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Short Communication

The subterranean spider *Cybaeus fujisanus* (Araneae: Cybaeidae) revisited: The first description of a male from Mt. Fuji, Japan

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ABSTRACT

A taxonomic account of the spider *Cybaeus fujisanus* Yaginuma, 1972, which was described on the basis of only a female holotype from a cave at the foot of Mt. Fuji, Japan, is revisited. A male specimen was collected near the type locality and is described for the first time. A redescription of the female using the holotype and other newly collected specimens is also provided. The conspecificity of the present male and female specimens was confirmed using nuclear *ITS-1* and mitochondrial *COI* sequences.

Introduction

Spiders of the genus *Cybaeus* L. Koch, 1868 are indigenous to three separate Holarctic regions (North America, Western Europe to the Caucasus, and the Far East) (World Spider Catalog 2021) and remarkably diversified in Western North America and Japan (Copley et al. 2009; Ihara 2009; Bennett et al. 2016, 2019, 2021a). It is noteworthy that some Japanese *Cybaeus* species were described from caves and considered fully troglobitic (Komatsu 1961, 1968; Yaginuma 1986). Those cave dwellers are generally characterized by their pale colors (Ihara 2009); additionally, *Cybaeus itsukiensis* Irie, 1998 is an eyeless species that has adapted to underground habitats (Irie 1998). Subterranean *Cybaeus* spiders can thus provide further insights into the adaptation to the underground and evolutionary history of *Cybaeus* spiders. However, our understanding of the systematic accounts of several hypogean *Cybaeus* species has been hampered because their descriptions were only based on either males or females. Accordingly, descriptions of the unknown male or female of these *Cybaeus* species are essential to elucidate their characteristics, as well as systematic accounts (e.g. Koike 2012).

Cybaeus fujisanus Yaginuma, 1972 is one such species of Japanese subterranean *Cybaeus*. This species was originally described on the basis of only a female specimen collected from a cave at the foot of Mt. Fuji, central Honshu, Japan (Yaginuma 1972). Therefore, male *C. fujisanus* have not been described (Ihara 2009). Moreover, detailed characteristics of the female spermathecae remain undocumented. We herein redescribe *C. fujisanus* on the basis of both sexes collected near the type locality and provide nuclear and mitochondrial gene sequences.

Material and methods

Samples and morphological observation

Cybaeus fujisanus specimens were collected near the type locality (Figure 1) in 2011 and 2012. One male, 14 female, and four juvenile specimens were preserved in 70% ethanol; legs of some specimens were removed and preserved in 99% ethanol for DNA extraction. Epigynes were dissected from three female specimens and then cleared to observe their internal structure following the method described by Matsuda et al. (2020). One left palp of the male specimen was detached from the carapace and examined. Examination of the specimens was conducted using a Leica M125C stereoscopic microscope (Leica Microsystems, Wetzlar, Germany). Images of the specimens were captured with the aid of a Leica MC170 HD digital camera (Leica Microsystems, Wetzlar, Germany) mounted on the Leica M125C and analyzed using Leica Application Suite v. 4.12 software (Leica Microsystems, Wetzlar, Germany).

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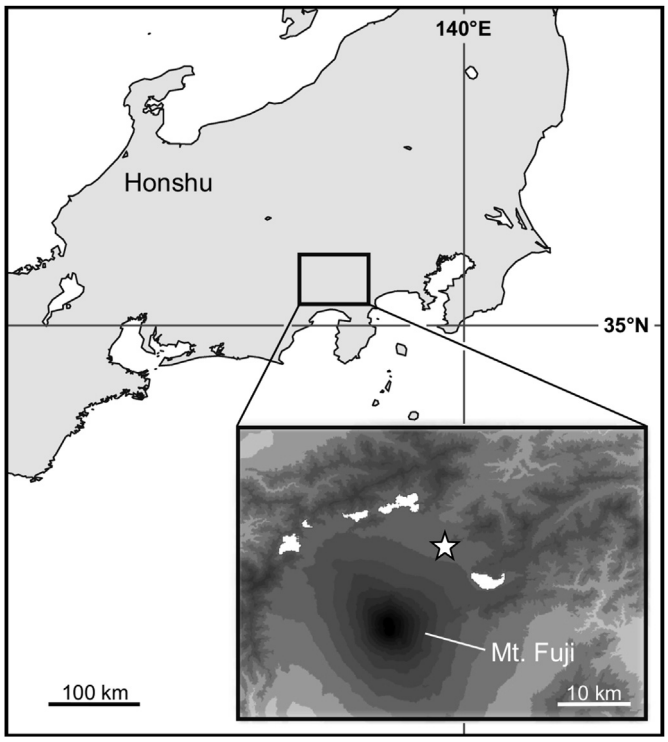

Figure 1. Map showing a collection locality of samples in the present study. Star indicates both the type locality of *Cybaeus fujisanus* Yaginuma and the location of the newly collected specimens. Shoreline data were based on Wessel and Smith (1996).

Measurements were taken to the nearest 0.01 mm using Leica Application Suite. Specimens examined in this study were deposited in the Zoological Collection of Kyoto University (KUZ). Additionally, the *C. fujisanus* holotype, which is preserved in the arachnological collection of the National Museum of Nature and Science, Tsukuba (NSMT), was also examined.

Terminology of morphological characters and the chaetotaxy of leg macrosetae follows Ihara et al. (2021). The following are the abbreviations used for macrosetae: p = prolateral; r = retrolateral; v = ventral; the number of macrosetae on each surface follows these abbreviations. The following are the abbreviations used for other characters: AER = anterior eye row; AME = anterior median eyes; BG = Bennett's gland; CD = copulatory duct; CL = carapace length; CP = copulatory pore; CW = carapace width; EM = embolus; FD = fertilization duct; PA = patellar apophysis; PCO = proximal arm of conductor; PER = posterior eye row; PME = posterior margin of epigynal plate (= 'anterior margin of atrium' in Bennett et al. 2021b); PP = primary pore; RTA = retrolateral tibial apophysis; SB = spermathecal base; SH = spermathecal head; SS = spermathecal stalk; TibIL = length of leg I tibia.

PCR and DNA sequencing

For confirming whether the examined male and female specimens belong to the same species, nuclear *internal transcribed spacer 1* (*ITS-1*) and mitochondrial *cytochrome c oxidase subunit I* (*COI*) were obtained from the male and one of the females. The sequences were deposited with the International Nucleotide Sequence Databases through the DNA Data Bank of Japan.

Methods for genomic DNA extraction and cycle sequencing reactions were as described by Matsuda et al. (2020); primer sets and conditions for the polymerase chain reactions (PCRs) and cycle sequencing reactions used in this study followed Sugawara et al. (2021). All PCRs were performed using a GeneAmp PCR System 9700 (Thermo Fisher Scientific, Waltham, MA, USA).

Taxonomic accounts

Family Cybaeidae Banks, 1892

Genus *Cybaeus* L. Koch, 1868
Type species: *Amaurobius tetricus* C.L. Koch, 1839.

Cybaeus fujisanus Yaginuma, 1972
(Figures 2–5)

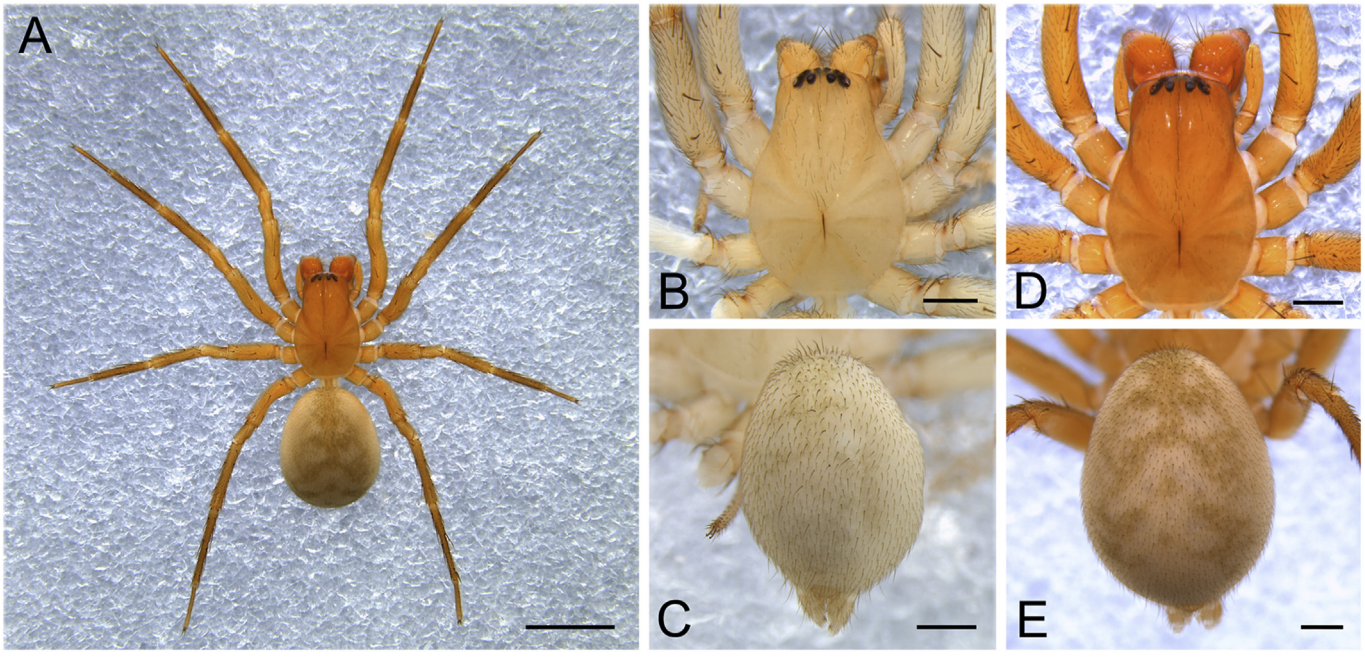

Figure 2. *Cybaeus fujisanus* Yaginuma, female (KUZ Z3926: A, D, E) and male (KUZ Z2412: B, C).: A, Habitus, dorsal view; B and D, prosoma, dorsal view; C and E, abdomen, dorsal view. <scale bar: 2 mm (A); 500 μm (B–E)>.

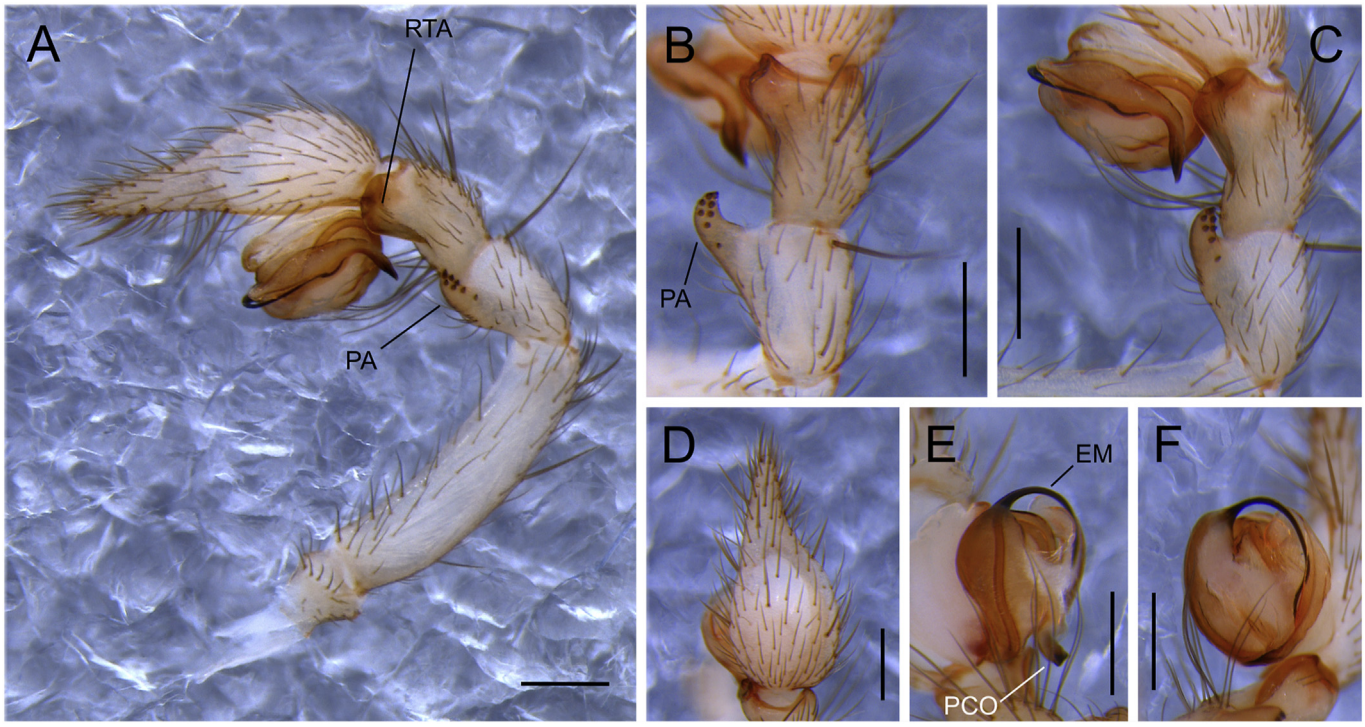

Figure 3. *Cybaeus fujisanus* Yaginuma, male (KUZ Z2412).: A, Left palp, retrolateral view; PA = patellar apophysis; RTA = retrolateral tibial apophysis; B, tibia and patellar of left palp, dorsal view; C, tibia and patellar of left palp, retrolateral view; D, cymbium of left palp, dorsal view; E, bulb of left palp, prolateral view; EM = embolus; PCO = proximal arm of conductor; F, bulb of left palp, retrolateral view. <scale bar: 250 μm (A-F)>.

Cybaeus fujisanus Yaginuma, 1972: 309–311, Figure 39; Ihara, 2009: 165, figure 2-2-30-187.

Amended diagnosis. "Medium-sized" Japanese *Cybaeus* (Figure 2). Both *C. fujisanus* sexes are most similar to those of *C. mellotteei* (Simon, 1886), *C. sanctus* (Kishida in Komatsu, 1942), *C. shingenni* Komatsu, 1968, and *C. jaanaensis* Komatsu, 1968 (see figures 2-2-30-36–47 in Ihara 2009). Females of *C. fujisanus* were clearly distinguished from the other species by their connected SH and SS (Figures 4 and 5; in Ihara 2009, see figures 2-2-30-36, 37 for *C. mellotteei*, figures 2-2-30-38, 39 for *C. sanctus*, figure 2-2-30-40 for *C. shingenni*, and figures 2-2-30-44, 45 for *C. jaanaensis*). Males of *C. fujisanus* can be distinguished by the PA shape (Figure 3A–C): the prolateral surface of the PA in *C. fujisanus* is more rounded, and the PA bears fewer peg setae than in *C. jaanaensis* (see figures 2-2-30-46, 47 in Ihara 2009); the PA in *C. fujisanus* is thicker with more peg setae than in *C. mellotteei* (see figure 2-2-30-41 in Ihara 2009), *C. sanctus* (see figure 2-2-30-42 in Ihara 2009), and *C. shingenni* (see figure 2-2-30-43 in Ihara 2009). Moreover, *C. fujisanus* were only found around its type locality and are relatively pale-colored.

Material examined. Holotype: 1♀, Kaneyama-fûketsu Cave, Fuji-yoshida City, Yamanashi Prefecture, Japan, 24 v 1970, NSMT-Ar 65. Additional specimens, from near the type locality, Kaneyama Waterfall, Fuji-yoshida City, Yamanashi Prefecture, Japan, 35.4571°N, 138.8068°E (Figure 1): 9♀♀, 14 xi 2011 (N. Koike), KUZ Z3925–Z3927; 3 juv., 4 ix 2012 (N. Koike), KUZ Z3928; 1♂5♀♀ and 1 juv., 26 ix 2012 (N. Koike), KUZ Z2412, Z2413, Z3930.

Type locality. Korin-no-fuketsu Cave (= Kaneyama-fûketsu Cave in Yaginuma 1972), Fuji-yoshida City, Yamanashi Prefecture, Japan (cave entrance: 35.4571°N, 138.8068°E).

Description. Male (KUZ Z2412: Figures 2B–C, 3). Measurements (mm): CL 2.12, CW 1.43; head 0.86 wide; abdomen 2.05 long, 1.57 wide; ocular area 0.22 long, 0.54 wide; sternum 1.04 long, 0.95 wide; CW/CL 0.68, TibIL/CL 0.79. Leg formula, IV > I > II > III; length

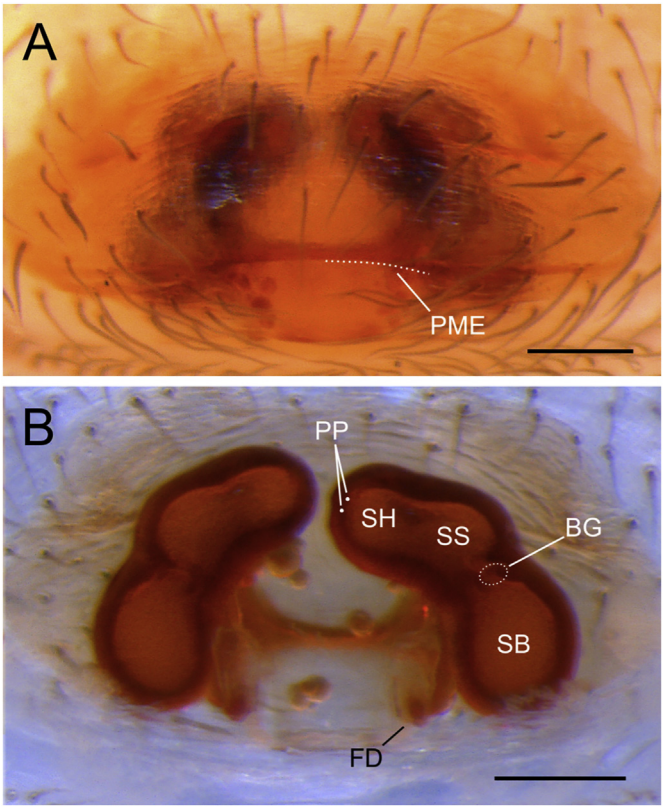

Figure 4. *Cybaeus fujisanus* Yaginuma, female (KUZ Z3926: A; KUZ Z3927: B).: A, Epigyne, ventral view; PME = posterior margin of epigynal plate; B, spermathecae, dorsal view; BG = Bennett's gland; FD = fertilization duct; PP = primary pore; SB = spermathecal base; SH = spermathecal head; SS = spermathecal stalk. <scale bar: 100 μm (A-B)>.

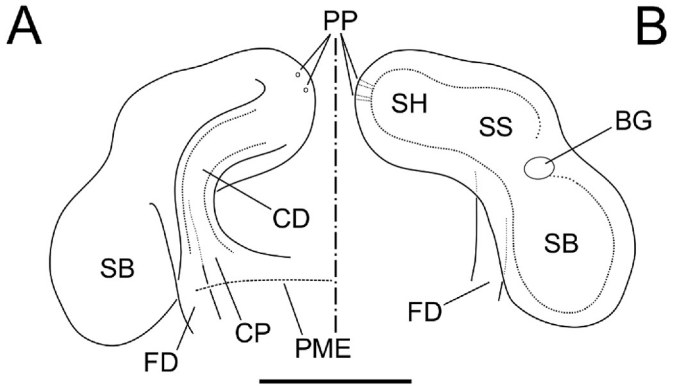

Figure 5. *Cybaeus fujisanus* Yaginuma, schematic drawing of the epigyne and spermathecae of a female specimen (KUZ Z3927).: A, Ventral view; CD = copulatory duct; CP = copulatory pore; FD = fertilization duct; PME = posterior margin of epigynal plate; SB = spermathecal base; PP = primary pore; B, dorsal view; BG = Bennett's gland; FD = fertilization duct; PP = primary pore; SB = spermathecal base; SH = spermathecal head; SS = spermathecal stalk. <scale bar: 100 μm (A-B)>.

of legs (femur + patella + tibia + metatarsus + tarsus): leg I 6.96 (1.86 + 0.62 + 1.68 + 1.63 + 1.17); leg II 6.69 (1.80 + 0.60 + 1.57 + 1.62 + 1.11); leg III 5.98 (1.66 + 0.57 + 1.24 + 1.57 + 0.95); leg IV 7.59 (1.96 + 0.62 + 1.70 + 2.11 + 1.20).

Carapace (Figure 2B). Head narrow, ca 0.60 × as wide as thoracic region; thoracic region almost as high as head. AER straight in frontal view; PER straight in dorsal view; AME smallest, < 1/2 diameter of other eyes; ocular area ca 2.5 × wider than long. Clypeus shorter than median ocular area.

Mouthparts. Chelicerae slightly geniculate, promargin of fang furrow with three teeth (median one largest), retromargin with two teeth and five denticles, and basally with lateral condyle. Labium wider than long.

Leg macrosetae (right). Leg I: tibia p2, r2, v2-2-2-2; metatarsus p4, r3, v2-2-3. Leg II: tibia p3, r2, v2-2-1-2; metatarsus p4, r3, v2-2-3.

Abdomen (Figure 2C). Oval; mid-posterior part widest. Colulus two groups of two setae.

Palp (Figure 3). PA distally pointed, directed anteriorly, dorsolateral surface relatively flat with eight peg setae. Tibia almost as long as patella; RTA plate-like, quadrangular, occupying 1/2 of length of tibia. Cymbium relatively wide, ca 2.1× longer than wide, slightly prolaterally expanded. Genital bulb circular in ventral view. Conductor: distal part short; PCO short, slightly curved. EM simple, originating and terminating, respectively, at ca 11 o'clock and ca 5 o'clock in ventral view.

Color (Figure 2B–C). Carapace: head pale beige; thoracic region pale beige, with radiating pale yellowish beige bands faintly. Chelicerae, maxillary lobe, and labium pale yellowish beige. Sternum pale beige. Legs pale beige to beige, without annulations. Abdomen: dorsally pale beige without clear chevron-like markings; ventrally pale beige.

Female (KUZ Z3926: Figures 2A and D-E, 4A; KUZ Z3927: Figures 4B, 5). Measurements (mm): CL 2.34, CW 1.52; head 1.04 wide; abdomen 3.12 long, 2.31 wide; ocular area 0.26 long, 0.63 wide; sternum 1.15 long, 1.00 wide. Leg formula, IV > I > II > III; length of legs (femur + patella + tibia + metatarsus + tarsus): leg I 6.52 (1.81 + 0.70 + 1.56 + 1.38 + 1.07); leg II 5.93 (1.63 + 0.66 + 1.38 + 1.33 + 0.92); leg III 5.42 (1.39 + 0.62 + 1.14 + 1.43 + 0.84); leg IV 6.87 (1.76 + 0.63 + 1.60 + 1.84 + 1.05).

Carapace (Figure 2D). Head ca 0.68 × as wide as thoracic region; thoracic region almost as high as head. AER straight in frontal view; PER straight in dorsal view; AME smallest, < 1/2 diameter of other eyes; ocular area ca. 2.4 × wider than long. Clypeus shorter than median ocular area.

Mouthparts. Chelicerae geniculate, promargin of fang furrow with 3 teeth (median one largest), retromargin with three teeth and four denticles, and basally with lateral condyle. Labium wider than long.

Leg macrosetae (right). Leg I: tibia p2, r0, v2-2-2-2; metatarsus p2, r1, v2-2-3. Leg II: tibia p4, r0, v2-2-1-2; metatarsus p3, r1, v2-2-3.

Abdomen (Figure 2E). Oval; mid-posterior part widest. Colulus two groups of three setae.

Genitalia (Figures 4 and 5). PME straight. Atrium located posteromedially on epigyne. CPs separated on both sides of atrium; CD short, curved and running anteriorly. SH and SS bulbous and almost continuous; SH with PP located medially; SS located laterally to SH; SB bulbous, directed posteriorly; BG located dorsally at connection between SS and SB. FD running from connection part between SS and SB, descending posteriorly, and then turned anterodorsally.

Color (Figure 2D and E). Carapace: head yellowish beige; thoracic region yellowish beige, with radiating darker yellowish beige bands faintly. Chelicerae, maxillary lobe, and labium yellowish brown. Sternum yellowish beige. Legs pale yellowish beige, without annulations. Abdomen: dorsally beige with pale beige chevron-like markings; ventrally pale beige.

Variation. Females. Measurements (mean ± 1SD, followed by ranges in parentheses; $n = 13$): CL 2.03 ± 0.19 (1.77–2.34), CW 1.33 ± 0.11 (1.20–1.52); CW/CL 0.66 ± 0.03 (0.63–0.73); TibIL 1.37 ± 0.15 (1.17–1.62); TibIL/CL 0.68 ± 0.02 (0.64–0.71).

DNA sequences. In total, four sequences were obtained: male (KUZ Z2412), *ITS-1* (LC622071; 673 bp) and *COI* (LC622070; 658 bp); and female (KUZ Z3930), *ITS-1* (LC622073; 673 bp) and *COI* (LC622072; 658 bp).

The respective nuclear *ITS-1* and mitochondrial *COI* sequences obtained from the male and female were completely identical. Therefore, the male and female clearly belong to the same species.

Remarks. The type locality of *C. fujisanus* was first stated in Yaginuma (1972) as "Kaneyama-fûketsu Cave," or Kaneyama Wind Cave. This cave is now called "Korin-no-fuketsu," or Korin Wind Cave, but is also known as "Korin-no-douketsu," or Korin Cave. The entrance of this cave is located approximately 30 m from "Kaneyama-no-taki," or Kaneyama Waterfall, which is also known as "Osano-no-taki," or Osano Waterfall. The location of this cave and waterfall corresponds to the type locality depicted in the original description (see figure 1 in Yaginuma 1972).

A holotype slide of the female genitalia (not shown; but see figure 2-2-30-187 in Ihara 2009) showed characteristics that are concordant with those of the specimens newly collected around Kaneyama Waterfall. Although Ihara (2009) showed that the CD of the holotype spermatheca turned before reaching the SH, the present examination of the holotype and newly collected females clarified that the CD of *C. fujisanus* directly ascends to the SH without a loop. According to the morphological characteristics and nuclear *ITS-1* and mitochondrial *COI* sequences, the newly collected male and females unquestionably represent *C. fujisanus*. Because these specimens were collected outside the cave, this species may inhabit underground habitats around the type locality given its pale coloration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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