Zieritz A, Bogan AE, Rahim KAA, Sousa R, Jainih L, Harun S, Razak NFA, Gallardo B, McGowan S, Hassan RB, Lopes-Lima M

Changes and drivers of freshwater mussel diversity and distribution in northern Borneo.

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Abstract

Human activities are threatening Borneo's unique biodiversity, but little is known on the status of freshwater invertebrates. We assessed changes in diversity and distribution of freshwater mussels (Bivalvia: Unionida) in northern Borneo, and identified drivers of present distribution and threats. Past distribution data were collected from literature and museum resources. Present distribution data were collected from 21 river basins, and 47 water quality, climatic, landscape and human variables explored as potential predictors of species presence/absence. Species delimitations were identified by morphology and COI barcoding, and haplotype networks generated. Our data indicate that over the past 50 years, four of originally five native species have become very rare or possibly locally extirpated. Since these four species are endemic to Borneo, other Bornean river basins should urgently be surveyed to identify any remaining populations. In the same time span, the non-native *Sinanodonta woodiana* has become the most widespread freshwater mussel in northern Borneo. The fifth native species was identified as *Rectidens sumatrensis* and found in four Sarawakian river basins, thus contradicting previous assumptions of an endemic Bornean *Rectidens* species. Although a number of stable *R. sumatrensis* populations are retained across Sarawak, the species' strong spatial contraction in mainland Sundaland and apparent low tolerance to eutrophication suggest that it is vulnerable to further habitat alteration. Our results indicate that Borneo's (endemic) freshwater invertebrate biodiversity is declining rapidly. Comprehensive surveys targeting an array of invertebrate and vertebrate taxa are needed to identify Borneo's freshwater biodiversity hotspots, where conservation efforts should be concentrated.

Keywords: extinction; deforestation; endemic species; invertebrates; Sundaland; Unionidae

Changes and drivers of freshwater mussel diversity and distribution in northern Borneo

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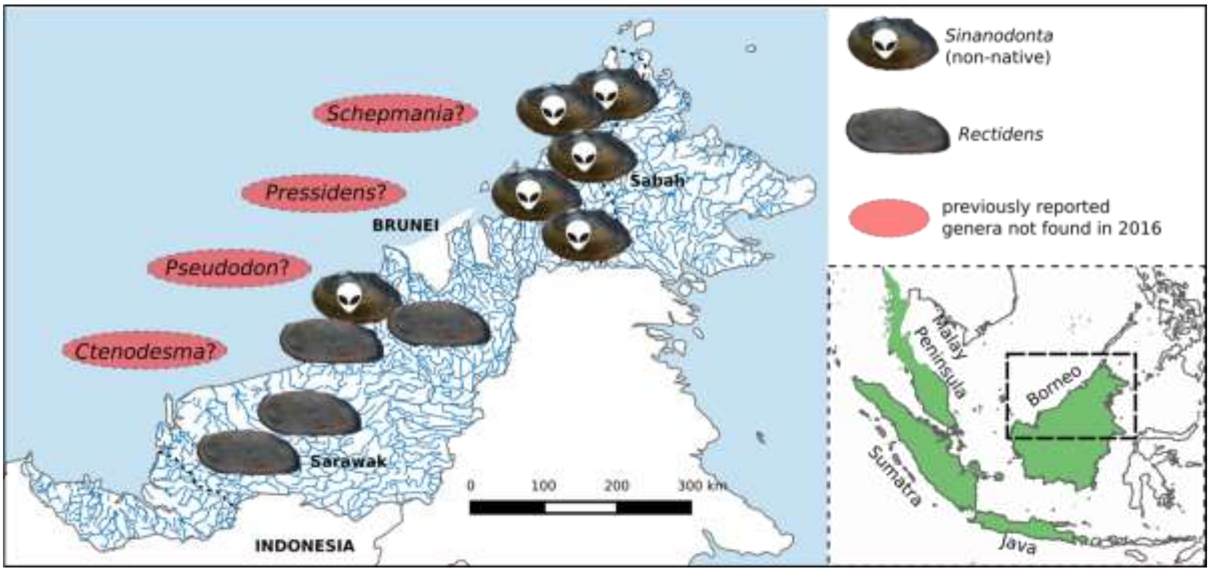

Graphical abstract Due to human pressure on freshwater habitats over the past 50 years, four of originally five native, endemic freshwater mussel species in northern Borneo have become extremely rare and possibly locally extirpated. In the same time span, the non-native *Sinanodonta woodiana* has become the most widespread freshwater mussel in northern Borneo. *Rectidens sumatrensis* is the only remaining native mussel that retains stable populations across Sarawak but is vulnerable to further habitat alteration, such as eutrophication.

1. Introduction

Freshwater biodiversity is declining at a rate far greater than terrestrial or marine ecosystems (Sala et al., 2000; Dudgeon et al., 2006; Strayer and Dudgeon, 2010). Meaningful conservation efforts, at the minimum, require knowledge on the diversity, distribution and habitat requirements of species. However, data on freshwater biodiversity is poor, so that undetected species extinctions are common, particularly for invertebrate taxa and in tropical habitats (Harrison and Stiassny, 1999; Dudgeon et al., 2006). At the same time, available data indicate that freshwater species-richness and levels of endemism peak in the tropics (Dudgeon et al., 2006 and references therein). Nevertheless, in contrast to terrestrial systems, the world's freshwater biodiversity hotspots "featuring an exceptional concentration of endemic species and experiencing exceptional loss of habitat" have yet to be identified (Myers et al., 2000).

For the designated terrestrial tropical biodiversity hotspot Sundaland, which includes the Malay Peninsula, Sumatra, Java and Borneo (Fig. 1), levels of freshwater biodiversity richness and endemism appear to be similarly high as for the terrestrial taxa studied by Myers et al. (2000). According to Mittermeier et al. (2005), this hotspot supports about 950 species of freshwater fish, 350 of which (representing about 3% of global diversity) are endemic to the region. Within Sundaland, Borneo hosts about 430 freshwater fish species, 164 of which are endemic, thus illustrating the particular importance of this island to global biodiversity conservation (Mittermeier et al., 2005; Abell et al., 2008). The region also appears to host a particularly high number of species of freshwater invertebrates, including crabs (Cumberlidge et al., 2009), Odonata (Kalkman et al., 2008) and Ephemeroptera (Sartori et al., 2003), although data in this respect are incomplete.

Knowledge on freshwater molluscs and specifically, freshwater bivalves, is poor across the tropics (Bogan, 2008; Zieritz et al., in press). The most species-rich freshwater bivalve order, the Unionida (also referred to as "freshwater mussels"), comprises about 800 species worldwide (Graf and Cummings, 2007), and has been identified as one of the most endangered groups of organisms (Bogan, 1993; Lopes-Lima et al., 2014; IUCN, 2016). Based on the scarce and outdated information available, Sundaland hosts about 35 Unionida species (4% of global diversity), approximately two thirds of which are endemic to Sundaland and one third is endemic to Borneo (Haas, 1969; Graf and Cummings, 2015; Zieritz et al., in press). For 75% of these species, IUCN conservation status assessment has not yet been attempted or could not be completed due to data deficiency (Zieritz et al., in press).

Present-day diversity and distribution of freshwater mussels in Sundaland differs markedly from that suggested by historical data for a number of reasons (Zieritz et al., 2016, in press): (1) lack of even historical data for much of the region; (2) the age of the vast majority of records (>50 years), which pre-date the period of rapid and intense human-induced habitat loss and alteration; and (3) lack of molecular data, which is required for reliable delimitation of boundaries of freshwater mussel species with high morphological variability. Molecular barcoding has, for example, led to the detection of a morphologically cryptic species and revealed a number of misidentifications of museum specimens in a recent survey of the freshwater mussels of Peninsular Malaysia (Zieritz et al., 2016). In addition, genetic sequence data can be used to unravel the biogeographic history of species and identify conservation management units within species (Palsbøll et al., 2007).

The drivers of freshwater mussel species distribution and, thereby, environmental requirements of freshwater mussels have been studied extensively in temperate freshwater habitats of North America and Europe (Strayer, 2008). In general terms, freshwater mussels are sensitive to (1) organic and inorganic pollution (Keller et al., 2007), (2) alteration of the hydrological regime and substrate characteristics of habitats by dams, land-use change or other means (Vaughn and Taylor, 1999; Strayer, 2008), (3) introduction of non-native species (Paunovic et al., 2006) and (4) lack of suitable host fish, which mussel larvae require for completion of their life cycle (Wächtler et al., 2001). In addition, climatic factors and over-exploitation may affect the presence/absence of freshwater mussels (Dudgeon, 2000; Allen et al., 2012; Zieritz et al., 2016).

The specific habitat requirements and threats differ considerably between freshwater mussel species and across different regions of the world. For example, whilst a number of endangered species in temperate Europe and North America are sensitive to sedimentation (Ricciardi and Rasmussen, 1999; Österling et al., 2010), freshwater mussels in Peninsular Malaysia were found to be affiliated with sites with high suspended sediment concentrations (Zieritz et al., 2016). However, more data are needed to even begin to understand the environmental requirements of, and threats to, freshwater mussels in Sundaland and other tropical regions. This research is particularly timely considering the rapid rates of habitat loss and alteration in this region caused by intense and widespread commercial logging and land-use change (Mittermeier et al., 2005; Langner et al., 2007; Miettinen et al., 2011), controversial hydroelectric projects (Lin 2003) and deliberate introduction of non-native species (Rahim et al., 2013).

Considering the global importance of Borneo's biodiversity, lack of knowledge on distribution patterns and potentially severe threats to this island's freshwater biodiversity, particularly freshwater mussels, this study aims (1) to assess past and present freshwater mussel diversity and distribution in northern Borneo; (2) to quantify genetic diversity and describe population genetic patterns of northern Bornean species across Sundaland, thereby identifying populations and freshwater bodies of particular conservation interest; and (3) to elucidate present drivers of distribution and threats to freshwater mussels in northern Borneo.

2. Materials and methods

2. 1. Study area

Species distribution and environmental data were collected across northern and central Sabah and Sarawak in 2016, situated within the Sundaland biodiversity hotspot (Fig. 1). The complete study area covers approximately 150,000 km² of northern Borneo, and spans from the Rajang basin in Sarawak in the west to the upper Sembakung, upper Kinabatangan and Bengkoka basins in Sabah to the east (Figs 1 and 2c, dashed lines). No sites within the borders of the ministate Brunei were surveyed. Geological conditions in the study area are mainly sedimentary and broadly characterised by tertiary sedimentary rocks in the lowland areas of Sarawak, older Cretaceous turbidites and melange in the upland areas of Sarawak, and Miocene flysch being prevalent in Sabah (Hutchison, 2005). The soils are predominantly peat and alluvium in lowland regions and clay-rich acrisols in the uplands. This results in generally moderate pH (~6-7.5) and conductivity (~10-50 µS cm^{-1}) values in rivers of Sarawak (e.g. Rajang (Ling et al., 2017b) and Baram (Ling et al., 2017a)), whilst in the rivers of Sabah, pH (~6.5-8.5) and conductivity (~50-140 µS cm^{-1}) tend to be higher (Cleophas et al., 2013). The study region includes a number of protected areas, most notably >3,000 km^2 of National Parks, including Crocker Range and Kinabalu in Sabah, and Gunong Mulu, Loagan Bunut, Rajang Mangroves, Bukit Tiban, Similajau, Lambir Hills, Gunong Buda and Niah in Sarawak (UNEP-WCMC and IUCN, 2017).

2.2. Sampling and voucher specimens

Historical data on freshwater mussel species distribution in the study area were gathered from different sources: (1) literature (Brandt, 1974; Drouet and Chaper, 1892; Haas, 1910, 1923, 1969, Simpson, 1900, 1914); (2) seven museum collections (Academy of Natural Sciences of Philadelphia; Florida Museum of Natural History; North Carolina Museum of Natural Sciences; Smithsonian National Museum of Natural History; Lee Kong Chian Natural

History Museum Singapore; BORNEENSIS collection, Universiti Malaysia Sabah; Museum of Zoology, University of Malaysia); (3) the Mussel-project database (Graf and Cummings, 2015), which provides photographs of a further 15 major collections; and (4) the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/).

We conducted present-day samplings of freshwater mussels in April, July and August 2016. We surveyed 72 sites, covering a diversity of freshwater habitats (rivers, streams, canals, rice-paddy run-offs and ponds) and 21 river basins (Fig. 1, Table 2). Sites within a continuous water body (e.g. river basin, lake) were at least 5 river-km apart from each other. At each site, we initially approached indigenous people and other locals and asked about the presence of freshwater mussels, which are widely used as a food source in the region (Zieritz et al., 2016). Interviewees were shown pictures/shells of the ten unionoid species historically recorded from northern Borneo and asked to identify the presence of particular species. Subsequently, we searched for freshwater mussels visually and hand-sampling for a minimum of 30 and up to 240 person-minutes, typically covering about 100 m river length (following Cummings et al., 2016). Although even 600 person-minutes of effort per site may be insufficient to detect some rare freshwater mussel species in North American rivers (Metcalfe-Smith et al., 2000; Huang et al., 2011; Reid, 2016), rivers in Sundaland show much lower mussel species-diversity per site and lack such extremely rare species (Zieritz et al., 2016).

Survey effort depended on a number of factors: (1) Pre-indication of freshwater mussels (species) by historical records and/or locals ("pre-indicated" or "not pre-indicated"); (2) Habitat visibility and sampling efficiency: clear visibility of substrate and lack of obstructions facilitating efficient scanning for mussels of a large area ("efficient") *vs*. low visibility and presence of obstructions (e.g. dead wood) or other physical conditions that resulted in inefficient surveying ("inefficient"); (3) Habitat homogeneity: discriminating up to three generalised microhabitat types (mud/silt, sand/gravel and macrophytes). "Pre-indicated efficient" sites were surveyed for a minimum of 90 person-minutes and either until respective freshwater mussels (species) were found or up to 120 person-minutes. "Pre-indicated inefficient" sites were surveyed for a minimum of 180 person-minutes and either until respective freshwater mussels (species) were found or up to 240 person-minutes. "Not pre-indicated efficient" sites were surveyed for 30 person-minutes per microhabitat. "Not pre-indicated inefficient" sites were surveyed for 60 person-minutes per microhabitat.

We collected foot snips (non-lethal to the mussels; Spicer et al., 2007) from a maximum of 25 specimens of each population and preserved in 100% ethanol. In addition, we preserved up to six whole animals as voucher specimens of each population in 95% ethanol. Voucher material from Sabah and Sarawak are deposited at the BORNEENSIS collection, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, and the collection of the Department of Aquatic Science, Universiti Malaysia Sarawak, respectively.

2.3. Measurement of water quality parameters

We measured (1) pH and (2) total dissolved solids (TDS), using a WP-81 Handheld TDS-pH-Temperature Instrumentation at each site before surveying for freshwater mussels. In addition, 300 ml-water samples were taken, of which at least 200 ml were filtered through pre-ashed GF/C filters, and filtered and unfiltered samples kept cool for subsequent determination of following parameters in the laboratory: (3) organic suspended solids concentration (OSS), measured by filtration of a given volume of water through a GF/C filter, and subsequent weighing of the filter after drying and loss-on-ignition for 4 hours at 550ºC; concentrations of (4) total phosphorus (TP), (5) soluble reactive phosphorus (SRP), (6) total ammonia nitrogen (TAN) and (7) Chlorophyll *a* (Chl *a*) were assessed using standard spectrophotometric/colorimetric methods (Lorenzen, 1967; Mackereth et al., 1989). Concentrations of (8) Ca^{2+}, (9) K^{+} and (10) Cl^{-} 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}).

2.4. Species identification and population genetics

Species identification through integrative morphological-molecular approach and sequencing of the mitochondrial cytochrome c oxidase 1 gene (COI) followed the same methodology as described in Zieritz et al. (2016). The F-type COI-gene (709 bp fragment) for at least one specimen of each population (species-site occurrence) was sequenced, and sequences deposited in GenBank. Haplotype networks were calculated for all sequences obtained in this study as well as sequences of other Sundaland and Wallacean populations of the same species available on Genbank as of November 2016 using TCS 1.21 (Clement et al., 2000), applying a threshold of 95% and visualised with tcsBU (Murias dos Santos et al., 2016). Sequence divergences (uncorrected p distance) were assessed using MEGA 7.0 (Tamura et al., 2013).

2.5. Associations of freshwater mussels with water quality, climatic, landscape and human parameters

As potential predictors of the presence/absence of freshwater mussels we explored a total of 47 water quality (10), climatic (20), landscape (10) and human (7) variables (Tables S1 and S2, Supplementary Material). Apart from water quality parameters, which were measured as explained above, we extracted the corresponding value of each potential predictor from each of the 72 sampling sites using Geographic Information Systems (Q-GIS v 10.2) and summarised the range of values observed in sampling sites inhabited by *R. sumatrensis*, *S. woodiana,* and without any of these mussels. Significant differences between the three groups were tested with non-parametric analyses of variance (Kruskal-Wallis test).

Given the high number of predictors initially considered, we first conducted analyses to select a subset of predictors. Details of the selection process can be consulted in Supplementary Material and consisted of three steps: 1-analyse pairwise Pearson correlations; 2-create a cluster of variables and select one or two predictors per cluster with correlation $r<|0.75|$ (Figs. S1 and S2); 3-analyse the deviance in species presence/absence explained by each predictor individually and select those able to explain >10% (Fig. S3). To investigate the deviance explained by each individual factor, we used Generalised Additive Models with the species presence/absence as response variable, each variable at a time as predictor, and a binomial family (logistic link). GAM was chosen instead of other regression procedures because of its ability to deal with nonlinear relationships between the response and the predictor (Guisan et al. 2002). In this case, we fitted GAM with three degrees of freedom to allow for linear, quadratic and cubic responses. Analyses and plots were conducted using packages "ClustofVar", "corrplot", "mgcv" and "ggplo2" in R.

Next, we tested whether sites with native species, invasive species and without freshwater mussels differed in their characteristics with a multivariate Redundancy Analysis (RDA) on the 17 variables identified using the protocol described before. RDA was calibrated using package "vegan" in R. We extracted the two first axes and used ANOVA and *post-hoc* Tukey HSD to test for significant differences among sites inhabited by *R. sumatrensis*, *S. woodinana* or none of them.

3. Results

3.1. Historical data

Previous data on Unionida from Borneo are scarce, of generally poor spatial resolution and old age (Fig. 1a, Table 1). For the study area, we could identify merely three historic species-site occurrences with a resolution <25x25 km cell size (i.e. *Rectidens lingulatus* at the

Niah River and the Niah Caves, and *Schepmania niewenhuisi* at Sepulut River), and another eight above this resolution (Fig. 1a). Based on these records, Unionida from the study area comprise five species and genera, *Pressidens exanthematicus*, *Pseudodon walpolei*, *R. lingulatus*, *Schepmania niewenhuisi* and *Sinanodonta woodiana* (Fig. 1a, Table 1a). In addition, *Ctenodesma borneensis* has been recorded from eastern Sabah (outside the study area) as well as unspecified locations in "Sarawak" (Table 1a, Fig. 1a); at least some of these latter locations are thus likely to fall inside our study area. With the exception of *S. woodiana*, which is not native to Borneo, these five species are considered endemic to the island of Borneo (Haas, 1969; Graf and Cummings, 2015) (Table 1a). According to historical records, *P. exanthematicus* and *R. lingulatus* are restricted to the study area (Table 1). Latest records of *C. borneensis*, *P. exanthematicus* (only found in Brunei) and *P. walpolei* date back more than 50 years, whilst *R. lingulatus*, *S. niewenhuisi* and *S. woodiana* were last found in the study area within the last 30 years (Table 1a).

Finally, four additional species have been recorded from Malaysian Borneo but only from locations outside the study area. These are *Pressidens insularis* and *Simpsonella gracilis* from Banggi Island off the Northeast coast of Sabah, *Pseudodon crassus* from the Sarawak river, and *Schepmania parcesculpta* from the lower Kinabatangan river (Fig. 1a, Table 1b).

3.2. Present species diversity

Our survey in 2016 confirmed the presence of freshwater mussels at 27 of 72 sites and nine of 21 river basins surveyed (Fig. 1b, Table 1a). Only one mussel species was present at all but three sites, where two species were found. In total, this amounted to 30 freshwater mussel populations (i.e. 30 species-site occurrences). For six of these populations, only recently dead specimens were found but presence of live mussels within the past year (possibly inaccessible at time of sampling) was confirmed by locals.

A total of 28 COI sequences were obtained from sampled specimens (Table 2). Combined with the morphological identifications, all sampled freshwater mussel populations (including dead shells) accounted for one native, *Rectidens sumatrensis*, and one non-native species, *S. woodiana*.

3.3. Present spatial distribution and genetic structure of populations

Rectidens sumatrensis was found at 15 sites from four river basins in Sarawak (Table 1, Fig. 1). The 17 COI-sequences obtained from these populations combined with the only other four available sequences from this species, all of which from the Perak river in Peninsular Malaysia (Zieritz et al., 2016), represented seven unique haplotypes. Genetic geographic structure was high among the main basins with low intra-basin diversity based on haplotype network and *p*-distance values within and among populations (Fig. 2a, Table 2a). The only exception was the Rajang basin, which appeared as divided into two clusters with haplotypes from the upper Rajang being more similar to haplotypes from the Suai basin than with the specimens from the lower Rajang (Figs. 2a and c). As expected, highest divergence was found between the Perak population and all Sarawakian populations (Fig. 2a, Table 2a).

Sinanodonta woodiana was the most widely distributed species, particularly common across northern Sabah, but also found in the Suai basin in Sarawak. In total, the species was found at seven river basins and 15 sites (Table 1, Figs 1 and 2c). The 11 sequences obtained from these populations combined with 14 sequences for this species from across Peninsular Malaysia (Zieritz et al., 2016) and two sequences from the island of Flores (Indonesia) represented ten unique haplotypes. No genetic geographic structure was detected based on COI haplotype network and *p*-distance values within and among populations (Fig. 2b and c, Table 2b). Of the five haplotypes occurring in the study area, all were present in Sabah. Three of these were unique to Sabah, one was also found in Peninsular Malaysia, and one was found both in Peninsular Malaysia and Sarawak, respectively.

3.4. Present associations of freshwater mussels with water quality, climatic, landscape, soil and human parameters

All of the *R. sumatrensis* populations were found in rivers, whereas 40% of *S. woodiana* populations were sampled from lakes or ponds. Out of 47 potential predictors of presence/absence of freshwater mussels, 17 variables were selected for modelling in a three-step process as explained in the Methods section. The selected predictors were seven indicators of water quality (Chl *a*, TDS, OSS, water pH, SRP, K and TAN), three climatic (annual mean and range of temperature, precipitation of the wettest quarter), three landscape (organic carbon content and %silt in soils, topographic roughness) and four human-related (% of anthropogenic land cover in a 10x10km cell, distance to transportation networks, dams and human populations).

High concentrations of chlorophyll *a* (Chl a), total dissolved solids (TDS), total ammoniacal nitrogen (TAN) and pH in the water were associated negatively with the occurrence of the native species and positively with the non-native in univariate GAMs (Fig. 3). Conversely, high precipitation and organic content of soils favour the native species over the non-native. Finally, human activities represented by the proportion of anthropogenic land cover, proximity to transportation networks (i.e. roads) and human populations increase the probability of presence of *S. woodiana* and decrease the probability of presence of *R. sumatrensis*.

R^2 was 0.49 ($R_{adj.}$= 0.33) in the RDA model, with the first two axes accounting for 97% variation in the dataset (Fig. 4). RDA1 was predominantly related to K^+-concentration in the water, separating sampling sites void of mussels (low K^+-concentrations) from sites inhabited by either native or non-native mussels (high K^+-concentrations; Tukey HSD test, $P<0.0001$). RDA2, on the other hand, was positively associated with water quality (negative correlation with Chl a, TAN, TDS, water pH), proportion of silt in the soil, precipitation, and distance to transportation and human populations. This axis clearly separated sites inhabited by native and non-native mussels, respectively (Tukey HSD test, $P<0.001$).

The land-use of sites currently occupied by *R. sumatrensis* has considerably changed over time: in 1950, 100% of sites were located on forested lands, but in 2000 53% have been converted into croplands. The change is even more intensive for sites colonised by *S. woodiana*, predominantly transformed to croplands (60%) and urban areas (20%).

4. Discussion

4.1. Past and present freshwater mussel diversity and distribution in northern Borneo

Based on historical records, at least five freshwater mussel species are native to the study area in northern Borneo, which additionally has been colonised by the non-native *S. woodiana* (Table 1). However, despite intensive and wide-ranging sampling efforts in 2016, four of the five native species (*Ctenodesma borneensis*, *Pressidens exanthematicus, Pseudodon walpolei* and *Schepmania niewenhuisi*) were not detected, indicating that these species have become very rare or even extirpated in the study area. All these species are considered endemic to Borneo.

More survey work, which should ideally include the deeper parts of rivers and lakes using grabs, dredges and/or scuba diving, is required to confirm the absence of these species in and beyond the study area. That said, local extirpation of one or more of these Bornean endemic freshwater mussel species would not be surprising. Historical records for these species are very scarce and old, dating back at least 50 years, with the exception of one record of *S. niewenhuisi* from 2003 (Table 1, Fig. 1). This suggests that these species may not have been particularly common even before any significant alterations to their habitats had occurred. Since the 1970s, the primary forests of Sarawak and Sabah have been logged at an unprecedented rate (Marsh and Greer, 1992; Reynolds et al., 2011). As a result, in 2013, merely 8% and 3% of land area in Sabah and Sarawak, respectively, were covered by intact forests (Bryan et al., 2013). Lowland areas, which provide important habitats for freshwater mussels in Peninsular Malaysia (Zieritz et al., 2016), have been particularly badly affected due to their accessibility. Deforestation in Borneo is known to have major effects on river ecosystems by increasing bank erosion and sediment yield, particularly of fine sediments, as well as altering pH, oxygen and other water quality parameters (Douglas et al., 1992; Chappell et al., 1999; Nor Zaiha et al., 2015). This has been shown to result in a decrease of both abundance and diversity of the benthic fauna and flora (Iwata et al., 2003; Lorion and Kennedy, 2009a). In the same time span, the hydrological regime of several rivers in the region, such as the Padas and the Rajang, have been substantially altered by hydroelectric dams (Hakim, 2009). Considering the known effects of impoundments, deforestation and land-use change on sedimentation and physico-chemical characteristics of rivers, particularly in the tropics (Douglas et al., 1992; Chappell et al., 1999; Nor Zaiha et al., 2015), it is conceivable that the profound alteration of their habitat has led to local extirpation of these mussel species.

The only native freshwater mussel species of northern Borneo that retained a considerable number of populations belongs to the genus *Rectidens*. However, contrary to previous assumptions that the Bornean *Rectidens* populations represent an endemic species, *Rectidens lingulatus* (Haas, 1969; Graf and Cummings, 2015), molecular data showed that all the Bornean populations belong to the same species that inhabits parts of the Perak river in Peninsular Malaysia, *Rectidens sumatrensis* (Zieritz et al., 2016). Consequently, there is no *Rectidens* species endemic to Borneo, and *Rectidens lingulatus* (Drouet & Chaper, 1892) has to be synonymised with *Rectidens sumatrensis* (Dunker, 1852). Our work represents first records of *R. sumatrensis* in the Rajang, Kemena and Suai basins (Table 1).

Besides *R. sumatrensis*, the non-native *S. woodiana* was the only other freshwater mussel species we could confirm for northern Borneo. Contrary to most native species, *S. woodiana* has spread considerably across northern Borneo over the past decades. Previously recorded only once from a market in Sabah about 10 years ago (Bogan and Schilthuizen, 2005), it is now present in at least six Sabahan and one Sarawakian river basins (Table 1). A similarly rapid spread of this species has been observed in Peninsular Malaysia (Zieritz et al., 2016). The ongoing spread of *S. woodiana* across Malaysia and probably much of remaining Sundaland has, in all likelihood, been driven by intentional introductions as a food source and for ornamental purposes (pers. obs.).

4.2. Phylogeographic patterns and conservation genetics

Rectidens sumatrensis COI haplotypes showed a strong geographic structure across river basins, with relatively low diversity within basins, as would be expected for populations inhabiting basins that have been separated for millions of years (de Bruyn et al., 2014). The fact that *R. sumatrensis* from the upper Rajang basin were more similar to haplotypes from the Suai basin than to those from the lower Rajang might suggest that the Suai basin used to be connected in the past with the middle reaches of the Rajang (Fig. 2c). The relatively high divergence between Peninsular Malaysian and Bornean populations might suggest the existence of two subspecies. However, sampling and sequencing of populations from the western range of this species' distribution, Sumatra and Java, will be required to fully answer this question.

In contrast to *R. sumatrensis*, *S. woodiana* did not exhibit any clear geographic structure of haplotypes. Two haplotypes present in Borneo were shared with Peninsular Malaysian populations. This pattern indicates that this species may have been repeatedly introduced to Borneo (and Peninsular Malaysia) and moved around within the island. Whilst *S. woodiana* is generally believed to be native to the Yangtze basin in China, the available Sundaland sequences do not closely match any of the *S. woodiana* sequences available on Genbank to date. The question of the exact origin of these introduced populations as well as whether this tropical invasive lineage in fact represents a separate species to the temperate invasive lineage (*sensu* Bolotov et al., 2016) thus remains unanswered.

4.3. Environmental requirements and present threats to mussel species in northern Borneo

Zieritz et al. (2016) recently showed that over the past 50 years, *R. sumatrensis* suffered a substantial contraction in distribution in Peninsular Malaysia. Whilst *R. sumatrensis* used to inhabit the Pahang as well as the Perak basins on the peninsula, surveys in 2015 confirmed the species' presence only from a single site in Perak (Zieritz et al., 2016). The present dataset does not indicate a similar range contraction of *R. sumatrensis* in Borneo, though this may in part be an artefact due to the restricted historical distribution data from the island. Our dataset significantly adds to our understanding of the ecological requirements of *R. sumatrensis*, which appears to be restricted to lowland rivers, is particularly sensitive to eutrophication and requires a pH close to 7 (Figs 3 and 4). Eutrophication, acidification and alkalinisation of freshwaters are linked to ongoing human disturbances in the region, such as deforestation, pollution, land-use change and fertilisation (Rodhe et al., 1988; Dudgeon, 2000; Luke et al., 2017). As a result, the probability of presence of *R. sumatrensis* populations increases with increasing distance to human activities (represented in Figs 3 and 4 by anthropogenic land-use, and proximity to transportation networks and human populations). It thus appears that the same factors that have apparently led to a substantial decrease and potential extirpation of up to four endemic Bornean freshwater mussels (see above) are continuing to threaten the remaining populations of *R. sumatrensis* in Borneo. Whilst our data suggest that *R. sumatrensis* may not be adversely affected by dams, this observation needs to be more thoroughly tested in higher-resolution studies at the reach scale. A better understanding of the effects of dams on mussels in tropical rivers is particularly urgent considering the number of large, controversial dams that are planned for the region despite the fact that the majority of the existing dams are not operating at full capacity due to moderate local energy demands (Hakim, 2009).

As in Peninsular Malaysia (Zieritz et al. 2016), *S. woodiana* occurs in a wide variety of habitats in Borneo, ranging from concrete ponds with very high nutritional content to fishing ponds, small streams and medium-sized rivers. The species showed a clear affinity to human activities, and populations were generally located close to roads and villages (Figs 3 and 4). Environmental data sampled at sites in northern Borneo confirmed the wide ecological niche and tolerance to pollution of this species, which was found at pH levels up to 9.2, turbidity levels up to 140 mg L^{-1}, organic suspended solids up to 42 mg L^{-1}, Chlorophyll *a* up to 140 µg L^{-1}, dissolved phosphate up to 700 µg L^{-1} and total ammoniacal nitrogen up to 1020 µg L^{-1} (Table S2).

Contrary to Europe and North America, where efforts are in place to minimise the spread and effects of harmful invasive non-native species (Gallardo et al., 2016; Zieritz et al., 2017 and references therein), no similar initiatives exist in Sundaland and most other parts of Asia. The rate of – mainly deliberate - introduction of non-native fish species to Malaysia has greatly increased over recent decades, causing serious ecological and economic harm (Rahim et al., 2013). Since most freshwater mussels require a fish host during their larval stage, the associated loss of native fish populations have been shown to indirectly lead to the loss of native freshwater mussel populations (Douda et al., 2013).

The threat posed by *S. woodiana* to native *R. sumatrensis* populations may currently be limited due to the distinct distribution and ecological niches of the two species. However, intentional spread such as we observed in Sabah could pose a significant threat to *R. sumatrensis* in the future. *R. sumatrensis* populations in the Suai river are already threatened by sympatrically living *S. woodiana*, which can outcompete native mussel populations in disturbed habitats (Paunovic et al., 2006; Sousa et al., 2014).

4.4. Conclusions

The present study indicates that northern Borneo's freshwater mussel fauna is severely threatened, with four out of five native species being very rare and possibly already extirpated from the study area. In order to identify any remaining populations of these species, all of which are considered endemic to Borneo, detailed surveying of river basins within our study area that we were not able to survey in the current work, as well as those adjacent to our study area (e.g. middle and lower reaches of the Kinabatangan and Sarawak rivers), is urgently needed. Freshwater habitats in Brunei, which has retained unlogged forest across about 54% of its land area (Bryan et al., 2013), might be of particular importance in this respect. Considering the difficult sampling conditions in Northern Borneo, novel molecular tools such as environmental DNA (Jerde et al., 2011) might be crucial in detecting rare species. If surviving populations of these species are found in the future, the respective reaches and river basins should be placed under immediate protection. This should be accompanied by in depth studies on the species' habitat requirements, including the identification of host fish species.

Rectidens sumatrensis is the most abundant native freshwater mussel species of northern Borneo. Nevertheless, the species is currently known from only one river basin in Peninsular Malaysia and four basins in Borneo, respectively. Comparison with historical data for this species, which include records from the Pahang river in eastern Peninsular Malaysia as well as an undefined location in Singapore, indicates a severe decrease in range of *R. sumatrensis* in mainland Sundaland. Combined with its apparent sensitivity to eutrophication and elevated turbidity levels, efforts should be directed towards minimising these disturbances to remaining *R. sumatrensis* populations. Based on the molecular data available to date, we propose four distinct management units for this species: (1) Perak, (2) lower Rajang/Kemena, (3) Suai and upper Rajang, and (4) Baram basins.

In the above mentioned basins, 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 nutrients and sediment from runoff (Lee et al., 2000; Gomi et al., 2006; Lorion and Kennedy, 2009b). Buffers have been shown to at least partly maintain fish species richness and functional diversity in a palm plantation in Kalimantan (Giam et al., 2015). Measures to mitigate adverse effects of dams on river fauna include modifying dam operations to provide more natural flows (Bednarek and Hart, 2005; Williams, 2008). The Suai basin unit is of particular concern due to its location within a palm oil plantation and the sympatric presence of *S. woodiana*. Halting or slowing down the spread of *S. woodiana* would require a campaign to inform the public about the potential threats this and other non-native species might pose to Borneo's unique freshwater biodiversity.

Our observation on declining freshwater mussel diversity in Borneo is in line with previous observations on declining fish and crab diversity in the region, whilst data on other invertebrate groups are rare (Dudgeon, 2000; Cumberlidge et al., 2009). Considering that freshwater mussels are recognised ecosystem engineers and predictors of benthic invertebrate diversity (Chowdhury et al., 2016), the observed decline in mussel biodiversity is likely to coincide with a loss of diversity in other taxonomic groups. Protection of the remaining freshwater biodiversity on this unique island will require a better understanding of the spatial distribution of this diversity and endemic species across taxa. This could be achieved through comprehensive surveys targeting an array of invertebrate and vertebrate taxa, which would allow identification of freshwater biodiversity hotspots, where conservation efforts should be concentrated.

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6. References

Abell, R., Thieme, M.L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S.C., Bussing, W., Stiassny, M.L.J., Skelton, P., Allen, G.R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J. V, Heibel, T.J., Wikramanayake, E., Olson, D., López, H.L., Reis, R.E., Lundberg, J.G., Sabaj Pérez, M.H., Petry, P., 2008. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. Bioscience 58, 403–414. doi:10.1641/b580507

Allen, D.J., Smith, K.G., Darwall, W.R.T., 2012. The Status and Distribution of Freshwater Biodiversity in Indo-Burma. IUCN, Cambridge, UK and Gland, Switzerland.

Bednarek, A.T., Hart, D.D., 2005. Modifying dam operations to restore rivers: ecological responses to Tennessee River dam mitigation. Ecol. Appl. 15, 997–1008. doi:10.1890/04-0586

Bogan, A.E., 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Hydrobiologia 595, 139–147.

Bogan, A.E., 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): A search for causes. Am. Zool. 33, 599–609. doi:10.1093/icb/33.6.599

Bogan, A.E., Schilthuizen, M., 2005. First report of the introduced freshwater bivalve, *Anodonta woodiana* (Lea, 1834) from the island of Borneo, Sabah, Malaysia. Ellipsaria 6, 5.

Bolotov, I.N., Bespalaya, Y. V, Gofarov, M.Y., Kondakov, A. V, Konopleva, E.S., Vikhrev, I. V, 2016. Spreading of the Chinese pond mussel, *Sinanodonta woodiana*, across Wallacea: One or more lineages invade tropical islands and Europe. Biochem. Syst. Ecol. 67, 58–

64. doi:http://dx.doi.org/10.1016/j.bse.2016.05.018

Brandt, R.A.M., 1974. The non-marine aquatic Mollusca of Thailand. Arch. fuer Molluskenkd. 105, 1–423.

Bryan, J.E., Shearman, P.L., Asner, G.P., Knapp, D.E., Aoro, G., Lokes, B., 2013. Extreme differences in forest degradation in Borneo: comparing practices in Sarawak, Sabah, and Brunei. PLoS One 8, e69679. doi:10.1371/journal.pone.0069679

Chappell, N.A., Ternan, J.L., Bidin, K., 1999. Correlation of physicochemical properties and sub-erosional landforms with aggregate stability variations in a tropical Ultisol disturbed by forestry operations. Soil Tillage Res. 50, 55–71. doi:http://dx.doi.org/10.1016/S0167-1987(98)00196-2

Chowdhury, G.W., Zieritz, A., Aldridge, D.C., 2016. Ecosystem engineering by mussels supports biodiversity and water clarity in a heavily polluted lake in Dhaka, Bangladesh. Freshw. Sci. 35, 188-199. doi:10.1086/684169.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659. doi:10.1046/j.1365-294x.2000.01020.x

Cleophas, N., Isidore, F., Han, L.K., Bidin, K., 2013. Water quality status of Liwagu River, Tambunan, Sabah, Malaysia. J. Trop. Biol. Conserv. 10, 67–73.

Cumberlidge, N., Ng, P.K.L., Yeo, D.C.J., Magalhães, C., Campos, M.R., Alvarez, F., Naruse, T., Daniels, S.R., Esser, L.J., Attipoe, F.Y.K., Clotilde-Ba, F.-L., Darwall, W., McIvor, A., Baillie, J.E.M., Collen, B., Ram, M., 2009. Freshwater crabs and the biodiversity crisis: Importance, threats, status, and conservation challenges. Biol. Conserv. 142, 1665–1673. doi:http://dx.doi.org/10.1016/j.biocon.2009.02.038

Cummings, K.S., Jones, H.A., Lopes-Lima, M., 2016. Rapid Bioassessment Methods for

Freshwater Molluscs, in: Larsen, T.H. (Ed.), Core Standardized Methods for Rapid Biological Field Assessment. Conservation International, pp. 185–207.

de Bruyn, M., Stelbrink, B., Morley, R.J., Hall, R., Carvalho, G.R., Cannon, C.H., van den Bergh, G., Meijaard, E., Metcalfe, I., Boitani, L., Maiorano, L., Shoup, R., von Rintelen, T., 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Syst. Biol. 63, 879–901. doi:10.1093/sysbio/syu047

Douda, K., Lopes-Lima, M., Hinzmann, M., Machado, J., Varandas, S., Teixeira, A., Sousa, R., 2013. Biotic homogenization as a threat to native affiliate species: fish introductions dilute freshwater mussel's host resources. Divers. Distrib. 19, 933–942. doi:10.1111/ddi.12044

Douglas, I., Spencer, T., Greer, T., Bidin, K., Sinun, W., Meng, W.W., 1992. The impact of selective commercial logging on stream hydrology, chemistry and sediment loads in the Ulu Segama rain forest, Sabah, Malaysia. Philos. Trans. Biol. Sci. 335, 397–406.

Drouet, H., Chaper, M., 1892. Voyage de M. Chaper a Bornéo. Unionidae. Mémoires la Société Zool. Fr. 5, 145–154.

Dudgeon, D., 2000. The ecology of tropical Asian rivers and streams in relation to biodiversity conservation. Annu. Rev. Ecol. Syst. 31, 239–263. doi:doi:10.1146/annurev.ecolsys.31.1.239

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, status, and conservation challenges. Biol. Rev. 81, 163–182.

Gallardo, B., Zieritz, A., Adriaens, T., Bellard, C., Boets, P., Britton, J.R., Newman, J.R., van

Valkenburg, J.L.C.H., Aldridge, D.C., 2016. Trans-national horizon scanning for invasive non-native species: a case study in western Europe. Biol. Invasions 18. doi:10.1007/s10530-015-0986-0

Giam, X., Hadiaty, R.K., Tan, H.H., Parenti, L.R., Wowor, D., Sauri, S., Chong, K.Y., Yeo, D.C., Wilcove, D.S., 2015. Mitigating the impact of oil-palm monoculture on freshwater fishes in Southeast Asia. Conserv. Biol. 29, 1357–1367. doi:10.1111/cobi.12483

Gomi, T., Sidle, R.C., Noguchi, S., Negishi, J.N., Nik, A.R., Sasaki, S., 2006. Sediment and wood accumulations in humid tropical headwater streams: Effects of logging and riparian buffers. For. Ecol. Manage. 224, 166–175. doi:http://dx.doi.org/10.1016/j.foreco.2005.12.016

Graf, D.L., Cummings, K.S., 2015. The Freshwater Mussels (Unionoida) of the World (and other less consequential bivalves), updated 5 August 2015. MUSSEL Project Web Site [WWW Document]. URL http://mussel-project.uwsp.edu/

Graf, D.L., Cummings, K.S., 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). J. Molluscan Stud. 73, 291–314.

Haas, F., 1969. Superfamilia Unionacea, in: Mertens, R., Henning, W. (Eds.), Das Tierreich (Berlin). de Gruyter & Co., Berlin, pp. 1–663.

Haas, F., 1923. Beiträge zu einer Monographie der asiatischen Unioniden. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 38, 129–203.

Haas, F., 1910. Die Unioniden, in: Küster, H.C. (Ed.), Systematisches Conchylien-Cabinet von Martini Und Chemnitz. Bauer und Raspe, Nürnberg, pp. 1–344.

Hakim, J., 2009. We are damned! Malaysia Today.

Harrison, I.J., Stiassny, M.L.J., 1999. The Quiet Crisis, in: MacPhee, R.D.E. (Ed.), Extinctions in Near Time: Causes, Contexts, and Consequences. Springer US, Boston, MA, pp. 271–331. doi:10.1007/978-1-4757-5202-1_12

Huang, J., Cao, Y., Cummings, K.S., 2011. Assessing sampling adequacy of mussel diversity surveys in wadeable Illinois streams. J. North Am. Benthol. Soc. 30, 923–934. doi:10.1899/10-172.1

Hutchison, C.S., 2005. Geology of north-west Borneo: Sarawak, Brunei and Sabah. Elsevier.

IUCN, 2016. IUCN Red List of Threatened Species. Version 2016-2. www.iucnredlist.org [WWW Document].

Iwata, T., Nakano, S., Inoue, M., 2003. Impacts of past riparian deforestation on stream communities in a tropical rain forest in Borneo. Ecol. Appl. 13, 461–473.

Jerde, C.L., Mahon, A.R., Chadderton, W.L., Lodge, D.M., 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conserv. Lett. 4, 150–157. doi:10.1111/j.1755-263X.2010.00158.x

Kalkman, V.J., Clausnitzer, V., Dijkstra, K.-D.B., Orr, A.G., Paulson, D.R., van Tol, J., 2008. Global diversity of dragonflies (Odonata) in freshwater. Hydrobiologia 595, 351–363. doi:10.1007/s10750-007-9029-x

Keller, A., Lydy, M., Ruessler, D.S., 2007. Unionid Mussel Sensitivity to Environmental Contaminants, in: Farris, J.L., Hassel, J.H. Van (Eds.), Freshwater Bivalve Ecotoxicology. CRC Press, Boca Raton, London, New York, pp. 151–167.

Langner, A., Miettinen, J., Siegert, F., 2007. Land cover change 2002–2005 in Borneo and the role of fire derived from MODIS imagery. Glob. Chang. Biol. 13, 2329–2340. doi:10.1111/j.1365-2486.2007.01442.x

Lee, K.-H., Isenhart, T.M., Schultz, R.C., Mickelson, S.K., 2000. Multispecies riparian buffers trap sediment and nutrients during rainfall simulations. J. Environ. Qual. 29, 1200–1205. doi:10.2134/jeq2000.00472425002900040025x

Ling, T.-Y., Soo, C.-L., Liew, J.-J., Nyanti, L., Sim, S.-F., Grinang, J., 2017a. Application of multivariate statistical analysis in evaluation of surface river water quality of a tropical river. J. Chem. 2017, 1–13. doi:10.1155/2017/5737452

Ling, T.-Y., Soo, C.-L., Phan, T.-P., Nyanti, L., Sim, S.-F., Grinang, J., 2017b. Assessment of water quality of Batang Rajang at Pelagus area, Sarawak, Malaysia. Sains Malaysiana 46, 401–411. doi:10.17576/jsm-2017-4603-07

Lopes-Lima, M., Teixeira, A., Froufe, E., Lopes, A., Varandas, S., Sousa, R., 2014. Biology and conservation of freshwater bivalves: past, present and future perspectives. Hydrobiologia 735, 1-13. doi:10.1007/s10750-014-1902-9

Lorenzen, C.J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346. doi:10.4319/lo.1967.12.2.0343

Lorion, C.M., Kennedy, B.P., 2009a. Relationships between deforestation, riparian forest buffers and benthic macroinvertebrates in neotropical headwater streams. Freshw. Biol. 54, 165–180. doi:10.1111/j.1365-2427.2008.02092.x

Lorion, C.M., Kennedy, B.P., 2009b. Riparian forest buffers mitigate the effects of deforestation on fish assemblages in tropical headwater streams. Ecol. Appl. 19, 468–479. doi:10.1890/08-0050.1

Luke, S.H., Barclay, H., Bidin, K., Vun Khen, C., Ewers, R.M., Foster, W.A., Nainar, A., Pfeifer, M., Reynolds, G., Turner, E.C., Walsh, R.P.D., Aldridge, D.C., 2017. The effects of catchment and riparian forest quality on stream environmental conditions across a tropical rainforest and oil palm landscape in Malaysian Borneo. Ecohydrology. 10: e1827.

doi:10.1002/eco.1827

Mackereth, F.J.H., Heron, J., Talling, J.F., 1989. Water Analysis: some Revised Methods for Limnologists. Ambleside: Freshwater Biological Association Special Publication.

Marsh, C.W., Greer, A.G., 1992. Forest Land-Use in Sabah, Malaysia: An Introduction to Danum Valley. Philos. Trans. R. Soc. London. Ser. B Biol. Sci. 335, 331–339. doi:10.1098/rstb.1992.0025

Metcalfe-Smith, J., Maio, J. Di, Staton, S.K., Mackie, G.L., 2000. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. J. North Am. Benthol. Soc. 19, 725–732.

Miettinen, J., Shi, C., Liew, S.C., 2011. Deforestation rates in insular Southeast Asia between 2000 and 2010. Glob. Chang. Biol. 17, 2261–2270. doi:10.1111/j.1365-2486.2011.02398.x

Mittermeier, R.A., Gil, P.R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J., Fonseca, G.A.B. da, 2005. Hotspots Revisited. Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. University of Chicago Press, Chicago, USA.

Murias dos Santos, A., Cabezas, M.P., Tavares, A.I., Xavier, R., Branco, M., 2016. tcsBU: a tool to extend TCS network layout and visualization. Bioinformatics 32, 627–628. doi:10.1093/bioinformatics/btv636

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858. doi:10.1038/35002501

Nor Zaiha, A., Mohd Ismid, M.S., Salmiati, Shahrul Azri, M.S., 2015. Effects of logging activities on ecological water quality indicators in the Berasau River, Johor, Malaysia. Environ. Monit. Assess. 187, 1–9. doi:10.1007/s10661-015-4715-z

Österling, M.E., Arvidsson, B.L., Greenberg, L.A., 2010. Habitat degradation and the decline of the threatened mussel *Margaritifera margaritifera*: influence of turbidity and sedimentation on the mussel and its host. J. Appl. Ecol. 47, 759–768. doi:10.1111/j.1365-2664.2010.01827.x

Palsbøll, P.J., Bérubé, M., Allendorf, F.W., 2007. Identification of management units using population genetic data. Trends Ecol. Evol. 22, 11–16. doi:10.1016/j.tree.2006.09.003

Paunovic, M., Csányi, B., Simic, V., Stojanovic, B., Cakic, P., 2006. Distribution of Anodonta (*Sinanodonta*) *woodiana* (Rea, 1834) in inland waters of Serbia. Aquat. Invasions 1, 154–160.

Rahim, K.A.A., Esa, Y., Arshad, A., 2013. The influence of alien fish species on native fish community structure in Malaysian waters. Kuroshio Sci. 7, 81–93.

Reid, S.M., 2016. Search effort and imperfect detection: Influence on timed-search mussel (Bivalvia: Unionidae) surveys in Canadian rivers. Knowl. Manag. Aquat. Ecosyst. 17.

Reynolds, G., Payne, J., Sinun, W., Mosigil, G., Walsh, R.P.D., 2011. Changes in forest land use and management in Sabah, Malaysian Borneo, 1990–2010, with a focus on the Danum Valley region. Philos. Trans. R. Soc. B Biol. Sci. 366, 3168–3176. doi:10.1098/rstb.2011.0154

Ricciardi, A., Rasmussen, J.B., 1999. Extinction rates of North American freshwater fauna. Conserv. Biol. 13, 1220–1222. doi:10.1046/j.1523-1739.1999.98380.x

Rodhe, H., Cowling, E., Galbally, I.A.N., Galloway, J., Herrera, R., 1988. Acidification and regional air pollution in the tropics, in: Rodhe, H., Herrera, R. (Eds.), Acidification in

Tropical Countries. John Wiley & Sons Ltd, New York, NY, pp. 3–39.

Sala, O.E., Stuart Chapin, F., III, Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global Biodiversity Scenarios for the Year 2100. Science 287, 1770–1774. doi:10.1126/science.287.5459.1770

Sartori, M., Derleth, P., Gattolliat, J.L., 2003. New data about the mayflies (Ephemeroptera) from Borneo, in: Gaino, E. (Ed.), Research Update on Ephemeroptera and Plecoptera. University of Perugia, Perugia, Italy, pp. 403–406.

Simpson, C.T., 1914. A Descriptive Catalogue of the Naiads, or Pearly Fresh-water Mussels. Part I-III. Bryant Walker, Detroit, Michigan.

Simpson, C.T., 1900. Synopsis of the naiades, or pearly fresh-water mussels. Proc. United States Natl. Museum 22, 501–872.

Sousa, R., Novais, A., Costa, R., Strayer, D.L., 2014. Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. Hydrobiologia 735, 233–251.

Spicer, J.F., Sobieski, R.J., Crupper, S.S., 2007. Testing a non-lethal DNA isolation technique for freshwater mussels that is compatible with Polymerase Chain Reaction (PCR) based studies. Trans. Kansas Acad. Sci. 110, 30–34.

Strayer, D.L., 2008. Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance. University of California Press, London.

Strayer, D.L., Dudgeon, D., 2010. Freshwater biodiversity conservation: recent progress and future challenges. J. North Am. Benthol. Soc. 29, 344–358. doi:10.1899/08-171.1

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729. doi:10.1093/molbev/mst197

UNEP-WCMC, IUCN, 2017. Protected Planet: The World Database on Protected Areas (WDPA) [WWW Document]. URL www.protectedplanet.net (accessed 1.1.17).

Vaughn, C.C., Taylor, C.M., 1999. Impoundments and the decline of freshwater mussels: a case study of an extinction gradient. Conserv. Biol. 13, 912–920. doi:10.1046/j.1523-1739.1999.97343.x

Wächtler, K., Mansur, M.C.D., Richter, T., 2001. Larval types and early postlarval biology in naiads (Unionoida), in: Bauer, G., Wächtler, K. (Eds.), Ecology and Evolution of the Freshwater Mussels Unionoida. Springer-Verlag, Berlin, pp. 93–125.

Williams, J.G., 2008. Mitigating the effects of high-head dams on the Columbia River, USA: experience from the trenches. Hydrobiologia 609, 241–251. doi:10.1007/s10750-008-9411-3

Zieritz, A., Bogan, A.E., Froufe, E., Klishko, O., Kondo, T., Kovitvadhi, U., Kovitvadhi, S., Lee, J.H., Lopes-Lima, M., Pfeiffer, J.M., Sousa, R., van Do, T., Vikhrev, I., Zanatta, D.T., in press. Diversity, biogeography and conservation of freshwater mussels (Bivalvia: Unionida) in East and Southeast Asia. Hydrobiologia 1–16. doi:10.1007/s10750-017-3104-8

Zieritz, A., Gallardo, B., Baker, S.J., Britton, J.R., van Valkenburg, J.L.C.H., Verreycken, H., Aldridge, D.C., 2017. Changes in pathways and vectors of biological invasions in Northwest Europe. Biol. Invasions 19, 269–282. doi:10.1007/s10530-016-1278-z

Zieritz, A., Lopes-Lima, M., Bogan, A.E., Sousa, R., Walton, S., Rahim, K.A.A., Wilson, J.-

J., Ng, P.-Y., Froufe, E., McGowan, S., 2016. Factors driving changes in freshwater mussel (Bivalvia, Unionida) diversity and distribution in Peninsular Malaysia. Sci. Total Environ. 571, 1069–1078. doi:10.1016/j.scitotenv.2016.07.098

Tables

Table 1. Conservation status and distribution data of freshwater mussel species that have been recorded in (a) the study area in Northern Borneo or (b) only from other parts of Malaysian Borneo.

				Presence in different regions of Borneo and approximate decade sampled last before this study					Presence in each river basin (from west to east, see Fig. 2(c) for location of basins)														
Species name	IUCN Conservation status	Number of records in Borneo (historic / 2016)	Presence outside Borneo	Sarawak	Sabah (mainland)	Brunei*	Banggi Island (Sabah)*$	Kalimantan*$	Sg. Sarawak*$	Sg. Rajang	Sg. Kemena	Sg. Suai	Sg. Niah*	Sg. Baram	Sg. Limbang*	Sg. Padas	Sg. Sembakung	Sg. Padang	Sg. Tuaran	Sg. Wariu	Sg. Bongan	Sg. Kinabatangan	Sg. Tungku*
(a) Species recorded from the study area (see Fig. 1 for extent of study area)																							
Ctenodesma borneensis (Issel, 1874)	NA	10/0	-	H^{1940}	H^{1960}			H^{1865}														H	
Pressidens exanthematicus (Küster, 1861)	NA	2/0	-			H^{1920}																	
Pseudodon walpolei (Hanley, 1871)	NA	15/0	-	H^{1950}		H^{1920}			H														

Rectidens sumatrensis[1] (Dunker, 1852)	DD	22/15	PM, SU, JA	B[1988]						C	C	C	H	B	H								H
Schepmania niewenhuisi (Schepman, 1898)	NA	6/0	-		H[2003]			H[1900]									H					H	H
Sinanodonta woodiana (Lea, 1834)	LC	1/15	wide-spread non-native	C	B[2003]							C				C	C	C	C	C	C		
(b) Species not recorded from study area but other parts of Malaysian Borneo																							
Pressidens insularis (Drouet, 1894)	NA	5/0	-				H[1930]																
Pseudodon crassus Drouet & Chaper, 1892	NA	2/0	-	H[1892]					H														
Schepmania parcesculpta (Martens, 1903)	NA	3/0	-		H[1964]			H[1908]														H	
Simpsonella gracilis (Lea, 1850)	NA	1/0	PH				H[1850]																
Total		67/30																					

* not surveyed in 2016

$ outside of study area

1 Bornean populations previously considered to represent a separate species *Rectidens lingulatus* (see text)

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; JA, Java; LC, least concern; NA, not assessed; PH, the Philippines; PM, Peninsular Malaysia; SU, Sumatra

Table 2. Pairwise genetic COI gene fragment distance matrix of (A) *Rectidens sumatrensis* and (B) *Sinanodonta woodiana* within and between river basins in Malaysia listed from west to east with Peninsular Malaysian and Bornean Malaysian basins separated by dashed line (uncorrected *p*-distance first column and below diagonal, and standard deviation second column and above diagonal, respectively).

(a) *Rs*	Genbank Accession Number(s)	Intra-basin		Inter-basin				
		p-dis	SD	Perak	Rajang	Kemena	Suai	Baram
Perak	KX051312-14, KX822664	0	0		0.005	0.006	0.005	0.005
Rajang	MG591492-99	0.004	0.001	0.021		0.001	0.003	0.004
Kemena	MG591500	-	-	0.022	0.002		0.004	0.004
Suai	MG591501-03	0	0	0.015	0.008	0.010		0.002
Baram	MG591504-08	0.001	0.001	0.017	0.010	0.012	0.002	

(b) *Sw*	Genbank Accession Number(s)	Intra-basin		Inter-basin															
		p-dis	SD	Perlis	Kedah	Perak	Tengi	Selang.	Langat	Kesang	Semerak	Terengganu	Pahang	Suai	Padas	Tuaran	Wariu	Sembakung	Bongan
Perl.	KX051323, KX051326, KX051327	0.002	0.002		0.002	0.002	0.001	0.004	0.001	0.001	0.001	0.004	0.002	0.001	0.003	0.002	0.001	0.003	0.001
Keda.	KX051322	-	-	0.002		0.000	0.003	0.005	0.003	0.003	0.003	0.005	0.002	0.003	0.005	0.000	0.003	0.005	0.003
Pera.	KX051319	-	-	0.002	0.000		0.003	0.005	0.003	0.003	0.003	0.005	0.002	0.003	0.005	0.000	0.003	0.005	0.003
Tengi	KX051325	-	-	0.001	0.004	0.004		0.003	0.000	0.000	0.000	0.003	0.002	0.000	0.003	0.003	0.000	0.003	0.000

Selan.	KX051315	-	-	0.005	0.007	0.007	0.004		0.003	0.003	0.003	0.005	0.004	0.003	0.005	0.005	0.003	0.005	0.003
Lang.	KX051324	-	-	0.001	0.004	0.004	0.000	0.004		0.000	0.000	0.003	0.002	0.000	0.003	0.003	0.000	0.003	0.000
Kesa.	KX051318, KX051321	0	0	0.001	0.004	0.004	0.000	0.004	0.000		0.000	0.003	0.002	0.000	0.003	0.003	0.000	0.003	0.000
Seme.	KX051316	-	-	0.001	0.004	0.004	0.000	0.004	0.000	0.000		0.003	0.002	0.000	0.003	0.003	0.000	0.003	0.000
Teren.	KX051328	-	-	0.005	0.007	0.007	0.004	0.007	0.004	0.004	0.004		0.004	0.003	0.005	0.005	0.003	0.005	0.003
Paha.	KX051317, KX051320	0.004	0.003	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.005		0.002	0.004	0.002	0.002	0.004	0.002
Suai	MG591509-11	0	0	0.001	0.004	0.004	0.000	0.004	0.000	0.000	0.000	0.004	0.002		0.003	0.003	0.000	0.003	0.000
Pada.	MG591512-14	0	0	0.005	0.007	0.007	0.004	0.007	0.004	0.004	0.004	0.007	0.005	0.004		0.005	0.003	0.000	0.003
Tuar.	MG591515	-	-	0.002	0.000	0.000	0.004	0.007	0.004	0.004	0.004	0.007	0.002	0.004	0.007		0.003	0.005	0.003
Wari.	MG591516-17	0	0	0.001	0.004	0.004	0.000	0.004	0.000	0.000	0.000	0.004	0.002	0.000	0.004	0.004		0.003	0.000
Semb.	MG591518	-	-	0.005	0.007	0.007	0.004	0.007	0.004	0.004	0.004	0.007	0.005	0.004	0.000	0.007	0.004		0.003
Bong.	MG591519	-	-	0.001	0.004	0.004	0.000	0.004	0.000	0.000	0.000	0.004	0.002	0.000	0.004	0.004	0.000	0.004	

273 # Figures

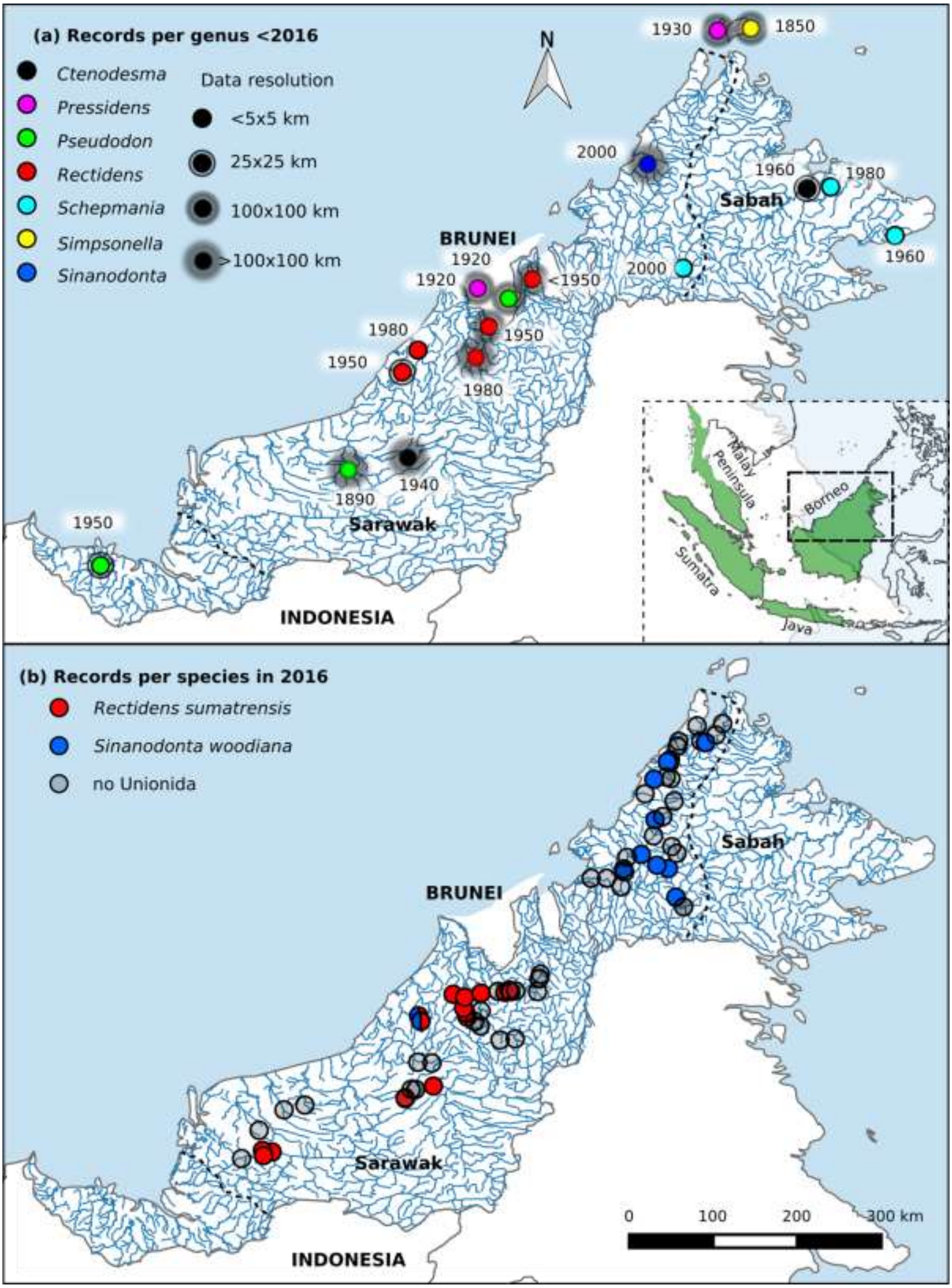

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Figure 1. Distribution records per freshwater mussel genus/species across Malaysian Borneo collected (a) before 2016, including decade of most recent record, and (b) in the course of the present study in April, July and August 2016. Insert shows position of study region within Sundaland (green area). Dashed lines indicate western and eastern borders of study area.

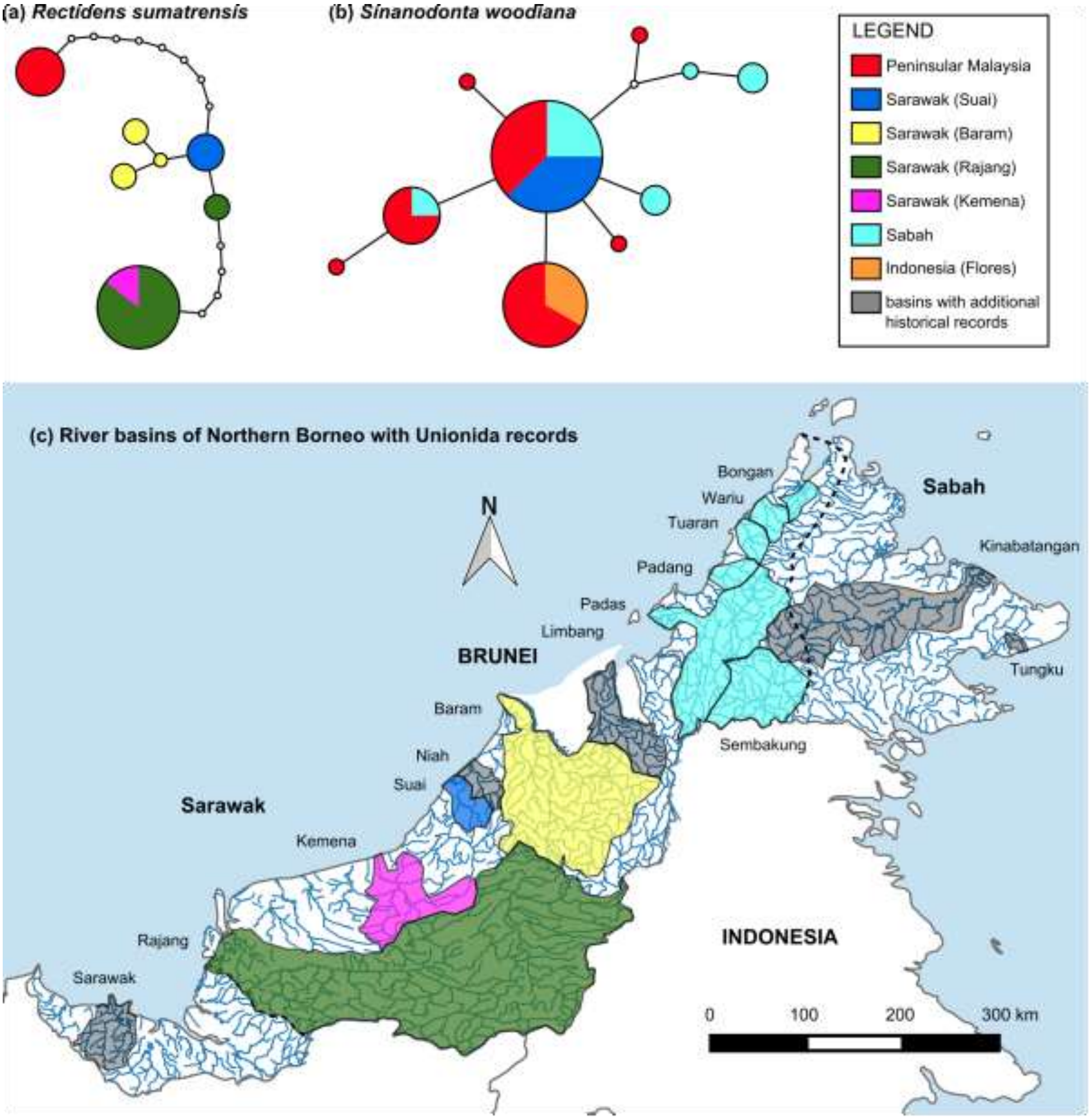

Figure 2. COI-Haplotype networks of (a) *Rectidens sumatrensis* (21 sequences) and (b) *Sinanodonta woodiana* (27 sequences) populations of Sundaland and Wallacea. Circle size is proportional to the observed haplotype frequencies, and white circles represent unobserved haplotypes and potential intermediates. (c) Location of northern Bornean river basins of analysed specimens and additional historical records. Dashed lines indicate western and eastern borders of study area. [colour should be used in print]

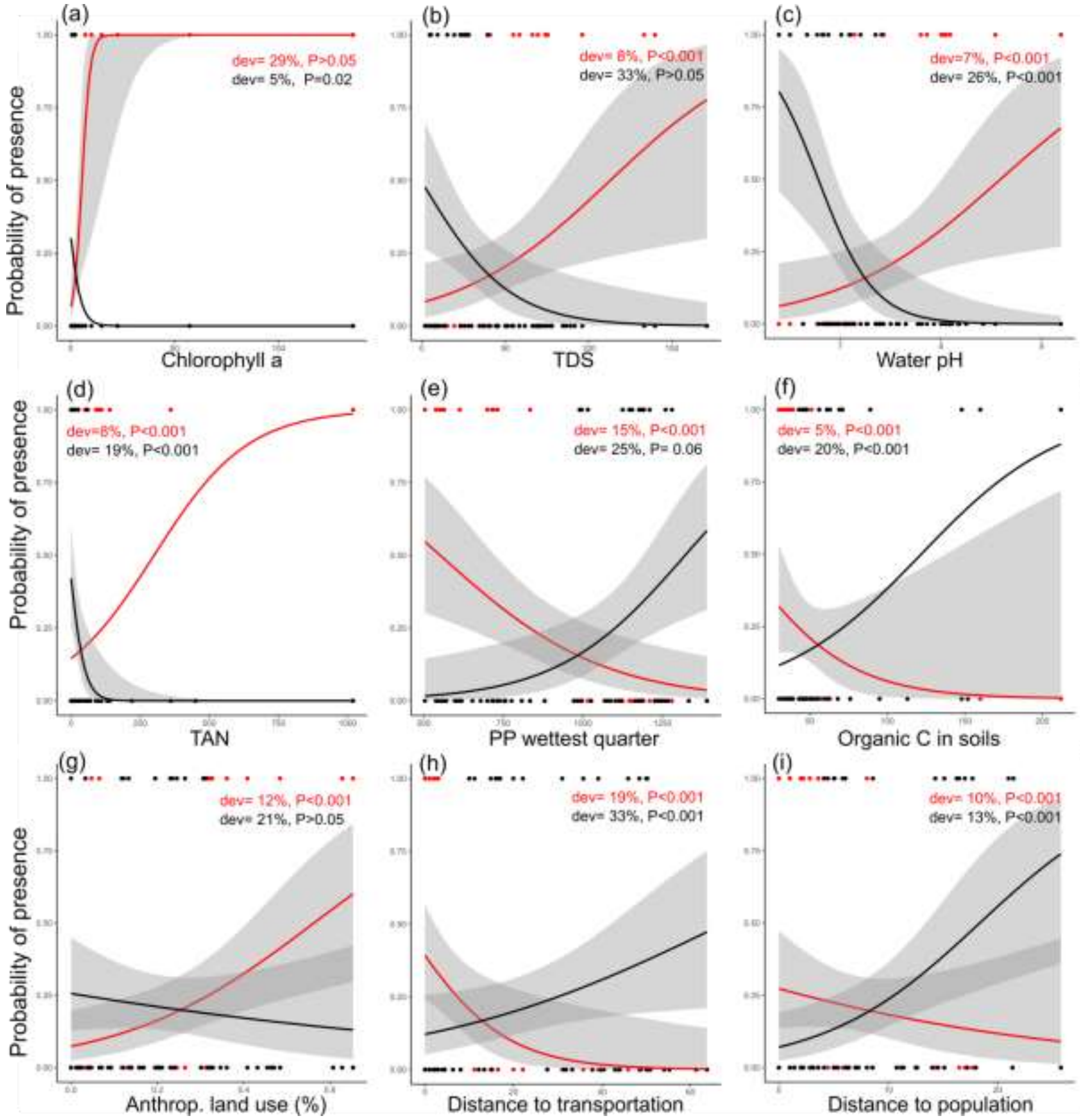

Figure 3. Response of the presence/absence of *Rectidens sumatrensis* (in black) and *Sinanodonta woodiana* (in red) to most important individual predictors identified in Fig. S3. The deviance explained and significance of each individual model is indicated in the upper right or left corner of each graph. See abbreviations of variables in Table S2.

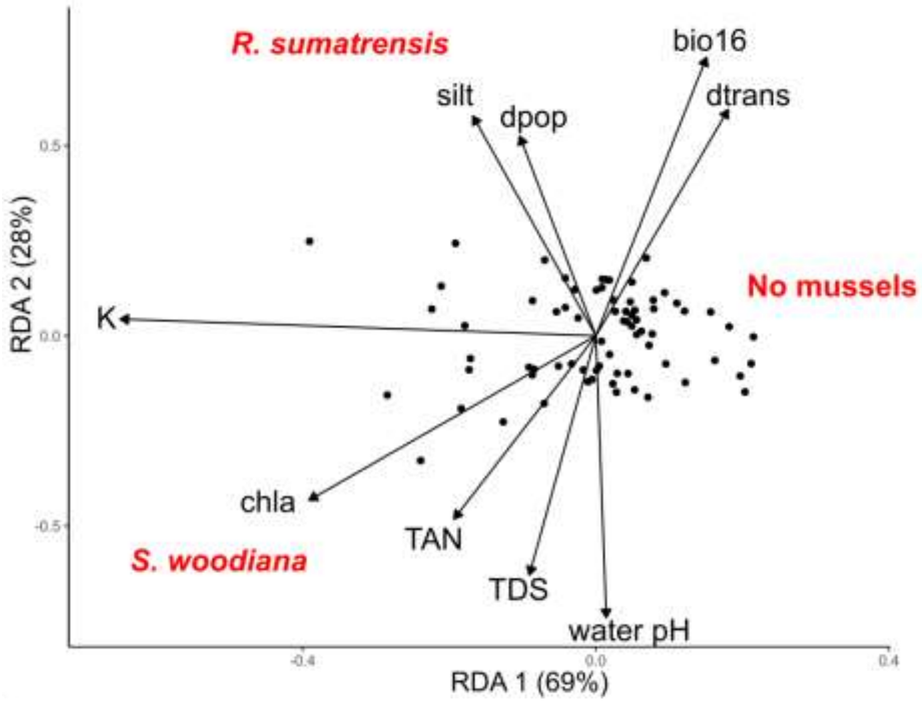

Figure 4. Redundancy analysis showing the influence of water quality, environmental and human factors on species composition in Borneo waterbodies. Sampling sites are represented by black dots. The probability of presence of species increases in the direction of their respective centroids. Only variables showing a weight ≥|0.40| in RDA1 or RDA2 are shown.