



TITLE:

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First record of the terrestrial amphipod, *Talitroides alluaudi* (Chevreux, 1896) (Crustacea, Amphipoda, Brevitalitridae), from Japan

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Abstract

We report *Talitroides alluaudi* (Chevreux, 1896) from Miyako Island, Ryukyu Islands, Japan. Although this terrestrial amphipod is distributed worldwide, including Indo-Pacific islands, Europe, and North and South America, the present specimens represent the first record of this species from Japanese islands. The cytochrome *c* oxidase subunit I (COI) sequence of a Miyako Island specimen was unique compared with the known COI sequences from Taiwanese and Bermudan populations.

Keywords

COI, Miyako Island, Talitroidea, Ryukyu Islands

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Introduction

Terrestrial amphipods known as talitrids—members of Talitroidea herein—have invaded various inland habitats (Bousfield 1984). They were previously classified into a single family, Talitridae; however, this family was recently split into seven families by Myers and Lowry (2020). *Talitroides* Bonnier, 1898 is a genus in the family Brevitalitridae, which is one of the seven newly erected families. This genus consists only of two species, *Talitroides alluaudi* (Chevreux, 1896) and *Talitroides topitotum* (Burt, 1934). *Talitroides topitotum* is globally distributed (Nascimento and Serejo 2016) and

was even reported from Okinawa Island in the Ryukyu Islands, Japan (Morino 2013). Although *T. alluaudi* was also recorded from various locations worldwide, to our knowledge, this species has not been found anywhere in the Japanese Archipelago, including the Ryukyu Islands. A review by Nascimento and Serejo (2016) and a study by Wildish et al. (2016) revealed that *T. alluaudi* inhabits islands in the Indian Ocean, southern Pacific and northern Atlantic, Hawaii, Israel, Europe, North America, and Brazil (Fig. 1A). In addition to those locations, a master's thesis (Chiang 2012), which was overlooked in the

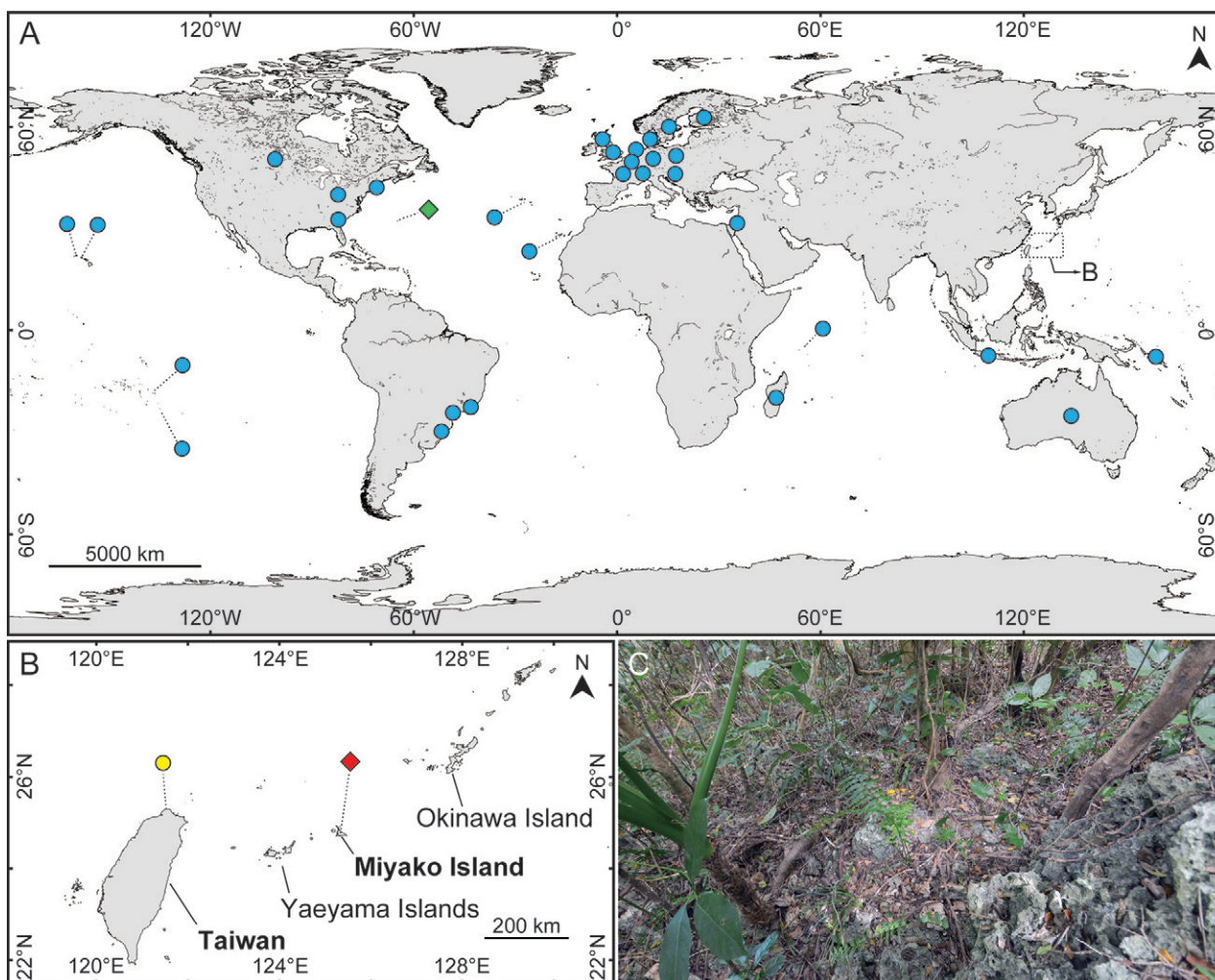


Figure 1. Collection localities and the habitat of *Talitroides alluaudi* (Chevreux, 1896). **A.** Worldwide map showing the collection localities in the preceding studies; the blue circles are from Nascimento and Serejo (2016); and the green diamond is from Wildish et al. (2016). **B.** Map showing the collection sites in East Asian islands; the red diamond denotes the new location; and the yellow circle is from Chiang (2012). **C.** Habitat in Miyako Island. Shoreline data were based on Wessel and Smith (1996).

previous studies, recorded this species from Taiwan (Fig. 1B).

In this study, we collected a lot of talitrids living inside rotten wood on Miyako Island in the Ryukyu Islands, Japan. We successfully identified all of them as *T. alluaudi* on the basis of both morphological characters and the mitochondrial DNA sequence of a specimen. Therefore, we report this species for the first time from Japan.

Methods

We collected the amphipods with rotten wood found in an inland forest, which is located ~2 km from the nearest beach, on Miyako Island, Ryukyu Islands, Japan (Fig. 1B, C) and brought them to the laboratory. In the laboratory, we separated the amphipods from rotten wood using a pooter, and then preserved them in 99% ethanol. We observed and dissected the specimens with a Leica M125C stereoscopic microscope. Detailed characteristics of the dissected specimens were inspected using an Olympus BX53 light microscope and the Leica M125C.

An image of the whole body was captured with a Leica MC170 HD digital camera mounted on the Leica M125C, and prepared using Leica Application Suite v. 4.12 software. Images of body parts were prepared with a Canon EOS Kiss X7 digital camera mounted on the BX53. We deposited the examined specimens in the Zoological Collection of Kyoto University (KUZ).

We determined a partial DNA sequence of mitochondrial cytochrome *c* oxidase subunit I (COI) of a dissected specimen (KUZ Z3771). We extracted genomic DNA from soft tissues of the specimen using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany). We conducted PCR and cycle sequencing reactions as described by Suzuki et al. (2017). The newly determined COI sequence was deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (DDBJ) (Table 1). We evaluated genetic variations of this COI sequence in MEGA 7.0.26 (Kumar et al. 2016) against the 15 *T. alluaudi* sequences registered in INSD—AB741765 (Chiang 2012); and KX360507, KX360513, KX360519, KX360524, KX360532, KX360533, KX360535, KX360538, KX360550, KX360565, KX

Table 1. Comparison of COI nucleotide variations in the 658 bp aligned positions between the present sequence and 15 *Talitroides alluaudi* sequences previously registered in INSD.

INSD accession number	Locality	Aligned position			Reference
		67	136	274	
LC597812	Miyako Island	T	C	T	Present study
AB741765	Taiwan	C	T	C	Chiang (2012)
KX360507 and 13 other sequences	Bermuda	C	T	C	Wildish et al. (2016)

360572, KX360574, KX360579, and KX360585 (Wildish et al. 2016)—(Table 1).

Results

Order Amphipoda

Superfamily Talitroidea Rafinesque, 1815

Family Brevitalitridae Myers & Lowry, 2020

Genus *Talitroides* Bonnier, 1898

Talitroides alluaudi (Chevreux, 1896)

Figure 2

New Japanese name: mijin-tsume-okatobimushi,

ミジンツメオカトビムシ

New record. JAPAN – Okinawa • Ryukyu Islands, Miyako Islands, Miyako Island; 24°48.77'N, 125°18.56'E; 18.X.2020; N. Sawada leg.; in rotten wood; 110 sex indet., KUZ Z3769; 1 ♀, KUZ Z3770; DDBJ: LC597812; 1 ♀, KUZ Z3771.

Identification. Body size small (KUZ Z3771: 5.3 mm; Fig. 2A). Antenna 1 flagellum 5-articulated (Fig. 2B). Antenna 2 flagellum 8 or 9-articulated (Fig. 2C).

Gnathopod 1, propodus simple, gradually narrowed to base of dactylus. Pereopods 3–7 cuspidactylate; pereopods 3 and 4, each dactylus with dentition (Fig. 2D, E); pereopod 5, dactylus with smooth prominence (Fig. 2F). Coxal gill of pereopod 6 L-shaped without lobes (Fig. 2G). Pleopod 1 outer ramus 9-articulated, inner ramus 3-articulated (Fig. 2H). Pleopod 2 outer ramus 9-articulated, inner ramus 1-articulated (Fig. 2I). Pleopod 3 lacking rami (Fig. 2J). Uropod 1 peduncle with simple distolateral robust seta. Uropod 2 peduncle with 3 posterodorsal robust setae on outer margin, middle one longest. Uropod 3 peduncle with lateral robust seta, ramus reduced.

The COI sequence obtained, which yielded 658 bp aligned positions, showed three different loci when compared with the known sequences from Taiwan and Bermuda (Table 1). The Taiwanese and Bermudan sequences, which showed 617, 635, or 658 bp aligned positions, were identical to each other. All substitutions detected between the Miyako individual and Taiwanese and Bermudan samples were synonymous.

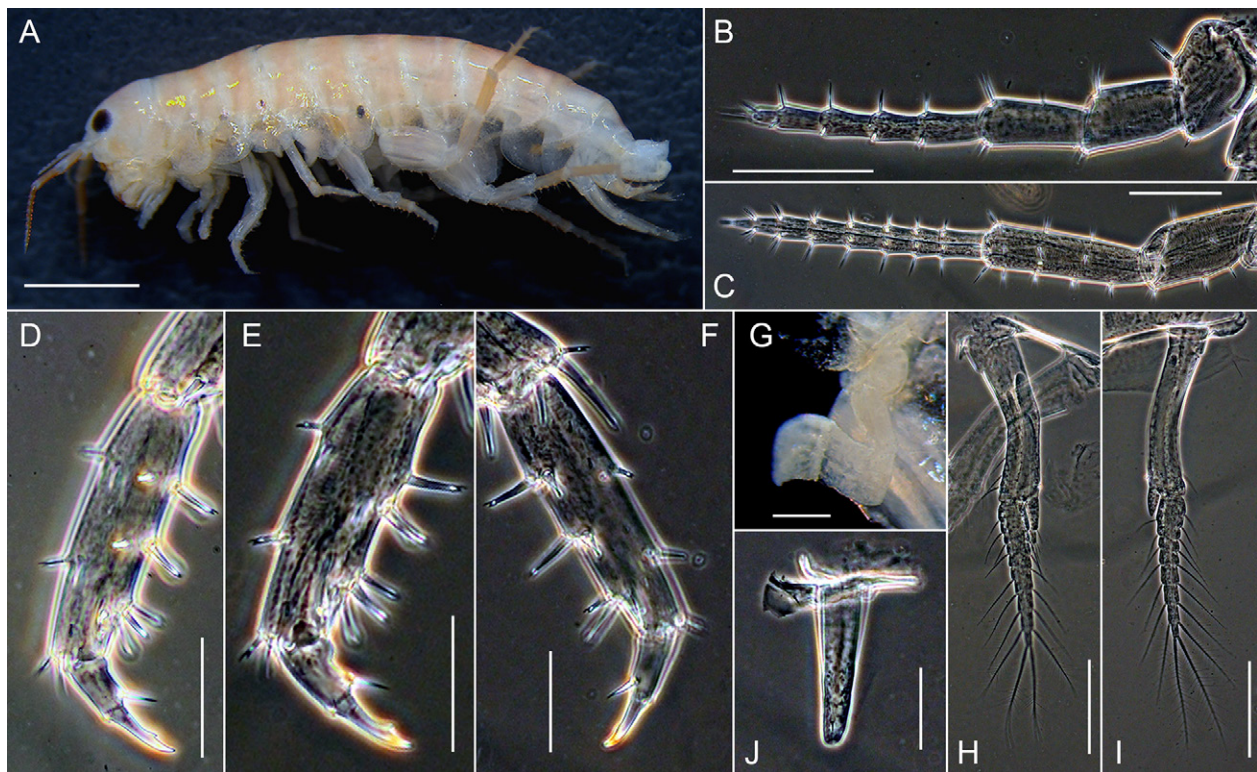


Figure 2. *Talitroides alluaudi* (Chevreux, 1896), female, 5.3 mm, KUZ Z3771. **A.** Habitus, lateral view. **B.** Antenna 1. **C.** Antenna 2. **D.** Propodus and dactylus of pereopod 3. **E.** Propodus and dactylus of pereopod 4. **F.** Propodus and dactylus of pereopod 5. **G.** Coxal gill of pereopod 6. **H.** Pleopod 1. **I.** Pleopod 2. **J.** Pleopod 3. Scale bars: A = 1 mm; B, C, G, H, I = 200 µm; D–F, J = 100 µm.

Discussion

The present specimens clearly showed diagnostic characters of *Talitroides*—cuspidactylate pereopods, reduction in rami of pleopods, L-shaped coxal gill of pereopod 6—and can be unquestionably identified as *T. alluaudi* because they possessed the following features: small body size whose body length ~7 mm, antenna 1 flagellum with ~5 articles, antenna 2 flagellum with fewer than 12 articles, pereopod 4 dactylus with dentation, and uropod 1 with simple distolateral robust setae (see Nascimento and Serejo 2016). Although several characteristics of the examined Miyako specimens differed from the *T. alluaudi* lectotype, i.e., antenna 1 flagellum with 5 articles, pereopod 3 dactylus nail with a sharp dentation posteriorly, and pereopod 5 dactylus nail with a slight prominence at middle on the anterior margin, those characteristics seem to be intraspecific variations of this species (Morino and Ortal 1993; Nascimento and Serejo 2016). Our genetic analysis of the COI sequences also corroborated that the *Talitroides* specimens on Miyako Island belong to *T. alluaudi*.

Around the Pacific Ocean, *T. alluaudi* has been reported from Southeast Asia (Stephensen 1935), the Australian region (Friend and Richardson 1986), Melanesia (Hurley 1975), French Polynesia (Stephensen 1935), the Hawaiian Islands (Bousfield and Howarth 1976; Richardson 1991), and Taiwan (Chiang 2012). This species inhabits Miyako Island and Taiwan, and therefore may also be distributed in other islands in the Ryukyu Islands, such as the Yaeyama Islands, which are located between Miyako Island and Taiwan. Although the Miyako and Taiwanese populations are geographically close to each other, their COI sequences showed that they were clearly distinct. Moreover, the Taiwanese sequence was similar to those obtained from Bermudan *T. alluaudi*. Given the global distribution of *T. alluaudi*, which was originally described from Paris, France (Chevreux 1896), this species is thought to have widely dispersed by synanthropic means (Nascimento and Serejo 2016; Wildish et al. 2016); moreover, its natural habitats remain unknown (Friend and Richardson 1986). The COI sequence results revealed the occurrence of genetic variations among geographical populations, which suggests that future analyses of genetic variations among *T. alluaudi* populations covering the distribution of this species may clarify the dispersal history of this species and may also elucidate whether *T. alluaudi* expands its range via human activities.

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Authors' Contributions

TT: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft. NS: Investigation, Resources, Writing – review & editing. TN: Supervision, Visualization, Writing – review & editing, Funding acquisition.

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