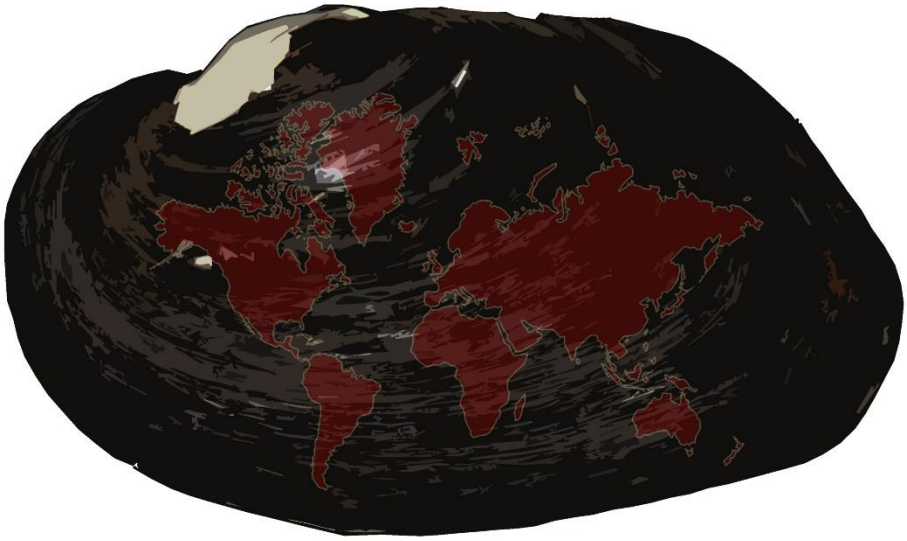

Combining phylogeny, systematics and ecology to advance the conservation of freshwater mussels (Bivalvia: Unionida)

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Doutoramento em Biodiversidade, Genética e Evolução
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2020

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Acknowledgments

This dissertation would not be possible without the help of my family that was supportive in so many ways to my “lunacy” in pursuing such an “extraordinary” research field such as biology and conservation of molluscs. I would like to thank to my supervisors for the help and critical analyses on the development of the thesis and to all of the members of the “Brave Mussel Warriors” team that cooperated and worked with me in so many hours of field and laboratory work, as well as in the writing and elaboration of the manuscripts. Additionally, this work would not be possible without the participation of all the co-authors of the manuscripts.

Abstract

The world is currently experiencing a biodiversity crisis, with many species facing the risk of extinction. This is particularly true for those in freshwater habitats, which are isolated between land and sea, and generally more exposed to human activities. Therefore, many freshwater groups of animals are now threatened with extinction and requiring urgent conservation measures. However, most current conservation efforts remain directed to charismatic terrestrial vertebrates, like mammals and birds. Although invertebrates dominate on Earth both in species richness and biomass, knowledge about these taxa is scarce and many groups need urgent conservation attention. This is the case of freshwater bivalves of the Unionida order, also known as freshwater mussels (FM). These bivalves are strictly freshwater inhabitants and originally dominated many freshwater habitats across the planet. They are important for the aquatic ecosystem functioning, playing key ecological roles and providing important ecosystem services to humans. They are also very interesting from the biological point of view, having a series of interesting traits that allow them to live in running water, like internal fertilization and parental care of their larvae. Especially, they have a unique life cycle in which FM larvae need to attach to a host (generally a fish) until adulthood, for nutrition but mainly for upstream dispersion. Another interesting feature is their rare form of mitochondrial inheritance, also called doubly uniparental inheritance (DUI), where the males inherit mitochondria from both parents. Male M- and female F-type mitochondrial lineages are highly divergent and are remarkable for the study of mtDNA evolution.

The overarching goal of this dissertation is to advance the conservation biology of freshwater mussels by combining research on phylogeny, systematics and ecology, showing how the integration of multiple research fields has practical implications to preserve highly endangered taxa. Specifically, the dissertation aims to: (i) understand the geographical diversity and conservation status patterns of freshwater bivalves, disclosing their main threats, and needs for conservation and research; (ii) highlight and discuss the importance of basic biological studies for the conservation of freshwater mussels; and (iii) accurately define species and integrate evolutionary patterns into conservation planning.

In **Chapter 1**, I start by introducing the global decline of biodiversity, focusing primarily on freshwater taxa and especially on the target taxonomic group of the dissertation, the freshwater mussels (FM). Then, their high ecological and economic importance are highlighted, as well as their unique biological features. I also discuss the importance of integrating basic research on species biology, taxonomy and phylogenetic patterns for conservation. Finally, the chapter presents the general and specific objectives of the thesis.

In **Chapter 2**, the geographical diversity patterns of freshwater bivalves were revised and their conservation status, threats, and the main needs for conservation and research are

analysed and discussed. **Chapter 2** reveals that among the several freshwater bivalve groups analysed, the most threatened by far are FMs, which support the main focus of the following chapters. We show that freshwater mussel diversity is geographically heterogeneous with two main hotspots of diversity, the Mississippi basin and associated basins in central North America, and the Indotropical rivers in Southeast Asia. The main global threats are associated with habitat degradation, while the most mentioned research needs globally refer to the need to collect baseline information on distribution, taxonomy, abundance, life-history traits, and threats. In terms of the needed conservation measures, protection and management of freshwater habitats are the most cited

The lack of baseline biological information identified in **Chapter 2** is then addressed in **Chapter 3**, which provides a baseline study on the Iberian dolphin mussel *Unio delphinus*. **Chapter 3** highlights the importance of basic biological studies for conservation planning and the potential use of biological traits as environmental indicators. For this, the distribution, growth, host-fish range and reproductive cycle of this species are described and discussed. *Unio delphinus* occupies the western Iberian River basins and, contrary to other well-known European FM species, is found to grow fast and to be short-lived. Their larvae may attach to most co-occurring fish species, but only native species were effective hosts. Based on these results, we reassessed its conservation status and provide recommendations on key conservation measures.

In **Chapters 4-8** the phylogenetic relationships among important groups of FMs are estimated and analysed, thereby addressing the need to accurately define conservation and management units, and to include evolutionary patterns in conservation planning. In **Chapters 4** and **6**, we estimated with a small number of molecular markers, the most comprehensive phylogenies so far for the most representative families of FMs in the northern hemisphere, the species-rich Unionidae and the threatened Margaritiferidae. The systematics and taxonomy within these families are updated with the help of an exhaustive search for diagnostic and synapomorphic, ecological and morphological characters. The distribution of the main groups inside those families is mapped and their biogeographic patterns discussed.

Chapter 5 focuses on a contentious group of North American species, that were originally lumped in a single genus, i.e. *Quadrula* sensu lato. We estimated its phylogeny and used molecular species delineation methods complemented by the assessment of ecological, morphological, and anatomical traits to revise the systematics and taxonomy of these species. Then we provide conservation guidance based on these results.

In **Chapters 7** and **8**, we updated the phylogenies within the Unionidae, this time with a multi-locus approach using whole mitogenomes. Given that many groups of FMs lack morphological diagnostic characters, it is important to develop new features that help us to characterize the main evolutionary history of FMs. The mitochondrial genome gene

arrangement is generally very conserved across taxa and eventual shifts in this order are rare in many taxa. In chapters 7 and 8 we also explored the use of mitogenome orders to be used as diagnostic of the higher-order taxonomic groups within FMs.

The present dissertation brings important advances in the basic biology, phylogeny, biogeography, and conservation of FMs globally. It presents more clear evolutionary relationships and biogeographical patterns among the main FM groups and highlights the biodiversity hotspots or areas where their levels of extinction risk, and species richness and genetic diversity are higher. Finally, this dissertation identifies the main knowledge gaps and threats for FM species to guide future research and conservation actions.

Keywords

Biogeography, Bivalvia, Conservation, Distribution, Doubly uniparental inheritance, Freshwater mussels, Growth, Host fish, Macroevolution, Mollusca, Phylogeny, Reproductive cycle, Species delineation, Taxonomic classification, *Unio delphinus*.

Resumo

A biodiversidade mundial está atualmente em crise, com muitas espécies em risco de extinção. Isto é particularmente verdadeiro para os organismos que habitam ambientes de água-doce, pois encontram-se isolados entre o mar e a terra e geralmente mais expostos às atividades humanas. Devido a esta situação, muitos grupos de animais dulçaquícolas estão agora ameaçados de extinção sendo necessárias medidas urgentes para a sua conservação. No entanto, a maioria dos esforços de conservação são geralmente direcionados para as espécies de vertebrados mais carismáticas, tais como os mamíferos e as aves. Embora na Terra os invertebrados sejam dominantes, tanto em número de espécies quanto em biomassa, o conhecimento sobre estes grupos é escasso e muitos deles precisam de atenção urgente no que respeita à sua conservação. Este é o caso dos bivalves de água-doce da ordem Unionida, também conhecidos como mexilhões de água-doce (MAD). Este grupo de bivalves ocorre apenas em água-doce e devido às suas elevadas abundâncias originais foram durante muito tempo um dos grupos taxonómicos dominantes em habitats de água-doce de todo o planeta. Os MAD são importantes para o funcionamento dos ecossistemas aquáticos pois desempenham papéis ecológicos cruciais e fornecem importantes serviços ecossistémicos aos seres humanos. Os MAD são também muito interessantes sob o ponto de vista biológico. Eles apresentam uma série de características interessantes que lhes permitem viver em água corrente, tais como a fertilização interna e cuidados parentais das suas larvas mas especialmente, um ciclo de vida único no qual as suas larvas precisam de se ligar a um hospedeiro (geralmente um peixe) até à idade adulta, para a sua nutrição, mas principalmente para dispersão a montante. Outra característica interessante dos MAD é que possuem uma forma rara de herança mitocondrial, também chamada herança duplamente uniparental, onde os machos herdam as mitocôndrias de ambos os pais. Essas linhagens mitocondriais herdadas dos pais (tipo M) e das mães (tipo F) são altamente divergentes e são muito interessantes para o estudo da evolução do ADN mitocondrial.

O objetivo principal desta dissertação é aumentar o conhecimento atual para a conservação de mexilhões de água-doce, combinando filogenia, sistemática e ecologia e mostrar como a integração destes vários campos de investigação tem implicações importantes na preservação de organismos em risco de extinção. Especificamente, a presente dissertação visa: (i) representar geograficamente os padrões de diversidade e estatutos de conservação dos bivalves de água-doce, revelando as suas principais ameaças e necessidades de conservação e investigação; (ii) destacar e discutir a importância de estudos biológicos básicos para a conservação de mexilhões de água-doce; e (iii) integrar metodologias de delimitação de espécies e padrões evolutivos na planificação de ações de conservação.

Começo o **Capítulo 1**, por introduzir o declínio global da biodiversidade, concentrando-me principalmente nos taxa de água-doce e, principalmente, no grupo taxonómico alvo da dissertação, os mexilhões de água-doce (MAD). Destaco a seguir, a sua elevada importância ecológica e económica, bem como as suas características biológicas únicas. Discuto posteriormente a importância para a conservação de integrar estudos básicos sobre a biologia de espécies com a taxonomia e padrões filogenéticos. Finalmente, o capítulo apresenta os objetivos gerais e específicos da tese.

No **Capítulo 2**, são revistos os padrões de diversidade geográfica dos bivalves de água-doce, os seus estatutos de conservação, bem como analisadas e discutidas as suas principais ameaças e as necessidades mais prementes para a sua conservação e investigação. O **Capítulo 2** revela que, dentre os vários grupos de bivalves de água-doce analisados, de longe os mais ameaçados são os MAD, que são o foco principal dos capítulos seguintes. Mostramos que a diversidade de mexilhões de água-doce é geograficamente heterogénea, com dois "hotspots" de diversidade: a bacia do Mississippi e sub-bacias associadas no centro da América do Norte e os rios Indotropicais no sudeste Asiático. As principais ameaças globais aos MAD estão associadas principalmente à degradação do habitat, enquanto os campos de investigação mais mencionados são a necessidade de obter informações básicas sobre a sua distribuição, taxonomia, abundância, e características da história de vida, bem como sobre as suas principais ameaças. Em termos das medidas de conservação necessárias, as mais citadas são a proteção e o a gestão sustentável dos habitats de água-doce que ocupam.

A falta de informação biológica básica identificada no **Capítulo 2** é então abordada no **Capítulo 3**, através de estudos sobre o MAD ibérico *Unio delphinus*. O **capítulo 3** destaca a importância de estudos biológicos básicos para a conservação e o uso potencial de algumas características biológicas como indicadores ambientais. Para esse efeito, caracterizamos e analisamos a sua distribuição, crescimento, gama de peixes hospedeiros e ciclo reprodutivo. O *Unio delphinus* ocupa as bacias ocidentais da Península Ibérica e, ao contrário de outras espécies europeias de MAD mais estudadas, cresce rapidamente e tem vida curta. As suas larvas conseguem afixar-se à maioria das espécies de peixes que ocorrem em simpatria, mas apenas as espécies de peixes nativos se revelaram hospedeiros eficazes. Com base nestes resultados, reavaliamos os seu estatuto de conservação e fornecemos recomendações sobre as principais medidas para a sua conservação.

Nos **Capítulos 4-8** foram estimadas e analisadas as relações filogenéticas entre grupos importantes de MAD, destacando também a necessidade de definir com precisão eventuais unidades de conservação e gestão e de incluir os padrões evolutivos encontrados, na sua conservação. Com um pequeno número de marcadores moleculares estimámos, nos **capítulos 4 e 6**, as filogenias mais abrangentes até então, para as famílias mais

representativas de MAD do hemisfério norte, a familia Unionidae que tem a maior riqueza de espécies, e a Margaritiferidae que contem o maior número de espécies ameaçadas. A sistemática e a taxonomia dessas famílias foi também atualizada com a ajuda de uma investigação exaustiva aos seus caracteres diagnósticos e sinapomórficos, ecológicos e morfológicos. A distribuição dos principais grupos dentro dessas famílias foi também mapeada e os seus padrões biogeográficos discutidos.

O **Capítulo 5** concentra-se num grupo polémico de espécies norte-americanas, originalmente agrupadas num único género, ou seja, *Quadrula* sensu lato. Estimamos a sua filogenia e revemos a sistemática e taxonomia dessas espécies usando métodos moleculares de delimitação de espécies, complementados pela avaliação de características ecológicas, morfológicas e anatómicas. Em seguida, fornecemos orientações para a sua conservação com base nesses resultados.

Nos **Capítulos 7 e 8**, atualizamos as filogenias dentro dos Unionidae, desta vez com uma abordagem com vários loci usando mitogenomas inteiros. Dado que muitos grupos de MAD carecem de caracteres-diagnóstico morfológicos, é importante desenvolver outros caracteres que nos ajudem a descrever a sua história evolutiva. A ordem em que os genes estão dispostos no genoma mitocondrial é geralmente muito conservado entre muitos grupos taxonómicos sendo que eventuais mudanças nessa ordem são geralmente raras. Nos **Capítulos 7 e 8**, exploramos então o uso das ordens genéticas dos mitogenomas como diagnóstico dos principais grupos taxonómicos de MAD.

A presente dissertação providencia importantes avanços para a biologia básica, filogenia, biogeografia e conservação de MAD a nível global. Clarifica as relações evolutivas e os padrões biogeográficos entre os principais grupos de MAD, destacando os seus ‘hotspots’ de diversidade. Por fim, esta dissertação identifica as principais lacunas no conhecimento e as ameaças para as espécies de MAD de forma a orientar ações futuras de investigação e conservação.

Palavras-chave

Biogeografia, Bivalvia, Conservação, Ciclo reprodutivo, Classificação taxonómica, Crescimento, Delimitação de espécies, Distribuição, Filogenia, Herança duplamente uni parental, Macroevolução, Mexilhões de água-doce, Moluscos, Peixe hospedeiro, *Unio delphinus*.

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List of Abbreviations

16S	16S ribosomal RNA gene
28S	28S ribosomal RNA gene
AF	Afrotropical
AU	Australasia
BI	Bayesian Inference
BIC	Bayesian information criterion
COI	Cytochrome oxidase subunit 1
DNA	Deoxyribonucleic acid
DUI	Doubly uniparental inheritance
FM	Freshwater mussel
GIS	Geographic Information System
H3	Histone H3 gene
IN	Indotropical
IUCN	International Union for the Conservation of Nature
MCMC	Markov-Chain Monte Carlo
ML	Maximum Likelihood
mtDNA	mitochondrial DNA
NA	Nearctic
ND1	NADH dehydrogenase subunit 1
nt	nucleotides
NT	Neotropical
PA	Palaearctic

CHAPTER 1

General introduction

1.1 The freshwater biodiversity crisis

Never in the world, so many humans used so many natural resources (Figs. 1 & 2). The exponential human growth over the last 2,000 years is coupled with the increase of consumptive biological resources per capita, resulting in increasing anthropogenic changes to natural environments (Sala et al 2000; IPBES 2019), as the major threat to biodiversity and ecosystem functioning (McKee et al 2003; McShane et al 2011). These impacts have already caused an extensive contraction of genetic, species and ecological diversity, with gene erosion and eradication, species extirpations and extinctions, and loss and irreversible transformation or destruction of many habitats around the globe (Butchart et al 2012; Ceballos et al 2015; Miraldo et al 2016; IPBES 2019). Therefore, we are living under a biodiversity crisis with unprecedented severity, since human life on Earth (Barnosky et al 2011). The extinction rates are far higher than baseline values and closer to those during major extinction events, and we are potentially experiencing the sixth mass extinction event (Barnosky et al 2011; Pievani 2014).

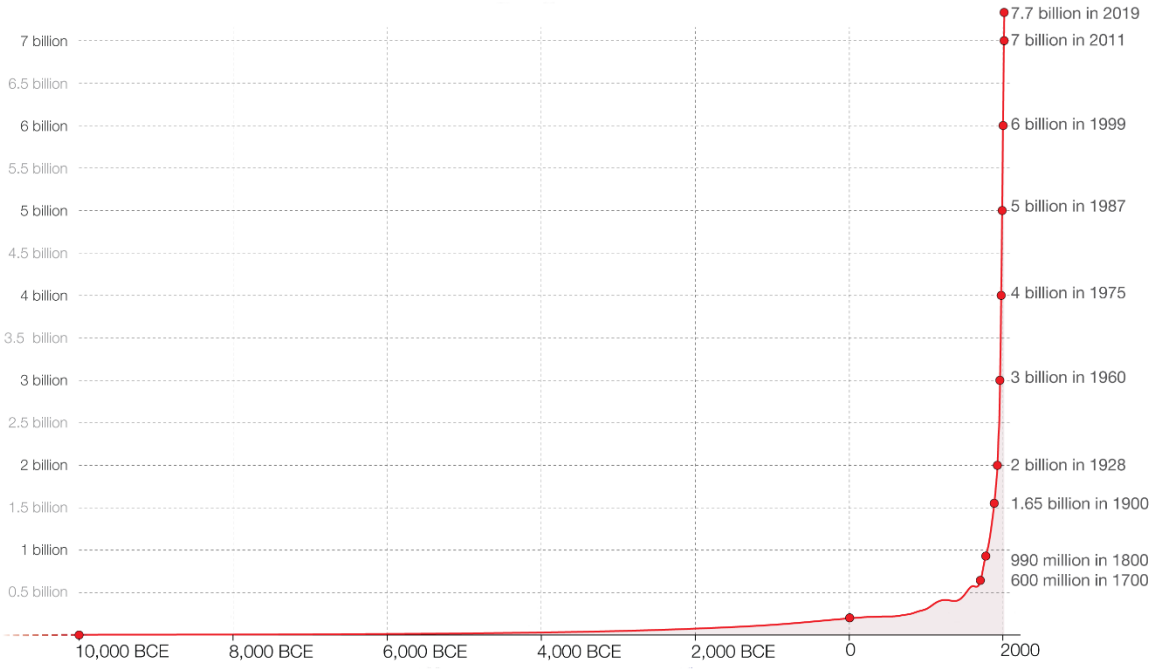

Figure 1. Size of the world human population over the last 12,000 years. Adapted from Roser et al (2019).

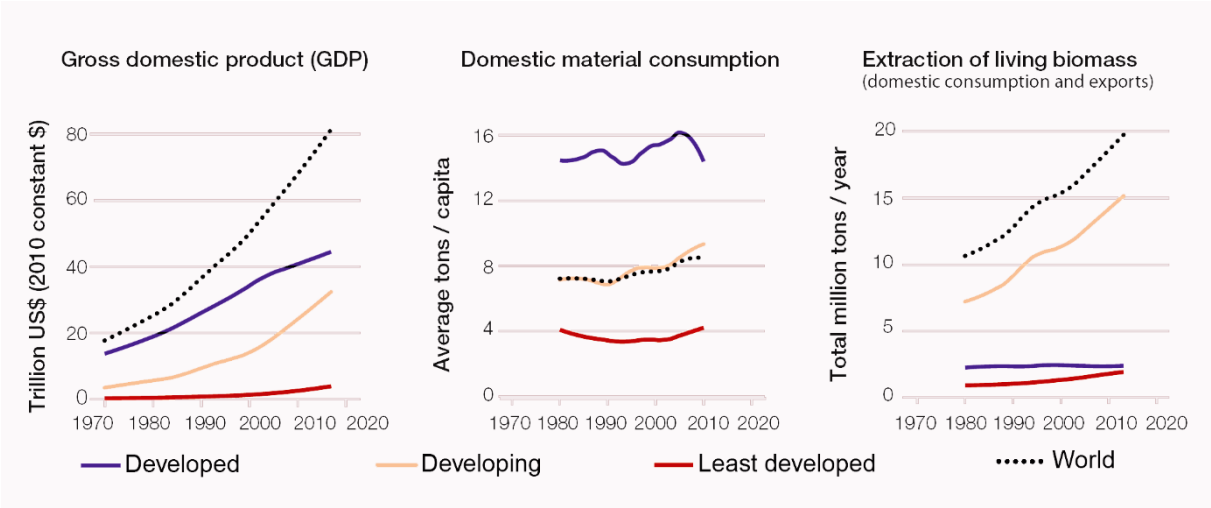

Figure 2. Changes of Gross domestic product (GDP), domestic material consumption, and extraction of living biomass (trillion USD at 2010 value) for groups of countries at distinct development levels. Adapted from IPBES (2019).

Diversity patterns are not homogeneous across the terrestrial, marine and freshwater realms (Dawson 2012). Although freshwater habitats only hold 0.01% of the global water volume and cover around 0.8% of the Earth's surface, they have a disproportionate species richness when compared with their terrestrial and marine counterparts (Collen et al 2014). For instance, they contain 40% of all fish species and a quarter of the global number of vertebrates (Dudgeon et al 2006). Freshwater habitats are, however, among the most threatened at the global level (Strayer & Dudgeon 2010; Vörösmarty et al 2010), given that they are rare, isolated by land and seawater, and generally located downhill of human settlements and therefore exposed to all kinds of runoff and human wastes (Strayer 2006). Freshwater water bodies have also been intensively modified for human purposes; for example, many rivers have been intubated, channelized and re-directed, and lakes dried or redesigned (Strayer & Dudgeon 2010). Water level, flow, and substrate have been exhaustively impacted by thousands of physical barriers such as dams, weirs, and floodgates, concrete embankments to prevent the overflow, and extraction of sands, gravels and other inert materials (Strayer 2006; Reid et al 2019). Therefore, freshwater habitats are suffering much higher biodiversity declines than those on marine or terrestrial realms (Dudgeon et al 2006; Reid et al 2019). This pattern can also be seen using the Living Planet Index developed by the World Wide Fund for Nature (WWF), where the freshwater species index dropped more sharply between 1970 and 2012 than the index for the marine or terrestrial populations (Fig. 3; WWF 2016).

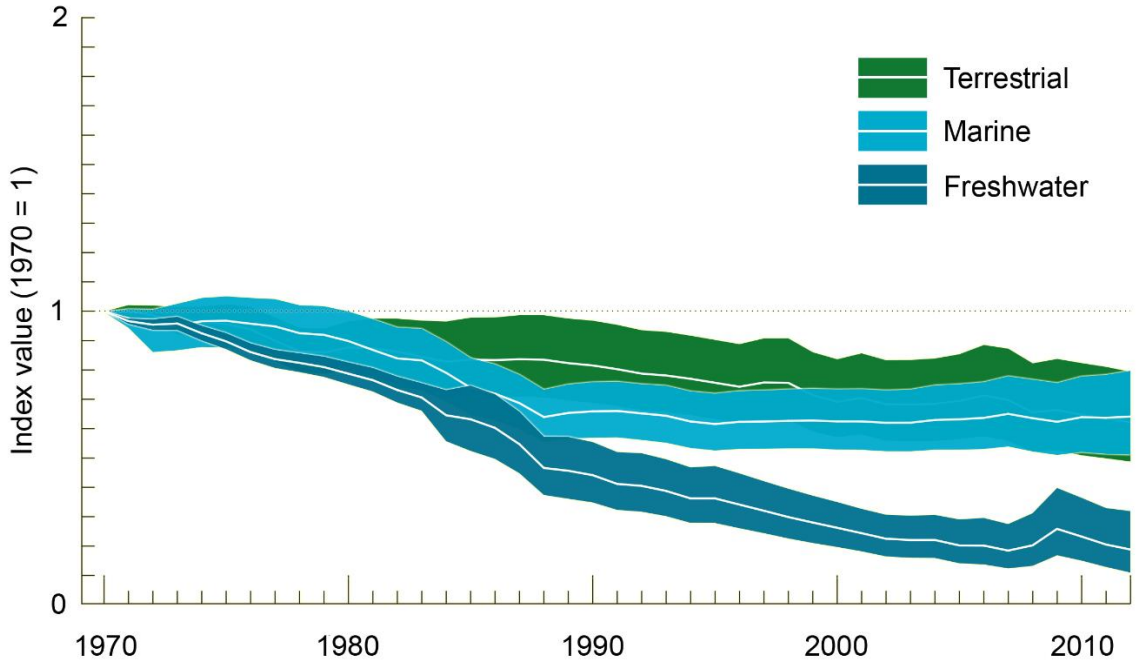

Figure 3. Evolution of the Living Planet Index (LPI) over the last decades. LPI is a measure of the state of the world's biological diversity based on population trends of species from terrestrial, freshwater and marine habitats. Adapted from WWF (2016).

1.2 Freshwater mussel diversity, importance, and conservation

Freshwater bivalves of the Unionida order, also known as Freshwater Mussels (FMs), freshwater clams or naiads, belong to an old (>200 Mya), big monophyletic group of molluscs that are strictly freshwater inhabitants and, for this reason, have a series of interesting adaptations to allow them to survive under constant flow (Strayer 2008; Haag 2012). Contrary to marine bivalves, FMs exhibit internal fertilization, parental care and, more interestingly, their specialized larvae (glochidia) need to attach to a host (mostly freshwater fish) for dispersion and nutrition until they metamorphose into juveniles and drop into the substrate (Graf & Cummings 2006; Barnhart 2008). Freshwater mussels play key ecological roles (e.g. water filtration, energy, and nutrient cycling, providing bioturbation or sediment mixing), and provide valuable ecosystem services to humans (e.g. increasing water transparency, source of protein, pearls and shell materials) (Howard & Cuffey 2006). This group of mussels has a wide distribution and they often dominate many freshwater habitats regarding both the number of individuals and biomass (Vaughn 2018). Unfortunately, FMs, like many other freshwater taxa, have suffered a massive global defaunation over the last centuries (Dirzo et al 2004; Lopes-Lima et al 2014) and are currently one of the most imperilled groups in the world (Fig. 4; Lydeard et al 2004; Ferreira-Rodriguez et al 2019).

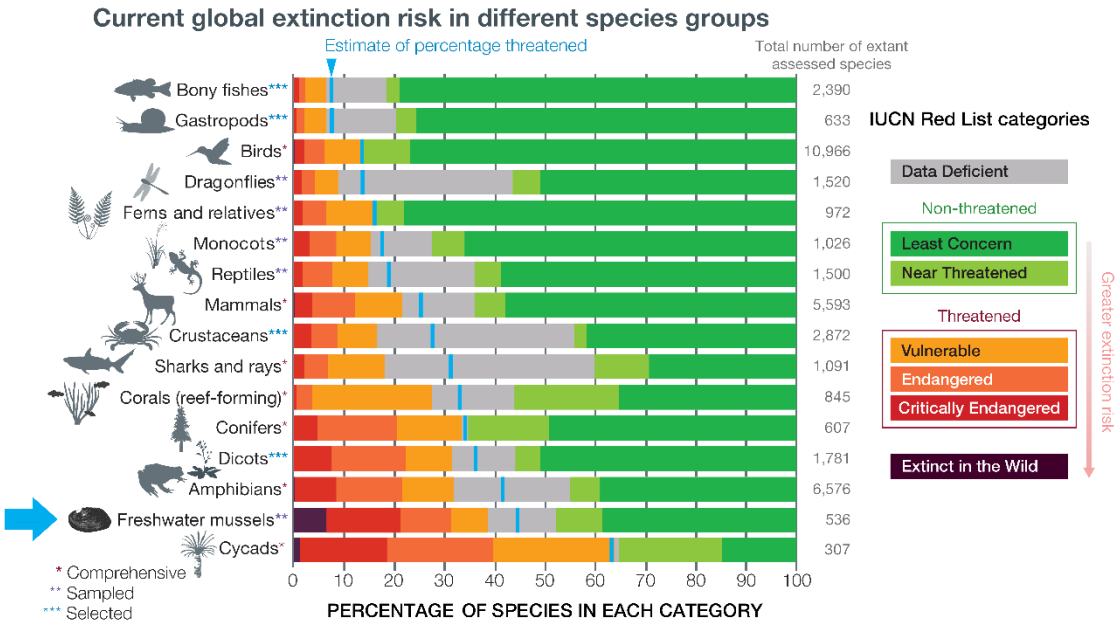

Figure 4. Current extinction risk in different species groups. Adapted from IPBES (2019).

This is further exacerbated by the fact that to complete their life cycle, these mussels are dependent on freshwater fish, which have also shown strong declines globally (Modesto et al 2017). Therefore, decline and extinction estimates of affiliate species such as FMs need to be recalibrated by taking the host species fluctuations and extinctions into account (Koh et al 2004). Based on the current IUCN Red List and if data deficient species are as threatened as non-data deficient species, 43% of all assessed FM species are currently threatened, with 13.2% being Critically Endangered and 6.3% already Extinct (Fig. 4; IUCN 2019). This situation has caused a substantial increase in research and conservation action dedicated to FMs since the emergence of ecological values during the 1970s (Haag 2012; Lopes-Lima et al 2014). These research and conservation efforts have been, however, concentrated in a handful of the more charismatic species (e.g., *Margaritifera margaritifera* and *Unio crassus* in Europe, and *Cumberlandia monodonta* in North America), and in more economically developed regions such as North America and Europe (Lopes-Lima et al 2014). Species in other parts of the world are still poorly known and their conservation status poorly evaluated (Lopes-Lima et al 2014; Ferreira-Rodriguez et al 2019).

Six families are currently recognized within Unionida based on morphological characters (Graf & Cummings 2007; Bogan 2008). Two of them are present in the northern hemisphere (Fig. 5): the Unionidae, which is by far the most speciose family of the order, with around 600 species, and the Margaritiferidae, which has a much lower species richness but with most species being at risk of extinction (IUCN 2019).

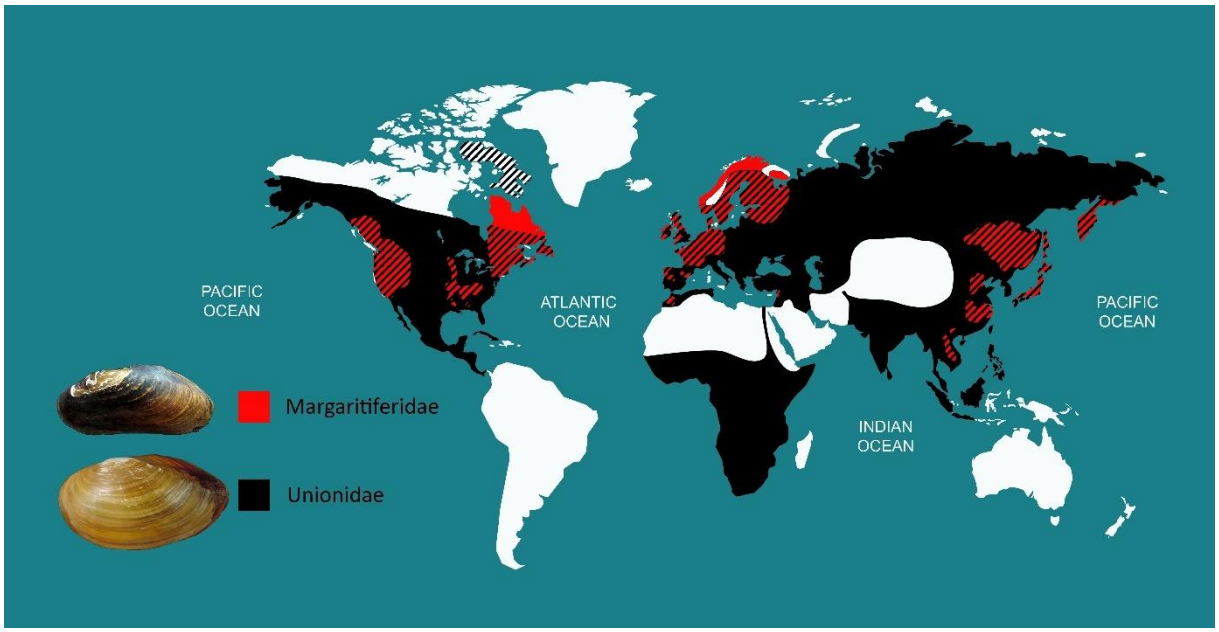

Figure 5. Global distribution map of the freshwater families Unionidae and Margaritiferidae. Adapted from Lopes-Lima et al (2017a, 2018).

Two of the families are present mainly in the southern hemisphere not crossing continental boundaries (Fig. 6), i.e. the Mulleriidae occurring in South America, and the Iridinidae in Africa.

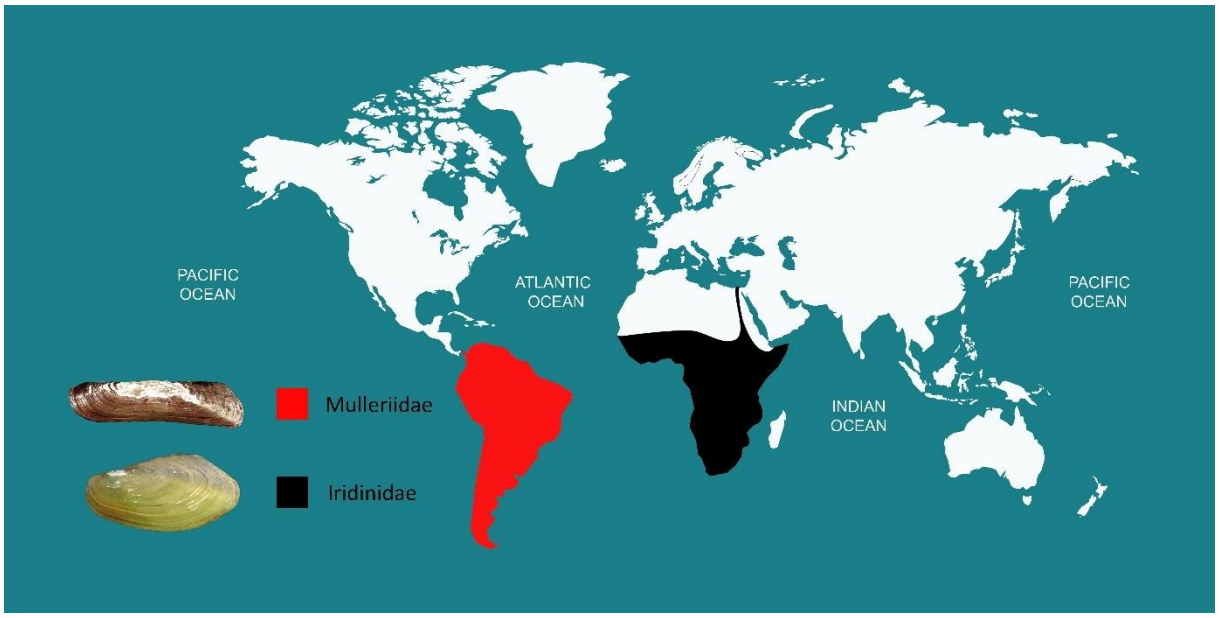

Figure 6. Global distribution map of the freshwater families Iridinidae and Mulleriidae. Adapted from Bogan (2008).

From the remaining two families, the Hyriidae is found in both South America and Australia, while the Etheriidae was originally thought to be composed by less than ten species in South America, Africa, and Asia, but due to recent revaluation is now considered restricted to Africa (Fig. 7; Bogan 2008).

Figure 7. Global distribution map of the freshwater families Hyriidae and Etheriidae. Adapted from Bogan (2008).

Although several phylogenetic studies have been developed over the last decades (e.g., Bogan & Hoeh 2000; Graf & Cummings 2006, 2007; Whelan et al 2011), the phylogeny within the order is far from stabilized and limited phylogenetic consensus has emerged, especially regarding to the early evolution of the Unionida (Graf 2013). Phylogenetic patterns within the families are also poorly understood due to the lack of sequenced taxa and limited robustness of available phylogenetic analyses (Huff et al 2004; Whelan et al 2011).

1.3 The need for baseline biological research

Accurate conservation status assessment and effective conservation actions require a profound knowledge about their target taxa and/or habitats (Lopes-Lima et al 2017b). However, baseline ecological and physiological data on most FM species is still scarce (Kindsvater et al 2018). Some features are almost unknown across all taxa, such as dispersal rates of larvae (hitchhiking on fish) or adults, and the mean and maximum distances a male can fertilize a female (Strayer et al 2004; Lopes-Lima et al 2017b). Other data are only available for a small number of species or populations and generally on a small-time scale, such as data on the distribution, population size, structure and trends, and demography (e.g. recruitment, mortality, and migration) (Ferreira-Rodriguez et al 2019). Also, life-history traits like lifespan, age at sexual maturity, reproduction stage timing, and fertility are poorly known, and many times wrongly extrapolated from data on better-known species, such as *Margaritifera margaritifera* in Europe or *Elliptio complanata* in North America (Lopes-Lima et

al 2014). Additionally, more extrinsic factors like the identification and availability of the fish host range, the main habitat requirements, and the sensitivity and responses to environmental stressors like habitat degradation and pollution, are requiring urgent research (Modestro et al 2017; Ferreira Rodriguez et al 2019). This lack of baseline biological data is hindering conservation efforts. For instance, the lack of knowledge on habitat requirements and sensitivity to habitat degradation does not allow us to understand how riverine or lacustrine habitats should be accurately rehabilitated to improve the status of FM populations. Also, the lack of knowledge on traits involved in the reproductive cycle, such as reproduction timing (fertilization, spawning, and larvae discharge), age of maturity, fertility, and host fish usage slows down the implementation of captive propagation programs, necessary for reintroduction and reinforcement of depleted populations (Patterson et al 2018). Species' baseline data are not only important for species-focused conservation and research. Meta-analyses and modelling studies on a wide spatial scale, depend on this type of data for designing bioregions, prioritizing areas for conservation and evaluating threats and other environmental factors affecting taxonomic groups, species assemblages and entire ecosystems (Kindsvater et al 2018).

1.4 Defining species boundaries and integrating phylogenetic diversity patterns in conservation ranking

Given that resources dedicated to conservation are limited, a careful selection of conservation targets is required (Moilanen & Arponen 2011). Species are globally considered the essential conservation units by policies and conservation status assessments (Fitzpatrick et al 2015). Therefore, it is crucial to thoroughly define species boundaries, which is not always an easy task, especially in FMs (Chong et al 2016; Inoue et al 2018). FMs' species delineation is many times difficult due to the lack of clear morphological diagnostic characters, high shell plasticity and morphological convergence among related species (Froufe et al 2016). For this reason, molecular analyses have been increasingly used to define species boundaries in these organisms (e.g. Chong et al 2016; Pfeiffer et al 2016; Inoue et al 2018). However, the molecular delimitation of cryptic species is fundamentally questioned by the continuous and dynamic nature of speciation (Chenuil et al 2019). There have been multiple ways and concepts on how to define species (revised in De Queiroz 2007) but, since the last decades of the 20th century, we have mostly used a reproductive isolation approach or the biological species concept (Mayr 1982), which is not always easy to demonstrate. However, many scientists are now increasingly using the monophyletic isolated lineages approach supported by multiple arguments or the unified species concept (De Queiroz 2005, 2007) which is easier to

characterize and easier to validate molecularly. Over the last decade, several analytical approaches on DNA sequences have been developed to identify species and define species boundaries (Luo et al 2018). Many of them relied on a standardized segment of the mitochondrial genome (a region of the cytochrome c oxidase subunit I, COI with around 600 nucleotides) (Hubert & Hanner 2015). The choice of COI over other mitochondrial or nuclear markers as the main molecular tag or barcode for each species, is due to several reasons: it has a high discrimination resolution, is generally easy to amplify even from small amounts or from degraded tissue, and standard protocols are available for amplification in a wide range of taxa (Hebert et al 2003). More recently, methods applied to coalescent trees are being increasingly applied not only to COI or single genes, but to multiple nuclear and mitochondrial markers (Luo et al 2018). The identification of these molecular operational taxonomic units or MOTUs allows scientists to identify and investigate hidden cryptic diversity and for species recognition to advance faster than in classical morphological approaches (Kekkonen & Hebert 2014).

Conservation targets and prioritization should also include the evolutionary history captured by specific sets of species or higher taxa, i.e. their phylogenetic diversity patterns (Winter et al 2013). For example, phylogenetically unique taxa are generally of higher priority for conservation; furthermore, metrics have already been developed to include phylogenetic diversity in species rankings for conservation attention, such as the Evolutionarily Distinct and Globally Endangered (EDGE) program developed by the London Zoological Society (Redding & Mooers 2006; Isaac et al 2007).

1.5 Objectives

The overall goal of this dissertation is to advance the conservation biology of freshwater mussels by combining research on phylogeny, systematics and ecology, showing how the integration of multiple research fields has practical implications to preserve highly endangered taxa. The specific objectives of this thesis are:

1. **To highlight the biodiversity hotspots of freshwater bivalves**, mapping the global species diversity and conservation status in the main freshwater bivalve ecoregions.

2. **To reveal the main gaps of knowledge, conservation and research needs of FMs**, using data from the IUCN Red List database.

3. **To show the importance of basic biological studies for conservation of freshwater mussels and environmental monitoring**, using a case study on several physiological and ecological traits of a poorly known Iberian endemic species, i.e. *Unio delphinus*.

4. **To demonstrate the importance of systematics and phylogenetic diversity for the conservation of freshwater mussels**, through a series of phylogenetic studies on several groups within the Unionida order.

5. **To test the use of molecular data to define important freshwater mussel taxa for conservation**, estimating the phylogenetic patterns and potential molecular operational taxonomic units.

6. **To test the use of mitochondrial genome arrangements as a diagnostic for freshwater mussel groups**, sequencing and assembling whole mitogenomes of selected species and mapping the retrieved gene arrangements in whole genome phylogenies.

7. To use information collected during the dissertation **to provide recommendations for freshwater mussel conservation**, at national, European and global scales.

1.6 Thesis structure

The dissertation was organised in nine chapters, which together address the general and specific objectives of the thesis listed in the previous section. The dissertation includes a general Introduction (**Chapter 1**) and a General Discussion (**Chapter 9**), together with seven chapters that correspond to seven scientific papers already published in international peer-reviewed journals. **Chapter 2** presents a review on the diversity, conservation status and knowledge gaps. Two of these main gaps are the lack of knowledge about basic biological traits and systematics clarification that are then addressed in **Chapter 3** and **Chapters 4-8**, respectively. Below, the main contents of each chapter included in the thesis are summarised.

Chapter 1 is an introduction to the main issues related to the dissertation theme. It starts by describing the global decline of biodiversity and then focus on the target taxonomic group of the dissertation – the freshwater mussels (FM) –, discussing its ecological and economic importance, but also its threatened status and conservation concern. Subsequently, the importance of basic biological research, taxonomy, and phylogenetic patterns for conservation are highlighted.

Chapter 2 addresses Objectives 1 and 2 by presenting a revision of the global diversity patterns of freshwater bivalves and their conservation status, threats, and research needs. Based on the results obtained, future paths for freshwater bivalve research are then suggested.

Chapter 3 addresses Objective 3 highlighting the importance of basic biological studies for conservation planning and the potential use of biological traits as environmental indicators. As a case study, the distribution, growth patterns, reproductive cycle, and host fish range of an Iberian endemic FM, the dolphin freshwater mussel *Unio delphinus* are presented.

Chapter 4 addresses Objective 4, providing a comprehensive, two marker (COI - cytochrome oxidase subunit I and the 28S ribosomal RNA) phylogeny of the most diverse Unionida family, the Unionidae. This phylogeny is complemented with distribution, morphological, anatomical and behavioural data to revise the systematics of the family.

Chapter 5 addresses Objectives 4 and 5, presenting single and two-marker (mtDNA COI - cytochrome c oxidase subunit I and ND1 - NADH dehydrogenase subunit 1) phylogenies of an imperilled group of North American Unionidae species that have historically been placed under a single genus, i.e. the genus *Quadrula*. Several molecular species delineation methods were used to define molecular operational taxonomic units (MOTUs) that were then tested with an integrative approach including ecological, behavioural and morphometric (Fourier Shape) analyses, and geographic distribution data.

Chapter 6 addresses Objective 4, revising the systematics of the whole Margaritiferidae family with five-marker (two mitochondrial: 16S ribosomal RNA and COI cytochrome c oxidase subunit I, and three nuclear 18S ribosomal RNA, 28S ribosomal RNA, and the H3 Histone 3 gene) phylogenies, coupled with morphological and ecological information. The distribution, potential origin and main biogeographic patterns of the family are also described.

Chapter 7 addresses Objective 4 and 6, providing newly sequenced female (F-) and male (M-) lineage whole mitochondrial genomes and a whole mitogenome phylogeny. In this chapter, we present the first published (M-) genome for the Margaritiferidae, revealing that the gene arrangements of both (F-) and (M-) type mitogenomes are unique within the order Unionida and can be used as molecular diagnostic characters for the family Margaritiferidae.

Chapter 8 addresses Objective 4 and 6, presenting a comprehensive phylogeny of the Unionidae using whole mitochondrial genomes. Each distinct mitogenome gene arrangement is mapped and dated, with most gene rearrangements coinciding with major extinction events. It is suggested that these evolutionary changes might be related to the pulsed-evolution theory. These phylogenetic results were then combined with an ancestral area reconstruction to describe the early diversification patterns and biogeography of the Unionidae. A new

systematics framework for the classification of the Unionidae was also proposed using the mitogenome phylogenetic results.

Chapter 9, presents the main conclusions of the dissertation, discussing the main findings, highlighting the major shortcomings and caveats of the studies, and proposing future pathways for research and conservation.

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CHAPTER 2

Freshwater bivalves' conservation, diversity, and research

Paper I

Conservation of freshwater bivalves at the global scale: diversity, threats and research needs.

Lopes-Lima M, Burlakova LE, Karatayev AY, Mehler K, Seddon M, Sousa R

Article published in *Hydrobiologia* **810**, 1-14 (2018).
DOI: 10.1007/s10750-017-3486-7

Conservation of freshwater bivalves at the global scale: diversity, threats and research needs

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Abstract

Bivalves are ubiquitous members of freshwater ecosystems and responsible for important functions and services. The present paper revises freshwater bivalve diversity, conservation status and threats at the global scale and discusses future research needs and management actions. The diversity patterns are uneven across the globe with hotspots in the interior basin in the United States of America (USA), Central America, Indian subcontinent and Southeast Asia. Freshwater bivalves are affected by multiple threats that vary across the globe; however, pollution and natural system (habitat) modifications being consistently found as the most impacting. Freshwater bivalves are among the most threatened groups in the world with 40% of the species being near threatened, threatened or extinct, and among them the order Unionida is the most endangered. We suggest that global cooperation between scientists, managers, politicians and the general public, and application of new technologies (new generation sequencing and remote sensing, among others) will strengthen the quality of studies on the natural history and conservation of freshwater bivalves. Finally, we introduce the articles published in this special issue of Hydrobiologia under the scope of the Second International Meeting on Biology and Conservation of Freshwater Bivalves held in 2015 in Buffalo, New York, USA.

Keywords

Bivalvia, Unionida, Venerida, IUCN Red List, Freshwater mussels, Conservation

Introduction

Freshwater ecosystems are among the most threatened on the planet facing unprecedented pressures related to the increase of human population and socioeconomic development (Dudgeon et al 2006; Vörösmarty et al 2010). Increasing anthropogenic pressure worldwide results in habitat loss, habitat modification and fragmentation, overexploitation of natural resources (including water), pollution, the introduction of invasive alien species (IAS) and climate change (Malmqvist & Rundle 2002; Strayer & Dudgeon 2010). The biodiversity crisis is one of the major consequences of steeply rising human demands, and among the animals with high extinction rates are freshwater bivalves (FBs) (Lydeard et al 2004; Strayer et al 2004; Régnier et al 2009; Lopes-Lima et al 2014, 2017a). The future survival of FBs is highly impaired and considering the large suite of ecosystem services they provide (Vaughn 2017) scientists, managers, politicians, and the general public need to strengthen their cooperation to conserve these species.

Whereas over the last years multiple studies have been published concerning the biology, ecology, and conservation of FBs, most of them were carried out in North America and Europe (Lopes-Lima et al 2014). Consequently, a great ignorance about basic aspects (e.g. distribution, diversity, abundance, population structure, and life cycle) concerning species inhabiting South America, Africa, and Asia persists and much more information is needed for these continents.

In the present paper, we compile data on FB diversity patterns, conservation status and threats from the International Union for Conservation of Nature (IUCN) database using a species list adapted from Graf & Cummings (2017) and mapped them in ecoregions adapted from Graf & Cummings (2007) and Haag (2010). We also briefly discuss research needs and urgent management actions that may help conserve these animals and introduce the articles published in this special issue resulting from the Second International Meeting on Biology and Conservation of Freshwater Bivalves held in 2015 in Buffalo, United States of America (USA).

Diversity patterns at the global scale

Freshwater bivalves are a polyphyletic group of animals restricted to freshwaters with a little over 1,200 described species (Bogan 2008; Bogan & Roe 2008; Graf 2013). The main core of the group (99%) is composed of freshwater mussels of the order Unionida (strictly freshwater) (72%) and species belonging to 7 families within the order Venerida (27%) (Fig. 1). The Venerida are composed mainly of families comprising 94% of the species the pea- or fingernail-clams Sphaeriidae (67%) and the Cyrenidae (27%), which include, for example, the invasive Asian clam *Corbicula fluminea* (Müller, 1774). The family Dreissenidae family (3%), well-known

to contain important invasive alien species (IAS) such as the quagga mussel *Dreissena bugensis* Andrusov, 1897 and the zebra mussel *Dreissena polymorpha* (Pallas, 1771), is also included in the order Venerida. The remaining handful of FB species are scattered among other essentially marine orders or families within the order Venerida (Fig. 1).

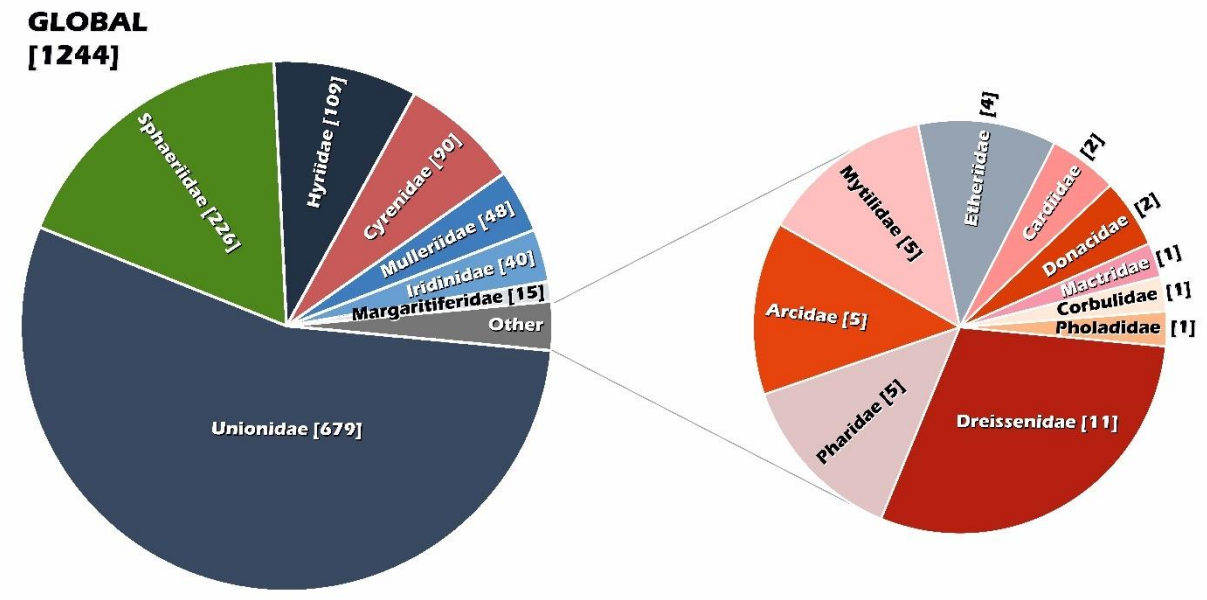

Figure 1 Global diversity of freshwater bivalves divided by families. The total number of species in brackets.

Freshwater bivalves are present in all continents except in glaciated (except few sphaeriid species) and desert areas, but the diversity patterns are not evenly distributed (Fig. 2). The diversity is higher in the Nearctic (NA), Neotropics (NT) and Indotropics (IN) with ≈25% species being found in each ecoregion. The Palaearctic (PA) and Afrotropics (AF) have a lower diversity (≈10%) with Australasia (AU) being the poorest ecoregion (≈5%) (Fig. 2A). There are also distinct distribution patterns across the main taxonomic groups. The Unionida is similar to the general pattern for all FBs, with 33% of the species inhabiting the NA and 6% inhabiting the PA (Fig. 2B). The distribution of pea clams is completely distinct with the hotspots of diversity being the NT (31%) and the PA (22%), while the remaining diversity is scattered among the other continents (Fig. 2C). Sphaeriids are also the only FB species that can live at the higher latitudes of the Arctic, such as the islands of Iceland, Greenland, Baffin, Svalbard and Novaya Zemlya (Schiøtte & Warén 1992; Bespalaya et al 2017). Finally, for Cyrenidae and a few other remaining species, the major diversity hotspot is in the IN that contains almost 70% of such species, followed by the PA (18%), while other ecoregions have a much lower diversity (Fig. 2D).

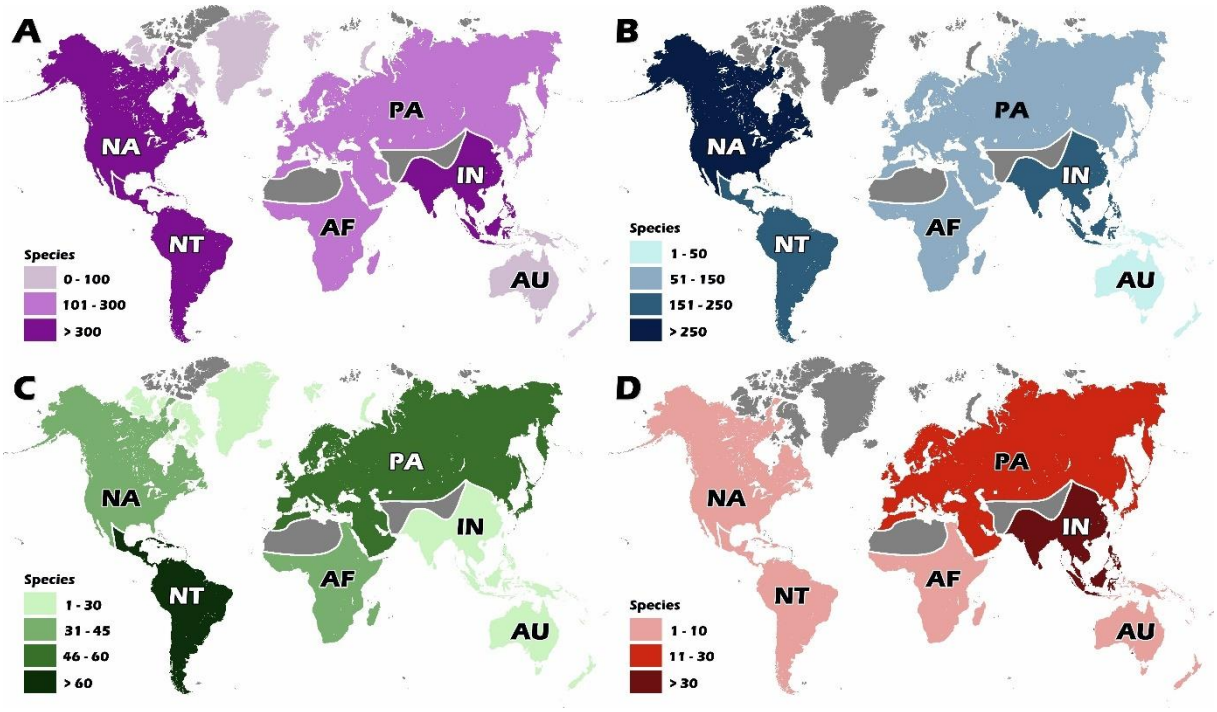

Figure 2 Diversity by ecoregions. **A** All freshwater bivalves; **B** Unionida; **C** Sphaeriidae; **D** Cyrenidae + remaining freshwater bivalve groups. Ecoregions adapted from Graf & Cummings (2007) and Haag (2010): NA Nearctic, NT Neotropical, PA Palaearctic, AF Afrotropical, IN Indotropical, AU Australasian. Glaciated and desert areas void of mussels in grey.

Diversity at an ecoregion scale is also not distributed evenly (Fig. 3). Within NA, the species diversity is generally higher in the interior basins, while in the NT the diversity is higher in Central America and the Orinoco, Amazon and Paraguay River basins (Fig. 3A). In the AF, the Congo River basin is richer in Unionida species (Fig. 3B), and the Nile and Eastern African River basins have a higher sphaeriid diversity (Fig. 3C). While the Western Palaearctic is quite diverse in sphaeriids and dreissenids, Laurasia has a higher diversity in the IN, from the Hindu to the Amur River basin (Figs. 3A, C and D). Within IN, the diversity of sphaeriids is higher in the Indian subcontinent, while in the Unionida and the remaining groups the diversity is higher in Indochina and Sundaland (Fig. 3). In AU, a higher number of species is found in the East (Fig. 3).

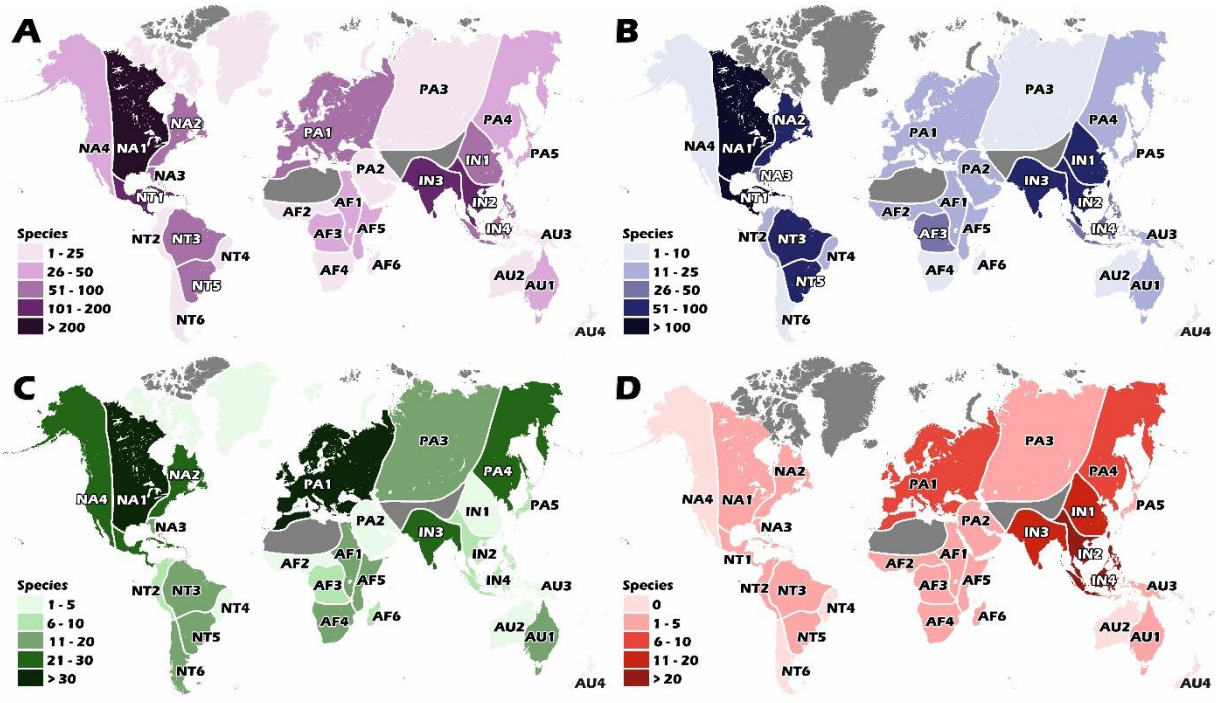

Figure 3 Diversity by ecoregions. **A** All freshwater bivalves; **B** Unionida; **C** Sphaeriidae; **D** Cyrenidae + remaining freshwater bivalve groups. Ecoregion subdivisions adapted from Graf & Cummings (2007) and Haag (2010): NA Nearctic, NT Neotropical, PA Palaearctic, AF Afrotropical, IN Indotropical, AU Australasian. Glaciated and desert areas lacking FBs are in grey.

Although specific diversity of FBs is similar in NA, NT and IN, there is a higher taxonomic diversity in the IN than in NA and NT. In the IN there are representative species of 5 orders and 10 families compared to the 2 orders and 4 families in the NA and 3 orders and 8 families in the NT (Fig. 4). Even within the most species-rich FB family, the Unionidae, the IN exhibits a much higher taxonomic diversity than all the other ecoregions, with representatives of all subfamilies of Unionidae occurring there, except for the NA Ambleminae.

We would like to stress that diversity patterns described above may be underestimated and may change substantially as a result of ongoing and future surveys in the less studied regions of Southeast Asia, Africa, NT, and AU. For example, Bolotov et al (2017) studying the FBs of a poorly known and remote basin (Sittaung) in Myanmar described two new genera and seven new species. Also, even in Europe and NA, which are the most well-studied regions, the knowledge of the diversity of Unionida is still undergoing considerable changes (e.g. Froufe et al 2016a,b, 2017; Araujo et al 2017; Lopes-Lima et al 2017a; Williams et al 2017; Smith et al 2018).

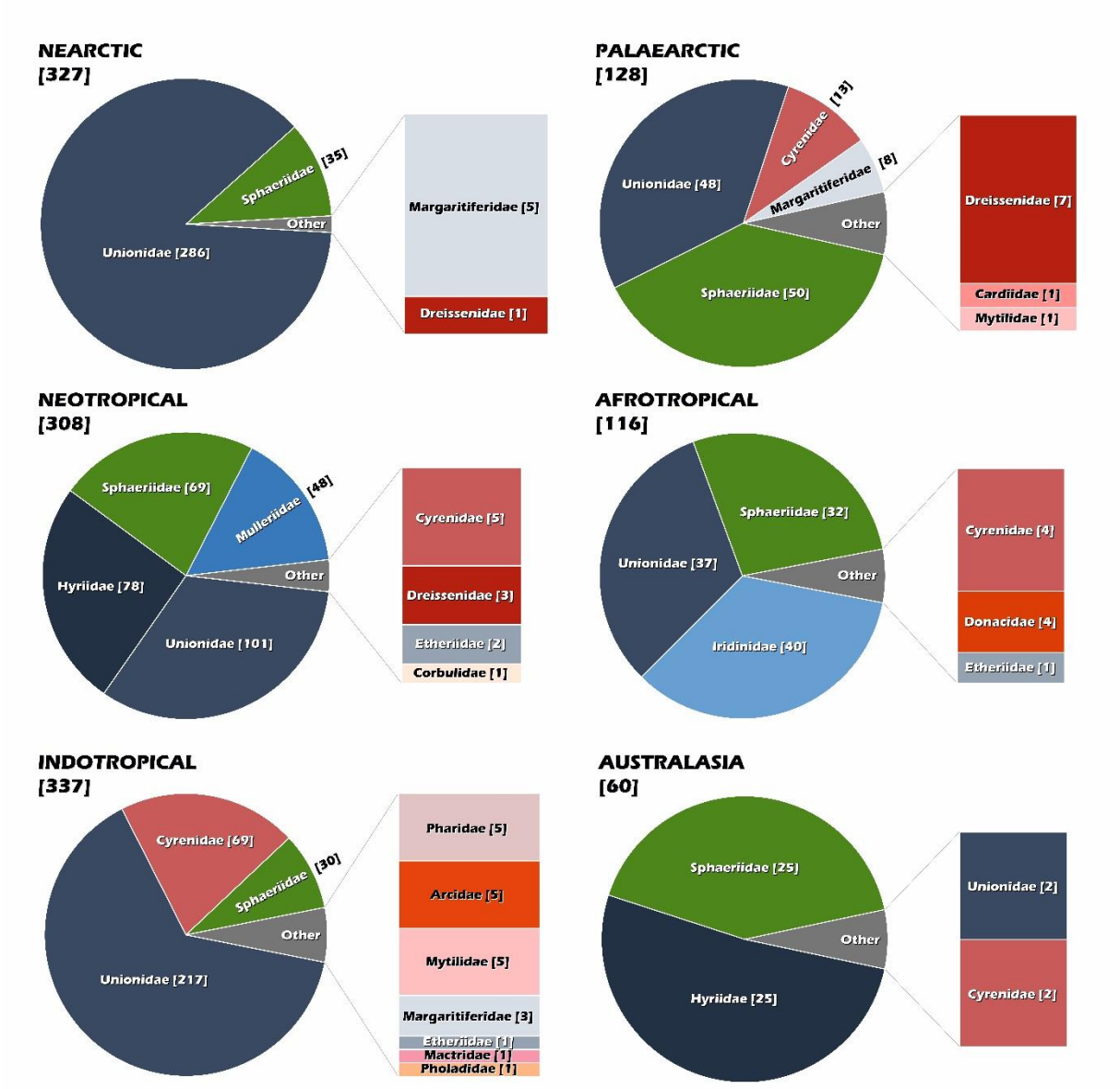

Figure 4 Taxonomic composition and diversity of freshwater bivalves in each ecoregion. The total number of species in brackets.

Conservation status and major threats

Freshwater bivalves are among the most threatened taxonomic groups in the world, with almost 40% of the species being near threatened, threatened or extinct (Fig. 5). However, this high imperilment is mainly due to the contribution of Unionida since not all groups are evenly threatened or assessed for conservation status (Fig. 5, top). Based on the number of assessed species, the highest percentage (45%) of near-threatened, threatened and extinct species (including 25 [2.8%] extinct or probably extinct species) is in Unionida, while only 14.5% of Sphaeriidae and 8.8% of Cyrenidae (plus all the other remaining species) have a near-threatened or threatened status (Fig. 5, top part). However, IUCN assessments are not evenly distributed across taxa and countries and FBs are a good example of this situation (Fig. 5).

Thus, a higher percentage of large and more conspicuous unionids has been assessed compared to other FB groups (Fig. 5, top). Some ecoregions (e.g. NA, AF, PA and IN) have a high percentage of species evaluated, while species from AU and especially NT have a very low Red List coverage (Fig. 5, bottom part). The percentage of threatened and near-threatened Unionida species is higher in NA (67%) and PA (52%) than in other ecoregions, with the lowest percentage (19%) in the IN (Fig. 5, bottom). This does not necessarily mean that fewer species are threatened in the IN, since this ecoregion has a much higher percentage of data deficient species, reflecting the lower level of knowledge and data on the threats available for IN species. On the other hand, almost half of the species have been assessed as of ‘‘least concern’’ in the AF, which might indicate a more favourable status of freshwater mussels in this ecoregion.

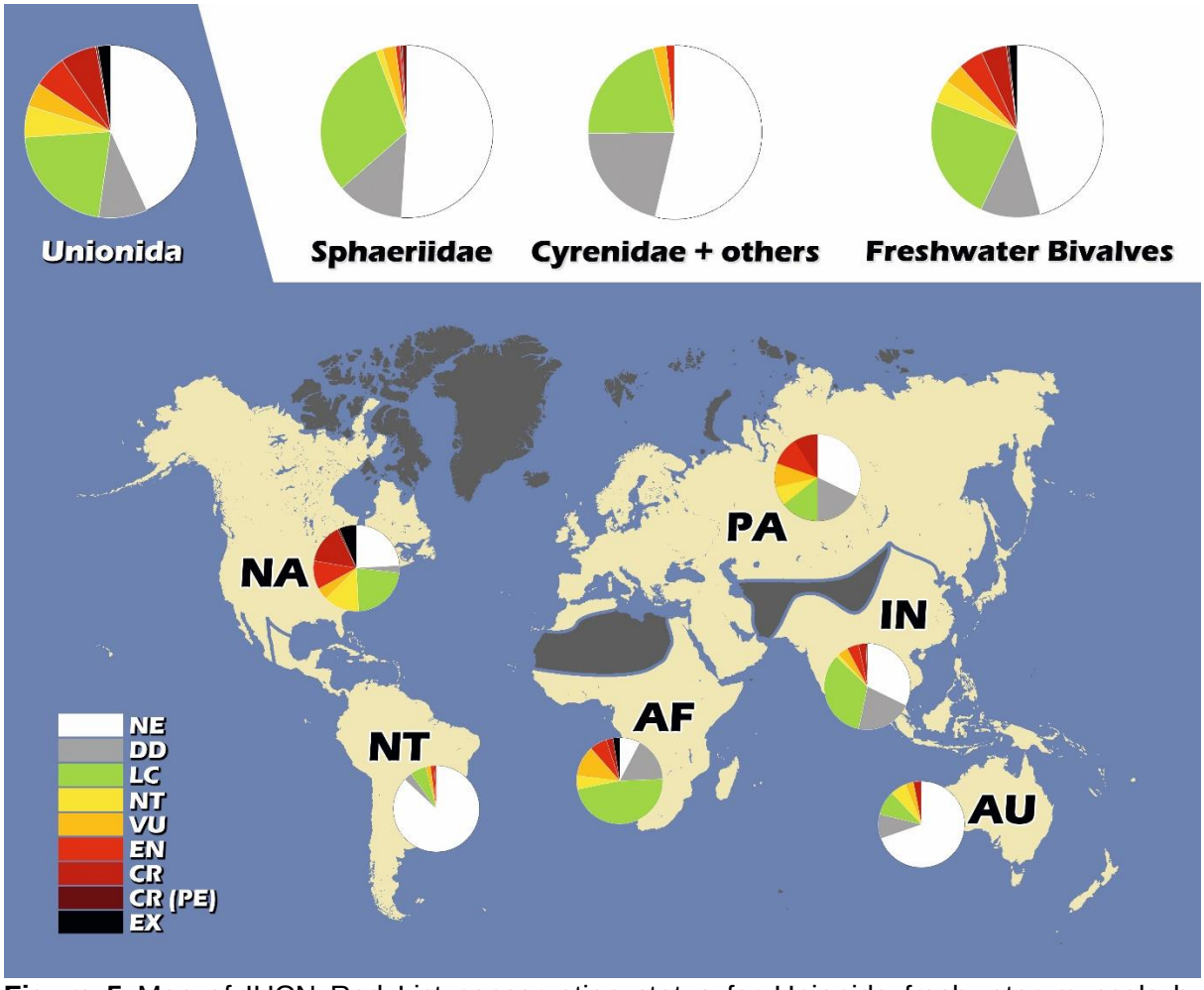

Figure 5 Map of IUCN Red List conservation status for Unionida freshwater mussels by ecoregions (bottom of the figure) and global conservation status for freshwater bivalves and each major freshwater bivalve group (top of the figure). Ecoregion subdivisions adapted from Graf & Cummings (2007) and Haag (2010): *NA* Nearctic, *NT* Neotropical, *PA* Palaearctic, *AF* Afrotropical, *IN* Indotropical, *AU* Australasian. On the scale bar: *NE* Not evaluated by the IUCN Red List; and the IUCN Red List categories: *DD* data deficient, *LC* least concern, *NT* near threatened, *VU* vulnerable, *EN* endangered, *CR* critically endangered, *CR* (*PE*) critically endangered probably extinct, *EX* extinct.

The IUCN Red List assessments are based on a set of five criteria: (A) population size reduction, (B) small geographic range, (C) small population size plus decline, (D) very small or restricted populations and (E) a quantitative analysis of extinction probability (IUCN 2012). Most of the near-threatened and threatened FB species have been assessed using criteria A and B and to a much lesser extent using criteria C and D (Fig. 6). Since criterion E needs comprehensive data on a wide range of features (e.g. demography, life history, habitat requirements, threats and management options), no FB species was ever evaluated using this criterion (Fig. 6). Most FB species have been assessed based on their population size reduction and geographic range contraction compared to a few species with very small distribution ranges. It is difficult to assign a threatened status using criterion D for most FB species due to their generally large distribution ranges.

The global pattern is similar in all ecoregions, except the NT and AF (Fig. 6). While the NT pattern may not be very representative of the ecoregion due to the few assessed species, in AF it reflects the poor knowledge about the population size and trends. This is due to the lack of research that is being done in the AF, where survey and monitoring studies are almost non-existent (Lopes-Lima et al 2014; Sousa et al 2016, 2017).

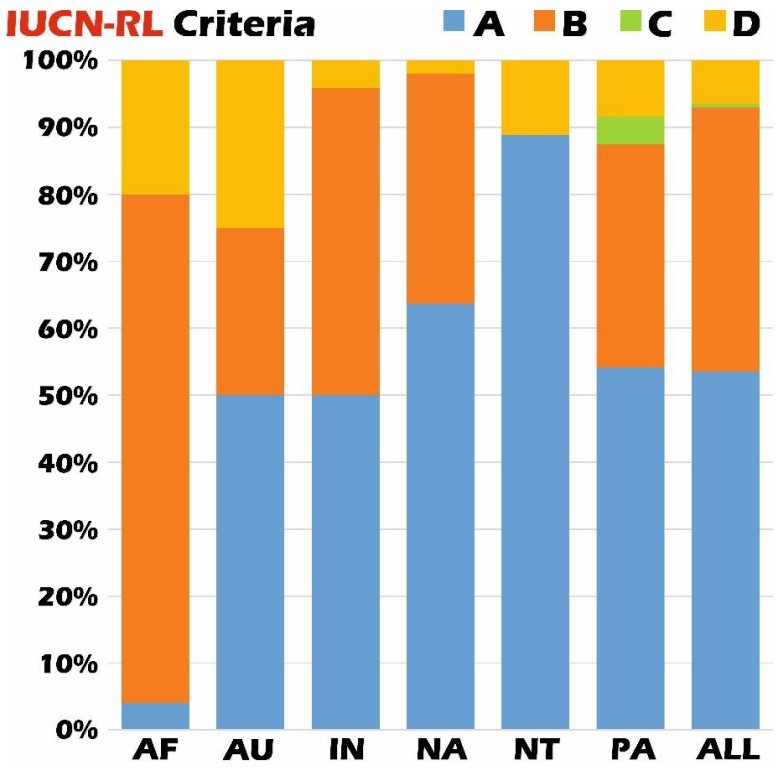

Figure 6 IUCN Red List criteria used for the assessment of freshwater bivalve species by ecoregions. Ecoregion subdivisions adapted from Graf & Cummings (2007) and Haag (2010): *AF* Afrotropical, *AU* Australasian, *IN* Indotropical, *NA* Nearctic, *NT* Neotropical, *PA* Palaearctic. IUCN Red List criteria: A population size reduction, B geographic range, C small population size and decline, D very small or restricted populations.

Freshwater bivalves are affected by multiple threats that range from natural system modifications to degradation, pollution, introduction of IAS, exploitation and human disturbance. Within the assessed FB species for the IUCN Red List, pollution is still the most recorded global threat comprising 42% of all threats (Fig. 7). Natural system (habitat) modifications such as the construction of dams and channels are the second most cited threat (20%), followed by urban development, exploitation, agriculture, climate change, mining, and IAS, together representing less than 10%. Other disturbances such as transport, recreational activities and geological events only play a minor role.

The relative percentage of recorded threats is generally similar across the main ecoregions with a few notable exceptions. For instance, in the NA and PA species seem to be less threatened by climate change than the tropical and southern hemisphere ecoregions. Conversely, in the more developed areas of the NA and the PA, habitat modifications seem to negatively affect more species in these ecoregions than in the AF and IN. Exploitation is a much more detrimental threat in the IN than elsewhere (Fig. 7). Harvesting of mussels for human consumption in East and Southeast Asia is a major economic activity; for example, in Vietnam, it may reach up to 50,000 tons per year in each major basin (Köhler et al 2012). Furthermore, the ratio of agriculture-related threats is higher in AU and PA, mainly due to water diversion and extraction.

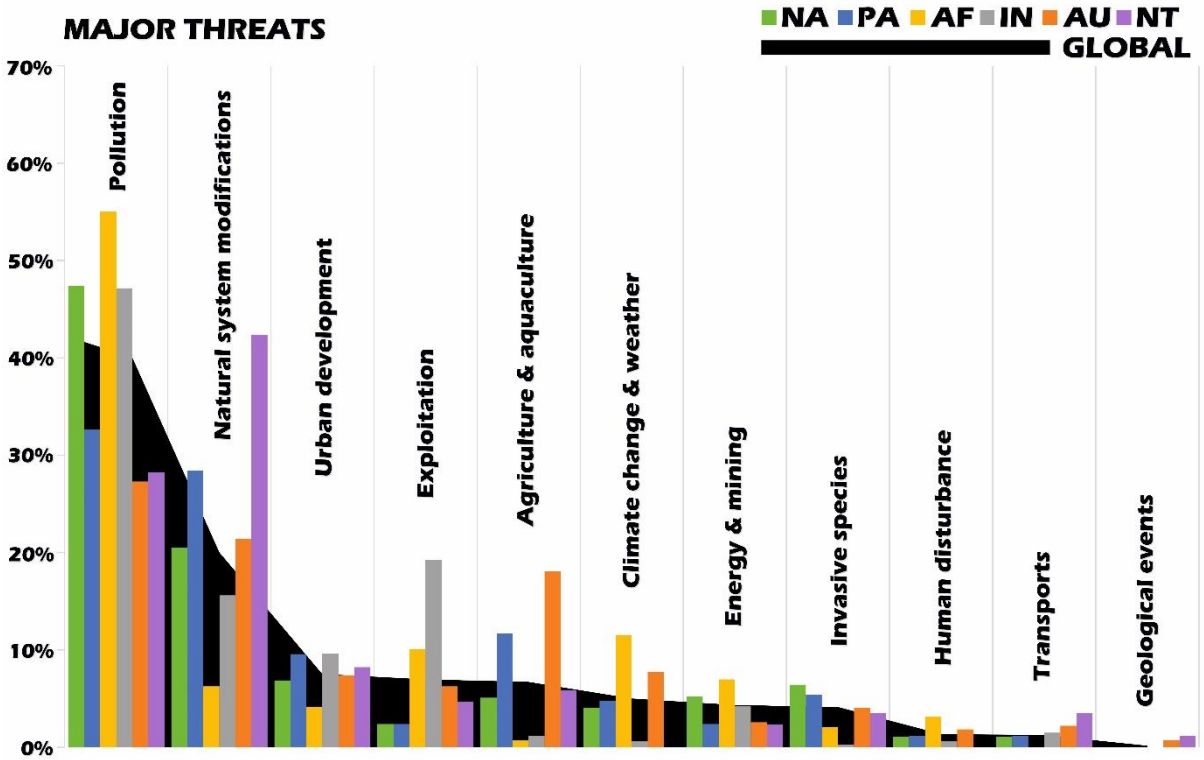

Figure 7 Main threats for freshwater bivalves recorded from the IUCN Red List database by ecoregions. Ecoregion subdivisions were adapted from Graf & Cummings (2007) and Haag (2010): *NA* Nearctic, *NT* Neotropical, *PA* Palaearctic, *AF* Afrotropical, *IN* Indotropical, *AU* Australasian.

Research and conservation actions needs

Many species of FB are still poorly understood, especially in Central America, Southeast Asia and Sundaland (Lopes-Lima et al 2014, 2017b). This lack of knowledge hampers their status assessment.

The IUCN database indicates that research needs are generally lower for the NA and PA compared to the other ecoregions, especially for the three top research needs, i.e. population size and distribution, identification of threats and life history (Fig. 8A). This may be explained by the stronger research effort and higher financial support available for North American and European studies. However, even in these ecoregions, basic data on distribution, population size, accurate identification of threats and basic life-history traits are still lacking for many species. Taxonomical data and knowledge on life-history traits are particularly needed for AF species. The same general trends in research needs can be seen for all species assessed by IUCN as well as for data-deficient species (Fig. 8B).

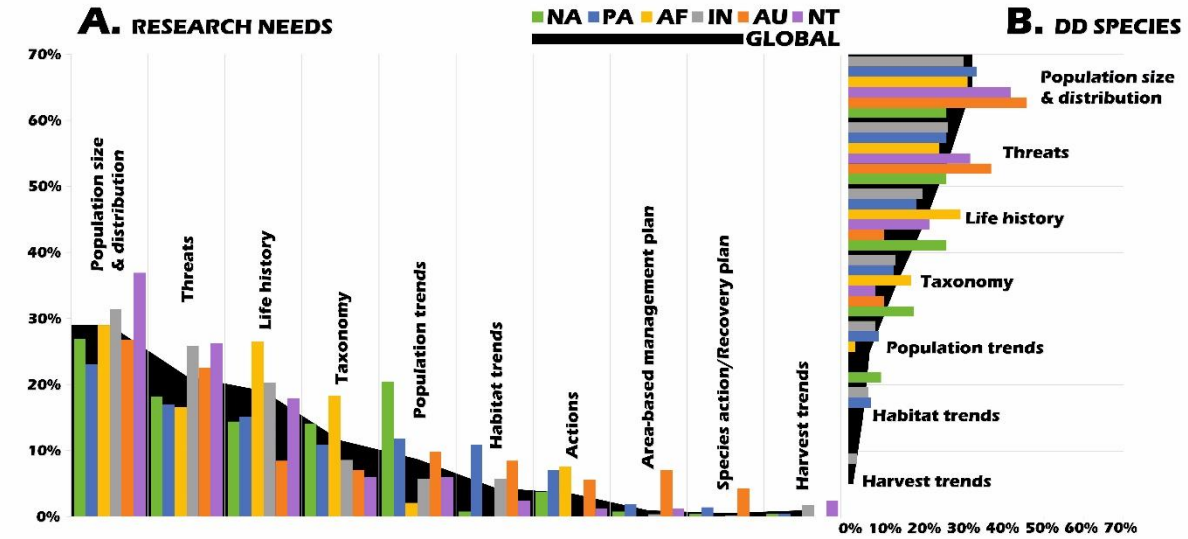

Figure 8 Research needs for freshwater bivalves recorded from the IUCN Red List database by ecoregions. **A** All assessed species in the IUCN Red List; **B** data-deficient species in the IUCN Red List. Ecoregion subdivisions adapted from Graf & Cummings (2007) and Haag (2010): *NA* Nearctic, *NT* Neotropical, *PA* Palaearctic, *AF* Afrotropical, *IN* Indotropical, *AU* Australasian. *DD* data-deficient species in the IUCN Red List.

Due to the high risk of extinction, many species urgently need worldwide conservation actions. Land and water protection was found to be the top conservation measure globally and throughout all ecoregions, but especially for IN species (Fig. 9). Land and water management is also shown to be one of the top priorities for FB conservation, particularly in the PA and AF ecoregions (Fig. 9). Other types of conservation actions showed quite distinct patterns among ecoregions. For example, stronger legislation is likely required for the AF, PA, NA, and AU, but law enforcement needs to be enhanced only in the AF and PA ecoregions. Moreover,

increasing awareness of the general public about the importance of conserving FB is quite essential for the PA and particularly in the IN. Special interest in species ex-situ propagation and reintroduction programs is exhibited for the NA, emphasising the vast knowledge already accumulated for many species in the ecoregion (Fig. 9).

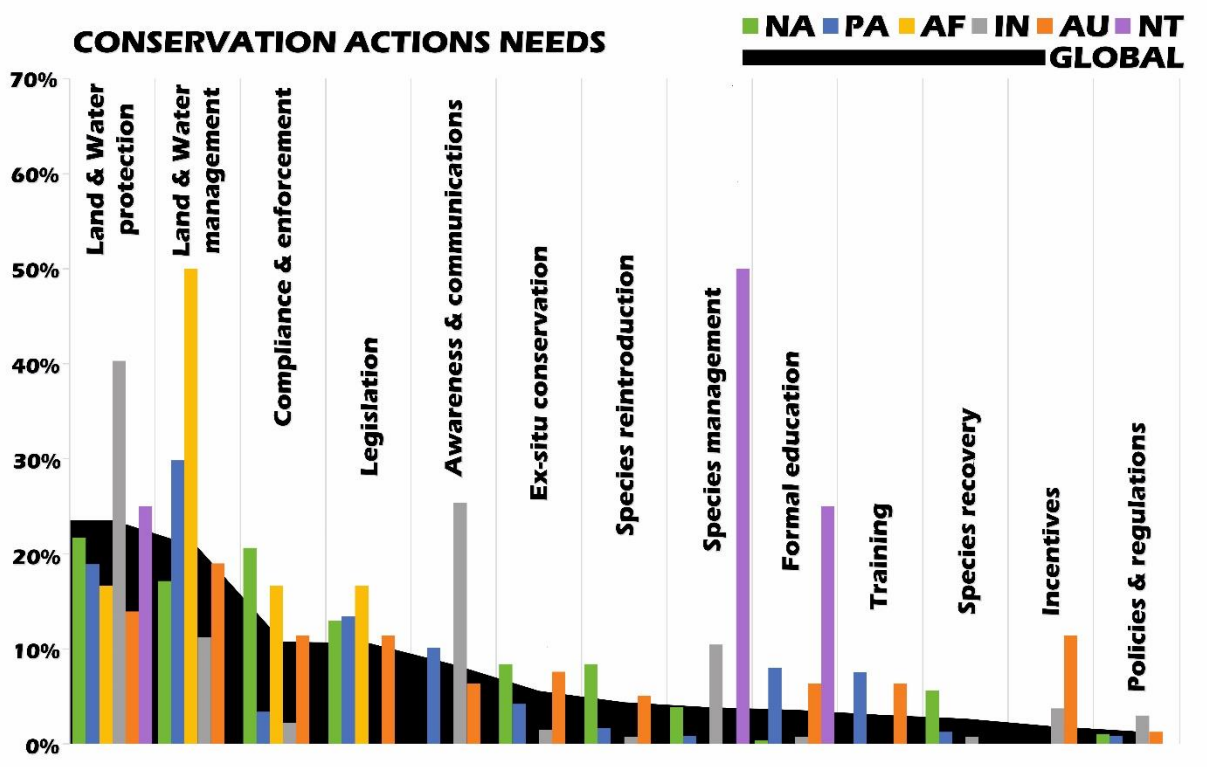

Figure 9 Conservation needs for freshwater bivalves extracted from the IUCN Red List database by ecoregions. Ecoregion subdivisions adapted from Graf & Cummings (2007) and Haag (2010): *NA* Nearctic, *NT* Neotropical, *PA* Palaearctic, *AF* Afrotropical, *IN* Indotropical, AU Australasian.

Although many research gaps and conservation needs have been identified in the last years, many recent technological advances can provide us with new insights that are needed for FB research. For example, new remote sensing techniques like underwater video and side-scan sonars may help survey FB populations and identify more favourable habitats (Powers et al 2014; Mehler et al 2016). The use of drones in semi-arid regions can aid in tracking and identifying the remaining pools after droughts where mussels take refuge. These technologies and the use of environmental DNA analyses may help gathering basic biological and ecological data on distribution and abundance, which are still missing for many species (Stoeckle et al 2016). More powerful genetics and morphometric tools are also increasingly available, for instance, new statistical tools for species delimitation using molecular and/or other types of data such as morphometry and anatomical traits (e.g. Froufe et al 2016b; Pfeiffer et al 2016). These tools are particularly important because many species present hidden cryptic diversity

(Froufe et al 2016b; Pfeiffer et al 2016). Additionally, next-generation sequencing is now allowing for quicker and less expensive robust phylogenies using methods like whole-transcriptome and whole-mitogenome analyses with a wide range of markers (Guerra et al 2017; Lopes-Lima et al 2017c). Furthermore, using reduced genome representations or snip analyses, it is now possible to get more information on the phylogeographic patterns of species and on the definition of conservation units (Catchen et al 2017; Desalle & Amato 2017).

While most of the global protected areas network is aimed at protecting essentially terrestrial vertebrates, the identification of sites to conserve freshwater vertebrates and invertebrates such as FB is also of crucial importance (Darwall et al 2011; Maceda-Veiga et al 2017). Using the IUCN Key Biodiversity Areas (KBAs) network (IUCN 2016) or new systematic conservation planning approaches (e.g. Hermoso et al 2015) may help to promote a better FB representation within protected area networks.

The proceedings of the Second International Meeting on Biology and Conservation of Freshwater Bivalves

All the research and conservation needs above summarised, make the facilitation of cooperation among scientists from different countries and continents particularly important. For example, recent reviews published by multinational teams of scientists provided vital baseline information about FB on different continents (e.g. Pereira et al 2014 for South America, Walker et al 2014 for Australia; Lopes-Lima et al 2017a for Europe; Williams et al 2017 for North America, and Zieritz et al 2017 for East and Southeast Asia). Additionally, intercontinental cooperative research is also becoming increasingly common (see for example Zieritz et al 2016; Lopes-Lima et al 2017b). To discuss the current and future research challenges and needs, the Second International Meeting on Biology and Conservation of Freshwater Bivalves was hosted by the Great Lakes Centre at SUNY Buffalo State College in Buffalo, New York, USA, from 4 to 8 October 2015, bringing together over 80 scientists from 19 countries and four continents (Europe, North America, South America, and Australia) (Burlakova et al 2017).

The present special issue in Hydrobiologia comprises a total of 34 papers (including this introductory note) summarising some of the information presented in this meeting. These papers cover a wide variety of topics, from a review of ecosystem services provided by freshwater mussels (Vaughn 2017) to papers describing the diversity patterns and conservation of Unionida in East and Southeast Asia (Zieritz et al 2017) as a result of international collaboration. Seven papers focus on different biological aspects of invasive bivalve species, including diversity changes by species substitution (Karatayev et al 2017), physiological aspects (Labecka & Domagala 2016), dispersion (Collas et al 2016), ecological effects on native bivalve species (Ferreira-Rodríguez et al 2016), low palatability to distinct

predators (Castro et al 2017), metabolite emission suppression in zebra mussels exposed to predation stress (Antoł et al 2017) and the use of a new sonar technology and underwater imagery analysis for the survey of FB in rivers (Mehler et al 2016). Propagation as a conservation tool was the subject of three studies: one about an improved method of in vitro culture of glochidia (Ma et al 2016), one introducing short-term breeding of the Endangered freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) as a new technique for the augmentation of declining populations (Moorkens 2017) and one revising the challenges in the conservation progress of *Margaritifera auricularia* (Spengler, 1793) (Prié et al 2017). Six papers used molecular tools to describe genetic structure or phylogeographic patterns of European (Feind et al 2017), North American (Hewitt et al 2016; Mathias et al 2016) and South American species (da Cruz Santos-Neto et al 2017); to reveal the uncommon doubly uniparental inheritance of mitochondria in a European species (Soroka and Burzynski 2017) and the sequencing of transcriptomic resources for an invasive species (Soroka et al 2017). The interaction between mussels and their host fishes was addressed in three papers that evaluate the effects of stress (Douda et al 2016), cross-immunity (Chowdhury et al 2017) and temperature (Schneider et al 2017) on the reproduction of freshwater mussels. Three papers describe distribution patterns with distinct spatial and temporal scales: the population trends of Unionidae in Romania (Sîrbu and Benedek 2017), the distribution of freshwater mussels and their host fishes in Texas (Dascher et al 2017) and a study that reconstructs the historical range and population size of the threatened species *Popenaias popeii* (Karatayev et al 2015). On a smaller scale, a study on the longitudinal variation in freshwater mussel assemblages within two rivers is presented by Chambers & Woolnough (2016), while Dittman et al (2017) evaluate the microhabitat and biology of the poorly studied pea clam *Sphaerium striatinum*. Two papers assess the growth of *M. auricularia* (Nakamura et al 2017) and juvenile freshwater pearl mussels *M. margaritifera* at the river scale (Cêrná et al 2017). One paper assesses the shell phenotypic plasticity of *Unio crassus* (Zajaç et al 2017). The influence of the flood pulses and near-bed hydrodynamics on freshwater mussels is evaluated by Callil et al (2017) and Sansom et al (2017), respectively. Finally, toxicology and archaeology are represented by a study of the effects of polycyclic aromatic hydrocarbons on unionid mussels (Archambault et al 2017) and the conservation implications of freshwater mussel remains in a Texan river (Popejoy et al 2016).

Conservation of FB requires urgent collaboration between scientists, managers, politicians and the general public, to share knowledge and efforts. An example of this collaboration is the International Meeting on Biology and Conservation of Freshwater Bivalves, but more efforts are necessary for the transfer of knowledge between scientists and the general public to raise awareness about the importance of FB conservation. These efforts can include, but not be limited to the increase in visibility of FB conservation issues in the media, better

engagement with local communities and stakeholders (e.g. providing training and lifelong learning opportunities like workshops for public, better information dissemination and accessibility of collaborative research even integrating participants from civil society into surveys and research projects), publications and additions to national collections.

Acknowledgements

We thank the Editor-in-Chief Prof. Dr. Koen Martens for his help and support and the coordinator of Springer Journals Editorial Office, Deepan Selvaraj, for his hard work and assistance in the editorial process of this Special Issue. This work was supported by FCT-Foundation for Science and Technology, Project 3599-Promote the Scientific Production and Technological Development and Thematic 3599-PPCDT by FEDER as part of the project FRESHCO: multiple implications of invasive species on Freshwater Mussel co-extinction processes (Contract: PTDC/AGRFOR/1627/2014). FCT also supported MLL under Grant (SFRH/BD/115728/2016).

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CHAPTER 3

Basic biological traits of *Unio delphinus*

Paper II

Setting the stage for new ecological indicator species: A holistic case study on the Iberian dolphin freshwater mussel *Unio delphinus* Spengler, 1793.

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Article published in *Ecological Indicators* **111**, 105987 (2020).
DOI: 10.1016/j.ecolind.2019.105987

Setting the stage for new ecological indicator species: A holistic case study on the Iberian dolphin freshwater mussel *Unio delphinus* Spengler, 1793.

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Abstract

Due to their sensitivity and dramatic declines, freshwater mussels are prime targets for conservation and environmental monitoring. For this, however, information is needed on life history and ecological traits, which is lacking in many taxa, including threatened species. Species recently described or recognized as valid are of particular concern, due to the shortage of even basic knowledge. A case in point is the recently recognized and Near Threatened dolphin freshwater mussel *Unio delphinus* Spengler, 1793, which is endemic to the western Iberian Peninsula and has suffered marked population declines. To overcome information gaps for *U. delphinus*, we carried out a holistic biological study across the species range, aiming to: i) estimate the area of occupancy (AOO) and extent of occurrence (EOO) based on updated distribution data taken from the literature and recent surveys; ii) estimate growth patterns from biometrical (shell dimensions and growth annuli) measurements taken on specimens from seven populations; iii) estimate sex ratios from gonad tissue biopsies collected on specimens from eight populations; iv) estimate gametogenesis through histological examination of gonad and gill tissues collected monthly for a year, from a single population; and v) determine host species from infestation trials of glochidia with co-occurring fish species. We estimated an EOO of 706 km2 and an AOO of 61 km2, which together with data on declines assigns the species to the Endangered category using IUCN criteria. *U*nio *delphinus* was found to grow faster and to be shorter-lived (up to 11 years, maturity at around 2 years old) than other European freshwater mussels. Growth and life span are similar across the range in lotic habitats, but different from that in lentic habitats. The larvae of *U. delphinus* may attach to most co-occurring fish species, but only native species were effective hosts. Native cyprinids, especially those from the genus *Squalius*, seem to be the primary hosts. Overall, the information provided contributes to a better conservation status assessment, selection of conservation and rehabilitation areas, guidance for the establishment of propagation programs and better timing for specimens' manipulation including monitoring and possible translocations. The framework presented here highlights the importance of basic biological studies to define good ecological and physiological status.

Keywords

Conservation, Unionida, Life-history traits, Growth, Host-fish

Introduction

The definition and requirements of ecological indicators have been subject to some debate and confusion, but indicator species are undoubtedly important components for ecosystem quality assessments (Heink and Kowarik 2010). A highly cited review by Carignan and Villard (2002) identified two ideal general qualities for indicator species: negative association with human disturbance, and habitat specialisation. Additional characteristics were described from previous studies (Noss et al 1997): the potential as an early warning system, the discrimination of the cause of change, the range of responses, and the cost-effectiveness of the survey. Potential indicator species are often at the same time keystone, area-limited 'umbrella', dispersal-limited, resource-limited, process-limited, or flagship species (Carignan and Villard 2002; Lambeck 1997; Noss et al 1997). However, even when matching all these criteria, there is still a need for several disparate indicators, since each species reacts to disturbances at different degrees and scales (Carignan and Villard 2002). Finally, for a species to be useful as an ecological indicator, it needs first and foremost to be very well studied, so that survey data allows distinguishing actual disturbance signals from variations that may be unrelated to the deterioration of ecological integrity (Carignan and Villard 2002).

The bivalves of the Unionida order, also known as freshwater mussels, are key elements of aquatic ecosystems (Lopes-Lima et al 2014, 2018). They play ecologically important roles such as bioturbation or sediment mixing, nutrient cycling and energy transfer from the water column to the bottom, among other processes (Vaughn 2018). However, this faunal group, like most others in freshwater ecosystems, has been declining dramatically over the last decades, with several species extinctions and many extirpations being reported (Lopes-Lima et al 2014, 2018). Freshwater mussels are very sensitive to human activities, but other intrinsic features increase the probability of extirpation or extinction. For instance, these organisms generally have a slow metabolism, taking at least a year to reach sexual maturity (Lopes-Lima et al 2017a). Also, they have a complex life cycle where larvae (glochidia) need to attach to specific fish hosts (Modesto et al 2018). Given their important ecological role, but also high sensitivity to habitat, water, and sediment quality, some freshwater mussel species simultaneously fulfil criteria for indicator, flagship, and umbrella species, making them important targets for environmental monitoring and conservation (Geist 2010; Lopes-Lima et al 2017a). Like some freshwater mussel species (e.g. *Margaritifera margaritifera*), umbrella species conservation strategies are directed towards wide home range species protecting other sympatric species (Geist 2010). This is due to the high sensitivity of freshwater mussels to environmental factors that can arise at different spatial scales, not only local but also regional such as the land-use and geological influence over the whole catchment area (Strayer et al 2004). Freshwater mussels are also highly valued for their rarity, beauty and interesting

behaviour (Strayer 2017), which added to their tight interspecific relationships and frequently high cultural value make them suitable 'flagship species' to raise support for freshwater habitat conservation (Caro 2010). Due to their unique and crucial roles in ecosystem functioning, and the high biomass in many habitats, they can also be considered ecosystem engineers, given their large physical effects on the ecosystem (Gutierrez et al 2003).

In Europe, 20 freshwater mussel species are currently recognized (Froufe et al 2016a,b, 2017; Lopes-Lima et al 2017a; Araujo et al 2018). Species richness is higher in central Europe but southern Europe presents a higher level of endemism and restricted-range species (Lopes-Lima et al 2017a). This is the case of the dolphin freshwater mussel *Unio delphinus* Spengler, 1793, which was considered a subspecies of the widespread and more common European *Unio pictorum* until its recent recognition as a valid distinct species (Araujo et al 2009). *Unio delphinus* has suffered a 30% range decline over the last decades, mainly due to habitat degradation, including pollution and changes in the hydrologic regime due to the presence of dams or other infrastructures, poor river management and water shortage (Araujo 2011). The Iberian Peninsula, as most of the regions within the Mediterranean biodiversity hotspot (Myers et al 2000), is suffering from water scarcity exacerbated by climate change and associated instability (Robson et al 2013; Cid et al 2017). Similar negative impacts were observed on the Iberian populations of other freshwater mussel species (Sousa et al 2012, 2018).

Invasive species are also pointed as one of the main threats to freshwater mussels (Sousa et al 2014). Introduced predators like mammals, fish, and crayfish are known to consume freshwater mussels and may cause local declines (Meira et al 2019; Sousa et al 2018, 2019). Zebra mussels *Dreissena polymorpha* and the Asian clam *Corbicula fluminea* can reach high densities in their invasive ranges and may outcompete native mussels, reducing their fitness and growth and increasing mortality rates (Sousa et al 2011; Bodis et al 2014; Lopes-Lima et al 2017a; Ferreira-Rodriguez et al 2019; Modesto et al 2019). Given that native mussels seem to depend on specific, and usually native fish host species to complete their life cycle, changes in the fish fauna can also have deep implications on the mussel populations (Douda et al 2013; Modesto et al 2018).

Over the last decades, there has been a rising awareness about the need to conserve freshwater ecosystems and taxa, accompanied by the increase of dedicated conservation funds, mainly in Europe and North America (Lopes-Lima et al 2017a, 2018). This has also boosted research on freshwater mussel conservation (Lopes-Lima et al 2014). However, in Europe, the majority of studies were concentrated on a small number of species present on the European Union (EU) Habitats Directive, disregarding most of the other freshwater mussels, especially those that were only recognized after the inception of that EU policy (Lopes-Lima et al 2018), as is the case for *U. delphinus*. On the other hand, most recent

research explores threats, management and conservation methods (Lopes-Lima et al 2014), but much less effort has been devoted to understand the underlying life-history traits that are essential for effective conservation planning (Lopes-Lima et al 2014, 2017a). These concerns have been raised in recent reviews that identify as top conservation research priorities acquiring information on life-history traits, abundance, distribution, and size structure (Lopes-Lima et al 2018, Ferreira-Rodriguez et al 2019).

Given the high sensitivity and filtering behaviour of freshwater mussels, they are many times colloquially mentioned as “aquatic canaries in the coalmine” or “livers of the rivers” (Cummings et al 2016). Demographic, physiological and behaviour features can be used to determine the status of a freshwater mussel population and therefore indicate potential environmental perturbation (Van Hassel & Farris 2007). Basic biological features of freshwater mussels have already been used to assess environmental disturbance in freshwater habitats, such as the effects of temperature and heated effluents, sewage, siltation, and impoundment (reviewed in Van Hassel & Farris 2007 and references therein). Many of the same characteristics that make freshwater mussels good sentinel organisms (e.g. sedentarism, large/easy to use, sensitive to disturbance, shells providing historical record, widely distributed, and bioaccumulation of pollutants) also make them well suited to use as indicators of ecological integrity in assessments of environmental impact, waterbody status monitoring, and assessments of environmental history (Van Hassel & Farris 2007). However, studies using freshwater mussels as biological indicators are still scarce (Lopes-Lima et al 2014) due to the limited knowledge about their life-history traits.

To face the dearth of life-history trait research and to set up a framework that can serve both as an example and base for future works, the present work applies a holistic approach to study *U. delphinus* and thus improve the efficacy of ongoing and future conservation measures and their use as environmental indicators. We update the distribution and revise the conservation status of *U. delphinus* focusing on eight *U. delphinus* populations to study the species growth and lifespan patterns throughout its range, determine and describe its reproductive cycle and sexuality, and identify its fish hosts.

Materials and methods

Distribution

Distribution data was compiled from the literature and personal data from the authors. The Extent Of Occurrence (EOO) and Area Of Occupancy (AOO) were estimated by two distinct methods. For estimating AOO, the number of occupied cells in a uniform 2 × 2 km grid, covering the entire range of the species taxon, was counted and then obtaining the total area of all occupied cells. This method is the one proposed in the most recent version of the IUCN

guidelines (IUCN Standards and Petitions Committee 2019) but, in our opinion, it overestimates the AOO for linear distributed species such as freshwater mussels. Therefore, we used another method to estimate AOO which better represents the known area occupied by the taxon. This method to estimate AOO was first used by Gomes-dos-Santos et al (2019) and consists in multiplying the mean width of the river by a longitudinal (along the river) 2 km buffer, for each record point and then tallying up the number of records. The mean river width was obtained per basin as the average of six equidistant points within the species range. As for EOO, the first method used was the least convex polygon, as the smallest polygon in which no internal angle exceeds 180 degrees and which contains all the sites of occurrence, as suggested by the IUCN guidelines (IUCN Standards and Petitions Committee 2019). However, since most of the species EOO is on land we feel that the terrestrial range should not be accounted for, and therefore we used also a second alternative method recently published by Gomes-dos-Santos et al (2019). This method consisted in multiplying the mean river width by the sum of the river length between species distribution records in each basin.

Sampling

Live specimens (n ≥ 30 per population) of *U. delphinus* with varied sizes were collected from eight populations on Atlantic Iberian river basins (Sabor River: 41.239625, -6.967942; Douro River: 41.152612, -7.765184; Mira Lagoon: 40.441897, -8.756483; Barrinha Lagoon: 40.450047, -8.797069; Mondego River: 40.204369, -8.361042; Ponsul River: 39.778456, -7.432322; Guadiana River: 38.831016, -7.085385; and Vascão River: 37.516950, -7.579433) (Fig. 1), chosen to represent a wide range of latitudes and habitats. All sites contain well-established and healthy populations of *U. delphinus* and display a good ecological and chemical status (Reis 2006; Oliveira et al 2007; SNIRH 2019) and never suffered from known acute events of pollution.

The shells and growth rings of all specimens were measured (see below Section 2.3 for further details) for seven of these eight populations (excluding the Ponsul River population). For the seasonal sexual development and determination of the age of maturity, 10 specimens of *U. delphinus* were collected from the Sabor River population each month for one year (Fig. 1). The mussels were transported in a refrigerated box and processed within 24 h. The mussels were anesthetized as described in Hinzmann et al (2013) and euthanized for histological inspection (see below Section 2.4 for further details). To minimize eventual negative impacts, the number of animals sacrificed was kept to a minimum.

Gonad tissue biopsies were collected for seven of these eight populations, (excluding the River Douro Population). All specimens measured and biopsied (see below Section 2.5 for further details), were then returned to their original locations.

For the host compatibility experiments, 14 native and 6 non-native fish species, representing the most common fish taxa with an overlapping distribution with *U. delphinus* (Oliveira et al 2007; Lopes-Lima et al 2017a) were collected by electrofishing. For the same experiment, six gravid mussels were collected from the Douro River population and transported to the laboratory (see below further details about hosts in Section 2.6).

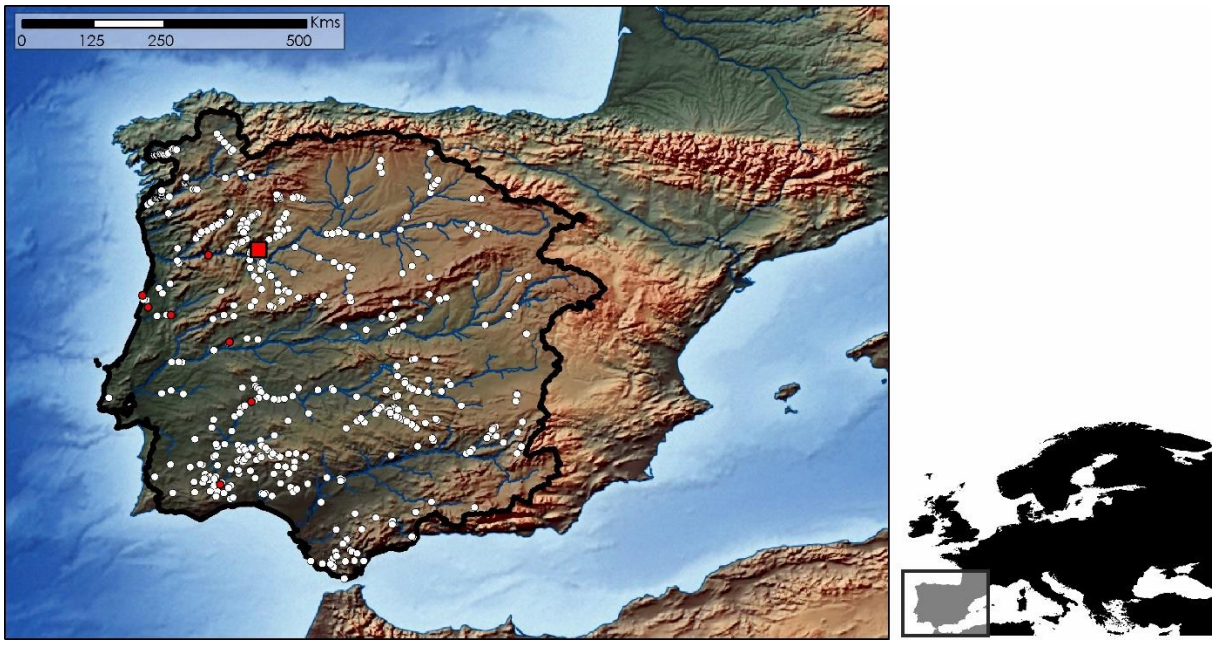

Figure 1 Map showing the known distribution records of *Unio delphinus* (white circles), sampling sites for growth and sex-ratio (all red markers), and sampling site for the evaluation of reproductive cycle (red square). Both maps are represented using the World Geodetic System 84 (WGS84) projection.

Shell dimensions (length, width, and height) and the annuli or growth rings were measured from each individual following Aldridge (1999). Individuals with shell abnormalities, incomplete annuli, and eroded shell umbos were excluded from the analysis. The length of the annuli dimensions was used to produce length-age plots for each population. Growth curves were constructed using the Von Bertalanffy equation (Bauer 1992; Aldridge 1999).

The Equation was used in the form: $L_{t+m} = cL_t + d$

Where:

L_t is the shell length at time t;

L_{t+m} is the shell length at time t + m;

m is the measurement collection interval (1 year in the present case, i.e. $m = 1$);

$c = e^{-km}$ being k the growth constant defining the rate at which the asymptotic length is reached;

$d = L\infty (1 - c)$, being $L\infty$ the asymptotic length.

For each population, the longest mussel length was used to calculate the maximum age at each site, using the following formula $t_m = -1/k.\ln[1 - (L_m/L\infty)]$ (Ziuganov et al 1994).

Analyses of Covariance (ANCOVA) implemented in PAST 3.25 (Hammer et al 2001) were then used to compare growth models.

Reproductive cycle histological procedure

The gonad and gill tissues were removed from each animal. Sex determination was first made from smears of fresh gonad tissue (across the whole gonad) observed under the microscope, and the macroscopic and microscopic aspects of the gonads were recorded. The gonad tissue samples were then fixed in Bouin's (Panreac) solution for a week, cut and inserted into histologic cassettes, and dehydrated in an ethanol gradient followed by xylene and paraffin impregnation overnight, using a Shandon Citadel 2000 Tissue Processor. To finalize, samples were included in paraffin blocks using a Shandon HistoCentre 2. Sections of 5-6 µm were made on a Leica RM2255 microtome and stained using standard H&E coloration (following Hinzmann et al 2013). Observations were made on an Olympus DX 41 with DP 70 camera. A division of the main stages of male and female gonadal development or Gonadal Development Index (GDI) was then established for *U. delphinus* based on most observed cases in the respective period, as described in Hinzmann et al (2013). For the determination of the embryonic development periods, the gills were also inspected.

Sex ratio distribution and age of maturity

All specimens were carefully opened with crossed pliers and four small tissue biopsies were collected with a biopsy needle across the foot to an Eppendorf tube, fixed and visualized under the microscope as explained above for the gonad sections. Sex was confirmed by the presence of male or female cells. The approximate age of maturity was determined using the inverse of Von Bertalanffy equation on the age of the younger individuals carrying gamete cells. To assess if sex ratios differed from the 1:1 expectation, a chi-square test was used for each of the seven populations analysed.

Fish hosts

Experiments assessing the infestation capability of *U. delphinus* glochidia with native and non-native fish species were conducted in July 2018. Extraction of the glochidia and infestation trials followed the methodology described in Douda et al (2013) with a minimal bath volume of 0.5 L per fish individual containing a mean ± SD of 1489 ± 150 viable glochidia, added to each tank. All fish were then separated by species in 40 L tanks (up to 3 fish per tank) to monitor the developmental success of *U. delphinus* glochidia. A 3-mm net was used on the bottom of each tank to avoid juvenile predation by fish. The tanks were part of a recirculation system

kept at 20 °C. Fish were fed daily with commercial fish food. Each tank was siphoned daily into filters (mesh size 100 µm), that were examined for the presence of glochidia and juvenile mussels. The proportion of successfully transformed juveniles (transformation rate) was calculated following Douda et al (2013), using the recorded number of juveniles and the initial number of attached glochidia (determined by the number of dead glochidia + viable juveniles counted from each tank post infestation). The cumulative number of degree-days was calculated by the sum of daily temperatures (all at 20 °C by controlled temperature) during glochidia attachment. This was determined by multiplying the daily temperature by the number of days during fish infestation. Five days after the last juvenile was recovered, we considered each trial (*N* = 56) to be complete. Fish were then checked for residual attached glochidia.

Results

Distribution

Unio delphinus populations were recorded in most Atlantic basins of the Iberian Peninsula, from the Ulla River in the north to the La Vega River basin in the south, near Gibraltar. It also occurs in few Mediterranean coastal basins east of Gibraltar until the Guadalhorce River basin near Malaga (Fig. 1; Appendix 1). The estimated EOO varies between 706 (multiplying the mean river width by the river length within the extremes of the species distribution in each basin) and 344,641 (using the minimum convex polygon EOO estimates) km^2. The AOO varies between 61 and 2,000 km^2 using the mean width of the river along the hydrographic network or 2 × 2 km grid overlay methods, respectively.

Growth and longevity

Individuals of all populations grew approximately up to 20 mm in the first year. Then the annual growth rate decreased steadily (Fig. 2). The maximum length measured revealed a major difference between lentic and lotic populations, varying in river populations between about 60-80 mm, while reaching over 100 mm in both lagoon populations. The von Bertalanffy growth parameters for all populations are represented in Table 1. The growth constant (k) is similar in populations from related habitats but are much higher for river than lagoon populations (Fig. 2; Table 1), indicating that the asymptotic length is reached sooner in the former.

Table 1
Growth parameters for Iberian *Unio delphinus* populations. $L\infty$ is calculated from the Wolford equation, L_{max} is the maximum observed length in the field. The maximum age was estimated from L_{max}

Population	Wolford plot	Growth constant (k)	Asymptotic length $L\infty$ (mm)	Maximum length L_{max} (mm)	Maximum age (years)
River Sabor	$y = 0.72x + 21.68$ ($R^2 = 0.996$)	0.33	76.88	69.08	7
River Douro	$y = 0.68x + 22.18$ ($R^2 = 0.993$)	0.38	69.85	68.8	11
Mira Lagoon	$y = 0.81x + 23.98$ ($R^2 = 0.996$)	0.22	125.04	106.95	9
Barrinha Lagoon	$y = 0.81x + 23.51$ ($R^2 = 0.991$)	0.21	125.04	104.94	9
River Mondego	$y = 0.67x + 24.10$ ($R^2 = 0.991$)	0.41	71.95	68.66	8
River Guadiana	$y = 0.71x + 23.39$ ($R^2 = 0.984$)	0.34	80.34	78.19	11
River Vascão	$y = 0.65x + 21.69$ ($R^2 = 0.998$)	0.44	61.63	58.98	7

As for the maximum age, populations from the larger rivers, i.e. Douro and Guadiana, attained the longest longevities (≈11 years) followed by the lagoon populations (≈9 years) and finally by populations colonizing smaller rivers (≈7-8 years) (Table 1). ANCOVA show significant differences in growth among all populations ($F = 9.4$, $p < 0.01$). No significant differences in growth were detected when considering separately lagoon (ANCOVA, $F = 0.07$, $p > 0.1$) and river (ANCOVA, $F = 1.71$, $p > 0.1$) populations.

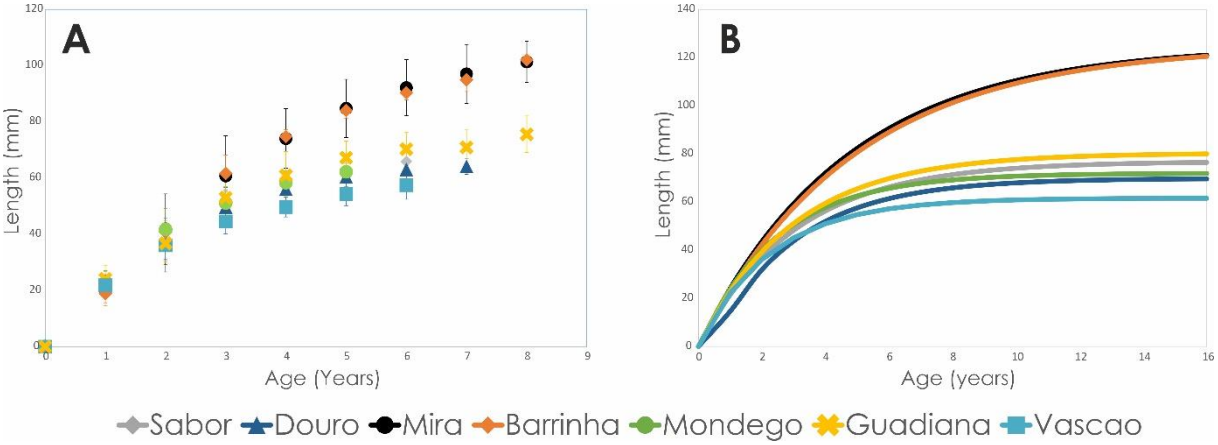

Figure 2 A - Size-at-age measurements of shell length; **B** - size as a function of bivalve age, modelled by the von Bertalanffy growth function.

Reproductive cycle

The general structure of the gonads of Unio delphinus

The gonads of *U. delphinus* fill most of the foot tissue surrounding the digestive tract. This species is strictly dioecious, as no case of hermaphroditism was detected. Macroscopically, gonad tissue presents sex-specific appearance and coloration. The gonad tissues of females are dark-yellow/orange and are denser and granular, due to the presence of mature oocytes (Fig. 3). The male gonad tissues have a lighter yellow coloration and were more fluid in terms of consistency (Fig. 4). The microscopic organization of the gonads consists of highly branched cell clusters (acini) surrounded by connective and muscular tissue. These acini were found full of gametes throughout the year, independent of sex.

Oogenesis

Female individuals presented follicles with reproductive cells in all development stages throughout the year and the reproductive cycle is biannual, continuous and uninterrupted. However, the prevalence of the different stages of development varies seasonally (see GDI sub-section below). The oogenesis was mainly divided into five continuous stages, according to the maturation stage of the gamete cell: oogonia, previtellogenic oocytes, early oocytes, oocytes and mature oocytes (Fig. 3).

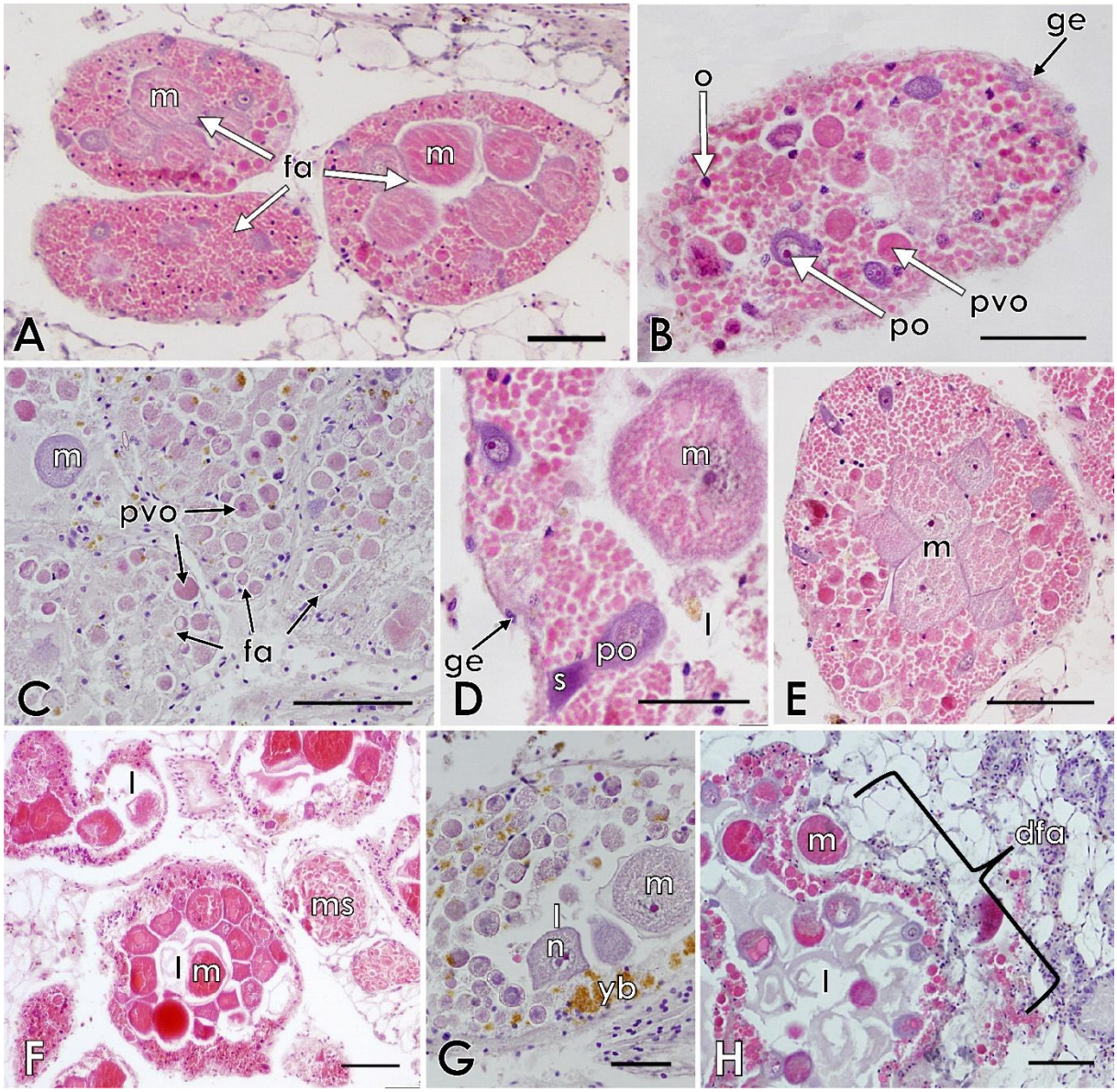

Figure 3 Histological sections from female gonads of *Unio delphinus* stained with Hematoxylin and Eosin (H&E). **A** - General aspect of female gonads (fa) organized in acini, in September the acini showing gonads at all development stages of oogenesis, with several mature oocytes (m) (scale bar 100 µm). **B** - Female acinus in September with a predominance of earlier stages of oogenesis: oogonia (o), previtellogenic oocytes (pvo), pedunculated oocytes (po), and the germinal epithelium (ge) are visible surrounding the germinative cells (scale bar 50 µm). **C** - Female acini in May also presented different development oogenesis stages, dominantly the earlier previtellogenic oocytes (pvo) surrounded by the germinative epithelium (scale bar 100 µm). **D** - Detail of female acinus in September showing pedunculated oocytes (po) with stalk (s) visible and mature oocyte in the lumen (l) (scale bar 50 µm). **E** - Mature acinus in September, full of mature oocytes (m) in the centre surrounded by earlier stages and germinative cells (scale bar 100 µm). **F** - Mature female acini with mature oocytes (m) released into the lumen (l), and muscle tissue (ms) (scale bar 100 µm). **G** - Female acinus in October with only a few mature oocytes (m) already in the lumen (l), one showing two nucleoli (n) in the nuclei, presenting still some early stages of oocytes and with several yellow bodies (yb), indicating early signs of degeneration (scale bar 50 µm). **H** - Degenerative female acinus (dfa), surrounded by an undifferentiated epithelium, but still presenting some stages of oocyte development (scale bar 100 µm).

Oogonia represent the first stage of the gamete development (Fig. 3B), corresponding to the smallest and rounder cells, located radially along the follicle next to the epithelial cells, with a diameter between 10 and 13 µm. The nucleus is not visible due to the disperse chromatin and the intense pink coloration is due to the cytoplasm acidophilic properties. This stage was rare or inexistent during the peak of maturation and release of mature oocytes (corresponding to stage 4 of the GDI, described below). In the next stage, previtellogenic oocyte (Fig. 3A and C), the size of the cells increased, and the nucleus can sometimes be differentiated. Previtellogenic oocytes still present a very acidophilic cytoplasm, and the size of the cells varied between 13 and 18 µm. These cells can be found in the periphery or slightly internal position in the follicle. The vitellogenic oocytes or early oocytes are bigger (20 - 30 µm cell diameter) than the previous stage, but the main difference is the presence of one or more (usually two) nucleolus in the nucleus and their localization that is more internal into the lumen of the follicle. However, early oocytes can still be linked to the germinal epithelium by a peduncle or stalk, as pedunculated oocytes (Fig. 3B and D). In the following stage, the oocytes, the shape of the cells become more irregular (Fig. 3A, D, E, and F), at this stage the cell length can vary between 50 and 60 µm, the nucleus and the nucleoli are visible in a central position, in some sections is possible to see many vesicles of reserve substances inside the cytoplasm. Finally, the mature oocytes or eggs can reach up to 100 µm length (cell diameter usually between 70 and 90 µm: Fig. 3A, D, E, and F). The aspect of these cells is more diffuse, presenting an acidophilic cytoplasm. The nucleus of mature oocytes is more difficult to observe in section, but when visible it is smaller and with more basophilic characteristics than the cytoplasm (Fig. 3G). At this stage, the gametes are concentrated in the lumen and fill the follicle (Fig. 3D, F, and G), giving little space for the other cell stages. It was also possible to observe some mature oocytes already in the ciliated gonoduct (not shown). After the major release events, it is possible to observe a few mature oocytes in the lumen; however, the acini show degenerative signs, being the integrity of the epithelium compromised (Fig. 3H).

Spermatogenesis

The process of maturation of the gamete cells is here described in four stages, although they occur simultaneously: spermatogonia, spermatocytes, spermatids, and spermatozoa, which corresponds to the mobile mature phase of the gametes (Fig. 4). The maturation of the cells in the male follicle is concentric, with the early stages located more in the periphery and the mature spermatozoa filling the centre of the lumen (Fig. 4A and E). Each cell stage shows a tendency to aggregate in clusters, especially the spermatocytes and the spermatids (Fig. 4C). In the case of spermatids, they were frequently found under the shape of morulae, where groups of 3-12 cells could be found together (Fig. 4C, D and E). Spermatogonia are the first stage of maturation of the male gametes (Fig. 4C and D). It corresponds to the larger cells with

a diameter between 6 and 9 µm and a more irregular shape. They were less frequently found in the sections. These cells were usually found in the periphery of the follicle, with lighter basophilic coloration. The interior of the spermatogonia is filled by the nucleus, with condensed chromatin. The spermatogonia then pass to a second stage, the spermatocyte, through mitotic division (Fig. 4C and D). With a spherical shape, smaller size, and a diameter between 4 and 6 µm, spermatocytes present dense chromatin that almost fills the whole cell, being the nuclear membrane hardly visible. These cells develop by meiosis into spermatids which are even smaller and rounder cells (3-4 µm diameter) (Fig. 4C and D). Spermatids are darkly marked with the dye, highly basophilic, and present a polyhedral shape and homogenous dark nucleus. These cells usually have a more internal distribution than the previous ones, and they are frequently organized in morulae, where clusters of 3 to more than 12 spermatids can be found. The spermatids develop then into the final stage of maturation, i.e. the spermatozoa (Fig. 4A, B, E, and F). Spermatozoa were present throughout the year (except in two organisms from August), but with the prevalence of the different development stages, varying seasonally (see GDI section below). In fresh samples the flagella were visible, and its activity registered. These cells present a rod shape in which the body length was approximately 3-5 µm and the flagella 10-20 µm. The spermatozoa with an oval shape had a very basophilic coloration, the cell diameter varies between 2.0 and 2.5 µm. In many sections, it was possible to observe the concentration of spermatozoa in the male ciliated gonoduct, ready for spawning (Fig. 4B). Associated with the reproductive cells it was always possible to observe the presence of several yellow-brownish bodies or granules in the follicles (Fig. 4C, D and E).

Gonadal Development Index

The Gonadal Development Index (GDI) along the year is summarized in Table 2.

Female

Following the reproductive cycle, it was possible to differentiate five different stages. Often the same organism presented follicles in different stages, making difficult the representation of cell maturation through time, which varied among individuals and seasons (Table 2).Stage 1 (early active) and Stage 2 (late active) correspond in average terms to the initial stages of gametes development, occurring in very short periods and almost simultaneously, just after the last stage of development, i.e. stage 5 (resorption) (Table 2). Stage 3 (mature) corresponds to the phase when gametes reach the maximum maturation. It occurred in two distinct periods, from January to May and in October (Table 2). This stage is also when follicles reach their maximum capacity, being full of mature oocytes, awaiting spawning. Stage 4 (spawned) is reached when many of the mature oocytes were already released (but some can still be found inside the follicles or the gonoduct).

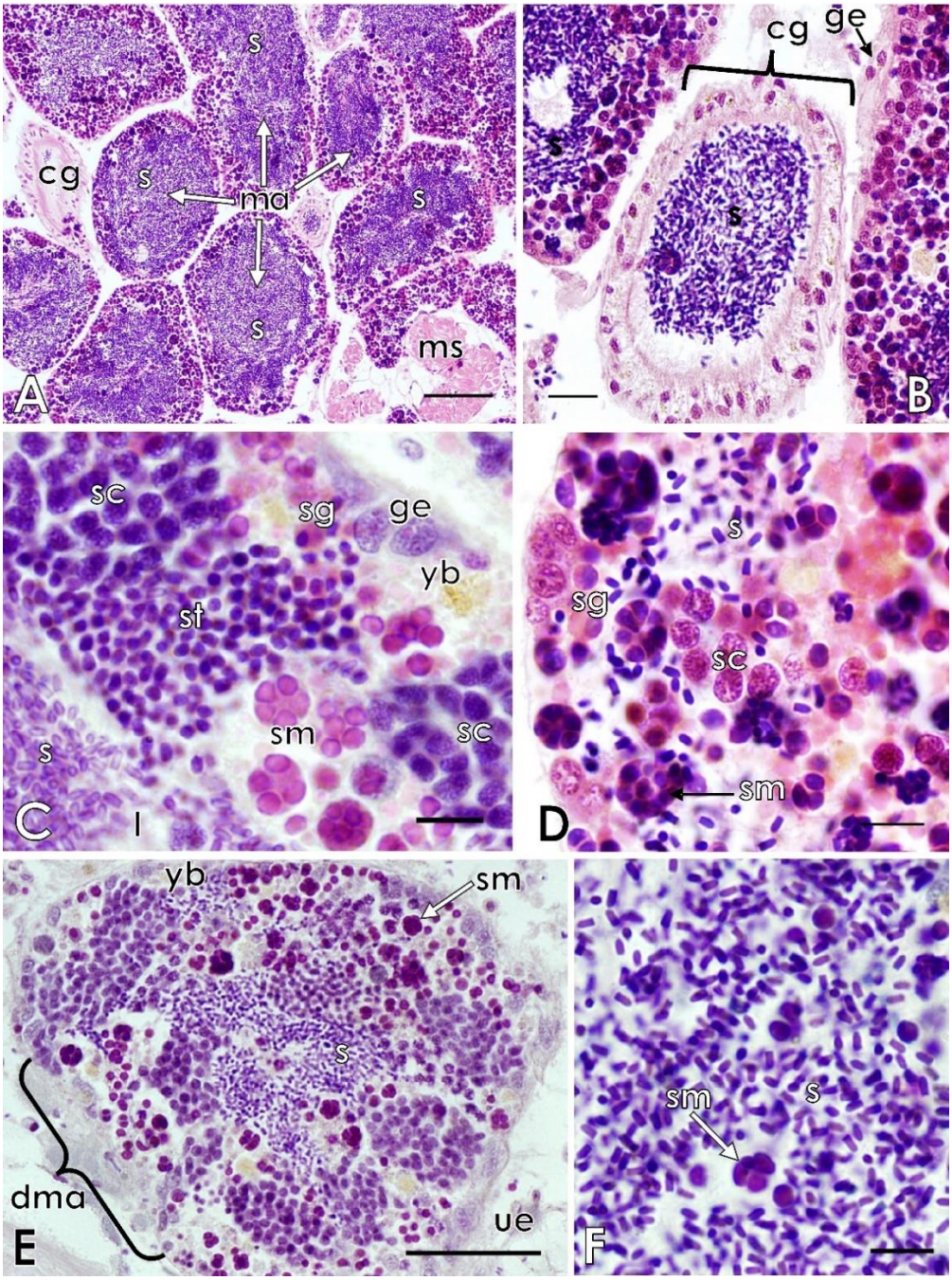

Figure 4 Histological sections from male gonads of *Unio delphinus* stained with Hematoxylin and Eosin (H&E). **A** - General aspect of male gonads (ma) organized in acini in January, with the acini showing gonads at all development stages of spermatogenesis, full with mature spermatozoa (s) in the lumen, with visible muscle tissue (ms) and portions of the ciliated gonoduct (cg) (scale bar 200 μm). **B** - Partial male acini in January, with male reproductive cells at different spermatogenesis stages and germinative epithelium (ge) visible, in the centre the ciliated gonoduct (cg) is full of mature spermatozoa (s) (scale bar 20 μm). **C** and **D** - Details of male acinus in October, where is possible to identify different development spermatogenesis stages, dominantly the earlier spermatogonia (sg), spermatocytes (sc), spermatids (st), sperm morulae (sm) and the last stage spermatozoa (s), not so abundant (scale bar 10 μm). **E** - Degenerative male acinus (dma) in August, at the beginning of the post-spawning period, lumen with already some free spaces, presenting some yellow bodies (yb) and surrounded by an undifferentiated epithelium, still presenting all stages of spermatozoa development (scale bar 100 μm). F - Mature spermatozoa (s) in March, few sperm morulae (sm) and other development stages (scale bar 10 μm).

Two peaks of maturation were registered during this stage, one larger from March to July and a shorter one from November to December (Table 2). Stage 5 (resorption) closely follows the previous stage, and it is characterized by the presence of empty, destroyed or degenerating follicles with some yellow bodies present (Table 2). In this last stage, the presence of some follicles already with gametes in the early stages and some free mature oocytes may also occur. This stage was dominant in August but occurred from May to September and from November to December. We only identified some organisms being exclusively in this stage during September and October (Table 2). Although the gametogenic activity never ceases completely, there is a decrease in activity during these months (Table 2).

Table 2
Monthly values of all identified Gonadal Development Index (GDI) stages in the male and female gonads, and presence/absence of eggs and larvae (glochidia) in the marsupium of *Unio delphinus*. See text for details on GDI.

Month	Female gonad (♀) stages	Male gonad (♂) stages	Gills (♀)
January	2, 3	4, 5	empty
February	2, 3	4, 5	empty
March	3, 4	3, 4, 5	empty
April	3, 4	3, 4, 5	eggs (rare)
May	3, 4, 5	3, 4, 5	eggs, glochidia
June	4, 5, 1, 2	2, 3, 4	eggs, glochidia
July	4, 5, 1, 2	2, 3, 4	eggs, glochidia
August	5, 1, 2	4, 5	glochidia
September	5, 1, 2	1, 2	eggs (residual)
October	1, 2, 3	1, 2, 3	eggs (residual)
November	4, 5	3, 4, 5	empty
December	4, 5	3, 4, 5	empty

Male

Due to the extreme variation among contemporaneously sampled individuals and even across follicles of the same individual, the distinction of the several spermatogenesis stages was more difficult than for oogenesis. Similarly, as females, all male individuals present mature follicles with gametes in a continuous cycle. Stage 1 (early active). Given that we never observed a complete cessation of the reproductive cycle, follicles containing only early stages of male gametes (spermatogonia and spermatocytes) were never found isolated in this stage. Follicles from this stage were rarely found in organisms that presented also follicles at stage 5 or in others that were already in stage 2 or 3 (April to May and September to October). Stage 2 (late active) corresponds to the period were all stages of maturation of the male gametes are present, except the mature spermatozoa that can be absent or rare. This stage was only detected in September and October. Stage 3 (mature) is characterized by the presence of all

maturation stages in the male follicles, the follicles are full of mature spermatozoa that fill the lumen. This stage occurred from March to May and from October to November, preceding spawning. It is characterized by an abundant presence of mature spermatozoa, but also by the high quantity of all the other stages (more in the periphery) and some yellow bodies. During this period some free spermatozoa may already be observed in the gonoduct. Stage 4 (spawned) occurred practically throughout the year from January to August (with peaks in March, May, and June) and from November to December. This stage is characterized by a decreasing presence of spermatozoa in the follicles and an increase of the other cell development stages, mainly spermatocytes and spermatids organized in morulae. During this phase, the gonoduct is full of spermatozoa and the follicles presented empty spaces inside, but no degenerative follicles were observed. Stage 5 (resorption) is almost inexistent, due to the continuity of the cycle, occurring only in few organisms in January, March, August, November, and December. This stage is characterized by the presence of empty follicles or in degeneration in the male reproductive tissue, by fewer reproductive cells and by the abundant presence of yellow bodies. There is a decrease in the number of follicles in the tissue, with some follicles already presenting the early maturation stages of male gametes.

Demibranchs

Unio delphinus females only use the outer pair of the female gills as a brooding chamber, or marsupium, for glochidia (Fig. 5A-5E). The species only kept glochidia in the marsupium during short periods (2-3 weeks) and may, therefore, be classified as tachytictic (short term brooders). When the gills are filled only with eggs, they began to swell, and their coloration is intense yellow (Fig. 5D). The brooding gills then become lighter and whitish as eggs mature into glochidia (Fig. 5B and 5E). Eggs were detected from April to July, with two peaks, one in April and one in June. Glochidia were detected from May to August, with two discharge peaks (May and August). The organization of the eggs in the marsupial gill is in small conglutinates with a feather shape (Fig. 5D). These conglutinates are generally composed of a variable content of eggs and/or glochidia that may change throughout the cycle. When glochidia become dominant before discharge, conglutinates become more diffuse and less evident.

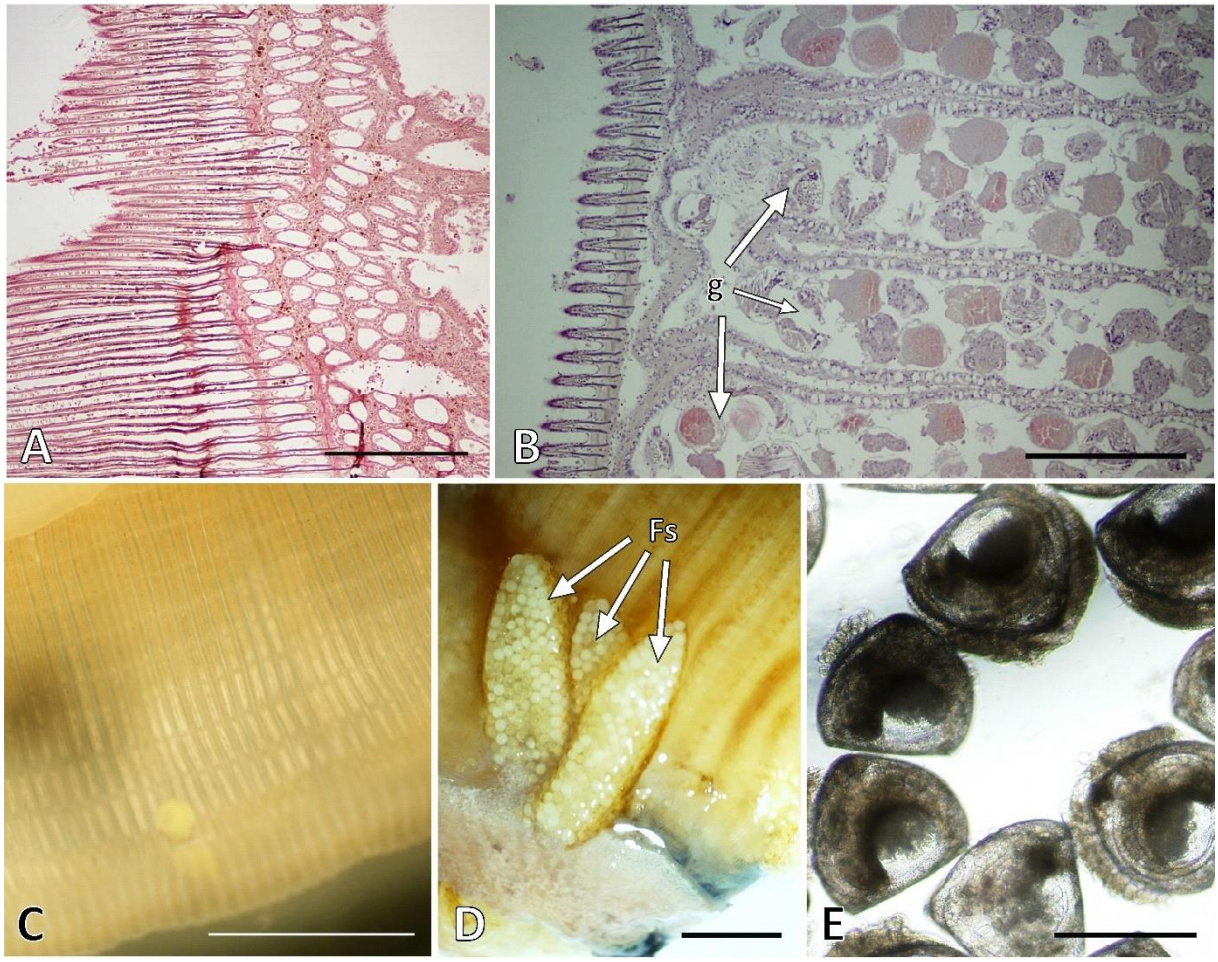

Figure 5 Histological sections from marsupial female gills of *Unio delphinus*, without and with glochidia stained with Hematoxylin and Eosin (H&E) (A and B); stereoscope images from the gills (C and D) and free glochidia (E). **A** - Histological section from marsupial female gill in April, devoid of offspring (scale bar 500 µm). **B** - Histological section from marsupial female gills in July, full of mature glochidia (g) (scale bar 500 µm). **C** - Marsupial gill at stereoscope, in March (scale bar 1 mm). **D** - Detail of gravid gill and feather-like conglutinate full of eggs (Fs) (scale bar 1 mm). **E** - Mature glochidia at the microscope, in June (scale bar 200 µm).

Sex ratio distribution and age of maturity

No significant differences were detected from the predicted 1:1 sex ratio in all populations (Table 3). No gametes were detected in individuals smaller than 41 mm (females) and 31 mm (males), corresponding to 2.5 years in females and 1.6 years for males, respectively. All individuals with size and age above these thresholds presented either female or male gametes in any stage of maturation.

Table 3
Sex distribution of selected Iberian populations of *Unio delphinus*

Population	♀ %	♂ %
Sabor River	47.2	52.8
Barrinha Lagoon	44.4	55.6
Mira Lagoon	50.0	50.0
Mondego River	47.4	52.6
Tejo River	48.6	51.4
Guadiana River	40.6	59.4
Vascão River	48.5	51.5

Hosts

The infestation trials showed that *U. delphinus* glochidia attach mainly to native cyprinids and the native brown trout *Salmo trutta fario* (Table 4; Fig. 6A). Conversely, glochidia were not as successful in attaching to non-native species, which showed in general much lower infestation rates (Table 4; Fig. 6A).

Unio delphinus glochidia successfully developed in 11 out of 14 native species tested (79%) (Table 4; Fig. 6B). In contrast, non-native fish species never produced any viable juvenile (Table 4; Fig. 6B). The glochidia transformation rates ranged from 0% for the native Southern Iberian spined-loach *Cobitis paludica*, the European eel *Anguilla Anguilla*, the flathead grey mullet *Mugil cephalus* and all non-native species, to 48.8% for the Northern Iberian chub *Squalius carolitertii* (Table 4; Fig. 7). Fully developed juveniles were collected from the tanks between 10 and 22 days post infestation, with the sum of daily temperatures during metamorphosis ranging from 240 to 440 degree-days (Table 4; Fig. 7).

Table 4
Fish species studied and host compatibility test results, including the number and mean (±SD) length of fish per species, mean initial number of attached glochidia, mean number of viable juveniles produced and transformation rate 'Transformation rate' indicates the proportion of *Unio delphinus* glochidia that successfully developed into juvenile mussels.

Fish Family	Fish Species	Fish (N)	Mean ± SD fish length (mm)	Mean (glochidia/fish)	Mean (juveniles/fish)	Transformation rate (%)
NATIVE						
ANGUILLIDAE	*Anguilla anguilla*	5	202.0 ± 47.6	5.0	0.0	-
COBITIDAE	*Cobitis paludica*	6	80.8 ± 5.8	16.7	0.0	-
CYPRINIDAE	*Achondrostoma oligolepis*	14	68.6 ± 9.1	24.2	1.5	5.8
	Luciobarbus bocagei	8	134.4 ± 23.1	160.8	111.7	41.0
	Luciobarbus comizo	16	90.2 ± 16.1	113.8	29.2	20.4
	Luciobarbus microcephalus	10	135.0 ± 25.1	138.9	40.1	22.4
	Luciobarbus steindachneri	12	133.3 ± 20.1	151.5	37.2	19.7
	Pseudochondrostoma duriense	7	132.0 ± 5.7	108.8	17.2	13.7
	Pseudochondrostoma polylepis	9	131.5 ± 21.1	89.5	11.9	11.7
	Squalius alburnoides	11	75.9 ± 7.0	85.1	50.9	37.4
	Squalius carolitertii	4	85.0 ± 9.1	197.2	188.3	48.8
	Squalius pyrenaicus	6	130.0 ± 7.9	97.5	80.9	45.3
MUGILIDAE	*Mugil cephalus*	3	160.0 ± 8.7	36.3	0.0	-
SALMONIDAE	*Salmo trutta fario*	5	100.0 ± 15.8	156.7	47.0	23.1
NON-NATIVE						
CENTRARCHIDAE	*Lepomis gibbosus*	13	83.8 ± 10.2	103.8	0.0	-
	Micropterus salmoides	3	123.3 ± 7.6	37.7	0.0	-
CYPRINIDAE	*Gobio lozanoi*	6	75.8 ± 10.2	31.3	0.0	-
ESOCIDAE	*Esox lucius*	3	171.6 ± 12.6	54.7	0.0	-
ICTALURIDAE	*Ameiurus melas*	3	103.5 ± 6.1	5.2	0.0	-
POECILIIDAE	*Gambusia holbrooki*	13	32.7 ± 3.9	3.1	0.0	-

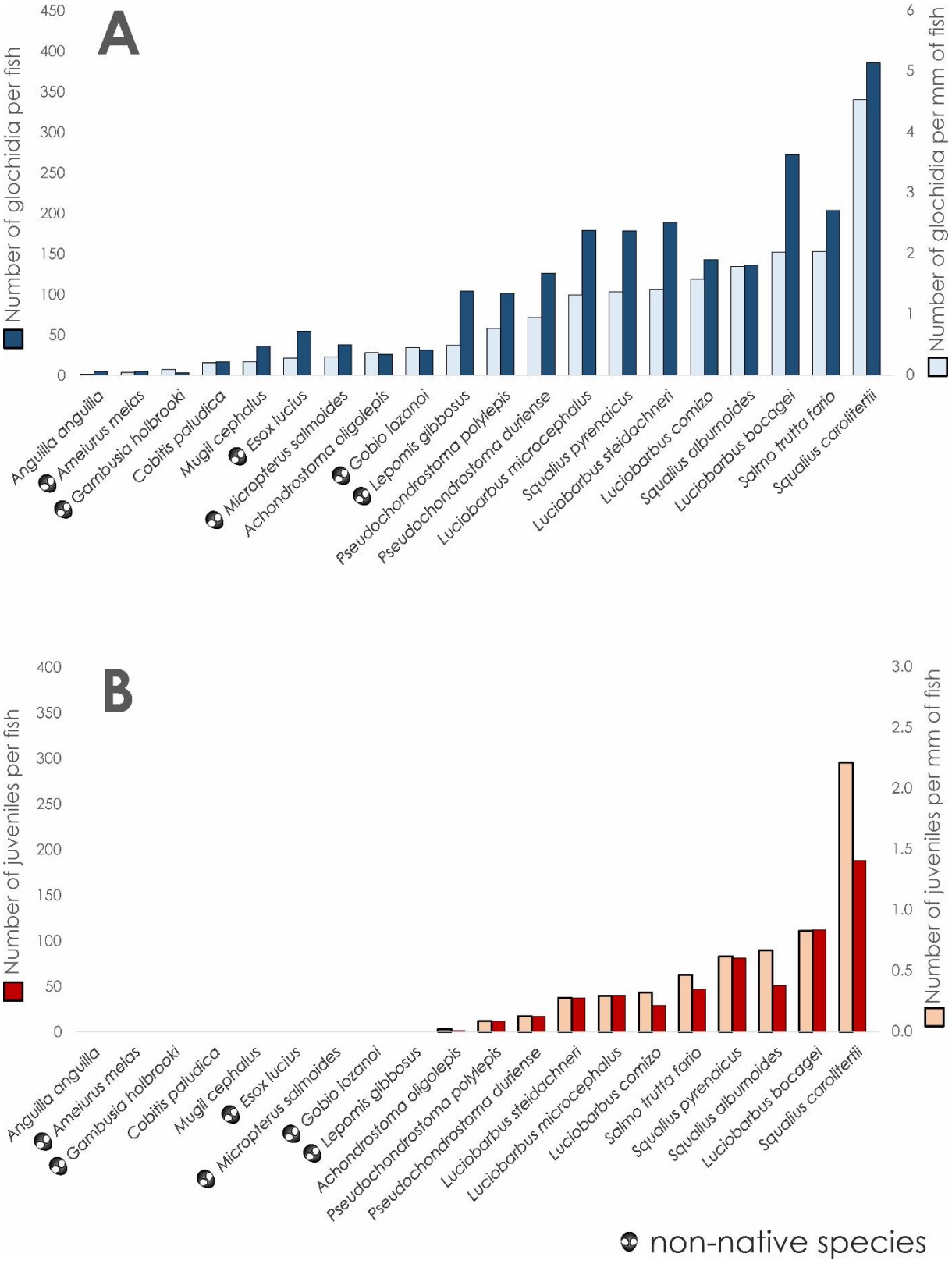

Figure 6 A - Mean glochidial infestation (i.e. the number of glochidia per fish and mm of fish) in all fish species; **B** - Effective transformation of glochidia into juveniles (i.e. the number of juveniles produced per fish and mm of fish) in all fish species.

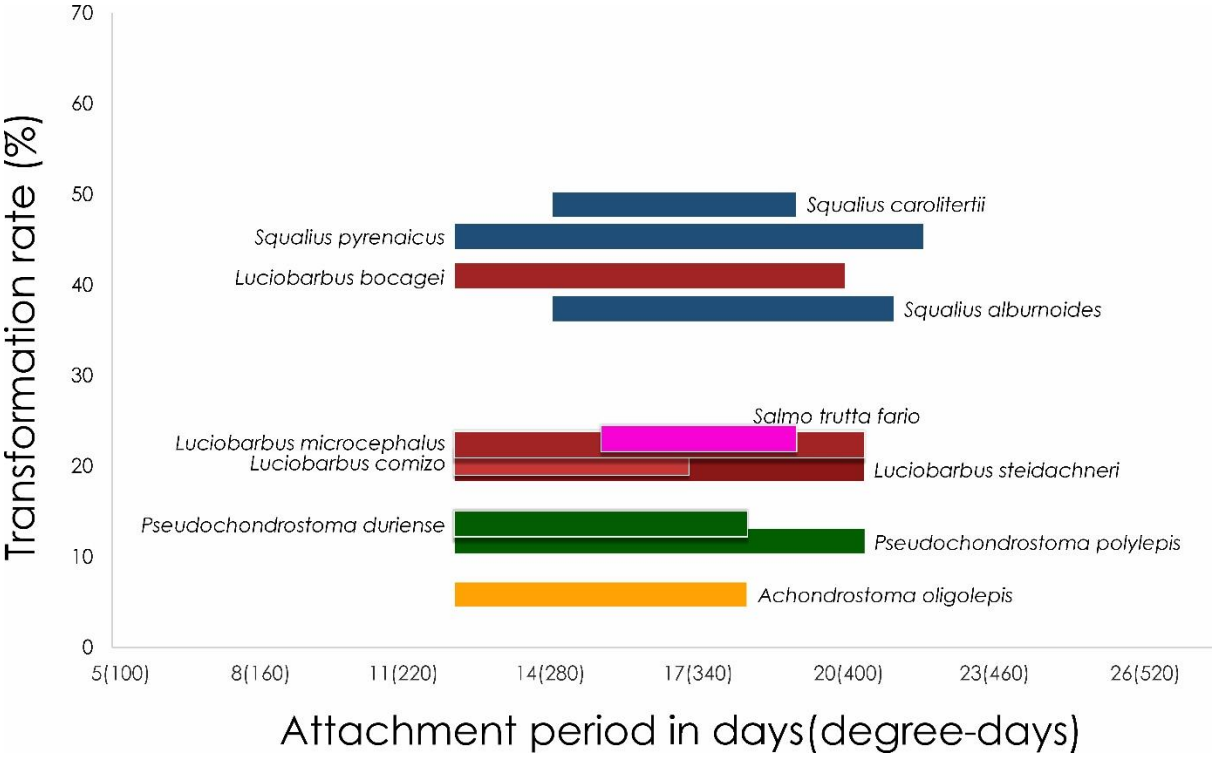

Figure 7 Glochidial transformation rate and attachment periods (shown in bars) per fish host species.

Discussion

This study describes for the first time the main life-history traits of the Iberian dolphin freshwater mussel *U. delphinus*. This information is vital to increase the basic knowledge about the biology and ecology of this species, and it may be used for mainstreaming this species as a valuable environmental indicator and to develop conservation management programs for its populations.

Distribution

Unio delphinus is widely distributed in the Atlantic coast of the Iberian Peninsula, mainly in its larger river basins, i.e. Minho, Douro, Tejo, Guadiana, and Guadalquivir (Fig. 1). This distribution is extended to some smaller river basins north of the Minho and east of the Guadalquivir. Populations south of the Tejo River basin are highly threatened due to habitat degradation and fragmentation, and water shortage. As previously described (Gomes-dos-Santos et al 2019), estimates of EOO and AOO are largely dependent on the method used. The larger estimates of AOO using the 2 × 2 km grid would allow the species to be listed as Vulnerable using the B criterion but we follow the suggested method of Gomes-dos-Santos et al (2019), using the mean length of the river as the best estimation method, that places the species as Endangered (Appendix 2).

Growth and longevity

The well-studied freshwater pearl mussel *Margaritifera margaritifera* and thick-shelled river mussel *Unio crassus* exhibit slow growth and may live up to 280 and 90 years, respectively (Lopes-Lima et al 2017a). The present study shows that the Iberian dolphin freshwater mussel *U. delphinus* presents a distinct growth pattern being a short-lived and fast-growing unionid species. These results corroborate the trends described within the tribe Unionini, where growth rates are generally faster and life spans shorter than most other unionid groups (Haag & Rypel 2011). A North-South latitudinal gradient has been previously reported for freshwater mussels growth, with several species showing slower growth but greater longevity for populations living at higher latitudes (Haag & Rypel 2011), including the European species *M. margaritifera* (San Miguel et al 2004) and *U. crassus* (Helama et al 2017). This pattern might be explained by the lower temperatures and shorter growth periods of the northern regions (Dunca & Mutvei 2001; Schone et al 2004). Comparing the obtained values of the growth constant K of the *U. delphinus* populations with previously published results from other *Unio* species, a marked inter-specific latitudinal gradient is evident: most populations of *Unio pictorum* from England and Russia (Aldridge 1999, Rizhinashvili 2008), and *U. crassus* from Central Europe (Hochwald 2001; Helama et al 2017) showed lower K values than those obtained here for *U. delphinus* and those previously published on the other Iberian *Unio* species, i.e. *Unio tumidiformis* (Reis & Araujo 2016) and the Middle eastern *Unio terminalis* (Ostrovsky et al 1993). Possibly due to the low number of sites tested and/or the much lower latitudinal distribution of *U. delphinus* compared with *U. crassus* or *M. margaritifera*, a north-south pattern was not evident for *U. delphinus* populations within Iberia, where growth and longevity showed no significant differences (Table 1). In contrast, the growth constant K was lower and maximum length higher in the populations from lentic than those from lotic habitats (Fig. 2; Table 1). Freshwater mussel growth is thought to be influenced by productivity and food availability, substrate type, water flow and exposure to wind and current (Haag & Rypel 2011 and references therein). The larger maximum size and lower K values of the Lagoon populations in Iberia should be related to the very high productivity and hydrological stability of these habitats (Varandas et al 2014), when comparing with the River populations. Despite the distinct growth patterns of Lagoon and River populations, the maximum age does not vary considerably between habitat types, ranging between 7 and 11 years old, average 9; Table 1). These values are within the lower end for the European *Unio* species range that has been reported from 5 to more than 50 and may even reach 90 years old for the *U. crassus* northern populations (Lopes-Lima et al 2017a and references therein).

The age of maturity determined in the Sabor population is higher for females (2.5 years) than for males (1.6 years), with a mean maturity value for the species around 2 years, similar to previously reported values for other *Unio* species (Lopes-Lima et al 2017a). Based on the

maximum longevity and age of maturity, the mean generation length of *U. delphinus* is about 5-6 years.

Reproductive cycle and sexual strategy

No hermaphroditism was detected, showing that *U. delphinus* is strictly dioecious, with a female-male ratio close to 1 but always with a dominance of males (Table 3). Histological studies on other freshwater mussels show that the sexes are typically separate, though hermaphroditism has been detected in some species (Van der Schalie 1970; Kat 2009). Nevertheless, the genus *Unio* seems to be strictly dioecious since no case of complete hermaphroditism has been previously detected for any species (e.g. Aldridge 1999; Cek & Sereflişan 2006; Lopes-Lima et al 2017a).

The reproductive cycle, characterized by the GDI, is continuous and uninterrupted with both male and female reproductive cells being found throughout the year. This contrasts with the reproductive cycle of the congeneric *U. terminalis* and *U. tumidiformis* that seems to exhibit a long and single reproductive cycle (Cek & Sereflişan 2006; Reis & Araujo 2016). Brooding and discharge periods overlap with what is known for most *Unio* species where brooding is coincident with the glochidia discharge period in the spring-summer months (i.e. generally between April and August; Lopes-Lima et al 2017a and references therein). No glochidia have been found after August which is coherent with results for other *Unio* species (Lopes-Lima et al 2017a). This might indicate that we failed to detect a second glochidia discharge period.

Unio delphinus, as all species within the subfamily Unioninae, is ectobranchous (Lopes-Lima et al 2017b), meaning that the females only use the outer pair of demibranchs as a brooding chamber for glochidia, also known as marsupium (Fig. 5). Glochidia mature and stay in the marsupium during short periods (2-3 weeks) and thus *U. delphinus* can be classified as tachytictic or short-term brooder. Glochidia are discharged by the exhalant aperture to the water column entangled in mucous threads, although feather-like constructs full of glochidia and eggs, also known as conglutinates, are also produced (Fig. 5d). However, as also seen in the congeneric *U. pictorum*, these conglutinates contain variable quantities of mature glochidia and its function is still uncertain, probably being released by females while under hypoxic stress, to increase ventilation (Aldridge 1999).

Fish hosts

As in all freshwater mussels, the life cycle of the Iberian dolphin freshwater mussel includes a parasitic stage, in which the larvae (glochidia) need to attach to fish to continue their development and metamorphose into a young juvenile (Modesto et al 2018). Therefore, it is crucial to understand the dynamic interactions between freshwater mussels and their fish hosts. Our study shows that *U. delphinus* glochidia may attach to all fish species on the trials

(Fig. 6; Table 4). However, it attached preferentially to native fish species, with the attachment rates to non-native species being much lower. Furthermore, the effective transformation rate of glochidia into juveniles only occurred in 10 cyprinid and 1 salmonid native fish species. Transformation rates were especially high in all fish species from the genus *Squalius*, followed by *Luciobarbus, Salmo* and *Pseudochondrostoma* species. This indicates a strong co-evolutionary relationship of *U. delphinus* with native co-occurring fish and especially with *Squalius* species. This link of the freshwater mussel genus *Unio* with cyprinids and especially *Squalius* species has been previously reported (Lopes-Lima et al 2017a). For example, except for the more divergent *Unio tumidus*, all European *Unio* species seem to use at least one species of *Squalius* as hosts (Lopes-Lima et al 2017a). Furthermore, *Squalius cephalus* seems to be the main host in some *U. crassus* populations (Taeubert et al 2012) and the Southern Iberian *U. tumidiformis* is only able to metamorphose in *Squalius* species (Reis et al 2014). The attachment period did not seem to vary much across the effective host species, occurring between 12 and 22 days post attachment at a constant temperature of 20 °C with the sum of daily temperatures during metamorphosis ranging from 240 to 440 degree-days (Fig. 7; Table 4). This speed of transformation seems to be lower than the southern Iberian *U. tumidiformis* (Reis et al 2014) and more like that reported to the more closely related species the Eastern Iberian *Unio mancus* (Araujo et al 2005).

Practical implications for conservation and environmental monitoring

Our results have important implications on future management and conservation actions for the Iberian dolphin freshwater mussel *U. delphinus.* The detailed compilation of distribution data and estimation of the EOO and AOO will allow for a more accurate assessment using IUCN categories and criteria (Appendix 2). Information about growth, age of maturity and life span are also especially critical in evaluating the risk of extinction of rare and threatened species, being important components of the evaluation criteria of IUCN Red-Listing. Unionid mussels were for long portrayed as long-lived and slow-growing organisms, but this has been increasingly demystified with species like *U. delphinus*, presenting distinct growth patterns and lifespan (Haag & Rypel 2011).

The information about the reproductive cycle here described is very important for future propagation programs and other conservation actions. For instance, gravid females should be searched mainly from late Spring to late Summer. Additionally, if the aim is getting a brood stock of cultivated mussels, only after two years would these mussels be ready for reproduction.

Although *U. delphinus* seems to have a continuous reproductive cycle, both oogenesis and spermatogenesis deaccelerate between August and March. Therefore, translocation and monitoring programs on this species ideally should be done in September before the rainy

season to minimize the impact on reproduction, and never during spring/summer where manipulation and transportation stress may lead to reduced reproductive intensity and ejection or abortion of larval content. Potential translocations should consider that enough time should be given to specimens to settle before the heavy torrential floods that generally occur from October onward, especially in the intermittent rivers in the South. Knowledge about the fish hosts is also crucial for the future of the species. As already reported in other Mediterranean species (e.g. *Unio foucauldianus*; Benaissa et al 2019), especially those with restricted ranges in southern Europe or North Africa, *U. delphinus* seems to be unable to transform in any of the non-native species. This turns the on-going biotic homogenization of the Iberian fish fauna due to the constant introduction of new non-native fish species in Iberian freshwater habitats (Clavero et al 2011, 2013; Anastacio et al 2019), one of the major threats to this mussel species. Furthermore, species like *Lepomis gibbosus* and *Micropterus salmoides* are now major components of many Iberian freshwaters. These fish are piscivorous and very aggressive, making nests at the banks, where *U. delphinus* generally aggregates (Authors pers. obs.). These invasive species maintain other native fish species far from the banks, potentially decreasing attachment success of *U. delphinus* larvae. Conversely, most larvae will attach to the non-native fish that will act as ecological sinks. The laboratory host fish studies here developed should be complemented with future field experiments to test which fish species are serving as the effective hosts and to better estimate the impact of invasive species.

Unio delphinus possesses many of the requirements for indicator species (Carignan & Villard 2002), and here we provide a solid and comprehensive ecological and developmental data baseline that will allow developing its potential even further. Besides its inherent conservation importance, information on the growth patterns and life-history traits here described should now be optimized for use as ecological indicators. This could be achieved, for instance, by comparing populations exposed to distinct disturbance types and levels. More comprehensive surveys of *U. delphinus* populations and varying compositions of co-occurring fish species could help to develop standardized metrics to assess the status and integrity of fish communities. Given that this mussel is unable to reproduce in most invasive fish, its decline or local extirpation can be a direct effect of the decline, extirpation or changes in the fish fauna. This already occurred in Lake Banyoles (Spain), where despite good abiotic conditions, all native fish are now extirpated and replaced by many other non-native species, with freshwater mussel populations declining and disappearing soon after (Garcia-Berthou et al 2000; Araujo et al 2015).

Conclusions

Although the study of life-history traits has now become old-fashioned and generally considered of local/regional interest or purely descriptive, conservation efforts are strongly hindered by the lack of this basic knowledge. Furthermore, many recent studies, e.g. state of the art modelling exercises and multispecies biological and biogeographical meta-analyses, that are attracting a lot of scientific attention, strongly rely on these basic biological data. The present study makes practical considerations about the conservation of a declining freshwater species endemic to one of the global biodiversity hotspots and highlights the need to go back to the basics and to promote the study of life-history traits of poorly studied taxa, especially those of conservation concern.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Portuguese FCT - Foundation for Science and Technology, Projects FRESHCO: Multiple implications of invasive species on Freshwater Mussel co-extinction processes (PTDC/AGR-FOR/1627/2014-04/SAICT/2015), MUSSELFLOW: Host-dependent evolution, ecology and conservation of freshwater mussels under varying hydrological conditions: consequences of climate change (PTDC/BIA-EVL/29199/2017), and ConBiomics: The missing approach for the Conservation of freshwater Bivalves Project N° NORTE-01-0145- FEDER-030286, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF. FCT also supported MLL under the grant (SFRH/BD/115728/2016), CM under the grant (SFRH/BD/111133/2015) and through Strategic Funding UID/Multi/04423/2019. PB was supported by EDP Biodiversity Chair.

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Appendix 1 Bibliography used to compile the distribution records of *Unio delphinus* which were complemented by personal data from all the authors.

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Appendix 2 Red List assessment of *Unio delphinus* Spengler, 1793

Scientific Name

Unio delphinus Spengler, 1793

Common Name

Iberian dolphin freshwater mussel

Taxonomic Notes

Unio populations from northwest Iberia were thought to belong to the widespread and more common European *Unio pictorum* and the species name *Unio delphinus* its junior synonym, until its recent recognition as a valid distinct species (Araujo et al 2009; Lopes-Lima et al 2017). *Unio* populations from Morocco, previously thought to be *U. delphinus,* belong to a separate species, the Moroccan endemic *Unio foucauldianus* Pallary, 1936 (Froufe et al 2016).

Red List Assessment

Endangered B2ab(ii,iii,iv)

Justification

The species has been declining significantly up to 30% over the last three generations, being this reported decline not enough to place the species in one of the threatened categories using criterion A (Araujo 2009). However, the species is severely fragmented with an estimated Area of Occupancy (AOO) of 61.45 Km^2 and exhibiting an observed continuing decline in terms of habitat, area of occupancy and number of populations. Therefore, the species is here listed as Endangered B2ab(ii,iii,iv).

Geographic Range

Unio delphinus populations have been recorded in most Atlantic basins of the Iberian Peninsula, from the Ulla river in the north to the Guadalquivir in the south and then east to the La Vega River basin near Gibraltar. It also occurs in few Mediterranean coastal basins east of Gibraltar until the Guadalhorce River basin near Malaga. Many populations have been recently extirpated due to dam construction in the Douro and Guadiana basins. Populations on the south have been disappearing due to decreased water levels.

Habitat and Ecology

It is generally found in the middle and lower sections of rivers and streams in a great variety of substrates from silt to sand and gravel, close to the banks (Araujo et al 2009). It may occasionally occur in standing water habitats like the Ruidera Lakes in Spain and Mira and Barrinha de Mira lagoons in Portugal. Larvae release occurs continuously from May to August, with two discharge peaks in May and August. The fish hosts are co-occurring native cyprinids and the native salmonid species the brown trout *Salmo trutta fario*.

Threats

Urban pollution, mainly in the coastal areas, has been the main cause of extirpation until the beginning of the 21st century. The species has been threatened by habitat loss and fragmentation due to the construction of dams and impoundments that continue to the present. In the south of Portugal and Spain, water shortage is the main threat due to the increasing demand for agriculture and urban purposes. This situation is further exacerbated by global warming scenarios that will likely induce irreversible trends of desertification. Given that *U. delphinus* seems to be unable to use non-native fish species as hosts, biotic homogenization and fish introductions may become a major threat to the species, soon.

Conservation Actions

This species would benefit from the restoration and maintenance of ecological flows in dams, wastewater treatment, river rehabilitation, and whole catchment management. The species should benefit from propagation programs for reintroduction in restored habitats.

Research Needs

Long term surveys are needed to monitor the population trends of the most important populations. Reproduction and ex-situ culture studies are needed for eventual propagation programs.

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CHAPTER 4

Phylogeny of the family Unionidae

Paper III

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

Lopes-Lima M, Froufe E, Do VT, Ghamizi M, Mock KE, Kebapçi Ü, Klishko O, Kovitvadhi S, Kovitvadhi U, Paulo OS, Pfeiffer JM, Raley M, Riccardi N, Şereflişan H, Sousa R, Teixeira A, Varandas S, Wu X, Zanatta DT, Zieritz A, Bogan AE

Article published in *Molecular Phylogenetics and Evolution* **106**, 174-191 (2017).
DOI: 10.1016/j.ympev.2016.08.021

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 these 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 behavioural characters used in traditional phylogenetic studies. No single morphological, anatomical or behavioural 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.

Keywords

Mollusca, Systematics, Freshwater mussels, Taxonomy, Classification

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 (Lydeard et al 2004; IUCN 2015). 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; Hoeh et al 1996, 2002a ; Breton et al 2007; Barnhart et al 2008), 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 & Roe 2008; Kohler et al 2012; IUCN 2015). The Unionidae is by far the most species-rich family within the order Unionida, with 620 species in 142 genera (Bogan & 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 & 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; Iredale 1934; Haas 1940), 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: Modell 1942, 1949, 1964; Haas 1969a,b). 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.

Table 1
Historical classification systems of the subfamilies and tribes now included in the Unionidae. (Blue) subfamilies; (red) tribes; (^{nn}) *nomen novum*; (*) regional study; (?) rank uncertain.

Simpson (1900/1914)	**Ortmann (1912)***	**Modell (1942)**	**Haas (1969a, 1969b)**	**Heard & Guckert (1970)***	**Brandt (1974)***
Unionidae	**Unionidae**	**Unionidae**	**Unionidae**	**Unionidae**	**Unionidae**
Unioninae	Unioninae	Unioninae	Alasmidontinae	Unioninae	Hyriopsinae
Hyriinae	Anodontinae	Anodontinae	Anodontinae	Anodontinae	Pseudodontinae
	Lampsilinae	Cafferiinae	Hyriinae	Lampsilinae	Parreysiinae
		Coelaturinae	Lampsilinae	Pleurobeminae	Rectidentinae
		Contradentinae	Quadrulinae	Popenaiadinae	Modellnaiinae
		Hyriinae	Unioninae		
		Hyriopsinae		**Amblemidae**	
		Lamellidentinae		Ambleminae	
		Lamprotulinae		Gonideinae	
		Nannonaiinae		Megalonaiadinae	
		Parreysiinae			
		Propehyridellinae			
		Quadrulinae			
		Rectidentinae			
		Elliptionidae			
		Alasmidontinae			
		Ambleminae			
		Elliptioninae			
		Lampsilinae			
		Pleurobeminae			
		Margaritiferidae			
		Heudeaninae			
		Pseudodontinae			

Table 1 (*cont.*)

Graf & Cummings (2007)	Bieler et al (2010)	Whelan et al (2011)	Carter et al (2011)	This Study
Unionidae	**Unionidae**	**Unionidae**	**Unionidae**	**Unionidae**
Unioninae	Unioninae	Unioninae	Unioninae	Unioninae
Unionini	Unionini	Unionini	Unionini	Unionini
Anodontini	Anodontini	Anodontini	Anodontini	Ambleminae
Ambleminae	Ambleminae	Ambleminae	Ambleminae	Amblemini
Amblemini	Amblemini	Amblemini	Amblemini	Lampsilini
Gonideini	Lampsilini	Lampsilini	Lampsilini	Pleurobemini
Lampsilini	?Oxynaiini	Pleurobemini	Pleurobemini	Quadrulini
Pleurobemini	Pleurobemini	Quadrulini	Quadrulini	Anodontinae
Quadrulini	Quadrulini	Gonideinae	Gonideinae	Anodontini
	Coelaturinae	Modellnaiinae	Modellnaiinae	Cristariini*nn*
	Gonideinae	Parreysiinae	Parreysiinae	Lanceolariini*nn*
	Modellnaiinae	Coelaturini	Rectidentinae	Gonideinae
	Parreysiinae	Oxynaiini		Gonideini
	Rectidentinae	Parreysiini		Chamberlainiini*nn*
		Lamellidentini		Lamprotulini
		Rectidentinae		Pseudodontini
				Parreysiinae
				Coelaturini
				Oxynaiini
				Parreysiini
				Lamellidentini
				Rectidentinae
				Rectidentini
				Contradentini
				?Modellnaiinae

Nevertheless, the work by Haas has been widely recognized as the more reliable in terms of representing generic and sub-generic distinctiveness and is considered fundamental in establishing the main genera of the Unionida and in particular, the Unionidae (Roe & 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 & 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 malacologists (see Graf 2007) and emergent Russian studies (Klishko et al 2014; Bolotov et al 2015).

A comprehensive molecular phylogenetic study of the Unionidae has not been attempted to date, primarily due to the difficulties in developing a dataset of enough geographical and species coverage. The first classification system using a phylogenetic framework was published by Heard & Guckert (1970; Table 1) for the North American Unionida fauna. Disregarding shell characters, these authors used a broad anatomical and reproductive behaviour 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. Davis et al 1977, 1981; Davis & Fuller 1981; Davis 1983, 1984; Hoeh et al 1998, 2001, 2002b, 2009; Roe & Hoeh 2003; Campbell et al 2005; Graf & Cummings 2006; Zanatta & Murphy 2006; Whelan et al 2011; Campbell & Lydeard 2012a,b; Pfeiffer & Graf 2013, 2015). 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. Nagel & Badino 2001; Roe & Hoeh 2003; Campbell & Lydeard 2012a,b). 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 (Whelan et al 2011; Graf 2013; Elderkin et al 2016) and Asia (Huang et al 2002; Zhou et al 2007; Pfeiffer & Graf 2013, 2015; Zieritz et al 2016).

Recent molecular phylogenetic studies have achieved considerable progress in describing the main divisions within the Unionidae (Graf & Cummings 2006; Whelan et al 2011; Campbell & Lydeard 2012a, 2012b; Pfeiffer & Graf 2013, 2015). The status of the North American Ambleminae with four recognized tribes has been recently confirmed (Table 1: Campbell et al 2005; Campbell & Lydeard 2012a,b). Studies including species from Africa and the Indotropics examined the subfamily Parreysiinae in detail and recognized several subfamilies (Table 1: Whelan et al 2011; Pfeiffer & Graf 2015). Despite the considerable recent progress (Huang et al 2002; Zhou et al 2007; Pfeiffer & Graf 2013, 2015), the vast majority of

unionid genera from the Eastern Palearctic and the Indotropics have never been analysed 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 Modellnaiinae with a single species from Thailand; the Rectidentinae with a Southeast Asian distribution; and two subfamilies, the Unioninae and Gonideinae, distributed through most of Asia, Europe, North Africa and west coast of North America.

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.

Materials and methods

Taxon sampling

All analysed taxa are listed in Table 2. Taxa were chosen to cover all available genera of Unionidae subfamilies. Exceptions were made concerning 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 & Lydeard 2012a,b; Campbell et al 2005; Whelan et al 2011). Taxa representatives 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 & Wheeler 2002).

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 ºC were used for COI (LCO1490 + HCO2198) and 28S; and 50 ºC for COI (LCO22me2 + HCO700dy2). Amplified DNA templates were purified and sequenced by the commercial company Macrogen using the same primers.

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, 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 & Standley 2013). The following GUIDANCE parameters were used: GUIDANCE score algorithm; 100 bootstrap replicates; a
sequence cut-off score of 0.0 (no sequence removal); a column cut off 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 (Darriba 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 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 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) using the previously selected models. Analyses were initiated with program-generated trees and four Markov chains with default incremental heating.

Table 2

Specimens analysed. [U]unkonwn country; *not generated from a single individual. Taxonomy follows Table 3.

Taxon	COI	28S	Country	Reference voucher specimen)
ANODONTINAE				
ANODONTINI				
Alasmidonta marginata Say, 1818	AF156502	AF400688	USA	Graf & Foighil 2000; Graf & Cummings 2006
Anodonta anatina (Linnaeus, 1758)	KX822632	KX822588	Russia	This study
Anodonta cygnea (Linnaeus, 1758)	KX822633	KX822589	Italy	This study
Anodonta nuttalliana Lea, 1838	KX822634	KX822590	USA	This study
Lasmigona compressa (Lea, 1829)	AF156503	DQ191414	USA	Graf & Foighil 2000; Graf & Cummings 2006
Pseudanodonta complanata (Rossmässler, 1835)	KX822661	KX822617	Ukraine	This study
Pyganodon grandis (Say, 1829)*	AF231734	AF305384	USA	Bogan & Hoeh 2000; Graf & Foighil 2000
Simpsonaias ambigua (Say, 1825)	KX822666	KX822622	USA	This study (NCSM30607)
Strophitus undulatus (Say, 1817)*	AF156505	DQ191415	USA	Graf & Foighil 2000; Graf & Cummings 2006
CRISTARIINI				
Anemina arcaeformis (Heude, 1877)	KF667530	KX822587	China	An et al 2016; this study
Cristaria plicata (Leach, 1814)	KX822637	KX822594	Russia	This study
Plethholophus tenuis (Griffith & Pidgeon, 1833)	KX822658	KX822614	Vietnam	This study (NCSM84924)
*Sinanodonta lucida (*Heude, 1877)	KX822667	KX822624	China	This study
Sinanodonta woodiana (Lea, 1834)	KX822668	KX822625	Vietnam	This study (NCSM84916)
LANCEOLARIINI				
Arconaia lanceolata (Lea, 1856)	NC_023955	KX822591	China	Wang et al 2016; this study
Lanceolaria gladiola (Heude, 1877)	KX822648	KX822605	China	This study
Lanceolaria grayana (Lea, 1834)	KX822649	KX822606	China	This study
Lanceolaria grayii (Griffith & Pidgeon, 1833)	KX822650	KX822607	Vietnam	This study (NCSM84945)
UNIONINAE				
UNIONINI				
Unio crassus Philipsson, 1788*	KC703878	KC703644	France	Prié & Puillandre 2014
Unio gibbus Spengler, 1793	KX822671	KX822629	Morocco	This study
Unio pictorum (Linnaeus, 1758)	KC429109	KC429447	[U](Europe)	Sharma et al 2013
Unio tumidus Philipsson, 1788	KX822672	KX822630	Ukraine	This study
incertae sedis (UNIONINAE)				
Aculamprotula tortuosa (Lea, 1865)	KX822631	KX822586	China	This study
Cuneopsis heudei (Heude, 1874)	KX822638	KX822595	China	This study

Taxon	COI	28S	Country	Reference (voucher specimen)
Cuneopsis pisciculus (Heude, 1874)	KX822639	KX822596	China	This study
Cuneopsis rufescens (Heude, 1874)	KX822640	KX822597	China	This study
Nodularia douglasiae (Griffith & Pidgeon, 1833)	KX822653	KX822610	China	This study
Nodularia nuxpersicae Dunker, 1848	KX822654	KX822611	Vietnam	This study (NCSM84990)
Schistodesmus lampreyanus (Baird & Adams, 1867)	KX822665	KX822621	China	This study
RECTIDENTINAE				
CONTRADENTINI				
Contradens contradens (Lea, 1838)	DQ191411	AF400692	[U](Asia)	Graf & Cummings 2006
Contradens semmelinki (Martens, 1891)	KX822636	KX822593	Vietnam	This study (NCSM84935)
Physunio modelli Brandt, 1974	KX822655	KX822612	Thailand	This study
Trapezoideus exolescens (Gould, 1843)	KP795036	KP795018	Laos	Pfeiffer & Graf 2015
RECTIDENTINI				
Ensidens ingallsianus (Lea, 1852)	KX822641	KX822598	Laos	This study (NCSM84889)
Ensidens sagittarius (Lea, 1856)	KP795033	KP795015	Cambodia	Pfeiffer & Graf 2015
Ensidens sp.	KX822642	KX822599	Laos	This study (NCSM84902)
Hyriopsis bialata Simpson, 1900	KX822643	KX822600	Thailand	This study
Hyriopsis desowitzi Brandt, 1974	KX822644	KX822601	Thailand	This study
Hyriopsis myersiana (Lea, 1856)	KX822645	KX822602	Thailand	This study
Rectidens sumatrensis (Dunker, 1852)	KX822664	KX822620	Malaysia	This study
GONIDEINAE				
CHAMBERLAINIINI				
Chamberlainia hainesiana (Lea, 1856)	KX822635	KX822592	Thailand	This study
Sinohyriopsis cumingii (Lea, 1852)*	HM347668	KX822623	China	unpublished; this study
LAMPROTULINI				
Lamprotula caveata (Heude, 1877)	KX822646	KX822603	China	This study
Lamprotula leaii (Griffith & Pidgeon, 1833)	KX822647	KX822604	China	This study
Potomida littoralis (Cuvier, 1798)	JN243905	JN243883	France	Whelan et al 2011
Pronodularia japanensis (Lea, 1859)	KX822659	KX822615	Japan	This study (NCSM27183)
GONIDEINI				
Gonidea angulata (Lea, 1838)*	DQ272371	AF400691	USA	Gustafson & Iwamoto 2005; Graf 2002
Leguminaia wheatleyi (Lea, 1862)	KX822651	KX822608	Turkey	This study
Microcondylaea bonellii (A. Ferussac 1827)	KX822652	KX822609	Italy	This study
Solenaia carinata (Heude, 1877)	KX822669	KX822626	China	This study

Taxon	COI	28S	Country	Reference (voucher specimen)
Solenaia oleivora (Heude, 1877)	KX822670	KX822627	China	This study
PSEUDODONTINI				
Pilsbryoconcha compressa (Martens, 1860)	KX822656	KX822613	Thailand	This study
Pilsbryoconcha exilis (Lea, 1838)*	KX822657	AF400693	Vietnam	Graf 2002; this study
Pseudodon cambodjensis (Petit de la Saussaye, 1865)	KX822660	KX822616	Thailand	This study
Pseudodon cumingii (Lea, 1850)	KX822662	KX822618	Laos	This study (NCSM84884)
Pseudodon mouhotii (Lea, 1863)	KX822663	KX822619	Laos	This study (NCSM84903)
incertae sedis (GONIDEINAE)				
Solenaia triangularis (Heude, 1885)	KJ434518	KX822628	China	This study
AMBLEMINAE				
AMBLEMINI				
Amblema plicata (Say, 1817)	APU56841	AF305385	USA	Hoeh et al 1998; Graf 2002
LAMPSILINI				
Actinonaias ligamentina (Lamarck, 1819)	AF156517	DQ191420	USA	Graf & Foighil 2000; Graf & Cummings 2006
Lampsilis cardium Rafinesque, 1820*	AF120653	AF305386	USA	Giribet & Wheeler 2002; Graf 2002
Villosa iris (Lea, 1829)	AF156524	DQ191422	USA	Graf & Foighil 2000; Graf & Cummings 2006
PLEUROBEMINI				
Elliptio complanata (Lightfoot, 1786)*	EU448173	JF899181	USA	Unpublished; Distel et al 2011
Elliptio dilatata (Rafinesque, 1820)*	AF156507	AF400690	USA	Graf & Foighil 2000; Graf 2002
Pleurobema sintoxia (Rafinesque, 1820)	AF156509	DQ191418	USA	Graf & Foighil 2000; Graf & Cummings 2006
QUADRULINI				
Quadrula quadrula (Rafinesque, 1820)	AF156511	DQ191417	USA	Graf & Foighil 2000; Graf & Cummings 2006
Quadrula verrucosa (Rafinesque, 1820)	DQ191413	DQ191416	USA	Graf & Cummings 2006
PARREYSIINAE				
COELATURINI				
Coelatura aegyptiaca (Cailliaud, 1827)	JN243892	JN243870	Egypt	Whelan et al 2011
LAMELLIDENTINI				
Lamellidens corrianus (Lea, 1834)	JN243903	JN243881	Burma	Whelan et al 2011
OXYNAIINI				
Oxynaia pugio (Benson, 1862)	JN243899	JN243879	Burma	Whelan et al 2011
PARREYSIINI				
Parreysia mandelayensis (Theobald, 1873)	JN243900	JN243876	Burma	Whelan et al 2011

Two independent runs of 24 × 10^6 generations were sampled at intervals of 1,000 generations producing a total of 24,000 trees. Burn-in was determined upon the convergence of log-likelihood and parameter values using Tracer 1.6 (Rambaut et al 2014).

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

Review of morphological, anatomical, and behavioural 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 analysed taxa. To characterize and compare glochidial size, the glochidial size index (Gln) was calculated following Davis & Fuller (1981) where Gln = glochidial shell length (μm) × shell height (μm) × 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.

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 (Zhadin 1938; Haas 1969a,b; Moskvicheva 1973a,b; Brandt 1974; Đang et al 1980; Clarke 1981; Zatravkin & Bogatov 1987; Subba Rao 1989; Bogatov & Starobogatov 1992; Howells et al 1996; Klishko 2001, 2003; Prozorova & Bogatov 2006; Cyr et al 2007; Vinarski et al 2007; Kondo 2008; Nedeau et al 2009; Bogatov 2012; Doucet-Beaupré et al 2012; He & Zhuang 2013). 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).

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 1,091 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 the other Unionidae.

The dataset including only Unionidae taxa spanned 70 species in 46 genera, with a total of 1,032 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 into 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).

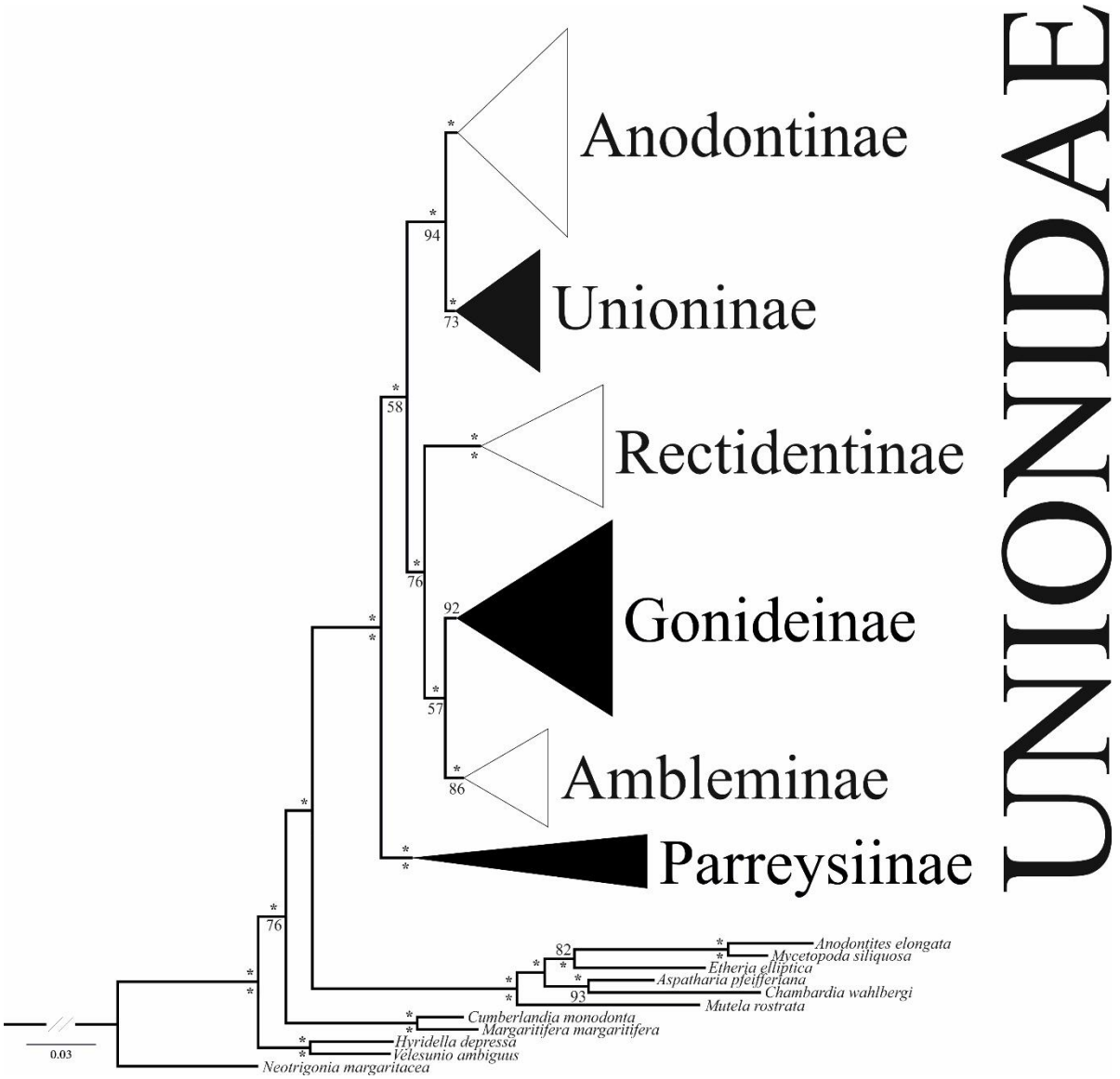

Figure 1 Phylogenetic tree of the Palaeoheterodonta obtained by Bayesian Inference (BI) and Maximum likelihood (ML) analyses of the first combined (COI + 28S) dataset. Support values above the branches are posterior probabilities (BI4) and bootstrap support (ML4) below. An asterisk (*) indicates nodes with P ≥ 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.

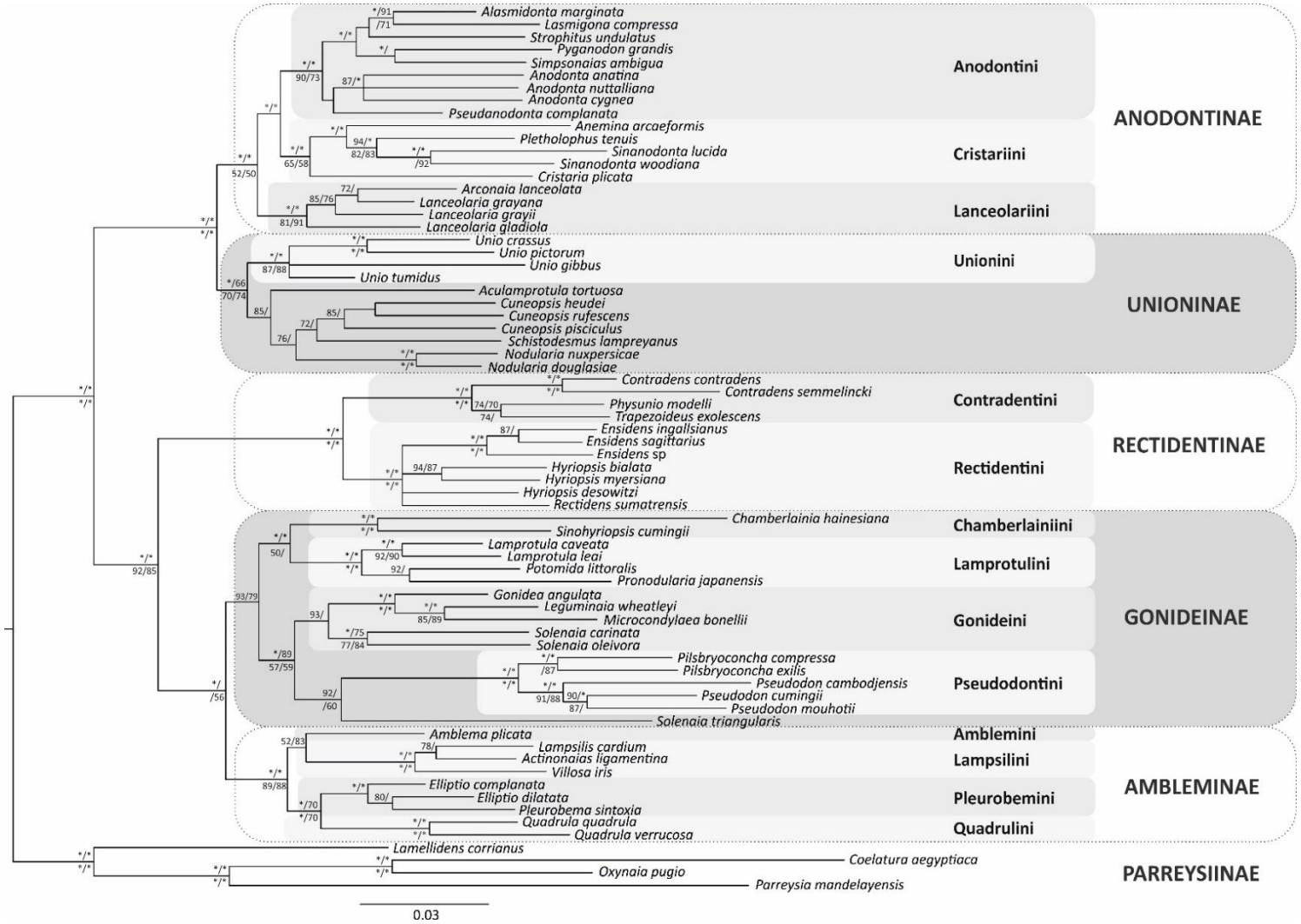

Figure 2 Phylogenetic tree of the Unionidae obtained by Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of the second combined (COI + 28S) dataset. Support values above the branches are posterior probabilities (BI4/BI2) and bootstrap support (ML4/ML2) below. An asterisk (*) indicates nodes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

In summary, our molecular phylogenetic analyses revealed a division of the Unionidae into 6 subfamilies and 18 tribes, three of which are named here for the first time. Revisions on the subfamilial and tribal classification within the Unionidae are discussed here along with several lower-level phylogenetic and taxonomic considerations.

To complement the present molecular analyses, seven morphological, anatomical and behavioural 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-behavioural character analysed 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 analysed 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).

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).

Table 3
Classification of the Unionidae based on the present analyses. (*) Not included in the present study.

ANODONTINAE Rafinesque, 1820
- **Anodontini** Rafinesque, 1820
- + Alasmidontini Rafinesque, 1820
- + Strophitini Starobogatov, 1970
- + Pseudanodontini Starobogatov, 1970
- + Brachanodonini Bogatov, Sayenko & Starobogatov, 2002
  - ****Arcidens*** Simpson, 1900 [+ *Arkansia* Ortmann & Walker 1912]
  - ***Alasmidonta*** Say, 1818
  - ***Anodonta*** Lamarck, 1799
  - ****Anodontoides*** Simpson in F.C Baker, 1898
  - ***Lasmigona*** Rafinesque, 1831
  - ***Pseudanodonta*** Bourguignat, 1876
  - ***Pyganodon*** Crosse & Fischer, 1894
  - ***Simpsonaias***, Frierson, 1914
  - ***Strophitus*** Rafinesque, 1820
  - ****Utterbackia*** F.C. Baker, 1927
- **Cristariini** Lopes-Lima, Bogan & Froufe, *Nom. Nov*.
  - ***Anemina*** Haas, 1969
  - ***Cristaria*** Schumacher, 1817
  - ***Pletholophus*** Simpson, 1900
  - ***Sinanodonta*** Modell, 1945
- **Lanceolariini** Froufe, Lopes-Lima & Bogan, *Nom. Nov*.
  - ***Arconaia*** Conrad, 1865
  - ***Lanceolaria*** Conrad, 1853

ANODONTINAE (*incertae sedis*)
- ****Pegias*** Simpson, 1900
- ****Simpsonella*** Cockerell, 1903

UNIONINAE Rafinesque, 1820
+ Cafferiinae Modell, 1942
- **Unionini** Rafinesque, 1820
- **+** Cafferiini Modell, 1942
  - ***Unio*** Philipsson in Retzius, 1788

UNIONINAE (*incertae sedis*)
- ***Aculamprotula*** Wu, Liang, Wang & Ouyang, 1998
- ****Acuticosta*** Simpson, 1900
- ***Cuneopsis*** Simpson, 1900
- ****Inversiunio*** Habe, 1991
- ****Lepidodesma*** Simpson, 1896
- ***Nodularia*** Conrad, 1853
- ****Rhombuniopsis*** Haas, 1920
- ***Schistodesmus*** Simpson, 1900

RECTIDENTINAE Modell, 1942
+ Hyriopsinae Modell, 1942
- **Contradentini** Modell, 1942
- + Physunioini Starobogatov, 1970
  - ***Contradens*** Haas, 1913
  - ***Physunio*** Simpson, 1900
  - ***Trapezoideus*** Simpson, 1900
- **Rectidentini** Modell, 1942
- **+** Limnoscaphini Lindholm, 1932
  - ***Ensidens*** Frierson, 1911

Hyriopsis Conrad, 1853
Rectidens Simpson, 1900
GONIDEINAE Ortmann, 1916
+ Leguminainae Starobogatov, 1970
Chamberlainiini Bogan, Froufe & Lopes-Lima, *Nom. Nov*.
Chamberlainia Simpson, 1900
Sinohyriopsis Starobogatov, 1970
Lamprotulini Modell, 1942
+ Psilunionini Starobogatov, 1970
Lamprotula Simpson, 1900
Potomida Swainson, 1840
Pronodularia Starobogatov, 1970
Gonideini Ortmann, 1916
+ Leguminaiini Starobogatov, 1970
Gonidea Conrad, 1857
Leguminaia Conrad, 1865
Microcondylaea Vest, 1866
Solenaia Conrad, 1869
Pseudodontini Frierson, 1927
Pseudodon Gould, 1844
Pilsbryoconcha Simpson, 1900
GONIDEINAE (*incertae sedis*)
****Discomya*** Simpson, 1900
****Inversidens*** Haas, 1911
Solenaia triangularis
AMBLEMINAE Rafinesque, 1820
Amblemini Rafinesque, 1820
Amblema Rafinesque, 1820
****Reginaia*** Campbell & Lydeard, 2012
Lampsilini Ihering 1901
+ Propterini Hannibal, 1912
+ Cyprogeniini Starobogatov, 1970
+ Dromini Starobogatov, 1970
+ Friersoniini Starobogatov, 1970
+ Glebulini, Starobogatov, 1970
+ Medionidinae Starobogatov, 1970
+ Pilaeini Starobogatov, 1970
+ Pileini Bieler et al 2010
+ Popenaiadini Heard & Guckert, 1970
+ Ptychobranchini Starobogatov, 1970
Actinonaias Crosse & Fischer, 1894
****Arotonaias*** von Martens, 1900
****Cyprogenia*** Agassiz, 1852
****Cyrtonaias*** Crosse & Fischer, 1894
****Dromus*** Simpson, 1900
****Ellipsaria*** Rafinesque, 1820
****Epioblasma*** Rafinesque, 1831
****Friersonia*** Ortmann, 1912
****Glebula*** Conrad, 1853
****Hamiota*** Roe & Hartfield, 2005
Lampsilis Rafinesque, 1820
****Lemiox*** Rafinesque, 1831
****Leptodea*** Rafinesque, 1820
****Ligumia*** Swainson, 1840
****Medionidus*** Simpson, 1900

Obliquaria Rafinesque, 1820
Obovaria Rafinesque, 1819
Plectomerus Conrad, 1853
Popenaias Frierson, 1927
Potamilus Rafinesque, 1818
Ptychobranchus Simpson, 1900
Toxolasma Rafinesque, 1831
Truncilla Rafinesque, 1819
Venustaconcha Frierson, 1927
Villosa Frierson, 1927

Pleurobemini Hannibal, 1912
+ Elliptionini, Modell, 1942
Elliptio Rafinesque, 1820
Elliptoideus Frierson, 1927
Fusconaia Simpson, 1900
Hemistena Rafinesque, 1820
Plethobasus Simpson, 1900
Pleurobema Rafinesque, 1819
Pleuronaia Frierson, 1927

Quadrulini Ihering, 1901
+ Megalonaiadini Heard & Guckert, 1970
Cyclonaias Pilsbry in Ortmann & Walker, 1922
Megalonaias Utterback, 1915
Quadrula Rafinesque, 1820
Tritogonia Agassiz, 1852
Uniomerus Conrad, 1853

AMBLEMINAE (*incertae sedis*)
Barynaias Crosse & Fischer, 1894
Delpinonaias Crosse & Fischer, 1894
Disconaias Crosse & Fischer, 1894
Martinsnaias Frierson, 1927
Micronaias Simpson, 1900
Nephritica Frierson, 1927
Nephronaias Crosse & Fischer, 1894
Pachynaias Crosse & Fischer, 1894
Psoronaias Crosse & Fischer, 1894
Psorula Haas, 1930
Reticulataus Frierson, 1927
Sphenonaias Crosse & Fischer, 1894

PARREYSIINAE Henderson 1935
Parreysiini Henderson, 1935
+ Diplasminae Modell, 1942
+ Hemisolasminae Starobogatov, 1970
Parreysia Conrad, 1853

Coelaturini Modell, 1942
+ Brazzaeini Leloup, 1950
+ Dentaspainiini Modell, 1964
+ Mweruellini Pain & F.R. Woodward, 1968
+ Prisodontopsini Pain & F.R. Woodward, 1968
+ Pseudaviculini Modell, 1942 [not available name, Bouchet & Rocroi, 2010]
+ Pseudospathini Leloup, 1950 [not available name, Bouchet & Rocroi, 2010]
+ Pseuodspathinae Starobogatov, 1970
Brazzaea Bourguignat, 1885
Coelatura Conrad, 1853
Grandidieria Bourguignat, 1885

Mweruella Haas, 1936
Nitia Pallary, 1924
Nyassunio Haas, 1936
Prisodontopsis Tomlin1928
Pseudospatha Simpson, 1900

Lamellidentini Modell, 1942
Lamellidens. Simpson, 1900

Oxynaiini Starobogatov, 1970
Oxynaia Haas, 1911
Radiatula Simpson, 1900
Scabies Haas, 1911

PARREYSIINAE (*incertae sedis*)
Germainaia Graf & Cummings, 2009

MODELLNAIINAE 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

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 into the 20th century (e.g. Ortmann 1910; Modell 1964; Haas 1969a,b; Heard & Guckert 1970; Davis & Fuller 1981). Subsequent studies demoted Anodontinae to a tribe within Unioninae due to the shared hooked type and subtriangular external shape of the glochidia (Graf 2002; Graf & Cummings 2007; Bieler et al 2010; Carter et al 2011). 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, following 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 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 tachytictia). 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).

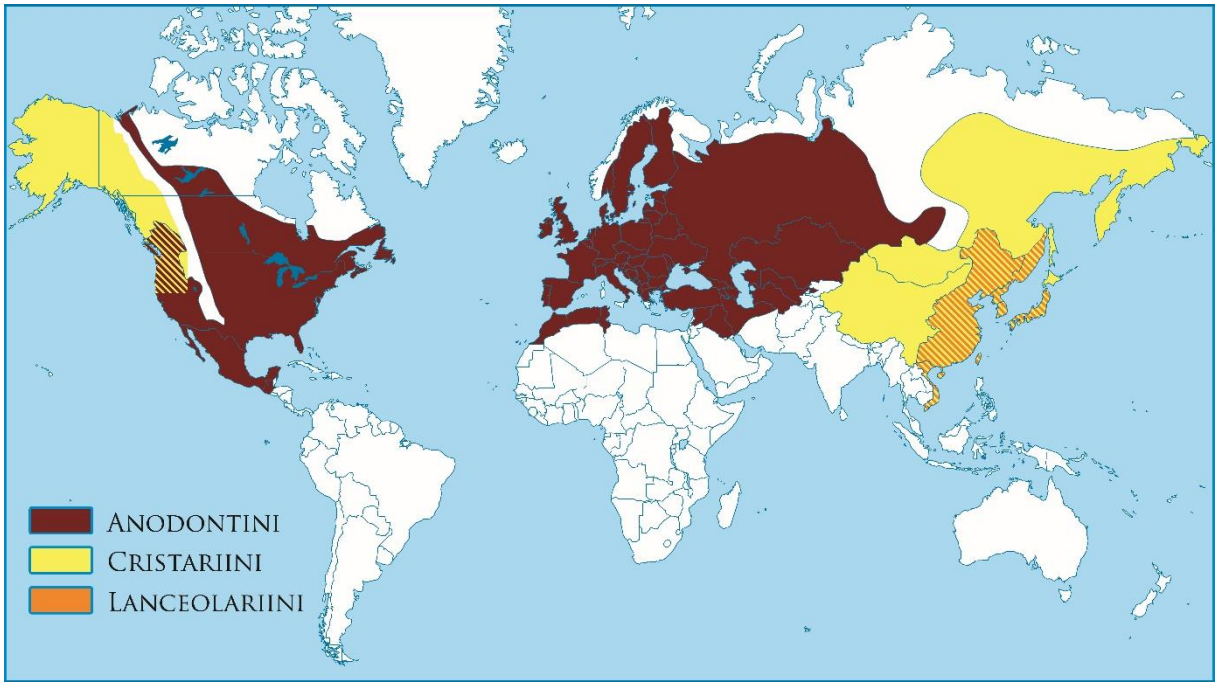

Figure 3 Distribution map of the subfamily Anodontinae.

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 analysed 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; Lydeard et al 1996; Zanatta et al 2007; Breton et al 2011; Inoue et al 2014). 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 (Haas 1969a,b; Graf and Cummings 2016), 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 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 type genus *Anodonta* that is also present on 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. arcaeformis*) 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.

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 includes 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 Simpson (1900, 1914), Đang et al (1980), and He & Zhuang (2013). Many species have been assigned to *Sinanodonta*, primarily by the Russian school of nomenclature (Haas 1969a; Graf 2007), but the validity of these placements should be tested using molecular tools. *Sinanodonta lucida* was first described as *Anodonta lucida* and then assigned to *Sinanodonta* (Đang et al 1980) but both generic attributions are still being used (e.g. Huang et al 2013; Pfeiffer & Graf 2013). Additionally, recent studies based on morphological data consider *S. lucida* as a synonym of *S. woodiana* (Graf & Cummings 2016; He & 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 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).

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,b; 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 anteroposterior 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).

Unioninae Rafinesque, 1820 phylogeny and tribal Classification

The Unioninae was 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 except 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) into several subfamilies using umbo sculpture as the main diagnostic character (Table 1). However, this character alone was unsuitable for this purpose and thus, these subfamilies were lumped back together until the emergence of modern phylogenetic approaches (Heard & Guckert 1970; Davis & Fuller 1981). Since then, several Asian and African genera have been reassigned to other subfamilies based on molecular phylogenetic analyses and morphology (Liu et al 1979; Huang et al 2002; Zhou et al 2007; Kondo 2008; Ouyang et al 2011; Pfeiffer & Graf 2013, 2015). Besides, 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 & Zatravkin, 1987 since the subfamily name Cuneopsinae Mongin, 1963 is not available (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 ectobranchous condition but see Araujo et al (2009) and Lopes-Lima et al (2016) 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. The brooding type is tachytictic or short term (Table C1). The Unioninae is 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. Besides, two *Unio* species have disjunct distributions, i.e. *Unio abyssinicus* in the Horn of Africa and *Unio caffer* in South Africa (Fig. 4).

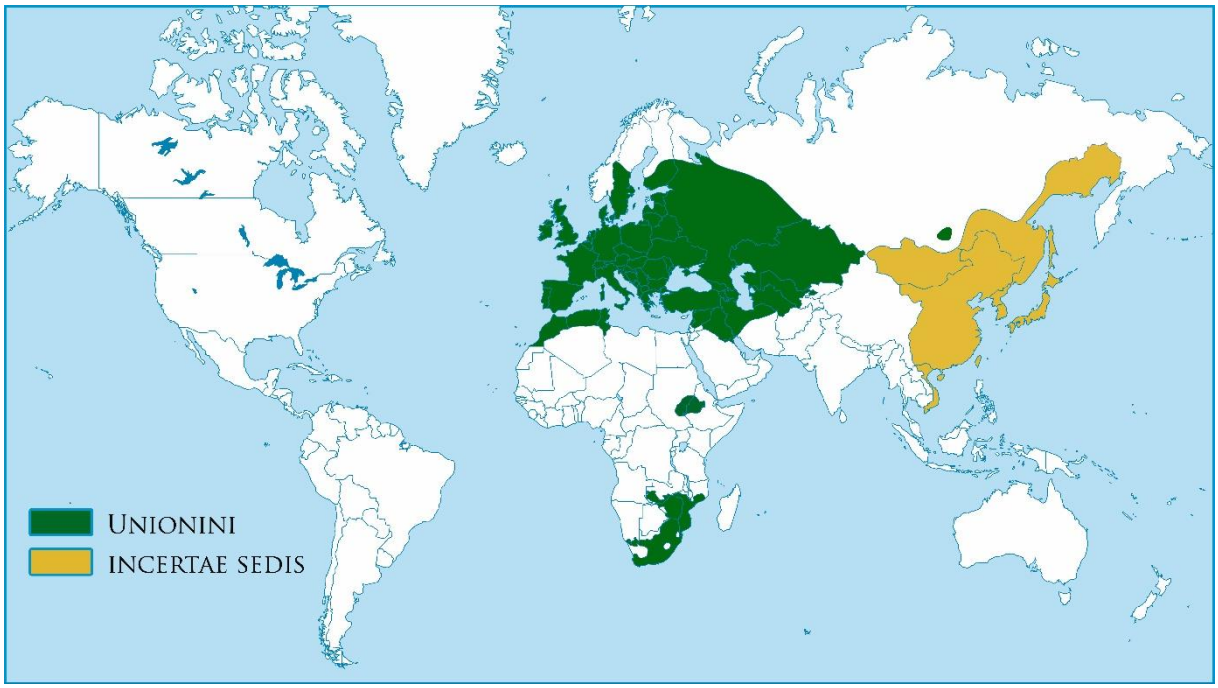

Figure 4 Distribution map of the subfamily Unioninae.

Tribe Unionini Rafinesque, 1820

Type Genus: *Unio* Philipsson in Retzius, 1788

Type Species: *Mya pictorum* Linnaeus, 1758

Comments: The Unionini contains 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 2017), 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. Also, three disjunct distributions are known, i.e. one in the Transbaikal region in Russia and two others in Sub-Saharan Africa (Fig. 4).

Rectidentinae Modell, 1942 phylogeny and tribal classification

The Rectidentinae originally included *Rectidens* as the type genus, as well as *Physunio* and *Ensidens* also including some eastern North American and Southeast Asian genera in this subfamily (e.g. *Lastena*, *Pyganodon,* and *Pilsbryoconcha*) (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 was 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), the priority of Rectidentinae was determined by the First Revisor action (Brandt 1974; Bieler et al 2010). Since the two tribes within the Rectidentinae show 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 south to Peninsular Malaysia and the Islands of Sumatra, Java, Borneo, and Sulawesi (Fig. 5).

Figure 5 Distribution map of the subfamily Rectidentinae.

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 from Indochina and the Island of Borneo and the Philippines (Modell 1942, 1964). Subsequently, all of these genera were reassigned to the Unioninae, except for *Simpsonella*, which was placed within the Anodontinae (Haas 1969a,b). More recently, *Caudiculatus* and *Pressidens* were once again placed within the Rectidentinae (Graf & 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, 1912 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 exolescens*). 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 tooth 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 & Graf 2015). The brooding type is ectobranchous, but the brooding period and length are unknown.

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

Tribe Rectidentini Modell, 1942

Type Genus: *Rectidens* Simpson, 1900

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

Comments: The Rectidentini includes the type genus *Rectidens* as well as *Hyriopsis* and *Ensidens*. 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 Đặng 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 numbers and shapes. Glochidia are of the unhooked elliptical type and intermediate sizes. The brooding type is ectobranchous or

tetragenous in *Hyriopsis* and tetragenous in *Ensidens* 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, Modellnaiinae, 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).

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 (Ortmann 1916, Heard & Guckert 1970), placed within other subfamilies such as the Pseudodontinae (Modell 1942) and the Unioninae (Haas 1969a,b), and in a separate tribe, i.e. Gonideini, within the Ambleminae (Graf 2002; Graf & 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 & Graf 2013, 2015). In the present work, the Gonideinae is 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).

Figure 6 Distribution map of the subfamily Gonideinae.

The second clade is 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 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 has a scattered distribution in the Northern Hemisphere, being present in restricted regions of the Palearctic, Indotropics and Western Nearctic (Fig. 6).

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 the 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).

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

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 was 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, except for *Pronodularia*, were reassigned to Quadrulinae (Haas 1969a,b). However, *Lamprotula*, *Pronodularia,* and *Potomida* were recently reassigned back to Gonideinae based on molecular, morphological and biogeographical studies (Pfeiffer & 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. 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). The 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).

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 *Solenaia*. *Solenaia* is not monophyletic, as *Solenaia triangularis* was not recovered within the Gonideini (see Pfeiffer & 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 & Cummings (2016) suggested the placement of these genera within the Gonideinae. In the present study, molecular data confirm the placement of these three genera within the Gonideini together with some representatives of the genus *Solenaia*.

Diagnosis: Shell shape is trapezoidal but much more elongated in *Solenaia*. Hinge teeth are small, vestigial or absent in *Solenaia*. 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. The 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. *Solenaia* occurs from eastern India to Myanmar, Thailand, North Vietnam and China (Fig. 6).

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 (Subba Rao 1989; Haas 1969a,b), and only recently were their relationships with the Gonideinae discussed (Whelan et al 2011; Pfeiffer and Graf 2015). 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 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. The 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 Java, Sumatra, and Borneo (Fig. 6).

Ambleminae Rafinesque, 1820, Parreysiinae Henderson, 1935 & Modellnaiinae Brandt, 1974

The Ambleminae and Parreysiinae were investigated in detail earlier (Campbell et al 2005; Whelan et al 2011; Campbell and Lydeard 2012a,b;) and thus not fully explored in the present study. The Modellnaiinae 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 phylogenetic analyses 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 Ambleminae, Parreysiinae, and Modellnaiinae (Table 3; Fig. 7). The Ambleminae are restricted to Canada and the United States east of the Rocky Mountains and extend south through Mexico to southern Panama. The Parreysiinae has a disjunct distribution in Africa and Southern Asia. In Africa, 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. *Germainaia* Graf & 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 Modellnaiinae (i.e. *Modellnaia siamensis*) is restricted to the middle section of the Mun River in Thailand (Fig. 7).

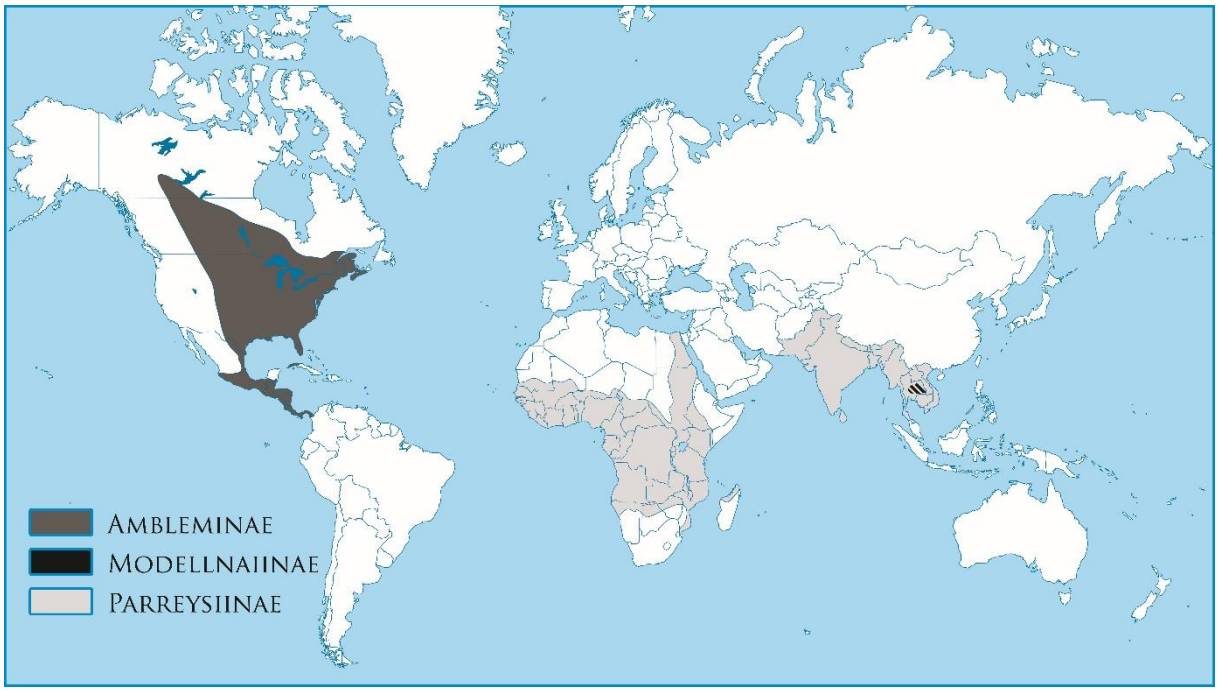

Figure 7 Distribution map of the subfamilies Ambleminae + Modellnaiinae + Parreysiinae.

Conclusions

Considering the high levels of the 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 Central America and the Indotropics. 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 these genera being sequenced for the first time. Furthermore, it includes 57 species from 35 genera, thereby tripling the number of analysed 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).

Acknowledgments

Financial support was provided by the Portuguese Foundation for Science and Technology (FCT) under grants to EF (SFRH/BPD/108445/2015) and MLL (SFRH/BD/115728/2016); the Vietnam Academy of Science and Technology under grant number IEBR.CBT.TS07/2015 for the Vietnamese field research; the Central Michigan University Poyang Lake Research Investment Fund and the Key Laboratory of Poyang Lake Wetland and Watershed Research Director's Open Fund of Jiangxi Normal University for procurement of and analysis of some Chinese specimens. The authors wish to thank: Jamie Smith, Collection manager of Molluscs, North Carolina Museum of Natural Sciences, Raleigh, North Carolina, for all of her efforts to ship tissue samples to be used in this project; the Confederated Tribes of the Umatilla Indian Reservation for providing access to specimens of *A. nuttalliana*.

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Supplementary Material

Appendix A

Concatenated (COI + 28S) FASTA file of the Palaeoheterodonta dataset.

Available online at http://dx.doi.org/10.1016/j.ympev.2016.08.021

Appendix B

Concatenated (COI + 28S) FASTA file of the Unionidae dataset.

Available online at http://dx.doi.org/10.1016/j.ympev.2016.08.021

Table C1
List of morphological, anatomical and behavioral characters used in traditional phylogenetic and systematic analyses of Unionida. (Gln) Glochidial size index; (Pse) Pseudocardinal teeth; (Lat) Lateral teeth; (L) Left valve; (R) Right valve. (*) Reported as bilaterally asymmetrical but see discussion in Pfeiffer & Graf (2015) (mostly likely unhooked like other *Pseudodon* species). Umbo sculpture classification follows Zieritz et al (2015).

Taxon	Glochidia shape	Gln	Brooding Period	Marsupium anatomy
ANODONTINAE	**triangular (hooked)**	**medium/large**	**various**	**ectobranchous**
ANODONTINI	**triangular (hooked)**	**large**	**bradytictic**	**ectobranchous**
Alasmidonta marginata	triangular (hooked)	large (0.115)	bradytictic	ectobranchous
Anodonta anatina	triangular (hooked)	large (0.122)	bradytictic	ectobranchous
Anodonta nuttalliana	triangular (hooked)	large (0.092)	bradytictic	ectobranchous
Anodonta cygnea	triangular (hooked)	large (0.096)	bradytictic	ectobranchous
Lasmigona compressa	asymmetric (hooked)	large (0.092)	bradytictic	ectobranchous
Pseudanodonta complanata	triangular (hooked)	large (0.099)	bradytictic	ectobranchous
Pyganodon grandis	triangular (hooked)	large (0.141)	bradytictic	ectobranchous
Simpsonaias ambigua	triangular (hooked)	medium (0.0645)	bradytictic	ectobranchous
Strophitus undulatus	triangular (hooked)	large (0.104)	bradytictic	ectobranchous (anterior sections)
CRISTARIINI	**triangular (hooked)**	**large**	**various**	**ectobranchous**

Anemina arcaeformis	triangular (hooked)	large (0.126)	bradytictic	ectobranchous
Cristaria plicata	triangular (hooked)	large (0.080)	bradytictic	ectobranchous
Plethholophus tenuis	triangular (hooked)	large (0.072)	unknown	unknown
Sinanodonta lucida	triangular (hooked)	large (0.190)	unknown	ectobranchous
Sinanodonta woodiana	triangular (hooked)	large (0.156)	tachytictic	ectobranchous
LANCEOLARIINI	**triangular (hooked)**	**medium**	**tachytictic**	**ectobranchous**
Arconaia lanceolata	triangular (hooked)	medium (0.037)	tachytictic	ectobranchous
Lanceolaria gladiola	triangular (hooked)	medium (0.041)	tachytictic	ectobranchous
Lanceolaria grayana	triangular (hooked)	medium (0.032)	tachytictic	ectobranchous
Lanceolaria grayii	unknown	unknown	unknown	ectobranchous
UNIONINAE	**triangular (hooked)**	**medium**	**tachytictic**	**ectobranchous**
UNIONINI	**triangular (hooked)**	**medium**	**tachytictic**	**ectobranchous**
Unio crassus	triangular (hooked)	medium (0.043)	tachytictic	ectobranchous
Unio gibbus	triangular (hooked)	medium (0.044)	Unknown	ectobranchous
Unio pictorum	triangular (hooked)	medium (0.042)	tachytictic	ectobranchous

Unio tumidus	triangular (hooked)	medium (0.042)	tachytictic	ectobranchous
***incertae sedis* (UNIONINAE)**				
Aculamprotula tortuosa	triangular (hooked)	medium (0.059)	tachytictic	ectobranchous
Cuneopsis heudei	triangular (hooked)	medium (0.054)	tachytictic	ectobranchous
Cuneopsis pisciculus	triangular (hooked)	medium (0.044)	tachytictic	ectobranchous
Cuneopsis rufescens	triangular (hooked)	medium (0.054)	tachytictic	ectobranchous
Nodularia douglasiae	triangular (hooked)	medium (0.043)	tachytictic	ectobranchous
Nodularia nuxpersicae	unknown	unknown	unknown	ectobranchous
Schistodesmus lampreyanus	triangular (hooked)	medium (0.034)	tachytictic	ectobranchous
RECTIDENTINAE	**asymmetrical; semi-elliptical (unhooked)**	**medium**	**unknown**	**ectobranchous; tetragenous**
CONTRADENTINI	**bilaterally asymmetrical**	**unknown**	**unknown**	**ectobranchous**
Contradens contradens	bilaterally asymmetrical	unknown	bradytictic	ectobranchous
Contradens semmelincki	unknown	unknown	unknown	unknown
Physunio modelli	unknown	unknown	unknown	ectobranchous
Trapezoideus exolescens	bilaterally asymmetrical	medium (0.047)	unknown	ectobranchous
RECTIDENTINI	**semi-elliptical (unhooked)**	**medium**	**unknown**	**ectobranchous; tetragenous**

Ensidens ingallsianus	semi-elliptical (unhooked)	unknown	bradytictic	tetragenous
Ensidens sp.	unknown	unknown	unknown	tetragenous
Ensidens sagittarius	unknown	unknown	unknown	unknown
Hyriopsis bialata	semi-elliptical (unhooked)	medium (0.040)	tachytictic	ectobranchous
Hyriopsis desowitzi	semi-elliptical (unhooked)	medium (0.025)	tachytictic	tetragenous
Hyriopsis myersiana	semi-elliptical (unhooked)	medium (0.040)	bradytictic	ectobranchous
Rectidens sumatrensis	semi-elliptical (unhooked)	unknown	unknown	tetragenous
GONIDEINAE	**semi-elliptical (unhooked)**	**medium**	**tachytictic**	**ectobranchous; tetragenous**
CHAMBERLAINIINI	**semi-elliptical (unhooked)**	**medium**	**various**	**ectobranchous**
Chamberlainia hainesiana	semi-elliptical (unhooked)	medium (0.043)	bradytictic	ectobranchous
Sinohyriopsis cumingii	semi-elliptical (unhooked)	medium (0.049)	tachytictic	ectobranchous
LAMPROTULINI	**semi-elliptical (unhooked)**	**medium**	**tachytictic**	**tetragenous**
Lamprotula caveata	semi-elliptical (unhooked)	medium (0.036)	tachytictic	tetragenous
Lamprotula leai	semi-elliptical (unhooked)	medium (0.036)	tachytictic	tetragenous

Potomida littoralis	semi-elliptical (unhooked)	medium (0.036)	tachytictic	tetragenous
Pronodularia japanensis	semi-elliptical (unhooked)	medium (0.040)	tachytictic	tetragenous
GONIDEINI	**semi-elliptical (unhooked)**	**medium**	**tachytictic**	**tetragenous**
Gonidea angulata	semi-elliptical (unhooked)	medium (0.036)	tachytictic	tetragenous
Leguminaia wheatleyi	semi-elliptical (unhooked)	unknown	unknown	unknown
Microcondylaea bonellii	semi-elliptical (unhooked)	medium (0.021)	tachytictic	tetragenous
Solenaia carinata	semi-elliptical (unhooked)	unknown	unknown	unknown
Solenaia oleivora	unknown	unknown	tachytictic	tetragenous
PSEUDODONTINI	**semi-elliptical (unhooked)**	**unknown**	**unknown**	**tetragenous**
Pilsbryoconcha compressa	unknown	unknown	bradytictic	unknown
Pilsbryoconcha exilis	semi-elliptical (unhooked)	medium (0.024)	unknown	tetragenous
*Pseudodon cambodjensis******	semi-elliptical (unhooked)	medium (0.050)	bradytictic	unknown
Pseudodon mouhotii	unknown	unknown	unknown	unknown
Pseudodon cummingii	semi-elliptical (unhooked)	unknown	unknown	tetragenous

Solenaia triangularis	unknown	unknown	unknown	unknown
AMBLEMINAE	**semi-elliptical (unhooked)**	**small; medium**	**various**	**ectobranchous; tetragenous**
AMBLEMINI	**semi-elliptical (unhooked)**	**medium**	**tachytictic**	**tetragenous**
Amblema plicata	semi-elliptical (unhooked)	medium (0.042)	tachytictic	tetragenous
LAMPSILINI	**semi-elliptical; axe-shaped (unhooked)**	**medium**	**bradytictic**	**ectobranchous (posterior sections)**
Actinonaias ligamentina	semi-elliptical; (unhooked)	medium (0.053)	bradytictic	ectobranchous (posterior sections)
Lampsilis cardium	semi-elliptical (unhooked)	medium (0.070)	bradytictic	ectobranchous (posterior sections)
Villosa iris	semi-elliptical (unhooked)	medium (0.067)	bradytictic	ectobranchous (posterior sections)
PLEUROBEMINI	**semi-elliptical (unhooked)**	**medium**	**tachytictic**	**ectobranchous**
Pleurobema sintoxia	semi-elliptical (unhooked)	medium (0.023)	tachytictic	ectobranchous
Elliptio complanata	semi-elliptical (unhooked)	medium (0.038)	tachytictic	ectobranchous
Elliptio dilatata	semi-elliptical (unhooked)	medium (0.044)	tachytictic	ectobranchous
QUADRULINI	**semi-elliptical (unhooked)**	**small**	**tachytictic**	**tetragenous**
Quadrula quadrula	semi-elliptical (unhooked)	small (0.007)	tachytictic	tetragenous
Quadrula verrucosa	semi-elliptical (unhooked)	small (0.010)	tachytictic	tetragenous

Table C1 (*cont.*)

Taxon	Shell shape	Umbo sculpture	Hinge structure	References
ANODONTINAE	**various**	**various**	**various**	
ANODONTINI	**ovate**	**various**	**teeth absent or vestigial**	
Alasmidonta marginata	triangular	double-looped, wrinkled	Pse: 2L, 1R (small); Lat: absent	Barnhart et al 2008; William et al 2008
Anodonta anatina	ovate	double-looped, pseudo-concentric, wrinkled	teeth absent	Hinzmann et al 2013; Pekkarinen & Englund 1995; Wächtler et al 2001
Anodonta nuttalliana	ovate	double-looped, nodulous	teeth absent	Heard 1975; Nedeau et al 2009
Anodonta cygnea	ovate	pseudo-concentric	teeth absent	Lima et al 2012; Pekkarinen & Englund 1995; Wächtler et al 2001
Lasmigona compressa	ovate	double-looped, wrinkled	Pse: 2L, 1R (smooth, low); Lat: 2L, 1R (thin); Interdental tooth in L valve;	Barnhart et al 2008
Pseudanodonta complanata	ovate	double-looped, nodulous	teeth absent	McIvor & Aldridge 2007; Pekkarinen & Englund 1995; Wächtler et al 2001
Pyganodon grandis	ovate	double-looped, nodulous, pseudo-concentric	teeth absent	Barnhart et al 2008
Simpsonaias ambigua	elongate	double-looped	Pse: 1L, 1R (small); Lat: absent or slight ridge	Clarke 1985
Strophitus undulatus	ovate	double-looped, pseudo-concentric	Pse: thickening only; Lat: absent	Barnhart et al 2008
CRISTARIINI	**ovate**	**various**	**teeth absent or vestigial**	

Anemina arcaeformis	ovate	pseudo-concentric	teeth absent	He & Zhuang 2013; Jeong et al 1993; Sayenko 2006
Cristaria plicata	ovate	pseudo-concentric	Pse: absent; Lat: 1L, 1R	He & Zhuang 2013; Heard 1977; Sayenko 2006; Wu et al 1999
Plethlophus tenuis	ovate	double-looped, pseudo-concentric	Pse: 1L, 1R (thin); Lat: 1L, 1R	He & Zhuang 2013; Inaba 1941; Simpson 1914
Sinanodonta lucida	ovate	double-looped, pseudo-concentric	teeth absent	He & Zhuang 2013; Wu et al 1999
Sinanodonta woodiana	ovate	double-looped, pseudo-concentric	teeth absent	Bogatov & Sayenko 2002; He & Zhuang 2013; Kondo 1987; Wächtler et al 2001; Wu et al 1999
LANCEOLARIINI	**lanceolate**	**nodulous**	**Pse: 2L, 2R; Lat: 2L, 1R**	
Arconaia lanceolata	lanceolate (twisted)	nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Simpson 1914
Lanceolaria gladiola	lanceolate	nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Wu et al 1999
Lanceolaria grayana	lanceolate	nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Kondo 1987; Wu et al 1999
Lanceolaria grayii	lanceolate	nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Simpson 1914
UNIONINAE	**various**	**various**	**various teeth well structured**	
UNIONINI	**elongate**	**various**	**Pse: 2L, 1/2R; Lat: 2L, 1R**	
Unio crassus	elongate, ovate	double-looped, nodulous	Pse: 2L, 2R (compressed); Lat: 2L, 1R	Pekkarinen & Englund 1995; Wächtler et al 2001

Unio gibbus	ovate	nodulous	Pse: 2L, 1R (lamellar); Lat: 2L, 1R (lamellar)	Araujo et al 2009; Khalloufi & Boumaïza 2009
Unio pictorum	elongate	nodulous	Pse: 2L, 2R (compressed); Lat: 2L, 1R	Pekkarinen & Englund 1995; Wächtler et al 2001
Unio tumidus	elongate	w-shaped, double-looped, nodulous, wrinkled	Pse: 2L, 2R (compressed); Lat: 2L, 1R	Pekkarinen & Englund 1995; Wächtler et al 2001
***incertae sedis* (UNIONINAE)**				
Aculamprotula tortuosa	ovate	nodulous	Pse: 2L, 1R; Lat: 2L, 1R	He & Zhuang 2013
Cuneopsis heudei	cuneiform	pseudo-concentric	Pse: 2L, 1R; Lat: 2L, 1R	He & Zhuang 2013
Cuneopsis pisciculus	cuneiform (twisted)	nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Wu et al 1999
Cuneopsis rufescens	cuneiform, elongate	nodulous	Pse: 2L, 1R; Lat: 2L, 1R	He & Zhuang 2013
Nodularia douglasiae	elongate, ovate	w-shaped, double-looped, wrinkled	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Kondo 1987; Wu et al 1999
Nodularia nuxpersicae	elongate, ovate	w-shaped, double-looped, wrinkled	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013
Schistodesmus lampreyanus	triangular	nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Shu et al 2012
RECTIDENTINAE	**various**	**various**	**various**	
CONTRADENTINI	**various**	**various**	**low number of thin teeth**	
Contradens contradens	ovate	V-shaped	Pse: 1L, 1R (thin/long); Lat: 1L, 1R (thin)	Brandt 1974; Panha 1990; Panha & Eongprakornkeaw 1995
Contradens semmelincki	elongate, ovate	V-shaped	Pse: 2L, 1R (thin, lamellar); Lat: 1R, 1L (thin)	Đặng et al 1980; Simpson 1914

Physunio modelli	ovate	W-shaped	Pse: 2L, 1R (thin, lamellar); Lat: 1R, 2L (thin)	Brandt 1974; Heard 1974
Trapezoideus exolescens	trapezoidal	pseudo-concentric	Pse: 1L, 1R (thin), Lat: 1R, 1L (thin)	Brandt 1974; Heard 1974; Panha & Eongprakornkeaw 1995; Pfeiffer & Graf 2015; Simpson 1914;
RECTIDENTINI	**various**	**various**	**various**	
Ensidens ingallsianus	cuneiform, elongate	double-looped, nodulous	Pse: 1/2L, 2R (thin/long); Lat: 2L, 1R (thin/long)	Brandt 1974; Heard 1977; Panha 1990; Panha & Eongprakornkeaw 1995
Ensidens sp.	elongate	double-looped, nodulous	Pse: 1/2L, 2R (thin/long); Lat: 2L, 1R (thin/long)	Brandt 1974; Heard 1977 Panha 1990; Panha & Eongprakornkeaw 1995
Ensidens sagittarius	elongate	double-looped, nodulous	Pse: 1/2L, 2R (thin/long); Lat: 2L, 1R (thin/long)	Brandt 1974; Heard 1977 Panha 1990; Panha & Eongprakornkeaw 1995
Hyriopsis bialata	elongate, with wings	pseudo-concentric	Pse: 2L, 2R (crenulate); Lat: 2L, 1R; interdentum sculptured	Brandt 1974; Chatchavalvanich et al 2006; Chumnanpuen et al 2011; Panha 1990; Panha & Eongprakornkeaw 1995
Hyriopsis desowitzi	ovate, with wings	pseudo-concentric	Pse: 2L, 2R (parallel ridges); Lat: 2L, 1R (short/narrow)	Brandt 1974; Duangsawang & Kovitvadhi 2009; Duangsawang et al 2008; Panha & Eongprakornkeaw 1995

Hyriopsis myersiana	ovate, with wings	nodulous	Pse: 1L, 1R; Lat 2L, 1R	Brandt 1974; Ortmann 1916; Panha 1990; Panha & Eongprakornkeaw 1995; Uthaiwan et al 2001
Rectidens sumatrensis	elongate	double-looped, pseudo-concentric	Pse: 2L, 2R (thin); Lat: 1L, 2R	Heard 1977; Thiele 1934
GONIDEINAE	**various**	**various**	**various**	
CHAMBERLAINIINI	**ovate, posterior wing**	**various**	**various**	
Chamberlainia hainesiana	ovate, with wings	nodulous	Pse: 2L (short, thick), 1R; Lat: 2L (small), 1R (strong)	Brandt 1974; Kovitvadhi & Kovitvadhi 2013; Panha 1993; Panha & Eongprakornkeaw 1995;
Sinohyriopsis cumingii	ovate, with wings	pseudo-concentric	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013; Wu et al 1999
LAMPROTULINI	**ovate**	**various**	**various, strong teeth**	
Lamprotula caveata	ovate	unknown	Pse: 2L, 1R; Lat: 2L, 1R	He & Zhuang 2013; Wu et al 1999
Lamprotula leai	ovate	double-looped, nodulous	Pse: 2L, 2R; Lat: 2L, 1R	He & Zhuang 2013
Potomida littoralis	ovate	w-shaped, double-looped	Pse: 2L, 1R (thick); Lat: 2L, 1R	Cek & Sereflişan 2011; Şereflişan et al 2009; Simpson 1914
Pronodularia japanensis	ovate	w-shaped, nodulous, wrinkled	Pse: 2L, 1R; Lat: 2L, 1R	Kondo 1982, 2008; Sayenko 2012
GONIDEINI	**trapezoidal; rectangular**	**various**	**absent or vestigial teeth**	
Gonidea angulata	trapezoidal	pseudo-concentric	Pse: 1L, 1R (small); Lat: absent	Heard 1974; Ortmann 1916; O'Brien et al 2013

Leguminaia wheatleyi	trapezoidal	double-looped, w-shaped, wrinkled	Pse: 1L, 1R (small); Lat: traces	Haas 1969
Microcondylaea bonellii	trapezoidal	double-looped	Pse: 1L, 1R (small); Lat: traces	Haas 1969; Nagel et al 2007
Solenaia carinata	rectangular	unknown	Pse: absent; Lat: traces	He & Zhuang 2013; Huang et al 2013
Solenaia oleivora	rectangular	unknown	Pse: absent; Lat: traces	He & Zhuang 2013; Wang et al 2015
PSEUDODONTINI	**various**	**double-looped**	**absent or vestigial teeth "v" shaped fossette**	
Pilsbryoconcha compressa	elongate	double-looped, nodulous, single-looped	teeth absent "v" shaped fossette	Brandt 1974; Panha 1990
Pilsbryoconcha exilis	elongate	double-looped	teeth absent "v" shaped fossette	Brandt 1974; Panha 1990; Panha & Eongprakornkeaw 1995
*Pseudodon cambodjensis****	rounded, triangular	unknown	Pse: 1L, 1R (small); "v" shape fossette	Brandt 1974; Panha 1990; Panha & Eongprakornkeaw 1995
Pseudodon mouhotii	elongate	unknown	Pse: 1L, 1R (small); "v" shape fossette	Brandt 1974
Pseudodon cummingii	ovate, posterior wing	double-looped, single-looped	Pse: 1L, 1R (small); "v" shape fossette	Brandt 1974; Panha 1990; Panha & Eongprakornkeaw 1995
***incertae sedis* (GONIDEINAE)**				
Solenaia triangularis	triangular	unknown	Pse: absent; Lat: traces	He & Zhuang 2013
AMBLEMINAE	**various**	**various**	**Pse: 2L, 1R; Lat: 2L, 1R**	
AMBLEMINI	**various**	**various**	**Pse: 2L, 1R; Lat: 2L, 1R**	

Amblema plicata	ovate, quadrate	double-looped, pseudo-concentric	Pse: 2L, 1R (heavy); Lat: 2L, 1R	Barnhart et al 2008
LAMPSILINI	**various**	**various**	**Pse: 2L, 1R; Lat: 2L, 1R**	
Actinonaias ligamentina	elliptical, inflated	double-looped	Pse: 2L, 1R (large); Lat: 2L, 1R	Barnhart et al 2008
Lampsilis cardium	ovate, inflated	double-looped	Pse: 2L, 1R; Lat: 2L, 1R	Barnhart et al 2008
Villosa iris	elliptical, elongate, compressed	double-looped	Pse: 2L, 1R (small); Lat: 2L, 1R	Barnhart et al 2008
PLEUROBEMINI	**various**	**various**	**Pse: 2L, 1R; Lat: 2L, 1R**	
Pleurobema sintoxia	triagular, ovate	double-looped, nodulous	Pse: 2L, 1R (heavy); Lat: 2L (short), 1R	William et al 2008
Elliptio complanata	trapezoidal, compressed	double-looped, pseudo-concentric	Pse: 2L, 1R (triangular); Lat: 2L, 1R (long)	Barnhart et al 2008
Elliptio dilatata	elongate	double-looped, pseudo-concentric	Pse: 2L, 1R (thick); Lat: 2L, 1R (thick)	Barnhart et al 2008
QUADRULINI	**rectangular**	**various**	**Pse: 2L, 1R; Lat: 2L, 1R**	
Quadrula quadrula	quadrate	w-shaped, nodulous, wrinkled	Pse: 2L (heavy), 1R; Lat: , 2L, 1R (long)	Barnhart et al 2008
Quadrula verrucosa	rectangular	w-shaped, double-looped, nodulous, pseudo-concentric	Pse: 2L (large), 1R; Lat: 2L (large, long), 1R	Kennedy & Haag 2005; William et al 2008

CHAPTER 5 Phylogeny, taxonomy and species of the genus *Quadrula*

Paper IV

Revisiting the North American freshwater mussel genus *Quadrula* sensu lato (Bivalvia Unionidae): Phylogeny, taxonomy and species delineation

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Article published in *Zoologica Scripta* **48**, 313-336 (2019).

DOI: 10.1111/zsc.12344

Revisiting the North American freshwater mussel genus *Quadrula* sensu lato (Bivalvia Unionidae): Phylogeny, taxonomy and species delineation

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Abstract

Freshwater mussels (Bivalvia, Unionidae) have suffered strong declines over the last century. High morphological plasticity of Unionidae causes disturbances in their systematics and taxonomy, hampering conservation efforts. Species that have historically been placed under the North American genus *Quadrula* have suffered from numerous taxonomic and species delineation problems since its inception. Four genera are presently recognized within *Quadrula* sensu lato, that is, *Cyclonaias*, *Quadrula, Theliderma*, and *Tritogonia*, but their phylogenetic basis remains incompletely tested. In the present study, we reconstructed several two-marker (mtDNA cytochrome *c* oxidase subunit I - COI and NADH dehydrogenase subunit 1 - ND1) phylogenies with newly collected specimens and all previously available sequences covering most species within this group. We then delineated the species within the group using an integrative approach with the application of molecular statistical methods, morphometric (Fourier Shape) analyses and geographic distribution data. Four clades corresponding to these genera were consistently recovered in all phylogenies. To validate the generic status of these clades, molecular analyses were complemented with morphological, anatomical and ecological data compiled from the literature. Several revisions are here proposed to the current systematics and taxonomy of these genera, including the synonymization of *Cyclonaias asperata* under *Cyclonaias kieneriana*; the inclusion of *Quadrula apiculata* and *Quadrula rumphiana* under *Quadrula quadrula*; the placement of *Quadrula nobilis* under *Tritogonia*; and finally the separation of the Mobile River basin populations of *Theliderma metanevra* as a new species, that is, *Theliderma johnsoni* n. sp. The conservation implications of the proposed changes are then discussed.

Introduction

Conservation programs and strategies are largely based on species as conservation units, making species delineation extremely important as a basic conservation tool (Prié et al 2012). However, taxon-based conservation strategies dedicated to the freshwater mussel family Unionidae, one of the world's most endangered taxa, are hindered by phylogenetic and taxonomic uncertainties (Lopes-Lima et al 2017). This is especially true within the most species-rich Unionidae subfamily, the North American Ambleminae. Across the most recent systematics studies, the Ambleminae is divided into five tribes (Pfeiffer et al 2019). However, polyphyly and inappropriate species boundaries have been revealed in some of these tribes, including the Quadrulini (Lydeard et al 2000; Serb et al 2003; Pfeiffer et al 2016). The quadruline freshwater mussels are distinctive animals producing thick quadrate shells, some of which are heavily sculptured. Shell morphology is highly variable within some species from this group, hindering unambiguous species identification or generic assignment. As shell morphology has been the original basis for Quadrulini systematics and taxonomy to date, the systematics and composition of this tribe have suffered a series of changes since its first description in the early 1900s (see Supporting Information Appendix S1 for an extensive taxonomic history of the Quadrulini). From the beginning of the 20th century, species that had been historically placed within the genus *Quadrula* sensu lato have been divided into four main species groups, that is, the *Quadrula* sensu stricto, the *pustulosa*, the *metanevra* and the *Tritogonia* species groups (Supporting Information Appendix S1). A molecular phylogeny of these taxa by Serb et al (2003) largely confirmed these groupings and recovered four clades: *Quadrula* sensu stricto, the *pustulosa* species group, the *metanevra* species group and a fourth clade including *Tritogonia verrucosa* and *Quadrula nobilis*. Although these four clades are commonly referred to as genera in regional checklists (Parmalee & Bogan 1998; Williams et al 2008; Howells 2013) the molecular, morphological and ecological evidence supporting these groups remains limited.

The present study is focused on re-examining the phylogeny, systematics and taxonomy of *Quadrula* sensu lato, here defined as including the species from the genera *Quadrula*, *Theliderma*, *Cyclonaias* and *Tritogonia* (Williams et al 2017). In detail, this study aims to: (a) estimate the phylogenetic relationships of specimens collected in Texas with all published Quadrulini sequences, using a two-marker approach (COI and ND1); (b) perform a comparative shell morphometry evaluation to complement the molecular results; (c) define species boundaries with a taxonomic revision of all analysed taxa; (d) test the four classical generic constructs and their evolutionary significance; and (e) describe the conservation implications of the obtained results.

Materials and Methods

Sample collection and materials examined

Specimens of quadruline mussels were collected from 50 sites across the state of Texas from 2003-2011 (Fig. 1). A total of 89 specimens were collected and placed in 99% ethanol for molecular analyses. Voucher specimens were labelled and deposited in the SUNY Buffalo State College Great Lakes Center collections, Buffalo, New York (BSGLC). The fieldwork was carried out with an appropriate Scientific Research Permit SPR-0503-300 issued by the Texas Parks and Wildlife Department. Additionally, dry shell specimens of the target nominal species were selected for morphometry from specimens deposited at the North Carolina Museum of Natural Sciences (NCMS) and BSGLC (See Supporting Information Table S1 for the examined lot numbers).

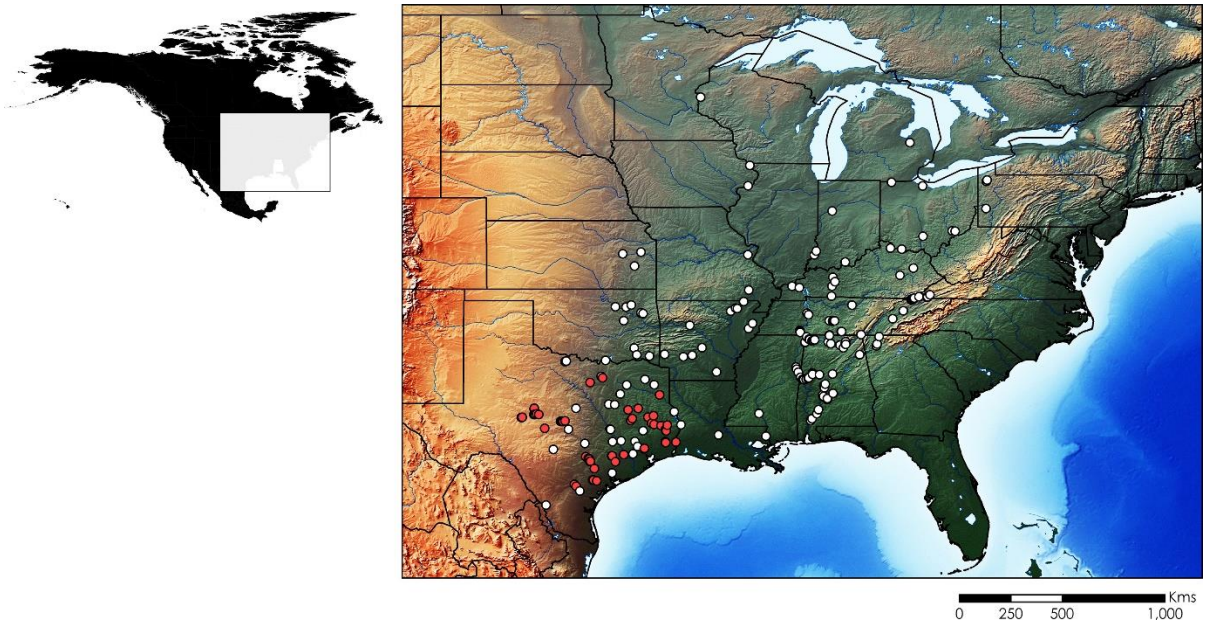

Figure 1 Map of all sampling sites for the present study; both tissue and shell materials in red; only shell materials in white

Sequencing, alignments and phylogenetic analyses

A total of 31 quadruline specimens, including all nominal taxa across the state of Texas, were selected for molecular analyses (Table 1). For each sample, genomic DNA extraction (Froufe et al 2014), amplification and bidirectional sequencing were carried out for the F-type mtDNA cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit 1 (ND1) genes.

For COI, the primers LCO_22me and HCO_700dy (Walker et al 2006) were used with an annealing temperature of 50°C and polymerase chain reaction (PCR) conditions as described in Froufe et al (2014).

Table 1.
List of newly sequenced specimens for Cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit 1 (ND1) datasets; nominal taxa, new identification, site, main basin, and COI and ND1 Haplotype number and Genbank references.

TAXON	NEW ID	RIVER	BASIN	GB (COI)	GB (ND1)
Quadrula petrina	*Cyclonaias necki*	San Marcos	S. Antonio/Guadalupe	MG969422	MK503297
Quadrula petrina	*Cyclonaias necki*	San Marcos	S. Antonio/Guadalupe	MG969423	MK503316
Quadrula petrina	*Cyclonaias necki*	San Marcos	S. Antonio/Guadalupe	MG969424	MK503317
Quadrula petrina	*Cyclonaias necki*	San Marcos	S. Antonio/Guadalupe	MG969425	MK503318
Quadrula petrina	*Cyclonaias petrina*	Concho	Colorado	MG969416	MK503293
Quadrula petrina	*Cyclonaias petrina*	Concho	Colorado	MG969417	MK503294
Quadrula petrina	*Cyclonaias petrina*	Concho	Colorado	MG969418	MK503295
Quadrula petrina	*Cyclonaias petrina*	San Saba	Colorado	MG969419	MK503311
Quadrula petrina	*Cyclonaias petrina*	San Saba	Colorado	MG969420	MK503312
Quadrula petrina	*Cyclonaias petrina*	San Saba	Colorado	MG969421	MK503313
Quadrula aurea	*Cyclonaias pustulosa*	San Marcos	S. Antonio/Guadalupe	MK503268	MK503296
Quadrula aurea	*Cyclonaias pustulosa*	San Antonio	S. Antonio/Guadalupe	MK503269	MK503298
Quadrula aurea	*Cyclonaias pustulosa*	San Antonio	S. Antonio/Guadalupe	MK503270	MK503299
Quadrula aurea	*Cyclonaias pustulosa*	San Antonio	S. Antonio/Guadalupe	MK503271	MK503300
Quadrula aurea	*Cyclonaias pustulosa*	San Antonio	S. Antonio/Guadalupe	-	MK503301
Quadrula aurea	*Cyclonaias pustulosa*	Guadalupe	S. Antonio/Guadalupe	MK503272	MK503302
Quadrula aurea	*Cyclonaias pustulosa*	Nueces	Nueces	MK503281	MK503314
Quadrula aurea	*Cyclonaias pustulosa*	Nueces	Nueces	MK503282	MK503315
Quadrula aurea	*Cyclonaias pustulosa*	Guadalupe	S. Antonio/Guadalupe	MK503283	MK503319
Quadrula aurea	*Cyclonaias pustulosa*	Guadalupe	S. Antonio/Guadalupe	MK503284	MK503320
Quadrula houstonensis	*Cyclonaias pustulosa*	Colorado	Colorado	MK503273	MK503303
Quadrula houstonensis	*Cyclonaias pustulosa*	Colorado	Colorado	MK503274	MK503304

Quadrula houstonensis	*Cyclonaias pustulosa*	Colorado	Colorado	MK503275	MK503305
Quadrula mortoni	*Cyclonaias pustulosa*	Sandy Creek	Neches	MK503276	MK503306
Quadrula mortoni	*Cyclonaias pustulosa*	Village Creek	Neches	MK503278	MK503308
Quadrula mortoni	*Cyclonaias pustulosa*	Trinity	Trinity	MK503286	MK503322
Quadrula mortoni	*Cyclonaias pustulosa*	Trinity	Trinity	MK503287	MK503323
Quadrula mortoni	*Cyclonaias pustulosa*	Trinity	Trinity	MK503288	MK503324
Quadrula nobilis	*Tritogonia nobilis*	Neches	Neches	MK503279	MK503309
Quadrula nobilis	*Tritogonia nobilis*	Neches	Neches	MK503280	MK503310
Quadrula nobilis	*Tritogonia nobilis*	Trinity	Trinity	MK503285	MK503321
Quadrula quadrula	*Quadrula quadrula*	Ohio	Ohio	MK503267	MK503291
Theliderma johnsoni	*Theliderma metanevra*	Alabama	Alabama	MK503289	-
Pleurobema riddellii	*Pleurobema riddellii*	Village Creek	Neches	MK503277	MK503307
Pleurobema oviforme	*Pleurobema oviforme*	Little Tennessee	Mississippi	-	MK503292
Anodonta nuttalliana	*Anodonta nuttalliana*	John Day	Columbia	MK503266	MK503290

ND1 was amplified using the PCR conditions and primers (Leu-uurF and LoGlyR) of Serb et al (2003). Sequences were obtained with the BigDye sequencing protocol (Applied Biosystems 3730xl) by Macrogen Inc., Korea. Forward and reverse sequences were edited and assembled using ChromasPro 1.7.4 (Technelysium, Tewantin, Australia). All new sequences have been deposited in GenBank (Table 1 and Supporting Information Tables S2 and S3). Three datasets were constructed as follows: one for COI, another for ND1 and a third concatenating COI and ND1. The COI and ND1 datasets included all newly sequenced individuals and all Quadrulini sequences available in the GenBank database for each gene (Supporting Information Tables S2-S4). The COI + ND1 dataset included all individuals sequenced for both COI and ND1 plus GenBank Quadrulini specimens with sequences available for the two genes (Supporting Information Table S4). For each of the three datasets, sequences of additional specimens were downloaded from Genbank and/or newly sequenced as outgroup (details in Supporting Information Tables S2-S4). The three datasets were aligned with the MAFFT multiple sequence alignment algorithm (Katoh & Standley 2013). Each gene alignment was then restricted to its unique haplotypes, retrieved using DnaSP v5.1.0.1 (Librado & Rozas 2009).

Phylogenetic analyses were then performed on the three datasets using Bayesian inference (BI) and maximum likelihood (ML). For the BI analyses, the best-fit models of nucleotide substitution were selected using JModelTest 2.1.10 (Darriba et al 2012) under the Bayesian information criterion. For each gene dataset, a three partition scheme was applied, one per gene codon, with the following selected models: COI (GTR + I + G, HKY, HKY + G), and ND1 (HKY + G, HKY + G, GTR + I + G). For the COI + ND1 dataset, a six-partition scheme was applied for the three codons of both COI and ND1 with the same models selected for the individual gene datasets. BI analyses were performed in MrBayes v3.2.6 (Ronquist et al 2012) implemented in CIPRES Science Gateway (Miller et al 2010). BI analyses were initiated with program-generated trees and four Markov chains with default incremental heating. Two independent runs of 30×10^6 generations were sampled at intervals of 1,000 generations producing a total of 30,000 trees. Burn-in was determined upon the convergence of log-likelihood and parameter values using Tracer 1.6 (Rambaut et al 2014).

For the ML analyses, the same partitioning scheme was applied for each dataset with the same model (GTR + G) for all partitions, and sequences were then analysed in RaxML 8.2.10 HPC Black Box (Stamatakis 2014) with 1,000 bootstrap replicates. Haplotype networks were calculated using TCS 1.21 (Clement et al 2000) with a threshold of 95%.

Molecular based species delineation methods

Five distinct molecular methods were applied to determine the number of molecular operational taxonomic units (MOTUs). All methods were applied to the COI, ND1 and concatenated (COI + ND1) datasets, except for the BIN system that relies only on COI. The

first two are distance-based, that is, the BIN system implemented in BOLD (Ratnasingham & Hebert 2013) and the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al 2012). For the BINs system, the COI dataset without the outgroups was analysed with the Cluster Sequences tool implemented in BOLD 4 (http://v4.boldsystems.org) (Ratnasingham & Hebert 2013). The ABGD species delineation tool was applied to all three datasets without outgroup using its online version (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with the default settings and the Kimura-2-parameter distance matrix (Puillandre et al 2012).

Two tree-based molecular species delineation methods were applied to all datasets, that is, the single threshold Generalized Mixed Yule Coalescent (GMYC) model (Fujisawa & Barraclough 2013) and the Bayesian implementation of the Poisson Tree Processes model (bPTP) (Zhang et al 2013). For the GMYC method, a Bayesian ultrametric phylogenetic tree was first generated in BEAST 2.4.6 (Bouckaert et al 2014) with the previously selected models for each partition and four independent runs of 20×10^6 Markov chain Monte Carlo (MCMC) generations, sampled every 1×10^3 generations. Convergence of the parameters was evaluated using Tracer 1.6 software (Rambaut et al 2014). The consensus tree was annotated using TreeAnnotator 2.4.6 (Bouckaert et al 2014). The consensus tree was loaded into the R software package “Species Limits by Threshold Statistics” (Ezard et al 2009) in R 3.2.0 (R Core Group available at http://www.r-project.org) and analysed using the single threshold model. For the bPTP, the BI phylogenetic trees previously obtained were used as input trees in the bPTP webserver (http://species.h-its.org) with 1×10^6 iterations of MCMC and 20% burn-in. Finally, a 95% statistical parsimony connection limit was used, by using TCS 1.21 (Clement et al 2000). Sequence divergences (uncorrected *p*-distance) were assessed using MEGA 7 (Kumar et al 2016).

Morphometry

For a detailed analysis of inter- and intraspecific variation in shell shape within the quadruline genera *Cyclonaias*, *Quadrula,* and *Theliderma*, we used Fourier Shape Analysis, as developed and explained by Crampton & Haines (1996). This method decomposes xy-coordinates of a shell outline into several harmonics, each of which is in turn explained by two Fourier coefficients. Xy-coordinates of the sagittal shell outline of 1,222 specimens from BSGLC and NCMS collections (739 specimens of *Cyclonaias* spp., 254 specimens of *Quadrula* spp. and 229 specimens of *Theliderma* spp.; Supporting Information Table S1) were obtained from digital photographs using the program IMAGEJ (Rasband 2008) and subjected to fast Fourier transformation using the program HANGLE, applying a smoothing normalization of 3 to eliminate high-frequency pixel noise. A preliminary analysis indicated that the first 10 harmonics described the outlines with sufficiently high precision. Discarding of the first harmonic, which does not contain any shape information, resulted in a set of 18 Fourier

coefficients per individual. Outlines of all specimens within each of the three genera were then rotated to maximum overlap by program HTREE, resulting in the final set of 18 Fourier coefficients per individual.

For visual examination of variation in shell shape within and between true and nominal species, principal component analysis was performed on the 18 Fourier coefficients of (a) all true species (recognized by the molecular species delineation methods, see results) of *Cyclonaias*, including a maximum of 50 specimens per species; (b) all nominal species of *Cyclonaias pustulosa*; (c) only *Cyclonaias kieneriana* and *Cyclonaias kleiniana*; (d) all nominal species of *Quadrula*; (e) all true species (recognized by the molecular species delineation methods, see results) of *Theliderma*; and (f) only *Theliderma metanevra* and *Theliderma johnsoni* n. sp. (see Supporting Information Appendix S2 for a detailed description of *T. johnsoni* n. sp.). Synthetic outlines of extreme and average shell shapes were drawn using program HCURVE as explained in Crampton & Haines (1996).

We assessed the rate of accurate identification of true and nominal species based on shell shape using linear discriminant analysis (LDA) on the 18 Fourier coefficients. To test for statistically significant differences in sagittal shell shape between species, multivariate analyses of variance (MANOVA) were run on the 18 Fourier coefficients. Pairwise Hotelling's post hoc tests were performed to identify significant differences between each pair of true/nominal species. Statistical analyses were performed in PAST v.3 (Hammer & Harper 2006).

Ecological, morphological and anatomical traits

An extensive bibliographic review of selected ecological, morphological and anatomical traits was accomplished for all species within *Quadrula* s.l. (Table 2; Supporting Information Table S5).

TABLE 2
List of morphological, anatomical and behavioural characters of *Cyclonaias*, *Quadrula*, *Theliderma,* and *Tritogonia* as recognized in the present study

	Sexual dimorphism	Shell Sulcus	Periostracal chevrons	Posterior ridge	GLN
Cyclonaias	NO	NO	NO	low rounded	0.05-0.09
Quadrula	NO	YES	NO	well developed	0.005-0.009
Theliderma	NO	NO[2]	YES	low rounded to prominent	0.03-0.04
Tritogonia	YES	YES	NO	well developed	0.009

	Mantle displays (magazines)			Reflexive release	Hosts
	Morphology	Size	Location (apertures)		
Cyclonaias	stomate-shaped	Small	Excurrent	YES	Ictaluridae (71%) Centrarchidae (24%) Acipenseridae (5%)
Quadrula	conical (knob-like)[1]	Large[1]	Excurrent[1]	NO*	Ictaluridae (67%) Centrarchidae (33%)
Theliderma	variable shape	Small	Excurrent	YES	Cyprinidae (72%) Centrarchidae (14%) Percidae (14%)
Tritogonia	slug-shaped*	Large*	Both*	NO*	Ictaluridae

Notes. GLN: mean glochidial size index. [a]Only analysed in one species. [b]For most species.

Results

Alignments and phylogenetic analyses

The COI dataset spanned 582 nucleotides (nt) and included 289 unique haplotypes (232 polymorphic and 192 parsimony informative sites). The ND1 dataset covered 619 bp with 339 unique haplotypes (297 polymorphic and 257 parsimony informative sites). Finally, the combined COI + ND1 dataset was 1,192 nt long and included 325 individual sequences (501 polymorphic and 427 parsimony informative sites). No insertions or deletions and no stop codons were observed in any of the datasets after translating all sequences to amino acids.

The results of the BI and ML phylogenetic analyses for the three datasets presented similar topologies (Table 3), thus only BI phylogenetic trees are shown in Figs. 2-4. In the COI phylogeny, the Quadrulini clade is monophyletic and well supported in the BI analyses. Within the Quadrulini clade, the *Megalonaias* + *Uniomerus* clade is sister to a clade including three well-supported subclades corresponding to the genera *Quadrula*, *Tritogonia*, and *Theliderma*, and a clade including all *Cyclonaias* sequences (Fig. 2).

TABLE 3
Results of repeatability clade analysis (RCA) of main clades corresponding to the preferred topology

Clades	Analyses	COI + ND1	COI	ND1
Quadrulini	BI	**100**	**100**	
	ML	**74**	55	
Quadrula sensu lato	BI	**100**	**100**	**100**
	ML	**98**	**93**	**90**
Cyclonaias	BI	**100**	**95**	**98**
	ML	**83**	35	68
Quadrula s.s.	BI	**100**	**100**	**100**
	ML	**100**	**99**	**99**
Theliderma	BI	**100**	**100**	89
	ML	**100**	**99**	**72**
Tritogonia	BI	**100**	**100**	**100**
	ML	**100**	**98**	**87**
C. infucata + *C. kleiniana* + *C. kieneriana*	BI	65	**97**	
	ML	55	37	
C. petrina + *C. nodulata* + *C. necki*	BI	**99**	**99**	**100**
	ML	**84**	51	**96**
C. pustulosa group	BI	**100**	**100**	89
	ML	**99**	64	45

Notes. In bold values higher than 95% (Bayesian Inference) and 70% (Maximum Likelihood).

The ND1 phylogeny recovered similar phylogenetic patterns to that obtained with COI. However, in these analyses, the Quadrulini is not monophyletic, with the remaining Ambleminae tribe clades, that is, Amblemini, Pleurobemini and Lampsilini clustering within the Quadrulini tribe clade (Fig. 3).

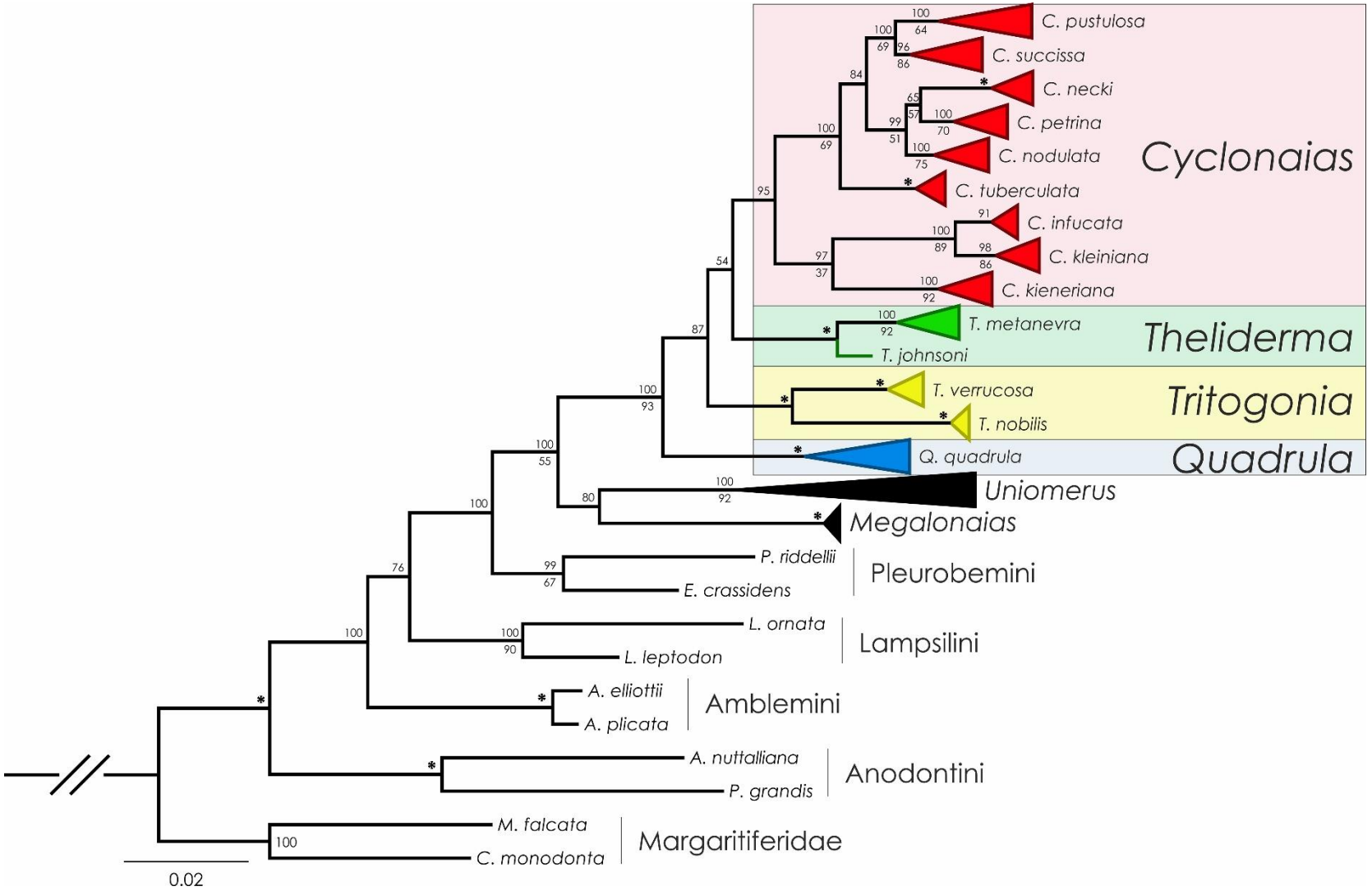

Figure 2 Bayesian consensus tree inferred from the cytochrome *c* oxidase subunit I (COI) gene fragment. The values above and below the nodes indicate Bayesian posterior probability (bpp) percentage and maximum likelihood bootstrap values (bs), respectively. Values over 95% are represented by an asterisk, and those <50% are not shown for clarity

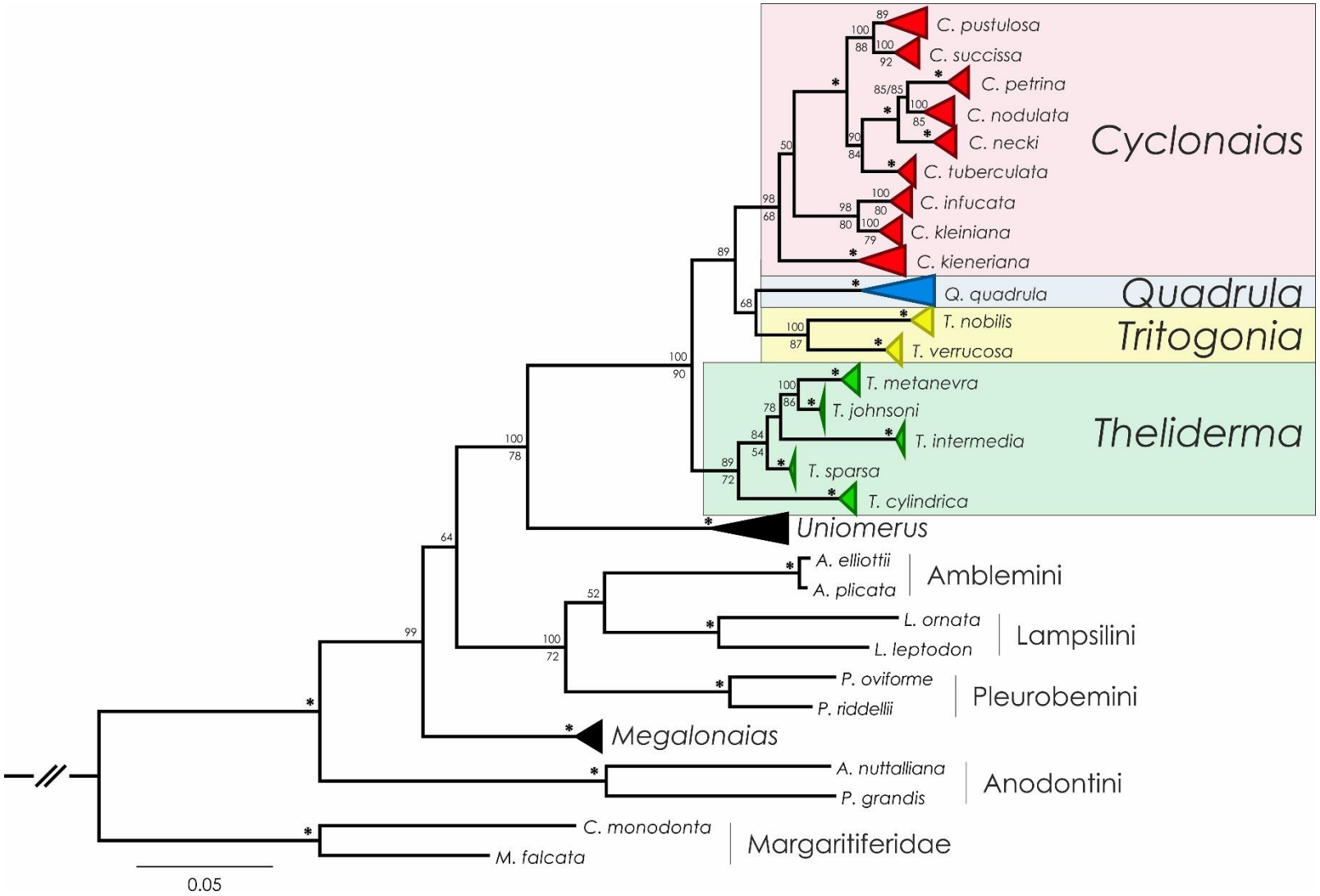

Figure 3 Bayesian consensus tree inferred from the NADH dehydrogenase subunit 1 (ND1) gene fragment. The values above and below the nodes indicate Bayesian posterior probability (bpp) percentage and maximum likelihood bootstrap values (bs), respectively. Values over 95% are represented by an asterisk, values below 50% are not shown for clarity

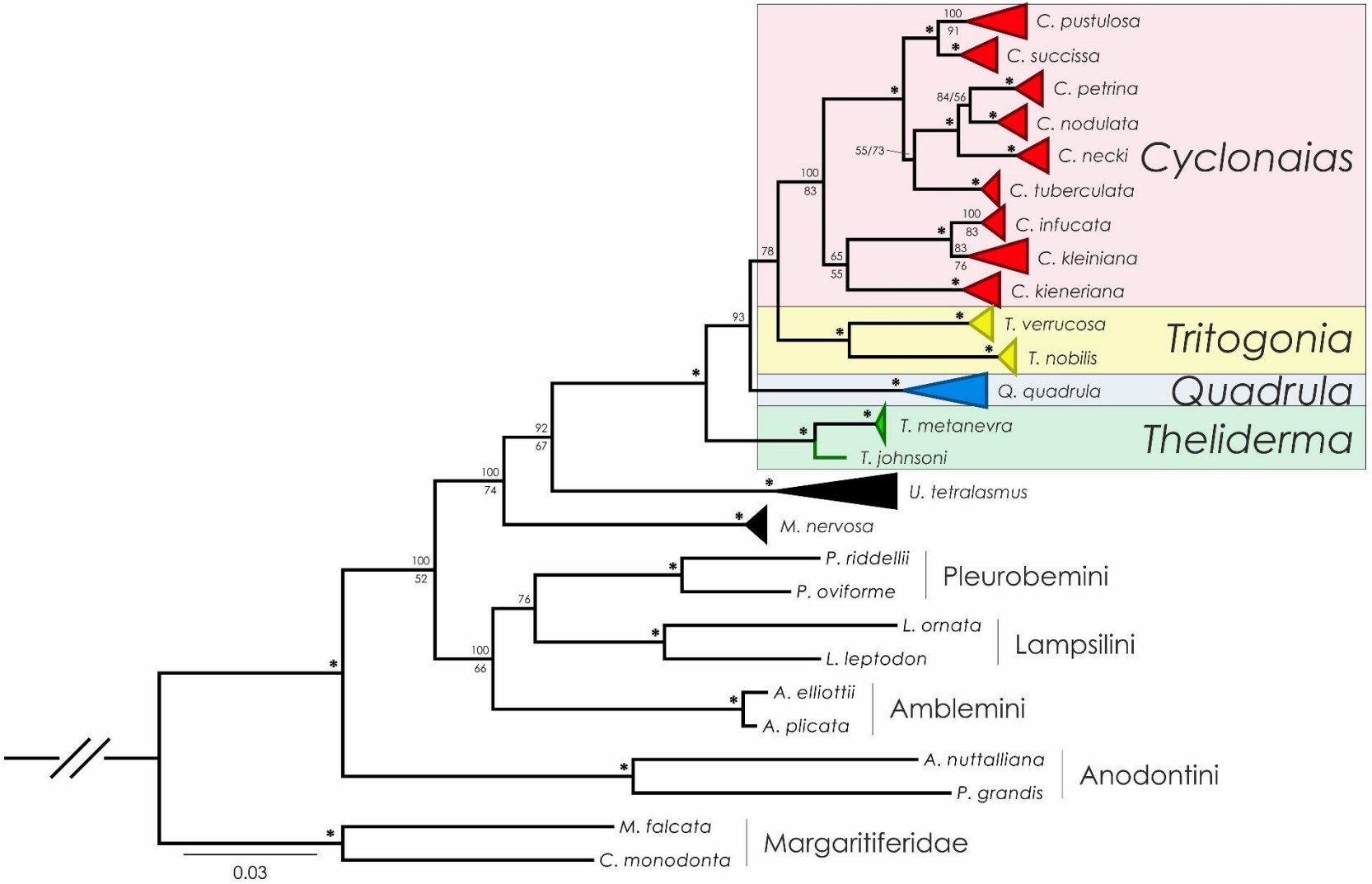

Figure 4 Bayesian consensus tree inferred from the NADH dehydrogenase subunit 1 (ND1) and cytochrome *c* oxidase I (COI) gene fragments concatenated dataset. The values above and below the nodes indicate Bayesian posterior probability (bpp) percentage and maximum likelihood bootstrap values (bs), respectively. Values over 95% are represented by an asterisk, values below 50% are not shown for clarity

The *Uniomerus* clade is sister to a clade containing the four remaining Quadrulini genera (i.e. *Quadrula*, *Tritogonia*, *Theliderma,* and *Cyclonaias*; Fig. 3). While *Cyclonaias*, *Quadrula* and *Tritogonia* are well supported, *Theliderma* has a low support value (Fig. 3). The COI + ND1 phylogeny shows Quadrulini as monophyletic with *Uniomerus* being sister to a clade comprising four well-supported clades (*Quadrula*, *Tritogonia*, *Theliderma,* and *Cyclonaias*; Fig. 4).

Cyclonaias

Within *Cyclonaias*, the clade labelled *C. pustulosa* includes specimens originally identified as *Cyclonaias aurea*, *Cyclonaias houstonensis*, *Cyclonaias mortoni*, *C. pustulosa*, and *Cyclonaias refulgens*.

Quadrula

All sequences from the nominal species *Quadrula quadrula*, *Quadrula apiculata*, and *Quadrula rumphiana* cluster within the *Q. quadrula* clade in all phylogenies (Figs. 2-4). However, both nominal species *Q. apiculata* and *Q. rumphiana* were found to be nested within *Q. quadrula* (Figs. 2-4). Both the COI and ND1 95% threshold haplotype networks of the *Q. quadrula* clade reveal a low number of mutations among the nominal taxa *Q. quadrula*, *Q. apiculata* and *Q. rumphiana* (Figs. 5a,b).

Theliderma

Not many COI sequences of *Theliderma* are represented in the COI dataset, and therefore in the COI and COI + ND1 phylogenies (Figs. 2 and 4). Nevertheless, in these phylogenies two distinct clades were obtained in sequences from specimens of *T. metanevra*: one corresponding to specimens from the Tennessee basin, and the other with specimens from the Mobile basin (Figs. 2 and 4). The ND1 phylogeny is better represented with all species recognized to date except for *Theliderma stapes* (Fig. 3).

Tritogonia

The sequences of specimens originally identified as *Q. nobilis* cluster together with those from *T. verrucosa* in all phylogenies forming a well-supported clade here assigned to *Tritogonia* (Figs. 2-4).

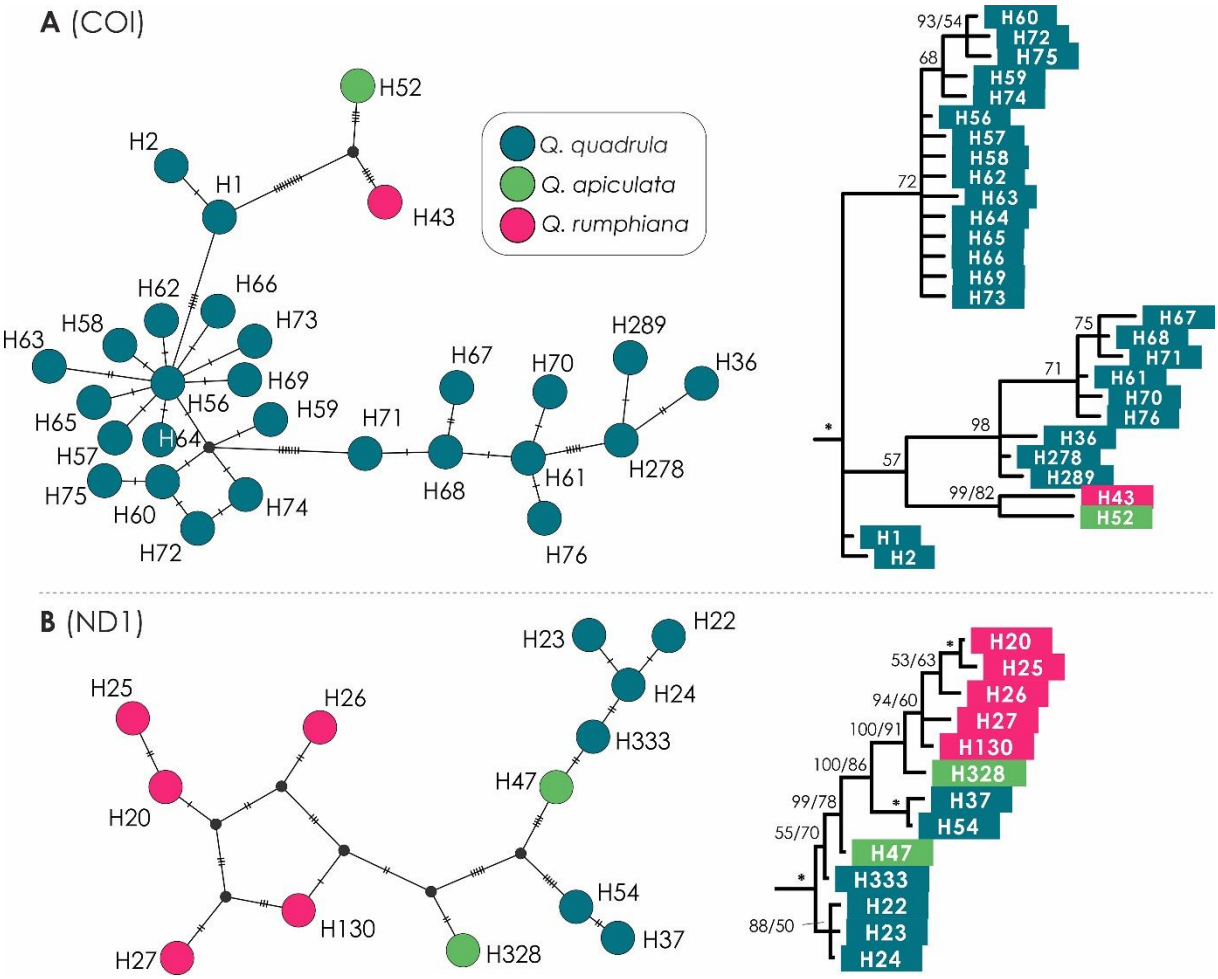

Figure 5 Haplotype (TCS) networks and uncollapsed *Quadrula* clade from Figures 2 and 3, showing the relationships of nominal species within the *Quadrula quadrula* group for (a) cytochrome *c* oxidase I (COI) and (b) NADH dehydrogenase subunit 1 (ND1)

Genetic divergence and species delineation methods

Cyclonaias

Pairwise uncorrected *p*-distance values among six of the nominal *Cyclonaias* species, *C. pustulosa*, *C. aurea*, *C. houstonensis*, *C. mortoni,* and *C. refulgens* were low (≤2% for both COI and ND1: Table 4).

Of the 14 putative *Cyclonaias* species, only nine were recognized as MOTUs based on a consensus of all species delineation methods, applied on the COI, ND1, and COI + ND1 datasets (Table 5). The pairwise uncorrected *p*-distance between these recognized *Cyclonaias* MOTUs varied between 2.8% (COI)/3.1% (ND1) and 11.2% (COI)/10.2% (ND1; Table 6). The uncorrected *p*-distance within each of the recognized MOTUs was ≤1.2% for COI and ≤1.6% for ND1 (Table 6).

Table 4
Pairwise genetic distance matrixes of nominal quadruline species of the genera *Cyclonaias*, *Quadrula*, *Theliderma*, and *Tritogonia*, using the original nominal taxa

	Within Groups		**Between** Groups										
	COI	ND1	***C. asperata***	***C. kieneriana***	***C. kleiniana***	***C. infucata***	***C. nodulata***	***C. petrina***	***C. necki***	***C. pustulosa***	***C. aurea***	***C. houstonensis***	***C. mortoni***
C. asperata	0.012	0.012		0.012	0.082	0.094	0.093	0.102	0.094	0.082	0.082	0.078	0.086
C. kieneriana	----	----			0.081	0.094	0.089	0.101	0.093	0.081	0.082	0.077	0.085
C. kleiniana	0.012	0.011	0.080	----		0.035	0.099	0.094	0.099	0.083	0.085	0.083	0.090
C. infucata	0.006	0.007	0.082	----	0.032		0.097	0.092	0.097	0.087	0.090	0.085	0.092
C. nodulata	0.006	0.009	0.077	----	0.088	0.083		0.038	0.040	0.063	0.063	0.064	0.062
C. petrina	0.007	0.006	0.076	----	0.095	0.090	0.028		0.047	0.063	0.062	0.064	0.061
C. necki	0.007	0.007	0.077	----	0.094	0.084	0.041	0.039		0.064	0.067	0.066	0.066
C. pustulosa	0.010	0.011	0.076	----	0.092	0.085	0.052	0.053	0.051		0.017	0.012	0.019
C. aurea	0.011	0.012	0.078	----	0.092	0.083	0.050	0.051	0.051	0.014		0.018	0.020
C. houstonensis	0.007	0.008	0.075	----	0.088	0.081	0.058	0.059	0.055	0.014	0.017		0.020
C. mortoni	0.013	0.012	0.075	----	0.086	0.079	0.052	0.054	0.055	0.020	0.019	0.020	
C. refulgens	0.015	0.010	0.074	----	0.091	0.084	0.052	0.052	0.052	0.014	0.014	0.017	0.020
C. succissa	0.011	0.011	0.081	----	0.094	0.085	0.048	0.044	0.054	0.036	0.033	0.041	0.037
C. tuberculata	0.006	0.006	0.078	----	0.088	0.090	0.050	0.056	0.062	0.058	0.056	0.064	0.055
Q. quadrula	0.014	0.012	0.112	----	0.110	0.103	0.096	0.097	0.098	0.108	0.104	0.112	0.109
Q. apiculata	----	0.018	0.105	----	0.096	0.096	0.093	0.089	0.095	0.100	0.099	0.103	0.100
Q. rumphiana	----	0.010	0.105	----	0.099	0.095	0.093	0.089	0.095	0.097	0.092	0.100	0.097
T. cylindrica	----	0.010	----	----	----	----	----	----	----	----	----	----	----
T. intermedia	----	0.003	----	----	----	----	----	----	----	----	----	----	----
T. metanevra	0.017	0.021	0.091	----	0.092	0.096	0.094	0.093	0.090	0.084	0.087	0.086	0.088
T. sparsa	----	0.002	----	----	----	----	----	----	----	----	----	----	----
T. verrucosa	0.007	0.008	0.096	----	0.105	0.093	0.102	0.104	0.098	0.105	0.107	0.104	0.105
T. nobilis	0.009	0.011	0.105	----	0.118	0.107	0.108	0.101	0.106	0.102	0.102	0.102	0.106

Table 4 (*cont.*)

	Between Groups											
	C. refulgens	***C. succissa***	***C. tuberculata***	***Q. quadrula***	***Q. apiculata***	***Q. rumphiana***	***T. cylindrica***	***T. intermedia***	***T. metanevra***	***T. sparsa***	***T. verrucosa***	***T. nobilis***
C. asperata	0.080	0.083	0.094	0.101	0.107	0.114	0.112	0.143	0.111	0.105	0.114	0.115
C. kieneriana	0.079	0.083	0.096	0.101	0.109	0.111	0.116	0.143	0.111	0.106	0.111	0.114
C. kleiniana	0.084	0.092	0.088	0.109	0.116	0.121	0.110	0.143	0.117	0.105	0.112	0.123
C. infucata	0.088	0.095	0.093	0.108	0.110	0.117	0.115	0.139	0.116	0.110	0.107	0.125
C. nodulata	0.059	0.064	0.055	0.123	0.129	0.134	0.129	0.144	0.121	0.113	0.118	0.126
C. petrina	0.058	0.064	0.065	0.127	0.131	0.136	0.125	0.140	0.121	0.110	0.122	0.130
C. necki	0.062	0.070	0.059	0.127	0.131	0.134	0.127	0.147	0.126	0.115	0.116	0.126
C. pustulosa	0.012	0.033	0.054	0.108	0.112	0.116	0.121	0.136	0.115	0.106	0.105	0.119
C. aurea	0.014	0.031	0.051	0.107	0.111	0.115	0.118	0.136	0.119	0.107	0.106	0.118
C. houstonensis	0.013	0.029	0.052	0.103	0.107	0.111	0.116	0.134	0.114	0.105	0.101	0.118
C. mortoni	0.017	0.030	0.050	0.111	0.115	0.119	0.126	0.137	0.118	0.107	0.106	0.118
C. refulgens		0.027	0.049	0.108	0.113	0.116	0.120	0.137	0.116	0.106	0.104	0.116
C. succissa	0.035		0.053	0.109	0.113	0.122	0.124	0.144	0.126	0.113	0.110	0.119
C. tuberculata	0.058	0.053		0.115	0.117	0.120	0.127	0.146	0.126	0.113	0.116	0.121
Q. quadrula	0.108	0.100	0.098		0.017	0.027	0.104	0.139	0.116	0.108	0.109	0.105
Q. apiculata	0.100	0.092	0.085	0.034		0.020	0.109	0.143	0.117	0.112	0.111	0.107
Q. rumphiana	0.097	0.088	0.084	0.034	0.015		0.112	0.145	0.119	0.117	0.110	0.116
T. cylindrica	----	----	----	----	----	----		0.106	0.086	0.079	0.122	0.126
T. intermedia	----	----	----	----	----	----	----		0.081	0.073	0.135	0.137
T. metanevra	0.088	0.095	0.083	0.101	0.090			0.096		0.040	0.115	0.126
T. sparsa	----	----	----	----	----	----	----	----	----		0.105	0.106
T. verrucosa	0.106	0.100	0.098	0.114	0.116	----	----	0.116	----	0.096		0.093
T. nobilis	0.102	0.099	0.114	0.110	0.116	----	----	0.114	----	0.114	0.085	

Table 5
Results of molecular species delineation methods

TAXA	COI					ND1				COI + ND1				CONSENSUS MOTUs
	BOLD	ABGD	TCS	bPTP	GMYC	ABGD	TCS (95%)	bPTP	GMYC	ABGD	TCS	bPTP	GMYC	
Cyclonaias														
C. asperata	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓	✗
C. kieneriana	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	✓
C. infucata	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C. kleiniana	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C. nodulata	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C. petrina	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C. necki	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C. pustulosa	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C. aurea	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
C. houstonensis	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗
C. mortoni	✗	✗	✗	✗	✓	✗	✗	✗	✓	✓	✓	✗	✓	✗
C. refulgens	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
C. succissa	✓	✓	✓	✓	✓	✗	✓	✗	✓	✓	✓	✓	✓	✓
C. tuberculata	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Quadrula	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Q. quadrula 1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Q. quadrula 2	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✓	✗	✓	✗
Q. quadrula 3	✓	✓	✗	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗
Q. apiculata	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✓	✗
Q. rumphiana	✗	✓	✗	✗	✗	✓	✓	✗	✗	✗	✗	✗	✓	✗
Theliderma														
T. cylindrica	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	✓
T. intermedia	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	✓
T. metanevra	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T. johnsoni n. sp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T. sparsa	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	✓
Tritogonia						✓	✓	✓	✓					
T. verrucosa	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
T. nobilis	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Notes. ✓**:** recognized as a molecular operational taxonomic unit (MOTU); ✗: not recognized as a MOTU; -: not analysed.

Table 6
Pairwise genetic distance matrixes of quadruline species of the genera *Cyclonaias*, *Quadrula*, *Theliderma*, and *Tritogonia*, as recognized in the present study

	Among groups		**Between groups**						
TAXA	COI	ND1	*C. kieneriana*	*C. infucata*	*C. kleiniana*	*C. nodulata*	*C. petrina*	*C. necki*	*C. pustulosa*
C. kieneriana	0.012	0.012		0.094	0.082	0.093	0.102	0.094	0.082
C. infucata	0.006	0.007	0.082		0.035	0.097	0.092	0.097	0.089
C. kleiniana	0.012	0.011	0.080	0.032		0.099	0.094	0.099	0.085
C. nodulata	0.006	0.009	0.077	0.083	0.088		0.038	0.04	0.063
C. petrina	0.007	0.006	0.076	0.090	0.095	0.028		0.047	0.062
C. necki	0.007	0.007	0.077	0.084	0.094	0.041	0.039		0.065
C. pustulosa	0.016	0.016	0.076	0.082	0.089	0.052	0.053	0.052	
C. succissa	0.011	0.011	0.081	0.085	0.094	0.048	0.044	0.054	0.036
C. tuberculata	0.006	0.006	0.078	0.090	0.088	0.050	0.056	0.062	0.057
Q. quadrula	0.017	0.019	0.112	0.103	0.109	0.096	0.096	0.098	0.107
T. cylindrica	----	0.010	----	----	----	----	----	----	----
T. intermedia	----	0.003	----	----	----	----	----	----	----
T. metanevra	0.009	0.005	0.090	0.095	0.091	0.093	0.094	0.090	0.086
T. johnsoni	----	0.002	0.093	0.099	0.096	0.095	0.092	0.088	0.088
T. sparsa	----	0.002	----	----	----	----	----	----	----
T. verrucosa	0.007	0.008	0.096	0.093	0.105	0.102	0.104	0.098	0.105
T. nobilis	0.009	0.011	0.105	0.107	0.118	0.108	0.101	0.106	0.103

	Between groups									
TAXA	*C. succissa*	*C. tuberculata*	*Q. quadrula*	*T. cylindrica*	*T. intermedia*	*T. metanevra*	*T. johnsoni*	*T. sparsa*	*T. verrucosa*	*T. nobilis*
C. kieneriana	0.083	0.095	0.107	0.112	0.143	0.111	0.111	0.105	0.114	0.115
C. infucata	0.095	0.093	0.112	0.115	0.139	0.117	0.115	0.110	0.107	0.125
C. kleiniana	0.092	0.088	0.115	0.110	0.143	0.118	0.116	0.105	0.112	0.123
C. nodulata	0.064	0.055	0.128	0.129	0.144	0.123	0.117	0.113	0.118	0.126
C. petrina	0.064	0.065	0.131	0.125	0.14	0.125	0.114	0.110	0.122	0.130
C. necki	0.07	0.059	0.13	0.127	0.147	0.129	0.12	0.115	0.116	0.126
C. pustulosa	0.031	0.052	0.112	0.121	0.136	0.119	0.112	0.106	0.105	0.118
C. succissa		0.053	0.114	0.124	0.144	0.129	0.118	0.113	0.11	0.119
C. tuberculata	0.053		0.117	0.127	0.146	0.126	0.126	0.113	0.116	0.121
Q. quadrula	0.100	0.097		0.108	0.141	0.122	0.109	0.112	0.110	0.110
T. cylindrica	----	----	----		0.106	0.088	0.082	0.079	0.122	0.126
T. intermedia	----	----	----	----		0.084	0.076	0.073	0.135	0.137
T. metanevra	0.096	0.083	0.102	----	----		0.035	0.042	0.117	0.129
T. johnsoni	0.094	0.085	0.094	----	----	0.032		0.036	0.109	0.121
T. sparsa	----	----	----	----	----	----	----		0.105	0.106
T. verrucosa	0.100	0.098	0.114	----	----	0.096	0.097	----		0.093
T. nobilis	0.099	0.114	0.110	----	----	0.115	0.107	----	0.085	

Quadrula

The pairwise uncorrected *p*-distance among all nominal *Quadrula* species varied from 1.4% (COI)/1.7% (ND1) to 3.4% (COI)/2.7% (ND1; Table 4). Considering the three datasets, only a single MOTU was consensually recognized for the *Quadrula* genus (Table 5) with a within the *p*-distance value of 1.7% for COI and 1.9% for ND1 (Table 6).

Theliderma

The pairwise uncorrected *p*-distance among all the nominal *Theliderma* species ranged between 4.0% and 10.6% for ND1(Table 4). The higher within *p*-distance recorded value was reached for *T. metanevra*, 1.7% for COI and 2.1% for ND1 (Table 4).

All originally described *Theliderma* species are here recognized as MOTUs with *T. metanevra* being further divided into two distinct MOTUs, that is, *T. metanevra* for specimens from the Tennessee River basin and *T. johnsoni* n. sp. from the Mobile River basin (Table 5). The *p*-distance values among the recognized *Theliderma* MOTUs varied between 3.5% and 10.1% for ND1 (Table 6). The *p*-distance within each of the recognized MOTUs was ≤0.9% for ND1 (Table 6).

Tritogonia

Our analyses revealed a complete consensus of two individual MOTUs within the *Tritogonia* genus (Table 5). The two recognized MOTUs *T. verrucosa* and *Tritogonia nobilis* exhibited high interspecific *p*-distance divergence, 8.5% (COI)/9.3% (ND1), and low intraspecific *p*-distance <0.9% for COI and <1.1% ND1 (Table 6).

Morphometry

Cyclonaias

Linear discriminant analysis on the 18 Fourier coefficients extracted through Fourier Shape Analysis for all *Cyclonaias* species recognized in this study assigned 75% of individuals to the correct species (Fig. 6a). Species that are particularly difficult to separate by shell shape are *C. kieneriana* and *C. pustulosa* (16% misidentifications), and *Cyclonaias infucata* and *C. kleiniana* (10%). Also, most true species differed significantly from each other in their shell shape as approximated by 18 Fourier coefficients, except for *C. infucata* and *C. kleiniana* (MANOVA, pairwise Hotelling's test $p = 0.742$), and *C. infucata* and *Cyclonaias necki* (MANOVA, pairwise Hotelling's test $p = 0.138$).

The proportion of *C. pustulosa* specimens correctly identified to the original nominal species within the *C. pustulosa* complex exceeded that of *Cyclonaias* specimens correctly identified to species level (see above), with 80% correct identifications (Fig. 6b). All nominal

species differed significantly from each other in their shell shape as approximated by 18 Fourier coefficients (MANOVA, pairwise Hotelling's tests $p < 0.05$).

Using only the nominal species *C. kieneriana* and *Cyclonaias asperata*, the Fourier coefficients differed significantly between *C. kieneriana* and its synonym *C. asperata* (MANOVA: $F_{18,82} = 2.094$, $p = 0.013$), and 95% of specimens were classified correctly based on shell shape through LDA (Fig. 6c).

Quadrula

Fourier coefficients differed significantly between the nominal species of *Quadrula* (MANOVA, pairwise Hotelling's tests $p < 0.05$; Fig. 6c). Seventy-six percent of specimens were assigned to the correct nominal species, with 21% and 11% of misidentifications between *Q. apiculata* versus *Q. quadrula* and *Q. rumphiana*, respectively.

Theliderma

Within the genus *Theliderma*, 91% of specimens were identified to the correct species (as they are here recognized) by LDA of Fourier coefficients (Fig. 6e). *Theliderma cylindrica*, characterized by its typical elongated-rectangular shape, was 100% correctly identified. Considerable difficulties in separation by shell shape were present for *Theliderma sparsa* versus *T. johnsoni* n. sp. (21% misidentifications) and *T. metanevra* (13%), respectively. Most true *Theliderma* species pairs differed significantly from each other in their shell shape except for *T. sparsa* versus *T. johnsoni* n. sp. (MANOVA, pairwise Hotelling's test: $p = 0.525$), *T. sparsa* versus *T. metanevra* ($p = 0.227$) and *T. stapes* and *T. johnsoni* n. sp. ($p = 0.427$; *p*-value could not be computed for the pair *T. sparsa* vs. *T. stapes* due to low replicate number).

When including the whole *Theliderma* dataset in LDA, only 5% of specimens of the pair *T. metanevra*/*T. johnsoni* n. sp. were assigned to the wrong clade (Fig. 6e). When using only the *T. metanevra* dataset, 11% of specimens were misidentified (Fig. 6f), though Fourier coefficients were significantly different between the two species (MANOVA: $F_{18,46} = 3.097$, $p = 0.001$).

Diagnostic characters of the classical genera within *Quadrula* s.l

Species within *Quadrula* and *Tritogonia* share several ecological and morphological traits but are distinct from those within *Cyclonaias* and *Theliderma* (Table 2; Supporting Information Table S5). *Quadrula* and *Tritogonia* species exhibit a marked sulcus that is absent in *Cyclonaias* and *Theliderma*, except for *T. sparsa* and *T. stapes* that may display shallow sulci (Table 2; Supporting Information Table S5).

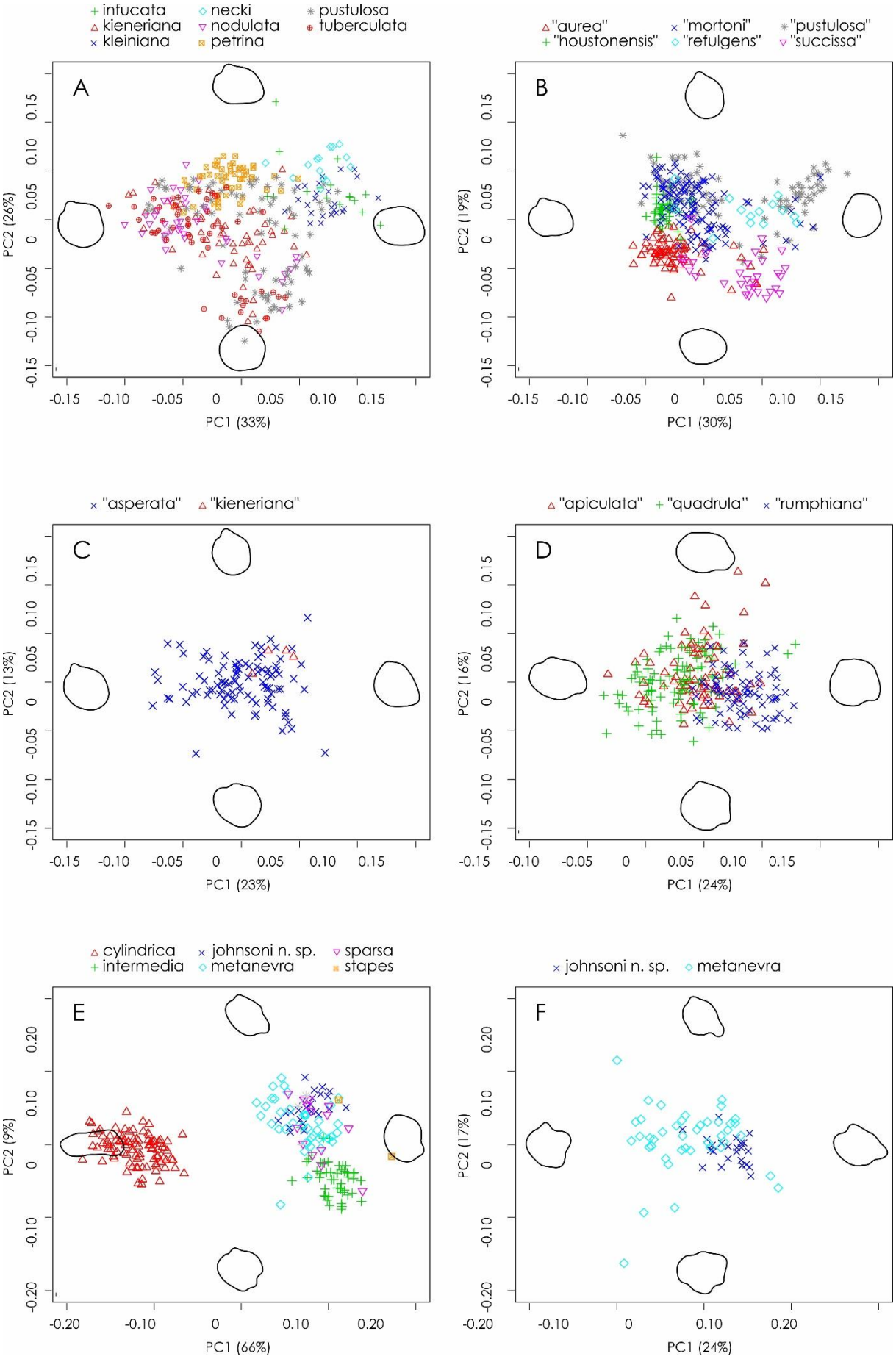
infucata
kieneriana
kleiniana
necki
nodulata
petrina
pustulosa
tuberculata
A
PC2 (26%)
PC1 (33%)
"aurea"
"houstonensis"
"mortoni"
"refulgens"
"pustulosa"
"succissa"
B
PC2 (19%)
PC1 (30%)
"asperata"
"kieneriana"
C
PC2 (13%)
PC1 (23%)
"apiculata"
"quadrula"
"rumphiana"
D
PC2 (16%)
PC1 (24%)
cylindrica
intermedia
johnsoni n. sp.
metanevra
sparsa
stapes
E
PC2 (9%)
PC1 (66%)
johnsoni n. sp.
metanevra
F
PC2 (17%)
PC1 (24%)

Figure 6 Shell outline principal component scores for the first two PC axes obtained from 18 Fourier coefficients of (a) all true species (recognized by molecular species delineation methods; see results) of *Cyclonaias*, including a maximum of 50 specimens per species; (b) all nominal species of *Cyclonaias pustulosa*; (c) only *Cyclonaias kieneriana* and *Cyclonaias asperata*; (d) all nominal species of *Quadrula*; (e) all true species (recognized by molecular species delineation methods; see results) of *Theliderma*; and (f) only *Theliderma metanevra* and *Theliderma johnsoni* n. sp. Synthetic shell outlines of "extreme" morphotypes are displayed with the anterior margin facing to the left and the dorsal margin to the top of the page

Quadrula and *Tritogonia* glochidial size index are ten times smaller than in *Cyclonaias* and *Theliderma* (Table 2; Supporting Information Table S5). *Quadrula* and *Tritogonia* also seem to share similar morphological and behavioural patterns of the mantle displays, also known as mantle magazines. While *Quadrula* and *Tritogonia* seem to exhibit large mantle displays with a non-reflexive glochidia release strategy when disturbed, *Cyclonaias* and *Theliderma* mantle displays are small and more inconspicuous and immediately expel their glochidial content when physically disturbed (Table 2; Supporting Information Table S5). However, some caution must be taken when interpreting this data since mantle displays were only studied in a small number of species.

Within *Quadrula* s.l. some of the analysed characters are exclusive and can be used to recognize some of the classically recognized genera *Cyclonaias*, *Quadrula*, *Theliderma,* and *Tritogonia* (Table 2; Supporting Information Table S5).

The presence of dark chevrons imprinted in the periostracum of shells is a trait that is exclusive of *Theliderma* species and can be used to recognize the genus within Quadrulini (Table 2; Supporting Information Table S5).

The stomate-shaped morphology of the mantle displays seems to be a diagnostic character for *Cyclonaias*, but laboratory studies on *C. asperata* (= *C. kieneriana*) did not observe any mantle display for this species (Haag & Staton 2003).

Theliderma hosts are mainly composed of small cyprinids while catfishes are the main hosts of the other three *Quadrula* s.l. genera (Table 2; Supporting Information Table S5). The mantle displays and glochidia of *Theliderma* are smaller than those of *Cyclonaias* (Table 2; Supporting Information Table S5).

Tritogonia verrucosa and *T. nobilis* are sexually dimorphic in shell shape, a trait that is unique within the Quadrulini and therefore diagnostic of the genus (Table 2; Supporting Information Table S5). Also, the mantle display mechanism of *T. verrucosa*, which involves the mantle to completely cover both the incurrent and excurrent aperture, is very distinct from those of all the other *Quadrula* s.l. species (Supporting Information Table S5). However, this trait needs to be verified for *T. nobilis* to be considered diagnostic of the genus.

Discussion

Phylogenetic relationships within *Quadrula* and generic support

The three BI and ML phylogenies (COI, ND1, and COI + ND1) obtained in the present study revealed a well-supported *Quadrula* sensu lato clade subdivided into four clades (mainly in the BI analyses), corresponding to the genera *Quadrula*, *Cyclonaias*, *Theliderma*, and *Tritogonia* (Figs. 2-4; Table 3). Furthermore, taxa in these clades exhibit coherent combinations of traits that in our opinion support the validity of their generic status as recently recognized by Williams et al (2017) (Figs. 2-4; Tables 3 and 5, Supporting Information Table S5).

The current molecular phylogenies cannot strongly support any suprageneric relationships (probably due to insufficient genetic marker representation) within *Quadrula* s.l. However, the morphological and ecological data here presented suggest common evolutionary origins for the genera *Quadrula* and *Tritogonia*, and for *Cyclonaias* and *Theliderma* (Table 2; Supporting Information Table S5). While *Quadrula* and *Tritogonia* include large reflexive mantle displays, miniaturized shell glochidia, and marked shell sulci, *Cyclonaias* and *Theliderma* species have small non-reflexive mantle displays, larger glochidia, and absent or shallow shell sulci (Table 2; Supporting Information Table S5).

The series of traits shared by *Quadrula* and *Tritogonia* are likely associated with maximizing attachment success to their main hosts, the catfishes (Table 2). These traits include large conspicuous mantle displays that do not respond to mechanical disturbance (but probably to another type of stimulus, for example, chemical, that might capitalize on the acute olfactory sense of their hosts) and miniaturized glochidia. *Tritogonia* species are the only *Quadrula* s.l. species that exhibit marked shell sexual dimorphism. This is probably a result of the presence of mantle displays that completely cover the incurrent and excurrent apertures of females, resulting in a distortion of their shells (Table 2, Supporting Information Table S5). On the other hand, a specialization in attracting small cyprinids and percids seems to have driven reproductive behaviour and morphology in *Theliderma* towards females that are generally completely buried with only the mantle display being visible (Sietman et al 2012). The displays of *Theliderma* are also more conspicuously displayed during the day favouring the visual predatory habits of cyprinids, which contrasts with the other three *Quadrula* s.l. genera who are generally displaying at night when feeding activity in catfishes is highest (Hove et al 2011). *Theliderma* species are unique within Quadrulini in the production of mucoid conglutinates (Haag 2012) and by presenting dark chevrons in the shells periostracum (Table 2; Supporting Information Table S5). The glochidia of *Theliderma* are also much bigger than those of *Tritogonia* and *Quadrula* and more similar in size to most of the other species within the Ambleminae (Table 2; Barnhart et al 2008). The large size of *Theliderma* glochidia can be related to the much lower fecundity of this genus when compared with the other *Quadrula* s.l.

genera (Haag 2012). *Cyclonaias* presents a set of reproductive features that are like those in *Theliderma* species. However, glochidial size in *Cyclonaias* is always larger than in *Theliderma,* and *Cyclonaias* exhibit a prevalence of catfish hosts rather than cyprinids and percids (Table 2). Adaptation to catfish hosts again is likely associated with the unique stomate-shaped mantle displays exhibited by *Cyclonaias* species (Table 2). The miniaturized glochidia shared by *Quadrula* and *Tritogonia* probably represent a derivation from the more primitive glochidial size of most amblemines (Barnhart et al 2008). On the other hand, preference for and related adaptations to catfish hosts seem to be ancestral for the Quadrulini, while preference for small cyprinids and percids in *Theliderma* is probably the derived state. A more robust multi-marker molecular approach is needed to get a clearer view of the evolutionary aspects of these interesting adaptations and to resolve the suprageneric relationships among *Quadrula* s.l. genera.

Phylogeny and systematics implications within the four *Quadrula* sensu lato genera

Cyclonaias

The present results confirm the results of a recent study on this genus (Johnson et al 2018) recognizing nine of the 14 *Cyclonaias* species listed by Williams et al (2017) as valid species (Table 7). However, we here consider *C. asperata* as a synonym of *C. kieneriana* due to the residual genetic divergence between these two taxa (ND1 *p*-distance <1%) and the fact that *C. kieneriana* (Lea, 1852) has priority over *C. asperata* (Lea, 1861). In contrast, Williams et al (2017) recognized both species based on their morphological distinctiveness and the fact that molecular evidence for synonymy was based on only one marker (ND1) from a single specimen. However, ND1 is a highly representative marker of overall mtDNA evolution in unionoid mussels (Fonseca et al 2016). Besides, the divergence between *C. asperata* and *C. kieneriana* sequences was very low. As a result, both ND1 (BI and ML) analyses were unable to resolve both species' phylogenies, and all ND1 species delineation methods were unable to separate the two species (Table 5), indicating that *C. asperata* should be synonymized under *C. kieneriana*. The morphometry results supported the distinct morphology of the two nominal species but very few *C. kieneriana* shells (*n* = 4) were available, preventing a comprehensive analysis (Fig. 6c). Although *C. asperata* has been reported from a much wider geographic range than *C. kieneriana*, both species are sympatric in the whole range of *C. kieneriana* suggesting that specimens previously described as *C. kieneriana* are smooth forms of the same species (Fig. 7).

Until recently, *Cyclonaias archeri* has been considered a subspecies of *C. asperata* (Turgeon et al 1998). However, since no sequences, tissues or shell specimens of *C. archeri*

were available for this study, we rely on Williams et al (2008, 2017) and recognize this species as separate from *C. asperata*, based on its distinct morphology.

Cyclonaias necki has recently been separated from *Cyclonaias petrina* based on molecular data (COI) and morphology (Burlakova et al 2018; Johnson et al 2018). The specific rank of *C. necki* is here confirmed by all species delineation methods used on each of the datasets (Table 5). The shell shape is also significantly different between *C. petrina* and *C. necki* (Fig. 6a), confirming observations of Burlakova et al (2018) and Johnson et al (2018) that *C. necki* shells are thinner, more compressed and more rectangular with a more distinct and prominent posterior ridge. Distribution ranges of the two species are exclusive, with *C. necki* being present only in the San Antonio/Guadalupe River basins, while *C. petrina* is restricted to the Colorado basin (Fig. 8; Burlakova et al 2018).

The present paper confirms the inclusion of four nominal species, that is, *C. aurea, C. houstonensis*, *C. mortoni*, *C. refulgens*, within *C. pustulosa* (Table 7) and *Cyclonaias succissa*, as a related but distinct species, as proposed by Johnson et al (2018). None of the phylogenies resolved them as monophyletic, and *p*-distance values among these taxa were very low (Table 4). All nominal species here synonymized with *C. pustulosa* have distinct and exclusive geographic distributions (Fig. 9). The molecular results suggest that *C. pustulosa* is divided into several morphotypes each in a distinct geographic area. These morphotypes are visible in the morphometry results and explain why these populations used to be considered distinct species (Fig. 7b).

The remaining *Cyclonaias* species recognized in the present study, that is, *C. infucata*, *C. kleiniana*, *C. kieneriana*, *Cyclonaias nodulata,* and *Cyclonaias tuberculata*, were always retrieved as well supported, divergent clades (Figs. 2-4), and recognized by all species delineation methods (Table 5). Furthermore, the shell shape is different among all these latter species, except for the pair *C. infucata* and *C. kleiniana*, which might be explained by their closer genetic relationship (Figs. 2-4; Table 6).

Table 7
Historical classification of species formerly assigned to *Quadrula*. * extinct.

Haas (1969a)	Graf & Cummings (2007)	Bogan (2013)
Quadrula		
Quadrula (Quadrula) q. quadrula	*Quadrula quadrula*	*Quadrula quadrula*
Quadrula (Quadrula) q. apiculata	*Quadrula apiculata*	*Quadrula apiculata*
Quadrula (Quadrula) q. rumphiana	*Quadrula rumphiana*	*Quadrula rumphiana*
Quadrula (Pustulosa) couchiana	*Amphinaias couchiana*	*Quadrula couchiana*
Quadrula (s.s.) quadrula fragosa	*Quadrula fragosa*	*Quadrula fragosa*
Cyclonaias		
Quadrula (Pustulosa) p. keineriana		*Quadrula kieneriana*
Quadrula (Pustulosa) p. pernodosa	*Amphinaias asperata*	*Quadrula asperata*
Fusconaia succissa succissa	*Quicucina infucata*	*Quadrula infucata*
Quincuncina securiformis kleiniana		*Quadrula kleiniana*
Quadrula (Pustulosa) archeri	*Amphinaias archeri*	
Quadrula (Pustulosa) nodulata	*Amphinaias nodulata*	*Quadrula nodulata*
Quadrula (Pustulosa) petrina	*Amphinaias petrina*	*Quadrula petrina*
Quadrula (Pustulosa) p. pustulosa	*Amphinaias pustulosa*	*Quadrula pustulosa*
Quadrula (Pustulosa) aurea	*Amphinaias aurea*	*Quadrula aurea*
	Amphinaias houstonensis	*Quadrula houstonensis*
Quadrula (Pustulosa) p. mortoni		*Quadrula mortoni*
Quadrula (Pustulosa) p. refulgens	*Amphinaias refulgens*	*Quadrula refulgens*
Fusconaia succissa succissa	*Fusconaia succissa*	*Quadrula succissa*
Cyclonaias tuberculata tuberculata	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*
Theliderma		
Orthonymus cylindricus	*Theliderma cylindrica*	*Quadrula cylindrica*
Orthonymus intermedius	*Theliderma intermedia*	*Quadrula intermedia*
Orthonymus m. metanevrus	*Theliderma metanevra*	*Quadrula metanevra*
Orthonymus m. tuberosus	*Theliderma tuberosa*	
	Theliderma sparsa	*Quadrula sparsa*
	Theliderma stapes	
Tritogonia		
Tritogonia verrucosa	*Tritogonia verrucosa*	Quadrula verrucosa
Quadrula (Quadrula) q. nobilis	*Quadrula nobilis*	*Quadrula nobilis*

Table 7 (*cont*.)

Williams *et al* (2017)	**This study**
Quadrula quadrula	1. *Quadrula quadrula*
Quadrula apiculata	+ *Quadrula apiculata*
Quadrula rumphiana	+ *Quadrula rumphiana*
Quadrula couchiana	2. *Quadrula couchiana**
Quadrula fragosa	3. *Quadrula fragosa*
Cyclonaias kieneriana	1. *Cyclonaias kieneriana*
Cyclonaias asperata	+ *Cyclonaias asperata*
Cyclonaias infucata	2. *Cyclonaias infucata*
Cyclonaias kleiniana	3. *Cyclonaias kleiniana*
Cyclonaias archeri	4. *Cyclonaias archeri*
Cyclonaias nodulata	5. *Cyclonaias nodulata*
Cyclonaias petrina	6. *Cyclonaias petrina*
	7. *Cyclonaias necki*
Cyclonaias pustulosa	8. *Cyclonaias pustulosa*
Cyclonaias aurea	+ *Cyclonaias aurea*
Cyclonaias houstonensis	+ *Cyclonaias houstonensis*
Cyclonaias mortoni	+ *Cyclonaias mortoni*
Cyclonaias refulgens	+ *Cyclonaias refulgens*
Cyclonaias succissa	9. *Cyclonaias succissa*
Cyclonaias tuberculata	10. *Cyclonaias tuberculata*
Theliderma cylindrica	1. *Theliderma cylindrica*
Theliderma intermedia	2. *Theliderma intermedia*
Theliderma metanevra	3. *Theliderma metanevra*
	4. *Theliderma johnsoni* n. sp.
Theliderma sparsa	5. *Theliderma sparsa*
	6. *Theliderma stapes*
Tritogonia verrucosa	1. *Tritogonia verrucosa*
Quadrula nobilis	2. *Tritogonia nobilis*

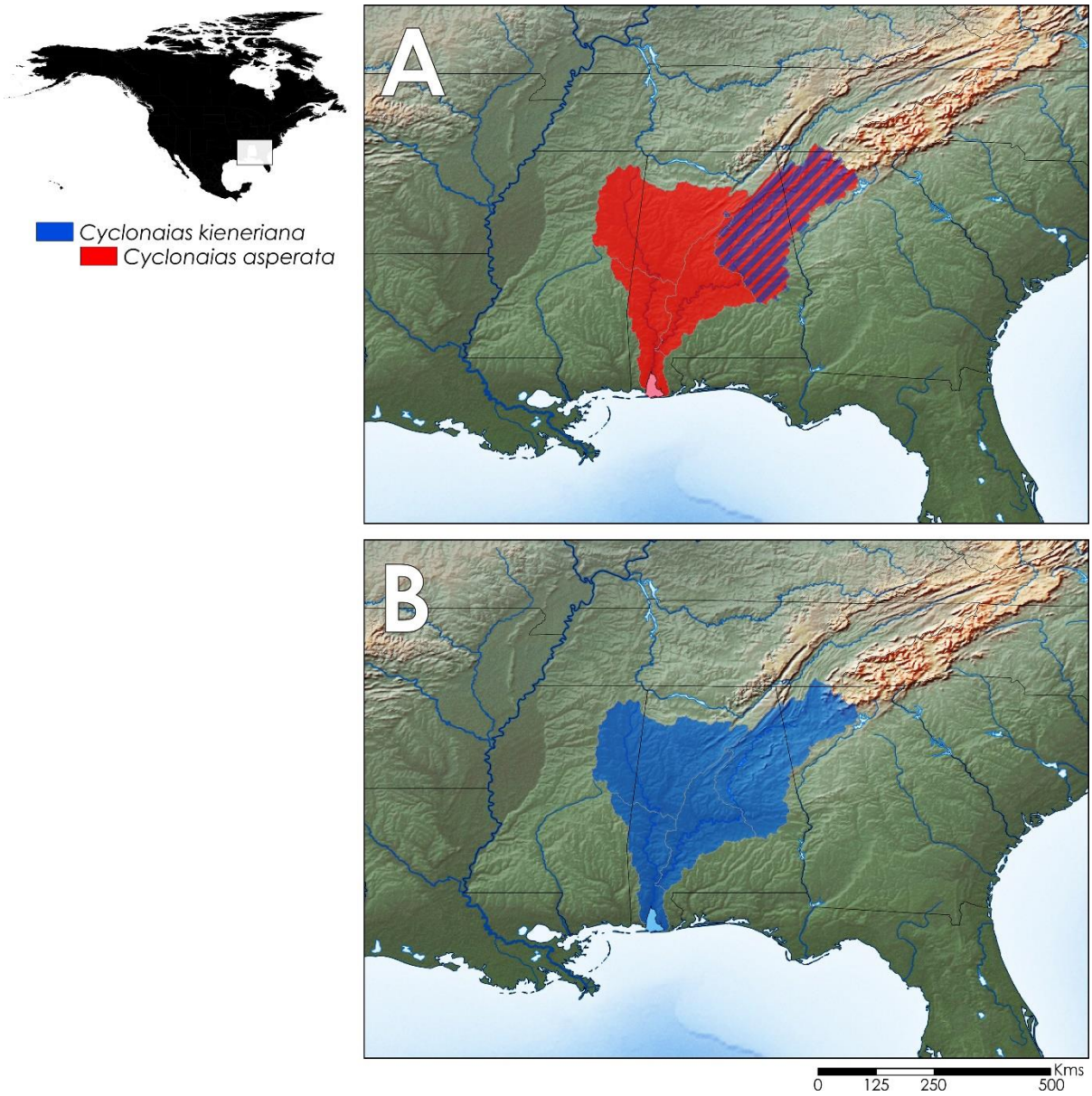

Figure 7 Distribution maps of (a) nominal species *Cyclonaias asperata* and *Cyclonaias kieneriana* before the present study and (b) of *C. kieneriana* as proposed in the present study

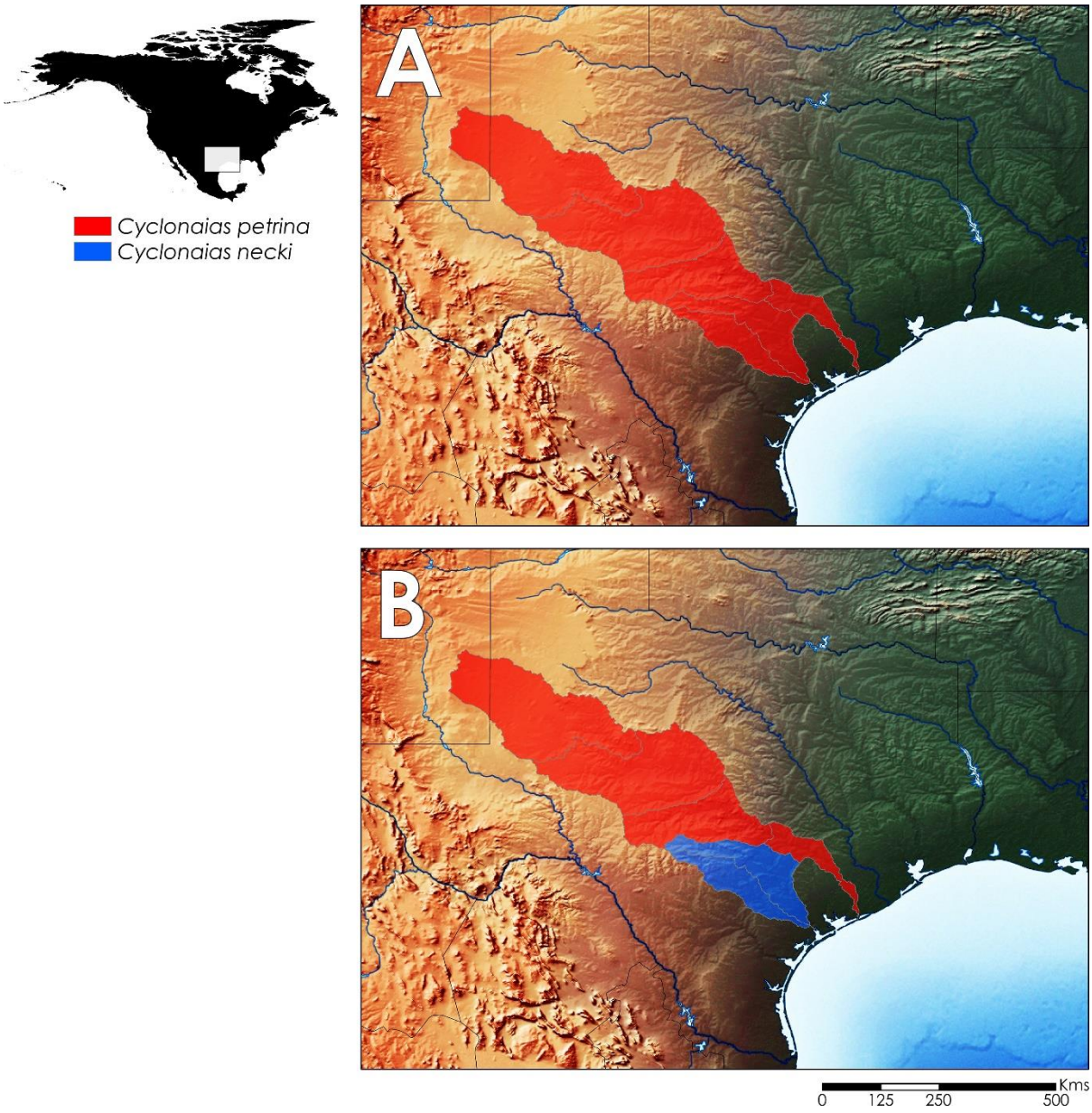

Figure 8 Distribution maps of (a) *Cyclonaias petrina* before Burlakova et al (2018) and (b) of *C. petrina* and *Cyclonaias necki* after Burlakova et al (2018) and Johnson et al (2018) findings also supported by the present study

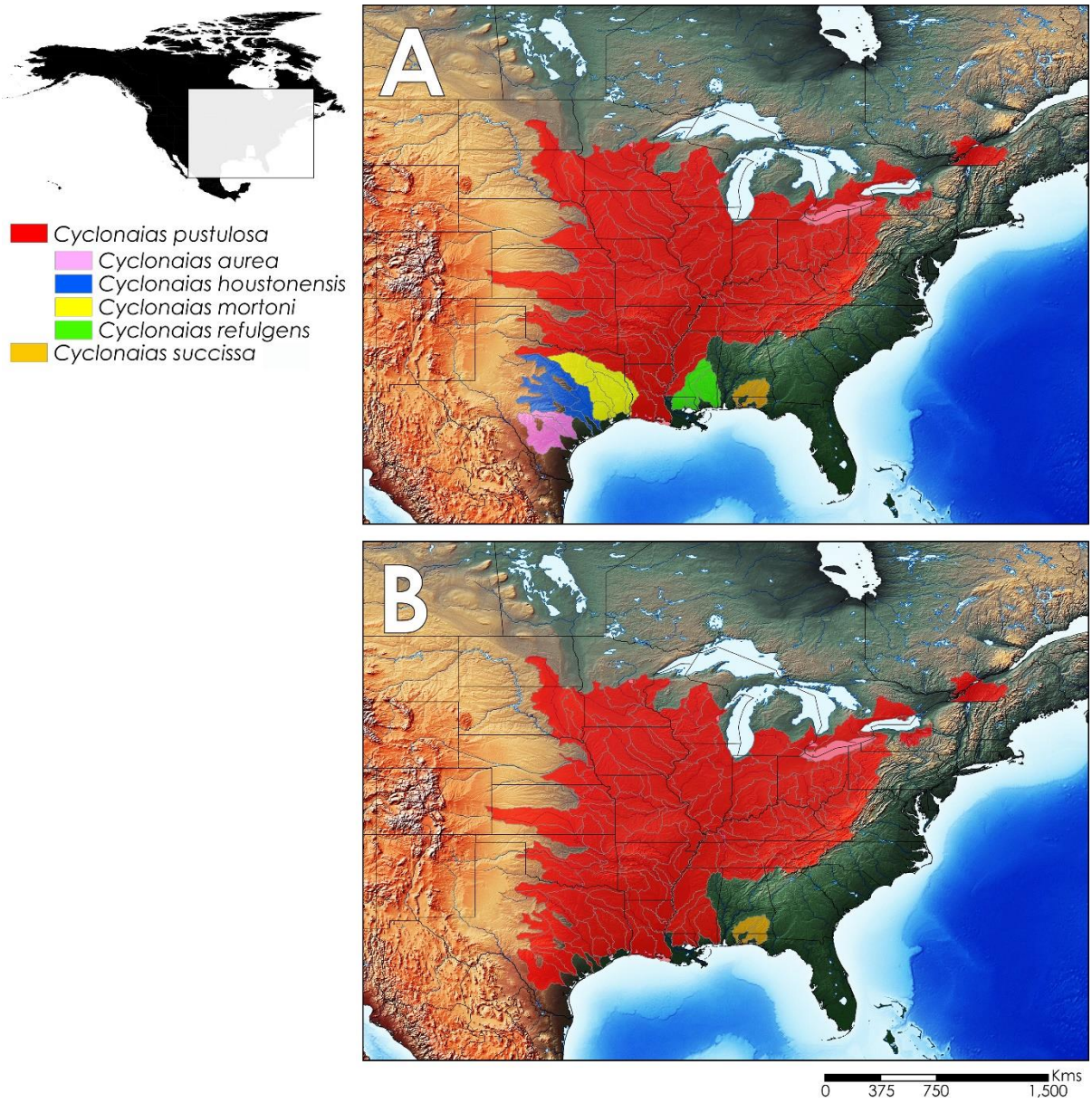

Figure 9 Distribution maps of (a) nominal species within the *Cyclonaias pustulosa* group and (b) of *C. pustulosa* and *Cyclonaias succissa* as confirmed by Johnson et al (2018) and the present study

Quadrula

In the absence of genetic data and shell materials for *Quadrula couchiana* and *Quadrula fragosa*, the first being most likely extinct (Williams et al 2017) and the second on the verge of extinction (Sietman 2003), we make no considerations about their systematics and accept both as valid within the *Quadrula* genus following Williams et al (2017).

We here synonymize *Q. apiculata* and *Q. rumphiana* under *Q. quadrula*. Although only a small number of sequences were available for *Q. apiculata* and *Q. rumphiana*, the level of divergence among these three nominal species is low for both markers (Table 4). Furthermore, in all phylogenies, *Q. quadrula* is paraphyletic, with *Q. apiculata* and *Q. rumphiana* falling inside the clade (Figs. 2-4). The level of divergence between these three nominal taxa is lower than

the divergence between the distinct clades of COI within *Q. quadrula* sensu stricto identified by Mathias et al (2018) and also retrieved here in the COI phylogeny and haplotype network (Figs. 2 and 6a). A specific rank for each of these divergent clades was rejected in that study due to the existence of gene-flow among them as shown by their microsatellite dataset (Mathias et al 2018).

The nominal species *Q. apiculata*, *Q. rumphiana* and *Q. quadrula* sensu stricto presented distinct shell shapes but only 76% of specimens were assigned to the correct nominal species (Fig. 6d). The slightly distinct shell morphology again suggests that distinct nominal species were assigned to regional forms despite the relative overlap in the distribution range of *Q. apiculata* with both *Q. quadrula* and *Q. rumphiana* (Fig. 10) that may also be related to the considerable overlap among shell shape forms (Fig. 6d).

Theliderma

Only two shells and no genetic material were available for *T. stapes*, since the species is very endangered and possibly extinct (NatureServe 2018). Until new evidence emerges, we, therefore, accept it as valid within the *Theliderma* genus following Williams et al (2017). Based on the molecular phylogenies and all species delineation methods, we recognize five additional species within *Theliderma*, that is, *T. cylindrica*, *Theliderma intermedia*, *T. metanevra*, *T. johnsoni* n. sp., and *T. sparsa* (Figs. 2-4; Tables 1 and 5). The nominal species *T. metanevra* is here divided into two distinct species, the *T. metanevra* sensu stricto with a Mississippi basin distribution and *T. johnsoni* n. sp. distributed within the Mobile basin (Fig. 11). The two species show high genetic divergence (3.2% for COI and 3.5% for ND1; Table 6). They also differ morphologically, presenting distinct shell shape with only 5%-11% of specimens being misidentified by Fourier analysis (Fig. 6e,f) as well as other morphological features (see Supporting Information Appendix S2).

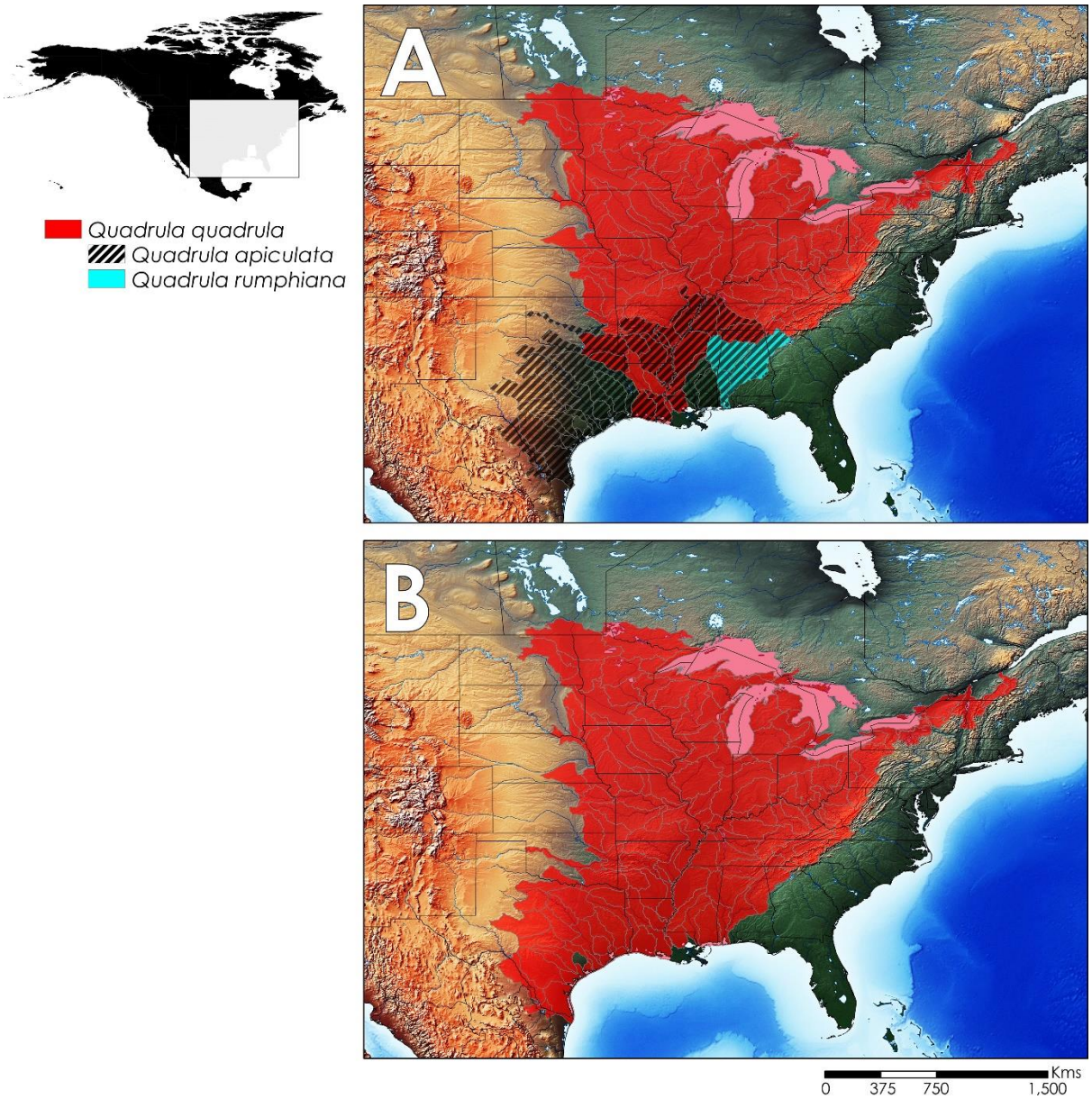

Figure 10 Distribution maps of (a) nominal species within the *Quadrula quadrula* group and (b) of *Quadrula quadrula* as proposed in the present study

Tritogonia

The position of *T. nobilis* could not be resolved in a previous single marker approach (Serb et al 2003) but in the present study, all phylogenies reveal a well-supported clade comprising *T. nobilis* and *T. verrucosa*. We, therefore, move the nominal species *Q. nobilis* into *Tritogonia* as *T. nobilis*. Until the end of the 20th century, *T. nobilis* was not recognized by most authors as a separate species from *Q. Quadrula* (Williams et al 2008). However, its placement under *Tritogonia* is not new as Simpson (1914) already used this designation. Both *T. nobilis* and *T. verrucosa* exhibit marked sexual dimorphism (Simpson, 1914; Williams et al 2008), which is a synapomorphy of the genera within the Quadrulini.

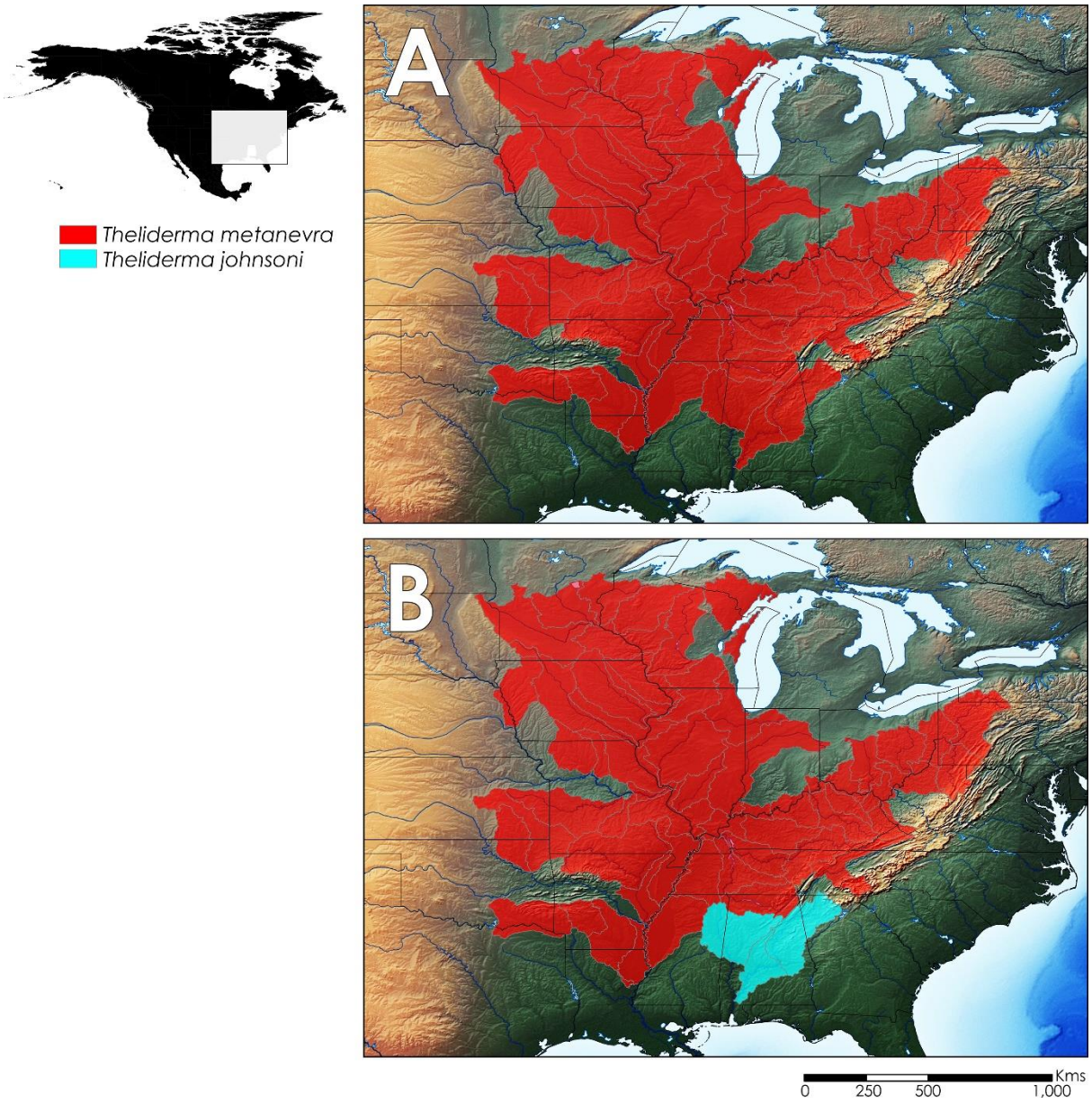

Figure 11 Distribution maps of (a) *Theliderma metanevra* before the present study and (b) after the present study divided in *T. metanevra* and *Theliderma johnsoni* n. sp

Conservation implications

Cyclonaias

As *C. asperata* is here synonymized under *C. kieneriana*, future conservation status assessment of *C. kieneriana* should include the distribution of *C. asperata* sensu stricto (Fig. 7), which would be expected to decrease the extinction risk of the species under the currently recognized systematics. The separation of *C. necki* from *C. petrina* will likely increase the extinction risk of both species as their distributions are even smaller than previously believed (Fig. 8) but see Johnson et al (2018) for detailed conservation implications. In contrast, the secure conservation status of *C. pustulosa* (Supporting Information Table S6) is here

strengthened by the inclusion of the nominal taxa *C. aurea*, *C. houstonensis*, *C. mortoni* and *C. refulgens* (Fig. 9; Table 7). However, due to their genetic uniqueness, the populations from Eastern Texas (originally identified as *C. mortoni*) should be managed independently.

Quadrula

Synonymization of the nominal species *Q. rumphiana* and *Q. apiculata* under *Q. quadrula* does not affect the conservation status of *Q. quadrula* due to the wide distribution areas and low extinction risk of the three forms. That said, subtler potential genetic differences between populations originally assigned to these species are likely to be revealed in future studies applying faster-evolving markers.

Theliderma

The conservation status of *T. metanevra* is currently considered as secure mainly based on the species' wide distribution range. However, considering that the Mobile basin populations represent a separate species (Fig. 11), i.e. *T. johnsoni* n. sp., the conservation statuses of *T. metanevra* and *T. johnsoni* n. sp. need to be re-assessed separately, and the two species need to be managed independently.

Acknowledgements

We would like to thank the Editor Dr. Per Ericson and the two reviewers Dr. Ivan Bolotov and Dr. John Pfeiffer for the help in a funding for Texas surveys was provided by the US Fish and Wildlife Service State Wildlife Grant Program through the Texas Parks and Wildlife Department (Contracts: T-15-P/2004-2006; 434301/2006-2007; 187549/2008-2010; 407709/2011-2012), and by the Texas Water Development Board (Contract: 0604830631/2006-2007) to AYK and LEB. Molecular analyses were developed under ConBiomics: the missing approach for the Conservation of Freshwater Bivalves Project No NORTE-01-0145-FEDER-030286, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds. FCT also supported MLL under Grant (SFRH/BD/115728/2016).

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Supplementary Materials

Supplementary Table 1
List of museum lots analysed for the morphometry: taxon, original identification, new identification, and lot catalogue number. BSGLC (SUNY Buffalo State College Great Lakes Center); NCSM (North Carolina Museum of Natural Sciences).

TAXON	ORIGINAL ID	NEW ID	CATALOGUE NUMBER
Cyclonaias	*C. asperata*	*C. kieneriana*	**NCSM:** 7117.1, 7117.2, 7117.3, 7117.4, 7117.5, 7118.1, 7118.2, 7118.3, 7118.4, 7118.5, 7118.7, 7118.8, 7166.1, 7166.2, 7166.3, 7166.4, 7324.1, 7324.2, 7324.3, 7324.4, 26973.1, 26973.2, 26973.3, 26973.4, 26973.5, 26973.6, 26973.7, 26973.8, 26973.9, 26981.1, 26981.2, 26981.3, 26983.1, 26983.2, 26983.3, 26983.4, 26983.5, 26983.6, 26983.7, 26983.8, 26987.1, 26987.2, 26994, 27003.1, 27003.2, 27003.3, 27003.4, 27003.5, 27003.6, 27003.7, 27003.8, 27008.1, 27008.2, 27008.3, 27008.4, 27008.5, 27020, 27155.1, 27155.2, 27155.4, 29403.1, 29403.2, 29403.3, 29403.4, 29630, 30258, 30962.1, 30962.2, 33704.1, 33704.2, 33704.3, 33704.4, 33704.5, 33704.6, 33704.7, 33704.8, 33705.1, 33705.2, 33706.1, 33706.2, 33706.3, 33706.4, 33706.5, 33706.6, 33706.7, 33707.1, 33707.2, 33707.3, 33707.4, 33707.5, 33708, 40672.1, 40672.2, 40672.3, 40672.4, 40672.5, 40830.2, 40830.4
	C. aurea	*C. pustulosa*	**NCSM:** 6898.1, 6898.2, 6898.3, 6898.4, 6898.5, 6898.6, 6923.1, 6923.2, 6923.3, 6923.4, 33720.1, 33720.2, 33721 **BSGLC:** 297, 825, 826, 829, 830, 832, 833, 834, 835, 1047, 1048, 1049, 1050, 1051, 1360, 1540, 1541, 1542, 1543, 1544, 1545, 1547, 1548, 1549, 1550, 1551, 1552, 1556, 1557, 1565, 1640, 1642, 1643, 1659, 1671, 1681, 1682, 1683, 1684, 1685, 1949, 2215, 2216, 2217, 2227, 2261, 2262, 2263, 2264, 2265, 2266, 2270, 2271, 1553, 1554, 1567, 1568, 1674, 2268, 2269, 3256, 3257
	C. houstonensis	*C. pustulosa*	**BSGLC**: 88, 89, 917, 918, 919, 945, 948, 971, 973, 974, 975, 976, 977, 979, 980, 981, 1007, 1008, 1032, 1711, 1712, 1715, 1716, 1717, 1765, 1766, 2129, 2130, 2131, 2135, 2136, 2137, 2138, 2139, 2178, 2179, 2180, 2181, 2182, 2184, 2185, 2186, 2187, 3246, 3247, 3248
	C. infucata	*C. infucata*	**NCSM:** 7182.1, 7182.2, 33759.1, 33759.1, 33759.2, 33759.3, 33759.4, 33759.5, 33759.6, 33759.7, 33759.8, 33759.9, 46806.1, 46806.4, 48448, 100656

C. kieneriana	*C. kieneriana*	**NCSM:** 6283.4, 7078.1, 45168.1, 45168.3
C. kleiniana	*C. kleiniana*	**NCSM:** 5564.1, 5564.2, 5564.3, 5564.4, 5564.5, 5564.6, 7183.1, 7183.1, 7183.11, 7183.12, 7183.13, 7183.14, 7183.15, 7183.2, 7183.3, 7183.5, 7183.6, 7183.7, 7183.8, 7183.9, 7184.1, 7184.2, 7184.3, 7184.4, 48450
C. mortoni	*C. pustulosa*	**NCSM:** 5595.1, 5595.1, 5595.2, 5595.3, 5595.4, 5595.5, 5595.6, 5595.7, 5595.8, 5595.9, 5677.1, 5677.1, 5677.11, 5677.13, 5677.14, 5677.2, 5677.3, 5677.4, 5677.5, 5677.6, 5677.7, 5677.8, 5677.9, 7483, 27280, 33732, 33733.1, 33733.2, 33733.3, 33739, 45803, 45804.1, 45804.2, 48451 **BSGLC:** 36, 37, 38, 39, 44, 45, 46, 63, 64, 90, 92, 94, 152, 345, 363, 364, 434, 436, 449, 542, 543, 558, 559, 560, 561, 562, 590, 618, 742, 743, 744, 747, 748, 749, 750, 752, 753, 754, 756, 757, 758, 759, 760, 761, 763, 764, 765, 766, 767, 768, 773, 774, 775, 844, 880, 924, 1231, 1776, 1871, 1872, 1873, 1884, 1885, 1886, 3224, 3225, 3226, 3227, 3228, 3229, 3230, 3231, 3232, 3233, 3234, 3235, 3236, 3237, 3238, 3239, 3240, 3241, 3242, 3243, 3244, 3245, 3249, 3251, 3258, 3259, 3260
C. necki	*C. necki*	**BSGLC:** 1670, 1672, 1673, 1952, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260
C. nodulata	*C. nodulata*	**NCSM:** 7227.1, 7227.3, 7228.1, 7228.2, 7229, 7230.1, 7230.2, 7230.3, 7230.4, 10064.11, 10064.13, 10064.14, 10064.15, 10064.16, 10795.1, 10795.4, 15666, 27412, 33711.1, 33711.1, 33711.2, 33711.3, 33711.4, 33711.5, 33711.6, 33711.7, 33711.8, 33711.9, 33712.1, 33712.2, 33712.3, 33712.4, 33712.5, 33712.6, 33735, 33738, 40784, 45800.1, 45800.2, 48379.1, 48379.2, 63794.1
C. petrina	*C. petrina*	**BSGLC:** 282, 283, 308, 579, 1027, 1028, 1029, 1030, 1031, 1041, 1584, 1585, 1605, 1606, 1615, 1616, 1617, 1619, 1620, 1620, 1622, 1623, 1626, 1627, 1630, 2127, 2128, 2133, 2134, 2140, 2142, 2143, 2147, 2148, 2150, 2151, 2153, 2154, 2155, 2156, 2157, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2172, 2173, 2174, 2175, 2176, 2177, 2233, 2247, 2248, 2249, 2250, 3254, 3255
C. pustulosa	*C. pustulosa*	**NCSM:** 5975.1, 5975.11, 5975.12, 5975.13, 5975.14, 5975.15, 5975.16, 5975.17, 5975.18, 5975.19, 5975.2, 5975.2, 5975.21, 5975.22, 5975.23, 5975.24, 5975.3, 5975.4, 5975.5, 5975.6, 5975.7, 5975.8, 5975.9, 6085.1, 6085.2, 6085.3, 6085.4, 6085.5, 7036.1, 7036.2, 7036.3, 27322, 27357.1, 27357.3, 27357.4, 30297, 33749, 35289, 43409.1, 43409.4, 43409.5, 43749, 45136.1, 45136.3, 45364.1, 45364.2, 45364.3, 45364.4, 45364.5, 45364.6, 45364.7, 45364.8, 46958, 47039.1, 48298.1, 48298.1, 48298.11, 48298.2, 48298.3, 48298.5, 48298.6, 48298.7, 48298.8, 48298.9, 61971, 63088,

			63088.1, 84539.1, 84539.2, 84539.3, 84541.1, 88100.1, 88100.2, 88100.3, 88100.4, 88100.5
	C. refulgens	*C. pustulosa*	**NCSM:** 33762, 33760.1, 33760.2, 33760.3, 33760.4, 33760.5, 33760.6, 33760.7, 33760.8, 33760.9, 33760.11, 33760.12, 33760.13, 33760.14, 33760.15
	C. succissa	*C. pustulosa*	**NCSM:** 7084, 7085.1, 7085.2, 7086.1, 7086.2, 7086.3, 7086.4, 7087.1, 7087.2, 7087.3, 7087.4, 7087.5, 7087.6, 7087.8, 27043.1, 27043.2, 27043.3, 27051.1, 27051.3, 27051.4, 27051.5, 27051.6, 27051.7, 27051.9, 27118, 27119.1, 27119.2, 27140.2, 48409.1, 48409.2
	C. tuberculata	*C. tuberculata*	**NCSM:** 5563.1, 5563.2, 5690, 5966.1, 5966.1, 5966.11, 5966.2, 5966.3, 5966.4, 5966.5, 5966.6, 5966.7, 5966.8, 5966.9, 6163.2, 6163.3, 6166, 6306, 6345.1, 6345.2, 6345.3, 6528, 6547, 6559.1, 6559.2, 6559.3, 6559.5, 6664.1, 6664.2, 6664.3, 6664.4, 6664.5, 6664.6, 6743.1, 6743.2, 6743.3, 6743.4, 6743.5, 6744, 6745, 6746.1, 6746.2, 6747, 6748, 6750, 6751, 6752, 6754.1, 6754.2, 6754.3, 6754.4, 7418, 7504, 7617.1, 7617.2, 7617.3, 7617.4, 27056, 27067, 27089.1, 27089.2, 27089.3, 27089.4, 27089.5, 27286, 27325.1, 27325.2, 27325.3, 27346.1, 27346.2, 27346.3, 27346.4, 27346.5, 27427.1, 27427.2, 33225.1, 33225.2, 33231, 33232.1, 33232.2, 33234, 43751, 44999.1, 46894.1, 48293.1, 48293.2, 48300.1, 48300.2, 48349, 48351.1, 48351.2, 48384, 63455, 63784.1, 84520.1, 84651, 87507.1, 87507.2, 87507.3, 87508.1, 87508.2, 87508.3, 87508.4, 100603, 102483.1, 102483.2, 102483.3, 102483.4, 102483.5, 102483.6, 102483.7
Quadrula			
	Q. apiculata	*Q. quadrula*	**NCSM:** 6090, 6091, 6092, 6095, 6096, 6097, 6098, 6281, 7251, 9876, 26990, 27154, 30365, 33692, 33693, 33694, 33695, 33696, 33697, 33699, 33700, 33701, 33702, 33703, 43664, 45106, 45119, 45131, 48443, 84523, 84542, 84618
	Q. quadrula	*Q. quadrula*	**NCSM:** 6186, 6196, 6200, 7109, 7110, 7112, 7113, 7114, 7115, 7521, 10794, 18085, 20441, 27012, 27077, 27085, 28981, 28998, 33741, 33751, 33752, 33753, 33755, 33756, 33757, 33758, 43411, 44372, 44377, 44389, 44399, 44431, 44458, 46881, 46888, 48368, 48369, 48449, 63086, 63793, 63798, 63799, 63800, 84010, 84390, 84519, 101762
	Q. rumphiana	*Q. quadrula*	**NCSM:** 6118, 6128, 6139, 6621, 6886, 6888, 6889, 6891, 6892, 40615, 45142, 45169, 45199, 47975, 102759

Theliderma			
	T. cylindrica	*T. cylindrica*	**NCSM:** 6402.1, 6402.1, 6402.2, 6402.3, 6402.4, 6402.5, 6402.6, 6402.7, 6402.8, 6500.1, 6500.2, 6500.3, 6500.4, 6500.5, 6906.1, 6906.2, 6954.1, 6954.2, 6954.3, 6957.1, 6957.2, 6959.1, 6959.2, 6959.3, 27227, 48047, 61191.1, 61191.2, 61191.3, 61191.4, 61191.5, 61191.6, 61191.7, 61191.8, 61192.1, 61192.2, 61192.3, 61192.4, 61193.1, 61193.1, 61193.11, 61193.12, 61193.2, 61193.5, 61193.6, 61193.7, 61193.8, 61193.9, 61259.14, 61259.15, 61259.2, 61259.21, 61259.28, 61259.3, 61259.33, 61259.36, 61259.5, 61259.6, 61270.1, 61270.1, 61270.13, 61270.2, 61270.3, 61270.4, 61270.6, 61270.7, 61270.8, 61270.9, 61274.1, 61274.2, 61275.2, 61275.3, 61275.4, 61275.6, 61288.1, 61288.2, 61290.1, 62994.1, 62994.2, 62994.3, 62994.4, 85056.1, 85056.2, 85056.3, 88455.11, 88455.14, 88455.15, 88455.16, 88455.2, 88455.24, 88455.3, 88455.6, 88455.8, 88458.1, 88458.2, 88464.1, 88502
	T. intermedia	*T. intermedia*	**NCSM:** 6338.1, 6338.2, 6338.3, 6338.4, 6338.5, 6338.6, 6338.7, 6338.8, 6338.9, 6899.1, 6899.2, 6900.1, 6900.2, 6900.3, 6900.4, 6900.5, 7616.1, 7616.2, 7616.3, 7616.4, 7616.5, 7616.6, 8850.3, 8850.4, 30512, 33715.1, 33715.2, 33716, 40857, 40914.1, 40914.2, 41039, 43683.2, 47044.2, 48445.1, 48445.2, 48980.1, 63220.1, 63221.1, 63221.2, 63221.3, 63223, 63224.1, 63224.2, 63224.3, 63225.1, 63225.2, 63225.3, 63226.1, 63227.1
	T. metanevra (Mobile basin)	*T. johnsoni*	**NCSM:** 7098.1, 7098.2, 7098.3, 7098.4, 7098.5, 7103, 7106.1, 7106.11, 7106.12, 7106.3, 7106.4, 7106.5, 7106.6, 7106.7, 7106.8, 26976, 27153.1, 27153.2, 33714, 33719, 33722, 46889.1, 46889.2
	T. metanevra (Tennessee basin)	*T. metanevra*	**NCSM:** 4412.1, 4412.2, 4606, 5972, 6080, 7100.1, 7100.2, 7101, 7102, 7104, 7105.1, 10765.1, 10765.2, 27428, 28995.1, 28995.2, 30298.1, 30298.2, 30298.3, 30522.1, 30522.2, 30522.3, 30522.4, 30522.5, 33718, 44361, 44776, 45824, 45841.1, 45841.2, 47051.1, 48447, 63145.2, 63145.4, 88505, 88517.1, 88517.2, 88523.1, 88523.2, 100604.1, 100604.2
	T. sparsa	*T. sparsa*	**NCSM:** 6919, 6334.1, 6334.2, 6920, 10188.6, 10188.7, 30916, 40915.1, 40915.2, 40915.3, 40919, 48362.1, 48362.2, 88695
	T. stapes	*T. stapes*	**NCSM:** 101849.1, 101849.2

Supplementary Table 2
List of specimens included in the Cytochrome c oxidase subunit I (COI) dataset; Haplotypes, GenBank references, original identification, new identification, voucher specimen, and respective study. BSGLC (SUNY Buffalo State College Great Lakes Center); FLMNH (Florida Museum of Natural History); NCSM (North Carolina Museum of Natural Sciences); UA (University of Alabama); UAUC (University of Alabama Unionid Collection); UAM (Auburn University Museum).

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
Quadrulini						
Cyclonaias						
	3	AF232805	*Quincucina infucata*	*Cyclonaias infucata*	UAUC919-926	Lydeard et al 2000
	4	AF232806	*Quincucina infucata*	*Cyclonaias infucata*	UAUC605	Lydeard et al 2000
	5	AF232807	*Quincucina infucata*	*Cyclonaias infucata*	UAUC561	Lydeard et al 2000
	257	MH633610	*Cyclonaias infucata*	*Cyclonaias infucata*	QkleOch001	Johnson et al 2018
	262	MH633615	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi007	Johnson et al 2018
	262	MH633621	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi015	Johnson et al 2018
	263	MH633616	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli009	Johnson et al 2018
	264	MH633617	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli010	Johnson et al 2018
	265	MH633618	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli011	Johnson et al 2018
	265	MH633620	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi014	Johnson et al 2018
	265	MH633623	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi017	Johnson et al 2018
	265	MH633625	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi020	Johnson et al 2018
	265	MH633627	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch022	Johnson et al 2018
	265	MH633647	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli034	Johnson et al 2018
	267	MH633622	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi016	Johnson et al 2018
	268	MH633624	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi019	Johnson et al 2018
	269	MH633626	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch021	Johnson et al 2018
	270	MH633628	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch024	Johnson et al 2018
	271	MH633629	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch026	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	272	MH633630	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi028	Johnson et al 2018
	282	MH633649	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi030	Johnson et al 2018
	283	MH633650	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi031	Johnson et al 2018
	284	MH633651	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi032	Johnson et al 2018
	285	MH633652	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi033	Johnson et al 2018
	255	MH633608	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla001	Johnson et al 2018
	256	MH633609	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla002	Johnson et al 2018
	258	MH633611	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla006	Johnson et al 2018
	259	MH633612	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla007	Johnson et al 2018
	260	MH633613	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla008	Johnson et al 2018
	288	MH633655	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla012	Johnson et al 2018
	6	AF232808	*Quincucina infucata*	*Cyclonaias kleiniana*	UAUC564	Lydeard et al 2000
	7	AF232809	*Quincucina infucata*	*Cyclonaias kleiniana*	UAUC567	Lydeard et al 2000
	261	MH633614	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw004	Johnson et al 2018
	276	MH633640	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw018	Johnson et al 2018
	281	MH633648	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw013	Johnson et al 2018
	287	MH633654	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw011	Johnson et al 2018
	15	MH362121	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua004	Johnson et al 2018
	15	MH362122	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua005	Johnson et al 2018
	15	MH362128	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua004	Johnson et al 2018
	15	MH362131	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua008	Johnson et al 2018
	15	MH362132	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua009	Johnson et al 2018
	15	MH362168	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua072	Johnson et al 2018
	15	MH362171	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua076	Johnson et al 2018
	15	MH362172	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua077	Johnson et al 2018
	15	MH362178	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua084	Johnson et al 2018
	15	MG969422	*Cyclonaias petrina*	*Cyclonaias necki*	BSGLC 1672	Burlakova et al 2018
	15	MG969423	*Cyclonaias petrina*	*Cyclonaias necki*	NCSM 65378	Burlakova et al 2018
	28	MH362119	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua001	Johnson et al 2018
	28	MH362120	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua003	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	28	MH362130	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua007	Johnson et al 2018
	28	MH362161	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua063	Johnson et al 2018
	28	MH362167	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua071	Johnson et al 2018
	28	MH362179	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua085	Johnson et al 2018
	28	MH362182	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua089	Johnson et al 2018
	28	MH362183	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua090	Johnson et al 2018
	28	MG969424	*Cyclonaias petrina*	*Cyclonaias necki*	BSGLC 2255	Burlakova et al 2018
	29	MG969425	*Cyclonaias petrina*	*Cyclonaias necki*	BSGLC 2256	Burlakova et al 2018
	53	MH362123	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua006	Johnson et al 2018
	53	MH362124	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua007	Johnson et al 2018
	53	KT285656	*Cyclonaias petrina*	*Cyclonaias necki*	FLMNH 441084	Pfeiffer et al 2016
	84	MH362125	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua008	Johnson et al 2018
	85	MH362126	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua002	Johnson et al 2018
	85	MH362139	*Cyclonaias necki*	*Cyclonaias necki*	QcouGua001	Johnson et al 2018
	86	MH362127	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua003	Johnson et al 2018
	87	MH362129	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua006	Johnson et al 2018
	87	MH362180	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua086	Johnson et al 2018
	95	MH362162	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua064	Johnson et al 2018
	96	MH362163	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua065	Johnson et al 2018
	97	MH362164	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua066	Johnson et al 2018
	98	MH362165	*Cyclonaias necki*	*Cyclonaias necki*	FmitGua021	Johnson et al 2018
	99	MH362166	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua069	Johnson et al 2018
	100	MH362169	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua074	Johnson et al 2018
	101	MH362170	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua075	Johnson et al 2018
	104	MH362181	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua088	Johnson et al 2018
	42	MH362115	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal022	Johnson et al 2018
	42	MH362116	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal023	Johnson et al 2018
	42	MH362118	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal025	Johnson et al 2018
	42	GU085316	*Cyclonaias nodulata*	*Cyclonaias nodulata*	Qnod1	Boyer et al 2011
	42	GU085317	*Cyclonaias nodulata*	*Cyclonaias nodulata*	Qnod2	Boyer et al 2011

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	77	MH362105	*Cyclonaias mortoni*	*Cyclonaias nodulata*	QmorRed050	Johnson et al 2018
	78	MH362112	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodNec017	Johnson et al 2018
	78	MH362113	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodNec019	Johnson et al 2018
	78	MH362106	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QaurNec113	Johnson et al 2018
	79	MH362107	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua012	Johnson et al 2018
	80	MH362108	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua013	Johnson et al 2018
	80	MH362110	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua015	Johnson et al 2018
	80	MH362111	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua016	Johnson et al 2018
	81	MH362109	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua014	Johnson et al 2018
	82	MH362114	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal021	Johnson et al 2018
	83	MH362117	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal024	Johnson et al 2018
	12	MG969416	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 1617	Burlakova et al 2018
	13	MH362135	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol011	Johnson et al 2018
	13	MH362136	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol012	Johnson et al 2018
	13	MH362147	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol030	Johnson et al 2018
	13	MH362156	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol041	Johnson et al 2018
	13	MH362184	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol097	Johnson et al 2018
	13	MG969417	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 1619	Burlakova et al 2018
	13	MG969418	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 1620	Burlakova et al 2018
	13	MG969421	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 2157	Burlakova et al 2018
	25	MH362133	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol009	Johnson et al 2018
	25	MH362134	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol010	Johnson et al 2018
	25	MH362137	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol018	Johnson et al 2018
	25	MH362138	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol019	Johnson et al 2018
	25	MH362142	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol024	Johnson et al 2018
	25	MH362145	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol028	Johnson et al 2018
	25	MH362146	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol029	Johnson et al 2018
	25	MH362149	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol033	Johnson et al 2018
	25	MH362152	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol036	Johnson et al 2018
	25	MH362153	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol038	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	25	MH362155	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol040	Johnson et al 2018
	25	MH362157	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol042	Johnson et al 2018
	25	MH362158	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol043	Johnson et al 2018
	25	MH362160	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol061	Johnson et al 2018
	25	MH362173	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol078	Johnson et al 2018
	25	MH362174	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol080	Johnson et al 2018
	25	MG969419	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 3254	Burlakova et al 2018
	25	MG969420	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 3255	Burlakova et al 2018
	88	MH362140	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol022	Johnson et al 2018
	89	MH362141	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol023	Johnson et al 2018
	90	MH362143	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol025	Johnson et al 2018
	91	MH362144	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol027	Johnson et al 2018
	91	MH362154	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol039	Johnson et al 2018
	92	MH362148	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol031	Johnson et al 2018
	92	MH362150	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol034	Johnson et al 2018
	93	MH362151	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol035	Johnson et al 2018
	94	MH362159	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol060	Johnson et al 2018
	102	MH362175	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol081	Johnson et al 2018
	102	MH362176	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol082	Johnson et al 2018
	103	MH362177	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol083	Johnson et al 2018
	14	MK503268	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1674	This study
	16	MH362191	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua013	Johnson et al 2018
	16	MH362202	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua041	Johnson et al 2018
	16	MH362246	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua090	Johnson et al 2018
	16	MH362248	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua092	Johnson et al 2018
	16	MK503269	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1553	This study
	17	MK503270	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1567	This study
	18	MK503271	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1568	This study
	19	MK503272	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1659	This study
	20	MH362255	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol003	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	20	MH362256	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol004	Johnson et al 2018
	20	MH362257	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol005	Johnson et al 2018
	20	MH362258	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol006	Johnson et al 2018
	20	MH362260	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol009	Johnson et al 2018
	20	MH362262	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra012	Johnson et al 2018
	20	MH362264	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra014	Johnson et al 2018
	20	MH362266	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra017	Johnson et al 2018
	20	MH362274	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra036	Johnson et al 2018
	20	MH362278	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra042	Johnson et al 2018
	20	MH362281	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra047	Johnson et al 2018
	20	KT285649	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	FLMNH 441135	Pfeiffer et al 2016
	20	MK503273	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	BSGLC 3246	This study
	20	MK503275	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	BSGLC 3248	This study
	20	MH362282	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol060	Johnson et al 2018
	20	MH362283	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol061	Johnson et al 2018
	20	MH362267	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpetCol026	Johnson et al 2018
	20	MH362279	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpetCol079	Johnson et al 2018
	21	MK503274	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	BSGLC 3247	This study
	22	MH362289	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab006	Johnson et al 2018
	22	MH362292	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri014	Johnson et al 2018
	22	MH362293	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri017	Johnson et al 2018
	22	MH362294	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri018	Johnson et al 2018
	22	MH362297	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec021	Johnson et al 2018
	22	MH362314	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec039	Johnson et al 2018
	22	MH362315	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec040	Johnson et al 2018
	22	MH362317	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec042	Johnson et al 2018
	22	MH362329	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri055	Johnson et al 2018
	22	MH362334	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab066	Johnson et al 2018
	22	MH362343	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec089	Johnson et al 2018
	22	MH362345	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ092	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	22	KT285655	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	FLMNH 441171	Pfeiffer et al 2016
	22	MK503276	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3249	This study
	23	MK503278	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3251	This study
	26	MH362211	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue050	Johnson et al 2018
	26	MH362216	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue058	Johnson et al 2018
	26	MK503276	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 3256	This study
	27	MH362233	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue075	Johnson et al 2018
	27	MK503282	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 3257	This study
	30	MK503283	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 2268	This study
	30	MK503284	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 2269	This study
	32	MK503286	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3258	This study
	33	MK503287	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3259	This study
	34	MK503288	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3260	This study
	37	MH362359	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC048	Johnson et al 2018
	37	MH362397	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo062	Johnson et al 2018
	37	MH362405	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF072	Johnson et al 2018
	37	MH362406	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF073	Johnson et al 2018
	37	MH362416	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua088	Johnson et al 2018
	37	GU085318	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	photo PP3	Boyer et al 2011
	37	EF033269	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	JC	Chapman et al 2008
	105	MH362185	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua001	Johnson et al 2018
	105	MH362213	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue052	Johnson et al 2018
	105	MH362215	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue057	Johnson et al 2018
	105	MH362225	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue067	Johnson et al 2018
	105	MH362227	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue069	Johnson et al 2018
	106	MH362186	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua004	Johnson et al 2018
	107	MH362187	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua006	Johnson et al 2018
	107	MH362192	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua014	Johnson et al 2018
	107	MH362193	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua015	Johnson et al 2018
	107	MH362203	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua042	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	107	MH362209	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua048	Johnson et al 2018
	107	MH362214	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue054	Johnson et al 2018
	107	MH362217	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue059	Johnson et al 2018
	107	MH362218	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue060	Johnson et al 2018
	107	MH362221	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue063	Johnson et al 2018
	107	MH362234	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue076	Johnson et al 2018
	107	MH362241	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue083	Johnson et al 2018
	107	MH362242	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue085	Johnson et al 2018
	107	MH362245	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue089	Johnson et al 2018
	107	MH362247	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua091	Johnson et al 2018
	107	MH362251	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua095	Johnson et al 2018
	107	MH362252	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua096	Johnson et al 2018
	108	MH362188	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua008	Johnson et al 2018
	108	MH362235	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue077	Johnson et al 2018
	108	MH362243	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue086	Johnson et al 2018
	108	MH362249	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua093	Johnson et al 2018
	109	MH362189	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua009	Johnson et al 2018
	109	MH362230	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue072	Johnson et al 2018
	110	MH362190	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua012	Johnson et al 2018
	111	MH362194	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua016	Johnson et al 2018
	112	MH362195	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua017	Johnson et al 2018
	113	MH362196	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua018	Johnson et al 2018
	114	MH362197	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua021	Johnson et al 2018
	115	MH362198	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue027	Johnson et al 2018
	115	MH362220	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue062	Johnson et al 2018
	115	MH362222	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue064	Johnson et al 2018
	115	MH362229	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue071	Johnson et al 2018
	115	MH362240	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue082	Johnson et al 2018
	116	MH362199	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue028	Johnson et al 2018
	116	MH362200	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue031	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	116	MH362212	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue051	Johnson et al 2018
	116	MH362219	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue061	Johnson et al 2018
	116	MH362226	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue068	Johnson et al 2018
	116	MH362228	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue070	Johnson et al 2018
	116	MH362231	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue073	Johnson et al 2018
	116	MH362232	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue074	Johnson et al 2018
	116	MH362237	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue079	Johnson et al 2018
	116	MH362244	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue088	Johnson et al 2018
	116	MH362328	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed055	Johnson et al 2018
	116	MH362375	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed025	Johnson et al 2018
	116	MH362391	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF041	Johnson et al 2018
	116	MH362410	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC081	Johnson et al 2018
	116	MH362414	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua086	Johnson et al 2018
	117	MH362201	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua040	Johnson et al 2018
	118	MH362204	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua043	Johnson et al 2018
	119	MH362205	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua044	Johnson et al 2018
	120	MH362206	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua045	Johnson et al 2018
	121	MH362207	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua046	Johnson et al 2018
	122	MH362208	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua047	Johnson et al 2018
	123	MH362210	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua049	Johnson et al 2018
	124	MH362223	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue065	Johnson et al 2018
	124	MH362239	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue081	Johnson et al 2018
	125	MH362224	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue066	Johnson et al 2018
	126	MH362236	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue078	Johnson et al 2018
	126	MH362238	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue080	Johnson et al 2018
	127	MH362250	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua094	Johnson et al 2018
	128	MH362253	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNec112	Johnson et al 2018
	129	MH362254	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol001	Johnson et al 2018
	130	MH362259	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol007	Johnson et al 2018
	131	MH362261	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra011	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	131	MH362269	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra020	Johnson et al 2018
	131	MH362271	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra023	Johnson et al 2018
	132	MH362263	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra013	Johnson et al 2018
	133	MH362265	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra015	Johnson et al 2018
	134	MH362268	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra019	Johnson et al 2018
	135	MH362270	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra021	Johnson et al 2018
	136	MH362272	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouCol029	Johnson et al 2018
	136	MH362284	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol062	Johnson et al 2018
	137	MH362273	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra035	Johnson et al 2018
	138	MH362275	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra037	Johnson et al 2018
	139	MH362276	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra038	Johnson et al 2018
	140	MH362277	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra041	Johnson et al 2018
	141	MH362280	*Cyclonaias hostonensis*	*Cyclonaias pustulosa*	QhouBra044	Johnson et al 2018
	142	MH362285	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol063	Johnson et al 2018
	143	MH362286	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab001	Johnson et al 2018
	144	MH362287	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab004	Johnson et al 2018
	145	MH362288	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab005	Johnson et al 2018
	146	MH362290	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab008	Johnson et al 2018
	147	MH362291	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri013	Johnson et al 2018
	148	MH362295	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec019	Johnson et al 2018
	148	MH362296	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec020	Johnson et al 2018
	149	MH362298	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec022	Johnson et al 2018
	149	MH362306	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec031	Johnson et al 2018
	150	MH362299	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec023	Johnson et al 2018
	151	MH362300	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec024	Johnson et al 2018
	151	MH362313	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec038	Johnson et al 2018
	151	MH362332	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri058	Johnson et al 2018
	151	MH362338	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ071	Johnson et al 2018
	152	MH362301	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec025	Johnson et al 2018
	153	MH362302	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec026	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	153	MH362370	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed020	Johnson et al 2018
	153	MH362377	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed027	Johnson et al 2018
	153	MH362390	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF040	Johnson et al 2018
	153	MH362409	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC080	Johnson et al 2018
	154	MH362303	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec028	Johnson et al 2018
	155	MH362304	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec029	Johnson et al 2018
	156	MH362305	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec030	Johnson et al 2018
	157	MH362307	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec032	Johnson et al 2018
	158	MH362308	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec033	Johnson et al 2018
	159	MH362309	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec034	Johnson et al 2018
	159	MH362312	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec037	Johnson et al 2018
	160	MH362310	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec035	Johnson et al 2018
	161	MH362311	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec036	Johnson et al 2018
	162	MH362316	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec041	Johnson et al 2018
	162	MH362342	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec088	Johnson et al 2018
	163	MH362318	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri043	Johnson et al 2018
	163	MH362319	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri044	Johnson et al 2018
	163	MH362333	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri059	Johnson et al 2018
	164	MH362320	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri046	Johnson et al 2018
	165	MH362321	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri047	Johnson et al 2018
	166	MH362322	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri048	Johnson et al 2018
	167	MH362323	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri049	Johnson et al 2018
	168	MH362324	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed051	Johnson et al 2018
	168	MH362327	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed054	Johnson et al 2018
	169	MH362325	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed052	Johnson et al 2018
	170	MH362326	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed053	Johnson et al 2018
	171	MH362330	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri056	Johnson et al 2018
	171	MH362340	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ073	Johnson et al 2018
	172	MH362331	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri057	Johnson et al 2018
	173	MH362335	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab068	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	174	MH362336	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ069	Johnson et al 2018
	175	MH362337	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ070	Johnson et al 2018
	176	MH362339	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ072	Johnson et al 2018
	176	MH362344	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ091	Johnson et al 2018
	177	MH362341	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec087	Johnson et al 2018
	178	MH362346	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ093	Johnson et al 2018
	179	MH362347	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ094	Johnson et al 2018
	180	MH362348	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed001	Johnson et al 2018
	180	MH362351	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed005	Johnson et al 2018
	180	MH362355	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed009	Johnson et al 2018
	180	MH362356	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed010	Johnson et al 2018
	181	MH362349	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed002	Johnson et al 2018
	182	MH362350	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed004	Johnson et al 2018
	182	MH362352	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed006	Johnson et al 2018
	183	MH362353	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed007	Johnson et al 2018
	184	MH362354	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed008	Johnson et al 2018
	185	MH362357	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi046	Johnson et al 2018
	185	MH362373	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed023	Johnson et al 2018
	186	MH362358	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi047	Johnson et al 2018
	187	MH362360	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed049	Johnson et al 2018
	188	MH362361	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QnodRed004	Johnson et al 2018
	189	MH362362	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QspeTri019	Johnson et al 2018
	190	MH362363	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi050	Johnson et al 2018
	191	MH362364	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi051	Johnson et al 2018
	192	MH362365	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi052	Johnson et al 2018
	193	MH362366	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi053	Johnson et al 2018
	194	MH362367	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi054	Johnson et al 2018
	195	MH362368	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi055	Johnson et al 2018
	196	MH362369	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi056	Johnson et al 2018
	197	MH362371	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed021	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	198	MH362372	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed022	Johnson et al 2018
	198	MH362379	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed029	Johnson et al 2018
	199	MH362374	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed024	Johnson et al 2018
	199	MH362381	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua031	Johnson et al 2018
	199	MH362389	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF039	Johnson et al 2018
	199	MH362393	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF057	Johnson et al 2018
	200	MH362376	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed026	Johnson et al 2018
	201	MH362378	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed028	Johnson et al 2018
	202	MH362380	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua030	Johnson et al 2018
	203	MH362382	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua032	Johnson et al 2018
	204	MH362383	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua033	Johnson et al 2018
	205	MH362384	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua034	Johnson et al 2018
	206	MH362385	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua035	Johnson et al 2018
	206	MH362386	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua036	Johnson et al 2018
	207	MH362387	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua037	Johnson et al 2018
	208	MH362388	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua038	Johnson et al 2018
	209	MH362392	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF043	Johnson et al 2018
	210	MH362394	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF058	Johnson et al 2018
	211	MH362395	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF059	Johnson et al 2018
	212	MH362396	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo061	Johnson et al 2018
	213	MH362398	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo063	Johnson et al 2018
	214	MH362399	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo064	Johnson et al 2018
	215	MH362400	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa065	Johnson et al 2018
	216	MH362401	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa067	Johnson et al 2018
	217	MH362402	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa068	Johnson et al 2018
	218	MH362403	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa070	Johnson et al 2018
	219	MH362404	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF071	Johnson et al 2018
	220	MH362407	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF074	Johnson et al 2018
	221	MH362408	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC079	Johnson et al 2018
	222	MH362411	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC082	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	223	MH362412	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua084	Johnson et al 2018
	224	MH362413	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua085	Johnson et al 2018
	225	MH362415	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua087	Johnson et al 2018
	226	MH362417	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua089	Johnson et al 2018
	227	MH362418	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QspeOua024	Johnson et al 2018
	228	MH362419	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas001	Johnson et al 2018
	228	MH362422	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas004	Johnson et al 2018
	229	MH362420	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas002	Johnson et al 2018
	230	MH362421	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas003	Johnson et al 2018
	231	MH362423	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas005	Johnson et al 2018
	232	MH362424	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl006	Johnson et al 2018
	233	MH362425	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl007	Johnson et al 2018
	234	MH362426	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl008	Johnson et al 2018
	235	MH362427	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl009	Johnson et al 2018
	236	MH362428	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl010	Johnson et al 2018
	237	MH362429	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho001	Johnson et al 2018
	238	MH362430	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho002	Johnson et al 2018
	239	MH362431	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho003	Johnson et al 2018
	240	MH362432	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho004	Johnson et al 2018
	240	MH362442	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho030	Johnson et al 2018
	241	MH362433	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucEsc013	Johnson et al 2018
	242	MH362434	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucYel017	Johnson et al 2018
	243	MH362435	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucYel018	Johnson et al 2018
	244	MH362436	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucYel019	Johnson et al 2018
	245	MH362437	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucEsc021	Johnson et al 2018
	246	MH362438	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucEsc022	Johnson et al 2018
	247	MH362439	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho027	Johnson et al 2018
	248	MH362440	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho028	Johnson et al 2018
	249	MH362441	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho029	Johnson et al 2018
	250	MH362443	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho031	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	251	MH362444	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho032	Johnson et al 2018
	252	MH362445	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho033	Johnson et al 2018
	253	MH362446	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho034	Johnson et al 2018
	254	MH362447	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho039	Johnson et al 2018
	38	GU085283	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	MMTC 150	Boyer et al 2011
	39	GU085284	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	photo Ctub2	Boyer et al 2011
	44	HM849070	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	H2789	Breton et al 2010
	44	HM230410	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	UAM1490	Campbell & Lydeard 2012
	44	MH633635	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	CtubTen003	Johnson et al 2018
	44	MH633636	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	CtubTen006	Johnson et al 2018
	45	HM849069	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	H1933	Breton et al 2010
	274	MH633637	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	CtubTen008	Johnson et al 2018
Quadrula						
	1	AF156511	*Quadrula quadrula*	*Quadrula quadrula*	UMMZ 265699	Graf & Foighil 2000
	1	MK503266	*Quadrula quadrula*	*Quadrula quadrula*	BIV0364	This study
	1	NIMUS820	*Quadrula quadrula*	*Quadrula quadrula*	MUS068.1	Biodiversity I. of Ontario
	1	NIMUS824	*Quadrula quadrula*	*Quadrula quadrula*	MUS070.1	Biodiversity I. of Ontario
	1	NIMUS886	*Quadrula quadrula*	*Quadrula quadrula*	MUS101.1	Biodiversity I. of Ontario
	1	NIMUS939	*Quadrula quadrula*	*Quadrula quadrula*	MUS127.1	Biodiversity I. of Ontario
	1	NIMUS955	*Quadrula quadrula*	*Quadrula quadrula*	MUS135.1	Biodiversity I. of Ontario
	2	AF231757	*Quadrula quadrula*	*Quadrula quadrula*		Bogan & Hoeh 2000
	36	EF033268	*Quadrula quadrula*	*Quadrula quadrula*	H1774	Chapman et al 2008
	43	HM230409	*Quadrula rumphiana*	*Quadrula quadrula*	UA20703	Campbell & Lydeard 2012
	52	MH633638	*Quadrula apiculata*	*Quadrula quadrula*	QapiRGr081	Johnson et al 2018
	52	KT285648	*Quadrula apiculata*	*Quadrula quadrula*	FLMNH 441088	Pfeiffer et al 2016
	56	KX853887	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ07	Mathias et al 2018
	56	KX853888	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ08	Mathias et al 2018
	56	KX853890	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ11	Mathias et al 2018
	56	KX853891	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ12	Mathias et al 2018
	56	KX853892	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ14	Mathias et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	56	KX853900	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ04	Mathias et al 2018
	56	KX853901	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ05	Mathias et al 2018
	56	KX853902	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ06	Mathias et al 2018
	56	KX853903	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ07	Mathias et al 2018
	56	KX853904	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ08	Mathias et al 2018
	56	KX853906	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ12	Mathias et al 2018
	56	KX853907	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ13	Mathias et al 2018
	56	KX853908	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ01	Mathias et al 2018
	56	KX853909	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ04	Mathias et al 2018
	56	KX853911	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ06	Mathias et al 2018
	56	KX853912	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ07	Mathias et al 2018
	56	KX853913	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ08	Mathias et al 2018
	56	KX853914	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ10	Mathias et al 2018
	56	KX853916	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ03	Mathias et al 2018
	56	KX853918	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ05	Mathias et al 2018
	56	KX853919	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ06	Mathias et al 2018
	56	KX853920	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ07	Mathias et al 2018
	56	KX853922	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ09	Mathias et al 2018
	56	KX853925	*Quadrula quadrula*	*Quadrula quadrula*	LMNRQQ08	Mathias et al 2018
	56	KX853927	*Quadrula quadrula*	*Quadrula quadrula*	LMNRQQ15	Mathias et al 2018
	56	KX853930	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ06	Mathias et al 2018
	56	KX853932	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ08	Mathias et al 2018
	56	KX853933	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ09	Mathias et al 2018
	56	KX853934	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ10	Mathias et al 2018
	56	KX853935	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ11	Mathias et al 2018
	56	KX853947	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ03	Mathias et al 2018
	56	KX853954	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ05	Mathias et al 2018
	56	KX853955	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ06	Mathias et al 2018
	56	KX853959	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ10	Mathias et al 2018
	56	KX853962	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ13	Mathias et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	56	KX853963	*Quadrula quadrula*	*Quadrula quadrula*	SLR1QQ02	Mathias et al 2018
	56	KX853964	*Quadrula quadrula*	*Quadrula quadrula*	SLR1QQ06	Mathias et al 2018
	56	KX853965	*Quadrula quadrula*	*Quadrula quadrula*	SLR1QQ10	Mathias et al 2018
	56	KX853967	*Quadrula quadrula*	*Quadrula quadrula*	SLR117	Mathias et al 2018
	56	KX853968	*Quadrula quadrula*	*Quadrula quadrula*	SLR116	Mathias et al 2018
	56	KX853969	*Quadrula quadrula*	*Quadrula quadrula*	SLR115	Mathias et al 2018
	56	KX853970	*Quadrula quadrula*	*Quadrula quadrula*	SLR118	Mathias et al 2018
	57	KX853889	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ09	Mathias et al 2018
	58	KX853893	*Quadrula quadrula*	*Quadrula quadrula*	BL06QQ15	Mathias et al 2018
	59	KX853894	*Quadrula quadrula*	*Quadrula quadrula*	BMC1QQ06	Mathias et al 2018
	60	KX853895	*Quadrula quadrula*	*Quadrula quadrula*	BMC1QQ07	Mathias et al 2018
	60	KX853897	*Quadrula quadrula*	*Quadrula quadrula*	BMC1QQ10	Mathias et al 2018
	60	KX853899	*Quadrula quadrula*	*Quadrula quadrula*	EFWRQQ10	Mathias et al 2018
	60	KX853938	*Quadrula quadrula*	*Quadrula quadrula*	MSWRQQ11	Mathias et al 2018
	60	KX853939	*Quadrula quadrula*	*Quadrula quadrula*	MSWR38	Mathias et al 2018
	60	KX853940	*Quadrula quadrula*	*Quadrula quadrula*	MSWR39	Mathias et al 2018
	60	KX853941	*Quadrula quadrula*	*Quadrula quadrula*	MSWR40	Mathias et al 2018
	60	KX853942	*Quadrula quadrula*	*Quadrula quadrula*	MSWR42	Mathias et al 2018
	60	KX853944	*Quadrula quadrula*	*Quadrula quadrula*	MSWR44	Mathias et al 2018
	60	KX853945	*Quadrula quadrula*	*Quadrula quadrula*	MSWR45	Mathias et al 2018
	60	KX853950	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ608	Mathias et al 2018
	60	KX853952	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ610	Mathias et al 2018
	60	KX853953	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ04	Mathias et al 2018
	60	KX853958	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ09	Mathias et al 2018
	60	KX853966	*Quadrula quadrula*	*Quadrula quadrula*	SLR113	Mathias et al 2018
	60	KX853972	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ04	Mathias et al 2018
	60	KX853974	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ06	Mathias et al 2018
	60	KX853976	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ08	Mathias et al 2018
	60	KX853978	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ10	Mathias et al 2018
	61	KX853896	*Quadrula quadrula*	*Quadrula quadrula*	BMC1QQ08	Mathias et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	61	KX853898	*Quadrula quadrula*	*Quadrula quadrula*	BMC1QQ12	Mathias et al 2018
	61	KX853917	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ04	Mathias et al 2018
	61	KX853943	*Quadrula quadrula*	*Quadrula quadrula*	MSWR43	Mathias et al 2018
	61	KX853946	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ02	Mathias et al 2018
	61	KX853956	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ07	Mathias et al 2018
	61	KX853957	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ08	Mathias et al 2018
	61	KX853961	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ12	Mathias et al 2018
	61	KX853971	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ03	Mathias et al 2018
	61	KX853973	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ05	Mathias et al 2018
	61	KX853977	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ09	Mathias et al 2018
	61	KX853979	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ11	Mathias et al 2018
	61	KX853982	*Quadrula quadrula*	*Quadrula quadrula*	WFWRQQ17	Mathias et al 2018
	62	KX853905	*Quadrula quadrula*	*Quadrula quadrula*	FLR1QQ11	Mathias et al 2018
	63	KX853910	*Quadrula quadrula*	*Quadrula quadrula*	GRMIQQ05	Mathias et al 2018
	63	KX853936	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ13	Mathias et al 2018
	64	KX853915	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ02	Mathias et al 2018
	65	KX853921	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ08	Mathias et al 2018
	65	KX853923	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ10	Mathias et al 2018
	66	KX853924	*Quadrula quadrula*	*Quadrula quadrula*	IRQRQQ11	Mathias et al 2018
	67	KX853926	*Quadrula quadrula*	*Quadrula quadrula*	LMNRQQ13	Mathias et al 2018
	68	KX853928	*Quadrula quadrula*	*Quadrula quadrula*	LMNRQQ16	Mathias et al 2018
	68	KX853937	*Quadrula quadrula*	*Quadrula quadrula*	MSWRQQ09	Mathias et al 2018
	69	KX853931	*Quadrula quadrula*	*Quadrula quadrula*	MR1QQ07	Mathias et al 2018
	70	KX853948	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ05	Mathias et al 2018
	71	KX853949	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ607	Mathias et al 2018
	72	KX853951	*Quadrula quadrula*	*Quadrula quadrula*	NFSRQQ609	Mathias et al 2018
	73	KX853960	*Quadrula quadrula*	*Quadrula quadrula*	SGMRQQ11	Mathias et al 2018
	74	KX853975	*Quadrula quadrula*	*Quadrula quadrula*	SNR1QQ07	Mathias et al 2018
	75	KX853980	*Quadrula quadrula*	*Quadrula quadrula*	WFWRQQ12	Mathias et al 2018
	76	KX853981	*Quadrula quadrula*	*Quadrula quadrula*	WFWRQQ15	Mathias et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	278	MH633643	*Quadrula quadrula*	*Quadrula quadrula*	QquaOhi009	Johnson et al 2018
	289	NC_013658	*Quadrula quadrula*	*Quadrula quadrula*	H1773	Breton et al 2009
Theliderma						
	50	JF326435	*Quadrula metanevra*	*Theliderma johnsoni*		Campbell & Lydeard 2012
	50	MK503289	*Quadrula metanevra*	*Theliderma johnsoni*	NCSM 30474.2	This study
	8	AF232823	*Quadrula quadrula*	*Theliderma metanevra*	UAUC145	Lydeard et al 2000
	40	GU085314	*Quadrula metanevra*	*Theliderma metanevra*	photo Qmet1	Boyer et al 2011
	41	GU085315	*Quadrula metanevra*	*Theliderma metanevra*	photo Qmet2	Boyer et al 2011
	277	MH633642	*Quadrula metanevra*	*Theliderma metanevra*	QmetTen003	Johnson et al 2018
	280	MH633646	*Quadrula metanevra*	*Theliderma metanevra*	QmetOhi005	Johnson et al 2018
Tritogonia						
	24	MK503279	*Quadrula nobilis*	*Tritogonia nobilis*	BSGLC 3253	This study
	24	MK503280	*Quadrula nobilis*	*Tritogonia nobilis*	BSGLC 1856	This study
	31	MK503285	*Quadrula nobilis*	*Tritogonia nobilis*	BSGLC 2313	This study
	11	AY655024	*Tritogonia verrucosa*	*Tritogonia verrucosa*	UAUC3195	Campbell et al 2005
	35	GU085322	*Tritogonia verrucosa*	*Tritogonia verrucosa*		Boyer et al 2011
	35	DQ191413	*Tritogonia verrucosa*	*Tritogonia verrucosa*	UMMZ 62984	Graf & Cummings 2006
	35	MH633641	*Tritogonia verrucosa*	*Tritogonia verrucosa*	QverOhi048	Johnson et al 2018
	54	KT285657	*Tritogonia verrucosa*	*Tritogonia verrucosa*	FLMNH 441208	Pfeiffer et al 2016
	275	MH633639	*Tritogonia verrucosa*	*Tritogonia verrucosa*	QverRed015	Johnson et al 2018
Uniomerus						
	46	HQ153528	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX01	McCartney et al 2016
	46	HQ153529	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX02	McCartney et al 2016
	46	HQ153530	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX03	McCartney et al 2016
	46	HQ153531	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX04	McCartney et al 2016
	46	HQ153532	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX05	McCartney et al 2016
	46	HQ153536	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX09	McCartney et al 2016
	46	HQ153582	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX55	McCartney et al 2016
	46	HQ153583	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX56	McCartney et al 2016
	46	HQ153584	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX57	McCartney et al 2016

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	46	HQ153587	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX60	McCartney et al 2016
	47	HQ153533	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX06	McCartney et al 2016
	47	HQ153585	*Uniomerus sp.*	*Uniomerus carolinianus*	wuspCOX58	McCartney et al 2016
	48	HQ153618	*Uniomerus carolinianus*	*Uniomerus carolinianus*	wuspCOX91	McCartney et al 2016
	48	HQ153619	*Uniomerus carolinianus*	*Uniomerus carolinianus*	wuspCOX92	McCartney et al 2016
	49	HQ153620	*Uniomerus carolinianus*	*Uniomerus carolinianus*	wuspCOX93	McCartney et al 2016
	49	HQ153621	*Uniomerus carolinianus*	*Uniomerus carolinianus*	wuspCOX94	McCartney et al 2016
	9	AY613846	*Uniomerus declivis*	*Uniomerus declivis*	UAUC3290	Campbell et al 2005
	55	KT285659	*Uniomerus declivis*	*Uniomerus declivis*	FLMNH 438312	Pfeiffer et al 2016
	51	JF326437	*Uniomerus tetralasmus*	*Uniomerus tetralasmus*		Campbell & Lydeard 2012
	273	MH633631	*Uniomerus tetralasmus*	*Uniomerus tetralasmus*	UtetCol005	Johnson et al 2018
	286	MH633653	*Uniomerus tetralasmus*	*Uniomerus tetralasmus*	UtetBaP011	Johnson et al 2018
Megalonaias						
	10	AY655007	*Megalonaias nervosa*	*Megalonaias nervosa*		Campbell et al 2005
	266	MH633619	*Megalonaias nervosa*	*Megalonaias nervosa*	MnerGua025	Johnson et al 2018
	279	MH633645	*Megalonaias nervosa*	*Megalonaias nervosa*	MnerOhi057	Johnson et al 2018
Amblemini		AY654991	*Amblema elliottii*	*Amblema elliottii*	UAUC2511	Campbell et al 2005
		DQ648099	*Amblema plicata*	*Amblema plicata*	AP33	Elderkin et al 2007
Lampsilini		NC_005335	*Lampsilis ornata*	*Lampsilis ornata*		Serb & Lydeard 2003
		NC_028522	*Leptodea leptodon*	*Leptodea leptodon*		Feng et al 2016
Pleurobemini		HQ153586	*Uniomerus sp.*	*Elliptio crassidens*	wuspCOX59	McCartney et al 2016
		MK503277	*Pleurobema riddellii*	*Pleurobema riddellii*	BIV2457	This study
Anodontini		MK503266	*Anodonta nuttalliana*	*Anodonta nuttalliana*	BIV0364	This study
		NC_013661	*Pyganodon grandis*	*Pyganodon grandis*		Breton et al 2009
Margaritiferidae		NC_034846	*Cumberlandia monodonta*	*Cumberlandia monodonta*		Guerra et al 2017
		NC_015476	*Margaritifera falcata*	*Margaritifera falcata*	H2912	Breton et al 2010

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Supplementary Table 3
List of specimens included in the NADH dehydrogenase subunit 1 (ND1) dataset; Haplotypes, GenBank references, original identification, new identification, and voucher/specimen and respective study. BSGLC (SUNY Buffalo State College Great Lakes Center); NCSM (North Carolina Museum of Natural Sciences); UA (University of Alabama); UAUC (University of Alabama Unionid Collection).

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
Quadrulini						
Cyclonaias						
	51	AY158810	*Quincuncina infucata*	*Cyclonaias infucata*	QiNfu2	Serb et al 2003
	53	AY655121	*Quincuncina infucata*	*Cyclonaias infucata*	UAUC3283	Campbell & Lydeard 2012
	307	MH633562	*Cyclonaias infucata*	*Cyclonaias infucata*	QkleOch001	Johnson et al 2018
	312	MH633567	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi007	Johnson et al 2018
	313	MH633568	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli009	Johnson et al 2018
	313	MH633602	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi031	Johnson et al 2018
	314	MH633569	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli010	Johnson et al 2018
	314	MH633603	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi032	Johnson et al 2018
	315	MH633570	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli011	Johnson et al 2018
	317	MH633572	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi014	Johnson et al 2018
	317	MH633578	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch021	Johnson et al 2018
	318	MH633573	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi015	Johnson et al 2018
	318	MH633574	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi016	Johnson et al 2018
	318	MH633575	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi017	Johnson et al 2018
	318	MH633577	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi020	Johnson et al 2018
	319	MH633576	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi019	Johnson et al 2018
	320	MH633579	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch022	Johnson et al 2018
	321	MH633580	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch024	Johnson et al 2018
	322	MH633581	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfOch026	Johnson et al 2018
	323	MH633582	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi028	Johnson et al 2018
	334	MH633599	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfFli034	Johnson et al 2018
	336	MH633601	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi030	Johnson et al 2018
	337	MH633604	*Cyclonaias infucata*	*Cyclonaias infucata*	QinfChi033	Johnson et al 2018
	7	AY158757	*Cyclonaias asperata*	*Cyclonaias kieneriana*	aspe792	Serb et al 2003
	8	AY158758	*Cyclonaias asperata*	*Cyclonaias kieneriana*	aspe784	Serb et al 2003

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	18	AY158768	*Cyclonaias asperata*	*Cyclonaias kieneriana*	aspe2712	Serb et al 2003
	19	AY158769	*Cyclonaias kieneriana*	*Cyclonaias kieneriana*	kieN334	Serb et al 2003
	29	AY158779	*Cyclonaias asperata*	*Cyclonaias kieneriana*	aspe333	Serb et al 2003
	48	AY158806	*Cyclonaias asperata*	*Cyclonaias kieneriana*	aspe2503	Serb et al 2003
	305	MH633560	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla001	Johnson et al 2018
	306	MH633561	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla002	Johnson et al 2018
	308	MH633563	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla006	Johnson et al 2018
	309	MH633564	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla007	Johnson et al 2018
	310	MH633565	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla008	Johnson et al 2018
	339	MH633607	*Cyclonaias asperata*	*Cyclonaias kieneriana*	QaspAla012	Johnson et al 2018
	41	AY158795	*Quincuncina infucata*	*Cyclonaias kleiniana*	QiNfu57	Serb et al 2003
	311	MH633566	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw004	Johnson et al 2018
	330	MH633592	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw018	Johnson et al 2018
	330	MH633606	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw011	Johnson et al 2018
	335	MH633600	*Cyclonaias kleiniana*	*Cyclonaias kleiniana*	QkleSuw013	Johnson et al 2018
	58	MH361785	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua004	Johnson et al 2018
	58	MH361786	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua006	Johnson et al 2018
	58	MH361788	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua008	Johnson et al 2018
	58	MH361789	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua009	Johnson et al 2018
	58	MH361822	*Cyclonaias necki*	*Cyclonaias necki*	FmitGua021	Johnson et al 2018
	58	MK503297	*Cyclonaias petrina*	*Cyclonaias necki*	BSGLC 1672	This Study
	58	MH361825	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua072	Johnson et al 2018
	58	MH361837	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua086	Johnson et al 2018
	71	MK503316	*Cyclonaias necki*	*Cyclonaias necki*	NCSM 65378	This Study
	72	MK503317	*Cyclonaias necki*	*Cyclonaias necki*	BSGLC 2255	This Study
	72	MH361776	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua001	Johnson et al 2018
	72	MH361787	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua007	Johnson et al 2018
	72	MH361818	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua063	Johnson et al 2018
	72	MH361823	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua069	Johnson et al 2018
	72	MH361836	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua085	Johnson et al 2018
	72	MH361839	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua089	Johnson et al 2018
	72	MH361840	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua090	Johnson et al 2018
	73	MK503318	*Cyclonaias necki*	*Cyclonaias necki*	BSGLC 2256	This Study

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	141	MH361777	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua003	Johnson et al 2018
	142	MH361778	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua004	Johnson et al 2018
	142	MH361779	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua005	Johnson et al 2018
	142	MH361784	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua003	Johnson et al 2018
	142	MH361819	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua064	Johnson et al 2018
	142	MH361820	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua065	Johnson et al 2018
	142	MH361838	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua088	Johnson et al 2018
	143	MH361780	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua006	Johnson et al 2018
	144	MH361781	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua007	Johnson et al 2018
	144	MH361782	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua008	Johnson et al 2018
	145	MH361796	*Cyclonaias necki*	*Cyclonaias necki*	QcouGua001	Johnson et al 2018
	145	MH361783	*Cyclonaias necki*	*Cyclonaias necki*	QspeGua002	Johnson et al 2018
	154	MH361821	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua066	Johnson et al 2018
	155	MH361824	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua071	Johnson et al 2018
	156	MH361826	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua074	Johnson et al 2018
	157	MH361827	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua075	Johnson et al 2018
	158	MH361828	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua076	Johnson et al 2018
	158	MH361835	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua084	Johnson et al 2018
	159	MH361829	*Cyclonaias necki*	*Cyclonaias necki*	QpetGua077	Johnson et al 2018
	5	AY158755	*Cyclonaias nodulata*	*Cyclonaias nodulata*	Nodu2595	Serb et al 2003
	6	AY158756	*Cyclonaias nodulata*	*Cyclonaias nodulata*	Nodu2592	Serb et al 2003
	126	GU085373	*Cyclonaias nodulata*	*Cyclonaias nodulata*	isolate 1	Boyer et al 2011
	126	MH361771	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal021	Johnson et al 2018
	126	MH361772	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal022	Johnson et al 2018
	126	MH361773	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal023	Johnson et al 2018
	126	MH361774	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal024	Johnson et al 2018
	126	MH361775	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodSal025	Johnson et al 2018
	127	GU085374	*Cyclonaias nodulata*	*Cyclonaias nodulata*	isolate 2	Boyer et al 2011
	137	MH361762	*Cyclonaias mortoni*	*Cyclonaias nodulata*	QmorRed050	Johnson et al 2018
	138	MH361763	*Cyclonaias aurea*	*Cyclonaias nodulata*	QaurNec113	Johnson et al 2018
	138	MH361769	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodNec017	Johnson et al 2018
	138	MH361770	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodNec019	Johnson et al 2018
	139	MH361764	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua012	Johnson et al 2018
	139	MH361765	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua013	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	139	MH361767	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua015	Johnson et al 2018
	139	MH361768	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua016	Johnson et al 2018
	140	MH361766	*Cyclonaias nodulata*	*Cyclonaias nodulata*	QnodOua014	Johnson et al 2018
	42	AY158798	*Cyclonaias petrina*	*Cyclonaias petrina*	Qpet2546	Serb et al 2003
	55	MK503293	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 1617	This Study
	55	MK503311	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 3254	This Study
	55	MK503312	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 3255	This Study
	55	MH361790	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol009	Johnson et al 2018
	55	MH361795	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol019	Johnson et al 2018
	55	MH361797	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol022	Johnson et al 2018
	55	MH361798	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol023	Johnson et al 2018
	55	MH361799	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol024	Johnson et al 2018
	55	MH361801	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol027	Johnson et al 2018
	55	MH361803	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol029	Johnson et al 2018
	55	MH361805	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol031	Johnson et al 2018
	55	MH361806	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol033	Johnson et al 2018
	55	MH361807	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol034	Johnson et al 2018
	55	MH361809	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol036	Johnson et al 2018
	55	MH361810	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol038	Johnson et al 2018
	55	MH361811	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol039	Johnson et al 2018
	55	MH361812	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol040	Johnson et al 2018
	55	MH361814	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol042	Johnson et al 2018
	55	MH361815	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol043	Johnson et al 2018
	55	MH361817	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol061	Johnson et al 2018
	55	MH361830	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol078	Johnson et al 2018
	55	MH361831	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol080	Johnson et al 2018
	55	MH361834	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol083	Johnson et al 2018
	56	MK503294	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 1619	This Study
	56	MK503295	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 1620	This Study
	69	MK503313	*Cyclonaias petrina*	*Cyclonaias petrina*	BSGLC 2157	This Study
	146	MH361791	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol010	Johnson et al 2018
	147	MH361792	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol011	Johnson et al 2018
	147	MH361793	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol012	Johnson et al 2018
	147	MH361804	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol030	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	148	MH361794	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol018	Johnson et al 2018
	149	MH361800	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol025	Johnson et al 2018
	150	MH361802	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol028	Johnson et al 2018
	151	MH361808	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol035	Johnson et al 2018
	152	MH361813	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol041	Johnson et al 2018
	153	MH361816	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol060	Johnson et al 2018
	160	MH361832	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol081	Johnson et al 2018
	160	MH361833	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol082	Johnson et al 2018
	161	MH361841	*Cyclonaias petrina*	*Cyclonaias petrina*	QpetCol097	Johnson et al 2018
	1	AY158745	*Cyclonaias aurea*	*Cyclonaias pustulosa*	aure1083	Serb et al 2003
	2	AY158752	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust2587	Serb et al 2003
	3	AY158753	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust2591	Serb et al 2003
	4	AY158754	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust2590	Serb et al 2003
	9	AY158759	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust866	Serb et al 2003
	12	AY158762	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust658	Serb et al 2003
	13	AY158763	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust2441	Serb et al 2003
	14	AY158764	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	mort1077	Serb et al 2003
	15	AY158765	*Cyclonaias aurea*	*Cyclonaias pustulosa*	aure1085	Serb et al 2003
	16	AY158766	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust2372	Serb et al 2003
	17	AY158767	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	pust1019	Serb et al 2003
	28	AY158778	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	mort2436	Serb et al 2003
	35	AY158788	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QREF405F	Serb et al 2003
	57	MK503296	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1674	This Study
	59	MK503298	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1553	This Study
	60	MK503299	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1567	This Study
	60	MK503301	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1554	This Study
	60	MK503314	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 3256	This Study
	60	MH361868	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue050	Johnson et al 2018
	60	MH361873	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue058	Johnson et al 2018
	60	MH361880	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue065	Johnson et al 2018
	60	MH361896	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue081	Johnson et al 2018
	61	MK503300	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1568	This Study
	62	MK503302	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 1659	This Study
	63	MK503303	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	BSGLC 3246	This Study

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	64	MK503304	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	BSGLC 3248	This Study
	64	MH361929	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol029	Johnson et al 2018
	64	MH361941	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol062	Johnson et al 2018
	65	MK503305	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	BSGLC 3247	This Study
	65	MH361912	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol003	Johnson et al 2018
	65	MH361914	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol005	Johnson et al 2018
	65	MH361917	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol009	Johnson et al 2018
	65	MH361918	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra011	Johnson et al 2018
	65	MH361919	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra012	Johnson et al 2018
	65	MH361921	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra014	Johnson et al 2018
	65	MH361926	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra020	Johnson et al 2018
	65	MH361928	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra023	Johnson et al 2018
	65	MH361930	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra035	Johnson et al 2018
	65	MH361931	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra036	Johnson et al 2018
	65	MH361932	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra037	Johnson et al 2018
	65	MH361934	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra041	Johnson et al 2018
	65	MH361935	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra042	Johnson et al 2018
	65	MH361937	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra044	Johnson et al 2018
	65	MH361939	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol060	Johnson et al 2018
	65	MH361940	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol061	Johnson et al 2018
	65	MH361942	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorCol063	Johnson et al 2018
	65	MH361924	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpetCol026	Johnson et al 2018
	65	MH361936	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpetCol079	Johnson et al 2018
	66	MK503306	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3249	This Study
	66	MH361944	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab004	Johnson et al 2018
	66	MH361962	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec030	Johnson et al 2018
	66	MH361969	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec037	Johnson et al 2018
	66	MH361972	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec040	Johnson et al 2018
	66	MH361973	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec041	Johnson et al 2018
	66	MH361992	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab068	Johnson et al 2018
	66	MH361999	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec088	Johnson et al 2018
	66	MH362045	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua038	Johnson et al 2018
	67	MK503308	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3251	This Study
	67	MH361958	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec025	Johnson et al 2018

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	70	MK503315	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 3257	This Study
	70	MH361890	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue075	Johnson et al 2018
	74	MK503319	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 2268	This Study
	74	MK503320	*Cyclonaias aurea*	*Cyclonaias pustulosa*	BSGLC 2269	This Study
	74	MH361862	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua044	Johnson et al 2018
	76	MK503322	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3258	This Study
	76	MH361996	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ072	Johnson et al 2018
	77	MK503323	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3259	This Study
	77	MK503324	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	BSGLC 3260	This Study
	78	DQ640237	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QPVT-ND1-01	Henley et al 2006
	78	FJ601230	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP11	Szumowski et al 2012
	78	MH362038	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua031	Johnson et al 2018
	78	MH362046	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF039	Johnson et al 2018
	79	DQ640238	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QPVT-ND1-02	Henley et al 2006
	80	DQ640239	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QPVT-ND1-03	Henley et al 2006
	81	DQ640240	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QPVT-ND1-04	Henley et al 2006
	82	DQ640241	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QPVT-ND1-05	Henley et al 2006
	83	FJ601221	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP2	Szumowski et al 2012
	83	MH362020	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi050	Johnson et al 2018
	84	FJ601222	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP3	Szumowski et al 2012
	84	FJ601248	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP14	Szumowski et al 2012
	84	FJ601253	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP1	Szumowski et al 2012
	85	FJ601223	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP4	Szumowski et al 2012
	86	MH361982	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed052	Johnson et al 2018
	86	FJ601224	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP5	Szumowski et al 2012
	87	FJ601225	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP6	Szumowski et al 2012
	88	FJ601226	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP7	Szumowski et al 2012
	89	FJ601227	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP8	Szumowski et al 2012
	89	FJ601228	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP9	Szumowski et al 2012
	89	FJ601237	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP18	Szumowski et al 2012
	89	FJ601241	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP22	Szumowski et al 2012
	89	FJ601243	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP8	Szumowski et al 2012
	89	FJ601245	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP11	Szumowski et al 2012
	89	FJ601250	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP18	Szumowski et al 2012

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	89	FJ601260	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP13	Szumowski et al 2012
	89	FJ601272	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP14	Szumowski et al 2012
	89	FJ601275	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP17	Szumowski et al 2012
	89	FJ601277	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP19	Szumowski et al 2012
	89	FJ601279	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	Q1MCOXA20	Szumowski et al 2012
	89	HM852934	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	BM20258	Breton et al 2010
	89	MH362016	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC048	Johnson et al 2018
	89	MH362065	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC079	Johnson et al 2018
	89	MH362070	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua085	Johnson et al 2018
	89	MH362073	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua088	Johnson et al 2018
	90	FJ601229	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP10	Szumowski et al 2012
	91	FJ601231	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP12	Szumowski et al 2012
	91	FJ601249	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP17	Szumowski et al 2012
	92	FJ601232	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP13	Szumowski et al 2012
	93	FJ601233	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP14	Szumowski et al 2012
	94	FJ601234	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP15	Szumowski et al 2012
	95	FJ601235	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP16	Szumowski et al 2012
	96	FJ601236	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP17	Szumowski et al 2012
	97	FJ601238	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP19	Szumowski et al 2012
	98	FJ601239	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP20	Szumowski et al 2012
	98	MH362031	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed024	Johnson et al 2018
	98	MH362058	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa067	Johnson et al 2018
	98	MH362063	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF073	Johnson et al 2018
	98	MH362071	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua086	Johnson et al 2018
	99	FJ601240	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	PP21	Szumowski et al 2012
	100	FJ601242	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP6	Szumowski et al 2012
	101	FJ601244	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP9	Szumowski et al 2012
	101	FJ601246	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP12	Szumowski et al 2012
	101	FJ601262	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP15	Szumowski et al 2012
	101	MH362077	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas002	Johnson et al 2018
	101	MH362049	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF043	Johnson et al 2018
	102	FJ601247	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP13	Szumowski et al 2012
	103	FJ601251	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP19	Szumowski et al 2012
	103	FJ601266	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP20	Szumowski et al 2012

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	103	FJ601267	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP1	Szumowski et al 2012
	104	FJ601252	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	SIP20	Szumowski et al 2012
	105	FJ601254	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP1	Szumowski et al 2012
	106	FJ601255	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP4	Szumowski et al 2012
	107	FJ601256	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP5	Szumowski et al 2012
	108	MH361981	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed051	Johnson et al 2018
	108	FJ601257	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP6	Szumowski et al 2012
	108	FJ601265	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP18	Szumowski et al 2012
	108	FJ601268	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP2	Szumowski et al 2012
	108	MH362085	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl010	Johnson et al 2018
	108	MH362066	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC080	Johnson et al 2018
	109	FJ601258	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP7	Szumowski et al 2012
	110	FJ601259	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP12	Szumowski et al 2012
	111	FJ601261	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP14	Szumowski et al 2012
	112	FJ601263	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP16	Szumowski et al 2012
	113	MH361985	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed055	Johnson et al 2018
	113	FJ601264	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	HP17	Szumowski et al 2012
	114	FJ601269	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP11	Szumowski et al 2012
	114	MH362059	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa068	Johnson et al 2018
	115	FJ601270	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP12	Szumowski et al 2012
	116	FJ601271	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP13	Szumowski et al 2012
	117	FJ601273	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP15	Szumowski et al 2012
	117	FJ601280	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	Q22MCOXA19	Szumowski et al 2012
	117	MH362067	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC081	Johnson et al 2018
	118	FJ601274	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP16	Szumowski et al 2012
	119	FJ601276	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP18	Szumowski et al 2012
	120	FJ601278	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	WRP20	Szumowski et al 2012
	132	HM849266	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	H2845	Breton et al 2010
	132	MH361925	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra019	Johnson et al 2018
	133	HM852935	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	BM20241	Breton et al 2010
	162	MH361842	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua001	Johnson et al 2018
	162	MH361870	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue052	Johnson et al 2018
	162	MH361872	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue057	Johnson et al 2018
	162	MH361882	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue067	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	162	MH361884	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue069	Johnson et al 2018
	163	MH361843	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua004	Johnson et al 2018
	163	MH361860	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua042	Johnson et al 2018
	164	MH361844	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua006	Johnson et al 2018
	164	MH361871	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue054	Johnson et al 2018
	164	MH361874	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue059	Johnson et al 2018
	164	MH361875	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue060	Johnson et al 2018
	164	MH361878	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue063	Johnson et al 2018
	164	MH361898	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue083	Johnson et al 2018
	164	MH361899	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue085	Johnson et al 2018
	164	MH361902	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue089	Johnson et al 2018
	164	MH361909	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua096	Johnson et al 2018
	165	MH361845	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua008	Johnson et al 2018
	165	MH361892	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue077	Johnson et al 2018
	165	MH361900	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue086	Johnson et al 2018
	165	MH361906	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua093	Johnson et al 2018
	166	MH361846	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua009	Johnson et al 2018
	167	MH361847	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua012	Johnson et al 2018
	168	MH361848	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua013	Johnson et al 2018
	168	MH361859	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua041	Johnson et al 2018
	168	MH361905	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua092	Johnson et al 2018
	169	MH361849	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua014	Johnson et al 2018
	169	MH361866	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua048	Johnson et al 2018
	170	MH361850	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua015	Johnson et al 2018
	171	MH361851	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua016	Johnson et al 2018
	172	MH361852	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua017	Johnson et al 2018
	173	MH361853	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua018	Johnson et al 2018
	174	MH361854	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua021	Johnson et al 2018
	175	MH361855	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue027	Johnson et al 2018
	175	MH361877	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue062	Johnson et al 2018
	175	MH361879	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue064	Johnson et al 2018
	175	MH361886	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue071	Johnson et al 2018
	175	MH361897	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue082	Johnson et al 2018
	176	MH361856	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue028	Johnson et al 2018

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	176	MH361857	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue031	Johnson et al 2018
	176	MH361869	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue051	Johnson et al 2018
	176	MH361876	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue061	Johnson et al 2018
	176	MH361883	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue068	Johnson et al 2018
	176	MH361885	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue070	Johnson et al 2018
	176	MH361888	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue073	Johnson et al 2018
	176	MH361889	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue074	Johnson et al 2018
	176	MH361894	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue079	Johnson et al 2018
	176	MH361901	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue088	Johnson et al 2018
	177	MH361858	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua040	Johnson et al 2018
	178	MH361861	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua043	Johnson et al 2018
	179	MH361863	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua045	Johnson et al 2018
	180	MH361864	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua046	Johnson et al 2018
	181	MH361865	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua047	Johnson et al 2018
	182	MH361867	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua049	Johnson et al 2018
	183	MH361881	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue066	Johnson et al 2018
	184	MH361887	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue072	Johnson et al 2018
	185	MH361891	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue076	Johnson et al 2018
	186	MH361893	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue078	Johnson et al 2018
	186	MH361895	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNue080	Johnson et al 2018
	187	MH361903	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua090	Johnson et al 2018
	188	MH361904	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua091	Johnson et al 2018
	189	MH361907	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua094	Johnson et al 2018
	190	MH361908	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurGua095	Johnson et al 2018
	191	MH361910	*Cyclonaias aurea*	*Cyclonaias pustulosa*	QaurNec112	Johnson et al 2018
	192	MH361911	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol001	Johnson et al 2018
	193	MH361913	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol004	Johnson et al 2018
	194	MH361915	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol006	Johnson et al 2018
	195	MH361916	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouCol007	Johnson et al 2018
	196	MH361920	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra013	Johnson et al 2018
	197	MH361922	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra015	Johnson et al 2018
	197	MH361933	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra038	Johnson et al 2018
	198	MH361923	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra017	Johnson et al 2018
	199	MH361927	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra021	Johnson et al 2018

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	200	MH361938	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QhouBra047	Johnson et al 2018
	201	MH361943	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab001	Johnson et al 2018
	202	MH361945	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab005	Johnson et al 2018
	203	MH361946	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab006	Johnson et al 2018
	204	MH361947	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab008	Johnson et al 2018
	205	MH361948	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri013	Johnson et al 2018
	206	MH361949	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri014	Johnson et al 2018
	207	MH361950	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri017	Johnson et al 2018
	208	MH361951	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri018	Johnson et al 2018
	208	MH361956	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec023	Johnson et al 2018
	208	MH361974	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec042	Johnson et al 2018
	208	MH361991	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSab066	Johnson et al 2018
	209	MH361952	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec019	Johnson et al 2018
	210	MH361953	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec020	Johnson et al 2018
	210	MH361955	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec022	Johnson et al 2018
	210	MH361963	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec031	Johnson et al 2018
	210	MH361966	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec034	Johnson et al 2018
	211	MH361954	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec021	Johnson et al 2018
	212	MH361957	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec024	Johnson et al 2018
	213	MH361959	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec026	Johnson et al 2018
	214	MH361960	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec028	Johnson et al 2018
	215	MH361961	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec029	Johnson et al 2018
	216	MH361964	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec032	Johnson et al 2018
	217	MH361965	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec033	Johnson et al 2018
	218	MH361967	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec035	Johnson et al 2018
	219	MH361968	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec036	Johnson et al 2018
	220	MH361970	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec038	Johnson et al 2018
	221	MH361971	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec039	Johnson et al 2018
	222	MH361975	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri043	Johnson et al 2018
	222	MH361976	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri044	Johnson et al 2018
	222	MH361990	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri059	Johnson et al 2018
	223	MH361977	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri046	Johnson et al 2018
	223	MH362033	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed026	Johnson et al 2018
	224	MH361978	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri047	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	225	MH361979	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri048	Johnson et al 2018
	226	MH361980	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri049	Johnson et al 2018
	227	MH361983	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed053	Johnson et al 2018
	228	MH361984	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorRed054	Johnson et al 2018
	229	MH361986	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri055	Johnson et al 2018
	230	MH361987	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri056	Johnson et al 2018
	231	MH361988	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri057	Johnson et al 2018
	232	MH361989	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorTri058	Johnson et al 2018
	232	MH361998	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec087	Johnson et al 2018
	233	MH361993	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ069	Johnson et al 2018
	234	MH361994	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ070	Johnson et al 2018
	234	MH362003	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ093	Johnson et al 2018
	235	MH361995	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ071	Johnson et al 2018
	236	MH361997	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ073	Johnson et al 2018
	237	MH362000	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorNec089	Johnson et al 2018
	237	MH362002	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ092	Johnson et al 2018
	238	MH362001	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ091	Johnson et al 2018
	239	MH362004	*Cyclonaias mortoni*	*Cyclonaias pustulosa*	QmorSaJ094	Johnson et al 2018
	240	MH362005	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed001	Johnson et al 2018
	240	MH362008	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed005	Johnson et al 2018
	240	MH362012	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed009	Johnson et al 2018
	240	MH362013	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed010	Johnson et al 2018
	241	MH362006	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed002	Johnson et al 2018
	242	MH362007	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed004	Johnson et al 2018
	242	MH362009	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed006	Johnson et al 2018
	242	MH362011	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed008	Johnson et al 2018
	243	MH362010	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed007	Johnson et al 2018
	244	MH362014	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi046	Johnson et al 2018
	244	MH362030	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed023	Johnson et al 2018
	245	MH362015	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi047	Johnson et al 2018
	246	MH362017	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed049	Johnson et al 2018
	247	MH362018	*Cyclonaias nodulata*	*Cyclonaias pustulosa*	QnodRed004	Johnson et al 2018
	248	MH362019	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QspeTri019	Johnson et al 2018
	249	MH362021	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi051	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	249	MH362053	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo061	Johnson et al 2018
	250	MH362022	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi052	Johnson et al 2018
	251	MH362023	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi053	Johnson et al 2018
	252	MH362024	*Cyclonaias houstonensis*	*Cyclonaias pustulosa*	QpusOhi054	Johnson et al 2018
	253	MH362025	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi055	Johnson et al 2018
	254	MH362026	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOhi056	Johnson et al 2018
	255	MH362027	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed020	Johnson et al 2018
	256	MH362028	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed021	Johnson et al 2018
	257	MH362029	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed022	Johnson et al 2018
	257	MH362036	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed029	Johnson et al 2018
	258	MH362032	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed025	Johnson et al 2018
	259	MH362034	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed027	Johnson et al 2018
	260	MH362035	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusRed028	Johnson et al 2018
	261	MH362037	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua030	Johnson et al 2018
	262	MH362039	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua032	Johnson et al 2018
	263	MH362040	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua033	Johnson et al 2018
	263	MH362056	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo064	Johnson et al 2018
	264	MH362041	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua034	Johnson et al 2018
	265	MH362042	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua035	Johnson et al 2018
	265	MH362043	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua036	Johnson et al 2018
	266	MH362044	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua037	Johnson et al 2018
	267	MH362047	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF040	Johnson et al 2018
	268	MH362048	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF041	Johnson et al 2018
	269	MH362050	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF057	Johnson et al 2018
	270	MH362051	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF058	Johnson et al 2018
	271	MH362052	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF059	Johnson et al 2018
	272	MH362054	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo062	Johnson et al 2018
	273	MH362055	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusNeo063	Johnson et al 2018
	274	MH362057	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa065	Johnson et al 2018
	275	MH362060	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOsa070	Johnson et al 2018
	276	MH362061	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF071	Johnson et al 2018
	277	MH362062	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF072	Johnson et al 2018
	278	MH362064	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStF074	Johnson et al 2018
	279	MH362068	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusStC082	Johnson et al 2018

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	280	MH362069	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua084	Johnson et al 2018
	281	MH362072	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua087	Johnson et al 2018
	282	MH362074	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QpusOua089	Johnson et al 2018
	283	MH362075	*Cyclonaias pustulosa*	*Cyclonaias pustulosa*	QspeOua024	Johnson et al 2018
	284	MH362076	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas001	Johnson et al 2018
	284	MH362079	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas004	Johnson et al 2018
	285	MH362078	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas003	Johnson et al 2018
	286	MH362080	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPas005	Johnson et al 2018
	286	MH362083	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl008	Johnson et al 2018
	287	MH362081	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl006	Johnson et al 2018
	288	MH362082	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl007	Johnson et al 2018
	289	MH362084	*Cyclonaias refulgens*	*Cyclonaias pustulosa*	QrefPrl009	Johnson et al 2018
	39	AY158792	*Fusconaia succissa*	*Cyclonaias succissa*	Fsucc8	Serb et al 2003
	50	AY158809	*Fusconaia succissa*	*Cyclonaias succissa*	Fsuc119	Serb et al 2003
	290	MH362086	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho001	Johnson et al 2018
	290	MH362103	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho034	Johnson et al 2018
	291	MH362087	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho002	Johnson et al 2018
	291	MH362089	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho004	Johnson et al 2018
	291	MH362099	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho030	Johnson et al 2018
	292	MH362088	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho003	Johnson et al 2018
	293	MH362090	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucEsc013	Johnson et al 2018
	294	MH362091	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucYel017	Johnson et al 2018
	295	MH362092	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucYel018	Johnson et al 2018
	296	MH362093	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucYel019	Johnson et al 2018
	297	MH362094	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucEsc021	Johnson et al 2018
	297	MH362096	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho027	Johnson et al 2018
	298	MH362095	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucEsc022	Johnson et al 2018
	299	MH362097	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho028	Johnson et al 2018
	300	MH362098	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho029	Johnson et al 2018
	301	MH362100	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho031	Johnson et al 2018
	302	MH362101	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho032	Johnson et al 2018
	303	MH362102	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho033	Johnson et al 2018
	304	MH362104	*Cyclonaias succissa*	*Cyclonaias succissa*	QsucCho039	Johnson et al 2018
	52	AY655088	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	UAUC3158	Campbell et al 2005

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	121	GU085342	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	Ctub2	Boyer et al 2011
	122	GU085343	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	MMTC 150	Boyer et al 2011
	131	HM849213	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	H2789	Breton et al 2010
	325	MH633587	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	CtubTen003	Johnson et al 2018
	326	MH633588	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	CtubTen006	Johnson et al 2018
	327	MH633589	*Cyclonaias tuberculata*	*Cyclonaias tuberculata*	CtubTen008	Johnson et al 2018
Quadrula						
	328	MH633590	*Quadrula apiculata*	*Quadrula quadrula*	QapiRGr081	Johnson et al 2018
	333	MH633595	*Quadrula quadrula*	*Quadrula quadrula*	QquaOhi009	Johnson et al 2018
	20	AY158770	*Quadrula rumphiana*	*Quadrula quadrula*	rump1044	Serb et al 2003
	22	AY158772	*Quadrula q. clade 2/3*	*Quadrula quadrula*	quad902	Serb et al 2003
	23	AY158773	*Quadrula q. clade 2/3*	*Quadrula quadrula*	quad1698	Serb et al 2003
	24	AY158774	*Quadrula q. clade 2/3*	*Quadrula quadrula*	quad1695	Serb et al 2003
	25	AY158775	*Quadrula rumphiana*	*Quadrula quadrula*	rump722	Serb et al 2003
	26	AY158776	*Quadrula rumphiana*	*Quadrula quadrula*	rump435	Serb et al 2003
	27	AY158777	*Quadrula rumphiana*	*Quadrula quadrula*	rump331	Serb et al 2003
	37	AY158790	*Quadrula q. clade 1*	*Quadrula quadrula*	QQU1045f	Serb et al 2003
	47	AY158805	*Quadrula apiculata*	*Quadrula quadrula*	Qapi2620	Serb et al 2003
	54	MK503291	*Quadrula q. clade 1*	*Quadrula quadrula*		This Study
	54	HM852936	*Quadrula q. clade 1*	*Quadrula quadrula*	BM19581	Breton et al 2010
	130	HM230421	*Quadrula rumphiana*	*Quadrula quadrula*	UA20703	Campbell & Lydeard 2012
	333	NC_013658	*Quadrula q. clade 2/3*	*Quadrula quadrula*	H1773	Breton et al 2009
Theliderma						
	33	AY158785	*Quadrula cylindrica*	*Theliderma cylindrica*	QCY2773	Serb et al 2003
	43	AY158800	*Quadrula cylindrica*	*Theliderma cylindrica*	Qcstr277	Serb et al 2003
	10	AY158760	*Quadrula intermedia*	*Theliderma intermedia*	iNte1512	Serb et al 2003
	30	AY158782	*Quadrula intermedia*	*Theliderma intermedia*	QINT2772	Serb et al 2003
	31	AY158783	*Quadrula intermedia*	*Theliderma intermedia*	QINT2775	Serb et al 2003
	44	AY158802	*Quadrula metanevra*	*Theliderma johnsoni*	Qmet1128	Serb et al 2003
	135	JF326448	*Quadrula metanevra*	*Theliderma johnsoni*		Campbell & Lydeard 2012
	21	AY158771	*Quadrula metanevra*	*Theliderma metanevra*	Qmet042	Serb et al 2003
	45	AY158803	*Quadrula metanevra*	*Theliderma metanevra*	Qmet954	Serb et al 2003
	125	GU085371	*Quadrula metanevra*	*Theliderma metanevra*	photo Qmet1	Boyer et al 2011
	125	GU085372	*Quadrula metanevra*	*Theliderma metanevra*	photo Qmet2	Boyer et al 2011

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER	STUDY
	332	MH633594	*Quadrula metanevra*	*Theliderma metanevra*	QmetTen003	Johnson et al 2018
	332	MH633598	*Quadrula metanevra*	*Theliderma metanevra*	QmetOhi005	Johnson et al 2018
	11	AY158761	*Quadrula sparsa*	*Theliderma sparsa*	spar1514	Serb et al 2003
	32	AY158784	*Quadrula sparsa*	*Theliderma sparsa*	QSP2761F	Serb et al 2003
Tritogonia						
	34	AY158786	*Quadrula nobilis*	*Tritogonia nobilis*	QNOB403	Serb et al 2003
	36	AY158789	*Quadrula quadrula*	*Tritogonia nobilis*	QQU145F	Serb et al 2003
	46	AY158804	*Quadrula nobilis*	*Tritogonia nobilis*	QNobi263	Serb et al 2003
	68	MK503309	*Quadrula nobilis*	*Tritogonia nobilis*	BSGLC 3253	This Study
	68	MK503310	*Quadrula nobilis*	*Tritogonia nobilis*	BSGLC 1856	This Study
	75	MK503321	*Quadrula nobilis*	*Tritogonia nobilis*	BSGLC 2313	This Study
	134	JF326447	*Quadrula nobilis*	*Tritogonia nobilis*		Campbell & Lydeard 2012
	38	AY158791	*Tritogonia verrucosa*	*Tritogonia verrucosa*	Tver040	Serb et al 2003
	49	AY158807	*Tritogonia verrucosa*	*Tritogonia verrucosa*	Tver2753	Serb et al 2003
	128	GU085382	*Tritogonia verrucosa*	*Tritogonia verrucosa*	isolate 1	Boyer et al 2011
	129	GU085383	*Tritogonia verrucosa*	*Tritogonia verrucosa*	isolate 2	Boyer et al 2011
	329	MH633591	*Tritogonia verrucosa*	*Tritogonia verrucosa*	QverRed015	Johnson et al 2018
	331	MH633593	*Tritogonia verrucosa*	*Tritogonia verrucosa*	QverOhi048	Johnson et al 2018
Uniomerus						
	136	JF326451	*Uniomerus tetralasmus*	*Uniomerus tetralasmus*		Campbell & Lydeard 2012
	324	MH633583	*Uniomerus tetralasmus*	*Uniomerus tetralasmus*	UtetCol005	Johnson et al 2018
	338	MH633605	*Uniomerus tetralasmus*	*Uniomerus tetralasmus*	UtetBaP011	Johnson et al 2018
Megalonaias						
	40	AY158794	*Megalonaias nervosa*	*Megalonaias nervosa*	MNerv266	Serb et al 2003
	123	GU085356	*Megalonaias nervosa*	*Megalonaias nervosa*	isolate 1	Boyer et al 2011
	124	GU085357	*Megalonaias nervosa*	*Megalonaias nervosa*	isolate 2	Boyer et al 2011
	124	MH633597	*Megalonaias nervosa*	*Megalonaias nervosa*	MnerOhi057	Johnson et al 2018
	316	MH633571	*Megalonaias nervosa*	*Megalonaias nervosa*	MnerGua025	Johnson et al 2018
Amblemini						
	Hap129	AY655086	*Amblema elliottii*	*Amblema elliottii*	UAUC2511	Campbell et al 2005
	Hap126	HM852922	*Amblema plicata*	*Amblema plicata*	BM19345	Boyer et al 2011
Lampsilini						
	Hap123	NC_005335	*Lampsilis ornata*	*Lampsilis ornata*		Serb et al 2003
	Hap124	NC_028522	*Leptodea leptodon*	*Leptodea leptodon*	LEPT20150810	Feng et al 2016

TAXON	HAP	REFERENCE	ORIGINAL ID	NEW ID	VOUCHER
Pleurobemini					
Hap125	MK503292	*Pleurobema oviforme*	*Pleurobema oviforme*		This Study
Hap108	MK503307	*Pleurobema riddellii*	*Pleurobema riddellii*		This Study
Anodontini					
Hap127	MK503290	*Anodonta nuttalliana*	*Anodonta nuttalliana*		This Study
Hap095	NC_013661	*Pyganodon grandis*	*Pyganodon grandis*		Breton et al 2009
Margaritiferidae					
Hap096	NC_034846	*Cumberlandia monodonta*	*Cumberlandia monodonta*	H3010	Guerra et al 2017
Hap128	NC_015476	*Margaritifera falcata*	*Margaritifera falcata*		Breton et al 2010

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Supplementary Table 4
List of specimens included in the concatenated Cytochrome c oxidase subunit I (COI) + NADH dehydrogenase subunit 1 (ND1) dataset; codes, original identification, new identification, and Genbank references.

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
Quadrulini				
Cyclonaias				
	Cyclonaias infucata	*Cyclonaias infucata*	AF232806	AY655121
	Cyclonaias infucata	*Cyclonaias infucata*	MH633615	MH633567
	Cyclonaias infucata	*Cyclonaias infucata*	MH633620	MH633572
	Cyclonaias infucata	*Cyclonaias infucata*	MH633621	MH633573
	Cyclonaias infucata	*Cyclonaias infucata*	MH633622	MH633574
	Cyclonaias infucata	*Cyclonaias infucata*	MH633623	MH633575
	Cyclonaias infucata	*Cyclonaias infucata*	MH633625	MH633577
	Cyclonaias infucata	*Cyclonaias infucata*	MH633624	MH633576
	Cyclonaias infucata	*Cyclonaias infucata*	MH633630	MH633582
	Cyclonaias infucata	*Cyclonaias infucata*	MH633649	MH633601
	Cyclonaias infucata	*Cyclonaias infucata*	MH633650	MH633602
	Cyclonaias infucata	*Cyclonaias infucata*	MH633651	MH633603
	Cyclonaias infucata	*Cyclonaias infucata*	MH633652	MH633604
	Cyclonaias infucata	*Cyclonaias infucata*	MH633616	MH633568
	Cyclonaias infucata	*Cyclonaias infucata*	MH633617	MH633569
	Cyclonaias infucata	*Cyclonaias infucata*	MH633618	MH633570
	Cyclonaias infucata	*Cyclonaias infucata*	MH633647	MH633599
	Cyclonaias infucata	*Cyclonaias infucata*	MH633626	MH633578
	Cyclonaias infucata	*Cyclonaias infucata*	MH633627	MH633579
	Cyclonaias infucata	*Cyclonaias infucata*	MH633628	MH633580
	Cyclonaias infucata	*Cyclonaias infucata*	MH633629	MH633581
	Cyclonaias infucata	*Cyclonaias infucata*	MH633610	MH633562

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias asperata	*Cyclonaias kieneriana*	MH633608	MH633560
	Cyclonaias asperata	*Cyclonaias kieneriana*	MH633609	MH633561
	Cyclonaias asperata	*Cyclonaias kieneriana*	MH633611	MH633563
	Cyclonaias asperata	*Cyclonaias kieneriana*	MH633612	MH633564
	Cyclonaias asperata	*Cyclonaias kieneriana*	MH633613	MH633565
	Cyclonaias asperata	*Cyclonaias kieneriana*	MH633655	MH633607
	Cyclonaias infucata	*Cyclonaias kleiniana*	AF232808	AY158795
	Quadrula kleiniana	*Cyclonaias kleiniana*	MH633614	MH633566
	Quadrula kleiniana	*Cyclonaias kleiniana*	MH633654	MH633606
	Quadrula kleiniana	*Cyclonaias kleiniana*	MH633648	MH633600
	Quadrula kleiniana	*Cyclonaias kleiniana*	MH633640	MH633592
	Cyclonaias necki	*Cyclonaias necki*	MG969422	MK503297
	Cyclonaias necki	*Cyclonaias necki*	MH362168	MH361825
	Cyclonaias necki	*Cyclonaias necki*	MH362128	MH361785
	Cyclonaias necki	*Cyclonaias necki*	MH362131	MH361788
	Cyclonaias necki	*Cyclonaias necki*	MH362132	MH361789
	Cyclonaias necki	*Cyclonaias necki*	MG969423	MK503316
	Cyclonaias necki	*Cyclonaias necki*	MG969424	MK503317
	Cyclonaias necki	*Cyclonaias necki*	MH362119	MH361776
	Cyclonaias necki	*Cyclonaias necki*	MH362161	MH361818
	Cyclonaias necki	*Cyclonaias necki*	MH362179	MH361836
	Cyclonaias necki	*Cyclonaias necki*	MH362182	MH361839
	Cyclonaias necki	*Cyclonaias necki*	MH362183	MH361840
	Cyclonaias necki	*Cyclonaias necki*	MH362130	MH361787
	Cyclonaias necki	*Cyclonaias necki*	MG969425	MK503318
	Cyclonaias necki	*Cyclonaias necki*	MH362165	MH361822
	Cyclonaias necki	*Cyclonaias necki*	MH362139	MH361796
	Cyclonaias necki	*Cyclonaias necki*	MH362126	MH361783
	Cyclonaias necki	*Cyclonaias necki*	MH362120	MH361777
	Cyclonaias necki	*Cyclonaias necki*	MH362121	MH361778

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias necki	*Cyclonaias necki*	MH362122	MH361779
	Cyclonaias necki	*Cyclonaias necki*	MH362123	MH361780
	Cyclonaias necki	*Cyclonaias necki*	MH362124	MH361781
	Cyclonaias necki	*Cyclonaias necki*	MH362125	MH361782
	Cyclonaias necki	*Cyclonaias necki*	MH362162	MH361819
	Cyclonaias necki	*Cyclonaias necki*	MH362163	MH361820
	Cyclonaias necki	*Cyclonaias necki*	MH362164	MH361821
	Cyclonaias necki	*Cyclonaias necki*	MH362166	MH361823
	Cyclonaias necki	*Cyclonaias necki*	MH362167	MH361824
	Cyclonaias necki	*Cyclonaias necki*	MH362169	MH361826
	Cyclonaias necki	*Cyclonaias necki*	MH362170	MH361827
	Cyclonaias necki	*Cyclonaias necki*	MH362171	MH361828
	Cyclonaias necki	*Cyclonaias necki*	MH362178	MH361835
	Cyclonaias necki	*Cyclonaias necki*	MH362172	MH361829
	Cyclonaias necki	*Cyclonaias necki*	MH362180	MH361837
	Cyclonaias necki	*Cyclonaias necki*	MH362129	MH361786
	Cyclonaias necki	*Cyclonaias necki*	MH362181	MH361838
	Cyclonaias necki	*Cyclonaias necki*	MH362127	MH361784
	Cyclonaias nodulata	*Cyclonaias nodulata*	GU085316	GU085373
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362115	MH361772
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362116	MH361773
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362118	MH361775
	Cyclonaias nodulata	*Cyclonaias nodulata*	GU085317	GU085374
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362105	MH361762
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362112	MH361769
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362113	MH361770
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362107	MH361764
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362108	MH361765
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362110	MH361767
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362111	MH361768

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362109	MH361766
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362114	MH361771
	Cyclonaias nodulata	*Cyclonaias nodulata*	MH362117	MH361774
	Cyclonaias petrina	*Cyclonaias petrina*	MG969416	MK503293
	Cyclonaias petrina	*Cyclonaias petrina*	MG969417	MK503294
	Cyclonaias petrina	*Cyclonaias petrina*	MG969418	MK503295
	Cyclonaias petrina	*Cyclonaias petrina*	MH362267	MH361924
	Cyclonaias petrina	*Cyclonaias petrina*	MH362279	MH361936
	Cyclonaias petrina	*Cyclonaias petrina*	MG969419	MK503311
	Cyclonaias petrina	*Cyclonaias petrina*	MG969420	MK503312
	Cyclonaias petrina	*Cyclonaias petrina*	MH362133	MH361790
	Cyclonaias petrina	*Cyclonaias petrina*	MH362138	MH361795
	Cyclonaias petrina	*Cyclonaias petrina*	MH362142	MH361799
	Cyclonaias petrina	*Cyclonaias petrina*	MH362146	MH361803
	Cyclonaias petrina	*Cyclonaias petrina*	MH362149	MH361806
	Cyclonaias petrina	*Cyclonaias petrina*	MH362152	MH361809
	Cyclonaias petrina	*Cyclonaias petrina*	MH362153	MH361810
	Cyclonaias petrina	*Cyclonaias petrina*	MH362155	MH361812
	Cyclonaias petrina	*Cyclonaias petrina*	MH362157	MH361814
	Cyclonaias petrina	*Cyclonaias petrina*	MH362158	MH361815
	Cyclonaias petrina	*Cyclonaias petrina*	MH362160	MH361817
	Cyclonaias petrina	*Cyclonaias petrina*	MH362173	MH361830
	Cyclonaias petrina	*Cyclonaias petrina*	MH362174	MH361831
	Cyclonaias petrina	*Cyclonaias petrina*	MG969421	MK503313
	Cyclonaias petrina	*Cyclonaias petrina*	MH362134	MH361791
	Cyclonaias petrina	*Cyclonaias petrina*	MH362135	MH361792
	Cyclonaias petrina	*Cyclonaias petrina*	MH362136	MH361793
	Cyclonaias petrina	*Cyclonaias petrina*	MH362147	MH361804
	Cyclonaias petrina	*Cyclonaias petrina*	MH362137	MH361794
	Cyclonaias petrina	*Cyclonaias petrina*	MH362140	MH361797

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias petrina	*Cyclonaias petrina*	MH362141	MH361798
	Cyclonaias petrina	*Cyclonaias petrina*	MH362143	MH361800
	Cyclonaias petrina	*Cyclonaias petrina*	MH362144	MH361801
	Cyclonaias petrina	*Cyclonaias petrina*	MH362154	MH361811
	Cyclonaias petrina	*Cyclonaias petrina*	MH362145	MH361802
	Cyclonaias petrina	*Cyclonaias petrina*	MH362148	MH361805
	Cyclonaias petrina	*Cyclonaias petrina*	MH362150	MH361807
	Cyclonaias petrina	*Cyclonaias petrina*	MH362151	MH361808
	Cyclonaias petrina	*Cyclonaias petrina*	MH362156	MH361813
	Cyclonaias petrina	*Cyclonaias petrina*	MH362159	MH361816
	Cyclonaias petrina	*Cyclonaias petrina*	MH362175	MH361832
	Cyclonaias petrina	*Cyclonaias petrina*	MH362176	MH361833
	Cyclonaias petrina	*Cyclonaias petrina*	MH362177	MH361834
	Cyclonaias petrina	*Cyclonaias petrina*	MH362184	MH361841
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503268	MK503296
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503269	MK503298
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503270	MK503299
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503271	MK503300
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503272	MK503302
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MK503273	MK503303
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MK503274	MK503304
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MK503275	MK503305
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362262	MH361919
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362264	MH361921
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362274	MH361931
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362278	MH361935
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362255	MH361912
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362257	MH361914
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362260	MH361917
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362282	MH361939

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362283	MH361940
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MK503276	MK503306
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MK503278	MK503308
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503281	MK503314
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362211	MH361868
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362216	MH361873
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503282	MK503315
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362233	MH361890
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503283	MK503319
	Cyclonaias aurea	*Cyclonaias pustulosa*	MK503284	MK503320
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MK503286	MK503322
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MK503287	MK503323
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MK503288	MK503324
	Cyclonaias refulgens	*Cyclonaias pustulosa*	EF033269	AY158788
	Quadrula pustulosa	*Cyclonaias pustulosa*	GU085318	FJ601222
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362185	MH361842
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362213	MH361870
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362215	MH361872
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362225	MH361882
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362227	MH361884
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362186	MH361843
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362187	MH361844
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362252	MH361909
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362214	MH361871
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362217	MH361874
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362218	MH361875
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362241	MH361898
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362242	MH361899
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362245	MH361902
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362188	MH361845

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362249	MH361906
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362235	MH361892
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362243	MH361900
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362189	MH361846
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362190	MH361847
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362191	MH361848
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362202	MH361859
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362248	MH361905
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362192	MH361849
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362209	MH361866
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362193	MH361850
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362194	MH361851
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362195	MH361852
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362196	MH361853
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362197	MH361854
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362201	MH361858
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362203	MH361860
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362204	MH361861
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362205	MH361862
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362206	MH361863
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362207	MH361864
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362208	MH361865
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362210	MH361867
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362246	MH361903
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362247	MH361904
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362250	MH361907
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362251	MH361908
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362253	MH361910
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362106	MH361763
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362198	MH361855

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362220	MH361877
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362222	MH361879
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362229	MH361886
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362240	MH361897
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362199	MH361856
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362200	MH361857
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362212	MH361869
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362219	MH361876
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362226	MH361883
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362228	MH361885
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362231	MH361888
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362232	MH361889
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362237	MH361894
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362244	MH361901
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362221	MH361878
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362223	MH361880
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362239	MH361896
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362224	MH361881
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362230	MH361887
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362234	MH361891
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362236	MH361893
	Cyclonaias aurea	*Cyclonaias pustulosa*	MH362238	MH361895
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362261	MH361918
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362269	MH361926
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362271	MH361928
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362263	MH361920
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362265	MH361922
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362266	MH361923
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362268	MH361925
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362270	MH361927

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362273	MH361930
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362275	MH361932
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362276	MH361933
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362277	MH361934
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362280	MH361937
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362281	MH361938
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362254	MH361911
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362256	MH361913
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362258	MH361915
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362259	MH361916
	Cyclonaias houstonensis	*Cyclonaias pustulosa*	MH362272	MH361929
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362284	MH361941
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362285	MH361942
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362295	MH361952
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362296	MH361953
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362297	MH361954
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362298	MH361955
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362306	MH361963
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362299	MH361956
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362300	MH361957
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362301	MH361958
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362302	MH361959
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362303	MH361960
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362304	MH361961
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362305	MH361962
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362307	MH361964
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362308	MH361965
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362309	MH361966
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362310	MH361967
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362311	MH361968

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362312	MH361969
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362313	MH361970
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362314	MH361971
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362316	MH361973
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362342	MH361999
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362317	MH361974
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362334	MH361991
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362294	MH361951
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362341	MH361998
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362343	MH362000
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362345	MH362002
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362324	MH361981
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362325	MH361982
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362326	MH361983
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362327	MH361984
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362328	MH361985
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362336	MH361993
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362337	MH361994
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362338	MH361995
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362339	MH361996
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362340	MH361997
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362344	MH362001
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362346	MH362003
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362347	MH362004
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362286	MH361943
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362287	MH361944
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362288	MH361945
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362289	MH361946
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362290	MH361947
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362335	MH361992

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362291	MH361948
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362292	MH361949
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362293	MH361950
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362318	MH361975
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362319	MH361976
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362333	MH361990
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362320	MH361977
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362321	MH361978
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362322	MH361979
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362323	MH361980
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362329	MH361986
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362330	MH361987
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362331	MH361988
	Cyclonaias mortoni	*Cyclonaias pustulosa*	MH362332	MH361989
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362361	MH362018
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362396	MH362053
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362397	MH362054
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362398	MH362055
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362399	MH362056
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362357	MH362014
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362373	MH362030
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362358	MH362015
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362363	MH362020
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362364	MH362021
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362365	MH362022
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362366	MH362023
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362367	MH362024
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362368	MH362025
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362369	MH362026
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362400	MH362057

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362401	MH362058
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362402	MH362059
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362403	MH362060
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362380	MH362037
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362381	MH362038
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362389	MH362046
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362382	MH362039
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362383	MH362040
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362384	MH362041
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362385	MH362042
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362386	MH362043
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362387	MH362044
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362388	MH362045
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362412	MH362069
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362413	MH362070
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362414	MH362071
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362415	MH362072
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362416	MH362073
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362359	MH362016
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362417	MH362074
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362348	MH362005
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362351	MH362008
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362355	MH362012
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362356	MH362013
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362349	MH362006
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362350	MH362007
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362352	MH362009
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362353	MH362010
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362354	MH362011
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362370	MH362027

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362371	MH362028
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362372	MH362029
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362379	MH362036
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362374	MH362031
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362375	MH362032
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362376	MH362033
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362377	MH362034
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362378	MH362035
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362360	MH362017
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362408	MH362065
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362409	MH362066
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362410	MH362067
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362411	MH362068
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362390	MH362047
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362391	MH362048
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362392	MH362049
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362393	MH362050
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362394	MH362051
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362395	MH362052
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362404	MH362061
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362405	MH362062
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362406	MH362063
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362407	MH362064
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362419	MH362076
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362422	MH362079
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362420	MH362077
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362421	MH362078
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362423	MH362080
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362424	MH362081
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362425	MH362082

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362426	MH362083
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362427	MH362084
	Cyclonaias refulgens	*Cyclonaias pustulosa*	MH362428	MH362085
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362418	MH362075
	Cyclonaias pustulosa	*Cyclonaias pustulosa*	MH362362	MH362019
	Cyclonaias succissa	*Cyclonaias succissa*	MH362429	MH362086
	Cyclonaias succissa	*Cyclonaias succissa*	MH362430	MH362087
	Cyclonaias succissa	*Cyclonaias succissa*	MH362431	MH362088
	Cyclonaias succissa	*Cyclonaias succissa*	MH362432	MH362089
	Cyclonaias succissa	*Cyclonaias succissa*	MH362442	MH362099
	Cyclonaias succissa	*Cyclonaias succissa*	MH362439	MH362096
	Cyclonaias succissa	*Cyclonaias succissa*	MH362440	MH362097
	Cyclonaias succissa	*Cyclonaias succissa*	MH362441	MH362098
	Cyclonaias succissa	*Cyclonaias succissa*	MH362443	MH362100
	Cyclonaias succissa	*Cyclonaias succissa*	MH362444	MH362101
	Cyclonaias succissa	*Cyclonaias succissa*	MH362445	MH362102
	Cyclonaias succissa	*Cyclonaias succissa*	MH362446	MH362103
	Cyclonaias succissa	*Cyclonaias succissa*	MH362447	MH362104
	Cyclonaias succissa	*Cyclonaias succissa*	MH362433	MH362090
	Cyclonaias succissa	*Cyclonaias succissa*	MH362437	MH362094
	Cyclonaias succissa	*Cyclonaias succissa*	MH362438	MH362095
	Cyclonaias succissa	*Cyclonaias succissa*	MH362434	MH362091
	Cyclonaias succissa	*Cyclonaias succissa*	MH362435	MH362092
	Cyclonaias succissa	*Cyclonaias succissa*	MH362436	MH362093
	Cyclonaias tuberculata	*Cyclonaias tuberculata*	MH633635	MH633587
	Cyclonaias tuberculata	*Cyclonaias tuberculata*	MH633636	MH633588
	Cyclonaias tuberculata	*Cyclonaias tuberculata*	MH633637	MH633589
	Cyclonaias tuberculata	*Cyclonaias tuberculata*	GU085283	GU085343
	Cyclonaias tuberculata	*Cyclonaias tuberculata*	GU085284	GU085342
	Cyclonaias tuberculata	*Cyclonaias tuberculata*	HM849070	HM849213

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
Quadrula				
	Quadrula rumphiana	*Quadrula quadrula*	HM230409	HM230421
	Quadrula apiculata	*Quadrula quadrula*	KT285648	AY158805
	Quadrula Quadrula	*Quadrula quadrula*	NC_013658	NC_013658
	Quadrula Quadrula	*Quadrula quadrula*	MK503267	MK503291
	Quadrula apiculata	*Quadrula quadrula*	MH633638	MH633590
	Quadrula Quadrula	*Quadrula quadrula*	MH633643	MH633595
Theliderma				
	Theliderma metanevra	*Theliderma johnsoni*	JF326435	JF326448
	Theliderma metanevra	*Theliderma metanevra*	GU085314	GU085371
	Theliderma metanevra	*Theliderma metanevra*	GU085315	GU085372
	Quadrula metanevra	*Theliderma metanevra*	MH633646	MH633598
	Quadrula metanevra	*Theliderma metanevra*	MH633642	MH633594
Tritogonia				
	Quadrula nobilis	*Tritogonia nobilis*	MK503279	MK503309
	Quadrula nobilis	*Tritogonia nobilis*	MK503280	MK503310
	Quadrula nobilis	*Tritogonia nobilis*	MK503285	MK503321
	Tritogonia verrucosa	*Tritogonia verrucosa*	AY655024	AY158791
	Tritogonia verrucosa	*Tritogonia verrucosa*	GU085322	GU085382
	Tritogonia verrucosa	*Tritogonia verrucosa*	MH633641	MH633593
	Tritogonia verrucosa	*Tritogonia verrucosa*	MH633639	MH633591
Uniomerus				
	Uniomerus tetralasmus	*Uniomerus tetralasmus*	JF326437	JF326451
	Uniomerus tetralasmus	*Uniomerus tetralasmus*	MH633653	MH633605
	Uniomerus tetralasmus	*Uniomerus tetralasmus*	MH633631	MH633583
Megalonaias				
	Megalonaias nervosa	*Megalonaias nervosa*	AY655007	AY158794
	Megalonaias nervosa	*Megalonaias nervosa*	MH633619	MH633571
	Megalonaias nervosa	*Megalonaias nervosa*	MH633645	MH633597

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
Amblemini				
	Amblema elliottii	*Amblema elliottii*	AY654991	AY655086
	Amblema plicata	*Amblema plicata*	DQ648099	HM852922

TAXON	ORIGINAL ID	NEW ID	GENBANK (COI)	GENBANK (ND1)
Lampsilini				
	Lampsilis ornata	*Lampsilis ornata*	NC_005335	NC_005335
	Leptodea leptodon	*Leptodea leptodon*	NC_028522	NC_028522
Pleurobemini				
	Pleurobema oviforme	*Pleurobema oviforme*	-	MK503292
	Pleurobema riddellii	*Pleurobema riddellii*	MK503277	MK503307
Anodontini				
	Anodonta nuttalliana	*Anodonta nuttalliana*	MK503266	ANBIV0364
	Pyganodon grandis	*Pyganodon grandis*	NC_013661	NC_013661
Margaritiferidae				
	Cumberlandia monodonta	*Cumberlandia monodonta*	NC_034846	NC_034846
	Margaritifera falcata	*Margaritifera falcata*	NC_015476	NC_015476

Supplementary Table 5
List of morphological, anatomical and behavioural characters analysed on specimens of *Quadrula s.l.*. GLN - mean glochidial size index. * only observed in laboratory conditions. Superscripts [1] occasionally, [2] shallow

	Sexual dimorphism	Periostracal chevrons	Shell sulcus	Shell shape	Mantle displays (magazines)		
					Morphology	Size	Location (apertures)
Quadrula	**NO**	**NO**	**YES**	**quadrate/rectangular**	**Conical (knob-like)**	**Large**	**Excurrent**
1. *Q. quadrula*	NO	NO	YES	quadrate	-----	-----	-----
+ *Q. apiculata*	NO	NO	YES	quadrate/rectangular	-----	-----	-----
+ *Q. rumphiana*	NO	NO	YES	quadrate	-----	-----	-----
2. *Q. couchiana*	NO	NO	YES		-----	-----	-----
3. *Q. fragosa*	NO	NO	YES	quadrate	Conical (knob-like)	Large	Excurrent
Cyclonaias	**NO**	**NO**	**NO**	**round/oval/quadrate**	**stomate-shaped**	**Small**	**Excurrent**
1. *C. kieneriana*	NO	NO	NO	oval/triangular	-----	-----	-----
+ *C. asperata*	NO	NO	NO	round/triangular	No modification*	-----	-----
2. *C. infucata*	NO	NO	NO	rectangular/triangular	-----	-----	-----
3. *C. kleiniana*	NO	NO	NO	round/quadrate triangular	-----	-----	-----
4. *C. archeri*	NO	NO	NO	quadrate	-----	-----	-----
5. *C. nodulata*	NO	NO	NO	round/quadrate	-----	-----	-----
6. *C. petrina*	NO	NO	NO	quadrate	-----	-----	-----
7. *C. necki*	NO	NO	NO	oval/quadrate	-----	-----	-----
8. *C. pustulosa*	NO	NO	NO	round/quadrate	stomate-shaped	Small	Excurrent

+ *C. aurea*	NO	NO	NO	round/quadrate	-----	-----	-----
+ *C. houstonensis*	NO	NO	NO	round/quadrate	-----	-----	-----
+ *C. mortoni*	NO	NO	NO	quadrate/			
+ *C. refulgens*	NO	NO	NO	round/oval	-----	-----	-----
9. C. succissa	NO	NO	NO	quadrate/rectangular	-----	-----	-----
10. *C. tuberculata*	NO	NO	NO	round/quadrate	stomate-shaped	Small	Excurrent
Theliderma	**NO**	**YES**	**OC[1]**	**round/quadrate/rectangular**	**Variate shape**	**Small**	**Excurrent**
1. *T. cylindrica*	NO	YES	NO	rectangular	white with orange ring	Small	Excurrent
2. *T. intermedia*	NO	YES	NO	round/quadrate	-----	-----	-----
3. *T. metanevra*	NO	YES	NO	quadrate/rectangular	polyp-like	Small	Excurrent
4. *T. johnsoni* n. sp.	NO	YES	NO	quadrate/rectangular	-----	-----	-----
5. *T. sparsa*	NO	YES	OC[1]	quadrate/rectangular	-----	-----	-----
6. *T. stapes*	NO	YES	YES[2]	triangular/quadraqte	-----	-----	-----
Tritogonia	**YES**	**NO**	**YES**	**elongate/rectangular**	**slug-shaped**	**Large**	**Both**
1. *T. verrucosa*	YES	NO	YES[2]	elongate/rectangular	slug-shaped	Large	Both
2. *T. nobilis*	YES	NO	YES	quadrate/rectangular	-----	-----	-----

Supplementary Table 5 (*cont.*)

Taxa	Reflexive release	Hosts	GLN	References
Quadrula	**NO**	**Ictaluridae (67%), Centrarchidae (33%)**	**0.005-0.009**	
1. *Q. quadrula*	NO	Ictaluridae (50%), Centrarchidae (50%)	0.007	Howard & Anson 1922 Schwebach et al 2002 Barnhart et al 2008
+ *Q. apiculata*	-----	-----	0.005	Parmalee & Bogan 1998 Barnhart et al 2008
+ *Q. rumphiana*	-----	-----	0.007	Barnhart et al 2008
2. *Q. couchiana*	-----	-----	-----	
3. *Q. fragosa*	NO	Ictaluridae	0.009	Steingraber et al 2004 Barnhart et al .2008 Hove et al 2012 Sietman et al 2012
Cyclonaias	**YES**	**Ictaluridae (71%), Centrarchidae (24%), Acipenseridae (5%)**	**0.05-0.09**	
1. *C. kieneriana*	-----	-----	-----	
+ *C. asperata*	-----	Ictaluridae	0.07	Barnhart et al 2008 Haag & Staton 2003
2. *C. infucata*	-----	-----	0.07	Williams et al 2008
3. *C. kleiniana*	-----	-----	0.07	Williams et al 2014
4. *C. archeri*	-----	-----	-----	
5. *C. nodulata*	-----	Ictaluridae (33%), Centrarchidae (67%)	0.05	Coker et al 1921 Surber 1913 Howard 1914 Parmalee & Bogan 1998 Barnhart et al 2008
6. *C. petrina*	-----	-----	-----	
7. *C. necki*	-----	-----	-----	

8. *C. pustulosa*	YES	Ictaluridae (67%), Centrarchidae (17%), Acipenseridae (17%)	0.07	Howard 1913, 1914 Surber 1913 Coker et al 1921 Howard & Anson 1922 Sietman et al 2012
+ *C. aurea*	-----	-----	-----	Howells et al 1996
+ *C. houstonensis*	-----	-----	-----	
+ *C. mortoni*				
+ *C. refulgens*	-----	-----	-----	
9. C. succissa	-----	Ictaluridae	0.06	Williams et al 2014
10. *C. tuberculata*		Ictaluridae	0.09	Hove et al 1994 Barnhart et al 2008 Williams et al 2008 Sietman et al 2012
Theliderma	**YES**	**Cyprinidae (72%), Centrarchidae (14%) Percidae (14%)**	**0.03-0.04**	
1. *T. cylindrica*		Cyprinidae (80%), Percidae (20%)	0.04	Yeager & Neves 1986 Barnhart et al 2008 Watters et al 2006, 2009
2. *T. intermedia*		Cyprinidae		Yeager & Saylor 1995
3. *T. metanevra*	YES	Cyprinidae (57%), Centrarchidae (29%), Percidae (14%)	0.03	Surber 1913, Howard 1914 Crownhart et al 2006 Hove et al 2011
4. *T. johnsoni* n. sp.	-----	-----	-----	
5. *T. sparsa*	-----	-----	-----	
6. *T. stapes*	-----	-----	-----	
Tritogonia	**NO**	**Ictaluridae**	**0.009**	
1. *T. verrucosa*	NO	Ictaluridae	0.009	Barnhart et al 2008 Hove et al 2011 Sietman et al 2012
2. *T. nobilis*	-----	Ictaluridae	-----	Howells 1997 Sietman et al 2012

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Supplementary Table 6
Species assigned *Quadrula sensu lato* according to the last comprehensive checklist of the United States (Williams et al 2017), conservation status by the International Union for Conservation of Nature (IUCN) Red List and by NaturServe, and legal protection status in the United States of America.

TAXON		CONSERVATION STATUS		PROTECTION
		IUCN	NATURSERVE	USFWS
Cyclonaias Pilsbry in Ortmann and Walker, 1922				
Cyclonaias archeri (Frierson, 1905)	Tallapoosa Orb		T1 - Critically Imperilled	petitioned under review
Cyclonaias asperata (Lea, 1861)	Alabama Orb	NT	G4 - Apparently Secure	
Cyclonaias aurea (Lea, 1859)	Golden Orb	NT	G1 - Critically Imperilled	petitioned candidate
Cyclonaias houstonensis (Lea, 1859)	Smooth Pimpleback	NT	G2 - Imperilled	petitioned candidate
Cyclonaias infucata (Conrad, 1834)	Sculptured Pigtoe	NT	G3 - Vulnerable	
Cyclonaias kieneriana (Lea, 1852)	Coosa Orb		G3 - Vulnerable	
Cyclonaias kleiniana (Lea, 1852)	Florida Mapleleaf		G2 - Imperilled	not listed
Cyclonaias mortoni (Conrad, 1835)	Western Pimpleback		T3 - Vulnerable	
Cyclonaias nodulata (Rafinesque, 1820)	Wartyback	LC	G4 - Apparently Secure	
Cyclonaias petrina (Gould, 1855)	Texas Pimpleback		G2 - Imperilled	petitioned candidate
Cyclonaias pustulosa (Lea, 1831)	Pimpleback	LC	G5 - Secure	
Cyclonaias refulgens (Lea, 1868)	Purple Pimpleback	NT	G3 - Vulnerable	
Cyclonaias succissa (Lea, 1852)	Purple Pigtoe	LC	G3 - Vulnerable	
Cyclonaias tuberculata (Rafinesque, 1820)	Purple Wartyback	NT	G5 - Secure	
Quadrula Rafinesque, 1820				
Quadrula apiculata (Say, 1829)	Southern Mapleleaf		G5 - Secure	
Quadrula couchiana (Lea, 1860)	Rio Grande Monkeyface	CR	GH - Possibly Extinct	
Quadrula fragosa (Conrad, 1835)	Winged Mapleleaf	CR	G1 - Critically Imperilled	Endangered
Quadrula nobilis (Conrad, 1854)	Gulf Mapleleaf		G4 - Apparently Secure	
Quadrula quadrula (Rafinesque, 1820)	Mapleleaf	LC	G5 - Secure	
Quadrula rumphiana (Lea, 1852)	Ridged Mapleleaf	LC	G4 - Apparently Secure	

Theliderma Swainson, 1840				
Theliderma cylindrica (Say, 1817)	Rabbitsfoot	NT	G3 - Vulnerable	Threatened
Theliderma intermedia (Conrad, 1836)	Cumberland Monkeyface	EN	G1 - Critically Imperiled	Endangered
Theliderma metanevra (Rafinesque, 1820)	Monkeyface		G4 - Apparently Secure	
Theliderma sparsa (Lea, 1841)	Appalachian Monkeyface	CR	G1 - Critically Imperiled	Endangered
Theliderma stapes (Lea, 1831)	Stirrupshell	CR	GH - Possibly Extinct	Endangered
Tritogonia Agassiz, 1852				
Tritogonia verrucosa (Rafinesque, 1820)	Pistolgrip		G4 - Apparently Secure	

Supplementary Appendix 1. History of Quadrulinae and the included genera

Rafinesque (1820) erected the subgenus *Obliquaria* (*Quadrula*) Rafinesque, 1820 and included eight species but did not designate a type species. Lea (1836, 1838, 1852, 1870) ignored *Quadrula* in his four editions of the Synopsis of *Unio*.

Theliderma Swainson, 1840 was introduced as a subgenus with seven species and no type species designated. Simpson (1900) subsequently designated *Unio lachrymosa* Lea, 1828 (=*quadrula* Rafinesque, 1820) as the type of *Theliderma*, thus making *Theliderma* a junior synonym of *Quadrula*. However, Simpson (1900) overlooked the prior designation of *metanevra* Rafinesque, 1820 as the type species of *Theliderma* by Gray (1847). Subsequently, *Quadrula, Tritogonia* Agassiz, 1852*, Orthonymus* Agassiz, 1852 and *Rotundaria* Rafinesque, 1820 were recognized by Agassiz (1852). To add to the confusion, Simpson (1900) used *metanevra* as the type species of *Quadrula*. Later, Simpson (1900, 1914) recognized *Quadrula* as a valid genus with *Q. metanevra* as the type species and placed *Quadrula* in the Unioninae Rafinesque, 1820.

The subfamily Quadrulinae was erected by Ihering (1901) in remarks reviewing Simpson's (1900) Synopsis. Ihering divided the Unionidae Rafinesque, 1820 into Unioninae, Quadrulinae Ihering, 1901 and Lampsilinae Ihering, 1901. Ihering in his definition of Quadrulinae included *Quadrula*, *Pleurobema* Rafinesque, 1819, *Obovaria* Rafinesque, 1819, *Cyprogenia* Agassiz, 1852, *Obliquaria* Rafinesque, 1820 and *Dromus* Simpson, 1900 (Table 1).

Ortmann (1910, 1911, 1912, 1919) and Walker (1918) followed Simpson (1900) and maintained *Quadrula* in the Unioninae. Hannibal (1912) recognized Quadrulinae von Ihering (1901) for *Quadrula*.

Ortmann & Walker (1922) sorted out some of the confusion surrounding some unionid names and relied on H.A. Pilsbry as an outside arbiter. They provided a concise history of the changes in the usage and the type species of *Quadrula* which had changed from *metanevra* to *quadrula* with the new taxonomic rules regarding absolute tautonymy. Ortmann & Walker (1922) recognized *Quadrula* with *Quadrula quadrula* as the type species and included six species, remarked that recognition of *Tritogonia* was a purely taxonomic question. *Cyclonaias* Pilsbry in Ortmann & Walker (1922) was erected for *Obliquaria tuberculata* Rafinesque, 1820. Since Ortmann & Walker (1922) assumed the type of *Theliderma* was *quadrula,* the next available name for the species group, including *metanevra* and *Unio cylindricus* Say, 1817*,* was *Orthonymus* Agassiz, 1852. However, since Gray (1847) designated *metanevra* as the type of *Theliderma*, this is the earliest available name for this species group.

Frierson (1927) placed *Quadrula* in the Unioninae, recognizing 10 subgenera in *Quadrula* including *Quadrula*, *Tritogonia*, *Bullata* Frierson, 1927, *Quincuncina* Ortmann in Ortmann & Walker 1922, *Orthonymus*, and *Cyclonaias*. The subgenus *Bullata* Frierson, 1927

was erected for the *Q. pustulosa* species group. Frierson (1927) in the printed errata, recognized *Bullata* Frierson, 1927 *non* Jousseaume, 1875 [Gastropoda] was preoccupied and coined *Pustulosa* Frierson, 1927 as the replacement name.

Graf & Cummings (2007) followed Haas (1969b) and Starobogatov (1970) in recognizing *Amphinaias* Crosse and Fischer in Fischer & Crosse, 1894 with *Unio couchiana* Lea, 1860 as the type species. *Amphinaias* was interpreted to include the taxa in the *Quadrula pustulosa* group. This action cannot be accepted as the shell shape of *Amphinaias couchiana* is more consistent with the *Quadrula quadrula* group and *Amphinaias* is considered a junior synonym of *Quadrula* (Williams et al 2017).

Considering *Unio pustulosa* Lea, 1831 and *tuberculata* Rafinesque, 1820 are found in the same clade (see Campbell & Lydeard 2012), the generic name *Pustulosa* Frierson, 1927 is a junior synonym of *Cyclonaias* Pilsbry in Ortmann & Walker 1922 (see Williams et al 2017). Expanding his work on central American taxa, Haas (1929) placed taxa in the Unionidae and divided the taxa among the subfamilies Lampsilinae, Anodontinae Rafinesque, 1820, Unioninae, and Quadrulinae. Haas (1929) placed species he assigned to *Crenodonta* Schülter, 1838 and *Rotundaria,* but did not describe or erect the subfamily. There was no designation of a type genus, however, he commented, he was putting the genus *Rotundaria* in the Quadrulinae. He provided no discussion of other genera included in the subfamily.

Modell (1942) was the first to clarify which genera might be considered to belong to the subfamily Quadrulinae Haas, 1929, not the subfamily concept of Ihering (1901) (Table 1).

Haas (1969a) defined the subfamily Quadrulinae Haas, 1929 and included 17 genera (Table 1). Five genera are Asian, one is European and two are Central American in distribution. They all have thick shells, often with some pustules and/or plications. This publication was followed in the same year by the section on Unionoida in the Treatise of Invertebrate Paleontology (Haas 1969b) where once again, Haas claimed authorship of Quadrulinae. Modern genera included in the Quadrulinae are the same except that two genera were moved to subgenera of *Amblema* Rafinesque, 1820 and one subgenus of *Quadrula* was changed, *Amphinaias* (Table 1).

Starobogatov (1970) treated the Quadrulini as belonging to the Quadrulinae, but placed it in the Amblemidae Rafinesque, 1820. His concept of the Quadrulinae included three tribes: Parreysiini Henderson, 1935 [primarily the subcontinent of India], Lamprotulini Modell, 1942 [Asia and southeast Asia] and Quadrulini [North and Central America]. These taxa are well sculptured, with the Lamprotulini and Quadrulini having sculptured, thick shells often with pustules and some plications. Only the taxa assigned to the Quadrulini are listed in Table 1.

Arguably, the first phylogenetic analysis of the Unionoidea was presented by Heard & Guckert (1971). They divided the Unionoidea into the Margaritiferidae Henderson, 1929, Amblemidae and the Unionidae. Amblemidae was divided among three subfamilies, Ambleminae

Rafinesque, 1820, Gonideinae Ortmann, 1916 and a new subfamily Megalonaiadinae Heard & Guckert, 1970. The Amblemidae included the Ambleminae with Quadrulinae Ihering, 1901 as a junior synonym. This subfamily included *Amblema* Rafinesque, 1820, *Elliptoideus* Frierson, 1927, *Fusconaia* Simpson, 1900, *Plectomerus* Conrad, 1853, *Quadrula*, *Quincuncina* and *Tritogonia* Agassiz, 1852.

Graf & Cummings (2007), Bouchet & Rocroi (2010) and Carter et al (2013) correctly used Quadrulini Ihering, 1901 and included seven genera (Table 1).

Modern molecular work on the unionid fauna has determined that the taxa in the Quadrulinae are assigned to the Ambleminae, a subfamily restricted to North America (Campbell & Lydeard 2012). Graf & Cummings (2007) listed eight genera in the Quadrulini. Asian taxa that historically have been placed in the Quadrulinae (Table 1), have recently been moved to other subfamilies and/or the Margaritiferidae (Lopes-Lima et al 2017, 2018; Huang et al 2018; Zieritz et al 2018).

Graf & Cummings (2007) provided a synthesis of published information. (Table 1) Williams et al (2017) provided the latest revised list of the unionid fauna of the United States and Canada and listed taxa assigned to the Quadrulini in six genera (Table 1). Graf & Cummings (2018) have updated the website that now matches Williams et al (2017), recognizing six genera in the Quadrulini (Table 1).

This study focused on *Quadrula sensu lato* and recognized four genera in what had been originally a single genus (Table 1). Currently, two species in this tribe are presumed extinct: *Theliderma stapes* (Lea, 1831) and *Quadrula couchiana*. (Williams et al 2008; Howells 2013).

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Supplementary Appendix 2. The description of *Theliderma johnsoni* n. sp.

Class: Bivalvia Linnaeus, 1758
Order: Unionida Gray, 1854
Family: Unionidae Rafinesque, 1820
Subfamily: Ambleminae Rafinesque, 1820
Tribe: Quadrulini von Ihering, 1901
Genus: *Theliderma* Swainson, 1840
Species: ***Theliderma johnsoni* n. sp. Bogan & Lopes-Lima (Figs. 1-8)**
Common name: Southern Monkeyface
Holotype NCSM 30474 (Figs. 1-2); (2 valves, 1 pair; body fixed in formalin and DNA COI Barcode, GenBank reference: MK503289) Alabama River, ARM 107.5, lower end of Wilcox Bar, 40 m from left descending bank, point estimated 2.57 air miles SSE centre of Yellow Bluff, [Coy Quad.]. Wilcox County, Alabama, geographic coordinates (WGS84; 31.93222, -87.45389); date collected**:** 19 July 2000; collectors: J.T. Garner, P. Kilpatrick.

Figures 1 (left) and **2** (right). *Theliderma johnsoni* n. sp. Holotype NCSM 30474, shell length 70.4 mm.

Paratypes: NCSM 7098, (10 valves, 5 pair), Tombigbee River at US 82, [2 air miles] W [centre] of Columbus, [Columbus South Quad.]. geographic coordinates (WGS84; 33.49342, -88.46031), 3 September 1978; **NCSM 7103**, (1 valve), Tombigbee River at US 82[US 45], [2 air] miles W [centre] of Columbus, [Columbus South Quad.], geographic coordinates (WGS84; 33.49342, 88.46031), 27 May 1978; **NCSM 7106** (Figs. 3-4), (22 dry valves, 11 pair), Tombigbee River [Columbus Lake], ca. 0.5 miles above MS 50 crossing, 7.4 air miles NW [centre] of Columbus, [Columbus North Quad.], geographic coordinates (WGS84; 33.59106, 88.48238), 3 September 1978; **NCSM 46889** (Figs. 5-6), (4 dry valves, 2 pair), Tombigbee River, about 9.5 [air] miles S [centre] of Columbus, 14 [air] miles ENE [centre] of Crawford, [Trinity Quad.]; geographic coordinates (WGS84; 33.36083, -88.38111), July 1972.; **NCSM**

33722, (2 dry valves, 1 pair), Alabama River (ARM 30.4), [ca. 3.7 air miles ESE centre town of Carlton], [Carlton Quad.]. geographic coordinates (WGS84: 31.33167, - 87.78389), 30 July 1996; **NCSM 26976**, (2 dry valves, 1 pair), Cahaba River, upstream of AL 14, right descending bank, [1.24 air miles NW centre Sprott], Sprott Quad. geographic coordinates (WGS84: 32.68700, -87.23883), 16 May 2001. **NCSM 27153** (Figs. 7, 8), (4 dry valves, 2 pair), Cahaba River, 1.5 [air] miles NNE [centre] of Heiberger, just E of CR 47, [Walter C Givhan Bridge], [Heiberger Quad.], geographic coordinates (WGS84: 32.77631, - 87.27242), 19 September 1990. **NCSM 33714**, (2 dry valves, 1 pair), Cahaba River, 2.0 [air] miles NNW [centre] Sprott, [Sprott Quad.], geographic coordinates (WGS84: 32.70699, -87.23769), 13 June 1993; **NCSM 33719**, (2 dry valves, 1 pair), Cahaba River, at first gravel bar below Walton Creek, [ca. 2.78 air miles SSE centre Harrisburg], [Harrisburg Quad.]; geographic coordinates (WGS84; 32.83972, -87.20361), 15 October 1993.

Figures 3 (left) and **4** (right). *Theliderma johnsoni* n. sp. Paratype NCSM 7106.10, shell length: 41.5 mm.

Figures 5 (left) and **6** (right). *Theliderma johnsoni* n. sp. Paratype NCSM 46889.1, shell length: 46.6 mm.

Figures 7 (left) and **8** (right). *Theliderma johnsoni* n. sp. Paratype NCSM 27153.2, shell length: 80.3

Etymology: *Theliderma johnsoni n. sp.* is named in honour of Dr. Paul D. Johnson in recognition of his significant contributions to the conservation, natural history and captive propagation of freshwater bivalves and freshwater gastropods. Paul is the head of Alabama Aquatic Biodiversity Center, Alabama Department of Conservation and Natural Resources, Marion, Alabama. The common name, Southern Monkeyface, is to note the close conchological similarity with the sister species, Monkeyface, *Theliderma metanevra* (Rafinesque, 1820), in the Mississippi River basin.

Diagnosis: *Theliderma johnsoni* n. sp. is distinguished from other unionid species by a combination of the following characters: thick shell, inflated, strong hinge teeth, broad interdentum; pallial line distant from ventral shell margin anteriorly, periostracum varies from yellowish, greenish-yellow to brown or black, usually without chevrons but may have green spots. Umbo is elevated above the hinge line, posterior ridge without pustules or knobs, elevated and narrow near the umbo, becoming broader ventrally, posterior and ventral margins emarginate anterior and poster to the posterior ridge. Based on high genetic divergence (uncorrected *p*-distance ≥3.2% COI and ≥3.5% ND1 from all *Theliderma* spp.) and Fourier analysis, (89-95 % of the time separated from *T. metanevra*) (Figs. 7E and 7F).

Description: Shell's length reaches about 100 mm, thick, quite inflated. Shell outline is quadrate to rhomboidal, anterior shell margin broadly rounded, dorsal shell margin straight posterior to the umbo, ventral shell margin broadly-rounded and slightly emarginate anterior to the posterior ridge, posterior margin straight to emarginate, posterior ridge is narrow and elevated dorsally, becoming broader posterior ventrally, lacking any large knobs on the ridge but may have marked growth lines. Posterior slope rather steep, becoming flattened posterior ventrally with an emargination at the posterior ventral margin of the posterior ridge, posterior slope usually covered with short plications, but no pustules, extending from posterior ridge to

the posterior margin. Umbo is narrow and raised dorsally, elevated above the hinge line, umbo sculpture unknown. Periostracum yellowish, greenish-brown to brown or black, becoming darker with age, this species may have green marks on the younger shell but not chevrons and older shells a uniform colour. Shell surface with pustules or elongate pustules covering disk of shell from the posterior ridge to anterior shell margin in younger specimens, becoming restricted to the disk near the posterior ridge and in large shells, pustules disappear completely. Pseudocardinal tooth large and single in right valve, often with a small blade-like tooth anterior to the main tooth and followed posteriorly by a less prominent denticle posterior to the large tooth, two large pseudocardinal teeth in left valve. Lateral teeth are short with a single heavy tooth in the right valve and a pair in the left valve. Interdental area flat and wide. Anterior adductor muscle scar is deep and smooth, pedal protractor muscle scar posterior and slightly ventral to anterior adductor muscle scar, separate from the adductor muscle scar, somewhat deep and smooth, anterior pedal retractor muscle scar located posterior dorsal margin of anterior adductor muscle scar and etched into the base of the pseudocardinal tooth, posterior adductor muscle scar shallow and faint, posterior pedal retractor muscle scar faint, may merge on dorsal edge of posterior adductor muscle scar. Pallial line distant from the ventral margin on the anterior portion of the shell, well impressed anteriorly becoming faint posteriorly, umbo cavity deep and open, nacre colour white.

Other Material Examined: Mobile Bay Basin, Tombigbee River. Mississippi, Lowndes County: NCSM 7103, (length 1 dry left valve), Tombigbee River at US 82[US 45], [2 air] miles W [centre] of Columbus, [Columbus South Quad.]. 33.49342^{o} N - 88.46031^{o} W, 27 May 1978.

Comparison with Similar Species: *Theliderma johnsoni* n. sp., resembles the shells of *Quadrula quadrula* (Rafinesque, 1820) and *Q. apiculata* (Say, 1829) [= *Q. quadrula*] which have pustules on the posterior ridge lacking in *T. johnsoni* n. sp., but has a pronounced distinct poster ridge lacking in these two species of *Quadrula* while *T. johnsoni* n. sp. lacks pustules. *Quadrula rumphiana* (Lea, 1852) [= *Q. quadrula*] has a well-defined posterior ridge without pustules but has a sulcus anterior to the posterior ridge and has pustules in the sulcus and on the shell disk. *Theliderma johnsoni* n. sp. lacks the sulcus anterior to the posterior ridge found in *Q. apiculata, Q. quadrula* and *Q. rumphiana*. *Theliderma stapes* (Lea, 1831) posterior ridge is smooth but the posterior slope is shorter and steeper than *T. johnsoni* n. sp.

Distribution: Currently known from the Mobile Bay basin including the Alabama, Cahaba and Coosa rivers occurring across eastern Mississippi, Alabama and north-western Georgia (See Williams et al 2008). The distribution of the Mobile Basin populations was reported as restricted

to the basin typically below the Fall Line in riverine reaches in the Alabama, Tombigbee and Cahaba rivers, while it extended up the Coosa River basin to Georgia (Williams et al 2008).

Habitat and Biology: *Theliderma johnsoni* n. sp. is found living from headwaters in the Coosa River Basin and below the Fall line in the Tombigbee, Cahaba and Alabama rivers. It lives in flowing waters and inhabits substrates with varying mixtures of gravel and sand.

Conservation Status: Williams et al (1993) listed *Quadrula metanevra* [now *Theliderma metanevra*] as Currently Stable. Since *T. johnsoni* n. sp. has been split from *T. metanevra*, *T. johnsoni* n. sp. will have to have a new conservation status designated. Williams et al (2008) reported: "there has been no evidence of robust recruitment in any Alabama population of *Q. metanevra* [both Tennessee and Alabama basins] during the past decade." This would suggest this species may warrant a listing as threatened.

Comparative material examined:

Quadrula apiculata

Mobile Bay Basin, Alabama River Drainage, Alabama River, Alabama, Monroe County:
NCSM 33694, (8 dry valves, 4 pair), Alabama River, ARM 82.5, [at Haines Island], [5.23 air miles W centre Franklin], [Franklin Quad.]. 31.72417 N - 87.49973 W, 31 July 1996.

Mobile Bay Basin, Alabama River Drainage, Alabama, Dallas County:
NCSM 45131, (12 dry valves, 6 pair), Bogue Chitto Creek, upstream of SR 22 bridge, [5.4 air miles ENE centre Safford], [Safford Quad.]. 32.30648 N -87.28007 W, 17 August 2006.

Pearl River Basin, Pearl River Drainage, Mississippi, Pearl River County:
NCSM 33700, (12 dry valves, 6 pair), East Pearl River, Moores Bayou, near Icebox Slough, [2.32 air miles W centre of Industrial], [Industrial Quad.]. 30.56943 N - 89.80167 W, 21-22 August 1995.

Matagorda Bay Basin, Colorado River Drainage, Texas, Llano County:
NCSM 6090, (2 dry valves,1 pair), Lake Buchanan (upper), [point estimated 4 air miles NE centre Bluffton], [Lake Buchanan Quad.]. 30.86692 N -98.44973 W, 5 May 1964.

Rio Grande Basin, Rio Grande Drainage, Texas, Webb County:
NCSM 6091, (2 dry valves, 1 pair), Lake Casa Blanca, [ca. 5.27 air miles NE center Laredo],

[Laredo East Quad.]. 27.54834 N -99.4.3367 W, 5 October 1994.

Quadrula quadrula

Mississippi River Basin, Arkansas River Drainage, Neosho River, Kansas, Woodson County:
NCSM 28981, (4 dry valves, 2 pair), Neosho River, below low head dam, on exposed bars and in the channel, in Neosho Falls, [point estimated 0.32 air miles ENE centre town of Neosho Falls], [Neosho Falls Quad.]. 38.0076 N -95.552 W, 10 October 2003.

Mississippi River Basin, Ohio River Drainage, Ohio River, Kentucky, McCracken County:
NCSM 7112, (2 dry valves, 1 pair) Ohio River, ca. 4 [air] miles above [NW centre] Paducah, [Paducah West Quad.]. 37.12126 N - 88.65439 W, 1980.

Mississippi River Basin, Ohio River Drainage, Tennessee River, Alabama, Limestone County:
NCSM 6186, (2 dry valves, 1 pair), Tennessee River, TRM 306, Decatur Boat Harbour, [point estimated 1.86 air miles S centre Whiteside], [Decatur Quad.]. 34.60722 N - 86.95805 W, 2 February 2000.

Quadrula rumphiana

Mobile Bay Basin, Alabama River Drainage, Coosa River, Conasauga River, Georgia, Murry-Whitfield County line:
NCSM 6888, (2 dry valves, 1 pair), Conasauga River at County Route 2, [point estimated at the town of Beaverdale], [Beaverdale Quad.]. 34.92127 N -84.84189 W, date collected unknown.

Mobile Bay Basin, Alabama River Drainage, Tombigbee River Drainage, Sipsey River, Alabama, Greene-Pickens County line:
NCSM 6139, (4 dry valves, 2 pair), Sipsey River, 0.5 kilometres downstream of bridge and railroad, along the right descending side of the river, [6.19 air miles S centre Aliceville], [Aliceville South Quad.]. 33.04139 N -88.13778 W, 8 June 1999.

Mobile Bay Basin, Alabama River Drainage, Tombigbee River Drainage, Alabama, Lowndes County:
NCSM 6886, (8 dry valves, 2 pair, 2 right and 2 left valves), Tombigbee River at [US 82/US

45], [2 air] miles W [centre] of Columbus, [Columbus South Quad.]. 33.49342 N -88.46031 W, 27 May 1978.

Theliderma metanevra

Mississippi River Basin, Tennessee River Drainage, Tennessee River, Tennessee, Hardin County:
NCSM 33718 (2 dry valves, 1 pair), Tennessee River, River Mile 201.3, [Counce Quad.], 35.1087 N -88.2973 W, 28 July 1988. NCSM 45841 (4 dry valves, 2 pair), [Tennessee River], TRM 195.5-197.3, Diamond Island area, [point estimated 5.7 air miles to 4.69 air miles NW centre Nixon], [Pittsburgh Landing Quad.]. start 35.18461 N -88.31265 W end 35.16167 N -88.3168 W, 14 February 1987.

Mississippi River Basin, Tennessee River Drainage, Tennessee River, Alabama, Marshall County:
NCSM 7104, (2 dry valves, 1 pair), Wheeler Reservoir [Wheeler Lake], ca. 1 mile below Guntersville Dam on the left shoreline, ca. 15 ft. below the high pool, Wheeler low plus, [8.2 air miles NNW centre Guntersville], [Guntersville Dam Quad.]. 34.42916 N -86.40913 W, 5 November 1979.

Mississippi River Basin, Tennessee River Drainage, Tennessee River, Alabama, Colbert County:
NCSM 6080, (2 dry valves, 1 pair), Tennessee River, TRM 249.0, tail end of 3rd island of the Buck Island complex, ca. 100 m. from the island, [point estimated 2.4 air miles ENE centre Pride], [Pride Quad.]. 34.73222 N -87.77861 W, 11 August 1999.
Mississippi River Basin, Black River Drainage, Black River, Missouri, Wayne County:
NCSM 48442, (2 dry valves, 1 pair), Black River, [point estimated at CR 417 crossing, 1.01 air miles S centre] Williamsville, [Williamsville Quad.]. 36.95672 N -90.54997 W, 4 July 1971.

Theliderma stapes

Mobile Bay Basin: Alabama River Drainage, Tombigbee River, Alabama, Pickens County:
NCSM 101849, (4 dry valves, 2 pair), Tombigbee River, [point estimated 6.15 air miles S centre Pickensville], River Mile 324.4, Memphis Landing, [Pickensville Quad.]. 33.13987 N 88.2858 W, 24 October 1976

Table 1
Measurements of *Theliderma johnsoni* **n. sp**. Type series.

Type Status	Museum acronym	Catalog number	River	State	Length	Height	Width
Holotype	NCSM	30474	Alabama	AL	70.4	59.1	47.0
Paratype	NCSM	7098.1	Tombigbee	MS	63.9	55.6	42.1
Paratype	NCSM	7098.2	Tombigbee	MS	81.1	66.1	46.0
Paratype	NCSM	7098.3	Tombigbee	MS	62.4	58.3	42.6
Paratype	NCSM	7098.4	Tombigbee	MS	36.5	31.1	21.5
Paratype	NCSM	7098.5	Tombigbee	MS	44.6	38.1	27.7
Paratype	NCSM	7106.1	Tombigbee	MS	78.5	64.8	44.5
Paratype	NCSM	7106.2	Tombigbee	MS	66.6	52.7	40.8
Paratype	NCSM	7106.3	Tombigbee	MS	71.7	58.7	47.7
Paratype	NCSM	7106.4	Tombigbee	MS	68.6	57.9	41.3
Paratype	NCSM	7106.5	Tombigbee	MS	61.1	50.4	41.9
Paratype	NCSM	7106.6	Tombigbee	MS	64.3	52.0	36.5
Paratype	NCSM	7106.8	Tombigbee	MS	54.3	44.9	26.5
Paratype	NCSM	7106.9	Tombigbee	MS	51.4	43.3	24.4
Paratype	NCSM	7106.10	Tombigbee	MS	45.1	40.1	29.3
Paratype	NCSM	7106.11	Tombigbee	MS	46.1	38.3	26.4
Paratype	NCSM	7106.12	Tombigbee	MS	40.7	33.9	23.8
Paratype	NCSM	26976	Cahaba	AL	95.3	73.8	57.4
Paratype	NCSM	27153.1	Cahaba	AL	70.6	59.3	37.5
Paratype	NCSM	27153.2	Cahaba	AL	80.3	68.1	42.5
Paratype	NCSM	33714	Cahaba	AL	74.7	71.6	40.2
Paratype	NCSM	33719	Cahaba	AL	54.5	46.7	32.8
Paratype	NCSM	33722	Alabama	AL	66.3	54.1	39.8
Paratype	NCSM	46889.1	Tombigbee	MS	46.6	39.8	27.4
Paratype	NCSM	46889.2	Tombigbee	MS	62.3	56.5	58.4

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CHAPTER 6

Phylogeny of the family Margaritiferidae

Paper V

Expansion and systematics redefinition of the most threatened freshwater mussel family, the Margaritiferidae

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Article published in *Molecular Phylogenetics and Evolution* **127**, 98-118 (2018).
DOI: 10.1016/j.ympev.2018.04.041

Expansion and systematics redefinition of the most threatened freshwater mussel family, the Margaritiferidae

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Abstract

Two Unionida (freshwater mussel) families are present in the Northern Hemisphere; the Margaritiferidae, representing the most threatened family, and the Unionidae, which include

several genera of unresolved taxonomic placement. The recent reassignment of the poorly studied *Lamprotula rochechouartii* from the Unionidae to the Margaritiferidae motivated a new search for other potential species of margaritiferids from members of *Gibbosula* and *Lamprotula*. Based on molecular and morphological analyses conducted on newly collected specimens from Vietnam, we here assign *Gibbosula crassa* to the Margaritiferidae. Additionally, we reanalysed all diagnostic characteristics of the Margaritiferidae and examined museum specimens of *Lamprotula* and *Gibbosula*. As a result, two additional species are also moved to the Margaritiferidae, i.e. *Gibbosula confragosa* and *Gibbosula polysticta*. We performed a robust five marker phylogeny with all available margaritiferid species and discuss the taxonomy within the family. The present phylogeny reveals the division of Margaritiferidae into four ancient clades with distinct morphological, biogeographical and ecological characteristics that justify the division of the Margaritiferidae into two subfamilies (Gibbosulinae and Margaritiferinae) and four genera (*Gibbosula*, *Cumberlandia*, *Margaritifera*, and *Pseudunio*). The systematics of the Margaritiferidae family is redefined as well as their distribution, potential origin, and main biogeographic patterns.

Keywords

Unionida, *Margaritifera*, *Lamprotula*, *Gibbosula*, Phylogeny, Bivalvia

Introduction

Unionida freshwater mussels: diversity and conservation status

The Unionida is the only strictly freshwater order of bivalves (Bogan 2008). It is an old and widespread order with approximately 800 described species in 180 genera (Bogan 2008). Six families are currently recognized within Unionida, but only the Unionidae and the Margaritiferidae are widespread in the Northern Hemisphere (Bogan 2008). While the Unionidae is extremely diverse (> 600 species), until the present study, only 12 species in one genus scattered across North America, Europe, North Africa, and Asia had been recognized within the Margaritiferidae (Bolotov et al 2016; Araujo et al 2017). Additionally, both families are declining globally and are highly endangered, especially the Margaritiferidae, where all species assessed with enough data present a near-threatened or threatened conservation status (IUCN 2018).

Taxonomical history of the Margaritiferidae and its diagnostic characters

Until the end of the twentieth century, the taxonomy and systematics of Unionida had been based primarily on conchological and anatomical characters (e.g. Haas 1969a; Parmalee & Bogan 1998; Watters et al 2009). Due to the better availability of Unionida specimens from North America and Europe, those from tropical and the Southern Hemisphere regions were relatively poorly studied (Simpson 1900, 1914; Ortmann 1921; McMichael & Hiscock 1958).

Early systematists encompassed all genera of freshwater mussels, including *Margaritana* (=*Margaritifera*) species, within the family Unionidae (Table 1: Lea, 1836, 1838, 1852, 1870; Simpson 1900, 1914; Frierson 1927). However, at the beginning of the twentieth century, Ortmann (1910) determined that some anatomical characters of some genera were distinct and of prime systematic value. This author erected a new taxon, first as a sub-family, Margaritaninae within Unionidae, but immediately after as a separate family, the Margaritanidae (=Margaritiferidae Henderson 1929, (1910)), both with the genus and species *Margaritana* (=*Margaritifera*) *margaritifera* (Linnaeus, 1758) as the type. As defined by Ortmann (1910, 1911a,b, 1912), the Margaritanidae presented distinct anatomical features from the other Unionidae species, including the lack of discrete apertures separated by mantle fusions, particular gill and marsupium structure, and glochidial (larval) shape (Table 2). Although at first other malacologists did not recognize Margaritiferidae as a separate family (e.g. Simpson 1914), soon it was accepted by most researchers (e.g. Henderson 1929), including in the comprehensive classification of the Unionida published by Haas (1969a,b).

Table 1
Comparison of Margaritiferidae classifications. Fossil genera excluded. (S) synonym. Superscripts: [1] Under tribe Heudeanini; [2] under subfamily Pseudodontinae; [3] under tribe Margaritiferini; [4] under tribe Leguminaiini

	Ortmann (1910)	Ortmann (1911a,b, 1912)	Frierson (1927)	Henderson (1929)	Thiele (1934)	Modell (1942)	Modell (1949)	Modell (1964)	Haas (1969a,b)	Starobogatov (1970)	Morrison (1975)	Boss (1982)	Starobogatov (1995)	Smith (2001)	Graf & Cummings (2007, 2017)	Huff et al (2004)	Bolotov et al (2016)	Araujo et al (2017)	This study
Margaritanidae		✓			✓														
Margaritaninae	✓		✓																
Margaritana	✓	✓	✓		✓														
(*Margaritana*)					✓							✓							
Margaritiferidae				✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Margaritiferinae						✓	✓	✓		✓		✓	✓						
Margaritifera				✓		✓		✓	✓	✓[3]	✓		✓	✓	✓	✓	✓	✓	✓
(*Margaritifera*)									✓								✓		
Margaritanopsis					s	✓		✓		✓[1]			✓	✓	s				s
(*Margaritanopsis*)									✓								✓		
Cumberlandia		✓	✓		✓	✓		✓		✓[3]		✓	✓	s	s		s		✓
(*Cumberlandia*)									✓										
Potomida					✓														
Pseudunio					s					✓[3]			✓	✓	s				✓
(*Pseudunio*)									✓								✓		
Dahurinaia									s	✓[1]			✓	s	s				s
Gibbosula											✓								✓
Ptychorhynchus						✓		✓[1]		✓[1]			✓						
Schalienaia										✓[3]			✓						s

	Ortmann (1910)	Ortmann (1911a,b, 1912)	Frierson (1927)	Henderson (1929)	Thiele (1934)	Modell (1942)	Modell (1949)	Modell (1964)	Haas (1969a,b)	Starobogatov (1970)	Morrison (1975)	Boss (1982)	Starobogatov (1995)	Smith (2001)	Graf & Cummings (2007, 2017)	Huff et al (2004)	Bolotov et al (2016)	Araujo et al (2017)	This study
Cucumerunioninae						✓	✓	✓		✓									
Cucumerunio						✓		✓											
Virgus						✓		✓											
Heudeaninae						✓	✓	✓		✓									
Heudeana						✓		✓		✓			✓						
Schepmania						✓		✓		✓			✓						
Ctenodesma						✓	✓	✓		✓			✓						
Pseudodontinae						✓	✓	✓		✓									
Pseudodon						✓		✓		✓									
Monodontina						✓	✓	✓		✓									
Nasus						✓		✓		✓									
Compsopseudodon						✓		✓											
Obovalis						✓		✓		✓									
Pseudodontopsis						✓		✓		✓[4]									
Leguminaia						✓	✓	✓		✓[4]									
Microcondylaea						✓		✓		✓[4]									
Leptanodonta						✓		✓		✓[4]									
Gonidea						✓	✓	✓		✓									
Arcidopsinae										✓									
Arcidopsis										✓									
Trapezoideus										✓									
Solenaia								✓[2]		✓									

Table 2
Characters used to define and diagnose Margaritiferidae. [1] papillae present only; [2] hinge teeth reduced.

Character	Ortmann (1910)	Ortmann (1911a, b)	Thiele (1934)	Modell (1942, 1949, 1964)
1. Diaphragm incomplete formed by gills	✓	✓		
2. Anterior end of inner gills distant from palps		✓	✓	
3. Branchial and anal siphons/apertures ill-defined not closed		✓		
4. Supra anal not separate	✓	✓	✓	✓ (1949)
5. Incurrent aperture with bifid or arborescent papillae		✓[1]		Elongate, unaffected (1949)
6. Gills no water tubes	✓	✓		✓ (1949)
7. Gills irregular scattered interlamellar connections	✓	✓	✓	✓ (1949)
8. Gills not fused with mantle posteriorly	✓	✓	✓	✓ (1949)
9. Marsupium in all four gills		✓	✓	✓ (1949)
10. Tachytictic				
11. Glochidia semilunate, hookless, irregular small teeth		✓	✓	✓ (1949)
12. Pedal elevators inconspicuous				
13. Anus located dorsal edge posterior adductor muscle				
14. Shell elongated		✓	✓	
15. Umbo low			✓	
16. Shell mostly compressed				
17. Shell with numerous folds/sculpture including pustules				some
18. Frequently concave ventral margin				
19. Shell with nacre				
20. Umbo sculpture angular un-joined chevron-like hooks				✓
21. Umbo sculpture weak concentric		✓	✓	
22. Maximum shell length				
23. Umbo cavity shallow				
24. Periostracum heavy, blackish		✓ (1911c)		
25. Shell aragonite				
26. Posterior lateral teeth tend to be reduced		✓[2]	✓	
27. Mantle attachment scars				
28. Conchiolin one layer				
29. Complete hinge teeth present				✓

Table 2 (*cont*.)

Character	Haas (1969a,b)	Heard & Guckert (1971)	Boss (1982)	Smith (2001)	Graf & Cummings (2006)	Araujo et al (2017)
1. Diaphragm incomplete formed by gills	✓	✓	✓	On mantle	✓	On mantle
2. Anterior end of inner gills distant from palps			✓			
3. Branchial and anal siphons/apertures ill-defined not closed	✓	✓		✓		
4. Supra anal not separate		✓	✓	✓		
5. Incurrent aperture with bifid or arborescent papillae				✓		✓
6. Gills no water tubes	✓	✓	✓			
7. Gills irregular scattered interlamellar connections		✓	✓	✓	✓	✓
8. Gills not fused with mantle posteriorly			✓	✓	✓	
9. Marsupium in all four gills	✓	✓	✓	✓		
10. Tachytictic			✓	✓		
11. Glochidia semi-lunate, hookless, irregular small teeth		✓	✓			
12. Pedal elevators inconspicuous					✓	✓
13. Anus located dorsal edge posterior adductor muscle					✓	✓
14. Shell elongated			✓			
15. Umbo low						
16. Shell mostly compressed	✓		✓	✓		
17. Shell with numerous folds/sculpture including pustules						
18. Frequently concave ventral margin			✓	✓		
19. Shell with nacre	✓					
20. Umbo sculpture angular un-joined chevron-like hooks	✓		✓			
21. Umbo sculpture weak concentric						
22. Maximum shell length			150mm	200mm		
23. Umbo cavity shallow	✓		✓			
24. Periostracum heavy, blackish			✓	✓		
25. Shell aragonite			✓			
26. Posterior lateral teeth tend to be reduced	✓		✓			
27. Mantle attachment scars				✓	✓	✓
28. Conchiolin one layer	✓			✓		
29. Complete hinge teeth present				✓		

In this fundamental work, the family Margaritiferidae was recognized with nine taxa (five species and four subspecies) under a single genus, *Margaritifera*, divided into four subgenera: *Margaritifera*, *Cumberlandia*, *Margaritanopsis,* and *Pseudunio*.

During the same period, alternative classifications were published (Modell 1942, 1949, 1964; Starobogatov 1970, 1995; Bogatov et al 2003) based only on few conchological characters that proposed a much larger number of taxa in the Margaritiferidae (Table 1). These studies were controversial and subsequently ignored by most malacologists (e.g. Boss 1982; Smith 2001, Graf & Cummings 2007). Since the beginning of this century, the family Margaritiferidae has been consistently restricted to around 12 species (Smith 2001; Huff et al 2004; Graf & Cummings 2006). Smith (2001), based on morphological characters only, divided the Margaritiferidae into three genera: *Pseudunio*, *Margaritifera*, and *Margaritanopsis*. Soon after, a molecular phylogenetic analysis was published using both nuclear and mitochondrial markers on seven Margaritiferidae species (Huff et al 2004). Although these phylogenetic analyses presented three clear clades, these did not agree with the genera previously defined by Smith (2001), causing Huff et al (2004) to conclude that the generic name *Margaritifera* should be considered for all species. In subsequent phylogenetic studies, the Margaritiferidae has been presented consistently as monophyletic, with a marked genetic structure and divided into three to four major clades; however, most authors have chosen not to discuss its generic assignment keeping *Margaritifera* as the single genus (Huff et al 2004; Graf & Cummings 2007; Araujo et al 2017). Nevertheless, many North American researchers continued to recognize *Cumberlandia* as a valid genus (e.g. Watters et al 2009; Haag 2012).

Recently, two comprehensive five loci molecular phylogenies on the Margaritiferidae documented several well-supported divergent clades. Bolotov et al (2016) recognized only three main clades, assigning them as subgenera (*Margaritanopsis*, *Margaritifera*, and *Pseudunio*) of *Margaritifera*, resembling the previous classification by Haas (1969a). Shortly afterward, Araujo et al (2017) described five major divergent clades within the Margaritiferidae but kept them under the same genus (*Margaritifera*).

Biogeography and diversification of the Margaritiferidae

The family Margaritiferidae has a broad but disjunct distribution range in the Northern Hemisphere (Smith 2001). It presents an enigmatic biogeographic pattern with species aggregations along the western and eastern continental margins and vast distribution gaps in inland areas (e.g. East Europe, Urals, and Siberia), possibly reflecting vicariance events driven by plate tectonics (Taylor 1988; Smith 2001; Huff et al 2004). Recently, Bolotov et al (2016) and Araujo et al (2017) reviewed available biogeographic schemes explaining the origin and expansion routes of the Margaritiferidae and independently provided new fossil-calibrated evolutionary models. However, the time and place of origin of the entire family remained

unclear (Bolotov et al 2016; Araujo et al 2017). The phylogenetic models placed the origin of the Margaritiferidae in the mid-Cretaceous (Bolotov et al 2016) or even in the Late Triassic (Araujo et al 2017). The strong temporal discordance between these fossil-calibrated phylogenies together with significant topological differences and low support values in several deep nodes suggest that both studies need additional taxon samples. Inclusion of *Pseudunio homsensis* from the Orontes River in Turkey, that had been missing from the previous phylogenetic studies (Bolotov et al 2016; Araujo et al 2017), did not help to obtain a fully resolved evolutionary reconstruction for the family, as it appears to be a close relative of *P. auricularius* (Vikhrev et al 2017). Additionally, previous analyses also lacked Margaritiferidae taxa from eastern China (i.e. between the Indo-China Peninsula and the Amur River; Smith 2001; Bolotov et al 2015, 2016). As has already been noted (Smith 2001; Bolotov et al 2015), the inclusion of newly discovered species from this vast range disjunction is crucial for developing a comprehensive understanding of the biogeography of the Margaritiferidae. Huang et al (2017) added molecular sequences of *Gibbosula rochechouartii* to the data set of Araujo et al (2017) and calculated an updated fossil-calibrated phylogeny placing the origin of the Margaritiferidae crown group in the Late Cretaceous but were not able to obtain a well-resolved biogeographic reconstruction.

A large number of fossil specimens assigned to the Margaritiferidae has been recovered in Europe, Middle Asia, China, Mongolia, Siberia, Japan, North America, and Africa (e.g. Henderson 1935; Modell 1957; Martinson 1982; Ma 1996; Fang et al 2009; Van Damme et al 2015; Bolotov et al 2016; Araujo et al 2017). However, recent phylogenetic models were calculated using a limited set of fossil calibrations because the true phylogenetic affinities of many fossil taxa remain unclear due to high conchological variability (Bolotov et al 2016; Araujo et al 2017; Huang et al 2017). The high taxonomic diversity of fossil margaritiferids disagrees with the limited number of extant taxa and likely reflects a lack of critical revisions in systematic palaeontology rather than multiple extinction events (Schneider & Prieto 2011; Bolotov et al 2016; Araujo et al 2017). Slow substitution rates in the Margaritiferidae (Bolotov et al 2016) allow us to expect rather delayed diversification processes within the family, although the diversification rates in margaritiferids have never been tested to date.

Historical description and classification of some *incertae sedis* Unionidae taxa

Although recent phylogenetic works have increased our knowledge on the position of many Unionida genera from the less studied African and Asian countries (e.g. Pfeiffer & Graf 2013 2015; Lopes-Lima et al 2017a; Bolotov et al 2017a,b), the most comprehensive revision of the Unionidae classification to date placed 42 genera as *incertae sedis* (Lopes-Lima et al 2017a). These included *Gibbosula* (Simpson 1900), whose type species was first described and illustrated by Wood (1815) as *Mya crassa* from an unknown locality in China and later classified

under *Gibbosula* (i.e. as *Gibbosula crassa*) within the Unionidae by Simpson (1900). A few years later, another specimen was found in southern China and described as a new species, i.e. *Unio* (*Quadrula*) *mansuyi* Dautzenberg & Fischer, 1908. Simpson (1914) placed this species under *Quadrula* and did not associate it with *G. crassa*. A third specimen was described in 1928 and added to *Gibbosula* (i.e. *Gibbosula confragosa* Frierson, 1928) based on conchological similarities with *G. crassa*. In his comprehensive classification of the Unionida, Haas (1969a,b) considered that *Gibbosula* had been superfluously created by Simpson and listed it as a synonym of *Lamprotula*, inside the Unionidae. Additionally, Haas (1969a) listed Dautzenberg & Fischer's species *Unio mansuyi* as a synonym of *Lamprotula crassa*.

Simpson (1914) was the first to notice that *G. crassa* presented some typical margaritiferid conchological features (i.e. mantle attachment scars), but due to other distinct characters (e.g. heavy shell, well-developed teeth, and deep umbo cavity), it was retained within the Unionidae. Later, Morrison (1975) also noted that *Gibbosula* had the same characters now known to characterize the Margaritiferidae. However, this information was overlooked by most malacologists who continued to follow Haas (1969a) and kept *G. crassa* and *G. confragosa* under *Lamprotula* (e.g. Prozorova et al 2005; Graf & Cummings 2007). Finally, some authors recently described conchological differences between the two *Gibbosula* species and *Lamprotula* and recognized *Gibbosula* as a separate genus within Unionidae (He & Zhuang 2013; Graf & Cummings 2018). Furthermore, based on conchological similarities, a third species of *Gibbosula* was recently described, i.e. *Gibbosula nanningensis* (Qian et al 2015).

The genus *Lamprotula* was recently revealed to be polyphyletic and divided into *Lamprotula* s.s. and *Aculamprotula* (Zhou et al 2007; Pfeiffer & Graf 2013). These authors also noted that all species of *Lamprotula* should be comprehensively analysed to clarify their status and relationships. For instance, based on molecular analyses, *Lamprotula rochechouartii* has been moved to Margaritiferidae (Huang et al 2017). Also, morphological and molecular characteristics of six specimens of *G. crassa* collected from Bang River, Cao Bang Province, Vietnam in 2016, suggested that the species did not belong to the Unionidae but to the Margaritiferidae (Bogan & Do 2016). The reassignment of these two Asian species (i.e. *L. rochechouartii* and *G. crassa*) from the Unionidae to the Margaritiferidae raises the question of whether there are other overlooked species of Margaritiferidae within this group. To address this issue, the congeneric *G. confragosa* and *L. rochechouartii* shell types were here analysed as well as other types of *Lamprotula* sp. for potentially misplaced margaritiferids.

Under these considerations, the present study aimed to: (i) perform a detailed morphological characterization of collected *G. crassa* specimens, and available museum specimens of all Margaritiferidae, *Lamprotula*, and *Gibbosula*; (ii) sequence and characterize

the whole F-type mitogenome of *G. crassa*; (iii) produce a robust phylogeny of the Margaritiferidae using five (nuclear and mitochondrial) markers and discuss the systematics and taxonomy within the family; (iv) compare anatomical, conchological and ecological characters within and among all retrieved clades; and (v) describe the potential origin and ancient radiations of the Margaritiferidae and detect the most probable ancestral geographic areas based on a new multi-locus fossil-calibrated phylogenetic model, using the most complete sampling of taxa to date and an expanded calibration dataset.

Materials and methods

Sampling and museum specimens

Six specimens of *G. crassa* were collected during a survey in northern Vietnam in the Bang River, Cao Bang Province, Vietnam, in 2016. Specimens were deposited as vouchers at the North Carolina Museum of Natural Sciences, United States of America (NCSM 102193, 102194) and the Institute of Ecology and Biological Resources, Hanoi, Vietnam (IEBR-FM 01-03). Museum specimens of *Gibbosula*, *Lamprotula*, and Margaritiferidae, including the type specimens of *Unio mansuyi* and *G. confragosa*, were analysed for morphology and/or genetics (Table 3 and Supplementary Table 1). Foot tissue samples were collected and preserved in 96% ethanol for DNA extraction.

DNA extractions, sequencing, assembly, and annotation

DNA was extracted from foot samples of two *G. crassa* individuals and other margaritiferid specimens (Table 3) following Froufe et al (2016). The complete F-type mitogenome of a single *G. crassa* sample was then sequenced and assembled using an established pipeline (Gan et al 2014). Mitochondrial gene annotations were performed using MITOS (Bernt et al 2013). The final tRNAs gene limits were rechecked with ARWEN (Laslett & Canbäck 2008). Finally, in-house scripts were applied to adjust the mtDNA protein-coding limits since MITOS seems to underestimate gene length. The whole mitogenome sequence has been deposited in GenBank (MH319826). The mitogenome was then visualized using GenomeVx (Conant and Wolfe 2008) (Supplementary Fig. 1). The mitochondrial 16S rRNA and Cytochrome c Oxidase I (COI), and the nuclear 18S rRNA, 28S rRNA, and Histone 3 (H3) gene fragments were amplified from the extracted gDNAs of both *G. crassa* and the remaining margaritiferid species, following the conditions described in Bolotov et al (2016) and Araujo et al (2017).

Phylogenetic analyses

Individual alignments were performed for each of the five markers: COI - 654 nt, 16S - 475 nt, 18S - 1778 nt, 28S - 307 nt, and H3 - 327 nt. Each alignment was constructed with up to two

representatives from all available Margaritiferidae species, including GenBank sequences (Table 3). Representative species from each of the families of the Unionida and *Neotrigonia*, Trigoniidae, the marine sister group of the Unionida (Giribet & Wheeler 2002), were included as outgroups (Table 3). All individual datasets were aligned using the standalone version of GUIDANCE2 (Sela et al 2015) with the MAFFT multiple sequence global pair alignment algorithm (Katoh & Standley 2013). The following GUIDANCE parameters were used: GUIDANCE score algorithm; 100 bootstrap replicates; and a column cut-off score of 0.8. Substitution saturation tests for all codon positions were accomplished in the protein-coding loci (COI, and H3) as implemented in DAMBE 6 (Xia 2017). Phylogenetic analyses were then performed by Bayesian Inference (BI) and Maximum Likelihood (ML) on 13 partitioned datasets from a single marker to a combination of markers as follows: (1) combined dataset 1: COI (3 codons) + 16S + 18S + 28S + H3 (3 codons); (2) combined dataset 2: COI + 16S + 18S + 28S + H3; (3) mtDNA 1: COI (3 codons) + 16S; (4) mtDNA 2: COI + 16S; (5) COI (3 codons); (6) COI; (7) 16S; (8) nDNA: 18S + 28S + H3 (3 codons); (9) nDNA: 18S + 28S + H3; (10) 28S; (11) 18S; (12) H3 (3 codons); and (13) H3. For the BI analyses, the best-fit models of nucleotide substitution for each partition were previously selected (Supplementary Table 2), under the Bayesian Information Criterion (BIC) using JModelTest 2.1.10 (Darriba et al 2012). BI analyses were performed in MrBayes v3.2.6 (Ronquist et al 2012) using the previously selected models. Analyses were initiated with program-generated trees and four Markov chains with default incremental heating. Two independent runs of 20×10^6 generations were sampled at intervals of 1,000 generations producing a total of 20,000 trees. Burn-in was determined upon the convergence of log-likelihood and parameter values using Tracer 1.6 (Rambaut et al 2014). For the ML phylogenetic analyses, sequences were analysed in RaxML 8.0.0 (Stamatakis 2014) with 1,000 bootstrap replicates, assuming a GTR + G + I model for each partition.

Morphological and ecological assessments

To evaluate the systematics within Margaritiferidae and detect other potential margaritiferid species, detailed conchological and anatomical characters were evaluated on newly collected *G. crassa* specimens and on museum specimens of *Gibbosula*, *Lamprotula* and Margaritiferidae, including the type specimens of *Unio mansuyi* and *G. confragosa*. Bibliographic data on the major ecological and physiological traits were also compiled for all margaritiferid species (Table 4). To characterize and compare glochidial size, the glochidial size index (GIn) was calculated following Lopes-Lima et al (2017a).

Table 3
List of specimens analysed, GenBank references, specimen number, locations, and museum voucher references. *not generated from a single individual.

Taxon	Specimen	COI	16S	18S	28S	H3
UNIONIDA						
MARGARITIFERIDAE						
GIBBOSULINAE						
Gibbosula crassa	1	MH293546	MH293536	MH293539	MH293542	MH293549
Gibbosula crassa	2	MH293547	MH293537	MH293540	MH293543	MH293550
Gibbosula laosensis	1	KU763224	KU763193	KU763255	KU763298	KU763342
Gibbosula laosensis	2	KU763225	KU763194	KU763256	KU763299	KU763343
Gibbosula rochechouartii	1	MF072498	MF072505	MF072519	MF072512	MF072526
Gibbosula rochechouartii	2	MF072502	MF072509	MF072523	MF072516	MF072530
MARGARITIFERINAE						
Cumberlandia monodonta	1	AY579131	AY579089	AY579105	AY579121	AY579144
Cumberlandia monodonta	2	MH293545	MH293535	MH293538	MH293541	MH293548
Margaritifera dahurica	1	KJ161516	KJ943526	KT343730	KT343738	AY579133
*Margaritifera dahurica**	2	KJ161520	KJ943527	KJ943531	MH293544	MH293551
Margaritifera falcata	1	AY579128	AY579085	AY579101	AY579117	AY579141
Margaritifera falcata	2	AY579127	AY579084	AY579100	AY579116	AY579140
Margaritifera hembeli	1	KU763218	KU763189	KU763250	KU763293	KU763336
Margaritifera hembeli	2	KU763219	KU763190	KU763251	KU763294	KU763337
Margaritifera laevis		KU763222	KU763192	KU763253	KU763296	KU763340
Margaritifera margaritifera	1	KU763227	KU763196	KU763258	KU763301	KU763345
Margaritifera margaritifera	2	AF303342	AF303301	KU763274	KU763317	KU763360
Margaritifera marrianae		KU763243	KU763214	KU763283	KU763326	KU763369
Margaritifera middendorffi	1	AY579124	AY579081	AY579092	AY579108	AY579134
Margaritifera middendorffi	2	KJ161547	KJ943528	KT343726	KT343735	MH293552
Pseudunio auricularius	1	AY579125	AY579083	AY579097	AY579113	AY579137
Pseudunio auricularius	2	AF303309	AF303274	KU763247	KU763290	KU763333
Pseudunio homsensis		KX550090	KX550092	KX550088	KX550086	MH293553
Pseudunio marocanus	1	EU429678	EU429689	KU763281	KU763324	KU763367

Pseudunio marocanus	2	EU429679	EU429691	KU763282	KU763325	KU763368
UNIONIDAE						
Lampsilis cardium		KX713472	KX713226	KX713305	KX713394	KX713547
Potomida littoralis		KP217871	KP217981	KU763287	KU763330	KU763373
Unio pictorum		KC429109	KC429266	KC429349	KC429447	KC429186
HYRIIDAE						
Hyridella australis		KX713467	KX713224	KX713301	KX713389	KX713545
Triplodon corrugatus		KX713505	KX713262	KX713352	KX713438	KX713585
Velesunio ambiguus		KC429106	KC429263	KC429346	KC429444	KC429183
MULLERIIDAE						
Anodontites elongata		KX713444	KX713190	KX713268	KX713357	KX713512
Lamproscapha ensiformis		KX713471	KX713225	KX713304	KX713393	KX713546
ETHERIIDAE						
Etheria elliptica		KX713462	KX713219	KX713296	KX713384	KX713540
IRIDINIDAE						
Aspatharia pfeifferiana		KC429107	KC429264	KC429347	KC429445	KC429184
Chambardia wahlbergi		KX713448	KX713202	KX713277	KX713365	KX713520
Mutela hargeri		KX713482	KX713237	KX713317	KX713405	KX713559
TRIGONIIDA						
TRIGONIIDAE						
Neotrigonia lamarckii		KC429105	KC429262	KC429345	KC429443	KC429182
Neotrigonia margaritacea		U56850	DQ280034	AF411690	AF411689	AY070155

Table 3 (*cont.*)

Taxon	Specimen	Location	Voucher
UNIONIDA			
MARGARITIFERIDAE			
GIBBOSULINAE			
Gibbosula crassa	1	Bang River, Cao Bang, Vietnam	IEBR-FM GC01
Gibbosula crassa	2	Bang River, Cao Bang, Vietnam	IEBR-FM GC03
Gibbosula laosensis	1	Mun River, Thailand	
Gibbosula laosensis	2	Luang Prabang, Laos	MNCN15.07/12038 (N1687)
Gibbosula rochechouartii	1	Poyang Lake, Yangtze, China	
Gibbosula rochechouartii	2	Poyang Lake, Yangtze, China	
MARGARITIFERINAE			
Cumberlandia monodonta	1	Missouri, USA	
Cumberlandia monodonta	2	Meramec River, Missouri, USA	
Margaritifera dahurica	1	Ilistaya River, Primorye, Russia	IEPN d0088/6
*Margaritifera dahurica**	2	Ilistaya River, Primorye, Russia	IEPN d0089/2
Margaritifera falcata	1	Idaho, USA	MCZ DNA100844
Margaritifera falcata	2	North Umpqua River, Oregon, USA	MCZ DNA100699
Margaritifera hembeli	1	Valentine Creek, Louisiana, USA	
Margaritifera hembeli	2	Brown Creek, Louisiana, USA	
Margaritifera laevis		Iwaizumi, Honshu, Japan	MNCN-FW1502-2
Margaritifera margaritifera	1	Locust Creek, Pennsylvania, USA	
Margaritifera margaritifera	2	Nore River, Ireland	MNCN-FW1490-1
Margaritifera marrianae		Hunter Creek, Alabama, USA	UAUC1651
Margaritifera middendorffi	1	Iturup, Kuril Islands, Russia	MCZ DNA100685
Margaritifera middendorffi	2	Nachilova River, Kamchatka, Russia	IEPN d0099/6
Pseudunio auricularius	1	Ebro River, Tarragona, Spain	MCZ DNA100674
Pseudunio auricularius	2	Canal Imperial, Zaragoza, Spain	MNCN-FW1238-12
Pseudunio homsensis		Karasu River, Turkey	
Pseudunio marocanus	1	Oum Er Rbia River, Morocco	MNCN-N1254
Pseudunio marocanus	2	Laabid River, Morocco	MNCN-N1264

UNIONIDAE		
Lampsilis cardium	Illinois, USA	BivAToL-421
Potomida littoralis	Cadiz, Spain	MNCN-N706
Unio pictorum	Thames River, UK	BivAToL-204
HYRIIDAE		
Hyridella australis	New South Wales, Australia	BivAToL-378
Triplodon corrugatus	Peru	BivAToL-380
Velesunio ambiguus	New South Wales, Australia	BivAToL-379
MULLERIIDAE		
Anodontites elongata	Peru	BivAToL-323
Lamproscapha ensiformis	Peru	BivAToL-382
ETHERIIDAE		
Etheria elliptica	Zambia	BivAToL-401
IRIDINIDAE		
Aspatharia pfeifferiana	Chambeshi River, Zambia	BivAToL-330
Chambardia wahlbergi	Zambia	BivAToL-405
Mutela hargeri	Zambia	BivAToL-401
TRIGONIIDA		
TRIGONIIDAE		
Neotrigonia lamarckii	North Stradbroke Island, Australia	BivAToL-97
Neotrigonia margaritacea	Tasmania, Australia	

Table 4
Biological and ecological characters. (Gln) glochidial size índex. Superscripts: ^{U} unknown; ^{R} rivers; ^{L} lakes.

	Host fish	Glochidia size (Gln)	Principal Habitats	Flow
G. confragosa	^{U}	^{U}	rivers-floodplain^{L}	^{U}
G. crassa	^{U}	^{U}	medium^{R}	moderate-strong
G. laosensis	^{U}	^{U}	headwaters^{R}	moderate-strong
G. rochechouartii	^{U}	^{U}	rivers-floodplain^{L}	slow-Moderate
G. polysticta	^{U}	^{U}	rivers-floodplain^{L}	slow-Moderate
C. monodonta	Hiodontidae	0.004	medium-large^{R}	moderate-strong
M. dahurica	Salmonidae	0.006	headwaters^{R}-large^{R}	moderate-strong
M. falcata	Salmonidae	0.006	headwaters^{R}-large^{R}	moderate-strong
M. hembeli	Esocidae	^{U}	headwaters^{R}	moderate
M. laevis	Salmonidae	0.004	headwaters^{R}-large^{R}	moderate-strong
M. margaritifera	Salmonidae	0.005	headwaters^{R}-large^{R}	moderate-strong
M. marrianae	Esocidae	0.002	headwaters^{R}	slow-moderate
M. middendorffi	Salmonidae	0.006	headwaters^{R}-large^{R}	slow-moderate
P. auricularius	Acipenseridae, Blenniidae, Gasterosteidae	0.018	middle-lower moderate-large^{R}	moderate-strong
P. homsensis	^{U}	^{U}	middle-lower moderate-large^{R}	slow-moderate
P. marocanus	^{U}	^{U}	middle-lower moderate-large^{R}	moderate-strong

Table 4 (*cont*.)

	Substrate	Water chemistry	References
G. confragosa	U	U	He & Zhuang 2013
G. crassa	boulder, cobble	hard	Bogan & Do 2016
G. laosensis	sand, grave, boulder	moderate-hard, oligotrophic	Bolotov et al 2014
G. rochechouartii	hard mud	soft-moderate	Do 2011a
G. polysticta	U	oligotrophic	Do 2011b
C. monodonta	under flat rocks, rock crevices	hard	S. McMurray pers com; Sietman et al 2017 Williams et al 2008
M. dahurica	sand, gravel	oligotrophic, soft	Bolotov et al 2015
M. falcata	sand, gravel	oligotrophic, soft	Nedeau et al 2009
M. hembeli	sand, gravel	oligotrophic, soft	Paul Johnson pers.com.
M. laevis	sand, gravel	oligotrophic, soft	Bolotov et al 2015
M. margaritifera	sand, gravel, cobble	oligotrophic, soft	Lopes-Lima et al 2017c
M. marrianae	sand, gravel	oligotrophic, soft	Paul Johnson pers.com.
M. middendorffi	sand, gravel	oligotrophic, soft	Bolotov et al 2015
P. auricularius	sand, gravel	hard	Prié et al 2010; Prié et al 2018
P. homsensis	silt	mesotrophic	Vikhrev et al 2017
P. marocanus	gravel, cobble	hard	Sousa et al 2016, 2018

Divergence time estimates

The acceptance of a global molecular clock to our multi-gene data set was estimated using the maximum likelihood test of MEGA6 (Tamura et al 2013), which revealed that the null hypothesis of equal evolutionary rate throughout the tree was rejected ($p < 0.001$). Thus, the time-calibrated haplotype-level Bayesian phylogeny was reconstructed in BEAST v. 1.8.4 based on multiple fossil calibration points using a lognormal relaxed clock algorithm with the Yule speciation process as the tree prior (Drummond et al 2006, 2012; Drummond & Rambaut 2007). Calculations were performed at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller et al 2010). A fossil-calibrated ultrametric tree was obtained using BEAST v. 1.8.4. Similar settings were assigned to nine partitions (3 codons of COI + 16S rRNA + 18S rDNA + 28S rDNA + three codons of H3) as in the MrBayes analyses. The eight fossil calibrations were used for timing the phylogeny (Supplementary Tables 3 and 4). Priors for out-group taxa were designated using a "Monophyly" option of BEAUti v. 1.8.4 (Drummond et al 2012) as follows: (Trigoniidae, (Unionida)). Four replicate BEAST searches were conducted, each with 30 million generations. The trees were sampled every 1,000th generation. The log files were checked visually with Tracer v. 1.6 for an assessment of the convergence of the MCMC chains and the effective sample size of parameters (Rambaut et al 2014). The first 10% of trees were discarded as an appropriate burn-in. Almost all ESS values were recorded as > 1,000, with a few values as > 250-800 and two values as > 100; the subsequent distributions were similar to the prior distributions. The resulting tree files from four independent analyses were compiled with LogCombiner v. 1.8.4 (Drummond et al 2012). The maximum clade credibility tree was obtained from 108,004 post-burn-in Bayesian trees using TreeAnnotator v. 1.8.4 (Drummond et al 2012).

Ancestral geographic area reconstructions

Ancestral geographic area patterns were tested using three different approaches, i.e. Statistical Dispersal-Vicariance Analysis (S-DIVA), Dispersal-Extinction Cladogenesis (Lagrange configurator, DEC), and Statistical Dispersal-Extinction Cladogenesis (S-DEC) implemented in RASP v. 3.2 (Yu et al 2015). The set of 108,004 fossil-calibrated binary trees that were combined from four runs of BEAST v. 1.8.4 (see above), was used for the ancestral area reconstruction. The user-specified, fossil-calibrated consensus tree, which was obtained based on this set of trees using TreeAnnotator v. 1.8.4 (see above), was used as a condensed tree. Outgroup sequences were removed from all datasets, using the appropriate option of RASP v. 3.2. Only a single sequence for each ingroup species was used for the analyses. Six possible geographic areas of the in-group taxa were coded as follows: (A) Southeast Asia; (B) East Asia; (C) western North America; (D) eastern North America; (E) Mediterranean Region (South Europe, Middle East, and Morocco); and (F) Europe. Seven geographically unreliable

distribution constraints were excluded from the input matrix as follows: Southeast Asia - western North America (AC), Southeast Asia - eastern North America (AD), Southeast Asia - Mediterranean Region (AE), Southeast Asia - Europe (AF), East Asia - eastern North America (BD), western North America - Mediterranean Region (CE), and western North America - Europe (CF). Geographic areas were assigned to the species as follows: Southeast Asia - *Gibbosula laosensis*, East Asia - *G. crassa*, *G. rochechouartii*, *Margaritifera dahurica*, *M. laevis*, and *M. middendorffi*, western North America - *M. falcata*, eastern North America - *Cumberlandia monodonta*, *Margaritifera marrianae*, and *M. hembeli*, and Mediterranean Region - *P. auricularius*, *P. homsensis*, and *Pseudunio marocanus*. Considering the broad trans-Atlantic distribution of *Margaritifera margaritifera*, we assigned the 'DEF' range for this species. The S-DIVA models were calculated with the following parameters: max areas=2; allow reconstruction with max reconstructions=100; max reconstructions for final tree=1000; and allowing extinctions. The DEC and S-DEC analyses were run with default settings and max areas=2. In addition to the evaluations obtained from each analysis separately, we used generalized results of all three modelling approaches, which were combined using an algorithm implemented in RASP v. 3.2.

Diversification rate analyses

The diversification rates were assessed based on the combined Bayesian phylogeny across the primary clades of the Margaritiferidae and the entire family. The set of 108,004 fossil-calibrated chronograms that were combined from four runs of BEAST v. 1.8.4 (see above) was used to construct semi-logarithmic lineage-through-time (LTT) plots in R-package 'ape' v. 4.0 (Paradis 2012; Popescu et al 2012) with the supplement of 'paleotree' v. 2.7 (Bapst 2012). We did not include a simulation for missing taxa (Pybus & Harvey 2000), because we assumed that our samples of the margaritiferid clades are nearly complete.

Two tests of a constant diversification rate for the endemic Indo-Chinese clades outlined above were calculated using 'ape' v. 4.0 based on the maximum clade credibility tree inferred from BEAST (Paradis 2012; Popescu et al 2012). First, the analysis of diversification with three survival models, i.e. a constant diversification model, a variable diversification rate through time (Weibull model), and diversification changes at a specified time point (Paradis 1997). The delta parameter from the constant rate model of Paradis (1997) was used as a mean diversification rate. Additionally, beta values of the Weibull model were tested where $\beta > 1$ suggests declining and $\beta < 1$ indicates an increasing rate of diversification. Second, the gamma statistic of Pybus & Harvey (2000) was applied. The null hypothesis of constant rate is rejected at the 5% level if a gamma statistic less than -1.645, which suggests a significantly decreasing rate of diversification through time (Pybus & Harvey 2000).

Results

Mitogenome characteristics

The length of the newly sequenced female mitogenome haplotype of *G. crassa* (16,196 nt) is within the typical range of Unionida. It includes the 13 protein-coding genes, the gender-specific ORF described for all Unionida mitogenomes with DUI system, 22 transfer RNA (tRNA) and 2 ribosomal RNA (rRNA) genes (Supplementary Fig. 1).

Phylogenetic analyses

The datasets included combinations of individual alignments (COI: 654 nt, 16S: 471 nt, 18S: 1778 nt, 28S: 309 nt, H3: 327 nt). No indels were observed and no stop codons were found after translating the sequences to amino acids in both COI and H3 datasets. All saturation tests showed significantly lower values of ISS than ISS.C (a critical value determined from computational simulation) indicating that the evaluated datasets (COI and H3) are not site saturated and are useful for phylogenetic comparisons. The resulting BI and ML trees of the concatenated (COI + 16S + 18S+ 28S + H3) datasets generated the same topology within the ingroup, being presented the topology of the BI with 9 partitions (Fig. 1). Except for the Iridinidae, paraphyletic in all analyses, all Unionida families are represented by well-supported monophyletic clades, including the Margaritiferidae (Fig. 1: Table 5). Within the Margaritiferidae, four well-supported clades can be found, identified here as Gibbosula, *Cumberlandia*, *Margaritifera*, and *Pseudunio* (Fig. 1; Table 5). In detail, a first division occurs between a *Gibbosula* clade (*G. rochechouartii* + *G. crassa* + *G. laosensis*) that is well-supported in the BI analysis and a clade encompassing all remaining species (Fig. 1; Table 5). This latter clade is further divided into the *Cumberlandia* clade (*C. monodonta*) + the *Pseudunio* clade (*P. auricularius* + *P. homsensis* + *P. marocanus*) and the *Margaritifera* clade (*M. margaritifera*, *M. dahurica*, *M. falcata*, *M. hembeli*, *M. laevis*, *M. marrianae*, and *M. middendorffi*) (Fig. 1; Table 5). The *Margaritifera* clade is further subdivided in the clade (*M. margaritifera* + *M. dahurica*) sister to the “Pacific” clade (*M. falcata* + (*M. hembeli* + *M. laevis* + *M. marrianae* + *M. middendorffi*) (Fig. 1; Table 5).

Morphological and ecological analyses

The literature review identified a total of 29 conchological, anatomical and physiological characters that are common to all analysed Margaritiferid species and can, therefore, be used to diagnose the family (Table 2).

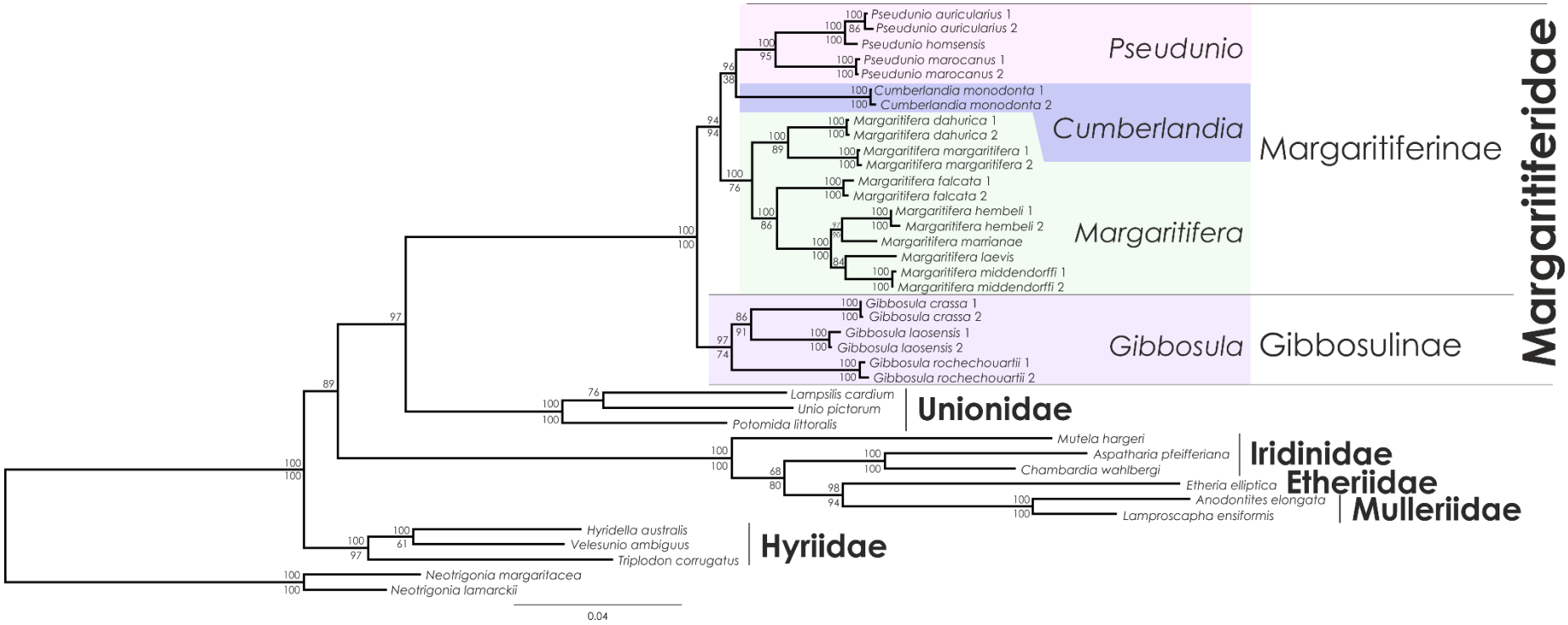

Figure 1 Phylogenetic tree of the Palaeoheterodonta obtained by Bayesian Inference (BI) and Maximum likelihood (ML) analyses of the combined (COI [3 codons] + 16S + 18S + 28S + H3 [3 codons]) dataset. Support values above the branches are posterior probabilities and bootstrap support below. Numbers after species names refer to specimen numbers (see Table 3).

Table 5.
Results of Repeatability Clade Analysis (RCA) of main clades corresponding to the preferred topology.

Clades	Analyses	Combined dataset		mtDNA				
		COI[3] + 16S + 18S + 28S + H3[3]	COI + 16S + 18S + 28S + H3	COI[3] + 16S	COI + 16S	COI[3]	COI	16S
Margaritifera	BI	**100**	**99**	**100**	**100**	**100**	**99**	58
	ML	**76**	**84**	**90**	**93**	58	**78**	24
'Pacific clade'	BI	**100**	**100**	-	-	**96**	83	-
	ML	**86**	**85**	-	-	62	61	-
Gibbosula	BI	**97**	**99**	**95**	89	-	-	78
	ML	**74**	64	65	-	52	-	61
Pseudunio	BI	**100**	**100**	**100**	**100**	**96**	75	64
	ML	**95**	**93**	**78**	**84**	-	**79**	42
Pseudunio +*Cumberlandia*	BI	**96**	**99**	-	-	50	-	-
	ML	38	47	-	50	-	39	-
Margaritiferidae	BI	**100**	**100**	**100**	**100**	**100**	**100**	72
	ML	**100**	**100**	**95**	**100**	**94**	**94**	**74**
Unionidae	BI	**100**	**100**	**100**	**100**	80	55	**99**
	ML	**100**	**100**	**97**	**99**	-	69	**81**
Etheriidae + *Mulleriidae* + *Iridinidae*	BI	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	ML	**100**	**100**	**100**	**100**	**100**	**100**	**94**
Hyriidae	BI	**100**	**100**	55	93	76	**98**	**97**
	ML	**97**	**98**	**76**	**75**	**70**	**72**	63

Table 5 (*cont*.)

Clades	Analyses	Nuclear					
		18S + 28S + H3[3]	18S + 28S + H3	18S	28S	H3[3]	H3
Margaritifera	BI	-	-	-	-	-	-
	ML	-	-	-	37	-	-
'Pacific clade'	BI	**100**	**100**	**100**	-	-	-
	ML	37	-	**98**	-	-	-
Gibbosula	BI	**98**	**99**	60	85	-	-
	ML	-	-	40	57	-	-
Pseudunio	BI	**100**	**100**	70	-	-	-
	ML	68	-	-	-	-	-
Pseudunio + *Cumberlandia*	BI	90	91	93	-	-	-
	ML	-	-	62	-	-	-
Margaritiferidae	BI	**100**	**100**	**100**	**100**	-	-
	ML	**100**	**100**	**100**	**100**	-	-
Unionidae	BI	**100**	**100**	**100**	**100**	-	-
	ML	**99**	**99**	**99**	**95**	-	-
Etheriidae + Mulleriidae + Iridinidae	BI	**100**	**100**	**100**	**100**	**100**	**100**
	ML	**100**	**100**	**100**	**91**	**98**	**97**
Hyriidae	BI	**100**	**100**	84	-	**100**	**100**
	ML	**93**	**93**	-	-	**82**	**91**

Graf & Cummings (2006) listed five morphological synapomorphies for Margaritiferidae, characters: 7 - gills irregular scattered interlamellar connections; 8 - gills not fused with mantle posterior; 12 - pedal elevator muscle scars inconspicuous; 13 - anus located dorsal edge of posterior adductor muscle; and 27 - mantle attachment scars (Table 2). However, only three historically recognized characters, i.e. characters 7, 13 and 27, are synapomorphies of the Margaritiferidae since all other characters can be found in other members of the Unionida, outside the Margaritiferidae. In this study, we identified a new synapomorphy for the Margaritiferidae, i.e. papillae on the external surface of the excurrent aperture. Also, two molecular characters are also synapomorphic, i.e. the F- and M- mitogenome gene orders (Lopes-Lima et al 2017b).

Inspection of the conchological features revealed a few similarities across all species (Table 6). Mantle attachment scars were found consistently in all analysed specimens and nacre colour was generally white with the only exceptions being the purple nacre of *M. falcata* and *M. laevis*, and the peach colour in the umbonal region of *G. laosensis* (Table 6). Interestingly, most of the inspected characters were distinct and consistent with the four clades retrieved with the phylogenetic analyses (i.e. *Gibbosula*, *Cumberlandia*, *Margaritifera*, and *Pseudunio*; Table 6). While thin shells are typical for *Cumberlandia*, thin to medium thick shells can be found in all species of *Margaritifera*. Except for *G. laosensis*, the remaining species belonging to *Pseudunio* and *Gibbosula* have ponderous, thick shells. All species within *Cumberlandia*, *Margaritifera,* and *Pseudunio* have shallow and open umbo cavities (e.g. Fig. 2). Conversely, all species of *Gibbosula* have deep, compressed umbo cavities (e.g. Fig. 2), except for *G. laosensis* (Table 6). Pseudocardinal teeth are also distinct among the clades (Fig. 2); while *Gibbosula* and *Pseudunio* species present large teeth (again except for *G. laosensis*), *Margaritifera* presents peg-like smaller teeth, and those in *Cumberlandia* are reduced (Fig. 2). The lateral teeth are consistently well-developed in most species across the clades, with a few exceptions (Table 6). However, the lateral teeth of species within *Pseudunio* and *Gibbosula* present vertical striations (except for *P. auricularius*), while this character is absent or visible only on the posterior end of laterals of *Cumberlandia* and *Margaritifera* species. Shell surface sculpture is also distinct across the genera (Table 6). Species within *Cumberlandia*, *Pseudunio* and *Margaritifera* are generally smooth, without any sculpture, the only exceptions being *M. hembeli* and *M. marrianae*, which present plications on the posterior slope and onto the posterior disk. A distinct pattern can be seen in *Gibbosula*, where all species, except *G. laosensis*, are strongly sculptured with pustules, plications or both (Table 6).

Table 6.
Analysed conchological characters of Margaritiferidae species. Superscripts: [1] W-shaped pustules on umbo and onto disk; [2] plications on posterior slope, posterior disk; [3] plications on the posterior slope, pustules on umbo and disk.

	Shell thickness	**Mantle attachment scars**	**Umbo pocket**	**Pseudocardinal teeth**	**Lateral teeth**
G. confragosa	thick	present	deep open	large	well-developed
G. crassa	thick	present	deep compressed	large	well-developed
G. laosensis	medium	present	shallow open	peg-like	Reduced
G. polysticta	thick	present	deep compressed	large	well-developed
G. rochechouartii	thick	present	deep compressed	large	well-developed
C. monodonta	thin	present	shallow open	reduced	reduced
M. dahurica	medium	present	shallow open	peg-like	reduced
M. falcata	thin-medium	present	shallow open	peg-like	well-developed
M. hembeli	medium	present	shallow open	peg-like	well-developed
M. laevis	medium	few	shallow open	peg-like	Reduced
M. margaritifera	thin-medium	present	shallow open	peg-like	well-developed
M. marrianae	thin-medium	present	shallow open	peg-like	well-developed
M. middendorffi	medium	present	shallow open	peg-like	well-developed
P. auricularius	thick	present	shallow open	large	well-developed
P. homsensis	thick	few	shallow open	large	well-developed
P. marocanus	thick	present	shallow open	large	well-developed

Table 6 (*cont*.)

	Lateral teeth sculpture	Umbo sculpture	Nacre colour	Ventral margin	Shell shape	Surface sculpture
G. confragosa	reduced	unknown	white	slight convex	oval	yes[1]
G. crassa	yes	unknown	white	slight convex	rectangular	yes[2]
G. laosensis	yes	unknown	white, peach umbo area	slight concave	elongate	no
G. polysticta	yes	unknown	white	convex	oval	yes[2]
G. rochechouartii	yes	unknown	white	straight convex	rectangular	yes[3]
C. monodonta	no	Concentric bars	white	concave	elongate	no
M. dahurica	no	unknown	white	straight	elongate	no
M. falcata	no	unknown	purple	straight slight concave	elongate	no
M. hembeli	posterior end	unknown	white	straight slight concave	elongate	yes[2]
M. laevis	posterior end	unknown	white	straight slight concave	elongate	no
M. margaritifera	no	Concentric bars	white	straight slight concave	elongate	no
M. marrianae	posterior end	Concentric almost double looped[1]	white	straight	elongate	yes[2]
M. middendorffi	posterior end	unknown	white	straight	elongate	no
P. auricularius	no	concentric bars	white	concave	elongate oval	no
P. homsensis	yes	unknown	white	straight concave	elongate oval	no
P. marocanus	yes	concentric bars	white	straight concave	elongate oval	no

Table 7.
Anatomical characters. *Not analysed for anatomy.

	Incurrent aperture	Excurrent aperture	Papillae	Anal position	Gill attachment	Gill structure	Labial Palp	Foot muscle pigmented	Diaphragm
G. confragosa*	-----	-----	-----	-----	-----	-----	-----	-----	-----
G. crassa	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
G. laosensis	arborescent	crenulated	-----	Posterior dorsal	anterior	Interrupted	falcate	yes	ridge
G. polysticta*	-----	-----	-----	-----	-----	-----	-----	-----	-----
G. rochechouartii	arborescent	crenulated	-----	posterior dorsal	unknown	interrupted	unk.	yes	ridge
C. monodonta	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
M. dahurica	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
M. falcata	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
M. hembeli	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
M. laevis	arborescent	crenulated	-----	Posterior dorsal	anterior	Interrupted	falcate	yes	ridge
M. margaritifera	arborescent	crenulated	no	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
M. marrianae	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
M. middendorffi	arborescent	crenulated	yes	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
P. auricularius	arborescent	crenulated	-----	Posterior dorsal	anterior	Interrupted	falcate	yes	ridge
P. homsensis	arborescent	crenulated	-----	Posterior dorsal	anterior	interrupted	falcate	yes	ridge
P. marocanus	arborescent	crenulated	yes	Posterior dorsal	anterior	Interrupted	falcate	yes	ridge

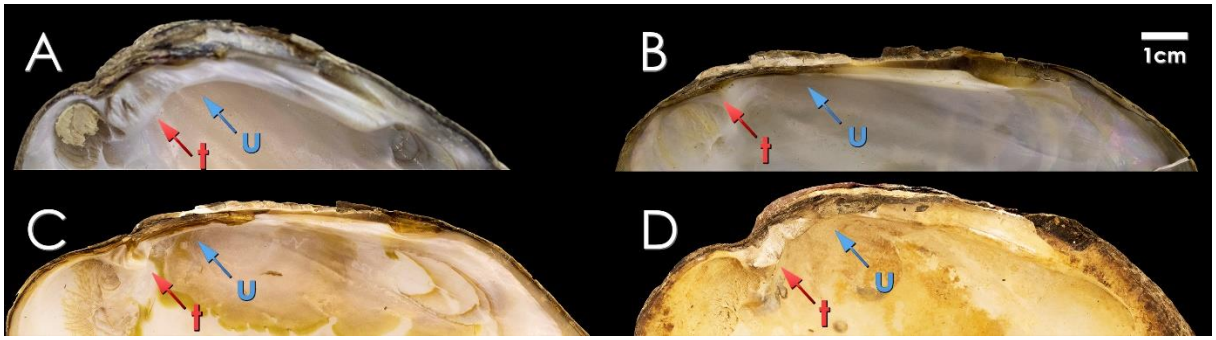

Figure 2 Hinge plate and umbo cavity of Margaritiferidae. A - *Gibbosula crassa* (NCSM 102194.2), B - *Cumberlandia monodonta* (NCSM 55359.18), C - *Margaritifera margaritifera*, (NCSM 5771.1) D - *Pseudunio auricularius* (NCSM 44514.2). t - pseudocardinal teeth, u - umbo cavities.

Shell shape is also distinct among the four clades: species within *Gibbosula* present a typically convex ventral margin and a variable shell shape; *Cumberlandia* have a concave ventral margin and elongated shape; *Margaritifera* shells are elongated and typically straight to slightly concave ventral margin; and finally *Pseudunio* shells are elongated-oval with a straight to concave ventral margin (Table 6). The umbo in most of the examined shells was eroded and therefore hindered a proper analysis of its sculpture. Nevertheless, concentric bars in the umbo were present in all species, where this feature was visible (Table 6). All the soft body anatomical traits were similar in all analysed species (Table 7).

The ecological and other biological characters analysed here also corroborate the existence of four genera (Table 4). The host fishes of *Margaritifera* species belong exclusively to the Salmonidae and the closely related Esocidae, while the hosts for *Pseudunio* and *Cumberlandia* do not belong to these fish families (Table 4). *Cumberlandia* uses two species of Hiodontidae, while members of three unrelated families of fish are found to be suitable for *P. auricularius* (Table 4). As for the other two species of *Pseudunio*, no hosts have yet been identified but no salmonid species occur sympatrically within their current known distribution (Table 4). The fish hosts for *Gibbosula* species are all unknown, although at least for the Southeast Asian taxa (*G. laosensis* and *G. crassa*) do certainly not include Salmonidae, since this family does not occur in this area (Table 4). The glochidia size of *P. auricularius* is much larger than those of *Margaritifera* and *Cumberlandia*. Since the glochidia of the other two *Pseudunio* and all *Gibbosula* species are undescribed, its utility for systematics still needs to be confirmed (Table 4). The habitat preferences are also distinct among the genera. While *Margaritifera* species prefer oligotrophic soft-water rivers and are more prevalent in headwaters, *Pseudunio* generally inhabits the middle to lower sections of moderate to hard-water mesotrophic rivers. *Cumberlandia* seems to occur in habitats like those of *Pseudunio* (Table 4). However, contrary to all the other genera it is mostly found in a very particular

microhabitat, i.e. under large flat rocks or in rock crevices (Table 4). *Gibbosula* seems to be much more plastic in its habitat preferences (Table 4) although the ecological features of most species need to be more thoroughly studied.

Origin and ancient radiations of the Margaritiferidae

The combined results of the biogeographic modelling (S-DIVA, DEC, and S-DEC approaches) based on the fossil-calibrated chronogram obtained from the relaxed molecular clock analyses returned a robust ancestral area reconstruction for the primary clades of the Margaritiferidae (Figs. 3 and 4, Supplementary Fig. 2, and Table 8). The model suggests that the Margaritiferidae Most Recent Common Ancestor (MRCA) was widespread across the eastern part of Laurasia (probability 55.0%). The S-DIVA, DEC, and S-DEC models support the same scenario (probability 53.3-58.3%). The origin of the crown group of the family was placed in the Jurassic (mean age 172 Ma, 95% HPD 168-178 Ma). Based on the combined biogeographic model, the Gibbosulinae MRCA most likely originated in East Asia (probability 78.6%), with a subsequent vicariance event separating the Southeast Asian species *G. laosensis* (probability 79.9%). The origin of the crown group of the subfamily is placed in the mid-Cretaceous (mean age ~103 Ma, 95% HPD 86-131 Ma).

The Margaritiferinae MRCA most likely evolved in the East Laurasia (East Asia + Mediterranean Region, probability 62.0%), with the crown group of the subfamily originating in the Late Jurassic (mean age ~151 Ma, 95% HPD 132-170 Ma). Among Margaritiferinae clades, the crown group of the *Cumberlandia* + *Pseudunio* clade most likely originated in the Early Cretaceous (mean age ~135 Ma, 95% HPD 129-146 Ma) within the Mediterranean region, with subsequent dispersal to eastern North America followed by a vicariance event (probability 45.0%). In contrast, S-DIVA model suggests a rather primary broad range of the MRCA across the Mediterranean Region and eastern North America followed by vicariance (probability 100%). The crown group of *Pseudunio* originated in the Mediterranean Region (probability 99.9%) in the Eocene (mean age 47 Ma, 95% HPD 35-66 Ma).

The crown group of *Margaritifera* is of Late Cretaceous origin (mean age 86 Ma, 95% HPD 51-131 Ma) and most likely evolved in East Asia (probability 52.4%). The sister species pair of *M. dahurica* and *M. margaritifera* diverged in the mid-Eocene (mean age 42 Ma, 95% HPD 34-57 Ma) via a dispersal event forming a continuous trans-Eurasian range of their MRCA followed by a vicariance event (probability 70.4%). The origin of the 'Pacific' clade, i.e. *M. falcata*, *M. laevis*, *M. middendorffi*, *M. hembeli*, and *M. marrianae*, is placed near the Palaeocene - Eocene boundary (mean age 57 Ma, 95% HPD 46-73 Ma). The diversification of this group was largely associated with several dispersal and vicariance events via the Beringian land bridge (probability 49.2-86.0%).

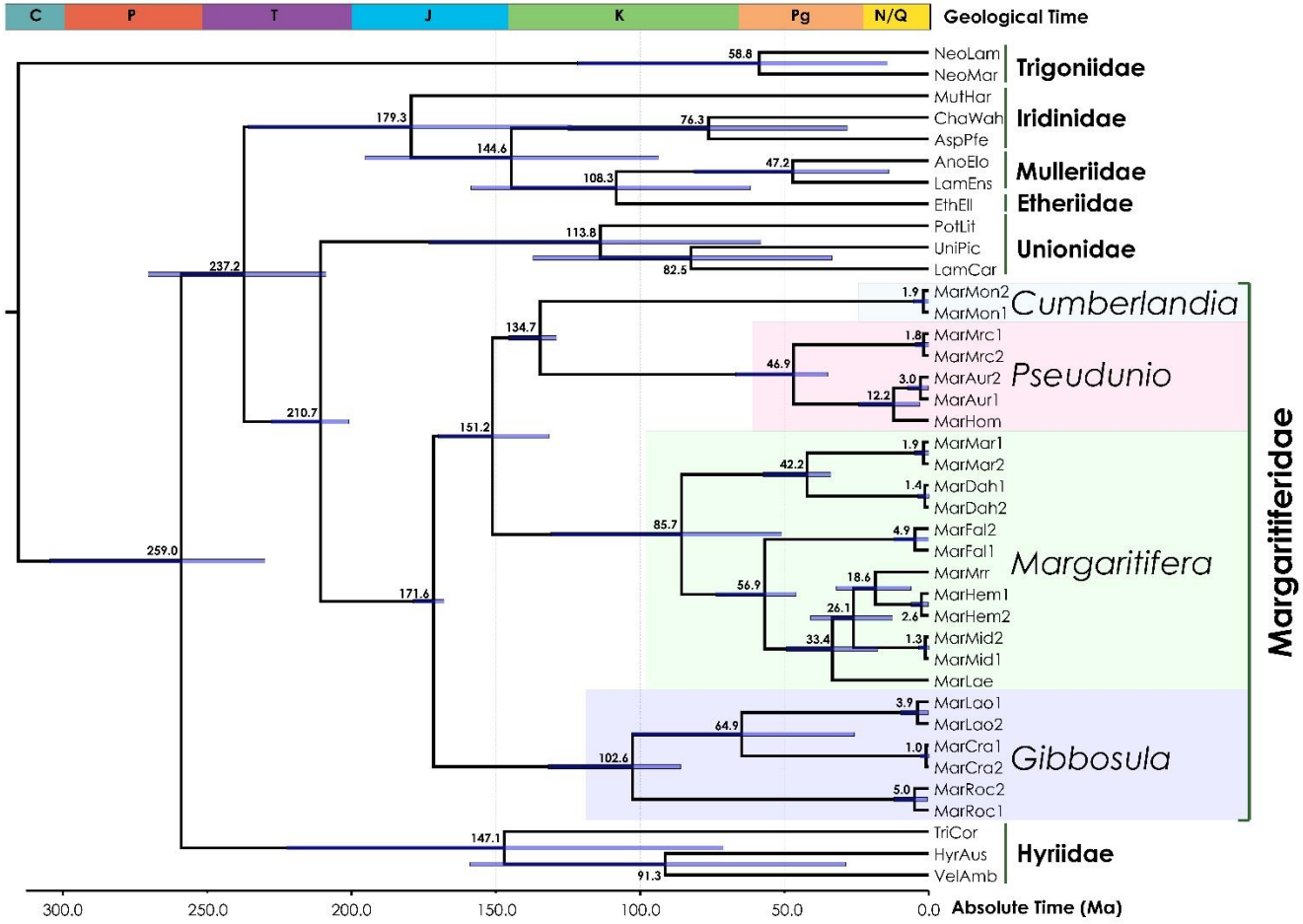

Figure 3 Fossil-calibrated ultrametric chronogram of the Margaritiferidae calculated under a lognormal relaxed clock model and a Yule process speciation implemented in BEAST 1.8.4 and obtained for the complete data set of mitochondrial and nuclear sequences (nine partitions: three codons of COI + 16S rRNA + 18S rDNA + 28S rDNA + three codons of H3). Bars indicate 95% confidence intervals of the estimated divergence times between lineages (Ma). Black numbers near nodes are mean ages (Ma). Stratigraphic chart according to the International Commission on Stratigraphy 2015.

Table 8.
The most probable ancestral areas of the primary clades within Margaritiferidae inferred from three different statistical modelling approaches. High support values (probability≥70%) are highlighted in bold. *Mediterranean + Eastern North America.

Clades	Ancestral areas	Biogeographic events	Probability of ancestral areas (%)			
			S-DIVA	DEC	S-DEC	Combined results
Margaritiferidae	E. Asia + Mediterranean	Dispersal	58.3	53.3	53.4	55.0
Gibbosulinae (*Gibbosula*)	E. Asia	Dispersal	**100.0**	67.6	68.2	**78.6**
G. laosensis - G. crassa	E. Asia + SE. Asia	Vicariance	**100.0**	**71.2**	68.6	**79.9**
Margaritiferinae (*Margaritifera* + *Pseudunio* + *Cumberlandia*)	E. Asia + Mediterranean	Vicariance	41.7	**73.4**	**71.0**	62.0
Margaritifera	E. Asia	Dispersal	65.0	49.1	43.1	52.4
M. dahurica - M. margaritifera	E. Asia + Europe	Dispersal + Vicariance	50.0	**81.4**	**79.9**	**70.4**
M. falcata - M. laevis (Pacific clade)	E. Asia + W. North America	Vicariance	**100.0**	**81.4**	**76.7**	**86.0**
M. laevis - M. middendorffi	E. Asia	Dispersal	**97.3**	63.2	67.3	49.2
M. middendorffi - M. hembeli	E. Asia + W. North America	Dispersal + Vicariance	33.3	66.0	63.8	54.4
M. hembeli - M. marrianae	W. North America + E. North America	Dispersal + Extinction	33.3	40.5	41.9	38.2
Pseudunio + *Cumberlandia*	Mediterranean	Dispersal + Vicariance	**100.0***	64.6	**70.5**	45.0
Pseudunio	Mediterranean	Intra-area radiation	**100.0**	**100.0**	**99.7**	**99.9**
P. auricularius - P. homsensis	Mediterranean	Intra-area radiation	**100.0**	**100.0**	**100.0**	**100.0**

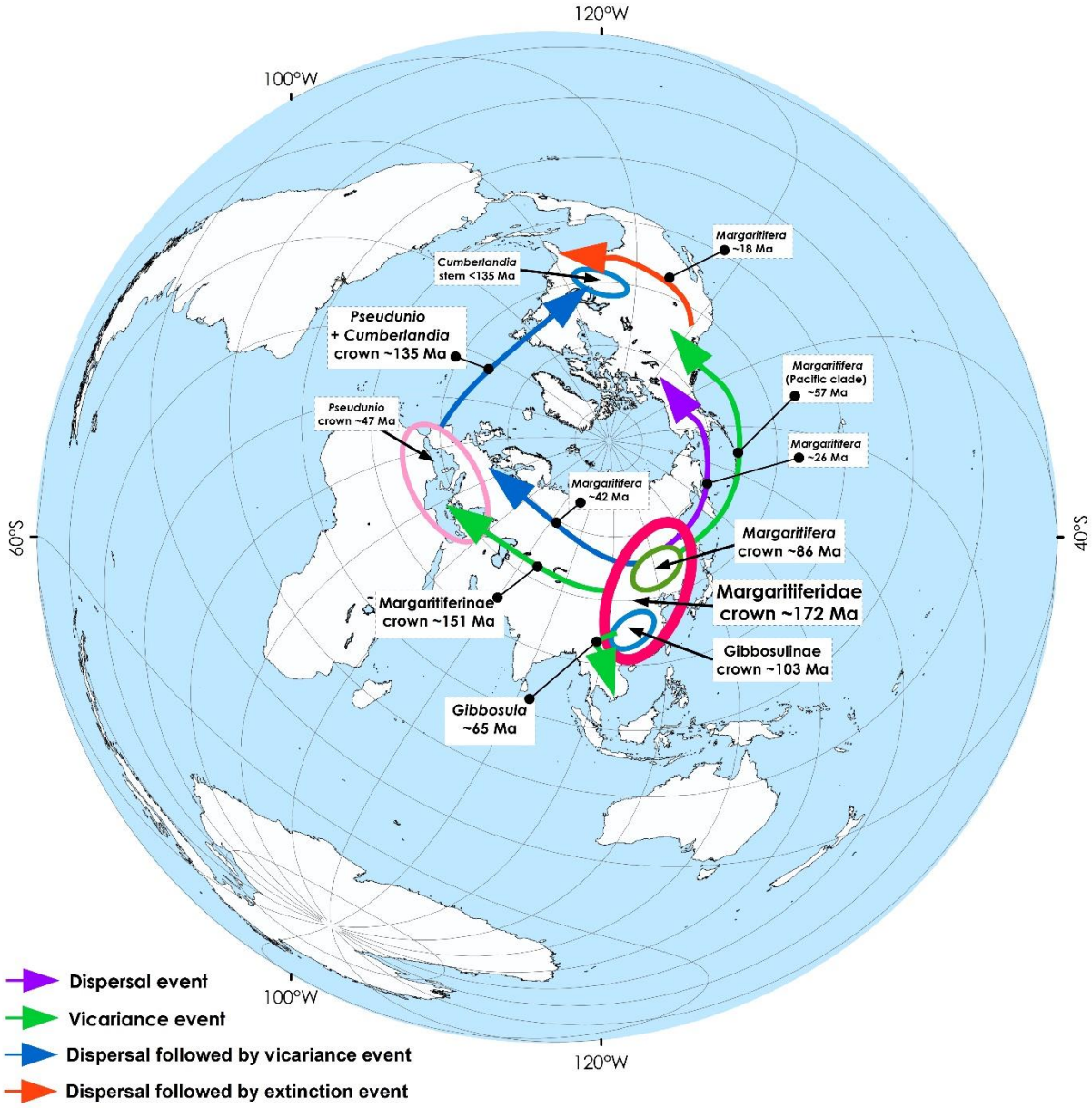

Figure 4 Simplified scheme of origin and expansion routes inferred across clades of the Margaritiferidae. The black numbers show the mean age of putative expansion events obtained from the multi-locus fossil-calibrated phylogenetic model (see Fig. 3 for details). Circles indicate the putative places of origin of the family and several clades. The map was created using ESRI ArcGIS 10 software (www.esri.com/arcgis); the topographic base of the map was created with ESRI Data and Maps.

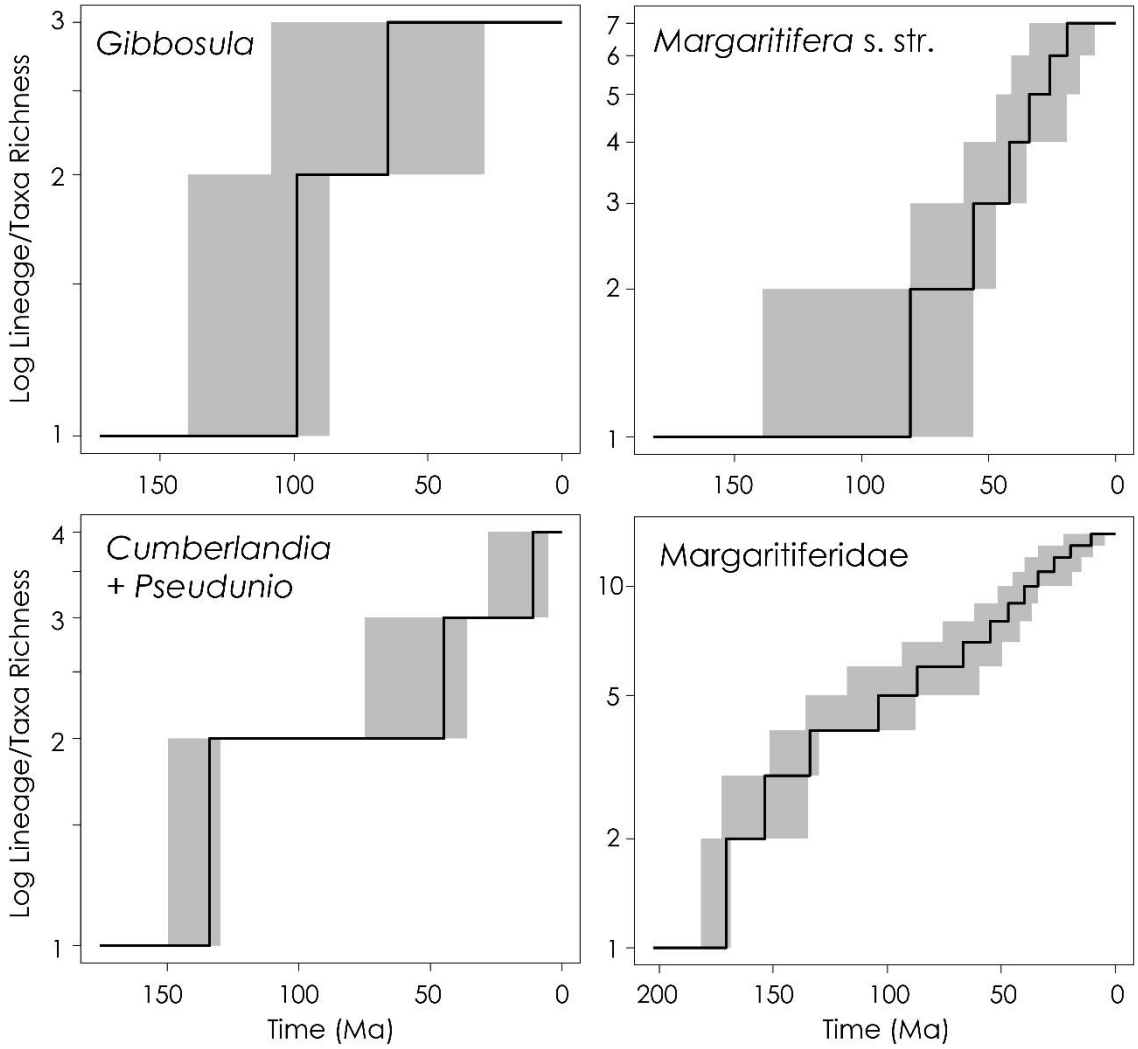

Figure 5 Semilogarithmic lineage-through-time (LTT) median plots of chronograms estimated from 108,004 post-burn-in Bayesian trees for the primary Margaritiferidae clades, including *Gibbosula*, *Cumberlandia* + *Pseudunio*, *Margaritifera*, and the entire family. The grey filling indicates 95% confidence intervals.

Diversification rates

The lineage-through-time modelling suggests extremely slow diversification rates in the Margaritiferidae (Fig. 5). The constant-rate test suggests that all clades diversified under the pure-birth (constant) model (Supplementary Table 5).

Discussion

Definition of the Margaritiferidae

Since the first definition of the Margaritiferidae by Ortmann, its supposed diagnostic characters have varied considerably (Table 2). Graf & Cummings (2006), based on a molecular (COI +

28S) and morphological phylogeny, revised margaritiferid synapomorphies noting that there was no previous consensus on characters diagnosing the family Margaritiferidae. These authors retained only five morphological synapomorphies, two conchological (characters 12 and 27, Table 2) and three anatomical (characters 7, 8, and 13, Table 2) characters. All other analysed characters were considered plesiomorphic (Graf & Cummings 2006). The main synapomorphies of the family were again re-evaluated by Araujo et al (2017) (Table 2). They rejected Graf & Cummings (2006) character 12 and considered character 27 as the only conchological synapomorphy for the Margaritiferidae. These authors retained anatomical characters 7, 8, and 13, but were not able to fully evaluate the anal position in all taxa (see Table 2). Other characters previously used to characterize Margaritiferidae were found in other genera of the Unionidae (Table 2). Finally, a recent mitogenomics study provided the F- and M- type gene-orders of the Margaritiferidae as two additional synapomorphic diagnostic characters (Lopes-Lima et al 2017b).

In the present study, 29 analysed characters were common to all margaritiferid species and therefore can be used to diagnose the family (Table 2). However, only six, i.e. characters 7, 13, and 27 (Table 2), the papillae on the external surface of the excurrent aperture, plus both mitogenome orders are synapomorphies of the Margaritiferidae. All the other characters can be found on other members of the Unionida and *Neotrigonia*, outside the Margaritiferidae.

Expansion of Margaritiferidae

Based on morphological and molecular evidence, the family Margaritiferidae is here expanded to 16 species and separated into two subfamilies (i.e. Margaritiferinae and Gibbosulinae) and four genera (i.e. *Pseudunio*, *Cumberlandia*, *Margaritifera*, and *Gibbosula*) (Fig. 1; Table 9; Supplementary Table 6).

Until recently, two different species of *Gibbosula* used to be recognized. Firstly, the type species *G. crassa* was described by Wood (1815) from a specimen collected in an unknown location in China. Since then, only a few specimens *of G. crassa* or its synonym *Unio mansuyi* have been collected, almost a hundred years ago, in the Bang River, Pearl/Zhu River basin, either in China or Vietnam. During recent surveys, the species was rediscovered but seems to be quite rare and restricted to the middle stretches of Bang River in Cao Bang Province, Vietnam. The second previously recognized species within *Gibbosula* is *G. confragosa*, described by Frierson from a single specimen, collected in an uncertain location in north China. Although Prozorova et al (2005) stated that this species was present in the Yangtze and other Eastern Chinese basins, there is no current evidence of its occurrence in the Yangtze basin.

Table 9
Margaritiferidae systematics and taxonomy.

Margaritiferidae Henderson, 1929

Gibbosulinae Bogan, Bolotov, Froufe, Lopes-Lima, *nom. nov.*

Gibbosula Simpson, 1900*,*
Gibbosula confragosa Frierson, 1928
Gibbosula crassa (Wood, 1815)
Gibbosula laosensis (Lea, 1863), *comb. nov.*
Gibbosula polysticta (Heude, 1877), *comb. nov.*
Gibbosula rochechouartii (Heude, 1875), *comb. nov.*

,

Margaritiferinae Henderson, 1929

***Cumberlandi*a** Ortmann, 1912
Cumberlandia monodonta (Say, 1829)

Margaritifera Schumacher, 1816
Margaritifera dahurica (Middendorff, 1850)
Margaritifera falcata (Gould, 1850)
Margaritifera hembeli (Conrad, 1838)
Margaritifera laevis (Haas,1910)
Margaritifera margaritifera (Linnaeus, 1758)
Margaritifera marrianae Johnson, 1983
Margaritifera middendorffi (Rosen, 1926)

Pseudunio Haas, 1910
Pseudunio auricularius (Spengler, 1793)
Pseudunio homsensis (Lea, 1864)
Pseudunio marocanus (Pallary, 1928)

Since *G. confragosa* original description, only one specimen has been collected and described, i.e. a specimen from Lake Baiyangdian, Hai River basin, Hebei province, northern China, previously incorrectly labelled as *U. microstictus* (He & Zhuang 2013). Besides the shell surface sculpture differences, the disjunct distribution of *G. confragosa* suggests a distinct specific rank. The newly found specimens and shells of *G. crassa* from Vietnam, here analysed in detail, feature the characteristics diagnostic and synapomorphies of the Margaritiferidae (Tables 6 and 7). Additionally, the F-type whole mitogenome sequence of one of the specimens collected revealed the typical gene order of the Margaritiferidae (Supplementary Fig. 1), which is unique to this family (Lopes-Lima et al 2017b). The phylogenetic analyses also confirm the inclusion of *G. crassa* in the Margaritiferidae family, forming a well-supported clade (BI only) with *G. laosensis* and *G. rochechouartii*. The shells of *G. confragosa* and *G. polysticta* present mantle attachment scars exclusive to the Margaritiferidae and were therefore included in the Margaritiferidae (Fig. 1; Table 6) and assigned to *Gibbosula* due to similarities in shell characteristics with the type species, *G. crassa* (Table 6). An additional *Gibbosula* species was

recently described, *Gibbosula nanningensis* (Qian et al 2015). No specimens of this species were available for evaluation, but based on the description, i.e. the absence of mantle attachment scars and its distinct morphology, we reject its assignment to *Gibbosula* and therefore to the Margaritiferidae. A detailed systematics description of the species within *Gibbosula* is presented in Supplementary Appendix 1. Most of the earlier works on the systematics of margaritiferid genera have failed to retrieve monophyletic clades based on morphological characters alone (Huff et al 2004). More recently, authors showed that previous generic assignments were inconsistent with the molecular phylogenetic patterns (Huff et al 2004; Bolotov et al 2016; Araujo et al 2017). Whilst all these studies recognized *Margaritifera* as the single genus within the Margaritiferidae, the rationale for this generic assignment is not always clear. Bolotov et al (2016) suggested that the clades found should be assigned to distinct subgenera but maintained *Margaritifera* as a monotypic genus due to the morphological similarity and moderate level of genetic divergence among the clades.

In the present study, four well-supported clades (mainly in the BI analyses) were consistently retrieved using the most comprehensive Margaritiferidae data set analysed to date (Fig. 1, Table 5). The divergence of these clades, corresponding to the subgenera identified by Bolotov et al (2016), is older (from late Jurassic to early Cretaceous) than previously believed due to the inclusion of new species and improvements in the fossil calibration (see details below). The present study further revealed a set of consistent morphological, biological and ecological features characteristic to each of the clades. Based on these results, each clade was assigned to a separate generic rank (Fig. 1). The genus *Gibbosula* includes the species *G. crassa*, *G. confragosa*, *G. laosensis*, *G. polysticta*, and *G. rochechouartii* (Fig. 1; Supplementary Table 6). The morphological and ecological features of *Gibbosula* are consistently more distinct from the other three genera (Tables 6 and 7). This agrees with the molecular phylogeny developed here, which presents two main clades, one with all *Gibbosula* species and another including (*Margaritifera* + (*Cumberlandia* + *Pseudunio*) (Fig. 1; Table 5). Due to their old divergence (late Jurassic, see below) and clear morphological differences, a subfamily rank was assigned to each of these two clades, i.e. Margaritiferinae and Gibbosulinae Bogan, Bolotov, Froufe, Lopes-Lima, new subfamily. Distribution of the two Margaritiferidae subfamilies is mutually exclusive, with the Gibbosulinae being restricted to East and Southeast Asia, while the Margaritiferinae are widespread throughout the rest of the Holarctic (Fig. 6).

Figure 6 Distribution map of the Margaritiferidae.

Systematics

Margaritiferidae Henderson, 1929 (Ortmann, 1910)

Type genus: *Margaritifera* Schumacher, 1816

Type species: *Mya margaritifera* Linnaeus, 1758

Type Locality: "Habitat in totius orbis arctici cataractis" [Arctic habitat in the entire world cataracts] (Linnaeus, 1758).

Comments: This family was split from the Unionidae and four more species were moved from the Unionidae, refining the definition of the family and the variation in shell shape, anatomy and geographic distribution.

Diagnosis: Shell shape varies from elongated to rectangular or oval, shell thickness varies from thin to very thick. The posterior ridge of the shell varies from low and rounded to well-developed and posterior slope with or without plications, maximum shell length about 200 mm. Umbo sculpture presents angular un-joined chevron-like hooks but Zieritz et al (2015) have referred to this sculpture as double looped. Periostracum colour varies from a dark green to typically black. Lateral teeth vary from vestigial to well-defined with vertical sculptures on all or the posterior portion of the teeth. Pseudocardinal teeth vary from peg-like in both valves to thick and massive. Umbo pocket varies from shallow and open to deep and compressed (Fig. 2). Lateral mantle attachment scars are present in varying numbers inside of the pallial line. Nacre varies from white to purple. Mantle free around edges of the animal. Apertures open without any mantle fusions to separate the incurrent, excurrent or supra-anal apertures. Branchial and supra-branchial areas not separated posteriorly by gills, but by a diaphragm

comprised of a ridge of mantle tissue. Incurrent aperture with arborescent papillae and in at least one species has simple papillae on the external side of incurrent aperture mantle surface typically along the length of the aperture. Excurrent aperture smooth or crenulated, lacking papillae, external side of excurrent aperture mantle surface typically has small papillae along the length of the aperture. Gills attached to the visceral mass only anteriorly. Labial palps falcate in outline. Interlamellar gill connections are “irregularly scattered or forming irregular oblique row, or incomplete septa which run obliquely to the direction of the gill filaments” (Heard & Guckert 1970). Gills lack water tubes. Marsupium occupies all four gills. Muscular section of the food pigmented either dark red or black. Anus is located on the posterior dorsal margin of the posterior adductor muscle. This family is a short term brooder or tachytictic. Most species are dioecious with only a few listed as hermaphroditic or having hermaphroditic populations. Fish hosts, when known, are Salmonidae, Esocidae, Acipenseridae, Blenniidae, Gasterosteidae, and Hiodontidae, with each margaritiferid genus being restricted to a single or few host fish families. Female and male mitochondrial genome orders are unique for Margaritiferidae and different from Unionidae.

Distribution: The family is found in North America north of Mexico, Western and Northern Europe, western North Africa in Morocco, western Middle-East in Syria, Turkey and Lebanon, Southeast Asia and north to eastern Russia and Japan (Fig. 6).

Subfamily Margaritiferinae Henderson, 1929

Type genus: *Margaritifera* Schumacher, 1816

Type species: *Mya margaritifera* Linnaeus, 1758

Type Locality: “Habitat in totius orbis arctici cataractis” [Arctic habitat in the entire world cataracts] (Linnaeus, 1758).

Comments: This subfamily contains three genera: *Margaritifera*, *Cumberlandia* and *Pseudunio*. Species of *Cumberlandia* and *Margaritifera* have thin to medium-thick, elongated shells, while *Pseudunio* has thick shells and well-developed teeth. All have a shallow open umbo cavity. The three genera use different fish families as hosts.

Diagnosis: Shell shape elongate, with a concave or straight ventral margin. Shell thin to moderately thick or thick, posterior ridge rounded. Shell surface smooth or with plications on the posterior slope and the posterior edge of the shell disk. Umbo sculpture is listed as concentric bars but usually eroded. Umbo pocket shallow and open (Fig. 2). Nacre colour usually white but may also be purple. Lateral teeth usually well-developed but may be reduced; some species have vertical sculpture. Pseudocardinal teeth are peg-like to large (Fig. 2). Fish hosts when known are Salmonidae, Esocidae, Acipenseridae, Blenniidae, Gasterosteidae, and Hiodontidae, with host fish families being mutually exclusive to each margaritiferine genus.

Distribution: This subfamily is Holarctic in distribution including North America, Europe, Morocco, Turkey, Syria and Lebanon, China, Japan, and eastern Russia (Fig. 6).

***Cumberlandia* Ortmann, 1912**

Type species: *Unio monodonta* Say, 1829

Type locality: "at the falls of the Ohio, on the rocky flats which are exposed in a low state of the water" (Say, 1829).

Type specimen: The type specimen of *Unio monodonta* appears to be lost (Watters et al 2009).

Comments: This large, arcuate shell is distinctive in shape, being very thin shelled and living in fast water usually under large flat rocks. It has been recognized as different from the typical *Margaritifera* and based on the gill structure, Heard & Guckert (1971) erected a subfamily for this genus.

Diagnosis: Shell shape elongate usually with a convex ventral margin, shell is thin, shell surface is smooth except for growth arrest line, posterior ridge rounded. Lateral teeth reduced to a slight rounded ridge. Pseudocardinal teeth are reduced (Fig. 2). Umbo cavity open and shallow (Fig. 2). Interlamellar gill connections were described as "scattered and in interrupted rows but developed as continuous septa which run obliquely forward" (Heard & Guckert 1970). Fish hosts are Hiodontidae.

Distribution: "*Cumberlandia monodonta* occurs in the Mississippi Basin from southern Minnesota and Wisconsin south to the Ouachita River drainage in south-central Arkansas, and in the Ohio River drainage from Ohio and West Virginia downstream to the mouth of the Ohio River, including some tributaries" such as the Tennessee and Cumberland River drainages (Williams et al 2008) (Fig. 6).

***Margaritifera* Schumacher, 1816**

Type species: *Mya margaritifera* Linnaeus, 1758

Type locality: "Habitat in totius orbis arctici cataractis" [Arctic habitat in the entire world cataracts]. (Linnaeus, 1758).

Type specimens: There exists a specimen in the Linnean Society of London, Box No. LSL 22, Dance label image Ref. G-M 00101251. Dance was uncertain this was a Linnean specimen, so the listing by Graf & Cummings (2018) may be invalid. There are two additional lots in the Linnean Collection, Uppsala University, Museum of Evolution, Zoology Section (Uppsala University 1999) which are potentially part of the syntype series (UUZM 2018).

Comments: *Margaritifera* is the most widespread genus within the family with a Pacific, Atlantic and central Eurasian distribution. Since Bolotov et al (2016), the Japanese endemic

M. togakushiensis (Kondo & Kobayashi 2005) has been considered a synonym of *M. middendorffi* based on morphology and phylogenetic data.

Diagnosis: Shell shape elongate, usually with concave ventral margin. Shell is thin to moderately thick. Posterior ridge rounded. Shell surface smooth except for growth arrest lines. Lateral teeth are distinct and peg-like. Pseudocardinal teeth vary from well-developed to reduced (Fig. 2). Umbo cavity shallow and open (Fig. 2). Nacre colour typically white but purple in *M. falcata* and in some *M. laevis* individuals. Host fish are species of the Salmonidae or Esocidae for two species restricted to the Gulf Coast of the United States. (Table 4).

Distribution: The genus *Margaritifera* is widespread across North America, Western Europe, China, Japan and eastern Russia (Fig. 6).

***Pseudunio* Haas, 1910**

Type species: *Unio sinuata* Lamarck, 1819 = *Unio auricularius* Spengler, 1793

Type locality: “Habite dans le Rhin, la Loire, et les autres grandes rivières du continent européen tempéré et austral” [Lives in the Rhine, the Loire and other great rivers of continental Europe] (Lamarck, 1819).

Type specimen: The Mollusc Collection, Muséum d’Histoire Naturelle, Genève contains one valid syntype of *Unio sinuata* Lamarck, 1819 MHNG-MOLL-50572 and 3 possible syntypes MHNG-MOLL-50573. Lamarck had only three specimens in total so at least one of these specimens is not a valid type. Dr. Tardy noted the specimens in lot 50573 measured 104 to 117mm while Lamarck listed a range of sizes from 140 to 145mm (Tardy, Pers. Comm.). The type of *Unio auricularius* was first listed and figured by Lister (1685) and is pre-Linnean. Spengler (1793) validated this species. There is a lectotype in lot ZMUC Biv-315 (Knudsen et al 2003). [Zoological Museum, University of Copenhagen, Copenhagen, Denmark].

Comments: Placement of the three species here assigned to *Pseudunio* have often been assigned to *Margaritifera*. However, in the phylogeny presented herein, they form a separate clade from *Margaritifera*, using a different suite of host fish families.

Diagnosis: Shell shape elongate oval. Shells thick. Posterior ridge rounded. Umbo sculpture is concentric bars. Posterior slope smooth. Shell surface is smooth. Lateral teeth are well developed, and most have vertical striations. Pseudocardinal teeth are large and well developed (Fig. 2). Umbo cavity open and shallow (Fig. 2). Fish hosts include species of the Acipenseridae, Blenniidae, and Gasterosteidae (Table 4).

Distribution: Species assigned to *Pseudunio* presently occur in rivers in northern Morocco, the Iberian Peninsula, France, southern Turkey, Syria, Lebanon, and formerly part of England, Italy, Germany and the Netherlands (Fig. 6).

Subfamily Gibbosulinae Bogan, Bolotov, Froufe and Lopes-Lima, new subfamily

Type genus: *Gibbosula* Simpson, 1900

Type species: *Mya crassa* Wood, 1815

Type locality: unknown (Wood, 1815); but listed as China, freshwater (Wood, 1825)

Comments: All the taxa included in this subfamily clade except for *G. laosensis* were historically included in the Unionidae. The only previous reference recognizing that *Gibbosula* belonged in the Margaritiferidae was by Morrison (1975). Transferring these four taxa from the Unionidae to the Margaritiferidae has changed our understanding of the range in morphological characteristics (including shell shape and anatomy) within this family.

Diagnosis: Shell shape ranges from elongate to rectangular or oval. Shell moderately thick to thick. Posterior ridge rounded to rather sharp. Shell surface is smooth with growth arrest rings or with the posterior slope marked with heavy plications and the disk of the shell covered with pustules or w-shaped nodules. Umbo sculpture is unknown. Lateral teeth well developed with vertical sculpture. Pseudocardinal teeth well developed and large (Fig. 2). Umbo pocket deep and compressed (Fig. 2) and one species with the pocket shallow and open. Nacre colour is white to some with peach colour. Fish hosts for this subfamily are unknown (Table 6).

Distribution: Species assigned to *Gibbosula* occur or used to occur in the upper Mekong River basin in Thailand, Laos, Vietnam, the Bang River in the Pearl River basin of Vietnam, the middle Sittaung River basin in Myanmar, the Yangtze River basin of southern China and one species from North China (Fig. 6).

***Gibbosula* Simpson, 1900**

Type species: *Mya crassa* Wood, 1815

Type locality: unknown (Wood, 1815); but listed as China, freshwater (Wood, 1825:12)

Type specimens: *Mya crassa* types are unknown; *Unio* (*Quadrula*) *mansuyi* Dautzenberg & Fischer 1908, a junior synonym, lectotype MNHN-MP-0136 here designated.

Comments: *Gibbosula* now contains five species, is restricted to Southeast Asia and northeast China. *Margaritanopsis laosensis* is included in *Gibbosula*, but conchologically resembles *Margaritifera* and *Cumberlandia* with a thin, elongate smooth shell rather than the thick rectangular or oval sculptured shells of the other species assigned to this genus. As *Gibbosula nanningensis* Qian, Fang & He 2015, does not conform to the diagnosis of *Gibbosula* and has simple papillae and not arborescent papillae in the incurrent aperture, it is here transferred to the genus *Lamprotula*, Unionidae.

Diagnosis: Shell shape varies from rectangular, oval to elongate in *G. laosensis*. Ventral margin varies from concave in *G. laosensis* to rounded or convex. Shell thickness ranges from medium-thick in *G. laosensis* to thick. Posterior ridge varies from rounded especially in *G. laosensis* to rather sharp. Umbo sculpture is unknown. Posterior slope has plications but is

smooth in *G. laosensis*. Shell surface is smooth, with plications or covered with pustules of various shapes. Lateral teeth are typically well developed except for the reduced teeth in *G. laosensis* and have vertical striations. Pseudocardinal teeth are usually large and well developed (Fig. 2), except in *G. laosensis* where they are peg-like. Umbo cavity deep and compressed (Fig. 2) or open and shallow as in *G. laosensis*. Nacre colour is typically white. Fish hosts are unknown (Table 4).

Distribution: Species assigned to *Gibbosula* occur in rivers of northern Thailand, Laos, central Myanmar, western Vietnam, northern Vietnam in the headwaters of Pearl River system, tributaries of the Yangtze River basin in southern China, and north China (Fig. 6).

Origin and diversification of the Margaritiferidae

In this study, we provide an updated fossil-calibrated phylogeny of the Margaritiferidae, which includes almost all known members of the family, except for *G. confragosa* and *G. polysticta*. These new results suggest that East Asia was the most likely place of origin of the Margaritiferidae. Although the statistical biogeographic models assume that the crown group of the family was widely distributed across the East Laurasia (East Asia + Mediterranean), the fossil evidence shows an East Asian origin for both the stem and the crown group (e.g. Chen 1984; Jingshan et al 1993; Ma 1994, 1996; Jiang et al 2005; Pan and Sha 2009; Fang et al 2009; Yao et al 2011), i.e. the region of the Yangtze Plate and the adjoining complex of small terraces that formed the present Tibetan Plateau (Van Damme et al 2015). Additionally, †*Shifangella margaritiferiformis* Liu & Luo 1981 from the Late Triassic deposits of China (Fang et al 2009) is here proposed as a fossil member of the crown group of Margaritiferidae + Unionidae, most likely representing a separate ancestral family (Supplementary Tables 3 and 4). This agrees with Graf et al (2015) and Skawina & Dzik (2011), who suggested that pre-Jurassic freshwater bivalves may represent the stem-groups of modern unionoid clades. Bolotov et al (2017a) showed that the Unionidae most likely originated in East and Southeast Asia, which is consistent with the hypothesis of an Asian origin for both families.

Concerning the combined results of our fossil-calibrated and biogeographic modelling, we suggest that the Margaritiferidae family originated in East Asia (Figs. 3 and 4) in the mid-Jurassic, most likely simultaneously with the Unionidae (Bolotov et al 2017a). We advance that †*Palaeomargaritifera guangyuanensis* Ma, 1984 comb. res. from the Middle Jurassic deposits of Sichuan is the earliest known fossil member of the family (Supplementary Tables 3 and 4). This dating is not consistent with the three earlier fossil-calibrated models (Bolotov et al 2016; Araujo et al 2017; Huang et al 2017). Bolotov et al (2016) placed the origin of Margaritiferidae in the mid-Cretaceous but did not use any fossil calibrations for the deep nodes, which led to a possible underestimation of the family age. In contrast, Araujo et al (2017) suggested that the family originated in the Late Triassic based on the age of †*Shifangella*, which is the most

probable MRCA of Margaritiferidae and Unionidae (Supplementary Tables 3 and 4). Huang et al (2017) assigned †*Shifangella* as a stem calibration for the Margaritiferidae and placed the origin of the family crown group in the Late Cretaceous that is close to the dating of Bolotov et al (2016).

The divergence between Gibbosulinae and Margaritiferinae in the Late Jurassic represented the earliest split within the Margaritiferidae. The Gibbosulinae, a local clade of East Asian origin, diversified during the Late Cretaceous possibly via connections between the paleo-river systems of East and Southeast Asia. We suggest that †*Gibbosula tibetica* (Gu, 1976) comb. nov. from the Late Cretaceous deposits of the Tibetan Plateau could be considered the earliest known fossil member of the Gibbosulinae (Supplementary Tables 3 and 4). Whilst Bolotov et al (2016) hypothesized that *G. laosensis* clustered with *C. monodonta*, this was not confirmed in our phylogeny. This discrepancy can be explained by the absence of other members of the Gibbosulinae in the reconstruction by Bolotov et al (2016). The external resemblance between *G. laosensis* and *C. monodonta* that was a subject of long-term discussion (Walker 1910; Smith 2001; Bolotov et al 2016) is surely a result of morphological convergence. Interestingly, both clades (Gibbosulinae and Pseudunio + Cumberlandia) include species with narrow, elongated shells (*G. laosensis* and *C. monodonta*) as well as broad, rounded shells (*G. crassa*, *G. rochechouartii*, *P. homsensis*).

The Margaritiferinae MRCA had a continuous range from East Asia to the Mediterranean Region in the Late Jurassic, which was most likely facilitated by host fish dispersal within a continuous paleo-river system or along the Tethys coastal line (Hou and Li 2017). The earliest history of this clade is well documented via fossil records from Jurassic deposits of North Africa and Europe (Delvene et al 2013, 2016; Van Damme et al 2015). † "*Margaritifera*" *crosthwaitei* (Newton, 1909) from the Late Jurassic deposits of Egypt and †*Asturianaia soudanensis* (Van Damme & Bogan, 2015) comb. nov. from the Middle to Late Jurassic deposits of Niger are the earliest fossil members from North Africa that could be assigned to this clade (Van Damme et al 2015). Fossils identified as "*Margaritifera*" cf. *valdensis* (Mantell, 1844) are known from the Late Jurassic deposits of Spain (Delvene et al 2013, 2016). Three additional Late Jurassic margaritiferid species were recently described from Spain: †*Asturianaia colunghensis* Delvene, Munt, Piñuela & García-Ramos, 2016, †*A. lastrensis* Delvene, Munt, Piñuela & García-Ramos, 2016 and †"*Margaritifera*" *lagriega* Delvene, Munt, Piñuela & García-Ramos, 2016 (Delvene et al 2016).

The MRCA of *Pseudunio* + *Cumberlandia* clade most likely originated in the Mediterranean Region and dispersed to eastern North America with a subsequent vicariant event in the Early Cretaceous. †*Paraheudeana idubedae* (Palacios & Sánchez, 1885) from the Early Cretaceous deposits of Spain appears to be the earliest known member of the crown group of this clade (Supplementary Tables 3 and 4). The evolutionary history of *Pseudunio*

was associated with the intra-Mediterranean radiation from the mid-Eocene to mid-Miocene. Our results support the assumption of Bolotov et al (2016) that the split between *P. auricularius* and *P. marocanus* was well before the Messinian Salinity Crisis (MSC). Additionally, the new model indicates that the split between *P. auricularius* and *P. homsensis* most likely preceded this paleogeographic event. In contrast, the divergence between *Unio* species in Morocco and Iberia was coincident with the MSC (Froufe et al 2016). The earliest fossils resembling the extant *Cumberlandia* are known from the Early Cretaceous deposits in North Africa: †*C. rhazensis* (Mongin, 1968) comb. nov. and †*C. saharica* (Mongin, 1968) comb. nov. (Van Damme et al 2015).

Margaritifera is the most widespread and diverse group of recent margaritiferids. This clade most likely originated in East Asia in the Late Cretaceous. The earliest fossils that may belong to this clade are known from the mid-Cretaceous deposits of Mongolia: †*Margaritifera elongata* (Martinson, 1982) comb. nov., †*M. sainshandensis* (Martinson, 1982) comb. nov. and †*M. glabra* (Kolesnikov, 1956) comb. nov. (Supplementary Table 3). However, the first two species together with nine additional fossil taxa from Mongolia were considered synonyms of †*Unio longus* (Zhu, 1976) from China (Sha et al 2006). A detailed discussion of the fossil taxa taxonomy is beyond the scope of the present investigation, but it should be mentioned that Sha et al (2006) provided their revision without studies of the type series of the synonymized species. Our reconstruction of the diversification patterns within this clade is largely congruent with the multiple trans-Beringian exchange model developed by Bolotov et al (2015, 2016) and is supported by numerous fossil records (Supplementary Table 3). An expanded sampling of species from the 'Pacific' clade (*M. falcata*, *M. laevis*, *M. middendorffi*, *M. hembeli*, and *M. marrianae*) indicates the possibility of an extinction event that closes the gap between East Asian *M. middendorffi* and its relatives from southeastern North America, i.e. *M. hembeli* and *M. marrianae*. Previously, Bolotov et al (2016) suggested that an additional *Margaritifera* species could be within this gap following the hypothesis of Taylor (1988) regarding vicariate forms of Margaritiferidae on both sides of the Pacific. However, Taylor's unnamed taxon is a morphological form of *M. falcata*, which differs by nacre colour (white with salmon spots) but is not genetically different from the typical violet-nacre form (our unpubl. data). The new fossil-calibrated model also supports the hypothesis that the Mekong and Yangtze unionoid faunas must have developed as independent radiations during the entire Cenozoic (Schneider et al 2013; Bolotov et al 2017a,b) because *G. laosensis* (Mekong River basin) and *G. crassa* (Pearl River basin) split ~65 Ma ago, and the *G. laosensis* + *G. crassa* subclade diverged from *G. rochechouartii* (Yangtze) ~103 Ma ago. The two largest paleo-Mekong radiations in the Unionidae most likely originated in the Early Cenozoic (mean age=51-55 Ma) or even pre-Cenozoic (mean age=65-71 Ma) (Bolotov et al 2017a,b). These results are following the concept of long-lived (ancient) rivers, suggesting that several large rivers on Earth may have

existed for long-term periods comparable with geological epochs (Bolotov et al 2017a). The present results highlight that the placement of several Jurassic and Early Cretaceous margaritiferid species within the genus *Margaritifera* (e.g. Delvene et al 2013, 2016; Van Damme et al 2015) needs to be revised because these taxa most likely represent ancestral fossil lineages that are not directly associated with the crown group of the latter genus despite their morphological similarity. The description of two fossil species from the same deposit based on small conchological differences, a common procedure in systematic palaeontology (e.g. Delvene et al 2016), most likely leads to the overestimation of the actual diversity of fossil taxa, e.g. Margaritiferidae, because the sympatric occurrence of several closely related species is an unusual phenomenon. The co-occurrence of *M. laevis* and *M. middendorffi* in several rivers of Japan, South Kuriles and Sakhalin Island (Bolotov et al 2015, 2016; Araujo et al 2017) is the only example of such a secondary sympatry known to date, whereas distribution ranges of the other species reflect a drainage-dependent allopatric speciation model without clear secondary contact zones. This evolutionary pattern suggests a limited number of ancestral fossil lineages not only by the single confirmed extinction event but also by the slow substitution and diversification rates within the family. Modelling results suggest delayed diversification rates in the Margaritiferidae (Fig. 5 and Supplementary Table 5) that are consistent with findings for the Indo-Chinese Unionidae, which also reveal slow diversification rates (Bolotov et al 2017a). Indeed, the rates in margaritiferids are ~2.5 times slower compared with the Unionidae (Bolotov et al 2016). These results may be associated with slower rates of molecular evolution in the Margaritiferidae, which support the hypothesis of a possible link between delayed diversification and slow molecular evolution in freshwater mussels (Bolotov et al 2017a), although this enigmatic pattern requires further investigation.

Conclusions

The current study supports the increase of extant margaritiferid species to 16 and suggests their division into two subfamilies and four genera. Since a better understanding of phylogenetic diversity is central for determining conservation priorities (Lopes-Lima et al 2017c, 2018), the results reported here may be important in the definition of future management strategies devoted to the conservation of margaritiferid species. The inclusion of *G. crassa*, *G. polysticta*, *G. rochechouartii*, and *G. confragosa* in the Margaritiferidae, confirms the family as the most threatened among unionoids (IUCN 2018). The first three mentioned species have a threatened status (IUCN 2018), while *G. confragosa* has never been evaluated (IUCN 2018). All four “new” margaritiferids seem to have small distribution ranges and are affected by multiple impacts (IUCN 2018). Further studies on the Margaritiferidae should include basic ecological and physiological research, collecting data on distribution, abundance,

habitat preferences, host fish identification, and reproductive cycles, as well as a phylogenomic approach to complement the current phylogenetic evaluation. Finally, a complete revision of numerous fossil margaritiferid taxa is necessary for the future development of reliable phylogenetic, phylogenomic and biogeographic reconstructions.

Acknowledgments

The authors would like to thank the editor Dr. Perez-Losada and two anonymous reviewers for their valuable suggestions and comments. M. Caballer MNHN e-RECOLNAT (ANR-11-INBS-0004) and Ms. Virginie Heros, molluscs, Museum National d'Histoire Naturelle, Paris are acknowledged for the photographs of the types of *Unio mansuyi*, *Unio rochechouartii*, and *Unio affinis* used in this paper and their assistance with specimens in their collection. Ms. Krasimira (Kasey) Seizova, Drexel University Co-op student working in the Malacology Department, Academy of Natural Sciences of Drexel University, Philadelphia took the photographs of the type specimen of *Gibbosula confragosa*. These pictures were taken for the ANSP type project and used with the permission of Dr. Gary Rosenberg. Pictures of the NCSM specimen of *Gibbosula crassa* were taken by Ms. Raquel Fagundo and Ms. Joanna Cox; those of the Margaritiferid hinge plates were taken by Macauley Whiting, all in the Non-Molluscan Invertebrate Unit, North Carolina Museum of Natural Sciences, Raleigh, NC with permission of Dr. Bronwyn Williams. Jeffrey T. Garner, Florence, Alabama assisted with the USNM ledgers. He Jing, Shell Discoveries and ConchBooks are acknowledged for the permission to reproduce the figures from Qian et al 2015 for the description of *Gibbosula nanningensis*. This work was supported by FCT - Foundation for Science and Technology, Project 3599 - Promote the Scientific Production and Technological Development and Thematic 3599-PPCDT by FEDER as part of the project FRESHCO: Multiple implications of invasive species on Freshwater Mussel co-extinction processes (contract: PTDC/AGRFOR/1627/2014). FCT also supported MLL (SFRH/BD/115728/2016) and EF (SFRH/BPD/108445/2015). The Russian Ministry of Education and Science (project no. 6.2343.2017/4.6), the Federal Agency for Scientific Organizations (project no. 0409-2015-0143), the Presidium of the Russian Academy of Sciences (scientific program no. 52), and the Russian Foundation for Basic Research, RFBR (project no. 17-45-290066) supported INB, MYG, AVK, and IVV. The Nagao Natural Environment Foundation (NEF) supported for VTD research project "The study of Unionoida biodiversity and conservation status in Bang and Ky Cung River basins in Northeast Vietnam".

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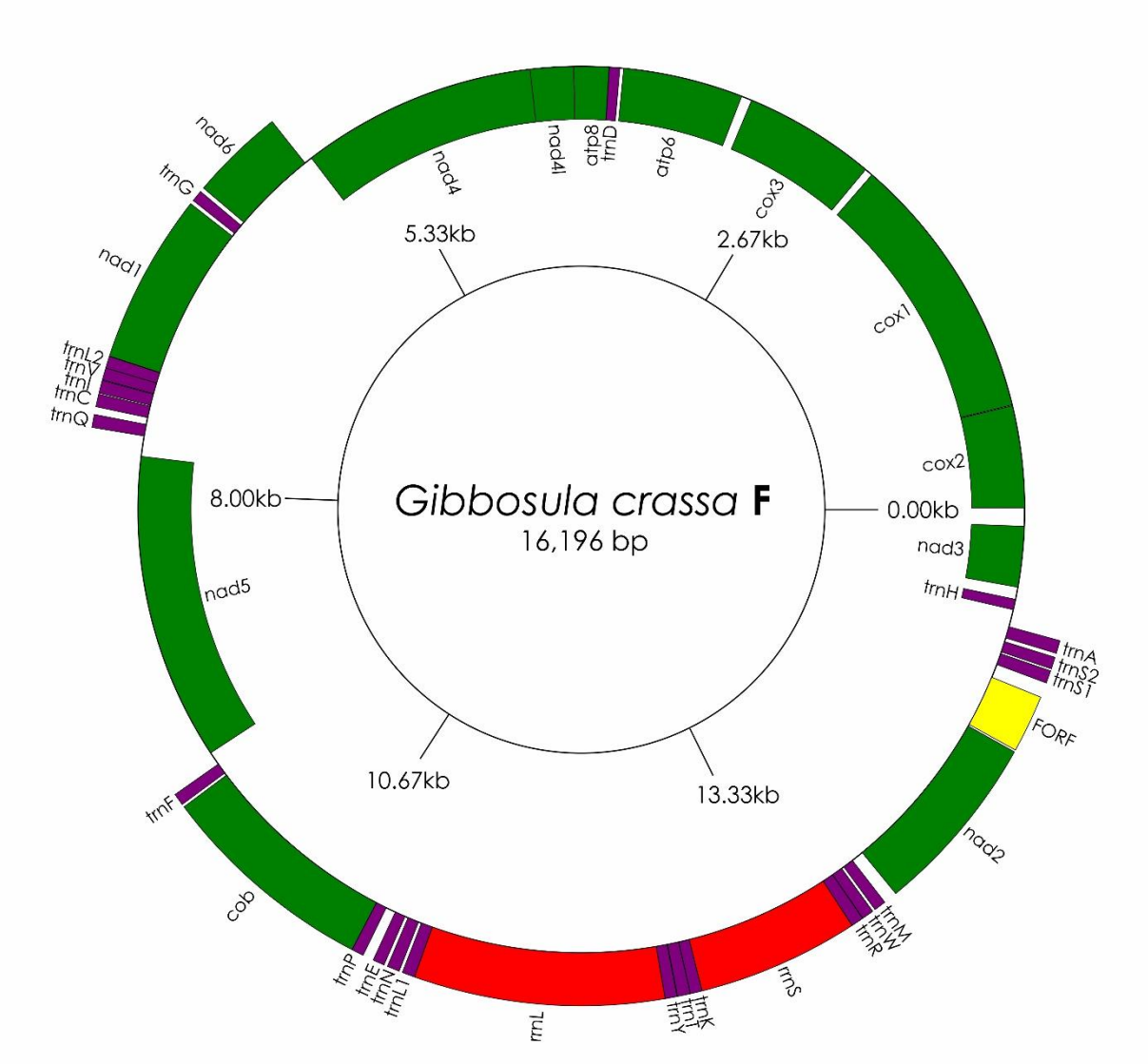
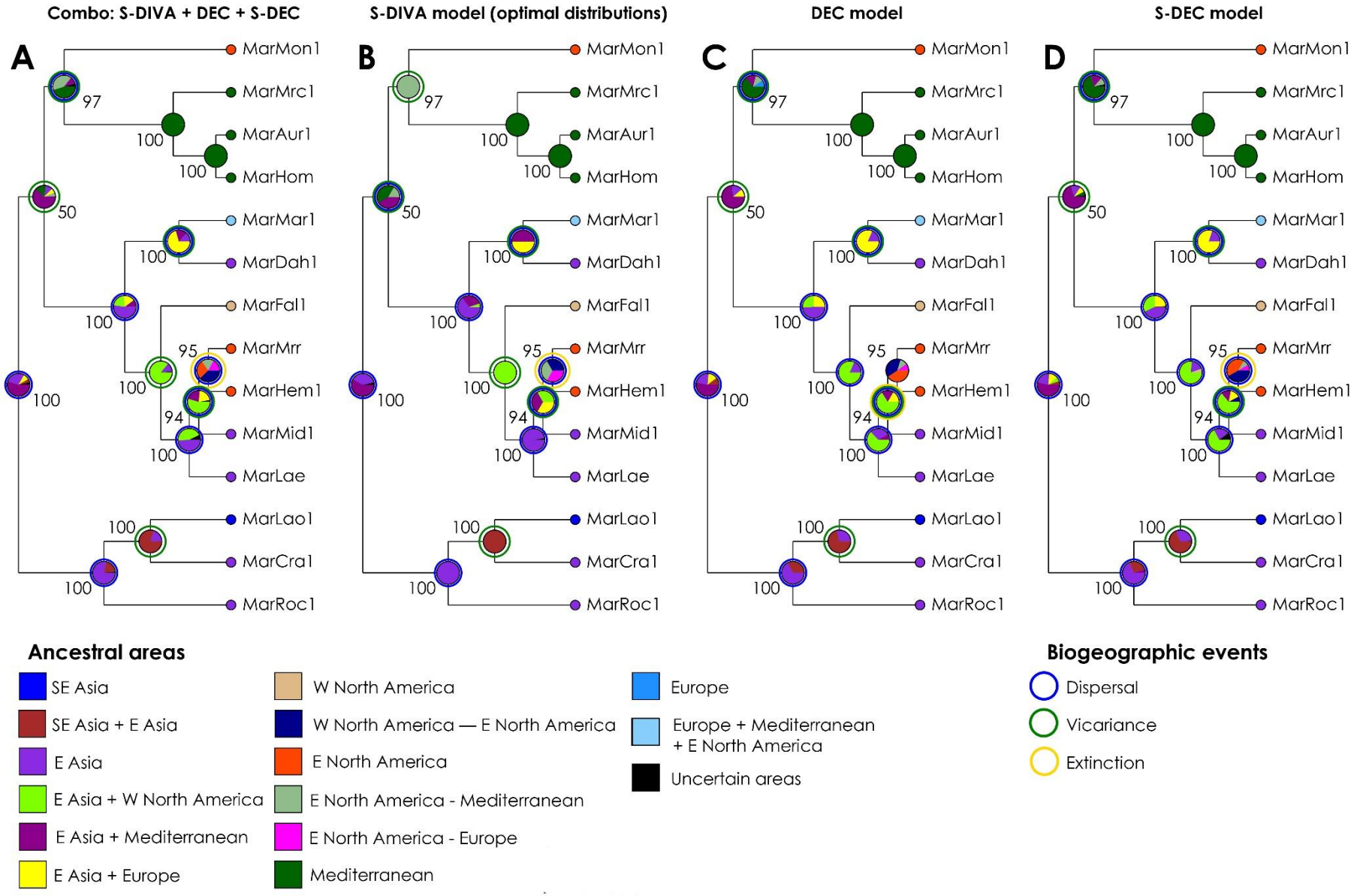
Supplementary material



Supplementary Figure 1 Gene map of the F-type mitochondrial genome of *Gibbosula crassa*. Genes positioned inside the circle are encoded on the heavy strand, and genes outside the circle are encoded on the light strand. Colour codes: small and large ribosomal RNAs (red), transfer RNAs (purple); F-specific open reading frame (yellow); protein-coding genes (green).


Combo: S-DIVA + DEC + S-DEC
S-DIVA model (optimal distributions)
DEC model
S-DEC model
A
B
C
D
MarMon1
MarMrc1
MarAur1
MarHom
MarMar1
MarDah1
MarFal1
MarMrr
MarHem1
MarMid1
MarLae
MarLao1
MarCra1
MarRoc1
Ancestral areas
SE Asia
SE Asia + E Asia
E Asia
E Asia + W North America
E Asia + Mediterranean
E Asia + Europe
W North America
W North America — E North America
E North America
E North America - Mediterranean
E North America - Europe
Mediterranean
Europe
Europe + Mediterranean + E North America
Uncertain areas
Biogeographic events
Dispersal
Vicariance
Extinction

Supplementary Figure 2 Historical biogeography of the Margaritiferidae inferred from three different statistical modelling approaches, including (A) the combined results of SDIVA, DEC, and S-DEC; (B) S-DIVA; (C) DEC; and (D) S-DEC, calculated under a lognormal relaxed clock model and a Yule process speciation implemented in BEAST 1.8.4 and obtained for the complete data set of mitochondrial and nuclear sequences (nine partitions: three codons of COI + 16S rRNA + 18S rDNA + 28S rDNA + three codons of H3). Pie chaps near nodes indicate probabilities of certain ancestral areas. Colour circles on the tip nodes indicate the range of each species. Black numbers near nodes are BPP values inferred from BEAST.

Supplementary Table 1.
Specimens examined for conchological and anatomical features. ANSP - Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA; MNHN - Muséum national d'Histoire Naturelle, Paris, France; NHMUK - Natural History Museum, London, UK; NCFM - Nanchang Freshwater Mollusk Collection, Nanchang University, Nanchang, Jiangxi Province, China; NCSM - North Carolina Museum of Natural Sciences, Raleigh, NC, USA; RMBH - Russian Museum of Biodiversity Hotspots, Federal Center for Integrated Arctic Research, Russian Academy of Sciences, Arkhangelsk, Russia.

Taxa	Conchological	Anatomical
Gibbosula confragosa	ANSP 145237	-----
Gibbosula crassa	MNHN-IM-2000-1743, 33100, 33101; NCSM 102193, 102194	NCSM 102193, 102194
Gibbosula laosensis	NCSM 83059	RMBH biv0136/3
Gibbosula polysticta	MNHN MP 3387	MNHN MP 3387
Gibbosula rochechouartii	NCSM 6468, NCFM 092142	NCFM 092142
Cumberlandia monodonta	NCSM 28549, 33041, 55345, 55350, 55359	NCSM 30377, 34969, 87784, 100543
Margaritifera dahurica	NCSM 27972	RMBH biv0165/2; NCSM 27972
Margaritifera falcata	NCSM 48386, 48393	NCSM 27974, 29455, 41056, 41059
Margaritifera hembeli	NCSM 28946, 45395	NCSM 100550
Margaritifera laevis	NCSM 102192	RMBH d0078/9
Margaritifera margaritifera	NCSM 5771,7329	NCSM 28288, 28944, 83212
Margaritifera marrianae	NCSM 33045, 62914, 62915	NCSM 30343, 30371, 46348
Margaritifera middendorffi + *M. togakushiensis*	NCSM 48955	RMBH biv0167/1; NCSM 48955
Pseudunio auricularius	NCSM 44514	MNHN-IM-2013-62643
Pseudunio homsensis	RMBH biv0176	RMBH biv0176; NHMUK 1936.10.3
Pseudunio marocanus	NCSM 85240, 102909, 102910	NCSM 1012909, 102910

Supplementary Table 2.
Best-fit models of nucleotide substitution for each partition based on Bayesian Information Criteria (BIC) using JMODELTEST 2.1.10 (Darriba et al 2012) for the Bayesian inference analyses.

Partition	Model
COI	HKY + G + I
COI codon 1	GTR + G + I
COI codon 2	F81 + G
COI codon 3	HKY + G
16S	GTR + G + I
18S	GTR + G + I
28S	GTR + G + I
H3	K80 + G
H3 codon 1	JC
H3 codon 2	JC
H3 codon 3	K80 + G

Supplementary Table 3.
List of characteristic examples of fossil records supporting the primary phylogenetic clades of freshwater bivalves identified in the present study.

Clades	**Group [dating following our fossil-calibrated model]**	**Ancestral genera [stratigraphic dating]**
Unionida	Crown [near Permian - Triassic boundary]	†*Silesunio* Skawina & Dzik, 2011 [early Late Triassic]
Margaritiferidae + Unionidae	Crown [Late Triassic]	†*Shifangella* Liu & Luo, 1981 [Late Triassic]
Margaritiferidae	Crown [Middle Jurassic]	†*Palaeomargaritifera* Ma, 1984 **stat. res.** [Middle Jurassic]
Gibbosulinae (*Gibbosula*)	Crown [mid-Cretaceous]	*Gibbosula* Simpson, 1900 [mid-Cretaceous]
G. laosensis - G. crassa	n/a	*Gibbosula* Simpson, 1900
Margaritiferinae (*Margaritifera* + *Pseudunio* + *Cumberlandia*)	Stem/Crown [Middle to Late Jurassic]	†*"Margaritifera"* [Late Jurassic]
Pseudunio + *Cumberlandia*	Stem [Late Jurassic to Early Cretaceous] Crown [Early Cretaceous]	†*Asturianaia* Delvene, Munt, Piñuela & García-Ramos, 2016 [Late Jurassic] †*Paraheudeana* Starobogatov, 1970 ?†*Protelliptio* Russell, 1934 [Early Cretaceous]
Cumberlandia	Stem [from Early Cretaceous]	?*Cumberlandia* Ortmann, 1912 [Early Cretaceous]
Pseudunio	Crown [Eocene]	*Pseudunio* Haas, 1910 [Lower Oligocene]
P. auricularius - P. homsensis	Crown [Miocene]	*Pseudunio* Haas, 1910
Margaritifera	Crown [Late Cretaceous]	*Margaritifera* Schumacher, 1816 [Late Cretaceous]
M. dahurica - M. margaritifera	Crown [Eocene]	*Margaritifera* Schumacher, 1816
Pacific clade (*M. laevis - M. falcata*)	Crown [near Paleocene - Eocene boundary]	*Margaritifera* Schumacher, 1816
M. laevis - M. middendorffi	Crown [near Eocene - Oligocene boundary]	*Margaritifera* Schumacher, 1816
M. middendorffi - M. hembeli	Crown [Oligocene]	*Margaritifera* Schumacher, 1816
M. hembeli - M. marrianae	Crown [Miocene]	*Margaritifera* Schumacher, 1816

Supplementary Table 3 (*cont.*)

Clades	Examples of ancestral species*	Reference
Unionida	†*S. parvus* Skawina & Dzik, 2011**	Skawina & Dzik (2011)
Margaritiferidae + Unionidae	†*S. margaritiferiformis* Liu & Luo, 1981**	Fang et al (2009)
Margaritiferidae	†*P. guangyuanensis* Ma, 1984 **comb. res.**, *P. angulata* (Ma, 1996) **comb. nov.**	Ma (1996), Fang et al (2009)
Gibbosulinae (*Gibbosula*)	†*G. tibetica* (Gu, 1976) **comb. nov.****	Ma (1996)
G. laosensis - G. crassa	n/a	n/a
Margaritiferinae (*Margaritifera* + *Pseudunio* + *Cumberlandia*)	†"*M.*" *crosthwaitei* (Newton, 1909)	Van Damme et al (2015)
Pseudunio + *Cumberlandia*	†*A. colunghensis* Delvene, Munt, Piñuela & García-Ramos, 2016, †*A. lastrensis* Delvene, Munt, Piñuela & García-Ramos, 2016, †*A. soudanensis* (Van Damme & Bogan, 2015) **comb. nov.**	Van Damme et al (2015), Delvene et al (2016)
	†*P. valdensis* (Mantell, 1844), †*P. idubedae* (Palacios & Sánchez, 1885)**	Van Damme et al (2015)
	P. biornatus (Russell, 1932), *P. douglassi* (Stanton, 1903), *P. hamili* (McLearn, 1929)	Skawina & Dzik (2011)
Cumberlandia	†*C. rhazensis* (Mongin, 1968) **comb. nov.**, †*C. saharica* (Mongin, 1968) **comb. nov.**	Van Damme et al (2015)
Pseudunio	†*Pseudunio* sp.**	Schneider & Prieto 2011), Araujo et al (2017)
P. auricularius - P. homsensis	†*P. flabellatus* (Goldfuss, 1837) **comb. nov.**, †*P. flabellatiformis* (Grigorowitch-Beresowski, 1915) **comb. nov.**	Bolotov et al (2016)
Margaritifera	†*M. sainshandensis* (Martinson, 1982) **comb. nov.**, †*M. elongata* (Martinson, 1982) **comb. nov.** [primary homonym of *Unio elongata* Lamarck, 1819], †*M. glabra* (Kolesnikov, 1956) **comb. nov.**	Martinson (1982)
M. dahurica - M. margaritifera	†*M. occulta* Maderny, 1972, †*M. martinsoni* Modell, 1964**	Bolotov et al (2016)
Pacific clade (*M. laevis - M. falcata*)	†*M. perdahurica* (Yokoyama, 1932)**, †*M. otatumei* (Suzuki, 1942), †*M. owadaensis* Noda, 1970, †*M. sinopae* (Cockerell, 1915), †*M. herrei* (Hannibal, 1912)	Henderson (1935); Modell (1957); Bolotov et al (2016)
M. hembeli - M. marrianae	*M. condoni* (White, 1885)	Modell (1957)

*The revision of numerous fossil species of the Margaritiferidae appears to be a complicated task and is well beyond the scope of the present study. Here we list characteristic examples of possible ancestral lineages supporting each primary clade of the family and provide several minor

taxonomic changes. Considering our new data on the extremely low diversification rates in the Margaritiferidae, high diversity of fossil species may be an artifact caused by shell shape variability. **Fossil calibrations (see Supplementary Table 4 for details). n/a - not available.

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Supplementary Table 4. List of fossil calibrations that were used in BEAST analyses

Calibration no.	MRCA	Description	Reference
Calibration 1	Unionida	Hard minimum age: 230 Ma, †*Silesunio parvus* Skawina & Dzik (2011) (Unionida: Silesunionidae). Diagnosis and phylogenetic placement: Elongated shell of small size does not exceed 50 mm and generalized morphology, with juvenile stage bearing concentric ribs parallel with the mantle margin (Skawina & Dzik 2011). Umbonal muscles tend to disperse over the anterior slope of the beaks. The Silesunionidae is a prospective earliest member of the order Unionida (Skawina & Dzik 2011). Stratigraphic horizon and locality: Lacustrine grey claystone and red finely grained mudstone bed within red-coloured fluviatile series of Late Carnian calcareous mudstones, Krasiejów, Opole Silesia, southern Poland (Skawina & Dzik 2011). Absolute age estimate: Late Carnian, 230 Ma, based on stratigraphy; 95% soft upper bound 273 Ma based on the age of †*Lyroschizodus orbicularis* Newell & Boyd 1975 (Trigoniida: Trigoniidae), the earliest known member of Trigoniidae (Newell & Boyd 1975). Prior settings: exponential distribution, mean (lambda) = 11.6, MRCA: *Unio pictorum* - *Velesunio ambiguus*.	Present study: New crown calibration
Calibration 2	Margaritiferidae + Unionidae	Hard minimum age: 201 Ma, †*Shifangella margaritiferiformis* Liu & Luo, 1981 in Liu (1981) (Unionida: unnamed ancestral family). Diagnosis and phylogenetic placement: Shell thin, large to very large; transversely elongate, ventral margin concave; elongate-oval to Margaritifera-shaped; equivalve, inequilateral; moderately to rather inflated. Posterior ridge strong, edge-form (Fang et al 2009). Umbonal region compressively flattened; beaks broad, not projecting or slightly rising above hinge margin, prosogyrous, situated rather anteriorly. Surface ornamented with regularly spaced concentric rings and growth lines, but with regular concentric rings only in preadult specimens; lunule absent; escutcheon developed, relatively wide; ligament opisthodetic. Hinge plate narrow, with unionid dentition, but teeth fine and smooth, parallel to hinge margin. Anterior adductor scar shallow, rounded, accompanied by a pedal scar at the upper posterior side. The genus was considered a member of Margaritiferidae (Fang et al 2009), but it seems to be rather a prospective ancestral lineage of both families. Stratigraphic horizon and locality: Second Member, Wuzhongshan Formation, Upper Triassic, Jinhe, Shifang, Sichuan, southwestern China (Fang et al 2009).	Present study: New crown calibration

Calibration no.	MRCA	Description	Reference
		Absolute age estimate: Triassic/Jurassic boundary, 201 Ma, based on stratigraphy; 95% soft upper bound 230 Ma based on calibration 2. Prior settings: exponential distribution, mean (lambda) = 7.9, MRCA: *Margaritifera marrianae* - *Potomida littoralis*.	
Calibration 3	Margaritiferidae	Hard minimum age: 168 Ma, †*Palaeomargaritifera guangyuanensis* (Ma, 1984) **comb. res.** Diagnosis and phylogenetic placement: Shell large; greatly elongate, with largest shell about 120 mm long and 44 mm high; length about 2.7 times as long as height; shell width about one-fifth as long as height; anterior margin rounded; posterodorsal margin nearly straight, obliquely passing into rounded posterior margin; posteroventral end prominently protracting backward; ventral margin long, with wide, shallow sinus; umbo broad and low, positioned at about two-ninths shell length from anterior; posterior ridge obtuse (Fang et al 2009). Shell flat, surface with irregular commarginal lines. Anterior pseudocardinal teeth strong, two in left valve and one in right valve; posterior lateral lamellar teeth, one in left valve and seemingly two in right valve; anterior adductor scar deep, elongate-oval, with arborescent-like striations; upper pedal scar deeper, lower one isolated and shallower; posterior adductor scar also shallow (Fang et al 2009). It seems to be a prospective stem lineage of extant Margaritiferidae. Stratigraphic horizon and locality: Third Member, Guangyuan Group, Middle Jurassic, Nanshan, Guangyuan, Sichuan, southwestern China (Fang et al 2009). Absolute age estimate: Bajocian/Bathonian boundary, 168 Ma, based on stratigraphy; 95% soft upper bound 201 Ma based on calibration 3. Prior settings: exponential distribution, mean (lambda) = 9, MRCA: *Gibbosula crassa* - *Margaritifera hembeli*.	Present study: New stem calibration
Calibration 4	*Pseudunio* + *Cumberlandia*	Hard minimum age: 129 Ma, †*Paraheudeana idubedae* (Palacios & Sánchez, 1885). Diagnosis and phylogenetic placement: The assignation of this species to the genus is based on the shell shape, notable mantle attachment scars on the inner side of the valve and the arborescent rugosity of the muscle adductor scars (Delvene & Araujo 2009; Van Damme et al 2015). It seems to be a prospective crown lineage of the extant *Pseudunio* + *Cumberlandia* clade. Stratigraphic horizon and locality: Hauterivian-Barremian Urbión Group, Valdehierro and Valdemadera sites, La Rioja Province, Cameros Basin, Spain (Delvene & Araujo 2009).	Present study: New crown calibration

Calibration no.	MRCA	Description	Reference
		Absolute age estimate: Hauterivian/Barremian boundary, 129 Ma, based on stratigraphy; 95% soft upper bound 168 Ma based on calibration 4. Prior settings: exponential distribution, mean (lambda) = 7.8, MRCA: *Pseudunio homsensis* - *Cumberlandia monodonta*.	
Calibration 5	*M. falcata* - *M. laevis*	Absolute age estimate: 46 Ma; 95% soft upper bound 92 Ma (twice the age of the fossil). Prior settings: exponential distribution, mean (lambda) = 12.5, MRCA: *Margaritifera falcata* - *M. laevis*.	Bolotov et al (2016): Crown calibration
Calibration 6	*M. margaritifera* - *M. dahurica*	Absolute age estimate: 34 Ma; 95% soft upper bound 68 Ma (twice the age of the fossil). Prior settings: exponential distribution, mean (lambda) = 9.3, MRCA: *Margaritifera margaritifera* - *M. dahurica*.	Bolotov et al (2016): Crown calibration
Calibration 7	*P. auricularius* - *P. marocanus*	Absolute age estimate: 35 Ma; 95% soft upper bound 70 Ma (twice the age of the fossil). Prior settings: exponential distribution, mean (lambda) = 9.5, MRCA: *Pseudunio auricularius* - *P. marocanus*.	Araujo et al (2017): Crown calibration
Calibration 8	*Gibbosula*	Hard minimum age: 86 Ma, † *Gibbosula tibetica* (Gu, 1976) **comb. nov.** Diagnosis and phylogenetic placement: The species differs by thick shell, massive hinge plate, well-developed pseudocardinal teeth, and the arborescent rugosity of the muscle adductor scars (Ma 1996; Data Base of Paleontological Fossils of Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences: lot nos. 30963, 30964, 30965, 30967, 30968, and 30969). It seems to be a crown lineage of the extant *Gibbosula*. Stratigraphic horizon and locality: Late Cretaceous Shigatse Group, Zhaxilin village, Shigatse Region, Tibet, China. Absolute age estimate: Coniacian/Santonian boundary, 86 Ma, based on stratigraphy; 95% soft upper bound 168 Ma based on calibration 4. Prior settings: exponential distribution, mean (lambda) = 22.3, MRCA: *Gibbosula rochechouartii* - *G. laosensis*.	Present study: New crown calibration

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Supplementary Table 5.
Diversification rate statistics for the Margaritiferidae and Unionidae clades.

Clade	Paradis's test of diversification with three survival models				The constant-rates test	
	Div. rate (delta ± s.e.)	LRT *p*-value (constant rate model vs. Weibull model)	Beta parameter of the Weibull model (± s.e.)	Selected model (by AIC)	Gamma statistic	*p*-value (two-sided)
Margaritiferidae	0.014±0.004	0.096	1.488±0.322	Weibull (variable rate through time)	-0.628	0.530
Gibbosula	0.012±0.008	0.037*	5.250±2.097	Weibull (variable rate through time)	-0.767	0.443
Pseudunio* + *Cumberlandia	0.015±0.009	0.703	1.203±0.566	Constant rate	0.945	0.345
Margaritifera	0.023±0.009	0.042*	2.135±0.618	Weibull (variable rate through time)	-0.838	0.402
Rectidentinae**	0.037±0.008	0.013*	1.603±0.256	Weibull (variable rate through time)	-1.612	0.107
Rectidentini[**,M]	0.036±0.011	0.018*	2.744±0.573	Weibull (variable rate through time)	-2.306	0.021*
Contradentini[M]	0.047±0.014	0.102	1.520±0.345	Constant rate	-0.747	0.455
Pseudodontinae[**,M]	0.038±0.010	0.022*	1.683±0.324	Weibull (variable rate through time)	-1.448	0.148

Superscripts: *variable diversification rate; **Data from Bolotov et al (2017a); [M]Mekong only.

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Supplementary Table 6
Margaritiferidae generic names, authorities, and type species.

Genus name and authority	Type species
Gibbosula Simpson, 1900	*Mya crassa* Wood, 1815
+*Margaritanopsis* Haas, 1910b[1]	*Unio laosensis* Lea, 1863
+(*Odhnerella*) Modell, 1964	*Unio rochechouartii* Heude, 1875
Cumberlandia Ortmann, 1912	*Unio monodonta* Say, 1829
Margaritifera Schumacher 1816[2]	*Mya margaritifera* Linn., 1758[2]
+*Margaritana* Schumacher, 1817[2]	*Mya margaritifera* Linn., 1758[2]
+*Damaris* "Leach" Swainson, 1823	*Mya margaritifera* Linn., 1758
+*Damalis* Leech, 1847 non Fabricius, 1805	*Mya margaritifera* Linn., 1758
+*Baphia* Mörch, 1853 non Gray, 1847	*Mya margaritifera* Linn., 1758
+*Margaritiferana* Fagot, 1893	*Unio elongata* Lamarck, 1819 = *Mya margaritifera* Linn., 1758
+*Kurilinaia* Bogatov & Zatravkin, 1988	*Dahurinaia kurilensis* Zatravkin & Starobogatov, 1984 = *M. laevis*
+*Dahurinaia* Starobogatov, 1970	*Unio dahuricus* Middendorff, 1850
+*Schalienaia* Starobogatov, 1970	*Unio hembeli* Conrad, 1838
+*Baryana* Locard, 1889[3] [nomen nudum]	*Unio baryus* "Bourg." Locard, 1889 [*nomen nudum*]
Pseudunio Haas, 1910a	*Unio sinuata* Lamarck, 1819 = *U. auricularius* Spengler, 1793
+*Potamida* Agassiz, 1846, *non* Brongniart, 1810	*Unio sinuata* Lamarck, 1819 = *U. auricularius* Spengler, 1793
+*Potodoma* Haas, 1969b, *non* Meigen, 1800	*Unio sinuata* Lamarck, 1819 = *U. auricularius* Spengler, 1793

[1]The date of publication for *Margaritanopsis* Haas has been used as 1912 (e.g. Haas 1969a; Smith 2001) and Haas 1969b and Graf & Cummings (2017) used 1910. The correct date of publication is based on the first publication of the generic name in association with a species name that occurs on Plate 12, figures 1-2 of Haas (1910) published in Lieferung 546, dated 1910 and signature 5, dated 13 August 1910 (see Bogan 2015). The text description of *Margaritanopsis* appears on pages 121-122 which was published in Lieferung 559, dated 1912 and signature 16 dated 13 February 1912 (see Bogan 2015).
[2]Spelling of *Margaritifera* and its type species, suppression of the generic name *Margaritana* and the use of Margaritiferidae have been stabilized by the ICZN Opinion 495 [1957].
[3]*Baryana* Locard, 1889 (Locard 1889:18) erected the group of *Baryana* "Bourguignat" in the genus *Unio* and declared the type of the group as" Le type de ce groupe est l'*Unio baryus* Bourg. de l'Euphrate." *Unio baryus* is the type species by original designation. The work is credited by

Locard to a Bourguignat manuscript. *Unio baryus* Bourg. in Locard is a manuscript name and is a *nomen nudum*. Graf (2010) noted *Unio sinuata* Lamarck, 1819 was the only other included species and observed this genus is invalid and a *nomen dubium*. *Baryana* has been used with *Unio sinuata* Lamarck as the type species in error, which would make it a senior synonym of *Pseudunio* Haas 1910 (Graf & Cummings 2017). Vinarski & Kantor (2016) include *Baryana* as a synonym of *Margaritifera* without comment on its nomenclatural status. All generic names were checked against the database of Neave (2018).

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CHAPTER 7

The unique mitogenomes of Margaritiferidae

Paper VI

The first Margaritiferidae male (M-type) mitogenome: mitochondrial gene order as a potential character for determining higher-order phylogeny within Unionida (Bivalvia)

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Article published in *Journal of Molluscan Studies* **83**, 249-252 (2017).
DOI: 10.1093/mollus/eyx009

The first Margaritiferidae male (M-type) mitogenome: mitochondrial gene order as a potential character for determining higher-order phylogeny within Unionida (Bivalvia)

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Introduction

The unionid family Margaritiferidae, comprising 12 extant species, is widely distributed across the northern hemisphere in North America, Europe and Asia (Bolotov et al 2016). Most species in this family have dramatically declined over the last century, with nine of the 12 species assessed as threatened in the most recent IUCN Red List (IUCN 2016). Among these is the Moroccan pearl mussel *Margaritifera marocana* (Pallary, 1918), considered one of the 100 most threatened species on the planet (Baillie & Butcher 2012). This species is now restricted to two small streams in the Oum Er Rbia and Sebou basins and conservation measures are urgently needed (Sousa et al 2016). Beyond the conservation concern, Unionida are also biologically interesting. They present an unusual mechanism of mitochondrial inheritance called doubly uniparental inheritance (DUI), in which all individuals have the typical maternally transmitted mtDNA (F-type), but the males possess in their germ cells a paternally inherited mtDNA instead (M-type) (Zouros et al 1994; Breton et al 2009). So far, DUI has been observed in over 100 species from four bivalve orders (Gusman, Azuelos & Breton 2017), including three families within Unionida, i.e. Unionidae, Hyriidae and Margaritiferidae (Walker et al 2006). However, to date, no whole M-type mitogenome has been published for any species belonging to the last two of these families.

The gene arrangement within mitogenomes is highly conserved in many taxonomic groups. For example, most vertebrates share the same gene order (Pereira 2000). In other faunal groups, like Bivalvia, the mitochondrial genome arrangement is more variable, although not many distinct gene orders have been described so far (Serb & Lydeard 2003). Still, in unionoids, mitogenome rearrangements seem to be rare events that are unlikely to be homoplastic. In this context, the mitogenome gene order might be used as an additional character for phylogenetic inference. However, its utility for the Unionida phylogeny has never been tested.

The order Unionida has 6 recognized families with around 800 species (Lopes-Lima et al 2014), but the phylogenetic relationships among these families are still not fully resolved (Graf 2013). This lack of coherence among studies has been consistently attributed to the low number of molecular markers used and insufficient taxon sampling (Bogan & Roe 2008; Graf 2013; Fonseca et al 2016).

Under the above-mentioned assumptions, the aims of the present study are to (1) sequence and analyse the whole M- and F-type mitogenomes of *M. marocana*, (2) infer the phylogenetic relationships among Unionoidea species using all both the F- and M-type mtDNA sequences publicly available and (3) determine the gene order of all analysed mitogenomes and evaluate its phylogenetic utility.

Materials and Methods

One male specimen deposited in the Muséum d'Histoire Naturelle de Marrakech (voucher MHNM16ZMB23) from the Laabid River (GPS WGS84: 32.142334, -7.027595) was dissected for the sampling of gonadal and mantle tissue. DNA extractions followed Froufe et al (2016). The complete M- and F-type mitogenomes were then sequenced, assembled and annotated using an established pipeline (Gan, Schultz & Austin 2014). The F and M mitogenomes have been deposited in the GenBank database under the accession numbers KY131953 and KY131954, respectively.

The two newly obtained *M. marocana* mitogenome sequences were aligned with all (43) M- and F-type Unionida mitogenome sequences available on GenBank as of March 2016, as well as with the F- and M-type mitogenomes of *Mytilus galloprovincialis* as outgroup (list of genomes and respective accession numbers used supplied on request). DNA (NUC) and amino acid (AA) sequences of all mtDNA protein-coding genes (PCGs) except ATP8, and the gender-specific open reading frames (M-ORF, H-ORF, and F-ORF) were used in the phylogenetic analyses. The sequences of each gene were aligned using MAFFT v. 7.304 (Katoh & Standley 2013) and trimmed with GUIDANCE v. 1.5 (Penn et al 2010; see Froufe et al 2016, for parameters used). The gene alignments were then concatenated, resulting in two alignments with the following length: 14,350 aligned nucleotide positions or 6,246 aligned amino acid + nucleotide positions (4,085 aligned amino acids positions and 2,161 aligned nucleotide positions from the rRNAs genes). The optimal partitioning scheme (i.e. the best set of nonoverlapping partitions that cover the whole alignment) for each alignment was selected using PartitionFinder v. 1.1.1 (Lanfear et al 2012) under the greedy algorithm with proportional branch lengths across partitions. The best substitution models of DNA and protein evolution for each partition were selected under the BIC ranking method (Schwarz 1978). The codon positions of the PCG and each rRNA were defined as the initial data blocks for the partitioning schemes search. Maximum likelihood (ML) phylogenetic inference was performed using RAxML v. 8.0.0 (Stamatakis 2014) with 100 rapid bootstrap replicates and 20 ML searches. Bayesian Inference (BI) was applied using MrBayes v. 3.2.1 (Ronquist et al 2012) with two independent runs (1×10^7 generations with a sampling frequency of 1 tree for every 100 generations), each with four chains (three hot and one cold). All runs reached convergence (average standard deviation of split frequencies < 0.01). The posterior distribution of trees was summarized in a 50% majority-rule consensus tree (burn-in of 25%).

Results and Discussion

The length of the two newly sequenced mitogenomes of *M. marocana*, 16,001 nt for the female haplotype and 17,562 nt for the male haplotype, is within the typical range for each sex-specific

haplotype within Unionida. The sequenced haplotypes include the 13 PCGs typically found in metazoan mitochondrial genomes, the sex-specific ORF described for all Unionida mitogenomes with DUI system (Breton et al 2009, 2011a) and 22 transfer RNA (tRNA) and two ribosomal RNA (rRNA) genes. The M-type genome is the largest sequenced to date within the Unionida. M-type genomes are generally larger than F-type genomes due to the larger size of the PCG COX2 and M-ORF in M-type genomes compared with COX2 and F-ORF in F-type genomes (Breton et al 2009). Four intergenic regions were identified in the *M. marocana* M-type genome between the following gene pairs: NAD3-tRNA (A) 106 nt, tRNA(H)-tRNA(Q) 411 nt, ND4L-tRNA(D) 255 nt and tRNA(D)-ATP8 498 nt. These regions were analysed to search for the M-ORF. The results of the blast search (Altschul et al 1997) retrieved a significant hit with another Margaritiferidae M-ORF (*Margaritifera monodonta*, *E*-value = 4e-34) and a Fickett test score of 1.201 (Fickett 1982; a score > 0.95 means the sequence is probably coding), suggesting that the M-ORF is located in the region between the genes ND4L and tRNA(D). The M-type mitogenome of *M. marocana* presents a novel gene order within Unionida (Fig. 1). The F-type mitogenome gene order is the same as already observed for the two previously available Margaritiferidae F-type mitogenomes (Breton et al 2011b; Yang et al 2015). All the phylogenies inferred in this study support the reciprocal monophyly of both (Unionidae + Margaritiferidae) F- and M-type lineages (Fig. 2 shows the topology of the BI-NUC tree; all other phylogenetic trees figures supplied on request). Additionally, the monophyly of Unionidae (both F- and M-type), Margaritiferidae (F-type) and all represented Unionidae subfamilies are well supported in all inferred mtDNA trees, except for the Unioninae, for which monophyly was only well supported in the BINUC tree. The remaining phylogenetic trees (BI-AA, ML-NUC, and ML-AA) showed conflicting results regarding the position of the clade comprising *Arconaia lanceolata* and *Lanceolaria grayana* (Fig. 2). These conflicting results have also been found in previous studies where different mitogenome phylogenetic methodologies revealed distinct tree topologies (Huang et al 2013; Fonseca et al 2016). Five distinct mtDNA gene orders have been detected in the present dataset, three in the F-type lineage and two in the M-type lineage. In the F-type lineage, gene order UF1 is shared by the Unionidae subfamilies Anodontinae, Ambleminae, and Unioninae, whereas gene orders UF2 and MF1 are found in the represented species of the subfamily Gonideinae and the family Margaritiferidae, respectively (Figs 1, 2). In the female lineage, there is only a difference between UF1 and MF1 in the location of tRNA(E) (Fig. 1). The gene order of UF2 is more distinct and might have resulted from a tandem duplication of the gene region between COX2 and tRNA(W) followed by random deletion of segments of the duplicated gene region (Doucet-Beaupré et al 2010). Between the M and F mitogenomes, the differences are in the location of tRNA(H) and the inversion of the ATP8-tRNA(D) region. An additional distinct location of tRNA(S1) is also found in margaritiferid M mitogenomes (Fig. 1).

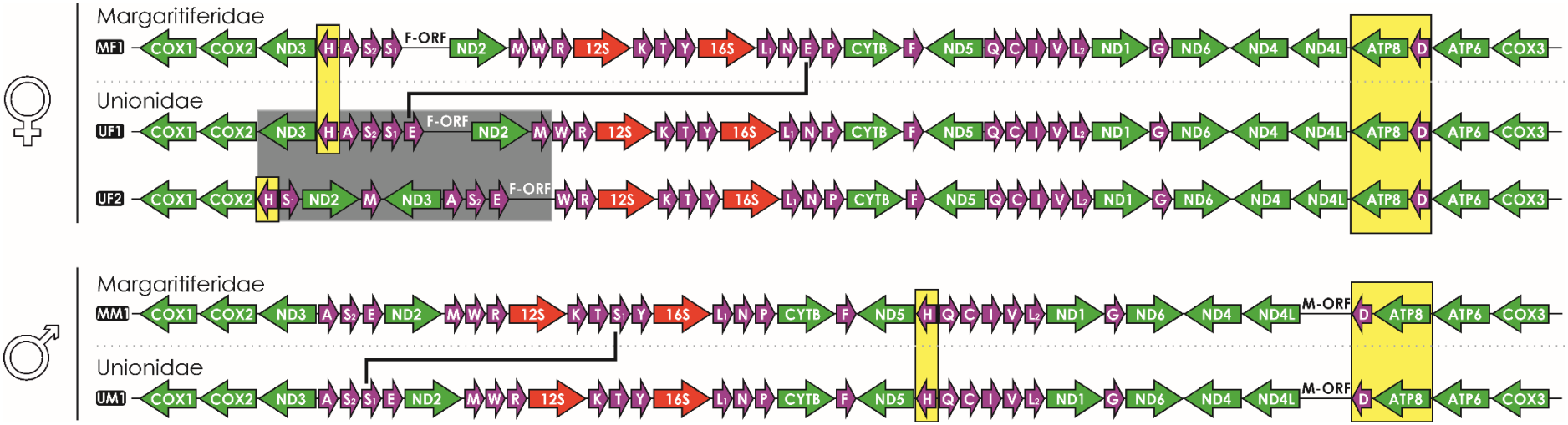

Figure 1 Diagrams of the five distinct gene orders detected in Unionida. In the female F-type lineage, three gene orders are depicted: Unionidae F-type 1 (UF1), Unionidae F-type 2 (UF2) and Margaritiferidae F-type 1 (MF1). In the male M-type lineage, two gene arrangements are shown: Unionidae M-type 1 (UM1) and Margaritiferidae M-type 1 (MM1). Continuous lines indicate different locations of genes between mitogenomes. Grey box highlights the gene rearrangement region between UF1 and UF2. Yellow boxes indicate the main differences in gene arrangement between female and male mitogenomes, tRNA (H) location and rearrangement of ATP8-tRNA(D) region.

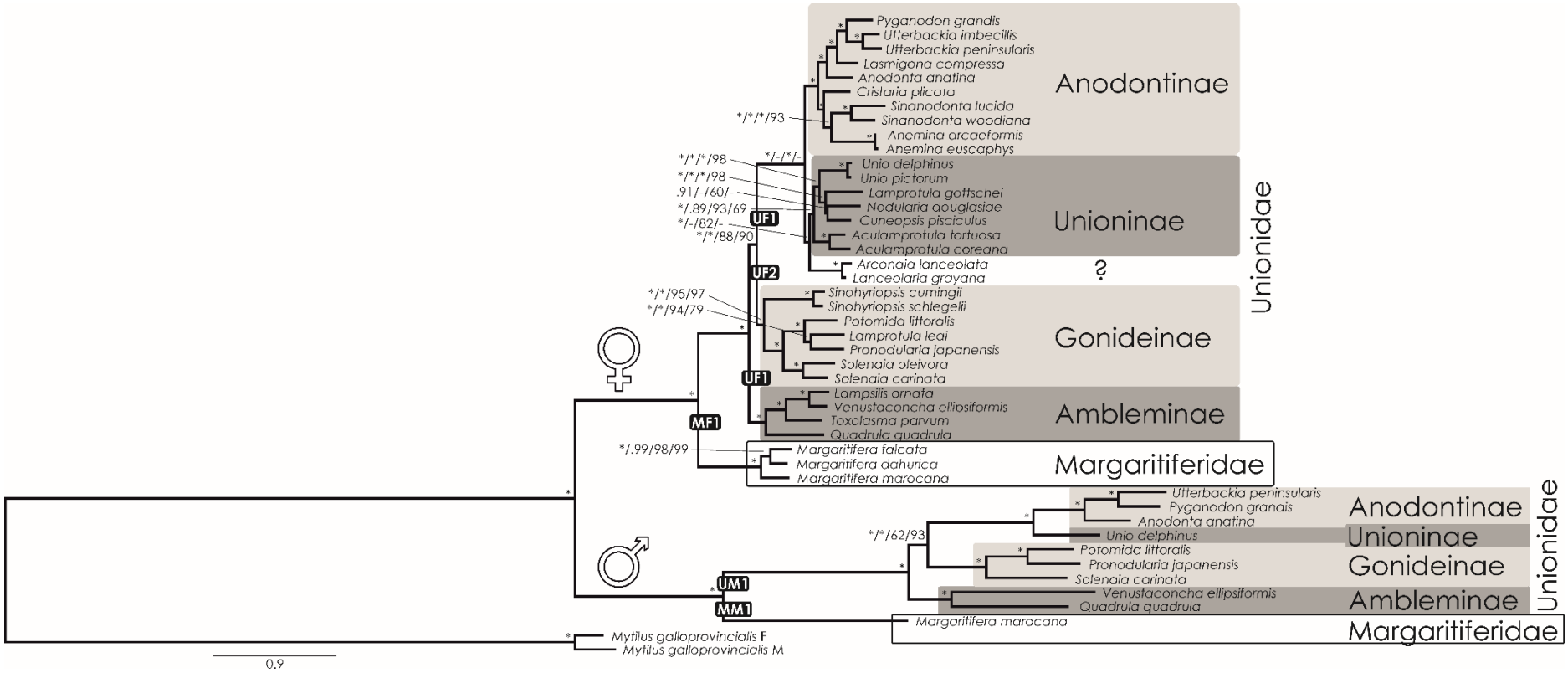

Figure 2 Phylogenetic (BI-NUC) tree of Unionida estimated from 14 concatenated individual mtDNA gene sequences (12 protein-coding and 2 rRNA genes). Values for branch support are represented in the following order: (1) Bayesian posterior probabilities (PP) for BI-NUC tree, (2) Bayesian PP for BI-AA tree, (3) ML bootstrap support (BS) values for ML-NUC and (4) ML BS values for ML-AA tree. Maximum support values (PP = 1, BS = 100) are represented by asterisks. All five distinct detected gene orders are mapped on the phylogeny branches (see Fig. 1 for gene order codes).

Mapping gene orders over the inferred mtDNA phylogeny suggests that UF1 might be ancestral within Unionidae and UF2 derived in the ancestral lineage of the Gonideinae. However, these hypotheses have limited support, because no mitogenome sequences, and therefore no gene order information, are available for three of the seven presently recognized unionid subfamilies. Future inclusion of mtDNA gene orders of these currently unrepresented subfamilies could change the inference of the ancestral and derived mtDNA gene orders within Unionidae. In the M-type, only one gene arrangement per family is obtained: UM1 for the Unionidae and MM1 for the Margaritiferidae. Since the Unionida are a very old order (Graf & Cummings 2007), and as a consequence of the several distinct mitogenome gene arrangements already found, it is likely that as novel mitogenomes from additional unionoid families and subfamilies become available, the corresponding gene orders might be useful to resolve their phylogenetic relationships within the order.

Acknowledgements

This work was partially supported by the Mohamed bin Zayed Species Conservation Fund under project 'Biodiversity and conservation of the critically endangered freshwater mussels in Morocco: ecogeographic, genetic and physiological information' (Reference 15256799), by the IUCN SOS Save Our Species fund under project 'Breeding the most endangered bivalve on Earth: *Margaritifera marocana*' (Ref. 2015B-015) and by the Portuguese Foundation for Science and Technology (FCT) under grants SFRH/BPD/70654/2010 (MMF), SFRH/BPD/108445/2015 (EF), and SFRH/BD/115728/2016 (MLL).

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CHAPTER 8

Evolution of mitogenome rearrangements in freshwater mussels

Paper VII

Mesozoic mitogenome rearrangements and freshwater mussel (Bivalvia: Unionoidea) macroevolution

Froufe F, Bolotov I, Aldridge DC, Bogan AE, Breton S, Gan HM, Kovitvadhi U, Kovitvadhi S, Riccardi N, Secci-Petretto G, Sousa R, Teixeira A, Varandas S, Zanatta D, Zieritz A, Fonseca MM, **Lopes-Lima M**

Article published in *Heredity* **124**, 182-196 (2020).
DOI: 10.1038/s41437-019-0242-y

Mesozoic mitogenome rearrangements and freshwater mussel (Bivalvia: Unionoidea) macroevolution

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Abstract

Using a new fossil-calibrated mitogenome-based approach, we identified macroevolutionary shifts in mitochondrial gene order among the freshwater mussels (Unionoidea). We show that the early Mesozoic divergence of the two Unionoidea clades, Margaritiferidae and Unionidae, was accompanied by a synchronous split in the gene arrangement in the female mitogenome

(i.e. gene orders MF1 and UF1). Our results suggest that this macroevolutionary jump was completed within a relatively short time interval (95% HPD 201-226 Ma) that coincided with the Triassic-Jurassic mass extinction. Both gene orders have persisted within these clades for ~200 Ma. The monophyly of the so-called “problematic” Gonideinae taxa was supported by all the inferred phylogenies in this study using, for the first time, the M- and F-type mitogenomes either singly or combined. Within Gonideinae, two additional splits in the gene order (UF1 to UF2, UF2 to UF3) occurred in the Mesozoic and have persisted for ~150 and ~100 Ma, respectively. Finally, the mitogenomic results suggest ancient connections between freshwater basins of East Asia and Europe near the Cretaceous-Paleogene boundary, probably via a continuous paleo-river system or along the Tethys coastal line, which are well supported by at least three independent but almost synchronous divergence events.

Introduction

The tempo, timing, and mode of evolution have attracted considerable debate among evolutionary biologists. Here we use a new approach using mitogenome rearrangements to investigate changes at the geological time scale in the speciose and imperilled freshwater mussels.

In many taxonomic groups, the gene arrangement within mitogenomes is highly conserved, e.g. many vertebrate groups share the same gene order (Pereira 2000). Other faunal groups, such as the Bivalvia, exhibit several different mitochondrial gene arrangements (e.g. Yuan et al 2012), which are the result of different mechanisms such as tandem duplication followed by gene loss (Boore 2000). Although local homoplastic arrangements have been identified in some invertebrate groups (e.g. Flook & Rowel 1995; Dowton & Austin 1999), complete gene orders generally remain unique and represent signatures with diagnostic value (Basso et al 2017), providing a powerful signal for inferring ancient evolutionary relationships (Boore 2000).

Among freshwater mussels of the order Unionida, which spans about 900 species and represents the major bivalve radiation in the freshwater environment (Lopes-Lima et al 2017a, 2018a), five mitogenome rearrangements have been described so far (Lopes-Lima et al 2017b). Within the superfamily Unionoidea (Margaritiferidae + Unionidae), the mitochondria are furthermore unusual in that two highly divergent mtDNA molecules exist in males (Female or F- and Male or M-type) as a result of Doubly Uniparental Inheritance (DUI) (Zouros et al 1994; Breton et al 2009). This contrasts with most animal taxa, which inherit their mtDNA exclusively through the maternal lineage and thus exhibit only F-type mtDNA. In Unionoidea males, M-type mtDNA is restricted to the gonadal tissue inherited from the paternal lineage, and F-type mtDNA is present in all somatic tissues transmitted from the maternal lineage and also in female gonadal tissue (Breton et al 2009; Froufe et al 2016; Fonseca et al 2016; Lopes-Lima et al 2017b).

In recent decades, complete mitochondrial genome sequences have been published for a wide range of taxa, enabling reconstruction of shallow and deep phylogenies in both vertebrates and invertebrates (e.g. Jacobsen et al 2014; Liu et al 2016). However, the number of available mitogenomes for Unionida is low, particularly for M-type genomes (Froufe et al 2016; Fonseca et al 2016; Lopes-Lima et al 2017b; Huang et al 2019). A further shortcoming is that published mitogenomes are restricted to only a few higher Unionida taxa, with no mitogenomes being available for several families and subfamilies. In fact, of the six recognized Unionida families (Lopes-Lima et al 2014), published mitogenomes are essentially restricted to the Unionoidea (Unionidae + Margaritiferidae) with a distribution predominantly within the Northern Hemisphere. While some studies have questioned the monophyly of the Unionoidea

(e.g. Combosch et al 2017; Whelan et al 2011) the most comprehensive recent studies, using either full mitogenomes (Huang et al 2019; Wu et al 2019) or hundreds of nuclear loci (Pfeiffer et al 2019) support its monophyletic status. Moreover, mitogenome-based Unionida phylogenies reconstructed to date have been based on either F- or M-type mitogenomes (Froufe et al 2016; Fonseca et al 2016; Lopes-Lima et al 2017b). Although in these studies the highly divergent F- and M-type mitogenomes recovered identical phylogenies, concatenated phylogenetic analyses of M- and F-type datasets would be expected to recover a more robust phylogeny.

The Unionidae is the most species-rich Unionida family, comprising 620 species in several subfamilies and distributed widely (Lopes-Lima et al 2017a). However, phylogenetic relationships within and between Unionidae subfamilies are still contentious and different phylogenies have been resolved with different analysed markers (e.g. Lopes-Lima et al 2017a; Bolotov et al 2017a).

One of the least studied Unionidae subfamilies, the Gonideinae, has a scattered distribution in the Northern Hemisphere (Lopes-Lima et al 2017a). Species in this subfamily have suffered major declines, and half of the assessed Gonideinae species are currently listed as Near Threatened or Threatened (IUCN 2019). Moreover, 70% of recognized Gonideinae species have either never been assessed or are listed as Data Deficient by the IUCN Red List (IUCN 2019), indicating an urgent need for research into this family's diversity, distribution, and ecology.

Another outcome of the general lack of data on Gonideinae is their unresolved phylogeny. Monophyly of this sub-family is disputed. The first molecular study to include the so-called "problematic" Gonideinae taxa (Graf 2002) only examined the type species, i.e. *Gonidea angulata* (Lea 1838). Subsequent studies included several additional Gonideinae taxa but the clade Gonideinae was never recovered as monophyletic (Graf & Cummings 2006; Whelan et al 2011; Pfeiffer & Graf 2013). More recently, multi-marker and mitogenomic approaches have consistently recovered Gonideinae as monophyletic (Huang et al 2013; Pfeiffer & Graf 2015; Fonseca et al 2016; Froufe et al 2016; Lopes-Lima et al 2017a,b). Bolotov et al (2017a,b) subsequently elevated one of the four Gonideinae tribes established by Lopes-Lima et al (2017a), i.e. Pseudodontini, to the subfamily level (i.e. Pseudodontinae).

A good understanding of the evolutionary biogeography of the Gonideinae can be fundamental for reconstructing patterns of connections of freshwater systems through space and time on a global scale. Our knowledge in this respect is still far from complete. The first biogeographic scenarios developed using Unionida data (e.g. Starobogatov 1970; Banarescu 1991) proved highly inaccurate, as they were mostly descriptive and based solely on the (dis-)similarity between unionid faunas. Furthermore, these scenarios were generated at a time when unionid taxonomy was poorly resolved and included numerous paraphyletic higher-order

taxa as well as nominal taxa, determined by shell shape rather than reliable indicators of true biological species (e.g. Bolotov et al 2017a; Konopleva et al 2017). Modern paleontology-based models seem to be much more reliable. Based on the fossil record from Vietnam, Schneider et al (2013) developed the hypothesis of the independent development of Unionida faunas in the Yangtze and Mekong basins, at least during the entire Cenozoic. Also, Van Damme et al (2015) showed that the African Early Cretaceous Unionida are representatives of Asian/Eurasian taxa with the lack of Gondwanan elements, while the African Jurassic assemblages are distinctly related to those in Eurasia.

Recently, a first statistical biogeographic model for the Unionidae at the global level indicated that the Unionidae most likely originated in Southeast and East Asia in the Jurassic, with the earliest expansions into North America and Africa (since the Albian), following the colonization of Europe and India (Bolotov et al 2017a). However, the Jurassic fossil record of western North America (for a review see Watters 2001) and Africa (Van Damme et al 2015) indicate that these continents were colonized before the Cretaceous. Additionally, two species-rich monophyletic mussel radiations with an early Cenozoic or even pre-Cenozoic origin were discovered within the paleo-Mekong catchment (Bolotov et al 2017a,b). These findings revealed that the largest river systems (e.g. the Mekong, Yangtze, and Mississippi) may represent ancient evolutionary hotspots of freshwater mussels (Scholz & Glaubrecht 2004; Wesselingh 2007).

Based on the most comprehensive data set of mitogenomes sampled to date, including eight newly sequenced mitogenomes, this paper aims to improve our understanding of the higher-order phylogeny and classification of Unionidae by the following: (1) testing the monophyly of the poorly known Gonideinae subfamily using both full F- and M- mitogenomes and, for the first time, mitogenomes concatenated; (2) estimating macroevolutionary patterns in freshwater mussels of the Unionidae using, for the first time, a fossil-calibrated mitogenomic approach; (3) estimating the timing of major divergence events and comparing them to those of mitogenome rearrangements; and (4) developing an updated integrative approach to the systematics of Unionidae, based on the mitogenomic results. This will allow the reconstruction of the potential origin and ancient radiations of the Unionidae and detect the most probable ancestral areas.

Methods

Sampling, DNA extractions, sequencing, assembly, and annotation

One male specimen of *Chamberlainia hainesiana*, *Microcondylaea bonellii*, *Pilsbryoconcha exilis* and *Monodontina vondembuschiana* were dissected for the sampling of gonadal (to recover M-type mtDNA) and mantle (to recover F-type mtDNA) tissues. DNA extractions

followed Froufe et al (2016). The complete M- and F-type mitogenome sequencing and assemblage followed Gan et al (2014), while annotations were performed using MITOS (Bernt et al 2013). The final limits of tRNA genes were rechecked with ARWEN (Laslett & Canbäck 2008). All F- and M-mitogenomes have been deposited in the GenBank database under the accession numbers MK994770-MK994777 and were visualized using GenomeVx (Conant & Wolfe 2008).

DNA (NUC) and amino acid (AA) sequences of all mtDNA protein-coding genes (PCG), except ATP8 and the gender-specific open reading frames (M-ORF, H-ORF, and F-ORF; Breton et al 2011), were used in the phylogenetic analyses. The sequences of each gene were aligned using MAFFT software (version 7.304, Katoh & Standley 2013) and trimmed with GUIDANCE2 (Sela et al 2015; see Froufe et al (2016) for the parameters used).

The gene alignments were then concatenated, resulting in two alignments with the following length: 13,449 aligned nucleotide positions and 3,870 aligned amino acid positions + 1,889 aligned nucleotide positions from the rRNA genes. The optimal partitioning scheme for each alignment was selected using PartitionFinder v. 2.1.1 software (Lanfear et al 2016) under the greedy algorithm with proportional branch lengths across partitions. The best substitution models of DNA and protein evolution for each partition were selected under the BIC ranking method (Schwarz 1978). The codon positions of the protein-coding genes and each rRNA were defined as the initial data blocks for the partitioning schemes search.

An additional data set was also created, concatenating both F- and M-type gene alignments, with the following length: 26,780 aligned nucleotide positions and 7,661 aligned amino acid positions + 3,797 aligned nucleotide positions from the rRNA genes. This alignment included 45 Unionida species plus *Mytilus galloprovincialis* as an outgroup (Table 1) using the same partitioning method and model selection as described above.

Phylogenetic analyses

All phylogenetic analyses were performed using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML analyses were performed using RAxML (v. 8.0.0, Stamatakis 2014) with 100 rapid bootstrap replicates and 20 ML searches. The BI was applied using MrBayes v. 3.2.7a (Ronquist et al 2012) with two independent runs (10^7 generations with a sampling frequency of 1 tree for every 100 generations), each with four chains (3 hot and 1 cold). All runs reached convergence (average standard deviation of split frequencies below 0.01). The posterior distribution of trees was summarized in a 50% majority-rule consensus tree (burn-in of 25%).

Table 1
List of specimens analysed (based on Lopes-Lima et al 2017a,b), GenBank references, and country

TAXON	CODE	F-TYPE GenBank	M-TYPE GenBank	COUNTRY
UNIONIDA				
UNIONIDAE				
AMBLEMINAE				
Lampsilis ornata	LamOrn	NC_005335	-	USA
Leptodea leptodon	LepLeo	NC_028522	-	China (Introduced)
Potamilus alatus	PotAla	KU559011	KU559010	China (Introduced)
Quadrula quadrula	QuaQua	NC_013658	FJ809751	USA
Toxolasma parvum	TaxPar	NC_015483	-	USA
Venustaconcha ellipsiformis	VenEll	FJ809753	NC_013659	USA
GONIDEINAE				
CHAMBERLAINIINI				
Chamberlainia hainesiana	ChaHai	MK994770	MK994771	Thailand
Sinohyriopsis cumingii	SinCum	NC_011763	KC150028	China
Sinohyriopsis schlegelii	SinSch	NC_015110	-	China (Introduced)
GONIDEINI				
Microcondylaea bonellii	MicBon	MK994772	MK994773	Italy
Ptychorhynchus pfisteri	PtyPfi	KY067440	-	China
Solenaia carinata	SolCar	NC_023250	KC848655	China
Solenaia oleivora	SolOle	NC_022701	-	China
LAMPROTULINI				
Lamprotula leai	LamLea	NC_023346	-	China
Lamprotula scripta	LamScr	NC_030258	-	China
Potomida littoralis	PotLit	NC_030073	KT247375	Portugal
Pronodularia japanensis	ProJap	AB055625	AB055624	Japan
PILSBRYOCONCHINI				
Pilsbryoconcha exilis	PilExi	MK994776	MK994777	Malaysia
Monodontina vondembuschiana	PseVon	MK994774	MK994775	Malaysia
UNIONINAE				

Aculamprotula tientsinensis	AcuTie	NC_029210	-	China
Aculamprotula coreana	AcuCor	NC_026035	-	South Korea
Aculamprotula tortuosa	AcuTor	NC_021404	-	China
Anemina arcaeformis	AneArc	NC_026674	-	China
Anemina euscaphys	AneEus	NC_026792	-	China
Anodonta anatina	AnoAna	NC_022803	KF030962	Poland
Cristaria plicata	CriPli	NC_012716	-	China
Cuneopsis pisciculus	CunPis	NC_026306	-	China
*'Lamprotula gottschei'**	LamGot	NC_023806	-	China
Lanceolaria grayana	LanGra	NC_026686	-	China
Lanceolaria lanceolata	ArcLan	NC_023955	-	China
Lasmigona compressa	LasCom	NC_015481	-	USA
Lepidodesma languilati	LepLan	NC_029491	-	China
Nodularia douglasiae	NodDou	NC_026111	-	China
Pyganodon grandis	PygGra	NC_013661	FJ809755	USA
Sinanodonta lucida	SinLuc	NC_026673	-	China
Sinanodonta woodiana	SinWoo	HQ283348	KM434235	China
Unio crassus	UniCra	KY290447	KY290450	Poland
Unio delphinus	UniDel	KT326917	KT326918	Portugal
Unio pictorum	UniPic	NC_015310	-	Poland
Unio tumidus	UniTum	KY021076	KY021073	Poland
Utterbackia imbecillis	UttImb	NC_015479	-	USA
Utterbackia peninsularis	UttPen	HM856636	NC_015477	USA
MARGARITIFERIDAE				
Margaritifera dahurica	MarDah	NC_023942	-	China
Margaritifera falcata	MarFal	NC_015476	-	USA
Pseudunio marocanus	PseMrc	KY131953	KY131954	Morocco
MYTILIDA				
Mytilus galloprovincialis	MytGal	AY497292	AY363687	Greece

Divergence time estimates

The time-calibrated mitogenomic phylogeny was reconstructed in BEAST v. 1.8.4 based on two reliable fossil calibrations (Supplementary Table 1) using a lognormal relaxed clock algorithm with the Yule speciation process as the tree prior (Drummond et al 2006, 2012; Drummond & Rambaut 2007). Calculations were performed at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller et al 2010). The sample of M-type mitogenomes was used as an outgroup. Similar settings to each gene partition as in the MrBayes analyses were specified but using a simplified evolutionary model (HKY; see Bolotov et al 2017a for details). Five replicate BEAST searches were conducted, each with 5×10^7 generations and a tree sampling every 5,000th generation. The log files were checked visually with Tracer v. 1.7 for an assessment of the convergence of the MCMC chains and the effective sample size of parameters (Rambaut et al 2018). The chains in one run did not reach the convergence and were excluded, the other runs were compiled with LogCombiner v. 1.8.4 (Drummond et al 2012) using an appropriate burn-in depending on the start of convergence of MCMC chains in each run. Most of ESS values were recorded as > 300, with a few ESS values > 100. The maximum clade credibility tree was obtained from the post-burn-in trees using TreeAnnotator v. 1.8.4 (Drummond et al 2012).

Ancestral gene order and ancestral area reconstructions

TreeREx (Bernt et al 2008) was used for inferring the most parsimonious putative ancestral gene orders and gene rearrangements along the obtained Unionidae F-haplotype phylogenetic sub-tree with the default settings (http://pacosy.informatik.uni-leipzig.de/185-0-TreeREx.html). Ancestral area reconstruction models were calculated for the Unionidae using three different approaches, i.e. Statistical Dispersal-Vicariance Analysis (S-DIVA), Dispersal-Extinction Cladogenesis (Lagrange configurator, DEC), and Statistical Dispersal-Extinction Cladogenesis (S-DEC) implemented in RASP v. 3.2 (Yu et al 2015) following Bolotov et al (2017a). Margaritiferidae were not used in this analysis due to the limited number of available mitogenomes. Four possible distribution areas of the in-group taxa were coded as follows: (A) Southeast Asia, (B) East Asia, (C) North America, and (D) Europe. From the input matrix, two geographically unreliable constrains (AC and AD) were excluded.

Results

Mitogenome characteristics and gene arrangements

All eight sequenced haplotypes include the 13 protein-coding genes (PCGs) typically found in metazoan mitochondrial genomes, the sex-specific ORF described for all Unionida mitogenomes with DUI system (Breton et al 2009, 2011) and 22 transfer RNA (tRNA) and two

ribosomal RNA (rRNA) genes (Fig. 1). As expected, the length of the four newly sequenced M-type mitogenomes is larger than the corresponding F-type (Breton et al 2009), ranging from 16,267 bp in *P. exilis* to 17,465 bp in *C. hainesiana*, while the F-type ranged from 16,020 bp in *M. bonellii* to 16,746 bp in *C. hainesiana* (Table 2). The A + T content, and GC and AT skews are similar in all sequenced species in both F- and M- mtDNA types, averaging around 60%, 37 (+) and -0.23 (+), respectively (Table 2).

The gene arrangements of *Microcondylaea bonellii*, *P. exilis,* and *Monodontina vondembuschiana* are identical to all Gonideinae mitogenomes available on GenBank (2018), named UF2 (Lopes-Lima et al 2017b). However, *C. hainesiana* has a new and distinct gene arrangement, here named UF3 (Fig. 2).

Phylogenetic analyses

All the phylogenies inferred in this study that are based on M and F mitogenomes alone (i.e. not combined) support the monophyly of Gonideinae (Fig. 3). Moreover, the four tribes Chamberlainiini, Gonideini, Lamprotulini, and Pilsbryoconchini, are also monophyletic in both M- and F-type trees (Fig. 3). The same results were obtained when using for the first time the M and F mitogenomes combined, despite the lower number of species (Fig. 4). The only unsupported result on the topology is seen in the relationship among the tribes Gonideini, Pilsbryoconchini and Lamprotulini in the ML AA data set (Fig. 4).

Ancestral gene order and ancestral area reconstructions

The TreeREx analysis indicated that the evolution of gene orders in the Unionidae F-type mtDNA is characterized by two independent events of tandem duplication and random loss (TDRL), with every ancestral gene order showing the highest consistency scores. The analysis suggests that the ancestral gene order for Unionidae F mitogenome is UF1, which is also found in the contemporary species of the subfamilies Ambleminae and Unioninae (Fig. 5). The fossil-calibrated mitogenomic analysis placed the split between the UF1 and MF1 gene orders in the Late Triassic (mean age = 208 Ma, 95% high posterior density (HPD) 201-226 Ma) (Fig. 6). The ancestral gene order of the Gonideinae species represented in our study is UF2, which results from a TDRL event of an mtDNA segment involving nad3, trnH, trnA, trnS2, trnS1, trnE, nad2, and trnM (Fig. 2 Box A). In UF2, the genes trnH, trnS1, nad2, and trnM pertain to the original segment, while the remaining genes-nad3, trnA, trnS2, and trnE-are present in the duplicated segment (Fig. 2 Box A). The fossil-calibrated model developed suggests that the UF1 and UF2 gene orders split near the Jurassic-Cretaceous boundary (mean age=149 Ma, 95% HPD 138-162Ma) (Fig. 6).

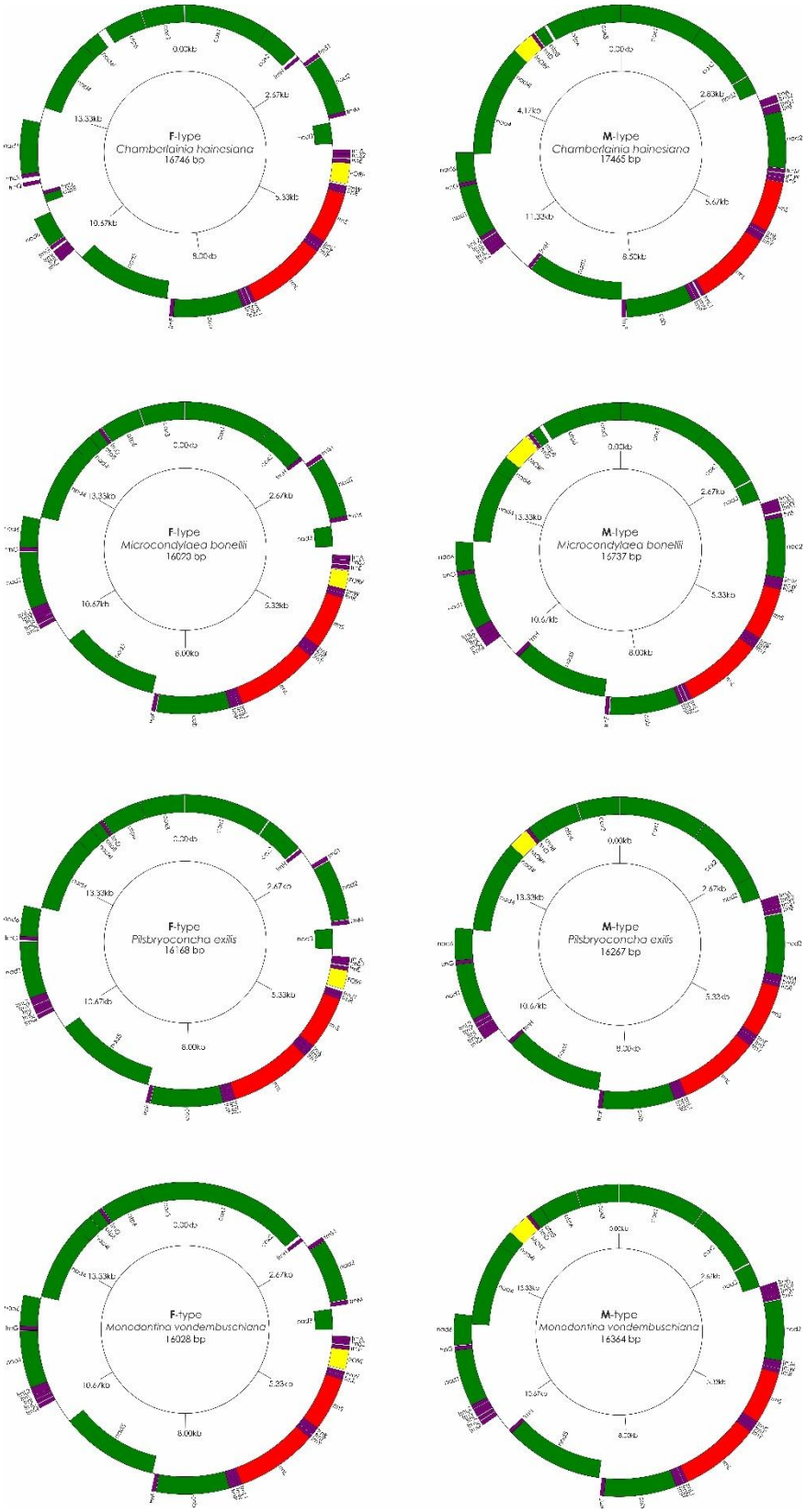

Figure 1 Gene maps of the F- and M-type mitochondrial genomes of *Chamberlainia hainesiana*, *Microcondylaea bonellii*, *Pilsbryoconcha exilis,* and *Monodontina vondembuschiana*. Genes positioned inside the circle are encoded on the heavy strand, and genes outside the circle are encoded on the light strand. Colour codes: Small and large ribosomal RNAs (red), transfer RNAs (purple), FORF F-specific open reading frame (yellow), MORF M-specific open reading frame (yellow), PCGs genes (green)

Table 2
Main structural features of the female (above) and male (below) transmitted mitochondrial genomes of Gonideinae species

	Chamberlainiini			Gonideini			
♀	***C. hainesiana***	*S. cumingii*	*S. schlegelii*	***M. bonellii***	*P. pfisteri*	*S. carinata*	*S. oleivora*
Tot. size (pb)	**16,746**	15,954	15,939	**16,020**	16,040	16,716	16,392
A + T %	**58.10**	60.24	60.30	**62.00**	60.77	60.89	59.93
GC (+) skew	**0.37**	0.36	0.35	**0.35**	0.36	0.39	0.37
AT (+) skew	**-0.29**	-0.23	-0.23	**-0.20**	-0.22	-0.22	-0.22
♂	***C. hainesiana***	*S. cumingii*		***M. bonellii***		*S. carinata*	
Tot. size (pb)	**17,465**	17,100		**16,737**		17,102	
A + T %	**62.35**	59.71		**59.79**		61.01	
GC (+) skew	**0.43**	0.41		**0.35**		0.38	
AT (+) skew	**-0.24**	-0.27		**-0.26**		-0.27	

	Lamprotulini				Pilsbryoconchini	
♀	*L. leai*	*L. scripta*	*P. littoralis*	*P. japanensis*	***P. exilis***	***M. vondembuschiana***
Tot. size (pb)	16,530	16,250	15,789	16,826	**16,168**	**16,028**
A + T %	60.28	58.95	58.23	57.20	**60.72**	**58.97**
GC (+) skew	0.37	0.36	0.36	0.36	**0.37**	**0.38**
AT (+) skew	-0.21	-0.23	-0.23	-0.21	**-0.22**	**-0.24**
♂			*P. littoralis*	*P. japanensis*	***P. exilis***	***M. vondembuschiana***
Tot. size (pb)			16,451	16,967	**16,267**	**16,364**
A + T %			58.93	57.12	**61.90**	**59.55**
GC (+) skew			0.34	0.36	**0.35**	**0.37**
AT (+) skew			-0.24	-0.25	**-0.25**	**-0.26**

Newly sequenced species are presented in bold

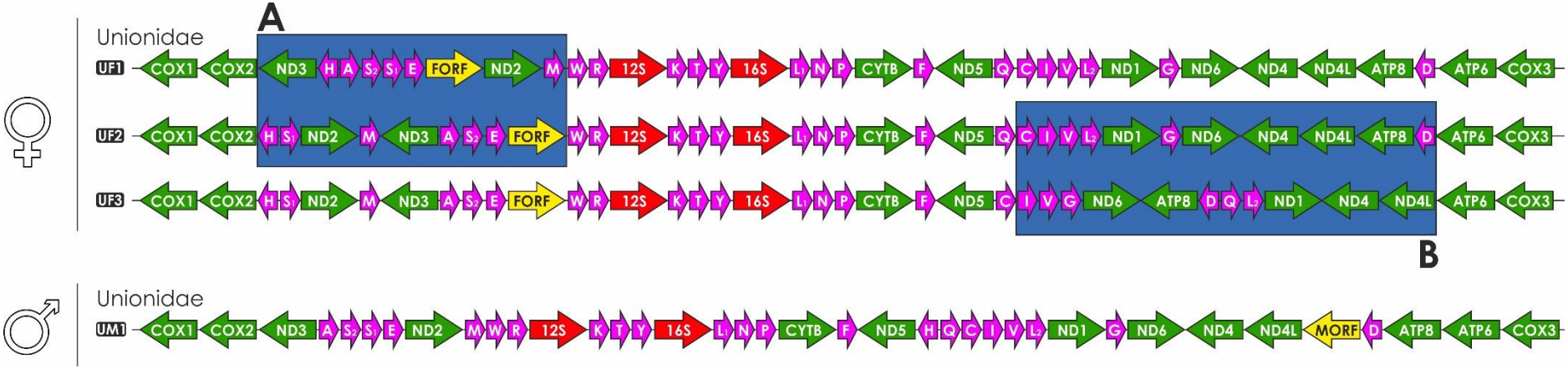

Figure 2 Diagrams of the four distinct gene orders known in Unionidae to date. In the F-type, three gene orders are depicted: UF1, UF2, and UF3. In the male M-type lineage, the only Unionidae gene arrangement is shown: M-type 1 (UM1). Blue boxes highlight the gene rearrangement region from UF1 to UF2 (Box A) and from UF2 to UF3 (Box B). Small and large ribosomal RNAs and transfer RNAs are depicted by one letter of the amino acid code; Arrow colour codes, follow Fig. 1

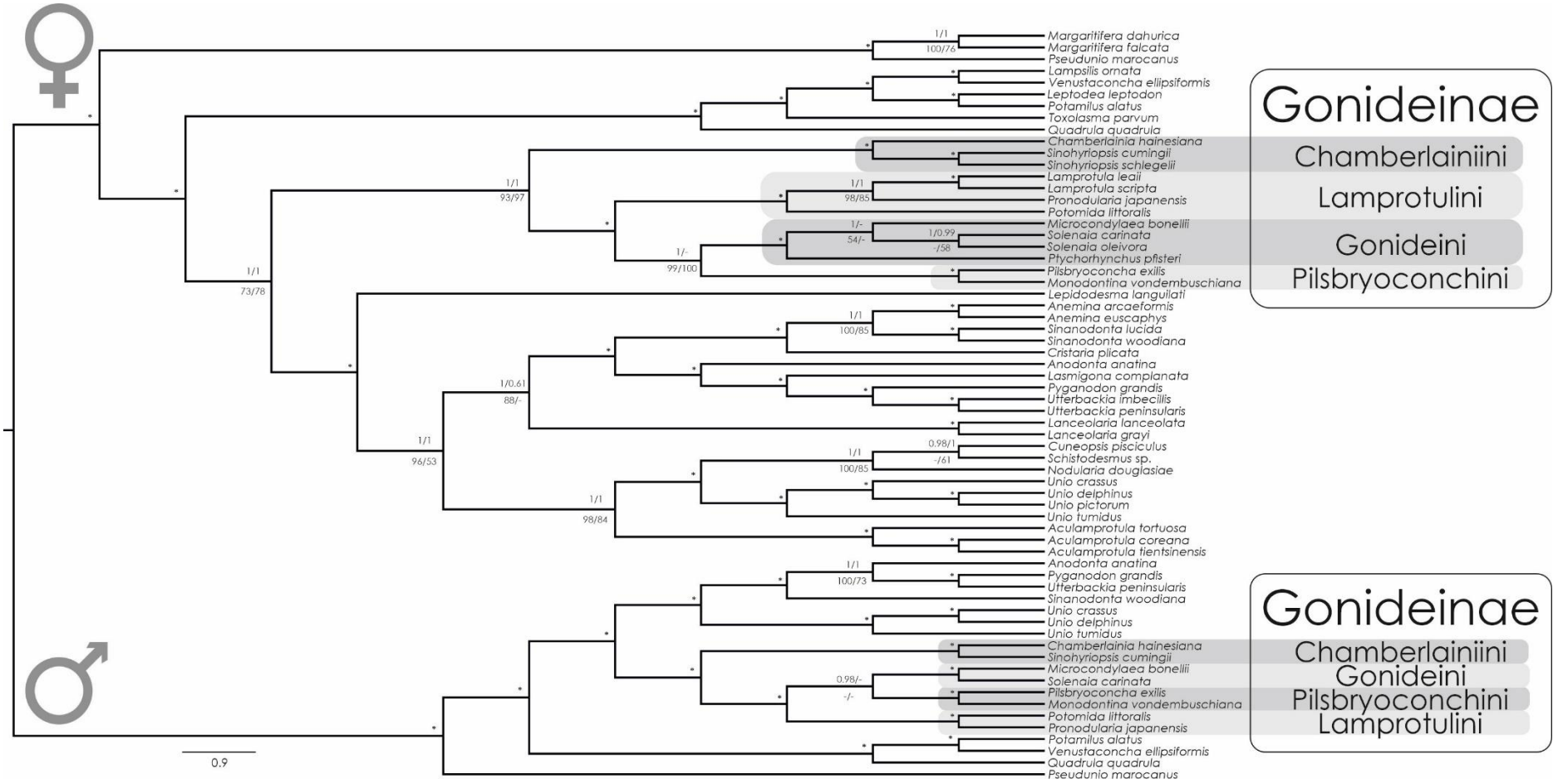

Figure 3 Phylogenetic (BI-NUC) tree of Unionida estimated from 14 concatenated individual mtDNA gene sequences (12 protein-coding and 2 rRNA genes). Values for branch support are represented in the following order: (1) Bayesian posterior probabilities (PP) for BI-NUC tree, (2) Bayesian PP for BI-AA tree, (3) ML bootstrap support (BS) values for ML-NUC and (4) ML BS values for ML-AA tree. Maximum support values (PP = 1, BS = 100) are represented by asterisks. Gonideinae subfamily and tribes are highlighted. For details see text. GenBank codes in Table 1

Finally, the UF3 gene order also arises after a TDRL event within Gonideinae (Fig. 2 Box B). It involved an mtDNA segment containing twelve genes: trnQ, trnC, trnI, trnV, trnL2, nad1, trnG, nad6, nad4, nad4l, atp8 and trnD. In UF3, the genes trnC, trnI, trnV, trnG, nad6, atp8, and trnD are retained in the original segment, whilst genes trnQ, trnL2, nad1, nad4, and nad4l were not lost in the duplicated one (Fig. 2 Box B). The fossil-calibrated model placed the split between the UF2 and UF3 gene orders in the Cretaceous near the Albian-Cenomanian boundary (mean age = 102 Ma, 95% HPD 77-124 Ma) (Fig. 6).

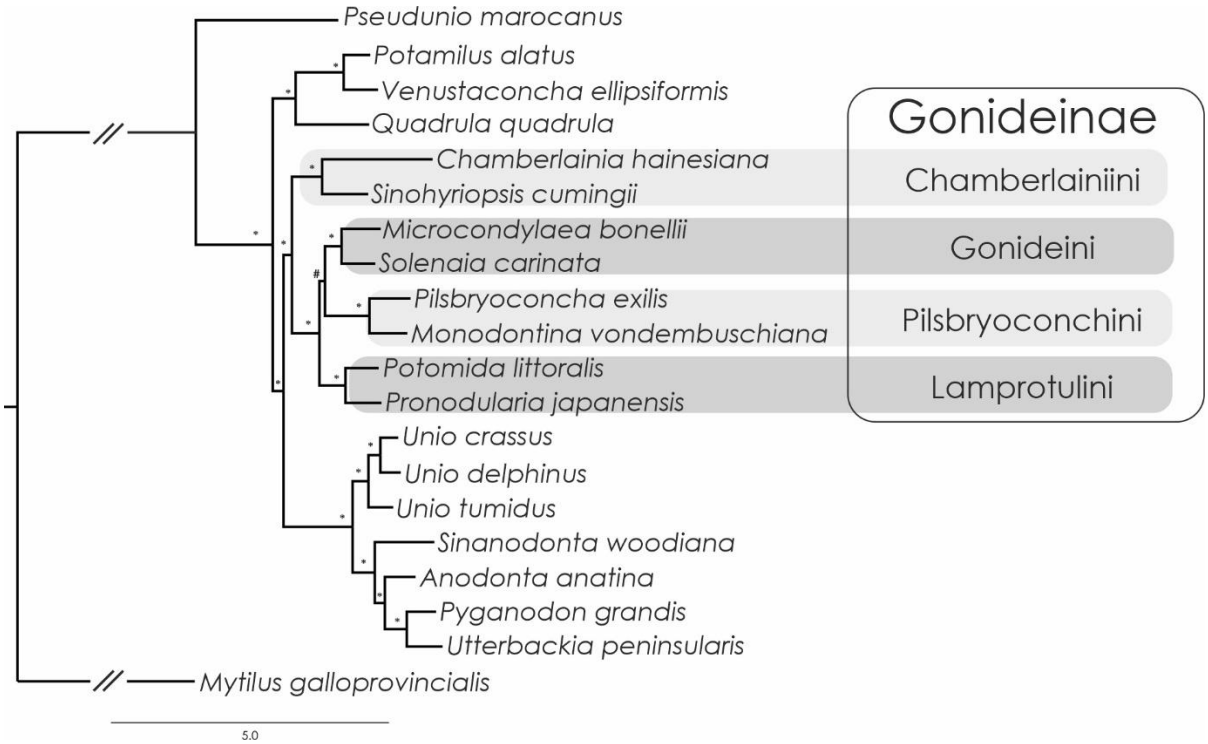

Figure 4 Phylogenetic (BI-NUC) tree of Unionida estimated from 28 concatenated individual mtDNA gene sequences (24 protein-coding and 4 rRNA genes) of the first combined Female + Male concatenated data set. Maximum branch support values (BI-NUC/BI-AA PP = 1; ML-NUC/ML-AA BS = 100) are represented by asterisks, while # represents the only non-supported branch by ML-AA tree. Gonideinae subfamily and tribes are highlighted. GenBank codes in Table 1

The combined ancestral area reconstruction model suggests that the Most Recent Common Ancestor (MRCA) of the crown group of the Ambleminae + (Gonideinae + Unioninae) clade used to be widely distributed across the supercontinent of Laurasia (probability 100%) (Fig. 7). The earliest split was between the Laurentian (Ambleminae) and Eurasian (Gonideinae + Unioninae) taxa. This vicariance event is placed in the Late Jurassic (mean age = 159 Ma, 95% HPD 155-170 Ma). Early diversification of the Gonideinae + Unioninae clade is placed within East Asia (probability 100%; Fig. 7). The origin of the MRCA of this large clade (mean age = 149 Ma, 95% HPD 138-162 Ma) and subsequent splitting into two subclades (mean ages of crown groups = 137 and 106 Ma and 95% HPD 123-152 and 90-124 Ma for Gonideinae

and Unioninae, respectively) most likely resulted from an intra-area radiation (probability 100% in each case) during the early Cretaceous. The Yangtze and Mekong unionid faunas have likely been separated since the Albian (mean ages = 95-102 Ma, 95% HPD 77-124 Ma) (Fig. 7).

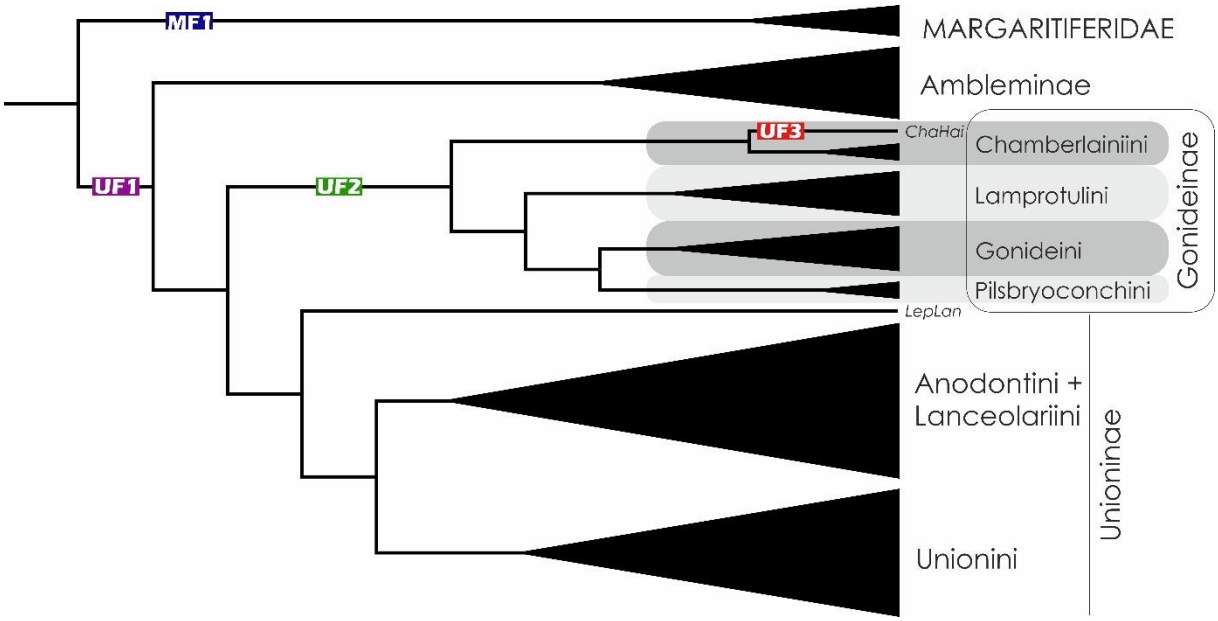

Figure 5 Unionidae F-haplotype phylogenetic sub-tree (BI-NUC) used to infer the most parsimonious putative ancestral gene orders and gene rearrangements mapped as MF1, UF1, UF2, and UF3 (see text for details). Margaritiferidae and all subfamily nodes were collapsed for visual purposes

Discussion

Phylogenetic patterns

The new mitogenomic results presented here place the Pilsbryoconchina subtribe (previously under the Pseudodontinae as described by Bolotov et al 2017a) as a subclade within the monophyletic Gonideinae in both the M- and F-type phylogenies. Our results are thus in agreement with the phylogeny recovered by Lopes-Lima et al (2017a), which is also supported by morphological data. However, the recovered results contradict that of Bolotov et al (2017a,b), which suggested elevation of the Pseudodontini to the subfamily level. Our results further indicate that the number of recognized subfamilies within the Unionidae is most likely lower than has been suggested by recent phylogenetic studies (Lopes-Lima et al 2017a,b; Bolotov et al 2017a,b).

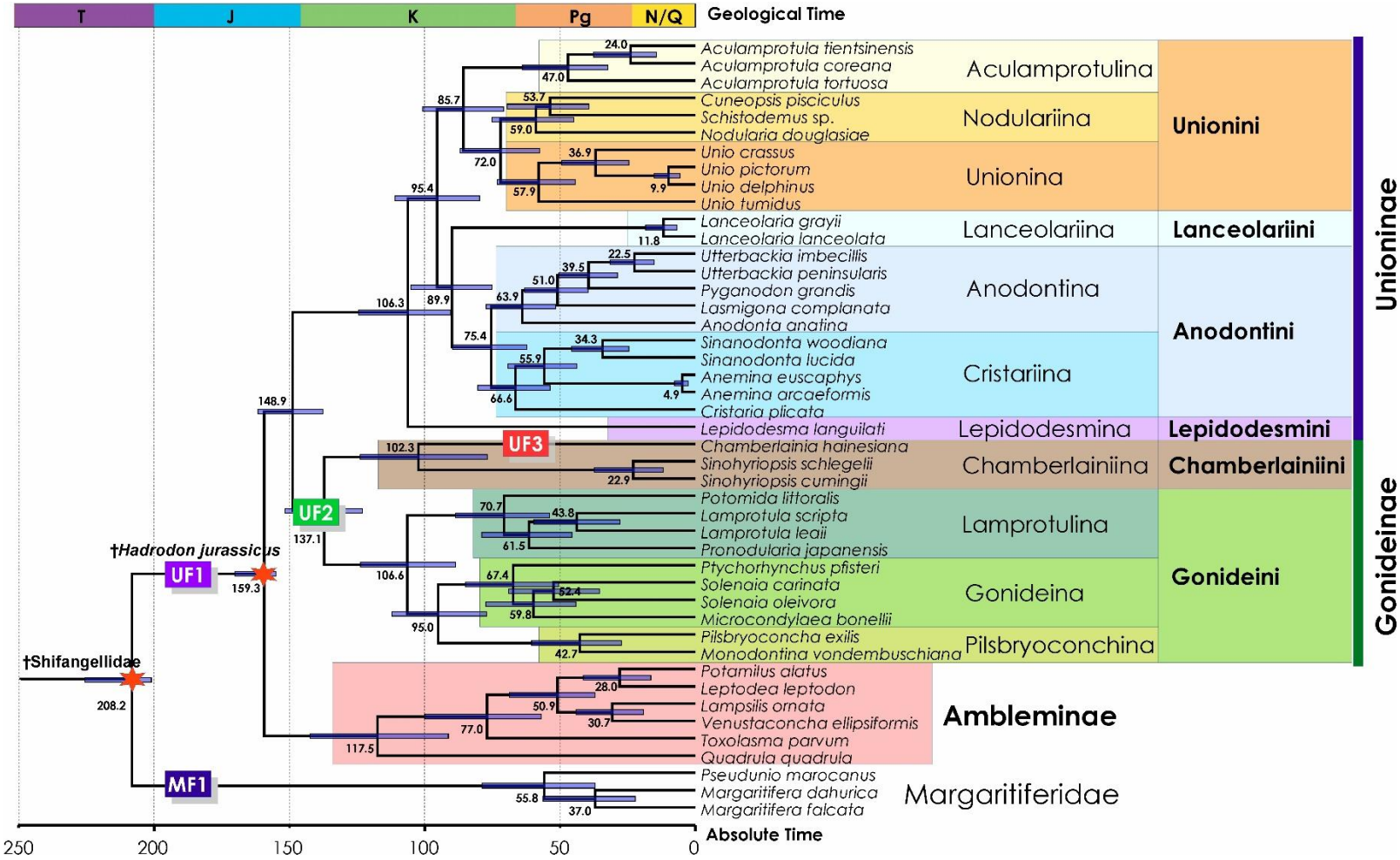

Figure 6 Time-calibrated mitogenomic phylogeny, an example of the three-level classification scheme (subfamilies, tribes, and subtribes) and evolution of the mitochondrial gene order in the Unionoidea. Fossil-calibrated ultrametric chronogram of the Unionoidea calculated under a lognormal relaxed clock model and a Yule process speciation implemented in BEAST and obtained for the complete mitogenome data set. The outgroup sample is not shown. Bars indicate 95% confidence intervals of the estimated divergence times between lineages (Ma). Black numbers near nodes are mean ages (Ma). Colour labels indicate the mitochondrial gene order (MF1, UF1, UF2, and UF3). Red asterisks indicate fossil calibrations (Supplementary Table 1). Stratigraphic chart according to the International Commission on Stratigraphy 2015

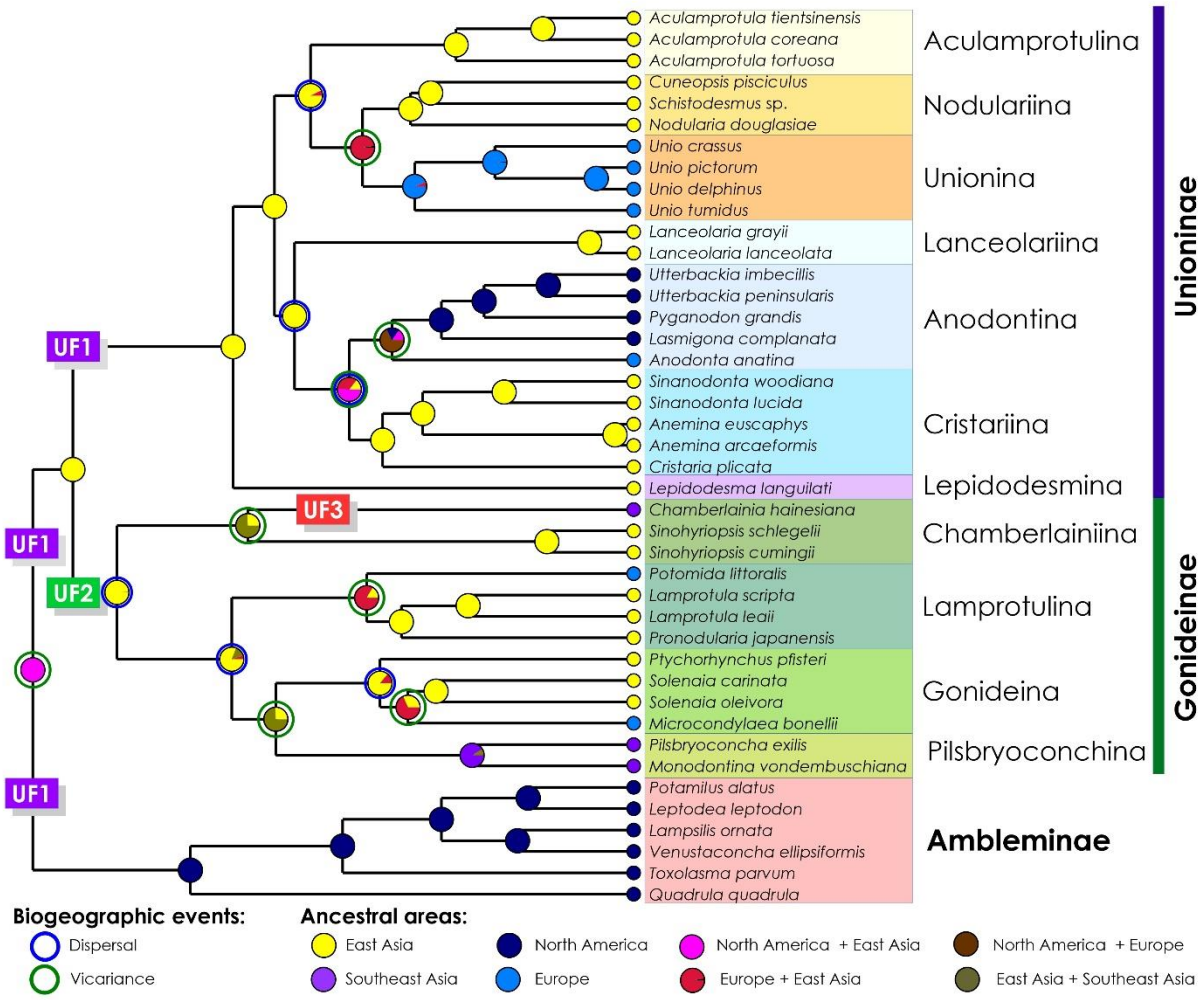

Figure 7 Historical biogeography of the Unionidae. This combined scenario has been inferred from three different statistical modelling approaches (S-DIVA, DEC, and S-DEC) based on the time-calibrated mitogenomic phylogeny (Fig. 6). Pie charts near nodes indicate probabilities of certain ancestral areas. Colour circles on the tip nodes indicate the range of each species. Colour labels indicate the mitochondrial gene order (UF1, UF2, and UF3)

The mitogenomic results fully support three large subfamily-level clades: Ambleminae, Gonideinae, and Unioninae. It is important to note that our analyses did not include members of the Parreysiinae and Rectidentinae. Nor did it include sequences of *Modellnaia siamensis*, the only species of the monotypic Modellnaiinae, which is characterized by several morphological and anatomical autapomorphies suggesting its separation within the Unionidae as a “phylogenetic relic” (Brandt 1974; Heard & Hanning 1978). Future studies including full mitogenomes of several taxa from Parreysiinae, Rectidentinae, and Modellnaiinae are needed to fully resolve the higher-level phylogeny of the global Unionidae. Our results highlight that resolving the systematics of a large, species-rich clade such as the Unionidae is a complex task. Previous taxonomic schemes for the Unionidae included only two levels of family-group names, i.e. subfamilies and tribes (reviews: Lopes-Lima et al 2017a; Bolotov et al 2017a,b). However, our whole mitogenome analyses reveal that despite the limited number of taxa

included, the Unionidae classification scheme could be better explained by including three levels of family-group names (i.e. subfamilies, tribes, and subtribes) to accurately reflect the presence of several levels of highly divergent clades within this family (Fig. 6). Subfamilies represent the largest clades that are fully supported by the mitogenomic approach (Fig. 7); some of which may be characterized by unique morphological synapomorphies, although several subfamilies have been supported by molecular data only (e.g. Lopes-Lima et al 2017a).

The most recent nuclear-based Unionoida phylogeny (using hundreds of nuclear protein-coding loci; Pfeiffer et al 2019) shows strong similarity to our findings concerning the relationships of both families and subfamilies. Moreover, mitogenome data currently available suggest that the Unionidae comprise seven (Lopes-Lima et al 2017a) or eight (Bolotov et al 2017a) subfamily clades. Of these, the Gonideinae (encompassing Pseudodontinae), Unioninae (encompassing the Anodontinae) and Ambleminae were well supported in the mitogenomic results obtained herein, whilst the validity and placement of the Parreysiinae, Rectidentinae and Modellnaiinae clades are yet to be confirmed by mitogenomic analyses.

The largest monophyletic clades, within each subfamily, exhibiting significant morphological synapomorphies and fully supported by the present mitogenomic results, are herein considered as tribes. Therefore, using these criteria, the Gonideinae comprise two tribes, i.e. Gonideini (trapezoidal to rectangular shells with none or only vestigial hinge teeth and tetragenous brooding type) and Chamberlainiini (round to oval shells, with a well-developed hinge structure and ectobranchous brooding type).

The subtribes represent smaller but distant clades within the tribes, comprising several genera or even a single highly divergent genus that usually does not reveal any unique synapomorphies but can be distinguished based on molecular characters. Based on data available to date, including the present results, the Gonideini comprise at least five subtribes, i.e. Chamberlainiina, Gonideina, Lamprotulina, Pilsbryoconchina and Pseudodontina (Lopes-Lima et al 2017a; Bolotov et al 2017a,b).

Macroevolutionary patterns of the Unionidae

The new mitogenomic analysis presented herein supports the hypothesis of an ancient Mesozoic origin and diversification of the Unionoidea (Taylor 1988; Ma 1996; Van Damme et al 2015; Bolotov et al 2016; Araujo et al 2017; Bolotov et al 2017a,b). The new results indicate that the Late Triassic split between the Margaritiferidae and Unionidae coincided approximately with the Triassic-Jurassic extinction that was one of the largest mass extinction events in the Phanerozoic (Watters 2001; Hesselbo et al 2002; Bogan & Weaver 2012; Percival et al 2017; Smithwick & Stubbs 2018). The divergence event between the two families was associated with the TDRL event leading to the formation of the two stable mitochondrial gene orders, i.e. MF1 and UF1, which have persisted without changes for ~200 Ma. However, there

were at least two additional Mesozoic splits in the mitochondrial gene order (i.e. UF1 vs. UF2 and UF2 vs. UF3) within the Unionidae, with UF2 and UF3 being restricted to a single subfamily, the Gonideinae. The first split coincided with the origin of this subfamily but the UF3 is a third, new and distinct gene arrangement derived from UF2 present in a single species, *Chamberlainia hainesiana*. These two mitochondrial gene orders have also persisted for long-term periods of ~150 and ~100 Ma for UF2 and UF3, respectively.

At least two splits in the mitochondrial gene order were associated with the origin of the MRCAs of large and diverse clades at the family (Unionidae vs. Margaritiferidae) or subfamily (Unioninae vs. Gonideinae) levels. Concerning this evidence, these TDRL events could be considered progressive evolutionary innovations because they lead to the formation of stable gene orders that have persisted within widely distributed and diverse clades for ~150-200 Ma. As for the mitogenome gene order, our ancestral state analyses suggest UF1 (in the Unionidae) as the ancestral gene order, which is maintained in the subfamilies Ambleminae and Unioninae sensu lato (Fig. 6). Additionally, it indicates that the evolution of F-type mtDNA gene orders is characterized by two independent events of TDRL (Moritz et al 1987; Boore 2000). One resulted in the evolution of UF2, present in the Gonideinae, and the other in UF3, within Gonideinae but restricted to *Chamberlainia hainesiana*. In contrast, all sequenced M-type Unionidae mitogenomes to date present the same gene order, i.e. UM1 (Lopes-Lima et al 2017b) (Fig. 2). Possibly this could be explained by the higher natural selection pressure and/or due to the tight control of the DUI system on the paternal mitochondrial inheritance. In summary, our results reveal that each TDRL event was followed by the stable long-term persistence of a mitochondrial gene order through evolving lineages (or even a single lineage, although the Chamberlainia clade may be under-sampled) and corresponds to the first reliable mitogenomic evidence supporting the evolutionary stasis in molecular traits of freshwater bivalves. However, this should be further explored using an expanded data set of mitochondrial genomes that may facilitate the understanding of how evolutionary rates have shifted across multiple genetic loci and how that corresponds to ecologically relevant traits.

Diversification and Biogeography

Combining our new fossil-calibrated mitogenomic analyses with robust ancestral area reconstruction provides new insights into early diversification patterns and biogeography of the Unionidae. According to our results, the Ambleminae + (Gonideinae + Unioninae) clade originated in the late Jurassic, with their MRCA distributed across Laurentia and Eurasia of the supercontinent of Laurasia. The split between the Ambleminae and Gonideinae + Unioninae clades was likely associated with a vicariance event driven by plate tectonics, i.e. the formation of the early Jurassic Transcontinental Laurasian Seaway (Bjerrum et al 2001). The Ambleminae is an entirely Laurentian subfamily, which diversified primarily through radiation

within the Mississippi drainage basin from the Early Cretaceous (Bolotov et al 2017a). In this context, a peculiar Unionidae fauna from the Late Jurassic of western North America (Watters 2001) appears to be ancestral lineages and stem groups of the Ambleminae + (Gonideinae + Unioninae) clade. The Gonideinae and the Unioninae (Unionini, Anodontini, Lanceolariini, and Lepidodesmini) (Fig. 6) originated in East Asia, most likely via intra-area radiation within the paleo-Yangtze River system during the Cretaceous (Fang et al 2009; Wang et al 2018). The Southeast Asian Gonideinae taxa (Mekong basin) were separated via several vicariance events in the Albian - Cenomanian, which may indicate the drainage rearrangement of paleo river systems of the Indochina Peninsula and surrounding terrains during this period (Wang et al 2018). The mitogenomic results suggest ancient connections between freshwater basins of East Asia and Europe near the Cretaceous-Paleogene boundary, probably via a continuous paleo-river system or along the Tethys coastal line (Hou & Li 2018), and this is also depicted in the Margaritiferinae subfamily within Margaritiferidae (Lopes-Lima et al 2018b). This pattern is well supported by at least three independent but almost synchronous divergence events: *Potomida* vs. *Lamprotula* and *Pronodularia*, *Microcondylaea* vs. *Solenaia*, and *Unio* vs. *Nodularia* and its relatives. During the same period, faunal exchange via the Beringian Land Bridge with subsequent vicariance events may also have started. The question of the origin of the family-clade, i.e. Unionidae, remains unanswered due to the lack of available mitogenomes of Parreysiinae and Rectidentinae, although combined COI, 28S and 16S data indicated that this family most likely originated within East or Southeast Asia (Bolotov et al 2017a).

The new results presented herein support the hypothesis that several of the largest river basins on Earth may represent so-called ancient (long-lived) rivers, the Unionida faunas of which have existed throughout long-term periods comparable with geological epochs (Bolotov et al 2017a; Lopes-Lima et al 2018b). The mitogenomic results suggest that the MRCA of the entire Gonideinae + Unioninae clade may have originated within the paleo-Yangtze drainage basin. This indicates that the modern Yangtze may be a system of at least Late Jurassic origin and a stable refugium for very ancient, relic lineages that have existed for approximately 150 Ma. The unionid fauna of the paleo- Mississippi system seems to be of Early Cretaceous origin (mean age of the crown group in our model) that has diversified for at least 120 Ma. The paleo-Mekong fauna appears to be younger as it likely separated from the paleo-Yangtze fauna in the Albian - Cenomanian, and its two largest monophyletic unionid radiations may have had a Late Cretaceous or Palaeocene origin (Bolotov et al 2017a,b). These results agree with the dating of divergence between two primary clades of the Southeast Asian cave spitting spiders that were separated at ~55 Ma by the paleo-Mekong River, which served as a biogeographic barrier (Luo & Li 2017).

Systematics

Based on the morphological evidence, we propose the putative MRCA of the Unionidae and Margaritiferidae as a new fossil family-level taxon in the Unionoidea.

Superfamily Unionoidea Rafinesque, 1820

Family †Shifangellidae Bolotov, Bogan, Lopes-Lima & Froufe fam. nov.

Type genus: †*Shifangella* Liu & Luo in Liu (1981)

Diagnosis: The Margaritiferidae and Unionidae are the most conchologically similar families to the †Shifangellidae. However, †Shifangellidae can be distinguished from the Margaritiferidae by having a weakly developed, narrow hinge plate (vs. typically well-developed and rather thick) and a shallow, smooth anterior adductor scar (vs. deep with arborescent-like striations), and from the Unionidae by an elongated *Margaritifera*-like shell with strongly concave ventral margin (vs. typically straight, rounded or slightly concave).

Distribution: Late Triassic, southwestern China (Sichuan).

Biology: This ancestral family likely had parasitic glochidial larvae like its descendants (ancestral state reconstruction, probability 100%).

Comments: Synonymy of the genus †*Palaeomargaritifera* Ma 1996 (Middle Jurassic, China) with †*Shifangella* suggested by Fang et al (2009) most likely erroneous because †*Palaeomargaritifera* has a well-developed, thick hinge plate, strong pseudocardinal teeth, and deep anterior adductor scar with arborescent-like striations supporting its original placement within the Margaritiferidae. The genus †*Dianoconcha* Guo, 1988 (Middle Jurassic, China), another synonym of †*Shifangella* proposed by Fang et al (2009), differs by a subtrapezoid, elongate-elliptical or rhomboid shell. This feature together with a narrow hinge plate and an observable but shallow anterior adductor scar suggest that it most likely belongs to the Unionidae. Concerning their age and diagnostic features mentioned above, †*Palaeomargaritifera* and †*Dianoconcha* appear to be the MRCAs of the crown groups of the Margaritiferidae and Unionidae, respectively. The family-level placement of several unionoid genera described from the Early Jurassic of China (e.g. †*Pseudomargaritifera* Ma 1996 and †*Solenoides* Ma 1996) is unclear and requires further revision; some of them might be members of the †Shifangellidae.

Conclusions

All the phylogenies inferred in this study using, for the first time, both the M- and F-mitogenomes individually and combined support the monophyly of the so-called “problematic” Gonideinae taxa. Moreover, the new mitogenomic results place the Pseudodontinae, as previously described by Bolotov et al (2017a), as a subclade within the monophyletic Gonideinae in both M- and F-type phylogenies. Additionally, the present work supports the

hypothesis of an ancient Mesozoic origin and diversification of the Unionoidea and reveals that each TDRL event was followed by the stable, long-term persistence of a mitochondrial gene order through evolving lineages and corresponds to the first reliable mitogenomic evidence supporting the evolutionary stasis in molecular traits of freshwater mussels. Finally, we propose a new systematics framework with three infrafamilial levels (i.e. subfamilies, tribes, and subtribes) that better explains the evolutionary patterns within the Unionidae. Future application of the phylogenetic mitogenome-based approach outlined here to Parreysiinae, Rectidentinae, and Modellnaiinae will be an important step to further resolve current taxonomic classification uncertainties within the Unionidae. Moreover, this study demonstrates the considerable potential for using comparative genomic techniques for unravelling patterns in the tempo, timing, and mode of evolutionary processes.

Data archiving

Sequence data have been submitted to GenBank accession numbers: MK994770-MK994777.

Acknowledgements

The authors wish to thank the Editor and the three anonymous reviewers for helpful remarks and suggestions that improved the quality of the manuscript. This research was developed under ConBiomics: the missing approach for the Conservation of Freshwater Bivalves Project N° NORTE-01-0145-FEDER-030286, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds (UID/Multi/04423/2019). FCT also supported MLL (SFRH/BD/115728/2016). The Russian Ministry of Education and Science (project no. 6.2343.2017/4.6), the Federal Agency for Scientific Organizations (project no. 0409-2015-0143), the Presidium of the Russian Academy of Sciences (scientific program no. 52), and the Russian Foundation for Basic Research, RFBR (project no. 17-45-290066) supported INB. Permits for fieldwork and sampling in Malaysia were issued by the Malaysian Ministry of Higher Education (FRGS/1/2015/WAB13/UNIM//1).

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Supplementary Material

Supplementary Table 1
List of fossil calibrations that were used in BEAST analyses

Calibration no.	MRCA	Description	Reference
Calibration 1	Margaritiferidae + Unionidae	**Hard minimum age**: 201 Ma, †*Shifangella margaritiferiformis* Liu & Luo, 1981 in Liu (1981) (Unionoidea: †Shifangellidae Bolotov, Bogan, Lopes-Lima & Froufe **fam. nov.**). **Diagnosis and phylogenetic placement**: A putative ancestor of the Margaritiferidae and Unionidae. The Margaritiferidae and Unionidae are the most conchologically similar families to the †Shifangellidae. However, †Shifangellidae can be distinguished from the Margaritiferidae by having a weakly developed, narrow hinge plate (vs. well-developed and rather hick) and a shallow smooth anterior adductor scar (vs. deep with arborescent-like striations), and from the Unionidae by an elongated Margaritifera-like shell with strongly concave ventral margin (vs. straight, rounded or slightly concave). **Stratigraphic horizon and locality**: Second Member, Wuzhongshan Formation, Upper Triassic, Jinhe, Shifang, Sichuan, southwestern China (Fang et al 2009). Absolute age estimate: Triassic/Jurassic boundary, 201 Ma, based on stratigraphy; 95% soft upper bound 230 Ma based on the age of †Silesunionidae, a prospective earliest member in the order Unionida (Skawina & Dzik 2011). **Prior settings**: exponential distribution, mean (lambda) = 7.9, MRCA: *Margaritifera marocana – Potomida littoralis*.	Present study: New crown calibration
Calibration 2	Ambleminae – (Gonideinae + Unioninae)	**Hard minimum age**: 155 Ma, †*Hadrodon jurassicus* Yen, 1952 (Unionoidea: Unionidae). **Diagnosis and phylogenetic placement**: A putative ancestral lineage of the Ambleminae – (Gonideinae + Unioninae) clade. This Laurentian unionid fossil is characterized by a broad hinge plate and strong lateral teeth in combination with its conspicuous undulating external surface (Yen 1952). Absolute age estimate: Late Jurassic, Morrison formation, Montrose County, Colorado, 155 Ma, based on stratigraphy (Yen 1952); 95% soft upper bound 201 Ma based on calibration 1. **Prior settings**: exponential distribution, mean (lambda) = 12.5, MRCA: *Quadrula quadrula – Unio crassus*.	Graf et al 2015: Crown calibration

CHAPTER 9
General Discussion

9.1 Main claims and highlights of the dissertation

This dissertation provides and integrates new knowledge about the biology, ecology, and distribution of freshwater mussels (FM), also providing considerable scientific advances on their phylogeny and systematics, thereby contributing to facilitate and guide future conservation planning and actions on FM. The FM biodiversity hotspots are delineated in the present dissertation and their main global threats, research, and conservation needs identified. One of these identified research needs, basic biological studies, is further demonstrated to be essential not only for conservation but also for the potential use of FM for environmental indication. We then show the importance of integrated morphological, ecological and molecular approaches to establish the evolutionary relationships among FM taxa at different taxonomic levels. Finally, we use the information gathered to provide guidance on future conservation actions.

9.2 Freshwater mussel hotspots, main threats, and conservation needs

The study of the diversity patterns of all freshwater bivalves highlighted the importance of several areas as diversity hotspots for the most diverse and threatened group, the Unionida or FMs (**Chapter 2**), as the Indotropical river basins that host a high taxonomic diversity of FMs. Over the last decades, this area has suffered from accelerated deforestation and freshwater habitat degradation, and thus deserves urgent conservation attention (Gallardo et al 2018). Furthermore, recent surveys indicate that FM species diversity is much higher than previously thought, making FM research in this area urgent (Bolotov et al 2017, 2019). The main knowledge gaps were also unveiled. Baseline data such as distribution, life-history, and taxonomy were the most cited research needs for FM species across the whole distribution, but especially in poorly studied areas (**Chapter 2**). In North America and Europe, FMs are being increasingly studied and protected (Lopes-Lima et al 2014). Nevertheless, long-term monitoring data to evaluate FM population trends and demographic features are still lacking, even in more developed regions (**Chapter 2**).

Most of the major identified threats to FMs were directly or indirectly connected to habitat degradation or modification, with pollution being generally pointed out as the main cause (**Chapter 2**). Although water quality and ecological status have been improving in many

developed countries, FM populations are still not reversing their decline status, with whole river catchment management producing better results (Lopes-Lima et al 2017). Nevertheless, in less developed areas with none or poor wastewater treatment, pollution is still a major cause of FM decline. Invasive species and climate change were still not considered as a major threat (**Chapter 2**). However, this should change shortly, given that recent reports show that these factors can have a major impact on freshwater mussels (Gallardo et al 2016). For instance, many restricted and threatened FM species are specialized in their host-fish usage (Modesto et al 2018). This was also shown for the Iberian dolphin mussel *Unio delphinus* which is not able to complete its life-cycle in any of the tested non-native fish (**Chapter 3**). Therefore, the increased biotic homogenization and freshwater fish introductions around the globe may become a serious threat to these mussels (Modesto et al 2018). Additional invasive species like other freshwater bivalves, mammals and crayfish have also been shown to have a deleterious effect on bivalves, either by competition or predation (Burlakova et al 2000; Skyrienė & Paulauskas 2012; Meira et al 2019). Species at the edge of their distribution or those living in semi-arid conditions have been shown to be seriously threatened. For instance, the Moroccan pearl mussel *Pseudunio marocanus* is now almost extinct due to water shortage (Sousa et al 2016). This species and others in northwest Africa are likely to be increasingly threatened due to the intensification of water consumption, combined with the on-going trends of increasing temperature, decreasing rainfall and instable climate (Sousa et al 2016). Freshwater mussel populations occupying their southern distribution limits, such as *Margaritifera margaritifera* in the Iberian Peninsula, are also particularly exposed to climate changes (Santos et al 2015) and associated increases in seasonal climatic instability (Sousa et al 2012, 2018; Nogueira et al 2019).

Ranking on the top of conservation needs, research on basic biological and ecological features is essential to effectively conserve and manage a species (**Chapter 2**). We demonstrated this in a case study on an Iberian FM species (**Chapter 3**). It was shown that the study of basic life-history traits and other basic biological and ecological features allows for a better estimation of FM species conservation status (**Chapter 3**) and that these traits can be used for ecological indication of freshwater habitats and communities. Furthermore, based on the study of these features, clear suggestions were given to improve the conservation success of potential actions on the target species, such as how to properly conduct population translocations or establish propagation programs and reintroductions.

The results of **Chapters 2 and 3** were not without potential limitations and shortcomings. The species richness patterns reviewed in **Chapter 2** were mapped on biogeographic regions adapted from Graf & Cummings (2007) for the world and revised for North America using Haag (2010). Although for North America a thorough compilation of species richness across the country coupled with a hierarchical cluster analysis produced

robust FMs' bioregions (Haag 2010), for the rest of the world the division made by Graf & Cummings (2007) is not clearly explained but seems to be based on the classical zoogeographic divisions subdivided by the main freshwater mussel assemblages. A more robust statistical analysis of the global diversity patterns was not attempted by these authors, probably due to the many inconsistencies and gaps of knowledge that still existed on the global FM phylogeny and taxonomy. Therefore, some of the bioregions chosen to map the diversity, threats, research, and conservation needs were possibly not accurate and should be revised with a proper statistical analysis.

Another potential limitation is the use of IUCN Red List data. Although around half of the freshwater bivalve species were already red listed at least once, their assessments are not homogeneous across regions. This means, for instance, that results obtained for South America or Australia were only based on very few species. Another limitation might be the lack of standardization of Red List assessments over the last decades. The Red List categories and criteria have changed since its inception, and the way species were analysed and the information included in each assessment changed considerably over the years, with recent assessments generally containing much more data, e.g. detailed distributions, threats, ecosystem services provided, among others.

For **Chapter 3** the conservation status of *U. delphinus* was updated based only on the species range and plausible threats using the B criterion of IUCN. Given that only very scarce information regarding population trends was available, it was not possible to use criteria A or C (**Chapter 2**). Although most of the fish species that co-occur with *U. delphinus* were used for the determination of its hosts, we were not able to collect a few of those fish species, being possible that other native or non-native species could also be good hosts for the reproduction of *U. delphinus.* Furthermore, all of the host fish studies were made under laboratory conditions and although the best hosts were species within the genus *Squalius*, field studies should be made in the future to indicate if larvae prefer these fishes *in situ* or if other species are more used. The growth experiments should have also been controlled for factors known to affect this parameter, like nutrients and calcium concentration, temperature, pH, etc. Since these parameters were not assessed, the differences in growth pattern evidenced by the lotic and lentic populations could only be inferred and speculated. This chapter also discusses its potential use for ecological indication, but this still needs to be tested with populations under different stresses or environmental conditions.

9.3 Linking systematics and phylogeny with conservation

The present dissertation also brings important advances in our knowledge about the evolutionary relationships among the major groups within the most speciose and the most threatened families of freshwater mussels, the Unionidae and the Margaritiferidae respectively. Due to their sessile condition and very low dispersal ability, freshwater mussels are interesting model taxa to use on biogeographic studies and reconstruction of historical hydrologic and geologic changes, such as the formation and development of the current river basins or the movement and connections of the Earth's landmasses (Graf & Cummings 2006). Therefore, it is very useful to use several layers of taxonomic complexity that help to explain and date these events and the biogeography of these organisms. Until the present dissertation, phylogenetic studies on FMs used a taxonomy with five layers within each family, i.e. species, genus, tribe, sub-family, and family (Graf & Cummings 2016). However, due to the lack of sampled species and access to whole specimens (for many species only shells are generally available in museum collections), many of them were left as *incertae sedis* (Graf & Cummings 2006; Whelan et al 2011). This classification framework was here applied to the results from the most comprehensive phylogeny performed until now in terms of sampled taxa, to revise the higher classification within both families. This classification divided the Unionidae into 6 subfamilies and 18 tribes, and the Margaritiferidae into two subfamilies (**Chapters 4 and 6**). The distribution of each subfamily and tribe was then mapped to highlight biogeographic patterns. We could see, with a few exceptions, that the Unionidae are divided into Eastern and Western Palaearctic subgroups by the Ural Mountains, with most tribes not sharing taxa from both sides (**Chapter 4**). A strong biogeographic divide was also seen in the Mekong basin that is not crossed by some major groups (**Chapter 4**). The Margaritiferidae also showed a biogeographic differentiation among its two subfamilies, with one occurring in southeast Asia and the other with a wide distribution area, probably due to the vagile nature of their migratory host fishes (Chapter 6). Considering these biogeographic patterns and risk of extinction, Margaritiferidae species are of extreme concern, mainly those within the genera *Gibbosula* in South-East Asia, *Pseudunio* around the Mediterranean, and *Cumberlandia* with a restricted range in North America, all highly threatened and of high phylogenetic distinctiveness (Chapter 6). Almost nothing is known regarding the population status, ecology and life-history traits of *Gibbosula* species which are very rare and not easily found (Do 2011a, 2011b; Thi Dieu Phuong 2011).

At a lower taxonomic scale, the phylogeny and species diversity of a North American group, that historically belonged to the *Quadrula* genus, were revised (Chapter 5). This group of species was traditionally lumped together under the genus *Quadrula* due to shell morphological similarities. However, until now there were many uncertainties regarding the identity, number of species and their generic assignment within this group. Our molecular results, complemented with a compilation of traits from the literature, supported the existence of 21 species in 4 genera. A new species to science, i.e. *Theliderma johnsoni*, was described

and important conservation implications of the taxonomical revision on the group were highlighted.

Some freshwater mussels have a very interesting and unique form of mitochondrial inheritance (only shared with few other bivalve groups), also called doubly uniparental inheritance (DUI), where males inherit mitochondria from both parents (Zouros et al 1994; Hoeh et al 2002). Each male has (F-) type mitochondria inherited from the mother in all cells, except in those from the germinative tissue that have (M-) type mitochondria and are inherited from the father (Breton et al 2007). In FMs, both F- and M- mitochondrial genome sequences are very divergent within a single individual, and even the gene arrangement of each mitogenome sequence is quite distinct (Doucet-Beaupré et al 2010). In this dissertation, all morphological and anatomical synapomorphies of the Margaritiferidae were compiled (**Chapter 6**), including the gene arrangement of both F- and M-type mitogenomes, which are unique to the family and may be used as a molecular diagnostic tool (**Chapter 7**). It was discovered that mitogenome rearrangements in FMs were rare events that occurred mainly during short periods and coincided with extinction events, stabilising thereafter during long periods (**Chapter 8**). The phylogenetic patterns obtained within the family Unionidae were consistent with other genomic phylogenies recently published by other authors in the meantime using hundreds of nuclear loci (Pfeiffer et al 2019). This suggests that we are getting closer to have a stabilized phylogeny of the main groups within Unionidae. Due to this reason, the classification system for this family was again revised introducing subtribes as an additional taxonomical layer (**Chapter 8**).

Several shortcomings and caveats can be identified from the results of **Chapters 4 to 8**, though they are unlikely to have affected significantly its results and key conclusions. These chapters provide a good phylogenetic basis on the major groups of the Unionidae and Margaritiferidae, which are complemented with other traits. However, some of the phylogenetic relationships among the higher taxa were not well supported, especially the relationships among the subfamilies. This should be related to the low number of molecular markers used, suggesting that additional markers are needed to reveal these ancient evolutionary relationships. Other recently produced phylogenies using the same low number of markers showed differences in these relationships that seem to be dependent on the number and composition of taxa (Pfeiffer et al 2019). Besides the low number of markers, taxa from several regions were never included in the Unionidae phylogeny and our knowledge of the whole group is still incomplete, with many species being still considered as *incertae sedis.* Furthermore, the biogeographic inferences accomplished in **Chapters 6 and 8** only considered the few taxa used for the phylogenetic analyses, and these patterns might change if more taxa are added to the models.

The present whole mitogenome analyses indicated that gene arrangements might be diagnostic of specific taxonomic groups, such as the families Unionidae and Margaritiferidae or specific subfamilies of the Unionidae (**Chapters 7 and 8**). However, whole mitogenome sequences are only available from a small number of species, and so these trends need to be confirmed with additional taxa from other regions and taxonomic groups. The phylogeny presented using whole mitogenomes (**Chapter 8**) was well supported but revealed a distinct topology regarding the main relationships among the Unionidae subfamilies captured in **Chapter 4**. Nevertheless, the mitogenome phylogenetic analyses are concordant with another recently published study with hundreds of nuclear markers across the whole genome (Pfeiffer et al 2019), indicating that the phylogeny should be stabilising, and we are closer to the true species tree.

Since more recent evolutionary diversification events needed to be retrieved, in the phylogenetic study of the species historically placed within *Quadrula* (**Chapter 5**), only mitochondrial markers were used. Given that mtDNA may suffer from introgression and hybridization events, with mtDNA evolution sometimes being distinct from the species evolution, all phylogenetic assumptions and molecular species delineation methods should now be confirmed with nuclear markers displaying distinct evolutionary rates.

9.4 Future research perspectives and conservation implications

The results of this thesis bring important conservation implications that vary across the globe. Wide and extensive surveys are needed in many regions, not only in less studied regions such as Africa, South America and Sundaland, but even in Europe and North America, where species inventories and population information is scarce and uneven across regions (Lopes-Lima et al 2014, 2017). To better estimate conservation status and to distinguish between natural fluctuations and anthropogenic declines, population trends should also be researched globally, emphasizing the need to establish long term monitoring programs for important FM populations, such as those included in the Long Term Ecological Research (LTER) Network (Knapp et al 2012).

FM species on the southern hemisphere in Africa, South America, and Australia have been neglected and need to be studied urgently to prevent future extinctions and extirpations. Basic biological traits and population information are almost non-existent for species in these regions and genetic diversity studies almost absent. The application of the framework here developed for the study of basic biological traits (chapter 3) in species from these regions should be pursued to better understand the ecological requirements and features of this poorly known fauna.

The use of FMs to monitor the environmental health of both freshwater habitats and their terrestrial surroundings has a great potential and should be developed significantly in the future. The efficacy of these traits and other potential biomarkers, e.g. biochemical or genetic, should then be tested in distinct impacted environments or to evaluate the success of eventual rehabilitation actions.

Another field of research that requires immediate attention is the selection of protected areas that include freshwater diversity (Suski & Cooke 2007). It has been shown that protected areas developed for terrestrial taxa fail to protect freshwater organisms (Darwall et al 2011). Also, there is a wide mismatch between the areas important for freshwater conservation and those within protected areas networks (Abraham & Kelkar 2012; Hermoso et al 2015). Therefore, FMs and other freshwater taxa should be included in future systematic conservation planning exercises to design more effective conservation or protected areas for freshwater diversity.

To help in the selection of the most diverse and important global areas for freshwater mussels, we should try to maximise species richness, but also to capture the higher phylogenetic diversity possible (Faith et al 2004). For that, we first need a robust phylogeny of the order using multiple markers and the higher number of taxa possible. The whole mitogenome phylogenies here presented and the multi-nuclear marker resources recently published by Pfeiffer et al (2019), seem to be close to the true species phylogeny. Therefore, a combined multi-marker approach using these two techniques should be accomplished in the future, with selected taxa from most genera within the order. At a higher resolution taxonomical scale, reduced genome representations like restriction site associated DNA markers (RAD-Seq) or genotyping by sequence (GBS) should be applied to reveal hidden cryptic diversity and test intraspecific mitochondrial lineages to have a clearer molecular definition of species. Given that in developing countries freshwater habitats are becoming increasingly impacted, it is essential to rapidly invest in the identification of species, evolutionarily significant units, and management units in these countries, especially on the identified hotspots such as the Indotropical region.

Knowing the higher taxa phylogeny and having most species identified will allow for the elaboration of robust biogeographic models and infer historical geological and hydrological patterns. Furthermore, the evolutionary relationships, distributions and conservation status patterns here obtained may be coupled with equivalent data from other freshwater taxa to select important areas for conservation and protection at several geographic levels.

By presenting an integrative approach combining ecological, distribution, morphological and genetic data, the present dissertation provides important advances on several research fields in freshwater mussels, from evolution and ecology to environmental

indication with important contributions for the global conservation planning of these highly threatened molluscs.

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