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Phylogeny of the most species-rich freshwater bivalve family (Bivalvia: Unionida: Unionidae): Defining modern subfamilies and tribes



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ABSTRACT

Freshwater mussels of the order Unionida are key elements of freshwater habitats and are responsible for important ecological functions and services. Unfortunately, these bivalves are among the most threatened freshwater taxa in the world. However, conservation planning and management are hindered by taxonomic problems and a lack of detailed ecological data. This highlights the urgent need for advances in the areas of systematics and evolutionary relationships within the Unionida. This study presents the most comprehensive phylogeny to date of the larger Unionida family, i.e., the Unionidae. The phylogeny is based on a combined dataset of 1032 bp (COI + 28S) of 70 species in 46 genera, with 7 of this genera being sequenced for the first time. The resulting phylogeny divided the Unionidae into 6 supported subfamilies and 18 tribes, three of which are here named for the first time (i.e., Chamberlainiini *nomen novum*, Cristariini *nomen novum* and Lanceolariini *nomen novum*). Molecular analyses were complemented by investigations of selected morphological, anatomical and behavioral characters used in traditional phylogenetic studies. No single morphological, anatomical or behavioral character was diagnostic at the subfamily level and few were useful at the tribe level. However, within subfamilies, many tribes can be recognized based on a subset of these characters. The geographical distribution of each of the subfamilies and tribes is also presented. The present study provides important advances in the systematics of these extraordinary taxa with implications for future ecological and conservation studies.

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1. Introduction

Understanding phylogenetic diversity is crucial for conservation prioritization of freshwater mussels (Bivalvia: Unionida), which are among the most threatened freshwater taxa in the world (IUCN, 2015; Lydeard et al., 2004). Due to their ecological and economic importance, interesting biological traits (e.g., a parasitic life with the reproductive dependence on a host fish and a particular form of mitochondrial inheritance called double uniparental inheritance; Barnhart et al., 2008; Breton et al., 2007; Hoeh et al., 1996, 2002a), scientific research on Unionida has grown in recent years (Haag, 2012; Lopes-Lima et al., 2014). However, taxon-based conservation efforts focused on the Unionidae are hindered by various phylogenetic and taxonomic uncertainties (e.g., Inoue et al., 2014; Pfeiffer et al., 2015), and many species, especially those outside of North America and Western Europe, have been assigned a Data Deficient status by the IUCN (Bogan and Roe, 2008; IUCN, 2015; Kohler et al., 2012).

The Unionidae is by far the most species rich family within the order Unionida, with 620 species in 142 genera (Bogan and Roe, 2008) widely distributed across the freshwater ecosystems of Europe, Asia, North America and Africa. The first classification of the global Unionidae fauna was attempted by Lea (1836, 1838, 1852, 1870), and later updated by Simpson (1900, 1914). These works, in which the marsupium (i.e., the gill structure where the eggs and larvae are brooded), anatomy, larvae type and umbo sculpture were used as key classification characters, divided the Unionidae into two subfamilies, Unioninae and Hyriinae (Table 1). Subsequently, A.E. Ortmann performed a series of studies on North American taxa including additional anatomical classification characters and divided the Unionidae into three subfamilies: Unioninae, Anodontinae and Lampsilinae (Table 1: Ortmann, 1910, 1911, 1912, 1919, 1921; Ortmann and Walker, 1922). In discussing his classification, Ortmann (1912) noted the inadequacy of shell characters to define families and subfamilies due to widespread convergences in shell morphology; a problem that was further discussed by Prashad (1931). Apart from regional works (e.g., Frierson, 1927; Haas, 1940; Iredale, 1934), little progress was made on Unionidae classification until the middle of the twentieth century, when Modell and Haas published their comprehensive classification systems (Table 1: Haas, 1969a,b; Modell, 1942, 1949, 1964). Both Haas and Modell classification systems used a set of morphological and anatomical characters, but relied heavily on shell morphology. Haas (1969a,b) classified the Unionidae into six subfamilies. One of these, i.e., the Hyriinae, combined species from South America and Australasia and would later be recognized as a distinct family. Modell (1942, 1949, 1964) developed a more complex and inflated classification system, which organized the Unionidae genera in distinct families and multiple subfamilies. Both authors' use of highly variable conchological characters for classification above the genus level led to incoherent associations. Nevertheless, the work by Haas has been widely recognized as the more reliable in terms of representing generic and subgeneric distinctiveness and is considered as fundamental in establishing the main genera of the Unionida and in particular, the Unionidae (Roe and Hoeh, 2003). Concurrent with the work of Haas (1969a,b) and Modell (1942, 1949, 1964), an even more inflated classification scheme was proposed by Starobogatov (1970) and Zatravkin and Bogatov (1987), who relied on conchological differences and focused on the curvature of the frontal section of the valves. This system is merely typological and was disregarded by most of the western school of malacologists (see Graf, 2007) and emergent Russian studies (Bolotov et al., 2015; Klishko et al., 2014).

A comprehensive molecular phylogenetic study of the Unionidae has not been attempted to date, primarily due to the difficulties in developing a dataset of sufficient geographical and species coverage. The first classification system using a phylogenetic framework was published by Heard and Guckert (1970; Table 1) for the North American Unionida fauna. Disregarding shell characters, these authors used a broad anatomical and reproductive behavior character set within a phylogenetic context. Their analyses resulted in the division of the North American Unionidae into two families and several subfamilies. The subsequent development of powerful molecular and statistical tools, providing a basis for more objective approaches, has led to the publication of several studies on unionid phylogeny (e.g., Campbell and Lydeard, 2012a, b; Campbell et al., 2005; Davis, 1983, 1984; Davis and Fuller, 1981; Davis et al., 1977, 1981; Graf and Cummings, 2006; Hoeh et al., 1998, 2001, 2002b, 2009; Pfeiffer and Graf, 2013, 2015; Roe and Hoeh, 2003; Whelan et al., 2011; Zanatta and Murphy, 2006). In many of these studies, unionid genera or species that had been identified by morphological characters were not consistent with those revealed through molecular phylogenetic analyses (e.g., Campbell and Lydeard, 2012a,b; Nagel and Badino, 2001; Roe and Hoeh, 2003). Although the vast majority of these molecular studies have focused almost exclusively on North American and European taxa, geographic and taxonomic sampling has recently increased, particularly in Africa (Elderkin et al., 2016; Graf, 2013; Whelan et al., 2011) and Asia (Huang et al., 2002; Pfeiffer and Graf, 2013, 2015; Zhou et al., 2007; Zieritz et al., 2016).

Recent molecular phylogenetic studies have achieved considerable progress in describing the main divisions within the Unionidae (Campbell and Lydeard, 2012a, 2012b; Graf and Cummings, 2006; Pfeiffer and Graf, 2013, 2015; Whelan et al., 2011). The status of the North American Ambleminae with four recognized tribes has been recently confirmed (Table 1: Campbell and Lydeard, 2012a,b; Campbell et al., 2005). Studies including species from Africa and the Indotropics examined the subfamily Parreysiinae in detail and recognized several subfamilies (Table 1: Pfeiffer and Graf, 2015; Whelan et al., 2011). Despite the considerable recent progress (Huang et al., 2002; Pfeiffer and Graf, 2013, 2015; Zhou et al., 2007), the vast majority of unionid genera from the Eastern Palearctic and the Indotropics have never been analyzed in a modern phylogenetic framework.

Based on bibliographical research, the classification of the Unionidae was recently reviewed, establishing the currently accepted subdivisions of the Unionidae (Carter et al., 2011; Table 1). This classification divided the family into six subfamilies: the Ambleminae with a North and Central American distribution; the Parreysiinae with a disjunct distribution primarily in Sub-Saharan Africa and the Indian subcontinent; the Modellinae with a single species from Thailand; the Rectidentinae with a South East Asian distribution; and two subfamilies, the Unioninae and Gonideinae, distributed through most of Asia, Europe, North Africa and west coast of North America.

In order to increase the success of ongoing and future management efforts and to inform conservation priorities more effectively, a better understanding of the evolutionary history of freshwater mussels is necessary. Our objective herein is to improve the understanding of unionid phylogeny through analysis of a combination of nuclear and mitochondrial molecular markers from a wide coverage of genera. In detail, this study aims to: (i) resolve the main phylogenetic relationships within the Unionidae; (ii) discuss the systematics, taxonomy and distribution of the recovered unionid subdivisions (subfamilies and tribes); and (iii) compare the obtained classification with those based on morphological characters.

Table 1

Historical classification systems of the subfamilies and tribes now included in the Unionidae. (Blue) subfamilies; (red) tribes; (^{nm}) *nomen novum*; (*) regional study; (?) rank uncertain.

Simpson (1900/1914)	Ortmann (1912)*	Modell (1942)	Haas (1969a, 1969b)	Heard and Guckert (1970)*	Brandt (1974)*	Graf and Cummings (2007)	Bieler et al. (2010)	Whelan et al. (2011)	Carter et al. (2011)	This Study
Unionidae	Unionidae	Unionidae	Unionidae	Unionidae	Unionidae	Unionidae	Unionidae	Unionidae	Unionidae	Unionidae
Unioninae	Unioninae	Unioninae	Alasmidontinae	Unioninae	Hyriopsinae	Unioninae	Unioninae	Unioninae	Unioninae	Unioninae
Hyriinae	Anodontinae	Anodontinae	Anodontinae	Anodontinae	Pseudodontinae	Unionini	Unionini	Unionini	Unionini	Unionini
	Lampsilinae	Hyriinae	Hyriinae	Lampsilinae	Parreysiinae	Anodontini	Anodontini	Anodontini	Anodontini	Ambleminae
		Coelaturinae	Lampsilinae	Pleurobeminiae	Rectidentinae	Ambleminae	Ambleminae	Ambleminae	Ambleminae	Amblemini
		Contradentinae	Quadrulinae	Popenaiadinae	Modellinae	Amblemini	Amblemini	Amblemini	Amblemini	Lampsilini
		Hyriinae	Unioninae			Gonideini	Lampsilini	Lampsilini	Lampsilini	Pleurobemini
		Hyriopsinae		Amblemidae		Lampsilini	?Oxynaiini	Pleurobemini	Pleurobemini	Quadrulini
		Lamellidentinae		Ambleminae		Pleurobemini	Pleurobemini	Quadrulini	Quadrulini	Anodontinae
		Lamprotulinae		Gonideinae		Quadrulini	Quadrulini	Gonideinae	Gonideinae	Anodontini
		Nannonaiinae		Megaloniaidinae			Coelaturinae	Modellinae	Modellinae	Cristarini^{nm}
		Parreysiinae					Gonideinae	Parreysiinae	Parreysiinae	Lanceolariini^{nm}
		Prophyridellinae					Modellinae	Coelaturini	Rectidentinae	Gonideinae
		Quadrulinae					Parreysiinae	Oxynaiini		Gonideini
		Rectidentinae					Rectidentinae	Parreysiini		Chamberlainiini^{nm}
								Lamellidentini		Lamprotulini
		Elliptionidae						Rectidentinae		Pseudodontini
		Alasmidontinae								Parreysiinae
		Ambleminae								Coelaturini
		Elliptioninae								Oxynaiini
		Lampsilinae								Parreysiini
		Pleurobeminiae								Lamellidentini
										Rectidentinae
		Margaritiferidae								Rectidentini
		Heudeaninae								Contradentini
		Pseudodontinae								?Modellinae

2. Materials and methods

2.1. Taxon sampling

All analyzed taxa are listed in Table 2. Taxa were chosen to cover all available genera of Unionidae subfamilies. Exceptions were made with regard to the North American subfamily Ambleminae (only up to three species per tribe were included) and the African/Asian subfamily Parreysiinae, since both of these subfamilies were studied in detail elsewhere (Campbell and Lydeard, 2012a,b; Campbell et al., 2005; Whelan et al., 2011). Taxa representative from all families of the subclass Palaeoheterodonta were also included (comprising all recognized Unionida families and from *Neotrigonia*, the marine sister group of the Unionida) (Giribet and Wheeler, 2002).

2.2. DNA extraction, amplification and sequencing

Whole genomic DNA was extracted from small foot tissue samples preserved in 96% ethanol using a standard high-salt protocol (Sambrook et al., 1989) or the Jetquick tissue DNA Spin Kit (Genomed) following the manufacturer's protocol. PCR conditions for both markers, the female lineages of mitochondrial cytochrome c oxidase subunit 1, COI (LCO22me2 + HCO700dy2; Walker et al., 2006, 2007; and LCO1490 + HCO2198; Folmer et al., 1994) and 28S ribosomal RNA (28S-RD1.3f and 28S-rD4b; Whiting, 2002) were described in Froufe et al. (2014). Annealing temperatures of 48 °C were used for COI (LCO1490 + HCO2198) and 28S; and 50 °C for COI (LCO22me2 + HCO700dy2). Amplified DNA templates were purified and sequenced by the commercial company Macrogen using the same primers.

2.3. Phylogenetic analyses

Two concatenated (COI + 28S) data sets were assembled, the Palaeoheterodonta dataset with representatives from each of the

families of the Palaeoheterodonta (Appendix A) and, in order to decrease the number of poorly aligned positions of the 28S, the Unionidae dataset with only representatives of the Unionidae (Appendix B). Both datasets were aligned using the stand-alone version of GUIDANCE (version 1.5, Penn et al., 2010) with the MAFFT multiple sequence alignment algorithm (version 7, Katoh and Standley, 2013). The following GUIDANCE parameters were used: GUIDANCE score algorithm; 100 bootstrap replicates; a sequence cutoff score of 0.0 (no sequence removal); a column cutoff score of 0.0 (no columns removal); global pair alignment. Incongruence Length Difference (ILD) tests were performed to investigate incongruence between them (Farris et al., 1994).

The best-fit models of nucleotide substitution under the corrected Akaike Information Criterion were selected using JModelTest 2.1.8 (Darrriba et al., 2012) for each partition, of the subsequent analyses.

For the Palaeoheterodonta dataset (Appendix A), a single scheme with 4 partitions was applied, model GTR + I + G was optimal for the first and third COI codon positions and for the whole 28S, while model F81 was optimal for the second COI codon positions. For the Unionidae dataset (Appendix B) more comprehensive analyses were executed including two distinct partitioning schemes; the first with two partitions corresponding to each gene fragment (COI and 28S) and the second with four partitions corresponding to the three codon positions of COI and one for 28S. For the scheme with 2 partitions, model GTR + I + G was optimal for both. For the scheme with 4 partitions, model GTR + I + G was optimal for the first COI codon positions and for the 28S, while model F81 was optimal for the second COI codon positions. Finally, model GTR + G was optimal for the third positions of COI.

Two different analyses were then performed on all partitioned schemes of the concatenated datasets using Bayesian Inference (BI) (BI2: 2 partitions, BI4: four partitions) and Maximum Likelihood analyses (ML) (ML2: 2 partitions, ML4: 4 partitions). BI analyses were performed in MrBayes v3.2.5 (Ronquist et al., 2012)

Table 2

Specimens analyzed. (^U) Unknown country; (*) not generated from a single individual. Taxonomy follows Table 3.

Taxon	COL	28S	Country	Reference	Voucher
UNIONIDAE					
ANODONTINAE					
ANODONTINI					
<i>Alasmidonta marginata</i> Say, 1818	AF156502	AF400688	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
<i>Anodonta anatina</i> (Linnaeus, 1758)	KX822632	KX822588	Russia	This study	
<i>Anodonta cygnea</i> (Linnaeus, 1758)	KX822633	KX822589	Italy	This study	
<i>Anodonta nuttalliana</i> Lea, 1838	KX822634	KX822590	USA	This study	
<i>Lasmigona compressa</i> (Lea, 1829)	AF156503	DQ191414	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
<i>Pseudanodonta complanata</i> (Rossmässler, 1835)	KX822661	KX822617	Ukraine	This study	
<i>Pyganodon grandis</i> (Say, 1829)*	AF231734	AF305384	USA	Bogan and Hoeh (2000); Graf and Foighil (2000)	
<i>Simpsonia ambigua</i> (Say, 1825)	KX822666	KX822622	USA	This study	NCSM30607
<i>Strophitus undulatus</i> (Say, 1817)*	AF156505	DQ191415	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
CRISTARIINI					
<i>Anemina arcaeformis</i> (Heude, 1877)	KF667530	KX822587	China	An et al. (2016); this study	
<i>Cristaria plicata</i> (Leach, 1814)	KX822637	KX822594	Russia	This study	
<i>Pletholophus tenuis</i> (Griffith & Pidgeon, 1833)	KX822658	KX822614	Vietnam	This study	NCSM84924
<i>Sinanodonta lucida</i> (Heude, 1877)	KX822667	KX822624	China	This study	
<i>Sinanodonta woodiana</i> (Lea, 1834)	KX822668	KX822625	Vietnam	This study	NCSM84916
LANCEOLARIINI					
<i>Arconaia lanceolata</i> (Lea, 1856)	NC_023955	KX822591	China	Wang et al. (2016); this study	
<i>Lanceolaria gladiola</i> (Heude, 1877)	KX822648	KX822605	China	This study	
<i>Lanceolaria grayana</i> (Lea, 1834)	KX822649	KX822606	China	This study	
<i>Lanceolaria grayii</i> (Griffith & Pidgeon, 1833)	KX822650	KX822607	Vietnam	This study	NCSM84945
UNIONINAE					
UNIONINI					
<i>Unio crassus</i> Philipsson, 1788*	KC703878	KC703644	France	Prié and Puillandre (2014)	
<i>Unio gibbus</i> Spengler, 1793	KX822671	KX822629	Morocco	This study	
<i>Unio pictorum</i> (Linnaeus, 1758)	KC429109	KC429447	^U (Europe)	Sharma et al. (2013)	
<i>Unio tumidus</i> Philipsson, 1788	KX822672	KX822630	Ukraine	This study	
Incertae sedis (UNIONINAE)					
<i>Aculamprotula tortuosa</i> (Lea, 1865)	KX822631	KX822586	China	This study	
<i>Cuneopsis heudei</i> (Heude, 1874)	KX822638	KX822595	China	This study	
<i>Cuneopsis pisciculus</i> (Heude, 1874)	KX822639	KX822596	China	This study	
<i>Cuneopsis rufescens</i> (Heude, 1874)	KX822640	KX822597	China	This study	
<i>Nodularia douglasiae</i> (Griffith & Pidgeon, 1833)	KX822653	KX822610	China	This study	
<i>Nodularia nuxpersicae</i> Dunker, 1848	KX822654	KX822611	Vietnam	This study	NCSM84990
<i>Schistodesmus lampreyanus</i> (Baird & Adams, 1867)	KX822665	KX822621	China	This study	
RECTIDENTINAE					
CONTRADENTINI					
<i>Conradens conradens</i> (Lea, 1838)	DQ191411	AF400692	^U (Asia)	Graf and Cummings (2006)	
<i>Conradens semmelinki fultoni</i> Haas, 1930	KX822636	KX822593	Vietnam	This study	NCSM84935
<i>Physunio modelli</i> Brandt, 1974	KX822655	KX822612	Thailand	This study	
<i>Trapezoideus exollescens</i> (Gould, 1843)	KP795036	KP795018	Laos	Pfeiffer and Graf (2015)	
RECTIDENTINI					
<i>Ensidents ingallsianus</i> (Lea, 1852)	KX822641	KX822598	Laos	This study	NCSM84889
<i>Ensidents sagittarius</i> (Lea, 1856)	KP795033	KP795015	Cambodia	Pfeiffer and Graf (2015)	
<i>Ensidents</i> sp.	KX822642	KX822599	Laos	This study	NCSM84902
<i>Hyriopsis bialata</i> Simpson, 1900	KX822643	KX822600	Thailand	This study	
<i>Hyriopsis desowitzi</i> Brandt, 1974	KX822644	KX822601	Thailand	This study	
<i>Hyriopsis myersiana</i> (Lea, 1856)	KX822645	KX822602	Thailand	This study	
<i>Rectidens sumatrensis</i> (Dunker, 1852)	KX822664	KX822620	Malaysia	This study	
GONIDEINAE					
CHAMBERLAINIINI					
<i>Chamberlainia hainesiana</i> (Lea, 1856)	KX822635	KX822592	Thailand	This study	
<i>Sinohyriopsis cumingii</i> (Lea, 1852)*	HM347668	KX822623	China	Unpublished; this study	
LAMPROTULINI					
<i>Lamprotula caveata</i> (Heude, 1877)	KX822646	KX822603	China	This study	
<i>Lamprotula leaii</i> (Griffith & Pidgeon, 1833)	KX822647	KX822604	China	This study	
<i>Potomida littoralis</i> (Cuvier, 1798)	JN243905	JN243883	France	Whelan et al. (2011)	
<i>Pronodularia japonensis</i> (Lea, 1859)	KX822659	KX822615	Japan	This study	NCSM27183
GONIDEINI					
<i>Gonidea angulata</i> (Lea, 1838)*	DQ272371	AF400691	USA	Gustafson and Iwamoto (2005); Graf (2002)	
<i>Leguminaia wheatleyi</i> (Lea, 1862)	KX822651	KX822608	Turkey	This study	
<i>Microcondylaea bonelli</i> (A. Ferussac 1827)	KX822652	KX822609	Italy	This study	
<i>Solenaia carinata</i> (Heude, 1877)	KX822669	KX822626	China	This study	
<i>Solenaia oleivora</i> (Heude, 1877)	KX822670	KX822627	China	This study	
PSEUDODONTINI					
<i>Pilsbryconcha compressa</i> (Martens, 1860)	KX822656	KX822613	Thailand	This study	
<i>Pilsbryconcha exilis</i> (Lea, 1838)*	KX822657	AF400693	Vietnam	Graf (2002); this study	
<i>Pseudodon cambodjensis</i> (Petit de la Saussaye, 1865)	KX822660	KX822616	Thailand	This study	
<i>Pseudodon cumingii</i> (Lea, 1850)	KX822662	KX822618	Laos	This study	NCSM84884
<i>Pseudodon mouhotii</i> (Lea, 1863)	KX822663	KX822619	Laos	This study	NCSM84903
Incertae sedis (GONIDEINAE)					
<i>Solenaia triangularis</i> (Heude, 1885)	KJ434518	KX822628	China	This study	

(continued on next page)

Table 2 (continued)

Taxon	COI	28S	Country	Reference	Voucher
AMBLEMINAE					
AMBLEMINI					
<i>Amblema plicata</i> (Say, 1817)	APU56841	AF305385	USA	Hoeh et al. (1998); Graf (2002)	
LAMPSILINI					
<i>Actinonaias ligamentina</i> (Lamarck, 1819)	AF156517	DQ191420	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
<i>Lampsilis cardium</i> Rafinesque, 1820*	AF120653	AF305386	USA	Giribet and Wheeler (2002); Graf (2002)	
<i>Villosa iris</i> (Lea, 1829)	AF156524	DQ191422	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
PLEUROBEMINI					
<i>Elliptio complanata</i> (Lightfoot, 1786)*	EU448173	JF899181	USA	Unpublished; Distel et al. (2011)	
<i>Elliptio dilatata</i> (Rafinesque, 1820)*	AF156507	AF400690	USA	Graf and Foighil (2000); Graf (2002)	
<i>Pleurobema sintoxia</i> (Rafinesque, 1820)	AF156509	DQ191418	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
QUADRULINI					
<i>Quadrula quadrula</i> (Rafinesque, 1820)	AF156511	DQ191417	USA	Graf and Foighil (2000); Graf and Cummings (2006)	
<i>Quadrula verrucosa</i> (Rafinesque, 1820)	DQ191413	DQ191416	USA	Graf and Cummings (2006)	
PARREYSIINAE					
COELATURINI					
<i>Coelatura aegyptiaca</i> (Cailliaud, 1827)	JN243892	JN243870	Egypt	Whelan et al. (2011)	
LAMELLIDENTINI					
<i>Lamellidens corrianus</i> (Lea, 1834)	JN243903	JN243881	Burma	Whelan et al. (2011)	
OXYNAIINI					
<i>Oxynaia pugio</i> (Benson, 1862)	JN243899	JN243879	Burma	Whelan et al. (2011)	
PARREYSIINI					
<i>Parreysia mandelayensis</i> (Theobald, 1873)	JN243900	JN243876	Burma	Whelan et al. (2011)	
TRIGONIIDAE					
<i>Neotrigonia margaritacea</i> (Lamarck, 1804)*	NMU56850	AF400695	Australia	Hoeh et al. (1998); Graf (2002)	
HYRIIDAE					
<i>Hyridella depressa</i> (Lamarck, 1819)	AF156496	AF305375	Australia	Graf and Foighil (2000); Graf (2002)	
<i>Velesunio ambiguus</i> (Philippi, 1847)	KC429106	KC429444	Australia	Sharma et al. (2013)	
MARGARITIFERIDAE					
<i>Cumberlandia monodonta</i> (Say, 1829)	AF156497	AF305382	USA	Graf and Foighil (2000); Graf (2002)	
<i>Margaritifera margaritifera</i> (Linnaeus, 1758)	KC429108	KC429446	^U (Europe)	Sharma et al. (2013)	
ETHERIIDAE					
<i>Etheria elliptica</i> Lamarck, 1807	KP184897	KP184873	Zambia	Graf et al. (2015)	
IRIDINIDAE					
<i>Aspatharia pfeifferiana</i> (Bernardi, 1860)	JN243885	JN243863	Zambia	Whelan et al. (2011)	
<i>Chambardia wahlbergi</i> (Krauss, 1848)	JN243886	JN243864	Zambia	Whelan et al. (2011)	
<i>Mutela rostrata</i> (Rang, 1835)	JN243884	JN243862	Egypt	Whelan et al. (2011)	
MULLERIIDAE					
<i>Anodontites elongata</i> (Swainson, 1823)	JN243888	JN243866	Peru	Whelan et al. (2011)	
<i>Mycetopoda siliquosa</i> (Spix & Wagner, 1827)	JN243887	JN243865	Peru	Whelan et al. (2011)	

using the previously selected models. Analyses were initiated with program-generated trees and four Markov chains with default incremental heating. Two independent runs of 24×10^6 generations were sampled at intervals of 1000 generations producing a total of 24,000 trees. Burn-in was determined upon convergence of log likelihood and parameter values using Tracer 1.6 (Rambaut et al., 2014).

For the ML phylogenetic analyses, sequences were analyzed in RaxML 8.0.0 (Stamatakis, 2014) where GTR + G + I model was assumed for each partition with 1000 bootstrap replicates.

2.4. Review of morphological, anatomical, and behavioral traits

A table of morphological characters commonly used for Unionidae systematics was constructed using a compilation of the available literature and direct observations of the analyzed taxa. To characterize and compare glochidial size, the glochidial size index (Gln) was calculated following Davis and Fuller (1981) where $Gln = \text{glochidial shell length } (\mu\text{m}) \times \text{shell height } (\mu\text{m}) \times 10^{-6}$. Gln was divided into three size classes: small (≤ 0.020), medium (> 0.020 and ≤ 0.070) and large (> 0.070). These classes were determined using all glochidia measurements collected for this study (Table C1) and those included in Barnhart et al. (2008) and Hoggarth (1999); the smaller size range of Quadrulini was used to define the class 'small'; the larger size range of Anodontini was used to define the class 'large'; and the 'medium' class size was defined with intermediate Gln values between the two other classes.

2.5. Distribution

Distribution maps were constructed using data available from the IUCN database (IUCN, 2015), the Mussel Project website (Graf and Cummings, 2016), the North Carolina Museum of Natural Sciences database (NCMNS, 2016), and additional reference works (Bogatov, 2012; Bogatov and Starobogatov, 1992; Brandt, 1974; Clarke, 1981; Cyr et al., 2007; Ð ng et al., 1980; Doucet-Beaupré et al., 2012; Haas, 1969a,b; He and Zhuang, 2013; Howells et al., 1996; Klishko, 2001, 2003; Kondo, 2008; Moskvicheva, 1973a,b; Nedeau et al., 2009; Prozorova and Bogatov, 2006; Subba Rao, 1989; Vinarski et al., 2007; Zatravkin and Bogatov, 1987; Zhadin, 1938). Because distribution data were gathered and compiled from very distinct sources, ranging from georeferenced data points, hydrographic basins and geographical regions or countries, the distributions on the maps are represented with various patterns (e.g., political borders or hydrographic basins).

3. Results and discussion

Previous phylogenetic studies of the Unionidae failed to include most of the genera, mainly those from the Eastern Palearctic and Indotropical ecoregions. We were able to clarify the phylogeny within Unionidae by the inclusion of samples from a wide coverage of genera and geographic distribution.

On both of the following (COI + 28S) datasets, no indels were observed in the COI alignments and no stop codons were found

after translating the sequences to amino acids. The ILD tests found no significant phylogenetic conflict between the COI and 28S for the Palaeoheterodonta ($p = 0.95$) and the Unionidae ($p = 0.94$) datasets.

The Palaeoheterodonta dataset (COI + 28S) included 81 species in 55 genera, with a total of 1091 bp (COI: 597 bp, 28S: 494 bp). Since the same topology in the supported nodes was obtained in the resulting phylogenetic trees, the BI4 (Bayesian Inference with 4 partitions, see methods) topology is here presented in Fig. 1. These analyses revealed the monophyly of the Unionidae in all analyses with six supported subfamilies supported by the BI analysis (Anodontinae, Unioninae, Rectidentinae, Gonideinae, Ambleminae, and Parreysiinae) showing the Parreysiinae as a sister clade to all of the other Unionidae.

The dataset including only Unionidae taxa spanned 70 species in 46 genera, with a total of 1032 bp (COI: 597 bp, 28S: 435 bp) aligned nucleotides. All resulting phylogenetic trees yielded the same topology up to the tribal level, being the BI4 (Bayesian Inference with 4 partitions, see methods) topology presented. Both BI topologies were generally associated with higher bootstrap support levels than ML topologies. Furthermore, the BI4 topology resulted in slightly higher bootstrap values than the BI2 topology, presumably due to distinct COI mutation rates.

The Unionidae is divided in two major clades, which are well supported in all analyses and partition schemes, i.e., Anodontinae + Unioninae and Rectidentinae + Gonideinae + Ambleminae (Fig. 2). At the subfamily level most clades are supported by the Bayesian analyses, with the Rectidentinae also being supported by both ML analyses (Fig. 2). At the tribal level the same trend is observed, with all four analyses supporting Contradentini, Rectidentini, Chamberlainiini, Lamprotulini, with the remaining tribes being supported mostly by BI analyses only.

The subfamily Anodontinae is divided into three tribes (i.e., Anodontini, Cristariini *nomen novum* and Lanceolariini *nomen novum*), and the subfamily Unioninae is not well resolved, with Unionini being the only supported tribe. Available tribe names for the currently unsupported group (sister to the Unionini) include Acuticostinae Starobogatov, 1967 and Nodulariinae Starobogatov and Zatravkin, 1987. The subfamily Rectidentinae is sister to Gonideinae + Ambleminae and encompasses two tribes (i.e., Contradentini and Rectidentini). Both Gonideinae and Ambleminae are divided into four tribes each (see Fig. 2). In summary, our molecular phylogenetic analyses revealed division of the Unionidae into 6 subfamilies and 18 tribes, three of which are named here for the first time. Revisions pertaining to the subfamilial and tribal classification within the Unionidae are discussed here along with a number of lower-level phylogenetic and taxonomic considerations.

To complement the present molecular analyses, seven morphological, anatomical and behavioral characters commonly used in traditional classifications of the Unionidae are summarized for each taxon in Table C1.

Glochidial shape is diagnostic in dividing the Anodontinae + Unioninae (triangular) and Rectidentinae + Gonideinae + Ambleminae (bilaterally asymmetrical or semi-elliptical) clades (Table C1). No single morpho-behavioral character analyzed herein is diagnostic of subfamilies within these clades. However, within subfamilies, certain tribes are characterized by unique diagnostic characters. Within Anodontinae, four characteristics (shell shape, hinge structure, glochidial size and brooding period) separate the Lanceolariini from the Anodontini + Cristariini. Additionally, all taxa within the Lanceolariini analyzed are characterized by nodulous umbo sculpture, although this morphological character is highly variable within all other subfamilies and tribes (Table C1). Within Rectidentinae, glochidial shape is diagnostic in separating the Contradentini (bilaterally asymmetrical) and Rectidentini

(semi-elliptical). Among the four tribes in Gonideinae, the Chamberlainiini taxa are unique in exhibiting ectobranchous marsupia (Table C1).

3.1. Classification system

Based on the present results, a new classification of the Unionidae is presented, including the description of three new tribes: Cristariini Lopes-Lima, Bogan and Froufe, 2016; Lanceolariini Froufe, Lopes-Lima and Bogan, 2016; and Chamberlainiini Bogan, Froufe and Lopes-Lima, 2016 (Table 3).

3.2. Anodontinae Rafinesque, 1820 phylogeny and tribal classification

The subfamily status of the Anodontinae was first defined by Rafinesque in 1820 and properly Latinized by Fleming in 1828. The subfamily status was retained in many of the classical classifications well in to the 20th century (e.g., Davis and Fuller, 1981; Haas, 1969a,b; Heard and Guckert, 1970; Modell, 1964; Ortmann, 1910). Subsequent studies demoted Anodontinae to a tribe within Unioninae due to the shared hooked type and subtriangular external shape of the glochidia (Bieler et al., 2010; Carter et al., 2011; Graf, 2002; Graf and Cummings, 2007). However, the rank change of Anodontinae into Anodontini has been recently disputed based on morphology discrepancies in glochidia morphology (Huang et al., 2013). Anodontinae and Unioninae are here recovered as sister clades and due to the ancient divergence of the two clades are herein considered as subfamilies, in accordance with traditional classifications. Within Anodontinae, we recognize three distinct tribes. In traditional classifications, this subfamily was characterized by a set of distinctive morphological (e.g., large and ovate thin shells, and large triangular and hooked glochidia), anatomical (e.g., demibranchs with perforated septa, secondary water tubes in the outer demibranchs, and marsupium in the external demibranch pair that distend laterally upon gravidity) and ecological (e.g., most species seem to be generalists concerning habitat and host fish) characters. Although all of the above characters are found in most of the species within Anodontini and Cristariini, the Lanceolariini present characters more similar to those of the Unioninae (i.e., shell size and form, glochidial size, and tachyctictia).

Members of the Anodontinae have a wide distribution in the Northern Hemisphere, not occurring in most of the Indotropical, and glaciated or desert regions (Fig. 3).

3.2.1. Tribe Anodontini Rafinesque, 1820

Type Genus: *Anodonta* Lamarck, 1799

Type Species: *Mytilus cygneus* Linnaeus, 1758

Comments: The Anodontini include one supported clade that contains all analyzed Anodontinae genera from Eastern North America, while the relationships among the *Anodonta* and *Pseudanodonta* species are not well resolved. The Anodontini encompass the genera *Alasmidonta*, *Anodonta*, *Lasmigona*, *Pseudanodonta*, *Pyganodon*, *Simpsonaias*, *Strophitus* (Fig. 2), *Anodontoides*, *Arcidens*, and *Utterbackia* (Table 3; Breton et al., 2011; Inoue et al., 2014; Lydeard et al., 1996; Zanatta et al., 2007). Due to the lack of molecular data, two genera usually assigned to this tribe, i.e., *Simpsonella* from the Philippines and *Pegias* from North America (Graf and Cummings, 2016; Haas, 1969a,b), are not included in the present molecular analyses. Placement of both these genera within the tribe thus remains to be tested by molecular methods. This will be of particular interest regarding *Simpsonella*, which has a disjunct distribution and has been placed within the Contradentini in other studies (Modell, 1942, 1964). We recovered two main clades within the Anodontini: one with Palearctic genera including the

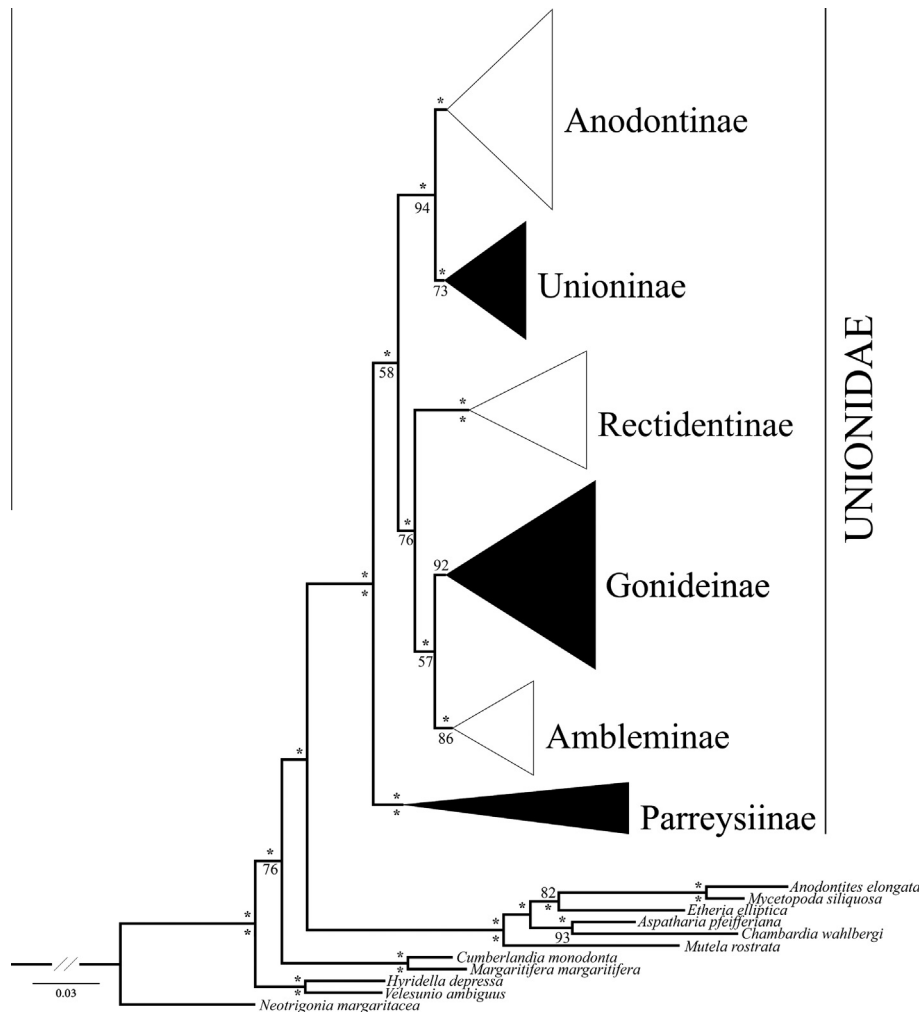


Fig. 1. Phylogenetic tree of the Paleoheterodonta obtained by Bayesian Inference (BI) and Maximum likelihood (ML) analyses of the first combined (COI + 28S) dataset. Support values above the branches are posterior probabilities (BI) and bootstrap support (ML) below. An asterisk (*) indicates nodes with $P \geq 95\%$ posterior probability or bootstrap support. Posterior probability (percentage) or bootstrap support with $P < 50\%$ were omitted for clarity. All subfamily nodes were collapsed for visual purposes.

type genus *Anodonta* that is also present in the West coast of North America and the other including all East coast North American genera (Figs. 2 and 3). The relationships among and within genera in each of these clades are not well resolved and should be further investigated.

Diagnosis: Shell is commonly thin and ovate to elongate but with some exceptions, mainly in *Alasmidonta* and *Lasmigona* spp. (Table C1). Hinge is generally toothless or with vestigial teeth in some genera e.g., *Alasmidonta*, *Lasmigona* and *Strophitus*. Umbo sculpture is varied and composed of double-looped and/or pseudo-concentric and/or single-looped ridges, which are sometimes wrinkled or nodulous. Glochidia are large, triangular, and ventrally hooked with spines (Table C1).

Distribution: The Anodontini have a disjunct distribution from the Western Palearctic to the Transbaikalia and on both North American coasts (Fig. 3). Almost all Eastern Asian Anodontinae species previously ascribed to *Anodonta* (e.g., *A. woodiana* and *A. arcaiformis*) have later been transferred to other genera that are now placed outside Anodontini (Haas, 1969a,b; Kondo, 2008). The only *Anodonta* species still recognized from East Asia, *Anodonta beringiana*, should be reassigned to the genus *Sinanodonta* (Chong et al., 2008). The presence of the tribe Anodontini in Central America and Middle East is pending further evaluation of the phylogenetic status of *Anodonta lurulenta* Morelet, 1849, *Anodonta pseudodopsis* Locard, 1883 and *Anodonta vescoiana* Bourguignat, 1856.

3.2.2. Tribe Cristariini Lopes-Lima, Bogan and Froufe, nomen novum

Type Genus: *Cristaria* Schumacher, 1817

Type Species: *Cristaria tuberculata* Schumacher, 1817; junior synonym of *Dipsas plicata* Leach, 1815.

Comments: The Cristariini include one supported clade composed by the genera *Anemina*, *Cristaria*, *Pletholophus* and *Sinanodonta* (Fig. 2; Table 3). The type genus *Cristaria* is not monophyletic in the current analyses and since *Cristaria plicata* is the type species, *Cristaria tenuis* is here reassigned to *Pletholophus* Simpson, 1900 following Đ ng et al. (1980), He and Zhuang (2013) and Simpson (1900, 1914). Many species have been assigned to *Sinanodonta*, primarily by the Russian school of nomenclature (Graf, 2007; Haas, 1969a), but validity of these placements should be tested using molecular tools. *Sinanodonta lucida* was first described as *Anodonta lucida* and then assigned to *Sinanodonta* (Đ ng et al., 1980) but both generic attributions are still being used (e.g., Huang et al., 2013; Pfeiffer and Graf, 2013). Additionally, recent studies based on morphological data consider *S. lucida* as a synonym of *S. woodiana* (Graf and Cummings, 2016; He and Zhuang, 2013). Due to the high genetic distance between these two taxa (12.3%; COI uncorrected *p*-distance), *Sinanodonta woodiana* and *Sinanodonta lucida* are here recognized as two distinct species. Finally, as mentioned above, *Anodonta beringiana*, although

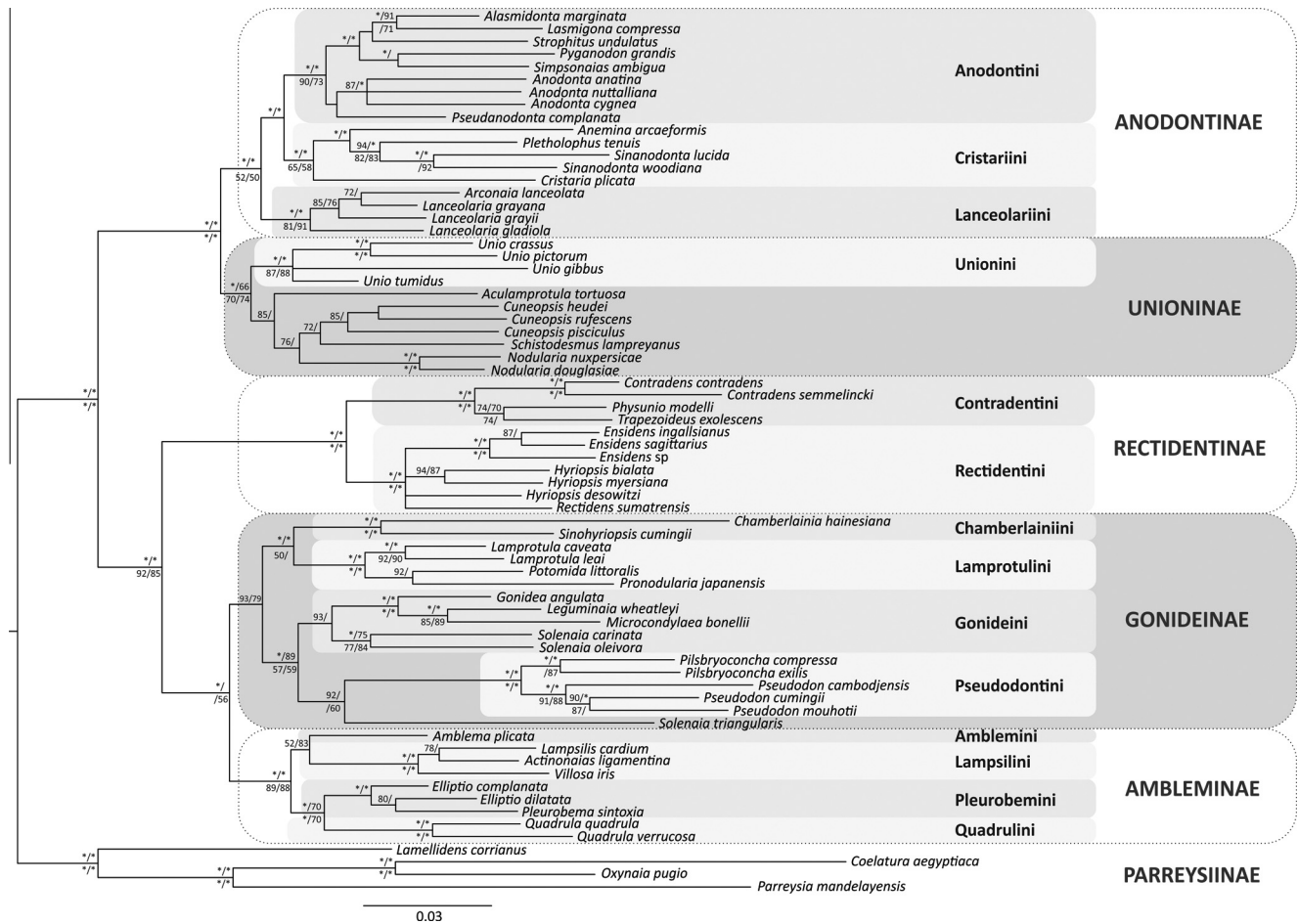


Fig. 2. Phylogenetic tree of the Unionidae obtained by Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of the second combined (COI + 28S) dataset. Support values above the branches are posterior probabilities (BI4/BI2) and bootstrap support (ML4/ML2) below. An asterisk (*) indicates nodes with $P \geq 95\%$ posterior probability or bootstrap support. Posterior probability or bootstrap support with $P < 50\%$ were omitted for clarity.

not included in the present analysis, should be placed within the Cristariini though its generic assignment remains to be investigated.

Diagnosis: Shell is usually thin, of elliptical to oval shape, with or without a posterior dorsal wing. Umbo rather low, sculpture usually consisting of pseudo-concentric folds that are nearly parallel to growth lines. Periostracum is usually rayed. Hinge is lacking in *Anemina* and *Sinanodonta*, but reduced lamellar lateral and pseudocardinal teeth may be present in *Cristaria* and *Pletholophus*.

Distribution: The native range of Cristariini spans from Indochina to China, Korea, Japan, the Sakhalin Island, Amur Basin, Kamchatka and Chukotka Peninsulas (in Russia) to the Aleutians and the Pacific Coastal Region of North America, where it may be found as far south as Oregon (Fig. 3).

3.2.3. Tribe Lanceolariini Froufe, Lopes-Lima and Bogan, nomen novum

Type Genus: *Lanceolaria* Conrad, 1853

Type Species: *Unio grayanus* Lea, 1834

Comments: The tribe Lanceolariini is sister to all other Anodontinae. Most of its shell morphological characteristics appear more similar to the subfamily Unioninae (e.g., well-developed hinge teeth, medium sized glochidia and tachytictia; Table C1). It is therefore not surprising that all previous classifications placed the genera of this tribe within the Unioninae rather than in

Anodontinae (e.g., Haas, 1969a, 1969b; Starobogatov, 1970). Lanceolariini encompasses two genera, i.e., *Arconaia* Conrad, 1865 and *Lanceolaria* Conrad, 1853, though this should be further investigated considering that our results indicate paraphyly of *Lanceolaria*, giving support for the monotypic status of Lanceolariini with *Lanceolaria* as the single genus.

Diagnosis: Shell is rather thick, of elongate or lanceolate shape and in some taxa, with antero-posterior torsion. Umbo is low and positioned near the anterior end. Umbo sculpture is strictly nodulous and usually restricted to the umbo area but in some cases more widespread. Pseudocardinal teeth are well developed and long; lateral teeth are straight and thick.

Distribution: Lanceolariini are restricted to Far East Asia, from the Amur River basin (Russia) to Japan, Korea, the Pacific basins of China and Vietnam (Fig. 3).

3.3. Unioninae Rafinesque, 1820 phylogeny and tribal classification

The Unioninae were one of the first defined subfamilies, and the subfamily level has been retained in all subsequent classifications of the Unionidae (Table 1). Until the middle of the 20th century, this subfamily encompassed almost all of the unionid genera of Europe, Africa and Asia with the exception of those assigned to Anodontinae (Haas, 1969a,b). It later became obvious that the Unioninae represented a simple collection of very distinct groups that were not related or similar in most of their characters. In this context, the Unionidae were subdivided by Modell (1942, 1964)

Table 3

Classification of the Unionidae based on the present analyses. (*) Not included in the present study.

ANODONTINAE Rafinesque, 1820	Solenia triangularis
Anodontini Rafinesque, 1820	AMBLEMINEAE Rafinesque, 1820
+ Alasmidontini Rafinesque, 1820	Amblemini Rafinesque, 1820
+ Strophitini Starobogatov, 1970	Amblema Rafinesque, 1820
+ Pseudanodontini Starobogatov, 1970	* Reginaia Campbell and Lydeard, 2012
+ Brachanodontini Bogatov, Sayenko and Starobogatov, 2002	Lampsilini Ihering 1901
* Arcidens Simpson, 1900 [+ <i>Arkansia</i> Ortmann & Walker 1912]	+ Propterini Hannibal, 1912
Alasmidonta Say, 1818	+ Cyprogeniini Starobogatov, 1970
Anodonta Lamarck, 1799	+ Dromini Starobogatov, 1970
* Anodontoides Simpson in F.C Baker, 1898	+ Friersoniini Starobogatov, 1970
Lasmigona Rafinesque, 1831	+ Glebulini, Starobogatov, 1970
Pseudanodonta Bourguignat, 1876	+ Medionidinae Starobogatov, 1970
Pyganodon Crosse & Fischer, 1894	+ Pilaeni Starobogatov, 1970
Simpsonaias , Frierson, 1914	+ Pileini Bieler et al. 2010
Strophitus Rafinesque, 1820	+ Popenaiadini Heard and Guckert, 1970
* Utterbackia F.C. Baker, 1927	+ Ptychobranchini Starobogatov, 1970
Cristariini Lopes-Lima, Bogan and Froufe, <i>Nom. Nov.</i>	Actinonaias Crosse & Fischer, 1894
Anemina Haas, 1969	* Arotonaias von Martens, 1900
Cristaria Schumacher, 1817	* Cyprogenia Agassiz, 1852
Pletholophus Simpson, 1900	* Cyrtonaias Crosse & Fischer, 1894
Sinanodonta Modell, 1945	* Dromus Simpson, 1900
Lanceolariini Froufe, Lopes-Lima and Bogan, <i>Nom. Nov.</i>	* Ellipsaria Rafinesque, 1820
Arconaia Conrad, 1865	* Epioblasma Rafinesque, 1831
Lanceolaria Conrad, 1853	* Friersonia Ortmann, 1912
ANODONTINAE (incertae sedis)	* Glebula Conrad, 1853
* Pegias Simpson, 1900	* Hamiota Roe & Hartfield, 2005
* Simpsonella Cockerell, 1903	Lampsilis Rafinesque, 1820
UNIONINAE Rafinesque, 1820	* Lemiox Rafinesque, 1831
+ Cafferriinae Modell, 1942	* Leptodea Rafinesque, 1820
Unionini Rafinesque, 1820	* Ligumia Swainson, 1840
+ Cafferriini Modell, 1942	* Medionidus Simpson, 1900
Unio Philipsson in Retzius, 1788	* Obliquaria Rafinesque, 1820
UNIONINAE (incertae sedis)	* Obovaria Rafinesque, 1819
Aculamprotula Wu, Liang, Wang & Ouyang, 1998	* Plectomerus Conrad, 1853
* Acuticosta Simpson, 1900	* Popenaias Frierson, 1927
Cuneopsis Simpson, 1900	* Potamilus Rafinesque, 1818
* Inversiumio Habe, 1991	* Ptychobranchus Simpson, 1900
* Lepidodesma Simpson, 1896	* Toxolasma Rafinesque, 1831
Nodularia Conrad, 1853	* Truncilla Rafinesque, 1819
* Rhombuniopsis Haas, 1920	* Venustaconcha Frierson, 1927
Schistodesmus Simpson, 1900	Villosa Frierson, 1927
RECTIDENTINAE Modell, 1942	Pleurobemini Hannibal, 1912
+ Hyriopsinae Modell, 1942	+ Elliptionini, Modell, 1942
Contradentini Modell, 1942	Elliptio Rafinesque, 1820
+ Physunioini Starobogatov, 1970	* Elliptioideus Frierson, 1927
Contradens Haas, 1913	* Fusconaia Simpson, 1900
Physunio Simpson, 1900	* Hemistena Rafinesque, 1820
Trapezoideus Simpson, 1900	* Plethobasus Simpson, 1900
Rectidentini Modell, 1942	Pleurobema Rafinesque, 1819
+ Limnoscapini Lindholm, 1932	* Pleuroaia Frierson, 1927
Ensidens Frierson, 1911	Quadrulini Ihering, 1901
Hyriopsis Conrad, 1853	+ Megaloniadini Heard and Guckert, 1970
Rectidens Simpson, 1900	* Cyclonaias Pilsbry in Ortmann & Walker, 1922
GONIDEINAE Ortmann, 1916	* Megalonaias Utterback, 1915
+ Leguminainae Starobogatov, 1970	Quadrula Rafinesque, 1820
Chamberlainiini Bogan, Froufe and Lopes-Lima, <i>Nom. Nov.</i>	* Tritogonia Agassiz, 1852
Chamberlainia Simpson, 1900	* Uniomerus Conrad, 1853
Sinohyriopsis Starobogatov, 1970	AMBLEMINEAE (incertae sedis)
Lamprotulini Modell, 1942	* Barynaias Crosse & Fischer, 1894
+ Psilunionini Starobogatov, 1970	* Delpinonaias Crosse & Fischer, 1894
Lamprotula Simpson, 1900	* Disconaias Crosse & Fischer, 1894
Potomida Swainson, 1840	* Martinsnaias Frierson, 1927
Pronodularia Starobogatov, 1970	* Micronaias Simpson, 1900
Gonideini Ortmann, 1916	* Nephritica Frierson, 1927
+ Leguminaiini Starobogatov, 1970	* Nephronaias Crosse & Fischer, 1894
Gonidea Conrad, 1857	* Pachynaias Crosse & Fischer, 1894
Leguminaia Conrad, 1865	* Psoronaias Crosse & Fischer, 1894
Microcondylaea Vest, 1866	* Psorula Haas, 1930
Solenia Conrad, 1869	* Reticulataus Frierson, 1927
Pseudodontini Frierson, 1927	* Sphenonaias Crosse & Fischer, 1894
Pseudodon Gould, 1844	PARREYSIINAE Henderson 1935
Pilsbryconcha Simpson, 1900	Parreysiini Henderson, 1935
GONIDEINAE (incertae sedis)	+ Diplasminae Modell, 1942
* Discomya Simpson, 1900	+ Hemisolasminae Starobogatov, 1970
* Inversidens Haas, 1911	Parreysia Conrad, 1853
	Coelaturini Modell, 1942
	+ Brazzaeini Leloup, 1950
	+ Dentaspainiini Modell, 1964

- + Mweruellini Pain and F.R. Woodward, 1968
- + Prisdontopsini Pain and F.R. Woodward, 1968
- + Pseudaviculini Modell, 1942 [not available name, Bouchet and Rocroi, 2010]
- + Pseudospathini Leloup, 1950 [not available name, Bouchet and Rocroi, 2010]
- + Pseudospathinae Starobogatov, 1970
 - **Brazzaea* Bourguignat, 1885
 - **Coelatura* Conrad, 1853
 - **Grandidieria* Bourguignat, 1885
 - **Mweruella* Haas, 1936
 - **Nitia* Pallary, 1924
 - **Nyassunio* Haas, 1936
 - **Prisdontopsis* Tomlin 1928
 - **Pseudospatha* Simpson, 1900
- Lamellidentini Modell, 1942
 - **Lamellidens* Simpson, 1900
- Oxynaiini Starobogatov, 1970
 - **Oxynaiia* Haas, 1911
 - **Radiatula* Simpson, 1900
 - **Scabies* Haas, 1911
- PARREYSIINAE (*incertae sedis*)
 - **Germainaia* Graf & Cummings, 2009
- MODELLNAINAE Brandt, 1974
 - **Modellnaia* Brandt 1974
- UNIONIDAE (*incertae sedis*)
 - **Arcidopsis* Simpson, 1900 [Arcidopsinae Starobogatov 1970]
 - **Caudiculatus* Simpson, 1900
 - **Ctenodesma* Simpson, 1900
 - **Diaurora* Cockerell, 1903
 - **Elongaria* Haas, 1913
 - **Gibbosula* Simpson, 1900
 - **Haasodonta* McMichael, 1956
 - **Harmandia* Rochebrune, 1882
 - **Pressidens* Haas, 1910
 - **Prohyriopsis* Haas, 1914
 - **Protunio* Haas, 1913
 - **Pseudodontopsis* Kobelt, 1913
 - **Pseudobaphia* Simpson, 1900
 - **Pseudomulleria* Anthony, 1907 [Pseudomulleriinae Starobogatov, 1970]
 - **Ptychorhynchus* Simpson, 1900
 - **Schepmania* Haas, 1912
 - **Unionetta* Haas, 1955

into several subfamilies using umbo sculpture as the main diagnostic character (Table 1). However, this character alone was clearly unsuitable for this purpose and thus, these subfamilies were lumped back together until emergence of modern phylogenetic approaches (Davis and Fuller, 1981; Heard and Guckert, 1970). Since then, several Asian and African genera have been reassigned to other subfamilies based on molecular phylogenetic analyses and morphology (Huang et al., 2002; Kondo, 2008; Liu et al., 1979; Ouyang et al., 2011; Pfeiffer and Graf, 2013, 2015; Zhou et al., 2007). In addition, many genera within this subfamily have never been characterized using a molecular approach.

In the present study, one well supported clade, i.e., the tribe Unionini, was obtained within the Unioninae. Phylogenetic relationships among the remaining genera are not well resolved. The phylogeny recovered *Aculamprotula* as sister to a clade including *Cuneopsis* + *Schistodesmus* + *Nodularia*, but with poor support. As a result, *Aculamprotula*, *Cuneopsis*, *Schistodesmus* and *Nodularia* were classified as *incertae sedis* within Unioninae. If future phylogenetic analyses that include additional taxa give support to the clade *Cuneopsis* + *Schistodesmus* + *Nodularia*, the available name would be *Nodulariini* Starobogatov and Zatravkin, 1987 since the subfamily name *Cuneopsinae* Mongin, 1963 is not an available name (Bieler et al., 2010). Furthermore, if in future studies the genus *Acuticosta* falls within this clade the earliest tribe name would change to *Acuticostini* Starobogatov, 1967.

As in the Anodontinae, the Unioninae present a strict ectobranchy condition, but see Araujo et al. (2009) and Lopes-Lima et al. (2016a) for unusual exceptions in some populations.

Marsupial demibranchs lack specialized characters present in Anodontinae. Hinge teeth are well-defined. Glochidia are hooked, triangular and of medium size. Brooding type is tachytictic or short term (Table C1). The Unioninae are one of the most widely distributed tribes, covering almost all of Europe and Northwest Africa, as well as Vietnam, China, Far East Russia, Korea, Japan and the Sakhalin Island. In addition, two *Unio* species have disjunct distributions, i.e., *Unio abyssinicus* in the Horn of Africa and *Unio caffer* in South Africa (Fig. 4).

3.3.1. Tribe Unionini Rafinesque, 1820

Type Genus: *Unio* Philipsson in Retzius, 1788

Type Species: *Mya pictorum* Linnaeus, 1758

Comments: The Unionini contain only one genus, i.e., *Unio*. This genus is divided into four main lineages, i.e., the *crassus*-, *pictorum*-, *gibbus*- and *tumidus*-lineages (Froufe et al., 2016a; Lopes-Lima et al., 2016b), all of which are represented in the present phylogeny. Whilst the *crassus*- and *pictorum*-lineages cluster together, relationships among this group and the other two *Unio* lineages are not well resolved (Fig. 2).

Diagnosis: The main shared characters of the Unionini are: ectobranchous; marsupial demibranch without any specialized character; presence of a well-defined hinge structure with two pseudocardinal and two lateral teeth on the left valve and one or two on the right; umbo sculpture W-shaped and/or double-looped bars, which in some cases become nodulous or wrinkled; tachytictia or short term brooding; and the hooked triangular glochidia of intermediate sizes (Table C1).

Distribution: The tribe has essentially a western Palearctic distribution, extending from Western Europe to European Russia and the Caspian basin. In addition, three disjunct distributions are known, i.e., one in the Transbaikal region in Russia and two others in Sub-Saharan Africa (Fig. 4).

3.4. Rectidentinae Modell, 1942 phylogeny and tribal classification

The Rectidentinae originally included *Rectidens* as the type genus, as well as *Physunio* and *Ensidents* also including some eastern North American and South Eastern Asian genera in this subfamily (e.g., *Lastena*, *Pyganodon* and *Pilsbryconcha*) (Modell, 1942, 1964), but these were subsequently reassigned to distinct subfamilies (Haas, 1969a). The present phylogeny reveals two well supported clades within Rectidentinae, i.e., the tribes *Contradentini* Modell, 1942 and *Rectidentini* Modell, 1942. The *Contradentini* were first described as a subfamily in the same study that defined Rectidentinae (Modell, 1942). Although the Rectidentinae, *Contradentinae* and *Nannonaiinae* were all described by Modell (1942), priority of Rectidentinae was determined by the First Reviser action (Bieler et al., 2010; Brandt, 1974).

Since the two tribes within the Rectidentinae show a wide variability in morphological and anatomical characters, none of these characteristics are distinctive on the subfamily level (Table C1). The Rectidentinae are restricted to South East Asia, i.e., from Eastern India to Myanmar, Thailand, Laos, Cambodia and Vietnam, and to Peninsular Malaysia, and the Islands of Sumatra, Java, Borneo and Sulawesi (Fig. 5).

3.4.1. Tribe *Contradentini* Modell, 1942

Type Genus: *Contradens* Haas, 1911

Type Species: *Contradens contradens* (Lea, 1838)

Comments: The *Contradentini* initially included the type genus *Contradens*, as well as *Caudiculatus*, *Pressidens* and *Simpsonella*, all

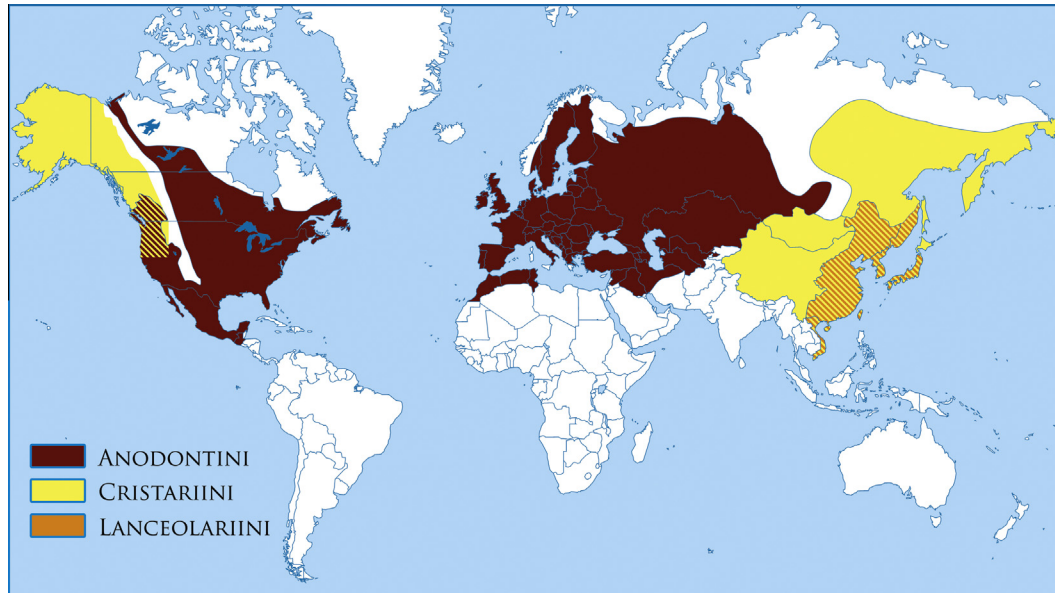


Fig. 3. Distribution map of the subfamily Anodontinae.

from Indochina and the Island of Borneo and the Philippines (Modell, 1942, 1964). Subsequently, all of these genera were reassigned to the Unioninae, with the exception of *Simpsonella*, which was placed within the Anodontinae (Haas, 1969a,b). More recently, *Caudiculatus* and *Pressidens* were once again placed within the Rectidentinae (Graf and Cummings, 2016). The present analyses recovered three genera in Contradentini, i.e., *Contradens*, *Physunio* and *Trapezoideus*. The phylogenetic relationships of the other genera, i.e., *Caudiculatus*, *Pressidens* and *Simpsonella* should be further investigated, since no sequence data are available at present. The date of publication of two genera, i.e., *Uniandra* Haas and *Contradens*, has been a source of confusion and has been clarified by Bogan (2015).

Diagnosis: Shell shape is variable, from rounded to elongate. Umbo sculpture ranges from v-shaped (e.g., in *Contradens contradens*) to w-shaped/double-looped/nodulous (e.g., in *Physunio superbus*) and pseudo-concentric ridges (e.g., in *Trapezoideus exollescens*). Hinge plate is well defined, with one lateral and one or two thin pseudocardinal teeth in the left valve, and one lateral and one pseudocardinal teeth in the right valve. Glochidia are bilaterally asymmetrical and are quite distinct from any other group of the Unionidae, rendering this trait diagnostic of the tribe (Pfeiffer and Graf, 2015). Brooding type is ectobranchous, but brooding period and length are unknown.

Distribution: The Contradentini have the same distribution in South East Asia as described above for the Rectidentinae (Fig. 5).

3.4.2. Tribe Rectidentini Modell, 1942

Type Genus: *Rectidens* Simpson, 1900

Type Species: *Unio lingulatus* Drouet and Chaper, 1892

Comments: The Rectidentini include the type genus *Rectidens* as well as *Hyriopsis* and *Ensidents*. Of the four *Hyriopsis* species included in this study, only *Hyriopsis cumingii* does not cluster with the type of the genus *Hyriopsis bialata*. Thus, *Hyriopsis cumingii* is here reassigned to *Sinohyriopsis Starobogatov, 1970*, with the type species *Unio cumingii* Lea, 1852 (see Ð ng et al., 1980). The remaining *Hyriopsis* species relationships, i.e., *Hyriopsis bialata*, *Hyriopsis desowitzi* and *Hyriopsis myersiana* are still unresolved.

Diagnosis: Shells are usually elongated and, in *Hyriopsis*, often with evident dorsal wings. Umbo sculpture is predominantly pseudo-concentric to double-looped or nodulous. Hinge structure is generally well defined with a variety of teeth number and shapes. Glochidia are of the unhooked elliptical type and of intermediate sizes. Brooding type is ectobranchous or tetragenous in *Hyriopsis* and tetragenous in *Ensidents* and *Rectidens* (Table C1). The semi-elliptical unhooked shape of Rectidentini glochidia distinguishes this tribe from the Contradentini. However, semi-elliptical unhooked glochidia are also present in other subfamilies (i.e., Gonideinae and Ambleminae, Modellinae, and Parreysiinae).

Distribution: Although the distribution of the Rectidentini significantly overlaps with that of the Contradentini, its range excludes Bangladesh and the Islands of Sulawesi and Sumatra (Fig. 5).

3.5. Gonideinae Ortmann, 1916 phylogeny and tribal classification

The Gonideinae was first described including only a single monotypic genus, i.e., *Gonidea angulata* (Lea, 1838), which had previously been assigned to Anodontinae (Ortmann, 1916). That species reassignment was based on the distinctive anatomical characters of *G. angulata*, which are unique among the North American unionid fauna (Ortmann, 1916). Since then, the phylogenetic position of *G. angulata* has changed many times. It has been recognized as a valid subfamily (Heard and Guckert, 1970; Ortmann, 1916), placed within other subfamilies such as the Pseudodontinae (Modell, 1942) and the Unioninae (Haas, 1969a, 1969b), and in a separate tribe, i.e., Gonideini, within the Ambleminae (Graf, 2002; Graf and Cummings, 2007), but always as a monotypic group. Recent molecular phylogenetic analyses have recovered *Gonidea* in a clade with several Old World genera (e.g., *Potomida*, *Pseudodon* and *Pronodularia*) and recognized that clade as the Gonideinae (Pfeiffer and Graf, 2013, 2015).

In the present work, the Gonideinae are recovered as a monophyletic subfamily that includes the type genus *Gonidea* from western North America, three Western Palearctic genera (i.e., *Leguminaia*, *Microcondylaea* and *Potomida*) and seven genera from East and Southeast Asia. The Gonideinae are here divided into two well supported clades. One includes two sister tribes, i.e., Chamberlainiini *nomen novum* and Lamprotulini (Fig. 2). The second clade is

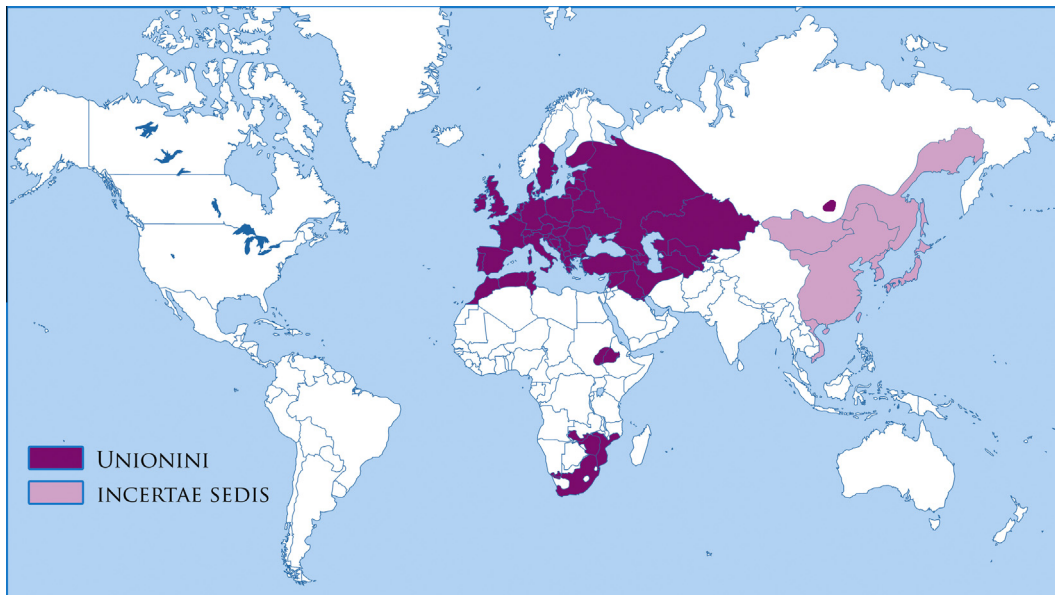


Fig. 4. Distribution map of the subfamily Unioninae.

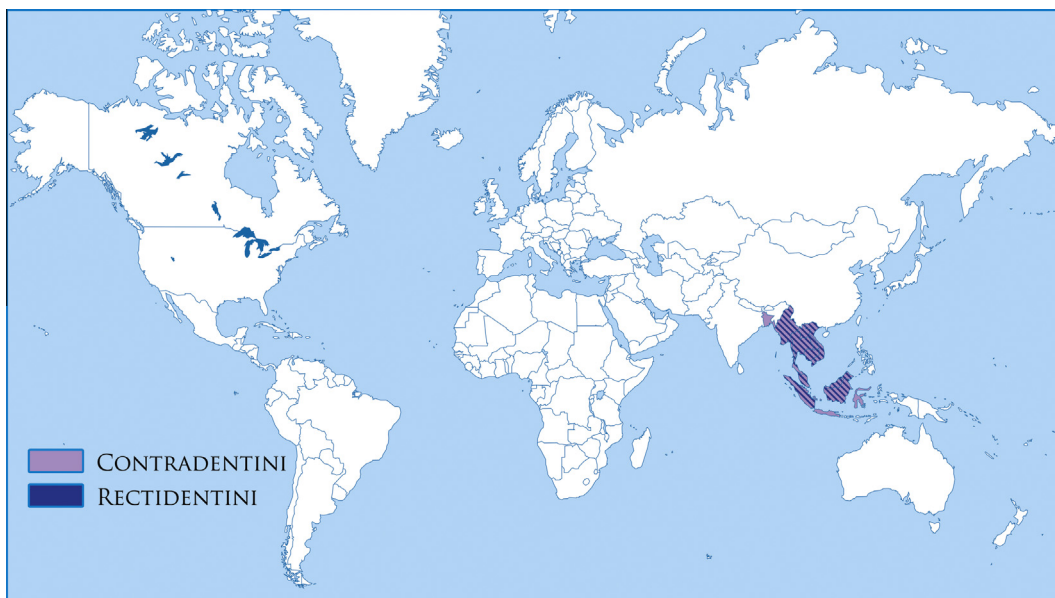


Fig. 5. Distribution map of the subfamily Rectidentinae.

composed of two tribes, i.e., the Gonideini and the Pseudodontini, and one isolated species, i.e., *Solenaia triangularis* (Fig. 2). No single morphological character is useful to diagnose the subfamily. All of the studied genera have medium sized semi-elliptical unhooked glochidia and are tachytictic, though the marsupium location varies among tribes (i.e., ectobranchous or tetragenous; Table C1). The Gonideinae have a scattered distribution in the Northern Hemisphere, being present in restricted regions of the Palearctic, Indotropics and Western Nearctic (Fig. 6).

3.5.1. Tribe Chamberlainiini Bogan, Froufe and Lopes-Lima, nomen novum

Type Genus: *Chamberlainia* Simpson, 1900

Type Species: *Unio hainesianus* Lea, 1856

Comments: Chamberlainiini *nomen novum* is here described for the first time and encompasses only two genera, i.e., the monotypic *Chamberlainia* and *Sinohyriopsis*. The latter includes *Sinohyriopsis cumingii*, previously assigned to *Hyriopsis* (see above), and *Sinohyriopsis schlegelii*, previously shown to be related to *S. cumingii* (Froufe et al., 2016b).

Diagnosis: Shell oval, elliptical to rhomboid, often with small anterior wing and posterior dorsal wing. Posterior ridge is rounded. Umbos are low. Umbo sculpture consisting of well-developed pseudo-concentric or nodulous ridges. Hinge with single pseudocardinal and lateral tooth in the right valve, and typically two pseudocardinal and lateral teeth in left valve. Glochidia, as in all Gonideinae, are unhooked and semi-elliptical in shape. Brooding type is ectobranchous and tachytictic. The Chamberlainiini the only ectobranchous tribe within the Gonideinae (Table C1).

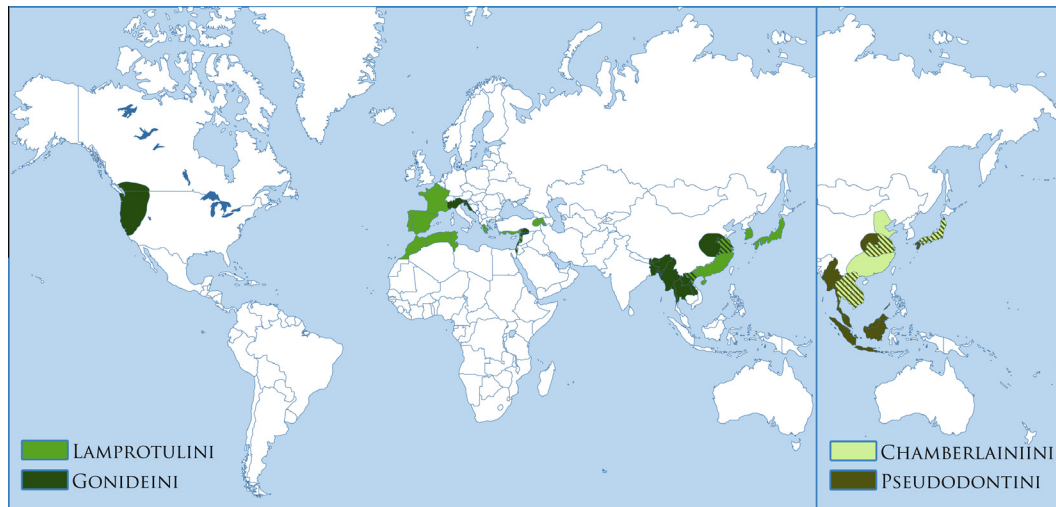


Fig. 6. Distribution map of the subfamily Gonideinae.

Distribution: Distribution of the Chamberlainiini is restricted to Indochina, the Huang He River basin in China, and Japan (Fig. 6).

3.5.2. Tribe Lamprotulini Modell, 1942

Type Genus: *Lamprotula* Simpson, 1900

Type Species: *Chama plumbea* Chemnitz, 1795

Comments: In addition to the type genus, the Lamprotulini include the western Palearctic *Potomida* and the Far East Asian *Pronodularia*. The Lamprotulini were first defined as a subfamily mainly based on characteristics of the umbo sculpture (Modell, 1942, 1964). It originally contained the genus *Discomya*, for which no genetic information is currently available, *Lamprotula*, *Potomida* and *Pronodularia*. Subsequently, all of these genera, with the exception of *Pronodularia*, were reassigned to Quadrulinae (Haas, 1969a, 1969b). However, *Lamprotula*, *Pronodularia* and *Potomida* were recently reassigned back to Gonideinae based on molecular, morphological and biogeographical studies (Pfeiffer and Graf, 2013, 2015; Whelan et al., 2011). The present study confirms the placement of these three genera within Lamprotulini.

Diagnosis: Shells are generally thick, and ovate to triangular in shape. Hinge with well-developed, strong teeth, generally three pseudocardinals and four laterals. Umbo sculpture consists of W-shaped to double-looped ridges, which sometimes become nodulous and/or wrinkled. Glochidia are semi-elliptical and unhooked, and of intermediate sizes (Table C1). Brooding type is tachytictic and tetragenous except for *Pronodularia*, which can be ectobranchous or tetragenous (Kondo, 1982) (Table C1). This tribe shares most of the traits with the other Gonideinae tribes, but all species present strong thick shells and well developed hinge teeth (Table C1).

Distribution: The Lamprotulini have a disjunct distribution, with *Potomida* presenting a patchy distribution in the Mediterranean region, *Lamprotula* being distributed from North Vietnam to North China and Korea, and *Pronodularia* restricted to Korea and Japan (Fig. 6).

3.5.3. Tribe Gonideini Ortmann, 1916

Type Genus: *Gonidea* Conrad, 1857

Type Species: *Anodon randalli* Trask, 1855 (Junior synonym of *Anodonta angulata* Lea, 1838)

Comments: The Gonideini are divided into two well supported clades, i.e., one encompassing the Western North American *Gonidea*, the Southern European *Microcondylaea* and the Middle Eastern *Leguminaia*, and the other with the Asian *Solenia*. *Solenia* is not monophyletic, as *Solenia triangularis* was not recovered within the Gonideini (see Pfeiffer and Graf, 2015). The type genus *Gonidea*, as well as *Leguminaia* and *Microcondylaea* were originally placed within the Pseudodontinae with other Asian genera (Modell, 1942), but were all subsequently reassigned to the Unioninae (Haas, 1969a,b). Starobogatov (1970) placed these genera in the Pseudodontinae within the Margaritiferidae. Only recently, based on biogeographic and morphological information, Graf and Cummings (2016) suggested the placement of these genera within the Gonideinae. In the present study, molecular data confirms the placement of these three genera within the Gonideini together with some representatives of the genus *Solenia*.

Diagnosis: Shell shape is trapezoidal but much more elongated in *Solenia*. Hinge teeth are small, vestigial or absent in *Solenia*. Umbo sculpture consists of pseudo-concentric, double-looped and/or W-shaped ridges, which are sometimes wrinkled. Glochidia are of intermediate sizes, semi elliptical and unhooked. Brooding type is tachytictic and tetragenous. Within the subfamily, the Gonideini are identified by a typical trapezoidal or rectangular shell shape, and a hinge without teeth or only vestigial teeth (Table C1).

Distribution: The tribe has a curious, disjunct distribution. While *Gonidea* is restricted to the west coast of North America, *Microcondylaea* only occurs from the Italian Peninsula to coastal Croatia in Europe, and *Leguminaia* is present in southeast Turkey and the Middle East. *Solenia* occurs from eastern India to Myanmar, Thailand, North Vietnam and China (Fig. 6).

3.5.4. Tribe Pseudodontini Frierson, 1927

Type Genus: *Pseudodon* Gould, 1844

Type Species: *Anodon inoscularis* Gould, 1844

Comments: This group was first named as a subfamily, Pseudodontinae, by Frierson (1927) and included the species *Pseudodon cambodjensis* and *Gonidea angulata*. It was then redefined, mainly using morphological characters, with *Pseudodon* as the type genus together with other genera including the North American *Gonidea* (Modell, 1942, 1964). All of these genera were then subsequently reassigned to the Unioninae subfamily (Haas, 1969a,b; Subba

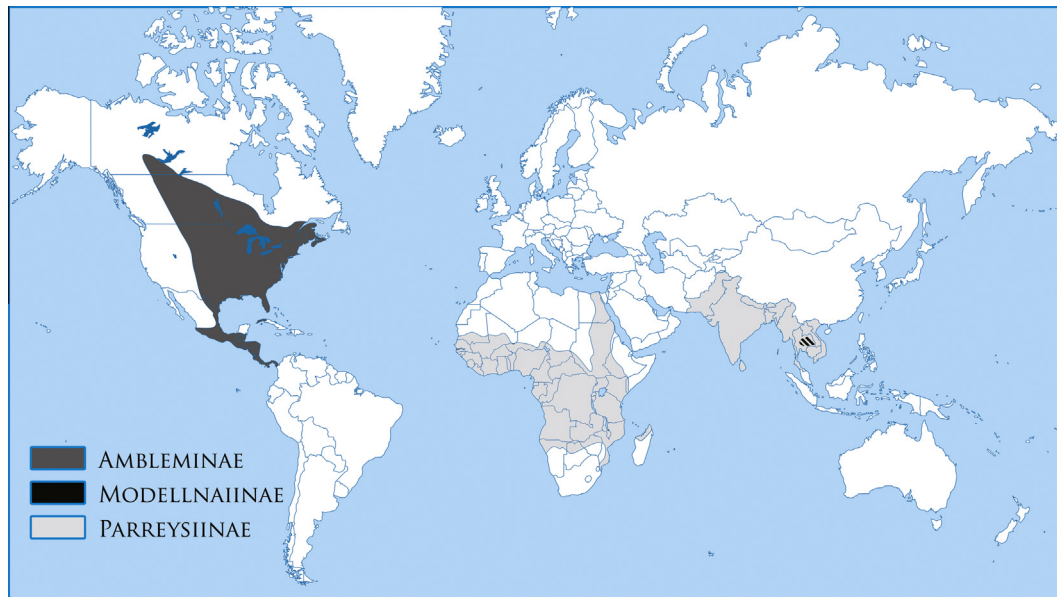


Fig. 7. Distribution map of the subfamilies Amblemninae + Modellnaininae + Parreysiinae.

Rao, 1989), and only recently were their relationships with the Gonideinae discussed (Pfeiffer and Graf, 2015; Whelan et al., 2011). The Pseudodontinae is here demoted to a tribe, Pseudodontini, within Gonideinae, being composed of only two monophyletic genera, i.e., the type genus *Pseudodon* and *Pilsbryoconcha* (Fig. 2).

Diagnosis: Shell shape is generally ovate in *Pseudodon* and more elongated in *Pilsbryoconcha*. Umbo sculpture is basically double-looped or W-shaped, with the anterior loops sometimes fading distally, so that only the posterior single-loop or a single row of nodes remains. Brooding type is tachytictic and tetragenous. Glochidia are unhooked and semi-elliptical. The representatives of this tribe present a characteristic “v” shaped fossette present at the posterior end of the hinge structure with small vestigial teeth, which are completely absent in *Pilsbryoconcha* (Table C1).

Distribution: The Pseudodontini are present in Myanmar, Malaysia, Thailand, Cambodia, Laos, Vietnam, China and Indonesia including the Islands of Java, Sumatra and Borneo (Fig. 6).

3.6. *Amblemninae* Rafinesque, 1820, *Parreysiinae* Henderson, 1935 and *Modellnaininae* Brandt, 1974

The Amblemninae and Parreysiinae were investigated in detail earlier (Campbell and Lydeard, 2012a,b; Campbell et al., 2005; Whelan et al., 2011) and thus not fully explored in the present study. The Modellnaininae is a monotypic subfamily defined by Brandt (1974) with *Modellnaia siamensis* as the only species. Its status as a subfamily has been retained by posterior classification systems based on its quite distinct morphological characters (Bieler et al., 2010; Carter et al., 2011; Whelan et al., 2011; this study). Unfortunately, this species has never been included in a phylogenetic analysis and no sample was available for the present study.

Based on these earlier works and the present classification system, distribution maps are here presented for Amblemninae, Parreysiinae and Modellnaininae (Table 3; Fig. 7). The Amblemninae are restricted to Canada and the United States east of the Rocky Mountains and extend south through Mexico to southern Panama. The Parreysiinae have a disjunct distribution in Africa and Southern Asia. In Africa, the Parreysiinae are found in the Nile River basin from the Nile delta south into East Africa and across sub-Saharan Africa south to Namibia and Mozambique. *Germainia* Graf and Cummings, 2009 from northwest Madagascar is treated here as

belonging to the Parreysiinae. In Asia, the Parreysiinae occur in Pakistan, India, Nepal, Myanmar, Thailand, Indonesia, Cambodia, Laos and Vietnam (Fig. 7). The Modellnaininae (i.e., *Modellnaia siamensis*) is restricted to the middle section of Mun River in Thailand (Fig. 7).

4. Conclusions

Considering the high levels of decline of freshwater mussel species worldwide, an understanding of the phylogenetic diversity is crucial for determining conservation priorities, especially in poorly explored regions such as the Central American and the Indotropics. In fact, conservation strategies should strive not only to maximize the current levels of biological diversity, but also to include phylogenetic patterns to maximize future levels of biodiversity. Furthermore, due to the increasing development and biotic homogenization in tropical areas (e.g., Malaysia and Indonesia) with dramatic negative implications on freshwater habitats, conservation and management efforts targeting freshwater taxa are urgently needed.

The present study is an important contribution to the definition of freshwater mussel diversity patterns, especially in the Indotropical and East Asian countries. Here, a phylogeny of the Unionidae is presented with the greatest generic and geographic coverage to date, based on a dataset comprising 70 species in 46 genera, 7 of this genera being sequenced for the first time. Furthermore, it includes 57 species from 35 genera, thereby tripling the number of analyzed taxa from Anodontinae, Unioninae, Rectidentinae and Gonideinae. Molecular phylogenetic analyses revealed the presence of 6 subfamilies in the Unionidae, divided into 18 tribes, 3 of which are described here for the first time. Although we compiled seven characters traditionally used in Unionidae systematics, no single one was found to be diagnostic at the subfamily level and few were useful at the tribe level (e.g., larval morphology for Contradentini). However, within subfamilies, many tribes can be characterized based on a subset of these characters.

Representing a major international collaborative effort, this study provides important advances in the systematics of these extraordinary taxa with implications for ecological and conservation studies (e.g., assessment of conservation status and distribution).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympbev.2016.08.021>.

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