GENETIC RELATIONSHIPS AMONG RECENT UNIONACEA (BIVALVIA) OF NORTH AMERICA[1]

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ABSTRACT

The purposes of this paper are to determine why there has been so little agreement among classifications of North American Unionacea, to test the Heard & Guckert (1971) assumptions that the number of marsupial demibranchs and length of breeding season serve to define higher taxa, to examine the congruency among major classifications of North American Unionacea, and to establish a classification resulting from a synthesis of data derived from molecular genetics, comparative anatomy, and zoogeography through time.

Immunoelectrophoretic studies of 52 species belonging to 27 genera were conducted. We scored the percent difference between pairs of taxa. Data were analyzed with multivariate techniques of the NT-SYS program. Emphasis was placed on results of multidimensional scaling, ordination, minimum spanning tree, and subsets.

On the basis of our results we determined that in the Nearctic Unionacea there are one family (Unionidae) and three, genetically very distinct subfamilies: Margaritiferinae, Anodontinae, and Ambleminae. The three subfamilies are clearly defined morphologically and immunologically. The Ambleminae are further divided into four tribes: Gonideini, Amblemini, Pleurobemini, Lampsilini. It is clear that both tetragenous and ectobranchous taxa have evolved in various clades. The ectobranchous genus *Elliptio* and tetragenous genus *Fusconaia* are closely related in the Pleurobemini, the ectobranchous genus *Cyclonaias* and tetragenous genus *Quadrula* are closely related in the Amblemini, and the tetragenous *Gonidea* is more closely related to the Lampsilini (which are ectobranchous) than to the Pleurobemini or Amblemini. The ectobranchous state has undergone parallel evolution, as have different lengths of breeding season.

Our classification and that of Ortmann (1910a) have the greatest congruence. We consider these classifications to reflect real clades more closely than other systems do, because both are based on all of the data available. We consider the other classifications to be artificial in that they are based on conchology alone or on the unjustified weighting of one or two key characters. We differ from Ortmann and all previous workers in establishing the Anodontinae as a taxon of equal standing with the Margaritiferinae as a second group and with all other North American Unionidae in the Ambleminae as a third.

INTRODUCTION

North American unionacean bivalves (unios, freshwater mussels, naiades) comprise one of the most diverse radiations of macroinvertebrates seen today in fresh water. There are about 50 nominal genera, which include over 225 species and subspecies (Heard & Guckert, 1971; Burch, 1973, 1975). Unios have dominated streambeds in terms of biomass and numbers of individuals, but decreasingly in this century. Centers of endemism and high species diversity are found in the eastern United States, e.g. the Ohio, Tennessee and Coosa-Alabama river drainages. Numbers of sympatric species literally paved the large-river shoals in the early 19th century. For example, Conrad (1834) reported the richest of all known localities, a section of the Tennessee River that later became known as Mussel Shoals. The shoals contained some 70 species packed valve to valve.

Even though the diversity and abundance of the unionid fauna stimulated a more than 80 year continuous outflow of systematic and taxonomic literature concerned with higher-category relationships among unionids (review by Heard & Guckert, 1971), there is little agreement among classifications today. Disparity among major classifications (reviewed in Appendix 1) seems to occur because 1) different sets of characters were used by dif-

ferent investigators; 2) a monothetic basis for classification was used by some; 3) there is the high probability that one or more "key" character-states has undergone parallel or convergent evolution, and 4) too few morphological characters with unique character-states exist (or have been found) that would enable a satisfactory comparison of taxa.

Classifications based primarily on shell characters persist to the present (Frierson, 1927; Modell, 1942, 1949, 1964; Haas, 1969 a, b). Some early classifications were based on additional characters of the soft parts, e.g. gill structure, marsupium, and glochidia (Simpson, 1896, 1900, 1914; Sterki, 1898, 1903). Ortmann (1910a, 1911, 1912b, 1916) extended the work of Simpson and Sterki by increasing the number of characters derived from soft-part morphology and integrated all available morphological data (i.e. on shell and soft parts). Use of shell characters for classification above the species level eventually was rejected. Hannibal (1912) stated that shell characters were of no use in establishing taxa above the generic level. Hannibal was followed by Heard & Guckert (1971), who stated: ". . . we have subjectively elected to ignore one entire array of characters (i.e., conchological features) and to suggest soft-part anatomy and reproductive habits as preeminent in describing phylogenies."

Heard & Guckert (1971) especially weighted two characters involving reproduction. These are the number of demibranchs used as the marsupium and the length of the breeding season. For example, they recognized two families, Amblemidae and Unionidae, on the basis that species with four marsupial demibranchs (tetragenous) belong to the former family while those with only the outer two demibranchs marsupial (ectobranchous) belong in the latter family. They created subfamilies on the basis of whether taxa are bradytictic (i.e. long-term breeders, retaining glochidia except in the Nearctic summer) or tachytictic (i.e. short-term breeders, retaining glochidia only in the Nearctic summer).

We initiated our work on higher-category relationships among North American unios in order to test the validity of the Heard & Guckert assumptions and classification. Because there is so little agreement among the major classifications, we suspected that one or more key character-states has undergone convergent or parallel evolution. We also suspected that one cannot excessively weight characters having to do with reproductive

strategies. For example, a strategy of bradytixis might occur again and again in different radiations of unionids. Likewise, the tetragenous and ectobranchous conditions feasibly could occur in different radiations. We noted that brooding young in the pallial oviduct of mesogastropods has arisen independently in several families of different superfamilies (Fretter & Graham, 1962).

Our suspicions are not without basis. For example, *Fusconaia masoni* (Conrad) was placed in the genus *Elliptio* by Haas (1969a) and relegated to *Pleurobema (Lexingtonia)* by Johnson (1970). *Elliptio* and *Pleurobema* were considered Unionidae by Ortmann (1910a, 1911, 1912b, 1919) and Heard & Guckert (1971). However, *F. masoni* is tetragenous and thus belongs to *Fusconaia* (Fuller, 1974). Except for the one character-state difference, one finds little difference between *F. masoni* and various species of *Elliptio* and *Pleurobema*. However, if the Heard & Guckert classification were followed, the species would have to be transferred from the ectobranchous Unionidae to the tetragenous Amblemidae on the basis of that one character-state.

We further suspected that unionid species have too few unique morphological character-states to permit an adequate phenetic or cladistic analysis of relationships. We admit that there may be more morphological characters, but these have yet to be discovered. Such characters probaby would have to be found by detailed comparative anatomical studies of internal organ systems. Because of the dearth of unique morphological character-states, we established a program to assess relationships on the basis of molecular genetics. In this paper we present a higher classification of North American Unionacea based on immunoelectrophoretic data and what morphological, paleontological, and zoogeographical data are available. In so doing, we assess not only the relationships among unionid taxa, but also the relative values of different approaches to unionacean taxonomy used to structure the various major classifications.

MATERIALS AND METHODS

Species studied

Fifty-two species, representing 27 genera, were studied (Table 1). These species were

TABLE 1. Fifty-two species of North American Unionacea alphabetized by the code designations used in this study. Localities are given with ANSP catalog numbers.

Code	Species	Locality	ANSP voucher no.
Ac	*Anodonta cataracta* (Say)	Gloucester Co., New Jersey	333526
Acr	*Actinonaias carinata* (Barnes)	Clark Co., Arkansas	341958
Ai	*Anodonta imbecillis* (Say)	Jenkins Co., Georgia	333563
Aip	*Anodonta implicata* (Say)	Hartford Co., Connecticut	334650
Ap	*Amblema perplicata* (Conrad)	Rapides Parish, Louisiana	334560
Apl	*Amblema plicata* (Say)	Clark Co., Arkansas	341939
Au	*Alasmidonta undulata* (Say)*	Hartford Co., Connecticut	334649
Aw	*Anodonta wahlametensis* (Lea)	Modoc Co., California	345880
Cp	*Carunculina parva* (Barnes)*	Rapides Parish, Louisiana	334564
Ct	*Cyclonaias tuberculata* (Raf.)*,+	Hancock Co., Tennessee	335048
Cu	*Cumberlandia monodonta* (Say)*,+	Hancock Co., Tennessee	341956
Eb	*Elliptio buckleyi* (Lea)	Putnam Co., Florida	334427
Ec	*E. complanata* (Lightfoot)	Gloucester Co., New Jersey	333527
Ec^2	***	Sussex Co., New Jersey	334428
Ec^3	***	Barnwell Co., South Carolina	333296
Ec^4	***	Barnwell Co., South Carolina	—
Ec^5	***	Wayne Co., Pennsylvania	339430
Ecr	*E. crassidens* (Lam.)*	Hancock Co., Tennessee	—
Ei	*E. icterina* (Conrad)	Jenkins Co., Georgia	333566
El	*E. lanceolata* (Lea)	Jenkins Co., Georgia	333565
El^2	***	Barnwell Co., South Carolina	—
El^3	***	Jenkins Co., Georgia	—
Ew	*E. waccamawensis* (Lea)	Columbus Co., North Carolina	339967
Fbb	*Fusconaia* cf. *flava* (Raf.)	Rapides Parish, Louisiana	334563
Fe	*F. ebena* (Lea)	Greene Co., Alabama	340626
Ff	*F. flava* (Raf.)	Hancock Co., Tennessee	335049
Fm	*F. masoni* (Conrad)	Jenkins Co., Georgia	333564
Ga	*Gonidea angulata* (Lea)*,+	Modoc Co., California	339965
Gr	*Glebula rotundata* (Lam.)*,+	Rapides Parish, Louisiana	334556
Lc	*Lasmigona costata* (Raf.)*	Hancock Co., Tennessee	335047
Lcl	*Lampsilis claibornensis* (Lea)	Lowndes Co., Mississippi	—
Lf	*Leptodea fragilis* (Raf.)	Hancock Co., Tennessee	335046
Lh	*Lampsilis hydiana* (Lea)	Rapides Parish, Louisiana	334558
Ln	*Ligumia nasuta* (Say)	Burlington Co., New Jersey	334251
Lo	*Lampsilis ovata* (Say)*	Hancock Co., Tennessee	335029
Lr	*L. radiata* (Gmelin)	Sussex Co., Delaware	339342
Lre	*Ligumia recta* (Lam.)*	Clark Co., Arkansas	340628
Ls	*Lampsilis splendida* (Lea)	Barnwell Co., South Carolina	334432
Lt	*L. teres* (Raf.)	Rapides Parish, Louisiana	334557
Lv	*L. ventricosa* (Barnes)	Clark Co., Arkansas	—
Mf	*Margaritifera falcata* (Gould)	Oregon	339339
Mg	*Megalonaias gigantea* (Barnes)*	Rapides Parish, Louisiana	334553
Mh	*Margaritifera hembeli* (Gould)	Rapides Parish, Louisiana	334426
Mm	*M. margaritifera* (L.)*	Schuylkill Co., Pennsylvania	334867
Pa	*Proptera alata* (Say)*	Hancock Co., Tennessee	335040
Pc	*Pleurobema cordatum* (Raf.)	Clark Co., Arkansas	340629
Pd	*Plectomerus dombeyanus* (Val.)*,+	Rapides Parish, Louisiana	334555
Ppu	*Proptera purpurata* (Lam.)	Clark Co., Arkansas	390630
Ps	*Ptychobranchus subtentum* (Say)	Hancock Co., Tennessee	335045
Qa	*Quadrula apiculata* (Say)	Evangeline Parish, Louisiana	339670
Qbb	*Q.* cf. *quadrula* (Raf.)*	Rapides Parish, Louisiana	334562
Qc	*Q. cylindrica* (Say)**	Hancock Co., Tennessee	335041
Qi	*Quincuncina infucata* (Conrad)	Crawford Co., Georgia	334539
Qp	*Quadrula pustulosa* (Lea)	Clark Co., Arkansas	340946
Tv	*Tritogonia verrucosa* (Raf.)*,+	Rapides Parish, Louisiana	339671
Ut^2	*Uniomerus tetralasmus* (Say)*	Rapides Parish, Louisiana	334561
Vd	*Villosa delumbis* (Conrad)	Jenkins Co., Georgia	333569
Vi	*V. iris* (Lea)	Hancock Co., Tennessee	335050

* = type-species   **type-species of *Orthonymus*
\+ = monotypic genus   ***used for analysis of conspecific populations

chosen among all those collected from various localities across the country because they were representative of each of the families and subfamilies recognized by Ortmann (1910a, 1911, 1912b, 1916, 1919), Modell (1942, 1949, 1964), and Heard & Guckert (1971). We collected and chose type-species whenever possible. We were able to study 18 type-species of the 27 genera (66.7%). These are marked with an asterisk in Table 1. Shells of each population are maintained as voucher specimens in the Academy of Natural Sciences of Philadelphia (ANSP); catalog numbers and locality data are given in Table 1.

Preparation of antigens and antigen bank

Foot muscle and gravid gill were used as a source of proteins. Gravid gills were carefully inspected for the presence of unionicolid mites in order to ensure that antigen preparations were not contaminated with mite antigens. Tissues were pooled from 12 to 60 individuals according to their size. The gravid gill extract essentially equaled glochidial extract because gill filament tissue added very little in terms of protein mg in comparison to the yield from the glochidia. In preparing extracts, 300 mg blotted foot tissue (cleaned of gonad) were homogenized in 1.5 cc buffer. Homogenization was accomplished by first subjecting the mixtures to a Waring Blender for two minutes and then to sonication-homogenization (via a Polytron) for two minutes per 30 ml. The homogenate was centrifuged at 6900 × g for 20 minutes; the supernatant was lyophilized (1 ml per 2 ml ampule). All prelyophilization operations were carried out at 1–3°C.

In this manner 100 to 300 ampules of lyophilized extract from each population were prepared for storage in freezers (at −20°C). The protein content of each lyophilized batch was determined by the folin reagent test (Daughady et al., 1952).

Antisera

Two rabbits (New Zealand white, virgin, female, 7–8 lbs) initially were used per mussel population. Lyophilized antigens were reconstituted with normal saline and injected subscapularly with an equal volume of Freund's complete adjuvent. There were two injection series (days 1, 3, 5, and 7; rest 3 weeks; repeat the series). Each injection contained 2 mg protein. We bled out the rabbits by heart puncture 4 days after the last injection.

Titre and Serum Quality—Antiserum quality was tested by immunoelectrophoresis (see below). Sera were kept and used in experiments if 10 or more precipitin arcs resulted in the homologous reaction. If an antiserum was discarded for producing too few antigen-antibody precipitin systems, two or more rabbits were used to produce specific antisera.

Controls—Each rabbit was bled from the ear prior to the first injection with antigen; the serum was tested for reactivity to molluscan antigen.

Absorption—An antiserum was absorbed with a heterologous antigen by adding 0.8 ml antiserum to an ampule of lyophilized antigen, swirling, leaving at room temperature for 30 minutes, refrigerating for 30 minutes, and centrifuging for 20 minutes (6900 × g).

Immunoelectrophoresis—The procedures used are those of Davis (1969) and Davis & Suzuki (1971), with some adjustments. The 2% agar noble contained 0.45% NaCl, 1:10,000 merthiolate, and half-strength barbital-acetate buffer of pH 8.2. Full-strength buffer contained 5.4 g Na-barbital, 4.3 g sodium acetate, and 58.2 ml 0.1 N HCl per liter.

Protein concentrations of antigens were adjusted to 6 mg/ml. Direct current of 6–8 v/cm across the slides was sustained for one hour.

Analysis of immunological data

Twelve slides were used in each experiment, of which two were controls, i.e. the homologous system with unabsorbed serum. We determined the number and position of each precipitin arc by comparing the experimental slides with control slides. In each experiment we absorbed the serum of the reference population (homologous system) with a heterologous antigen so that there were five sets of absorbed sera. The two wells punched in the agar on each slide were loaded with homologous and heterologous antigens, respectively. Absorbed antisera were used in the slots of the 10 non-control slides. Lack of arcs between the slots and the wells with the heterologous antigens indicated complete absorption. The number of arcs between the slot and the well with the homologous antigens indicated the number of anitgens unique to the reference species. The position of the arc identified the antigen.

We scored the percent difference between taxa. The average number of precipitin arcs was 12 with a range of 10 to 14. We analyzed

TABLE 2. Matrix giving the average percent difference of antigens in cross comparison of 14 taxa (14 sets of antigens × 14 sets of antisera).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Margaritifera falcata	1	0													
M. hembeli	2	30.5	0												
M. margaritifera	3	34.0	33.0	0											
Cumberlandia monodonta	4	39.0	24.5	20.5	0										
Quadrula cylindrica	5	61.0	41.5	45.0	50.0	0									
Fusconaia flava	6	58.0	36.5	56.0	40.5	35.0	0								
F. masoni	7	58.5	47.5	47.5	55.5	42.5	17.5	0							
Quadrula quadrula	8	64.5	49.5	66.5	45.5	14.0	22.5	21.5	0						
Megalonaias gigantea	9	52.0	43.5	66.5	50.0	22.5	29.5	13.0	20.5	0					
Cyclonaias tuberculata	10	65.0	41.5	54.0	58.0	23.0	22.5	26.5	12.0	16.0	0				
Elliptio buckleyi	11	63.0	47.5	60.5	60.5	37.5	26.5	27.0	26.5	26.5	34.5	0			
Proptera alata	12	61.5	45.5	50.0	45.5	30.0	31.5	25.5	35.5	31.5	31.5	28.5	0		
Villosa delumbis	13	61.0	50.0	45.5	56.5	27.5	32.5	36.5	33.3	37.5	29.0	45.5	32.5	0	
Anodonta cataracta	14	62.0	50.0	67.5	54.0	40.0	46.5	47.0	40.0	49.0	40.5	37.5	45.0	37.5	0

TABLE 3. Raw data (percent difference) for 21 antisera and 52 species (antigens). Key to abbreviations is given in Table 1. NC = no data available.

Spe-cies	Antisera																				
	1 Ac	2 Au	3 Ct	4 Cu	5 Eb	6 Ec2	7 Ecr	8 Ei	9 Fbb	10 Fm	11 Ga	12 Lo	13 Mg	14 Mh	15 Mf	16 Mm	17 Pa	18 Qbb	19 Qc	20 Tv	21 Ut2
Mf	70	56	58	33	63	50	50	56	53	45	70	75	41	25	0	50	50	66	50	50	50
Mm	60	50	58	25	63	41	56	50	46	45	50	60	75	0	36	0	41	58	50	50	58
Mh	50	56	50	33	45	50	50	41	40	54	50	56	66	0	36	33	41	58	50	50	58
Cu	50	56	58	0	63	41	50	50	40	45	70	64	50	16	45	16	41	50	50	56	50
Ga	50	50	41	58	NC	25	41	50	33	27	0	41	41	50	63	75	33	50	40	41	33
Ap	40	41	33	41	18	25	25	25	26	18	30	50	16	33	63	50	16	33	30	16	16
Fe	50	50	50	66	36	50	33	25	40	27	50	50	41	50	81	58	33	41	40	16	41
Ff	50	50	25	58	18	16	33	16	33	9	50	41	25	41	72	41	25	33	30	25	33
Fbb	40	41	25	41	27	25	50	16	0	9	40	41	33	33	63	66	33	25	40	41	41
Fm	40	41	25	66	36	33	41	16	27	0	50	41	8	41	72	50	25	16	30	25	33
Qbb	30	41	8	41	27	25	41	16	20	27	50	41	16	41	63	48	33	0	40	16	41
Qa	30	50	8	66	36	33	50	41	33	27	50	41	25	50	72	58	25	16	20	33	33
Qc	40	50	16	50	45	25	41	25	20	45	40	41	25	33	63	50	25	8	10	16	25
Pd	40	56	16	50	27	16	41	25	33	27	40	41	8	41	63	58	16	8	0	16	41
Qi	40	NC	16	58	36	NC	NC	NC	33	27	NC	NC	41	41	NC	58	25	25	20	25	33
Tv	40	50	25	58	27	41	50	25	40	45	40	50	41	41	90	50	33	16	30	0	41
Mg	40	56	16	50	27	16	50	33	26	18	50	41	0	41	63	48	25	25	20	25	41
Ct	40	50	0	58	36	16	41	25	20	27	30	41	16	33	72	48	25	16	30	25	25
Ec	50	41	25	41	18	0	33	8	25	9	30	50	25	33	63	33	25	25	20	16	41
Ei	40	50	25	66	18	16	33	0	33	27	40	50	50	50	72	58	25	33	30	33	41
Ei	40	50	16	50	0	16	33	8	33	18	30	50	41	41	63	75	25	33	30	16	41
Ew	50	50	41	66	18	8	33	25	33	18	50	50	41	50	63	66	33	50	40	25	41
Ecr	40	41	16	41	27	16	0	8	33	27	50	41	33	41	54	58	25	33	20	33	41

0	41	41	33	41	41	16	41	41	NC	41	41	33	41	NC	NC	41	NC	50	66	41	41	50	50	41	41	33	16	41
25	33	41	33	41	25	33	33	41	25	41	33	41	33	33	NC	33	NC	50	50	56	50	50	56	41	41	16	16	41
10	30	30	30	40	40	40	30	30	30	30	40	30	30	30		50	50	50	50	60	50	40	30	20	10	40		
25	33	33	25	41	33	33	41	41	25	33	41	41	NC	41	33	41	25	50	50	66	50	50	58	16	41	8	16	33
25	16	25	16	33	16	16	16	25	16	NC	25	0	8	40	40	40	25	41	41	33	41	33	50	25	8	33	25	41
58	50	41	50	50	66	50	50	58	41	66	41	50	50	66	50	50	58	75	66	66	58	50	66	58	58	50	59	58
72	72	63	63	63	72	81	72	81	72	63	72	63	72	72	72	63	63	54	63	63	72	63	63	63	72	62	81	
50	41	50	50	50	41	41	50	41	41	41	50	50	33	41	50	50	58	41	50	41	50	50	50	41	41	50		
33	33	50	33	41	58	41	33	41	45	41	33	33	41	50	41	33	25	58	50	50	58	41	50	41	41	27	8	41
41	56	41	33	41	25	16	0	25	16	25	33	41	33	16	NC	33	50	50	56	50	56	50	60	33	16	50	41	16
20	40	30	50	40	50	50	40	30	40	50	30	40	50	60	NC	50	30	50	50	60	60	60	70	40	40	40	NC	40
27	0	36	36	45	27	36	36	36	27	36	45	18	45	36	27	27	18	54	45	45	45	63	45	45	36	36	0	45
26	20	33	26	46	33	26	33	26	46	40	26	33	46	40	33	26	53	60	46	40	46	46	46	33	33	33	46	
41	25	33	33	41	33	16	33	16	NC	25	33	25	33	41	NC	33	NC	41	56	41	56	50	56	41	16	33	16	33
16	16	41	41	41	33	33	41	33	25	41	25	25	41	41	NC	33	NC	50	50	33	33	33	50	25	41	50	33	25
33	16	25	25	33	33	41	41	16	25	41	33	33	25	33	NC	25	25	50	50	41	50	50	33	16	25	8	33	
45	NC	45	18	27	36	36	27	36	NC	36	27	NC	54	45	36	0	45	54	45	45	45	NC	NC	NC	NC	NC		
50	50	50	50	75	58	50	58	50	58	58	41	50	66	33	50	58	58	50	66	50	50	50	50	50	50	50		
25	33	33	33	41	41	33	33	25	41	25	33	41	33	33	33	41	58	41	41	50	58	33	33	25	16	50		
41	41	41	56	41	50	33	50	50	41	56	50	41	50	50	NC	50	41	33	16	8	0	33	41	60	56	50	41	50
40	50	30	30	40	40	40	40	40	40	50	30	40	50	50	40	50	30	0	20	20	20	20	40	60	50	50	50	50

Ut Pc Cp Gr Lt Lcl Ls Lo Lh Lf Lr Ln Pa Ppu Vd Vi Ps Eb Ac Ai Aip Au Lc Aw Lre Acr Qp Apl Lv

the relationships among taxa by using multivariate analysis. Computations were made using the NT-SYS program (Rohlf et al., 1972) at the Uni-Coll Corporation, Philadelphia, or via a remote job-entry station to the Sun Oil Corporation Computer (IBM 370/168 VS2). Initially, three types of matrices were used: 1) the Mainardi (1959) immunological distance was used as a distance coefficient, 2) the distance between taxa was used as a distance coefficient (Table 2), and 3) and OTU × antiserum matrix was made where the 52 OTUs were antigens (i.e. species or populations of a given species) and the 21 antisera were treated as characters (Bashford et al., 1968). The percent arcs unique to the homologous system = percent difference that was used as a distance coefficient (Table 3). In the first two matrices, comparisons were made where there was an antiserum for each species.

In the analysis we standardized by rows (antisera) in order to produce a matrix of transformed distance coefficients. We employed the minimum spanning tree (MST) and "subsets" components. Character correlations were subjected to Principal Component Analysis (PCA) with components extracted until eigen-values became less than 1.0. A transposed matrix of the first three PCs with their character loading was post-multiplied by the standardized matrix in order to yield a matrix of OTU projections in the PCA space (Rohlf et al., 1972). The resulting PCA-based configuration portrays distance-ordered relationships well, but tends to distort close-relative relationships, which often are of critical interest to taxonomists (Rohlf, 1970; Webster, 1975). OTU locations in the 3-dimensioned PCA space were used as the initial configuration for a nonmetric multidimensional scaling (MDS) placement of taxonomic distances between OTUs (Kruskal, 1964). OTU configurations were adjusted after scaling by PCA analysis on a variance-covariance matrix obtained from the MDS-coordinates in order to realign the major trends of the variation in the reduced configuration space with the coordinate axes, while maintaining the accuracy of between-OTU distances in the ordination space (Rohlf et al., 1972). Distances between OTUs in the PCA- and MDS-spaces were found and compared with the matrix (cophenetic) correlation coefficient.

We placed no reliance on cluster analysis and phenograms to illustrate relationships. We emphasized ordination and MDS that are freed from the constraints of phenogram construction.

Comparison of taxa

Experiments were conducted by using foot muscle antigens in order to determine to what extent we could find differences between unionids and non-unionacean clams and between different conspecific populations of the same species. Results would be important bench marks for assessing differences between species. We also compared different populations of the same species by using glochidial antigens.

We compared three species of unionids with three species of marine bivalves by using the foot-muscle system. The comparisons involved four different taxonomic orders (Table 4).

TABLE 4. Classification according to Morton (1971) of marine species compared with the unionacean species used to test immunological congruity on the basis of foot-mussel antigens.

Class Bivalvia
    Order: Anisomyaria
        Superfamily: Mytilacea
            Genus and species: *Geukensia demissa* (Dillwyn)
    Order: Schizodonta
        Superfamily: Unionacea
            Genus and species: *Anodonta cataracta* Say
                                Elliptio complanata (Lightfoot)
                                Elliptio icterina (Conrad)
    Order: Heterodonta
        Superfamily: Veneracea
            Genus and species: *Mercenaria mercenaria* (Linné)
    Order: Adapedonta
        Superfamiy: Myacea
            Genus and species: *Mya arenaria* Linné

Annotations and terminology

Annotations, indicated by superscript number with a taxon, are given in numerical order in Appendix 2. Definitions of terms concerning breeding season and marsupial conditions are given in Appendix 3.

RESULTS

Comparison of unionids with marine species

As is seen in Table 5, only one or two antigens were shared in common by unionids and

TABLE 5. Congruity of marine and unionid species in percentage differences.

Marine species	Antisera		
	Ac^2 (10)	Ec (12)	Ei (12)
Mercenaria mercenaria	80	92	84
Geukensia demissa	80	92	92
Mya arenaria	80	92	92

( ), number of preciptin arcs. Coded names given in Table 1.

TABLE 6. Comparison of different populations of the same species on the basis of glochidial and foot-muscle antigen-antisera systems. Percentage difference is given.

Gravid Gill

Populations	Antisera	
	Ec^2 (12)	El (14)
Ec^3*	33	—
El^2	—	21
El^3	—	14

Foot-Muscle

Populations	Antisera		
	Ec^2 (12)	Ac^2 (10)	El (12)
Ec	0	—	—
Ec^3	0	—	—
Ec^4	0	—	—
Ec^5	0	—	—
Ac	—	0	—
El^2	—	—	0
El^3	—	—	0

( ) number of precipitin arcs.
*coded names given in Table 1.

species of other bivalve orders. Accordingly, the immunological comparisons among unionid taxa involve antigens that are primarily (> 85%) unique to the Unionidae.

Comparisons among populations of the same species

It was possible to demonstrate 14% to 33% difference among populations of the same species by using glochidial antigen-antibody systems; it was not possible to discriminate among populations of the same species by using foot-muscle systems (Table 6). Because the foot-muscle systems were the more conservative, investigations reported here were based on foot-muscle systems. Differences among taxa are differences above the conspecific population level.

Comparing species by using foot-muscle antigens

An initial multivariate assessment was made where a comparison involving an antiserum for each species was possible. This initial comparison involved 14 species. We used the Mainardi (1959) immunological distance and the average percent difference as distance coefficients. We abandoned use of the Mainardi distance coefficient because the results of ordination by using this distance were more distorted ($r = 0.809$) than results with the average distance (Table 2, $r = 0.922$). The results of ordination based on the average distance and the first two principal components are given in Fig. 1. The first two components accounted for 89.50% of the data. The correlation between the matrix of taxonomic distances and distances in the 3-dimensional MDS was excellent, i.e., 0.922; the stress was 0.213.

As can be seen from Fig. 1, there are three widely separated groups of taxa: 1) species considered on classical grounds to be Margaritiferidae (i.e., species of the nominal genera Margaritifera and Cumberlandia), 2) the single species of Anodonta, and 3) the cluster including Elliptio, Fusconaia, Megalonaias, Proptera, Quadrula, and Villosa. C. monodonta and Margaritifera margaritifera are in the same set; Cyclonaias tuberculata and Quadrula cf. Q. quadrula are in a set and more closely allied to each other than either is to Q. cylindrica. Megalonaias gigantea and F. masoni are in a set. Elliptio and Fusconaia are closely associated.

ANODONTA
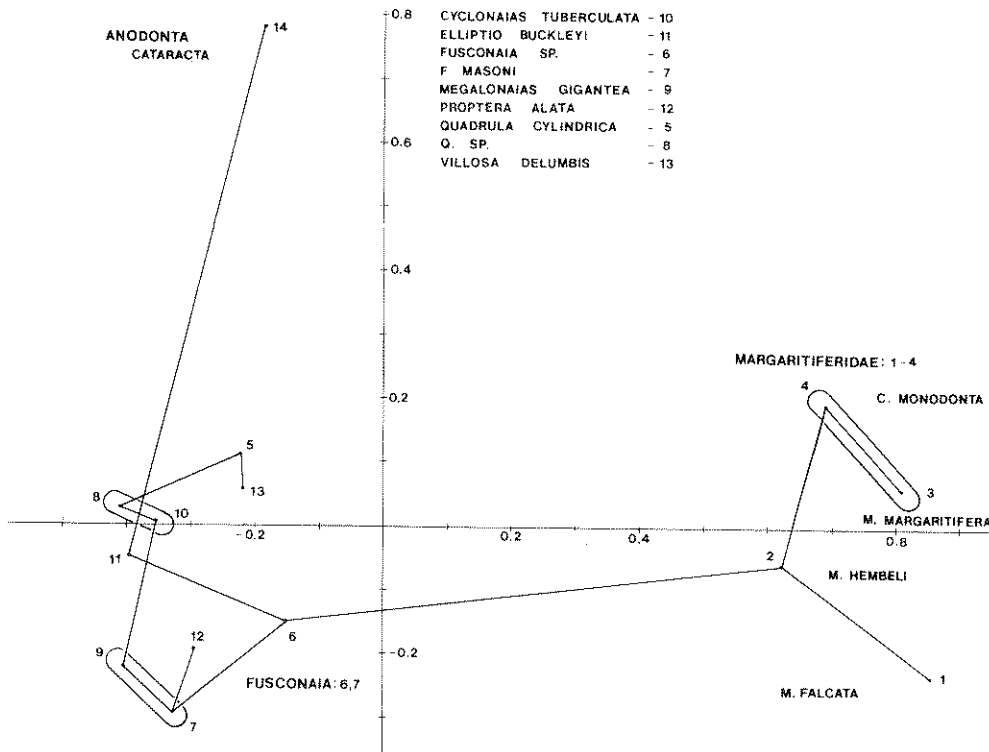CATARACTA

CYCLONAIAS TUBERCULATA	- 10
ELLIPTIO BUCKLEYI	- 11
FUSCONAIA SP.	- 6
F MASONI	- 7
MEGALONAIAS GIGANTEA	- 9
PROPTERA ALATA	- 12
QUADRULA CYLINDRICA	- 5
Q. SP.	- 8
VILLOSA DELUMBIS	- 13

MARGARITIFERIDAE: 1 - 4

C. MONODONTA

M. MARGARITIFERA

M. HEMBELI

M. FALCATA

FUSCONAIA: 6,7

FIG. 1. Ordination diagram in two dimensions showing relationships among 14 taxa via use of the minimum spanning tree and subsets. The data are based on cross comparisons using 14 sets of antisera and antigens. See text for details.

Results of comparing 52 OTUs × 21 antisera are shown in Fig. 2. The ordination involving the first two principal components accounted for 92.84 percent of the data. The correlation between the matrix of taxonomic distances and distances in the 2-dimensional MDS was excellent, i.e., 0.946; the stress was 0.205.

We again found three widely separated groups of species. These groups are essentially those seen in Fig. 2: 1) the *Margaritifera* group (quadrant I), 2) the *Anodonta* group (quadrant IV), and 3) a large mass of taxa linked together primarily in quadrants II and III.

A series of subsets encloses the species in the *Margaritifera* cluster. *Cumberlandia monodonta* is in a subset with *M. hembeli*; those two are in a large set with *M. margaritifera*. All four species are clustered in an inclusive set. In the *Anodonta* cluster *A. cataracta* is in a subset with *A. imbecillis. A. implicata* is in another subset with *Lasmigona costata.*

Because so many taxa are grouped in the third cluster and because this cluster is so distinct from the *Anodonta* and *Margaritifera* groups, we did a multivariate analysis of only those species and corresponding antisera from that third cluster. This involved a subset of the database (Table 7) of 15 antisera and 40 OTUs. We omitted data for *Quincuncina infucata* because we had too few data for this comparison. In this reduced set there were only 19 comparisons of 600 for which we had no data. The results of ordination involving the first two principal components are shown in Fig. 3. Only 78.97 percent of the data are represented. The correlation between the matrix of taxonomic distances and distances in the 3-dimensional matrix was 0.913; the stress was 0.378.

Three closely allied clusters are seen (Fig. 3) in quadrants I, II–III, and IV. Genera within these clusters are listed in Table 8. Two of the genera are found in two clusters: *Amblema* and *Fusconaia. A. plicata* is in cluster 1; *A.*

FIG. 2. Ordination diagram in two dimensions showing relationships among 52 taxa via the minimum spanning tree and subsets. The data are the percent difference when 52 taxa were analyzed using 21 sets of antisera. See text for details. Abbreviations are explained in Table 1.

perplicata, cluster 2. Three species of Fusconaia are in cluster 1; one, in cluster 3. Four species of two genera are emphasized in Table 8 because the species are not grouped in the same major cluster. Distance coefficients (d.c.) are given for those three species that are the least distant from each of the species in question. The closest relationship of A. plicata is with Elliptio buckleyi (d.c. = 0.620), its next is to Plectomerus dombeyanus of the 2nd cluster (d.c. = 0.97), and the third is to A. perplicata (2nd cluster; d.c., 1.095) or Glebula rotundata (3rd cluster, d.c. = 1.095). Note that in Fig. 3 the MST ties Glebula and Plectomerus together and A. perplicata to Plectomerus. On the basis of the interrelationships that revolve around Plectomerus, it is appropriate to consider the two species of Amblema to be part of cluster 2.

The situation with Fusconaia ebena of cluster 3 is different. Closest relationships of F. ebena are with Lampsilis, Ptychobranchus,

and Glebula, all of cluster 3. F. flava of cluster 1 has its closest relationship with Elliptio of cluster 1 (Table 8). We suspect experimental error in testing relationships with F. ebena (see Appendix 2, point 1).

Taxa that are grouped in subsets are listed in Table 9 with the taxonomic distances among them. There are only seven such subsets, and only two of these are found in cluster 3. The closest relationship in a subset involves Plectomerus dombeyanus and Megalonaias gigantea (d.c. = 0.588).

By use of the MST, groupings of taxa are shown that suggest relationships (Fig. 3) that will be discussed later. 1) In cluster 1 there are no clear associations of classically defined species of Elliptio or Fusconaia that result in an Elliptio cluster or a Fusconaia cluster. 2) Plectomerus, Megalonaias, and Amblema form a grouping in cluster 2. 3) Quadrula, Tritogonia, and Cyclonaias are linked in cluster 2. All species of Lampsilis are linked in cluster 3, and Villosa and Proptera

TABLE 7. Raw data (percent difference) for 15 antisera and 40 species (antigens). Key to abbreviations is given in Table 1. NC = no data available.

Spe-cies	Ct	Eb	Ecr	El	Fbb	Fm	Ga	Lo	Mg	Pa	Qbb	Qc	Tv	Ut	Ec
Ga	41	NC	41	50	33	27	0	41	41	33	50	40	41	33	25
Ap	33	18	25	25	26	18	30	50	16	16	33	30	16	16	25
Fe	50	36	33	25	40	27	50	50	41	33	41	40	16	41	50
Ff	25	18	33	16	33	9	50	41	25	33	33	30	25	41	16
Fbb	25	27	50	16	0	9	40	41	33	25	25	30	41	33	25
Fm	25	36	41	16	26	0	50	41	8	33	16	40	25	41	33
Qbb	8	27	41	16	20	27	50	41	16	25	0	20	16	33	25
Qa	8	36	50	41	33	27	50	41	25	25	16	10	33	25	33
Qc	16	45	41	25	20	45	40	41	25	25	8	0	16	41	25
Pd	16	27	41	25	33	27	40	41	8	16	25	20	25	33	16
Tv	25	27	50	25	40	45	40	50	41	33	25	20	0	41	41
Mg	16	27	50	33	26	18	50	41	0	25	25	20	25	41	16
Ct	0	36	41	25	20	27	30	41	16	25	16	30	25	25	16
Ec	25	18	33	8	20	9	30	50	25	25	33	30	33	41	0
El	25	18	33	0	25	27	40	50	50	25	33	30	16	41	16
Ei	16	0	33	8	33	18	30	50	41	25	33	30	16	41	16
Ew	41	18	33	25	33	18	50	50	41	33	50	40	25	41	8
Ecr	16	27	0	8	33	27	50	41	33	25	33	20	33	41	16
Ut	25	45	16	41	26	27	20	41	33	25	25	10	25	0	33
Pc	33	NC	16	25	20	0	40	56	33	16	25	30	33	41	16
Cp	33	45	41	33	33	36	30	41	50	25	33	30	41	41	25
Gr	33	18	41	33	26	36	50	33	33	16	25	30	33	33	25
Lt	33	27	41	41	46	45	40	41	41	33	41	30	41	41	33
Lcl	41	36	33	33	33	27	50	25	58	16	33	40	25	41	33
Ls	41	36	33	16	26	36	50	16	41	16	33	40	33	16	41
Lo	33	27	41	33	33	36	40	0	33	16	41	40	33	41	41
Lh	33	36	33	16	26	36	30	25	41	25	41	30	41	41	16
Lf	25	27	25	NC	46	27	40	16	45	16	25	30	25	NC	25
Lr	41	NC	41	25	40	36	50	25	41	NC	33	30	41	41	41
Ln	25	36	25	33	26	45	30	33	33	25	41	30	33	41	33
Pa	33	27	25	25	33	18	40	41	33	0	41	30	41	33	33
Ppu	41	NC	41	33	46	45	50	33	41	8	NC	40	33	41	25
Vd	33	54	41	41	40	36	60	16	50	40	41	30	33	NC	33
Ps	33	36	33	33	40	27	50	33	33	40	41	30	33	41	25
Eb	33	0	NC	NC	26	18	30	50	25	25	25	30	NC	NC	25
Lre	33	NC	25	41	46	45	40	33	41	25	16	40	41	41	33
Acr	33	NC	50	33	33	36	40	50	25	33	8	20	16	33	25
Qp	25	NC	50	33	33	36	40	50	25	33	8	20	16	33	25
Apl	16	NC	33	16	33	9	NC	41	8	25	16	10	16	16	8
Lv	50	NC	25	33	46	45	40	16	41	41	33	40	41	41	33

link to *L. radiata.* 4) *L. radiata* is central in the *Lampsilis* cluster. 5) *Gonidea* is a distinct subgroup of cluster 3, far removed from other species of that cluster (d.c. = 1.068).

DISCUSSION
Evolutionary trends: primitive and derived character-states

In the evolution of freshwater bivalves, the ecological transition from the sea through estuaries into rivers necessitated the survival of larval forms. The free-swimming veliger larvae had to be retained in the parent in order to prevent their destruction by being swept downstream or by osmotic shock. Two strategies evolved to accommodate retention of the veliger larvae; brooding young to the juvenile stage and brooding young to an early pre-pediveliger parasitic state.

The two larval retention strategies correlate with the size of the breeding adult for reasons documented by Hoagland (1975). Where one

TABLE 8. Listing of genera in each of the three clusters shown in Fig. 3. Distance coefficients are given showing the three species closest to species of those two genera apparently located in two different clusters. Coded names are given in Table 1.

	Distance coefficients		
	1.	2.	3.
Quadrant I (cluster 1)			
Amblema plicata	Eb (0.620)	Pd (0.970)	Apl (1.095) Gr (1.095)
Elliptio			
Fusconaia flava	Ea (0.816)	Ei (0.850)	El (0.870)
Pleurobema			
Uniomerus			
Quadrant IV (cluster 2)			
Amblema perplicata	Pd (0.893)	Qbb (1.042)	Ct (1.042)
Cyclonaias			
Megalonaias			
Plectomerus			
Quadrula			
Tritogonia			
Quadrants II–III (cluster 3)			
Actinonaias			
Carunculina			
Fusconaia ebena	Lcl (0.993)	Ps (1.005)	Gr (1.125)
Glebula			
Gonidea			
Lampsilis			
Ligumia			
Leptodea			
Ptychobranchus			
Proptera			
Villosa			

TABLE 9. Taxa included in the smallest subsets together with their taxonomic distance coefficient. Ranking is by lowest to highest taxonomic distances. The smaller the distance, the closer the relationship.

Taxonomic distance	Species pairs
0.588	Plectomerus dombeyanus × Megalonaias gigantea
0.620	Amblema plicata × Elliptio buckleyi
0.631	Elliptio icterina × E. lanceolata
0.658	Ligumia nasuta × Actinonaias carinata
0.697	Lampsilis radiata × Ptychobranchus subtentum
0.777	Fusconaia flava × Elliptio waccamawensis
0.870	Quadrula pustulosa × Q. cylindrica

niche dimension is small body size, a proportionally small amount of energy is available for reproduction, few young are produced, and these are brooded to the juvenile state; there is a high probability of individual survivorship of the young. When body size is large, reproduction is delayed until large body size is attained, and then proportionally large amounts of energy are available to produce numerous young that are released at an early larval stage; there is a low probability of survivorship to the young.

Native freshwater bivalves of North America are of two types: Unionacea, which are
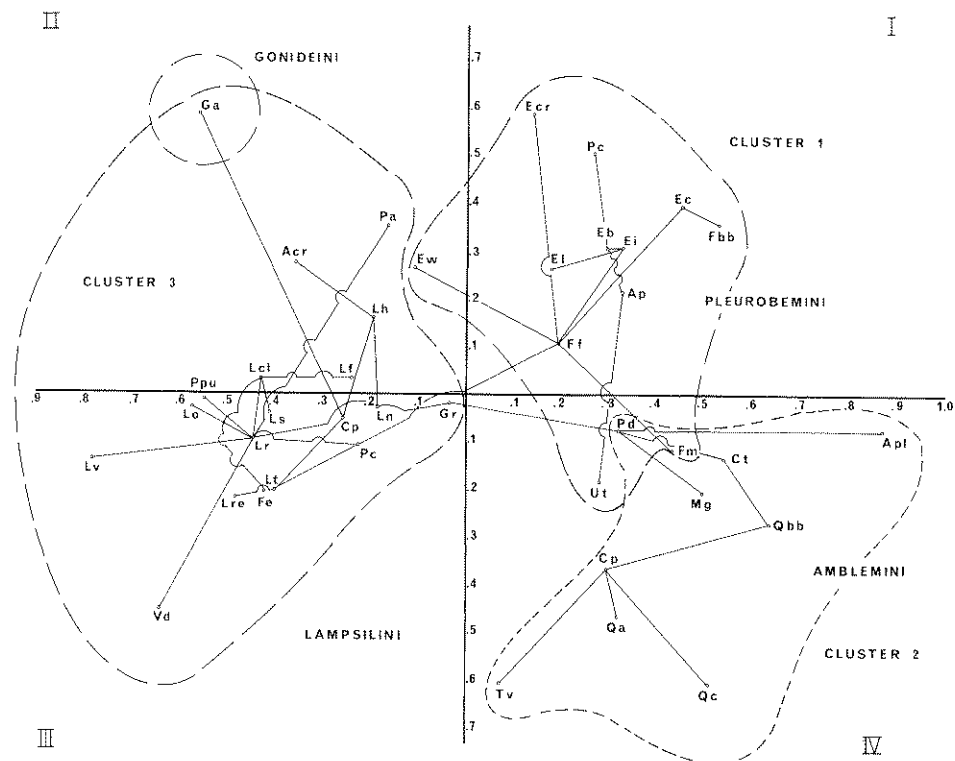
FIG. 3. Ordination diagram in two dimensions showing relationships primarily among those taxa clustered in quadrants II and III in Fig. 2 using only antigens and antisera pertaining to those taxa. Relationships are clarified by use of the minimum spanning tree and subsets. See text for details. Abbreviations are explained in Table 1.

large-bodied (most adult shells exceeding 8 cm length), and Sphaeriacea, which are small-bodied (adult shells usually are less than 12 mm in length). Sphaeriidae brood their young to the juvenile stage, whereas Nearctic Unionidae typically brood young to the glochidial (parasitic) stage. The Unionidae are sedentary as adults, and numerous species often live sympatrically side by side in the same river bed. Because of this sedentary life and the brooding of the young, it is not surprising that morphological characters serving to distinguish among species are few and that those soft-tissue characters that are useful involve structures of the demibranchs for housing the brooding young and structures of the mantle margins and pseudosiphonal regions, which interface with the aquatic environment for the purposes of pumping water and food into the animal, expelling water and waste, and getting the glochidia to the appropriate host.

Morphological character-states were considered primitive when they represented the simplest condition. Derived morphological character-states are those showing increased organization, complexity, and specialization. We follow Ortmann (1910a, 1911, 1912b), Heard & Guckert (1971), and Heard (1974) in considering *Margaritifera* to have the most primitive groundplan of all unionaceans. We suggest that taxa with this type of groundplan probably gave rise to all other Recent unionaceans.

Primitive character-states are designated with "P" in tables 10 and 11. The most derived states are designated "S". The consideration that the direction of evolution is from primitive to derived as defined here is consistent with the facts that *Margaritifera* is ancient, known from the Cretaceous, and has a Holarctic distribution, including representation in Southeast Asia (Heard, 1974; Smith, 1977). Representative of the most derived and specialized taxa, *Lampsilis* is known from the Oligocene and is endemic in North America.

In *Margaritifera*, demibranch lamellae are held apart by randomly arranged trunks of interlamellar connective tissue. The ctenidia

TABLE 10. Morphological character-states serving to define subfamilies of Unionidae employed in this paper.

Margaritiferinae
        *1. No true septa—P
        *2. No water tubes—P
        *3. Excurrent aperture entire—P
        *4. Diaphragm grossly incomplete—P
        *5. No additional connective tissue at distal margin of marsupial demibranch—P
        *6. Glochidia with irregular teeth
        *7. Glochidia without numerous spines—P
        8. Glochidia subspherical
        *9. Glochidia small[5]—P

Anodontinae
        1. With true septa (parallel to gill filaments)—S
        *2. With water tubes tripartite—S
        3. With supra-anal opening—S
        4. Diaphragm slightly incomplete—S
        *5. Additional connective tissue at distal margin of marsupial demibranch—S
        *6. Glochidia with hooks—S
        *7. Glochidia with numerous spines—S
        *8. Glochidia subtriangular
        *9. Glochidia large[5]—S

Ambleminae
        1. With true septa (parallel to gill filaments)
        2. Water tubes present, not tripartite
        3. With supra-anal opening, but excurrent aperture sometimes entire
        4. Diaphragm slightly incomplete
        5. No additional connective tissue at distal margin (= ventral margin) of marsupial demibranch
        *6. Glochidia without hooks** or teeth
        7. Glochidia without numerous spines
        8. Glochidial shape variable
        9. Glochidia medium sized[5]

  * most distinguishing character-states
  ** except *Proptera*
  5, see Appendix 2
  P, Primitive
  S, derived, specialized

thus lack water tubes, and the eggs and/or larvae are incubated in a flaccid sac. All four demibranchs are marsupial. When feeding and respiring, the animal exhibits a wide gape between the posterior ends of the valves, which leaves the soft tissues within vulnerable to disturbances from without. Associated with this gape is an extraordinary development of muscular arborescent papillae at the incurrent mantle aperture. *Margaritifera* lacks a separate supra-anal opening (i.e. there is no subdivision of the excurrent mantle aperture by fusion of the opposing mantle margins), and there is no clear demarcation of the anterior boundary of the incurrent aperture. Finally, at the posterior end of the gills, the diaphragm is incomplete and formed only by the ctenidia. The glochidia are tiny (about 50 μm long) and hookless (Baker, 1928).

The sac-like marsupia, tetragenous condi-tion, and posteriorly gaping valves are conditions associated with low species diversity and ecological restrictions to streams of pebble-cobble substrate with rapid flow of highly oxygenated water. That sac-like marsupia involve all demibranchs means that gravid gills are loaded with eggs and embryos, a condition that must interfere with respiration. Because there is little supporting storage structure within the gill to assure efficient packaging and protection, there probably is some vulnerability of the eggs and embryos to mechanical damage, especially as the posterior animal, including the gills, is exposed due to the wide shell gape and the lack of mantle sutures helping to protect the posterior region from the outside environment.

Morphologically derived character-states in other unionids involve increasing complexity of the interlamellar gill tissue and modifica-

TABLE 11. Morphological features characterizing the four tribes of the Ambleminae employed in this paper.

Gonideini
    1. Tetragenous (mostly or all—P) or ectobranchous (perhaps some)?
    *2. Perforated septa—P
    3. Marsupia not confined to restricted regions of the demibranchs—P
    4. No specialized mantle structures—P
    5. Marsupial water tubes do not extend beyond distal margins of demibranch lamellae—P
    6. Shells smooth—P

Lampsilini
    1. Ectobranchous—S
    2. Septa not perforated—S
    *3. Marsupia confined to restricted region of the demibranchs—S
    *4. Many taxa with specialized mantle structures (flaps, caruncles, etc.)—S
    5. Marsupial water tubes extend beyond distal margins of demibranch lamellae—S
    6. Shells mostly smooth

Pleurobemini
    1. Ectobranchous (mostly) or tetragenous
    2. Septa not perforated
    3. Marsupia rarely confined to restricted regions of the demibranchs
    4. No specialized mantle structures
    5. Marsupial water tubes do not extend beyond the distal margins of the demibranch lamellae
    *6. Shells smooth

Amblemini
    1. Tetragenous (mostly) or ectobranchous
    2. Septa not perforated**
    3. Marsupia not confined to restricted region of the demibranchs
    4. No specialized mantle structure
    5. Marsupial water tubes not extending beyond distal margins of the demibranch lamellae
    *6. Shells heavily sculptured (few exceptions)—S

  * distinguishing character-states(s)
  ** except for marsupium of *Megalonaias*
P, primitive
S, derived, specialized

tions of the mantle margin. Also, there are trends of reduction in the number of marsupial demibranchs and development of specialized regions of the gill for incubation of young.

There are several derived character-states of great importance. First, the scattered margaritiferoid interlamellar connectives were increased numerically. The advantage of more connectives probably is to increase the internal strength and stability of the gill and, therefore, the safety of its contents. Second, the connectives were organized into continuous walls (septa) that served to define and separate linear series of adjacent water tubes within the gills. Septa are perforate or imperforate. The perforate condition probably is the more primitive (Heard, 1974). Septa probably greatly increased structural support for the gills. Third, septa were aligned "vertically," i.e., parallel to the gill filaments. This vertical attachment along the filament strength-

ens the septum, and, as a simple exercise in geometry will demonstrate, less space within the demibranch is needed for parallel orientation of septa and filaments than is occupied by identically spaced septa oriented obliquely to the filaments. A reduction in space occupied by interlamellar tissue presumably would facilitate gas exchange. Fourth, a portion of the gill was set aside as a permanently modified marsupium whose interlamellar septa became thicker and more closely spaced than those in non-marsupial parts of the gill. This further reduction in the extent of the marsupium presumably facilitated respiration additionally. Indeed it may have been necessitated by proliferation of interlamellar tissue, at least in the case of very active mussels. There is a strong association between reduced marsupial size and the need for energy (and thus for oxygen). In what we regard as the most advanced Nearctic unionids (i.e.,

Lampsilis and its allies) are found the least extensive marsupia. These are consistently the most active of mussels, not only in terms of locomotion, but also because of the movements of specialized structures on the postbasal mantle margin (flaps, caruncles, etc., which are important in reproduction). In any case, thicker, closer-spaced septa in the marsupium strengthen it further and thus provide added protection for its contents (eggs and/or larvae).

These developments were accompanied by modifications of the bivalve hydrodynamic (water pumping) system. Modifications apparently were necessitated because development of the vertical water tube meant an increase in the distance that larvae would have to travel in order to escape the marsupium to the external environment; marsupial contents vertically evacuate the water tube and then perpendicularly traverse the excurrent pallial chamber before emission to the waterway through the excurrent mantle aperture. The necessary increase in propulsive hydrodynamic pressure was created by realizing or at least approximating a "closed" hydrodynamic system within the adult female mussel. Several devices were possible: stronger muscular adduction of the valves, close fit of the valves, increased fusion between apposing mantle margins, and/or posteriad extension of the diaphragm.

Morphology, immunology, and a new classification

The ordination diagrams (Figs. 2, 3) with MST and subsets indicate the classification given in Table 12. For three reasons, we argue that there are one family and three subfamilies. First, we see only two directions of morphological change from the primitive *Margaritifera* type, i.e. to the derived *Anodonta* and *Lampsilis* types. These are progressive changes within a single morphological groundplan. Few morphological changes, involving increased complexity, are needed to progress from a *Margaritifera*-type morphology to an anodontine type or to an amblemine type. We do not see abrupt differences among the three groundplans such as exist between the marine Cardiidae and Tridacnidae of the superfamily Cardiacea, for example, or between the marine families Pteriidae, Malleidae, and Pectinidae of the superfamily Pteriacea (see Yonge & Thompson, 1976).

Second, immunologically there are three distinct clusters, which correspond to the three morphologically defined Nearctic groups within the unionid morphological groundplan; the *Margaritifera*-type, the amblemine type, and the anodontine type (Tables 10 and 11). (Cladistic relationships among these types will be presented later.) We believe that immunologically, as well as morphologically, the three groups have equal weight. They might be interpreted as three families or as three subfamilies of a common family.

Of all the antigens discovered during our analyses only one or two were not unique to the freshwater mussels we used. This suggests strong immunological cohesion of this group. The average genetic distances among the three mussel subgroups were close to 50%. This reinforces the conclusion (above) that the three groups are not far apart genetically. Therefore we conclude that the three groups are best regarded as subfamilies within one family. The taxonomic results are Unionidae: Margaritiferinae, Anodontinae, and Ambleminae. The greatest difference is between the *Anodonta* and *Margaritifera* groups; the least between the *Amblema* and *Margaritifera* groups.

What is the relationship between immunoelectrophoretic genetic distance, as presented here, and taxonomic hierarchy? The relationship is not a simple one; there is no direct correspondence. Classifications traditionally have been based on comparative morphology. Increments of change in the taxonomic hierarchy follow discrete changes in morphological groundplans. Pronounced changes in morphology and behavior can occur rapidly with respect to geological time (Stanley, 1979). These changes, presumably under the control of regulatory genes, may involve few genetic changes involving regulatory genes, yet be pronounced enough to impress taxonomists that the taxon in question belongs in a different higher-category taxon from that of the taxon most closely related to the one in question. Such morphological change may not be accompanied by an equal amount of change in structural proteins. The now classic example is one involving man and chimpanzee. These animals are classified in different families on the basis of considerable morphological and behavioral divergence. However, the molecular genetic distance between man and chimpanzee is very small, essentially equal to the genetic distance among sibling

TABLE 12. Comparison of major classifications showing similarities and differences. Genera are not listed if the subfamily of an author is equivalent to the tribe or subfamily of Davis et al. (1978).

Davis et al. 1978[6]

A. Unionidae
 I. Margaritiferinae
  Margaritifera
  (+Cumberlandia)
 II. Anodontinae
  Anodonta
  Alasmidonta
  Lasmigona
 III. Lampsilinae
  1. Lampsilini
   Actinonaias
   Carunculina
   Glebula
   Lampsilis
   Leptodea
   Ligumia
   Proptera
   Ptychobranchus
   Villosa
  2. Gonideini
   Gonidea
  3. Elliptionini
   Elliptio
   Fusconaia
   Pleurobema
   Uniomerus[2]
  4. Amblemini
   Amblema (+Megalon-
     aias;
     Plectomerus)
   Cyclonaias
   Orthonymus (=Q_c)
   Quadrula
   Quincuncina[3]
   Tritogonia

Davis & Fuller[6] (this paper)

A. Unionidae
 I. Margaritiferinae
 II. Anodontinae
 III. Ambleminae
  1. Lampsilini
  2. Gonideini
  3. Pleurobemini
  4. Amblemini

Ortmann (1910a, 1916)

A. Unionidae
 I. Margaritiferinae
 II. Anodontinae
 III. Lampsilinae†
 IV. Gonideinae†
 V. Unioninae†
  Elliptio*
  Fusconaia*
  Pleurobema*
  Uniomerus*
  Amblema
  Megalonaias
  Plectomerus
  Cyclonaias
  Orthonymus
  Quadrula
  Quincuncina
  Tritogonia

Heard & Guckert (1971)

A. Margaritiferidae†
 I. Margaritiferinae
 II. Cumberlandinae†
B. Unionidae†
 I. Anodontinae
 II. Lampsilinae
 III. Pleurobeminae †
  Cyclonaias*
  Elliptio
  Pleurobema
  Uniomerus
 IV. Popenaiadinae†
  Popenaias buckleyi*
C. Amblemidae†
 V. Gonideinae†
 VI. Ambleminae†
  Amblema
  Fusconaia*
  Plectomerus
  Quadrula
  Quincuncina
  Tritogonia
 VII. Megalonaiadinae†
  Megalonaias

Modell (1942, 1949, 1964)

A. Margaritiferidae†
 I. Margaritiferinae
 II. Pseudodontinae†
  Gonidea
B. Elliptionidae†
 III. Alasmidontinae†
  Alasmidonta
  Lasmigona
 IV. Lampsilinae
 V. Pleurobeminae†
  Fusconaia
  Pleurobema
 VI. Elliptioniinae†
  Elliptio
  Uniomerus
  Popenaias
 VII. Ambleminae†
  Amblema
  Plectomerus
C. Unionidae
 VIII. Quadrulinae†
  Cyclonaias
  Megalonaias
  Quadrula
  Quincuncina
  Tritogonia
 IX. Anodontinae†
  Anodonta
 X. Rectidentinae†
  Pyganodon cataracta

A, etc., families.
I, etc., number of subfamilies.
1, etc., number of tribes.
†, supragenetic taxon that is not equivalent to our taxon, or a taxon we cannot agree to. See text for details.
*, genus belonging to a different suprageneric taxon considering the other genera with which it has been grouped, and considering generic placement in the Davis & Fuller classification.
superscript 2,3,6 See Appendix 2, Annotations.

species of other organisms (King & Wilson, 1975).

However, there is strong evidence that, once lineages have begun to diverge and are reproductively isolated, there is a rather regular increase in molecular genetic distance with increasing time (Fitch, 1976; Sarich, 1977). Further, there seem to be rapid and slow rates of certain loci in protein evolution that can be detected electrophoretically. The contribution of rapidly evolving loci should be completed in some six million years, while further increases in genetic distance are contributed by the slowly evolving loci (Sarich, 1977). Given the divergence of *Margaritifera, Anodonta,* and Ambleminae at least by the late Cretaceous (i.e. 60 million years ago), one would reasonably expect considerable genetic distance among these naiad taxa.

In the few immunoelectrophoretic studies such as this one, species of different gastropod families, but of the same superfamily, have differed from 40% to 80% and most have differed by 50% to 80% (Davis & Suzuki, 1971; Davis, 1978). Using allozyme electrophoretic analyses, and considering changes in I between different hierarchial levels in other organisms (Davis et al., 1980) we have found that genetic similarity between *Margaritifera* and our Ambleminae was greater (I = 27%) than we would expect if these taxa belonged to different families.

In summary, given the 47% immunoelectrophoretic genetic distance between our Margaritiferinae and Ambleminae and considering the great age of divergence of these taxa, we think it reasonable to consider them to belong to a single family, the Unionidae. This is supported by the cohesiveness of the unionid morphological groundplan, which includes a single larval type (the glochidium).

The three subfamilies are cohesive and distinct immunologically and morphologically. Morphological character-states that aid in distinguishing among these taxa are marked with an asterisk in Tables 10 and 11.

The Margaritiferinae have been discussed above in terms of character-states that have been considered primitive and that in some cases are unique to taxa of this subfamily.

The Anodontinae are defined, in part, by unique derived character-states, indicated in Table 10. These states have been discussed previously, for the most part (Ortmann, 1910b, 1911, 1912b; Heard, 1975). Known Anodontinae have an extraordinary type of glochidium, whose shell is subtriangular in lateral outline, usually large and powerful, and armed with spined hooks at the apex of the ventral margin. The powerful, well armed anodontine glochidium can sever soft host tissues (e.g. the gill filaments of fishes) and prematurely fall from the host to the streambed, where it will die. On the other hand, this type of glochidium fares well on tougher tissues (e.g. the fins and even the scales of fishes) and thus occupies a metamorphic niche that has been rarely exploited and perhaps chiefly vacated by weaker types of unionid glochidia.

The anodontine marsupium exemplifies Simpson's (1900, 1914) group Homogenae, yet it is of a unique type. During gravidity a marsupial water tube adopts a tripartite construction: secondary septa develop parallel to the inner and outer demibranch lamellae. The object presumably is to facilitate additional gas exchange for the marsupial contents. The dorsal margins of gravid water tubes are sealed by a film of connective tissue, whose purpose presumably is prevention of the escape of eggs and the premature escape of larvae. With one known exception, the egg mass is loosely structured. Premature loss of ova and larvae to the excurrent mantle chamber would be a great threat were it not for the dorsal tissue.

Gravid anodontine marsupia are greatly and uniquely swollen. This is facilitated by another unique feature, the development along the distal margins of the outer demibranchs of additional connective tissue during gravidity. This device permits the apposing lamellae to separate and move apart. The entire phenomenon necessitates the presence of the secondary water tubes (above) and probaby is a response to the need for space in order to incubate competitively large numbers of offspring, which are themselves exceptionally large, as noted earlier.

The typically loose structure of the egg and larval masses is caused by the great size of the marsupial contents, which cannot pack together so securely as can those small ova and glochidia of other unionid groups. On the other hand, the loose mass is an advantage because the adult does not have to overcome the inertia of a large mass during expulsion of marsupial contents. Instead, the glochidia can be pumped out singly or in small numbers. The advantage is of even greater value for very thin-shelled, low-density species, whose poor valve adduction and fit weaken the hydrodynamic system.

The Ambleminae are like the Anodontinae in that the water tubes parallel the gill fila-

ments; the posterior mantle is not united, but drawn together by the diaphragm, to effect functional separation of incurrent and excurrent apertures; the excurrent aperture is closed above, which effects a supra-anal opening separate from an anal one; and the diaphragm is almost complete and is formed entirely by the ctenidia. These two subfamilies differ in that most Ambleminae: Amblemini and some Ambleminae: Pleurobemini are tetragenous, whereas Anodontinae are ectobranchous; amblemine glochidia are hookless and are of various shapes and sizes but never so large as the anodontine glochidia or hooked in the same way; and amblemine water tubes are undivided at all times.

We distinguish four subgroups of Ambleminae: Gonideini, Lampsilini, Amblemini, and Pleurobemini (Table 11). There are few unique morphological character-states serving to define these tribes. The Gonideini have perforated septa and are tetragenous; the Lampsilini have specialized marsupial (in most taxa) and postbasal mantle modifications (many taxa) and are ectobranchous; the Pleurobemini have smooth shells and no specialized marsupial features or postbasal mantle structures and mostly are ectobranchous; and the Amblemini have heavily sculptured shells and mostly are tetragenous.

We have not employed the subfamily name Unioninae because we do not know how *Unio* s.s. of Europe relates to North American Unionidae. Ortmann (1912b) did not consider *Unio* s.s. to be equivalent to North American taxa that have similar morphology of shell and soft parts. Heard & Vail (1976) provided an excellent account of the morphology of *Unio*. *Unio* differs from our Ambleminae by having glochidia with hooks, perforated marsupial septa and imperforated non-marsupial septa, subtriangular and medium-sized glochidia. *Unio* and allied taxa have a smooth shell, undivided water tubes, and ectobranchous marsupium. The genetic relationship of *Unio* s.s. to our Ambleminae and Anodontinae must be determined before we can consider the use of this taxon name for a higher-category taxon below the level Unionidae. However, the groundplan of the Eurasian *Unio* is generally so like our Ambleminae: Pleurobemini type that we preserve the traditional usage of the name at the family level.

Comparison of classifications

Eleven classifications are given in Appendix 1. These classifications represent three very different types of approach, and much is to be learned from their study. An historical account of unionid classification is given in Appendix 4. The three approaches that have been used are based on 1) conchology only, 2) selected use of a few key characters, 3) use of all data available and asking questions about how character-states evolved and about the relationships of character-states to environments. Of all the classifications prior to our own, only Heard & Guckert (1971) clearly stated the basis for their classification. Accordingly, we will first contrast our classification with theirs and, by so doing, address various problems raised in the Introduction.

We reject the Heard & Guckert classification for three reasons.

First, they used the character "number of marsupial demibranchs" to establish families. They used the Amblemidae to accommodate all non-margaritiferine unionids with four marsupial demibranchs; those taxa with two outer demibranchs marsupial were considered Unionidae. On the basis of immunologically derived relationships, it is clear that the tetragenous condition has undergone parallel evolution and that reduction to two marsupial demibranchs has occurred at least four and possibly five times, i.e. at the origin of the *Anodonta* clade, during the evolution of recent Ambleminae, and within the lineages of the Amblemini and Pleurobemini; it possibly has occurred in the Gonideini.

It should not be surprising that reduction of marsupial demibranchs occurred several times once greater efficiency in the hydrodynamic system had been achieved. Any reduction in the space taken up with marsupial function would mean increased efficiency in respiration. Specializations in how glochidia are incubated and delivered to their hosts correlate with different reproductive strategies.

Second, they created two new subfamilies on the basis of length of breeding season: the Megalonaiadinae and the Popenaiadinae within their families Amblemidae and Unionidae, respectively. The new subfamilies contained only taxa that are bradytictic, whereas the other subfamilies of their Amblemidae and Unionidae are tachytictic. An examination of Fig. 3 clearly shows that there is no separate clade that separates *Megalonaias* from other taxa or so-called *Popenaias buckleyi* from its congeners (*Elliptio*). Heard & Guckert (1971) created their new subfamily concept Popenaiadinae primarily because of information about *E. buckleyi* (see Fuller, 1975). We do not, however, have biochemical data for *Popenaias*

popei (Lea), so this subfamily has not been fully invalidated (but see Fuller, 1975).

It clearly is unacceptable to create higher taxa on the basis of length of breeding season. As with the question of how many demibranchs and water tubes bear glochidia, the question of length of breeding season seems more correlated with reproductive strategy than with diverging clades. This character (length of breeding season) in most cases should not be used to assess taxonomic relationships among unionid taxa.

Third, they created the subfamily Cumberlandiinae to provide a subfamily for the monotype *Cumberlandia monodonta* (Say), which is the only margaritiferine sensu nostra of the Mississippi basin and is confined to it. This rank was based on a single character state, the proliferation of interlamellar connective tissue approximating true septa. We argue that *Cumberlandia* (described by Ortmann, 1912a) is not deserving of subfamily rank, and that it may best be considered a synonym of *Margaritifera*. On the basis of immunological data *C. monodonta* is in the same subset with *M. margaritifera* (Fig. 2). The MST shows that *C. monodonta* is intermediate in relationship between *M. margaritifera* and *M. hembeli. M. falcata* is more distantly related to *M. margaritifera* than is *Cumberlandia monodonta*. We consider these relationships to be accurate as shown because we had antisera for each of the four species and thus could make the appropriate cross-comparisons.

What distinguishes *Cumberlandia monodonta* from the other three species is the somewhat continuous, oblique "septa" of the former in contrast to the patternless interlamellar connectives of the latter. We consider this small modification of the "septum" to be indicative of a species difference in the *Margaritifera* complex and thus we consider *Cumberlandia*, on all data available, to be a synonym of *Margaritifera*. We see no reason to use subgeneric rank at all. We are especially confident of our conclusions because we have tested and examined every nominal Nearctic margaritiferine species: *Cumberlandia monodonta* of the Mississippi basin, *Margaritifera hembeli* of the Gulf drainage, *M. margaritifera* of the northern Atlantic drainage, and *M. falcata* of the Pacific drainage.

We compared our classifications with those of Ortmann (1910a, 1911, 1912a, 1916, 1919) and Modell (1942, 1949, 1964), as well as Heard & Guckert (Table 12). These classifications were chosen because Ortmann's work stands for the totally synthetic approach; Modell's is a sprawling classification based on shell characters; and the Heard & Guckert classification purposely ignores conchology and is essentially monothetic with some attention to reproductive characters.

It is clear that there is closest agreement between our classification and that of Ortmann, especially his earliest work (1910a). In his later work Ortmann (1911, 1912b, 1919) elevated the group of *Margaritifera* to familial status. Accordingly, in scoring divergence of other classifications from ours, we used a range for Ortmann's classification based on his earlier and later schemes. We arbitrarily gave 5 points for each family or subfamily that is not equivalent to our comparable family or family concept (Table 12, †) and one point for a genus that has been placed with another genus (genera) that belongs to another suprageneric taxon in our classification (Table 12, *). Ortmann's score is 19 or 29; the Heard & Guckert score, 48; Modell scores 55. On this basis, Modell's classification is the least satisfactory.

Modell (1942, 1949, 1964) established a strictly conchological classification of three families and 10 subfamilies with Nearctic members. Heard & Guckert (1971) and Heard (1974) were mostly correct in stating that shell characters typically do not correlate with soft-part characters. For the most part shell characters do not correlate with anatomical characters or genetic data, also we have discussed the reasons for rejecting a two- or three-family classification. *Gonidea* is not closely related to *Margaritifera* even though the shells are somewhat similar. *Alasmidonta* and *Anodonta* are closely related genera of the same subfamily, and neither is closely related to *Lampsilis* or *Elliptio*. Numerous other objections to Modell's scheme could be raised. We have discussed our far fewer objections to the Heard & Guckert classification. Our classification is closest to Ortmann's (1910a) original arrangement, i.e. one family and the subfamilies Margaritaninae (= Margaritiferinae), Unioninae, Anodontinae, and Lampsilinae. Indeed, the combination of his North American Unioninae, Lampsilinae, and (1916) Gonideinae equals our Ambleminae. Moreover, his Lampsilinae and Gonideinae have exact cognates in our amblemine tribes Lampsilini and Gonideini.

Our classification thus differs from Ortmann's in three significant ways. First, his

Unioninae comprised diverging clades, i.e. our Amblemini and Pleurobemini. Ortmann lacked the biochemical tools necessary to reveal genetic relationships and parallel evolution that are crucial to our concepts Pleurobemini and Amblemini. Second, by raising what we consider tribes to subfamily rank he implied greater morphological and genetic divergence among groups than we think justifiable. Third, no previous author recognized that the Anodontinae are as distinct and separate a group as indeed they are. While the anodontine taxa have unique morphological character-states that set them apart, these do not appear to present as great a magnitude of difference in comparison with the comparable character-states of our Ambleminae as in comparison with those of the Margaritiferinae. In other words, the Anodontinae have advanced beyond the Margaritiferinae about as far as the Ambleminae have done, but divergently.

Given the evolving differentiation of the gills as efficient marsupial chambers, one could argue on the basis of morphology that our Amblemini and Pleurobemini are the most primitive of Nearctic non-margaritiferinae taxa; they lack the complex mantle structures of some of our Lampsilini, and many species have heavy shells, some resembling those of Margaritifera. The anodontine species could have been considered (and indeed, have been so considered by some authors) as derived from our Ambleminae with advanced specialization that included a reduction from heavy to light shell, reduction of hinge, and further marsupial development to yield the tripartite water tube. This definitely has not been the case.

Considering all classifications and in summary we can make several points. 1) Wherever a monothetic basis for classification has been used, the classification places closely related taxa into artificial groupings. 2) Ortmann's approach is superior because he used all data available. He was interested not simply in a utilitarian classification, but also in obtaining answers to why and how morphology and habits yielded the amazing unionid diversity in North America. 3) The Heard & Guckert (1971) study is of particular value because it purposefully set up a classification following a stated approach. They provided clear-cut concepts that are amenable to establishing hypothesis that can be tested. 4) Heard & Guckert were mistaken in "subjectively electing . . . to ignore one array of fea-

tures (i.e., conchological features)" and in overemphasizing such reproductive aspects as number of marsupial demibranchs and length of breeding season.

Relationships within the Ambleminae

We discuss at some length relationships among certain taxa within the Ambleminae because of our extensive immunological data and the availability of a certain amount of anatomical data.

1. *Gonidea*—The placement of this genus in a taxonomic hierarchy has continuously been a problem to malacologists. On the basis of the shell it would be considered a magaritiferine. Modell (1942, 1949, 1964) considered this to be the case and relegated *Gonidea* to his Pseudodontinae of the Margaritiferidae. Ortmann (1916) considered *Gonidea* a member of the Unionidae: Gonideinae. Heard & Guckert (1971) placed *Gonidea* in their Amblemidae because the genus is tetragenous. They preserved Ortmann's (1916) Gonideinae for it because of its perforated septa. Heard (1974) reported that *Megalonaias* has perforated septa and considered them characteristic of primitive tetragenous taxa, such as *Gonidea, Megalonaias,* and *Pseudodon.*

Gonidea is immunologically more closely related to our core Ambleminae (Fig. 3). *Gonidea* diverges away from the Amblemini and Pleurobemini and can in no way be considered a member of the Margaritiferinae. With the *Anodonta* group removed from Ortmann's equal ranking of Anodontinae, Gonideinae, Unioninae, and Lampsilinae, we see that *Gonidea* deserves equal rank with Ortmann's generic groupings around *Lampsilis* (our Lampsilini) and around *Elliptio* and *Quadrula* (his Unioninae, our Pleurobemini and Amblemini). Because *Gonidea* has vertical septa and a complete diaphragm, in contrast to the primitive margaritiferine groundplan, we consider its perforations a primitive condition that has been sustained in the Gonideini and, by parallel evolution, in *Megalonaias* of the Amblemini. It is possible that this west coast North American genus is most closely related to the tetragenous genera of Asia (Heard, 1974). Further investigations are necessary to assess such a suggested relationship. Should this proposed link to Asia be correct, it is probable that the Gonideini would deserve subfamiy ranking.

2. The *Elliptio-Fusconaia-Pleurobema* problem. Immunologically and morphologically there is little basis for taxonomically separating these genera. *Pleurobema* and *Elliptio* are ectobranchous, and *Fusconaia* is tetragenous. However, species assigned to these taxa do not sort into two or three immunologically separate clusters. Clarification of relationships within the Pleurobemini will depend on molecular genetic studies using taxa only in this group, plus more sets of Pleurobemini antisera. As seen in Tables 13 and 14, when the 12 traditional genera of Pleurobemini and Amblemini are compared by using eight characters, *Fusconaia* differs from *Elliptio* in three character-states and from *Pleurobema* in two. *Fusconaia* differs from both genera in having brightly colored tissues and in being tetragenous. *Elliptio* differs from both *Fusconaia* and *Pleurobema* in having simple, not dentritic, incurrent papillae.

We shall retain these genera until such a study has been completed. The genera are tentatively defined as follows: *Elliptio*, ectobranchous, simple incurrent papillae, shells more or less elongate with beaks placed well anterior and not prominent; *Pleurobema*, ectobranchous, dentritic incurrent papillae, shells subtriangular to rhomboid with beaks anterior or subanterior and prominent; and *Fusconaia*, tetragenous, dentritic incurrent papillae, shell much as in *Pleurobema*.

However these taxa are defined in the future, we note that so-called *Elliptio, Fusconaia,* and *Pleurobema* are very closely related genetically.

3. Linkages in the Lampsilini—The immunological data (Figs. 2, 3) show that 1) the closest relationship of the Anodontinae to the Ambleminae is via the genus *Lampsilis*, specifically *L. teres*, 2) *L. teres, Ptychobranchus subtentum,* and *L. radiata* form the core of related taxa from which other taxa fan out. 3) The Pleurobemini and Amblemini are closely related to each other and there does not appear to be an extensive divergence among genera of these tribes. 4) *Gonidea* ties into *L. teres* via *Carunculina parva.*

Because the relationships indicated by the MST represent genetic relationships among living taxa, and as new taxa are studied and added to the data matrix, one would expect shifts in relationships from those seen in Figs. 2 and 3. Accordingly, one should not consider the overall MST patern to represent evolutionary pathways. For example, *Lampsilis teres* did not evolve from *Lasmigona costata.* Also,

as additional anodontine taxa are studied, the linkage between the Anodontinae and Ambleminae might not be between *Lasmigona costata* and *Lampsilis teres.* Realizing that with additional data there will be shifts in associations of taxa along the MST, we can still say quite a lot about general relationships among groups of genera in the Ambleminae. It is clear that the amblemine clade is ancient and that the genus *Lampsilis,* of all taxa within this clade, is most closely related to the Anodontinae.

4. The *Amblema-Plectomerus-Megalonaias* complex—Immunological data indicate a close relationship among species of these three genera. *Megalonaias* and *Plectomerus* are especially closely allied within the same subset (Table 10). There is a remarkable piece of morphological evidence that corroborates the implied close genetic relationships of these three taxa. Arborescent incurrent papillae are characteristic of our Amblemini (*Quadrula* s.s. and *Quincuncina* have dentritic papillae); incurrent papillae of the simple type do occur, but *only* in the trio of genera in question. In short, these form a natural group within the Amblemini.

Plectomerus and *Megalonaias* are immunologically so closely related as to suggest congeneric status. There are data supporting and against congeneric status. The supporting data would include *Amblema* with them in a common genus because all three have large, strong, thick, heavy shells with plicate sculpture (three different character-states). In view of the above data, these taxa are allied on the basis of four distinct character-states (3 shell, 1 soft-part) additional to those serving to define the Lampsilini (in addition to the close immunological relationship).

Differences occur. *Megalonaias* possesses a somewhat unusual beak sculpture (i.e. it persists until after adult sculpture has begun and thus intermingles with it). *Megalonaias* exhibits perforate gill septa, at least in the gravid female (Heard, 1974). Both character-states are considered primitive. Unfortunately, *Plectomerus* has not been studied adequately in regard to these character-states. *Amblema* beak sculpture is separate from the disc of the adult shell; it has no known perforate septa.

Given the total evidence available, given the comparisons in Tables 13 and 14, and considering the morphological changes one expects to see in adaptive radiation (Davis, 1979), we consider it worthwhile to make a

TABLE 13. Comparison of 12 genera of the Lampsilinae: Pleurobemini and Amblemini by using eight characters and 21 character-states. [*Q. (Orthonymus) cylindrica* represents a discrete genus here because it differs so greatly from *Quadrula* s.s.]

	Amblema	Megalonaias	Plectomerus	Quadrula s.s.	Q. cylindrica	Cyclonaias	Tritogonia	Quincuncina	Elliptio	Fusconaia	Pleurobema	Uniomerus
1. Incurrent papillae simple (0) dendritic (1) arborescent (2)	0	0	0	1	2	2	2	1	0	1	1	1
2. Excurrent papillae weak or absent (0) well developed (1)	1	1	1	1	0	0	0	1	1	1	1	0
3. Tetragenous (0, 1)*	1	1	1	1	1	0	1	1	0	1	0	0
4. Supra-anal opening (0, 1)	1	1	1	1	1	0	1	1	1	1	1	1
5. Septa not perforated (0) weak, but imperforate (1) perforate (2)	0	2	0	0	0	0	0	0	0	0	0	1
6. Tissues brightly colored (0, 1)	0	0	0	0	1	0	0	0	0	1	0	0
7. Sculpture smooth (0) plicate (1) pustulate (2)	1	1/2**	1	0/2	2	2	2	2	0	0	0	0
8. Shell pustulate: in chevron pattern (1) random (2) 1 row large pustules (3) 2 rows pustules (4)	0	0	0	2/4	3	2	2	1	0	0	0	0

*given only (0, 1), 0—does not have, 1—has the character state.
**juvenile only.

TABLE 14. Comparison of genera given in Table 13 by the number of shared character states.

	Amblema	Megalonaias	Plectomerus	Quadrula s.s.	Q. cylindrica	Cyclonaias	Tritogonia	Quincuncina	Elliptio	Fusconaia	Pleurobema	Uniomerus
Amblema	8	7	8	5	3	2	4	5	6	5	5	3
Megalonaias		8	7	4	2	1	3	3	5	4	4	3
Plectomerus			8	5	3	2	4	5	6	5	5	3
Quadrula s.s.				8	3.5	3	5	6.5	4.5	5.5	5.5	3.5
Q. cylindrica					8	4	6	4	2	4	2	2
Cyclonaias						8	6	3	2	1	3	3
Tritogonia							8	5	3	3	3	3
Quincuncina								8	4	5	5	3
Elliptio									8	5	7	5
Fusconaia										8	6	4
Pleurobema											8	6
Uniomerus												8

hypothesis that these three taxa are congeneric and that the synonymy is:

Amblema Rafinesque, 1820
+ *Plectomerus* Conrad, 1853
+ *Megalonaias* Utterback, 1916

5. The *Cyclonaias-Quadrula-Tritogonia* complex—We studied at least five Amblemini taxa that have complex pustulate shell sculpture: *Quadrula* spp., *Q. (= Orthonymus) cylindrica*, *Cyclonaias tuberculata*, *Tritogonia verrucosa*, and *Quincuncina infucata*. Admittedly some species traditionally assigned to *Quadrula* have smooth or nearly smooth shells. However, as noted in the discussion of *Q. cylindrica* (below), the genus *Quadrula* has yet to be defined with precision. Also, subgenera such as *Q. (Bullata)* Frierson [represented by *Q. pustulosa* (Lea)] may or may not have validity.

Quadrula cylindrica differs from the other species of *Quadrula* we studied in 4.5 morphological character-states (shell and soft parts) (Tables 13, 14). *Q. cylindrica* has arborescent incurrent papillae such as occur in the Margaritiferinae and *Gonidea* of the Ambleminae; these papillae are considered to represent a primitive character-state. Excurrent papillae are absent (contrast *Quadrula* s.s. and *Quincuncina*); tissues are various shades of browns and blacks (contrast uncolored tissues of other genera studied here except *Fusconaia* and *Margaritifera*). Other differences involve shell sculpture. On the basis of molecular genetics (immunological distance coefficients), the closest relationships *are* with other species of *Quadrula* [*Q. pustulosa* (0.870); *Q.* cf. *quadrula* (Qbb, 0.902); *Q. apiculata* (1.04) and then *Plectomerus* (1.04)]. Because *Q. cylindrica* differs from *Quadrula* s.s. in three anatomical (soft part) character-states, we consider *Q. cylindrica* to typify a distinct genus, *Orthonymus*.

Cyclonaias, a monotypic genus, closely resembles *Quadrula* conchologically. Of the Amblemini genera, only *Cyclonaias, Orthonymus,* and *Tritogonia* have arborescent papillae and no (or poorly developed) excurrent papillae. *Cyclonaias* differs from all other Amblemini studied by us in being ectobranchous and having an entire excurrent aperture. The immunological distance coefficients among *C. tuberculata* and the five closest species are, in increasing order: *Plectomerus* (0.780); *Quadrula* cf. *quadrula* (Qbb, 0.826): *Megalonaias* (0.966); *Q. pustulosa* (1.04);

and *Q. apiculata* (1.05). No immediate genetic relationship to *Orthonymus* or *Tritogonia* is indicated. Because *Cyclonaias* differs from *Quadrula* in three morphological character-states and from *Amblema* (plus synonyms) in at least six character-states (Tables 13, 14), we shall maintain *Cyclonaias* as a discrete genus.

Tritogonia is maintained as a separate genus because it has arborescent papillae, not in *Quadrula* s.s., and a different shell sculpture (Table 13). Its closest immunological relationships are with *Quadrula pustulosa* (0.870) and *Orthonymus cylindrica* (0.219) and then with non-Amblemini, e.g. *Elliptio lanceolata* (1.257) and *Lampsilis teres* (1.263).

6. Lampsilini—The Lampsilini are unique among the Unionidae in that the marsupial water tubes extend beyond the distal margins of the demibranch lamellae; the marsupia show externally marked sulci, not the smooth pads as in the homogenous taxa (tetragenous or ectobranchous); and discrete areas of the outer demibranch are marsupial in the great majority of species.

It is reasonable to assume that in the evolution of the Lampsilini there were independent origins of some of the marsupial types and that some developed from others. For example, it is improbable that the mesogenous condition (Appendix 3) was modified to produce the heterogeneous condition, because the two are structurally different and occupy different parts of the outer demibranchs. Nevertheless, there is a progression from primitive to specialized character-states.

Presumably the most primitive state is longenous: the entire length of the demibranch is marsupial, and the distad distension of the water tubes is slight. This is not a very successful state; only two genera have been assigned to the Lampsilini: Longenae (a possible subtribal concept): *Friersonia* and *Cyrtonaias*. We have not studied these genera immunologically. Additional information about them occurs in Ortmann (1912b), Heard & Guckert (1971), and Fuller (1975).

A condition possibly derived from the longenous is the ptychogenous state. Here the marsupium extends the full length of the demibranch, but only the ventral portions of the water tubes are marsupial. Additional space for incubation of larvae is created by a distad distension of the water tubes that is greater than that in the Longenae: the tubes

are somewhat distended laterally and from front to rear, which causes a folded ("ptychogenous") condition such that the lower border of the demibranch is furbelowed. Only *Ptychobranchus*, the only genus of Ptychogenae, has this condition (Fig. 3).

The eschatigenous condition (in the lone genus of Eschatigenae, *Dromus*, not studied here) resembles the ptychogenous type in being limited to the ventral border of the demibranch, but is unique in consisting of a series of several discontiguous sacs formed by a distad distension of the marsupial water tubes that exceeds that in ptychogenous mussels.

The mesogenous condition (in the Mesogenae, *Obliquaria* and *Cyprogenia*, not studied here) involves great distad distension of several contiguous water tubes in the middle of the demibranch; the distensions exceed those in the eschatigenous marsupium, and in *Cyprogenia* they are so long that they must coil in order to remain within the mantle cavity and thus be protected by the shell.

The heterogenous condition is restriction of the marsupium to the posterior (or even the postbasal) portion of the demibranch. All other Lampsilini are Heterogenae.

Results of our immunological analysis of Heterogenae are represented in Fig. 3. The eventual addition of other taxa to the analysis doubtless will change this portrayal in some respects and will permit greater confidence in all the results of that time. At present, however, the picture has some features that are gratifyingly in keeping with morphological evidence; there are, also, some relationships that are mystifying. In the former category, there is, for example, the radiation of several *Lampsilis* and *Villosa* from *L. radiata*. This is not surprising, because of the similarity of the two genera and because this species is not an advanced member of the genus (the mantle flap is essentially ribbon-like, unlike the fully developed piscine type seen in *L. ovata*). Also of note is that *L. teres* is not a part of this radiation, because its postbasal mantle margin questionably forms a flap of the sort exemplified by *L. ovata,* the type-species of the genus *and* a member of the radiation from *L. r. radiata,* and because its beak sculpture, also, is atypical of the genus. A further interesting aspect of *L. teres* is its immunological alliance to *Ligumia recta* (Rafinesque), which it resembles morphologically so much that it long was the accepted practice to place *Ligumia* in *Lampsilis*. One concludes that in at least some cases conchological evidence is more meaningful than has been recognized in many years.

The opposite point is indicated in some other cases. For example, *Ligumia recta* and *L. nasuta* have been considered congeneric because of their similar shells, but they are not closely allied in our immunological analysis. We cannot be confident that we fully understand these two species' relationship. We feel even more uncertainty about our results concerning *Lampsilis hydiana*. This species seems morphologically to be related to (or even part of) the sprawling *Lampsilis r. radiata* complex, but immunolologically it not only is not part of the radiation centered in that subspecies, but also lies a great genetic distance from it. Entirely unexpected results, such as these, strongly suggest the need (and some directions) for further study.

These remarks about relationships within the Lampsilini serve to illustrate some of the apparent strengths and weaknesses of our analysis. The same point can be made about the indicated relationships between the Lampsilini and other tribes and subfamilies. We think it significant that *Ptychobranchus* (Ptychogenae) is both the genus of Lampsilini studied by us that has been considered most generalized by some (e.g., Ortmann, 1912b) and the one that serves as the immunological connector to the Ambleminae: Pleurobemini and (though *Lampsilis teres*) to the Anodontinae. Similarly, the pathway between the Lampsilini and the Ambleminae: Amblemini lies through *Glebula,* a monotypic, generalized genus in the Heterogenae. We by no means anticipate that these details would remain unmodified in the event of an analysis of a larger number and variety of taxa, but we find it intuitively satisfying that rather unspecialized animals are the connections of the present scheme.

Adaptive radiation and success

Changes from primitive to derived character-states presumably indicate entrances into new adaptive zones and the establishment of new groundplans that engendered adaptive radiation. In considering the success of groups with various groundplans, we are concerned with 1) the extent of a given adaptive radiation, i.e. the number of species radiating with a given morphological groundplan; 2) the geographic range and abundance of these species, and 3) the competitive ability of the

species that enables coexistence with other, sympatric unionid species.

As discussed previously, the critical factors for unionid success appear to involve aspects of reproduction and respiration that depend on hydrodynamic efficiency; the critical factor for increased hydrodynamic efficiency is the bivalve diaphragm. The diaphragm is a collection of tissue that more or less separates the incurrent and excurrent portions of the mantle cavity. The unionid diaphragm is incomplete, i.e. the separation of incurrent and excurrent mantle cavities is imperfect and a tightly closed hydrodynamic system is thus impossible. In the Margaritiferinae this difficulty is exacerbated because the gill extends posteriad far short of the posterior mantle margin and only the gill bars effect separation of the two cavities. As a result, there is leakage between them, which must cause a physiological disadvantage, but also correlates well with other primitive aspects of margaritiferine morphology, namely, the large foot and gaping valves.

The latter two features obviously are related, and they serve further to weaken the margaritiferine hydrodynamic system. On the other hand, the large foot helps in negotiating the gravels and rock interstices favored by margaritiferines. The apapillose character-state of the excurrent posterior mantle aperture is considered by us to be the primitive condition and perhaps correlates with a weak pumping system because papillae would impede exit of waste particles and larvae expelled by the weak excurrent stream.

By comparison to other unionid groups the Margaritiferinae are not very successful. They are holarctic with representation in southeast Asia. They have a fossil record extending from the upper Cretaceous (Haas, 1969b). However, they are few species (five or six), which apparently belong to only one genus. Most of the species are restricted to cool, highly oxygenated water and gravel or rocky substrate. The species are most frequently found in soft-water upland streams without other species of unionids.

The Anodontinae have some unique character-states. These complement any decision, based on whatever kind of evidence, that *Anodonta* and its kin are a distinct unionid group. The subfamily is holarctic in distribution, as is the Margaritiferinae The widespread distribution patterns suggest that the two subfamilies predate some groups of the Ambleminae that are entirely restricted to

North America. The Anodontinae have had a far greater success than have the Margaritiferinae. The genus *Anodonta* is represented by several nominal subgenera (including, no doubt, at least some legitimate biological entities). The Nearctic is the area of greatest anodontine survival and speciation, as apparently is the case for the Margaritiferinae. The Anodontinae may have been successful elsewhere, as well, as is suggested by the great similarity of *Alasmidonta arcula* (Lea) of the Altamaha River, George, U.S.A., to *Unio languilati* of China (see Johnson, 1970, and Heude, 1875).

The Anodontinae are similar (yet hardly identical) to the Margaritiferinae, but vastly different from other Nearctic Unionidae, in exhibiting almost no hitherto discernible generic differences of soft-tissue anatomy. Soft-tissue diversification has been the key to success of the Ambleminae, even though there have been some correlative conchological adjustments. However, evolution among the Anodontinae appears to have involved essentially only the shell. Accordingly, in North America (where genera that are morphologically anodontine are numerous) there exists a conchological range from the heavily hinged and completely dentate *Lasmigona complanata* through the paper-thin and edentulous *Anodonta imbecillis*. The genus *Alasmidonta* (perhaps including *Unio languilati*) and its complex of at least five nominal subgenera represent an intermediate step in this evolutionary progression. The conchological characters of this genus include pseudocardinal dentition and more or less well developed lateral teeth. Our point is that this group includes character variation that probably is too great to justify inclusion in a single genus. For example, one such species, *Alasmidonta (Prolasmidonta) heterodon* (Lea) recently had its subgenus (of which the species is Ortmann's (1914) monotype) raised to generic level (Fuller, 1977). The correct systematic placement of this species is most uncertain, but it remains symbolic of the difficulties in classifying morphologically equivocal animals whose genetic affinities have not been immunologically well established.

The conchological diversity and the soft-tissue conservatism of the Anodontinae have been reviewed. This peculiar combination of trends in characters probably justifies our suspicion that this subfamiy's morphological features are mainly unique and war-

rant unusual taxonomic treatment. However, this standpoint does not exhaust the roster of anodontine peculiarities.

The modern Anodontinae are more species-rich and morphologically diversified than the modern Margaritiferinae, but this serves to dramatize the apparent pattern of anodontine differential extinction—or lack of initial success. Several of the Nearctic anodontine genera are monotypic, and the list probaby will increase as a result of further research because some of the other genera have numerous monotypic nominal subgenera, some of which probaby deserve generic rank. Only *Anodonta* itself has speciated with much success, and only this genus exhibits wide ecological and geographical ranges. Anodontine failure strengthens the supposition of the subfamily's antiquity and early derivation from other Unionidae.

Subtribal groups of Ambleminae: Lampsilini that are based on the longenous, ptychobranchous, eschatigenous, and mesogenous marsupial types include only six genera, of which *Obliquaria* and *Dromus* are monotypic. *Cyprogenia, Friersonia,* and *Cyrtonaias* presently include at most two species each. These marsupial conditions and corresponding subtribes are not correlated with success as measured by large radiations of species or numerous genera. *Cyrtonaias tampicoensis* (Lea) and the monotype *Obliquaria reflexa* Rafinesque are successful in the Gulf of Mexico drainage of Texas and Mexico and in the Gulf drainage and the Mississippi River basin, respectively, but the other species of these groups probably never have had geographically successful ranges. More specifically, several of these species have been restricted to the Cumberlandian and/or Ozarkian faunas (see van der Schalie & van der Schalie, 1950). *Dromus* and *Cyprogenia* are limited to one or both biogeographical provinces. It probably is significant that the Longenae are geographically separated from the others of these unsuccessful groups.

The Heterogenae are successful. Their success correlates with the marsupial restriction to the posterior section of the demibranch (see p. 242). One quarter of the naiad species recognized as having invaded or reinvaded the Canadian interior basin since the most recent (Wisconsin) glaciation are heterogeneous Lampsilini (Clarke, 1973). As another example, the *Lampsilis radiata* complex probably is the geographically most widely ranging group of Nearctic naiades. In order to accomplish its geographic range, the complex must have wide ecological tolerances, as well.

The Heterogenae include most of the genera of Lampsilini. However, even within this, the most specialized and by far the most successful Lampsilini group, there are different degrees of morphological development and of success. There is a morphological gradient corresponding to the joint theme of greatly reducing the amount of outer demibranch that is marsupial and of locating the marsupium at the posterior end of the demibranch.

The more specialized heterogeneous genera have a swollen reniform marsupium (when charged) in the postbasal corner of the outer demibranch. The nearby postbasal mantle margin is modified in various ways that serve as attractants for piscine hosts of unionid larvae. For example, the posbasal margins of *Lampsilis* are piscine in character; the implication is that predatory (or merely grazing) fish species will attack the "prey" represented by the mussel's mantle margins and will be showered with glochidia *if,* as is often true in the case of heterogenous genera, discharge of parasitic larvae is through the marsupial wall and not through the excurrent mantle aperture.

These morphological adjustments have been accompanied by ethological adaptations, as well. For example, the female of some (perhaps all) *Lampsilis* is able to orient herself so that her marsupium's proximity to the host fish is optimized and the movement of her posbasal mantle margins (piscine flaps) are capable of attracting a host.

Whether or not all Lampsilini: Heterogenae can coordinate with potential hosts is not known, but complementary structural and behavioral strategies are clear. The incorporation of behavioral factors into the reproductive process not only probably is the key to the success of the Heterogenae, but also provides a key to classification of the group. The fact that the postbasal portion of the outer demibranch is marsupial defines this group, but there are other variables that are of use in classification, e.g. pigmentation, size and shape of the egg mass, and lamellar coverage of the egg mass.

An example of a problem in a classification that uses such characters is *Unio ochraceus* Say. This species was long classified as a *Lampsilis,* which it clearly is not, because it has no mantle flaps, as recognized by Morrison (1975), who considered this species a

Leptodea. Bereza & Fuller (1975) pointed out that the number and structure of the egg masses of this species are not similar to those of *Leptodea.* No one has yet proposed a generic name for this species.

As a group, however, the Heterogenae are character-poor. This not only has created taxonomic problems, but also makes tracing the group's radiation very difficult on morphological grounds alone. Nevertheless, immunological evidence is somewhat compensating.

Zoogeography through time

The Unionacea are known with some degree of authority from the Triassic (review by Walker, 1910; Modell, 1942; Haas, 1969b). They are perhaps known from the upper Devonian (Smith, 1977). The Unionacea were widely spread in Pangaea; the presumably primitive family of Hyriidae of Australasia and western South America remained in Gondwanaland continents; the Unionidae, essentially in Laurasian continents. African Unionidae are either due to an original Gondwanaland stock or derived from a later invasion from Eurasia (see Heard, 1974).

The greatest diversity of naiades today is found in the Atlantic drainages of the Old and New Worlds. The implication is that the area of initial radiation of the ancestors of modern naiades lay in that portion of Pangaea where the Atlantic rift began in the Mesozoic. Whether or not the Unionacea (a nearly global group) and the Mutelacea (a Gondwanaland element) have a derivative relationship is unresolved, though not at issue here.

The essential identity of soft-tissue plan in all Unionacea suggests that a common stock existed in Pangaea. The acquisition of four or five derived morphological character-states separating the more specialized non-margaritiferine Unionidae from the Margaritiferinae must have occurred before the breakup of Pangaea, as is evidenced by the modern distribution of Margaritiferinae, Anodontinae, and African and Asian taxa related to North American Ambleminae.

The *Margaritifera* group is of Laurasian origin and has a modern relict distribution in Laos and the Holarctic. It is inconceivable that this ephemeral, at present largely unsuccessful group, confined essentially to uplands, could have achieved its present distribution entirely by post-Pangaean land bridges. Walker (1910) argued persuasively that *Margaritifera* evolved in Asia and reached western North America via the Bering land bridge in the Miocene or early Pliocene, and that *Margaritifera* reached eastern North America via the Greenland bridge. Pangaean-Asian origin of *Margaritifera* subsequently affected by plate tectonics and dispersal over Pliocene to Pleistocene land bridges was endorsed by Smith (1977).

The Anodontinae, also, are Holarctic and confined to the northern hemisphere. Only *Anodonta* is known with certainty to be represented in Eurasia. There is a pronounced conchological similarity of certain Nearctic *Alasmidonta* to at least one species of eastern Asia, which accordingly is considered anodontine. The geographic distribution of Anodontinae is in Europe, Asia (Oriental zoogeographic province), and North America; this indicates a widespread Laurasian distribution.

The modern proliferation of anodontine genera is in North America. Only *Anodonta* is widespread, commonly encountered, species-rich, and biologically adaptable. There are at least three, chronologically differing interpretations of the occurrence of Eurasian *Anodonta* (or very similar forms): 1) The anodontine radiation, including *Anodonta,* was complete prior to the breakup of Laurasia; 2) the greatest anodontine cladogenesis occurred in North America, but *Anodonta* was widespread in Laurasia before North America separated from the pangaean supercontinent; 3) *Anodonta* spread to Asia from eastern North America prior to the rise of the Rocky Mountains and subsidence of the Bering land bridge.

Walker (1910) considered Nearctic *Anodonta* of the Pacific drainage to be of Asiatic origin. Heard (1974) considered primitive progenitors of modern Anodontinae (e.g., *Strophitus*) to have originated in Asia and spread via the Bering land bridge into North America. Following Walker, it is highly probable that *Anodonta,* as ancient as *Margaritifera,* had its origin in the same pangaean region as *Margaritifera,* and dispersed via the same general routes.

Some taxa in our Ambleminae questionably originated in the Triassic age and certainly existed in the Cretaceous. *Margaritifera* and the Anodontinae are known with certainty from the Cretaceous. Simpson (1896) noted a "remarkable similarity" between the unionid faunas of North America and southeastern Asia, plus the Tertiary faunas of both Europe

and Asia. Walker (1910) stated that there is "no doubt but that the characteristic (unionid) fauna of North America is descended from the Upper Cretaceous species, which then lived" in certain western U.S.A. states, as is evidenced by the fossil record. Walker (1910) noted the strong resemblance of Oriental Unionidae and those of North American Cretaceous to early Tertiary North American fossil unionids. Given the evidence, one reasonably assumes that the breakup of Pangaea did isolate a segment of early unionid stock in North America and that these isolates gave rise to most of the current endemic North American fauna. Only much later did some Asian stock reach western North America via the Bering bridge or eastern North America from Europe.

Members of the Ambleminae: Pleurobemini and Gonideini have morphological affinities to certain African and Eurasian taxa. These are reviewed by Heard & Guckert (1971) and Heard (1974); a few will be mentioned here. *Brazzaea anceyi* Bourguignat, of Africa, was grouped in the Gonideinae (Heard & Guckert, 1971) because it had been reported (Bloomer, 1931a) to be tetragenous, with distinct supra-anal opening, and with continuous, but perforated septa. *Lamellidens marginalis* (Lamarck) from India is ectobranchous, yet with perforated septa (Bloomer, 1931b). Because tetragenous or ectobranchous taxa may occur in any tribe, we provisionally place this taxon in the Gonideini. Heard & Guckert listed several Southeast Asian taxa with perforated septa that they considered Amblemidae and we provisionally consider as Gonideini.

The tribe Lampsilini is uniquely North American and, with the possible exception of a morphologically somewhat Lampsilini element in the Pacific drainage (Dwight Taylor, personal communication), is entirely confined to the Atlantic drainages. It is probable that the Lampsilini radiation occurred only since the complete independence of North America. Of the five morphologically defined sub-Lampsilini groupings of taxa that have been proposed, four are comparative failures, but the fifth, the Heterogenae, dominates the entire Atlantic drainage faunas in terms of numbers of genera and species and in terms of ecological success. This great success is attributed to the sum of morphological character-states that are unique to the Lampsilini in general and to the Heterogenae in particular.
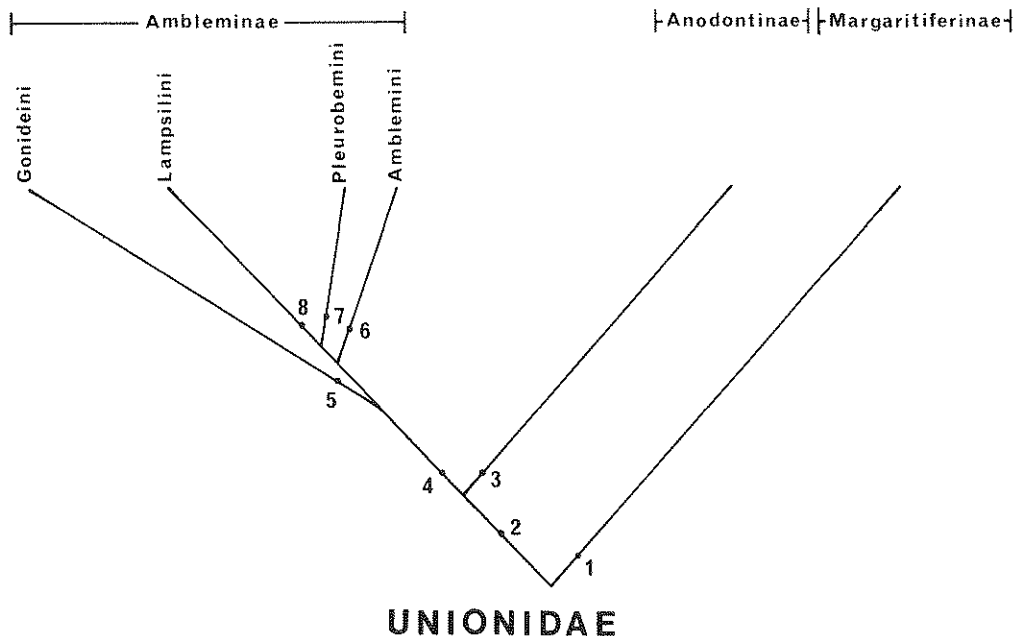

FIG. 4. Cladogram portraying relationships among unionid taxa. Numbered points are discussed in the text, p. 247.

Cladistic relationships

A cladogram (Fig. 4) was constructed on the basis of our immunological results, morphology, the fossil record, and zoogeography. As implied by the cladogram, there was divergence within proto-unionid stock before Gondwanaland split up in the late Mesozoic. The proto-unionid stock would have had the generalized, *Margaritifera*-type anatomy and would have been tetragenous. Divergence gave rise to proto-*Margaritifera* (point 1, Fig. 4) and to a lineage that gained some morphological advances, i.e. development of septa and water tubes parallel to the gill filaments, creation of the diaphragm and supra-anal aperture. The septa probably were perforated (point 2, Fig. 4).

The unionids with these derived morphological character-states diverged before Gondwanaland split up and yielded yet again two lineages. One of these, the proto-Anodontinae (point 3, Fig. 4), developed tripartite water tubes, became ectobranchous, and developed hooks on the glochidia. One eventual taxon (*Strophitus*) retained perforated septa.

The other lineage (point 4, Fig. 4) remained tetragenous and had undivided water tubes with hookless glochidia and perforated septa. This lineage diverged, yielding proto-Gonideini (point 5, Fig. 4) prior to the breakup of Pangaea. This clade is primarily tetragenous and has perforated septa.

There was, also, rapid divergence that formed the lineages of the 1) proto-Amblemini (point 6, Fig. 4), where the taxa are primarily tetragenous, one species group has perforated septa, and several species have arborescent incurrent papillae, as in the Margaritiferinae and the lineage of the 2) Pleurobemini (point 7, Fig. 4), where the taxa primarily are ectobranchous, without perforated septa, and without arborescent papillae.

Last, the proto-Lampsilini (point 8, Fig. 4) evolved; they are uniquely North American, totally ectobranchous, and with the most specialized character-states of marsupial development and mantle modifications.

The cladogram is consistent with the ordination diagrams based on immunological data given in Figs. 2, 3.

ACKNOWLEDGEMENTS

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APPENDIX 1. A list of some important unionacean classifications. Note the profound influence of Ortmann's work on most subsequent systems.

Simpson (1900, 1914)	Ortmann (1910a, 1911, 1916)	Hannibal (1912)

Unionidae
  Unioninae
    Heterogenae
    Digenae
    Mesogenae
    Ptychogenae
    Eschatigenae
    Diagenae
    Homogenae
    Tetragenae
	Margaritiferidae
	Unionidae
	  Gonideinae
	  Unioninae
	  Anodontinae
	  Lampsilinae
		Margaritiferidae
		Unionidae
		  Unioninae
		  Anodontinae
		Lampsilidae
		  Lampsilinae
		  Propterinae
		Quadrulidae
		  Quadrulinae
		  Pleurobeminae

Frierson (1927)	**Modell (1942, 1949, 1964)**	**Morrison (1955)**

Unionidae
  Margaritiferinae
  Unioninae
  Alasmidontinae
  Anodontinae
  Lampsilinae
	Margaritiferidae
	  Margaritiferinae
	Unionidae
	  Quadrulinae
	  Rectidentinae
	  Anodontinae
	Elliptionidae
	  Pleurobeminae
	  Elliptioninae
	  Alasmidontinae
	  Ambleminae
	  Lampsilinae
		Margaritiferidae
		Unionidae
		  Unioninae
		  Alasmidontinae
		  Anodontinae
		Amblemidae
		  Ambleminae
		  Lampsilinae

APPENDIX 1. (Continued)

Haas (1969a,b)	Heard & Guckert (1971)	Clarke (1973)
Margaritiferidae	Margaritiferidae	Margaritiferidae
Unionidae	Margaritiferinae	Unionidae
Unioninae	Cumberlandiinae	Ambleminae
Quadrulinae	Unionidae	Anodontinae
Alasmidontinae	Unioninae	Alasmidontini
Anodontinae	Pleurobeminae	Anodontini
Lampsilinae	Popenaiadinae	Lampsilinae
	Anodontinae	
	Lampsilinae	
	Amblemidae	
	Gonideinae	
	Ambleminae	
	Megalonaiadinae	

Davis et al. (1978)	Davis & Fuller (this study)
Unionidae	Unionidae
Margaritiferinae	Margaritiferinae
Anodontinae	Anodontinae
Lampsilinae	Ambleminae
Lampsilini	Lampsilini
Gonideini	Gonideini
Elliptionini	Pleurobemini
Amblemini	Amblemini

APPENDIX 2. Annotations.

1. *Fusconaia ebena* (Lea)

Shells of the species that we call *F. ebena* conform to the type-concept of *F. ebena*. We cannot, at this time, explain the close relationship of this species to *Lampsilis* (Fig. 3). We must obtain fresh *F. ebena*, examine the anatomy, and retest the relationship. We suspect either experimental error in this case or a species with Lampsilini anatomy within a *Fusconaia*-appearing shell.

2. *Uniomerus*

In the first analysis of taxa here relegated to the Ambleminae (matrix of 15 antisera × 41 sets of antigens), *Uniomerus tetralasmus* was linked by the minimum spanning tree to *Quincuncina infucata* (see annotation no. 3). Because we were missing 46.6% of the data for *Q. infucata* in the matrix of cross comparisons, we reran the analysis without the data for *Q. infucata*. We had anti-*Q. infucata* antisera and data for all but three comparisons

(no data for *Leptodea fragilis, Villosa delumbis, Elliptio buckleyi*).

In the reanalysis (15 × 40 matrix), *U. tetralasmus* was, on examination of the matrix of distance coefficients, most clearly related to 1) *Elliptio buckleyi* (.749), 2) *E. complanata* (.893), and 3) *Fusconaia flava* (.982). On this basis *Uniomerus* is classified as a genus of the tribe Pleurobemini.

3. *Quincuncina*

We did not have data for 7 of the 15 comparisons of the marix of 15 antisera × 41 sets of antigens (OTUs). Given this lack of data, the closest relationships seen in the matrix of taxonomic distances were: *Elliptio crassidens* (.726), *Leptodea fragilis* (.786), *Elliptio lanceolata* (.891), *Tritogonia verrucosa* (.895), *Quadrula apiculata* (.896). The minimum spanning tree showed connections of *L. fragilis, E. crassidens, U. tetralasmus,* and *Q. apiculata* to all other taxa in the Amblemini and through *L. fragilis* to all other taxa in the Lampsilini.

The net result indicates that *Quincuncina*

should be provisionally placed in the Amblemini. Verification of this placement is dependent on filling in the missing data permitting a more precise analysis of relationships. The placement in the Amblemini agrees with the grouping of genera in the Ambleminae sensu Heard & Guckert (1971) if one excludes *Fusconaia* (we have no data for *Elliptoideus*) but includes *Megalonaias* which does not deserve separate ranking in a subfamily (Megalonaiadinae).

4. Hooked glochidia

There are two types of hooked glochidia. The large single pair of hooks at the periphery of the glochidial shells of Anodontinae are not homologous with the two pairs of hooks, one pair at each side of the glochidial shells of *Proptera*. The hook morphology is quite different in the two taxa.

5. Glochidium size

We used a glochidial index (Gln) for size, where the Gln = the height of the glochidium (Hmm) × the length of the glochidium (Lmm). The glochidium of the Margaritiferinae is small (Ortmann, 1911; Baker, 1928). "Small" is defined as Gln = < .0036. The glochidia of the Anodontinae are "large": the average Gln = about 0.1000. The range is from 0.078 in *Alasmidonta* to 0.1225 in *Anodonta corpulenta*. Most species have a Gln > 0.0900. The glochidia of the Ambleminae are "medium" sized where the average Gln = about 0.047. The smallest was that of *Quadrula quadrula* (Gln = 0.007; note that Gln of *Q. pustulosa* was 0.0736); the largest was that of *Cyclonaias tuberculata* (Gln = 0.0867). Most had a Gln between 0.02 and 0.06 (16 of 24 = 66.7%). None was as small as seen in the Margaritiferinae; only two species (8%) had a glochidium size as large as the smallest glochidium size of the Anodontinae (*Cyclonaias tuberculata* and *Megalonaias gigantea* of our Amblemini).

6. Change in nomenclature

Lampsilinae was changed to Ambleminae; Elliptionini was changed to Pleurobemini for reasons of nomenclatural priority (see Heard & Guckert, 1971, and Haas, 1969a,b).

APPENDIX 3. Glossary of terms. In the following definitions the noun is followed by its adjective in parentheses.

Bradytixis (bradytictic): long term breeder; retains larvae in demibranchs except in Nearctic summer.

Diagenae (diagenous): ectobranchous group whose ovisacs are transverse to the demibranchs (only in *Strophitus* of Anodontinae).

Digenae (digenous): ectobranchous; two outer demibranchs are marsupial.

Ectobranch (ectobranchous): digenous; defined above.

Eschatigenae (eschatigenous): sub-tribal taxon of Lampsilini where the lower part of the posterior region of the demibranch is marsupial. Demibranch not folded; eschatigenous state.

Heterogenae (heterogenous): subtribal taxon of Lampsilini where the posterior section of the demibranch is marsupial; heterogenous state.

Homogenae (homogenous): entire outer demibranch loads with glochidia forming a smooth pad; Anodontinae; Ambleminae, Gonideini, Pleurobemini, Amblemini, and Lampsilini: Longenae (in part).

Longenae (longenous): subtribal taxon of Lampsilini where the lower region of the demibranch is marsupial; longenous state.

Mesogenae (mesogenous): sub-tribal taxon of the Lampsilini where the middle section of the demibranch is marsupial.

Ptychogenae (ptychogenous): sub-tribal taxon of the Lampsilini where the lower part of outer demibranch is marsupial and folded.

Tachytixis (tachytictic): short-term breeder; retains larvae in demibranchs only in Nearctic summer.

Tetragenae (tetragenous): four demibranchs are marsupial and homogenous.

APPENDIX 4. Historical account of unionid classification.

One should consult Heard & Guckert (1971) for additional historical information.

Lea (1858, 1863), although using an erroneous and simplistic classification of his own devising, nevertheless wrote and illustrated many soft-tissue descriptions and thus was

the first to develop this category of data. Had Lea not overlooked the possibility that his observations could be applied to a revolutionary new type of classification, he might have become *the* important figure in the history of naiad systematics; instead, that mantle eventually fell to Simpson and ultimately to Ortmann. Sterki (1898, 1903) partly succeeded where Lea had missed his opportunity; he recognized that soft-tissue characters could be important in unionid classification, but he did not exploit this realization, perhaps because of his greater interest in the Sphaeriidae. He indicated that unionids should be classified on the basis of characters involving reproductive structures such as the marsupial demibranchs, the specialized marsupial areas of some demibranchs, the glochidial morphology, and duration of breeding season.

Simpson (1900, 1914) published not only the first comprehensive account of global naiad systematics, but also the first naiad classification that purposely incorporated soft-tissue data. Moreover, his classification arranged taxa according to marsupial characters, thus preparing the way for more sophisticated work by Ortmann. Finally, Simpson's work is especially important for our study because so many of his observations (some of them unique and no longer replicable because of extinction) concern Nearctic unionids. Simpson's works not only were prodigious, but also marked the turning point in the history of studying freshwater mussels. They pointed the way from totally inadequate 19th century conchological schemes towards Ortmann's future classifications.

Simpson's classification involved a single family and subfamily (Unionidae: Unioninae for Nearctic naiades), plus numerous further subdivisions, of the same rank, which today can be construed as tribes. The great weakness of the classification is that it is primarily monothetic, based on where the gills are loaded with glochidia in gravid females, and that his goal was a utilitarian classification. For example, Simpson (1900, 1914) was aware of the essential morphological peculiarities of the Margaritiferinae, but classified them in his tribe "Homogenae" with all other naiades of his acquaintance that exhibit a marsupium occupying the entirety of the outer demibranch.

Ortmann (1910a) was the first to ask fundamental questions about how the organisms related to themselves and to their environ-

ments. He was the first to make a synthesis of all data available while questioning how morphological structure related to function. He integrated data from shell, soft tissues, behavior, and environments. His result (1910a) was an original classification of one family and three subfamilies (Unionidae: Margaritiferinae [= Margaritaninae in those days], Unioninae, Lampsilinae). Subsequently, Ortmann (1911) raised his "Margaritaninae" to family rank and (1916) created another unionid subfamiy, Gonideinae, for *Gonidea angulata* (Lea) of the Pacific drainage of North America. These were Ortmann's last (and only) changes of family-group taxa in comparison to his (1910a) original scheme.

Ortmann correctly interpreted the unique morphological character-states that set apart the higher taxa that include the groups of 1) *Margaritifera,* 2) *Anodonta,* 3) *Lampsilis,* 4) *Gonidea.* His grouping in the Unioninae (our Pleurobemini and Amblemini) included taxa with four as well as two marsupial demibranchs. The marsupium is not confined to restricted region of the gills as in his Lampsilinae, and taxa do not have unique mantle structures below the branchial openings as in many Lampsilinae. It is with Ortmann's Unioninae that we find, as did Heard & Guckert (1971), need for re-evaluation.

Most subsequent classifications involve alternate interpretations of the groups of *Lampsilis* and *Anodonta.* Hannibal (1912) recognized four families of Nearctic naiades (Appendix 1). His Unionidae is a partial subscription to Simpson's Homogenae; the marsupia in his subfamilies Unioninae and Anodontinae are homogeneous. His Unioninae comprise taxa in our Ambleminae: Pleurobemini (partim); his Anodontinae essentially are Ortmann's and ours. His Lampsilidae are our Ambleminae: Lampsilini. He created a subfamily for *Proptera,* presumably because of that genus' "ax-head" shaped glochidium. His Quadrulidae equals our Amblemini (partim) and Pleurobemini (partim). His Quadrulinae probably equals our Amblemini; his Pleurobeminae, our Pleurobemini (partim).

In summary of Hannibal's contribution, he anticipated our division of Ortmann's Unioninae into district groups of Pleurobemini and Amblemini. Overall, however, his system is one of gross taxonomic inflation. For example, there is no justification for a higher category based on *Proptera* (Fig. 3).

Frierson (1927) divided the Nearctic

Unionidae into five subfamilies. Only two items of his arrangement differ significantly from ours. His Unioninae is that of Ortmann and depends on the Eurasian concept of *Unio*. As Heard & Guckert (1971) have shown, this concept does not adequately accommodate the relevant New World naiades, which we interpret as the tribes Amblemini and Pleurobemini. Our second objection to Frierson's arrangement is his Alasmidontinae. We consider the relevant genera as evolutionary stages within a single subfamiy Anodontinae (Fig. 2).

Modell (1942, 1949, 1964) is an atavism to 19th century conchology. He created a highly controversial scheme that is monothetic, i.e. based almost solely upon a single discriminant, beak sculpture. Heard & Guckert (1971) have fully discussed the artificiality of the Modell classification. Remarkably, our Anodontinae, which we seemingly rightly regard as an integrated group both morphologically and immunologically are distributed by Modell between two families and subfamilies, the Unionidae: Anodontinae and the Elliptionidae: Alasmidontinae. We reject Modell's classification.

Morrison's (1955) classification is primarily based on the monothetic notion that the nature of the glochidial shell is the key to naiad classification. He opted for a three-family arrangement (Appendix 1). The Unionidae are taxa with hooked glochidia and divided into three subfamilies: Alasmidontinae, Anodontinae, Unioninae. Morrison's Amblemidae (our tribes Amblemini, Pleurobemini, Lampsilini) are equal to our Amblemidae minus *Gonidea*; his Amblemini are, excepting the European *Unio*, equal to Ortmann's Unioninae.

Morrison's classification is rejected because it is taxonomically inflated, separates morphologically and immunologically allied groups, exhibits the problems of a classification based on monothetic concepts, and, as in many of his published ideas about naiades, is supported by little or no evidence. His work is, however, laudable because very often he employed ecological information in framing his ideas.

Haas (1969a) and Clarke (1973) published classifications that are essentially rearrangements of Morrison's (1955).