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Source: American Malacological Bulletin, 32(1):1-31. 2014.

Published By: American Malacological Society

DOI: <http://dx.doi.org/10.4003/006.032.0103>

URL: <http://www.bioone.org/doi/full/10.4003/006.032.0103>

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Molecular phylogeny, evolution of shell shape, and DNA barcoding in Polygyridae (Gastropoda: Pulmonata), an endemic North American clade of land snails

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Abstract: A hypothesis of relationships among subfamilies, tribes, genera, and species of Polygyridae was established by Ken Emberton in 1995, using shell, behavioral, allozyme, and soft anatomical characters. We tested this hypothesis using four mitochondrial and two nuclear loci. We present data from 418 polygyrid individuals sequenced for one to six loci, including 110 named species (out of 294 nominal taxa) from 21 of the 24 recognized genera. We carried out phylogenetic and DNA barcoding analyses to examine relationships at the family, genus, and species-level. In our analyses, the subfamilies are not supported as monophyletic groups. The tribes Mesodontini, Ashmunellini and Vespericolini were recovered as monophyletic, while all other tribes were paraphyletic. Regardless of analysis method, we found a close, well-supported relationship between Mesodontini and Triodopsini, two tribes that were distantly related in Emberton's hypothesis. Most genera were recovered as monophyletic with the notable exceptions of *Cryptomastix* Pilsbry, 1839, *Mesodon* Rafinesque in Férussac, 1821, and *Neohelix* von Ihering, 1892. Of the species for which we had multiple individuals, populations of 27 formed monophyletic groups on our phylogenies, while 47 did not, indicating an urgent need for revisionary taxonomy at all levels of classification in this family.

Key words: land snails, phylogeny, morphology

Some of the most commonly encountered and visible native land snails in North America are species in the land snail family Polygyridae (Pilsbry 1940, Emberton 1994). This endemic North American family contains 294 nominal species (Richardson 1986, Turgeon *et al.* 1998, Dourson 2011, 2012, Perez 2011, Thompson 2011), three of which are known to be federally endangered (United States Fish and Wildlife Service 2012) and five of which are considered to be problematic invasive species (Dundee 1974, Robinson 1999). In this study, we had three aims: 1) evaluate the current morphology-based phylogeny and classification of the family using a new molecular phylogeny; 2) evaluate the current species-level classification using a molecular phylogeny and DNA barcoding methods; and 3) in light of our new phylogenetic hypothesis, examine the evolution of shell characteristics that had previously been reported to be convergent and the utility of genital characters in higher level classification.

What are the relationships among subfamilies, tribes, and genera within Polygyridae?

Most work on Polygyridae has focused at the species-level, much of it describing new species, distributions, and ecology,

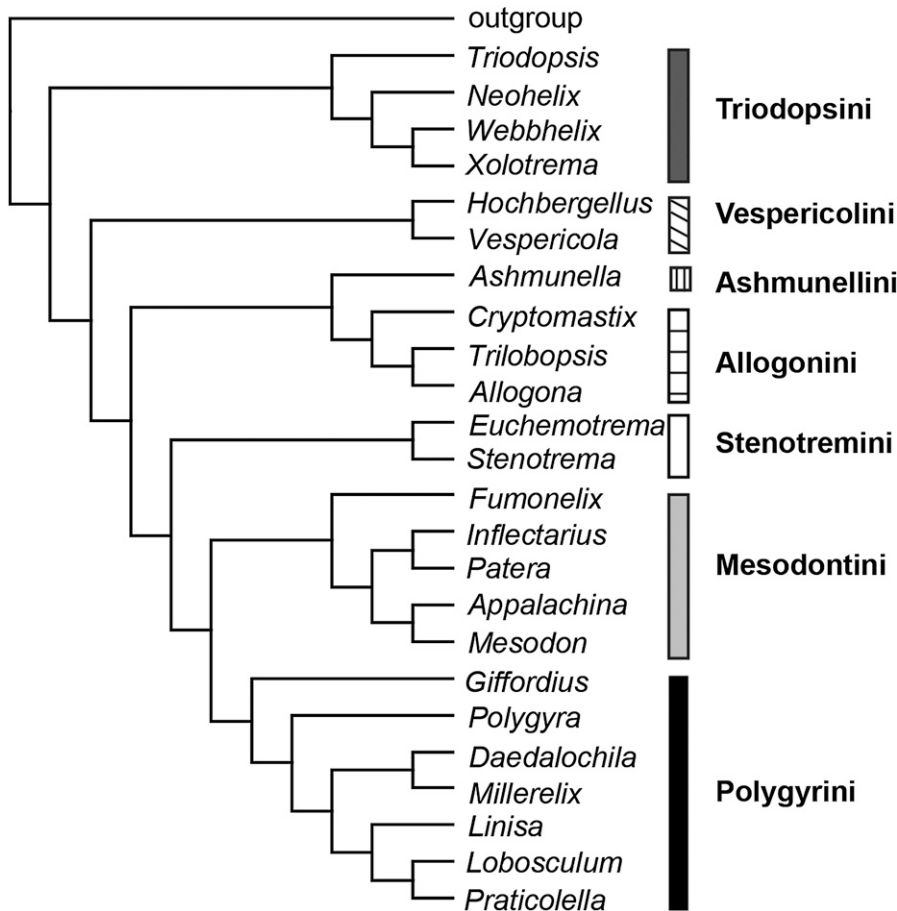
while relatively few authors have addressed the higher-level systematics of the group. Polygyridae was first established as a subfamily of Helicidae by Pilsbry (1894) who started to divide the species into genera and sections using what was, for the time, a remarkably wide variety of character sets including shell morphology and genital anatomy and less often pallial organ, jaw, and radular morphology. Prior to that point, species were usually treated as members of the genus *Helix* L., 1758, which encompassed nearly all snails with helicoid shells world-wide. Pilsbry (1930) raised the group to familial rank, established two subfamilies, Triodopsinae and Polygyrinae (Pilsbry 1940), and continued organizing species into subfamilies, generic, and sub-generic groupings culminating in the first large-scale taxonomic revision of the Polygyridae (Pilsbry 1940). This established the species groups that are still recognized today. However, Pilsbry's (1940) work included only species found north of Mexico (excluding ~20% of the described species in the family that are now known). Most works that focused on the Mexican fauna have been relatively limited in geographic area and taxonomic scope (Pilsbry 1948a, b, Solem 1957, Correa-Sandoval 1992, 1993, 1999, Naranjo-García 2003).

The most comprehensive treatments of the Mexican fauna focus on species descriptions and distributions and predate much of Pilsbry's reorganization of the polygyrids (Fischer and Crosse 1870-1878, Strebel 1875, von Martens 1890-1901). Because the Mexican polygyrids have not been treated in the same detail as their northern counterparts, some species still cannot be unequivocally assigned to genus (Thompson 2011).

Pilsbry's revisions focused on determining species groups and less on the relationships of these groups with each other. Emberton (1988, 1991, 1994), using a more comprehensive sampling of genital anatomy, especially penial sculpture in combination with allozyme data, developed the first comprehensive hypotheses of phylogenetic relationships within genera of Polygyridae. Emberton (1995b) refined this hypothesis using a Hennigian approach to form a hypothesis of relationships among tribes, genera, and subgenera in Polygyridae (Fig. 1). Emberton's binary data matrix produced a single tree with most nodes supported by one synapomorphy. This cladogram forms the basis for our current understanding of higher level relationships within the Polygyridae and Emberton's

studies provide a solid framework of testable hypotheses (Emberton 1988, 1991, 1994, 1995a, b).

A recent study showing incongruence between Emberton's phylogeny and molecular evidence at both the species- and genus-level (Perez 2011) indicates the need for reassessment of both species- and higher-level phylogeny of Polygyridae using additional independent characters, especially molecular characters. Convergences in shell characters appear to be widespread in land snails including Polygyridae (Asami 1988, Emberton 1995a, b), which led Emberton to focus his studies on genital morphology. However, genital morphology, especially penial sculpture, also appears to evolve rapidly (Wade *et al.* 2007) and while that rapid evolution makes this character set ideal for looking at species-level relationships, it is also likely to be prone to homoplasy, especially at generic and higher-level relationships. While details of the sexual apparatus are likely to reflect species differences, classifications built using multiple characters (Tillier 1989) from independent and congruent datasets, including molecular evidence, are likely to be more robust, especially at higher taxonomic levels.



Species-level relationships within the polygyrids: Filling out the tips of the polygyrid tree.

Species delineation in polygyrids is based primarily on shell characters and can be extremely difficult. Land snails are well known for high within-species variation in shells, while at the same time they are remarkable for also having close shell convergences among groups and species (Emberton 1995a). These difficulties may result in large part from their unique biology. Terrestrial snails are sensitive to desiccation and are often restricted to moist microhabitats that may limit shell shapes and lead to shell convergences (Emberton 1995a, Wiens *et al.* 2006). They are also poor dispersers with no life stage capable of actively traveling long distances. Therefore, their populations are likely to experience limited gene flow allowing populations to evolve in relative isolation compared to more vagile species. In fact, terrestrial gastropods in general are notorious for having high levels of intraspecific variability in shell morphology (Goodfriend 1986, Chiba 1993). More specifically, shell morphology in some land snails has been shown to have a strong genetic component (Cook 1965, Murray and Clarke 1968, Gould and

Figure 1. Tree of relationships of polygyrid genera and tribes from the morphology-based classification of Emberton (Emberton 1995b).

Woodruff 1990, Davison and Clarke 2000) and, thus, might represent local adaptation (Teshima *et al.* 2003). However, other studies demonstrate that shell characters can be highly plastic in some species; shell variation may be influenced by moisture, temperature, predation, population density, environmental calcium levels (reviewed in Goodfriend 1986), or altitude (Burla and Stahel 1983, Engelhard and Silk 1994, Welter-Schultes 2000). Finally, excluding both genetic and environmental components, several researchers have found snails with high degrees of shell variation that, when examined with molecular data, are found to represent a single, highly variable species (Hillis *et al.* 1991, Wilke and Falniowski 2001, Teshima *et al.* 2003) with apparently random variability in shell shape or color.

Alpha-level taxonomy of the polygyrids is based in large part on relatively few shell characters, especially in groups other than Mesodontini and Triodopsini, which were revised by Emberton (Emberton 1988, 1991). Most species descriptions since Pilsbry's (1940) revision are based primarily on shell characters, a few in combination with genitalic characters, and other than Perez (2011), have not been named in the context of generic or group revisions. Because of high intraspecific variation and interspecific convergences in shell morphology, a shell-only approach to taxonomic revisions is not likely to be successful. For example, Vagvolgyi's (1968) revision of *Triodopsis s.l.* Rafinesque, 1819 relied entirely on shell characters and did not support recognizing some species which were later supported using genital anatomy (Emberton 1988). Some authors have attributed variation in shell characters to hybridization (Vagvolgyi 1968, Hubricht 1983) however, convincing evidence for hybridization is lacking.

One possible explanation that has not received the same attention is that what appear to be high levels of intraspecific variation may be the result of poorly defined species, cryptic species, or unrecognized species complexes resulting from poor taxonomy. Some species are identified using single shell characters and the efficacy of these characters has never been independently evaluated. For example, *Inflectarius rugeli* (Shuttleworth, 1852) differs from *Inflectarius inflectus* (Say, 1821) in having a recessed palatal tooth, however both these species are highly variable for other shell characters (Pilsbry 1940) and the genus includes several species in which palatal and basal lamellae are reduced or absent. In this case, as in most other polygyrids, relatively few populations have been studied in detail, and genital anatomy is known only for several individuals from a few populations. Given the lack of knowledge of geographic variation in both shell and genital morphology, it is impossible to assess whether variation in shell characters represents within-species variation or species complexes. To be sure, multiple characters sampled from across the range of each species are needed. In light of these challenges, we believe a careful reevaluation of the species-

level classification of Polygyridae is warranted and we propose that DNA data are an ideal independent data set to test species boundaries.

A number of problems have made detailed taxonomic revisions of polygyrid groups difficult. Even the relatively well sampled Eastern United States contains large regions that are poorly sampled (Hubricht 1985). Sampling in the Western United States is sparser and sampling in Mexico is mostly restricted to the type localities of species (Thompson 2011). As a result, fluid-preserved material of many species is limited, precluding the use of genital, molecular, pallial, and frequently, jaw and radular characters in taxonomic revisions. In many cases, species descriptions lack illustrations and precise locality information. This is especially true in many Mexican species that were described from material collected during large European museum expeditions, which often labeled type collections with simply "Mexico" (Pfeiffer 1841, 1848, Binney 1851, 1857, Fischer and Crosse 1870-1878, Strebel 1875, Strebel and Pfeffer 1880, von Martens 1890-1901). These collections were described in multiple series of large volumes that were often not immediately available to researchers in other countries, resulting in common species being described several times. Contributing to the modern difficulties in taxonomy, several polygyrid type specimens were lost during World War II (Dance 1986).

Sampling initiated, in part for this study, is starting to provide specimens to address some of these problems. Perez (2011) used both molecular and morphometric analyses to recognize one new species and revise several others in the polygyrid genus, *Praticolella* von Martens, 1892. That study found several additional unrecognized species that remain to be described and some incongruence between molecular and morphological datasets. Compared to some other polygyrid genera, *Praticolella* has received a great deal of taxonomic attention (Vanatta 1915, Pilsbry 1936, Webb 1967, Neck 1977, Hubricht 1983, Perez 2011), yet there are still many unrecognized species. This led us to question the accuracy of current species-level classification in Polygyridae and to evaluate it using a molecular phylogeny and DNA barcoding methods.

DNA barcoding in Polygyridae

DNA barcoding relies on a short (~600 base pair) sequence of the cytochrome *c* oxidase subunit 1 (CO1) gene to provide a practical, species-level identification tool (Hebert *et al.* 2003b, Kress and Erickson 2008). This method relies on a "barcoding gap" to recognize species-level distinctions, a frequently used standard is interspecific variation ten times the mean intraspecific variation. The broad utility of DNA barcodes has been shown across plant and animal groups (Hebert *et al.* 2003b, Grant and Linse 2009, Hausmann *et al.* 2011, Park *et al.* 2011, Siddall *et al.* 2012), though recent studies

have shown them to perform poorly in groups with low vagility and corresponding levels of geographic structure (Bergsten *et al.* 2012). While the limitations of barcoding and other DNA taxonomy methods are well known (Prendini 2005, Packer *et al.* 2009), most studies recognize barcoding as a useful method for identifying groups of interest, however the utility of this method in land snails is unclear for the reasons detailed below.

A large body of literature exists on the subject of snail species with extremely divergent intraspecific population structure in mtDNA sequence (Guiller *et al.* 1994, Thomaz *et al.* 1996, Schilthuizen *et al.* 1999, Backeljau *et al.* 2001, Pfenniger and Posada 2002, Haase *et al.* 2003, Hugall *et al.* 2003, Pinceel *et al.* 2005, Davison *et al.* 2009). Thomaz *et al.* (1996) found high levels of intraspecific sequence divergence (12.9%) in a mitochondrial gene (16S) within two species of land snails, *Cepaea nemoralis* (L., 1758) and *Cornu aspersum* (Müller, 1774). High levels of intraspecific genetic divergence have also been observed in *Candidula* Kobelt, 1871 (Pfenniger and Posada 2002), *Discus* Fitzinger, 1833 (Ross 1999), *Arion* Moquin-Tandon, 1855 (Pinceel *et al.* 2005), and *Euhadra* Pilsbry, 1890 (Watanabe and Chiba 2001). Johnson *et al.* (1988) and Thomaz *et al.* (1996) concluded that the most likely explanation for the observed levels of divergence is the low vagility of land snails, which produces a population structure consisting of many isolated demes with infrequent migration, leading to deep divergences within species. Thomaz *et al.* additionally suggested that low vagility contributes to the long-term maintenance of ancient mtDNA lineages (*i.e.*, long coalescence times). This inverse relationship of vagility and intraspecific divergence is seen in freshwater snails (Perez *et al.* 2005) and other animal groups (Lanzaro *et al.* 1993, Ditchfield 2000, Pabijan and Babik 2006, Hebert *et al.* 2010). Alternatively, some of these populations may represent independent evolutionary lineages. In this study, we examine whether a DNA barcoding approach is useful for species delineation in Polygyridae, a taxonomic group with low vagility.

Shell and genitalia character evolution in Polygyridae

Our understanding of the evolutionary history of morphological characteristics depends on accurate phylogenetic hypotheses. In one example of evolution of convergent shell morphologies, members of different polygyrid genera have completely indistinguishable shells when they occur in sympatry (Emberton 1991, 1995b) *e.g.*, *Mesodon normalis* (Pilsbry, 1900) and *Neohelix major* (A. Binney, 1837). Currently, to separate these species reliably, one must examine internal anatomy. Molecular characters are likely to be useful, and external soft part characters might eventually be found to separate them. Nevertheless, shell characters are not sufficient to separate them. In this study, we examine two sets of shell characters in Polygyridae: apertural denticles and carinate peripheries and two genitalia

characteristics: insertion point of the penial retractor muscle and presence of a penial sheath.

In an examination of the phylogenetic utility of denticle characters in Thai gastrocoptine land snails, Tongkerd *et al.* (2004) found that denticle characteristics performed very poorly as generic-level characters, and cautioned against the unquestioned use of denticle characters in gastropod classification, hypothesizing that ecological transitions can lead to rapid modification of the denticle apparatus. There are several hypotheses regarding the function and evolution of apertural denticles, each proposing that there could be strong adaptive significance to the presence and extent of apertural denticles, with these structures serving one or more purposes. However, the presence of denticles is not a fixed condition in some polygyrid species; for example, *Praticolella mobilia* (I. Lea, 1841), *P. lawae* (J. Lewis, 1874), and *Mesodon thyroidus* (Say, 1816) all have mixed populations with some individuals possessing a large parietal tooth while others lack any hint of a tooth. Hypotheses of the adaptive significance of denticles can be best examined only within a broad phylogenetic context including family- and species-level relationships.

Many species of land snail show variation in the form of their body whorl periphery. In polygyrids, this variation ranges from a completely rounded (globose) shell to a sharply angular keel or flattened shell. The keeled form has been suggested to evolve through paedomorphic retention of juvenile shell angularity (Gould 1969, 1971). Other authors (Cook and Pettitt 1979) suggested that this aspect of shell shape is an adaptation against crushing, *i.e.*, keeled shells are more resistant to crushing than rounded shells. Solem and Climo (1985) suggested that keeled shells are associated with open ground with deep leaf litter, but in some groups of snails keels are associated with limestone substrates (Alonso *et al.* 1985). It has also been proposed that flat shells allow for deeper penetration into sheltered areas and are important in arid lands for aestivation and escape from desiccation (Goodfriend 1986, Pfenniger and Magnin 2001). Teshima *et al.* (2003) found serial convergent attainment of a flat/keeled shell shape from a globose ancestor in populations of *Aimohelix editha* (A. Adams, 1868) and it is uncertain whether polygyrids present other examples of this kind of convergence.

In this study, we use a six-gene phylogeny to determine the sister group of Polygyridae, test the current morphology-based family classification with molecular data, examine relationships within the family- to the species-level, and examine evolution of two shell characters. Using DNA barcoding methods, we also evaluate the current species-level classification of Polygyridae and examine whether barcoding is a useful approach to species delineation in Polygyridae.

MATERIALS AND METHODS

Taxon sampling

Our phylogenetic and barcoding analyses used a matrix comprising data from 418 individuals (“418-taxon” matrix/tree; complete list in Supplemental Table 1) to examine genera and species in Polygyridae, or a matrix comprising a subset of 39 individuals (“39-taxon” matrix/tree) intended to examine relationships among tribes. Of the 294 named polygyrid species, we sampled 110 species, 37.4% of the family. This includes 110 named species and 21 of the 24 genera (87.5%), all of the seven tribes, and members of both subfamilies. In this review, we focus on the Tribe-level of classification to allow more resolution than the subfamily-level and to facilitate discussion of larger groups than genus (Fig. 2). Following is a list of each genus with the number of species we sampled compared with the total number of extant named species in that genus: *Allogona* Pilsbry, 1939, 3 of 4; *Appalachina* Pilsbry, 1940, 2 of 2; *Ashmunella* Pilsbry and Cockerell, 1899, 9 of 44; *Cryptomastix* Pilsbry, 1939, 7 of 11; *Daedalochila* Beck, 1837, 9 of 22; *Euchemotrema* Archer, 1939, 2 of 5; *Fumonelix* Emberton, 1991, 3 of 10; *Giffordius* Pilsbry, 1930, 0 of 2; *Hochbergellus* Roth and Miller, 1992, 0 of 1; *Inflectarius* Pilsbry, 1940, 4 of 11; *Linisa* Pilsbry, 1930, 1 of 30; *Lobosculum* Pilsbry, 1930, 1 of 2; *Mesodon* Rafinesque in Férussac, 1821, 8 of 10; *Millerelix* Pratt, 1891, 2 of 14; *Neohelix* von Ihering, 1892, 6 of 8; *Patera* Albers, 1850, 5 of 14; *Polygyra* Say, 1818, 2 of 5; *Praticolella*, 11 of 15; *Stenotrema* Rafinesque, 1819, 13 of 27; *Trilobopsis* Pilsbry, 1939, 2 of 5; *Triodopsis*, 15 of 28; *Vespericola* Pilsbry, 1939, 5 of 16; *Webbhelix* Emberton, 1988, 0 of 2; *Xolotrema* Rafinesque, 1819, 4 of 5.

DNA sequence generation

For most specimens, total genomic DNA was extracted from several milligrams of foot tissue by digestion with hexadecyltrimethylammonium bromide (CTAB) lysis buffer (Saghai-Marouf *et al.* 1984) and proteinase K and then purified by phenol: chloroform extraction according to standard procedures (Palumbi *et al.* 1991); some specimens were extracted using DNAzol® (Molecular Research Center, Inc.) following manufacturer’s protocols. We obtained up to six genes for the individuals sequenced. The six genes we amplified included four mitochondrial loci: cytochrome oxidase subunit 1 (COI or *cox1*) using primers: COIH2198, COIL1490 (Folmer *et al.* 1994), cytochrome b (cytb or *cob*) using primers Ucob151F and Ucob270R (Merritt *et al.* 1998), large ribosomal subunit (16S or *rrnL*) using primer sets: 16sar or d16sar, 16sbr, d16sbr or 16SL2510-deg, 16SH3080-deg (Palumbi *et al.* 1991, Gellar *et al.* 1997, Perez 2011), and small ribosomal subunit (12S or *rrnS*) using primers SR-N-14588 (12Sai) and SR-J-14233 (12Sbi) (Simon *et al.* 1994) and two nuclear loci: one protein-coding, histone H3 (H3) using primers: H3F and H3R (Hillis

et al. 1996) and one ribosomal large ribosomal subunit (28S or LSU) using primers: VI and X (Hillis *et al.* 1996). PCR was carried out in three labs according to various cycling procedures; the standard procedures are detailed in Meyer (2003), Anderson and Smith (2005), and Perez (2011). Additional samples were sequenced for COI by the Consortium for the Barcode of Life (www.barcodeoflife.org) using their standard methods. After PCR purification using Qiagen gel-extraction kits, both strands were sequenced using the PCR primers on an ABI3100 automated genetic analyzer. Contigs were assembled in Sequencher™ 4.0.5 (Gene Codes Corporation, Ann Arbor, MI) or CodonCode Aligner (©CodonCode Corporation).

Phylogenetic analyses

What are appropriate outgroups for these analyses?

Several previous molecular phylogenetic studies have included polygyrid exemplars; these studies could provide guidance regarding selection of appropriate outgroups for a study of polygyrid phylogeny. Phylogenies for stylommatophoran (Wade *et al.* 2001, 2006), helicoid (Wade *et al.* 2007), and Australian camaenid (Hugall and Stanisc 2011) land snails based on partial nuclear ribosomal RNA cluster data suggest that Polygyridae is a member of Helicoidea and is closely related to members of Bradybaenidae, Camaenidae, Helicidae, Helminthoglyptidae and Hygromiidae. In particular, a clade comprising representatives of the East Asian camaenid genera *Coniglobus* (Pilsbry and Hirase, 1905) and *Satsuma* (Adams, 1861) was found to be sister to a clade of three polygyrids (*Mesodon thyroidus*, *Neohelix alleni* (Sampson, 1883) and *Vespericola columbianus* (I. Lea, 1838)) in these phylogenies.

To provide an initial evaluation of the appropriate outgroup(s) for a phylogenetic analysis of Polygyridae, all non-polygyrid sigmurethan sequences available in GenBank as of April 19, 2012, were downloaded and parsed into FASTA files by gene with the Perl script GenBankStrip v2.0 (Bininda-Emonds 2005). Loci for which polygyrid data were available—COI, cytb, 16S, and 12S—were retained for further analyses. Preliminary Maximum Likelihood (ML) analyses of a multilocus data set comprising ~500 polygyrid operational taxonomic units (OTUs) and ~1000 outgroup OTUs (results not shown) supported the hypothesis that Polygyridae is a member of Helicoidea, with Camaenidae as the likely sister group (see above). For all other subsequent analyses, four helicoid species were selected as outgroups: *Cornu aspersum* (Helicidae), *Amplichagada mitchelliana* Solem, 1981, *Carinotrachia admirale* (Köhler, 2010), and *Satsuma jacobii* Pilsbry, 1900 (all Camaenidae). These four species were chosen because COI and 16S data were available in GenBank for all four, and they allow rooting of the polygyrid phylogeny (with *Cornu aspersum* as the most distant outgroup) while representing each major clade within

Camaenidae found in preliminary analyses, potentially mitigating problems with long branches amongst the outgroups.

Polygyrid and outgroup data for each of the four loci with sufficient data—COI, cytochrome b, 16S and 12S—were initially aligned using Muscle (Edgar 2004) with default parameters. Note, these are all mitochondrial loci. For the two rRNA data sets (12S and 16S), Randomized Axelerated Maximum Likelihood (RAxML) (Stamatakis 2006) was used to eliminate identical sequences from these initial Muscle alignments. The resulting alignments were then aligned in RNASalsa (Stocsits *et al.* 2009) under default parameters, using a structure constraint for *Apis mellifera* L., 1758 (honeybee). Alignments for all four

loci were concatenated into a single data set in Mesquite v. 2.75 (Maddison and Maddison 2011). Polygyrid sequences were concatenated only if they were generated from the same specimen. For the outgroup species, sequences for each locus were taken from GenBank from different individuals within the same species. All polygyrid specimens not identified to species were deleted from the data set. This yielded a data set comprising four outgroup taxa and 434 polygyrid OTUs representing all polygyrid genera except *Giffordius*, *Hochbergellus* and *Webbhelix*. This combined data set was analyzed in RAxML with the data treated as a single partition (*i.e.*, unpartitioned) and partitioned by gene and gene/codon position (resulting in eight

partitions—12S; 16S; COI first, second and third positions; cytochrome b first, second and third positions). Partitioned (1522 replicates) and unpartitioned ML bootstrap (2481 replicates) and BKL (best-known likelihood) tree searches (unpartitioned = 1000; partitioned = 1244 search replicates) were run using the GTRCAT model, with the final BKL tree topology evaluated under the general time reversible+gamma model.

The data set described above was also edited to allow for more thorough tree searches and to reduce the amount of missing data. This smaller data set (39 individuals) was generated by retaining only those polygyrid OTUs for which at least COI and 16S data were available (with a few exceptions; three genera—*Cryptomastix*, *Fumonelix*, and *Vespericola*—were represented by specimens for which only COI or COI and 12S had been sequenced) and eliminating OTUs of questionable ID, as determined by an unusual placement (*i.e.*, distant from congeneric sequences) in trees resulting from analyses of the full combined data set. This reduced combined data set was analyzed in RAxML (both partitioned and unpartitioned, as described above) and MrBayes 3.2 (Ronquist *et al.* 2012). Bayesian analyses consisted of four independent runs, each with four chains (one cold, three heated) for 10 million generations. A convergence diagnostic (the average standard deviation of split frequencies with a 25% burn-in) was used to stop the analyses automatically once topological convergence across all four runs

- Family Polygyridae
 Subfamily Polygyrinae
 Tribe Polygyrini
Daedalochila auriculata (Say, 1818)
Daedalochila avara (Say, 1818)
Daedalochila delecta (Hubricht, 1976)
Daedalochila hausmani (Jackson, 1948)
Daedalochila hippocrepis (Pfeiffer, 1848)
Daedalochila peninsulæ (Pilsbry, 1940)
Daedalochila postelliana (Bland, 1859)
Daedalochila subclausa (Pilsbry, 1899)
Daedalochila uvulifera (Shuttleworth, 1852)
Daedalochila uvulifera bicornuta (Pilsbry, 1900)
Linisa tamaulipasensis (I. Lea, 1857)
Lobosculum pustuloides (Bland, 1858)
Millerelix mooreana (W. G. Binney, 1858)
Millerelix plicata (Say, 1821)
Polygyra cereolus (Mühlfeld, 1816)
Polygyra septemvoiva Say, 1818
Praticolella berlandieriana (Moricand, 1833)
Praticolella candida Hubricht, 1983
Praticolella flavescens (Weigmann in Pfeiffer, 1856)
Praticolella griseola (Pfeiffer, 1841)
Praticolella jejuna (Say, 1821)
Praticolella martensiana (Pilsbry, 1907)
Praticolella mexicana Perez, 2011
Praticolella mobiliana I. Lea, 1841
Praticolella pachyloma (Menke, 1847)
Praticolella taeniata Pilsbry, 1940
Praticolella trimatris Hubricht, 1983
 Tribe Stenotremiini
Euchemotrema fraternum (Say, 1824)
Euchemotrema hubrichti (Pilsbry, 1940)
Stenotrema altispira (Pilsbry, 1894)
Stenotrema barbigerrum (Redfield, 1856)
Stenotrema cohuttense (G. H. Clapp, 1914)
Stenotrema deceptum (G. H. Clapp, 1905)
Stenotrema depilatum (Pilsbry, 1895)
Stenotrema edwardsi (Bland, 1856)
Stenotrema exodon (Pilsbry, 1900)
Stenotrema exodon turbiniella (Clench and Archer, 1933)
Stenotrema hirsutum (Say, 1817)
Stenotrema magnifumosum (Pilsbry, 1900)
Stenotrema piula (Pilsbry, 1900)
Stenotrema spinosum (I. Lea, 1830)
Stenotrema stenotrema (Pfeiffer, 1842)
 Tribe Mesodontini
Appalachina chillhoweensis (J. Lewis, 1870)
Appalachina sayana (Pilsbry, 1906)
Fumonelix roanensis Dourson, 2012
Fumonelix wheatleyi (Bland, 1860)
Fumonelix wheatleyi clingmanicus (Pilsbry, 1904)
Infectarius ferrisi (Pilsbry, 1897)
Infectarius infectus (Say, 1821)
Infectarius rugeli (Shuttleworth, 1852)
Infectarius smithi (G. H. Clapp, 1905)
Infectarius subpalliatius (Pilsbry, 1893)
Mesodon altivagus (Pilsbry, 1900)
Mesodon andrewsae W. G. Binney, 1879
Mesodon clausus (Say, 1821)
Mesodon elevatus (Say, 1821)
Mesodon mitchellianus (I. Lea, 1839)
Mesodon normalis (Pilsbry, 1900)
Mesodon thyroidus (Say, 1816)
Mesodon zaletus (A. Binney, 1837)
Patera appressa (Say, 1821)
Patera clarki (I. Lea, 1858)
Patera laevior (Pilsbry, 1940)
Patera perigrapta (Pilsbry, 1894)
Patera sargentiana (C.W. Johnson and Pilsbry, 1892)
 Subfamily Triodopsinae
 Tribe Allogonini
Allogona lombardii A. G. Smith, 1943
Allogona profunda (Say, 1821)
Allogona ptychophora (A. D. Brown, 1870)
Cryptomastix devia (Gould, 1846)
Cryptomastix germana (Gould, 1851)
Cryptomastix magnidentata (Pilsbry, 1940)
Cryptomastix mullani (Bland and J.G. Cooper, 1861)
Cryptomastix mullani hemphilli (W. G. Binney, 1886)
Cryptomastix mullani latilabris (Pilsbry, 1940)
Cryptomastix mullani olneyae (Pilsbry, 1891)
Cryptomastix mullani tuckeni (Pilsbry and Henderson, 1930)
Triodopsis penitens (Hanna and Rixford, 1923)
Triodopsis trachypepla (S.S. Berry, 1933)
 Tribe Ashmunellini
Ashmunella animasensis Vagvolgyi, 1974
Ashmunella ashmuni (Dall, 1897)
Ashmunella auriculata Vagvolgyi, 1974
Ashmunella hebardii Pilsbry and Vanatta, 1923
Ashmunella mearsii (Dall, 1895)
Ashmunella organensis Pilsbry, 1936
Ashmunella pseudodonia (Dall, 1897)
Ashmunella rhyssa (Dall, 1897)
Ashmunella todseni Metalf and Smartt, 1977
 Tribe Triodopsini
Neohelix albolabris (Say, 1817)
Neohelix alleni (Sampson, 1883)
Neohelix alleni fuscolabris (Pilsbry, 1903)
Neohelix dentifera (A. Binney, 1837)
Neohelix major (A. Binney, 1837)
Neohelix solemi Emberton, 1988
Triodopsis alabamensis (Pilsbry, 1902)
Triodopsis anteridon Pilsbry, 1940
Triodopsis burchi Hubricht, 1950
Triodopsis fallax (Say, 1825)
Triodopsis fallax affinis (Hubricht, 1954)
Triodopsis fraudulenta (Pilsbry, 1894)
Triodopsis fulciden Hubricht, 1952
Triodopsis hopetonensis (Shuttleworth, 1852)
Triodopsis juxtidentis (Pilsbry, 1894)
Triodopsis palustris Hubricht, 1958
Triodopsis soelneri (J.B. Henderson, 1907)
Triodopsis tennesseensis (Walker and Pilsbry, 1902)
Triodopsis tridentata (Say, 1816)
Triodopsis vannonstrandi (Bland, 1875)
Triodopsis vulgata Pilsbry, 1940
Xolotrema carolinense (I. Lea, 1834)
Xolotrema denotatum (Ferussac, 1821)
Xolotrema fosteri (F.C. Baker, 1921)
Xolotrema obstrictum (Say, 1821)
 Tribe Vespericolini
Vespericola columbianus (I. Lea, 1838)
Vespericola entrichius (S.S. Berry, 1933)
Vespericola megasoma (Pilsbry, 1928)
Vespericola pinicola (S.S. Berry, 1916)
Vespericola shasta (S.S. Berry, 1921)

Figure 2. List of species examined in this analysis with species authority. Names follow Turgeon *et al.* (1998).

was achieved. For both partitioned and unpartitioned Bayesian analysis, sampling across the GTR model space was performed during the Markov Chain Monte Carlo (MCMC) analysis using the command “lset nst = mixed rates = gamma”. This eliminated the need for *a priori* model selection.

Shell and genitalia character evolution in Polygyridae

Ancestral states for several characters of interest were reconstructed using MP, ML, and reversible-jump MCMC (RJ-MCMC) methods on trees resulting from analyses of the 39-taxon set. To test Emberton's hypothesis of convergent evolution in shell form in Polygyridae, we reconstructed ancestral states for two conchological characters—shell outline (character states: *flat* [0] or *round* [1]) and shell teeth (character states: *no teeth* [0], *any tooth or blade* [1], or *three teeth* [2]). To test Emberton's reliance on genitalic characteristics for inferring tribe-level relationships, we reconstructed states for two genitalic characters—the position of the penial retractor muscle insertion (character states: *on penis apex* [0], *on vas deferens* [1]) and presence of a penial sheath (character states: *no sheath* [0], *diaphanous sheath* [1], *sheath present* [2]). In some cases, intrageneric (and even intraspecific) variability exists for these characters; species were coded with all the character states present in any member of the genus, so they appear as polymorphic (e.g., 0 and 1) as appropriate. MP and ML inferences were performed under default settings in Mesquite v. 2.74 (Maddison and Maddison 2011) on the BKL topologies generated in RAxML for the unpartitioned and partitioned data sets. Two ML models were used for ancestral state reconstruction – the Markov k-state 1 parameter (Mk1) model and the Asymmetrical Markov k-state 2 parameter (AsymmMk) model. The shell teeth character could not be analyzed under ML in Mesquite, because multiple taxa are polymorphic for this character and, thus, cannot be analyzed under either model.

To take phylogenetic uncertainty into account, the states of these characters for the ancestor of Polygyridae and the ancestor of a clade comprising the tribes Mesodontini and Triodopsini were also estimated using reversible-jump MCMC methods in BayesMultiState, part of the BayesTraits 1.0 package (Pagel *et al.* 2004; Pagel and Meade 2006). The ~90K post burn-in trees resulting from partitioned Bayesian analyses of the reduced combined data set were evaluated in BayesMultiState. To test whether a particular state is supported for a given character at each node of interest, model likelihoods were calculated with each alternative state fixed at each node using the ‘fossil’ command. All RJ-MCMC analyses were run for 100 million generations and sampled every 100 generations, with results from the first 10 million generations discarded as burn-in. All RJ-MCMC analyses were performed three times to assess stability of the results. Exponential priors were used, seeded on uniform hyperprior

distributions with intervals of 0 to 10 (this interval was selected using results from initial ML analyses in BayesMultiState). Rate deviation parameters were adjusted to achieve acceptance rates of 20–40%. To compare support for alternative character states at a given node, the logarithm of the harmonic mean of the likelihoods of all post burn-in samples was recorded under each alternative fossilization and the harmonic means were compared using log-Bayes factors, with the following test statistic: $2(\log[\text{harmonic mean under the better model}] - \log[\text{harmonic mean under the worse model}])$. Following Kass and Raftery (1995), differences between harmonic means of 0–2 were interpreted as insignificant support for the better model, while differences of 2–6 indicate positive support, and differences of 6–10 indicate strong support for the better model.

DNA barcoding in Polygyridae

The COI sequences generated by the authors were added to polygyrid sequences downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). The data set was aligned using Muscle (Edgar 2004) in Molecular Evolutionary Genetics Analysis, MEGA 5.05 (Tamura *et al.* 2011). Sequences that had no overlap with a majority of other sequences were excluded, leaving us with a data set of 706 aligned positions for 383 individuals. We calculated genetic distances using the Kimura two-parameter model (K2P, Kimura 1980). The K2P model is the standard for DNA barcode studies, where distances are assumed to be relatively low (Hebert *et al.* 2003a). The following K2P distances were calculated in MEGA: mean within genus, and pair-wise distances within and between species in single genera. Since little is known about the systematics of polygyrid subspecies, we treated each subspecies as a distinct species for barcoding.

To look for a barcoding gap, we compared the within- and between-species K2P distances within each genus and grouped the results. A potential ‘gap,’ where interspecific and intraspecific distances overlapped minimally, was observed at a K2P distance of 0.05, corresponding to 28 differences in our aligned dataset. Using barcodes from two of our well-sampled genera (*Daedalochila* and *Praticolella*), we used jMOTU (Jones *et al.* 2011) to assign individuals to molecular operational taxonomic units (MOTUs). This approach attempts to identify taxa based on their barcodes independent of recognizing species (Blaxter *et al.* 2005). This method may also compensate for systematic bias in groups where the number of expected taxa is potentially much greater than the number of taxa described (Blaxter 2003). We ran jMOTU on each generic dataset separately, using a cut-off point of 28 base differences to delineate taxa, along with a 95% BLAST identity filter and 60% minimum sequence length alignment overlap. Each analysis was repeated twenty times to determine if there was any difference among analytical runs. We additionally

conducted a species profile analysis (*sensu* Barrett and Hebert 2005) by randomly selecting one sequence from each species represented in this study. This yielded 112 terminals, to which an aligned NCBI sequence (AY546270) for *Cepaea nemoralis* (Linnaeus, 1758) was added as an outgroup (Steinke *et al.* 2004). We analyzed this dataset using K2P neighbor-joining in MEGA in order to calculate a guide tree. The fewer taxa included in a tree, the more difficult it is to place new additions into the correct group (Zwickl and Hillis 2002); this method provided the most rigorous test of the ability of COI sequences to distinguish among species. We then used a random subset of 100 of the remaining sequences and added each to the species profile sequences, analyzed them using K2P neighbor-joining, and scored whether or not conspecifics grouped together.

RESULTS

Sequence Data

We obtained sequences for all polygyrid genera except three genera, *Webbhelix*, *Giffordius*, and *Hochbergellus*. For two of these we were unable to obtain samples, for *Webbhelix* we could not extract high-quality DNA from existing samples. The number of individuals sequenced for each locus are as follows: COI, 401 individuals; 12S and cytb, 68 individuals;

16S, 122 individuals, H3, 39 individuals; and 28S, 21 individuals (one or more loci were sequenced from 418 individuals). Amounts of sequence data generated for each locus were: COI, 650 bp; cytb, 350–400 bp; 12S, 350 bp; 16S, 400–500 bp; H3, 350 bp; 28S, 300 bp. There were 1086 parsimony-informative characters and 766 constant characters (out of 2141 total characters) in the full combined data set and 969 parsimony-informative and 1486 constant characters (out of 2697 total characters) in the reduced combined data set. The extra ~500 bp in the reduced combined data set were due to inadvertent retention of full-length cytochrome b and 16S sequences from *Cornu aspersum* and *Carinotrachia admirale*, respectively; as these ~500 bp were missing for all other taxa in the data set, they were phylogenetically uninformative, and had no effect on analyses of this data set.

What are the relationships among subfamilies, tribes, and genera within Polygyridae?

Maximum likelihood analysis of all genes for 39 individuals representing all seven polygyrid tribes and most of the 24 polygyrid genera resulted in a single highest likelihood tree, shown in Fig. 3 (henceforth the “39-taxon tree”). For the well-supported nodes (> 50% bootstrap support), there is no conflict among individual gene trees. We found moderate (66%) ML bootstrap support for a monophyletic Polygyridae. The subfamily Polygyrinae is paraphyletic with respect to

Table 1. Ancestral state reconstructions for the two shell characters in the polygyrid ancestor and the Mesodontini+Triodopsini ancestor under maximum parsimony, maximum likelihood, and reversible-jump MCMC. State with highest likelihood in RJ-MCMC analyses (harmonic mean averaged across three runs) shown in bold; no asterisk = no significant difference between states based on Bayes factor comparison, * = positive support for state with highest likelihood, ** = strong support for state with highest likelihood. Asterisks indicating support apply only to RJ-MCMC.

Ancestor (Code)	Character	MP	ML	RJ-MCMC	Bayes Factor
Polygyridae (P)	Shell outline	Flat	Flat	Flat: -25.70101 Globose: -25.68581	
	Shell teeth	Equivocal	—	None: -24.59939167 Any: -23.60205933* Three: -23.89132	0.578521333
	Position of penial retractor	—	—		2.857383333
	Penial sheath	—	—		5.957600667
Mesodontini+Triodopsini (MT)	Shell outline	Flat	Flat	Flat: -24.07905 Globose: -24.54811	
	Shell teeth	Any	—	None: -25.68374067 Any: -23.00440633* Three: -25.57937233	5.149932
	Position of penial retractor	—	—	On penis apex: -18.563499 On vas deferens: -17.13480733*	5.332564667
	Penial sheath	—	—	No sheath: -27.75355167 Diaphanous sheath: -25.567186 sheath: -21.54416233**	8.046047333

Triodopsinae. The subfamily Triodopsinae is also not monophyletic. Only three tribes are supported as monophyletic, Vespericolini, Mesodontini and Ashmunellini. A clade comprising *Praticolella*+*Lobosculum*+*Linisa*+*Polygyra*+*Daedalochila* is sister to a clade comprising the rest of Polygyridae, including *Millerelix*, rendering Polygyrini paraphyletic. There is good support for a sister relationship between *Daedalochila* and *Polygyra* (95% bootstrap support). Ashmunellini is the next split in the tree, followed by a major dichotomy splitting the other members of the family into an Allogonini+Vespericolini+Stenotremeni clade and a Triodopsini+Mesodontini clade (75% bootstrap support). In the Allogonini+Vespericolini+Stenotremeni clade, relationships among tribes are poorly resolved. Allogonini is found to be paraphyletic with *Allogona* sister to the rest of the Allogonini+Vespericolini+Stenotremeni clade, while one member of *Cryptomastix* forms a clade with *Vespericola* (64%) and two other species of *Cryptomastix* group with *Euchemotrema* (< 50%). Stenotremeni is also non-monophyletic, with the representative of *Euchemotrema* not forming a clade with *Stenotrema*; however, this relationship has low support (< 50%). In the Triodopsini+Mesodontini clade, there is stronger support for several relationships. Mesodontini is recovered as a monophyletic group, with *Triodopsis* (Triodopsini) as its sister group (< 50%). The other genera in Triodopsini—*Xolotrema* and *Neohelix*—form a well-supported clade (95%). Within the Triodopsini+Mesodontini clade (75% support), *Appalachina* is found nested within *Mesodon* (65%) (rendering *Mesodon* paraphyletic unless *Appalachina* is reduced to a subgenus within *Mesodon*), and one *Neohelix* species is closer to *Xolotrema* (89%) than to the other *Neohelix* (rendering *Neohelix* paraphyletic). For the 39-taxon dataset, results of the partitioned analysis are shown in Fig. 3. The partitioned and unpartitioned analyses had only one node difference with > 50% bootstrap support. In the unpartitioned analysis (not shown), the clade containing *Cryptomastix*+*Vespericola* switched positions with the clade of *Ashmunella* with 66% bootstrap support. This relationship is uncertain in both analyses.

Species-level relationships within the polygyrids: Filling out the tips of the polygyrid tree.

This tree included data from 418 polygyrid individuals sequenced for 1–6 loci (Fig. 4). Here we present the results of the partitioned analysis, then discuss any differences with the unpartitioned analysis. To avoid a large amount of text that will be overly redundant with Fig. 4, significant results will be highlighted for each genus and species, especially relationships that are not observed or supported in the 39-taxon analysis.

The partitioned ML analysis of the 418-individual tree resulted in a single tree. Of the 110 species, 36 species are represented by only a single individual. Of the species for which

more than one individual are included, 27 form monophyletic groups, and 47 do not. Of the 21 genera included, two are represented by only a single species. In genera for which we have several species represented in the tree, 13 are monophyletic and 6 are not monophyletic. In Polygyrini (Figs. 4H–K), two genera—*Lobosculum* and *Linisa*—are only represented by one species. *Lobosculum* is sister to *Millerelix*, and this clade is sister to *Praticolella*. *Linisa* shows a close relationship to *Pra. martensiana* Pilsbry, 1907 (98%). The *Linisa*+*Pra. martensiana* relationship, the position of *Praticolella mobiliana* (sister to *Polygyra*+*Daedalochila*, 52%), and the position of *P. jejuna* (Say, 1821) (sister to the rest of Polygyrini; 51%, Fig. 4K) all indicate that *Praticolella* is polyphyletic. In *Polygyra* (98%), the two species included appear completely mixed in one large clade, with some subclades showing structure and support (Fig. 4I–J). In *Daedalochila* (Figs. 4H–I), some species are supported as monophyletic such as *D. uvulifera* (Shuttleworth, 1852) (95%), *D. avara* (Say, 1818) (100%), and *D. delecta* (Hubricht, 1976) (95%), but many are not. As seen in the 39-taxon analysis, there is support (78%) for a sister relationship between *Polygyra* and *Daedalochila*. A group of three individuals, representing two species of *Trilobopsis* (Fig. 4F), is sister to *Euchemotrema* or *Euchemotrema*+*Stenotrema*, both members of, of *Stenotremeni*. However, *Trilobopsis* is not recovered as monophyletic. The two genera of *Stenotremeni* are both monophyletic. In *Euchemotrema*, *E. hubrichti* (Pilsbry, 1940) is monophyletic (97%), but *E. fraternum* (Say, 1824) is not. In *Stenotrema*, some species are monophyletic, but others, including *S. exodon* (Pilsbry, 1900), *S. stenotrema* (Pfeiffer, 1842), and *S. deceptum* (G. H. Clapp, 1905), are not. A member of Allogonini (Fig. 4G), *Cryptomastix germana* (Gould, 1851), is sister to Vespericolini (*Vespericola*). For the most part, these genera are monophyletic, with the exception of *C. germana* which groups with *Vespericola* (63%). The species of *Vespericola* are monophyletic, however two species of *Cryptomastix* are not. The other genus of Allogonini, *Allogona*, is sister to *Vespericola*+*Cryptomastix* as seen in the 39-taxon tree. *Allogona* is a monophyletic group (90%) as are all the species within it, all with high bootstrap support. As seen in the 39-taxon analysis, Triodopsini and Mesodontini are closely related and mixed, with *Inflectarius*, a member of Mesodontini, sister to *Triodopsis*. In *Xolotrema* and *Neohelix*, most of the species are not monophyletic (Fig. 4E). This is also the case in *Inflectarius* and *Triodopsis*. *Fumoneelix* was split into two lineages; however, there was minimal bootstrap support for this relationship, so this genus may actually be monophyletic (Fig. 4D). It is sister to *Mesodon* (72%), which forms a monophyletic group including the genus *Appalachina*. In *Appalachina* all the species are monophyletic. In the rest of *Mesodon*, one species, *M. normalis* (95%) is distinct while others appear mixed in several clades (Figs. 4C–D). *Patera*, a member of Mesodontini, includes one

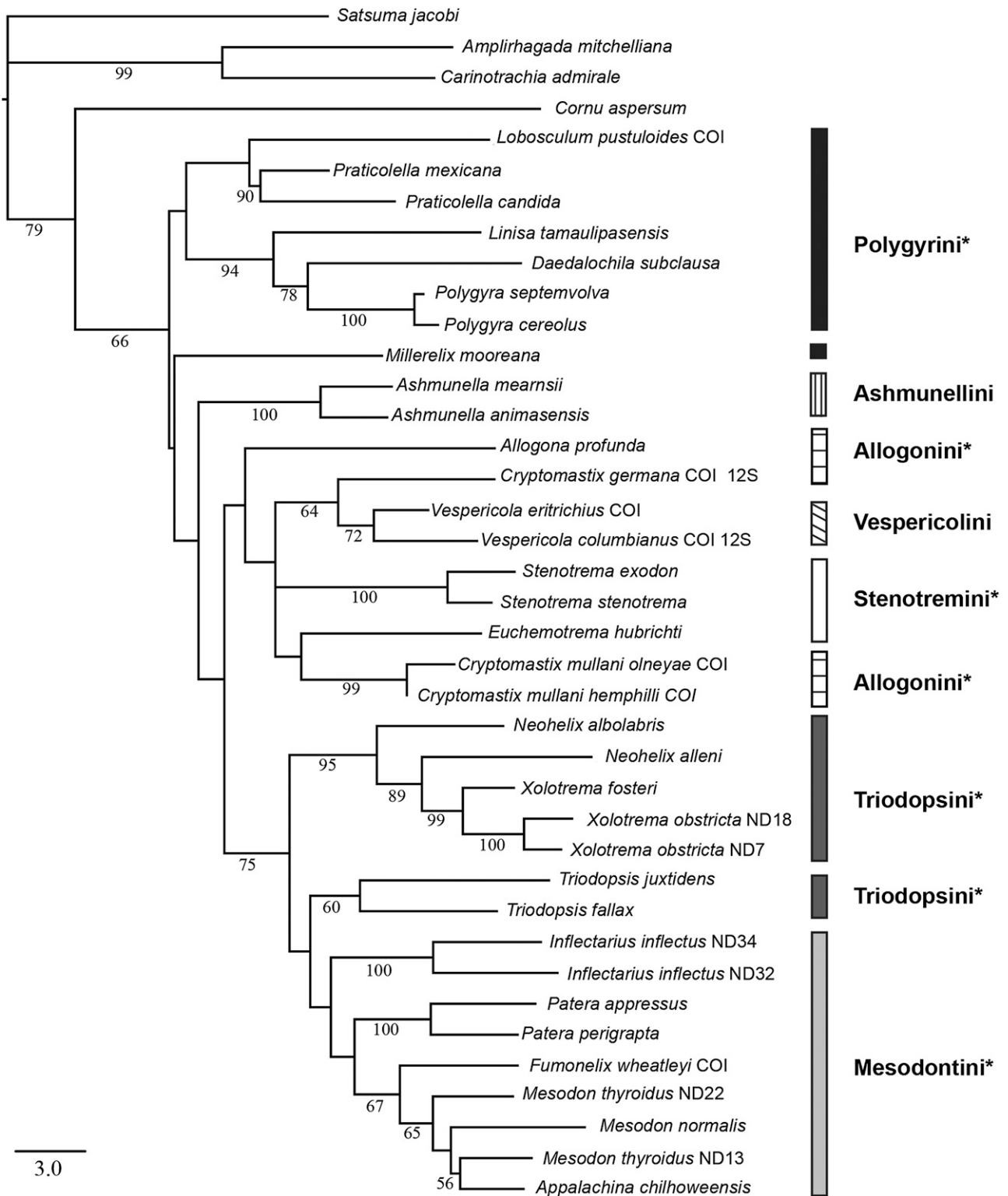


Figure 3. Maximum likelihood phylogram of the partitioned analysis of the 39-taxon data matrix (bootstrap values > 50% shown above branches). Tribes not recovered as monophyletic in this analysis are indicated with an asterisk. Bars are colored or patterned similarly to Figure 1 for comparison.

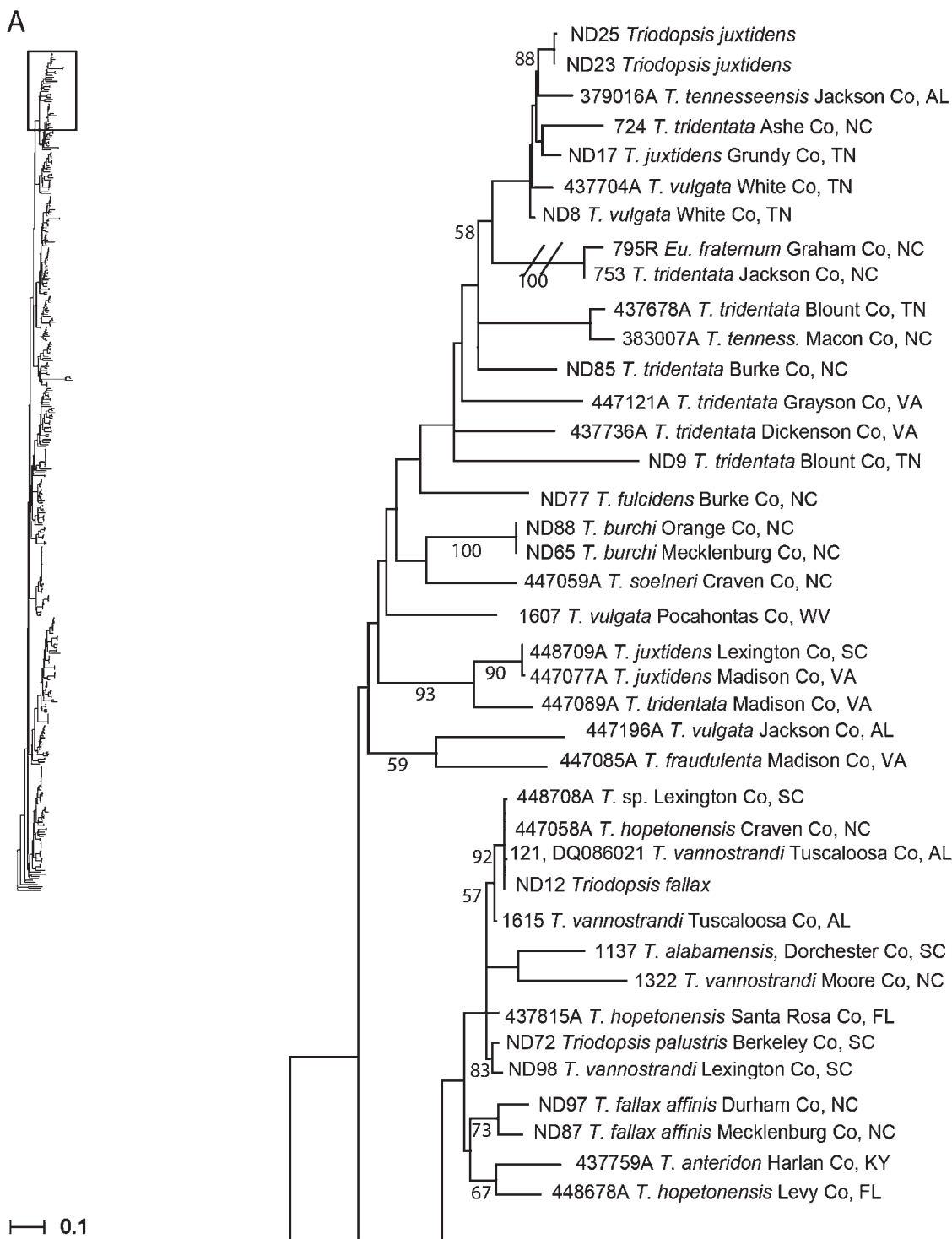
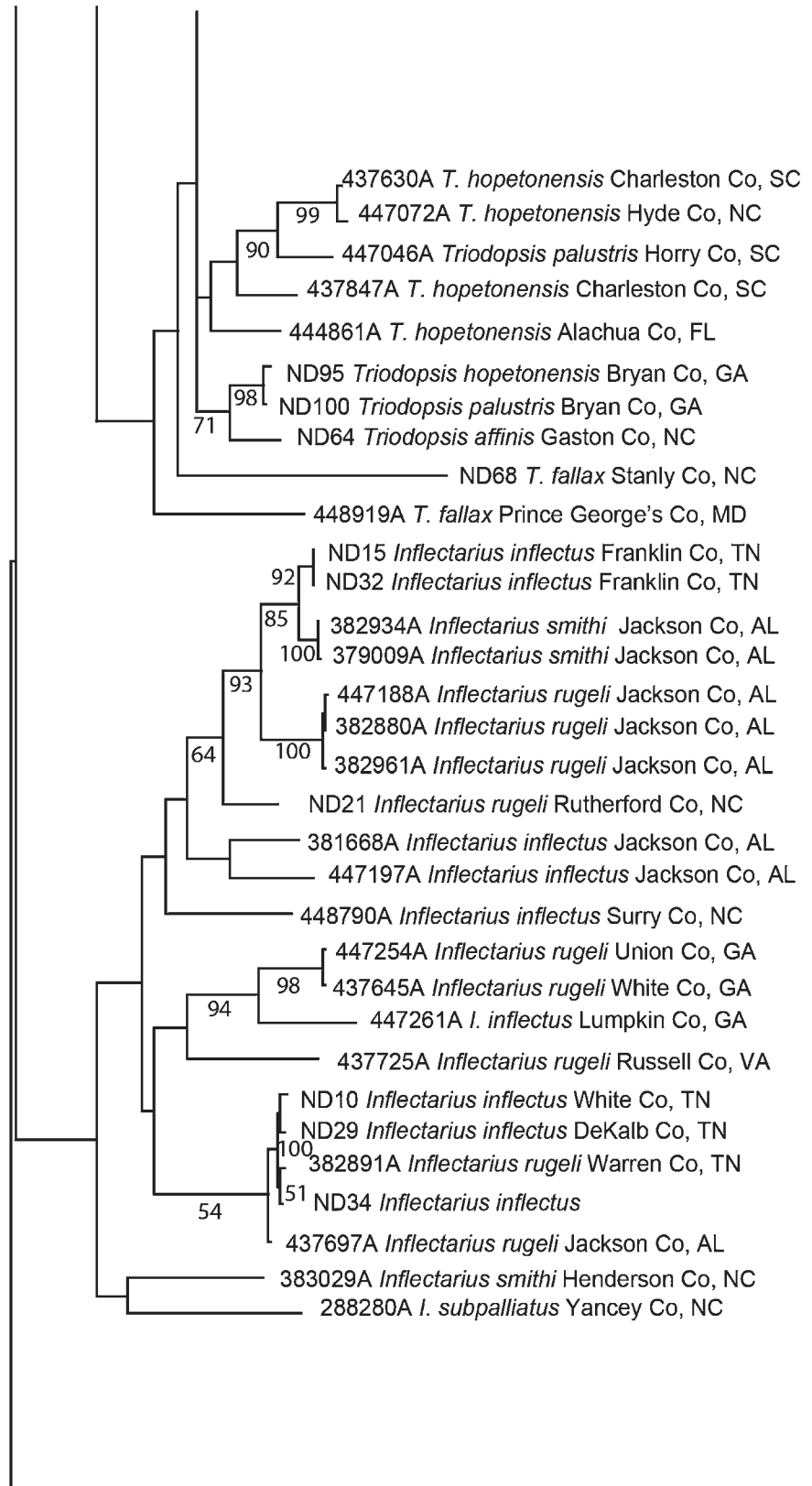
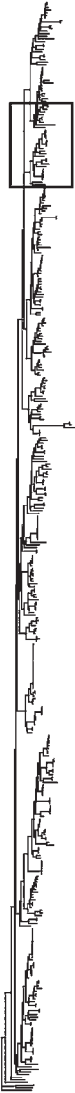


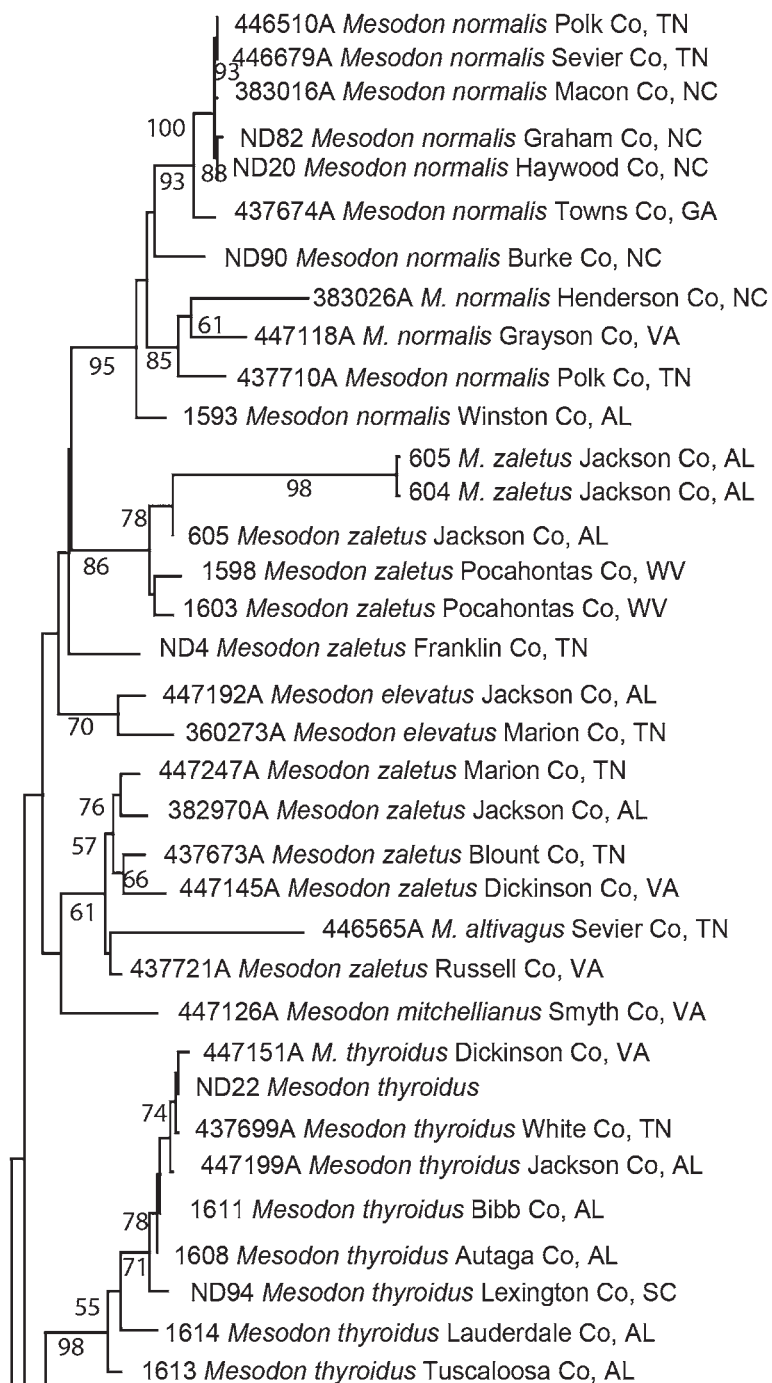
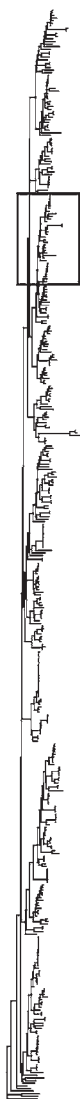
Figure 4A–K. Maximum likelihood phylogram of the partitioned analysis of the 418-taxon data matrix, (bootstrap values > 50% shown below branches). Terminals are labeled with putative species identification, GenBank, or DNA numbers (which are the initial number series given to the specimen before museum deposition), and the county and state of collection. A few names are abbreviated to fit the page. A guide tree is provided on each page to orient the viewer to the position of that page in the bigger tree. A few branches were shortened to fit the page; these are indicated with two diagonal lines. An accurate indication of the branch length can be viewed in the guide tree.

B



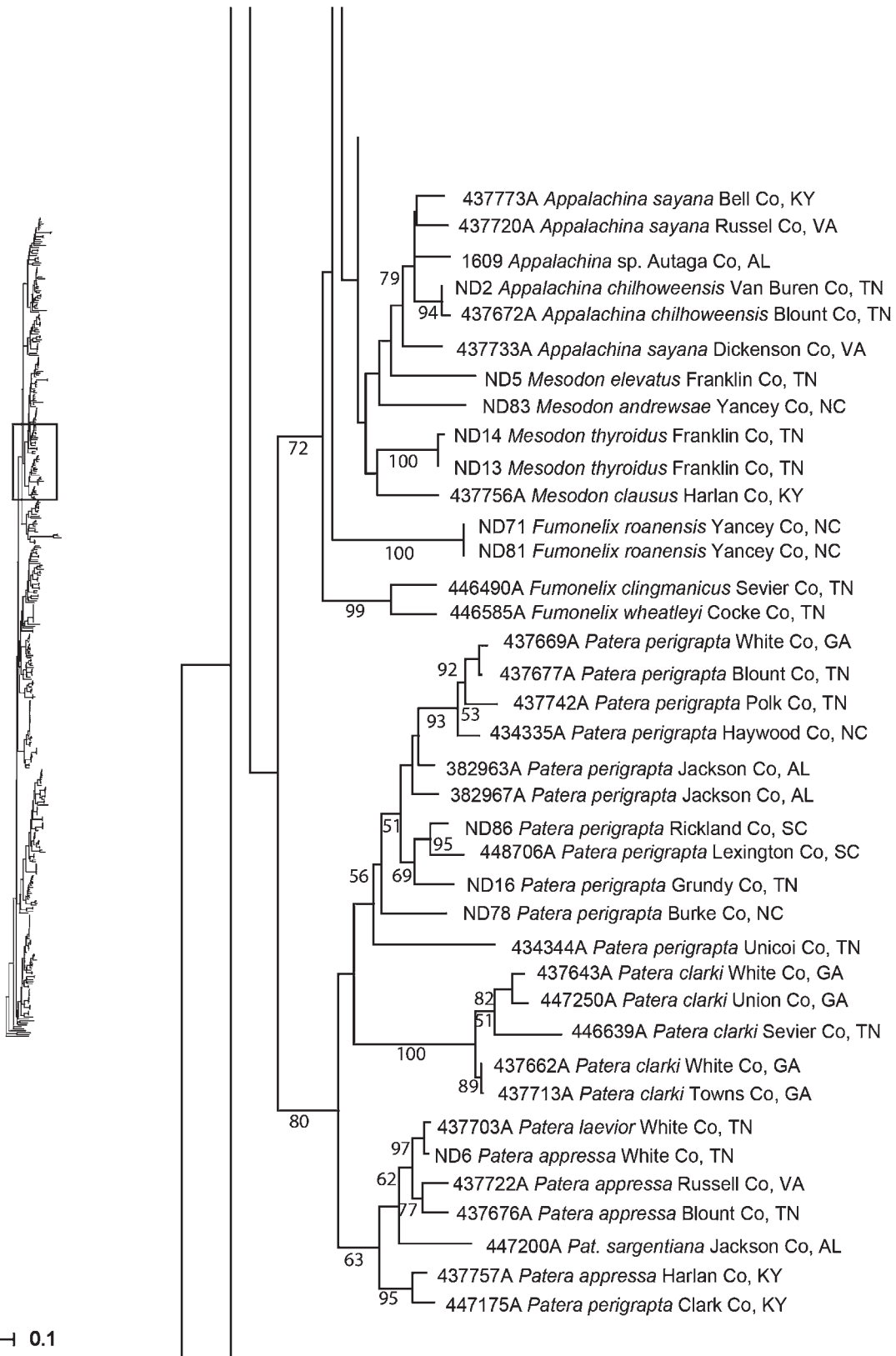
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C

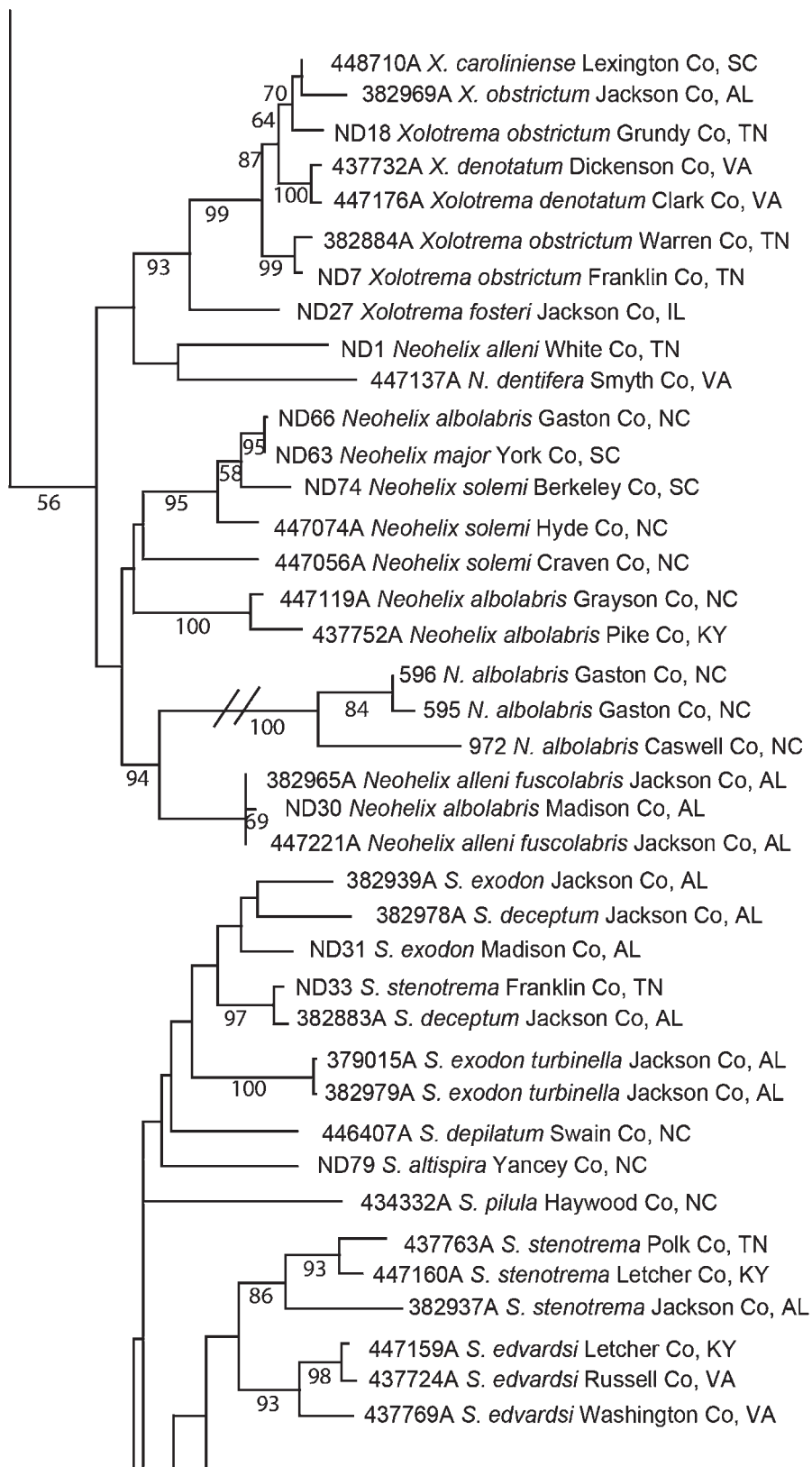
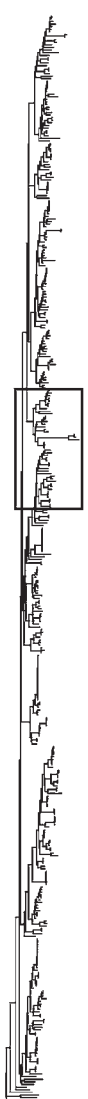


0.1

D

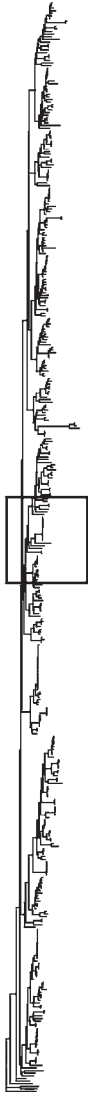
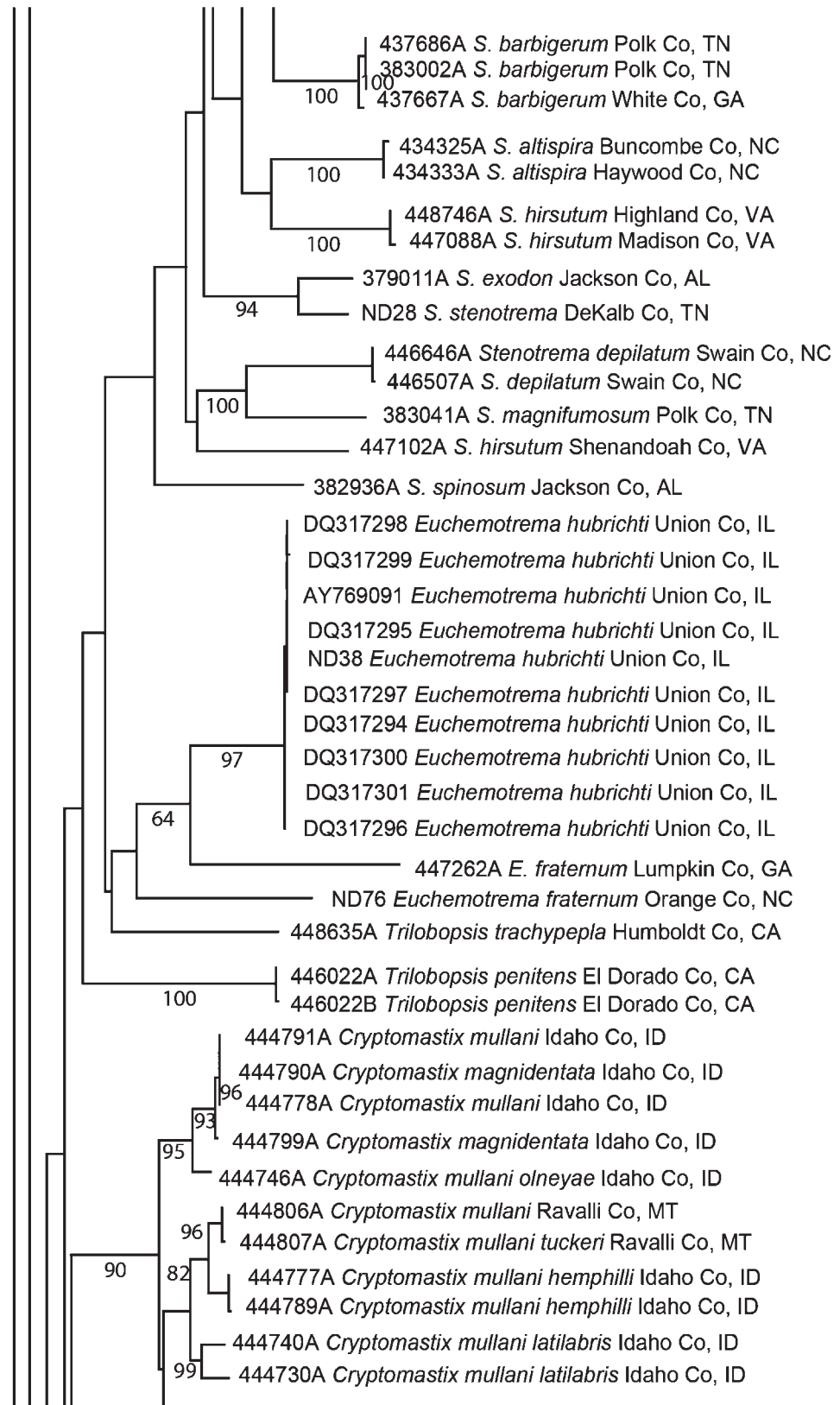


E

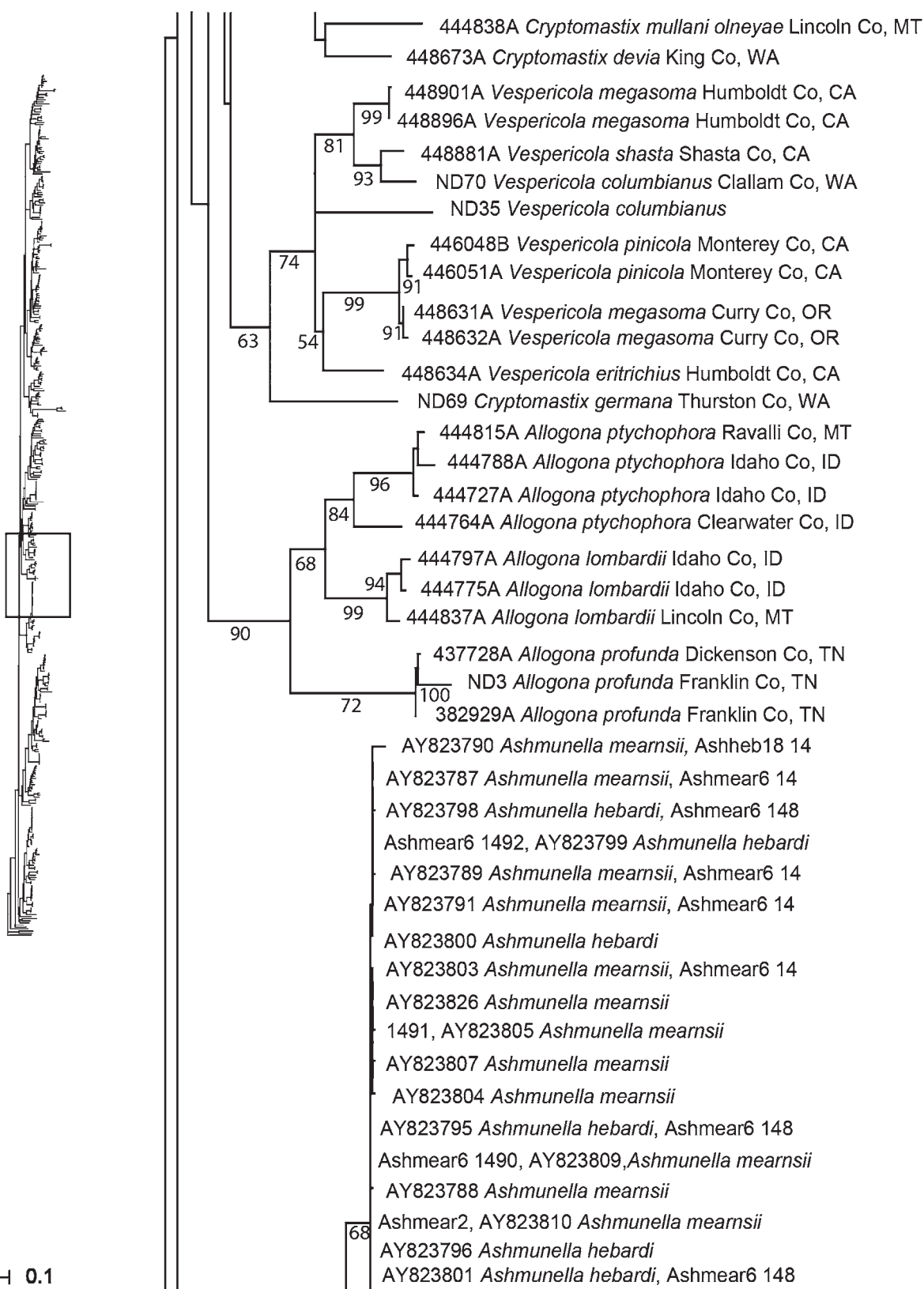


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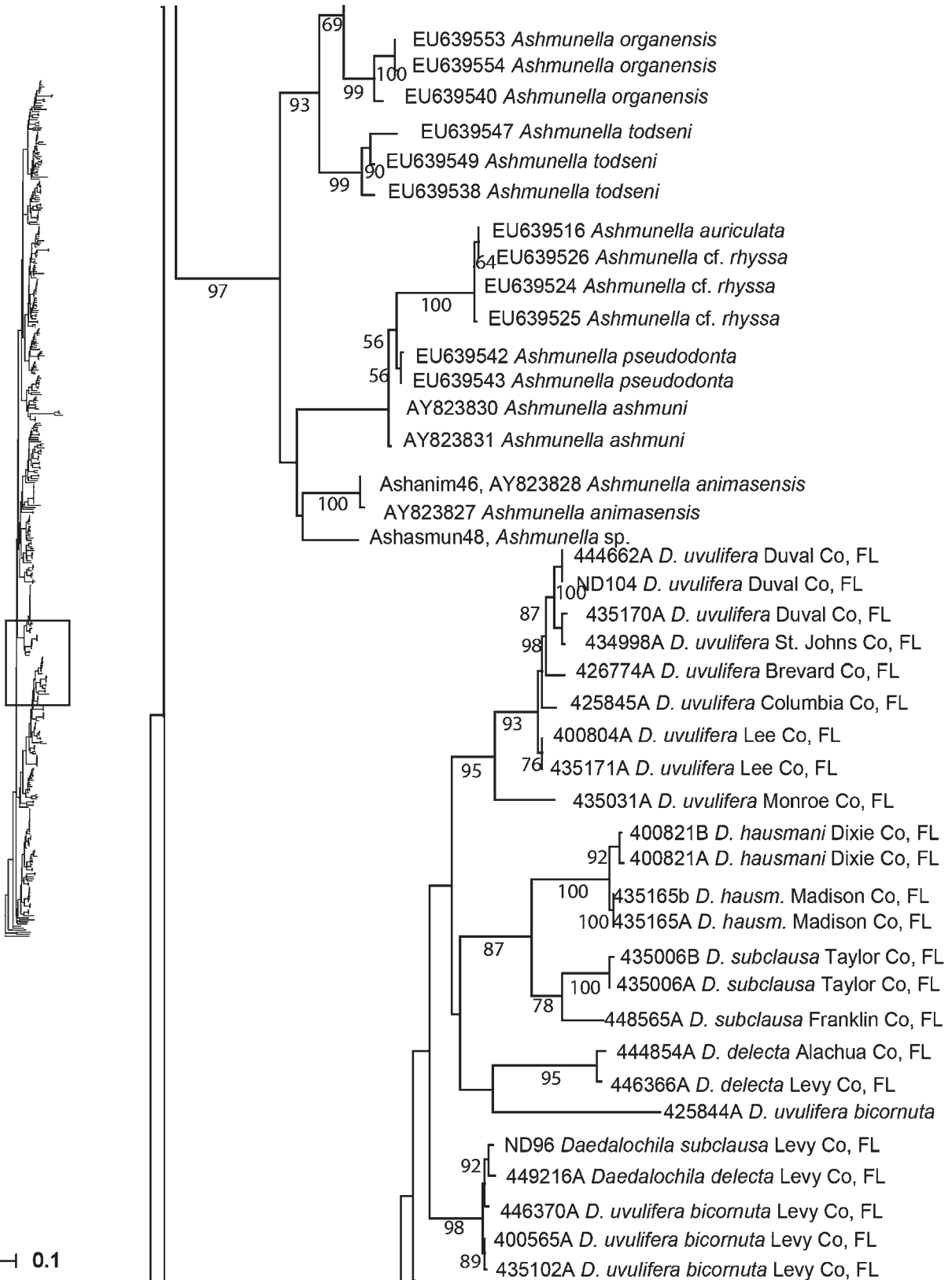
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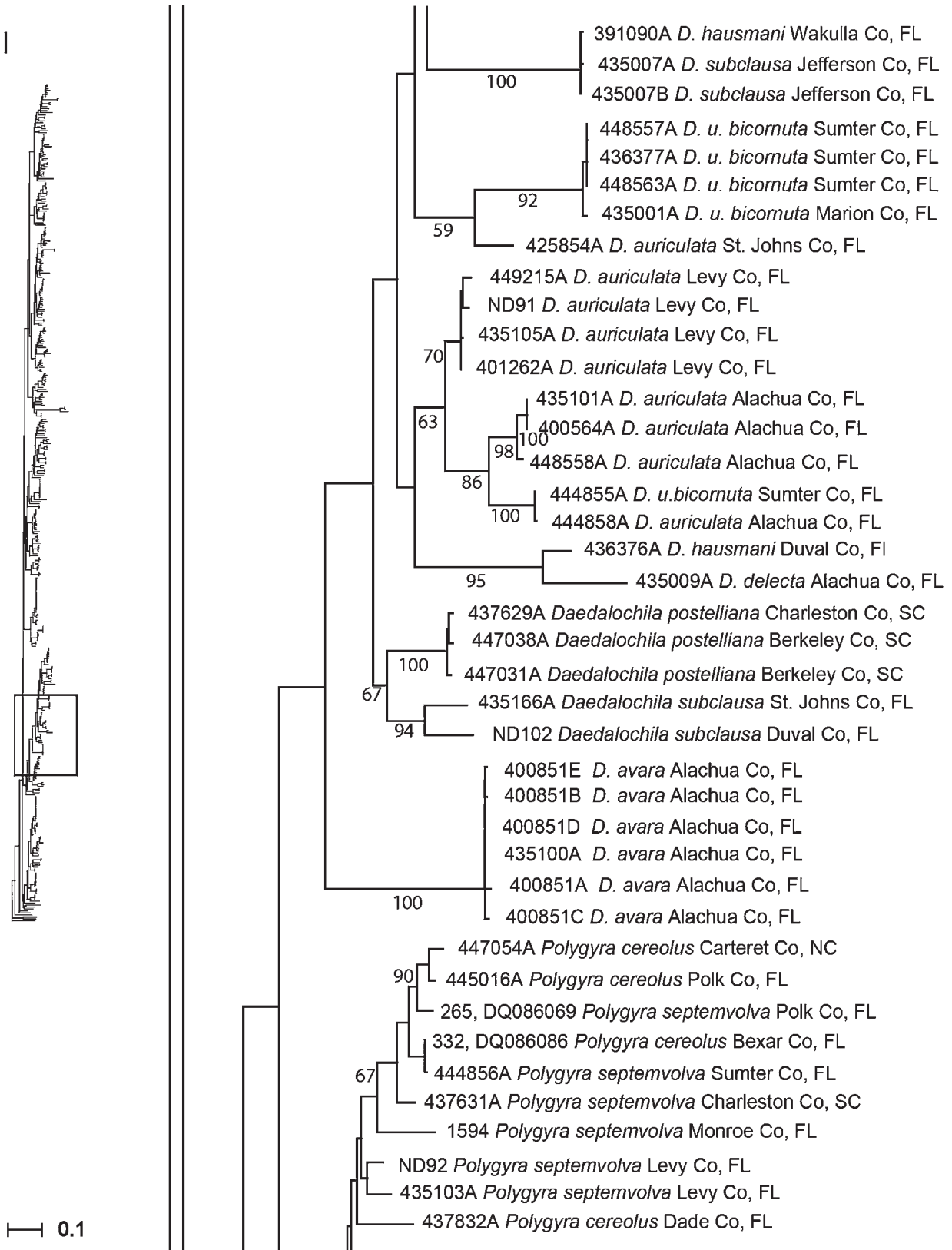
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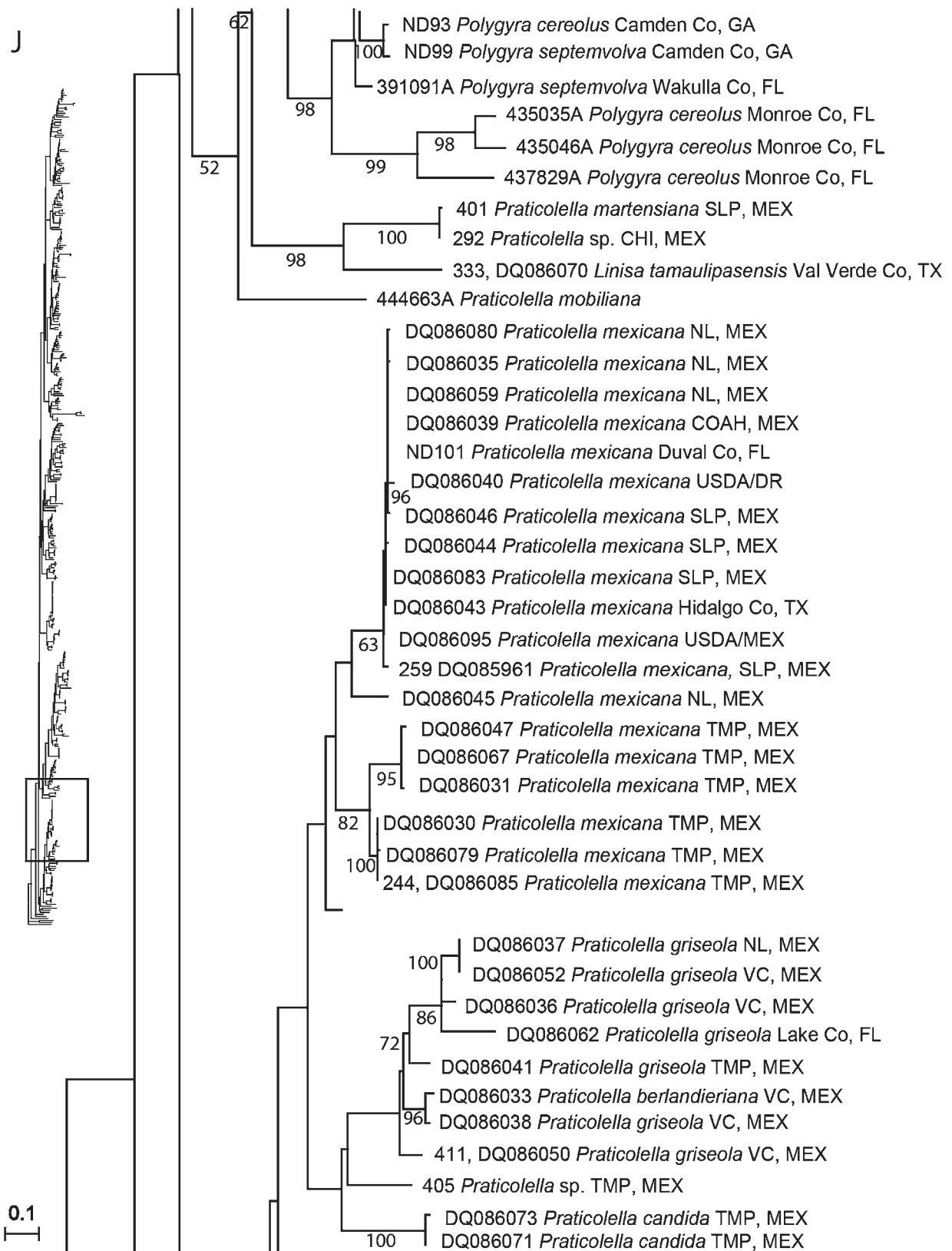


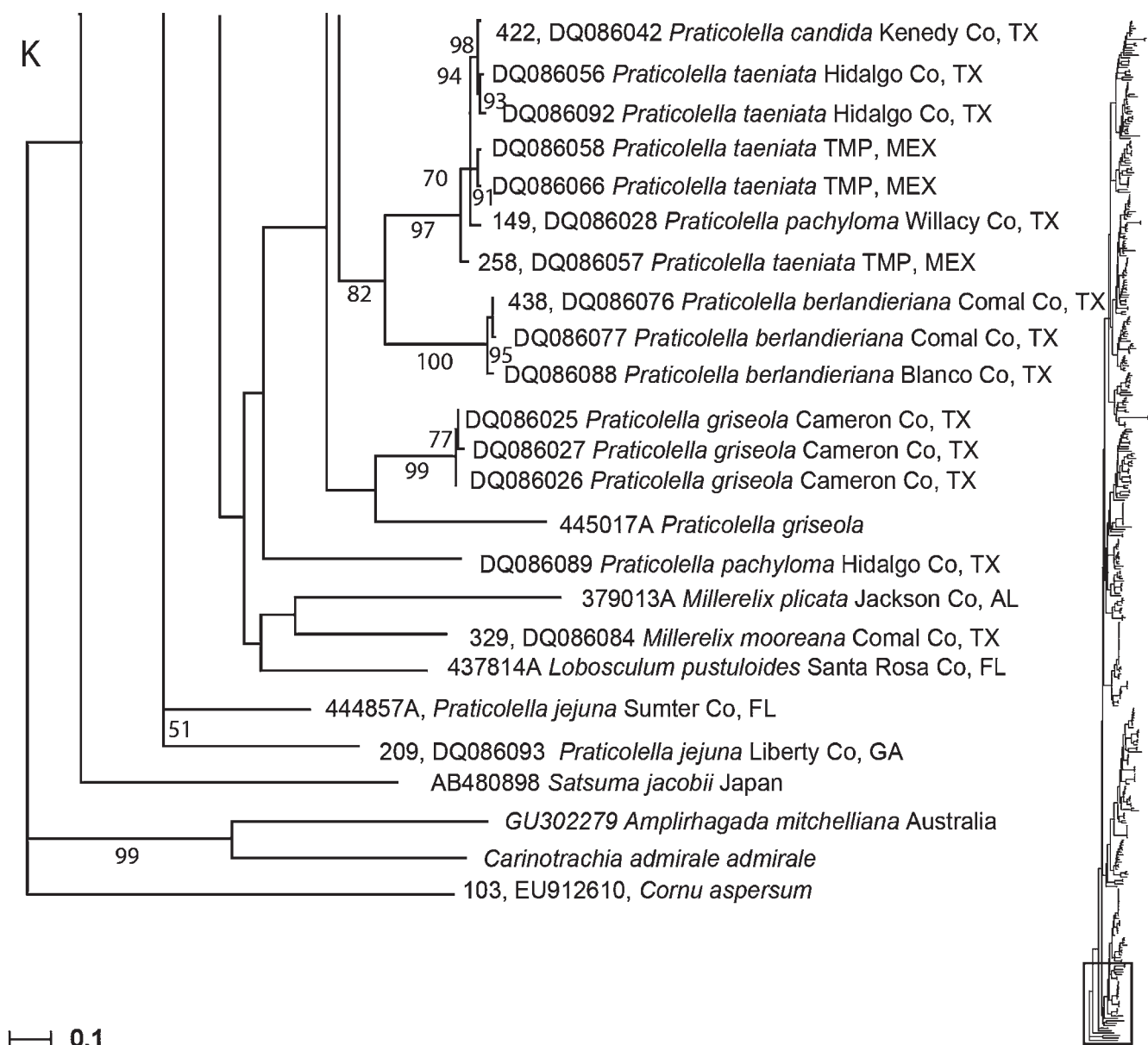
H



0.1







(Fig. 4D) species that was recovered as monophyletic, *Pat. clarki* (I. Lea, 1858) (100%). *Patera perigrapta* (Pilsbry, 1894) (56%) was monophyletic except for one individual from Kentucky that grouped with an individual of *Pat. appressa* (Say, 1821). A single individual of *Pat. sargentiana* (C. W. Johnson and Pilsbry, 1892) is embedded within the *P. appressa* clade. In Ashmunellini (Fig. 4G–H), some species are quite distinct, but a large mixed clade (68%) containing *Ashmunella mearnsii* (Dall, 1895) and *Ash. hebardei* Pilsbry and Vanatta, 1923 showed little distinction among species.

In the unpartitioned tree (not shown), relationships of one weakly (66%) supported node of the backbone of the tree differed from those seen in the partitioned tree. However,

species-level relationships remained the same. In the unpartitioned analysis, *Ashmunella* and *Allogona* swap positions, with *Allogona* sister to a clade comprising most of Polygyridae (except Polygyrini). Other relationships remain the same.

Shell and genitalia character evolution in Polygyridae

Ancestral states were reconstructed for two shell characters (Fig. 5) and two genitalia characters (Suppl. Fig. 1). Results are shown on the partitioned tree topology. The closest relatives of Polygyridae as inferred in the outgroup analysis all have globose shells, but the polygyrid ancestor was inferred to have a flat shell in MP and ML reconstructions in Mesquite (Table 1). By contrast, the RJ-MCMC analysis

found the globose shape had highest likelihood for this node, but the Bayes factor for this comparison was not significant. If we assume a flat-shelled polygyrid ancestor, a globose shell has evolved at least four and up to six times in Polygyridae within five of the tribe-level clades (Polygyrini, Stenotrematini, Allogonini, Vespericolini, Triodopsini, and Mesodontini). All analyses support a flat-shelled ancestor for the Mesodontini+Triodopsini clade.

For the apertural denticle (teeth) characters, the outgroups included in this analysis lack teeth, and MP and ML analyses found the state at the base of the polygyrid tree to be equivocal, however, the RJ-MCMC analysis found positive support for a tridentate polygyrid common ancestor. From a tridentate common ancestor, the presence of fewer than three teeth evolved, and from that state, the toothless condition appears to have evolved at least four times in Polygyrini, Vespericolini, Triodopsini, and Mesodontini. If we consider just the Mesodontini+Triodopsini clade, MP and RJ-MCMC analyses found support for a common ancestor bearing 1–2 teeth.

For the two genitalia characteristics, we found a great deal of character state change across polygyrids. The outgroups have a penial sheath, and it appears polygyrids evolved from an ancestor with a penial sheath and the lack of a penial sheath evolved at least three times and possibly as many as eight times. The reconstruction is ambiguous in parts so this number is not certain. The position of the penial retractor muscle is also highly variable, with the outgroups having an insertion point on the vas deferens and the Bayesian analysis weakly supporting a vas deferens

insertion point for Polygyridae. This is strongly supported for Mesodontini+Triodopsini. If we presume penial retractor inserting on the vas deferens is the ancestral character state for the group, insertion on the penis apex has evolved from 3–10 times.

DNA barcoding Polygyridae

Mean intraspecific distance was less than an order of magnitude smaller than mean distance between congeneric species, suggesting no barcoding gap existed in our dataset. The smallest overlap between intra-specific and inter-specific distances existed at a K2P distance of 0.05 (Table 2, Fig. 6). The genetic distance, calculated using the K2P model, within genera ranged from 0.061 in *Euchemotrema* to 0.253 in *Daedalochila*. Analysis of the 56 *Daedalochila* barcodes yielded 25 MOTUs for the ten species represented (Fig. 7); 27 MOTUs were recovered for the 61 *Praticolella* barcodes from ten species (not shown). Each of the 112 species in our profile tree (Suppl. Fig. 2) possessed a unique COI sequence. The COI profile tree identified 62 of the 100 random test individuals correctly by grouping them with their conspecific sequence.

DISCUSSION

What are the relationships among subfamilies, tribes, and genera within Polygyridae?

In our analyses, the subfamilies Polygyrinae and Triodopsinae are not recovered as monophyletic groups. Among

Table 2. Genetic distance calculated using K2P from barcoding analysis. For each polygyrid genus mean distance between species and within species are shown. For the species with less than 0.01 genetic divergence, the species pairs are listed in the last column.

Genus	Mean distance between species in the genus	Mean distance within species in the genus	Species pairs with ≤ 0.01 distance
<i>Allogona</i>	17.0%	6.0%	
<i>Ashmunella</i>	14.2%	1.0%	<i>mearnsii-hebardi</i> <i>auriculata-rhyssa</i>
<i>Cryptomastix</i>	12.8%	7.3%	<i>mullani-magnadentata</i>
<i>Daedalochila</i>	26.6%	14.8%	<i>delecta-peninsulae</i> <i>auriculata-bicornuta</i> <i>subclausa-hausmani</i>
<i>Euchemotrema</i>	21.8%	0.9%	
<i>Inflectarius</i>	20.6%	19.1%	<i>inflectus-rugeli</i>
<i>Mesodon</i>	19.8%	11.3%	
<i>Neohelix</i>	19.7%	14.3%	
<i>Patera</i>	19.0%	13.5%	
<i>Polygyra</i>	15.7%	15.2%	<i>cereolus-septemvolvla</i>
<i>Praticolella</i>	19.7%	9.4%	
<i>Stenotrema</i>	20.8%	12.2%	
<i>Triodopsis</i>	19.6%	16.0%	most species
<i>Vespericola</i>	13.7%	10.5%	
<i>Xolotrema</i>	14.9%	11.4%	

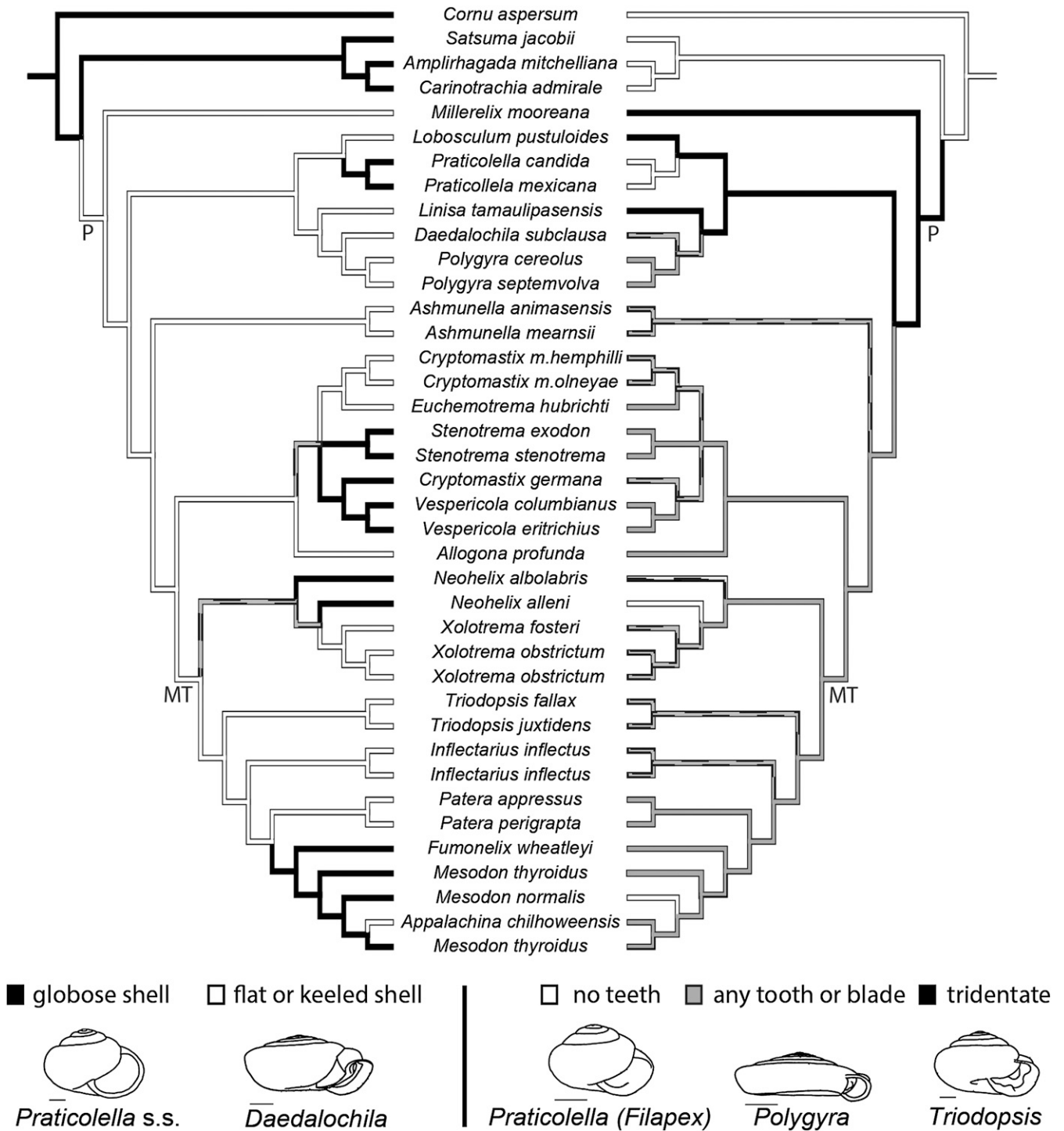


Figure 5. Ancestral state reconstruction for two shell morphological characters, shell outline, and apertural teeth. Character states are assigned for all the species in the genus. L: Shell outline, hollow bars = flat or keeled shell, filled = globose shell; R: apertural teeth, hollow branches = no teeth, black filled branches = tridentate, gray filled branches = any tooth or blade. Dashed lines indicate ambiguous reconstruction. MT indicates the Mesodontini+Triodopsini clade. P indicates Polygyridae.

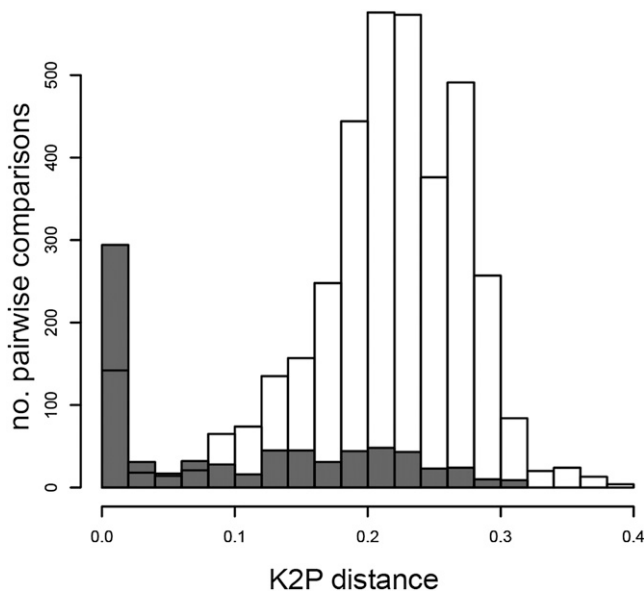


Figure 6. Histogram of pairwise K2P distances between (hollow bars) and within (grey bars) all polygyrid species in the barcoding analysis.

the tribes, Ashmunellini, and Vespericolini are monophyletic. Mesodontini is monophyletic, albeit with < 50% bootstrap support. All other tribes are paraphyletic. In all analyses we find a sister relationship between Mesodontini and Triodopsini, two tribes that were quite distantly related in Emberton's phylogenies. Polygyrini is a paraphyletic assemblage.

The phylogenetic hypothesis resulting from the molecular analysis of relationships (Fig. 3) within Polygyridae differs from the phylogenetic hypothesis of Emberton (1995b) (Fig. 1) in relationships of tribes as well as genera. Emberton proposed Triodopsini as sister to the rest of the family, whereas in our molecular trees, this position is occupied by Polygyrini. Emberton proposed Polygyrini as sister to Mesodontini, however we find Triodopsini and Mesodontini to have a sister relationship. Within Polygyrini, Emberton's proposed close relationship between *Praticolella* and *Lobosculum* was recovered in the molecular analyses. However, another of Emberton's predictions, that *Linisa* and *Lobosculum* were sisters, was not recovered; in our analysis *Linisa* is closer to *Polygyra*+*Daedalochila*. Within Triodopsini, the close relationship of *Xolotrema* and *Neohelix* was supported, but *Triodopsini* was sister to Mesodontini, not *Xolotrema*+*Neohelix* as proposed by Emberton.

Mesodontini was found to represent a monophyletic group. Within Mesodontini, the close relationship of *Mesodon* and *Appalachina* was supported, in fact, *Appalachina* was found to be nested within a species of *Mesodon*. It appears that *Appalachina* is a subgroup of *Mesodon* as initially proposed by Pilsbry (1940). Emberton proposed a sister relationship of

Patera+*Fumonelix* (sister to *Mesodon*+*Appalachina*). We found instead *Fumonelix* sister to *Mesodon*+*Appalachina*, with *Patera* sister to that entire clade. Within Allogonini, Emberton proposed a sister relationship between *Allogona*+*Trilobopsis*, with *Cryptomastix* sister to that clade. We did not have a representative of *Trilobopsis* in the 39-taxon analysis due to limited sequence data, so by that hypothesis we would expect to find *Cryptomastix*+*Allogona*. Instead, we found *Allogona* sister to an unresolved clade comprising three tribes: Allogonini, Vespericolini, and Stenotremeni, with no bootstrap support. In the 418-taxon tree, we do have three individuals of *Trilobopsis* represented; all three display a close relationship to Stenotremeni. One species of *Cryptomastix* was sister to *Vespericola* (our sole representative of Vespericolini as we did not have *Hochbergellus*) and the other species were sister to *Euchemotrema* (Stenotremeni). *Euchemotrema* and *Stenotrema* were expected to be sister taxa as the sole genera in Stenotremeni, but a monophyletic Stenotremeni was not recovered in either analysis. Emberton proposed Ashmunellini to be sister to Allogonini, but we found Ashmunellini sister to a clade comprising most of the polygyrids. Most genera where we have multiple species sampled are monophyletic, except *Cryptomastix*, *Neohelix*, and *Mesodon* (which has *Appalachina* nested within). However, this analysis does not include all members of each genus, specifically *Praticolella*, where previous work (Perez 2011) found the subgenus *Eduardus* Pilsbry, 1930 more closely related to *Linisa* and the subgenus *Filapex* Pilsbry, 1940 close to *Lobosculum*. A similar pattern may be found with more complete sampling within each genus.

Species-level relationships within the polygyrids: filling out the tips of the polygyrid tree.

Our analyses include ~35% of the named, extant polygyrid species. This is the largest molecular examination of this family to date and the only published examination of any group larger than a species complex (Perez 2011). While we found marked disagreement with Emberton's hypotheses on family and tribe relationships, many of the genus-level revisions he made were supported by this analysis, although six of the 21 genera are not found to be monophyletic. At the species-level, only 27 of the species represented by more than one individual were monophyletic. This indicates substantial taxonomic work will be required in Polygyridae for the classification of the family to adequately capture the diversity and evolutionary history of the group.

In Polygyrini, several classification problems are observed: *Linisa* has a close relationship to *Praticolella martensiana*, *Pra. jejuna* (Say, 1821) is sister to the rest of Polygyrini, and *Pra. mobiliana* I. Lea, 1841) (subgenus *Farragutia* Vanatta, 1915) is sister to *Polygyra*. This indicates that *Praticolella* is polyphyletic, with each subgenus of *Praticolella* (except *Praticolella sensu stricto*)



Figure 7. Neighbor-joining tree of K2P distances for *Daedalochila*. Boxes indicate individual molecular operational taxonomic units (MOTUs) identified by jMOTU.

grouping with other genera. In *Praticolella*, considerable diversity remains to be described. In *Polygyra*, the two species of this genus that are represented in our data set are completely mixed or misidentified, drawing into question the distinction between *Polygyra cereolus* (Mühlfeld, 1816) and *Polygyra septemvolva* Say, 1818. This is perhaps not surprising; there has long been discussion in the malacological community on the difficulty of distinguishing these species (Cheatum and Fullington, 1971). However, simply synonymizing these two taxa is not a good

solution, as some clades are quite distinct, for example, the clade containing *Polygyra cereolus* 437829A, which appears to be a lineage unique to the Florida Keys. In *Daedalochila*, three species are monophyletic while five are not (60%).

Of the polygyrid species that have more than one individual represented in the tree, 63% of the species are not monophyletic. Some portion of this may be attributable to misidentification. These snails were all identified by authors Perez, Pearce, or Slapcinsky, all of whom have 10+ years' experience working on polygyrids, but some errors could have occurred. This may be a greater source of error in some genera such as *Triodopsis* (1 of 10 species monophyletic) or *Mesodon* (1 of 5), which are particularly taxonomically problematic, with some species distinguished by slightly different, non-diagnostic shell characteristics. Some of this lack of monophyly might be attributed to an underestimation of species diversity, as documented in Perez (2011) where the number of species in *Praticolella* will likely double with adequate taxonomic attention. This could also be the case in other polygyrid genera. It is apparent that a few species have been oversplit; an example might be *Ashmunella mearnsii* and *Ash. heberdi*, which appear to have no distinction at the loci we examined, although this single line of evidence does not conclusively demonstrate they should be synonymized. Finally, we cannot rule out introgression or incomplete lineage sorting as sources of apparent lack of monophyly.

As seen in Fig. 3, *Triodopsini* and *Mesodontini* are closely related (75%) and will require some taxonomic work to disentangle. *Inflectarius*, currently classified as a member of *Mesodontini*, is sister to *Triodopsis*, a member of *Triodopsini*. In *Xolotrema*, *Neohelix*, and *Inflectarius*, none of the species are monophyletic. This is also nearly the case in *Triodopsis* (1 of 10). *Fumonelix* was split into two lineages; however, there was no bootstrap support for this relationship, so this genus may be monophyletic. This study finds *Appalachina* completely within *Mesodon*, which is a monophyletic group if *Appalachina* is included. This finding could mean that *Appalachina* should be subsumed in *Mesodon* or that there are multiple

generic-level lineages in this group. In Triodopsini+Mesodontini, the generic classification (Emberton 1988, 1991) was supported.

The conclusions that can be drawn for species-level taxonomy with this analysis are limited by incomplete sampling for most species. A complete picture of species-level diversity requires multiple individuals from multiple populations including the entire geographic range of the species, which this analysis does not include for most taxa. For example, *Daedalochila avara* (Fig. 4I) appears to be a monophyletic species; however, all the individuals in the analysis are from the same population in Florida. The inclusion of more populations may increase the complexity of this picture of *D. avara*. With this caveat, we can make a few observations about general trends among polygyrid species. For many of the species in the tree, the nominal taxon is not monophyletic; however, deep clades support a geographically unified set of individuals. For example, *Polygyra cereolus*+*Pol. septemvolva* form two distinct clades, one includes only representatives from the Florida Keys, the other includes individuals from localities ranging along the coast from Florida, Georgia, North Carolina, South Carolina, and Texas. Future taxonomic work can build on this initial analysis, but will need to include broader sampling for each member of each genus along with individuals from type localities.

Shell and genitalia character evolution in Polygyridae

Most reconstructions of ancestral character states (Table 1: MP and ML) found the common ancestor of the polygyrids likely had a flat shell and three apertural teeth. From this flat, tridentate shell, a variety of shell shapes evolved. From a flat ancestor, a globose shell has evolved between four and six times, that number will require better resolution of relationships among genera to pinpoint. Globose shells have evolved in the genera *Praticolella*, *Euchemotrema*, *Cryptomastix*, and *Vespericola*. The switch in this part of the tree from flat to globose may have happened only once; final determination will require better resolution in this part of the tree to be sure. Globose shells have also evolved in Triodopsini in *Neohelix*, which was not recovered as monophyletic and may represent two separate transitions to a globose shape. Finally, within Mesodontini, *Fumonelix* and *Mesodon* have globose shells. Interestingly, in *Mesodon*, *Appalachina* appears to have re-evolved a flat shell.

Our analyses suggest that the ancestral polygyrid had three apertural teeth. From a tridentate ancestor, the toothless condition has evolved at least once in *Praticolella* (our data), but probably more than once, as the *Praticolella* subgenera appear to have convergently attained a toothless condition (Perez 2011). The tridentate condition is retained in *Millerelix*, *Lobosculum*, and *Linisa*. In Polygrini, one lineage also evolved the 1–2 tooth condition (*Daedalochila*+*Polygyra*).

The common ancestor of all the polygyrids except Polygrini appears to have had 1–2 teeth or blades, and from that phenotype, a toothless aperture evolved at least three times: *Vespericola columbiana* in Vespericolini; *Neohelix* in Triodopsini; and some members of the genus *Mesodon*, including *Appalachina chilhoweensis* (J. Lewis, 1870) in Mesodontini. From the 1–2 toothed condition, a tridentate aperture re-evolved on at least one occasion in *Triodopsis*.

If we consider Mesodontini and Triodopsini, several interesting patterns are uncovered. These tribes were not considered close relatives previously (Emberton 1994); Triodopsini was proposed to be sister to the rest of Polygyridae. Therefore, our conclusions about character evolution among these two tribes will necessarily be very different than Emberton's. The common ancestor of this clade is reconstructed with a flat shell and 1–2 teeth. From that point, a globose shell has evolved 2–3 times. One lineage, *Triodopsis*, has evolved a tridentate shell, and *Mesodon* has evolved a toothless or single-tooth condition. The tooth state is ambiguously reconstructed in *Mesodon* as there is great variability among species in this genus. Some species consistently have teeth, but in some species, tooth number varies within and among populations.

Emberton (1988, 1991, 1994, 1995a, b) described extensive convergence in shell characteristics among genera in two tribes: *Mesodon* (Mesodontini) and *Neohelix* (Triodopsini). The convergence is so close that distinguishing individuals of these species where they occur sympatrically requires dissection of the penial structures to look for genus-level diagnostic criteria. Our analyses support this hypothesis of shell convergence. Another proposal of Emberton (1988) was that of conchological stasis within genera. Rather than conchological stasis, we find several instances where shell outline and tooth number varies within genera. For example, *Vespericola* and *Mesodon* include both toothed and untoothed species. In *Mesodon*, there is also a lineage with flat shells—*Appalachina*.

The significance, if any, of these two shell characteristics is unknown. Several authors have proposed adaptive hypotheses for apertural teeth and shell shape. The hypothesis with the strongest support (correlative) appears to be the link between flat shells and rocky, or vertical, crevice habitats (Goodfriend 1986, Emberton 1988). Increasing size of apertural teeth (degree of apertural obstruction) has been shown to correlate positively with ground moisture in several species of *Triodopsis* (Vagvolgyi 1968, Emberton 1988). It is also possible that shell shape and tooth number varies randomly among Polygyridae and does not have adaptive significance, or that these characters are adaptive, but evolve so rapidly that their ancestral states cannot be reconstructed easily.

Characters of the genital anatomy especially penis texture are often used to determine species-level relationships.

However, other characters of the genital system are assumed to be more slowly evolving, for example, the presence of structures like the dart sac, epiphallus, and penial sheath. These characters are often used to define higher-level relationships among stylommatophorans. In many cases, only one or a few of these characters define groups. Some groups are defined by the presumed loss of these characters as in the Camaenidae. However, molecular evidence suggests that loss in complex structures has happened many times in closely related groups and that the dart sac has been lost multiple times in the camaenid/bradybaenid lineage (Wade *et al.* 2007). Traditionally, higher-level relationships among Polygyridae are largely defined by genital characters. For example, Triodopsini have a penial sheath with the retractor muscle inserted on the vas deferens and Mesodontini lack a penial sheath and have the retractor muscle inserted on the apex of the penis.

In our analysis, the Mesodontini and Triodopsini are sister clades (75% bootstrap) and not distantly related as suggested by their genital anatomy. Although our ancestral reconstructions of these traits were equivocal, it is clear that the penial sheath has been lost more than once; once in the Polygyrini and again in the Mesodontini. In addition to that, the penial sheath has been reduced at least once to a diaphanous sheath in *Euchemotrema* and *Stenotrema*. Similarly, the apical insertion of the retractor muscle does not appear to be an informative character in determining the relationship of the Polygyrini and Mesodontini to each other. Although our ancestral reconstruction is ambiguous, it is clear this character is homoplasious. Single or small groups of characters are insufficient to determine higher-level relationships within the Polygyridae.

DNA barcoding Polygyridae

Analysis of the Folmer *et al.* (1994) COI region suggested that DNA barcoding was not an effective tool in discriminating among polygyrid species given the current taxonomic state of the group. No evidence of the 'barcoding gap' seen in many taxa (Hebert *et al.* 2003a, 2003b, 2004) was found; instead, there was considerable overlap between intraspecific and interspecific K2P distances (Fig. 6) similar to some other studies (Meyer and Paulay 2005, Wiemers and Fiedler 2007). The high intraspecific distances were easily visualized in the *Daedalochila* MOTU analysis (Fig. 7), and the overlap in distances was reflected in the low success rate (62%) of guide tree identifications using neighbor-joining. The failure of barcodes to delimit species and genera in Polygyridae may be the result of many factors, including but not limited to taxonomically poorly understood clades, incomplete sampling, the presence of closely related sister lineages, species that have undergone introgressive hybridization, species where interspecific and intraspecific variation overlap, and species sharing ancestral polymorphisms

(Baker *et al.* 2009). Land snails also do not disperse readily, and DNA barcodes often perform poorly in groups with low vagility and corresponding levels of geographic structure (Bergsten *et al.* 2012). While the limitations of barcoding and other DNA taxonomy methods are well known (Prendini 2005, Packer *et al.* 2009), most studies recognize barcoding as a useful method for identifying groups of interest. Our barcoding results, in addition to phylogenetic analysis results, indicate a great deal of needed taxonomic work.

Future polygyrid work

We began this study with some indications that relationships among Polygyridae were poorly understood; unfortunately, these results support this initial impression to a greater extent than we had expected. Most of the results presented show incongruence with previous morphology-only approaches to polygyrid systematics and taxonomy that have relied on relatively few characters. Clearly more characters and character sets are required for an accurate picture of species boundaries and relationships among species and higher taxa in this family. Molecular characters add some resolution to these relationships, but much of the tree of the family remains poorly resolved. Testing hypotheses of the mechanisms driving shell character evolution will require a more fully-resolved phylogeny. Our work uncovered substantial problems with the current species-level taxonomy of polygyrids; in fact, most of the species examined were not monophyletic. This lack of monophyly is pervasive in our representatives of polygyrid species, even though we did not sample the entire geographic extent of most species.

Extensive taxonomic work will need to be carried out to determine if this lack of monophyly is due to cryptic or poorly characterized but real species or biological processes like introgression, convergence, or incomplete lineage sorting. Barcoding may be of limited utility when the taxonomy of a group is in an embryonic state. However, sequence data generated in barcoding efforts can help point out groups that can be targeted for taxonomic study using a wider suite of characters, thus, enhancing our understanding of species-level relationships and the efficacy of barcoding. Some potential next steps for a better understanding of polygyrids would begin with geographically complete, population-level sampling of species shown to be non-monophyletic using morphological and molecular characteristics in order to begin to stabilize species-level boundaries. Higher-level relationships might prove difficult to resolve. The use of additional molecular markers with different levels of variability may prove useful to resolving the backbone of the tree. While relationships among polygyrids appear to be at a low-point in our understanding, we hope this work provides a foundation for future taxonomic and systematic work that will enlighten our

understanding of the evolution of this common, charismatic group of snails.

ACKNOWLEDGMENTS

Collections for this project began by the authors in 1997. Over that length of time, a great number of people and institutions supported the authors with collections, lab work, and monetary support. The following institutions supported the authors or provided research funding: NSF-IGERT (DGE-9972810 to A. K. Ward), the University of Alabama, the SPIRE program at UNC-Chapel Hill (NIGMS-MORE division-GM 00678), Cliff Cunningham lab at Duke University, Faculty Research Grant at the University of Wisconsin at La Crosse, Texas Academy of Science, Malacological Society of London, Conchologists of America, American Malacological Society, American Museum of Natural History, Delaware Museum of Natural History, North Carolina Museum of Natural Sciences, Howard Hughes Medical Institute Undergraduate Science Education grant (to ULM), NSF to FEA (DEB-0235794), SIU REACH Program, and the Saluki Scholars Program. The following individuals provided assistance in museum, lab, or field: J. J. Apodaca, K. Auffenberg, A. Bogan, S. A. Clark, B. Coles, K. Mugford Davis, Z. Felix, F. Fontanella, D. Furr, K. Gallant, D. Garate, J. Godwin, B. Henry, J. Laurila, H. G. Lee, T. G. Littleton, P. Marquez, L. McCutchen, S. McGregor, J. Nekola, J. B. Pollock, D. Robinson, B. Roth, J. Smith, N. E. Strenth, P. Super, F. G. Thompson, A. S. and R. W. Van Devender, J. Walker, R. Werren, R. Winters, and F. Zimmerman. Mexican specimens were collected under permit 7146 to Dr. A. Correa Sandoval, Universidad Nacional Autónoma de México at Victoria.

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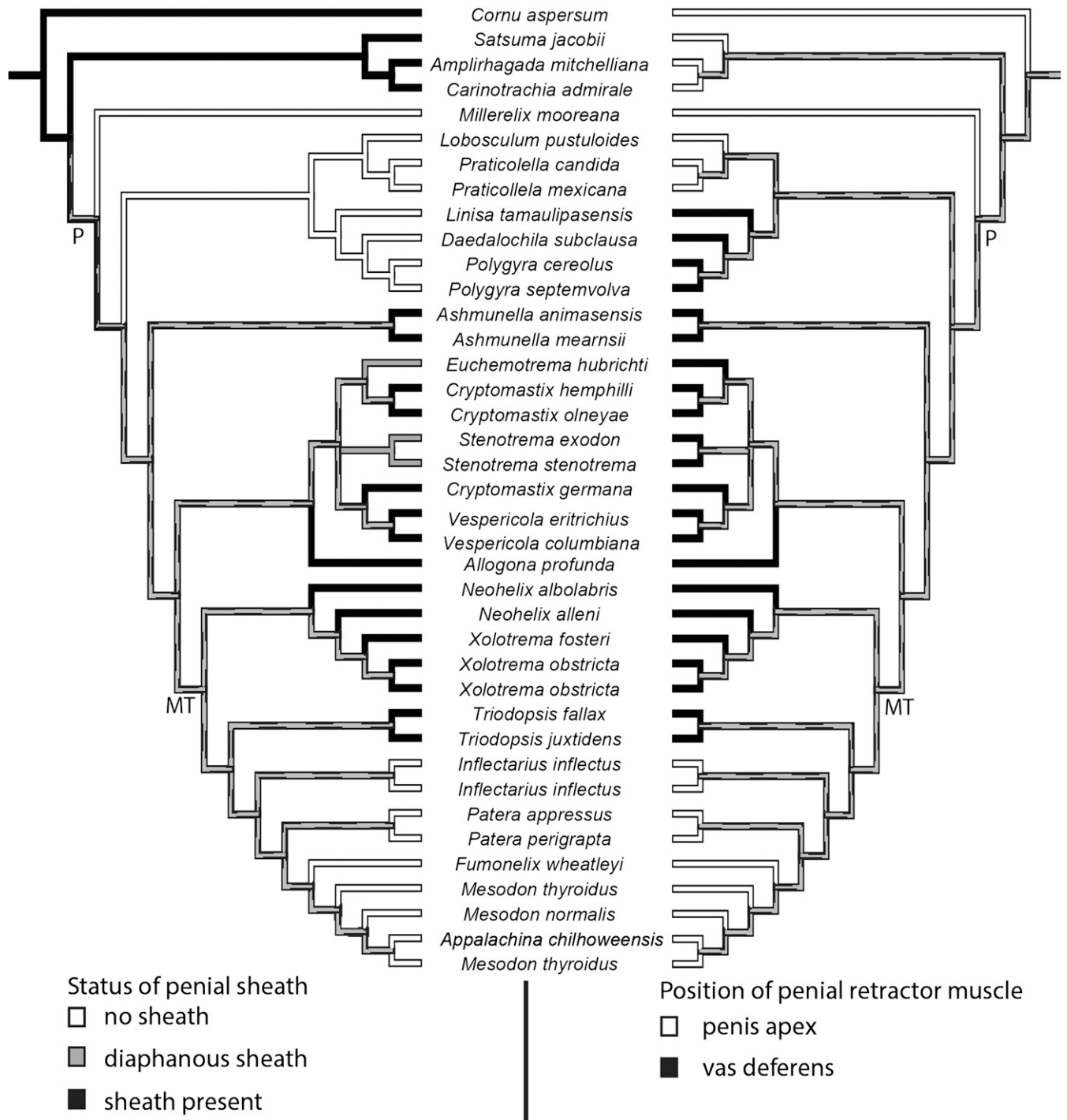
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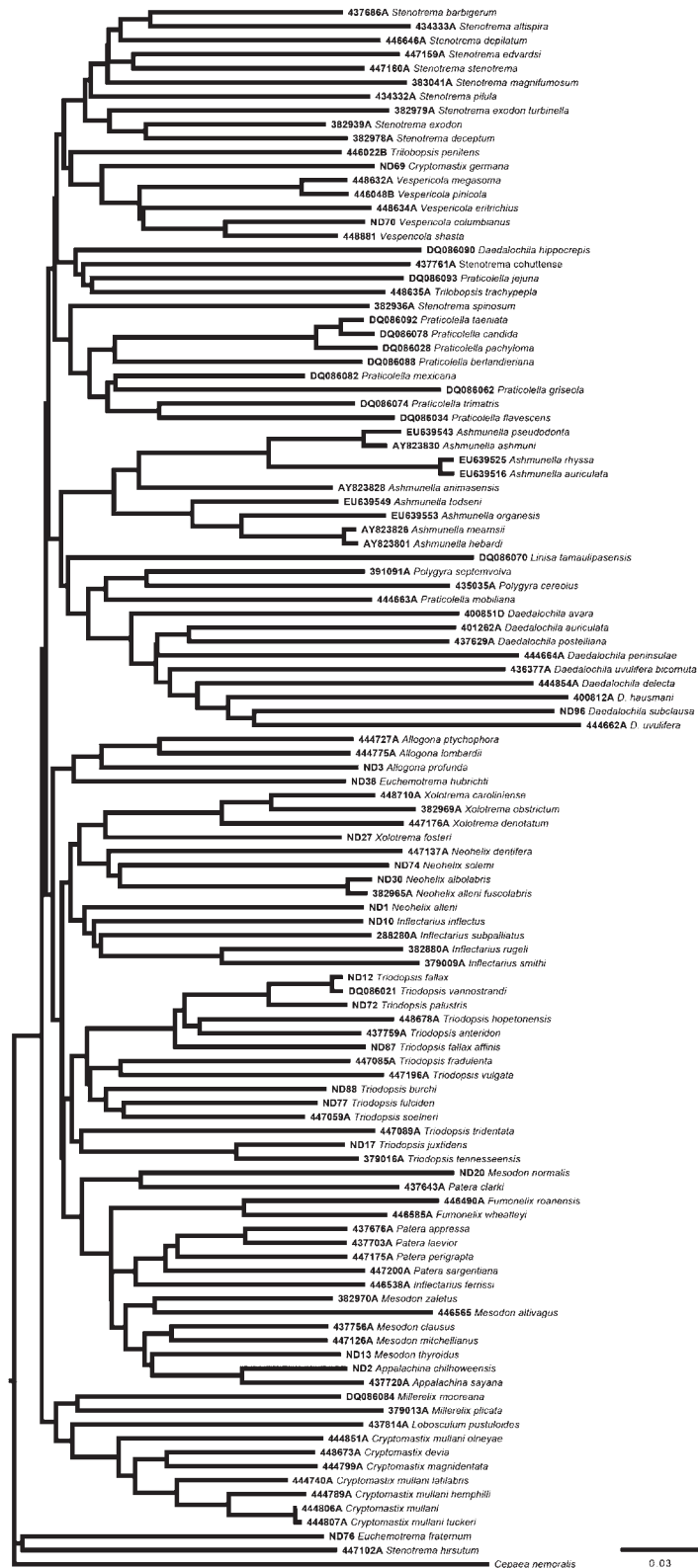
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Submitted: 18 January 2013; **accepted:** 28 June 2013; **final revisions received:** 9 October 2013



Supplemental Figure 1. Ancestral state reconstruction for two genitalia characters, status of penial sheath and position of penial retractor muscle. L: penial sheath, hollow bars = no sheath, gray = diaphanous sheath, black filled = sheath present; R: position of penial retractor muscle, hollow branches = penis apex, black filled branches = vas deferens. Dashed lines indicate ambiguous reconstruction. MT indicates the Mesodontini+Triodopsini clade. P indicates Polygyridae.



Supplemental Figure 2. K2P neighbor-joining guide tree for DNA barcoding.

Supplemental Table 1. A list of the specimen collection and voucher information for all of the individuals sequenced. Data are not shown for individuals appearing in previously published papers (Guiller and Madec 2010, Hoso *et al.* 2010, Koehler 2010, Perez 2011). North Carolina Museum of Natural Sciences (NCMNS), Carnegie Museum of Natural History (CMNH), Florida Museum of Natural History, University of Florida (UF). Spelling was retained from museum labels. UF museum numbers with an A at the end indicate specimens preserved in alcohol.

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Allogona lombardii</i>		UF444775A		State Road 12 at Rye Patch Creek, Idaho Co, ID
<i>Allogona lombardii</i>		UF444797A		State Road 12 at Dipper Creek, Idaho Co, ID
<i>Allogona lombardii</i>		UF444837A		Kootenai Falls, just upstream of falls, Lincoln Co, MT
<i>Allogona profunda</i>	ND3	123554		Sherwood, Franklin Co, TN
<i>Allogona profunda</i>		UF382929A		Walls of Jericho Trail, 0.5 km NW of Clark Cemetery, Jackson Co, AL
<i>Allogona profunda</i>		UF437728A		Breaks Interstate Park, Grassy Creek Gorge, Dickenson Co, VA
<i>Allogona ptychophora</i>		UF444727A		White Bird, junction of Old Route 95 and White Bird Creek, Idaho Co, ID
<i>Allogona ptychophora</i>		UF444764A		Clearwater National Forest, Moose Creek Road 10 km E of Kelly Forks Ranger Station, Clearwater Co, ID
<i>Allogona ptychophora</i>		UF444788A		State Road 12 at Canyon Creek, Idaho Co, ID
<i>Allogona ptychophora</i>		UF444815A		Sleeping Child Road, 4 km NW of Forest Road 273, Ravalli Co, MT
<i>Amplirhagada mitchelliana</i>		Koehler 2010	GU302279	Koehler 2010
<i>Appalachina chilhoweensis</i>	ND2	123553		Campus Gulf, Van Buren Co, TN
<i>Appalachina chilhoweensis</i>		UF437672A		Happy Valley, Abrams Creek Campground, Blount Co, TN
<i>Appalachina sayana</i>		UF437720A		Hayters Gap, State Route 80, Russell Co, VA
<i>Appalachina sayana</i>		UF437733A		Breaks Interstate Park, Grassy Creek Gorge, Dickenson Co, VA
<i>Appalachina sayana</i>		UF437773A		Clear Creek Springs, abandoned railroad along Clear Creek, 1 km NE of US Route 119, Bell Co, KY
<i>Appalachina</i> sp.	1609	CMNH 101763	JX839900, JX839919	Little Mulberry Creek off of CR 37, Autauga Co, AL
<i>Ashmunella animasensis</i>	Ashanim46		AY823828	Locality not posted on GenBank
<i>Ashmunella animasensis</i>			AY823827	Locality not posted on GenBank

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Ashmunella ashmuni</i>			AY823831	Locality not posted on GenBank
<i>Ashmunella ashmuni</i>			AY823830	Locality not posted on GenBank
<i>Ashmunella auriculata</i>			EU639516	Locality not posted on GenBank
<i>Ashmunella hebaridi</i>	Ashmear6		AY823799	Locality not posted on GenBank
<i>Ashmunella hebaridi</i>	Ashmear6		AY823798	Locality not posted on GenBank
<i>Ashmunella hebaridi</i>	Ashmear6		AY823795	Locality not posted on GenBank
<i>Ashmunella hebaridi</i>			AY823801	Locality not posted on GenBank
<i>Ashmunella hebaridi</i>			AY823800	Locality not posted on GenBank
<i>Ashmunella hebaridi</i>			AY823796	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashmear6		AY823805	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashheb18		AY823790	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashmear2		AY823810	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashmear6	1490	AY823809	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashmear6		AY823791	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashmear6		AY823787	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>	Ashmear6		AY823789	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>			AY823803	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>			AY823826	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>			AY823807	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>			AY823804	Locality not posted on GenBank
<i>Ashmunella mearnsii</i>			AY823788	Locality not posted on GenBank
<i>Ashmunella organensis</i>			EU639540	Locality not posted on GenBank
<i>Ashmunella organensis</i>			EU639553	Locality not posted on GenBank
<i>Ashmunella organensis</i>			EU639554	Locality not posted on GenBank
<i>Ashmunella pseudodontata</i>			EU639542	Locality not posted on GenBank
<i>Ashmunella pseudodontata</i>			EU639543	Locality not posted on GenBank
<i>Ashmunella rhyssa</i>			EU639525	Locality not posted on GenBank
<i>Ashmunella rhyssa</i>			EU639524	Locality not posted on GenBank
<i>Ashmunella rhyssa</i>			EU639526	Locality not posted on GenBank
<i>Ashmunella</i> sp.	Ashasmun48			Locality not posted on GenBank

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Ashmunella todseni</i>			EU639538	Locality not posted on GenBank
<i>Ashmunella todseni</i>			EU639547	Locality not posted on GenBank
<i>Ashmunella todseni</i>			EU639549	Locality not posted on GenBank
<i>Carinotrachia admirale</i>		Koehler 2010	GU302298	Koehler 2010
<i>Cornu aspersum</i>		Guiller and Madec 2010	EU912610	Guiller and Madec 2010
<i>Cryptomastix devia</i>		UF448673A		Seattle, Discovery Park, King Co, WA
<i>Cryptomastix germana</i>	ND69	123593		Millersylvania State Park, Thurston Co, WA
<i>Cryptomastix mullani hemphilli</i>		UF444777A		State Road 12 at Rye Patch Creek, Idaho Co, ID
<i>Cryptomastix mullani hemphilli</i>		UF444789A		State Road 12 at Canyon Creek, Idaho Co, ID
<i>Cryptomastix mullani latilabris</i>		UF444730A		White Bird, junction of Old Route 95 and White Bird Creek, Idaho Co, ID
<i>Cryptomastix mullani latilabris</i>		UF444740A		John Day Road, just E of junction with Route 95, 5 km S of Lucile, Idaho Co, ID
<i>Cryptomastix magnidentata</i>		UF444790A		State Road 12 at Canyon Creek, Idaho Co, ID
<i>Cryptomastix magnidentata</i>		UF444799A		State Road 12 at Dipper Creek, Idaho Co, ID
<i>Cryptomastix mullani</i>		UF444778A		State Road 12 at Rye Patch Creek, Idaho Co, ID
<i>Cryptomastix mullani</i>		UF444791A		State Road 12 at Canyon Creek, Idaho Co, ID
<i>Cryptomastix mullani</i>		UF444806A		Bitterroot National Forest, Forest Road 49 at junction with Forest Road 5720, Ravalli Co, MT
<i>Cryptomastix mullani olneyae</i>		UF444746A		W bank of the South Fork of the Clearwater River, 8 km S of Stites, Idaho Co, ID
<i>Cryptomastix mullani olneyae</i>		UF444838A		Kootenai Falls, just upstream of falls, Lincoln Co, MT
<i>Cryptomastix mullani tuckeri</i>		UF444807A		Bitterroot National Forest, Forest Road 49 at junction with Forest Road 5720, Ravalli Co, MT
<i>Daedalochila auriculata</i>	ND91	123609		Otter Creek, Levy Co, FL
<i>Daedalochila auriculata</i>		UF400564A		Lochloosa, Lochloosa Boat Ramp, Cypress Lake Edge, Alachua Co, FL
<i>Daedalochila auriculata</i>		UF401262A		Devils Hammock, State Route 24 at Wacasassa River Crossing, Levy Co, FL

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Daedalochoila auriculata</i>		UF425854A		Picolata, just S of County Road 208, 4.2 mi E of State Road 13, St. Johns Co, FL
<i>Daedalochoila auriculata</i>		UF435101a		Lochloosa, Lochloosa Boat Ramp, Alachua Co, FL
<i>Daedalochoila auriculata</i>		UF435105a		Devil's Hammock, junction of State Route 24 and Waccasassa River, Levy Co, FL
<i>Daedalochoila auriculata</i>		UF444858A		Paynes Prairie, Alachua Sink Trail, near Alachua Sink, Alachua Co, FL
<i>Daedalochoila auriculata</i>		UF448558A		County Road 476, 6.9 mi W of US Highway 301 (Bushnell), W side of road, Sumter Co, FL
<i>Daedalochoila auriculata</i>		UF449215A		US Route 19, 0.3 miles N of State Route 24, right bank of Otter Creek, Levy Co, FL
<i>Daedalochoila avara</i>		UF400851A		Lochloosa, Lochloosa Boat Ramp, Cypress Lake Edge, Alachua Co, FL
<i>Daedalochoila avara</i>		UF400851B		Lochloosa, Lochloosa Boat Ramp, Cypress Lake Edge, Alachua Co, FL
<i>Daedalochoila avara</i>		UF400851C		Lochloosa, Lochloosa Boat Ramp, Cypress Lake Edge, Alachua Co, FL
<i>Daedalochoila avara</i>		UF400851D		Lochloosa, Lochloosa Boat Ramp, Cypress Lake Edge, Alachua Co, FL
<i>Daedalochoila avara</i>		UF400851E		Lochloosa, Lochloosa Boat Ramp, Cypress Lake Edge, Alachua Co, FL
<i>Daedalochoila avara</i>		UF435100A		Lochloosa, Lochloosa Boat Ramp, Alachua Co, FL
<i>Daedalochoila uvulifera bicornuta</i>		UF400565A		Gulf Hammock, County Route 362 at Mule Creek Crossing, Levy Co, FL
<i>Daedalochoila uvulifera bicornuta</i>		UF425844A		9.4 mi NE of Aripeka, E side of County Road 597, 0.2 mi S of State Road 50, Hernando Co, FL
<i>Daedalochoila uvulifera bicornuta</i>		UF435001A		Stumpknockers Restaurant, County Road 200, at the Withlacoochee River, Marion Co, FL
<i>Daedalochoila uvulifera bicornuta</i>		UF435102a		Gulf Hammock, junction of County Road 326 and Mule Creek, Levy Co, FL
<i>Daedalochoila uvulifera bicornuta</i>		UF436377A		Sumterville, County Road 475, 5 km S of County Road 470, Sumter Co, FL
<i>Daedalochoila uvulifera bicornuta</i>		UF444855A		3 km S of Bushnell, Sumter Co, FL

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Daedalochila uvulifera bicornuta</i>		UF446370A		Gulf Hammock, Post Office at junction of US Highway 19/98 and County Road 326, Levy Co, FL
<i>Daedalochila uvulifera bicornuta</i>		UF448557A		Sumterville, County Road 475, 3.1 mi S of County Road 470, E side of road, Sumter Co, FL
<i>Daedalochila uvulifera bicornuta</i>		UF448563A		Lake Panasoffkee, County Road 470, 0.4 mi SSE Marsh Bend Outlet, W side of road at culvert, Sumter Co, FL
<i>Daedalochila delecta</i>		UF435009A		Paynes Prairie, south entrance, Alachua Co, FL
<i>Daedalochila delecta</i>		UF444854A		Paynes Prairie State Preserve, Bolens Bluff Trail, Alachua Co, FL
<i>Daedalochila delecta</i>		UF446366A		Otter Creek, junction of US Highway 19/98 and State Road 24, Levy Co, FL
<i>Daedalochila delecta</i>		UF449216A		US Route 19, 4.8 miles S of State Route 24, right bank of Otter Creek, Levy Co, FL
<i>Daedalochila hausmani</i>		UF391090A		US Route 98, 2.2 km W of Hells Half Acre Road, Wakulla Co, FL
<i>Daedalochila hausmani</i>		UF400821A		Pumpkin Swamp, US Route 98, 2 km WNW of State Route 358, Dixie Co, FL
<i>Daedalochila hausmani</i>		UF400821B		Pumpkin Swamp, US Route 98, 2 km WNW of State Route 358, Dixie Co, FL
<i>Daedalochila hausmani</i>		UF435165A		County Road 14, 8 km NE of State Route 55, Madison Co, FL
<i>Daedalochila hausmani</i>		UF435165b		County Road 14, 8 km NE of State Route 55, Madison Co, FL
<i>Daedalochila hausmani</i>		UF436376A		Jacksonville, Imeson Road, 0.3 km N of Commonwealth Avenue, Duval Co, FL
<i>Daedalochila postelliana</i>		UF437629A		Francis Marion National Forest, Forest Route 212 ca. 4 km E of Honey Hill, Charleston Co, SC
<i>Daedalochila postelliana</i>		UF447031A		Francis Marion National Forest, Forest Route 212 at tributary of Wambaw Creek, Berkeley Co, SC
<i>Daedalochila postelliana</i>		UF447038A		Francis Marion National Forest, Forest Route 212, E of Honey Hill, Berkeley Co, SC
<i>Daedalochila subclausa</i>	ND102	123620		Jacksonville, Duval Co, FL

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Daedalochila subclausa</i>	ND96	123614		Otter Creek, Levy Co, FL
<i>Daedalochila subclausa</i>		UF435006A		US Route 98 at Enconfina River Boat Ramp, 2.8 km W of Cow Creek Road, Taylor Co, FL
<i>Daedalochila subclausa</i>		UF435006B		US Route 98 at Enconfina River Boat Ramp, 2.8 km W of Cow Creek Road, Taylor Co, FL
<i>Daedalochila subclausa</i>		UF435007A		US Route 98, 3.5 km W of Aucilla River, Jefferson Co, FL
<i>Daedalochila subclausa</i>		UF435007B		US Route 98, 3.5 km W of Aucilla River, Jefferson Co, FL
<i>Daedalochila subclausa</i>		UF435166a		W side of County Road 13A, 1.1 km N of Gracewood Lane, St. Johns Co, FL
<i>Daedalochila subclausa</i>		UF448565A		Tate's Hell Wildlife Management Area, Buck Siding Road, 0.01 mi E bridge at intersection with Car Body Road, Franklin Co, FL
<i>Daedalochila uvulifera</i>	ND104	123622		MorocCo. Shrine, Duval Co, FL
<i>Daedalochila uvulifera</i>		UF400804A		Sanibel, 0.4 mi E of Lindgren, Just S of Campus Turf, Sandalfoot Condos, 671 E Gulf Drive, Lee Co, FL
<i>Daedalochila uvulifera</i>		UF425845A		Lake City, Lake City Municipal Airport, S side of US Highway 90, 0.3 mi E of County Road 245, Columbia Co, FL
<i>Daedalochila uvulifera</i>		UF426774A		Canaveral Peninsula, Canaveral Air Force Station/Kennedy Space Center, both sides of Lighthouse Road, just NW launch pad number 17, Brevard Co, FL
<i>Daedalochila uvulifera</i>		UF434998A		Nine Mile Road at St. Marks Pond Boulevard, 1.3 km SSW of US Highway 1, St. Johns Co, FL
<i>Daedalochila uvulifera</i>		UF435031a		Long Pine Key, National Key Deer Refuge, Blue Hole, Monroe Co, FL
<i>Daedalochila uvulifera</i>		UF435170a		Oceanway, 11800 block of Alta Drive, just N of Donato Drive, just SE of Rushing Branch, Duval Co, FL

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Daedalochila uvulifera</i>		UF435171a		Sanibel Island, Sandalfoot Condominiums, East Gulf Drive, 0.5 km SSW of Lighthouse Road, Lee Co, FL
<i>Daedalochila uvulifera</i>		UF444662A		Jacksonville, St. Johns Industrial Parkway, pond behind Ameritape Building, Duval Co, FL
<i>Euchemotrema fraternum</i>	795REufrat	NCMNS 41438-2	JX839911	Nantahala National Forest, Horse Cove Campground near campsite 13 and surrounding woods along road, Graham Co, NC
<i>Euchemotrema fraternum</i>	ND76	123597		Efland RR track, Orange Co, NC
<i>Euchemotrema fraternum</i>		UF447262A		Chestatee River bank, 4.5 km SSW of Dahlonga, Lumpkin Co, GA
<i>Euchemotrema hubrichti</i>	ND38	missing		Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317294	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317297	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317298	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317299	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317295	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317300	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317301	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			DQ317296	Larue-Pine Hills RNA, Union Co, IL
<i>Euchemotrema hubrichti</i>			AY769091	Larue-Pine Hills RNA, Union Co, IL
<i>Fumonelix clingmanicus</i>		UF446490A		Great Smoky Mountains National Park, heading towards Georgia along Appalachian Trail from Clingmans Dome, approximately 250 m north off AT, Sevier Co, TN
<i>Fumonelix roanensis</i>	ND71	missing		Mt. Mitchell, Yancey Co, NC
<i>Fumonelix roanensis</i>	ND81	missing		Mt. Mitchell, Yancey Co, NC
<i>Fumonelix wheatleyi</i>		UF446585A		Great Smoky Mountains National Park, off Snake Den Trail, Cocke Co, TN
<i>Inflectarius inflectus</i>	ND10	missing	missing	Cedar Creek, White Co, TN
<i>Inflectarius inflectus</i>	ND15	123564		Sherwood, Franklin Co, TN
<i>Inflectarius inflectus</i>	ND29	123575		Sligo Bridge, DeKalb Co, TN

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Inflectarius infectus</i>	ND32	123578		Sherwood, Franklin Co, TN
<i>Inflectarius infectus</i>	ND34	123580		Walls of Jericho Wildlife Management Area, Polly Anne Spring, 1.4 km W of Jericho, Jackson Co, AL
<i>Inflectarius infectus</i>		UF381668A		State Route 35, 1 km SW of Woodville, Jackson Co, AL
<i>Inflectarius infectus</i>		UF447197A		Jackson Co, AL
<i>Inflectarius infectus</i>		UF447261A		Chestatee River bank, 4.5 km SSW of Dahlonega, Lumpkin Co, GA
<i>Inflectarius infectus</i>		UF448790A		Elkin, junction of Route 268 and Little Bend Trail, Surry Co, NC
<i>Inflectarius rugeli</i>	ND21	missing		Rainbow Falls Trailhead, Rutherford Co, NC
<i>Inflectarius rugeli</i>		UF382880A		County Route 171, 0.2 km E of County Route 170, Jackson Co, AL
<i>Inflectarius rugeli</i>		UF382891A		Rock Island State Park, Blue Hole, Warren Co, TN
<i>Inflectarius rugeli</i>		UF382961A		County Route 170, 0.7 km N of County Route 171, Jackson Co, AL
<i>Inflectarius rugeli</i>		UF437645A		State Route 348 at Hogpen Gap, White Co, GA
<i>Inflectarius rugeli</i>		UF437697A		Old US Highway 70, 5.9 km E of Sparta, White Co, TN
<i>Inflectarius rugeli</i>		UF437725A		Hayters Gap, State Route 80, Russell Co, VA
<i>Inflectarius rugeli</i>		UF447188A		County Road 298, 0.5 km SW of County Road 98, Jackson Co, AL
<i>Inflectarius rugeli</i>		UF447254A		Blood Mountain, Byron Reese Trail, 15.5 km SSE of Blairsville, Union Co, GA
<i>Inflectarius smithi</i>		UF379009A		Walls of Jericho Wildlife Management Area, Polly Anne Spring, 1.4 km W of Jericho, Jackson Co, AL
<i>Inflectarius smithi</i>		UF382934A		Walls of Jericho Trail, 0.5 km NW of Clark Cemetery, Jackson Co, AL
<i>Inflectarius smithi</i>		UF383029A		Lake Summit, Lake Summit Road at Small Creek and Ravine on S Shore, Henderson Co, NC
<i>Inflectarius subpalliatius</i>		UF288280A		W Slope of Bald Mountain, 2 mi SSW of Spivey Gap, Yancey Co, NC

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Linisa tamaulipasensis</i>		Perez 2011	DQ086070	Perez 2011
<i>Loboscutum pustuloides</i>		UF437814A		Milton, Tomahawk Landing Road at Coldwater Creek, Santa Rosa Co, FL
<i>Mesodon altivagus</i>		UF446565A		Great Smoky Mountains National Park, between Appalachian Trail and Boulevard Trail, Sevier Co, TN
<i>Mesodon andrewsae</i>	ND83	123602		Mt. Mitchell, Yancey Co, NC
<i>Mesodon clausus</i>		UF437756A		US Route 119, 0.3 km WSW of State Route 2010, Harlan Co, KY
<i>Mesodon elevatus</i>	ND5	123556		Sherwood, Franklin Co, TN
<i>Mesodon elevatus</i>		UF360273A		W Bank of Battle Creek at Junction of Ladds Cove Road and Interstate 24, Marion Co, TN
<i>Mesodon elevatus</i>		UF447192A		County Road 298, 0.5 km SW of County Road 98, Jackson Co, AL
<i>Mesodon mitchellianus</i>		UF447126A		State Route 16, 8.5 km E of Marion, Smyth Co, VA
<i>Mesodon normalis</i>	1593	CMNH 101732	JX839894, JX839913	Sipsey wilderness, Bankhead national forest, Winston Co. AL
<i>Mesodon normalis</i>	ND20	missing		Purchase Knob, Haywood Co, NC
<i>Mesodon normalis</i>	ND82	123601		Big Flat Gap area, Graham Co, NC
<i>Mesodon normalis</i>	ND90	123608		South Mt. State Park, Burke Co, NC
<i>Mesodon normalis</i>		UF383016A		Nantahala Gorge, N of Beechertown Parking Lot, Macon Co, NC
<i>Mesodon normalis</i>		UF383026A		Lake Summit, Lake Summit Road at Small Creek and Ravine on S Shore, Henderson Co, NC
<i>Mesodon normalis</i>		UF437674A		Brasstown Bald, stone wall along abandoned path below observatory, Towns Co, GA
<i>Mesodon normalis</i>		UF437710A		Ocoee Gorge, US Route 64 at Goforth Creek, Polk Co, TN
<i>Mesodon normalis</i>		UF446510A		Great Smoky Mountains National Park, at bottom of Andrew's Bald by trail sign, Swain Co, NC
<i>Mesodon normalis</i>		UF446679A		Great Smoky Mountains National Park, off 441, had to cross river, Sevier Co, TN

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Mesodon normalis</i>		UF447118A		Comers Rock Recreation Area, Camping Site, Grayson Co, VA
<i>Mesodon thyroideus</i>	1608Mesthyr	CMNH 101763	JX839899, JX839918	Little Mulberry Creek, Autaga Co, AL off of CR 37, 10Sept01, KEP, JS
<i>Mesodon thyroideus</i>	1611Mesthyr	CMNH 101765	JX839901, JX839920	Oakmulgee Creek, Bibb Co, AL land next to creek at Hwy 82 bridge, 12Sept01, KEP, JS
<i>Mesodon thyroideus</i>	1613Mesthyr	CMNH 101757	JX839902, JX839921	Flower garden @ 13 Windsor Dr., Tuscaloosa, Tuscaloosa Co, AL, 30Sept2001, S. McGregor coll.
<i>Mesodon thyroideus</i>	1614Mesthyr	CMNH 101760	JX839903, JX839922	McKinley Avenue, Florence, Lauderdale Co, AL, 14Oct01, S. McGregor
<i>Mesodon thyroideus</i>	ND13	123562		Sherwood, Franklin Co, TN
<i>Mesodon thyroideus</i>	ND14	123563		Sherwood, Franklin Co, TN
<i>Mesodon thyroideus</i>	ND22	123568		Sherwood, Franklin Co, TN
<i>Mesodon thyroideus</i>	ND94	123612		
<i>Mesodon thyroideus</i>		UF437699A		Parking lot, Lexington Co, SC
<i>Mesodon thyroideus</i>		UF447151A		Old US Highway 70, 5.9 km E of Sparta, White Co, TN
<i>Mesodon thyroideus</i>		UF447199A		Breaks Interstate Park, Grassy Creek Trail, Dickinson Co, VA
<i>Mesodon zaletus</i>	604	CM123478	JX839907	State Route 35, 1 km SW of Woodville, Jackson Co, AL
<i>Mesodon zaletus</i>	605	CM123479	JX839908	Along Road on steep bluff, along SR117 3rd mi S Tennessee River, Sand Mtn, Jackson Co, AL
<i>Mesodon zaletus</i>	1598	CMNH 101738	JX839896, JX839915	Along Road on steep bluff, along SR117 3rd mi S Tennessee River, Sand Mtn, Jackson Co, AL
<i>Mesodon zaletus</i>	1603	CMNH 101753	JX839897, JX839916	WV1, Pocahontas Co, WV
<i>Mesodon zaletus</i>	ND4	123555		WV1, Pocahontas Co, WV
<i>Mesodon zaletus</i>		UF382970A		Sherwood, Franklin Co, TN
<i>Mesodon zaletus</i>		UF437673A		Walls of Jericho Trail, 1 km W of Jericho, Jackson Co, AL
<i>Mesodon zaletus</i>		UF437721A		Happy Valley, Abrams Creek Campground, Blount Co, TN
<i>Mesodon zaletus</i>				Hayters Gap, State Route 80, Russell Co, VA

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Mesodon zaletus</i>		UF447145A		Breaks Interstate Park, Grassy Creek Trail, Dickinson Co, VA
<i>Mesodon zaletus</i>		UF447247A		Battle Creek, Ladds Cove Road, 0.5 km NNW of Battle Creek Road, Marion Co, TN
<i>Millerelix mooreana</i>		Perez 2011	DQ086084	N bank of Guadalupe River, 3.3 miles WNW of jct of loop 337 and River Road in New Braunfels, Comal Co. TX
<i>Millerelix plicata</i>		UF379013A		Walls of Jericho Wildlife Management Area, Polly Anne Spring, 1.4 km W of Jericho, Jackson Co, AL
<i>Neohelix albolabris</i>	595Neoalbo	CM123476	JX839905, JX839924	N. of Lake, Crowder's Mountain State Park, Gaston Co, NC
<i>Neohelix albolabris</i>	596Neoalbo	CM123477	JX839906	N. of Lake, Crowder's Mountain State Park, Gaston Co, NC
<i>Neohelix albolabris</i>	972Neoalbo	NCMNS 41248-0	JX839912	Wildlife viewing area, approximately 3 miles S of Yanceyville and 0.5 miles W of Highway 62, Caswell Co, NC
<i>Neohelix albolabris</i>	ND30	123576		Mt. Sano, Madison Co, AL
<i>Neohelix albolabris</i>	ND66	123590		Mt. Holly, Homestead Dr., Gaston Co, NC
<i>Neohelix albolabris</i>		UF437752A		State Route 80, 3 km E of Elkhorn City, Pike Co, KY
<i>Neohelix albolabris</i>		UF447119A		Comers Rock Recreation Area, Camping Site, Grayson Co, VA
<i>Neohelix alleni</i>	ND1	missing	missing	Cedar Creek, White Co, TN
<i>Neohelix dentifera</i>		UF447137A		Route 603, 0.5 km SW of Beaver Pond, Smyth Co, VA
<i>Neohelix alleni fuscolabris</i>		UF382965A		Walls of Jericho Trail, 1 km W of Jericho, Jackson Co, AL
<i>Neohelix alleni fuscolabris</i>		UF447221A		State Route 79, 6.8 km N of Hytop, Jackson Co, AL
<i>Neohelix major</i>	ND63	123587		Fort Mill Residence, York Co, SC
<i>Neohelix solemi</i>	ND74	123595		McConnells Landing, Berkeley Co, SC
<i>Neohelix solemi</i>		UF447056A		Great Lake at end of Great Lake Road, Craven Co, NC
<i>Neohelix solemi</i>		UF447074A		Ocracoke Island, Hammock Trail NW of airport, Hyde Co, NC

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Patara</i>		UF448706A		Cayce, Thomas Newman Boat Ramp on the Congaree River, Lexington Co, SC
<i>Patara</i>		UF447200A		State Route 35, 1 km SW of Woodville, Jackson Co, AL
<i>Patara</i>		UF437669A		Spoilane Creek floodplain along State Road 75, 7 km N of Helen, White Co, GA
<i>Polygyra</i>	332	Perez 2011	DQ086086, DQ086001	NE side of Denny Conference Building Southwest foundation for Biomedical research, San Antonio, Bexar Co. Kingsland Wood, Camden Co, GA
<i>Polygyra</i>		123611		
<i>Polygyra</i>	ND93	UF435035a		Long Pine Key, National Key Deer Refuge, Blue Hole, Monroe Co, FL
<i>Polygyra</i>		UF435046a		Fat Deer Key, Curry Hammock State Park, Monroe Co, FL
<i>Polygyra</i>		UF437829A		Big Pine Key, Monroe Co, FL
<i>Polygyra</i>		UF437832A		Biscayne Bay National Park, Elliot Key, Spite Road near park dock, Dade Co, FL
<i>Polygyra</i>		UF445016A		Hesperides, junction Boy Scout Camp Road and State Route 60, Polk Co, FL
<i>Polygyra</i>		UF447054A		Cedar Point, Cedar Point Tideland Trail, Carteret Co, NC
<i>Polygyra</i>	265	Perez 2011	DQ086069, DQ085984	Hillsborough State Park, Polk Co, FL
<i>Polygyra</i>	1594	CMNH 101733	JX839895, JX839914	Plantation Key off of HW1, Monroe Co, FL, MRRM 87, 4/10/03 KEP
<i>Polygyra</i>	ND92	123610		Otter Creek, Levy Co, FL
<i>Polygyra</i>	ND99	123617		Kingsland:wood, Camden Co, GA
<i>Polygyra</i>		UF391091A		US Route 98, 2.2 km W of Hells Half Acre Road, Wakulla Co, FL
<i>Polygyra</i>		UF435103a		Gulf Hammock, junction of County Road 326 and Mule Creek, Levy Co, FL
<i>Polygyra</i>		UF437631A		Francis Marion National Forest, Forest Route 212 ca. 4 km E of Honey Hill, Charleston Co, SC
<i>Polygyra</i>		UF444856A		3 km S of Bushnell, Sumter Co, FL
<i>Praticolella</i>		Perez 2011	DQ086088	Perez 2011

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Patara appressa</i>		UF437676A		Happy Valley, Abrams Creek Campground, Blount Co, TN
<i>Patara appressa</i>		UF437722A		Hayters Gap, State Route 80, Russell Co, VA
<i>Patara appressa</i>		UF437757A		US Route 119, 0.3 km WSW of State Route 2010, Harlan Co, KY
<i>Patara appressa</i>	ND6	missing		Cedar Creek, White Co, TN
<i>Patara clarki</i>		UF437643A		State Route 348 at Hogpen Gap, White Co, GA
<i>Patara clarki</i>		UF437662A		Spoilane Creek floodplain along State Road 75, 7 km N of Helen, White Co, GA
<i>Patara clarki</i>		UF437713A		Brasstown Bald, stone wall along abandoned path below observatory, Towns Co, GA
<i>Patara clarki</i>		UF446639A		Great Smoky Mountains National Park, off Bull Head Trail, Sevier Co, TN
<i>Patara clarki</i>		UF447250A		Blood Mountain, Byron Reese Trail, 15.5 km SSE of Blairsville, Union Co, GA
<i>Patara laevior</i>		UF437703A		Old US Highway 70, 5.9 km E of Sparta, White Co, TN
<i>Patara perigrapta</i>	ND16	123565		Beersheba, Grundy Co, TN
<i>Patara perigrapta</i>	ND78	123599		South Mt. State Park, Burke Co, NC
<i>Patara perigrapta</i>	ND86	123605		Riverbanks Zoo, Richland Co, SC
<i>Patara perigrapta</i>		UF382963A		County Route 170, 0.7 km N of County Route 171, Jackson Co, AL
<i>Patara perigrapta</i>		UF382967A		Walls of Jericho Trail, 1 km W of Jerichoo, Jackson Co, AL
<i>Patara perigrapta</i>		UF434335a		Carter Mountain Road, 2 km SW of Betsy's Gap, Haywood Co, FL
<i>Patara perigrapta</i>		UF434344a		Unaka Springs, cold air slope 1 km W of town, Unicoi Co, TN
<i>Patara perigrapta</i>		UF437668A		Spoilane Creek floodplain along State Road 75, 7 km N of Helen, White Co, GA
<i>Patara perigrapta</i>		UF437677A		Happy Valley, Abrams Creek Campground, Blount Co, TN
<i>Patara perigrapta</i>		UF437742A		Ocoee Gorge, US Route 64 at powerplant, Polk Co, TN
<i>Patara perigrapta</i>		UF447175A		4 mile Road, 1.6 km NE of Ford, Clark Co, KY

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Praticolella berlandieriana</i>		Perez 2011	DQ086076	Perez 2011
<i>Praticolella berlandieriana</i>		Perez 2011	DQ086077	Perez 2011
<i>Praticolella berlandieriana</i>		Perez 2011	DQ086033	Perez 2011
<i>Praticolella candida</i>		Perez 2011	DQ086078	Perez 2011
<i>Praticolella candida</i>		Perez 2011	DQ086042	Perez 2011
<i>Praticolella candida</i>		Perez 2011	DQ086073	Perez 2011
<i>Praticolella candida</i>		Perez 2011	DQ086071	Perez 2011
<i>Praticolella flavescens</i>		Perez 2011	DQ086034	Perez 2011
<i>Praticolella flavescens</i>		Perez 2011	DQ086065	Perez 2011
<i>Praticolella flavescens</i>		Perez 2011	DQ086049	Perez 2011
<i>Praticolella flavescens</i>		Perez 2011	DQ086087	Perez 2011
<i>Praticolella flavescens</i>		Perez 2011	DQ086063	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086050	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086062	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086036	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086037	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086052	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086041	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086038	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086095	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086025	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086027	Perez 2011
<i>Praticolella griseola</i>		Perez 2011	DQ086026	Perez 2011
<i>Praticolella griseola</i>		UF445017A		Hesperides, junction Boy Scout Camp Road and State Route 60, Polk Co, FL
<i>Praticolella jejuna</i>		UF281388	DQ086093, DQ086008	St. Catherine Island, Liberty Co, GA
<i>Praticolella jejuna</i>		UF444857A		Bushnell, Lincoln Park, Sumter Co, FL
<i>Praticolella martensiana</i>		UF2837965	DQ086016	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086057	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ085982, DQ086067	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086046, DQ085961	Perez 2011

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Stenotrema altispira</i>	ND79	123600		Mt. Mitchell, Yancey Co, NC
<i>Stenotrema altispira</i>		UF434325a		Big Ivy, Forest Route 74, 2 km NW Douglas Falls, Buncombe Co, NC
<i>Stenotrema altispira</i>		UF434333a		Carter Mountain Road, 2 km SW of Betsy's Gap, Haywood Co, FL
<i>Stenotrema barbigerum</i>		UF383002A		Ocoee Gorge, Goforth Creek Trail near Parking Lot, Polk Co, TN
<i>Stenotrema barbigerum</i>		UF437667A		Spoilcane Creek floodplain along State Road 75, 7 km N of Helen, White Co, GA
<i>Stenotrema barbigerum</i>		UF437686A		Ocoee Gorge, US Route 64 at Goforth Creek, Polk Co, TN
<i>Stenotrema deceptum</i>		UF382883A		County Route 171, 0.2 km E of County Route 170, Jackson Co, AL
<i>Stenotrema deceptum</i>		UF382978A		Walls of Jericho Trail, 1 km W of Jericho, Jackson Co, AL
<i>Stenotrema depilatum</i>		UF446407A		Mount Kephart, 0.7 km S of Icewater Spring Cabin, Swain Co, NC
<i>Stenotrema depilatum</i>		UF446507A		Great Smoky Mountains National Park, approx. 100m off trail, near Clingmans Dome parking lot, Swain Co, NC
<i>Stenotrema depilatum</i>		UF446646A		Great Smoky Mountains National Park, 150m downhill from Clingmans Dome road, Swain Co, NC
<i>Stenotrema edwardsi</i>		UF437724A		Hayters Gap, State Route 80, Russell Co, VA
<i>Stenotrema edwardsi</i>		UF437769A		Damascus, S bank of Laurel Creek at intersection with S Greenway Avenue, Washington Co, VA
<i>Stenotrema edwardsi</i>		UF447159A		Pine Mountain, County Route 1679, 1.4 km W of US Route 119, Letcher Co, KY
<i>Stenotrema exodon</i>	ND31	123577		Gurley Mt, Madison, AL
<i>Stenotrema exodon</i>		UF379011A		Walls of Jericho Wildlife Management Area, Polly Anne Spring, 1.4 km W of Jericho, Jackson Co, AL
<i>Stenotrema exodon</i>		UF382939A		McCoy Mountain, State Road 35, 2 km W of Lim Rock, Jackson Co, AL
<i>Stenotrema hirsutum</i>		UF447088A		Shenandoah National Park, Little Stone Man Parking, Madison Co, VA

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Praticolella mexicana</i>		Perez 2011	DQ086083	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086043	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086040	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086080	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086035	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086059	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086039	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086046	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086044	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086045	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086031	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086047	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086067	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086085	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086030	Perez 2011
<i>Praticolella mexicana</i>		Perez 2011	DQ086079	Perez 2011
<i>Praticolella mobiliana</i>		UF444663A		Jacksonville, Imeson Road at Pritchard Road, Duval Co, FL
<i>Praticolella pachyloma</i>		Perez 2011	DQ086089	Perez 2011
<i>Praticolella pachyloma</i>		Perez 2011	DQ086028	Perez 2011
<i>Praticolella</i> sp.		UF254639	DQ086032, DQ085946	Perez 2011
<i>Praticolella</i> sp. (405)		Perez 2011	DQ085986, DQ086071	19 km E of Soto de la Marina, TMP, MEX
<i>Praticolella taeniata</i>		Perez 2011	DQ086058	Perez 2011
<i>Praticolella taeniata</i>		Perez 2011	DQ086066	Perez 2011
<i>Praticolella taeniata</i>		Perez 2011	DQ086056	Perez 2011
<i>Praticolella taeniata</i>		Perez 2011	DQ086092	Perez 2011
<i>Praticolella trimatris</i>		Perez 2011	DQ086074	Perez 2011
<i>Praticolella trimatris</i>		Perez 2011	DQ086075	Perez 2011
<i>Praticolella mexicana</i>	ND101	123619		Jacksonville, Regency Square Blvd., Duval Co, FL
<i>Satsuma jacobii</i>		Hoso <i>et al.</i> 2010	AB480898	Hoso <i>et al.</i> 2010

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Stenotrema</i>		UF447102A		Woodstock, County Road 663 at creek, Shenandoah Co, VA
<i>Stenotrema</i>		UF448746A		US Route 250 at summit of Lantz Mountain, 2.5 km W of Hightown, Highland Co, VA
<i>Stenotrema</i>		UF383041A		Ocoee Gorge, Frog Mountain Wilderness Trail Head, Polk Co, TN
<i>Stenotrema</i>		UF434332a		Carter Mountain Road, 2 km SW of Betsy's Gap, Haywood Co, FL
<i>Stenotrema</i>		UF382936A		McCoy Mountain, State Road 35, 2 km W of Lim Rock, Jackson Co, AL
<i>Stenotrema</i>	ND28	123574		Sligo Bridge, DeKalb Co, TN
<i>Stenotrema</i>	ND33	123579		Sherwood, Franklin Co, TN
<i>Stenotrema</i>		UF382937A		McCoy Mountain, State Road 35, 2 km W of Lim Rock, Jackson Co, AL
<i>Stenotrema</i>		UF437763A		N
<i>Stenotrema</i>		UF447160A		Pine Mountain, County Route 1679, 1.4 km W of US Route 119, Letcher Co, KY
<i>Stenotrema</i>		UF379015A		Walls of Jericho Wildlife Management Area, Polly Anne Spring, 1.4 km W of Jericho, Jackson Co, AL
<i>Stenotrema</i>		UF382979A		Walls of Jericho Trail, 1 km W of Jericho, Jackson Co, AL
<i>Trilobopsis</i>		UF446022A		Auburn, State Route 49 at American River Confluence, El Dorado Co, CA
<i>Trilobopsis</i>		UF446022B		Auburn, State Route 49 at American River Confluence, El Dorado Co, CA
<i>Trilobopsis</i>		UF448635A		Redwood Drive, 1.7 km NNE of Redway, Humboldt Co, CA
<i>Triodopsis</i>	ND64	123588		Bessemer City, Jason Court Cul-de-sac, Gaston Co, NC
<i>Triodopsis</i>	ND87	123606		Junction at W. Morehead ST. and S. Summit Ave, Mecklenburg Co, NC
<i>Triodopsis</i>	ND97	123615		Durham, near lake in Woodlake subdivision, Durham Co, NC
<i>Triodopsis</i>		UF448708A		Cayce, Thomas Newman Boat Ramp on the Congaree River, Lexington Co, SC

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Triodopsis alabamensis</i>	1137Talab	NCMNS 42096-0	JX839892	Near junction Interstate Highway 26 and SC 453, Harleyville exit along Railroad tracks and road S and W of Interstate, Dorchester Co, SC.
<i>Triodopsis anteridon</i>		UF437759A		US Route 119, 0.3 km WSW of State Route 2010, Harlan Co, KY
<i>Triodopsis burchi</i>	ND65	123589		Charlotte, Reedy Creek Nature Preserve, Rocky River Rd, Mecklenburg Co, NC
<i>Triodopsis burchi</i>	ND88	123607		Beech/Maple Woods, Orange Co, NC
<i>Triodopsis fallax</i>	ND12	123561		unknown
<i>Triodopsis fallax</i>	ND68	123592		Norwood, Silver Spr Rd, Stanly Co, NC
<i>Triodopsis fallax</i>		UF448919A		Riverdale Park, Rivertech Court, 0.15 km SSW of River Road, Prince George's Co, MD
<i>Triodopsis fraudulenta</i>		UF447085A		Shenandoah National Park, Little Stone Man Parking, Madison Co, VA
<i>Triodopsis fulcidens</i>	ND77	123598		South Mt. State Park, Burke Co, NC
<i>Triodopsis hopetonensis</i>	ND95	123613		Pine Woods, Bryan Co, GA
<i>Triodopsis hopetonensis</i>		UF437630A		Francis Marion National Forest, Forest Route 212 ca. 4 km E of Honey Hill, Charleston Co, SC
<i>Triodopsis hopetonensis</i>		UF437815A		Milton, Tomahawk Landing Road at Coldwater Creek, Santa Rosa Co, FL
<i>Triodopsis hopetonensis</i>		UF437847A		Daniel Island, Charleston Co, SC
<i>Triodopsis hopetonensis</i>		UF444861A		High Springs, 1.5 km E of town, Alachua Co, FL
<i>Triodopsis hopetonensis</i>		UF447058A		Great Lake at end of Great Lake Road, Craven Co, NC
<i>Triodopsis hopetonensis</i>		UF447072A		Ocracoke Island, beach access NW of airport, Hyde Co, NC
<i>Triodopsis hopetonensis</i>		UF448678A		Seahorse Key, Levy Co, FL
<i>Triodopsis juxtidentis</i>	ND17	123566		Beersheba, Grundy Co, TN
<i>Triodopsis juxtidentis</i>	ND23	123569		
<i>Triodopsis juxtidentis</i>	ND25	123571		
<i>Triodopsis juxtidentis</i>		UF447077A		Shenandoah National Park, Stony Man Trail, Madison Co, VA

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Triodopsis juxtidentis</i>		UF448709A		Cayce, Thomas Newman Boat Ramp on the Congaree River, Lexington Co, SC near GA 144, Bryan Co, GA
<i>Triodopsis palustris</i>	ND100	123618		
<i>Triodopsis palustris</i>	ND72	missing		
<i>Triodopsis palustris</i>		UF447046A		McConnells Landing, Berkeley Co, SC State Route 905, NE of Hammond, Horry Co, SC
<i>Triodopsis soehneri</i>		UF447059A		Catfish Lake Road, SW of W Prong of Brice Creek, Craven Co, NC
<i>Triodopsis tennesseensis</i>		UF379016A		Walls of Jericho Wildlife Management Area, Polly Anne Spring, 1.4 km W of Jericho, Jackson Co, AL
<i>Triodopsis tennesseensis</i>		UF383007A		Nantahala Gorge, N of Beechertown Parking Lot, Macon Co, NC
<i>Triodopsis tridentata</i>	724Ttrid	CM123481	JX839909	Fern trail at New River State Park, Ashe Co, NC
<i>Triodopsis tridentata</i>	753Ttrid	CM123482	JX839910	242 Oak Forest Dr. Cullowee, Jackson Co, NC
<i>Triodopsis tridentata</i>	ND85	123604		South Mt. State Park, Burke Co, NC
<i>Triodopsis tridentata</i>	ND9	123559		Sherwood, Franklin Co, TN
<i>Triodopsis tridentata</i>		UF437678A		Happy Valley, Abrams Creek Campground, Blount Co, TN
<i>Triodopsis tridentata</i>		UF437736A		Breaks Interstate Park, Grassy Creek Gorge, Dickenson Co, VA
<i>Triodopsis tridentata</i>		UF447089A		Shenandoah National Park, Little Stone Man Parking, Madison Co, VA
<i>Triodopsis tridentata</i>		UF447121A		Comers Rock Recreation Area, Camping Site, Grayson Co, VA
<i>Triodopsis vannostrandii</i>	121Trioan	CM123480	DQ086021	Road to SRW quarry, Vance, Tuscaloosa Co, AL
<i>Triodopsis vannostrandii</i>	1322Tvanno	NCMNS 41867-2	JX839893	Fort Bragg, off firebreak 5, near King Road, Moore Co, NC
<i>Triodopsis vannostrandii</i>	1615Tvann	missing	JX839904, JX839923	34 Windsor Drive, Tuscaloosa, Tuscaloosa Co, AL
<i>Triodopsis vannostrandii</i>	ND98	123616		Cayce, Lexington Co, SC
<i>Triodopsis vulgata</i>	1607	CMNH 101754	JX839898, JX839917	WV1, Pocahontas Co, WV, 8/03 KEP
<i>Triodopsis vulgata</i>	ND8	123558		Cedar Creek, White Co, TN
<i>Triodopsis vulgata</i>		UF437704A		Old US Highway 70, 5.9 km E of Sparta, White Co, TN

Supplemental Table 1. (Continued)

Species Name	DNA #	Museum Number	GenBank #'s	Collection Locality
<i>Triodopsis</i>		UF447196A		State Route 35, 1 km SW of Woodville, Jackson Co, AL
<i>Vespericola columbianus</i>	ND35	missing		side of L. Sutherland, Clallam Co, WA
<i>Vespericola columbianus</i>	ND70	123594		Redwood Drive, 1.7 km NNE of Redway, Humboldt Co, CA
<i>Vespericola eritrichius</i>		UF448634A		Harris Beach, 2.8 km NW of Brookings, Curry Co, OR
<i>Vespericola megasoma</i>		UF448631A		Harris Beach, 2.8 km NW of Brookings, Curry Co, OR
<i>Vespericola megasoma</i>		UF448632A		Harris Beach, 2.8 km NW of Brookings, Curry Co, OR
<i>Vespericola megasoma</i>		UF448896A		Trinidad, bluff above Luffenholtz Beach, Humboldt Co, CA
<i>Vespericola megasoma</i>		UF448901A		Trinidad, Patrick's Point Drive at Forestry Fire Station, Humboldt Co, CA
<i>Vespericola pinicola</i>		UF446048B		Big Sur, US Route 1, 0.5 km N of Posts, Monterey Co, CA
<i>Vespericola pinicola</i>		UF446051A		Pebble Beach, Morse Botanical Reserve, Monterey Co, CA
<i>Vespericola shasta</i>		UF448881A		Fenders Ferry Road, 5 km SE of McCloud Bridge, Shasta Co, CA
<i>Xolotrema caroliniense</i>		UF448710A		Cayce, Thomas Newman Boat Ramp on the Congaree River, Lexington Co, SC
<i>Xolotrema denotatum</i>		UF437732A		Breaks Interstate Park, Grassy Creek Gorge, Dickenson Co, VA
<i>Xolotrema denotatum</i>		UF447176A		4 mile Road, 1.6 km NE of Ford, Clark Co, KY
<i>Xolotrema fosteri</i>	ND27	123573		97 Conifer Lane, Murphysboro, Jackson Co, IL
<i>Xolotrema obstrictum</i>	ND18	123567		Beersheba, Grundy Co, TN
<i>Xolotrema obstrictum</i>	ND7	123557		Sherwood, Franklin Co, TN
<i>Xolotrema obstrictum</i>		UF382884A		Rock Island State Park, Blue Hole, Warren Co, TN
<i>Xolotrema obstrictum</i>		UF382969A		Walls of Jericho Trail, 1 km W of Jericho, Jackson Co, AL