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Rediscovery of the troglobitic midget-cave spiders *Masirana glabra* (Komatsu 1957) with redescription of the male and first description of the unknown female (Araneae: Leptonetidae)

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Abstract — The species *Masirana glabra* (Komatsu 1957) is redescribed based on fresh specimens collected in the type locality, 65 years after its original description. The female is recorded and described for the first time. Habitus and genitalia of both sexes are illustrated in detail and the DNA barcode of the species is provided. Additional notes on the ecology of the species are reported. Our morphological and ecological observations further support *M. glabra* as a true troglobiont.

Key words — Barcode, caves, Kochi Prefecture, Shikoku, subterranean environment, troglobiont.

Introduction

Leptonetidae, also known as midget-cave spiders or mashiragumo（マシラグモ科）, is a family of tiny and slender spiders currently numbering 368 species and 21 genera worldwide (WSC 2022). Several leptonetid species are related to caves or cave-like habitats like screes, mines and artificial tunnels (Ledford et al. 2021). They often show different degrees of morphological adaptations to the life in the subterranean environment such as depigmentation, reduction of eyes or elongation of legs (Culver & Pipan 2019). Numerous cave-related species have limited geographic distribution being often restricted to few or even a single cave.

In Japan, Leptonetidae are relatively well diversified numbering 51 species and subspecies shared between two genera: *Falcileptoneta* Komatsu 1970 (27 species, 1 subspecies) and *Masirana* Kishida 1942 (20 species, 3 subspecies) (Tanikawa 2021, WSC 2022). Despite being an important component of the Japanese cave fauna, the taxonomy, ecology, and microhabitat preference of several Japanese leptonetid species are still poorly studied being known only by few records. In particular, 11 species (~22% of all the Japanese leptonetids) are described based on a single sex only, among them several cave-adapted species (WSC 2022).

The troglobitic species *Masirana glabra* (Komatsu 1957) is a good example of a cave endemic yet poorly-known species of Japanese leptonetid. This spider was described based on a single male collected in 1957 from Saruda-do cave in Kochi prefecture, Shikoku Is. No further specimens were recorded after the original description and the female is still considered unknown (Komatsu 1957, 1961). Pictures of male palp currently remain based on drawings only (Komatsu 1974) and images of female genitalia are missing.

While surveying subterranean environments in Kochi prefecture, we had the opportunity to collect some fresh specimens of *M. glabra* from the type locality cave. Among this material two females were also collected. With this work we aim to redescribe the species and describe the female for the first time. We report the species barcode, notes on its ecology, and detailed pictures of the genitalia of both sexes in order to help future identifications and studies of the subterranean leptonetids in Japan.

Material and methods

The specimens used in this study were collected by hand using tweezers and immediately put in pure ethanol (99%) for morphological and future molecular analysis. Environmental variables inside the cave (temperature and humidity) were recorded using an As One TH-220 Portable thermo-hygrometer. A Nikon SMZ1270 microscope with a Canon EOS60D digital camera mounted on it hosted in the Systematic Zoology Laboratory, Department of Biological Sciences,

Tokyo Metropolitan University, Japan (TMU) was used to observe and photograph the samples. Female genitalia were dissected using a sharp needle and cleared immerging the sample in lactic acid for 30 minutes. Photos of the female internal genitalia were taken using a Nikon Optiphot-2 biological microscope with the same model of digital camera mounted on it. Photos were merged using Helicon Focus v.7 image stacking software (https://www.heliconsoft.com) and improved with Adobe Photoshop CC v.20.0.6 (https://www.photoshop.com/). The left palp of the male is illustrated. All measurements are reported in millimeters. Leg measurements are reported as follows: total length (femur, patella, tibia, metatarsus, tarsus). Protocols and instruments used for DNA extraction, amplification and sequencing follow Suzuki & Ballarin (2020). The primer couple LCO1490 (forward) GGTCAACAAATCATCATAAAGATATTGG and HCO2198 (reverse) TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al. 1994) was used to amplify the barcode region. The following abbreviations are used in the text and figures: ALE = anterior lateral eyes; AT = atrium; CO = copulatory opening; E = embolus; MS = median sclerite; PL = prolateral lobe; PLE = posterior lateral eyes; PME = posterior median eyes; PS = prolateral sclerite; RS = retrolateral sclerite; S = spermathecae; SS = spermathecae stalk. All samples are deposited in the personal collection of the first author.

Results

Taxonomy

Class Arachnida Cuvier 1812
Order Araneae Clerck 1757
Family Leptonetidae Simon 1890
Genus *Masirana* Kishida 1942

Masirana glabra (Komatsu 1957)
[Japanese name: Ishikawa-mashiragumo, イシカワマシラグモ]
(Figs. 1–18)

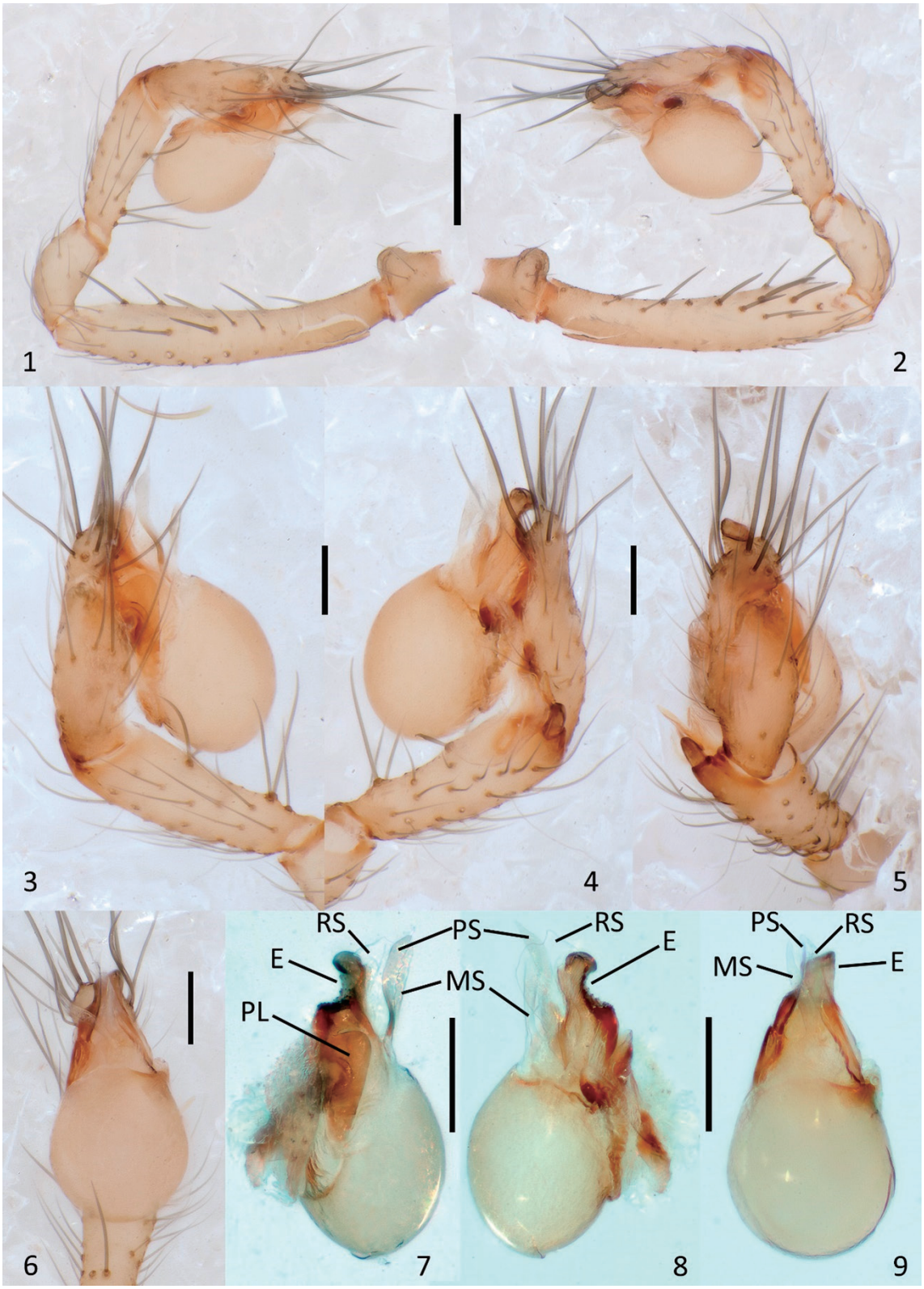
Sarutana glabra Komatsu 1957: 70, f. 3A–C (♂).
Sarutana glabra: Komatsu 1961: 18, f. 10A–D (♂).
Sarutana glabra: Komatsu 1974: 27, f. 20, 29 (♂).
Masirana glabra: Irie & Ono 2009: 119, f. 99–100 (♂).

DNA Barcode. 5’-TACTTTATATTTTATTTTTGGGGTGTGGTCTGCTATGTTAGGGACTGCTATGAGTGTGTTGATTCGGGTTGAGTTGGCACAGGGAGGTAGCTTAATTGGTAATGATCATCTTTATAATGTTATTGTTACGGCTCATGCGTTTGTTATGATTTTTTTTATGGTTATGCCTATTTTAATTGGGGGGTTTGGTAATTGATTGGTACCTTTAATAATTGGGGCTCCGGATATGGCTTTTCCTCGAATGAATAATTTGAGATTTTGGTTATTACCCCCGTCTTTGTTTTTGTTGATTGTTTCGGGTATGGTAGAAATGGGGGTGGGTACGGGTTGAACCATTTATCCGCCGTTAGCATCTTTTTTGGGTCATTCCTTTGTTTCTGTTGATTTTACCATTTTTTCTTTGCATTTGGCCGGGGCTTCGTCTATTATGGGGGCTATTAATTTTATTACTACCATTATTAATATGCGTGTTAGTGGAATGAGATTTGATAAGGTTTCTTTGTTTGTTTGATCTGTTTTGATTACTGCAGTATTGTTGTTGTTATCTCTGCCTGTGCTAGCGGGGGCTATTACTATGTTATTGACTGATCGTAATTTTAATACGTCTTTTTTTGACCCTGCTGGGGGAGGGGATCCAATTTTGTTTCAGCATCTATT-3’ (GenBank accession number: ON637943)

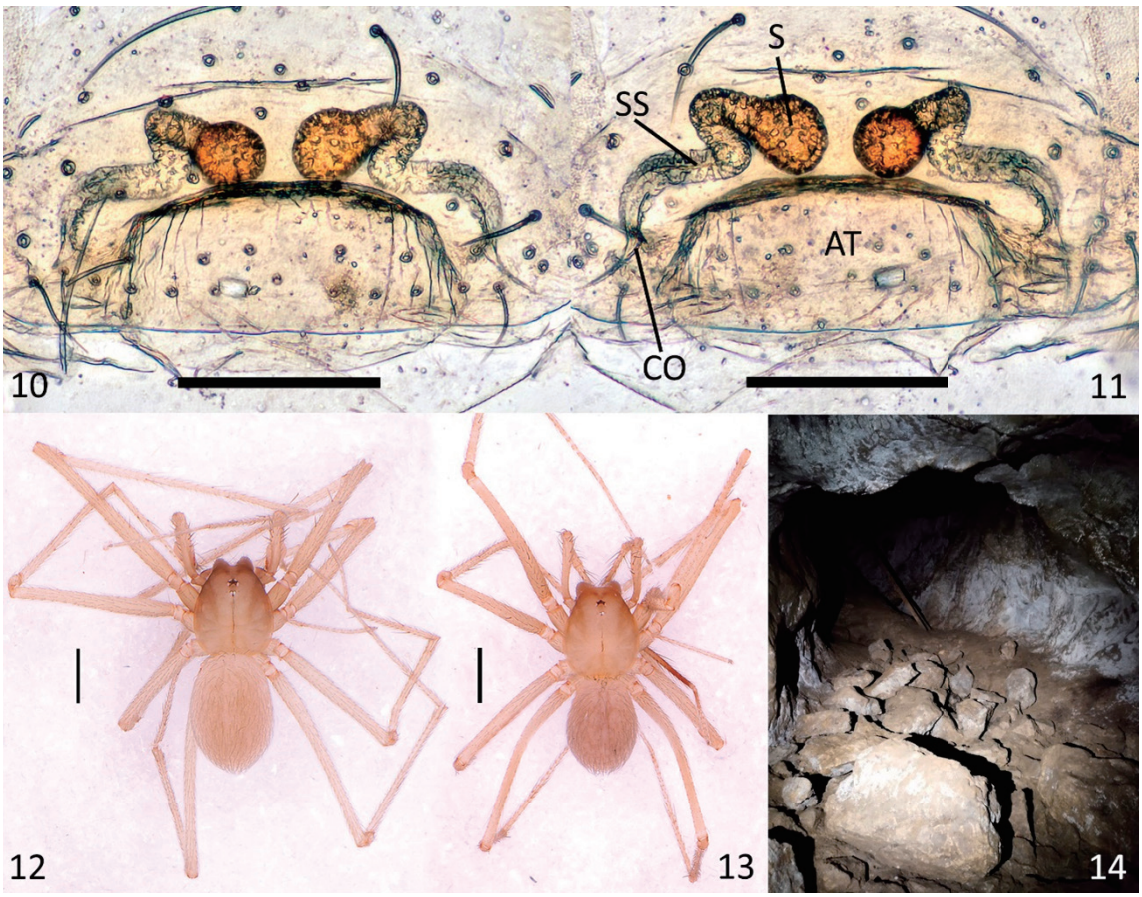
Material examined. JAPAN - 1♂, 2♀♀, Shikoku Is., Kochi Pref., Takaoka-gun, Hidaka-mura, Okina, Saruda-do cave（猿田洞）, 106 m a.s.l., (33.51573°N, 133.35760°E), humid area with rocks in the dark zone of the upper branch of the cave, 04 March 2022, Ballarin F. & Kutsukake J. leg.

Diagnosis. Species similar to *M. kawasawai* (Komatsu 1970) from Arakura-do cave in the same prefecture. Male of *M. glabra* can be easily distinguished from male of *M. kawasawai* by the shape of the antero-retrolateral tibial apophyses having a wide, lobate ventral apophysis and a short, blunt dorsal apophysis in contrast with sharper, more sclerotized or leaf-like ventral apophysis or more pointed and slimmer dorsal apophysis in *M. kawasawai*. Male of *M. glabra* can also be separated from male of congeneric species by the shape of embolus, slimmer, S-shaped, headed dorsally and with a serrated dorsal margin rather than thicker, straighter, headed frontally or lacking a serrated dorsal margin in *M. kawasawai* and other *Masirana* species (see Figs. 3–8 vs. figs. 15–17 in Komatsu 1970 and figs. 15–19, 21–28 in Komatsu 1974 or figs. 1–3 in Nishikawa 1986). Female of *M. glabra* are distinguished by congeneric species by the shape of spermathecae and stalks being rounded and with more twisted stalks in contrast with elongated spermathecae or less convoluted stalks in other species (see Figs. 10–11 vs. fig. 5 in Nishikawa 1986)

Redescription of male. Habitus as in Fig. 12. Total length: 1.91, Prosoma 0.87 long, 0.73 wide. Carapace uniformly very light brownish, frontal margins slightly darker. Median groove, cervical grooves and radial furrows faint and poorly visible. Row of short hairs along the median groove. Cephalic area poorly defined, slightly raised from the carapace. Sternum uniformly light brownish. ALE = 0.04, PLE = 0.03, PME = 0.02, ALE-PLE = 0.01, PLE-PME = 0.03. Chelicera, maxillae, and labium uniformly brownish, darker than the carapace. Promargin of chelicera with a row of 8 teeth, retromargin with a row of 7 tiny denticles. Maxillae distally dilatated with a bulge protruding externally (Figs. 15, 17). Six eyes reduced in size (Fig. 15), some eyes strongly reduced in some specimens (see also Komatsu 1957). Legs uniformly light brownish with sparce hairs. Leg formula: I, IV, II, III. Leg measurements as follow: I = 7.09 (1.98, 0.28, 2.27, 1.66, 0.90), II = 5.52 (1.58, 0.27, 1.66, 1.24, 0.77), III = 4.31 (1.23, 0.24, 1.12, 1.05, 0.67), IV = 5.94 (1.77, 0.25, 1.79, 1.36, 0.77). Opisthosoma uniformly light brownish covered with short hairs. Palp as in Figs. 1–9. Femur with two ventral rows of long, robust spines. Tibia elongated, approximately one and half the length of patella.



Figs. 1–9. Male palp of *Masirana glabra*. 1, whole palp, prolateral view; 2, ditto, retrolateral view; 3, tip of the palp, prolateral view; 4, ditto, retrolateral view; 5, ditto, dorsal view; 6, ditto, ventral view; 7, bulb of the palp, prolateral view; 8, ditto, retrolateral view; 9, ditto, ventral view. Abbreviations: E = embolus; MS = median sclerite; PL = prolateral lobe; PS = prolateral sclerite; RS = retrolateral sclerite. Scale bars = 0.2 mm (1–2); 0.1 mm (3–9).



Figs. 10–14. Male and female of *Masirana glabra.* 10, internal genitalia of female, ventral view; 11, ditto, dorsal view; 12, habitus of male, dorsal view; 13, habitus of female, dorsal view; 14, habitat of *Masirana glabra* inside Saruda-do cave where the specimens were collected. AT = atrium; S = spermathecae; CO = copulatory opening; SS = spermathecae stalk. Scale bars = 0.1 mm (10–11); 0.5 mm (12–13).

Few sparce long hairs on the ventral border, three on the proximal-ventral margin and one on the mid-ventral margin. Two apophyses on the retro-distal margin of tibia, dorsal apophysis cylindrical, short and stocky, ventral apophysis wide and lobate, with an elongated recess in the center and ending dorsally with a phylliform, S-shaped branch (Figs. 2, 4–5). Cymbium relatively short and stocky with several long spines on the apex and prolateral margin (Figs. 3–6). Bulb with three ribbon-like, translucent sclerites: median sclerite (MS) long and thin, dorso-ventrally flattened and headed antero-prolaterally; prolateral sclerite (PS) laterally flattened with a slightly sclerotized dorsal edge, slightly warped and headed frontally; retrolateral sclerite (RS) wide and transparent, wrapping the other sclerites on the retrolateral side. Embolus (E) strongly sclerotized, S-shaped when observed laterally, with a blunt tip headed dorsally toward the cymbium and a serrated dorsal margin (Figs. 7–9 and figs. 22, 29 in Komatsu 1974). For the description of the male see also Komatsu (1961), pages 18–19.

Description of female. Habitus as in Fig. 13. Total length: 1.65, Prosoma 0.82 long, 0.66 wide. Coloration of prosoma, legs and opisthosoma as in male. Maxillae lacking a distal bulge (Fig. 18). Eyes reduced as in male, PME often more reduce than anterior eyes and barely visible in some specimens (Fig. 16). ALE = 0.03, PLE = 0.03, PME = 0.01, ALE-PLE = 0.01, PLE-PME = 0.02. Other characteristics as in male. Leg formula: I, IV, II, III. Leg measurements as follows: I = 6.71 (1.80, 0.30, 2.11, 1.52, 0.98), II = 5.09 (1.46, 0.26, 1.53, 1.11, 0.73), III = 4.03 (1.17, 0.22, 1.01, 0.96, 0.67), IV = 5.87 (1.75, 0.26, 1.72, 1.31, 0.83). Internal genitalia as in Figs. 10–11. Atrium (AT) wide, trapezoidal. Spermathecae stems (SS) starting at the sides of the atrium, reaching the spermathecae (S) with a slightly S-convoluted course heading frontally and internally toward the atrium. Spermathecae small and rounded, close to each other and slightly bent posteriorly (Figs. 10–11).

Female size variation. Total length: 1.65–1.73, Prosoma 0.82–0.84 long, 0.66–0.69 wide.

Distribution. Endemic to Saruda-do cave in Kochi Pref., Shikoku Is., Japan. Known only from the type locality.

Notes on habitat and ecology. Specimens used in this study were collected in the dark zone of the upper branch of the cave, approximately 30 m far from the secondary entrance, in a humid area (temp: 15°C, humidity: 91.8%) with clay and large rocks on the floor (Fig. 14). Adult and juveniles were observed spinning simple irregular webs in narrow recesses of the walls of the cave and in empty spaces between the rocks on the floor. No specimens of *Masirana*



Figs. 15–18. Detail of male and female of *Masirana glabra.* 15, prosoma of male, frontal view; 16, prosoma of female, frontal view; 17, maxillae of male, ventral view; 18, maxillae of female, ventral view. Scale bars = 0.2 mm.

glabra have been found in the lower and drier branches or in the earlier traits (entrance and twilight zones) of the cave. It is worth noting that, according to Mr. Seiichi Ozaki (Hidaka-mura Board of Education), there was much less precipitation than usual during the months prior to the survey. Therefore, it is possible that the cave was drier than normally, causing the suitable microhabitat of the species to shrink inside the cave.

Komatsu (1961, pp. 40, 43) suggested that *Masirana glabra* is a permanent cave-dwelling spider only living in the inner section of the cave. Our observations mentioned above further support *M. glabra* as a true troglobiont. In addition, *M. glabra* shows clear morphological adaptations for the life in dark, subterranean habitats such as depigmentation, reduction of eyes and elongation of legs.

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