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J. Moll. Stud. (2000), **66**, 157–170

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THE EVOLUTION OF BROODING CHARACTERS AMONG THE FRESHWATER PEARLY MUSSELS (BIVALVIA: UNIONOIDEA) OF NORTH AMERICA

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(Received 13 January 1999, accepted 16 July 1999)

ABSTRACT

Brooding characters have figured prominently in the classification of North American freshwater pearly mussels (Bivalvia: Unionoidea). The purpose of our study was to evaluate phylogenetic hypotheses of brooding character evolution in order to test homology statements suggested by earlier taxonomic systems of the Unionoidea. Parsimony analysis of partial COI sequences from 29 species of freshwater mussels and 13 outgroups were used to derive a phylogeny. Thirteen brooding characters (*e.g.*, brooding period, marsupium arrangement, structure of interlamellar septa, *etc.*) were traced onto this phylogeny. Results indicate that long-term brooding (bradytictia) is the derived state among North American freshwater mussels; short-term brooding (tachytictia) is plesiomorphic. Bradytictia evolved independently in the Anodontinae and Lampsilini, with unique morphological modifications derived in those clades to facilitate long-term brooding.

INTRODUCTION

The extraordinary life cycle of the Unionoidea (Bivalvia: Paleoheterodonta: Unionoida) has been well-studied (*e.g.*, Coker, Shira, Clark & Howard, 1921; Kat, 1984; Graf, 1998), and much has been made of the systematic value of variation in both the mechanics of their life history and morphology of their various semaphoronts (Simpson, 1900, 1914; Ortmann, 1911a, 1912b; Parodiz & Bonetto, 1963; Haas, 1969, 1969B; Heard & Guckert, 1971; Davis & Fuller, 1981; Lydeard, Mulvey & Davis, 1996; Roe & Lydeard, 1998; Graf, 2000). The characters associated with parental care (and reproduction, in general) have been widely employed to define taxa within the Unionoidea. Especially important in past classifications of the more than 290 species of North American freshwater mussels are brooding period (*i.e.*, the length of time embryos and larvae are brooded) and arrangement of the marsupium within the females' demibranchs. Our objective was to test hypotheses of brooding character evolution in order to evaluate their effectiveness in recovering phylogeny. Do similarities in brooding characters among North American freshwater mussel taxa represent homology or homoplasy?

Two general patterns of brooding have long been recognized among the Unionoidea of North America: short-term and long-term (Sterki, 1895, 1898; Ortmann, 1909; reviewed in Graf, 1997 and Heard, 1998). Ortmann (1911b) coined the terms **tachytictic** and **bradytictic** for each of these brooding types, respectively. Tachytictic (short-term brooding) mussels spawn their gametes in the spring, with embryos and larvae brooded in the females' marsupial demibranchs only until they have fully developed into parasitic larvae, the **glochidia**. The larvae are then released to the water to infect their host fish and complete their metamorphosis. The whole sequence of events is generally completed over the course of the late spring and summer, with certain exceptions (see below). Bradytictic (long-term brooding) mussels, in contrast, spawn in the late summer, brood their glochidia over the winter, and release them in the early spring. The fundamental distinction is that bradytictic mussels continue to brood their larvae long after they are infectious (Coker *et al.*, 1921; Kat, 1984). Variation in the brooding patterns of North American mussels has been attributed to climate, especially ice ages (Sterki, 1903; Ortmann, 1909; Graf, 1997), as well as to synchronize with seasonal host activity (Zale & Neves, 1982).

There is also significant variation in morphological characters associated with parental care. In the Unionoidea, as with the freshwater Sphaeriidae and Corbiculidae (both Bivalvia: Heterodonta), larvae are brooded within the

interlamellar spaces of the ctenidia (McMahon, 1991). The portion of the female's ctenidia that serve as brood spaces, the **marsupium**, varies from only a limited portion of the outer demibranchs, to the entire outer pair, to all four demibranchs (Ortmann, 1911b, 1912b). There are also fine structural differences in the development of interlamellar connections among and within the different marsupial arrangements (Ortmann, 1911b; Heard & Vail, 1976).

Early on, malacologists recognized the correlation between brooding period and morphology, and they felt that anatomical specializations associated with long-term ovovivipary of larvae were of special systematic significance (Table 1).

> 'Having correlated physiological function with anatomical and morphological structures, we may rest assured, that we have discovered an essential principle in the development of the Najades, and we may say with all confidence that a systematic arrangement, which is founded upon such structures, which we are able to understand, must be the correct one.' (Ortmann, 1911b: 305)

The extent to which these characters were perceived as homologies, however, varied from taxonomist to taxonomist. This is reflected in their disparate classifications (Table 2; also reviewed in Davis & Fuller, 1981). There has, however, been widespread agreement that the Margaritiferidae, because of their morphological simplicity, are the most 'primitive' unionoideans (Ortmann, 1912b; Heard & Guckert, 1971; Davis & Fuller, 1981). The brooding characters of margaritiferids, thus, are taken to be the plesiomorphic condition among the freshwater mussels, in general. This assumption has not been tested phylogenetically.

Any discussion of character evolution within the Unionoidea must be based on a phylogenetic hypothesis that reflects the evolutionary history of the group. Although the classifications of Ortmann (1911b, 1912b), Heard & Guckert (1971), and Davis & Fuller (1981) each have strong points, no single one of these is suitable to test hypotheses of brooding character evolution among the freshwater mussels of North America. A fundamental drawback of these studies is their lack of outgroups to objectively polarize the direction of character evolution (Wiley, 1980). Also, interpreting the classifications of the authors cited above from a phylogenetic perspective may not always be appropriate. After all, it may not have been their intention to recognize only monophyletic taxa (*i.e.*, groups composed of all of the descendents of a common ancestor). For example, the

Table 1. Brooding characters of bradytictic and tachytictic freshwater mussels of North America.

	bradytictia	tachytictia
brooding period	long	short
marsupial demibranchs	the outer pair or less	the outer pair or sometimes all four
ctenidial brooding modifications	gravid marsupium expands tripartite water tubes, *etc.*	none

Table 2. Synopsis of the classifications of Ortmann (1912b), Heard & Guckert (1971), and Davis & Fuller (1981; Lydeard *et al.*, 1996) for North American Unionoidea.

Ortmann	Heard & Guckert	Davis & Fuller
MARGARITIFERIDAE	MARGARITIFERIDAE	UNIONIDAE
UNIONIDAE	Margaritiferinae	Margaritiferinae
Unioninae	Cumberlandinae	Anodontinae
Anodontinae	AMBLEMIDAE	Ambleminae
Lampsilinae	Ambleminae	Amblemini
	Megalonaiadinae	Pleurobemini
	UNIONIDAE	Lampsilini
	Unioninae	
	Pleurobeminae	
	Anodontinae	
	Lampsilinae	

classification of Heard & Guckert (1971), from a cladistic vantage, is at odds with their own evolutionary tree (their Figure 1).

Lydeard *et al.* (1996) published the first cladistic phylogeny of the Unionoidea. Their study greatly improved the resolution of intergeneric relationships among the freshwater mussels of North America and also supported certain aspects of Davis & Fuller's (1981) classification. However, their use of the edible blue mussel, *Mytilus edulis* (Linnaeus), as the sole outgroup does not allow for a discussion of brooding character evolution among freshwater mussels. Although possibly a meaningful outgroup for molecular characters, no logical criterion exists to make homology statements about the morphological characters of the Unionoida and those of *Mytilus* (their Table 3).

A fundamental difficulty of arranging the freshwater mussels of North America into natural groups is the apparent lack of informative morphological characters shell, adult and larval gross anatomical, and, especially, brooding characters have been exploited in the past, but these are of poor quantity and quality. For our study, we reconstructed the phylogeny of the Unionoidea using a fragment of the mitochondrial gene encoding cytochrome *c* oxidase, subunit I (COI). We sampled a wide range of taxa. Not only representatives of the major groups of North American unionoideans, but also *Unio* (from both Europe and Africa), non-unionoidean freshwater pearly mussels, and several other bivalves. This includes *Neotrigonia*, the putative marine outgroup of all freshwater Unionoida (Thiele, 1934; Newell, 1969; Boss, 1982; Hoeh, Black, Gustafson, Bogan, Lutz & Vrijenhoek, 1998; but see Newell & Boyd, 1975 and Morton, 1987). Tracing brooding characters onto this molecular phylogeny allows independent tests of hypotheses of morphological evolution. Specifically, we set out to test the homology of bradytictia and of ctenidial morphological modifications associated with parental care among the Lampsilini and Anodontinae.

METHODS AND MATERIALS

Acquisition of Nucleotide Sequences

Partial COI mitochondrial gene sequences were obtained both from GenBank (National Center for Biotechnology Information, National Institutes of Health; http://www.ncbi.nlm.nih.gov) (n = 15) and by direct sequencing (n = 38); four sequences were acquired directly from the literature (Hoeh, Sewart, Sutherland & Zouros, 1996b; Ó Foighil, Gaffney, Wilbur & Hillbish, 1998) (Table 3). Specimens were preserved by freezing them at −70°C or by fixation in 95% ethanol. Whenever possible, two individuals per species were sequenced, and all representative haplotypes were included in the analyses.

Total cellular DNA was extracted from mantle or foot tissue using a QIAmp Tissue Kit (QIAGEN). A COI fragment roughly 680 nt long was amplified by the polymerase chain reaction (PCR) from each specimen using the primers LCO1490 (5′-ggtcaacaaatcataaagatattgg-3′) and HCO2198 (5′-taaacttcagggtgaccaaaaaatca-3′), or a modified version of the latter lacking 6 bases from the 5′ end (Folmer, Black, Hoeh, Lutz & Vrijenhoek, 1994). Each run of 44 cycles [17× @ (30 sec 94°C denaturing, 60 sec 60°C − 1°/cycle annealing, 60 sec 72°C extension), 27× @ (30 secs 94°C denaturing, 30 sec 43°C annealing, 1 min 72°C extension)] included a negative control.

Double-stranded PCR products were stained with ethidium bromide, isolated on 1% agarose gels, excised under UV light, and purified using a QIAquick (QIAGEN) Gel Extraction Kit. Both strands of amplified products were directly cycle-sequenced using 'Big Dye' Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Applied Biosystems, Inc.) with the same primers as above (47°C annealing temperature of LCO1490, 43°C for HCO2198) and electrophoresed on an ABI 377 automated DNA sequencer. The sequences were aligned using the CLUSTAL option of Sequence Navigator 1.0.1 (Kececioglu & Myers, 1994).

Initially, only females were utilized as sources of mtDNA in order to avoid potential complications associated with doubly-uniparental mitochondrial inheritance among freshwater mussels (Hoeh *et al.*, 1996b). Once heteroplasmy was determined not to be a problem for direct sequencing of somatic tissue (*e.g.* foot, mantle), males and mussels of undetermined sex were also included.

Phylogenetic Analyses

Parsimony analyses (heuristic searches, 20 random sequence additions, tree-bisection-reconnection) were performed using PAUP* (Swofford, 1998). Besides the unweighted analysis, a protocol of iterative reweighting of characters based on their Rescaled Consistency Index (*RC*) was also performed (Farris, 1969, 1989). In all analyses, *Lumbricus, Macrobdella, Placobdella*, and *Drosophila* were defined as outgroups.

To gauge the 'robustness' of the resulting trees, both Jackknife and Bremer-Decay Index values were calculated. Jackknifing (50% character deletion each replication; 200 replications, heuristic searches of 10 random additions) provides a rough quantification of the amount of support throughout the data set for a particular node. Bremer-Decay Indices (BDI) were calculated using TreeRot (Sorenson, 1996), which creates a constraint file for PAUP*. For each node, BDI indicate the difference in length of the next shortest tree without that node. The greater the BDI, the better support for that node (Bremer, 1995).

Table 3. Taxa for which cytochrome *c* oxidase subunit I fragments were obtained. Taxonomy follows Heard & Guckert (1971), Davis & Fuller (1981), Boss (1982) and Graf (2000). All source-specimens for novel sequences collected and identified by DLG unless noted (†). GB = GenBank Accession #; UMMZ = University of Michigan Museum of Zoology voucher.

Taxon	Source (GenBank Accession #, references, *etc.*)
OUTGROUPS: ANNELIDA & INSECTA	
Lumbricus terrestris Linn., 1758	GB U24570, Boore & Brown, 1995
Placobdella parasitica (Say, 1824)	GB AF003261, Siddall & Burreson, unpublished
Macrobdella decora (Say, 1824)	GB AF003271, Siddall & Burreson, unpublished
Drosophila melanogaster Meigen, 1830	GB U37541, several sources
MOLLUSCA: BIVALVIA: PTERIOMORPHA	
Crassostrea gigas (Thunberg, 1793)	Ó Foighil *et al.*, 1998
Crassostrea virginica (Gmelin, 1791)	Ó Foighil *et al.*, 1998
Mytilus edulis Linn., 1758	GB U68773, Hoeh *et al.*, 1997
Modiolus modiolus (Linn., 1758)	GB U56848, Hoeh *et al.*, 1998
HETERODONTA	
Corbicula fluminea (Mueller, 1774)	GB U47647, Baldwin *et al.*, 1996
Dreissena polymorpha (Pallas, 1771)	GB U47653, Baldwin *et al.*, 1996
Mercenaria mercenaria (Linn., 1758)	GB U47648, Bladwin *et al.*, 1996
Rangia cuneata (Gray, 1831)	GB U47652, Baldwin *et al.*, 1996
PALEOHETERODONTA: TRIGONIOIDA	
Neotrigonia margaritacea (Lam., 1804)	*GB U56850, Hoeh* et al., 1998
UNIONOIDA: ETHERIOIDEA: IRIDINIDAE	
Mutela rostrata (Rang, 1835)	GB U56849, Hoeh *et al.*, 1998
HYRIIDAE	
Hydrella depressa (Lam., 1819) (n = 2)	GB AF156496, UMMZ 265691 (MB+)
UNIONOIDEA: MARGARITIFERIDAE	
Margaritifera margaritifera (Linn., 1858)	GB U56847, Hoeh *et al.*, 1998
Cumberlandia monodonta (Say, 1829) (n = 2)	GB AF156497–AF156498, no voucher available (MH†)
UNIONIDAE: UNIONINAE	
Unio pictorum (Linn., 1757)	GB AF156499, no voucher available (KR†)
Unio caffer Krauss, 1848 (n = 2)	GB AF156500–AF156501, UMMZ 265692 (CC†)
ANODONTINAE	
Anodonta cygnea (Linn., 1758)	GB U56842, Hoeh *et al.*, 1998
Strophitus undulatus (Say, 1817) (n = 2)	GB AF156505, UMMZ 265693–265694 (RM)
Alasmidonta marginata Say, 1818 (n = 2)	GB AF156502, UMMZ 265695
Lasmigona compressa (Lea, 1829)	GB AF156503, UMMZ 265696
Pyganodon fragilis (Lam, 1819)	Hoeh *et al.*, 1996b
Pyganodon grandis (Say, 1829)	GB AF156504, UMMZ 265697 (RM†)
AMBLEMINAE: AMBLEMINI	
Amblema plicata (Say, 1817) (n = 2)	GB AF156512, UMMZ 265698 (RM) GB U56841, Hoeh *et al.*, 1998
Quadrula quadrula (Raf., 1820)	GB AF156511, UMMZ 265699 (RM)
PLEUROBEMINI	
Elliptio dilatata (Raf., 1820) (n = 2)	GB AF156506, UMMZ 265700 (RM) GB AF156507, UMMZ 265701 (RM)
Fusconaia flava (Raf., 1820) (n = 2)	GB AF156510, UMMZ 265702 (RM) & Hoeh *et al.*, 1996b
Pleurobema coccineum (Conrad, 1836) (n = 2)	GB AF156508, UMMZ 265703 (RM) GB AF156509, UMMZ 265704 (RM)

Table 3. (*Continued*)

LAMPSILINI	
Truncilla truncata Raf., 1820	GB AF156513, UMMZ 265705
Ptychobranchus fasciolaris (Raf., 1820)	GB AF156514, UMMZ 265706
Lampsilis cardium Raf., 1820 (n = 2)	GB AF156518–AF156519, UMMZ 265707 (RM†)
Lampsilis fasciola Raf., 1820 (n = 2)	GB AF156520, UMMZ 265708 (RM)
Lampsilis siliquoidea (Barnes, 1823) (n = 2)	GB AF156521–AF156522, UMMZ 265709 (RM†)
Ligumia nasuta (Say, 1817) (n = 2)	GB AF156515, UMMZ 265710 (LC)
Ligumia recta (Lam., 1819) (n = 2)	GB AF156516, UMMZ 265711
Villosa iris (Lea, 1829) (n = 2)	GB AF156523, UMMZ 265712 (RM†)
	GB AF156524, UMMZ 265713 (CG)
Villosa vanuxemensis (Lea, 1838) (n = 2)	GB AF156525–AF156526, UMMZ 265714 (CG)
Actinonaias carinata (Barnes, 1823)	GB AF156517, UMMZ 265715
Epioblasma brevidens (Lea, 1834)	GB AF156527, UAUC 509 (KR†)
Epioblasma triquetra (Raf., 1820) (n = 2)	GB AF156528, UMMZ 265716 (RM)

Specimens received from:
CC = C. Cambray, Rhodes U, Grahamstown, South Africa: CG = C. Gatenby, Virginia Polytechnic Inst., Blacksburg, USA; KR = K. Roe, U of Alabama, Tuscaloosa, USA; LC = L. Cooley, U of Michigan, Ann Arbor, USA; MB = M. Bryne, U of Sydney, Australia; MH = M. Hove, U of Minnesota, St. Paul, USA; RM = R. Mulcrone, U of Michigan, Ann Arbor, USA.

Thirteen brooding characters (Table 4) were traced onto the COI phylogeny using PAUP*. Transformation series were followed using both PAUP* and MacClade 3.07 (Maddison & Maddison, 1997).

RESULTS

Thirty-three novel COI haplotypes were acquired from 38 individuals representing 26 species of freshwater mussels (Table 3). These were added to 19 COI sequences obtained from GenBank and the literature. Those sequences from external sources were generally shorter than ours. All 52 sequences were aligned into a matrix of 653 characters, 413 of which were found to be parsimony informative (the aligned matrix is available from the authors). Pair-wise, dinucleotide sequence comparisons among all taxa revealed saturation with respect to transversions and transitions, especially among outgroup comparisons (Fig. 1). There was also significant among-site mutation rate variation (*e.g.*, >60% of the observed changes occurred at 3rd codon positions). This has been observed before (*e.g.*, Brown, Prager, Wang & Wilson, 1982) and has been suggested to confound phylogenetic analysis, especially parsimony (Felsenstein, 1978; Meyer, 1994). Our results, however, demonstrate, as has been recently supported by Yang (1998), that there is sufficient phylogenetic signal regardless of the homoplasy introduced by among-site variation in rates and strong biases for certain state changes.

The strict consensus of the three equally most-parsimonious trees (3034 steps, Consistency Index = *CI* = 0.312) recovered from the unweighted analysis is shown in Figure 2A. This COI tree agrees closely with the mitochondrial 16S phylogeny published by Lydeard *et al.* (1996) for the taxa they included. The Paleoheterodonta (*Neotrigonia* + Unionoida), was found to be monophyletic, and this result is well-supported. The remaining bivalves also formed a clade. These results support a sister-group relationship between the Trigonioida and the Unionoida (Thiele, 1934; Taylor, Kennedy & Hall, 1969; Boss, 1982; Smith, 1983; Healy, 1989, Hoeh *et al.*, 1998).

The Unionidae is composed of three clades: the Unioninae, Anodontinae, and Ambleminae; the latter subfamily has been further subdivided by Davis & Fuller (1981) into the Lampsilini, Pleurobemini, and 'Amblemini.' These are synonymous with the Lampsilinae, Pleurobeminae, and 'Ambleminae' of Heard & Guckert (1971), respectively. Of these, only the Lampsilini and Pleurobemini are monophyletic. No support is found for the familial taxa of Heard & Guckert (1971) (Table 2) nor the inclusion of the North American Pleurobemini, *Quadrula*, or *Amblema* among the Unioninae of Ortmann (1912b). The Unionidae is sister to the Margaritiferidae, and the two comprise a monophyletic Unionoidea.

A single tree (3036 unweighted steps, *CI* = 0.312) was resolved by iteratively re-weighting all characters according to their *RC* (Farris, 1969, 1989) (Fig. 2B). It differs from the unweighted consensus tree (Fig. 2A) only in its

Table 4. Matrix and diagnoses of brooding characters among the Paleoheterodonta. Character states were determined from direct observation of specimens and from the literature (Baker, 1928; Bloomer, 1932; Darragh, 1998; Heard & Vail, 1976; Kraemer, 1970; McMichael & Hiscock, 1958; Morton, 1987; Ortmann, 1911b, 1912a, 1912b, 1913–1916, 1918a, 1918b, 1918c, 1921, 1923–1924; Smith, 1979). A gap ('–') indicates inapplicable characters. See text for a discussion of character coding.

	CHARACTER MATRIX												
	1	2	3	4	5	6	7	8	9	10	11	12	13
N. margaritacea	0	0	–	–	0	–	–	–	–	–	0	–	0
M. rostrata	1	1	1	–	1	1	0	1	0	0	0	?	0
H. depressa	1	1	1	–	2	2	0	0	0	0	0	?	0
M. margaritifera	1	1	0	–	0	0	–	0	0	0	0	?	0
C. monodonta	1	1	0	–	0	0	–	0	0	0	0	?	0
U. pictorum	1	1	2	0	1	2	0	0	0	0	0	0	0
U. caffer	1	1	2	0	1	2	0	0	0	0	0	0	0
A. cygnea	1	1	2	0	1	1	1	0	1	0	0	1	0
S. undulatus	1	1	2	0	1	1	1	0	1	0	0	1	0
A. marginata	1	1	2	0	1	1	1	0	1	0	0	1	0
L. compressa	1	1	2	0	1	1	1	0	1	0	0	1	0
P. fragilis	1	1	2	0	1	1	1	0	1	0	0	1	0
P. grandis	1	1	2	0	1	1	1	0	1	0	0	1	0
A. plicata	1	1	0	–	1	1	0	0	0	0	0	0	0
Q. quadrula	1	1	0	–	1	1	0	0	0	0	0	0	0
E. dilatata	1	1	2	0	1	1	0	0	0	0	0	0	0
F. flava	1	1	0	–	1	1	0	0	0	0	0	0	0
P. coccineum	1	1	2	0	1	1	0	0	0	0	0	0	0
T. truncata	1	1	2	1	1	1	0	0	1	1	1	1	0
P. fasciolaris	1	1	2	1	1	1	0	0	1	1	1	1	0
L. cardium	1	1	2	1	1	1	0	0	1	1	1	1	1
L. fasciola	1	1	2	1	1	1	0	0	1	1	1	1	1
L. siliquoidea	1	1	2	1	1	1	0	0	1	1	1	1	1
L. nasuta	1	1	2	1	1	1	0	0	1	1	1	1	1
L. recta	1	1	2	1	1	1	0	0	1	1	1	1	1
V. iris	1	1	2	1	1	1	0	0	1	1	1	1	1
V. vanuxemensis	1	1	2	1	1	1	0	0	1	1	1	1	1
A. carinata	1	1	2	1	1	1	0	0	1	1	1	1	0
E. brevidens	1	1	2	1	1	1	0	0	1	1	1	1	1
E. triquetra	1	1	2	1	1	1	0	0	1	1	1	1	1

CHARACTER DIAGNOSES

Brooding and Life History Characters

1. Habitat. 0 = marine; 1 = freshwater.
2. Parental care. 0 = none, fertilization is presumably external; 1 = female broods embryos and larvae in ctenidial marsupium.
3. Demibranchs occupied by marsupium. 0 = all four; 1 = inner pair only; 2 = outer pair only.
4. Outer marsupial demibranch. 0 = entire demibranch marsupial or nearly so; 1 = a restricted portion of the demibranch marsupial.
5. Interlamellar connections of non-marsupial demibranchs, including those of males. 0 = none or scattered; 1 = complete septa; 2 = perforated septa.
6. Interlamellar connection of marsupial demibranchs. 0 = absent or scattered;' 1 = complete septa; 2 = perforated septa.
7. Marsupial water tubes. 0 = undivided; 1 = divided by lateral septa ('tripartite').
8. Interlamellar septa of marsupium. 0 = without a swelling protruding into the water tubes; 1 = bearing a 'marked swelling.'
9. Edge of marsupium. 0 = remains sharp when gravid; 1 = expands greatly when gravid.
10. Ventral extent of marsupium. 0 = ventral margin of marsupium does not extend past the non-marsupial portion; 1 = ventral margin of marsupium extends past the non-marsupial portion.
11. Larval discharge. 0 = larvae discharged out the excurrent aperture with the respiratory current; 1 = larvae discharge through the ventral margin of the demibranch and out the incurrent aperture.
12. Brooding period. 0 = tachytictic (short), 1 = bradytictic (long)
13. Mantle ventral to the incurrent aperture. 0 = smooth or weakly elaborated; 1 = elaborated with conspicuous papillae or a ribbon-like flap.

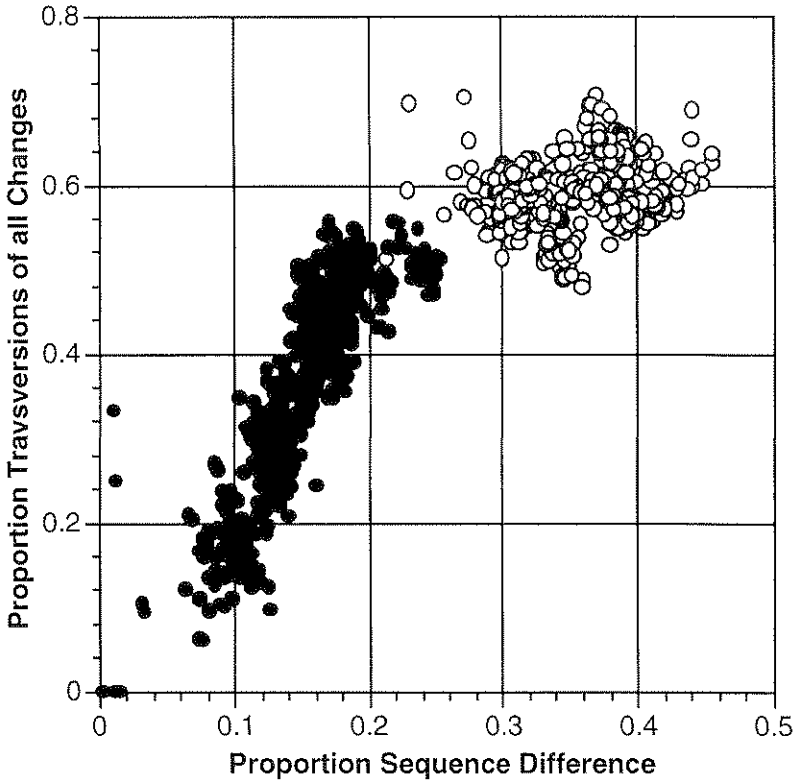

Figure 1. Proportion transversions of all changes vs. proportion sequence difference among pair-wise comparisons of all haplotypes. Open circles indicate outgroup comparisons; dots represent comparisons within the Paleoheterodonta.

complete resolution of the Lampsilini. The topology of that clade, however, is distinct from any of the three individual trees recovered in the unweighted analysis. Based on our analyses, we take the re-weighted tree (Fig. 2B) to be the best corroborated phylogeny of the Paleoheterodonta presently available. We do, however, acknowledge that some nodes are weakly supported and may be subject to change with additional data.

Assuming that Figure 2B is the 'true' tree, Figure 3 depicts the pattern of character evolution among the 13 brooding characters listed in Table 4. Character transformations are described in the Appendix. Seven brooding characters are shown to be unambiguous synapomorphies (CI = 1.0): freshwater habitat [character 1, see Table 4] and brooding [2] (synapomorphies of the Unionoida); 'marked swelling' protruding into the water tubes [8] (*Mutela* and other Iridinidae); tripartite water tubes [7] (Anodontinae); restriction of the marsupium to a portion of the outer demibranchs [4], ventral extension of the marsupium [10], and larval discharge through the ventral margin of the marsupium [11] (Lampsilini). The six remaining characters exhibit homoplasy in varying degrees, including brooding period [12] (CI = 0.500) and number of marsupial demibranchs [3] (CI = 0.400) which have figured prominently in past classifications (Ortmann, 1912b; Heard & Guckert, 1971).

DISCUSSION

Evolution of Brooding Among Freshwater Bivalves

The evolution of brooding among bivalves is correlated with colonization of freshwater habitats from a marine environment. The phylogeny in Figure 2 indicates three independent bivalve invasions of freshwater: the Unionoida, *Cor-*

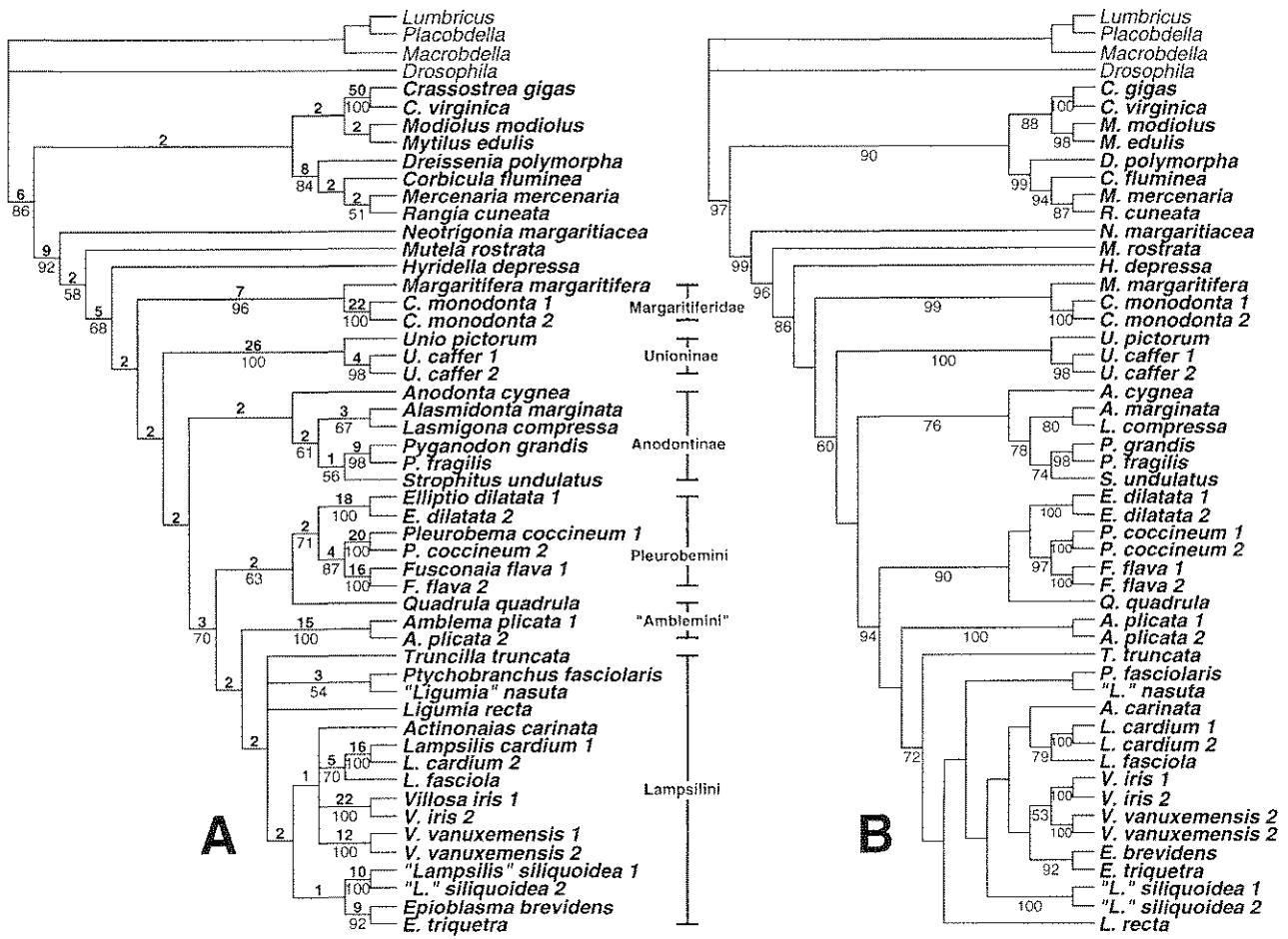

Figure 2. Results of phylogenetic analyses. A: Strict consensus of unweighted analysis (3 trees, all 3034 steps with $CI = 0.312$). B: Single tree resolved by interatively re-weighting characters according to their RC (3036 unweighted steps, $CI = 0.312$). Values above the branches are Bremer-Decay Indices. Those below indicate the percent Jackknife support, if greater than 50%.

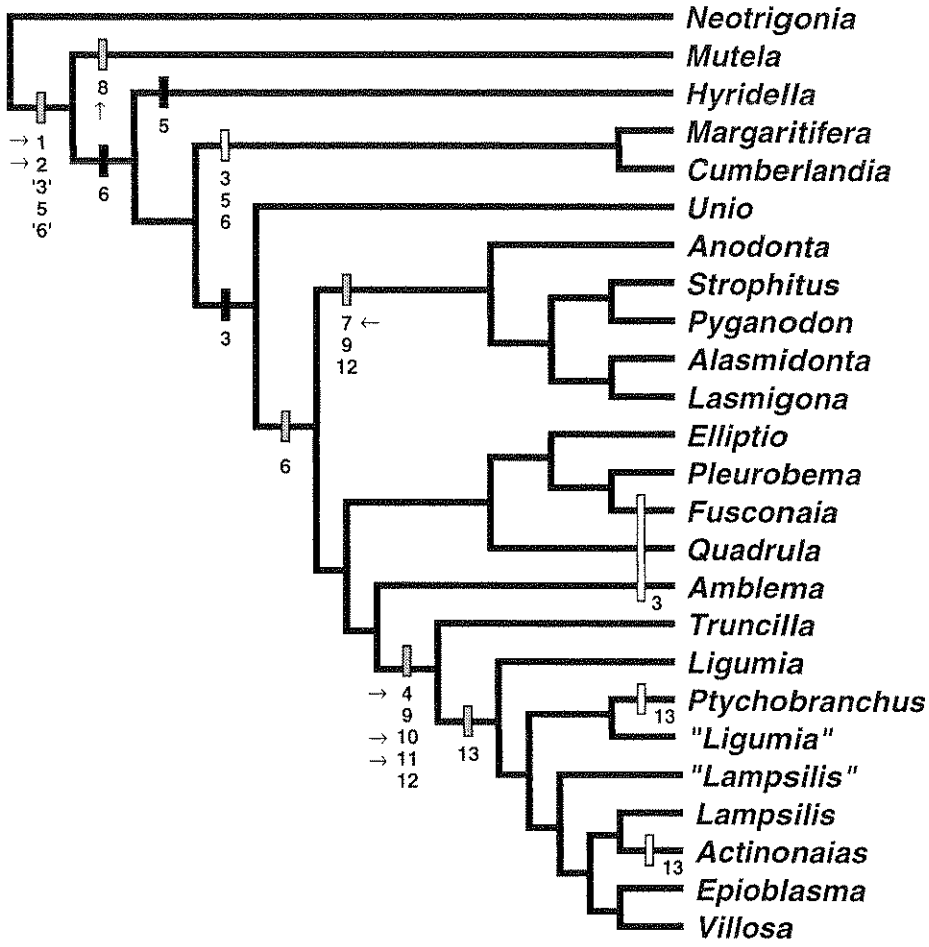

Figure 3. Brooding character transformations traced on the phylogeny of the Paleoheterodonta. Character numbers refer to those listed in Table 4. Shaded boxes indicate character acquisition (gray and black for states 1 and 2, respectively), white boxes identify character losses (character state 0). An arrow ('→') indicates unambiguous character transformations ($CI = 1.0$). Characters 3 and 6 have pleisiomorphic states other than '0'. See text for discussion.

bicula fluminea, and *Dreissena polymorpha*. Sphaeriids are a fourth group to do so independently (*e.g.*, Park & Ó Foighil, 1998, 2000). Among these taxa, only *Dreissena* has not evolved ovovivipary. It retains its plesiomorphic veliger. However, *Dreissena* may have infiltrated freshwater environments only as recently as the Pleistocene (McMahon, 1991).

In marine environments, the stereotypical bivalve larval form is a planktonic veliger (Brusca & Brusca, 1990), and passive dispersal of this veliger or other planktonic larva is the principle means of distribution. In a freshwater stream environment, such a strategy is disadvantageous—reliance upon buoyant, microscopic larvae for dispersal would allow bivalves to colonize only downstream habitats and eventually fall back into the ocean. Sphaeriids and corbiculids have overcome this problem by abandoning a planktonic larval stage in favour of direct-development of offspring within their brood chambers (McMahon, 1991). Indirect development has persisted among the Unionoida, although passive dispersal by water currents has been swapped for distribution by the host fishes of their parasitic glochidia (Coker *et al.*, 1921; Kat, 1984). Direct development has been secondarily derived in a few unionoid

lineages (*e.g.*, Kondo, 1990; Parodiz & Bonetto, 1963).

Evolution of Brooding Pattern Among North American Unionoidea

Among the Unionoidea of North America, two general patterns of brooding have been observed: bradyticia (long-term brooding) and tachyticia (short-term brooding) (reviewed in Graf, 1997 and Heard, 1998). Sterki (1903), Ortmann (1912b), and Heard & Guckert (1971) considered brooding period to be of principle importance in their classifications of the freshwater mussels of North America. Davis & Fuller (1981) and Lydeard *et al.* (1996: 1601) argued that brooding period lacked value as a phylogenetic character, suggesting that, '. . . the bradytictic and tachytictic conditions have evolved several times . . .' Our data (Fig. 3) clearly indicate that, among North American freshwater mussels, bradytictia is a derived condition, having evolved twice independently: once in the Anodontinae and once in the Lampsilini. The plesiomorphic condition among the Unionidae is tachyticia, as noted by Heard (1998). The brooding data on the non-North American taxa is sparse, but the Hyriidae and Iridinidae apparently breed all year or during the austral summer (reviewed in Watters, 1994).

As discussed by Graf (1997), much of the confusion regarding the systematic value of brooding period has been caused by differing definitions of long-term and short-term brooding among systematists, especially by confusing them with their original descriptors: winter-brooding and summer-brooding, respectively (Lefevre & Curtis, 1910, 1912). For example, *Megalonaias* has been regularly listed among the bradytictic mussels (Utterback, 1916; Heard & Guckert, 1971; Lydeard *et al.*, 1996; Heard, 1998) because it broods in the late fall and winter (Woody & Holland-Bartels, 1993). However, it is a short-term brooder (*i.e.*, glochidia are not brooded after they are infectious) and might thus be dubbed 'winter-tachytictic.' Although not included in our analysis, Lydeard *et al.* (1996) found *Megalonaias* to be sister to *Quadrula*, another tachytictic genus.

There has been similar confusion surrounding the Margaritiferidae. Heard & Guckert (1971), Davis & Fuller (1981), and Lydeard *et al.* (1996) considered the them to be bradytictic, while Sterki (1903), Connor (1909), and Ortmann (1912b) considered those mussels to be tachytictic. Watter's (1994) review of infection periods for margaritiferids, as well as Howard (1915) and Gordon & Smith's (1990) reports of multiple broods for *Cumberlandia*, suggest that margaritiferids are facultatively bradytictic. Heard (1998) considered the Margaritiferidae to be 'sequentially tachytictic' while Graf (1997) suggested that unionid terminology might best be reserved solely for the Unionidae. Obviously, more life history data are needed from margaritiferids and other mussel species to resolve this problem. For our analysis, margaritiferid brooding pattern was coded as unknown ('?', character 12 in Table 4).

Evolution of Marsupium Morphologies

As noted by Ortmann (1911b, 1912b; also see Heard & Guckert, 1971 and Graf, 1997), certain morphological novelties have been associated with bradytictia. Among these are the number and arrangement of marsupial demibranchs as well as modifications of the marsupium to facilitate long-term brooding (Table 1). Our analyses (Fig. 3, Appendix), however, suggest that (1) tachytictia and tetrageny (use of all four demibranchs for brooding) are not correlated and that (2) the brooding modifications of the bradytictic clades are not homologous.

The plesiomorphic condition of the Unionidae is a tachytictic [12] mussel employing only the outer pair of demibranchs for brooding [3] (Fig. 3). Within the Unionidae, use of all four demibranchs is shown to be a derived condition among certain Ambleminae. Ortmann (1912b) noted in *Amblema* and *Quadrula* that the septa of the outer marsupial demibranchs are more crowded than those of the inner demibrachs. This further supports the hypothesis that tetrageny evolved secondarily from an ectobranchous (*i.e.*, using only the outer demibranchs as marsupia) condition in which the septa are more crowded in marsupial demibranchs than they are in those that are non-marsupial (Ortmann, 1911b, 1912b).

The plesiomorphic marsupial arrangement of the Unionoidea is ambiguous (Fig. 3 and Appendix). Our phylogenetic analyses suggest that the 'primitive' margaritiferid condition may actually be derived. Besides being tetragenous [3], *Margaritifera* and *Cumberlandia* have reduced the septa of their demibranchs to sparse interlamellar junctions [5,6]. A reduction hypothesis would also apply to other presumed 'primitive' characters among the Margaritiferidae, such as loss of a supra-anal aperture and atrophy of the diaphragm dividing the mantle cavity (Ortmann, 1912b; Baker, 1928; Heard & Guckert, 1971; Davis & Fuller, 1981).

This hypothesis of margaritiferid specialization vs. plesiomorphy could be tested by adding taxa to the analysis that would intersect the branch between the Hyriidae and Unionoidea (Fig. 2 and 3). Hoeh *et al.* (1996a) reported that the African *Caelatura* was the most basal unionoidean in their analysis. *Caelatura* is tetranenous, as are *Gonidea*, *Brazzaea*, and *Parreysia* of the western U.S., Africa, and India, respectively (Bloomer, 1931, 1932; Ortmann, 1911a, 1916), but other tropical Unionoidea are known to use only one or the other pair of demibranchs as marsupia (Ortmann, 1911a; Brandt, 1974; Kondo, 1990). These freshwater mussels all have septa dividing the demibranchs into water tubes, though the septa are perforated. Contrary to present classification (*e.g.*, Haas, 1969a, 1969b; Brandt, 1974; Boss, 1982), these tropical unionoideans (including *Gonidea*) may represent a radiation independent of the temperate taxa that we have included here and may provide insights into the plesiomorphic condition of the Unionoidea.

Having their gas exchange and feeding organs clogged with developing offspring for extended periods is an obvious physiological disadvantage to a gravid mussel (*e.g.*, Tankersley, 1996). Besides gross morphological changes in marsupial arrangement, the freshwater mussels of North America have also undergone several structural specializations to alleviate this strain. Both the Anodontinae and Lampsilini have convergently augmented the base of the lamellae of the marsupium with tissue to allow for great expansion when the mussel is gravid [9]. In the case of the Lampsilini, this tissue is further modified to allow the marsupium to extend beyond the ventral margin of the demibranch [10] and for the expulsion of glochidia through that tissue [11] rather than via the suprabranchial space. In most Lampsilini, the marsupium is limited to only a portion of the marsupial demibranch [4], but the actual configuration varies among genera (numerous figures in Ortmann, 1912b).

While the Lampsilini tend to limit the number of water tubes reserved for brooding, the Anodontinae divided the water tubes themselves. Each water tube of the gravid marsupium is divided by a pair of lateral septa running parallel to the axis of the ctenidium (figured in Ortmann, 1911b). These 'tripartite' water tubes [7], with the embryos and larvae brooded only in the center compartment, allow the respiratory and feeding current to flow freely through the lateral compartments. And so, as long-term brooding has evolved separately in the two bradytictic clades, each has derived unique specializations to accommodate it.

These results bear upon the characters classically employed to diagnose unionoidean taxa. While some brooding characters were found to be unambiguous synapomorphies diagnosing clades within the Paleoheterodonta and Unionoida, brooding period and, especially, the arrangement of marsupial demibranchs were found to be of limited systematic value. Bradytictia evolved independently in both the Anodontinae and Lampsilini (Fig. 3), so long-term brooding can not be considered homologous among all bradytictic mussels. Rather, long-term brooding may be a convergent adaptation to temperate winters in these two clades (see discussions in Graf, 1997 and Heard, 1998).

Marsupial arrangement has figured prominently in past classifications of the North American Unionoidea. Although the plesiomorphic marsupial arrangement of the Unionoidea is ambiguous, the hypothesis that tetrageny is the primitive condition among the Unionoida can be rejected. Our analysis suggests that using all four demibranchs for brooding may be a derived condition, but this hypothesis is in need of further testing. This may be best achieved by including tropical unionoideans in future phylogenetic analyses.

ACKNOWLEDGMENTS

We would especially like to thank Renée Sherman Mulcrone for the numerous specimens she provided. M. Bryne, C. Cambray, C. Gatenby, M. Hove, L. Reich-Cooley, and K. Roe also provided specimens which we would not otherwise have been able to acquire. The phylogenetic aspects of this work greatly benefited from discussions with J. Ast, W. Fink, J. Sparks, M. Siddall, and S. Webb. Useful suggestions were also provided by an anonymous reviewer. This study was funded by NSF grant 9617689 to Diarmaid Ó Foighil.

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APPENDIX

CHARACTER STATISTICS AND TRANSFORMATIONS

Steps refers to the number of transformations each character undergoes on a given tree; *CI* and *RC* are the Consistency and Rescaled Consistency Indices, respectively. A dagger ('†') indicates that the *RC* is taken to be unity when the Retention Index is undefined (Farris, 1989). Character statistics are also provided for the ensemble of all brooding characters. Transformations are depicted in Figure 3.

Character	Steps	CI	RC	Transformation
1	1	1.000	1.000†	Synapomorphy of the Unionoida.
2	1	1.000	1.000†	Synapomorphy of the Unionoida.
3	5	0.400	0.250	*1* is the primitive condition among the Unionoida. *0* a synapomorphy of the Margaritiferidae. *2* a synapomorphy of the Unionidae, with three independent reversions to *0* in *Amblema*, *Fusconaia*, and *Quadrula*. The plesiomorphic state of the Unionoidea is ambiguous.
4	1	1.000	1.000	Synapomorphy of the Lampsilini.
5	3	0.667	0.444	*1* is a synapomorphy of the Unionoida, with a transition to *2* in *Hyridella* and reversion to *0* in the Margaritiferidae.
6	3	0.667	0.533	*1* is the primitive condition among the Unionoida. *2* is a synapomorphy of (*Hyridella* + Unionoidea). There is reversion to *0* in the Margaritiferidae and *1* in (Anodontinae + Ambleminae).
7	1	1.000	1.000	Synapomorphy of the Anodontinae.
8	1	1.000	1.000†	Synapomorphy of *Mutela* and other Iridinidae.
9	2	0.500	0.469	Arises independently as synapomorphies of both the Anodontinae and Lampsilini.
10	1	1.000	1.000	Synapomorphy of the Lampsilini.
11	1	1.000	1.000	Synapomorphy of the Lampsilini.
12	2	0.500	0.455	Arises independently as synapomorphies of both the Anodontinae and Lampsilini.
13	3	0.333	0.278	Arises within the Lampsilini, but is lost independently in *Ptychobranchus* and *Actinonaias*.
All	25	0.640	0.584	