Evolution of bilaterally asymmetrical larvae in freshwater mussels (Bivalvia: Unionoida: Unionidae)

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Received 1 January 2015; revised 2 March 2015; accepted for publication 9 April 2015

Bilaterally asymmetrical glochidia (i.e. bivalved parasitic larvae bearing a large marginal appendage on a single valve) have been reported from five Asian freshwater mussel genera belonging to two separate subfamilies, the Gonideinae (i.e. Pseudodon, Solenaia, and Physunio) and Rectidentinae (i.e. Contradens and Trapezoideus). This classification requires that the bilaterally asymmetrical glochidium-bearing mussels are not monophyletic, and suggests that this atypical larval morphology evolved twice in the same geographic region. Although homoplastic glochidium characters are known (e.g. marginal appendages and size), we hypothesized that bilaterally asymmetrical glochidia represent a novel morphological synapomorphy. We tested the monophyly of the mussels bearing bilaterally asymmetrical glochidia using a molecular matrix consisting of representatives from all six freshwater mussel families and three molecular markers (28S, 16S, and COI). Bayesian inference, maximum likelihood, and ancestral state reconstruction were employed to estimate the phylogeny and larval trait transformations. The reconstructed phylogeny rejects the monophyly of the asymmetrical glochidium-bearing mussels and resolves two putative origins of asymmetrical glochidia; however, ancestral state reconstruction supports asymmetrical glochidia as a synapomorphy of only one supraspecific taxon of the Rectidentinae. In the Gonideinae, asymmetrical glochidia were autapomorphic of Pseudodon cambodjensis (Petit, 1865). That is, no other taxa resolved among the Gonideinae had bilaterally asymmetrical glochidia, including other Pseudodon species. We describe how the alleged intrageneric glochidial variation in Pseudodon, and in the other genera of the Gonideinae reported to have asymmetrical glochidia (i.e. Solenaia and Physunio), challenge the resolved convergence of asymmetrical glochidia. Our results are discussed in the context of freshwater mussel larval evolution, patterns in life-history traits, and the classification of freshwater mussels generally.


doi: 10.1111/zoj.12282


INTRODUCTION

Freshwater mussels (Bivalvia: Unionoida) have evolved various life-history traits adapted to parasitizing their obligate host fishes, including phagomimicry, host capture, and larvae with various types of marginal appendages (e.g. ‘hooks’ and ‘teeth’). Many of these life-history traits, particularly those associated with the larval stage, have been important in reconstructing the evolutionary history of freshwater mussels (Ortmann, 1912; Hoggarch, 1999; Graf & Cummings, 2006; Barnhart, Haag & Roston, 2008; Haag, 2012). Systematic malacology has clearly documented the cladistic utility of freshwater mussel larval characters, and has converged on similar hypotheses of larval evolution (Lydeard, Mulvey & Davis, 1996; Roe & Lydeard, 1998; Graf, 2000, 2002; Hoeh, Bogan & Heard, 2001; Hoeh et al., 2002; Roe & Hoeh, 2003; Graf & Cummings, 2006). Despite largely concordant hypotheses of larval
trait transformation, the current theory of freshwater mussel larval evolution is incomplete, as it focuses exclusively on larvae with bilaterally symmetrical bodyplans. Although bilaterally asymmetrical larvae were described nearly a century ago (Ortmann, 1916), and have been reported from a wide taxonomic distribution (Panha & Eongprakornkeaw, 1995; Dein et al., 2008), they have yet to be considered from an evolutionary perspective. We set out to incorporate this atypical morphology into the current theory of freshwater mussel larval evolution by testing the monophyly of the bilaterally asymmetrical glochidium-bearing mussels.

Freshwater mussel larvae can be divided into two general morphologies: (1) glochidia – small (50–350 μm) calcified bivalved parasites that possess a single adductor muscle and lack a visceral mass and foot; and (2) lasidia – small (80–150 μm) uncalcified trilobed parasites with a long (30× body width) larval thread (Wächtler, Mansur & Richter, 2001; Graf & Cummings, 2006). The glochidium-bearing mussels (families Hyriidae, Margaritiferidae, and Unionidae) are more species-rich (> 80% of all freshwater mussel species) and geographically widespread (all continents except Antarctica) than are the lasidium-bearing mussels (families Etheriidae, Iridinidae, and Mycetopodidae) (Graf & Cummings, 2007). Among the over 750 species of glochidium-bearing mussels there are five major types of glochidia: (1) unhooked; (2) hooked with basal spines; (3) S-shaped hooked; (4) axe-head shaped; and (5) bilaterally asymmetrical. Each of these types of glochidia, with the exception of S-shaped hooked, is represented in the most species-rich freshwater mussel family, the Unionidae.

The plesiomorphic larval condition among the Unionidae is the unhooked type, and it is the most geographically (North America, Eurasia, and Africa) and taxonomically (subfamilies Amblemminae, Parreysiinae, Gonideinae, Rectidentinae, and Modellainae) widespread glochidium morphology (Graf & Cummings, 2006; Graf & Cummings, 2007). Hooked glochidia with basal spines and axe head-shaped glochidia are both secondarily derived forms of the unhooked type, and are synapomorphic of the subfamily Unioninae (26 genera) and the genus Potamilus (six species), respectively (Graf & Cummings, 2006). Unlike the other major types of glochidia, the homology and origin(s) of bilaterally asymmetrical glochidia have yet to be tested.

Bilaterally asymmetrical glochidia, referred to as ‘unequivalse, unequilateral’ by Panha & Eongprakornkeaw (1995) and Dein et al. (2008), are characterized by having a large marginal appendage on only one of the two larval valves (Fig. 1). Bilaterally asymmetrical glochidia have been reported from five Asian genera: Contradens Hass, 1913; Trapezoideus Simpson, 1900; Physunio Simpson, 1900; Pseudodon Gould, 1844; and Solenaea Conrad, 1869 (Ortmann, 1916; Panha & Eongprakornkeaw, 1995; Dein et al., 2008). The current classification recognizes these five genera as constituents of two separate subfamilies, the Gonideinae (i.e. Pseudodon, Solenaea, and Physunio) and the Rectidentinae (i.e. Contradens and Trapezoideus) (Bieler, Carter & Coan, 2010; Whelan, Geneva & Graf, 2011; Huang et al., 2013a). This classification requires that the bilaterally asymmetrical glochidium-bearing mussels are not monophyletic, and suggests that this unusual larval morphology evolved twice in the same geographic region. Although non-homologous glochidium characters are known (e.g. marginal appendages and size), we hypothesize that bilaterally asymmetrical glochidia evolved once and represent a novel morphological synapomorphy.

Figure 1. Bilaterally asymmetrical glochidium of: A, Contradens sp. (UMMZ 304653); B, Physunio eximius (10×; ANSP 3612).

MATERIAL AND METHODS

TAXON AND CHARACTER SAMPLING

To test the monophyly of the bilaterally asymmetrical glochidium-bearing mussels we sampled representatives from all major glochidium types and family group-level taxa of the Unionidae. Representatives of marine and freshwater bivalve families were included as an out-group to polarize character transformations and interpret larval evolution in the Unionidae.

Three molecular markers were used to reconstruct the phylogeny: the nuclear-encoded large ribosomal subunit rDNA (28S) and two mitochondrial genes, the protein-coding cytochrome c oxidase subunit I (COI) and the large ribosomal subunit rRNA gene (16S). Tissue was preserved in RNAlater (Qiagen) or 95% ethanol and extracted using a QIAamp DNA Mini Kit (Qiagen). Primers for polymerase chain reaction (PCR) and cycle sequencing are as follows: 28S – D23F, 5′-GGAGGTTTCAAGAGTACGTG-3′, and D4RB, 5′-TGTTAGACTCCTTGGTCCGTG-3′ (Park & Ó Foighil, 2000); COI – LCO1490, 5′-GGTCAACAAATCAT
AAAGATATTGG-3', and HCO2198, 5'-TAAACTTCAGGG TGACCAAAAAATCA-3' (Folmer et al., 1994); 16S, 16Sar-L-myt, 5'-CGACTGTGTTACAAAAACAT-3', and 16Sbr-H-myt, 5'-CCGGTCTGAACCTCAGTGT-3' (Lydeard et al., 1996). PCR was performed in 25-μL reactions using the following volumes and reagents: H2O (13.4 μL), 5× Green GoTaq® Flexi Buffer (5.0 μL; Promega), 25-mM MgCl (2.5 μL), 10-mM dNTP and primers (1.0 μL), 5-U μl−1 GoTaq® DNA polymerase (0.1 μL; Promega), and DNA template (1 μL). Bidirectional Sanger sequencing was performed on an ABI 3100 automatic sequencer at the University of Alabama. Previously published sequences were also included in our analyses from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/; Table 1). Mitochondrial genomes were used when available, and chimeric terminal taxa (i.e. loci not generated from the same individual) were avoided wherever possible.

ALIGNMENT AND PHYLOGENETIC ANALYSIS
Nuclear and mitochondrial rDNA loci (28S and 16S) were aligned independently using ClustalW (Larkin et al., 2007), as implemented in MESQUITE 2.75 (Maddison & Maddison, 2011), and PRANK (Löytynoja & Goldman, 2005), as implemented in PRANKSTER (http://www.ebi.ac.uk/goldman-srv/prank/prankster/). COI was translated into amino acids using MESQUITE and aligned using ClustalW. All three loci were analysed independently and in combination under maximum likelihood (ML) and Bayesian inference (BI).

The ML analyses were performed in RAxML 7.0.3 ( Stamatakis, 2006) using 1000 standard ML searches and 2000 rapid bootstraps. BI analyses were performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) on the TeraGrid (two runs with eight chains, 24 × 10^6 generations, sampling every 1000 trees, and omitting the first 8000 as burn-in) using the Cipres Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Convergence of the two runs was confirmed using the average of the standard deviations of the split frequencies and the potential scale reduction factor (PSRF). COI was analysed as a single partition, as well as three partitions (one partition/codon position), in both ML and BI analyses. We maintained the same model of DNA evolution (GTR + GAMMA + I) for all ML and BI analyses. Bipartition congruence and conflict in the combined analyses, alignment methods (PRANK and ClustalW), and the independent partitions (28S, COI, and 16S) was assessed using the clade conflict analysis of Whelan et al. (2011).

Larval characters were coded from the literature (Ortmann, 1916; Prashad, 1918a, b, 1919; Thiele, 1934; Woodward, 1969; Panha & Eongprakornkeaw, 1995; Hoggarth, 1999; Wu et al., 1999; Graf & Cummings, 2006) and the examination of specimens. A roughly time-calibrated ultrametric tree was generated using the optimal topology and the ‘chronopl’ function (semi-parametric penalized-likelihood method) in the R package ‘ape’ (Sanderson, 2002; Paradis, Claude & Strimmer, 2004). The ML ancestral state reconstruction was implemented in MESQUITE using the MK1 model on the aforementioned ultrametric tree.

MICROSCOPY
Light and scanning electron microscopy were used to determine the glochidium morphology of Contradens sp., Pseudodon cumingii (Lea, 1850), Solenaiola soleniformis (Benson, 1836), and Physuo eximius (Lea, 1856). Larval tissues were digested using a modified protocol from Hoggarth (1999). Micrographs were captured at the University of Alabama Optical Analysis Facility on a Nikon Eclipse 501 compound light microscope (Melville, New York) and a Hitachi S-2599 scanning electron microscope (Tokyo, Japan).

RESULTS
Using standard light and electron microscopy we confirmed that Contradens sp. (UMMZ 304653) and Physuo eximius (ANSP 3612) have bilaterally asymmetrical glochidia (Fig. 1). We observed unhooked glochidia in Pseudodon cumingii (UMMZ 304648) and Solenaiola soleniformis (BMNH 1926.1.26).

We generated sequences from representatives of each major glochidium morphology, including three of the five genera reported to have bilaterally asymmetrical glochidia (i.e. Pseudodon, Contradens, and Trapezoideus). Molecular data were generated from representative genera of all six families of the Unionoida and five of the six subfamilies of the Unionidae (the monotypic subfamily Modellnaiae was not available for study) (Table 1).

The molecular matrix consists of 42 terminal taxa, each of which is represented by three loci (Table 1). Seventeen, 18, and 19 novel sequences were generated for 28S (average sequence length of 425 nt), COI (624 nt), and 16S (479 nt), respectively. Over 70% of the species analysed were represented by a single individual. Two combined molecular matrices were assembled: ClustalW-aligned (1659 nt) and PRANK-aligned (1925 nt) matrices. The average percentages of gaps per taxon were 14.9 and 10.1% in the 28S and 16S ClustalW alignments, respectively, and 23.1 and 35.8% in the 28S and 16S PRANK alignments, respectively. Both matrices contained the same partial COI alignment truncated to 627 nt. The average of the standard deviations of split frequencies in all BI analyses were <0.0055 and the PSRF for all parameters was <1.001, indicating clear convergence of the two runs.
<table>
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<tr>
<th>Taxon</th>
<th>Species</th>
<th>COI</th>
<th>C1</th>
<th>C2</th>
<th>Source Locality Larval type</th>
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* Asterisks indicate that not all loci were generated from a single individual. Taxonomy follows Graf & Cummings (2007).
The PRANK-aligned data set resolved tree topologies with consistently higher likelihoods in comparison with the ClustalW-aligned data set for all individual and combined analyses. Partitioning COI into codon positions resolved tree topologies with higher likelihoods. The optimal topology (i.e. the one with the highest likelihood) was generated in ML from the PRANK 5 partition data set (Fig. 2).

The taxa reported to have bilaterally asymmetrical glochidia are resolved in two distantly related clades in all independent and combined analyses (Fig. 2). We resolve *Contradens contradens* (Lea, 1838) + *Contradens* sp. + *Trapezoideus exolescens* (Gould, 1843) (= *Contradens* group) among the subfamily Rectidentinae, and each has been previously or is herein reported to have asymmetrical glochidia (Ortmann, 1916; Panha & Eongprakornkeaw, 1995; Fig. 1). The *Contradens* group was strongly supported (bootstrap, BS 100; posterior probability, PP 100) as sister to *Ensidens*, which has unhooked glochidia (Thiele, 1934; Woodward, 1969; Panha & Eongprakornkeaw, 1995). *Pseudodon cambodjensis* (Petit, 1865) was the only taxon with bilaterally asymmetrical glochidia resolved among the Gonideiniae. All other Gonideiniae representatives in the analysis have unhooked (six) or unknown (three) glochidia.

Topological conflicts, defined here as relationships that were not supported (BS < 70; PP < 90) in the optimal topology but were well supported (BS ≥ 70; PP ≥ 90) in another analysis, are described in Table 2. Eighteen clades were found to have conflicts but none bore on the polyphyly of the asymmetrical glochidium-bearing mussels. Seven clades were identified as having conflicts between the alignment methods and 11 clades had conflict between at least one partition and the combined PRANK 5 partition alignment (Table 2). All of the unionid subfamilies were supported as monophyletic in the combined analyses of both alignments, except for the Gonideiniae (Table 2: clades 4–6). The ML and BI combined analyses resolved the glochidium-bearing mussels as paraphyletic, with the lasidium-bearing mussels sister to a clade comprised of the

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**Figure 2.** Most likely topology recovered from the maximum-likelihood (ML) PRANK 5 partition analysis. Inset (A) indicates branch lengths of the transformed cladogram (B). Values above and below the branches indicate ML bootstrap support and Bayesian inference (BI) posterior probability, respectively. Filled boxes represent the reported larval morphologies of each taxon (C).

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Table 2. Conflicts of topological support between the optimal topology and various alignments and partitions

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<th>ML</th>
<th>BI</th>
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</tr>
<tr>
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</tr>
<tr>
<td>4</td>
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<tr>
<td></td>
<td>RECTIDENTINAE+AMBLEMINAE+UNIONINAE)</td>
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<td></td>
</tr>
<tr>
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<td>(Gonidea, Pronodularia, Lamprotula, Potomida,</td>
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<td>45</td>
</tr>
<tr>
<td></td>
<td>AMBLEMINAE+UNIONINAE)</td>
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<tr>
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<td>AMBLEMINAE)</td>
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<td>7</td>
<td>(Etheria, Chambardia)</td>
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<td>81</td>
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<td>P. vondembuschianus)</td>
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<tr>
<td>18</td>
<td>(Pseudodon cumingii, P. mouhotii)</td>
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<td>37</td>
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</table>

Entries in bold indicate relationships supported (BS ≥ 70; PP ≥ 90) in an individual partition or combined ClustalW alignment, but not supported (BS < 70; PP < 90) in combined PRANK alignment.
Margaritiferidae and Unionidae (Table 2: clade 1). The ML PRANK 28S-only analysis generated the only topology that supported the monophyly of the glochidium-bearing mussels (Table 2: clade 2).

Ancestral state reconstruction resolved asymmetrical glochidia as a synapomorphy of the Contradens group (Fig. 3: node 7). Bilaterally asymmetrical glochidia were not diagnostic of any supraspecific taxon of the Gonideinae, but they were autapomorphic for Pseudodon cambodjensis. The plesiomorphic glochidium condition of Pseudodon was strongly supported as the unhooked type, although larval descriptions were unavailable for three of the five Pseudodon species included in our phylogenetic analysis (Fig. 3: node 8). The plesiomorphic glochidium condition of both the Rectidentinae (P = 94.7) and the Gonideinae (P > 99) is unhooked.

**DISCUSSION**

Our reconstructed phylogeny rejects the monophyly of the bilaterally asymmetrical glochidium-bearing mussels (Fig. 2). The taxa reported to have bilaterally asymmetrical glochidia were resolved in two clades corresponding to the subfamilies Rectidentinae (i.e. Contradens contradens + Contradens sp. + Trapezoideus exolescens = Contradens group) and Gonideinae (i.e. Pseudodon cambodjensis). The polyphyly of the bilaterally asymmetrical glochidium-bearing mussels suggests two independent larval transitions to the bilaterally asymmetrical bodyplan in the same geographic region; however, Pseudodon cambodjensis was the only taxon reported to have asymmetrical glochidia resolved among the Gonideinae. All other representatives of the subfamily included in our phylogenetic

**Figure 3.** Ancestral state reconstruction of larval morphologies in the Unionoida. Node numbers correspond to a schematic of the hypothesized ancestral larval morphology and pie charts depicting the proportional likelihood of ancestral states.
analysis have unhooked (six species) or unknown glochidia (three species), including other *Pseudodon* species. We describe how the alleged intrageneric glochidial variation in *Pseudodon*, and in the other two Gonideinae genera reported to have asymmetrical glochidia (i.e. *Solenaia* and *Physunio*), challenge the resolved convergence of asymmetrical glochidia.

Our phylogenetic analyses and ancestral state reconstruction strongly support bilaterally asymmetrical glochidia as a synapomorphy of the *Contradens* group. The sequenced individual of *Contradens* sp. has bilaterally asymmetrical glochidia (Fig. 1A) and is strongly supported in a clade with other bilaterally asymmetrical glochidium-bearing mussels (i.e. *C. contradens* and *T. exolescens*; Fig. 2). The *Contradens* group is well supported as sister to *Ensidens*, which has unhooked glochidia (Thiele, 1934; Woodward, 1969; Panha & Eongprakornkeaw, 1995). *Ensidens* also differs from the *Contradens* group in that it broods glochidia in all four demibranchs (tetrigenous), whereas *T. exolescens*, *Contradens* sp., and *C. contradens* each brood in the outer demibranchs only (ectobranchous; Ortmann, 1916; Thiele, 1934; Woodward, 1969; Kovitvadhi & Kovitvadhi, 2012; for an exception in *Trapezoideus*, see Prashad 1921). The combination of bilaterally asymmetrical glochidia and ectobranch may be diagnostic of the *Contradens* group, and could be useful in determining what other taxa belong to that clade.

Asymmetrical glochidia are not resolved as diagnostic of any supraspecific taxon of the Gonideinae, but they are autapomorphic of *Pseudodon cambodjensis* (Fig. 2). We question the single report of asymmetrical glochidia in *Pseudodon cambodjensis* based on the fact that its congeners have unhooked glochidia (Prashad, 1919; Panha & Eongprakornkeaw, 1995; herein). Moreover, the other two genera of the Gonideinae reported to have asymmetrical glochidia (i.e. *Solenaia* and *Physunio*) also have constituent species with unhooked glochidia (Prashad, 1918b; Panha & Eongprakornkeaw, 1995; Huang et al., 2013a; herein). That is, all three putative genera of Gonideinae reported to bear bilaterally asymmetrical glochidia have at least two species with unhooked glochidia.

Intrageneric glochidium variation has been reported in several Asian genera (e.g. *Inversidens*, *Lamprotula*, and *Pseudodon*), but each of these traditional generic alignments have subsequently been shown to be polyphyletic (Kondo & Yamashita, 1980; Kondo, 1998, 2008; Wu et al., 1999; Zhou et al., 2007; Pfeiffer & Graf, 2013). We suspect that the alleged intrageneric glochidium variation in *Pseudodon*, *Solenaia*, and *Physunio* may also be products of unnatural classifications, and/or are spurious reports of bilaterally asymmetrical glochidia. We discuss the alleged glochidial variation in each of these genera in the context of their relevance to the potential homology of asymmetrical glochidia.

*Pseudodon cambodjensis* is the only *Pseudodon* species reported to have bilaterally asymmetrical glochidia, whereas *Pseudodon cumingii* and *Pseudodon salweniana* (Gould, 1844) each have unhooked glochidia (Prashad, 1919; Panha & Eongprakornkeaw, 1995; herein). Despite sampling *Pseudodon* species reported to have unhooked and asymmetrical glochidia (i.e. *P. cumingii* and *P. cambodjensis*, respectively), we resolve *Pseudodon* as monophyletic, albeit with low and variable support (Table 2: clade 15). The monophyly of the sampled *Pseudodon* taxa in this analysis suggests: (1) both unhooked and asymmetrical glochidia are present in *Pseudodon*; (2) the report of bilaterally asymmetrical glochidia in *P. cambodjensis* is erroneous; or (3) the reports of unhooked glochidia in *P. cumingii* and *P. salweniana* are erroneous. The most likely scenario, in our opinion, is that the report of asymmetrical glochidia in *P. cambodjensis* is spurious, and may be a product of species misidentification or glochidium contamination. The examination of more *Pseudodon* material is needed to determine the presence or absence of bilaterally asymmetrical glochidia in the genus, as well as more fully test its monophyly and supraspecific relationships, which show significant conflict (Table 2: clades 15–18).

Various *Solenaia* species have been resolved among the Gonideinae (Ouyang et al., 2011; Huang et al., 2013a, b), but none have been reported to bear asymmetrical glochidia. Huang et al. (2013a) advocated for the placement of *Solenaia carinatus* (Heude, 1877) in the Gonideinae, in part because of the presence of unhooked glochidia; however, they did not discuss the implications of that justification relative to the systematic position of *Solenaia khwaenoiensis* Panha & Deein, 2003, the only species of *Solenaia* reported to have bilaterally asymmetrical glochidia (Deein et al., 2008). Prashad (1919) reported unhooked glochidia in the Indian species, *S. soleniformis* (confirmed herein), and designated it as the type of a new genus, *Balivantia*, to distinguish it from the Southeast Asian type species of *Solenaia, Solenaia emarginata* (Lea, 1860); however, recent taxonomic treatments regard *Balivantia* as a junior synonym of *Solenaia* (Subba Rao, 1989; Graf & Cummings, 2007; Ramakrishna & Dey, 2007). Based on the presence of species with unhooked (i.e. *S. soleniformis* and *S. carinatus*) or asymmetrical glochidia (i.e. *S. khwaenoiensis*), we suspect *Solenaia* is not monophyletic and is in need of generic revision.

*Physunio*, the only other genus reported to bear asymmetrical glochidia, has yet to be included in a phylogenetic analysis, and its classification among the Gonideinae is untested. Divergent life-history traits

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within the genus challenge its monophyly and the placement of at least some of its constituents in the subfamily Gonideinae. Physunio superbus (Lea, 1843) and P. eximius both possess asymmetrical glochidia and ectobranchous brooding (Panha & Eongprakornkeaw, 1995; Kovitvadhi & Kovitvadhi, 2012; Fig. 1B; J. Pfeiffer, pers. observ.), a combination of traits we hypothesize to be diagnostic of the Contradens group. Physunio ferrugineus Annandale, 1918 and Physunio micropteroides Annandale, 1918 are also ectobranchous, but have unhooked glochidia, similar to several mussels in the subfamily Parreysiinae (e.g. some Lamellidens; Ghosh, 1918; Prashad, 1918b). We suspect that the apparent diversity of life-history traits within Physunio is spurious and is rather a product of a reclassification that does not reflect shared common ancestry (i.e. Physunio is not monophyletic).

The variation in glochidium morphologies within both Solenaia and Physunio occurs among species endemic to different freshwater mussel faunal regions (i.e. Indo-Burma, South China, and Southeast Asia; Graf & Cummings, 2007; Bogan, 2008), further suggesting the non-monophyly of these genera. Solenaia soleniformis, Solenaia carinatus, Physunio ferrugineus, and Physunio micropteroides are endemic to Indo-Burmese or Yangtze–Huang faunal regions, and have unhooked glochidia (Prashad, 1918b, 1919; Huang et al., 2013a), whereas Solenaia kwaenoinensis, Physunio superbus and Physunio eximius are endemic to the Southeast Asian freshwater mussel faunal region, and have asymmetrical glochidia (Panha & Eongprakornkeaw, 1995; Dein et al., 2008; Fig. 1). We hypothesize that the endemic Southeast Asian Solenaia and Physunio species with asymmetrical glochidia belong to the Contradens group, and the Indo-Burmese and Chinese species in these genera are likely to belong to the Parreysiinae or Gonideinae. This phylogenetic scenario would support the homology of asymmetrical glochidia. In order to definitively test the homology of bilaterally asymmetrical glochidia, the larval morphologies, monophyly, and supraspecific relationships of Solenaia, Physunio, and Pseudodon must be investigated.

ADAPTIVE CONVERGENCE OF GLOCHIDIUM MARGINAL APPENDAGES

Large glochidium marginal appendages have evolved at least three times in the Unionoida (Fig. 3: nodes 2, 6, and 7), and may represent an adaptive convergence. The marginal appendage of bilaterally asymmetrical glochidia in the Contradens group may serve a function similar to the marginal appendages in the Unioninae and Hyriidae. In comparison with unhooked glochidia, which are generally gill parasites, glochidia with marginal appendages are better adapted to parasitizing the fins of their hosts (Lefèvre & Curtis, 1910; Hoggarth & Gaunt, 1988; Bauer, 1994, 2001; Shadoan & Dimock, 2000; Jansen, Bauer & Zahner-Meike, 2001). Although the life-history traits of the bilaterally asymmetrical glochidium-bearing mussels are poorly understood, at least one member of the Contradens group (i.e. C. contradens) has also been observed to parasitize the fins of its host (Panha, 1990: fig. 11).

The presence or absence of glochidium marginal appendages may be correlated with several other life-history traits, including host infection strategy and host utilization. Many representatives of the Unioninae and Hyriidae generally (although the latter is less well characterized): (1) broadcast glochidia that have (2) marginal appendages and (3) parasitize the fins of (4) a broad range of host fishes (Walker et al., 2001; Barnhart et al., 2008; Haag, 2012; Klunzinger et al., 2012). The Contradens group may share a similar suite of characters, including broadcasting glochidia (no apparent morphological adaptations for attraction), fin parasitism, and glochidia with a marginal appendage (Ortmann, 1916; Panha, 1990; Panha & Eongprakornkeaw, 1995; Dein et al., 2008).

Many representatives of the Ambelminae (e.g. most Lampsiilini, and many Pleurobemini and Quadrulini) use a largely different suite of life-history traits that include: (1) using host attraction strategies to (2) infect the gills of (3) comparably fewer hosts with (4) unhooked glochidia (Barnhart et al., 2008; Haag, 2012). There are, however, many taxa that do not follow these two generalized suites of life-history traits, and these exceptions may be useful for testing hypotheses of life-history correlation and convergence. For example, Unio crassus (Retzius, 1788) appears to have converged on a suite of life-history traits similar to those found in some clades of the Ambelminae, despite being nested within the Unioninae. Unlike many members of the Unioninae, U. crassus infects the gills of an intermediate number of hosts with glochidia having reduced marginal appendages via a unique ‘spurting’ host infection strategy (Hochwald, 2001; Vicentini, 2005; Taubert, Gum & Geist, 2012; Reis et al., 2014). On the other hand, some representatives of the Ambelminae (e.g. Potamilus and some ‘Elliptio’) may have some traits similar to those of the Unioninae and Hyriidae, including glochidia with marginal appendages (although smaller) and broadcasting glochidia (Roe, Simons & Hartfield, 1997; O’Brien, Williams & Hoggarth, 2003; Haag, 2012). These exceptions may represent independent origins of several life-history traits and could suggest that infection strategy, host utilization, encystment location, and glochidium morphology are part of a correlated suite of characters.

The correlation of life-history traits is an interesting and potentially informative pattern that deserves
explicit testing rather than conjecture. Rigorous testing of the evolution, correlation, and functional significance of freshwater mussel life-history traits would facilitate biologically relevant inferences across unionoid diversity (e.g. elucidate host use patterns and infection strategy) that could improve conservation efforts (e.g. predict species loss and determine conservation priorities) (Sæther, Ringsby & Røskaft, 1996; Haag, 2012); however, such inferences will be limited as long as they are based on incomplete data from polyphyletic regional groups (e.g. North American or Southeast Asian unionoids). We hope this study will promote the investigation of understudied freshwater mussel groups, and facilitate a more inclusive interpretation of freshwater mussel life-history traits and diversity.

ACKNOWLEDGEMENTS

This research was funded by a grant from the National Science Foundation (DEB-AToL-0732903), and was facilitated by a Jessup Fellowship awarded to J.M.P. from the Academy of Natural Sciences of Drexel University. We would like to thank Jon Albett, Kevin Cummings, Maurice Kottelat, Amanda Lawless, Christina Piotrowski, Gary Rosenberg, Jeremy Tiemann, Jeff Wilkinson, and especially James Stockel, each of whom provided specimens or facilitated access to them. Paul Johnson, Art Bogan, Larry Page, Zach Randall, and two anonymous reviewers improved earlier versions of this article.

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