

A new species of whip spider, *Weygoldtia hainanensis* sp. nov., from Hainan, China (Arachnida: Amblypygi: Charinidae)

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Abstract

To date, only one species of whip spider has been recorded in China. Here, we describe a new species, *Weygoldtia hainanensis* sp. nov., from Hainan, China. The new species is morphologically similar to *W. davidovi* (Fage, 1946) and *W. consonensis* Miranda *et al.* 2021, but can be distinguished with a combination of the following characters: 26 segments in tibia I, 6-7 teeth on chelicerae, distitibia IV trichobothria sc and sf series each with 10-11 trichobothria. To validate our morphological inferences and support the erection of *W. hainanensis* sp. nov. as a new species, we sequenced the COI gene region for two individuals and performed molecular phylogenetic analyses. The inferred phylogenetic trees placed the new species within *Weygoldtia* and highlighted the evolutionary distinction between *W. hainanensis* sp. nov. and currently described whip spiders. The type specimens are deposited in the Museum of Biology, East China Normal University (ECNU).

Key words: taxonomy, biodiversity, karst, island

Introduction

Amblypygi (whip spiders) is a small order of arachnids known to inhabit different habitats such as under rocks, trees, and inside caves (Rahmadi *et al.* 2010). Whip spiders are characterized by a flattened body and forceful raptorial pedipalps with sharp spines that vary in numbers and arrangements. The first pair of legs are extremely elongated and antenniform (Foelix & Hebets 2001; Garwood *et al.* 2017). Charinidae is the most diverse and widely distributed family in extant whip spiders with 132 species (Miranda *et al.*, 2021b). Recent studies show three genera belong to the Charinidae: *Charinus* Simon 1892, *Sarax* Simon 1892, and *Weygoldtia* Miranda *et al.*, 2018 (Miranda *et al.* 2018, 2021a, 2021b). *Charinus* is distributed in the Americas, Africa, and Oceania, with 94 species (Miranda *et al.* 2021b). *Sarax* contains 36 species which are found in East Africa, the Arabian Peninsula to South- East Asia (Miranda *et al.* 2021b). *Weygoldtia* has only two described species which have been recorded in Cambodia, Laos, and Vietnam (Miranda *et al.* 2021b).

Studies have shown that the fauna of Amblypygi in some regions is understudied, especially southeast Asia (Rahmadi *et al.* 2010; Seiter *et al.* 2015; Miranda & Reboleria 2019). For instance, only one species (Charontidae: *Charon ambreae* Reveillion & Pierre-Olivier 2018) has previously been described from China (Reveillion & Pierre-

Olivier 2018). However, in recent biodiversity surveys conducted in Hainan Island, we found an unknown Charinidae species. This is the second record of an Amblypygi and the first record of the family in China (Figs. 1, 2). Here, we describe this new species based on morphological and molecular data, and test its phylogenetic position within the Charinidae tree of life (Miranda *et al.* 2021a, 2021b).

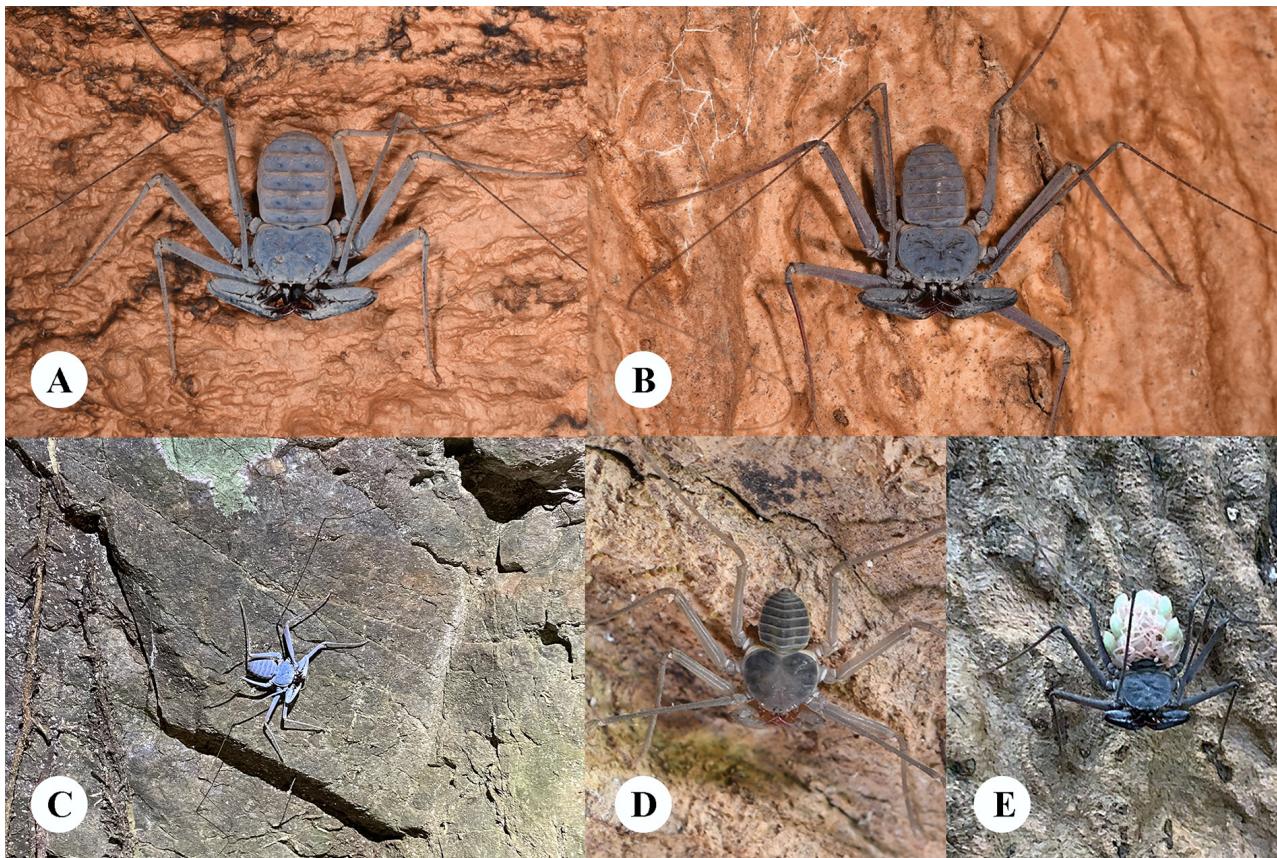


FIGURE 1. Dorsal habitus of *Weygoldtia hainanensis* sp. nov. from Hainan, China. A: Female; B: Male; C: A female on the wall of a cliff; D: Immature; E: Female carrying hatching praenymphae.

Material and methods

Sampling and scanning electron microscopy (SEM). We collected specimens by conducting biodiversity surveys in karst areas of Hainan Island at night. Specimens were collected and preserved in 75% ethanol and deposited in the Museum of Biology, East China Normal University (ECNU). Specimens were dried and photographed using a Leica M125 stereo microscope and a SC2000 digital CMOS Camera in the lab. Chelicerae were photographed in 75% ethanol. Male gonopods were dissected and used for critical point drying and SEM through the following procedures: Fixation with 2.5% glutaraldehyde and then rinsed by 0.1 M PB three times, fifteen minutes each time. Samples were then fixed for two hours using 2.1% potassium osmate for two hours and rinsed by 0.1 M PB three times, fifteen minutes each time. The dehydration was performed through an ethanol series of concentration 30%, 50%, 70%, 80%, 95%, and 100%, fifteen minutes each, and repeated 100% ethanol dehydration for three times. The SEM was performed with a Hitachi S-4800 scanning electron microscope.

DNA extraction and gene amplification. Total genomic DNA was extracted from tissue of the abdomen. We amplified the *COI* gene region (635 bp) using the amplification primers U1m3 TCWACWAATCATAAAGACATT-GGAAC and L1pyc CTATRATDGCRRAATACDGCTCCTA (Arabi *et al.* 2010). Amplification conditions for PCR were as follows: pre-denaturation for 2 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 45°C, 30 s at 72 °C, then a final extension at 72 °C for 7 min. All PCR products were sequenced directly with the same primers. GenBank accession numbers are provided in results.

Phylogenetic analyses. The phylogenetic position of *Weygoldtia hainanensis* sp. nov. was examined in the context of the currently described Charinidae species. A molecular dataset was assembled with sequences from

Miranda *et al.* (2021a). This study presented the phylogeny which is to date the most comprehensive species-level tree within the Charinidae. The authors of that study extensively discussed the phylogenetic relationships between species in the group based on multiple sources of evidence. Here, we focus on inferring the phylogeny of the same set of species. However, we also include sequences for two individuals that are described here. Our phylogenetic analyses are exclusively based on gene sequences.

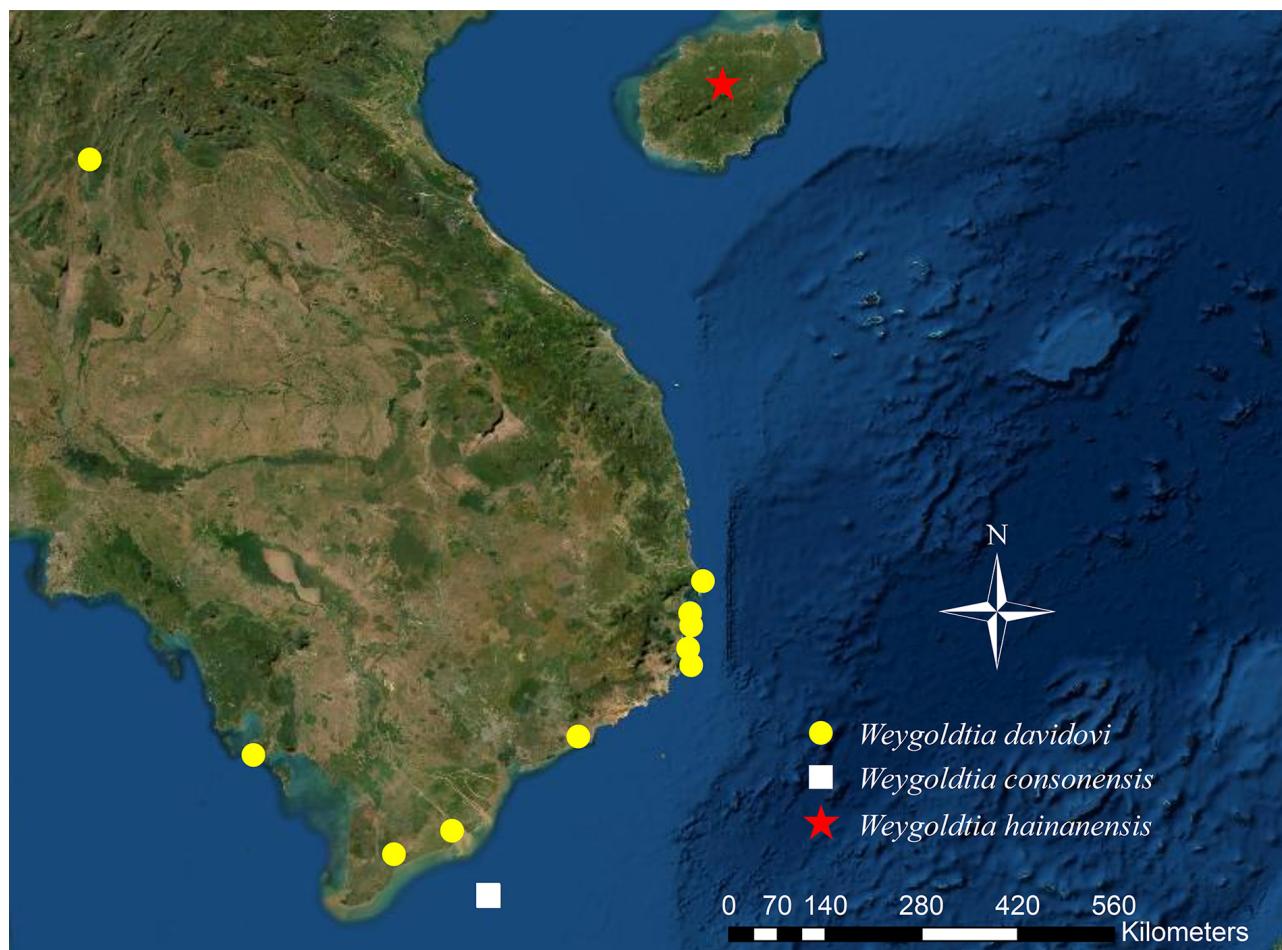


FIGURE 2. Distribution of the *Weygoldtia* species. Yellow circle, *Weygoldtia davidovi* (Fage, 1946); White square, *Weygoldtia consonensis* Miranda *et al.*, 2021; Red star, *Weygoldtia hainanensis* sp. nov.

Miranda *et al.* (2021a) analyzed the phylogenetic position of 31 Amblypygi species (four outgroup taxa) using sequence data for 51 individuals. Their molecular sampling included regions of *COI*, *I2S*, *I6S*, *I8S*, and *28S*. For each gene region, we retrieved sequences from GenBank (Appendix S2 of Miranda *et al.* 2021a). Next, we included the two newly generated sequences for *Weygoldtia hainanensis* sp. nov. to the *COI* unaligned sequence dataset. Sequences within each of the analyzed gene regions were aligned using MAFFT version 7 under default parameters (Katoh & Standley 2013). The resulting alignments were concatenated using the SuperMatrix function in the *evo-biR* R package version 1.1 (Blackmon & Adams 2015). Phylogenetic analyses on the resulting concatenated dataset were conducted using maximum likelihood and Bayesian methods.

Maximum likelihood trees were estimated using IQ-TREE version 2 (Minh *et al.* 2020) to infer the phylogenetic relationships among species sampled in our dataset. IQ-TREE analyses were run in the W-IQ-TREE webserver (Trifinopoulos *et al.* 2016). Analyses were performed using a gene-based partitioning strategy for the dataset. Best-fitting models were identified for each partition using ModelFinder (Kalyaanamoorthy *et al.* 2017), also implemented in IQ-TREE. We allowed rate heterogeneity through both Gamma (+G) and invariable sites (+I). Branch support was assessed based on 1,000 bootstrap replicates and all the remaining parameters were set to default (Fig. 3). Finally, we used MrBayes version 3.2.7 (Huelskenbeck & Ronquist 2001) to infer the phylogenetic relationships under a Bayesian framework. We used the GTR+I+G model for each gene region in the concatenated dataset (based on ModelFinder), analyzed two independent MCMC runs, each consisting of a total 10 million generations. The

maximum clade credibility tree was constructed using 90% of the posterior samples and convergence was assessed based on ESS>200.

Terminologies. Terminology used to describe carapace, abdomen, legs, pedipalps and chelicera follows Harvey and West (1998), Wolff *et al.* (2015) and Miranda *et al.* (2021b). Terminology used to describe the male genitalia follows Giupponi & Kury (2013). LoD = *lobus dorsalis*; LoL 1= *lobus lateralis primus*; LoL 2= *lobus lateralis secundus*. Fi=*fistula*

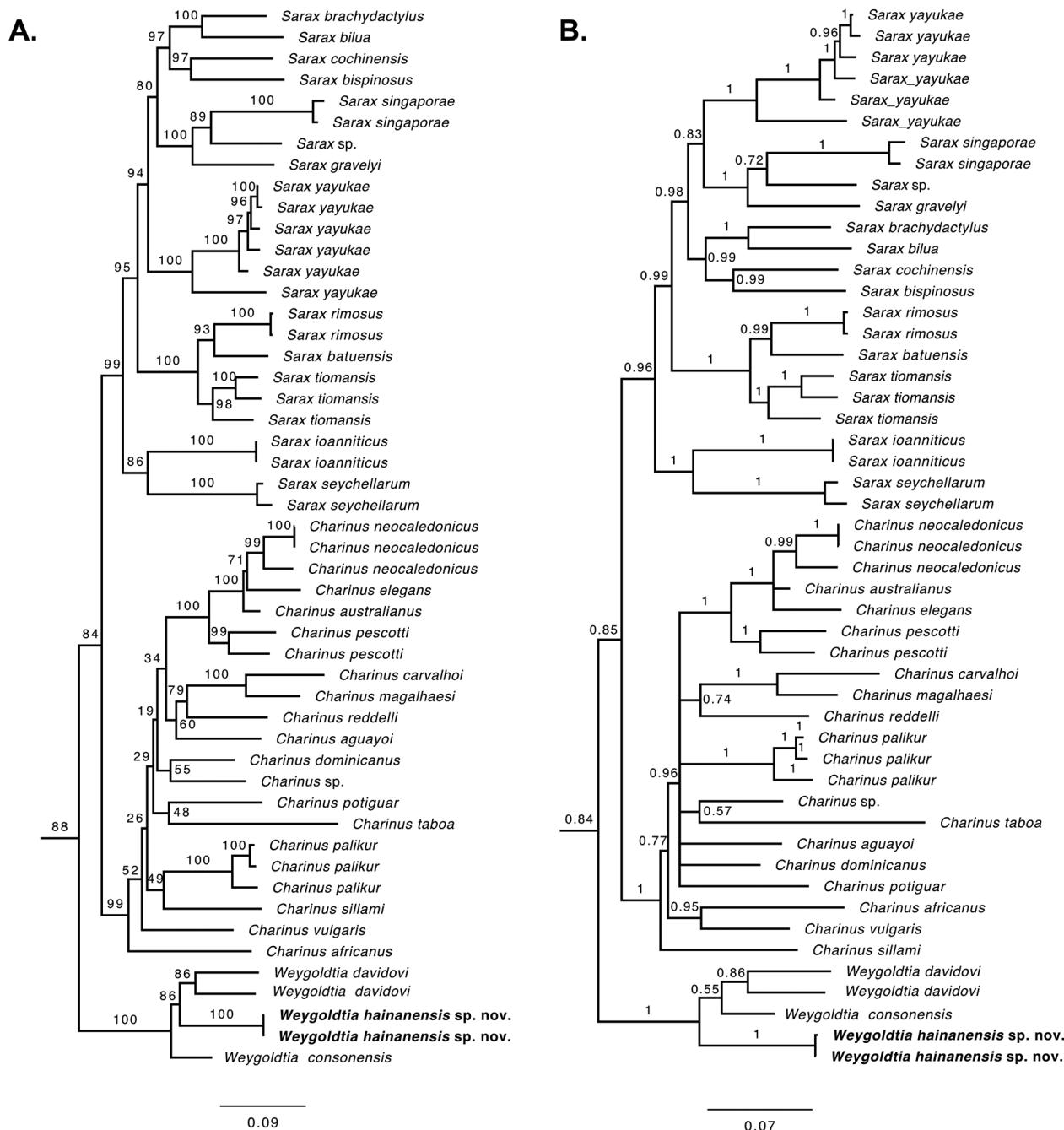


FIGURE 3. Phylogenetic relationships of charinid whip spiders highlighting the topology of *Weygoldtia hainanensis* sp. nov. (boldfaced). Phylogenetic analysis inferred under maximum likelihood (A) and Bayesian inference (B) based on the molecular dataset of Miranda *et al.* (2021). Support values are based on 1,000 bootstrap replicates (A) or posterior probabilities (1 million generations and 10% post-burnin). Branch lengths in substitutions per site (see scale under trees). Only relationships between species of Charinidae are presented; other Amblypygi analyzed as outgroups are *Damon diadema* Simon 1876, *Phryinus longipes* Pocock 1894, *Baalrog sbordonii* Brignoli 1973 and *Mastigoproctus giganteus* Lucas 1835.

Result

Class Arachnida Cuvier, 1812

Order Amblypygi Thorell, 1883

Suborder Euamblypygi Weygoldt, 1996

Family Charinidae Quintero, 1986

Genus *Weygoldtia* Miranda, Giupponi, Prendini & Scharff, 2018

Key to the identification of *Weygoldtia* based on Miranda et al. (2021b)

1. Pedipalp femur with five dorsal spines; pedipalp patella with six dorsal spines; cheliceral claw with 7–9 teeth; leg IV distitibia with six or seven *sc* trichobothria and seven or eight *sf* trichobothria *Weygoldtia davidovi* (Fage, 1946)
- Pedipalp femur with four dorsal spines; pedipalp patella with five dorsal spines; cheliceral claw with 6–7 teeth; leg IV distitibia with 9–11 *sc/sf* trichobothria 2
2. Tibia I with 26 segments, cheliceral claw with 6–7 teeth; leg IV distitibia with 10–11 trichobothria for each *sc* and *sf* trichobothria series *Weygoldtia hainanensis* sp. nov.
- Tibia I with 25 segment, cheliceral claw with 6 teeth; leg IV distitibia with 9 trichobothria for each *sc* and *sf* trichobothria series *Weygoldtia consonensis* Miranda et al., 2021

Diagnosis. *Weygoldtia hainanensis* sp. nov. can be distinguished from *W. davidovi* (Fage, 1946) through the following combination of characters: tibia I with 26 segments, whereas *W. davidovi* has 25 segments; basal region of tritosternum with high number of setae; *W. hainanensis* has four dorsal spines on pedipalpal femur, whereas *W. davidovi* has five; 6–7 teeth on the chelicerae claw of *W. hainanensis*, whereas *W. davidovi* has 7–9 teeth; distitibia IV trichobothrium *sc* and *sf* series each with 10–11 trichobothria in *W. hainanensis*, while *W. davidovi* has 7–8 trichobothria in the same series. *W. hainanensis* can be distinguished from *Weygoldtia consonensis* Miranda et al. 2021 through the combination of the following characters: tibia I of *W. hainanensis* with 26 segments, while *W. consonensis* has 25 segments; the distitibia IV trichobothria *sc* and *sf* series each with 10–11 trichobothria in *W. hainanensis*, while *W. consonensis* has 9 trichobothria; pedipalp of *W. hainanensis* with 15–20 setae in the ventral row of the cleaning organ, while *W. consonensis* has 24–30 setae in the ventral row of the cleaning organ; *W. hainanensis* has 6–7 teeth on the chelicerae claw, while *W. consonensis* has 6 teeth (Miranda et al. 2018, 2021b). LoL1 of male gonopod is cuspid with many spiniform projections.

Weygoldtia hainanensis Zhu, Li & He sp. nov.

Figs. 4–8

Holotype. ECNU-IV-0002, adult male, Changjiang, Dongfang City, Hainan Island, China, 300 m a.s.l., rainforest, over a high cliff, 18-iii-2021, Zhu Xiao-Yu leg. *COI* gene sequence No. MZ352109.

Paratype. ECNU-IV-0001, adult female, same data as holotype. *COI* gene sequence No. MZ352110.

Measurements of both female and male are provided in Table 1.

Carapace. Calamine blue with many tiny granulations eventually distributed at the surface. Anterior and posterior region bend downwards to the lateral eye, creating concave shape. Lateral and anteromedial eyes small, well developed and in grey and white coloration respectively. Curved carina presents between lateral eyes and carapace margin, and straight carina anterior to lateral eyes. Lateral ocular tubercles black, anteromedial eyes grey. Straight crest anterior to lateral eyes present. Three pairs of furrows reach the middle line; fovea is oval. Frontal margins with small granulations and 6 setae (Fig. 4B).

Sternum. Three well sclerotized segments. Tritosternum with round base, elongated and conical, about ten setae on basal region (more dense setae on basal region in female). Pair of setae close to apical setae. Tetrasternum rounded, convex, with four setae. Pentasternum rounded, convex, subequal to tetrasternum, with five setae. All setae on sternum rufous (Fig. 5C).

TABLE 1. Measurements (mm) of the carapace, abdomen, pedipalp segments, and femora of the legs of two specimens.

	ECNU-IV-0001		ECNU-IV-0002	
Sex	Female		Male	
Total length with chelicerae	13.5		14.4	
Carapace				
Maximum width	6.6		7.8	
Maximum length	4.5		5.1	
Abdomen				
Maximum width	5.5		5.8	
Maximum length	7.3		7.2	
	Right	Left	Right	Left
Pedipalp lengths				
Trochanter	1.1	1.4	1.2	1.3
Femur	4.2	4.1	5.9	5.5
Patella	4.1	4.8	6.2	6.4
Tibia	1.7	1.8	1.8	1.7
Tarsus and claw	2.2	2.0	2.5	2.1
Femur length				
Leg I	13.9	14.5	16.5	16.7
Leg II	7.4	7.4	9.1	8.8
Leg III	8.8	8.9	10.1	10.3
Leg IV	7.3	7.5	8.4	
No. segments tibia I	26		26	
No. segments tarsus I	45		45	
No. segments basitibia IV	4		4	

Abdomen. Flat, oblong and blue. Many tiny punctuations evenly distributed on the surface, smaller than those on the carapace. Only ten setae on male genital operculum (many short setae on female genital operculum). Ventral sac presents. Abdomen narrower than carapace (Fig. 4B, 7B, 7C).

Chelicera. Dorsum covered with many fine setae and six frontal setae (ectal view). All setae brown. Cheliceral furrow with four internal teeth. First distal tooth (upper) bifid, and Ia slightly bigger than Ib. Second tooth slightly bigger than the third one. Tooth length: IV > II > Ia > Ib > III. Claw with seven denticles (female has six denticles). The size of denticles gradually increases away from the tip (Fig. 5A).

Pedipalp. Blue with tips of spines red. *Trochanter*: with many rufous setae on antero-dorsal side, with two sub-equal ventral spines and two dorsal setiferous tubercles. Many long setae between spines at the same side, six setae near margin of the trochanter near coxa. Female with fewer setae than male. *Femur*: finely granulated. Four dorsal spines, decreasing in length. One short dorsal spine between spine I and spine II, three short dorsal spines between spine II and spine III, one short dorsal spine between spine III and spine IV, one short dorsal spine after spine IV distally. One prominent setiferous tubercle between spine I and proximal margin. Four ventral spines, spine I distal to spine II, half in length. Spine II to spine IV decreasing in length, small short ventral spine between each primary spine series. Spine II two thirds of spine I, spine III two thirds of spine II. Two setae on spine I. Several setae in postero-dorsal margin. Female has no secondary dorsal spines between dorsal primary spine series. *Patella*: finely granulated. Five dorsal spines decreasing in length. Prominent spine distal to spine I. Two short dorsal spines after spine V distally. Four ventral spines, I > II > IV > III. Spine II two thirds of spine I, spine III two thirds of spine II. One short ventral spine between spine I and spine II, in the middle, two short ventral spines after spine IV distally. One short ventral spine distal to spine I, one third in length. Many setae on the postero-dorsal side, opposite to spines. *Tibia*: Two large dorsal spine present with many rufous setae near the bottom of the spine, one ventral spine. Several setiferous tubercles at the antero-dorsal side of the tibia. Many setae on the postero-dorsal side, opposite to

spines. *Tarsus*: Three small subequal dorsal blunt spines. The internal surface next to tibia covered with a fine line of small setae near base of the cleaning organ (that line of setae is much shorter in female). Many long setae randomly distributed throughout the segment. Ventral row of cleaning organ with 15-20 setae (Fig. 6A, B).

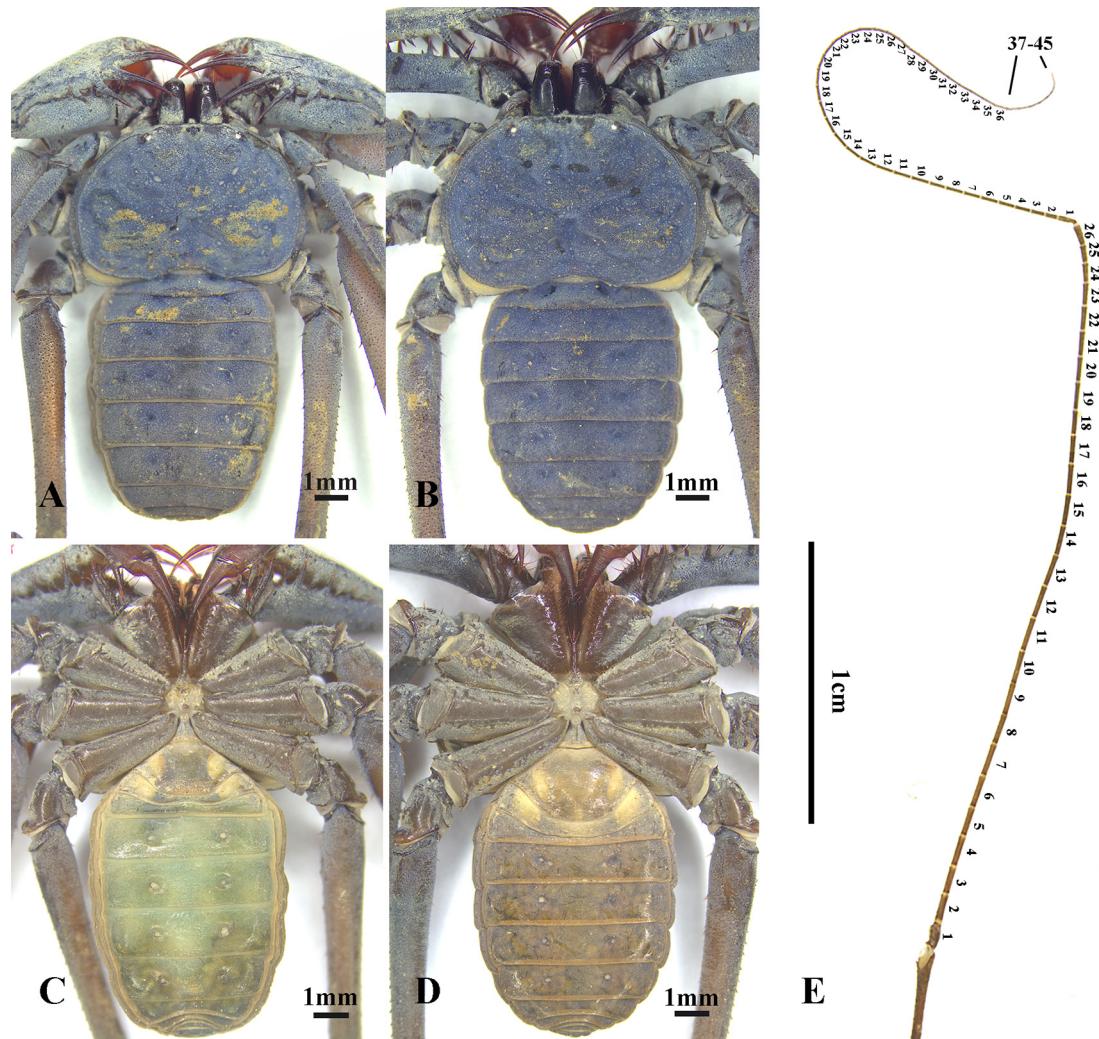


FIGURE 4. *Weygoldtia hainanensis* sp. nov. from Hainan, China. A: Female, dorsal; B: Male, dorsal; C: Female, ventral; D: Male, ventral; E: Leg I tibia and tarsus of an adult female, dorsal.

Legs. All setose, calamine blue. All setae are rufous. Leg I elongated, 26 segments in tibia, 45 segments in tarsus (Fig. 4E). Basitibia IV with four well sclerotized margins. Walking-leg tarsi with arolium (Fig. 5D, E). Difference in length shown in Table 1. Distitibia IV trichobothria sc and sf series each with 10 - 11 trichobothria.

Color pattern. Pedipalp, carapace, abdomen, and legs mostly calamine blue. The blue on distal regions of legs is lighter, and gradually turns grey. Tarsus and claws on the pedipalp are in grey coloration. Chelicera brown, spines on the pedipalp black, tips light red. All setae are rufous and brown (Fig. 4A, B).

Male genitalia. Paired lobes covered ventrally by genital operculum. LoL1 cuspid with many small spiculate projections, most submarginal projection has the greatest length. LoL2 larger than LoL1 with hairy-like ends. The surface of LoD has many spiniform projections, smaller than those of LoL1. Surface of Fi has a large smooth area (Fig. 8). Female gonopods were collapsed and could not be described properly.

Distribution: China (Hainan).

Etymology: This species is named after Hainan Island.

Natural history: *Weygoldtia hainanensis* sp. nov. is only found in karst topography of Hainan Island. Both males and females were collected in March at night (30°C and 75% humidity). The whip spiders were spotted on huge flat cliffs with deep narrow cracks. When disturbed and threatened, they moved rapidly into the cracks nearby, and sometimes jumped onto the ground to hide under fallen leaves. We found individuals distributed randomly on the cliff varying from 0.5–3.0 m in heights. They were tolerant to conspecific individuals, and sometimes could

be found in close proximity around 30 cm to each other. They were found to co-occur with other arthropods, such as Mygalomorphae (*Cyriopagopus hainanus* Liang *et al.* 1999), Thelyphonida O.P-Cambridge 1872, and Scolopendromorpha Latreille 1817. In the lab, they show preference to prey on the house crickets, *Acheta domesticus* Linnaeus 1758. Their egg sac contains 12 eggs, about 1.5 mm in diameter. At 25°C, the egg development takes 113 days. The hatching praenymphae have white carapaces, light green abdomens, and light pink appendages (Fig. 1E). They stay on the back of the adult female for about 3 days. Then, they molt into protonymphae and change into gray color, about 3-4 mm in length (Fig. 1D).

Phylogenetic position. Higher-level phylogenetic relationships were equivalent between our study and the trees presented in Miranda *et al.* (2021a, b). The monophyly of major groups including the Charinidae (bootstrap=88%; posterior probability [pp hereafter]=0.84), *Sarax* (bootstrap=99%; pp=0.96) and *Charinus* (bootstrap=99%; pp=1) were highly supported as found in Miranda *et al.* (2021a). Phylogenetic relationships between congeneric species in *Weygoldtia* and *Charinus* were also similar between our study and the tree presented in Miranda *et al.* (2021a, b). Specifically, the reciprocal monophyly of the samples analyzed in this study and assigned to *Weygoldtia hainanensis* sp. nov. was recovered and highly supported (bootstrap=100%; pp=1). Furthermore, *Weygoldtia hainanensis* sp. nov. is consistently found nested within *Weygoldtia*, and this clade is highly supported by both Bayesian and maximum likelihood inference frameworks (bootstrap=100%; pp=1). However, the relationships within *Weygoldtia* were not resolved by both maximum likelihood and Bayesian inference approaches. For instance, while *W. hainanensis* sp. nov. is recovered to be sister of *W. davidovi* under maximum likelihood analyses (bootstrap support=86%), Bayesian analyses recovered a weakly supported single clade for both *W. consonensis* and *W. davidovi* (pp=0.55) (Fig. 3).

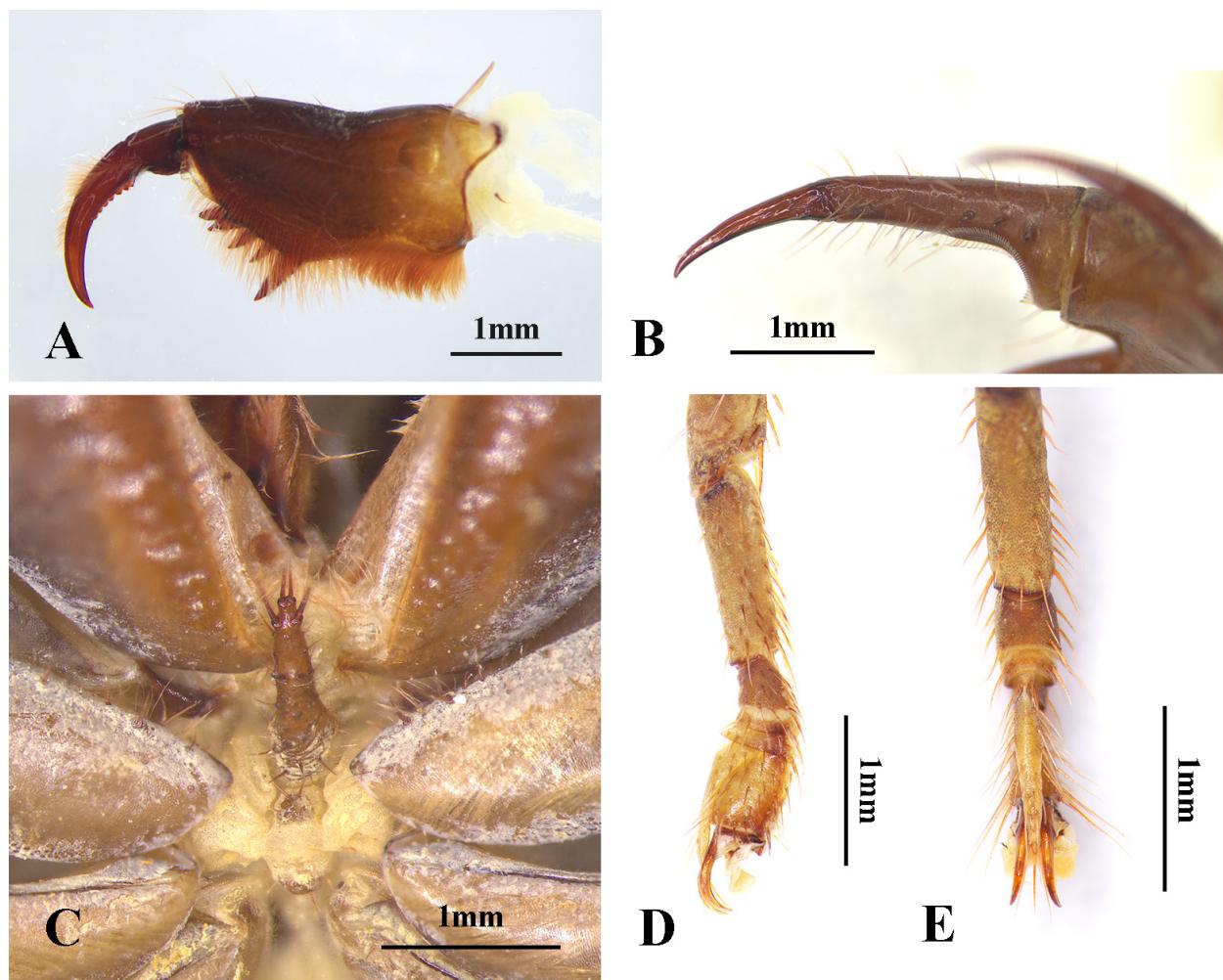


FIGURE 5. *Weygoldtia hainanensis* sp. nov. A: Male chelicera, ectal view; B: Pedipalp tarsus and claw, lateral view; C: Sternum, ventral view; D: Walking-leg tarsi with arolium, ectal view; E: Walking-leg tarsi with arolium, dorsal view.



FIGURE 6. A: Pedipalp, dorsal view; B: Pedipalp, ventral view.

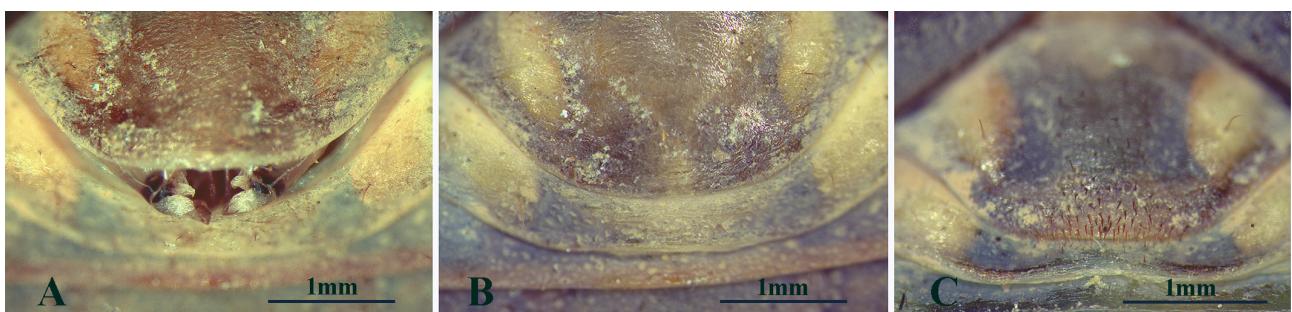


FIGURE 7. *Weygoldtia hainanensis* sp. nov. A: Male genital operculum and gonopod, ventral view; B: Male genital operculum, ventral view; C: Female genital operculum, ventral view.

Discussion

We used molecular phylogenetics and independent morphological evidence to study the evolutionary relationship of a new species of *Weygoldtia* and other described species in Charinidae. Consistent with the morphological inferences, our molecular phylogenetic analyses demonstrated *W. hainanensis* sp. nov. in a monophyletic clade with two previously described *Weygoldtia* species. Overall, our study supports the newly found population of whip spiders as a new species of *Weygoldtia*. Although the phylogenetic analyses strongly supported the placement of *W. hainanensis* sp. nov. within *Weygoldtia* (bootstrap=100%; pp=1), more data is needed to fully resolve the phylogenetic relationships within this genus. To our knowledge, whip spiders have not been recorded in mainland China. The

first record of whip spiders in China is *Charon ambreae*, which was found in Orchid Island of Taiwan (Reveillion & Pierre-Olivier 2018). *Weygoldtia hainanensis sp. nov.* provides the second species record of Amblypygi in China.

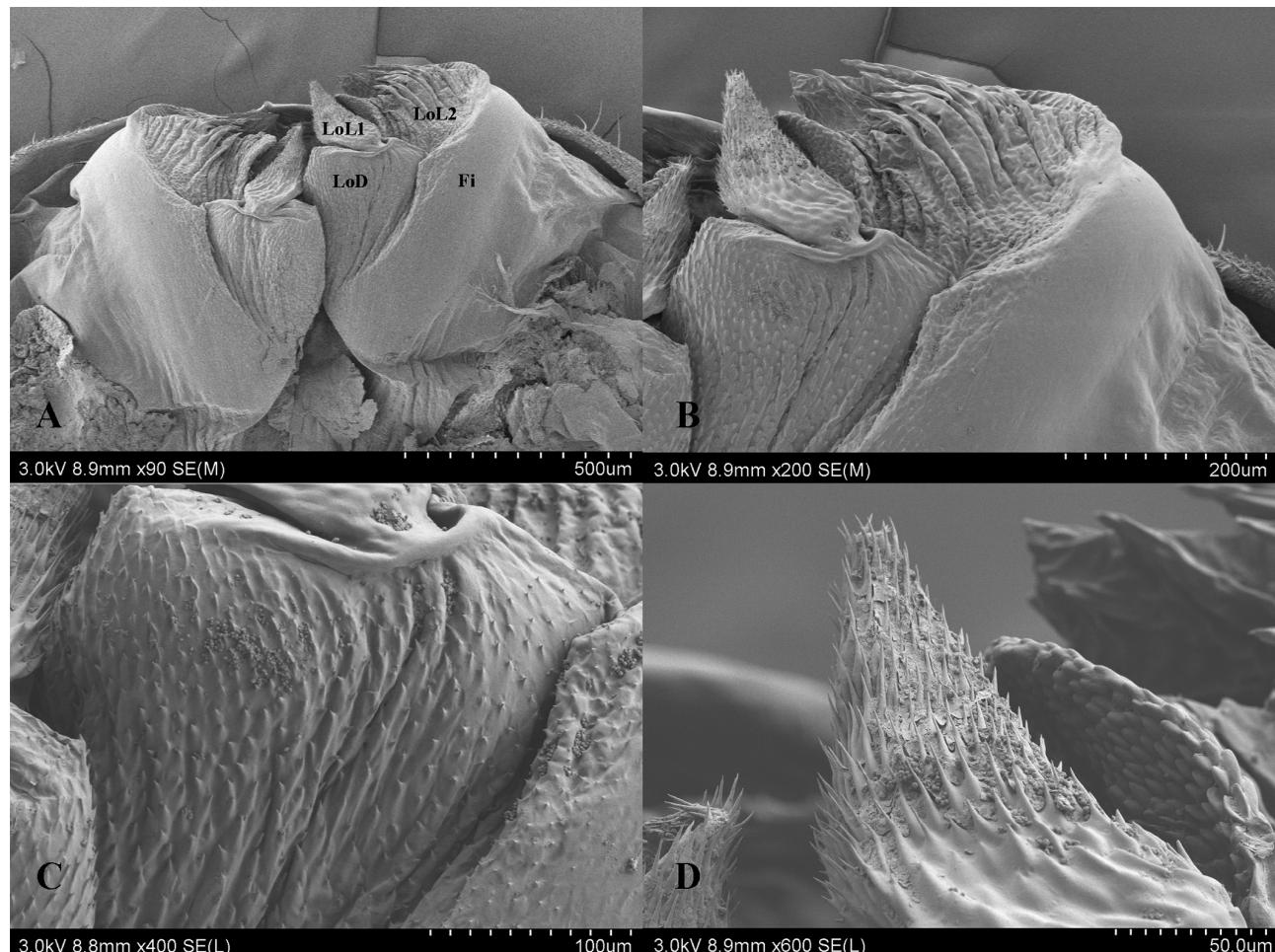


FIGURE 8. Male gonopod of *Weygoldtia hainanensis sp. nov.* from Hainan, China. A: Dorsal view; B: Detail of ornamentation of LoD, LoL 2, LoL 1, and Fi; C: Detail of ornamentation of LoD; D: Detail of ornamentation of LoL 1 (Details in Terminologies).

Weygoldtia hainanensis sp. nov. is only found in a small region of karst topology of Hainan. Similar to other endemic species in Hainan, their population size is likely to be small. Given that in many areas, whip spiders are popular exotic pets (Weygoldt 2009), conservation efforts of this species and their habitat are needed. The discovery of this species provides another case for karst topology in southeast Asia as biodiversity hotspots. For example, five new species of cave geckos *Goniurosaurus* Barbour 1908 were discovered in karst area in southern China over the last two years. (Qi *et al.* 2020a, 2020b; Zhu *et al.* 2020a, 2020b; Zhu *et al.* 2021). Future research in this area will continue to enrich our understanding of biodiversity and provide practical solutions for conservation in this region.

Weygoldtia hainanensis sp. nov. has only been collected in Hainan Island. The other two described *Weygoldtia* species are found in Cambodia, Laos, and Vietnam (Miranda *et al.* 2021). This new species provides the northernmost distribution for the genus and its distribution does not overlap with other *Weygoldtia*. The current biogeographical pattern of *Weygoldtia* is not surprising given the geographical history of Hainan Island. Hainan was connected to Guangxi and northern Vietnam as part of western Indochina Peninsula during the Mesozoic (Zhu 2016; Liang 2018). In the Early Cretaceous (~ 120 MYa), it drifted to approximately its current location (Liu & Morinaga 1999; Zhu 2016). This geographical history results in plants and animals found in Hainan are more similar to those found in northeastern Indochina compared to northwestern Indochina or southeastern China (Bain & Hurley 2011; Zhu 2016; Xu *et al.* 2017; Liang 2018). For example, the flora of Hainan Island is most similar to that of Vietnam, with 100% and 89.0% similarities at family and genus levels respectively, and then followed by Guangxi (Zhu 2016). Similarly, the Crested Gibbon *Nomascus hainanus* Thomas 1892, which is only found in Hainan, is more closely related to *N. nasutus* Künckel d'Herculais 1884 from northeastern Vietnam rather than *N. concolor* Harlan 1826

from west of the Red River (Bain & Hurley 2011). Overall, the biogeographical pattern of *Weygoldtia* is consistent with many plants and animals found in Hainan Island and the Indochina Peninsula. The discovery of *Weygoldtia hainanensis* sp. nov. expanded the habitat range of *Weygoldtia* and leaves a big gap in their current distribution. This discovery suggests potential new species remain to be discovered in suitable habitats of the Indochina Peninsula and southern China. Future surveys in these areas will fulfill this gap and provide a better understanding of the evolutionary history of whip spiders in this region.

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