



Molecular phylogeny and taxonomic revision of two enigmatic freshwater mussel genera (Bivalvia: Unionidae *incertae sedis*: *Harmandia* and *Unionetta*) reveals a diverse clade of Southeast Asian Parreysiinae

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ABSTRACT

The Indotropical freshwater mussel assemblage has more genera of uncertain subfamily-level position (i.e. genera *incertae sedis*) than all other regional faunas. Of the 16 genera *incertae sedis* in the Indotropics, only two, *Harmandia* and *Unionetta*, are distributed in the mainland southeast Asia subregion. Resolving the enigmatic systematic position of *Harmandia* and *Unionetta* would finally establish a complete subfamily-level classification for the freshwater mussel genera of southeast Asia and facilitate more comprehensive evaluation of regional freshwater mussel diversity and distribution. Molecular phylogenetic reconstructions using the nuclear-encoded *large ribosomal subunit rRNA* (28S) and the mitochondrial protein-coding *cytochrome c oxidase subunit I* (COI) recovered *Harmandia* and *Unionetta* in the Indo-Afrotropical subfamily Parreysiinae, and more specifically as members of the tribe Indochinellini (formerly Oxynaiini). *Harmandia* is shown to be polyphyletic with its former species belonging to three distantly related clades of the Unionoida (i.e. Parreysiinae, Rectidentinae and Hyriidae). *Unionetta* was likewise not recovered as monophyletic, despite the strong morphological similarities and close geographic proximity of the sequenced individuals. Based on a synthesis of molecular, morphological and biogeographic data, the taxonomy and geographic distributions of *Harmandia* and *Unionetta* are revised. These results are discussed in the context of the dispersal of the Parreysiinae into Southeast Asia and the species-level diversity of the Indochinellini in the Mekong River.

INTRODUCTION

The freshwater mussel family Unionidae (Bivalvia: Unionoida) is the most species-rich freshwater bivalve family, representing more than half of global freshwater bivalve species (Graf, 2013). The majority of unionid species-level diversity is distributed in two globally important freshwater biodiversity hotspots, the Nearctic (about 300 species) and the Indotropics (about 220 species) (Graf & Cummings, 2007a; Abell *et al.*, 2008; Zieritz *et al.*, 2018). Despite similar degrees of regional species-level richness, the Southeast Asian freshwater mussel fauna has received disproportionately less systematic attention than that of North America (Graf, 2013). As a consequence, the Indotropical freshwater mussel assemblage has more genera of uncertain subfamily-level position (i.e. genera *incertae sedis*) than all other regional faunas.

Several recent molecular systematic studies have helped to clarify the phylogeny of Indotropical freshwater mussels (Zhou *et al.*, 2007; Whelan, Geneva & Graf, 2011; Pfeiffer & Graf, 2013, 2015; Lopes-Lima *et al.*, 2017; Bolotov *et al.*, 2017a, b) and reduced the number of genera *incertae sedis* in the region by half, from 32 to 16 (Graf & Cummings, 2007a; Lopes-Lima *et al.*,

2017). These genera *incertae sedis* limit our understanding of the diversification, morphological evolution and biogeography of the freshwater mussel clades in the region.

These 16 genera *incertae sedis* are unevenly distributed across the Indotropics, with the majority being restricted to the poorly characterized drainages of the Sunda Islands (7) and China and northern Vietnam (5). The four remaining genera *incertae sedis* are distributed on the Indian subcontinent (2) and mainland Southeast Asia (2). The present study focuses on the two enigmatic genera, *Harmandia* Rochebrune, 1881 and *Unionetta* Haas, 1955, of mainland Southeast Asia. That area is a global hotspot of unionid subfamily-level diversity (Zieritz *et al.*, 2018) and has been hypothesized to be the centre of origin for multiple freshwater mussel radiations (Bolotov *et al.*, 2017a). Resolving the enigmatic systematic position of *Harmandia* and *Unionetta* would finally establish a complete subfamily-level classification for the freshwater mussel genera of southeast Asia and facilitate more comprehensive evaluation of regional freshwater mussel diversity and distribution.

The objective of the present study is to determine the systematic position and taxonomic validity of each of the nominal species of

Harmandia and *Unionetta*, and to describe their morphological characteristics and geographic distributions. These results are discussed in the context of the dispersal of the Parreysiinae into Southeast Asia and the species-level diversity of the tribe Indochinellini Bolotov, Pfeiffer, Vikhrev *et* Konopleva in Bolotov *et al.*, 2018 (formerly Oxynaiini) in the Mekong River.

MATERIAL AND METHODS

Taxon and character sampling

The initial test of the family- and subfamily-level position of *Harmandia* and *Unionetta* was performed by including a representative individual of the type species of *Harmandia* (*H. somboriensis* Rochebrune, 1881) and of *Unionetta* (*Unio fabagina* Deshayes in Deshayes & Jullien, 1874) in the unionoid phylogeny of Lopes-Lima *et al.* (2017). *Harmandia munensis* Brandt, 1974 and a molecularly divergent specimen of *U. fabagina* (i.e. ?*Unionetta fabagina*) were also included in the initial phylogenetic reconstruction. To understand more completely the systematic position of *Harmandia* and *Unionetta* within the Parreysiinae, genera were sampled from across the geographical range of the subfamily, representing each of the recognized tribes from the Indian subcontinent and mainland Southeast Asia (Parreysiini Henderson, 1935, Lamellidentini Modell, 1942, Leopardreysiini Vikhrev, Bolotov *et* Kondakov in Bolotov *et al.*, 2017 and Indochinellini Bolotov, Pfeiffer, Vikhrev *et* Konopleva in Bolotov *et al.*, 2018), and Africa (Coelaturini Modell, 1942). Taxon sampling focused on the Parreysiinae of mainland Southeast Asia, with a particular emphasis on the Mekong River fauna (Table 1). Two molecular markers were used to reconstruct the phylogeny: the nuclear-encoded *large ribosomal subunit rRNA* (28S) and the mitochondrial protein-coding *cytochrome c oxidase subunit I* (COI). Tissues were preserved in 95% ethanol and extracted using a DNeasy Blood & Tissue kit (Qiagen). Primers for polymerase chain reaction (PCR) and sequencing were as follows: 28S – D23F, GAGAGTTCAAGAGTACGTG, and D4RB, TGTTAGACTCCTTGCTCCGTGT (Park & O’Foighil, 2000); COI – dgLCO-1490, GGTCAACAAATCAT AAAGAYATYGG, and dgHCO-2198, TAAACTTCAGGGT GACCAAARAAYCA (Meyer, 2003).

PCR was performed in 25- μ l reactions using the following reagents and volumes: H₂O (17.75 μ l), 5 \times MyTaq Reaction Buffer (5 μ l; Bioline), primers (0.5 μ l), MyTaq Red DNA polymerase (0.25 μ l) and DNA template (1 μ l). Bidirectional Sanger sequencing was performed at the University of Florida Interdisciplinary Center for Biotechnology Research. Raw chromatograms were assembled into bidirectional consensus sequences and edited using Geneious v. 6.1.2 (<http://www.geneious.com>; Kearse *et al.* 2012).

Phylogenetic analysis

Consensus sequences were aligned in Mesquite v. 3.10 (Maddison & Maddison, 2016) using ClustalW (Larkin *et al.*, 2007). PartitionFinder v. 1.1.1 (Lanfear *et al.*, 2012) was used to determine the most likely partitioning scheme and models for nucleotide substitution using the models available in MrBayes under the greedy algorithm using linked partitions. Loci were analysed in concatenation using maximum likelihood (ML) and Bayesian inference (BI). BI analyses were performed with MrBayes v. 3.2.6 (Ronquist & Huelsenbeck, 2003; Ronquist *et al.*, 2012) using the Cipres Science Gateway (Miller, Pfeiffer & Schwartz, 2010). MrBayes was implemented using two runs of four chains for 20×10^6 generations, sampling every 1000 trees and omitting the first 5000 as burn-in. Convergence of the two runs was monitored by the average standard deviation of split frequencies and the potential scale reduction factor (PSRF) and effective sample size (ESS) of the estimated parameters. ML analyses were performed using RAXMLGUI (Silvestro

& Michalak, 2012) using 100 tree searches, autoMRE bootstrapping and the GTRGAMMA model of nucleotide evolution.

Morphological methods

Four characters associated with the marsupium, larval and mantle morphology were analysed for *Harmandia somboriensis*, *H. munensis* and *Unionetta fabagina* using dissecting and compound microscopes. These characters were selected as they have previously been demonstrated to be useful in diagnosing suprageneric freshwater mussel clades (Graf & Cummings, 2006a; Bolotov *et al.*, 2018). Representatives of each of the tribes of the Parreysiinae were also analysed, as well as taxa relevant to previous classifications of *Harmandia* and *Unionetta* (Table 2). Literature sources were used to score traits for taxa in which no anatomical material was available (*Parreysia*: Ortmann, 1910; Prashad, 1918; *Nodularia*: Bolotov *et al.*, 2018; *Triplodon*: Pimpão, 2011).

Biogeographic methods

The geographic distributions of *Harmandia* and *Unionetta* were determined by examining (and digitizing) the Southeast Asian freshwater mussel collections at thirteen natural history museums (AMNH, ANSP, NHMUK, UF, FMNH, INHS, MCZ, MNHN, NCSM, SMF, SMRL, UMMZ, USNM). Institutional abbreviations follow Sabaj (2016): AMNH, American Museum of Natural History; ANSP, Academy of Natural Sciences at Drexel University; NHMUK, Natural History Museum, London; UF, Florida Museum of Natural History; FMNH, Field Museum of Natural History, Chicago; INHS, Illinois Natural History Survey; MCZ, Museum of Comparative Zoology, Harvard University; MNHN, Muséum National d’Histoire Naturelle, Paris; NCSM, North Carolina Museum of Natural Sciences; SMF, Senckenberg Forschungsinstitut und Naturmuseum; SMRL, Applied Malacology Center and Mollusk Museum, Bangkok; UMMZ, University of Michigan Museum of Zoology; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Photographed specimens and the associated textual information (e.g. catalogue number, locality, collector, species identification, etc.) were normalized and incorporated into the MUSSEL Project Database (MUSSELpdb) following methods described previously (Graf & Cummings, 2006b, 2007a, b, 2009, 2011). More information about the data model, management and dissemination of the MUSSELpdb is available at the MUSSEL Project Web Site (www.mussel-project.net/). Dot distribution maps were produced using ArcMap v. 10.2.2 (<http://www.esri.com>). Freshwater ecoregions follow Abell *et al.* (2008).

RESULTS

Phylogenetic results

The expanded reanalysis of the matrix of Lopes-Lima *et al.* (2017) resolved *Harmandia somboriensis*, *Unionetta fabagina*, and ?*U. fabagina* within the Parreysiinae, whereas *H. munensis* was recovered among the Rectidentinae (Fig. 1). The two-gene Parreysiinae-focused matrix included 69 individuals and an alignment of 1149 nt (Table 1). All individuals were represented by both COI and 28S sequences, except for eight with COI alone. The COI alignment (av. 646 nt) contained no indels or stop codons. The 28S alignment (av. 403 nt) contained an average of 18.4% of gaps per taxon. The following partitioning scheme and models of nucleotide substitution were implemented in the BI analysis: COI_1 = GTR + I + G, COI_2 = F81, COI_3 = HKY + G, 28S = GTR + G. Convergence of the BI runs was supported by the average standard deviation of split frequencies (0.00215), average PSRF values (1.000) and high ESS values (>8000). The BI and ML reconstructions recovered identical supraspecific topologies (Fig. 2). The

Table 1. Individuals included in the phylogenetic reconstructions with catalogue numbers, GenBank accession numbers, original source and generalized locality information.

Taxon	Catalogue no. (tissue)	COI	28S	Source	Freshwater ecoregion
Margaritiferidae					
<i>Margaritifera margaritifera</i>	UA 21019	JN243891	JN243869	Whelan et al. (2011)	–
Parreysiini					
<i>Parreysia corrugata</i>	RNB1	KJ872809	–	GenBank	Western Ghats
<i>Parreysia corrugata</i>	RNB11	JQ861229	–	GenBank	Southern Deccan Plateau
<i>Parreysia favidens</i>	RNB12	JQ861238	–	GenBank	Western Ghats
Leoparreysiini					
<i>Leoparreysia bhamoensis</i>	UA 20722	JN243900	JN243876	Whelan et al. (2011)	Sittaung – Irrawaddy
<i>Leoparreysia olivacea</i>	UMMZ 304641	KP795022	KP795005	Pfeiffer & Graf (2015)	Sittaung – Irrawaddy
<i>Leoparreysia tavoyensis</i>	UMMZ 304640	JN243901	JN243877	Whelan et al. (2011)	Sittaung – Irrawaddy
Lamellidentini					
<i>Lamellidens corrianus</i>	UMMZ 304642	JN243903	JN243881	Whelan et al. (2011)	Sittaung – Irrawaddy
<i>Lamellidens generosus</i>	UA 20727	JN243902	JN243880	Whelan et al. (2011)	Sittaung – Irrawaddy
Coelaturini					
<i>Coelatura aegyptiaca</i>	ANSP 416304	JN243894	JN243872	Whelan et al. (2011)	Nile Delta
<i>Nitia teretiuscula</i>	ANSP 416305	JN243897	JN243875	Whelan et al. (2011)	Nile Delta
<i>Prisodontopsis aviculaeformis</i>	UMMZ 304379	KJ081180	KJ081193	Graf et al. (2014)	Bangweulu – Mweru
Rectidentini					
<i>Trapezoideus comptus</i> (= <i>Harmandia munensis</i>)	UF 507396 (ICH-00474)	MH350968	MH351013	This study	Kratie – Stung Treng
Indochinellini					
<i>Harmandia somboriensis</i>	UF 507791 (ICH-00810)	MH350952	MH351001	This study	Khorat Plateau
<i>Harmandia somboriensis</i>	UF 507791 (ICH-00811)	MH350953	MH351002	This study	Khorat Plateau
<i>Harmandia somboriensis</i>	UF 507831 (ICH-00889)	MH350959	MH351008	This study	Khorat Plateau
<i>Harmandia somboriensis</i>	UF 507831 (ICH-00890)	MH350960	MH351009	This study	Khorat Plateau
<i>Unionetta fabagina</i>	UF 507399 (ICH-00489)	MH350930	MH350979	This study	Kratie – Stung Treng
<i>Unionetta fabagina</i>	UF 507399 (ICH-00490)	MH350931	MH350980	This study	Kratie – Stung Treng
<i>Unionetta fabagina</i>	UF 507399 (ICH-00491)	MH350932	MH350981	This study	Kratie – Stung Treng
<i>Unionetta fabagina</i>	UF 507399 (ICH-00492)	MH350933	MH350982	This study	Kratie – Stung Treng
<i>Unionetta fabagina</i>	UF 507399 (ICH-00493)	MH350934	MH350983	This study	Kratie – Stung Treng
<i>Unionetta fabagina</i>	UF 507399 (ICH-00494)	MH350935	MH350984	This study	Kratie – Stung Treng
? <i>Unionetta fabagina</i>	UF 507378 (ICH-00401)	MH350962	MH351011	This study	Kratie – Stung Treng
<i>Scabies crispata</i>	UF 507693 (2012-0428)	MH350921	MH350970	This study	Mae Khlong
<i>Scabies crispata</i>	UF 507590 (2014-0030)	MH350922	MH350971	This study	Mae Khlong
<i>Scabies crispata</i>	UF 507682 (2012-0409)	MH350920	MH350969	This study	Chao Phraya
<i>Scabies crispata</i>	UF 507712 (ICH-00655)	MH350940	MH350989	This study	Chao Phraya
<i>Scabies crispata</i>	UF 507521 (ICH-02195)	MH350964	–	This study	Chao Phraya
<i>Scabies mandarinus</i>	UF 507434 (ICH-00593)	MH350937	MH350986	This study	Mekong Delta
<i>Scabies mandarinus</i>	UF 507450 (ICH-00630)	MH350939	MH350988	This study	Mekong Delta
<i>Scabies mandarinus</i> (= <i>Scabies crispata sensu Pfeiffer & Graf, 2015</i>)	UMMZ 304646	KP795023	KP795006	Pfeiffer & Graf (2015)	Mekong Delta
<i>Scabies mandarinus</i>	UF 507551 (ICH-02254)	MH350965	–	This study	Eastern Gulf of Thailand Drainages
<i>Scabies nucleus</i>	UF 507738 (ICH-00706)	MH350943	MH350992	This study	Khorat Plateau
<i>Scabies nucleus</i>	UF 507738 (ICH-00707)	MH350944	MH350993	This study	Khorat Plateau
<i>Scabies phaselus</i>	UF 507876 (ICH-02099)	MH350967	–	This study	Mae Khlong
<i>Scabies phaselus</i>	UF 507651 (2014-0683)	MH350927	MH350976	This study	Chao Phraya
<i>Scabies phaselus</i>	UF 507730 (ICH-00689)	MH350941	MH350990	This study	Chao Phraya
<i>Scabies phaselus</i>	UF 507657 (2014-0692)	MH350963	MH351012	This study	Lower Lancang
<i>Scabies phaselus</i>	UF 507734 (ICH-00696)	MH350942	MH350991	This study	Lower Lancang
<i>Scabies phaselus</i>	UF 507608 (2014-0582)	MH350924	MH350973	This study	Khorat Plateau
<i>Scabies phaselus</i>	UF 507848 (ICH-01163)	MH350961	MH351010	This study	Khorat Plateau

Continued

Table 1. *Continued*

Taxon	Catalogue no. (tissue)	COI	28S	Source	Freshwater ecoregion
<i>Scabies phaselus</i> (= <i>Radiatula aff humilis</i> 2 sensu Bolotov et al., 2017a)	biv_124_1	KX865850	KX865722	Bolotov et al. (2017a)	Khorat Plateau
<i>Scabies phaselus</i>	UF 507441 (ICH-00612)	MH350938	MH350987	This study	Mekong Delta
<i>Scabies anceps</i>	UF 507385 (ICH-00428)	MH350928	MH350977	This study	Kratie – Stung Treng
<i>Scabies anceps</i>	UF 507394 (ICH-00456)	MH350929	MH350978	This study	Kratie – Stung Treng
<i>Scabies anceps</i>	UF 507431 (ICH-00583)	MH350936	MH350985	This study	Mekong Delta
<i>Scabies scobinatus</i>	UF 507782 (ICH-00796)	MH350951	MH351000	This study	Khorat Plateau
<i>Scabies scobinatus</i>	UF 507825 (ICH-00879)	MH350958	MH351007	This study	Khorat Plateau
<i>Scabies songkramensis</i>	UF 507634 (2014-0658)	MH350926	MH350975	This study	Khorat Plateau
<i>Scabies songkramensis</i>	UF 507775 (ICH-00768)	MH350948	MH350997	This study	Khorat Plateau
<i>Scabies songkramensis</i>	UF 507766 (ICH-00779)	MH350949	MH350998	This study	Khorat Plateau
<i>Scabies songkramensis</i>	UF 507781 (ICH-00793)	MH350950	MH350999	This study	Khorat Plateau
<i>Scabies songkramensis</i>	UF 507803 (ICH-00835)	MH350955	MH351004	This study	Khorat Plateau
<i>Radiatula pilata</i>	UF 507760 (ICH-00740)	MH350945	MH350994	This study	Khorat Plateau
<i>Radiatula pilata</i>	UF 507752 (ICH-00743)	MH350946	MH350995	This study	Khorat Plateau
<i>Radiatula pilata</i>	UF 507761 (ICH-00754)	MH350947	MH350996	This study	Khorat Plateau
<i>Radiatula pilata</i>	UF 507794 (ICH-00817)	MH350954	MH351003	This study	Khorat Plateau
<i>Radiatula pilata</i>	UF 507807 (ICH-00843)	MH350956	MH351005	This study	Khorat Plateau
<i>Radiatula pilata</i>	UF 507813 (ICH-00853)	MH350957	MH351006	This study	Khorat Plateau
<i>Radiatula caerulea</i>	UF 507572 (ICH-02312)	MH350966	–	This study	Lower & Middle Salween
<i>Radiatula caerulea</i> (= <i>Radiatula aff caerulea</i> sensu Bolotov et al., 2017a)	biv_108_1	KX865835	KX865709	Bolotov et al. (2017a)	Sittaung – Irrawaddy
<i>Radiatula bonneaudii</i>	UMMZ 304645	JN243898	JN243878	Whelan et al. (2011)	Sittaung – Irrawaddy
<i>Radiatula bonneaudii</i> (= <i>Parreysia cf. burmana</i> sensu Bolotov et al., 2017a)	biv_107_3	KX865840	KX865712	Bolotov et al. (2017a)	Sittaung – Irrawaddy
<i>Radiatula humilis</i>	UF 507600 (2014-0570)	MH350923	MH350972	This study	Khorat Plateau
<i>Radiatula humilis</i>	UF 507616 (2014-0604)	MH350925	MH350974	This study	Khorat Plateau
<i>Radiatula humilis</i> (= <i>Radiatula aff humilis</i> 1 sensu Bolotov et al., 2017a)	biv_126_1	KX865844	KX865716	Bolotov et al. (2017a)	Khorat Plateau
<i>Radiatula khadakvaslaensis</i>	RNBI7	JQ861244	–	GenBank	Western Ghats
<i>Indochinella pugio</i>	UMMZ 304644	JN243899	JN243879	Whelan et al. (2011)	Sittaung – Irrawaddy
<i>Indochinella pugio</i> (= <i>Scabies crispata</i> sensu Bolotov et al., 2017a)	biv_147_3	KX865852	KX865724	Bolotov et al. (2017a)	Lower & Middle Salween

monophyly of the Indochinellini was moderately supported (94 PP and 67 BS) and that clade was sister to the African tribe Coelaturini. *Harmandia*, *Unionetta*, *Scabies* and *Radiatula* were all recovered as nonmonophyletic, but the relationships among these taxa were poorly supported.

Morphological results

Twenty species were scored for four morphological characters and were used to diagnose some of the recovered suprageneric clades and corroborate the higher-level classification of *Harmandia* and *Unionetta* (Table 2). *Harmandia somborensis* and *U. fabagina* both possess unhooked glochidia, tetragenous brooding and complete fusion of the ascending lamella of the inner demibranch to the visceral mass, a combination of characters unique to Parreysiinae (excluding Lamellidentini) (Bolotov et al., 2018). *Harmandia munensis* is ectobranchous and has bilaterally asymmetrical glochidia, which unambiguously place it in the Contradentini (Rectidentinae) (Pfeiffer & Graf, 2015; Lopes-Lima et al., 2017).

Biogeographic results

The MUSSELPdb contained 6917 digitized lots from Asia, and of the 1450 records from the Mekong drainage only nine and 16 lots of *Harmandia* and *Unionetta* were discovered, respectively. Many of these lots had previously been identified as *Scabies* Haas, 1911, *Contradens* Haas, 1911, *Trapezoideus* Simpson, 1911 or Unionidae sp. *Harmandia somborensis* and *U. fabagina* are both widely distributed in the Mekong River drainage from central Cambodia to northern Laos (Fig. 4). Each species is found primarily in the Mekong River main stem and its larger tributaries (i.e. Tonle Sekong/Sre Pok, Mun, Songkhram and Ou).

SYSTEMATIC DESCRIPTIONS

Harmandia Rochebrune, 1881

Harmandia Rochebrune, 1881: 45 (type species *Harmandia somborensis* Rochebrune, 1881, monotypy).

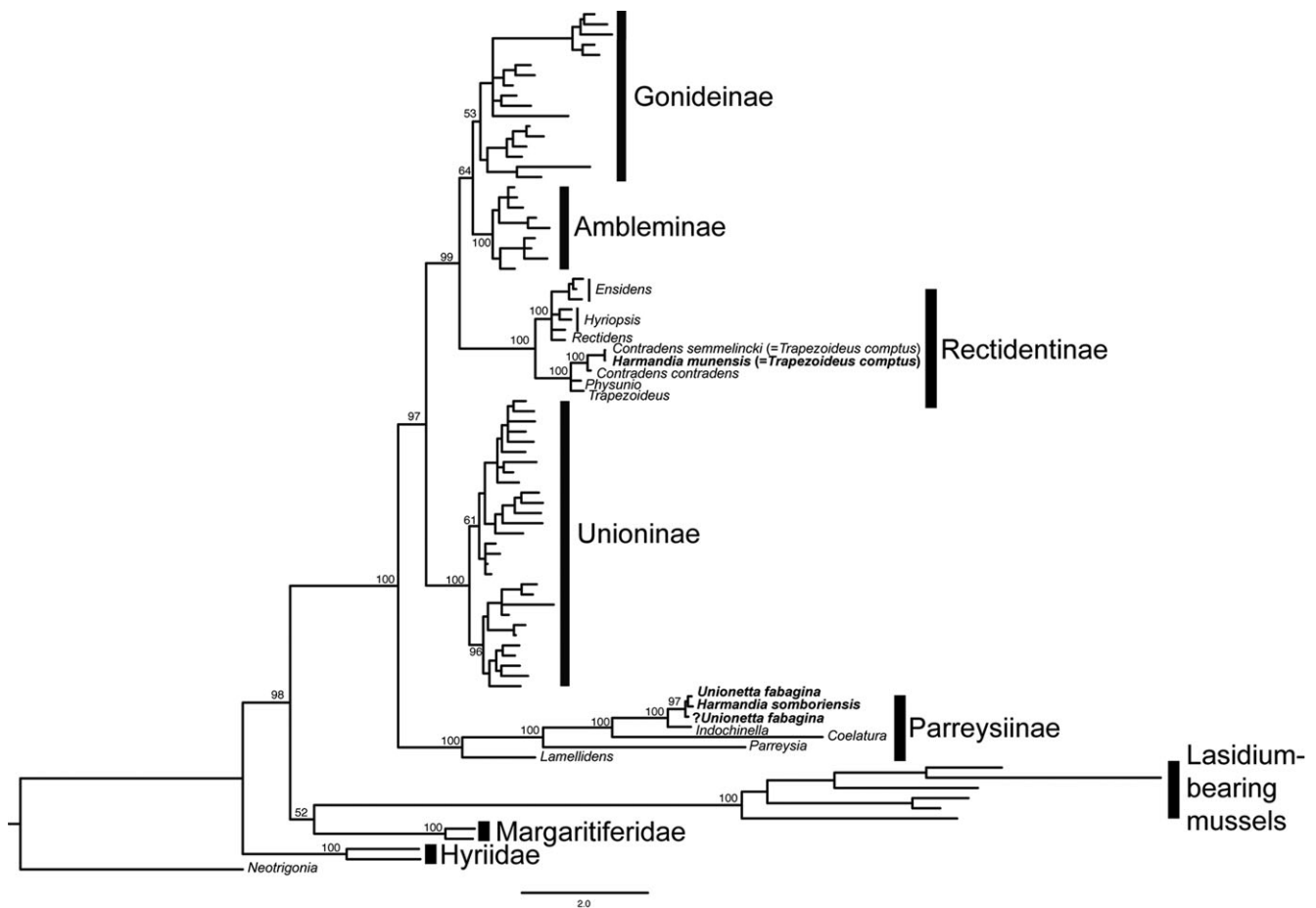


Figure 1. Phylogeny of the Unionoida with phylogenetic positions of *Harmandia* and *Unionetta* highlighted in bold. Bayesian posterior probabilities at nodes.

Diagnosis: *Harmandia* is distinguished from other representatives of the Indochinellini by having a trapezoidal shell outline (*vs* ovate), a truncated anterior end (*vs* bluntly pointed), strong lateral compression (*vs* moderately inflated) and a gradual posterior slope (*vs* moderately to very steep).

Description: Shell moderately thin; shell disc with strong V-shaped and zigzag corrugations; outline trapezoidal; anterior margin truncate, posterior broadly rounded, dorsal and ventral margins straight; strongly compressed; posterior ridge rounded, posterior slope gradual; umbo hardly elevated above hinge line; periostracum yellow to brown, often with green posterior slope; lateral teeth strong, moderately short, 2 in left valve (LV), 1 in right valve (RV); pseudocardinal teeth strong, triangular, 2 in LV, 1 in RV; supra-anal and excurrent apertures smooth, excurrent aperture with many simple papillae; mantle connection between supra-anal and excurrent apertures very short; mantle ventral to incumbent aperture with many short papillae; ascending lamella of inner demibranch completely fused to visceral mass; brooding tetragnous; glochidia unhooked.

Distribution: Mekong River drainage.

Remarks: Much of the disagreement regarding the systematic position of *Harmandia* has focused on its inclusion in the families Unionidae or Hyriidae. The prevailing hypothesis had been that *Harmandia* belongs to the Hyriidae (Thiele, 1934; Modell, 1964; Haas, 1969a, b; Vokes, 1980; Vaught, 1989; Millard, 2001), while fewer authors have considered it in the Unionidae (Brandt, 1974;

Graf & Cummings, 2007a; Lopes-Lima *et al.*, 2017). These conflicting classifications stemmed from the assumed monophyly of *Harmandia* and the fact that shell characters cannot unambiguously distinguish most members of the two families. *Harmandia somboriensis* is clearly a unionid, supported by our molecular phylogeny (Fig. 1) and the presence of a supra-anal aperture (an unambiguous synapomorphy of the Unionidae) and unhooked glochidia, whereas the Hyriidae have no supra-anal aperture and S-shaped hooked glochidia (Table 2). However, *H. castelneui* Rochebrune, 1904, previously a junior synonym of *H. somboriensis* and known only from its type specimens, does appear to be a hyriid. Rochebrune (1904) described *H. castelneui* from specimens collected during the *Voyages de Castelneau* in 1847 and stated the type locality to be ‘Cochinchine’ (i.e. southern Vietnam). However, to our knowledge, all the specimens from the 1847 *Voyages de Castelneau* were collected in South America, including the types of at least nine other nominal species. Given that the types of *H. castelneui* were also likely collected from South America, and its morphological similarity to other Hyriidae (Fig. 3C, D), *H. castelneui* is considered a junior synonym of *Triplodon corrugatus* (Lamarck, 1819).

The only other species attributed to *Harmandia* is *H. munensis* Brandt, 1974, but this taxon clearly belongs to the tribe Contradentini (Rectidentinae) based on the molecular phylogeny (Fig. 1) and the possession of asymmetrical glochidia (Table 2), a synapomorphy of the Contradentini (Pfeiffer & Graf, 2015; Lopes-Lima *et al.*, 2017). *Harmandia munensis* is herein considered a junior synonym of *Trapezoideus comptus* (Deshayes in Deshayes & Jullien, 1874), based on their conchological similarities (Fig. 3E–G). *Harmandia* is therefore recognized as being monotypic.

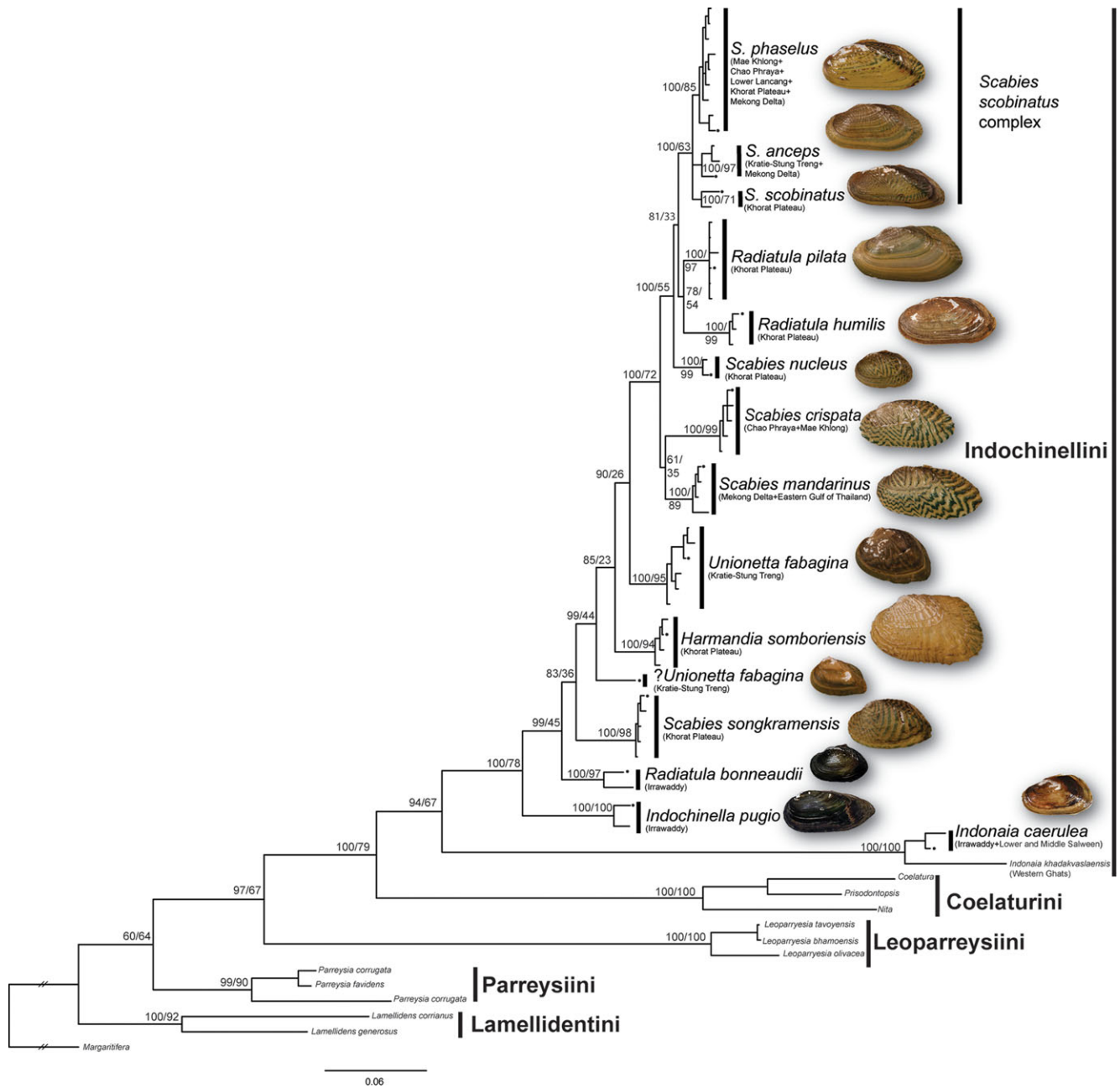


Figure 2. Concatenated COI+28S phylogeny of the Parreysiinae. Posterior probabilities and bootstrap support at nodes (PP/BS). The listed freshwater ecoregions refer to the area in which the samples were collected. Photographs of specimens correspond to terminals with dots in the adjacent clade.

***Harmandia somboriensis* Rochebrune, 1881**

(Fig. 3A, B)

Harmandia somboriensis Rochebrune, 1881: 46; pl. 1, figs 1–4 (Rapides de Sombor-Sombor [Mekong River, Sambour, Kratie Province, Cambodia; 12.7744°N, 105.96311°E]; holotype MNHN-IM-2000-1789).

Material examined: MNHN-IM-2000-1789 (*n* = 1), Mekong River, Sombor, Kratie Province, Cambodia [12.7744°N, 105.96311°E]; UF 507791 (*n* = 5), Mekong River, Ban Tha Pho on Rt 212, Bueng Kan Province, Thailand [18.339°N, 103.721°E]; UF 507831 (*n* = 2), Mekong River, near Muk River confluence, Mukdahan Province, Thailand [16.546°N, 104.732°E]; ANSP

349499 (*n* = 8), Mun River, Nong Bua, Si Sa Ket Province, Thailand [15.1330°N, 104.52°E]; ANSP 349504 (*n* = 1), Mekong River, Ban Dan, Ubon Ratchathani Province, Thailand [15.324°N, 105.493°E]; ANSP 349505 (*n* = 1), Mekong River, Khemmarat, Ubon Ratchathani Province, Thailand [16.044°N, 105.223°E]; MNHN IM-2014-6878 (*n* = 1), Nam Ou, Hat Kho, Luang Prabang Province, Laos [20.10392°N, 102.2813°E]; SMF 220526 (*n* = 1), Mekong River, Champassak, Champassak Province, Laos [14.90097°N, 105.88887°E]; SMF 220527 (*n* = 1), Mun River, Rasi Salai, Si Sa Ket Province, Thailand [15.34466°N, 104.15476°E].

Diagnosis: Same as for genus.

Description: Same as for genus.

Table 2. Summary of morphological observations and taxonomic classification.

Taxon	Source	Aperture	ALIDFVM	Brooding	Glochidia	Higher classification
<i>Tripodon corrugatus</i> (Lamarck, 1819) (= <i>Harmandia castelneau</i>)	Pimpão (2011)	No supra-anal	Complete	Endobranchous	S-shaped hooked	Hyriidae
<i>Margaritifera falcata</i> (Gould, 1850)	UF 271468	No supra-anal	Anterior end only	Tetragenous	Unhooked	Margaritiferidae
<i>Lamellidens marginalis</i> (Lamarck, 1819)	CAS 180833	Supra-anal	Complete	Ectobranchous	Unhooked	Parreysiinae: Lamellidentini
<i>Parreysia corrugata</i> (Lamarck, 1819)	Ortmann (1910); Prashad (1918)	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Parreysiini
<i>Leoparreysia burmana</i> (Blanford, 1869)	CAS 180831	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Leoparreysiini
<i>Coelatura</i> sp.	UF 510905	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Coelaturini
<i>Harmandia somboriensis</i> Rochebrune, 1881	UF 507831, UF 507791	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Indochinellini
<i>Unionetta fabagina</i> (Deshayes in Deshayes & Jullien, 1874)	UF 507399	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Indochinellini
<i>Radiatula caerulea</i> (Lea, 1831)	UF 507572	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Indochinellini
<i>Scabies crispata</i> (Gould, 1843)	UF 507693, UF 507682	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Indochinellini
<i>Radiatula pilata</i> (Lea, 1866)	UF 507813, UF 507807	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Indochinellini
<i>Indochinella pugio</i> (Benson, 1862)	CAS 180796, CAS 189963	Supra-anal	Complete	Tetragenous	Unhooked	Parreysiinae: Indochinellini
<i>Rectidens sumatrensis</i> (Dunker, 1852)	UF 410001	Supra-anal	Anterior end only	Ectobranchous	?	Rectidentinae: Rectidentini
<i>Contradens contradens</i> (Lea, 1838)	UF 507874, UF 507591	Supra-anal	Anterior end only	Ectobranchous	Asymmetrical	Rectidentinae: Contradentini
<i>Trapezoideus comptus</i> (Deshayes in Deshayes & Jullien, 1874) (= <i>Harmandia munensis</i>)	UF 507396, UF 507833	Supra-anal	Anterior end only	Ectobranchous	Asymmetrical	Rectidentinae: Contradentini
<i>Nodularia douglasiae</i> (Griffith & Pidgeon, 1833)	Bolotov et al., (2018)	Supra-anal	Anterior end only	Ectobranchous	Triangular hooked	Unioninae
<i>Nodularia micheloti</i> (Morlet, 1886)	NCSM 84920, NCSM 84425	Supra-anal	Anterior end only	Ectobranchous	Triangular hooked	Unioninae
<i>Pseudodon vondembuschiana</i> (Lea, 1840)	UF 507565, UF 507438	Supra-anal	Anterior end only	?	?	Gonideinae: Pseudodontini
<i>Pilsbryoconcha</i> sp.	UF 507453	Supra-anal	Anterior end only	Tetragenous	Unhooked	Gonideinae: Pseudodontini
<i>Chamberlainia hainesiana</i> (Lea, 1840)	UF 507722, UF 507872	Supra-anal	Complete	?	?	Gonideinae: Chamberlainiini

Abbreviation: ALIDFVM, ascending lamella of inner demibranch fusion to visceral mass.

Distribution: *Harmandia somboriensis* is geographically widespread in the Mekong River (Fig. 4). Previously reported only from Sambour, Cambodia, and Champasak and Khong Island, Laos (Brandt, 1974), *H. somboriensis* is known from at least nine localities distributed across Cambodia, Thailand and Laos and in three of the five freshwater ecoregions of the Mekong drainage (Lower Lancang, Khorat Plateau and Kratie-Stung Treng).

Remarks: *Harmandia somboriensis* is uncommon in the world's natural history collections and the species was collected at only two of 135 recent collection localities in the Mekong drainage (Pfeiffer & Page, unpublished data). It remains unclear if populations of this species are stable, in decline, or simply infrequently detected.

Unionetta Haas, 1955

Unionella Haas, 1913: 37 (type species *Unio fabagina* Deshayes in Deshayes & Jullien 1874, original designation; junior homonym of *Unionella* Etheridge, 1888).

Unionetta Haas, 1955: 212 (replacement name for *Unionella* Haas, 1913).

Unionea 'Haas, 1955' Hass, 1969b: N435 (unavailable; misspelling).

Diagnosis: *Unionetta* is distinguished from other representatives of the Indochinellini by its triangular to quadrate outline (*vs* ovate) and very short and steep posterior slope (*vs* long and moderately steep). *Scabies nucleus* and some young *S. songkramensis* may resemble *Unionetta*, but the former two species have fine, even V-shaped sculpturing on the shell disc.

Description: Shell moderately thick; shell disc smooth or with thick variable ridges (e.g. concentric, nodulose, double looped) and irregular V-shaped sculpturing; moderately inflated; outline triangular to quadrate; anterior margin truncate to bluntly pointed, posterior broadly rounded, dorsal and ventral margins straight to slightly curved; posterior ridge short, moderately sharp; posterior slope steep; umbo elevated above hinge line; periostracum yellow, green to brown; lateral teeth strong, moderately short, 2 in LV, 1 in RV; pseudocardinal teeth strong, triangular, 2 in LV, 1 in RV;



Figure 3. Shells of *Harmandia somboriensis* (A, B), *Triplodon corrugatus* (C, D), *Trapezoideus comptus* (E–G) and *Unionetta fabagina* (H–L). **A.** *H. somboriensis* Rochebrune, 1881 (holotype, MNHN-IM-2000-1789). **B.** *H. somboriensis* (UF 507831: sequenced specimen, ICH_00890). **C.** *T. corrugatus* (*Harmandia castelneau* Rochebrune, 1904, syntype, MNHN-IM-2000-1655). **D.** *T. corrugatus* (MNHN IM-2014-6881). **E.** *T. comptus* (*Unio comptus* Deshayes in Deshayes & Jullien, 1874, syntype, MNHN-IM-2000-1661). **F.** *T. comptus* (*Harmandia munensis* Brandt, 1974, holotype SMF 220828). **G.** *T. comptus* (UF 507396: sequenced specimen, ICH_00474). **H.** *U. fabagina* (*Unio fabagina* Deshayes in Deshayes & Jullien, 1874, syntype MNHN-IM-2000-1694). **I.** *Unionetta fabagina* (UF 507399: sequenced specimen, ICH_00492). **J.** *Unionetta fabagina* (*Unio broti* Deshayes in Deshayes & Jullien, 1874, syntype MNHN-IM-2000-1652). **K.** *U. fabagina* (UF 507399: sequenced specimen, ICH-00494). **L.** ?*Unionetta fabagina* (UF 507378: sequenced specimen, ICH_00401).

supra-anal and excurrent apertures smooth, excurrent aperture with many simple papillae; mantle connection between supra-anal and excurrent aperture moderately long; mantle ventral to incurrent aperture with many short papillae; inner demibranch completely fused to visceral mass; brooding tetragenous; glochidia unhooked.

Distribution: Mekong River drainage.

Remarks: *Unionetta* Haas, 1955 was initially described as *Unionella* Haas, 1913 and subsequently renamed because the latter is a junior homonym of a fossil unionoid described by Etheridge, 1888. *Unionetta* had generally been considered a representative of the

Unioninae (Thiele, 1934; Haas, 1969a, b; Starobogatov, 1970; Vokes, 1980; Vaught, 1989; Millard, 2001), although the taxa included in these groupings varied widely. However, Modell (1942, 1964) and Brandt (1974) classified *Unionetta* among the Parreysiinae, and that hypothesis is well supported in the molecular phylogeny (Fig. 1) and the morphological assessment (Table 2).

Despite the morphological similarities (Fig. 3) and close geographic proximity (about 60 km) of the seven sequenced *Unionetta* specimens, the genus was not recovered as monophyletic (Fig. 2). A single molecularly divergent specimen, ?*U. fabagina* from the Tonle Sekong watershed (Fig. 4), rendered *U. fabagina* nonmonophyletic. Morphologically, the ?*U. fabagina* specimen closely

resembles other small *U. fabagina* specimens, including the type of *Unio broti* (Fig. 3). Further research is necessary to determine if this molecular lineage is aberrant or worth formal taxonomic recognition. For the time being, we consider the two lineages to represent a single species, *U. fabagina*.

***Unionetta fabagina* (Deshayes in Deshayes & Jullien, 1874)**

(Fig. 3H–L)

Unio fabagina Deshayes in Deshayes & Jullien, 1874: 128, pl. 7, figs 4–6 (rivage du Mekong, à Sombor [Mekong River, Sambour, Kratie Province, Cambodia, 12.7744°N, 105.96311°E]; syntype MNHN-IM-2000-1694).

Unio broti Deshayes in Deshayes & Jullien, 1874: 129, pl. 7, figs 1–3 (rivage du Mekong à Sombor [Mekong River, Sambour, Kratie Province, Cambodia, 12.7744°N, 105.96311°E]; syntype MNHN-IM-2000-1652).

Unio molleuri Morlet, 1891: 238, 242, pl. 7, fig. 4 (Vallée du Mékong; syntype MNHN-IM-2000-1751; syntype SMF 3599).

Material examined: MNHN-IM-2000-1694 ($n = 1$), Mekong River, Sambor, Kratie Province, Cambodia [12.7744°N, 105.96311°E]; MNHN-IM-2000-1652 ($n = 1$), Mekong River, Sambor, Kratie Province, Cambodia [12.7744°N, 105.96311°E]; MNHN-IM-2000-1751 ($n = 1$), MNHN IM-2014-6879, ($n = 1$), Vallée du Mékong; SMF 3599 ($n = 1$), Nam Ou, Ban Maungxun, Luang Prabang Province, Laos [20.32242°N, 102.43534°E]; ANSP 120637 ($n = 1$), Siam; ANSP 336450 ($n = 2$), Mekong River, Khong Island, Muang Khong, Champasak Province, Laos [14.11689°E, 105.85619°N]; NHMUK 20180150 ($n = 3$), SMF 193871 ($n = 3$), USNM 786230, ($n = 3$), Huai Mae Un, Sri Songkram, Nakhon Phanom Province, Thailand [17.62316°N, 104.23926°E]; SMF 198393 ($n = 2$), Mekong River, Ban Dan, Ubon Ratchathani Province, Thailand [15.32385°N, 105.49303°E]; SMF 220833 ($n = 1$), SMRL 5872 ($n = 3$), Maenam Songkram, Sri Songkram, Nakhon Phanom, Thailand [17.63444°N, 104.24555°E]; MB54/1 ($n = 1$), Sa Thay River, Cambodia-Vietnam boarder [*c.* 13.998°N, 107.444°E]; UF 507399 ($n = 11$), Mekong River, 5.17 miles upstream of Mekong and Sekong confluence, Stung Treng Province, Cambodia [13.59515°N, 106.01447°E]; UF 507378 ($n = 1$), Tonle Srepok River, on road from Stung Treng to Ban Lung, Ratanakiri Province, Cambodia [13.44339°E, 106.60329°N].

Diagnosis: Same as for genus.

Description: Same as for genus.

Distribution: *Unionetta fabagina* is geographically widespread in the Mekong River (Fig. 4). The species is known from at least seven localities distributed across Cambodia, Vietnam, Thailand, Laos and three of the five ecoregions in the Mekong drainage (Lower Lancang, Khorat Plateau and Kratie-Stung Treng; Fig. 4).

Remarks: *Unionetta fabagina* was collected at only two of 135 recent sampling localities in the Mekong drainage (Pfeiffer & Page, unpublished data). However, the species was locally abundant at one site (14% of the qualitative survey), especially among the submerged root mats of emergent macrophytes in flowing water (Fig. 5).

Unionetta fabagina has recently been reported in the ornamental pet trade in Singapore, a global hub of the aquarium industry; this has raised regional conservation concerns in relation to overharvesting and introduction of nonnative species (Chan, 2008; Ng et al., 2016). However, the reports of *U. fabagina* were misidentified specimens of *Corbicula* sp. (personal observation); as such, there are no known pet trade-associated conservation concerns for this species.

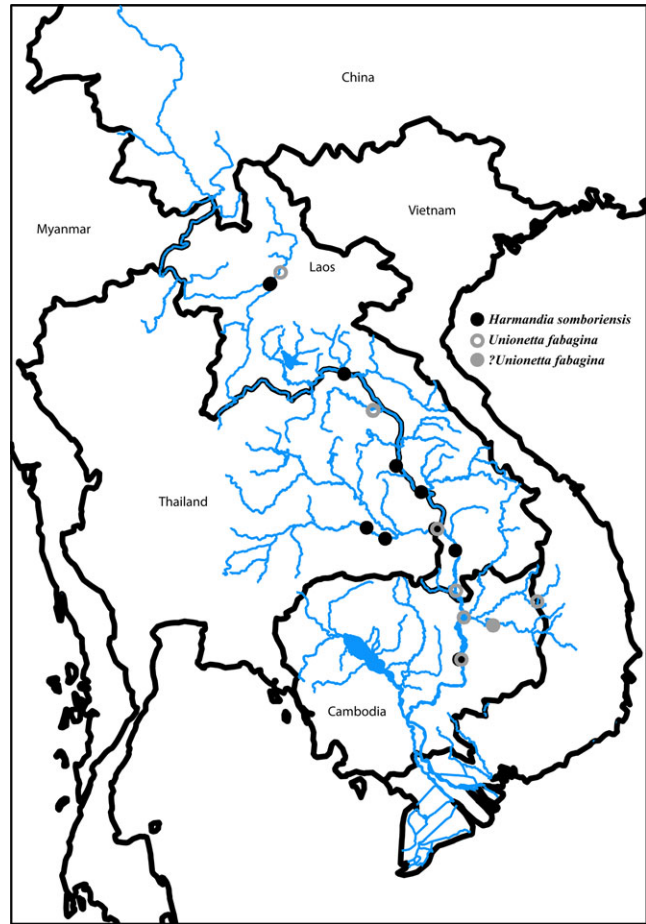


Figure 4. Distribution map of *Harmandia somboriensis*, *Unionetta fabagina* and ?*Unionetta fabagina*. Only Mekong River drainage shown for clarity.

DISCUSSION

The initial test of the phylogenetic position of *Harmandia somboriensis* and *Unionetta fabagina* recovered both taxa as members of the Indo-Afrotropical subfamily Parreysiinae (Fig. 1). The Parreysiinae-focused phylogeny (Fig. 2) and morphological assessment (Table 2) determined *Harmandia* to be polyphyletic with its putative species belonging to three distantly related clades of the Unionoidea: *H. somboriensis* (type species) in the Parreysiinae (Unionidae); '*H.* castelneai' in the Hyriidae (= *Triplodon corrugatus*); '*H.* munensis' in the Rectidentinae (Unionidae; = *Trapezoides comptus*). *Unionetta* was likewise not recovered as monophyletic, despite the strong morphological similarities and close geographic proximity of the sequenced individuals.

The systematic position of the Mekong endemic *Harmandia* and *Unionetta* among the Afrotropical and Indotropical Parreysiinae requires reappraisal of the subfamily's biogeography and diversification.

The Parreysiinae of Southeast Asia

The Parreysiinae are primarily distributed on the Gondwanan fragments of Africa and the Indian subcontinent (and adjacent Myanmar), with a relatively minor portion of the subfamily thought to extend east of the Salween drainage and into mainland Southeast Asia (Lopes-Lima et al., 2017; Bolotov et al., 2017a, b; Zieritz et al., 2018). Testing the systematic position of *Harmandia* and *Unionetta* has revealed that the Parreysiinae are taxonomically and morphologically more diverse in mainland Southeast Asia



Figure 5. Mekong River near Sambour, Cambodia (13.59515°N, 106.01447°E). Box highlights habitat where *Unionetta fabagina* was abundant.

Table 3. Indochinellini species reported from the Mekong River.

Taxon	Zieritz <i>et al.</i> (2018)	This study	Summary of update
<i>Radiatula humilis</i> (Lea, 1856)	Present	Present	–
<i>Radiatula pilata</i> (Lea, 1866)	Present	Present	–
<i>Scabies nucleus</i> (Lea, 1856)	Present	Present	–
<i>Scabies phaselus</i> (Lea, 1856)	Present	Present	–
<i>Scabies songkramensis</i> Kongim & Panha in Kongim <i>et al.</i> , 2015	Present	Present	–
<i>Harmandia somboriensis</i> Rochebrune, 1881	–	Present	Subfamily position resolved
<i>Unionetta fabagina</i> (Lea, 1838)	–	Present	Subfamily position resolved
<i>Scabies anceps</i> (Deshayes in Deshayes & Jullien, 1874)	–	Present	Removed from synonymy of <i>Contradens gratusus</i>
<i>Scabies scobinatus</i> (Lea, 1856)	–	Present	Removed from synonymy of <i>Scabies crispata</i>
<i>Scabies mandarinus</i> (Morelet, 1864)	–	Present	Removed from synonymy of <i>Scabies crispata</i>
<i>Scabies crispata</i> (Gould, 1843)	Present	–	Distributed west of Mekong River in SE Asia
<i>Oxynaia gladiator</i> (Ancy, 1881)	Present	–	Misidentification (MNHN IM-2014-6880)
<i>Oxynaia micheloti</i> ((Morlet, 1886)	Present	–	Incorrect interpretation of locality (FMNH 20402, NCSM 84425)

than recently proposed; consisting of four (nonmonophyletic) genera and at least 11 species (Table 3). All of the Parreysiinae taxa distributed east of the Salween drainage are recovered in a shallow clade (albeit with low support), nested within the larger Parreysiinae, which are otherwise distributed west of the Salween (Fig. 2). The phylogenetic position and monophyly of the mainland Southeast Asian Parreysiinae clade, in combination with the available fossil evidence and broader biogeographic patterns of the subfamily, suggest a recent eastward dispersal event into the region, followed by rapid molecular and morphological diversification.

This hypothesized dispersal event into mainland Southeast Asia is difficult to reconcile with some recent biogeographic hypotheses proposed for the Parreysiinae, especially their ancient origin in adjacent western Indochina. Bolotov *et al.* (2017a) generated a comprehensive, multilocus, time-calibrated phylogeny of the Unionidae and proposed a global biogeographic model for the family, providing an evolutionary and biogeographic framework on which to build future research. As part of their detailed global biogeographic analysis, these authors hypothesized that the crown group of the Parreysiinae originated in western Indochina (*c.* 150 Ma), colonized

Africa via ‘vicariance’ (*c.* 95 Ma), colonized India via dispersal across ancient land bridges connecting western Indochina and India (*c.* 60 Ma) and that a small clade of mainland Southeast Asian Parreysiinae (3 species) was a product of a comparatively recent vicariant event (*c.* 12 Ma) (Bolotov *et al.*, 2017a). This biogeographic scenario requires a dramatic westward migration from western Indochina onto Gondwanan fragments starting more than 100 Ma, all the while there were no major biogeographic events in the hypothesized centre of origin for over 130 Myr, until a recent vicariant event that gave rise to the adjacent mainland Southeast Asian Parreysiinae clade.

An ancient western Indochinese origin of the Parreysiinae appears to be in conflict with the fact that the early-diverging lineages of the subfamily are primarily distributed in the western Indotropics (Parreysiini and Lamellidentini), the existence of a comparatively recent mainland Southeast Asian clade and that the oldest known fossils of three Parreysiinae lineages on Gondwanan fragments all postdate collision with Laurasia (Coelaturini: 7.5 Ma; *Parreysia*: 9 Ma; *Lamellidens*: 9 Ma; Bolotov *et al.*, 2017a and references therein). An alternative hypothesis is that the Parreysiinae started to diversify in western Eurasia and dispersed south to Africa,

and east to India, Myanmar and eventually mainland Southeast Asia, after final contact of the Gondwanan fragments with the Eurasian plate. Taxon sampling focused on Burmese representatives, and the exclusion of fossil calibration points for the Parreysiinae, appear to have biased the hypotheses of Bolotov *et al.* (2017a) towards older and more easterly origins of the Parreysiinae. Definitive tests of these alternative hypotheses require more comprehensive sampling of the western Indotropical Parreysiinae.

The demonstration of a diverse clade of Southeast Asian Parreysiinae is an important step towards a more complete understanding of the biogeography and phylogenetic relationships of the Parreysiinae and deserves further study. Knowledge of the historical biogeography of the Parreysiinae will improve as the phylogenetic positions of other poorly understood taxa from the Indotropics are established. Of particular importance are the phylogenetic affinities of *Conradens subcircularis* (Brandt, 1974) and *Elongaria trompi* (Drouet & Chaper, 1892). *Conradens subcircularis* was described from the middle Mekong (Brandt, 1974) and is currently classified in the Rectidentinae. However, *C. subcircularis* looks remarkably similar to species of the Leoparreysiini (Parreysiinae), which are otherwise known only from the Salween River and westwards, and may represent an additional lineage of the Parreysiinae that dispersed into mainland Southeast Asia. An enigmatic Bornean taxon, *E. trompi* (Drouet & Chaper, 1892), strongly resembles representatives of the Indochinellini with which it shares a straight ventral margin and similar sculpturing on the shell disc. If *E. trompi* is shown to be a member of the Indochinellini, this would substantially extend the southern distribution of the Parreysiinae, from mainland Southeast Asia to the Sunda Islands. Besides its potential biogeographic implications, *E. trompi* is also of taxonomic interest since *Unio trompi* is the name-bearing type of the Nannonaiini Model, 1942 and would have priority over Indochinellini if they were shown to represent the same clade.

The Indochinellini of the Mekong River

Based on a thorough review of voucher specimens and the literature, Zieritz *et al.* (2018) reported eight Indochinellini species in the Mekong drainage (Table 3), but also recognized that the data on the distribution and diversity of the Indochinellini were generally older in comparison with those in many other family-group taxa in the region (their fig. 4A). The current study updates the species-level diversity and biogeography of the Indochinellini of the Mekong River. The phylogeny of the Southeast Asian Parreysiinae recovered ten morphologically recognizable species-level clades in the Mekong River drainage (Fig. 2), five of which were not recognized by Zieritz *et al.* (2018) (Table 3). The validity of species in the *S. scobinatus* complex requires further examination, but there are clear patterns of morphological and genetic variation within the Mekong drainage consistent with the recognition of three species. All of the Indochinellini species previously reported to occur in the Mekong drainage were recovered here, with the exception of two. However, records of these two species, *Oxyaia gladiator* (Ancey, 1881) and *O. micheloti* (Morlet, 1886), in the Mekong were based on historically misidentified specimens or on incorrect interpretations of locality (Table 3) and they are now recognized as members of the genus *Nodularia* (Bolotov *et al.*, 2018). This assessment has helped to clarify the species-level diversity of the Indochinellini in the Mekong River, but much remains to be done to confirm their generic classification, because the supraspecific relationships and diagnostic characters of these clades remain unclear.

Several of the Indochinellini of mainland Southeast Asia show a much more complex biogeographic pattern in comparison to what has recently been proposed for the regional fauna. Bolotov *et al.* (2017a) suggested that the freshwater mussel fauna of Southeast Asia is comprised almost exclusively of single-drainage endemics, with only three of 66 Indotropical species being distributed in more than one drainage. The “drainage-shaped distribution pattern” and “exceptionally high levels of [drainage] endemism”

proposed by Bolotov *et al.* (2017a: 4, 7, respectively) suggest significant independence and isolation of the freshwater systems of mainland Southeast Asia, which is inconsistent with the dynamic geoclimatic history of the region and its many hypothesized palaeodrainages (reviewed by Rainboth, Vidthayanon & Yen, 2012), and with the distribution patterns observed here.

Considering just the 11 mainland Southeast Asian Indochinellini species focused on here (Table 3), three were confirmed to be distributed in multiple drainages: *Scabies crispata*: Petchaburi, Mae Khlong, Chao Phraya, Bang Prakong; *S. phaseolus*: Mae Khlong, Chao Phraya, Mekong; *S. mandarinus*: Chanthaburi, Mekong (Table 1; Fig. 2). Even in the taxonomically restricted sample, there are clear cases of recent faunal exchange between six currently independent drainages. Bolotov *et al.* (2017a) are correct in that Southeast Asia has many narrowly endemic freshwater mussel species, but it appears that their geographically sparse sampling may have overestimated the proportion of single-drainage endemics and underestimated the importance of interbasin faunal exchanges in shaping freshwater mussel distribution in the region. Furthermore, an emphasis on drainage-based patterns of endemism neglects the important biogeographic barriers within a drainage. For example, the Mekong drainage is composed of five freshwater ecoregions, each of which has a characteristic assemblage of aquatic species, many of which are unique to just that ecoregion (e.g. Khorat Plateau: *S. nucleus*, *S. songkramensis*, *Radiatula humilis*). Increased geographic sampling, especially in areas of hypothesized stream capture and historical confluence, is likely to reveal more nuanced biogeographic patterns that reflect the fluidity of the freshwater boundaries in Southeast Asia.

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