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AUTHOR(S):

Sugawara, Yusuke; Koike, Naoki; Nakano, Takafumi

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A new species of *Cybaeus* with short genitalia from central Honshu, Japan (Araneae: Cybaeidae)

Yusuke Sugawara^{1,2*}, Naoki Koike^{1,3} & Takafumi Nakano¹

¹Department of Zoology, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

²E-mail: sugawara@zoo.zool.kyoto-u.ac.jp

³982 Minamichitose-machi, Nagano 380-0822, Japan

*Corresponding author

Abstract — Spiders of the genus *Cybaeus* are diverse in Japan and exhibit extensive morphological variation of their genitalia among species. Among Japanese *Cybaeus*, several species possess an elongated embolus in males and elongated spermathecae in females. Here, we describe *Cybaeus iharai* sp. nov. from Nagano Prefecture, central Honshu, Japan, which possesses a short embolus in males and short copulatory ducts in females. In addition, DNA sequences of the new species were obtained for future phylogenetic analyses.

Key words — Arachnida, *Cybaeus iharai* sp. nov., RTA clade, spider, taxonomy

Introduction

Spiders of the genus *Cybaeus* L. Koch 1868 are diverse in western North America and the Japanese Archipelago (Copley et al. 2009); to date, more than 90 *Cybaeus* species have been described from Japan (World Spider Catalog 2022). The taxonomic status of *Cybaeus* species inhabiting western Honshu Island, Japan has been continuously investigated since the 2000s (e.g., Ihara 2003; Ihara & Nojima 2004). However, species richness and taxonomic accounts of *Cybaeus* species indigenous to central Honshu have only been investigated by Kobayashi (2006), and dozens of species likely remain undescribed.

Cybaeus spiders show morphological variation of their genital organs (Ihara 2007, 2008). The majority of Japanese *Cybaeus* possess a simple embolus and spermathecae, each of which consists of three distinct bulbous parts: the spermathecal head, stalk, and base. However, several species, e.g., *C. melanoparvus* Kobayashi 2006, *C. monticolus* Kobayashi 2006, and *C. hikidai* Ihara, Koike & Nakano 2021, possess a remarkably elongated embolus and spermathecae. Therefore, the genitalia of Japanese *Cybaeus* were classified into two types based on these features in a previous study (Ihara et al. 2021): ‘Type 1,’ which refers to common genitalia, and ‘Type 2,’ which refers to elongated genitalia. However, the function of each genital part and copulatory behaviors of *Cybaeus* spiders are unclarified; thus, the evolution of elongated genitalia in Type 2 species has not been fully revealed.

An unidentified *Cybaeus*, which possesses Type 1 spermathecae, was collected from Nagano Prefecture, central

Honshu, Japan. They possess a short embolus in males and short copulatory ducts in females. Here, we describe it as a new species, and briefly discuss its short genitalia. Additionally, DNA sequences were obtained for future phylogenetic analyses.

Materials and methods

Samples and morphological examination

Cybaeus spiders were collected from central Honshu Island, Japan (Fig. 1) between 2010 and 2013. Specimens were preserved in 70% ethanol, and legs of some specimens were removed and preserved in 99% ethanol for DNA extraction. Epigynes were dissected from female specimens and then cleared to observe their internal structure following the method described by Matsuda et al. (2020). Examination of the specimens was conducted using a stereoscopic microscope (M125C, Leica Microsystems, Wetzlar, Germany). Images of the specimens were captured with a Leica MC170 HD digital camera mounted on the Leica M125C and analyzed using Leica Application Suite (LAS) v. 4.12. Measurements were taken to the nearest 0.01 mm in LAS. All specimens examined in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

Terminology of morphological characters and the chaetotaxy of leg macrosetae followed Ihara et al. (2021). The abbreviations for macrosetae: p, prolateral; r, retrolateral; v, ventral. The abbreviations 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; EPM,

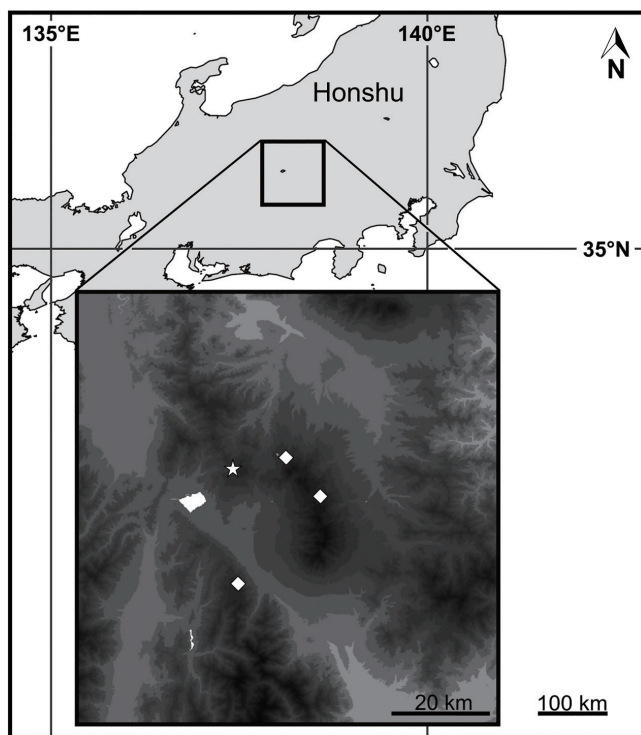


Fig. 1. Map showing collection localities of samples examined in the present study. Star denotes the type locality of *Cybaeus iharai* sp. nov.; diamonds denote the localities of *C. iharai* sp. nov. Shoreline data were based on Wessel and Smith (1996).

epigynal plate posterior margin; FD, fertilization duct; PA, patellar apophysis; PCO, proximal arm of conductor; PER, posterior eye row; 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

The following four markers were obtained from the holotype male to aid future phylogenetic studies: nuclear internal transcribed spacer 1 (ITS-1), nuclear histone H3 (H3), nuclear 28S rRNA (28S), and mitochondrial cytochrome *c* oxidase subunit I (COI). Additionally, ITS-1 and COI sequences from a female paratype were obtained to confirm the conspecificity of male and female specimens examined in this study. The sequences were deposited with the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan.

Genomic DNA extraction and cycle sequencing reaction methods were conducted as described by Matsuda et al. (2020); primer sets and conditions for the polymerase chain reactions and cycle sequencing reactions used in this study followed Sugawara et al. (2021).

Taxonomy

Genus *Cybaeus* L. Koch 1868

Cybaeus iharai sp. nov.

[Japanese name: Ihara-namihagumo]
(Figs. 2–5)

Diagnosis. “Small-sized” Japanese *Cybaeus* in central Honshu (Fig. 2A). *Cybaeus iharai* sp. nov. was only found around its type locality (Fig. 1), and no particularly similar small-sized species have been described from the adjacent areas. Both sexes of *C. iharai* sp. nov. are clearly distinguished from the other small-sized Japanese *Cybaeus* species by their genital characters. Males of *C. iharai* sp. nov. are clearly distinguished by its spatula-like PA with two corners on its distal part and with ca. 10 peg setae in dorsal view (Fig. 3A–C), and by its short embolus with bent distal tip. Females of *C. iharai* sp. nov. are distinguished by its spermathecae (Figs. 4–5), especially its remarkably short CDs running anteriorly and curved dorsally, ducts from SH running laterally to SS, similar-sized SS and SB, and its overall arrangements of SH, SS, and SB.

Material examined. Holotype: ♂, KUZ Z4023; Showa Temple, Mt. Kirigamine, Shimosuwa, Nagano Pref., Japan; 36.1017°N, 138.1585°E; 28 Aug. 2010, collected by Naoki Koike.

Paratypes: 1♀, KUZ Z4021, same collection data as for holotype. 1♀, KUZ Z4022, same collection data as for holotype. 1♂, KUZ Z4024, same collection data as for holotype. 1♂, KUZ Z4025; Gosensui Natural Park, Mt. Tateshina, Tateshina, Nagano Pref., Japan; 36.1282°N, 138.2921°E; 15 Aug. 2012. 1♂, KUZ Z4026; Mt. Nyukasa, Fujimi, Nagano Pref., Japan; 35.8991°N, 138.1733°E; 17 Aug. 2012. 1♀, KUZ Z4027, same collection data as KUZ Z4026. 2♂ and 2♀, KUZ Z4028; near Shirakoma Pond, Sakuho, Nagano Pref., Japan; 36.0593°N, 138.3606°E; 10 Aug. 2013. All specimens were collected by Naoki Koike.

Type locality. Showa Temple, Mt. Kirigamine, Shimosuwa, Nagano Pref., Japan (36.1017°N, 138.1585°E).

Description. *Male* (holotype, KUZ Z4023: Figs. 2B–C, 3). Measurements (mm): CL 2.11, CW 1.52; head 0.88 wide; abdomen 2.48 long, 2.10 wide; ocular area 0.27 long, 0.52 wide; sternum 1.12 long, 0.99 wide; CW/CL 0.72, TibIL/CL 0.68. Leg formula, IV > I > II > III; length of legs (femur + patella + tibia + metatarsus + tarsus): leg I 6.02 (1.61 + 0.70 + 1.44 + 1.31 + 0.96); leg II 5.58 (1.54 + 0.67 + 1.26 + 1.19 + 0.92); leg III 5.07 (1.39 + 0.58 + 1.02 + 1.27 + 0.81); leg IV 6.72 (1.80 + 0.65 + 1.50 + 1.76 + 1.01).

Carapace (Fig. 2B). Head narrow, ca. 0.58× 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. 2.0× wider than long. Clypeus shorter than median ocular area.

Mouthparts. Chelicerae slightly geniculate, promargin of fang furrow with 3 teeth (median one largest), retromargin

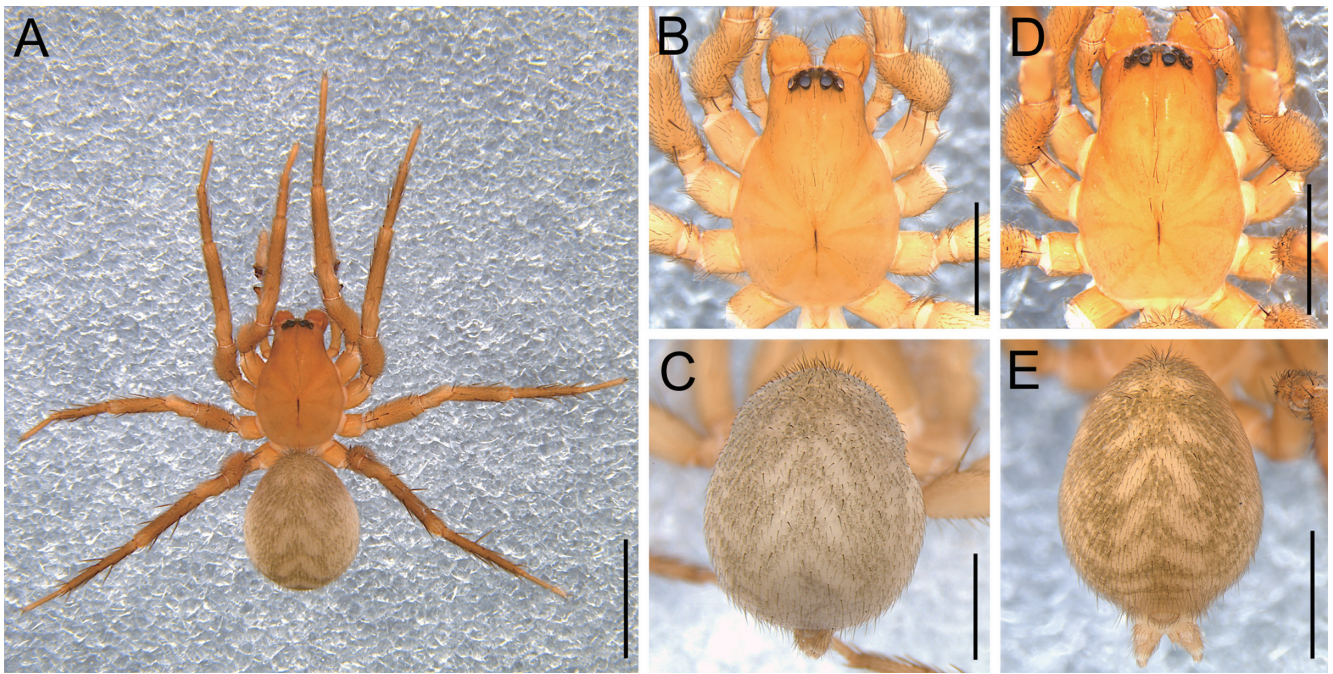


Fig. 2. *Cybaeus iharai* sp. nov., male paratype (KUZ Z4024; A), male holotype (KUZ Z4023; B–C), and female paratype (KUZ Z4021; D–E). A, habitus, dorsal view; B, D, prosoma, dorsal view; C, E, abdomen, dorsal view. Scales = 2 mm (A); 1 mm (B–E).

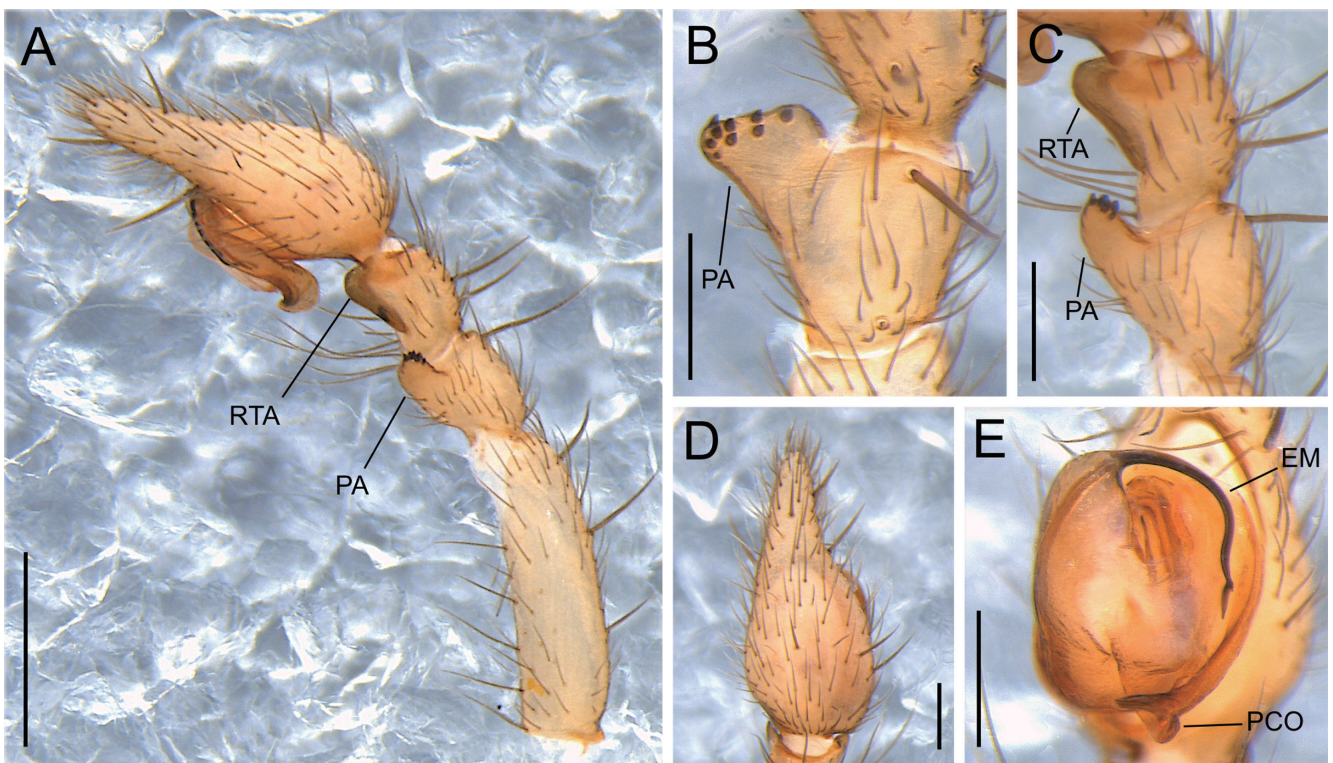


Fig. 3. *Cybaeus iharai* sp. nov., male holotype (KUZ Z4023). A, left palp, retrolateral view; B, patella of left palp, dorsal view; C, tibia and patella of left palp, retrolateral view; D, cymbium of left palp, dorsal view; E, bulb of left palp, ventral view. Abbreviations: EM, embolus; PA, patellar apophysis; PCO, proximal arm of conductor; RTA, retrolateral tibial apophysis. Scales = 500 μ m (A); 200 μ m (B–E).

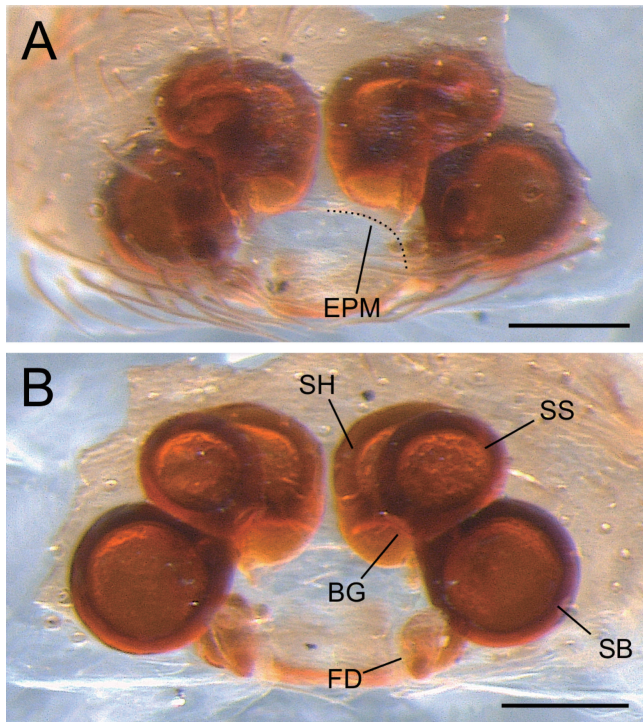


Fig. 4. *Cybaeus iharai* sp. nov., female paratype (KUZ Z4021). A, epigyne, ventral view; B, spermathecae, dorsal view. Abbreviations: BG, Bennett's gland; EPM, epigynal plate posterior margin; FD, fertilization duct; SB, spermathecal base; SH, spermathecal head; SS, spermathecal stalk. Scales = 100 μ m.

with 2 teeth and 5 denticles, and basally with lateral condyle. Labium wider than long.

Leg macrosetae (left legs). Leg I: tibia p3, r1, v2-2-2; metatarsus p1, r1, v2-2-3. Leg II: tibia p2, r0, v2-2-1-2; metatarsus p2, r1, v2-2-3.

Abdomen (Fig. 2C). Colulus 1 seta on each side.

Palp (Fig. 3). PA directed anteriorly, spatula-like in dorsal view, dorsal surface flat, anterodorsal surface with 9 peg setae, anterior margin almost straight, perpendicular to palpal axis. Tibia as long as patella; RTA plate-like, occupying 3/4 of length of tibia. Cymbium relatively wide, ca. 2.2 \times longer than wide, slightly expanded prolaterally. Genital bulb almost circular in ventral view. Conductor: distal part short; PCO short, curved prolaterally. EM short, distal tip bent, originating and terminating, respectively, at ca. 12 o'clock and ca. 4 o'clock in ventral view.

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

Female (paratype, KUZ Z4021: Figs. 2D–E, 4–5). Measurements (mm): CL 1.97, CW 1.29; head 0.88 wide; abdomen 2.20 long, 1.65 wide; ocular area 0.25 long, 0.55 wide; sternum 0.96 long, 0.90 wide; CW/CL 0.65, TibIL/CL 0.58. Leg formula, IV > I > II > III; length of legs (femur + patella

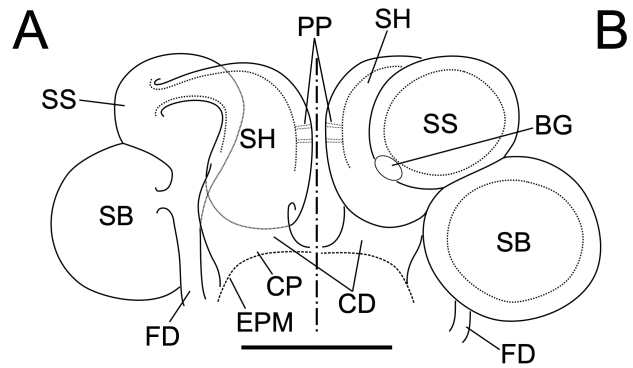


Fig. 5. *Cybaeus iharai* sp. nov., schematic drawing of the epigyne and spermathecae of a female paratype (KUZ Z4021). A, ventral view; B, dorsal view. Abbreviations: BG, Bennett's gland; CD, copulatory duct; CP, copulatory pore; EPM, epigynal plate posterior margin; FD, fertilization duct; PP, primary pore; SB, spermathecal base; SH, spermathecal head; SS, spermathecal stalk. Scales = 100 μ m.

+ tibia + metatarsus + tarsus): leg I 4.81 (1.35 + 0.58 + 1.15 + 1.00 + 0.72); leg II 4.44 (1.29 + 0.57 + 0.99 + 0.90 + 0.68); leg III 4.10 (1.13 + 0.54 + 0.81 + 0.99 + 0.63); leg IV 5.33 (1.41 + 0.57 + 1.19 + 1.39 + 0.78).

Carapace (Fig. 2D). Head ca. 0.68 \times as wide as thoracic region; thoracic region height slightly shorter than head. AER straight in frontal view; PER straight in dorsal view; AME smallest, < 1/2 diameter of other eyes; ocular area ca. 2.2 \times wider than long. Clypeus shorter than median ocular area.

Mouthparts. Chelicerae geniculate, promargin of fang furrow with 3 teeth (median one largest), retromargin with 4 teeth and 4 denticles, and basally with lateral condyle. Labium wider than long.

Leg macrosetae (left legs). Leg I: tibia p1, r0, v2-2-1; metatarsus p1, r0, v2-2-2. Leg II: tibia p2, r0, v1-1-1-0; metatarsus p1, r0, v2-2-3.

Abdomen (Fig. 2E). Colulus 1 seta on each side.

Genitalia (Figs. 4–5). EPM curved. Atrium located posteromedially on epigyne. CPs separated on anterior side of atrium; CD thick and short, running anteriorly and curved dorsally. SH developed, SH with PP located medially; SS bulbous, located dorsally to SH; SB bulbous, directed laterally; BG located on SS posteromedially around connection between SS and SB; FD running from duct between SS and SB, descending posteriorly, and then turned anterodorsally.

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

Variation. **Males.** Measurements (mean \pm 1SD, followed by ranges in parentheses; n = 6): CL 2.15 \pm 0.04 (2.08–2.19), CW 1.50 \pm 0.04 (1.44–1.54); CW/CL 0.70 \pm 0.01 (0.68–0.72); TibIL 1.40 \pm 0.05 (1.34–1.45); TibIL/CL 0.65 \pm 0.02 (0.63–0.68). Legs longer than those of females. Dorsal surface of PA with 9–12 peg setae. Distal tip of EM bent in all

specimens.

Females. Measurements (mean \pm 1SD, followed by ranges in parentheses; $n = 5$): CL 2.05 ± 0.07 (1.97–2.15), CW 1.34 ± 0.06 (1.28–1.42); CW/CL 0.65 ± 0.01 (0.64–0.67); TibIL 1.12 ± 0.06 (1.06–1.18); TibIL/CL 0.55 ± 0.04 (0.49–0.58).

Distribution. This species inhabits the high montane area (ca. 1500–2100 m in elevation) around Lake Suwa, i.e., Mt. Kirigamine, the Yatsugatake Mountains, and the northern Akaishi Mountains, in Nagano Pref., Japan (Fig. 1).

DNA sequences. In total, six sequences were obtained: male holotype (KUZ Z4023), four sequences, ITS-1 (INSD accession number, LC668570; 681 bp), H3 (LC668568; 328 bp), 28S (LC668565; 790 bp), and COI (LC668566; 763 bp); and female paratype (KUZ Z4021), two sequences, ITS-1 (LC668569; 442 bp) and COI (LC668567; 763 bp).

According to these nuclear ITS-1 and mitochondrial COI sequences, the male and female specimens examined clearly belonged to the same species that was newly described in this study. Their overlapping aligned positions of ITS-1 and COI sequences (442 bp and 763 bp, respectively) were completely identical.

Etymology. The specific name is dedicated to Dr. Yoh Ihara, who has contributed to systematics of Japanese *Cybaeus*.

Remarks. Several specimens were collected under rotten trees in dry forests; therefore, this species may prefer epigeic habitat despite its relatively pale coloration.

Sister species and close congeners of *C. iharai* sp. nov. have not been elucidated yet; thus, insights into the evolutionary history of the short genitalia of the new species cannot be discussed here. However, the embolus (Fig. 3E) and copulatory ducts (Figs. 4A, 5A) of *C. iharai* sp. nov., which are among the shortest of the Type 1 species, may provide new insights into the evolutionary history of *Cybaeus* genital length. Additionally, the distal tip of the embolus is bent in all male specimens (Fig. 3E). Future systematic studies and elucidation of genital organ function may provide a better understanding of the evolution of the variable features of *Cybaeus* genitalia.

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