessor* **stat. r.** as a separate genus and it is resurrected based on the combination of new DNA, morphological, behavioral and ecological data.

Twenty-three *Aphaenogaster* species (Hymenoptera: Formicidae) occur in North America. While morphology and ecology define most species, the species limits of a group in the Eastern United States are unclear. In particular, the morphological and behavioral characters once thought to define *A. carolinensis*, *A. picea* and *A. rudis* do not associate with their hypothesized species limits. These observations suggest that these species are not monophyletic. We therefore tested the monophyly of *Aphaenogaster* in the context of molecular phylogenetic analyses. We used DNA data from five genes: CO1, CAD, EF1αF2, Long-wavelength Rhodopsin and Wingless to reconstruct phylogenies for 44 *Aphaenogaster* and outgroup species.

In the resulting trees, reconstructed using parsimony and Bayesian inference, species boundaries associate with well-supported monophyletic clades of individuals collected from multiple locations. For example, *A. carolinensis* was monophyletic and a missing CAD intron was a diagnostic trait for the clade. However, some clades were unresolved, and *A. picea* and *A. rudis* were not monophyletic. Given the short branch lengths, these results suggest that these ants have likely recently radiated, and lack of gene lineage sorting explains the non-monophyly of species. Conversely, these results may indicate that clades of multiple species represent fewer but morphologically varied species. Additional biological information concerning pre- and post-mating barriers is needed before a complete revision of species boundaries for *Aphaenogaster*.

Aphaenogaster Mayr 1853, contains 227 species worldwide (Bolton 2006) with 23 valid North American species, several species of which are hard to separate based on morphology alone (Umphrey 1996). The difficulty in identifying some of these species is due to limited diagnostic characters and to the lack of a comprehensive illustrated key. A recent analysis returned three species from *Aphaenogaster* to *Novomessor*, thus making *Aphaenogaster* in North America monophyletic (DeMarco and Cognato 2015). While many species have easily identifiable morphological characters, some east coast species within the *A. rudis* clade in North America are difficult to differentiate. Two of these species, *A. carolinensis* and *A. miamiana*, can be diagnosed using DNA. The gene CAD was missing an intron in those taxa. Four additional taxa, all identified morphologically as *A. rudis*, were found to be polyphyletic (DeMarco and Cognato, in prep, or see Chapter 2).

This work is dedicated to my husband for his unfailing support during the time it took to do my research and complete my dissertation. I will be forever grateful.

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Please note that figures have been edited to conform to the Michigan State University dissertation formatting guidelines. Please refer to resulting publications for the final version of the figures.

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CHAPTER 1

INTRODUCTION TO *APHAENOGASTER* (HYMENOPTERA: FORMICIDAE)

Ecological and behavioral diversity of *Aphaenogaster*

Aphaenogaster Mayr 1853 contains 227 species worldwide (Bolton, 2006) with 23 valid North American species, reduced from 31 original species descriptions. The North American taxa have not been taxonomically reviewed in over 60 years (Creighton 1950). Umphrey (1996) attempted to discriminate a complex group of ten sibling species of the *Aphaenogaster fulva-rudis-texana* complex in northeastern US with karyotypes and morphology. He concluded that karyotypes provided the best, but imperfect, means for species diagnosis. He acknowledged that DNA would ultimately prove useful as a definitive method for separating these groups.

Aphaenogaster has been a popular genus for many studies including biology and natural history (Lubertazzi 2012), tool use (Fellers and Fellers 1976), communication (Menzel and Marquess 2008), interactions with other ant taxa (Bewick et al. 2014) and temperature tolerance (Warren and Chick 2013).

In Connecticut, Lubertazzi (2012) found nesting sites for ants in the *Aphaenogaster rudis* group in soil, in rotting wood, under rocks and in leaf litter. Nests in soil were shallow and had a single entrance. Lubertazzi (2012) planted 25 artificial wooden nests at 3 different sites and followed them through an entire year. Seventeen nests survived; all but one contained a queen. Half the nests produced males, but only 3 produced female alates. The smallest nest contained 183 and the largest nest 1033 workers, with an average nest size of 613 individuals. He measured foraging distances by placing baits randomly in a 10 m square area and following workers back to the nests. The average foraging distance was 57 cm. *Aphaenogaster* behavior was observed as timid around other ant species, and they did not defend foraging territories. These ants laid a trail pheromone (Attygalle et al. 1998) using their poison gland to recruit nest

mates to food items. They fed on small invertebrates including termites (Buczkowski and Bennett 2007), eliaosome bearing seeds (Heithaus et al. 2005 and Clark and King 2012) and even mushrooms (Carroll, et al. 1981). Luburtazzi (2012) also observed caste attributes. He found that winged reproductives left the nest between late July and mid-August. Mated queens and brood overwintered in the soil, and workers began foraging in early spring. They are some of the earliest foragers observed in the forest. Larvae hatched from eggs in about 20 days, there are four larval instars, with an average larval period of 28 days, and the pupal stage lasts 16 days. Total time from egg to ecolsion averaged 64 days. Workers fed first instar larvae a liquid diet from food stored in their crop, while later instars were able to ingest solid foods. Haskins (1960) observed queens in *Aphaenogaster picea* able to survive 8-13 years.

Not all *Aphaenogaster* species nest in the same habitats. *Aphaenogaster treatae* nests in open, sandy fields under clumps of grass or lichen (Talbot, 1954). *Aphaenogaster megommata* is a nocturnal ant in the deserts of southwest US and nests in sand. *Aphaenogaster tennesseensis* is a social parasite that enters the nests of *Aphaenogaster rudis* and *A. fulva* (Creighton, 1950). The *A. tennesseensis* queen is attractive to the workers in the *A. rudis* and *A. fulva* nests, and they unknowingly raise her eggs as their own (Creighton, 1950). *Aphaenogaster tennesseensis* have a wide geographical range, from Virginia south to Florida, and east to Iowa and Nevada. They are morphologically distinct in that they lack hairs on the mesosoma and gaster (Ellison, et al. 2012). *Aphaenogaster mariae* Forel occurs in Virginia and Mississippi but is rarely collected. It is an arboreal species with a starburst pattern of striae on the first gastral tergite (Ellison et al. 2012). *Aphaenogaster ashmeadi* and *A. treatae* are identified by the size of a lobe at the base of the scape (Creighton, 1950). Other NA *Aphaenogaster* do not have this lobe. *Aphaenogaster ashmeadi* is found throughout the southeast, while *A. treatae* occurs from the southeast north into

Michigan. *Aphaenogaster lamellidens* is also morphologically distinct with a tooth or lobe on the frontal carina that is rearward facing towards the back of the head (Creighton, 1950). They nest in similar habitats as the rest of the northeastern *Aphaenogaster* species with nests in soil, under rocks and in rotting pine and oak logs. They are found throughout the southeast.

Substrate vibration generating behavior has been observed in ants in the ant genera *Messor* (Grasso et al. 1999), *Novomessor* (Markl and Holldobler 1978), *Atta* (Roces and Holldobler 1996), and *Solenopsis* (Rauth and Vinson 2006). Ants in these genera use stridulation to create sounds in response to the discovery of a food source. Menzel and Marquess (2008) also observed substrate vibration generating behavior in *Aphaenogaster.* They described this behavior and its causes in *Aphaenogaster carolinensis*. This ant produces vibrations by striking, then dragging its mandible across a substrate surface. They concluded that this behavior was not in response to food, but a reaction to the presence of non-nest mate conspecifics and to a lesser extent, ants from other species.

Bewick et al. (2014) observed interactions between *A. rudis* and two other ant species, *Prenolepis imparis* and *Nylanderia faisonensis*. The three species were chosen because of different nest sizes and different feeding habits. Bewick et.al (2014) compared specific species traits (food discovery rate, food clearance rate, body mass, dominance hierarchy and thermal niche), the effect and interaction of interspecific competition and climate change on community composition. Equalizing discovery rates, food clearance rates and dominance had a negative effect on *A. rudis* and *P. impairis*, but a positive effect on *N. faisonensis*. Equalizing body mass had the opposite effect. Compared to the other traits, loss of thermal niches had less of an effect on the evenness of species distribution (but the most severe effect on local coexistence), with increases for *A. rudis* and *P. impairis* and a decrease for *N. faisonensis*. The overall conclusion

was that climate change would have a negative effect on *P. impairis* (known as the winter ant), but also surprisingly on *N. faisonensis*, which is active during the summer months. *Aphaenogaster rudis* faired the best and Bewick et al. (2014) predicted that the three community species would decrease to *A. rudis* and *P. impairis*.

Warren and Chick (2013) examined data over a 38-year period of upward movement for *A. rudis* and *A. picea* along the southern end of the Appalachian Mountain chain in Georgia. In 1974, 100% of *Aphaenogaster* ants at 900 m elevation were *A. picea.* By 2012, 25% of the *Aphaenogaster* ants at 900 m were *A. rudis* and only 75% were *A. picea*. Warren and Chick (2013) also tested thermal tolerance of individuals of both species. The absolute temperature range for *A. picea* was a minimum of -0.5 °C and a maximum of 42.5°C. The absolute temperature range for *A. rudis* was a minimum of 2 °C and a maximum of 43.5°C. These results indicate possible changes in insect species as climates increase in temperature, due to the differeing thermal tolerance levels for both species.

Systematics of *Aphaenogaster* and preliminary cladistics analysis of morphology

Aphaenogaster species systematics has been difficult for the lack of morphologically diagnostic and phylogenetically informative characters. To demonstrate the need for additional characters (i.e., DNA), I conducted a parsimony analysis using morphological characters gleaned from previous studies (Creighton 1950, Ward 1985 and Coovert 2005). 42 morphological characters were coded for 25 *Aphaenogaster* and 10 outgroup species (Chapter 1, Table 1). The phylogenetic analysis resulted in 5 parsimonious trees; the strict consensus of the trees was mostly unresolved (Chapter 1, Fig. 1). There was relatively high support for the clades containing the outgroups, but no support for *Aphaenogaster* relationships.

Given the lack of phylogenetically informative morphological characters, DNA was utilized to potentially help resolve the phylogeny. Previous studies in ant systematics utilized a number of genes to resolve relationships among ant taxa. We used one mitochondrial gene (Cytochrome oxidase I), which was used for taxa within a genus (Branstetter 2012, Lucky 2011) and four nuclear genes that have provided resolution at higher levels (Brady et al. 2006, Moreau et al. 2013).

The purpose of this dissertation is to reconstruct *Aphaenogaster* phylogeny with molecular characters to elucidate relationships within the genus. Chapter One incorporated a small sample of 44 taxa to show that previously, *Aphaenogaster* was polyphyletic within North America. *Novomessor*, which includes three species, was resurrected, making *Aphaenogaster* in North America a monophyletic clade. Chapter Two examined a larger sample of 123 taxa and showed a number of taxa as monophyletic, but some samples, identified as *Aphaenogaster rudis,* were polyphyletic. Chapter Three provided a revised key to *Aphaenogaster* in North America and included DNA data for definitive diagnosis of some species.

CHAPTER 2

PHYLOGENETIC ANALYSIS OF *APHAENOGASTER* SUPPORTS THE RESURRECTION OF *NOVOMESSOR* (HYMENOPTERA: FORMICIDAE)

Abstract

The ant genus *Aphaenogaster* Mayr is an ecologically diverse group that is common throughout much of North America. *Aphaenogaster* has a complicated taxonomic history due to variability of taxonomic characters. *Novomessor* Emery was previously synonymized with *Aphaenogaster,* which was justified by the partial mesonotal suture observed in *A. ensifera* Forel. Previous studies using Bayesian phylogenies with molecular data suggest *Aphaenogaster* is polyphyletic. Convergent evolution and retention of ancestral similarities are two major factors contributing to non-monophyly of *Aphaenogaster*. Based on 42 multi-state morphological characters and five genes, we found *Novomessor* more closely related to *Veromessor* Forel and that this clade is sister to *Aphaenogaster*. Our results confirm the validity of *Novomessor* **stat. r.** as a separate genus and it is resurrected based on the combination of new DNA, morphological, behavioral and ecological data.

Introduction

The ant genus *Aphaenogaster* Mayr, 1853 is a speciose group, which has not been taxonomically reviewed in over 60 years (Creighton 1950). *Aphaenogaster* contains 227 worldwide species (Bolton 2006) with 23 valid North American species reduced from 31 original species descriptions. They are an ecologically diverse group that is common throughout much of North America (Creighton 1950). They occur in deciduous forests, open grassy areas, pine barrens and sand hills. Ecologically, they are general scavengers, feeding on a variety of arthropods, and other small invertebrates; they are also keystone seed dispersers in mesic forests of eastern North America (Lubertazzi 2012). Many species live in dead wood and promote

decomposition and nutrient recycling (Warren and Bradford 2012).

Aphaenogaster has a complicated taxonomic history due to variability of taxonomic characters. Mayr (1853) described the genus based on two new species from Italy, *A. sardoa* Mayr, 1853 and *A. senilis* Mayr, 1853. Mayr (1863) later moved the genus into *Atta* (Fabricius, 1804) as a subgenus. Emery (1895) removed *Aphaenogaster* from *Atta* and placed it as a subgenus of *Stenamma* Westwood, 1839. Emery (1908) decided it merited generic status. Umphrey (1996) addressed the complicated *Aphaenogaster fulva-rudis-texana* complex using morphometric characters and karyotypes to identify ten taxa that included six previously recognized species and four undescribed species. He concluded that additional DNA data was needed to define and diagnose these groups. Recent inclusion of DNA data in Bayesian phylogenetic analyses resolved *Aphaenogaster* as polyphyletic, including *Messor* Forel, 1890 and *Stenamma* (Brady et al. 2006, Moreau and Bell 2013). Ward (2011) suggested that convergent evolution and retention of ancestral similarities were two major factors contributing to polyphyly of *Aphaenogaster*.

Aphaenogaster taxonomy was further complicated with the description of *Novomessor* Emery, 1915 and *Veromessor* Forel, 1917. Brown (1974) synonymized *Novomessor* with *Aphaenogaster* and returned two species, *N. ensifera* Forel, 1899 and *N. manni* Wheeler and Creighton, 1934, to *Aphaenogaster*. Based on this synonymy, he reduced *Novomessor* to a subgenus of *Aphaenogaster.* Hölldobler et al. (1976) resurrected *Novomessor* to generic status; however, Bolton (1982 and 2003) considered *Novomessor* as a junior synonym of *Aphaenogaster* and *Veromessor* as a junior synonym of *Messor*. The synonymy of *Novomessor* with *Aphaenogaster* was justified by the partial mesonotal suture in *A. ensifera* (Brown 1974). The *Novomessor* lineage of three species *A. albisetosa* Mayr, 1886, *A. cockerelli* André, 1893

and *A. ensifera,* has several distinct morphological characters, as well as behaviors and habitat preferences. Two of the species, *A. albisetosa* and *A. cockerelli*, do not have a mesonotal suture and the three species differ from most other North American *Aphaenogaster* by their large size (2x). They inhabit desert environments, but forage in the morning (Sanders and Gordon 2002), as compared to other desert species of *Aphaenogaster* that forage at night (personal observation of BBD). *Aphaenogaster albisetosa* and *A. cockerelli* also exhibit a stridulating behavior not observed in other cogeners (Hölldobler et al. 1978). They drag their abdomen over sand to recruit nestmates to help with prey. In addition, Hölldobler et al. (1976) suggested the resurrection of *Novomessor* based on the presence of a new complex exocrine gland in the *Novomessor* species. Ward et al. (2014) recently resurrected *Veromessor* based on DNA evidence.

The preponderance of morphological, ecological and behavioral differences suggests the validity of *Novomessor*. However, monophyly of *Novomessor* has not been tested, which is necessary for the delimitation of a genus. The close relationship between *Aphaenogaster, Messor*, *Veromessor* and *Novomessor* has made molecular tools crucial to understanding the relationships between these taxa. In this study, we test the monophyly of *Novomessor* in phylogenetic analyses using molecular, morphological, ecological and behavioral data from a sample of North American *Aphaenogaster* species.

Materials and Methods

Specimens were collected at a number of North American localities, including wooded areas of eastern and central US, and the western deserts. Additional specimens were borrowed from colleagues and institutions (Table 1.1). Historically poorly collected areas were

specifically targeted, such as the Michigan Upper Peninsula. Specimens were collected using an aspirator and baits (peanut butter and pecan shortbread cookies) and stored in 100% ethanol at -80 °C. At least 12 workers per nest were collected at each site. Specimens were deposited at the A.J. Cook Arthropod Research Collection, Michigan State University, East Lansing, Michigan. The following 42 morphological characters/indices (Ward 1985, Bolton 1994, Lucky and Ward 2010, Brady and Ward 2005) were scored for the phylogenetic analysis (Table 1.2). All multistate characters are unordered and each character is based on workers. Sculpture terms are from Harris (1979).

Morphological characters

1. *Cephalic index* (head width / head length): (0) 0.99 mm or less; (1) 1 mm; (2) 1.01 mm +.

2. *Frontal triangle*: (0) shiny; (1) striated; (2) punctate; (3) finely punctate; (4) absent.

3. *Lobe at base of scape*: (0) lobe absent; (1) lobe flat and thin (as seen from side); length not more than 1/5 scape; (2) lobe thick (as seen from side); length usually 1/4 scape or longer; (3) small angled extension at scape base.

4. *Sculpturing on mandible*: (0) striated; (1) punctate.

5. *Apical 4 segments paler in color than rest of antenna*: (0) no; (1) yes (Coovert 2005).

6. *Antennal segment number*: (0) 10 segments; (1) 12 segments.

7. *Antennal segment color*: (0) same as head; (1) lighter than head; (2) darker than head.

8. *Antennal club*: (0) antennal funiculi without a differentiated club; (1) antennal funiculi terminate in weak 4-segmented club; (2) antennal funiculi terminate in 3-segmented club; (3) antennal funiculi terminate in 2-segmented club.

9. *Psammophore*: (0) no hairs under head; (1) psammophore present; (2) some long hairs under head; but not a complete psammophore.

10. *Antennal scape index*: (0) 0.99 or less; (1) 1; (2) 1.01 - 2.0; (3) 2.01 - 3.00; (4) 3.01+.

11. *Palp formula indicates the number of maxillary and labral palp segments, respectively*. (0) 2,2; (1) 4,3; (2) 5,3; (3) 6,4.

12. *Ocular index* (eye length x eye width / head width): (0) 0.001 - 0.009; (1) 0.010 - 0.039; (2) 0.040 - 0.07 ;(3) 0.071 +.

13. *Clypeal margin shape*: (0) emarginated; (1) slightly emarginated; (2) straight; (3) emarginate notched); (4) bicarinate without teeth; (5) bicarinate with teeth.

14. *Sculpture pattern on head*: (0) fine rugae; (1) long rugae; (2) long wavy rugae; (3) coarse rugae; (4) coarse sculpturing; (5) shiny; (6) long to transverse rugae; (7) punctate; (8) finely punctate.

15. *Sculpture location on head*: (0) to occiput; (1) to top of eyes; (2) to bottom of eyes; (3) none.

16. *Posterior border of clypeus with deep; semicircular impression*: (0) no; (1) yes.

17. *Anterior edge of mesonotum rising abruptly above adjacent portion of pronotum*: (0) no; (1) yes.

18. *Mesosoma lacking erect hairs*: (0) no hairs present; (1) hairs present.

19. *Sculpturing on pronotum*: (0) punctate; (1) finely punctate; (2) coarsely punctate; (3) fine rugae; (4) coarse rugae; (5) fine transverse rugae; (6) coarse transverse rugae; (7) coarse sculpturing; (8) shiny.

20. *Sculpturing on mesonotum*: (0) punctate; (1) finely punctate; (2) coarsely punctate; (3) fine rugae; (4) coarse rugae; (5) fine transverse rugae; (6) coarse transverse rugae; (7) coarse sculpturing; (8) shiny.

21. *Sculpturing on propodeum*: (0) punctate; (1) finely punctate; (2) coarsely punctate; (3) fine rugae; (4) coarse rugae; (5) fine transverse rugae; (6) coarse transverse rugae; (7) coarse

sculpturing; (8) shiny.

22. *Propodeal spines*: (0) spines absent; (1) spines present; (2) spines present but small; (3) spines present but very small; (4) spines present; but thin; (5) spines present, but small and triangular.

23. *Spine index* (Spine width/Spine length): (0) = 0; (1) 0.01 - 0.85; (2) 0.86 - 1.2; (3) 1.21 - 3.0; (4) 3.01 +.

24. *Coxae sculpturing*: (0) shiny; (1) finely punctate; (2) punctate; (3) 1st coxa finely punctate; others shiny; (4) fine rugae.

25. *Coxae color* (compared to mesosoma): (0) same; (1) lighter; (2) darker.

26. *Leg color* (compared to mesosoma): (0) same; (1) lighter; (2) darker.

27. *Weber's length*: (0) 0.75 - 0.99 mm; (1) 1.0 - 1.6 mm; (2) 1.61 - 2.0 mm; (3) 2.01 - 3.00 mm; (4) 3.01 - 3.99 mm; (5) > 4.00 + mm.

28. *Promesonotal suture*: (0) no; (1) yes; (3) indistinct

29. *Striae on first gastral tergite*: (0) no striae; (1) Striae present.

30. *Erect hairs on gastral tergite and sternite*: (0) no; (1) yes.

31. *Appressed hairs on gastral tergite and sternite*: (0) no; (1) many; (2) sparse.

32. *Metasoma color compared to mesosoma*: (0) same; (1) lighter; (2) darker.

33. *Gaster color compared to head*: (0) same; (1) lighter; (2) darker.

34. *Petiole/postpetiole*: (0) petiole only; (1) Petiole and postpetiole.

35. *Petiole index* (petiole length/ petiole height): (0) 1.0 - 1.24;(1) 1.25 - 1.55; (2) 1.56 - 1.8; (3)

1.81 +.

36. *Postpetiole index* (postpetiole length/ postpetiole height): (0) none; (1) 0.70 - 0.99 mm; (2) 1.00 - 1.25; (3) 1.26 +.

37. *Hind femur length*: (0) 0.8 - 0.99 mm; (1) 1.00 - 1.99 mm; (2) 2.00 - 2.99 mm; (3) 3.00 - 3.99 mm; (4) 4.00 mm +.

38. *Outer face of frontal lobe bearing a flange which projects rearward in the form of a tooth*: (0) No tooth; (1) tooth present.

39. *Mandible slender and triangular with outer margin not strongly curving toward midline*: (0) no; (1) yes.

40. *Spine shape*: (0) none; (1) angled back; (2) angled back; thin; (3) small right angle; (4) angled up; (5) triangular; (6) angled back and curved in; (7) angled back; small; (8) angled up and small; (9) curved back.

41. *Spine angle*: (0) 180°; (1) 120° +; (2) 130° +; (3) 140° +; (4) 150° +; (5) 160° +.

42. Petiole constricted with junction at gaster: (0) none; (1) slight constriction; (2) strong constriction;
(3) no postpetiole.

Molecular characters

Molecular data were assembled for genetic loci, which were phylogenetically informative for ant genera (Brady et al. 2006, Ward et al. 2010) (Table 1.3). DNA was extracted from ants preserved in 100% ethanol using a silica-based spin column procedure (Qiamp, Qiagen Inc., Santa Clara, CA), following the manufacturer's tissue protocol. Specific regions of

mitochondrial (CO1, 650 base pairs or bp) and nuclear DNA [carbomoylphosphate synthase (CAD, 816 bp), Elongation factor 1-alpha F2(EF2, 517 bp), Long Wavelength Rhodopsin(LWR, 560 bp) and Wingless(WG, 428 bp)] were amplified via polymerase chain reaction (PCR). The total number of base pairs for all genes was 2972. For the mitochondrial gene CO1, the annealing temperature was 50°C, and for the nuclear genes CAD and LWR were 54°C, for EF2, 53°C and for WG, 58°C. These loci were amplified following published protocols (Table 1.3). After PCR, unincorporated deoxyribonucleotide triphosphates (dNTPs) and oligonucleotides were removed from PCR reactions with Exo-SAP (http=//www.usbweb.com/) and directly sequenced on an ABI 3700 automated sequencer using a BigDye (Applied Biosystems, Inc., Foster City, CA) fluorescent chemistry reaction, with both sense and anti-sense strands sequenced for all individuals. Sequences were aligned using Sequencher® version 5.2 and deposited in Genbank (Table 1.2). CO1 sequences were produced for all taxa. The following taxa were missing sequences: CAD, *Camponotus pennsylvanicus* (DeGeer, 1773)*, Formica glacialis* Wheeler, 1908*, Veromessor andrei* (Mayr, 1886)*, Messor bouvieri* Bondroit, 1918*, Myrmica latifrons* Stärke*,*1927, *Aphaenogaster balcanica* (Emery, 1898)*, A. boulderensis* Smith, 1941*, A. floridana* Smith, 1941*, A. huachucana, A. mutica* Pergande, 1896*, A. patruelis* Forel, 1886*, A. tennesseensis* (Mayr,1862), *A. texana* Wheeler 1915, *A. treatae* Forel, 1886 and *A. umphreyi* Deyrup and Davis, 1998*;* EF2, *V. andrei* and *A. huachucana* Creighton, 1934; LWR, *Solenopsis aurea* Wheeler, 1906, *A. boulderensis,* one individual of *A. picea* Wheeler, 1908. *A. texana,* and *A. uinta* Wheeler, 1917; WG, one individual of *A. ashmeadi* (Emery, 1895)*,* and *A. tennesseensis*.

Phylogenetic analysis was performed using the computer software TNT (Goloboff et al. 2008). The analysis used the new technology search in TNT that included four search models:

ratchet (Nixon 1999), sectorial searches, drifting and fusing. Default settings were used except for ratchet, which was set at 10 perturbations and 200 iterations. Bootstrap analysis used resampling, with 1000 replicates. Bremer support was preformed with the script Bremer.run from the TNT wiki website (http://tnt.insectmuseum.org/index.php/Bremer_Support).

We also inferred a phylogeny with likelihood with RAxML (Stamatakis 2014) and Bayesian analysis With Mr. Bayes via the CIPRES Gateway (Huelsenbeck and Ronquist 2001, Miller et al. 2010). For both analyses, data were partitioned by gene, and codon position (Castoe et al. 2004), with models of evolution applied independently to each partition (Nylander et al. 2004). We used MrModeltest 3.7 (Nylander 2004) for the selection of partition-specific substitution models for the nucleotide data using the Akaike Information Criterion in order to decrease the potential of over parameterizating the models although complex models often perform as well or better than simpler models (Nylander et al. 2004). We followed guidelines to make credible Bayesian inferences (Bollback 2002, Huelsenbeck and Ronquist 2001). The best-fit model for all genes was GTR + I + G.

Results

Using TNT (Goloboff et al. 2008) a morphological matrix was constructed with 42 characters and 43 taxa. Analysis of these data resulted in five most parsimonious trees. The consensus of these trees was unresolved and showed no support except for the outgroups. The three species of the *Novomessor* lineage, *A. albisetosa*, *A. cockerelli* and *A. ensifera* grouped with *Aphaenogaster* (Figure 1.1). *Veromessor* was polyphyletic and *Messor* was within *Aphaenogaster*. This illustrates the unreliability of using only morphological characters within this group. However, morphological characters are diagnostic for these genera, including scape

index and the amount of constriction between the postpetiole and the gaster (see Key).

Parsimony with morphology and DNA, in addition to maximum likelihood and Bayesian analyses with DNA only, resolved a monophyletic *Novomessor,* which was sister to the *Veromessor* species, and with *Novomessor* and *Veromessor* clades as sister to *Aphaenogaster* (Figs. 2, 3). Since maximum likelihood and Bayesian analysis resulted in a nearly identical topology, only the parsimony Bayesian analyses are shown. The European *Messor* species were imbedded within the *Aphaenogaster* clade. The relationships among the *Novomessor* and *Veromessor* species were well supported (Figures 1.2, 1.3). There was variable support for the subclades within the *Aphaenogaster* clade (Figures 1.2, 1.3). These results confirm that *Novomessor* **stat. r.** is monophyletic and is resurrected from synonomy under *Aphaenogaster*.

Identification key to included genera

This key is modified from Creighton (1950) for the following 4 genera.

1a. Scape index (scape length/head width) is less than 1, psammophore often present……2

1b. Scape index greater than 1, psammophore absent……3

2a. New World species with small to large propodeal spines……*Veromessor*

2b. Old World species with no or small propodeal spines……*Messor*

3a. Distinct promesonotal suture, postpetiole constricted at connection to gaster…… *Aphaenogaster*

3b. Indistinct promesonatal suture, postpetiole not constricted at connection to gaster……*Novomessor*

Discussion

The lack of resolution of the morphology-based tree is not surprising because of the limited number of variable characters found for *Aphaenogaster*. However, resolution was

recovered where expected; for the outgroup species and *Novomessor*. The outgroup species have several apomorphic characters that separate them from *Aphaenogaster*. *Formica* Linneaus 1758 and *Camponotus* Mayr, 1861 are in Formicinae, and have a petiole, but no post-petiole. *Myrmica* Latreille, 1804 lacks a distinct peduncle, making the petiole shorter. *Veromessor* species in this analysis have a complete psammophore, or a fringe of long hairs beneath the head (*V. andrei* and *V. julianus* (Pergande, 1894)), while *Messor* species in this analysis have an incomplete psammophore or few long hairs beneath the head, including *M. bouvieri* and *M. denticornis* Forel, 1910. *Stenamma* has a bicarinate clypeus.

Along with morphology, several DNA, behavioral and habitat characters are diagnostic for *Novomessor*. *Novomessor albisetosa*, *N. cockerelli* and *N. ensifera* are found in xeric habitats, while most North American *Aphaenogaster* are found in woodland or field habitats. *Novomessor albisetosa* and *N. cockerelli* are abundant at low mid-altitudes in arid habitats (Wheeler and Creighton 1934). Both species form conspicuous nests with sloppy gravel craters, and the workers are active from late afternoon into the night hours (Wheeler and Creighton 1934). They feed on seeds, plant material and dead or dying insects. *Novomessor ensifera* is known only from Mexico, and nests in soil that consists of many large stones buried in coarse sand (Kannowski 1954). Kannowski (1954) also did not observe plant material, but only dead insects in their nests. Some *Aphaenogaster* occur in open, grassy habitats, pine barrens, and sand hills. Most Eastern *Aphaenogaster* species build nests in soil, sand or under rocks, but in forest habitats, nests may also be found in rotten logs, branches, stumps, and occasionally live trees. *Aphaenogaster texana*, which is found in the southwest, occurs at a higher elevation, and is found in dead logs or under rocks, which differs from those in the *Novomessor* lineage, but is similar to many of the remaining North American *Aphaenogaster* species. Additionally, there are

several desert dwelling species within *Aphaenogaster* including *A. boulderensis*, *A. huachucana*, *A. megommata* Smith, 1963, and *A. uinta*. Morphologically, characters of the promesonatal suture, and the postpetiole, are diagnostic for *Novomessor*. Reproductive characters such as the forewing venation (Brown 1974) are potentially diagnostic but these characters need further examination in a future study concerning *Aphaenogaster*.

This study provides another example of molecular phylogenies elucidating generic boundaries for taxonomically challenging groups like *Aphaenogaster*. The molecular/morphological-based phylogenies provide a strong justification for the delimitation and recognition of *Novomessor* as for other ant genera in recent studies. *Stenamma* was shown to form two separate clades (Holarctic and Middle American regions) using a ten gene concatenated dataset (Branstetter 2012). Blaimer (2012) synonymized five of 13 former subgenera of *Crematogaster* Lund, 1831 under *C.* (*Orthocrema*), and the remaining eight under *Crematogaster* sensu stricto. Using five genes and morphology, Blaimer (2012) concluded that there was a deep divergence event between *Crematogaster* and *Orthocrema* Santschi, 1918, and provided a key to separate these subgenera based on morphology. Lucky and Ward (2010) and Lucky (2011) also provided the first molecular and morphological phylogeny of *Leptomyrmex* Mayr, 1862. They separated *Leptomyrmex* into two clades, "micro-" and "macro-" *Leptomyrmex*. The "macro" species have wingless queens and are found in Australia, New Caledonia and New Guinea. The "micro-" *Leptomyrmex* species are found only in southeast Australia. Additionally, nine subspecies were elevated to species status (Lucky and Ward 2010). Given precedence set by these studies, we resurrect *Novomessor* based on its monophyly, nucleotide differences, and morphological diagnostic characters. Furthermore, our results (Figures 1.2, 1.3) and others (Brady et al. 2006, Moreau and Bell 2013) indicate that *Aphaenogaster* is polyphyletic with

inclusion of the European species of *Messor* which are sister to *A. japonica* (Figures 1.2, 1.3). Additional phylogenetic study and subsequent generic revision are needed to resolve the polyphyly of *Aphaenogaster*.

Genus *Novomessor* Emery, 1915

(Complete taxonomic references for *Novomessor* in Bolton 2006)

Diagnosis: Morphological characters that separate *Novomessor* from *Aphaenogaster* include a head width and length each of greater than 2 mm, and a striated frontal triangle above the clypeus. The intraocular distance is 1.4 mm or greater. The distance between the tips of the spines is greater than 0.56 mm and the spine length is 1 mm or longer. The Weber's length is 3 mm or greater, and the promesonotal suture is indistinct or absent. Characters that diagnose *Messor* from *Novomessor* include a large metasternal process in *Messor*, which is smaller in *Novomessor* and a quadrate head in *Messor*, which is elongate in *Novomessor*. In addition, *Novomessor* has no constriction of the postpetiole as the gaster, *Messor* and *Veromessor* have a slight constriction and *Aphaenogaster* has a strong constriction.

Description: Workers in *Novomessor* are 8-8.5 mm in length and reddish brown in color. The head in all three species is longer than it is wide, the mesosoma has long transverse rugae and the gaster is darker than the head. *Novomessor albisetosa* and *N. cockerelli* have long hairs under the head resembling a psammophore, while *Novomessor ensifera* has only short hairs. They have well-developed propodeal spines, averaging 1 mm in length. *Novomessor albisetosa* and *N. cockerelli* can be difficult to distinguish from each other, but the long wavy rugae on the head end at the top of the eyes in *N. cockerelli* and extend to the occiput in *N. albisetosa*.

Distribution: *Novomessor albisetosa* and *N. cockerelli* occur in southeastern Arizona,

southern New Mexico, southwestern Texas and northern Mexico at elevations from 150 to 300 m. *Novomessor cockerelli* is found on the desert floor, with large, crater-like nest entrances surrounded by coarse gravel. *Novomessor albisetosa* is found near *N. cockerelli*, but their nests occur in the desert foothills, under flat rocks or stones, and surrounded by gravel. *Novomessor ensifera* has only been found in Mexico, in sandy soil with large stones present (Kannowski 1954).

Biology: Both *N. cockerelli* and *N. albisetosa* forage late in the day and into evening, and feed upon small insects, seeds and bits of plant tissue (Cook, 1953). Kannowski (1954) describes a different foraging pattern for *N. ensifera*. He observed them foraging in the early morning and late afternoon, and also only observed them feeding on insects, but no plant material. He also found no plant pieces or seeds in their nests.

Included Species

Novomessor albisetosa (Mayr, 1886), new combination (restored status)

Novomessor cockerelli (André, 1983), new combination (restored status)

Novomessor ensifera (Forel, 1899), new combination (restored status)

Novomessor manni (Wheeler and Creighton, 1934) junior synonym of *N. ensifera*

CHAPTER 3

A MULTIPLE GENE PHYLOGENY REVEALS POLYPHYLY AMONG EASTERN NORTH AMERICAN *APHAENOGASTER* SPECIES (HYMENOPTERA: FORMICIDAE)

Abstract

Twenty-three *Aphaenogaster* species (Hymenoptera: Formicidae) occur in North America. While morphology and ecology define most species, the species limits of a group in the Eastern United States are unclear. In particular, the morphological and behavioral characters of *A. carolinensis*, *A. picea* and *A. rudis* overlap. These observations suggest that these three species are not monophyletic. We therefore tested the monophyly of *Aphaenogaster* in the context of molecular phylogenetic analyses. We used DNA data from five genes: CO1, CAD, EF1αF2, Long-wavelength Rhodopsin and Wingless to reconstruct phylogenies for 44 *Aphaenogaster* and outgroup species. In the resulting trees, reconstructed using parsimony and Bayesian inference, species boundaries associated with well-supported monophyletic clades of individuals in most of the 23 North American *Aphaenogaster* collected from multiple locations. However, some clades were unresolved, and both *A. picea* and *A. rudis* were not monophyletic. Although this may indicate that clades of multiple species represent fewer but morphologically varied species, given the short branch lengths, the lack of resolution may reflect the fact that these ants have recently radiated, and a lack of gene lineage sorting explains the non-monophyly of species. Additional biological information concerning pre- and post-mating barriers is needed before a complete revision of species boundaries for *Aphaenogaster*.

Introduction

The number of recognized ant species worldwide increases each year. Hölldobler & Wilson (1990) estimated that there were 8800 described species. By 2007, Fisher & Cover

(2007) reported 12,000, and currently AntWeb (http://www.antweb.org) posts almost 16,000 valid species and subspecies. Ants are ubiquitous in many ecosystems, and ecologically dominant as predators, scavengers and herbivores (Wilson & Hölldobler, 2005). Although ants make up approximately 2 percent of the known global insect fauna, they comprise at least one third of its biomass (Wilson & Hölldobler 2005). In the tropics, they can make up to 94% of the biomass of tropical rainforest canopies (Davidson *et al.* 2003). Consequently, ants play an important role in the environment and their study depends on a thorough understanding of their diversity.

The woodland ant genus *Aphaenogaster* includes important seed dispersers in North American forests, and has been the focus of a number of ecological and evolutionary studies (Lubertazzi 2012, Warren & Chick 2013, Bewick *et al.* 2014). Previous systematic studies focused on species descriptions (Kiran *et al.* 2008, Shattuck 2008, Longino & Cover 2004); however, the magnitude of species diversity of *Aphaenogaster* is unclear due to few and conserved distinguishing morphological characters.

Aphaenogaster have expanded frontal carinae that partially or wholly cover the antennal insertions (Creighton 1950). All members of this genus have 12-segmented antennae with a long scape, well-developed eyes, a two-segmented petiole, and (usually) distinct propodeal spines (Coovert 2005). Approximately 18 morphological characters vary among 23 *Aphaenogaster* species (Creighton 1950, Umphrey 1996, Coovert 2005). Ward (1985) replaced total body length with Weber's length. Recently, DeMarco & Cognato (2015) identified an additional 15 diagnostic characters, including the cephalic index, shape of the base of the antennal scape, length of propodeal spines and sculpturing on the head and thorax. The base of the antennal scape is particularly important, due to the wide range of shapes observed in different species.

Current identification keys are based on the workers. Genitalia have not been described for most species, except in Boudinot (2013), and original species descriptions have insufficient information concerning queens and males.

The study of ant diversity has largely been based on morphological characters, although in the last decade molecular characters provided crucial information for the determination of generic and species limits (Branstetter 2012, LaPolla *et al.* 2010, Moreau & Bell 2013). Early genetic studies revealed variability in chromosome number and enzymatic variation among different ant species, which suggested potential taxonomic utility of molecular characters (Whelden & Haskins 1953, Imai 1966, Tomaszewski *et al*. 1973, Pamilo *et al.* 1975). Indeed, chromosomal and allozyme variation exist for *Aphaenogaster* species and among populations (Crozier 1977). For example, *A. rudis* Enzmann, from the coastal plains of the US were n=20 and nearly fixed for an esterase allele, while montane specimens were either n=18 or n=22 and had variable allele frequencies (Crozier 1977).

Umphrey (1996) attempted to discriminate a complex group of ten sibling species of the *Aphaenogaster fulva-rudis-texana* complex with karyotypes and morphology. Karyotyping of 223 colonies from 63 localities, mostly in Eastern North America, identified 10 genetic forms including *A. rudis, A. picea* (Wheeler)*, A. miamiana* Wheeler*, A. carolinenesis* Wheeler*, A. texana* Wheeler*, A. fulva* Roger and four undescribed taxa. Chaetotaxy and a morphometric analysis using 12 characters including characters such as head width, scape length, spine length, and distance between the spines yielded little additional diagnostic information. While there was some variability in size, shape and color, this variation was confounded by variation within a colony or species. For example, *A. rudis* was morphologically similar to other species occurring in the same habitat. Umphrey (1996) concluded that karyotypes provided the best, but imperfect,

means for species diagnosis. He acknowledged that DNA would ultimately prove useful as a definitive method for separating these groups.

A phylogeny based on DNA characters could define the relationships among *Aphaenogaster* species and further diagnose North American species. A recent Bayesian phylogeny testing the placement of the genus demonstrated non-monophyly of *Aphaenogaster*, as a clade of *Aphaenogaster* species grouped with species in two other genera, *Messor* and *Stenamma* (Brady *et al*. 2006, Branstetter 2012). Ward (2011) suggested that convergent evolution and retention of ancestral similarities were two major factors contributing to this non-monophyly. *Aphaenogaster* monophyly was resolved, in part, with the resurrection of *Novomessor* (DeMarco & Cognato 2015). There are no published phylogenies based on DNA or morphological data that focus on the species relationships within *Aphaenogaster* despite the apparent need (Umphrey 1996, Lubertazzi 2012, Ward 2011, Ward *et al.* 2015), particularly for the "*fulva-rudis-texana"* complex, as described by Umphrey (1996). In this study, we use sequence data from five genes to reconstruct a phylogeny for 44 *Aphaenogaster* and outgroup species. The resulting trees support some previously recognized groups, but also reveal polyphyly among specimens identified as *A. rudis*.

Materials and Methods

Previous species concepts for *Aphaenogaster* were mainly morphological and genetic (Crozier 1977, Umphrey 1996). Our phylogenetic approach necessitated a phylogenetic species concept, founded in hypothesis testing (Hey 2006). Thus we tested the monophyly of the currently recognized *Aphaenogaster* species. Non-monophyly of species suggested the need for the revision of species boundaries.

Ant collecting occurred in the eastern and central US forests and grasslands, and the western forests and deserts. For hypothesis generating purposes, four additional samples were included in the analysis from Costa Rica, Greece, Japan and Madagascar to begin to understand the relationships between North American and worldwide *Aphaenogaster*. Specimens were collected into 100% ethanol using an aspirator and baits (peanut butter and pecan shortbread cookies) for analysis. GPS coordinates were recorded for all sites. At least 12 ants per nest were collected, and 10 nests were sampled for within a 3 km radius to assess intraspecific variation at a local level. Reproductive forms were collected when possible. Eight representatives from each nest were pinned. Specimens collected were vouchered in the A.J. Cook Arthropod Research Collection at Michigan State University (Table 1). Other individuals were stored in 100% ethanol at -80 °C for future DNA analysis.

A molecular data set was assembled using genetic loci identified in a previous study of ant phylogeny (Brady *et al.*, 2006), including the nuclear protein coding genes wingless, long-wavelength rhodopsin, elongation factor 1α F2 and the mitochondrial protein-coding gene COI. The gene CAD was also used (Ward *et al.* 2010). DNA was extracted from 22 of 23 currently recognized species of *Aphaenogaster* ants plus outgroups using a silica-based spin column procedure (Qiamp, Qiagen Inc., Santa Clara, CA), following the manufacturer's tissue protocol. Specific regions of mitochondrial and nuclear DNA were amplified via polymerase chain reaction (PCR). All PCR cocktails consisted of a total volume of 25µl and included 14.25-17.25 µl ddH20, 2.5µl 10X PCR buffer (Qiagen), 1.0µl 25mM MgCl2 (Qiagen), 0.5µl dNTP mix (Qiagen), 2-5µl DNA template, 0.25µl HotStar Taq DNA polymerase (Qiagen). PCR reactions were performed as specified by DeMarco and Cognato (2015). After PCR, unincorporated deoxyribonucleotide triphosphates (dNTPs) and oligonucleotides were removed from PCR

reactions with Exo-SAP (http://www.usbweb.com/category.asp?cat=pcr&id=78200) and directly sequenced on an ABI 3700 automated sequencer using a BigDye (Applied Biosystems, Inc., Foster City, CA) fluorescent chemistry reaction. Both sense and anti-sense strands were sequenced for all individuals.

Phylogenetic parsimony analysis was performed using the computer software PAUP* (Swofford 2003). Bootstrap analysis used resampling, with 1000 replicates. Bremer support was performed with TreeRoot v.2.0 (Sorenson 1999) with partition Bremer support for all genes. A phylogeny was inferred with likelihood with RAxML (Stamatakis 2014) via the CIPRES Gateway (Huelsenbeck & Ronquist 2001, Miller *et al.* 2010) with 1000 bootstrap replicates. A phylogeny was also inferred with Bayesian analysis with Mr. Bayes via the CIPRES Gateway (Huelsenbeck & Ronquist 2001, Miller *et al.* 2010). We followed guidelines to make credible Bayesian inferences (Bollback 2002, Huelsenbeck & Ronquist 2001). Data were partitioned by gene and codon position (Castoe *et al.* 2004), with models of evolution applied independently to each partition (Nylander *et al.* 2004). We used MrModeltest 3.7 (Nylander 2004) for the selection of partition-specific substitution models for the nucleotide data using the Akaike Information Criterion in order to decrease the potential of over-parameterization of the models. The best-fit model for all genes was GTR + I + G.

Results

All analyses recovered similar phylogenies and the Bayesian phylogeny was mostly resolved. Most species represented by more than one individual were monophyletic and had relatively high branch support, except *A. rudis*, *A. carolinensis, A. picea, A. huachucana* and *A. uinta* (Figs. 1, 3 and 4). The parsimony tree differed compared to the likelihood and Bayesian trees with a polyphyletic *A. texana* (Figs. 1, 3 and 4). The likelihood and Bayesian trees differed

by the positions of *A. carolinensis,* and the *A. ashmeadi* (Emery) and *A. treatae* Forel clades (Figs. 3 and 4). As indicated by the partition Bremer values, COI provided most of the support followed by EF1α2 (Table 2, Fig. 2). The other genes (CAD, LWR and WG) provided little support or conflicted with CO1 and EF1α2 as indicated by negative values. An intron was missing from *A. carolinensis* and *A. miamiana* CAD sequences.

There was strong support for the outgroup taxa in the Formicinae with *Camponotus* and *Formica* sister to the remaining taxa. This was also true for most of the Myrmicinae, including *Solenopsis*, *Stenamma*, *Myrmica*, and *Novomessor*. *Veromessor* was sister to the European *Messor* species and *Aphaenogaster swammerdami*. *Aphaenogaster swammerdami* was the only species not within the *Aphaenogaster* clade. *Aphaenogaster araneoides*, from Costa Rica and an undescribed species (JTL-001) from Mexico were sister to the other *Aphaenogaster* species. *Aphaenogaster japonica* Forel, from Japan, was within the NA *Aphaenogaster* clade, as was *A. balcanica* (Emery), from Greece.

Most of species collected west of the Rocky Mountains were grouped together near the outgroup species. *Aphaenogaster uinta* Wheeler, like *A. huachucana* Creighton, was polyphyletic. *Aphaenogaster occidentalis* (Emery) from Washington, Utah and Colorado formed a monophyletic clade. There was strong support for the clade including *A. tennesseensis* (Mayr) and *A. mariae* Forel. The clade containing *A. fulva* and *A. umphreyi,* is completely separate from the *A. rudis* species complex.

Aphaenogaster floridana Smith and *A. flemingi* Smith were sister to the *A. picea* and *A. rudis* clades. *Aphaenogaster picea* individuals were found two clades, one containing mostly northern *A. picea* samples and the other individuals were in the *A. rudis* clade. *Aphaenogaster*

rudis was not monophyletic, and appeared in 4 clades (Figs. 3,4). Taxa in the largest *A. rudis* clade included *A. rudis*, *A. carolinenesis* and *A. picea*, in addition to *A. miamiana, A. lamellidens* Mayr and *A. texana,* and the clade was sister to *A. ashmeadi* and *A. treatae*.

Discussion

We tested the monophyly of *Aphaneogaster* in the context of a multi-gene phylogenetic analysis. In the resulting phylogenies, species boundaries associated with well-supported monophyletic clades of individuals for 10 of 16 NA *Aphaenogaster* species. Many of these monophyletic species contained morphological diagnostic characters discovered by previous taxonomic studies. For example, *A. tennesseensis* lacks setae on the mesosoma and gaster and is a nest parasite of *A. rudis* and *A. fulva* (Creighton 1950, Ellison *et al.* 2012). *Aphaenogaster mariae* is an arboreal species with a starburst pattern of striae on the first gastral tergite (Ellison *et al.* 2012). *Aphaenogaster floridana* is the only southeastern species lacking propodeal spines and nests in sandy soil in pine forests in North Carolina and Florida (Creighton 1950). *Aphaenogaster flemingi* is diagnosed by a shiny exoskeleton and thin propodeal spines (Creighton 1950). *Aphaenogaster fulva* and *A. umphreyi* have upward pointing spines, and can be separated from each other by the reduced eyes in *A. umphreyi* (Deyrup & Davis 1998). There is no pattern to the type or the magnitude of difference among morphological characters that diagnosis species; they can be obvious like the lack of spines or subtle like the pattern of striae.

Polyphyly of the remaining six species is an issue of concern because given the criteria of monophyly, our phylogeny suggests the recognition of fewer species. The large clade of *A. rudis* also includes *A. ashmeadi, A. carolinensis*, *A. lamellidens*, *A. miamiana, A. texana* and *A. treatae,* and the placement of *A. rudis* individuals are scattered in six separate clades among

these other species (Fig. 1). Other instances of paraphyly occur with *A. fulva-A. umphreyi*, *A. texana - A. huachucana, A. uinta* and *A. picea.* It is tempting to synonymize these species in order to preserve monophyly. However, many of the included species are well-supported subclades with morphological and behavioral diagnostic characters. For example, the presence and size of lobe at the base of the scape diagnoses *A. ashmeadi* and *A. treatae,* which are well-supported monophyletic species (Creighton 1950). In other cases the diagnostic character is minor, as with the smaller eye, which characterizes *A. umphreyi* from *A. fulva* (Deyrup and Davis 1998). In addition, there are other potential molecular differences that could diagnose species. For example, *A. carolinensis* and *A. miamiana* lack a 300 bp CAD intron as compared to most other *Aphaenogaster* species. The remaining clades of individuals (e.g. *A. rudis*) may represent unrecognized species that await the discovery of diagnostic characters. Morphology of reproductive adults and nest architecture could provide these characters (Tschinkel 2011, Boudinot 2013).

Moreover, there are a number of alternative reasons for the apparent polyphyly in the *A. rudis* clade of NA *Aphaenogaster*, which would argue against abandoning existing nomenclature without additional evidence. First, there may be an insufficient amount of phylogenetically informative data for complete resolution. Although we sampled five genes known to resolve ant phylogenies, only 1102 of 2967 characters were phylogenetically informative and most of the phylogenetic support derived from COI and EF1αF2 (Table 2). The other genes gave little or negative support, which is a pattern observed in other insect phylogenies (e.g., Damgaard & Cognato 2003, Danforth *et al.* 2004). Doubling the number of sampled genes or increasing the number of nucleotides to the 100,000's via phylogenomic methods may help to resolve this issue, as they have provided resolution for other taxa (Peterson, *et al.* 2012, Ward & Sumnicht

2012).

It is also possible that recent species radiation could explain the low resolution due to a lack of lineage sorting of gene lineages, as demonstrated with gallwasps (Rokas *et al.* 2003) and *Formica* ants (Goropashnaya *et al.* 2004). Although the estimated age of this genus is 44 million years (Moreau *et al.* 2006), the relatively short branches and minimal COI sequence variation (mean 2.85%) observed for the *A. rudis* clade (not including the larger *A. picea* clade) suggest more recent origins of the species. A possible Pleistocene origin of these species during the expansion and contraction of glaciers could have contributed to the isolation of populations by altitude and latitude in northeastern US, as has been shown for many other taxa (Cognato *et al.* 2003, Maroja *et al.* 2007, Lecocq *et al.* 2013). Potentially, pre- and post-mating isolating mechanisms such as chromosomal rearrangements may have developed in glacial refugia and contributed to *Aphaenogaster* speciation. Potentially karyotype number may diagnose species boundaries, because much chromosomal variation exists within subfamilies, genera and even *Aphaenogaster* (Umphrey 1996, Menezes *et al.* 2013, Cardoso *et al.* 2014), and could be important in the generating reproductive isolation (Lorite & Palomeque 2010). This is consistent with the observation that distinct karyotypes associate with geographic distributions (Umphrey, 1996). For example, populations of western *A. picea* have n = 17, while eastern populations have n = 18. Our specimens of *A. picea* occur in two clades (Fig. 2, 3, 4) but unfortunately, other than one sample, *A. rudis* (# 43), we do not have associated karyotype numbers for our specimens. It is unknown whether members of the *A. rudis* clade with different karyotypes produce viable offspring or if other isolating mechanisms exist. Identification of these mechanisms and the possibility of a speciation gene, such as those that cause hybrid male sterility in *Drosophila*, could help resolve *Aphaenogaster* species relationships (Gomes & Civetta 2014). Obviously,

more study is needed to resolve the non-monophyly of *A. rudis* and other species, provide diagnostic characters, and to determine the existence of pre- or post- mating barriers among the species. Thus, a revision of *Aphaenogaster* is premature.

CHAPTER 4

APHAENOGASTER (HYMENOPTERA: FORMICIDAE) OF NORTH AMERICA: A KEY TO SPECIES USING MORPHOLOGY AND DNA

Abstract

Aphaenogaster Mayr 1853, contains 227 species worldwide (Bolton 2006) with 23 valid North American species, several species of which are hard to separate based on morphology alone (Umphrey 1996). The difficulty in identifying some of these species is due to limited diagnostic characters and to the lack of a comprehensive illustrated key. A recent analysis returned three species from *Aphaenogaster* to *Novomessor*, thus making *Aphaenogaster* in North America monophyletic (DeMarco and Cognato 2015). While many species have easily identifiable morphological characters, some east coast species within the *A. rudis* clade in North America are difficult to differentiate. Two of these species, *A. carolinensis* and *A. miamiana*, can be diagnosed using DNA. The gene CAD was missing an intron in those taxa. Four additional taxa, all identified morphologically as *A. rudis*, were found to be polyphyletic (DeMarco and Cognato, in prep, or see Chapter 2).

Introduction

Aphaenogaster Mayr 1853, contains 227 species worldwide (Bolton 2006) with 23 valid North American species, several species of which are hard to separate based on morphology alone (Umphrey 1996). The difficulty in identifying some of these species is due to limited diagnostic characters and to the lack of a comprehensive illustrated key. A recent analysis returned three species from *Aphaenogaster* to *Novomessor*, thus making *Aphaenogaster* in North America monophyletic (DeMarco and Cognato 2015). While many species have easily identifiable morphological characters, some east coast species within the *A. rudis* clade in North America are difficult to differentiate. Two of these species, *A. carolinensis* and *A. miamiana*, can

be diagnosed using DNA. The gene CAD was missing an intron in those taxa. Four additional taxa, all identified morphologically as *A. rudis*, were found to be polyphyletic (DeMarco and Cognato, in prep, or see Chapter 2).

Aphaenogaster has been a popular genus for many studies including biology and natural history (Lubertazzi 2012), tool use (Fellers and Fellers 1976), communication (Menzel and Marquess 2008), interactions with other ant taxa (Bewick et al. 2014) and temperature tolerance (Warren and Chick 2013). *Aphaenogaster* also have a variety of interesting behaviors. They laid trail pheromones (Attygalle et al. 1998) using their poison glands to recruit nest mates to food items. They fed on small invertebrates including termites (Buczkowski and Bennett 2007), eliaosome bearing seeds (Heithaus et al. 2005 and Clark and King 2012) and even mushrooms (Carroll et al. 1981). Haskins (1960) observed longevity in *A. picea* with queens able to survive 8-13 years. Menzel and Marquess (2008) observed substrate vibration generating behavior in *A. carolinensis.* A worker would strike a substrate with its mandible and drag it across the surface. This behavior was in response to the presence of non-nest mate conspecifics and ants from other species.

Recently, *Aphaenogaster* species have become the focus of climate change studies. Bewick et al. (2014), observed interactions among *A. rudis* and two other ant species, *Prenolepis imparis* and *Nylanderia faisonensis*. They tested for the importance of different species traits (food discovery rate, food clearance rate, body mass, dominance hierarchy and thermal niche), how climate change affected community composition and how interspecific competition mediated shifts in community composition in response to climate change. The overall conclusion was that climate change would have a negative effect on *P. impairis* (known as the winter ant), but also surprisingly on *N. faisonensis*, which is more active during the summer months.

Aphaenogaster rudis faired the best and Bewick et al. (2014) predicted that the three community species would decrease to two species including *A. rudis* and *P. impairis*. *Nylanderia faisonensis* would be expatriated from its current range.

Warren and Chick (2013) examined data over a 38-year period of upward movement in elevation for *A. rudis* and *A. picea* along the southern end of the Appalachian Mountain chain in Georgia. They found 100% of *Aphaenogaster* ants at 900 m elevation in 1974 were *A. picea.* In 2012, 25% of the *Aphaenogaster* ants present were *A. rudis* and only 75% were *A. picea*. They also tested thermal tolerance of individuals by increasing and decreasing temperatures. Their results indicate possible changes in insect species as climates increase in temperature.

The studies described above, and others necessitate the ability to identify *Aphaenogaster* species in research. It is thus timely to create a comprehensive identification key for NA *Aphaenogaster* species, given their increased use as indicator species in climate change studies.

Materials and Methods

Collecting occurred in areas across North America, including the eastern and central US, and the western deserts. Specimens were collected using an aspirator and baits (peanut butter and shortbread cookies with pecans) to collect specimens. GPS coordinates were recorded for all sites. Additional specimens were examined from the following museums: The Museum of Comparative Zoology at Harvard University, The Smithsonian, The Field Museum in Chicago, Mississippi State University, University of Michigan and the California Academy of Sciences. Specimens were vouchered in the A.J. Cook Arthropod Research Collection at Michigan State University as pinned and frozen samples.

Most taxa can be identified using characters included in the key. Additional morphological characters can be found in DeMarco and Cognato (2015). DNA data is required to separate *Aphaenogaster carolinensis* and *A. miamiana* from *A. rudis*. Both of these taxa are missing an intron in the gene CAD (carbomoylphosphate synthase). See an example in Genbank (sample number KJ9205520). There are 545 base pairs in *Aphaenogaster carolinensis* and *A. miamiana*. *Aphaenogaster rudis* contains 762 base pairs. Methods for sequencing this gene are in DeMarco and Cognato (in prep).

Specimens were also photographed using a Canon EOS 5D Mark II camera with a Canon Macro Pro lens (MP-E 65mm, 1-2.8, 1-5x). The images were taken using EOS Utility and Zerene stacker in combination with Stackshot. The stacks were montaged using Helicon Focus.

Glossary

Many terms used in ant identification are unfamiliar to other entomologists and to non-entomologists needing to identify ant taxa. Therefore, a brief glossary of terms is included (Bolton 1994, Fisher and Cover 2007).

Clypeus – the anterior sclerite of the head. The edge may be smooth, emarginate or notched.

Eye Size: variable (Figure 3.4)

Frontal carina – A pair of longitudinal ridges on the head, behind the clypeus.

Gaster – Abdominal segments four through seven, when a petiole and postpetiole are present.

Mesosoma – The second tagma of an ant's body, including the thorax and propodeum.

Metasoma – The third tagma of an ant's body, including the petiole, postpetiole (when present) and gaster.

Petiole – The second abdominal segment, reduced and isolated into a separate segment.

Piceous – Meaning pitch, or darkly colored.

Postpetiole - The third abdominal segment (in some ant taxa), reduced and isolated into a separate segment.

Pronotum – The first tergite of the thorax.

Propodeal spines – spines present, extending from the propodeum. (Figure 3.3)

Propodeum – Morphologically, the first tergite of the abdomen, but forming the back of the mesosoma.

Punctate – With numerous fine pits.

Rugae – wrinkled ridges, often forming parallel lines.

Rugose – containing rugae.

Scape – elongate basal section of antenna.

Spine shape – variable (Figure 3.2)

Spiracle – An orifice of the tracheal system. The propodeal spiracle is used to compare to the propodeal spines.

Striae – fine lines.

Tergite – the upper sclerite of a segment.

Identification Key to *Aphaenogaster* species

Some characters are from Creighton (1950) and Coovert (2005), including striae, frontal carina with rearward facing tooth, antennal scape shape, and color of last four antennal segments.

1 Striae at base of first gastral tergite,

	arboreal species (Figure 3.1)	*Aphaenogaster mariae*
1’	No striae on first gastral tergite, not arboreal	2
2 (1)	Propodeal spines absent	3
2’	Propodeal spines present (even if small)	4
3(2)	Gaster same color as head and mesosoma,	
	range = AL, FL, GA, MS, NC, SC	*Aphaenogaster floridana*
3’	Gaster darker than head and mesosoma	
	range = AZ, CA, CO, NM, MEX	*Aphaenogaster boulderensis*
4 (2)	No setae on mesosoma or metasoma,	
	spines strongly curved back,	
	wide range from NE to northern FL	*Aphaenogaster tennesseensis*
4’	Setae on mesosoma and metasoma, spines straight,	
	curved in or not strongly curved back	5
5(4)	Lobe at base of scape	6
5’	No lobe at base of scape	7
6(5)	Lobe one-fifth the length of the scape (Figure 3.3)	
	range = AL, FL, GA, LA, MS, NC, SC, TN	*Aphaenogaster ashmeadi*
6’	Lobe one-fourth the length of scape (Fig. 3)	
	range = FL, IL, MI, MO, MS, NC, TN, VA	*Aphaenogaster treatae*
7(5)	Color light yellow in color, large eyes	
	range = AZ, CA, NV	*Aphaenogaster megommata*
7’	Color varies from light brown to piceous,	
	eyes not large	8

8(7)	Frontal carina with rearward-facing tooth	
	range = southeastern states	*Aphaenogaster lamellidens*
8’	Frontal carina without rearward-facing tooth	9
9(8)	Spines pointed upward from propodeum,	
	anterior edge of pronotum above mesonotum	10
9’	Spines angled back or reduced, anterior edge	
	of pronotum equal to or below mesonotum	11
10(9)	Eyes reduced, reduced hind tibial spurs	
	RARE species (FL, AL)	*Aphaenogaster umphreyi*
10’	Eyes normal size, normal hind tibial spurs	
	range = AL, IL, MN, MS, NC, NJ, TN, VA, WS	*Aphaenogaster fulva*
11(9)	Spines thin (Fig. 3) head and mesonotum shiny,	
	light brown in color, range = FL, LA, NC, MS	*Aphaenogaster flemingi*
11’	Spines variable, head and mesonotum not shiny,	
	color variable	12
12(11)	Spine length less than or equal to diameter of	
	propodeal spiracle	13
12’	Spine length greater than diameter of spiracle.	15
13(12)	Body unicolorous brown to black, with lighter legs	
	range = southern CA, MEX (Baja Sur)	*Aphaenogaster patruelis*
13’	Head and mesosoma light brown/tan, gaster dark	14
14(13)	Head rounded (wider at occiput), clypeus notched	
	range = MEX (Baja Sur)	*Aphaenogaster mutica*

14’	Head rectangular, clypeus emarginate	
	range = CA, ID, NV, UT	*Aphaenogaster uinta*
15(12)	Spine shape triangular,	
	(See Fig. 2, like A. huachucana)	
	barely longer than width of propodeal spiracle	
	Scape with small triangular extension at base	
	range = AZ, NM	*Aphaenogaster huachucana*
15’	Spine shape not triangular,	
	longer than width of spiracle	16
16(14)	Head narrowed posteriorly into neck, with collar	
	RARE, only known from MEX	*Aphaenogaster mexicana*
16’	Head not narrowed posteriorly	17
17(16)	Last four antennal segments lighter in color	
	(except some forms in Canada) range = Northeast	
	plus GA, NC,TN, and WV at higher altitudes	*Aphaenogaster picea* clade
17’	Antenna unicolorous	18
18(17)	Mesosoma with fine rugae	
	range = BC(Canada), CA, CO, OR, UT, WA, WY	*Aphaenogaster occidentalis*
18’	Mesosoma punctate or coarsely rugose	19
19(18)	Dorsum of head with coarse rugae	
	range = AR, AZ, MO, NM, OK, TX	*Aphaenogaster texana*
19’	Dorsum of head with fine rugae	20
20(19)	Posterior border of head moderately pointed	

	RARE species, found only in New Mexico	*Aphaenogaster punctaticeps*
20’	Posterior border of head rounded to flattened	21
21(20)	Propodeal spines curved slightly inward	
	(dorsal view), coarse rugae on mesosoma,	
	Range = Al, FL, NC (CAD intron absent)	*Aphaenogaster miamiana*
21’	Propodeal spines straight, fine rugae	
	or punctate on mesosoma	22
22(21)	Light to medium brown	
	range = NC to MS (CAD intron absent)	*Aphaenogaster carolinensis*
22’	Medium to dark brown	
	Widely distributed throughout East coast,	
	from Georgia to Massachusetts	
	and west to Minnesota (CAD intron present)	*Aphaenogaster rudis* clades

Overview of species

***Aphaenogaster ashmeadi* (Emery)** (Figure 3.5.)

Taxonomic history:

Stenamma (Aphaenogaster) treatae var. ashmeadi Emery, C. 1895d: p. 302 (worker) U.S.A.

Combination in *Aphaenogaster*: (Wheeler 1913).

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Raised to species and senior synonym of *A. hardeni*: (Creighton 1950).

Type locality: Florida (holotype at USNM).

Aphaenogaster ashmeadi is similar to *A. treatae*, but has a smaller lobe at the base of the scape. The lobe is one-fifth the length of the scape. Specimens examined were collected from AL, FL, GA, LA, MS, NC, SC and TN. They range in color from reddish brown to dark brown.

***Aphaenogaster boulderensis* Smith** (Figure 3.6.)

Taxonomic history:

Aphaenogaster (Attomyrma) boulderensis Smith, M. R. 1941: p. 120 (worker) U.S.A.

Type locality: Arizona: Horseshoe Island in Mead Lake, beneath a lava rock. (holotype at USNM).

Aphaenogaster boulderensis is one of the NA *Aphaenogaster* species without propodeal spines. The head and mesosoma are light brown, and the gaster is dark brown. The antennal scapes pass the occipital margin by one-third the length of the scape. Specimens examined were collected from AZ, CA, NM, TX, UT, and Mexico.

***Aphaenogaster carolinensis* Wheeler** (Figure 3.7.)

Taxonomic history:

Aphaenogaster texana var. carolinensis Wheeler, W. M. 1915: p. 414 (worker, queen)U.S.A.

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Subspecies of *Aphaenogaster texana*: (Creighton 1950).

Raised to species: (Umphrey 1996).

Type locality: North Carolina: Tyron, in open woods under stones (Holotype at Harvard MCZ).

Aphaenogaster carolinensis was described as similar to *A. texana*, but with shorter spines and directed further backwards. This research finds overlapping morphological characters with both

A. texana and *A. rudis*. DNA analysis is necessary to confirm identification by a missing intron in the gene CAD (DeMarco and Cognato, in prep). Specimens examined were collected from NC and MS.

***Aphaenogaster flemingi* Smith** (Figure 3.8.)

Taxonomic history:

Aphaenogaster texana ssp. flemingi Smith, M. R. 1928: p. 275 (worker) U.S.A.

Raised to species: (Creighton 1950).

Senior synonym of *Aphaenogaster macrospina* (Smith 1958).

Type locality: Mississippi: at A and M College, in a stump (Holotype at USNM).

Aphaenogaster flemingi has slender, upward pointing propodeal spines, feeble sculpturing on the mesosoma and an overall shiny appearance. Specimens examined were collected from FL, LA, MS and NC.

***Aphaenogaster floridana* Smith** (Figure 3.9.)

Taxonomic history:

Aphaenogaster (Attomyrma) floridana Smith, M. R. 1941: p. 118 (worker) U.S.A.

Type locality: Florida.

Aphaenogaster floridana is one of the NA *Aphaenogaster* species without propodeal spines. The gaster is not significantly darker than the head and mesosoma (compared to *A. boulderensis*). They are found in sandy pine scrub and mixed hardwood forest. Specimens examined were collected from AL ,FL, GA and NC.

***Aphaenogaster fulva* Roger** (Figure 3.10.)

Taxonomic history:

Aphaenogaster fulva Roger, J. 1863: p. 190 (worker) U.S.A.

Combination in *Stenamma* (*Aphaenogaster*): (Emery 1895).

Combination in *Aphaenogaster*: (Wheeler 1913).

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Senior synonym of *Aphaenogaster rubida* (Brown 1949).

Current subspecies: nominal plus *Aphaenogaster fulva azteca*.

Type locality: "North America" (Holotype: unknown).

Aphaenogaster fulva is diagnosed by the spines pointing upward from propodeum and the anterior edge of pronotum above mesonotum. They are similar to *A. umphreyi*, but have larger eyes and larger hind tibial spurs. The last four antennal segments are lighter in color. Specimens examined were collected from AL, IL, MN, MS, NC, NJ, TN, VA and WS.

***Aphaenogaster huachucana* Creighton** (Figure 3.11.)

Taxonomic history:

Aphaenogaster (Attomyrma) huachucana Creighton, W. S. 1934: p. 189 (worker) U.S.A.

Current subspecies nominal plus *crinimera*.

Type locality: Arizona (Holotype missing from USNM, Syntype at Harvard MCZ).

Aphaenogaster huachucana is similar in appearance to *A. texana*, but is larger and found nesting in rocky ledges as opposed to *A. texana* that can be found under logs and rocks. The antennal scapes pass the occipital margin by one-third the length of the scape.Specimens examined were collected from AZ and NM.

***Aphaenogaster lamellidens* Mayr** (Figure 3.12.)

Taxonomic history:

Aphaenogaster lamellidens Mayr, G. 1886: p. 444 (worker, queen, male) U.S.A.

Combination in *Stenamma* (*Aphaenogaster*) (Emery 1895).

Combination in *Aphaenogaster* (Wheeler 1913).

Combination in *Aphaenogaster* (*Attomyrma*) (Emery 1921).

Senior synonym of *Aphaenogaster nigripes* (Creighton 1950).

Type locality: Virginia (Holotype missing, syntype at Harvard MCZ).

Aphaenogaster lamellidens has dark legs compared to the rest of the body and the frontal carina has a rearward-facing tooth. Specimens examined were collected from AL, AR, FL, LA, MO, MS, NC, SC, TN and VA.

***Aphaenogaster mariae* Forel** (Figure 3.13.)

Taxonomic history:

Aphaenogaster mariae Forel, A. 1886: p. 4 (worker) U.S.A.

Combination in *Stenamma* (*Aphaenogaster*): (Emery 1895).

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Type locality: Florida (Holotype at Naturhistorisches Museum in Vienna, Austria)

Aphaenogaster mariae has the diagnostic character of a starburst of striae (Ellison, et al. 2012) at the base of the gaster. They also have very rugose sculpturing on the mesosoma. This species is arboreal, and has only been collected on trunks of live trees at bait. One nest was observed in a treehole. Specimens examined were collected from FL, MD, MS, NJ and TN.

***Aphaenogaster megommata* Smith** (Figure 3.14.)

Taxonomic history:

Aphaenogaster (Attomyrma) megommatus Smith, M. R. 1963: p. 244 (worker) U.S.A.

Type locality: Nevada: One mi N Camp Foster, Pyramid Lake, Washoe Co. (Holotype at USNM).

Aph*aenogaster megommata* is a desert species that forages only at night. They have huge eyes and miniscule propodeal spines. They are pale yellow in color. Specimens examined were collected from AZ, CA and NV.

Aphaenogaster *mexicana* (Pergande) (Figure 3.15.)

Taxonomic history:

Ischnomyrmex mexicanum Pergande, T. 1896: p. 893 (worker) MEXICO.

Combination in *Aphaenogaster* (*Ischnomyrmex*): (Forel 1899).

Combination in *Aphaenogaster* (*Deromyrma*): (Emery 1915).

Type locality: Mexico: Tepic (Lectotype at CAS).

Aphaenogaster mexicana is rarely collected, but can be distinguished from other species in North America due to the head being narrowed posteriorly into a neck with a collar. The antennal scapes pass the occipital margin by one-half the length of the scape.

***Aphaenogaster miamiana* Wheeler** (Figure 3.16.)

Taxonomic history:

Aphaenogaster (Attomyrma) texana miamiana Wheeler, W. M. 1932: p. 5 (worker, queen, male) U.S.A.

Raised to species: (Creighton 1950).

Type locality: Florida (Holotype at AMNH).

Aphaenogaster miamiana is within the *A. rudis* clade but can be distinguished by the more rugose sculpturing on the head and mesosoma, and by a missing intron in the gene CAD (DeMarco and Cognato, in prep.). Specimens examined were collected from FL and NC. This species was previously only known from Florida.

***Aphaenogaster mutica* Pergande** (Figure 3.17.)

Taxonomic history:

Aphaenogaster mutica Pergande, T. 1896: p. 891 (worker) MEXICO.

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Type locality: Mexico: Baja California Sur, Jose del Cabo (Holotype at CAS).

Aphaenogaster mutica has a head and mesosoma that are light brown, with a dark gaster. The head is rounded (wider at occiput), with a notched clypeus. Specimens examined were collected from Mexico.

***Aphaenogaster occidentalis* (Emery)** (Figure 3.18.)

Taxonomic history:

Aphaenogaster occidentalis Emery, C. 1895d: p. 301 (worker) U.S.A.

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Senior synonym of *A. borealis* (Creighton, 1950).

Raised to species (Hunt and Snelling 1975).

Type locality: Washington: Pullman City (Holotype at Harvard MCZ).

Aphaenogaster occidentalis has relatively short spines and scapes. This is the only species that is a pest in Washington homes. Specimens examined were collected from WA, UT and CO. The range extends further East than previously recorded.

***Aphaenogaster patruelis* Forel** (Figure 3.19.)

Taxonomic history:

Aphaenogaster patruelis Forel, A. 1886: xli (worker) U.S.A.

Combination in *Stenamma* (*Aphaenogaster*): (Emery 1895).

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Subspecies of *A. subterreanea* (Emery 1895), Revived status as species (Wheeler 1904).

Senior synonym of *A. willowsi* (Creighton, 1950), of *A. bakeri* (Smith 1979).

Current subspecies: nominal plus *carbonaria*.

Type locality: Mexico: Guadelupe Island (Holotype at CAS).

Aphaenogaster patruelis ranges in color from dark brown to black, with lighter legs. The spines are minute, less than the width of the propodeal spiracle. Specimens examined were collected from CA and Mexico.

***Aphaenogaster picea* (Wheeler)** (Figure 3.20.)

Taxonomic history:

Stenamma (Aphaenogaster) fulvum piceum Wheeler, W. M. 1908: p. 621 (worker, queen, male)

(Emery, C. 1895 first available use of name)

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Subspecies of *A. fulva* (Buren 1944), of A. *rudis* (Creighton, 1950), but Creighton incorrect as *A. picea* is senior name.

Raised to species (Bolton 1995).

Type Locality: Connecticut (Holotype unknown)

Aphaenogaster picea is diagnosed by the last four antennal segments being lighter in color than the rest of the antenna, by its piceous color and northern ranges in North America. Species from NC, TN, and WV occur at higher elevations. Other samples are from MI, MA, MN, OH, PA and NY.

***Aphaenogaster punctaticeps* MacKay** (Figure 3.21.)

Taxonomic history:

Aphaenogaster punctaticeps MacKay, W. P. 1989: p. 47 (worker) U.S.A.

Type Locality: New Mexico: Jornada Experimental Range, Dona Ana Co. (Holotype at USNM).

Aphaenogaster punctaticeps is similar to *A. texana*, but with the posterior border of head moderately pointed as opposed to rounded. This is a rare species, found only in New Mexico.

***Aphaenogaster rudis* Enzmann** (Figure 3.22.)

Taxonomic history:

Aphaenogaster fulva rudis Enzmann, J. 1947: p. 150 (worker, queen) U.S.A.

(Emery, 1895 first available use of name).

Raised to species (Creighton, 1950), but Creighton incorrect as *A. picea* is senior name.

Raised to species (Umphrey 1996).

Type locality: Virginia (Holotype unknown).

This is the most commonly found *Aphaenogaster* species on the east coast. It is polyphyletic and ranges in color from light to dark brown. The last four antennal segments are not lighter in color. It cannot be distinguished from *A. carolinensis* without the gene CAD, which has an intron that *A. carolinensis* is missing. (DeMarco and Cognato, in prep.) Specimens examined are from AR, FL, GA, MI, MN, NC, NJ, OH, PA and VA.

***Aphaenogaster tennesseensis* (Mayr)** (Figure 3.23.)

Taxonomic history:

Atta tennesseensis Mayr, G. 1862: p. 743 (worker) U.S.A.

Combination in *Aphaenogaster* (Roger 1863).

Combination in *Stenamma* (*Aphaenogaster*): (Emery 1895).

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Senior synonym of *A. subrubra* (Mayr 1886), of *A. laevis* (Mayr 1886) and of *A. ecalcaritum* (Creighton 1950).

Type locality: Tennessee (Holotype at Naturhistorisches Museum, Vienna, Austria). This ant is easily diagnosed by its lack of hair on the mesosoma and metasoma, and by the propodeal spines that curve back towards the gaster. Specimens examined are from IA, MI, MN, OH, NE andVA.

***Aphaenogaster texana* Wheeler** (Figure 3.24.)

Taxonomic history:

Aphaenogaster texana Wheeler, W.M. 1915: p. 306 (worker, queen, male) U.S.A.

(Emery, C. 1895 first available use of name).

Senior synonym of A. furvescens, A. silvestrii.

Type locality: Texas (type unknown).

Aphaenogaster texana was described as similar to *A. carolinensis*, but with longer spines and directed further upwards. This research finds overlapping morphological characters with both *A. texana* and *A. rudis*. DNA data indicates that *A. texana* occurs west of the Mississippi River. Specimens examined are from AR, AZ, MO and TX.

***Aphaenogaster treatae* Forel** (Figure 3.25.)

Taxonomic history:

Aphaenogaster treatae Forel, A. 1886b: p. xl (worker, queen, male) U.S.A.

Combination in *Stenamma* (*Aphaenogaster*): (Emery 1895).

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Senior synonym of *A. wheeleri* (Creighton 1950).

Type locality: New Jersey: Vineland (Lectotype at CAS).

Aphaenogaster treatae is similar to *A. ashmeadi*, but has a larger lobe at the base of the scape. The lobe is one-fourth the length of the scape. Specimens examined were collected from FL, IL, MI, MO, MS, NC, TN and VA. They range in color from light to dark brown.

***Aphaenogaster uinta* Wheeler** (Figure 3.26.)

Taxonomic history:

Aphaenogaster uinta Wheeler, W. M. 1917: p. 517 (worker, queen, male) U.S.A.

Combination in *Aphaenogaster* (*Attomyrma*), (Emery 1921).

Type locality: Utah: East Mill Creek, Salt Lake County (Holotype at Harvard MCZ).

Aphaenogaster uinta is one of several *Aphaenogaster* species with a lighter head and mesosoma, and darker gaster. They have large eyes, very short propodeal spines and scapes that extend just beyond the occiput of the head. They live between thin layer of limestone in arid areas. Specimens examined were collected from AZ, CA and NV.

***Aphaenogaster umphreyi* Deyrup & Davis** (Figure 3.27.)

Taxonomic history:

Aphaenogaster umphreyi Deyrup, M.; Davis, L. 1998: p. 88 (worker) U.S.A.

Type locality: Florida: Putnam County, Sandhill habitat (Holotype at Harvard MCZ).

Aphaenogaster umphreyi is diagnosed by the spines pointing upward from propodeum and the anterior edge of pronotum above mesonotum. They are similar to *A. fulva*, but have smaller eyes and smaller hind tibial spurs. The last four antennal segments are not lighter in color. Specimens examined were collected from FL.

APPENDICES

APPENDIX A

Tables and Figures for Chapter 2

Table 1.1. Specimens included in current analysis with associated localities and Genbank numbers.

Taxon name	DNA extraction codes	Collection locations	Genbank CO1 #	Genbank CAD #	Genbank EF2 #	Genbank LWR #	Genbank WG #
A. araneoides	Aphara01	CR: La Selva	KJ920514	KJ920549	KJ920579	KJ920615	KJ920650
A. ashmeadi 7	Aphash07	USA: North Carolina	KJ920515	KJ920550	KJ920581	KJ920616	N/A
A. ashmeadi 12	Aphash12	USA: North Carolina	KJ920516	KJ920551	KJ920580	KJ920617	KJ920651
A. balcanica	Aphbal01	Greece: Kefalonia	KJ920517	N/A	KJ920582	KJ920618	KJ920652
A. boulderensis	Aphbou01	USA: California	KJ920518	N/A	KJ920583	N/A	KJ920653
A. carolinenesis	Aphcar01	USA: Mississippi	KJ920519	KJ920552	KJ920584	KJ920619	KJ920654
A. flemingi	Aphfle01	USA: Florida	KJ920522	KJ920555	KJ920587	KJ920622	KJ920657
A. floridana	Aphflo01	USA: Florida	KJ920523	KJ920556	KJ920588	KJ920623	KJ920658
A. fulva 5	Aphful05	USA: North Carolina	KJ920524	KJ920557	KJ920590	KJ920624	KJ920660
A. fulva 7	Aphful07	USA: North Carolina	KJ920525	KJ920558	KJ920589	KJ920625	KJ920659
A. huachucana	Aphhua01	USA: Arizona	KJ920526	N/A	N/A	KJ920626	KJ920661
A. japonica	Aphjap01	Japan: Chugoku	KJ920527	KJ920559	KJ920591	KJ920627	KJ920662
A. lamellidens	Aphlam01	USA: Virginia	KJ920528	KJ920560	KJ920592	KJ920628	KJ920663
A. mariae	Aphmar01	USA: Virginia	KJ920529	KJ920561	KJ920593	KJ920629	KJ920664
A. megommata	Aphmeg01	USA: Nevada	KJ920530	KJ920562	KJ920594	KJ920630	KJ920665
A. miamiana	Aphmia01	USA: Florida	KJ920531	KJ920563	KJ920595	KJ920631	KJ920666
A. mutica	Aphmut01	Mexico: Baja CA Sur	KJ920532	N/A	KJ920596	KJ920632	KJ920667
A. occidentalis	Aphocc01	USA: Washington	KJ920533	KJ920564	KJ920597	KJ920633	KJ920668
A. patruelis	Aphpat01	USA: California	KJ920534	N/A	KJ920598	KJ920634	KJ920669
A. picea 1	Aphpic01	USA: Michigan	KJ920535	KJ920565	KJ920599	KJ920635	KJ920670
A. picea 6	Aphpic06	USA: Minnesota	KJ920536	KJ920566	KJ920600	KJ920636	KJ920671
A. picea 39	Aphpic39	USA: Massachusetts	KJ920537	KJ920567	KJ920601	N/A	KJ920672
A. rudis 2	Aphrud02	USA: Michigan	KJ920538	KJ920568	KJ920602	KJ920637	KJ920673
A. rudis 4	Aphrud04	USA: Ohio	KJ920539	KJ920569	KJ920603	KJ920638	KJ920674
A. rudis 8	Aphrud08	USA: New Jersey	KJ920540	KJ920570	KJ920604	KJ920639	KJ920675

Table 1.1. (cont'd).

Taxon name	DNA extraction codes	Collection locations	Genbank CO1 #	Genbank CAD #	Genbank EF2 #	Genbank LWR #	Genbank WG #
A. swammerdami	Aphswa01	Madagascar: Antsiranana	JQ742635	JQ742579	EF013388	EF013546	JQ742891
A. tennesseensis	Aphten01	USA: Virginia	KJ920541	N/A	KJ920605	KJ920640	N/A
A. texana	Aphtex01	USA: Arizona	KJ920542	KJ920571	KJ920606	KJ920641	KJ920676
A. treatae	Aphtre01	USA: Michigan	KJ920543	N/A	KJ920607	KJ920642	KJ920677
A. uinta	Aphuin01	USA: California	KJ920544	KJ920572	KJ920608	N/A	KJ920678
A. umphreyi	Aphump01	USA: Florida	KJ920545	N/A	KJ920609	KJ920643	KJ920679
C.pennsylvanicus	Campen01	USA: Michigan	KJ920508	N/A	KJ920573	KJ920610	KJ920644
F. glacialis	Forgla01	USA: Michigan	KJ920509	N/A	KJ920574	KJ920611	KJ920645
Me. bouvieri	Mesbou01	Spain: Mallorca	JQ742637	JQ742581	EF013447	EF013590	JQ742893
Me. denticornis	Mesden01	S Africa: Western Cape	JQ742636	JQ742580	EF013446	EF013589	JQ742892
My. latifrons	Myrlat01	USA: Massachusetts	KJ920510	N/A	KJ920576	KJ920613	KJ920647
N. albisetosa	Novalb01	USA: Arizona	KJ920513	KJ920548	KJ920578	KJ920614	KJ920649
N. cockerelli	Novcoc01	USA: Arizona	KJ920520	KJ920553	KJ920585	KJ920620	KJ920655
N. ensifera	Novens01	Mexico: Guerrero	KJ920521	KJ920554	KJ920586	KJ920621	KJ920656
So. aurea	Solaur01	USA: Arizona	KJ920512	KJ920547	KJ920577	N/A	KJ920648
St. diecki	Stedie01	USA: Minnesota	JQ742647	JQ742591	JQ742693	JQ742738	JQ742903
V. andrei	Verand01	USA: California	DQ074325	N/A	N/A	HE963100	HE963097
V. juliana	Mesjul01	Mexico: Baja CA Sur	KJ920511	KJ920546	KJ920575	KJ920612	KJ920646

A = Aphaenogaster
C = Camponotus
F = Formica
Me = Messor
My = Myrmica
N = Novomessor
So = Solenopsis
St = Stenamma
V = Veromessor

Table 1.2. Morphological character state matrix for *Aphaenogaster* and outgroup species

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Species																						
A. araneoides	0	2	0	0	0	1	0	0	0	4	2	3	1	2	0	0	0	1	3	3	0	2
A. ashmeadi 7	0	0	1	0	0	1	1	0	0	2	2	2	1	0	0	0	0	1	0	0	0	1
A. ashmeadi 12	0	1	0	0	1	1	2	0	0	2	2	1	0	0	0	0	1	1	0	0	0	1
A. balcanica	0	1	3	0	1	1	0	0	0	2	2	1	0	0	0	0	0	1	3	3	3	1
A. boulderensis	0	1	0	0	0	1	0	0	0	2	2	1	0	0	1	0	0	1	1	3	3	2
A. carolinensis	0	0	0	0	0	1	0	0	0	2	2	1	0	2	0	0	0	1	0	0	0	1
A. flemingi	0	0	3	0	0	1	0	0	0	2	2	1	1	7	0	0	0	1	1	1	1	1
A. floridana	0	0	3	0	0	1	0	0	0	2	2	2	1	0	0	0	0	1	1	1	1	2
A. fulva 5	0	1	0	0	1	1	2	0	0	2	2	1	0	0	0	0	1	1	0	0	0	1
A. fulva 7	0	0	0	0	1	1	2	0	0	2	2	1	0	0	0	0	1	1	0	0	0	1
A. huachucana	0	1	3	0	0	1	0	0	0	2	2	1	0	0	0	0	0	1	0	0	0	1
A. japonica	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	0	1	8	0	8	1
A. lamellidens	0	0	0	0	0	1	0	0	0	2	2	2	0	0	0	0	0	1	0	0	0	1
A. mariae	0	0	0	2	0	1	0	0	0	2	2	1	0	4	0	0	0	1	7	7	7	1
A. megommata	0	1	3	0	0	1	2	0	0	2	2	3	3	0	1	0	0	1	8	1	5	1
A. miamiana	0	1	0	0	0	1	0	0	0	2	2	1	0	4	0	0	0	1	2	2	2	1
A. mutica	0	1	0	0	0	1	0	0	0	0	2	1	3	0	1	0	0	1	1	3	1	1
A. occidentalis	0	1	3	0	0	1	0	0	0	0	2	1	0	2	1	0	0	1	3	3	0	1
A. patruelis	1	0	0	0	0	1	0	0	0	0	2	1	0	0	2	0	0	1	1	1	1	1
A. picea 1	0	0	0	0	1	1	0	0	0	2	2	1	0	0	2	0	0	1	1	1	5	1
A. picea 6	0	0	0	0	1	1	0	0	0	2	2	1	0	0	2	0	0	1	1	0	0	1
A. picea 39	0	0	0	0	1	1	0	0	0	2	2	1	0	0	1	0	0	1	1	1	1	1
A. rudis 2	0	0	0	0	0	1	1	0	0	2	2	1	0	0	2	0	0	1	0	0	0	1

Table 1.2 (cont'd).

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Species																						
A. tennesseensis	0	0	0	0	1	1	0	0	0	2	2	1	1	0	2	0	0	0	0	0	4	1
A. texana	1	0	3	0	0	1	0	0	0	2	2	1	0	0	2	0	0	1	0	0	0	1
A. treatae	0	0	2	0	0	1	0	0	0	2	2	2	0	0	2	0	0	1	5	5	5	1
A. uinta	2	1	0	0	0	1	0	0	0	2	2	2	0	2	1	0	0	1	1	3	3	1
A. umphreyi	0	0	0	0	0	1	0	0	0	2	2	1	0	3	0	0	1	1	4	0	4	1
C. pennsylvanicus	1	3	0	3	0	1	0	0	0	1	3	3	0	8	0	0	0	1	1	1	1	0
F. glacialis	0	2	0	0	0	1	0	0	0	1	3	2	2	8	0	0	0	1	1	1	1	0
Me. bouvieri	2	3	0	0	0	1	0	2	1	0	2	1	1	0	0	0	0	1	3	4	4	2
Me. denticornis	2	3	0	0	0	1	0	2	2	0	2	3	1	0	0	0	0	1	3	4	4	2
My. latifrons	0	0	0	0	0	1	0	1	0	0	2	1	2	1	0	1	0	1	4	4	4	1
N. albesitosa	1	2	0	0	0	1	0	0	0	2	2	2	1	2	0	0	0	1	1	4	4	1
N. cockerelli	0	2	0	0	0	1	0	0	0	0	2	2	2	2	1	0	0	1	1	4	4	1
N. ensifera	0	2	0	0	0	1	0	0	0	2	2	2	2	6	1	0	0	1	5	5	5	1
So. aureus	0	0	0	0	0	0	1	3	0	0	0	0	5	5	3	0	0	1	8	8	8	0
St. diecki	0	0	0	0	0	1	1	1	0	0	2	0	4	1	0	2	0	1	6	6	6	1
V. andrei	2	1	3	0	0	1	0	2	1	0	2	1	1	1	0	0	0	1	4	4	4	1
V. julianus	0	1	0	0	0	1	0	1	1	0	2	1	1	1	0	0	0	1	4	6	6	1

Table 1.2 (cont'd).

Character	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Species																				
A. araneoides	1	2	0	0	5	1	0	1	1	0	0	1	1	2	4	0	1	3	1	2
A. ashmeadi 7	2	1	0	0	3	1	0	1	0	0	0	1	1	2	2	0	1	1	4	2
A. ashmeadi 12	2	4	1	0	2	1	0	1	2	2	2	1	2	3	1	0	1	1	2	2
A. balcanica	2	1	3	1	0	0	3	1	0	1	1	0	0	1	1	2	2	0	1	2
A. boulderensis	0	1	0	0	3	1	0	1	0	2	2	1	1	1	2	0	1	3	1	2
A. carolinensis	2	1	1	1	2	1	0	1	1	0	0	1	1	3	1	0	1	1	6	2
A. flemingi	2	1	1	1	3	1	0	1	2	2	0	1	1	1	4	0	1	2	4	0
A. floridana	0	0	0	0	3	1	0	1	0	0	0	1	3	3	2	0	1	3	1	2
A. fulva 5	2	4	1	0	2	1	0	1	2	2	2	1	2	2	1	0	1	4	2	2
A. fulva 7	2	4	1	1	2	1	0	1	2	2	2	1	1	2	1	0	1	4	2	2
A. huachucana	2	0	0	0	3	1	0	1	2	0	0	1	2	3	2	0	1	5	3	2
A. japonica	3	0	1	1	3	1	0	1	2	0	2	1	1	2	2	0	1	5	3	2
A. lamellidens	2	1	1	1	3	1	0	1	1	1	1	1	1	3	1	1	1	1	3	2
A. mariae	3	0	1	0	2	1	1	1	2	1	1	1	0	2	1	0	1	1	6	2
A. megommata	2	0	0	0	3	1	0	1	2	0	0	1	2	1	2	0	1	5	1	2
A. miamiana	2	1	1	1	2	1	0	1	0	0	0	1	1	3	1	0	1	6	3	2
A. mutica	1	1	0	0	3	1	0	1	2	0	0	1	1	2	2	0	1	3	1	2
A. occidentalis	2	1	0	0	2	1	0	1	2	2	2	1	1	1	1	0	1	7	3	2
A. patruelis	3	1	0	0	2	1	0	1	0	0	0	1	1	1	1	0	1	7	2	2
A. picea 1	2	1	1	1	2	1	0	1	1	0	0	1	1	2	1	0	1	1	5	2
A. picea 6	2	0	1	1	2	1	0	1	1	0	0	1	1	2	1	0	1	1	5	2
A. picea 39	2	0	1	1	2	1	0	1	1	0	0	1	1	1	1	0	1	1	5	2
A. rudis 2	2	0	1	1	2	1	0	1	2	0	0	1	1	2	1	0	1	1	4	2
A. rudis 4	3	0	1	1	3	1	0	1	2	0	0	1	1	2	1	0	1	1	4	2
A. rudis 8	2	1	1	1	2	1	0	1	2	0	0	1	1	2	1	0	1	1	4	2
A. swammerdami	1	9	3	0	0	0	5	1	0	1	1	0	0	2	1	4	4	0	1	2
A. tennesseensis	3	1	1	0	3	1	0	0	1	1	1	1	1	1	1	0	1	9	5	2

Table 1.2 (cont'd).

Character	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Species																				
A. texana	3	1	0	0	3	1	0	1	1	0	0	1	2	3	2	0	1	7	3	2
A. treatae	2	1	1	1	3	1	0	1	1	0	0	1	0	2	2	0	1	1	4	2
A. uinta	2	0	0	0	3	1	0	1	2	2	2	1	1	3	1	0	1	5	1	2
A. umphreyi	1	1	0	0	3	1	0	1	2	0	0	1	1	2	1	0	1	4	2	2
C. pennsylvanicus	0	5	0	0	4	0	0	1	1	0	0	0	3	0	3	0	2	0	0	3
F. glacialis	0	1	0	0	3	0	0	1	1	0	0	0	3	0	3	0	2	0	0	3
Me. bouvieri	3	3	1	1	0	0	3	1	0	1	1	0	0	1	1	2	2	0	2	2
Me. denticornis	2	3	1	1	0	0	4	1	0	1	0	0	0	1	1	2	3	0	2	2
My. latifrons	2	1	0	0	2	0	0	1	1	0	0	1	0	1	1	0	2	1	3	2
N. albesitosa	1	2	0	2	4	0	0	1	0	2	2	1	2	1	3	0	1	1	6	0
N. cockerelli	3	1	0	0	5	0	0	1	0	2	2	1	1	3	4	0	1	1	6	1
N. ensifera	1	1	0	0	5	0	0	1	0	2	2	1	1	1	4	0	1	1	4	0
So. aureus	0	0	0	0	0	0	0	1	1	0	2	1	1	1	0	0	2	0	0	2
St. diecki	3	0	1	1	1	0	0	1	1	0	0	1	1	2	0	0	2	8	2	2
V. andrei	2	1	3	1	0	0	3	3	0	1	0	0	0	1	1	1	2	0	2	1
V. julianus	1	1	1	0	3	3	0	1	1	2	2	1	2	3	2	0	2	4	2	1

A = Aphaenogaster
C = Camponotus
F = Formica
Me = Messor
My = Myrmica
N = Novomessor
So = Solenopsis
St = Stenamma
V = Veromessor

Table 1.3. PCR primers used for the amplification of gene loci.

Gene	Primer	Sequence	Source
CO1	LCO	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer, *et al.* (1994)
	HCO	TAA ACT TCA GGT GAC CAA AAA ATC A	Folmer, *et al.* (1994)
CAD	CD892F	5'- GGYACCGGRCGTTGYTAYATGAC -3'	Ward, *et al.* (2010)
	CD1491R	5'- GCCGCARTTNAGRGCRGTYTGYCC -3'	Ward, *et al.* (2010)
EF1-alpha F2	F2-557F	5'- GAACGTGAACGTGGTATYACSAT -3'	Brady, *et al.* (2006)
	F2-1118R	5'- TTACCTGAAGGGGAAGACGRAG -3'	Brady, *et al.* (2006)
LW Rhod	LR143F	5'- GACAAAGTKCCACCRGARATGCT -3'	Ward & Downie (2005)
	LR639ER	5'- YTTACCGRTTCCATCCRAACA -3'	Ward & Downie (2005)
Wingless	Wg578F	5'- TGCACNGTGAARACYTGCTGGATGCG -3'	Ward & Downie (2005)
	Wg1032R	5'- ACYTCGCAGCACCARTGGAA -3'	Ward & Downie (2005)

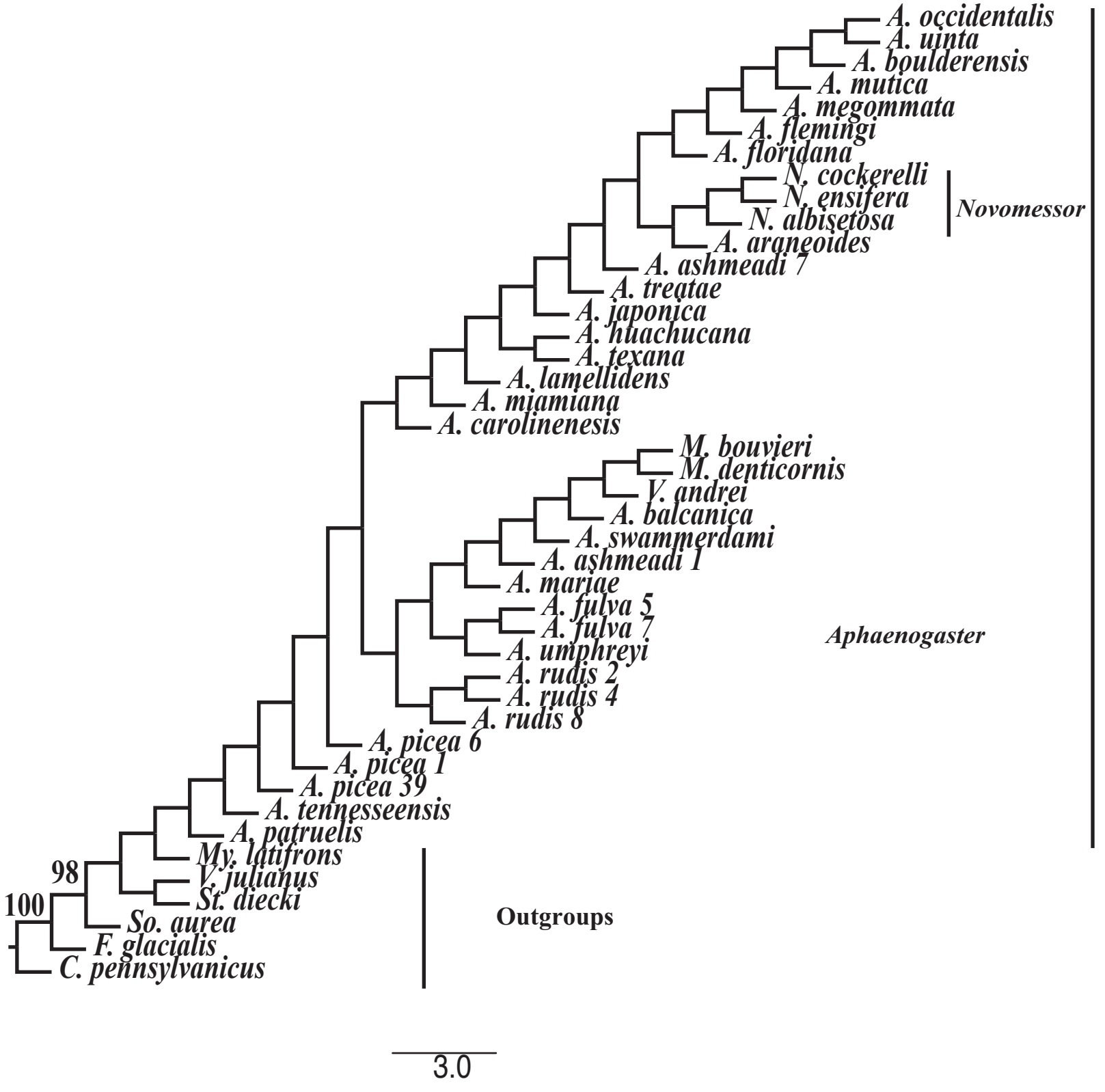


Figure 1.1. One of 5 most parsimonious trees reconstructed for 43 taxa with morphology data in a TNT analysis. Bootstrap values are above the branches. Clades without bootstrap values were not resolved in a strict consensus tree. A. = *Aphaenogaster*, C. = *Camponotus*, F. = *Formica*, M. = *Messor*, My. = *Myrmica*, N. = *Novomessor*, So. = *Solenopsis*, St. = *Stenamma*, V. = *Veromessor.*

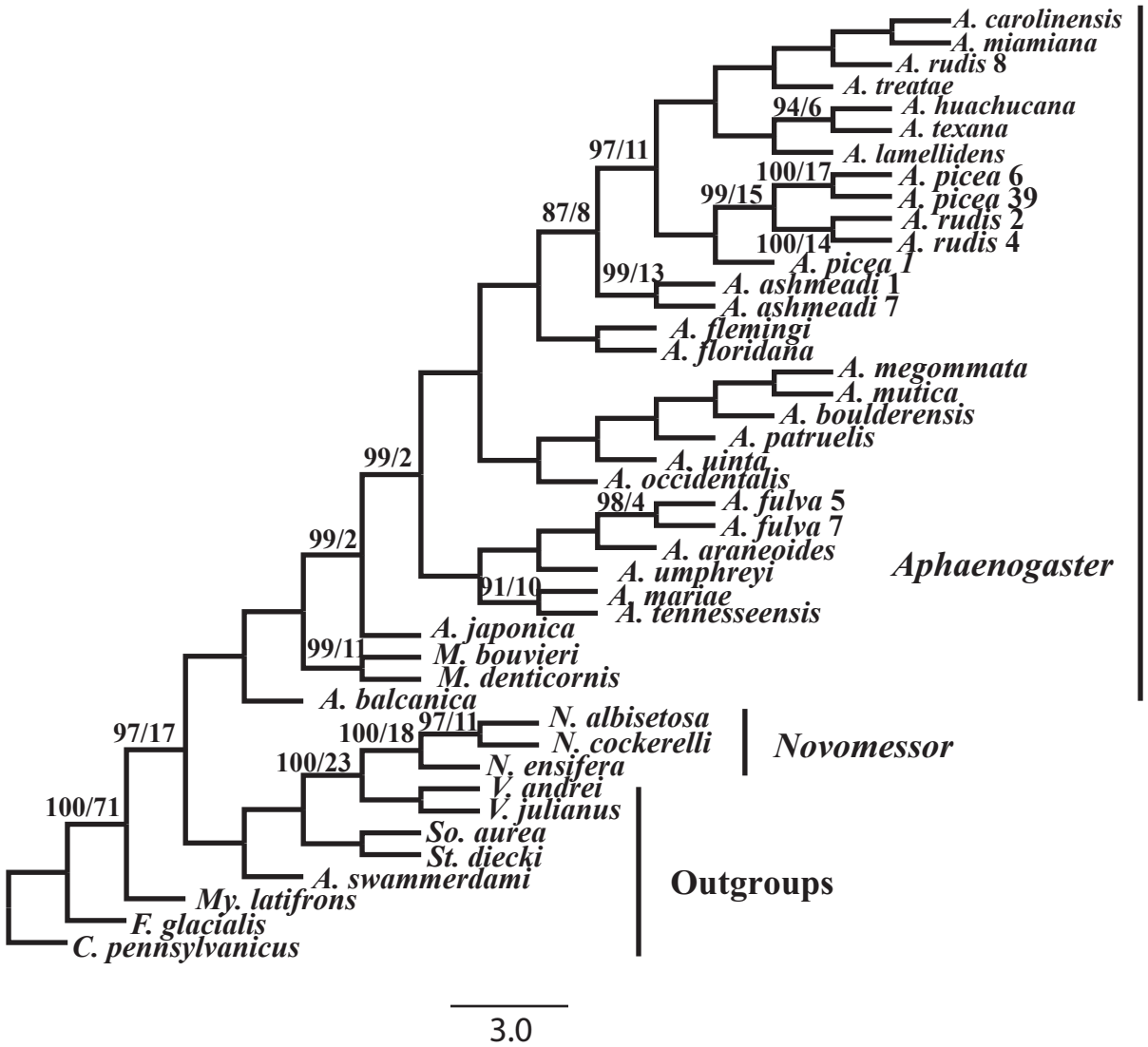


Figure 1.2. One most parsimonious tree reconstructed for 43 taxa with morphology and DNA data (CO1, CAD, EF2, LWR, WG) of 43 in a TNT analysis. Bootstrap values/Bremer support are above the branches. A. = *Aphaenogaster*, C. = *Camponotus*, F. = *Formica*, M. = *Messor*, My. = *Myrmica*, N. = *Novomessor*, So. = *Solenopsis*, St. = *Stenamma*, V. = *Veromessor*.

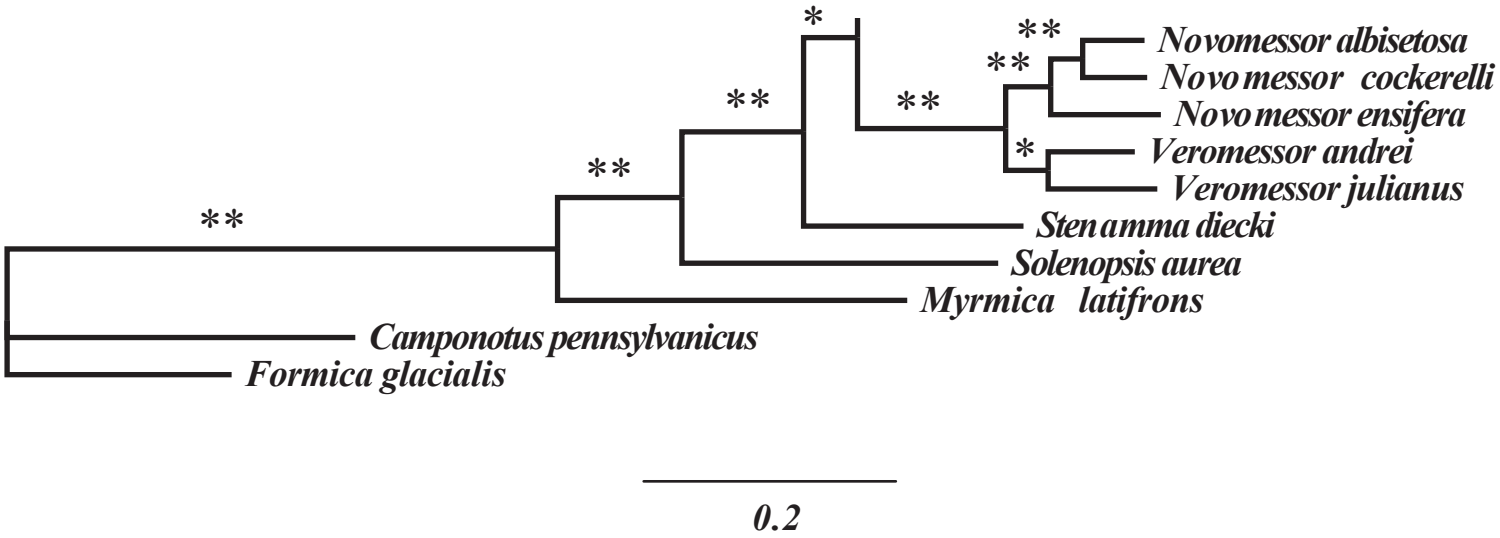
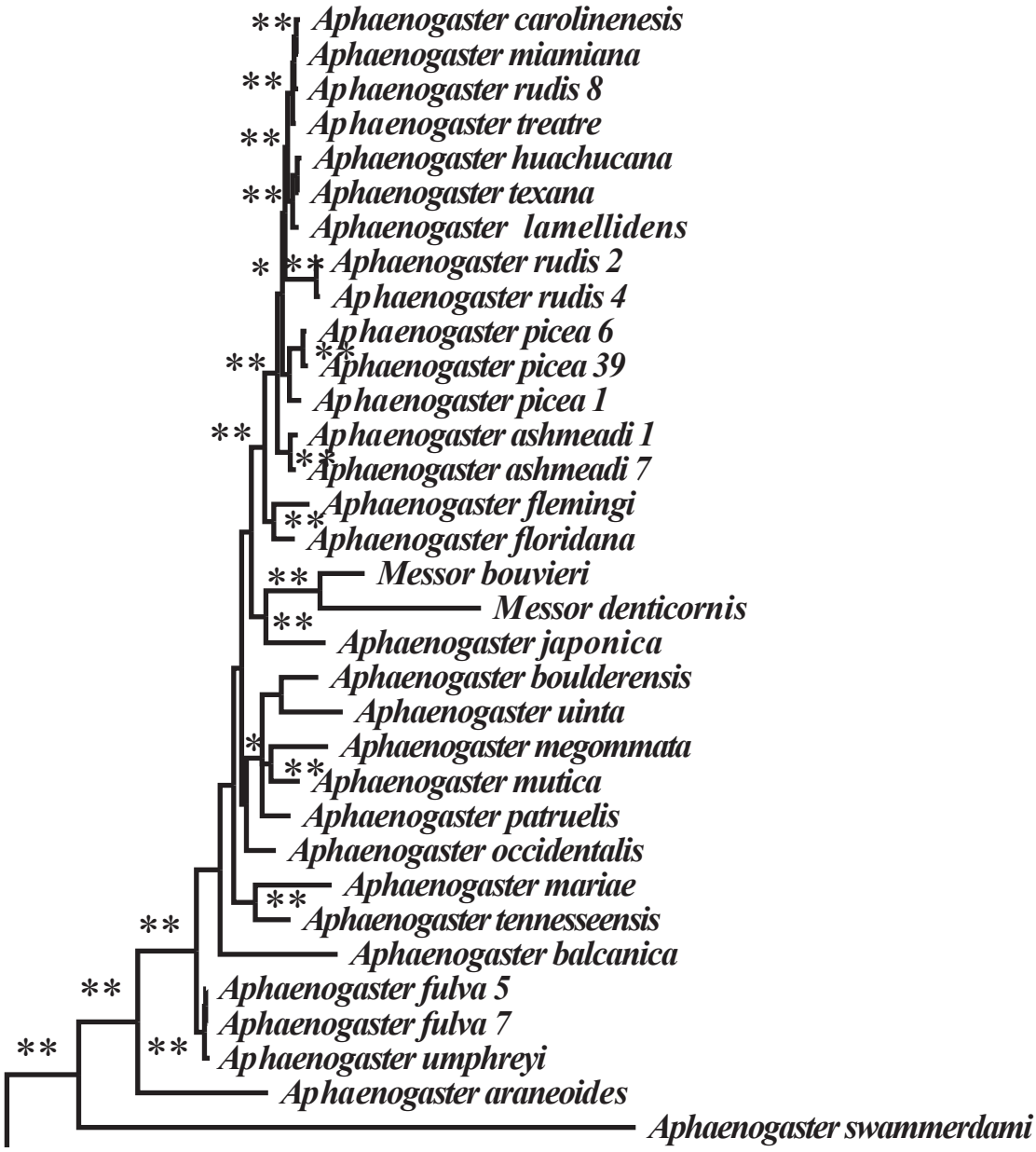


Figure 1.3. Bayesian majority rule consensus tree reconstructed for 43 taxa with morphology and five genes (CO1, CAD, EF2, LWR, WG) in a Mr. Bayes analysis, Posterior probabilities values greater than 90% are above the branches (* > 90%, **= 100%). Data were partitioned by gene and codon position and and analyzed with a best-fit GTR + I + G model, 20 million generations and a burn-in of 5,000,000 generations *Novomessor* and outgroups.

Figure 1.3. (cont'd).



APPENDIX B

Tables and Figures for Chapter 3

Table 2.1. *Aphaenogaster* and outgroup specimens with associated localities and Genbank numbers

Taxon name, author and specimen number	Genbank COI#	Genbank CAD #	Genbank EF1αF2 #	Genbank LWR #	Genbank WG #
Aphaenogaster araneoides Emery	KJ920514	KJ920549	KJ920579	KJ920615	KJ920650
Aphaenogaster ashmeadi(Emery) 7	KJ920515	KJ920550	KJ920581	KJ920616	N/A
Aphaenogaster ashmeadi 10	KP730068	N/A	KP730150	KP860353	N/A
Aphaenogaster ashmeadi 12	KJ920516	KJ920551	KJ920580	KJ920617	KJ920651
Aphaenogaster balcanica (Emery)	KJ920517	N/A	KJ920582	KJ920618	KJ920652
Aphaenogaster boulderensis Smith	KJ920518	N/A	KJ920583	N/A	KJ920653
Aphaenogaster carolinenesis Wheeler 1	KJ920519	KJ920552	KJ920584	KJ920619	KJ920654
Aphaenogaster carolinenesis 2	KP730069	KP860427	KP730151	KP860354	KP730229
Aphaenogaster carolinenesis 3	KP730070	KP860428	KP730152	KP860355	KP730230
Aphaenogaster carolinenesis 12	KP730071	KP860429	KP730153	KP860356	KP730231
Aphaenogaster carolinenesis 16	KP730072	KP860430	KP730154	KP860357	KP730232
Aphaenogaster flemingi Smith	KJ920522	KJ920555	KJ920587	KJ920622	KJ920657
Aphaenogaster floridana Smith 1	KJ920523	KJ920556	KJ920588	KJ920623	KJ920658
Aphaenogaster floridana 6	KP730073	N/A	KP730155	KP860358	KP730233
Aphaenogaster fulva 1	KP730074	KP860431	KP730156	KP860359	KP730234
Aphaenogaster fulva 4	KP730075	KP860432	KP730157	KP860360	KP730235
Aphaenogaster fulva 5	KJ920524	KJ920557	KJ920590	KJ920624	KJ920660
Aphaenogaster fulva 6	KP730076	KP860433	KP730158	KP860361	KP730236
Aphaenogaster fulva 7	KJ920525	KJ920558	KJ920589	KJ920625	KJ920659
Aphaenogaster fulva 10	KP730077	KP860434	KP730159	KP860362	KP730237
Aphaenogaster fulva 11	KP730078	KP860435	KP730160	KP860363	KP730238
Aphaenogaster fulva 12	KP730079	KP860436	KP730161	KP860364	KP730239
Aphaenogaster fulva 13	KP730080	KP860437	KP730162	KP860365	KP730240
Aphaenogaster fulva 17	KP730081	KP860438	KP730163	KP860366	KP730241
Aphaenogaster fulva 18	KP730082	KP860439	KP730164	KP860367	KP730242
Aphaenogaster huachucana Creighton 1	KJ920526	N/A	N/A	KJ920626	KJ920661
Aphaenogaster huachucana 4	KP730083	KP860440	KP730165	KP860368	KP730243

Table 2.1. (cont'd).

Taxon name, author and specimen number	Genbank COI#	Genbank CAD #	Genbank EF1αF2 #	Genbank LWR #	Genbank WG #
Aphaenogaster japonica Forel	KJ920527	KJ920559	KJ920591	KJ920627	KJ920662
Aphaenogaster lamellidens Mayr 1	KJ920528	KJ920560	KJ920592	KJ920628	KJ920663
Aphaenogaster lamellidens 2	KP730084	KP860441	KP730166	KP860369	KP730244
Aphaenogaster mariae Forel 1	KJ920529	KJ920561	KJ920593	KJ920629	KJ920664
Aphaenogaster mariae 2	KP730085	KP860442	KP730167	KP860370	KP730245
Aphaenogaster megommata Smith	KJ920530	KJ920562	KJ920594	KJ920630	KJ920665
Aphaenogaster miamiana Wheeler 1	KJ920531	KJ920563	KJ920595	KJ920631	KJ920666
Aphaenogaster miamiana 2	KP730086	KP860443	KP730168	KP860371	KP730246
Aphaenogaster miamiana 3	KP730087	KP860444	KP730169	KP860372	KP730247
Aphaenogaster miamiana 5	KP730088	KP860445	KP730170	KP860373	KP730248
Aphaenogaster mutica Pergande	KJ920532	N/A	KJ920596	KJ920632	KJ920667
Aphaenogaster occidentalis (Emery) 1	KJ920533	KJ920564	KJ920597	KJ920633	KJ920668
Aphaenogaster occidentalis 4	KP730089	KP860446	KP730171	KP860374	KP730249
Aphaenogaster occidentalis 5	KP730090	KP860447	KP730172	KP860375	KP730250
Aphaenogaster patruelis Forel	KJ920534	N/A	KJ920598	KJ920634	KJ920669
Aphaenogaster picea (Wheeler) 1	KJ920535	KJ920565	KJ920599	KJ920635	KJ920670
Aphaenogaster picea 2	KP730091	KP860448	KP730173	KP860376	KP730251
Aphaenogaster picea 3	KP730092	KP860449	KP730174	KP860377	KP730252
Aphaenogaster picea 4	KP730093	KP860450	KP730175	KP860378	KP730253
Aphaenogaster picea 6	KJ920536	KJ920566	KJ920600	KJ920636	KJ920671
Aphaenogaster picea 15	KP730094	KP860451	KP730176	KP860379	KP730254
Aphaenogaster picea 16	KP730095	KP860452	KP730177	KP860380	KP730255
Aphaenogaster picea 19	KP730096	KP860453	KP730178	KP860381	KP730256
Aphaenogaster picea 21	KP730097	KP860454	KP730179	KP860382	KP730257
Aphaenogaster picea 23	KP730098	KP860455	KP730180	KP860383	KP730258
Aphaenogaster picea 26	KP730099	KP860456	KP730181	KP860384	KP730259
Aphaenogaster picea 27	KP730100	KP860457	KP730182	KP860385	KP730260
Aphaenogaster picea 28	KP730101	KP860458	KP730183	KP860386	KP730261

Table 2.1. (cont'd).

Taxon name, author and specimen number	Genbank COI#	Genbank CAD #	Genbank EF1αF2 #	Genbank LWR #	Genbank WG #
Aphaenogaster picea 30	KP730102	KP860459	KP730184	KP860387	KP730262
Aphaenogaster picea 31	KP730103	KP860460	KP730185	KP860388	KP730263
Aphaenogaster picea 32	KP730104	KP860461	KP730186	KP860389	KP730264
Aphaenogaster picea 36	KP730105	KP860462	KP730187	KP860390	KP730265
Aphaenogaster picea 37	KP730106	KP860463	KP730188	KP860391	KP730266
Aphaenogaster picea 39	KJ920537	KJ920567	KJ920601	N/A	KJ920672
Aphaenogaster rudis Enzmann 2	KJ920538	KJ920568	KJ920602	KJ920637	KJ920673
Aphaenogaster rudis 4	KJ920539	KJ920569	KJ920603	KJ920638	KJ920674
Aphaenogaster rudis 6	KP730107	KP860464	KP730189	KP860392	KP730267
Aphaenogaster rudis 8	KJ920540	KJ920570	KJ920604	KJ920639	KJ920675
Aphaenogaster rudis 10	KP730108	KP860465	KP730190	KP860393	N/A
Aphaenogaster rudis 12	KP730109	KP860466	KP730191	KP860394	KP730268
Aphaenogaster rudis 14	KP730110	KP860467	KP730192	KP860395	KP730269
Aphaenogaster rudis 15	KP730111	KP860468	KP730193	KP860396	N/A
Aphaenogaster rudis 16	KP730112	KP860469	KP730194	KP860397	KP730270
Aphaenogaster rudis 17	KP730113	KP860470	KP730195	N/A	KP730271
Aphaenogaster rudis 19	KP730114	KP860471	KP730196	KP860398	KP730272
Aphaenogaster rudis 28	KP730115	KP860472	KP730197	KP860399	KP730273
Aphaenogaster rudis 29	KP730116	KP860473	KP730198	KP860400	KP730274
Aphaenogaster rudis 32	KP730117	KP860474	N/A	KP860401	KP730275
Aphaenogaster rudis 33	KP730118	KP860475	N/A	KP860402	KP730276
Aphaenogaster rudis 34	KP730119	KP860476	KP730199	KP860403	N/A
Aphaenogaster rudis 41	KP730120	KP860477	KP730200	KP860404	KP730277
Aphaenogaster rudis 43	KP730121	KP860478	KP730201	KP860405	KP730278
Aphaenogaster rudis 44	KP730122	KP860479	KP730202	N/A	KP730279
Aphaenogaster rudis 45	KP730123	KP860480	KP730203	KP860406	N/A
Aphaenogaster rudis 46	KP730124	KP860481	KP730204	KP860407	KP730280
Aphaenogaster rudis 47	KP730125	KP860482	KP730205	KP860408	KP730281

Table 2.1. (cont'd).

Taxon name, author and specimen number	Genbank COI#	Genbank CAD #	Genbank EF1αF2 #	Genbank LWR #	Genbank WG #
Aphaenogaster rudis 48	KP730126	KP860483	KP730206	KP860409	KP730282
Aphaenogaster rudis 49	KP730127	KP860484	KP730207	KP860410	KP730283
Aphaenogaster rudis 50	KP730128	KP860485	KP730208	KP860411	KP730284
Aphaenogaster rudis 51	KP730129	KP860486	KP730209	KP860412	KP730285
Aphaenogaster swammerdami Forel	JQ742635	JQ742579	EF013388	EF013546	JQ742891
Aphaenogaster tennesseensis (Mayr) 1	KJ920541	N/A	KJ920605	KJ920640	N/A
Aphaenogaster tennesseensis 2	KP730130	N/A	KP730210	KP860413	KP730286
Aphaenogaster tennesseensis 5	KP730131	N/A	KP730211	KP860414	KP730287
Aphaenogaster tennesseensis 6	KP730132	KP860487	KP730212	KP860415	KP730288
Aphaenogaster texana Wheeler 1	KP730133	N/A	KP730213	N/A	KP730289
Aphaenogaster texana 3	KJ920542	KJ920571	KJ920606	KJ920641	KJ920676
Aphaenogaster texana 4	KP730135	KP860488	KP730215	KP860416	KP730291
Aphaenogaster texana 5	KP730136	KP860489	KP730216	KP860417	KP730292
Aphaenogaster texana 6	KP730137	KP860490	KP730217	KP860418	KP730293
Aphaenogaster texana 7	KP730138	KP860491	KP730218	KP860419	KP730294
Aphaenogaster treatae Forel 1	KJ920543	N/A	KJ920607	KJ920642	KJ920677
Aphaenogaster treatae 4	KP730139	N/A	KP730219	KP860420	KP730295
Aphaenogaster uinta Wheeler 1	KJ920544	KJ920572	KJ920608	N/A	KJ920678
Aphaenogaster uinta 2	KP730140	N/A	KP730220	KP860421	KP730296
Aphaenogaster umphreyi Deyrup & Davis 1	KJ920545	N/A	KJ920609	KJ920643	KJ920679
Aphaenogaster umphreyi 2	KP730141	KP860492	KP730221	KP860422	KP730297
Camponotus castanaeus (Latreille)	KP730061	N/A	KP730143	KP860349	KP730223
Camponotus nearcticus Emery	KP730062	N/A	KP730144	KP860350	N/A
Camponotus pennsylvanicus (DeGeer)	KJ920508	N/A	KJ920573	KJ920610	KJ920644
Formica glacialis Wheeler	KJ920509	N/A	KJ920574	KJ920611	KJ920645
Formica subintegra Wheeler	KP730063	N/A	KP730145	N/A	KP730224
Formica subsericea Say	KP730064	N/A	KP730146	KP860351	KP730225
Messor bouvieri Bondroit	JQ742637	JQ742581	EF013447	EF013590	JQ742893

Table 2.1. (cont'd).

Taxon name, author and specimen number	Genbank COI#	Genbank CAD #	Genbank EF1αF2 #	Genbank LWR #	Genbank WG #
Messor denticornis Forel	JQ742636	JQ742580	EF013446	EF013589	JQ742892
Myrmica incompleta Provancher	KP730065	N/A	KP730147	N/A	N/A
Myrmica latifrons Stärcke	KJ920510	N/A	KJ920576	KJ920613	KJ920647
Novomessor albisetosa (Mayr)	KJ920513	KJ920548	KJ920578	KJ920614	KJ920649
Novomessor cockerelli André 1	KJ920520	KJ920553	KJ920585	KJ920620	KJ920655
Novomessor cockerelli André 2	KP730066	KP860424	KP730148	KP860352	KP730226
Novomessor ensifera Forel	KJ920521	KJ920554	KJ920586	KJ920621	KJ920656
Solenopsis aurea Wheeler	KJ920512	KJ920547	KJ920577	N/A	KJ920648
Solenopsis invicta Buren	KP730067	KP860425	KP730149	N/A	KP730228
Stenamma diecki Emery	JQ742647	JQ742591	JQ742693	JQ742738	JQ742903
Veromessor andrei (Mayr)	DQ074325	N/A	N/A	HE963100	HE963097
Veromessor julianus (Pergande)	KJ920511	KJ920546	KJ920575	KJ920612	KJ920646
JTL-001 (undescribed species)	KP730142	N/A	KP730222	KP860423	KP730298

Table 2.2. Bootstrap and partitioned bremer support values that correspond to the label nodes in the parsimony phylogeny (Fig. 2).

	Bootstrap	COI	CAD	EF2	LWR	Wg	Sum
Node 1	62	-0.1	0.0	0.1	0.0	0.1	0.0
Node 2	51	0.1	0.0	0.0	0.0	-0.1	0.0
Node 3	62	0.0	-2.0	2.9	0.0	0.0	1.0
Node 4	<50	0.1	0.0	0.1	0.0	-0.1	0.0
Node 5	64	0.9	0.0	-0.1	0.0	0.1	1.0
Node 6	67	0.6	0.0	0.1	0.0	0.3	1.0
Node 7	85	2.8	0.0	0.0	0.0	0.1	3.0
Node 8	61	1.0	0.0	-0.1	0.0	0.1	1.0
Node 9	60	0.0	0.0	-0.1	0.0	1.1	1.0
Node 10	84	1.7	0.0	0.1	0.0	0.1	2.0
Node 11	97	7.8	0.0	-0.5	-0.4	0.1	7.0
Node 12	59	-0.6	0.0	0.0	2.0	-0.3	1.0
Node 13	85	4.0	0.0	0.0	0.0	0.0	4.0
Node 14	98	3.4	0.0	0.0	1.0	0.7	5.0
Node 15	<50	-0.6	0.7	0.0	0.8	0.1	1.0
Node 16	<50	-1.4	1.0	0.1	1.6	-0.3	1.0
Node 17	<50	-0.9	0.9	-0.1	1.2	-0.1	1.0
Node 18	<50	-0.4	0.8	-0.1	0.5	0.2	1.0
Node 19	<50	0.3	0.0	0.0	0.0	-0.3	0.0
Node 20	<50	-0.7	0.0	0.0	0.0	0.7	0.0
Node 21	63	0.7	0.0	0.0	0.0	0.3	1.0
Node 22	<50	-0.5	0.0	0.0	0.0	0.6	0.0
Node 23	58	1.2	0.0	-0.1	0.0	-0.1	1.0
Node 24	<50	-0.6	0.0	0.0	2.0	-0.3	1.0
Node 25	55	-0.2	1.5	1.4	0.0	0.3	3.0
Node 26	55	-0.2	1.6	1.2	-0.2	0.6	3.0
Node 27	86	2.8	0.0	0.0	0.0	0.2	3.0
Node 28	85	0.0	2.0	0.9	0.0	0.1	3.0
Node 29	74	0.2	0.0	-0.1	0.9	-0.1	1.0
Node 30	<50	-0.1	0.0	0.1	0.0	0.1	0.0
Node 31	91	13.2	-5.5	0.2	0.0	0.2	8.0
Node 32	81	0.0	1.0	-0.1	0.0	0.1	1.0
Node 33	100	11.4	0.1	0.3	0.0	0.2	12.0
Node 34	100	11.0	0.0	-0.4	1.0	0.4	12.0
Node 35	96	6.0	0.1	0.9	0.0	0.0	7.0
Node 36	54	1.9	0.0	-1.1	0.0	0.2	1.0
Node 37	52	-0.8	1.5	1.7	-0.4	1.1	3.0
Node 38	61	-0.5	0.0	0.4	0.0	0.1	0.0

Table 2.2 (cont'd).

	Bootstrap	COI	CAD	EF2	LWR	Wg	Sum
Node 39	<50	-0.3	0.0	0.2	0.0	0.1	0.0
Node 41	52	2.0	0.0	-0.2	-1.0	0.2	1.0
Node 42	<50	0.2	-0.5	0.0	0.0	1.3	1.0
Node 43	<50	0.1	0.2	0.1	0.0	0.6	1.0
Node 44	<50	0.2	1.4	0.0	0.0	-0.6	1.0
Node 45	<50	0.0	0.4	0.5	0.0	0.2	1.0
Node 46	93	-0.1	1.8	0.6	0.0	0.7	3.0
Node 47	<50	0.0	1.2	-0.5	0.0	0.3	1.0
Node 48	<50	0.1	0.0	0.0	0.0	0.0	0.0
Node 49	<50	0.0	0.0	0.0	0.0	0.0	0.0
Node 50	<50	1.2	0.0	-0.1	0.0	-0.1	1.0
Node 51	54	0.1	0.0	-0.1	0.0	1.0	1.0
Node 52	66	-0.1	1.0	-0.1	0.0	0.1	1.0
Node 53	98	0.0	8.7	-0.1	0.0	1.4	10.0
Node 54	<50	6.8	-3.0	0.2	0.0	0.0	4.0
Node 55	<50	0.3	0.0	0.8	0.0	-0.1	1.0
Node 56	90	21.6	-5.9	-1.1	-0.9	-1.7	12.0
Node 57	87	41.8	-17.0	-5.6	-3.1	-8.1	8.0
Node 58	88	1.4	0.0	-0.5	0.0	0.1	1.0
Node 59	<50	1.5	0.0	-0.2	0.0	-0.3	1.0
Node 60	100	9.1	0.0	3.0	0.0	0.9	13.0
Node 61	<50	6.7	0.1	2.4	2.0	0.8	12.0
Node 62	100	7.0	0.0	0.2	1.0	-0.1	8.0
Node 63	100	16.1	13.0	-0.1	1.0	1.0	31.0
Node 64	<50	0.6	0.0	0.8	0.0	0.7	2.0
Node 65	62	3.3	0.0	-0.1	0.0	0.8	4.0
Node 66	<50	-0.4	0.0	1.0	0.0	1.4	2.0
Node 67	<50	0.2	0.0	0.9	0.0	0.9	2.0
Node 68	52	2.6	0.0	0.6	0.0	0.8	4.0
Node 69	<50	0.1	0.0	2.9	1.0	1.0	5.0
Node 70	<50	-0.9	0.0	0.9	0.0	1.0	1.0
Node 71	<50	0.4	0.0	-0.1	0.0	0.6	1.0
Node 72	<50	-1.1	0.0	0.9	0.0	1.2	1.0
Node 73	80	0.9	0.0	0.2	0.0	0.0	1.0
Node 74	73	1.4	-1.0	0.2	0.0	0.4	1.0
Node 75	100	40.2	-7.1	-2.2	-1.3	-3.5	26.0
Node 76	100	13.8	1.0	1.9	0.0	0.3	17.0
Node 77	94	8.2	0.0	-0.2	1.0	0.9	10.0
Node 78	84	34.4	-16.0	-5.2	-3.0	-8.2	2.0

Table 2.2 (cont'd).

	Bootstrap	COI	CAD	EF2	LWR	Wg	Sum
Node 79	63	-0.1	0.0	-0.1	0.0	1.2	1.0
Node 80	61	1.4	0.0	-0.1	0.0	-0.3	1.0
Node 81	64	2.0	0.0	-1.1	0.0	0.1	1.0
Node 82	69	1.6	0.0	-0.6	0.0	0.0	1.0
Node 83	75	1.9	0.0	-1.1	0.0	0.1	1.0
Node 84	92	1.3	0.9	2.8	0.0	0.0	5.0
Node 85	<50	-4.0	6.0	8.9	0.7	2.5	14.0
Node 86	<50	34.3	-16.0	-5.1	-3.0	-8.2	2.0
Node 87	<50	34.7	-16.0	-5.5	-3.0	-8.2	2.0
Node 88	<50	8.2	0.0	5.9	1.0	7.9	23.0
Node 89	99	27.5	2.5	-3.4	-1.5	-2.1	23.0
Node 90	89	22.2	4.0	-3.3	-7.0	-4.0	12.0
Node 91	100	24.2	-6.0	7.0	0.0	1.8	27.0
Node 92	65	-0.1	2.0	0.1	0.0	0.0	2.0
Node 93	100	47.3	8.0	3.9	0.0	-0.2	59.0
Node 94	98	12.2	-3.0	0.0	3.0	1.9	14.0
Node 95	100	9.0	4.0	1.0	-1.0	8.0	21.0
Node 96	89	21.7	0.2	-2.3	-2.8	-4.8	12.0
Node 97	97	42.0	-16.5	13.7	-4.1	-15.0	20.0
Node 98	68	6.7	0.0	1.9	1.0	-0.7	9.0
Node 99	90	34.3	-16.0	-5.0	-3.0	-8.3	2.0
Node 100	100	12.8	0.0	17.0	0.0	0.2	30.0
Node 101	84	2.9	0.0	3.0	0.0	0.1	6.0
Node 102	100	29.9	0.0	11.0	8.0	0.1	49.0
Node 103	81	2.4	0.0	0.9	0.0	0.7	4.0
Node 104	100	25.7	0.0	10.3	0.0	13.9	50.0
Node 105	100	16.0	0.0	54.0	0.0	0.0	70.0
Node 106	100	21.0	0.0	22.9	1.0	8.1	53.0
Totals		751.1	-73.1	142.9	-5.7	-6.1	809.0

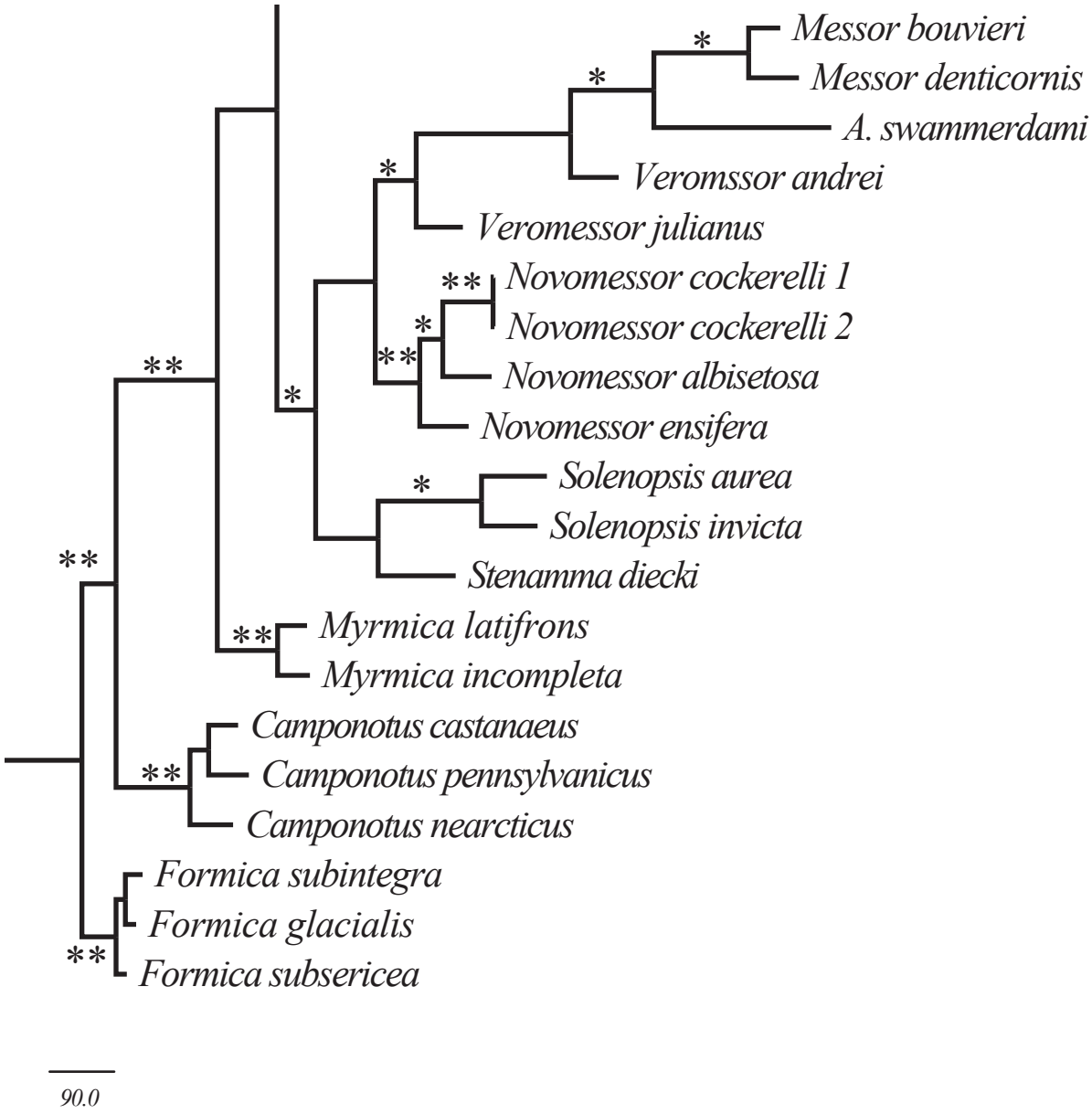
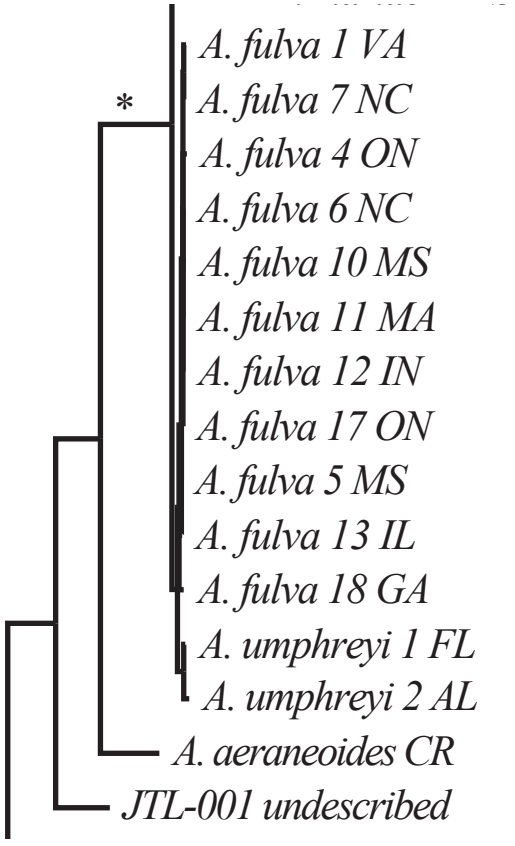


Figure 2.1. One of 64,525 MPT reconstructed for 123 taxa of *Aphaenogaster* and outgroups with DNA data and analysis of 5 genes in PAUP*. Bootstrap values greater than 90% are above the branches (* > 90%, **= 100%). A. = *Aphaenogaster*. Specimen numbers and states/ provinces where collected are displayed next to each sample. The names of non-monophyletic species correspond to specific colors.

Figure 2.1. (cont'd).



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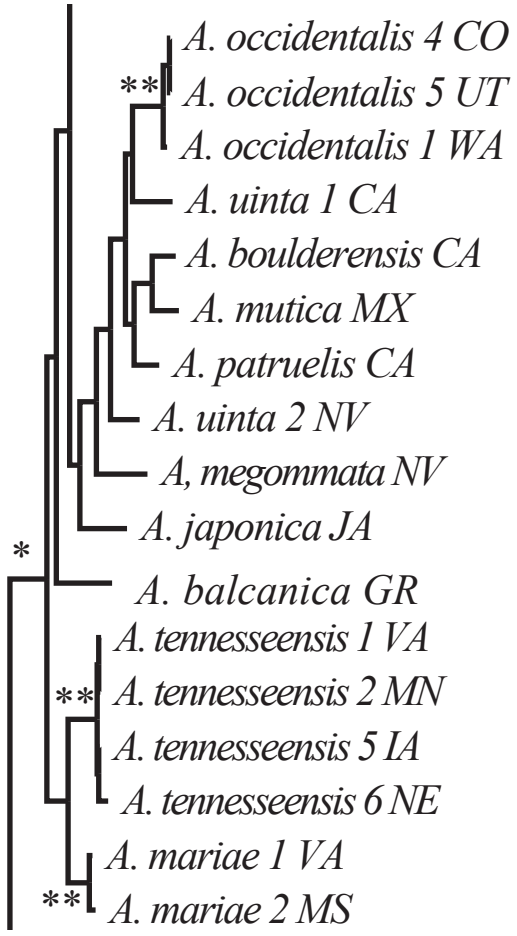


Figure 2.1. (cont'd).

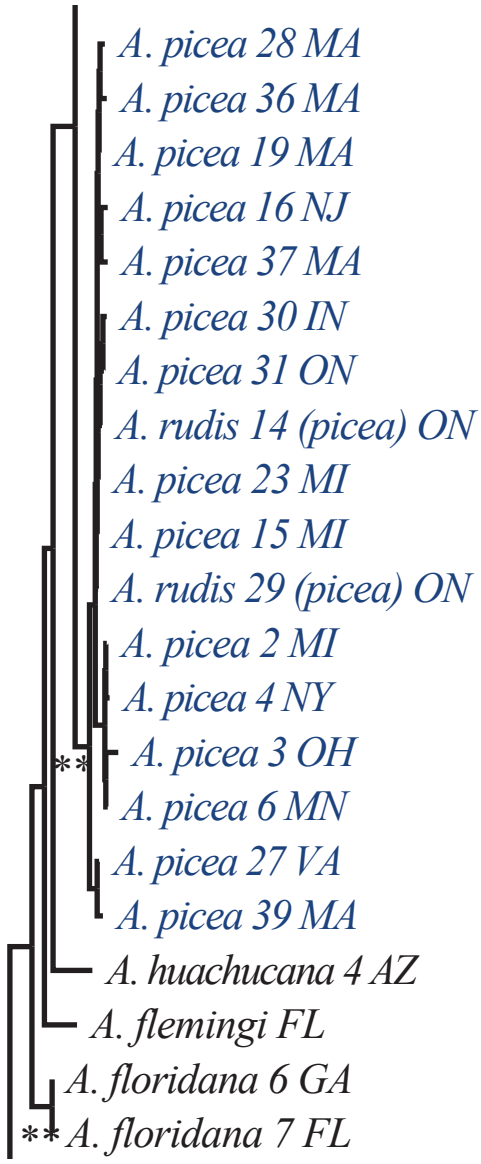


Figure 2.1. (cont'd).

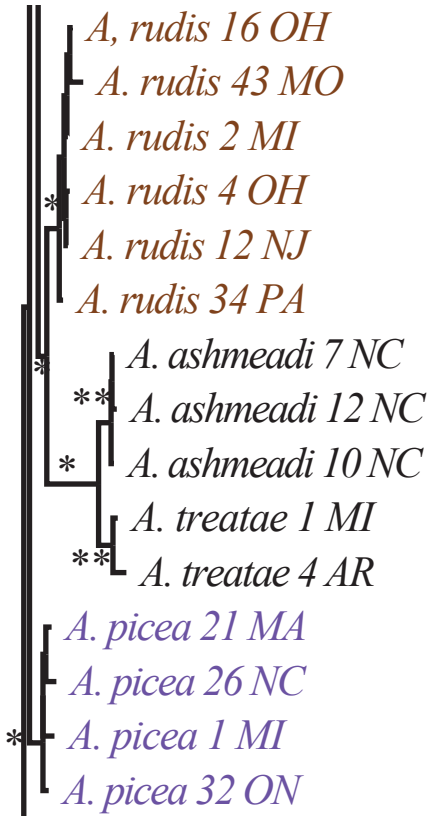


Figure 2.1. (cont'd).

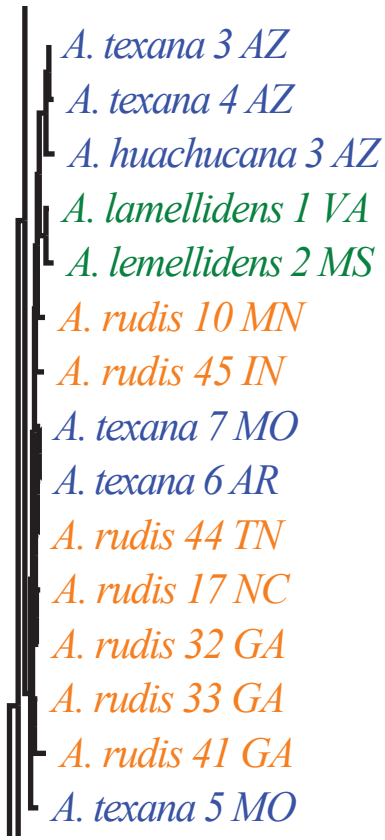
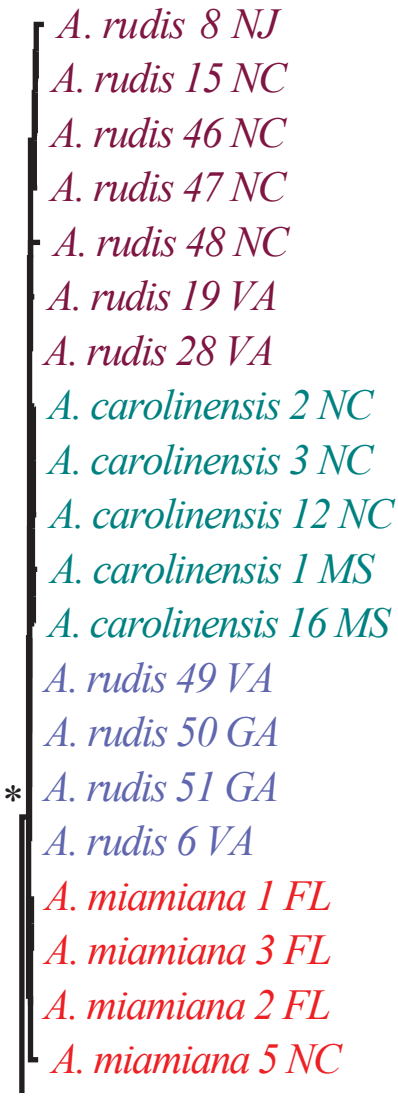


Figure 2.1. (cont'd).



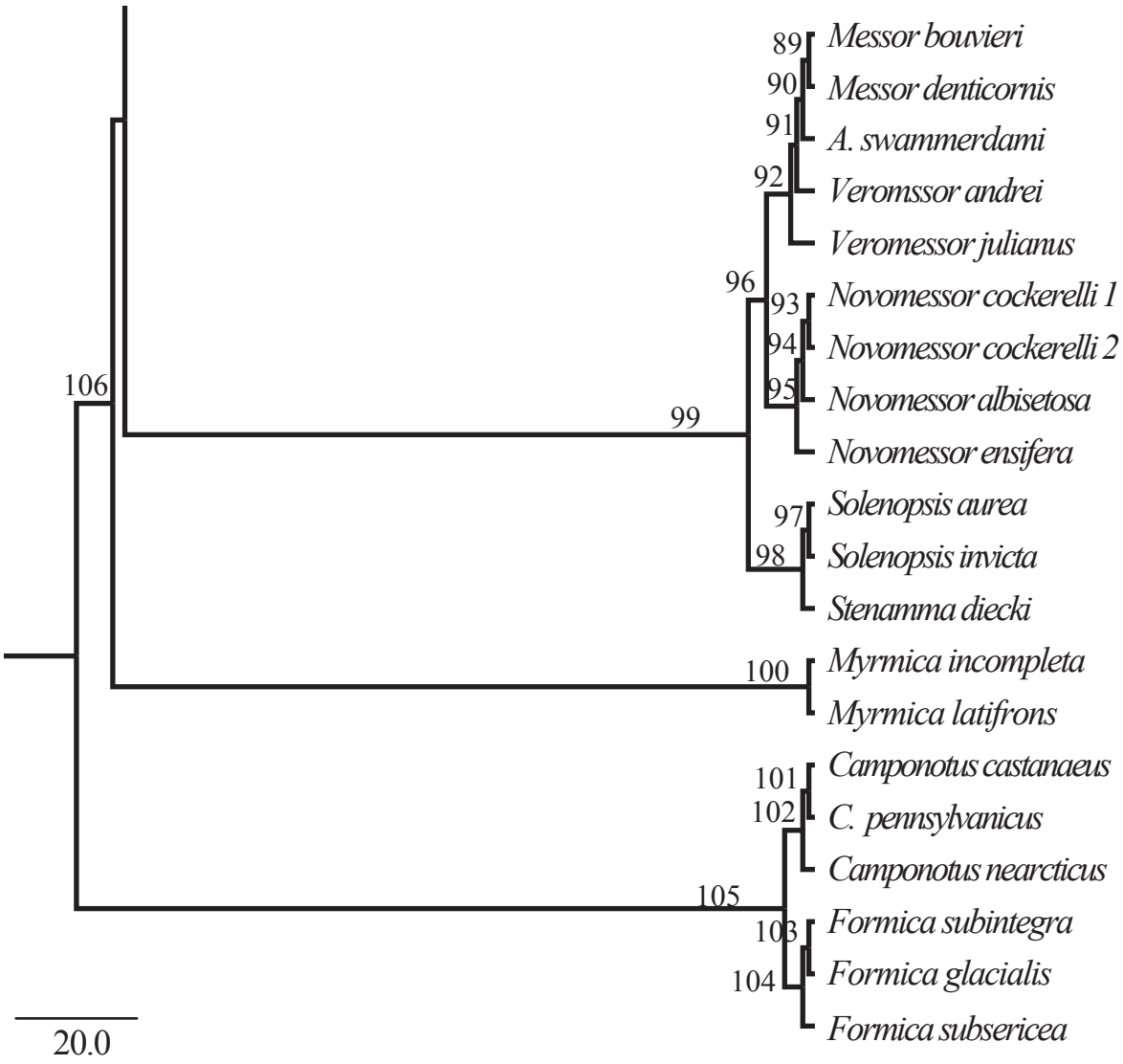
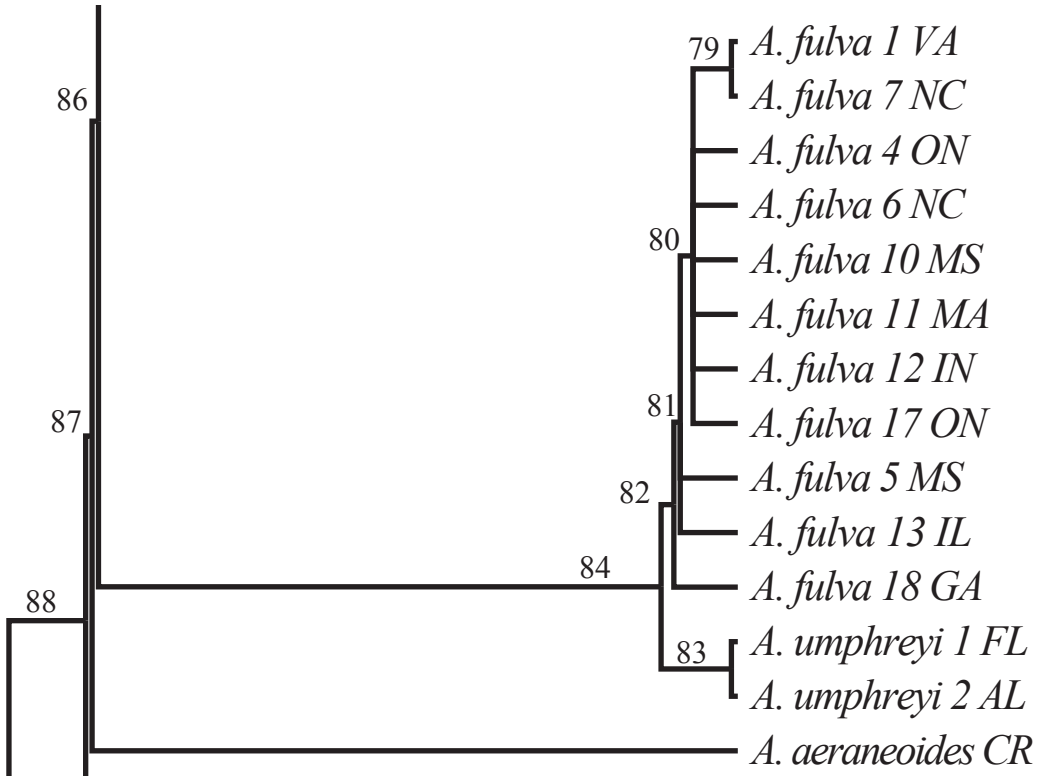


Figure 2.2. One of 64,525 MPT shown as a cladogram reconstructed for 123 taxa of *Aphaenogaster* and outgroups with DNA data and analysis of 5 genes in PAUP*. Node numbers are above the branches. A. = *Aphaenogaster*. Specimen numbers and states/provinces where collected are displayed next to each sample. The names of non-monophyletic species correspond to specific colors.

Figure 2.2. (cont'd).



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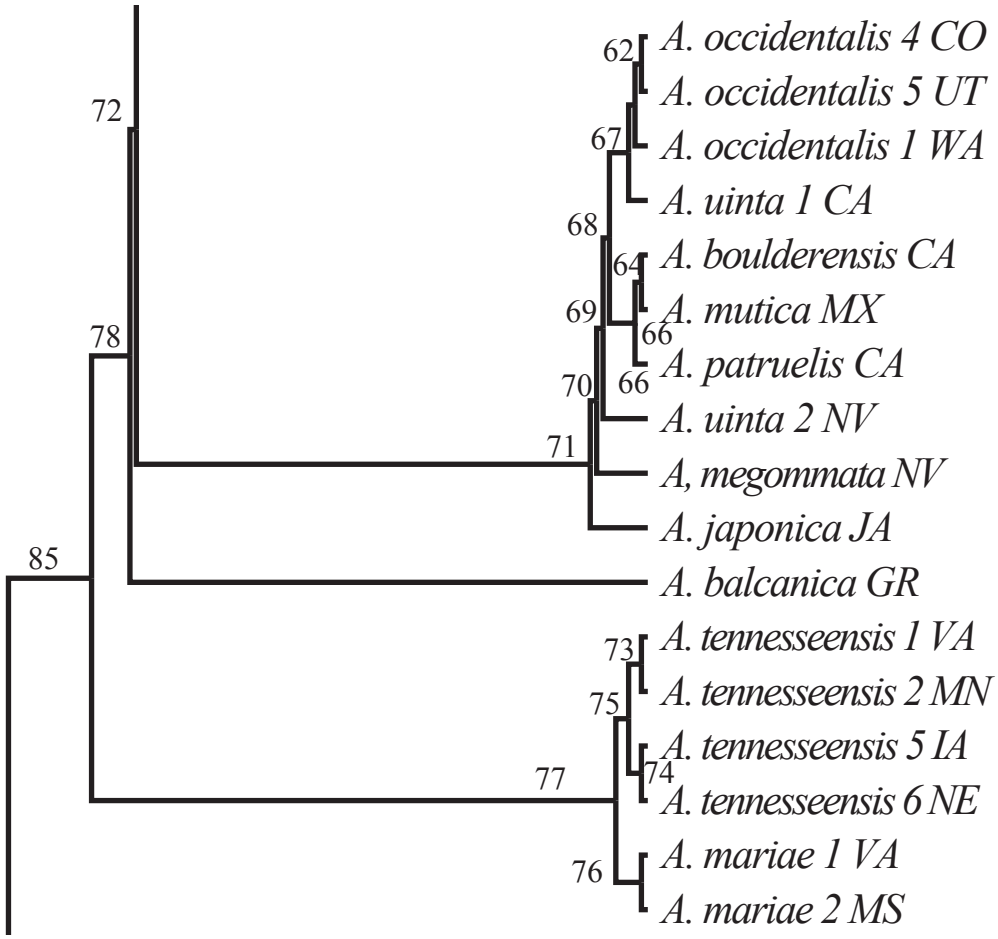


Figure 2.2. (cont'd).

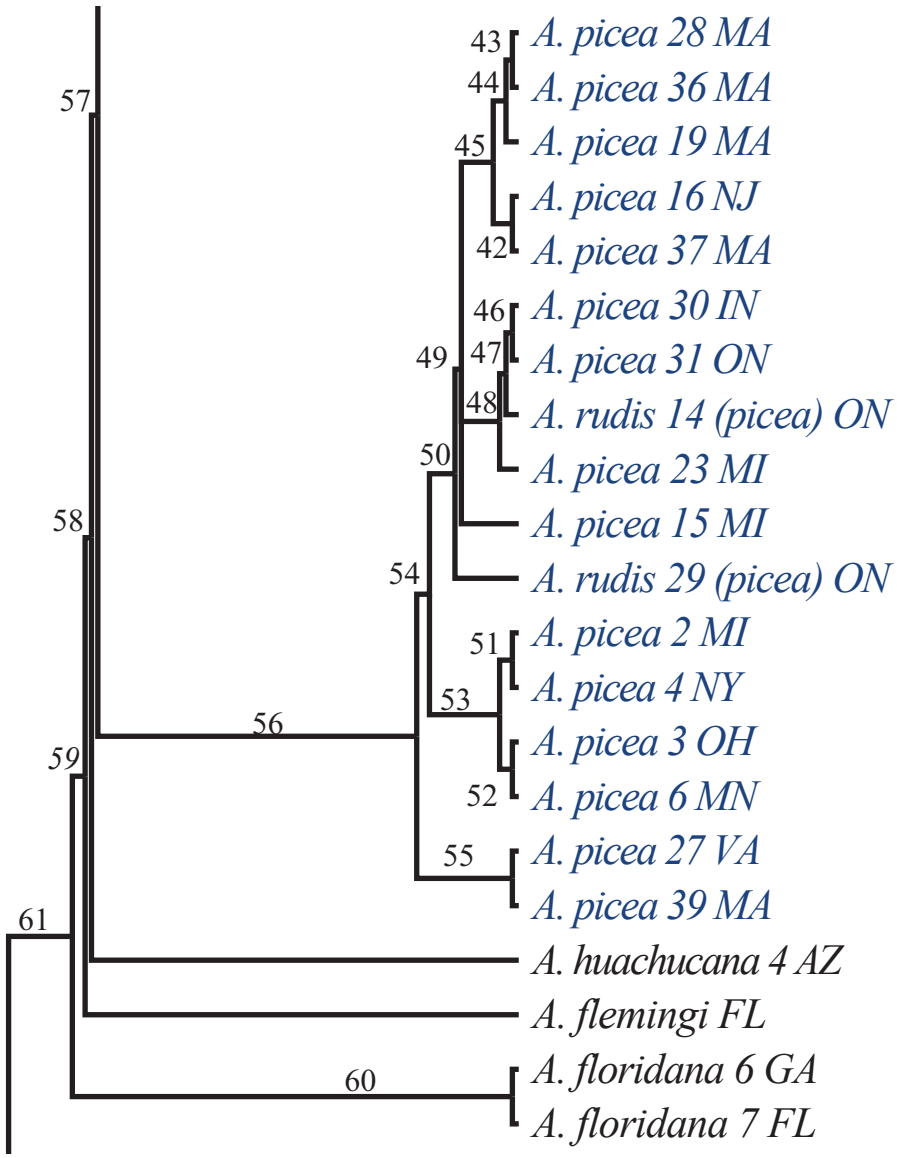


Figure 2.2. (cont'd).

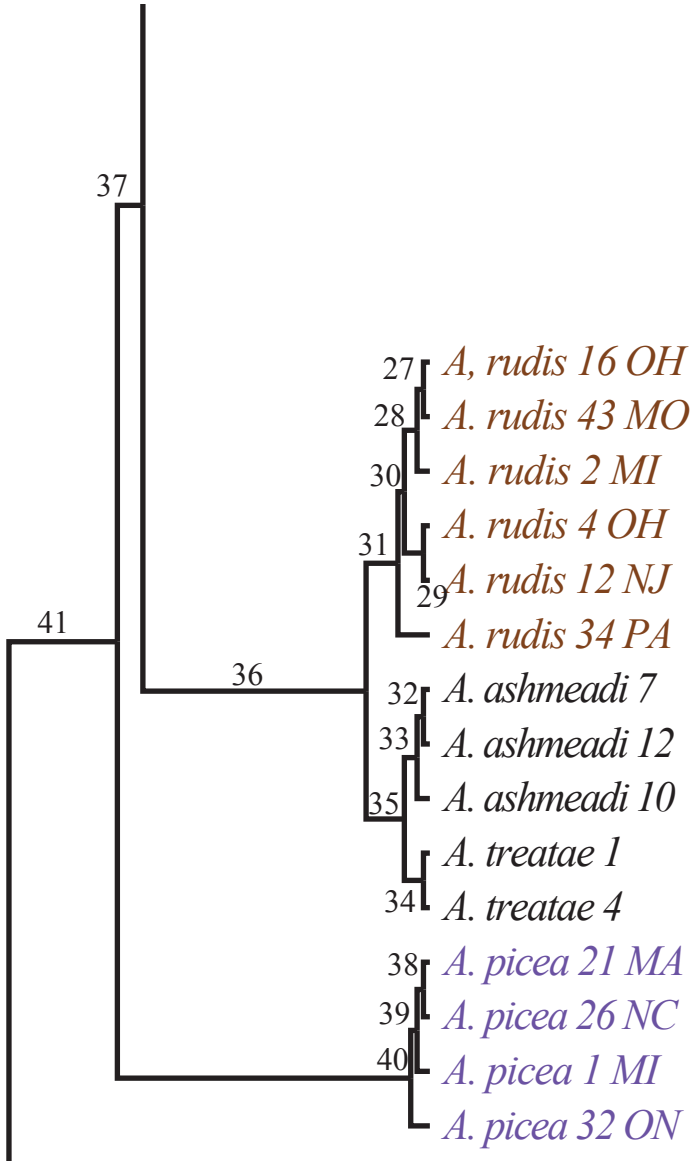


Figure 2.2. (cont'd).

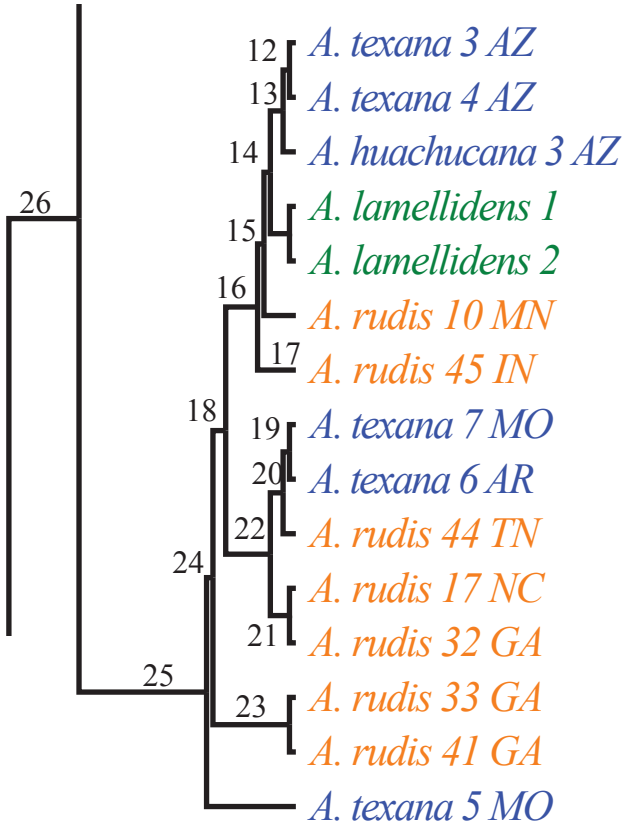
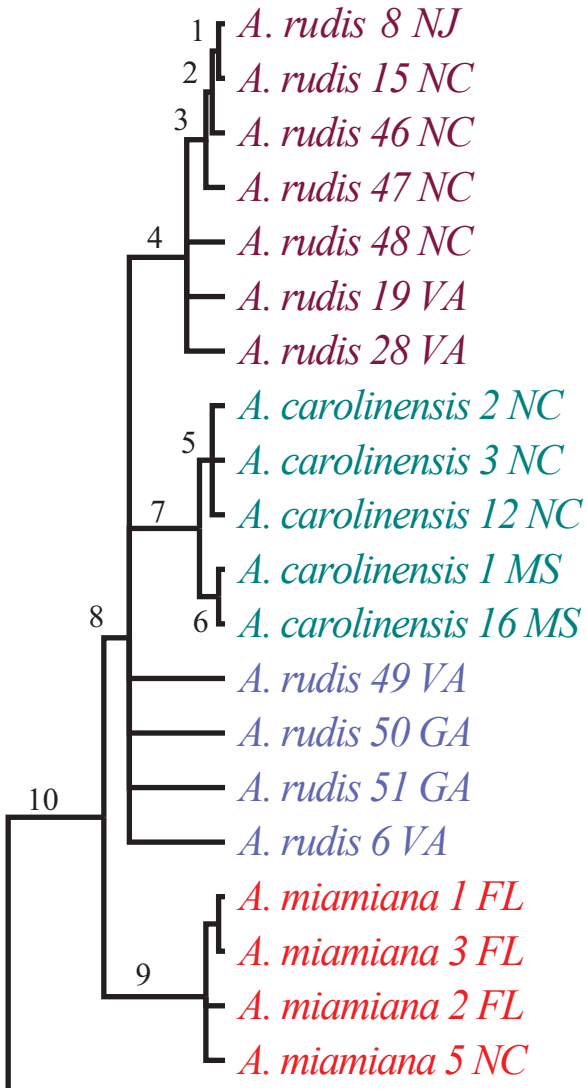


Figure 2.2. (cont'd).



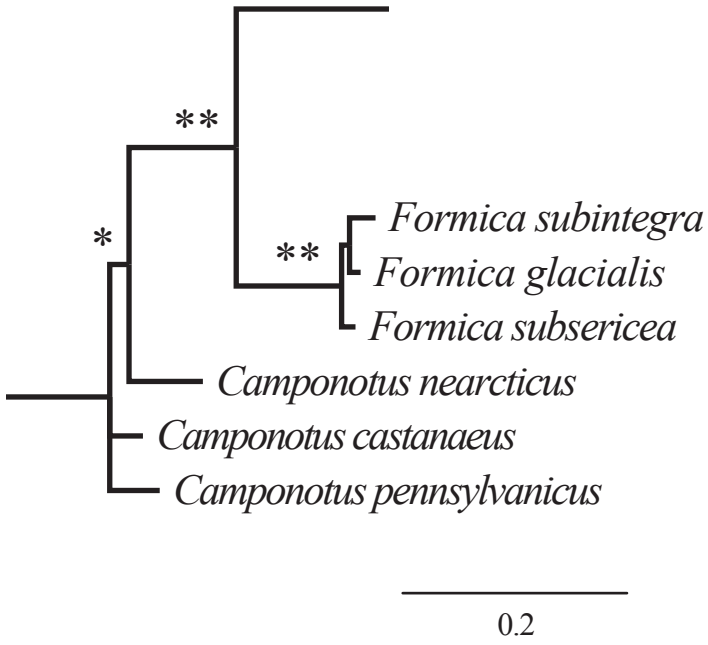
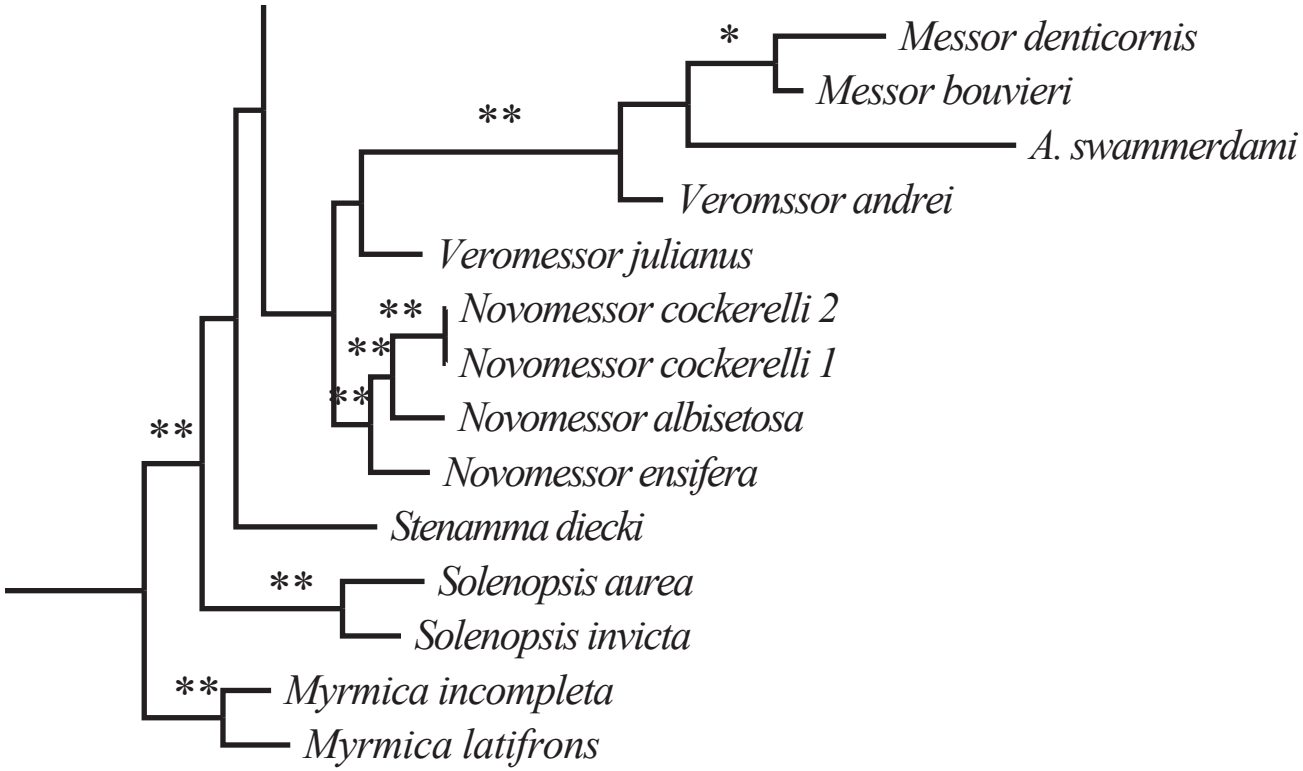


Figure 2.3. Maximum likelihood tree reconstructed for 123 taxa with DNA data and analysis of 5 genes in a RAxML analysis. Bootstrap values greater than 90% are above the branches (* > 90%, **= 100%). A. = *Aphaenogaster*. Specimen numbers and states/ provinces where collected are displayed next to each sample. The names of non-monophyletic species correspond to specific colors.

Figure 2.3. (cont'd).



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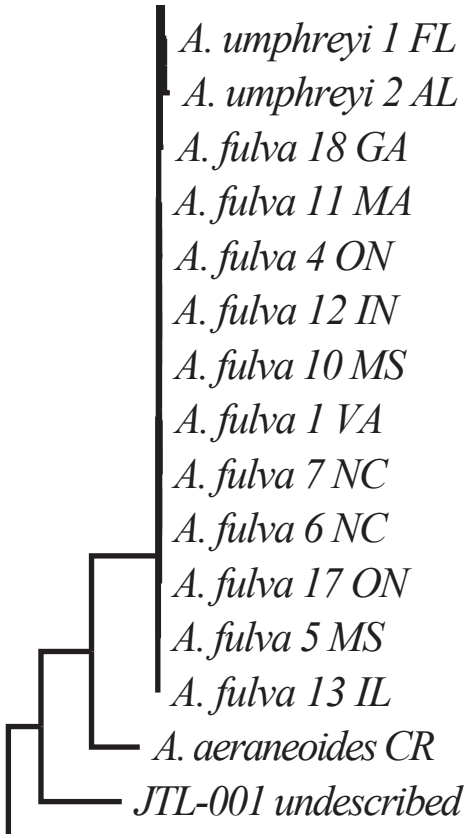


Figure 2.3. (cont'd).

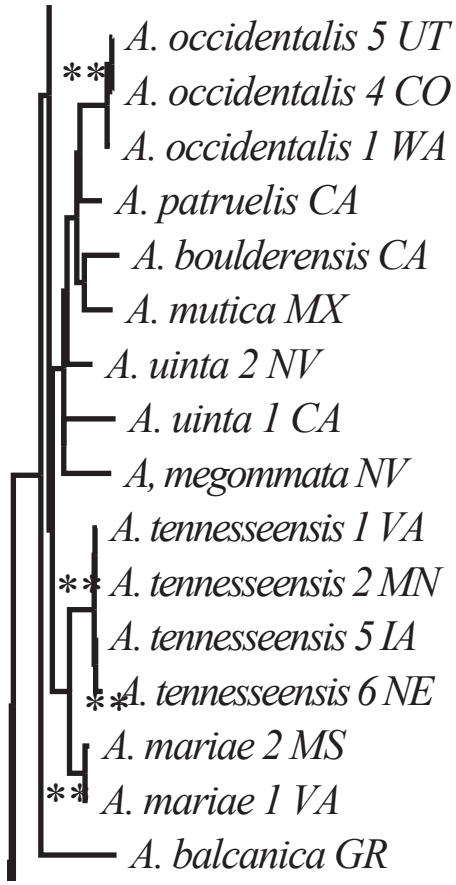


Figure 2.3. (cont'd).

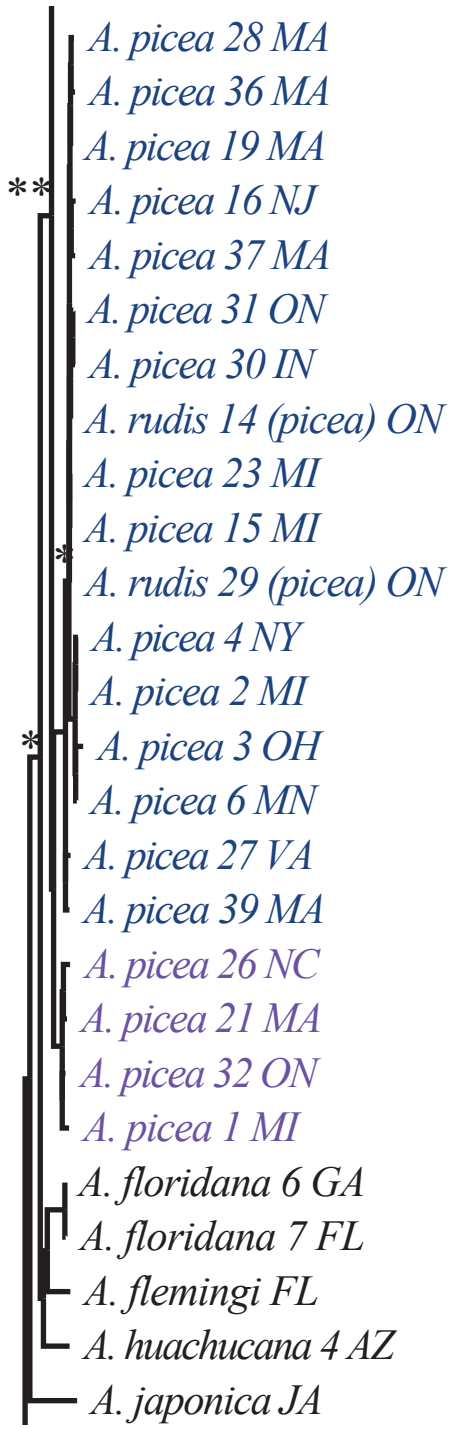


Figure 2.3. (cont'd).

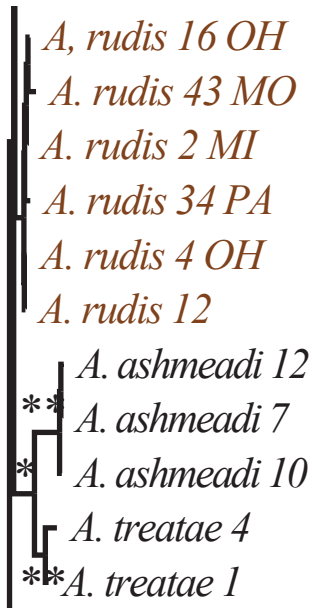


Figure 2.3. (cont'd).

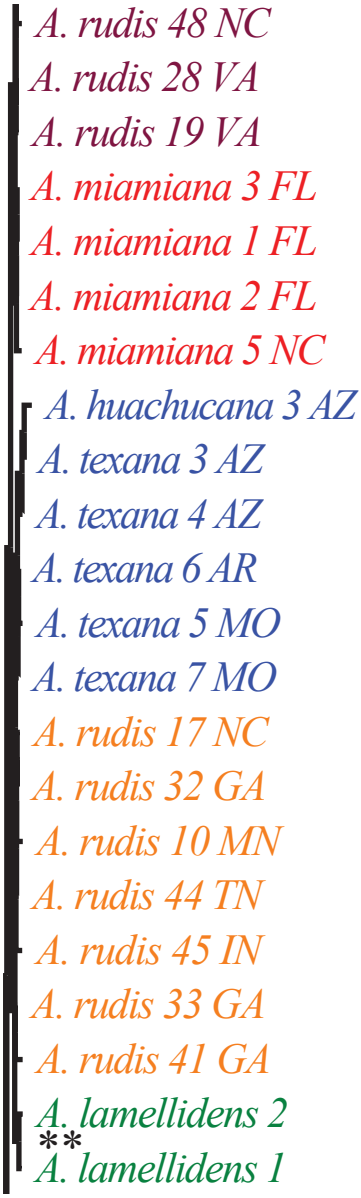
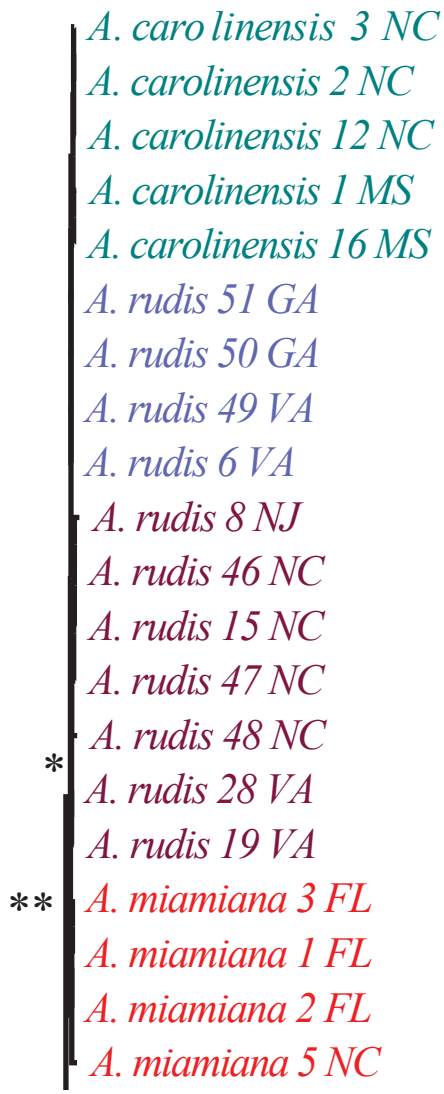


Figure 2.3. (cont'd).



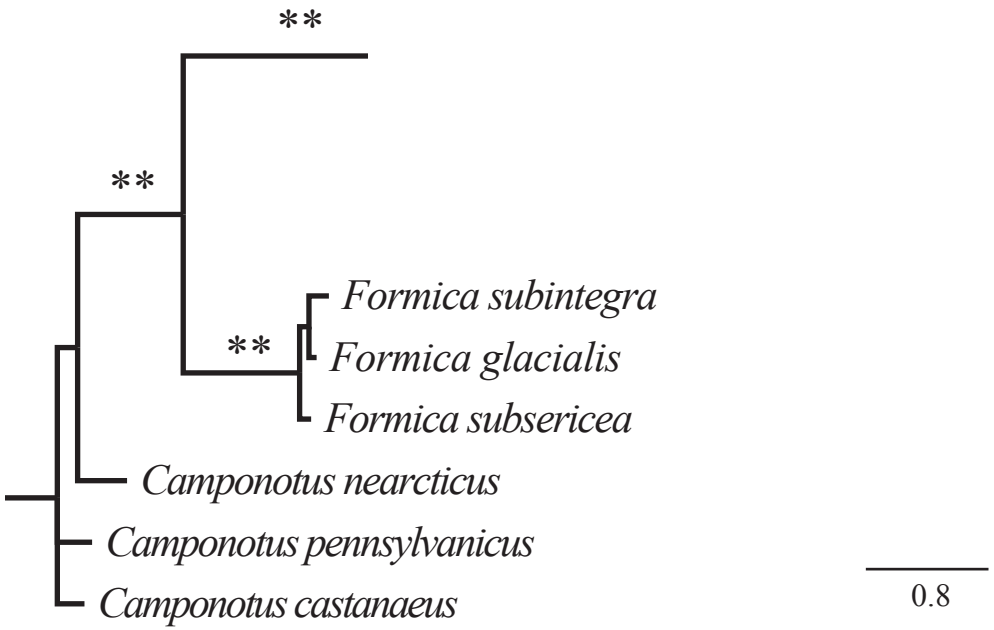


Figure 2.4. Bayesian majority rule consensus tree reconstructed for 123 taxa with morphology and five genes in a Mr. Bayes analysis, Posterior probabilities values greater than 90% are above the branches (* > 90%, **= 100%). Data were partitioned by gene and codon position and analyzed with a best-fit GTR + I + G model, 30 million generations and a burn-in of 7,500,000 generations. A. = *Aphaenogaster*. Specimen numbers and states/ provinces where collected are displayed next to each sample. The names of non-monophyletic species correspond to specific colors.

Figure 2.4. (cont'd).

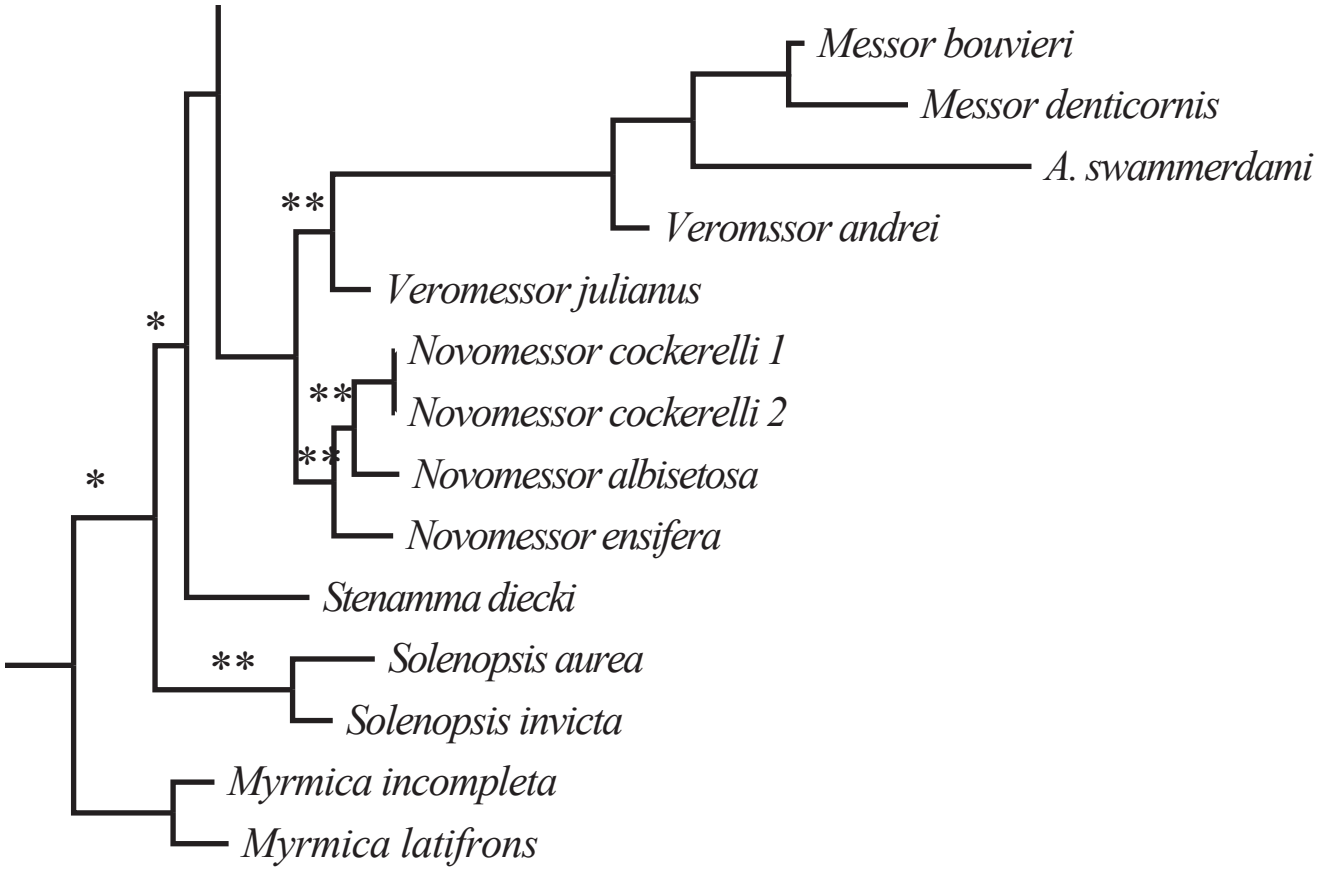


Figure 2.4. (cont'd).

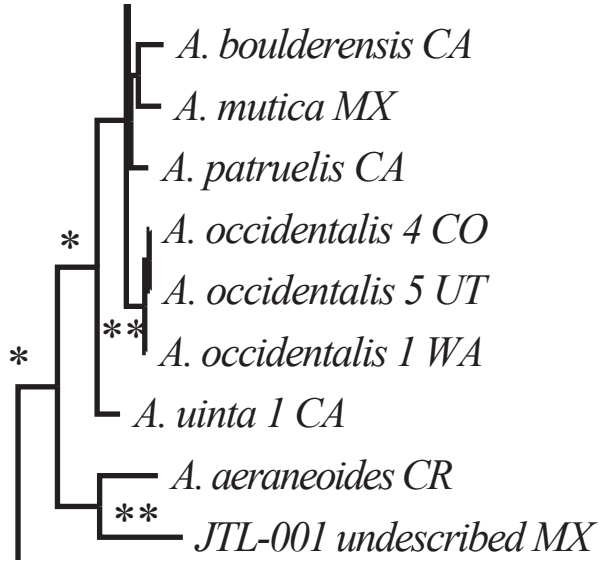


Figure 2.4. (cont'd).

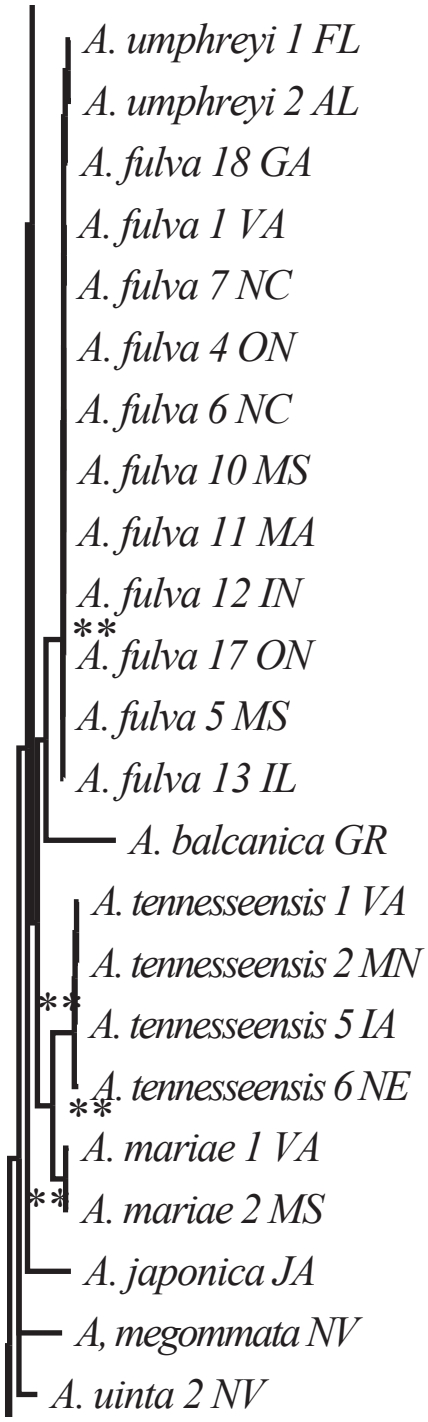


Figure 2.4. (cont'd).

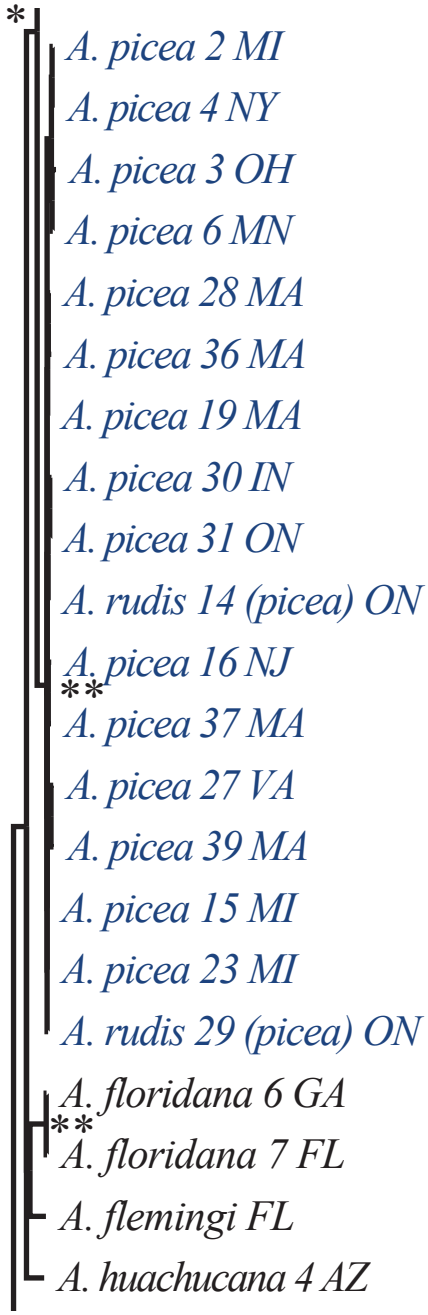


Figure 2.4. (cont'd).

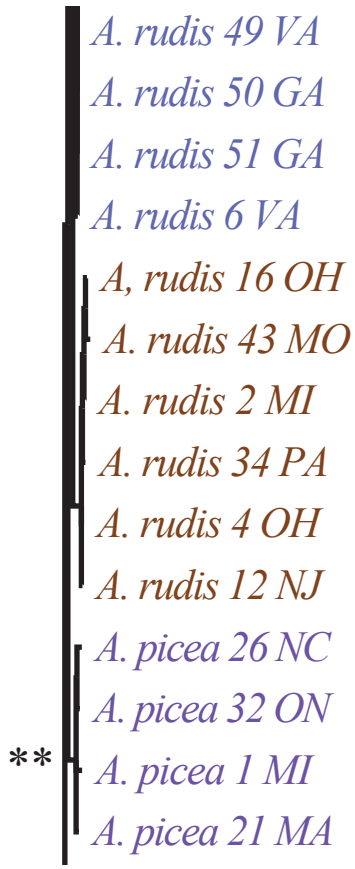


Figure 2.4. (cont'd).

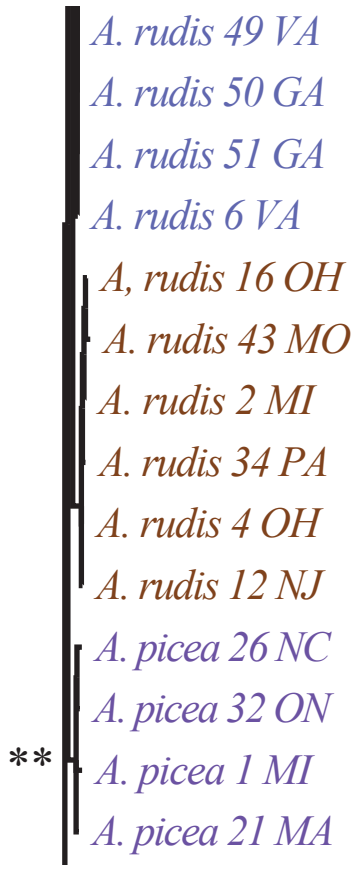
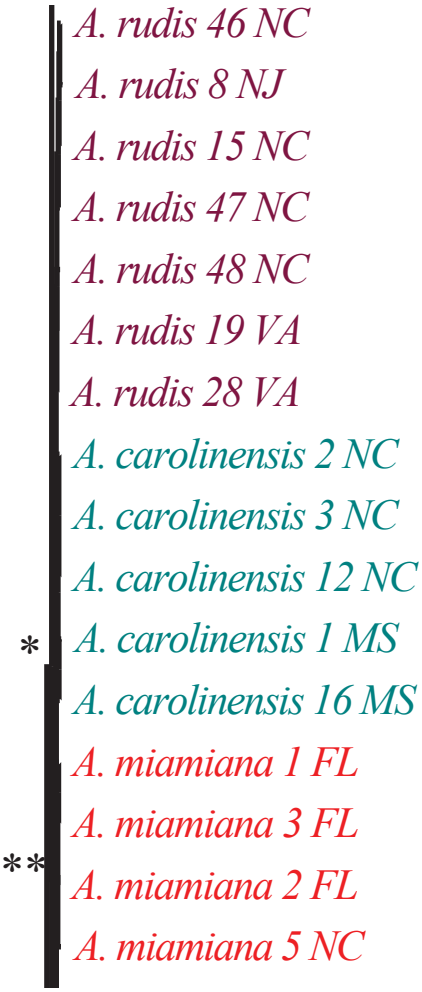


Figure 2.4. (cont'd).



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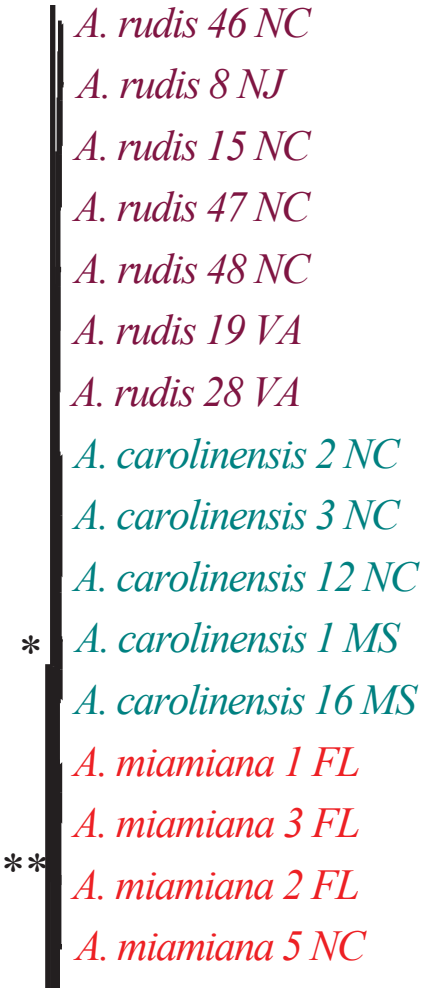
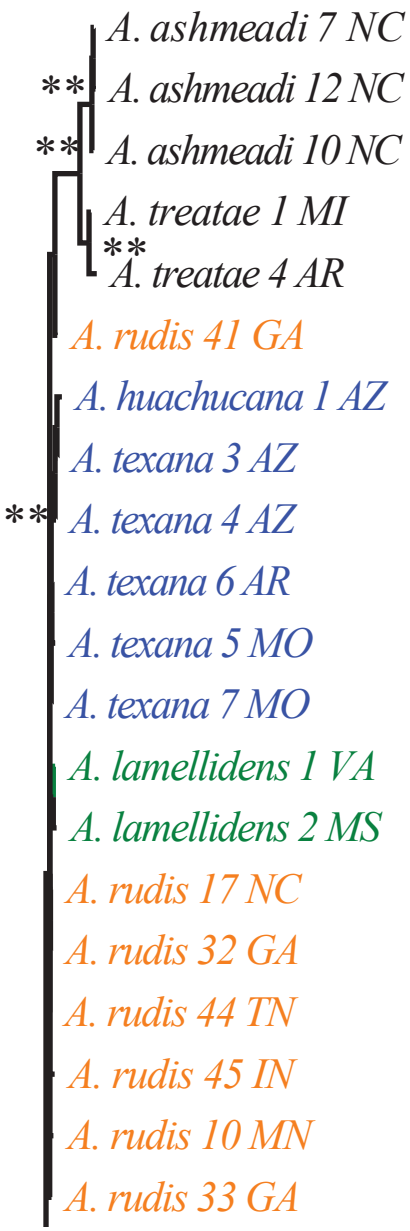


Figure 2.4 (cont'd).



APPENDIX C:

Tables and Figures for Chapter 4

Table 3.1. A list of *Aphaenogaster* species known from North America

Genus *Aphaenogaster*
Aphaenogaster ashmeadi (Emery 1895)
Aphaenogaster boulderensis Smith 1941
Aphaenogaster carolinensis Wheeler 1915
Aphaenogaster flemingi Smith 1928
Aphaenogaster floridana Smith 1941
Aphaenogaster fulva Roger 1863
Aphaenogaster huachucana Creighton 1934
Aphaenogaster lamellidens Mayr 1886
Aphaenogaster mariae Forel 1886
Aphaenogaster megommata Smith 1963
Aphaenogaster *mexicana* (Pergande 1896)
Aphaenogaster miamiana Wheeler 1932
Aphaenogaster mutica Pergande 1896
Aphaenogaster occidentalis (Emery 1895)
Aphaenogaster patruelis Forel 1886
Aphaenogaster picea (Wheeler 1908)
Aphaenogaster punctaticeps MacKay 1989
Aphaenogaster rudis Enzmann 1947
Aphaenogaster tennesseensis (Mayr 1862)
Aphaenogaster texana Wheeler 1915
Aphaenogaster treatae Forel 1886
Aphaenogaster uinta Wheeler 1917
Aphaenogaster umphreyi Deyrup & Davis 1998

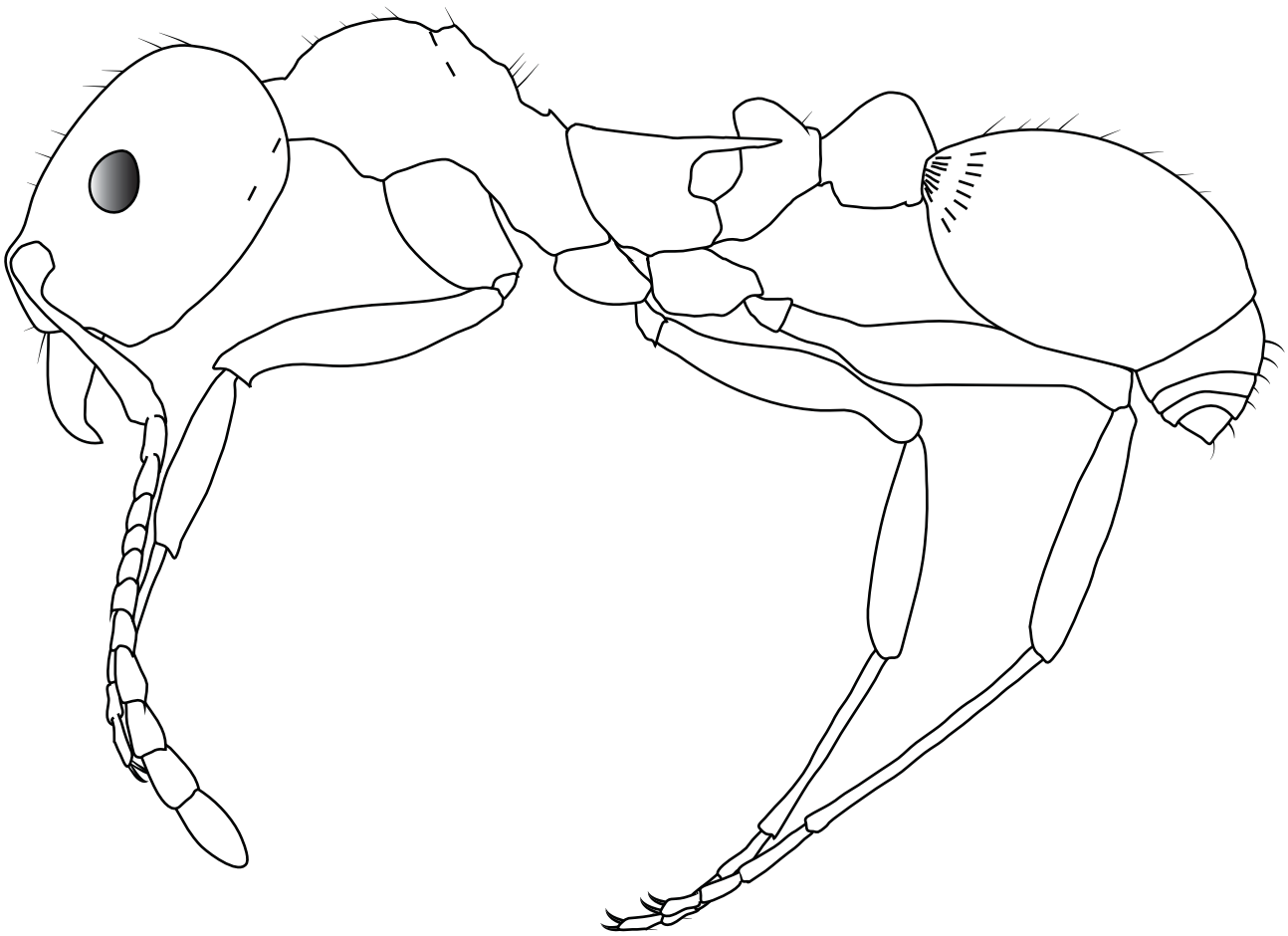
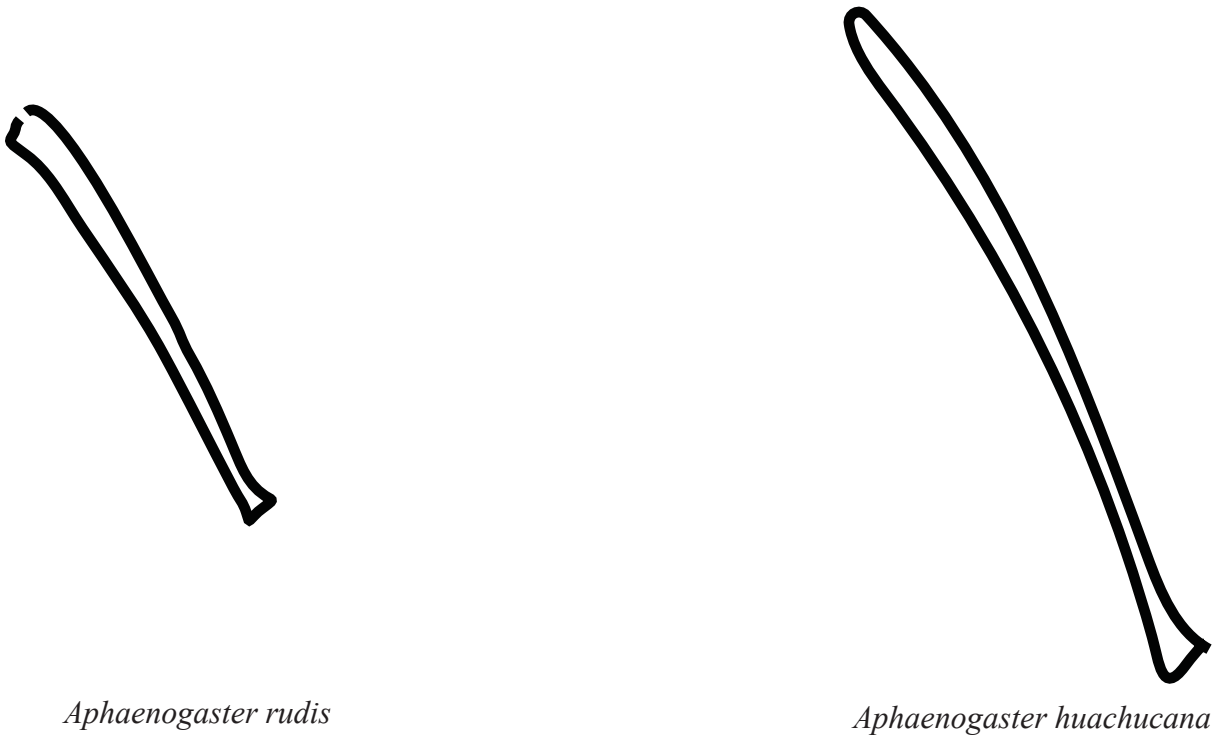


Figure 3.1. Lateral view of *Aphaenogaster mariae* showing striae on first gastral tergite.



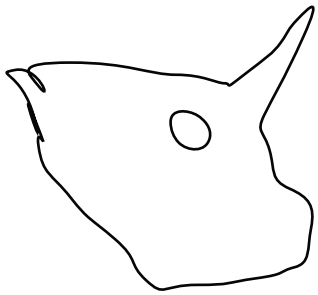
Aphaenogaster rudis

Aphaenogaster huachucana

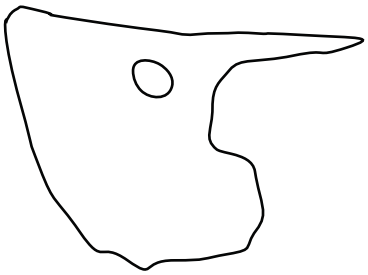
Aphaenogaster treatae

Aphaenogaster ashmeadi

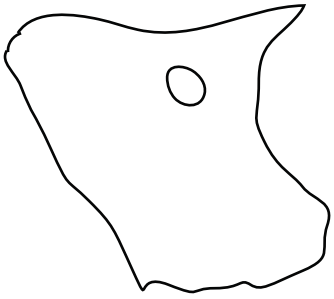
Figure 3.2. Scape shapes for 4 species of *Aphaenogaster*.



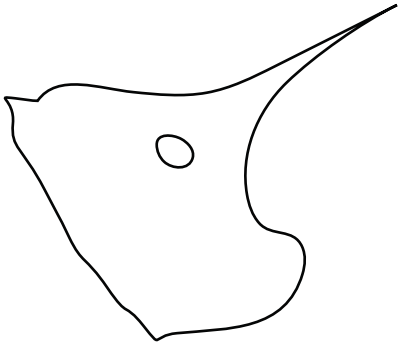
Aphaenogaster fulva



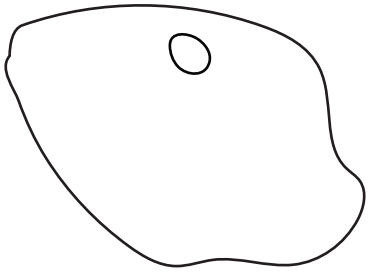
Aphaenogaster mariae



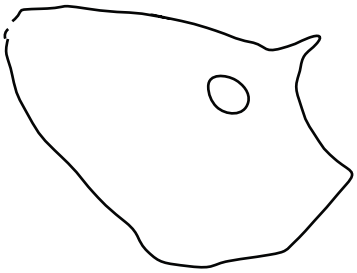
Aphaenogaster rudis



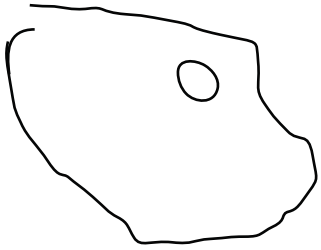
Aphaenogaster tennesseensis



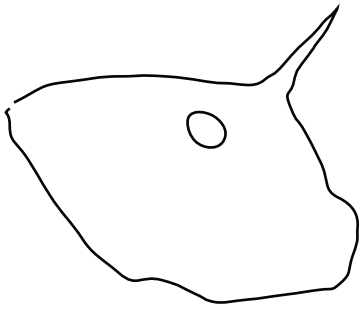
Aphaenogaster boulderensis



Aphaenogaster texana

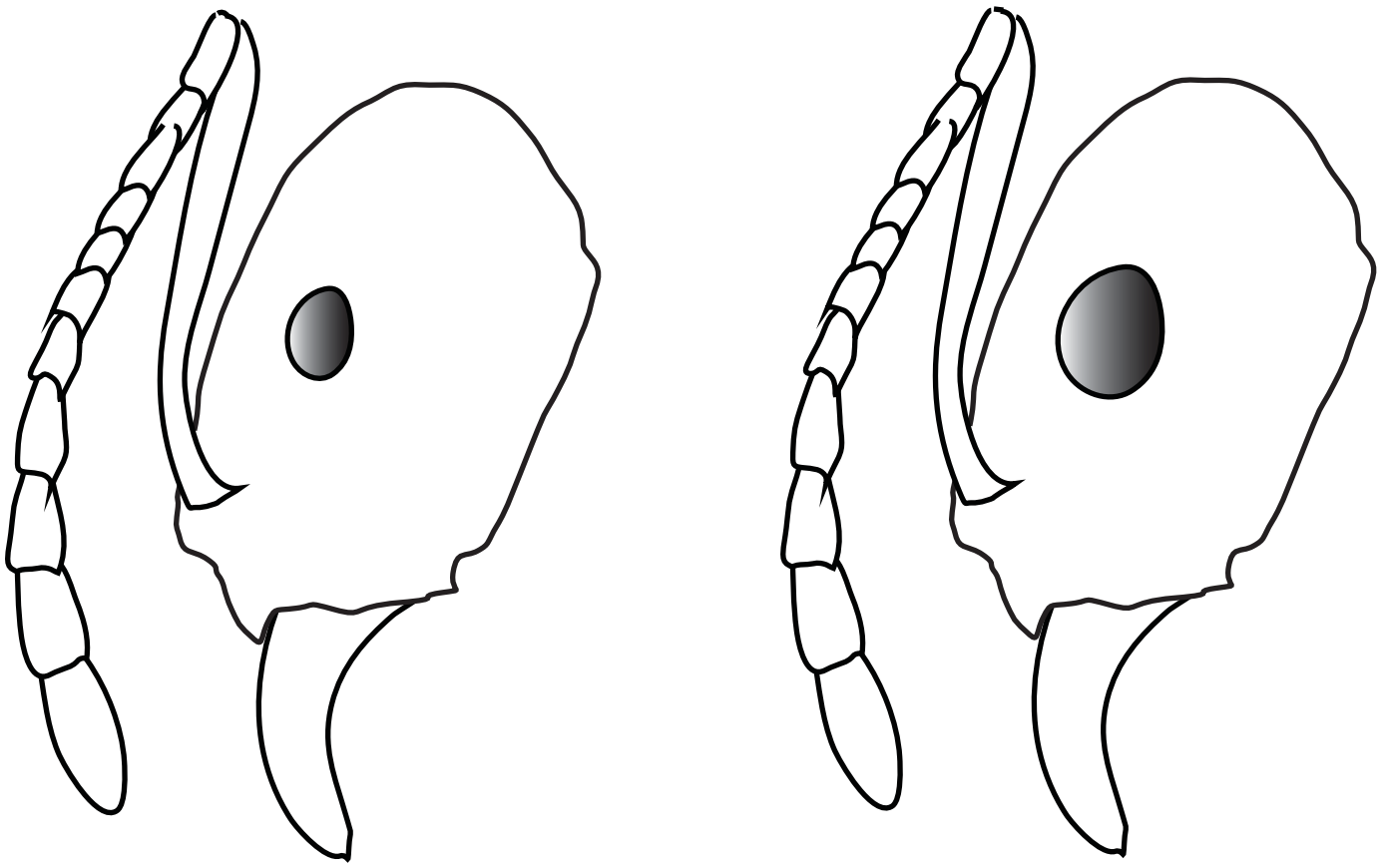


Aphaenogaster huachucana



Aphaenogaster flemingi

Figure 3.3. Propodeal spine shape and angle for 8 *Aphaenogaster* species.



Aphaenogaster umphreyi

Aphaenogaster fulva

Aphaenogaster rudis

Aphaenogaster megommata

Figure 3.4. Relative eye size for 4 species of *Aphaenogaster*.

Figure 3.5. Lateral, head and dorsal views of *Aphaenogaster ashmeadi* (Emery).

Figure 3.6. Lateral and head views of *Aphaenogaster boulderensis* Smith.

Figure 3.7. Lateral, head and dorsal views of *Aphaenogaster carolinensis* Wheeler.

Figure 3.8. Lateral, head and dorsal views of *Aphaenogaster flemingi* Smith.

Figure 3.9. Lateral, head and dorsal views of *Aphaenogaster floridana* Smith.

Figure 3.10. Lateral, head and dorsal views of *Aphaenogaster fulva* Roger.

Figure 3.11. Lateral, head and dorsal views of *Aphaenogaster huachucana* Creighton.

Figure 3.12. Lateral, head and dorsal views of *Aphaenogaster lamellidens* Mayr.

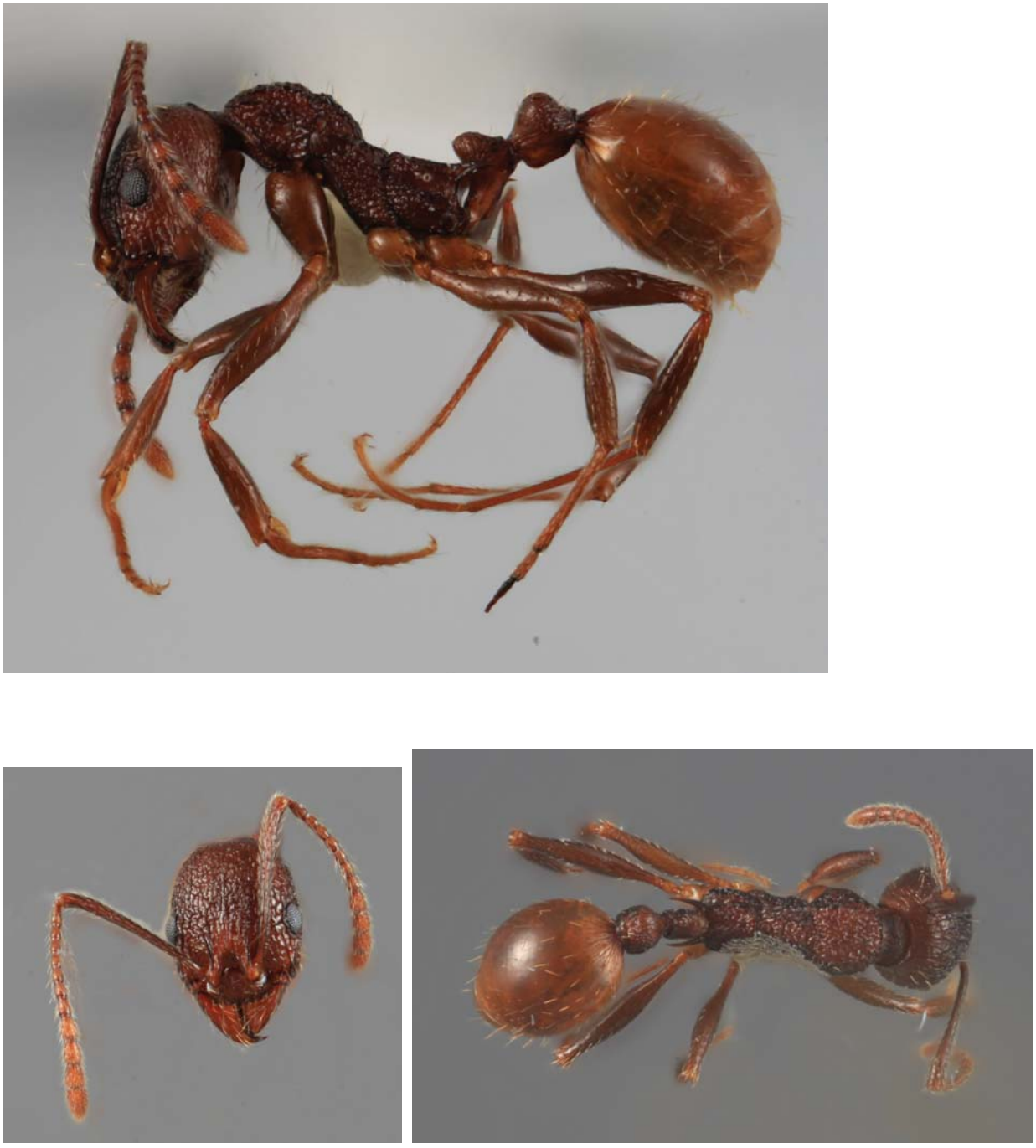


Figure 3.13. Lateral, head and dorsal views of *Aphaenogaster mariae* Forel.

Figure 3.14. Lateral, head and dorsal views of *Aphaenogaster megommata* Smith.

(photos from Antweb)

Figure 3.15. Lateral, head and dorsal views of Aphaenogaster *mexicana* (Pergande).

Figure 3.16. Lateral, head and dorsal views of *Aphaenogaster miamiana* Wheeler.

Figure 3.17. Lateral, head and dorsal views of *Aphaenogaster mutica* Pergande.

Figure 3.18. Lateral, head and dorsal views of *Aphaenogaster occidentalis* (Emery).

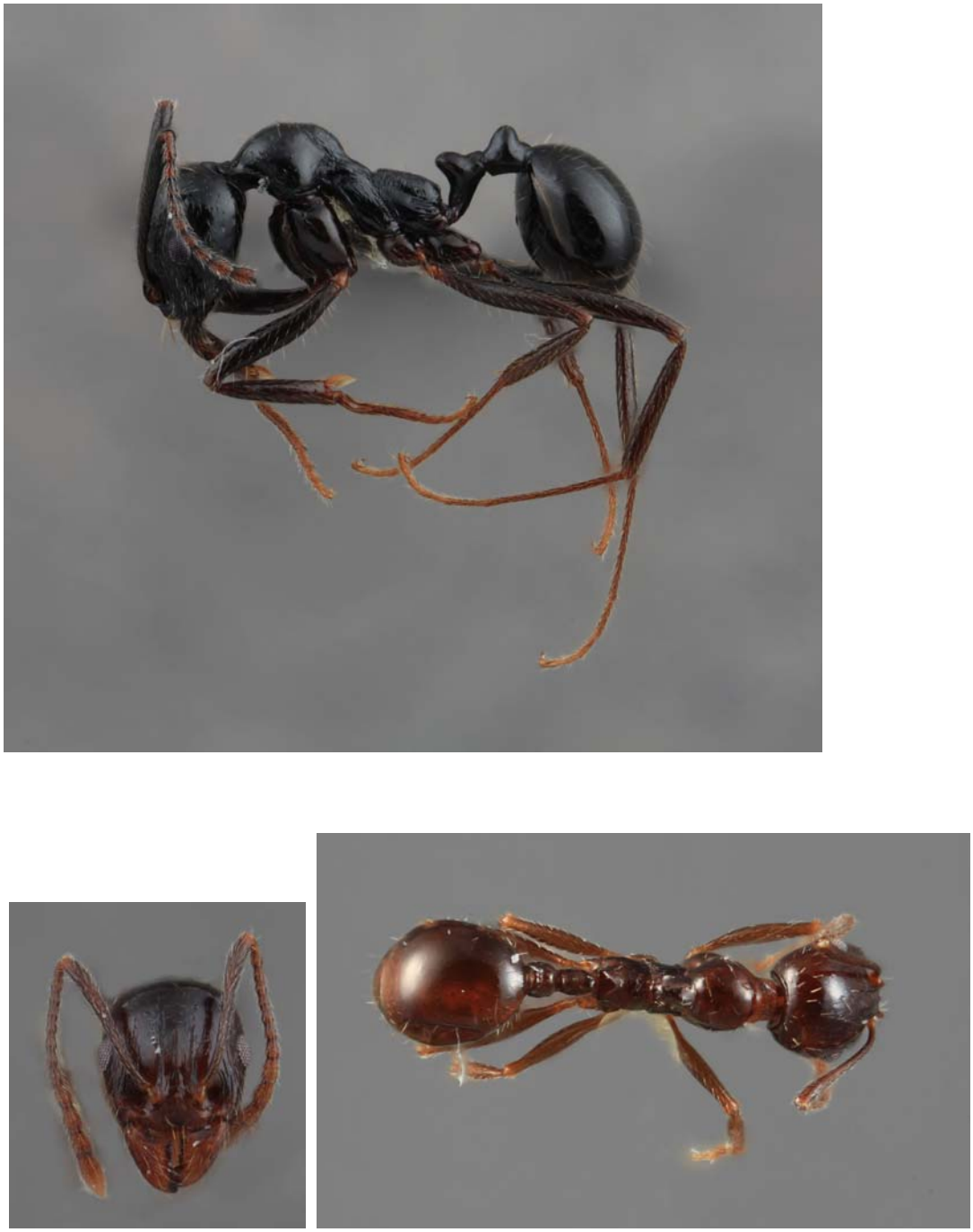


Figure 3.19. Lateral, head and dorsal views of *Aphaenogaster patruelis* Forel.

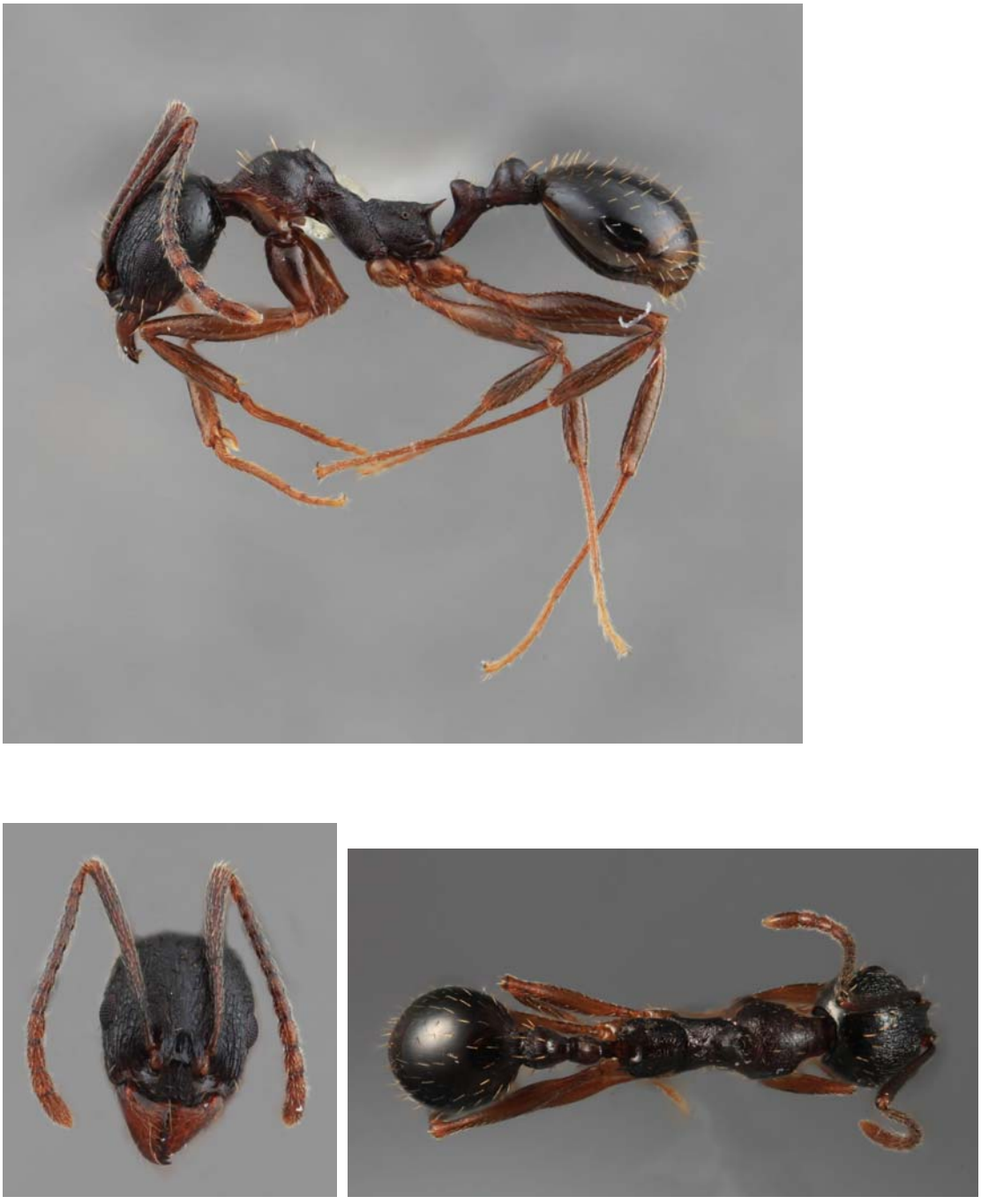
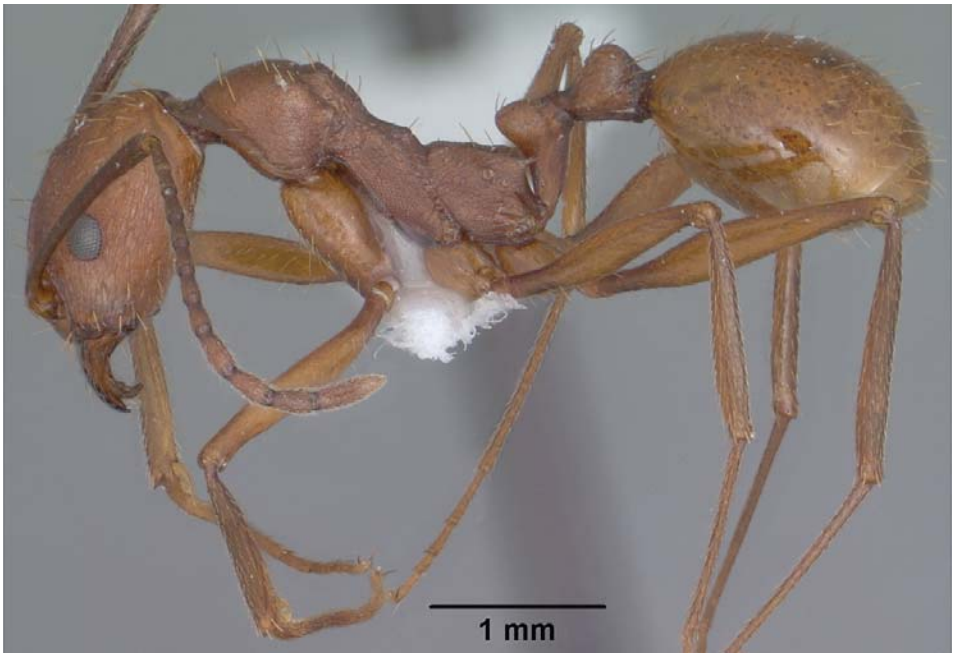
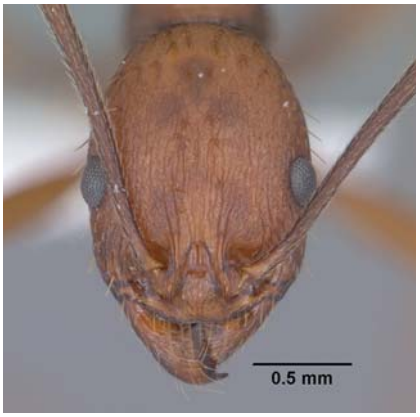
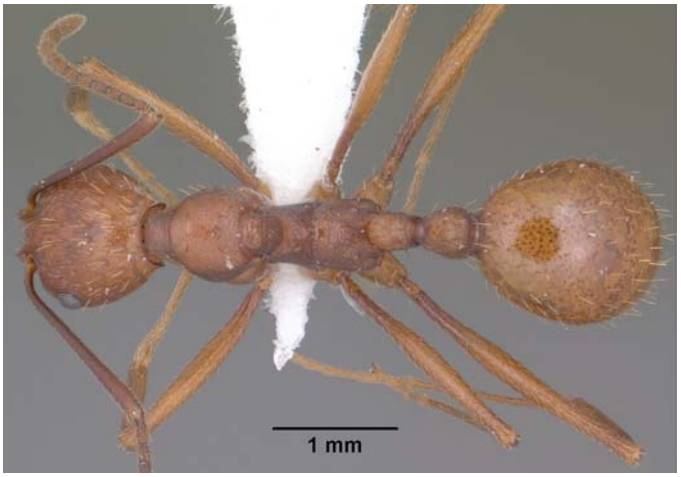


Figure 3.20. Lateral, head and dorsal views of *Aphaenogaster picea* (Wheeler).







(photo from AntWeb, photo by Jen Fogarty)

Figure 3.21. Lateral, head and dorsal views of *Aphaenogaster punctaticeps* MacKay.

Figure 3.22. Lateral, head and dorsal views of *Aphaenogaster rudis* Enzmann.

(Mayr).

Figure 3.23. Lateral, head and dorsal views of *Aphaenogaster tennesseensis*

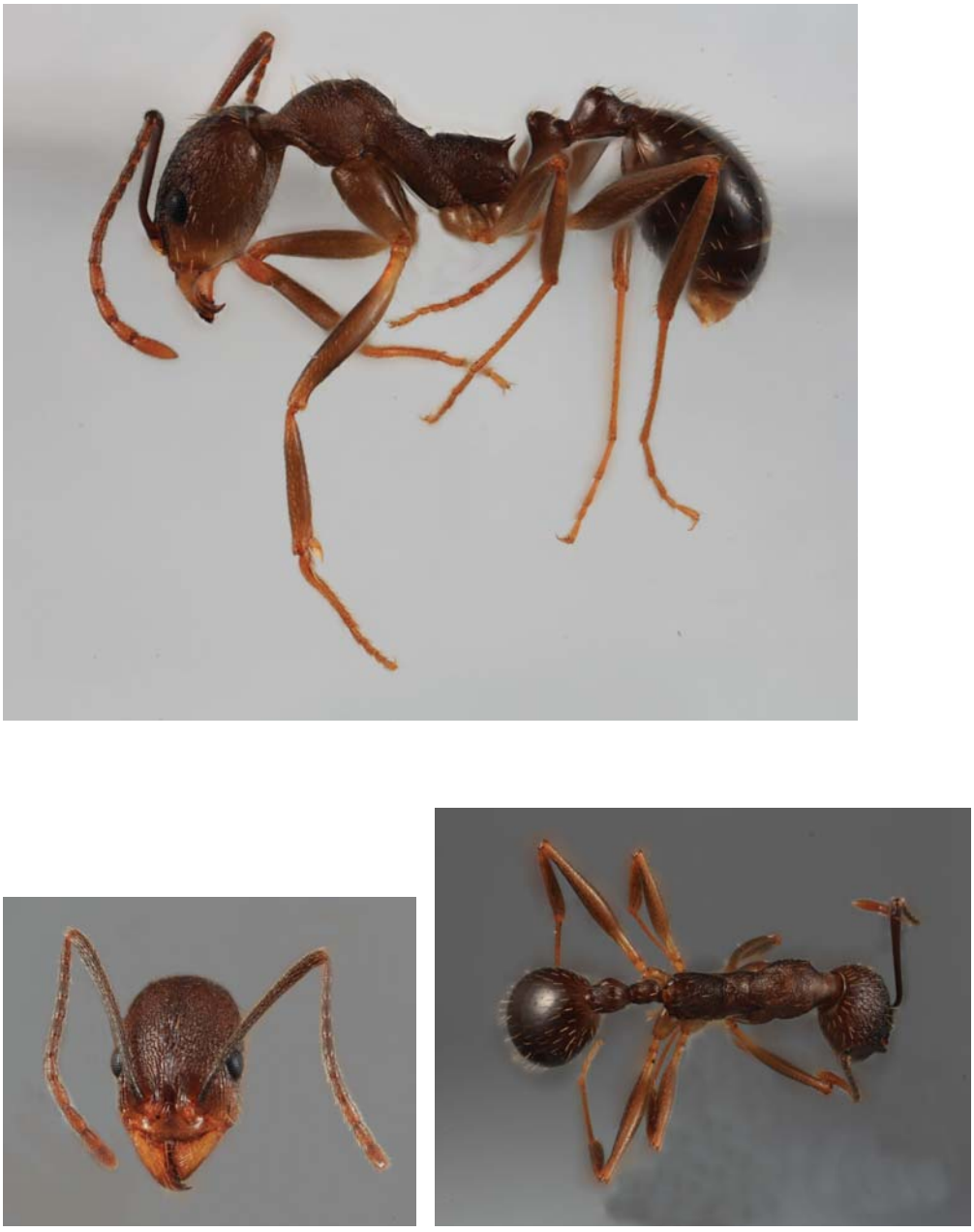


Figure 3.24. Lateral, head and dorsal views of *Aphaenogaster texana* Wheeler.

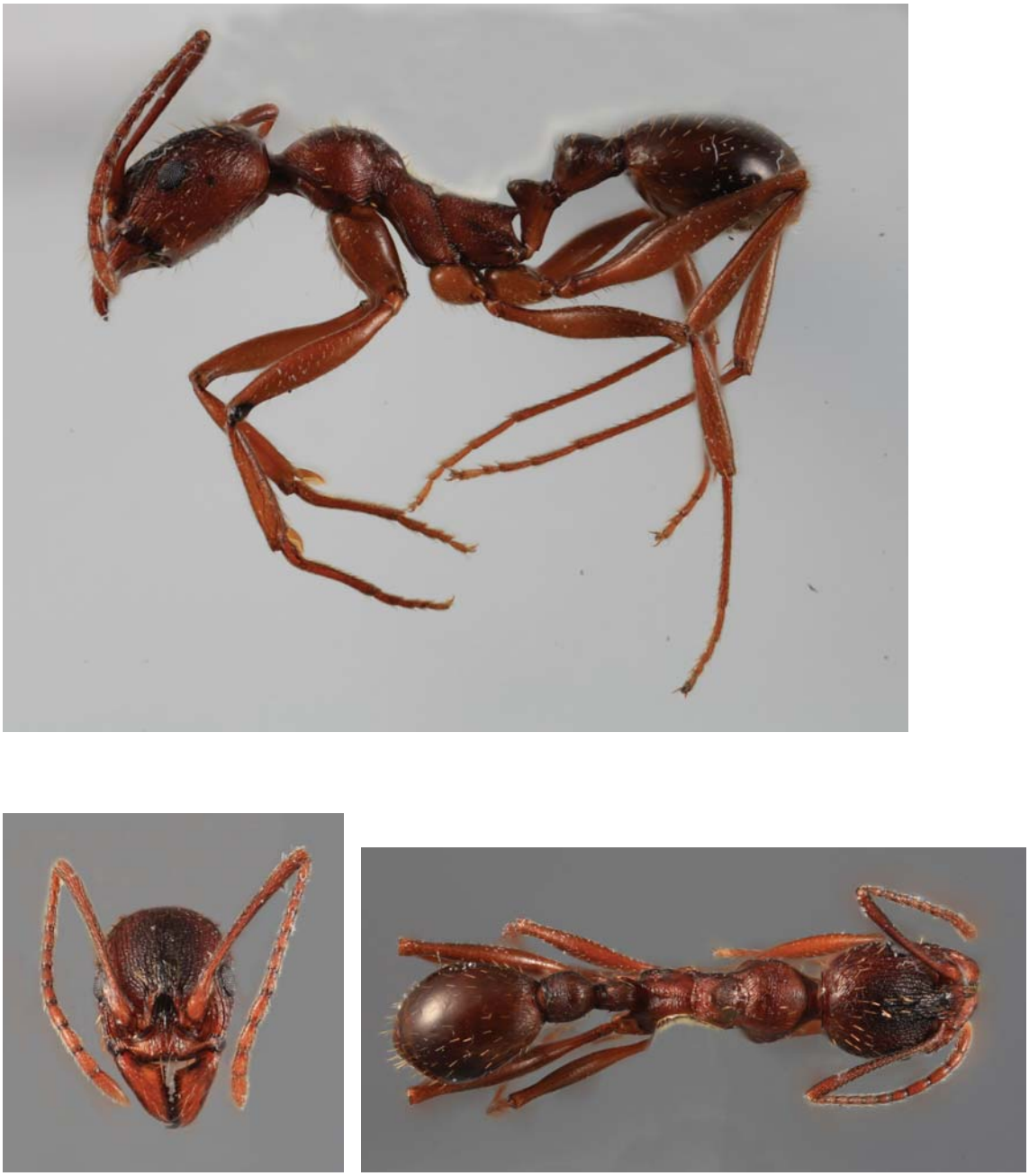


Figure 3.25. Lateral, head and dorsal views of *Aphaenogaster treatae* Forel.

Figure 3.26. Lateral, head and dorsal views of *Aphaenogaster uinta* Wheeler.

Figure 3.27. Lateral, head and dorsal views of *Aphaenogaster umphreyi* Deyrup & Davis.

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