PROCEEDINGS OF THE ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA 156: 211-216 JUNE 2007

Reassessment of the phylogenetic relationships among *Anodonta*, *Pyganodon*, and *Utterbackia* (Bivalvia: Unionoida) using mutation coding of allozyme data

DAVID T. ZANATTA, ANDRE NGO, AND JOHAN LINDELL

University of Toronto, Department of Ecology and Evolutionary Biology, 25 Willcocks Street, Toronto, Ontario Canada M5S3B2

ABSTRACT.— The use of molecular markers has greatly increased our understanding of unionoid systematics. However, it is critical that their use in phylogenetic studies be conducted with the correct methodologies in order to ensure that the correct interpretations of evolutionary history are made. The phylogenetic relationships of a selection of *Anodonta* were investigated by Hoeh (1990), who found variation in 23 allozyme loci. These allozymes were coded using the presence/absence of alleles, yielding 67 characters used in a phylogenetic analysis. The resulting phylogeny was used as evidence to recommend the elevation of *Pyganodon* and *Utterbackia* to full generic status. Since the publication of Hoeh (1990) the coding of characters using the presence/absence of alleles has been shown to be invalid and has been superseded by mutation coding, with the locus as the character. The phylogenetic analysis of 20 characters, coded using mutation coding, yielded two equally parsimonious trees and an interpretation markedly different from that of Hoeh (1990). Both trees supported the monophyly of *Pyganodon* and *Utterbackia*. However, the genus *Anodonta* was paraphyletic with respect to both *Pyganodon* and *Utterbackia*. The one Eurasian species (*Anodonta cygnea*) was resolved as the sister of the remaining ingroup taxa, including *Pyganodon, Utterbackia*, and the North American *Anodonta*. These findings lead to a taxonomic problem, requiring further phylogenetic analysis of the Anodontinae. In order to test the phylogenetic hypotheses presented herein, we strongly recommend the construction of a phylogeny for all anodontine taxa using a combination of mitochondrial and nuclear DNA sequences.

Keywords: allozymes, phylogenetics, character coding, *Anodonta*, *Pyganodon*, *Utterbackia*, freshwater mussels, Unionoida, Anodontinae

INTRODUCTION

Molecular markers have greatly increased our understanding of unionoid systematics. Allozymes and DNA sequencing have been used to reevaluate both higher and lower level phylogenies and classifications of unionoids (e.g., Campbell et al., 2005; Roe and Hoeh, 2003, and references therein). However, even with the advancement of molecular data available for systematic studies, it remains critically important that correct phylogenetic methodologies are used to resolve species relationships.

The genera *Anodonta* Lamarck, 1799; *Pyganodon* Crosse and Fisher, 1893; and *Utterbackia* Baker, 1927 belong to the Holarctic unionid subfamily Anodontinae. Early classifications of *Anodonta sensu lato* were based on morphological characters (Heard and Guckert, 1970; Kat, 1983). However, many of the shell characters are extremely plastic, making identifications of species of *Anodonta s. l.* very difficult. Accordingly, the phylogenetic relationships of a selection of *Anodonta s. l.* were investigated with molecular characters by Hoeh (1990), who found variation in 23 allozyme loci. These allozymes were coded using the presence/absence of alleles, yielding 67 characters used in a phylogenetic analysis. The resulting phylogeny was used as evidence to recommend the elevation of *Pyganodon* and *Utterbackia* to full generic status.

Hoeh (1990) suggested that using the allele as the character was preferred over using the locus as the character (Buth, 1984) because it did not ignore potential phylogenetic information contained in shared alleles. This reasoning is flawed, as the additional characters provided by treating the presence or absence of an individual allele as a character do not necessarily reflect the evolution of new states. Precence/absence coding of characters allows parallel losses of an allele to be treated as a unifying character. Additionally, some of the extra "information" provided by presense/absence coding serves to disproportionally weight polymorphic loci (Murphy, 1993; Murphy and Doyle, 1998; Murphy and Lovejoy, 1998). Hoeh (1990) also stated that his preference for coding the allele as the character provided for much higher resolution in the resultant phylogeny. Choosing a methodology based on the resulting phylogeny is circular reasoning and violates the rules of parsimony (Brooks and McLennan, 2002). Coding the locus as the character often results in different topologies from topologies derived from the allele being coded as the character (Altaba, 1997; Meier, 1994; Murphy, 1993; Murphy and Doyle, 1998; Murphy and Lovejoy, 1998). Hoeh (1990) was correct in his contention that coding the locus as the character may significantly increase the number of equally parsimonious

trees (EPT). However, the single tree resolved by presence/absence coding may not be one of the group of EPTs resolved by using the locus as the character (Murphy and Doyle, 1998). We recognize that adequate descriptions of methods for coding the locus as the character (Murphy and Doyle, 1998) were not introduced until after Hoeh's (1990) paper was published. Nevertheless, in order to test the phylogenetic hypothesis for *Anodonta* proposed by Hoeh (1990), we have reanalyzed his allozyme dataset using mutation coding as described by Murphy and Doyle (1998).

METHODS

We recoded the allozyme dataset of (Hoeh, 1990) using the locus as the character (Murphy and Doyle, 1998), yeilding 20 characters (Table 1). The original allozyme dataset used in Hoeh (1990) is reprinted for comparison in Table 2. Based on mutation coding, 4 of the 23 allozyme loci (CAT-2, FH, PGD, and SOD) were found to be uninformative as all of the alleles found in the ingroup taxa were also found in the outgroup taxa. In order to code the locus ACP, it had to be split into two characters, as some character states of this highly polymorphic locus required additive coding and others required non-additive coding. *Lasmigona costata*, *L. complanata,* and *L. compressa* were used as outgroup taxa. A maximum parsimony analysis of the 20 allozymic characters was performed using an heuristic search with 100 replications of random stepwise additions using PAUP* v.4.0b10 (Swofford, 1998). Nodal support was assessed with Bremer decay analysis (Bremer,

Table 1. Character matrix of allozyme data from Hoeh (1990), coded using mutation coding (Murphy and Doyle, 1998). Only potentially phylogenetically informative characters are included in the matrix.

Character	PGM	GPD	ACPa	ACPb	GAPDH	GPT	ICD-2	MDH-1	MDH-2	AO
P. cataracta (Say, 1817)	0	1	?	2	0	0	0	1	1	1
P. gibbosa (Say, 1824)	2	1	2	1	0	0	0	1	1	1
P. grandis (Say, 1829)	0	1	0	0	0	0	0	0	1	0
P. lacustris (Lea, 1857)	0	1	?	2	0	0	0	1	1	0
P. fragilis (Fleming, 1828)	0	1	0	0	0	0	0	1	1	1
A. implicata (Say, 1829)	1	0	0	0	0	1	0	1	2	0
A. suborbiculata (Say, 1831)	0	0	2	1	0	1	0	1	2	1
A. couperiana (Lea, 1840)	0	0	2	0	1	1	0	1	2	1
*A. heardi** (Gordon & Hoeh, 1995)	2	0	2	0	1	1	0	1	2	1
A. cygnea (Linnaeus, 1758)	1	1	0	0	0	0	1	1	0	0
A. kennerlyi (Lea, 1860)	0	0	0	0	0	1	0	1	0	1
U. peggyae (Johnson, 1965)	0	0	1	0	0	0	1	1	1	1
U. imbecillis (Say, 1829)	0	0	1	0	0	0	1	1	1	1
L. compressa• (Lea, 1829)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
L. complanata• (Barnes, 1823)	0	0	0	0	0	?	0	0	0	0
L. costata• (Rafinesque, 1820)	0	0	0	0	0	?	0	0	0	0

Character	CAT-1	ADH	B-GUR	ICD-1	EST	FDP	GOT	LAP	PEP-1	PEP-2
P. cataracta (Say, 1817)	2	0	0	0	0	0	0	0	0	2
P. gibbosa (Say, 1824)	2	0	0	0	0	0	0	1	0	2
P. grandis (Say, 1829)	2	1	2	1	1	0	0	1	0	2
P. lacustris (Lea, 1857)	2	0	2	0	0	0	0	1	0	2
P. fragilis (Fleming, 1828)	2	1	2	0	0	0	1	1	0	2
A. implicata (Say, 1829)	1	0	1	1	1	1	1	1	0	1
A. suborbiculata (Say, 1831)	0	0	0	1	0	1	1	0	0	1
A. couperiana (Lea, 1840)	0	0	0	1	0	1	1	0	0	1
*A. heardi** (Gordon & Hoeh, 1995)	1	0	0	1	0	1	1	0	1	1
A. cygnea (Linnaeus, 1758)	0	1	2	0	0	0	0	0	1	1
A. kennerlyi (Lea, 1860)	0	0	1	1	0	0	1	0	0	1
U. peggyae (Johnson, 1965)	0	0	0	0	0	0	0	1	0	1
U. imbecillis (Say, 1829)	0	0	0	0	0	0	1	1	0	1
L. compressa• (Lea, 1829)	0	?	0	0	0	0	0	0	0	0
L. complanata• (Barnes, 1823)	0	0	0	0	0	0	0	0	0	0
L. costata• (Rafinesque, 1820)	0	0	0	0	0	0	0	0	0	0

**Anodonta heardi* is a recently described species (Gordon and Hoeh, 1993-1994) called *A. "couperiana"* by Hoeh (1990).

• Outgroup taxa.

Table 2. Character matrix of allozyme data from Hoeh (1990), coded using presence absence coding.

Allozyme Locus	PGM				GPD			ACP						SOD				FH		GAPDH		GPT		PGD		ICD-2		MDH-1		MDH-2			AO
Character No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**
P. cataracta	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	0	1	1	0
P. gibbosa	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	0	1	0	1	0	0	1	1	1	0	0	1	1	0
P. grandis	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1	0	0	1	1	0	0	0	1	0	1
P. lacustris	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1
P. fragilis	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1	0	1	0	0	1	1	0
A. implicata	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0
A. suborbiculata	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0	1	0
A. couperiana	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
A. heardi	0	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
A. cygnea	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	1	0	0	0
A. kennerlyi	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0
U. peggyae	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0
U. imbecillis	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0
L. compressa•	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	1	0	0	?	?
L. complanata•	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	?	?	0	1	0	1	1	0	1	0	0	0	1
L. costata•	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	0	1	1	0	?	?	1	0	0	1	0	0	1	0	0	?	?

Allozyme Locus	CAT-2			CAT-1			ADH				B-GUR			ICD-1		EST		FDP			GOT				LAP			PEP-1			Pep-2		
Character No.	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**67**
P. cataracta	1	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1
P. gibbosa	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1
P. grandis	0	0	1	1	1	0	0	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1
P. lacustris	0	0	1	1	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1
P. fragilis	0	1	0	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1
A. implicata	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	1	0	1	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0
A. suborbiculata	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
A. couperiana	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
A. heardi	0	0	1	0	0	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0
A. cygnea	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0
A. kennerlyi	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0
U. peggyae	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	?	0	0	1	0	0	1	1	0	0
U. imbecillis	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1	0	0
L. compressa•	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0
L. complanata•	0	0	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0
L. costata•	1	1	0	0	1	1	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0

• Outgroup taxa.

1988; Bremer, 1994) by using AutoDecay v.4.0.2 (Eriksson, 1998) in combination with PAUP*.

RESULTS

The phylogenetic analysis yielded two EPTs. The strict consensus of these is presented in Fig 1A. Both EPTs (50 steps, CI=0.54, RI=0.70, RC=0.38) supported the monophyly of *Pyganodon* and *Utterbackia*. However, the genus *Anodonta* was paraphyletic with respect to both *Pyganodon* and *Utterbackia*. The one Eurasian species (*Anodonta cygnea*) was resolved as the sister of the remaining ingroup taxa, including *Pyganodon, Utterbackia*, and the North American *Anodonta*. The relationships within the North American *Anodonta sensu stricto* were fully resolved, though the relationships among the species of *Pyganodon* were not.

The topology constructed using Hoeh's (1990) allozyme data matrix (Fig. 1B; 156 steps, CI=0.43, RI=0.60, RC=0.26) differed from the topology constructed using mutation coding (Fig. 1A). The most significant differences between the two topologies were the relationships among the species of *Anodonta*. The analysis based on presence/absence coding yielded a monophyletic *Anodonta* (Fig. 1B). However, when using mutation coding, *Anodonta* was paraphyletic with respect to both *Pyganodon* and *Utterbackia* (Fig 1A). Additionally, the relationships within *Pyganodon* were not resolved when using mutation coding

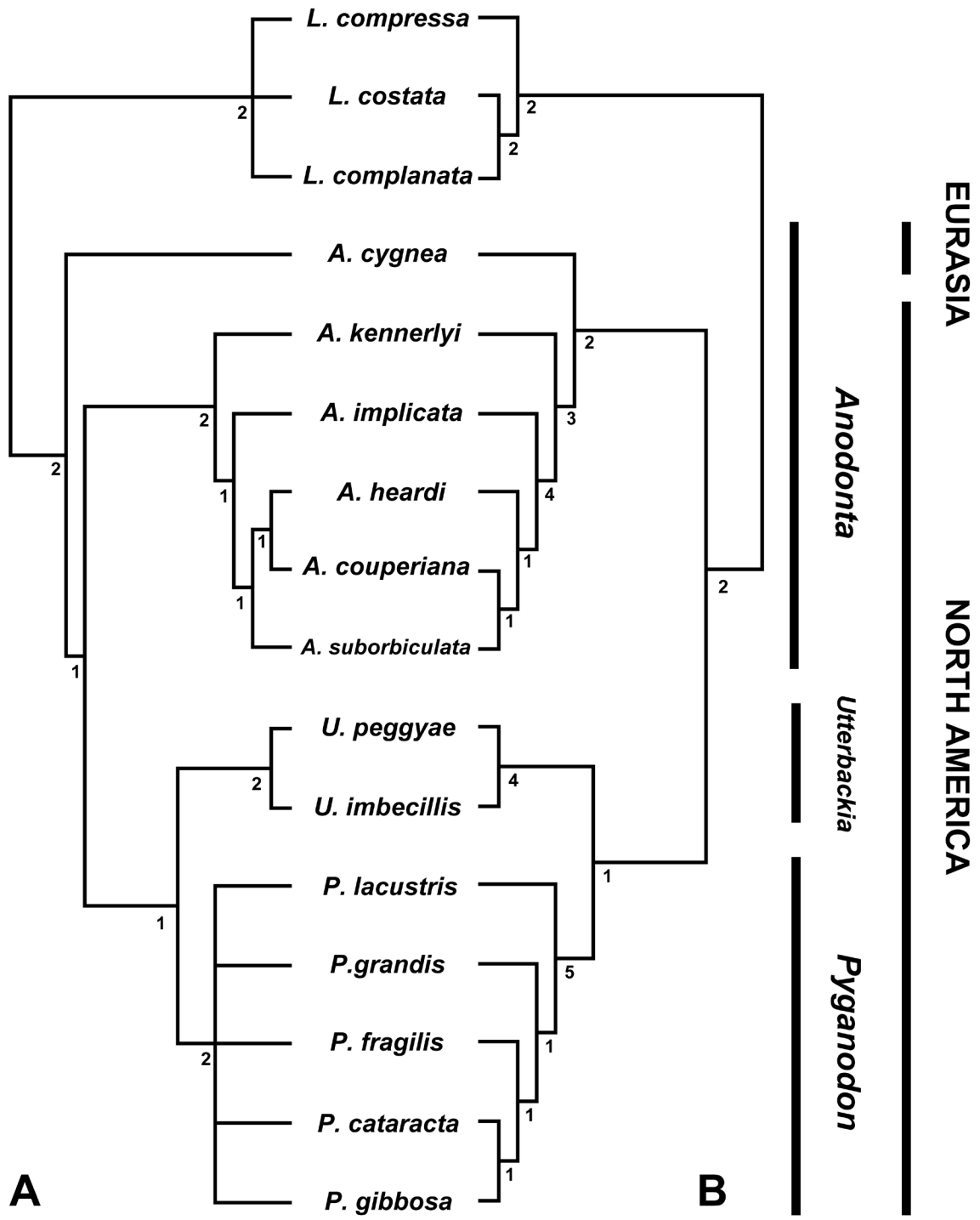

Fig. 1. Consensus (**A.**) of two equally parsimonious trees using the locus as the character (50 steps, CI=0.54, RI=0.70, RC=0.38) and (**B.**) the single most parsimonious tree using the allele (presence/absence) as the character (Hoeh, 1990) (156 steps, CI=0.43, RI=0.60, RC=0.26). Decay indices (Bremer, 1994) are shown below the nodes.

(Fig. 1A), but were fully resolved in the topology based on presence/absence coding (Fig. 1B). The relationships among species of *Pyganodon* recovered using presence/absence coding (Fig. 1B) was not among the EPTs recovered using mutation coding. The relationships between the taxa of North American *Anodonta* also differed. With mutation coding a sister relationship between *A. heardi* and *A. couperiana* was recovered (Fig. 1A), while *A. suborbiculata* was resolved as the sister group to *A. couperiana* using presence/absence coding (Fig. 1B).

The two phylogenies were not found to be significantly different (P>0.05), although these tests may be limited due to the low number of characters used. The mutation coded phylogeny is 50 steps and the Hoeh's (1990) original phylogeny is 53 steps using the mutation coded dataset. The phylogenies were statistically compared under a maximum parsimony framework using the parametric Kishino-Hasegawa (P=0.0828), non-parametric Templeton (0.0833), and non-parametric winning-sites tests (P=0.2500) in PAUP* v4.0b10.

DISCUSSION

The monophyly of both Pyganodon and *Utterbackia* are supported by our analysis. However, the paraphyly of *Anodonta* with respect to both *Pyganodon* and *Utterbackia* causes a taxonomic problem. Because the Eurasian *Anodonta cygnea* is the type species, the generic name is tied to it, requiring either the elevation of the monophyletic North American clade of *Anodonta* (Fig. 1A) to generic rank or the demotion of *Pyganodon* and *Utterbackia* to subgeneric rank and returning them to the *Anodonta*. However, before any taxonomic changes can be recommended, a phylogeny with increased taxon sampling of both Eurasian and Western North American *Anodonta* is required.

We recognize that allozymes are generally viewed as outdated markers. However, few highly taxon-inclusive phylogenetic studies have been published for the Anodontinae using DNA sequence data. The new allozyme topology presented herein does not conflict with the few limited DNA sequence-based phylogenies available for comparison (e.g., Graf and O'Foighil 2000; Huang et al. 2002; Köllersjö et al., 2005; King et al. 1999). The only DNA sequence-based phylogeny published that includes a mix of North American and Eurasian anodontine taxa is by Huang et al. (2002). Using mitochondrial DNA (16S rRNA), Huang, et al. (2002) resolved a topology that was most compatible with the tree besed on mutation coding (Fig. 1A). Preferably, sequences from several genes should be evaluated as single-gene phylogenies are more likely to be misleading (Funk and Omland, 2003). The DNA sequencing-based phylogenies listed above also suggest that *Lasmigona* spp. are inappropriate as outgroups for *Anodonta* as it seems that *Anodonta s. s.* may not be monophyletic, with *Lasmigona* spp. sharing a more recent common ancestor with *Pyganodon* and *Utterbackia*. Hence, we strongly recommend the construction of a complete phylogeny for all Anodontinae using a combination of mitochondrial and nuclear DNA sequences, with hypotheses being made for morphological character evolution (e.g. loss of hinge dentition). This would provide a thorough test of the phylogenetic hypotheses suggested by both Hoeh (1990) and herein.

REFERENCES

Altaba, C.R. 1997. Phylogeny and biogeography of midwife toads (Alytes, Discoglossidae): A reappraisal. Contributions to Zoology 66: 257-262.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795-803.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10: 295-304.

Brooks, D.R. and McLennan, D.A. 2002. The Nature of Diversity. University of Chicago Press, Chicago, IL.

Buth, D.G. 1984. The application of electrophoretic data in systematics. Annual Review of Ecology and Systematics 15: 501-522.

Campbell, D.C., Serb, J.M., Buhay, J.E., Roe, K.J., Minton, R.L., and Lydeard, C. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. Invertebrate Biology 124: 131-164.

Eriksson, T. 1998. AutoDecay version 4.0 (program distributed by the author). Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm. http://www.bergianska.se/index_forskning_soft.html. [Accessed May 1, 2006]

Funk, D.J. and Omland, K.E. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology and Systematics 34: 397-423.

Gordon, M.E. and Hoeh, W.R. 1993-1994. *Anodonta heardi*, a new species of freshwater mussel (Bivalvia: Unionidae) from the Apalachicola River system of the southeastern United States. Walkerana 7: 265-273.

Graf, D.L. and O'Foighil, D. 2000. The evolution of brooding characters among the freshwater pearly mussels (Bivalvia: Unionoidea) of North America. Journal of Molluscan Studies 66: 157-170.

Heard, W.H. and Guckert, R.H. 1970. A re-evaluation of the recent Unionacea (Pelecypoda) of North America. Malacologia 10: 333-355.

Hoeh, W.R. 1990. Phylogenetic relationships among eastern North American *Anodonta* (Bivalvia: Unionidae). Malacological Review 23: 63-82.

Huang, Y., Liu, H., Wu, X., and Ouyang, S. 2002. Testing the relationships of Chinese freshwater Unionidae (Bivalvia) based on analysis of partial mitochondrial 16S rRNA sequences. Journal of Molluscan Studies 68: 358-363.

Köllersjö, M., Von Proschwitz, T., Lundberg, S., Eldenäs, P., and Erséus, C. 2005. Evaluation of ITS rDNA as a complement to mitochondrial gene sequences for phylogenetic studies in freshwater mussels: an example using Unionidae from northwestern Europe. Zoologica Scripta 34: 415-424.

Kat, P.W. 1983. Genetic and morphological divergence among nominal species of North American *Anodonta* (Bivalvia: Unionidae). Malacologia 23: 361-374.

King, T.L., Eackles, M.S., Gjetvaj, B., and Hoeh, W.R. 1999. Intraspecific phylogeography of *Lasmigona subviridis* (Bivalvia: Unionidae): conservation implications of range discontinuity. Molecular Ecology 8: S65-S78.

Meier, R. 1994. On the inappropriateness of presence/ absence recoding for nonadditive multistate characters in computerized cladistic analyses. Zoologischer Anzeiger 232: 201-212.

Murphy, R.W. 1993. The Phylogenetic Analysis of Allozyme Data: Invalidity of Coding Alleles by Presence/Absence and Recommended Procedures. Biochem. Syst. Ecol. 21: 25-38.

Murphy, R.W. and Doyle, K.D. 1998. Phylophenetics: Frequencies and Polymorphic Characters in Genealogical Estimation. Systematic Biology 47: 737-761.

Murphy, R.W. and Lovejoy, N.R. 1998. Punctuated Equilibrium or Gradualism in the Lizard Genus *Sceloporus*? Lost in Plesiograms and a Forest of Trees. Cladistics 14: 95-103.

Roe, K.J. and Hoeh, W.R. 2003. Systematics of Freshwater Mussels (Bivalvia: Unionoida). *In* Molecular Systematics and Phylogeography of Mollusks. Smithsonian Books, Washington DC, USA. pp. 91-122.

Swofford, D.L. 1998. PAUP*: Phylogenetic Analysis Using Parsimony, version 4.0. Sinauer Associates, Sunderland, Massachusetts.