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PHYLOGENETIC RELATIONSHIPS AMONG EASTERN NORTH AMERICAN *ANODONTA* (BIVALVIA: UNIONIDAE)

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ABSTRACT

This study presents estimates of the genetic differentiation and phylogenetic relationships among 13 presumptive species of *Anodonta*. These estimates are based on allozymic and allozymic+morphological data, respectively. The combined allozymic and morphological data base yields the following hypothesis of relationships for the species of *Anodonta* considered in this work: *((((((A. cataracta, A. gibbosa) (A. lacustris, A. grandis)) A. fragilis) (A. imbecilis, A. peggyae)) (A. cygnea (A. kennerlyi (A. implicata (A. "couperiana" (A. couperiana, A. suborbiculata))))))*. From the evidence presented in this report, a number of conclusions are suggested. (1) Gross conchological divergence is poorly correlated with allozymic divergence in *Anodonta*. Conchologically similar species (*e.g., A. cataracta* and *A. implicata; A. imbecilis* and *A. couperiana)* can be very divergent allozymically. Conchologically divergent species (*e.g., A. suborbiculata* and *A. couperiana*) can display relatively little allozymic divergence. These data suggest that, in *Anodonta*, allozymes and conchology are not evolving in concert. This finding together with the ecophenotypic plasticity of *Anodonta* present substantial problems for species delineation and phylogenetic analysis based on traditional conchological approaches. (2) As a corollary of the first conclusion, certain similarities in conchology have been misleading as far as diagnosing monophyletic groups in *Anodonta* due to their convergent or plesiomorphic nature. Raised umbos, independently acquired in the ancestor of *A. implicata* and in the ancestor of the *A. cataracta, A. gibbosa, A. grandis, A. lacustris, A. fragilis* clade, was the characteristic previously used to diagnose the polyphyletic subgenus *Pyganodon*. Low umbos, symplesiomorphic in *A. imbecilis, A. peggyae, A. suborbiculata*, and *A. couperiana*, was the characteristic previously used to diagnose the polyphyletic subgenus *Utterbackia*. (3) In the revised classification, *Pyganodon* comprises *A. cataracta, A. gibbosa, A. grandis, A. lacustris*, and *A. fragilis*, *Utterbackia* comprises *A. imbecilis* and *A. peggyae*, and *Anodonta sensu stricto* comprises *A. cygnea, A. kennerlyi, A. implicata, A. "couperiana", A. suborbiculata*, and *A. couperiana*. (4) These three highly differentiated clades within what has been recognized previously as *Anodonta sensu lato* should be considered taxa of generic rank.

Key words: allozymes, *Anodonta*, cladistics, classification, morphology, phylogenetics, Unionidae.

INTRODUCTION

The Holarctic freshwater mussel genus *Anodonta* Lamarck 1799 comprises approximately 63 recognized species (Simpson, 1914), 16 of which are in North America north of Mexico (Burch, 1975). The North American species exhibit a considerable array of variation in morphology, breeding systems, and life history characteristics. Interspecific morphological variation occurs in adult (*e.g.*, see Burch, 1975; Fig. 1) and glochidial (*e.g.*, see Rand & Wiles, 1982; Hoggarth, 1988) conchology as well as in the internal anatomy (Kat, 1983a, 1986) of *Anodonta*. The great majority of North American unionid species are gonochoric (dioecious) with the remainder being simultaneous hermaphrodites (*e.g.*, see van der

Schalie, 1970). This predominance of the gonochoric breeding system is also apparent in the genus *Anodonta*, of which only one North American species, *A. imbecilis* Say 1829 is a simultaneous hermaphrodite. Life history variation encompasses some of the most interesting features of *Anodonta* biology. Both multivoltine and univoltine reproductive patterns have been observed within and among species (*e.g.*, see Allen, 1924, Heard, 1975).

This array of interspecific variability invites evolutionary explanations. However, in order to test hypotheses of process, the evolutionary relationships among the species of *Anodonta* must be estimated. In particular, there is a need for an estimate of cladogenic pattern based on the analysis of multiple characters (Eldredge & Cracraft, 1980; Wiley, 1981). The resultant phylogenetic hypothesis will enable the testing of hypotheses addressing the evolution of morphology, breeding systems, and life history characteristics (Fink, 1982).

Species limits and species level relationships within unionid genera are almost entirely based on conchological characters. Due to the phenotypic plasticity of shell shape, suspected high levels of conchological convergence, and the relative paucity of informative anatomical and conchological characters, species limits as well as interspecific relationships within the family Unionidae are, in general, poorly understood. Some of the limitations involved in using conchological characters in unionid classification have been discussed previously (*e.g.*, see Heard & Guckert, 1970; Heard, 1974; Davis, 1982, 1983, 1984). Within the genus *Anodonta*, many species-grouping schemes have been proposed (*e.g.*, see Simpson, 1900, 1914; Frierson, 1927; Haas, 1969; Kat, 1983a). Three subgenera (*Anodonta sensu stricto*, *Pyganodon* Crosse & Fischer 1893, and *Utterbackia* F.C. Baker 1927) comprising the North American species have been referred to in recent works on North American *Anodonta* (*e.g.*, see Johnson, 1970, 1972, 1980; Heard, 1975; Kat, 1983a). These subgenera are based on a limited number of possibly plesiomorphic conchological features such as relative inflation of the umbo and general shell shape.

The integration of data sets, such as those produced by comparative studies of freshwater mussel internal anatomy (Kat, 1983a, 1983c, 1986; Smith, 1980, 1986), shell ultrastructure (Kat, 1983b, 1986), glochidial morphology (Rand & Wiles, 1982; Clarke, 1981b, 1985; Hoggarth, 1988), karyology (Jenkinson, 1983), and molecular characteristics (Baagoe *et al.*, 1985; Hvilsom & Pedersen, 1988; Davis, 1983, 1984; Davis & Fuller, 1981; Davis *et al.*, 1981; Kat & Davis, 1984; Kat, 1983a, 1983c, 1986), is needed to independently test hypotheses of species identity and relationships in *Anodonta* that are often based on a few unpolarized conchological features (Davis, 1983; Kat, 1983a). To that end, this study presents estimates of the genetic differentiation and phylogenetic relationships among 13 presumptive species of *Anodonta*. These estimates are based on allozymic and allozymic + morphological data, respectively. Included in this study are representative species from each of the three subgenera currently recognized for North American *Anodonta*.

MATERIALS AND METHODS

Shells representing the 16 species included in this study (13 species of *Anodonta*, three species of *Lasmigona* as outgroup taxa) are shown in Fig. 1. A list of the species, with sampling localities, voucher specimen numbers and sample sizes for the allozyme analyses is presented in Table 1. The type species of *Anodonta s.s.* and *Utterbackia*, namely *A. cygnea* (Linnaeus 1758) and *A. imbecilis*, are among the species included in this study. Except in the case of *A. peggyae* Johnson 1965 vs. *A. imbecilis*, all interspecific comparisons

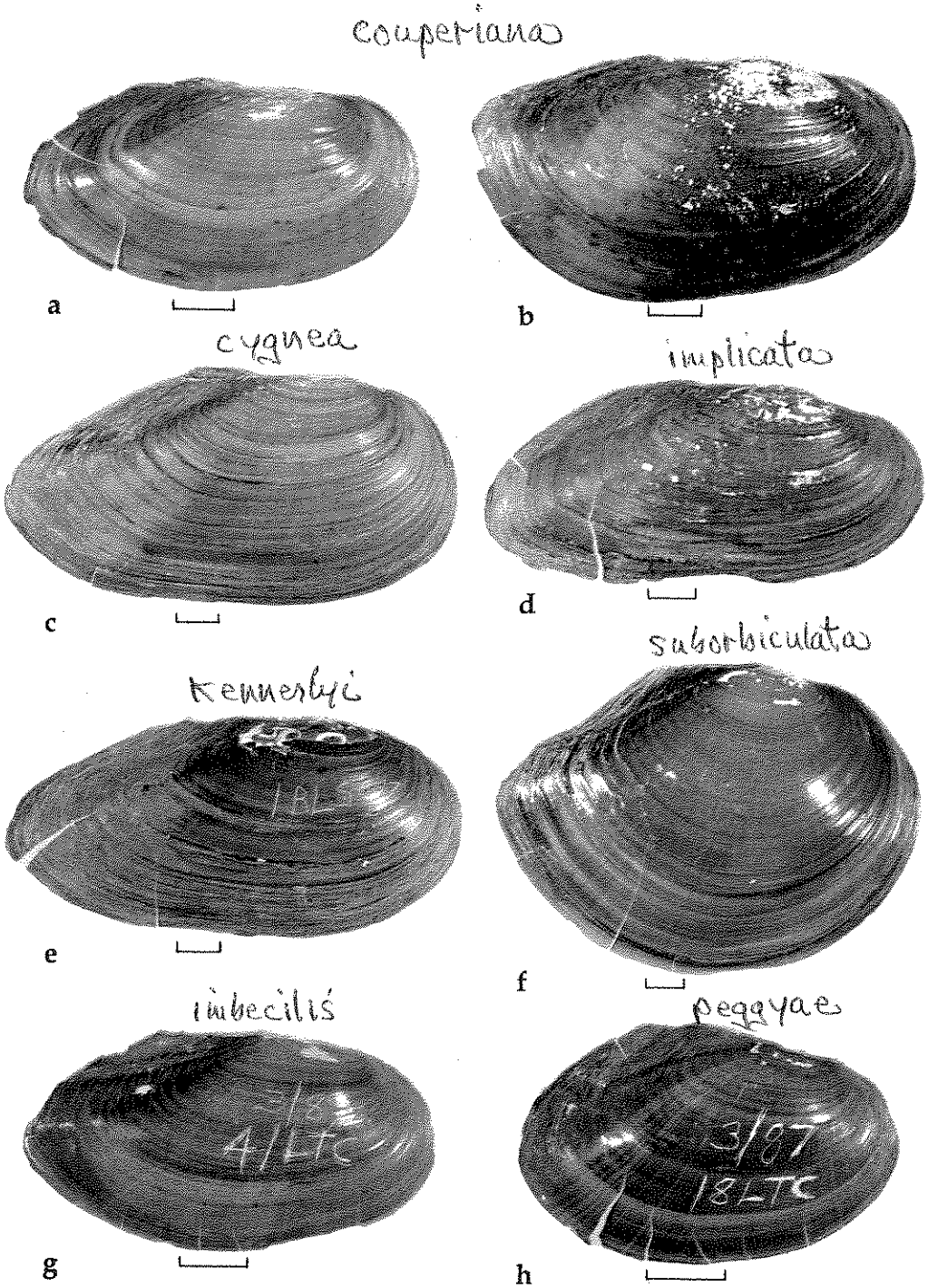

FIG 1. Shells of representative individuals of the *Anodonta* and *Lasmigona* species used in the allozyme analyses. Locality data are presented in Table 1. **a**, *A. couperiana*; **b**, *A. "couperiana"*; **c**, *A. cygnea*; **d**, *A. implicata*; **e**, *A. kennerlyi*; **f**, *A. suborbiculata*; **g**, *A. imbecilis*; **h**, *A. peggyae*. Measurement lines = 1 cm.

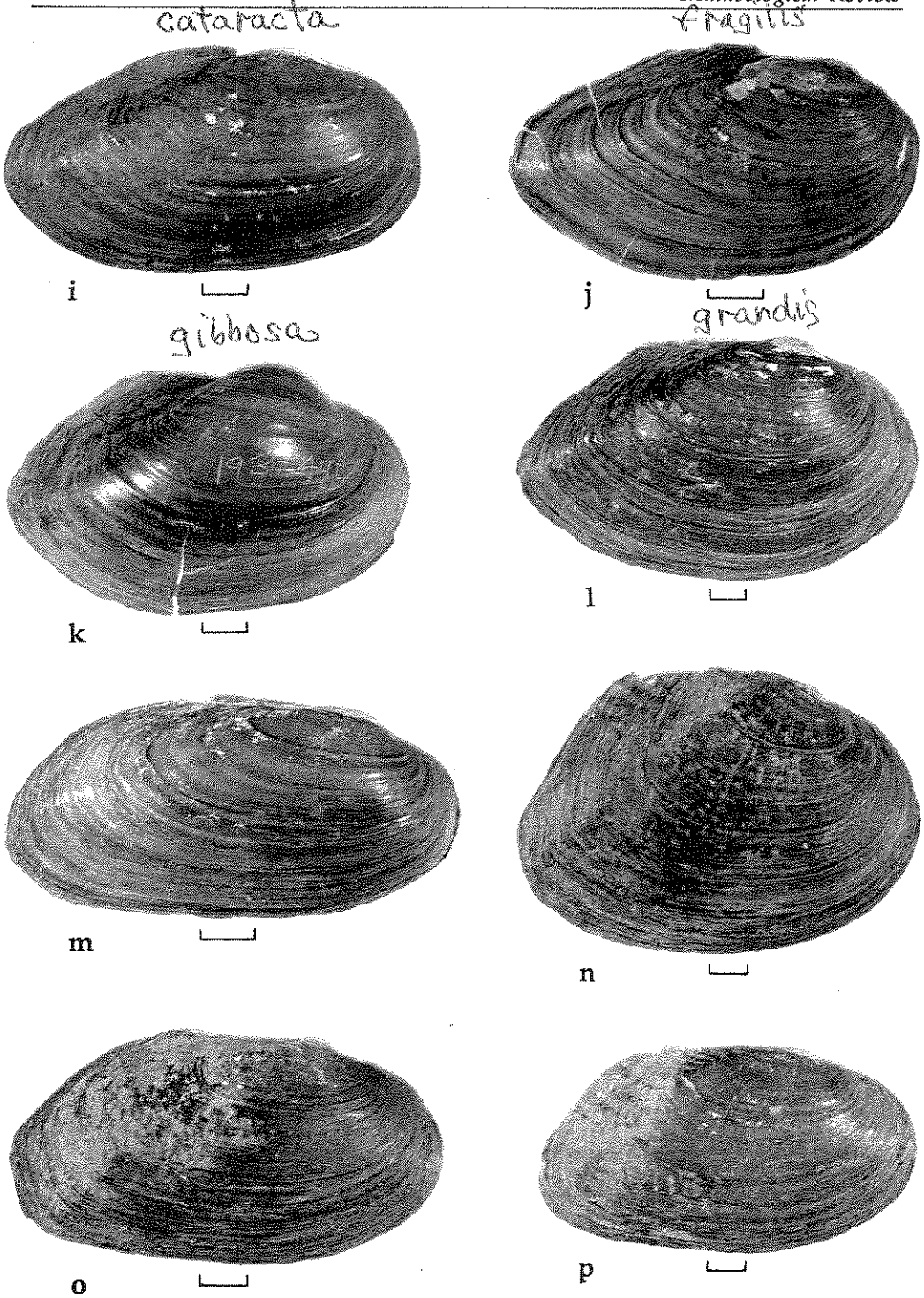

FIG. 1 (cont.). **i**, *Anodonta cataracta*; **j**, *A. fragilis*; **k**, *A. gibbosa*; **l**, *A. grandis*; **m**, *A. lacustris*; **n**, *Lasmigona complanata*; **o**, *L. compressa*; **p**, *L. costata*. Measurement lines = 1 cm.

TABLE 1. Species designation, sample size, collection locality information, traditional subgeneric assignment (*Anodonta* only) and University of Michigan Museum of Zoology voucher specimen catalog numbers for the specimens used in the allozyme portion of this study.

Species	Sample size	Collection locality	Catalog number
Genus *Anodonta*			
Subgenus *Anodonta s.s.*			
A. cygnea	1	Budworth Mere, Cheshire, England	250710
A. kennerlyi	2	Beaver Lake, Skagit Co., Wash.	250711
Subgenus *Pyganodon*			
A. cataracta	5	Pickering Cr., Phoenixville, Chester Co., Pa.	250699
A. fragilis	5	~~Wells Gully on~~ Bird's Pond, Whitbourne, Nfld., Canada	250704
A. gibbosa	4	Ocmulgee R., Ben Hill-Coffee Co. line, Ga.	250707
A. grandis	5	Mill Cr., below Starve Hollow Lake, Jackson Co., Ind.	250709
A. implicata	5	Canals off of the Connecticut R., Hampden Co., Conn.	250700
A. lacustris	4	Lancaster Lake, Cheboygan Co., Mich.	250701
Subgenus *Utterbackia*			
A. couperiana	5	St. Johns R., Florida Rt. 192 bridge, Brevard Co., Fla.	250706
A. "couperiana"	1	Apalachicola R., Chattahoochee, Gadsden Co., Fla.	250708
A. imbecilis	5	Coe's Landing, Lake Talquin, Leon Co., Fla.	250702
A. peggyae	5	Coe's Landing, Lake Talquin, Leon Co., Fla.	250705
A. suborbiculata	4	Ponds off the Yazoo R., Yazoo Co., Miss.	250703
Genus *Lasmigona*			
L. complanata	5	Black R., Sanilac Co., Mich.	250696
L. compressa	5	Goose Cr., Brooklyn, Jackson Co., Mich.	250698
L. costata	5	Black R., Sanilac Co., Mich.	250697

were based on individuals from allopatric populations. After specimen collection, gill tissues were excised and cleaned of macroscopic parasites and debris, frozen on liquid nitrogen, and subsequently stored at -70°C. Gill tissues were homogenized with a glass pestle in 1.5 ml microcentrifuge tubes. The gill tissues contained sufficient water to eliminate the need for homogenization buffer. The resultant homogenate was centrifuged at 13,605 x g for 10 minutes at 4°C. Supernatants were aliquoted and stored at -70°C until use.

TABLE 2. Enzymes assayed (substrates in parentheses), number of loci scored per enzyme, enzyme abbreviation, Enzyme Commission number, and gel buffer systems used in the allozyme portion of this study.

Enzyme	No. loci scored	Abbreviation	E.C. no.	Buffer system
Acid phosphatase	1	ACP	3.1.3.2	MC 6.0
Aconitase	1	ACON	4.2.1.3	MC 6.0
Alcohol dehydrogenase (octanol)	1	ADH	1.1.1.1	HC 7.0
Aldehyde oxidase (benzaldehyde)	1	AO	1.2.3.1	MC 6.0
Catalase	2	CAT	1.11.1.6	EBT 8.6
Esterase (alpha naphthyl acetate)	1	EST	3.1.1.1	MC 6.0
Fructose diphosphatase	1	FDP	3.1.3.11	MC 6.0
Fumarate hydratase	1	FH	4.2.1.2	TMME 7.4
Beta glucuronidase	1	B-GUR	3.2.1.31	MC 6.0
Glutamate-oxaloacetate transaminase	1	GOT	2.6.1.1	LiOH 8.3
Glutamate-pyruvate transaminase	1	GPT	2.6.1.2	TC 8.0
Glyceraldehyde-phosphate dehydrogenase	1	GAPDH	1.2.1.12	TC 8.0
Glycerol-3-phosphate dehydrogenase	1	GPD	1.1.1.8	MC 6.0
Isocitrate dehydrogenase	2	ICD	1.1.1.42	MC 6.0, TMME 7.4
Leucine aminopeptidase	1	LAP	3.4.11.1	LiOH 8.3
Malate dehydrogenase	2	MDH	1.1.1.37	EBT 8.6
Peptidase (L-leucylglycyl-glycine & L-valyl-L-leucine)	2	PEP	3.4.11	LiOH 8.3
Phosphoglucomutase	1	PGM	2.7.5.1	MC 6.0
6-Phosphogluconate dehydrogenase	1	PGD	1.1.1.44	TMME 7.4
Superoxide dismutase	1	SOD	1.15.1.1	TMME 7.4

Electrophoresis was carried out on 12% starch gels (51 g Connaught starch in 425 ml of gel buffer) using six electrophoretic buffer systems. Twenty-four presumptive loci were scored from 21 enzyme systems. The actual loci scored and electrophoretic buffer systems used per locus are listed in Table 2. The references for the electrophoretic buffer systems used are as follows: EBT 8.6 (Wurzinger, 1980), TMME 7.4 (Spencer *et al.*, 1964), MC 6.0 (Clayton & Tretiak, 1972), HC 7.0 (Brewer, 1970), LiOH 8.3 (Ashton & Braden, 1961), TC 8.0 (Selander *et al.*, 1971). Stain recipes are after Shaw & Prasad (1970), Siciliano & Shaw (1976) and Wurzinger (1980). Assignment of allelic identity was based on comparisons in adjacent gel lanes. To estimate levels of genetic differentiation among the taxa, Nei genetic distances (Nei, 1972, 1978) were calculated from allele frequency data using a computer program written in BASIC (Dowling & Moore, 1984).

Coding of the allozyme data set for phylogenetic analysis used the allele (presence/absence) as the character. This coding scheme was preferred over an alternative scheme (*i.e.*, coding the locus as the character; Buth, 1984) because it does not ignore the potential phylogenetic information contained in shared alleles and it provided for much higher resolution in the resultant cladograms of this study and others (*e.g.*, see Dowling & Brown, 1989). Highly resolved cladograms (*i.e.*, hypotheses of relationship) are desirable because they are more susceptible to falsification. Morphological characters (stomach anatomy characters after Kat (1983a), glochidial characters after Hoggarth (1988)) were coded as either binary or unordered multistate characters. In adherence to the principle of total evidence (*e.g.*, see Miyamoto, 1985; Kluge, 1989), allozymic and morphological characters compose a single data matrix. However, exploration of the individual data sets (*i.e.*, allozyme and morphology) is desirable because of potential insights afforded by comparisons of separate allozyme and morphology based cladograms with cladograms derived from total evidence (*e.g.*, see Miyamoto, 1985; Hillis, 1987). Only the phylogenetically informative characters (*i.e.*, those characters shared by at least two but less than 15 species) were entered into the data matrix. The cladistic analyses of the allozymic, morphological and allozymic+morphological data sets were carried out with the microcomputer version of PAUP (Phylogenetic Analysis Using Parsimony, version 2.4.1, written by D. Swofford, Illinois Natural History Survey, Champaign, Illinois). For the analysis of the morphological data set, a strict consensus cladogram (Rohlf, 1982) was calculated by the CONTREE program (D. Swofford). Rooting was accomplished by using *Lasmigona complanata* (Barnes 1823), *L. compressa* (Lea 1829), and *L. costata* (Rafinesque 1820) as outgroup taxa.

RESULTS

The electrophoretic analysis of allozymes detected 100 electromorphs for the 24 presumptive loci. Nei's genetic distance and the number of loci with fixed allelic differences between pairs of *Anodonta* species are presented in Table 3. The range of Nei's genetic distances and number of loci with fixed allelic differences (out of 24 loci) are 0.087-1.634 and 2-18, respectively. The allozymic evidence suggests that *A. "couperiana"* (Apalachicola River), *A. fragilis* Lamarck 1819, and *A. peggyae* are specifically distinct from *A. couperiana* Lea 1842, *A. cataracta* Say 1817, and *A. imbecilis*, respectively.

The data matrix of the 82 informative characters used for phylogenetic analysis is presented in Table 4. Using only the allozyme characters (1-67), PAUP found a single, fully resolved, most parsimonious tree of 156 steps with a consistency index of 0.429 (Fig. 2). The analysis of the morphological characters (68-82) found 100 equally parsimonious trees of 40 steps, each with a consistency index of 0.700. The CONTREE program (D. Swofford) was used to produce a strict consensus tree (Rohlf, 1982) from the 100 equally parsimonious trees (Fig. 3). Using the complete data set (characters 1-82), PAUP found a single, fully resolved, most parsimonious tree of 201 steps with a consistency index of 0.473 (Fig. 4). The allozyme and overall analyses suggest that, at least with respect to

TABLE 3. Genetic distances (above the diagonal; Nei 1978) and the number of allozyme loci (out of 24) with no shared alleles (below the diagonal) among 13 species of *Anodonta*.

	cataracta	*gibbosa*	*grandis*	*lacustris*	*fragilis*	*implicata*	*suborbic.*	*couper.*	*"couper."*	*cygnea*	*kennerlyi*	*peggyae*	*imbecilis*
cataracta	-	.246	.406	.325	.295	1.022	.846	1.059	1.127	1.217	1.227	.765	.678
gibbosa	3	-	.291	.348	.326	.994	.815	1.044	1.022	1.175	1.173	.707	.623
grandis	7	4	-	.301	.481	.981	.945	1.102	1.288	1.129	1.196	.747	.747
lacustris	6	5	5	-	.372	1.075	1.075	1.466	1.530	1.133	1.634	.653	.653
fragilis	6	5	7	7	-	.847	.896	1.015	1.054	1.165	1.291	.769	.602
implicata	15	13	13	15	13	-	.444	.387	.326	1.239	.669	1.210	.955
suborbiculata	13	12	13	15	14	8	-	.136	.244	.972	.608	.866	.684
couperiana	15	14	14	18	15	7	3	-	.136	1.232	.617	1.232	.981
"couperiana"	16	14	16	18	15	6	5	3	-	.960	.676	1.211	.960
cygnea	17	16	16	16	16	16	15	17	15	-	.881	.875	.875
kennerlyi	17	16	16	18	17	12	11	11	12	14	-	.988	.785
peggyae	13	11	12	10	13	17	14	17	17	14	15	-	.087
imbecilis	12	10	12	10	11	15	12	15	15	14	13	2	-

TABLE 4. Character matrix including allozyme (characters 1-67) and morphological (characters 68-82) data. Only phylogenetically informative characters are included in the matrix. **Allozyme characters** (abbreviations for allozyme loci as per Table 2): 1-4, PGM; 5-7, GPD; 8-13, ACP; 14-17, SOD; 18-19, FH; 20-21, GAPDH; 22-23, GPT; 24-25, PGD; 26-27, ICD-2; 28-29, MDH-1; 30-32, MDH-2; 33-34, AO; 35-37, CAT-2; 38-41, CAT-1; 42-44, ADH; 45-47, B-GUR; 48-49, ICD-1; 50-51, EST; 52-54, FDP; 55-58, GOT; 59-61, LAP; 62-64, PEP-1; 65-67, PEP-2. **Morphological characters**: 68-76, after Kat (1983a; characters 1-9); 77-80, after Hoggarth (1988; characters 2,5,9,13); 81, umbo typically above hinge line; 82, strongly double-looped beak sculpture. Missing data are represented by a "?".

character #	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	35	36	37	38	39	40	41
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	1	0	0	1	1	0	0
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	0	0	1	1	0	0	0
grandis	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	0	0	1	1	1	0	0
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	0	0	1	1	0	1	0
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	0	1	0	1	0	0	0
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	0	0	1	0	0	0	1
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	0	0	1	0	0	1	0
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	0	0	1	0	1	0	0
"couperiana"	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	0	0	1	0	0	0	1
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	0	0	0	0	0	0	1	0	0	0	0
kennerlyi	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0
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	0	0	1	0	0	1	0
imbecilis	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	0	0	1	0	0	1	0
compressa	0	0	1	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	?	?	0	0	1	0	0	1	0
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	0	0	1	0	0	1	0
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	?	?	1	1	0	0	1	1	0

character #	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	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
cataracta	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	0	0	1	0	0	0	0	1	0	1	0	1	3	1	1
gibbosa	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	0	0	2	0	0	0	1	1	1	?	?	?	?	1	1
grandis	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	0	0	0	1	0	0	0	1	0	1	0	1	3	1	1
lacustris	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	?	?	?	?	?	?	?	?	1	0	1	3	1	1
fragilis	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	0	1	0	1	0	1	1	0	0	?	?	?	?	1	0
implicata	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	1	2	0	2	2	2	2	0	2	1	1	0	0	1	0
suborbiculata	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	0	?	?	?	?	?	?	?	?	0	1	0	5	0	0
couperiana	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	1	1	2	0	0	?	?	?	?	0	0
"couperiana"	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0
cygnea	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	2	0	0	1	0	0	1	0	1	3	0	0
kennerlyi	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	?	?	?	?	?	?	?	?	?	1	0	1	0	0	0
peggyae	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	1	1	2	1	1	1	2	0	2	?	?	?	?	0	0
imbecilis	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	1	1	0	1	1	1	1	0	2	0	0	1	4	0	0
compressa	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	?	?	?	?	?	?	?	?	?	0	1	2	3	1	1
complanata	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	?	?	?	?	?	?	?	?	?	0	0	3	2	1	1
costata	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	?	?	?	?	?	?	?	?	?	1	1	3	1	1	0

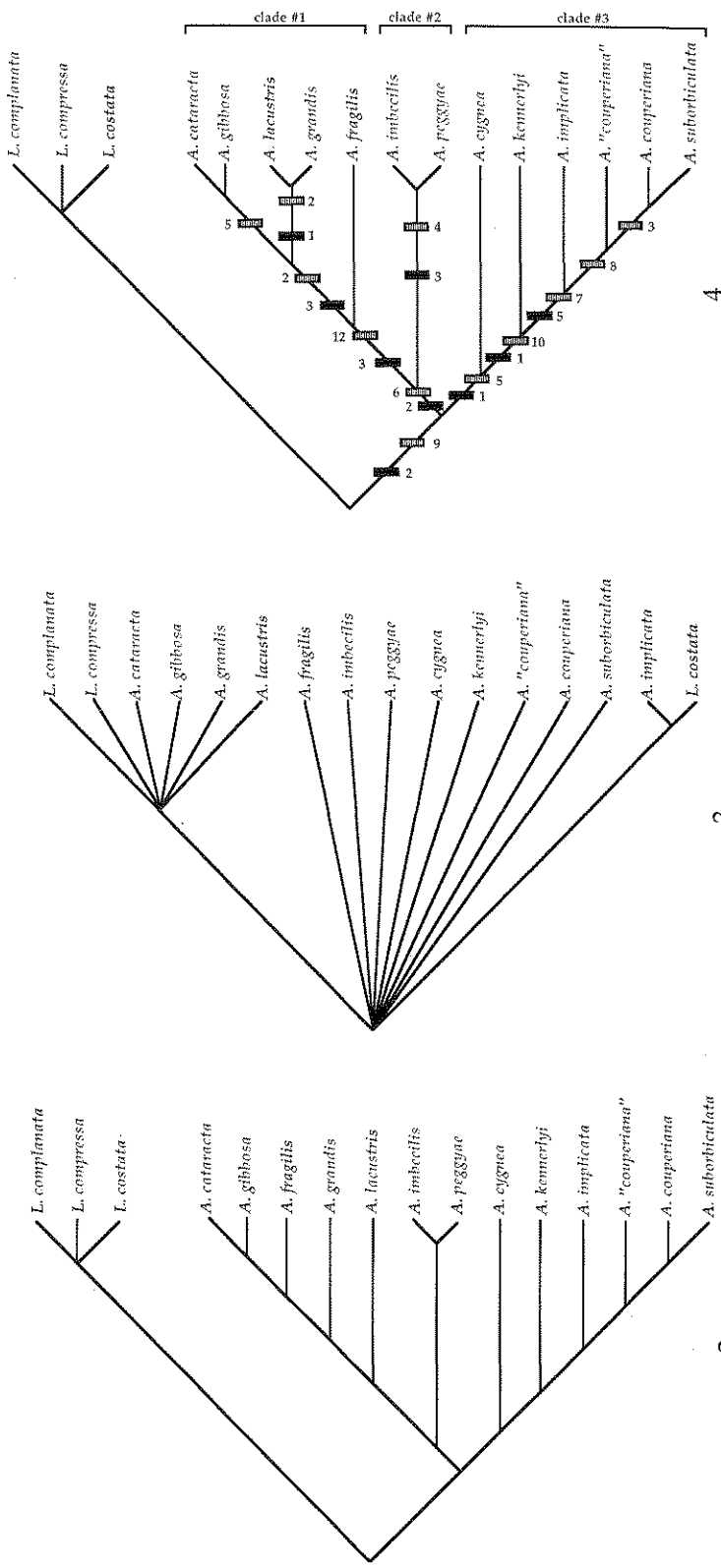

FIG 2. Most parsimonious tree produced by cladistic analysis of the allozyme data (characters 1-67, Table 4). Tree length = 156 steps; Consistency index = 0.429. FIG. 3. Consensus tree produced by cladistic analysis of the morphological data (characters 68-82, Table 4). FIG. 4. Most parsimonious tree produced by cladistic analysis of the complete data matrix (allozymes+morphology; characters 1-82, Table 4). Tree length = 201 steps; Consistency index = 0.473. Numerals represent the number of unique (solid bars) and homoplasious (striped bars) synapomorphies diagnosing each node. The brackets denote three distinct clades within *Anodonta*.

the three species of *Lasmigona* used for the outgroup, *Anodonta* is monophyletic. Furthermore, these two analyses support three major clades (*i.e.*, monophyletic groups) within *Anodonta* namely, (1) [*A. cataracta, A. fragilis, A. gibbosa* Say 1824, *A. grandis* Say 1829, and *A. lacustris* Lea 1857 (=*A. "marginata" sensu* F.C. Baker, see Hoeh & Burch, 1989)], (2) [*A. imbecilis* and *A. peggyae*], and (3) [*A. couperiana, A. cygnea, A. implicata* Say 1829, *A. kennerlyi* Lea 1860, *A. suborbiculata* Say 1831, and *A. "couperiana"*]. Clades number one and two (Fig. 4) are sister groups.

DISCUSSION

Methodological Questions

The individual parsimony analyses of the allozymic, morphological, and combined data sets each produced a different topology (Figs. 2, 3 and 4, respectively). The cladograms resulting from the allozyme characters (1-67, Fig. 2) and the allozyme+morphology characters (1-82, Fig. 4) are completely resolved and both support the monophyly of *Anodonta*.

Unlike the previous two analyses, the strict consensus cladogram based on the morphological characters (68-82, Fig. 3) is poorly resolved and does not support the monophyly of *Anodonta*. This low level of resolution can be attributed to the relatively small number of morphological characters (15) in relation to the number of taxa (13) in this data set.

Discrepancies among cladograms are often resolved using consensus or combination techniques (Hillis, 1987). Strict consensus cladograms (*e.g.*, see Rohlf, 1982) are constructed by finding the clades in common between fundamental cladograms while combination cladograms are derived from parsimony analyses of combined data matrices (*e.g.*, see Miyamoto, 1985). Consensus techniques emphasize taxonomic congruence while combination techniques emphasize character congruence (Kluge, 1989). With consensus techniques, the relative strength of character evidence among data sets is sacrificed for taxonomic stability across fundamental cladograms (Miyamoto, 1985). Confidence in a phylogenetic hypothesis is commensurate with the number of independent congruent characters diagnosing a particular clade (Kluge, 1989) and therefore, combination techniques, which emphasize character congruence, should be the preferred method for integrating multiple data sets. Since there are no clades in common between the allozyme-based cladogram (Fig. 2) and the morphology-based cladogram (Fig. 3), the production of a strict consensus cladogram results in a completely unresolved polytomy. However, using these same data in a combination approach results in a single most parsimonious, fully resolved cladogram (Fig. 4). Despite the concern that relatively large data sets (*e.g.*, molecular) may swamp out the phylogenetic signal contained in relatively small data sets (*e.g.*, morphology) (Kluge, 1983), the addition of the relatively small and seemingly uninformative (based on Fig. 3) morphological data set significantly altered the topology of the *Anodonta cataracta, A. fragilis, A. gibbosa, A. grandis, A. lacustris* clade (*cf.* Figs. 2 and 4). If we adhere to the principle of total evidence (see Kluge, 1989), the cladogram derived from the combined data matrix (*i.e.*, Fig. 4) should be considered the most highly corroborated hypothesis of relationship for the species of *Anodonta* considered in this work, and as such, will be used to interpret character evolution in this group.

Even though there is a highly significant negative correlation between the consistency index of Kluge & Farris (1969) and the number of taxa included in a

study (Archie 1989, Sanderson & Donoghue, 1989), the relatively low consistency index for the allozyme analysis (C.I.=0.429) suggests a large degree of homoplasy exists in this data set. From the highly resolved cladograms (Figs. 2 and 4) and the character congruence obtained (Fig. 4), there is little doubt that this data set contains phylogenetic information. Moreover, this level of resolution using predominantly allozymes seems remarkable given the Holarctic distribution and antiquity of *Anodonta* (dating at least from the Cretaceous; Henderson, 1935; Haas, 1969).

The extremely high levels of allozyme divergence between some of the species of *Anodonta* obtained in this study suggest that it may be inappropriate to use this type of data base in systematic studies of a broader nature within the Anodontinae. Cladistic analyses of allozyme data may prove informative in phylogenetic studies of specific and generic level relationships within unionid tribes endemic (=relatively recent origin?) to North America (*e.g.*, Lampsilini, Davis & Fuller 1981).

Species Delineation

Various authors have either explicitly or implicitly questioned the specific-level distinction of *Anodonta fragilis* (Clarke & Rick, 1963, Clarke, 1981a), *A. "couperiana"* (Florida panhandle; Johnson, 1969, 1970), and *A. peggyae* (van der Schalie, 1966, 1970) from *A. cataracta*, *A. couperiana*, and *A. imbecilis*, respectively. The allozymic evidence confirms that significant levels of divergence exist between each of these three species pairs.

Anodonta fragilis

The subordination of *Anodonta fragilis* as a subspecies of *A. cataracta* was proposed by Clarke & Rick (1963). Their assessment was based on beak sculpture intermediates observed in Nova Scotia. Typical *A. fragilis* may be restricted to Newfoundland and Labrador while typical *A. cataracta* occurs in Atlantic Slope Drainages from New Jersey south to the Gulf of Mexico (Johnson, 1970; Clarke, 1981a). Kat (1983a, 1983b) performed comparative analyses of allozymes, stomach anatomy, and conchiolin layer microstructure on *A. fragilis* from Nova Scotia and *A. cataracta* from Delaware and New Jersey. He reported an average Nei genetic distance (based on 14 loci) of 0.502 with six fixed allelic differences. Kat's anatomical and conchiolin layer analyses corroborated this relatively high degree of differentiation (Kat, 1983a, 1983b, 1986; Kat & Davis, 1984). The data presented here [Nei's D=0.295 with six fixed allelic differences (Table 3)] support the distinction of *A. fragilis* from *A. cataracta*. In addition, the position of *A. fragilis* relative to *A. cataracta* on the cladogram (Fig. 4) does not support the subspecific relationship postulated for these two taxa by Clarke & Rick (1963). *Anodonta cataracta* and *A. fragilis* do not constitute a monophyletic group. Given the evidence presented above, the observations of beak sculpture intermediates in Nova Scotia by Clarke & Rick (1963) present a paradox. Beak sculpture intermediates are consistent with a hybridization hypothesis. Three data sets, which do not display intermediate phenotypes (*i.e.*, allozymes, stomach anatomy, conchiolin-layer microstructure), are unequivocal in rejecting a hybridization hypothesis. Different data sets (*e.g.*, mitochondrial DNA) should be explored for additional evidence that might bear on this phenomenon.

Anodonta "couperiana"

Johnson (1965) considered *Anodonta couperiana* to be restricted to the southern Atlantic Slope and peninsular Florida regions. After examining specimens collected from the Apalachicola and Ocklockonee river systems (Florida panhandle) by W. H. Heard, Johnson (1969) extended the range of *A. couperiana* to include these two drainages. In a later study, Heard (1975) reported a marked difference in sexuality between two populations of *A. couperiana*; one from the Apalachicola River and the other from the Myakka River (peninsular Florida). The Myakka River population was made up of males and females while the Apalachicola River population contained females and hermaphrodites. This is suggestive of some genetic divergence between the Apalachicola River and peninsular Florida populations of *A. couperiana*. The allozymic evidence reported in this paper (D=0.136 with three fixed differences, Table 3) is consistent with this view. This represents the same level of allozymic differentiation observed between the conchologically very distinct *A. couperiana* and *A. suborbiculata* (Fig. 1). The reproductive and allozymic divergence of the Apalachicola River form of *A. couperiana* (*i.e., A. "couperiana"*) from the peninsular Florida form is evidence consistent with specific level recognition for the former. This hypothesis is supported by the non-monophyly of *A. couperiana* and *A. "couperiana"* (Fig. 4) and the substantial degree of conchological differentiation between the two forms (Fig. 1). The latter, as yet undescribed, species will be formally described in a subsequent paper (Gordon & Hoeh, in preparation).

Anodonta peggyae

Initially described by Johnson (1965) as a distinct species, *Anodonta peggyae* was regarded as a southern race of *A. imbecilis* by van der Schalie (1966, 1970). Recognizing the characteristic shell features, distinctive geographical range, and different sexual composition, Heard (1975) supported the specific level validity of *A. peggyae*. Kat's (1983a) comparative analysis of *Anodonta* stomach morphology also corroborates the distinction of *A. peggyae* from *A. imbecilis*. The data presented in this paper indicate relatively little genetic divergence between *A. peggyae* and *A. imbecilis* (D=0.087, with two fixed allelic differences, Table 3). However, the syntopic distribution of these two taxa (Table 1), together with the fixed allelic differences, argues for the rejection of the hypothesis of a common gene pool and, therefore, substantiates the specific level distinction of *A. peggyae* from *A. imbecilis*. The low levels of allozymic and morphological differentiation between these two species suggest a relatively recent divergence. Since the gonochoric *A. peggyae* is hypothesized to be the sister taxon to the hermaphroditic *A. imbecilis* (Fig. 4), an examination of the fine scale phylogenetic relationships among multiple populations of these two species in conjunction with parallel studies of ecological, genetic, life history, and morphological variation may elucidate factors involved in the transition from gonochorism to simultaneous hermaphroditism.

Even though there is a strong correspondence in the relative levels of allozymic and conchological divergence observed between *Anodonta imbecilis* and *A. peggyae* (*cf.* Fig. 1 and Table 3), this is not the general pattern for the species of *Anodonta* examined in this study. For example, the conchologically similar *A. cataracta* and *A. implicata* are highly differentiated based on allozymes (Nei's D=1.022, Table 3). Similarly, *A. imbecilis* and *A. couperiana*, which often bear a

striking conchological resemblance (Fig. 1), are very different allozymically (Nei's D=0.981, Table 3). Furthermore, a high degree of conchological divergence does not necessarily correspond to a high degree of allozymic differentiation. The conchological uniqueness of *A. suborbiculata* (Fig. 1) has led to its placement in a species "group" separate from all other species of *Anodonta* (*e.g.*, see Simpson 1914). However, relatively little allozymic differentiation has occurred between it and *A. couperiana* (Nei's D=0.136, Table 3). A similar finding of high conchological divergence accompanied by relatively little allozymic differentiation was observed between *Lampsilis radiata* and *L. fullerkati* (Kat, 1983c). The data from the present report suggest that in *Anodonta*, allozymes and conchology are not evolving in concert (*cf.* King & Wilson, 1975).

Interspecific Relationships

Frierson (1927) and Johnson (1970) have assigned *Anodonta kennerlyi* to the subgenus *Anodonta s.s.* Lamarck 1799 (type species, *Anodonta cygnea* (Linnaeus)). This grouping of western North America *Anodonta* (*e.g., A. kennerlyi*) with the *A. cygnea*-like Palearctic species has considerable historical precedent (Simpson, 1895, 1900, 1914; Walker, 1910, 1917; Hannibal, 1912). *Anodonta s.s.* is diagnosed by low, relatively non-inflated umbos and relatively thick, usually rayless shells. *Anodonta s.s.* is broadly distributed across the Palearctic and western North America.

Recent authors (Johnson, 1970, 1972, 1980; Heard, 1975, Kat, 1983a) have placed *A. couperiana, A. peggyae,* and *A. suborbiculata* in the subgenus *Utterbackia* F. C. Baker 1927 (type species, *Anodonta imbecilis* Say). This taxon is commonly diagnosed by relatively thin, often rayed, shells with low, relatively non-inflated, umbos. As regarded by these authors, *Utterbackia* is restricted to the area east of the North American continental divide (northeastern Mexico to southern Ontario, Canada). Using a phenogram based on characteristics of *Anodonta* stomach morphology (eight species were compared, *A. suborbiculata* was not examined), Kat (1983a) implied that *A. couperiana, A. imbecilis,* and *A. peggyae* were more similar to each other than to the other analyzed species. This finding is consistent with traditional concepts of *Utterbackia.*

The low, flattened, umbos and purported hermaphroditism shared between *Anodonta s.s.* and *Utterbackia* were used as criteria for including *A. imbecilis* in *Anodonta s.s.* by Morrison (in Walter, 1956). Bloomer (1934) and Heard (1975) have shown that neither the species in *Anodonta s.s.* nor *Utterbackia* are uniformly hermaphroditic. Johnson (1970, 1972) believed that convergence was responsible for the umbonal similarity between the species in *Anodonta s.s.* and *Utterbackia.* However, based on the analysis of stomach morphology, Kat's (1983a) phenogram indicated that *A. cygnea* is most similar to the *A. couperiana-A. imbecilis-A. peggyae* cluster.

Anodonta cataracta, A. fragilis, A. gibbosa, A. grandis, A. implicata, and *A. lacustris* have been placed by recent authors (Johnson, 1970; Heard, 1975; Kat, 1983a) in the subgenus *Pyganodon* Crosse & Fischer 1894 (type species, *Anodonta globosa* Lea). *Pyganodon* is commonly diagnosed by the presence of more or less high, relatively inflated umbos on North American species. The Eurasian species with inflated umbos have been assigned to *Anodonta s.s.*

Due to the relative paucity and ecophenotypic plasticity of conchological characters as well as their somewhat subjective nature, the monophyly of each of the currently recognized subgenera within *Anodonta, i.e., Anodonta s.s., Pyganodon,* and *Utterbackia,* is questionable. Since anatomical and/or reproduc-

tive differences between *Anodonta s.s.* and *Pyganodon* had not been demonstrated, Clarke (1973) doubted the validity of these subgeneric groupings. Using comparisons of stomach morphology, allozymes, and conchiolin microstructure, Kat (1983a, 1983b) suggested that *A. implicata* was distinct from *A. cataracta, A. gibbosa,* and *A. grandis* and should not be included in *Pyganodon*. Davis (1984) suggested separate generic status for *A. implicata*. These recent works suggest that *Pyganodon* may not be a monophyletic group. However, this argument is predominantly based on the high levels of divergence between *A. implicata* and the other examined species of *Anodonta* rather than on a comparison of *A. implicata* with potential outgroups. A relatively high level of divergence is not a sure indicator of non-monophyly.

The phylogenetic hypothesis derived from the complete data matrix of this study (Fig. 4) suggests that *Anodonta s.s., Pyganodon,* and *Utterbackia,* as conventionally construed, are not monophyletic groups. *Pyganodon* and *Utterbackia* are polyphyletic taxa, while *Anodonta s.s.* is paraphyletic (*sensu* Oosterbroek 1987). The cladogram suggests that inflated umbos, the diagnostic characteristic for *Pyganodon,* had two independent origins, namely, (1) in an ancestor of *A. implicata* and (2) in the lineage that gave rise to the *A. cataracta, A. fragilis, A. gibbosa, A. grandis, A. lacustris* clade (Fig. 4). This finding substantiates the proposed exclusion of *A. implicata* from *Pyganodon* (Kat 1983a, 1983b; Davis, 1984) and illustrates the problematic nature of conchological convergence in freshwater mussels as hypothesized by Davis (1984).

Highly corroborated hypotheses of convergence offer an opportunity to gain insight on the possible adaptive significance of the convergent characteristics (*e.g.,* see Ridley, 1983). Thin shells and small body size are hypothesized as adaptations of marine bivalves for life in soft muds (Stanley, 1970). These characteristics decrease density and increase substratum support, respectively. All species of *Anodonta* lack hinge teeth and have relatively thin shells while some species (*e.g., A. imbecilis, A. peggyae,* and *A. couperiana)* reach relatively small adult size. The general habitat for *Anodonta* is one of low current velocity lotic and lentic freshwater systems where depositional processes often create soft substratum (*e.g.,* see Parmalee, 1967). The lack of hinge teeth and presence of thin shells together with, at least in some species, small adult body size, is likely advantageous to *Anodonta* for life in such habitats. Since increased surface area for a given mass yields increased support from the substratum (Stanley, 1970), inflated umbos may be advantageous for relatively large species that live in soft mud.

The cladogram in Fig. 4 also allows the inference that the low umbos shared by *Utterbackia* and *Anodonta s.s.* are the result of the retention of a plesiomorphic character state. The earliest *Anodonta* fossils (Upper Cretaceous) have been placed in the subgenus *Anodonta s.s.* (Haas, 1969). This assignment is consistent with the hypothesis that the possession of low umbos is the plesiomorphic condition in *Anodonta*. The similarity in beak sculpture, noted by Clarke (1973), among *A. cygnea, A. kennerlyi,* and *A. fragilis,* the presence of multi-voltine reproductive cycles in *A. imbecilis* (Allen, 1924), *A. peggyae* (Heard, 1975), and *A. cygnea* (data presented in Bloomer, 1934, 1935), and the similarity in stomach anatomy among *A. cygnea, A. fragilis,* and *A. imbecilis* (Kat, 1983a) may also be interpreted as symplesiomorphic conditions.

As mentioned above, taxonomic opinion suggests that Eurasian and western North American species of *Anodonta* are more closely related to each other than either is to eastern North American species of *Anodonta* (*e.g.,* see Simpson, 1895, 1900, 1914; Walker, 1910, 1917; Hannibal, 1912; Taylor, 1988). Hannibal (1912)

claimed that western and eastern North American species of *Anodonta* were independently derived from very divergent progenitor stocks. The phylogenetic hypothesis presented in this report (Fig. 4) does not support either opinion. The hypothesized sister species status of the western North American *A. kennerlyi* to the eastern North American clade of *A. implicata, A. "couperiana", A. couperiana,* and *A. suborbiculata* was not anticipated prior to this study.

Classification of *Anodonta*

In order to facilitate the study of evolutionary processes, the taxonomic recognition of clades (monophyletic groups), as the products of evolution, is a necessity (*e.g.*, see Cracraft, 1983). The level of genetic divergence among clades 1, 2 and 3 (Fig. 4, Table 5) is extremely high for intrageneric comparisons. For perspective, these levels of divergence are here compared with published data on allozymic divergence among other taxa within the Unionidae (Davis *et al.*, 1981; Davis, 1984). The mean Nei (1972) genetic distances reported by Davis (1984) in pair-wise comparisons among three genera within the Pleurobemini and among four genera within the Amblemini were 0.243 and 0.651, respectively. Furthermore, Davis (1984) estimated the mean genetic distance among three tribes within the Ambleminae to be 0.954. Even this level of inter-tribal differentiation is less than two of the three pair-wise estimates for the inter-clade genetic divergence within *Anodonta* (Table 5). The extremely high levels of inter-clade genetic divergence within *Anodonta* support the generic level recognition of these clades.

TABLE 5. Mean genetic distances (above the diagonal) and mean number of loci (out of 24) with no alleles shared (below the diagonal) between species from the three clades within *Anodonta* (Figure 4).

	Clade #1	Clade #2	Clade #3
Clade #1	---	0.694	1.118
Clade #2	11.4	---	0.969
Clade #3	15.2	14.8	---

From a nomenclatural standpoint, the traditional names, *i.e., Pyganodon, Utterbackia,* and *Anodonta,* should be applied at the generic level for clades 1, 2 and 3 (Fig. 4), respectively. However, two reservations regarding this taxonomic restructuring need to be mentioned. (1) The type species of *Pyganodon, A. globosa,* was not included in the present analysis. If *Pyganodon* is to be used as the generic designation for clade 1 (Fig. 4), *A. globosa* must be a member of that clade. Simpson (1914) placed *A. globosa* in his *A. grandis* group. Furthermore, the presence of inflated umbos and double-looped beak sculpture, both derived characteristics, suggests that *A. globosa* is a member of the *A. cataracta, A. gibbosa, A. grandis, A. lacustris* clade (Fig. 4). Unless subsequent phylogenetic analyses show otherwise, the phylogenetic position of *A. globosa* is assumed to be in clade 1 and, therefore, the name *Pyganodon* can be applied to clade 1 (Fig. 4). (2) A hypothesis of phylogenetic relationship among *Anodonta*-like taxa which

is predominantly based on eastern North American species may be a poor estimate of the actual phylogeny. The inclusion of additional species (from Eurasia and western North America) or other populations of species currently represented in this study may affect the resulting hypothesis of evolutionary relationships (*e.g.*, see Gauthier *et al.*, 1988). Further investigations on a geographically representative array of *Anodonta*-like taxa, based on internal anatomy, shell morphology and molecular analyses, will provide the evidence required for the corroboration or refutation of the evolutionary relationships and taxonomic restructuring proposed here.

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