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Original Article

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High endemic freshwater mussel (Bivalvia: Unionida) diversity in western Borneo, with description of three new species

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ABSTRACT

The freshwater mussels (Bivalvia: Unionida) of the biodiversity hotspot Sundaland are experiencing severe anthropogenic threats, whilst their diversity and distribution remain poorly understood. Here, we present the first modern-day data on Unionida diversity and distribution across western Borneo. Mussels were surveyed and collected in the upper Kapuas and Pawan river basins in West Kalimantan, Indonesia, and the Sambas Besar, Sarawak and Batang Sadong river basins in Sarawak, Malaysia. DNA sequencing (*COI* + 16S + *ND1* + 18S + 28S) and morphological analyses were conducted to delineate and identify species, and reconstruct phylogenetic relationships and population genetics. Specimens belonged to six native genera and nine species, of which seven are endemic to Borneo and three are new to science, i.e. *Ctenodesma mawonae* Zieritz *et al.*, sp. nov., Sarawak basin, and *Ctenodesma bersinara* Zieritz *et al.*, sp. nov., and *Rectidens lauris* Zieritz *et al.*, sp. nov., both Pawan basin. The monotypic genera *Caudiculatus* and *Discomya* were phylogenetically highly divergent from other known Gonideinae taxa, potentially indicating a separate tribe (for *Caudiculatus*) and subfamily (for *Discomya*). In addition, we report new records of the non-native *Sinanodonta pacifica* Heude, 1878 in the Batang Sadong and Kapuas river basins.

Keywords: Borneo; freshwater bivalves; freshwater mussels; Mollusca; Sundaland; Unionidae

INTRODUCTION

Freshwater mussels of the order Unionida represent the most species-rich radiation of freshwater bivalves, comprising 958 of the >1200 extant species (Graf and Cummings 2021). For a long time, North America was considered the epicentre of Unionida species diversity, with >300 species. However, recent efforts focused on the long-neglected Unionida fauna of South-East Asia, including extensive specimen collecting and DNA sequencing, has resulted in a significant increase in the recorded Unionida species' richness of that region, as well as the recognition of several new higher taxa (Graf and Cummings 2021). The majority of these taxonomic changes were from mainland South-East Asia (Konopleva *et al.* 2019, Bolotov *et al.* 2020, Jeratthitikul *et al.* 2021, Pfeiffer *et al.* 2021).

In contrast, the Unionida fauna of the biodiversity hotspot Sundaland, spanning the Malay peninsula and the islands of Borneo, Sumatra, and Java, has received considerably less recent expeditionary work and systematic research, the exception being northern Borneo (Zieritz *et al.* 2018, 2020, 2021). That work, based on surveys at 139 sites (and interviews at another 50+ sites) across an area of approximately 100 000 km², revealed that the northern Bornean freshwater mussel fauna has declined over

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the past 50+ years, largely owing to industrial-scale deforestation and conversion to oil palm plantations, resulting in the destruction and alteration of freshwater habitats. Today, only one native Unionida species appears to have retained stable population sizes, i.e. *Rectidens sumatrensis* (Dunker, 1852), which was found in several river basins in north-western Borneo (i.e. in the Malaysian State of Sarawak). In addition, the non-native *Sinanodonta pacifica* (Heude, 1878) (=*S.* cf. *woodiana*) (Douda *et al.* 2024) is particularly widespread and abundant in the north-east (i.e. in the Malaysian State of Sabah) and thrives in anthropogenic habitats (Gallardo *et al.* 2018). Although 15 out of the 20 currently recognized Unionida species from Borneo are endemic, only three of these, i.e. *Schepmania* sp., *Khairuloconcha lunbawangorum* Zieritz *et al.*, 2021 and *Khairuloconcha sahanae* Zieritz *et al.*, 2021, were found in these surveys from only a single site each, all situated within (largely) undisturbed rainforest streams (Zieritz *et al.* 2020, 2021). Both *Khairuloconcha* species were new to science, and the genus was established as the sister to *Ctenodesma borneensis* (Issel, 1874). In addition, a new tribe Ctenodesmaini (subfam. Gonideinae, fam. Unionidae) was erected for *Ctenodesma* (western Borneo) + *Khairuloconcha* (northern Borneo) as sister to Rectidentini + Contradentini (Zieritz *et al.* 2021, International Commission on Zoological Nomenclature 2022).

Whilst the work in northern Borneo has highlighted severe shortcomings in, and improved our understanding of, the species and phylogenetic diversity of the Bornean Unionida fauna, the majority of the island, including all of Indonesian Borneo (=Kalimantan), remains very poorly understood. Historical records suggest that the river basins of western Borneo, most notably the Kapuas basin in West Kalimantan, host a particularly diverse and highly endemic unionid fauna, with nine species from eight genera (Table 1).

Here we present the first modern-day data on freshwater mussel diversity and distribution across western Borneo, here defined as river basins emptying into the South China Sea ranging from the Lupar River in the north to the Sungai Kendawangan in the south. Based on a multilocus DNA sequence dataset, this paper aims to (i) determine the number, identity, and evolutionary relationships of species, (ii) discuss the distribution of these species, and, in light of these findings, (iii) discuss the importance of the region as a freshwater biodiversity hotspot that requires urgent conservation attention.

MATERIALS AND METHODS

Study area

Fieldwork was conducted at 16 sites in West Kalimantan (Indonesia) in August 2019, specifically in the upper Kapuas and the Pawan river basins, and four sites in western Sarawak (Malaysia) in July 2022, specifically in the Sambas Besar, the Sarawak, and the Batang Sadong river basin (Fig. 1). Selection of study sites was informed by available historical data and local knowledge. Study sites were situated within the freshwater ecoregions of 'Kapuas' and 'north-western Borneo' (Abell *et al.* 2008). Geological units at the sites are dominated by a mixture of Triassic–Eocene metamorphics, volcanic and sedimentary rocks (NW Kalimantan domain), Permo-Triassic granites and metamorphics (Embuoi Complex), and Cenozoic-aged sediments (Schwaner Mountains) (Moss 1998). There are three main protected areas situated in the study areas, i.e. Danau Sentarum National Park (1320 km^2 surface area) in the northern Kapuas basin, Gunung Palung National Park (900 km^2) nearby the Laur River basin, and Bungo Range National Park (65 km^2) in Western Sarawak (UNEP-WCMC and IUCN 2023) (Fig. 1).

Field surveys

Survey methodology followed Zieritz *et al.* (2020). In brief, mussels were sampled by hand and net. Voucher specimens and tissue snips were collected and preserved in absolute ethanol and deposited at the Fahutan Kapuas Hulu Collection, Tanjungpura University, Indonesia (FKH; for specimens collected in West Kalimantan), and the Faculty of Resource Science and Technology Collection, Universiti Malaysia Sarawak, Malaysia (FRST; for specimens collected in Sarawak). Except for vouchers, all other specimens were returned to their habitat.

Species' identification, and morphological and phylogenetic analyses

Collected specimens were identified to species' level through an integrative morphological-molecular approach. DNA was extracted from 44 samples (one to three specimens per population) from the study area for DNA barcoding using a standard high-salt protocol (Sambrook *et al.* 1989) or the Jetquick tissue DNA Spin Kit (Genomed) following the manufacturer's protocol (Supporting Information, Table S1–S4). For molecular phylogenetic reconstructions, DNA was additionally extracted from seven other taxa representing the major groups within the Unionidae (Supporting Information, Table S1). Female lineages of mitochondrial cytochrome *c* oxidase subunit I (*COI*) were amplified and sequenced in both directions from all extracts using primer pair LCO22me2 + HCO700dy2 (Walker *et al.* 2006, 2007). For one specimen of each native species and a selected group of additional Unionida taxa (Supporting Information, Table S1), additional sequences were generated for the large ribosomal subunit rRNA 16S (16SL + 16SH; Palumbi *et al.* 1991), the mtDNA *ND1* (Leu-uurF and LoGlyR; Serb *et al.* 2003), the small ribosomal subunit rDNA 18S (18Sa2.0 + 9R; Giribet *et al.* 1996), and the large ribosomal subunit 28S (28S-RD1.3f and 28S-rD4b; Whiting 2002). Polymerase chain reaction conditions (PCRs) followed Froufe *et al.* (2016) and Bolotov *et al.* (2016) for 18S, with annealing temperatures of 48°C and 50°C (only *ND1*). Amplified DNA templates were purified and sequenced by the commercial company Macrogen using the same primers. Sequences were assembled in MEGA X (Kumar *et al.* 2018) and deposited in GenBank (accession numbers in Supporting Information, Tables S1–S4).

Five-gene phylogenies

Alignments were constructed for each marker using newly sequenced individuals and representatives from available taxa belonging to other described tribes within the subfamily Gonideinae, as well as representatives of other Unionida subfamilies (Supporting Information, Table S1). Sequences from species belonging to the Unionidae subfamily Ambleminae and the Margaritiferidae were included as an outgroup. Each marker dataset was aligned using the standalone version of GUIDANCE2 (Sela *et al.* 2015) and the MAFFT multiple

Table 1. Freshwater mussel (Bivalvia: Unionida: Unionidae) species native to western Borneo (i.e. river basins emptying into the South China Sea ranging from the Lupar River in the north to the Sungai Kendawangan in the south), including details on their type locality and distribution across Borneo and globally. Data collated from the mussel-project https://mussel-project.uwsp.edu/fmuotwaolcb.

Taxa	Type locality	Distribution
Unionidae		
incertae sedis		
[a]*Discomya radulosa* (Drouët and Chaper, 1892)	Sebroang River (Kapuas River basin, West Kalimantan, Borneo)	western Borneo and Sumatra
Gonideinae		
incertae sedis		
[a]*Caudiculatus caudiculatus* (Martens, 1867)	Danau Sriang (=Danau Seriang), Pulo Matjan, (Kalimantan) Borneo (Kapuas River basin)	western Borneo
Contradentini		
[a]*Lens lugens* (Drouët and Chaper, 1892)	Kapuas River, Semitau, Borneo	western Borneo
Ctenodesmaini		
[b]*Ctenodesma bersinara* Zieritz, Rahim, Prayogo and Lopes-Lima sp. nov.	Sungai Rempangi (Pawan Drainage), 17 km SSW of Randau, Ketapang Regency, West Kalimantan, Indonesia	western Borneo
[a]*Ctenodesma borneensis* (Issel, 1874)	'north-western Borneo', near Mampawa (Sungai Mempawah basin) and at Danau Seriang (Kapuas River basin)	western Borneo
[b]*Ctenodesma mawonae* Zieritz, Rahim, Lopes-Lima and Pfeiffer sp. nov.	Sungai Sebua Jebung (Sarawak Drainage), 3 km SSW of Bau, Kuching division, Sarawak, Malaysian Borneo	western Borneo
Rectidentini		
Elongaria trompi (Drouët and Chaper, 1892)	Kapuas River, Semitau, Borneo	Kapuas basin, western Borneo
Hyriopsis velthuizeni (Schepman, 1896)	Mandai River near Nanga Kalis (Kapuas River basin, West Kalimantan, Borneo)	western Borneo and Sumatra
[b]*Rectidens lauris* Zieritz, Anwari, Diba and Pfeiffer sp. nov.	Sungai Rempangi (Pawan Drainage), 17 km SSW of Randau, Ketapang Regency, West Kalimantan, Indonesia	western Borneo
[a]*Rectidens sumatrensis* (Dunker, 1852)	Danau Luar, Sumatra	Sumatra, Malay peninsula, Borneo, Java
Pseudodontini		
Pseudodon aeneolus Drouët and Chaper, 1892	Sebroang River, Kapuas River basin, Semitau, (West Kalimantan) Borneo	western Borneo
[a]*Pseudodon walpolei* (Hanley, 1871)	Sarawak, (Malaysian) Borneo	western Borneo

[a] Species detected in the present study.
[b] Species described in the present study.

sequence alignment algorithm (v.7; Katoh and Standley 2013) with default parameters. Subsequently, a concatenated dataset comprising *COI*, *ND1*, 16S, 18S, and 28S was created for the phylogenetic analyses. The optimal number of partitions and substitution models were selected for Bayesian inference (BI) using PartitionFinder2 with a greedy search approach, MrBayes model set, and the Bayesian information criterion (Lanfear *et al.* 2017). For maximum likelihood (ML), the best-fit model was determined using ModelFinder (Kalyaanamoorthy *et al.* 2017). BI analyses were conducted using MrBayes 3.2.7a (Ronquist *et al.* 2012) with program-generated trees and four Markov chains with default incremental heating. Two independent runs of 20 × 106 generations were sampled at intervals of 1000 generations, resulting in a total of 20 000 trees. Burn-in was determined by assessing the convergence of log-likelihood and parameter values using TRACER 1.7.1 (Rambaut *et al.* 2018). ML analyses were conducted using IQ-TREE 2.1.1 (Minh *et al.* 2020) with initial tree searches, followed by 10 independent runs and 10 000 ultrafast bootstrap replicates.

COI phylogenies and species' delineation

COI datasets were generated for each of the genera *Rectidens*, *Lens*, and *Pseudodon* for further phylogenetic analysis, phylogeographic interpretation and species delineation. For each genus, the respective *COI* dataset includes all newly sequenced individuals, as well as all previously published sequences available in GenBank. Due to the large number of sequences available for the genus *Lens*, the *Lens COI* dataset was reduced to unique haplotypes (Supporting Information, Table S4). The full *COI* datasets of the genera *Pseudodon* and *Rectidens*, as well as the reduced *COI* dataset of *Lens*, were analysed using ML and BI methods, using the same software and parameters as for the five-gene phylogenies. Sequences from *Ensidens ingallsianus*, *Solenaia emarginata*, and *Namkongnaia inkhavilayi* were selected as outgroup for phylogenetic analyses of *Rectidens*, *Lens* and *Pseudodon*, respectively (Supporting Information, Tables S1–S3).

Three different methods were applied to each of the *Rectidens*, *Lens*, and *Pseudodon COI* datasets to determine the number of

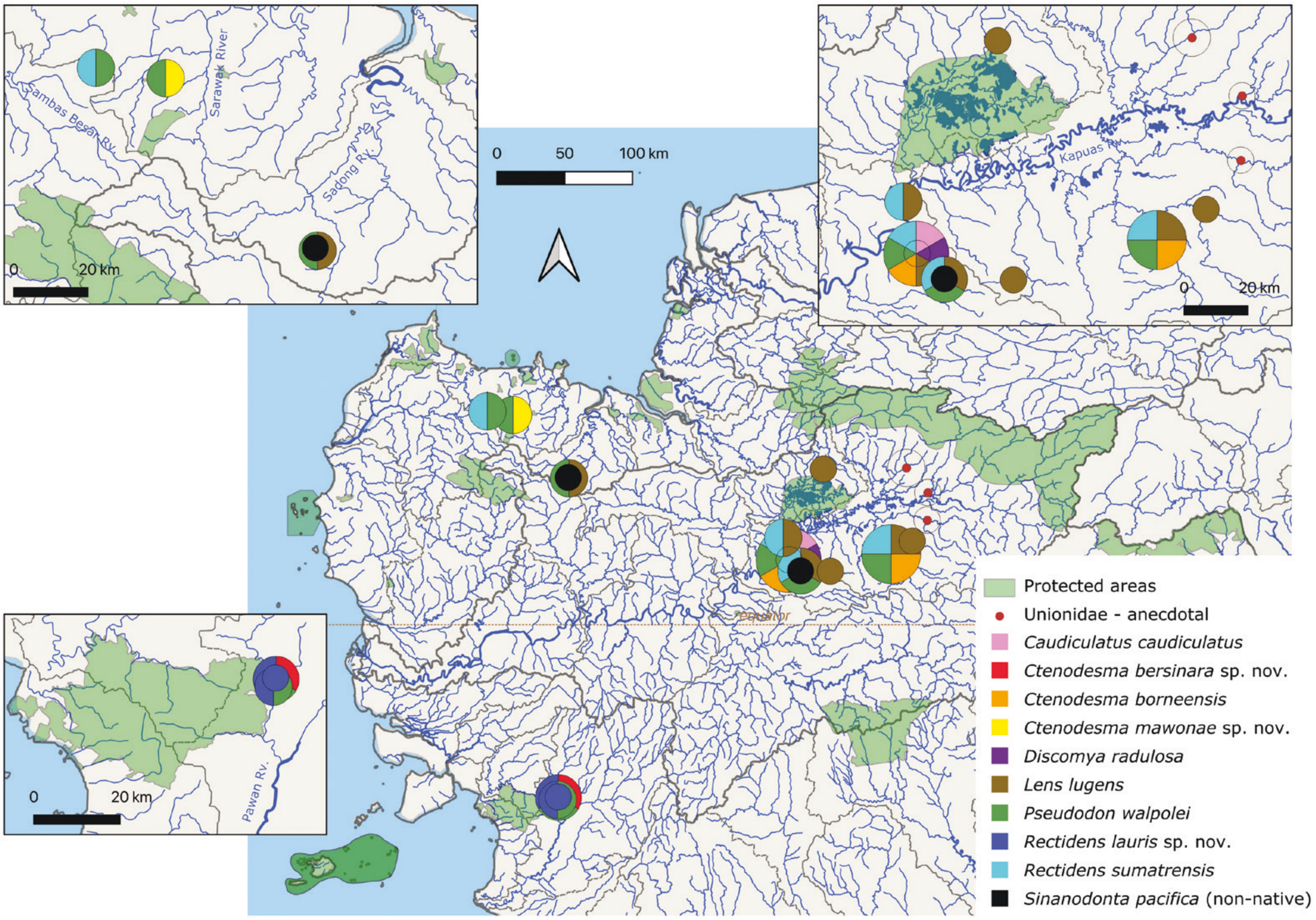

Figure 1. Map of western Borneo, showing sites and species of freshwater mussels (Bivalvia: Unionida) found and collected during fieldwork in 2019 and 2022. Protected Areas shapefiles derived from UNEP-WCMC and IUCN (2023). HydroBASINS (Lehner and Grill 2013) shown as solid (level 4) and dashed (level 6) lines.

molecular operational taxonomic units (MOTUs). The barcode index number (BIN) system was applied to each *COI* dataset without the outgroup using the cluster sequences tool as implemented in BOLD 4 (http://v4.boldsystems.org) (Ratnasingham and Hebert 2013). Assemble species by automatic partitioning (ASAP) was applied to the *COI* dataset without the outgroup using its online version (https://bioinfo.mnhn.fr/abi/public/asap/), employing the default settings and the Kimura 2-parameter (K2P) distance matrix (Puillandre *et al.* 2021). The third method used haplotype network reconstructions in TCS 1.21 (Clement *et al.* 2000) with a statistical parsimony limit of 95%. MEGA X (Kumar *et al.* 2018) was used to estimate sequence divergence (uncorrected *p*-distance) and examine genetic differentiation between lineages. TCS 1.21 was also used to create haplotype networks for interpreting the phylogeography of *Rectidens*, *Lens*, and *Pseudodon*, with the same parameters as above.

RESULTS

Description of the molecular dataset

The size, number of haplotypes, and parsimony-informative sites of all four datasets (one *COI* + *ND1* + 16S + 18S + 28S and three *COI* individual genus datasets), together with the partition schemes and nucleotide substitution models for all analyses, are presented in the Supporting Information, Table S5. The size of each individual gene fragment in the combined matrix of five loci was as follows: 649 nt (*COI*) + 883 nt (*ND1*) + 514 nt (16S) + 643 nt (18S) + 780 nt (28S). No indels or stop codons were found in the *ND1* nor on any of the *COI* datasets. Convergence of the BI runs was supported by the average deviation of split frequencies (<0.001), average PSRF values (1.000), and high ESS values (>500). Both BI and ML five-loci phylogenies show similar topologies with no major differences in the nodes including the target taxa (Fig. 2).

Species' delineation, distribution, phylogenies, and haplotype networks

Collected specimens belong to six native genera and nine species, i.e. *Caudiculatus caudiculatus* (Martens, 1867) (Fig. 3), ***Ctenodesma bersinara*** Zieritz *et al.*, **sp. nov.** (urn:lsid:zoobank.org:act:8F46E62E-BF34-4011-A329-803B170241C4) (Figs 4 and 5), ***Ctenodesma mawonae*** Zieritz *et al.*, **sp. nov.** (urn:lsid:zoobank.org:act: D095249A-306B-46FC-8F37-8A29C52451B1) (Figs 4 and 5), *Ctenodesma borneensis* (Fig. 4), *Discomya radulosa* (Drouët and Chaper, 1892) (Fig. 3), *Lens*

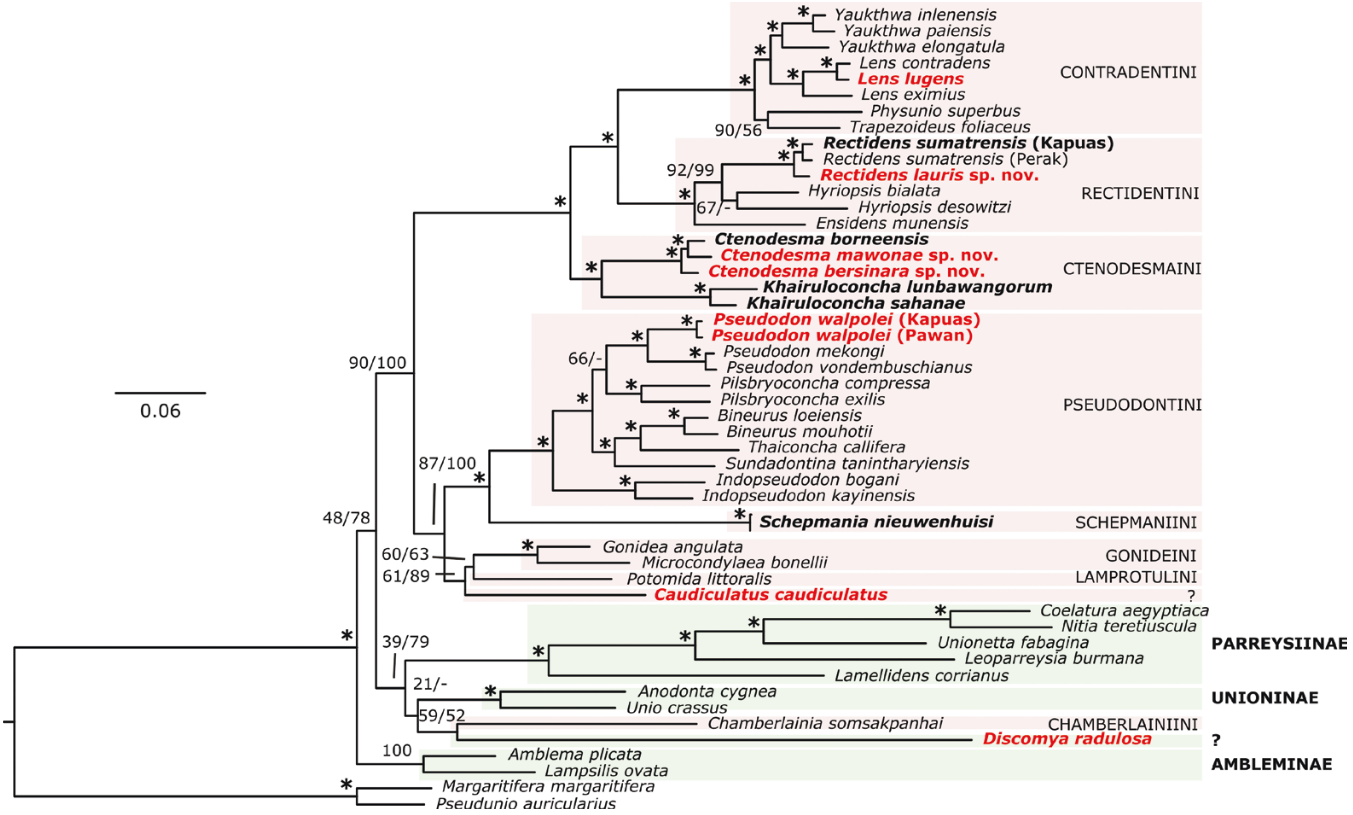

Figure 2. Maximum likelihood (ML) phylogenetic tree inferred from the combined (*COI* + 16S + *ND1* + 18S + 28S) dataset. Values at nodes indicate Bayesian inference (BI) posterior probability percentage/ML ultrafast bootstrap values. Support values >95% for both phylogenetic analyses are indicated by an asterisk. Support values marked with '-' indicate nodes that differ in the BI tree. Species present in Borneo in bold. Species for which DNA sequences are first reported in the present paper in red. Gonideinae clades marked in red; non-Gonideinae clades marked in green.

lugens (Drouët and Chaper, 1892) (Fig. 3), *Pseudodon walpolei* (Hanley, 1871) (Fig. 3), ***Rectidens lauris*** Zieritz *et al.*, **sp. nov.** (urn:lsid:zoobank.org:act: DC3289A3-C7D9-468A-BA56-7E3C739BD609) (Figs 4 and 5), and *R. sumatrensis* (Dunker, 1852) (Table 1; Figs 1, 2). All of these species are endemic to Borneo except *D. radulosa*, which is historically also known from a site in Sumatra (Table 1; Fig. 3), and *R. sumatrensis*, which is also known from the Malay peninsula, Sumatra, and Java (Table 1; Fig. 6B). The BI and ML *COI* phylogenies for *Rectidens*, *Lens*, and *Pseudodon* show a strong concordance, with two unresolved nodes concerning the populations of *R. sumatrensis* from Borneo (Fig. 6A) with the remaining populations and another within the *P. walpolei* clade (Fig. 7A).

Caudiculatus and *Discomya*

Individuals of *Caudiculatus* and *Discomya* were collected from only one site in the River Sebroeang in the upper Kapuas River basin (Figs 1, 3; Supporting Information, Figs S1, S2), and our data represent the first DNA sequences for both of these genera. *Discomya* was recovered as a highly divergent lineage sister to *Chamberlainia somsakpanhai* Kongim *et al.*, 2023, but this sister relationship had very limited support (Fig. 2). *Caudiculatus* was recovered as a highly divergent lineage sister to Lamprotulini + Gonideini but with limited support (Fig. 2).

All other native species belong to genera within the subfamily Gonideinae with high support (Fig. 2). In addition, our dataset includes sequences of the non-native invasive genus *Sinanodonta*, native to East Asia and member of the subfamily Unioninae.

Ctenodesma

Ctenodesma is endemic to Borneo and currently comprises two accepted species, i.e. *C. borneensis* from western Borneo and *C. scheibeneri* Haas, 1927 known from a single locality in southern Borneo (i.e. Sungai Batoe, South Kalimantan; Supporting Information, Fig. S3). These two species are morphologically quite divergent, and the monophyly of the genus has been questioned (Pfeiffer *et al.* 2021). We collected live specimens of *Ctenodesma* from three drainages in Borneo (i.e. Kapuas, Pawan, and Sarawak River basins; Fig. 1; Supporting Information, Fig. S3). We collected *C. borneensis* from its type locality (Kapuas River basin; Zieritz *et al.* 2021) (Table 1) and two tributaries of the Kapuas River, i.e. Sungai Sebroeang (alternative spellings: Seberuang, Sebroang) and Sungai Bunut. Specimens collected outside the Kapuas River basin represent two new *Ctenodesma* species, separated by an uncorrected *p*-distance (*COI*) of 6.2%, and divergent from *C. borneensis* by 5.9% (*C. mawonae*), and 4% (*C. bersinara*) using the same metrics. Both of these species are known from only a single population each: First, *C. mawonae* (Fig. 4) is known from only two specimens collected from Sungai Sebua Jebung in the Sarawak River basin, Jambusan, Bau, Sarawak, Malaysian Borneo, situated to the north of the Kapuas River basin and emptying in the South China Sea in the northern

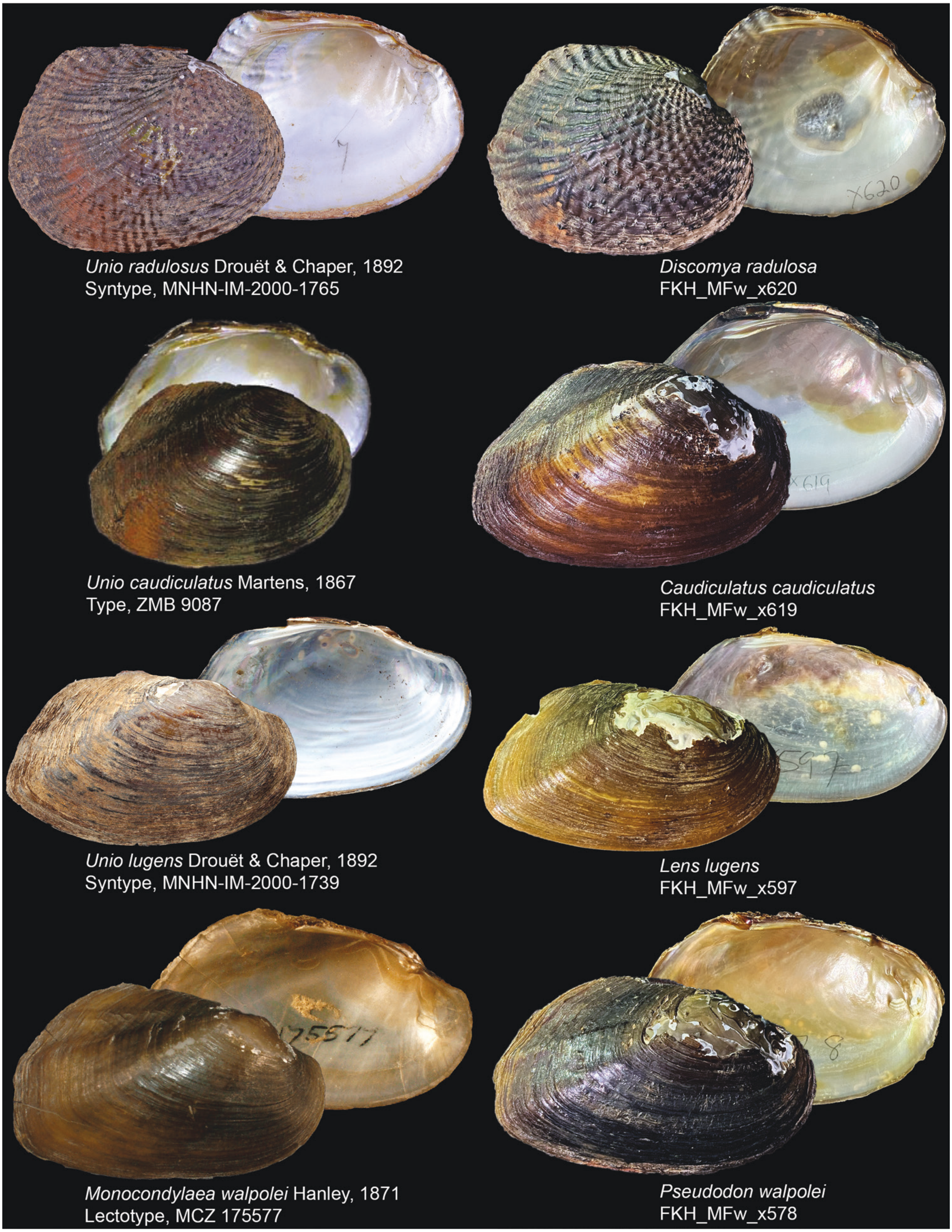

Figure 3. Types and sequenced specimens of *Discomya radulosa*, *Caudiculatus caudiculatus*, *Lens lugens*, and *Pseudodon walpolei*. Abbreviations: FKH, Fahutan Kapuas Hulu Collection, Tanjungpura University, Indonesia; MCZ, Museum of Comparative Zoology, Cambridge, USA; MNHN, Muséum National d'Histoire Naturelle, Paris, France; ZMB, Museum für Naturkunde, Berlin, Germany

Bornean coast (Figs 1, 5A; Table 1; Supporting Information, Fig. S3). The stream is situated in one of the few remaining patches of dense secondary forest and appears to have suffered from a pollution event in 2021, which has strongly decreased abundance of freshwater mussels at the site (pers. observation, K.A.A. Rahim). Second, *C. bersinara* (Fig. 4) is known from only a single specimen collected from the Sungai Rempangi, a tributary of the Laur River, which flows into the Pawan River in West Kalimantan, Indonesia (Figs 1, 5B; Table 1; Supporting Information, Fig. S3). Again, the site is situated in a patch of relatively intact rainforest, albeit with considerable deforestation and land-use change to palm oil plantation occurring in the immediate vicinity.

Rectidens

Currently, one species of *Rectidens* is recognized, i.e. *R. sumatrensis*, with an assumed distribution across Sumatra, Java, the Malay peninsula, and northern and western Borneo (Pfeiffer *et al.* 2021) (Table 1). Our molecular dataset confirms the conspecific status of *Rectidens* populations of Peninsular Malaysia and northern and north-western Borneo, and expands the species' confirmed distribution to the river basins of western Sarawak and the Kapuas basin in West Kalimantan (Figs 1, 6). However, populations from the Laur River catchment, Pawan River basin, West Kalimantan, situated south of the Kapuas River basin, belong to a new species, described herein as *R. lauris* (Figs 4, 6). *Rectidens lauris* is separated from *R. sumatrensis* by a mean value of 3.55% (uncorrected *p*-distance of *COI*). Considering the geographic vicinity of confirmed *R. sumatrensis* populations in the Perak River basin in Peninsular Malaysia to the type locality 'Danau Luar, Sumatra' of *R. sumatrensis* (Table 1), we retain the name *R. sumatrensis* for this lineage but note that DNA sequence data from the species' type locality will be needed to confirm whether this taxonomic name is correct.

We collected *R. lauris* live from two tributaries of the Sungai Laur, i.e. the type locality Sungai Rempangi (Fig. 5B) and the Sungai Melayu. In addition, a shell of the species was collected at Sungai Belantik, also a tributary of the Laur River.

The haplotype network analysis is consistent with the strong geographic structure of haplotypes as described in by Zieritz *et al.* (2018), with different haplotypes being present in each of the six river basins where this species was collected and sequenced thus far (Fig. 6B). The most divergent haplotype of the dataset occurs in the Perak River basin in Peninsular Malaysia.

Lens

Currently, two species of *Lens* are presumed to occur in Borneo: *Lens semmelinki* (Martens, 1891), known from two coastal drainages in southern Borneo, and *L. lugens*, known only from the Kapuas drainage (Pfeiffer *et al.* 2021). Our *COI* data show that all the sequenced *Lens* specimens we collected from Borneo belong to one species, morphologically identified as *L. lugens*, with 2.2% *p*-distance to *L. micropterus* (Supporting Information, Table S6), its sister-species (Fig. 7A). Live specimens were collected from seven sites in the Kapuas River basin, including the main stem at Semitau, three southern tributaries of the Kapuas (i.e. the Sungai Sebroeang, Sungai Embau, and Sungai Bunut) and one site from Danau Sentarum to the north of the Kapuas mainstem. In addition, the species was found at one site in Sarawak, specifically in the Sungai Nyanuh at Kampung Lobang Batu, Silabur, Serian in the Batang Sadong basin (sympatric with *M. walpolei*) (Fig. 1; Supporting Information, Fig. S4), thereby expanding the known range of *L. lugens* beyond the Kapuas River basin. The three haplotypes present in our dataset were found in the Kapuas River basin, whilst the most common haplotype (Haplotype 2; Fig. 7B) in the Kapuas River basin was the only one found in Sarawak (Supporting Information, Fig. S4).

Finally, it should be noted that all four species' delineation methods additionally considered the *Lens* populations from north-west Peninsular Malaysia as separate to their sister-species *Lens contradens* (Lea, 1838) (Fig. 7A). Further work, including additional sampling in the area and morphological analysis, is needed to confirm whether this clade indeed represents a distinct species.

Pseudodon

Currently, two species of *Pseudodon* are recognized from Borneo, i.e. *Pseudodon walpolei* (as *Monocondylaea walpolei* Hanley, 1871), with the type locality 'Sarawak, Borneo', and *Pseudodon aeneolus* Drouët and Chaper, 1892, with the type locality 'Sebroeang River, Kapuas, Semitau, Borneo' (Table 1). No molecular sequence data from this genus have been available to date from Borneo.

We collected *Pseudodon* specimens from eight sites and five river basins in western Borneo (Figs 1, 8B), including the type localities of *P. walpolei* and *P. aeneolus*, respectively: three populations in Sarawak (from west to east) in the Sungai Skibang 1 at Serikin, Sibau, Sungai Sambas Besar River basin, Sungai Sebua Jebung at Jambusan, Bau, Sarawak River basin (Fig. 5A), and Sungai Nyanuh at Kampung Lobang Batu, Silabur, Serian, Batang Sadong basin; three populations in the Kapuas River basin, i.e. two sites in the Sebroeang River catchment, and Sungai Temuyuk at Kampung Temuyuk, Sungai Bunut catchment; and two populations in the Laur River catchment, a tributary of the Pawan River basin, i.e. at Sungai Melayu and Sungai Rempangi (Fig. 5B).

Our *COI* data indicate that all populations belong to a single species that is endemic to western Borneo, with a distribution ranging from western Sarawak, including the Sarawak River basin, in the north to at least the Kapuas River basin and the Laur River basin in West Kalimantan in the south (Figs 1, 8; Table 1; Supporting Information, Table S7). Considering that the distribution of this clade includes the type locality of *P. walpolei*, and the sequenced specimens match the morphological description of this species (see below), we consider this species as *P. walpolei*. Historical data of yet unsequenced populations indicate that *Pseudodon* is distributed south until at least the Barito River basin in Central and South Kalimantan (Fig. 8B), but DNA sequence data will be required to clarify whether these populations belong to *P. walpolei* or a different *Pseudodon* species.

The haplotype network analysis confirmed a strong geographic structure of haplotypes, with different haplotypes being present in each of the five river basins where this species was collected and sequenced thus far (Fig. 8B). Highest genetic diversity was observed in the Kapuas basin, especially in the Sebroeang catchment. More population sampling will be needed to more completely resolve the distribution of *P. walpolei* haplotypes and biogeographic history of this species.

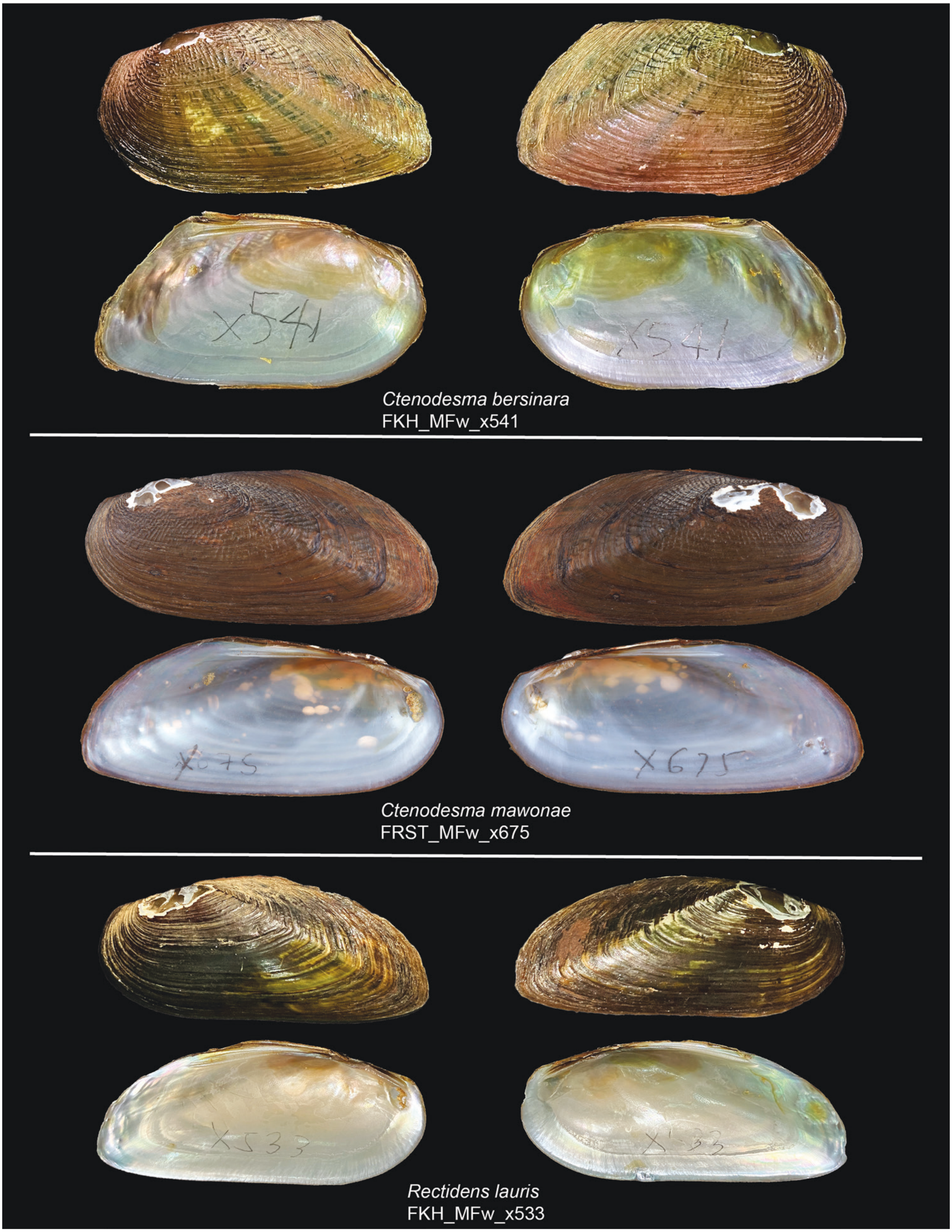

Figure 4. Holotypes of *Ctenodesma bersinara*, Sungai Rempangi, Pawan River basin, West Kalimantan, Indonesian Borneo; *Ctenodesma mawonae*, Sungai Sebua Jebung, Sarawak River basin, Jambusan, Bau, Sarawak, Malaysian Borneo; and *Rectidens lauris,* Sungai Rempangi, Pawan River basin, West Kalimantan, Borneo. Abbreviations: FKH, Fahutan Kapuas Hulu Collection, Tanjungpura University, Indonesia; FRST, Faculty of Resource Science and Technology Collection, Universiti Malaysia Sarawak, Malaysia.

Figure 5. Images of type localities of (A) *Ctenodesma mawonae*, i.e. Sungai Sebua Jebung in the Sarawak River basin, Jambusan, Bau, Sarawak, Malaysian Borneo, and (B) *Ctenodesma bersinara* and *Rectidens lauris*, i.e. Sungai Rempangi, Pawan River basin, West Kalimantan, Indonesian Borneo.

Sinanodonta

We are reporting two new records of the non-native species *S. pacifica* (=*Sinanodonta* cf. *woodiana*—tropical lineage) (Douda *et al.* 2024), i.e. in a slow-moving arm of the Sungai Redan at Kampung Lobang Batu, Silabur, Serian, in the Batang Sadong basin, Malaysia, and in a fish pond near Sungai Bulan in the Sungai Sebroeang catchment of the Kapuas River basin, Indonesia (Fig. 1; GenBank accession numbers PP697612-14). The specimen from Sarawak, Malaysia, has haplotype 1 of Douda *et al.* (2024: fig. 2), which was also found in several other sites in Malaysian Borneo and, in 2010, a small group of fishponds in New Jersey, USA, where it has been successfully eradicated. The specimens from the Kapuas basin, Indonesia, have haplotype 10 of Douda *et al.* (2024: fig. 2), which was previously detected in Java, Peninsular Malaysia, Singapore, Philippines, Japan, Taiwan and Costa Rica. It seems therefore likely that occurrences in Indonesian and Malaysian Borneo may represent separate invasion events.

Taxonomic accounts

Ctenodesma bersinara Zieritz *et al.*, **sp. nov.**

(Fig. 4)

Type locality: Sungai Rempangi (Pawan Drainage), 17 km SSW of Randau, Ketapang Regency, West Kalimantan, Indonesia (Fig. 5B).

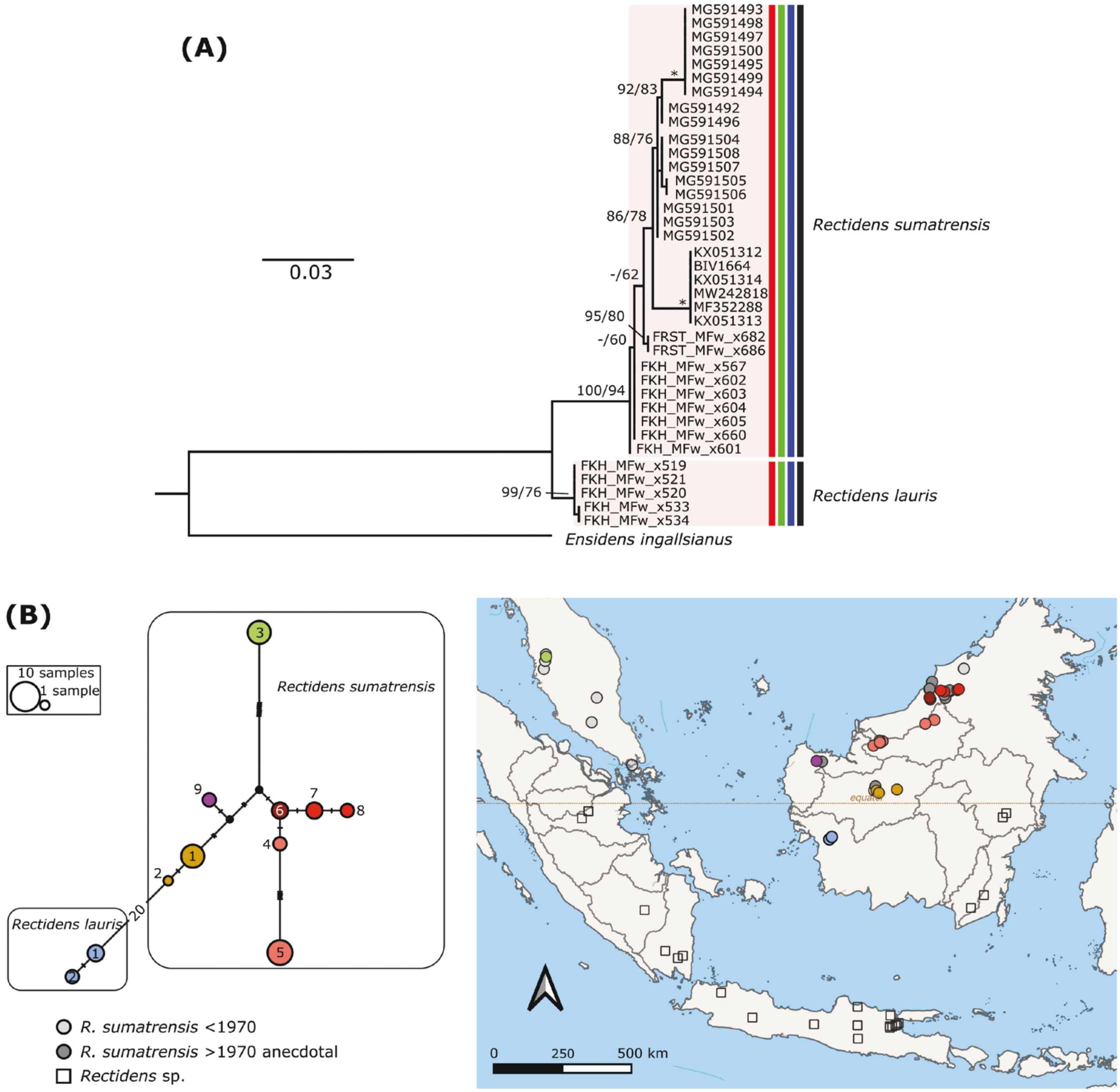

Figure 6. (A) maximum likelihood (ML) phylogenetic tree and (B) haplotype network of *Rectidens* inferred from the *COI* dataset. Values at nodes indicate Bayesian inference (BI) posterior probability percentage/ML ultrafast bootstrap values. Support values >95% for both phylogenetic analyses are indicated by an asterisk. Support values marked with '-' indicate nodes that differ in the BI tree.

Type: Holotype, FKH_MFw_x541.

Material examined: One lot: FKH_MFw_x541.

Diagnosis: Morphologically very similar to other *Ctenodesma* species but is less elongate, has a sharper posterior ridge, and strong green rays on posterior slope and shell disk.

Description: Shell thin, with fine wrinkles extending from posterior ridge, fine sculpture on dorsal third of shell disk; subrhombodial shell outline with slightly biangulate posterior slope; ventral margin slightly rounded, dorsal margin angled upwards, umbo even with hinge line; periostracum yellowish brown with green rays of variable thickness. Pseudocardinal teeth, elongate, moderately thick, one tooth in left valve, two teeth in right valve. Lateral teeth long, straight, two in left valve, one in right valve; Nacre bluish white, with yellowish brown tint near umbo and posterior slope. Soft anatomy unavailable for study.

Distribution: Known only from the type locality in West Kalimantan (Fig. 5B).

Etymology: The species is named after its strong green rays using the Bahasa Indonesian word '*bersinar*', meaning 'radiating'.

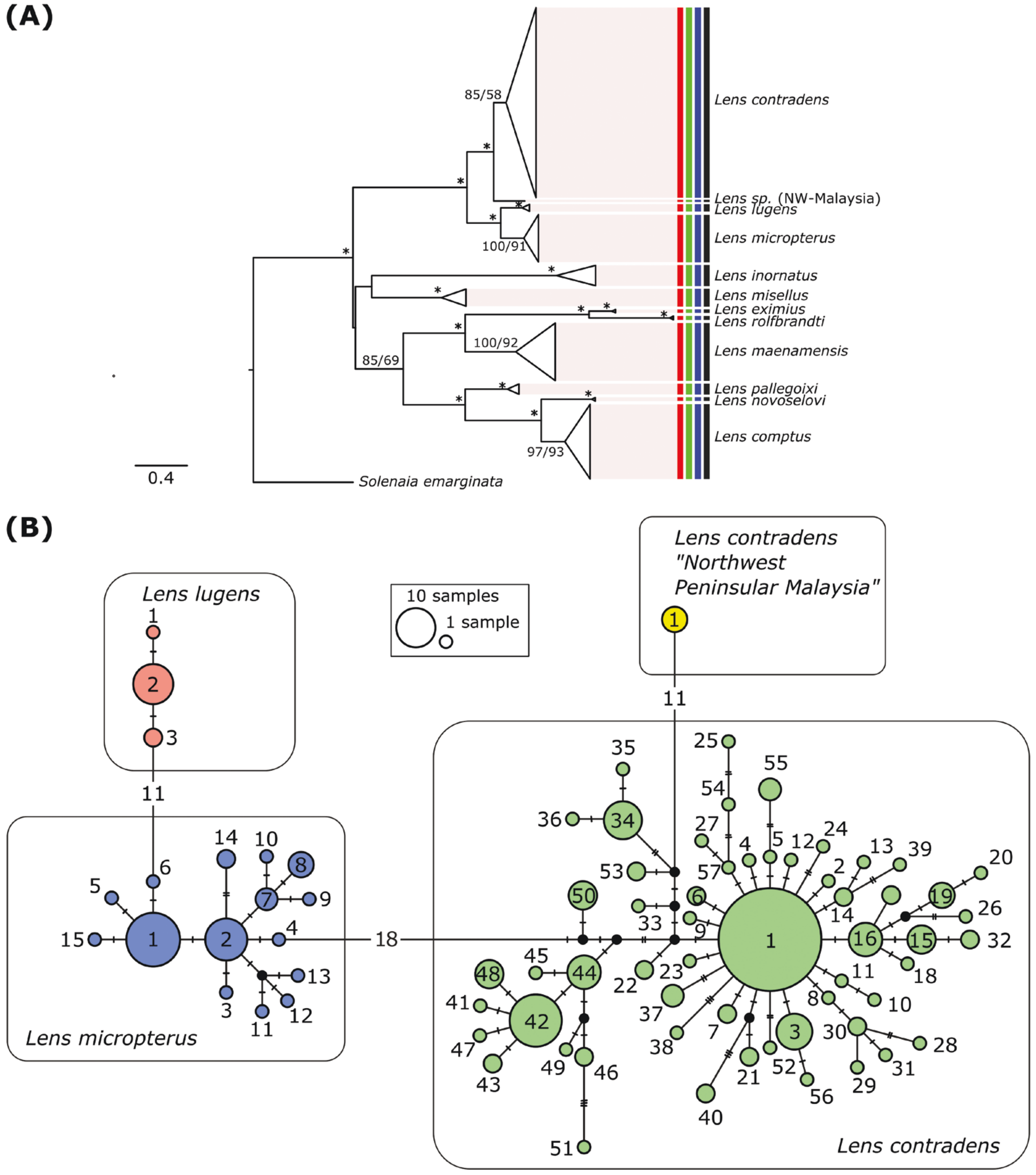

Figure 7. (A) phylogenetic tree of *Lens*, and (B) haplotype network of *the L. contradens–lugens–micropterus* clade inferred from the *COI* dataset.

Remarks: This species is known from a single small specimen (~40 mm), making meaningful morphological description of the species very difficult.

Ctenodesma mawonae Zieritz *et al.*, sp. nov.

(Fig. 4)

Type locality: Sungai Sebua Jebung (Sarawak Drainage), 3 km SSW of Bau, Kuching division, Sarawak, Malaysian Borneo (Fig. 5A).

Type: Holotype, FRST_MFw_x675. Paratype, FRST_MFw_x674, same locality as holotype.

Material examined: Five lots: FRST_MFw_x675, FRST_MFw_x674, MNHN MP 2723, MCZ 174117, BMNH MP D022.

Diagnosis: Morphologically very similar to other *Ctenodesma* species but is more elongate and has a less developed posterior ridge.

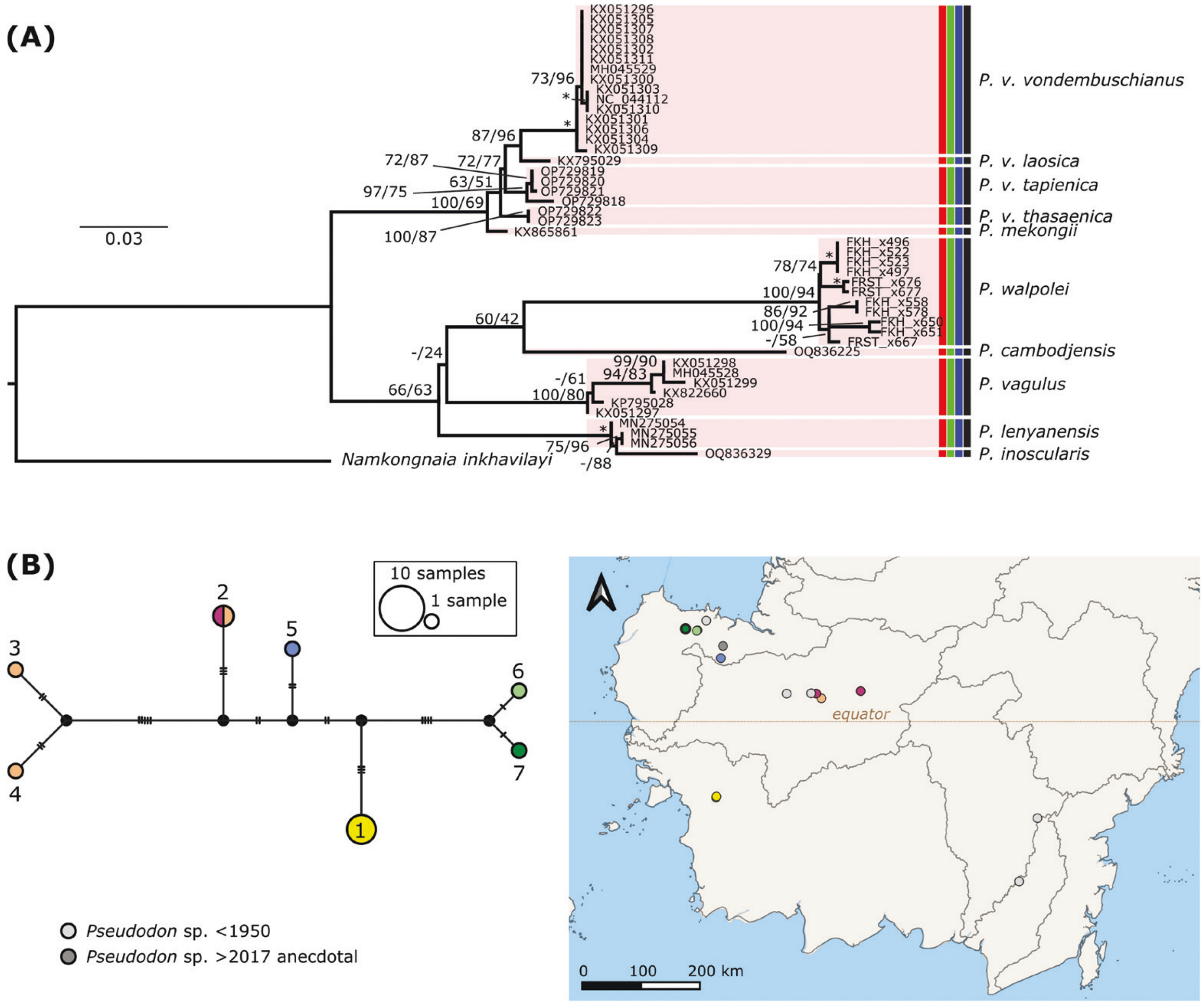

Figure 8. (A) maximum likelihood (ML) phylogenetic tree of *Pseudodon* and (B) haplotype network of *P. walpolei* inferred from the *COI* dataset. Values at nodes indicate Bayesian inference (BI) posterior probability percentage/ML ultrafast bootstrap values. Support values >95% for both phylogenetic analyses are indicated by an asterisk. Support values marked with '-' indicate nodes that differ in the BI tree.

Description: Shell thin, with fine wrinkles extending from posterior ridge, fine sculpture on dorsal third of shell disk; elliptical shell outline with slightly biangulate posterior slope; ventral margin slightly rounded, dorsal margin angled slightly upwards; umbo slightly elevated above hinge line; periostracum brown to yellow, with or without green rays of variable thickness. Pseudocardinal teeth, thick, fractured, one tooth in left valve, two teeth in right valve. Lateral teeth long, straight, two in left valve, one in right valve; Nacre blueish white, with peach tint near the umbo. Soft anatomy unavailable for study.

Distribution: Known from the type locality in Sarawak (Fig. 5A) and three lots from 'Sarawak'.

Etymology: The species is named in honour of Ms Dora Mawon, an amateur naturalist belonging to the local indigenous Bidayuh tribe, who assisted us during fieldwork at the type locality.

Rectidens lauris Zieritz *et al.*, **sp. nov.**

(Fig. 4)

Type locality: Sungai Rempangi (Pawan Drainage), 17 km SSW of Randau, Ketapang Regency, West Kalimantan, Indonesia.

Type: Holotype, FKH_MFw_x533. Paratype, FKH_MFw_x519, Sungai Melayu [Pawan Drainage], 18.7km SSW of Randau, Ketapang Regency, West Kalimantan, Indonesia.

Material examined: Two lots: FKH_MFw_x533, FKH_MFw_x519.

Diagnosis: Morphologically very similar to *Rectidens sumatrensis*.

Description: Shell thin, elongate shell outline; dorsal margin slightly rounded; ventral margin straight; posterior slope slightly biangulate; umbo even with hinge line; periostracum, yellowish/greenish brown. Psedocardinal teeth, long, thick, one tooth in left valve, two teeth in right valve. Lateral teeth long, curved, two in left valve, one in right valve; Nacre white, with peach tint near the umbo. Soft anatomy unavailable for study.

Distribution: Known from only two localities in the Pawan drainage in West Kalimantan (Fig. 5).

Etymology: The species is named after the river catchment of its type locality, i.e. the Laur River basin, and the ethnic group indigenous to this area, i.e. 'Dayak Laur'.

DISCUSSION

Our study revealed very high levels of phylogenetic diversity and endemism of freshwater mussels in western Borneo, rendering this region a hotspot of freshwater biodiversity within the Sundaland biodiversity hotspot and, thus, of exceptional importance for global freshwater conservation. Of the now 12 species reported from the study area (ranging from the Lupar River in the north to the Sungai Kendawangan in the south), nine are local endemics (Table 1). The species belong to eight genus-, six tribe- and two subfamily-level clades, thus demonstrating an exceptional high-level taxonomic diversity. A similar pattern has been observed for cyprinid fish, with the Kapuas River alone hosting four families, 50 genera, and 140 species of cypriniforms (Sulaiman and Mayden 2012).

Although only 20 sites in total were surveyed by us, three new species were discovered, suggesting that further sampling efforts in Borneo, including in Central, East, and North Kalimantan, may be expected to reveal additional species not yet known to science. This appears to be a particularly likely scenario for the Bornean endemic genera *Ctenodesma* and its sister *Khairuloconcha*, which to date comprise four and two species, respectively, all of which are known from a single river and, in some cases, a single site each (Zieritz *et al.* 2021). Species of these genera are small-sized and generally found in low-order streams in undisturbed forest habitats. As discussed in Zieritz *et al.* (2021), this may at least partly explain the diversification history of these genera on Borneo, as restriction to low-order streams would be expected to lead to restricted dispersal and thus, reproductive isolation of populations. Additional sampling will also be needed to unravel the taxonomic and phylogenetic status of *Elongaria trompi* (Drouët and Chaper, 1892) and *Hyriopsis velthuizeni* (Schepman, 1896), and to identify any remaining populations. Both species are known from the study area but have not been recorded for around 100 years (Graf and Cummings 2023).

Our data include the first DNA sequences for the monotypic genera *Caudiculatus*, endemic to Borneo, and *Discomya*, endemic to Borneo and Sumatra. Based on conchological characteristics, Pfeiffer *et al.* (2019) and Graf and Cummings (2021) tentatively placed both genera in the Gonideinae and specifically, *Caudiculatus* in the tribe Chamberlainiini and *Discomya* in the tribe Lamprotulini. Our five-gene phylogeny revealed that both of these hypotheses are wrong and that each of the two taxa represents a unique clade that is highly divergent from other extant taxa, with *Caudiculatus* probably representing its own tribe and *Discomya* its own subfamily. In addition to the Schepmaniini, which are also endemic to Borneo (Zieritz *et al.* 2021), these clades would, therefore, represent two further highly divergent molecular lineages from Borneo. However, we refrain from describing these new taxa at this point, because the positions of these taxa in our phylogeny were poorly supported (and resulted in a non-monophyletic Gonideinae), and because recognizing higher level taxa based on poorly supported topologies can create instability in classifications (Pfeiffer *et al.* 2019). Phylogenomic analysis will be needed to provide a more robust understanding of the phylogenetic position of *Caudiculatus* and *Discomya*, and together with a comprehensive analysis of the conchology and soft anatomy, enable identification of the sister groups and synapomorphies of these clades.

Whilst the specific ecological requirements of freshwater mussels in Borneo are still insufficiently understood, native species were found exclusively at sites of relatively undisturbed freshwater habitat, featuring natural, multispecies riparian vegetation (as opposed to oil palm plantations often dominating in lowland areas of the region), and no visible evidence of pollution or excessive sediment input (pers. obs.). However, as in most of Sundaland, such natural, functioning river habitats are becoming increasingly rare and threatened in western Borneo, mostly owing to the ongoing expansion of oil palm plantations. For example, whilst the lake Danau Sentarum and its immediate surrounding area are protected due to its status as a National Park (Fig. 1), the rivers connected to this lake system, i.e. the Kapuas River and tributaries, are unprotected, and much of the land has already been committed for future oil palm plantations ('Oil palm concessions'. Accessed through Global Forest Watch on 21/02/2024. www.globalforestwatch.org). Deforestation and conversion of natural riparian vegetation to agricultural monocultures profoundly alters and degrades riverine habitats by increasing erosion, water temperature, input of fine sediment, pollutants and nutrients (Douglas 1996, Koning *et al.* 2017, Luke *et al.* 2017, Chellaiah and Yule 2018). Native freshwater mussel communities have been shown to be particularly sensitive to these environmental changes (Zieritz *et al.* 2016, 2018, Gallardo *et al.* 2018), which can, therefore, be expected to have devastating effects on the mussel fauna in the Kapuas and other rivers studied here.

To an extent, the negative effects of oil palm plantations on freshwater biodiversity can be mitigated by maintaining the legally required riparian buffer zones (Giam *et al.* 2015, Luke *et al.* 2019), but the specific characteristics and dimensions of these can vary greatly and, thus, their effectiveness. Sites and rivers hosting particularly rare, endemic species, such as the Sebroeang River, the Laur River and their tributaries in West Kalimantan, and the Sungai Sebua Jebung in Bau, Sarawak, therefore urgently require special protection to ensure that these species are not lost globally. In addition to any on-the-ground conservation actions, outreach programmes are needed to raise awareness of the region's outstanding diversity of mussels and other freshwater taxa.

SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

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AUTHOR CONTRIBUTIONS

Conceptualization: Manuel Lopes-Lima, Alexandra Zieritz. Formal analysis: Manuel Lopes-Lima, John Pfeiffer, Alexandra Zieritz. Funding acquisition: Elsa Froufe, Manuel Lopes-Lima, John Pfeiffer, Alexandra Zieritz. Investigation: Muhammad Anwari, Farah Diba, Manuel Lopes-Lima, John Pfeiffer, Hari Prayogo, Khairul Rahim, Alexandra Zieritz. Methodology: Manuel Lopes-Lima, Alexandra Zieritz. Project administration: Elsa Froufe, Alexandra Zieritz. Resources: all authors. Writing—original draft preparation: Manuel Lopes-Lima, John Pfeiffer, Alexandra Zieritz. Writing—review and editing: all authors.

DATA AVAILABILITY

DNA sequence data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/nucleotide/, and can be accessed with the accession numbers available in this article. The GPS location data for freshwater mussel populations underlying this article cannot be shared publicly for conservation reasons. The data will be shared on reasonable request to the corresponding author. All other data underlying this article are available in the article and in its online supplementary material.

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