RE-ANALYSIS CONFIRMS THE POLYPHYLY OF *LAMPROTULA* SIMPSON, 1900 (BIVALVIA: UNIONIDAE)

JOHN M. PFEIFFER, III[1] AND DANIEL L. GRAF[2]

[1]*Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA; and*
[2]*Department of Biology, University of Wisconsin-Stevens Point, Stevens Point, WI 54481, USA*

Correspondence: D.L. Graf; e-mail: dgraf@uwsp.edu

ABSTRACT

Zhou *et al.* (2007, *Acta Zoologica Sinica*, **53**: 1024–1030) reported the eastern Asian freshwater mussel genus *Lamproula sensu lato* Simpson, 1900 (Unionidae) to be polyphyletic and advocated a revision of the genus- and family-level classifications. However, their taxon sampling and analyses were insufficient to infer accurately the systematic placement of the resultant clades. We reanalysed their mtDNA ND1 and 16S sequences in a broader phylogenetic context. In addition to nine putative species of *Lamprotula s. l.*, we sampled genera from five of six unionid subfamilies as well as two outgroup families (34 species in total). Both character partitions were analysed separately and in combination under maximum parsimony and maximum likelihood, and combined matrices were also examined using Bayesian inference. Our results confirm support for polyphyly among the species of *Lamprotula s. l.*, with genus-level clades recovered in two different unionid subfamilies. The taxonomic implications of recognizing two genera, *Lamprotula sensu stricto* (subfamily Gonideinae) and *Aculamprotula* Wu, Liang, Wang & Ouyang, 1999 (subfamily Unioninae), are discussed, as are anomalies discovered in the published data. It is concluded that, while *Lamprotula s. l.* is polyphyletic, a more comprehensive revision is necessary to determine the valid names for these two genera.

INTRODUCTION

Freshwater mussels (order Unionoida) represent the richest radiation of bivalves into fresh waters, with more than 850 species worldwide (Graf & Cummings, 2007; Bogan, 2008). The order is represented by six Recent families, the Unionidae being the most species-rich and geographically widespread (Graf & Cummings, 2007). The Unionidae are Holarctic in distribution, extending south into the Neo-, Afro- and Indotropics. These molluscs are of practical importance for their imperiled conservation status, due not only to the degradation of freshwater ecosystems generally but also to overharvest for their nacre (Strayer, 2006; Strayer & Dudgeon, 2010). Freshwater mussels are of zoological interest for their unique life cycles, involving both parental care and larval parasitism upon fishes (Cummings & Graf, 2009; Fritts *et al.*, 2013). Associated life history characters—such as the arrangement of the marsupial demibranchs in which larvae are brooded, brooding period and larval morphology—have traditionally been applied to diagnose family-group-level taxa among freshwater mussels (Ortmann, 1912; Heard & Guckert, 1971; Heard, 1974; Davis & Fuller, 1981). However, for many genera, taxonomic placement has been inferred using only shell characters in the absence of soft-anatomical and behavioural data.

The species of the eastern Asian freshwater mussel genus *Lamprotula* Simpson, 1900 (about 27 spp., from Korea south to

northern Vietnam) have recently been revised to comprise two genera in separate subfamilies of the Unionidae. *Lamprotula sensu lato* had traditionally been allied to Nearctic genera such as *Quadrula* Rafinesque, 1820, due to the shared occurrence of heavy, sculptured shells, tetrageny (i.e. brooding in all four ctenidial demibranchs) and unhooked-type glochidia (parasitic larvae) (Simpson, 1914; Haas, 1969a,b). However, some Chinese species of *Lamprotula s. l.* have been discovered to be ectobranchous (brooding in only the outer pair of demibranchs) with hooked-type glochidia. Based on these characters, Wu *et al.* (1999) divided the species among two genera: *Lamprotula sensu strico* (subfamily Ambleminae) and *Aculamprotula* (subfamily Unioninae) (Fig. 1). This novel genus- and family-group level classification was supported by the molecular phylogenetic analyses of Zhou *et al.* (2007), but has been only infrequently applied in the published literature (e.g. Reichard, Liu & Smith, 2007).

While Zhou *et al.* (2007) recovered cladistic support for the separation of *Lamprotula s. l.* species between two subfamilies of the Unionidae, the generality of their conclusions was undermined by insufficient sampling as well as their analytical approach. Specifically, (1) ingroup sampling was too sparse to resolve accurately the placement of *Lamprotula s. s.*, (2) outgroup sampling did not convincingly root the Unionidae, (3) two different mitochondrial character sets (ND1 and 16S) were assembled with nonoverlapping taxon sets and (4) the two matrices were only analysed separately, each under different
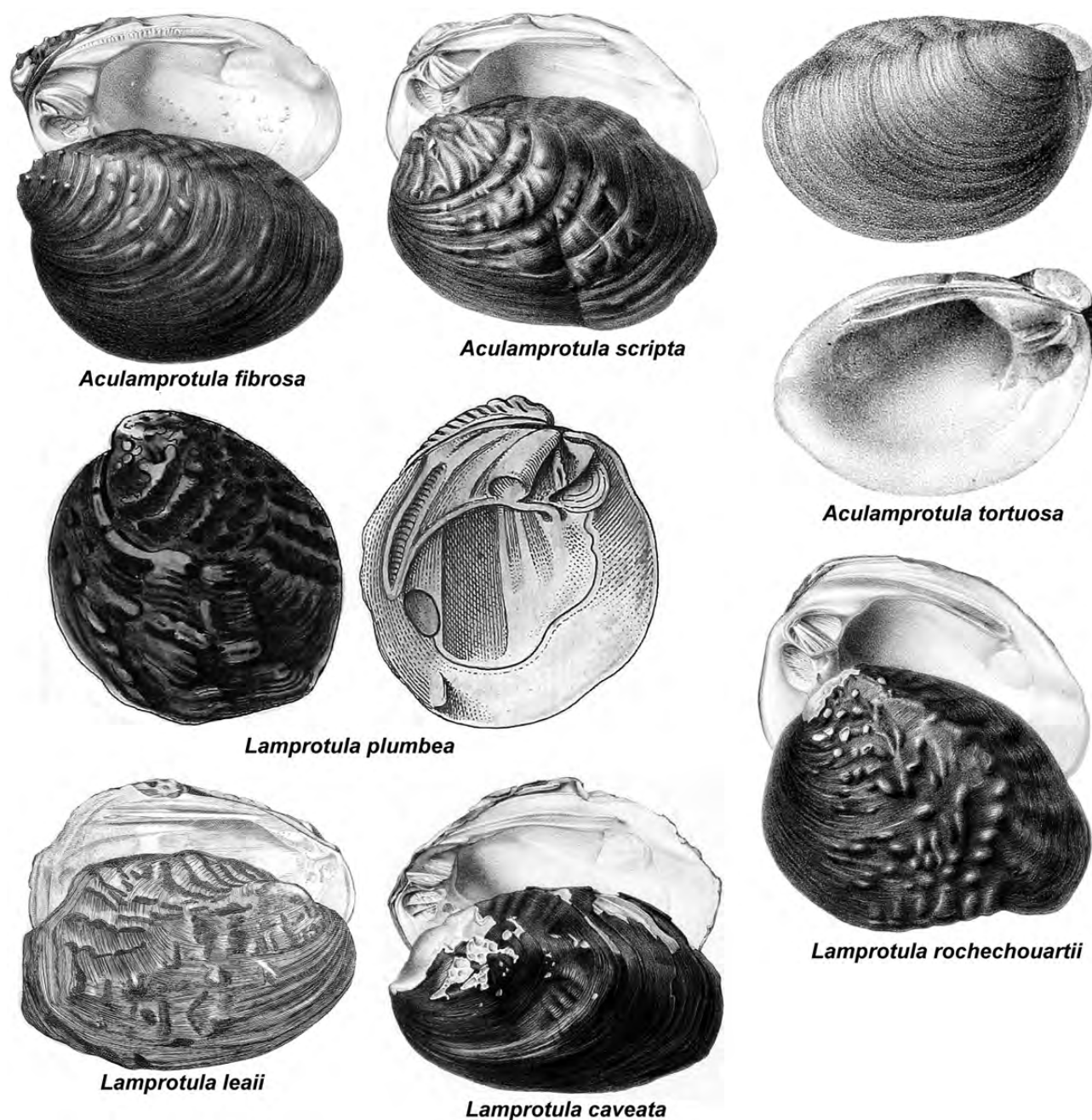
Figure 1. Original figures of representative *Aculamprotula* and *Lamprotula s. s.* species. *Aculamprotula fibrosa* (Heude, 1874−1885: pl. 11, fig. 22), *A. scripta* (Heude, 1874−1885: pl. 3, fig. 8), *A. tortuosa* (Lea, 1865) (figured by Lea, 1868: pl. 39, fig. 98), *L. plumbea* (Dillwyn, 1817) (figured by Chemnitz, 1795: pl. 203, figs 1991, 1992), *L. leaii* (Gray, 1833) (figured by Griffith & Pidgeon, 1833: pl. 21, fig. 1), *L. caveata* (Heude, 1874−1885: pl. 24, fig. 53) and *L. rochechouartii* (Heude, 1874−1885: pl. 5, fig. 13). All images reproduced from the Biodiversity Heritage Library (http://www.biodiversitylibrary.org/).

optimality criteria (phenetic distance and parsimony). Our objective herein is to reanalyse the published *Lamprotula s. s.* and *Aculamprotula* mtDNA sequences in the context of broader taxon sampling and likelihood-based methods, in order to test the classification of Zhou *et al*. (2007).

MATERIAL AND METHODS

Taxon sampling

Ingroup taxa were chosen to represent the subfamilies of the Unionidae, as well as the tribes of the Unioninae and

Ambleminae, as proposed by Bieler, Carter & Coan (2010) and modified by Whelan, Geneva & Graf (2011) (Table 1). The outgroup consisted of species of the freshwater mussel families Margaritiferidae and Hyriidae.

We studied the same target mitochondrial markers applied by Zhou *et al*. (2007): segments of the NADH dehydrogenase subunit I (ND1) and the large ribosomal subunit (16S). Previously published nucleotide sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/), relying upon mitochondrial genomes when possible. New sequences were generated following standard PCR and dye-terminator sequence protocols (Graf & Ó Foighil, 2000). Primers for amplification and

Table 1. Unionoid taxa analysed, GenBank accession numbers and sources of sequences. Taxonomy follows Graf & Cummings (2007) and Whelan *et al.* (2011), as updated on the MUSSEL Project Web Site (http://www.mussel-project.net/). Voucher specimens for new sequences are deposited in the University of Michigan Museum of Zoology (UMMZ), Academy of Natural Sciences of Drexel University (ANSP), and the Field Museum of Natural History (FMNH).

Taxon	ND1	16S	Sources
Family HYRIIDAE			
Velesunio ambiguus (Philippi, 1847)	KF011249	KF011257	FMNH 337195 (BivAToL-379), Nepean R., New South Wales
Family MARGARITIFERIDAE			
Margaritifera margaritifera (L., 1758)	EF446105	AF303297	Machordom *et al.* (2003); Kneeland & Rhymer (2007)
Family UNIONIDAE			
Subfamily UNIONINAE, Tribe UNIONINI			
Unio pictorum (Linnaeus, 1758)	NC_015310	NC_015310	Soroka & Burzyński (2010)
Nodularia douglasiae (Gray, 1833)	DQ077896	DQ073824	Zhou *et al.* (2007)
Acuticosta chinensis (Lea, 1838)	DQ077897	DQ073812	Zhou *et al.* (2007)
Aculamprotula fibrosa (Heude, 1877)	DQ156187	DQ073828	Zhou *et al.* (2007)
A. scripta (Heude, 1875)	DQ156179	DQ073829	Zhou *et al.* (2007)
A. tientsinensis (Crosse & Debeaux, 1863)	DQ156177	DQ073826	Zhou *et al.* (2007)
A. tortuosa (Lea, 1865)	DQ077894	DQ156203	Zhou *et al.* (2007)
A. zonata (Heude, 1883)	DQ156178	DQ073827	Zhou *et al.* (2007)
Tribe ANODONTINI			
Sinanodonta woodiana (Lea, 1834)	HQ283348	HQ283348	Soroka (2010)
Cristaria plicata (Leach, 1814)	FJ986302	FJ986302	Jiang *et al.* (2010)
Strophitus undulatus (Say, 1817)	GU085377	AY238491	Krebs, Vlasceanu & Tevesz (2003); Boyer *et al.* (2011)
Pyganodon grandis (Say, 1829)	NC_013661	NC_013661	Breton *et al.* (2009)
Lasmigona compressa (Lea, 1829)	NC_015481	NC_015481	Breton *et al.* (2011)
Subfamily AMBLEMINAE, Tribe AMBLEMINI			
Amblema plicata (Say, 1817)	AY158796	U72548	Lydeard *et al.* (1996), Serb *et al.* (2003)
Tribe QUADRULINI			
Quadrula quadrula (Rafinesque, 1820)	NC_013658	NC_013658	Breton *et al.* (2009)
Tribe PLEUROBEMINI			
Elliptio dilatata (Rafinesque, 1820)	DQ385872	U72557	Lydeard *et al.* (1996), Campbell *et al.* (2005)
Tribe LAMPSILINI			
Lampsilis ornata (Conrad, 1835)	NC_005335	NC_005335	Serb & Lydeard (2003)
Truncilla truncata Rafinesque, 1820	GU085380	AY655080	Krebs *et al.* (2003), Boyer *et al.* (2011)
Subfamily GONIDEINAE			
Gonidea angulata (Lea, 1838)	AY655099	KF011258[*]	Campbell *et al.* (2005)
Hyriopsis cumingii (Lea, 1852)	NC_011763	NC_011763	RefSeq
H. schlegelii (Martens, 1861)	HQ641406	HQ641406	Genbank
Lamprotula caveata (Heude, 1877)	DQ077898	DQ073820	Zhou *et al.* (2007)
L. caveata (2)	KF011250	KF011259	UMMZ 304345, L. Donghu, China
L. cornuumlunae (Heude, 1883)	DQ077900	DQ073818	Zhou *et al.* (2007)
L. leaii (Gray, 1833)	DQ077901	DQ073821	Zhou *et al.* (2007)
L. rochechouartii (Heade, 1875)	DQ156183	DQ073819	Zhou *et al.* (2007)
Potomida littoralis (Cuvier, 1798)	KF011251	KF011260	UMMZ 304348, Ognon R., France
Pronodularia japanensis (Lea, 1859)	AB055625	AB055625	Genbank
Pseudodon inoscularis (Gould, 1844)	KF011252	KF011261	UMMZ 304349, Pursat Prov., Cambodia
P. cambodjensis (Petit, 1865)	KF011253	KF011262	UMMZ 304350, Pursat R., Cambodia
Subfamily PARREYSIINAE			
Coelatura aegyptiaca (Cailliaud, 1827)	KF011254	KF011264	ANSP 416304, Nile R., Egypt
Lamellidens generosus (Gould, 1847)	KF011255	KF011263	UMMZ 304346, Chindwin R., Burma
Subfamily RECTIDENTINAE			
Trapezoideus exolescens (Gould, 1843)	KF011256	KF011265	UMMZ 304347, Nam Ou R., Laos

[*]A small tissue biopsy was provided from a living individual of *G. angulata* by Lora Nield (Ministry of Forests, Lands and Natural Resources Operations, British Columbia). No voucher specimen is available.

sequencing were as follows: ND1 Leu-uurF-tggcagaaaagtgcatca-gattaaagc and NIJ-12073-tcggaattctccttctgcaaagtc (Serb, Buhay & Lydeard, 2003); 16S 16Sar-L-myt-cgactgtttaacaaaaacat and 16Sbr-H-myt-ccgttctgaactcagctcatgt (Lydeard, Mulvey & Davis 1996). Ribosomal 16S sequences were aligned using the CLUSTAL and PRANK algorithms with the respective default settings (http://www.ebi.ac.uk/Tools/msa/), with only minor adjustments by eye. Protein-coding ND1 sequences were translated into amino acids with Mesquite v. 2.75 (Maddison & Maddison, 2011) and nucleotides were aligned manually by codon position.

Both character partitions were analysed separately and in combination to recover optimal topologies under both maximum parsimony (MP) and maximum likelihood (ML). In addition, the combined datasets were analysed using Bayesian Inference (BI). A partition homogeneity test (ILD; Farris *et al.*, 1995) was performed using PAUP* v. 4b10 (Swofford, 2002) to confirm the phylogenetic compatibility of the two partitions. MP analyses were conducted with PAUP* using a heuristic search with 100 random sequence additions. Branch support was estimated with 2000 bootstrap replications (heuristic searches, 10 random sequence additions each). For likelihood-based analyses, the optimal model for each partition was explored using jModelTest (Posada, 2008), although model application was limited by the software employed. All ML analyses were performed using the rapid bootstrap method (2000 replicates) in RAxML v. 7.0.3 (Stamatakis, 2006) under GTR + Γ + I. The same model was applied for BI using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) on the TeraGrid (2 runs, 8 chains each, 21×10^6 generations) via the Cipres Portal (Miller, Pfeiffer & Schwartz, 2010). Sufficient mixing of the chains was monitored using the average of the standard deviations of the splits frequencies (<0.01) and stationarity was verified using AWTY (Wilgenbusch, Warren & Swofford, 2004). For both ML and BI, protein-coding ND1 was analysed both as a single partition and as three partitions with unlinked codon positions, and 16S was examined using alternative CLUSTAL and PRANK alignments. Patterns of topological support among the various MP, ML and BI analyses were evaluated following the methods of Whelan *et al.* (2011) to determine areas of conflicting signal among the gene fragments, alignments and/or optimality criteria. Templeton (Wilcoxon signed-ranks) and Shimodaira–Hasegawa (S-H) tests (Felsenstein, 2003) were implemented in PAUP* and RAxML, respectively, to compare statistically the optimal topologies based on the combined character partitions with constraint trees requiring the monophyly of *Lamprotula s. l.* (= *Lamprotula s. s.* + *Aculamprotula*).

RESULTS

Thirty-five combined sequences representing 34 putative species in 25 genera were assembled into two separate matrices: CLUSTAL-aligned 16S + ND1 and PRANK-aligned 16S + ND1 (available from the corresponding author). Both matrices

Table 2. Statistics for combined analyses of unionoid sequences under MP, ML and BI. The resultant phylogram from the combined analysis of PRANK-aligned 16S + ND1 with four partitions is shown in Figure 2.

	PRANK-16S + ND1			CLUSTAL-16S + ND1		
MP Analyses	total	16S	ND1	total	16S	ND1
no. of taxa	35			35		
no. of characters	1032	524	508	841	333	508
informative characters	408	150	258	404	156	258
trees	6			4		
islands	2			3		
tree length	2682	704.67*	1977.33*	2810	829*	1981*
RC	0.1547	0.2509*	0.1257*	0.1480	0.2094*	0.1251*
ILD(*P*)	0.146			0.192		
Lamprotula s. l. monophyly constraint						
trees	3			15		
tree length	2725	718.67*	2006.33*	2864	857.27*	2006.73*
RC	0.1481	0.2379*	0.1207*	0.1406	0.1927*	0.1207*
Templeton (*P*)	<0.001			<0.01		
	PRANK-16S + ND1			CLUSTAL-16S + ND1		
ML Analyses	2 partitions	4 partitions		2 partitions	4 partitions	
-ln likelihood	11425.9656	11072.6381		11546.0333	11186.5806	
tree length	3.8972	5.2592		4.8462	6.4117	
Lamprotula s. l. monophyly constraint						
-ln likelihood	11542.4677	11190.0563		11678.0485	11318.6193	
S-H (*P*)	<0.05	<0.05		<0.05	<0.05	
	PRANK-16S + ND1			CLUSTAL-16S + ND1		
BI Analyses	2 partitions	4 partitions		2 partitions	4 partitions	
mean -ln likelihood	11398.07	11097.66		11517.45	11215.04	
mean tree length	7.7858	8.6884		8.5036	9.4175	
trees sampled	7326	7879		9414	8582	
95% credibility set	5926	6479		8014	7182	

*Mean values of partition tree length and Rescaled Consistency Index (RC) on combined analysis topologies.
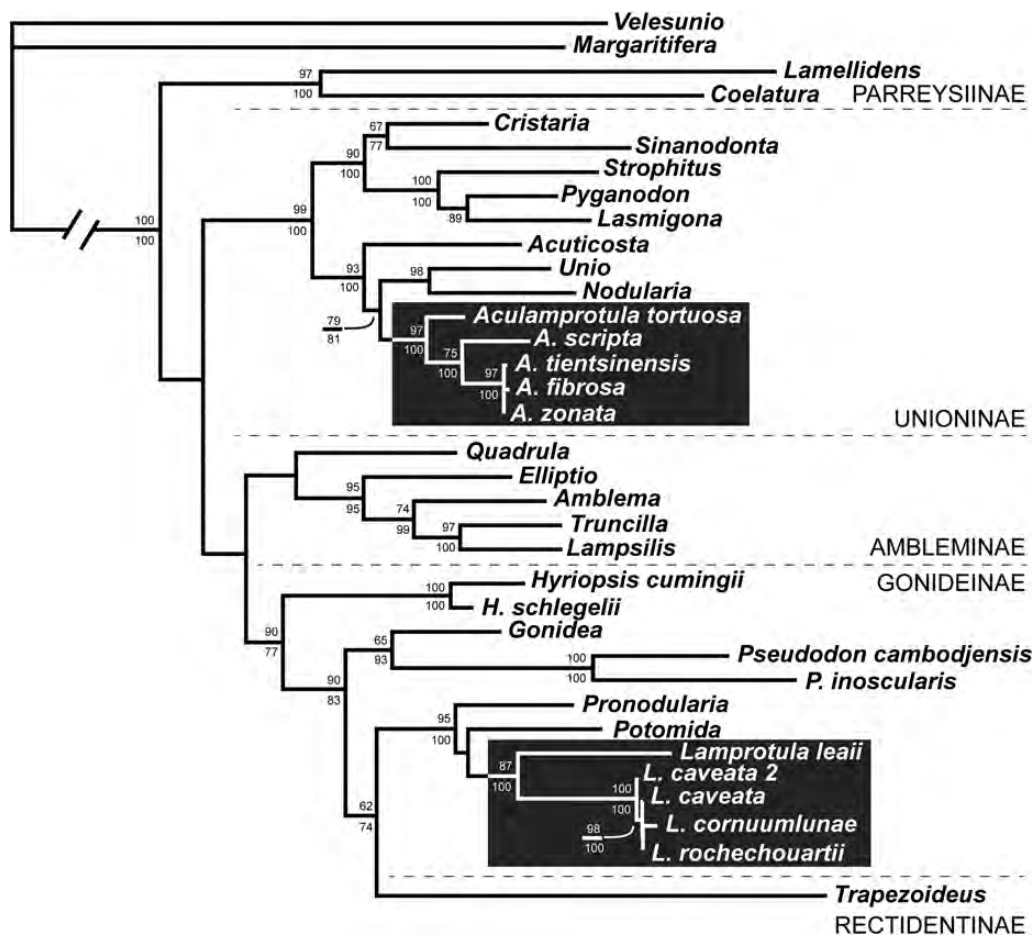
Figure 2. ML phylogram of unionoid taxa from the combined analysis of PRANK-aligned 16S + ND1 with four partitions. Numbers above the branches are ML bootstrap values ≥50%; below are ≥70% BI clade confidence posterior probabilities. Dark boxes highlight the species of *Aculamprotula* and *Lamprotula s. s.*

included the same ND1 alignment truncated to 508 characters. The CLUSTAL and PRANK alignments of 16S differed in length (333 and 524, respectively) owing to the difference in the treatment of insertion–deletion (gaps) by the two algorithms. Included taxa represented five of the six unionoid subfamilies recognized by Whelan *et al*. (2011).

The results of the combined analyses of both matrices are shown in Table 2 (the MP and ML analyses of the individual partitions are not shown). Partition homogeneity tests of both alignments found no significant conflict in the phylogenetic signal between the two gene fragments ($P > 0.05$). The resultant topology with the lowest overall –ln likelihood was obtained from the analysis of the combined PRANK-aligned 16S + ND1 matrix with four unlinked partitions (i.e. 16S and the three ND1 codon positions) (Fig. 2). *Aculamprotula* was recovered as monophyletic and sister to the (*Unio* + *Nodularia*) clade in the Unioninae, while *Lamprotula s. s.* was placed in a well-supported clade with *Pronodularia* and *Potomida* in the Gonideinae. Topologies constraining the monophyly of *Lamprotula s.l.* (= *Lamprotula s. s.* + *Aculamprotula*) were found to be significantly worse ($P < 0.05$) under both MP Templeton tests and ML S-H tests (Table 2).

The clade support values obtained from MP and ML bootstrap and BI analyses of all partitions analysed separately and in combination were examined to identify clades with high support (≥0.70 bootstrap, ≥0.95 posterior probability) that lacked high support in the preferred tree in Figure 2. Only 11 clades were found that indicated conflicting phylogenetic signal among the

different genes, alignments and/or optimality criteria (Table 3), and none contradict the relevant results obtained from the topology in Figure 2. That is, no well-supported alternatives to the polyphyly of *Lamprotula s. l.*, the monophyly of either *Lamprotula s. s.* or *Aculamprotula*, or the sister groups of either clade were supported by any of our analyses.

DISCUSSION

The results of our re-analysis confirm the polyphyly of *Lamprotula s. l.*, but they do not support the classification favoured by Zhou *et al*. (2007: Figs 1 and 2). Broader outgroup and ingroup sampling revealed that their conclusions were biased by the taxa in their analyses and an outmoded classification paradigm for the Unionidae. While branch support from our combined analyses of the two mitochondrial markers is weak among the subfamilies, those clades (or segments of them) are well supported. Our analyses resolve the taxonomic positions of both *Lamprotula s. s.* and *Aculamprotula*, and they have broader implications for the evolving phylogenetic classification of the Unionidae. However, as we discuss below, the topology in Figure 2 raises questions about the species identity of the terminals in both the *Lamprotula s. s.* and *Aculamprotula* clades, and important problems remain to be solved with regard to generic nomenclature.

The type species of *Aculamprotula*, *A. fibrosa* (Heude, 1875), has hooked-type glochidia and broods only in the outer demibranchs (Wu *et al*., 1999). These characters in combination are

Table 3. Summary of topological results from the ML, MP and BI analyses of unionoid taxa. Table lists the clades with high support that are not supported in the phylogeny shown in Figure 2 (ML, PRANK-aligned 16S + ND1 with 4 partitions; bootstrap values underlined). ML and MP bootstrap ≥70% and BI clade confidence ≥95% shown in bold.

	Dataset alignment	Combined analyses				16S		ND1	
		PRANK		CLUSTAL		PRANK	CLUSTAL		
No. clade	No. of partitions	4	2	4	2			3	1
ML (Combo/PRANK/4 partitions) vs ML analyses									
1.	(*Lamprotula, Potomida*)	37	55	35	65	—	—	**78**	**70**
2.	(*Lamprotula, Potomida, Pronodularia, Trazoideus*)	62	63	**83**	**82**	60	**72**	25	44
3.	(*L. cornuumlunae, L. caveata* 2)	—	—	—	—	—	—	58	**86**
4.	(*L. cornuumlunae, L. rochechouartii*)	55	62	53	61	**94**	**81**	—	—
5.	(*L. rochechouartii, L. caveata*)	45	37	46	39	—	09	**98**	**98**
6.	(*Gonidea, Pseudodon*)	65	**78**	**82**	**80**	**91**	**77**	—	13
7.	AMBLEMINAE	23	31	**82**	**72**	—	**80**	36	22
8.	(*Cristaria, Sinanodonta*)	67	**78**	**87**	**79**	**72**	**90**	21	21
9.	(*Pyganodon, Strophitus*)	36	14	11	06	62	**73**	—	—
10.	(*Pyganodon, Lasmigona*)	49	**81**	67	**81**	24	16	47	65

	Dataset alignment	ML PRANK	Combined Analyses PRANK	Combined Analyses CLUSTAL	16S PRANK	16S CLUSTAL	ND1
ML (Combo/PRANK/4 partitions) vs MP analyses							
5.	(*L. rochechouarti, L. caveata*)	45	**80**	**82**	—	08	**89**
11.	(*L. rochechouartii, L. caveata, L. caveata* 2)	—	52	55	—	—	**87**
6.	(*Gonidea, Pseudodon*)	65	63	45	**72**	52	13
7.	AMBLEMINAE	23	11	52	—	**76**	11

	dataset alignment no. of partitions	ML PRANK 4	Combined PRANK 4	Combined PRANK 2	CLUSTAL 4	CLUSTAL 2
ML (Combo/PRANK/4 partitions) vs BI analyses						
7.	AMBLEMINAE	23	58	25	**98**	**99**
10.	(*Pyganodon, Lasmigona*)	49	89	**95**	66	79

diagnostic for the subfamily Unioninae (Graf & Cummings, 2006), and their discovery was the impetus for separating that species from others like *Lamprotula caveata* that have unhooked-type glochidia and tetragenous brooding (Wu *et al.*, 1999). The placement of *Aculamproula* among the Unioninae is robust, based on morphological and molecular synapomorphies.

To date, the subfamilial placement of *Lamprotula s. s.* has varied. Conflicting arrangements have been proposed (e.g. Modell, 1964; Haas, 1969a,b; Starobogatov, 1970), but until recently no consensus had been reached. A paraphyletic 'Ambleminae' (or sometimes 'Quadrulinae') had been applied to cover all the unionids with unhooked-type glochidia (Graf & Cummings, 2006, 2007). Bieler *et al.* (2010) proposed a novel comprehensive subfamilial classification of the Unionidae, and this system has been variously supported and refined by subsequent phylogenetic analyses (Whelan *et al.*, 2011), including the current study. Contrary to Zhou *et al.* (2007), the Ambleminae is now restricted to genera in eastern North America and Mesoamerica. *Lamprotula s. s.* is well supported as a member of the Gonideinae (Fig. 2), a widespread taxon ranging from Europe (e.g. *Potomida* Swainson, 1840) through eastern Asia (e.g. *Hyriopsis* Conrad, 1853) to Pacific North America (only *Gonidea angulata* among Recent taxa). The genus *Trapezoideus* Simpson, 1900 has been assigned to the Rectidentinae (Brandt, 1974; Subba Rao, 1989), but our analyses recover it among the species of the Gonideinae (Fig. 2). Our phylogenetic study is the first to find good support for the placement of *Gonidea* Conrad,

1857 among the taxa of Eurasia (see also Campbell *et al.*, 2005, Graf & Cummings, 2006; Whelan *et al.*, 2011) and we have resolved the subfamily of *Lamprotula s. s.*

The constituent species of *Lamprotula s. s.* and *Aculamprotula* are based largely upon the taxon sampling of Zhou *et al.* (2007): those species recovered as part of the Unioninae are assigned to *Aculamprotula* and the rest have been retained in *Lamprotula s. s.* We know of no published work that lists all valid species of these genera, although various Internet initiatives have adopted this classification (e.g. http://fada.biodiversity.be/, http://mussel-project.net/). Closer consideration of these taxa raises important questions about the species composition of both genera, with implications for the generic nomenclature.

The identifications of the terminals applied by Zhou *et al.* (2007) and herein indicate five species of *Aculamprotula* and four of *Lamprotula s. s.* (Table 1). However, our results suggest fewer phylogenetic species (Fig. 2). Polytomies and short branch lengths among *Aculamprotula fibrosa*, *A. zonata* and *A. tientsinensis* (average uncorrected p distance = 0.010) and *Lamprotula caveata*, *L. cornuumlunae* and *L. rochechouartii* (0.016) are consistent with each of those shallow clades representing single evolutionary entities. We are loath to apply a phenetic threshold to revise species taxonomy. Furthermore, lacking larger sample sizes and range-wide revisions including examination of type specimens, it is impossible to distinguish if it is the nominal species or merely the identifications that are invalid. No voucher specimens were reported by Zhou *et al.* (2007) and all of their samples were

reported to be from the same locality ('Poyang Lake, Jiangxi'). Although it is well supported that taxa formerly assigned to *Lamprotula* belong to a separate clade among the Unioninae, these results bring into question the current species composition of both genera and the broader applicability of these results.

What is known of the morphology of *Aculamprotula scripta* challenges its inclusion in both that genus and the subfamily Unioninae. *Aculamprotula fibrosa* and other unionine mussels generally possess subtriangular hooked-type glochidia and brood their larvae only in the outer pair of demibranchs (Wu *et al.*, 1999; Graf & Cummings, 2006). *Aculamprotula scripta* has been reported to have unhooked-type glochidia and a tetragenous marsupium, as observed among the species of *Lamprotula s. s.* (Park & Kwon, 1993; Wei *et al.*, 1994; Wu *et al.*, 1999). This suggests misidentification of either the single specimen sequenced by Zhou *et al.* (2007) or those for which the reproductive biology was reported. Moreover, inclusion of *A. scripta* in *Aculamprotula*, as currently constituted, necessitates revision of the generic nomenclature. *Unio scriptus* Heude, 1875 is the type species of *Scriptolamprotula* Modell, 1964, an older available name for the genus.

The question of whether *Aculamprotula* or *Scriptolamprotula* is the correct name for this clade may be moot. The phylogenetic position of the type species of *Lamprotula*, *Chama plumbea* Dillwyn, 1817 (Simpson, 1900), has not been studied and that species is unknown beyond the figures by Chemnitz (1795: pl. 203, figs 1991, 1992). *Lamprotula plumbea* is conchologically similar to the type of *Aculamprotula* (*A. fibrosa*), both having prominent umbos and serrated lateral teeth (Fig. 1). Should *L. plumbea* be discovered to fall among those species assigned to *Aculamprotula*, the name *Lamprotula* would follow it, and the species now classified in the latter genus would move to the next available name, *Gibbosula* Simpson, 1900. The actual phylogenetic position of *Lamprotula crassa* (Wood, 1815), the type species of *Gibbosula*, is also poorly understood, so one of several other available generic names may prove valid once all the species of *Lamprotula s. l.* and related genera have been rigorously evaluated. These unanswered questions highlight the futility of applying provincial taxon sampling to the revision of species-rich clades with broad geographical distributions.

Zhou *et al.* (2007) demonstrated that *Lamprotula s. l.* is polyphyletic, but our re-analysis of their data in a global phylogenetic context has revealed that the taxonomic situation is not as clear as it might seem. This confusion is further evidence that the family-, genus- and species-level taxonomy of the freshwater mussels of eastern Asia, based largely on the century-old works of Simpson (1900, 1914) and Haas (1910–1920, 1924), is in need of revision to reflect accurately their evolutionary history. *Lamprotula s. l.* is not the first such traditional genus to be split up among different subfamilies following determination of soft-anatomy and larval characters of the constituent species (e.g. Kondo, 1998). Too much of what we 'know' about freshwater mussel evolutionary relationships was extrapolated in the pre-cladistic era from homoplastic conchological characters.

These problems have implications beyond freshwater mollusc systematics. Accurate knowledge of the patterns of both species and phylogenetic diversity are valuable for informing conservation priorities for freshwater ecosystems (Faith, 1992; Darwall & Vié, 2005). Freshwater mussels provide a valuable complement to fishes for identifying diversity hotspots (Graf & Cummings, 2011), but only when their evolutionary relationships are well understood. Given the ongoing biodiversity crisis we can no longer afford to rely on pre-cladistic provincial taxonomic arrangements. Revisionary studies are difficult and time consuming, but they are necessary if systematic malacology is going to serve productively the conservation-research community. We hope this study will inspire a more comprehensive analysis of *Aculamprotula*, *Lamprotula* and freshwater mussels generally.

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