



TITLE:

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AUTHOR(S):

Takahashi, Tomoyuki; Morino, Hiroshi; Tomikawa,
Ko; Lai, Yi-Te; Nakano, Takafumi

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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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6 Tomoyuki Takahashi^a, Hiroshi Morino^b, Ko Tomikawa^c, Yi-Te Lai^d, Takafumi
7 Nakano^{a,*}

8

9 Hiroshi Morino: <https://orcid.org/0000-0003-4672-121X>

10 Ko Tomikawa: <https://orcid.org/0000-0003-1521-9016>

11 Yi-Te Lai: <https://orcid.org/0000-0003-4906-1202>

12 Takafumi Nakano: <https://orcid.org/0000-0001-6107-2188>

13

14 ^a *Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-*
15 *8502, Japan*

16 ^b *Department of Zoology, National Museum of Nature and Science, Tsukuba, Ibaraki*
17 *305-0005, Japan*

18 ^c *Graduate School of Humanities and Social Sciences, Hiroshima University,*
19 *Higashihiroshima, Hiroshima 739-8524, Japan*

20 ^d *Department of Life Science, National Taiwan University, Taipei 10617, Taiwan*

21

22 * Corresponding author at: Department of Zoology, Graduate School of Science, Kyoto
23 University, Kyoto 606-8502, Japan.

24 *E-mail address:* nakano@zoo.zool.kyoto-u.ac.jp (T. Nakano)

25

26 ABSTRACT

27

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.

50 **1. Introduction**

51
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

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*

107

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, epigeal 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.

127

128 2.2. *PCR and DNA sequencing*

129

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*

140

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.

161

162 2.4. Ancestral character state reconstruction

163

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 epigeal 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 epigeal
 189 talitrids concurrently inhabiting the island, in contrast to the evolutionary relationships
 190 found between the troglobitic and epigeal *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 (= masculopod) 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

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 *Pyatakoveestia*—(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 *Pyatakoveestia*. While *Kokuborchestia* is indigenous to the
271 northern part of Japan (Morino and Miyamoto, 2015c), *Pyatakoveestia* 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 *Pyatakoveestia* 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 epigeal 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

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

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388

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390

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404

405 **Appendix A. Supplementary material**

406

407 Supplementary data to this article can be found online at
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409

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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 ≥ 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

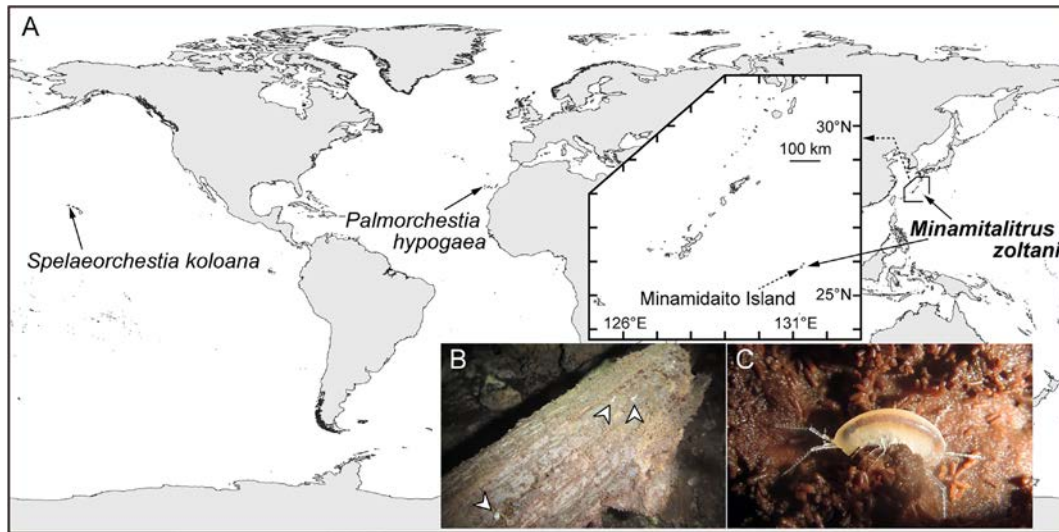
535 **Table 1**

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

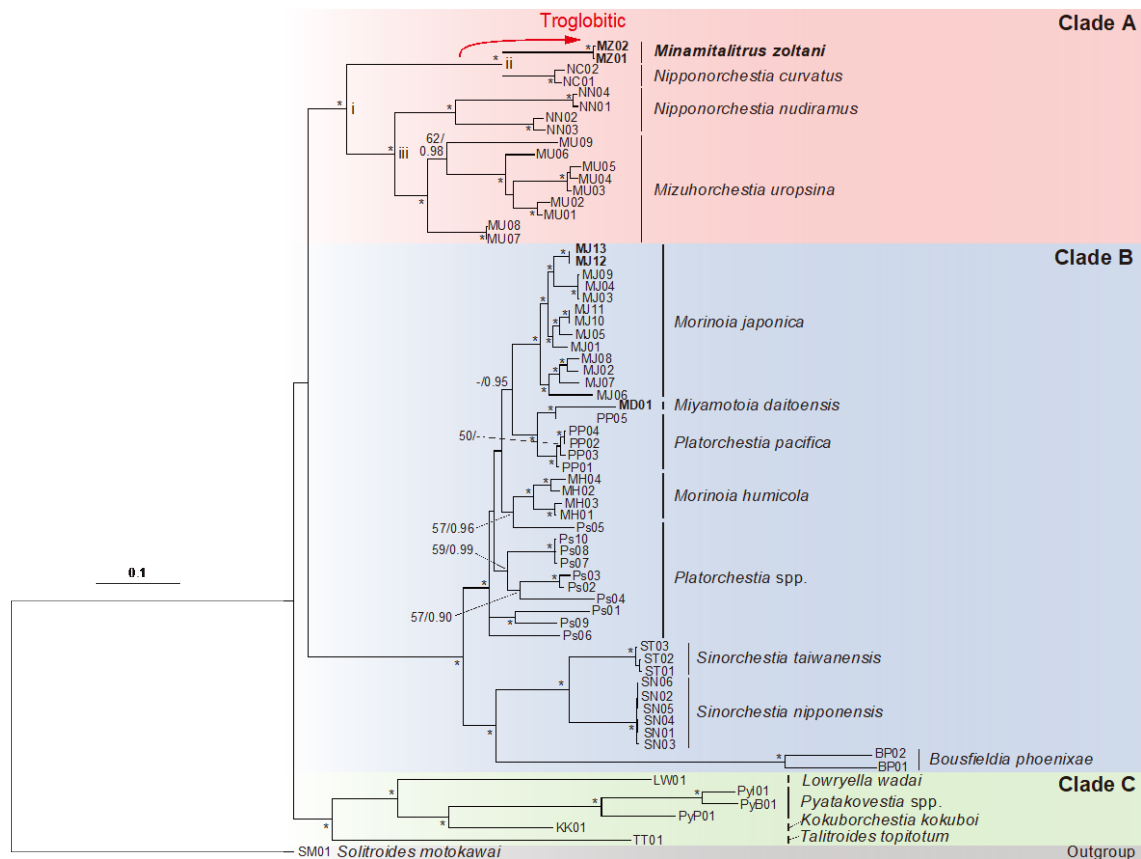
Nodes/Species	Maxilliped palp segment 2		Male gnathopod 2	
	lobate	non-lobate	mascupod	femipod
Node i	0.9774	0.0226	0.9994	0.0006
Node ii	0.9993	0.0007	0.9562	0.0438
Node iii	0.9156	0.0844	1.0000	0.0000
<i>Minamitalitrus zoltani</i>	1.0000	0.0000	0.0000	1.0000
<i>Nipponorchestia curvatus</i>	1.0000	0.0000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

540

541



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*.
548



549
550 **Fig. 2.** Molecular phylogenetic position of the troglitic *Minamitalitrus zoltani*, based
551 on Bayesian inference for 2751 bp alignment positions of nuclear histone H3, 28S
552 rRNA, and mitochondrial COI and 16S rRNA markers. Samples collected from
553 Minamidaito Island are shown in bold. Numbers on nodes indicate bootstrap (BS)
554 values for maximum likelihood $\geq 50\%$ and Bayesian posterior probabilities (BPP) \geq
555 0.90. An asterisk denotes the node with BS $\geq 70\%$ and BPP ≥ 0.95 . Roman numerals (i-
556 iii) indicate the nodes with the ancestral state reconstruction result summarized in Table
557 1.