

# First report of partial albinism in the blue lobster *Panulirus inflatus* (Bouvier, 1895) from the Mexican Pacific (Crustacea, Decapoda, Palinuridae)

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## Abstract

The first case of partial albinism registered in the Mexican Pacific by the blue lobster *Panulirus inflatus* is presented. The specimen was collected on the southern coast of Jalisco known as Punta “El Estrecho”. It constitutes one of the few registered cases of albinism in invertebrates from the Eastern Tropical Pacific.

## Keywords

crustaceans, Eastern Tropical Pacific, Palinuridae, Spiny Lobster, western America

## Introduction

Albinism is a genetic condition, the result of a mutation on the gene that codifies the enzyme tyrosinase, and results in losing the capacity to synthesize melanin, the same pigment that is responsible for the color of skin and hair in animals; therefore the anomaly causes the organisms to adopt a white coloration on the skin and hair and have red eyes (Griffiths et al. 2000). According to the phenotypic characteristic of the individuals, this type of mutation can be expressed in four ways: a) albinism, which is the complete loss of pigmentation throughout the body, b) dilution, where

the tonality of the color is reduced as well as other natural pigments, c) squizocroism where the pigment is not expressed but does not affect any other pigments, and d) leucism that is translated in the loss of color of the skin and hair without affecting the soft parts (Buckley 1982). In the latter case, the animals present white skin and hair (Miller 2005, Garcia-Morales et al. 2010). This condition has been recorded in different groups of marine animals like echinoderms (Kehas et al. 2005, Fernández-Rivera et al. 2015), crustaceans (James 2005), fishes (Evangelista-Leal et al. 2013), and sharks (Sancho-Vazquez et al. 2015). Chromatic anomalies have also been registered such as partial albinism, which involve the lack of pigmentation of different body regions (Sancho-Vazquez et al. 2015).

*Panulirus* is a genus that includes representatives of species known as spiny, rock, or blue lobsters. Most species of the genus have a large worldwide commercial importance (Holthuis 1991, Hendrickx 1995). The blue lobster, *P. inflatus* (Bouvier, 1895), which constitutes the object of this contribution, is frequently observed on rocky reefs with crystalline waters and to 30 m depth (Briones-Fourzan and Lozano-Alvarez 2003). The species is considered to be endemic to the west coast of Mexico, within a specific geographic range from Bahía Magdalena, Baja California, to the Gulf of Tehuantepec, Oaxaca, Mexico (Butler et al. 2011).

## Materials and methods

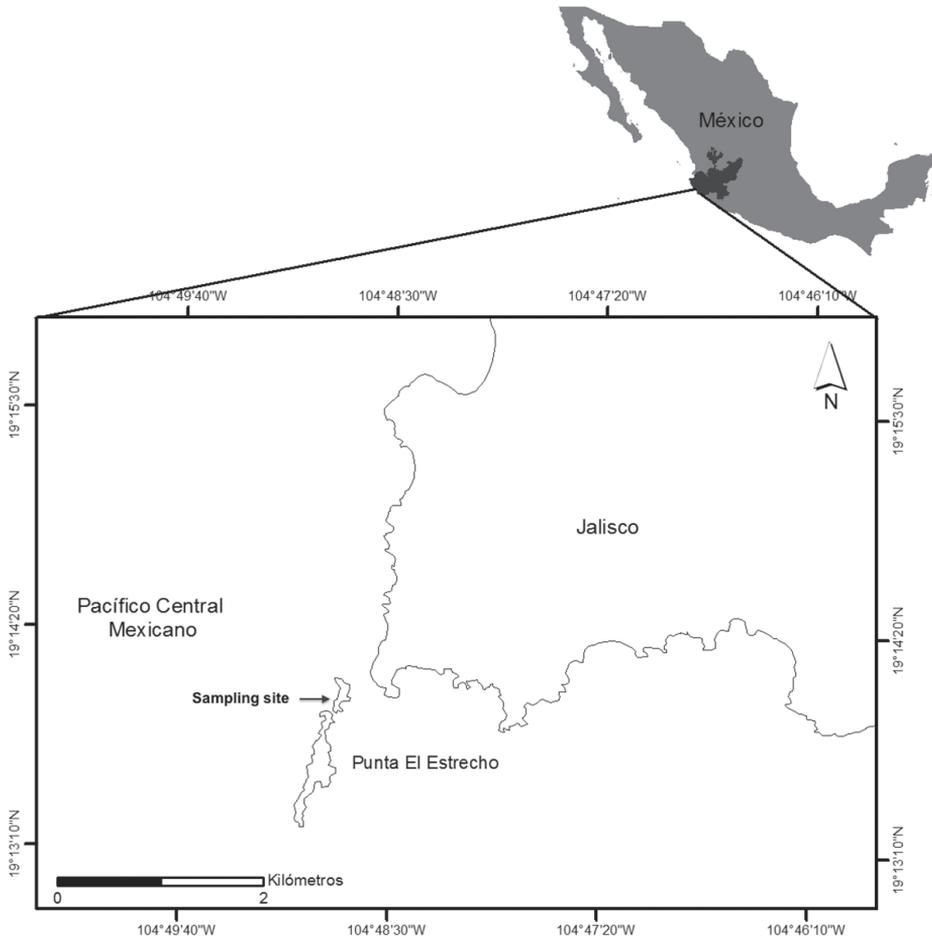
A specimen of *Panulirus inflatus* was collected from the coastal waters on a site known as Punta El Estrecho, on the southern coast of the state of Jalisco, Mexico (19°06'11"N; 104°29'12"W; Figure 1). The specimen was captured using the hook fishing technique during night submersions at a depth of 15 m on predominantly rocky bottoms using scuba diving on 05 July 2016.

Due to the peculiar characteristics of the specimen, the fisherman who captured it donated the individual to the Universidad de Guadalajara. The specimen was identified using specialized literature (Holthuis 1991, Hendrickx 1995) and preserved in formaldehyde at 10% for a week. The specimen was then washed and transferred to a permanent container in 70% alcohol in the local Invertebrates Collection assigning the following catalogue number: CIDEDSZC1008.

## Results

The specimen examined was a male with a total length of 24 cm and a weight of 300 g. Other measurements taken included: length and width of the cephalothorax 9 cm and 6 cm; length and width of the tail 11 cm and 6 cm; length and width of the claw 4 cm and 3 cm.

Lack of pigmentation was observed on different parts of the body and appendages. Appendages lacking pigments included the flagella of the right and left antennules, the

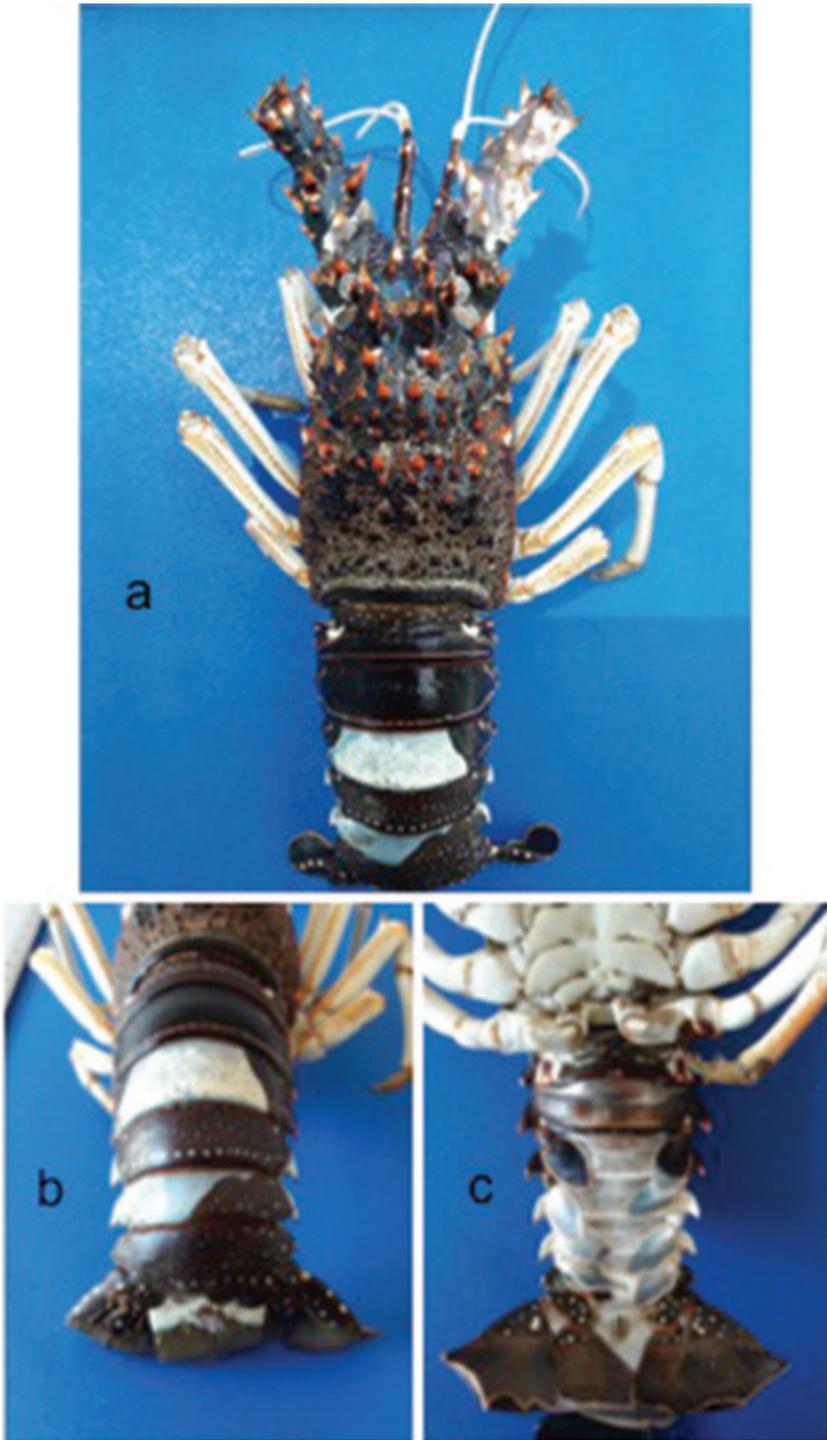


**Figure 1.** Map of the sampling area, Punta El Estrecho, Jalisco, México.

right antenna, the left and right chelipeds, and all pereopods (Figure 2a). The abdominal somites III and V were partly devoid of pigmentation dorsally and somites III to VI lacked pigmentation ventrally (Figs. 2b, c). The cephalothorax lacked pigment only on the ventral part (Figure 2c). The third left pleopod was partially pigmented and pairs of pleopods IV–VI lacked pigmentation (Figure 2c). The telson partially lacked pigmentation on the dorsal and totally on ventral side (Figure 2b, c).

## Discussion

The albinism phenomenon has been reported on vertebrates, and elasmobranches. However, the data are scarce regarding invertebrates (Fernández-Rivera et al. 2015).



**Figure 2.** Blue Lobster *Panulirus inflatus*. **a** Complete dorsal view **b** Partial dorsal view **c** Partial ventral view.

Isolated cases of albinism on invertebrates have been reported by Kehas et al. (2005) in the land snail *Planorbella trivolvis*, James (2005) observed this phenomenon in juveniles of crabs *Cancer pagurus*, and Fernández-Rivera et al. (2015) found two specimens with albinism of sea cucumber *Isostichopus fuscus* in the Gulf of California, México. As far as we know, the only reported case of albinism in lobsters refers to *Panulirus japonicus* (Okamoto and Misyuku 1998).

According to Evangelista-Leal et al. (2013), the occurrence of albinism in fishes can be caused by three factors: random genetic alteration, an effect of marine pollution, or genetic alterations due to the size of the populations. Further studies are required in different species displaying albinism to understand the factors that cause this phenomenon and to evaluate if abnormal pigmentation can be used as an indicator of the quality of the habitat or populations in special situations. In benthic organisms such as species of crustaceans, pigmentation of the body is a vital factor for survival because the organisms use camouflage to avoid predators, when it confused with other animals or their environment (Tan and Richer De Forges 1993).

It must be highlighted that during the previous year in which the specimen was captured an event of the El Niño South Oscillation in the Mexican tropical Pacific occurred (National Oceanic and Atmospheric Administration 2015, Jacox et al. 2016); however, there is no evidence to associate the observed albinism to this oceanographic situation.

## Acknowledgements

The authors are grateful to J. Osiel Arteaga Aguilar, the scuba diver for it's generous donation of the specimen to the invertebrate collection of the DEDSZC-CUCSUR of the University of Guadalajara. We are also grateful to Alejandra Valdez Cibrian for drawing the map and Ramiro Flores Vargas for his editorial assistance.

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# Taxonomic study of the *Pinelema bailongensis* species group with descriptions of six new species from China (Araneae, Telemidae)

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## Abstract

The *Pinelema bailongensis* Wang & Li, 2012 species group of the spider family Telemidae Fage, 1913 from Guangxi and Guizhou, China is revised. Previously, this species group contained two species: *P. bailongensis* and *P. xiushuiensis* Wang & Li, 2016. In this study, four species are transferred from *Telema* Simon, 1882 to *Pinelema* Wang & Li, 2012, and treated as members of the *P. bailongensis* species group. They are *P. cordata* (Wang & Li, 2010) **comb. n.**, *P. liangxi* (Zhu & Chen, 2002) **comb. n.**, *P. strentarsi* (Lin & Li, 2010) **comb. n.** and *P. zhewang* (Chen & Zhu, 2009) **comb. n.** Additionally, six new species belonging to the species group are described: *P. cheni* Zhao & Li, **sp. n.** (♂♀), *P. huoyan* Zhao & Li, **sp. n.** (♂♀), *P. lizhuang* Zhao & Li, **sp. n.** (♂♀), *P. wangshang* Zhao & Li, **sp. n.** (♂♀), *P. wenyang* Zhao & Li, **sp. n.** (♂♀) and *P. yunchuni* Zhao & Li, **sp. n.** (♂♀). A key to males is provided.

## Keywords

Haplogynae, new combination, spider, *Telema*

\* Both authors contributed equally to this work.

## Introduction

Telemidae Fage, 1913 currently contains 79 species in ten genera worldwide (World Spider Catalog 2018). It has fragmented distributions in rainforest or karst caves of tropical Africa, Eurasia, and the New World (Song et al. 2017a). *Pinelema* Wang & Li, 2012, the second largest genus of the family, occurring in karst caves or leaf litter. A total of 15 *Pinelema* species was known from China and Vietnam before the current study.

The *P. bailongensis* species group, characterized by a distinctly long embolus relative to the bulb, currently contains only two species: *P. bailongensis* Wang & Li, 2012 and *P. xiushuiensis* Wang & Li, 2016. The species group is restricted to karst caves in southern Guizhou and western Guangxi, China. In this paper, four species are transferred from *Telema* Simon, 1882 to *Pinelema*, and six new species are described.

## Material and methods

The individuals of *Pinelema bailongensis* species group studied here are from the cave expeditions in southern China during last decade.

All specimens were examined and measured using a LEICA M205 C stereomicroscope. All measurements are given in millimeters. Leg measurements are as follows: total length (femur, patella, tibia, metatarsus, tarsus). The habitus, left male palp, and receptacle were photographed using an Olympus C7070 digital camera. Female genitalia were removed and treated in lactic acid before being photographed. Images were combined using Helicon Focus image stacking software. For SEM images, the left male palp was photographed using a Hitachi SU8010 Environmental Scanning Electron Microscope.

To confirm the stable morphology of male palps for each species in the *P. bailongensis* species group, photos of additional individuals are provided in the supplementary material. Genetic distances were obtained for four to five individuals of each species. Genomic DNA was extracted from the prosomas of females. Standard barcode COI (650 bp) was amplified using the primer pair LCO1490 (5'-GGTCAACAAATCAT-AAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The PCR protocol consisted of initial denaturing of 95 °C for 5 min, 5 cycles of 95 °C for 30 s, 45 °C for 30 s, and 72 °C for 30 s, then 35 cycles of 95 °C for 30 s, 51 °C for 30 s, and 72 °C for 30 s, with a final extension of 72 °C for 5 min. All PCR positive products were purified and sequenced by Tianyihuiyuan Biotech Co., Ltd (Beijing, China) using an ABI 3730 automated sequencer. Raw ABI sequences were edited by hand in BioEdit (Hall 1999). Uncorrected pairwise distances between species in the *P. bailongensis* species group were calculated using MEGA 5.0 (Tamura et al. 2011). All sequences are deposited in GenBank, and the accession numbers are listed in Table 1.

References to figures in the cited papers are listed in lowercase (figure or figs); figures from this paper are noted with an initial capital (Figure or Figs). The following abbreviations are used in the text or figures:

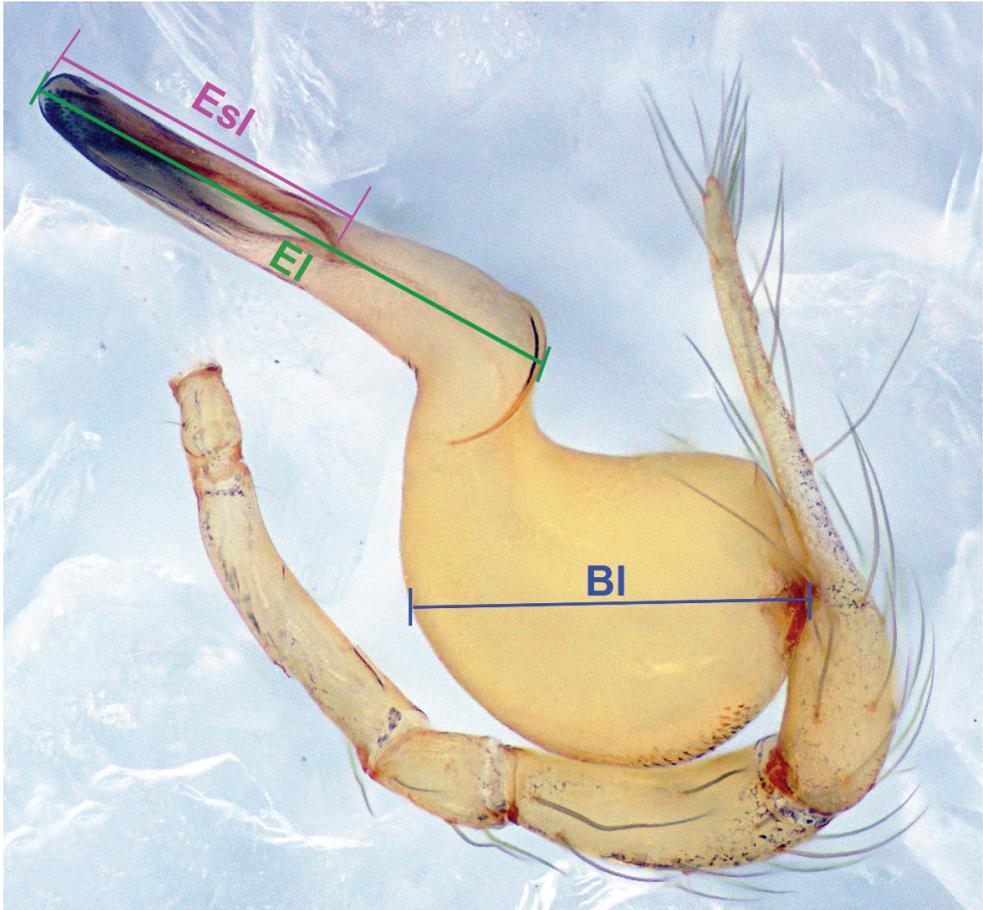
**Table 1.** GenBank accession numbers and mean inter-specific uncorrected p-distances of species in the *P. bailongensis* species group from COI data.

	Species	GenBank accession number	1	2	3	4	5	6	7	8	9	10	11
1	<i>P. bailongensis</i>	MH643817– MH643821											
2	<i>P. cheni</i>	MH643822– MH643825	0.169										
3	<i>P. cordata</i>	MH643826– MH643830	0.151	0.149									
4	<i>P. huoyan</i>	MH643831– MH643835	0.168	0.163	0.164								
5	<i>P. liangxi</i>	MH643836– MH643840	0.146	0.161	0.154	0.155							
6	<i>P. lizhuang</i>	MH643841– MH643844	0.151	0.169	0.149	0.177	0.153						
7	<i>P. strentarsi</i>	MH643845– MH643849	0.161	0.164	0.158	0.154	0.170	0.165					
8	<i>P. wangshang</i>	MH643850– MH643853	0.160	0.166	0.159	0.154	0.151	0.162	0.158				
9	<i>P. wenyang</i>	MH643854– MH643858	0.156	0.167	0.160	0.154	0.177	0.159	0.169	0.162			
10	<i>P. xiushuiensis</i>	MH643859– MH643863	0.137	0.146	0.169	0.149	0.134	0.154	0.139	0.166	0.162		
11	<i>P. yunchuni</i>	MH643864– MH643867	0.169	0.147	0.171	0.133	0.145	0.164	0.135	0.153	0.150	0.146	
12	<i>P. zbewang</i>	MH643868– MH643872	0.142	0.155	0.122	0.154	0.143	0.152	0.148	0.157	0.154	0.146	0.128

- Bl** Bulb length, the bulbal bisector line from the junction of the bulb and cymbium to the distal ridge of bulb (blue line in Figure 1).
- Ca** Cymbial apophysis.
- El** Embolus length, the bisector line of the embolus from base to tip (green line in Figure 1).
- El/Bl** The ratio of El to Bl. The gap between the El/Bl ratio ranges between two species should be larger or equal to the El/Bl ratio range within each species.
- Em** Embolus.
- Es** Embolic slit.
- Esl** Es length (purple line in Figure 1).
- Esl/El** The ratio of the Esl and El. The gap between the Esl/El ratio ranges between two species should be larger or equal to the Esl/El ratio range within each species.
- Pa** Papillae on bulb proximo-retrolaterally.
- Re** Receptacle.
- Sr** Spiral ridge of embolus.

Abbreviations of institutes:

- IZCAS** Institute of Zoology, Chinese Academy of Sciences, Beijing, China.
- MHBU** Museum of Hebei University, Baoding, China.
- MLR** Maolan National Natural Reserve, Libo, Guizhou, China.



**Figure 1.** *Pinelema bailongensis*, male palp, retrolateral view showing bulb length (Bl), embolus length (El) and embolic slit length (Esl).

## Taxonomy

Family *Telemidae* Fage, 1913

Genus *Pinelema* Wang & Li, 2012

**Type species.** *Pinelema bailongensis* Wang & Li, 2012 from Guangxi, China.

**Composition.** The total number of *Pinelema* species has increased to 25. They are *P. bailongensis* Wang & Li, 2012, *P. cheni* Zhao & Li, sp. n., *P. cordata* (Wang & Li, 2010) comb. n., *P. cunfengensis* Zhao & Li, 2017, *P. curcici* Wang & Li, 2016, *P. damtaoensis* Zhao & Li, 2018, *P. huobaensis* Wang & Li, 2016, *P. huoyan* Zhao & Li, sp. n., *P. laensis* Zhao & Li, 2018, *P. liangxi* (Zhu & Chen, 2002) comb. n., *P. lizhuang* Zhao & Li, sp. n., *P. nuocnutensis* Zhao & Li, 2018, *P. pacchanensis* Zhao & Li, 2018, *P. podiensis* Zhao & Li, 2017, *P. qingfengensis* Zhao & Li, 2017, *P. spirulata* Zhao & Li,

2018, *P. strentarsi* (Lin & Li, 2010) comb. n., *P. wangshang* Zhao & Li, sp. n., *P. wenyang* Zhao & Li, sp. n., *P. xiezi* Zhao & Li, 2018, *P. xiushuiensis* Wang & Li, 2016, *P. yaosaensis* Wang & Li, 2016, *P. yunchuni* Zhao & Li, sp. n., *P. zhenzhuang* Zhao & Li, 2018, and *P. zhewang* (Chen & Zhu, 2009) comb. n.

**Diagnosis and descriptions.** See Wang and Li (2016) and Zhao et al. (2018).

**Distribution.** China (Guangxi, Guizhou, Yunnan), Vietnam (Vinh Phuc, Quang Binh, Phu Tho, Bac Kan).

### *Pinelema bailongensis* species group

**Diagnosis.** Species of *Pinelema bailongensis* species group can be distinguished from all other *Pinelema* species by embolus distinctly longer than bulb and by endogyne U-shaped, J-shaped or spiral. In contrast, embolus of other *Pinelema* species shorter than bulb and endogyne bag-like.

**Distribution.** China (western Guangxi, southern Guizhou).

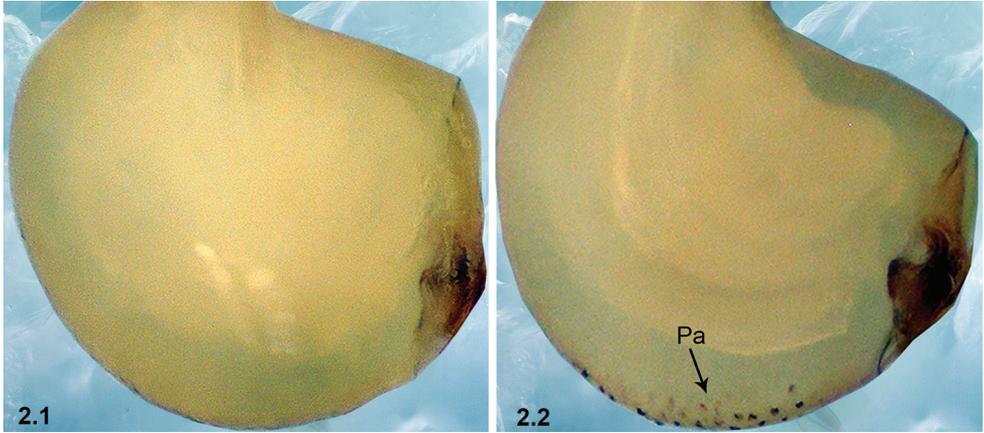
**Comments.** *Pinelema bailongensis* species group is recognized as the first species group in *Pinelema* according to the long embolus relative to the bulb. Morphologically, the species in this group have quite simple and almost identical copulatory organs, only proportions of the bulb and its parts can help to separate the species, endogynes can not be used for distinguishing species (except *P. bailongensis* and *P. wangshang* Zhao & Li, sp. n.). Genetically, each species in this group owns very significant difference comparing the congeners (Table 1).

### Illustrated key to males of the *Pinelema bailongensis* species group

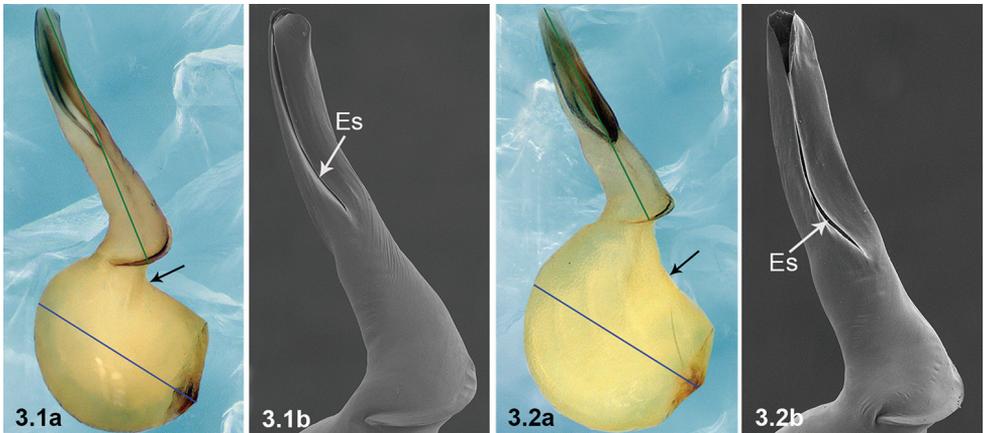
1	Eyes present (1.1).....	2
–	Eyes absent (1.2).....	8



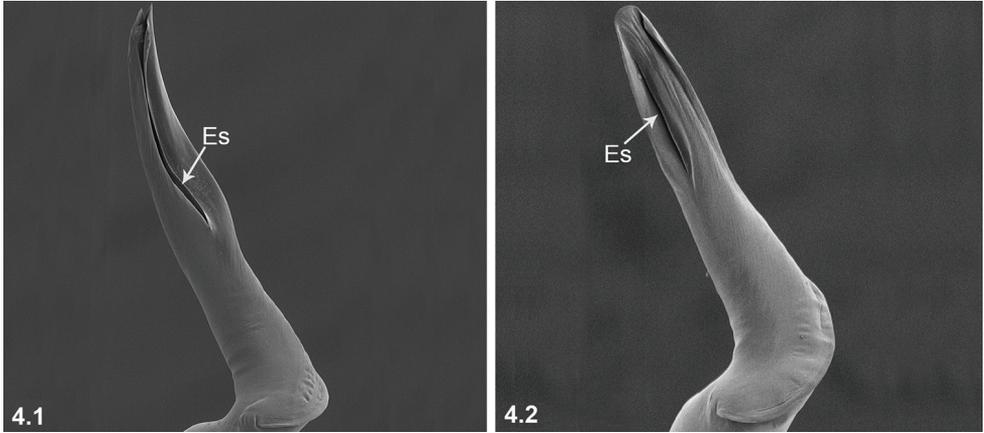
- 2 Bulb without papillae proximo-retrolaterally (2.1) ..... 3
- Bulb with papillae proximo-retrolaterally (2.2) ..... 4



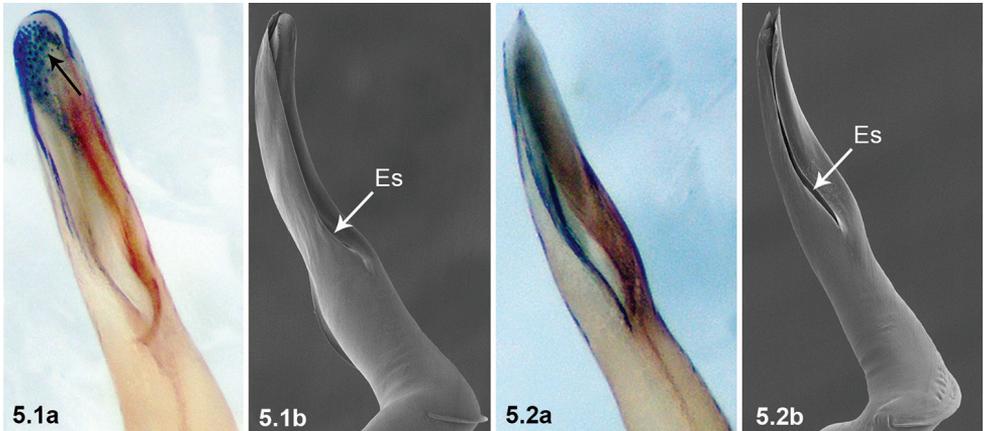
- 3 Bulb curved dorso-distally (arrowed in 3.1a); EsI/El ratio: 0.51–0.55 (n = 5, mean: 0.53) (3.1b); El/Bl ratio: 1.47–1.54 (n = 5, mean: 1.49) ..... *P. wenyang* Zhao & Li, sp. n.
- Bulb curved dorso-medially (arrowed in 3.2a); EsI/El ratio: 0.63–0.67 (n = 6, mean: 0.65) (3.2b); El/Bl ratio: 1.24–1.31 (n = 6, mean: 1.27) ..... *P. lizhuang* Zhao & Li, sp. n.



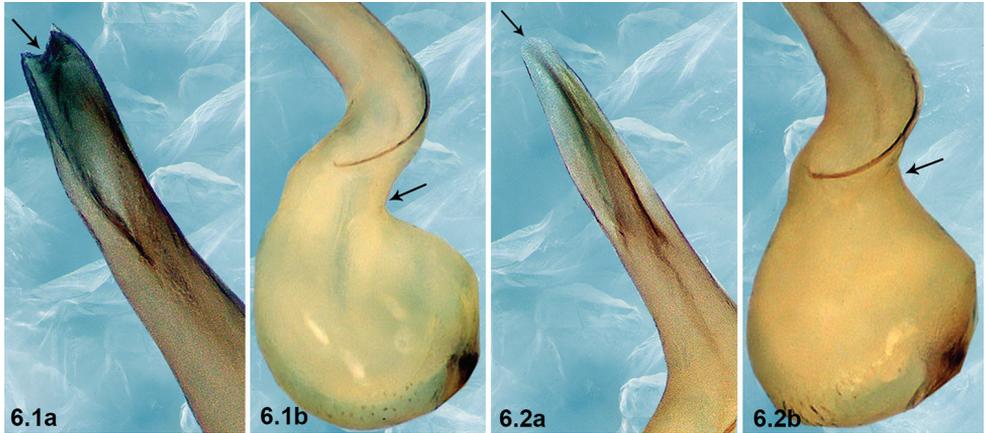
- 4 Esl/El ratio: 0.51–0.62 (n = 10) (4.1) ..... 5
- Esl/El ratio: 0.33–0.45 (n = 14) (4.2) ..... 6



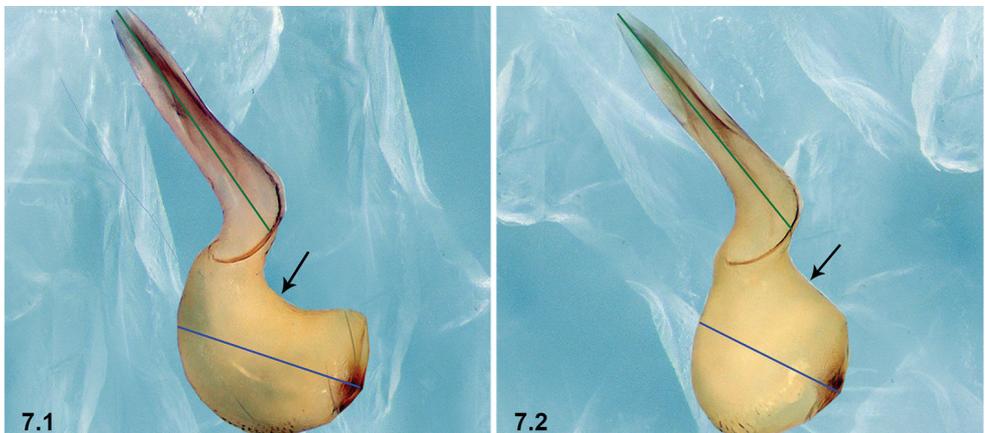
- 5 Embolus with small apophyses distal-retrolaterally (arrowed in 5.1a); Esl/El ratio: 0.58–0.62 (n = 5, mean: 0.60) ..... *P. bailongensis* Wang & Li, 2012
- Embolus without small apophyses distal-retrolaterally (5.2a); Esl/El ratio: 0.51–0.54 (n = 5, mean: 0.52) ..... *P. cordata* (Wang & Li, 2010)



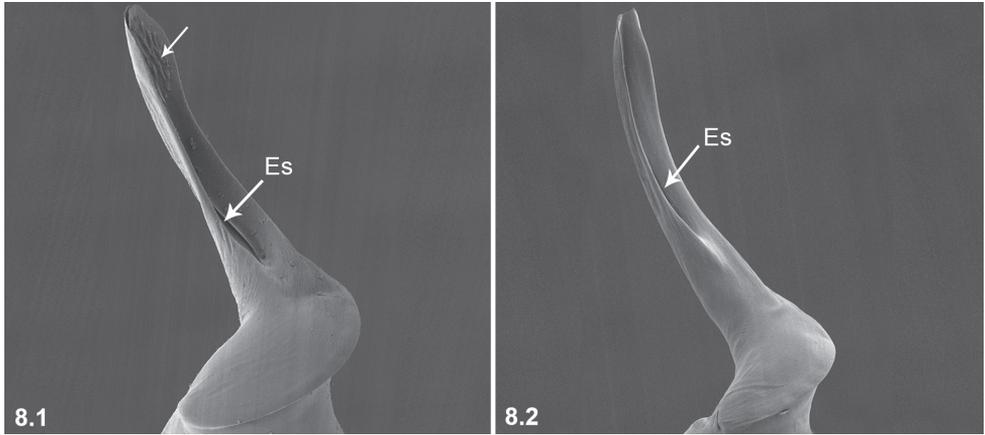
- 6 Embolus with a bifurcated tip (arrowed in 6.1a); bulb with a right-angled bend dorso-subdistally (arrowed in 6.1b) ..... *P. cheni* Zhao & Li, sp. n.
- Embolus with a slightly arch-shaped tip (arrowed in 6.2a); bulb with an obtuse-angled bend dorso-distally (arrowed in 6.2b) ..... 7



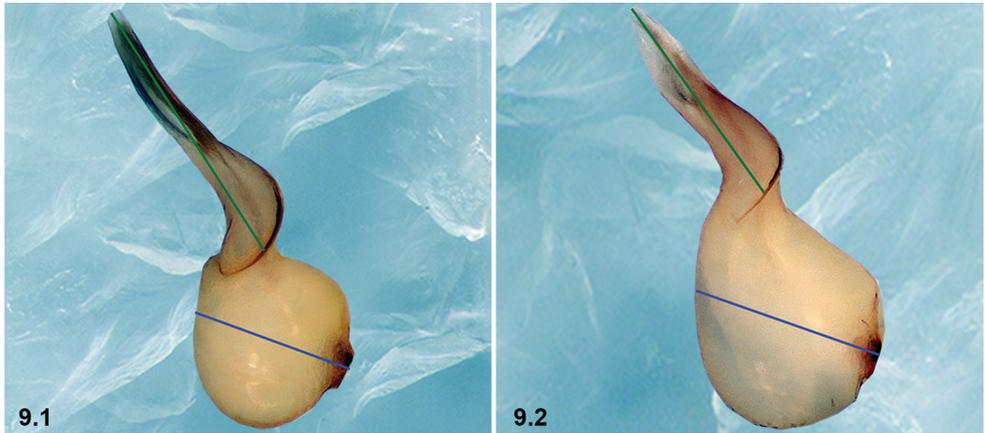
- 7 Bulb kidney-shaped and curved dorso-medially (arrow in 7.1), El/Bl ratio 1.37–1.45 (n = 5, mean: 1.41) (7.1) ..... *P. wangshang* Zhao & Li, sp. n.
- Bulb pear-shaped and not curved dorso-medially (arrow in 7.2), El/Bl ratio 1.78–1.82 (n = 4, mean: 1.80) (7.2) ..... *P. yunchuni* Zhao & Li, sp. n.



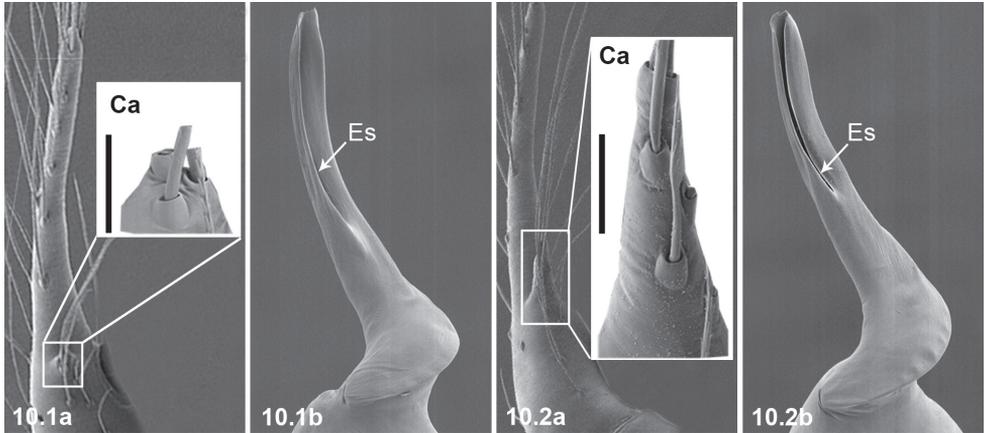
- 8 Embolus with small apophyses distal-retrolaterally (arrowed in 8.1); Esl/El ratio 0.72–0.78 (n = 5, mean: 0.76)..... ***P. buoyan* Zhao & Li, sp. n.**
- Embolus without small apophyses distal-retrolaterally (8.2); Esl/El ratio 0.48–0.63 (n = 18) .....



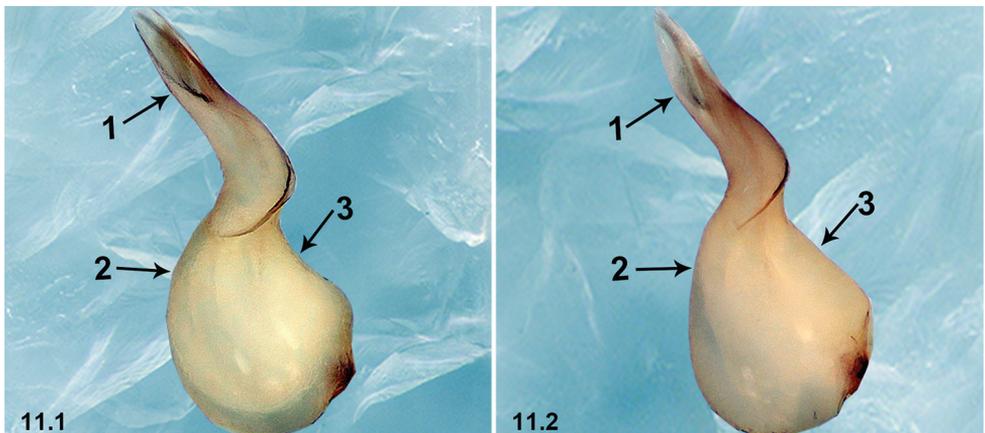
- 9 El/Bl ratio: 1.58–1.83 (n = 9) (9.1)..... **10**
- El/Bl ratio: 1.14–1.25 (n = 9) (9.2)..... **11**



- 10 Ca short (10.1a, scale bar 0.2 mm); Es/El ratio: 058–0.63 (n = 4, mean 0.60) (10.1b)..... *P. zhewang* (Chen & Zhu, 2009)
- Ca long (10.2a, scale bar 0.2 mm); Es/El ratio: 050–0.52 (n = 5, mean 0.51) (10.2b)..... *P. xiushuiensis* Wang & Li, 2016



- 11 Embolus straight (arrow 1 in 11.1); bulb protruding ventro-subdistally (arrow 2 in 11.1) and slightly curved dorso-medially (arrow 3 in 11.1)..... *P. strentarsi* (Lin & Li, 2010)
- Embolus curved (arrow 1 in 11.2); bulb not protruding ventro-subdistally (arrow 2 in 11.2) and not curved dorso-medially (arrow 3 in 11.2)..... *P. liangxi* (Zhu & Chen, 2002)



***Pinelema bailongensis* Wang & Li, 2012**

Figs 1, 31

*Pinelema bailongensis*: Wang and Li 2012: 82, figs 1–17 (♂♀); Song et al. 2017b: 85, figs 7A, 8A, 9A, 10A, 11A, 12A (♂).

**Material examined.** Holotype ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Baise Prefecture, Pingguo County, Bailong Cave, 23°19.094'N, 107°34.387'E, 111 m, 1.VIII.2009, C. Wang & Z. Yao leg. Paratypes (IZCAS): 1♂ and 4♀, same data as holotype.

**Other material examined.** 5♂ and 5♀ (molecular vouchers, IZCAS), same data as holotype.

**Diagnosis.** *Pinelema bailongensis* resembles *P. cordata* (see Figure 5 and Wang and Li 2010: 9, figs 11–15) but can be distinguished by following characters: small apophyses present on embolus distal-retrolaterally (see Song et al. 2017b: figure 8A and Wang and Li 2012: figure 4) (apophyses absent on embolus distal-retrolaterally in *P. cordata*), larger Esl/El ratio (0.58–0.62, n = 5, mean: 0.60, Suppl. material 1: Figure S1) (smaller Esl/El ratio 0.51–0.54, n = 5, mean: 0.52 in *P. cordata*), and shape of receptacle spiral (see Wang and Li 2012: figs 8, 11) (receptacle U-shaped in *P. cordata*).

**Description.** El/Bl ratio 1.40–1.48 (n=5, mean: 1.43, Suppl. material 1: Figure S1), Esl/El ratio 0.58–0.62 (n = 5, mean: 0.60, Suppl. material 1: Figure S1). For more detailed descriptions see Wang and Li (2012) and Song et al. (2017b).

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

***Pinelema cheni* Zhao & Li, sp. n.**

<http://zoobank.org/909EE4D6-1A37-4BC2-A146-4280791CFED1>

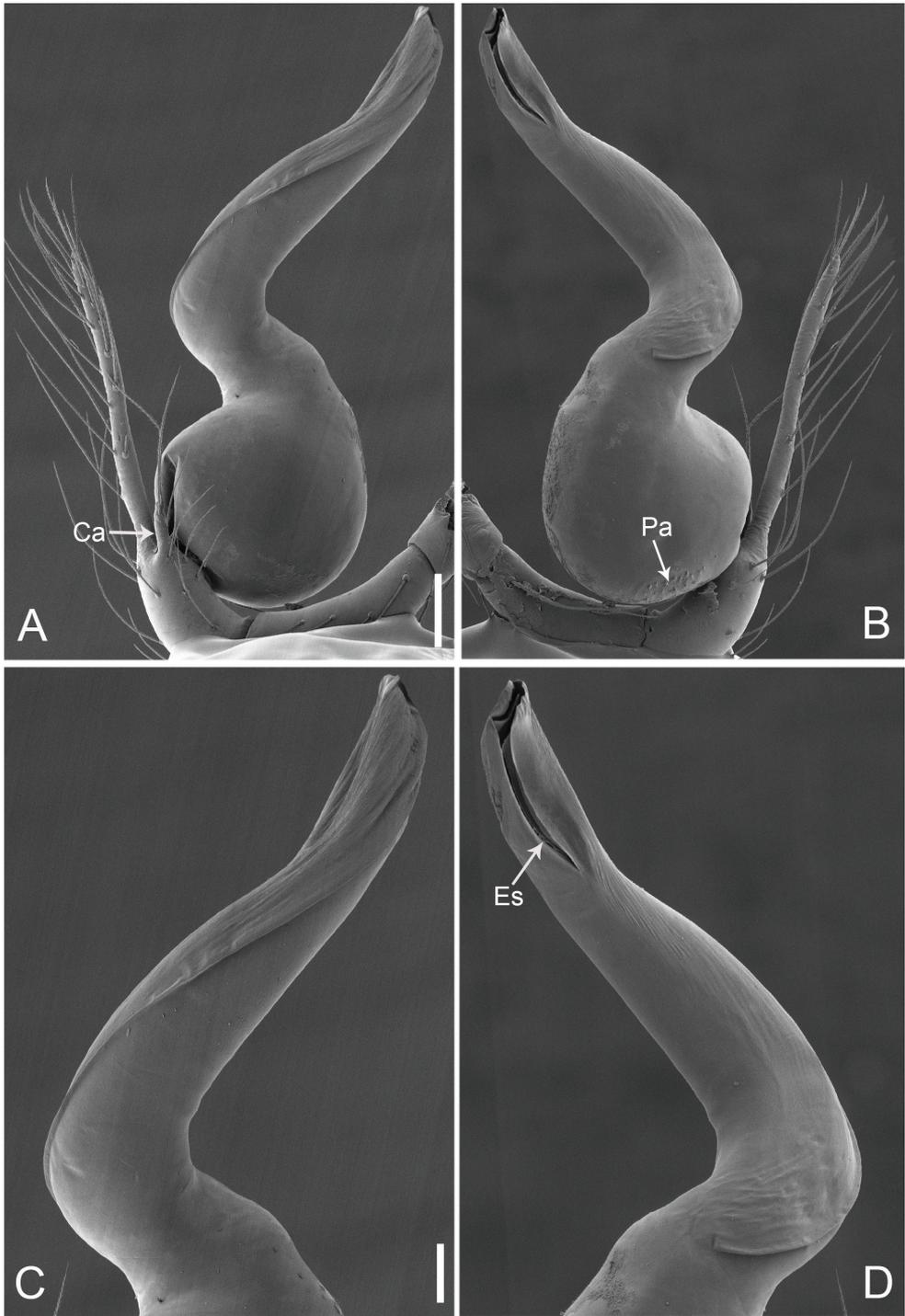
Figs 2–4, 31

**Type material. Holotype** ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Hechi Prefecture, Du'an County, Gaoling Town, Jiangzhong Village, Huoyan Cave, 24°01.820'N, 108°04.720'E, 243 m, 11.II.2015, Y. Li and Z. Chen leg. **Paratypes** (IZCAS): 3♂ and 3♀, same data as holotype.

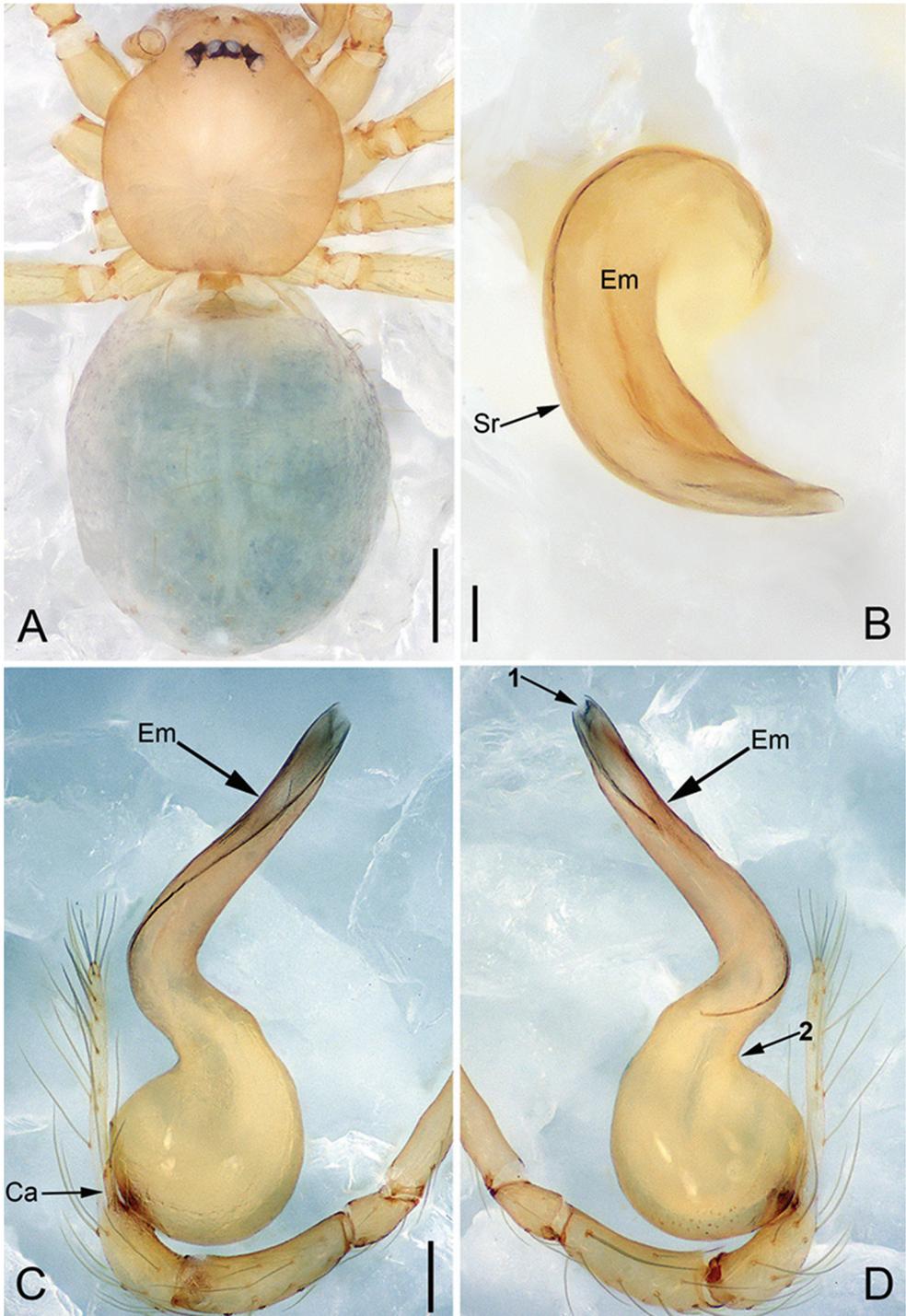
**Other material examined.** 4♀ (molecular vouchers, IZCAS), same data as holotype.

**Etymology.** The specific name is a patronym in honor of the collector Zhigang Chen.

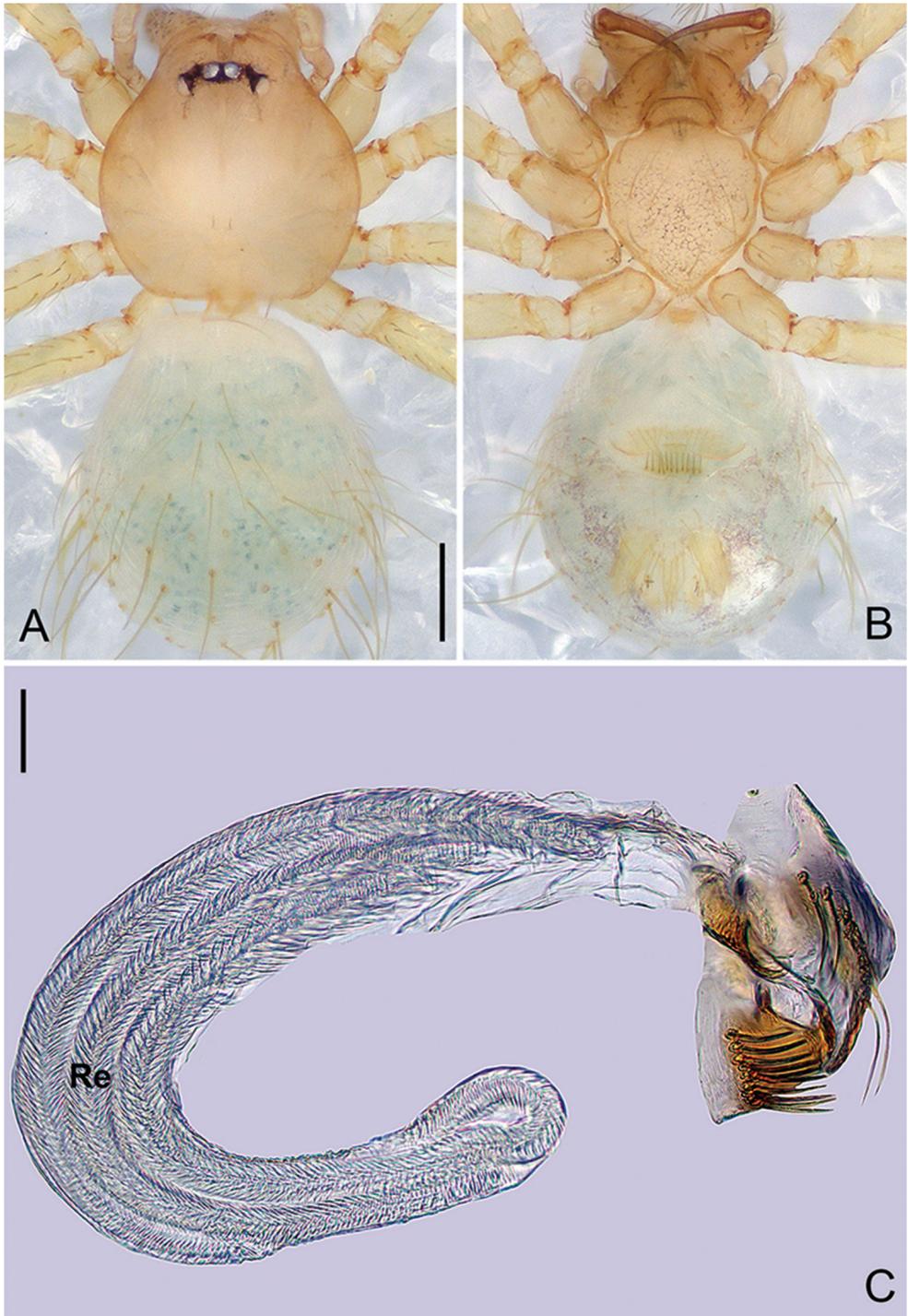
**Diagnosis.** *Pinelema cheni* Zhao & Li, sp. n. can be easily distinguished from all other congeners by following characters: bifurcated tip of embolus (arrow 1 in Figure 3D) (arch-shaped tips on emboli in other congeners), right-angled bend on bulb dorso-subdistally (arrow 2 in Figure 3D) (no right-angled bend on bulbs in other congeners), and smaller Esl/El ratio (0.33–0.37, n = 5, mean: 0.34, Suppl. material 1: Figure S2) (larger Esl/El ratio 0.42–0.78 in other congeners).



**Figure 2.** *Pinelema cheni* Zhao & Li, sp. n., male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 3.** *Pinelema cheni* Zhao & Li, sp. n., male holotype. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C-D**).



**Figure 4.** *Pinelema cheni* Zhao & Li, sp. n., female paratype. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

**Description. Male (holotype):** Total length 1.38. Carapace 0.57 long, 0.51 wide. Abdomen 0.79 long, 0.69 wide. Carapace light brown (Figure 3A). Six eyes ringed with black, clypeus height 0.07, ocular quadrangle 0.17 wide (Figure 3A). Chelicerae, legs, labium, and endites yellow. Sternum light brown with sparse setae. Leg measurements: I 4.58 (1.36, 0.22, 1.48, 0.95, 0.57); II 3.80 (1.13, 0.22, 1.19, 0.75, 0.51); III 2.74 (0.81, 0.20, 0.79, 0.50, 0.44); IV 3.42 (1.06, 0.22, 0.97, 0.71, 0.46). Abdomen light blue with several long setae.

Palp: Tibia 2.8 times longer than patella, cymbium 2.0 times longer than tibia, cymbial apophysis cone-shaped (Figs 2A, 3C); bulb with papillae proximo-retrolaterally (Figure 2B) and a right-angled bend dorso-subdistally (arrow 2 in Figure 3D); embolus tube-shaped, with a bifurcated tip (arrow 1 in Figure 3D), spiral ridge dark brown (Figure 3B), El/Bl ratio 1.65 (Figure 3D), Esl/El ratio 0.37 (Figure 3D).

**Female:** Total length 1.30. Carapace 0.59 long, 0.54 wide. Abdomen 0.71 long, 0.59 wide. Coloration as in male and abdomen with many long setae (Figure 4A, B). Six eyes, well developed, clypeus height 0.10, ocular quadrangle 0.17 wide (Figure 4A). Leg measurements: I 4.38 (1.31, 0.21, 1.39, 0.86, 0.61); II 3.72 (1.13, 0.21, 1.13, 0.71, 0.54); III 2.73 (0.84, 0.19, 0.78, 0.48, 0.44); IV 3.28 (1.03, 0.21, 0.96, 0.62, 0.46). Insemination duct thinner than receptacle (Figure 4C); receptacle with multiple membranous tubes, U-shaped (Figure 4C).

**Variation.** In 4♂ paratypes: El/Bl ratio 1.69–1.78, Esl/El ratio 0.33–0.36.

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

### *Pinelema cordata* (Wang & Li, 2010), comb. n.

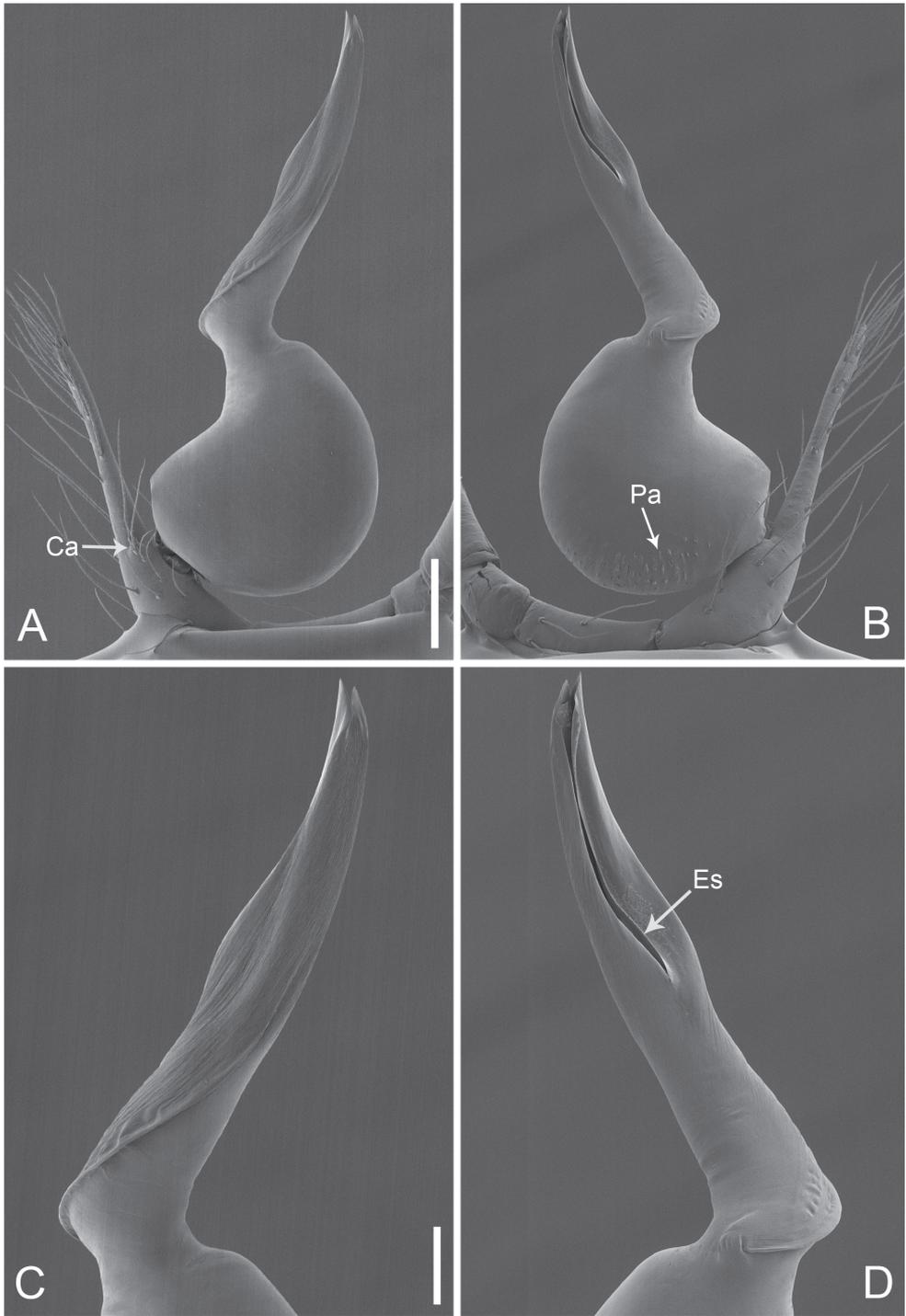
Figs 5, 31

*Telema cordata*: Wang and Li 2010: 9, figs 11–15 (♂♀).

**Material examined.** Holotype ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Nanning Prefecture, Wuming County, Yiling Cave, 23°02.374'N, 108°17.529'E, 151 m, T: 23 °C, RH: 86%, 31.VII.2009, C. Wang and Z. Yao leg. Paratypes (IZCAS): 1♂ and 4♀ (IZCAS), same data as holotype.

**Other material examined.** 5♂ and 5♀ (molecular vouchers, IZCAS), same data as holotype.

**Diagnosis.** This species resembles *P. bailongensis* (see Figure 1, Wang and Li 2012: 82, figs 1–17 and Song et al. 2017b: 85, figs 7A, 8A, 9A, 10A, 11A, 12A) but can be distinguished by following characters: small apophyses absent on embolus distal-retrolaterally (Figure 5D) (small apophyses present on embolus distal-retrolaterally in *P. bailongensis*), smaller Esl/El ratio (0.51–0.54, n = 5, mean: 0.53, Suppl. material 1: Figure S3) (larger Esl/El ratio 0.58–0.62, n = 5, mean: 0.60 in *P. bailongensis*), and receptacle U-shaped (see Wang and Li 2010: figure 13C) (receptacle spiral in *P. bailongensis*).



**Figure 5.** *Pinelema cordata* comb. n., male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).

**Description.** Male palp: Bulb with papillae proximo-retrolaterally (Figure 5B); El/Bl ratio 1.51–1.54 ( $n = 5$ , mean: 1.52, Suppl. material 1: Figure S3), Esl/El ratio 0.51–0.54 ( $n = 5$ , mean: 0.52, Suppl. material 1: Figure S3). For more detailed descriptions, see Wang and Li (2010).

**Comments.** This species is transferred to *Pinelema* because it shares similar morphological characters with *P. bailongensis*, such as the long, tube-shaped embolus (see Figure 5 and Wang and Li 2010: figs 11A–B, 12B), the presence of a distinct cymbial apophysis in the male palp prolaterally (see Figure 5A and Wang and Li 2010: figure 11B), and the U-shaped and medially strongly curved receptacle (see Wang and Li 2010: figure 13).

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

***Pinelema buoyan* Zhao & Li, sp. n.**

<http://zoobank.org/FED63C61-C96B-460D-A338-8B2C142EE4B8>

Figs 6–8, 31

**Type material.** **Holotype** ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Hechi Prefecture, Du'an County, Gaoling Town, Jiangzhong Village, Huoyan Cave, 24°01.820'N, 108°04.720'E, 243 m, 11.II.2015, Y. Li and Z. Chen leg. **Paratypes** (IZCAS): 4♂ and 6♀, same data as holotype.

**Other material examined.** 5♀ (molecular vouchers, IZCAS), same data as holotype.

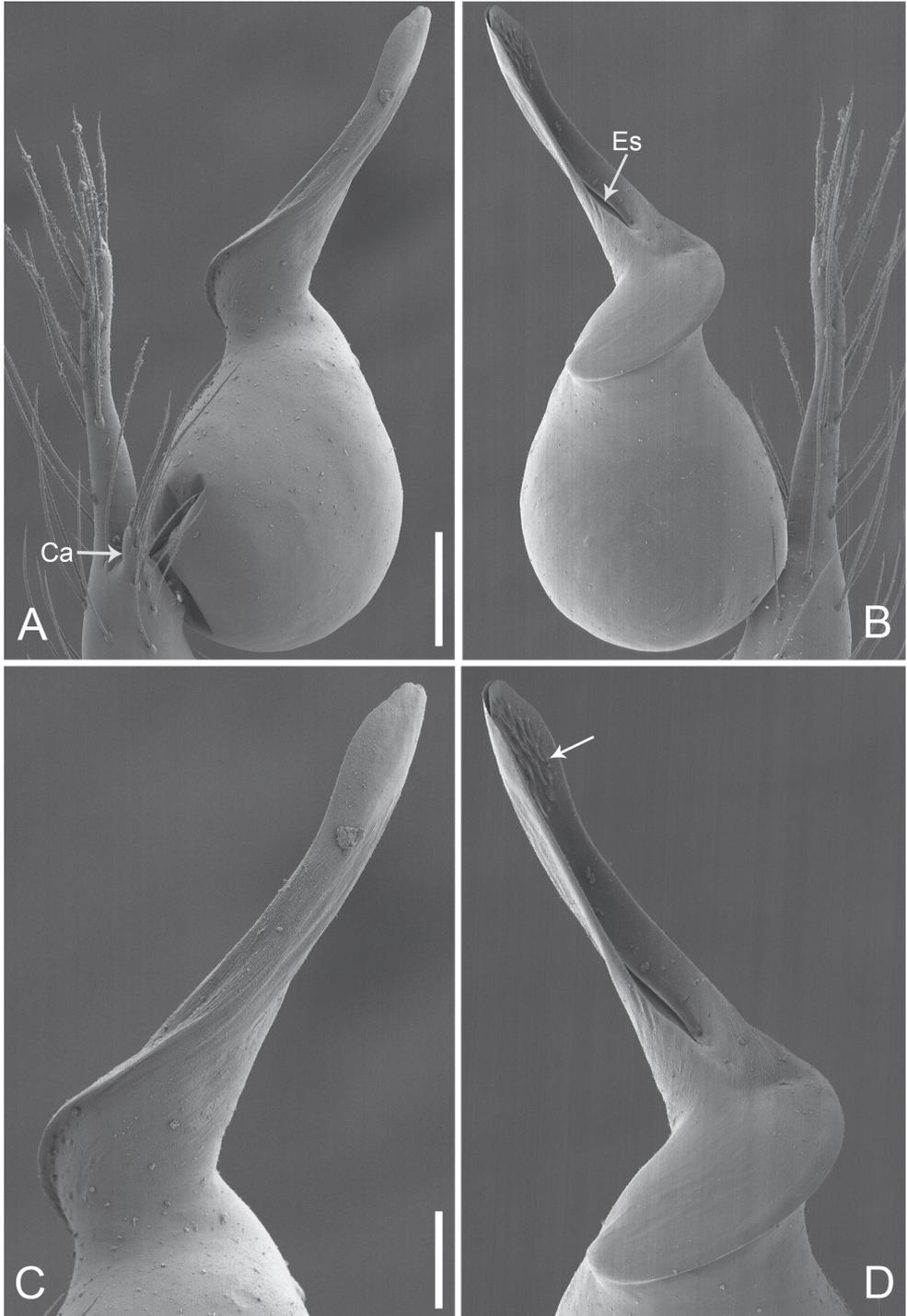
**Etymology.** The species epithet refers to the type locality; noun.

**Diagnosis.** *Pinelema buoyan* Zhao & Li, sp. n. differs from all other congeners of *P. bailongensis* species group by following combination of characters: eyeless (Figure 7A), embolus with small distal-retrolateral apophyses (arrow in Figure 6D), and Esl/El ratio 0.72–0.78 ( $n = 5$ , mean: 0.76). In other congeners, combination of relative characters are either eyeless and no apophyses, or eyes distinct and no apophyses, or eyes distinct and apophyses present, and Esl/El ratio 0.33–0.67.

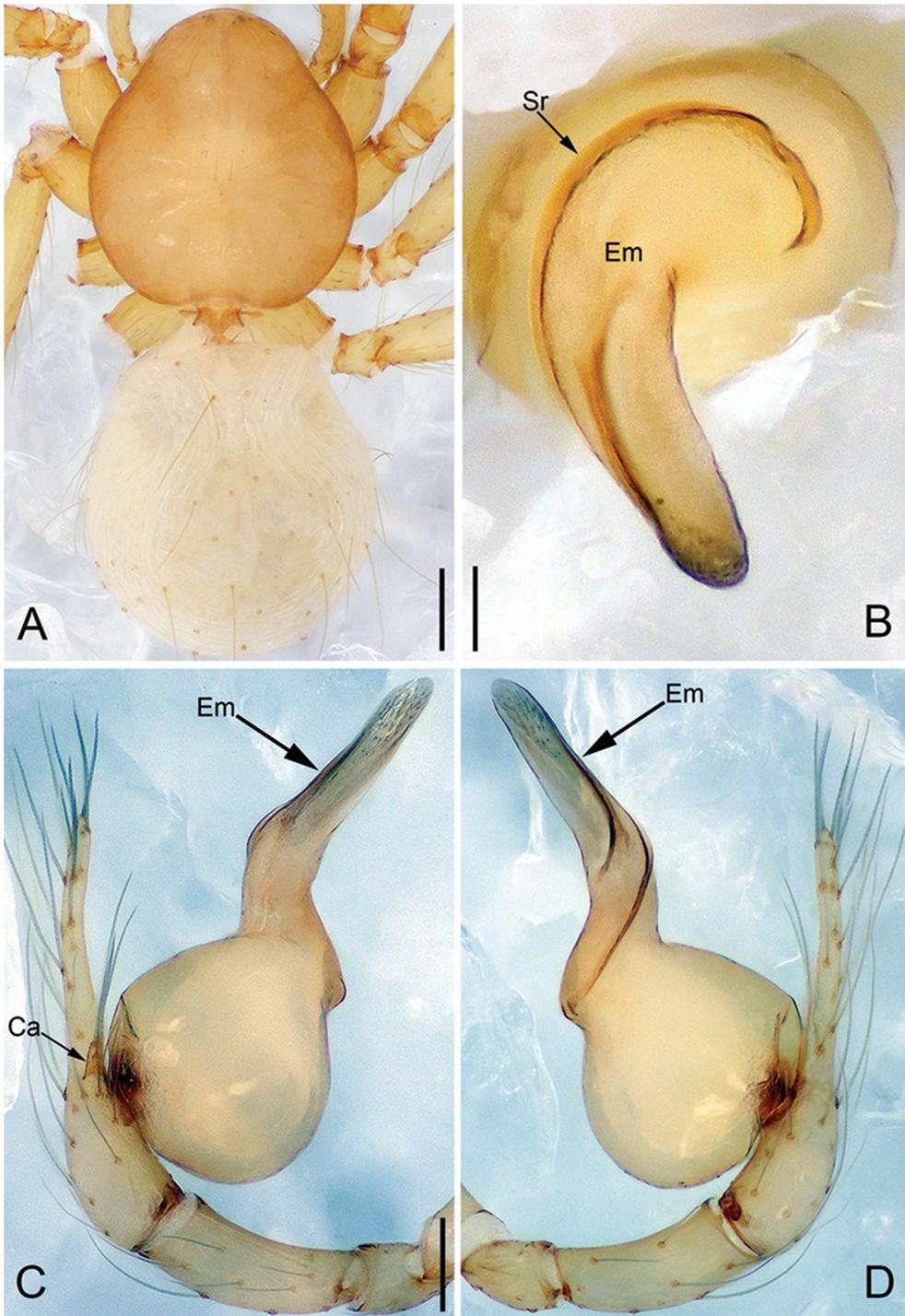
**Description. Male (holotype):** Total length 1.58. Carapace 0.75 long, 0.64 wide. Abdomen 0.84 long, 0.71 wide. Carapace brown (Figure 7A). Eyeless (Figure 7A). Chelicerae, legs, labium, and endites brown. Sternum bright brown with sparse setae. Leg measurements: I 6.23 (1.84, 0.28, 1.96, 1.39, 0.76); II 5.42 (1.64, 0.27, 1.70, 1.18, 0.63); III 3.53 (1.28, 0.22, 1.18, 0.85, 0.53); IV 4.98 (1.60, 0.22, 1.44, 1.10, 0.62). Abdomen pale yellow with a few long setae (Figure 7A).

Palp: Tibia 2.0 times longer than patella, cymbium 2.2 times longer than tibia, cymbial apophysis dark brown and cone-shaped (Figure 7C); bulb shaped as in Figure 7D; embolus with numerous small apophyses distally (arrow in Figure 6D), spiral ridge brown (Figure 7B), El/Bl ratio 1.42, and Esl/El ratio 0.76.

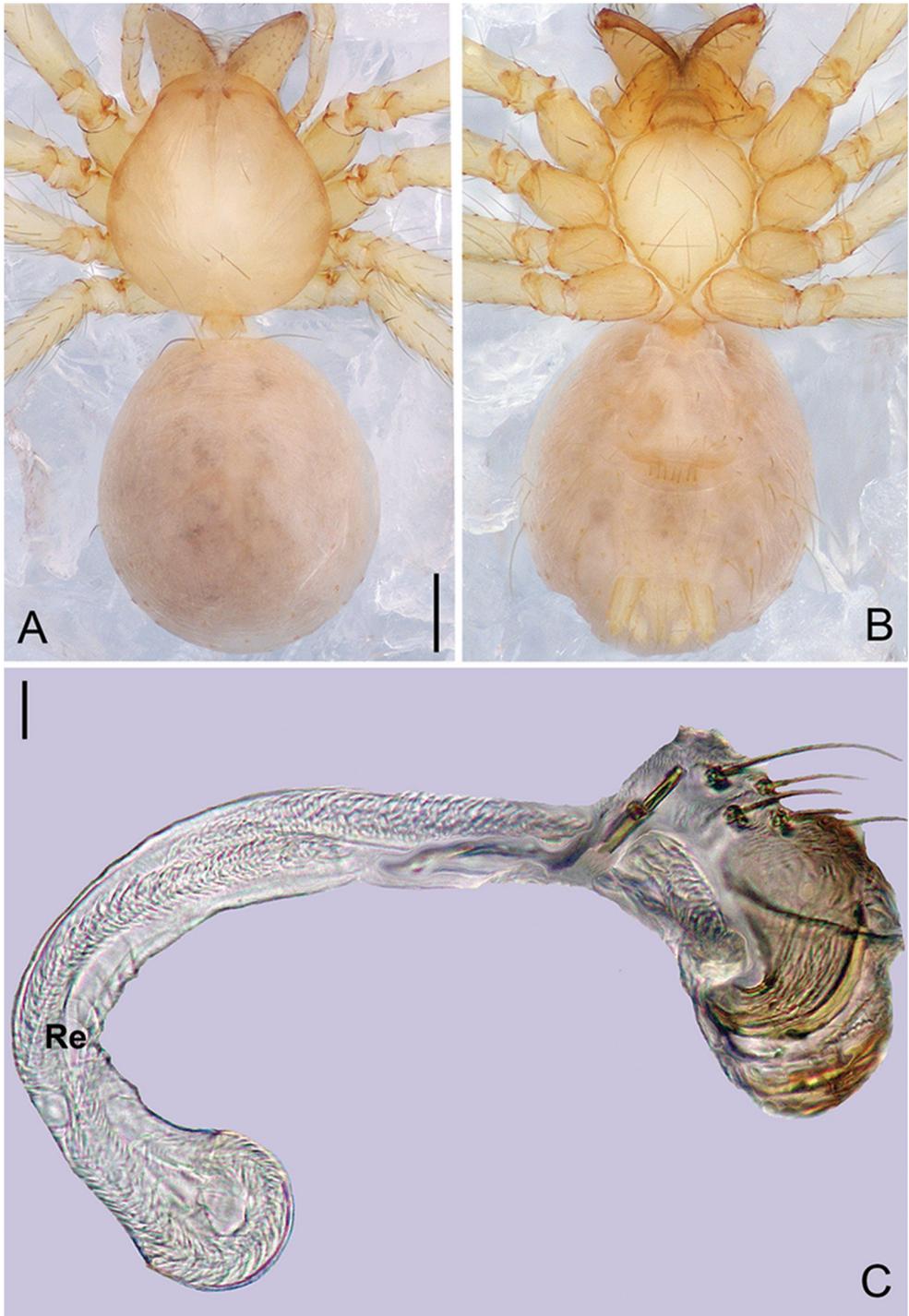
**Female:** Total length 1.48. Carapace 0.65 long, 0.58 wide. Abdomen 0.85 long, 0.71 wide. Coloration as in male (Figure 8A, B). Leg measurements: I 5.89 (1.76, 0.25, 1.88, 1.25, 0.75); II 5.26 (1.62, 0.25, 1.64, 1.08, 0.67); III 3.75 (1.19, 0.23,



**Figure 6.** *Pinelema huoyan* Zhao & Li, sp. n., male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 7.** *Pinelema huoyan* Zhao & Li, sp. n., male holotype. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C-D**).



**Figure 8.** *Pinelema huoyan* Zhao & Li, sp. n., female paratype. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

1.13, 0.73, 0.47); IV 4.64 (1.50, 0.24, 1.34, 0.96, 0.60). Abdomen light brown. Insemination duct with a membranous tube (Figure 8C); receptacle as wide as insemination duct, U-shaped, slightly swollen at end (Figure 8C).

**Variation.** In 4♂ paratypes: El/Bl ratio 1.38–1.43, Esl/El ratio 0.72–0.78.

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

***Pinelema liangxi* (Zhu & Chen, 2002), comb. n.**

Figs 9–11, 31

*Telema liangxi*: Zhu and Chen 2002: 82, figs 1–7 (♂♀); Chen and Zhu 2009: 1709, figure 3E, M–N (♂♀).

**Type material.** holotype ♂ (MHBU), China, Guizhou Province, Qiannan Prefecture, Libo County, Maolan National Nature Reserve: Liangxi Cave, 24.5°N, 100.2°E, 5.XI.1999, H. Chen leg. Paratypes (MHBU): 2♀, same data as holotype. Not examined.

**Material examined.** 5♂ and 8♀ (including five molecular vouchers, IZCAS) from the type locality, 25°12'N, 108°00'E, 15.III.2011, C. Wang and L. Lin leg.

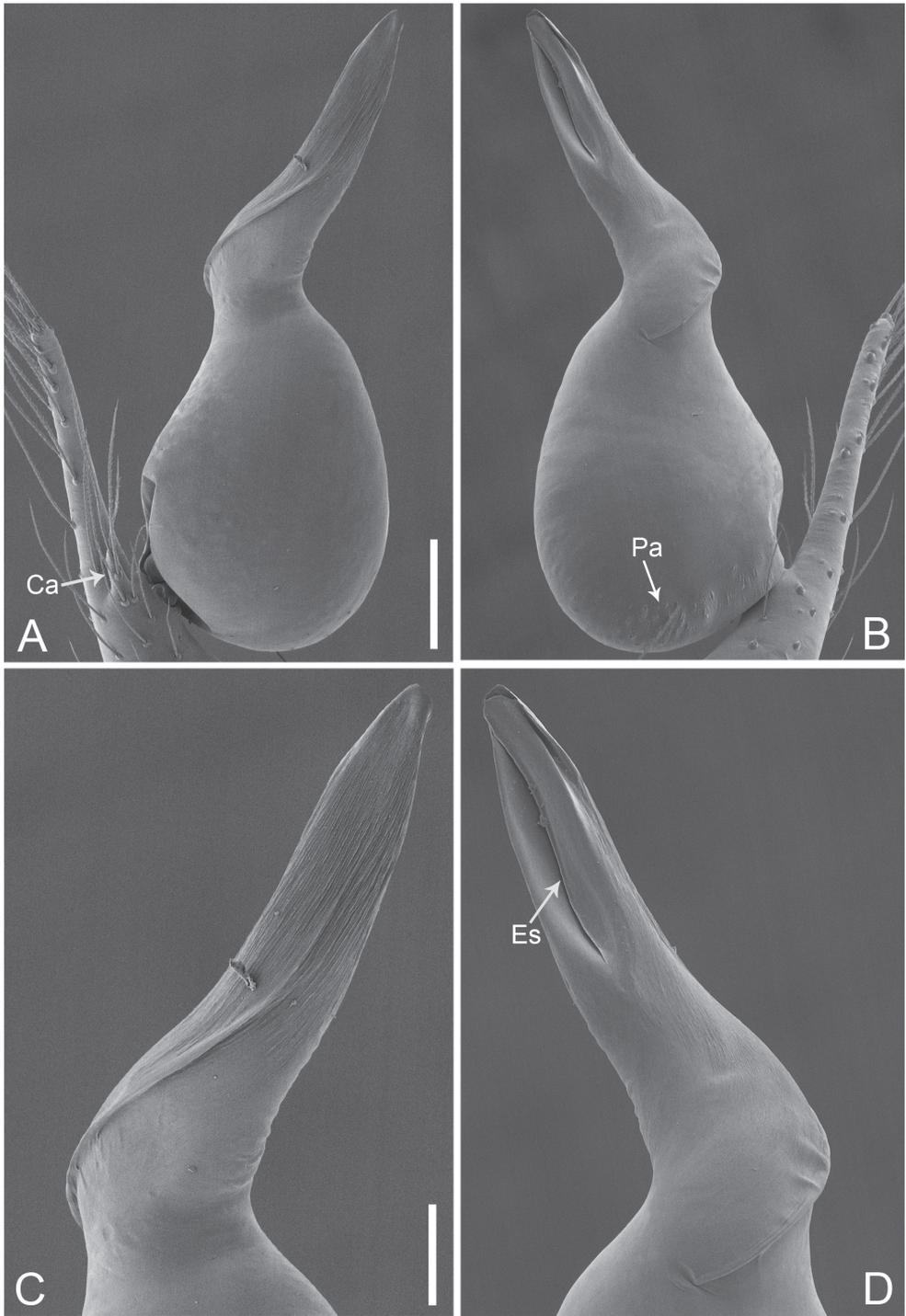
**Diagnosis.** *Pinelema liangxi* resembles *P. strentarsi* (see Figs 15–17 and Lin and Li 2010: 23, figs 14–15) but can be distinguished by following characters: embolus curved (arrow 1 in Figure 10D) (embolus straight in *P. strentarsi*), bulb not protruding ventro-subdistally (arrow 2 in Figure 10D) (bulb protruding ventro-subdistally in *P. strentarsi*), and bulb not curved dorso-medially (arrow 3 in Figure 10D) (bulb curved dorso-medially in *P. strentarsi*).

**Description.** Male palp: Cymbial apophysis light brown (Figure 10C); bulb with a few papillae proximo-retrolaterally (Figure 9B); El/Bl ratio 1.14–1.25 (n = 5, mean: 1.20, Suppl. material 1: Figure S5), Esl/El ratio 0.49–0.51 (n = 5, mean: 0.50, Suppl. material 1: Figure S5). Endogyne: receptacle thin and long, U-shaped (see Figure 11C and Chen and Zhu 2009: figure 3M, N). For more detailed descriptions, see Zhu and Chen (2002).

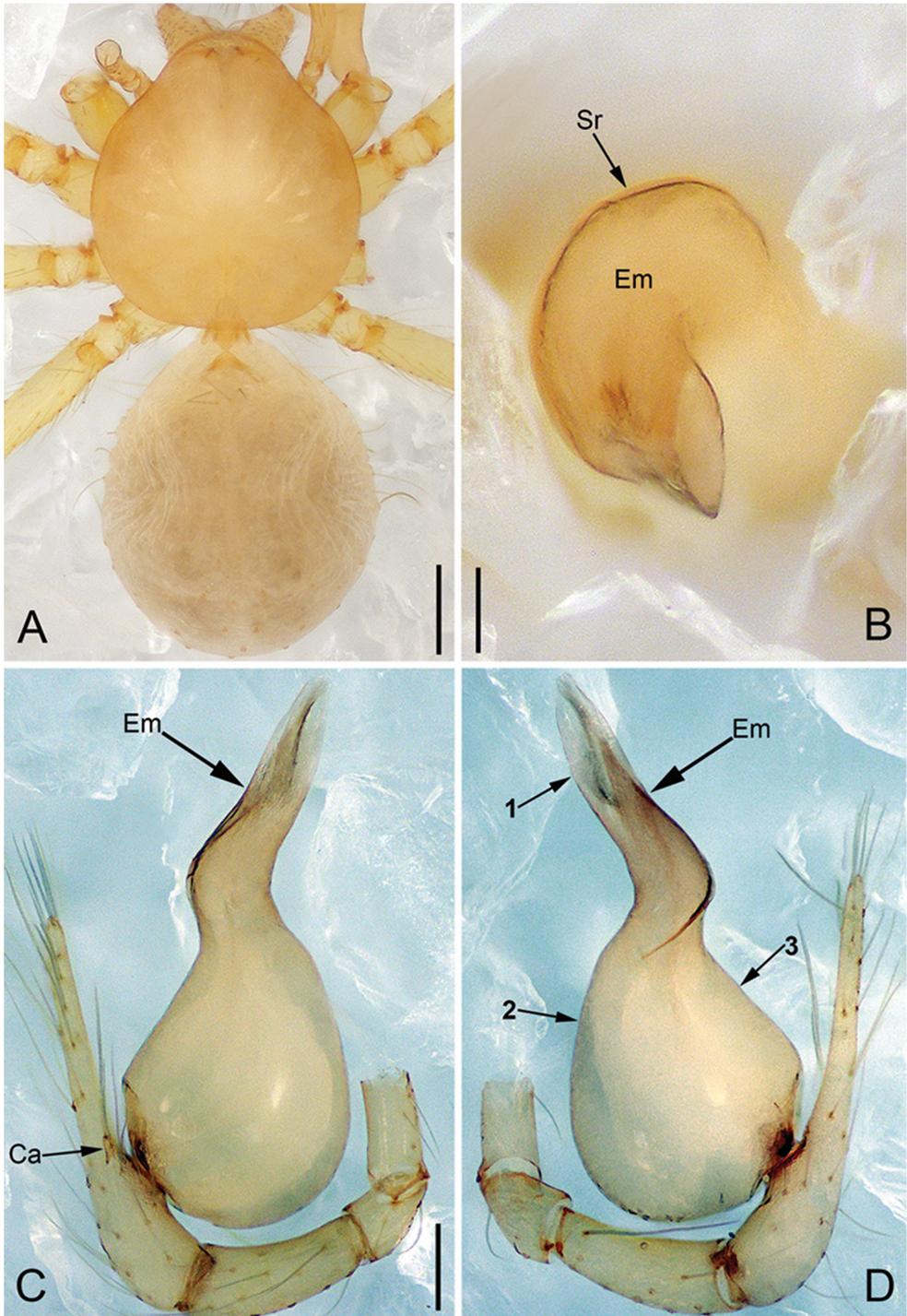
**Comments.** Because this species shares similar morphological characters with *P. bailongensis*, such as the long, tube-shaped embolus (see Figs 9A–D, 10C–D and Zhu and Chen 2002: figs 5–6), the presence of a distinct cymbial apophysis in the male palp prolaterally (see Figs 9A, 10C and Zhu and Chen 2002: figure 5), and the U-shaped and medially strongly curved receptacle (Figure 11C), it is transferred to *Pinelema*.

**Remarks.** The coordinates of the type locality of this species in Zhu and Chen (2002) is wrong because they refer to the place located approximately 700 kilometers from Liangxi Cave. The coordinate information reported here is confirmed by Dr H. Chen who collected type material.

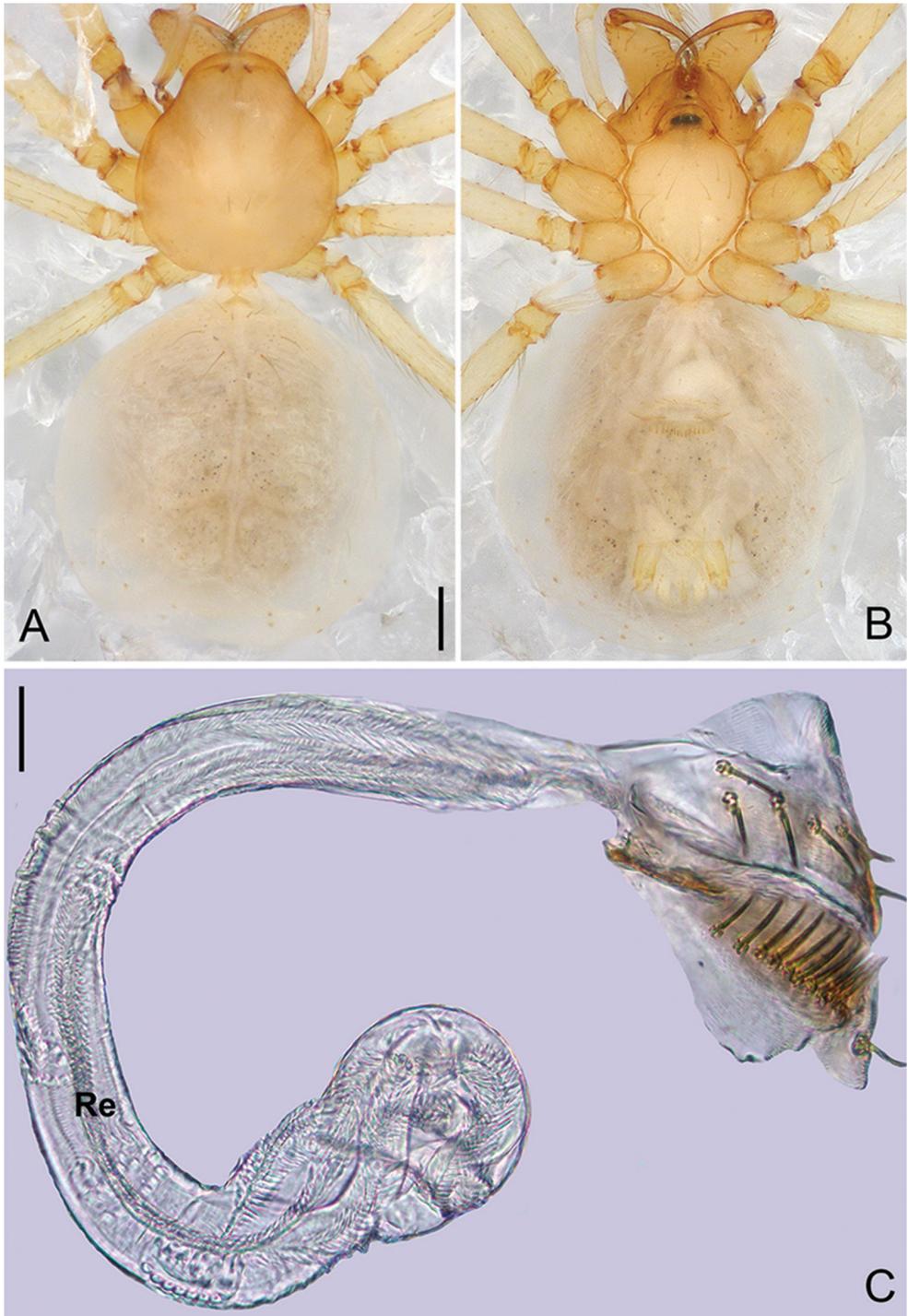
**Distribution.** China (Guizhou, Figure 31), known only from the type locality.



**Figure 9.** *Pinelema liangxi*, male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 10.** *Pinelema liangxi*, male. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C-D**).



**Figure 11.** *Pinelema liangxi*, female. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

***Pinelema lizhuang* Zhao & Li, sp. n.**

<http://zoobank.org/8C9BA784-6DBB-4DA7-B7A8-FAB0F9015D28>

Figs 12–14, 31

**Type material.** **Holotype** ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Hechi Prefecture, Nandan County, Liuzai Town, Lizhuang Village, Cave without a name, 25°18.490'N, 107°22.910'E, 828 m, 28.I.2015, Y. Li and Z. Chen leg. **Paratypes** (IZCAS): 5♂ and 5♀, same data as holotype.

**Other material examined.** 4♀ (molecular vouchers, IZCAS), same data as holotype.

**Etymology.** The species epithet refers to the type locality; noun.

**Diagnosis.** This species resembles *P. wenyang* Zhao & Li, sp. n. (Figs 21–23) but can be distinguished by following characters: bulb curved dorso-medially (Figure 13D) (bulb curved dorso-subdistally in *P. wenyang* Zhao & Li, sp. n.), larger Esl/El ratio (0.63–0.67, n = 6, mean = 0.65, Suppl. material 1: Figure S6) (smaller Esl/El ratio 0.51–0.55, n = 5, mean = 0.53 in *P. wenyang* Zhao & Li, sp. n.), and smaller El/Bl ratio (1.24–1.31, n = 6, mean: 1.27, Suppl. material 1: Figure S6) (larger El/Bl ratio 1.47–1.54, n = 5, mean: 1.49 in *P. wenyang* Zhao & Li, sp. n.).

**Description. Male (holotype):** Total length 1.20. Carapace 0.50 long, 0.51 wide. Abdomen 0.67 long, 0.56 wide. Carapace pale brown (Figure 13A). Six eyes ringed with black (Figure 13A), clypeus height 0.11, ocular quadrangle 0.15 wide. Chelicerae, legs, labium, and endites light yellow with dark brown dots. Sternum dark brown with sparse setae. Leg measurements: I 3.83 (1.13, 0.19, 1.18, 0.78, 0.55); II 3.15 (0.92, 0.19, 0.96, 0.59, 0.49); III 2.29 (0.67, 0.16, 0.63, 0.43, 0.40); IV 2.36 (0.87, 0.18, 0.78, 0.53, 0.42). Abdomen purple-bluish (Figure 13A).

Palp: Tibia 2.9 times longer than patella, cymbium 2.0 times longer than tibia, cymbial apophysis cone-shaped (Figure 13C); bulb hemispherical (Figure 13C, D); spiral ridge brown (Figure 13B), El/Bl ratio 1.26 (Figure 13D), the distal part of embolus black (Figure 13C, D), and Esl/El ratio 0.65 (Figure 13D).

**Female:** Total length 1.39. Carapace 0.50 long, 0.48 wide. Abdomen 0.85 long, 0.73 wide. Coloration as in male (Figure 14A–B). Six eyes, well-developed, clypeus height 0.11, ocular quadrangle 0.17 wide. Leg measurements: I 3.42 (1.00, 0.19, 1.05, 0.65, 0.53); II 2.86 (0.85, 0.19, 0.85, 0.53, 0.44); III 2.06 (0.63, 0.19, 0.53, 0.37, 0.34); IV 2.68 (0.85, 0.19, 0.76, 0.48, 0.40). Abdomen purple-bluish (Figure 14A). Insemination duct as wide as receptacle (Figure 14C); receptacle C-shaped with several membranous tubes inside (Figure 14C).

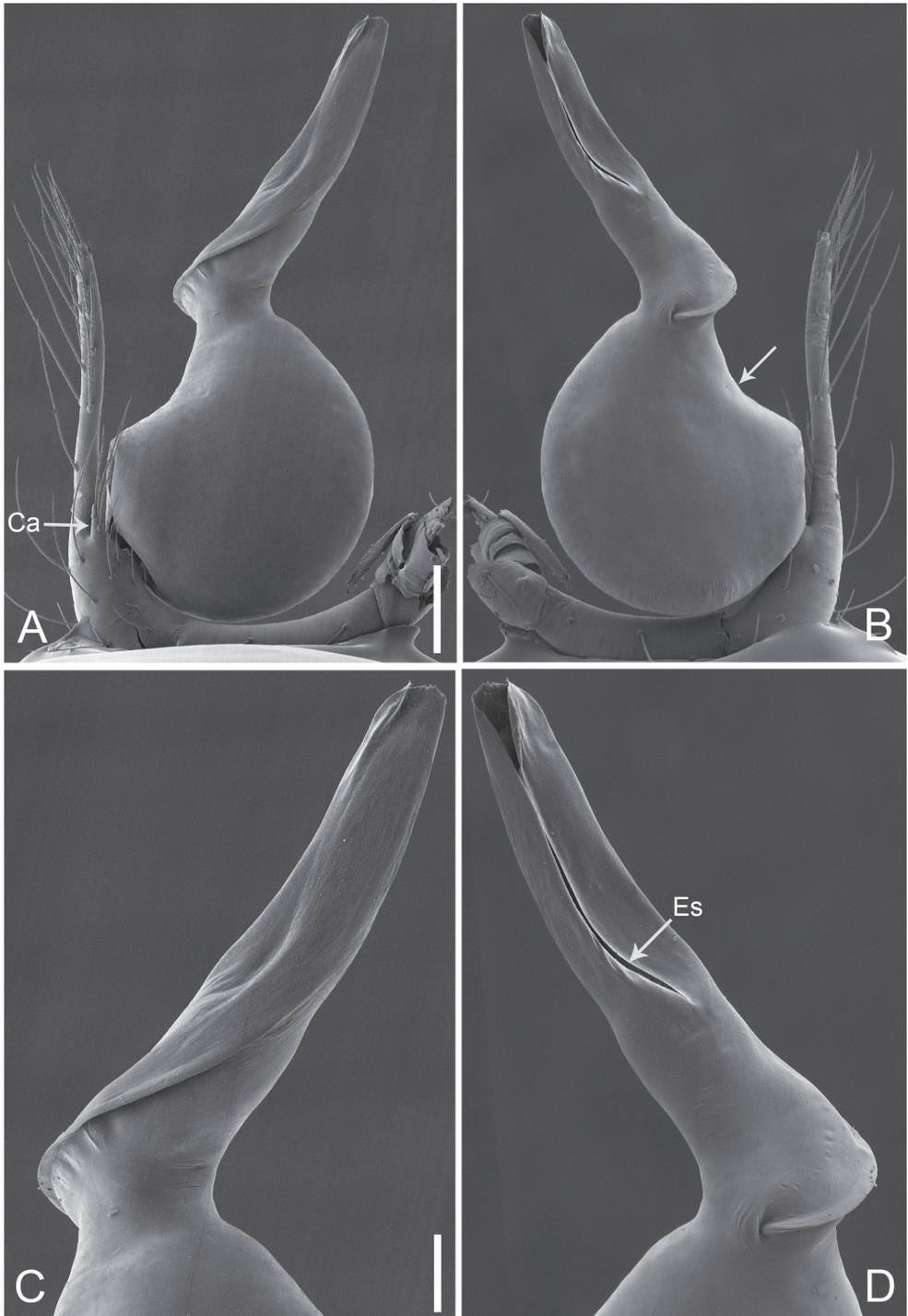
**Variation.** In 5♂ paratypes: El/Bl ratio 1.24–1.31, Esl/El ratio 0.63–0.67.

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

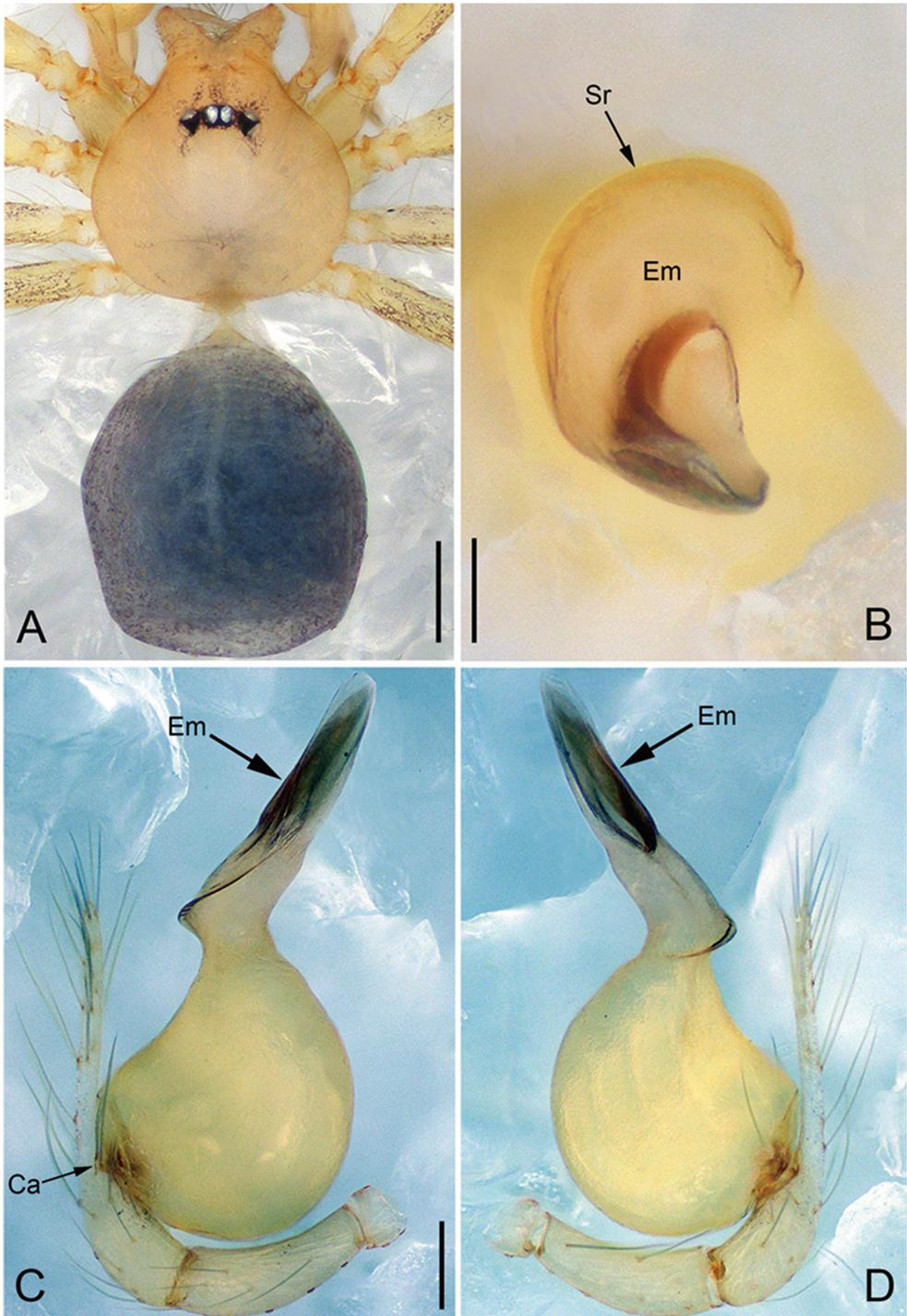
***Pinelema strentarsi* (Lin & Li, 2010) comb. n.**

Figs 15–17, 31

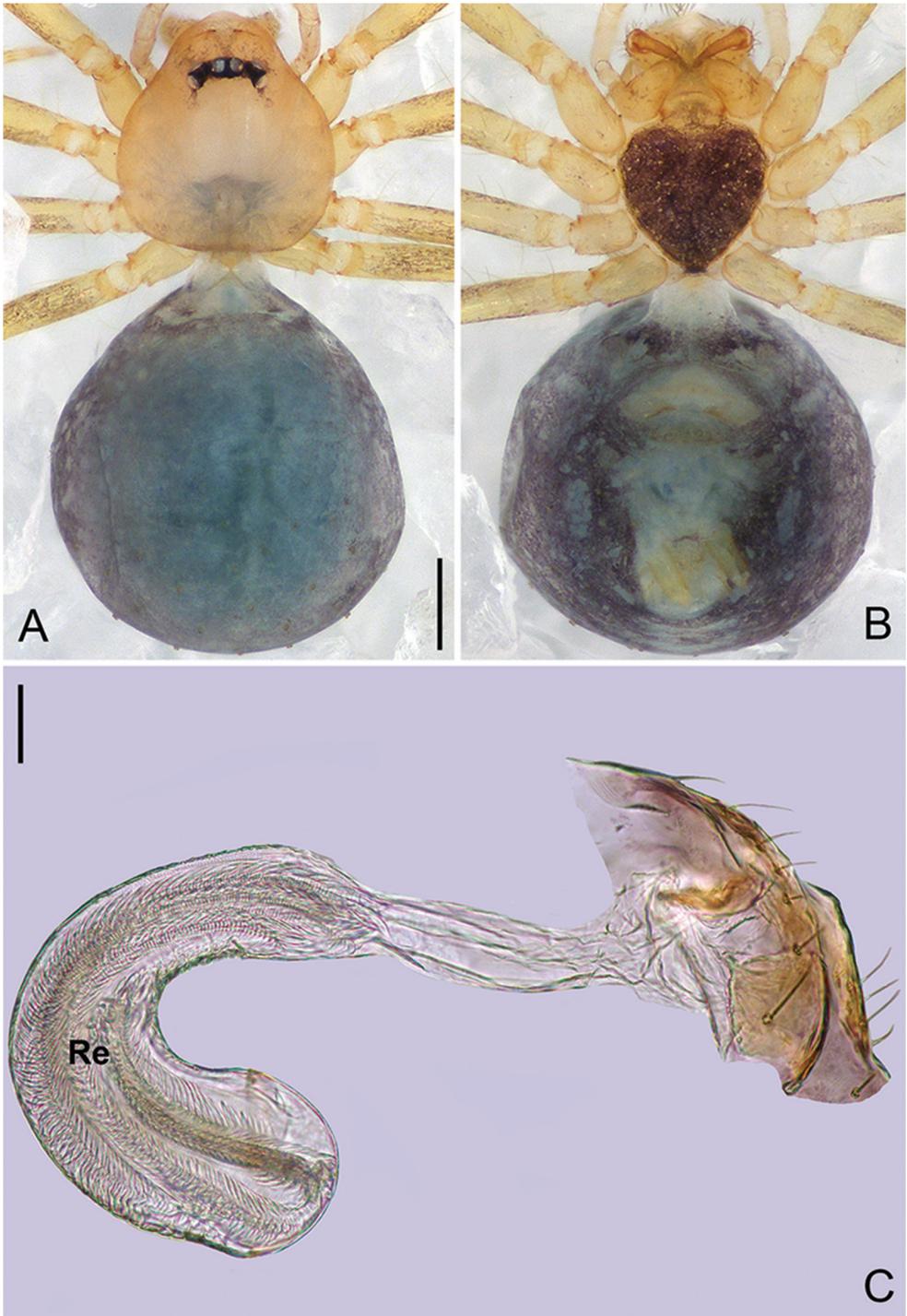
*Telema strentarsi*: Lin and Li 2010: 23, figs 14–15 (♂♀).



**Figure 12.** *Pinelema lizhuang* Zhao & Li, sp. n., male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 13.** *Pinelema lizhuang* Zhao & Li, sp. n., male holotype. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C-D**).



**Figure 14.** *Pinelema lizhuang* Zhao & Li, sp. n., female paratype. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

**Type material examined.** Holotype ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Hechi Prefecture, Dahua County, Jiangnan Township, Huangniu Cave, 23°55.120'N, 107°37.479'E, 8.III.2007, J. Liu and Y. Lin leg. Paratypes: 1♂ and 6♀ (IZCAS), same data as holotype.

**Other material examined.** 4♂ and 5♀ (molecular vouchers, IZCAS) from the type locality, 175 m, 10.IV.2017, Z. Chen leg.

**Diagnosis.** This species resembles *P. liangxi* (see Figs 9–11, Zhu and Chen 2002: 82, figs 1–7 and Chen and Zhu 2009: 1709, figure 3E, M–N) but can be differentiated by following characters: embolus straight (arrow 1 in Figure 16D) (embolus curved in *P. liangxi*), bulb protruding ventro-subdistally (arrow 2 in Figure 16D) (bulb not protruding ventro-subdistally in *P. liangxi*), and bulb slightly curved dorso-medially (arrow 3 in Figure 16D) (bulb not curved dorso-medially in *P. liangxi*).

**Description.** Male palp: Cymbial apophysis finger-like (Figs 15A, 16C); spiral ridge pale brown (Figure 16B), El/Bl ratio 1.15–1.21 (n = 4, mean: 1.18, Suppl. material 1: Figure S7), Esl/El ratio 0.48–0.49 (n = 4, mean: 0.48, Suppl. material 1: Figure S7), Female endogyne: insemination duct wider than receptacle, receptacle comma-shaped (Figure 17C). For more detailed descriptions, see Lin and Li (2010).

**Comments.** This species is transferred to *Pinelema*, because it shares similar morphological characters with *P. bailongensis*, such as the long, tube-like embolus (see Figs 15A–D, 16C–D and Lin and Li 2010: figure 14D–F), the presence of a distinct cymbial apophysis in male palp prolaterally (see Figs 15A, 16C and Lin and Li 2010: figure 14E), and the U-shaped and medially strongly curved receptacle (see Figure 17C and Lin and Li 2010: figure 15F).

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

### *Pinelema wangshang* Zhao & Li, sp. n.

<http://zoobank.org/4EE41668-F748-4EC3-AA54-FAF109BB407B>

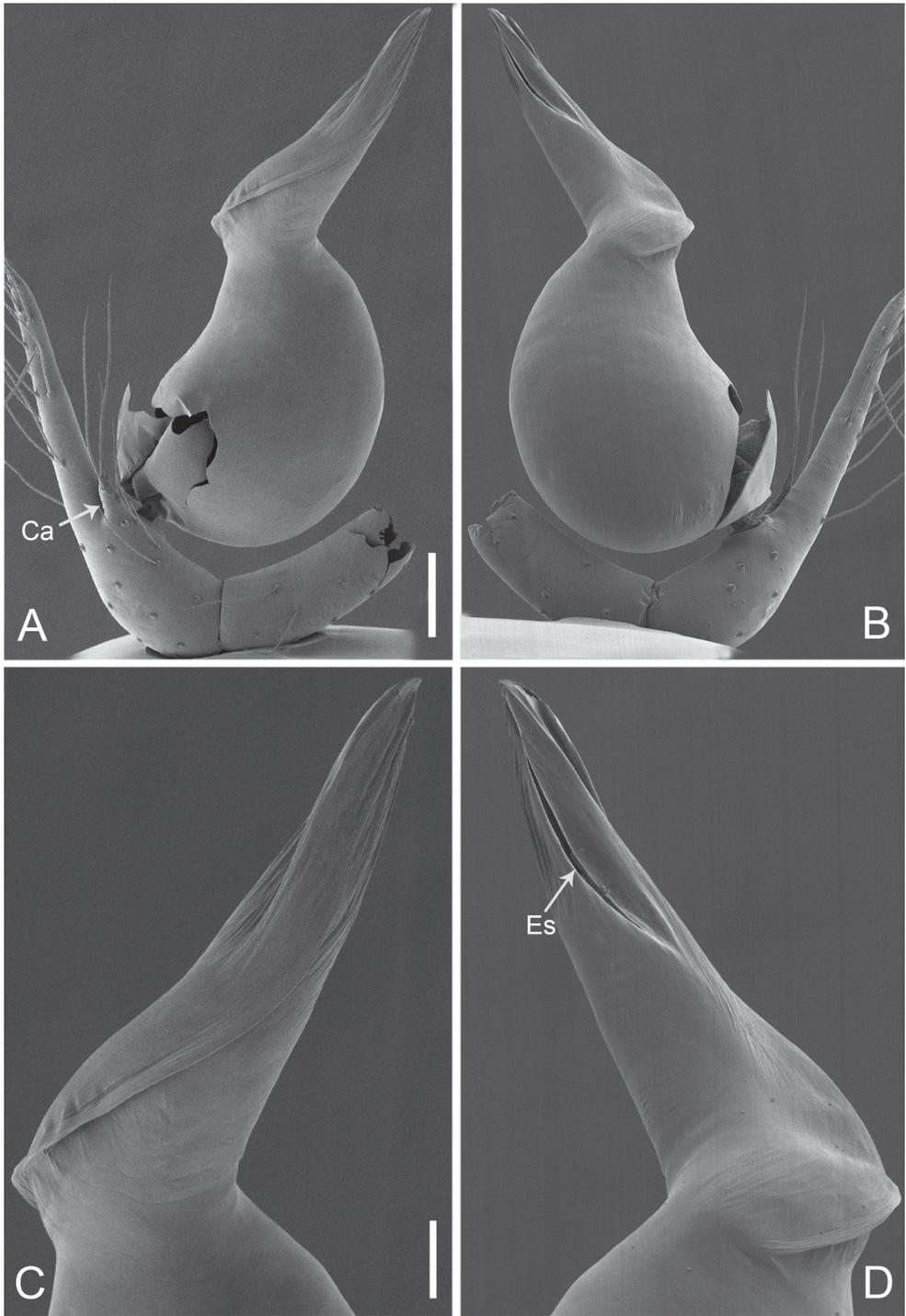
Figs 18–20, 31

**Type material. Holotype** ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Hechi Prefecture, Nandan County, Lihu Town, Wangshang Village, Wangshang Cave, 25°05.300'N, 107°38.420'E, 602 m, 1.II.2015, Y. Li and Z. Chen leg. **Paratypes** (IZCAS): 4♂ and 5♀, same data as holotype.

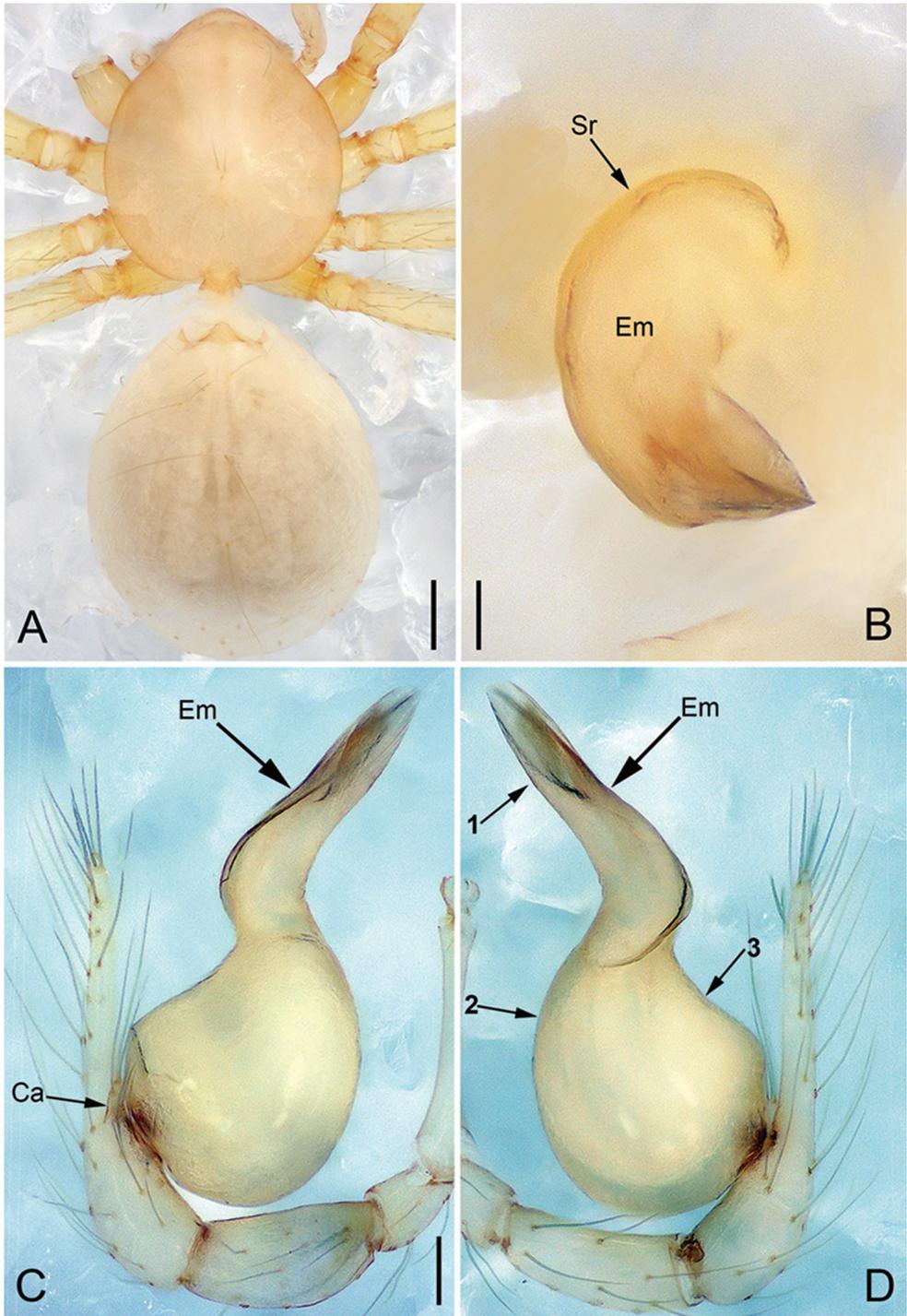
**Other material examined.** 4♀ (molecular vouchers, IZCAS), same data as holotype.

**Etymology.** The species epithet refers to the type locality; noun.

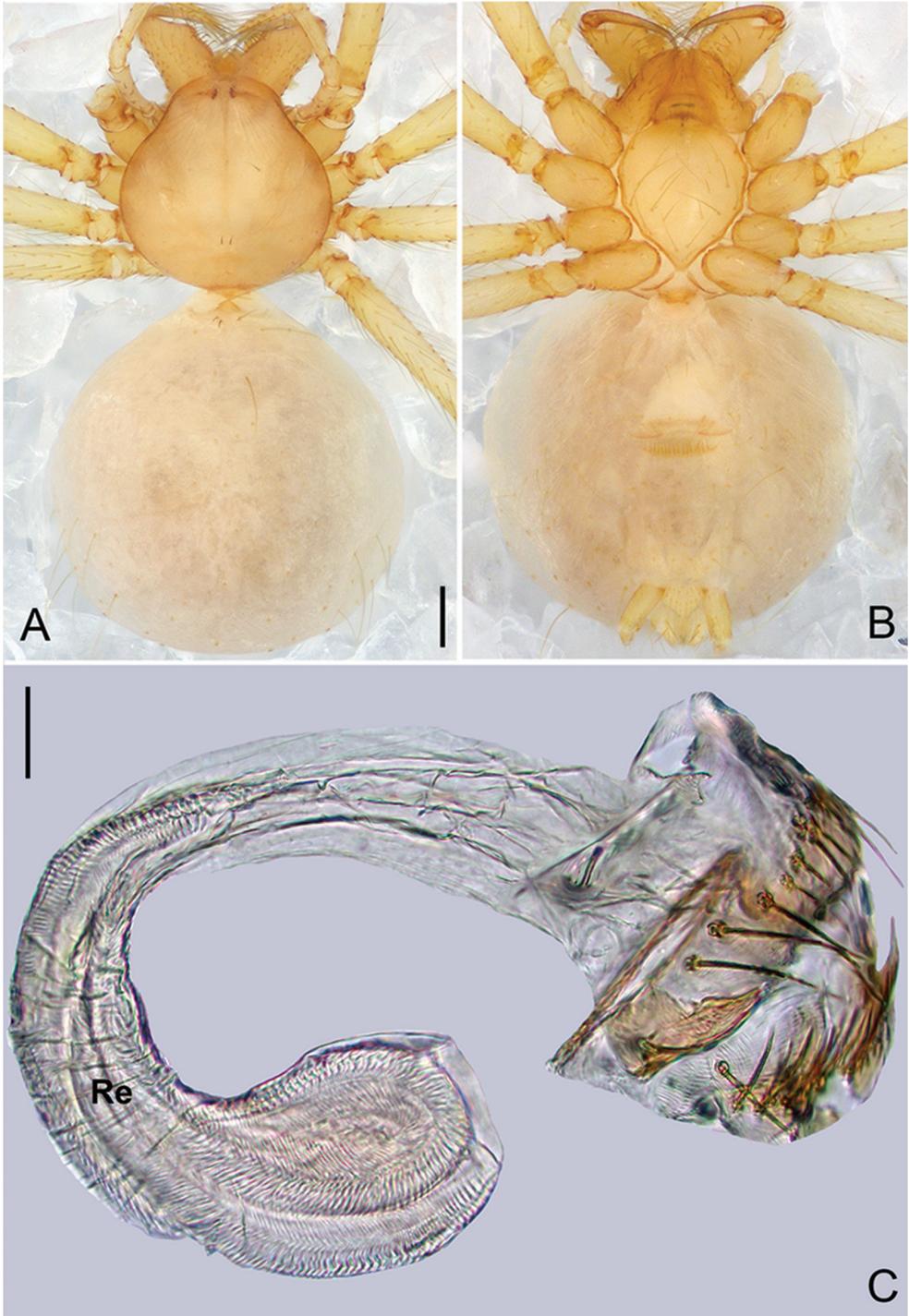
**Diagnosis.** This species resembles *P. yunchuni* Zhao & Li, sp. n. (Figs 25–27) but can be distinguished by following characters: bulb kidney-shaped (Figs 18A, B, 19C, D) (bulb pear-shaped in *P. yunchuni* Zhao & Li, sp. n.), curved dorso-medially (Figs 18A, B, 19C, D) (bulb not curved dorso-medially in *P. yunchuni* Zhao & Li, sp. n.), smaller El/Bl ratio (1.37–1.45, n = 5, mean: 1.41, Suppl. material 1: Figure S8) (larger El/Bl ratio 1.78–1.82, n = 4, mean: 1.80 in *P. yunchuni* Zhao & Li, sp. n.),



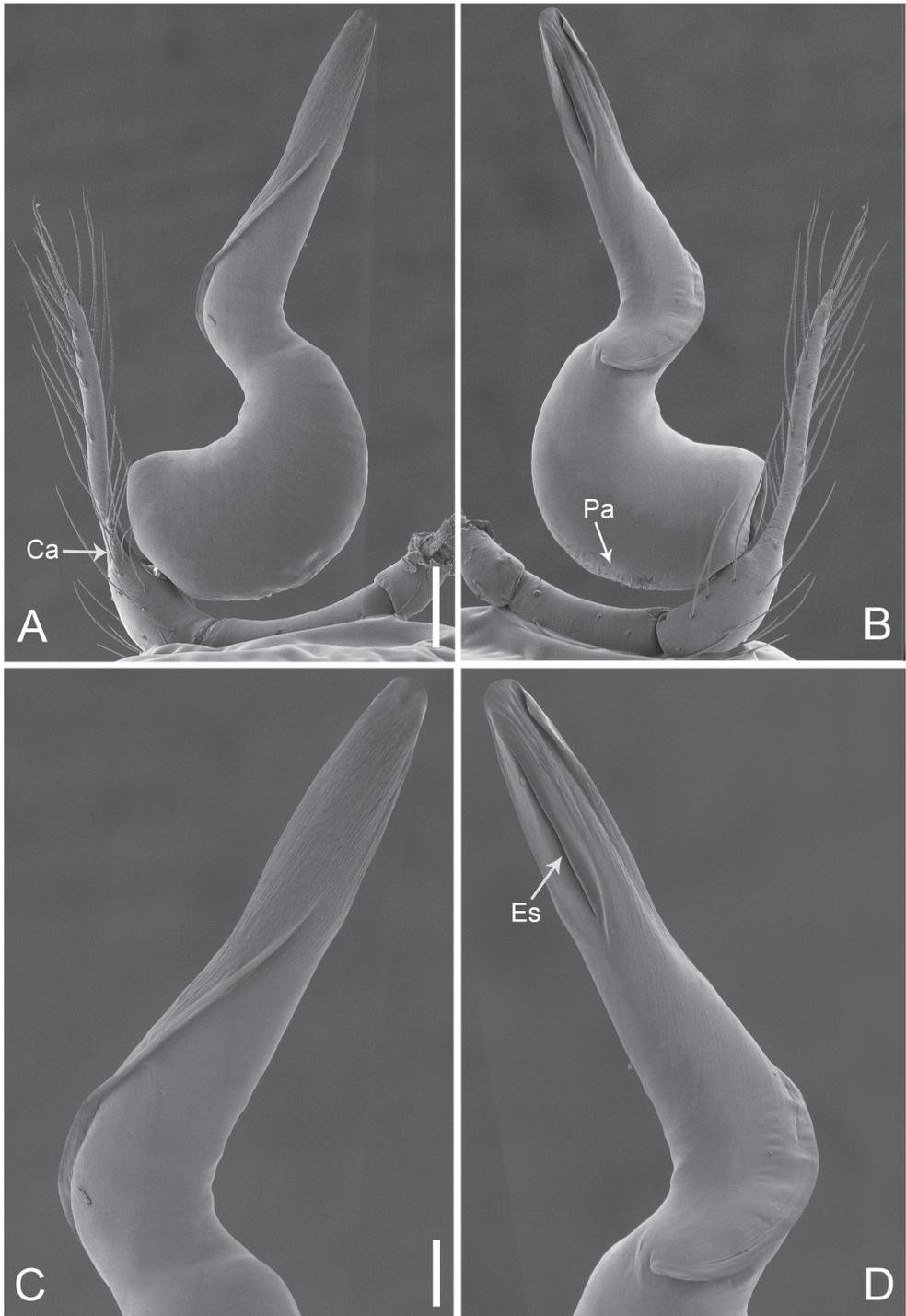
**Figure 15.** *Pinelema strentarsi*, male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



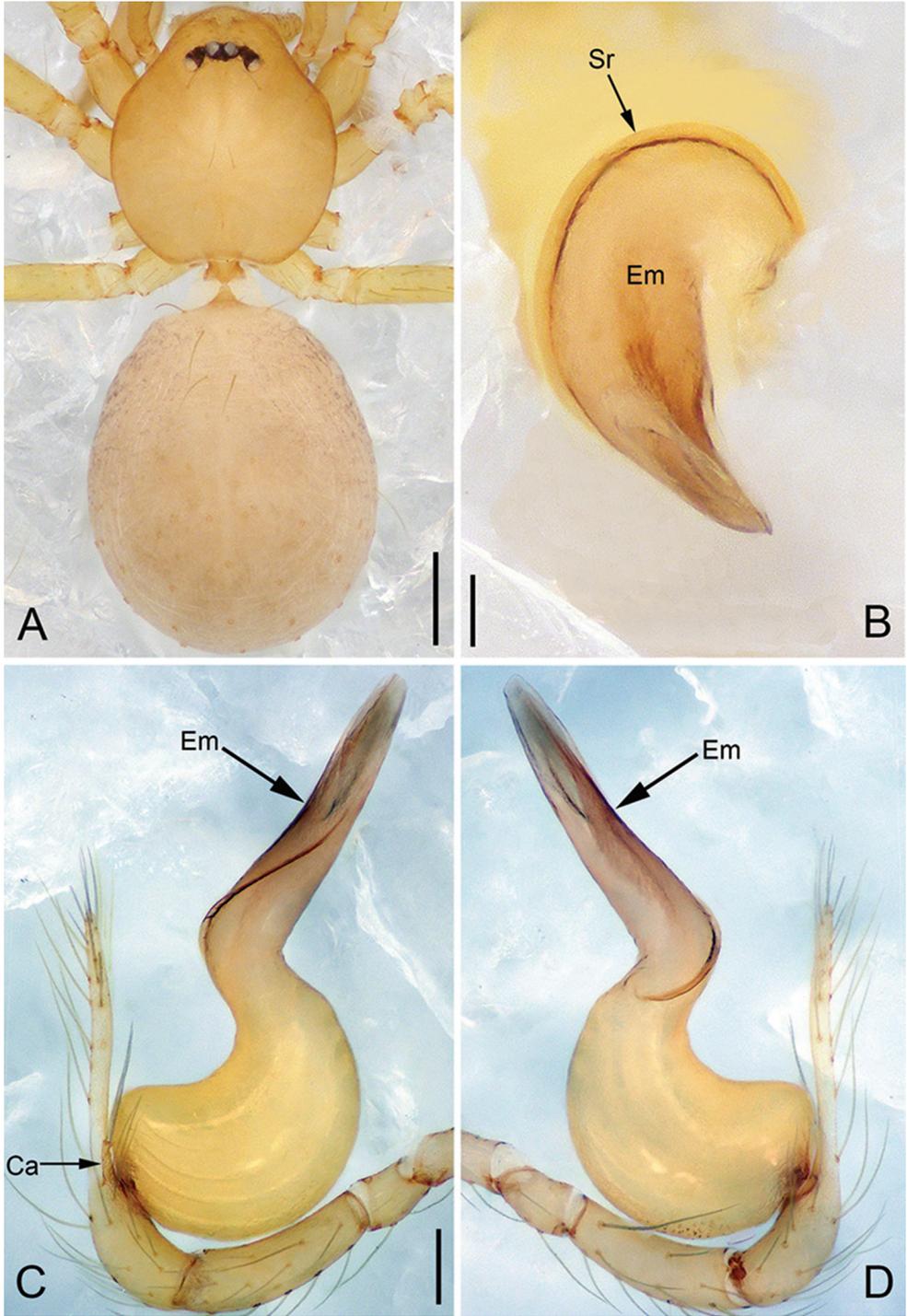
**Figure 16.** *Pinelema strentarsi*, male. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C-D**).



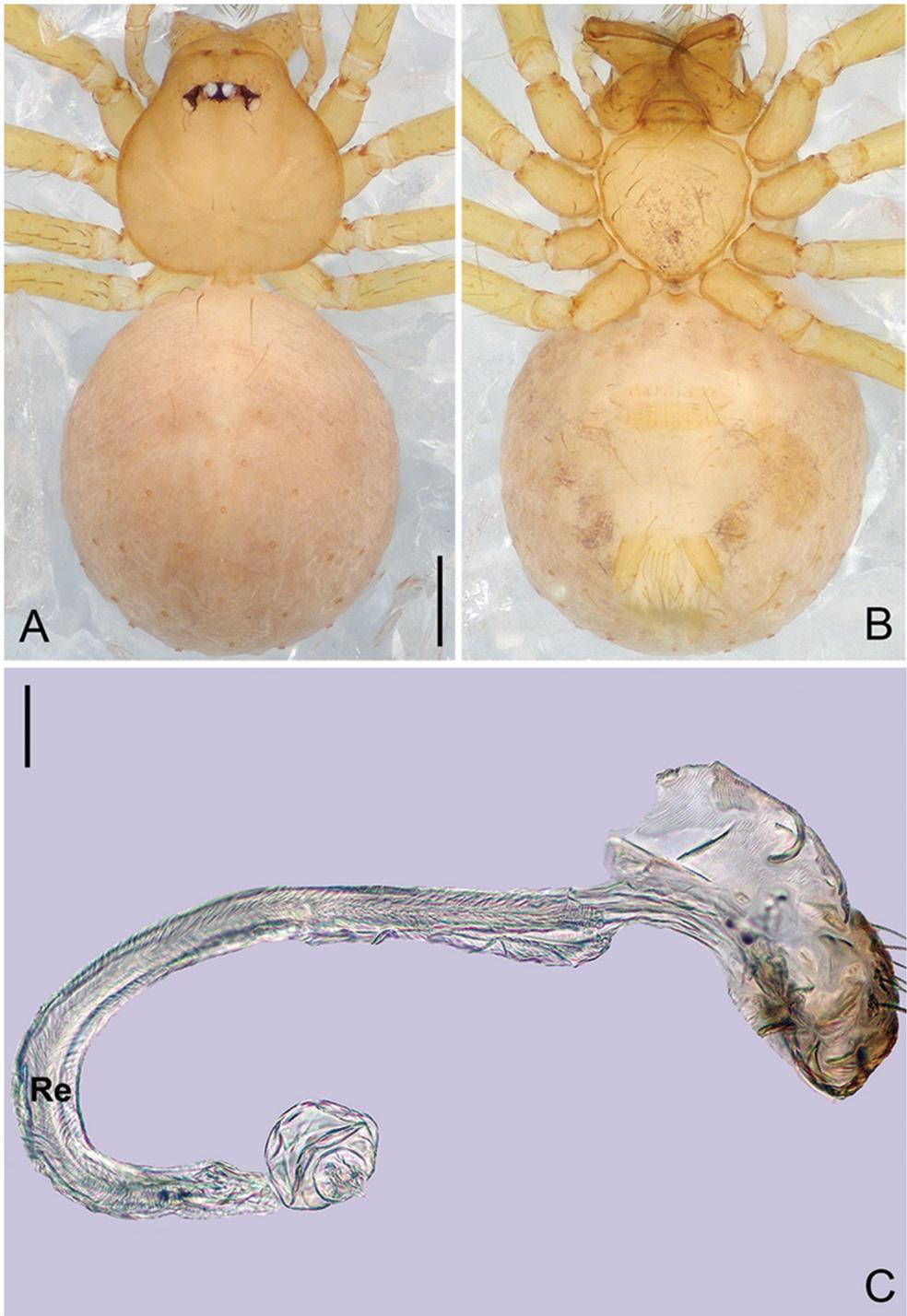
**Figure 17.** *Pinelema strentarsi*, female. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).



**Figure 18.** *Pinelema wangshang* Zhao & Li, sp. n., male. **A** Palp, proteral view **B** Palp, retrolateral view **C** Embolus, proteral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 19.** *Pinelema wangshang* Zhao & Li, sp. n., male holotype. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C–D**).



**Figure 20.** *Pinelema wangshang* Zhao & Li, sp. n., female paratype. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

and receptacle J-shaped (Figure 20C) (receptacle U-shaped in *P. yunchuni* Zhao & Li, sp. n.).

**Description. Male (holotype):** Total length 1.39. Carapace 0.54 long, 0.48 wide. Abdomen 0.79 long, 0.60 wide. Carapace brown (Figure 19A). Six eyes ringed with black, clypeus height 0.08, ocular quadrangle 0.15 wide (Figure 19A). Chelicerae, legs, labium, and endites yellow. Sternum light brown. Leg measurements: I 4.41 (1.25, 0.19, 1.41, 0.94, 0.62); II 3.78 (1.11, 0.19, 1.20, 0.75, 0.53); III 2.72 (0.85, 0.18, 0.76, 0.50, 0.43); IV 3.29 (1.05, 0.19, 0.96, 0.63, 0.46). Abdomen oval and light brown (Figure 19A).

Palp: Tibia 3 times longer than patella, cymbium 2 times longer than tibia, cymbial apophysis long and brown (Figure 19C); bulb with a U-shaped curve dorso-medially (Figs 18A–B, 19C–D) and with papillae proximo-retrolaterally (Figure 18B); embolus long and tube-like, spiral ridge distinct and brown (Figure 19B), El/Bl ratio 1.37 (Figure 19D), and Esl/El ratio 0.43 (Figure 19D).

**Female:** Total length 1.38. Carapace 0.54 long, 0.51 wide. Abdomen 0.84 long, 0.76 wide. Coloration as in male (Figure 20A–B). Six eyes ringed with black, clypeus height 0.11, ocular quadrangle 0.17 wide (Figure 20A). Leg measurements: I 4.22 (1.27, 0.18, 1.33, 0.85, 0.59); II 3.68 (1.20, 0.19, 1.08, 0.71, 0.50); III 2.69 (0.85, 0.18, 0.75, 0.50, 0.41); IV 3.37 (1.03, 0.19, 1.00, 0.67, 0.48). Insemination duct short (Figure 20C); receptacle J-shaped, as narrow as insemination duct, end globular (Figure 20C).

**Variation.** In 4♂ paratypes: El/Bl ratio 1.37–1.45, Esl/El ratio 0.42–0.43.

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

### *Pinelema wenyang* Zhao & Li, sp. n.

<http://zoobank.org/81E4223F-A71B-4A14-A615-A7C26306C085>

Figs 21–23, 31

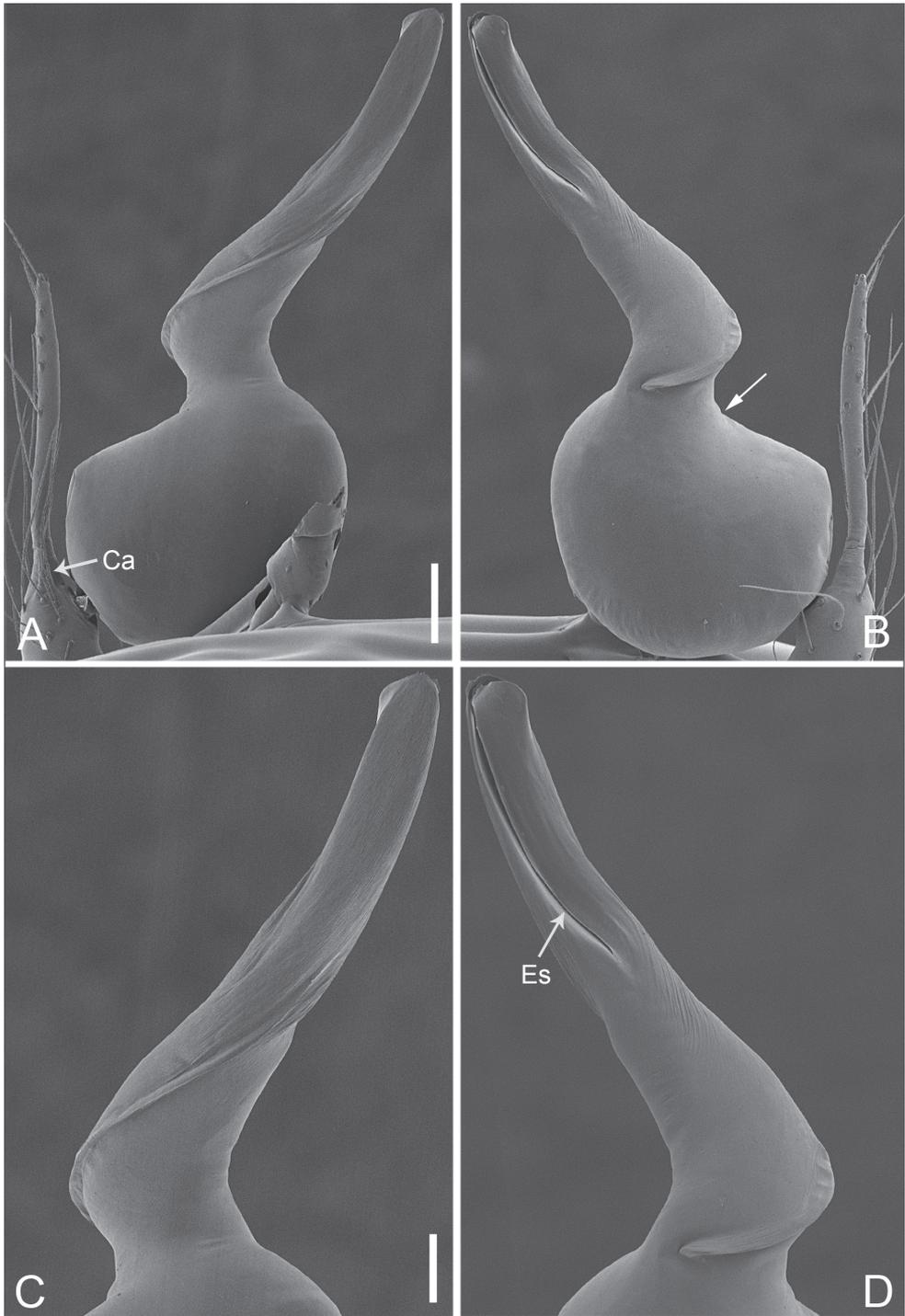
**Type material. Holotype** ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Chongzuo Prefecture, Taiping Township: Wenyang Cave, 22°26.792'N, 107°24.134'E, 180 m, 13.IV.2017, Z. Chen leg. **Paratypes** (IZCAS): 4♂ and 5♀, same data as holotype.

**Other material examined.** 5♀ (molecular vouchers, IZCAS), same data as holotype.

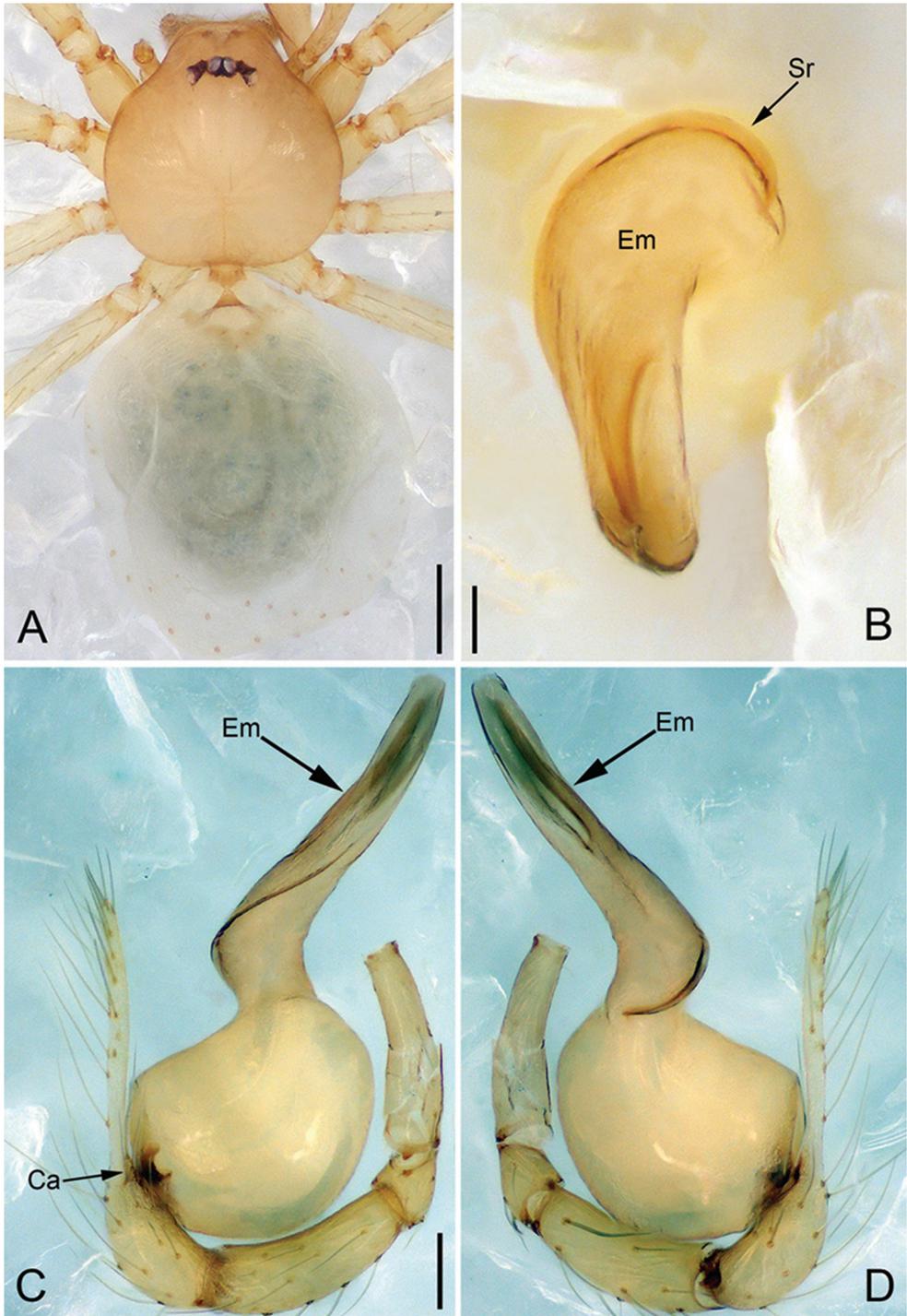
**Etymology.** The species epithet refers to the type locality; noun.

**Diagnosis.** This species resembles *P. lizhuang* Zhao & Li, sp. n. (Figs 12–15) but can be distinguished by following characters: bulb curved dorso-distally (arrow in Figure 21B) (bulb curved dorso-medially in *P. lizhuang* Zhao & Li, sp. n.), smaller Esl/El ratio (0.51–0.55, n = 5, mean: 0.53, Suppl. material 1: Figure S9) (larger Esl/El ratio 0.63–0.67, n = 6, mean: 0.65 in *P. lizhuang* Zhao & Li, sp. n.), and larger El/Bl ratio (1.45–1.54, n = 5, mean: 1.49, Suppl. material 1: Figure S9) (smaller El/Bl ratio 1.23–1.29, n = 6, mean: 1.27 in *P. lizhuang* Zhao & Li, sp. n.).

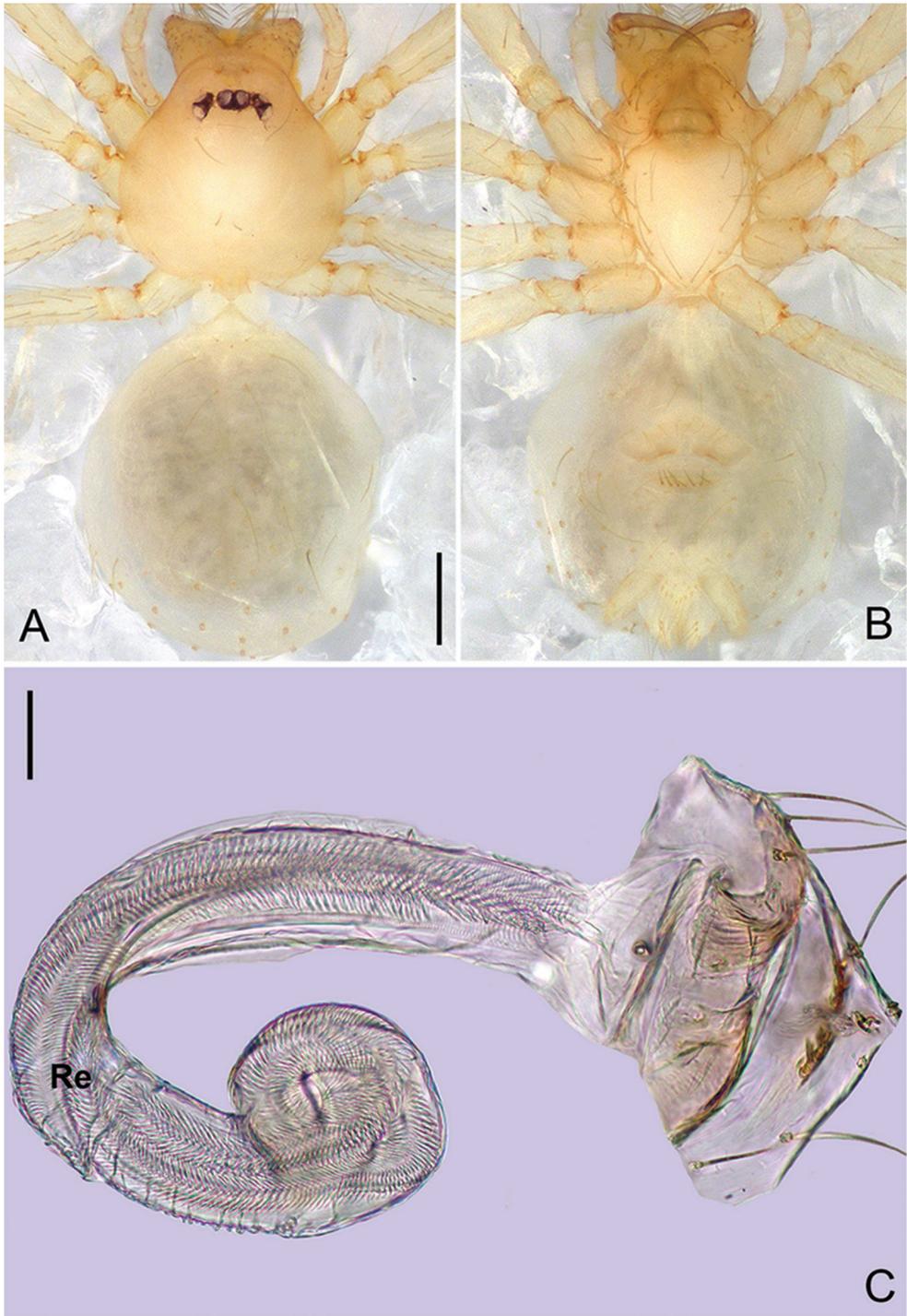
**Description. Male (holotype):** Total length 1.33. Carapace 0.54 long, 0.53 wide. Abdomen 0.79 long, 0.68 wide. Carapace light brown (Figure 22A). Six eyes ringed with black, clypeus height 0.08, ocular quadrangle 0.16 wide (Figure 22A). Chelicerae, legs, labium, and endites light yellow. Sternum light brown. Leg measurements: I 4.55 (1.31, 0.22, 1.45,



**Figure 21.** *Pinelema wenyang* Zhao & Li, sp. n., male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 22.** *Pinelema wenyang* Zhao & Li, sp. n., male holotype. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C–D**).



**Figure 23.** *Pinelema wenyang* Zhao & Li, sp. n., female paratype. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

0.94, 0.63); II 3.86 (1.13, 0.22, 1.19, 0.75, 0.57); III 2.93 (0.85, 0.20, 0.90, 0.53, 0.45); IV 3.34 (1.06, 0.22, 0.95, 0.63, 0.48). Abdomen grey with sparse, long setae (Figure 22A).

Palp: Tibia 3.2 times longer than patella, cymbium 2.1 times longer than tibia, cymbial apophysis approximately cone-shaped (Figs 21A, 22C); bulb strongly protruding ventro-subdistally (Figure 22D) and curved dorso-distally (arrow in Figure 22D); spiral ridge brown (Figure 22B), El/Bl ratio 1.54 (Figure 22D), and Esl/El ratio 0.52 (Figure 22D).

**Female:** Total length 1.33. Carapace 1.53 long, 0.50 wide. Abdomen 0.80 long, 0.67 wide. Coloration as in male (Figure 23A–B). Six eyes, well developed, clypeus height 0.09, ocular quadrangle 0.17 wide (Figure 23A). Leg measurements: I 4.23 (1.25, 0.19, 1.33, 0.83, 0.63); II 3.60 (1.08, 0.19, 1.13, 0.67, 0.53); III 2.69 (0.86, 0.17, 0.75, 0.48, 0.43); IV 3.16 (1.03, 0.18, 0.91, 0.59, 0.45). Insemination duct as wide as receptacle (Figure 23C); receptacle comma-shaped with a globular end (Figure 23C).

**Variation.** In 4♂ paratypes: El/Bl ratio 1.45–1.50, Esl/El ratio 0.51–0.55.

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

### *Pinelema xiushuiensis* Wang & Li, 2016

Figs 24, 31

*Pinelema xiushuiensis*: Wang and Li 2016: 556, figs 9–12 (♂♀).

**Material examined.** Holotype ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Baise Prefecture, Pingguo County, Xingning Village, Xiushui Cave, T: 24°C, RH: 90%, 23°34.048'N, 107°40.777'E, 285 m, 3.VIII.2009, C. Wang and Z. Yao leg. Paratypes: 1♂ and 4♀ (IZCAS), same data as holotype. 5♂ and 5♀ (molecular vouchers, IZCAS), same data as holotype.

**Diagnosis.** This species resembles *P. zhewang* (see Figs 28–30 and Chen and Zhu 2009: 1709, figure 3A–D, F–L, O–Q) but can be differentiated by following characters: cymbial apophysis long (Figure 24A) (cymbial apophysis very short in *P. zhewang*) and smaller Esl/El ratio (0.50–0.52, n = 5, mean: 0.51, Suppl. material 1: Figure S10) (larger Esl/El ratio 0.58–0.63, n = 4, mean: 0.60 in *P. zhewang*).

**Description.** Male palp: Embolus bent (Figure 24A–D), El/Bl ratio 1.58–1.73 (n = 5, Suppl. material 1: Figure S10), and Esl/El ratio 0.50–0.52 (n = 5, Suppl. material 1: Figure S10). For more detailed descriptions, see Wang and Li (2016).

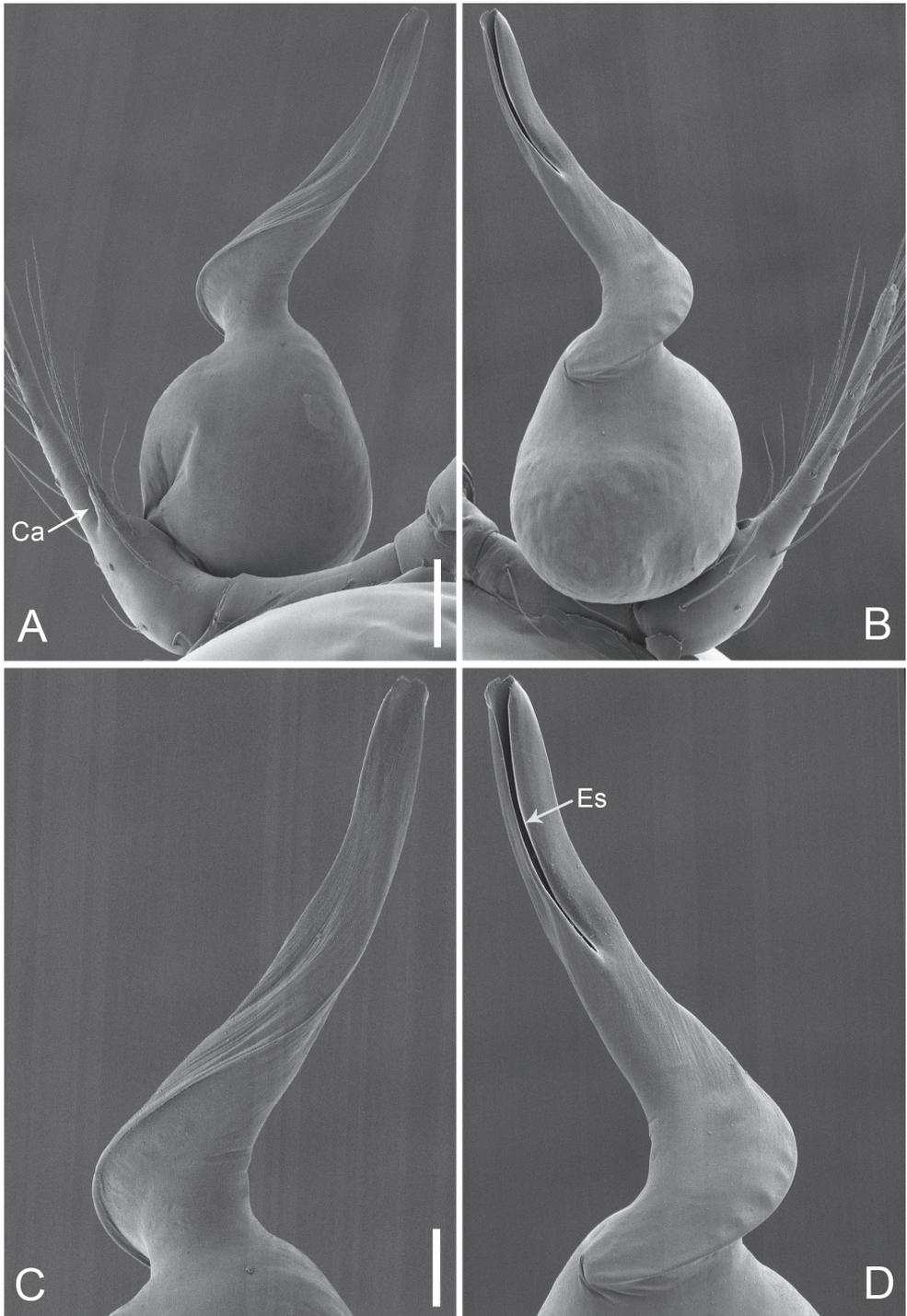
**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

### *Pinelema yunchuni* Zhao & Li, sp. n.

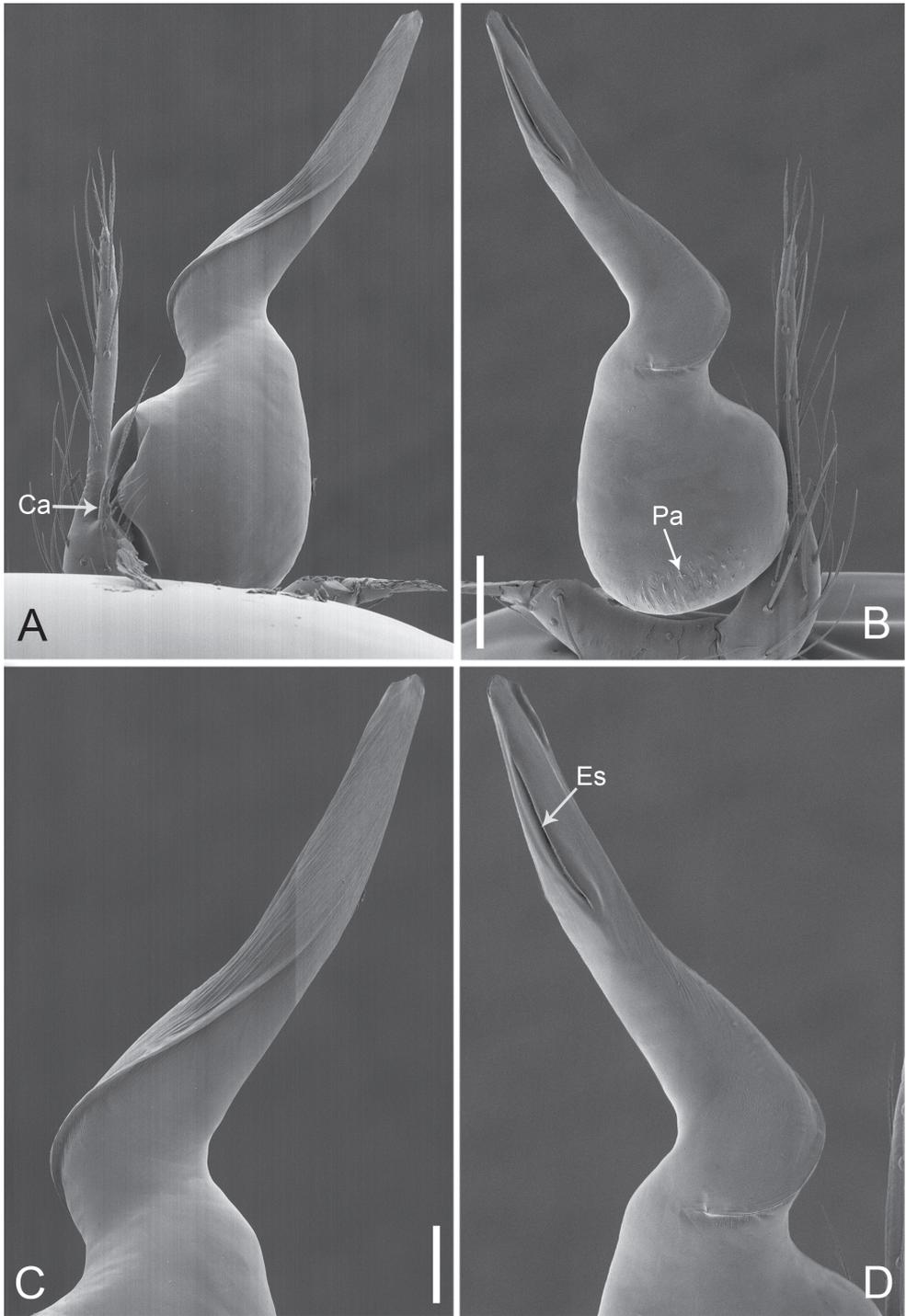
<http://zoobank.org/56E92945-7F34-47D1-9E91-2ED4C8B2EF46>

Figs 25–27, 31

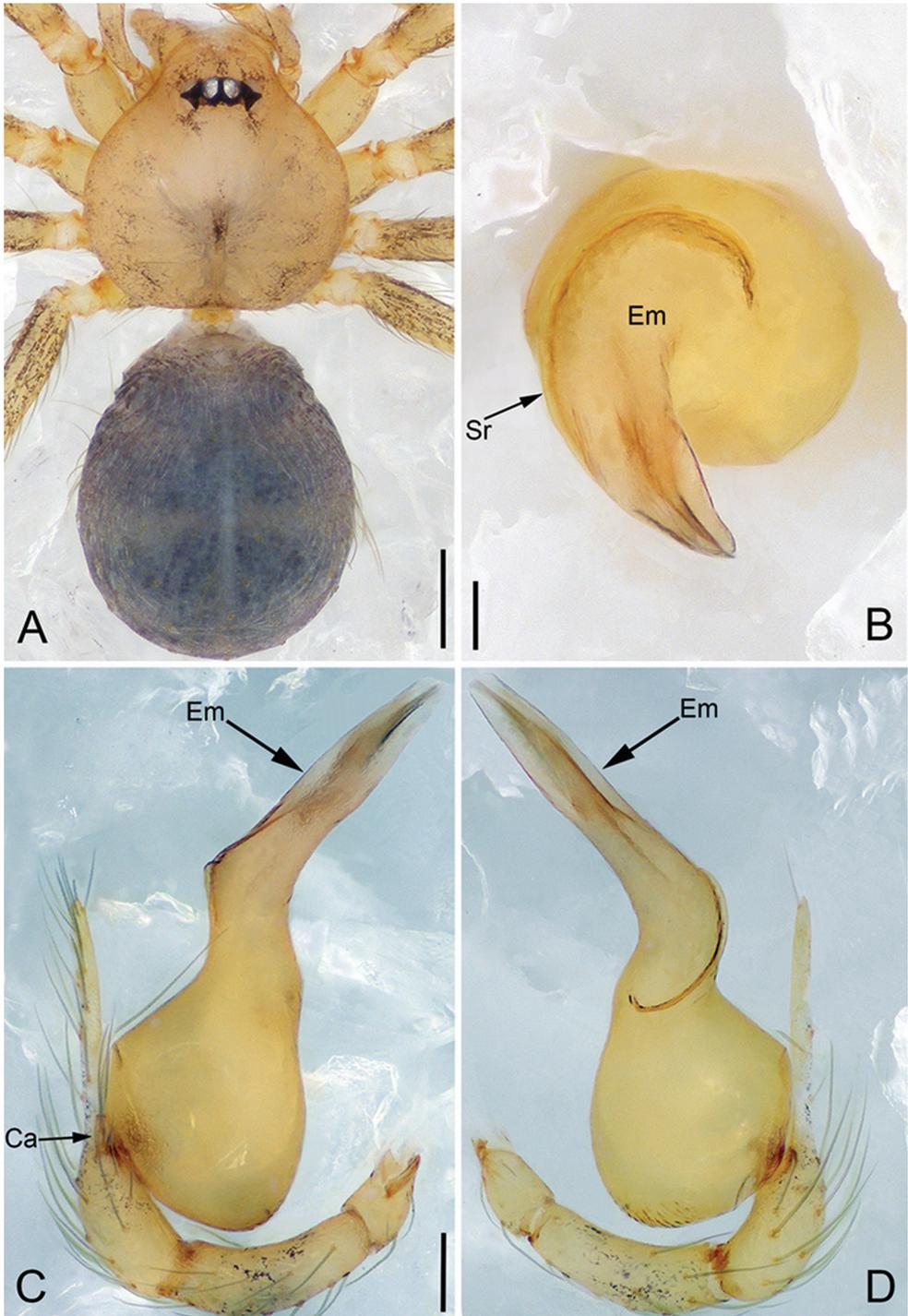
**Type material.** Holotype ♂ (IZCAS): China, Guangxi Zhuang Autonomous Region, Hechi Prefecture, Du'an County, Gaoling Town, Sanlian Village, Cave without a



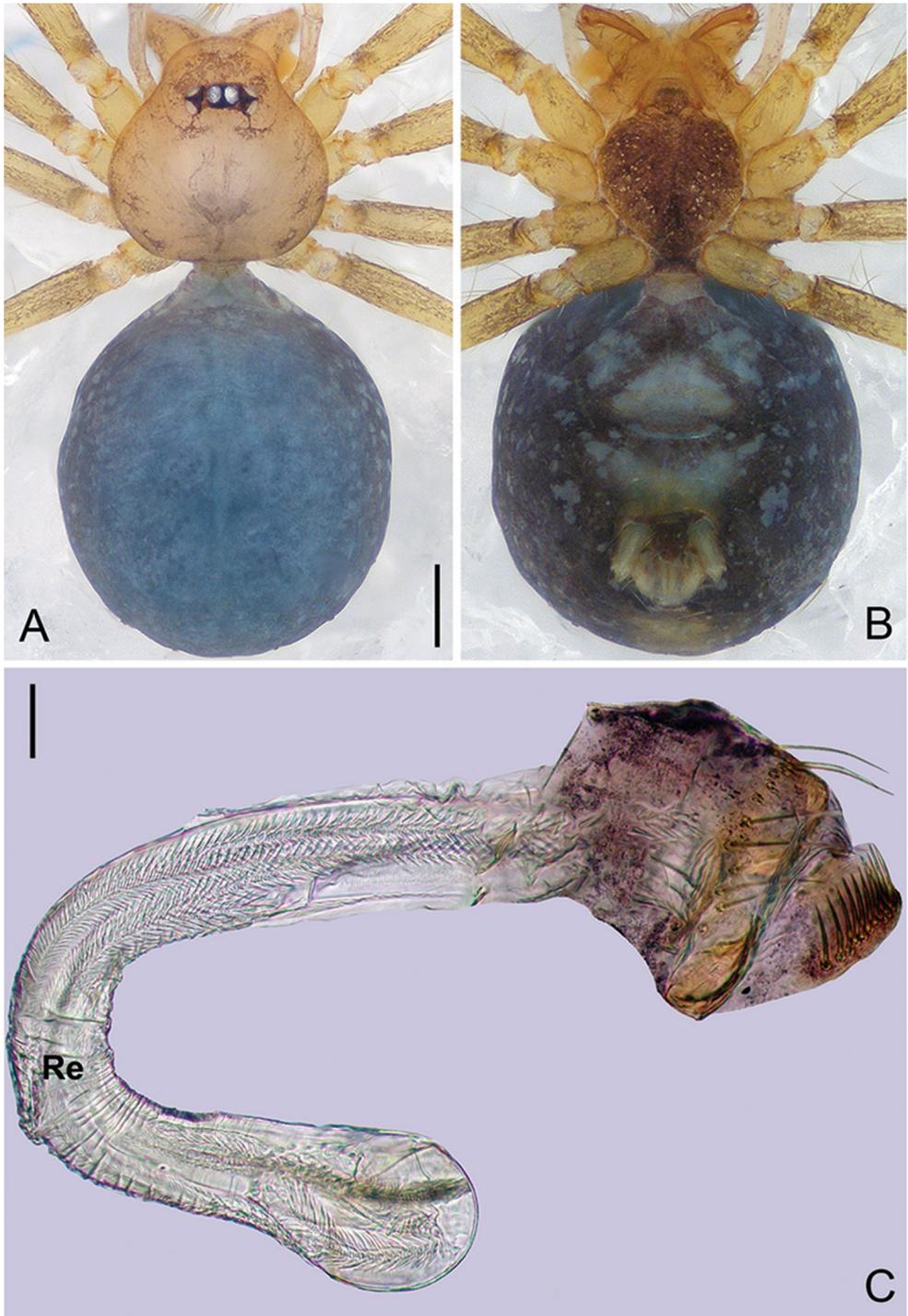
**Figure 24.** *Pinelema xiushuiensis*, male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 25.** *Pinelema yunchuni* Zhao & Li, sp. n., male. **A** Palp, proteral view **B** Palp, retrolateral view **C** Embolus, proteral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 26.** *Pinelema yunchuni* Zhao & Li, sp. n., male holotype. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C–D**).



**Figure 27.** *Pinelema yunchuni* Zhao & Li, sp. n., female paratype. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).

name, 24°02.340'N, 108°03.720'E, 225 m, 12.III.2015, Y. Li and Z. Chen leg. **Paratypes** (IZCAS): 3♂ and 5♀, same data as holotype.

**Other material examined.** 5♀ (molecular vouchers, IZCAS), same data as holotype.

**Etymology.** The specific name is a patronym in honor of the collector Yunchun Li.

**Diagnosis.** This species resembles *P. wangshang* Zhao & Li, sp. n. (Figs 18–20) but can be distinguished by following characters: bulb pear-shaped (Figs 25A, B, 26C, D) (bulb kidney-shaped in *P. wangshang* Zhao & Li, sp. n.), not curved dorso-medially (Figure 26C, D) (bulb curved dorso-medially in *P. wangshang* Zhao & Li, sp. n.), larger El/Bl ratio (1.78–1.82, n = 4, mean: 1.80, Figs 25B, 26D) (smaller El/Bl ratio 1.37–1.45, n = 5, mean: 1.41 in *P. wangshang* Zhao & Li, sp. n.), and receptacle U-shaped (Figure 27C) (receptacle J-shaped in *P. wangshang* Zhao & Li, sp. n.).

**Description. Male (holotype):** Total length 1.27. Carapace 0.56 long, 0.53 wide. Abdomen 0.68 long, 0.56 wide. Carapace light brown with black speckles (Figure 26A). Six eyes ringed with black, clypeus height 0.10, ocular quadrangle 0.17 wide (Figure 26A). Chelicerae, legs, labium, and endites light brown. Sternum dark brown. Leg measurements: I 4.43 (1.33, 0.22, 1.41, 0.86, 0.61); II 3.63 (1.08, 0.21, 1.14, 0.67, 0.53); III 2.64 (0.81, 0.19, 0.76, 0.45, 0.43); IV 3.21 (1.04, 0.19, 0.94, 0.59, 0.45). Abdomen blue with sparse setae (Figure 26A).

Palp: Tibia 2.3 times longer than patella, cymbium 2.0 times longer than tibia, cymbial apophysis brown and cone-shaped (Figs 25A, 26C); bulb nearly pear-shaped (Figs 25A–B, 26C–D) with papillae proximo-retrolaterally (Figure 25B); spiral ridge brown (Figure 26B), El/Bl ratio 1.81 (Figure 26D), Esl/El ratio 0.44 (Figure 26D).

**Female:** Total length 1.52. Carapace 0.61 long, 0.56 wide. Abdomen 0.89 long, 0.89 wide. Coloration and pattern as in male (Figure 27A–B). Six eyes, well developed, clypeus height 0.12, ocular quadrangle 0.17 wide (Figure 27A). Leg measurements: I 4.35 (1.33, 0.22, 1.39, 0.70, 0.71); II 3.54 (1.08, 0.20, 1.10, 0.63, 0.53); III 2.62 (0.80, 0.19, 0.75, 0.48, 0.40); IV 3.28 (1.03, 0.20, 1.01, 0.61, 0.43). Insemination duct as wide as receptacle (Figure 27C); receptacle U-shaped, slightly swollen at end (Figure 27C).

**Variation.** In 3♂ paratypes: El/Bl ratio 1.78–1.82, Esl/El ratio 0.43–0.45.

**Distribution.** China (Guangxi, Figure 31), known only from the type locality.

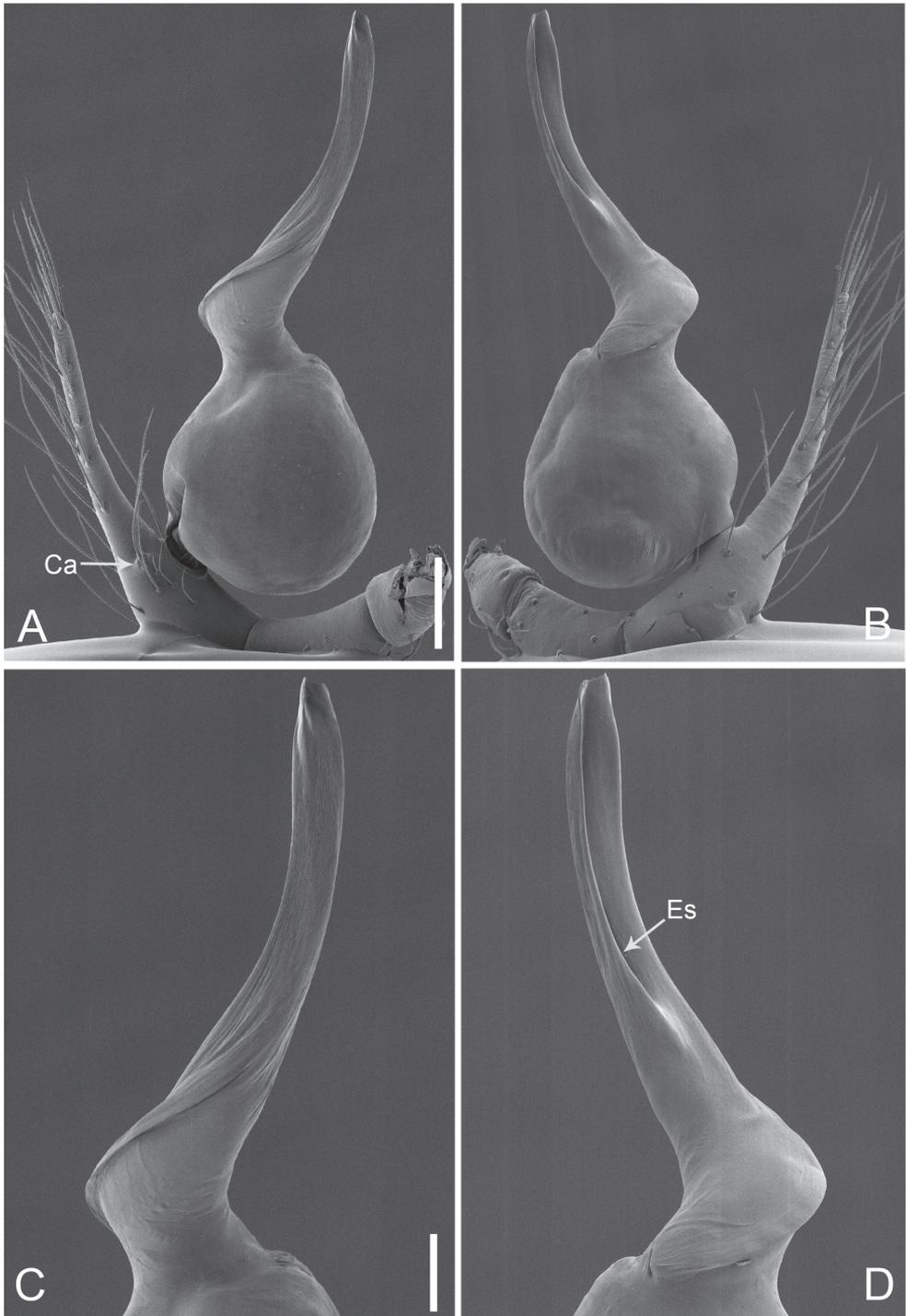
### *Pinelema zhewang* (Chen & Zhu, 2009) comb. n.

Figs 28–31

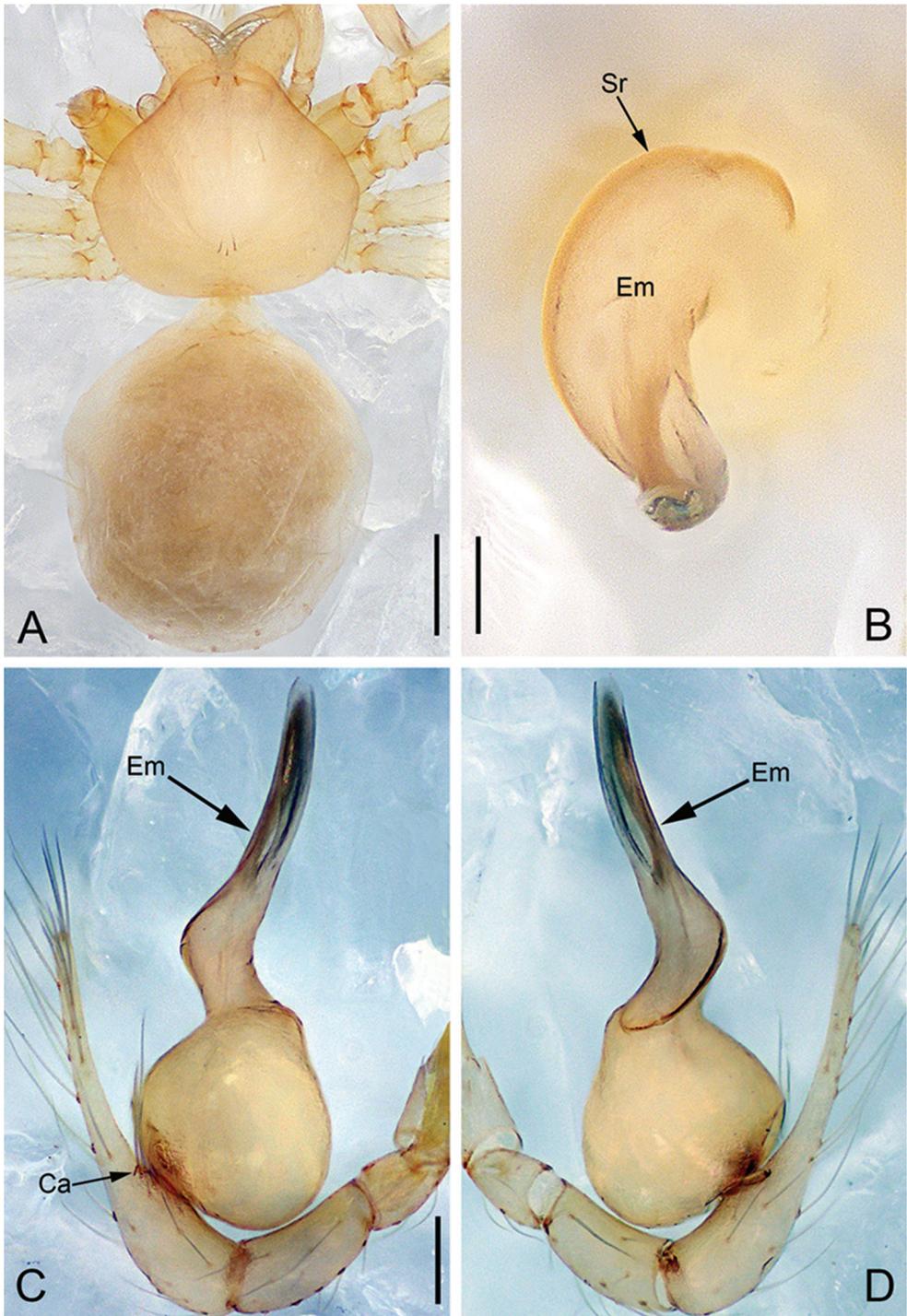
*Telema zhewang*: Chen and Zhu 2009: 1709, figure 3A–D, F–L, O–Q (♂♀).

**Type material.** Holotype ♂ (MLR): China, Guizhou Province, Qianxinan Prefecture, Ceheng County, Qingping Township, Zhewang village, Zhoujia cave, 600 m, 25°11.000'N, 105°55.000'E, 5.XI.1999, H. Chen and Y. Zhang leg. Paratypes: 13♂ and 22♀ (MHBU), same data as holotype. Not examined.

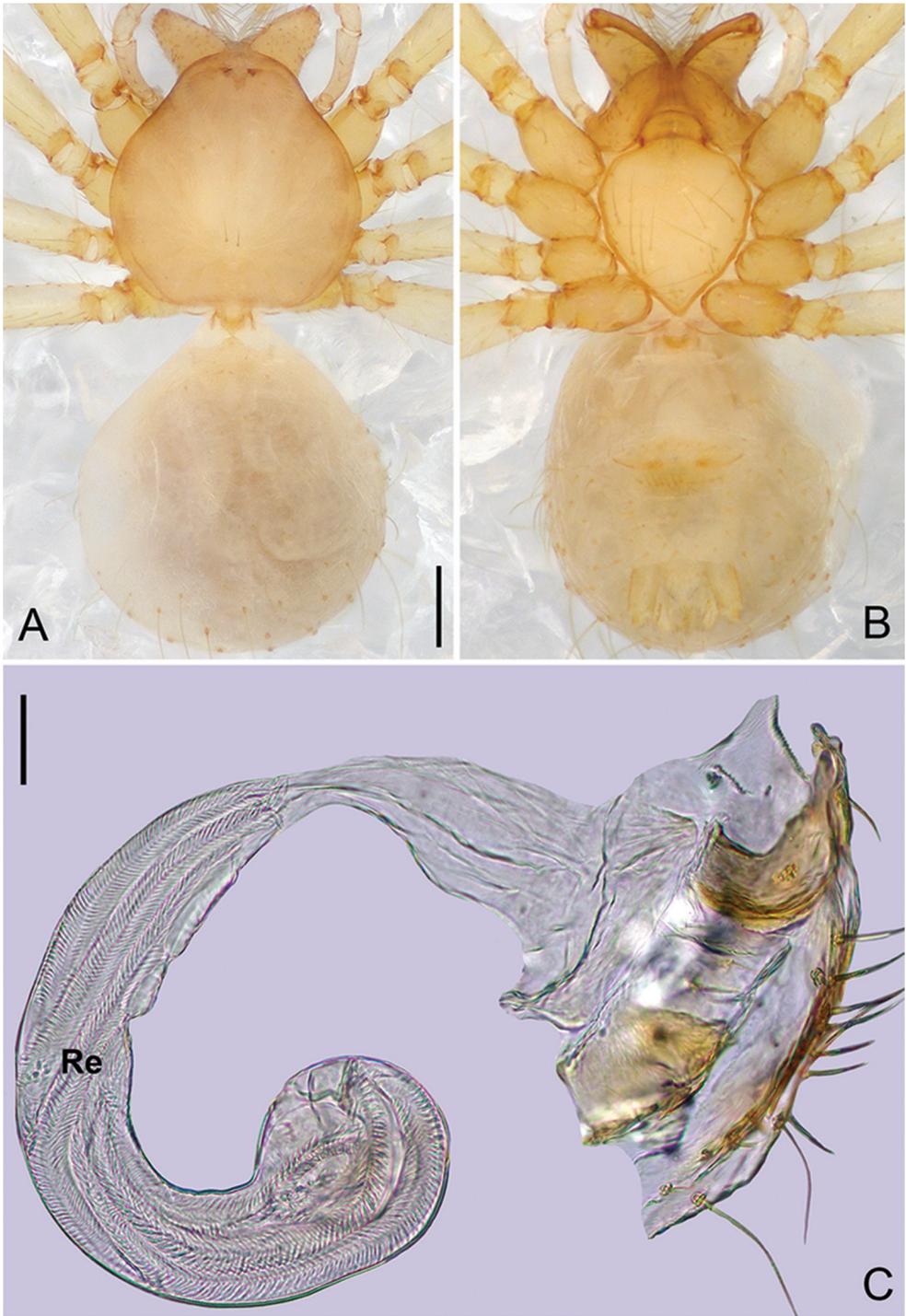
**Material examined.** 4♂ and 8♀ (molecular vouchers, IZCAS) from the type locality, 10.III.2011, C. Wang and L. Lin leg.



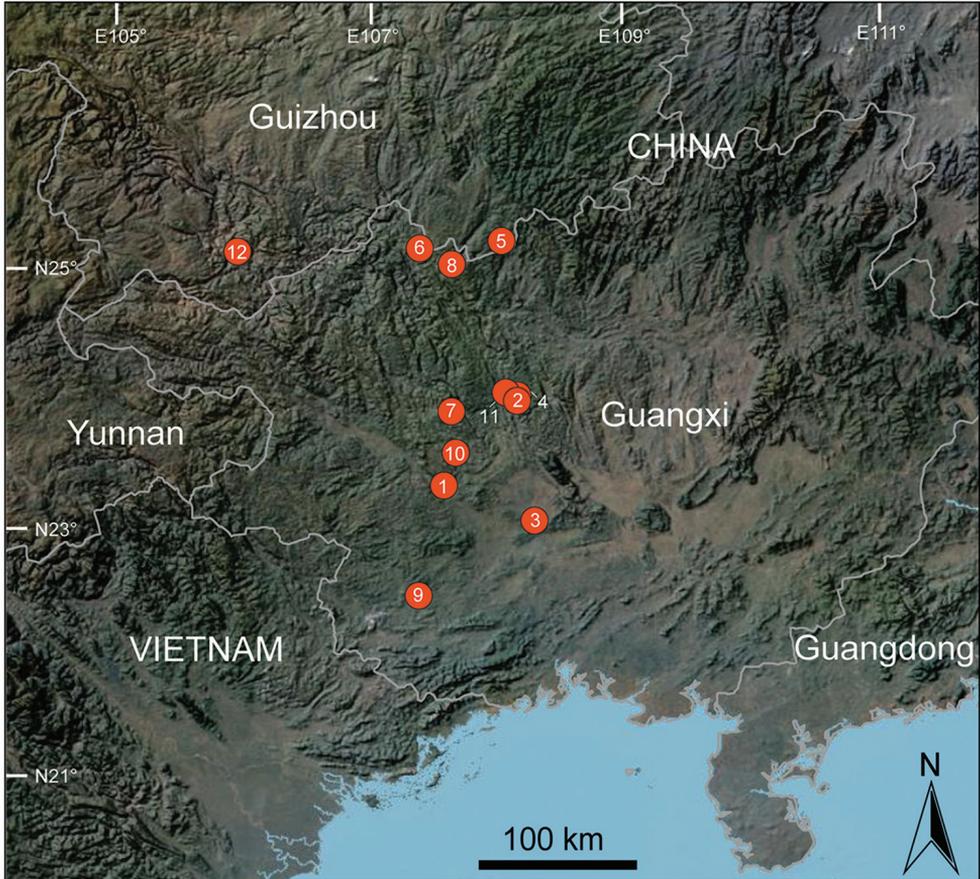
**Figure 28.** *Pinelema zhewang*, male. **A** Palp, prolateral view **B** Palp, retrolateral view **C** Embolus, prolateral view **D** Embolus, retrolateral view. Scale bars: 0.1 mm (**A–B**), 0.05 mm (**C–D**).



**Figure 29.** *Pinelema zhewang*, male. **A** Habitus, dorsal view **B** Embolus, apical view **C** Palp, prolateral view **D** Palp, retrolateral view. Scale bars: 0.2 mm (**A**), 0.05 mm (**B**), 0.1 mm (**C-D**).



**Figure 30.** *Pinelema zhewang*, female. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Endogyne, lateral view. Scale bars: 0.2 mm (**A–B**), 0.05 mm (**C**).



**Figure 31.** Distribution records of *Pinelema bailongensis* species group: **1** *P. bailongensis* **2** *P. cheni* Zhao et Li, sp. n. **3** *P. cordata* **4** *P. huoyan* Zhao & Li, sp. n. **5** *P. liangxi* **6** *P. lizhuang* Zhao & Li, sp. n. **7** *P. stren-tarsi* **8** *P. wangshang* Zhao & Li, sp. n. **9** *P. wenyang* Zhao & Li, sp. n. **10** *P. xiushuiensis* **11** *P. yunchuni* Zhao & Li, sp. n. **12** *P. zhewang*.

**Diagnosis.** This species resembles *P. xiushuiensis* (see Figure 24 and Wang and Li 2016: 556, figs 9–12) but can be distinguished by following characters: cymbial apophysis very short (Figs 28A, 29C) (cymbial apophysis long in *P. xiushuiensis*) and larger  $Esl/El$  ratio (0.58–0.63,  $n = 4$ , mean: 0.60, Suppl. material 1: Figure S12) (smaller  $Esl/El$  ratio 0.50–0.52,  $n = 5$ , mean: 0.51 in *P. xiushuiensis*).

**Description.** Male palp: Embolus bent (Figs 28A–D, 29C–D), spiral ridge brown (Figure 29B),  $El/Bl$  ratio 1.73–1.83 ( $n = 4$ , mean: 1.76, Suppl. material 1: Figure S12);  $Esl/El$  ratio 0.58–0.63 ( $n = 4$ , mean: 0.60, Suppl. material 1: Figure S12). Female endogyne: insemination duct as wide as receptacle (Figure 30C); receptacle U-shaped, slightly swollen at end (Figure 30C). For more detailed descriptions, see Chen and Zhu (2009).

**Comments.** This species shares a combination of morphological characters with *P. bailongensis*, such as the long, tube-shaped embolus (see Figs 28A–D, 29C–D and Chen and Zhu 2009: figure 3C–D), the presence of a cymbial apophysis in the male palp prolaterally (see Figs 28A, 29C and Chen and Zhu 2009: figure 3F), and a U-shaped and medially strongly curved receptacle (see Figure 30C and Chen and Zhu 2009: figure 3J–L), so this species is transferred to *Pinelema*.

**Distribution.** China (Guizhou, Figure 31), known only from the type locality.

## Acknowledgements

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## Supplementary material I

### Figures S1–S12. Left male palps of *Pinelema bailongensis* species group, retrolateral view

Authors: Huifeng Zhao, Zhiyuan Yao, Yang Song, Shuqiang Li

Data type: specimens data

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# A first phylogenetic analysis reveals a new arboreal tarantula genus from South America with description of a new species and two new species of *Tapinauchenius* Ausserer, 1871 (Araneae, Mygalomorphae, Theraphosidae)

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## Abstract

Based on molecular and morphological phylogenetic analyses a new genus of Theraphosidae is described, *Pseudoclamoris* **gen. n.** *Tapinauchenius gigas* and *Tapinauchenius elenae* are transferred to *Pseudoclamoris* and a new species of *Pseudoclamoris* from the Amazon Region is described: *P. burgessi* **sp. n.** Two new species of *Tapinauchenius* from the Caribbean are described: *T. rasti* **sp. n.** and *T. polybotes* **sp. n.** *Tapinauchenius subcaeruleus* is considered a *nomen dubium*. Psalmopoeinae subfamily is diagnosed based on molecular and morphological phylogenies, and *Pseudoclamoris* **gen. n.** and *Ephebopus* Simon, 1892 are included. A taxonomic key for Psalmopoeinae genera *Tapinauchenius*, *Pseudoclamoris*, *Psalmopoeus*, and *Ephebopus* is provided.

## Keywords

arboreal, morphology, tarantula, phylogenomics, Psalmopoeinae

## Introduction

The Theraphosidae are among the largest Mygalomorphae and along with the Theraphosinae, members of the Aviculariinae remain of great interest to arachnologists due to their unresolved phylogenetic relationship, exhibiting extreme diversity all over south America.

The genera *Tapinauchenius* Ausserer, 1871 and *Psalmopoeus* Pocock, 1895 have never been reviewed or revised before, even though new species have been described in recent years (Mendoza 2014). Ausserer (1871) defined the genus *Tapinauchenius* for the already-described species *Mygale plumipes* C. L. Koch in 1842, based on the absence of stridulatory organ. The newly designated type species, *Tapinauchenius plumipes*, was only known from a mature male until Becker (1879) described the female of the species as *Avicularia deborri*, which later got referred as *T. plumipes* by Schmidt (1994a), based on morphological similarities and same type locality of Paramaribo, Suriname. Simon (1892) placed *Mygale sanctivincentii* Walckenaer, 1837 in *Tapinauchenius*: *T. sanctivincentii* is only known from a single female specimen, of which the type is apparently lost (Rollard, pers. comm.). *Tapinauchenius latipes* was described by L. Koch in 1875, based on a single male specimen from Venezuela. However, this species is supposed to differ from *T. plumipes* by its ocular alignment, but Koch did not specify in which way these differ exactly and no further diagnosis was provided. The female of *T. latipes* was subsequently described by Schiapelli and Gerschman (1945). Two years later, Caporiacco (1947) described *Tapinauchenius concolor* from Guyana, but erroneously placed it in *Pachistopelma*. This placement was found to be incorrect by Bertani (2012), who transferred the species to *Tapinauchenius*, referring to its type specimen as an immature male. Caporiacco (1954) described *Tapinauchenius gigas* from French Guyana based on a female specimen, while Schmidt (1994b) described the male of this species. In the following years, Schmidt worked extensively on the taxonomy of Theraphosidae and described a number of additional species of *Tapinauchenius*, namely *Tapinauchenius elenae* Schmidt, 1994 from Ecuador, *Tapinauchenius brunneus* Schmidt, 1995 from the Amazon region of Mato Grosso, Brazil, based on a single male specimen and *Tapinauchenius cupreus* Schmidt & Bauer, 1996 from Ecuador. Schmidt (1995b) also described *Tapinauchenius purpureus* from French Guyana from both sexes, but the species was later found to be a junior synonym of *Tapinauchenius violaceus* (Mello-Leitão, 1930) by West et al. (2008). Bauer and Antonelli (1997) described *Tapinauchenius subcaeruleus* from Ecuador, based on material from pet trade lacking further information on the exact collection locality. Auer et al. (2007) presented a summary of all known *Tapinauchenius* available in the pet trade and provided valuable comments and suggestions regarding the groupings in the genus. For the first time, two groups within *Tapinauchenius* were recognized, based on the colouration of juvenile specimens. These groups were the *gigas* group, with ontogenetic pattern change, comprising *T. gigas*, *T. elenae* and *T. subcaeruleus*, and the *plumipes* group, without ontogenetic pattern change, consisting of *T. plumipes*, *T. latipes*, *T. violaceus*, *T. sanctivincentii*, and *T. cupreus*. West et al. (2008) showed images of lyrate setae on prolateral palpal coxa in an unidenti-

fied *Tapinauchenius* species from Peru but did not provide further comments besides the SEM images of the mentioned structure. Bertani (2012) included *Tapinauchenius* and *Psalmopoeus* in his cladistic analysis and placed them within Aviculariinae while describing new genera and species in the same subfamily.

The latest changes to the supposed sister taxon of *Tapinauchenius*, *Psalmopoeus*, were made by Mendoza (2014), who described *Psalmopoeus victori* from Mexico, providing some insight on the northernmost distribution of the Psalmopoeinae clade. Mendoza (2014) followed Bertani (2012) by placing *Psalmopoeus*, *Tapinauchenius*, and *Ephobopus* Simon, 1892 in the Aviculariinae. In the most recent study on Aviculariinae, Fukushima and Bertani (2017) used an in-depth morphological cladistic analysis to justify the placement of *Psalmopoeus*, *Tapinauchenius*, and *Ephobopus* in Aviculariinae. Contrary to their results, the proposals of Samm and Schmidt (2010), Lüddecke et al. (2018) and Turner et al. (2018) are followed here by placing *Psalmopoeus*, *Tapinauchenius*, as well as *Pseudoclamoris* gen. n. in Psalmopoeinae. Molecular based data from Lüddecke et al. (2018), Turner et al. (2018), and the work presented here strongly suggest a close phylogenetic relationship between Psalmopoeinae and Schismatothelinae, while morphological data further revealed that these groups possibly share several synapomorphies.

During the process of revising *Tapinauchenius* and *Psalmopoeus*, significant morphological traits in certain species of the genus *Tapinauchenius* were revealed (see Figs 2, 13). These features have been studied and were included in the morphological cladistic analysis. In addition, tissue samples were sequenced for molecular phylogenetic analyses. Combined analyses lead to the establishment of a new genus to accommodate two *Tapinauchenius* species: *Pseudoclamoris* gen. n., comprising *Pseudoclamoris gigas* comb. n., *Pseudoclamoris elenae* comb. n., and *Pseudoclamoris burgessi* sp. n. Morphological and genetic material from two Caribbean Islands lead to the description of two new species of *Tapinauchenius*: *Tapinauchenius rasti* sp. n. from Union Island, Lesser Antilles and *Tapinauchenius polybotes* sp. n. from the island of Saint Lucia, Lesser Antilles.

## Materials and methods

Measurements were taken with an ocular micrometre of a Nikon SMZ645 binocular microscope. Measurements were made along the central axis of the measured structures and are given in millimetres. Measurements of leg and palpal segments were taken dorsally. The eye measurements were taken from the widest spans of the lens, AME in dorsal view, ALE, PLE, and PME in dorsolateral view. Measurement of the total body length, including cephalothorax and abdomen without spinnerets, were made using a digital caliper. As measurements of total body length include non-sclerotized tissue of the abdomen, they should be considered to be approximates only.

Appendage measurements were based on right appendages (unless otherwise stated); palpal tibia & leg I – retrolateral, legs III and IV – prolateral, extent of metatarsal scapulation – ventral. The lengths of the leg articles were taken from the mid-proximal

point of articulation to the mid-distal point of the article (sensu Coyle 1995, Bond et al. 2012, Bond and Godwin 2013, and Hamilton et al. 2016).

Genitalia were prepared according to von Wirth (2006) and photographed with a Canon EOS 6D DSLR camera body using the Macro Photo MP-E 65mm f/2.8 1–5× Manual Focus Lens for EOS mounted on a Cognisys Automatic Stacking Rail. Thirty (3×) photographs were taken of each specimen in ethanol under glass and stacked using Zerene Stacking Software. The scientific picture plate layout follows Fukushima and Bertani (2018).

The extent of the tarsal and metatarsal scopulae on the ventral side of both leg segments was expressed as a percentage of the total length of the segment from the apical end. Terminology used to describe the male palpal bulb structures follows Bertani (2000). Maps were made with SimpleMapp, an online tool to produce species distribution maps (Shorthouse 2010).

### **Molecular techniques and phylogenomic analyses**

An important aspect of this work involves the genetic studies carried out. The following primers used for the polymerase chain reactions (PCRs) were used in this paper. The primers LCO-1490 (5' GGT CAA CAA ATC ATA AAG ATA TTG G 3') and HCO-2198 (5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3') (Folmer et al. 1994) target a 710-base pairs fragment of the COI mitochondrial region. The primer pair 16SAL-Tarantula-F1 (5' GTG CTA AGG TAG CAY AAT 3') and 16S-Tarantula-R2 (5' TAA TTC AAC ATC GAG GTC 3') (Lüddecke et al. 2018) amplify a fragment of about 270 base pairs of the mitochondrial 16S rRNA gene. Finally, the primers 28SO F (5' TCG GAA GGA ACC AGC TAC TA 3') and 28SC R (5' GAA ACT GCT CAA AGG TAA ACG G 3') (Hedin and Maddison 2001) were used to amplify a fragment of 760–800 base pairs of the nuclear 28S rRNA gene.

A negative control that contained no DNA was included in every PCR round to check for cross-contamination. PCR products were run on agarose gels and imaged under UV light to verify the amplicon size. The PCR products were bi-directionally sequenced using the PCR primers. Electropherogram analysis and overlapping was conducted using Geneious 8.1.8. During the electropherogram analysis, the primer annealing regions and the low-quality regions at both ends of each electropherogram were trimmed (error probability limit of 0.03).

Specimens used in this first phylogenetic approach of this subfamily are listed in Supplementary Data, originating partly from pet trade and wild caught specimens. All specimens sampled by use of non-lethal techniques (leg autotomy) appeared to undergo very little stress. All specimens survived the respective procedures (until preserved for vouchers). All data (molecular, morphological, etc.) used to establish these species hypotheses have been deposited in the Dryad Data Repository (doi:10.5061/dryad.k6483cr). GenBank data for Theraphosidae outgroup has been used, namely *Selenocosmia javanensis* (MG273512.1), *Theraphosa apophysis* (KY017414.1) and *Pocillotheria metallica* (KY016161.1).

Maximum likelihood analyses were conducted using RAxML version 8.2 with the GTRGAMMA model and 1000 rapid bootstrap replicates. Bayesian analyses were conducted with MrBayes 3.2.6 on the CIPRES portal (Miller et al. 2010). Analyses were run for 10 million generations, logging every 1000 generations. Analyses were carried out on the authors' computers for crosscheck reference in order to prevent any wrong settings for calculation. Figure 16 shows the preferred tree which is used for discussion.

Specimens from the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt (**SMF**), Museum für Naturkunde der Humboldt-Universität, Berlin (**ZMB**), Museum National d'Histoire Naturelle, Paris (**MNHN-AR**), and the personal collection of the author (**MHCOL**) were examined as part of this work. The general description format follows Hamilton et al. (2016), with modifications, mainly of setae and trichobothria patterns, which were not studied in detail here. The description format of Hamilton et al. (2016) was used to evaluate morphological variation when more material was available.

Abbreviations (see Hamilton et al. 2016: fig. 3):

<b>Cl</b>	length of the carapace;	<b>P4</b>	patella IV length;
<b>Cw</b>	width of the carapace;	<b>T4</b>	tibia IV length;
<b>LBl</b>	labial length;	<b>M4</b>	metatarsus IV length;
<b>LBw</b>	labial width;	<b>A4</b>	tarsus IV length;
<b>F1</b>	femur I length (retrolateral aspect);	<b>PTI</b>	palpal tibia length (retrolateral aspect);
<b>F1w</b>	femur I width;	<b>PTw</b>	palpal tibia width;
<b>P1</b>	patella I length;	<b>SC3</b>	ratio of the extent of metatarsus III scopulation (length of scopulation/ventral length of metatarsus III);
<b>T1</b>	tibia I length;	<b>SC4</b>	ratio of the extent of metatarsus IV scopulation (length of scopulation/ventral length of metatarsus IV);
<b>M1</b>	metatarsus I length;	<b>AER</b>	Anterior Eye Row;
<b>A1</b>	tarsus I length;	<b>PER</b>	Posterior Eye Row;
<b>F3</b>	femur III length (prolateral aspect);	<b>MPT</b>	most parsimonious tree;
<b>F3w</b>	femur III width;	<b>CI/ci</b>	consistency index;
<b>P3</b>	patella III length;	<b>RI/ri</b>	retention index;
<b>T3</b>	tibia III length;	<b>hi</b>	homoplasy index;
<b>M3</b>	metatarsus III length;	<b>G-fit</b>	Goloboff fits.
<b>A3</b>	tarsus III length;		
<b>F4</b>	femur IV length (prolateral aspect);		
<b>F4w</b>	femur IV width;		

Material examined (for detailed information to examined and sequenced material, see Suppl. material 1): Male holotype and female paratype of *Tapinauchenius elenae* from Tena, Ecuador, SMF (37349), leg. Hirsch, VIII, 1992; examined. Female lectotype of *Tapinauchenius gigas* from French Guyana, MNHN (AR14637), leg. Sammler; and male from French Guyana, SMF (38050), leg. Verdez; examined. Male holotype

(38042) and female paratype (38046) of *Tapinauchenius cupreus* from Ecuador, SMF, leg. Bullmer; examined. Female holotype and male paratype of *Psalmopoeus langensbuchi* from Caripe, Venezuela, SMF (58086), leg. Langensbucher; examined. Male holotype of *Tapinauchenius brunneus* from Mato Grosso, Brazil, SMF (38008), leg. Ockert; examined. 2 male syntypes (legs sep.), 1 male bulb (sep.) of *Tapinauchenius plumipes* from Suriname, ZMB (MYR 2044); examined. Female holotype (38042) and male paratype (38046) of *Tapinauchenius violaceus* from French Guyana, SMF, leg. Braunshausen, 1994; examined.

Other material examined: *Tapinauchenius elenae*: female exuviae (SMF 57952). *Tapinauchenius gigas*: 1 female (SMF 38050), 3 females (MHCOL\_00131, 00112, 00121) and 4 males (MHCOL\_00201, 00161, 00174, 00189). *Tapinauchenius cupreus*: female exuviae (SMF 57925, SMF 58281), 3 females (MHCOL\_0012, 0097, 027) and 2 males (MHCOL\_0289, 0301). *Psalmopoeus langensbuchi*: 3 females (MHCOL\_00055, 00198, 00401) and 3 males (MHCOL\_00501, 00512, 00518). *Tapinauchenius plumipes*: 3 females (MHCOL\_0192, 0211, 0312) and 4 males (MHCOL\_0089, 0092, 0212, 0415). *Tapinauchenius violaceus*: 3 females (MHCOL\_0152, 0243, 0442) and 2 males (MHCOL\_0163, 0288).

## Phylogenetics

Members of the family Theraphosidae are known for their morphological homogeneity (see Raven 1985, Goloboff 1993, Bertani 2001, Bond et al. 2012). Considering this, it is extremely difficult to find characteristics that offer the required level of stability and uniqueness to be useful for analyses. For example, similarly to Fukushima and Bertani (2017), certain ratios between the extremities (such as legs and palps) and the carapace itself were set out to apply. However, these were found not suitable for further analyses.

As shown by Fukushima and Bertani (2017), a revision based solely on morphological characters is extremely difficult, as the variation within characters is often high. It has been shown that an approach that combines classical morphological taxonomy with complementary techniques, such as DNA sequencing, is more accurate and reliable due to the larger amount of data to work with (Bond et al. 2012; Hamilton et al. 2016).

## Morphological analysis

A data matrix consisting of 19 taxa and 30 unordered, parsimony informative characters (Table 1) was analyzed in PAUP\* 4.0a162 Swofford (2018) under the maximum parsimony criterium at 10 replicates with random addition sequence using tree bisection reconnection (TBR). Bootstrap support was evaluated at 1000 replicates after successive reweighting according to the mean ci at a base-weight of 10. Table 1 and 2 together with Figure 17 are displaying the results of this work.

**Table 1** Character step matrix used for cladistic analysis.

Taxa	1										2										3									
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
<i>Stenoterommata</i>	0	0	-	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0
<i>Sason</i>	0	2	-	0	-	0	0	1	0	0	0	0	?	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
<i>Antillena</i>	1	4	-	1	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	3	0	0	1	0	3	1	0	1	1	0
<i>Avicularia</i>	1	3	-	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	3	0	0	1	1	2	0	1	1	2	0	1
<i>Caribena</i>	1	4	-	1	0	0	1	1	0	0	0	0	0	1	1	1	1	1	3	0	0	1	1	3	1	0	0	1	0	1
<i>Pachistopelma</i>	1	3	-	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	3	0	0	1	1	2	1	0	0	2	0	1
<i>Ephebopus</i>	1	1	1	2	1	1	1	0	0	0	0	0	1	1	1	1	1	0	3	0	1	1	1	0	0	1	1	0	1	0
<i>Holothele</i>	1	1	1	0	0	1	1	0	0	1	1	1	1	1	0	0	1	0	2	1	1	0	0	2	0	0	0	0	0	0
<i>Schismatothele</i>	1	1	0	0	0	1	1	0	0	1	1	1	1	1	0	0	1	0	2	1	1	0	0	4	0	0	0	0	0	0
<i>P. gigas</i>	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1	1	1	3	0	1	1	1	2	0	1	1	0	0	0
<i>P. elenae</i>	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1	1	1	3	0	1	1	1	2	0	1	1	0	0	0
<i>P. burgessi</i>	1	1	1	0	1	1	1	0	1	0	0	0	1	1	1	1	1	1	3	0	1	1	1	2	0	1	1	0	0	0
<i>P. irminia</i>	1	1	0	0	1	1	1	0	2	0	0	0	1	1	1	1	1	1	3	0	1	1	0	1	0	0	0	0	0	0
<i>P. reduncus</i>	1	1	0	0	1	1	1	0	2	0	0	0	1	1	1	1	1	1	3	0	1	1	1	0	0	0	0	0	0	0
<i>P. cambridgei</i>	1	1	0	0	1	1	1	0	2	0	0	0	1	1	1	1	1	1	3	0	1	1	0	1	0	0	0	0	0	0
<i>T. plumipes</i>	1	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1	1	0	3	0	1	1	0	4	0	0	1	0	0	0
<i>T. rasti</i>	1	1	2	0	1	1	1	0	0	0	0	0	1	1	1	1	1	0	3	0	1	1	0	4	0	0	1	0	0	0
<i>T. polybotes</i>	1	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1	1	0	3	0	1	1	0	4	0	0	1	0	1	0

**Table 2** Character changes connected to nodes in preferred topology resulting from cladistic analysis; ambiguous changes in italics.

Node	Character change(s)
33 > <i>Stenoterommata</i>	2 (1<->0), 19 (0<->1), 20, (0<->1)
33 > <i>Sason</i>	2 (1>2), 8 (0>1)
33 > 32	1 (0>1), 6 (0>1), 7 (0>1), 14 (1>0), 17 (0>1), 19 (1>2), 21 (0>1)
32 > 30	5 (0>1), 15 (0>1), 16 (0>1), 19 (2>3), 20 (1>0), 22 (0>1)
30 > 27	3 (0>1), 27 (0>1)
27 > 25	23 (0>1), 26 (1>0)
25 > 24	18 (0>1), 24 (0>2)
24 > 21	2 (1>3), 4 (0>1), 5 (1>0), 6 (1>0), 13 (1>0), 21 (1>0), 28 (0>2), 30 (0>1)
21 > 20	25 (0>1), 26 (1>0), 27 (1>0)
20 > 19	2 (3>4), 8 (0>1), 24 (2>3), 28 (2>1)
19 > <i>Antillena</i>	23 (1>0), 27 (0>1)
24 > 23	9 (0>1)
25 > <i>Ephebopus</i>	4 (0>2), 29 (0>1)
27 > 26	24 (0>4)
26 > <i>T. rasti</i> sp. n.	3 (1>2)
26 > <i>T. polybotes</i> sp. n.	29 (0>1)
30 > 29	9 (0>2), 18 (0>1)
29 > 28	24 (0>1)
29 > <i>P. reduncus</i>	23 (0>1)
32 > 31	10 (0>1), 11 (0>1), 12 (0>1), 24 (0>2)
31 > <i>Holothele</i>	3 (0>1)
30 > <i>Schismatothele</i>	24 (2>4)

## Characters

Characters from Guadanucci (2014) (2, 3, 5, 8, 9, 10, 11, 12, 13, 15, 15, 18, 19, 20, 21, 22, 23) and Fukushima and Bertani (2017) (1, 6, 7, 14, 16, 17, 24, 25, 26, 27, 28, 29, 30) were combined and slightly modified (2, 3, 9, 23, 29). Character 4 was added in order to account for the autapomorphy (presence of urticating setae on palpal femora) of *Ephobopus*, alongside the presence of abdominal urticating setae in Aviculariinae. Abdominal urticating setae have also been acquired in Theraphosinae, whereas the state scored for *Ephobopus* is unique among Theraphosidae:

- (1) Cymbial lobes: (0) distinct from each other; (1) similar to each other.
- (2) Male tibial apophysis: (0) absent; (1) present, prolateral branch well developed with spine, Guadanucci et al. (2007: figs. 10 and 11); (2) present, weakly developed, with megaspine, Guadanucci (2014: fig. 16); (3) present, composed of spiniform setae, von Wirth and Striffler (2005: fig. 26); (4) present, prolateral branch well developed and fused with retrolateral branch; Bertani (2001: figs. 22 – 23).
- (3) Male tibial spur: number of spines on Rap: (0) 1 short and strong spine on the inner face and 1 short and strong spine on top; (1) 1 short and strong spine on the inner face and 2 short and strong spines on outer face; (2) 1 short and strong spine on the inner face and 1 short and 3 strong spines on outer face (Figure 11c).
- (4) Urticating setae: (0) absent; (1) present on opisthosoma; (2) present on palpal femur.
- (5) Multilobular spermathecae: (0) absent; (1) present, Guadanucci (2007: Figure 6).
- (6) Spermathecae cuticula sclerotization: (0) slightly (thin and soft); (1) strongly sclerotized.
- (7) Anterior maxillary lobe (anterior ventral corner, Raven 1994): (0) not produced; (1) produced.
- (8) Number of maxillary cuspules: (0) several (more than 30); (1) few (less than 15).
- (9) Lyra on prolateral maxilla. 1/4<sup>th</sup> of the surface: (0) absent; (1) field of needle-like setae (Figure 2); (2) curved lyriform setae (Figure 3).
- (10) Labial cuspules located: (0) on a flat area; (1) on a raised area.
- (11) Labial cuspules density: (0) weakly dense, spread over the labium; (1) strongly dense, concentrated at the apical portion (approximately 200 cuspules).
- (12) Sternal anterior bulge: (0) absent; (1) present.
- (13) Armed Tarsal Claw: (0) absent; (1) present.
- (14) Third tarsal claw: (0) absent; (1) present.
- (15) Leg spines: (0) present on tibiae and metatarsi; (1) absent on tibiae and metatarsi.
- (16) Tarsal and metatarsal scopula laterally projected: (0) absent; (1) present.
- (17) Tarsal scopula I: (0) divided, Guadanucci (2011a: fig. 2); (1) undivided.
- (18) Tarsal scopula IV: (0) divided; (1) undivided.
- (19) Tarsal trichobothria disposition: (0) absent; (1) small compact group, Guadanucci (2012: fig. 1); (2) two rows, Guadanucci (2012: fig. 47); (3) U-shaped, Guadanucci (2012: fig. 39).

- (20) Tibial thickened trichobothria: (0) absent; (1) present.
- (21) Tibial clavate trichobothria: (0) absent; (1) present.
- (22) Tibial clavate trichobothria disposition: (0) straight row, Guadanucci (2012: fig. 189a); (1) compact group, Guadanucci (2012: fig. 161).
- (23) Ontogenetic change of colour pattern: (0) pattern remains practically the same; (1) pattern presents drastic changes.
- (24) Dorsal abdominal pattern in juveniles: (0) homogeneous; (1) herringbone; (2) central longitudinal black stripe with 5–6 lateral stripes, connecting or not with the central stripe; (3) central longitudinal reddish stripe inside a dark area with zigzag border; (4) longitudinal central stripe of a different colour of remaining abdomen.
- (25) Body colouration in juveniles: (0) matte/dull (brown or grey); (1) iridescent (green or blue).
- (26) Colouration of tarsi in juveniles: (0) same colour of other articles; (1) black.
- (27) Distribution of abdominal setae in females: (0) homogeneous; (1) heterogeneous, with long guard-setae grouped on lateral and dorso anterior areas.
- (28) Proximal part of embolus in frontal view, shape: (0) straight; (1) slightly curved; (2) strongly curved.
- (29) Proximal part of embolus in frontal view, state: (0) no curvature; (1) strongly S-shaped curvature, West et al. (2008: Figs. 7–9).
- (30) Anterior eye row: (0) straight; (1) procurved.

## Results

Morphology based searches in PAUP\* resulted in a single most parsimonious trees (length = 58, Ci = 0.7241, Ri = 0.8298; Figure 17; character changes see Table 2, character diagnostics see Table 3). Within Theraphosidae, Psalmopoeinae and Aviculariinae share a number of synapomorphies in characters 5 (Spermathecal receptacles), 15 (leg spines absent on tibiae and metatarsi), 16 (tarsal and metatarsal scopula laterally projected) and 22 (tibial clavate trichobothria disposition), resulting in a good support for this grouping (100). *Psalmopoeus* is found monophyletic and well-supported (99) based on synapomorphies in characters 9 (lyra on prolateral maxilla; see West et al. 2008) and 18 (tarsal scopula IV), the latter with a parallelism in *Pseudoclamoris*+Aviculariinae. *Psalmopoeus cambridgei* and *Psalmopoeus irminia* differ mainly by their colouration and there is convincing evidence (97) these two are sister taxa, as their juveniles share the same state in character 24 (dorsal abdominal pattern in juveniles). Character 24 was found to introduce homoplasy, but not because the unique state found in these two species of *Psalmopoeus*, but due to ambiguous changes in Schismatothelinae. The character should be further tested in this group to account for its stability correctly (in prep.). *Psalmopoeus reduncus* differs from the other species by its apomorphy in character 23 (ontogenetic change of colour pattern), with a parallelism in *Ephobopus*+*Pseudoclamoris*+Aviculariinae and a reversal in *Antillena*. *Tapinauchenius* and other

**Table 3.** Character diagnostics for preferred topology resulting from cladistic analysis.

Character	Ci	Ri	G-fit
1	1.000	1.000	1.000
2	1.000	1.000	1.000
3	0.500	0.500	0.600
4	1.000	1.000	1.000
5	0.500	0.833	0.750
6	0.500	0.800	0.750
7	1.000	1.000	1.000
8	0.500	0.500	0.750
9	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
16	1.000	1.000	1.000
17	1.000	1.000	1.000
18	0.500	0.857	0.750
19	1.000	1.000	1.000
20	0.500	0.500	0.750
21	0.500	0.800	0.750
22	1.000	1.000	1.000
23	0.333	0.714	0.600
24	0.667	0.750	0.600
25	1.000	1.000	1.000
26	0.500	0.750	0.750
27	0.333	0.750	0.600
28	1.000	1.000	1.000
29	0.500	0.000	0.750
30	1.000	1.000	1.000

Psalmopoeinae+Aviculariinae share a single synapomorphy in character 27 (distribution of abdominal setae in females), but as this character seems to be rather homoplastic (ci = 0.333), support for this grouping was rather low (61), as to be expected. However, the state in character 3 (male tibial apophysis: number of spines on Rap), with a parallelism in *Holothele* Karsch, 1879, might be another synapomorphy to support this grouping. *Tapinauchenius* is found monophyletic based on character 24 (dorsal abdominal pattern in juveniles), but as the same state can also be found in *Schismatothele*, this monophyly is not well supported (74). *T. rasti* sp. n. males differ from those of the other *Tapinauchenius* species by the number of spines on Rap based on character 3, whereas *T. polybotes* sp. n. has a distinct curvature of its embolus, character 29, otherwise only found in *Ephobopus* (ci = 0.5). *Ephobopus*, *Pseudoclamoris* gen. n. and the Aviculariinae share synapomorphies in characters 23 (ontogenetic change of colour pattern) and 26 (colouration of tarsi in juveniles), a grouping that is not well

supported (62), due to both of these characters exhibiting homoplasy. Urticating setae on palpal femora are found to be an autapomorphy of *Ephobopus*, whereas abdominal urticating setae are a synapomorphy of Aviculariinae with a possible parallelism in Theraphosinae. *Pseudoclamoris* and Aviculariinae share the same states in characters 18 (tarsal scopula IV) and 24 (dorsal abdominal pattern in juveniles). As both of these characters introduce homoplasy, this is found another of the not so well supported groupings (75). *Pseudoclamoris* is found monophyletic and forms a more stable grouping (85) based on their unique state in character 9 (lyra on prolateral maxilla). The possibility of the structure found in *Pseudoclamoris* being an earlier stage of a lyra found in *Psalmopoeus* was tested (cotype ord: 9) and resulted in four trees (length = 59, Ci = 0.7119, Ri = 0.8247), each one step longer than the presented topology resulting in a less resolved strict consensus, while the presented topology was still found part of the treespace. A lyra can also be found in Dipluridae Simon, 1889, *Idiommata* Ausserer, 1871 and Selenocosmiinae Simon, 1889 (Raven 1985). The lyra in Selenocosmiinae might become more complex, but is possibly secondarily lost in parts of this subfamily. While certainly being homoplastic in larger context, the development of such a lyra in Selenocosmiinae might shed some light on how to evaluate this character in Psalmopoeinae. *P. gigas* and *P. elenae* are found sister taxa based on the number of spines on Rap and form a rather stable grouping (76). Developments in Aviculariinae resemble those presented by Fukushima and Bertani (2017); also see Table 2.

Spermatheca, male palpal bulb, and tibial apophysis shape as well as somatic characters (except colouration traits) of *Psalmopoeus*, *Tapinauchenius* and *Pseudoclamoris* gen. n. are very similar among different populations and species. Therefore, as occurring in *Aphonopelma* (see Hamilton et al. 2016) and *Avicularia* (see Fukushima and Bertani 2017) it is very probable that we can only access the real diversity of this clade using multiple approaches for an accurate definition of specific and generic boundaries.

Morphologically cryptic species are an increasingly recurrent problem on traditional zoological taxonomy (Satler et al. 2013). Boundaries of many Psalmopoeinae species could not be delimited using the current morphological tools and data here.

## Molecular results

In general, the combined gene tree has a strong support in both BA and ML (100/100) for the subfamily of Psalmopoeinae and shows two distinct lineages within Schismatothelinae.

*Tapinauchenius polybotes* sp. n. displays as a sister species to *T. cupreus* even though their geographic locations, based upon their type locality, are hundreds of miles apart. *Tapinauchenius rasti* sp. n., which is geographically next to *T. polybotes* sp. n. and *T. plumipes*, shows as a distinct lineage within the grouping of *Tapinauchenius* forming a strong support for the genus (100/100)

The genus *Ephobopus* shows close relationship to *Tapinauchenius* in BA (97) but not in ML (40), making the placement of it rather difficult and unresolved at this

stage. Further sampling of this genus is needed to clarify the correct placement regarding their relationship within the subfamily Psalmopoeinae.

*Pseudoclamoris* gen. n. forms a unique clade within the Psalmopoeinae subfamily with strong support of 100/97. At this stage it is unclear whether *Pseudoclamoris* gen. n. is the sister genus of *Tapinauchenius* or *Psalmopoeus*, as it's not possible to determine the evolutionary progress of the stridulatory organ. The presence of needle-like field of setae on the proximal maxilla, described here, is unique within Psalmopoeinae and clearly differentiates this genus from other genera. Phylogenomic analysis also show no close relationship to Selenocosmiinae subfamily, therefore the scoring of stridulatory lyra in morphology cladistic is questionable in general.

Based on our analysis, *Ephebopus* appears to be the sister group to *Tapinauchenius* as also shown by West et al. (2008). Therefore, the definition of Psalmopoeinae must be altered to accommodate *Pseudoclamoris* gen. n. and *Ephebopus*, as it fits the result of the morphologically based cladistic analysis by West et al. (2008), as well as the two approaches presented here.

Even though *Psalmopoeus* is not in focus in this work, analysis of several species of *Psalmopoeus* might lead to a possible paraphyly of the genus. *P. cambridgei* and *P. irminia* show distinct morphological characteristics (curvature of stridulatory organ, Mendoza (2014b: Figs. 16 and 17) and juvenile colouration and ontogenetic pattern change differs from other species of the genus) which supports the hypothesis of an independent evolutionary lineage. The type species of *Psalmopoeus*, *P. cambridgei*, was not examined and therefore no taxonomical changes are proposed for this genus.

As a comparison, Fukushima and Bertani (2018) illustrated *Guyrita cerrado* Guadanucci et al. 2007, both male and female characteristics. Primary and secondary copulation organs in both male and female specimens closely resemble those found in Psalmopoeinae, while the spermatheca morphology matches the one found in *P. elenae* comb. n. In male specimens, it features the megaspine PL on tibia of leg I. (Fukushima et al. 2018: fig. 20; also see Figure 8b in this work) which is present in Psalmopoeinae. Furthermore, the abdominal pattern is almost identical to the younger stages of *Pseudoclamoris* gen. n. species, while fading away when reaching maturity (Figure 13), although remaining present in *G. cerrado*.

According to the results presented here, phylogenetic relationship corresponds with the shape of both tibial apophysis and embolus. Aviculariinae genera form a clade with slender and strongly procurved embolus, as well as a lack of tibial apophysis while Psalmopoeinae genera are the sister group to the ground-dwelling Schimatothelinae with which they share similar structures, such as palpal bulb with long embolus bearing no keels and two tibial apophyses distally on the leg I. This grouping supports the hypothesis by Ortiz and Francke (2017) stating that “general shape of the pedipalpal bulb, types and position of the keels on the bulb, size of the tibia I accessory apophysis and spermatheca shape” are primary features for species groups and usable for species delimitation.

Morphological and DNA-based studies, including neotropical Ischnocolinae, are urgently needed to resolve the relationship of these taxa, as they are possibly more

closely related than previously thought. It is necessary to find new morphological characteristics and, combined with molecular, geographic and ecological data, to undertake a more extensive and integrative analysis of Psalmopoeinae and possibly related taxa, as mentioned above (Hüsser et al. in prep).

Even though this contribution is a crucial step to better understand the diversity of Psalmopoeinae and their close relatives, information remains incomplete due to gaps in sampling, both molecular and morphological. The erection of the new genus *Pseudoclamoris* to include former *Tapinauchenius* species remains stable in both molecular and morphological analyses and thus lead to a better understanding of evolution of certain morphological characters, including stridulatory setae.

## Taxonomy

**Araneae Clerck, 1757**

**Mygalomorphae Pocock, 1892**

**Theraphosidae Thorell, 1869**

**Psalmopoeinae Samm & Schmidt, 2010**

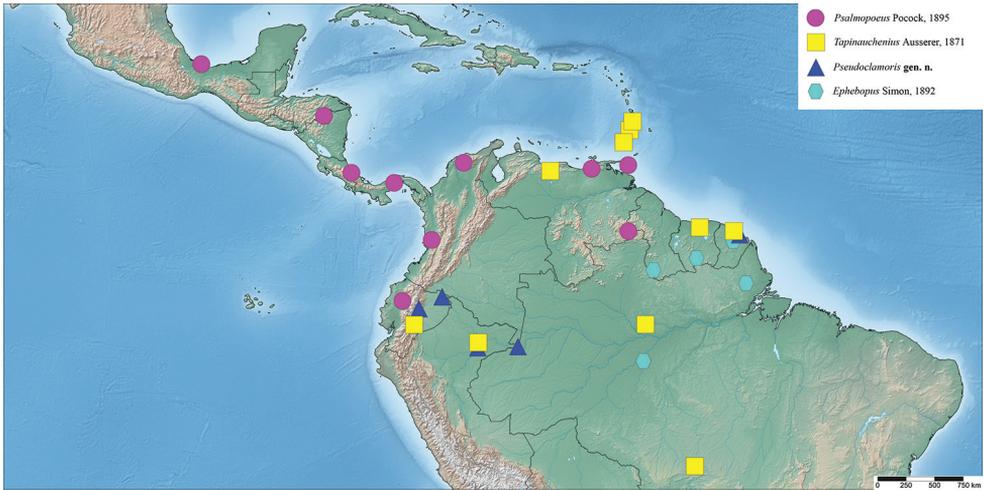
**Included genera.** *Ephobopus*, *Psalmopoeus*, *Pseudoclamoris*, *Tapinauchenius*

**Diagnosis.** The subfamily Psalmopoeinae is diagnosed by following synapomorphies as defined by Samm and Schmidt (2010), altered to fit the results presented here:

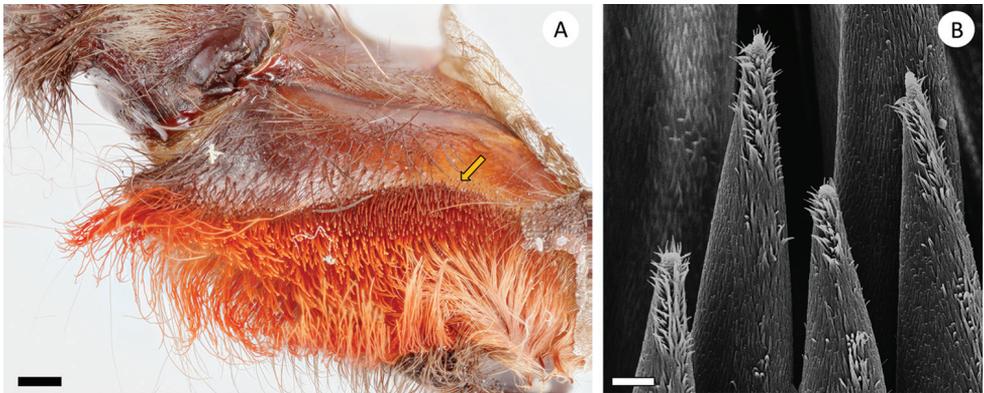
Psalmopoeinae can be distinguished from other new world subfamilies (save Aviculariinae) by their scopulae on anterior tarsi and metatarsi being extended laterally, giving a spatulate appearance and the absence of leg spines on tibiae and metatarsi. They differ from Aviculariinae (except females of *Pachistopelma* and *Iridopelma marcoi* Bertani, 2012), as well as Theraphosinae by the lack of urticating setae on the opisthosoma. Females can further be distinguished from Schismatothelinae by their completely separated spermathecae. Males can easily be distinguished from those of Aviculariinae by the presence of two tibial apophyses on leg I.

**Description.** Legs aspinose or with few apical spines on ventral tibiae and metatarsi; metatarsi and tarsi with scopulae very extended laterally, mainly on anterior legs, giving a spatulate appearance. Spermathecae consisting of two completely separated stalks. Male palpal bulb with long embolus without keels. Males with two tibial apophyses distally on the leg I. Type V urticating setae on prolateral palpal femur present (*Ephobopus*), or absent (all others). Stridulatory organ present (*Psalmopoeus*, *Pseudoclamoris*) or absent (all others). Legs weakly spined or aspinose, tarsi as broad as, or broader than metatarsi. Arboreal (*Psalmopoeus*, *Pseudoclamoris*, *Tapinauchenius*) and fossorial (*Ephobopus*, fossorial only as adults) species.

**Distribution.** Mexico, Central America, north of South America and the Caribbean Islands.



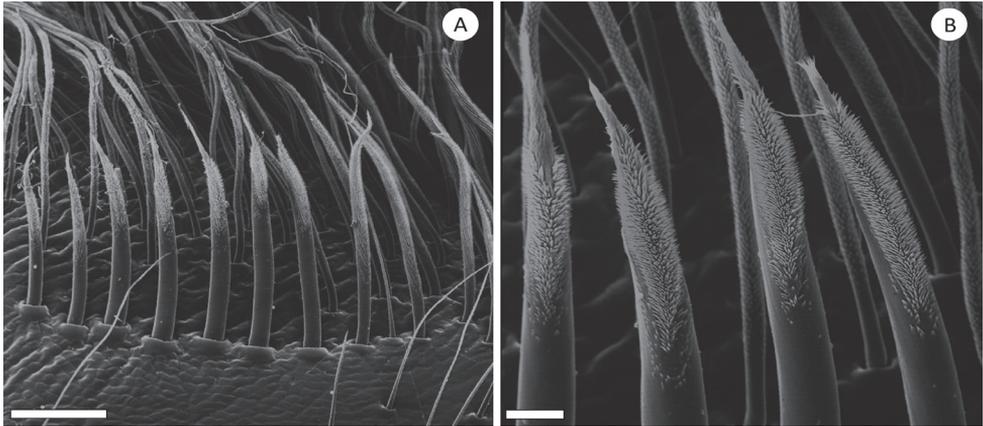
**Figure 1.** Distribution map of Psalmopoeinae.



**Figure 2.** Field of needle-like setae on the proximal maxilla of *Pseudoclamoris* gen. n. **A** overview of the structure **B** detailed structure. Scale bar: 10  $\mu$ m.

**Key to Psalmopoeinae genera**

- 1 Prolateral palpal femora with field of urticating setae .....*Ephebopus*
- Urticating setae absent ..... 2
- 2 Prolateral maxillae without lyra..... *Tapinauchenius*
- Prolateral maxillae with lyra ..... 3
- 3 Lyra consisting of small field of needle-shaped bristles .....  
.....*Pseudoclamoris* gen. n.
- Lyra oval, consisting of short-shafted paddles; sometimes with distal blades..  
.....*Psalmopoeus*



**Figure 3.** Lyra, proximal maxilla of *Psalmopoeus* **A** overview of the structure **B** detailed structure. Scale bar: 10  $\mu$ m.

Araneae Clerck, 1757

Mygalomorphae Pocock, 1892

Theraphosidae Thorell, 1869

Psalmopoeinae Sann & Schmidt, 2010

*Pseudoclamoris* gen. n.

<http://zoobank.org/653225B7-2454-4F04-BD70-F7CBBAE7AD29>

Figs 4–6, 13, 15b

**Type species.** *Tapinauchenius gigas* Caporiacco, 1954, herein designated.

**Species included.** *Pseudoclamoris gigas* (Caporiacco, 1954), comb. n., *Pseudoclamoris elenae* (Schmidt, 1994), comb. n., and *Pseudoclamoris burgessi* sp. n.

**Etymology.** The genus name derives from the Greek *pseudo* meaning false and the Greek *clamoris* meaning screaming/shouting translating into a “false screaming” group of Theraphosidae with the herein described feature of needle-like setae on the proximal maxilla, since the behaviour of *Pseudoclamoris* species resembles the one of *Psalmopoeus*, but no sound is audible when the specimens stridulate in defence posture. Gender is masculine.

**Diagnosis.** Species of *Pseudoclamoris* differ from all known Psalmopoeinae, save *Psalmopoeus*, by the presence of a stridulatory organ on prolateral maxillae. They differ from *Psalmopoeus* by the specific shape of the stridulatory organ consisting of a field of needle-like bristles on the proximal maxilla (Figure 2) (vs. maxillary lyra oval in form, covering 1/4<sup>th</sup> of surface, consisting of short shafted paddles with/without distal blades as in *Psalmopoeus*, compare with Mendoza 2014: figs. 3, 12–17). They furthermore differ from *Ephobopus* by the absence of urticating setae on palpal femora. Juveniles of *Pseudoclamoris* can be distinguished from those of *Tapinauchenius* by their ontogenetic

colour change (Figure 15) and further in the first two instars by their distinct black heringbone pattern on a bright, slightly orange coloured opisthosoma in combination with a dark/black coloured metatarsus, while other leg segments are bright/brown coloured.

**Description.** Carapace longer than wide with cephalic region slightly raised and convex. Striae well marked, fovea deep and straight. Chelicerae without rastellum. Eye tubercle distinct and wider than long, anterior eye row is (slightly) procurved and clypeus absent. Labium subquadrate, slightly wider than long, with numerous (100–300) cuspules concentrated on the anterior half. Maxillae subrectangular with anterior lobe distinctly produced into a conical form, with the inner angle bearing numerous cuspules (more than 100). Sternum longer than wide with posterior sigillae submarginal. STC with median row of a few small teeth. All leg tarsi and anterior metatarsi fully scopulated, Mt III scopulated at distal half and Mt IV scopulated only at distal third. Scopulae on anterior tarsi and metatarsi extended laterally resulting in a spatulate appearance. Femur IV without retrolateral scopula.

***Pseudoclamoris burgessi* sp. n.**

<http://zoobank.org/59A4D644-6FFC-493F-81EE-0881CD798AD7>

Figs. 4–6

**Material examined.** Male holotype and female paratype from Leticia, Colombia deposited in SMF, leg. Auer, 2010; examined.

**Other material examined.** 1 female (MHCOL\_00182) and 1 male (MHCOL\_00201) from Iquitos, Peru in collection, leg. Auer; examined

**Etymology.** The specific epithet is a noun in apposition as a recognition to Joseph Burges, USA who collected important material for this study.

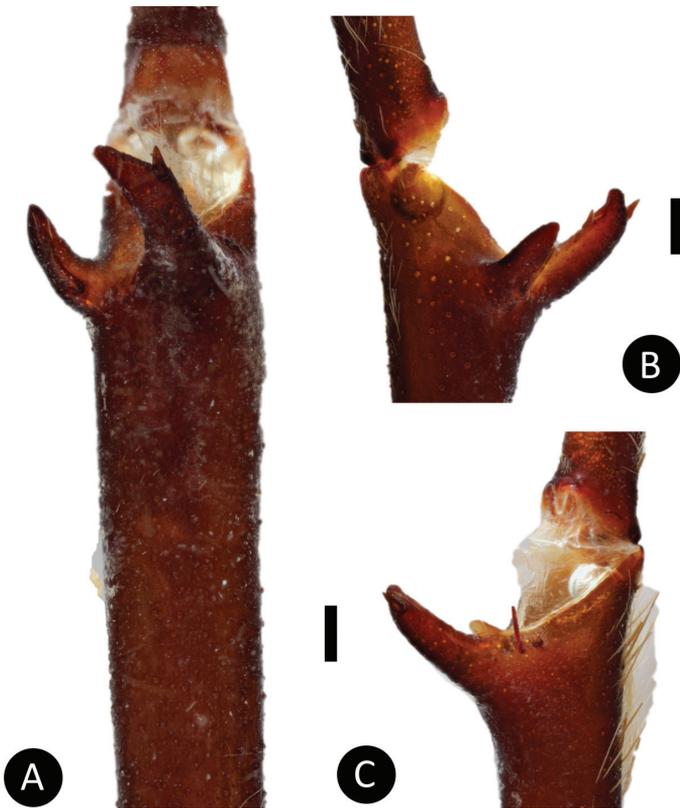
**Diagnosis.** Females of *Pseudoclamoris burgessi* differ from all other species of the genus by the lack of reddish setae on legs and opisthosoma (compare Figure 4a with Figure 13). From *P. gigas* comb. n. by the type locality and the shape of spermatheca where dual lobes appear to be more apart (compare with Schmidt 2003: figs. 6c, 614). Females differ from *P. elenae* comb. n. by the shape of spermatheca (dual lobed instead of multilobed as seen in *P. elenae* comb. n., Fig 1 in Schmidt 1994b). Males of *P. burgessi* differ from those of other *Pseudoclamoris* species by the presence of an additional spine located distally on Rap.

**Description of male holotype.** *Specimen preparation and condition.* The specimens (raised in captivity) were offspring of a wild caught specimen that were originally collected from a burrow and preserved in 80% ethanol. The original colouration of the caught specimen is faded due to the preservation. Right legs I, III, IV, and right pedipalp were removed for measurements and photographs and stored in vial with specimen. Tissue for DNA was extracted.

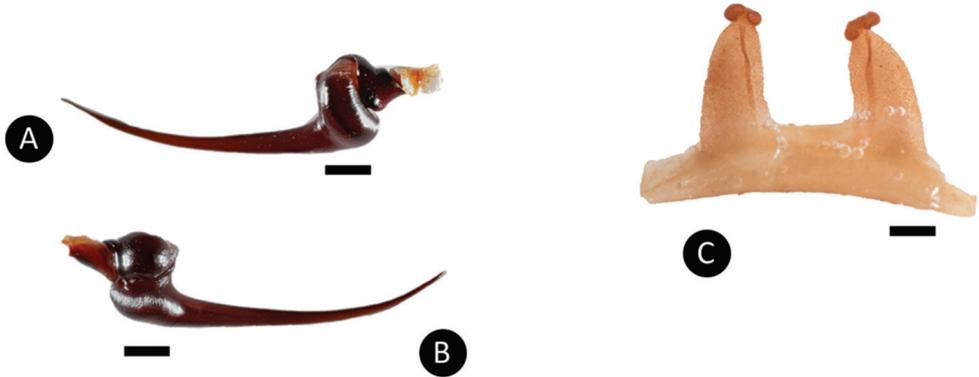
*General colouration:* The specimen is faded black/brown in colour *Cephalothorax:* Carapace 6.541 mm long and 5.214 mm wide; densely clothed with faded pubescence; appressed to the surface; fringe covered in long setae not closely appressed to the sur-



**Figure 4.** *Pseudoclamoris burgessi* sp. n. (habitus) **A** female specimen **B** male specimen. Scale bar: 5mm.  
**Figure 5.** *Pseudoclamoris burgessi* sp. n., male holotype – tibial apophyses **A** ventral **B** retrolateral **C** pro-lateral. Scale bar: 5mm.



**Figure 5.** *Pseudoclamoris burgessi* sp. n., male holotype – tibial apophyses **A** ventral **B** retrolateral **C** pro-lateral. Scale bar: 5mm.



**Figure 6.** *Pseudoclamoris burgessi* sp. n., male holotype and female paratype **A** retrolateral view of palpal bulb **B** proximal view of palpal bulb **C** spermathecae dorsal view. Scale bar: 5mm.

face; hirsute appearance; foveal groove is medium deep and straight. The pars cephalica region rises very gradually from the foveal groove on a straight plane towards the ocular area; AER is procurved; PER is recurved; clypeus extends slightly on a curve. LBl 1.512 and LBw 2.891; sternum hirsute, clothed with faded brown, densely packed, short setae *Abdomen*: Densely clothed in short black/brown pubescence with numerous longer, lighter setae interspersed (generally red or orange *in situ*)

*Legs*: Hirsute; densely clothed in faded brown pubescence. Metatarsus I is straight. F1 14.123; F1w 3.142; P1 6.142; T1 11.521; M1 10.212; A1 6.125; F3 12.012; F3w 3.042; P3 4.062; T3 7.524; M3 8.145; A3 5.214; F4 13.012; F4w 3.042; P4 5.012; T4 11.102; M4 12.051; A4 5.105; femur III is normal. All tarsi are fully scopulate. Extent of the metatarsal scopulation, leg III (SC3) = 55% and leg IV (SC4) = 78%. Two ventral spinose setae are on metatarsus III, five ventral spinose setae on metatarsus IV, one prolateral spinose seta on tibia I, and one megaspine on the apex on the retrolateral branch of the tibial apophyses. *Coxa I*: Prolateral surface is covered by fine, hair-like setae. *Tibia I*: two apophyses that do not originate from a common base; Pap short and strong, with one short spine on inner face; the Rap is well developed, broad at its base with one short and strong spine on the inner face and two short and strong spines distally. *Pedipalps*: Hirsute, densely clothed in the same setal colour as the other legs, with numerous longer ventral setae, one spinose seta at the apical, prolateral femur and three prolateral spinose setae on the palpal tibia; PT1 7.012, PTw 2.125. When extended, embolus tapers with a curve to the retrolateral side; embolus slender, no keels; distinct dorsal and ventral transition from bulb to embolus

**Description of female paratype.** *Specimen preparation and condition*: The examined specimens, raised in captivity, are offspring of wild caught specimen that were collected live from a burrow, and preserved in 80% ethanol. The original colouration has faded due to the preservation. A 50 mg tissue sample was extracted for DNA analysis. The genital plate with spermathecae was removed and cleared, then stored in a vial with the specimen.

**General colouration:** faded black/brown. **Cephalothorax:** Carapace is 17.456 mm long, and 16.245 mm wide; densely clothed with short faded black/brown pubescence, closely appressed to the surface, the fringe densely covered in slightly longer setae; foveal groove is medium deep and slightly procurved. The pars cephalica region gently rises from the thoracic furrow, arching anteriorly toward the ocular area; AER is slightly procurved, PER is very slightly recurved; clypeus extends forward on a curve. LBl 1.627 and LBw 2.982; sternum is covered with short faded setae. The abdomen is densely clothed dorsally in short faded black setae with longer, lighter setae (generally red or orange in situ). **Spermathecae:** paired and separate, with capitate bulbs widening towards the bases and not fused, two lobes on each end. **Legs:** are densely clothed in short faded black/brown pubescence. F1 16.145; F1w 4.234; P1 8.241; T1 13.356; M1 11.278; A1 7.252; F3 11.412; F3w 3.845; P3 6.265; T3 9.156; M3 11.589; A3 6.256; F4 14.163; F4w 4.167; P4 7.532; T4 12.356; M4 13.578; A4 6.521. All tarsi are fully scopulate. Extension of metatarsal scopulation: Leg III (SC3) = 58% and leg IV (SC4) = 79%. There is one ventral and one prolateral spinose seta on metatarsus III, and four ventral spinose setae and one prolateral spinose seta on metatarsus IV. **Coxa I:** prolateral surface is covered by very thin tapered and fine, hair-like setae. **Pedipalps:** densely clothed in the same setal colour as the other legs, with one spinose seta on the apical, prolateral femur, four prolateral (two at the apical, prolateral border with the tarsus) spinose setae and one ventral spinose seta on the tibia.

**Distribution and natural history.** Lowland rainforest of the Amazon region of the countries of Colombia and Peru. Ecuador as distribution is highly likely due to the occurrence very close to the border of Ecuador in Leticia, Columbia. *Pseudoclamoris burgessi* is sympatric with *Tapinauchenius* sp. in the same mentioned region (Auer, pers. comm.)

**Remarks.** No threat through poaching or smuggling of animals out of the country of origin is to be expected, since *P. burgessi* is bred in the pet-trade all around the world for several years now. Egg sacs of *P. burgessi* typically contain between 80 and 220 spiderlings, depending on the size of the female. (pers. obs. and Rast pers. comm.)

## Araneae Clerck, 1757

### Mygalomorphae Pocock, 1892

#### Theraphosidae Thorell, 1869

#### Psalmopoeinae Salm & Schmidt, 2010

#### *Tapinauchenius* Ausserer, 1871

**Type species.** *Tapinauchenius plumipes* (C. L. Koch, 1842).

**Species included.** *Tapinauchenius brunneus* Schmidt, 1995, *Tapinauchenius cupreus* Schmidt & Bauer, 1996, *Tapinauchenius latipes* L. Koch, 1875, *Tapinauchenius sanctivincenti* (Walckenaer, 1837), *Tapinauchenius violaceus* (Mello-Leitão, 1930), *Tapinauchenius rasti* sp. n., *Tapinauchenius polybotes* sp. n.

**Diagnosis.** Differs from all other Psalmopoeinae genera by the lack of a stridulatory organ palpal coxa, prolateral and from *Ephebopus* by the absence of urticating setae on palpal femora.

**Description.** Carapace longer than wide with cephalic region slightly raised and convex. Striae well marked, fovea deep and straight. Chelicerae without rastellum. Eye tubercle distinct and wider than long, anterior eye row is straight and clypeus absent. Labium subquadrate, slightly wider than long, with numerous (100–300) cuspules concentrated on the anterior half. Maxillae subrectangular with anterior lobe distinctly produced into a conical form, with the inner angle bearing numerous cuspules (more than 100). Sternum longer than wide with posterior sigillae submarginal. STC with median row of a few small teeth. All leg tarsi and anterior metatarsi fully scopulated, Mt III scopulated at distal half and Mt IV scopulated only at distal third. Scopulae on anterior tarsi and metatarsi extended laterally resulting in a spatulate appearance. Femur IV without retrolateral scopula.

**Remarks.** Species of the genus *Tapinauchenius* do not show any ontogenetic pattern change. Juveniles are uniformly coloured in a dark-grey with faint bluish tone (Figure 15a).

***Tapinauchenius polybotes* sp. n.**

<http://zoobank.org/12D484B9-3C64-4E80-8251-E2C41F91C90E>

Figs 7–9

**Material examined.** Male holotype and female paratype from Saint Lucia, Lesser Antilles deposited in SMF, leg. Sanchez, don. B Rast, 2013; examined.

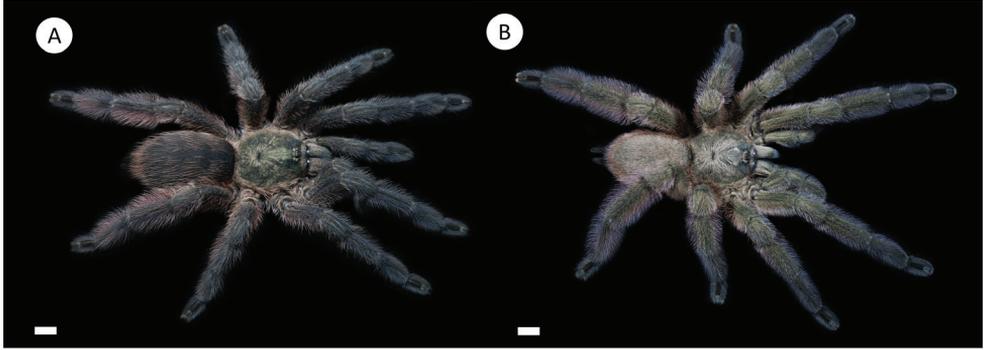
**Other material examined.** 1 female (MHCOL\_0034) and 1 male (MHCOL\_0048)

**Etymology.** The specific epithet is a noun in apposition, referring to the giant Polybotes originating in the Greek mythology and is in reference to the large size of the species compared to congeners of the genus and subfamily in general.

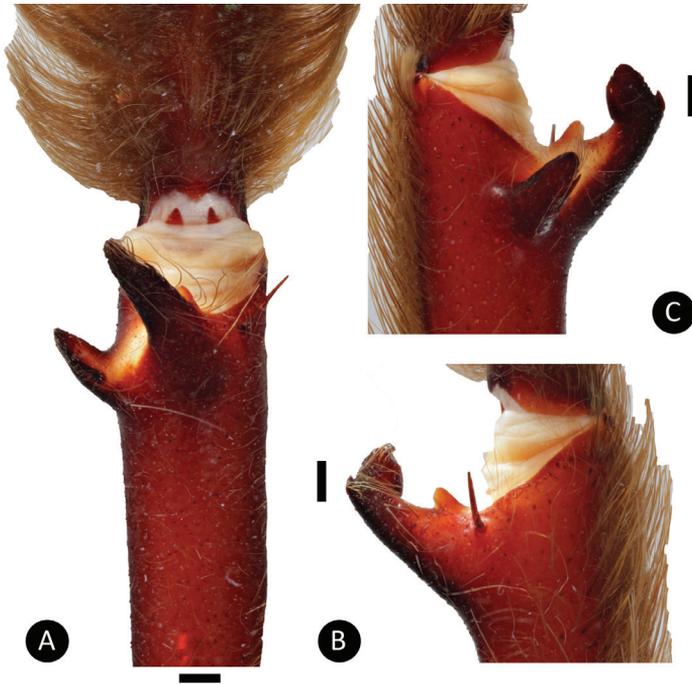
**Diagnosis.** *Tapinauchenius polybotes* differs from all other *Tapinauchenius* species by its large overall size in both male and female specimens and the fact that it's only known from the type locality, with high possibility of endemism due to its location.

Males additionally differ from all other *Tapinauchenius* species by having the embolus strongly S-curved to retrolateral side in apical fourth (Figure 9c), otherwise only known from species of *Ephebopus*, as shown by West et al. (2008: Figs 7–9).

**Description of male holotype.** *Specimen preparation and condition:* Adult female collected at the type locality in 2010 by A Sanchez. In captivity, female built an egg sack of which the specimens (holotype and paratype) were raised to adulthood and then donated to the author by B Rast, collected alive as adult specimens, preserved in 80% ethanol; original colouration faded due to preservation. Right legs I, III, IV, and right pedipalp removed for measurements and photographs; stored in vial with specimen. Tissue for DNA was extracted.

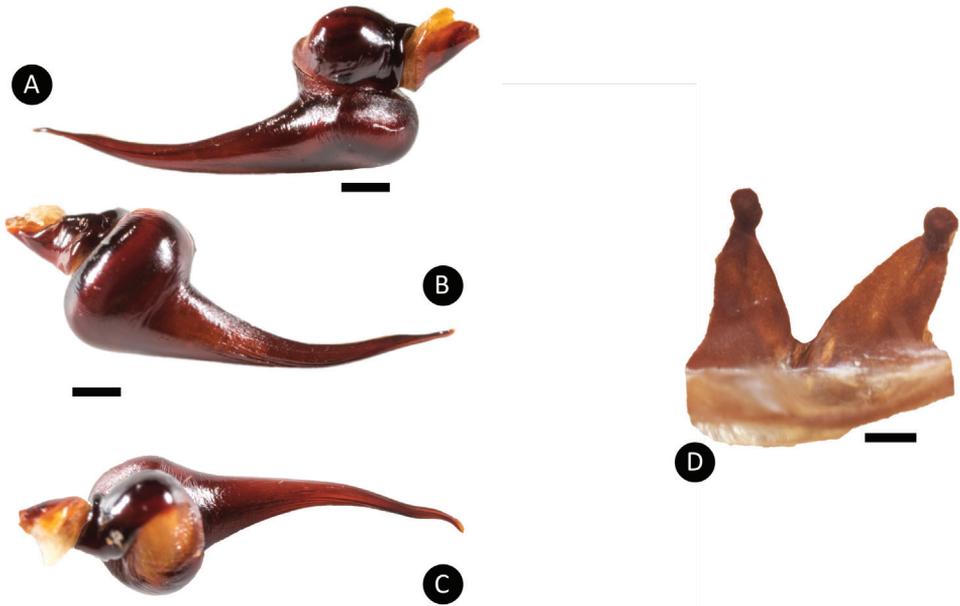


**Figure 7.** *Tapinauchenius polybotes* sp. n., (habitus) **A** female specimen, paratype **B** male specimen, holotype. Scale bar: 5mm.



**Figure 8.** *Tapinauchenius polybotes* sp. n., male holotype – tibial apophyses **A** ventral **B** retrolateral **C** prolateral. Scale bar: 5mm.

*General colouration:* Faded black/blueish. *Cephalothorax:* Carapace 19.421 mm long, 16.412 mm wide; densely clothed with faded pubescence, appressed to surface; fringe covered in long white setae not closely appressed to surface, hirsute appearance; foveal groove medium deep and straight; pars cephalica region rises very gradually from foveal groove on a straight plane towards the ocular area; AER procurved, PER recurved; clypeus extends slightly on a curve; LBl 3.314, LBw 3.021; sternum hirsute,



**Figure 9.** *Tapinauchenius polybotes* sp. n., male holotype and female paratype **A** retrolateral view of palpal bulb **B** prolateral view of palpal bulb **C** distal view of palpal bulb **D** spermathecae dorsal view. Scale bar: 5mm.

clothed with faded, densely packed, short setae. *Abdomen*: Densely clothed in short black/brown pubescence with numerous longer, lighter setae. *Legs*: Hirsute; densely clothed in faded pubescence. Metatarsus I straight. F1 16.962; F1w 4.232; P1 9.171; T1 14.816; M1 12.983; A1 7.151; F3 13.892; F3w 3.895; P3 7.462; T3 11.122; M3 9.834; A3 7.167; F4 17.916; F4w 4.641; P4 8.266; T4 13.873; M4 16.368; A4 8.143; femur III is normal. All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 60%; leg IV (SC4) = 78%. Two ventral spinose setae on metatarsus III; five ventral spinose setae on metatarsus IV; one prolateral spinose seta on tibia I; one megaspine on the apex on the retrolateral branch of the tibial apophyses. *Coxa I*: Prolateral surface covered by fine, hair-like setae. *Tibia I*: two apophyses that do not originate from a common base, Pap short and strong, with one short spine on inner face; the Rap is well developed, broad at its base with one short and strong spine on the inner face and two short and strong spines on top; *Pedipalps*: Hirsute; densely clothed in the same setal colour as the other legs, with numerous longer ventral setae; one spinose seta at the apical, prolateral femur and three prolateral spinose setae on the palpal tibia. PT1 7.145, PTw 2.145. Palpal bulb large, globular, short slender embolus tapering slightly apically, two times longer than the tegulum, “S” shapely curved to retrolateral side on apical fourth (Figure 9c). The embolus base shows a clear separation from the tegulum, with the width of the embolus base  $\frac{3}{5}$  of the tegulum height.

**Description of female paratype.** *Specimen preparation and condition*: Origin same as holotype; collected alive, preserved in 80% ethanol. The original colouration has faded due to the preservation. A 50 mg tissue sample was extracted for DNA analysis.

**General colouration:** faded black. **Cephalothorax:** carapace is 20.14 mm long and 16.542 mm wide. It is densely clothed with short faded black/brown pubescence closely appressed to surface, the fringe is densely covered in slightly longer setae; foveal groove is medium deep and slightly procurved; cephalic region gently rises from the thoracic furrow, arching anteriorly toward the ocular area. AER is slightly procurved; PER very slightly recurved; clypeus extends forward on a curve; LBl 3.514, LBw 3.014. The sternum is covered with short faded setae. **Abdomen:** densely clothed dorsally in short faded black setae with longer, lighter setae (generally red *in situ*). **Spermathecae:** paired and separate, with capitate bulbs widening towards the bases and not fused. **Legs:** densely clothed in short faded black/blue pubescence. F1 17.132; F1w 5.142; P1 9.214; T1 15.212; M1 13.213; A1 7.211; F3 14.212; F3w 4.215; P3 7.512; T3 11.32; M3 10.132; A3 7.321; F4 18.217; F4w 5.241; P4 8.576; T4 14.576; M4 16.678; A4 8.213. All tarsi are fully scopulate. Extent of the metatarsal scopulation: leg III (SC3) = 66% and leg IV (SC4) = 54%. Ventral and prolateral spinose setae on metatarsus IV, five ventral spinose setae and one prolateral spinose seta on metatarsus IV. **Pedipalps:** densely clothed in the same setal colour as the other legs.

**Distribution and natural history.** Only known from the island of Saint Lucia, Lesser Antilles.

**Remarks.** In pet trade, specimens labelled as *Tapinauchenius sanctivincenti* and *Tapinauchenius* cf. *sanctivincenti* belong to the same species described here as *Tapinauchenius polybotes* sp. n. Pet trade material originates from the mother of the type material of the species herein described. Shortly after introduction to the hobby, the species was available under the name of *Tapinauchenius* cf. *sanctivincenti* and *Tapinauchenius* sp. “St. Lucia”.

No threat through poaching or smuggling of animals out of the country of origin is to be expected, since *T. polybotes* is bred successfully in the pet trade all around the world since 2012. Egg sac of *T. polybotes* typically contain between 80 and 190 spiderlings, depending on the size of the female. (pers. obs. and B Rast pers. comm.)

***Tapinauchenius rasti* sp. n.**

<http://zoobank.org/4261DE10-B337-43EB-A137-AE34FA7589CE>

Figs. 10–12

**Material examined.** Male holotype and female paratype from region around Mt. Taboi, Union Island, St. Vincent and the Grenadines, Lesser Antilles deposited in SMF, leg. Burgess, don. B Rast, 2013; examined.

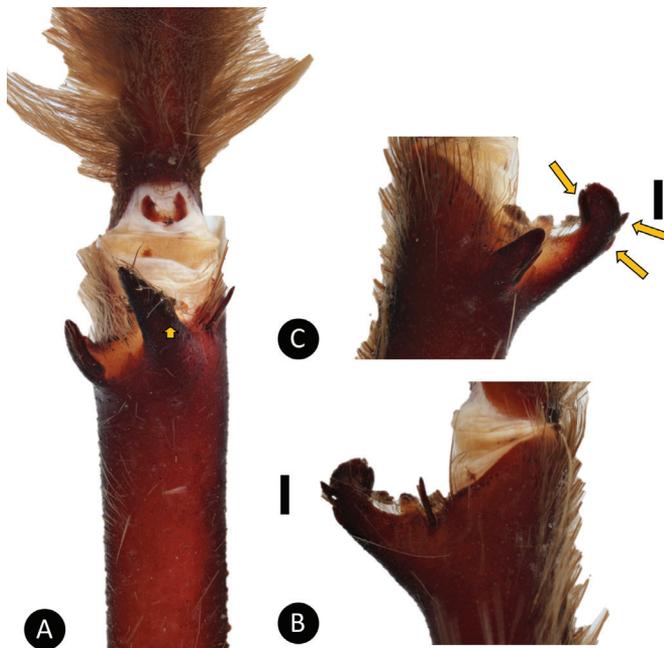
**Other material examined.** 1 female (MHCOL\_0087) and 2 males (MHCOL\_0076, 0065).

**Etymology.** The specific epithet is a noun in apposition as a recognition to Bastian Rast, Switzerland, who guided the author in his early years of tarantula research and is still tremendously supportive of the author’s work.

**Diagnosis.** *Tapinauchenius rasti* sp. n. differs from all known *Tapinauchenius* by their type locality and unique colouration in adult females. Males furthermore differ

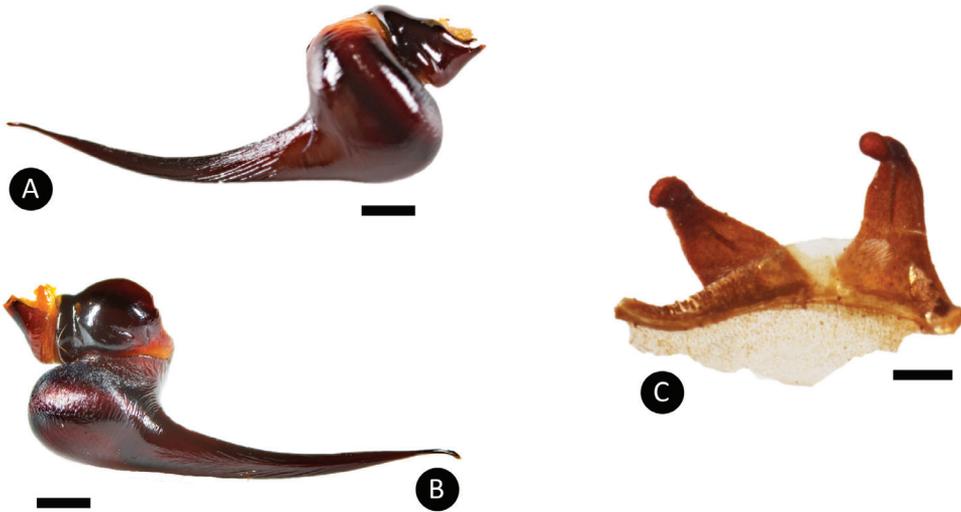


**Figure 10.** *Tapinauchenius rasti* (habitus) **A** female specimen, paratype **B** male specimen, holotype. Scale bar: 5mm.



**Figure 11.** *Tapinauchenius rasti*, male holotype – tibial apophyses **A** ventral **B** retrolateral **C** prolateral. Scale bar: 5mm.

from those of *T. polybotes* by their slenderer embolus and from all other *Tapinauchenius* species by having a Rap with three short and strong spines (Figure 11c). Females can be distinguished from those of all other *Tapinauchenius* by their adult colouration (see



**Figure 12.** *Tapinauchenius rasti* sp. n., male holotype and female paratype **A** retrolateral view of palpal bulb **B** prolateral view of palpal bulb **C** spermathecae dorsal view. Scale bar: 5mm.

Figure 10), having a brightly green coloured cephalothorax while its abdomen and legs are of blue to violet colour with longer reddish setae on legs III and IV.

**Description of male holotype.** *Specimen preparation and condition:* In captivity, female built an egg sack from which the specimens (holotype and paratype) were raised to adulthood and donated to the author by B Rast, collected alive, preserved in 80% ethanol. The original colouration has faded due to the preservation. A 50 mg tissue sample was extracted for DNA analysis. Right legs I, III, IV, and right pedipalp removed for measurements and photographs; stored in vial with specimen. Tissue for DNA was extracted.

*General colouration:* Faded black/blueish. *Cephalothorax:* Carapace 16.421 mm long, 14.412 mm wide; densely clothed with faded pubescence, appressed to surface; fringe covered in long white setae not closely appressed to surface, hirsute appearance; foveal groove medium deep and straight; pars cephalica region rises very gradually from foveal groove on a straight plane towards the ocular area; AER procurved, PER recurved; clypeus extends slightly on a curve; LBl 2.104, LBw 2.231; sternum hirsute, clothed with faded, densely packed, short setae. *Abdomen:* Densely clothed in short black/brown pubescence with numerous longer, lighter setae.

*Legs:* Hirsute; densely clothed in faded pubescence. Metatarsus I straight. F1 14.961; F1w 4.032; P1 5.714; T1 13.210; M1 11.973; A1 6.013; F3 10.753; F3w 3.832; P3 4.692; T3 9.072; M3 11.312; A3 5.173; F4 13.112; F4w 4.252; P4 5.342; T4 13.102; M4 13.552; A4 5.981; femur III is normal. All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 59%; leg IV (SC4) = 72%. Two ventral spinose setae on metatarsus III; five ventral spinose setae on metatarsus IV; one prolateral spinose seta on tibia I; one megaspine on the apex on the retrolateral branch of the tibial apophyses. *Coxa I:* Prolateral surface covered by fine, hair-like setae. *Tibia I:*

two apophyses that do not originate from a common base, Pap short and strong, with one short spine on inner face; the Rap is well developed, broad at its base with one short and strong spine on the inner face and three short and strong spines on top (see arrows Figure 11c); *Pedipalps*: Hirsute; densely clothed in the same setal colour as the other legs, with numerous longer ventral setae; one spinose seta at the apical, prolateral femur and three prolateral spinose setae on the palpal tibia. PT1 7.123, PTw 2.154. Palpal bulb large, globular, short slender embolus tapering slightly apically. When extended, the embolus tapers with a curve to the retrolateral side; no keels; distinct dorsal and ventral transition from bulb to embolus.

**Description of female paratype.** *Specimen preparation and condition*: offspring of wild-caught specimen raised in captivity, specimen collected live from burrow 50 mg tissue sample extracted for DNA analysis. Genital plate with spermathecae removed and cleared, stored in vial with specimen.

*General colouration*: Faded black/brown. *Cephalothorax*: Carapace 16.553 mm long, 13.634 mm wide; metallic green colouration in live specimens, densely clothed with short faded black/brown pubescence closely appressed to surface; fringe densely covered in slightly longer setae; foveal groove medium deep and slightly procurved; pars cephalica region gently rises from thoracic furrow, arching anteriorly toward ocular area; AER procurved, PER very slightly recurved; clypeus extends forward on a curve; LBl 2.221, LBw 2.521; sternum hirsute, clothed with short faded setae. *Abdomen*: Densely clothed dorsally in short faded black setae with longer, lighter setae (generally red in situ). *Spermathecae*: Paired and separate, with capitate bulbs widening towards the bases; not fused. *Legs*: Hirsute; densely clothed in short faded blue pubescence; F1 14.432; F1w 4.012; P1 7.212; T1 11.413; M1 9.312; A1 5.512; F3 10.857; F3w 4.132; P3 6.142; T3 8.581; M3 9.731; A3 5.321; F4 13.214; F4w 4.235; P4 7.125; T4 11.235; M4 12.456; A4 6.236. All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 58%; leg IV (SC4) = 74%.

One ventral and one prolateral spinose seta on metatarsus III; four ventral spinose setae and one prolateral spinose seta on metatarsus IV. *Coxa* I: Prolateral surface covered by very thin tapered and fine, hair-like setae. *Pedipalps*: Densely clothed in the same setal colour as the other legs; one spinose seta on the apical, prolateral femur, four prolateral (two at the apical, prolateral border with the tarsus) spinose setae and one ventral spinose seta on the tibia.

**Distribution and natural history.** Only known from Union Island, Caribbean. All adult specimens were observed on larger/older growth trees and used cavities and “knot holes” as retreats. One subadult specimen observed under loose bark. Retreats were typically silk-lined around the entrance. Sub adults were seen on large and small diameter trees as well as bromeliads, rocks and one occurrence on the ground. Females showed parental care. Two different females observed with spiderlings in and around retreat entrance. (pers. comm. Joseph Burgess).

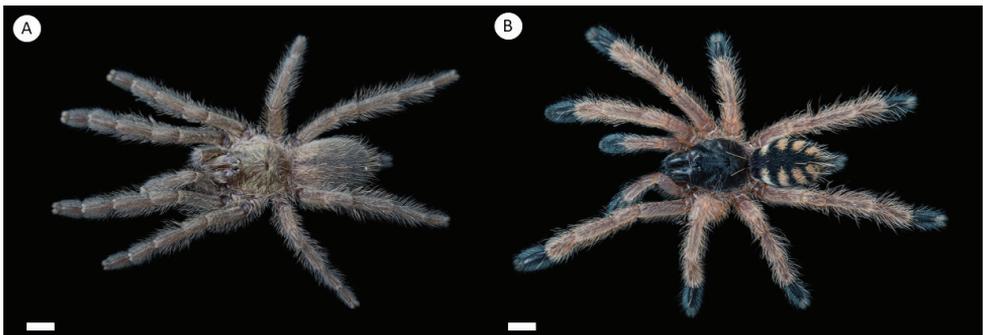
**Remarks.** In the pet trade, specimens labelled as *Tapinauchenius* sp. “Caribbean Diamond” and *Tapinauchenius* sp. “Union Island” belong to the same species described here as *Tapinauchenius rasti* sp. n.



**Figure 13.** *Pseudoclamoris* spp. (habitus) **A** female of *Pseudoclamoris elenae* comb. n. **B** female specimen of *Pseudoclamoris gigas* comb. n. Scale bar: 5mm.

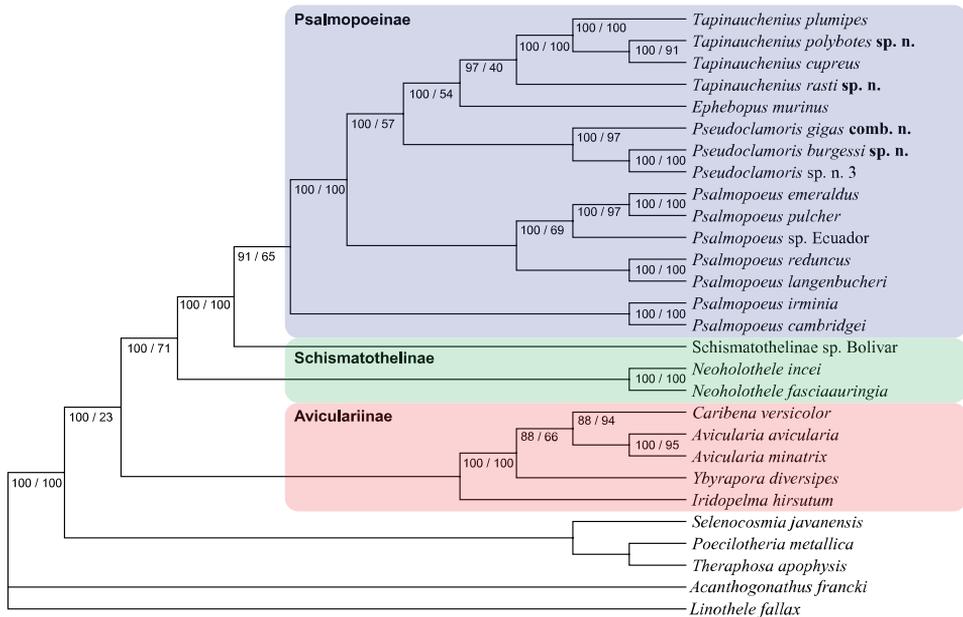


**Figure 14.** Cheliceral strikers of *Tapinauchenius violaceus* **A** overview **B** detailed view. Scale bars: 20  $\mu\text{m}$  (**A**); 100  $\mu\text{m}$  (**B**).



**Figure 15.** Ontogenetic pattern change **A** juvenile of *Tapinauchenius* **B** juvenile of *Pseudoclamoris*. Scale bar: 5mm.

Legend: MrBayes (BA) / RAxML (ML)



**Figure 16.** Phylogenetic tree of Psalmopoeinae species and close relatives, based upon combined CO1, 16S and 28S genomic region analysis, combined with ML and BA values according to defined parameters.

No threat through poaching or smuggling of animals out of the country of origin is to be expected, since *T. rasti* sp. n. is bred successfully in the pet-trade all around the world since 2012. Egg sac of *T. rasti* sp. n. typically contain between 80 and 190 spiderlings, depending on the size of the female. (pers. obs. and Rast pers. comm.)

## Species inquirenda

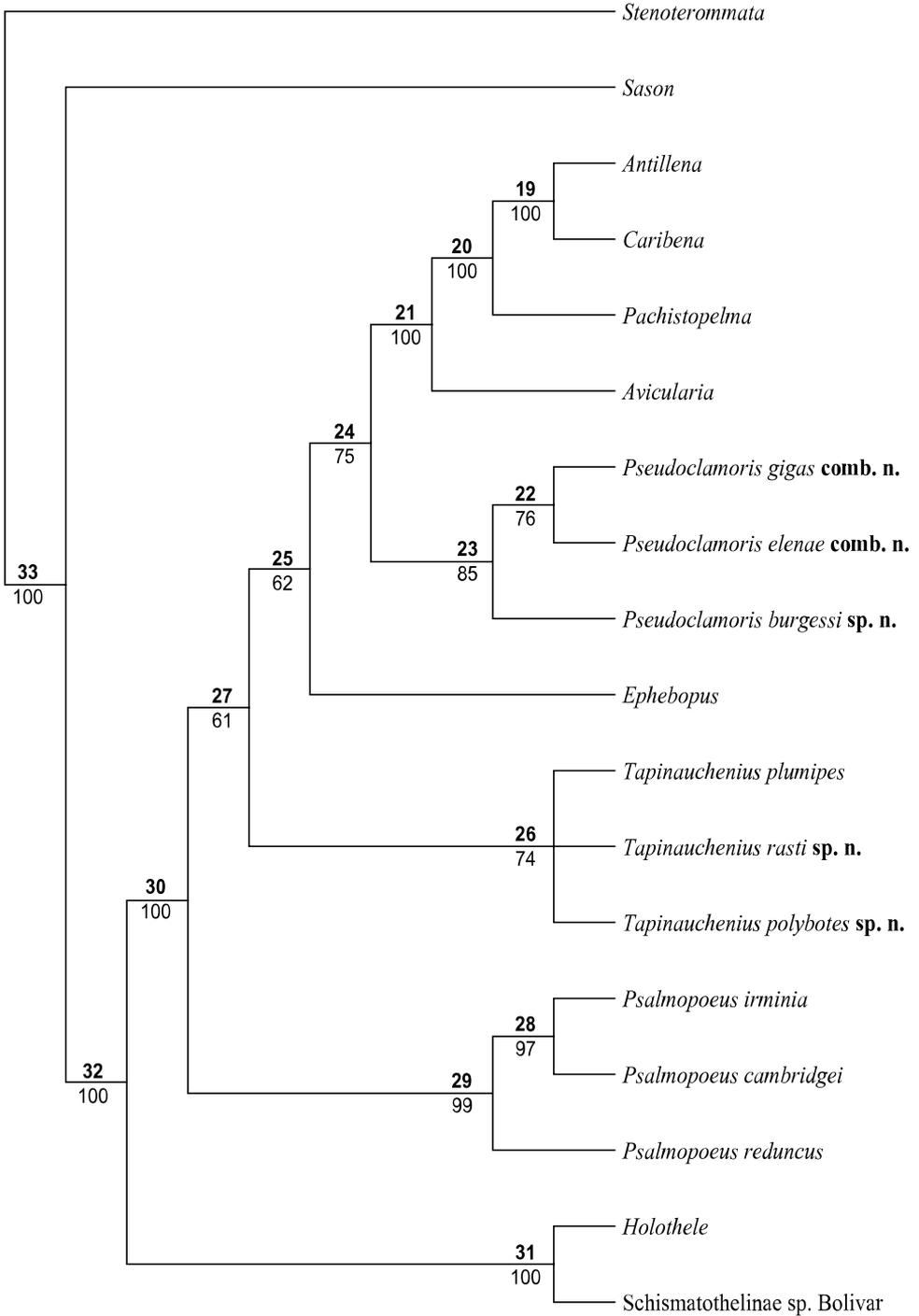
### *Tapinauchenius sanctivincenti* (Walckenaer, 1837)

*Mygale sancti-vincentii* Walckenaer, 1837: 216 (Df).

*Tapinauchenius sanctivincenti* Simon, 1892e: 553.

*Tapinauchenius sanctivincenti* FO Pickard-Cambridge, 1896: 745, pl. 34, f. 21.

**Remarks.** The type material, presumably deposited in MNHN, could not be located in the museum collection and is considered lost by the curator (Rollard, pers. comm.). Due to the given type locality of St. Vincent Island it is possible to allocate new material in order to designate a neotype (in prep.).



**Figure 17.** Single tree resulting from cladistic analysis (Length = 58, Ci = 0.7241, Ri = 0.8298)

## Nomen dubium

### *Tapinauchenius subcaeruleus* Bauer & Antonelli, 1997

*Tapinauchenius subcaeruleus* Bauer & Antonelli, 1997: 429, f. 1–3 .

**Remarks.** The original description by Bauer and Antonelli (1997) stated the authors intended to deposit a female holotype at SMF. However, this holotype could not be located in the collections and has never been deposited there (Jäger, pers. comm.). As there is no holotype and the description does not allow for a precise diagnosis, and as further characterisation of the species and the provided type locality (Ecuador without specification) are too broad, it is impossible to allocate new material. Consequently, the species is herein considered a *nomen dubium*.

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This manuscript and its outcome are dedicated to Myrtha Müller, my grandmother, who sadly is not able to see the results of this research, may her soul rest in peace. I would like to thank my parents Charlotte and Marcel and my brother Florian Hüsser, as well as the rest of my family for their ongoing and boundless support over the years. Furthermore, I am especially grateful for the support shown by Yanick Frey, Nadja Koch and, last but not least, Katharina Birchler. Without them this work would not have been possible.

Invaluable gratitude goes to the worldwide tarantula community for donating material and first-hand insights about breeding and the conservation of Theraphosidae outside their natural environment, where habitat destruction is still the most significant threat. Joseph Burgess, Alejandro Sanchez, Hans-Werner Auer, Frank Schneider, Bastian Rast, Gian Sposato, Frank Schneider, Michael Fischer, Yvonne Kindle, Martin Gamache, Patrick Schuhmacher, Jakub Skwobronek, Maria and Laszlo Gudenus are thanked for donating specimens. Bastian Rast is acknowledged for his ongoing friendship and great support, including the opportunity to use his photographs for assembling figures 4, 7, 10, and 15, in addition to his valuable donations. Thanks to Dr. Rainer Foelix for his guidance, input, and possibility to operate the SEM and assemble illustrations for this manuscript. Neue Kantonsschule Aarau (NKA) is thanked for the use of the SEM. Christian Andre, to whom I am grateful for in-depth conversations regarding Theraphosidae systematics and encouraging me to think further and to not blindly rely on already-published data. Bastian Drolshagen is thanked for his support regarding (technical) questions and cladistics within the subfamily Theraphosidae and for his comments on an early version of the manuscript. Thanks to Georg Bommer from Inter-Networking AG and DataGovernance Technologies Ltd for providing a perfect working environment, work-life balance, and support. Dirk Weinmann and Steffen Esche are thanked for their open-minded discussions about Theraphosidae tax-

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## **Supplementary material I**

### **Dryad Data Info**

Authors: Martin Hüsser

Data type: measurement

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# *Stigmella multispicata* Rocienė & Stonis, an Asian leafminer on Siberian elm, now widespread in eastern North America (Lepidoptera, Nepticulidae)

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## Abstract

*Stigmella multispicata* Rocienė & Stonis, 2014, previously known from the single male holotype from Primorye, Russia, is reported as a new invasive species mining leaves of Siberian elm, *Ulmus pumila* L., in eastern North America. Both adults and leafmines have been reported from many sites as unidentified Nepticulidae since 2010. Crucial for the identification was a match of the DNA barcode of a single larva collected on *Ulmus pumila* in Beijing with adults from North America. The single larva constitutes a new record for China. *Stigmella multispicata* is closely related to the European *S. ulmivora* (Fologne, 1860), feeding likewise on *Ulmus*, but differs in details of external morphology and genitalia, particularly in the female, where *S. multispicata* has a remarkable elongated narrow ovipositor, suitable for oviposition in underside hairy leaf vein axils, where all mines start. In North America *S. multispicata* is the only *Ulmus*-feeding nepticulid with green larvae. Currently the species is known from USA: Illinois, Indiana, Iowa, Maryland, Massachusetts, Minnesota, New York, Ohio, Tennessee, Wisconsin, and Canada: Ontario and Québec. In Sagaponack, on Long Island, New York, larvae have been reported to occur en masse on Siberian elms from at least two sites. The current distribution could be reconstructed thanks also to many

online photographs from observation websites. The species is redescribed, with the first descriptions of female, larva, and leafmine, and compared with *S. ulmivora*, which is fully redescribed. The two native North American nepticulid *Ulmus* leafminers, *S. apicalbella* (Chambers, 1873) and *Ectoedemia ulmella* (Braun, 1912), are diagnosed and new provincial and state records are provided. A key to linear mines on *Ulmus* in North America is provided. We suspect that trade of live plants through nurseries played a role in the sudden spread of this invasive species.

### Keywords

Canada, China, DNA barcoding, invasive, key, pest species, *Stigmella ulmivora*, *Ulmus pumila*, United States

## Introduction

The North American insect fauna has been enriched by an influx of numerous alien species. Most invasions in temperate North America originate in Europe, far fewer in Asia (Mattson et al. 1994, 2007). Among phytophagous insects on woody plants, the number reported in 2007 as arriving from Europe is 310 species, compared with only 77 coming from Asia (Mattson et al. 2007). The increasing global travel and transport of goods results in an ever-growing number of established alien insect species, estimated to be more than 40 over a five-year period (Work et al. 2005). While the number and proportion of non-native Lepidoptera is not as large as in some other insect groups, around 250 of the 5431 named Canadian and Alaskan species (4.6%) are thought to be introduced (Pohl et al. 2018). Thanks to DNA barcoding, previously overlooked introductions have been recognized, particularly in taxonomically poorly studied groups (Landry et al. 2013). Here we report another case of a lepidopterous insect introduced from Asia, where DNA barcoding played an essential role in the identification.

Beginning around 2010, James Vargo found numerous specimens of an unknown *Stigmella* species that he had not seen before, while light collecting on his property in Indiana, USA. Material was sent to specialists Don Davis and David Wagner, neither of whom recognized the moths as belonging to a known American species. EJvN got some of these specimens when borrowing unidentified Nepticulidae from the Mississippi Entomological Museum in 2010, and also failed to name them. A near match of the DNA barcode with a larva collected in 2013 from a leafmine on *Ulmus* in Beijing, China, was striking, but initially regarded as coincidental.

In October 2015, DOG received a sample of green nepticulid larvae from Ms. Lee Foster in Sagaponack on Long Island, Suffolk County, New York, that were descending en masse on silk strands from Siberian elm (*Ulmus pumila* L.) (Figs 21–25). Since the two known North American Nepticulidae on elm (see below) have yellow larvae, he contacted EJvN for identification. It soon was clear by comparing DNA barcodes and the emerging moths that these were the same species as the one found in Indiana. The property in Indiana also has several old Siberian elm trees, but leafmines were not seen until 2018 (J. Vargo, pers. comm. to EJvN). Meanwhile, more records with

the same DNA barcode turned up in the BOLD database (Ratnasingham and Hebert 2007). The match with the Chinese elm miner now made more sense, and the male genitalia and external features appeared to match the illustrations of the recently described *Stigmella multispicata* Rocienė & Stonis, 2014, based on a single male holotype from Primorye, Russia (Stonis and Rocienė 2014). It was therefore likely that we were dealing with a recent invasion. Also, several online photos of leafmines and adults, including on BugGuide (2018) and iNaturalist (2018), could now be matched with *S. multispicata* (Table 1).

The Nepticulidae (pygmy moths) of North America were revised almost 40 years ago (Wilkinson 1979, 1981, Wilkinson and Scoble 1979, Wilkinson and Newton 1981; Newton and Wilkinson 1982), but these studies were based on the relatively poor material then available in some museum collections only; no new fieldwork was carried out, and the publications are hampered by the lack of good color illustrations of moths and leafmines. In recent years, extensive collecting and genetic analyses have shown that the nepticulid fauna of North America is much richer than previously thought and also that more revisionary work of old types is needed. The first results of this research were summarized in the recent catalogues of global and Canadian Nepticulidae (van Nieukerken et al. 2016, van Nieukerken 2018). Also, a revival of leafmine studies, partly made possible through online resources (BugGuide 2018, Moth Photographers Group 2018) resulted in an influx of new distribution records.

The Nepticulidae of East Asia are best known from Far East Russia, especially the Primorye region, by studies of J. Stonis (formerly R. Puplesis) and his students (Rocienė and Stonis 2013, Stonis and Rocienė 2013). The study on Chinese nepticulids is still in its infancy [two papers deal with species feeding on Fagaceae (van Nieukerken and Liu 2000, Stonis et al. 2013)] whereas studies on the Japanese fauna are now progressing (Kemperman et al. 1985, Hirano 2013, 2014, Yagi and Hirowatari 2017).

We will here redescribe *Stigmella multispicata*, compare it with the European *S. ulmivora* (Fologne, 1860) and the other North American species of Nepticulidae feeding on *Ulmus*, and discuss the probability of its invasion from Asia into North America.

## Material and methods

### Material

The material of *S. multispicata* from the United States and Canada originates from several sources; the few adult specimens from Canada were collected during the School Malaise Trap Program (Steinke et al. 2017). Leafmines in China were collected by EJVN during a collaboration between groups in The Netherlands and China in the 1980s (van Driel and van Nieukerken 1985, van Nieukerken and Liu 2000) and again during a 2013 visit of EJVN to Beijing and Tianjin. More data were obtained from internet searches and barcoded specimens on BOLD (Ratnasingham and Hebert 2007).

**Table 1.** Online photographs of adults and leafmines of *Stigmella multispicata*. Identifications by E.J. van Niekerken. All leafmines on *Ulmus pumila*.

Stage	State	County/City	Locality	Date	Observer	url
<b>Canada</b>						
1 adult *	Ontario	Toronto	Yarmouth Gardens	26.vii.2015	D. Beadle	<a href="https://www.inaturalist.org/observations/9482752">https://www.inaturalist.org/observations/9482752</a>
1 adult	Ontario	Toronto	Yarmouth Gardens	18.vii.2016	D. Beadle	<a href="https://www.inaturalist.org/observations/9542202">https://www.inaturalist.org/observations/9542202</a>
<b>United States</b>						
1 ♀	Illinois	Cook	Skokie, Balaban House	24.viii.2017	John & Jane Balaban	<a href="https://bugguide.net/node/view/1439557">https://bugguide.net/node/view/1439557</a>
ca 8 mines	Illinois	Lisle	The Morton Arboretum	4.x.2010	Bruce J. Marlin	<a href="http://www.cirrusimage.com/tree_Siberian_Elm.htm">http://www.cirrusimage.com/tree_Siberian_Elm.htm</a>
ca. 3 young mines	Illinois	Urbana	residential area	4.ix.2010	John Hilty	<a href="http://www.illinoiswildflowers.info/trees/plants/sb_elm.html">http://www.illinoiswildflowers.info/trees/plants/sb_elm.html</a>
3 mines	Iowa	Shelby	south of Elk Horn	19.x.2016	M.J. Hatfield	<a href="https://bugguide.net/node/view/1362960">https://bugguide.net/node/view/1362960</a>
1 ♂	Iowa	Winneshiek	100 Acre Wood	27.vii.2014	M.J. Hatfield	<a href="https://bugguide.net/node/view/966519">https://bugguide.net/node/view/966519</a>
1 ♂ **	Iowa	Winneshiek	Plymouth Rock	26.ix.2014	M.J. Hatfield	<a href="https://bugguide.net/node/view/1028011">https://bugguide.net/node/view/1028011</a>
old mines***	Maryland	Baltimore City	Herring Run Park	15.x.2013	Thomas Wilson	<a href="https://bugguide.net/node/view/906301">https://bugguide.net/node/view/906301</a>
1 adult	Minnesota	Hennepin	Minneapolis	19.viii.2013	Bill Johnson	<a href="https://bugguide.net/node/view/813155">https://bugguide.net/node/view/813155</a>
1 adult	Ohio	Pickaway	Orient	7.viii.2012	Gregory Raterman	<a href="https://bugguide.net/node/view/686805">https://bugguide.net/node/view/686805</a>
1 adult	Tennessee	Davidson	Nashville	25.vii.2010	Steven Loftin	<a href="https://bugguide.net/node/view/433176">https://bugguide.net/node/view/433176</a>
1 adult	Wisconsin	Dane	Cross Plains	4.ix.2010	Ilona L.	<a href="https://bugguide.net/node/view/451465">https://bugguide.net/node/view/451465</a>

\* The single photographed adult was said to be part of a “mini invasion” at a light on this night.

\*\* This specimen was sent to the authors and is also listed under material examined.

\*\*\* According to the observer the tree was either *Ulmus parvifolia* or *pumila*, we think it is *U. pumila*.

Material of the other species discussed here mostly originates from the collections of the Naturalis Biodiversity Center, Leiden, Netherlands. The Material examined section provides only the basic locality data, all details on registration numbers, genitalia slides, collectors etc. are provided in the Suppl. material 1.

### Abbreviations for depositories, etc.

<b>BIN</b>	Barcode Index Number (Ratnasingham and Hebert 2013)
<b>BIOUG</b>	Biodiversity Institute of Ontario, University of Guelph, Canada
<b>BOLD</b>	Barcode of Life Data Systems ( <a href="http://www.barcodinglife.com/">http://www.barcodinglife.com/</a> )
<b>CSEC</b>	C.S. Eiseman Research Collection
<b>MEM</b>	Mississippi Entomological Museum, Starkville, Mississippi, United States
<b>RMNH</b>	Naturalis Biodiversity Center, Zoological collections, Leiden, The Netherlands
<b>USNM</b>	National Museum of Natural History, Smithsonian Institution, Washington DC, United States
<b>ZIN</b>	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia

### Methods

For the collection of leafmines in China see van Nieukerken and Liu (2000). Since the collecting of adults and mines in North America was done by several different persons, there are no general methods to describe here.

Genitalia were prepared according to our standard procedures, usually including DNA extraction, which were described earlier in detail (van Nieukerken 1985, van Nieukerken et al. 2010).

Measurements of genitalia were obtained from digital images, using calibrated scaling in the Zeiss AxioVision software; we used a 20× objective for male genitalia and 10× or 20× for female genitalia. Capsule length was measured from vinculum to middle of uncus; valva length from tip of posterior process to ventral edge, excluding the sublateral process; phallus length was measured along the sclerotized tube, from tip, excluding any protruding vesica parts. Total bursa length includes all of the internal genitalia from cloaca to anterior edge of bursa; apophyses were measured from abdominal tip to anterior tip of apophyses. Genitalia measurements are rounded off to the nearest 5 µm. Forewing length was measured from tip of fringe to attachment on thorax, with a Zeiss SV11 stereo-microscope at a magnification of 20×. Antennal segment counts include scape and pedicel; they were counted on photographs or directly under the same stereo microscope.

Photographs of moths were made with an AxioCam MRc 5 digital camera attached to a motorized Zeiss SteREO Discovery V12, using the Module Extended Focus, Zeiss AxioVision software, to prepare a picture in full focus from a Z-stack of ca 10 to 40 individual photos. Leafmines were photographed by EJvN with an AxioCam HRC camera on a Zeiss Stemi SV11 stereo-microscope, without extended focus. Photos by

CSE were taken either with a Canon EOS Rebel XSi SLR digital camera, and MP-E 65 mm macro lens (Figure 34), or with a Nikon D50 digital camera and AF Micro Nikkor 105mm lens (Figure 39) and with a Macro Twin Lite MT-24EX flash unit. Genitalia were photographed with an MRC 5 camera on a manually operated Zeiss Axioskop H, without using extended focus. Photographs were edited with Adobe Photoshop® (various versions), avoiding any change to the real object, but backgrounds are cleaned of excess debris and artifacts by using healing brush and clone tools; tone and contrast are adjusted and some sharpening is used.

Our methodology for DNA barcoding has been described in other papers (van Nieukerken et al. 2012a, 2012c, Dooreneewerd et al. 2015, 2016). We present a Neighbor Joining tree, with KP2 distances, of the selected taxa, made with tools provided by BOLD Systems (Ratnasingham and Hebert 2007). The DNA barcode data as used here are given in detail in the public BOLD dataset DS-STMULT (*Stigmella multispicata* on *Ulmus*, <https://doi.org/10.5883/DS-STMULT>).

## Taxonomy

### *Stigmella multispicata* Rocienė & Stonis

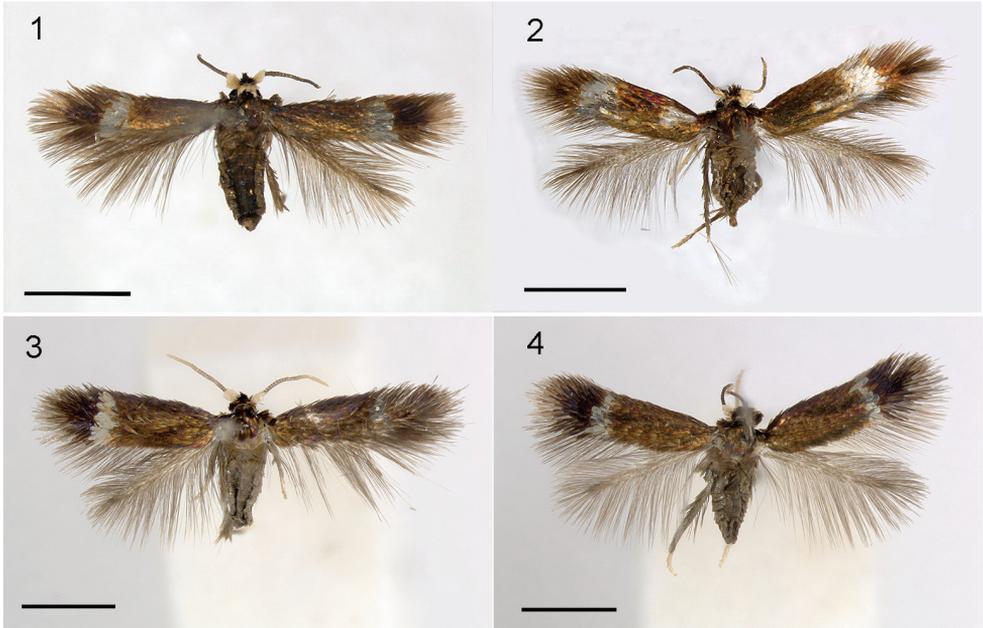
Figures 1, 2, 5–9, 12, 14, 16, 18, 19–26, 28, 29

*Stigmella multispicata* Rocienė & Stonis in Stonis & Rocienė, 2014: 205. Holotype ♂, Russia, Primorskiy Krai, 20 km E Ussuriysk, Gornotayezhnoe, Biological Station, 8.viii.2011, leg. A. Rocienė, genitalia slide no. AG427 (ZIN) [not examined].

*Stigmella multispicata*; van Nieukerken et al. 2016: 106, 173 [moved to *S. ulmivora* group].

**Diagnosis.** In North America *S. multispicata* is the only *Stigmella* species with the combination of black frontal tuft, white collar, and single fascia. *Stigmella quercipulchella* (Chambers, 1882) is relatively similar, but has an additional silver patch at tornus, is slightly larger and has more strongly purple reflections across the forewings. This combination of characters is also diagnostic in East Asia, but there remains a possibility that similar species will be discovered. From the closely related European *S. ulmivora* it differs by the white collar (dark in *ulmivora*) and the entirely dark antennae (those of *S. ulmivora* have the distal 7–8 flagellomeres white); *S. ulmivora* is also slightly larger and has more antennal segments. The female of *S. multispicata* differs from all more or less similar species by the obvious long ovipositor, visible even without dissection.

Male genitalia differ from those of *S. ulmivora* in the shallowly indented uncus and the very short sublateral processes of the transtilla; the female genitalia are easily recognized by the long apophyses; the ductus spermathecae has no spines in contrast to *S. ulmivora*. Some species in the *S. rhamnella* group have superficially similar male genitalia, but they have no juxta, and the moths are externally very different.



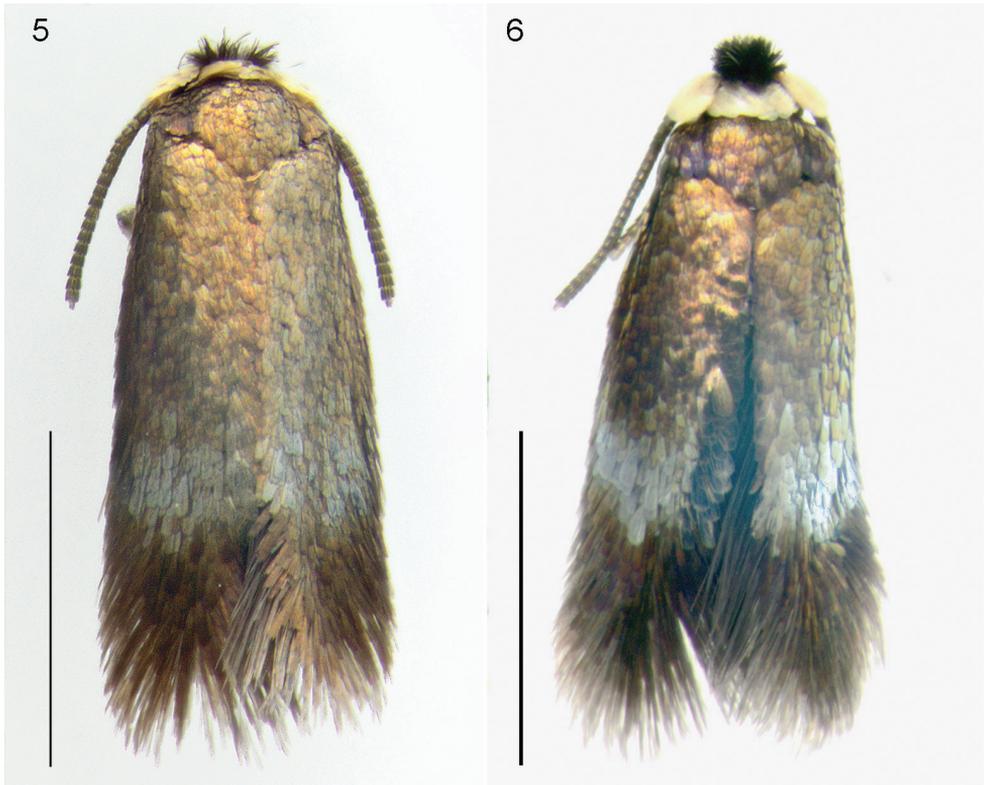
**Figures 1–4.** *Stigmella* species, adult habitus. **1** *S. multispicata*, male, USA, Indiana, RMNH.INS.24511 **2** *S. multispicata*, female, USA, Indiana, 10.viii.2010 **3** *S. ulmivora*, male, Netherlands, RMNH.INS.15496 **4** *S. ulmivora*, female, Netherlands, RMNH.INS.15497. Scale bars 1 mm. Photographs C. Doorenweerd (1), E.J. van Nieuwerkerken.

*Stigmella multispicata* leafminers are characterized by the egg placement on the leaf underside in vein axils, larval exit on leaf underside, and green to blue-green larval color.

**Redescription.** Male (Figs 1, 5, 6). Forewing length 1.8–1.9 mm ( $1.8 \pm 0.1$ , 9), wingspan 3.7–4.1 mm. Head: frontal tuft black, collar cream white. Scape cream white. Antenna fuscous, short, with 21–24 segments ( $22.8 \pm 1.1$ , 5), ratio to forewing length 12–13 segments/mm. Thorax and forewing shining fuscous bronze, in some lights appearing greenish, a silver fascia at 2/3, apex darker fuscous, terminal cilia concolorous, underside dark fuscous. Hindwing grey-brown. Abdomen brown, without visible anal tufts.

Female (Figure 2). Forewing length 1.8–2.2 mm ( $1.9 \pm 0.2$ , 7), wingspan 4.0–4.3 mm. Antenna with 18–20 segments ( $18.3 \pm 0.8$ , 6), ratio to forewing length 8–11 segments/mm. Otherwise as male, abdomen with conspicuous long protruding ovipositor, with small anal tufts.

Male genitalia (Figs 7–9, 12). Capsule length 190–235  $\mu\text{m}$  ( $215.6 \pm 23.0$ , 3), 1.1–1.2 $\times$  as long as wide. Vinculum anteriorly with pointed and anteriorly protruding lateral corners. Uncus with shallow lobes, widely separated. Gnathos with widely separated posterior processes, running parallel. Valva length 170–185  $\mu\text{m}$  ( $177.2 \pm 5.0$ , 3), narrow, 2.5–3.0 $\times$  as long as wide, distally becoming narrower, slightly curved inwards, transtilla with pointed corners, sublateral processes almost absent (Figure 12). Juxta



**Figures 5–6.** *Stigmella multispicata*, dead male moths, unmounted, both from USA, Iowa. **5** 26.ix.2014 **6** 27.vii.2014. Scale bars: 1 mm. Photographs M.J. Hatfield.

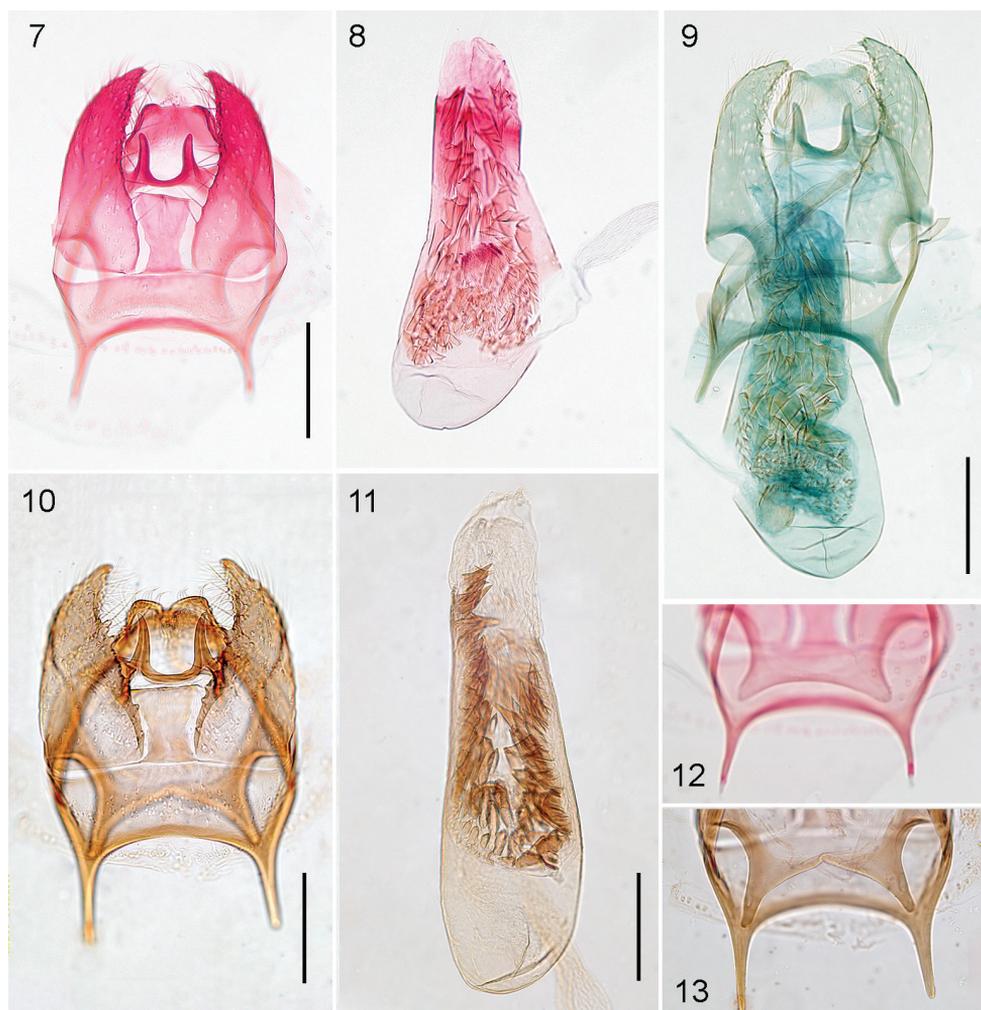
present, haltere-shaped, with triangular point distally. Phallus 320–330  $\mu\text{m}$  ( $323.4 \pm 4.8$ , 3), 2.3–2.9 $\times$  as long as wide; vesica with many relatively stout cornuti, varying from long-pointed to broadly triangular, with anterior cornuti smaller.

Female genitalia (Figs 14, 16, 18). No anal papillae; T8 narrow, anterior and posterior apophyses long and narrow, anterior ones longer (ca 290  $\mu\text{m}$ ) than posterior (ca 235  $\mu\text{m}$ ). Bursa length ca 810  $\mu\text{m}$ ; accessory sac strongly curved. Corpus bursae completely covered with relatively distinct pectinations; accessory sac and vestibulum without sclerotizations. Ductus spermathecae originating from accessory sac, with many narrow and indistinct convolutions.

Larva (Figs 23, 25, 26). Head-capsule length 290  $\mu\text{m}$ , width 315  $\mu\text{m}$ .

**Biology.** *Host plants.* *Ulmus pumila* L. (Ulmaceae), Siberian elm, a widespread tree in East Asia, cultivated globally in temperate climates, widely planted in North America. Vacated mines presumably representing this species were also collected in China on *Ulmus macrocarpa* Hance, Large-fruited elm.

*Leafmine* (Figs 19–22). Egg always deposited in vein axils on leaf underside, beneath the trichomes; leafmine a long narrow upper side gallery or corridor mine, running through leaf, usually not along veins and not crossing midrib; slightly curved, but



**Figures 7–13.** *Stigmella* species, male genitalia in ventral view. **7, 8, 12** *S. multispicata*, slide EvN4511, RMNH.INS.24511 **9** *S. multispicata*, slide JCK8417 **10, 11, 13** *S. ulmivora*, slide VU0440, RMNH.INS.20440. **12** and **13** show the difference in transtilla. Scale bars: 100  $\mu$ m. Photographs E.J. van Nieukerken.

many mines make a U-turn near the end. Frass initially in narrow medial black line, later becoming contorted, brown and almost filling mine. Larval exit on leaf underside.

*Larva* (Figs 23, 25, 26). Bright green to blue-green, probably feeding with venter upwards (analogy with *S. ulmivora*, but not positively observed); head capsule translucent brown. Larvae descending by silken threads, sometimes en masse, spinning a brown cocoon on debris.

*Life history.* Larvae and leafmines found in China in October; in North America larvae were observed from 15 June to mid-July and from 19 October to 6 November. Moths were found on 26 May and from 8 July throughout August to 6 September

(with a peak between 10 and 15 August), a few late records from 22 and 26 September and 2 October. Moths reared from October mines emerged in the laboratory between 25 March and 19 May. The species has at least two annual generations, maybe more. Adults are frequently found at light.

**Distribution** (Figs 28, 29). Presumed to be native in Russia: Primorye and China: Beijing. Almost certainly introduced in North America: Canada: Ontario, Québec; United States: Illinois, Indiana, Iowa, Maryland, Massachusetts, Minnesota, New York, Ohio, Tennessee, Wisconsin. The species has been found in the urban environment, in farmland and in more natural habitats. Table 1 lists the online photographs that we recognized as representing *S. multispicata*.

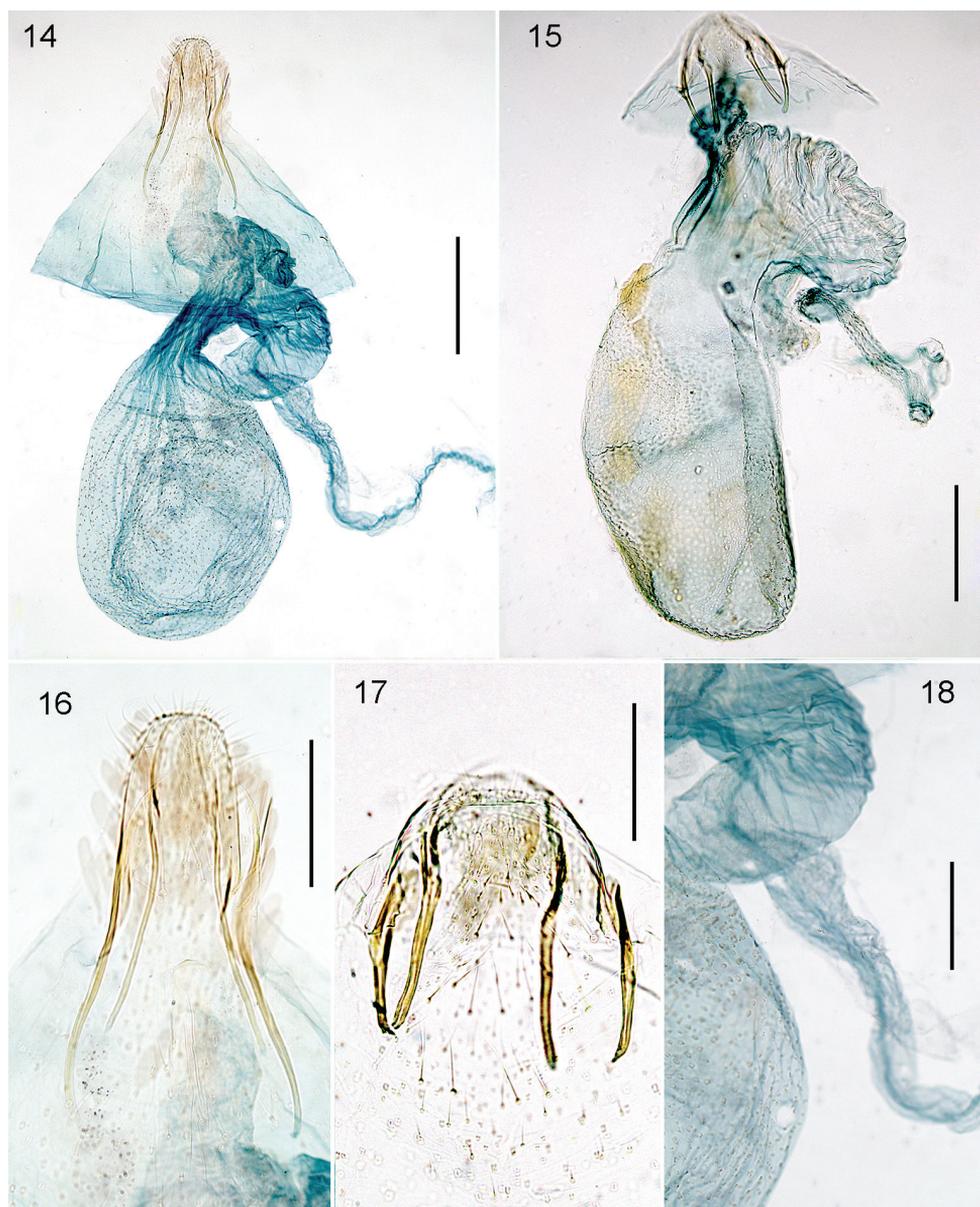
**DNA barcodes.** All eight barcodes belong to BIN BOLD:ACP7362. All North American barcodes are 100% identical; the single Chinese one differs in 11 basepairs (1.7%). The nearest neighbor, at 6.3%, is *Stigmella ulmivora* (Figure 44).

**Remarks.** The extensive collections of Chinese microlepidoptera of Nankai University were searched in vain for this species (Li Houhun, personal communication to EvN). It is possible that specimens can be found in other collections in China, such as the Zoological Institute in Beijing.

**Material examined.** 19♂, 26♀, 3 sex undetermined, 5 larvae, mines. **China:** 1 larva (green, dried out, destructively extracted for DNA), China, Beijing, Beijing Botanical garden – Wofosi, E.J. van Nieukerken & S. Richter, 17.x.2013, EvN no 2013117–M, *Ulmus pumila*, N40.00417, E116.20419, 108 m, RMNH.INS.30070; 1 mine from which previous larva was taken, RMNH.INS.43922; 1 mine with dead larva, same data, EvN no 2013117–H, RMNH.INS.43923 (all RMNH).

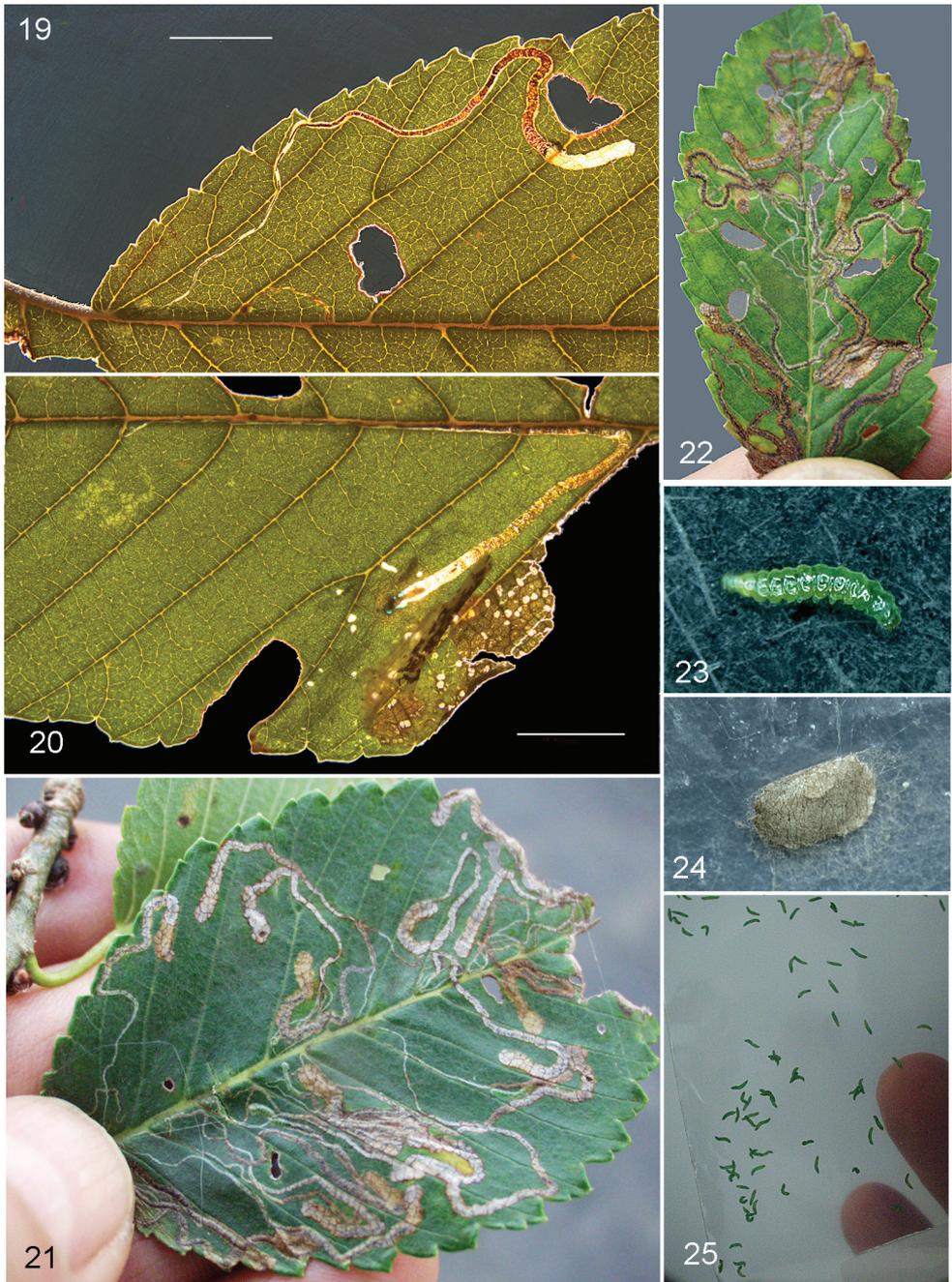
**Canada:** 1♂, 1♀ (in ethanol 96%), Ontario, Toronto, Etobicoke School of the Arts, EQP–CLL–602, Brad Schumacher, 22–28.ix.2014, GMP#05745, Malaise trap, N43.631, W79.504, 109 m, BIOUG16150–E04, BIOUG16150–E05 (BIOUG); 1 vacated leafmine, Ontario, Ottawa, Bayview Rd., E.J. van Nieukerken, 12.vii.2018, *U. pumila*, amidst 100's of mines of *Orchestes steppensis*, EvN no 2018080–H, N45.40819, W75.72474, RMNH.INS.45003 (RMNH); 4 vacated mines, Québec, Montreal, Old Montreal, Avenue de l'Hotel de Ville, E.J. van Nieukerken, 1.viii.2018, *U. pumila*, EvN no 2018101–H, N45.50894, W73.55604, RMNH.INS.45004 (RMNH).

**United States:** 1♀, Indiana, St. Joseph Co., J. Vargo, 26.v.2010, N 41.37'46.2"–W 86.08'13.9", [N41.62950, W86.13719] (USNM); 1♂, same locality, 2.viii.2010 (USNM); 5♂, 4♀, same locality, 10.viii.2010, genitalia slides JCK8416, JCK8417 (♂), JCK8617, RMNH.INS.15499 (RMNH, MEM, USNM); 1♂, same locality, 13.viii.2010, genitalia slide EvN4511, RMNH.INS.24511 (RMNH); 1♂, same locality, 11.viii.2010 (MEM); 1♀, same locality, 13.viii.2010 (USNM); 5♂, 16♀, same locality, 15.viii.2010 (MEM, RMNH); 1♀, same locality, 28.viii.2010 (MEM); 1♂, 2♀, same locality, 6.ix.2010 (USNM); 1♂ (abdomen missing), Indiana, Pulaski Co., Jasper–Pulaski FWA, J. Vargo, 4.viii.2010, 41 09' 31.0"N 086 58' 42.6"W [N41.15861, W86.9785] (USNM); 1♂, Iowa, Winneshiek Co., Plymouth Rock, Black light in a planted prairie near woodlands along the Upper Iowa River, M.J. Hatfield, 26.ix.2014, N43.4376, W92.0041, Genitalia slide EvN5052, RMNH.INS.25052

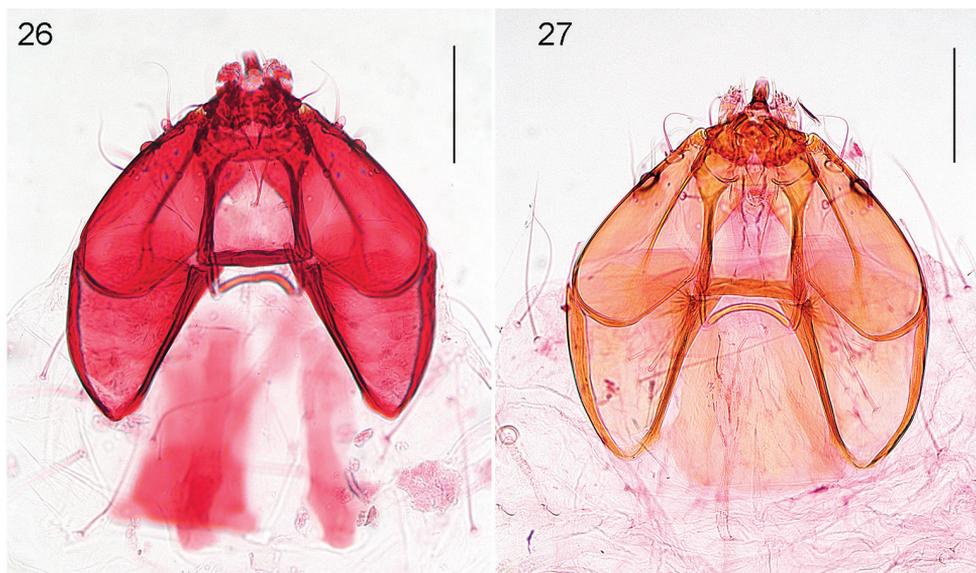


**Figures 14–18.** *Stigmella* species, female genitalia. **14, 16, 18** *S. multispicata*, slide JCK8416 **15** *S. ulmivora*, slide VU1638, RMNH.INS.21638 **17** *S. ulmivora*, slide VU1769, RMNH.INS.21769. Scale bars: 200  $\mu$ m (14, 15), 100  $\mu$ m. Photographs E.J. van Nieukerken.

(RMNH); 14 leafmines (13 vacated, 1 with dead larva), Massachusetts, Franklin Co., Sunderland, N42.498380, W72.544853, C.S. Eiseman, 22.vii.2018, *U. pumila* (CSEC); 4 larvae (in Tissue collection, ethanol 96%,  $-80^{\circ}$ , 1 preparation), New York, Suffolk Co., Sagaponack, Ms. Lee Foster, 21.x.2015, *U. pumila*, N40.93, W72.28,



**Figures 19–25.** *Stigmella multispicata*, immature stages and leafmines on *Ulmus pumila*. **19** leafmine of barcoded larva, RMNH.INS.30070. China, Beijing **20** leafmine with dead larva, same locality **21–25** all from NY, Sagaponack, 23.x.2015 and 28.x.2015 (cocoon) **25** showing large number of emerged larvae on plastic sheet. Scale bars: 5 mm. Photographs E.J. van Nieuwerkerken (**19**, **20**), D.O. Gilrein (**21–25**).



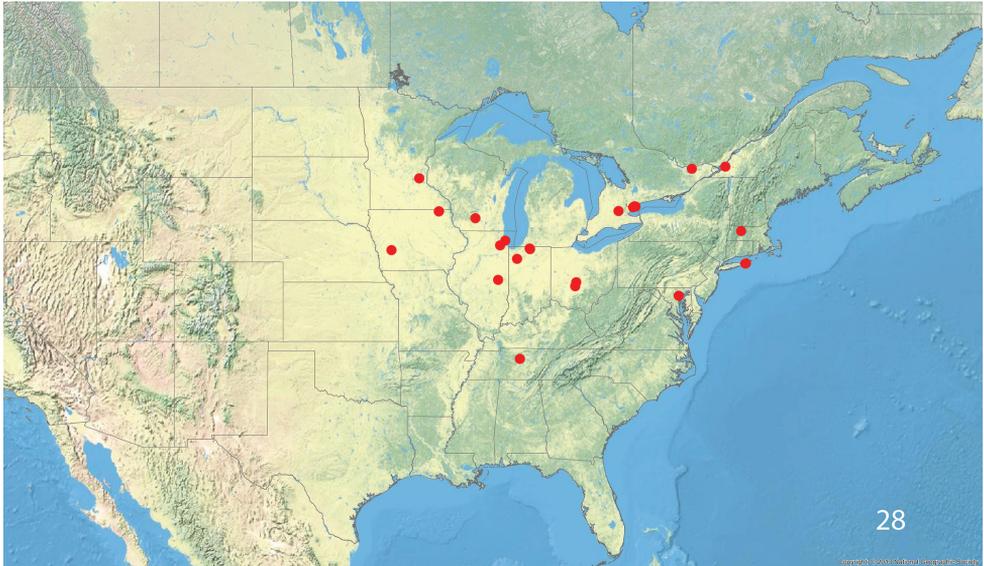
**Figures 26–27.** *Stigmella* species, larval headcapsules, dorsal view. **26** *S. multispicata*, RMNH.INS.30698 **27** *S. ulmivora*, RMNH.INS.18871. Scale bars: 100  $\mu$ m. Photographs E.J. van Nieuwerkerken.

RMNH.INS.30697, RMNH.INS.30698 (extracted), larval preparation RMNH.INS.30698.P, RMNH.INS.30699, RMNH.INS.30700 (RMNH); 3 adults (in capsule), same data, emerged 25.iii & 19.v.2016, (RMNH); 1♂, Ohio, Franklin Co., Hillard, D.J. Shetlar, 21.vii. 2016, N40.007, W83.1738, Genitalia slide EvN4901, RMNH.INS.24901; 1♂, same locality, 1.ix.2016, RMNH.INS.15533 (RMNH).

**Tentative ID, most likely this species. China:** 4 tenanted mines (rearing failed), Beijing, Xiangshan, Wofosi and botanical garden, E.J. van Nieuwerkerken & J.W. van Driel, 1.x.1984, EvN no 18–1–1K, Hills with deciduous shrub and low trees, *U. macrocarpa*, N39.983, E116.2, 100–500 m, RMNH.INS.44328; 1 vacated mine, same data, EvN no 18–1–1H, RMNH.INS.44330.

**Other data, material not examined. Canada** (Data from BOLD, barcode identification): 1 adult, Ontario, Waterloo region, Kitchener, Crestview Public School, EQP-CLL-863, Sherrie Cochrane, 2.x.2015, GMP#08378, N43.454, W80.44, 334m, BIOUG25491–E12 (BIOUG); 1 adult, Ontario, Toronto, Eastdale CI, EQP-CLL-605, David Servos, 2.x.2015, GMP#08428, N43.666, W79.349, 89, BIOUG25505–D03 (BIOUG).

**United States** (Observations, personal communications to authors): 4 larvae/mines, Indiana, St. Joseph Co., J. Vargo, 15–29.vi.2018; 120 adults, St. Joseph Co., Mishawaka, J. Vargo, 8.vii.2018, light trap; larvae descending en masse from trees, New York, Suffolk Co., Sagaponack, Ms. Lee Foster, 21.x.2015, *U. pumila*, N40.93, W72.28; larvae still present, same locality, 8.xi.2015; larvae, same locality, Mike Harmon, 16.vii.2016; larvae, same locality, 19.x.2016; larvae descending en masse from trees, New York, Suffolk Co., Wainscott, 3.xi.2015, Mike Harmon, *U. pumila*, N40.94, W72.24.



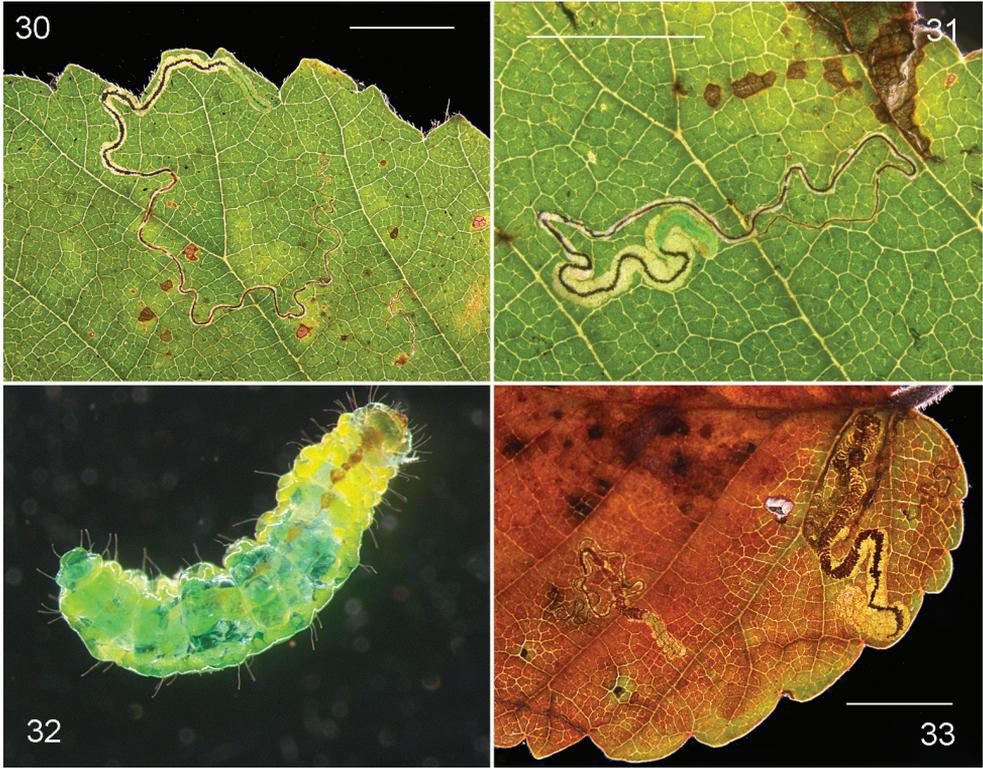
Figures 28–29. *Stigmella multispicata*, distribution.

***Stigmella ulmivora* (Fologne) Beirne**

Figures 3, 4, 10, 11, 13, 15, 17, 27, 30–33

*Nepticula ulmivora* Fologne, 1860: 199. Syntypes, Belgium, Brussels region, reared from *Ulmus*, 1859, emerged 1860, Fologne [probably lost].

*Stigmella ulmivora*; Beirne 1945: 199 [recombination]; van Nieuwerkerken et al. 2016: 105 [full synonymy].



**Figures 30–33.** *Stigmella ulmivora*, leafmines and larvae on *Ulmus minor*, Greece. **30–32** Akhaia, Strofilia, 4.xi.2011, larva RMNH.INS.29046 **33** Ilía, Olympia, 5.xi.2011. Scale bars: 5 mm. Photographs E.J. van Nieuwerkerken.

**Diagnosis.** *Stigmella ulmivora* can be separated from *S. multispicata* by the slightly larger size, the dark collar, and the antennae with the terminal 7–8 flagellomeres white. In Europe and North America there are no other *Stigmella* species with the same combination of characters. The male genitalia are very similar to those of *S. multispicata*, but have a deeper indentation in the uncus, and longer and more distinct sublateral processes of the transtilla. The female differs by the blunt ovipositor and the spiny ductus spermathecae.

Leafmines differ from those of *S. multispicata* by the egg position not being in vein axils; in Europe mines are inseparable from those of *S. ulmiphaga* (Priessecker, 1942). Due to the variability of mines of *S. ulmivora*, they sometimes are difficult to separate from those of *S. lemniscella* (Zeller, 1839), from which the yellow larva emerges through the leaf upper side, not the underside as in *S. ulmivora*.

**Redescription.** Male (Figure 3). Forewing length 2.1–2.5 mm ( $2.3 \pm 0.1$ , 10), wingspan 4.6–5.2 mm. Head: frontal tuft black, collar cream white. Scape cream white. Antenna fuscous, terminal 7–8 flagellomeres completely white, with 25–29 segments ( $26.7 \pm 1.6$ , 10), ratio to forewing length 10–13 segments/mm ( $11.6 \pm 0.9$ , 10).

Thorax and forewing shining fuscous bronze, a silver fascia at 2/3, apex darker fuscous, terminal cilia concolorous, underside dark fuscous. Hindwing grey-brown. Abdomen brown, no visible anal tufts.

Female (Figure 4). Forewing length 1.9–2.6 mm ( $2.3 \pm 0.2$ , 10), wingspan 4.2–5.4 mm. Antenna with 19–23 segments ( $21.0 \pm 1.2$ , 8), ratio to forewing length 8–11 segments/mm ( $9.3 \pm 1.0$ , 8). Otherwise as male, abdomen with conspicuous long protruding ovipositor, with small anal tufts.

Male genitalia (Figs 10, 11, 13). Capsule length 190–210  $\mu\text{m}$  ( $203.8 \pm 10.2$ , 4), ca 0.9 $\times$  as long as wide. Vinculum anteriorly with pointed and anteriorly protruding lateral corners. Uncus distinctly bilobed, lobes adjacent. Gnathos with widely separated posterior processes, running parallel. Valva length 180–185  $\mu\text{m}$  ( $182.4 \pm 0.7$ , 4), rather narrow, 2.0–2.4 $\times$  as long as wide, distally becoming narrower, slightly curved inwards, transtilla with pointed distinct sublateral processes (Figure 13). Juxta present, haltere-shaped. Phallus 275–440  $\mu\text{m}$  ( $351.1 \pm 68.4$ , 4), 2.0–3.3 $\times$  as long as wide; vesica with many relatively stout cornuti, varying from long and pointed to broadly triangular, with anterior cornuti smaller.

Female genitalia (Figs 15, 17). No anal papillae; T8 rounded, not elongated, anterior and posterior apophyses short, almost equal in length, anterior ones ca 170–210  $\mu\text{m}$ , posterior ca 185–200  $\mu\text{m}$ . Bursa length ca 770–930  $\mu\text{m}$ ; accessory sac strongly curved. Corpus bursae completely covered with relatively distinct pectinations; accessory sac and vestibulum without sclerotizations. Ductus spermathecae originating from accessory sac, basally wide and covered with many spines, with several narrow and indistinct convolutions.

Larva (Figs 27, 32). Head-capsule (n=2) length 300–310  $\mu\text{m}$ , width 300–315  $\mu\text{m}$ .

**Biology.** *Host plants.* *Ulmus minor* Mill., *U. glabra* Huds., *Ulmus* spp. (Ulmaceae). Reared specimens labeled as coming from *Acer* were from cocoons found on trunks of that tree; therefore this cannot be considered a host record.

*Leafmine* (Figs 30–33). Egg on leaf underside, against a vein. Mine a highly variable gallery, ranging from short and filled with dense frass in thick leaves (usually in the sun) to long and narrow, often partially following a vein, with frass either linear or becoming contorted, mines sometimes much winding. Larval exit on leaf underside.

*Larva* (Figure 32). Bright green, feeding with venter upwards; head capsule translucent brown. Larvae descending by silken threads, spinning a brown cocoon on debris or on tree trunks.

*Life history.* Bivoltine, or possibly partially univoltine in northern parts of Europe. Larvae in June to early July, again in August to November. Adults recorded from May (a single April record) to early July and again in August.

**Distribution.** Widespread throughout Europe, east to the Volga region in Russia (Johansson and Nielsen 1990, van Nieukerken 2017). The species occurs both in natural habitats and on trees in cities, often in large numbers (EJvN, personal observations).

**DNA barcodes.** We have 13 barcodes from across Europe, all belonging to BIN BOLD:AAI0023, with some variation, a maximum distance for the Greek barcodes of 2.41% to the rest. The nearest neighbor, at 6.31%, is *Stigmella multispicata* (Figure 44).

**Material examined. Adults:** 25♂, 50♀. **Croatia:** 1♂, Krk, Kappelje, 17.viii.2001; 1♀, Krk, Mt. Hlam, loc. Branusine, 15.viii.2012. **Germany:** 1♀, Berlin; 1♂, Thüringen, Bad Blankenburg, Muschelkalk, 1.vii.1986; 1♀, Thüringen, Bad Blankenburg, Schwarzatal, 5.vii.1986. **Italy:** 1♂, Cuneo, Entracque, ca 1 km SE, Il Bosco, 16.viii.2007, la on *Ulmus*; 1♂, 1♀, same locality, 13.x.2008, la on *Ulmus*; 1♀, Latina, Monti Aurunci, 4 km NW Castelforte, 22–23.vi.1969. **Netherlands:** 2♂, 2♀, Gelderland, Wageningen, *Ulmus*, e.l. 19.v.1977; 1♀, ibidem, larva x.1989, *Ulmus*; 2♂, 3♀, Noord-Brabant, Breda, v.1877; 1♀, ibidem, e.l. 10.vi.1877, “acer pseudop.”; 2♂, 1♀, ibidem, e.l. 25.vi.; 1♀, 3 damaged adults, ibidem, v.1878; 1♀, Noord-Holland, Amsterdam, 2.viii.1937; 1♂, Noord-Holland, Amsterdam, Koloniaal instituut, 7.viii.1937; 1♀, Noord-Holland, Amsterdam, *Ulmus*, e.p. 30.vi.1929; 1♀, ibidem, *Ulmus* e.l. 27.iv.1942; 5♀, ibidem, *Ulmus* e.l. 29.vii–2.viii.1943; 1♀, Noord-Holland, Amsterdam N.W., *Ulmus*, 16.viii.1942; 1♂, 4♀, ibidem, *Ulmus* e.l. 2–14.viii.1947; 1♀, ibidem, *Ulmus* e.l. 6.vi.1948; 1♀, ibidem, *Ulmus* e.l. 11.vi.1948; 1♀, Noord-Holland, Bussum, 16.iv.1934; 1♂, Noord-Holland, Castricum, 15.viii.1979, *Ulmus* e.l. 13.vi.1980; 6♂, 9♀, Noord-Holland, Hilversum, *Ulmus* e.l. 4–24.vi.1943; 4♀, ibidem, *Ulmus* e.l. 17.v.–6.vi.1945; 1♀, Noord-Holland, Overveen, 7.vi.1929; 1♂, ibidem, 1.vii.1929; 2♀, Zuid-Holland, Den Haag, *Ulmus* e.l. 9.v–10.vi.1865; 1♀, Zuid-Holland, s Gravenhage, 29.v; 1♀, Zuid-Holland, Lexmond, 3.viii.1999; 1♂, Zuid-Holland, Rottm. [Rotterdam], e.p. 22.vi.1901; 1♂, ibidem, e.l. 24.vi.1864; 1♂, ibidem, e.l. 13.vii. 1870; 1♂, ibidem, 21.vi.1877; 1♀, ibidem, pupa in iv, e.p. 21.vii.1879; 1♀, ibidem, 27.v.1877. **Poland:** 1♂, Silesia, Wroclaw (Breslau), e.l. iii.1882.

**Leafmines and larvae.** When no hostplant is given, read *Ulmus* sp. – **Czech Republic:** 1 mine, Moravia, Lednice, 3 km SW, forest near lake, 3.x.1992. **France:** 4 larvae, vacated mines, Alpes-Maritimes, Saorge, 0.8 km SW, vallée de Roya, 9.x.2008, *U. minor*; 1 vacated mine, Alpes-Maritimes, Tende, ca 1 km S, E. slope, 10.x.2008, *U. glabra*; mines, Bouches-du-Rhône, Aix-en-Provence, Parc Jourdan, 12–16.x.1983; several vacated mines, Cher, Villeneuve-sur-Cher, 30.vii.2009, *U. minor*; vacated mines, Drôme, Beaurières, 4 km W: Marais des Boulignons, 21.viii.2002; 1 vacated mine, Eure, Le Marais Vernier, la Vallée, 5.x.2017; 2 vacated mines, Finistère, Presqu’île de Crozon, ca 4 km E Crozon, l’Aber, 12.vii.2006; 1 vacated mine, Haut-Rhin, Colmar, 3 km SW, Le Neuland, 26.ix.2002; 1 larva, 4 mines, Haut-Rhin, Lapoutroie, La Bohle, 21.x.2002, *U. glabra*; 3 vacated mines, Indre-et-Loire, Huismes, N of Contebault, along C17, 8.x.2017; several vacated mines, Lozère, Marjoab, 2.5 km SW Meyrueis, 22.vii.2009, *U. minor*; 3 vacated mines, Lozère, Barre-des-Cévennes, 26.vii.2009, *U. minor*; vacated mines, Pyrénées-Orientales, Port-Vendres, near railway station, 28.vii.1982. **Germany:** 1 vacated mine, Brandenburg, Erkner, 9–11.ix.2007; several vacated mines, Saarland, Neunkirchen, Zoo, 30.ix.2004; 1 vacated mine, Sachsen-Anhalt, Dornburg, 2 km S, along Elbe, 19.vii.2014. **Greece:** 2 larvae, many mines, Akhaia, Strofilia, S Kalogria, 4.xi.2011, *U. minor*; 6 larvae, several mines, Ilía, Olympia, archeological site, 5.xi.2011, *U. minor*. **Italy:** 1 vacated mine, Bolzano, Vinschgau, Prad an Stilfser Joch, Suldenbach banks, 27.vii.2005; 1 larva, many mines, Cuneo, Entracque, ca 1 km SE, Il Bosco, 16.viii.2007; several mines, Cuneo, Entracque, ca 1 km SE, Il Bosco, 13.x.2008; 3 vacated mines, Roma, Trevignano Romano, 17.ix.2005. **Monaco:** 3 vacated mines, Monaco Ville, E slope of Palais Princier, 9.viii.2007. **Netherlands:** mines, Gelderland,

Winterswijk, Bekendelle, along Slinge, 1.x.1979; 1 larva, mines, Gelderland, Nijmegen, Winkelsteeg, 11.x.2008; mines, Limburg, Eys, railway near Piepert, 11.ix.1979; mines, Limburg, Thorn, klooster Bethanien, 24.ix.1979; mines, Limburg, Gulpen, NW, holle weg, 8.x.1979; 1 mine, Limburg, Thorn: Baarstraat, 3.x.1988; mines, Noord-Holland, Castricum, hedge along road, 15.viii.1979; mines, Noord-Holland, Castricum, hedge along road, 15.viii.1979; mines, Noord-Holland, Amstelveen, Beneluxbaan, median reserve, 21.ix.1979; several vacated mines, Overijssel, Weerribben, Ossenzijl, Venebosch, 27.viii.2011; several vacated mines, Zeeland, Middelburg N., Brigdamseweg, 2.viii.2009; 1 larva, 1 mine, Zuid-Holland, Den Haag, Waalsdorpervlakte, 7.x.2007; mines, Zuid-Holland, Leiden W, experimental garden University, 5.vii.1979; mines, ibidem, 5.ix.1979; mines, Zuid-Holland, Wassenaar, Meijendel, near Kijfhoek, 18.ix.1979; 1 larva, Zuid-Holland, Oegstgeest, Rhijngest, 23.ix.1997. **Portugal:** 1 mine, Tras-os-Montes, PN Montesinho, Salgueiros, Vallone das Furnas, 8 km N Vinhais, 30.vii.2001. **Romania:** a few vacated mines, Brasov, Braşov, Mt. Tâmpa, 2.viii.2011. **Sweden:** several vacated mines, Bohuslan, Svenneby, Valön Nature Reserve, 7.viii.2008.

### North American *Ulmus* leafminers

Previously only two Nepticulidae were known to feed on *Ulmus* in North America: *Stigmella apicialbella* (Chambers, 1873) and *Ectoedemia ulmella* (Braun, 1912). This is a much poorer fauna than the seven European species (van Nieukerken 1986, Puplesis 1994), and in Asia the number is probably still higher, but for several species that are potentially *Ulmus* feeders the hosts are as yet unknown.

Identification of the North American Nepticulidae mines and adults reared from *Ulmus* is straightforward. For convenience we provide a key that distinguishes these from other insects that form partially or entirely linear mines. Primary blotch mines on elm are formed by additional species of Lepidoptera (Coleophoridae: *Coleophora*; Gracillariidae: *Cameraria*, *Phyllonorycter*), Coleoptera (Buprestidae: *Brachys*; possibly also Curculionidae: *Tachygonus*), and Hymenoptera (Tenthredinidae: *Fenusia*). For a complete key, see Eiseman (2018).

### Key to North American *Ulmus* (linear) leafmines

- 1 Linear-blotch mine formed in spring; egg inserted in the leaf .....2
- Linear or linear-blotch mine formed in summer or fall; egg deposited on the leaf surface .....3
- 2 Mine originating at a scar on the underside of the leaf midrib; frass forming a central line in the linear portion; larva with a head capsule, pupating in a globular cocoon within the mine..... ***Orchestes* spp.** (Coleoptera: Curculionidae)
- Mine typically originating near the leaf margin, not associated with a scar; frass indistinct in the linear portion; larva without a head capsule, exiting the mine to pupate..... ***Agromyza aristata* Malloch, 1915** (Diptera: Agromyzidae)

- 3 Mine approx. 2 cm long and less than 1 mm wide, mostly following the midrib and one or two lateral veins; frass filling the width of the mine except for the terminal several mm, in which no frass is deposited; larva emerging to feed externally in patches on the lower leaf surface.....  
..... *Bucculatrix* (Lepidoptera: Bucculatricidae)  
Note: *Bucculatrix eclecta* Braun, 1963 has been reared from elm but no details of the mine were recorded (Braun 1963). The above description is based on mines of an undetermined species collected on *Ulmus alata* in NC by T. S. Feldman and on *U. pumila* in MA by C. S. Eiseman.
- Mine longer than 2 cm and eventually more than 1 mm wide, with extended portions that do not follow veins; frass at least initially forming a narrow central line; larva feeding as a miner throughout its development ..... 4
- 4 Mine distinctly linear throughout its length; egg usually on the lower leaf surface; cocoon never spun within the mine..... 5
- Mine contorted to form a blotch in the later portion, although parts of the mine may be linear; egg often on the upper leaf surface; cocoon of overwintering generation sometimes spun within the mine (Figs 40–43) .....  
..... *Ectoedemia ulmella* (Lepidoptera: Nepticulidae)
- 5 Egg deposited on either leaf surface, may be next to a vein but not in an axil; larva yellow, exiting through the upper epidermis; on native elms (Figs 36–39)..... *Stigmella apicalbella*
- Egg deposited on the lower leaf surface, in a vein axil and beneath the trichomes; larva green, exiting through the lower epidermis; on Siberian elm (Figs 19–22) ..... *Stigmella multispicata*

### *Stigmella apicalbella* (Chambers) Newton & Wilkinson

Figures 34, 36–39

*Nepticula apicalbella* Chambers, 1873: 127.

*Stigmella apicalbella*: Newton and Wilkinson 1982: 413.

**Diagnosis.** *Stigmella apicalbella* (Figure 34) can easily be recognized by the combination of a yellow head, white collar, and forewing with a narrow medial white fascia and an apical triangular white spot extending into the fringe. The basal half of the forewing is a bit more brown or fuscous, whereas the apical part is almost black with coarse scaling. The male genitalia are remarkably “bulky” and do not resemble any other North American species (Newton and Wilkinson 1982).

**Biology.** *Host plants.* *Ulmus americana* L., *U. alata* Michx., *U. rubra* Muhl. (= *fulva* Michx.), *U. thomasii* Sarg. (= *racemosa* D. Thomas), *Ulmus* spp. (Ulmaceae) (Braun 1917, Newton and Wilkinson 1982). *Ulmus alata* constitutes a new host record.

*Leafmine* (Figs 36–39). Egg on either leaf surface, may be against a vein, but never in leaf axil. Mines linear, usually rather straight, partly following veins, or more con-



**Figures 34–35.** North American *Ulmus* feeding Nepticulidae, adults. **34** *Stigmella apicialbella*, Massachusetts, Hampshire Co., Northampton **35** *Ectoedemia ulmella*, male, Tennessee, Blount Co., Townsend. Photographs C. Eiseman, E.J. van Nieukerken.

torted; frass variable, from narrow linear to contorted, green or brown, sometimes completely filling the mine. Larval exit on leaf upperside.

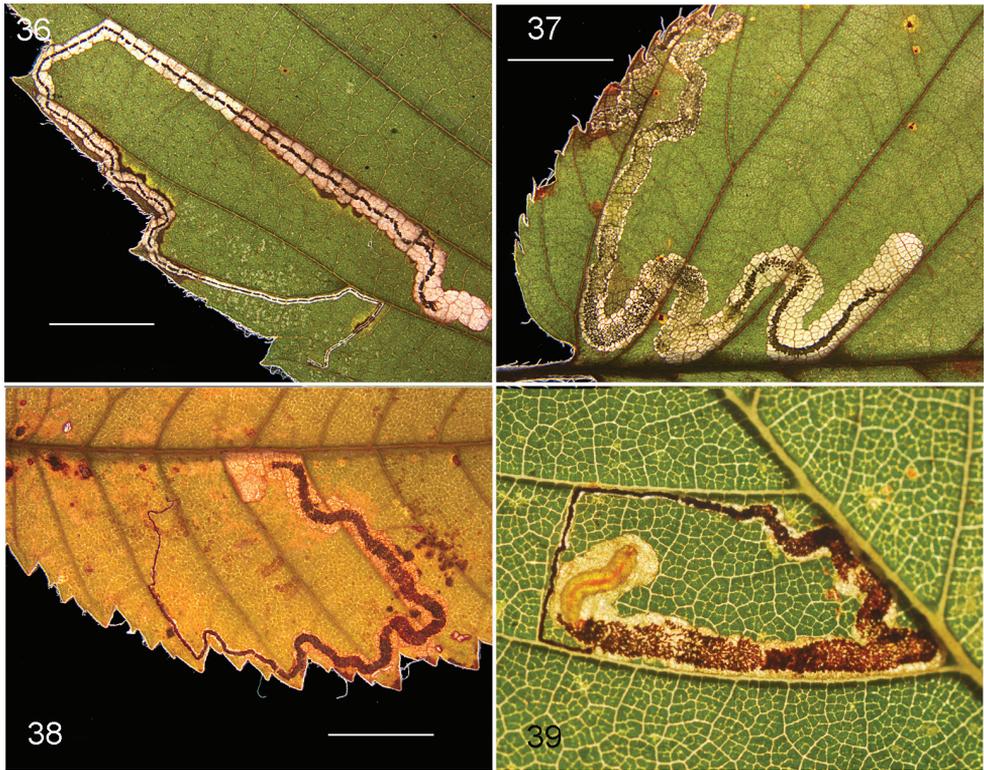
*Larva* (Figure 39). Yellow, feeding with dorsum upwards; head capsule brown. Larva spinning a brown cocoon on debris.

*Life history.* Bivoltine, or possibly trivoltine (Braun 1917). Larvae in June to early July, again in August to October. Adults recorded from April to early July and again in August.

**Distribution.** Widespread in Eastern North America, positive records from: Canada: New Brunswick, Ontario (BOLD: BIOUG33718-A12), Quebec (van Nieukerken 2018), USA: Alabama\*, Connecticut\*, Georgia\*, Illinois, Indiana, Kentucky (Chambers 1873), Massachusetts, Mississippi\*, New York\*, North Carolina, Ohio (Braun 1917), Tennessee, Vermont. States without reference are new records: from states with asterisk we have as yet only seen vacated mines; from the other states the occurrence is confirmed by adults or DNA barcodes of larvae.

**DNA barcodes.** We have three DNA barcodes, all with BIN BOLD: ACG9146 (Figure 44).

**Remarks.** The leafmines of this species are remarkably variable. Since we have not seen any adults or DNA barcodes yet from the southernmost states, the possibility that some of these mines represent other taxa cannot be excluded.



**Figures 36–39.** *Stigmella apicialbella*, leafmines. **36** Vacated mine, Canada, Québec, Brome-Missisquoi, RMNH.INS.40376 **37** Vacated mine, USA, Connecticut, Litchfield Co., Canaan, RMNH.INS.43576 **38** Vacated mine, USA, Mississippi, Oktibbeha Co., Black Prairie Reserve, RMNH.INS.43158 **39** Mine with larva, USA, Tennessee, Obion Co. Scale bars 5 mm. Photographs E.J. van Nieuwerkerken, C. Eiseman (39).

**Material examined. Canada:** 1 larva, several mines, Québec, Brome-Missisquoi, St Armand, Étang Streit, 7.ix.2015, *Ulmus americana*; 1♀, Québec, Gatineau, Aylmer, 18 rue Washington, 19.v.1998. **United States:** several vacated mines, Alabama, Monroe Co., Haines Island Park, along Alabama River, 12.x.2010, *Ulmus*; several vacated mines, Connecticut, Litchfield Co., Canaan, Page Road near Falls Village, 11.ix.2011, *U. americana*; 8 vacated mines, Georgia, Murray Co., Chattahoochee Nat. Forest, E of Chatsworth, GA rd 52, 14.x.2010, *U. alata*; 1♂, Indiana, St. Joseph Co., 25.v.2010; 1♂, ibidem, 13.viii.2010; 3♂, ibidem, 15.viii.2010; 1♂, Kentucky [Kenton Co., Covington], Lectotype; 1 adult, Massachusetts, Hampshire Co., Northampton, Northampton Bikeway west of King St., 13.ix.2013, *Ulmus*, emerged 22.v.2014; 3 vacated mines, Mississippi, Oktibbeha Co., Black Prairie Reserve, nr 16<sup>th</sup> Section Rd, 6.x.2010, *U. alata*; 1 vacated mine, Mississippi, Winston Co., Tombigbee Nat. Forest, Noxubee Hills trailhead, 7.x.2010, *U. alata*; 2 vacated mines, New York, Essex Co, S Wilmington, W branch Ausable river, 13.ix.2011, *U. americana*; 1 larva, mines, Tennessee, Obion Co., Reelfoot Lake, 17.xi.2012, *Ulmus*; 1 larva, 2 mines, Vermont, Addison Co., Addison, Dead Creek WMA, 16.ix.2011,

*U. americana*; 1 vacated mine, Vermont, Chittenden Co., Burlington, Colchester Bog, 5.ix.2015, *U. americana*.

*Online photographs:* **Canada:** vacated mine, New Brunswick, York Co., Fredericton, 28.viii.2015, Christopher Adam, <https://bugguide.net/node/view/1162297>, **United States:** 1 adult, Illinois, Cook Co., Glencoe, 22.v.2017, James F. Steffen, <https://bugguide.net/node/view/1373817>; mine with larva, North Carolina, Durham Co., Durham, Pelham Road, 25.vi.2016, *U. alata*, Tracy S. Feldman, <https://bugguide.net/node/view/1247382>, plus many records of vacated mines from NC.

### ***Ectoedemia ulmella* (Braun) Wilkinson & Scoble**

Figures 35, 40–43

*Nepticula ulmella* Braun, 1912: 87.

*Ectoedemia ulmella*: Wilkinson and Scoble 1979: 91.

**Diagnosis.** *Ectoedemia ulmella* (Figure 35) can easily be distinguished from the *Ulmus*-feeding *Stigmella* spp. by the small collar with hair-scales only (*Stigmella* spp. have lamellar scales), combined with the medial white fascia and pale fringe. Other species of *Ectoedemia* may look similar, but males of *E. ulmella* stand out by the brown androconial scales on hindwing upperside. For genitalia see Wilkinson and Newton (1981).

**Biology.** *Host plants.* *Ulmus americana* L., *U. alata* Michx., *U. rubra* Muhl. (= *fulva* Michx.), *U. thomasi* Sarg. (= *racemosa* D. Thomas) (Ulmaceae) (Braun 1917, Wilkinson and Newton 1981). *Ulmus alata* constitutes a new host record.

*Leafmine* (Figs 40–43). Egg usually on upper leaf surface, often against a vein. Early mine a narrow linear tract with broken narrow linear frass, sometimes filled with frass, at first often winding, then straighter, often following a vein, later mine abruptly widening into an irregular blotch with scattered frass. Larval exit on leaf upper side, or cocoon spun inside mine, often in center of blotch (Braun 1917) (Figure 43).

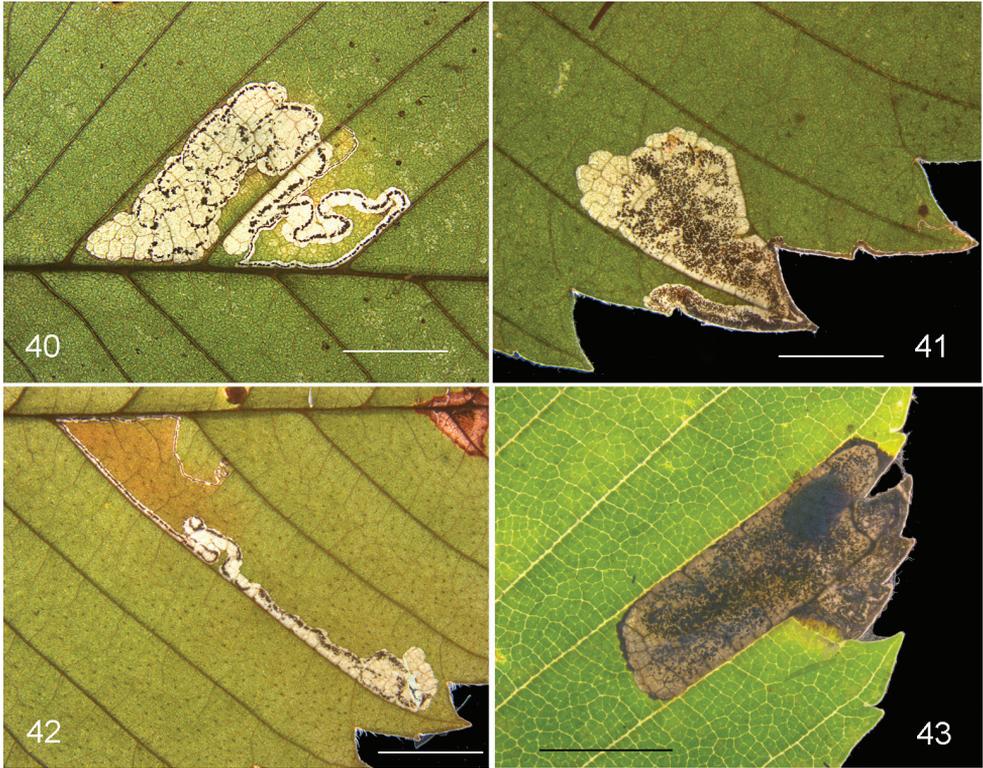
*Larva.* Pale yellowish white, feeding with venter upwards, ganglia usually obvious; head capsule translucent brown. Larva spinning brown cocoon inside mine or on debris.

*Life history.* Larvae found from July to early October, possibly in two generations, but it is also possible that this represents one extended generation. Adults recorded from May to August. From larvae collected in August, the adults emerged the following year.

**Distribution.** Widespread in Eastern North America, positive records from: Canada: New Brunswick\*, Ontario, Quebec, USA: Alabama\*, Florida\*, Kentucky, Maine, Maryland\*, Massachusetts\*, Mississippi\*, New York, North Carolina\*, Ohio, Pennsylvania, Tennessee\*, Vermont\* (Braun 1917, Wilkinson 1981, Wilkinson and Newton 1981, asterisks indicate new records).

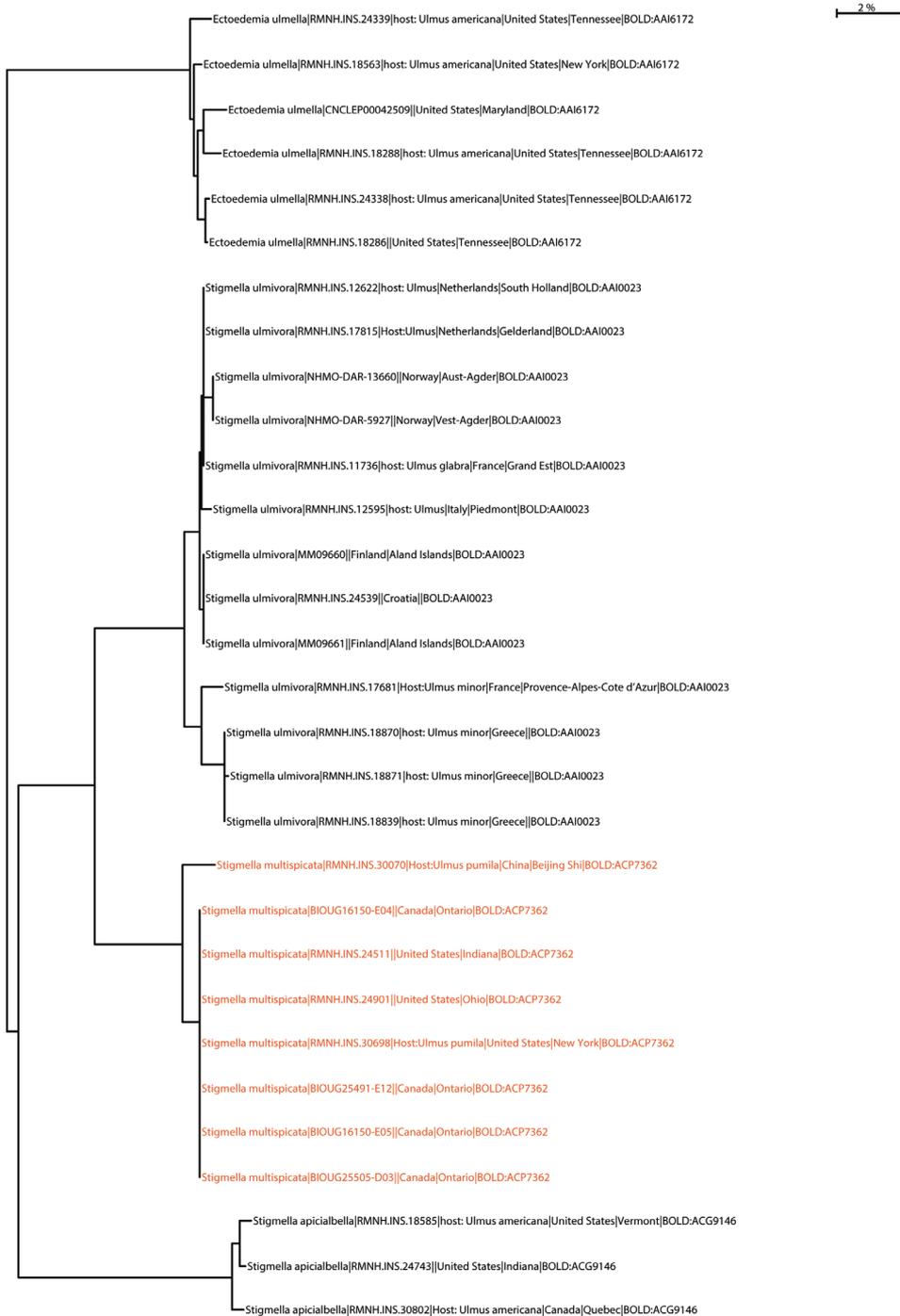
**DNA barcodes.** We have six DNA barcodes, all with BIN BOLD: AAJ6172 (Figure 44).

**Remarks.** In BOLD there are two specimens from Texas with a distant but related DNA barcode (BOLD:ABX4315); we have not yet checked whether these also belong to *E. ulmella*.



**Figures 40–43.** *Ectoedemia ulmella*, leafmines. **40, 41** Vacated mines, Canada, Québec, Brome-Missisquoi, RMNH.INS.40373 **42** mine, larva taken out, barcoded, USA, New York, Essex Co, S Wilmington, larva RMNH.INS.18563. **43** Mine with cocoon, RMNH.INS.43636. Scale bars: 5 mm. Photographs E.J. van Nieuwerkerken.

**Material examined. Canada:** 2♂, 1♀, 1 leafmine, Ontario, La Passe, e.l. 22.ii–26.iii.1971, *Ulmus americana*; 4 leafmines, Ontario, Ottawa, 28.vii–7.viii.1955, *U. americana*; 1♀, 5 leafmines, Ontario, Overbrook, 22.vii–7.viii.1955, *U. americana*; 2 larvae, several mines, Québec, Brome-Missisquoi, St Armand, Étang Streit, 7.ix.2015, *U. americana*; 4 vacated mines, Québec, Gatineau, Aylmer, Deschênes, Ottawa rivershore, 12.ix.2015, *U. rubra*; 1♂, Québec, Gatineau, Aylmer, 11.vi.1989; 1♀, ibidem, 7.vii.1989; 1♂, Québec, Gatineau, Aylmer, 48 rue Notre-Dame, 1.viii.1997; 1♂, 1♀, Québec, Gatineau, Aylmer, 18 rue Washington, 21–22.vi.1999; 3♂, 4♀, 11 leafmines, Québec, Kingsmere, 10–11.ix.1955, e.l. 12.v–17.vii.1956, *U. rubra*; 5 leafmines, Québec, Kingsmere, 1971, *U. americana*; 4 leafmines, Québec, Quyon, 1969, *U. americana*. **United States:** several vacated mines, Alabama, Monroe Co., Haines Island Park, along Alabama River, 12.x.2010, *Ulmus*; several mines, Florida, Volusia Co., Lake Woodruff NWR, Mud Lake Road, 24.ix.2016, *U. americana*; 1♀, Maryland, Calvert Co., Calvert, Scientists Cliffs, 2179 Bluebell Road, 3.viii.2007; vacated mines, Massachusetts, Hampshire Co., Northampton, Northampton Bikeway west of King St., 13.ix.2013, *Ulmus*; several vacated mines, Mississippi, Oktibbeha Co.,



**Figure 44.** Neighbor Joining tree of DNA barcodes of the treated species. The labels mention: Taxon name, Sample id (collection registry), hostplant (when available), Country, State or Province, BIN number.

Black Prairie Reserve, nr 16<sup>th</sup> Section Rd, 6.x.2010, *U. alata*; 1 larva, several mines, New York, Essex Co., S Wilmington, W branch Ausable river, 13.ix.2011, *U. americana*; several mines, New York, Essex Co, Wilsboro, Noblewood Park, 14.ix.2011, *U. americana*; 1♂, New York, Tompkins Co., Cornell Univ., e.l. 14.viii.1994, *Ulmus*; 3 vacated mines, North Carolina, Haywood Co., NP Great Smoky Mts, Big Creek area, 28.ix.2010, *U. alata*; 3 larvae, several mines, Tennessee, Blount Co., NP Great Smoky Mts, Rich Mountain Gap, 2.x.2010, *U. americana*; 1♂, 2♀, several mines, Tennessee, Blount Co., Townsend, Laurel Valley, 3.x.2010, e.l. 11–19.iv.2011, *U. americana*; 1 vacated mine, Vermont, Addison Co., Addison, Dead Creek WMA, 16.ix.2011, *U. americana*; 2 vacated mines, Vermont, Chittenden Co., Burlington, Colchester Bog, C. Eiseman & J. Blyth, 5.ix.2015, *U. americana*.

*Online photographs:* **Canada:** vacated mines, New Brunswick, York Co., Fredericton (Lincoln Trail), 9.ix.2014, Christopher Adam, <https://bugguide.net/node/view/1042642>.

## Discussion

Morphology and DNA barcoding show clearly that the North American and Asian populations of *Stigmella multispicata* are conspecific, suggesting a recent invasion by this species. We think that the invasion must have been from Asia to North America and not the other way around. Although the North American fauna is not yet completely known, a species that is now so abundant and widespread would have been hard to miss by earlier collectors, but not a single older specimen has been found in collections. Also its occurrence on the introduced Siberian elm and (so far) absence from native elm species speaks in favor of an Asian alien. The close relationship between *S. multispicata* and the western Palearctic *S. ulmivora* in the Palearctic *S. ulmivora* species group (van Nieukerken et al. 2016), further supports its Palearctic origin, even though we have no older records than the empty leafmines collected in Beijing in 1984. The DNA barcoding results show that the North American population is slightly different from the single barcode from China: Beijing. This could mean that the source for the invasion is somewhere else in East Asia.

The date of introduction is difficult to assess. Our oldest North American records are from 2010, but since those already are numerous, the species must have been well established several years before that. Since nepticulids are able to spin their cocoons on many substrates, including tree trunks, branches or other objects, we assume that an introduction of larvae or pupae inside cocoons with plants of Siberian elm, or even another plant or object, is most likely; transport of insect invaders with live plant material is considered a major pathway for exotic herbivores (Liebhold et al. 2012, Brockerhoff and Liebhold 2017). Current global traffic has resulted in several recent examples of introductions of leafminers from North America into Europe and vice versa (van Nieukerken et al. 2012b, 2012d, Landry et al. 2013). As far as we know this is only the second instance of a leafmining moth from Asia introduced into North America, the

other instance being *Caloptilia triadicae* Davis, 2013 on the Chinese Tallow Tree *Triadica sebifera* (L.) (Davis et al. 2013). We suspect that the apparent rapid spread of *S. multispicata* may have been aided by the nursery trade. A quick internet search found nurseries in Illinois and Tennessee that will ship Siberian elm plants almost anywhere in the US; although phytosanitary certificates are typically required by states receiving nursery stock, inspection of plants may be less common after they arrive at their destination and even careful inspection of potted or bare-root plants could easily miss the small cocoons. It was recently shown that trade amongst tree nurseries throughout Europe contributed to the spread of the citrus longhorn beetle, *Anoplophora chinensis* (Forster, 1771) (Eschen et al. 2015).

There is a parallel with another invasive Asian leafminer, the weevil *Orchestes steppensis* Korotyaev, 2016 (Korotyaev 2016), also a leafminer of Siberian elm. The species was first found in North America around 2003, then misidentified as the European *O. alni* (Linnaeus, 1758) (Anderson et al. 2007, Looney et al. 2012), and has since spread widely over the United States, including the west (Looney et al. 2012). Also in Asia this species has probably been spreading, and is reported as a local pest species in China (Li et al. 2017). It is possible that *S. multispicata* is also more widespread in Asia, but overlooked due to the limited number of people studying lepidopterous leafminers, and the fact that the mines in small numbers are inconspicuous.

The Siberian elm was introduced in the United States around 1860 and is widely planted for windbreaks and lumber, and now itself an invasive species, particularly in pastures, roadsides and prairies throughout the Midwest and Great Plains regions (Swearingen and Barger 2016). It is uncertain whether *S. multispicata* also will colonize American elm species; the American species of *Ulmus* are in a phylogenetically different subgenus, *Oreoptelea* (Spach) Planchon, whereas all Palearctic species belong to subgenus *Ulmus* L. (Wiegrefe et al. 1994). Larvae of the Siberian Elm specialist *Orchestes steppensis* have not yet been confirmed on American elm species (Looney et al. 2012), but adults have been found feeding on *U. americana* (Anderson et al. 2007), and in Ottawa in 2018 we observed some *Orchestes* mines on *U. americana* in association with abundant *O. steppensis* mines on *U. pumila*. Another invasive Palearctic leafminer, the tenthredinid *Fenusa ulmi* Sundevall, 1844, has been recently noted to colonize the indigenous North American species of *Ulmus* (Anonymous 2015).

At the time of writing, *Stigmella multispicata* already is widespread in eastern North America, from eastern New York to western Iowa, and from Minnesota and Québec to Tennessee. Although the collected material gives a good insight already, online observation websites have been very helpful in providing a quick survey of the distribution, as this source alone was responsible for five of the state records. So far, *S. multispicata* does not appear to have risen to the status of a damaging pest. Although Siberian elm is no longer widely planted as an amenity tree, many specimens remain in urban and residential areas, where the descending larvae and aesthetic damage could be a concern. At this point the damage by *S. multispicata* is local, and probably not yet a great problem. In most places, the mines of *O. steppensis* outnumber those of *S. multispicata*. We advise to follow a prudent course and monitor both species simultaneously to document their spread and impact.

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## Supplementary material I

### Specimen data of *Stigmella multispicata* and other *Ulmus* mining Nepticulidae

Authors: Erik J. van Nieukerken, Daniel Owen Gilrein, Charles S. Eiseman

Data type: Microsoft Excel (xlsx)

Explanation note: The 288 records are the records of specimens examined and records obtained from online sources, of the taxa treated in this paper and supplement the presented Material examined.

Records are listed alphabetically by Species, Country, State, Province, AdminDivision2, AdminDivision3, Locality. Records contain BOLD Process ID's and GenBank accession numbers.

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Link: <https://doi.org/10.3897/zookeys.784.27296.suppl1>



# BumbleKey: an interactive key for the identification of bumblebees of Italy and Corsica (Hymenoptera, Apidae)

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## Abstract

BumbleKey is a matrix-based, interactive key to all 45 species of bumblebees of Italy and Corsica. The key allows to identify adult males and females (queens and workers) using morphological characters. The key is published online, open-access, at <http://www.interactive-keys.eu/bumblekey/default.aspx>.

## Keywords

*Bombus*, bumblebee, identification tool, interactive key, Italy

## Introduction

The genus *Bombus* Latreille, 1802 includes around 260 species of eusocial bees, mostly distributed in temperate and cold regions of the northern hemisphere (Williams 2018). Of the 70 European species, 45 have been recorded from Italy and Corsica (Rasmont 1983, Intoppa et al. 1995, Rasmont and Adamski 1995, Rasmont and Is-erbyt 2010–2013, Lecocq et al. 2015). Bumblebees are showy, large and loud insects, known to the public and easily recognizable as bees, and they are considered keystone species (Goulson 2010).

In the last decades, an apparent population decline of many species worldwide has been observed (Goulson et al. 2008, Williams and Osborne 2009, Goulson 2010, Rasmont et al. 2015). Even if the causes are still not clear, this decline is probably driven by a complex combination of factors like the reduction and fragmentation of natural habitats (especially for mountain species), climate changes and heat waves, use of pesticides, and reduction and alteration of floral resources. On the other hand, some species, the most generalist and heat tolerant ones, are expanding their ranges (Rasmont et al. 2015).

Bumblebees are pollinators of great ecological and commercial importance throughout much of the temperate world; the industry of greenhouse crop pollination is worth billions of dollars each year (Velthuis and Doorn 2006).

In Italy ecological studies focused on the effects of climate change and anthropogenic pressure on bumblebee communities, on their role as pollinators, or on the ecology of bumblebees in general, are very few. This is due, at least in part, to the difficulty in identifying these insects to species level by non-specialists. Moreover, the actual occurrence and the detailed distribution of bumblebee species in Italy are poorly known. In this respect, information is based on scattered, occasional, and often old or unreliable data that needs to be updated.

Surprisingly, however, bumblebees are difficult to identify to species level even for experienced taxonomists. They show great intraspecific variability in colouration pattern and a remarkable regional interspecific colouration pattern convergence, which is usually explained in terms of Müllerian mimicry (Plowright and Owen 1980, Williams 2007). Otherwise, they are morphologically monotonous showing only subtle differences in diagnostic character states (Michener 2007).

Available keys, all dichotomic, for the identification of West-Palaeartic bumblebees are hardly accessible for non-specialists, often lacking of adequate iconography supporting character states assessment (Pittioni 1939, Løken 1973, 1984, Amiet 1996, Intoppa et al. 2009, Gorczade et al. 2010, Intoppa et al. 2014). BumbleKey, a matrix-based interactive key for the identification of Italian bumblebees, is intended to be a flexible, practical and easily accessible tool, that could be useful to increase the interest for these bees and to facilitate and foster different kind of studies (ecological, conservation, etc.).

## **Project description**

### **Study area**

The study area includes Italy (political boundaries) and Corsica. For practical reasons, the area has been divided into four regions: Alps, Apennine mountains, Italian islands (Sicily and minor islands), Tuscan Archipelago, Sardinia and Corsica (Fig. 1). Coordinates are between 35°28'56"N and 47°07'04"N Latitude, and between 8°12'08"E and 18°32'15"E Longitude.



**Figure 1.** Map of the four regions in which the study area is divided.

### Taxonomic coverage

The key includes all the 45 species of bumblebees that have been recorded for the study area (Rasmont and Adamski 1995, Intoppa et al. 1995, 2009, Lecocq et al. 2015, Martinet et al. 2018). A few species, i. e. *Bombus confusus* Schenck, *B. pomorum* (Panzer), *B. veteranus* (Fabricius), have not been collected in the last several decades and their presence in Italy is doubtful and should be confirmed by new records. The single published record of *B. magnus* Vogt and the two records of *B. distinguendus* Morawitz are based on misidentifications. *Bombus xanthopus* Kriechbaumer and *B. renardi* Radoszkowski, traditionally considered as Corsican subspecies of *B. terrestris* (Linnaeus) and *B. lucorum* (Linnaeus) respectively (Estoup et al. 1996, Williams et al. 2012, Lecocq et al. 2013, Williams 2018), are here treated at rank of species according to Lecocq et al. (2015).

### List of the terminal taxa included in the current version of the identification key (last update 10 April 2018)

*Bombus alpinus* (Linnaeus, 1758); *B. argillaceus* (Scopoli, 1763); *B. barbutellus* (Kirby, 1802); *B. bohemicus* Seidl, 1837; *B. brodmannicus* Vogt, 1909; *B. campestris* (Panzer,

1802); *B. confusus* Schenck, 1859; *B. cryptarum* (Fabricius, 1775); *B. distinguendus* Morawitz, 1869; *B. flavidus* Eversmann, 1852; *B. gerstaeckeri* Morawitz, 1882; *B. hor-torum* (Linnaeus, 1761); *B. humilis* Illiger, 1806; *B. hypnorum* (Linnaeus, 1758); *B. inexpectatus* (Tkalcu, 1758); *B. jonellus* (Kirby, 1802); *B. konradini* Reinig, 1965; *B. lapidarius* (Linnaeus, 1758); *B. lucorum* (Linnaeus, 1761); *B. magnus* Vogt, 1911; *B. mendax* Gerstaecker, 1869; *B. mesomelas* Gerstaecker, 1869; *B. monticola* Smith, 1849; *B. mucidus* Gerstaecker, 1869; *B. muscorum* (Linnaeus, 1758); *B. norvegicus* (Sparre-Schneider, 1918); *B. pascuorum* (Scopoli, 1763); *B. pomorum* (Panzer, 1805); *B. pratorum* (Linnaeus, 1761); *B. pyrenaeus* (Pérez, 1879); *B. quadricolor* (Lepeletier, 1832); *B. renardi* Radoszkowski, 1884; *B. ruderarius* (Muller, 1776); *B. ruderatus* (Fabricius, 1775); *B. rupestris* (Fabricius, 1793); *B. sichelii* Radoszkowski, 1859; *B. soroensis* (Fabricius, 1776); *B. subterraneus* (Linnaeus, 1758); *B. sylvarum* (Linnaeus, 1761); *B. sylvestris* (Lepeletier, 1832); *B. terrestris* (Linnaeus, 1758); *B. vestalis* (Geoffroy in Fourcroy, 1758); *B. veteranus* (Fabricius, 1793); *B. wurflenii* Radoszkowski, 1859; *B. xanthopus* Kriechbaumer, 1870

### Applicability and operational methods

The key is designed to allow species-level identification of adult bumblebee specimens, both males and females. The sex of the specimen must be assessed as a first step, as explained in the *Instructions* section of the key. Specimens should be pinned, and the male genitalia capsule must be pulled out from the metasoma. No dissection or further special preparation are required. All the characters can be examined by means of a stereomicroscope, with incident light and at convenient magnification. Some of the most obvious colour pattern traits can be even assessed to the naked eye.

Thanks to the non-hierarchic structure of the key, the identification of damaged specimens, or part of them, is to some extent possible.

### General features of characters used in the key

Among the characters traditionally employed in the relevant literature as diagnostic, the ones that are too difficult to observe or to evaluate, as well as those whose evaluation is too subjective or time consuming (e. g. complex measurements) have been excluded. All the characters and states have been verified and evaluated on a reference collection of specimens of all the 45 species included in the key. The reference specimens consist of males, queens, and workers of each species collected on purpose by the authors or preserved in the following collections: Museo di Zoologia, Sapienza Università di Roma, Rome, Italy (MZUR), Museo Civico di Zoologia, Rome, Italy, and M. Mei personal collection (Rome).

A total of 50 diagnostic characters have been selected, 10 relevant to both males and females, 25 relevant only to females, and 15 relevant only to males. 19 characters are binary, and 31 are multistate with 3-9 alternative states each, for a grand total of 185

states. No character is dependent on the state of another; therefore, their applicability is not constrained, the only limitation being the sex of the specimen to identify.

In many instances, especially in the colouration pattern section, more than one state has been assigned to a character for a given species, to cover most of the intraspecific variability or to avoid possible subjectivity in the assessment of certain character states.

For cryptic species, i. e. those in the so-called “*Bombus lucorum*-complex” (Bossert 2015), and for morphologically very similar species, it could be difficult or impracticable to end up a single taxon using the characters in the key. In such cases, additional diagnostic information is given in the relevant species files.

### **List of the characters used in the key**

**PATTERN:** face and clypeus hair colour; upperside of the head (vertex) hair colour; bands on mesosoma; mesopleurae main colour; wing colour; hind tibia cuticle colour; hind tibia hair colour; bands on metasoma; metasomal tergites 4-7 main colour; metasomal tergites 4-7 colour arrangement.

**HEAD:** [F] antenna, antennomeres A3-A5; [F] antenna – antennal segment A3 ratio; [M] antenna – median antennomeres (A5-A9); [M] eye; [F] ocello-ocular area, sculpture; [F] ocello-ocular area, unpunctured and shining areas; [F] oculo-malar area; [M] oculo-malar area; [F] clypeus, shape; [F] labrum, overall shape; [F] labrum, median furrow and lateral tubercles (overall shape); [F] labrum, median furrow; [F] labrum, lamella shape; [F] labrum, lamella dimension; [F] mandible, number of teeth; [F] mandible, shape; [F] mandible, anterior keel; [F] mandible, sulcus obliquus (posterior groove); [F] mandible, incisura (distal notch); [M] mandible, “beard”.

**MESOSOMA:** [F] mid basitarsus, distal posterior corner; [F] mid basitarsus, erect hairs on the outer surface; [F] hind tibia, outer surface; [M] hind tibia, outer surface; [F] hind tibia, corbicula; [M] hind tibia, posterior fringe hairs; [F] hind basitarsus, proximal posteriorly-directed process; [F] hind basitarsus: length of posterior fringe’s hairs.

**METASOMA:** [F] metasomal tergite 6 (T6); [F] metasomal sternite 2 (S2); [F] metasomal sternite 6 (S6), keels; [M] volsella and gonostylus; [M] volsella, dorsal view; [M] volsella, overall shape; [M] volsella, process of the inner margin; [M] gonostylus, dorsal view; [M] gonostylus, inner process; [M] penis valve, head, dorsal view; [M] penis valve, shaft; [M] gonocoxa, apex, dorsal view.

### **Iconography**

Every character state is illustrated by 1 to 4 pictures (214 in total). For each species, a minimum of 5 pictures (dorsal female and male habitus, frontal view of female and male head, and male genitalia) have been provided in the species window. For most species, however, multiple pictures (up to 16) are available, in order to illustrate the range of colour-pattern intraspecific diversity or important diagnostic morphological details. The morphological terminology used in the key is illustrated by original line drawings.

## Software technical specifications

*Platform:* Framework.Net

*Web Server:* Microsoft Internet Information Service 6.0

*Programming language:* C#

*Application version:* MOSCHweb 1.0

*Data base:* Microsoft SQL Server

*Data:* 1.0beta

*Language:* English

*License for use of the key:* Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

*Use of the primary data:* Primary data are available from the authors by agreement.

*Web Location:* <http://www.interactive-keys.eu>

## Software technical features

BumbleKey interface is simple and intuitive (Fig. 2). The chosen software is MOSCH, originally developed for Diptera Tachinidae, and later used for other groups (Cerretti et al. 2012, Bonato et al. 2014).

- 1 – *Instructions:* a button that refers to a dedicated window, where the user can find information about how to use the key.
- 2 – *Morphology:* a button that refers to a dedicated window (Fig. 3), with drawings illustrating the terminology used in the key and pictures of different shades of hair colours. Images are obtainable just by hovering over entries.
- 3 – *Bibliography:* a button that refers to a dedicated window, illustrating a selection of papers used for the creation of the key.
- 4 – *How to cite:* a button that refers to a dedicated window, illustrating how user should cite the key.
- 5 – *Species window:* an updating real-time box showing all the species that share selected character states. The name of the species is followed by the author name and the year of description. Clicking on a species name, a new window opens to show more information about that species (Fig. 4). The *taxon window* shows binomial name and subgenus, main synonyms, recorded subspecies in the study area, distribution worldwide and in the study area, similar species with additional diagnostic characters, remarks, and pictures. Each species picture can be opened in a dedicated window, for example to be compared with other pictures, by clicking on its name in the *double temporary view* section.
- 6 – *Regions:* a button that refers to a dedicated window, showing a map of the four regions in which the study area is divided.

- 7 – *Regions menu*: the user can select the region of origin of the specimen to reduce the query to the taxa belonging to that region. By default, all the regions are selected.
- 8 – *Body parts bar*: a bar with buttons that allow the user to quickly reach the desired section. Characters are arranged in four sections: pattern (10 characters), head (20 characters), mesosoma (8 characters), and metasoma (12 characters).
- 9 – *Refresh*: a button that clears the checkboxes for characters and regions.
- 10 – *Character window*: a window with all the characters used in the key and pictures of all the states. Each state picture can be opened in a dedicated window, for example to be compared with other pictures, by clicking on its name in the *double temporary view* section. The characters can be used in any order, and the more selective ones are highlighted in green. BumbleKey allows for uncertainty to be expressed by the selection of more than one state for each character.
- 11 – *Selected choice box*: an updating real-time box showing the chosen characters and state selected by the user, ordered as they appear in the *Character window*. This represents an ID-code which is linked to the specimen under examination
- 12 – *Export data*: a button allowing the user to export in TXT format the terminal taxon/taxa, followed by the list of selected states in the form of a code, together with the specification of the used BumbleKey version. This code serves as a record of the character states used to achieve a specimen identification.

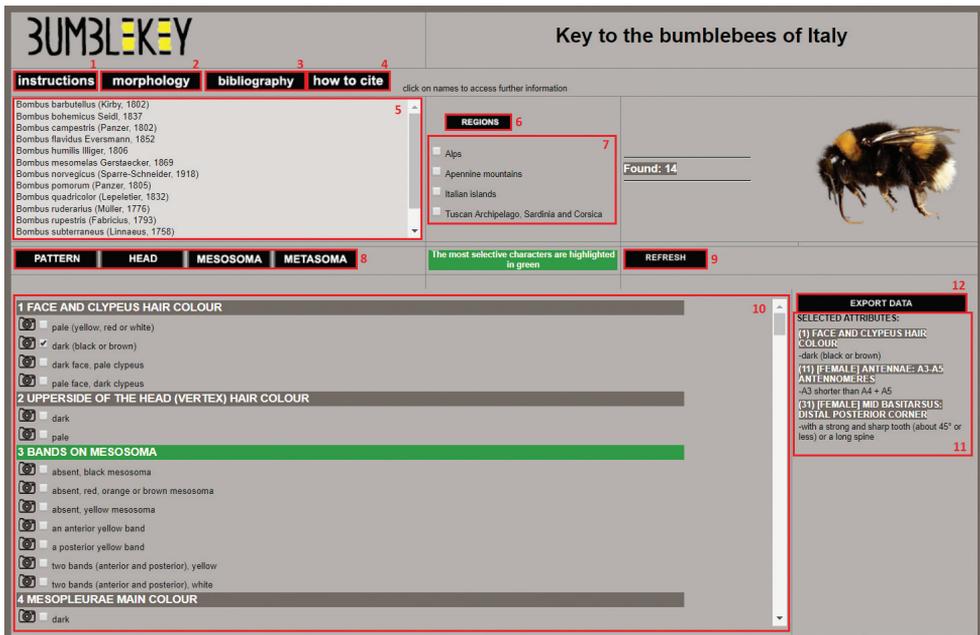


Figure 2. Interactive key main page.

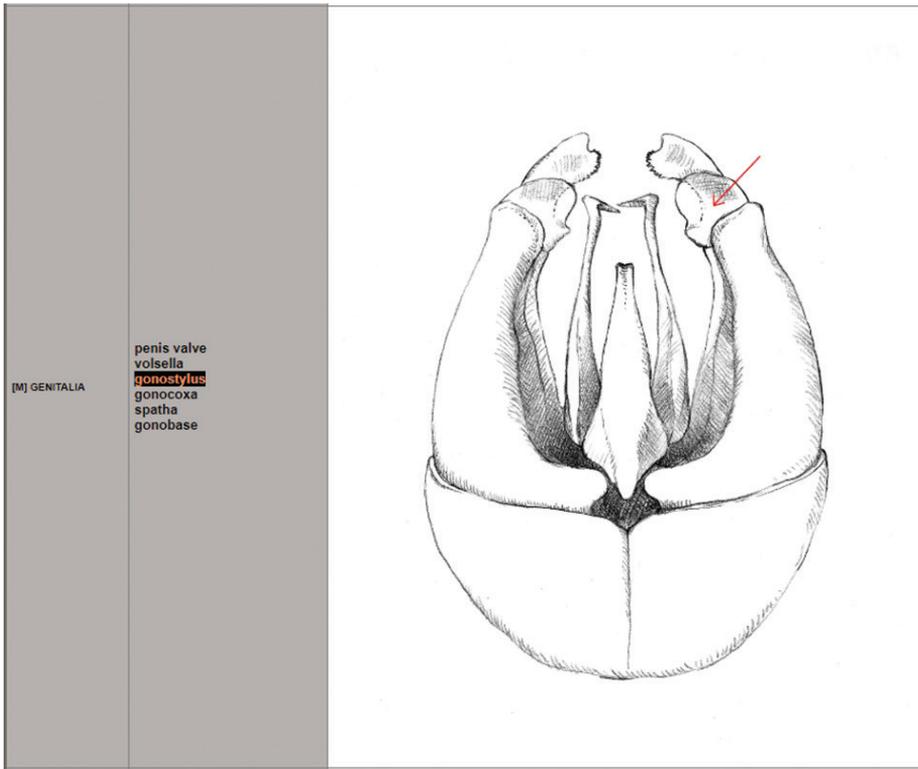


Figure 3. Interactive image window for morphology.

 <b>Bombus pascuorum</b>	
<b>BINOMIAL NAME (W/SUBGEN):</b>	<i>Bombus (Thoracobombus) pascuorum</i> (Scopoli, 1763)
<b>SYNONYMS:</b>	<i>Apis pascuorum</i> Scopoli, 1763 <i>Apis agrorum</i> Fabricius, 1787 nec Schrank, 1781 <i>Apis floralis</i> Gmelin in Linnaeus, 1790 <i>Apis italica</i> Fabricius, 1793 <i>Bombus agrorum</i> : Walckenaer, 1802 <i>Bombus italicus</i> : Fabricius, 1804 <i>Bombus pascuorum</i> : Illiger, 1806 <i>Bombus floralis</i> : Illiger, 1806 <i>Bombus thoracicus</i> Spinola, 1806 <i>Bombus agrorum</i> var. <i>pascuorum</i> : Schmiedeknecht, 1882 <i>Bombus Fairmairei</i> Friese, 1887 nec Sichel 1864
<b>RECORDED SUBSPECIES (STUDY AREA):</b>	<i>B. p. floralis</i> (Gmelin, 1790) <i>B. p. frey-gessneri</i> (Vogt, 1909) <i>B. p. maculatus</i> (Vogt, 1909) <i>B. p. meliofacies</i> (Vogt, 1909) <i>B. p. pascuorum</i> (Scopoli, 1763) <i>B. p. siciliensis</i> Tkalcu, 1977
<b>DISTRIBUTION (GLOBAL):</b>	Palearctic.
<b>DISTRIBUTION (STUDY AREA):</b>	Alps, Apennine mountains, Italian islands (no Sardinia), Corsica.
<b>SIMILAR SPECIES:</b>	<i>Bombus humilis</i> , <i>Bombus muscorum</i> Identification of females of these three species (pale forms) can be difficult. <i>Bombus pascuorum</i> : (F) long and irregular hair on thorax; sometimes, few black hair on T1-T5; pale hair on T6. <i>Bombus humilis</i> : (F) short and even hair on thorax; many black hair on T1-T5; black hair on T6. <i>Bombus muscorum</i> : (F) very short and almost like shaved off hair on thorax; no black hair on T1-T5; black hair on T6.
<b>REMARKS:</b>	One of the most common bumblebee in Italy.
 Pictures available: 9	
	

Figure 4. Example of taxon window.

## Conclusions and future work

MOSCHweb is an open-access web application, but it is not open source. The application can be modified or updated only by, or in agreement with, the authors of this paper. The authors will keep updated both the web application and the data matrix, by improving encoded descriptions of terminal taxa or by adding possible new taxa to the matrix. Indeed, one of the future desirable developments of BumbleKey will be to better document the chromatic and morphological variation of every species and to extend the key to include all the European and possibly West-Palaeartic species. This would be very difficult with a traditional, dichotomous tool, but with MOSCHweb the problem would be easily solved by augmenting the database with the addition of new data strings to the matrix.

The taxonomy and systematics of Italian and European bumblebees are thoroughly studied and relatively stable (Cameron et al. 2007, Rasmont and Iserbyt 2010–2013). However, recent revisions and phylogeographic studies have led to several taxonomic changes (Rasmont et al. 2005, Williams et al. 2008, Lecocq et al. 2011, Lecocq et al. 2015, Martinet et al. 2018). Moreover, possible faunal changes can result from the increase of species ranges due to climate change (Neumayer 2004, Jenič et al. 2010, Šima and Smetana 2012, Martinet et al. 2015) or from the introduction of species or subspecies from other regions as a consequence of the commercialization of bumblebee colonies for pollination (Ings et al. 2010). When necessary, the key could be easily augmented or modified to cope with such minor changes too. Any modification will be reported at <http://www.interactive-keys.eu>.

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# Chiropteran diversity in the peripheral areas of the Maduru-Oya National Park in Sri Lanka: insights for conservation and management

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## Abstract

In Sri Lanka, there are 31 species of bats distributed from lowlands to mountains. To document bat diversity and their habitat associations, 58 roosting sites in Maduru-Oya National Park periphery were surveyed. Fifteen bat species were recorded occupying 16 different roosting sites in this area. Among all the species recorded, *Rhinolophus rouxii* was the most abundant species per roosting site whereas *Kerivoula picta* was the least abundant. A road-kill specimen similar to genus *Phoniscus* was found during the survey, a genus so far only documented in Southeast Asia and Australasia. Although our study area provided habitats for a diverse chiropteran community, the colony size per roost was remarkably low. Although our study area is supposedly a part of the park's buffer zone, many anthropogenic activities are threatening the bat community: felling large trees, slash-and-burn agriculture, excessive use of agrochemicals, vengeful killing, and subsidized predation. We strongly recommend adoption of wildlife-friendly sustainable land management practices in the buffer zone such as forest gardening, agroforestry (alley cropping, mixed-

cropping), and integrated farming. Bat conservation in this region should take a landscape-scale conservation approach which includes Maduru-Oya National Park and other surrounding protected areas into a regional conservation network. Extents of undisturbed wilderness are dramatically declining in Sri Lanka; thus, future conservation efforts must be retrofitted into anthropocentric multiuse landscapes and novel ecosystems like areas surrounding Maduru-Oya National Park.

### Keywords

behavior, conservation, habitat associations, roosting sites, species richness, threats

## Introduction

Sri Lanka is a small (65,610 km<sup>2</sup>), Indian Oceanic tropical island providing habitats for a rich mammalian diversity containing 126 species including 23 (~18%) endemic species (de Silva Wijeyeratne 2016; Leowinata and Luk 2016; Ministry of Environment 2012). The island provides habitats for a number of charismatic mega-mammals, such as, the Asian elephant, Sri Lankan leopard, sloth bear, and Old-World monkeys (de Silva Wijeyeratne 2014; Weerakoon and Goonatilake 2006). The majority of Sri Lanka's mammalian diversity is comprised of small and medium-sized mammals, including 31 species of chiropterans that belongs to 14 yinpterochiropteran and 18 yangochiropteran species (Leowinata and Luk 2016; Yapa 2017). Currently, 18 (58%) Sri Lankan bat species are "nationally threatened" (five Critically Endangered, five Endangered, and eight Vulnerable) (Ministry of Environment 2012). As evident from the southeast-Asian island Singapore where 72% of chiropterans have gone extinct, island bats are more vulnerable to anthropogenic stressors and habitat loss (Lane et al. 2006; Mickleburgh et al. 2002). Due to greater access to roosts and diversified food niches, woodlands and forests are the primary habitats for most bats, although a handful of synanthropic species inhabit built-up urban environments (Avila-Flores and Fenton 2005; Gehrt and Chelsvig 2003). Bats are among the most imperiled mammals, particularly in the tropical realm including Sri Lanka, due to decline in prey availability, pesticide use, roost destruction, and deforestation (Mickleburgh et al. 2002; Ministry of Environment 2012).

Availability and diversity of roosting sites are paramount elements of life history of bats. A number of critical biological functions, such as reproduction, postnatal care, predator avoidance, and thermoregulation are provisioned by roosting sites (Campbell et al. 2006; Chaverri and Kunz 2010; Kunz and Lumsden 2003; Lewis 1995). Although bats in general are known to occupy a diverse range of habitats for roosting, studies conducted in Sri Lanka have mostly reported caves as suitable roosting sites (Kusuminda et al. 2013; Rubsamen et al. 2004; Yapa and Ratnasooriya 2006; Yapa 1992). Although behavior, echolocation, and trophic ecology of bats have been satisfactorily explored in Sri Lanka (Neuweiler et al. 1987; Pavey et al. 2001; Schmidt et al. 2011), much remain unknown about selection of roosting sites and habitat associations. Thus, surveying roosting sites helps understanding habitat associations of bats in areas of interest.

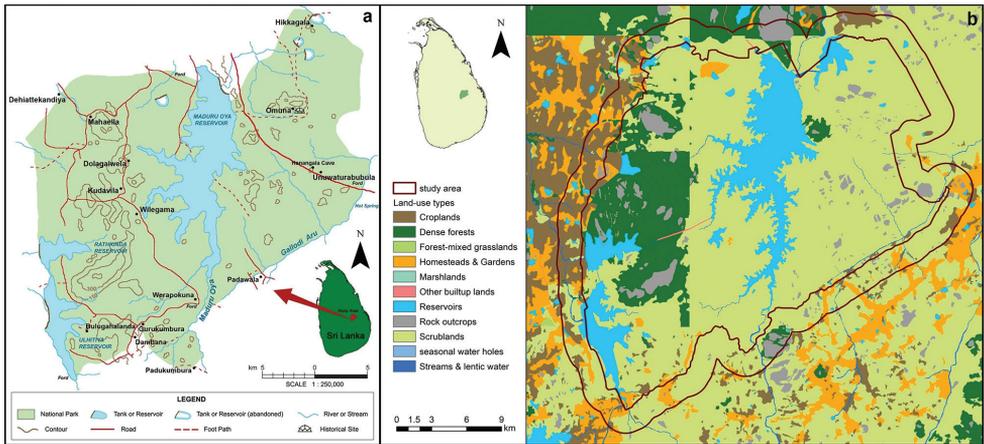
There is a pressing need for local inventories of bat diversity to map species distributions throughout the island, which is critical for conservation assessments. Amongst the remaining Sri Lankan forest cover, the dry-mixed evergreen forests are largely secondary in origin, the most extensive and embedded in rural-agricultural landscapes (covering 21% of the island's land area) (FAO 2010a; b); yet these habitats remained relatively unexplored in terms of bat diversity. Therefore, documenting bat diversity in less-explored habitats is crucial for updating bat distribution ranges and to inform conservation planning. Although conservation lands provide substantial immunity against habitat degradation, bat communities occupying outside protected areas could be highly vulnerable to anthropogenic threats. Thus, investigating bat diversity and their use of roosting sites are salient for conservation.

Our specific objectives in this study were to (1) document species richness of bats in the periphery of Maduru-Oya National Park; (2) investigate their roosting site selection and identify local and landscape-scale land-cover types that influence presence of bats in potential roosting sites. Surveying habitats outside conservation lands is of conservation importance for several reasons. First, species distribution ranges may not be restricted by conventional protected area boundaries, thus bat occupancy can extend into the peripheral habitats of Maduru-Oya National Park. Thus, field surveys are necessary to confirm species occupancy outside the park. Secondly, we attempted to identify suitable bat habitats outside the park and thereby help re-delineation and management of a park buffer zone. Third, although bat habitats inside the park are protected from human disturbances and habitat loss, the same cannot be said for habitats of the park periphery. Therefore, documenting bat diversity and threats outside the park can help develop wildlife-friendly habitat management practices.

## **Materials and methods**

### **Study area**

Peripheral landscapes of Maduru-Oya National Park (~58,850 ha; 7.3833–7.5833N and 81.033–81.3333E) comprise diverse habitat mosaics including woodlands, teak plantations, scrublands, grasslands, home gardens, croplands, lakes and reservoirs, streams, marshlands, and a variety of build-up environments (Figure 1). The main vegetation type of the area is tropical dry mixed-evergreen (semi-evergreen) forests (Gunatilleke and Gunatilleke 1990). Teak plantations and unprotected woodlands in these areas are subjected to repeated slash-and-burn agriculture (Gabadage et al. 2015). Located in the dry zone lowlands (annual average precipitation <2,000 mm, annual average temperature 28.7 °C; elevation < 500 m), our study area is influenced by the northeastern monsoon rain (October – late January) (Green 1990; Survey Department of Sri Lanka 2007). Maduru-Oya National Park falls within Mahaweli Development Area, which is a government-sponsored, large-scale socioeconomic development scheme (Gabadage et al. 2015). Reforestation after abandonment of ancient civilizations and slash-and-burn



**Figure 1.** **a** the local topography, road network, local towns and hydrology and **b** land–used types (the 2km-wide study area is delineated by solid lines) in and around Maduru-Oya National Park.

farming practices in our study area have resulted in large extents of secondary forests and scrublands (Pemadasa 1990). The local topography is mostly comprised of lowlands (30–150 m in elevation range), an isolated residual mountain (485 m), an 8 km long vegetated rocky outcrop, and granite caves (Figure 1).

## Field survey

We conducted this survey for 2.5 years (May 2014 to December 2016) and surveyed 58 roosting sites found within a 2 km-wide peripheral area around Maduru-Oya National Park. Surveying roosting sites is highly effective and time-efficient for assessing bat diversity in a given region (Phelps et al. 2016). We surveyed each roosting site both day (0800–1400 h) and night (1900–0100 h) and revisited each roosting site twice in two different years. Based on visual encounter surveys and using headlamps, we searched through a diverse range of roosting sites such as caves (11), rock crevices (4), rocky ledges (2), large tree crowns (5), tree hollows (8), abandoned buildings (8), homesteads (2), culverts (3), bridges (5), banana plantations (6), and paddy fields (4). At each roosting site, we recorded the species found and the relative abundance of each species based on direct roost count. Using an endoscope camera and an 8 mm illuminated camera head (Work zone – China), we documented bat occupancy inside tree cavities. We captured flying bats using hoop-nets (Circumference 2.5 m) and released them at site of capture after identification. We identified bats using published standard keys and guides (Bates and Harrison 1997a; Corbet and Hill 1992; Phillips 1980; Srinivasulu et al. 2010) and photographed with an SLR digital camera (Cannon EOS 60D). In addition to roosting site surveys, we documented road-kill bats on a 10 km stretch of a double lane highway (the major highway found within the park periphery) by walking along the highway on both edges of the road while making visual observa-

tions (both on the road as well as the road verges) for bat carcasses. If a road-kill bat was found, we identified the bat to the highest possible taxonomic level. After identification, we moved the carcasses away from the road to avoid secondary road-kills. We repeated the road-kill survey six times in a given year throughout the study period. Our study conforms to the guidelines of American Society of Mammologists; the IACUC committee of Bridgewater State University approved our research procedure.

## Data analyses

To test for differences in species compositions among different roosting sites, we ran a Multiple Response Permutation Procedures (MRPP) treating species composition of bats as response variable and different types of roosting sites as predictor variables. Here, we ran 500 permutations and used Bray-Curtis Index to calculate the distance matrix. To study patterns of roosting site selection by different species of bats, we constructed an ordination based on NMDS (non-metric multidimensional scaling). We calculated the distance measures from Bray Curtis index for different bat species. We extracted two axes with the lowest final stress that best ordinated the roosting sites in species space through 500 iterations with random starting configurations. To select dimensionality with the lowest stress, we ran a Monte Carlo test (60 runs from real data, and 60 runs from randomized data). We constructed an ordination plot with optimal axes to visualize patterns of roosting site selection. We used *R*-Studio (vegan and mgcv packages) for all statistical analyses (*R* Core Team 2017; *R*-Studio Team 2016).

To investigate the influence of land-use and land-cover types around potential roosting sites on presence of bats (using ArcGIS Pro 2.2) we extracted land-use and land-cover data from a reclassified global land cover data (European Space Agency 2017) for a 500 m (local scale) and a 5 km-radius (landscape-scale) around surveyed roosting sites. We classified each of the roosting site into two binary responses, bats present (through direct and indirect evidence) versus bats absent, and constructed a binomial (logit function) generalized additive model (GAM) where the binary response of presence or absence of bats was treated as the response variable and percentage land-cover types at 500 m and 5 km radii around each roosting site as the predictor variables.

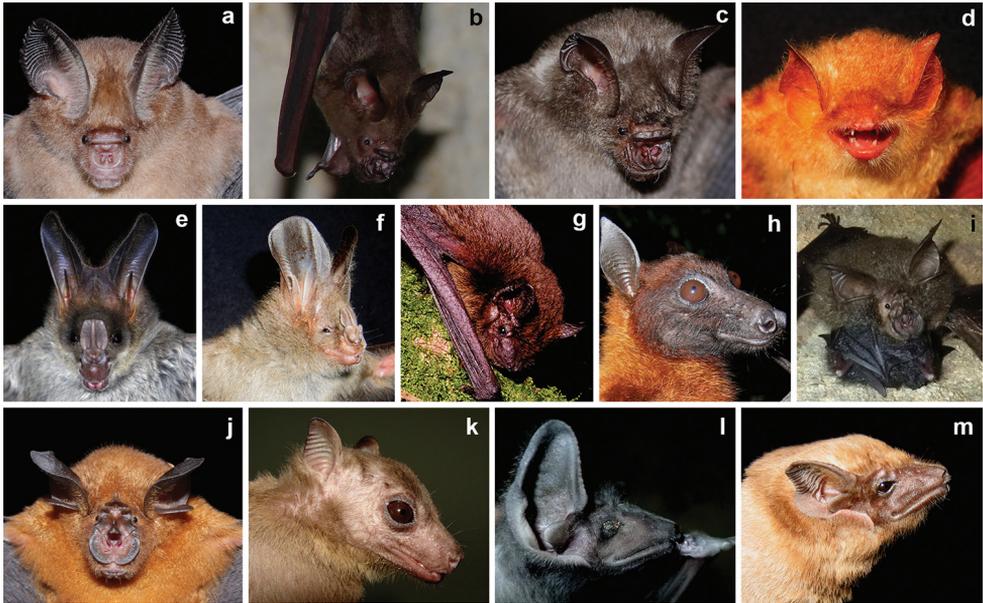
## Results

### Species richness of the bat community

We recorded a total of 15 species of bats including 10 yinpterochiropteran and five yangochiropteran species representing six chiropteran families (Table 1, Figure 2). The bat species richness we documented around Maduru-Oya National Park accounted for 71% of yinpterochiropteran species and 29% of yangochiropteran species of Sri Lanka (Figure 2). Bats occupied a variety of roosting sites, including the canopy of large trees,

**Table 1.** Roosting sites used by different bat species in the peripheral areas of Maduru-Oya National Park, Sri Lanka and relative abundance of each bat species at each type of roosting site. The number of sightings indicates the number of different days on which each bat species was present at a given roosting site. Superscripts denote national conservation status LC: least concerned, NT: near threatened, VU: vulnerable, EN: endangered. The global conservation status for all species was “least concerned”. Two more species (*Cynopterus sphinx* and *Phoniscus cf. jagorii*) were only recorded as dead specimens.

Family	Species	Trophic guild	Roosting site	Total no. of sightings	Avg. no. of individuals (std. dev.)	Used for day or night roosting?
Pteropodidae	<i>Pteropus giganteus</i> <sup>LC</sup>	Frugivore	Large tree	11	22.54 (4.39)	Both
	<i>Rousettus leschenaulti</i> <sup>LC</sup>	Frugivore	Abandoned building	34	15.60 (1.30)	Both
Hipposideridae	<i>Hipposideros ater</i> <sup>LC</sup>	Insectivore	Abandoned building	10	5.56 (2.45)	Night
	<i>Hipposideros lankadina</i> <sup>VU</sup>	Insectivore	Underneath bridge	11	4.09 (2.34)	Night
	<i>Hipposideros speoris</i> <sup>LC</sup>	Insectivore	Cave	18	16.90 (0.32)	Both
			Cave	18	29.08 (4.25)	Both
Vespertilionidae	<i>Kerivoula picta</i> <sup>NT</sup>	Insectivore	Cave	18	49.56 (4.12)	Both
			Banana shrub	21	2.13 (0.99)	Day
Megadermatidae	<i>Pipistrellus tenuis</i> <sup>LC</sup>	Insectivore	Hollow tree	10	6.78 (4.18)	Both
			Underneath bridge	26	28.62 (2.09)	Night
	<i>Megaderma lyra</i> <sup>VU</sup>	Carnivore	Underneath bridge	26	22.4 (2.35)	Night
			Abandoned building	34	66.33 (3.89)	Both
Rhinolophidae	<i>Megaderma spasma</i> <sup>VU</sup>	Insectivore	Cave	09	5.11 (1.69)	Both
	<i>Rhinolophus beddomi</i> <sup>VU</sup>	Insectivore	Abandoned building	34	285.00 (5.50)	Both
Emballonuridae	<i>Taphozous longimanus</i> <sup>EN</sup>	Insectivore	Cave	09	40.14 (3.02)	Both
			Cave	34	9.73 (5.36)	Both
	<i>Taphozous melanopogon</i> <sup>VU</sup>	Insectivore	Cave	15	17.10 (6.51)	Both



**Figure 2.** Bat species recorded in the periphery of Maduru-Oya National Park. **a** *Hipposideros ater* **b** *Hipposideros lankadiva* **c** *Hipposideros speoris* **d** *Kerivoula picta* **e** *Megaderma lyra* **f** *Megaderma spasma* **g** *Pipistrellus tenuis* **h** *Pteropus giganteus* **i** *Rhinolophus beddomei* **j** *Rhinolophus rouxii* **k** *Rousettus leschenaulti* **l** *Taphozous longimanus* **m** *Taphozous melanopogon*.

tree cavities and hollow trees, abandoned buildings, underneath bridges, caves, and banana plants (Figure 3). Although we surveyed 58 potential roosting sites, only 15 sites had bats. Even though we did not encounter bats in 14 more sites, we documented indirect evidence for bat occupancy— such as characteristic bat odor and presence of fresh guano. We had neither direct nor indirect evidence for bat occupancy in the rest of 29 sites. The roosting sites we surveyed were overwhelmingly used for day roosting. However, *Hipposideros ater*, *Megaderma lyra*, and *Hipposideros lankadiva* were only found in their respective roosting sites during night visits. *Rousettus leschenaulti* used the same roosting site both day and night. Among all species, *Rhinolophus rouxii* was, on average, the most abundant species per roosting site (250–300) whereas *Kerivoula picta* was the least abundant (1–3). All bat species we recorded in this survey were considered “least concerned” in the Global IUCN Red List. In contrast, national conservation assessments of Sri Lanka listed six species “threatened” (one endangered and five vulnerable) and one species “near threatened” (Table 1).

We recorded three bats as road-kills, and identified two of them as *Cynopterus sphinx* and *Rhinolophus rouxii* (Figure 4). A third specimen was found along the highway we surveyed (7.724550°N and 81.212600°E) on August 9, 2015 (0822 h). External morphological characteristics (Table 2, Figure 5)— long tragus with a prominent notch, wing membrane attachment to the ankle, and short golden hair on forearms and hands— suggested that the specimen could belong to genus *Phoniscus* which is



**Figure 3.** Habitats of bats in the peripheral areas of Maduru-Oya National Park. **a** a cave in Dananjaya Gala **b** rocky outcrops surrounded by forests **c** scrublands with temporary pools **d** small canal inside the forest **e** a cave nearby the Maduru-Oya reservoir **f** a historical cave in Damminna **g** a cave nearby Henanigala **h** under a large bridge.

restricted to Southeast Asia and Australasia (Table 2). We named this specimen as *Phoniscus cf. jagorii* given its close resemblance to Peter's trumpet-eared bat (*Phoniscus jagorii*) (Figure 5). Due to extensive damage to the facial structure, we could not make any detailed accounts on the dentition.

### Selection of roosting sites and habitat associations

We noted four different types of roosting sites frequently occupied by bats in our study area. These included caves of different sizes, different locations of large mature trees and banana trees, abandoned buildings, and underneath of bridges (Table 1); all roosting sites were located within or in proximity to (140 m) dense forests or sparse woodlands. Fig trees (*Ficus* spp.) were exclusively used by tree-roosting bats. Roosting trees had a dense canopy with no low-lying branches. The bats occupied branches approximately 3 m above the ground level. Roosting bats were not found inside home gardens, agricultural lands (with the exception of banana plantations), or open habitats such as grasslands or scrublands. Of the 16 active roosting sites we surveyed, seven of them were caves. Multiple species occupied a single type of roosting sites therefore roost selection may not be species-specific. For instance, three species occupied caves while another two species used abandoned buildings for roosting (Table 1). However, roost-sharing was only observed in two instances during our survey; *Megaderma lyra* and *Hipposideros lankadiva* roosted underside of a concrete bridge (height 3 m from the streambed) over a slow-flowing stream with a dense forest cover along the banks while *Taphozous longimanus* and *Rhinolophus beddomei* roosted in the same cave located in a woodland habitat. For all four species, roost-sharing was only observed during the dry season.

The MRPP analyses showed significant differences in species composition of bats across different roosting sites (Chance corrected within group agreement = 0.14, expected  $\delta = 0.73$ , observed  $\delta = 0.62$ ,  $p = 0.001$ ). The NMDS ordination reached a stable solution (mean stress = 0.08) after 500 iterations and produced two axes. The



**Figure 4.** Dead specimens recorded in the peripheral areas of Maduru-Oya National Park **a** *Cynopterus sphinx* **b** *Phoniscus* cf. *jagorii* **c** *Rhinolophus rouxii* **d** *Pteropus giganteus*.

Monte Carlo randomization also suggested that the two axis solution was optimal (stress in randomized data = 0.26,  $p < 0.05$ ). The stress level we reached (closer to 0.05 and less than 0.1) in our stable solution with two dimensions suggests a good fit of our dissimilarity object. The ordination plots (axes 1 and 2) indicated substantial segregation of the different species based on the roosting sites selected (Figure 6). *Megaderma lyra* and *Hipposideros lankadiva* ordinated exclusively with underside of bridges. *Rousettus leschenaulti*, *Rhinolophus rouxii*, *Hipposideros ater*, and *Megaderma spasma* ordinated in close association with the abandoned buildings (although some of them ordinated outside the 95% confidence interval). *Hipposideros speoris*, *Rhinolophus beddomei*, *Taphozous longimanus*, and *Taphozous melanopogon* closely ordinated with the caves. *Pipistrellus tenuis*, *Pteropus giganteus*, and *Kerivoula picta*, ordinated with trees. However, 95% confident ellipses for roosting locations overlapped substantially.

The GAM we constructed indicated that both the percent cover of crop-mixed natural vegetation and percent forest cover at 500 m radius are of significantly important predictors of bat presence in potential roosting sites (Table 3; adjusted model correlation coefficient = 0.412; deviance explained = 45.3%). Although percent cover of agricultural lands within a 5 km radius had a relatively greater coefficient estimator, its impact was marginally insignificant.

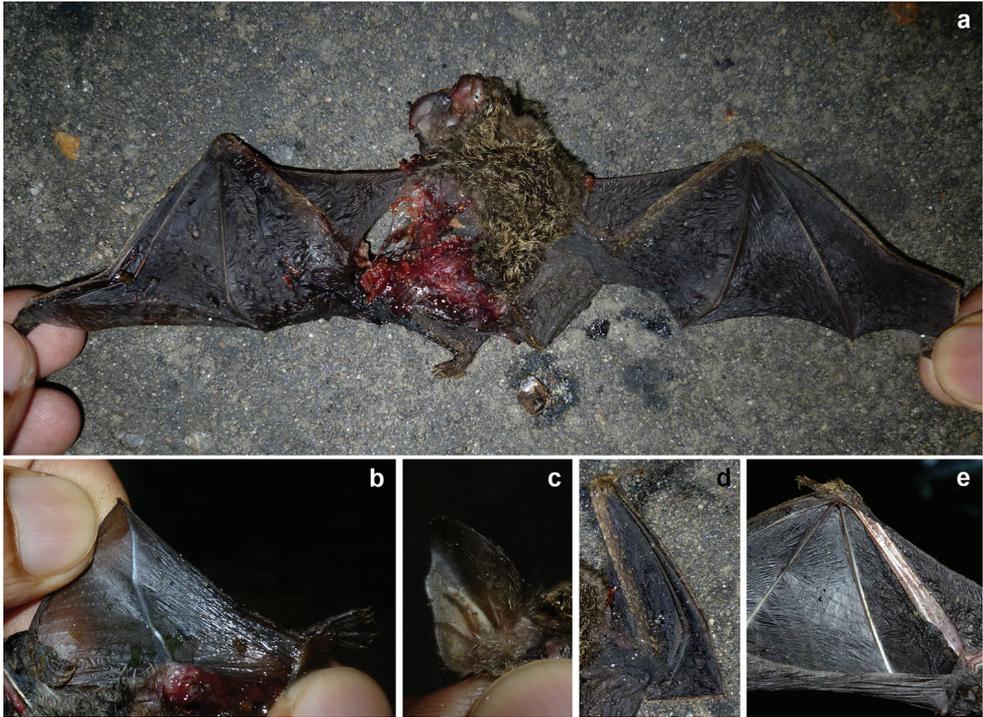
## Discussion

The bat species richness in the peripheral areas of Maduru-Oya National Park is remarkable as this bat community represents six of the seven Sri Lankan chiropteran families and ~50% of the island's total bat species (Bates and Harrison 1997a, Yapa 2017). The species richness we documented is comparable to or greater than the diversity recorded from other similar regions of Sri Lanka. For instance, baseline biodiversity surveys at Wasgamuwa National Park (located in the same bioclimatic region as Maduru-Oya area) documented seven species of bats (Department of Wildlife Conservation 2007). Kusuminda et al. (2013) reported six species of bats in a

**Table 2.** Morphological characteristics and morphometric features (mm) of the road-killed specimen (*Phoniscus* cf. *jagorzi*) from Peripheral areas of Maduru-Oya National Park, *Phoniscus jagorzi*, *Kerivoula hardwickii*, and *Kerivoula picta* from Asia (Bates and Harrison 1997a; Blanford 1888-91; Corbet and Hill 1992; Dobson 1848–1895; Francis 2008a; Hill 1965; Phillips 1980; Tomes 1858).

Character	Unidentified road-kill specimen ( <i>Phoniscus</i> cf. <i>jagorzi</i> )	<i>Phoniscus jagorzi</i>	<i>Kerivoula hardwickii</i>	<i>Kerivoula picta</i>
Muzzle	The facial structure is damaged beyond characterization	Extremity of the mussel is projecting; covered with hair	Covered with hair; moderately small. Short, and pointed	Moderately small; long and rather pointed; densely covered with long hair which overhangs the mouth
Ears	Large, few short hair at the base of ears; Tip of the ear rounded with a few short hairs (ear length: 14.03; ear width: 11.20)	Funnel-shaped large ears with rounded tips; two slight concavities—one just below the apex and another at the center of the posterior margin	Ears naked and relatively large, funnel shaped, tip rounded, inner margins regularly convex from base to tip, outer margins deeply concave immediately below the tip, the lower portions regularly convex. a prominent notch on tip of the posterior edge.	Moderately long; separate and distinctly funnel-shaped, bluntly pointed at tips; inner and outer margins terminating close together and giving the impression of almost complete cups; partially covered with short hair on the outer side
Tragus	Long, narrowing gradually to a point and a deep notch present on the posterior edge (tragus length: 8.44; tragus width: 2.11)	Tragus rather broad at base and tapers to an acute point; white with deep notch on posterior edge	Tragus very long, narrow, and attenuated, outer straight-sided and sharply pointed	Very long, slender, grey in color; terminating in fine points
Dorsal area of the body	Fur golden brown, black, and hair banded with four colors; dark grey–brown bases, then a buff band, then dark brown, then golden tips	Overall, golden brown and black; fur with four bands of color including a pale tip; dark brown or blackish-brown at the base, followed by buff, then brown, and finally golden or whitish-yellow tips; the paler tips are more pronounced on the ventral surface	Hair very soft and of moderate length; confined to the body and ceasing abruptly, both on the upper and the lower sides. General color of the upper parts rufescent brown although a few hairs could be pale-tipped; the hairs of the head, shoulders and mantle unicolored, those of the lower back and hinder parts generally, with the basal portions dark grey	Fur rather long, dense, and woolly; Extending slightly onto the membranes near the body; dorsal fur orange or tawny–red
Ventral area of the body	Fur paler grey to dark brown with golden tips	Under parts paler with slightly greyer tips	Lower parts, light rufescent–fawn, with the basal portions of the hairs dark brownish grey and paler grey hair tips; membranes and ears unicolored, semi-transparent blackish brown	Compared to dorsum; ventral hair is paler and yellowish

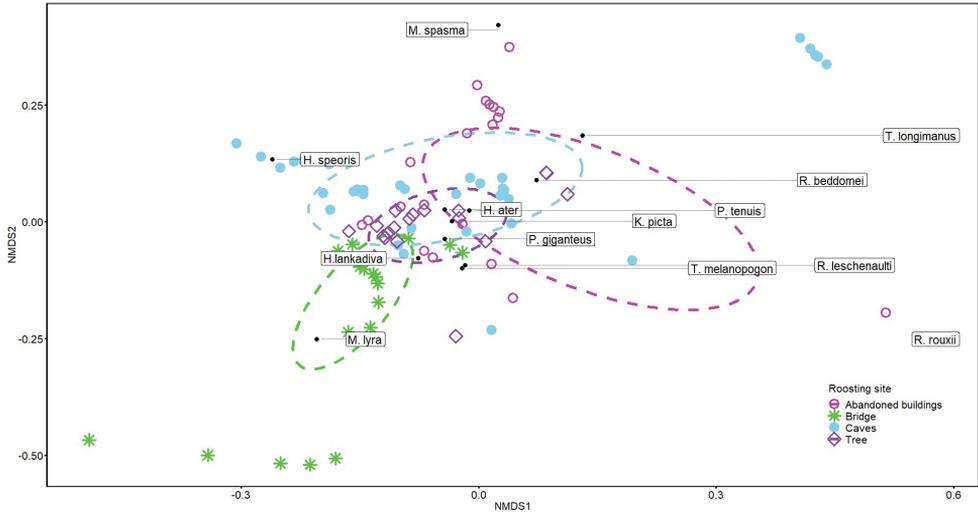
Character	Unidentified road-kill specimen ( <i>Phoniscus</i> cf. <i>jagoriti</i> )	<i>Phoniscus jagoriti</i>	<i>Kerivoula hardwickii</i>	<i>Kerivoula picta</i>
Ante-brachial membrane	Naked	Naked	Semi-transparent, thin in texture	Sparsingly but visibly covered with many minute hairs on both surfaces; bright orange to scarlet in color.
Wing membrane	Naked and well developed. Attached to the ankle	Attached to the ankle	Arise from the base of the outer toes. Brown-colored but nearly transparent; upper surface almost naked, expect for a thin spread of small hair.	Moderately long and broad with the membrane attached to the base of the toes; orange to bright scarlet along the length of the forearms and fingers the rest is black
Interfemoral membrane	Ventral side naked, dorsal side is partly covered with hair; membrane is well developed and semi-transparent	Hair very short and almost invisible; the margins are mostly naked; sometimes with sparse sprinkle of hair	Naked, well developed; thin in texture and semi-transparent. Long scattered hair present on femur and tibia, feet are almost naked; no prominent fringe on the posterior boarder but scattering hair may be present	Sparsingly but visibly covered with many minute hairs on both surfaces; bright orange to scarlet in color
Tail	Tip of the tail projects slightly (tail length: 40.54)	Tip of the tail projects slightly	Tail considerably shorter than the head and body	Long tail (as long as the head and the body) entirely contained within the interfemoral membrane
Radio-metacarpal pouch	Absent	Absent	Absent	Absent
Forearms and hand	The metacarpals and digits covered with short, golden hair (Forearm length: 37.64; thumb + claw length: 7.33; 2 <sup>nd</sup> -5 <sup>th</sup> metacarpal lengths: 37.37, 36.80, 34.32, 35.04)	Short shiny yellow hairs along forearm and fingers	Hair absent on forearms or hands	No hair on forearms; Hair on metacarpals and digits are sparse and scattered. Bright orange color on all fingers and metacarpals
Feet	Few short golden hairs present (tibial length: 18.70; foot length: 09.58)	Short hair present on the hind feet	Feet small, equipped with relatively long, sharp claws, with a few short hairs on the toes	Feet small, densely-covered with short reddish-brown hairs and equipped with sharp small claws
Calcar	Long; covered with a dense fringe of short golden hairs (calcar length: 11.22)	Calcar relatively long; No hair on the calcar	Calcar long, extending approximately two-thirds of the distance from the ankle to the tail	Well developed with no lobes; covered with a dense fringe of short reddish hairs



**Figure 5.** Road killed specimen of *Phoniscus* cf. *jagorii* with key characters **a** wingspan with the dorsal body color **b** Interfemoral membrane is well developed and semi-transparent (parts of the interfemoral membrane was damaged) **c** Long, tapering, notched tragus **d** forearm and digits covered with short, golden hair **e** ventral aspect of the wing.

Buddhist monastery which encompassed extensive secondary forests with numerous caves. In the intermediate zone of Sri Lanka, Yapa (1991) found five bat species in a cave complex. However, compared to other tropical roosts, colony sizes we reported were remarkably low (100,000–500,000 cf. ~300) (Rubsamen et al. 2004; Yapa 1992). Our survey also confirmed presence of several rare and threatened bat species outside protected areas in Sri Lanka (*Hipposideros lankadiva*, *Kerivoula picta*, *Taphozous longimanus*, *T. melanopogon* and *Rhinolophus beddomei*). Thus, our study calls for science informed management and conservation of habitats around Maduru-Oya National Park.

Our discovery of *Phoniscus* cf. *jagorii* in our survey is noteworthy. The genus *Phoniscus* is currently known from four species, three of which are from Southeast Asia, New Guinea, and Eastern coast of Australia (Corbet and Hill 1992; Hutson et al. 2001; Wilson and Reeder 2005). This genus has not so far been recorded from Indian sub-continent or nearby south-Asian islands, thus our observation might be the first documentation of the genus *Phoniscus* for South Asia. *Phoniscus* species are forest dependent (found in both primary and secondary forests) and are found mostly in lowland rain-forests, dry dipterocarp forests, and semi-evergreen forests (Francis 2008b). The closest



**Figure 6.** NMDS Ordination of Bat association with different roosting sites in Maduru-Oya National Park periphery. The ellipses represent 95% confident intervals around the centroids. *Cynopterus sphinx* and *Phoniscus cf. jagorii*, which were only recorded as dead specimens, were not included in the ordination.

**Table 3.** Local (500 m radius around the roosting site) and landscape-scale (5 km radius around the roosting site) predictors of bat presence at potential roosting sites derived from a binomial generalized additive model (no large wetlands or aquatic land cover types were found within a 500 m radius).

Land-use variable	Coefficient estimate		z		p	
	500 m	5 km	500 m	5 km	500 m	5 km
Agricultural lands	0.52	-3.10	0.97	-1.30	0.33	0.19
Scrublands	-0.32	0.47	-0.96	0.49	0.34	0.62
Crop-mixed scrublands & woodlands	0.60	-0.84	2.10	-0.90	0.04*	0.37
Other vegetation mosaics	0.75	-0.71	1.54	-0.87	0.12	0.39
Forests (woody vegetation)	1.38	-0.67	2.81	-0.76	0.005 **	0.44
Wetlands and other open water bodies	n/a	0.13	n/a	0.22	n/a	0.82

species match to the specimen we found – *Phoniscus jagorii* (Peter’s Trumpet-eared Bat)– have been documented throughout Southeast Asia, including both the mainland and the Malayan archipelago: Bali, Borneo, Java, Laos, Lombok, Peninsular Malaysia, Samar, South China, South East Asia, Sulawesi, Thailand, and Vietnam (Beolens et al. 2009; Francis 2008b; Thong et al. 2006). *Phoniscus jagorii* is a low-flyer (Thong et al. 2006), thus could be susceptible to vehicular collisions, which supports our documentation. The damage sustained by the facial structure of our specimen precluded proper species identity. Given the geographical disjunction between Sri Lanka and *Phoniscus* biogeography, our specimen could represent a new species (Pipat Soisook, [Prince of Songkla University, Hat Yai, Songkhla, Thailand] and Chelmala Srinivasulu [Osma-

nia University, Hyderabad, India] pers. comm. [September 2017]). We recommend further sampling via mist-netting, bioacoustic surveys, active surveys in roosting sites, eDNA-surveys, and molecular phylogenetic analyses to confirm the taxonomic status of this specimen (Walker et al. 2016).

Nearly all the active roosting sites we documented were located within or in proximity to dense forests. These observations suggested that most bats of Maduru-Oya area are forest dependent. Our GAM for species presence also indicated forest dependency of these bats in terms of roost selection. Forest habitats provide a diverse array of high-caloric and nutrient-rich food resources for foraging bats. Our inferences about forest-dependency of bats are consistent with surveys conducted elsewhere in Sri Lanka and other humid tropical regions (Furey et al. 2010; Neuweiler et al. 1987; Phillips 1980). However, the forested habitats outside Maduru-Oya National Park are fragmented into isolated, smaller patches. Studies on Palaeotropical forests have shown that bats roosting in tree cavities and foliage can be more susceptible to habitat fragmentation due to loss of suitable roosting sites and negative consequences of edge effect (Struebig et al. 2008). Furthermore, most insectivore bats we recorded in our study are narrow-space foraging guilds that possess ecomorphological specializations (wing dimensions) and echolocation signals to forage in high-clutter forest interiors; such guilds can be affected by reduced availability of foraging areas as well as edge effect (Kingston 2010; Lane et al. 2006; Struebig et al. 2010).

Although we visited >50 potential roosting sites, only a quarter of those had bats at least once during our surveys. Also, a number of caves we visited had indirect evidence (guano) for bat presence. These observations may indicate lower site fidelity of bats at our study area where bats may shift between different roosting sites. Such high roost lability can be attributed to lower cost in commuting to foraging grounds, high familiarity with multiple roosts that vary in microclimatic conditions, greater availability of high-quality roosts; nest lability also ensures increased cross breeding potential, reduced predation risk, and lowered parasitic loads (Lewis 1995). Besides, bats change roost location based on their life-history stage (pregnancy vs. lactation vs. post-lactation), which is particularly common among female bats (Lausen and Barclay 2002). Alternatively, low-roost occupancy we documented (only 52% of the roosting sites surveyed had either direct or indirect evidence for bat occupancy) can be an artifact of high selectivity over roosting sites. Bats have high preference to roosting sites with multiple entry points, greater complexity of the roost interior, larger roosting area, broader interior temperature range, lack of anthropogenic disturbances both inside and outside the roosting site (Boyles 2007; Phelps et al. 2016). Moreover, the extent of modified land-cover types at both local and landscape-level around the roosting site can also influence the species composition of bats of a given roosting site.

Access to suitable roosting sites is a critical element for bats' life-history functions. Human-occupied landscapes usually contains forest preserves, mature woody vegetation, and buildings, therefore, may provide shelters for bat roosting compared to agricultural landscapes under intensive commercial farming which may lack a diverse array of roosting sites (Gehrt and Chelsvig 2003). Microclimatic stability and thermoregu-

latory advantages, protection from inclement weather and predators, nursing young, and grooming are some benefits conferred from roosting sites; social behaviors, such as determination of hierarchies, competition, cooperation, and recruiting females into “harems” are also critical ethological elements of roost selection (Campbell et al. 2006; Chaverri and Kunz 2010; Lewis 1995; Schmidt et al. 2011; Storz and Kunz 1999; Tan et al. 1997). Selection of optimal roost have profound fitness consequences for bats as roost conditions cater to their diversified functional attributes, physiological optima, life-history specifications, and social integrity (Campbell et al. 2006).

Although our survey covered potential roosts in agricultural habitats, none of those were occupied by bats, except for *Kerivoula picta* we documented from a banana plantation. Reduced overall bat activities (foraging and roosting) have been reported from agricultural landscapes in tropical Southeast Asia as well as the northern template of American Midwest (Gehrt and Chelsvig 2003). Lack of dense tree cover, monotypic vegetation, pesticide use, prey scarcity, intensive crop management activities, and limited access to water may have rendered agricultural habitats unsuitable for bats (Gehrt and Chelsvig 2003). Although we did not find active bat roosts in proximity to human settlements, our overall study area has substantial human occupancy and built-up environments, yet, provides suitable roosts for bats. Similarly, remarkably high bat activities have been documented in human-inhabited urban and suburban landscapes with sizable woodlots with suitable roosting sites (such as large mature trees, less-used buildings), reliable water sources, and suitable foraging grounds such as woodland edges and urban parks (Gaisler et al. 1998; Gehrt and Chelsvig 2003; Sparks et al. 2005).

Caves appeared to be the preferred roosting sites for most bats in our study area; similar habitat preferences have also been reported elsewhere in Sri Lanka (Yapa and Ratnasooriya 2006; Yapa et al. 2011; Yapa 1992) as well as throughout the Indo-Malayan realm, especially in the karst ecosystems of southeastern Asia (Furey et al. 2010). Caves serve as microclimatically-stable, predator-safe roosting habitats for both adults and juveniles (Chaverri and Kunz 2010; Kunz and Lumsden 2003; Yapa et al. 2011). Being endotherms, occupying thermally optimal environments yields bats with energetically-efficient metabolism. In dry tropical environments, bats use caves for aestivation and in the temperate zone for hibernation (Lewis 1995). In monsoon-dependent dry zone of Sri Lanka, water is a limited resource as most of the surface waters are ephemeral. However, some of the bat-occupied caves we surveyed provide year-round access to water making those caves suitable roosting sites for bats. Our observations on roost-sharing was limited to two instances (*Hipposideros lankadiva* and *Megaderma lyra* underneath a bridge and *Taphozous longimanus* and *Rhinolophus beddomei* in the same cave). In stark contrast, roost-sharing has been frequently observed throughout both the Old World and the New World (Bates and Harrison 1997b; Eckrich and Neuweiler 1988; Rubsamen et al. 2004). Greater availability of suitable roosting sites in Maduru-Oya area may have negated the need for sharing refugia.

Members of the family Pteropodidae (Old World Fruit bats) mostly roost on large, mature trees and deserted buildings as confirmed by our study (Chaverri and Kunz 2010). They also exhibit a wide variation in roosting sites including foliage of large-

leaved trees, dead or dry palm fronds, seed strings, in bark recesses, and aerial roots (Campbell et al. 2006; Digana et al. 2003; Kunz and Lumsden 2003; Storz and Kunz 1999; Tan et al. 1997) although our survey did not reveal comparable observations. Being predominately frugivorous, roosting on large fruiting trees may provide easy access to food for pteropodid bats. Dense canopy and large, well-grown branches of mature trees also provide stable roosting substrates and protection from predators. Moreover, roost selection of some Old World fruit bats is biased towards certain tree species and prefers riparian trees in undisturbed forests (Mildenstein et al. 2005). Although tent constriction have been observed among tree-roosting bats, we made no such observations in our survey (Chaverri and Kunz 2010; Digana et al. 2003). Roosting sites we surveyed were mostly used as day roosts indicating extensive nocturnal activity which also agrees with general activity patterns of tropical bats that have lengthy daily activities from dawn to dusk (Yapa et al. 2011).

Presence of bats in potential roosting sites was only significantly influenced by forest cover and crop-mixed natural vegetation cover (woodland and scrubland mosaics with multiple types of crops) whereas the former was the most impactful predictor. Both of those predictors were only significant at local (500 m radius around the roosting site) scale not at the landscape-scale. Importance of mixed vegetation mosaics, particularly those embedded with polyculture practices (comparable to analog forests and permaculture systems) as a local-scale land-cover predictor warrant further investigation. This may indicate use of such vegetation mosaics by open-space and edge foragers. In contrast to our findings, a multitude of other studies have underscored the importance of landscape-scale features for bat occupancy in potential roosting sites (Gaisler et al. 1998; Gehrt and Chelsvig 2003; Sparks et al. 2005). Our study area was predominantly covered by forests at landscape scale, thus, forest cover across a 5 km radius may have been less variable among different roosting sites.

### **Conservation challenges and recommendations**

Mahaweli Development scheme and subsequent expansion of human settlements, and agricultural intensification have resulted in tremendous habitat transformations in the landscape structure in Maduru-Oya area (Ekayanake 1987; Manatunge et al. 2008). Such socioeconomic schemes resulted in several novel anthropogenic disturbances, such as felling large trees for lumber, burning grasslands for livestock, reduced tree cover, lack of habitat connectivity, and agricultural expansion which are detrimental for local bat fauna (Furey et al. 2010; Gaikwad et al. 2012; Mickleburgh et al. 2002; Mildenstein et al. 2005). Moreover, slash-and-burn farming destroys lower vegetation cover and fire consumes snags, tree cavities, and cluttered foraging grounds (Hutson et al. 2001; Mickleburgh et al. 2002). In the recent decades, application of broadcast pesticides for vector control has substantially increased in Maduru-Oya area (Amerasinghe et al. 1991), which can impact the bats' prey base (Mickleburgh

et al. 2002; Weerakoon and Goonatilake 2006). Snag removal and demolition of alternative roosting sites (abandoned huts, barns, and mines) are also detrimental for bats (Hutson et al. 2001; Lane et al. 2006). Folklores combined with perceived fear of diseases may lead to vengeful killing in our study area (Dickman and Hazzah 2016; Klimpel and Mehlhorn 2016). Pteropodids are considered pests by fruit farmers, thus are targeted for extermination in our study area. Large bats have long been exploited as bush meat in Indian oceanic islands, which was consistent with our occasional observations at Maduru-Oya National Park periphery (Mickleburgh et al. 2009; Mickleburgh et al. 2002).

We recommend a landscape-scale approach for bat conservation of Maduru-Oya area, which entails conservation of roosting sites and associated habitats, particularly forest patches and aquatic habitats both within the national park and peripheral wilderness (Avila-Flores and Fenton 2005; Gehrt and Chelsvig 2003; Jaberg and Guisan 2001). Bat roosting sites also provide habitats for other species, such as snakes, geckos, rodents, toads, and invertebrates. Cave systems in our study area also have cultural, aesthetic, historical, paleontological, and geological importance, thus conservation of these caves also confers multitude of benefits (Kingston 2010; Mickleburgh et al. 2002). We propose adoption of wildlife-friendly land management practices in Maduru-Oya peripheral areas. Although much of our study area is considered the buffer zone of the park, neither management actions nor legislative enforcements are implemented there (Department of Wildlife Conservation 2004). We suggest zonation of land uses and limiting human activities in and around bat roosting sites. Introduction of organic farming and permaculture might minimize agrochemical use while increasing the habitat heterogeneity of the buffer zone. Agroforestry systems that include forest gardening, alley cropping, intercropped fruit and nut bearing trees for shade and fodder, forested riparian buffers, and tree-planted hedgerows as windbreaks will make agro-pastoral systems amicable for bats since such landscape elements are critical for bat activities (Harvey and Villalobos 2007; Hochegger 1998; Jacob and Alles 1987; Long and Nair 1999). Bats have high fidelity to landscapes with multiple suitable roosting sites (Avila-Flores and Fenton 2005; Lewis 1995). Thus, maintaining redundancy in suitable roosting sites provides insurance against loss of primary roosts (Mager and Nelson 2001).

Different species of bats we documented differed markedly in their natural histories. For instance, *Rhinolophus rouxii* forages in the foliage of dense forests (Phillips 1980; Rubsamen et al. 2004) while *Hipposideros speoris* is equipped to forage in dense scrublands, woodlands, river channels, and wetlands (Bates and Harrison 1997a; Pavey et al. 2001). Furthermore, critical resources required for bats are distributed throughout the landscape, and these resource demands vary seasonally, among variable life-history stages, and between sexes (Broders and Forbes 2004; Jaberg and Guisan 2001). Thus, conservation of Maduru-Oya bats should focus on managing a mosaic of interconnected habitats including forest patches, lake fringes, wetlands, river channels, and riparian buffers. Aquatic habitats provide profitable

food resources (high-density insect swarms); forest patches provide suitable roosting sites while forested landscapes are used as refuge against predators (Broders and Forbes 2004; Jaberger and Guisan 2001). Woodlots of different vegetation types, snags of variable decay classes, caves of different sizes, and isolated mature trees should be systematically protected.

Our study underscored the importance of conservation outside protected areas. Previous studies have also highlighted the importance of “trees outside the forests” for biodiversity conservation (Long and Nair 1999). Maduru-Oya National Park periphery is dominated by secondary vegetation types ranging from scrublands to seasonal mixed-deciduous evergreen forests, yet, supported a diverse assemblage of bats. Importance of such novel ecosystems for overall biodiversity conservation in Sri Lanka is not trivial (Pethiyagoda 2012; Pethiyagoda and Manamendra-Arachchi 2012). Since our study was focused synanthropic bat communities, we hope that our study provides a foundation for exploring conservation potential in anthropocentric environments.

Chiropterans are salient for multiple ecological functions, regulating invertebrate populations, serving as a prey-base, seed dispersal, and pollination, thus conservation of bats is imperative for healthy ecosystems (Digana et al. 2000; Eckrich and Neuweiler 1988; Jayasekara et al. 2003; Medellín et al. 2000; Neuweiler et al. 1987). Agriculture is the main livelihood of Maduru-Oya area where insectivorous bats can keep agricultural pests in check and effectively regulate medically important pests such as mosquitos. Given their critical ecosystem services, bats can be considered as both a keystone species (especially in cave and subterranean ecosystems) and an umbrella species that ensure conservation of whole landscapes (Mildenstein et al. 2005; Phelps et al. 2016). Our findings will also contribute towards developing species distribution maps, Red List assessments, conservation prioritizations, and influence local land management around Maduru-Oya National Park.

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