Article

A new species of whip spider, *Sarax sinensis* sp. nov., from Fujian, China (Arachnida: Amblypygi: Charinidae)

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Abstract

To date, only two whip spider species have been recorded in China. We describe a new species, *Sarax sinensis* **sp. nov.**, from Fujian, China. This species is morphologically similar to *S. ioanniticus* (Kritscher, 1959), *S. israelensis* (Miranda *et al.*, 2016), and *S. seychellarum* (Kraepelin, 1898), but can be distinguished by the combination of the following characters: 35 segments in leg I tarsus, eight teeth on cheliceral claw, and four dorsal and ventral spines respectively on pedipalp femur. To examine the evolutionary history of *S. sinensis* **sp. nov.**, we sequenced *12S*, *16S*, and *COI* gene regions of our specimens and inferred its phylogenetic position. The inferred phylogenetic trees placed the new species within *Sarax*, with its closest relative being distributed across the western Asia. The type specimens are deposited in the Museum of Biology, East China Normal University (ECNU).

Key words: taxonomy, biodiversity, arachnology, tailless whip scorpion

Introduction

Amblypygi Thorell, 1883 (whip spiders), is an order of arachnids with a dorsoventrally flattened body, robust pedipalps, and antenniform first pair of legs (Foelix & Hebets 2001; Garwood *et al*. 2017; Miranda *et al*. 2021a). Whip spiders live in various habitats such as under rocks, tree barks, in crevices, and inside caves (Weygoldt 2000; Rahmadi *et al*. 2010). There are five families currently accepted within the Amblypygi. Among these, Charinidae Quintero, 1986, is the most species rich, exhibiting also the widest geographical distribution (i.e. occupying the intertropical zone on all continents; Miranda *et al*. 2018, 2021a, 2021b). Recent studies have shown that Charinidae is composed of only three genera: *Charinus* Simon, 1892, *Sarax* Simon, 1892, and *Weygoldtia* Miranda *et al*., 2018 (Miranda *et al*., 2018, 2021b). Furthermore, recent morphological- and molecular-based phylogenetic evidence suggests that *Sarax* and *Charinus* are sister groups (Miranda *et al*. 2018, 2021a). *Charinus* contains 94 species that distribute in the Americas, Africa, and Oceania (Miranda *et al*. 2021b). *Sarax,* containing 36 species, is only found in the Old World such as East Africa, the Arabian Peninsula, and South-East Asia (Miranda *et al*. 2021b). Finally, *Weygoldtia* includes three species that are restricted to Vietnam, Cambodia, Laos, and the Hainan Island of China (Miranda *et al*. 2018, 2021b; Zhu *et al*. 2021).

A recent study erected a new species of *Weygoldtia* from Hainan, China. This species has only been found in karst topography (Zhu *et al*. 2021). Therefore, this biogeographical pattern has left a major distribution gap of Am-

bylpygi species in southern China. In recent biodiversity surveys, we found a previously undescribed Charinidae species in Fujian, China (Fig. 1, 2). This is the third record of an Amblypygi and the second record of the family in China. Here, based on morphological and molecular data, we described the new collected specimens as a new species. We further use molecular sequences to examine the phylogenetic placement of this species in relation to the rest of the Amblypygi (Miranda *et al.* 2021a, 2021b).

FIGURE 1. Dorsal habitus of *Sarax sinensis* **sp. nov.** from Fujian, China. A: Immature of unknown sex (photographed in the field); B: Adult female carrying an egg sac.
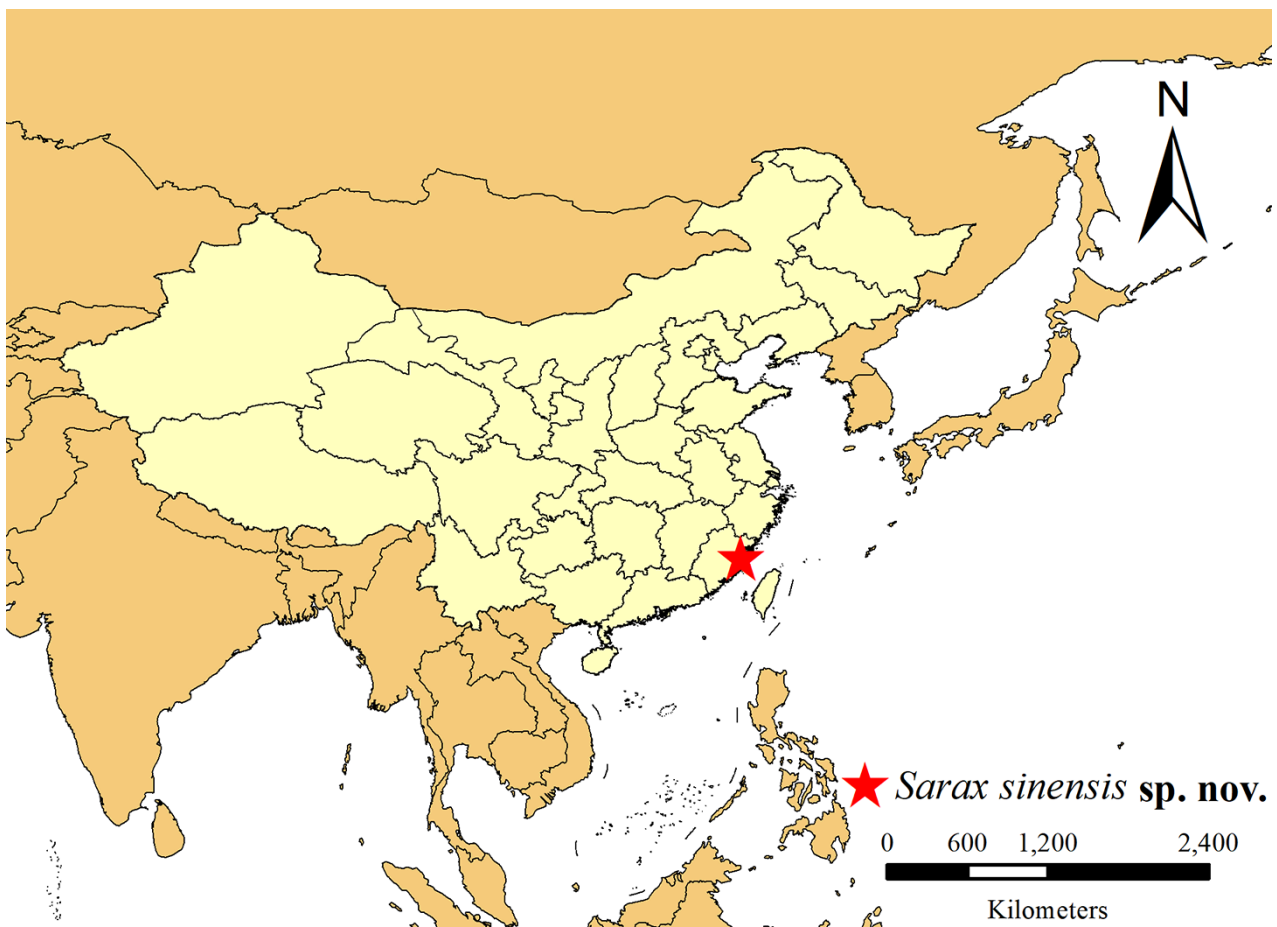
FIGURE 2. Distribution of the *Sarax* species. *Sarax sinensis* **sp. nov.** is found in Fujian, China.

Material and methods

Sampling and scanning electron microscopy (SEM). We conducted biodiversity surveys at nighttime in Fujian. Nine specimens were collected in the field. Three individuals were raised in captivity for describing their natural history (one adult male, one adult female, and one immature unknown sex). The other six specimens were preserved in 75% ethanol and deposited in the Museum of Biology, East China Normal University (ECNU). We dried and photographed specimens using a Leica M125 stereomicroscope and an SC2000 digital CMOS Camera. Chelicerae were photographed in 75% ethanol. Male gonopods were dissected and used for critical point drying and SEM using 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. Tissue was performed through an ethanol series of concentrations 30%, 50%, 70%, 80%, 95%, and 100%, fifteen minutes each, and repeated 100% ethanol dehydration 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 the tissue of the abdomen. We amplified three gene regions: *COI* (635 bp), *12S* (374bp), and *16S* (523bp). Primers for *COI* gene were U1m3 TCWACWAATCATAAAGACATTGGAAC and L1pyc CTATRATDGCRAATACDGCTCCTA (Arabi *et al*. 2010). Amplification conditions for *COI* 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. Primers for 12S and 16S gene were 12Sai AAACTAGGATTAGATACCCTATTAT 12Sbi AAGAGCGACGGGCGATGTGT (Kocher *et al*. 1989) and 16Sbr CTCCGGTTTGAACTCAGATCA16Sar CGCCTGTTTATCAAAAACAT (Simon *et al*. 1994) respectively. Amplification conditions for *12S* and *16S* genes included: pre-denaturation for 2 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 50°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 the Table 1.

TABLE 1. GenBank accession number for each sequence used in this study.

| Specimen	*COI*	*12S*	*16S*
ECNU-IV-0003	ON103514	ON112296	ON112299
ECNU-IV-0004	ON103515	ON112297	ON112300
ECNU-IV-0005	ON103516	ON112298	ON112301

Phylogenetic analyses. We inferred the phylogenetic position of *Sarax sinensis* **sp. nov.** in the Amblypygi phylogeny using molecular data. For this, we included new sequences for *Sarax sinensis* **sp. nov.** into the original sequence alignments used in Zhu *et al*. (2021). In the later study, the authors assembled a molecular dataset consisting of sequences that were originally examined by Miranda *et al*. (2021a) along with sequences for a newly described species, *Weygoldtia hainanensis* (Zhu *et al*. 2021). The phylogenetic tree presented in Zhu *et al*. (2021) is, thus, to date, the most comprehensive species-level phylogeny for the Charinidae. A detailed discussion of the phylogenetic relationships within the clade is provided in Miranda *et al*. (2021). Tree topologies in both Miranda *et al*. (2021a) and Zhu *et al*. (2021) are congruent. Here, we focus on inferring the phylogeny of the same set of species sampled in Zhu *et al*. (2021), but including sequences for two individuals assigned to *Sarax sinensis* **sp. nov.** described in this study. Thus, the species-level sampling in this study includes two more species relative to Miranda *et al*. (2021a; i.e. *Sarax sinensis* **sp. nov.** and *Weygoldtia hainanensis*).

In Zhu *et al*. (2021), we analyzed the phylogenetic position of 32 Amblypygi species (four outgroup taxa) using sequence data for 53 individuals. Molecular sampling included regions of *COI*, *12S*, *16S*, *18S*, and *28S*. For each gene region, we retrieved sequences from GenBank following the accession numbers listed in Appendix 3 from Miranda *et al*. (2021a). Additionally, we included sequences for *Sarax sinensis* to the unaligned *COI* sequence dataset. In Zhu *et al*. (2021), sequences within each of the analyzed gene regions were aligned using MAFFT version 7 under default parameters (Katoh & Standley 2013). In this study, we include sequences for two *Sarax sinensis* **sp. nov.** individuals in the sequence alignments generated in Zhu *et al*. (2021). For this, we used the --addlong argument in MAFFT version 7 to include the new *12S*, *16S*, and *COI* sequence for *Sarax sinensis* **sp. nov.**. We allowed the direction of the new sequences to be adjusted by MAFFT if necessary. Finally, the resulting alignments for *COI*, *12S*, *16S*, *18S*, and *28S* resulting from adding two sequences from *Sarax sinensis* **sp. nov.** to the existing alignment analyzed in Zhu *et al*. (2021), were concatenated using the SuperMatrix function in the evobiR R package version

1.1 (Blackmon and Adams 2015). Phylogenetic analyses on the resulting concatenated dataset were conducted using both maximum likelihood and Bayesian methods.

We used 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 using the -p argument in IQ-TREE. Best-fitting models and partitioning strategy were identified for the dataset using ModelFinder using the -m MFP+MERGE argument (Kaly-aanamoorthy *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 (Huelsenbeck and 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 of 10 million generations. The maximum clade credibility tree was constructed using 90% of the posterior samples and convergence was assessed based on ESS>200.
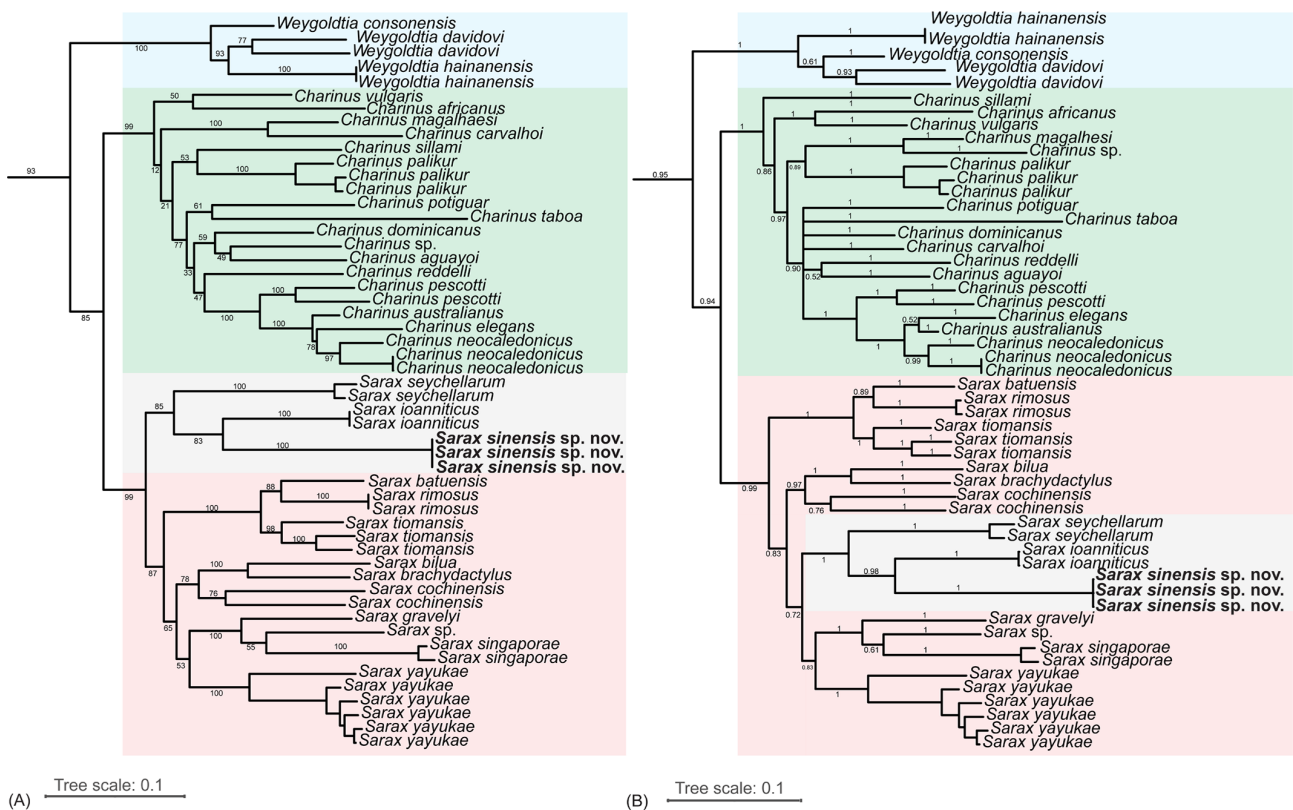
FIGURE 3. Phylogenetic relationships of charinid whip spiders highlighting the phylogenetic position of *Sarax sinensis* **sp. nov.** (boldfaced). Phylogenetic analysis inferred under maximum likelihood (A) and Bayesian inference (B) based on the molecular dataset of Miranda *et al.* (2021) and Zhu *et al.* (2021). Support values are based on 1,000 bootstrap replicates (A) and posterior probabilities (B; 1 million generations and 10% post-burnin). Branch lengths in substitutions per site (see scale under trees). Only relationships between species of Charinidae are shown. Other Amblypygi analyzed as outgroups but not shown in the figure are *Damon diadema* Simon 1876, *Phrynus longipes* Pocock 1894, *Baalrog sbordonii* Brignoli 1973, and *Mastigoproctus giganteus* Lucas 1835. Blue represents *Weygoldtia*; Green represents *Charinus*; Grey represents *Sarax* of the West Asia Clade; Pink represents *Sarax* of the East Asia Clade.

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

Result

Class Arachnida Cuvier, 1812

Order Amblypygi Thorell, 1883

Suborder Euamblypygi Weygoldt, 1996

Family Charinidae Quintero, 1986

Genus *Sarax* Simon, 1892

Key to the identification of the species of *Sarax* in Africa, the Middle East, and South Asia revised from Miranda *et al*. (2021b)

Diagnosis. *Sarax sinensis* **sp. nov.** can be distinguished from *S. ioanniticus* (Kritscher, 1959) by the combination of following characters: four dorsal and ventral spines on pedipalp femur (vs. five dorsal and ventral spines in *S. ioanniticus*), 35 articles in leg I tarsus (vs. 37 articles in leg I tarsus). It differs from *S. israelensis* by fewer dorsal spines on pedipalp patella (five in *S. israelensis*), fewer articles in leg I tarsus (vs. 37 articles in leg I tarsus). It is different from *S. seychellarum* (Kraepelin, 1898) with, fewer dorsal spines on pedipalp femur (three spines in *S. seychellarum*), 35 articles in leg I tarsus (vs. 37 segments in *S. seychellarum*), and presence of ventral sac (vs. absent in *S. seychellarum*). *S. sinensis* is also distinct to *S. omanensis* (Delle Cave, Gardner & Weygoldt, 2009) and *S. stygochthobius* (Weygoldt & Van Damme, 2004) by the present of median eyes, for those two species lack median eyes. It can be differed from *S. cochinensis* (Gravely, 1915) and *S. dhofarensis* (Weygoldt *et al*., 2002) with two spines on pedipalp tarsus (vs. one spine in *S. cochinensis* and *S. dhofarensis*). It has eight teeth on cheliceral claw and three pseudo-articles on leg IV basitibia, whereas *S. socotranus* (Weygoldt *et al*, 2002) has twelve teeth on cheliceral claw and only two pseudo-articles on leg IV basitibia. Differs from *S. bispinosus* (Nair, 1934) by bearing more teeth on the cheliceral claw (8 in the new species vs. 4 in *S. bispinosus*), less articles in tarsus I (34–35 in the new species and 39 in *S. bispinosus*), and less spines on pedipalp patella dorsal (4 in the new species and 5 in *S. bispinosus*). Differs

from *S. abbatei* (Delle Cave, 1986) by the well-developed eyes, in contrast to reduced eyes in *S. abbatei*. Differs from *S. pakistanus* (Weygoldt, 2005) by the eye development (well developed in the new species while weakly developed in *S. pakistanus*) and the number of spines on pedipalp patella dorsal (4 in the new species and 3 in *S. pakistanus*). Differs from *S. bengalensis* (Gravely, 1911) by the shape and size of ventral projection between spine I and distal margin; it is a long spine in the new species and a setiferous tuber in *S. bengalensis*.

Sarax sinensis Wu, Zhu, Li & He sp. nov.
Figs. 4–9

Holotype. ECNU-IV-0004, adult male, Fuzhou City, Fujian Province, China, 26.03 °N , 119.30 °E, 39m a.s.l., Yu Lin-rui and Wang Ya-fei leg.

Paratype. All immature, unknown sex, same data as holotype, ECNU-IV-0003, ECNU-IV-0005, ECNU-IV-0006, ECNU-IV-0007.

Carapace. Seven frontal setae; frontal process triangular. Small granules densely and evenly scattered between ocular triads and among sulci. Median eyes and median ocular tubercle well developed; pair of setae on median ocular tubercle; lateral eyes well developed, pale. Three pairs of furrows reach the middle line; fovea oval (Figs. 4A, 5A).


FIGURE 4. *Sarax sinensis* **sp. nov.** from Fujian, China. Holotype, ECNU-IV-0004, male. A: Dorsal; B: Ventral.

Sternum. Three well-sclerotized segments. Tritosternum with round base, elongated and conical, ten setae on the basal region. Pair of setae close to apical setae, in the middle of the projection. Tetrasternum rounded, convex, with a pair of setae. Pentasternum rounded, convex, smaller than tetrasternum, with a pair of setae (Fig. 6A).

Abdomen. Flat and oblong. Many tiny punctuations evenly distributed on the surface, smaller than those on the carapace. Genital operculum with 37 setae randomly on the surface. Ventral sac presents. Abdomen narrower than the carapace (Figs. 4A, 4B, 5B).

Chelicera. Dorsum covered with many fine setae and three frontal setae (ectal view). Cheliceral furrow with four internal teeth. First distal tooth (upper) bifid; Ia and Ib subequal. Second tooth subequal to the first and third ones. Fourth tooth longest. Claw with eight denticles (mesal view) (Figs. 6B, 6C).
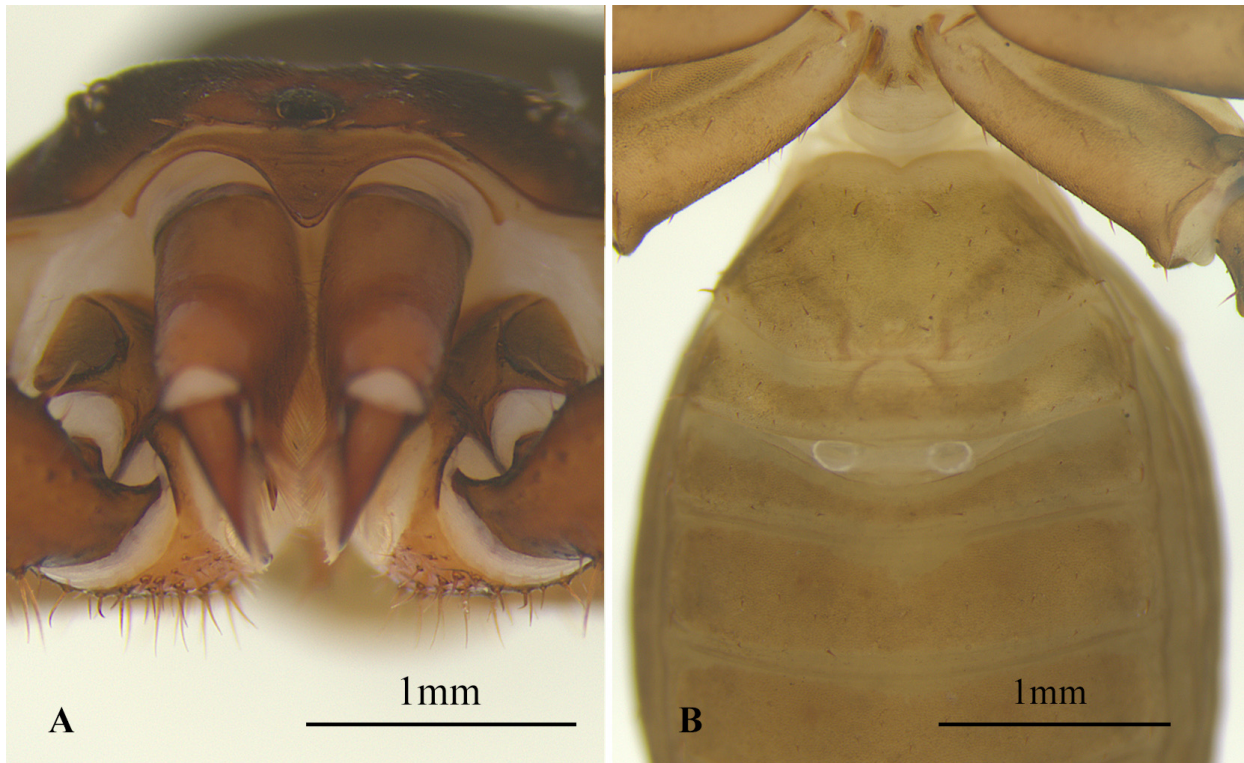
FIGURE 5. *Sarax sinensis* **sp. nov.** A: Frontal process of holotype, ECNU-IV-0004; B: Ventral sac of paratype ECNU-IV-0006.

FIGURE 6. *Sarax sinensis* **sp. nov.** Holotype, ECNU-IV-0004, male. A: Sternum, ventral view; B: Chelicera, mesal view; C: Chelicera, ectal view.

Pedipalp. Coxa: Fourteen setae encircle around the carina. Many fine setae on the ventral surface and denser near the ventral mesal region. Trochanter: many rufous setae on antero-dorsal side. Two small subequal ventral spines. Ventral apophysis small and setiform. Femur: finely granulated. Four dorsal spines, decreasing in length. One prominent setiferous tubercle between spine I and proximal margin. Four ventral spines, decreasing in length. Patella: finely granulated. Four dorsal spines. Prominent spine distal to spine I. Three setae on spine I. One seta on spine II. One short secondary spine between spine III and IV. Spine II three-fourths of spine I, spine III half of spine II, spine IV one-third of spine III. Three ventral spines, decreasing in length. Spine II two-thirds of spine I, and spine III half of spine II. Many setae on posterodorsal side, opposite to spine series. Tibia: two large dorsal spines present with many rufous setae near the bottom of the spine, one ventral spine. Many setae on the posterodorsal side, oppo-

site to spines. Tarsus: two dorsal spines. Distal spine long, about one-third length of tarsus, proximal spine one-third length of the distal spine. Many long setae randomly distributed throughout the segment. Ventral row of cleaning organ with 25–30 setae (Figs. 7A, 7B, 7D, 8B, 8C).
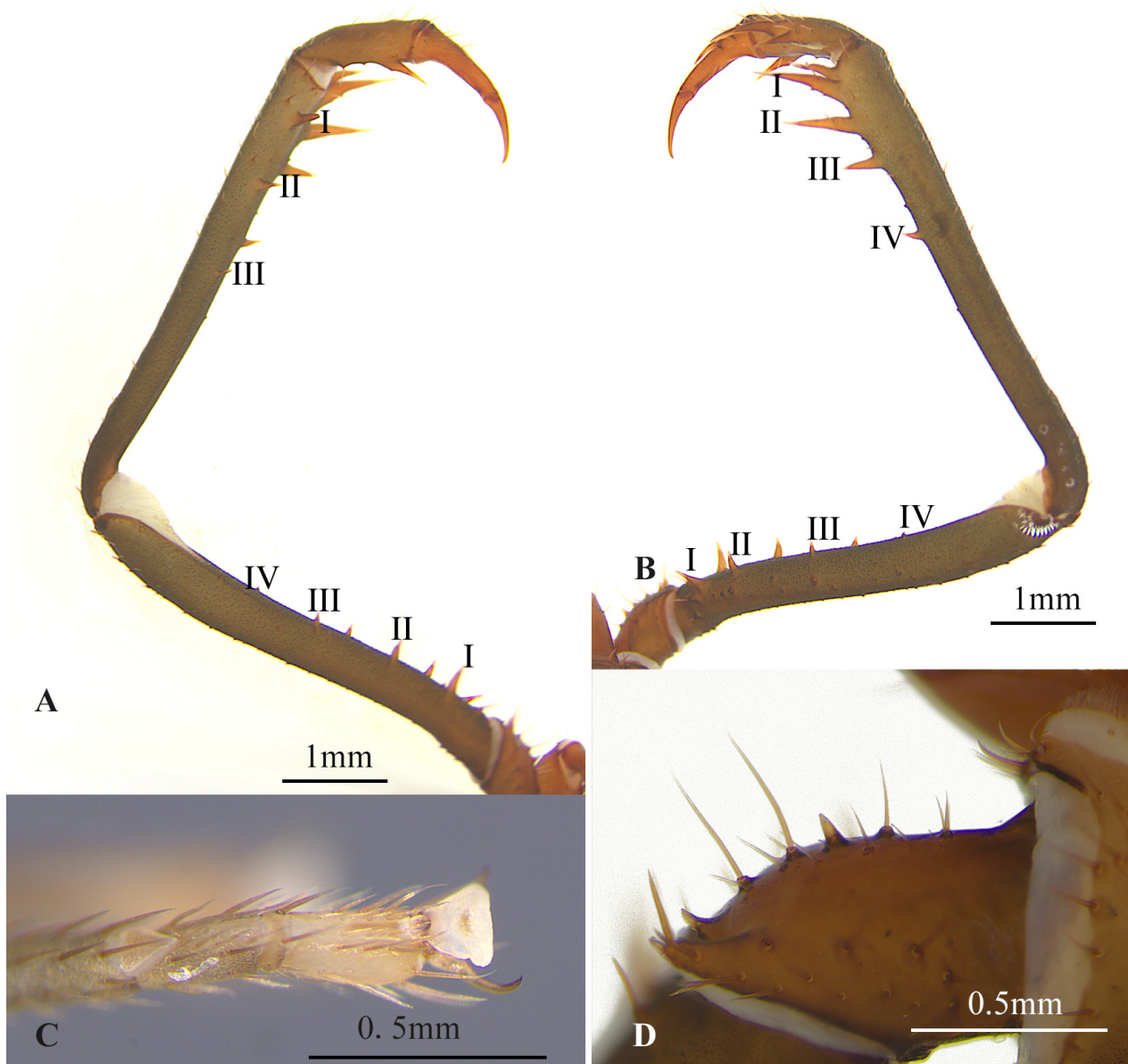
FIGURE 7. *Sarax sinensis* **sp. nov.** Holotype, ECNU-IV-0004, male. A: Pedipalp, ventral view; B: Pedipalp, dorsal view; C: Walking-leg tarsi with arolium, ventral view; D: Pedipalp ventral apophysis, ventral view.

Legs. All setose. Leg I elongated, 20 segments in tibia, 34–35 segments in tarsus. Basitibia IV with three pseudo-articles. Walking-leg tarsi with arolium. Distitibia IV trichobothria *sc* and *sf* series each with 5 trichobothria (Figs. 7C, 8A, 8D).

Color pattern. Pedipalp, carapace, abdomen, and legs mostly dark gray. The margin of carapace pinkish. Spines on the pedipalp black, tips light red. All setae brown (Figs. 4A, 4B).

Male genitalia. Paired lobes covered ventrally by genital operculum. LoL1 with 2 grooves and small spines at the base. LoL2 larger than LoL1 with many small spiculate projections. LoD cuspid with smooth surface at the tip. The surface of LoD base with many spiniform projections, larger than those of LoL2. Spiniform projections wider and 3-5 projections share a base near the bottom of LoD. Surface of Fi has a large smooth area with about fifteen grooves parallel with each other diagonally (Fig. 9).

Measurements: see Table 2.

TABLE 2. Measurements (mm) of the carapace, abdomen, pedipalp segments, and femora of the legs of the holotype (ECNU-IV-0004).
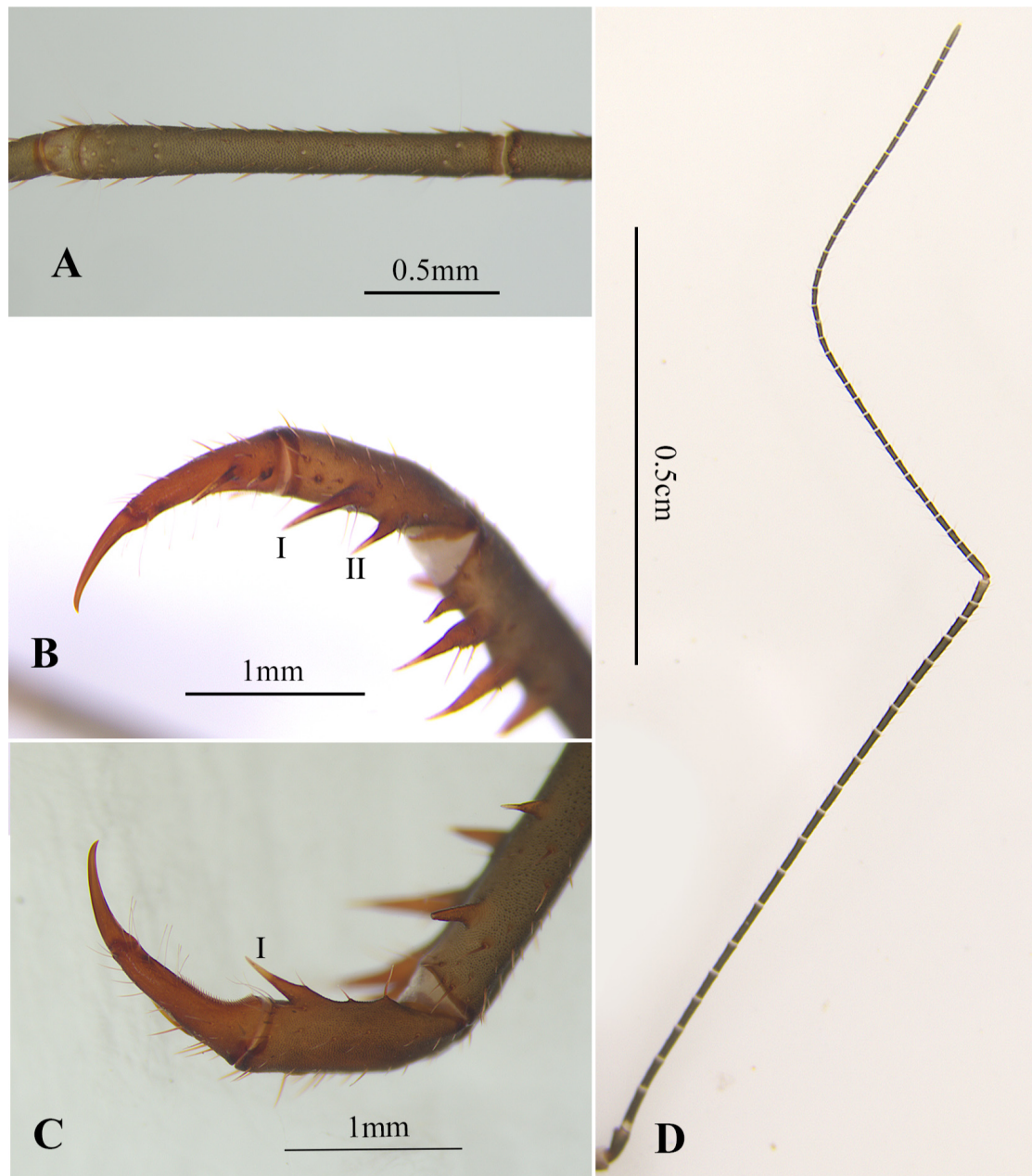
	ECNU-IV-0004	
Sex	Male	
Total length with chelicerae	6.86	
Carapace		
Maximum width	3.31	
Maximum length	2.26	
Abdomen		
Maximum width	2.74	
Maximum length	3.75	
	Right	Left
Pedipalp lengths		
Trochanter	0.62	0.60
Femur	4.12	4.07
Patella	4.82	4.77
Tibia	1.10	1.02
Tarsus and claw	1.48	1.47
Femur length		
Leg I	22.94	22.98
Leg II	9.89	9.98
Leg III	10.98	11.02
Leg IV	12.49	12.68
No. segments tibia I	21	21
No. segments tarsus I	35	34
No. segments basitibia IV	3	3

Distribution. China (Fujian)

Etymology. The species is named after China. This is the first Amblypygi species found in the mainland of China. The other two species described from China are restricted to islands.

Natural History: *Sarax sinensis* **sp. nov.** is found in Fuzhou city of Fujian, China. They inhabit abandoned houses and hide under rocks and crevices on walls. The range of their habitats remains to be studied. This species is oviparous, and the female carries the egg sac that contains 12–13 eggs. The egg sac has a transparent membrane that encircles spherical white eggs. During postembryonic growth, pedipalps develop allometrically.

Phylogenetic position. Higher-level phylogenetic relationships were equivalent between our study and the trees presented in both Miranda *et al*. (2021) and Zhu *et al*. (2021; Figure 3). The monophyly of major groups including the Charinidae (bootstrap=93%; posterior probability [pp hereafter]=0.94), *Sarax* (bootstrap=99%; pp=0.98) and *Charinus* (bootstrap=99%; pp=1) were highly supported as found in Miranda *et al*. (2021). Phylogenetic relationships between congeneric species in *Weygoldtia* and *Charinus* were also similar between this study and the phylogenetic trees presented in Zhu *et al*. (2021), and Miranda *et al*. (2021). Specifically, the reciprocal monophyly of the samples analyzed in this study and assigned to *Sarax sinensis* **sp. nov.** was recovered and highly supported (bootstrap=100%; pp=1). Furthermore, *Sarax sinensis* **sp. nov.** is consistently found nested within *Sarax*. We recovered *Sarax ioanniticus* as the sister species to *Sarax sinensis* **sp. nov.** in both Bayesian and maximum likelihood inference frameworks (bootstrap=83%; pp=0.97).
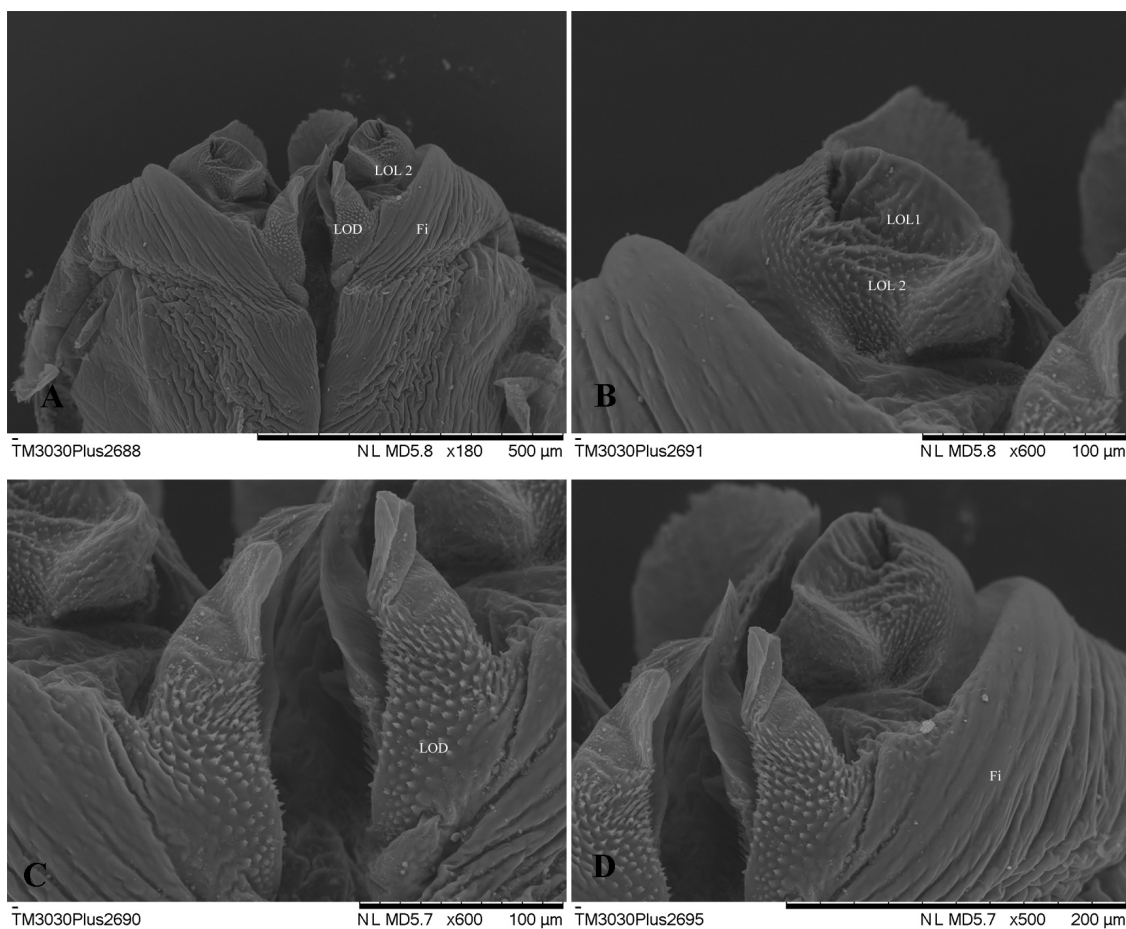
FIGURE 8. *Sarax sinensis* **sp. nov.** Holotype, ECNU-IV-0004, male. A: Trichobothria on distitibia IV; B: Pedipalp tarsus and claw, dorsal view; C: Pedipalp tarsus and claw, ventral view; D: Leg I tibia and tarsus of an adult male, dorsal.

Discussion

This paper presents the description of a new species of *Sarax* from southeastern China and its phylogenetic position in the Charinidae. Based on morphology, *Sarax sinensis* **sp. nov.** is distinct from its congeners by the numbers of tarsal segments, cheliceral teeth, and dorsal and ventral spines on the pedipalp femur. Using morphological and molecular approaches, a recent study inferred two major lineages of *Sarax* (the West Asia Clade and East Asia Clade) which are consistent with their distributions (Miranda *et al.* 2021a). Species in the West Asia clade are found in the Middle East, Northeastern Africa, and South Asia. The East Asia clade is restricted in Southern Asia and on Malay Archipelagos (Miranda *et al.* 2021a,b). Morphologically, species in the West Asia clade exhibit a higher maximum number of cheliceral teeth and fewer segments in leg I tarsus compared to species in the East Asia clade (Miranda *et al.* 2021b). Based on morphology, *Sarax sinensis* **sp. nov.** is more similar to the West Asia clade, as it has fewer segments on leg I tarsus, and more than six teeth on the cheliceral claw (Miranda *et al.* 2021a,b). Consistent with

the morphological differences, our molecular phylogenetic analysis placed *Sarax sinensis* **sp. nov.** in the West Asia clade, and sister to *Sarax ioanniticus*. Overall, molecular and morphological analyses support this whip spider population as a new species of *Sarax* and closely related to species in the West Asia Clade.

Previous studies show the diversity of Amblypygi is underestimated, especially in Asia and Southeast Asia (Rahmadi *et al.* 2010; Seiter *et al.* 2015; Miranda & Reboleria 2019; Zhu *et al.*, 2021). For instance, only two Amblypygi species have been described in China: *Charon ambreae* Reveillion & Pierre-Olivier, 2018 and *Weygoldtia hainanensis* Zhu *et al.*, 2021 (Reveillion & Pierre-Olivier 2018; Zhu *et al.* 2021). To date, no *Sarax* species have been recorded in China. However, large geographical ranges and complex topographies in China are likely to provide suitable habitats for Amblypygi including *Sarax* (Miranda *et al.* 2021b; Badgley 2017). To our knowledge, *Sarax sinensis* **sp. nov.** is only found in Fujian, China. This is the first Amblypygi species found and described from mainland China, rather than on islands (Reveillion & Pierre-Olivier 2018; Zhu *et al.* 2021). The discovery of *Sarax sinensis* **sp. nov.** expands the distribution of *Sarax* in the West Asia Clade from Eastern India to Southeastern China. This leaves a gap in their current distribution across southern China: from western Yunnan to eastern Guangdong. Future surveys in these areas will be needed to discover potential new species and provide a more comprehensive understanding of the biogeography and evolutionary history of whip spiders in Asia.

FIGURE 9. Male gonopod of *Sarax sinensis* **sp. nov.** from Fuzhou, China. Holotype, ECNU-IV-0004, male. A: Dorsal view; B: Detail of ornamentation of LoL1 and LOL2; C: Detail of ornamentation of LoD; D: Detail of ornamentation of Fi (Details in Terminologies).

Acknowledgment

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