



Evolution of active host-attraction strategies in the freshwater mussel tribe Lampsilini (Bivalvia: Unionidae)

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Abstract

Most freshwater mussels (Bivalvia: Unionoida) require a host, usually a fish, to complete their life cycle. Most species of mussels show adaptations that increase the chances of glochidia larvae contacting a host. We investigated the evolutionary relationships of the freshwater mussel tribe Lampsilini including 49 of the approximately 100 extant species including 21 of the 24 recognized genera. Mitochondrial DNA sequence data (COI, 16S, and ND1) were used to create a molecular phylogeny for these species. Parsimony and Bayesian likelihood topologies revealed that the use of an active lure arose early in the evolution of the Lampsilini mussels. The mantle flap lure appears to have been the first to evolve with other lure types being derived from this condition. Apparently, lures were lost independently in several clades. Hypotheses are discussed as to how some of these lure strategies may have evolved in response to host fish prey preferences. © 2006 Published by Elsevier Inc.

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1. Introduction

Freshwater mussels (also known as unionids, naiads, or clams) are filter feeding bivalve molluscs from the order Unionoida. They have a specialized and unique life history in that most species require a host fish for their glochidium larvae (Nedeau et al., 2000; Parmalee and Bogan, 1998). Many species of mussels show adaptations that probably increase the chances of glochidia contacting a suitable host fish. The most conspicuous of these adaptations are the modified mantle flaps, mantle papillae, and large glochidial packages (superconglutinates) that are displayed by gravid females of the tribe Lampsilini (Haag and Warren, 1999). These remarkable adaptations often appear to mimic prey of host fish for the mussel (Haag et al., 1995) and have been shown to elicit attacks by potential hosts (Haag and Warren, 1999).

A wide diversity of mantle derived lures are used by species from the tribe Lampsilini to actively attract a host.

Mantle lures in these species are useful to attract predacious fish since several species of *Lampsilis* (e.g., *L. siliquoides*, *L. cardium*, and *L. fasciola*) actively pulse uniquely pigmented mantle flaps that often look remarkably like a small fish (Haag and Warren, 1999; personal observations; see Appendix A for video of *Lampsilis ovata* and *L. cardium* using mantle flap lures). The rainbow mussel (*Villosa iris*) uses a mantle display and behaviour that mimics a crayfish, an equally elaborate strategy for attracting a host (Appendix A). Most species of *Lampsilis* and *V. iris* use predacious bass (*Micropterus* sp.) as primary hosts (Coker et al., 1921; Watters and O'Dee, 1997a,b; Zale and Neves, 1982). The species of the critically imperiled genus *Epioblasma* use brightly coloured and textured mantle pads often in combination with micro-lures that appear to mimic the cerci of an aquatic insect to attract their hosts (Jones, 2004). These often very elaborate lures appear to be in close association with their darter hosts (genera *Etheostoma* and *Percina*). An amazing behaviour has been observed in *Epioblasma*; that the pad structures are used to trap the host in order to assist in the infestation by the glochidia (Jones, 2004; Appendix A).

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Several lamspsiline species also use active lures not derived from the mantle. Species from the genera *Ptychobranthus*, *Cyprogenia*, and *Dromus* use elaborate conglutinates (packages of glochidia) that resemble fish fry, worms, or the pupae of aquatic insects (Watters, 1999; Appendix A). It was only very recently that superconglutinates were first documented in the genus *Hamiota* (Haag et al., 1995; Appendix A). These non-mantle derived active lures remain attached to the female mussel and are manipulated through the use water currents in order to actively attract a host fish.

We investigated the maternal history of the freshwater mussel tribe Lampsilini (*sensu* Campbell et al. (2005), Lydeard et al. (1996), and Davis and Fuller (1981)). This group has evolved the most radical lures found among freshwater mussels. We have used our phylogeny to evaluate the evolution of lures. Furthermore, because many freshwater mussels are at extreme risk of extinction (Bogan, 1993; Lydeard et al., 2004; Ricciardi and Rasmussen, 1999), we put the use of lures and host attraction strategies in a phylogenetic context, as evolutionary interpretations on the life history are critical to their survival. Forty-nine of the approximately 100 recognized species, from 21 of the 24 recognized genera in the tribe (Roe and Hartfield, 2005; Turgeon et al., 1998; Williams et al., 1993) were included in this analysis.

2. Methods

2.1. Molecular techniques

Specimens of *Epioblasma torulosa rangiana*, *Lampsilis fasciola*, *Venustaconcha ellipsiformis*, and *Villosa fabalis* were collected by DTZ from the Allegheny River, Pennsylvania, USA; the Thames River, Ontario, Canada; Buffalo Creek, Iowa, USA; and the Sydenham River, Ontario, Canada, respectively. Due to the rarity and permit regulations, shell vouchers were only taken for *V. fabalis* (ROM Cat M10428). Photographs were taken as vouchers of the other animals. Mantle tissues were collected in the field using the protocols of Berg et al. (1995) and Naimo et al. (1998). Tissues were maintained at -80°C .

Total genomic DNA was extracted from $\sim 15\text{mg}$ of frozen tissue samples by standard phenol extraction or with a Purgene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN, USA). For *E. t. rangiana*, *V. ellipsiformis*, and *V. fabalis*, mitochondrial DNA (mtDNA) sequences were obtained for a 630bp fragment of COI using primers LCO1490 and HCO2198 from Folmer et al. (1994) and an 894bp complete sequence of the ND1 gene using primers Leu-uurF and LoGlyR developed by Serb et al. (2003). For *L. fasciola*, only the ND1 sequence was obtained. PCR's were done in a 25.45 μl solution containing 1.0 μl of genomic DNA, 0.2 μl of each dNTP at 0.1 mM, 1.0 μl of each primer at 1.0 μM , 2.5 μl of $10\times$ PCR buffer, 0.15 μl of *Taq* polymerase, and 19 μl of dH_2O . Each PCR run (92°C for 2 min; 5 cycles of 92°C for 40s, 40°C for 40s, 72°C for 90s; 25 cycles of 92°C for 40s, 50°C for 40s, 72°C for 90s; 72°C for 10 min; and hold at 4°C) included a negative control.

Double-stranded PCR products were visualized using 1.0% agarose gels stained with ethidium bromide or Sybr Green II. PCR products were purified using a Microcon YM-100 (Millipore, Inc.) or QIAquick DNA purification kits. The 5' end of the amplified products were cycle-sequenced using 'Big Dye' Terminator Cycle Sequencing Ready Reaction (Perkin-Elmer Applied Biosystems, Inc.) with the primers LCO1490 or Leu-uurF (50°C annealing temperature) and visualized on an ABI 377 or ABI 3100 automated DNA sequencer.

2.2. Phylogenetic analyses

Partial sequences for COI, 16S rRNA, and ND1 were taken from GenBank for an additional 49 species from the tribe Lampsilini and 9 outgroup taxa: *Cumberlandia monodonta* (Margaritiferidae); *Pyganodon grandis* (Unionidae: Anodontinae); *Fusconaia flava*, *Lexingtonia dollabelloides*, *Hemistena lata*, *Elliptio dilatata*, *Pleurobema clava* (Unionidae: Ambleminae: Pleurobemini), *Amblema plicata*, and *Popenaias popeii* (Unionidae: Ambleminae: Amblemini) were taken from GenBank (Table 1). The sequences were concatenated then aligned using Clustal X (v. 1.83) and checked by eye.

Lure strategy and specific lure morphology were used as two morphological characters and added to the data matrix. We defined lure strategy as the method by which a mussel attracts a host: either active or passive. We defined active host-attraction as luring behaviour controlled by movement of the mussel (e.g., a flap of the mantle) or water currents affecting glochidial packages while remaining attached to the mussel for a period of time (e.g., elaborate conglutinates and superconglutinates). We defined passive host-attraction as broadcast release of glochidia or small packages of glochidia that do not remain attached to the mussel. Specific lure morphology was defined as the actual type of lure the mussel used (Table 2; Appendix B). The character states for lure strategy were: active lure present and active lure absent. The character states for specific lure morphology were: no active lure; elaborate conglutinates; active caruncles; active valve gaping; superconglutinate; elaborate mantle pad "trap"; and active mantle flap (described further in Appendix B).

A maximum parsimony analysis was performed via an heuristic search with 1000 replications of random stepwise additions using PAUP* v4.0b10 (Swofford, 1998). To gauge the robustness of the resulting trees, both Bootstrap (using PAUP* v4.0b10) and Bremer-Decay Index values were calculated. Bremer-Decay Indices (Bremer, 1994) were calculated using AutoDecay (Eriksson, 1998). Bootstrapping used 1000 replications and heuristic searching with 10 random stepwise additions.

A second phylogenetic analysis, Bayesian inference (Huelsenbeck and Ronquist, 2001), used MrBayes v3.0b4. The two morphological characters were omitted from this analysis as they are incompatible with MrBayes' file formatting. The initial model of evolution for MrBayes

Table 1

Taxa for which cytochrome *c* oxidase subunit I (COI), 16S rRNA, and NADH dehydrogenase (ND1) fragments were obtained from GenBank (<http://www.ncbi.nlm.nih.gov>) and sequencing

Species	GenBank Accession No(s).			Studies
	COI	16S	ND1	
Outgroup species				
<i>Cumberlandia monodonta</i> (Say, 1829)	AF231753	AY579089		Bogan and Hoeh (2000), Huff et al. (2004)
<i>Fusconia flava</i> (Rafinesque 1820)	AF232822	AY655042	AY613793	Lydeard et al. (2000), Campbell et al. (2005)
<i>Popenaias popeii</i> (Lea, 1857)	AY655020	AY655073	AY655118	Campbell et al. (2005)
<i>Pyganodon grandis</i> (Say, 1829)	AF156504	AY238490		Graf and O'Foighil (2000), Krebs et al. (2003)
<i>Amblyma plicata</i> (Say, 1817)	AF156512	U72548	AY158796	Graf and O'Foighil (2000), Lydeard et al. (1996), Serb et al. (2003)
<i>Lexingtonia dollabelloides</i> (Lea, 1840)	AY655004	AY655051	AY655106	Campbell et al. (2005)
<i>Hemistena lata</i> (Rafinesque, 1820)	AY613825	AY655046	AY613796	Campbell et al. (2005)
<i>Elliptio dilatata</i> (Rafinesque, 1820)	AF156506	U72557	AY655094	Graf and O'Foighil (2000), Lydeard et al. (1996), Campbell et al. (2005)
<i>Pleurobema clava</i> (Lamarck, 1819)	AY655013	AY655060	AY613802	Campbell et al. (2005)
Ingroup species				
Tribe Lampsilini				
<i>Actinonaias ligamentina</i> (Lamarck, 1819)	AF156517	AY655027	AY655085	Graf and O'Foighil (2000), Campbell et al. (2005)
<i>Actinonaias pectorosa</i> (Conrad, 1834)	AY654990	AY655028		Campbell et al. (2005)
<i>Cyprogenia stegaria</i> (Rafinesque, 1820)	AY654992		AY655089	Campbell et al. (2005)
<i>Cyrtonaias tampicoensis</i> (I. Lea, 1838)	AF231749	AY655032	AY655090	Hoeh et al. (2001), Campbell et al. (2005)
<i>Dromus dromas</i> (I. Lea, 1834)	AY654993	AY655033		Campbell et al. (2005)
<i>Ellipsaria lineolata</i> (Rafinesque, 1820)	AY654994		AY655092	Campbell et al. (2005)
<i>Epioblasma brevidens</i> (I. Lea, 1831)	AF156527	AY655036	AY094378	Graf and O'Foighil (2000), Campbell et al. (2005), Buhay et al. (2002)
<i>Epioblasma capsaeformis</i> (I. Lea, 1834)	AY094372	AY655037	AY094382	Buhay et al. (2002), Campbell et al. (2005)
<i>Epioblasma florentina walkeri</i> (Wilson and Clark, 1914)	AY094374		AY094384	Buhay et al. (2002)
<i>Epioblasma triquetra</i> (Rafinesque, 1820)	AF156528		AY094375	Graf and O'Foighil (2000), Buhay et al. (2002)
<i>Epioblasma torulosa rangiana</i> (Rafinesque, 1839)	DQ220724		DQ220720	This study
<i>Glebulia rotundata</i> (Lamarck, 1819)	AF231729	AY655044	AY613795	Hoeh et al. (2001), Campbell et al. (2005)
<i>Hamiota altilis</i> (Conrad, 1834)	AF385108	AF385132	AY655101	Roe et al. (2001), Campbell et al. (2005)
<i>Hamiota australis</i> (Simpson, 1900)	AF385101	AF385125		Roe et al. (2001)
<i>Hamiota perovalis</i> (Conrad, 1834)	AF385096	AF385120		Roe et al. (2001)
<i>Hamiota subangulata</i> (I. Lea, 1840)	AF385104	AF385128		Roe et al. (2001)
<i>Lampsilis cardium</i> (Say, 1831)	AF120653			Giribet and Wheeler (2002)
<i>Lampsilis fasciola</i> (Rafinesque, 1820)	AF156520		DQ220721	Graf and O'Foighil (2000), This study
<i>Lampsilis ornata</i> (Conrad, 1835)	AF385112	AF385136	AY158748	Roe et al. (2001), Serb et al. (2003)
<i>Lampsilis ovata</i> (Say, 1817)	AF385111	AY655048	AY613797	Roe et al. (2001), Campbell et al. (2005)
<i>Lampsilis siliquoidea</i> (Barnes, 1823)	AF156522	AY498703	AY158747	Graf and O'Foighil (2000), Campbell et al. (2005), Serb et al. (2003)
<i>Lampsilis teres</i> (Rafinesque, 1820)	AF385113	AF385137	AY655102	Roe et al. (2001), Campbell et al. (2005)
<i>Lemiox rimosus</i> (Rafinesque, 1831)	AY655002	AY655049	AY655104	Campbell et al. (2005)
<i>Leptodea fragilis</i> (Rafinesque, 1820)	AF049519	AY238483		Roe and Lydeard (1998), Krebs et al. (2003)
<i>Leptodea leptodon</i> (Rafinesque, 1820)	AY655003	AY655050	AY655105	Campbell et al. (2005)
<i>Ligumia nasuta</i> (Say, 1817)	AF156515	AY655052		Graf and O'Foighil (2000), Campbell et al. (2005)
<i>Ligumia recta</i> (Lamarck, 1819)	AF156516	AF385134		Graf and O'Foighil (2000), Roe et al. (2001)
<i>Medionidus acutissimus</i> (Lea, 1831)	AY655005	AY655054	AY655107	Campbell et al. (2005)
<i>Medionidus conradicus</i> (Lea, 1834)	AY655006		AY158746	Campbell et al. (2005)
<i>Obliquaria reflexa</i> (Rafinesque 1820)	AY655008	AY655055	AY655108	Campbell et al. (2005)
<i>Obovaria jacksoniana</i> (Frierson, 1912)	AY655009		AY655109	Campbell et al. (2005)
<i>Obovaria olivaria</i> (Rafinesque, 1820)	AF232812	AF232787		Lydeard et al. (2000)
<i>Obovaria unicolor</i> (I. Lea, 1845)	AF232811	AF232786		Lydeard et al. (2000)
<i>Obovaria subrotunda</i> (Rafinesque, 1820)	AY655010	AY655056		Campbell et al. (2005)
<i>Potamilus alatus</i> (Say, 1817)	AF231752	AY238484	AY655119	Bogan and Hoeh (2000), Krebs et al. (2003), Campbell et al. (2005)
<i>Potamilus amphichaenus</i> (Frierson, 1898)	AF049517			Roe and Lydeard (1998)
<i>Potamilus inflatus</i> (I. Lea, 1831)	AF049506			Roe and Lydeard (1998)
<i>Potamilus ohioensis</i> (Rafinesque, 1820)	AF049515			Roe and Lydeard (1998)
<i>Potamilus purpuratus</i> (Lamarck, 1819)	AF406804	U72573		Roe and Lydeard (1998); Lydeard et al. (1996)
<i>Ptychobranchus fasciolaris</i> (Rafinesque, 1820)	AF156514	AY655075	AY655120	Graf and O'Foighil (2000), Campbell et al. (2005)

(continued on next page)

Table 1 (continued)

Species	GenBank Accession No(s).			Studies
	COI	16S	ND1	
<i>Toxolasma lividus</i> (Rafinesque, 1831)	AF231756			Bogan and Hoeh (2000)
<i>Toxolasma parvus</i> (Barnes 1823)	AY655022	AY238482	AY655123	Campbell et al. (2005), Krebs et al. (2003)
<i>Toxolasma texasiensis</i> (Lea, 1857)	AY655023	AY655078	AY655124	Campbell et al. (2005)
<i>Truncilla truncata</i> (Rafinesque, 1820)	AF156513	AY655089	AY655125	Graf and O'Foighil (2000), Campbell et al. (2005)
<i>Venustaconcha ellipsiformis</i> (Conrad, 1836)	DQ220725	AY655082	DQ220722	This study, Campbell et al. (2005)
<i>Venustaconcha pleasii</i> (Marsh, 1891)	AY655026		AY655126	Campbell et al. (2005)
<i>Villosa fabalis</i> (I. Lea, 1831)	DQ220726		DQ220723	This study
<i>Villosa iris</i> (I. Lea, 1829)	AF156524	AY655083	AY655127	Graf and O'Foighil (2000), Campbell et al. (2005)
<i>Villosa vanuxemensis</i> (I. Lea, 1838)	AF156526	AY655084		Graf and O'Foighil (2000), Campbell et al. (2005)

(Huelsenbeck and Ronquist, 2001) was determined by comparing 24 models of evolution in MrModeltest 2.2 (Nylander, 2004). MrBayes was run using 1,000,000 generations, sampling every 100 generations (10,000 trees total), and the most likely tree was calculated using posterior probabilities with a burn-in of 40,000 generations (400 trees). A 50% majority-rule consensus tree was constructed from the remaining 9600 trees.

The parsimony and Bayesian trees were compared using the parametric Kishino-Hasegawa (KH) (Kishino and Hasegawa, 1989), non-parametric Templeton (Templeton, 1983), and non-parametric winning-sites tests (Prager and Wilson, 1988). A likelihood version of the KH test and the Shimodaira-Hasegawa test (SH) (Shimodaira and Hasegawa, 1999) was run in PAUP* v4.0b10 using the best-fit model selected by MrModeltest 2.2 to determine the most likely tree.

The implications for the evolution of lure morphologies within the Lampsilini were determined by mapping the lure strategies (Table 2) on the topologies generated in the maximum parsimony and Bayesian analyses using MacClade v4.05 (Maddison and Maddison, 1997).

3. Results

Sequences from fragments of the mitochondrial genes COI and ND1 were generated from *Epioblasma torulosa rangiana*, *Venustaconcha ellipsiformis*, and *V. fabalis* and an ND1 sequence was generated from *L. fasciola* (Table 1). These sequences were combined with the COI, 16S and ND1 sequences obtained from GenBank (Table 2). Two short variable regions in the 16S gene (64 bp total) were excluded from the analysis because positional homology was unclear. The final alignment had 2027 characters of which 606 were found to be potentially phylogenetically informative. The aligned data matrices are available from the authors. Many taxa do not have complete datasets available for analysis; therefore the topology, resolution, and/or support values may change with the missing data or additional taxa added to the analysis.

A strict consensus of the ten equally most parsimonious trees was constructed (4166 steps, CI=0.32, RI=0.46, RC=0.15; Fig. 1). Monophyly of the tribe Lampsilini was supported. However, the tree showed a high degree of poly-

phyly of genera within the Lampsilini. The monophyletic genera represented by more than one species included *Epioblasma*, *Hamiota*, *Potamilus*, *Toxolasma* and *Venustaconcha*. The genera *Actinonaias*, *Lampsilis*, *Leptodea*, *Ligumia*, *Medionidus*, *Obovaria*, and *Villosa* were all resolved as being paraphyletic or polyphyletic. Decay indices and bootstrapping generally did not strongly support most clades. Decay indices showed that only 1 or 2 additional steps were required to break many relationships in the tree. Bootstrapping likewise did not support many of the clades beyond genus level. Within the Lampsilini, good support was obtained for the following: clades consisting of *Epioblasma* + *Venustaconcha* + *Obovaria*; *Leptodea* + *Potamilus*; *Truncilla* + *Ellipsaria*; and *Cyprogenia* + *Dromus* (Fig. 1).

A 50% majority-rule consensus of 9600 Bayesian likelihood trees (burn-in=400 trees, mean log likelihood = -19,593) was constructed from the dataset (4247 steps, CI=0.31, RI=0.43, RC=0.13; Fig. 2). The initial model of evolution selected by MrModeltest 2.2 (Nylander, 2004) was the General Time Reversible model with invariant sites and gamma correction (GTR+I+ Γ). Most of the same relationships were resolved as in the parsimony tree (Fig. 1). Like the parsimony tree, *Epioblasma* + *Obovaria* + *Venustaconcha* formed a well-supported clade; the group of *Leptodea* + *Potamilus* were monophyletic; *Truncilla* + *Ellipsaria* formed a clade; and *Cyprogenia* and *Dromus* were sister taxa. The Bayesian topology also resolved well-supported clades for: Lampsilini, *Cyrtonaias* + *Glebula* + *Obliquaria*, the genus *Toxolasma* and *Toxolasma* + *V. fabalis*, *Medionidus* + *Lemiox* + *Ptychobranthus* + *Cyprogenia* + *Dromus*, and *Lampsilis teres* + the genus *Hamiota* (Fig. 2).

Comparisons of the parsimony and Bayesian topologies were made using the parsimony based parametric KH and the non-parametric Templeton and winning-sites tests. The parsimony and Bayesian topologies were not found to be of significantly different lengths at the $\alpha=0.05$ level ($P=0.1110$ for the KH test, $P=0.1123$ for the Templeton test, and $P=0.1073$ for the winning-sites test). Using a maximum likelihood version of the KH and SH tests (with 1000 bootstrap replicates) to determine the most likely tree based on the GTR+I+ Γ model of evolution selected by MrModeltest 2.2 (Nylander, 2004), the Bayesian topology was found to be the most likely tree ($P=0.001$ for the KH test and $P=0.001$ for the SH test).

Table 2

Active host-attraction (0 = absent, 1 = present) and lure morphology (0 = no lure, 1 = elaborate conglutinate, 2 = active valve gaping, 3 = worm-like caruncle, 4 = superconglutinate, 5 = active mantle “trap”, 6 = active mantle flap) mapped onto molecular phylogeny (Figs. 3 and 4)

Species	Active host-attraction strategy	Lure morphology	Source
Outgroup species			
<i>Cumberlandia monodonta</i> (Say, 1829)	0	0	http://unionid.missouristate.edu
<i>Fusconaia flava</i> (Rafinesque 1820)	0	0	Personal observations, http://unionid.missouristate.edu
<i>Popenaias popeii</i> (Lea, 1857)	0	0	Smith et al. (2003)
<i>Pyganodon grandis</i> (Say, 1829)	0	0	Lydeard et al. (1996), Davis and Fuller (1981)
<i>Amblema plicata</i> (Say, 1817)	0	0	Personal observations
<i>Lexingtonia dollaballoides</i> (Lea, 1840)	Unknown	Unknown	
<i>Hemistena lata</i> (Rafinesque, 1820)	Unknown	Unknown	
<i>Elliptio dilatata</i> (Rafinesque, 1820)	0	0	Personal observations
<i>Pleurobema clava</i> (Lamarck, 1819)	0	0	Personal observations
Ingroup species			
Tribe Lampsilini			
<i>Actinonaias ligamentina</i> (Lamarck, 1819)	0	0	Graf and O’Foighil (2000), personal observations
<i>Actinonaias pectorosa</i> (Conrad, 1834)	0	0	M. McGregor, Kentucky Dept. of Fish and Wildlife Resources, personal communication
<i>Cyprogenia stegaria</i> (Rafinesque, 1820)	1	1	Jones and Neves (2002), http://unionid.missouristate.edu
<i>Cyrtonaias tampicoensis</i> (I. Lea, 1838)	0	0	R. Howells, Texas Parks and Wildlife Department, personal communication
<i>Dromus dromas</i> (I. Lea, 1834)	1	1	Jones et al. (2004)
<i>Ellipsaria lineolata</i> (Rafinesque, 1820)	1	2	M. Davis, Minnesota DNR, personal communication
<i>Epioblasma brevidens</i> (I. Lea, 1831)	1	5	Jones (2004)
<i>Epioblasma capsaeformis</i> (I. Lea, 1834)	1	5	Jones (2004)
<i>Epioblasma florentina walkeri</i> (Wilson and Clark, 1914)	1	5	Jones (2004)
<i>Epioblasma torulosa rangiana</i> (Rafinesque, 1839)	1	5	Jones (2004)
<i>Epioblasma triquetra</i> (Rafinesque, 1820)	1	5	Jones (2004)
<i>Glebula rotundata</i> (Lamarck, 1819)	0	0	R. Howells, Texas Parks and Wildlife Department, personal communication
<i>Hamiota altilis</i> (Conrad, 1834)	1	4 or 6	Roe et al. (2001); Roe and Hartfield (2005)
<i>Hamiota australis</i> (Simpson, 1900)	1	4 or 6	Roe et al. (2001); Roe and Hartfield (2005)
<i>Hamiota perovalis</i> (Conrad, 1834)	1	4 or 6	Roe et al. (2001), Roe and Hartfield (2005)
<i>Hamiota subangulata</i> (I. Lea, 1840)	1	4 or 6	Roe et al. (2001), Roe and Hartfield (2005)
<i>Lampsilis cardium</i> (Say, 1831)	1	6	http://www.biosci.ohio-state.edu/~molluscs/OSUM2/gallery.htm , Personal observations
<i>Lampsilis fasciola</i> (Rafinesque, 1820)	1	6	Personal observations
<i>Lampsilis ornata</i> (Conrad, 1835)	1	6	Haag and Warren (2003)
<i>Lampsilis ovata</i> (Say, 1817)	1	6	http://www.biosci.ohio-state.edu/~molluscs/OSUM2/gallery.htm , Personal observations
<i>Lampsilis siliquoidea</i> (Barnes, 1823)	1	6	Graf and O’Foighil (2000), Davis and Fuller (1981), Personal observations
<i>Lampsilis teres</i> (Rafinesque, 1820)	1	6	Lydeard et al. (1996), Davis and Fuller (1981)
<i>Lemiox rimosus</i> (Rafinesque, 1831)	1	6	J. Jones, Virginia Tech, personal communication
<i>Leptodea fragilis</i> (Rafinesque, 1820)	0	0	Simpson (1914), Personal observations
<i>Leptodea leptodon</i> (Rafinesque, 1820)	0	0	Simpson (1914)
<i>Ligumia nasuta</i> (Say, 1817)	1	6	D. Strayer, Institute of Ecosystem Studies, personal communication
<i>Ligumia recta</i> (Lamarck, 1819)	1	6	Barnhart and Baird (2000), Personal observations
<i>Medionidus acutissimus</i> (Lea, 1831)	1	6	Haag and Warren (2003)
<i>Medionidus conradicus</i> (Lea, 1834)	1	6	M. McGregor, Kentucky Dept of Fish and Wildlife Resources, personal communication
<i>Obliquaria reflexa</i> (Rafinesque 1820)	0	0	Personal observations,
<i>Obovaria jacksoniana</i> (Frierson, 1912)	Unknown	Unknown	
<i>Obovaria olivaria</i> (Rafinesque, 1820)	0	0	A. Martel, Caanadian Museum of Nature, personal communication
<i>Obovaria unicolor</i> (I. Lea, 1845)	0	0	Haag and Warren (2003)
<i>Obovaria subrotunda</i> (Rafinesque, 1820)	0	0	Personal observations
<i>Potamilus alatus</i> (Say, 1817)	0	0	Simpson (1914), Personal observations

(continued on next page)

Table 2 (continued)

Species	Active host-attraction strategy	Lure morphology	Source
<i>Potamilus amphichaenus</i> (Frierson, 1898)	0	0	Inferred from Simpson (1914)
<i>Potamilus inflatus</i> (ILea, 1831)	0	0	Inferred from Simpson (1914)
<i>Potamilus ohiensis</i> (Rafinesque, 1820)	0	0	Simpson (1914), Personal observations
<i>Potamilus purpuratus</i> (Lamarck, 1819)	0	0	Inferred from Simpson (1914)
<i>Ptychobranhus fasciolaris</i> (Rafinesque, 1820)	1	1	Watters (1999), Personal observations
<i>Toxolasma lividus</i> (Rafinesque, 1831)	1	3	M. McGregor, Kentucky Dept of Fish and Wildlife Resources, personal communication
<i>Toxolasma parvus</i> (Barnes 1823)	1	3 or 6	Burch (1973), T. Watters, Ohio State University, personal communication
<i>Toxolasma texasiensis</i> (Lea, 1857)	1	3	R. Howells, Texas Parks and Wildlife Department, personal communication
<i>Truncilla truncata</i> (Rafinesque, 1820)	1	2	M. Davis, Minnesota DNR, personal communication
<i>Venustaconcha ellipsiformis</i> (Conrad, 1836)	1	6	Hove and Anderson (1997)
<i>Venustaconcha pleasii</i> (Marsh, 1891)	1	6	http://unionid.missouristate.edu
<i>Villosa fabalis</i>	1	6	http://www.biosci.ohio-state.edu/~molluscs/OSUM2/gallery.htm , personal observations
<i>Villosa iris</i> (ILea, 1829)	1	6	http://unionid.missouristate.edu
<i>Villosa vanuxemensis</i> (ILea, 1838)	1	6	Inferred from Ortmann (1921)

The parsimony and Bayesian topologies do not differ significantly under a parsimony framework. However, under likelihood settings the Bayesian tree is significantly more likely using either the KH or more conservative SH test (Goldman et al., 2000). The two trees are quite similar in topology. Considering the two lure characters, the lure strategy character requires six steps in both the parsimony and Bayesian topologies (Fig. 3), while the specific lure morphology character is one step shorter in the Bayesian topology—10 versus 11 steps, respectively (Fig. 4).

The host-attraction strategies (Fig. 3) and known specific lure types (Fig. 4) were mapped on the parsimony (Figs. 3A and 4A) and Bayesian (Figs. 3B and 4B) trees. The parsimony topology suggested a single evolution of active host-attraction strategies with secondary loss of active lures in several clades (e.g., the clade of *Leptodea* + *Potamilus*). The ancestral state for host-attraction strategy in lamsilines was equivocal between no lure and active mantle flap in the Bayesian tree. The Bayesian topology did not refute the hypotheses of lure evolution made in the parsimony tree. The major differences between the parsimony and Bayesian topologies was the placement of the clade of *Leptodea* + *Potamilus* and at the base of the lamsilines with the placement of *Cyrtonaias* + *Glebula*, *Obliquaria*, *V. fabalis*, and *Toxolasma*. Other minor differences lay in the placement of some terminal taxa (see Figs. 1 and 2).

4. Discussion

This phylogenetic analysis of the Lamsilini provides the best-resolved and most complete hypothesis of lamsilini evolution proposed to date. Although many of the basal nodes are not well-supported by bootstrapping and decay indices, or Bayesian posterior probabilities, they are corroborated by other phylogenetic studies of unionoid

systematics (Campbell et al., 2005; Hoeh et al., 2001; Lydeard et al., 1996). We recognize that this topology could change when more taxa and sequence data are added to the analysis.

4.1. Evolution of lures in the Lamsilini

The presence of an active host-attraction strategy (lures) appears to have occurred early in the evolution of lamsilines as some form of a mantle flap. The parsimony tree (Fig. 4A) showed that the mantle flap lure evolved in an ancestral lamsilini sister to *Cyrtonaias* + *Glebula*. Mapping the host-attraction strategies onto both the parsimony and Bayesian topologies revealed that active host-attraction strategies evolved early in the Lamsilini. This is best-resolved in the parsimony tree where lures first appear in the common ancestor of *V. fabalis* (Appendix A) and the remaining lamsilines (exclusive of *Cyrtonaias* and *Glebula*). The ancestral state of lure strategies could not be elucidated in the Bayesian topology (Fig. 4B), as the ancestral state was equivocal. The first lures appear to be some type of mantle flap, with all other lures being derived from this character state (Fig. 4A).

Both trees reveal a common ancestry between *Truncilla truncata* and *Ellipsaria lineolata*, linked by their nearly identical lure strategies (Fig. 4). Species of *Truncilla* and the monotypic genus *Ellipsaria* have not been known to use any kind of mantle flap to attract a host (Ortmann, 1919; Simpson, 1914). However, during recent nighttime observations in the St. Croix River (Minnesota/ Wisconsin, USA), SCUBA divers found gravid female *T. truncata* and *E. lineolata* at the surface of the substrate. When touched or disturbed by a diver the animal would open its shell widely revealing a bright white marsupial gill loaded with glochidia (M. Davis, Minne-

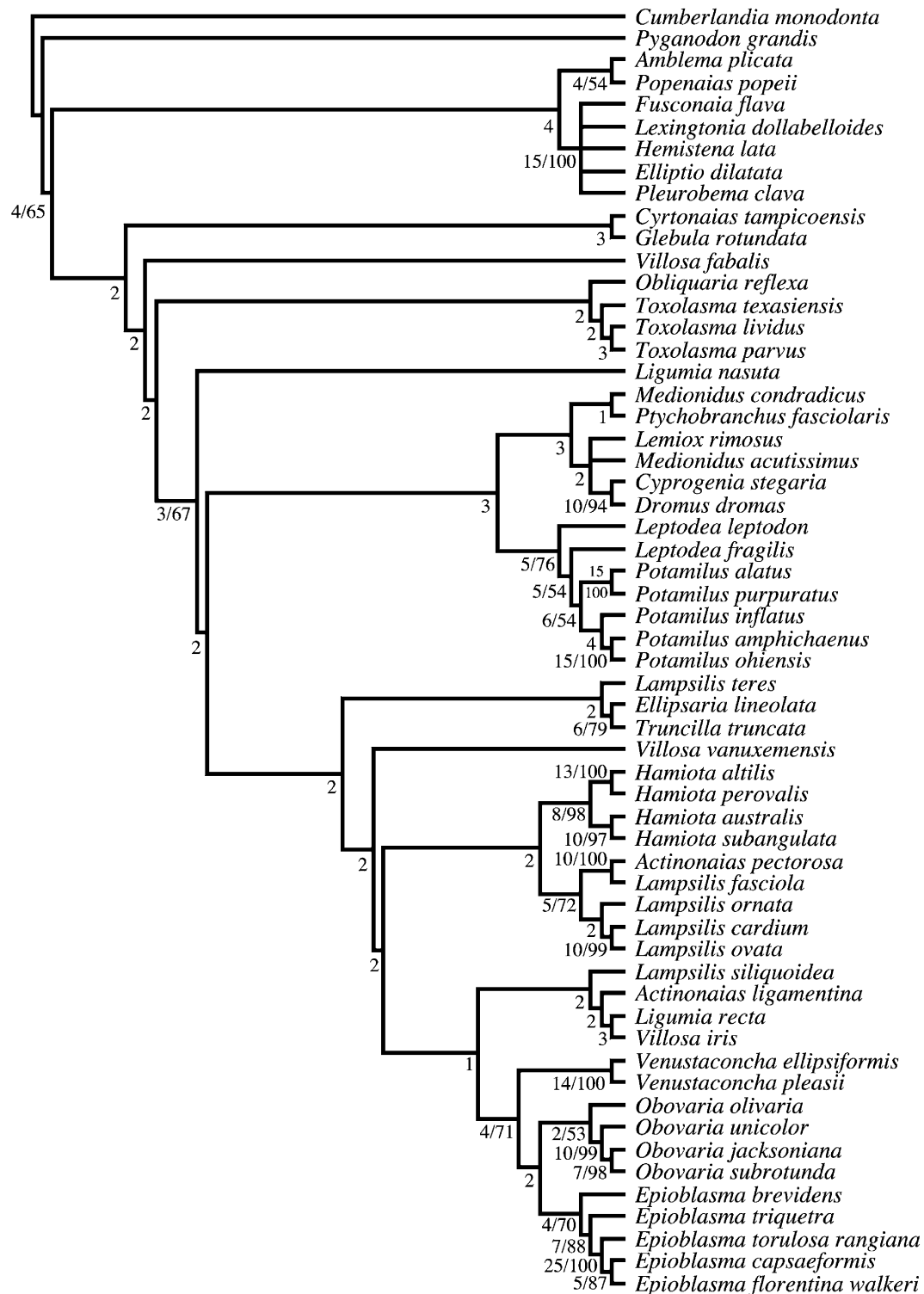


Fig. 1. A strict consensus of the 10 equally parsimonious trees (4166 steps, CI = 0.32, RI = 0.46, RC = 0.15) using an unweighted maximum parsimony analysis of the COI, 16S, and ND1 sequence data and two morphological characters of the tribe Lampsilini. The numbers at the nodes indicate the proportion of replications (1000 replicates with 10 random stepwise additions) that a particular clade occurred in the bootstrap analysis (only proportions greater than 50% are shown) and the Bremer–Decay Indices.

sota Dept. of Natural Resources, personal communication). This behaviour may lure a passing host as they brush by the mussels in search of prey. The sauger (*Stizostedion canadense*) and freshwater drum (*Aplodinotus*

grunniens) are the known hosts for *T. truncata* (Wilson, 1916) and freshwater drum is the only known host for *E. lineolata* (Coker et al., 1921). Although no active flapping of a mantle or papillae was observed, this appears to



Fig. 2. A 50% majority-rule consensus tree created through Bayesian inference (10,000 trees, burn-in = 400 trees, mean log likelihood = -19,593) constructed from the COI, 16S and ND1 sequence data of the tribe Lampsilini. The numbers shown at the nodes are the calculated posterior probabilities (greater than 50%), indicating the proportion of trees that these nodes appeared.

be active luring behaviour that is a synapomorphy for these species. Sequence data and lure observations for other species of *Truncilla* (i.e., *T. donaciformis* and *T. macrodon*) need to be included in a lampsilini phylogeny to confirm the relationship of *Truncilla* and *Ellipsaria*.

Most of the Lampsilini use some form of packaging of glochidia in addition to an active lure (e.g., movement of the mussel to attract a host). However, none of the described conglutinates are as elaborate or manipulated by the mussel and water currents as those of *Ptychobranthus*

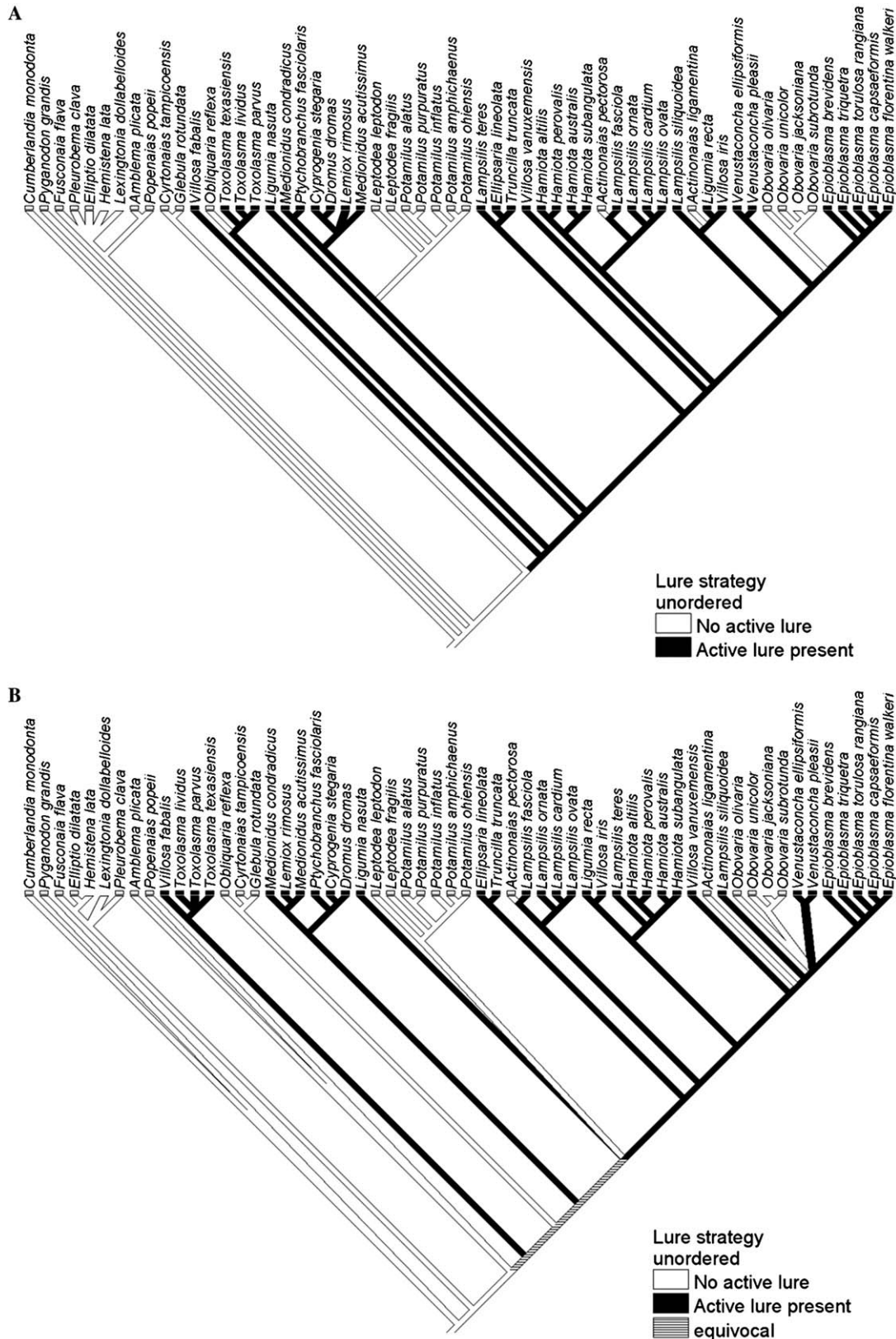


Fig. 3. Generalized host-attraction strategies mapped onto the phylogenies resolved using (A) maximum parsimony and (B) Bayesian likelihood.

(Watters, 1999), *Cyprogenia* (Jones and Neves, 2002), and *Dromus* (Jones et al., 2004). The conglutinates of *Ptychobranchus* resemble fish fry, complete with pigmentation

resembling eyes and lateral lines or brightly coloured mimics of insect larvae such as simuliids or chironomids (Watters, 1999; Appendix A). The inclusion of sequence

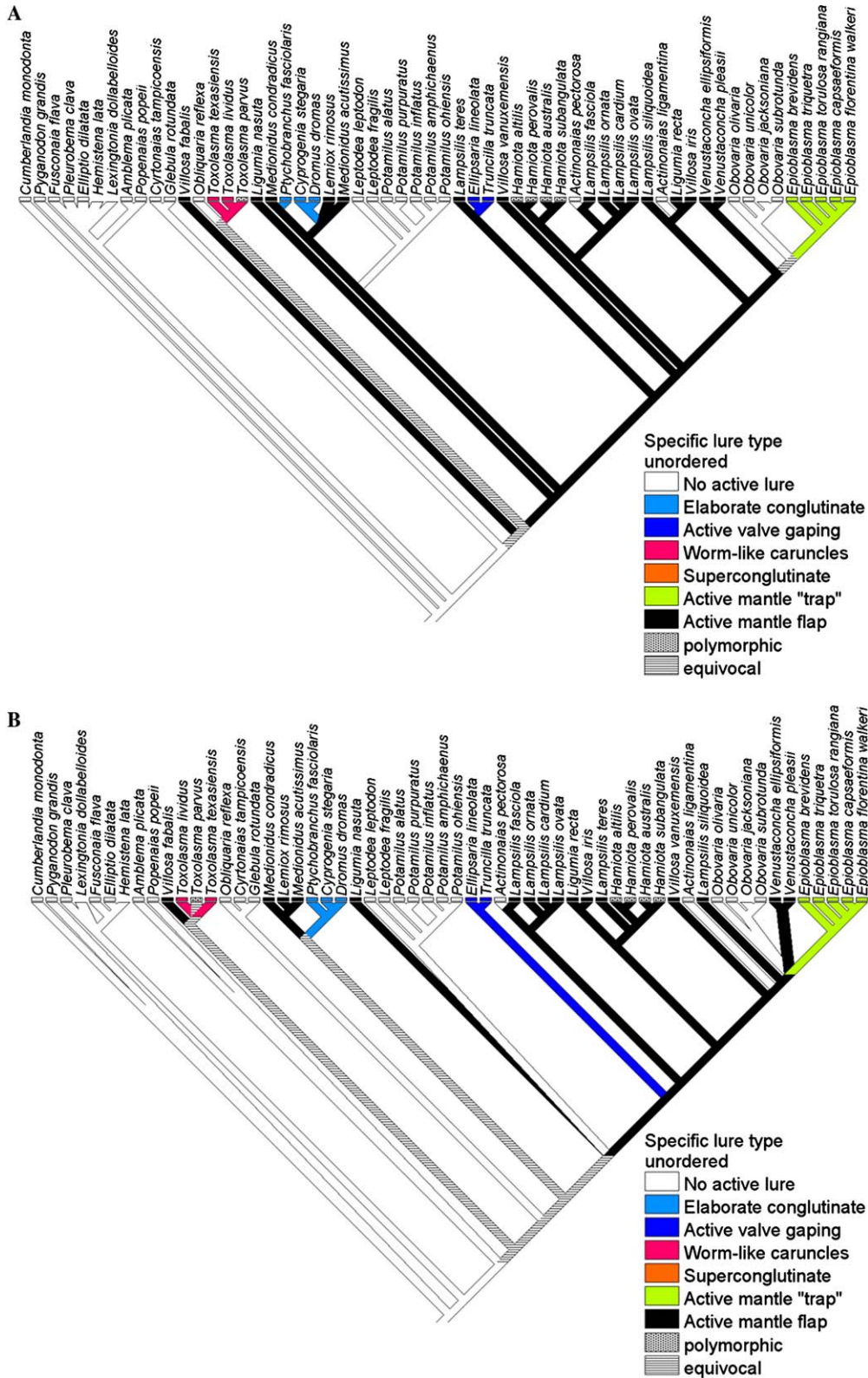


Fig. 4. Specific lure morphologies mapped on the phylogenies resolved using (A) maximum parsimony and (B) Bayesian likelihood.

data from other species of *Ptychobranchius* (*P. greeni*, *P. jonesi*, *P. occidentalis*, and *P. subtenum*) should strengthen the phylogenetic placement of this genus. Closely related *Cyprogenia* and the monotypic genus *Dromus* both use

large, often brightly pigmented, worm-like conglutinates that are actively manipulated by the mussel or water currents to attract a host (Jones and Neves, 2002; Jones et al., 2004; Appendix A). When this character state is mapped

onto the phylogeny, use of a mantle flap lure was secondarily and independently lost in these taxa. This loss could be attributed to the development of these elaborate conglutinates that negated the benefit of a metabolically expensive flapping lure.

The association of *Potamilus* + *Leptodea* clade is one of the better-supported clades. The loss of an active lure links all members of this group. Virtually all of the species in this group exclusively use the freshwater drum (*Aplodinotus grunniens*) as a host (Barnhart et al., 1998; Coker and Surber, 1911; Howard, 1913; Roe et al., 1997; Surber, 1913). Therefore, it is possible that *A. grunniens*, being primarily a benthivorous molluscivore (Scott and Crossman, 1973), would not be attracted to a flapping fish-like or worm-like lure. As with *Ptychobranchus*, *Cyprogenia* and *Dromus*, this loss could be attributed to the lack of usefulness in having a metabolically expensive active lure.

The superconglutinate-producing mussels of the genus *Hamiota* form a well-supported, monophyletic clade in both the parsimony and Bayesian analyses. Roe et al. (2001) investigated the species level relationships but did not give a phylogenetic hypothesis of evolution for the superconglutinate-producing mussels in relation to a large number of lamspsiline taxa. Both the parsimony and Bayesian analyses have the superconglutinate-producing clade arising from lamspsilines that use active mantle flaps. The superconglutinate lure (Appendix A) was only first observed in the mid-1990's—after more than 175 years of unionoid systematics research (Fleischmann, 1997). *Hamiota* also have a structure, resembling the mantle flaps observed in species of *Lampsilis* and *Villosa*, along the posterior mantle edge and may use this as a mantle flap in addition to the more conspicuous and unique superconglutinate lure (Roe and Hartfield, 2005). Before the superconglutinate lure strategy was discovered (Haag et al., 1995), older, morphologically based taxonomies had placed these species with *Lampsilis* and *Villosa*. Based on their monophyly, molecular data and unique lure strategy and morphology, Roe and Hartfield (2005) proposed that this group be reclassified as the new genus *Hamiota*. Our analyses support the recognition of the new genus.

4.2. Systematic and taxonomic implications

In the parsimony tree (Fig. 1) *Cyrtonaias tampicoensis* and *Glebula rotundata* form a clade sister to the much larger group of lamspsilines. Campbell et al. (2005) show these species to be lamspsilines. However, Campbell et al. (2005) suggest that a greater sampling from Central American species of *Cyrtonaias* may be necessary to better resolve and add support to the base of the lamspsiline tree. Neither *C. tampicoensis*, nor *G. rotundata*, are known to use a lure (R. Howells, Texas Parks and Wildlife Division, personal communication). However, the presence of specialized gill structures place these taxa in close affinity to the Lampsilini (Hoeh et al., 2001; Howells et al., 1996). The parsimony and

Bayesian trees differ in the placement of these taxa. By mapping the host-attraction strategies onto the parsimony topology, it appears these taxa diverged prior to the evolution of active host lures found in most of other lamspsilines. However, the Bayesian topology shows the lure-using *V. fabalis* + *Toxolasma* clade as sister to the remaining lamspsilines.

The parsimony and Bayesian topologies differ on the relationship of *Toxolasma* and *V. fabalis*. The Bayesian analysis reveals *V. fabalis* to be the sister to *Toxolasma*, while the parsimony tree shows *V. fabalis* to be sister to the ancestor of the remaining lamspsilines (exclusive of *Cyrtonaias* + *Glebula*). The results of our analyses are concurrent to those of J.E. Buhay (Brigham Young University, personal communication) who found that *Villosa* shows a high degree of polyphyly with *V. fabalis*, a sister taxon to the remaining *Villosa* and other lamspsilines. Further investigations of these relationships and lures used in this clade are warranted.

Species of *Lampsilis* show the highest degree of polyphyly in our phylogenetic hypotheses. The inclusion of *Lampsilis* within several other mixed clades (Figs. 1 and 2) make the current classification quite problematic. Most of these species are known to use some form of mantle flap as a lure, although such lure use appears to have been secondarily and independently lost in species of *Actinonaias*, *Obovaria*, and *Truncilla* + *Ellipsaria*. Some of the most elaborate mantle flap lures are found in *Lampsilis* and *Villosa* (Haag et al., 1995). Many *Lampsilis* have lures that closely mimic small fish, even going so as far as having large eyespots, a lateral line, and fins (Appendix A). An indication of the “true *Lampsilis*” (by the inclusion of the type species, *L. ovata*) is shown by the high support values given to the group of *L. cardium*, *L. fasciola*, *L. ovata*, and *L. ornata*, and *Actinonaias pectorosa* in both the parsimony and Bayesian topologies. Increased taxon sampling and sequence data should clear the confusion that remains in *Lampsilis*.

The genus *Ligumia* has long been a conundrum in unionoid systematics. Early on, taxonomists had considered *L. recta* and *L. nasuta* as congeneric because of their similar shells (Ortmann, 1919; Simpson, 1914). Our analysis refutes this taxonomy and is consistent with the analyses of Davis and Fuller (1981), Graf and O'Foighil (2000), and Campbell et al. (2005). The Bayesian analysis places *L. nasuta* in a polytomy sister to *Potamilus* + *Leptodea* and all remaining lamspsilines (exclusive of *Cyrtonaias*, *Glebula*, *Obliquaria*, *V. fabalis*, *Toxolasma*, *Medionidus*, *Lemiox*, *Ptychobranchus*, *Cyprogenia*, and *Dromus*) and the parsimony tree places it as a sister taxon to the ancestor of virtually all of the remaining lamspsilines (exclusive of *Cyrtonaias*, *Glebula*, *Obliquaria*, *V. fabalis*, and *Toxolasma*). Neither topology place it as sister taxon to *L. recta*. Because *L. recta* is the type species of the *Ligumia*, *L. nasuta* would require designation into a currently existing or newly described genus. *Ligumia subrostrata* is thought to be closely related to *L. nasuta*. *Ligumia subrostrata* has yet to be sequenced; these data could better resolve the phylogenetic placement and taxonomic classification of *L. nasuta*.

The *Epioblasma* form a well-supported clade further strengthened by their unique and incredible lures and host infestation behaviour. The well-supported phylogeny of *Epioblasma* (including the type species *E. t. rangiana*) produced by our parsimony and Bayesian analyses largely agrees with classifications and phylogenies created by Johnson (1978), Buhay et al. (2002), and Jones (2004). Species of *Epioblasma* show extreme sexual dimorphism. Females of many *Epioblasma*, like many other freshwater mussels from the tribe Lampsilini, use a lure along the posterior margin of the shell to attract a potential host fish. Females from Johnson's (1978) subgenus *Torulosa* have elaborate and often a brightly coloured, spongy mantle pad (Appendix A). Some of these species have one or more micro-lures that mimic the cerci of an aquatic insect. These lures entice darters (*Etheostoma* and *Percina*) close enough to parasitize them with glochidia (Jones, 2004). Many *Epioblasma* use their mantle pads and shells like a trap to capture a potential host long enough to parasitize with glochidia (Jones, 2004; Appendix A). The parsimony tree shows the mantle pad 'trap' structures were derived from other mantle flap lures (Fig. 4A).

On the basis of lure morphology, *Epioblasma*, *Venustaconcha*, and *Obovaria* are not similar. Few comparisons of reproductive structures (i.e., gill marsupia or glochidia) and host fish usage in *Venustaconcha* and *Obovaria* exist to support the topologies we are presenting. In terms of lure strategy, species of *Venustaconcha* have a lure that consists of active flapping small papillae around the excurrent siphon (Hove and Anderson, 1997; Appendix A). *Obovaria unicolor* does not use an active lure (Haag and Warren, 2003). Field observations of *O. subrotunda* (by DTZ) and *O. olivaria* (A. Martel, Canadian Museum of Nature, personal communication) have not revealed any obvious lure behaviour. Thus, it is likely that closely related *O. jacksoniana* does not use an active lure either. Mapping the lure strategies of lampsiline mussels onto our topologies revealed that the lure might have been secondarily lost in all species of *Obovaria*. Mirroring Campbell et al. (2005), efforts should be made (if possible) to sequence DNA and lure strategy observations from *O. retusa*, the nearly extinct type species of *Obovaria*. These data are required to determine the validity of the genus and the ancestral state of lure strategies.

Our placement of *Venustaconcha ellipsiformis* does not agree with the recently published phylogeny of Campbell et al. (2005). The difference might have owed to an error in identification. We found that *V. ellipsiformis* was sister to *V. pleasii*. This was supported with high bootstrap values and Bayesian posterior probabilities. Campbell et al. (2005) state that the sequence for *V. pleasii* may have been a mislabeled *Obovaria olivaria*. However, it appears from our results that the labeling was either correct or it was another *V. ellipsiformis*. The locality for the animals we collected, eastern Iowa, was outside the range for *V. pleasii* (Oesch, 1984). We are confident in the identification of our samples of *V. ellipsiformis*, as our samples were independently identified by an expert (D. Woolnough, Iowa State University; shell photographs available upon request).

We found that the use of active lures in the tribe Lampsilini had a single origin. Strategies of host-attraction appeared to have evolved to exploit predator-prey relationships and feeding guilds of host fish (Haag and Warren, 1999). The use of an active lure was an amazing adaptive response by lampsiline freshwater mussels to the problem of finding a host for their glochidial larvae. Lures appear to have been secondarily lost several times in the phylogeny (i.e., *Potamilus* + *Leptodea*, *Actinonaias ligamentina*, *A. pectorosa*, *Obovaria*, and possibly *Obliquaria* + *Cyrtonaias* + *Glebula*), and in some cases a mantle flap lure was replaced by elaborate conglutinates (i.e., *Ptychobranthus*, *Dromus*, and *Cyprogenia*). Because the host-glochidia interaction is critical to the life cycle of freshwater mussels, this makes the host-attraction stage among the most important stages in the life history of freshwater mussels. The diversity and function of these lures deserve further study and description. We hope that this research will further the understanding of evolution and life histories in these increasingly imperiled organisms.

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Appendix A

Photos and videos of lampsiline host-attraction strategies available on the World Wide Web

Species	URL
<i>Cyprogenia aberti</i>	http://unionid.missouristate.edu/gallery/cyprogenia/fanshell.htm
<i>Epioblasma torulosa rangiana</i>	http://unionid.missouristate.edu/gallery/Epioblasma/riffleshell_5MB.wmv
<i>Epioblasma triquetra</i>	http://unionid.missouristate.edu/gallery/Epioblasma/gallery_snuffbox_1.wmv
<i>Hamiota perovalis</i>	http://unionid.missouristate.edu/gallery/L_perovalis/perovalis-4.mpg

(continued on next page)

Appendix A (continued)

Species	URL
<i>Lampsilis ovata</i>	http://www.biosci.ohio-state.edu/~molluscs/OSUM2/images/DSCN1707.MOV
<i>Lampsilis cardium</i>	http://www.biosci.ohio-state.edu/~molluscs/OSUM2/images/DSCN1704.MOV
<i>Ptychobranthus occidentalis</i>	http://unionid.missouristate.edu/gallery/ouachita/kidneyshell.htm
<i>Ptychobranthus subtentum</i>	http://unionid.missouristate.edu/gallery/Psubtentum/fluted.htm
<i>Venustaconcha ellipsiformis</i>	http://unionid.missouristate.edu/gallery/venustaconcha/venus.mpg
<i>Villosa fabalis</i>	http://www.biosci.ohio-state.edu/~molluscs/OSUM2/images/fabalis.wmv
<i>Villosa iris</i>	http://unionid.missouristate.edu/gallery/Villosa_iris/villosa_iris_movie.htm

Appendix B

Definition of host-attraction strategies described in text and Table 2

Host-attraction strategy	Definition
No active lure	No demonstrated movement or use of water currents by the female mussel to attract a potential host
Elaborate conglutinate	Use of a large conglutinate (package of glochidia) with structures mimicking eyes, head, and coloration of a potential prey item and manipulation of the package by the mussel or water currents to entice a host attack
Active valve gaping	Gravid females actively position themselves high in the substrate followed by opening of the valves of a gravid female upon contact with a potential host, revealing bright white gill marsupia
Worm-like caruncles	Small actively moving worm-like extensions of the mantle
Superconglutinate	The contents of both marsupial gills are extruded simultaneously within a mucus jacket, and remain tethered to the female by a clear mucus cord. The mucus jacket has eyespots and is pigmented mimicking the lateral line of a fish. Water currents cause the tethered package to appear to move like a prey fish
Active mantle “trap”	Females gape their valves widely (sometimes with small moving lures mimicking the cerci of aquatic insects). Upon disturbance by a potential host investigating the lures or gape, the valves rapidly snap shut and a mantle pad inflates capturing the host in order to infest it with glochidia. After infestation the host is released
Active mantle flap	Highly diverse, pigmented and/ or papillose structures that are flapped vigorously by the female mussel

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