1 *Short communication*

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3 Molecular phylogenetic position of *Minamitalitrus zoltani* elucidates a further

4 troglobisation pattern in cave-dwelling terrestrial amphipods (Crustacea: Talitridae)

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25

26 ABSTRACT

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28 Talitrids are a highly diverse group of amphipod crustaceans that have colonized

29 various terrestrial habitats. Three genera have successfully adapted to cave habitats on

30 islands in the Pacific and Atlantic Oceans. However, the evolutionary origin of the

31   Pacific troglobitic talitrids has remained unknown. We estimate the phylogenetic

32   position of the troglobitic *Minamitalitrus zoltani*, which inhabits limestone caves on

33   Minamidaito Island in the Northwestern Pacific, on the basis of the traditional multi-

34   locus dataset. For the analyzed talitrids, we also reconstruct ancestral states of the

35   maxilliped palp and male gnathopod 2. Our results indicate that *Minamitalitrus zoltani*

36   is sister to the epigean *Nipponorchestia curvatus* with a deep divergence.

37   *Nipponorchestia curvatus* inhabits coastal habitats in Japan, but is not indigenous to

38   Minamidaito Island. A previous study estimated that the Atlantic troglobitic species had

39   invaded subterranean habitats multiple times, but we provide new insight into the

40   troglobisation history in talitrids. We also recover secondary shifts of character states of

41   the maxilliped palp and male gnathopod 2 within the lineage composed of

42   *Minamitalitrus* and its phylogenetically close genera. Our findings highlight the need

43   for the genus-level reclassification of these genera; we split *Nipponorchestia* into two

44   genera, establishing a new genus for *Nipponorchestia nudiramus*.

45

46   *Keywords:* Phylogeny, Ancestral state reconstruction, Insular cave, Taxonomy,

47   *Yamatorchestia* gen. nov.

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50   **1. Introduction**

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52   The crustacean Talitridae is the only amphipod taxon that has successfully

53   invaded land all over the world, excluding Antarctica (Bousfield, 1984). The talitrids'

54   wide distribution has been interpreted based on its evolutionary history. Several studies

55   suggested an old Pangean origin (Bousfield, 1984; Lowry and Myers, 2019); and Myers

56   and Lowry (2020) hypothesized it originated no later than the Jurassic. Conversely, a

57   molecular phylogenetic study dated the terrestrialization of talitrids to the Palaeogene

58   suggesting their young age (Copilaş-Ciocianu et al., 2020). The divergence time of this

59   terrestrial group among amphipods thus remains controversial. Talitrids have clearly

60   diversified by adapting to various types of terrestrial habitats (Bousfield, 1984), and

61   extant talitrids are presently classified into ca. 120 genera (see Myers and Lowry, 2020).

62   Based on a morphological cladistic analysis with careful evaluation of apomorphies

63   within these diverse genera, the family-level classification of talitrids was revisited, and

64   the family Talitridae was split into seven families (Myers and Lowry, 2020). However,

65   the molecular phylogenetic backbone for this classification has not yet been established.

66   While morphological features of the talitrids (e.g., pereopod dactyli and male

67   second gnathopod) have been treated as taxonomic and evolutionary key characters

68   (e.g., Bousfield, 1982; Lowry and Coleman, 2012), habitat preferences have also been

69   categorized to understand the evolution of their colonization of various terrestrial

70   habitats. As talitrids inhabit semiterrestrial environments—intertidal zones and marshes

71   to inland forests—, Bousfield (1982) categorized them into four types based on habitat

72   preferences: palustral talitrids, beach fleas, sand hoppers, and land hoppers. This

73   ecological grouping has been amended by subsequent studies, and the latest framework

74   divides talitrids into 10 types (Lowry and Myers, 2019): marsh-, beach-, driftwood-,

75   sand-, field-, ground-, riparian-, forest-, moss- and cave-hoppers. The cave-hopper,

76   specialized in subterranean habitats, represents a notable ecological group among the

77   talitrids.

78   Six talitrid genera were categorized as cave-hoppers by Lowry and Myers

79   (2019) and, among these cave-hoppers, four species belonging to four genera were

80   deemed to be truly troglobitic because they lack eyes. Except for the enigmatic Chinese

81   *Houlia bousfieldi*, whose exact habitats remain uncertain (Hou and Li, 2003), the other

82   three species are indigenous to insular caves (Fig. 1A; coastline data based on Wessel

83   and Smith, 1996); that is, *Palmorchestia hypogaea* inhabits lava tubes on La Palma in

84   the Canary Islands, East-Central Atlantic (Stock and Martin, 1988), *Spelaeorchestia*

85   *koloana* is known from a lava tube on Kauai in the Hawaiian Islands, Central Pacific

86   (Bousfield and Howarth, 1976), and *Minamitalitrus zoltani* is known from a limestone

87   cave on Minamidaito Island in the Daito Islands of Japan, Northwestern Pacific (White

88   et al., 2013). Villacorta et al. (2008) revealed that *P. hypogaea* and its epigean congener

89   *Palmorchestia epigaea*, which is also endemic to La Palma, did not form respective

90   monophyletic lineages, suggesting that their epigean ancestors had invaded subterranean

91   habitats multiple times on the island. However, the evolutionary origin of the Hawaiian

92   *S. koloana* and the Japanese *M. zoltani* remains uninvestigated.

93   Elucidating phylogenetic relationships between the epigean and troglobitic

94   talitrids is thus essential for understanding how the troglobisation and habitat transitions

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95 have occurred in these terrestrial amphipods. Here, we estimate the phylogenetic

96 position of the Japanese *M. zoltani* among the Far Eastern talitrid taxa based on the

97 traditional multi-locus dataset consisting of the nuclear histone H3 and 28S rRNA, and

98 mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA sequences. Because, to

99 our knowledge, molecular phylogenetic relationships among the talitrid genera endemic

100 to the Far Eastern islands have never been clarified, this study also establishes a

101 phylogenetic backbone for these terrestrial amphipods, and may shed light on the

102 evolution of their morphological and ecological transitions.

103

104 **2. Material and methods**

105

106 *2.1. Sampling and morphological examination*

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108   Terrestrial amphipods on Minamidaito Island in the Daito Islands, which belong

109 to the Ryukyu Islands, Japan (Fig. 1A), were mainly collected using a pooter. Although

110 *Minamitalitrus zoltani* individuals were not found in their type locality, Hoshinodo

111 Cave (cave entrance: 25.85765°N, 131.22432°E), specimens could be collected from

112 the other limestone cave on the island, Akiba Subterranean-lake Cave (approximate

113 cave entrance: 25.84°N, 131.25°E) on 30 October 2019. *Minamitalitrus zoltani*

114 individuals dwelt and fed on rotten wood in the cave (Fig. 1B, C). During the same

115 survey, epigean talitrids were also collected from Minamidaito Island (Supplementary

116 Table S1) and morphologically identified as *Morinoia japonica* (first record from the

117 island) and *Miyamotoia daitoensis* based on previous studies (Morino, 2015, 2020). The

118 specimens were fixed in 70% ethanol in the field. In the laboratory, muscle tissue or

119 pereopods were removed from the specimens for DNA extraction. The specimens were

120 dissected and examined morphologically using a Leica M125C stereoscopic microscope

121 and an Olympus BX53 light microscope.

122   Along with the specimens from Minamidaito Island, 62 talitrid samples were

123 collected from various locations in Japan and Taiwan (Supplementary Table 1) and also

124 preserved in either 70% or 99% ethanol. These specimens comprised samples collected

125 from the type localities of several species and were identified according to Morino

126 (2015) with the aid of specific taxonomic literature.

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2.2. PCR and DNA sequencing

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130   The phylogenetic position of *M. zoltani* among the Far Eastern talitrids was

131   estimated based on two nuclear—histone H3 (H3) and 28S ribosomal RNA (28S)—and

132   two mitochondrial—cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA

133   (16S)—markers. Genomic DNA was extracted from pereopods or soft tissues of the

134   talitrids using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany).

135   Methods used for the PCR and cycle sequencing reactions were as described by Suzuki

136   et al. (2017). In total, 266 sequences were newly obtained in the present study and

137   deposited in GenBank (Supplementary Table 1).

138

139   *2.3. Molecular phylogenetic analyses*

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141   A Vietnamese talitrid, *Solitroides motokawai*, was selected as an outgroup

142   according to Myers and Lowry (2020), and its DNA sequences were obtained from

143   GenBank. The alignments of H3 and COI were trivial, as no indels were observed. The

144   28S and 16S sequences were aligned using MAFFT v7.543 L-INS-i (Katoh and

145   Standley, 2013) The lengths of H3, 28S, COI and 16S were 328, 1330, 658 and 435 bp,

146   respectively. The concatenated sequences thus yielded 2751 bp of alignment positions.

147   Phylogenetic trees were reconstructed using maximum likelihood (ML) and

148   Bayesian inference (BI). The best-fit partition scheme and models were identified based

149   on the Bayesian information criterion using PartitionFinder v2.1.1 (Lanfear et al., 2017)

150   with the "all" algorithm: for H3 1st and 2nd positions, TRNEF + I (ML), or GTR + I

151   (BI); for H3 3rd position, K80 + G; for 28S, GTR + I + G; for COI 1st position, TRNEF

152   + I + G (ML), or GTR + I + G (BI); for COI 2nd position, K81UF + I + G (ML), or

153   GTR + I + G (BI); for COI 3rd position, GTR + G; and for 16S, HKY + I + G. The ML

154   phylogenetic tree was calculated using IQ-TREE v2.0.5 (Minh et al., 2020) with non-

155   parametric bootstrapping (BS) conducted with 1000 replicates. BI tree and Bayesian

156   posterior probabilities (PP) were estimated using MrBayes v3.2.7a (Ronquist et al.,

157   2012). Two independent runs for four Markov chains were conducted for 10 million

158   generations, and the tree was sampled every 100 generations. The parameter estimates

159 and convergence were checked using Tracer v1.7.1 (Rambaut et al., 2018), and the first

160 30001 trees were discarded based on the results.

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162 *2.4. Ancestral character state reconstruction*

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164 Morphological character states regarding maxilliped palp segment 2 (or article 2;

165 see Myers and Lowry, 2020) and gnathopod 2 in males were gathered from previous

166 studies (Chou and Lee, 1996; Lowry and Myers, 2019; Myers and Lowry, 2020). The

167 character states of the maxilliped palp segment 2 were coded as "lobate" or "non-

168 lobate", and those of the male gnathopod 2 as developed "mascupod" or undeveloped

169 "femipod" (Supplementary Table 1). These morphological character states were mapped

170 on the resulting tree generated from the MrBayes BI analysis. Ancestral states were

171 reconstructed under the likelihood model (Markov k-state 1 parameter model) using the

172 Trace Character History option implemented in Mesquite v3.61 (Maddison and

173 Maddison, 2019).

174

175 **3. Results and discussion**

176

177 The BI tree (mean ln $L = -25855.475$; Fig. 1) had an identical topology to the

178 ML tree (ln $L = -25815.938$; not shown). Partial results of the ancestral state

179 reconstruction (ASR) are summarized in Table 1, and all results are illustrated in

180 Supplementary Fig. 1.

181 The obtained phylogenies showed that the Far Eastern talitrids analyzed herein

182 were split into three distinct clades (denoted hereinafter as Clades A–C). Clades A and

183 B formed a monophyletic lineage, but this relationship was unsupported in both

184 analyses (BS = 57%, PP = 0.55). While the subterranean *Minamitalitrus zoltani*

185 (samples: MZ01 and MZ02) was a member of Clade A, the two epigean species on

186 Minamidaito Island—*Morinoia japonica* (MJ12 and MJ13) and *Miyamotoia daitoensis*

187 (MD01)—belonged to Clade B (Fig. 1). The results thus clarified that the troglobitic

188 talitrid on Minamidaito Island does not share its common ancestor with the epigean

189 talitrids concurrently inhabiting the island, in contrast to the evolutionary relationships

190 found between the troglobitic and epigean *Palmorchestia* talitrids on La Palma

191   (Villacorta et al., 2008).

192   Three epigean species of *Nipponorchestia* and *Mizuhorchestia* belonged to

193   Clade A along with *M. zoltani*. The monophyly of these three genera was fully

194   supported in both analyses (BS = 99%, PP = 1.0). The phylogenies showed that *M.*

195   *zoltani* formed a monophyletic lineage with *Nipponorchestia curvatus* (BS = 100%, PP

196   = 1.0); *Nipponorchestia nudiramus* and *Mizuhorchestia urospina* formed another fully

197   supported clade (BS = 100%, PP = 1.0). *Nipponorchestia nudiramus* and *Miz. urospina*

198   are indigenous to forest habitats (= forest-hoppers; Lowry and Myers, 2019) in lowland

199   to montane areas on the Japanese main islands and adjacent islets (Morino, 2014;

200   Morino and Miyamoto, 2015a). *Nipponorchestia curvatus*, which belongs to the field-

201   hopper category as defined by Lowry and Myers (2019), inhabits coastal vegetation on

202   the Japanese main islands as well as forest habitats on small islets in the Ryukyu, Izu

203   and Ogasawara Islands, and Tsushima Island (Morino and Miyamoto, 2015a). The

204   approximate distributions of the *Nipponorchestia* and *Mizuhorchestia* species are

205   provided in Supplementary Fig. 2.

206   The monophyly of *M. zoltani* and *N. curvatus* may highlight another

207   troglobisation history that arose in talitrids. It has been suggested that *N. curvatus* is

208   capable of transoceanic dispersal since this species occurs on islands in the Ryukyu and

209   Ogasawara Islands, which are distant from Japan's main islands, and because its

210   distribution on the Japanese main islands is limited to coastal habitats (Morino and

211   Miyamoto, 2015a). Aside their deep phylogenetic divergence with *N. nudiramus* and

212   *Miz. urospina*, the present analyses also reveal the low genetic diversity between the

213   two specimens of *N. curvatus* collected from the Izu Peninsula (Honshu, Japan) and

214   Tanegashima Island (the Ryukyu Islands; approximately 850 km from the Izu

215   Peninsula). It is thus possible that *N. curvatus* can reach distant locations by

216   transoceanic dispersal like other several coastal talitrids that are known to raft using

217   driftwood (see Wildish, 2017). Furthermore, we observed that *M. zoltani* is tightly

218   associated with wood in the cave habitat (Fig. 1B). Accordingly, the results suggest the

219   most recent common ancestor (MRCA) of *M. zoltani* and *N. curvatus* was a *curvatus*-

220   like epigean species inhabiting coastal regions; the ancestor of *M. zoltani* had rafted to

221   Minamidaito Island, and then invaded and adapted to cave habitats on the island.

222   It is evident that *M. zoltani* has secondarily lost its eyes as a result of

223   troglobisation. In addition to its eyeless state, this cave-hopper possesses a gnathopod 2

224   that is not sexually dimorphic—that is, its males possess an undeveloped female-like (=

225   femipod) gnathopod 2. The ASR result unquestionably clarified that the sexual

226   dimorphism of gnathopod 2 has also been lost in *M. zoltani*. Because males of the other

227   troglobitic species, *Palmorchestia hypogaea* and *Spelaeorchestia koloana*, also have a

228   femipod gnathopod 2 (Stock and Martin, 1988; Bousfield and Howarth, 1976), the loss

229   of sexual dimorphism in gnathopod 2 may be correlated with the adaptation to cave

230   habitats in talitrids. The mating behavior of talitrids, of which males bear the developed

231   (= mascupod) gnathopod 2, is different from that of species with the femipod male

232   gnathopod 2 (see Lowry and Coleman, 2012). As the first step toward a better

233   understanding of the evolutionary history of this morphological trait, therefore, the

234   mating behaviors of cave-hoppers should be investigated. Future studies should also

235   aim to estimate the divergence time of this cave-hopper, which will lead us to a better

236   understanding of its troglobisation history.

237   The phylogenetic and ASR results clarified that the non-lobate segment 2 of

238   maxilliped palp has evolved independently in talitrids. The family Brevitalitridae was

239   recently erected by Myers and Lowry (2020) based on a morphological cladistic

240   analysis. The non-lobate maxilliped palp segment 2 was treated as one of the key

241   characters of this family-group taxon. Therefore, *Mizuhorchestia* and *Talitroides* were

242   classified within the Brevitalitridae along with other seven genera including *Bousfieldia*

243   (Myers and Lowry, 2020), although the type species of *Bousfieldia*, *B. phoenixae*,

244   unquestionably possesses a lobate maxilliped palp segment 2 (Chou and Lee, 1996).

245   Our findings support the hypothesis that *Mizuhorchestia* is phylogenetically close to

246   *Nipponorchestia* (Morino and Miyamoto, 2015a), and suggest that the MRCA of *Miz.*

247   *urospina* and *N. nudiramus* possessed the lobate maxilliped palp segment 2. The present

248   results exemplify how morphological features, which have been treated as key

249   taxonomic characters in talitrids, have frequently evolved independently in this

250   terrestrial amphipod group. Also, the non-lobate maxilliped palp segment 2 in *Miz.*

251   *urospina* is distinct from those of some other genera in the Brevitalitridae (e.g.,

252   *Brevitalitrus* and *Talitroides*) in that in the former it is broad, while in the latter it is

253   slender (see Miyamoto and Morino, 2012; Morino, 2013, 2014).

254   Our results also reveal the noteworthy phylogenetic relationships of the other

8

255   Far Eastern talitrids. The monophyly of the *Platorchestia* complex—*Platorchestia*,

256   *Miyamotoia* and *Morinoia*—, *Sinorchestia* and *Bousfieldia* was fully supported (= Clade

257   B), and the sister relationship between the sand-hopper *Sinorchestia* and the forest-

258   hopper *Bousfieldia* was also supported (BS = 90%, PP = 0.99). However, our

259   phylogenies fail to support the monophyly of *Morinoia*, which was established by

260   Lowry and Myers (2019) for three Far Eastern species previously classified as

261   *Platorchestia*. Our findings highlight the need to assess the phylogenetic relationships

262   among the genera split out *Platorchestia* in recent studies (e.g., Lowry and Myers,

263   2019; Morino, 2020) based on broad taxonomic sampling. Clade C was composed of a

264   cosmopolitan *Talitroides topitotum* and three genera—*Lowryella*, *Kokuborchestia* and

265   *Pyatakovestia*—(BS = 90%, PP = 0.99). *Lowryella wadai* is the only known species of

266   the genus indigenous to restricted marsh habitats of the southern part of the Japanese

267   main islands (Morino and Miyamoto, 2016). The morphological similarities between

268   *Lowryella* and *Nipponorchestia* as well as *Mizuhorchestia* were suggested by Morino

269   and Miyamoto (2016), but nonetheless, this marsh-hopper talitrid shares the MRCA

270   with *Kokuborchestia* and *Pyatakovestia*. While *Kokuborchestia* is indigenous to the

271   northern part of Japan (Morino and Miyamoto, 2015c), *Pyatakovestia* talitrids are

272   widely distributed in seashore habitats of the Japanese main islands, Primorsky (Russia)

273   and the Korean Peninsula, and also occur at almost all adjacent islets (Morino and

274   Miyamoto, 2015b). The evolutionary and biogeographic histories of *Lowryella*,

275   *Kokuborchestia* and *Pyatakovestia* will be subject to future studies.

276   Our findings depict the backbone phylogeny of the Far Eastern talitrid genera

277   and also elucidate the phylogenetic position of the cave-hopper *M. zoltani* among these

278   talitrids; however, our phylogenetic dataset could not cover all the genera and species

279   indigenous to this region. Our results also reveal the deep divergence between the

280   troglobitic *M. zoltani* and its sister species *N. curvatus*. Future study should assess the

281   phylogenetic position of the Hawaiian *Spelaeorchestia* to determine whether it may

282   have evolved from epigean talitrids inhabiting the island of Kauai, like *Palmorchestia*,

283   or from a lineage deeply diverge from its sister species, like *Minamitalitrus*. Our study

284   shows that the four species of *Minamitalitrus*, *Nipponorchestia* and *Mizuhorchestia*,

285   respectively, formed a distinctive lineage within their monophyletic group (= Clade A).

286   These results affirm the need for a genus-level taxonomic revision of these genera.

287 Because *Nipponorchestia* was revealed to be non-monophyletic, a new genus is herein

288 established for *N. nudiramus* by the first and second authors; therefore, the diagnosis of

289 *Nipponorchestia* is amended from the preceding one (Morino and Miyamoto, 2015a).

290 Additionally, *Mizuhorchestia* is affiliated with the Talitridae. The phylogenetic analyses

291 also reveal each of *N. nudiramus* and *Miz. urospina* is composed of the divergent

292 lineages suggesting that they contain several cryptic species. Their species-level

293 taxonomy and evolutionary history should be investigated by future studies.

294

295 **4. Taxonomy**

296

297 **Family Talitridae Rafinesque**

298   **Subfamily Talitrinae Rafinesque sensu Myers and Lowry (2020)**

299   **Genus *Minamitalitrus* White, Lowry, and Morino, 2013**

300   **Diagnosis.** See White et al. (2013).

301   **Type species.** *Minamitalitrus zoltani* White, Lowry, and Morino, 2013, fixed by

302 original designation (White et al., 2013). Genus monospecific.

303   **Genus *Nipponorchestia* Morino and Miyamoto, 2015a**

304   **Amended diagnosis.** Body size medium, eyes small to medium. Antenna 1

305 reaching end of peduncular segment 4 of antenna 2; peduncle longer than flagellum,

306 peduncular segments 1–3 subequal in length; flagellum with 3 articles, article 1 slightly

307 curved, article 2 longest. Antenna 2 in male not incrassate, flagellum longer than

308 peduncle. Mandible left lacina 4-dentate. Maxilliped palp segments 2 (= carpus) and 3

309 (= propodus) broad, mediodistally lobate, segment 4 (= dactylus) distinct, reduced,

310 inserted apically on segment 3.

311   Gnathopod 1 strongly subchelate in both sexes; in male, merus, carpus and

312 propodus with pellucid lobes, propodus lateral surface with a row of elongate setae; in

313 female, carpus and propodus lacking pellucid lobe. Gnathopod 2 in male, propodus

314 powerfully subchelate, dactylus attenuated apically; in female, basis slender, propodus

315 mitten-shaped. Pereopods 3–7, cuspidactylate (bicuspidate), propodus locking robust

316 setae developed. Coxa of pereopod 4 wider than deep. Coxa of pereopod 6, posterior

317 lobe smoothly curved. Pereopods 6 and 7 in male not incrassate. Coxal gills of

318 pereopods 2 and 6 large, lobate at midway. Coxal gill of pereopod 6 without leaf-shaped

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319 lobe. Oostegites with simple-tipped setae along middle to distal margin.

320 Epimeral plates lacking marginal pits. Pleopods, peduncle marginally bare or

321 weakly robust-setose; rami moderately reduced. Uropod 1, distolateral robust seta on

322 peduncle longer than subdistal one; inner ramus with dorso-marginal robust setae; outer

323 ramus marginally bare. Uropod 2, rami subequal in length, marginally robust-setose.

324 Uropod 3, peduncle truncate or narrowing distally; ramus short. Telson lobes each with

325 lateral and apical robust setae.

326 **Type species.** *Nipponorchestia curvatus* Morino and Miyamoto, 2015a, fixed by

327 original designation (Morino and Miyamoto, 2015a). Genus monospecific.

328 **Genus *Yamatorchestia* Takahashi and Morino, gen. nov.**

329 **Diagnosis.** Body size medium, eyes medium. Antenna 1 reaching end of

330 peduncular segment 4 of antenna 2; peduncle longer than flagellum; flagellum with 4

331 articles, geniculated between articles 2 and 3, article 3 longest. Antenna 2 in male not

332 incrassate, flagellum longer than peduncle. Mandible left lacina 4-dentate. Maxilliped

333 palp segments 2 (= carpus) and 3 (= propodus) broad, mediodistally lobate, segment 4

334 (= dactylus) distinct, reduced, inserted apically on segment 3.

335 Gnathopod 1 strongly subchelate in both sexes; in male, merus lacking pellucid

336 lobes, carpus and propodus with pellucid lobes, propodus lateral surface with a row of

337 elongate setae; in female, carpus with small pellucid lobe. Gnathopod 2 in male,

338 propodus powerfully subchelate, dactylus attenuated apically; in female, basis slender,

339 propodus mitten-shaped. Pereopods 3–7, cuspidactylate (bicuspidate), propodus locking

340 robust setae well-developed. Coxa of pereopod 4 wider than deep. Coxa of pereopod 6,

341 posterior lobe smoothly curved. Pereopods 6 and 7 in male not incrassate. Coxal gills of

342 pereopods 2 and 6 large (especially in pereopod 6), lobate at midway, attenuated

343 distally. Coxal gill of pereopod 6 with leaf-shaped lobe. Oostegites with simple-tipped

344 setae distally.

345 Epimeral plates lacking marginal pits. Pleopods, peduncle marginally bare or

346 weakly robust-setose; rami moderately reduced. Uropod 1, distolateral robust seta on

347 peduncle longer than subdistal one; inner ramus with dorso-marginal robust setae; outer

348 ramus marginally bare. Uropod 2, rami subequal in length, marginally robust-setose.

349 Uropod 3, peduncle truncate or narrowing distally; ramus short. Telson lobes each with

350 lateral and apical robust setae.

351 **Type species.** *Nipponorchestia nudiramus* Morino and Miyamoto, 2015a, fixed

352 by the present designation.

353 **Etymology.** A compound noun of feminine gender derived from "Yamato", an

354 old name for Japan, especially Nara Prefecture, which is a part of the distribution of the

355 type species, and the generic name "*Orchestia*".

356 **Remarks.** *Yamatorchestia* is discriminated from three phylogenetically close

357 three genera—*Mizuhorchestia*, *Nipponorchestia* and *Minamitalitrus*—by the following

358 combination of characters (see Morino, 2015): 1) coxal gill on pereopod 6 large, with a

359 leaf-shaped lobe, 2) flagellum of antenna 1 4-articulate, and 3) outer ramus of uropod 1

360 marginally bare. This new genus can be clearly distinguished from *Morinoia*, another

361 talitrid genus inhabiting similar habitats around the distribution of *Yamatorchestia*, by

362 the display of a developed robust distolateral seta on uropod 1 peduncle (vs. small

363 simple seta in *Morinoia*; Lowry and Myers, 2019).

364 Although *Yamatorchestia* currently consists only of its type species *Y.*

365 *nudiramus*, the genus may be composed of at least two genetically divergent species.

366 **Genus *Mizuhorchestia* Morino, 2014**

367 **Diagnosis.** See Morino (2014).

368 **Type species.** *Mizuhorchestia urospina* Morino, 2014, fixed by original

369 designation (Morino, 2014).

370 **Remarks.** The genus *Mizuhorchestia* should be placed within the Talitrinae in

371 the family Talitridae sensu Myers and Lowry (2020). The genus is currently

372 monospecific, but may contain several undescribed species.

373

374 This work has been registered in ZooBank with the registration number

375 urn:lsid:zoobank.org:pub:1DB3E8F3-D204-4814-8383-5E9D64590E99.

376

377 **CRediT authorship contribution statement**

378

379 **Tomoyuki Takahashi:** Conceptualization, Formal analysis, Investigation,

380 Resources, Data curation, Writing - original draft. **Hiroshi Morino:** Conceptualization,

381 Writing - review & editing, Supervision. **Ko Tomikawa:** Investigation, Resources, Data

382 curation, Writing - review & editing. **Yi-Te Lai:** Investigation, Resources, Writing -

383 review & editing. **Takafumi Nakano:** Conceptualization, Methodology, Formal

384 analysis, Investigation, Resources, Writing - original draft, Writing - review & editing,

385 Supervision, Project administration, Funding acquisition.

386

404

405 **Appendix A. Supplementary material**

406

407   Supplementary data to this article can be found online at

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508

509   **Fig. 1.** (A) Map showing the distribution of the troglobitic talitrids, *Minamitalitrus*

510   *zoltani*, *Palmorchestia hypogaea* and *Spelaeorchestia koloana*. The type locality of

511   *Houlia bousfieldi* remains unclarified. (B) Habitat of *Minamitalitrus zoltani* in the

512   Akiba Subterranean-lake Cave on Minamidaito Island. Arrowheads indicate talitrids on

513   rotten wood. (C) Lateral view of a live individual of *Minamitalitrus zoltani*.

514

515   **Fig. 2.** Molecular phylogenetic position of the troglobitic *Minamitalitrus zoltani*, based

516   on Bayesian inference for 2751 bp alignment positions of nuclear histone H3, 28S

517   rRNA, and mitochondrial COI and 16S rRNA markers. Samples collected from

518   Minamidaito Island are shown in bold. Numbers on nodes indicate bootstrap (BS)

519   values for maximum likelihood $\geq$ 50% and Bayesian posterior probabilities (BPP) $\geq$

520   0.90. An asterisk denotes the node with BS $\geq$ 70% and BPP $\geq$ 0.95. Roman numerals (i–

521   iii) indicate the nodes with the ancestral state reconstruction result summarized in Table

522   1.

523

524   **Supplementary Fig. 1.** Bayesian inference tree of *Minamitalitrus zoltani* and the other
525   Far Eastern talitrid genera showing ancestral states of the maxilliped palp segment 2 (=
526   carpus) and male gnathopod 2. Ancestral states were reconstructed by the one-parameter
527   Mk1 model implemented in Mesquite. Results of the ancestral state reconstruction at the
528   nodes within Clade A are provided in Table 1.
529
530   **Supplementary Fig. 2.** Approximate ranges of *Minamitalitrus zoltani*, *Nipponorchestia*
531   *curvatus*, *Nipponorchestia nudiramus*, and *Mizuhorchestia urospina*. Distribution data
532   are based on Morino (2014), Morino and Miyamoto (2015a) and the present study.
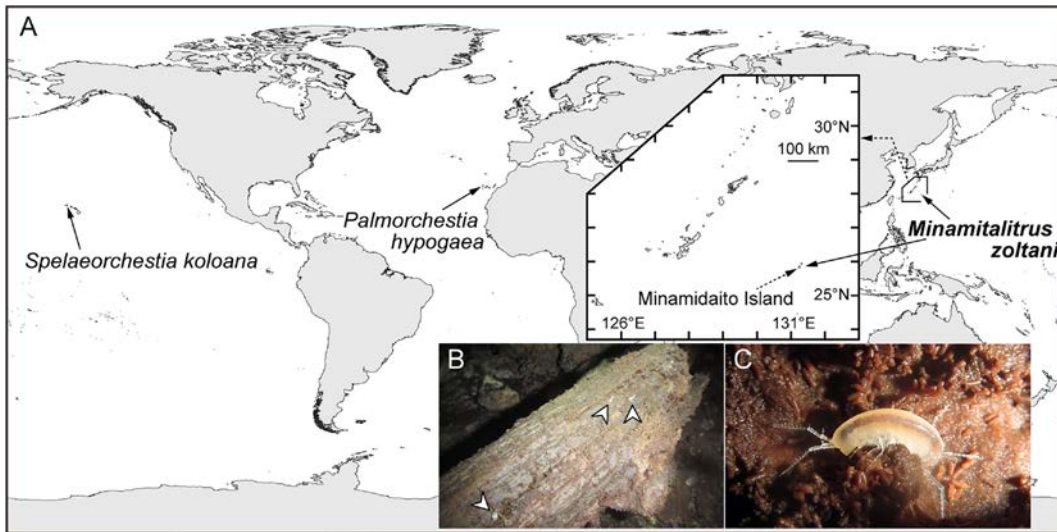533   Coastline data are based on Wessel and Smith (1996).
534

Table 1

Ancestral character estimation of morphological traits of nodes i–iii shown in Fig. 2 and four talitrid species. Probabilities are estimated using a maximum likelihood approach based on the one-parameter Mk1 model for the character states of maxilliped palp segment and male gnathopod 2.

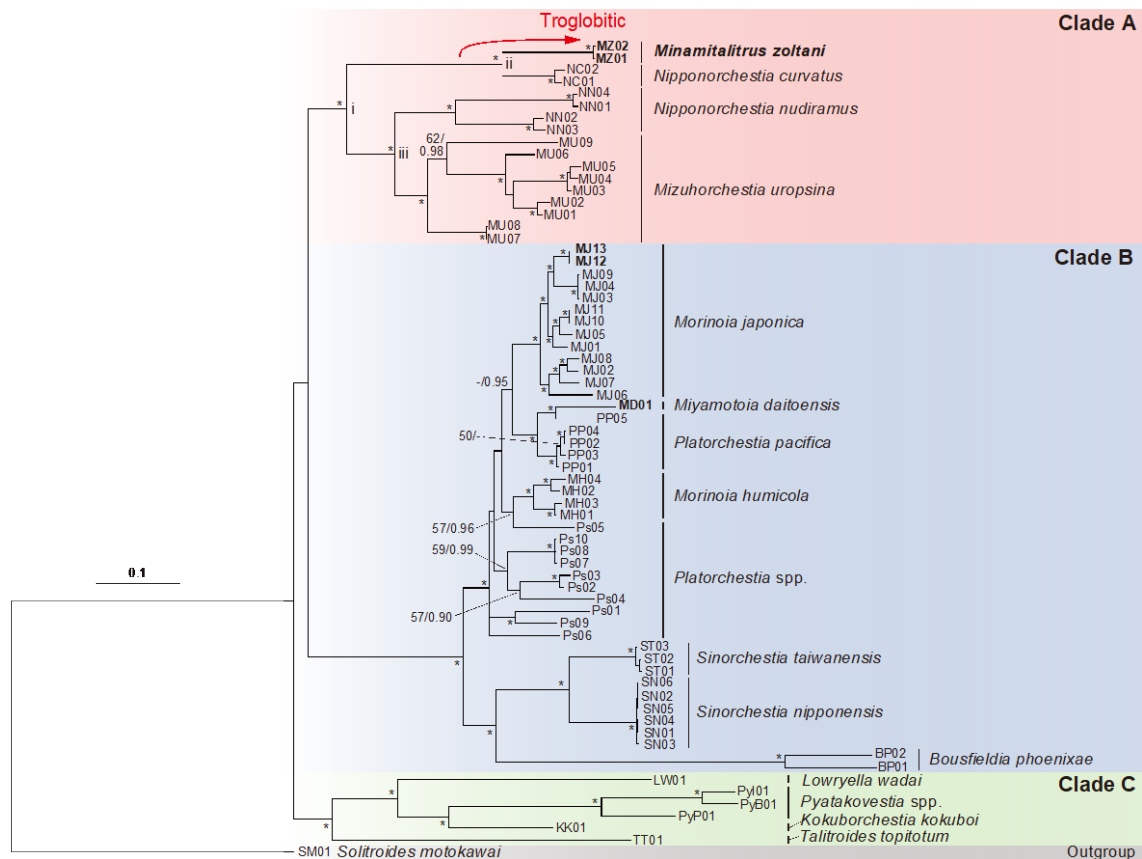| Nodes/Species	Maxilliped palp segment 2		Male gnathopod 2	
	lobate	non-lobate	mascupod	femipod
Node i	0.9774	0.0226	0.9994	0.0006
Node ii	0.9993	0.0007	0.9562	0.0438
Node iii	0.9156	0.0844	1.0000	0.0000
Minamitalitrus zoltani	1.0000	0.0000	0.0000	1.0000
Nipponorchestia curvatus	1.0000	0.0000	1.0000	0.0000
Nipponorchestia nudiramus	0.9916	0.0084	0.9999	0.0001
Mizuhorchestia urospina	0.0273	0.9727	1.0000	0.0000

542
543 **Fig. 1.** (A) Map showing the distribution of the troglobitic talitrids, *Minamitalitrus*
544 *zoltani*, *Palmorchestia hypogaea* and *Spelaeorchestia koloana*. The type locality of
545 *Houlia bousfieldi* remains unclarified. (B) Habitat of *Minamitalitrus zoltani* in the
546 Akiba Subterranean-lake Cave on Minamidaito Island. Arrowheads indicate talitrids on
547 rotten wood. (C) Lateral view of a live individual of *Minamitalitrus zoltani*.
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Fig. 2. Molecular phylogenetic position of the troglobitic *Minamitalitrus zoltani*, based
on Bayesian inference for 2751 bp alignment positions of nuclear histone H3, 28S
rRNA, and mitochondrial COI and 16S rRNA markers. Samples collected from
Minamidaito Island are shown in bold. Numbers on nodes indicate bootstrap (BS)
values for maximum likelihood ≥ 50% and Bayesian posterior probabilities (BPP) ≥
0.90. An asterisk denotes the node with BS ≥ 70% and BPP ≥ 0.95. Roman numerals (i–
iii) indicate the nodes with the ancestral state reconstruction result summarized in Table
1.