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# Redescription of the Subterranean Millipede *Antrokoreana gujoensis* (Diplopoda: Julida: Nemasomatidae) from Central Honshu, Japan

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A subterranean millipede species, *Antrokoreana gujoensis* Masuda, 2010, is redescribed based on specimens newly collected around its type locality, in the limestone area of Mino Terrane on Honshu Island, Japan. Males of *A. gujoensis* were originally described as having no penes, but nonetheless, the examined specimens clearly demonstrate that this species possesses the penes behind leg-pair 2. An emended diagnosis of this species and a key to all eight currently recognized species of *Antrokoreana* is provided.

**Key Words:** Myriapoda, Juliformia, cave-dweller, SEM images, cytochrome *c* oxidase subunit I.

## Introduction

Julidan millipedes of the nemasomatid genus *Antrokoreana* Verhoeff, 1938, which inhabit the Korean Peninsula and Japanese Archipelago, currently are classified into eight species (Kato et al. 2022). A previous cladistic analysis based on morphological features estimated *Antrokoreana* as forming a distinct lineage that is a sister to the other nemasomatid genera (Enghoff 1985); moreover, a recent molecular phylogenetic analysis showed the non-monophyletic relationship between *Antrokoreana* and the clade consisting of *Nemasoma* C. L. Koch, 1847 and *Thalassissobates* Verhoeff, 1908 (Kato et al. 2022). Thus, based on the available systematic studies, it is unclear whether the genus *Antrokoreana* belongs to the family Nemasomatidae.

Three species of *Antrokoreana* are considered indigenous to the central part of Honshu Island, Japan, i.e., *A. asuwaensis* Kato, Umemura, and Nakano, 2022, *A. gujoensis* Masuda, 2010 and *A. uenoi* Haga, 1956 (Takashima and Haga 1956; Masuda 2010; Kato et al. 2022). Although *A. gujoensis* was originally described as lacking penes in the males (Masuda 2010), this character seems to be questionable since male gonopores of all julidan millipedes open through penes behind leg-pair 2 (Enghoff et al. 2015). Therefore, the taxonomic account of *A. gujoensis* is revisited based on newly collected specimens to clarify the morphological characteristics of this species. Additionally, partial DNA sequences of nuclear 28S rRNA, and mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rRNA are determined to aid future phylogenetic studies of *Antrokoreana* species. The COI sequences are also used to confirm the conspecificity of the examined specimens.

## Materials and Methods

**Samples and morphological examination.** *Antrokoreana* millipedes were collected from four caves near the type locality in Gifu Prefecture, Honshu, Japan (Fig. 1), in 2021–2022. Specimens were fixed and preserved in ~80% ethanol. In the laboratory, the legs of some specimens were removed to extract DNA.

Examination, dissection, and drawing of the specimens were performed using a stereoscopic microscope with a drawing tube (Leica M125C) and a light microscope (Olympus BX53). Images were captured using a Leica MC170 HD digital camera mounted on the Leica M125C and prepared

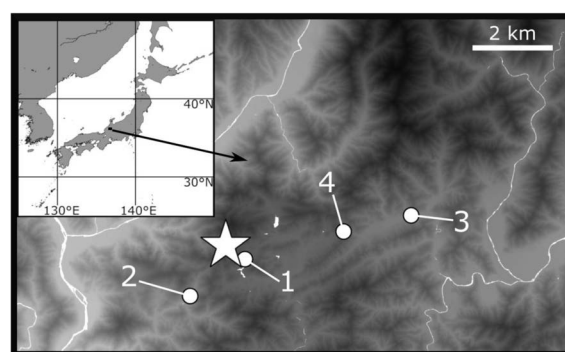


Fig. 1. Map showing the type locality of *Antrokoreana gujoensis* and the collection localities of samples in the present study. The star denotes the type locality of the species (Kumaishi-do Cave). Names of localities are shown in Tables 1, 2. Shoreline data of the inset map is based on Wessel and Smith (1996), and elevation data is on the Fundamental Geospatial Data (DEM) 5m Mesh (elevation) by Geospatial Information Authority of Japan (<https://fgd.gsi.go.jp/download/menu.php>).

Table 1. DNA sequences of *Antrokoreana gujoensis* obtained in the present study. The information on the voucher specimens includes the sex, locality (locality numbers, see Fig. 1) and the International Nucleotide Sequence Database (INSD) accession numbers.

Voucher	Sex	Locality (#)	INSD accession#		
			28S	COI	16S
KUZ Z4034	Male	Kumaishi-kanko-do Cave (1)	LC739350	LC733526	LC733533
KUZ Z4341	Male	Miyama Limestone Cave (4)	LC739351	LC733527	LC733534
KUZ Z4347	Female	Miyama Limestone Cave (4)	LC739352	LC733528	LC733535
KUZ Z4360	Male	Otaki Limestone Cave (2)	LC739353	LC733529	LC733536
KUZ Z4362	Female	Otaki Limestone Cave (2)	LC739354	LC733530	LC733537
KUZ Z4365	Male	Gujo Limestone Cave (3)	LC739355	LC733531	LC733538
KUZ Z4366	Female	Gujo Limestone Cave (3)	LC739356	LC733532	LC733539

Acronym: KUZ, Zoological Collection of Kyoto University.

using Leica Application Suite v. 4.12 software, or with an Olympus DP23 digital camera mounted on the Olympus BX53. The head, mouthparts, legs, and genital organs of six specimens were dehydrated through a graded ethanol series, and dried using hexamethyldisilazane (Nation 1983). Samples were then sputter-coated with gold (JEOL JFC-1200 Fine Coater) and observed by scanning electron microscopy (JEOL JSM-5800LV).

Specimens examined in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

**PCR, DNA sequencing and analyses.** Partial sequences of the nuclear 28S rRNA (474–517 bp) and the mitochondrial markers COI (658 bp) and 16S rRNA (425 bp) were determined from seven specimens. Methods for genomic DNA extraction, polymerase chain reactions, and cycle sequencing reactions are as described by Kato et al. (2022). In total, 21 sequences were newly obtained and they have been deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (Table 1).

Pairwise comparison of uncorrected *p*-distances for COI sequences of this species (658 bp) was calculated using MEGAX (Kumar et al. 2018).

## Taxonomy

Genus *Antrokoreana* Verhoeff, 1938

*Antrokoreana gujoensis* Masuda, 2010

[Japanese name: Gujo-tateune-horayasude]

(Figs 2–8)

*Antrokoreana gujoensis* Masuda, 2010: 29–32, figs 1, 2; Masuda 2021: 31 (list).

**Emended diagnosis.** This species is clearly distinguishable from all congeners by each distal tip of the posterior gonopods elongate and rake-shaped with 3 or 4 branched-processes (bps; Fig. 7B, C) and its basal part with 2 tulip-leaf shaped processes (tsps; Fig. 7B, C) which do not reach the distal end of the posterior gonopod, differs further from its closest congener, *A. takakuwai* (Verhoeff, 1941), by its possession of elongate distal tips of posterior gonopods, of which the basal processes do not reach the distal end of each

posterior gonopod (compared with short distal tips of posterior gonopods in *A. takakuwai*; Shinohara 1973).

**Type locality.** Kumaishi-do Cave (cave entrance: ~35.74°N, ~136.99°E), Gujo, Gifu Prefecture, Japan (Masuda 2010).

**Material examined.** In total 32 specimens collected from Gujo, Gifu Prefecture, Japan, were examined: KUZ Z4034, immature male, Kumaishi-kanko-do Cave (cave entrance: 35.74340°N, 136.99746°E; near the type locality), 2 December 2021, leg. Takafumi Nakano; KUZ Z4340–Z4346, 7 males, KUZ Z4347–Z4358, 12 females, and KUZ Z4359, immature female, Miyama Limestone Cave (cave entrance: 35.74971°N, 137.02514°E), 12 March 2022, leg. Taiga Kato (TK) and Kohei Kawamura (KK); KUZ Z4360, Z4361, 2 males, and KUZ Z4362–Z4364, 3 females, Otaki Limestone Cave (cave entrance: 35.73494°N, 136.98211°E), 12 March 2022, leg. TK and KK; KUZ Z4365, male, and KUZ Z4366–Z4370, 5 females, Gujo Limestone Cave (cave entrance: 35.75343°N, 137.04408°E), 8 May 2022, leg. KK.

**Description.** *Size and number of body rings.* Males: 21.5–29.2 mm long, vertical diameter of largest body ring 1.0–1.8 mm; body with 32–45 podous rings + 1 apodous rings + telson. Females: 22.7–33.9 mm long, vertical diameter of largest body ring 1.2–2.3 mm; body with 32–40 podous rings + 1–2 apodous rings + telson.

*Head* (Fig. 3). Without ommatidia (Figs 2, 3A); with 4 frontal setae. Labrum (Fig. 3B) with 2–4 labral teeth, 2–7 supralabral setae and 15–22 labral setae. Mandibular cardo with 1 seta; mandibular stipes without setae, unexpanded in males. Gnathal lobe (Fig. 3C–E): external tooth with 4 cusps; internal tooth with 6 cusps (Fig. 3C); 6 (4 complete + 2 incomplete) rows of pectinate lamellae (Fig. 3D), posterior teeth of each lamella with 2 finger-like branches (fbs) (Fig. 3E). Gnathochilarium (Fig. 3F) with triangular promontum. Lamellae linguales each with 6 setae in row, stipites each with 3 distolateral setae, without short medial setae. Antennae (Fig. 3G–I), in male (KUZ Z4341), 4.6 mm long, ~3.2× longer than vertical diameter of largest body ring, in female (KUZ Z4347), 3.0 mm long, ~2.1× longer than vertical diameter of largest body ring, with 4 apical sensilla. Lengths of antennomeres I–VIII (VIII = apical sensilla) (in mm), in male (KUZ Z4341): 0.17 (I), 0.57 (II), 0.69 (III), 0.64 (IV), 0.66 (V), 0.54 (VI), 0.44 (VII) and 0.09 (VIII). Length/width ratio of antennomeres I–VII, in male



Fig. 2. *Antrokoreana gujoensis*, female (A: KUZ Z4363) and male (B: KUZ Z4340). A, Habitus of live animal in natural habitat; B, habitus of live animal (photograph taken by T. Jonishi), scale bar: 5 mm.

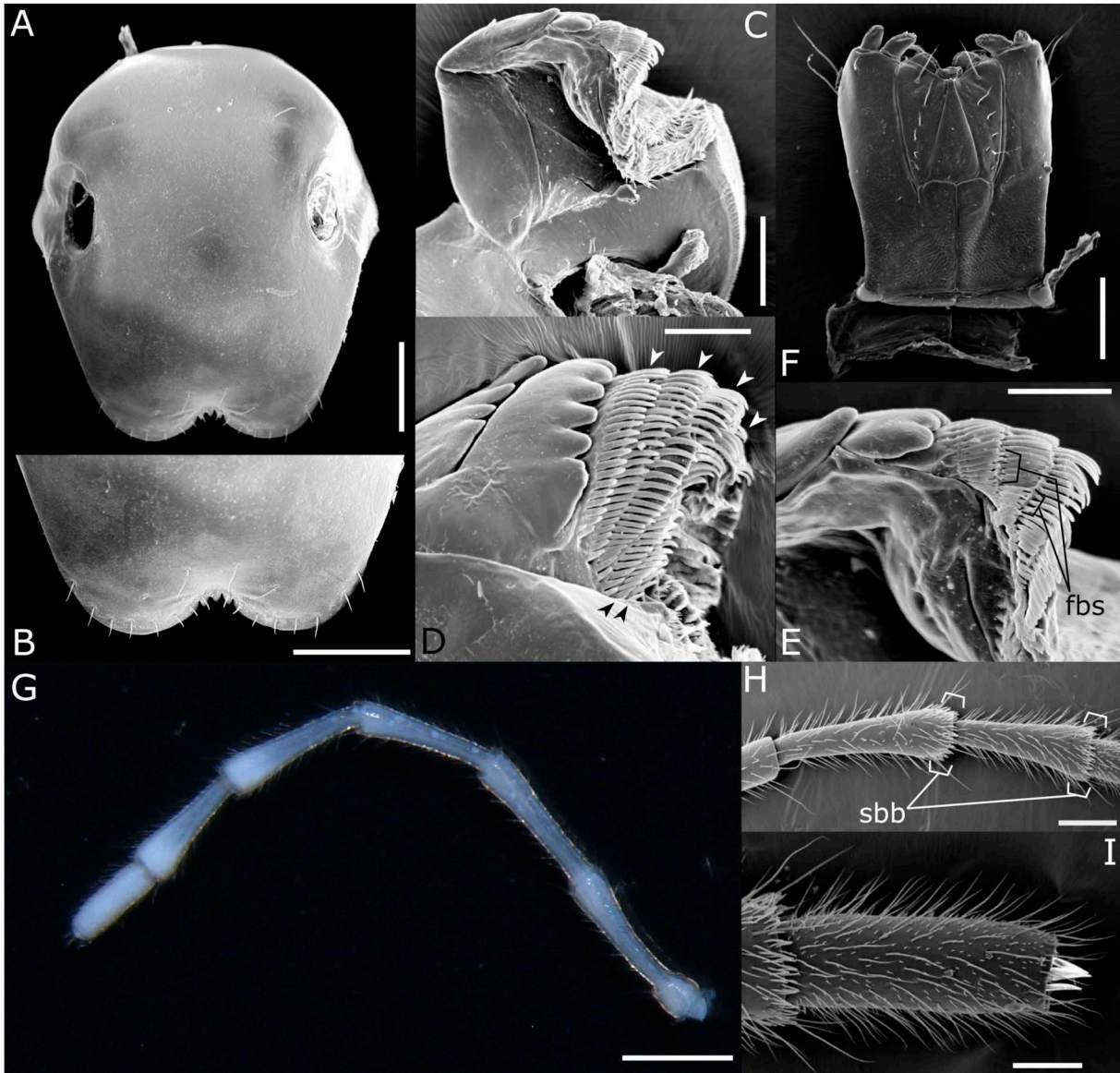


Fig. 3. *Antrokoreana gujoensis*, males (A–E, H, I: KUZ Z4341; F, G: KUZ Z4343). A, Head, frontal view; B, labrum, frontal view; C, right mandible, ventral view; D, mandibular gnathal lobe of left mandible, anterior view; white arrowheads indicate complete pectinate lamellae, black arrowheads donate incomplete lamellae; E, mandibular gnathal lobe of right mandible, posterior view; F, gnathochilarium, ventral view; G, right antenna, sub-mesal view; H, antennomeres V–VI, showing a corolla of large sensilla basiconica bacilliformia at each distal margin; I, antennomere VII, showing short sensilla chaetica and longer sensilla trichodea. Abbreviations: fbs, finger-like branches; sbb, sensilla basiconica bacilliformia. Scale bars: A, B, 250  $\mu$ m; C, I, 100  $\mu$ m; D, E, 50  $\mu$ m; F, H, 200  $\mu$ m; G, 500  $\mu$ m.

(KUZ Z4341): 1.0 (I), 3.4 (II), 3.7 (III), 4.4 (IV), 4.1 (V), 3.3 (VI) and 3.2 (VII). Antennomeres V–VI (Fig. 3H) each with terminal corolla of large sensilla basiconica bacillifor-

mia (sbb); antennomere VII (Fig. 3I) whole surface covered with numerous short sensilla chaetica among longer sensilla trichodea.

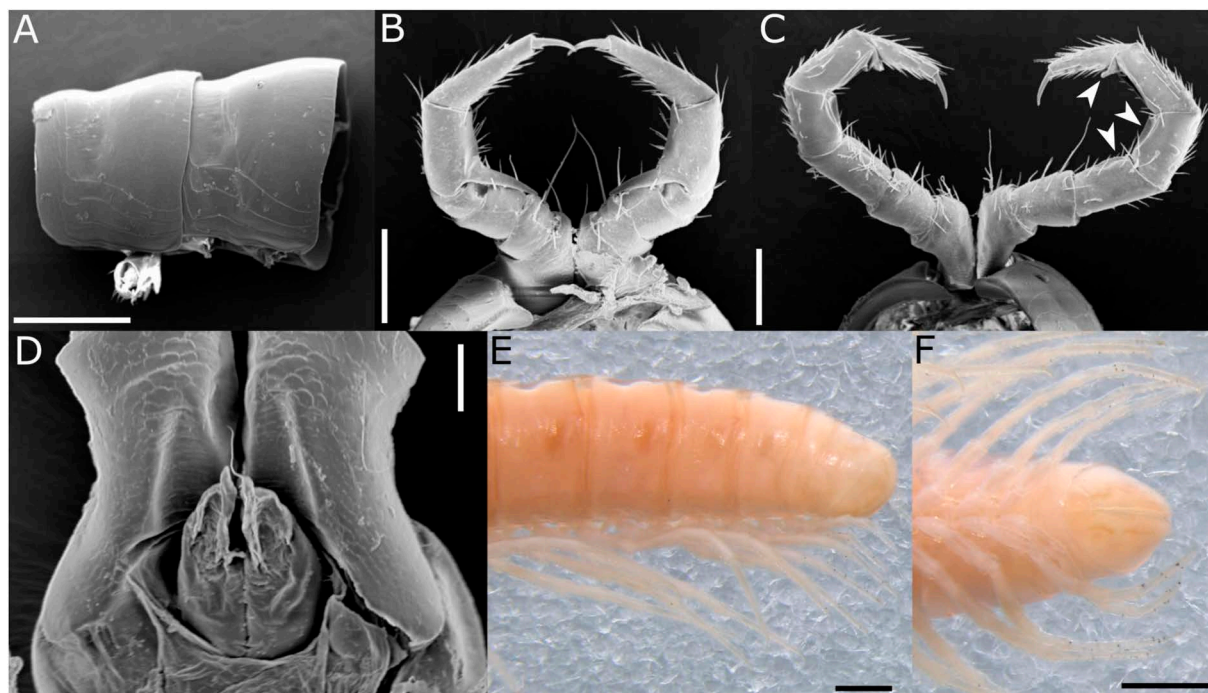


Fig. 4. *Antrokoreana gujoensis*, males (A, D: KUZ Z4343; B, C, E, F: KUZ Z4341). A, Body ring 5 and 6, lateral view; B, first leg pair, posterior view; C, third leg pair, posterior view, white arrowheads indicate adhesive pads; D, penes, posterior view; E, caudal end with 4 podous rings + 1 apodous ring + telson, lateral view; F, telson, ventral view. Scale bars: A, E, F, 500  $\mu$ m; B, C, 200  $\mu$ m; D, 50  $\mu$ m.

**Collum.** With whorl of 8 setae located about one-tenth of collum length from posterior margin.

**Body rings** (Fig. 4A). Metazona dorsal surface smooth, ventral surface with 4–7 longitudinal striae; with posterior marginal whorl of very short setae, length of mid-body setae  $\sim$ 1.8% of vertical diameter of rings; 1 pair of ozopores present at center of lateral surface of each metazona of 6th ring to ultimate podous ring (Fig. 2). Sternum of each ring, free from pleurotergum, with wing-like expansions anteriorly.

**Legs** (Fig. 4B–D). In KUZ Z4341, mid-body legs  $\sim$ 2.2 $\times$  longer than vertical diameter of ring; relative podomere lengths (prefemur to claw): 1.0:1.75:0.9:1.0:1.3:0.4 (leg-pair 10). Claw slender,  $\sim$ 4.1 $\times$  longer than height (KUZ Z4341); with accessory claw. In males, tibia of leg-pair 1 with 1 long spine directed proximad (Fig. 4B). Femur, post-femur, and tibia of following legs each with adhesive pad (Fig. 4C). Penes (Fig. 4D) shorter than coxae of leg-pair 2, each with 0 or 1 microsetae apically.

**Telson** (Fig. 4E, F). Preanal ring without projection, with marginal whorl of setae and 5 additional dorsal setae in front of marginal whorl. Anal valves rounded, each with 7 setae; subanal scale semi-circular, without any modifications, with 2 setae.

**Gonopods** (Figs 5–7). Both anterior and posterior gonopods projecting ventrad. Anterior gonopods (Figs 5, 6): each coxal process (cp) not expanded distally, distal part rounded (Fig. 6C), with several microsetae on posterior surface (Fig. 5A), posterior part curved mesad (Fig. 5B); telopodite (tl) slender, finger-shaped, composed of single article, with several long setae on distal margin, field of spine-like setae absent; each flagellum (fl) with minute spinules (sps) distally (Fig. 6D). Posterior gonopods (Fig. 7) slender, straight, with

1 longitudinal furrow, field of spine-like setae absent; anterior surface covered with minute scale-like processes (slps); posterior surface smooth, with 4 long bristles (lbs) on distal part, reaching apex of each posterior gonopod; distal tip elongate and rake-shaped with 3 or 4 branched-processes (bps), and its basal part with 2 tulip-leaf shaped processes (tsp), which do not reach the distal end.

**Vulvae** (Fig. 8). Pyriform, located inside body ring 3. Operculum (op) slightly longer than bursa, anterior surface of distal part with 4 setae located in 2 longitudinal rows (Fig. 8A, B). Bursa with 3–5 setae around central funnel. Receptaculum seminis (rs) single, long, claviform (Fig. 8C).

**Coloration.** In life, yellowish brown with the defense glands visible by transparency as reddish spots (Fig. 2). In ethanol, yellowish white.

**Genetic distances.** The male and female specimens in each cave shared identical COI sequences, and therefore clearly belong to the same species.

The COI sequence from the specimen collected from Kumaishi-kanko-do Cave and those from specimens collected from Otaki Limestone Cave were identical. The COI uncorrected *p*-distances are shown in Table 2. The COI uncorrected *p*-distance was 1.2% between the specimens collected from Kumaishi-kanko-do Cave and from Gujo Limestone Cave, 1.7% between the specimens collected from Kumaishi-kanko-do Cave and from Miyama Limestone Cave, and 0.5% between the specimens collected from Gujo Limestone Cave and from Miyama Limestone Cave.

**Distribution.** Known from seven caves located in the Mino Terrane in Gifu Prefecture, Japan: six caves, i.e., Kumaishi-do Cave (type locality), Okugo-do Cave, Miyama Limestone Cave (= Sakabe-do Cave) (Masuda 2010, 2021),

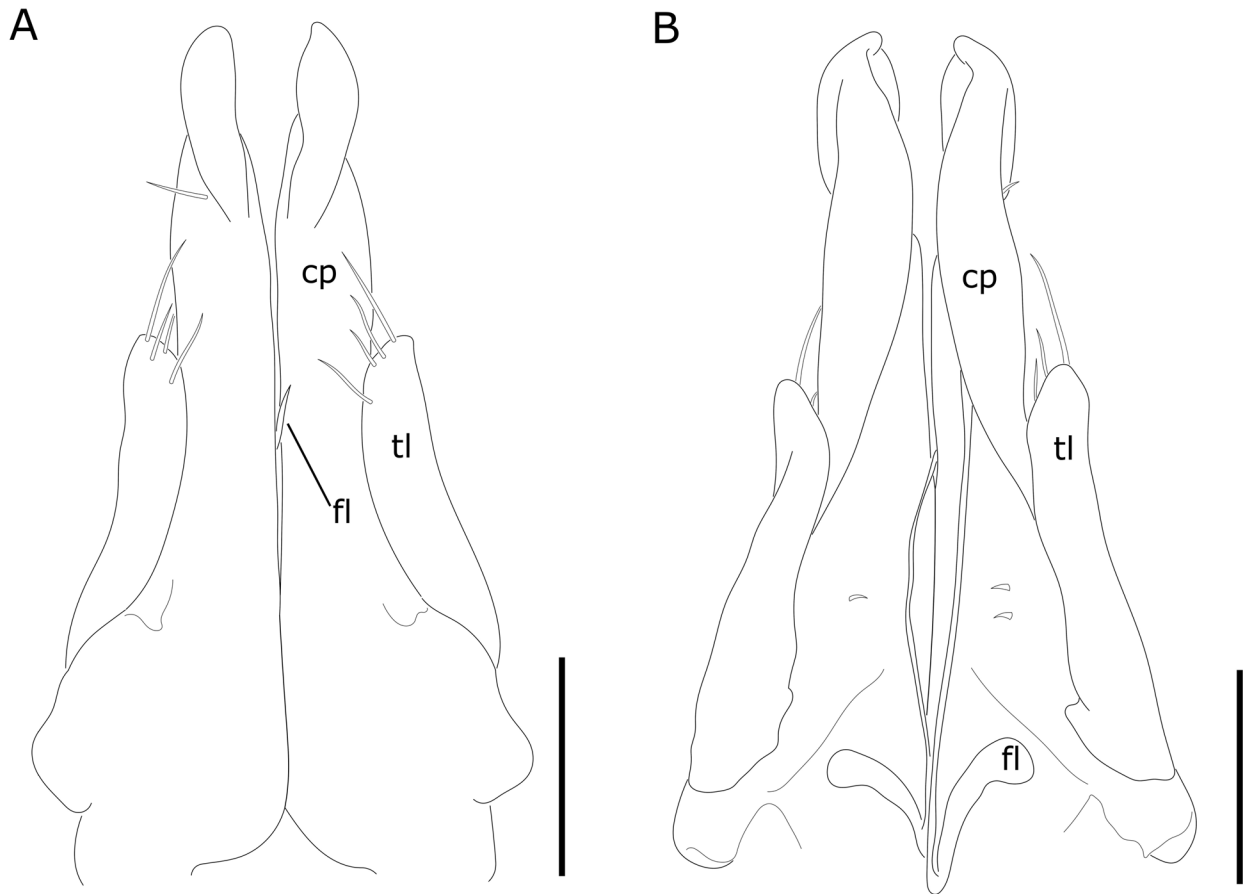


Fig. 5. *Antrokoreana gujoensis*, male (KUZ Z4341). A, Anterior gonopods, anterior view; B, anterior gonopods, posterior view. Abbreviations: cp, coxal process; fl, flagellum; tl, telopodite. Scale bars: A, B, 250  $\mu$ m.

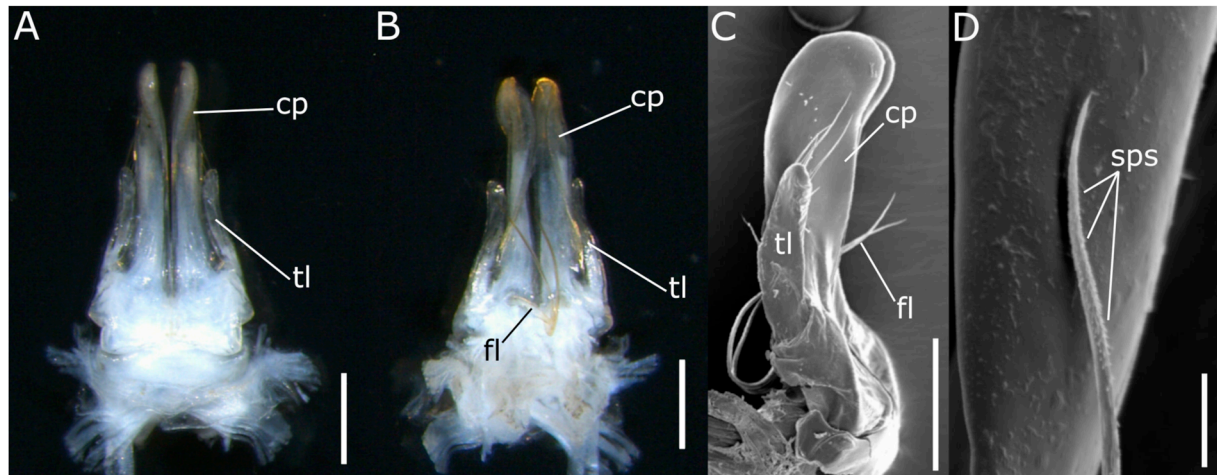


Fig. 6. *Antrokoreana gujoensis*, males (A, B: KUZ Z4341; C: KUZ Z4343; D: KUZ Z4344). A, Anterior gonopods, anterior view; B, anterior gonopods, posterior view; C, anterior gonopods, right lateral view; D, tip of right flagellum of anterior gonopods. Abbreviations: cp, coxal process; fl, flagellum; sps, spinules; tl, telopodite. Scale bars: A–C, 250  $\mu$ m; D, 25  $\mu$ m.

Gujo Limestone Cave, Kumaishi-kanko-do Cave, Otaki Limestone Cave at Gujo, and Inui-no-ana Cave at Yamagata (Masuda 2010, 2021).

**Remarks.** The present specimens unquestionably belong to *Antrokoreana* given that they possess the diagnostic feature of the genus defined by Enghoff (1985), i.e., the an-

tennomere VII covered with short sensilla chaetica among longer sensilla trichodea. Additionally, the present male specimens clearly possess a pair of penes behind the coxae of leg-pair 2, but nonetheless, all the specimens collected from the caves at Gujo can be undoubtedly identified as *A. gujoensis* because the shape of their posterior gonopods is

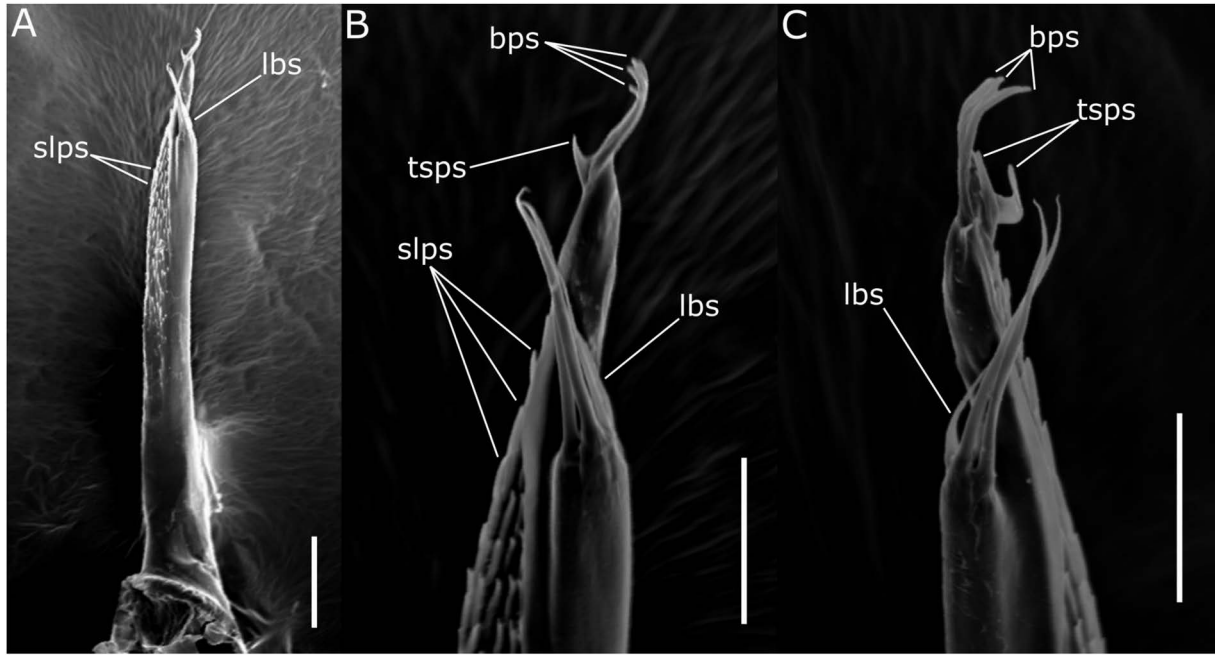


Fig. 7. *Antrokoreana gujoensis*, males (A, B: KUZ Z4365; C: KUZ Z4360). A, Right posterior gonopod, lateral view; B, apical part of right posterior gonopod, lateral view; C, apical part of left posterior gonopod, lateral view. Abbreviations: bps, branched-processes; lbs, long bristles; slps, scale-like processes; tsps, tulip-leaf shaped processes. Scale bars: A, 100  $\mu$ m; B, C, 50  $\mu$ m.

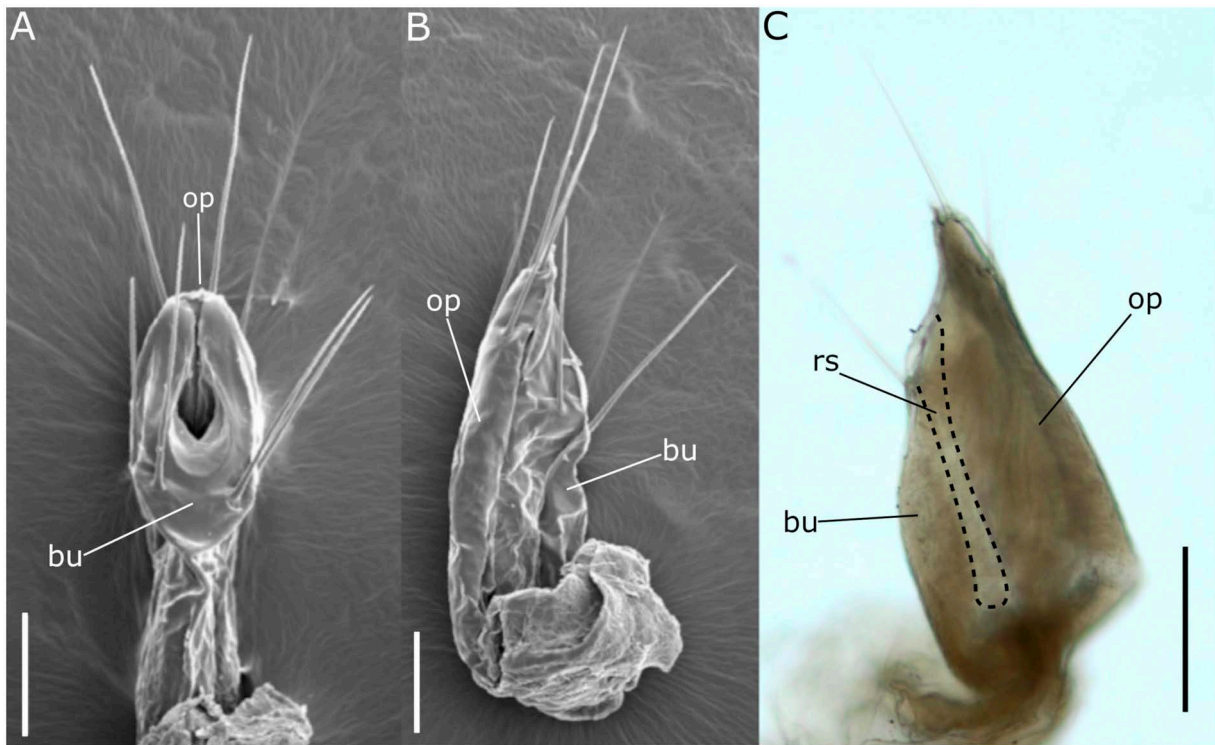


Fig. 8. *Antrokoreana gujoensis*, females (A, B: KUZ Z4349; C: KUZ Z4347). A, Right vulva, posterior view; B, left vulva, mesal view; C, left vulva, lateral view, broken line outlines the receptaculum seminis. Abbreviations: bu, bursa; op, operculum; rs, receptaculum seminis. Scale bars: A, B, 100  $\mu$ m; C, 200  $\mu$ m.

coincident with that of *A. gujoensis* as described by Masuda (2010: fig. 2B). Indeed, the presence of a pair of penes in males is consistent with the placement of the species in the julidan family Nemasomatidae. Based on the morphological

characters provided in the original description of *A. gujoensis* (Masuda 2010), the species was indistinguishable from *A. takakuwai* which is endemic to montane habitats around Mt. Fuji in Honshu (Shinohara 1973).

Table 2. Uncorrected *p*-distances (%) for COI sequences among the specimens of *Antrokoreana gujoensis* collected from different caves (locality numbers, see Fig. 1) in central Honshu, Japan.

Locality	1	2	3
1. Kumaishi-kanko-do Cave			
2. Otaki Limestone Cave	0.0		
3. Gujo Limestone Cave	1.2	1.2	
4. Miyama Limestone Cave	1.7	1.7	0.5

A recent study of *Antrokoreana* showed that *A. asuwaensis* possesses 4 complete + 2 incomplete pectinate lamellae of the mandibular gnathal lobes, a triangular promentum of gnathochilarium, minute spinules on the distal tips of flagella of anterior gonopods, and a long claviform receptaculum seminis of vulva, suggesting the possibility that these features would be shared with the other congeners (Kato et al. 2022). The present results corroborate this hypothesis, since *A. gujoensis* also possesses these morphological features.

The COI sequences from the specimens collected from the two western caves (Kumaishi-kanko-do Cave and Otaki Limestone Cave; Fig. 1) were identical. In contrast, the COI uncorrected *p*-distances between the specimens inhabiting the two western caves and those in the two eastern caves (Gujo Limestone Cave and Miyama Limestone Cave; Fig. 1) were 1.2%–1.7%. The results suggest gene flow between the *Antrokoreana* millipedes in the two western caves.

### Key to Species of *Antrokoreana* Based on Adult Males

The identification key to the *Antrokoreana* species was already provided by Kato et al. (2022), however, the key did not include *A. gujoensis*, given that its original description stated that this species lacks penes in the male. The following key is updated from Kato et al. (2022) and includes *A. gujoensis*, and thus covers all eight currently recognized species of the genus *Antrokoreana*.

1. Anterior margin of anterior gonopods convex; posterior gonopods arcuate. . . . . *A. arcuata* Murakami, 1969
  - Anterior margin of anterior gonopods nearly straight; posterior gonopods straight or slightly bent . . . . . 2
2. Telopodites of anterior gonopods 2 articulate. . . . . 3
  - Telopodites of anterior gonopods with a single article . . . . . 4
3. Coxae of leg-pair 2 with many recurved setae . . . . . *A. uenoi* Haga, 1956
  - Posterior surface of leg-pair 2 coxae asetose . . . . . 5
4. Apex of posterior gonopods with uncinat process, with fine seta at middle of inner margin . . . . . *A. uncinata* Murakami, 1969
  - Posterior gonopods without uncinat process or fine seta. . . . . 6
5. Body length of mature individuals exceeding 20 mm; coxal processes of anterior gonopods usually with several basal spinules . . . . . *A. gracilipes* Verhoeff, 1938

- Body length of mature individuals less than 20 mm; coxal processes of anterior gonopods without basal spinule. . . . . *A. gamooi* Takakuwa, 1941
- 6. Distal part of anterior gonopods concave . . . . . *A. asuwaensis* Kato, Umemura, and Nakano, 2022
  - Distal part of anterior gonopods rounded . . . . . 7
- 7. Basal processes of distal tip of posterior gonopods reaching distal tip . . . . . *A. takakuwai* (Verhoeff, 1941)
  - Basal processes of distal tip of posterior gonopods not reaching distal tip . . . . . *A. gujoensis* Masuda, 2010

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### Authors Contributions

Taiga Kato: Conceptualization; Resources; Investigation; Formal analysis; Visualization; Writing – original draft. Takafumi Nakano: Conceptualization; Supervision; Resources; Investigation; Writing – original draft; Writing – review & editing; Project administration; Funding acquisition.

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### Declarations

**Competing interests.** The authors declare no conflicts of interest.

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