

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

Pacific troglobitic talitrids has remained unknown. We estimate the phylogenetic position of the troglobitic *Minamitalitrus zoltani*, which inhabits limestone caves on Minamidaito Island in the Northwestern Pacific, on the basis of the traditional multi-locus dataset. For the analyzed talitrids, we also reconstruct ancestral states of the maxilliped palp and male gnathopod 2. Our results indicate that *Minamitalitrus zoltani* is sister to the epigean *Nipponorchestia curvatus* with a deep divergence. *Nipponorchestia curvatus* inhabits coastal habitats in Japan, but is not indigenous to Minamidaito Island. A previous study estimated that the Atlantic troglobitic species had invaded subterranean habitats multiple times, but we provide new insight into the troglobisation history in talitrids. We also recover secondary shifts of character states of the maxilliped palp and male gnathopod 2 within the lineage composed of *Minamitalitrus* and its phylogenetically close genera. Our findings highlight the need for the genus-level reclassification of these genera; we split *Nipponorchestia* into two genera, establishing a new genus for *Nipponorchestia nudiramus*.

Keywords: Phylogeny, Ancestral state reconstruction, Insular cave, Taxonomy, *Yamatorchestia* gen. nov.

1. Introduction

The crustacean Talitridae is the only amphipod taxon that has successfully invaded land all over the world, excluding Antarctica (Bousfield, 1984). The talitrids' wide distribution has been interpreted based on its evolutionary history. Several studies suggested an old Pangean origin (Bousfield, 1984; Lowry and Myers, 2019); and Myers and Lowry (2020) hypothesized it originated no later than the Jurassic. Conversely, a molecular phylogenetic study dated the terrestrialization of talitrids to the Palaeogene suggesting their young age (Copilaş-Ciocianu et al., 2020). The divergence time of this terrestrial group among amphipods thus remains controversial. Talitrids have clearly diversified by adapting to various types of terrestrial habitats (Bousfield, 1984), and extant talitrids are presently classified into ca. 120 genera (see Myers and Lowry, 2020). Based on a morphological cladistic analysis with careful evaluation of apomorphies

within these diverse genera, the family-level classification of talitrids was revisited, and the family Talitridae was split into seven families (Myers and Lowry, 2020). However, the molecular phylogenetic backbone for this classification has not yet been established.

While morphological features of the talitrids (e.g., pereopod dactyli and male second gnathopod) have been treated as taxonomic and evolutionary key characters (e.g., Bousfield, 1982; Lowry and Coleman, 2012), habitat preferences have also been categorized to understand the evolution of their colonization of various terrestrial habitats. As talitrids inhabit semiterrestrial environments—intertidal zones and marshes to inland forests—, Bousfield (1982) categorized them into four types based on habitat preferences: palustral talitrids, beach fleas, sand hoppers, and land hoppers. This ecological grouping has been amended by subsequent studies, and the latest framework divides talitrids into 10 types (Lowry and Myers, 2019): marsh-, beach-, driftwood-, sand-, field-, ground-, riparian-, forest-, moss- and cave-hoppers. The cave-hopper, specialized in subterranean habitats, represents a notable ecological group among the talitrids.

Six talitrid genera were categorized as cave-hoppers by Lowry and Myers (2019) and, among these cave-hoppers, four species belonging to four genera were deemed to be truly troglobitic because they lack eyes. Except for the enigmatic Chinese *Houlia bousfieldi*, whose exact habitats remain uncertain (Hou and Li, 2003), the other three species are indigenous to insular caves (Fig. 1A; coastline data based on Wessel and Smith, 1996); that is, *Palmorchestia hypogaea* inhabits lava tubes on La Palma in the Canary Islands, East-Central Atlantic (Stock and Martin, 1988), *Spelaeorchestia koloana* is known from a lava tube on Kauai in the Hawaiian Islands, Central Pacific (Bousfield and Howarth, 1976), and *Minamitalitrus zoltani* is known from a limestone cave on Minamidaito Island in the Daito Islands of Japan, Northwestern Pacific (White et al., 2013). Villacorta et al. (2008) revealed that *P. hypogaea* and its epigean congener *Palmorchestia epigaea*, which is also endemic to La Palma, did not form respective monophyletic lineages, suggesting that their epigean ancestors had invaded subterranean habitats multiple times on the island. However, the evolutionary origin of the Hawaiian *S. koloana* and the Japanese *M. zoltani* remains uninvestigated.

Elucidating phylogenetic relationships between the epigean and troglobitic talitrids is thus essential for understanding how the troglobisation and habitat transitions

have occurred in these terrestrial amphipods. Here, we estimate the phylogenetic position of the Japanese *M. zoltani* among the Far Eastern talitrid taxa based on the traditional multi-locus dataset consisting of the nuclear histone H3 and 28S rRNA, and mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA sequences. Because, to our knowledge, molecular phylogenetic relationships among the talitrid genera endemic to the Far Eastern islands have never been clarified, this study also establishes a phylogenetic backbone for these terrestrial amphipods, and may shed light on the evolution of their morphological and ecological transitions.

2. Material and methods

2.1. Sampling and morphological examination

Terrestrial amphipods on Minamidaito Island in the Daito Islands, which belong to the Ryukyu Islands, Japan (Fig. 1A), were mainly collected using a pooter. Although *Minamitalitrus zoltani* individuals were not found in their type locality, Hoshinodo Cave (cave entrance: 25.85765°N, 131.22432°E), specimens could be collected from the other limestone cave on the island, Akiba Subterranean-lake Cave (approximate cave entrance: 25.84°N, 131.25°E) on 30 October 2019. *Minamitalitrus zoltani* individuals dwelt and fed on rotten wood in the cave (Fig. 1B, C). During the same survey, epigean talitrids were also collected from Minamidaito Island (Supplementary Table S1) and morphologically identified as *Morinoia japonica* (first record from the island) and *Miyamotoia daitoensis* based on previous studies (Morino, 2015, 2020). The specimens were fixed in 70% ethanol in the field. In the laboratory, muscle tissue or pereopods were removed from the specimens for DNA extraction. The specimens were dissected and examined morphologically using a Leica M125C stereoscopic microscope and an Olympus BX53 light microscope.

Along with the specimens from Minamidaito Island, 62 talitrid samples were collected from various locations in Japan and Taiwan (Supplementary Table 1) and also preserved in either 70% or 99% ethanol. These specimens comprised samples collected from the type localities of several species and were identified according to Morino (2015) with the aid of specific taxonomic literature.

2.2. PCR and DNA sequencing

The phylogenetic position of *M. zoltani* among the Far Eastern talitrids was estimated based on two nuclear—histone H3 (H3) and 28S ribosomal RNA (28S)—and two mitochondrial—cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA (16S)—markers. Genomic DNA was extracted from pereopods or soft tissues of the talitrids using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany). Methods used for the PCR and cycle sequencing reactions were as described by Suzuki et al. (2017). In total, 266 sequences were newly obtained in the present study and deposited in GenBank (Supplementary Table 1).

2.3. Molecular phylogenetic analyses

A Vietnamese talitrid, *Solitroides motokawai*, was selected as an outgroup according to Myers and Lowry (2020), and its DNA sequences were obtained from GenBank. The alignments of H3 and COI were trivial, as no indels were observed. The 28S and 16S sequences were aligned using MAFFT v7.543 L-INS-i (Katoh and Standley, 2013). The lengths of H3, 28S, COI and 16S were 328, 1330, 658 and 435 bp, respectively. The concatenated sequences thus yielded 2751 bp of alignment positions.

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). The best-fit partition scheme and models were identified based on the Bayesian information criterion using PartitionFinder v2.1.1 (Lanfear et al., 2017) with the “all” algorithm: for H3 1st and 2nd positions, TRNEF + I (ML), or GTR + I (BI); for H3 3rd position, K80 + G; for 28S, GTR + I + G; for COI 1st position, TRNEF + I + G (ML), or GTR + I + G (BI); for COI 2nd position, K81UF + I + G (ML), or GTR + I + G (BI); for COI 3rd position, GTR + G; and for 16S, HKY + I + G. The ML phylogenetic tree was calculated using IQ-TREE v2.0.5 (Minh et al., 2020) with non-parametric bootstrapping (BS) conducted with 1000 replicates. BI tree and Bayesian posterior probabilities (PP) were estimated using MrBayes v3.2.7a (Ronquist et al., 2012). Two independent runs for four Markov chains were conducted for 10 million generations, and the tree was sampled every 100 generations. The parameter estimates

and convergence were checked using Tracer v1.7.1 (Rambaut et al., 2018), and the first 30001 trees were discarded based on the results.

2.4. Ancestral character state reconstruction

Morphological character states regarding maxilliped palp segment 2 (or article 2; see Myers and Lowry, 2020) and gnathopod 2 in males were gathered from previous studies (Chou and Lee, 1996; Lowry and Myers, 2019; Myers and Lowry, 2020). The character states of the maxilliped palp segment 2 were coded as “lobate” or “non-lobate”, and those of the male gnathopod 2 as developed “mascupod” or undeveloped “femipod” (Supplementary Table 1). These morphological character states were mapped on the resulting tree generated from the MrBayes BI analysis. Ancestral states were reconstructed under the likelihood model (Markov k-state 1 parameter model) using the Trace Character History option implemented in Mesquite v3.61 (Maddison and Maddison, 2019).

3. Results and discussion

The BI tree (mean $\ln L = -25855.475$; Fig. 1) had an identical topology to the ML tree ($\ln L = -25815.938$; not shown). Partial results of the ancestral state reconstruction (ASR) are summarized in Table 1, and all results are illustrated in Supplementary Fig. 1.

The obtained phylogenies showed that the Far Eastern talitrids analyzed herein were split into three distinct clades (denoted hereinafter as Clades A–C). Clades A and B formed a monophyletic lineage, but this relationship was unsupported in both analyses (BS = 57%, PP = 0.55). While the subterranean *Minamitalitrus zoltani* (samples: MZ01 and MZ02) was a member of Clade A, the two epigeal species on Minamidaito Island—*Morinoia japonica* (MJ12 and MJ13) and *Miyamotoia daitoensis* (MD01)—belonged to Clade B (Fig. 1). The results thus clarified that the troglobitic talitrid on Minamidaito Island does not share its common ancestor with the epigeal talitrids concurrently inhabiting the island, in contrast to the evolutionary relationships found between the troglobitic and epigeal *Palmorchestia* talitrids on La Palma

(Villacorta et al., 2008).

Three epigean species of *Nipponorchestia* and *Mizuhorchestia* belonged to Clade A along with *M. zoltani*. The monophyly of these three genera was fully supported in both analyses (BS = 99%, PP = 1.0). The phylogenies showed that *M. zoltani* formed a monophyletic lineage with *Nipponorchestia curvatus* (BS = 100%, PP = 1.0); *Nipponorchestia nudiramus* and *Mizuhorchestia urospina* formed another fully supported clade (BS = 100%, PP = 1.0). *Nipponorchestia nudiramus* and *Miz. urospina* are indigenous to forest habitats (= forest-hoppers; Lowry and Myers, 2019) in lowland to montane areas on the Japanese main islands and adjacent islets (Morino, 2014; Morino and Miyamoto, 2015a). *Nipponorchestia curvatus*, which belongs to the field-hopper category as defined by Lowry and Myers (2019), inhabits coastal vegetation on the Japanese main islands as well as forest habitats on small islets in the Ryukyu, Izu and Ogasawara Islands, and Tsushima Island (Morino and Miyamoto, 2015a). The approximate distributions of the *Nipponorchestia* and *Mizuhorchestia* species are provided in Supplementary Fig. 2.

The monophyly of *M. zoltani* and *N. curvatus* may highlight another troglobisation history that arose in talitrids. It has been suggested that *N. curvatus* is capable of transoceanic dispersal since this species occurs on islands in the Ryukyu and Ogasawara Islands, which are distant from Japan's main islands, and because its distribution on the Japanese main islands is limited to coastal habitats (Morino and Miyamoto, 2015a). Aside their deep phylogenetic divergence with *N. nudiramus* and *Miz. urospina*, the present analyses also reveal the low genetic diversity between the two specimens of *N. curvatus* collected from the Izu Peninsula (Honshu, Japan) and Tanegashima Island (the Ryukyu Islands; approximately 850 km from the Izu Peninsula). It is thus possible that *N. curvatus* can reach distant locations by transoceanic dispersal like other several coastal talitrids that are known to raft using driftwood (see Wildish, 2017). Furthermore, we observed that *M. zoltani* is tightly associated with wood in the cave habitat (Fig. 1B). Accordingly, the results suggest the most recent common ancestor (MRCA) of *M. zoltani* and *N. curvatus* was a *curvatus*-like epigean species inhabiting coastal regions; the ancestor of *M. zoltani* had rafted to Minamidaito Island, and then invaded and adapted to cave habitats on the island.

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

troglobisation. In addition to its eyeless state, this cave-hopper possesses a gnathopod 2 that is not sexually dimorphic—that is, its males possess an undeveloped female-like (= femipod) gnathopod 2. The ASR result unquestionably clarified that the sexual dimorphism of gnathopod 2 has also been lost in *M. zoltani*. Because males of the other troglobitic species, *Palmorchestia hypogaea* and *Spelaeorchestia koloana*, also have a femipod gnathopod 2 (Stock and Martin, 1988; Bousfield and Howarth, 1976), the loss of sexual dimorphism in gnathopod 2 may be correlated with the adaptation to cave habitats in talitrids. The mating behavior of talitrids, of which males bear the developed (= mascupod) gnathopod 2, is different from that of species with the femipod male gnathopod 2 (see Lowry and Coleman, 2012). As the first step toward a better understanding of the evolutionary history of this morphological trait, therefore, the mating behaviors of cave-hoppers should be investigated. Future studies should also aim to estimate the divergence time of this cave-hopper, which will lead us to a better understanding of its troglobisation history.

The phylogenetic and ASR results clarified that the non-lobate segment 2 of maxilliped palp has evolved independently in talitrids. The family Brevitalitridae was recently erected by Myers and Lowry (2020) based on a morphological cladistic analysis. The non-lobate maxilliped palp segment 2 was treated as one of the key characters of this family-group taxon. Therefore, *Mizuhorchestia* and *Talitroides* were classified within the Brevitalitridae along with other seven genera including *Bousfieldia* (Myers and Lowry, 2020), although the type species of *Bousfieldia*, *B. phoenixae*, unquestionably possesses a lobate maxilliped palp segment 2 (Chou and Lee, 1996). Our findings support the hypothesis that *Mizuhorchestia* is phylogenetically close to *Nipponorchestia* (Morino and Miyamoto, 2015a), and suggest that the MRCA of *Miz. urospina* and *N. nudiramus* possessed the lobate maxilliped palp segment 2. The present results exemplify how morphological features, which have been treated as key taxonomic characters in talitrids, have frequently evolved independently in this terrestrial amphipod group. Also, the non-lobate maxilliped palp segment 2 in *Miz. urospina* is distinct from those of some other genera in the Brevitalitridae (e.g., *Brevitalitrus* and *Talitroides*) in that in the former it is broad, while in the latter it is slender (see Miyamoto and Morino, 2012; Morino, 2013, 2014).

Our results also reveal the noteworthy phylogenetic relationships of the other

Far Eastern talitrids. The monophyly of the *Platorchestia* complex—*Platorchestia*, *Miyamotoia* and *Morinoia*—, *Sinorchestia* and *Bousfieldia* was fully supported (= Clade B), and the sister relationship between the sand-hopper *Sinorchestia* and the forest-hopper *Bousfieldia* was also supported (BS = 90%, PP = 0.99). However, our phylogenies fail to support the monophyly of *Morinoia*, which was established by Lowry and Myers (2019) for three Far Eastern species previously classified as *Platorchestia*. Our findings highlight the need to assess the phylogenetic relationships among the genera split out *Platorchestia* in recent studies (e.g., Lowry and Myers, 2019; Morino, 2020) based on broad taxonomic sampling. Clade C was composed of a cosmopolitan *Talitroides topitotum* and three genera—*Lowryella*, *Kokuborchestia* and *Pyatakoveestia*—(BS = 90%, PP = 0.99). *Lowryella wadai* is the only known species of the genus indigenous to restricted marsh habitats of the southern part of the Japanese main islands (Morino and Miyamoto, 2016). The morphological similarities between *Lowryella* and *Nipponorchestia* as well as *Mizuhorchestia* were suggested by Morino and Miyamoto (2016), but nonetheless, this marsh-hopper talitrid shares the MRCA with *Kokuborchestia* and *Pyatakoveestia*. While *Kokuborchestia* is indigenous to the northern part of Japan (Morino and Miyamoto, 2015c), *Pyatakoveestia* talitrids are widely distributed in seashore habitats of the Japanese main islands, Primorsky (Russia) and the Korean Peninsula, and also occur at almost all adjacent islets (Morino and Miyamoto, 2015b). The evolutionary and biogeographic histories of *Lowryella*, *Kokuborchestia* and *Pyatakoveestia* will be subject to future studies.

Our findings depict the backbone phylogeny of the Far Eastern talitrid genera and also elucidate the phylogenetic position of the cave-hopper *M. zoltani* among these talitrids; however, our phylogenetic dataset could not cover all the genera and species indigenous to this region. Our results also reveal the deep divergence between the troglobitic *M. zoltani* and its sister species *N. curvatus*. Future study should assess the phylogenetic position of the Hawaiian *Spelaeorchestia* to determine whether it may have evolved from epigean talitrids inhabiting the island of Kauai, like *Palmorchestia*, or from a lineage deeply diverge from its sister species, like *Minamitalitrus*. Our study shows that the four species of *Minamitalitrus*, *Nipponorchestia* and *Mizuhorchestia*, respectively, formed a distinctive lineage within their monophyletic group (= Clade A). These results affirm the need for a genus-level taxonomic revision of these genera.

Because *Nipponorchestia* was revealed to be non-monophyletic, a new genus is herein established for *N. nudiramus* by the first and second authors; therefore, the diagnosis of *Nipponorchestia* is amended from the preceding one (Morino and Miyamoto, 2015a). Additionally, *Mizuhorchestia* is affiliated with the Talitridae. The phylogenetic analyses also reveal each of *N. nudiramus* and *Miz. urospina* is composed of the divergent lineages suggesting that they contain several cryptic species. Their species-level taxonomy and evolutionary history should be investigated by future studies.

4. Taxonomy

Family Talitridae Rafinesque

Subfamily Talitrinae Rafinesque sensu Myers and Lowry (2020)

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

Diagnosis. See White et al. (2013).

Type species. *Minamitalitrus zoltani* White, Lowry, and Morino, 2013, fixed by original designation (White et al., 2013). Genus monospecific.

Genus *Nipponorchestia* Morino and Miyamoto, 2015a

Amended diagnosis. Body size medium, eyes small to medium. Antenna 1 reaching end of peduncular segment 4 of antenna 2; peduncle longer than flagellum, peduncular segments 1–3 subequal in length; flagellum with 3 articles, article 1 slightly curved, article 2 longest. Antenna 2 in male not incrassate, flagellum longer than peduncle. Mandible left lacina 4-dentate. Maxilliped palp segments 2 (= carpus) and 3 (= propodus) broad, mediodistally lobate, segment 4 (= dactylus) distinct, reduced, inserted apically on segment 3.

Gnathopod 1 strongly subchelate in both sexes; in male, merus, carpus and propodus with pellucid lobes, propodus lateral surface with a row of elongate setae; in female, carpus and propodus lacking pellucid lobe. Gnathopod 2 in male, propodus powerfully subchelate, dactylus attenuated apically; in female, basis slender, propodus mitten-shaped. Pereopods 3–7, cuspidactylate (bicuspidate), propodus locking robust setae developed. Coxa of pereopod 4 wider than deep. Coxa of pereopod 6, posterior lobe smoothly curved. Pereopods 6 and 7 in male not incrassate. Coxal gills of pereopods 2 and 6 large, lobate at midway. Coxal gill of pereopod 6 without leaf-shaped

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

Epimeral plates lacking marginal pits. Pleopods, peduncle marginally bare or weakly robust-setose; rami moderately reduced. Uropod 1, distolateral robust seta on peduncle longer than subdistal one; inner ramus with dorso-marginal robust setae; outer ramus marginally bare. Uropod 2, rami subequal in length, marginally robust-setose. Uropod 3, peduncle truncate or narrowing distally; ramus short. Telson lobes each with lateral and apical robust setae.

Type species. *Nipponorchestia curvatus* Morino and Miyamoto, 2015a, fixed by original designation (Morino and Miyamoto, 2015a). Genus monospecific.

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

Diagnosis. Body size medium, eyes medium. Antenna 1 reaching end of peduncular segment 4 of antenna 2; peduncle longer than flagellum; flagellum with 4 articles, geniculated between articles 2 and 3, article 3 longest. Antenna 2 in male not incrassate, flagellum longer than peduncle. Mandible left lacina 4-dentate. Maxilliped palp segments 2 (= carpus) and 3 (= propodus) broad, mediodistally lobate, segment 4 (= dactylus) distinct, reduced, inserted apically on segment 3.

Gnathopod 1 strongly subchelate in both sexes; in male, merus lacking pellucid lobes, carpus and propodus with pellucid lobes, propodus lateral surface with a row of elongate setae; in female, carpus with small pellucid lobe. Gnathopod 2 in male, propodus powerfully subchelate, dactylus attenuated apically; in female, basis slender, propodus mitten-shaped. Pereopods 3–7, cuspidactylate (bicuspidate), propodus locking robust setae well-developed. Coxa of pereopod 4 wider than deep. Coxa of pereopod 6, posterior lobe smoothly curved. Pereopods 6 and 7 in male not incrassate. Coxal gills of pereopods 2 and 6 large (especially in pereopod 6), lobate at midway, attenuated distally. Coxal gill of pereopod 6 with leaf-shaped lobe. Oostegites with simple-tipped setae distally.

Epimeral plates lacking marginal pits. Pleopods, peduncle marginally bare or weakly robust-setose; rami moderately reduced. Uropod 1, distolateral robust seta on peduncle longer than subdistal one; inner ramus with dorso-marginal robust setae; outer ramus marginally bare. Uropod 2, rami subequal in length, marginally robust-setose. Uropod 3, peduncle truncate or narrowing distally; ramus short. Telson lobes each with lateral and apical robust setae.

Type species. *Nipponorchestia nudiramus* Morino and Miyamoto, 2015a, fixed by the present designation.

Etymology. A compound noun of feminine gender derived from “Yamato”, an old name for Japan, especially Nara Prefecture, which is a part of the distribution of the type species, and the generic name “*Orchestia*”.

Remarks. *Yamatorchestia* is discriminated from three phylogenetically close three genera—*Mizuhorchestia*, *Nipponorchestia* and *Minamitalitrus*—by the following combination of characters (see Morino, 2015): 1) coxal gill on pereopod 6 large, with a leaf-shaped lobe, 2) flagellum of antenna 1 4-articulate, and 3) outer ramus of uropod 1 marginally bare. This new genus can be clearly distinguished from *Morinoia*, another talitrid genus inhabiting similar habitats around the distribution of *Yamatorchestia*, by the display of a developed robust distolateral seta on uropod 1 peduncle (vs. small simple seta in *Morinoia*; Lowry and Myers, 2019).

Although *Yamatorchestia* currently consists only of its type species *Y. nudiramus*, the genus may be composed of at least two genetically divergent species.

Genus *Mizuhorchestia* Morino, 2014

Diagnosis. See Morino (2014).

Type species. *Mizuhorchestia urospina* Morino, 2014, fixed by original designation (Morino, 2014).

Remarks. The genus *Mizuhorchestia* should be placed within the Talitrinae in the family Talitridae sensu Myers and Lowry (2020). The genus is currently monospecific, but may contain several undescribed species.

This work has been registered in ZooBank with the registration number urn:lsid:zoobank.org:pub:1DB3E8F3-D204-4814-8383-5E9D64590E99.

CRedit authorship contribution statement

Tomoyuki Takahashi: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft. **Hiroshi Morino:** Conceptualization, Writing - review & editing, Supervision. **Ko Tomikawa:** Investigation, Resources, Data curation, Writing - review & editing. **Yi-Te Lai:** Investigation, Resources, Writing -

review & editing. **Takafumi Nakano:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2020.106984>.

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Fig. 1. (A) Map showing the distribution of the troglobitic talitrids, *Minamitalitrus zoltani*, *Palmorchestia hypogaea* and *Spelaeorchestia koloana*. The type locality of *Houlia bousfieldi* remains unclarified. (B) Habitat of *Minamitalitrus zoltani* in the Akiba Subterranean-lake Cave on Minamidaito Island. Arrowheads indicate talitrids on rotten wood. (C) Lateral view of a live individual of *Minamitalitrus zoltani*.

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 $\geq 50\%$ and Bayesian posterior probabilities (BPP) ≥ 0.90 . An asterisk denotes the node with BS $\geq 70\%$ and BPP ≥ 0.95 . Roman numerals (i–iii) indicate the nodes with the ancestral state reconstruction result summarized in Table 1.

Supplementary Fig. 1. Bayesian inference tree of *Minamitalitrus zoltani* and the other Far Eastern talitrid genera showing ancestral states of the maxilliped palp segment 2 (= carpus) and male gnathopod 2. Ancestral states were reconstructed by the one-parameter Mk1 model implemented in Mesquite. Results of the ancestral state reconstruction at the nodes within Clade A are provided in Table 1.

Supplementary Fig. 2. Approximate ranges of *Minamitalitrus zoltani*, *Nipponorchestia curvatus*, *Nipponorchestia nudiramus*, and *Mizuhorchestia urospina*. Distribution data are based on Morino (2014), Morino and Miyamoto (2015a) and the present study. Coastline data are based on Wessel and Smith (1996).

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
<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

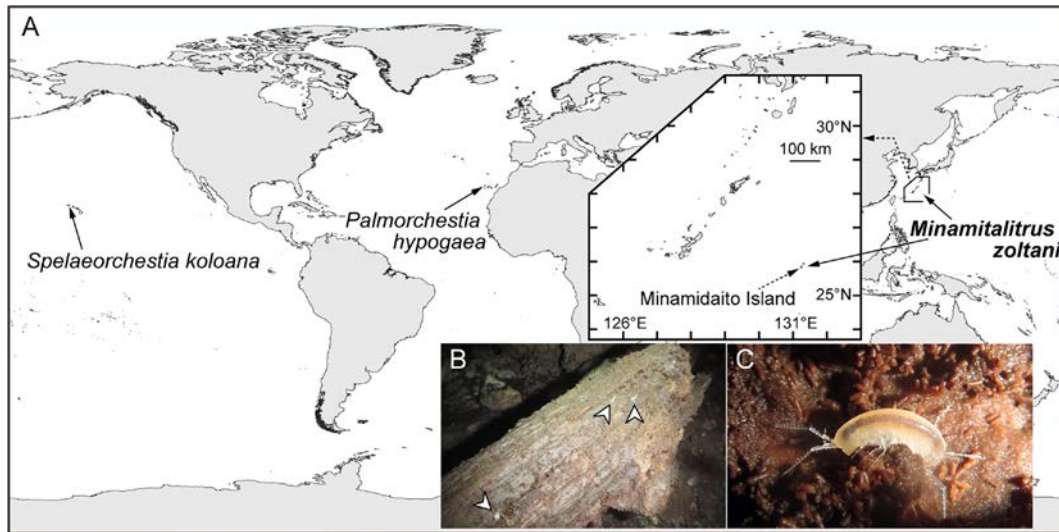


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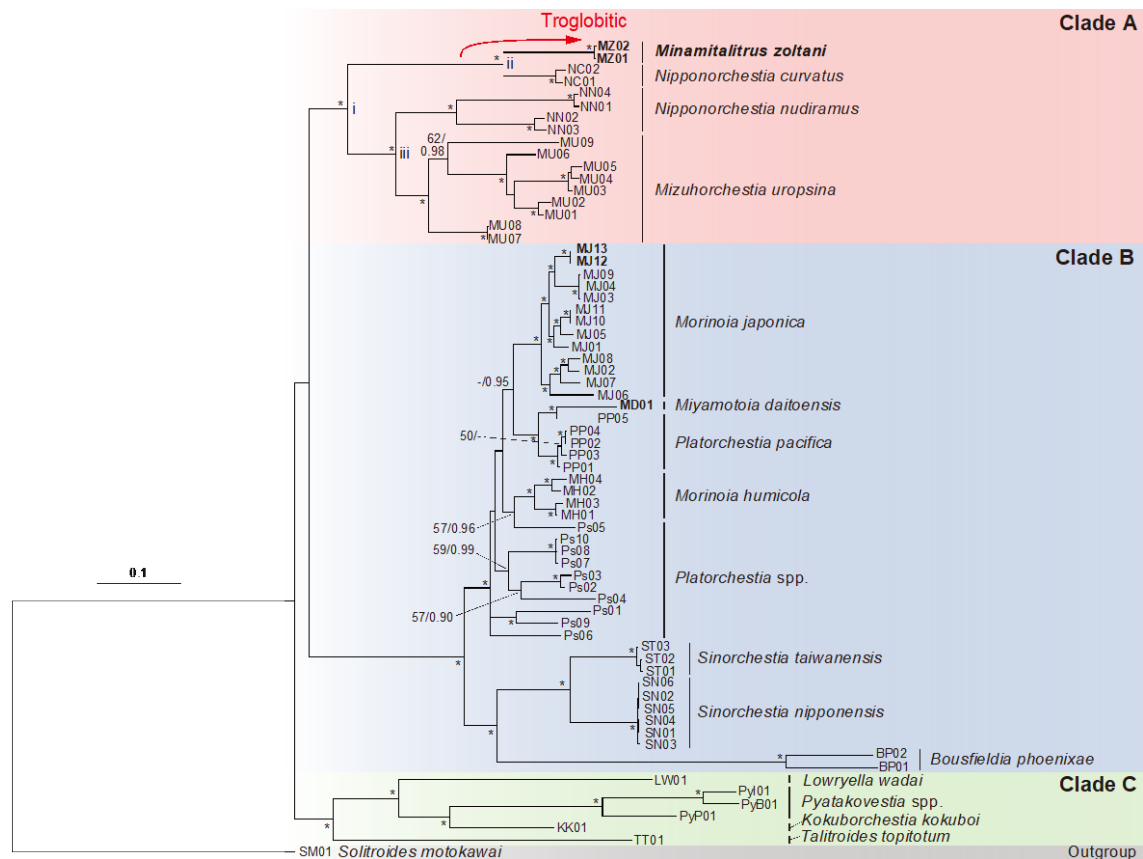


Fig. 2. Molecular phylogenetic position of the troglomorphic *Minamitalitrus zoltani*, based on Bayesian inference for 2751 bp alignment positions of nuclear histone H3, 28S rRNA, and mitochondrial COI and 16S rRNA markers. Samples collected from Minamidaito Island are shown in bold. Numbers on nodes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