Burmoniscus kitadaitoensis Nunomura, 2009 (Crustacea, Isopoda, Oniscidea) from southern Japan, a junior synonym of B. meeusei (Holthuis, 1947)

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Abstract
Re-examination of the holotype of Burmoniscus kitadaitoensis Nunomura, 2009 from Kitadaitojima Island, southern Japan reveals that this species is a junior synonym of B. meeusei (Holthuis, 1947). Partial regions of mitochondrial COI, 12S and 16S rRNA genes, and nuclear 18S and 28S rRNA genes were detected for species identification in the future.

Keywords
Kitadaitojima Island, mitochondrial DNA, nuclear DNA, Philosciidae, terrestrial isopods

Introduction
Burmoniscus Collinge, 1914 can be dominant in terrestrial isopod communities in subtropical forests of East Asia (Ma et al. 1991). Therefore clarifying the taxonomic status of Burmoniscus species is important to understanding species diversity of isopod communities in these habitats. Thirteen species in the genus Burmoniscus were reported from Japan (Nunomura 2011), but their taxonomic status is still confused (Karasawa and Honda 2012).
**Burmoniscus meeusei** (Holthuis, 1947) was first described as *Chaetophiloscia meeusei* Holthuis, 1947, based on specimens found from a greenhouse at the Royal Botanic Gardens, Kew, United Kingdom. Later, Taiti and Ferrara (1991) found this species in Hawaii and transferred it to the genus *Burmoniscus*. Since then this species has been found in Taiwan and Brazil (Kwon and Jeon 1993; Araujo et al. 1996). *Burmoniscus meeusei* can be distinguished from congeneric species by a small lobe on the inner margin of the apical part of the male pleopod 1 endopodite, a triangular distal part on the male pleopod 1 exopodite, and the round apex of the pleotelson (see Holthuis 1947).

*Burmoniscus kitadaitoensis* Nunomura, 2009 was described from specimens collected on Kitadaitojima Island, southern Japan, where it was supposed to be endemic because it was never reported from other areas (Nunomura 2011). In the original description, Nunomura (2009) compared the morphological features of *B. kitadaitoensis* to those of *B. okinawaensis* (Nunomura, 1986) and *B. daioensis* (Nunomura, 1986), and he concluded that *B. kitadaitoensis* was an undescribed species. The figures of Nunomura (2009), however, show that the male pleopod 1 exopodite of *B. kitadaitoensis* has a similar shape to that of *B. meeusei* (e.g., Holthuis 1947). However, the small lobe of the male pleopod 1 endopodite is not illustrated in the figures.

The aim of this study is to examine the holotype of *B. kitadaitoensis* and clarify its taxonomic status. Moreover, partial sequences of the mitochondrial COI, 12S and 16S rRNA genes, and nuclear 18S and 28S rRNA genes are detected for DNA markers of species identification.

**Materials and methods**

**Sample collection**

The holotype of *B. kitadaitoensis* was deposited in Toyama Science Museum (male, TOYA-Cr 14899). We examined the holotype, but the specimen was dissected and in bad condition. However, we were able to observe some parts as follows: male pleopod 1 endo- and exopodites, pleopod 2 endo- and exopodites, male pereiopods 1 and 7, genital papilla, epimera of pereionite 7, and pleotelson. Four specimens were also collected from Kitadaitojima Island (type locality) and Amamioshima Island and were used for measurements of the coordinate of the noduli laterales and molecular analysis. The voucher specimens are deposited in the collection of Kitakyushu Museum of Natural History and Human History (KMNH-IvR), Kitakyushu, Fukuoka Prefecture, Japan.

**Morphology**

The male pleopods 1 and 2, genital papilla, and male pereiopods 1 and 7 of the holotype, and the position of noduli laterales of specimens collected from Kitadaitojima
Island were examined using a Nikon Eclipse E400 microscope (magnification of 40–400×). The epimera of pereionite 7 and pleotelson of the holotype were examined using an Olympus SZH-10 microscope (magnification of 7–64×). A color image was produced from multi-focused montage images using a digital microscope VHX-2000 (KEYENCE Corporation).

**Molecular analysis**

The partial sequences of mitochondrial cytochrome oxidase subunit I (COI), mitochondrial 12S and 16S ribosomal RNA (rRNA) genes, and nuclear 18S and 28S rRNA genes were determined for identifying this species in the future. DNA extraction and PCR amplification are described in Karasawa and Honda (2012). The primers and the accession numbers are shown in Tables 1 and 2, respectively.

**Table 1.** PCR primers used in this study.

| Genes | Primer | Sequences (5’ to 3’) | Source |
|-------|--------|----------------------|--------|
| Forward | | | |
| COI | LCO1490 | GGTCACAACAAATCATAAAGATATTG | Folmer et al. (1994) |
| 12S | 12Sai | AAAAAATCAAGATATTG | Palumbi (1996) |
| 16S | 16Sar-int-sf | GCCGCAGTATHCTRACGTGGCT | Parmakelis et al. (2008) |
| 18S | 18Sforward | TACCTGGTTGATCCTGCCAG | Maraun et al. (2009) |
| 28S | D3A | GACCCGTCTTGAACACCGGA | Litvaitis et al. (1994) |
| Reverse | | | |
| COI | HCO2198 | TAAACTTCAGGGTGACCAAAAAATCA | Folmer et al. (1994) |
| 12S | 12Sbi | AAGAGCGAGCGGGCCATG | Palumbi (1996) |
| 16S | 16Sbr | CCGGTACTGAACGTACAGCTACGT | Klossa-Kilia et al. (2006) |
| 18S | 18S614r | TCCAAC TACGAGCTTTTAAACC | Maraun et al. (2009) |
| 28S | D3B | TCGGAAAGGGACAGCTACTA | Litvaitis et al. (1994) |

**Table 2.** Locality, DDBJ accession numbers and voucher specimens of *Burmoniscus meeusei*.

| Locality | COI | 12S | 16S | 18S | 28S | Voucher specimens |
|----------|-----|-----|-----|-----|-----|------------------|
| Minami, Kitadaito Village, Kitadaitojima Island, Okinawa Prefecture, Japan | AB889795 | AB889798 | AB889801 | AB889804 | AB889807 | KMNHIvR 50072 |
Results

Genus *Burmoniscus* Collinge, 1914

*Burmoniscus meeusei* (Holthuis, 1947)

http://species-id.net/wiki/Burmoniscus_meeusei

Figs 1–3

*Chaetophiloscia meeusei* Holthuis, 1947: p.124–130, Figs 1–2.

*Brumoniscus meeusei*: Taiti and Ferrara 1991, p.212, Figs 7–8; Kwon and Jeon 1993, p.142–143, Fig. 7; Araujo et al. 1996, p.118–120, Figs 15–21.

*Brumoniscus kitadaitoensis* Nunomura, 2009, p.79–81, Fig. 3; Nunomura 2011, p.60.

Syn. n.

Material examined. Holotype of *B. kitadaitoensis*, TOYA-Cr-14899, male, dissected, near Daitogu, Kitadaitojima Island, Okinawa Prefecture, Japan, 25th November 2006, Noboru Nunomura leg; non types, 2 male, KMNH-IvR 500720 and 500721, 25.9314°N, 131.3094°E, Minami, Kitadaito Village, Kitadaitojima Island, Okinawa Prefecture, Japan, 30th June 2012, Takeshi Goto leg.; non type, 1 male, KMNH-IvR 500722, 25.9444°N, 131.3021°E, Daitogu, Kitadaito Village, Kitadaitojima Island, Okinawa Prefecture, Japan, 30th June 2012, Takeshi Goto leg.; non type, 1 male, KMNH-IvR 500723, 28.3560°N, 129.3935°E, Yamato Village, Amamioshima Island, Kagoshima Prefecture, Japan, 12th September 2012, Shigenori Karasawa leg.

Remarks. *Burmoniscus meeusei* is characterized by the male pleopod 1 endopodite slender and gradually narrowing to the apex (Fig. 1A) with a small lobe on the inner margin close to the apex (Fig. 1B); the male pleopod 1 exopodite with triangular posterior point bent outward and inner margin evenly convex (Fig. 1C); the male pleopod 2 endopodite slender (Fig. 1D) and exopodite trapezoidal (Fig. 1E); genital papilla elongated and simple (Fig. 1F); male pereiopods 1 and 7 without particular modifications (Fig. 2A, B); the pereonite 7 with postero-lateral corners at obtuse angle (Fig. 3A); and the apex of pleotelson broadly rounded (Fig. 3B).

On the holotype of *B. kitadaitoensis*, a small lobe was found on the inner margin of the male pleopod 1 endopodite, although this character was not shown in the original description (Fig. 3P in Nunomura 2009). Moreover, the other morphological characters including the co-ordinate of the noduli laterales (Fig. 4) are consistent with those of *B. meeusei* (see Figs 1 and 2 in Holthuis 1947; Figs 7 and 8 in Taiti and Ferrara 1991; Fig. 7 in Kwon and Jeon 1993; Figs 15–21 in Araujo et al. 1996). Thus the present study considers *B. kitadaitoensis* as a junior synonym of *B. meeusei*.

Distribution. *Burmoniscus meeusei* was previously reported from the United Kingdom (greenhouses), Hawaii, Brazil, Taiwan (Schmalfuss 2004) and Japan. In Japan this species has been collected from Kitadaitojima Island only (Nunomura 2009, 2011), but the present study found the species on Amamioshima Island, which is about 300 km from Kitadaitojima Island.
Figure 1. *Burmoniscus kitadaitoensis*, male, holotype, TOYA-Cr 14899. A, B Pleopod 1 endopodite C pleopod 1 exopodite D pleopod 2 endopodite E pleopod 2 exopodite F genital papilla. Scale bars: A, C–E 200 μm, B 50 μm.
Figure 2. *Burmoniscus kitadaitoensis*, male, holotype, TOYA-Cr 14899. A Pereiopod 1 B pereiopod 7. Scale bar: 200 μm.

Figure 3. *Burmoniscus kitadaitoensis*, male, holotype, TOYA-Cr 14899. A Left epimeron of pereonite 7 B telson. Scale bars: 300 μm.
Burmoniscus kitadaitoensis Nunomura, 2009 (Crustacea, Isopoda, Oniscidea)...

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DNA sequences. The COI, 12S rRNA, 16S rRNA, 18S rRNA and 28S rRNA alignments comprised 653, 354, 453, 675 and 635 bp, respectively. With the exception of the 12S rRNA gene, there is no difference in the four genes among the specimens collected from Kitadaitojima and Amamioshima Islands. Only one 12S rRNA gene base varied between the specimens collected from the two islands.

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