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# Taxonomic notes on leptonetid spiders from the Ryukyu Archipelago with the description of two new species and the first record of the genus *Longileptoneta* from Japan (Araneae: Leptonetidae)

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# Abstract

The species belonging to the family Leptonetidae living in the Ryukyu Archipelago, Southwestern Japan, are herein discussed. Two species, *Longileptoneta yamasakii* **sp. nov.** and *Masirana suzukii* **sp. nov.**, are described as new to science on the basis of both male and female. The new species were collected in caves in Yonaguni-jima Is. and Okinawa-honto Is., respectively the westernmost and largest islands of the Ryukyu arc. *Longileptoneta yamasakii* **sp. nov.** represents the first record of the genus *Longileptoneta* Seo, 2015 from Japan. The male and female of another leptonetid species previously known from the Ryukyus, *Masirana longipalpis* Komatsu, 1972, are redescribed and illustrated in detail. The internal genitalia of the female are shown and described for the first time.

Key words: caves, endemic species, Ishigaki-jima Island, Okinawa-honto Island, sinkhole, subterranean environment

# Introduction

Leptonetidae Simon, 1890, commonly known as midget-cave spiders, is a family of tiny, six-eyed (lacking in caveadapted species), slender haplogynae spiders numbering 370 species and 22 genera worldwide (WSC 2022). These spiders are mainly distributed in Central-North America, the Mediterranean region, and in Eastern Asia. Most of the leptonetid species show troglophilic habits building delicate space-webs in dark and moist habitats. They usually dwell in caves, screes, mines, and other cave-like environments with stable humidity and temperature, although some species can also inhabit empty spaces in moist forest leaf litter or under superficial stones (Ledford et al. 2021). In Eastern Asia, eight genera and 236 species of Leptonetidae are known to occur: Falcileptoneta Komatsu, 1970 (62 species from China, Korea, Japan, and Taiwan), Jingneta Wang & Li, 2020 (9 species, all from China), Leptoneta Simon, 1872 (16 species, all from Korea), Leptonetela Kratochvíl, 1978 (105 species, all from China), Longileptoneta Seo, 2015 (14 species from China and Korea), Masirana Kishida, 1942 (27 species from Korea, Japan, and Taiwan), Rhyssoleptoneta Tong & Li, 2007 (2 species, all from China), and the recently described monospecific genus Yueleptoneta Yang, Tong & Bian, 2022 from China (Wang et al. 2020; Yang et al. 2022; WSC 2022). In contrast, in Japan only two genera of Leptonetidae have been reported so far, numbering a total of 51 species and subspecies: Falcileptoneta (27 species, 1 subspecies) and Masirana (20 species, 3 subspecies) (Tanikawa 2022; Ballarin & Eguchi 2022; WSC 2022). Despite being an important component of the Japanese spider fauna, several of the Japanese leptonetid species are still poorly studied, being described on the basis of a single sex or lacking proper illustrations of internal female genitalia (Ballarin & Eguchi 2022).

Among the Japanese leptonetids, only two species, *Falcileptoneta okinawaensis* Komatsu, 1972 and *Masirana longipalpis* Komatsu, 1972, are currently recorded in the islands forming the Ryukyu Archipelago in Southwestern Japan. Both these species are found in caves. In particular, *M. longipalpis* shows extensive morphological adaptations to the subterranean lifestyle having reduced eyes, elongated legs and palps, and lacking pigmentation. Despite the fact that the Ryukyus are known to host a diverse and scientifically interesting endemic cave fauna, including several

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spider species (Machida *et al.* 2001, Shimojana 1977), the study of Leptonetidae in the islands of this archipelago has been long neglected. With the exclusion of the original descriptions by Komatsu (1972) and the records reported in the extensive preliminary research on cave spiders by Shimojana (1977), no other studies on leptonetids have been conducted in the Ryukyus. Both recorded leptonetid species lack modern, detailed photographs of their diagnostic characters, as their recognition currently relies only on the original drawings of the male palps and habitus. In addition, in both cases, the shape of internal genitalia of the female has never been illustrated or described.

While collecting spiders in caves in the Ryukyus, we had the opportunity to find several leptonetid spiders, including a large population of an unknown species dwelling near the entrance of a small cave in Yonaguni-jima Is., the westernmost inhabited island of the Ryukyu arc. Another unknown species was collected in a cave in Okinawahonto Is., the largest island of the Ryukyus, located at the central part of the archipelago. A careful morphological examination of genitalia of these two species revealed them as new to science. In addition, the new species from Yonaguni-jima Is. showed the diagnostic character of *Longileptoneta* Seo, 2015, a genus previously recorded only from mainland China and Korea (Seo 2015; Wang 2020; WSC 2022) and thus representing the first record of this genus for Japan. The aim of this work is to make a preliminary update on the leptonetid fauna in the Ryukyu Archipelago, describing the new species, illustrating the diagnostic character of *M. longipalpis*, including the unknown internal genitalia of the female, and providing the barcodes of the species.

# Material and methods

Specimens were collected by hand in caves and preserved in 99% ethanol for morphological and future molecular analysis. Samples were examined under a Nikon SMZ1270 microscope in the Systematic Zoology Laboratory, Department of Biological Sciences, Tokyo Metropolitan University, Japan (TMU) and photographed with a mounted Canon EOS60D digital camera. Internal genitalia were dissected with a sharp needle, cleared in lactic acid for 30 minutes and photographed using the same Nikon camera mounted on a Nikon Optiphot 2 biological microscope. Photos were prepared with Helicon Focus v.7 image stacking software (https://www.heliconsoft.com) and digitally edited with Adobe Photoshop CC v.20.0.6 (https://www.photoshop.com/). The left palp of males is illustrated. All measurements are given in millimeters. Leg measurements are given as follows: total length (femur, patella, tibia, metatarsus, tarsus). The following abbreviations are used in the text and figures (after Wang *et al.* 2020): ALE= anterior lateral eyes; AC = apical constriction; AT = atrium; CO = copulatory opening; E = embolus; MS = median sclerite; PA = patellar apophyses; PL = prolateral lobe; PLE = posterior lateral eyes; PME = posterior median eyes; PS = prolateral sclerite; RS = retrolateral sclerite; S = spermathecae; SS = spermathecae stalk, TA = tibial apophyses, TS = tarsal spur.

The following primers were used to amplify the barcode region: LCO1490 (forward) GGTCAACAAATCAT CATAAAGATATTGG (Folmer *et al.* 1994) and HCO2198 (reverse) TAAACTTCAGGGTGACCAAAAAATCA (Folmer *et al.* 1994) or CHR2 (reverse) GGATGGCCAAAAAATCAAAATCAAAATG (Barrett & Herbert 2005). Protocols and equipment used for extraction, amplification, and sequencing follows Suzuki & Ballarin (2020) and Ballarin & Eguchi (2022). Despite several attempts and the use of different primers we failed to sequence the barcode of *Masirana suzukii* **sp. nov.** the resulting sequence being noisy and difficult to read. All vouchers used in this study are preserved in the National Museum of Nature and Science, Tokyo, Japan (NMST), the Museum of Nature and Human Activities, Hyogo (MNHAH), the Ryukyu University Museum Fujukan, Okinawa, Japan (RMUF), the Civic Museum of Natural History of Verona, Italy (MSNVR), and in the personal collection of Francesco Ballarin (FBPC).

#### Results

# Taxonomy

Class Arachnida Cuvier, 1812

Order Araneae Clerck, 1757

#### Family Leptonetidae Simon, 1890

Genus Longileptoneta Seo, 2015 (Japanese name: nagamashiragumo ナガマシラグモ)

Type species: Longileptoneta songniensis Seo, 2015 from Korea.

Distribution. China, Japan (new record), Korea.

#### Longileptoneta yamasakii sp. nov.

(Japanese name: yamasakinagamashiragumo ヤマサキナガマシラグモ) Figs. 1A-G, 2A-F, 3A-D

#### DNA barcode. GenBank accession number: OP680015.

**Material examined.** *A* **Holotype. JAPAN: Okinawa Pref.: Yonaguni-jima Is.,** Yaeyama-gun, Yonaguni-cho, unnamed short cave inside a deep, shadowed sinkhole, 24.45872°N, 122.95618°E, 23 m a.s.l., under stones and in mud crevices at the entrance of the cave, 02 March 2021, F. Ballarin & K. Eguchi leg. (NSMT-Ar 22244).

**Paratypes. JAPAN**—same data as the holotype 13, 192 (52 NSMT-Ar 22245; 13, 92MNHAH-B6-000402; 52 RMUF); same locality, 102 (62 FBPC; 42 MSNVR-Ar028–031), 05 March 2021, all F. Ballarin & K. Eguchi leg.

**Etymology.** The new species is a patronym in honor to our colleague and friend Takeshi Yamasaki (Museum of Nature and Human Activities, Hyogo Prefecture, Japan), for his contribution to the study of arachnology and for kindly helping with field collections in Japanese caves.

**Diagnosis.** The male of *Longileptoneta yamasakii* **sp. nov.** can be distinguished from the male of the similar *L. gutan* Wang & Li, 2020 and *L. shenxian* Wang & Li, 2020 or any other congeners by the following combination of unique characters: presence of a pair of lanceolate apophyses (PA) on the retrodistal part of the patella (reduced to normal, sharp spines in *L. gutan* and *L. shenxian*; cf. Figs. 1B, D, 3B vs. figs. 8D and 12C in Wang *et al.* 2020) and a robust and strongly sclerotized prolateral sclerite (PS) (thinner PS in *L. gutan* and *L. shenxian*, or transparent and less sclerotized in other congeners (cf. Figs. 1A, G, 3A vs. figs. 8C and 12C in Wang *et al.* 2020). In addition, the new species can be recognized by the general shape of the other palpal sclerites when the bulb is observed ventrally or dorsally (differently shaped in *L. gutan, L. shenxian* and in other congeners; cf. Figs. 1G, 3C vs. figs. 8B and 12B in Wang *et al.* 2020). The female of *L. yamasakii* **sp. nov.** is distinguished from the female of *L. gutan, L. shenxian* and other congeneric species by the shape of internal genitalia having less twisted ducts (SS) and spermathecae (S) headed toward to each other (vs. more coiled SS and S headed more frontally in *L. gutan* and *L. shenxian* or usually smaller S in other congeners; cf. Figs. 2A, B, 3D vs. figs. 9C and 13C in Wang *et al.* 2020). The dorsal pattern, having clear dark stripes on the opisthosoma, and the general shape of genitalia both help to quickly distinguish *L. yamasakii* **sp. nov.** from any other leptonetid species living in the Ryukyus.

Description. Male (holotype). Habitus as in Fig. 2C. Total length: 2.52; prosoma 1.03 long, 0.93 wide. Carapace dark brown with a lighter central area less visible in alive specimens (Fig. 2F). Median groove, cervical grooves and radial furrows distinct. Cephalic area poorly defined, slightly raised from carapace. Six eyes all well-developed. ALE = 0.06, PLE = 0.05, PME = 0.05, ALE-PLE = 0, PLE-PME = 0.02. Chelicera, labium and maxillae uniformly brownish. Promargin of chelicera bearing a row of 8 denticles; denticles absent on retromargin. Sternum uniformly dark brown. Legs uniformly brown. Leg formula: I, IV, II, III. Leg measurements (leg II partially missing): I = 9.43 (2.57, 0.36, 2.98, 2.47, 1.05), II = ? (1.83, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 1.46, 0.88), IV = 7.84 (2.11, 0.34, -), III = 5.8 (1.60, 0.25, 1.61, 0.25), III = 5.8 (1.60, 0.25), III = 5.8 (1.60,2.38, 2.02, 0.99). Opisthosoma greyish with two rows of 4–5 dark transversal stripes gradually merging to each other toward the posterior part of opisthosoma. Palp as in Figs. 1A-G, 3A-C. Femur with a row of long and robust spines on ventral margin, additional strong spines on the prolateral and dorsal margins. Patella elongated, bearing a pair of robust, lanceolate apophyses (PA) on retrodistal margin. Tibia short, approx. half of length of patella, with a tubular, robust apophysis on retrodistal margin with a spine on its apex and another spine at its base (TA) (Figs. 1B, 3B). Cymbium with medial depression and several long and robust dorsal spines headed prolaterally. Bulb with three sclerites: prolateral sclerite (PS) spine-like, robust and heavily sclerotized; median sclerite (MS) long and laminar, twisted apically; retrolateral sclerite (RS) flat and wrinkled, sclerotized at its basal trait and wrapped around embolus. Embolus (E) sclerotized at its base, distally leaf-like and transparent, ending with a long, narrow lobe (Figs. 1C–G and 3A–C).



**FIGURE 1.** Male palp of *Longileptoneta yamasakii* **sp. nov.** (holotype). A palp, prolateral view; **B** same, retrolateral view; **C** tip of the palp, prolateral view; **D** same, ventral view; **E** same, retrolateral view; **F** same, dorsal view; **G** bulb of the palp, dorsal view. Abbreviations: E = embolus; MS = median sclerite; PS = prolateral sclerite; RS = retrolateral sclerite. Scale bars: A-F = 0.2 mm; G = 0.1 mm.

Female (based on one of the paratypes). Habitus as in Figs.2 D–F. Total length: 2.70, Prosoma 1.01 long, 0.91 wide. Similar to male for coloration and pattern. Frontal view of cephalic area as in Fig. 2E. ALE = 0.06, PLE = 0.05, PME = 0.05, ALE-PLE = 0, PLE-PME = 0.02. Leg formula: I, IV, II, III. Leg measurements: I = 5.62(1.47, 0.23, 1.71, 1.38, 0.83), II = 4.12 (1.16, 0.23, 1.17, 0.95, 0.61), III = 3.47 (0.96, 0.20, 0.92, 0.83, 0.56), IV = 4.78 (1.30, 0.22, 1.45, 1.16, 0.65). Opisthosoma wrinkled in the frontal part (Fig. 2E). Other characters as in male. Internal genitalia as in Fig. 2A, B, 3C. Atrium (AT) wide, triangular; spermathecae stalk (SS) reaching spermathecae (S) with a slight S-shaped course. Spermathecae oval, separated from each other by two and 2/3 of their diameter, slightly headed toward each other and slightly bent posteriorly toward AT.



FIGURE 2. male and female of *Longileptoneta yamasakii* sp. nov. A internal genitalia of female, ventral view; B same, dorsal view; C habitus of male, dorsal view (holotype); D habitus of female, dorsal view (one of the paratypes); E female cephalic area, frontal view; F habitus of the female in life; G habitat where the species was collected. Scale bars: A, B, E = 0.2 mm; C, D = 0.5 mm



**FIGURE 3.** male and female of *Longileptoneta yamasakii* **sp. nov. A** male palp, prolateral view; **B** same, retrolateral view **C** bulb, ventral view; **D** internal genitalia of female. Abbreviations: AC = apical constriction; AT = atrium; E = embolus; MS = median sclerite; PA = patellar apophyses; PL = prolateral lobe; PS = prolateral sclerite; RS = retrolateral sclerite; S = spermatheca; SS = spermatheca stalk; TA = tibial apophyses. Scale bars: A, B, D = 0.2 mm, C = 0.1 mm

Size variation: Male (based on 2 specimens): total length: 2.52–2.61, Prosoma 1.03–1.06 long, 0.92–0.96 wide. Female (based on 5 specimens): total length: 2.51–2.70, Prosoma 0.91–1.01 long, 0.89–0.91 wide.

Distribution. Known only from the type locality (Fig. 10).

**Habitat.** Cave-like habitats. The new species was found spinning small sheet-webs in mud and rock crevices on the ground and under dead wood at the entrance of a short cave opening at the bottom of a humid, shadowed sinkhole covered with subtropical vegetation (Fig. 2G).

**Remarks.** Longileptoneta yamasakii **sp. nov.** is locally abundant. The population numbered tens of specimens, often spinning webs in crevices close to each other, but occurring only in a small area of few square meters near the entrance of the short cave in the type locality. The species clearly shows troglophilic preferences, however it retains a full pigmentation and large, functional eyes. Thus, it lacks any troglomorphic characters typical of species deeply adapted to a subterranean life-style like in others leptonetids living in Ryukyus caves (e.g., *M. longipalpis*). This suggests that *L. yamasakii* **sp. nov.** might also inhabit screes or external habitats, especially if stable and moist. However, despite intensive collections by the authors in the surroundings of the type locality, in the leaf litter of forests covering the central area of Yonaguni-jima Is., no specimens of this or any other leptonetid species were collected.

### Genus Masirana Kishida, 1942

Type species. Masirana cinevacea Kishida, 1942 from Japan.

Distribution. Japan, Korea, Taiwan.

#### Masirana suzukii sp. nov.

(Japanese name: tiragamahinamashiragumoティラガマヒナマシラグモ) Figs. 4A–F, 5A–E, 6A–E.

Material examined. ♂ Holotype. JAPAN: Okinawa Pref.: Okinawa-honto Is., Kunigami-gun, Nakijin-son, Tiragama cave (ティラガマ = Tametomo no horaana, 為朝の洞穴), 26.684°N, 128.005°E, 55 m a.s.l., short and rather humid cave, 17 November 2020, F. Ballarin leg. (NSMT-Ar 22246).

**Paratypes. JAPAN** - same data as the holotype, 23, 12 (23, 12 RMUF; 13, 12 FBPC).

**Etymology.** The new species is a patronym in honor to our colleague and friend Yuya Suzuki (United Graduate School of Agricultural Sciences, Kagoshima University, Japan). Yuya is a young and promising arachnologist actively working on ethology, ecology and taxonomy of Japanese spiders, including cave species from the Ryukyus.

**Diagnosis.** Species closely related to *Masirana changlini* (Zhu & Tso, 2002) from Taiwan. The male of *M. suzukii* **sp. nov.** can be distinguished from the male of *M. changlini* by the different number of denticles on the chelicera (a single row of 9 denticles on the promargin in *M. suzukii* **sp. nov.** vs. two rows of denticles, 6 on the promargin and 7 on the retromargin, in *M. changlini*; cf. Fig. 6D vs. fig. 3 in Zhu & Tso 2002) and by the slimmer and longer tip of the cymbium bearing a short, stronger spine (vs. a shorter, tougher tip of cymbium lacking any thick apical spine in *M. changlini*; cf. Figs. 4B, C–F, 6A, B vs. figs. 4, 5 in Zhu & Tso, 2002). In addition, the embolus of the new species shows a more rounded apex in contrast with a pointy apex in *M. changlini* (cf. Figs. 4B, 6B vs. fig. 4 in Zhu & Tso 2002). The male of *M. suzukii* **sp. nov.** can be easily separated from the male of *M. longipalpis* by the different length of the palpal femur and tibia (cf. Figs. 4A, B vs. 7A, B). The female of *M. suzukii* **sp. nov.** is distinguished from the female of other congeners, including *M. longipalpis*, by the different shape of internal genitalia (e.g., smaller and rounded spermathecae (S) in contrast with larger, sac-like S in *M. longipalpis*, cf. Figs. 5A, B and 6E vs. Figs. 8E, F).

**Description.** Male (holotype). Habitus as in Fig. 5C. Total length: 1.66; prosoma 0.72 long, 0.63 wide. Carapace brownish with sightly lighter central area. Median groove, cervical grooves and radial furrows distinct. Cephalic area poorly defined, slightly raised from carapace. Sternum uniformly brownish. Six eyes all well-developed. ALE=0.05, PLE=0.05, PME=0.05, ALE-PLE=0.01, PLE-PME=0.03. Chelicera, labium and maxillae uniformly brownish. Chelicera bearing a single row of 8 denticles on promargin; denticles on retromargin missing (Fig. 6D). Legs uniformly light brown. Leg formula: I, IV, II, III. Leg measurements: I = 4.67 (1.42, 0.22, 1.74, 1.38, 0.91), II = 3.75 (1.06, 0.22, 1.07, 0.81, 0.59), III = 3.09 (0.87, 0.21, 0.80, 0.73, 0.48), IV = 4.03 (1.19, 0.23, 1.26, 1.03, 0.59). Opisthosoma yellowish with faint dorsal marks. Male palp as in Figs. 4A–F, 6A–C. Femur with several long and robust spines on its ventral and dorsal margins. Patella and tibia both elongated, approximately of the same length. Two apophyses (TA) on retrodistal margin of tibia close to each other; ventral apophysis large and lanceolate; dorsal apophysis spine-like, larger at its base and ending with a sharp and long tip (Figs. 4D, E and 6B). Cymbium bearing several long and thin spines, ending pointy and with a robust spine at its apex (= tarsal spur). Bulb with two

sclerite: median sclerite (MS) spine-like, long and thin; prolateral sclerite (PS) wide and transparent, ribbon-like and wrapped around MS. Embolus (E) long and robust, thread-like and laterally flattened, ending with a rounded tip slightly curved dorsally (Figs. 4C–F and 6A–C).

Female (one of the paratypes). Habitus as in Fig. 5D, E. Total length: 1.59; prosoma 0.69 long, 0.63 wide. General coloration and pattern as in male. Frontal view of cephalic area as in Fig. 5E. ALE=0.05, PLE=0.05, PME=0.05, ALE-PLE=0.01, PLE-PME=0.03. Leg formula: I, IV, II, III. Leg measurements: I = 4.69 (1.28, 0.23, 1.42, 1.09, 0.67), II = 3.51 (1.01, 0.21, 0.95, 0.77, 0.57), III = 3.03 (0.85, 0.23, 0.76, 0.69, 0.50), IV = 4.09 (1.12, 0.22, 1.19, 0.95, 0.61). Opisthosoma brownish or yellowish, strongly wrinkled in the frontal part (Fig. 5E). Other characters as in male. Internal genitalia as in Fig. 5A, B, 6E. Atrium (AT) wide, cup-shaped. Spermathecae stalk (SS) starting at sides of atrium, first bending inward with a comma course, then reaching spermathecae (S) after one convolution. Spermathecae small and round, separated from each other by two and a half of their diameter.



**FIGURE 4.** Male palp of *Masirana suzukii* **sp. nov.** from Okinawa. **A** palp, prolateral view **B** same, retrolateral view; **C** tip of palp, prolateral view; **D** same, retrolateral view; **E** same, dorsal view; **F** same, ventral view. Scale bars: A, B = 0.2 mm; C-F = 0.1 mm.



**FIGURE 5.** Female and male of *Masirana suzukii* **sp. nov.** A internal genitalia of female, ventral view; **B** same, dorsal view; **C** habitus of male, dorsal view; **D** habitus of female, dorsal view **E** same, frontal view. Scale bars: A, B, E = 0.2 mm, C, D = 0.5 mm.

Size variation: Male (based on 4 specimens): total length: 1.55–1.66, Prosoma 0.65–0.72 long, 0.63–0.66 wide. Female (based on 2 specimens): total length: 1.59–1.70, prosoma 0.69–0.72 long, 0.63–0.66 wide.

Distribution. Known only from the type locality (Fig. 10).

**Habitat.** Caves. The new species was found in empty spaces under stones and in recesses of the floor in the twilight zone of a short and humid cave.

**Remarks.** *M. suzukii* **sp. nov.** shows reduced pigmentation and faint dorsal color pattern. Despite lacking any real troglobitic adaptation, the collecting environment suggests troglophilic habits. However, we do not exclude that this species might also inhabit screes and other shallow subterranean habitats or even external environments (e.g., forest litter). According to Shimojana (1977, pg. 347) *Falcileptoneta okinawaensis* was also recorded from the same cave where *M. suzukii* **sp. nov.** was collected. Despite extensive collections inside the cave, we could not find any other leptonetid species. In addition, the records of *F. okinawaensis* by Shimojana in Tiragama cave were based on females only. Due to the external similarities of females in Leptonetidae and the lack of information about the internal genitalia of these species, it is possible that such records refer to misidentified samples of *M. suzukii* **sp. nov.** 



**FIGURE 6.** male and female of *Masirana suzukii* **sp. nov. A** male palp, prolateral view; **B** same, retrolateral view **C** bulb, ventral view; **D** male chelicera, internal view; **E** internal genitalia of female. Abbreviations: AT = atrium; E = embolus; MS = median sclerite; PL = prolateral lobe; PS = prolateral sclerite; S = spermatheca; SS = spermatheca stalk; TA = tibial apophyses; TS = tarsal spur. Scale bars: <math>A-D = 0.1 mm, E = 0.2 mm.

# Masirana longipalpis Komatsu, 1972

Figs.7A-F, 8A-I, 9A-D

*Masirana longipalpis* Komatsu, 1972: 83, f. 6-9 ( $\overset{\wedge}{\bigcirc} \overset{\circ}{\downarrow}$ ). *M. longipalpis* Shimojana, 1977: 347, f. 3 (♂).

## DNA barcode. GenBank accession number: OP680016 (specimen from Nisshudo cave).

Material examined. JAPAN-Okinawa Pref., Okinawa-honto Is.: Kunigami-gun: Motobu-cho: Shinzato, Abuntogama cave  $(\mathcal{P}\mathcal{T} \vee \vdash \mathcal{H}\mathcal{T})$ , 26.70075°N, 127.89205°E, 40 m a.s.l., slightly humid cave, twilight and dark zone, 4∂, 4♀ (3∂, 3♀ FBPC; 1∂, 1♀ MSNVR), 16 Nov. 2020, F. Ballarin leg.; Kin-cho, Kin-Kannonji Temple, Nisshudo cave (日秀洞 = Kannonji cave, 観音寺鍾乳洞) (type locality), 26.45530°N, 127.92170°E, 71 m a.s.l., large and humid cave, twilight and dark zone (temp: 20.3°C, hum: 98.7%), 33, 112 (23, 62 FBPC; 13, 32 MNHAH; 22MSNVR), 15.V.2022, F. Ballarin & M. Araki leg.—Ishigaki-jima Is.: Ishigaki-shi: Tonoshiro, Fukubukuîzâ cave (7 クブクイーザー, 第1洞), 24.36533°N, 124.17721°E, 66 m a.s.l., long cave humid with a small creek, dark zone, 1♂ (MNHAH), 9 Nov. 2020, F. Ballarin leg.; same locality, 1∂, 9♀ (FBPC), 11 Nov. 2020, F. Ballarin leg.

Type locality. Nisshu-do cave, Kin-cho, Kunigami-gun, Okinawa-honto Is., Okinawa Pref.

Diagnosis. The male of *Masirana longipalpis* can be easily distinguished from the male of *M. suzukii* sp. nov. and any other congeners by the unique shape of palp having an extremely elongated femur and tibia which are much shorter in other congeners (e.g., cf. Figs. 7A, B vs. 4A, B). The female of *M. longipalpis* can be easily separated by the female of other congeners, including M. suzukii sp. nov., by the large, sac-like spermathecae which are much smaller, or more rounded in other congeners (e.g., cf. Figs. 8E, F vs. 5A, B).

Redescription of male (based on specimen from Abuntogama cave). Male habitus as in Fig. 8G. Total length: 1.55; prosoma 0.69 long, 0.60 wide. ALE=0.04, PLE=0.04, PME=0.04, ALE-PLE=0, PLE-PME=0.04. Leg formula: I, IV, II, III. Leg measurements: I = 7.71 (2.45, 0.25, 2.2, 1.75, 1.06), II = 5.4 (1.81, 0.24, 1.89, 0.96, (0.50), III = 5.23 (1.49, 0.25, 1.45, 1.23, 0.81), IV = ? (2.08, 0.24, -). Palp as in Fig. 7A–F. Palpal femur extremely elongated, bearing some long and robust spines on its ventral margin, smaller sparce spines on dorsal and lateral margins. Patella long; tibia extremely elongated, approximately 3 and half time longer than patella. Two apophyses (TA) on the retrodistal margin of tibia close to each other: ventral apophysis large and lanceolate; dorsal apophysis spine-like, larger at its base and ending with a sharp and long tip. Cymbium with several long spines on dorsal and distal margin, headed antero-prolaterally, ending with a long spine (= tarsal spur) similar to the others. Bulb with two transparent sclerite; median sclerite (MS) long and tread-like, twisted in middle trait; retrolateral sclerite (RS) wide and transparent, ribbon-like and wrinkled, wrapped around embolus. Embolus (E) long and robust, ending with a lightly serrated tip curved dorsally. See also Komatsu (1972) for a detailed description of the male.

Description of female (based on specimens from Abuntogama cave). Habitus as in Fig. 8H, I. Total length: 1.87; prosoma 0.80 long, 0.73 wide. Carapace uniformly yellowish. Median groove, cervical grooves and radial furrows barely visibly. Cephalic area poorly defined, slightly raised from carapace. Frontal view of cephalic area as in Fig. 8I. Six eyes all reduced, PME strongly reduced (level of eye degeneration differs among specimens examined). ALE=0.05, PLE=0.04, PME=0.03, ALE-PLE=0, PLE-PME=0.05. Chelicera, labium and maxillae uniformly yellowish. Chelicera bearing a single row of 5-6 denticles on promargin, first 2-3 proximal teeth smaller than the others; denticles on retromargin missing. Palps elongated. Sternum uniformly yellowish. Legs uniformly yellowish. Leg formula: I, IV, II, III. Leg measurements: I = 10.19 (2.81, 0.32, 3.17, 2.43, 1.46), II = 7.99 (2.18, 0.29, 2.31, 1.74, 1.47), III = 6.19 (1.73, 0.31, 1.71, 1.49, 0.95), IV = 8.24 (2.34, 0.31, 2.52, 1.95, 1.12). Opisthosoma greyish with faint pattern of slightly darker marks, wrinkled in the frontal part (Fig. 8I). Internal genitalia as in Fig. 8E, F. Atrium (AT) triangular with a wide base. Spermathecae stalk (SS) narrow, starting from sides of atrium, heading first frontally then turning outward and inward before reaching the top of spermathecae. Spermathecae (S) very large, sac-like, headed posteriorly.

**Remarks on variation:** Size variation: male (based on 3 specimens from Abuntogama cave): total length: 1.55–1.90, prosoma 0.69–0.8–long, 0.60–0.74 wide; female (based on 5 specimens from Abuntogama cave): total length: 1.56-2.1, prosoma 0.67-0.88 long, 0.63-0.77 wide.

Species with reduced eyes, depigmentation and elongation of legs and palps. The degree of troglomorphic adaptations differs among populations living in different caves or different islands. Eyes reduction varies from no reduction to totally absent (e.g., compare Figs. 8C vs. 8D and 8I, see also Shimojana 1977, pg. 348). According to Shimojana (1977, pg. 363) the populations from some caves in Okinawa-honto Is. have the highest degree of



FIGURE 7. Male palp of *Masirana longipalpis* from Abutogama cave, Okinawa-honto. A palp, prolateral view; B same, retrolateral view; C tip of palp, prolateral view; D same, retrolateral view; E same, dorsal view; F same, ventral view. Scale bars: A, B = 0.2 mm; C-F = 0.1 mm.



**FIGURE 8.** male and female of *Masirana longipalpis*. A male from Nisshu-do cave, Okinawa-honto Is., tip of palp retrolateral view; **B** same, ventral view; **C** female from Nisshu-do cave, frontal view; **D** habitus of female from Fukubukuîzâ cave, Ishigakijima Is., dorsal view; **E** internal genitalia of female from Abuntogama cave, ventral view; **F** same, dorsal view; **G** habitus of male from Abuntogama cave, dorsal view; **H** same, habitus of female; **I** same, frontal view. Abbreviations: AT = atrium; E = embolus; MS = median sclerite; RS = retrolateral sclerite; S = spermathecae; SS = spermathecae stalk; TA = tibial apophyses. Scale bars: A-B = 0.1 mm, C, E, F, I = 0.2; D, G, H = 0.5 mm.

eyes reduction. Pigmentation and dorsal pattern of opisthosoma is variable, with populations totally or partially depigmented (e.g., cf. Figs. 8G, H and Figs. 9A–D) and other having a clearer pattern (see Fig. 8D). Specimens from different caves also show differences in thickness of embolus and its tip, ranging from thin and sharp to large and stocky (e.g., cf. Figs. 7D, F vs. 8A, B). Additional differences can be observed in the length of male palp with some populations having the femur considerably longer than tarsus + patella while in others these segments have approximately the same length (cf. Figs. 7A, B vs. fig. 8 in Komatsu 1972).



**FIGURE 9.** male and female of *Masirana longipalpis* from Okinawa in life **A** male from Nisshu-do cave, lateral view; **B** same, female; **C** same, female frontal view (photos by F. Ballarin); D female in its natural habitat (photo by Y. Suzuki)

**Habitat.** Caves. *M. longipalpis* can be found in the twilight zone and, more commonly, in the humid and dark zone in the deep of the cave. It usually spins small webs in the crevices and among the rocks at the base of the cave walls or on the cave floor. The populations can be locally abundant being distributed in a large area inside the cave; in other cases the specimens occupy only a limited section of the cave where the microclimate is more favorable for this species (F. Ballarin pers. obs.).

**Distribution.** Endemic to the Central and Southern Ryukyus (Fig.10). Widely distributed in the central and southern Ryukyus occurring in several islands: Aguni-jima Is., Hamahiga-shima Is., Ie-jima Is., Ike-shima Is., Ishigaki-jima Is., Kouri-jima Is., Kume-jima Is., Miyagi-jima Is., Okinawa-honto Is., and Tonaki-jima Is. (Shimojana 1977; Tanikawa & Sasaki 1999) (Fig. 10).

**Remarks.** Although *M. longipalpis* is relatively common and recorded from several caves in central and southern Ryukyus, during our surveys we could not find this species in some of the localities where its presence was historically documented. It is possible that the microclimate conditions of some of the caves might have changed along the years due to the increasing urbanization and consequent human activities (Y. Suzuki pers. comm.).



**FIGURE 10.** Known records of Leptonetidae in the Ryukyu Archipelago and location of the related caves. Legend: pink hexagon = *Longileptoneta yamasakii* **sp. nov.**; blue dot = *Masirana. suzukii* **sp. nov.**; yellow dots = *M. longipalpis*; red squares = *Falcileptoneta okinawaensis*. Type localities of the species are highlighted with an internal black dot.

# Genus Falcileptoneta Komatsu, 1970

Type species. Leptoneta striata (Oi, 1952) from Japan

Distribution. China, Japan, Korea, Taiwan.

# Falcileptoneta okinawaensis Komatsu, 1972

*Falcileptoneta okinawaensis* Komatsu, 1972: 82, f. 1-5 ( $\stackrel{\circ}{\bigcirc} \stackrel{\circ}{\ominus}$ ). *F. okinawaensis* Shimojana, 1977: 346, f. 4D ( $\stackrel{\circ}{\bigcirc}$ ).

**Type locality**. Shimuku-gama cave, Namihira village, Yomitan-son, Nakagami-gun, Okinawa-honto Is., Okinawa Pref. (26.40247°N, 127.73133°E).

**Description.** See Komatsu, 1972 for the description of the male (detailed description of the female habitus and genitalia missing).

Distribution. Endemic to Okinawa-honto Is. (Fig.10).

**Remarks.** This troglophilic species is endemic to Okinawa-honto Is. and reported from few caves in the Central-Southern part of the island (Fig. 10). It is apparently absent in other islands of the Ryukyu arc (Shimojana 1977) and no external records are currently known. We failed to collect any specimens of *F. okinawaensis* during our surveys in the Ryukyus despite searching in some of the recorded localities including the type locality cave. Consequently, we postpone the study of this species to future works when some fresh samples of both sexes are available. Apparently, *F. okinawaensis* can cohabits in the same caves with the more widely distributed *M. longipalpis* (see Shimojana 1977). We cannot directly confirm this observation since we only collected the latter species during our surveys. Nevertheless, the two species show different degrees of morphological adaptations to the subterranean environment, with *F. okinawaensis* being apparently less adapted. Thus, they can possibly occupy different sections of the same cave and different niches avoiding direct competition. Additional ecological studies on the micro-habitat preference of *M. longipalpis* and *F. okinawaensis* are necessary to confirm this hypothesis.

#### **Discussion and conclusions**

Herein we increase the general knowledge on the diversity of the family Leptonetidae in Japan, providing a preliminary update focused on the Ryukyu arc and reporting two new species and a new recorded genus. The new record of *Longileptoneta* from Japan suggests a wider distribution of this genus in Eastern Asia. Due to its distribution covering mainland China, Korea and now Southern Ryukyus, it is possible that other *Longileptoneta* species may also occur in Taiwan or in other islands of the Ryukyu and Japanese archipelagoes.

Among the leptonetid species in the Ryukyu Archipelago, Masirana longipalpis shows a wide distribution and a high level of morphological and genetic divergence among populations from different islands or different caves (for details see "Remarks on variation" of *M. longipalpis*). Such divergence may suggest that this species is in fact a complex of species, a hypothesis that deserves to be tested more in detail in the future. Due to the limited number of specimens and localities investigated, in the present study we prefer to follow a conservative approach and temporarily consider M. longipalpis as a single species. Shimojana (1977, pg. 349) reported possible unknown species of Leptonetidae from other islands belonging to the Amami-group in Central Ryukyus (e.g., Yoron-jima Is., Kikai-jima Is., Tokuno-shima Is.). So far, we did not have the opportunity to collect or study any material from this area, thus the presence of additional new species from those islands still needs to be confirmed. However, some females of unrecorded leptonetid species have been collected by us from deep screes in northern Okinawa-honto Is. (Yambaru National Park) and Kume-jima Is., as well as by other researchers in Aka-jima Is., Amami-Ôshima Is. and in central and northern Okinawa-honto Is. (Y. Suzuki, pers. comm.). Preliminary molecular analysis and morphological comparison of internal genitalia of some of these samples suggest them as belonging to putative new species. In the absence of additional deeper analysis we prefer to postpone the study and potential description of these species to a future work. Nevertheless, these preliminary results clearly suggest that the real diversity of the family Leptonetidae in the Ryukyu Archipelago is probably still widely underestimated. In addition, most of the Japanese species collected in mainland Japan and currently included in the genera Masirana or Falcileptoneta have never been revised after their original descriptions in the '70s. Meanwhile new genera of Leptonetidae have been established in Eastern Asia based on newly collected species from China and Korea. A modern revision of the Japanese leptonetids is thus mandatory to clarify the systematic position of the Japanese species or the presence of further undescribed taxa. New collections and the use of different techniques (e.g., integrative taxonomy combining both morphology and molecular data) may help to shed new light on the endemic subterranean spider fauna of the Japanese islands.

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#### References

Ballarin, F. & Eguchi, K. (2022) 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). *Acta Arachnologica*, 71 (1), 53–58.

https://doi.org/10.2476/asjaa.71.53

Barrett, R.D.H. & Hebert, P.D.N. (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, 83, 481–491.

https://doi.org/10.1139/z05-024

- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Yang, K.W., Li, H.C., Tong, Y.F. & Bian, D.J. (2022) A new genus and species of leptonetid spiders (Araneae, Leptonetidae) from Guangdong Province, China. *Biodiversity Data Journal*, 10 (e80219), 1–8.
- https://doi.org/10.3897/BDJ.10.e80219 Komatsu, T. (1972) Two new cave spiders from Okinawa Island (genera *Falcileptoneta* and *Masirana*, Leptonetidae). *Acta Arachnologica*, 24 (2), 82–85.

https://doi.org/10.2476/asjaa.24.82

- Ledford, J., Derkarabetian, S., Ribera, C., Starrett, J., Bond, J.E., Griswold, C. & Hedin, M. (2021) Phylogenomics and biogeography of leptonetid spiders (Araneae: Leptonetidae). *Invertebrate Systematics*, 35 (3), 332–349. https://doi.org/10.1071/IS20065
- Machida, H. & Kawana, T., Nagaoka, S., Ota, Y. & Moriwaki, H. (2001) *Japanese Geology. Vol.* 7. Kyushu and Nansei Islands, Tokyo University Press, Tokyo, 355 pp
- Seo, B.K. (2015) Four new species of the genera Masirana and Longileptoneta (Araneae, Leptonetidae) from Korea. Korean Journal of Environmental Biology, 33 (3), 306–313. https://doi.org/10.11626/KJEB.2015.33.3.306
- Shimojana, M. (1977) Preliminary report on the cave spider fauna of the Ryukyu Archipelago. *Acta Arachnologica*, 27 (Spec. No.), 337–365.
  - https://doi.org/10.2476/asjaa.27.Specialnumber 337
- Suzuki, Y. & Ballarin, F. (2020) Nesticus kosodensis Yaginuma, 1972 bona species. Molecular and morphological separation from N. latiscapus Yaginuma, 1972 with notes on cave scaffold-web spiders subspecies in Japan (Araneae, Nesticidae). Subterranean Biology, 35, 79–96.

https://doi.org/10.3897/subtbiol.35.53933

Tanikawa, A. & Sasaki T. (1999) List of spiders from Okinawa Prefecture. Kishidaia, 76, 61-101.

- Tanikawa, A. (2022) A Check List of Japanese Spiders. Version 2022 R1. Available from: http://www.asahi-net.or.jp/~dp7atnkw/japan.pdf (accessed 30 September 2022)
- Wang, C.X., Li, S.Q. & Zhu, W.H. (2020). Taxonomic notes on Leptonetidae (Arachnida, Araneae) from China, with descriptions of one new genus and eight new species. *Zoological Research*, 41 (6), 684–704. https://doi.org/10.24272/j.issn.2095-8137.2020.214
- World Spider Catalog (2022) World Spider Catalog Version 22.5. Natural History Museum Bern, Bern. Available from: http:// wsc.nmbe.ch (accessed 30 September 2022)

https://doi.org/10.24436/2