1 **Title**: A new genus and two new, rare freshwater mussel (Bivalvia: Unionidae) species

2 endemic to Borneo are threatened by ongoing habitat destruction

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4 **Authors**: Alexandra Zieritz[1,2], Leonardo Jainih[3], John Pfeiffer[4], Khairul Adha A. Rahim[5], Hari

5 Prayogo[6], Muhammad Sofwan Anwari[6], Arman Hadi Fikri[3], Farah Diba[6], Hussein Taha[7],

6 Zohrah Sulaiman[8], Elsa Froufe[9], Manuel Lopes-Lima[9,10]

7

8 **Affiliations**:

9 [1] School of Geography, University of Nottingham, Nottingham, UK

10 [2] School of Environmental and Geographical Sciences, University of Nottingham Malaysia

11 Campus, Malaysia

12 [3] Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu,

13 Sabah, Malaysia

14 [4] National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

15 [5] Faculty of Resource Science & Technology, Universiti Malaysia Sarawak, Kota Samarahan,

16 Sarawak, Malaysia

17 [6] Faculty of Forestry, Tanjungpura University, Pontianak, West Kalimantan, Indonesia

18 [7] Environmental and Life Sciences Programme, Faculty of Science, Universiti Brunei

19 Darussalam, Bandar Seri Begawan, Brunei Darussalam

20 [8] Universiti Teknologi Brunei, Bandar Seri Begawan, Brunei Darussalam

21 [9] CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research,

22 University of Porto, Matosinhos, Portugal

23 [10] CIBIO/InBIO – Research Center in Biodiversity and Genetic Resources, University of Porto,

24 Vairão, Portugal

25

ORCID

Farah Diba 0000-0002-3906-6168; Manuel Lopes-Lima 0000-0002-2761-7962; John Pfeiffer

0000-0001-5368-0589; Alexandra Zieritz 0000-0002-0305-8270

Author contributions

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

Abstract

41

42   1. Most of the Bornean endemic freshwater mussel (Unionida) species known to date have not

43   been recorded by science for the past 50 years or more, owing to a lack of research effort and

44   presumed population losses.

45   2. This study assesses current patterns and recent changes in the diversity and distribution of

46   freshwater mussels in northeastern Borneo. Physical surveys and interviews were conducted at

47   24 sites, and anecdotal evidence for current or historical presence of mussels was collected for

48   a further 13 sites.

49   3. Native species, i.e. *Schepmania* sp. and *Khairuloconcha sahanae* gen. & sp. nov., were only

50   found in one small stream of the Kinabatangan River basin within the Gomantong Forest

51   Reserve, whilst the non-native *Sinanodonta* cf. *woodiana* was common across the study area.

52   4. Molecular phylogenetics (5 genes) of the native taxa, including comparative material from

53   West Kalimantan and Sarawak, revealed (1) the presence of a new genus with two new, rare

54   species, i.e. *Khairuloconcha lunbawangorum* sp. nov. in the Limbang River basin and *K.*

55   *sahanae* in the Kinabatangan River basin; (2) that *Khairuloconcha* and *Ctenodesma* form the

56   Bornean endemic tribe Ctenodesmini trib. nov.; and (3) that *Schepmania* represents another

57   Bornean endemic tribe Schepmaniini trib. nov.

58   5. Both *Khairuloconcha* gen. nov. species are known from a single stream each and are

59   apparently restricted to forest stream habitats where they occur in very low densities.

60   *Schepmania* appears to have a severely contracted range in the Kinabatangan and adjacent

61   basins. We urgently call for full protection of the currently known sites of *K. lunbawangorum*

62   and *K. sahanae*, and development of an Action Plan to save the Bornean freshwater mussel

63   fauna.

64

67

1. Introduction

Borneo exhibits an exceptionally high rate of endemism of freshwater mussels (Bivalvia: Unionida), with 13 of the 18 currently recognised native species being restricted to this island, all of which belong to the family Unionidae (see Table 1 in Zieritz et al., 2020b). However, most of these species have not been recorded for several decades, and their taxonomy is based mostly on shell morphology as DNA sequence data is lacking (Figure 1a). The 13 currently recognised Bornean endemic freshwater mussel species are primarily known from specimens collected in the 19th and early 20th century. Only a few records (<15) of Bornean freshwater mussels have been reported in the second half of the 20th century (Graf & Cummings, 2021a), including four records from Sabah (northern Borneo) collected in 1962-1964 and identified as *Ctenodesma borneensis* (Issel, 1874) (FMNH 116932 [FMNH = Field Museum of Natural History, Chicago, USA]), *Schepmania niewenhuisi* (Schepman, 1898) (FMNH 116933, FMNH 118893) and *Schepmania parcesculpta* (Martens, 1903) (USNM 656126 [USNM = National Museum of Natural History, Washington, USA]).

The first DNA sequence data of Bornean freshwater mussels was generated in 2016, when our team commenced a series of targeted expeditions across northern Borneo, comprising the Malaysian states of Sarawak and Sabah, and Brunei (Figure 1a) (Gallardo et al., 2018; Zieritz et al., 2018c; Zieritz et al., 2020b). That work revealed that, with the exception of the relatively common species *Rectidens sumatrensis* (Dunker, 1852), all native and particularly endemic species appear to be rare and, in some cases, may even be already extirpated. Out of 115 sites surveyed between 2016 and 2018, endemic Bornean mussels were found only at a single site in a small stream within a patch of intact rainforest (Zieritz et al., 2020b). That population, of which only three individuals were found, was identified as *C. borneensis* based on the species' small size, elongated shell shape, yellowish/brown epidermis with green rays and fine sculpture

92 on the shell disc, and morphological comparison to the type specimen of *Unio plicatulus* Lea,

93 1859 (Figure 2).

94 Due to the paucity of data, the reasons for the declines of native Bornean freshwater mussels

95 are not well understood. However, there is little doubt that the industrial-scale deforestation

96 and land-use change from primary dipterocarp forest to secondary forest and ultimately,

97 agricultural monocultures (now predominantly oil palm) since the 1960s has been a major

98 driver of freshwater mussel population losses on the island (Bryan et al., 2013; Gallardo et al.,

99 2018). These practises result in high levels of soil erosion, strongly increasing sediment yield,

100 and organic and inorganic pollution (*via* agricultural run-off) of rivers (Figures 3b and d)

101 (Douglas, 1996; Igwe & Onyegbado, 2007; Rulli et al., 2019), all of which negatively affects

102 freshwater mussels directly by degrading habitat quality and/or indirectly by reducing host fish

103 populations that they require to complete their life cycles (Wächtler, Mansur & Richter, 2001;

104 Wilkinson et al., 2018; Zieritz et al., 2018b). Other potential drivers of declines in Borneo's

105 freshwater mussel populations include pollution from domestic and industrial sewage,

106 hydrological alterations, mining, climate change and invasive species (Dudgeon et al., 2006;

107 Zieritz et al., 2018b; Sundar et al., 2020).

108 Whilst native mussel species have declined on the island, humans have repeatedly introduced

109 and are actively spreading the non-native *Sinanodonta* cf. *woodiana* (Lea, 1834) for food and

110 ornamental purposes (Zieritz et al., 2018c). A native to the Yangtze River basin, *S.* cf.

111 *woodiana* was first reported in Borneo from a market in Sabah (Bogan & Schilthuizen, 2004)

112 and is now the most common and widespread unionid species on Borneo, particularly in Sabah

113 (Figure 1a). In 2018, another non-native species, *Sinanodonta lauta* (Martens, 1877), native to

114 Japan, was recorded for the first time in Borneo from a pond in Lawas district (Zieritz et al.,

115 2020b). These non-native species may exacerbate the negative effects of anthropogenic habitat

116 degradation on native species due to interspecific competition and by inducing cross-resistance

117 of host fish (Donrovich et al., 2017).

118 As highlighted by Zieritz et al. (2020b), more survey work is urgently needed to rapidly locate

119 and subsequently protect any remaining populations of native freshwater mussels in Borneo.

120 Considering the vast area of virtually unsurveyed habitat (Figure 1a), initial focus should

121 therefore be put on regions with historical records of freshwater mussels. In Malaysian Borneo,

122 this specifically includes the river basins of eastern Sabah, including the Kinabatangan River

123 (Figure 1). In Indonesian Borneo (=Kalimantan), where no DNA sequence data of freshwater

124 mussels are available to date, historical records are particularly concentrated in the Kapuas

125 River in West Kalimantan, the Mahakam River in East Kalimantan, and the river basins of

126 South Kalimantan (including the Barito) (Figure 1a). These surveys should also involve

127 gathering data on past occurrences of freshwater mussel populations, which are crucial for

128 determining recent changes in species diversity and distribution ranges. Whilst data availability

129 in the published literature and museum records are often insufficient in this respect, local

130 ecological knowledge can provide an alternative source of this information, especially in

131 Southeast Asia (Silvano & Valbo-Jørgensen, 2008; Zieritz et al., 2018c; Zieritz et al., 2020b),

132 where freshwater mussels are widely being used as a source of food and material (Zieritz et al.,

133 2018a).

134 The present study aims to assess current patterns and recent changes in the diversity and

135 distribution of freshwater mussels in northeastern Borneo, focusing on the Kinabatangan River

136 basin, through field surveys and interviews. In addition, comparative material from West

137 Kalimantan and Sarawak is also discussed, which was collected in the course of our ongoing,

138 larger effort towards unravelling these patterns across the whole of Borneo. Molecular

139 phylogenetic analyses (five genes) of sampled individuals recovered several previously

140 undescribed lineages. Two new tribes are described, i.e. Ctenodesmini trib. nov. and

141 Schepmaniini trib. nov., as well as a new genus, i.e. *Khairuloconcha* gen. nov., and two new

142 species, i.e. *K. lunbawangorum* sp. nov. and *K. sahanae* sp. nov., both of which are endemic

143 to Borneo and are known from only a single stream each. Specific threats to these endemic

144 mussel species are discussed, and actions towards their conservation and that of the Bornean

145 freshwater mussel fauna in general are recommended.

2. Methods

2.1. Study area

148 Fieldwork was conducted in eastern Sabah in the Kinabatangan and Danum River basins as

149 well as the surrounding coastal basins (Figure 1). Study sites were situated within the

150 freshwater ecoregions of Borneo Highlands and Northeastern Borneo (Abell et al., 2008).

151 Dominating geological units are ophiolitic and sedimentary rocks in the upper Kinabatangan

152 and Danum catchments, and Miocene melanges and sedimentary rocks in the lower river

153 catchments (Balaguru & Nichols, 2004). The area features several protected areas, including

154 the Kinabatangan Wildlife Sanctuary (260 km^2), the Protection Forest Reserves of Gomantong

155 (51 km^2) and Tawai (227 km2), and the Virgin Jungle Reserve Kabili Sepilok (43 km^2) (UNEP-

156 WCMC & IUCN, 2014-2020). Whilst the region is renowned for its exceptional biodiversity

157 and a popular nature tourism destination, land-use conversion from the natural forest into

158 commercial logging forest and later into oil palm plantation has reduced primary forest to

159 narrow strips along the riparian corridor (Goh, 2017).

2.2. Field surveys

161 Field surveys in Sabah were conducted during the dry season from 9-16 May 2019, when water

162 levels are lowest and access to mussels is therefore optimal. A total of 32 sites were surveyed.

163 Survey methodology followed that by Zieritz et al. (2020b). Briefly, at each site, covering about

164 100-300 m river length, at least three local individuals were initially interviewed to ask about

165 safe access to sampling sites, presence of Estuarine Crocodiles (*Crocodylus porosus*), and

166   historical and current presence of mussels. Interviewees were thereby shown photographs or,

167   if available, shells of mussel genera known from Borneo and asked to identify any that used to

168   be or are present at the site. Where access was safe (24 sites), environmental parameters known

169   to affect tropical mussel distribution (Zieritz et al., 2016; Gallardo et al., 2018) were recorded:

170   (1) temperature and (2) pH readings were taken using a YSI ProDSS Water Quality

171   Multiparameter probe. Water samples were taken with acid-washed, dried sampling bottles,

172   and 300-500mL filtered through a pre-weighed Whatman GF/F filter for analysis of (3) total

173   suspended solids (TSS), (4) nitrate (NO_3-N), total ammoniacal nitrogen (TAN) and (5) soluble

174   reactive phosphorus (SRP) on the day of sampling (Eaton et al., 2005). TSS was calculated as

175   the difference between the initial weight of the filter paper and after drying to constant weight

176   at 105°C. Concentrations of NO_3-N, TAN and SRP were determined from filtered water

177   samples by ascorbic acid, cadmium reduction and salicylate method, respectively, using a

178   calorimeter (Nutrient Auto Analyzer, HACH, DR900) (HACH, 2012).

179   Mussel surveys were conducted by hand and net until genera identified by respective locals

180   were found or for at least one person hour, following the same protocol as described in Zieritz

181   et al. (2018c). Where mussels were found, voucher specimens and tissue snips were collected

182   and preserved in absolute ethanol and deposited at the BORNEENSIS Collection, Institute for

183   Tropical Biology and Conservation, Universiti Malaysia Sabah (BOR/MOL) (Appendix).

184   Except for vouchers, all other specimens were returned to their habitat. Additional interviews

185   were conducted at an ad-hoc basis at markets, homestays and a biological field centre.

186   On 25 August 2019, *Ctenodesma borneensis* were collected from its putative type locality

187   "northwestern Borneo" ("Nordwestliches Borneo" in von Martens (1867) incorrectly translated

188   to "Nord-Est Borneo" by Issel (1874)), specifically from Sungai (River) Seberuang (alternative

189   spellings: Sebruang, Sebroang), Kapuas River basin, West Kalimantan (Figure 1a). A voucher

190   specimen (FKH-MFw0001) was deposited at the Fahutan Kapuas Hulu collection,

191   Tanjungpura University, Pontianak, West Kalimantan, Indonesia (FKH) (Appendix).

192   **2.3. Species identification, and morphological and phylogenetic analysis**

193   Collected specimens were identified to species-level through an integrative morphological-

194   molecular approach. Genomic DNA was extracted from tissue samples of the newly collected

195   and other selected available specimens (Appendix), using a standard high-salt protocol

196   (Sambrook, Fritsch & Maniatis, 1989) or the Jetquick tissue DNA Spin Kit (Genomed)

197   following the manufacturer's protocol. Female lineages of mitochondrial cytochrome c oxidase

198   subunit 1 (COI) were amplified and sequenced in both directions from all extracts using primer

199   pair LCO22me2 + HCO700dy2 (Walker et al., 2006; Walker et al., 2007). For native species,

200   additional sequences were generated for the large ribosomal subunit rRNA 16S (16SL + 16SH;

201   Palumbi et al., 1991); the mtDNA ND1 (Leu-uurF and LoGlyR; Serb, Buhay & Lydeard,

202   2003); the small ribosomal subunit rDNA 18S (18Sa2.0 + 9R; Giribet et al., 1996); and the

203   large ribosomal subunit 28S (28S-RD1.3f and 28S-rD4b; Whiting, 2002). PCR

204   conditions followed Froufe et al. (2016) and Bolotov et al. (2016) for 18S, with annealing

205   temperatures of 48°C and 50°C (only ND1). As the selected primer pair failed to amplify the

206   16S fragment in both *Khairuloconcha* species, amplification using an alternative primer pair

207   (i.e. primers 16SA + 16SB; Palumbi, 1996) was then attempted. Amplified DNA templates

208   were purified and sequenced by the commercial company Macrogen using the same primers.

209   Sequences were cleaned up in program MEGA X (Kumar et al., 2018) and deposited on

210   Genbank (Accession Numbers in Appendix).

211   For COI barcoding and to identify the generic position of each sequenced individual, a

212   preliminary COI alignment with all newly sequenced individuals and representatives from

213   most previously published unionid genera was produced and analysed with a Bayesian

214   Inference (BI) phylogenetic approach, using MrBayes 3.2.7a (Ronquist et al., 2012) with

215 20×10^6 generations sampled at intervals of 1,000 generations on a single partition with model

216 GTR+I+G.

217 Five alignments corresponding to the sequenced markers were then constructed with the newly

218 sequenced individuals and representatives from available taxa belonging to the four described

219 tribes within the subfamily Gonideinae (Appendix). Sequences from species belonging to the

220 unionid subfamily Ambleminae and the Margaritiferidae were included as an outgroup

221 following the relationships recovered in Pfeiffer, Breinholt & Page (2019). Each marker dataset

222 was aligned using the stand-alone version of GUIDANCE2 (Sela et al., 2015) with the MAFFT

223 multiple sequence alignment algorithm (version 7; Katoh & Standley, 2013) using default

224 parameters. As the 16S fragment of the two *Khairuloconcha* species failed to amplify (see

225 Results Section), two datasets were assembled for phylogenetic analyses: (1) a concatenated

226 COI+ND1+16S+18S+28S dataset coding 16S nucleotides of *Khairuloconcha* as "missing

227 data"; and (2) a concatenated COI+ND1+18S+28S dataset. The best-fit number of partitions

228 and substitution models were then selected for Bayesian Inference (BI) with PartitionFinder2

229 using a greedy search approach, MrBayes model set and the Bayesian Information Criterion

230 (Lanfear et al., 2017); and for Maximum Likelihood (ML) with ModelFinder

231 (Kalyaanamoorthy et al., 2017). BI analyses implemented in MrBayes 3.2.7a (Ronquist et al.,

232 2012) were initiated with program-generated trees and four Markov chains with default

233 incremental heating. Two independent runs of 20×10^6 generations were sampled at intervals

234 of 1,000 generations, producing a total of 20,000 trees. Burn-in was determined upon the

235 convergence of loglikelihood and parameter values using Tracer 1.7.1 (Rambaut et al., 2018).

236 Maximum-Likelihood analyses implemented in IQ-TREE 2.1.1 (Minh et al., 2020) were

237 conducted with initial tree searches, followed by 10 independent runs and 10,000 ultrafast

238 bootstrap replicates.

239 The genus- and species-level nomenclatural acts proposed in this publication were registered

240 at ZooBank under the Life Sciences Identifier - urn:lsid:zoobank.org:pub:C357B255-63D1-

241 4F87-9F51-FB5B8B061235. This included material from the collection of the Faculty of

242 Resource Science & Technology, Universiti Malaysia Sarawak (FRST).

3. Results

3.1. Diversity and distribution

245 Full surveys were conducted at 24 sites (Figure 1b). Live specimens were found at two of these

246 sites, whilst only shells were collected from another two sites. In addition, anecdotal evidence

247 for current or historical presence of mussels was collected for a further 13 sites (Figure 1b).

248 Native species were only found at one site, in a small stream (locally referred to as Sungai

249 Dayang) situated within the Gomantong Forest Reserve draining initially into the River

250 Menanggul and ultimately, the lower Kinabatangan River (Figures 1b and 3a; pers. comm. R.

251 Amandus, Gomantong Forest Reserve). Presence of mussels was initially confirmed by Forest

252 Reserve staff, and numerous empty shells were found by us within the first ten person-minutes

253 of surveying. However, live specimens were only found after about 15 person-hours in a single

254 dense aggregation at the stream edge in mud-sandy substrate underneath roots of bank

255 vegetation (Figure 3a). Within about 20 person-minutes, 47 *Schepmania* sp. and 1

256 *Khairuloconcha sahanae* sp. nov. (see Taxonomy section below for details) were collected. In

257 addition, >80 shells of *Schepmania* sp. and 5 shells of *K. sahanae* were found. In comparison

258 to other study sites, conditions at this site were characterised by colder water temperature

259 (26.3ºC *vs*. an average of 30.4ºC and 29.5ºC at sites with *S*. cf. *woodiana* and without unionids,

260 respectively), low pH (6.8 *vs*. 7.3 and 7.1), and high concentrations of NO_3-N (0.04 mg L^{-1} *vs*.

261 0.02 and 0.01) and SRP (0.49 mg L^{-1} *vs*. 0.21 and 0.19) (Figure 4).

262 No live specimens, shells or anecdotal evidence for current presence of native mussel species

263 were found at any other study site, but anecdotal evidence for historical presence of

264   *Schepmania* sp. was collected from three independent sources (Figure 1b): (1) a female vendor

265   (~60 years of age) selling freshwater shellfish at Beluran town market stated that several

266   decades ago, *Schepmania* sp. used to be present in small, sandy streams near "Kampung Kepod,

267   Batu 54", situated in the drainage of the coastal River Sapi (Figure 1b), but has disappeared;

268   (2) a female vendor (~40 years of age) selling fish at Kinabatangan market stated that

269   *Schepmania* sp. used to be present in some of the small tributaries of the Kinabatangan in this

270   area, but disappeared several decades ago; (3) the owner of a small homestay south of the town

271   of Kinabatangan (male, ~50 years of age) also stated that *Schepmania* sp. used to be present in

272   this area, but has not been seen for several decades.

273   Live specimens of *Sinanodonta* cf. *woodiana* were collected in a small tributary of Sungai

274   Imbak, situated within an oil palm plantation within the upper Kinabatangan catchment

275   (Figures 1b and 3b). Densities were moderate, and seven specimens were collected within

276   approximately one person-hour. Shells of this species were additionally found in Sungai

277   Tongod, also in the upper Kinabatangan catchment, and in the coastal Sungai Matamba basin

278   near the city of Lahad Datu (Figures 1b and 3c). Anecdotal records of current *S.* cf. *woodiana*

279   presence were also collected from other tributaries of the upper, middle and lower

280   Kinabatangan, the Danum river basin, and across coastal river basins, i.e. Sungai Sendala,

281   Sungai Segaliud and Sungai Suanlamba, which could not be surveyed due to the presence of

282   crocodiles (Figures 1b and 3d).

283   **3.2. COI barcoding**

284   The newly sampled specimens from eastern Sabah belonged to three different species, and the

285   initial COI-BI tree positioned all except *Sinanodonta* cf. *woodiana* within the Gonideinae. The

286   *Sinanodonta* cf. *woodiana* sequence was 100% identical to that of specimens x284, x289, x294,

287   x354, x363 and x372 (Genbank Accession Numbers MG591509-11, MG591516-17,

288   MG591519) originating from other river basins of Sabah and Sarawak, i.e. Sungai Wariu,

289 Sungai Bongan and Sungai Suai. COI sequences of the remaining species were highly

290 divergent from available sequences on Genbank, with the closest matches being members of

291 the Gonideinae, i.e. *Gonidea angulata* (e.g. MN957829) for *Schepmania* cf. *niewenhuisi* with

292 84.1% similarity, and "*Ctenodesma borneensis*" (=*Khairuloconcha lunbawangorum*;

293 MN900788-90 [request for correcting species IDs will be sent to Genbank after acceptance of

294 this manuscript]) for *Khairuloconcha sahanae* with 93.7%. Finally, the best matches for

295 *Ctenodesma borneensis* collected from the type locality in the Kapuas River basin were

296 *Hyriopsis bialata* (MG025690) and *Contradens* (=Lens; Pfeiffer et al., 2021) sp. (KX865925)

297 with 87.9% similarity, respectively.

3.3. Phylogenetic position of Bornean endemic species

299 Both of the primer pairs applied failed to amplify the 16S fragment of the two *Khairuloconcha*

300 species. Topologies of ML and BI trees were identical for concatenated

301 COI+ND1+16S+18S+28S and COI+ND1+18S+28S datasets, respectively. Topologies of trees

302 for concatenated COI+ND1+16S+18S+28S and COI+ND1+18S+28S datasets were identical

303 with respect to the positions of the Bornean endemic species (Figure 5 and Suppl. Material 1).

304 The two *Khairuloconcha* species were recovered as sister to *Ctenodesma borneensis*, with

305 strong nodal support (PP/UF = 0.99/91 and 0.92/88 for COI+ND1+16S+18S+28S and

306 COI+ND1+18S+28S, respectively), together forming the Bornean endemic tribe Ctenodesmini

307 trib. nov. The Ctenodesmini were in turn consistently recovered as sister to the Contradentini

308 + Rectidentini again with strong nodal support. COI uncorrected *p*-distances were 6.47%

309 between *K. lunbawangorum* and *K. sahanae*, 13.25% between *C. borneensis* and *K.*

310 *lunbawangorum*, and 13.71% between *C. borneensis* and *K. sahanae*, respectively.

311 The two sequenced *Schepmania* cf. *niewenhuisi* specimens exhibited identical sequences and

312 were consistently recovered as a previously unrecognised and divergent sister-group to the

313 Pseudodontini that is herein described as the tribe Schepmaniini trib. nov..

314

3.4. Taxonomy

Family Unionidae Rafinesque, 1820

Subfamily Gonideinae Ortmann, 1916

Tribe Schepmaniini trib. nov. Lopes-Lima, Pfeiffer & Zieritz

Type Genus: *Schepmania* Haas, 1910

Diagnosis: The Schepmaniini is distinguished from all other Gonideinae taxa in southeast Asia

(Chamberlainiini, Contradentini, Ctenodesmini, Pseudodontini, Rectidentini) by its ovate shell

outline, the presence of a strongly sculptured posterior slope and largely smooth shell disk,

robust pseudocardinal and lateral teeth, and an obvious ligamental fossette.

Distribution: Eastern Borneo

Genera:

   Schepmania Haas, 1910

Remarks: The higher-level classification of *Schepmania* has varied substantially over the past

50 years, having been placed in the Margaritiferidae (Starobogatov, 1970; Bogatov, Prozorova

& Starobogatov, 2003), Unionidae *incertae sedis* (Graf & Cummings, 2007; Lopes-Lima et al.,

2017; Zieritz et al., 2018b), and the tribe Lamprotulini (Unionidae, Gonideinae) (Pfeiffer,

Breinholt & Page, 2019; Graf & Cummings, 2021b). Our phylogeny robustly recovers

Schepmania as a member of a previously unrecognised lineage of the Gonideinae that we

describe herein as the Schepmaniini trib. nov.

This new tribe includes only the genus *Schepmania*, which consist of two species, *Schepmania*

niewenhuisi (Schepman, 1898) and *Schepmania parcesculpta* (Martens, 1903) (Haas, 1969;

Graf & Cummings, 2007; Zieritz et al., 2018b; Zieritz et al., 2020b; Graf & Cummings, 2021b).

These two taxa are morphologically very similar but are thought to be distinguished based on

the degree of sculpturing on the posterior ridge — with *S. niewenhuisi* being more heavily

339 sculptured than *S. parcesculpta* (Figure 2) (Haas, 1969). However, the minor reported

340 differences between these putative species are based on few specimens and there is doubt about

341 whether the two nominal taxa represent distinct species (Haas, 1969). The sequenced

342 specimens clearly belong to the genus *Schepmania* but because of uncertainties surrounding

343 the validity and distributions of *S. niewenhuisi* and *S. parcesculpta*, we cannot confidently

344 assign our specimens to either species. Further research focused on specimens from near the

345 type localities of *S. niewenhuisi* (Bloe-oe, East Borneo [=Mahakam River, East Kalimantan,

346 Borneo, Indonesia]) and *S. parcesculpta* (Guleh-Fluss, Ost Borneo [=river near Sangkulirang,

347 East Kalimantan, Borneo, Indonesia]) will be necessary to resolve the systematics of

348 *Schepmania*.

349

350 **Tribe Ctenodesmini trib. nov. Pfeiffer, Zieritz, Rahim & Lopes-Lima**

351 **Type Genus:** *Ctenodesma* Simpson, 1900

352 **Diagnosis:** The Ctenodesmini is morphologically very similar to its sister group, which

353 consists of the tribes Contradentini and Rectidentini. The Ctenodesmini can be distinguished

354 from the Contradentini by its tetragenous brooding condition (*vs*. ectobranchous brooding in

355 Contradentini). The Ctenodesmini can be distinguished from the Rectidentini by its sculptured

356 shells disc (*vs*. smooth in Rectidentini).

357 **Distribution:** Borneo

358 **Genera:**

359   *Ctenodesma* Simpson, 1900

360   *Khairuloconcha* Zieritz, Lopes-Lima and Pfeiffer gen. nov.

361 **Remarks:** The higher-level classification of *Ctenodesma* has also varied substantially over the

362 past 50 years, including placement in the Margaritiferidae (Starobogatov, 1970; Bogatov,

363 Prozorova & Starobogatov, 2003), the subfamily Unioninae (Unionidae) (Haas, 1969),

364 Unionidae *incertae sedis* (Graf & Cummings, 2007; Lopes-Lima et al., 2017; Zieritz et al.,

365 2018b), a member of the Rectidentini (Unionidae, Gonideinae) (Pfeiffer, Breinholt & Page,

366 2019; Graf & Cummings, 2021b; Pfeiffer et al., 2021) or an unnamed sister-group to the

367 Rectidentini (Zieritz et al., 2020b). The topology recovered here suggests that *Ctenodesma* and

368 its sister taxon *Khairuloconcha* represent a previously unrecognised tribe-level clade of the

369 Gonideinae, described here as the Ctenodesmini trib. nov.

370 ***Khairuloconcha* gen. nov. Zieritz, Lopes-Lima & Pfeiffer**

371 **Type species:** *Khairuloconcha lunbawangorum*

372 **Diagnosis:** Morphologically similar to its sister clade *Ctenodesma* but tends to have a sharper

373 posterior ridge with nodulous sculpturing especially dorsally (*vs.* rounded and smooth posterior

374 ridge in *Ctenodesma*), and has faint zigzag or irregular sculpture on the posterior slope (*vs.*

375 uniform plications in *Ctenodesma*).

376 **Description:** Ovate shell outline, dorsal and ventral margins straight, largely parallel, dorsal

377 margin angled slightly upwards, anterior end broadly rounded, posterior end narrowly rounded

378 to broadly rounded. Posterior ridge somewhat distinct, often with small nodulous sculpture.

379 Posterior slope gradual, bi- or triangulate with faint zigzag or irregular sculpturing. Yellowish-

380 brown periostracum, occasionally with two or three green rays associated with ridges on

381 posterior slope. Umbo slightly elevated above hinge line. Umbo sculpture zigzag with small

382 nodules. Left valve with two straight, long, thin, slightly diverging lateral teeth. Left valve with

383 one long, thick, crenulate or finely fractured pseudocardinal tooth. Right valve with one

384 straight, long, thin lateral tooth. Right valve with two long pseudocardinal teeth, posterior

385 pseudocardinal tooth much longer and thicker than the anterior pseudocardinal tooth. Nacre

386 iridescent, faintly bluish or purplish, translucent.

387 **Distribution:** northern Borneo

388 **Remarks:** Morphologically very similar to *Ctenodesma* (Figure 2) but is molecularly very

389 divergent (13.25-13.71% uncorrected p-distance; Figure 5).

390 **Etymology:** The species is named in honour of Dr. Khairul Adha A. Rahim, one of Borneo's

391 leading aquatic biologists and Senior Lecturer at the Universiti Malaysia Sarawak.

392

393 *Khairuloconcha lunbawangorum* **sp. nov. Zieritz, Rahim, Taha & Pfeiffer**

394 **Type locality:** A small stream near Kuala Medalam (local name: Sungai Kemadi), Limbang

395 drainage, Sarawak, Borneo, Malaysia (Figure 2c in Zieritz et al., 2020b).

396 **Types:** Holotype, FRST_MFw_X439 (transferred from UNIMAS X439 of Zieritz et al., 2020b

397 [UNIMAS = Universiti Malaysia Sarawak]). Paratypes, FRST_MFw_X438 (n=2, transferred

398 from UNIMAS X438 and X440 of Zieritz et al., 2020b), same location as holotype.

399 **Diagnosis:** *Khairuloconcha lunbawangorum* is distinguished from its sister species

400 *Khairuloconcha sahanae* by its more narrowly pointed posterior end (*vs.* broadly rounded in

401 *K. sahanae*) and its less sharply defined and less nodulous posterior ridge (*vs.* sharper and more

402 nodulous posterior ridge in *K. sahanae*) and less obvious sulcus.

403 **Description:** Ovate shell outline, dorsal and ventral margins straight, largely parallel, dorsal

404 margin angled slightly upwards, anterior end broadly rounded, posterior end narrowly rounded.

405 Posterior ridge somewhat distinct, often with small nodulous sculpture. Posterior slope gradual,

406 bi- or triangulate with faint zigzag or irregular sculpturing, Yellowish-brown periostracum,

407 occasionally with two or three green rays associated with ridges on posterior slope,

408 occasionally with faint green rays on shell disc. Umbo slightly elevated above hinge line. Umbo

409 sculpture zigzag with nodules. Left valve with two straight, long, thin, slightly diverging lateral

410 teeth. Left valve with one long, thick, crenulate or finely fractured pseudocardinal tooth. Right

411 valve with one straight, long, thin lateral tooth. Right valve with two long pseudocardinal teeth,

412 posterior pseudocardinal tooth much longer and thicker than the anterior pseudocardinal tooth.

413 Nacre iridescent, faintly bluish, translucent. Excurrent aperture longer than incurrent, multiple

414 rows of small conical papillae. Incurrent aperture smooth, shorter than excurrent. Tetragenous

415 brooding.

416 **Distribution:** Sungai Kemadi in Limbang River basin.

417 **Etymology:** The species is named in honour of the Lun Bawang people, an ethnic group

418 indigenous to parts of the Bornean highlands, including the type locality.

419 **Remarks:** This species is known from only 3 specimens from one location. Sampling

420 additional populations and individuals will be necessary to more completely describe the

421 species' distribution and morphological variation.

422

423 *Khairuloconcha sahanae* **sp. nov. Zieritz, Jainih & Lopes-Lima**

424 **Type locality:** A small stream (Sungai Dayang) within the Gomantong Forest Reserve,

425 Kinabatangan drainage, Sabah, Borneo, Malaysia (Figure 3a).

426 **Types:** Holotype, BOR/MOL 14416. Paratype, FMNH 116932 (n = 1), Gomantong, Sandakan

427 Residency, North Borneo.

428 **Diagnosis:** *Khairuloconcha sahanae* is distinguished from its sister species *Khairuloconcha*

429 *lunbawangorum* by its more broadly rounded posterior end (*vs.* narrowly rounded in

430 *Khairuloconcha lunbawangorum*) and its more sharply defined and more nodulous posterior

431 ridge (*vs.* rounder and less nodulous posterior ridge in *K. lunbawangorum*) and a more obvious

432 sulcus.

433 **Description:** Ovate shell outline, dorsal and ventral margins straight, largely parallel, dorsal

434 margin angled slightly upwards, anterior end broadly rounded, posterior end broadly rounded.

435 Slight sulcus in shell disc. Posterior ridge distinct, with small nodulous sculpture. Posterior

436 slope gradual, triangulate with faint irregular sculpturing. Yellowish-brown periostracum.

437 Umbo very slightly elevated above hinge line. Umbo sculpture zigzag with nodules. Left valve

438 with two straight, long, thin, slightly diverging lateral teeth. Left valve with one long, thick,

439 crenulate or finely fractured pseudocardinal tooth. Right valve with one straight, long, thin

440 lateral tooth. Right valve with two long pseudocardinal teeth, posterior pseudocardinal tooth

441 much longer and thicker than the anterior pseudocardinal tooth. Nacre iridescent, faintly

442 purplish, translucent.

443 **Distribution:** Tributaries of lower Kinabatangan River basin but known only from one stream.

444 **Etymology:** The species is named in honour of the late Dr. Sahana Harun, a dedicated lecturer

445 and freshwater ecologist at the Institute of Tropical Biology and Conservation, Universiti

446 Malaysia Sabah, Malaysia.

447 **Remarks:** This species is known from only two specimens (BOR/MOL 14416 and FMNH

448 116932). Both lots were collected from the Gomantong Hill area, which features only one

449 stream (Sungai Dayang; pers. comm. R. Amandus, Gomantong Forest Reserve); however, the

450 exact sampling location of FMNH 116932 is unknown. Sampling additional populations and

451 individuals will be necessary to more completely describe the species' distribution and

452 morphological variation.

453 ## 4. Discussion

454 ### 4.1. A new genus and two new, rare freshwater mussel species endemic to Borneo

455 Our study reveals the presence of a new genus of freshwater mussel endemic to Borneo that is

456 comprised of two rare species with highly restricted distributions, i.e. *Khairuloconcha*

457 *lunbawangorum* within the Limbang River basin, Sarawak, and *K. sahanae* within the

458 Kinabatangan River basin, Sabah. These species are very poorly understood and records of

459 both have been previously identified as *Ctenodesma borneensis* (UNIMAS X438-X440 = *K.*

460 *lunbawangorum;* FMNH 116932 = *K. sahanae*). Molecular phylogenetic analysis revealed that

461 *Khairuloconcha* is a divergent lineage sister to *C. borneensis*, and can morphologically be

462 discriminated from *C. borneensis* by its sharper posterior ridge with nodulous sculpturing

463 (especially dorsally) and a faint zigzag or irregular sculpture on the posterior slope rather than

464 uniform plications. Based on the limited distribution data available to date, *Khairuloconcha*

465 appears to be present in northern Borneo, whilst *Ctenodesma* may be restricted to western

466 Borneo.

467 Both *Khairuloconcha* species as well as *Schepmania* sp. were found only at a single site each,

468 with *K. lunbawangorum* and *K. sahanae* occurring in very low densities (i.e. 3 specimens per

469 4 person-hours and 1 specimen per 15 person-hours, respectively). Their habitats were similar,

470 i.e. cool headwater streams situated in dense primary or secondary forest with natural bank

471 vegetation, and characterised by near neutral pH. Nutrient concentrations were, however,

472 elevated at both sites and especially at Gomantong (see Figure 4).

473 The Sungai Dayang, where *K. sahanae* was found, is located in the Class I – Protection Forest

474 Reserve 'Gomantong' (Figure 6a) and as such, is protected by law from any form of land

475 conversion or timber exploitation. As this stream exclusively receives water from the

476 Gomantong Caves and surrounding hills within the Forest Reserve (pers. comm. R. Amandus,

477 Gomantong Forest Reserve), the elevated nutrient concentrations in the water possibly stem

478 from excrements of the dense avian and mammalian fauna of the forest (Lundberg &

479 McFarlane, 2012; and references therein). However, the Gomantong Forest Reserve is small-

480 sized (approx. 3,297 ha Class I + 1,816 ha Class VI in the centre, including the Gomantong

481 Caves) and is surrounded by large areas of oil palm plantations with very high rates of

482 historical, recent and ongoing deforestation (Figure 6a). The nearest oil palm plantation and

483 mill are located less than 3 and 15 km from the site where *K. sahanae* was found, respectively

484 (Figure 6a). Considering the very low population density observed, it is therefore possible that

485 this species is already threatened by ongoing habitat deterioration, potentially associated with

486 logging and land-use change activities in the surrounding catchment.

487 The situation is particularly alarming for *K. lunbawangorum*, as its single known population is

488 situated in an unprotected area that has been allocated for an industrial-scale oil palm plantation

489 (Figure 6b). Whilst the population was found in a stream within a secondary forest,

490 deforestation is ongoing and land-use conversion to oil palm plantation has already commenced

491 in the immediate vicinity of the site (Figure 6b), presenting an immediate threat of extinction

492 to *K. lunbawangorum*.

493 **4.2. Evolutionary biogeography**

494 *Khairuloconcha* appears to have undergone a recent divergence (speciation), a diversification

495 pattern that is not common in the freshwater mussel genera native to Borneo, which based on

496 currently available data, are frequently represented by a single species on the island (e.g.

497 *Caudiculatus caudiculatus* (Martens, 1867), *Discomya radulosa* (Drouët & Chaper, 1892),

498 *Elongaria trompi* (Drouët & Chaper, 1892), *Hyriopsis velthuizeni* (Schepman, 1896),

499 *Pressidens exanthematicus* (Küster, 1861)). We suspect that this may be related to the habitat

500 and host fish requirements of species within *Khairuloconcha*, which, in contrast to most other

501 known freshwater mussels on Borneo, appears to be restricted to headwater streams (Zieritz et

502 al., 2018c; Zieritz & Lopes-Lima, 2018). Utilising headwater fish species as hosts for their

503 parasitic larvae (glochidia) would be expected to strongly restrict dispersal between river

504 (sub)basins, thus potentially leading to reproductive isolation of populations and ultimately,

505 divergence of species. Potential *Khairuloconcha* spp. host fish species include the cyprinids

506 *Nematabramis borneensis*, *Nematabramis everetti* and *Rasbora* spp., all of which are

507 commonly found in headwater streams of Sarawak and Sabah (Sulaiman & Mayden, 2012; Ng

508 et al., 2017). The fact that the only two *Khairuloconcha* populations known to date represent

509 separate species suggests that more, yet undescribed species of this genus and potentially other

510 endemic mussel taxa may exist in other headwater streams across Borneo, particularly those

511   situated within functioning rainforest habitat. It should be a priority to locate such populations

512   and facilitate the protection of their habitats.

513   The Bornean endemic freshwater mussel taxa sequenced to date fall into two newly described

514   clades that are endemic to Borneo, i.e. Schepmaniini and Ctenodesmini. Our dataset thus

515   increases the number of Gonideinae tribes from six (Pfeiffer, Breinholt & Page, 2019) to eight

516   and provides further evidence for the importance of Borneo as an evolutionary hotspot for

517   freshwater biodiversity (de Bruyn et al., 2014). A recent fossil-calibrated mitogenome

518   phylogeny (Zieritz et al., 2020a) placed the split of the Contradentini and Rectidentini, and the

519   Gonideini and Pseudodontini, at 79 and 95 mya, respectively. Assuming these node ages, the

520   two Bornean endemic tribes split from their respective sister tribes with a predominantly

521   mainland-Southeast Asian distribution sometime in the late-Cretaceous, i.e. the Ctenodesmini

522   from Contradentini + Rectidentini, and the Schepmaniini from Pseudodontini (Figure 5).

523   According to palaeogeographical reconstructions (Figure 3.4 in Hall, 2012), at that time,

524   western Borneo was connected by land (i.e. the Sunda Shelf) with today's mainland Southeast

525   Asia as well as eastern Sumatra and Java, with significant elevations (highlands) from the

526   Belitung Islands in the west to what is now the lower Kapuas River in the east. These highlands

527   may have created an effective dispersal barrier for freshwater mussel populations, which could

528   have led to a divergence of the Ctenodesmini and Schepmaniini in the river basin(s) east of

529   these highlands from their respective sister clades.

530   **4.3. Recommendations for conservation**

531   Our anecdotal evidence collected indicates that historically, at least *Schepmania* sp. probably

532   used to be widely distributed across the lower Kinabatangan basin and adjacent coastal basins,

533   but that most of these populations have been lost within the last 50 years or so. These

534   populations may have been extirpated in the course of deforestation campaigns in Sabah, which

535   reached industrial scale and devastation in the 1980s and 1990s, and are particularly intense in

536 the Kinabatangan basin (Seda, 1993; Bryan et al., 2013). A similar situation is likely to be true

537 for *K. sahanae*, but this species is small and difficult to discriminate from young *Schepmania*

538 sp. for the laymen, preventing us from gathering reliable information on its historical

539 distribution.

540 In light of the rapid pace of destruction of forest stream habitat on Borneo, it is of utmost

541 urgency that forest stream and other freshwater habitats across the island are surveyed to

542 identify yet unknown populations and potentially, even species, of endemic Unionidae, and

543 characterise and monitor their distribution and population characteristics. Particular attention

544 should be paid to surveying rivers in protected areas or other areas of largely undisturbed

545 habitat, including the National Parks of Gunung Buda and Gunung Mulu in Sarawak, and

546 Danau Sentarum and Sebangau National Parks in Kalimantan. Speed of data collection could

547 be significantly increased by complementing traditional physical surveys with environmental

548 DNA (metabarcoding) surveys, which could also be employed at otherwise inaccessible sites

549 (e.g. due to presence of crocodiles) but has rarely been used in the tropics (Ruppert, Kline &

550 Rahman, 2019; Prié et al., 2020). Wherever possible, these surveys should also include

551 collection of data on mussel ecology and biology, including those on habitat requirements,

552 potential threats and population structure, which will provide a critical basis on which targeted

553 conservation measures can be developed.

554 Considering the limited resources available for this herculean task, a considerable number of

555 populations and species will likely have already disappeared before they will have been located

556 and subsequently, protected. Parallel to ongoing survey efforts, we therefore recommend that

557 an Action Plan to save the Bornean endemic freshwater mussel species should be urgently

558 developed and implemented. First and foremost, to maximise the chances of survival of *K.*

559 *lunbawangorum*, the Sungai Kemadi, including its surrounding remaining forest habitats

560 (Figure 6b), should immediately be placed under legal protection (e.g. declaration as a 'High

561 Conservation Value Area') that prohibits deforestation and landuse conversion to agricultural

562 land. Considering the very low population sizes of both *Khairuloconcha* populations,

563 environmental conditions and population characteristics (including levels of recruitment)

564 should be regularly monitored at both Sungai Kemadi and Sungai Dayang. Ideally, these

565 measures of habitat protection and further survey efforts should be accompanied by captive

566 breeding programs for reintroduction and augmentation. This will require identification of host

567 fish species and data gathering on reproductive cycle and specific habitat requirements.

568 Wider conservation actions across all Bornean states should focus on mitigating the impacts of

569 deforestation and palm plantations by establishing riparian buffer zones, which are required by

570 law (i.e. Sabah 5-20 m, Sarawak 5-50 m, Indonesia 50-200 m, depending on river width) but

571 are often not implemented (Figures 3b and d) (Luke et al., 2019). Outreach programmes,

572 including Citizen Science Projects, will be needed to improve awareness of stakeholders,

573 decision makers and the general public about Borneo's unique freshwater mussel diversity and

574 the important ecosystem services they provide (Irvine et al., 2016; Zieritz et al., 2018a).

575

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587   **Conflict of interest**

588   The authors have no conflict of interest to declare.

589

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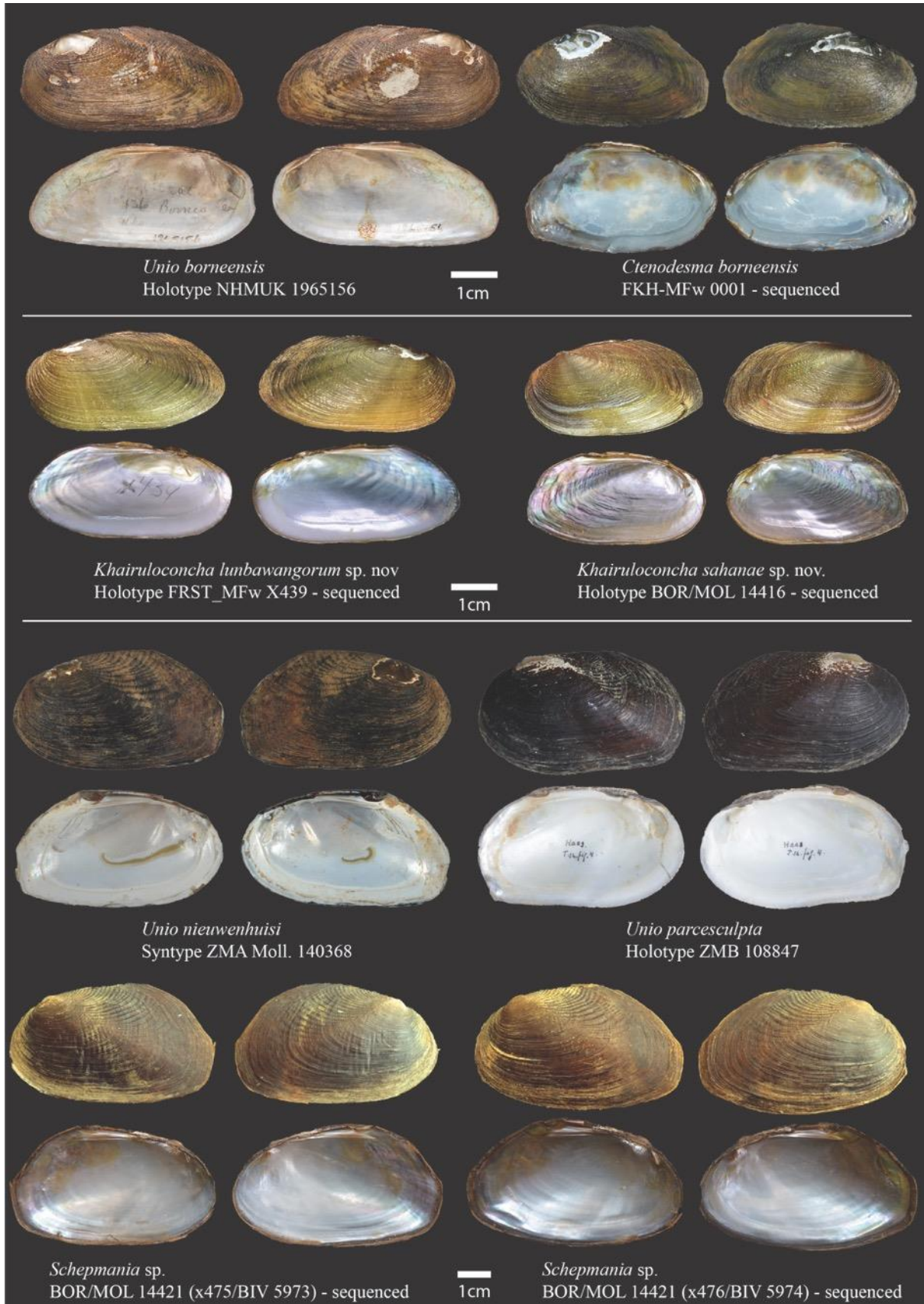
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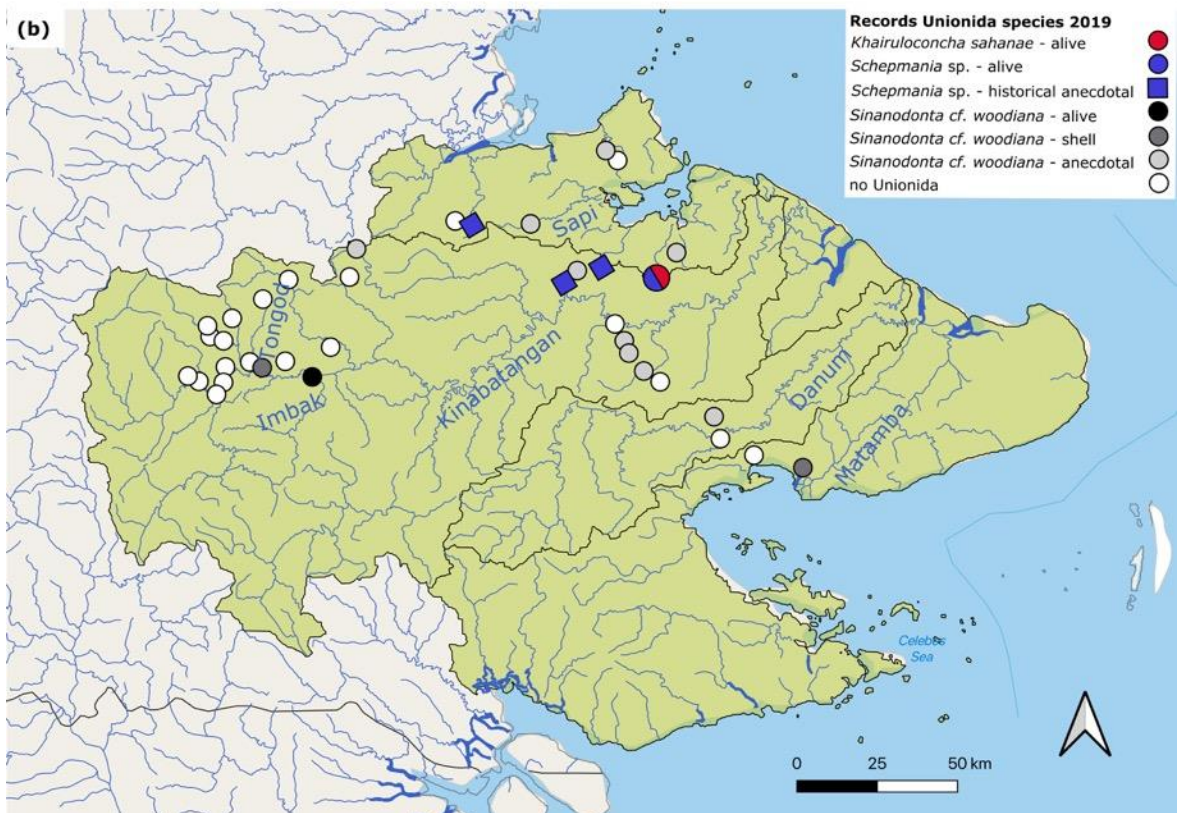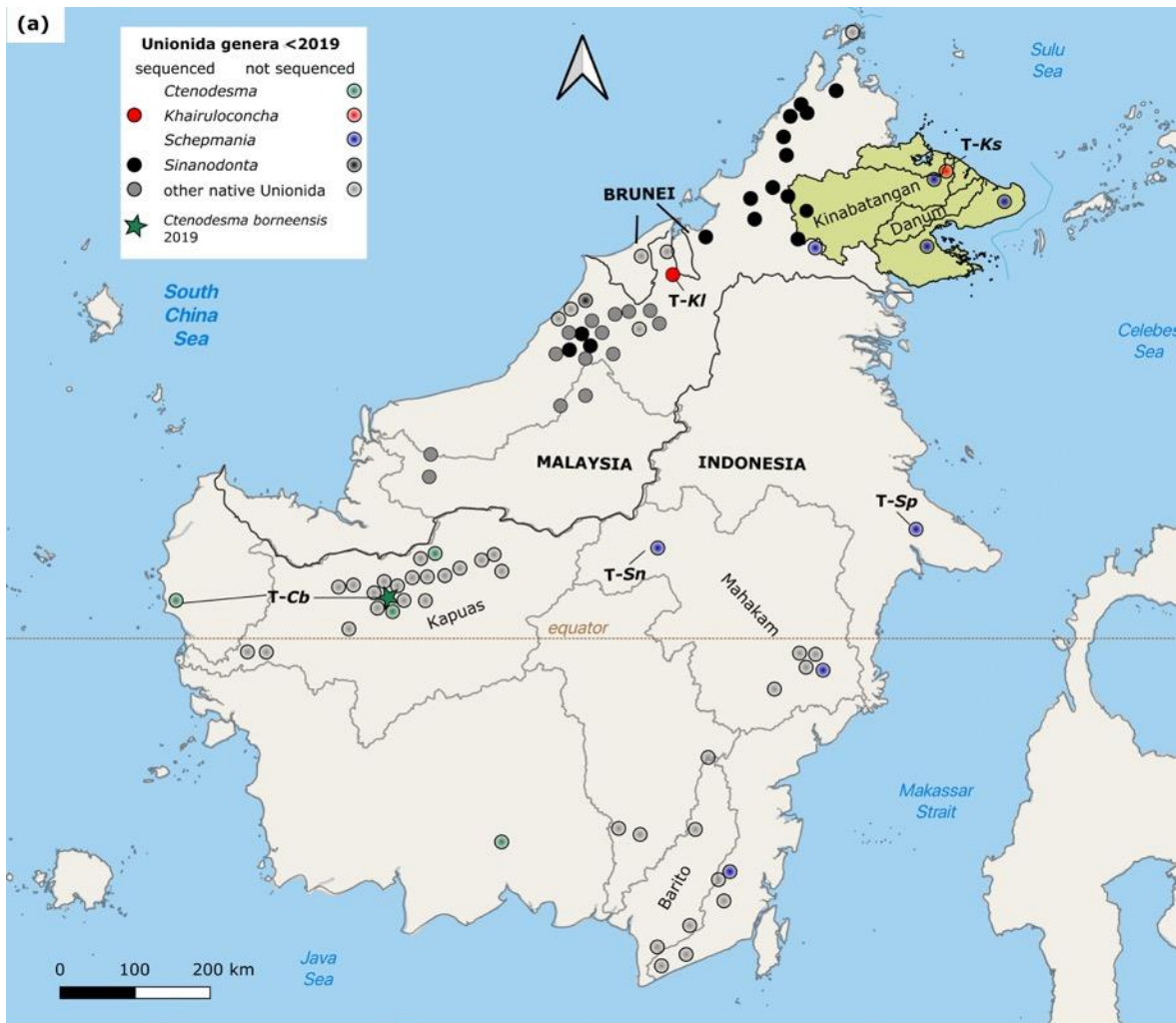
807   **Figure 1**. (a) Locations of freshwater mussel (Bivalvia: Unionida) genus-level records (pre-

808   2019) in Borneo and locality of collection of *Ctenodesma borneensis* in Kapuas basin,

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810   https://bioportal.naturalis.nl, including only records that were georeferencable at

811   HydroBASINS level 4 (Lehner & Grill, 2013) or higher. (b) Locations of study sites and

812   species-level records collected in Sabah in 2019. Green shading, level 6 HydroBASINS where

813   surveys were conducted; grey outline, level 4 HydroBASINS outside of study area.

814   Abbreviations: T-Cb, Type locality of *Ctenodesma borneensis*, T-Kl, Type locality of

815   *Khairuloconcha lunbawangorum* sp. nov.; T-Ks, Type locality of *Khairuloconcha sahanae* sp.

816   nov.; T-Sn, Type locality of *Schepmania niewenhuisi*; T-Sp, Type locality of *Schepmania*

817   *parcesculpta*.

818

Unio borneensis
Holotype NHMUK 1965156

1cm

Ctenodesma borneensis
FKH-MFw 0001 - sequenced

Khairuloconcha lunbawangorum sp. nov
Holotype FRST_MFw X439 - sequenced

1cm

Khairuloconcha sahanae sp. nov.
Holotype BOR/MOL 14416 - sequenced

Unio nieuwenhuisi
Syntype ZMA Moll. 140368

Unio parcesculpta
Holotype ZMB 108847

Schepmania sp.
BOR/MOL 14421 (x475/BIV 5973) - sequenced

1cm

Schepmania sp.
BOR/MOL 14421 (x476/BIV 5974) - sequenced

Figure 2. Type specimens and sequenced specimens of the treated Ctenodesmini trib. nov. and Schepmaniini trib. nov. taxa.

822

Figure 3. Photographs of sites in eastern Sabah where freshwater mussel occurrences were confirmed by physical surveys or interviews in May 2019. (a) Stream in Gomantong Forest Reserve, where *Schepmania* sp. and *Khairuloconcha sahanae* sp. nov. were collected alive; (b) tributary of River (Sungai) Imbak, where *Sinanodonta* cf. *woodiana* was collected alive; (c) River (Sungai) Tongod, where *S.* cf. *woodiana* shells were collected; (d) River (Sungai) Lokan, where *S.* cf. *woodiana* presence was confirmed anecdotally in interviews but could not be confirmed by us in physical surveys.

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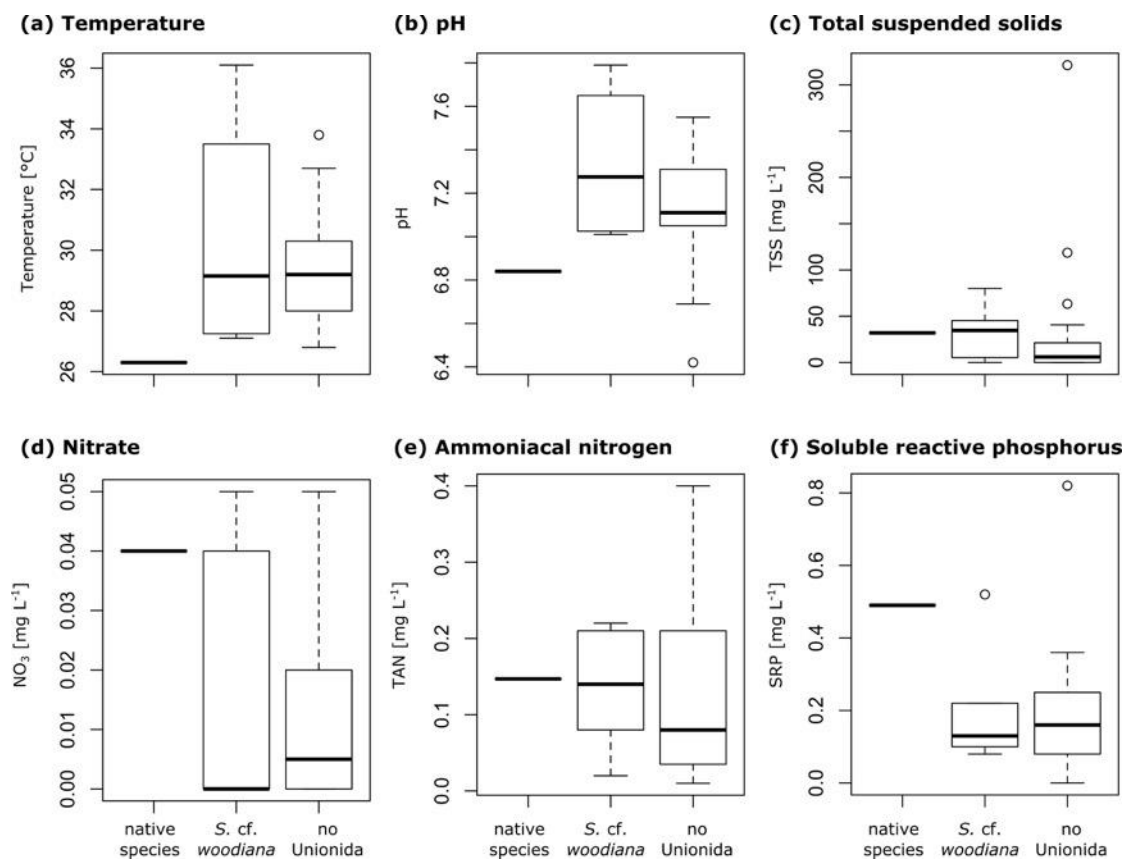
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832 **Figure 4.** Variation in water quality parameters in May 2019 across 24 study sites in eastern

833 Sabah with current occurrence of native freshwater mussel species *Khairuloconcha sahanae*

834 sp. nov. and *Schepmania* sp. (one site), non-native *Sinanodonta* cf. *woodiana* (five sites), and
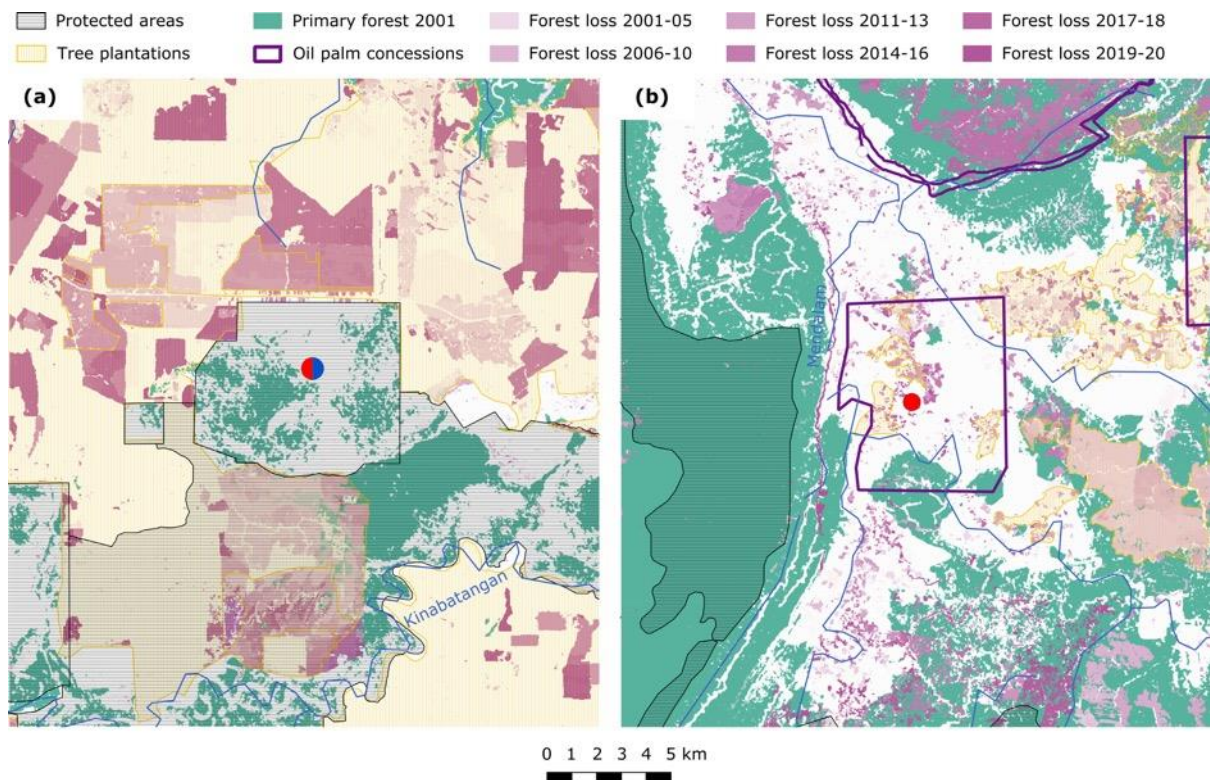
835 no Unionida (18 sites).

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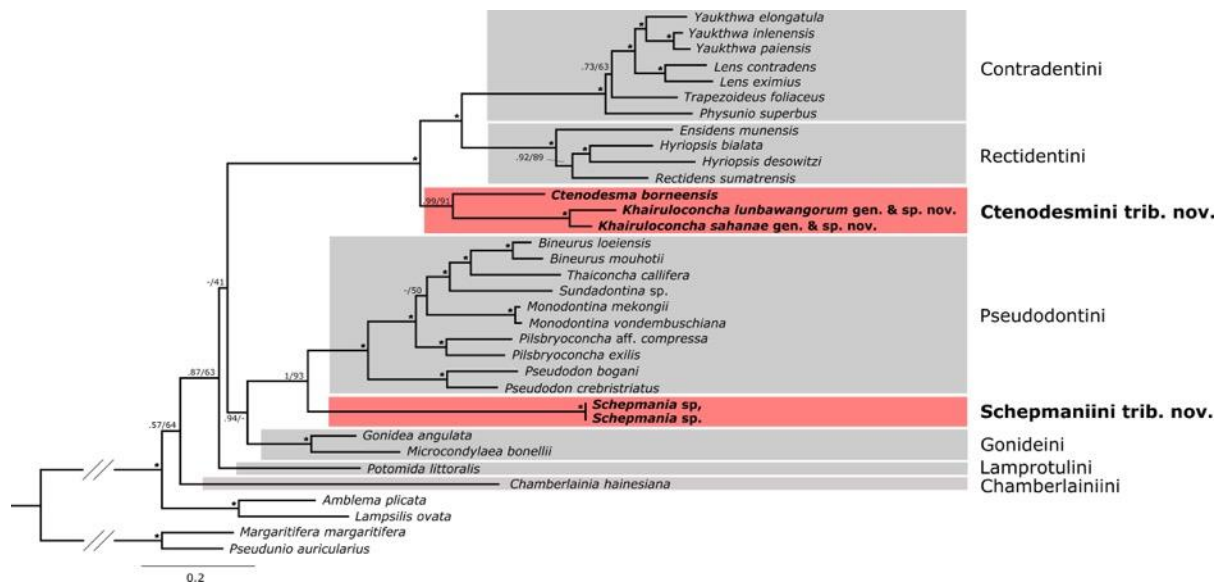
Figure 5. Maximum Likelihood phylogenetic reconstruction (COI + ND1 + 16S + 18S + 28S) of the Gonideinae (shaded) with support values listed as posterior probabilities / ultrafast bootstraps (PP/UF). Values of PP and UF ≥ 95 are marked with ''*''. Newly sequenced, Bornean endemic specimens in bold and shaded in red.

842

843

844 **Figure 6.** Landcover and landuse of areas surrounding the two recently (i.e. in 2018 and 2019)

845 confirmed locations of endemic freshwater mussel species populations, i.e. (a) *Khairuloconcha*

846 *sahanae* sp. nov. (red) and *Schepmania* sp. (blue) at the Gomantong Protection Forest Reserve,

847 and (b) *Khairuloconcha lunbawangorum* sp. nov. (red) in the upper Limbang River catchment.

848 Data sources: Forest loss, Hansen/UMD/Google/USGS/NASA (Hansen et al., 2013); Oil palm

849 concessions, World Resources Institute (2014); Primary forest 2011, Turubanova et al. (2018);

850 Protected areas, UNEP-WCMC & IUCN (2014-2020); Tree plantations, Harris, Goldman &

851 Gibbes (2019).

852