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Phylogenetic Relationships of the Cochliopinae (Rissooidea: Hydrobiidae): An Enigmatic Group of Aquatic Gastropods

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Phylogenetic analysis based on a partial sequence of the mitochondrial cytochrome *c* oxidase subunit I gene was performed for 26 representatives of the aquatic gastropod subfamily Cochliopinae, 6 additional members of the family Hydrobiidae, and outgroup species of the families Rissoidae and Pomatiopsidae. Maximum-parsimony analysis yielded a single shortest tree which resolved two monophyletic groups: (1) a clade containing all cochliopine taxa with the exception of *Antroselates* and (2) a clade composed of *Antroselates* and the hydrobiid genus *Amnicola*. The clade containing both of these monophyletic groups was depicted as more closely related to members of the family Pomatiopsidae than to other hydrobiid snails which were basally positioned in our topology. New anatomical evidence supports recognition of the cochliopine and *Antroselates–Amnicola* clades, and structure within the monophyletic group of cochliopines is largely congruent with genitalic characters. However, the close relationship between the Pomatiopsidae and these clades is in conflict with commonly accepted classifications and suggests that a widely accepted scenario for genitalic evolution in these snails is in need of further study.

INTRODUCTION

The cosmopolitan gastropod family Hydrobiidae is the largest group of freshwater mollusks, with several thousand species placed in more than 300 genera (Kabat and Hershler, 1993). Despite the ubiquity and ecological importance of these small, locally abundant snails, the biology of few species has been thoroughly investigated and the systematics of the group remains unstable at all ranks. The divergent classifications that have been offered for the hydrobiids (Kabat and Hershler, 1993) have not been well tested as there is no rigorously proposed analysis of relationships that includes more than a trivial sampling of this large group (e.g., Altaba, 1993; Ponder *et al.,* 1993; Ponder, 1999). Phylogenetic reconstructions of these animals have been hampered by a paucity of apparent synapomorphies (Thompson, 1984), putatively extensive homoplasy (Davis, 1988; Hershler and Thompson, 1992), and difficulties in reconciling homology (Hershler and Ponder, 1998). Whereas a recent survey and reassessment of characters (Hershler and Ponder, 1998) may pave the way for more fruitful analyses that use morphological criteria, the study of hydobiid phylogeny clearly would benefit from infusion of additional data sets, and we are utilizing DNA sequences of mitochondrial genes for this purpose.

One of our goals is to assess the phylogenetic relationships of the major groups of hydrobiid snails. In this paper we focus on the subfamily Cochliopinae, which is composed of 34 genera and more than 260 species and is distributed within tropical and temperate America and several regions of the Old World (Hershler and Thompson, 1992; Altaba, 1993; Hershler and Velkovrh, 1993; Hershler, 1999). The Cochliopinae was early conceived as a small group of squat-shelled hydrobiids (Taylor, 1966; Starobogatov, 1970). More recently, Hershler and Thompson (1992) reconstituted the subfamily as a larger group of diverse-shelled taxa diagnosed primarily by the partial or complete separation of the sperm-conducting channel (sperm tube) from the female glandular oviduct and the absence of a glandular duct within the male penis. However, monophyly of the cochliopines was not established in this nonphylogenetic treatment and was considered suspect because these diagnostic characters are not unique in the Hydrobiidae—amnicolines share with cochliopines a diaulic arrangement of the female genitalia (Hershler and Thompson, 1988) and most hydrobiids lack an accessory glandular duct in the penis. The phylogenetic relationships of the cochliopines also are uncertain. Several other rissooidean families have di-

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aulic female genitalia (e.g., Pomatiopsidae, Stenothyridae) and a minority of workers have suggested that cochliopines are more closely related to these than to hydrobiids (Starobogatov, 1970; Ioganzen and Starobogatov, 1982; Starobogatov and Sitnikova, 1983). Others have argued that diauly was independently evolved by the cochliopines within the Hydrobiidae (Davis *et al.,* 1982; Ponder, 1988; Hershler and Ponder, 1998) and this is reflected in most of the recent classifications of these snails. The single detailed analysis of rissooidean phylogeny did not resolve this issue as its terminals were composite representations of individual families (Ponder, 1988), and a more recent analysis of a small number of taxa supports the latter hypothesis (Ponder, 1999).

In a recent paper, which focused on analysis of phylogenetic relationships of species of the genus *Tryonia* by use of partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, eight cochliopine genera were shown to be a monophyletic group relative to two Australian hydrobiid outgroups (Hershler *et al.,* 1999). Here we analyze partial sequences of this same gene from a broader spectrum of cochliopine and other taxa to more fully assess monophyly of the subfamily and to examine its relationships to other rissooidean snails. We also contrast phylogenetic structure within the cochliopines with previously proposed morphological groupings (Hershler and Thompson, 1992) and briefly discuss biogeographic implications of our data.

MATERIALS AND METHODS

Specimens

For this study we utilized partial mtCOI sequences of 26 cochliopine species placed in 18 genera. Ten of these sequences are newly reported herein and others were previously reported by Hershler *et al.* (1999). We have sampled cochliopines from all of the major geographic regions where these snails occur except tropical Africa (to which the genus *Lobogenes* is endemic), and we included in our analysis two or more representatives of each of the three subgroups of cochliopines proposed by Hershler and Thompson (1992). For 17 of the cochliopine genera we analyzed 1 or 2 species and 7 species were analyzed for *Tryonia* to encompass the phylogenetic diversity of these snails previously revealed by mtCOI sequences (Hershler *et al.,* 1999). The genus name is used in parentheses for those species of *Tryonia* that are being transferred to other genera based on molecular and anatomical data (Hershler, 2001). To test monophyly of the Cochliopinae and to determine its phylogenetic position within the Rissooidea, we sampled representatives of the hydrobiid subfamily Amnicolinae and the family Pomatiopsidae, which both share with cochliopines a diaulic arrangement of the female genitalia. We also sampled four additional hydrobiids that include representatives of two other commonly accepted subfamilies (Lithoglyphinae, Nymphophilinae) and two putatively plesiomorphic hydrobiids (*Hydrobia* and *Phrantela*). Trees were rooted with *Rissoina,* a representative of the primitive marine rissooidean family Rissoidae (*fide* Ponder, 1985, 1988, Fig. 4). We also utilized several mtCOI sequences of pomatiopsids and rissoids reported by Davis *et al.* (1998). Taxa are listed in Table 1. Samples not assigned to species represent novelties whose generic placements were based on our morphological study of voucher material. Voucher material (UF, Florida Museum of Natural History, University of Florida; USNM, National Museum of Natural History, Smithsonian Institution) is cited in Table 1 for taxa whose sequences are newly provided herein.

Laboratory Methods

Snails were collected alive and placed directly in 90% ethanol. Genomic DNA was extracted from these specimens with the CTAB method (Bucklin, 1992). A segment of the mitochondrial cytochrome *c* oxidase subunit I gene was amplified via polymerase chain reaction (PCR) with primer pairs COIL1490 and COIH2198 (Folmer *et al.,* 1994) or COIL1492 and COIH2390. The COIL1492 (5′TCA ACA AAT CAT AAA GAT ATT GGT AC3′) was a modification of COIL1490 that we developed for hydrobiid snails. The COIH2390 (5′ATA GTA GCC GCA GTA AAA TAA GC3′) was based on published mtDNA sequences of several mollusks (*Cypraea, Katharina,* and *Mytilus;* GenBank) and our unpublished sequences of hydrobiid snails.

Approximately 100 ng of genomic DNA was used as a template for double-stranded PCRs in a 25-μl reaction solution containing each dNTP at 125 μM, each primer at 0.5 μM, 0.5 unit *Taq* polymerase (Applied Biosystems, Inc.), and 5 μl 5$\times$ optimizer kit buffer F (Invitrogen, Inc.). Reactions were amplified for 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The PCR product was incubated at 37°C for 15 min and then at 85°C for another 15 min with 0.5 unit shrimp alkaline phosphatase (SAP; Amersham) and 5 unit exonuclease I (ExoI; Amersham) to remove remaining primers and nucleotides. Approximately 30–70 ng of cleaned PCR product served as a template in a cycle sequencing reaction that used the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Inc.). The following cycling conditions were used: 96°C for 4 min and then 30 cycles of 94°C for 15 s, 50°C for 5 s, and 60°C for 4 min. The cycle sequencing product was cleaned with the ethanol precipitation method and then run on an ABI 310 sequencer. Sequences were edited and aligned with Sequencher and checked by eye. Sequences were deposited under GenBank Accession Nos. AF354758–AF354774.

TABLE 1

List of Taxa Analyzed

Taxa	Location	Accession No.
Cochliopinae		
Antroselates spiralis	Harrison Cave Spring, Harrison Co., IN (USNM 883970)	AF354758
[1]*Aphaostracon* sp.	Alexander Springs, Lake Co., FL (AS); Lake Panasoffkee, Sumter Co., FL (LP)	
Aroapyrgus sp.	Rio Jagual, 2.0 km southwest of Guabalá, Chiriquí Prov., Panama (UF 271930)	AF354759
Cochliopa sp.	Rio Jagual, 2.0 km southwest of Guabalá, Chiriqui Prov., Panama (UF 271928)	AF354762
Cochliopina riograndensis	Sycamore Creek, Hwy 277 crossing, Val Verde Co., TX (USNM 905042)	AF354760
Durangonella coahuilae	Spring, west of Sierra San Marcos, Cuatro Cienegas, Coahuila, Mexico (USNM 854953)	AF354761
[3]*Eremopyrgus eganensis*	Steptoe Valley, north of Ely, White Pine Co., NV	
[1]*Heleobia dalmatica*	Spring Vrilo, Pirovac, Sibenik, Croatia	
[1]*Heleobops docimus*	Chisholm Point, Grand Cayman Island, United Kingdom	
Lithococcus multicarinatus	Rio Cayapas, south of Burbón, Esmeraldas Prov., Ecuador (UF 271943)	AF354763
[1]*Littoridinops monroensis*	Fort Gates Ferry, St. Johns Co., FL	
[1]*Littoridinops palustris*	Marsh, 4.0 km southwest of Yankeetown, Levy Co., FL	
[1]*Mexipyrgus carranzae*	Mojarral West Laguna, Cuatro Cienegas, Coahuila, Mexico	
Mexithauma quadripaludium	Laguna Churince, Cuatro Cienegas, Coahuila, Mexico (USNM 863501)	AF354764
[1]*Onobops jacksoni*	Marsh, 4 km southwest of Yankeetown, Levy Co., FL	
[1]*Pyrgophorus platyrachis*	Lithia Springs, Hilsborough Co., FL	
Spurwinkia salsa	Spurwink River, Cape Elizabeth, Cumberland Co., ME (USNM 883966)	AF354765
[1]*Tryonia aequicostata*	Lake Eustis, Lake Co., FL	
[1]*Tryonia clathrata*	Moapa National Wildlife Refuge, Clark Co., NV	
[1]*Tryonia rowlandsi*	Grapevine Springs, Death Valley, CA	
[1]*"Tryonia" alamosae*	Spring 100 m west of Ojo Caliente, Socorro Co., NM	
[1]*"Tryonia" brevissima*	Lake Panasoffkee, Sumter Co., FL	
[1]*"Tryonia" kosteri*	Lost River, Chaves Co., NM	
[1]*"Tryonia" robusta*	Travertine Springs, Death Valley, Inyo Co., CA	
Zetekina sp. 1	Creek, 8.0 km south-southeast of Sona, Veraguas Prov., Panama (UF 271908)	AF354766
Zetekina sp. 2	Rio Jagual, 2.0 km southwest of Guabalá, Chiriquí Prov., Panama (UF 271926)	AF354767
Other Hydrobiidae		
Amnicola limosa (Amnicolinae)	Scarborough, Cumberland Co., ME (USNM 883963)	AF354768
Amnicola dalli (Amnicolinae)	Juniper Springs, Marion Co., FL (USNM 863500)	AF354769
Hydrobia acuta	Lagoon 6, Suffolk, East Anglia, United Kingdom (USNM 892204)	AF354773
Lithoglyphus naticoides (Lithoglyphinae)	River Ipel, Sahy, Slovakia (USNM 892077)	AF354770
Nymphophilus minckleyi (Nymphophilinae)	Laguna Churince, Cuatro Cienegas, Coahuila, Mexico (USNM 863502)	AF354771
[1]*Phrantela marginata*	Tributary of Thirteen Mile Creek, Tasmania, Australia	
Outgroups		
[2]*Gammatricula chinensis* (Pomatiopsidae)	Zhejiang Province, Kaiwa Co., China	
[2]*Oncomelania hupensis* (Pomatiopsidae)	Hubei Province, Han Yang Co., China	
Pomatiopsis lapidaria (Pomatiopsidae)	Hudson River, Cruger Island, Columbia Co., NY (USNM 883968)	AF354774
[2]*Tricula* sp. (Pomatiopsidae)	Sichuan Province, Dayi Co., China	
Rissoina fasciata (Rissoidae)	Long Reef, Collaroy, Sydney N, NSW, Australia (USNM 894317)	AF354772
[2]*Setia turriculata* (Rissoidae)	1 km west of Nessebar, Bulgaria	

[1] Sequence reported by Hershler *et al.* (1999).
[2] Sequence reported by Davis *et al.* (1998).
[3] Reported as *Tryonia* n.sp. 2 in Hershler *et al.* (1999).

Analyses

Base compositional differences were evaluated with the χ^2 test for all positions and for the third codon positions only. Only single representatives of divergent genera were included in homogeneity tests to limit the correlation due to shared ancestry. Compositional heterogeneity was also examined by estimation of the compositional distance between pairs of taxa with the equation

$$D = \sum_i (p_i - q_i)^2 / \sum_i [2P_i(1 - P_i)/n],$$

where the frequencies of the four bases (i = G, A, T, C) in two sequences being compared are p_i and q_i; $P_i = (p_i +$

q_i)/2; and n is the number of nucleotides in the comparison (Gillespie, 1986; Martin and Bermingham, 1998).

Mutational saturation for each codon was examined by the plotting of the absolute number of transitions (TS) and transversions (TV) against uncorrected genetic distance (p-distance) and by the plotting of p-distance against inferred distance (TrN distance, HKY85 distance) (Berbee *et al.,* 1995; Griffiths, 1997; Siemer *et al.,* 1998).

No intraspecific sequence differences were observed in *Amnicola dalli, Amnicola limosa, Durangonella coahuilae, Lithococcus multicarinatus,* and *Tryonia aequicostata* ($n = 2$ in each case). Thus, one specimen per taxon was used in our phylogenetic analysis (except for *Aphaostracon* sp.).

Minimum-length trees were determined with weighted parsimony (Swofford, 2000). Various weighting schemes were employed. In all cases, the heuristic search option with 10 replications of random stepwise additions was used to search for minimum-length trees. Uninformative characters were ignored and zero-length branches were collapsed. Bootstrapping (Felsenstein, 1985) with 1000 replicates was employed to assess branch support on the resulting trees.

Based on our analysis of nucleotide substitution patterns, genetic distances were corrected for multiple substitutions using (a) Kimura's two-parameter method for all substitutions, (b) a maximum-likelihood HKY substitution model (Hasegawa *et al.,* 1985) which assumes a transition to transversion ratio of 2.45 and a gamma shape parameter of 0.3 (estimated from an initial tree with PAUP 4.0*), and (c) Log determinants. Topologies were generated from matrices of corrected genetic distances with the neighbor-joining algorithm (Saitou and Nei, 1987).

RESULTS

Nucleotide composition was typical of a mitochondrial gene (Collins *et al.,* 1996; Hershler *et al.,* 1999). Average base frequencies for the total data set were 24.8% A, 38.4% T, 17.3% C, and 19.5% G. Base frequencies were homogeneous across all sites ($\chi^2 = 28.31$, $df = 39$, $P = 0.89$), but not at the third codon positions ($\chi^2 = 122.6$, $df = 39$, $P = 0.00$) (Table 2). We further examined base frequency heterogeneity at third codon positions by estimating compositional distance for all pairwise comparisons (Table 3). Our results indicated an alternative preference for either A or G nucleotides, suggesting that this problem can be alleviated by the downweighting of transitions at third codon positions.

Tests indicated no mutational saturation at the first and second codon positions. For the third codon position, multiple hits occurred, TS leveled off in the first test, and the plotted line deviated from the identity line in the second and third tests.

TABLE 2

Nucleotide Composition at Third Codon Positions for Representatives of 14 Genera

	A	C	G	T
Aroapyrgus	0.4049	0.0341	0.0537	0.5073
Durangonella	0.3204	0.0437	0.1165	0.5194
Hydrobia	0.3085	0.0945	0.1393	0.4577
Lithococcus	0.3447	0.0485	0.0971	0.5097
Lithoglyphus	0.4829	0.0976	0.0537	0.3658
Nymphophilus	0.3889	0.1263	0.0707	0.4141
Onobops	0.3932	0.0291	0.0631	0.5146
Pyrgophorus	0.3495	0.0486	0.1019	0.5000
"Tryonia" brevissima	0.4175	0.0534	0.0485	0.4806
Tryonia clathrata	0.3883	0.0243	0.0631	0.5243
"Tryonia" robusta	0.4272	0.0291	0.0388	0.5049
Zetekina	0.3073	0.0683	0.1610	0.4634
Pomatiopsis	0.3544	0.0485	0.0874	0.5097
Rissoina	0.3855	0.0279	0.1564	0.4302
Average	0.3646	0.0528	0.0998	0.4828

Given this pattern, we treated our data by downweighting TS 25, 50, and 75% in the third codon position, equally weighting all substitutions, and employing a model which uses maximum transition to transversion ratios at each codon position (18:1:27). (The percentages indicate the weight applied to transversions relative to transitions, and the three numbers [in parentheses] indicate weightings applied to first, second, and third codon positions, respectively.) When TS in the third codon position were downweighted 25%, a single shortest tree was produced from the parsimony analysis of the 617 aligned DNA sequences containing 247 phylogenetically informative base positions (Fig. 1; TL = 4907, CI = 0.30). In this topology a clade composed of all but one of the cochliopines and a clade composed of the remaining cochliopine *Antroselates* and two species of the hydrobiids *Amnicola* were well supported. A sister relationship between the *Antroselates* + *Amnicola* clade and *Lithoglyphus* received weak bootstrap support (51%) and a clade containing these taxa and four pomatiopsid species in turn was sister to the cochliopine clade. Within the monophyletic group of cochliopines *"Tryonia" robusta* was sister to all remaining species, which are grouped in five clades. Clades I (composed of *Onobops, Heleobops,* and *Heleobia*) and V (*Mexipyrgus* and three species of *Tryonia*) were well supported, whereas clades II (*Aroapyrgus, Cochliopa, Cochliopina, Lithococcus,* and *Mexithauma*), III (*Eremopyrgus* and *Zetekina*), and IV (*Aphaostracon, Durangonella, Littoridinops, Pyrgophorus, Spurwinkia,* and three species of *Tryonia*) had less support (58, 20, and 48%, respectively).

When TS in the third codon position were downweighted 50%, a single most parsimonious tree was produced (TL = 4145, CI = 0.29). Tree topology differed from that of Fig. 1 in that *"Tryonia" robusta* was

TABLE 3

Pairwise Compositional Distances at Third Codon Positions for Representatives of 14 Genera

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Aroapyrgus*													
2 *Durangonella*	**1.96**												
3 *Hydrobia*	**3.74**	**1.13**											
4 *Lithococcus*	0.99	0.18	**1.27**										
5 *Lithoglyphus*	**5.13**	**9.29**	**7.26**	**7.25**									
6 *Nymphophilus*	**2.95**	**3.97**	**2.18**	**2.88**	**1.97**								
7 *Onobops*	0.05	**1.45**	**3.37**	0.68	**5.93**	**3.26**							
8 *Pyrgophorus*	0.98	0.24	**1.12**	0.02	**6.65**	**2.57**	0.69						
9 *"Tryonia" brevissima*	0.22	**2.67**	**3.63**	**1.46**	**3.28**	**1.82**	0.45	**1.34**					
10 *Tryonia clathrata*	0.13	**1.36**	**3.56**	0.67	**6.73**	**3.76**	0.03	0.73	0.68				
11 *"Tryonia" robusta*	0.14	**3.11**	**5.09**	**1.86**	**4.72**	**3.39**	0.33	**1.82**	0.25	0.46			
12 *Zetekina*	**3.99**	0.95	0.18	**1.30**	**8.31**	**3.20**	**3.49**	**1.13**	**4.13**	**3.62**	**5.42**		
13 *Pomatiopsis*	0.68	0.36	**1.55**	0.03	**6.74**	**2.70**	0.43	0.05	**1.10**	0.44	**1.42**	**1.65**	
14 *Rissoina*	**2.84**	**2.29**	**1.79**	**1.95**	**4.69**	**2.73**	**2.67**	**1.56**	**2.64**	**2.97**	**3.59**	**1.40**	**2.05**

Note. Values in excess of 1 indicate significant compositional divergence (Gillespie, 1986) and are shown in boldface.

sister to the group composed of clades II, III, IV, and V, and *Spurwinkia salsa* was sister to the clade composed of *"T." brevissima, "T." alamosae,* and *Aphaostracon* sp.

When TS in the third codon position were down-weighted 75% and when all substitutions received equal weight, 11 and 14 trees of shortest length were found, respectively. A 50% majority rule tree was generated from these equally parsimonious trees and was identical to the tree shown in Fig. 1 except for aspects of phylogenetic structure within the cochliopine clade. Clade V was sister to clades I and II in the former analysis and *"Tryonia" robusta* was sister to clades III and IV in the later analysis.

Weighted parsimony analysis, using the 18:1:27 scheme, also yielded a single parsimonious tree. Tree topology differed from that of Fig. 1 in that *"Tryonia" robusta* was sister to clade IV, and clades II and III were sister to the group composed of clades I, IV, and V. These alternative placements are not surprising given the generally weak support for relationships among the five clades within the Cochliopinae.

Neighbor-joining topologies were determined from matrices of genetic distances corrected for multiple hits with different methods. The three resulting trees were identical to that shown in Fig. 1 except that the species relationships differed within Clade IV, and the clade composed of *Lithoglyphus naticoides, Antroselates spiralis, Amnicola limosa,* and *Amnicola dalli* was sister to the cochliopine clade instead of to the four pomatiopsid species.

DISCUSSION

Systematics

Our data reveal the cochliopines to be paraphyletic owing to the highly supported sister relationship between the cochliopine *Antroselates* and *Amnicola.* In *Antroselates* (and closely similar *Antrobia*) the duct to the female albumen gland issues from the sperm tube (Fig. 2D), whereas in other cochliopines the albumen gland duct issues from the oviduct prior to its junction with the sperm tube, with a distinct duct of variable length (Sdu) connecting this region of the oviduct with the sperm tube (Figs. 2A–2C). This difference lead Hershler and Thompson (1992, p. 118) to suggest that these two genera may represent "a small clade separate from the general cochliopine radiation." Whereas a close relationship with *Amnicola* was not previously suspected because *Antrobia* and *Antroselates* lack the penial gland diagnostic of the Amnicolinae, this relationship is supported by our new determination that *Amnicola* shares the condition of the albumen gland duct in these two genera as described above (Figs. 2D and 2E; Hershler and Thompson, 1988, Fig. 8b re *Amnicola limosa* is incorrect in this regard). The difference between this and the state of the albumen gland duct in other cochliopines can be subtle (compare Fig. 2A with Figs. 2D and 2E) or quite obvious in cochliopines in which the opening to the albumen gland is separated from the sperm tube by a fairly long section of duct (Figs. 2B and 2C), but nevertheless the distribution of these states is completely congruent with our topology and we suggest that these characters may be synapomorphies for the cochliopine and the Amnicolinae + *Antroselates* clades.

Paraphyly of the Hydrobiidae in this topology, owing to in-group placement of pomatiopsids and the rissoid *Setia,* renders familial assignment of the cochliopines enigmatic as this clade is not closely related to basally positioned hydrobiids, which include the type species of the type genus of the family, *Hydrobia acuta.* Note that within the context of the single, preliminary analysis of the phylogenetic relationships of higher taxa of rissooidean snails (Ponder, 1988), the morphological basis for

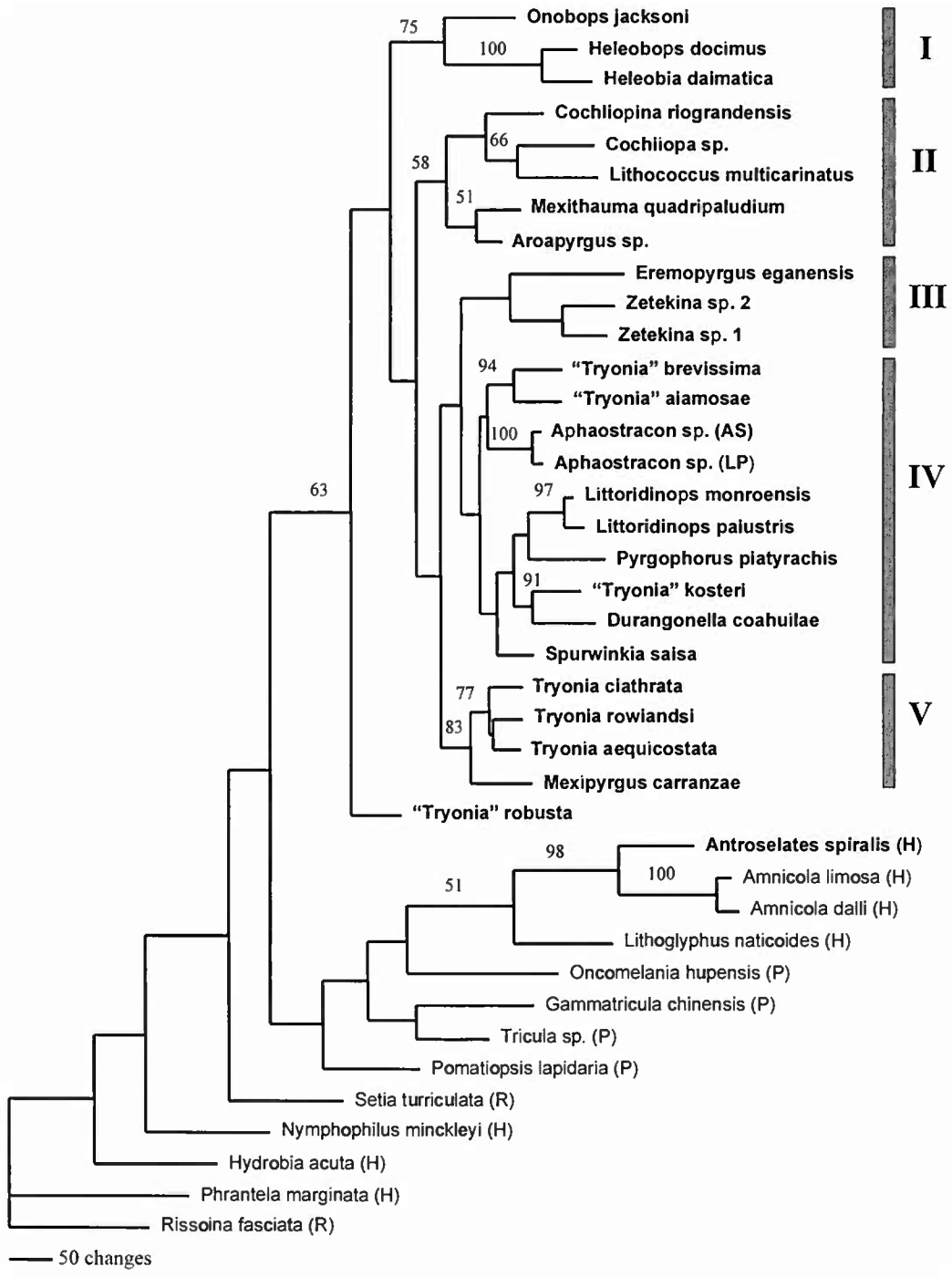

FIG. 1. Shortest length tree based on maximum-parsimony analysis of mtCOI sequence data. CI, 0.30; TL, 4907. Bootstrap percentages are given when ≥50%. Boldfaced species are currently placed in the subfamily Cochliopinae. Familial assignments are given (in parentheses) for noncochliopine taxa. H, Hydrobiidae; P, Pomatiopsidae; R, Rissoidae.

recognizing the Hydrobiidae as a clade was weak (e.g., two synapomorphies consisting of a parallelism and a reversal) and it is not surprising to us that our data show this group, as currently constituted (*fide* Ponder and Warén, 1988), to be paraphyletic.

Our topology indicates a close relationship among the cochliopines, amnicolines, and pomatiopsines, which is congruent with a common origin of female diauly in these groups (as opposed to an independent origin in the cochliopines), with a reversal to the presumably primitive monaulic condition occurring in *Lithoglyphus*. This hypothesis conflicts with ontoge-

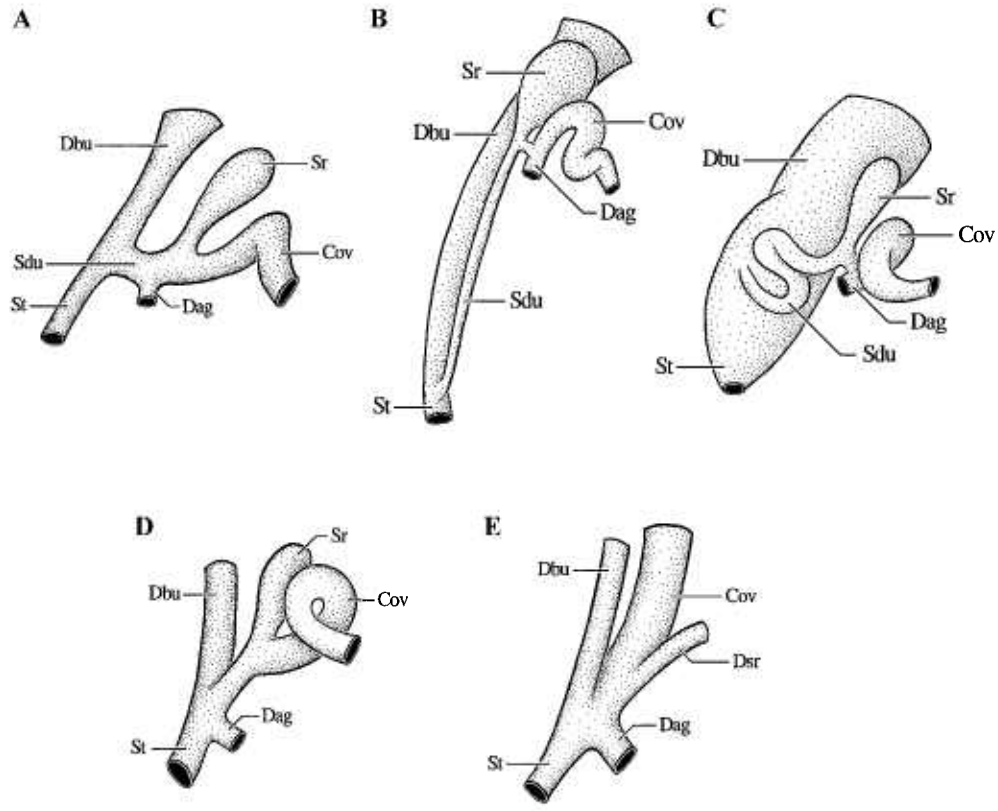

FIG. 2. Schematic drawings of distal female genitalia showing differences in the origin of the albumen gland duct (Dag). (A) *Cochliopa* sp., UF 271928. (B) *Littoridinops palustris,* USNM 892071. (C) *Mexipyrgus carranzae,* USNM 850278. (D) *Antroselates spiralis,* USNM 858333. (E) *Amnicola limosa,* USNM 883963. Cov, coiled section of oviduct; Dbu, bursal duct; Dsr, seminal receptacle duct; Sdu, sperm duct; Sr, seminal receptacle; St, sperm tube.

netic and anatomical evidence that diauly is not homologous in the pomatiopsids and Cochliopinae + Amnicolinae (Davis *et al.,* 1976; Hershler and Ponder, 1998), although this evidence needs to be studied in more detail. A denser sampling of taxa outside of the cochliopine clade also is needed to determine whether the position of the pomatiopsids in our topology is stable and we are pursuing this within the context of a detailed evaluation of hydrobiid monophyly based on DNA sequences for several genes (Liu *et al.,* 1997). The importance of sufficient sampling is highlighted by a recent study of mtCOI sequences of rissooidean snails which depicted the hydrobiids and pomatiopsids as distinct clades (Davis *et al.,* 1998). The single hydrobiid genus included (*Hydrobia*) in that analysis is positioned basal to the portion of our topology that reveals a close relationship between other hydrobiids and pomatiopsids and thus the conclusions of Davis *et al.* (1998) differ from this study with respect to the phylogenetic relationships between these two families.

Phylogenetic structure of the Cochliopinae is largely congruent with the three informal groupings proposed by Hershler and Thompson (1992) on the basis of morphological characters that are unique within the Hydrobiidae. *Heleobia dalmatica* and *Heleobops docimus,* members of the *Heleobia* group, which is diagnosed by the possession of apocrine penial glands, form a strongly supported (100%) monophyletic unit within clade I. Clade II is composed of members of the *Cochliopa* group, diagnosed by a simple penis having a smooth elongate filament strongly demarcated from the folded base, although inclusion of *Lithococcus multicarinatus* is enigmatic as this species has a completely different and unique penial morphology. All members of the weakly supported monophyletic group composed of clades III, IV, and V belong to the *Littoridina* group, diagnosed by a long sperm duct, with the exception of *Aphaostracon,* which has a short duct. The short duct is shared by clades I and II and is interpreted by us as the primitive condition within the cochliopine clade. Phylogenetic structure within this monophyletic group also is largely concordant with genitalic morphology. In species of clades III and V the sperm duct is coiled behind the posterior pallial wall, whereas in species of clade IV the sperm duct is straight and extends into the pallial cavity, except for *Aphaostracon* (as noted above) and *Pyrgophorus* (which has a coiled duct). Additional, more slowly

evolving molecular markers will be necessary to better resolve the phylogenetic relationships among clades I–V and permit further evaluation of congruence with genitalic variation.

Biogeographic Considerations

Our revision of the systematic relationships of *Antrobia* and *Antroselates* resolves a vexing biogeographic problem associated with the cochliopines. These two genera are endemic to cave streams of the upper Mississippi River basin, whereas other cochliopines of eastern North America are restricted to the coastal margins (Hershler and Thompson, 1992). We are not aware of any historical process that might have effected vicariance of or snail dispersal between these broadly disjunct areas within an appropriate time frame (e.g., post-Paleozoic; Hershler and Thompson, 1992). On the other hand, local origin of *Antroselates* and *Antrobia* associated with invasion of cave habitats in the central United States is consistent with a sister relationship with *Amnicola,* which is represented by both epigean and subterranean species in this region (Hubricht, 1971, 1979).

Our topology confirms the trans-Atlantic distribution of the cochliopines owing to the in-group placement of *H. dalmatica,* which is distributed along the Adriatic coast. (This is the only representative of the Old World cochliopines that we analyzed.) A detailed analysis of cochliopine biogeography in relation to pertinent paleogeographic events is deferred until a fuller set of taxa is sequenced, as the Old World and South American faunas in particular are underrepresented.

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