J. Moll. Stud. (2000), **66**, 233–257

A MOLECULAR PHYLOGENY OF NORTH AMERICAN PLEUROCERIDAE (GASTROPODA: CERITHIOIDEA) BASED ON MITOCHONDRIAL 16S rDNA SEQUENCES

WALLACE E. HOLZNAGEL and CHARLES LYDEARD
Biodiversity and Systematics, Department of Biological Sciences, University of Alabama, Box 870345, Tuscaloosa, Alabama 35487
(Received 26 January 1999; accepted 14 October 1999)

ABSTRACT

The Pleuroceridae Fischer, 1885, is one of three freshwater gastropod families currently recognized in the superfamily Cerithioidea Férussac, 1819 (Mollusca: Caenogastropoda Cox, 1960). Despite considerable literature justifying various proposed generic names of North American pleurocerids, no study has been conducted examining phylogenetic relationships of the recognized genera. In an effort to expand our understanding of evolutionary relationships of North American pleurocerid genrea, we examined a large portion of the mitochondrial 16S rRNA gene among 32 extant North American taxa. Multiple sequence alignment of the amplified region for our taxa resulted in a matrix consisting of 900 nucleotides including insertions and deletions. Based on analysis of nucleotide substitution patterns, we employed two approaches in our phylogenetic analysis: (1) all substitutions received equal weighting and (2) transversions were weighted 2X and 4X transitions to compensate for transition saturation among distantly related taxa. The molecular phylogeny based on the mitochondrial 16S rDNA sequences supports the monophyly of *Pleurocera* Rafinesque, 1819, *Elimia* H.&A. Adams, 1854, and *Juga* H.&A. Adams, 1854, but depicts the genera *Lithasia* Haldeman, 1840, and *Leptoxis* Rafinesque, 1819, as polyphyletic. The genus *Pleurocera* is sister to *Elimia*, which in turn is sister to a paraphyletic assemblage including representatives of *Leptoxis*, *Lithasia*, and the monotypic genus *Io* Lea, 1831. *Juga*, a genus restricted to west of the North American continental divide is the basal-most clade and is sister to all the aforementioned genera found east of the continental divide.

INTRODUCTION

The Pleuroceridae is one of three freshwater gastropod families currently recognized in the superfamily Cerithioidea (Mollusca: Caenogastropoda). The current classification scheme is largely the product of Morrison (1954), who disassembled Thiele's (1929) Melaniidae, which included all freshwater certithioidean taxa into the Melanopsidae H.&A. Adams, 1854. Thiaridae Troschel, 1857, and Pleuroceridae. This classification was based on an examination of reproductive anatomy and egg mass characteristics. Recent phylogenetic studies of cerithioideans based on limited anatomical material (Houbrick, 1988; Ponder, 1991; Glaubrecht, 1996, 1998) indicate that the three families should be treated as poorly defined, but separate evolutionary entities until further systematic work is conducted. The large heterogeneous Thiaridae (sensu Morrison, 1954) is putatively sister to the marine Planaxidae Gray, 1850, while Melanopsidae is putatively sister to Pleuroceridae (Houbrick, 1988; Ponder, 1991).

The composition of the poorly-defined Pleuroceridae is uncertain. Most malacologists agree that the Pleuroceridae includes genera from North America (Houbrick, 1988; Burch, 1980, 1982, 1988), but disagree about the placement of Meso-American, Asian, and African genera. For example, Morrison (1954) included certain African (e.g., *Potadoma* Swainson, 1840) and Asian (e.g., *Paludomus* Swainson, 1840) taxa in his concept of the Pleuroceridae, but gave no justification for doing so. Brown (1994) rejected the pleurocerid assignment of all African cerithioidean gastropods and placed them in the family Thiaridae. Starobogatov (1970) and Ponder & Warén (1988) suggested all North American taxa be referred to as Pachychilidae Troschel, 1857, operating under the assumption that the Middle American genus *Pachychilus* Lea & Lea, 1850, was closely related to North American genera. Recent morphological (Glaubrecht, Ponder, & Healy, unpublished) and preliminary molecular studies (Lydeard & Holznagel, unpublished) suggest that North American pleurocerid genera constitute a monophyletic group, and that *Pachychilus* is more closely related to some members of the Melanatriinae Thiele, 1921, which are thought to form a distinct taxon separate from the Thiaridae (*sensu stricto*) (Glaubrecht, 1999). Regrettably, too little anatomical and other biological data on a global scale are available to know (Houbrick, 1988; Dazo, 1965) whether some Asian taxa like the genera *Semisulcospira* Troschel, 1857, and *Hua* Chen, 1943, belong in the Pleuroceridae. However, material is now being gathered and studies are now ongoing (Matthias Glaubrecht, pers. comm.; Lena Sayenko & Larisa Prozorova, pers. comm.).

The classification scheme typically used for North American Pleuroceridae is that presented by Burch (1980, 1982, 1988), which admittedly was not intended to be a systematic monographic treatment, but a means to identify species and their distributions. Burch (1980, 1982, 1988) considered North American Pleuroceridae to be comprised of seven genera—*Elimia*, *Gyrotoma*, *Io*, *Juga*, *Leptoxis*, *Lithasia*, and *Pleurocera*. Previous classification schemes differ largely in the assignment of generic names. For example, some investigators used *Goniobasis* Lea, 1862a, instead of *Elimia*, *Apella* Mighels in Anthony, 1843, instead of *Gyrotoma*, and *Anculosa* Say, 1821, instead of *Leptoxis*. Aside from name differences, Burch's classification scheme differs from an earlier synopsis of the family presented by Walker (1918). These differences include *Eurycaelon* Lea, 1864, which was recognized by Walker but synonymized by Burch with *Leptoxis* while *Juga* was recognized by Burch but synonymized with *Goniobasis* by Walker. Burch justified his name choice, and we follow his recommendations as an initial working hypothesis.

Despite considerable literature justifying various proposed generic names and groupings of North American pleurocerids, no studies have been conducted examining the monophyly and phylogenetic relationships of the recognized genera. Virtually all taxonomic studies on North American pleurocerids have been done within a genus and are mostly due to Goodrich (e.g., 1922, 1924, 1928, 1931, 1934a, b, c, 1935a, b, 1937, 1938, 1941) and that of a few other investigators (e.g., Adams, 1900, 1915; Rosewater, 1960) based on shell and opercular characters. Recently, Lydeard, Holznagel, Garner, Hartfield & Pierson (1997) examined generic relationships of North American pleurocerids within the Mobile River basin of Alabama, Georgia, Mississippi, and Tennessee using molecular data, but this study included only three of six extant genera (*Gyrotoma* is now presumed extinct; Stein, 1976; Burch, 1982; Lydeard & Mayden, 1995; Turgeon, Quinn, Bogan, Coan, Hochberg, Lyons, Mikkelsen, Neves, Roper, Rosenberg, Roth, Scheltema, Thompson, Vecchione & Williams, 1998). In an effort to extend our understanding of evolutionary relationships among North American pleurocerid genera in this study, we examined a 900-bp fragment of the mitochondrial (mt) 16S rRNA gene among 32 extant North American pleurocerid species or subspecies. We believe such a phylogeny will provide a valuable phylogenetic framework from which further more detailed systematic studies can be conducted.

MATERIALS AND METHODS

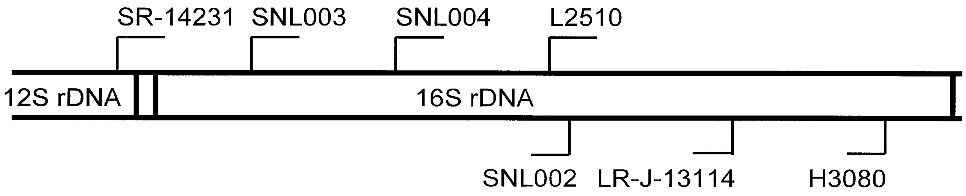
Taxa Examined

Of the 36 specimens listed in Table 1, 34 specimens, considered the ingroup, represented 32 species or subspecies of pleurocerids. *Melanoides tuberculata* (Müller, 1774) (Thiaridae) and *Melanopsis praemorsa* Linnaeus, 1758, (Melanopsidae) were chosen as outgroup representatives. The family Melanopsidae is hypothesized to be sister to Pleuroceridae, while the Thiaridae is thought to be a more basal member of Cerithioidea (Houbrick, 1988; Lydeard *et al.*, unpubl.). Voucher specimens are deposited at North Carolina State Museum of Natural Sciences. The source of material and GenBank sequence accession numbers for specimens examined are shown in Table 1.

Sequence Procurement, Alignment, and Analysis

Genomic DNA was isolated from frozen or ethanol preserved tissue samples (typically the entire head of the snail) by standard chloroform/phenol extraction. Mitochondrial (mt) DNA sequences were obtained for the amplified segment of the mitochondrial 16S rRNA gene using the primers shown in Fig. 1. We initially used primers L2510, alias 16sar-L and H3080, alias 16sbr-H (Palumbi, Martin, Romano, McMillan, Stice & Grabowski, 1991), to amplify a ca 550-bp fragment of the 3′ half of the 16S rRNA gene. From these data, we designed LR-J-13114 (5′-tgttcctyagtcgccccaac-3′) and SNL002 (Lydeard *et al.*, 1997). To amplify the 5' half of the 16S rRNA gene we used Sr-14231 (Lydeard *et al.*, 1997) which is located in the 12S rRNA gene beyond the 5' end of the 16S rRNA gene, with SNL002 to amplify this stretch of DNA. Because this fragment was very large, ca 1100-bp, we designed two internal primers, SNL003 and SNL004 (5′-ccttccaagtagaaagatta-3′ and 5′-cyttttgtatcatggtttagc-3′), as sequencing primers.

Approximately 50–500 ng of genomic DNA provided template for double-stranded amplifications via PCR in 25 μl of Tris (67 mM, pH 8.8) containing 6 mM $MgCl_2$, 1 mM of each dNTP, 1mM of each primer, and *Taq* polymerase (1.25 units, Perkin-



External Primers		
SR-14231	5'-acatatcgcccgtcactctc-3'	(Lydeard *et al.*, 1997)
SNL002	5'-aaatgattatgctacctttgc-3'	(Lydeard *et al.*, 1997)
L2510	5'-cgcctgtttaacaaaaacat-3'	(Palumbi *et al.*, 1991)
H3080	5''-acgtgatctgagttcagaccgg-3'	(Palumbi *et al.*, 1991)
Internal Primers		
SNL003	5'-ccttccaagtagaaagatta-3'	This Study
SNL004	5'-cyttttgtatcatggtttagc-3'	This Study
LR-J-13114	5'-tgttcctyagtcgccccaac-3'	This Study

Figure 1. A schematic diagram showing the position of primers and the sequence of the primers used in this study. See text for actual amplification strategies used.

Elmer-Cetus). The amplification regime consisted of 30 cycles, denaturing at 92° C for 40 s, annealing at 52°C for 60 s, and extension at 72°C for 90 to 150 s. Single-stranded DNA was produced for sequencing via asymmetric amplification (Gyllensten & Erlich, 1988) using low-melt agarose (FMC BioProducts) purified double-stranded PCR product as template. Reaction conditions for asymmetric PCR were the same with the following exceptions, one primer was held limiting and the final volume of the reaction cocktail was increased to 50 μl. Single stranded amplification was performed during the above described parameters. Following purification by centrifugal filtration (Millipore Ultra-free-MC 30,000), single-stranded DNA was sequenced by dideoxy chain termination using Sequenase Version 2.0 (Amersham Life Science) following manufacturer's protocol. A schematic diagram of the amplification and sequencing primers is shown in Fig. 1. The radiolabeled sequencing reaction products were run on 6% polyacrylamide gel (Long Ranger, FMC BioProducts) from 2 to 4.5 hours. Following electrophoresis, all gels were vacuum-dried and exposed to X-ray film for 48 to 120 hrs. Sequences were initially entered into the software program XESEE (Ver. 3.0, Cabot & Beckenbach, 1989). A visual alignment was constructed by identifying corresponding stems and loops to the hypothesized secondary structure of gastropod sequences from *Cacozeliana lacertina* (Gould, 1861) and *Paracrostoma paludiformis* (Yen, 1939) (Lydeard, Holznagel, Schnare, & Gutell, 2000).

Before conducting a phylogenetic analysis, it is necessary to examine the nucleotide substitution patterns exhibited among taxa. Based on the observed patterns of substitution, decisions can be made determining approximate step matrices or weighting schemes to be employed in the phylogenetic analysis. Therefore, we constructed bivariate plots of the number of transitions (TS) and transversions (TV) versus genetic distances (*p*-distance, uncorrected for multiple hits) for all pairwise comparisons. Absolute genetic distance (*p*) and numbers of TS and TV were calculated using the software program MEGA (Kumar, Tamura & Nei, 1993).

The phylogenetic analysis was conducted using maximum parsimony (MP) of the orthologous sequences using the heuristic search option (10 replicates) of PAUP* (version 4.0b.1 Swofford, 1998). We employed the following options in PAUP*: uninformative characters were ignored, only minimal trees were kept, gaps were treated as missing, and zero length branches were collapsed. A bootstrap analysis (Felsenstein, 1985) with 500 iterations was conducted to estimate the internal stability of the matrix. In addition, a skewness test statistic (gl) was calculated based on the distribution of tree lengths of a random sample of 10,000 topologies. Data matrices with a strong phylogenetic signal are significantly more structured than random data (Hillis & Huelsenbeck, 1992).

RESULTS

Sequence Variation

Multiple sequence alignment of the amplified region from our selected taxa resulted in a matrix consisting of 900 characters (Fig. 2)

Table 1. Species and locales of specimens used in this study following classification proposed by Burch (1980)*. NCSM = North Carolina State Museum of Natural Sciences.

Taxon	Locality	NCSM Voucher number	GenBank Accession number
Pleuroceridae			
Elimia			
Elimia olivula group			
E. cylindracea (Conrad, 1834b)	Noxubee Creek, Noxubee Co., MS	NCSM-P-4661	U73765
E. olivula (Conrad, 1834a)	Alabama River, Monroe Co., AL	NSCM-P-4664	U73766
E. showalteri (Lea, 1861b)	Cahaba River Shelby Co., AL	NSCM-P-4665	U73767
Elimia haysiana group			
E. alabamensis (Lea, 1861b)	Coosa River, Coosa Co., AL	NSCM-P-4658	U7361
E. haysiana (Lea, 1843)	Coosa River, Elmore Co., AL	NSCM-P-4662	U73763
Elimia hydei group			
E. hydei (Conrad, 1834b)	Black Warrior River, Jefferson Co., AL	NSCM-P-4663	U7364
Elimia vanuxemiana group			
E. caelatura caelatura (Reeve, 1860)	Choccolocco Creek, Calhoun Co., AL	NSCM-P-4659	AF100988
Elimia catenaria group			
E. crenatella (Lea, 1862b)	Cheaha Creek, Talladega Co., AL	NSCM-P-4660	U7362
Elimia virginica group			
E. virginica (Say, 1817)	Conodoguinet Creek, Cumberland Co., PA	NSCM-P-4666	AF100989
Lithasia			
Subgenus *Lithasia*			
L. geniculata geniculata Haldeman, 1840	Duck River, Maury Co., TN	NSCM-P-4681	AF100995
L. geniculata fuliginosa (Lea, 1842)	Duck River, Maury Co., TN	NSCM-P-4680	AF100996
Subgenus *Angitrema*			
L. duttoniana (Lea, 1841)	Duck River, Maury Co., TN	NSCM-P-4678	AF100998
L. armigera (Say, 1821) $n = 2$	Harpeth River, Davidson Co., TN	NSCM-P-4678	AF100998
Io (monotypic)			
Io fluvialis (Say, 1825)	Holston River, Sullivan Co., TN	NSCM-P-4667	AF100999
Pleurocera			
Pleurocera prasinatum group			
P. annuliferum (Conrad, 1934b)	Black Warrior River, Jefferson Co., AL	NSCM-P-4685	U73772
P. vestitum (Conrad, 1834b)	Little Cahaba River, Bibb Co., AL	NSCM-P-4691	U73775
P. prasinatum #1 (Conrad, 1934a)	Yellowleaf Creek, Shelby Co., AL	NSCM-P-4689	U73774
P. prasinatum #2 (Conrad, 1934a)	Coosa River, Elmore Co., AL	NSCM-P-4688	U73773

Pleurocera pyrenellum group			
P. pyrenellum (Conrad, 1834b)	Piney Creek, Limestone Co., AL	NSCM-P-4690	AF100990
Pleurocera unicale group			
P. unciale hastatum (Anthony, 1854)	Holston River, Sullivan Co., TN	NSCM-P-4687	AF100993
Pleurocera canaliculatum group			
P. canaliculatum filum (Lea, 1845)	Duck River, Maury Co., TN	NSCM-P-4686	AF101991
Pleurocera acuta group			
P. acuta acuta Rafinesque, 1831	White River, Ozark C., MO	NSCM-P-4684	AF100994
Subgenus *Strephobasis*			
P. walkeri Goodrich, 1928	Shoal Creek, Lauderdale Co., AL	NSCM-P-4692	AF100992
Leptoxis			
Subgenus *Mudalia*			
L. virgata (Lea, 1841)	Clinch River, Hancock Co., TN	NSCM-P-4677	AF101000
Subgenus *Athearnia*			
L. crassa anthonyi (Redfield, 1854)	Sequatchie River, Marion Co., TN	NSCM-P-4672	AF101001
Subgenus *Leptoxis*			
L. ampla (Anthony, 1855)	Little Cahaba River, Bibb Co., AL	NSCM-P-4671	U73768
L. picta (Conrad, 1834a)	Alabama River, Monroe Co., AL	NSCM-P-4673	U73769
L. plicata (Conrad, 1834b)	Black Warrior River, Jefferson, Co., AL	NSCM-P-4674	U73770
L. praerosa (Say, 1821)	Harpeth River, Davidson Co., TN	NSCM-P-4675	AF101002
L. taeniata (Conrad, 1834b)	Choccoloco Creek, Talladega Co., AL	NSCM-P-4676	U73771
Juga			
Subgenus *Juga*			
J. plicifera (Lea, 1838)	McKenzie River, Lane Co., OR	NSCM-P-4669	AF101004
J. silicula (Gould, 1847)	Oak Creek, Benton Co., OR	NSCM-P-4670	AF101003
Subgenus *Oreobasis*			
J. nigrina (Lea, 1856)	Unnamed spring, Douglas Co., OR	NSCM-P-4668	AF101005
Thiaridae			
Melanoides			
Melanoides tuberculata (Müller, 1774)	Drainage ditch, Gainesville, Alachua Co., FL	NSCM-P-4682	AF101006
Melanopsidae			
Melanopsis praemorsa Linnaeus, 1758	Granada, Andalusia, Spain	NSCM-P-4683	U73776

*We recognize that Burch's classification may not adequately reflect phylogeny, but use it as a working hypothesis.

```
                           1           .           .           .           .           .          60
E. olivula                 GTGTCGATGAGAAA----ATAAT----TATAAAATA--------TTAAT-----------
E. haysiana                ..............----.....----.........--------.....-----------
E. alabamensis             ..............----.....----.........--------.....-----------
E. cylindracea             ..............----.....----.........--------.....-----------
E. caelatura caelatura     ..............----.....----.....G...--------.....-----------
E. crenatella              NNN...........----.C...----.........--------.....-----------
E. showalteri              ..............----.....----.........--------C....-----------
E. hydei                   ...C.....G....----G....----..C......--------C....-----------
E. virginica               .......CA.....----....AC---.......C.--------.....-----------
P. annuliferum             ........A.....----...C.----.........--------.....-----------
P. pyrenellum              ........A.....----.....----.........--------.....-----------
P. vestitum                ........A.....----.....----.........--------.....-----------
P. canaliculatum filum     ........A.....----.....----.........--------.....-----------
P. walkeri                 ........A.....----.....----.........--------.....-----------
P. prasinatum #1           ........A.....----G....----.........--------..G..-----------
P. prasinatum #2           ........A.....----.....----.........--------.....-----------
P. unciale hastatum        ........A.....----G....----.G.......--------.....-----------
P. acuta acuta             ........A.....----..GG.----.....G...--------C....-----------
L. geniculata geniculata   .......CA....G----G...A----.....G...--------.....-----------
L. geniculata fuliginosa   .......CA....G----G...A----.....G...--------.....-----------
L. duttoniana              .......CA....G----G...A----.....G...--------.....-----------
L. armigera                ........A.A...----GC.G.----.....G...--------C.G..-----------
Io fluvialis               NNNNNNNNNNNNNN----NNNNN----NNNNNG...--------.....-----------
L. virgata                 ......G.A.....----G....----.........---------....-----------
L. crassa anthonyi         ......G.A.....----G....----.........--------.....-----------
L. praerosa                ......GGA.....----G....----.....T...--------....A-----------
L. plicata                 ......GAA.....-----....----..G......--------...G.-----------
L. ampla                   ......GCTG....-----C..C----.........--------C....-----------
L. taeniata                ......GCTG....-----C..C----.........--------C....-----------
L. picta                   ......GGA....G----.G...---TA......C.--------.....-----------
Juga silicula              ......GG..A...---TT.G.-----.T..T....--------.....-----------
Juga plicifera             ......GG..A..G---TT.G.-----.T..T....--------.....-----------
Juga nigrina               ......GG..A..G---CT.G.-----.T..T....--------.C...-----------
Melanopsis praemorsa       ......GG..A..----CGAC.GTGT-.T.G.T..---------C..-------------
Melanoides tuberculata     ...CT-.AAGC.C-ACAATGG..T-----......TAAAGTAAT....AATTCTTATTAT


                           61          .           .           .           .           .         120
E. olivula                 -TATTTCATA-AAATA-TTTCTC-GATTAATTTTTTTGAGGATAAGCTCGGAAAAA--AG
E. haysiana                -.........-.....-......-..........................A.....--..
E. alabamensis             -.........-.....-......-..........................A.....--..
E. cylindracea             -.....T...-.....-......-................................--..
E. caelatura caelatura     -.....T...-.G..G-......-..........................A.....--..
E. crenatella              -.....TG..-.....-.C....-A.........................A.....--..
E. showalteri              -.....T...-....G-......-..........................A.....--..
E. hydei                   -.....TG..-....G-......-A.........................A.....--..
E. virginica               -.G...T...-.GG..-.....T-..........................A.....--..
P. annuliferum             -.....T...-.....-.....T-C...............................--..
P. pyrenellum              -.....T...-.....-.....T-C..........C...................G--..
P. vestitum                -.....T...-.....-.....T-C..........C....................--..
P. canaliculatum filum     -.....T...-.G...-.....T-...........................G....--..
P. walkeri                 -.....T...-.G...-.....T-................................--..
P. prasinatum #1           -.....T...-.....-.....T-..........................A.....--..
P. prasinatum #2           -.....T...-.....-.....T-................................--..
P. unciale hastatum        -.....T...-.....-.....T-................................--..
P. acuta acuta             -.....T...-....G-.....T-................................--..
L. geniculata geniculata   -.....T...-.GG..-C.....-............C.............AG....--G.
L. geniculata fuliginosa   -.....T...-.GG..-C.....-............C.............AG....--G.
L. duttoniana              -.....T...-.GG..-C.....-............C.............AG....--G.
L. armigera                -.....T...-....C-...T.T-A...........C.............A....C-ATA
Io fluvialis               -.....T...-.G.NN-N....T-..........................AG....--..
L. virgata                 -.....T...-....G-.....T-..........................A....T-C..
L. crassa anthonyi         -.....T...-....G-....CTC..........................A....T-C..
```

Figure 2. An aligned data matrix of 900 bp of mitochondrial 16S rDNA sequence for 33 pleurocerid snails and 2 outgroup species, *Melanoides tuberculata* and *Melanopsis praemorsa*. Dashes correspond to gaps and N's are missing data. See Table 1 for complete taxon labels.

```
L. praerosa                 -.....T...-.....-.....TTACC.G.C.....C...................-ATT
L. plicata                  -.....T...-....G-.....T-ATC.G.G.....C.............A.....--TT
L. ampla                    -..A..TTACAG....-....CT-TGC.........C.............A.....--..
L. taeniata                 -..A..TTACAG....-....CT-TGC.........C.............A.....--..
L. picta                    -.G...T...-....G-C..T.T-T.C.........G............C.....T--..
Juga silicula               -....AT...T.G..TA...TC.--.C........C.............A......--..
Juga plicifera              -....AT...T.G..TA...TC.--.C......................AA.....--..
Juga nigrina                -....AT...T....GC...TC.--.C......................A......--..
Melanopsis praemorsa        A...CAA.--T..T.TC-..T..-CGCC..A...C.C.............AG....--..
Melanoides tuberculata      TA....T.----------G.-CTAAT.GG.C.C.................A..GTTGTT.

                            121      .         .         .         .         .       180
E. olivula                  TTAAGAAATT--TTACT-AATTTAGGTTATTATGTGGGCTTAAAATTGGCCATCATAAGA
E. haysiana                 ..........--.....-..........................................
E. alabamensis              ..........--.....-.......A........................T.........
E. cylindracea              ..........--.....-....................................G.....
E. caelatura caelatura      ..........--.....-............C...................T.........
E. crenatella               ..........--.....-......A..........T..............A.........
E. showalteri               ..........--.....-................................A.........
E. hydei                    ......G.A.--...T.-....C.....G.C...................T.........
E. virginica                ......CG..--...T.-G.........G......A................C...G...
P. annuliferum              ......T...--..G..-G.........G......A........................
P. pyrenellum               ......T...--.....-G.........G......A........................
P. vestitum                 ......T...--.....-G.........G.......A.......................
P. canaliculatum filum      ......T...--.....TG.........G.......................C.......
P. walkeri                  ......T...--.....TG.........G.......................C.......
P. prasinatum #1            ......T...--..GT.-G.........G...............................
P. prasinatum #2            ......T...--..GT.-G.........G...............................
P. unciale hastatum         ......T...--..GT.-G.........G...............................
P. acuta acuta              ......T...--.....-G.........G...............................
L. geniculata geniculata    ......T...--.....--.....AA..G.......................C.......
L. geniculata fuliginosa    ......T...--.....--.....AA..G.......................C.......
L. duttoniana               ......T...--.....--.....AA..G.......................C.......
L. armigera                 .....GT..---....ACC..AATA..........A.......G...A.T......G...
Io fluvialis                .....GT...--.....--.C....A..G.......................C.......
L. virgata                  ......T...--..TT.-G...C.AA..GC.........................C.G..
L. crassa anthonyi          ......TT..--..TT.-G.....AA..GC...........................G..
L. praerosa                 ......T.AA--.ATT.-.TAA.TAA..GC............G....A........TG..
L. plicata                  .....GT..---...A.-....A.AA....C................A............
L. ampla                    ......T...--..TAG--T.AA.AT..G..GC..........T..C...T..TG.....
L. taeniata                 ......T...--..TAGTT.AAATTTG.G..GC..A.......T......T...G.....
L. picta                    ......T..A--..TTA--.....AA...C.........C...G...A............
Juga silicula               ......T...--..T---....A.A...G..GC...................C.....A.
Juga plicifera              ......T...--..T---....A.A...G..GC...................C.....A.
Juga nigrina                ......T...--..T---....A.A...G..GC...................C.....A.
Melanopsis praemorsa        .C....T..C--.AT--A.GC....T..G..G...........G...........CG...
Melanoides tuberculata      ..G..GGT.ATA...----T..A.AA...C.G.......C.......A.......C..-.

                            181      .         .         .         .         .       240
E. olivula                  GTTTGTTATAAAACAATAAT-CTTAATATTTAAGATAAATATATTTTATT---CTAATTT
E. haysiana                 ....................-.............................---.......
E. alabamensis              ....................-.............................---.......
E. cylindracea              ....................-.C...........................---.......
E. caelatura caelatura      ....................-T............................---.......
E. crenatella               ...............GC...-T...G....................C...---......C
E. showalteri               ...............GC...-T...G....................C...---.......
E. hydei                    ................C...-.......C....A.............T..---.......
E. virginica                ........C....T...G..-.....G......A................---....C..
P. annuliferum              .............T..C...-.C..........A................---.......
P. pyrenellum               ....................-.C..........A................---.......
P. vestitum                 ................C...-............A................---.......
P. canaliculatum filum      ................C..C-.CC.........A..............C.---....C..
P. walkeri                  ................C..C-.CC.........A..............C.---....C..
P. prasinatum #1            ................C...-.CC.........A................---.......
P. prasinatum #2            ................C...-.CC.........A................---.......
```

Figure 2. (*Continued*).

```
P. unciale hastatum        ................C...-.C..........A................---.......
P. acuta acuta             ................G...-............A...........A....---.......
L. geniculata geniculata   ................C...-T...G.......A....C.........C.---......C
L. geniculata fuliginosa   ................C...-T...G.......A....C.........C.---......C
L. duttoniana              ................C...-T...G.......A....C.........C.---......C
L. armigera                ....................-TA..C...C...A................---......A
Io fluvialis               ...............GC...TT.....T.C...A......GNNNNNNNN.---....A..
L. virgata                 ...............GCGG.-T...........A.........G......---.....A.
L. crassa anthonyi         ...............GCGG.-T...........A.........G......---.......
L. praerosa                ......C........GCGT.-T...G.GACC..A................---.......
L. plicata                 ...............G.GTATT......A....A..G...........CA---....A..
L. ampla                   .............A......-T.......C...A.........A......---....C..
L. taeniata                .............A......-T.....GCC...A...G.....A......---....C..
L. picta                   A...................-GCA......A..A............AT..---....C..
Juga silicula              .............T.....C-T....ATC.A..A.............GA.---....CA.
Juga plicifera             .............T.....C-T....ACC.A..A.............GA.---...GCA.
Juga nigrina               .............T......-T....GCC.A..A...G..........A.---....C..
Melanopsis praemorsa       ................CT..-AAA..AGCA...A..TT..C..G...TG.---....C..
Melanoides tuberculata     .............T..C..AGTA..TA.AAAT.TTATT....G.AGC.CCATAGA.....

                           241       .         .         .         .         .      300
E. olivula                 TTT--ACAGAAATAAAGACCTCAATTAATAAATACCTTATACCTATGCTAGGATGAGTAT
E. haysiana                ...T-.......................................................
E. alabamensis             ...--............................G..........................
E. cylindracea             ...T-............................G..........................
E. caelatura caelatura     ...T-.................G..........GTT........................
E. crenatella              CC.--.............T..............G..........................
E. showalteri              ...T-.................G..........G..........................
E. hydei                   AC.T-............GTGCTG.....................................
E. virginica               ...--............GT..T........G..G.....C....................
P. annuliferum             ...--..........GA..TAT...........GTT..G.G...................
P. pyrenellum              ...--..........GA..TAT...........GTT..G.G...................
P. vestitum                ...T-..........GA..TAT...........GTT..G.G...................
P. canaliculatum filum     ...--..........G...TAT...........GTT..G.G...................
P. walkeri                 ...--..........G...TAT...........GTT..G.G...................
P. prasinatum #1           ...--..........GA..TAT......C...CGTT....G...................
P. prasinatum #2           ...T-..........GA..TAT......C...CGTT....G...................
P. unciale hastatum        ...T-..........GA..TAT......C...CGTT....G...................
P. acuta acuta             ..CT-.T..G.......C.TCT........G..GT.......T.................
L. geniculata geniculata   AC.--............G.A.T...C.CC..T.G..A..CG...................
L. geniculata fuliginosa   AC.--............G.A.T...C.CC..T.G..A..CG...................
L. duttoniana              AC.--............G.A.T...C.CC..T.G..A..CG...................
L. armigera                C.A--............GT.AT...A.....TG.....CC...........A........
Io fluvialis               .C.--............G.A...C.C..C..T.G..A...G...................
L. virgata                 ...--...........AG.T.TG.C..TC....G.T...C..........A.........
L. crassa anthonyi         C..--...........AG.TCT.....TC....G.T...C..........A.........
L. praerosa                ...--..TA.......AG.T.T.....GA....G.T.A.C....................
L. plicata                 ..CCT.TC..........TA.T.....TA.G...TT.A.C....................
L. ampla                   ...--.T..............T...C....T..GT...GC..........A.........
L. taeniata                ...--.T..............T...C....T..GT...GC..........A.........
L. picta                   A..--.....G....GAG...T..C.T...G..G.T..............A.........
Juga silicula              C..--..G..G.C..GA..T....C...AGT.AGTT...CG...................
Juga plicifera             C..--..G.GG....GA..T....C...AGT.AGTT...CG...................
Juga nigrina               C..--..G..G....GA....T..C...AGT.AGTT...CG...................
Melanopsis praemorsa       C..--...A.G.....AG.TCT....CC..G.GG.T...............A........
Melanoides tuberculata     A.A--.G.AC.....TC---.A..C...A.G..TTAA...G...................

                           301       .         .         .         .         .      360
E. olivula                 TAAAACTTTTTATATTCT---AAGAAAGTTTTATGTAT--T-TTTCTTCAAAAAAAA--T
E. haysiana                ..................---.................--.-...............--.
E. alabamensis             ..................---.................--.-...............--.
E. cylindracea             ................T.---.................--.-...............--.
E. caelatura caelatura     ................T.---.............A...--.-.......T.......--.
E. crenatella              .........A....C.T.---...............-.--.-.......T.......--.
E. showalteri              .........A......T.---.................--.-.......T.......--.
```

Figure 2. (*Continued*).

```
E. hydei                    ......C.-A.....AT.---.............A..---.A.......T.......--.
E. virginica                .....T.CCG...G.AT.---...........C.A..C--.-........G......--.
P. annuliferum              .....T..A.....AT.---.............A.G.--.-..........G....--.
P. pyrenellum               .....T..G.....AT.---.............A...--.-.............G.--.
P. vestitum                 .....T..G......T----...............-.--.-........G....G.--.
P. canaliculatum filum      .....T..G...G.GT.---.............A..---.-.............G.--.
P. walkeri                  .....T..G...G.GT.---.............A..---.-.............G.--.
P. prasinatum #1            .....T....C...CGT.---.............A...--.-.......CGGG..G.--.
P. prasinatum #2            .....T....C...CGT.---.............A...--.-.......C.GG..G.--.
P. unciale hastatum         .....T...AC....GT.---.............A...--.-.......T.GG..G.--C
P. acuta acuta              .....TC.-AC..GCAT.---.............A...--.-.......T..T....--.
L. geniculata geniculata    ......CA.A.....AT.---.............A..---CA.....A.C.......AA.
L. geniculata fuliginosa    ......CA--.....AT.---.............A..---CA.....A.C.......AA.
L. duttoniana               ......CA--.....AT.---.............A..---CA.....A.C.......A-.
L. armigera                 ......CA.A...G.GTAAA-C.....A.AA...A..AT-.-.....A...G..G.CA--
Io fluvialis                ..G....A.A.NNN.GG.---TT....A...A.......--.-....AA.T.T...TG--.
L. virgata                  .....T.C.GC....AA.---......A.......A...--.-.......T.......--.
L. crassa anthonyi          .....TCC.AC....AA.---.............A...--.-.......T.......--.
L. praerosa                 ........-AC....AT.ATT.....C-.....CAAG.--AT.......G.......--.
L. plicata                  .....AACA.A..TACT----G.....-......AA..--GT.......TT.....T--A
L. ampla                    ......G.A.....GGT.---G.....A-..A..C.T.--.-.C..C.AG.....----C
L. taeniata                 ......G.A.....GGT.---G...G.A-..A..C.T.--.-.C..C.AG.....----C
L. picta                    CT......ACATA.AAT----.G....--.....A.G.T-.A.......T.......--A
Juga silicula               .....ACAAC.T...A.----......A.C..T.A.T.--.-.........G......--.
Juga plicifera              .....ACAAC.T...A.----......A.C..T.C.T.--.-.........GGG....--.
Juga nigrina                .....ACAAC.T...A.----......A.C..T.A.T.--.-...........G....--.
Melanopsis praemorsa        ..C..-CCGC.C..AAA----G.A....--....C...TA.-...T.AT.....GC.--C
Melanoides tuberculata      ..T..AA..A.G....-------A.GTA---A.AC..G--.AC..---.T.......--.

                            361      .         .         .         .         .       420
E. olivula                  ATTAATTGAATTAAATTAGTTAAAAGGAACTCGGCAAAATTAATGCTTTCGCCTGTTTAT
E. haysiana                 ............................................................
E. alabamensis              ...G........................................................
E. cylindracea              ...G........................................................
E. caelatura caelatura      .......A....................................................
E. crenatella               .C.G..CA....................................................
E. showalteri               ......CA....................................................
E. hydei                    .......A..............C.....................................
E. virginica                ......CA..C...........G......T..A...........................
P. annuliferum              .....CCA..CC...C......G...............T..T..T.G..T..........
P. pyrenellum               ......CA..CC...C.G...TG...............T.....................
P. vestitum                 ......CA..CC...C......G...............T.C...................
P. canaliculatum filum      ......CA..CC..........G...............T.C...................
P. walkeri                  ......CA..CC..........G...............T.C...................
P. prasinatum #1            ...G..CA...C..........G......T........T.....................
P. prasinatum #2            ...G..CA...C..........G...............T.....................
P. unciale hastatum         ...G..CA...C..........G...............T.....................
P. acuta acuta              ......CA...C..........G...............T.C.....A.C...........
L. geniculata geniculata    ...G...T..............G...............T.....................
L. geniculata fuliginosa    ...G...T..............G...............T.....................
L. duttoniana               ...G...T..............G...............T.....................
L. armigera                 .....C.T.GG.......AA..G.........................C...........
Io fluvialis                .......A..............G...............C.....................
L. virgata                  .......A.G........A...G...............T...G.....C...........
L. crassa anthonyi          .C.......G........A...G...............T...G.....C...........
L. praerosa                 .......A.G............G...............T.........C...........
L. plicata                  T..CTA.T..............G.................A...TGC.CT..........
L. ampla                    ....G.....C.......A.A.................T.C.GC................
L. taeniata                 ....G.............A.A.................T.C.GC....C...........
L. picta                    TC.....T.G.....A..A...................T...G.................
Juga silicula               ...TG.A...AA......A..GG...............T...G.................
Juga plicifera              ...T..A...AA......A...G...............T...G.................
Juga nigrina                ...T.AA...A.G.....A...G...............T...G.TG..............
Melanopsis praemorsa        ...TT.CA..ACT..A......G...............T.A.G.....C...........
Melanoides tuberculata      ...TT..AT..-......AAG.G......T.T......TA.--..T-.............
```

Figure 2. (*Continued*).

```
                            421       .         .         .         .         .      480
E. olivula                  CAAAAACATGGCTCTCTGAATTCATTTTTATAGAGAGTCAGGCCTGCCCAGTGA-ATAAT
E. haysiana                 ......................................................-.....
E. alabamensis              ......................................................-.....
E. cylindracea              ......................................................-.....
E. caelatura caelatura      ......................T...............................-.....
E. crenatella               ..................G...A.....-....G....................-.....
E. showalteri               ......................A.....-....G....................-.....
E. hydei                    ......................T...............................-.....
E. virginica                ..................CC..T.....-..........C..............-.A...
P. annuliferum              ...............T..T...T..C..-...A.....................-.....
P. pyrenellum               ...............T..C...T..C..-...A.....................-.....
P. vestitum                 ...............T..C...T.....-...A.....................-...G.
P. canaliculatum filum      ...............T..C...T.....-...A.....................-.....
P. walkeri                  ...............T..C...T.....-...A.....................-.....
P. prasinatum #1            ...............T..C...T.....-...A.....................-.....
P. prasinatum #2            ...............T..C...T.....-...A.....................-.....
P. unciale hastatum         ...............T..C...T.....-...A.....................-.....
P. acuta acuta              ...............T..C..A...........G....................-.....
L. geniculata geniculata    ..................T...T.....C.........................-....C
L. geniculata fuliginosa    ..................T...T.....C.........................-....C
L. duttoniana               ..................T...T.....C.........................-....C
L. armigera                 ...............T...G..AT..AAA...A.....................-....A
Io fluvialis                ..................T...-.....C..........GA.............-.....
L. virgata                  ...............T..TG..TTA.......A.....................-.....
L. crassa anthonyi          ...............T..T...TTA.......A.....................-.....
L. praerosa                 ...............T..C...T....G-...A.....................C.....
L. plicata                  ...............T..TG..A....AA...A........A............--....
L. ampla                    ...............T......AT....-...A.....................A..TTA
L. taeniata                 ...............T......AT....-.........................A..TTA
L. picta                    ...............T..C...A...A.-...AG....................T..TTA
Juga silicula               ..................TT.A...AAA.....G.....GA.............-..TT-
Juga plicifera              ..................TT.A...AAA.....G.....GA.............-..TT-
Juga nigrina                ..................CT.A...AAA.....G.....GA.............-..TT-
Melanopsis praemorsa        ...............T.....AATCACG....A......GA.............-..TT-
Melanoides tuberculata      ...............T....GAG.ACAA-...A......GAA.......G....-..T..

                            481       .         .         .         .         .      540
E. olivula                  ATT-T-AACGGCCGCGGTACTCTGACCGTGCAAAGGTAGCATAATCATTTGCCCTGTAAT
E. haysiana                 ...-.-......................................................
E. alabamensis              ...-.-......................A..........................A....
E. cylindracea              ...-.-.................................................A....
E. caelatura caelatura      ...-.-...............................................T.A....
E. crenatella               ...-.-...............................................T.A....
E. showalteri               ...-.-...............................................T.A....
E. hydei                    ...-.-...............................................T.A....
E. virginica                ...-.-...............................................T.A....
P. annuliferum              ...-.-...............................................T.A....
P. pyrenellum               ...-.-...............................................T.A....
P. vestitum                 ...-.-...............................................T.A....
P. canaliculatum filum      ...-.-...............................................T.A....
P. walkeri                  ...-.-...............................................T.A....
P. prasinatum #1            ...-.-...............................................T.A....
P. prasinatum #2            ...-.-...............................................T.A....
P. unciale hastatum         ...-.-...............................................T.A....
P. acuta acuta              ...-.-...............................................T.A....
L. geniculata geniculata    ...-.-...............................................T.A....
L. geniculata fuliginosa    ...-.-...............................................T.A....
L. duttoniana               ...-.-...............................................T.A....
L. armigera                 ...-.-...............................................T.A....
Io fluvialis                ...-.-...............................................T.A....
L. virgata                  ...-.-..................................T............T.A....
L. crassa anthonyi          ...-.-...............................................T.A....
L. praerosa                 ...-C-.................A.............................T.A....
L. plicata                  ...-C-.................A.............................T.A....
L. ampla                    C-.T.-...............................................T.A....
```

Figure 2. (*Continued*).

```
L. taeniata                 C-.T.-...............................................T.A....
L. picta                    T-.A--.........A....C.....T........................T.T.A....
Juga silicula               --.-.A...............................................T.A....
Juga plicifera              --.-.A...............................................T.A....
Juga nigrina                --.-.A...............................................T.A....
Melanopsis praemorsa        --.-.-....................................TTA........T.A....
Melanoides tuberculata      T-.-.-..............C................................T.A....

                            541        .         .         .         .         .      600
E. olivula                  TGAGGGCTAGTATGAATGGTTTAACGAAAGCAATACTGTCTCCTTTCTATTTTCAAGAAC
E. haysiana                 ............................................................
E. alabamensis              ............................................................
E. cylindracea              ............................................................
E. caelatura caelatura      ...A........................................................
E. crenatella               ...A.......................................................T
E. showalteri               ...A.......................................................T
E. hydei                    ...A.......................G...............................T
E. virginica                ...A.......................G...............................T
P. annuliferum              ...A.............................................C..........
P. pyrenellum               ...A.............................................C..........
P. vestitum                 ...A.............................................C..C.......
P. canaliculatum filum      ...A.............................................C..........
P. walkeri                  ...A..............................C..............C..........
P. prasinatum #1            ...A......C......................................C.........T
P. prasinatum #2            ...A......C......................................C.........T
P. unciale hastatum         ...A......C......................................C.........T
P. acuta acuta              ...A.......................G.....................G.........T
L. geniculata geniculata    ...A.......................G................................
L. geniculata fuliginosa    ...A.......................G................................
L. duttoniana               ...A.......................G................................
L. armigera                 ...A........................................A.T.............
Io fluvialis                ...A.......................G................................
L. virgata                  ...A.............................C............T.............
L. crassa anthonyi          ...A.............................C............T.............
L. praerosa                 ...A....C.............G....G.......................C.TT....T
L. plicata                  ...A.......................G..T..............A.....C.TT....A
L. ampla                    ...A.......................G....GC........T...T.....C.......
L. taeniata                 ...A..................G....G....GC........T...T.............
L. picta                    ...A.A................G.........CCT.......T...T......T.....T
Juga silicula               ...A..................G....G.....C............T.............
Juga plicifera              ...A..................G....G.....C............T.............
Juga nigrina                ...A..................G....G.A................T.............
Melanopsis praemorsa        ...A..................G....G....GC...........A...C...TT.....
Melanoides tuberculata      ...A......C.....G...C.G.....................C.T......TT....A

                            601        .         .         .         .         .      660
E. olivula                  TTAACCTTTAGGTGAAGAGGCCCAAATTAAATTGAAGGACAAGAAGACCCTGTCGAGCTT
E. haysiana                 ............................................................
E. alabamensis              ............................................................
E. cylindracea              ......................T....A................................
E. caelatura caelatura      ......................T.....T...............................
E. crenatella               ......................T....GT...............................
E. showalteri               ......................T....CT...............................
E. hydei                    ......................T.....T...............................
E. virginica                ......................T.....T...............................
P. annuliferum              ............................T.......................A.......
P. pyrenellum               ............................T...............................
P. vestitum                 ............................T...............................
P. canaliculatum filum      ............................T...............................
P. walkeri                  ............................T...............................
P. prasinatum #1            ............................T...............................
P. prasinatum #2            ............................T...............................
P. unciale hastatum         ............................T...............................
P. acuta acuta              ......................T.....T...............................
L. geniculata geniculata    ............................T...............................
```

Figure 2. (*Continued*).

```
L. geniculata fuliginosa     ............................T...............................
L. duttoniana                ............................T...............................
L. armigera                  ......................T.....T...............................
Io fluvialis                 ......................T.....T...............................
L. virgata                   ......................T.....T...............................
L. crassa anthonyi           ......................T.....T...C...........................
L. praerosa                  ......................T.....T...............................
L. plicata                   ......................T....GC...............................
L. ampla                     ......................T.....TT..............................
L. taeniata                  ......................T.....TT..............................
L. picta                     .......C..............T.G...CT..............................
Juga silicula                .....T................T.....TT.....................A........
Juga plicifera               .....T................T.....TT.....................A........
Juga nigrina                 .....T................T.....TT.....................A........
Melanopsis praemorsa         ......................T.....TT....................C.........
Melanoides tuberculata       .....T.A..............T.T....T..............................

                             661      .         .         .         .         .       720
E. olivula                   A-AAAGACACTATAGAAGTCC-AT--ATATTTTTAAATAAATTTTCTAT-TAAGCTTTTT
E. haysiana                  .-...................-..--.......................-..........
E. alabamensis               .-...................-..--.......................-..........
E. cylindracea               .-...................-..--.......................-..........
E. caelatura caelatura       .-.................T.-..--..................C....-..........
E. crenatella                .-.................T.-..--...C..............A....-.G........
E. showalteri                .-...................-..--...G..............A....-.G........
E. hydei                     .-.......T.........A.-..--.......................-..........
E. virginica                 .-..........C......TG-..--......A.........C....G.-..........
P. annuliferum               .-.....T.TC........TT-..--.........G........C....TA.........
P. pyrenellum                .-.....T.T.........TT-..--.........G.....AAGA....-.G........
P. vestitum                  .-.....T.T.........TT-..--.........G........C....TA.........
P. canaliculatum filum       .-.....T...........TT-..--..................C..G.TA.........
P. walkeri                   .-.....T...........TT-..--.........G........C..G.TA.........
P. prasinatum #1             .-.....T...........TT-..--.........G........C....TA.........
P. prasinatum #2             .-.....T...........TT-..--.........G........C....TA.........
P. unciale hastatum          .-.....T...........TT-..--.........G.......CC....TA.........
P. acuta acuta               .-.....A..C......T.TT-..--.........G........C....-..........
L. geniculata geniculata     .-........C......C.T.-..--..................A....-..........
L. geniculata fuliginosa     .-........C......C.T.-..--..................A....-..........
L. duttoniana                .-........C......C.T.-..--..................A....-..........
L. armigera                  .-...........G.....TT-..--...A.A...G.....A.AA.C..-..........
Io fluvialis                 .-..........C..G...T.-..--...C.............C.....-..........
L. virgata                   .-................CTT-..--...C...................-..........
L. crassa anthonyi           .-.........G......CTT-..--...C...................-..........
L. praerosa                  .-.....T.T..A......TT-..--.....................T.-..........
L. plicata                   .-.......T..G...G..TGTG.--...C..A.........-A...C.-..........
L. ampla                     .-.................TT-..--....ACC..............G.-..........
L. taeniata                  .-.................TT-..--....A.C..............G.-..........
L. picta                     .-.....T.....G.....T.-..--....A.C.............CG.-..........
Juga silicula                .-.......T.........TT-..--......C................-..........
Juga plicifera               .-.......T.........TT-..--......C................-.G........
Juga nigrina                 .-.......T.........TT-..--.......................-.G........
Melanopsis praemorsa         .-.......T.G.......TG-..--...AC.A..G...G..A.....C-..........
Melanoides tuberculata       TA...TTA.A.G...GT..AA-..TAT..ACAAA--..C...GAA...CA.C.CA.....

                             721      .         .         .         .         .       780
E. olivula                   AGTTGGGGCGACTAAGGAACAAAAAAAGCTTCTTGT-AAT-AACCAAATGTCCAAGTTTT
E. haysiana                  ....................................-...-...................
E. alabamensis               ....................................-...-...................
E. cylindracea               ....................................-...-..G.....A..........
E. caelatura caelatura       ....................................-...-...T...............
E. crenatella                ..................................A.-...-.......C.CT........
E. showalteri                ..................................A.-...-..T....C..T........
E. hydei                     ..................................AA-..A-...T...CACA.....A..
E. virginica                 ..................................A.-.G.-..ATT..CACG........
P. annuliferum               ..................................A.-...-..ATG..CAA..GG.....
```

Figure 2. (*Continued*).

```
P. pyrenellum               ..................................A.-...-..ATG..CAA..TG.....
P. vestitum                 ..................................A.-...-..AT...CAA..GG.....
P. canaliculatum filum      .....................GG...........AC-...-..AT...CAA..GG.....
P. walkeri                  .....................G............AC-...-..AT...CAA..GG.....
P. prasinatum #1            ..................................A.-...-..A..G.CAA..GG.....
P. prasinatum #2            ..................................A.-...-..A..G.CAA..TG.....
P. unciale hastatum         ..................................A.-...-..A....CAA..GG.....
P. acuta acuta              .A.........T....................C.AC-..A-..AT...CAA...G.....
L. geniculata geniculata    ......................T...........T.-..C-..A....CAGA..G.....
L. geniculata fuliginosa    ......................T...........T.-..C-..A....CAGA..G.....
L. duttoniana               ......................T...........T.-..C-..A....CAGA..G.....
L. armigera                 ..................................A.-.TA-..AT.G.CA.A..G.....
Io fluvialis                ..................................T.-..C-C.A....CAAT..G.....
L. virgata                  ..................................A.-...-..AT...CAAT..G.....
L. crassa anthonyi          ..................................AC-...-..AT...CAAT..G.....
L. praerosa                 ....................................-..A-..A..G.CTAT..G.....
L. plicata                  ..................................A.AG.A-..A-.G.CCGT........
L. ampla                    ..................................AA-GCA-..A....CA.T........
L. taeniata                 ..................................AA-GTA-..A....CA.T........
L. picta                    ................................C.A.-..A-........CGTT.GA....
Juga silicula               ...................................CTG..--.AT.G.CAGT..T....A
Juga plicifera              ...................................CT...--.AT.G.CAGT..T....A
Juga nigrina                ...................................CTG..--.AT..GCAGT..T....A
Melanopsis praemorsa        ..................................A.T...--.AT.C.CAGA..G..AAA
Melanoides tuberculata      ............G..........C..........T.-.T.TTTAT....T.AT.GA...A

                            781         .         .         .         .         .    840
E. olivula                  GATCCGGTAATAACCGATTAAGGAAATTAGTTACCGCAGGGATAACAGCATAATCTCTCT
E. haysiana                 ............................................................
E. alabamensis              ..........A.................................................
E. cylindracea              ............................................................
E. caelatura caelatura      ..........A.................................................
E. crenatella               ............................................................
E. showalteri               .......C...............G....................................
E. hydei                    .......C.............A......................................
E. virginica                .............T.....G........................................
P. annuliferum              ......A......T.......A.G............T.......................
P. pyrenellum               ......A......T.......A.G....................................
P. vestitum                 ......A......T.......A.G...............................C....
P. canaliculatum filum      ......A......T.......T.G....................................
P. walkeri                  ......A......T.......T.G....................................
P. prasinatum #1            ......A.G....TT........G....................................
P. prasinatum #2            ......A......T.........G....................................
P. unciale hastatum         ......A.G....T.........G....................................
P. acuta acuta              ......A......T..............................................
L. geniculata geniculata    .....................A.G....................................
L. geniculata fuliginosa    .....................A.G....................................
L. duttoniana               .....................A.G....................................
L. armigera                 ............G.....CT.A.G....................................
Io fluvialis                ............G..........G....................................
L. virgata                  .......C....G.....CC...G....................................
L. crassa anthonyi          ......AC.TN.G.....CT...G....................................
L. praerosa                 .........T..T......T.A.G....................................
L. plicata                  .........T..T....C.T.A......................................
L. ampla                    ........G...G.....CT.A......................................
L. taeniata                 ........G...G......T.A.G....................................
L. picta                    .....A....C.G.T......TAG....................................
Juga silicula               ......A......T....C..A..............T.......................
Juga plicifera              ......A......T....C..A..............T.......................
Juga nigrina                ......A......T....C..A..............T.......................
Melanopsis praemorsa        ......A.-...GT....C..A......................................
Melanoides tuberculata      .....A.C-.A.G.T.....GAA..................................CT.
```

Figure 2. (*Continued*).

```
                           841      .         .         .         .         .       900
E. olivula                 TGAGAGACCTAATCGAAAGAGGGGGTTGTGACCTCGATGTTGGACTAGAATATCCTAAAG
E. haysiana                ............................................................
E. alabamensis             ............................................................
E. cylindracea             ............................................................
E. caelatura caelatura     .........C..................................................
E. crenatella              .........A.......................................NNNNNNNNNNN
E. showalteri              ............................................................
E. hydei                   ............................................................
E. virginica               ............................................................
P. annuliferum             ............................................................
P. pyrenellum              ............................................................
P. vestitum                ............................................................
P. canaliculatum filum     ............................................................
P. walkeri                 ............................................................
P. prasinatum #1           ............................................................
P. prasinatum #2           ............................................................
P. unciale hastatum        ............................................................
P. acuta acuta             ............................................................
L. geniculata geniculata   .A.......A..................................................
L. geniculata fuliginosa   .A.......A..................................................
L. duttoniana              .A.......A..................................................
L. armigera                ...................................................G........
Io fluvialis               CA.......A..................................................
L. virgata                 .......T....................................................
L. crassa anthonyi         .......T..................................................NN
L. praerosa                ......T........................................A............
L. plicata                 .......T................C........................G..........
L. ampla                   ............................................................
L. taeniata                ...............................................A............
L. picta                   .......T....................................................
Juga silicula              ............................................................
Juga plicifera             ............................................................
Juga nigrina               ............................................................
Melanopsis praemorsa       ............................................AT..............
Melanoides tuberculata     .................AG.....................................G...
```

Figure 2. (*Continued*).

including insertions and deletions (indels). Length variation was observed among the taxa ranging from 833 to 844 bp. Of the 900 characters examined, 446 (49.6%) were variable and 314 (34.9%) were phylogenetically informative among all taxa including the outgroup.

Pairwise percent sequence differences corrected for multiple hits by the two-parameter method of Kimura (1980) are as follows. Interspecific values for the genus *Lithasia* ranged from 0.0% (*L. geniculata geniculata* (Haldeman, 1840), *L. geniculata fuliginosa* (Lea, 1842), and *L. duttoniana* (Lea, 1841)) to 14.77% (*L. duttoniana* vs. *L. armigera* (Say, 1821)); for *Elimia* 0.12% (*E. olivula* (Conrad, 1834a) vs. *E. haysiana* (Lea, 1843)) to 11% (*E. crenatella* (Lea, 1862b) vs. *E. virginica* (Say, 1817)); for *Leptoxis* 2.69% (*L. virgata* (Lea, 1841) vs. *L. crassa anthonyi* (Redfield, 1854)) to 22.76% (*L. picta* (Conrad, 1834a) vs. *L. plicata* (Conrad, 1834b)); for *Pleurocera* 0.47% (*P. canaliculatum filum* (Lea, 1845) vs. *P. walkeri* (Goodrich, 1928) to 8.32% (*P. acuta acuta* Rafinesque, 1831 vs. *P. pyrenellum* (Conrad, 1834b)); for *Juga* 1.66% (*J. silicula* (Gould, 1847) vs. *J. plicifera* (Lea, 1838)) to 4.12% (*J. silicula* vs. *J. nigrina* (Lea, 1856)). The low and high range values for the intergeneric sequence comparisons are shown in Table 2. The lowest value for an ingroup comparison is between *Io* and *Lithasia* (7.25%) while the highest ingroup difference is between *Leptoxis* and *Juga* (22.96%). The lowest nucleotide sequence difference between an ingroup and an outgroup taxon was 19.88% between *Juga* (ingroup) and *Melanopsis* (outgroup) while the highest nucleotide sequence difference was between the ingroup *Leptoxis* and the outgroup *Melanoides* (33.29%).

A scatterplot (Fig. 3) of pairwise genetic sequence differences (*p*-distance) versus the absolute number of transitions and transversions among all pleurocerids and the outgroup taxa reveal some interesting patterns. From zero to about 15% sequence difference, we observed a clear separation of TS and TV with each increasing in a roughly linear fashion.

Table 2. Estimated percentage nucleotide sequence difference among pairwise comparisons of North American pleurocerid genera based on Mitochondrial 16S rDNA sequences.

	Elim	Io	Juga	Lept	Lith	Pleu	Mp	Mt
Elim		9.28	15.69	9.86	9.14	7.97	20.83	27.79
Io	12.33		17.35	11.33	7.25	11.09	22.00	27.99
Juga	18.38	17.68		16.30	16.73	15.14	19.88	29.38
Lept	19.62	20.25	22.96		11.69	9.62	20.65	29.02
Lith	16.54	15.82	22.05	21.24		10.01	22.24	29.73
Pleu	11.60	13.29	17.51	19.24	16.23		20.07	29.48
Mp	24.67	22.00	20.06	26.19	23.47	21.57		33.14
Mt	32.25	57.99	29.99	33.29	30.43	30.66	33.14	

Note. Percentage sequence values were corrected for multiple hits using Kimura's two-parameter model (Kimura, 1980). Values above the diagonal are the low range values and the values below the diagonal are the high range values. Genus abbrevations: Elim, *Elimia*; Io, *Io*; Juga, *Juga*; Lept, *Leptoxis*; Lith, *Lithasia*; Pleu, *Pleurocera*; Mp, *Melanopsis*; Mt, *Melanoides*.

These values correspond to those within a genus (excluding some *Leptoxis* pairwise comparisons) and the intergeneric comparisons between *Elimia*, *Io*, *Lithasia*, and *Pleurocera*. For greater than 15% sequence difference, TS starts to level off and TV continues to increase in a linear fashion. At this point, an overlap between the numbers of TS and TV occurs with the TV actually exceeding the number of TS in some instances. These greater distance values correspond to some intrageneric comparisons within the genus *Leptoxis*, and intergeneric comparisons between *Elimia*, *Io*, *Lithasia*, *Pleurocera* and *Leptoxis*, and ingroup-outgroup comparisons. The reduction in the rate of TS increase beyond a genetic distance of about 20% indicates that TS may not provide reliable phylogenetic information due to saturation at this level. Around 17% the number of TS appears to be saturating. The 17% to 27% sequence difference corresponds to comparisons between the ingroup and *Melanoides* and *Melanopsis*, the outgroup.

Base Compositional Bias

Base compositional bias, which is unequal proportions of the four bases (G, A, T, C), is common in DNA sequences. Table 3 summarizes the base composition for all 35 specimens. All taxa exhibit a high proportion of A and T (35.68 and 32.89% average, respectively) and a low portion of C and G (13.99 and 17.42% average, respectively).

It is important to consider base composition when conducting a phylogenetic analysis because when base compositional bias varies among taxa potentially unreliable phylogenies may result. Most analytical methods, including parsimony, maximum-likelihood, and neighbor-joining, tend to group sequences of similar nucleotide compositions together regardless of their evolutionary history (Lockhart, Steel, Hendy & Penny, 1994). A Chi-square test of homogeneity of base frequency across taxa as implemented in PAUP* (Swofford, 1998) revealed no significant differences in base frequency ($P = 1.0000$).

Phylogenetic Analysis

Based on the analysis of nucleotide substitution patterns discussed previously, we employed two different approaches in our phylogenetic analysis: (1) all substitutions received equal weighting and (2) transversions were weighted 2X and 4X transitions to compensate for saturation occurring among distantly related taxa.

Maximum parsimony analysis in which base substitutions were equally weighted resulted in a single most parsimonious tree (TL = 1310, CI = 0.528, gl = −0.578; Fig. 4). The molecular phylogeny (Fig. 4) is highly resolved and supports the monophyly of *Elimia* and *Pleurocera*, which are sister taxa. Three *Lithasia* taxa (*L. geniculata geniculata*, *L. g. fuliginosa*, and *L. duttoniana*) form a monophyletic group, all possessing identical sequences, and this group is sister to the monotypic genus *Io*. Together, the three *Lithasia* + *Io* are sister to *Elimia* + *Pleurocera*. The next four basal-most clades include: *Leptoxis virgata* + *L. crassa anthonyi*, *L. ampla* (Anthony, 1855) + *L. taeniata* (Conrad, 1834b) + *Lithasia armigera*, *Leptoxis praerosa* (Say, 1821) + *L. plicata*, and *L. picta*. The three *Juga*

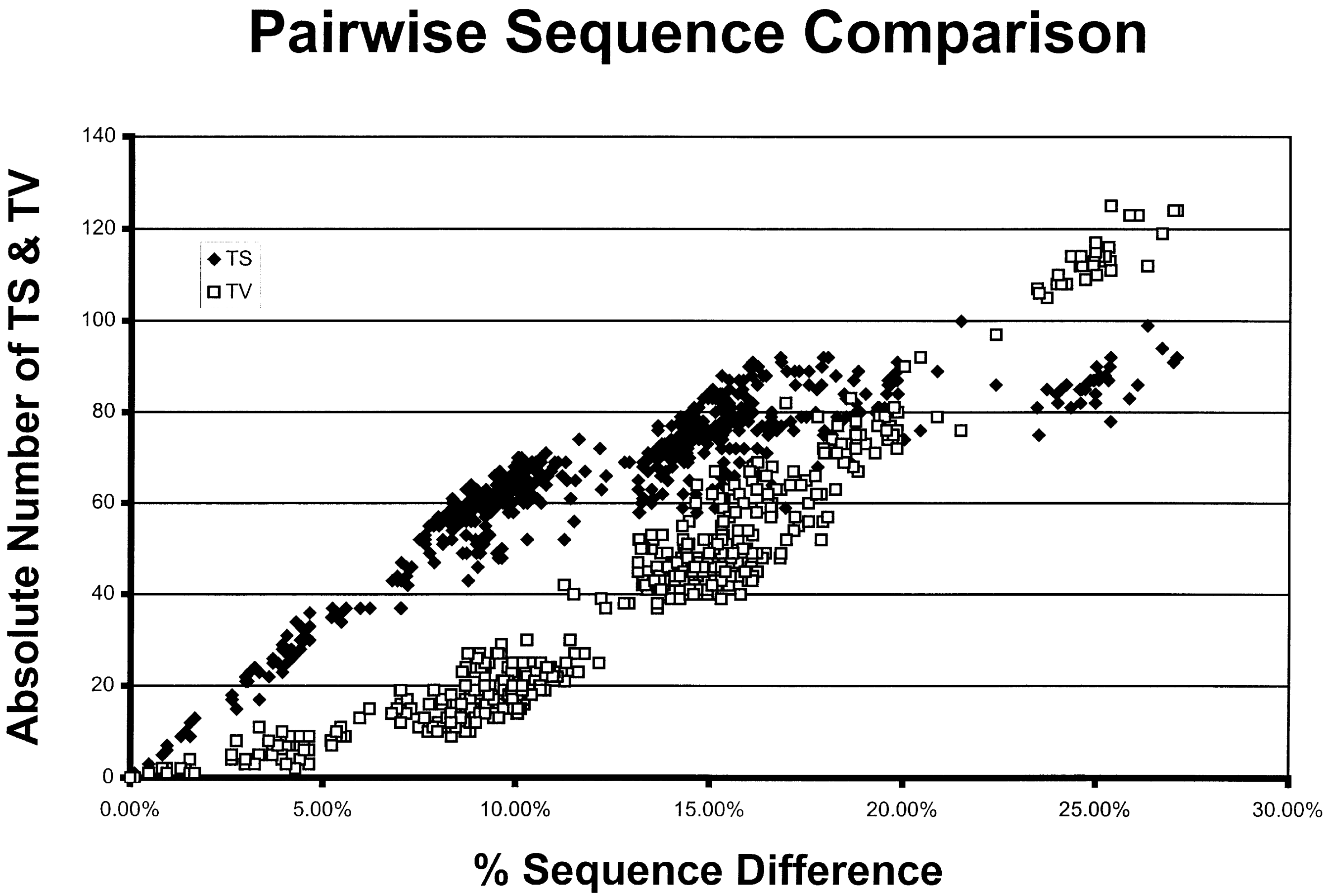


Figure 3. Scatterplot showing a pairwise sequence comparison of absolute number of transitions and transversions against percentage sequence difference (p-distance). Transitions are filled diamonds, transversions are open squares.

Table 3. Base composition of the taxa examined in this study

	% A	% C	% G	% T
Elimia olivula	36.07	13.92	16.78	33.21
Elimia haysiana	36.14	13.91	16.64	22.39
Elimia alabamensis	36.42	13.81	16.66	33.09
Elimia cylindracea	36.02	13.55	17.00	33.41
Elimia caelatura caelatura	35.91	12.96	16.76	34.36
Elimia crenatella	36.20	14.21	16.88	32.68
Elimia showalteri	35.95	13.57	17.26	33.21
Elimia hydei	36.90	13.81	16.78	32.50
Elimia virginica	35.00	14.16	18.09	32.73
Pleurocera annuliferum	35.95	13.09	16.78	34.16
Pleurocera pyrenellum	35.99	13.11	17.16	33.73
Pleurocera vestitum	35.75	13.46	17.04	33.73
Pleurocera canaliculatum filum	35.23	13.81	17.73	33.21
Pleurocera walkeri	35.23	13.92	17.61	33.21
Pleurocera prasinatum #1	35.00	13.33	17.85	33.81
Pleurocera prasinatum #2	35.31	13.55	17.36	33.76
Pleurocera unciale hastatum	35.19	13.43	17.71	33.65
Pleurocera acuta acuta	35.71	13.57	17.50	33.21
Lithasia geniculata geniculata	35.91	15.33	17.24	31.51
Lithasia geniculata fuliginosa	35.87	15.37	17.28	31.46
Lithasia duttoniana	35.80	15.39	17.30	31.50
Lithasia armigera	37.91	14.10	17.18	30.80
Io fluvialis	34.33	15.23	18.22	32.21
Leptoxis virgata	35.11	13.69	17.38	33.81
Leptoxis crassa anthonyi	34.92	14.18	17.16	33.73
Leptoxis praerosa	34.95	13.86	17.29	33.88
Leptoxis plicata	36.59	13.23	17.40	32.77
Leptoxis ampla	35.16	14.83	17.70	32.29
Leptoxis taeniata	34.60	14.43	18.25	32.69
Leptoxis picta	36.03	13.84	17.06	33.05
Juga silicula	35.52	13.87	17.82	32.75
Juga plicifera	35.16	13.87	18.06	32.89
Juga nigrina	35.28	13.75	17.94	33.01
Melanopsis praemorsa	34.45	16.44	19.20	29.89
Melanoides tuberculata	37.09	13.19	17.83	31.86
MEAN	35.68	13.99	17.42	32.89
Chi-Square = 29.8218	(df = 102)	P = 1.00000		

species form a clade that is sister to all the aforementioned clades.

Maximum parsimony analysis weighting transversions (TV) 2× transitions(TS) resulted in two equally most parsimonious trees (TL = 1803, gl = −0.869). A strict consensus tree of the two most parsimonious trees is shown in Fig. 5. One of the two most parsimonious trees is equivalent to the topology obtained when TS and TV were weighted equally (Fig. 4). The other most parsimonious tree placed *Leptoxis plicata* + *L. praerosa* as sister to *L. ampla* + *L. taeniata* + *Lithasia armigera*. Weighting TV 4× TS resulted in two most parsimonious trees (TL = 2787; Gl = −0.87). One of the two trees is equivalent to one of the most parsimonious trees obtained when weighting TV 2× TS, and the other most parsimonious tree differs in the more basal position of the *Leptoxis praerosa* + *L. plicata* clade and *Lithasia armigera* being sister to *Leptoxis praerosa* + *L. plicata* instead of *L. ampla* + *L. taeniata*.

Bootstrap values for most shallower nodes were relatively high (2/3rds of nodes > 70%) for all three phylogenetic analyses (see Figs 4 & 5). Hillis & Bull (1993) suggested that bootstrap values of 70 or more percent may correspond to confidence limits approaching 95% under conditions of equal rates of change and reasonably low internodal change. Deeper nodes in all instances exhibited less support as indicated by bootstrap values below 50%.

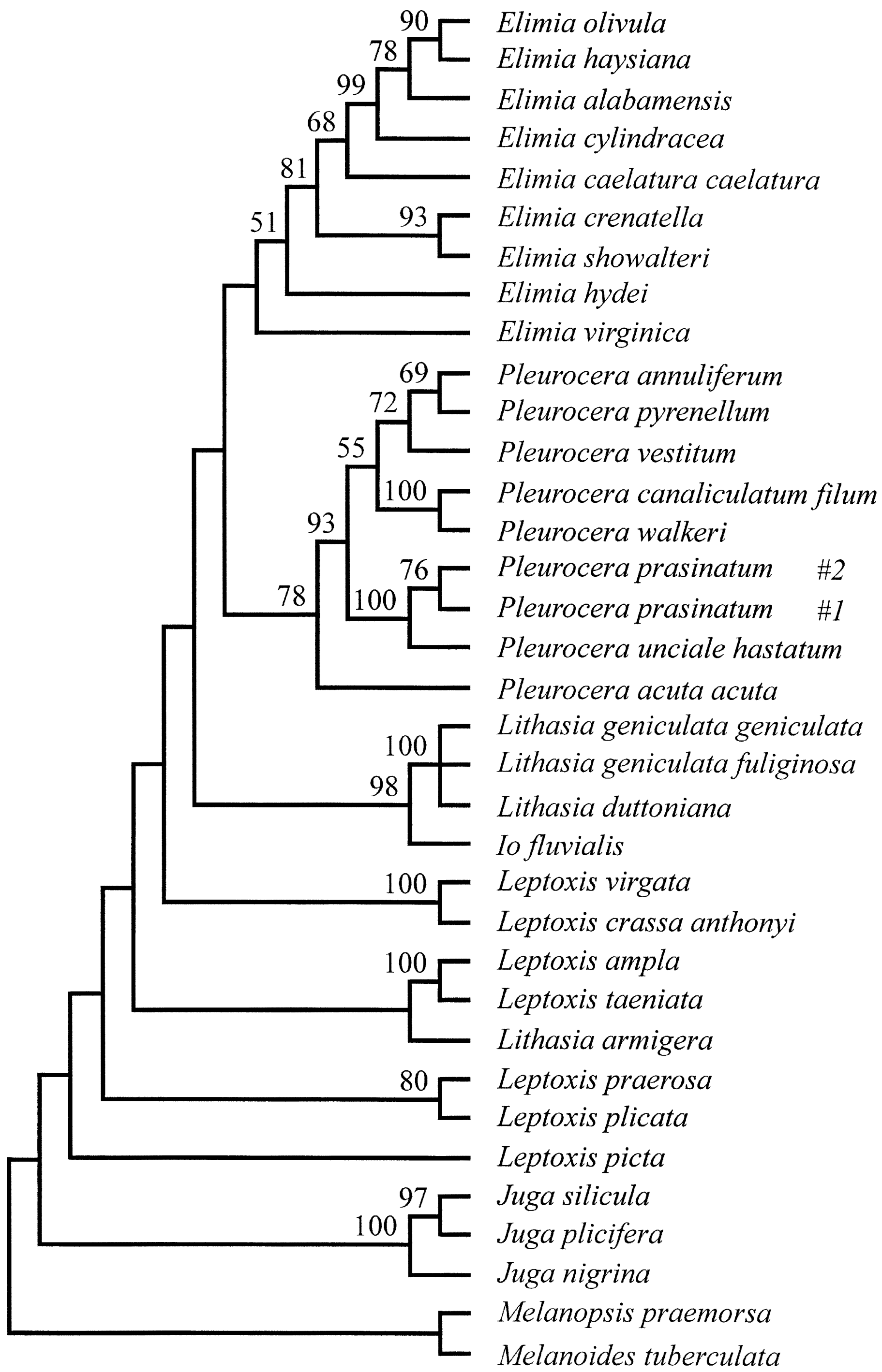


Figure 4. The single most parsimonious tree generated by maximum parsimony analysis in which all base substitutions were equally weighted (TL = 1310, CI = 0.528). Bootstrap values are shown above nodes having support greater than 50%.

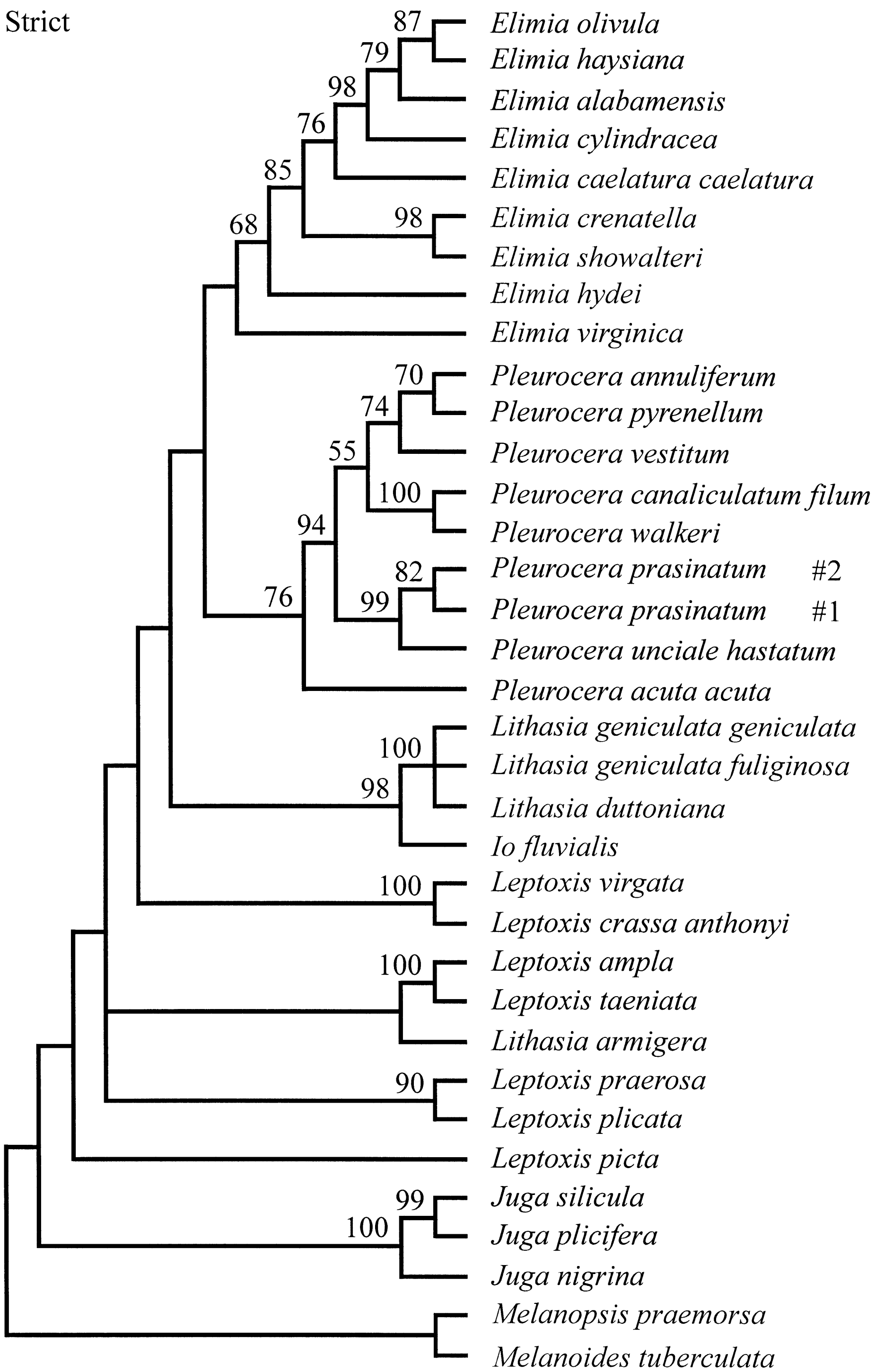


Figure 5. Strict consensus of two equally parsimonious trees generated when transversions (TV) are weighted 2× transitions (TS) (TL = 1803). Bootstrap values are shown above nodes having support greater than 50%.

DISCUSSION

The current generic groups of pleurocerids are based on shell characteristics, although there is a considerable overlap among some species between certain genera. The genus *Io* is characterized as possessing a large, fusiform shell with the anterior end of the aperture prolonged into a long canal (Burch, 1988). *Io* is currently considered monotypic, although Tryon (1873) listed five species of *Io*—two smooth or somewhat tuberculated and three spinose. Adams' (1915) comprehensive examination revealed that *Io* conchological variation is clinal, with head-water forms typically smooth and downstream forms being spinose. Further molecular studies can be used to determine if *Io* is in fact monotypic. *Io* is closely related to two species of *Lithasia* examined in our study, as hypothesized by Davis (1974).

The genus *Pleurocera* is characerized with the anterior or 'basal' end of the aperture prolonged into a short canal, producing an auger-shaped base to the shell (Burch, 1988). The molecular phylogeny based on the mitochondrial 16S rRNA gene sequences supports the monophyly of *Pleurocera*. Of the eight taxa included in our study, *Pleurocera acuta acuta*, the type species of *Pleurocera*, is the basal-most species. The relationships among the remaining taxa do not reflect any regional or traditional taxonomic grouping. For example, *P. annuliferum* (Conrad, 1834b) of the Mobile River basin is sister to *P. pyrenellum* of the Tennessee River basin, which in turn is sister to *P. vestitum* of the Mobile River basin. Furthermore, *P. annuliferum*, *P. vestitum* (Conrad, 1834b), and *P. prasinatum* (Conrad, 1834a) of the *P. prasinatum* group do not constitute a monophyletic group. All this is supported by the high bootstrap values shown at the various nodes within the *Pleurocera* clade (Figs 4 & 5). One taxonomic entity that was considered a subgenus of *Lithasia* by Tryon (1873), but later moved to *Pleurocera* by Pilsbry & Rhoads (1896) is *Strephobasis* Lea, 1861a. Goodrich (1928) agreed with Pilsbry's placement of *Strephobasis* in *Pleurocera*, and noted that the demarcations between species of the subgenus *Strephobasis* and other non-*strephobasis Pleurocera* species is exceedingly faint. Although the type species of *Strephobasis* (*Melania plena* Anthony, 1854) was not included in our study, the single *Strephobasis* representative, *P. walkeri*, is nested within the clade including other *Pleurocera* species.

The genus *Elimia* is extremely diverse conchologically. We follow Burch (1982) in using *Elimia* in place of the better known synonym *Goniobasis*. Walker (1918) characterizes the genus as possessing a shell of medium size, dextral, spiral, imperforate; smooth, longitudinally plicate, transversely striate or tuberculate; thick, solid ovate-conic to elongate turreted; aperture subrhomboidal, subangular at the base but not canaliculate; columella smooth, not twisted; lip simple and acute. These shell differences also describe pleurocerid species west of the North American Continental Divide resulting in the synonymy of *Juga* with *Elimia* by most pleurocerid systematists (e.g., Tryon, 1873; Walker, 1918; Rosewater, 1960). Burch (1982, 1988) chose to recognize the genus *Juga*, but the only difference used in his identification key to separate the two genera is whether they are found east or west of the continental divide. The molecular phylogenetic hypothesis (Fig. 4) supports the monophyly of the genus *Elimia* as delimited by Burch (1982, 1988), but it is quite likely that further taxonomic sampling will reveal the genus is para- or polyphyletic. There are several problematic species not examined in this study, such as *Elimia interrupta* (Haldeman, 1840) that exhibit conchological traits similar to other genera like *Lithasia*, which may more appropriately be assigned to other genera once more detailed studies are conducted (e.g., Arthur E. Bogan, pers. comm.). In concert, the genus *Juga* (as delimited by Burch 1980, 1982, 1988) is supported as being monophyletic based on the mtDNA-based phylogeny (Figs 4 & 5). Interestingly, *Juga* is not closely related to *Elimia*, but is sister to all genera east of the Continental Divide. Most investigators have united these two most distantly related taxa on the basis of shell characters revealing the degree to which their shell characters have converged.

The genus *Lithasia* is characterized by being conic, subglobose, ovate, or cylindrical with the surface of most species sculptured with obtuse spines or prominent nodules. The columellar margin of the aperture is thickened, meeting the anterior lip with a channel or strong angle, and a calloused thickening usually on the parietal wall at the posterior end of the aperture (Walker, 1918). The mitochondrial 16S rRNA gene tree united three *Lithasia* taxa representing two different subgenera—*Angitrema* (*Lithasia duttoniana*) and *Lithasia* (*L. geniculata geniculata*, *L. g. fuliginosa*). However, the type species of the genus *Lithasia*, *L. armigera*, was depicted as being more closely related to *Leptoxis ampla* and *Leptoxis taeniata* than to the other three

Lithasia species. Constraining the tree to produce a monophyletic *Lithasia* results in a tree that is 14 steps longer, but this topology is not significantly different from the most parsimonious tree ($P = 0.08$; Templeton's, 1873a, b sign rank test as implemented in PAUP*). The odd placement of *L. armigera* was confirmed by sequencing another specimen of *L. armigera*. Interestingly, the three species of *Lithasia* that were depicted as monophyletic have identical sequences and come from the same locality. Given the conservative nature of the mitochondrial 16S rRNA gene, it is not a surprise that the two subspecies of *Lithasia geniculata* do not differ, but perhaps *L. duttoniana* of the subgenus *Angitrema* is a product of hybridization with *Lithasia geniculata*. One of us and a colleague (R. Minton and CL, unpubl.) are conducting a systematic review of the entire genus *Lithasia* including more detailed population-level analyses and appropriate outgroup taxa to attempt to resolve this unusual finding.

Members of the genus *Leptoxis* are characterized by a medium to small, subglobose, globosely or broadly conic, or ovate shell (Burch, 1988). The molecular-based phylogeny (Fig. 4) depicts the genus as polyphyletic. The subgenus *Athearnia* Morrison, 1971 represented by *Leptoxis crassa anthonyi*, is sister to the subgenus *Mudalia* Haldeman, 1840, represented by *Leptoxis virgata*. The subgenus *Athearnia* is comprised of a single species and two subspecies. *Leptoxis crassa anthonyi* is federally listed as endangered and *Leptoxis crassa crassa* (Haldeman, 1841) is presumed extinct (Turgeon *et al*., 1998). *Athearnia* has been treated as a distinct genus (*Eurycaelon*) by some previous systematists (e.g., Tryon, 1873) and more recently in an allozyme study comparing *Leptoxis crassa anthonyi* with *Leptoxis praerosa* (Dillon & Ahlstedt, 1997). The mitochondrial 16S data reveals (Fig. 4) that the subgenus *Leptoxis* is polyphyletic. Constraining the tree to produce a monophyletic *Leptoxis* produces a tree that is 18 steps longer and is significantly different from the most parsimonious tree ($P < 0.05$). However, constraining the tree to allow *Lithasia armigera* to be nested within an otherwise monophyletic *Leptoxis* results in a tree of 11 steps longer and this is not statistically different from the most parsimonious tree. Given the limited taxonomic sampling in *Mudalia*, it would be best to await further data and a thorough review of the genus in conjunction with *Lithasia*, before making any formal taxonomic recommendations.

The genus *Gyrotoma* is distinguished by the presence of a posterior slit along the last whorl suture junction (Burch, 1988). The genus was restricted to the Coosa River of Alabama and is presumed extinct (Burch, 1988, Lydeard & Mayden, 1995; Turgeon *et al*., 1998) due to the inundation of its shoal habitat from the building of a series of impoundments during the early to mid-1900s. Regrettably, we have been unable to obtain DNA from the limited number of samples of *Gyrotoma* we have obtained that still have desiccated bodies intact. Perhaps, however, improved methods of DNA extraction will increase the likelihood of yielding DNA in the future.

Lydeard *et al*. (1997) examined the phylogenetic relationships of three Mobile Basin pleurocerid genera using mitochondrial 16S rDNA sequences and found support for the monophyly of Mobile basin *Pleurocera*, *Elimia*, and the paraphyly of *Leptoxis*. Like the present study, *Pleurocera* is sister to *Elimia*, and a paraphyletic assemblage of *Leptoxis* is sister to *Pleurocera* + *Elimia*. The present study, which includes other pleurocerid taxa from other drainages, reveals that the Mobile River basin taxa do not constitute a monophyletic assemblage.

Species Richness, Taxonomy, and Conservation

Burch (1980) provided species accounts for North American pleurocerid species and subspecies, including recognized 'morphs' or 'forms' as follows: *Elimia* (111 taxa), *Gyrotoma* (6 taxa), *Io* (1 taxon), *Juga* (12 taxa), *Leptoxis* (25 taxa), *Lithasia* (14 taxa), *Juga* (12 taxa) and *Pleurocera* (31 taxa). Burch (1988) expressed concern that the number of taxa may not reflect valid species due to the lack of any thorough systematic treatment for most genera. Indeed, the number of nominal pleurocerid species is approximately 1,000 largely due to the efforts of Isaac Lea and J. G. Anthony, who described nearly 500 and 150 species, respectively, in the early 1800s. Tryon (1873) was the first to synthesize the work done up until that time on pleurocerids and synonymized liberally, collapsing the number of species to ca. 500. Goodrich (e.g., 1922, 1924, 1934a-c, 1935a,b) continued the trend eventually resulting in the numbers reported by Burch (1980). One specific example is the synonymy of 113 nominal forms with *Pleurocera canaliculata* (Rosewater, 1960) based on an interpretation that all conchological variation represented intraspecific-level variation, but this still needs testing. Unfortunately, the lack of a thorough modern taxonomic monograph on pleurocerids has created problems in their effective management.

Pleurocerids represent one of the most endangered groups of organisms in North America with 33 species presumed extinct (Bogan, Pierson & Hartfield, 1995; Lydeard & Mayden, 1995; Neves, Bogan, Williams, Ahlstedt & Hartfield 1997; Turgeon *et al*. 1998). In the state of Alabama, U.S.A., 65% of the 147 caenogastropod snail species are thought to be either extinct, endangered, or threatened (Lydeard & Mayden, 1995). However, given the uncertain taxonomy, the number of extinctions may actually be much different. Lydeard, Yoder, Holznagel, Thompson & Hartfield (1998) found little support for the monophyly of several species of *Elimia* that are distributed throughout a single drainage basin (Coosa River, Alabama). These disjunct evolutionary entities may actually represent different species. In an analogous situation, two modern, systematic reviews of the problematic hydrobiid genus *Pyrgulopsis* Call & Pilsbry, 1886, (Hershler, 1994, 1998) resulted in the description of 131 species, whereas previously only five species of *Pyrgulopsis* were reported in Burch (1988). Clearly, modern, systematic monographic treatments are needed to discover and describe pleurocerid species diversity. We are now beginning these efforts using both traditional and molecular methods. The molecular phylogeny described herein provides an important framework from which to build.

ACKNOWLEDGEMENTS

We gratefully acknowledge S. Ahlstedt, N.H. Anderson, N. Baldwin, A.E. Bogan, T. Frest, J. Garner, M. Glaubrecht, P. Hartfield, R.D. Oesch, M. Pierson, and F.G. Thompson for providing specimens used in this study. A.E. Bogan, P. Churchill, R.L. Mayden, H. Smith-Summerville, F.G. Thompson, P.J. West, colleagues of the Advanced Systematics Discussion Group and two anonymous reviewers provided helpful comments on the manuscript. This research was supported in part by Research Grants Committee Award (2-67858) from the University of Alabama, National Science Foundation (DEB-9707623) to CL, and a contract with the U.S. Department of Interior (1448-0004-04-929).

REFERENCES

ADAMS, C.C. 1900. Variation in *Io. Proceedings of the American Association for the Advancement of Science*, **49**: 208-225, pls i-xxuii.

ADAMS, C.C. 1915. The variations and ecological distribution of the snails of the genus *Io. Memoirs of the National Academy of Sciences*, **12**: 1-92., pls. 1-60.

ADAMS, H. & ADAMS, A. 1853. *The genera of Recent Mollusca; arranged according to their organization*, Vol. 1. John Van Voorst, London, 256 pp.

ANTHONY, J.G. 1843. [Published letter on *Schizostoma* and *Melatoma*.] *Proceedings of the Academy of Natural Sciences of Philadelphia*, **1**: 251.

ANTHONY, J.G. 1854. Descriptions of new fluviatile shells of the genus *Melania* Lamarck, from the western states of North America. *Annual Report of the Lyceum of Natural History of New York*, **6**: 80-130.

ANTHONY, J.G. 1855. Descriptions of new species of *Ancylus* and *Anculosa*, from the western states of North America. *Annual Report of the Lyceum of Natural History of New York*, **6**: 158-160.

BOGAN, A.E., PIERSON, J.M. & HARTFIELD, P. 1995. Decline in the freshwater gastropod fauna in the Mobile Basin. *Our Living Resources: A report to the Nation on the distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems* (E.T. LaRoe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac, eds.), pp. 249-252, U.S. Department of the Interior, National Biological Service, Washington, DC.

BROWN, D.S. 1994. *Freshwater snails of Africa and their medical importance*. Revised 2nd ed. Taylor & Francis, London.

BURCH, J.B. 1980. North American Freshwater Snails. Species list, ranges and illustrations. *Walkeriana*, **1**: 81-215.

BURCH, J.B. 1982. North American Freshwater Snails. Identification keys, generic synonymy, supplemental notes, glossary, references, index. *Walkeriana*, **1**: 217-365.

BURCH, J.B. 1988. North American Freshwater Snails. Introduction, systematics, nomenclature, identification, morphology, habitats, distribution. *Walkeriana*, **2**: 1-80.

CABOT, E.L. & BECKENBACH, A.T. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Cabios*, **5**: 233-234.

CALL, R.E. & PILSBRY, H.A. 1886. On *Pyrgulopsis*, a new genus of rissoid mollusk, with descriptions of two new forms. *Proceedings of the Davenport Academy of Natural Science*, **5**: 9-14.

CONRAD, T.A. 1834a. Description of some new species of fresh water shells from Alabama, Tennessee. *American Journal of Science and Arts*, **25**: 338-343. [Reprinted in *Sterkiana*, 1963, 49-52.]

Conrad, T.A. 1834b. *New fresh water shells of the United States, with coloured illustrations, and a monograph of the genus* Anculotus *of Say; also a synopsis of the American naiades*. Judah Dobson, Philadelphia, 76pp.

COX, L.R. 1960. Thoughts on the classification of the Gastropoda. *Proceedings of the Malacological Society of London*, **33**: 239-261.

DAVIS, G.M. 1974. *Report on the rare and endangered status of a selected number of freshwater Gastro-*

poda from southeastern U.S.A. Unpublished report submitted to the Fish and Wildlife Service, U.S. Dept. of Interior, Washington, D.C., 15 December 1974, 51 pp., maps.

DAZO, B.C. 1965. The morphology and natural history of *Pleurocera acuta* and *Goniobasis livescens* (Gastropoda: Cerithiacea: Pleuroceridae). *Malacologia*, **3**: 1-80.

DILLON, R.T. & AHLSTEDT, S.A. 1997. Verification of the specific status of the endangered Anthony's River Snail, *Athearnia anthonyi*, using allozyme electrophoresis. *Nautilus*, **110**: 97-101.

FELSENSTEIN, J.F. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**: 783-791.

FÉRUSSAC, J.B.L. D'A DE. 1819. *Histoire naturelle général et particuliére des mollusques terrestres et fluviatiles*. Paris, 128 pp, 162 pls.

FISCHER, P. 1885. *Manuel de conchyliologie et de paléontologie conchyliologique ou histoire naturelle des mollusques vivants et fossiles (suivi d'un appendice sur les Brachiopodes par D.P. Oehlert)*. F. Savy, Paris, pp 689-784.

GLAUBRECHT, M. 1996. *Evolutionsökologie und Systematik am Beispiel von Süß-und Brackwasserschnecken (Mollusca: Caenogastropoda: Cerithioidea): Ontogenese-Strategien, Paläontologische Befunde und Historische Zoogeographie*. Backhuys Publishers, Leiden, The Netherlands.

GLAUBRECHT, M. 1998. Wrestling with homology: evolution of vivipary in tropical freshwater gastropods (Cerithioidea: Thiaridae sensu lato). *Abstracts of the World Congress of Malacology, Washington DC*, (Bieler, R. & Mikkelsen, P.M., eds). p. 123. Field Museum of Natural History, Chicago.

GLAUBRECHT, M. 1999. *Systematics and the evolution of vivipary in tropical freshwater gastropods (Cerithioidea: Thiaridae sensu lato)*. Courier Forschungsinstitut Senckenberg Frankfurt a.M., vol. 203.

GOODRICH, C. 1922. The *Anculosae* of the Alabama River drainage. *Miscellaneous Publications, Museum of Zoology, University of Michigan*, **7**: 1-57 pls. 1, 2.

GOODRICH, C. 1924. The genus *Gryotoma. Miscellaneous Publications, Museum of Zoology, University of Michigan*, **12**: 1-29, pls. 1, 2.

GOODRICH, C. 1928. *Strephobasis*: a section of *Pleurocera. Occasional Papers, Museum of Zoology, University of Michigan*, **192**: 1-15, pls. 1, 2.

GOODRICH, C. 1931. The pleurocerid genus *Eurycaelon. Occasional Papers, Museum of Zoology, University of Michigan*, **223**: 1-9, pl. 1.

GOODRICH, C. 1934a. Studies of the gastropod family Pleuroceridae—I. *Occasional Papers, Museum of Zoology, University of Michigan*, **286**: 1-17, pl. 1.

GOODRICH, C. 1934b. Studies of the gastropod family Pleuroceridae—II. *Occasional Papers, Museum of Zoology, University of Michigan*, **295**: 1-6.

GOODRICH, C. 1934c. Studies of the gastropod family Pleuroceridae—III. *Occasional Papers, Museum of Zoology, University of Michigan*, **300**: 1-11.

GOODRICH, C. 1935a. Studies of the gastropod family Pleuroceridae—IV. *Occasional Papers, Museum of Zoology, University of Michigan*, **311**: 1-11.

GOODRICH, C. 1935b. Studies of the gastropod family Pleuroceridae—V. *Occasional Papers, Museum of Zoology, University of Michigan*, **318**: 1-12.

GOODRICH, C. 1937. Studies of the gastropod family Pleuroceridae—VI. *Occasional Papers, Museum of Zoology, University of Michigan*, **347**: 1-12.

GOODRICH, C. 1938. Studies of the gastropod family Pleuroceridae—VII. *Occasional Papers, Museum of Zoology, University of Michigan*, **376**: 1-12.

GOODRICH, C. 1941. Pleuroceridae of the small streams of the Alabama River system. *Occasional Papers, Museum of Zoology, University of Michigan*, **428**: 1-30.

GOULD, A.A. 1847. [Descriptions of *Melania*, from the collection of the Exploring Expedition.] *Proceedings of the Boston Society of Natural History*, **2**: 222-225.

GOULD, A.A. 1861. On the specific distribution of faunae far removed from one another. *Proceedings of the Boston Society of Natural History*, **7**: 98.

GRAY, J.E. 1850. Explanation of plates and list of genera. In: *Figures of Molluscous Animals, Selected from various Authors. Etched for the Use of Students, Vol. 4*, by M.E. Gray. 124 pp. Longman, Brown, Green and Longman, London.

GYLLENSTEN, U.B. & ERLICH, H.A. 1988. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. *Proceedings of the National Academy of Sciences of the USA*, **85**: 7652-7656.

HALDEMAN, S.S. 1840. *A monograph of the Limniades and other freshwater univalve shells of North America*. Suppl. to No. 1, 1840, pp. 1-3. J. Dobson, Philadelphia.

HALDEMAN, S.S. 1841. *A monograph of the Limniades and other freshwater univalve shells of North America*. No. 3, 1841, [*Limnea*], pp. 1-16. J. Dobson, Philadelphia.

HERSHLER, R. 1994. A review of the North American freshwater snail genus *Pyrgulopsis* (Hydrobiidae). *Smithsonian Contributions to Zoology*, **554**: 115 pp.

HERSHLER, R. 1998. A systematic review of the hydrobiid snails (Gastropoda: Rissooidea) of the Great Basin, western United States. Part I. Genus *Pyrgulopsis. Veliger*, **41**: 1-132.

HILLIS, D.M. & BULL, .J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, **42**: 182-192.

HILLIS, D.M. & HUELSENBECK, J.P. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity*, **83**: 189-195.

HOUBRICK, R.S. 1988. Cerithioidean phylogeny. In: *Prosobranch phylogeny: Proceedings of a symposium held at the 9th International Malacological Congress, Edinburgh, 1986 (Ponder, W.F., ed.).* Malacological Review, Suppl. **4**: 88-128.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**: 111-120.

KUMAR, S., TAMURA, K. & NEI, M. 1993. *MEGA: Molecular Evolutionary Genetics Analysis, Institute* *of Molecular Evolutionary Genetics*. Pennsylvania State University, University Park.

LEA, I. 1831. Description of a new genus of the family Melaniana of Lamarck. *Transactions of the American Philosophical Society*, **4**: 122-123.

LEA, I. 1838. Description of New Freshwater and Land Shells. *Transactions of American Philosophical Society*, **6**: 1-154.

LEA, I. 1841. New Fresh Water and Land Shells. *Proceedings of the American Philosophical Society*, **2**: 11-15.

LEA, I. 1842. Description of New Fresh Water and Land Shells. *Transactions of the American Philosophical Society*, **8**: 163-250.

LEA, I. 1843. Continuation of Paper on Fresh Water Shells. *Proceedings of the American Philosophical Society*, **2**: 241-243.

LEA, I. 1845. Description of New Fresh Water and Land Shells. *Proceedings of the American Philosophical Society*, **4**: 162-168.

LEA, I. & LEA, H.C. 1850. Description of a New Genus of the Family Melaniana, and of many New Species of the Genus *Melania*, chiefly collected by Hugh Cuming, Esq., during his Zoological Voyage in the East, and now first described. *Proceedings of the Zoological Society of London*, **8**: 179-197.

LEA, I. 1856. Description of New Fresh-water Shells from California. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **8**: 80-81.

LEA, I. 1861a. Description of a new genus (*Strephobasis*) of the family Melanidae, and three new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **13**: 96.

LEA, I. 1861b. Description of forty-nine New Species of the Genus *Melania. Proceedings of the Academy of Natural Sciences of Philadelphia*, **13**: 117-123.

LEA, I. 1862a. Description of a new genus (*Goniobasis*) of the family Melanidae and eighty-two new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **14**: 262-272.

LEA, I. 1862b. New Melanidae of the United States. *Journal of the Academy of Natural Sciences of Philadelphia*, **5**: 217-356.

LEA, I. 1864. Description of a New Genus of the Family Melanidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **16**: 2-3.

LINNAEUS, C.A. 1758. *Systema Naturae, per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentilis, synonymis, locis*. 10th edition. Laurentii Salvii, Holmiae. 824pp.

LOCKHART, P.J., STEEL, M.A., HENDY, M.D. & PENNY, D. 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. *Molecular Biology and Evolution*, **11**: 605-612.

LYDEARD, C. & MAYDEN, R.L. 1995. A diverse and endangered aquatic ecosystem of the southeastern United States. *Conservation Biology*, **9**: 800-805.

LYDEARD, C., HOLZNAGEL, W.E., GARNER, J., HARTFIELD, P. & PIERSON, M. 1997. A molecular phylogeny of Mobile River drainage pleurocerid Snails (Caenogastropoda: Cerithioidea). *Molecular Phylogenetics and Evolution*, **7**: 117-128.

LYDEARD, C., YODER, J.H., HOLZNAGEL, W.E., THOMPSON, F.G. & HARTFIELD, P. 1998. Phylogenetic utility of the 5′-half of Mitochondrial 16S rDNA gene sequences for inferring relationships of *Elimia* (Cerithioidea: Pleuroceridae). *Malacologia*, **39**: 183-193.

LYDEARD, C., HOLZNAGEL, W.E., SCHNARE, M.N. & GUTELL, R.R. 2000. Phylogenetic analysis of molluscan Mitochondrial LSU rDNA sequences and secondary structure. *Molecular Phylogenetics and Evolution*, (In Press).

MORRISON, J.P.E. 1954. The relationships of Old and New World Melanians. *Proceedings of the United States National Museum*, **103**: 357-394, pl. 11.

MORRISON, J.P.E. 1971. *Athearnia*, a new name for a genus of pleurocerid snails. *Nautilus*, **84**: 110-111.

MÜLLER, O.F. 1774. *Vermium terrestrium et fluviatilium, seu animalium infusoriorum, helminthicorum et testaceoum, non marinorum, succincta historia*. Heineck et Faber, Havniae et Lipsiae. Vol. 2, xxxvi, 214 pp.

NEVES, R.J., BOGAN, A.E., WILLIAMS, J.D., AHLSTEDT, S.A. & HARTFIELD, P.W. 1997. Status of aquatic mollusks in the southeastern United States: A downward spiral of diversity. In: *Aquatic Fauna in Peril: The Southeastern perspective* (G.W. Benz and D.E. Collins, eds), 43-86. Special Publication 1, Southeastern Aquatic Research Institute, Lenz Design & Communications, Decatur, Georgia.

PALUMBI, S., MARTIN, A., ROMANO, S., MCMILLAN, W.O., STICE, L. & GRABOWSKI, G. 1991. *A simple fools guide to PCR*, Privately distributed, Honolulu, Hawaii.

PILSBRY, H.A. & RHOADS, S.N. 1896. Contributions to the Biology of Tennessee, No. 4 Mollusks. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **48**: 487-506.

PONDER, W.F. 1991. The anatomy of *Diala*, with an assessment of its taxonomic position (Mollusca: Cerithiodea). In: *Proceedings of the Third International Marine Biological Workshop*: *The marine flora and fauna of Albany, Western Australia* (Wells, F.E., Walker, D.I., Kirkman H., and R. Lethbridge eds), Vol. 2, 499-519. Western Australian Museum, Perth.

PONDER, W.F. & WARÉN, A. 1988. Classification of the Caenogastropoda and Heterostropha—a list of the family-group names and higher taxa. *Malacological Review*, Suppl. **4**: 288-326.

RAFINESQUE, C.S. 1819. De 70 nouveaux genres d'animaux découverts dans l'intérieur des Etats-Unis d'Amérique, durant l'année 1818. *Journal de Physique, de Chimie, d'Histoire Naturelle et des Arts*, **88**: 423-428.

RAFINESQUE, C.S. 1831. *Enumeration and account of some remarkable natural objects in the cabinet of Prof. Rafinesque, in Philadelphia; being animals, shells, plants and fossils, collected by him in North America.* C.S. Rafinesque, Philadelphia. 8 pp.

REDFIELD, J.H. 1854. Descriptions of new species of shells. *Annual report of the Lyceum of Natural History of New York*, **6**: 130-132.

REEVE, L.A. 1869. *Conchologia Iconica: or illustrations of the shells of molluscous animals*. Lovell Reeve, Covent Garden, London.

ROSEWATER, J. 1960. *Geographic variation of* Pleurocera canaliculata *(Mollusca: Prosobranchia) in the Ohio River System*. Unpublished Doctoral Thesis. Harvard University, Cambridge, Massachusetts. 224 pp.

SAY, T. 1817. Descriptions of new species of land and fresh water shell of the United States. *Journal of the Academy of Natural Sciences of Philadelphia*, **1**: 276-284.

SAY, T. 1821. Descriptions of the univalve shells of the United States. *Journal of the Academy of Natural Sciences of Philadelphia*, **2**: 149-179.

SAY, T. 1825. Descriptions of some new species of fresh water and land shells of the United States. *Journal of the Academy of Natural Sciences of Philadelphia*, **5**: 119-131.

SHUTTLEWORTH, R.J. 1845. Über *Gyrotoma*, eine neue Gattung der Melaniana, Gasteropoda Pectinibranchiata. *Mittheilung der naturforschenden Gesellschaft in Bern*, 85-88.

STEIN, C.B. 1976. *Gastropods*. Pages 21-41 in Endangered and threatened species of Alabama, Bulletin no. 2. University of Alabama Museum of Natural History, Tuscaloosa.

STAROBOGATOV, Y.I. 1970. *Mollusk fauna and the zoogeographic partitioning of continental waterbodies of the globe*. [In Russian.] Nauka, Leningrad Branch of the Academy of Science, U.S.S.R., Leningrad, 372 pp.

SWAINSON, W. 1840. [A Treatise on Malacology; or the natural classification of shells and shellfish.] Longman, Brown, Green, and Longmans, Paternoster Row, London.

SWOFFORD, D. 1998. *PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.0b1,* Computer program distributed by the Illinois Natural History Survey, Champaign, Ill.

TEMPLETON, A.R. 1983a. Convergent evolution and non-parametric inferences from restriction fragment and DNA sequence data. In: *Statistical Analysis of DNA sequence data* (B. Weir, ed.), 151-179. Marcel Dekken, New York.

TEMPLETON, A.R. 1983b. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. *Evolution*, **37**: 221-244.

THIELE, J. 1921. Zur Systematik der Mollusken. *Archiv für Molluskenkunde*, **53**: 140-163.

THIELE, J. 1929. *Handbuch der systematischen Weichtierkunde*. Part 1. Gustav Fischer, Jena, 376 pp.

TROSCHEL, F.H. 1856-93. *Das Gebiß der Schnecken zur Bergründung einer natürlichen Classification*. Vol. 1, 252 pp., Vol. 2, 409 pp. Nicolaische Verlagsbuchhandl., Berlin. [Vol. 1, pp. 73-112, 1857].

TRYON, G.W., JR. 1873. Land and Freshwater Shells of North America. Part IV. Strepomatidae. *Smithsonian Miscellaneous Collections*, No. 253: i-iv, 1-435.

TURGEON, D.D., QUINN, J.F., BOGAN, A.E., COAN, E.V., HOCHBERG, F.G., LYONS, W.G., MIKKELSEN, P.M., NEVES, R.J., ROPER, C.F.E., ROSENBERG, G., ROTH, B., SCHELTEMA, A., THOMPSON, F.G., VECCHIONE, M. & WILLIAMS, G.D. 1998. *Common and scientific names of aquatic invertebrates from the United States and Canada: Mollusks* (2nd ed.). American Fisheries Society Special Publication **26**.

WALKER, B. 1918. A synopsis of the classification of the freshwater Mollusca of North America, north of Mexico, and a catalogue of the more recently described species, with notes. *Miscellaneous Publications, Museum of Zoology, University of Michigan*, **6**: 1-213.