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Research article

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Two new species of the genus *Macrothele* Ausserer, 1871 from Yunnan Province, China (Araneae: Macrothelidae)

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Abstract. Two new species of the genus *Macrothele* Ausserer, 1871 are described from Yunnan Province, China: *Macrothele nullispine* sp. nov. and *M. auriculata* sp. nov. Detailed descriptions, photos of morphological characteristics, DNA barcode sequences and genetic distances of the new species are provided.

Keywords. New species, DNA barcode, *Macrothele*, Yunnan.

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Introduction

The genus *Macrothele* Ausserer, 1871 of the family Macrothelidae, Simon, 1892 is a genus of mygalomorph spiders with venom, considered medically significant. So far, 49 described species of Macrothelidae have been reported worldwide: 43 species in *Macrothele*, and 6 species in *Vacrothele* Tang & Yang, 2022. *Macrothele* is found in Asia, Europe, and Africa (World Spider Catalog 2023), while *Vacrothele* Tang & Yang, 2022 is restricted to Asia only (Tang *et al.* 2022).

Macrothele generally live on slopes or cliffs with good ventilation and unobstructed drainage. The retreats of *Macrothele* and *Vacrothele* are quite different, with the former using existing rock

crevices along roadsides or cracks in soil slopes for nesting, and the latter making burrows in loose soil.

The two new species of *Macrothele* proposed here were collected from Yunnan Province, China. The results of morphological classification and DNA sequencing analyses suggest they are not known to science so far, and are herein named *Macrothele nullispine* sp. nov. and *M. auriculata* sp. nov. Type specimens are deposited in the Institute of Entomocetics Research, Dali University, China (DUIER).

Material and methods

Sample processing

All examined specimens were preserved in 80% ethanol. The left male palps were removed from the specimens. After taking habitus photos first, the palpal bulb was then separated from the distal part of the palpal tibia and stored in 80% ethanol; the female spermathecae were first dissected from the opisthosoma using a forceps and a dissecting needle, and then treated in 10% NaOH for 24 hours to dissolve the non-sclerotized surrounding tissue. Specimens were examined and measured with



Fig. 1. Distribution of *Macrothele nullispine* sp. nov. and *Macrothele auriculata* sp. nov. (red triangles) (the green triangles show other species of *Macrothele* spiders distributed in Yunnan Province, China, with a total of 15 species, including *M. bannaensis* Xu & Yin, 2001, *M. cangshanensis* Z.B. Yang, Zhao, Zhang & Z.Z. Yang, 2018, *M. jingzhao* Chen, Jiang & Yang, 2020, *M. jinlin* Z.B. Yang, Zhao, Zhang & Z.Z. Yang, 2018, *M. menglunensis* Li & Zha, 2013, *M. multispine* Wang, Li & Yang, 2019, *M. yani* Xu, Yin & Griswold, 2002, *M. yongshengensis* Z.B. Yang, Zhao & Z.Z. Yang, 2019, *M. yunlingensis* Z.B. Yang, Zhao & Z.Z. Yang, 2019, *M. sanheensis* Tang, Zhao & Yang, 2020, *M. undata* Tang, Zhao & Yang, 2020, *M. arcuata* Tang, Zhao & Yang, 2020, *M. mingsheng* Wu & Z.Z. Yang, 2022, *M. washanensis* Wu & Z.Z. Yang, 2022 and *M. wuliangensis* Wu & Z.Z. Yang, 2022).

Olympus SZX16 and Leica M205A stereo microscopes and an Olympus CX33 compound microscope. All measurements are in millimeters. Leg measurements are given as following: total length (femur + patella + tibia + metatarsus + tarsus).

Abbreviations

ALE	=	anterior lateral eyes
AME	=	anterior median eyes
PLE	=	posterior lateral eyes
PLS	=	posterior lateral spinnerets.
PME	=	posterior median eyes
PMS	=	posterior median spinnerets

DNA extraction, amplification, and sequencing

Four specimens were selected for DNA extraction (*M. nullispine* sp. nov.: 2 females; *M. auriculata* sp. nov.: 1 male, 1 female). Each sample took about 10 mg of spider leg tissue. DNA extraction was conducted using Tiangen blood/cell/tissue/genomic DNA extraction kit. After extraction, PCR amplification of the COI gene was conducted using the universal primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCOoutout (5'-GTAAATATATATGRTGDGCTC-3') (Folmer *et al.* 1994). PCR reaction system of 50 µL: Gold Mix (green) 45 µL, 2 µL each of forward and reverse primers, 1 µL of DNA template. Amplification procedure: 98°C pre-deformation for 2 min; 98°C for 10 s, 46°C for 10 s, 72°C for 10 s for 30 cycles; 72°C extension for 1 min. The PCR amplification products were detected by 1.0% agarose gel electrophoresis and the results were visualized using a Bio-Rad gel imaging system. The sequencing quality was observed using SeqMan (DNASTAR, Inc. ver. 7.1.0.44), the sequences were proofread, spliced, and then subjected to BLAST homology analysis in the NCBI database. Genetic distances between species were calculated using MEGA 7 (Kumar *et al.* 2016), and the resulted sequences were uploaded to NCBI to obtain GenBank numbers.

Results

Taxonomy

Order Araneae Clerck, 1757
Family Macrothelidae Simon, 1892
Genus *Macrothele* Ausserer, 1871

Macrothele nullispine sp. nov.

urn:lsid:zoobank.org:act:3781E457-652E-4BC3-96B6-542AD4BFC339

Figs 2–5

Diagnosis

This new species is similar to *Macrothele arcuata* Tang, Zhao & Yang, 2020 (Tang *et al.* 2020), but differs by the male embolus and the bulb not strongly bent in the new species, and the embolus gradually tapering down from the bulb, with a curved apex (Fig. 3D–G), and the palpal tibia without spines (Fig. 3A–C); male chelicerae ventrally with 9 short and stout teeth (Fig. 2D); female copulatory ducts long, with a wider base and an inwardly curved proximal end, spermathecae small and nearly droplet-like (Fig. 4D–E). Conversely, the embolus and bulb are strongly bent, the end of the embolus is straight in; and the female copulatory ducts are longer the *M. arcuata*.

Etymology

The new species name is a noun in apposition, referring to the male palpal tibia lacking spines.

Material examined

Holotype

CHINA • ♂; Yunnan Province, Shidian County, He Yuan Town, Shiping Highway; 24°44'26" N, 99°8'49" E; 1891 m a.s.l.; 1 Oct. 2019; Zizhong Yang and Yunfen Xiong leg.; DNA voucher no. HYY;

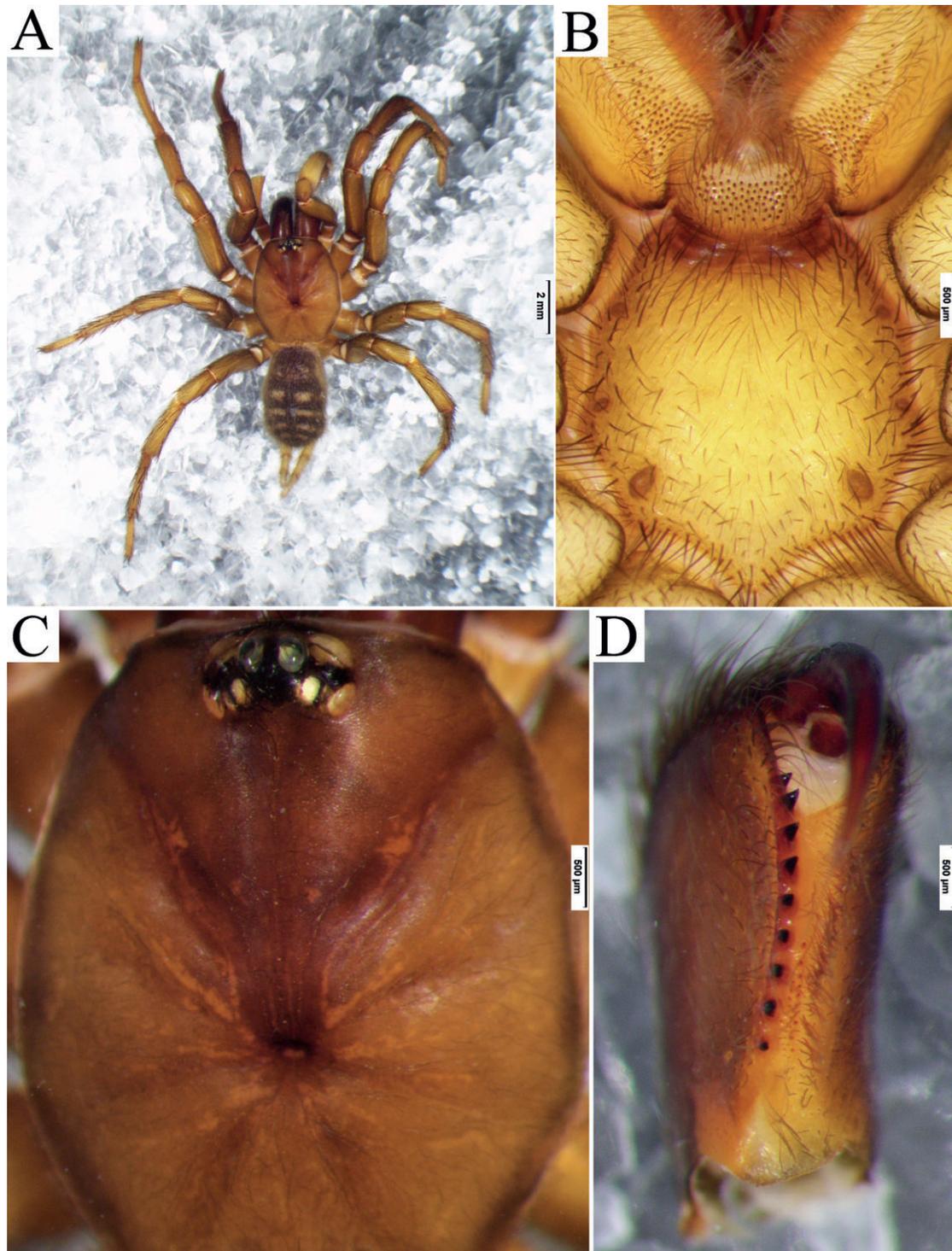


Fig. 2. *Macrothele nullispine* sp. nov., holotype, ♂ (Mn-hy-m01), ventral view. **A.** Body. **B.** Cuspules and sternum. **C.** Fovea. **D.** Left chelicera.

♀ GenBank accession no.: OQ724523; Mn-hy-f01; DNA voucher no. SPGL01; ♀ GenBank accession no.: OQ724526; Mn-hy-f02.

Paratypes

CHINA • 3 ♂♂ (juveniles), 11 ♀♀ (4 juveniles); same collection data as for holotype • 3 ♀♀ (1 juvenile); Yunnan Province, Shidian County, He Yuan Town, near the village entrance of Da Menzhai Village; 24°41'35.59" N, 99°7'31.97" E; 1430 m a.s.l.; 24 Dec. 2020; Zizhong Yang and Chaowu Yang leg. • 2 ♀♀; Jiangxi Province, Jinggangshan; 8 Sep. 1982; Jiafu Wang leg.

Description

Male (holotype, see Figs 2–3)

MEASUREMENTS. Total length 9.81: cephalothorax 4.99 long, 4.23 wide; opisthosoma 4.70 long, 2.91 wide.

PROSOMA. Carapace brown, brown hairs. Fovea pit-shaped (Fig. 2C). Chelicerae brown, with 9 short and stout promarginal teeth, 9 small retromarginal teeth, and 18 tiny teeth within fang furrow. Labium yellow at base and end; maxillae yellow medially, colloquial laterally; labium and maxillae with numerous cuspules; maxillae 1.48 long, and cuspule area length 0.97. Sternum yellow and with three pairs of sigilla.

PALP. Palpal tibia spinose. Palpal bulb suborbicular, joint of embolus and bulb not strongly bent in new species, embolus gradually tapers down from bulb, with curved end, 2.75 long.

EYES AND SPINNERETS. Both eye rows recurved in dorsal view. Eye sizes and inter-distances: AME 0.16, ALE 0.40, PME 0.21, PLE 0.27; ALE–AME 0.09, AME–AME 0.15, ALE–PLE 0.10, PLE–PME 0.13, PME–PME 0.37. Eye area 0.65 long, 1.15 wide.

LEGS. Brown, hirsute. Leg formula: 4321. Leg measurements as follows: I 11.02 (3.27, 1.56, 2.37, 2.16, 1.66); II 12.41 (3.12, 2.04, 2.46, 2.89, 1.90); III 12.92 (3.24, 3.64, 2.27, 2.96, 1.81); IV 13.27 (2.94, 1.98, 2.63, 3.82, 1.90).

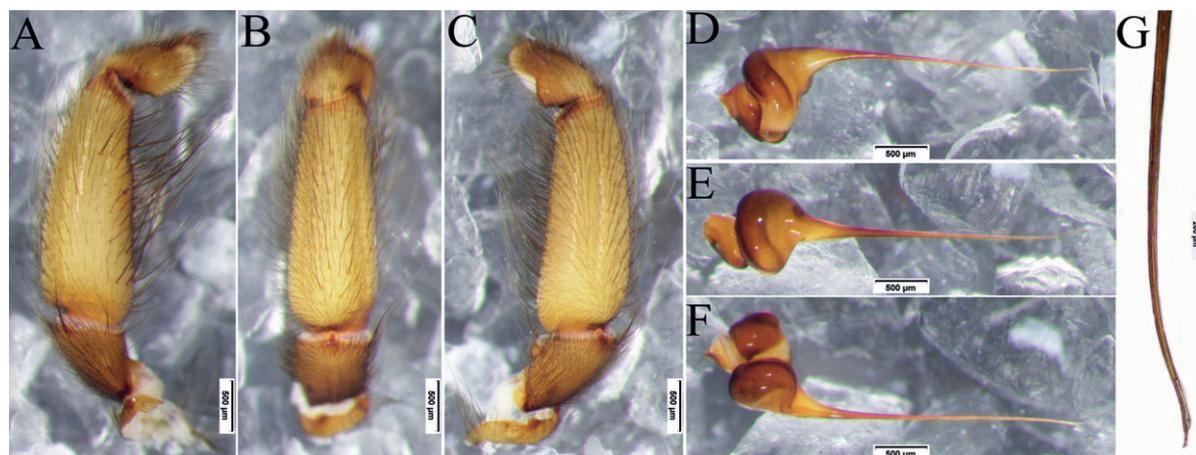


Fig. 3. *Macrothele nullispine* sp. nov., holotype, ♂ (Mn-hy-m01). **A.** Left palp tibia, inside view. **B.** Same, dorsal view. **C.** Same, lateral view. **D–G.** Left palpal embolus. **D.** Inside view. **E.** Dorsal view. **F.** Lateral view. **G.** The end.

OPISTHOSOMA. Brown, hirsute. Spinnerets: PMS one segment, 0.83 long, 0.26 wide, PMS–PMS 0.76; PLS three segments, PLS 5.20 long (1.49, 1.79, 1.92).

Female (see Fig. 4)

MEASUREMENTS. Total length 10.80: cephalothorax 5.63 long, 4.51 wide; opisthosoma 5.41 long, 3.51 wide.

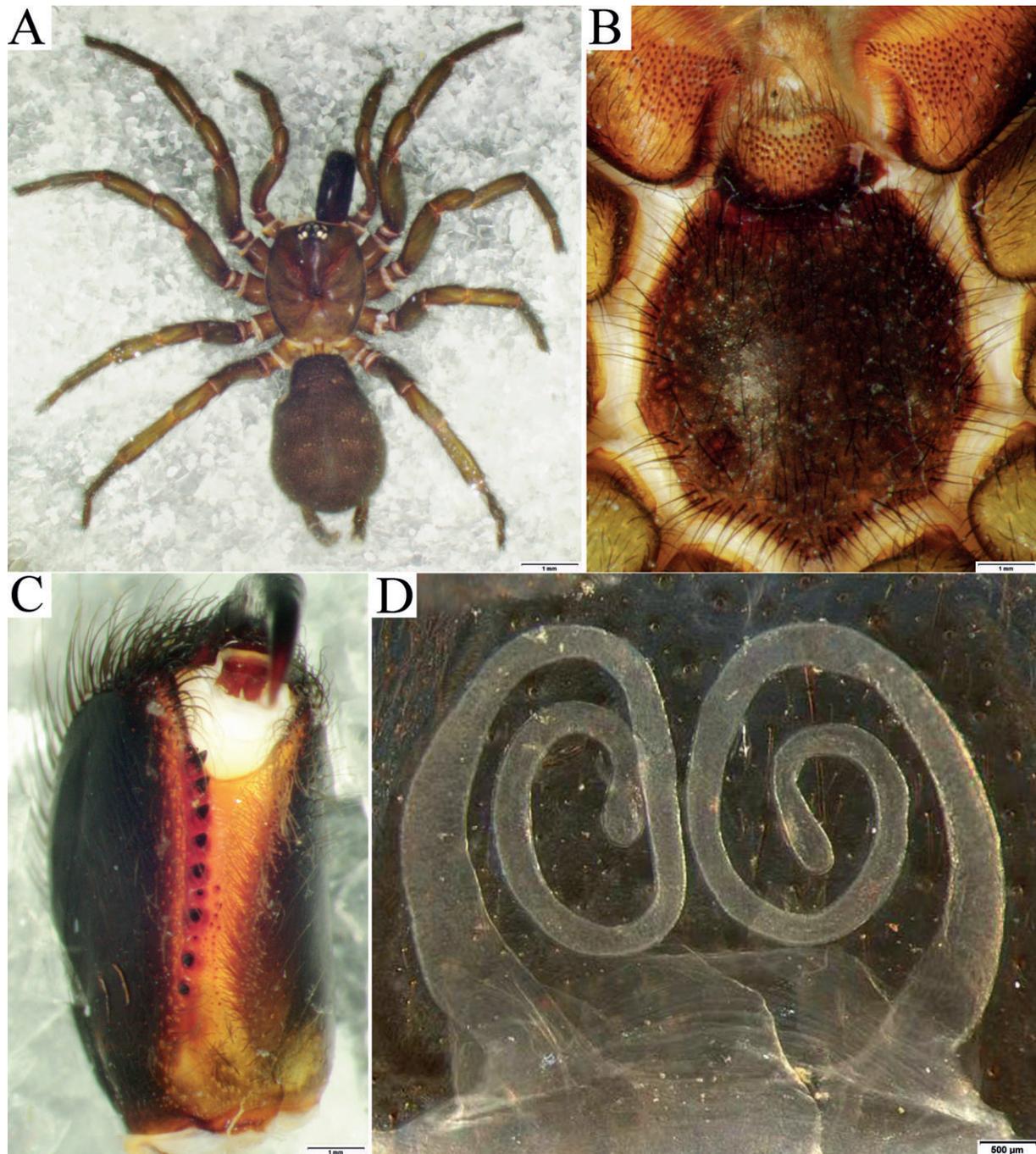


Fig. 4. *Macrothele nullispine* sp. nov., paratype, ♀ (Mn-hy-f01). **A.** Body. **B.** Left chelicera, ventral view. **C.** Cusps and sternum. **D.** Genitalia, dorsal view.

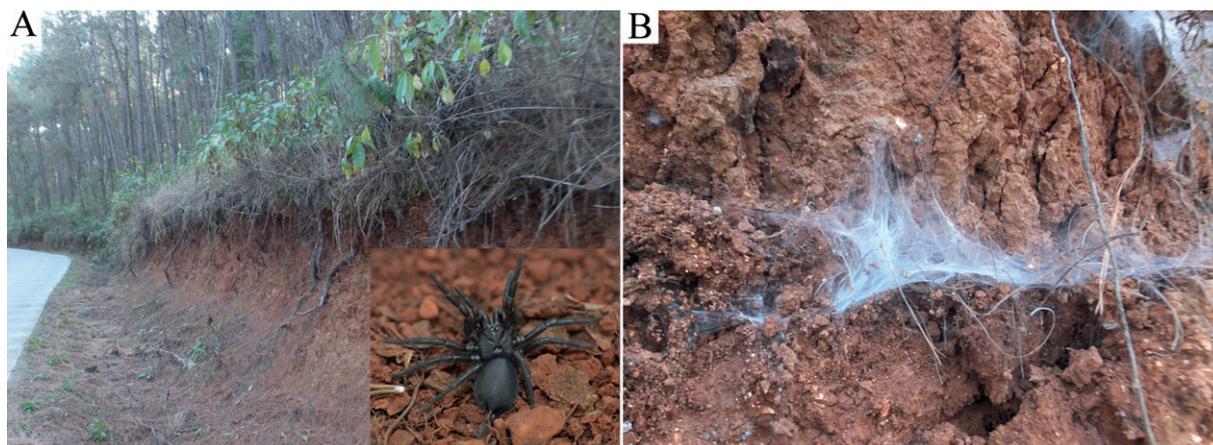


Fig. 5. *Macrothele nullispine* sp. nov. **A.** Microhabitat and living female. **B.** Web.

PROSOMA. Chelicerae brown, with 10 stout promarginal teeth, 14 small retromarginal teeth, and 18 tiny teeth within fang furrow. Labium and maxillae with numerous cuspules; maxillae length 1.54, and cuspules area length 1.02.

EYES AND SPINNERETS. Eye sizes and inter-distances: AME 0.18, ALE 0.34, PME 0.23, PLE 0.27; AME–AME 0.19, ALE–AME 0.11, ALE–PLE 0.13, PME–PME 0.52, PLE–PME 0.08. Eye area 0.75 long, 1.27 wide.

LEGS. Legs brown with many spines and hairs. With spines. Leg formula: 4321. Leg measurements as follows: I 9.86 (2.57, 1.55, 2.77, 1.70, 1.27); II 11.4 (3.10, 1.47, 2.73, 2.24, 1.86); III 11.59 (3.13, 1.29, 2.39, 2.63, 2.15); IV 13.39 (3.57, 1.56, 2.55, 3.29, 2.42).

SPERMATHECAE. Receptacula apically small and nearly teardrop-shaped, copulatory ducts long, broad at base and inward bending. Spinnerets: PMS 1.25 long, 0.43 wide, PMS–PMS 1.03; PLS 3.46 long (1.45, 0.93, 1.08).

Natural history

The spiders mainly reside in existing stone crevices or caves along highways, soil slopes, gaps in tree bark, and under fallen leaves.

Distribution

Yunnan Province, China (Shidian City).

Macrothele auriculata sp. nov.

urn:lsid:zoobank.org:act:8A57AE32-43C2-4381-AA8C-368282C5EB69

Figs 6–9

Diagnosis

This new species is similar to *Macrothele guihzouensis* Hu & Li, 1986 (Wu *et al.* 2022), but differs by the male embolus being slender, slightly curved from the middle with a pointed apex in the new species (Fig. 7D–F); the palp tibia with six prolateral spines and three dorsal spines (Fig. 7A–B), the retrolateral face of the palpal trochanter has no lyrate organ; female copulatory ducts long (Fig. 8D). Conversely, the

embolus is short, stout, and straight in *M. guihzouensis*, and with a flattened apex; the retrolateral face of the palpal trochanter has a lyrate organ; and the female copulatory ducts are short.

Etymology

The new species name is a noun in apposition and refers to the auricle-like female genitalia.

Material examined

Holotype

CHINA • ♂; Yunnan Province, Binchuan County, Jizu Mountain; 25°57'40" N, 100°22'40" E; 2406 m a.s.l.; 15 Sep. 2021; Zizhong Yang, Lijun Ding, Jiasen Wei and Yaping Chang leg; DNA voucher

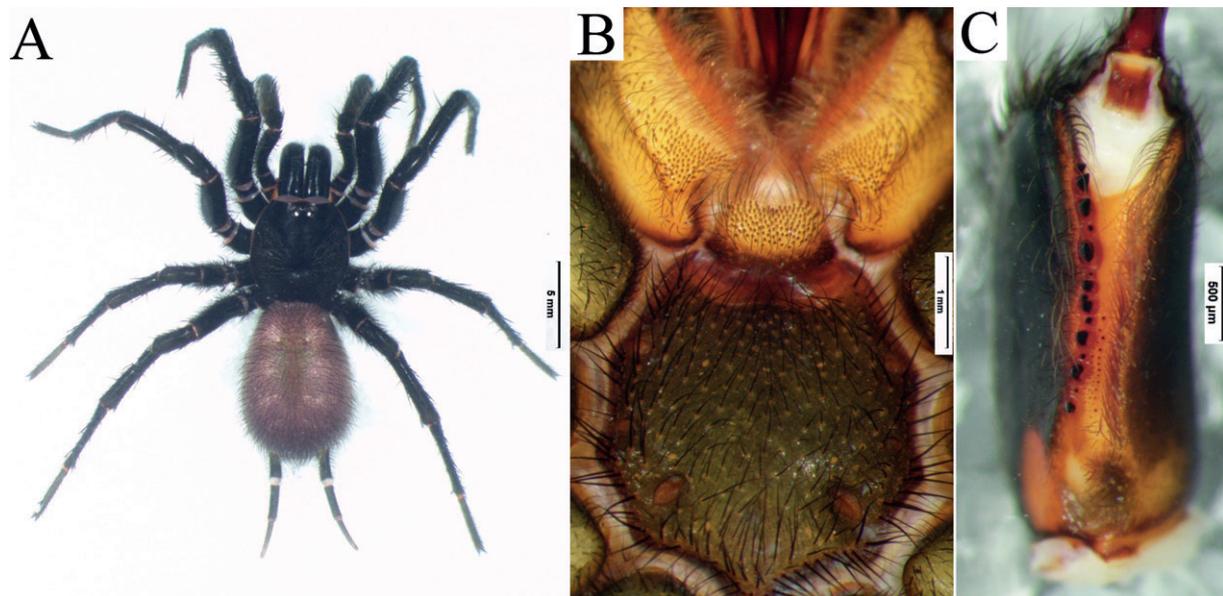


Fig. 6. *Macrothele auriculata* sp. nov., holotype, ♂ (Ma-kh-m01). A. Body. B. Cuspules and sternum. C. Left chelicera, ventral view.



Fig. 7. *Macrothele auriculata* sp. nov., holotype, ♂ (Ma-kh-m01). A–C. Left palp tibia. A. Inside view. B. Dorsal view. C. Lateral view. D–G. Left palpal embolus. D. Inside view. E. Dorsal view. F. Lateral view. G. The end.

no. JZS02; ♀: GenBank accession no. OQ724525; Ma-kh-f01; DNA voucher no. gx1; ♂: GenBank accession no. OQ692411; Ma-kh-m01.

Paratypes

CHINA – Yunnan Province • 1 ♂, 2 ♀♀; same collection data as for holotype • 1 ♀; Binchuan County, Jizu Mountain, near Yulong Waterfall; 25°57'50.3" N, 100°22'11.8" E; 1 Jul. 2018; Wenjie Zhang leg.

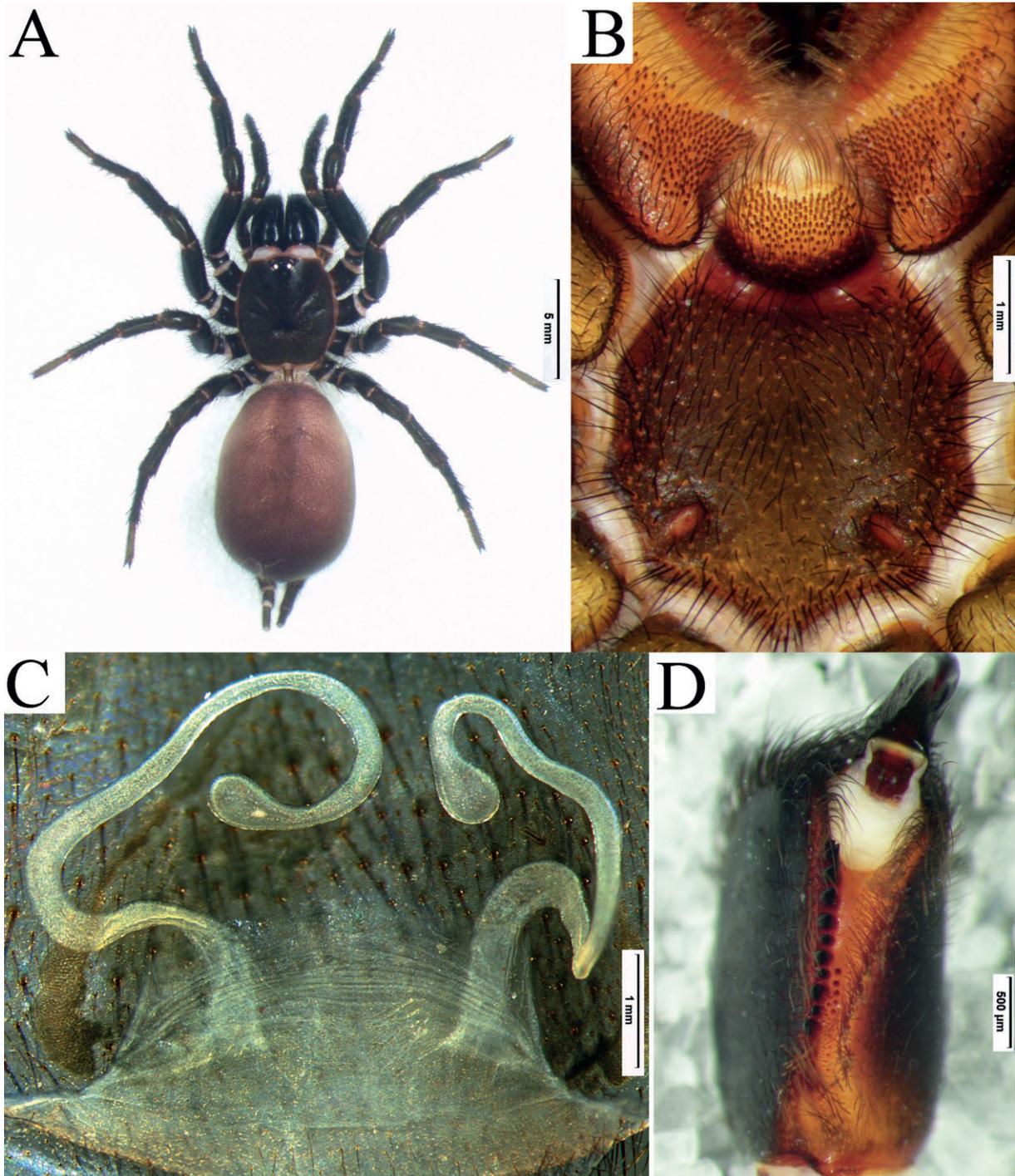


Fig. 8. *Macrothele auriculata* sp. nov., paratype, ♀ (Ma-kh-m01). **A.** Body. **B.** Cuspules and sternum. **C.** Left chelicera, ventral view. **D.** Genitalia, dorsal view.

• 1 ♂, 1 ♀; Binchuan County, Kang he Highway; 25°56'28" N, 100°22'27" E; 1905 m a.s.l.; 10 Jul. 2021; Yongming You, Lijun Ding, Xiaoliang Gu and Yaying Wu leg.

Description

Male (holotype, Figs 6–7)

MEASUREMENTS. Total length 18.10: cephalothorax 6.95 long, 5.59 wide; opisthosoma 8.66 long, 6.02 wide.

PROSOMA. Carapace grey-black, gray hairy. Fovea pit-shaped. Chelicerae black, with 10 short and stout promarginal teeth, 12 small retromarginal teeth, 25 tiny teeth within fang furrow. Labium brown at base and end, maxillae internal colour lighter, maroon laterally; labium and maxillae with numerous cuspules; maxillae length 1.48, and cuspule area length 0.97. Sternum dark brown, with three pairs of sigilla.

PALP. Palp tibia with 6 prolateral and 3 dorsal spines, retrolateral face of palpal trochanter without lyrate organ. Embolus slender, slightly curved from middle and tapered at end, 4.75 long.

EYES AND SPINNERETS. Both eye rows recurved. Eye sizes and inter-distances: AME 0.27, ALE 0.41, PME 0.28, PLE 0.37; ALE–AME 0.12, AME–AME 0.27, ALE–PLE 0.11, PLE–PME 0.10, PME–PME 0.62. Eye area 0.88 long, 1.60 wide.

LEGS. Brown, hirsute. Spines. Leg formula: 4312. Leg measurements as follows: I 17.68 (4.17, 1.86, 4.02, 4.53, 3.10); II 16.26 (4.04, 1.95, 4.31, 3.19, 2.77); III 17.97 (4.36, 1.46, 3.95, 5.05, 3.15); IV 20.75 (5.09, 1.34, 4.65, 5.83, 3.84).

OPISTHOSOMA. Gray-brown, hirsute. Spinnerets: PMS one segment, 1.56 long, 0.36 wide; PMS–PMS 1.14; PLS three segments, PLS 6.80 long (2.48, 2.07, 2.25).

Female (Fig. 8)

MEASUREMENTS. Total length 17.52: cephalothorax 7.11 long, 5.67 wide; opisthosoma 11.43 long, 8.46 wide.

PROSOMA. Chelicerae brown, with 11 stout promarginal teeth, 12 small retromarginal teeth, and 13 tiny teeth within fang furrow. Labium and maxillae with numerous cuspule; maxillae length 1.54, and cuspules area length 1.02.

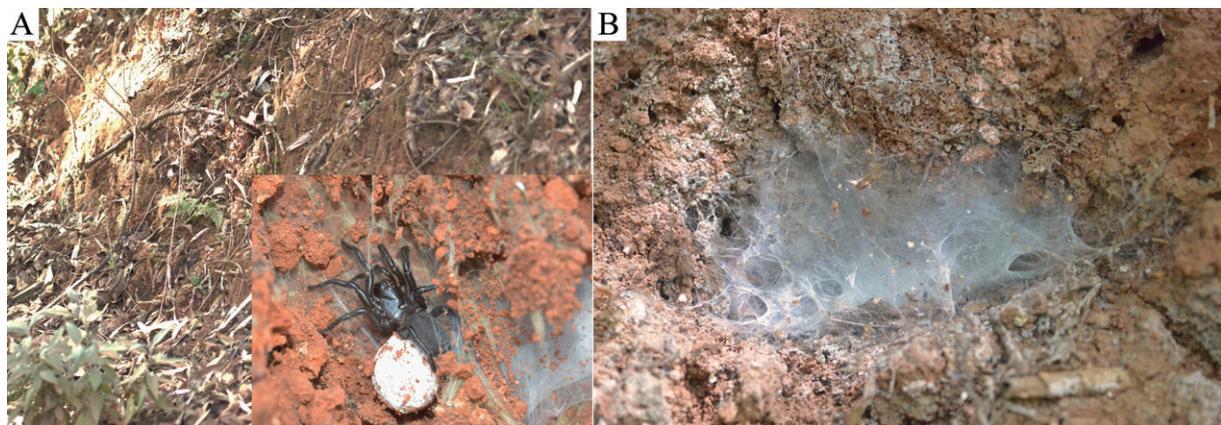


Fig. 9. *Macrothele auriculata* sp. nov. A. Microhabitat, living female and egg. B. Web.

Table 1. Data of six samples of *Macrothele* Ausserer, 1871 and their DNA barcodes (COI).

Species	Individual No.	Gender	Length (bp)	Collecting information	GenBank number
<i>M. nullispine</i> sp. nov.	Mn-hy-f01	female	717	Heyuan Town, Shidian County, Dali City	OQ724523
	Mn-hy-f02	female	753	Shiping Highway, Shidian County, Dali City	OQ724526
<i>M. auriculata</i> sp. nov.	Ma-kh-m01	male	686	Jizu Mountain, Binchuan County, Dali City	OQ692411
	Ma-kh-f01	female	682	Jizu Mountain, Binchuan County, Dali City	OQ724525
<i>M. arcuata</i> Tang, Zhao & Yang, 2020	gx1-m	male	614	Mengnuo Town, Longling County	OQ692566
<i>M. guizhouensis</i> Hu & Li, 1986	gx2-f	female	642	Mengnuo Town, Longling County	OQ724524
	gz-f	female	680	Pu Village, Meitan County, Guizhou Province	OP363144

EYES AND SPINNERETS. Eye sizes and interdistances: AME 0.31, ALE 0.42, PME 0.24, PLE 0.38; AME–AME 0.18, ALE–AME 0.14, ALE–PLE 0.16, PME–PME 0.70, PLE–PME 0.09. Eye area 0.89 long, 1.65 wide.

LEGS. Legs black-brown, hairy. With spines. Leg formula: 4321. Leg measurements as follows: I 14.90 (4.55, 2.75, 3.21, 2.62, 1.77); II 14.98 (4.02, 2.56, 2.91, 3.21, 2.28); III 15.61 (4.52, 2.49, 2.42, 3.73, 2.45); IV 18.02 (4.80, 2.75, 3.62, 4.20, 2.65).

SPERMATHECAE. Spermathecal receptacles apically small with globular apex, copulatory ducts long. Spinnerets: PMS 1.92 long, 0.54 wide, PMS–PMS 1.58; PLS 8.62 long (2.68, 2.82, 3.12).

Natural history

The spiders mainly reside in existing stone crevices or caves along highways, soil slopes, gaps in tree bark, and under fallen leaves.

Distribution

Yunnan Province, China (Dali City).

DNA barcoding

A total of four samples are determined for *Macrothele nullispine* sp. nov. (2 ♀♀) and *M. auriculata* sp. nov. (♀, ♂), the DNA barcode sequences were uploaded to NCBI. Also, the COI sequences of *M. arcuata* Tang, Zhao & Yang, 2020 (♀, ♂) and *M. guizhouensis* Hu & Li, 1986 (♀) were obtained from GenBank (see Table 1 for accession numbers).

Discussion

Table 2 shows that the intraspecific genetic distance between the two samples of *M. nullispine* sp. nov. is 0.002, which is less than 2%, the interspecific genetic distance between *M. nullispine* and *M. arcuata* Tang, Zhao & Yang, 2020 is 0.544, significantly greater than the intraspecific genetic distance, so there

Table 2. Intraspecific genetic distances in two samples of *Macrothele nullispine* sp. nov. based on the p-distance mode.

GenBank number	Species	Mn-hy-f01	Mn-hy-f02	gx1-m	gx2-f
OQ724523	Mn-hy-f01				
OQ724526	Mn-hy-f02	0.002			
OQ692566	gx1-m	0.544	0.544		
OQ724524	gx2-f	0.544	0.544	0.000	

Table 3. Intraspecific genetic distances in two samples of *Macrothele auriculata* sp. nov. based on the p-distance mode.

GenBank number	Species	Ma-kh-m01	Ma-kh-f01	gz-f
OQ692411	Ma-kh-m01			
OQ724525	Ma-kh-f01	0.001		
OP363144	gz-f	0.187	0.186	

is a clear gap between the two samples (Hebert *et al.* 2004). Therefore, we consider *M. nullispine* sp. nov. as a new species.

Table 3 shows that the intraspecific genetic distance between the two samples of *M. auriculata* sp. nov. is 0.001, which is less than 2%, the interspecific genetic distance between *M. auriculata* and *M. guizhouensis* Hu & Li, 1986 is 0.186~0.187, significantly greater than the intraspecific genetic distance, there is a clear gap between the two samples. Therefore, we consider the *M. auriculata* as a new species.

Morphologically, the two new species described are different from other species. In addition to morphological observations, the interspecific genetic distance was calculated, and the results indicate that both species are indeed new species in the genus *Macrothele*.

Author contributions

Conceptualization, Y.Z., Z.Z.Y.; methodology, all authors; project administration, Z.Z.Y.; identification, Z.Z.Y.; original draft preparation, M.M.Z.; review and editing, Y.Y.W. and Z.Z.Y. All authors have read and agreed to the published version of the manuscript.

Data availability statement

All DNA barcode numbers in the text are available for use on NCBI.

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