

A new species of *Cybaeus* L. Koch, 1868 (Araneae, Cybaeidae) with simple genitalia from central Japan is the sister species of *C. melanoparvus* Kobayashi, 2006 with elongated genitalia

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Abstract

Spiders of the genus *Cybaeus* L. Koch, 1868 exhibit two major centers of diversity: Western North America and Japan. Several Japanese *Cybaeus* possess an elongated embolus in the male palp and elongated tubular spermathecae in the female genitalia. Here we describe *Cybaeus koikei* sp. nov. from central Honshu, Japan, which has an unelongated embolus and bulbous spermathecae. Phylogenetic analyses using nuclear and mitochondrial gene markers clearly support the monophyly of *C. koikei* sp. nov. and *Cybaeus melanoparvus* Kobayashi, 2006, a species with elongated genitalia. Both species share a similar habitus and a cluster of robust setae on the lateral surface of the male palpal patella. The latter is considered a synapomorphy for *C. koikei* sp. nov. and *C. melanoparvus*. A supplementary description of the spermathecae of *C. melanoparvus* is also provided.

Key Words

Arachnida, *Cybaeus koikei* sp. nov., molecular phylogeny, RTA clade, spermatheca

Introduction

Epigeal spiders of the genus *Cybaeus* L. Koch, 1868 are remarkably diverse in Western North America and the Japanese Archipelago (Copley et al. 2009; Ihara 2009a; Bennett et al. 2016, 2019, 2021). To date, 91 of the 174 known *Cybaeus* species have been described from Japan (World Spider Catalog 2021) and they have been classified according to the body length of mature individuals (Ihara 2004): ‘small-sized’ (< ca. 5 mm), ‘medium-sized’ (ca. 5–10 mm), and ‘large-sized’ (> ca. 10 mm).

The extraordinary species-richness of Japanese *Cybaeus* may be caused by ecological attributes, such as poor dispersal abilities. *Cybaeus* spiders are not known to disperse via ballooning (Copley et al. 2009) and this may lead to range restriction in many species. Closely related species of *Cybaeus* generally share a similar body size and genital morphology but their ranges are parapatric and do

not overlap (see fig. 1 in Ihara 2009b). Accordingly, these species may be classified as species groups (= ‘superspecies’ in Ihara 2008) but the monophyly of each group is yet to be confirmed by molecular phylogenetic analyses.

The morphological diversity of genitalia in *Cybaeus* is also noteworthy (Ihara 2007, 2008). A previous study divided the genitalia of Japanese *Cybaeus* into two types based on features of the embolus and spermathecae (‘types 1 and 2’ by Ihara et al. 2021). In ‘type 1’ the embolus is not elongated and each spermathecae consists of three distinct bulbous parts: the spermathecal head, the stalk, and the base. In ‘type 2’ the embolus is elongated and the spermathecal head and stalk form an elongated tubular duct but the spermathecal base remains bulbous. The spermathecal head (= ‘secondary spermatheca’ in Ramírez 2014) of *Cybaeus* can be distinguished by the presence of primary pores on its surface (Bennett 1992, 2006). In *Cybaeus*, additionally, Bennett’s gland, which

is a round-shaped patch of developed gland pores on the spermathecae, is typically located at the connection between the spermathecal base (= ‘primary spermatheca’ in Ramírez 2014) and the stalk (Bennett 1992; Ramírez 2014). Therefore, the homology of spermathecal structures can be determined by the relative position of primary pores and Bennett’s gland. The ‘type 2’ spermathecal head can also be recognized by examining the primary pore positions (Ihara et al. 2021). The number of species that bear the ‘type 2’ genitalia is small and eight, or possibly nine, species have been recognized to date (Kobayashi 2006; Ihara 2009a; Ihara et al. 2021): six species inhabiting the Ryukyu Islands, plus *C. melanoparvus* Kobayashi, 2006 in central Honshu, *C. ishikawai* (Kishida, 1940) (in Komatsu 1940) on Shikoku Island, and possibly *C. monticola* Kobayashi, 2006 in central Honshu. Since ‘type 2’ genitalia are exclusive to some Japanese species, it is considered to be apomorphic at the species level although this condition may have evolved several times independently (Ihara et al. 2021) and the closest relatives of each ‘type 2’ species have not yet been fully established.

The *Cybaeus* fauna in central Honshu, especially around Lake Biwa, was well documented by Kobayashi (2006). However, an unidentified ‘small-sized’ *Cybaeus* was collected recently from montane habitats on the western side of Lake Biwa. This unidentified species shows external similarity with *C. melanoparvus* but possessed bulbous ‘type 1’ spermathecae. Here

we describe it as a new species, *Cybaeus koikei* sp. nov., and analyze its phylogenetic position with special focus on the relationship between *C. koikei* sp. nov. and *C. melanoparvus*.

Materials and methods

Samples and morphological observation

Cybaeus spiders were collected from central Honshu (Fig. 1) between 2007 and 2020. All specimens were collected either by hand or using a pooter. Where possible, geographical coordinates of the collection sites were obtained using a portable GPS unit (Garmin eTrex, Garmin Ltd., Olathe, KS). Specimens were preserved in 70% ethanol but 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 the aid of a Leica MC170 HD digital camera mounted on the Leica M125C, and analyzed using 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

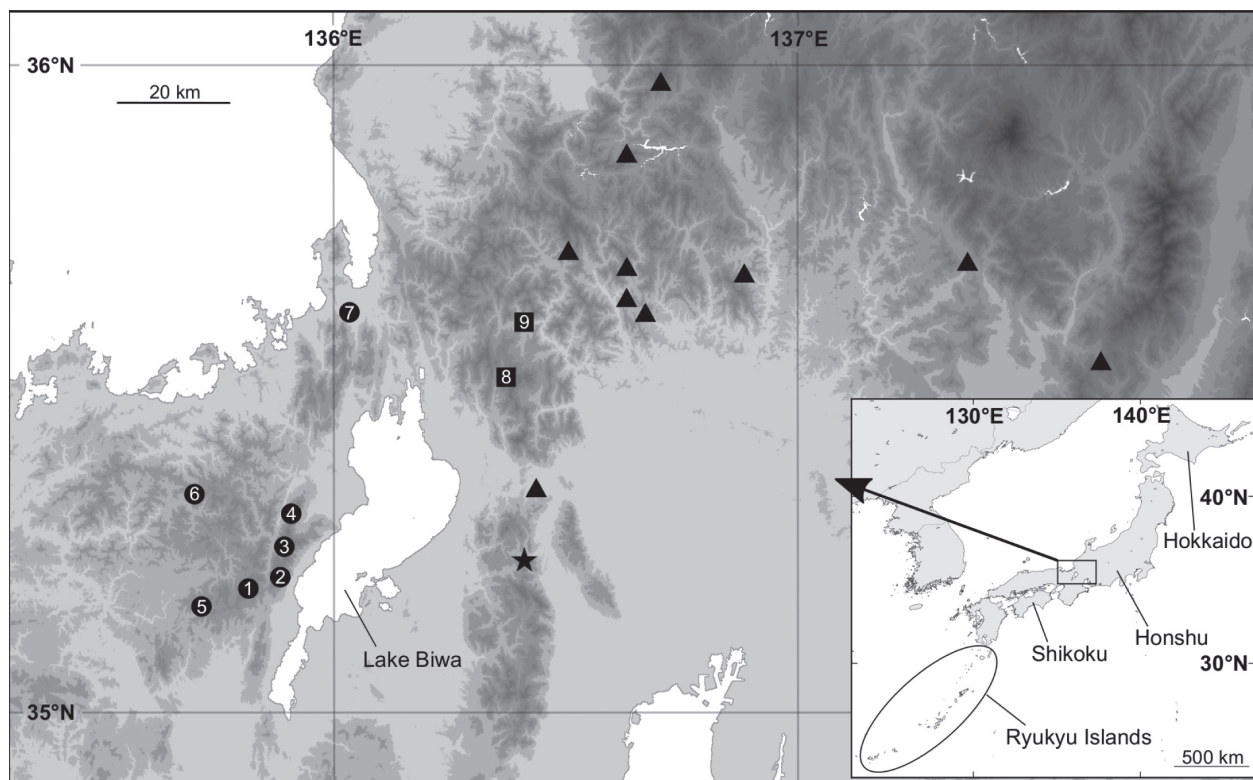


Figure 1. Map showing collection localities of samples in the present study. Circles, locations of the present specimens of *Cybaeus koikei* sp. nov.; squares, locations of the present specimens of *Cybaeus melanoparvus* Kobayashi; triangles, known localities of *C. melanoparvus* in Kobayashi (2006); star, the type locality of *C. melanoparvus* (Kobayashi 2006). The symbols and numbers correspond to those in Fig. 3. Shoreline data were based on Wessel and Smith (1996).

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Terminology of morphological characters and the chaetotaxy of leg macrosetae follows Ihara et al. (2021); abbreviations for macrosetae: **p**, prolateral; **r**, retrolateral; **v**, ventral. 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; **PP**, primary pore; **RTA**, retrolateral tibial apophysis; **SB**, spermathecal base; **SH**, spermathecal head; **SS**, spermathecal stalk; **TibIL**, length of leg I tibia.

Molecular phylogenetic analyses

Three nuclear and three mitochondrial markers for phylogenetic analyses were selected following previous studies (Matsuda et al. 2020; Ihara et al. 2021): (1) 28S rRNA, (2) internal transcribed spacer 1 (ITS-1), (3) histone H3, (4) cytochrome *c* oxidase subunit I (COI), (5) 12S rRNA, and (6) 16S rRNA. However, 12S and 16S sequences could not be obtained from *C. koikei* sp. nov. and *C. melanoparvus*. Methods for genomic DNA extraction and cycle sequencing (CS) reactions were as described by Matsuda et al. (2020); primer sets and conditions for the polymerase chain reactions (PCR) and CS reactions used in this study are also listed in Suppl. material 1: Table S1. All PCRs were performed using a GeneAmp PCR System 9700 (Thermo Fisher Scientific, Waltham, MA, USA) using an Ex Taq Polymerase Kit (Takara Bio Inc., Kusatsu, Japan). In total, 20 sequences were newly obtained in this study and deposited with the International Nucleotide Sequence Databases (INSD) through the DNA Databank of Japan.

Phylogenetic relationships of the *Cybaeus* spiders were inferred based on the dataset comprising 28S, ITS-1, H3, COI, 12S and 16S sequences. In addition to the newly obtained 8 sequences of *C. koikei* sp. nov. and *C. melanoparvus*, 85 sequences of 17 species (see dataset in Ihara et al. 2021) were also included in our dataset (Table 1). *Cybaeus striatipes* Bösenberg & Strand, 1906 was treated as the outgroup *a priori* because this species seems to be a close relative of Eurasian-continental *Cybaeus* species (see Ihara 2009a). The alignments of H3 and COI were trivial and no indels were observed. The ITS-1, 12S, and 16S sequences were aligned using MAFFT L-INS-i v. 7.475 (Katoh and Standley 2013) and the 28S sequences were aligned using MAFFT G-INS-i. The fragments of 28S, ITS-1, H3, COI, 12S, and 16S were 792, 769, 328, 763, 335, and 441 bp, respectively. The concatenated sequences thus yielded 3428 bp of aligned positions.

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). The best-fit partition scheme and models for both analyses were identified based on the corrected Akaike information criterion (AICc) using PartitionFinder v. 2.1.1 (Lanfear et

al. 2017) and the 'greedy' algorithm (Lanfear et al. 2012). The selected partition scheme and models are shown in Suppl. material 2: Table S2. The ML phylogenetic tree was calculated using IQ-TREE v. 1.6.12 (Nguyen et al. 2015) with non-parametric bootstrapping (BS) conducted with 1000 replicates. BI tree and Bayesian posterior probabilities (PP) were estimated using MrBayes v. 3.2.7a (Ronquist et al. 2012) with 15 million generations and tree sampling every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut et al. 2018), and the first 30001 trees were discarded based on the results.

To confirm whether males and females of the present specimens belong to *C. koikei* sp. nov., and also to recognize their geographic structures, genetic relationships among 12 samples of *C. koikei* sp. nov. and two individuals of *C. melanoparvus* were inferred using relatively fast-evolving nuclear ITS-1 sequences. A neighbor network analysis was conducted using Neighbor-Net (Bryant and Moulton 2004) implemented in SplitsTree v. 4.16.2 (Huson and Bryant 2006) based on uncorrected *p*-distances. The ITS-1 sequences were aligned using MAFFT L-INS-i, and the length was 681 bp of aligned positions.

Results

Molecular analyses

The obtained BI (mean $\ln L = -10901.95$; Fig. 2) and ML ($\ln L = -10848.99$; not shown) trees had almost identical topologies, and were almost identical to those of the previous analyses (Ihara et al. 2021). The monophyly of *C. koikei* sp. nov. and *C. melanoparvus* was strongly supported (BS = 100%, PP = 1.0) but the sister-group of this clade remains unclear since the monophyly of a lineage including *C. koikei* sp. nov., *C. melanoparvus*, and *C. daimonji* Matsuda, Ihara & Nakano, 2020 was not fully supported (BS = 63%, PP = 0.70).

The SplitsTree network (Fig. 3) using ITS-1 sequences showed all males and females forming a cluster, which was clearly separated from that of *C. melanoparvus*, confirming that all specimens belong to *C. koikei* sp. nov. Although specimens of *C. koikei* sp. nov. collected from its type locality and adjacent locations formed a cluster, no further geographic structure was detected among the present specimens of *C. koikei* sp. nov. Interestingly, one sample of *C. koikei* (KUZ Z3761) and two samples of *C. melanoparvus* were geographically close to each other, but they showed the greatest genetic divergence.

Taxonomy

Family Cybaeidae Banks, 1892

Genus *Cybaeus* L. Koch, 1868

Type species. *Amaurobius tetricus* C.L. Koch, 1839.

Table 1. Samples of the *Cybaeus* species used for the molecular analyses. The information on the voucher is accompanied by the collection locality and the INSD accession numbers. Sequences marked with an asterisk (*) were obtained for the first time in this study. Samples marked with two asterisks (**) were only used for the neighbor network analysis.

Taxa	Voucher #	Locality	INSD #					
			28S rRNA	ITS-1	histone H3	COI	12S rRNA	16S rRNA
<i>C. koikei</i> sp. nov.	KUZ Z3744	Hanase Pass, Kyoto, Honshu	LC601900*	LC601903*	LC601902*	LC601901*		
<i>C. koikei</i> sp. nov.**	KUZ Z2364	Hanase Pass, Kyoto, Honshu		LC601897*				
	KUZ Z3741	Hanase Pass, Kyoto, Honshu		LC601898*				
	KUZ Z3743	Hanase Pass, Kyoto, Honshu		LC601899*				
	KUZ Z3745	Yokotani Valley, Shiga, Honshu		LC601904*				
	KUZ Z3746	Yokotani Valley, Shiga, Honshu		LC601905*				
	KUZ Z3747	Yokotani Valley, Shiga, Honshu		LC601906*				
	KUZ Z3748	Yokotani Valley, Shiga, Honshu		LC601907*				
	KUZ Z3754	Mt. Shirataki, Shiga, Honshu		LC601908*				
	KUZ Z3757	Mt. Shirataki, Shiga, Honshu		LC601909*				
	KUZ Z3758	Mt. Horai, Shiga, Honshu		LC601910*				
	KUZ Z3761	Mt. Nosaka, Fukui, Honshu		LC601911*				
<i>C. melanoparvus</i> Kobayashi, 2006	KUZ Z3764	Sakauchihirore, Gifu, Honshu	LC601893*	LC601896*	LC601895*	LC601894*		
<i>C. melanoparvus</i> Kobayashi, 2006 **	KUZ Z3765	Mt. Bungen, Shiga, Honshu		LC601892*				
<i>C. aikana</i> Ihara, Koike & Nakano, 2021	KUZ Z2137	Mt. Yuwandake, Amamioshima Island	LC552248		LC552250	LC552249	LC552246	LC552247
<i>C. amamiensis</i> Ihara, Koike & Nakano, 2021	KUZ Z2120	Mt. Yuwandake, Amamioshima Island	LC552236	LC552239	LC552238	LC552237	LC552234	LC552235
<i>C. ashikitaensis</i> (Komatsu, 1968)	KUZ Z2213	Itsuki, Kumamoto, Kyushu	LC552192	LC552195	LC552194	LC552193	LC552191	
<i>C. daimonji</i> Matsuda, Ihara & Nakano, 2020	KUZ Z2755	Mt. Daimonjiyama, Kyoto, Honshu	LC529207	LC529208	LC529206	LC529209	LC529211	LC529210
<i>C. fuujinensis</i> (Komatsu, 1968)	KUZ Z2199	Fujindo Cave, Kumamoto, Kyushu	LC552187	LC552190	LC552189	LC552188	LC552186	
<i>C. gotoensis</i> (Yamaguchi & Yaginuma, 1971)	KUZ Z2251	Iana Cave, Fukuejima Island, Goto Islands	LC552201	LC552204	LC552203	LC552202		
<i>C. hikidai</i> Ihara, Koike & Nakano, 2021	KUZ Z2106	Mt. Nagodake, Okinawajima Island	LC552264	LC552267	LC552266	LC552265	LC552262	LC552263
<i>C. ishikawai</i> (Kishida, 1940)	KUZ Z2715	Near Ryugado Cave, Kochi, Shikoku	LC552276	LC552278	LC552277		LC552274	LC552275
<i>C. itsukiensis</i> Irie, 1998	KUZ Z2184	Tsuzurasedo Cave, Kumamoto, Kyushu	LC552182	LC552185	LC552184	LC552183		
<i>C. kodama</i> Ihara, Koike & Nakano, 2021	KUZ Z2141	Hanayama Trail, Yakushima Island	LC552215	LC552218	LC552217	LC552216		
<i>C. kompiraensis</i> (Komatsu, 1968)	KUZ Z2317	Kompirado Cave, Kochi, Shikoku	LC552179	LC552181	LC552180	LC552178		
<i>C. kunisakiensis</i> Ihara, 2003	KUZ Z2303	Mt. Futagosan, Oita, Kyushu	LC552197	LC552200	LC552199	LC552198	LC552196	
<i>C. kumadori</i> Ihara, Koike & Nakano, 2021	KUZ Z2143	Mt. Yaguradake, Kuroshima Island, Mishima Islands	LC552225	LC552228	LC552227	LC552226	LC552223	LC552224
<i>C. okumurai</i> Ihara, Koike & Nakano, 2021	KUZ Z2719	Kunigami, Tanegashima Island	LC552280	LC552282	LC552281		LC552279	
<i>C. striatipes</i> Bösenberg & Strand, 1906	KUZ Z2718	Mt. Rausudake, Shari, Hokkaido	LC552174	LC552177	LC552176	LC552175		
<i>C. tokunoshimensis</i> Ihara, Koike & Nakano, 2021	KUZ Z2113	Mt. Inokawadake, Tokunoshima Island	LC552253	LC552256	LC552255	LC552254	LC552251	LC552252
<i>C. yakushimensis</i> Ihara, Koike & Nakano, 2021	KUZ Z2138	Shiratani-unsuiyko Valley, Yakushima Island	LC552207	LC552209	LC552208		LC552205	LC552206

Cybaeus koikei sp. nov.

<http://zoobank.org/30DB7CE2-EDD2-49D7-9CD4-F508B82FACFC>

Figures 4–7

Diagnosis. ‘Small-sized’ Japanese *Cybaeus*. Both sexes of *C. koikei* are most similar to those of *C. melanoparvus*. Although males of these two species resemble each other in lacking a palpal PA and having a palpal tibia that is almost as long as the palpal patella (Figs 6C, 8D), they can be clearly distinguished by their simple and small palpal bulb (Fig. 6B) whereas *C. melanoparvus* have a well-developed palpal bulb (Fig. 8B). Females of *C. koikei* are clearly distinguishable by their relatively simple spermathecae (Fig. 6E); *C. melanoparvus* has highly complex spermathecae (Fig. 8F).

Material examined. Holotype: JAPAN • ♂; Kyoto Prefecture, Kyoto City, Sakyo, Hanase Pass; 35°9.98'N, 135°47.58'E; 14 Oct. 2020; T. Nakano leg.; under a stone along a mountain stream; KUZ Z3744. **Paratypes:** JAPAN • 1 ♀; same locality as for holotype; 35°09.85'N, 135°47.51'E; 14 Oct. 2020; T. Nakano leg.; under a stone

along a forest trail; KUZ Z3743 • 1 ♂; same locality as for holotype; 35°9.65'N, 135°47.19'E; 24 Sep. 2012; N. Koike leg.; KUZ Z2364 • 1 ♂; same data as for preceding; NSMT-Ar 20964 • 8 ♀♀; same data as for preceding; KUZ Z2365 • 1 ♀; same data as for preceding; KUZ Z3741; 1 ♀; same data as for preceding; NSMT-Ar 20965.

Additional specimens: JAPAN • 1 ♂; Kyoto Prefecture, Nantan City, Ashiu; 35°17.97'N, 135°44.19'E; 14 Nov. 2007; N. Koike leg.; KUZ Z2335 • 1 ♀; Kyoto Prefecture, Kyoto City, Kita, Kumogahata-detanicho; 35°7.92'N, 135°42.7'E; 7 Nov. 2012; N. Koike leg.; KUZ Z2419 • 4 ♀♀; same data as for preceding; KUZ Z3742 • 1 ♂; Shiga Prefecture, Takashima City, Yokotani Valley; 35°18.34'N, 135°53.96'E; 21 Oct. 2020; Y. Sugawara leg.; under a stone along a mountain stream; KUZ Z3748 • 1 ♀; same data as for preceding; KUZ Z3745 • 1 ♀; same data as for preceding; KUZ Z3747 • 1 juv.; same data as for preceding; KUZ Z3746 • 1 ♀; same locality as for holotype; 35°9.46'N, 135°47.24'E; 16 Oct. 2019; K. Matsuda leg.; under a stone along a mountain stream; KUZ Z2824 • 2 ♀♀; same locality as for holotype; 18 Jun. 2008; N. Koike leg.; KUZ Z3751

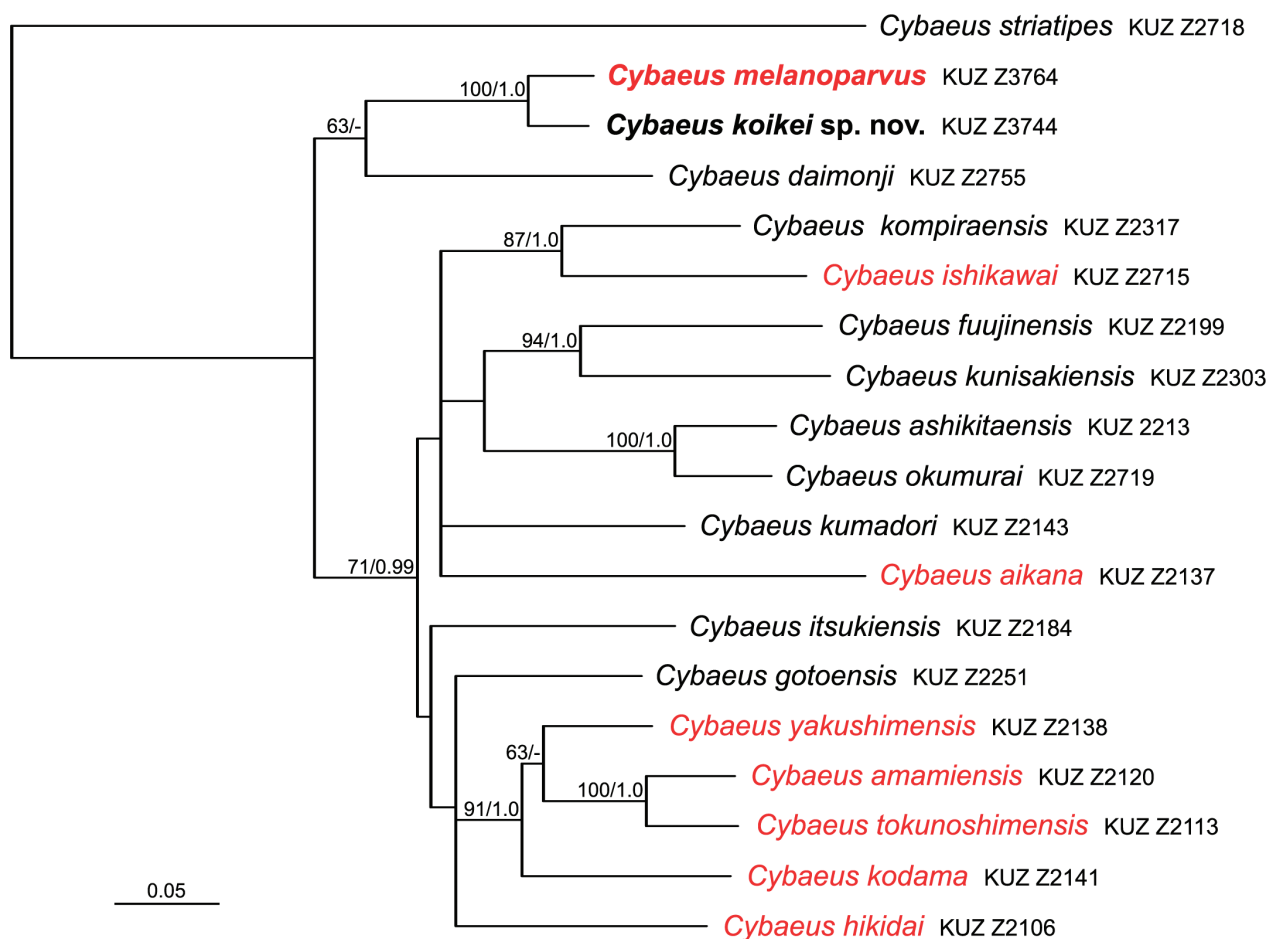


Figure 2. Bayesian inference tree (mean $\ln L = -10901.95$) for 3428 bp of 28S rRNA, ITS-1, H3, COI, 12S rRNA and 16S rRNA markers. Numbers on nodes represent ML bootstrap values and Bayesian posterior probabilities. Species names colored in black and those in red denote that they possess 'type 1', and 'type 2' genitalia, respectively.

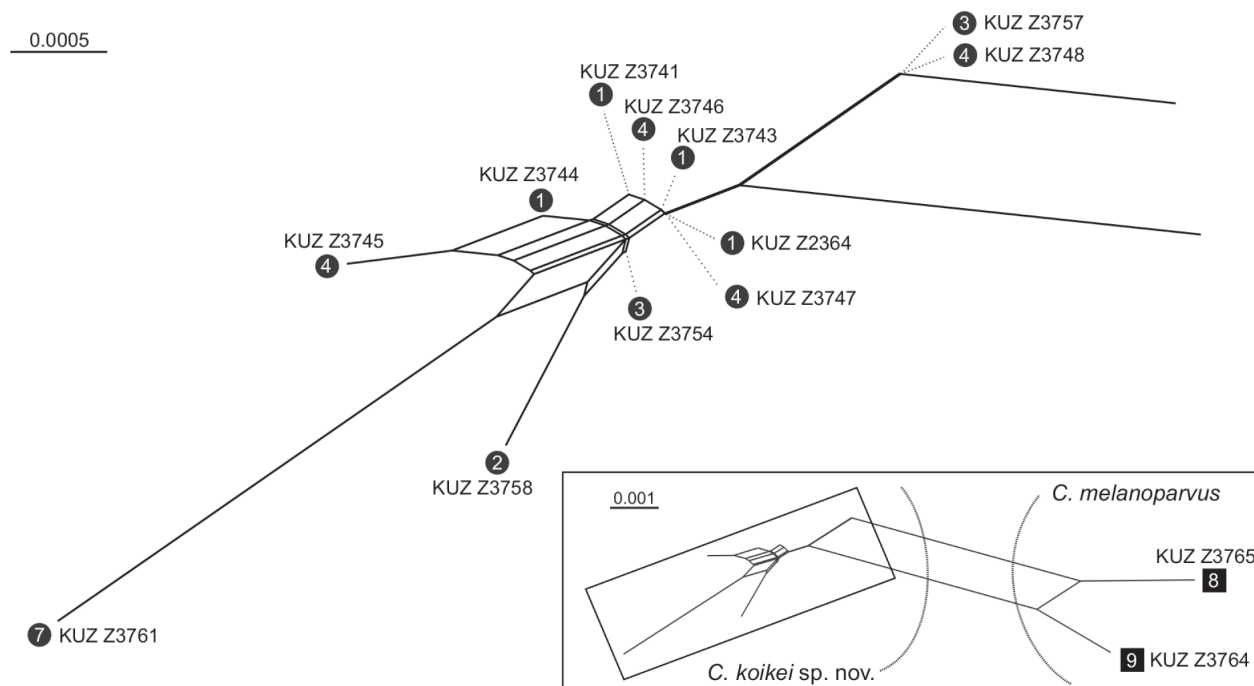


Figure 3. Neighbor-Net based on uncorrected p -distances for 681 bp of ITS-1 sequences. Circles denote *Cybaeus koikei* sp. nov., and squares indicate *Cybaeus melanoparvus* Kobayashi. The symbols and numbers correspond to those in Fig. 1.



Figure 4. *Cybaeus koikei* sp. nov. and its retreat. **A.** Dorsal view of holotype male (KUZ Z3744); **B.** Retreat of paratype female (KUZ Z3743); photo taken at the type locality; **OP**, opening of retreat. Scale bars: 1 mm (**A.**); 5 mm (**B.**).

• 1 ♀; same locality as for holotype; 30 Nov. 2008; N. Koike leg.; KUZ Z3752 • 1 ♂; Kyoto Prefecture, Kyoto City, Sakyo, Ohara-kochihiracho, Amida-ji Temple; 35°8.27'N, 135°49.64'E; 2 Nov. 2008; N. Koike leg.; sifted from fallen leaves; KUZ Z3753 • 1 ♂; Shiga Prefecture, Otsu City, Mt. Shirataki; 35°14.61'N, 135°52.57'E; 24 Oct. 2012; N. Koike leg.; KUZ Z3754 • 3 ♂♂; same data as for preceding; KUZ Z3755 • 6 ♀♀; same data as for preceding; KUZ Z3756 • 1 ♀; same data as for preceding; KUZ Z3757 • 4 ♂♂; Shiga Prefecture, Otsu City, southeast of Mt. Horai; 35°11.81'N, 135°53.49'E; 24 Oct. 2012; N. Koike leg.; KUZ Z3759 • 1 ♀; same data as for preceding; KUZ Z3758 • 6 ♀♀; same data as for preceding; KUZ Z3760 • 5 ♂♂; Fukui Prefecture, Tsuruga City, Mt. Nosaka; 35°36.16'N, 136°1.66'E; 25 Oct. 2012; N. Koike leg.; along a forest trail; KUZ Z3762 • 1 ♀; same data as for preceding; KUZ Z3761 • 9 ♀♀; same data as for preceding; KUZ Z3763.

Type locality. Japan, Kyoto Prefecture, Kyoto City, Sakyo, Hanase Pass (35°9.98'N, 135°47.58'E).

Description. Male (holotype, KUZ Z3744; Figs 4A, 5A, B). *Measurements* (mm). CL 2.04, CW 1.53; head 0.91 wide; abdomen 1.71 long, 1.32 wide; ocular area 0.27 long, 0.59 wide; sternum 0.95 long, 0.93 wide. Leg

formula, IV > I > II > III; length of legs (femur + patella + tibia + metatarsus + tarsus): leg I 6.03 (1.70 + 0.68 + 1.44 + 1.41 + 0.80); leg II 5.58 (1.55 + 0.64 + 1.28 + 1.33 + 0.78); leg III 4.96 (1.41 + 0.58 + 0.93 + 1.26 + 0.77); leg IV 6.31 (1.67 + 0.60 + 1.41 + 1.75 + 0.87).

Carapace (Fig. 5A). Head narrow, ca. 0.59× as wide as thoracic region; thoracic region almost as high as head. AER straight in frontal view; PER almost straight in dorsal view; AME smallest, < 1/2 diameter of other eyes; ocular area ca. 2.1× wider than long. Clypeus shorter than median ocular area.

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

Leg macrosetae. Leg I: tibia p4, r0, v2-2-2-2; metatarsus p4, r1, v2-2-2. Leg II: tibia p3, r0, v2-2-2-2; metatarsus p4, r2, v2-2-3.

Abdomen (Fig. 5B). Oval; mid-posterior part widest. Colulus with two groups of 1 or 2 setae.

Palp (Fig. 6A–C). Patella without PA, lateral surface covered with cluster of robust setae. Tibia almost as long as patella; RTA plate-like, occupying 2/3 of length of tibia. Cymbium ca. 2× longer than wide, slightly expanded prolaterally. Genital bulb circular in ventral view. Conductor: distal part unelongated; proximal arm short, hooked. Embolus simple, originating and terminating, respectively, at ca. 11 o'clock and ca. 5 o'clock in ventral view.

Color (Fig. 5A, B). Carapace: head brown, with reticulate dark brown markings; thoracic region yellowish brown, with radiating dark brown bands. Chelicerae, maxillary lobe and labium yellowish brown. Sternum beige. Legs beige to yellowish brown, darker distally, with pale to dark brown annulations. Abdomen: dorsally dark olive-brown with pale brown chevron-like markings; ventrally pale brown.

Female (paratype, KUZ Z3743; Fig. 5C, D). *Measurements* (mm). CL 2.17, CW 1.51; head 1.00 wide; abdomen 2.03 long, 1.35 wide; ocular area 0.31 long, 0.66 wide; sternum 1.00 long, 0.98 wide. Leg formula, IV > I > II > III; length of legs (femur + patella + tibia + metatarsus + tarsus): leg I 5.39 (1.57 + 0.62 + 1.34 + 1.20 + 0.66); leg II 5.05 (1.54 + 0.60 + 1.13 + 1.14 + 0.64); leg III 4.51 (1.35 + 0.55 + 0.89 + 1.10 + 0.61); leg IV 5.71 (1.64 + 0.60 + 1.30 + 1.45 + 0.72).

Carapace (Fig. 5C). Head ca. 0.66× as wide as thoracic region; thoracic region slightly higher than head. AER almost straight in frontal view; PER slightly recurved in dorsal view; AME smallest, < 1/2 diameter of other eyes; ocular area ca. 2.1× wider than long. Clypeus shorter than median ocular area.

Mouthparts. Chelicerae slightly geniculate, teeth and denticles of fang furrow not observable and covered by long setae; basally with lateral condyle. Labium wider than long.

Leg macrosetae and abdomen (Fig. 5D). Same as for male holotype.

Genitalia (Figs 6D, E, 7). Posterior margin of epigynal plate weakly 'M'-shaped. Atrium located posteromedially on epigyne and with 2 distinct copulatory openings.

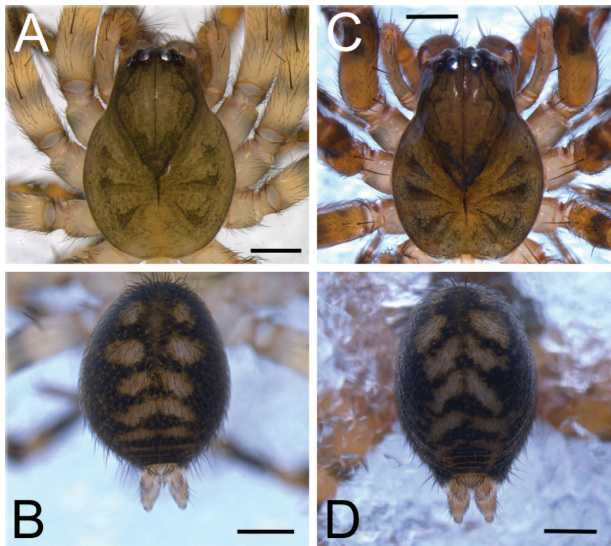


Figure 5. *Cybaeus koikei* sp. nov., male holotype (KUZ Z3744: A, B) and female paratype (KUZ Z3743: C, D). A, C. Prosoma, dorsal view; B, D. Abdomen, dorsal view. Scale bars: 500 µm.

CPs separated on both sides of atrium; CD long running anteromedially. Each of SH and SS slightly bulbous; SH small, with a few primary pores anteromedially; SS running posteromedially; SB large, globular, directed anterolaterally; Bennett's gland located anteriorly at connection between SS and SB.

Colour (Fig. 5C, D). Carapace: head brown, with reticulate dark brown markings; thoracic region yellowish brown, with radiating dark brown bands. Chelicerae, maxillary lobe and labium dark yellowish-brown. Sternum light yellowish-brown. Legs pale brown to yellowish brown, darker distally, with medium to dark brown annulations. Abdomen:

dorsally dark olive-brown with pale brown chevron-like markings; ventrally light yellowish-brown to brown.

Variation. Males. *Measurements* (mean±1SD, followed by ranges in parentheses; n = 19, including holotype and paratypes): CL 2.05±0.11 (1.87–2.23), CW 1.50±0.10 (1.33–1.65); CW/CL 0.73±0.03 (0.70–0.82); TibIL 1.45±0.07 (1.31–1.59); TibIL/CL 0.71±0.02 (0.68–0.74). Legs longer than those of females.

Females. *Measurements* (n = 42, including paratypes): CL 2.06±0.17 (1.67–2.31), CW 1.41±0.12 (1.06–1.57); CW/CL 0.68±0.02 (0.64–0.75); TibIL 1.30±0.12 (1.04–1.51); TibIL/CL 0.63±0.03 (0.53–0.68).

Etymology. The specific name is dedicated to Mr Naoki Koike who assembled the large collection of Japanese *Cybaeus* spiders that is now kept at Kyoto University, including the specimens of this new species.

Remarks. This species constructs a 'V-shaped' retreat (Fig. 4B), which is the most common type of retreat among Japanese *Cybaeus* (Ihara 2006, 2009b; Ihara et al. 2021). Because all specimens collected in 2019 and 2020 were found under stones, it is possible that this species prefers to construct its retreat on the underside of stones rather than in decaying substrates.

According to the nuclear ITS-1 sequences obtained from the holotype male (KUZ Z3744) and the paratype female (KUZ Z3743), the males and females examined in this study clearly belong to the same species newly described here. Their ITS-1 sequences through the overlapping aligned positions (451 bp) are completely identical to each other.

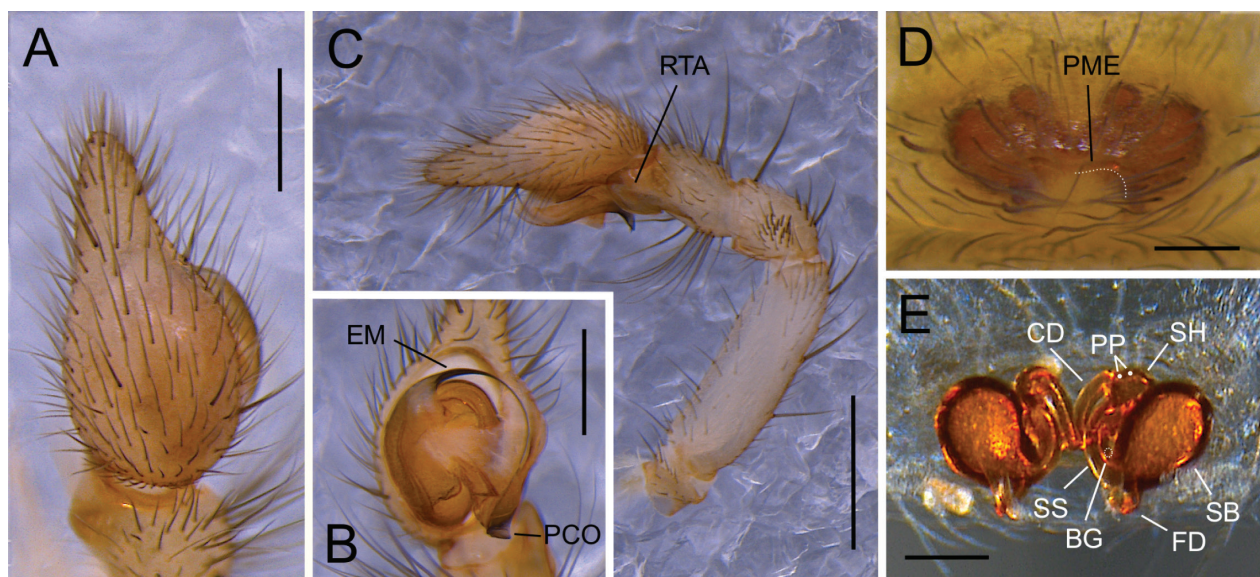


Figure 6. *Cybaeus koikei* sp. nov., male holotype (KUZ Z3744: A–C) and female paratype (KUZ Z3743: D, E). A. Cymbium of left palp, dorsal view; B. Bulb of left palp, ventral view; EM, embolus; PCO, proximal arm of conductor; C. Left palp, retrolateral view; RTA, retrolateral tibial apophysis; D. Epigyne, ventral view; PME, posterior margin of epigynal plate; E. Spermathecae, dorsal view; BG, Bennett's gland; CD, copulatory duct; FD, fertilization duct; PP, primary pore; SB, spermathecal base; SH, spermathecal head; SS, spermathecal stalk. Scale bars: 250 µm (A, B.); 500 µm (C.); 100 µm (D, E.).

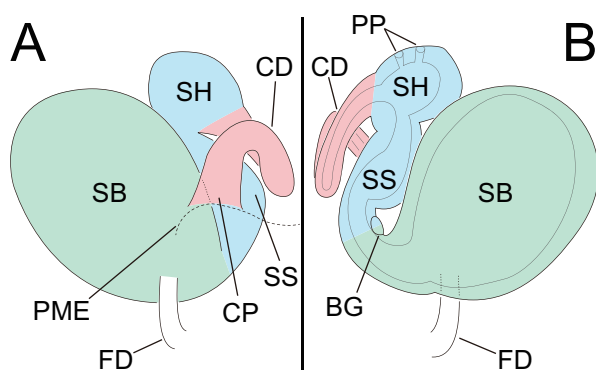


Figure 7. *Cybaeus koikei* sp. nov., schematic drawing of epigyne and spermathecae based on female paratype (KUZ Z3743). **A.** Ventral view; **CD**, copulatory duct; **CP**, copulatory pore; **FD**, fertilization duct; **PME**, posterior margin of epigynal plate; **SB**, spermathecal base; **SH**, spermathecal head; **SS**, spermathecal stalk; **B.** Dorsal view; **BG**, Bennett's gland; **CD**, copulatory duct; **FD**, fertilization duct; **PP**, primary pore; **SB**, spermathecal base; **SH**, spermathecal head; **SS**, spermathecal stalk. Duct from CP to before SH colored in red; SH to before SB colored in blue; SB colored in green. Scale bar: 50 μ m.

Two species, *C. daimonji* and *C. kiiensis* Kobayashi, 2006 whose spermathecae are grouped into 'type 1', occur in sympatry with *C. koikei* sp. nov. through montane habitats on the western side of Lake Biwa. However, they are clearly distinguishable by body size: the body

length of *C. koikei* sp. nov. reaches ca. 4 mm whereas that of *C. daimonji* and *C. kiiensis* reaches ca. 6 mm and ca. 2.5 mm, respectively (Kobayashi 2006; Matsuda et al. 2020). Additionally, males of *C. koikei* sp. nov. are conclusively distinguishable from those of *C. daimonji* and *C. kiiensis* by the lack of PA on its palp. Males of both *C. daimonji* and *C. kiiensis* possess a PA on their pedipalp (Kobayashi 2006; Matsuda et al. 2020).

Cybaeus melanoparvus Kobayashi, 2006

Figures 8, 9

Cybaeus melanoparvus Kobayashi, 2006: 41–42, figs 53–57; Ihara 2009a: 167, figs 2-2-30-228–230.

Material examined. JAPAN • 1 ♂, 1 ♀; Gifu Prefecture, Ibi-gun, Ibigawa Town, Sakauchihirore; 35°36.77'N, 136°25.16'E; 27 Oct. 2012; N. Koike leg.; KUZ Z3764 • 2 ♂♂, 2 ♀♀; Shiga Prefecture, Maibara City, Mt. Bungen; 35°30.51'N, 136°23.42'E; 26 Oct. 2012; N. Koike leg.; KUZ Z3765.

Supplementary description of female spermathecae (Figs 8F, 9). CD, SH, and SS continuous tubular, and similar in diameter from CP through to SB; this duct starting from CP running on ventral side anteriorly, then bending posteromedially and coiling once toward anterior tip; this

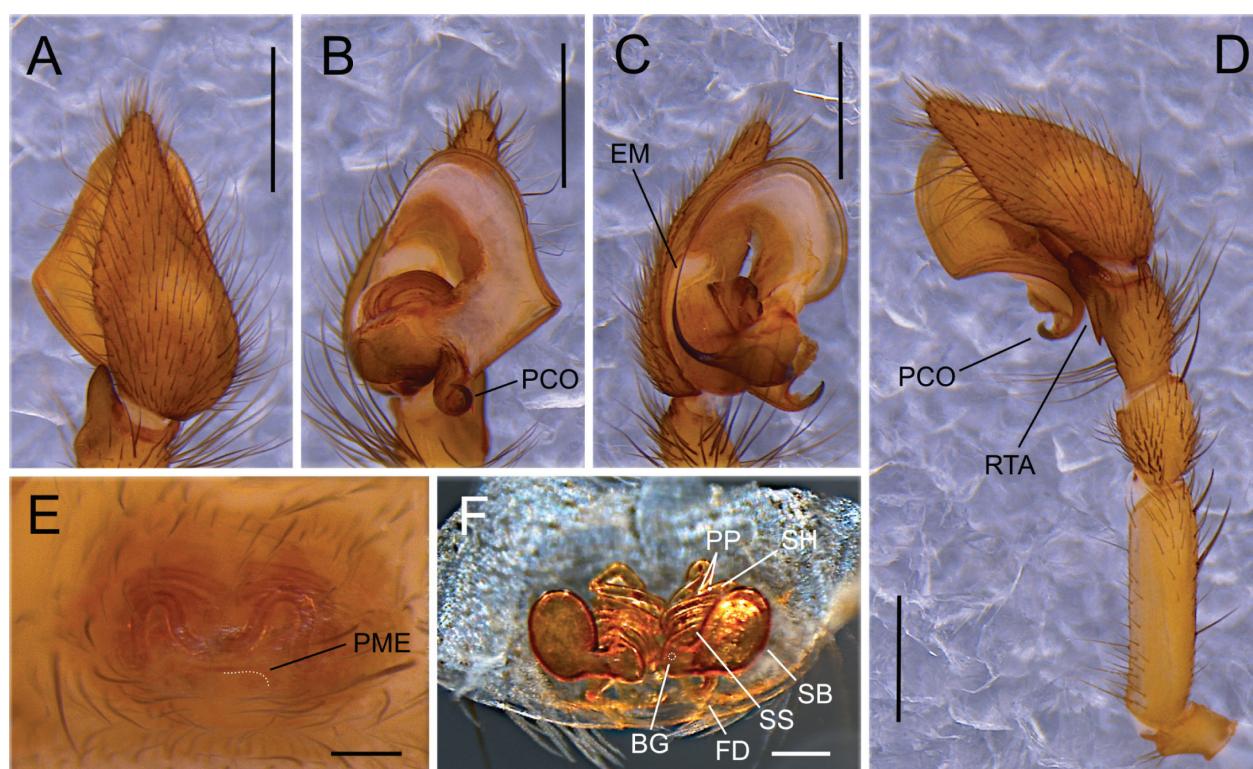


Figure 8. *Cybaeus melanoparvus* Kobayashi, from Mt. Bungen, Shiga Prefecture (KUZ Z3765), male (**A–D**) and females (**E, F**). **A.** Cymbium of left palp, dorsal view; **B.** Bulb of left palp, ventral view; **PCO**, proximal arm of conductor; **C.** Bulb of left palp, prolateral view; **EM**, embolus; **D.** Left palp, retrolateral view; **PCO**, proximal arm of conductor; **RTA**, retrolateral tibial apophysis; **E.** Epigyne, ventral view; **PME**, posterior margin of epigynal plate; **F.** Spermathecae, dorsal view; **BG**, Bennett's gland; **FD**, fertilization duct; **PP**, primary pore; **SB**, spermathecal base; **SH**, spermathecal head; **SS**, spermathecal stalk. Scale bars: 500 μ m (**A–D**); 100 μ m (**E, F**).

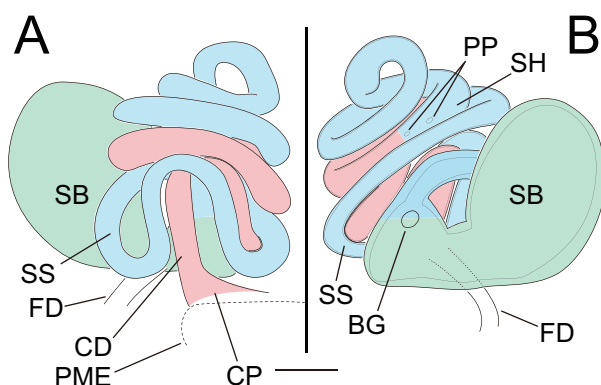


Figure 9. *Cybaeus melanoparvus* Kobayashi, schematic drawing of epigyne and spermathecae, based on female specimen (KUZ Z3765). **A.** Ventral view; **CD**, copulatory duct; **CP**, copulatory pore; **FD**, fertilization duct; **PME**, posterior margin of epigynal plate; **SB**, spermathecal base; **SH**, spermathecal head; **B.** Dorsal view; **BG**, Bennett's gland; **FD**, fertilization duct; **PP**, primary pore; **SB**, spermathecal base; **SS**, spermathecal stalk; **SS**, spermathecal stalk. Duct from CP to before PP colored in red; duct from PP to before SB colored in blue; SB colored in green. Scale bar: 50 µm.

duct then turning at anterior tip, coiling 2.5 times in the opposite direction, undulating twice on ventral side, and then a half coil to SB; PP located on dorsal side, 1 coil before turning point at anterior tip; SB globular, extending anterolaterally, and expanded at connection with SS; BG well-developed, located dorsally at connection between SS and SB; each FD not running from basal part of SB, but extending from small atrium located beneath SB.

Remarks. The present specimens were unquestionably identified as *C. melanoparvus* by features of the male palp and female genitalia; that is, the remarkably developed conductor and complicated tubular spermathecae.

The present study could successfully document the positions of PP and BG in the spermathecae of this species for the first time. Because PPs were located at the dorsal surface of the duct near its anterior tip, this tubular structure, which was referred to as an ‘anterior duct’ by Kobayashi (2006), must comprise CD, and also SH and SS.

Discussion

Cybaeus koikei sp. nov. shares generally similar external features with *C. melanoparvus*; that is ‘small-sized’ body length and blackish color, as well as a cluster of robust setae on the lateral surface of the male palpal patella (see also Kobayashi 2006). Although the new species does not possess ‘type 2’ female spermathecae, the morphological similarities between the two species suggest that they are closely related to each other, forming a species-group. Our molecular phylogenies support the monophyly of *C. koikei* sp. nov. and *C. melanoparvus* and thus, they corroborate their close relationship. The present findings

of remarkably different genitalia between the ‘type 1’ *C. koikei* sp. nov. and ‘type 2’ *C. melanoparvus* represent an exception because members of the other species groups have been identified mainly based on similarities in their genitalia (Ihara 2009b).

Given the close sister relationship between *C. koikei* sp. nov. and *C. melanoparvus*, their morphological similarities, especially the cluster of robust setae on the palpal patella, are probably synapomorphies between the two species. The positions of primary pores and Bennett's glands in their spermathecae are noticeable as well. Although the tubular spermathecae of *C. melanoparvus* are complex, features of its primary pores and spermathecal base are essentially concordant with those of *C. koikei* sp. nov.; the primary pores of both species are located mid-anteriorly and their globular spermathecal bases are directed antero-laterally. These similarities imply that the spatial positions of the spermathecal head and base in *C. melanoparvus* have not been drastically changed from their most recent common ancestor.

The geographical range of *C. koikei* sp. nov. and *C. melanoparvus* is also noteworthy. The new species inhabits montane forests along the western side of Lake Biwa and *C. melanoparvus* is known only from montane regions at the eastern side of the lake (Fig. 1). These data suggest a parapatric distribution for both species and a distributional boundary somewhere at the northern side of the lake.

Our previous study (Ihara et al. 2021) and the results shown herein also confirm the monophyly of the ‘type 1’ species *C. kompiraensis* (Komatsu, 1968) and ‘type 2’ species *C. ishikawai* (Komatsu 1968; Ihara 2009a); however both species were deeply divergent based on the phylogenies and the closest relative of the ‘type 2’ *C. ishikawai* probably remains unknown. Close relatives of six ‘type 2’ species endemic to the Ryukyu Islands also remain unresolved (Ihara et al. 2021). Another possible ‘type 2’ species *Cybaeus monticola* might be an eastern neighbor of *C. melanoparvus* that inhabits montane regions in central Honshu. Although the phylogenetic position of *C. monticola* remains uninvestigated, the morphological features of *C. monticola*, including a palpal patella with an apophysis and posteriorly directed spermathecal base (Kobayashi 2006), suggest that this species may not be closely related to *C. koikei* sp. nov. and *C. melanoparvus*. Nevertheless, further investigations into the underlying phylogeny and comparative morphology of Japanese *Cybaeus* are essential to elucidate their evolutionary history.

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References

- Banks N (1892) A classification of the North American spiders. The Canadian Entomologist 24: 88–97. <https://doi.org/10.4039/Ent2488-4>
- Bennett RG (1992) The spermathecal pores of spiders with special reference to dictynoids and amaurobioids (Araneae, Araneomorphae, Araneocladia). Proceedings of the Entomological Society of Ontario 123: 1–21.
- Bennett RG (2006) Ontogeny, variation, and synonymy in North American *Cybaeus* spiders (Araneae: Cybaeidae). The Canadian Entomologist 138: 473–492. <https://doi.org/10.4039/n06-804>
- Bennett R[G], Copley C, Copley D (2016) *Cybaeus* (Araneae: Cybaeidae): the Nearctic species of the Holarctic clade. Zootaxa 4164: 1–67. <https://doi.org/10.11646/zootaxa.4164.1.1>
- Bennett R[G], Copley C, Copley D (2019) *Cybaeus* (Araneae: Cybaeidae): the *adenes* species group of the Californian clade. Zootaxa 4711: 245–274. <https://doi.org/10.11646/zootaxa.4711.2.2>
- Bennett R[G], Copley C, Copley D (2021) *Cybaeus* (Araneae: Cybaeidae): the *aspenicolens* species group of the Californian clade. Zootaxa 4926: 224–244. <https://doi.org/10.11646/zootaxa.4926.2.4>
- Bösenberg W, Strand E (1906) Japanische Spinnen. Abhandlungen Herausgegeben von der Senckenbergischen Naturforschenden Gesellschaft 30: 93–422.
- Bryant D, Moulton V (2004) Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. Molecular Biology and Evolution 21: 255–265. <https://doi.org/10.1093/molbev/msh018>
- Copley CR, Bennett R, Perlman SJ (2009) Systematics of Nearctic *Cybaeus* (Araneae: Cybaeidae). Invertebrate Systematics 23: 367–401. <https://doi.org/10.1071/IS09001>
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23: 254–267. <https://doi.org/10.1093/molbev/msj030>
- Ihara Y (2003) Geographic differentiation of the *miyosii*-group of *Cybaeus* (Araneae: Cybaeidae) in western Japan, with descriptions of two new species. Acta Arachnologica 52: 103–112. <https://doi.org/10.2476/asjaa.52.103>
- Ihara Y (2004) Descriptions of large- and medium-sized species of the genus *Cybaeus* (Araneae: Cybaeidae) from the Tohoku district, northern Honshu, Japan. Acta Arachnologica 53: 35–51. <https://doi.org/10.2476/asjaa.53.35>
- Ihara Y (2006) *Cybaeus jinsekiensis* n. sp., a spider species with protogynous maturation and mating plugs (Araneae: Cybaeidae). Acta Arachnologica 55: 5–13. <https://doi.org/10.2476/asjaa.55.5>
- Ihara Y (2007) Diversity in genital morphology and geographic differentiation patterns in the spider genera *Cybaeus* and *Arcuphantes* (Araneae) in the Chugoku Mountains, Japan. Taxa, Proceedings of the Japanese Society of Systematic Zoology 22: 20–30. [In Japanese] https://doi.org/10.19004/taxa.22.0_20
- Ihara Y (2008) Species diversity and geographic differentiations of reproductive organs and body size in the genus *Cybaeus* (Araneae: Cybaeidae) in Japan. Acta Arachnologica 57: 87–109. [In Japanese] <https://doi.org/10.2476/asjaa.57.87>
- Ihara Y (2009a) Cybaeidae. In: Ono H (Ed.) The Spiders of Japan: with Keys to the Families and Genera and Illustrations of the Species. Tokai University Press, Hadano, 152–168. [In Japanese]
- Ihara Y (2009b) Revision of the *Cybaeus hiroshimaensis*-group (Araneae: Cybaeidae) in western Japan. Acta Arachnologica 58: 69–85. <https://doi.org/10.2476/asjaa.58.69>
- Ihara Y, Koike N, Nakano T (2021) Integrative taxonomy reveals multiple lineages of the spider genus *Cybaeus* endemic to the Ryukyu Islands, Japan (Arachnida: Araneae: Cybaeidae). Invertebrate Systematics 35: 216–243. <https://doi.org/10.1071/IS20070>
- Irie T (1998) A new eyeless spider of the genus *Cybaeus* (Araneae: Cybaeidae) found in a limestone cave of Kyushu, Japan. Acta Arachnologica 47: 97–100. <https://doi.org/10.2476/asjaa.47.97>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kobayashi T (2006) Ten new species of the genus *Cybaeus* (Araneae: Cybaeidae) from central Honshu, Japan. Acta Arachnologica 55: 29–44. <https://doi.org/10.2476/asjaa.55.29>
- Koch CL (1839) Die Arachniden. Getreu nach der Natur abgebildet und beschrieben. Sechster Band. Zeh'schen Buchhandlung, Nürnberg, 156 pp.
- Koch L (1868) Die Arachnidengattungen *Amaurobius*, *Caelotes* und *Cybaeus*. Abhandlungen der Naturhistorischen Gesellschaft zu Nürnberg 4: 1–52.
- Komatsu T (1940) On five species of spiders found in the Ryugado Cave, Tosa Province. Acta Arachnologica 5: 186–195. <https://doi.org/10.2476/asjaa.5.186>
- Komatsu T (1968) Cave Spiders of Japan. II. *Cybaeus*, *Dolichocybaeus* and *Heterocybaeus* (Cybaeinae). Arachnological Society of East Asia, Osaka, 38 pp.
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. <https://doi.org/10.1093/molbev/msw260>
- Matsuda K, Ihara Y, Nakano T (2020) Description of a new species of *Cybaeus* (Araneae: Cybaeidae) from central Honshu, Japan. Species Diversity 25: 145–152. <https://doi.org/10.12782/specdiv.25.145>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. <https://doi.org/10.1093/molbev/msu300>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67: 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Ramírez MJ (2014) The morphology and phylogeny of dionychnan spiders (Araneae: Araneomorphae). Bulletin of the American Museum of Natural History 390: 1–374. <https://doi.org/10.1206/821.1>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Wessel P, Smith WHF (1996) A global, self-consistent, hierarchical, high-resolution shoreline database. Journal of Geophysical Research. Solid Earth 101: 8741–8743. <https://doi.org/10.1029/96JB00104>

World Spider Catalog (2021) World Spider Catalog. Version 22.0. Species list for *Cybaeus*. Natural History Museum Bern. <https://wsc.nmbe.ch/specieslist/686> [25 March 2021]

Yamaguchi T, Yaginuma T (1971) The fauna of the insular lava caves in west Japan. VIII. Araneae (Part 2). Bulletin of the National Science Museum (Tokyo) 14: 171–180.

Supplementary material 1

Table S1

Authors: Yusuke Sugawara, Yoh Ihara, Takafumi Nakano
Data type: molecular data

Explanation note: Primers for polymerase chain reactions (PCR) and cycle sequencing reactions, and PCR conditions used in this study.

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Link: <https://doi.org/10.3897/zse.97.64473.suppl1>

Supplementary material 2

Table S2

Authors: Yusuke Sugawara, Yoh Ihara, Takafumi Nakano

Data type: molecular data

Explanation note: The selected partitioning schemes and molecular evolutionary models for the phylogenetic analyses.

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Link: <https://doi.org/10.3897/zse.97.64473.suppl2>