Journal of Molluscan Studies Advance Access published 18 September 2007

A MOLECULAR PHYLOGENY OF PHYSIDAE (GASTROPODA: BASOMMATOPHORA) BASED ON MITOCHONDRIAL DNA SEQUENCES

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(Received 14 August 2006; accepted 19 April 2007)

ABSTRACT

The family Physidae (Pulmonata: Basommatophora) is a group of freshwater hermaphroditic snails that have a Holarctic distribution with extension into Central and South America. Despite considerable literature justifying various taxonomic schemes and groupings, no classification has been proposed using modern phylogenetic methods. In an effort to expand what is known concerning the evolutionary relationships of Physidae, we examined a portion of the mitochondrial 16S rRNA and cytochrome *c* oxidase subunit I genes among 66 specimens representing 28 taxa. The molecular phylogeny based on mitochondrial sequences supports the monophyly of the family Physidae. Six major clades were uncovered in the analysis, corresponding to differences in penial morphology. These six groups include the following recommended phylogenetic species and species groups: *Aplexa elongata* (Say), *Aplexa* 1 group; *Physa marmorata* Guilding, *Aplexa* 3 group; *P. fontinalis* (Linneaus), *P. jennessi* Dall and *P. vernalis* Taylor & Jokinen, type a group; *P. gyrina* Say and *P. 'ancillaria'* Say, type b group; *P. acuta* Draparnaud, *P. spelunca* Turner & Clench, *P.* species A and *P. zionis* Pilsbry, type c group; and *P. pomilia* Conrad and *P. hendersoni* Clench, type bc group.

INTRODUCTION

The freshwater family Physidae (Pulmonata: Basommatophora) has a Holarctic distribution, extending into Central and South America. Physids have been introduced around the world and figure prominently in aquatic ecosystems, particularly in lentic habitats. Physid diversity is centred in North America, where they are the most abundant and widespread freshwater gastropods (Burch, 1982). Physidae are hermaphrodites and can be distinguished from other pulmonates by the following characteristics: a high-spired sinistral shell, radula with teeth in V-shaped rows, simple jaw with no lateral processes, and lack of both haemoglobin and pseudobranchia. Other unique characteristics of many species of Physidae are an extended mantle edge that can partly cover the shell, as well as the presence of a preputial gland (Te, 1978).

Physid classification has been fluid and is still in a state of considerable flux (Taylor, 2003). Nineteenth-century workers (Walker, 1918) generally recognized two genera, the monotypic *Aplexa* Fleming, 1820 and the speciose *Physa* Draparnaud, 1805, which was divided into two 'sections': *Physa s.s.* (shell smooth) and *Costatella* Dall, 1870 (shell longitudinally costate). Baker (1928) placed all North American physids in the genus *Physella* Haldeman, 1843 separate from the European genus *Physa* based on anatomical differences in the mantle between European and North American species. *Physella* was further divided into two subgenera, *Physella s.s.* and *Physodon* Haldeman, based on penial morphology, and shell and columella characteristics (Baker, 1928). Thiele (1931–1935) and Zilch (1959–1960) recognized only the two older genera, *Aplexa* and *Physa*, with two subgenera under the former (*Aplexa s.s.* and *Stenophysa* Martens, 1898) and four subgenera under the latter (*Physa s.s.*, *Alampetista* Zilch, *Costatella* and *Petrophysa* Pilsbry). Starobogatov (1967) created two new subfamilies, Aplexinae with three genera and Physinae with four.

One influential monograph of the Physidae was the doctoral dissertation of Te (1978) who examined 85 taxa of physids (78 of these were nominal species, the rest represented populations that Te felt were unique) using 71 morphological characters (37 shell and 34 anatomical characters). Although the dissertation was never published, a modified version of Te's (1978) classification was used in an influential guide to the North American freshwater snails (Burch & Tottenham, 1980; Burch, 1988). Te (1978) relied almost exclusively on the penial complex and associated characters to make his primary groupings: *Aplexa* group ('*Aplexa*-type' penial complex with six variations), *fontinalis* group ('*Physa* type-a' penial complex with no variation), *gyrina* group ('*Physa* type-b' penial complex with five variations), *acuta* group ('*Physa* type-c' penial complex with four variations) and *cubensis* group ('*Physa* type-bc' penial complex with three variations). Then considering both shell and anatomical characters, Te used both the 'simgra' technique (Estabrook, 1966) and the character compatibility method (Estabrook, 1972) to provide a phenetically based classification scheme for the family. He suggested two subfamilies, Aplexinae and Physinae, the former with genera *Aplexa* and *Stenophysa* and the latter with genera *Physa* and *Physella*. Te also noted that other ways to interpret his systematic results as a classification were to use *Stenophysa* as a separate genus of Physinae (instead of Aplexinae) or *Stenophysa* could be the sole genus of Stenophysinae. *Physella* was further divided by Te into three subgenera: *Physella*, *Petrophysa* and *Costatella*, with *Costatella* further divided into two sections: *Alampetista* having penial morphology c and *Costatella* having penial morphology bc. Te considered the monotypic subgenus *Petrophysa* to be the most differentiated physid, having both an unusual morphology and ecology. *Physella* (*Petrophysa*) *zionis* was initially grouped by Te with those having the *cubensis* bc penial complex.

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 doi:10.1093/mollus/eym021

Table 1. Penial group, the proposed new classification, Te's (1978) classification as adopted by Burch (1988), and Taylor's (2003) classification.

Penial group	Genus	Species	Te (1978)/Burch (1988) classification	Taylor (2003) classification
Aplexa variety 1	*Aplexa*	*elongata*	*Aplexa elongata*	Aplexini *Sibirenauta elongates*
Aplexa variety 3	*Physa*	*marmorata*	*Stenophysa marmorata*	Stenophysini *Stenophysa marmorata*
a	*Physa*	*fontinalis*	*Physa fontinalis*	Physini *Physa fontinalis*
	Physa	*jennessi*	*Physa jennessi*	Physini *Beringophysa jennessi*
	Physa	*vernalis*	not applicable	Physini *Laurentiphysa vernalis*
b	*Physa*	'*ancillaria*'	*Physella (Physella) ancillaria*	Physellini *Physella ancillaria*
			Physella (Costatella) integra brevispira	Haitini *Haitia integra*
			Physella (Physella) magnalacustris	Physellini *Physella vinosa*
			Physella (Physella) parkeri	Physellini *Archiphysa parkeri*
	Physa	*gyrina*	*Physella (Physella) gyrina*	Physellini *Physella gryina*
			Physella (Physella) gyrina aurea	Physellini *Physella gyrina*
			Physella (Physella) gyrina microstoma	Physellini *Physella gyrina*
			Physella (Costatella) johnsoni	Physellini *Physella gyrina*
			Physella (Physella) microstriata	Physellini *Utahphysa microstriata*
			Physella (Physella) utahensis	Physellini *Physella gyrina*
			Physella (Physella) wrighti	Physellini *Physella gyrina*
bc	*Physa*	*hendersoni*	*Physella (Costatella) hendersoni*	Haitini *Haitia pomilia*
	Physa	*pomilia*	*Physella (Costatella) heterostropha pomilia*	Haitini *Haitia pomilia*
c	*Physa*	*acuta*	*Physella (Costatella) acuta*	Haitini *Haitia acuta*
			Physella (Costatella) cubensis	Haitini *Haitia cubensis*
			Physella (Costatella) heterostropha	Haitini *Haitia acuta*
			Physella (Costatella) heterostropha cupreonitens	Haitini *Haitia mexicana*
			Physella (Costatella) integra	Haitini *Haitia integra*
			(*Physella (Costatella) integra billingsii*)	(Haitini *Haitia integra*)
			Physella (Costatella) integra niagarensis	Physinae *incertae sedis*
			Physella (Costatella) virgata	Haitini *Haitia mexicana*
	Physa	*spelunca*	*Physella (Costatella) spelunca*	Haitini *Haitia spelunca*
	Physa	species A	not applicable	Not applicable
	Physa	*zionis*	*Physella (Petrophysa) zionis*	Physellini *Petrophysa zionis*

Taylor (2003) recently provided a new classification scheme of the Physidae. However, unlike Te (1978) who ultimately considered both shell and anatomical characters, Taylor's final classification was primarily based on the penial complex. Taylor (2003) assumed that changes in penial morphology were progressive, and classified the family into grades and clades based on whether groups possess primitive or specialized characteristics. Although a phylogeny was provided, this was really a dichotomous trellis diagram, not a cladogram. Following Starobogatov (1967) and Te (1978), Taylor treated Aplexinae and Physinae as subfamilies. The subfamilies were each divided into new tribes and the tribes into genera, many newly described (Taylor, 2003). Table 1 shows a comparison of Te's (1978) classification (as adopted by Burch, 1982, 1988; Burch & Tottenham, 1980) and Taylor's (2003) classification scheme for the individuals used in this study.

To investigate evolutionary relationships of the Physidae, we sequenced portions of two mitochondrial genes from 66 individuals of 28 taxa, representing six distinct morphological groups.

MATERIAL AND METHODS

Collection, dissection and organization into penial morphology groups

Physids were obtained from many geographic areas including 24 type or near type localities and placed directly in 95% ethanol (see Appendix for locality information). Representative individuals were dissected to ascertain their penial morphology. Using Te (1978) as an initial framework, the material was partitioned into taxonomic groups based on presence or absence of a penial gland, the number and proportion of penial sheaths, and whether certain sections of the penial sheaths were glandular or nonglandular, as follows: subfamily Aplexinae: *Aplexa* variation 1 group (no preputial gland with a one-part glandular penial sheath, 1 individual); *Aplexa* variation 3 group (no preputial gland with a one-part nonglandular penial sheath, 2 individuals); subfamily Physinae: type a group (preputial gland present with a one-part glandular penial sheath, 5 individuals); type b group (preputial gland present with a two-part penial sheath having both glandular and nonglandular regions, 26 individuals); type bc group (preputial gland present with a one- to two-part penial sheath with both glandular – much smaller – and nonglandular regions, 7 individuals, including *Physa pomilia*); type c group (preputial gland present with a one-part nonglandular penial sheath, 25 individuals, including *P. cubensis* and *P. zionis*). Unlike Te (1978), we placed both *P. cubensis* and *P. zionis* in the type c group instead of the type bc penial morphology typical of other members of the group (e.g. *P. hendersoni*) based on the dissections. Similarly, based on dissections, we placed *P. pomilia* in the bc group instead of the type c group as originally designated by Te (1978). Figure 1 shows schematic diagrams of the penial morphology for each representative group.

Additionally, 10 specimens were included as representatives of other freshwater basommatophoran families (Ancylidae, Lymnaeidae, Planorbidae; see Appendix). The marine putative basommatophoran *Siphonaria* sp. and two stylommatophorans (*Euhadra herklotsi* and *Albinaria coerulea*) were included as outgroup taxa (Hatzoglow, Rodakis & Lekanidou, 1995; Yamazaki *et al.*, 1997).

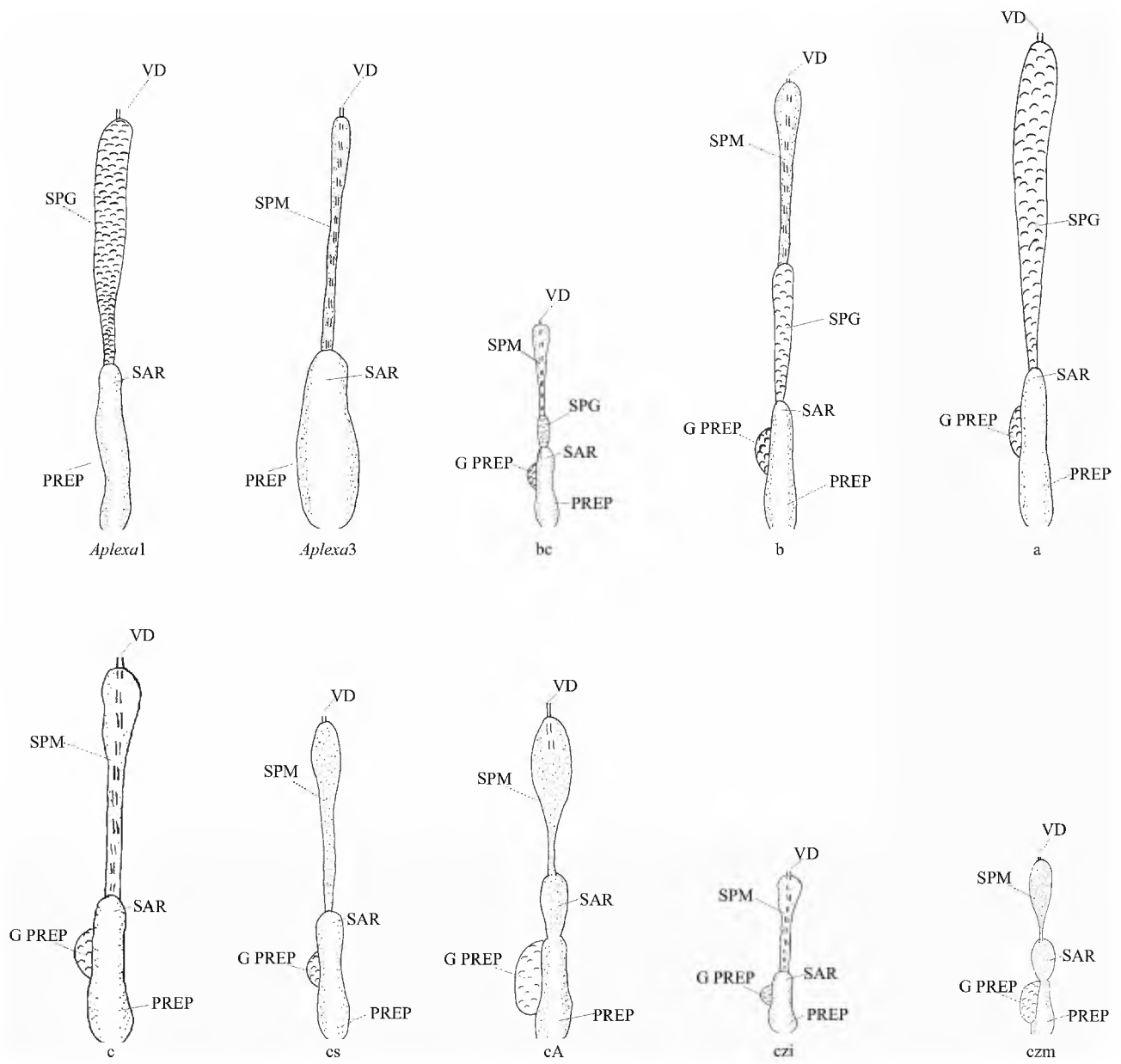

Figure 1. The six phylogenetic groups and their respective penial complexes. The genus *Aplexa* here represents *Aplexa* type 1 penial morophology. The genus *Physa* has five distinct penial morphologies: *Aplexa* 3, c, bc, b and a. The variation within type c is shown in the bottom row: c = typical penial c morphology, cs = penial morphology found in *Physa spelunca*, cA = penial morphology found in *Physa* species A, czi = penial morphology found in immature *Physa zionis* and czm = penial morphology found in mature *Physa zionis*. Abbreviations: PREP, preputium; G PREP, preputial gland; SPG, the glandular portion of penial sheath; SPM, muscular portion of the penial sheath; VD, vas deferens; and SAR, sarcobelum (underneath what is drawn). The penis is easily visible in the muscular portion of the penial sheath and becomes the vas deferens outside the penial sheath(s).

DNA extraction

Genomic DNA was isolated from head tissues or whole animals using standard phenol/chloroform methods. Mitochondrial DNA sequences were obtained for a 650 base-pair segment of the mitochondrial cytochrome *c* oxidase subunit I (COI, the primers used were LCO1490: 5′-ggtcaacaaatcataaagatattgg-3′ and LCO2198: 5′-taaacttcagggtgaccaaaaaatca-3′ from Folmer *et al.*, 1994) and a 550 base-pair segment of the mitochondrial 16S rRNA (the primers used were L2510: 5′-cgcctgtttatcaaaaacat-3′ and H3080: 5′-acgtgatctgagttcagaccgg-3′ from Palumbi *et al.*, 1991). Double-stranded amplifications via PCR were generated using 50–500 ng of template genomic DNA in 25 μl volumes (10 mM Tris, 50 mM KCl, 2.5 mM $MgCl_2$, 1 μM each primer, 0.1 mM each dNTP, 1.5 U Taq DNA polymerase; Fisher Scientific). The amplification regime began with a denaturation at 92°C for 2 min followed by 35 cycles of the following: denaturation at 92°C for 40 s, annealing at 52°C for 60 s (16S)/ 50°C for 60 s (COI), and extension at 68°C for 90 s. Double-stranded products were concentrated using Millipore Ultrafree MC filters and provided the template for cycle sequencing using the ABI BigDye kit following manufacturer's instructions. Reactions were purified using Quiagen DyeEx spin columns and sequenced on an ABI 3100 genetic analyser.

Phylogenetic analysis

Sequence data were aligned by eye for COI. For 16S, the sequence data were aligned initially using Clustal W (Thompson, Higgins & Gibson, 1994) and subsequently adjusted by eye using molluscan secondary structure models for 16S rRNA to identify conserved stems and loops (Lydeard *et al.*, 2000) in BioEdit (Hall, 1999). Since both 16S and COI are mitochondrial genes, sequence data from both were combined for three separate phylogenetic analyses (parsimony, likelihood and distance) using a putative basal basommatophoran (*Siphonaria* sp.) and two stylommatophorans (*Euhadra herklotsi* and *Albinaria coerulea*) to root the trees (Wade & Mordan, 2000). Two separate Baysian analyses were performed on the COI and 16S data sets. Portions of the loop region were excised from the 16S data due to ambiguities of alignment. Also, one section (from 3 to 15 bp) of COI was excised where two taxa (*Gyraulus parvus* and *Siphonaria* sp.) have extra bases. This truncated data set consisted of 1,122 bases (463 of 16S and 659 of COI, including indels).

In preliminary parsimony runs, the heuristic search did not get past the first replicate before running out of memory. Consequently, the maximum number of trees retained in any one replicate of the analysis was set to 20,000 for each of the 100 random replicates. Otherwise, default settings were used. Of the 1,122 total sites in the combined analysis, 554 were potentially phylogenetically informative according to the parsimony criterion. Alignment gaps were treated as missing character states and only minimal-length trees were retained (one tree held at each step during stepwise addition). A bootstrap analysis was performed using 10,000 pseudoreplicates and these values were mapped onto the resulting strict consensus tree. We used version 4.0b10 of PAUP* (Swofford, 2001) to perform the parsimony analysis.

An optimal model for sequence evolution was determined using Modeltest 3.0 (Posada & Crandall, 1998). The TVI + I+G model was selected as being the most appropriate model of nucleotide substitution with the assumed proportion of invariable sites being 0.1646 and the gamma distribution shape parameter being 0.5057. Using the optimal base-pair substitution model, a likelihood analysis was performed. A bootstrap was performed using 100 bootstrap replicates with the optimality criterion set to parsimony (addition sequence was simple, number of trees held at each step during stepwise addition was one, and the TBR branch-swapping algorithm was used). The maximum number of trees held at each bootstrap replicate was preset to 20,000, which was more than needed for most of the replicates performed. All characters were given equal weight and multistate taxa were interpreted as uncertain. We used version 4.0b10 of PAUP* (Swofford, 2001) to perform the likelihood analysis.

Using the same optimal model for sequence evolution determined by Modeltest 3.0 (Posada & Crandall, 1998), the TVI + I+G model was used to estimate DNA distances, a neighbour-joining tree was constructed with 10,000 bootstrap pseudoreplicates using version 4.0b10 of PAUP* (Swofford, 2001).

MrBayes v3.0B4 (Ronquist & Huelsenbeck, 2003) was used for the Baysian analyses. The Baysian inference was based on the posterior probabilities guided by the General Times Reversible model. The COI and 16S gene portions were analysed separately due to the limitations of computer memory. There were four separate Monte Carlo Markov chains and the number of generations was preset to 10,000,000 with the first 10,000 generations excluded from the analysis for both runs. The burnin value was sufficient for stable likelihood tree values for each analysis. Probabilities were calculated for each node.

Since COI is a coding region of the mtDNA genome, a coding block was used for COI Baysian inference. The data were partitioned by codon and the GTR (General Times Reversible) model was used for each defined partition within the 594-base-pair segment used.

A noncoding block was used for the 16S gene portion (528 base-pairs included). The GTR model was used to infer the Baysian phylogeny.

RESULTS

Phylogenetic analyses

Parsimony analysis of the 1,122 molecular characters resulted in 20,000 trees (set by the analysis) of tree length 3,417. The consistency index was 0.3705. The homoplasy index was 0.6295. The consistency index excluding uninformative characters was 0.3297. The homoplasy index excluding uninformative characters was 0.6703. The retention index was 0.7310. The rescaled consistency index was 0.2708. A strict consensus tree of all equally parsimonious trees is shown in Figure 2 along with bootstrap values. Both the Likelihood and Distance methods used the TVM + I+G model as selected by Modeltest (Posada & Crandall, 1998) and are shown in Figures 3 and 4, respectively. The separate COI and 16S Baysian analyses are shown in Figure 5 (A and B, respectively).

The molecular phylogeny supports a monophyletic freshwater Basommatophora, Lymnaeidae and Physidae (all analyses). The ancylid *Laevapex fuscus* (labelled scAB1), nested within the Planorbidae (all analyses), which together are sister to the Physidae in all analyses except the likelihood and the COI Baysian analysis where the Lymnaeidae were sister to the Physidae.

The putatively circumboreal *Aplexa elongata* (*Aplexa* variation 1) is the most basal member of the family Physidae, followed by an unresolved polytomy containing all Physinae taxa plus *Stenophysa marmorata* (*Aplexa* variation 3) of the subfamily Aplexinae, rendering each subfamily paraphyletic. The 16S Baysian analyses place *S. marmorata* as the second most basal group of the family Physidae and potentially outside the rest of the Physinae, but still paraphyletic with regards to the *Aplexa* penial morph (variations 1 and 3). Within the 'Physinae' + *S. marmorata* clade are five major subclades: (1) *S. marmorata*, (2) penial morphology a complex, (3) penial morphology b complex, (4) penial morphology bc complex and (5) penial morphology c complex. The only exception is the COI Baysian analysis which did not resolve the difference between penial morphology a and *Aplexa* penial variation 3.

Within the penial morphology a complex, all three included members form monophyletic groups: *Physa fontinalis*, *P. jennessi* and *P. vernalis*. *Physa fontinalis* and *P. jennessi* are more closely related to each other than either is to *P. vernalis*. In the Baysian COI analysis, *P. vernalis* was sister to *S. marmorata*, rendering the penial morphology a group paraphyletic (Fig. 5A). In all other analyses, penial morphology a formed a monophyletic group nested within the Physinae (Figs 2–4 and 5B).

The nominally diverse *gyrina* complex is monophyletic (Figs 2–5) and includes two subclades in most phylogenetic analyses (the exception being the COI Baysian analysis where the two subclades became paraphyletic, Fig. 5A), tentatively referred to as 'typical' species and 'globose' species. The members of the 'typical' clade, including *P. gyrina* from its type locality, are well suited to water temperatures that are either elevated by position in the water column (Clampitt, 1970), thermal springs (Clench, 1926; Wethington & Guralnick, 2004), or artificially from thermal effluent (Agersborg, 1929). The individuals included in the 'globose' clade are generally large as adults (between 10.6–25.6 mm) with globose shells (some with pronounced shoulders as is *Physa parkeri* Currier) and are found in much cooler water than those of the other clade. Within the 'typical' clade, *Physa gyrina*, *P. gyrina microstoma*

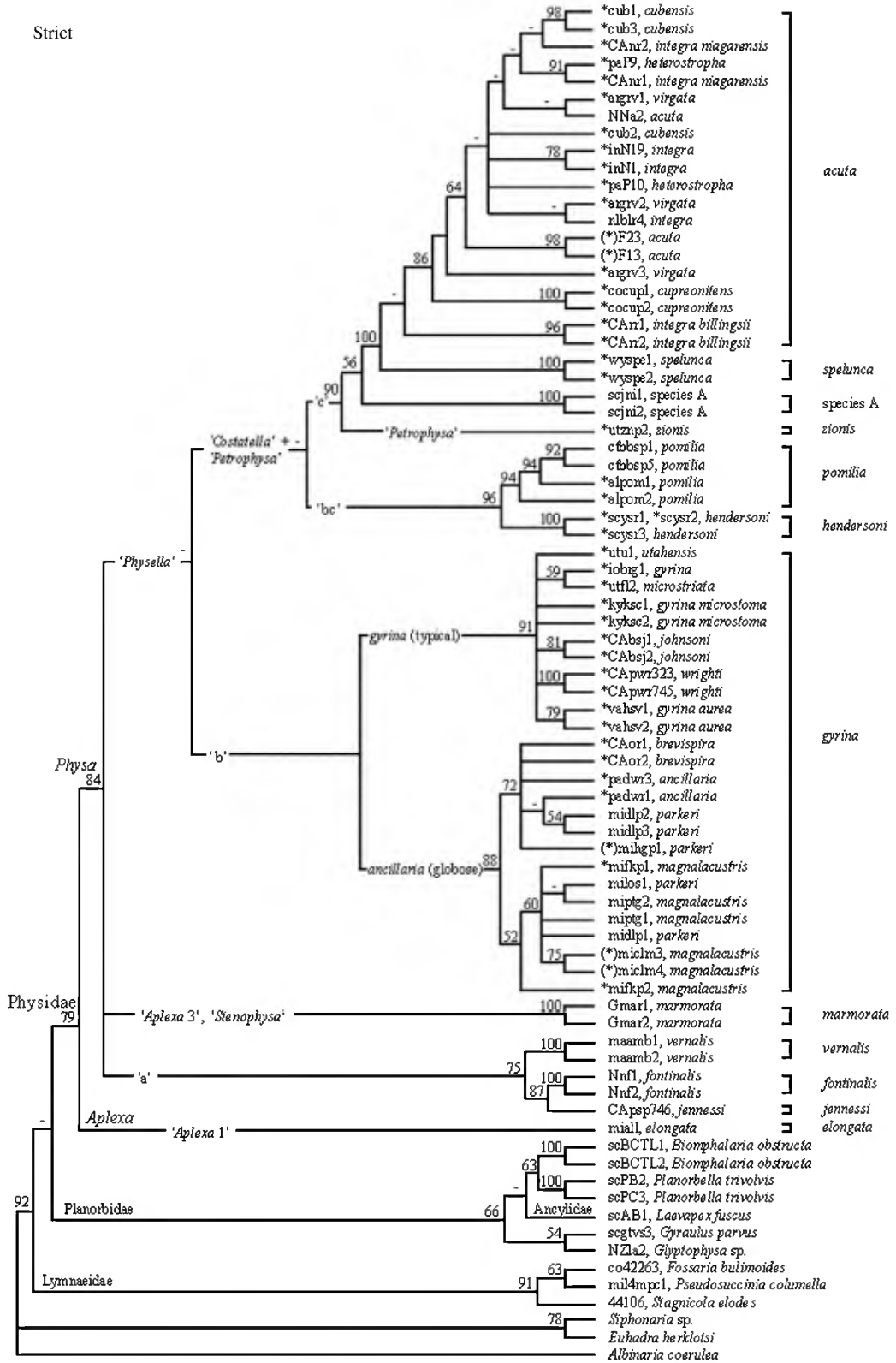

Figure 2. Strict consensus tree of all equally parsimonious trees (tree length = 3,417) for the Physidae sequence data. Bootstrap values are located above stems. Nodes are labelled by penial morphology and taxonomic category. The node labelled '*Costatella*' + '*Petrophysa*' is equivalent to Te's (1978) subgenera *Costatella* Dall + *Petrophysa* Pilsbry. The node labelled '*Physella*' is equivalent to Baker's (1928) genus *Physella* Haldeman. Nodes labelled *gyrina* (typical) and *ancillaria* (globose) within the *gyrina* clade show separation between the two morphotypes despite the small genetic distance. Taxon labels with * are from type localities and with (*) are from near type localities.

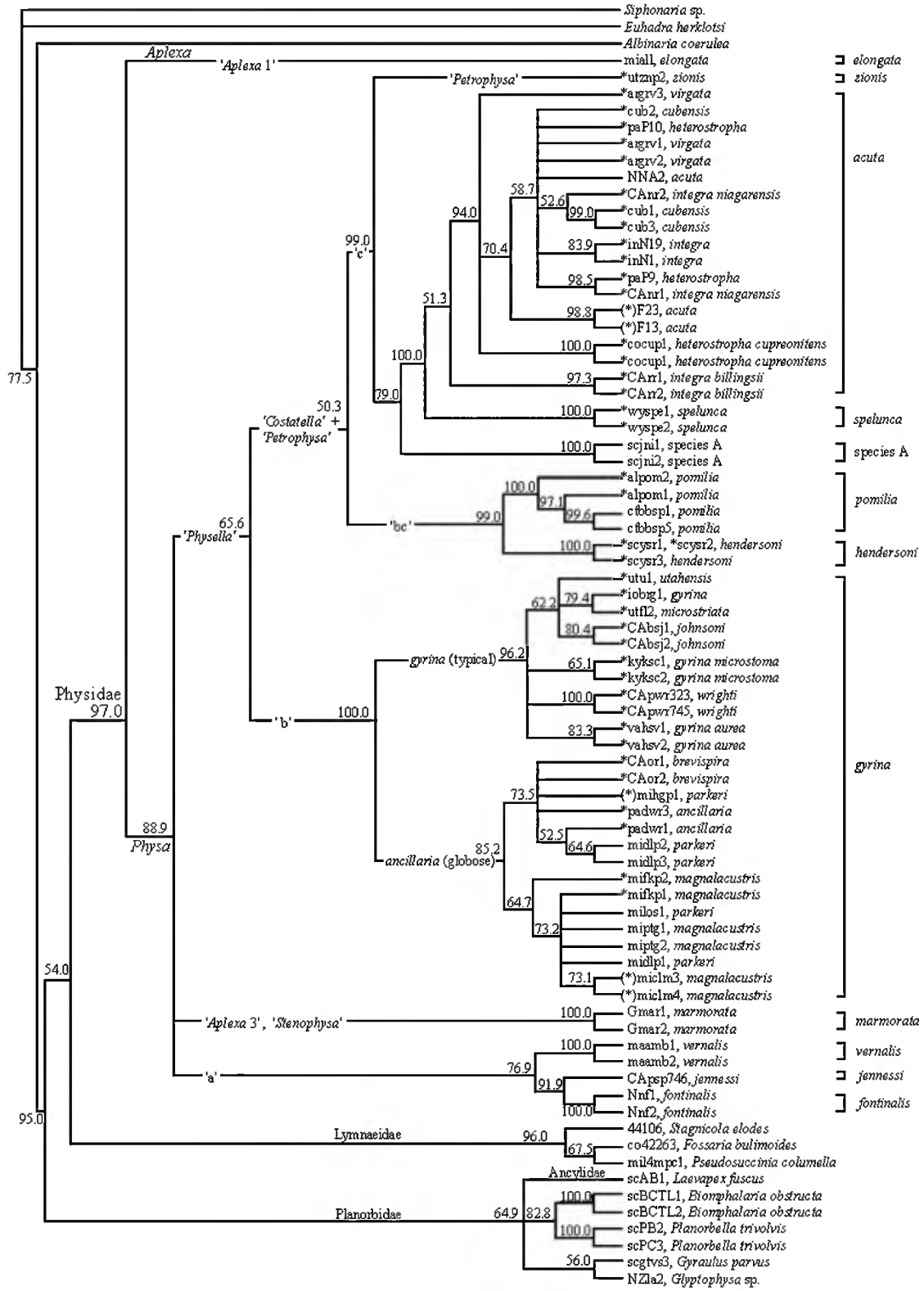

Figure 3. The tree generated by the likelihood analysis of the Physidae sequence data. Bootstrap values from likelihood analysis (using the TVI + I+G model selected by Modeltest) are given for each node. Nodes are labelled by penial morphology and taxonomic category. The node labelled '*Costatella*' + '*Petrophysa*' is equivalent to Te's (1978) subgenera *Costatella* + *Petrophysa*. The node labelled '*Physella*' is equivalent to Baker's (1928) genus *Physella*. Nodes labelled *gyrina* (typical) and *ancillaria* (globose) within the *gyrina* clade show separation between the two morphotypes despite the small genetic distance. Taxon labels with * are from type localities and with (*) from near type localities.

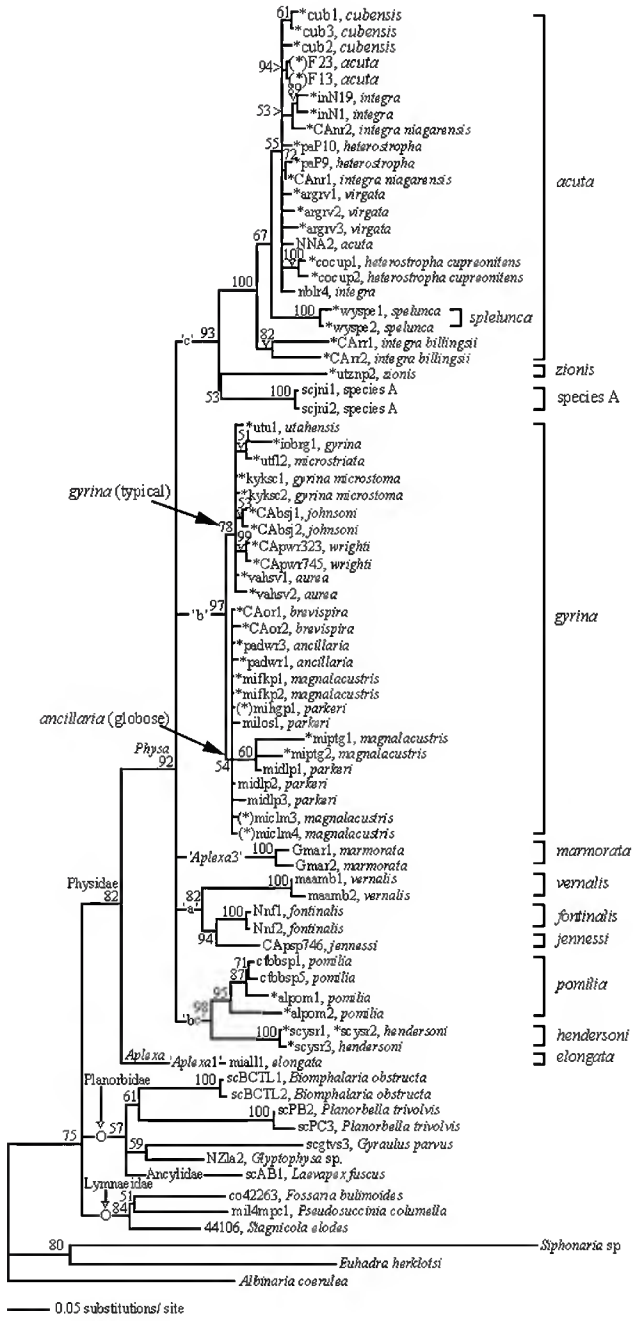

Figure 4. Neighbour-joining analysis for the Physidae sequence data set with each penial morphology group highlighted. The TVI + I+G model was selected by Modeltest. Bootstrap values are provided for each group. Nodes are labelled by penial morphology and taxonomic category. There is no node equivalent to Baker's (1928) genus *Physella* or Te's (1978) subgenera *Costatella* + *Petrophysa*. Nodes labelled *gyrina* (typical) and *ancillaria* (globose) within the *gyrina* clade show separation between the two morphotypes despite the small genetic distance. Taxon labels with * are from type localities and with (*) from near type localities.

and *P. microstriata* are paraphyletic, but *P. johnsoni*, *P. wrighti* and *P. gyrina aurea* are monophyletic. The sampling of *Physa utahensis*, of the typical clade, was not sufficient to determine whether it is monophyletic. However, the level of genetic differentiation among the 'typical' nominal species is only about 6% for the combined 16S rRNA and COI on average and the monophyly of all nominal species is not supported in all phylogenetic analyses (Wethington & Guralnick, 2004). Within the 'globose' subclade, none of the nominal species were monophyletic including *P. brevispira*, *P. ancillaria*, *P. parkeri*, nor *P. magnalacustris*.

Within the penial morphology bc complex, both *Physa pomilia* and *P. hendersoni* are monophyletic with *P. pomilia* of the southeastern USA (Alabama) more closely related to the *P. pomilia* of the northeastern USA (Connecticut) than to the *P. hendersoni* of the southeastern USA (South Carolina).

None of the following nominal species was monophyletic within the nominally diverse penial morphology c complex: *Physa acuta*, *P. cubensis*, *P. heterostropha*, *P. integra*, nor *P. virgata*. But *P. heterostropha cupreonitens*, *P. integra billingsii*, *P. spelunca*, two specimens from John's Island (species A), and *P. zionis* were monophyletic. The two most basal members of the penial morphology c group, the specimens from John's Island and *P. zionis*, both possess a modified version of the type c penial complex, having a more pronounced and opaque sarcobelum (especially *P. zionis*). (See Fig. 1 for comparisons of penial morphology within the type c penial complex)

DISCUSSION

The molecular phylogeny supports the monophyly of Physidae, which was presumed previously based on a combination of anatomical characters associated with the mantle edge, jaw and radula (Te, 1978). The most closely related family-group to Physidae appears to be Planorbidae + Ancylidae in most analyses. Hubendick (1978) argued that the latter two families should be combined into a single family named Ancyloplanorbidae based on similarity of anatomical features. Our mitochondrial gene-based molecular phylogeny supports the recognition of a single family and is consistent with a recent molecular study on planorbids (Morgan *et al.*, 2002).

Te (1975, 1978) relied almost exclusively on the penial complex and associated characters to make his five primary groupings: (1) '*Aplexa*-type' penial complex with six variations including *Stenophysa*; (2) '*Physa* type-a' penial complex with no variation; (3) '*Physa* type-b' penial complex with five variations; (4) '*Physa* type-bc' penial complex with three variations; and (5) '*Physa* type-c' penial complex with four variations. The molecular phylogeny based on mitochondrial gene sequences supports recognition of the groups having type a, b, bc and c penial morphology, respectively, but the *Aplexa* group (represented here by one individual having variation 1 and two individuals having variation 3) should be separated into at least two unrelated groups (representatives of Te's *Aplexa* variations 2, 4, 5 and 6 not included).

'Aplexa-*type*' *penial complex (variations 1 and 3)*

'*Aplexa*-type' penial morphology is actually a composite of six morphologically distinct variations, which Te (1975, 1978) and Taylor (2003) united in the subfamily Aplexinae based solely on the absence of a preputial gland, which is a plesiomorphic condition, shared by the rest of freshwater basommatophorans (Hubendick, 1978). Molecular data places *Aplexa elongata* (*Aplexa* 1 penial morphology) as the most basal member of Physidae, while *Stenophysa marmorata* (*Aplexa* 3 penial morphology) is generally nested within Physinae (Figs 2–5). Thus, absence of a preputial gland is not a synapomorphy uniting *Stenophysa* plus *Aplexa*, and the *Aplexa* complex should be divided to recognize the separation of these two groups. A major difference between *Aplexa* and *Stenophysa* lies in the character of their single penial sheath: *Aplexa* has a glandular penial sheath, whereas that of *Stenophysa* is muscular (Te, 1978; Taylor, 2003). More genetic sampling is required to examine the diversity and relationships of these two groups.

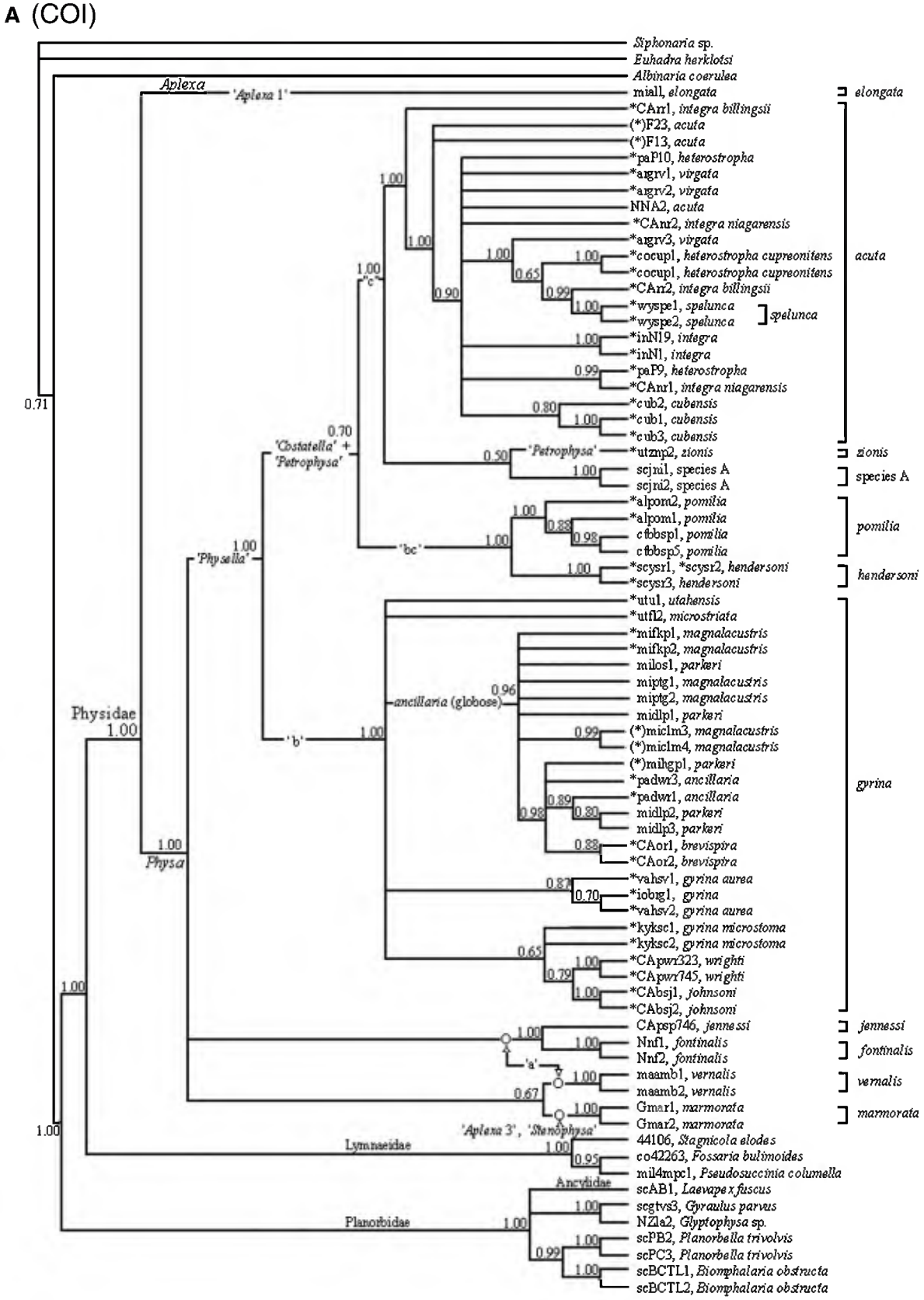

Figure 5. Two trees inferred by Baysian analysis. **A.** COI. **B.** 16S rRNA. The node labelled '*Costatella*' + '*Petrophysa*' is equivalent to Te's (1978) subgenera *Costatella* + *Petrophysa*. The node labelled '*Physella*' is equivalent to Baker's (1928) genus *Physella*. Nodes labelled *gyrina* (typical) and *ancillaria* (globose) within the *gyrina* clade show separation between the two morphotypes despite the small genetic distance. Note that there is no node labelled *gyrina* for COI (Fig. 5A) as this group is paraphyletic in this analysis.

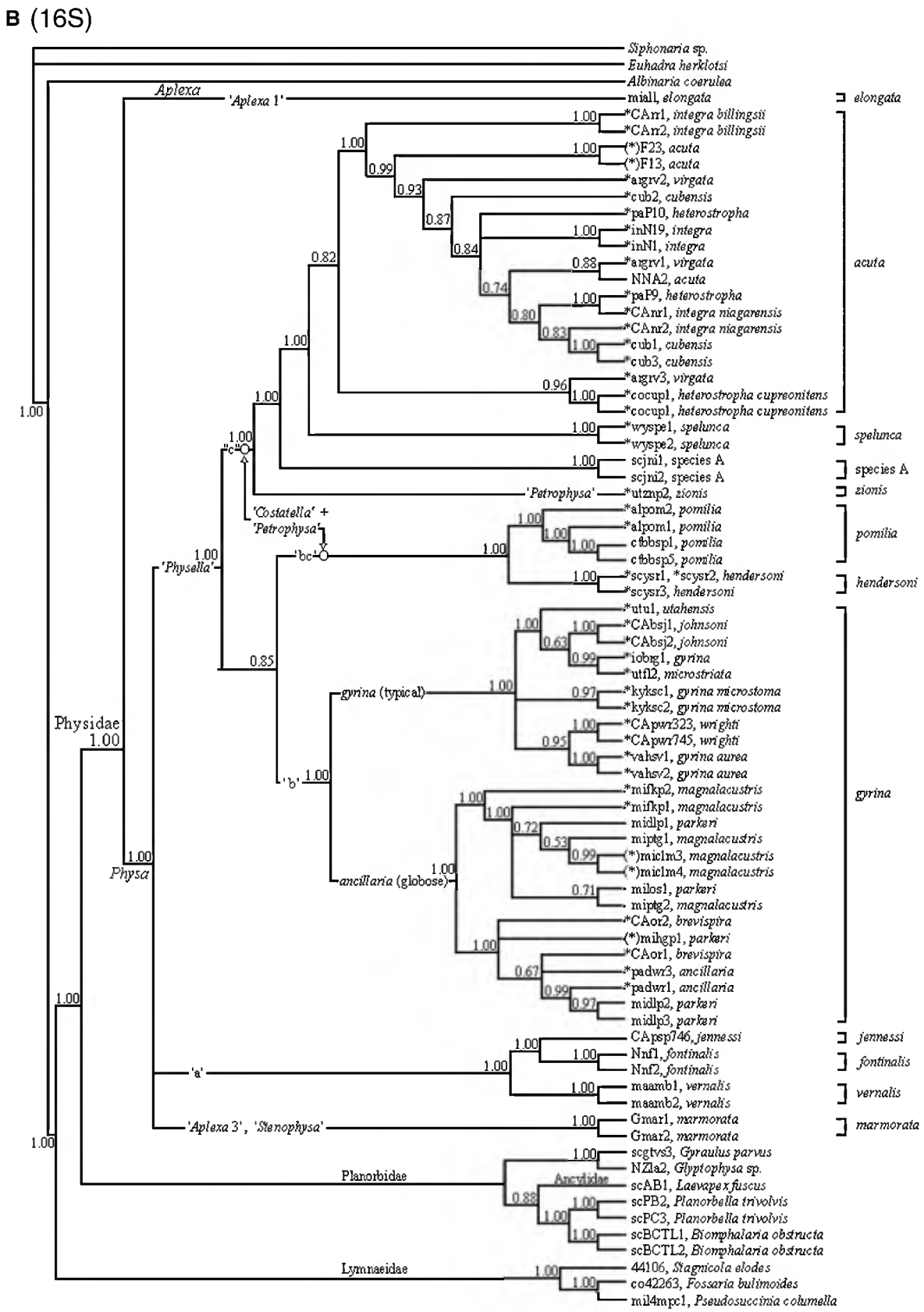

Figure 5. *Continued*

'Physa *type-a' penial complex*

The monophyly of the *fontinalis* group, which exhibits a Holarctic distribution, is generally supported by each molecular analysis (Figs 2–5). The exception is the COI Baysian analysis where the type a penial morphology was made paraphyletic by the placement of *Physa marmorata* as sister to *P. vernalis* (Fig. 5A). The original description of *P. vernalis* (Taylor & Jokinen, 1984) mentions that the penial sheath had a nonglandular region near the preputium (also noticed in our dissections) and that the appearance of the shells resembled juvenile *Aplexa* sp. *Physa jennessi* is sister to *P. fontinalis* and in turn these two species are sister to *P. vernalis*. *Physa vernalis*, which was described from the northeastern USA (Taylor & Jokinen, 1984), possesses the 'type-a' penial morphology, which supports its placement in the *fontinalis* group by Taylor & Jokinen (1984). Taylor (2003) described a new genus, *Laurentiphysa* with *P. vernalis* as the type species. It includes the newly described species *L. chippevarum* Taylor from the Great Lakes region of northern Wisconsin. Taylor (2003) also recognized the monotypic genus *Beringophysa* Starobogatov & Budnikova, 1976, for *Physa jennessi*. There is no apparent phylogenetic or taxonomic value in recognizing three separate genera within the closely related *fontinalis* group.

'Physa *type-b' penial complex*

The *gyrina* group includes Taylor's (2003) tribe Physellini and three of his seven genera, *Physella*, *Utahphysa* and *Archiphysa* (Figs 2–5). Our sampling was not broad enough to comment on the other two genera Taylor named from Central America and included in his tribe Physellini (*Chiapaphysa* and *Ultraphysella*). We find no support for inclusion of *Petrophysa* in the Physellini or for separation of the three genera *Physella*, *Utahphysa* and *Archiphysa*. Taylor's monotypic genus *Costatella* belongs to the type bc penial morphology complex, instead of within Taylor's tribe Physellini (Te, 1978; A.R. Wethington, personal observation).

Our inclusion of *P. brevispira* in the *gyrina* group is based on penial morphology and DNA sequence data of specimens collected from the type locality. *Physa brevispira* was described by Lea in 1864 from the Ottawa River in Canada, based solely on shell characters, and has traditionally been considered a subspecies of *P. integra* (Burch, 1982, 1988; Burch & Tottenham, 1980) following Te (1978). *Physa johnsoni* was placed in the penial morphology c group by Burch (1982, 1988) and Burch & Tottenham (1980), again based on Te (1978), but it is of the penial morphology group b (Taylor, 2003; Wethington & Guralnick, 2004).

We find that the penial morphology group b can be divided into two distinct groups, the 'typical' (*P. gyrina*) and 'globose' (*P. ancillaria*) shelled forms, in all analyses except the COI Baysian analysis (Figs 2–5). *Physa gyrina* includes the following species and subspecies: *P. gyrina*, *P. gyrina aurea*, *P. gyrina mictrostoma*, *P. johnsoni*, *P. microstriata*, *P. utahensis* and *P. wrighti*. *Physa ancillaria* includes the following species and subspecies: *P. ancillaria*, *P. brevispira*, *P. magnalacustris* and *P. parkeri*. These two groups may differ ecologically. The 'typical' group (*P. gyrina*) includes taxa that were originally described from hot springs, such as *P. johnsoni*, *P. wrighti* and *P. gyrina aurea*. These taxa have a more tightly coiled shell than those of the 'globose' group (*P. ancillaria*). *Physa gyrina* is known to be tolerant of heated waters (Clench, 1926; Agersborg, 1929; Clampitt, 1970; Wethington & Guralnick, 2004). However, *P. parkeri* (of the globose group) is almost impossible to culture in the laboratory and requires conditions cooler than room temperature (Dillon & Wethington, 2006b).

Many members of the 'globose' group are larger than those of the 'typical' group, besides having a more globose shell. However, *P. utahensis* of the 'typical' group is both large and globose. Clench (1925) originally described *P. utahensis* as a subspecies of *P. lordi* Baird (which we predict should be in the 'globose' group = *P. ancillaria*), but it would seem more correct for it to be a subspecies of *P. gyrina*. Utah Lake, the type locality of *P. utahensis*, is shallow (mean depth, 2.74 m) and hot during the summer (average annual temperatures 15–25°C; http://www.waterquality.utah.gov/watersheds/lakes/utahlake.pdf) in contrast to the habitats of the more northern 'globose' group. For instance, Douglas Lake (Michigan) where *P. parkeri* can be found, is deep (mean depth, 5.5 m) and stays cold throughout the year, even in the summer (summer temperatures were 16.6°C averaged over the years 1921–1949; http://www.umich.edu/ ~ umbs/research/dlprofile.pdf; http://www.umich.edu/ ~ umbs/research/profiles.htm). Also, it is known that shell shape in physids can be influenced by both biological (DeWitt, 1998; DeWitt, Sih & Hucko, 1999; DeWitt, Robinson & Wilson, 2000) and environmental (Burnside, 1998; Britton & McMahon, 2004) factors despite underlying genetics.

The penial morphology of both 'typical *gyrina*' and 'globose *ancillaria*' groups is similar, with each member having a preputial gland and two penial sheaths, the first sheath being glandular and the second nonglandular. Te (1978) separated members within the *Physa* type b penial morphology group based on penile sheath ratios, but we did not see any basis for this in the mtDNA data.

Dillon & Wethington (2006a) found no reproductive isolation among six taxa having *gyrina*-type penial morphology (which includes the following nominal species: *P. aurea*, *P. gyrina*, *P. microstriata* and *P. utahensis* of the typical *gyrina* form and *P. ancillaria* and *P. parkeri* of the globose *ancillaria* form), but conclusions were rendered tentative by life history differences so broad as to obstruct the culture of control populations. However, given the small genetic distance separating *P. gyrina* from *P. ancillaria* (and the paraphyly of the group in the COI Baysian analysis, Fig. 5A), it is possible that this represents one species that is diverse both morphologically and ecologically. Dillon & Wethington (2006b) could find no distinction between *P. gyrina* and either *P. parkeri* or *P. magnalacustris* (as a subspecies of *P. sayii*) in a survey of genetic variation at seven allozyme loci in nine populations sampled from Michigan. Unpublished sequence data show a geographic signal that corresponds with *P. gyrina* and *P. ancillaria*, with the latter being restricted to more northern climes and the former found in both northern and more southern climes, although one population (a small ditch near Wagner Falls, Michigan) supported both sequences (A.R. Wethington, unpublished).

'Physa *type-bc' penial complex*

Within the *pomilia* complex, both *Physa pomilia* and *P. hendersoni* are phylogenetically distinct (Figs 2–5). The *P. pomilia* of Connecticut appears to be more closely related to the *P. pomilia* of Alabama than either is to the *P. hendersoni* of South Carolina. Experimental breeding data gathered by Dillon, Robinson & Wethington (2007) suggest that *P. hendersoni* and *P. pomilia* are conspecific, as originally suggested by Clench (1925).

In the parsimony run (Fig. 2), likelihood run (Fig. 3) and COI Baysian run (Fig. 5A), the bc penial morphology group was sister to the penial morphology c group (which here includes *P. zionis*) as predicted by Te's (1978) morphological data set, but with moderate to no bootstrap support. The sister relationship among the three groups (see Figs 2, 3, 5A and B) correlates with Te's subgenera *Costatella* plus *Petrophysa*, ignoring the placement of *P. zionis* as more closely related to the c group instead of the bc group as Te (1978) predicted. However support for the subgenera *Costatella* + *Petrophysa* is lost in the distance analysis (Fig. 4). All analyses support Te's (1978) section *Costatella*

(Figs 2–5) with high bootstrap support. The bc penial morphology group appears to be separate from all other groups based on the molecular phylogeny instead of being included with the c penial morphology group as in Taylor (2003), Burch (1982, 1988) and Burch & Tottenham (1980).

Te (1978) was incorrect in his placement of *P. cubensis* and *P. zionis* in the bc penial group, and *P. pomilia* in the c penial group, but was correct in his placement of *P. hendersoni* and *P. costata* in the bc penial group. Other members that Te placed in his *Physa* bc group that were not included in our analysis are: *P. ariomis*, *P. bermudeai*, *P. floridana* and *P. peninsularis*. If Te was correct, the range of the *pomilia* group extends further through the Southeastern USA and possibly into the Caribbean. Taylor (2003) incorrectly placed *Physa costata* Newcombe (first described from Clear Lake, California), as the sole member of the genus *Costatella*, into his tribe Physellini. Based on penial morphology, *P. costata* should be placed in the bc penial morphology group (as predicted by Te, 1978; A.R. Wethington, personal observation) together with *P. pomilia* and *P. hendersoni*.

'Physa *type-c*' *penial complex*

The molecular phylogeny supported recognition of the penial morphology c group (equivalent to Te's, 1978, section *Alampetista*) (Figs 2–5). Within the nominally diverse *P. acuta* complex, none of the following nominal species were monophyletic: *P. acuta*, *P. cubensis*, *P. heterostropha*, *P. integra*, or *P. virgata*. Dillon *et al.* (2002) found that *P. acuta*, *P. heterostropha* and *P. integra* all interbreed and are able to produce a successful F1 generation with no depression of egg laying compared to incross controls, as well as a successful F2, which suggests that they all represent one biological species. Similarly, *P. virgata* shows no reproductive isolation from *P. acuta* (Dillon *et al.*, 2005). Wethington (2003 and unpublished) also showed that the three nominal species *P. acuta*, *P. heterostropha* and *P. integra* are one phylogenetic species based on allozyme and mitochondrial DNA data. Based on the failure of the six topotypic nominal species and subspecies *P. acuta*, *P. heterostropha*, *P. integra*, *P. virgata*, *P. cubensis* and *P. integra niagarensis* to constitute an exclusive clade, it appears that only one phylogenetic species is involved, to which the valid name *P. acuta* would apply. Inclusion of *P. cubensis* under the name *P. acuta* is in accordance with Paraense & Pointier (2003) who showed that *P. cubensis* is morphologically indistinguishable from *P. acuta* based on anatomy, including penial morphology. So, not only should *P. cubensis* be reassigned to the penial c morphology group from Te's (1978) penial bc morphology group (our *pomilia* species group), but it should also be synonymized with *P. acuta* based on the molecular phylogeny.

Setting aside the COI Baysian analysis, three nominal species within the *acuta* complex were monophyletic (Figs 2–5) including *P. integra billingsii* (CArr1 and CArr2), *P. spelunca* (wyspe1 and wyspe2) and *P. heterostropha cupreonitens* (cocup1 and cocup2). *Physa cupreonitens* was sister to the phylogenetic species *P. acuta* in the parsimony and likelihood analyses (Figs 2, 3), but nested within the *acuta* clade in the distance analysis and both Baysian analyses (Figs 4, 5A, 5B), so we tentatively consider it to be synonymous with *P. acuta*. *Physa billingsii* appeared to be phylogenetically distinct in most of the analyses, but is likely to be synonymous with *P. acuta*. In the COI Baysian analysis (Fig. 5A) *P. billingsii* was paraphyletic. *Physa spelunca* was phylogenetically distinct; it has a unique ecology, living in a heated spring within a cave filled with toxic sulphuric gas and feeding primarily on bacteria (Turner & Clench, 1974). *Physa spelunca* also has very little mtDNA genetic diversity when more individuals are sampled (M. Porter, personal communication). It is possible that *P. spelunca* is a valid species as suggested by Wethington & Guralnick (2004), although this is not consistent with its placement within *P. acuta* in the COI Baysian analysis (Fig. 5A).

Physa *species A*

A physid population from John's Island (South Carolina) consistently appeared basal within the penial morphology c group (Figs 2–5), even when five more individuals from the population were added (16S mtDNA only). There is postmating reproductive isolation between this population and the population of *P. acuta* from Charles Towne Landing (R.T. Dillon, personal communications); both populations are from Charleston County. Other populations of species A have recently been reported from South Carolina (R.T. Dillon and A.R. Wethington, unpublished). The collective data suggest that it is an undescribed species.

Physa zionis

Physa zionis also fell basally within the penial morphology c group (Figs 2–5). The ecology of *P. zionis* is strikingly different from all other physids as it crawls vertically on the rock face where seepage occurs, along the narrows in Zion National Park. These physids reach maturity at a small size (<5 mm in length) and lay correspondingly small egg masses (between 1 and 4 eggs per capsule (C. Rogers, personal communications), as compared to as many as 200 or more eggs in some *P. acuta* (A.R. Wethington, personal observations)). *Physa zionis* was originally placed in the subgenus *Petrophysa* by its discoverer, Pilsbry (Chamberlain & Jones, 1929). The characters separating *Petrophysa* from the subgenus *Physella* are a nondigitate mantle, and the radula teeth having few, large cusps and numerous small, interstitial cusps. Te and Taylor disagree about its penial anatomy; Te (1978) suggested that *P. zionis* has a '*Physa* type-bc' penial morphology while Taylor (2003) suggested that it has a '*Physa* type-b' penial morphology. Both authors maintain the name *Petrophysa*, as either a subgenus (Te, 1978) or genus (Taylor, 2003). Neither author was correct with regard to its penial morphology as *P. zionis* has a modified '*Physa* type-c' penial morphology, with the sarcobelum becoming more inflated in more mature specimens. There are physids (*P. gyrina*) that are sympatric with *P. zionis*, but are in ponds, swampy areas and the Virgin River that runs through the narrows. There does not seem to be any reason to keep the subgenus *Petrophysa* for *P. zionis*. Instead, *P. zionis* is more properly placed in the same group as *P. acuta*.

Novel patterns in penial morphology

The penial morphology of the *acuta* group shows east-west variation, with the eastern forms (*P. acuta*, *P. heterostropha* and *P. integra*) having transparent, muscular penial sheaths while the sheaths of the western forms (*P. virgata* and *P. spelunca*) are less transparent and more opaque.

There is a superficial resemblance in penial morphology between members of the bc penial morphology group and *P. spelunca*, *P.* species A and *P. zionis*, with two separate components to the penial apparatus in each. The members of the *pomilia* group have a *Physa* type-bc penial complex with two separate penial sheaths or sections within one penial sheath (the glandular portion being smaller than the muscular portion). *Physa spelunca*, *P.* species A and *P. zionis* all have a modified *Physa* type-c penial complex with an inflated sarcobelum in comparison with *P. acuta*. *Physa zionis* has the largest sarcobelum of the three, roughly equal in length to the preputium in mature

adults (Fig. 1). Within *P. zionis* there seems to be an age/size component to the degree of variation from a standard type *Physa* type-c penis. Smaller specimens of *P. zionis* had penial morphologies indistinguishable from the typical *Physa* type-c penis (Fig. 1).

Molecular phylogeny and systematics of Physidae

The separation of Aplexinae and Physinae sensu Starobagotov (1967) or *Aplexa* and *Physa sensu* Thiele (1931–1935) and Zilch (1959–1960) is supported if *Stenophysa* is included with the Physinae (or *Physa*) instead of the Aplexinae (or *Aplexa*). The plesiomorphic character, lack of a preputial gland, in Te's (1978) *Aplexa* group is not a synapomorphy uniting *Aplexa* and *Stenophysa* within Aplexinae. Instead, the sole *Stenophysa* representative was nested within the Physinae. It would appear that *S. marmorata* (here renamed *Physa marmorata*) retained a plesiomorphic character, resulting in its previous erroneous taxonomic placement. This placement of *Stenophysa* (*Aplexa* 3 penial morphology) within the Physinae was suggested by Te (1978) as a possible alternative.

Baker (1900,1928) suggested that the penial morphology a group should be referred to as the genus *Physa* Draparnaud and the remaining Physinae as the genus *Physella* Haldeman, based on anatomical differences in the mantle edge. This classification scheme was subsequently followed by Te (1978),Burch & Tottenham (1980), Burch (1982,1988) and Burch & Yung (1992). Based on the topology of the mtDNA-based parsimony phylogeny, the genera *Physa* (penial morphology a) and *Physella* (penial morphologies b + bc + c) as proposed by Baker (1900,1928) are supported by the strict consensus tree, but with little to no bootstrap (Fig. 2). The bootstrap support for Baker's *Physella* is only 65.6 in the likelihood analysis (Fig. 3), but the posterior probability is 1.00 in both Baysian analyses (Figs 5A, B), but there is no support for the genus in the distance analysis (Fig. 4), with the two genera becoming paraphyletic.

Ignoring the placement of *P. zionis*, Te's (1978) subgenus *Costatella* (*acuta* + *pomilia* groups) is recovered in the mtDNA-based parsimony phylogeny (Figs 2, 3, 5A), but not in the neighbour-joining phylogeny (Fig. 4) or the 16S Baysian analysis (Fig. 5B). Te's (1978) sections *Alampetista* and *Costatella* are recovered in all analyses, but are not united under his subgenus *Costatella* in the neighbour-joining analysis (Fig. 4) or 16S Baysian analysis (Fig. 5B).

It seems that each penial morph represents a unique species or species group, but that Taylor's (2003) higher-order classification does not seem warranted. Taylor's subfamily ranks are paraphyletic due to the placement of *S. marmorata* (*Aplexa* 3 penial morphology). Each penial morphology represented here correlates loosely with Taylor's (2003) tribe designations: penial morphology a represents the tribe Physini, penial morphology b the tribe Physellini, penial morphology c the tribe Haitini, penial morphology *Aplexa* 3 the tribe Stenophysini, and penial morphology *Aplexa* 1 the tribe Aplexini. Taylor did not correctly distinguish all members having penial morphology bc, and he improperly placed the two genera *Costatella* and *Petrophysa* in his tribe Physellini.

In this study, we examined six general penial morphology types which correspond to separate species or species groups: *Aplexa* variation 1 (*A. elongata*), *Aplexa* variation 3 (*P. marmorata*), type a (*P. fontinalis*, *P. jennessi*, *P. vernalis*), type b (*P. gyrina*, '*P. ancillaria*'), type bc (*P. pomilia*, *P. hendersoni*) and type c (*P. acuta*, *P. spelunca*, *P.* species A, *P. zionis*). We uncovered as many as thirteen phylogenetic units that approximate species (Table 1, Figs 1–5), but further study may narrow this to as few as nine species. There is evidence, for instance, that *P. ancillaria* is a junior synonym of *P. gyrina* (Dillon & Wethington, 2006b; R.T. Dillon & A.R. Wethington, unpublished data), and that *P. hendersoni* may be a junior synonym of *P. pomilia* (Dillon *et al.*, 2007).

CONCLUSIONS

The molecular phylogeny supports six groups based on penial morphology (Figs 1–5; Table 1). These six groups (Fig. 1) correlate well with reproductive isolation experiments conducted by Dillon and colleagues. No evidence of reproductive isolation was found among six populations within *Physa acuta* (Dillon *et al.*, 2002), or among six taxa of penial morphology b (Dillon & Wethington, 2006a), but complete reproductive isolation was found between *P. acuta* and *P. gyrina* (Dillon, Earnhardt & Smith, 2004). R.T. Dillon (personal communications, 2003) has expanded these results to document reproductive isolation (in varying degrees) among *Aplexa elongata*, *P. acuta*, *P.* species A, *P. gyrina* and *P. pomilia*.

These six morphological groups correspond to the following recommended species: *A. elongata*; *P. marmorata*; *P. fontinalis*, *P. jennessi* and *P. vernalis* of penial morphology a; *P. gyrina* (including *P. gyrina*, *P. aurea*, *P. microstoma*, *P. johnsoni*, *P. microstriata*, *P. utahensis* and *P. wrighti*) and possibly *P. ancillaria* (including *P. ancillaria*, *P. brevispira*, *P. magnalacustris* and *P. parkeri*) of penial morphology b; *P. acuta* (including *P. acuta*, *P. billingsii*, *P. cubensis*, *P. cupreonitens*, *P. heterostropha*, *P. integra*, *P. niagarensis* and *P. virgata*), *P. spelunca*, *P.* species A and *P. zionis* of penial morphology c; and *Physa pomilia* and *P. hendersoni* of penial morphology bc (Table 1). The number of physid species in North America has been over-estimated. The data presented here show that of the 28 nominal species included, no more than 12, plus one new species (John's Island *Physa*), are valid.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the following people for reading early drafts of the paper and providing input: Rob Dillon, Laura Gough, Amy Ward, Phil Harris, Philippe Jarne, J. P. Pointier, Peter Mordan, Ellen Strong, Sarah Joyce, Stephanie Clark, Jill Detwiler and an anonymous reviewer. The following people provided assistance in the field, ranging from collecting to identification aid: Susan Wethington, Zelda Wethington, Jennifer Stephens, Tom Smith, Matt Rhett, Steve Finch, Megan Porter, Christopher Rogers, Elliot Rogers, Jeffrey Sides, Deb Kirkland, Stephanie Clark, Nirmala Karnik, Nang Sechanixay, Katy Metzner-Roop, Andrew Roop, Bryan Dillon, Rob Dillon, Andrew Lydeard, Eileen Jokinen and Jack Burch. The following people assisted indirectly in collecting efforts: Tim Roop, Jordan Roop, Charles Wethington, Curt Lively and Lynda Delph. The following people sent specimens that were used in this study: Curt Lively, Roy Anderson, Philippe Jarne, Doug Smith, Eileen Jokinen, Rob Dillon, David Maceira and Sophia B. Twitchell, Jen Buhay, Charles Pacas, David Prescott, Rob Guralnick, Dwayne Lepitzki and Elinor Michel. We would also like to acknowledge funding sources: NSF (awarded to Lydeard, Dillon and Strong); Conchologists of America and Western Society of Malacologists (awarded to Wethington); the University of Alabama Graduate Association and Biology Department, and the Multi-User Equipment Grant from NSF (awarded to Lydeard, Mayden, Powell and Harris (DBI-007-351). This manuscript represents part of Amy Wethington's dissertation research at the University of Alabama. This manuscript was completed while Charles Lydeard served as a Program Officer at the National Science Foundation under the Intergovermental Personnel Agreement Act and was supported in part by the IR/D program.

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Appendix. Information about each specimen name used in the mtDNA analyses.

Specimen name	genbank no. CO1, 16S	Family	First classified as	Taxon name	Locality
44106	EU038352, EU038305	Lymnaeidae		*Stagnicola elodes* (Say, 1821)	
Albinaria				*Albinaria coerulea* (Rossmassler)	See Hatzoglow *et al.*, 1995 (acc. no. NC_001761)
alpom1	EU038353, EU038306	Physidae	*Physa pomilia* Conrad, 1834	*Physa pomilia*	Randons Creek, near Claiborne, Monroe County,
alpom2	EU038354, EU038307				Alabama [31°32′24″N, 87°30′56″W] (type locality)
argrv1	AY651170, AY651209	Physidae	*Physa virgata* Gould, 1855	*Physa acuta*	Gila River, Arizona (type locality)
argrv2	AY651171, AY651210				
argrv3	EU038355, EU038308				
CAbsj1	AY651172, AY651211	Physidae	*Physa johnsoni* Clench, 1926	*Physa gyrina*	Middle Spring, Hot Sulphur Springs, Banff, Alberta,
CAbsj2	AY651173, AY651212				Canada [51°10′N, 115°34′W] (type locality)
CAnr1	EU038356, EU038309	Physidae	*Physa niagarensis* Lea, 1864	*Physa acuta*	Niagara River, Canada [43°15′38″N, 79°04′27″W]
CAnr2	EU038357, EU038310				(type locality)
CAor1	EU038358, EU038311	Physidae	*Physa brevispira* Lea, 1864	*Physa* '*ancillaria*'	Ottawa River, Ottawa, Ontario, Canada.
CAor2	EU038359, EU038312				(type locality)
CApsp746	AF346758, AF346746	Physidae	*Physa jennessi* Dall, 1919	*Physa jennessi*	Alberta, Canada
CApwr323	AF419322, AF419323	Physidae	*Physella wrighti* Te & Clarke, 1985	*Physa gyrina*	Alpha Stream, Liard Hot Springs Provincial Park,
CApwr745	AF346757, AF346745				British Columbia, Canada (type locality)
CArr1	EU038360, EU038313	Physidae	*Physa billingsii* Heron, 1880	*Physa acuta*	Billing's Bridge, near Ottawa [45°25′N, 75°42′W]
CArr2	EU038361, EU038314				Ontario, Canada (type locality)
co42263	EU038362, EU038315	Lymnaeidae		*Fossaria bulimoides* (Lea)	Mesa County, Colorado
					[39°04′47″N, 107°59′11″W]
Cocup1	AY651183, AY651221	Physidae	*Physa cupreonitens* Cockerell, 1889	*Physa acuta*	Hot springs at Wellsville, Colorado
Cocup2	AY651184, AY651222				[38°29′12″N, 105°54′34″W] (type locality)
ctbbsp1	EU038363, EU038316	Physidae	*Physa pomilia* Conrad, 1834	*Physa pomilia*	Beaver Brook State Park, Windham County,
ctbbsp5	EU038364, EU038317				Connecticut [41°44′01″N, 72°07′35″W]
Cub1	EU038365, EU038318	Physidae	*Physa cubensis* Pfeiffer, 1839	*Physa acuta*	Cuba (Santiago de Cuba)(type locality)
Cub2	EU038366, EU038319				
Cub3	EU038367, EU038320				
Euhadra				*Euhadra herklotsi* (Martens)	See Yamazaki *et al.*, 1997
F13	EU038368, EU038321	Physidae	*Physa acuta* Draparnaud, 1805	*Physa acuta*	The Rieutort Wadi in Saint-Martin de Londres,
F23	AY65118, AY651223				25 km north of Montpellier, France
					[43°47′N, 03°44′W] (near type locality)
Gmar1	EU038369, EU038322	Physidae	*Physa marmorata* Guilding, 1828	*Physa marmorata*	Guadeloupe, Etang gommier
Gmar2	EU038370, EU038323				
inN1	EU038371, EU038324	Physidae	*Physa integra* Haldeman, 1841	*Physa acuta*	New Harmony, Indiana [38°08′01″N, 87°56′11″W]
inN19	EU038372, EU038325				(type locality)
Iobrg1	AY651187, AY651225	Physidae	*Physa gyrina* Say, 1821	*Physa gyrina*	Boyer River, north of Council Bluffs Iowa.
					(type locality)
Kyksc1	EU038373, EU038326	Physidae	*Physa microstoma* Haldeman, 1840	*Physa gyrina*	Silver Creek, 1.5 mi down Arbuckle Rd., Madison
Kyksc2	EU038374, EU038327				County, Kentucky [37°39′55″N, 84°28′15″W]
					(type locality)

Continued

Appendix. *Continued*

Specimen name	genbank no. CO1, 16S	Family	First classified as	Taxon name	Locality
maamb1	EU038375, EU038328	Physidae	*Physa vernalis* Taylor Jokinen, 1985	*Physa vernalis*	Bristol County, Massachusettes [41°55′45″N,
maamb2	EU038376, EU038329				71°04′31″W]
mil4mpc1	AY651206, AY651244	Lymnaeidae		*Pseudosuccinea columella* (Say)	Four Mile Lake, Michigan
miall1	EU038377, EU038330	Physidae	*Physa elongata* Say, 1821	*Aplexa elongata*	Oakland County, Michigan [42°33′N, 83°31′35″W]
miclm3	EU038378, EU038331	Physidae	*Physa magnalacustris* Walker, 1901	*Physa* '*ancillaria*'	Crystal Lake, Benzie County, Michigan
miclm4	EU038379, EU038332				(near type locality)
midlp1	EU038380, EU038333	Physidae	*Physa parkeri* Currier, 1868	*Physa* '*ancillaria*'	North Fish Tail Bay, Douglas Lake, Cheboygan
midlp2	EU038381, EU038334				County, Michigan [45°33′47″N, 84°40′35″W]
midlp3	EU038382, EU038335				
mifkp1	EU038383, EU038336	Physidae	*Physa magnalacustris* Walker, 1901	*Physa* '*ancillaria*'	Lake Michigan at Frankfort, Benzie County,
mifkp2	EU038384, EU038337				Michigan [44°38′01″N, 86°14′04″W] (type locality)
mihgp1	EU038385, EU038338	Physidae	*Physa parkeri* Currier, 1868	*Physa* '*ancillaria*'	Higgins Lake, Roscommon County, Michigan.
					(near type locality)
milos1	EU038386, EU038339	Physidae	*Physa parkeri* Currier, 1868	*Physa* '*ancillaria*'	Long Lake, Michigan
miptg1	EU038387, EU038340	Physidae	*Physa magnalacustris* Walker, 1901	*Physa* '*ancillaria*'	Lake Michigan at Petosky, Michigan.
miptg2	EU038388, EU038341				Emmet County [45°22′55″N, 84°57′41″W]
nblr4		Physidae	*Physa integra* Haldeman, 1841	*Physa acuta*	Loup River, Nebraska
NNa2	EU038389, EU038342	Physidae	*Physa acuta* Draparnaud, 1805	*Physa acuta*	Netherlands
NNf1	AY651189, AY651227	Physidae	*Bulla fontinalis* Linnaeus, 1758	*Physa fontinalis*	Netherlands
NNf2	AY651190, AY651228				
NZla2	EU038390, EU038343	Planorbidae		*Glyptophysa* sp.	Lake Alexandria, New Zealand
padwr1	EU038391, EU038344	Physidae	*Physa ancillaria* Say, 1825	*Physa* '*ancillaria*'	Delaware River near Easton, Pennsylvania
padwr3	EU038392, EU038345				[40°41′23″N, 75°12′18″W] (type locality)
paP9	AY651193, AY651231	Physidae	*Physa heterostropha* Say, 1817	*Physa acuta*	Schuykill River, tributary of Delaware River at
paP10	AY651192, AY651230				Philadelphia, Pennsylvania [39°58′50″N,
					75°12′14″W] (type locality)
scAB1	EU038393, EU038346	Ancylidae		*Laevapex fuscus* (Adams)	Pond at Golf Course off Bee's Ferry Road,
					Charleston County, SC [32°49′59″N, 80°04′13″W]
Scbctl1	AY651207, AY651245	Planorbidae		*Biomphalaria obstructa*	Charles Towne Landing, Charleston County, SC
Scbctl2	AY651207, AY651245				[32°48′17″N, 79°59′12″W]
Scgtvs3	EU038394, EU038347	Planorbidae		*Gyraulus parvus* (Say)	TV Station, Charleston County, SC
					[32°47′51″N, 79°53′45″W]
scjni1	EU038395, EU038348	Physidae	*Physa sp.*, new species	*Physa* species A	Agricultural ditch on Jenkin's Farm, John's Island,
scjni2	EU038396, EU038349				SC [32°40′N, 80°03′W]
scpb2	AY651208, AY651246	Planorbidae		*Planorblla trivolvis* (Say, 1817)	Pond at Golf Course off Bee's Ferry Road,
					Charleston County, SC [32°49′59″N, 80°04′13″W]
scpc3	EU038397, EU038350	Planorbidae		*Planorbella trivolvis* (Say)	Pond near Charles Towne Landing, Charleston
					County, SC [32°48′25″N, 79°59′24″W]
scysr1	AY651194, AY651232	Physidae	*Physa hendersoni* Clench (1925)	*Physa hendersoni*	Yemassee, Hampton County, South Carolina
scysr2	AY651195, AY651233				[32°42′24″N, 87°24′07″W] (type locality)
scysr3	AY651196, AY651234				
Siphonaria				*Siphonaria* sp.	

utfl2	EU038398, EU038351	Physidae	*Aplexa microstriata* Chamberlain & Berry, 1930	*Physa gyrina*	Fish Lake, Sevier County, Utah. (type locality)
utznp2	AY651198, AY651236	Physidae	*Physa zionis* Pilsbry, 1926	*Physa zionis*	Zion National Park, The Narrows on canyon wall, Utah [37°09′54″N, 113°00′40″W] (type locality)
vahsv1	AY651201, AY651239	Physidae	*Physa aurea* Lea, 1838	*Physa gyrina*	Hot Springs, Bath County, Virginia
vahsv2	AY651202, AY651240				[37°59′58″N, 79°49′55″W] (type locality)
wyspe1	AY651204, AY651242	Physidae	*Physa spelunca* Turner & Clench, 1974	*Physa spelunca*	Lower Kane Cave, near Kane, about 12 miles east
wyspe2	AY651205, AY651243				of Lovell, on east side of Big Horn River, Wyoming. (type locality)