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Factors driving changes in freshwater mussel (Bivalvia, Unionida) diversity and distribution in Peninsular Malaysia

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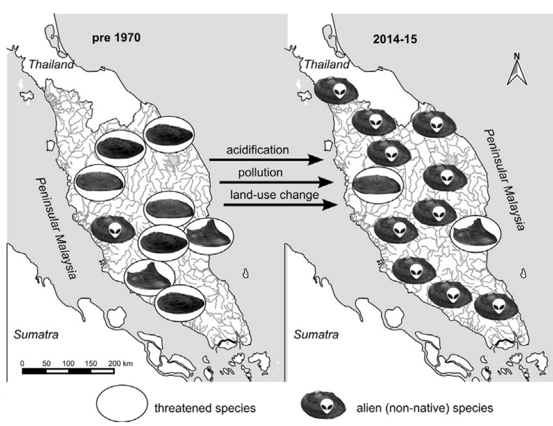
HIGHLIGHTS

- We conducted the first assessment of freshwater mussels in Peninsular Malaysia.
- We found ten species, two of which had not been previously recorded.
- Three species are acutely threatened due to restricted and declining distributions.
- Main threats to this fauna are human-induced acidification and eutrophication.
- We recommend establishing riparian buffers and improving waste water treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater mussels (Bivalvia, Unionida) fulfil important ecosystem functions and are one of the most threatened freshwater taxa globally. Knowledge of freshwater mussel diversity, distribution and ecology in Peninsular Malaysia is extremely poor, and the conservation status of half of the species presumed to occur in the region has yet to be assessed. We conducted the first comprehensive assessment of Peninsular Malaysia's freshwater mussels based on species presence/absence and environmental data collected from 155 sites spanning all major river catchments and diverse habitat types. Through an integrative morphological-molecular approach we recognised nine native and one widespread non-native species, i.e. *Sinanodonta woodiana*. Two species, i.e. *Pilsbryoconcha compressa* and *Pseudodon cambodjensis*, had not been previously recorded from Malaysia, which is likely a result of morphological misidentifications of historical records. Due to their restriction to single river catchments and declining distributions, *Hyriopsis bialata*, possibly endemic to Peninsular Malaysia, *Ensidens*

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Endemic species
Eutrophication
Land-use change
Rare species

ingallsianus, possibly already extinct in the peninsula, and *Rectidens sumatrensis*, particularly require conservation attention. Equally, the Pahang, the Perak and the north-western river catchments are of particular conservation value due to the presence of a globally unique freshwater mussel fauna. Statistical relationships of 15 water quality parameters and mussel presence/absence identified acidification and nutrient pollution (eutrophication) as the most important anthropogenic factors threatening freshwater mussel diversity in Peninsular Malaysia. These factors can be linked to atmospheric pollution, deforestation, oil-palm plantations and a lack of functioning waste water treatment, and could be mitigated by establishing riparian buffers and improving waste water treatment for rivers running through agricultural and residential land.

1. Introduction

Freshwater mussels (Bivalvia, Unionida) inhabit aquatic habitats across the globe, serving crucial ecological functions, such as water clearance by filtration, nutrient transport between the water column and the benthos, bioturbation and oxygenation of sediments, and providing habitat for other organisms (Vaughn and Hakenkamp, 2001; Chowdhury et al., 2016). Particularly in Asia, freshwater mussels are also used by humans as a food source (A. Zieritz, pers. obs.), and as raw materials for a variety of end products ranging from cosmetics to jewellery and medicine (Fiske and Shepherd, 2007; Jian-Ping et al., 2010). Unfortunately, owing to habitat deterioration, loss and fragmentation, river flow alterations and introduction of non-native species, freshwater mussels are also considered to be amongst the most threatened freshwater animals in the world (Bogan, 1993; Ricciardi and Rasmussen, 1999; Lydeard et al., 2004). Of the 517 freshwater mussel species that have been assessed by the International Union for Conservation of Nature (IUCN), 6% are extinct, 13% critically endangered, 10% endangered, 7% vulnerable, 9% near threatened and 37% least concern (IUCN, 2016). The remaining 95 species (i.e. 18%), 45 of which are from South and Southeast Asia, were assigned "data deficient" status. This highlights the lack of, and urgent need for, basic data on the distributions and ecological requirements of the freshwater mussel species of this region.

For Peninsular Malaysia, historical data on freshwater mussel diversity and distributions are scarce but indicate the presence of at least ten species (Van Benthem Jutting, 1960; Berry, 1963, 1974; Brandt, 1974; Graf and Cummings, 2015) (Table 1). However, present-day diversity and distribution are likely to differ markedly from that based on these previous records due to a number of reasons. (1) The vast majority of available historical records date back several decades to centuries. Considering that habitat loss, fragmentation and modification, associated with growing human populations, industrialisation and changes in land use, as well as expanding ranges of non-native invasive species, have been shown to considerably alter freshwater mussel diversity and distribution across the globe (Strayer et al., 2004; Shea et al., 2013; Jones and Byrne, 2014; Lopes-Lima et al., 2014; Lopes-Lima et al., 2016), similar patterns can be expected for Peninsular Malaysia. (2) Some parts of the country (e.g. Terengganu) are practically unexplored with respect to freshwater mussels. Through targeted surveys, we may therefore expect to detect previously unrecorded freshwater mussel populations. (3) Due to the extreme morphological variability of freshwater mussels (Zieritz et al., 2010), accurate delimitation of species boundaries and species identification by morphological means are notoriously difficult. Molecular techniques have helped reveal misidentifications, over-naming (i.e. splitting) of morphologically highly variable species and under-naming (i.e. lumping) of morphologically similar, cryptic species of freshwater mussels (Hebert et al., 2003; Zieritz et al., 2012; Froufe et al., 2016). Considering that freshwater mussel records for Peninsular Malaysia have been exclusively based on morphological identifications, we expect that an integrative morphological-molecular approach of species identification will significantly alter our understanding of freshwater mussel biogeography in the region.

Due to the lack of data, about half of the freshwater mussel species recorded from Peninsular Malaysia have either been assigned "data deficient" status or are yet to be assessed by the IUCN (Table 1). No freshwater mussel species known from Peninsular Malaysia are currently protected by law, and no Species Action Plans or monitoring programmes are in place. This lack of protection is in contrast to other regions such as North America and Europe, where each year, a great amount of money is dedicated to mussel conservation (e.g. projects within the EU-LIFE program towards the conservation of *Margaritifera margaritifera* populations have received several million € from the EU to date; http://ec.europa.eu/environment/life/project/Projects/ (Lopes-Lima et al., 2016)).

The threats to freshwater mussels and their ecosystems in Peninsular Malaysia – as in other regions of Southeast Asia – are many and severe. Since publication of Brandt (1974), the last significant body of work for the region's freshwater mussels, Malaysia's human population has more than doubled from 12 to 30 million (http://www.worldbank.org/). The steep population growth was accompanied by rapid economic growth, urban expansion, and a 10-fold increase in GDP per capita from 800 to 11,000 USD (http://www.worldbank.org/). The collateral environmental effects of this "industrial modernisation" have included the loss of 20% of Malaysia's ancient forests between 1975 and 2005 (Wicke et al., 2011). Particularly in Peninsular Malaysia, much of the logged area was converted to oil palm plantations, which in 2011, covered 20% (25,000 km^2) of the land (Mahat, 2012). To further satisfy the increasing energy demands, numerous small and several large dams have been constructed in most major river catchments of the Peninsula (Marmulla, 2001).

All these activities have severely impacted many Malaysian freshwater ecosystems by altering flow regimes, disrupting longitudinal connectivity, and increasing pollution, sedimentation and eutrophication (Marmulla, 2001; Allen et al., 2012; Ngah and Othman, 2012; Nor Zaiha et al., 2015). Sources of nutrients (in particular phosphorus and nitrogen) and pollutants (e.g. heavy metals) include domestic and industrial sewage, agricultural runoffs, mining, housing and road construction, logging, factories and motor boats. As a result, the water quality of rivers has declined sharply since the early 1990s (Ngah and Othman, 2012). Over 60% of Malaysia's lakes are known to suffer from nutrient enrichment, resulting in phytoplankton and macrophyte blooms (Sharip and Zakaria, 2008; Mamum and Zainudin, 2013). Heavy metal concentrations in, for example, Lake Kenyir (Syaripuddin et al., 2014) and Lake Chini (Ebrahimpour and Mushrifah, 2008), frequently exceed levels that are lethal to freshwater mussels (Naimo, 1995). Almost half of the rivers are polluted with ammoniacal nitrogen (Mamum and Zainudin, 2013). Other potential threats to freshwater mussels in the region include other forms of habitat degradation, flow modification, over-exploitation, invasive species and loss of host fish populations that mussels require for successful reproduction (Dudgeon, 2000; Wächtler et al., 2001; Allen et al., 2012).

This study aims to collect baseline data that will facilitate the development of future conservation efforts of the freshwater mussels (Unionida) of Peninsular Malaysia. The main objectives of this study are (1) to determine the diversity and distribution of freshwater mussels; (2) to assess water quality requirements of freshwater mussels in

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Table 1
Conservation status, distributions (per Federated State), museum (Museum of Zoology, University of Malaya), Barcode Index and GenBank accession numbers, and genetic (COI) distances of freshwater mussel species reported from Peninsular Malaysia.

Species name	IUCN conservation status	Number of historic and present-day records	Perlis	Kedah	Perak	Kelantan	Terengganu	Pahang	Selangor/KL	Negeri Sembilan	Malacca	Johor	Museum accession number	Barcode Index Number	GenBank accession numbers	Maximum intraspecific distance (%)	Minimum interspecific distance (%)
Contradens contradens Lea, 1838[a]	LC	30/22	B	B	B	B		B		B	C	B	MZUM(BIV) 0001–22	ACX7839, ACX9052	KX051243-KX051272, KX051294	2.94	10.13
Ensidens ingallsianus Lea, 1852	LC	8/0			H	H		H			H		NA	NA	NA	NA	NA
Hyriopsis bialata Simpson, 1900[b]	NA	16/2						B			H		MZUM(BIV) 0023–24	ACX8996	KX051273-KX051274	0	9.98
Physunio superbus (Lea, 1843)	LC	1/4						B					MZUM(BIV) 0025–28	ACX8746	KX051275-KX051282	0.34	9.88
Pilsbryoconcha compressa (Martens, 1860)	NA	0/2			C								MZUM(BIV) 0029–30	ACX8982	KX051283-KX051285	N/A	8.9
Pilsbryoconcha exilis (Lea, 1838)[a]	LC	3/6	B	C		C				C			MZUM(BIV) 0031–36	ACX8478, ACX8706	KX051286-KX051291	3.07	8.33
Pseudodon cambodjensis (Petit de la Saussaye, 1865)	DD	0/3			C	C							MZUM(BIV) 0037–39	ACW0418	KX051297-KX051299	2.05	8.35
Pseudodon chaperi (Morgan, 1885)	NA	8/0	H		H	H							NA	NA	NA	NA	NA
Pseudodon cumingii (Lea, 1850)	NA	7/3	C	C	H								MZUM(BIV) 0040–42	ACX1690	KX051292, KX051293, KX051295	0.35	10.65
Pseudodon vondembuschianus (Lea, 1840)	LC	4/8		H	C			C		B		C	MZUM(BIV) 0043–50	ACX7840	KX051296, KX051300-KX051311	0.7	2.56
Rectidens sumatrensis Dunker, 1852	DD	7/1			B			H					MZUM(BIV) 0051	ACX8645	KX051312-KX051314	0	13.29
Sinanodonta woodiana (Lea, 1834)	LC	1/19	C	C	C	C	C	C	B	C	C	C	MZUM(BIV) 0052–65	ACX1363	KX051315-KX051328	0.56	3.08
Total		85/70															

Abbreviations: B, taxon recorded both historically and in current assessment; C, taxon recorded only in current assessment; DD, data deficient; H, taxon recorded only historically; IUCN, International Union for Conservation of Nature; LC, least concern; NA, not assessed.

[a] *Contradens contradens* and *Pilsbryoconcha exilis* are regarded as single species, respectively, for reasons explained in the text.

[b] Currently listed as LC, but considering that molecular data show that the Malaysian populations in fact comprise a separate species (A.Z., M.L., A.B., R.S., J.W., U. Kovitvadhi, S. Kovitvadhi; unpublished data [see Appendix S2]), conservation status of the Peninsular Malaysian species requires re-assessment.

general and any species-specific requirements; (3) to identify the most vulnerable freshwater mussel species and (4) to identify freshwater bodies of particular conservation interest. To that end, historical and present-day data are reviewed and assessed from across all provinces and major river systems in Peninsular Malaysia.

2. Materials and methods

2.1. Study area

Peninsular Malaysia, covering a total land area of 130,268 km^2, forms part of Sundaland, which includes Borneo, Java and Sumatra (Hutchison and Tan, 2009). Geological conditions in the region are characterised by granite bedrock dominating the inland and upland areas and limestone deposits at the margins of the peninsula (Hutchison and Tan, 2009). This results in freshwater systems ranging from ion-dilute, very soft-water (<5 mg L^{-1}) habitats with low pH (<6) and low ion (e.g. Ca^{2+} < 1 mg L^{-1}) availability to moderately hard-water (<100 mg L^{-1}) habitats with higher pH (>7) and ion concentrations (e.g. Ca^{2+} > 30 mg L^{-1}) (Ministry of Natural Resources and Environment Malaysia, 2009).

2.1.1. Sampling and voucher specimens

We collated historical distribution data of freshwater mussels in Peninsular Malaysia from the mussel-project database at http://mussel-project.uwsp.edu/, which provides photographs of specimens from 17 major museum collections (Graf and Cummings, 2015). Due to the large number of local names for most rivers, we analysed historical data on the Federated State level.

We conducted present day-sampling of freshwater mussels between November 2014 and October 2015. We surveyed 155 sites, covering a diversity of freshwater habitats (rivers, streams, canals, rice-paddy run-offs, lakes, reservoirs and ponds) and all major river catchments in Peninsular Malaysia (Fig. 1, Table 2). Sites within a continuous water body (e.g. river catchment, lake) were at least 2 km apart from each other. At each site, we initially approached locals and asked about the presence of freshwater mussels. This allowed us to assess the chance of missing freshwater mussels, particularly when water levels were high. Subsequently, we searched for freshwater mussels visually (glass-bottom buckets) and hand-sampling for a minimum of 10 and up to 120 person-minutes, typically covering about 100 m river length (Cummings et al., 2016). Survey effort thereby depended on a

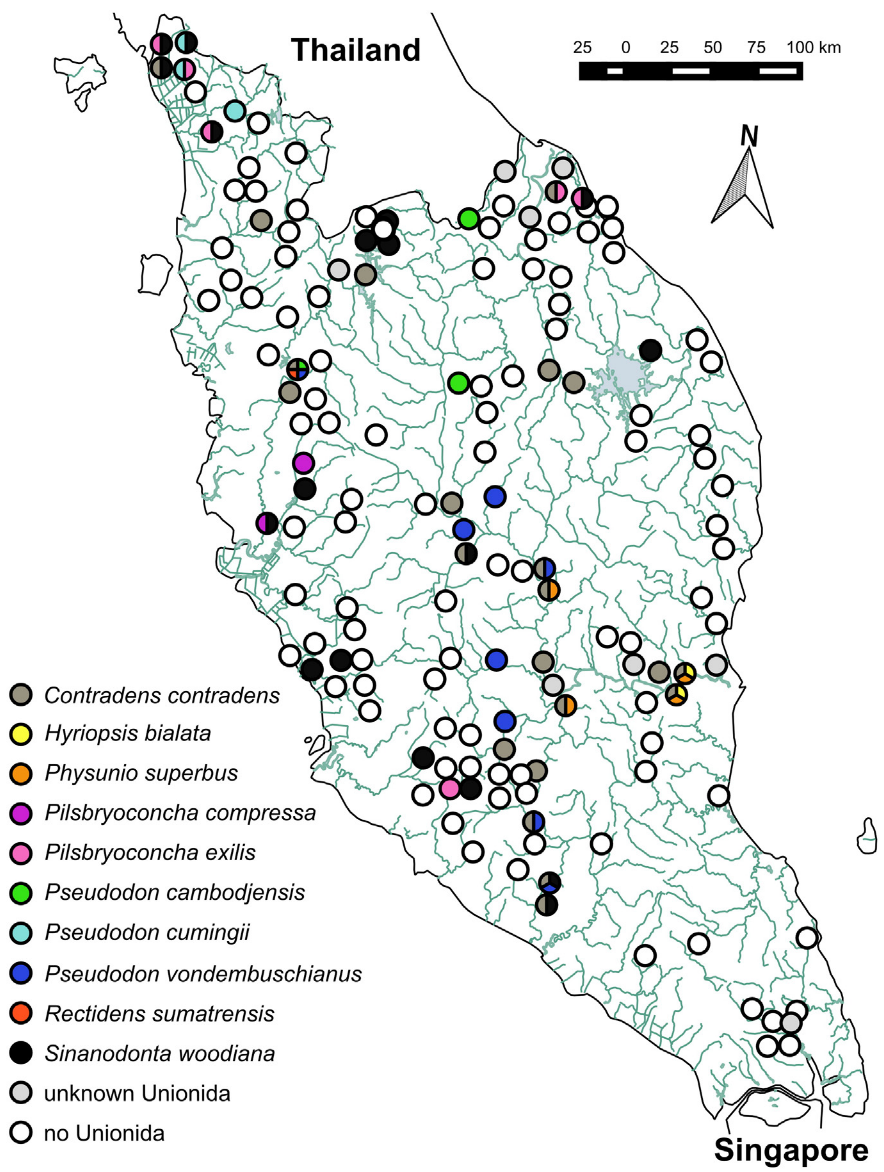

Fig. 1. Distribution of freshwater mussel species across 155 sites in Peninsular Malaysia as recorded from November 2014 to October 2015. Size of pie chart wedges does not reflect relative abundance of species.

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Table 2
Present-day freshwater mussel species composition of Peninsular Malaysian river catchments.

River catchment	*Sinanodonta woodiana*	*Pilsbryoconcha exilis*	*Pilsbryoconcha compressa*	*Pseudodon vondembuschianus*	*Pseudodon cambodjensis*	*Pseudodon cumingii*	*Contradens contradens*	*Physunio superbus*	*Hyriopsis bialata*	*Rectidens sumatrensis*	Region
Perlis River	X	X				X	X				North-western catchments
Kedah River	X	X				X					
Muda River							X				
Perak River	X		X	X	X		X			X	Perak River
Tengi River	X										
Selangor River (abandoned mining pool)	X										
Langat River (Lake Semenyih)	X										Central-western catchments
Muar River				X			X				
Kesang River	X			X			X				South-western catchments
Golok River					X						
Kelantan River					X		X				
Semerak River	X	X			–		X				North-eastern catchments
Terengganu River (Lake Kenyir)	X										
Pahang River (*P. exilis* from man-made fish pond only)	X	X		X			X	X	X		Pahang River

number of factors. (1) Historical records and/or local knowledge: sites where presence of freshwater mussel (species) was indicated by historical records and/or locals were surveyed either until respective freshwater mussels (species) were found or for a minimum of 60 min. (2) Habitat visibility: less time was spent surveying at sites where the substrate could easily be seen and a large area could thus be swiftly scanned for mussels, compared to sites where this was not the case. (3) Habitat homogeneity: sites with different types of microhabitats were surveyed for longer to allow for adequate surveying of all microhabitat types. (4) Sampling efficiency: sites that were difficult to move in (e.g. due to presence of dead wood) or where adequate mussel habitat was difficult to survey (e.g. due to steep banks) were searched for comparatively longer periods to account for inefficient surveying. We collected foot snips (non-lethal to the mussels; Spicer et al., 2007) from a maximum of 25 specimens per population and preserved in 100% ethanol. In addition, we preserved up to six voucher specimens (i.e. whole animal) per population in 95% ethanol and deposited at the Museum of Zoology, University of Malaya, Kuala Lumpur.

2.2. Measurement of water quality parameters

We measured the following water quality parameters at each site before surveying for freshwater mussels: (1) dissolved oxygen saturation (DOS), (2) specific conductivity (SPC) and (3) pH, using a YSI Professional Plus multi-probe. In addition, water samples were taken, partially filtered through pre-ashed GF/C filters, and kept cool for subsequent determination of following parameters in the laboratory: (4) total suspended solid (TSS) concentrations, measured by filtration of a given volume of water through a pre-weighed GF/C filter and subsequent weighing of the dried filter, and (5) weight-percentage of the organic portion of TSS (ORG), estimated by subsequent loss-on-ignition analysis at 550 °C; concentrations of (6) total phosphorus (TP), (7) soluble reactive phosphorus (SRP), (8) nitrate (NO_3^-), (9) total ammonia nitrogen (TAN) and (10) silicate (SiO_4^{2-}) were assessed using standard spectrophotometric/colorimetric methods (Mackereth et al., 1989). Concentrations of (11) Na^+, (12) Mg^{2+}, (13) K^+ and (14) Ca^{2+} were determined through ion chromatography (IC) analysis using a Metrohm Basic 792 ion chromatography system (Metrosep A Supp 4-250 column, with 1 mmol sodium bicarbonate and 3.2 mmol of sodium carbonate eluent at 1.0 mL min^{-1}). (15) River widths (width) were measured in Google Maps.

2.3. Identification of species

We identified species using an integrative taxonomic approach, combining a comparison of morphological characters against descriptions by Brandt (1974) and photographs of museum specimens from the mussel-project database (Graf and Cummings, 2015) with DNA barcoding (Hebert et al., 2003). One exception was some specimens of the well-known, non-native *Sinanodonta woodiana*, which were unambiguously identified by AB, AZ, ML and RS by morphology alone. For the other species, genomic DNA was extracted from at least one specimen per population (i.e. species-site occurrence) using a Nucleospin tissue kit (Machery-Nagel) following the manufacturers' instructions. A fragment of cytochrome *c* oxidase I (COI) mtDNA was PCR amplified with universal primers (Folmer et al., 1994) and standard PCR protocol (Wilson, 2012) from each DNA extract. PCR products were Sanger sequenced using the PCR primers.

The COI DNA barcodes, specimen collection details and photographs were submitted to the Barcode of Life Datasystems (BOLD; Ratnasingham and Hebert, 2007), where they are available in the public dataset DS-FMPM. The DNA barcodes were automatically sorted into Barcode Index Numbers (BINs) by BOLD (Ratnasingham and Hebert, 2013). BINs are molecular operational taxonomic units (MOTUs) delimited through Refined-single linkage (RESL) analysis based on patterns on connectivity without a fixed threshold, and have shown high congruence with traditionally recognised species in previous studies (Hausmann et al., 2013; Ratnasingham and Hebert, 2013).

2.4. Statistical analyses

We tested whether sites with and without freshwater mussels differed in their water quality characteristics by conducting (1) a MANOVA on the 15 water quality parameters as dependent variables, (2) a *t*-test on the Linear Discriminant (LD) obtained by Linear Discriminant Analysis (LDA) of the 15 water quality parameters, and (3) *t*-tests on the first three Principal Components (PCs) obtained by Principal Component Analysis (PCA) on the 15 water quality parameters.

Water quality requirements of Peninsular Malaysian freshwater mussels in general were elucidated by examining which of the 15 parameters contributed the most to LD and those PCs that showed significant differences between sites with and without freshwater mussels. To reveal differences and similarities in water quality requirements of species, we then performed a Canonical Correspondence Analysis (CCA) of the 15 water quality parameters and presence/absence data per species (excluding sites without freshwater mussels).

For all parametric tests, non-normal data were normalised through box-cox transformation. LDA and MANOVA were performed with missing values replaced by averages for each variable. PCA and CCA were performed in PAST v.3. All other statistical analyses were performed in R3.1.1.

3. Results

3.1. Species diversity

Freshwater mussels were found and sampled at 55 of 155 surveyed sites, comprising 13 river catchments in Peninsular Malaysia (Fig. 1, Table 2). This amounted to 78 populations (i.e. 78 species-site occurrences). The presence of freshwater mussels at eight sites is anecdotal, i.e. was confirmed by at least two independent local sources but could not be confirmed by sampling due to high water levels. As a consequence, the identity of the species present at these eight sites could not be determined (Fig. 1, light grey dots).

A total of 87 COI sequences were obtained (see Table 1 for BOLD and GenBank accession numbers) and sorted into 12 BINs by BOLD. However, when combined with the morphological identifications we take a conservative approach and recognise 10 species (*Contradens contradens* and *Pilsbryoconcha exilis* both were split into two BINs; Table 1). Previous researchers have reported intraspecific variation in COI sequences in several freshwater mussel species exceeding 2.5% (uncorrected p-distance), especially those with broad spatial distributions (e.g. *Anodonta anatina* (Froufe et al., 2014), *Lampsilis australis* (Roe et al., 2001)). A taxonomic revision of the freshwater mussels of Southeast Asia is ongoing by our research group, but is outside the scope of the current paper.

Based on our integrative taxonomic approach, the 70 sampled freshwater mussel populations accounted for nine native and one non-native species: (1) *Sinanodonta woodiana* (non-native to Peninsular Malaysia), (2) *Pilsbryoconcha exilis*, (3) *Pilsbryoconcha compressa*, (4) *Pseudodon cambodjensis*, (5) *Pseudodon cumingii*, (6) *Pseudodon vondembuschianus*, (7) *Contradens contradens*, (8) *Hyriopsis bialata*, (9) *Physunio superbus* and (10) *Rectidens sumatrensis*.

3.2. Spatial distribution of species

S. woodiana was the most widely distributed species, being sampled at ten river catchments and 19 sites (Fig. 1, Table 2), followed by *C. contradens*, which was sampled from seven river catchments and 22 sites. The three *Pseudodon* species and *P. exilis* showed fairly restricted distributions and were sampled in two to three river catchments and three to eight sites, respectively. The remaining four species, i.e. *P. compressa*, *P. superbus*, *H. bialata* and *R. sumatrensis*, were highly restricted in distribution and found at only one river catchment, respectively. *R. sumatrensis* was found at only a single site in the Perak River.

3.3. Associations of freshwater mussels with water quality parameters

MANOVA and LDA of the 15 water quality parameters revealed significant differences between sites with and without freshwater mussels (MANOVA Pillai test: F = 2.197, num df = 15, den df = 139, P = 0.009; *t*-test on LD: t = −2.817, df = 153, P = 0.006). The water quality parameters that contributed most to LD were SPC (35%), ORG (18%), TSS (11%), width (10%), Ca^{2+} (8%) and pH (6%). This indicates that freshwater mussels tended to occur at sites with relatively high specific conductivity (mussel sites: 80.3 ± 48.0 μS cm^{-1}; no mussel sites: 64.2 ± 74.5 μS cm^{-1}), high TSS concentrations (mussel sites: 79 ± 140 mg L^{-1}; no mussel sites: 51 ± 136 mg L^{-1}) with low particulate organic content (mussel sites: 38 ± 27%; no mussel sites: 44 ± 25%), large width (for rivers) (mussel sites: 93 ± 152 m; no mussel sites: 29 ± 35 m), and sites that were at the top end of the spectrum for measured Ca^{2+} concentrations (mussel sites: 13.1 ± 13.3 mg L^{-1}; no mussel sites: 10.0 ± 9.5 mg L^{-1}) and pH (mussel sites: 6.6 ± 0.5; no mussel sites: 6.1 ± 0.8) (Appendix S1).

Pronounced differences in the water quality parameters of sites with and without freshwater mussels were confirmed by PCA (Fig. 2). A significant difference between sites with and without freshwater mussels was found in PC2 (*t*-test: $t = 2.015$, df = 153, P = 0.046) and PC3 (*t*-test: $t = 3.532$, df = 153, P = 0.001) but not in PC1 (*t*-test: $t = 1.037$, df = 153, P = 0.302). PC2 explained 16.1% of the variance and was predominantly influenced by TSS, ORG and PO_4^{3-}. PC3 explained 11.3% of the variance and was predominantly influenced by (river) width, pH and TAN concentrations. PC1 (not depicted in Fig. 2) explained 26.8% of the variance in the dataset and was predominantly influenced by concentrations of cations (i.e. K^+, Ca^{2+}, Na^+ and Mg^{2+}) in the water. Thus, freshwater mussels are more likely to be found at sites with relatively low TAN (mussel sites: 51 ± 87 μg L^{-1}; no mussel sites: 264 ±

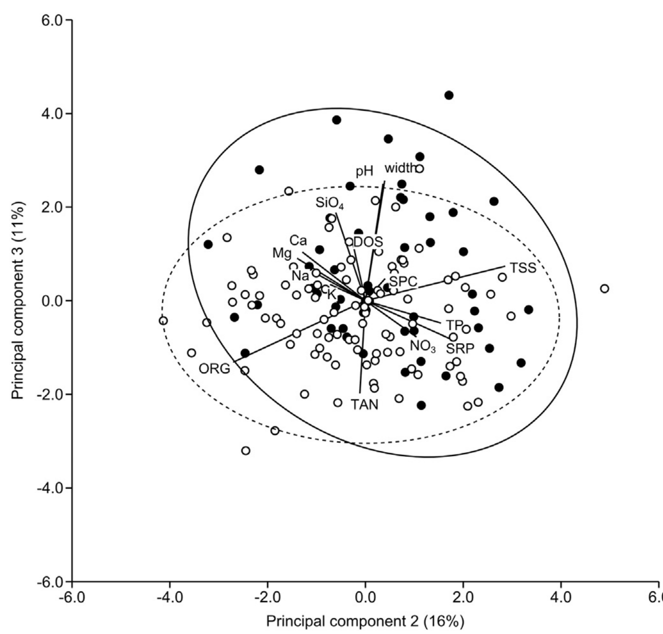

Fig. 2. Ordination diagram of PC2 and PC3 for 155 sites in Peninsular Malaysia without freshwater mussels (white dots, dashed 95% confidence ellipses) and with freshwater mussels (black dots, continuous 95% confidence ellipses). Abbreviations: Ca, calcium; DOS, dissolved oxygen saturation; K, potassium; Mg, magnesium; Na, sodium; NO_3, nitrate; ORG, weight-percentage of the organic portion of TSS; SiO_4, silicate; SPC, specific conductivity; SRP, soluble reactive phosphorus; TAN, total ammonia nitrogen; TP, total phosphorus; TSS, total suspended solids; width, river width.

642 μg L^{-1}) and low PO_4^{3-} (mussel sites: 24 ± 21 μg L^{-1}; no mussel sites: 45 ± 151 μg L^{-1}) (Fig. 2, Appendix S1), besides confirming the importance of TSS, ORG, pH and river width in determining freshwater mussel distribution.

3.4. Water quality requirements of freshwater mussel species

CCA revealed that some freshwater mussel species differed significantly from each other in their water quality requirements (Fig. 3). *R. sumatrensis* and *P. cambodjensis*, and to a lesser extent, *P. vondembuschianus*, were associated with rivers with relatively high (6.5–7.3) pH and high (4.5–12.6 μg L^{-1}) SiO_4^{2-} concentrations, relatively low TP (13–138 μg L^{-1}), low Na^+ (0.5–6.6 mg L^{-1}) and low K^+ concentrations (0.3–3.9 mg L^{-1}), and relatively low TSS concentrations (8–87 mg L^{-1}) with high organic content (18–53%). *H. bialata* and *P. superbus* also clearly preferred high pH (6.9–7.5), as well as large rivers (182–652 m wide), relatively high TSS concentrations (46–174 mg L^{-1}) with low organic matter content (15–17%). *S. woodiana* is situated at the opposite end of the CCA plot, and is the species that is most tolerant to acidic conditions (pH range: 5.5–7.4). The remaining species, i.e. *Pseudodon cumingii*, *P. exilis*, *P. compressa* and *C. contradens*, clustered in the centre of the CCA plot, thus indicating indistinct water quality requirements.

4. Discussion

The present study represents the first comprehensive assessment of the diversity and distribution of freshwater mussels of Peninsular Malaysia and may serve as a reference for developing conservation strategies for this important fauna. We found one non-native and nine native species, many of which exhibit very restricted distributions. Two of these species had not been recorded from Peninsular Malaysia before. On the other hand, three species reported in the historical records were not found in the current assessment. Consequently, our data indicate that the distributions of several freshwater mussel species within Peninsular Malaysia have changed considerably over recent decades, and suggest that historical data on freshwater mussel diversity and distribution in Peninsular Malaysia suffer from widespread morphological misidentifications.

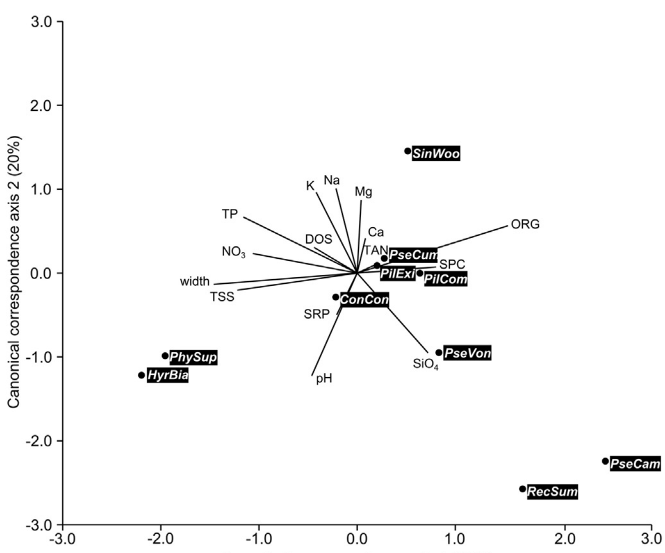

Fig. 3. Ordination diagram of CCA for ten species of freshwater mussels (presence/absence data; white letters on black background) with respect to 15 water quality parameters (black letters) at 55 sites in Peninsular Malaysia. Environmental triplots amplified ×3 for better visualisation. Abbreviations: Ca, calcium; *ConCon*, *Contradens contradens*; *HyrBia*, *Hyriopsis bialata*; DOS, dissolved oxygen saturation; K, potassium; Mg, magnesium; Na, sodium; NO_3, nitrate; ORG, weight-percentage of the organic portion of TSS; *PhySup*, *Physunio superbus*; *PilExi*, *Pilsbryoconcha exilis*; *PilCom*, *Pilsbryoconcha compressa*; *PseCam*, *Pseudodon cambodjensis*; *PseCum*, *Pseudodon cumingii*; *PseVon*, *Pseudodon vondembuschianus*; *RecSum*, *Rectidens sumatrensis*; SiO_4, silicate; *SinWoo*, *Sinanodonta woodiana*; SPC, specific conductivity; SRP, soluble reactive phosphorus; TAN, total ammonia nitrogen; TP, total phosphorus; TSS, total suspended solids; width, river width.

4.1. Changing freshwater mussel diversity and distribution in Peninsular Malaysia

The distributions of at least three freshwater mussel species appear to have contracted in Peninsular Malaysia over the past decades, as we were unable to confirm these species in at least one of the Federal States in which they had been recorded before (Table 2). The extreme case is *E. ingallsianus*, previously recorded from at least four States, but not found at any of the 155 sites surveyed in 2014–15. The apparent disappearance of this species in Peninsular Malaysia is particularly surprising when considering the relatively recent dates of some of the historic records, including 1977 in Lake Chini (British Museum of Natural History, no Acc. No.) and 1965 in the Perak River (The Academy of Natural Sciences of Drexel University, Acc. No. 389,074). Due to its alleged widespread distribution across much of Southeast Asia, *E. ingallsianus* is currently considered as "Least Concern", despite a lack of information on its ecological requirements (Do and Bogan, 2012a).

The two other species observed to have contracting distributions in Peninsular Malaysia, i.e. *R. sumatrensis* and *H. bialata*, are characterised by very restricted distributions globally and a severe lack of ecological data. *R. sumatrensis* is known from Malaysia and Indonesia (including the islands of Borneo and Sumatra) (Graf and Cummings, 2015) and listed as "Data Deficient" (Bogan, 2011). In Peninsular Malaysia, where we found *R. sumatrensis* only at a single site in the Perak River, this species had not been collected since 1958 (Lake Chenderoh, Museum of Comparative Zoology Harvard University, Acc. No. 228,705). *H. bialata* is currently regarded as fairly widespread and common in Southeast Asia, explaining its current "Least Concern" status (Do and Bogan, 2012b). However, molecular data (A.Z., M.L., A.B., R.S., J.W., U. Kovitvadhi, S. Kovitvadhi; unpublished data [see Appendix S2]) indicate that the Malaysian populations, which are restricted to the lower reaches of the Pahang River, represent a separate, cryptic species that is probably endemic to the peninsula. Pending further assessments of reproductive status and population sizes, we argue that against this background, the conservation status of the Malaysian *H. bialata* requires re-assessment.

Difficulties in delimiting and identifying freshwater mussel species by morphology alone also appear to be the main cause for the mismatch of historical and present-day distributions of *Pilsbryoconcha* and *Pseudodon* species in and across Peninsular Malaysia. Of the two *Pilsbryoconcha* and three *Pseudodon* species recorded in the present study, only *Pilsbryoconcha exilis*, *Pseudodon cumingii* and *Pseudodon vondembuschianus* had been previously recorded from the peninsula. *Pilsbryoconcha compressa* and *Pseudodon cambodjensis* were considered restricted to the Mekong catchment (Graf and Cummings, 2015). On the other hand, *Pseudodon chaperi*, which had been considered native to Peninsular Malaysia (Graf and Cummings, 2015), was not found in the present study (Table 1). These discrepancies between historical and recent species records of *Pilsbryoconcha* and *Pseudodon* in Peninsular Malaysia are likely caused by the extensive morphological variability within freshwater mussel genera (Zieritz and Aldridge, 2009; Zieritz et al., 2010; Zieritz and Aldridge, 2011), although differences in sampling effort between the present and previous surveys may also have played a role. A comprehensive revision of *Pilsbryoconcha* and *Pseudodon* through a combined molecular-morphological approach is required to resolve the real diversity and biogeography of these genera in Southeast Asia.

Our records increase the known range of three native freshwater mussel genera, i.e. *Contradens*, *Pilsbryoconcha* and *Pseudodon*, by one, four and two States in Peninsular Malaysia, respectively (Table 1).

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Whilst there is no indication of artificial propagation by human vectors for most of these species, some of the new *P. exilis* records may represent human-induced range expansion. This is particularly true for a *P. exilis* record from a man-made fish pond in the State of Negeri Sembilan. This population was highly divergent in COI from all other Peninsular Malaysian populations (Table 1), indicating that it was most likely introduced through stocking of mussel larvae-infested host fish from a catchment outside Peninsular Malaysia.

Human vectors are also responsible for the introduction of *S. woodiana* (the Chinese Pond Mussel), which was first recorded in Malaysia in 1965 in an abandoned mining pool in Kuala Lumpur (Museum of Comparative Zoology Harvard University, Acc. No. 266,180). Not a single record from this species in Peninsular Malaysia is available until 50 years later, when we recorded it in all of the States surveyed. In fact, *S. woodiana* is now the most widespread freshwater mussel species in Peninsular Malaysia, occurring in various types of water bodies ranging from abandoned mining pools to large lakes, and small and medium-sized rivers. *S. woodiana* have also been introduced into Borneo (Bogan and Schilthuizen, 2005). The widespread occurrence of *S. woodiana* is reason for concern, as the species is known for continuing range expansion in Europe and has shown to outcompete native species, particularly in heavily modified and artificial habitats (Paunovic et al., 2006; Sousa et al., 2014). To what extent this has taken place in Peninsular Malaysia over the past 50 years is impossible to answer. However, considering their sympatric occurrences and shared ecological niches, *Pilsbryoconcha* spp. and *Pseudodon cumingii* may be particularly threatened by interspecific competition of *S. woodiana* in Peninsular Malaysia.

4.2. Water quality requirements and threats

Our results indicate that in general, freshwater mussels in Peninsular Malaysia typically inhabit sites with relatively high conductivity, high pH, high Ca^{2+} concentration, high total suspended solids load with low organic content, and large river width (only tested for river sites). Other parameters that appear to prevent mussel presence include elevated total ammonia nitrogen and/or phosphate concentrations. It thus appears that freshwater mussel distribution in Peninsular Malaysia is strongly affected by the geological conditions in the region, which are characterised by granite bedrock dominating the inland and upland areas and limestone deposits only at the margins of the peninsula (Hutchison and Tan, 2009). This results in rivers and lakes of generally low pH (i.e. 2.8–7.5 in our study; Appendix S1), low buffering capacity and low specific conductivity due to low ion availability (e.g. Ca^{2+} concentration 1.0–66.8 mg L^{-1} in our study; Appendix S1). That said, due to the low buffering capacity of most freshwater ecosystems in Peninsular Malaysia, freshwater mussels in the region will be particularly sensitive to further acidification. Acidification as well as TAN and phosphorous pollution, are clearly linked with human activities including atmospheric deposition of anions (Rodhe et al., 1988), ammonia-based fertilisation of oil palm plantations and other agricultural land (Nelson et al., 2011), and insufficient treatment of industrial and domestic waste water, particularly in rural and sub-urban areas (Mamum and Zainudin, 2013).

The high sensitivity of freshwater mussels to ammonia, particularly during their larval and juvenile stages, is well established (Augspurger et al., 2003; Keller et al., 2007). In fact, mussels are one of the most ammonia-sensitive freshwater organisms and the reason why the U.S. Environmental Protection Agency (2013) reduced their Criteria Continuous Concentrations (i.e. the highest concentration of a pollutant to which aquatic life can be exposed for an extended period of time) from 1.2 to 0.26 mg TAN^{-L} at pH 8.0 and 4.5 to 1.9 mg TAN^{-L} at pH 7.0. The lack of freshwater mussels at sites in Peninsular Malaysia exceeding 0.44 mg TAN^{-L} at pH 5.5–7.5 further supports the extreme sensitivity of freshwater mussels to ammonia in a tropical country. Our observation that freshwater mussels in Peninsular Malaysia tend to inhabit sites with higher suspended sediment load was, on the other hand, contrary to our expectations, as previous studies have shown the detrimental effects of sedimentation on these filter-feeding molluscs (Ricciardi and Rasmussen, 1999).

Of the species that are of particular conservation concern due to their restricted and/or contracting distributions, our data indicate that *H. bialata* and *R. sumatrensis*, both of which were found only in large rivers, appear to be particularly sensitive to acidification. *R. sumatrensis* additionally appears as sensitive to elevated P and, contrary to most other unionoid species in Peninsular Malaysia, suspended sediment concentrations. In the tropics, increased sedimentation in rivers is heavily linked with deforestation of primary dipterocarp forest and associated land-use change to agricultural land, which has been shown to increase sediment yield up to 20 times (Nik, 1988; Douglas, 1996; Ngah and Othman, 2012). Finally, although our data do not indicate specific water quality requirements of *P. compressa*, which was found at pH levels as low as 6.25, further human-induced acidification and habitat degradation might lead to loss of the few and geographically restricted Peninsular Malaysian populations of this species.

4.3. Recommendations for conservation

Considering their restricted and declining distributions, combined with a severe lack of ecological data, we strongly recommend targeted surveys to assess the detailed distribution, ecological requirements, reproductive status and host fish requirements of *E. ingallsianus*, *H. bialata* and *R. sumatrensis*. Such an exercise needs to include an assessment of the molecular diversity of these species in order to facilitate optimal conservation of their genepools. In addition, changes in the distribution of *S. woodiana* should be continuously monitored, and potential negative effects on sympatric native species, in particular *Pilsbryoconcha* spp. and *Pseudodon cumingii*, need to be assessed.

Due to the presence of freshwater mussel species with very restricted distributions, the Pahang catchment (with *H. bialata* and *P. superbus* possibly endemic here globally and for Peninsular Malaysia, respectively; Fig. 1), the Perak catchment (with *R. sumatrensis* endemic here for Peninsular Malaysia), and the north-western river catchments of Peninsular Malaysia (with *Pseudodon cumingii* possibly endemic here for Peninsular Malaysia) are of particular importance and conservation value. At the very least, in these catchments, riparian buffers should be established for rivers passing through agricultural and residential lands, as they have shown to be effective in considerably reducing loads of nitrogen, phosphorus and sediment from agricultural and other runoff (Lee et al., 2000; Gomi et al., 2006; Lorion and Kennedy, 2009). Effect of deforestation on increasing sediment yield can additionally be minimised by e.g. regulating sediment yield to rivers by buffer strips (Douglas, 1996). Halting or slowing down the spread of *S. woodiana* will first and foremost require a campaign to inform the public about the threats this and other non-native species pose to Malaysia's unique freshwater biodiversity.

A summary of measured water quality parameters (mean ± SD) (range) (Appendix S1), and *Hyriopsis* species divergence and phylogenetic tree (Appendix S2) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author. Supplementary data associated with this article can be found in the online version, at doi: http://dx.doi.org/10.1016/j.scitotenv.2016.07.098.

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