Description of a New Species of Cybaeus (Araneae: Cybaeidae) from Central Honshu, Japan

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A new spider species, Cybaeus daimonji, from Kyoto, western-central Honshu, Japan is described based on both sexes. The shape of epigyne indicates that this new species is close to C. communis Yaginuma, 1972, C. kirigaminensis Komatsu, 1963, C. maculosus Yaginuma, 1972 and C. shinkaii (Komatsu, 1970), which are distributed in eastern to central Honshu.

Nuclear internal transcribed spacer 1, 28S ribosomal RNA and histone H3 as well as mitochondrial cytochrome c oxidase subunit I, 12S ribosomal RNA and 16S ribosomal RNA sequences of the new species are provided for future phylogenetic studies.

Key Words: Arachnida, RTA clade, epigeic, retreat, Mt. Daimonjiyama, molecular identification.

Introduction

Cybaeus L. Koch, 1868 is the type genus of Cybaeidae, a member of the “marronoid clade” of the RTA clade (Wheeler et al. 2017) the members of which possess a retrolateral tibial apophysis (RTA) on the male palp (Coddington and Levi 1991; Griswold et al. 1999, 2005). Cybaeus spiders are epigeic, inhabiting moist woodlands, and are widely distributed in the Holarctic region, i.e., Europe to Caucasus and the southern Far East in the Palearctic and eastern and western North America in the Nearctic (Bennett 2017; World Spider Catalog 2020). The genus now comprises 161 species (World Spider Catalog 2020), and is most diverse in Far East Asia, especially on the Japanese Archipelago and in western North America (Copley et al. 2009). In Japan, 82 species of Cybaeus are known so far (World Spider Catalog 2020). In addition, 14 species have been described from the Korean Peninsula (Seo 2016), and five species have been described from the Russian Far East and Kuril Islands (Marusik and Logunov 1991; Marusik and Kovblyuk 2011). These figures illustrate the significant diversity of the genus (over 100 species) in Far East Asia.

To help delimit the diversity of this species-rich taxon, informal species-groups have been recognized for the Japanese and North American Cybaeus species based on characters of the male palp and female genitalia (Ihara 2003b, 2007, 2008, 2009a, b; Ihara and Nojima 2004; Copley et al. 2009; Bennett et al. 2016, 2019). In addition, the Japanese species have been conveniently divided into three groups according to body length of mature spiders (Ihara 2004) as defined by Roth (1993): body length less than 5.0 mm (“small-sized”), body length ranging from 5.0 mm to 10 mm (“medium-sized”), and body length greater than 10 mm (“large-sized”). Based on a combined molecular and morphological analysis, a phylogenetic backbone of Holarctic and Californian clades for North American and European Cybaeus species has been established (Copley et al. 2009; Bennett et al. 2016, 2019); similar work has not yet been done for the Japanese Cybaeus species.

An unidentified “medium-sized” Cybaeus, collected from the montane area of the eastern part of Kyoto City, central Honshu Island, Japan, is described as a new species in this study. In addition, its DNA sequences of several nuclear and mitochondrial genetic markers are provided to aid future phylogenetic studies to determine its phylogenetic position within the Japanese Cybaeus fauna.

Materials and Methods

Sampling and morphological examination. During the period October to November in 2019, Cybaeus spiders were collected from 13 locations in montane regions in the north to eastern parts of Kyoto City, Honshu, Japan. When possible, geographical coordinates for the collection sites were obtained using a Garmin eTrex® GPS unit. Specimens were preserved in 70% ethanol; legs of some specimens were removed and preserved in 99% ethanol for DNA extraction.

Epigynes were dissected from several female specimens, and cleared with proteinase K (100 µg/ml) at 37°C for 8–10 hours, or at 55°C for 2–3 hours to observe the internal structure. Morphological examination of the specimens was conducted using a Leica M125C stereoscopic microscope. Images of the specimens were captured with the aid of a Leica MC170 HD digital camera mounted on the Leica
M125C, and prepared using a Leica Application Suite (LAS) v. 4.12 software. Measurements were taken to the nearest 0.01 mm using LAS. Specimens examined in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

Terminology of morphological characters follows Bennett (2005, 2017) and Bennett et al. (2016, 2019), nonetheless, a conductor is adopted for the structure on the bulb, which was defined as a proximal arm of the regular apophysis in these studies. The chaetotaxy and arrangement of leg spines follow Komatsu (1968); abbreviations: p, prolateral; r, retrolateral; and v, ventral.

**PCR and DNA sequencing.** The methodology for extraction of genomic DNA from leg muscle was modified from Nakano (2012). Primer sets for the polymerase chain reactions (PCR) and cycles sequencing (CS) reactions used for nuclear histone H3 (H3), internal transcribed spacer 1 (ITS-1), and mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S) were shown in Nakano et al. (2017). Those for nuclear 28S ribosomal RNA (28S) were 28SO (Hedin and Maddison 2001) and 28SrCy500 (\(5’\-GCTCTTTACAAGACCCATGGA-3’\)), and 28Sa and 28Sb (Whiting et al. 1997); and that for mitochondrial 12S ribosomal RNA (12S) was 12Sai and 12Sbi (Simon et al. 1990, 1994). The primer 28SrCy500 was newly designed at the Primer3 website (Koressaar and Remm 2007; Untergasser et al. 2012).

All PCR reactions were performed using a GeneAmp PCR System 9700 (Applied Biosystems; ABI), or a GeneAtlas (ASTEC) using an Ex Taq Polymerase Kit (Takara Bio Inc.). The PCR mixtures were heated to 94°C for 6 min, followed by 35 cycles at 94°C (10 s), 40°C for COI and 16S or 50°C for the other markers (20 s), and 72°C (42 s), with a final extension at 72°C for 6 min. The amplified DNA fragments were purified using polyethylene glycol (20% PEG 6000) precipitation.

All samples were sequenced in both directions. The CS reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (ABI). Each CS reaction mixture was incubated at 96°C for 2 min, followed by 40 cycles of 96°C (10 s), 50°C (5 s), and 60°C (42 s). The products were collected by ethanol precipitation and sequenced on an ABI 3130xl (10 s), 50°C (5 s), and 60°C (42 s). The products were collected by ethanol precipitation and sequenced on an ABI 3130xl Genetic Analyzer. The obtained sequences were edited using DNA BASER (Heracle Biosoft S.R.L.). The DNA sequences obtained in this study were deposited with the International Nucleotide Sequence Database Collaboration through the DNA Data Bank of Japan.

**Taxonomy**

Genus *Cybaeus* L. Koch, 1868

*Cybaeus daimonji* sp. nov.

[New Japanese name: Daimonji-namihagumo]

(Figs 1–5)

**Diagnosis.** "Medium-sized" Japanese *Cybaeus*. Females of *C. daimonji* sp. nov. resemble those of *C. communis* Yaginuma, 1972, *C. kirigaminensis* Komatsu, 1963, *C. maculosus* Yaginuma, 1972 and *C. shinkaii* (Komatsu, 1970), which also are "medium-sized" and have a similar postero medi ally located atrium and inverted V-shaped spermathecae [figs 2-2-30-18–23 in Ihara (2009a)]. However, females of *C. daimonji* sp. nov. can be discriminated from those of the other four species by the copulatory ducts running toward the medial part of respective spermathecae, while in the other four congers the copulatory ducts run directly toward the respective spermathecal heads [for *C. communis*, based on an unpublished observation by Yoh Ihara; for *C. kirigaminensis*, pl. 4, fig. H in Komatsu (1963); for *C. maculosus*, fig. 38 in Yaginuma (1972); and for *C. shinkaii*, fig. 4 in Komatsu (1970)]. Males of *C. daimonji* sp. nov. possess a palpal tibia, which is shorter than the palpal patella, and thus can be clearly discriminated from those of *C. communis* [fig. 37 in Yaginuma (1972)], *C. kirigaminensis* [pl. 4, fig. D in Komatsu (1963)] and *C. shinkaii* [fig. 2-2-30-17 in Ihara (2009a)] bearing a tibia that is longer than the patella [see also figs 2-2-30-14–16 in Ihara (2009a)].

**Material examined.** Holotype: KUZ Z2753 (Fig. 1A), male, under rotten wood in Mt. Daimonjiyama, Sakyo ku, Kyoto City, Kyoto Prefecture, Japan (35.027462°N, 135.801530°E), Kenji Matsuda (KM), 1 November 2019. Paratypes (in total 13 specimens collected from the type locality by KM): 6 males, KUZ Z2754 (35.027378°N, 135.801555°E), KUZ Z2755 (35.027462°N, 135.801530°E), KUZ Z2756–Z2757 (35.027280°N, 135.801641°E), KUZ Z2758 (35.026685°N, 135.801831°E), KUZ Z2759 (35.024971°N, 135.802875°E), and 4 females, KUZ Z2761 (35.027302°N, 135.801637°E), KUZ Z2762 (35.027124°N, 135.801608°E), KUZ Z2763 (35.025709°N, 135.802005°E), KUZ Z2764 (35.025709°N, 135.802005°E), 1 November 2019; 3 females,

![Fig. 1. *Cybaeus daimonji* sp. nov. A, dorsal view of holotype male (KUZ Z2753); B, retreat of non-type male specimen (KUZ Z2782), from Mt. Hieizan, Kyoto, Japan, without scale. Abbreviation: OP, opening of retreat.](image-url)
A new Cybaeus from Japan


Description. Males. Measurements [KUZ Z2753 (holotype)]. Body length 6.27; carapace 3.04 long, 2.21 wide; abdomen 2.98 long, 2.46 wide; sternum 1.52 long, 1.38 wide; labium 0.45 long, 0.45 wide. Leg formula, IV > I > II > III; length of legs (femur + patella + tibia + metatarsus + tarsus): leg I 10.24 (2.56 + 0.88 + 2.54 + 2.45 + 1.81); leg II 9.29 (2.25 + 0.88 + 2.29 + 2.29 + 1.58); leg III 8.45 (2.24 + 0.80 + 1.92 + 2.16 + 1.33); leg IV 11.11 (2.78 + 0.90 + 2.66 + 3.14 + 1.63).

Carapace (Fig. 2A): head narrow, 0.57 times as wide as thoracic region; thoracic region as high as head. Anterior median eyes smallest, approximately one-half diameter of other eyes; anterior eye row straight in frontal view; posterior eye row almost straight in dorsal view; ocular area twice as wide as long. Clypeus length 0.62 times length of median ocular area in holotype. Chelicerae geniculate, promarginal fang furrow with 3 teeth, retromargin with 4 teeth and 5 denticles (= small teeth), and basal with lateral condyle. Abdomen (Fig. 2B) globular.
Leg spination: Leg I: tibia p3, r3 (left) or 2 (right), v2-2-2-2; metatarsus p4 (left) or 3 (right), r3 (left) or 2 (right), v2-2-3. Leg II: tibia p3, r3 (left) or 2 (right), v2-2-1-2; metatarsus p4, r4 (left) or 3 (right), v2-2-3.

Palp [KUZ Z2755 (paratype)] relatively slender (Fig. 3A). Patellar apophysis prominent on retrolateral anterior margin of patella, extended distally, slightly arched dorsally, triangular in dorsal view, distodorsal surface with 6–7 (left) or 6–8 (right) peg setae (Fig. 3B). Tibia slightly shorter than patella, retrolateral tibial apophysis (RTA), plate-like, occupying most of length of tibia, distal margin slightly extended (Fig. 3B). Cymbium slender, prolaterally unexpanded (Fig. 3C), 2.7 times longer than wide; 0.9 times as long as femur, 1.3 times as long as patella+tibia. Bulb elliptic (Fig. 4A); embolus simple, curved, originated and terminated, respectively, at ca. 11 o'clock and ca. 4 o'clock positions in ventral view (Fig. 4A, B); conductor simple, triangular, small (Fig. 4B, C).

Females. Measurements [KUZ Z2760 (paratype)]. Body length 6.02; carapace 3.14 long, 2.19 wide, head 1.47 wide; abdomen 3.28 long, 2.51 wide; sternum 1.47 long, 1.34 wide; labium 0.42 long, 0.48 wide. Legs shorter than those of male; leg formula, IV > I > II > III; length of legs (femur+patella+tibia+metatarsus+tarsus): leg I 7.96 (2.29+0.72+2.14+1.74+1.07); leg II 7.57 (2.29+0.69+1.82+1.65+1.12); leg III 6.69 (1.95+0.69+1.50+1.62+0.93); leg IV 8.51 (2.13+0.80+2.11+2.22+1.25).

Carapace (Fig. 2C) longer than that of male: head 0.67 times as wide as thoracic region, slightly wider than that of male. Abdomen (Fig. 2D) oval, slightly larger than that of male.

Leg spination. Leg I: tibia p2, v2-2-2-2; metatarsus p1, r2 (left) or 1 (right), v2-2-2. Leg II: tibia p3 (left) or 4 (right), v2-2-1-2; metatarsus p3, r2 (left) or 1 (right), v2-2-3.

Genitalia [KUZ Z2764 (paratype)]. Atrium slightly concave, located posteromedially on epigyne, anterior margin slightly curved, with two distinct copulatory openings (Fig. 5A). Copulatory ducts, running anterolaterally toward medial part of respective spermathecae (Fig. 5A, C). Paired spermathecae inverted V-shaped in ventral view; each spermatheca consisting of 3 well-defined parts, head, stalk and base (Fig. 5B, C); head globular, located anteromedially above epigynum, with 2 simple pores ventromedially, heads well separated from each other; stalk cylindrical, loosely coiled, with developed Bennett’s gland at junction with base; base larger than head or stalk, globular, located posterolaterally above epigynum. Pair of fertilization ducts narrow, running posteromedially (Fig. 5B, C).

Variation. Males. Measurements (mean±1SD, followed by ranges in parentheses; n=23, including holotype): body length 5.26±0.42 (4.40–6.27); carapace length 2.91±0.15 (2.48–3.14), width 2.14±0.13 (1.81–2.35), head width 1.26±0.08 (0.99–1.36); abdomen length 2.45±0.27 (2.00–2.98), width 1.81±0.24 (1.33–2.46); leg I 9.40±0.54 (7.97–10.24); leg II 8.89±0.52 (7.36–9.78); leg III 7.92±0.43 (6.61–8.72); leg IV 10.07±0.57 (8.30–11.10). Leg I meta-
tarsus with v2-2-3 spines (v2-2-1 only on right leg of KUZ Z2758). Distodorsal surface of patellar apophysis with 5–10 (left) or 5–11 (right) (usually 6, or 7) peg setae.

**Females.** Measurements [mean ± 1SD, followed by ranges in parentheses; n = 19, including KUZ Z2760: for body length, abdomen length and width, n = 7 (KUZ Z2760, Z2761, Z2763, Z2765, Z2783, Z2790, Z2794)]; body length 5.90 ± 0.54 (5.36–6.43); carapace length 3.13 ± 0.19 (2.78–3.52), width 2.18 ± 0.13 (2.02–2.29), head width 1.49 ± 0.09 (1.34–1.58); abdomen length 3.37 ± 0.39 (2.85–3.71), width 2.38 ± 0.37 (1.82–2.70); leg I 8.15 ± 0.39 (7.60–8.59); leg II 7.59 ± 0.43 (6.94–8.26); leg III 6.90 ± 0.41 (6.03–7.44); leg IV 8.89 ± 0.49 (8.19–9.44). Leg I metatarsus with v2-2-3 spines, but sometimes with v2-2-2 spines. Spermathecal head location slightly variable, located from anterior margin to almost middle part of epigynum, but heads always separated.

**Coloration.** Carapace yellowish brown with reticulate olive-black markings on lateral sides of head, and with radical olive-black bands on thorax. Chelicerae, maxillae, labium and sternum yellowish brown; chelicerae deeper than others. Legs also yellowish brown, but paler than carapace, with olive black annulations. Dorsum of abdomen olive black with dull-yellowish broken chevrons (Fig. 2).

**Retreat.** This species constructs a silken tube-like retreat, often V-shapes, with an opening at each end (Fig. 1B) and silk signal threads extending from each opening.

**Distribution.** This species appears to be restricted to a very small montane area of approximately 1.5 km² located on the western side of the southernmost part of Lake Biwa, Japan (Fig. 6). Spiders identified as *Cybaeus* daimonji* sp. nov. were collected from the western side of the Higashiyama-Sanjuroppo mountains. The confirmed northernmost habitat occupied by the species was located at Mt. Hieizan and the southernmost collection site was the type locality, Mt. Daimonjyama.

**DNA sequences.** In total 11 sequences were determined: paratype male (KUZ Z2755), six sequences, ITS-1 (LC529208; 676 bp), 28S (LC529207; 790 bp), H3 (LC529206; 328 bp), COI (LC529209; 658 bp), 12S (LC529211; 332 bp), and 16S (LC529210; 439 bp); and paratype female (KUZ Z2764), five sequences, ITS-1 (LC529214; 676 bp), 28S (LC529213; 790 bp), H3 (LC529212; 328 bp), COI (LC529215; 658 bp), and 12S (LC529216; 332 bp).

According to the nuclear ITS-1 and mitochondrial COI sequences obtained from the paratype male and paratype female, the males and females examined in this study clearly belong to the same species newly described in the present study. The ITS-1 sequences obtained from the male and female are almost concordant with each other (675/676 bp),
and their COI sequences are identical.

**Etymology.** The specific name is derived from the type locality, Mt. Daimonjiyama. The specific name is from a Japanese word, and thus treated as indeclinable.

**Remarks.** Females of *C. daimonji* sp. nov. may be most likely to be confused with females of *C. shinkaii* in their spermathecal characteristics. In addition to the feature of copulatory ducts, however, females of *C. daimonji* sp. nov. are distinguishable from those of *C. shinkaii* by spermathecal heads that are separated [vs. heads contiguous; fig. 2-2-30-23 in Ihara (2009a)]. Although males of *C. maculosus* remains unknown, Ihara (2009a) suggested a possibility that *C. maculosus* might belong to the same species as *C. communis*. Therefore, it is highly possible that males of *C. daimonji* sp. nov. are also distinguishable from those of *C. maculosus* by characteristics of the palpal tibia.

In addition to the four “medium-sized” congeners, i.e., *C. communis*, *C. kirigaminensis*, *C. maculosus*, and *C. shinkaii*, males of *C. daimonji* sp. nov. and the other “medium-sized” *C. tajimaensis* Ihara and Nojima, 2004 share similar RTA characteristics, but the former can be distinguished from the latter by a triangular patella apophysis with peg setae concentrated distodorsally, an elliptic bulb, and a simple triangular conductor [vs. a trapezoidal patella apophysis with peg setae widely distributed dorsally, an almost circular bulb, and a complex hook-like conductor in *C. tajimaensis*; figs 8E, 18 in Ihara and Nojima (2004)]. Although males of *C. daimonji* sp. nov. and the medium-sized *C. tottoriensis* Ihara, 1994 share the simple triangular conductor, *C. daimonji* sp. nov. differs from *C. tottoriensis* in its distally extended patellar apophysis with distodorsal peg setae [vs. retrolaterally extended patellar apophysis with peg setae laterally in *C. tottoriensis*; figs 11–13 in Ihara (1994)]. Females of *C. daimonji* sp. nov. can be unquestionably differentiated from females of *C. tajimaensis* and *C. tottoriensis* by their epigynal features.

**Discussion**

Over half of the Japanese species of *Cybaeus*, ca. 45 of 82 species (based on Yoh Ihara unpublished observation), build silken, tube-like retreats. Four types of retreat morphologies have been described, and of these, V-shaped with two openings is the most common (Ihara 2006). V-shaped with three openings, Y-shaped with three openings, and hexagonal with three openings (Komatsu 1961, 1968; Ihara 2003a, 2009b) are less common types of retreat. All described retreats of Japanese *Cybaeus* have silk signal threads attached to the openings. *Cybaeus daimonji* sp. nov. usually constructs the most common type of retreat. *Cicurina bryantae* Exline, 1936 (Hahniidae), a species indigenous to the southern Appalachians in southeastern North America, constructs a similar V-shaped retreat; the retreat of that species, however, does not have signal threads associated with its openings (Bennett 1985).

Previous systematic studies of the Nearctic species of *Cybaeus* have demonstrated the highly species-specific morphology of male palpal characteristics within informal morphology-based species-groups whereas the females within those species-groups share generally similar genital morphology (Bennett 2006; Copley et al. 2009; Bennett et al. 2016, 2019). Therefore, it is probable that *C. daimonji* sp. nov. is related to *C. communis* and the three other allied species with which it shares a very similar epigyne. Since any DNA sequences of *C. communis* and the three allied species have not been deposited yet, the phylogenetic relationships of these species may be clarified by future studies incorporating the DNA sequences of *C. daimonji* sp. nov. provided in this study.

**Key to Cybaeus daimonji sp. nov. and Congeners with a Similar Epigyne**

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<td>2. Palpal tibia shorter than palpal patella</td>
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<td>– Palpal tibia longer than palpal patella</td>
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<td>3. Apophysis of palpal patella without peg setae</td>
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<td>– Apophysis of palpal patella with few peg setae</td>
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<td>4. Copulatory ducts running toward medial part of respective spermathecae</td>
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<td>– Copulatory ducts running directly toward respective spermathecal heads</td>
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<td>5. Anterior margin of atrium slightly curved, spermathecal heads slightly recurved</td>
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<td>– Anterior margin of atrium almost straight, spermathecal heads globular</td>
<td>11</td>
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<td>6. <strong>C. kirigaminensis</strong> and <em>C. shinkaii</em></td>
<td>12</td>
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<td>7. <strong>C. communis</strong></td>
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<td>8. <em><strong>C. maculosus</strong></em></td>
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* Males of *C. maculosus* remains unknown [see Remarks above and Ihara (2009a)], and thus were not included in this key; ** although they had been diagnosed by characteristics of the palpal tibia and patella, and spermathecae, *C. kirigaminensis* and *C. shinkaii* were deemed to be hardly distinguishable by their morphological characteristics (Ihara 2009a); *** when epigynal features of *C. communis* were provided for the first time, it was also stated that females of *C. communis* and *C. maculosus* were morphologically indistinguishable (Ihara 2009a).

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References


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