A molecular phylogeny of the Chinese *Sinopoda* spiders (Sparassidae, Heteropodinae): Implications for taxonomy (#57907)

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A molecular phylogeny of the Chinese *Sinopoda* spiders (Sparassidae, Heteropodinae): Implications for taxonomy

He Zhang Equal first author, [1], **Yang Zhong** Equal first author, [2], **Yang Zhu** [1], **Ingi Agnarsson** Corresp., [1, 3], **Jie Liu** Corresp. [1, 2]

[1] The State Key Laboratory of Biocatalysis and Enzyme Engineering of China, School of Life Sciences, Hubei University, Wuhan, Hubei, China

[2] School of Nuclear Technology and Chemistry & Biology, Hubei University of Science and Technology, Xianning, Hubei, China

[3] Department of Biology, University of Vermont, Burlington, Vermont, United States of America

Corresponding Authors: Ingi Agnarsson, Jie Liu
Email address: iagnarsson@gmail.com, sparassidae@aliyun.com

Sinopoda spiders are a diverse group which is dispersal limited and remarkably sympatric among related species, which often results in misidentification and incorrect matching of sexes. In order to understand the evolutionary relationships and revise the taxonomy problems in this genus, we offer the first molecular phylogeny of *Sinopoda*. Our results strongly support the monophyly of *Sinopoda* and its sister relationship with *Spariolenus* and reject the monophyly of the *S. okinawana* species group. We establish three new species groups based on both molecular and morphological data. Our phylogeny also illuminates some taxonomic issues and clarifies species compositions: 1. Supporting the newly revised matching of sexes in *S. longiducta* and *S. yaanensis* by *Zhong et al*., *2019*. 2. The original description of *S. campanacea* included mismatched sexes. *S. changde* is proposed as a junior synonymy of *S. campanacea*, while the original female '*S. campanacea*' is here described as a new species: *S. papilionaceous* Agnarsson & Liu **sp. nov.**. 3. The original description of *S. serpentembolus* contained mismatched sexes. The female is considered as *S. campanacea*, while we here report the correctly matched females of *S. serpentembolus*. 4. We describe one additional new species: *S. wuyiensis* Agnarsson & Liu **sp. nov.**. Our first molecular phylogeny of Sinopoda provides a tool for comparative analyses and a solid base for the future biodiversity and taxonomic work on the genus.

A molecular phylogeny of the Chinese *Sinopoda* spiders (Sparassidae, Heteropodinae): Implications for taxonomy

He Zhang[1,*], Yang Zhong[2,*], Yang Zhu[1], Ingi Agnarsson[1, 3], Jie Liu[1, 2]

[1]The State Key Laboratory of Biocatalysis and Enzyme Engineering of China, College of Life Sciences, Hubei University, Wuhan, Hubei, China

[2]School of Nuclear Technology and Chemistry & Biology, Hubei University of Science and Technology, Xianning, Hubei, China

[3]Department of Biology, University of Vermont, Burlington, VT, USA.

[*]These authors contributed equally to this work.

Corresponding Author:
Jie Liu
No. 368 Youyi Road, Wuchang District, Wuhan, Hubei, 430062, China
Email address: sparassidae@aliyun.com

Ingi Agnarsson
316 Marsh Life Science Building, Burlington, VT, 05405-0086, USA
Email address: iagnarsson@gmail.com

Abstract

Sinopoda spiders are a diverse group with 132 species endemic to East Asia, South Asia and Southeast Asia, which are dispersal limited and remarkable in showing extensive sympatry among related species. Along with morphological similarity among species, such sympatry often results in taxonomic problems–in particular misidentification and incorrect matching of sexes. In order to understand the evolutionary relationships among these species, and revise the taxonomy problems in this genus, we offer the first molecular phylogeny of *Sinopoda* and its relatives within the subfamily Heteropodinae. Our results strongly support the monophyly of *Sinopoda* and its sister relationship with *Spariolenus Simon, 1880* and reject the monophyly of the *S. okinawana* species group. We also establish three new species groups based on both molecular and morphological data, with providing detailed descriptions and photos. Our phylogeny also illuminates some taxonomic issues and clarifies species compositions: 1. Supporting the newly revised matching of sexes in *S. longiducta* and *S. yaanensis* by *Zhong et al., 2019* who both are supported as monophyletic respectively. 2. The original description of *S. campanacea* included mismatched sexes. The holotype male *S. campanacea* instead matches specimens of *S. changde*. We therefore propose *S. changde* as a junior synonymy of *S. campanacea*, while the female '*S. campanacea*' from the original description is here described as a new species: *S. papilionaceous Agnarsson & Liu* **sp. nov.**. 3. The original description of *S. serpentembolus* contained mismatched sexes. The female is considered as belonging to *S. campanacea*, while we here report for the first time the correctly matched females of *S. serpentembolus*. 4. We describe one additional new species: *S. wuyiensis Agnarsson & Liu* **sp. nov.**. Our first molecular phylogeny of *Sinopoda* provides a tool for comparative analyses and a solid base for the future biodiversity and taxonomic work on the genus.

Introduction

Taxonomy can be challenging in groups that have undergone relatively little morphological change through the speciation processes. This is especially true when there is extensive sympatry among related species, a rather rare phenomenon (*Agnarsson et al., 2016*). While the majority of spider taxonomy is still purely based on morphological data, integrative approaches are critical to address taxonomy in such challenging groups (Bond et al., submitted). A good example of the

kind of taxonomic chunundrum readily solved by molecular phylogenetics is the correct matching of sexes in similar sympatric species.

With 132 species, *Sinopoda Jäger*, *1999*, is the fourth largest genus of the family Sparassidae. China is the hotspot of diversity and endemicity of this genus, but it is also widely distributed in East Asia (81 species in China, Japan, and Korea), Southeast Asia (50 species in Brunei, Indonesia, Laos, Malaysia, Myanmar, Thailand and Vietnam) and South Asia (1 species in India). *Sinopoda* spiders prefer humid habitats in mountainous forests, and are common in leaf litter, rock crevices, caves, and on tree bark (*Grall & Jäger, 2020; Jäger, 1999, 2012; Liu, Li & Jäger, 2008; Zhang, Zhang & Zhang, 2015*). Some cave dwelling *Sinopoda* spiders (such as *S. guap Jäger*, *2012*, *S. steineri Jäger*, *2012* and others) exhibit obviously troglomorphic features including reduced eyes and pale body. Among them, *S. caeca Grall & Jäger*, *2020* and *S. scurion Jäger*, *2012* are the only known blind (eyeless) huntsman spiders in the world. With preference for cryptic habitats *Sinopoda* spiders do not appear to engage in frequent long distance dispersal and have not been directly recorded dispersing through air on silk threads-or ballooning (*Bell et al., 2005*).

Sinopoda spiders, as nocturnal hunters, are often known only by single sex due to the difficulty to collect mature pairs in the field. To date, over half of the known species (66) were described based on a single sex (*World Spider Catalog 2021*), and the matching sex remains unknown. In addition, sympatry among species is extensive, and multiple species can be collected at a single site such as Hengduan Mountains and Mountains surrounding the Sichuan Basin (Fig. 1). Therefore, mismatches or wrong identifications are common in this genus. For example, *Zhong et al. (2019)* considered *S. longiducta* as mismatched and transferred the female to *S. yaanensis* based on collection data and morphological characters. However, neither match has ever been tested phylogenetically.

To date, no molecular phylogeny has been published for this genus, or any group within it. A single study (*Moradmand*, *Schonhofer & Jäger*, *2014*) included one *Sinopoda* species grouping it with the genera *Heteropoda* Latreill, 1804 and *Spariolenus* Simon, 1880. The monophyly of this genus and its known species groups (*S. chiangmaiensis*-group and *S. okinawana*-group) (*Grall & Jäger, 2020; Jäger & Ono, 2002*) remains to be tested. *Sinopoda* (the prefix "*sino*" means "belonging to China"), unsurprisingly has its center of diversity in China where more than 50% of *Sinopoda* species occur. In the past ten years, a series of surveys

on Chinese *Sinopoda* spiders were conducted by the team from Hubei University and yielded numerous specimens of known and new species. This is our fourth paper on Chinese *Sinopoda* spiders (*Zhong, Cao & Liu 2017; Zhong et al., 2018, 2019*) and provides the first molecular phylogenetic estimate of *Sinopoda* spiders, with the following aims: 1. To test the monophyly of *Sinopoda* and *S. okinawana*-group respectively. 2. To investigate phylogenetic relationships among species and establish new species groups by combining molecular and morphological evidences. 3. To revise matching of sexes in putatively mismatched species and report on potentially new species based on molecular and morphological evidence.

Materials & Methods

Taxon sampling

Spiders were sampled from China between 2008 and 2018 and deposited in the Centre for Behavioural Ecology and Evolution (CBEE), College of Life Sciences, Hubei University. Most of these individuals were collected by the members of our laboratory and others were provided by the colleagues from Southwest University. A total of 856 individuals from 12 Provinces (Fujian, Gansu, Guizhou, Hainan, Henan, Hubei, Hunan, Jiangxi, Liaoning, Shanxi, Sichuan and Yunnan), 1 Municipality (Chongqing) and 1 Autonomous Regions (Xizang Autonomous Region) were collected from the field. Every specimen was given a unique identification number ('S' number). Species were initially sorted by morphological characterizes and stored in 70% ethanol for morphological work and in 100% ethanol for molecular analyses. In total, we included 70 specimens of the genus *Sinopoda* for molecular analyses including three individuals from Genbank. Individual data (including species name, sample locations and GenBank Accession Numbers) are provided in Supplement 1.

In the present paper, 10 species from 13 individuals were used as outgroups (including *Barylestis occidentalis* (*Simon*, *1887*), *H. davidbowie Jäger*, *2008*, *H. jugulans* (*L. Koch*, *1876*), *H. languida Simon*, *1887*, *H. renibulbis Davies*, *1994*, *H. venatoria* (*Linnaeus*, *1767*), *Pandercetes spSD662*, *Pseudopoda confusa Jäger et al.*, *2006*, *P. prompta* (*O. Pickard-Cambridge*, *1885*), *Spariolenus iranomaximus Moradmand* & *Jäger*, *2011*). This choice was guided by the recent phylogenetic result (*Moradmand, Schonhofer & Jäger, 2014*) and these five genera all belong to the subfamily Heteropodinae. We retrieved molecular data on 16 species from Genbank (Supplement 1).

117

118 **Molecular protocols**

119 One or two legs of each individual (depending on the size of specimens) were used to extract
120 total genomic DNA. DNA extraction was achieved with the Universal Genomic DNA Kit
121 (CWBIO, Beijing, China). We use a target gene approach including both mitochondrial and
122 nuclear genes. Six loci were targeted with different degrees of variability. Two mitochondrial
123 genes (two regions including 16S ribosomal RNA gene (16S) and cytochrome c oxidase subunit
124 1 (COI)) and four nuclear genes (protein-coding histone H3 (H3), 18S ribosomal RNA gene
125 (18S), 28S ribosomal RNA gene (28S) and Internal Transcribed Spacer 2 (ITS2)) are used in this
126 research. Primers (*Folmer et al., 1994; Simon et al., 1994; White et al., 1990*) and PCR
127 conditions are shown in Table 1. Multiple primers were employed in the amplification of a large
128 region of COI (approximately 1.2 kb). These primers include the pairs LCOI1490 and
129 HCOI2198, and Jerry and C1-N-2776. Fragments were sequenced by the companies of Tsingke
130 Biological Technology (Wuhan, China) and Sunny Biotechnology Company Limited (Shanghai,
131 China) in both directions. Sequences were assembled and edited using the Chromaseq module in
132 Mesquite (*Maddison & Maddison, 2011a; Maddison & Maddison, 2011b*) employing Phred and
133 Phrap (*Green, 1999; Green & Ewing, 2002*). After assembly, to all sequences were blasted
134 against Genbank (National Center for Biotechnology Information (NCBI)) to verify they all
135 belonged to the family Sparassidae.
136

137 **Phylogenetic analyses**

138 All sequences were aligned with MAFFT (*Katoh, 2013*) on XSEDE in parallel on the
139 Cyberinfrastructure for Phylogenetic Research Project (CIPRES Science Gateway) at the UC
140 San Diego Supercomputing Center (*Miller, Pfeiffer & Schwartz, 2010*). Other large analyses
141 were performed also using this platform.
142 Considering the lack of gaps, we used the L-INS-i method to align the protein-coding genes
143 H3 and the COI. We verified absence of stop codons by translating sequences to amino acids. In
144 virtue of the highly variable structure of ribosomal RNA genes, the ambiguously aligned regions
145 were excluded by using the E-INS-i method to align the following four genes: 16S, 18S, 28S,
146 ITS2 (*Wheeler et al., 2016*). We concatenated these six aligned genes in Mesquite.

Two analytical methods (Maximun Likehood and Bayesian) were used to estimate the phylogenetic relationships. In all analyses, we treated the gaps and ambiguous as missing data. Trees for each target genes were also reconstructed. Bayesian inference analyses were performed via the parallel MrBayes 3.2.6 (*Ronquist et al., 2012*) on XSEDE. Due to the highly substitution rates of the third position, protein-coding genes (COI and H3) were implemented three different partition schemes, namely as COI-1st, COI-2nd, COI-3rd, H3-1st, H3-2nd and H3-3rd. For sensitivity analyses of the multilocus dataset, six genes were divided into ten data partitions, the jModelTest2 on XSEDE (2.1.6) (*Darriba et al., 2012*) were used to choose the most suitable and best-fit models for mtDNA and nuDNA, according to the Akaike information criterion (AIC) (*Posada & Buckley, 2004*). The model parameters were estimated during the analyses and the choice by the jModelTest2 on XSEDE (2.1.6). For 16S, 28S, COI-2nd and ITS2, we used the model of GTR + I + G. The best model for 18S is GTR. GTR + G for COI-3rd and H3-1st. HKY + I + G model were used for the partitions of COI-1st. HKY + G for H3-3rd. For H3-2nd, we used the model of HKY. For every analysis, $5*10^7$ generations were run for two simultaneous independent analyses with four Markov Chains (one cold and three heated) and every 1000th states were saved for the current tree file. Based on the TRACER v1.7.1 (*Rambaut & Drummond, 2007*), all the results for the posterior distributions of the parameters had an Effective Sample Size (ESS) $\geq$ 200. The first 25% trees (1.25×10^7 generations) of every run were discarded as burn in. Maximum likelihood (ML) analyses were performed using GARLI 2.01 on XSEDE (*Zwickl, 2006*). Dataset was partitioned with the same as the Bayesian analysis.

Taxonomy

Specimens were examined with an Olympus SZX16 stereomicroscope; details were further investigated with an Olympus BX51 compound microscope. Epigyna were cleared in proteinase K at 56 °C to dissolve non-chitinous tissues. Photos were taken with Leica M205C stereomicroscope and Olympus BX51 equipped with a Micropublisher 3.3 RTV camera (QImaging, Surrey, BC, Canada). The digital images depicting the habitus and genital morphology were a composite of multiple images taken at different focal planes along the Z axis and assembled using the software package Helicon Focus 3.10.

Leg measurements are shown as: total length (femur, patella, tibia, metatarsus, tarsus). Numbers of spines are listed for each segment in the following order: prolateral, dorsal,

retrolateral, ventral (in femora and patellae ventral spines are absent and fourth digit is omitted in the spination formula). All measurements are in millimeters.

Abbreviations used in the text: ALE, anterior lateral eyes; AME, anterior median eyes; AB, anterior bands; AW, anterior width of prosoma; C, conductor; CH, clypeus height; dRTA, dorsal retrolateral tibial apophysis; E, embolus; EA, embolic apophysis; FD, fertilization duct; GA, glandular appendage; LL, lateral lobes; LS, lobal septum; MS, membranous sac; PLE, posterior lateral eyes; PME, posterior median eyes; PL, prosoma length; PP, posterior part of spermathecae; PW, prosoma width; ST, subtegulum; SP, spermophor; T, tegulum; vRTA, ventral retrolateral tibial apophysis; I, II, III, IV, legs I to IV. Collections: CBEE, Centre for Behavioural Ecology and Evolution, College of Life Sciences, Hubei University, Wuhan, China.

Nomenclatural acts

According to the International Commission on Zoological Nomenclature (ICZN), the electronic version of this article in portable document format (PDF) will represent a published work a. The new species names contained in the electronic version are effectively published under that Code from the electronic edition alone. This article and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub: DE32C06B-FB70-497D-A690-74DED52939DB.

Results & Discussion

Phylogenetic inference and classification

Our full DNA matrix contains 83 individuals, 70 of which belongs to 38 *Sinopoda* species including about one-third (29 %) of the known species and one new species. For outgroups we included 13 individuals belonging to 10 species in 5 genera of the subfamily Heteropodinae. The aligned sequences amounted to 460bp for 16S (65 individuals), 818bp for 18S (42 individuals), 698bp for 28S (79 individuals), 1155bp for COI (79 individuals), 330bp for H3 (74 individuals), 386bp for ITS2 (66 individuals). The phylogenetic trees from the two phylogenetic methods (Bayesian inference and Maximum Likelihood) were highly consistent with relatively high

posterior probabilities (PP) and bootstrap values (BS). Hence, we showed the nodal supports with these two analyses together (Fig. 2) on the BI topology. In general, Bayesian posterior probabilities were slightly higher than ML bootstrap supports. The monophyly of *Sinopoda* was robustly supported (PP 1.00, BS 100%). *Spariolenus* was supported as the sister group of *Sinopoda* (PP 0.93, BS 100%), which is not consistent with former study based on the only previous molecular analyses of the subfamily (*Moradmand, Schonhofer & Jäger, 2014*). The phylogeny suggested the polyphyly of the *S. okinawana*-group, as *S. nuda* is far removed from the remaining group members. We established three new species groups, according to the phylogeny, all supported by morphological and molecular characters: *S. anguina*-group (Fig. 3, PP 1.00, BS 92%), *S. globosa*-group (Fig. 4, PP 0.97, BS 83%) and *S. tumefacta*-group (Fig. 5, PP 1.00, BS 95%). These are diagnosed, described and illustrated in detail in the following taxonomy part. Male and female individuals from the Tianping Mountain belonging to *S. campanacea* and *S. changde* were analysed. Male *S. campanacea* form a tight monophyletic cluster with the female *S. changde* (PP 0.98, BS 97%). As the *S. campanacea* holotype is male, we therefore propose *S. changde* as a **new synonym** of *S. campanacea*. The originally described female of *S. campanacea* is considered as a new species: *S. papilionaceous Agnarsson & Liu* ***sp. nov.*** which is redescribed in current paper according to the original illustration (*Wang*, 1*990*: 7, Figs. 4–5) For *S. serpentembolus*, the male of *S. serpentembolus* from Shanxi Province and female individuals from Henan Province are monophyletic (PP 1.00, BS 100%). However, females differ significantly from the originally matched female of *S. serpentembolus* (*Zhang et al*., *2007*: 251, Figs. 5–6) based on morphological data. Therefore, we revised the *S. serpentembolus* as following: 1. The correct female of *S. serpentembolus* is reported for the first time. 2. The originally mismatched female of *S. serpentembolus* is found to be similar to *S. campanacea* where we tentatively place it. We note, however, that it shows some morphological differences with *S. campanacea* and further molecular data is needed to clarify its placement. Meanwhile, our result support the recent revised matching of sexes in *S. longiducta* and *S. yaanensis* by *Zhong et al. (2019)* as both species are monophyletic (*S. longiducta* PP 1.00, BS 99%; *S. yaanensis* PP 1.00, BS 97%).

Sinopoda anguina group, new group

(Figure 3)

Diagnosis. This group can be recognized by the following combination of characters: 1. Embolic apophysis developed, with a semicircular membrane distally, tip of embolic apophysis significantly longer than embolic tip. 2. Ventral RTA blunt, clavate-shaped in lateral view, dorsal RTA slightly longer than ventral RTA, with sharp end. 3. Epigynal pockets running from posterior-lateral to medio-anterior. 4. Margins of lobal septum straight, almost extending to the posterior margin of epigyne, roughly forming a triangle in the median epigyne. 5. Posterior part of spermathecae reduced, significantly narrower than glandular appendage. 6. Internal ducts running parallel along median line.

Species included. Twelve species are included in this group: *S. anguina Liu*, *Li* & *Jäger*, *2008* (♂♀), *S. bifurca Grall* & *Jäger*, *2020* (♂♀), *S. bispina Grall* & *Jäger*, *2020* (♂), *S. fornicata Liu*, *Li* & *Jäger*, *2008* (♀), *S. improcera Zhong et al.*, *2019* (♂♀), *S. lata Zhong et al.*, *2019* (♀), *S. longicymbialis Grall* & *Jäger*, *2020* (♂♀), *S. mamillata Zhong*, *Cao* & *Liu*, *2017* (♂♀), *S. nanphagu Grall* & *Jäger*, *2020* (♀), *S. phiset Grall* & *Jäger*, *2020* (♀), *S. rotunda Grall* & *Jäger*, *2020* (♀) and *S. tuber Grall* & *Jäger*, *2020* (♀).

Distribution. China (Yunnan) (Fig. 2), Brunei (Belait district), Myanmar (Southern Shan State) and Thailand (Chiang Mai Province).

Sinopoda globosa group, new group

(Figure 4)

Diagnosis. This group can be recognized by the following combination of characters: 1. Subdistal embolus with a triangular projection. 2. Ventral RTA wide, blunt, dorsal RTA thinner, longer. 3. Internal ducts running parallel along median line. 4. Spermathecae with ovate posterior parts.

Species included. Six species are included in this group: *S. globosa Zhang*, *Zhang* & *Zhang*, *2015* (♂♀), *S. longiducta* (♂♀), *S. mi Chen* & *Zhu*, *2009* (♂♀), *S. ovata Zhong et al.*, *2019* (♂♀), *S. triangula Liu*, *Li* & *Jäger*, *2008* (♂♀) and *S. yaanensis* (♂♀).

Distribution. China, Mountains around Sichuan Basin (Chongqing, Guizhou, Sichuan, Shanxi, Yunnan) (Fig. 2).

Sinopoda globosa group, new group

(Figure 5)

Diagnosis. This group can be recognized by the following combination of characters: 1. Embolus filiform, almost straight with a reduced embolic apophysis. 2. Ventral RTA well developed and strong, dorsal RTA longer than ventral RTA. 3. Lateral lobes fused, with almost horizontal margins anteriorly. 4. Posterior parts of spermathecae swollen.

Species included. Six species are included in this group: *S. crassa Liu*, *Li* & *Jäger*, *2008* (♀), *S. dehiscens Zhong et al.*, *2019* (♀), *S. erromena Zhong et al.*, *2019* (♀), *S. tumefacta Zhong et al.*, *2019* (♂♀), *S. yanlingensis Zhong et al.*, *2019* (♂♀) and *S. yaojingensis Liu*, *Li* & *Jäger*, *2008* (♂♀).

Distribution. China (Hunan, Jiangxi, Yunnan) (Fig. 2).

***Sinopoda campanacea* (Wang, 1990)**

Heteropoda campanacea Wang, *1990*: 7, Figs. 1–5 (Description of male and mismatched female).

Sinopoda campanacea Song, *Zhu* & *Chen*, *1999*: 469, Figs. 269O, 270A (Description of male and mismatched female).

Sinopoda campanacea Jäger, *1999*: 21 (Transfer from *Heteropoda*).

Sinopoda campanacea Yin et al., *2012*: 1238, Figs. 663a–e (Description of male and mismatched female).

Sinopoda changde Zhong et al., *2019*: 19, Figs. 13A–E, 14A–F, 15A–D (Description of male and female), **New synonym**.

Material examined. 5 males and 14 females (CBEE) from Hupingshan National Nature Reserve (N30.11°, E110.78°), Changde City, Hunan Province, CHINA, 1395 m, 2017.VI.16 to 2020.VIII.1, Yang Zhong & Yang Zhu leg. 5 males and 5 females (CBEE) from Tianpingshan Scenic Area (N29.79°, E110.09°), Zhangjiajie City, Hunan Province, China, 1503 m, 2017.VI.20, Yang Zhong & Yang Zhu leg. 4 males and 2 females (CBEE) from Taibai Mountain (N34.06°, E107.89°), Tangyu Town, Mei County, Baoji City, Shanxi Province, China, 1340 m, 2017.V.10, Yang Zhong & Zichang Li leg.

Diagnosis. *S. campanacea* is similar to *S. serpentembolus* in having strongly curved and sheet-shaped embolic apophysis, developed RTA with short and broad vRTA, longer dRTA in male, the epigyne with anterio-lateral margins of lateral lobes almost parallel with posterior margin of epigyne in female, but can be distinguished from the latter by the following characters: 1. The

sperm duct of *S. campanacea* is almost straight, but significantly curved in *S. serpentembolus*. 2. The tegular apophysis is absent in *S. campanacea*, but present and located posteriorly in *S. serpentembolus*. 3. The glandular appendages are widely separated from posterior part of internal duct system in *S. campanacea*, but distinctly close with each other in *S. serpentembolus*.

Description. For details see *S. changde Zhong et al. (2019)*.

Remarks. *S. changde* is proposed as the new synonym of *S. campanacea* based on the following reasons: 1. *S. changde* was also collected in Tianpingshan Scenic Area where the holotype of *S. campanacea* located (Fig. 2). 2. The same RTA, the curved and sheet-shaped embolic apophysis, the short and slender embolic tip indicate that the male of *S. changde* belongs to *S. campanacea*. 3. *Zhong et al*. *(2019)* indicated the main difference between *S. changde* and *S. campanacea* was the palpal tegulum covering proximal part of embolus in *S. changde sp. nov.* but not in *S. campanacea*, we found it was due to the photos taken at different angle. 4. The main difference between *S. changde* and *S. campanacea* is in the female genitalia, while the mached *S. changde* individuals including two females and one male from different localities are strongly monophyletic by the molecular phylogeny (Fig. 2). Therefore, the original matched female of *S. campanacea* may be another species which is proposed as a new species in the following part.

***Sinopoda papilionacea* Agnarsson & Liu sp. nov.**

urn:lsid:zoobank.org:act:FE072552-5B7A-4E32-967B-45A265B38BCA

Heteropoda campanacea Wang, *1990*: 7, Figs. 4–5 (Description of mismatched female).

Sinopoda campanacea Song, *Zhu* & *Chen*, *1999*: 469, Fig. 270A (Description of mismatched female).

Sinopoda campanacea Jäger, *1999*: 21 (Transfer from *Heteropoda*).

Sinopoda campanacea Yin et al., *2012*: 1238, Figs. 663d–e (Description of mismatched female).

Holotype (not examined). CHINA: Hunan Province: female, Tianping Mountain, Sangzhi County, Zhangjiajie City, 1984.VIII.21, Jiafu Wang & Yongjing Zhang leg. We didn't collect this species in the type locality and the type specimen described by *Wang (1990)* has been lost, therefore, we designate the female specimen described by *Wang (1990)* as holotype, another female as paratype in current paper.

Paratype. 1 female, same data as the holotype, not examined.

Etymology. The specific name is derived from the Latin adjective *papilionaceus*, *-a, -um*, meaning "butterfly-shaped", referring to the papilionaceous shape of internal duct systems.

Diagnosis. This new species can be distinguished from other *Sinopoda* species by the papilionaceous shape of internal duct systems based on the original illustrations from *Wang* (1990: 7, Fig. 5).

Description (based on the illustrations and description from *Wang*, *1990*). Mediaum sized Heteropodinae. PL 5.8, PW 4.8; OL 6.1, OW 4.0. Cheliceral furrow with 3 anterior and 4 posterior teeth. Dorsal prosoma deep yellowish-brown, with a yellow spot in the middle part. Fovea and redial furrows distinctly dark brown. Dosal opisthosoma brownish black, and a yellow triangular macula in posterior part. Ventral opisthosoma yellow.

Female genitalia: Epigynal field wider than long, without anterior bands. Lobal septum narrow. Anterior and posterior margins of lateral lobes almost parallel. Internal ducts system anteriorly touching each other at the median line but posteriorly and widely separated. Posterior part of internal duct system slightly wider than anterior part. Fertilization ducts arising posterio-laterally (*Wang*, *1990*: 7, Figs. 4–5).

Distribution. Hunan (Zhangjiajie) Province, China.

Remarks. We didn't examine the holotype specimen because the type specimens may be lost. It is easily identified as a new species according to its special internal duct system based on the original illustrations.

Sinopoda serpentembolus Zhang, Zhu, Jäger & Song, 2007

(Figures 8 and 9)

Sinopoda serpentembolus Zhang et al., *2007*: 251, Figs. 1–6 (Description of male, female may be *S. campanacea*).

Sinopoda serpentembolus Zhu & Zhang, *2011*: 418, Figs. 298A–F (Description of male, female may be *S. campanacea*).

Material examined. 3 males and 5 females (CBEE) from Baotianman National Nature Reserve (N33.50°, E111.93°), Neixiang County, Nanyang City, Henan Province, China, 1300 m, 2017.VI.16, Yang Zhong & Zichang Li leg. 2 males and 4 females (CBEE) from Laojunshan Scenic Area (N33.74°, E110.63°), Luanshan County, Luoyang City, Henan Province, China, 860 m, 2017.IV.27, Yang Zhong & Zichang Li leg. 8 males (CBEE) from Taibai Mountain (N34.06°,

E107.89°), Tangyu Town, Mei County, Baoji City, Shanxi Province, China, 1340 m, 2017.V.10, Yang Zhong & Zichang Li leg.

Diagnosis. See the above diagnosis under *S. campanacea*.

Description. Male. See *Zhang et al. (2007)*.

Female (from Baotianman, China). Mediaum sized Heteropodinae. PL 5.4, PW 4.8; AW 3.0; OL 5.7, OW 3.4. Eyes: AME 0.20, ALE 0.35, PME 0.23, PLE 0.36, AME–AME 0.24, AME–ALE 0.10, PME–PME 0.40, PME–PLE 0.54, AME–PME 0.42, ALE–PLE 0.46, CH AME 0.24, CH ALE 0.28. Spination: Palp: 131, 001, 2121, 1014; Fe: I–III 323, IV 331; Pa: I–IV 001; Ti: I–III 2026, IV 2226; Mt: I–II 1014, IV–IV 3036. Measurements of palps and legs: Palps 6.6 (2.1, 0.8, 1.3, –, 2.4); I 15.2 (4.6, 1.6, 3.8, 3.9, 1.3); II 15.7 (4.8, 1.8, 4.1, 3.8, 1.2); III 13.0 (4.1, 1.4, 3.1, 3.2, 1.2); IV 14.3 (4.1, 1.7, 3.7, 3.5, 1.3). Leg formula: II-I-IV-III. Cheliceral furrow with 3 anterior and 4 posterior teeth, and with ca. 22 denticles. Dorsal prosoma deep yellowish-brown, with yellow submarginal transversal band posteriorly, fovea and redial furrows distinctly dark brown. Dosal opisthosoma yellow-brown, covered by brown hairs. Ventral opisthosoma uniformly yellowish-brown with some irregular. Legs yellowish-brown, with dark setae.

Female genitalia: Epigynal field wider than long, without anterior bands and slit sensilla. Lobal septum anteriorly around 4/5 of epigyne width, anterior part wider than midian part. Lateral lobes fused. Anterior part of internal ducts system diverging. Glandular appendages long and distinctly curved. Posterior part of spermathecae, bulging laterally, fertilization ducts arising posterio-laterally (Figs. 8 A–B).

Remark. The originally matched female is very similar to *S. campanacea* in having the epigyne with anterio-lateral margins of lateral lobes almost parallel with posterior margin of epigyne, short glandular appendages, longer posterior parts of internal duct systems and having the same distribution in Taibai Mountain. However, there are also some subtle differences between them as follows: 1. The lobal septum is narrower than that of *S. campanacea*. 2. The paired internal ducts are juxtaposed medially but widely separated in *S. campanacea*. It is difficult to be sure if this is intraspecific variation or represents two species based only on morphological data. We tentatively place this specimen in *S. campanacea* here. This problem may be dealt with when we collect the fresh individuals for the molecular phylogeny in the future.

Distribution. Henan (Nanyang, Luoyang) Province and Shanxi (Baoji) Province, China (Fig. 2).

Sinopoda wuyiensis Agnarsson & Liu sp. nov.

(Figure 7)

urn:lsid:zoobank.org:act:E7566CCC-DE55-49A5-B9AF-0EFC5A906AB5

Holotype. CHINA: Fujian Province: female (CBEE) from Wuyishan National Reserve (N27.58°, E117.48°), Wuyishan City, 1300 m, 2013.VII.17, Xiaowei Cao & Yang Zhong leg.

Paratypes. 6 females (CBEE), same data as for holotype.

Etymology. 'Wuyi' refers to the type locality of this species, Wuyishan National Reserve.

Diagnosis. This new species can be separated from other *Sinopoda* species by the following combined characters: 1. The lateral lobes fused with each other posteriorly, with their anterior and posterior margins almost parallel. 2. Posterior part of internal duct system almost same wide as anterior part.

Description. Male. Unknown.

Female (holotype). Mediaum sized Heteropodinae. PL 5.6, PW 5.0; AW 2.7; OL 6.6, OW 3.8. Eyes: AME 0.22, ALE 0.35, PME 0.24, PLE 0.36, AME–AME 0.27, AME–ALE 0.12, PME–PME 0.37, PME–PLE 0.52, AME–PME 0.43, ALE–PLE 0.46, CH AME 0.24, CH ALE 0.26. Spination: Palp: 131, 101, 2121, 1012; Fe: I–III 323, IV 331; Pa: I–IV 101; Ti: I–II 2126, III–IV 2326; Mt: I–II 1014, IV–IV 3036. Measurements of palps and legs: Palps 7.4 (2.5, 1.1, 1.4, –, 2.4); I 18.0 (5.2, 2.0, 4.6, 4.6, 1.6); II 19.9 (5.7, 2.6, 5.6, 4.6, 1.5); III 16.3 (4.8, 2.2, 4.4, 3.4, 1.5); IV 17.6 (5.0, 2.2, 4.7, 4.2, 1.5). Leg formula: II-I-IV-III. Cheliceral furrow with 3 anterior and 4 posterior teeth, and with ca. 20 denticles. Dorsal prosoma yellowish-brown, medio-laterally with brown semicircular-patten, posterior margins dark, with shallow fovea and redial furrows. Dosal opisthosoma greyish-brown with three pairs of dark patches laterally. Ventral opisthosoma yellowish-brown. Legs yellowish-brown, with dark spots (Figs. 7C–D).

Female genitalia: Epigynal field wider than long, with thin anterior bands. Lobal septum anteriorly around 1/8 of epigyne width. Lateral lobes fused. Internal ducts partly running parallel along the median line. Glandular appendages as wide as posterior part of spermathecae, anterior part of internal duct system narrower than posterior part. Fertilization ducts arising posterio-laterally (Figs. 7A–B).

Distribution. Fujian (Mt. Wuyishan) Province, China (Fig. 2).

Conclusions

We provide the first phylogenetic analysis of *Sinopoda*, focusing on the Chinese species. Our analysis strongly supports the monophyly of *Sinopoda*. According to a previous study (*Moradmand*, *Schonhofer* & *Jäger*, *2014*), *Sinopoda* was hypothesized to group with *Heteropoda* and *Spariolenus*, however, we find support to the sister relationship between *Sinopoda* and *Spariolenus*. Further sampling of Heteropodinae genera will be necessary to further clarify the placement of *Sinopoda*. Our main goal here is to discuss the relationships within *Sinopoda.* We reject the monophyly of the *S. okinawana*-group and establish three new groups: *S. anguina*-group, *S. globosa*-group and *S. tumefacta*-group basing on both molecular and morphological data (Figs. 2–5). *S. okinawana*-group contains 11 described species, seven of which are included here. This group was established mainly based on the male genital characters (reduced embolic apophysis and ventral RTA), because the females are all highly similarity (*Jäger* & *Ono*, *2002*; *Zhong et al.*, *2018*). Our phylogeny indicates that the reduced embolic apophysis is not a synapomorphic character because it is also occurred in *S. tumefacta* and *S. yaanensis.* In addition, the embolic apophysis is totally absent in *S. longshan Yin et al*., *2000* and *S. nuda Liu*, *Li* & *Jäger*, *2008.* The data suggests that both the embolic apophysis and the RTA evolve rapidly in this genus and may thus not be reliable for group diagnostics. Furthermore, generally speaking, the characters of male palp may not be the best evidence to guide classification at the higher level. This conclusion is consistent with the finding in Australian huntsman spiders (*Agnarsson* & *Rayor*, *2013*). Our phylogeny well supports the establishment of *S. anguina*-group which includes 12 species in fact distributed in southern area of the Ailao Shan-Red River Fault zone (Southeast Asia, Hengduan Moutain of Yunnan) though only four species are contained in our investigation. The other two new species groups (*S. globosa*-group and *S. tumefacta*-group) are also well supported, especially the *S. globosa*-group distributed in the mountains surrounding the Sichuan Basin. This group shows clear morphological diagnostic features: subdistal embolus with a triangular projection in male palp, spermathecae with ovate posterior parts in female vulva. Meanwhile, our phylogeny also provides implications for the taxonomy and classification on the species level clarifying recent taxonomic rematching of sexes (*Zhang, Zhang & Zhang, 2015; Zhong et al., 2019*) and supporting further rematching of sexes and recircumscription of some species, and the description of two new species. The main reason for the prevalence of mismatches in *Sinopoda* is similar morphology among species and extensive sympatry in the genus. For example, we collected up to five *Sinopoda* species in a single locality in Wuyi

Mountain of Fujian Province. Our results indicate that care must be taken in the sex matching of *Sinopoda* spiders and ultimately matching based on field data and morphology should be tested using molecular phylogenetic evidence. In other words, *Sinopoda* provides a strong argument for the importance of integrative taxonomy, approaching species delimitation and description using multiple lines of evidence (Bond et al., submitted). Our first molecular phylogeny of *Sinopoda* provides a solid base for the biodiversity and taxonomic work in the genus, and a tool for comparative studies, such as analyses of biogeographical patterns in this genus.

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Figure 1

Habitats of *Sinopoda* spiders (A–C) and a map of localities where the known species of *Sinopoda* distributed in the world (D).

Every dot represents one locality. Red color represents the localities did not include in our analyses. Blue color represents the sample collection place involved in this project.

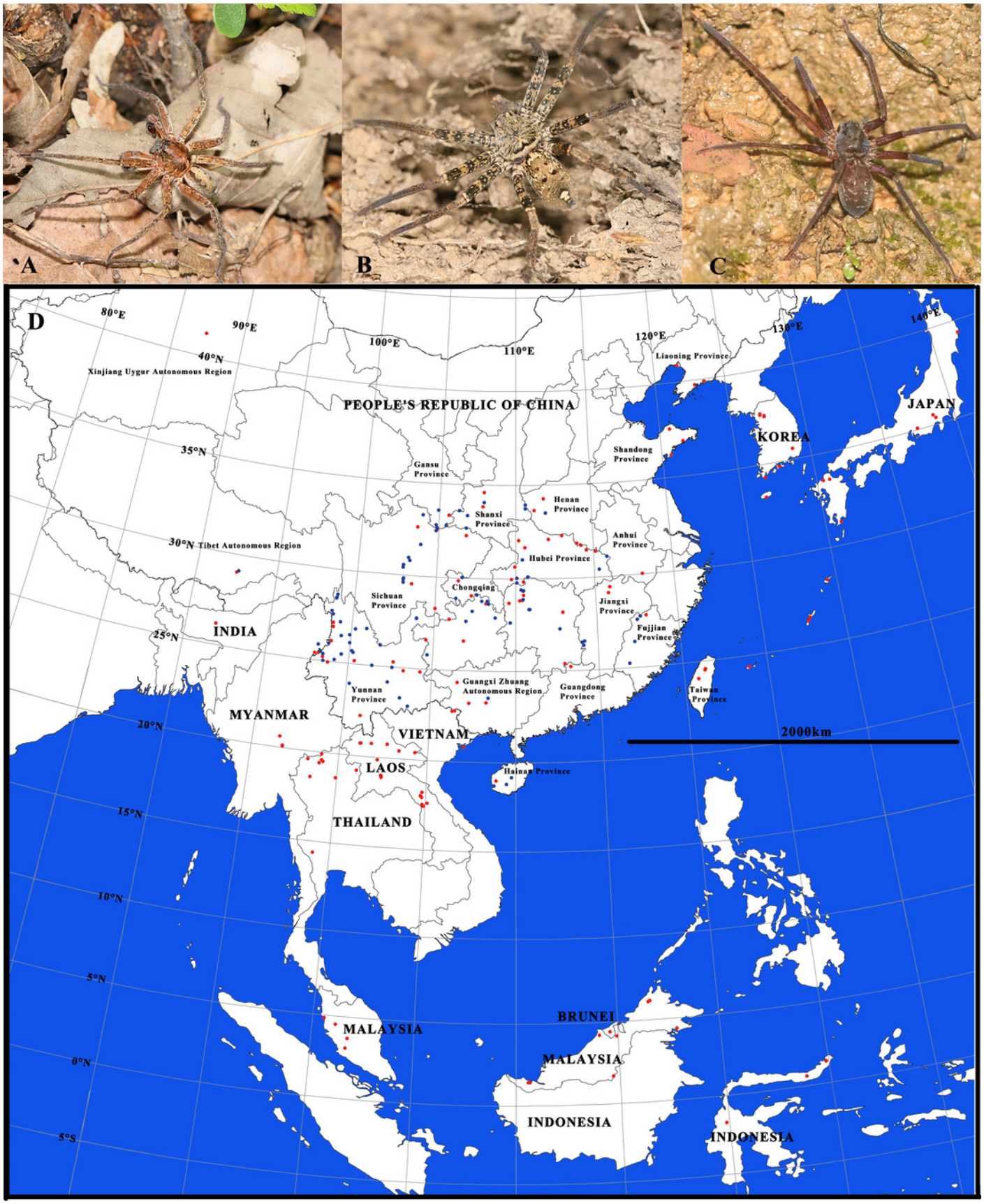
A
B
C
D
80°E
90°E
100°E
110°E
120°E
130°E
140°E
40°N
35°N
30°N
25°N
20°N
15°N
10°N
5°N
0°N
5°S
Xinjiang Uygur Autonomous Region
PEOPLE'S REPUBLIC OF CHINA
Liaoning Province
JAPAN
KOREA
Shandong Province
Gansu Province
Henan Province
Shanxi Province
Anhui Province
Tibet Autonomous Region
Hubei Province
Chongqing
Sichuan Province
Jiangxi Province
Fujjian Province
INDIA
Guangxi Zhuang Autonomous Region
Guangdong Province
Taiwan Province
Yunnan Province
MYANMAR
VIETNAM
2000km
LAOS
Hainan Province
THAILAND
BRUNEI
MALAYSIA
MALAYSIA
INDONESIA
INDONESIA

Figure 2

Summary of phylogenetic analysis of the genus *Sinopoda* and the sample current distribution in Asia.

(A) Combined results of the phylogenetic analysis based on seven gene fragments. Numbers on nodes are MrBayes Bayesian Inference posterior probability (pp); bootstrap value (BP) from Garli analyses is indicated as solid stars for values >95%, open stars >50–95%. Branch lengths are scaled in relation to the number of substitutions per site. (B) Map with sampling localities of the genus *Sinopoda* and its major lineages. Different colors refer to different groups. *S. anguina*-group (orange), *S. globosa*-group (purple), *S. tumefacta*-group (cyan) and *S. okinawana*-group (red). (1. *S. abstrusa* Zhong *et al.*, 2019; 2. *S. aequalis* Zhong et al., 2019; 3. *S. altissima* (Hu & Li, 1987); 4. *S. anguina*; 5. *S. apiculiformis* Zhong et al., 2019; 6. *S. brevis* Zhong et al., 2019; 7. *S. wuyiensis* Agnarsson & Liu sp. nov.; 8. *S. campanacea* ("*changde*"); 9. *S. cochlearia* Zhang, Zhang & Zhang, 2015; 10. *S. columnaris* Zhong et al., 2019; 11. *S. dehiscens*; 12. *S. erromena*; 13. *S. fasciculata* Jäger, Gao & Fei, 2002; 14. *S. globosa*; 15. *S. grandispinosa* Liu, Li & Jäger, 2008; 16. *S. guangyuanensis* Zhong et al., 2018; 17. *S. hamata* (Fox, 1937); 18. *S. horizontalis* Zhong, Cao & Liu, 2017; 19. *S. improcera*; 20. *S. koreana* (Paik, 1968); 21. *S. lata*; 22. *S. liui* Zhong, Cao & Liu, 2017; 23. *S. longiducta*; 24. *S. longshan*; 25. *S. mamillata*; 26. *S. wangi* Song & Zhu, 1999; 27. *S. nuda*; 28. *S. ovata*; 29. *S. pengi* Song & Zhu, 1999; 30. *S. pyramidalis* Zhong et al., 2019; 31. *S. serpentembolus*; 32. *S. stellatops* Jäger & Ono, 2002; 33. *S. tengchongensis* Fu & Zhu, 2008; 34. *S. tham* Jäger, 2012; 35. *S. triangula*; 36. *S. tumefacta*; 37. *S. yaanensis*; 38. *S. yanlingensis*).

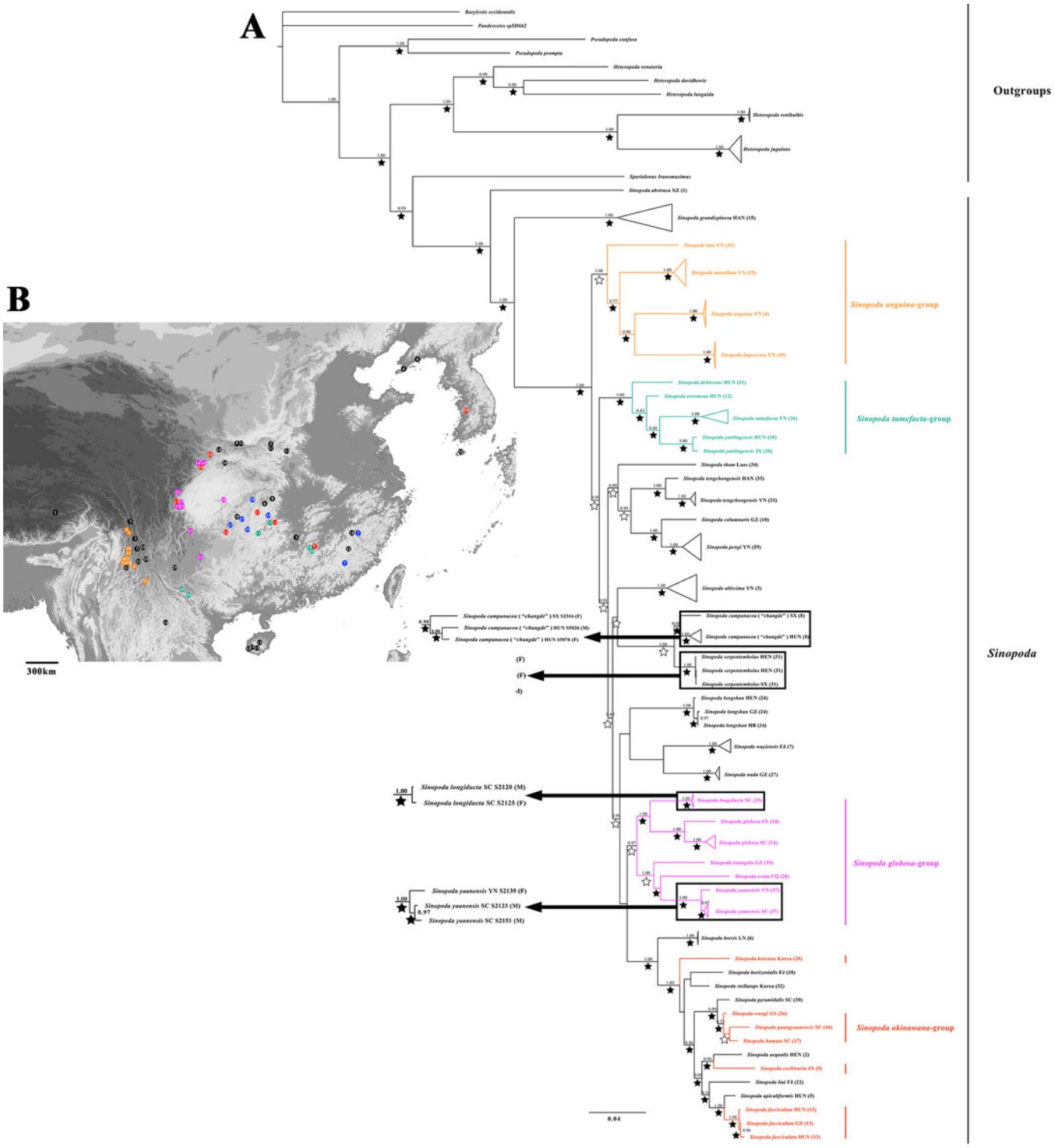
A
Outgroups
Sinopoda anguina-group
Sinopoda tumefacta-group
Sinopoda
Sinopoda globosa-group
Sinopoda okinawana-group
B
300km
0.04

Figure 3

Members of *Sinopoda anguina*-group.

Sinopoda anguina (A, female epigyne, ventral; E, female vulva, dorsal; I, left male palp, ventral; J, left male palp, retrolateral); *Sinopoda improcea* (B, female epigyne, ventral; F, female vulva, dorsal; K, left male palp, ventral; L, left male palp, retrolateral); *Sinopoda lata* (C, female epigyne, ventral; G, female vulva, dorsal); *Sinopoda mamillata* (D, female epigyne, ventral; H, female vulva, dorsal; M, left male palp, ventral; N, left male palp, retrolateral). Scale bars: 0.5 mm.

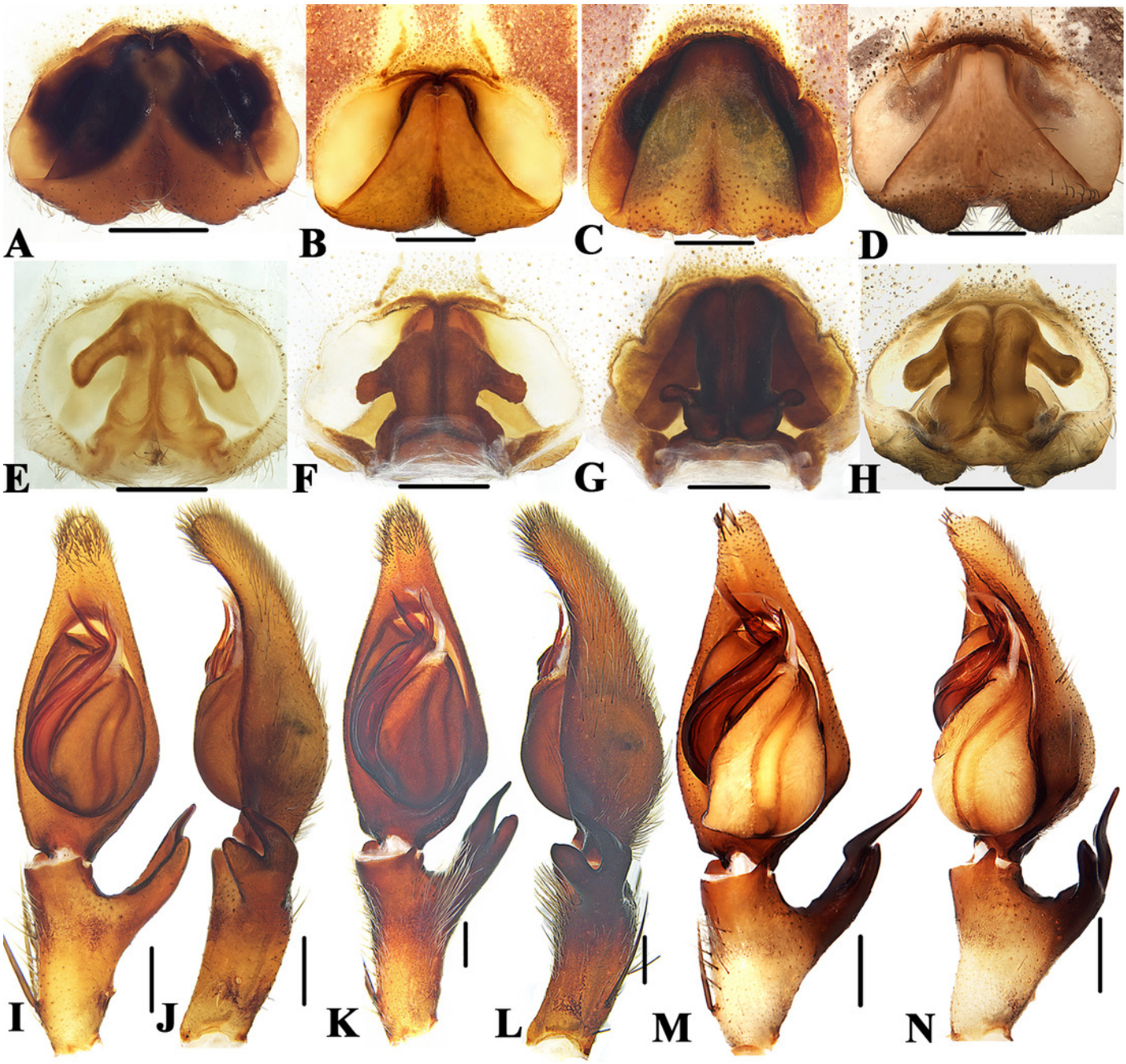
A
B
C
D
E
F
G
H
I
J
K
L
M
N

Figure 4

Members of *Sinopoda globosa*-group.

Sinopoda globosa (A, female epigyne, ventral; F, female vulva, dorsal; K, left male palp, ventral; P, left male palp, retrolateral); *Sinopoda longiducta* (B, female epigyne, ventral; G, female vulva, dorsal; L, left male palp, ventral; Q, left male palp, retrolateral); *Sinopoda ovata* (C, female epigyne, ventral; H, female vulva, dorsal; M, left male palp, ventral; R, left male palp, retrolateral); *Sinopoda triangula* (D, female epigyne, ventral; I, female vulva, dorsal; N, left male palp, ventral; S, left male palp, retrolateral); *Sinopoda yaanensis* (E, female epigyne, ventral; J, female vulva, dorsal; I, left male palp, ventral; J, left male palp, retrolateral). Scale bars: 0.5 mm.


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T

Figure 5

Members of *Sinopoda tumefacta*-group.

Sinopoda dehiscens (A, female epigyne, ventral; E, female vulva, dorsal); *Sinopoda erromera* (B, female epigyne, ventral; F, female vulva, dorsal); *Sinopoda tumefacta* (C, female epigyne, ventral; G, female vulva, dorsal; I, left male palp, ventral; J, left male palp, retrolateral); *Sinopoda yanlingensis* (D, female epigyne, ventral; H, female vulva, dorsal; K, left male palp, ventral; L, left male palp, retrolateral). Scale bars: 0.5 mm.

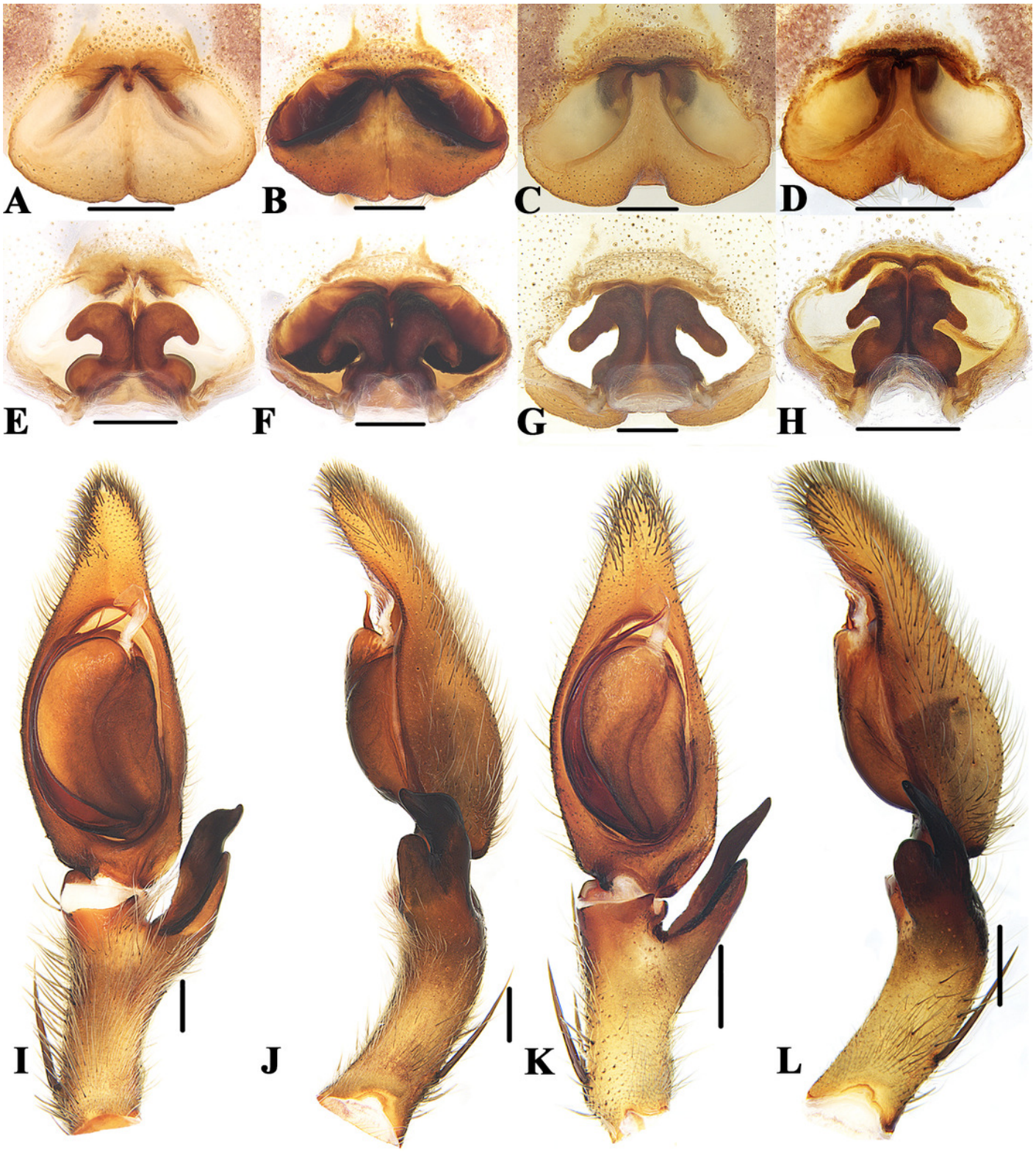
A
B
C
D
E
F
G
H
I
J
K
L

Figure 6

Members of *Sinopoda okinawana*-group (collected in this project)

Sinopoda cochlearia (A, female epigyne, ventral; B, female vulva, dorsal; C, left male palp, ventral; D, left male palp, retrolateral); *Sinopoda fasciculata* (E, female epigyne, ventral; F, female vulva, dorsal; G, left male palp, ventral; H, left male palp, retrolateral); *Sinopoda guangyuanensis* (I, female epigyne, ventral; J, female vulva, dorsal; K, left male palp, ventral; L, left male palp, retrolateral); *Sinopoda hamata* (M, female epigyne, ventral; N, female vulva, dorsal; O, left male palp, ventral; P, left male palp, retrolateral); *Sinopoda wangi* (Q, female epigyne, ventral; R, female vulva, dorsal; S, left male palp, ventral; T, left male palp, retrolateral). Scale bars: 0.5 mm

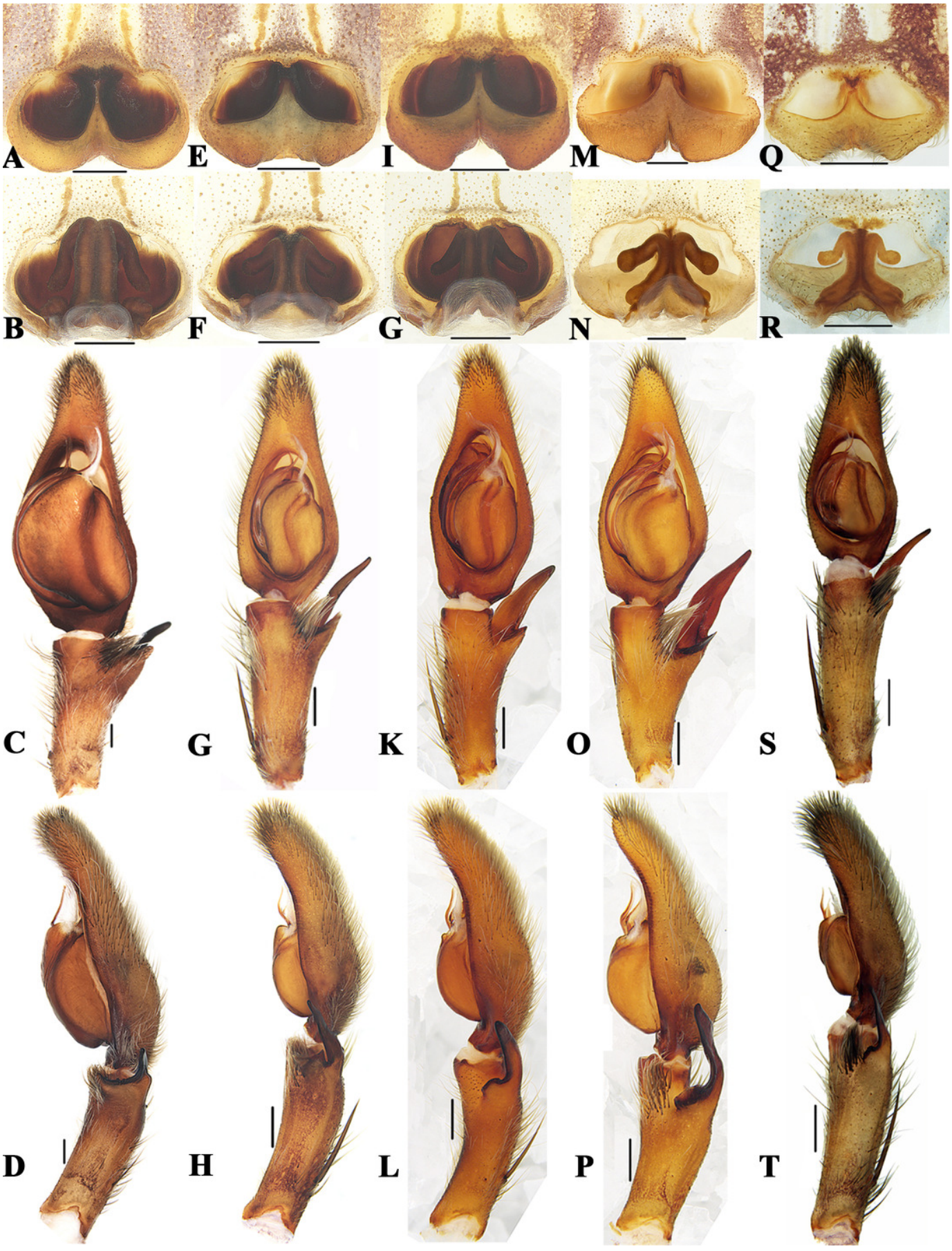
A
E
I
M
Q
B
F
G
N
R
C
G
K
O
S
D
H
L
P
T

Figure 7

Sinopoda wuyiensis Agnarsson & Liu sp. nov..

A, epigyne, ventral; B, vulva, dorsal; C–D, female habitus (C, dorsal; D, ventral). Abbreviations: AB, anterior bands; FD, fertilization duct; GA, glandular appendage; LL, lateral lobes; LS, lobal septum; MS, membranous sac; PP, posterior part of spermathecae; Scale bars: A–B 0.5 mm; C–D 2 mm.

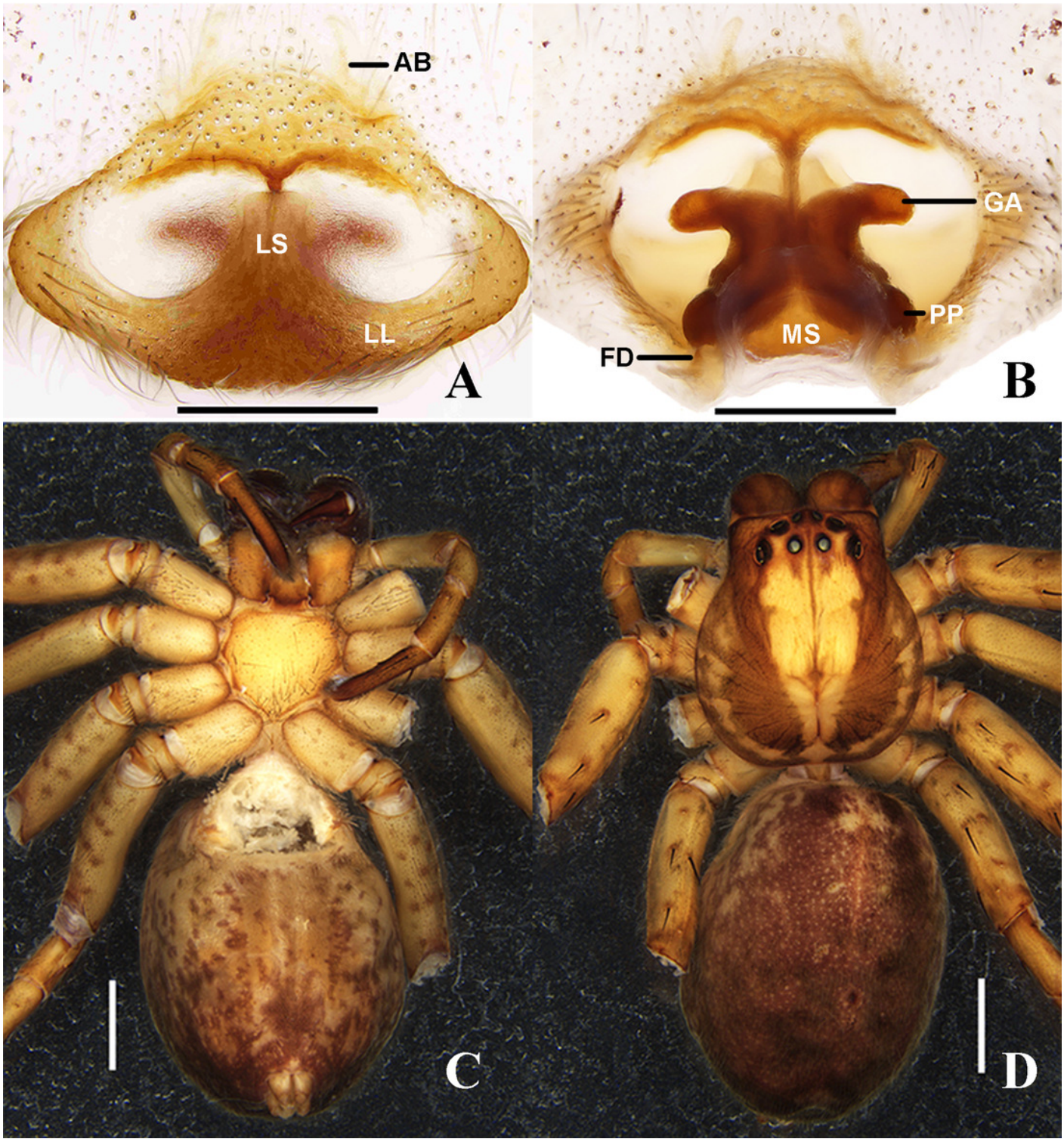
AB
LS
LL
A
GA
PP
MS
FD
B
C
D

Figure 8

Sinopoda serpentembolus (Zhang *et al.*, 2007).

A, epigyne, ventral; B, vulva, dorsal; C–D, female habitus (C, dorsal; D, ventral). Abbreviations: AB, anterior bands; FD, fertilization duct; GA, glandular appendage; LL, lateral lobes; LS, lobal septum; MS, membranous sac; PP, posterior part of spermathecae; Scale bars: A–B 0.5 mm; C–D 2 mm.

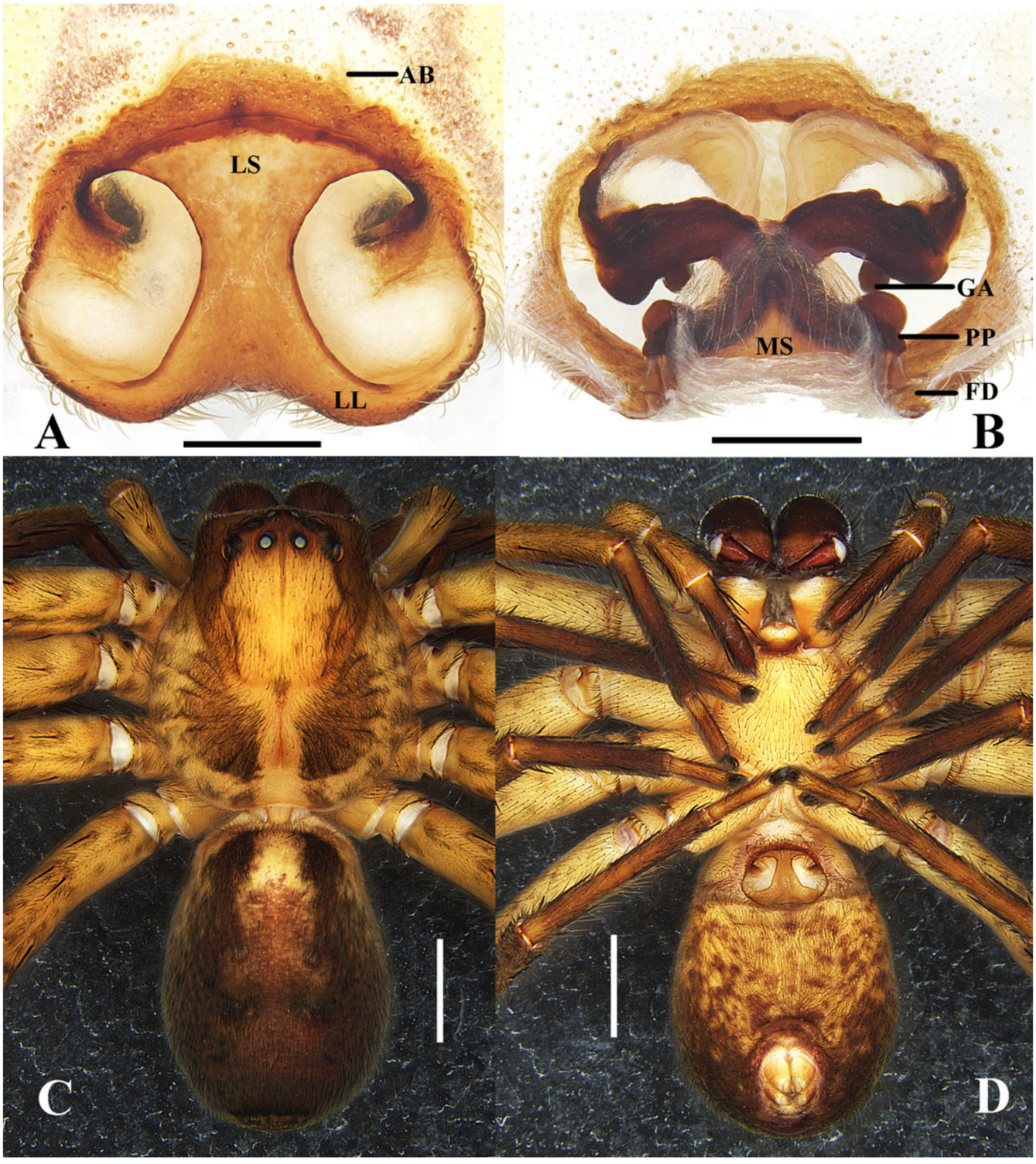
AB
LS
LL
A
GA
PP
MS
FD
B
C
D

Figure 9

Sinopoda serpentembolus (Zhang *et al.*, 2007).

A–C, left male palp (A, prolateral; B, ventral; C, retrolateral); D, left male palpal tibia, retrolateral; E–F, male habitus (E, dorsal; F, ventral). Abbreviations: C, conductor; dRTA, dorsal retrolateral tibial apophysis; E, embolus; EA, embolic apophysis; SP, spermophor; ST, subtegulum; T, tegulum; vRTA, ventral retrolateral tibial apophysis; Scale bars: A–D 0.5 mm; E–F 2 mm.

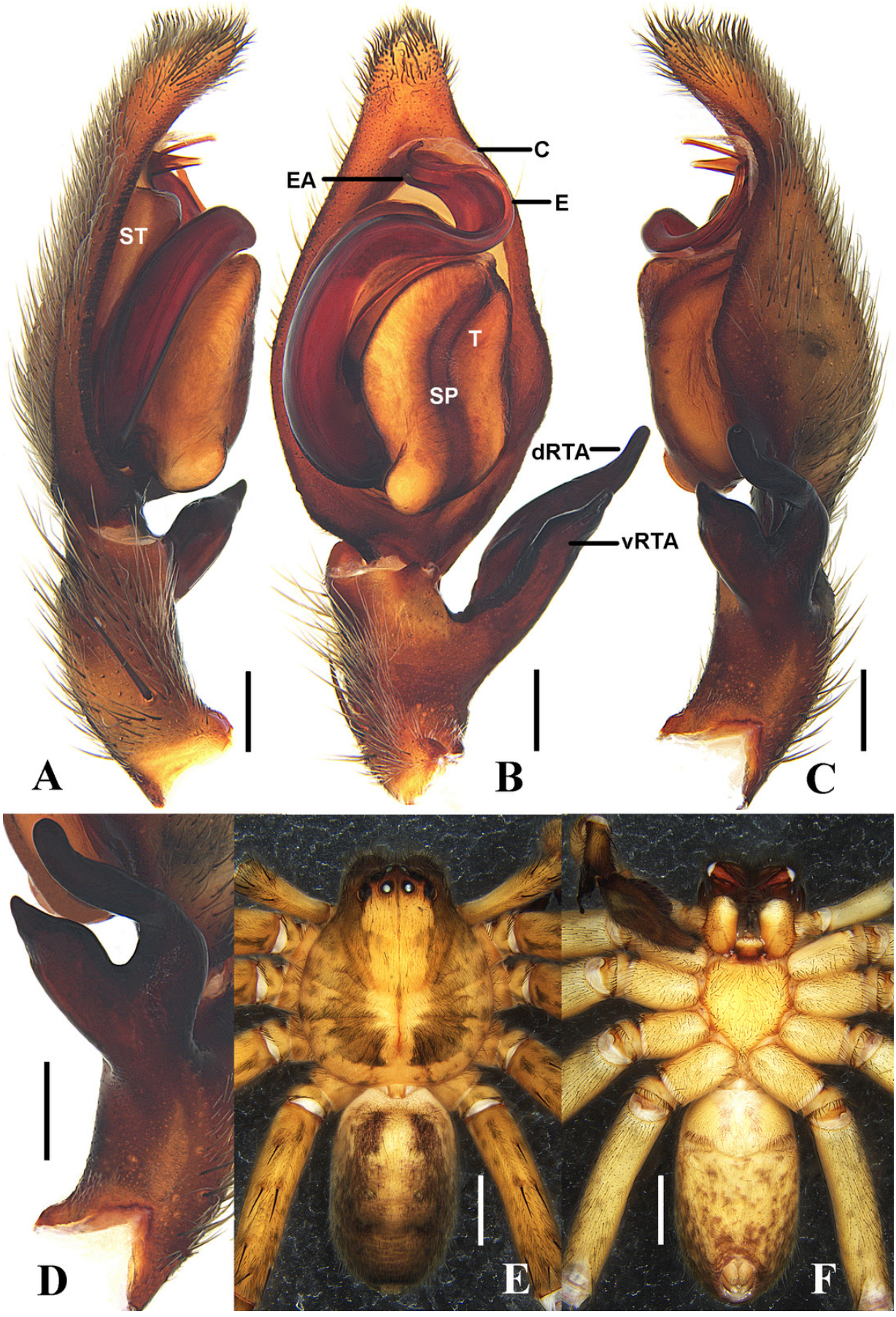
ST
EA
C
E
T
SP
dRTA
vRTA
A
B
C
D
E
F

Table 1(on next page)

Molecular markers and primers used for amplification.

The amplification was performed in 50 µl final volume containing 18 µl of ultra-pure water (dd H_2O), 25 µl of I-5™ 2X High-Fidelity Master Mix, 2 µl of each primer (100 pmol/µl), 3 µl of the genomic spider DNA templates. PCR settings list Initial Denaturation (ind), followed by /n cycles (Denaturation: de, Primer Annealing: pra, Primer Elongation: pre), and one Terminal Elongation (tee). (Temperature in °C following by time in seconds)

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Marker	Primer name	Premier sequence (5'→ 3')	PCR settings
16S	16SA	CGCCTGTTTACCAAAAACAT	ind98 (120s), [de 98(10s), pra 52(15s), pre72(15s)/35], tee72(120s)
	16SB	CCGGTTTGAACTCAGATC	
18S	18S5f	GCGAAAGCATTTGCCAAGAA	ind98 (120s), [de 98(10s), pra 57(15s),pre72(15s)/35], tee72(120s)
	18S9r	GATCCTTCCGCAGGTTCACCTAC	
28S	28SC	GGTTCGATTAGTCTTTCGCC	ind98 (120s), [de 98(10s), pra 55(15s),pre72(15s)/35], tee72(120s)
	28SO	GAAACTGCTCAAAGGTAAACGG	
COIf	LCOI1490	GGTCAACAAATCATAAAGATATTGG	ind98 (120s), [de 98(10s), pra 47(15s),pre72(15s)/35], tee72(120s)
	HCOI2198	TAAACTTCAGGGTGACCAAAAAATCA	
COIr	Jerry	CAACATTTATTTTGATTTTTTGG	ind98 (120s), [de 98(10s), pra 52(15s),pre72(15s)/35], tee72(120s)
	C1-N-2776	GGATAATCAGAATATCGTCGAGG	
H3	H3aF	ATGGCTCGTACCAAGCAGACVGC	ind98 (120s), [de 98(10s), pra 56(15s),pre72(15s)/35], tee72(120s)
	H3aR	ATATCCTTRGGCATRATRGTGAC	
ITS2	ITS4	TCCTCCGCTTATTGATATGC	ind98 (120s), [de 98(10s), pra 50(15s),pre72(15s)/35], tee72(120s)
	ITS5.8	GGGACGATGAAGAACGCAGC	

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