

Three new species of western California springsnails previously confused with *Pyrgulopsis stearnsiana* (Caenogastropoda, Hydrobiidae)

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Abstract

We describe three new, allopatric species of springsnails (genus *Pyrgulopsis*) from western California (*P. lindae*, *P. ojaiensis*, *P. torrida*) that were previously identified as *P. stearnsiana*. The new species are differentiated from *P. stearnsiana* and each other both by mtCOI sequences (3.9–9.9%) and details of penial morphology. We also provide a phylogeny with increased sampling which confirms a previous finding that *P. stearnsiana sensu stricto* is paraphyletic relative to two other California species (*P. diablensis*, *P. giulianii*). Our molecular and morphological evidence suggests that *P. stearnsiana* paraphyly is an artifact of conservative taxonomy, however additional studies utilizing rapidly evolving genetic markers will be needed to confidently tease apart the cryptic diversity in this widely ranging springsnail. The new species described herein are narrowly distributed and vulnerable to anthropogenic stressors. The single known population of *P. torrida* may have become extirpated between 2000 and 2015.

Keywords

Gastropoda, United States, freshwater, taxonomy, conservation

Introduction

Pyrgulopsis Call & Pilsbry, 1886 is a large genus (139 species; Hershler et al. 2014a) of hydrobiid gastropods (commonly known as springsnails) that is distributed in springs and other groundwater-dependent habitats throughout much of western North America from the Missouri River headwaters and Rio Grande Basin to the Pacific margin, and from the lower Columbia River to the Rio Nazas-Rio Aguanaval basin (Hershler et al. 2014b). Most of these tiny snails have very narrow geographic ranges consisting of a single spring, spring system or local watershed (Hershler et al. 2014b). Molecular studies have shown that several of the more widely ranging members of this genus are composites of divergent lineages. This is the third in a series of papers that revises the taxonomy of these species (Hershler et al. 2013, Hershler et al. 2014a).

Pyrgulopsis stearnsiana (Pilsbry, 1899) (= *Paludestrina stearnsiana* Pilsbry, 1899) was described for small (2.6 mm), narrowly umbilicate, ovate-conic shells from “near Oakland” (type locality), two additional localities in the San Francisco Bay area, and “Tuolumne County” (located along the western flank of the Sierra Nevada). Taylor (1981) subsequently expanded the range of *P. stearnsiana* to include a large portion of the central and southern California coast. Hershler (1994) provided a detailed description and illustrations of *P. stearnsiana* from Palo Seco Creek in Oakland and emended the diagnosis by adding details of penial morphology. A recent phylogenetic analysis resolved mtCOI sequences from six *P. stearnsiana* populations into four evolutionarily distinct, allopatric lineages (Hershler and Liu 2010). One of the lineages was composed of specimens from just north of Oakland (Wildcat Canyon, San Pablo Creek drainage) and two localities in central California coastal drainages; two regional congeners (*P. diablensis* Hershler, 1995; *P. giulianii* Hershler and Pratt, 1990) were also nested within this clade (also see Liu et al. 2003). The other lineages were single populations from the Sierra Nevada foothills, and near the southern edge of the species’ range. Here we detail previously unreported morphological differences among these lineages and describe three of them as new species based on our combined (molecular and morphological) evidence. We also provide a molecular phylogeny with additional sampling in the *P. stearnsiana* clade and discuss the taxonomic status of this group.

Methods

During 2014 and 2015 we sampled 18 additional *P. stearnsiana* populations in the San Francisco Bay area (including one in Oakland) and central and southern California coastal drainages (Fig. 1, Pst1-Pst18). Some of these are new localities while the others are previously known records documented in museum collections and/or an unpublished monograph on the genus *Fontelicella* Gregg & Taylor, 1965 (a junior synonym of *Pyrgulopsis*) by Wendell Gregg and Dwight Taylor. Specimens were collected by hand or with a small sieve and preserved in 90% (non-denatured) ethanol in the field for mtDNA analysis. Portions of the larger samples were anaesthetized with menthol

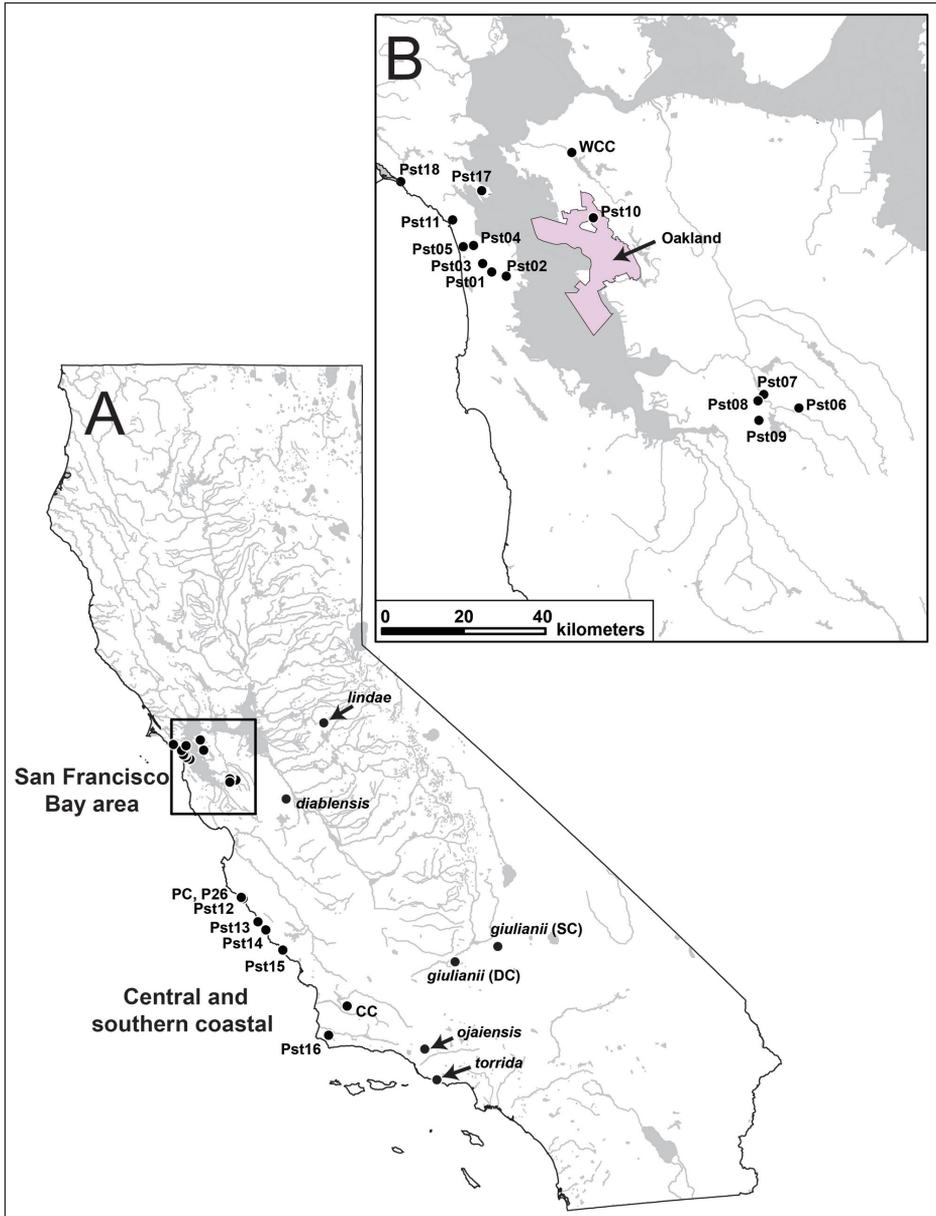


Figure 1. Map of California (A) and San Francisco Bay area (B, area occupied by rectangle in A) showing the collection localities for samples of *P. stearnsiana* (and closely related *P. diablensis* and *P. giulianii*) and the three new species (highlighted by arrows) that were used in the molecular analysis. Specimen codes are from Suppl. material 1.

crystals (for 13 hours), fixed in dilute formalin (10% of stock solution), and preserved in 70% ethanol for subsequent anatomical study. GPS coordinates were taken at each (snail-positive) site using a hand-held unit (Garmin Oregon 450t).

Genomic DNA was extracted from entire snails (3–6 specimens per sample) using a CTAB protocol (Bucklin 1992); each specimen was analyzed for mtDNA separately. LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify a 710 base pair (bp) fragment of cytochrome *c* oxidase subunit I (COI), and ND43F and RND592F (Liu et al. 2003) were used to amplify a 550 bp fragment of NADH dehydrogenase subunit I (NDI). Amplification conditions and sequencing of amplified polymerase chain reaction product followed Liu et al. (2003). Sequences were determined for both strands and then edited and aligned using SEQUENCHER™ version 5.0.1. The 76 newly sequenced specimens (69 COI and 72 NDI sequences) were analyzed together with previously published sequences of *P. stearnsiana* (Hershler et al. 2003, Liu et al. 2003, Hershler and Liu 2010) and 11 congeners from California and southwestern Nevada; the collecting localities for the *P. stearnsiana*, *P. diablensis* and *P. giulianii* samples are shown in Figure 1. The type species of the eastern North American genus *Marstonia* (a close relative of *Pyrgulopsis*; Hershler et al. 2003) was used to root the resulting trees. One example of each haplotype detected in a given sample was used in our analyses. Sample information for the sequences that were included in our analysis is detailed in Suppl. material 1.

We analyzed the COI and NDI datasets both separately and combined. MRMODELTEST 2.3 (Nylander 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for this analysis. Phylogenetic relationships were inferred by Bayesian analysis using MRBAYES 3.1.2 (Huelsenbeck and Ronquist 2001). Metropolis-coupled Markov chain Monte Carlo simulations were run with four chains (using the model selected through MRMODELTEST) for 5,000,000 generations, and Markov chains were sampled at intervals of 10 generations to obtain 500,000 sample points. We used the default settings for the priors on topologies and the GTR + I + G model parameters selected by MRMODELTEST as the best fit model. At the end of the analysis, the average standard deviation of split frequencies was less than 0.01 and the Potential Scale Reduction Factor (PSRF) was 1, indicating that the runs had reached convergence. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree with the first 25% of the samples removed to ensure that the chain sampled a stationary portion. Genetic distances (maximum composite likelihood) within and between species/lineages were calculated using MEGA6 (Tamura et al. 2013), with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of missing data.

The material collected during the course of this study was deposited in the National Museum of Natural History (USNM) collection. Asterisked lots are vouchers for the new mtDNA sequences reported herein. Other relevant material from the USNM, Academy of Natural Sciences of Philadelphia (ANSP), Bell Museum of Natural History (BellMNH), and Santa Barbara Museum of Natural History (SBMNH) was also examined during the course of this study. Specimens of *P. stearnsiana sensu stricto* that were examined during the course of this study are listed in Suppl. material 2. Large adults were used for shell measurements. The total number of shell whorls was counted (WH) for each specimen; and the height and width of the entire shell (SH, SW), body

whorl (HBW, WBW), and aperture (AH, AW) were measured from camera lucida outline drawings (Hershler 1989). Three ratios were generated from the raw data (SW/SH, HBW/SH, AH/SH). Descriptive statistics were generated using SYSTAT FOR WINDOWS 11.00.01 (SSI 2004). Other methods of morphological study were routine (Hershler 1994, Hershler 1998); descriptive penial terminology is from Taylor (1987) and Hershler (1994, 1998). Inasmuch as we have limited material for the new species, we have only provided brief taxonomic descriptions that are focused on diagnostic aspects of morphology.

Results

The Bayesian analysis of the COI dataset (Fig. 2) resolved specimens of *P. stearnsiana sensu lato* into four distinct, allopatric lineages. These four lineages were also delineated in the otherwise poorly resolved NDI and combined (COI + NDI) Bayesian trees (not shown). The sister relationships of the four lineages of *P. stearnsiana sensu lato* were not well supported. One of the lineages (referred to herein as the “*P. stearnsiana* clade”) contained the newly sequenced specimens and other snails conforming to *P. stearnsiana* as currently diagnosed—i.e., having a narrowly umbilicate and ovate-conic shell with medium convex whorls, and an elongate penial filament and very small penial lobe with a single gland along its distal edge (Hershler 1994). *Pyrgulopsis diablensis* and *P. giulianii* were nested in this marginally supported (93% posterior probability) clade as in the previous published analyses. The other three lineages (consisting of single populations) are substantially divergent genetically, differing from *P. stearnsiana* and each other by 3.9–9.9% for COI (Table 1); and are further differentiated by penial morphology (Fig. 3). (Note that we sequenced NDI for one of these lineages, described as *P. ojaiensis* below, which differed from *P. stearnsiana sensu stricto* by 6.0 +/- 1.0% for this marker; Table 2.) Our findings that these lineages are both genetically divergent and morphologically diagnosable suggests that they are distinct species, which we describe below.

Systematic descriptions

Family Hydrobiidae

Subfamily Nymphophilinae

Genus *Pyrgulopsis* Call & Pilsbry, 1886

The three new species are assignable to *Pyrgulopsis* based on morphology, e.g., presence of a single seminal receptacle, diffuse mantle pigmentation, superficial position of the bursa copulatrix and its duct on the albumen gland (Liu and Hershler 2005); and molecular phylogenetic evidence.

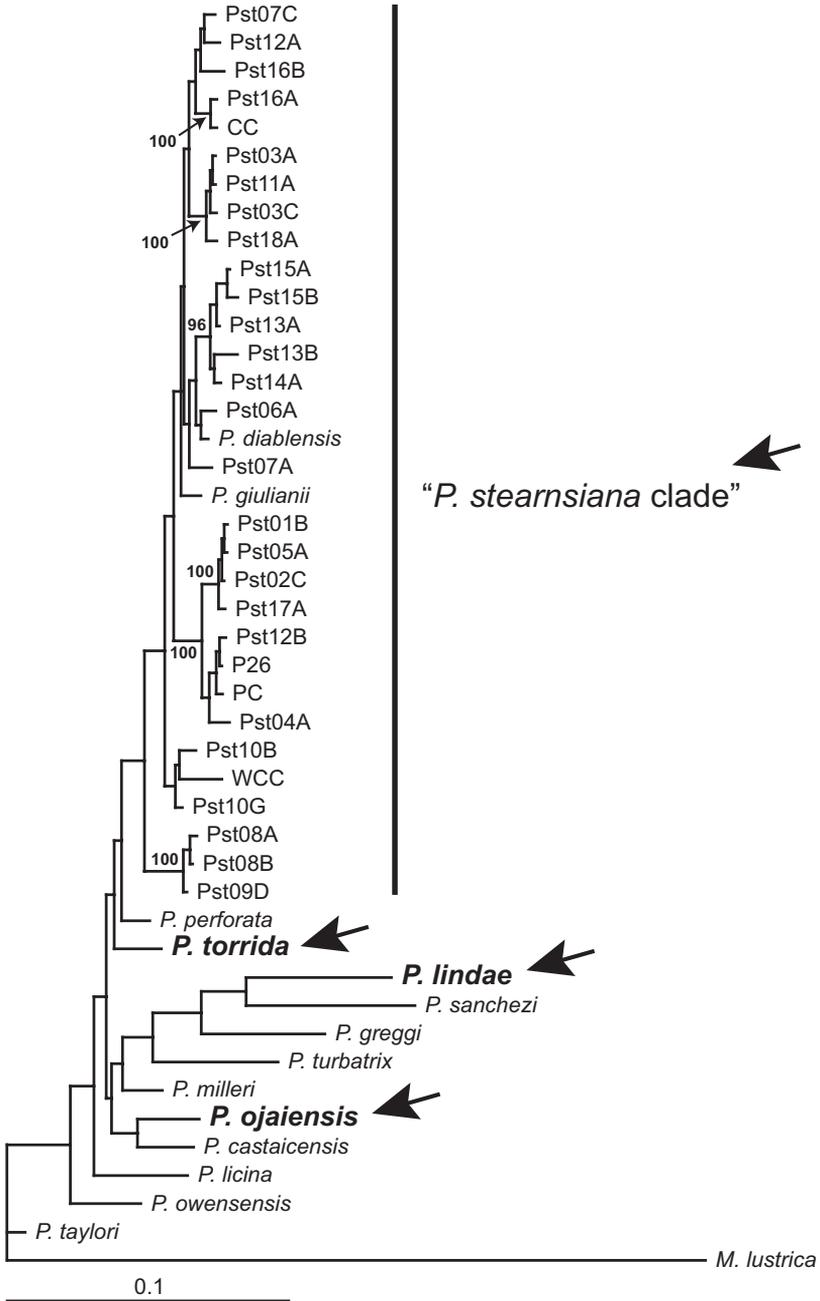


Figure 2. Bayesian tree based on the COI dataset. The four lineages of *P. stearnsiana sensu lato* are identified by arrows. Posterior probabilities for nodes are shown when >95%. Specimen codes are from Suppl. material 1.

Table 1. Per cent COI sequence divergence among *Pyrgulopsis* species included in the phylogenetic analyses. Values are mean +/- standard deviation.

	<i>stearnsiana</i>	<i>diablensis</i>	<i>giulianii</i>	<i>lindae</i>	<i>ojaiensis</i>	<i>torrida</i>
<i>stearnsiana</i>	2.0 +/- 0.3					
<i>diablensis</i>	1.5 +/- 0.3	-				
<i>giulianii</i>	1.9 +/- 0.4	1.1 +/- 0.4	-			
<i>lindae</i>	9.6 +/- 1.2	10.2 +/- 1.3	9.7 +/- 1.3	-		
<i>ojaiensis</i>	5.4 +/- 0.8	4.8 +/- 0.9	5.6 +/- 0.9	9.9 +/- 1.2	-	
<i>torrida</i>	3.9 +/- 0.7	3.5 +/- 0.8	3.5 +/- 0.8	9.4 +/- 1.2	4.8 +/- 0.9	-
other species	3.5–9.4	2.6–9.1	3.1–9.4	9.0–10.8	3.8–11.7	2.8–9.2

Table 2. Per cent NDI sequence divergence among *Pyrgulopsis* species included in the molecular phylogenetic analyses. Data are not available for *P. lindae* and *P. torrida*. Values are mean +/- standard deviation.

	<i>stearnsiana</i>	<i>diablensis</i>	<i>giulianii</i>	<i>ojaiensis</i>
<i>stearnsiana</i>	2.4 +/- 0.4			
<i>diablensis</i>	2.0 +/- 0.4	-		
<i>giulianii</i>	2.3 +/- 0.4	1.5 +/- 0.5	0.8 +/- 0.4	
<i>ojaiensis</i>	6.0 +/- 1.0	6.0 +/- 1.1	6.0 +/- 1.1	-
other species	5.2–10.4	5.1–10.5	4.6–10.5	5.4–11.3

***Pyrgulopsis lindae* Hershler, Liu, Babbitt, Kellogg & Howard, sp. n.**

<http://zoobank.org/2C71096A-39EE-4808-AAB9-EEE6D6787D92>

Figs 3A, 4

Pyrgulopsis stearnsiana.—Hershler and Liu 2010 (in part).

Types. Holotype, USNM 905258, San Domingo Creek, 3.8 km up flow from Dogtown along San Domingo Road, Calaveras County, California, 38.14122°N, 120.53920°W, 6/26/2000, R. Hershler. Paratypes, *USNM 1254709 (one dry shell and six alcohol-preserved specimens), from same lot.

Referred material. California. *Calaveras County*: ANSP 158719, Santo Domingo (probably San Domingo) Creek Valley, N (north) of Murphys, no coordinates available, 9/11/1929. *Tuolumne County*: BellMNH 20821, Salvada Gulch (37.87062°N, 120.41987°W), 11/9/1966.

Diagnosis. A medium-sized congener (maximum shell height, 3.3 mm) having an ovate-conic shell. Distinguished from other regional species in having a penial gland along the outer edge of the filament. Further differentiated from *P. stearnsiana* in having a ventral gland on the penis, and a larger penial lobe and terminal gland.

Description. Shell (Fig. 4A–B, Table 3) ovate-conic, spire slightly longer than shell width in largest specimens, whorls 4.00–4.75. Teleoconch whorls medium convex, sometimes weakly shouldered. Aperture ovate, slightly angled above; parietal lip complete, nearly straight, narrowly disjunct, thin or slightly thickened; umbilicus ab-

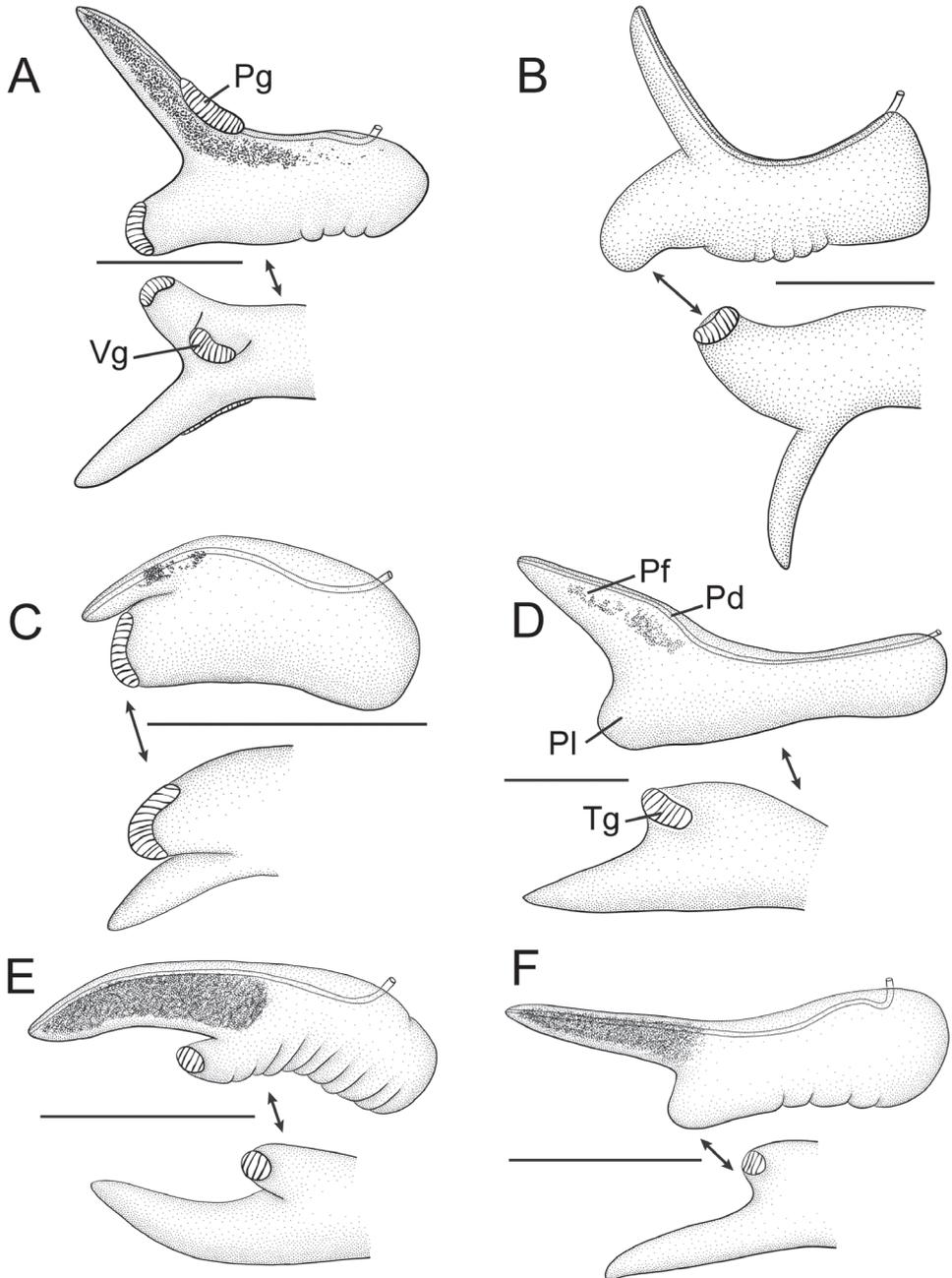


Figure 3. Penes (dorsal, ventral surfaces). **A** *P. lindae* sp. n., USNM 1257409 **B** *P. ojaiensis* sp. n., SBMNH 460496 **C** *P. torrida* sp. n. USNM 1120443 **D, E, F** *P. stearnsiana* USNM 1297168, USNM 1252041, USNM 905251, respectively. Scale bars: 250 μ m. **Pd** penial duct **Pf** penial filament **Pg** penial gland **Pl** penial lobe **Tg** terminal gland **Vg** ventral gland.

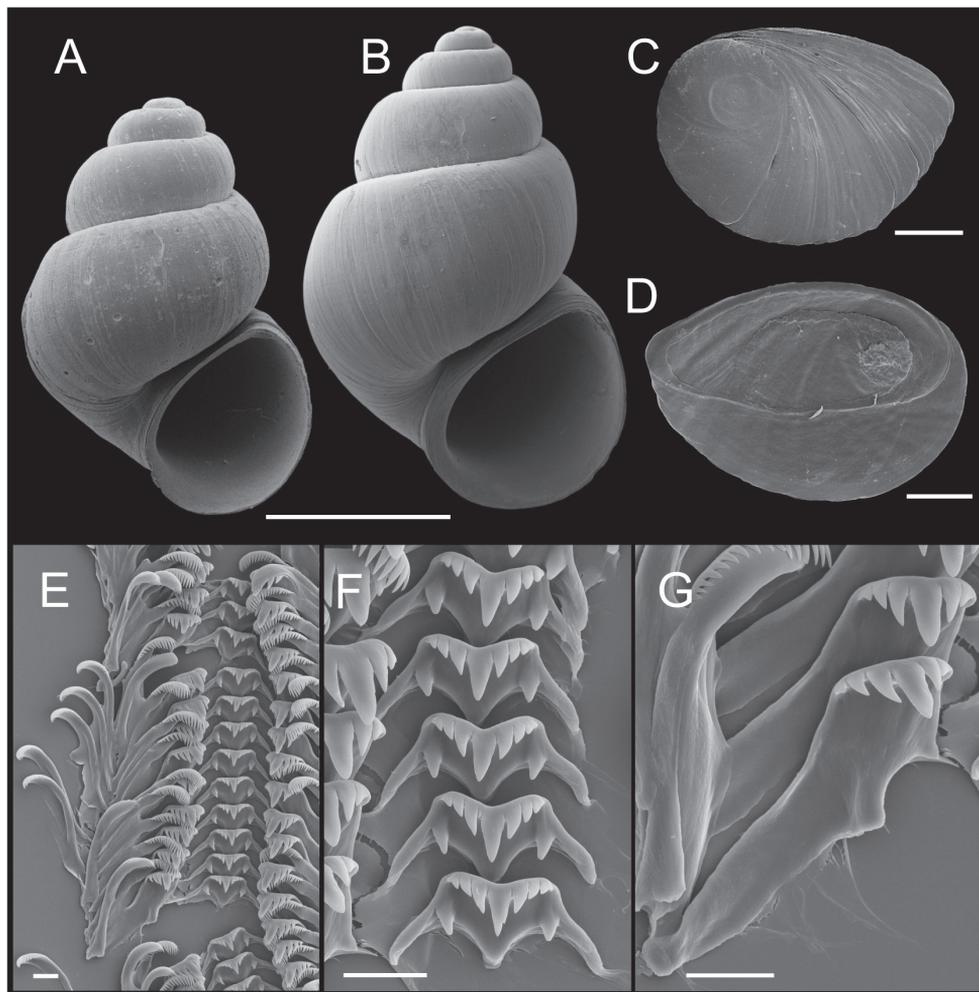


Figure 4. Shells, opercula and radula, *P. lindae* sp. n. **A** Holotype, USNM 905250 **B** Shell, BellMNH 20821 **C, D** Opercula (outer, inner sides), BellMNH 20821 **E** Portion of radular ribbon, BellMNH 20821 **F** Central teeth, BellMNH 20821 **G** Lateral teeth, BellMNH 20821. Scale bars: **A–B** = 1.0 mm; **C–D** = 200 μ m; **E–G** = 10 μ m.

sent or very small. Outer lip thin, orthocone. Teleoconch whorls sculptured with numerous irregular spiral striae.

Operculum (Fig. 4C–D) as for genus; muscle attachment margin thickened on inner side. Radula (Fig. 4E–G) as for genus; dorsal edge of central teeth concave, lateral cusps three–four, basal cusp one. Lateral teeth having two cusps on inner and three cusps on outer side. Inner marginal teeth with 15–20 cusps, outer marginal teeth with 22–28 cusps. Radula data are from BellMNH 20821.

Penis (Fig. 3A) medium-sized; filament darkly pigmented, medium length, narrow, tapering; lobe medium-sized, rectangular, slightly oblique; penial gland narrow,

Table 3. Shell parameters for *P. lindae*. Measurements are in mm.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
Holotype, USNM 905258										
	4.25	2.46	1.61	1.79	1.43	1.06	0.94	0.65	0.73	0.43
BellMNH 20821 (n=17)										
Mean	4.56	2.83	1.83	2.08	1.57	1.21	1.10	0.65	0.74	0.43
S.D.	0.17	0.21	0.09	0.13	0.09	0.07	0.07	0.04	0.03	0.02
Range	4.25–	2.65–	1.63–	1.87–	1.40–	1.09–	1.01–	0.59–	0.69–0.77	0.39–
	4.75	3.33	1.95	2.34	1.76	1.31	1.18	0.68		

positioned along outer edge of filament basally; terminal gland narrow, curved, overlapping both dorsal and ventral sides of lobe; ventral gland small, narrow, curved, borne on short stalk near base of lobe. Penial data are from USNM 905259 (5 specimens), BellMNH 20821 (3 specimens).

Etymology. This species is named for Linda Lee Crisostomo who provided invaluable field assistance and logistical support for this project. We propose that “San Domingo pyrg” be used as the common name for this species.

Distribution and habitat. *Pyrgulopsis lindae* is known from three geographically proximate localities in the upper Calaveras and upper Tuolumne River basins. The type locality is a moderate-size stream of about one meter depth; specimens were found on emergent macrophytes near the banks. The second locality in San Domingo Valley is an old record (1929) based on dry shells. The place name for the third locality, “Salvada Gulch,” is no longer in use, but is shown on older maps (e.g., USGS Chinese Camp 15-minute quadrangle [1948]) as being located just to the east of Chinese Camp near the western edge of Don Pedro Reservoir. The geographic coordinates given on the original labels for the Salvada Gulch sample (BellMNH 2081) suggest that the collecting locality was the small stream just to the south of Shawmut Road.

Conservation status. *Pyrgulopsis lindae* was found only rarely in San Domingo Creek in 2000; when re-visited in 2015 the creek consisted of a few pools separated by long, dry reaches; we were unable to sample these habitats as they were on fenced (private) land. The Salvada Gulch population has not been surveyed since it was first collected in 1966.

***Pyrgulopsis ojaiensis* Hershler, Liu, Babbitt, Kellogg & Howard, sp. n.**

<http://zoobank.org/91F33C91-EEFB-4517-9255-740113055AF0>

Figs 3B, 5

Pyrgulopsis stearnsiana.—Hershler and Liu 2010 (in part).

Types. Holotype, SBMNH 74347, Sisar Creek, Santa Paula Canyon, 3.4 km up flow from Sulphur Springs, Ventura County, California, 34.43213°N, 119.12414°W, 1/7/1962, W. B. Miller. Paratypes, SBMNH 460496 (19 dry shells and ca. 100 alcohol preserved specimens), from same lot.

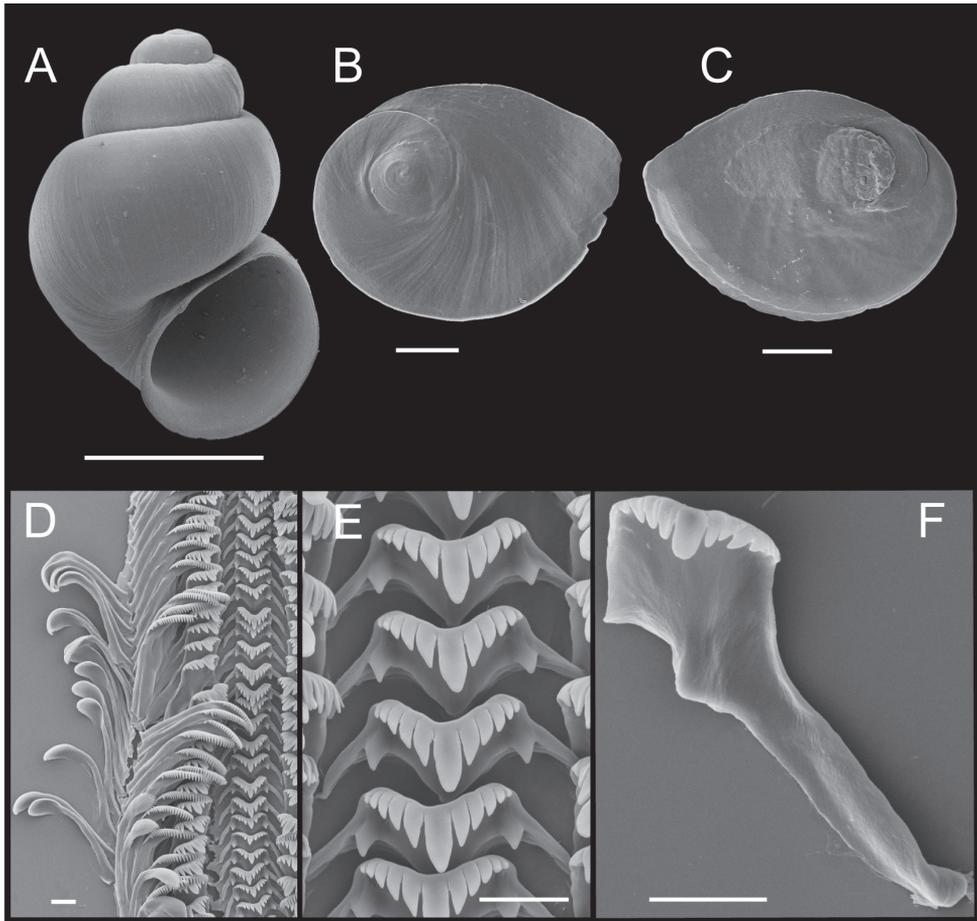


Figure 5. Shells, opercula and radula, *P. ojaiensis* sp. n. **A** Holotype, SBMNH 74347 **B, C** Opercula (outer, inner sides), SBMNH 460496 **D** Portion of radular ribbon, SBMNH 460496 **E** Central teeth, SBMNH 460496 **F** Lateral teeth, SBMNH 460496. Scale bars: **A** = 1.0 mm; **B–C** = 200 μ m; **D–F** = 10 μ m.

Referred material. California. *Ventura County*: *USNM 905259, USNM 1287762, *ibid.*, 6/23/2000, 6/26/2015.

Diagnosis. A medium-sized congener (maximum shell height, 3.1 mm) having an ovate-conic shell. Distinguished from closely similar *P. stearnsiana* and *P. torrida* (described below) in having an oblique penial filament and larger penial lobe. Further differs from *P. torrida* in having a longer penial filament and smaller terminal gland.

Description. Shell (Fig. 5A, Table 4) ovate-conic, whorls 4.00–4.25. Teleoconch whorls medium convex, narrowly shouldered. Aperture ovate, slightly angled above; parietal lip complete, nearly straight, narrowly disjunct, last 0.25 whorl sometimes separated, thin or slightly thickened; umbilicus small. Outer lip thin, weakly prosocline or orthocline. Teleoconch smooth aside from collabral growth lines.

Table 4. Shell parameters for *P. ojaiensis*. Measurements are in mm.

WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
Holotype, SBMNH 70437									
4.00	2.51	1.72	1.95	1.50	1.17	1.07	0.69	0.78	0.47

Operculum (Fig. 5B–C) as for genus; inner side nearly smooth. Radula (Fig. 5D–F) as for genus; dorsal edge of central teeth concave, lateral cusps four–seven, basal cusp one. Lateral teeth having three–four cusps on inner and four–five cusps on outer side. Inner marginal teeth with 20–27 cusps, outer marginal teeth with 25–37 cusps. Radula data are from SBMNH 7437.

Penis (Fig. 3B) medium-sized (pigmentation unknown), filament medium length, narrow, oblique, tapering; lobe medium-sized, rectangular, oblique; terminal gland small, narrow, positioned along ventral edge of lobe. Penial data are from SBMNH 7437 (6 specimens).

Etymology. The species name is a geographical epithet referring to Ojai Valley, the upper portion of which is drained by Sisar Creek. We propose “Sisar pyrg” as the common name for this species.

Distribution and habitat. Endemic to the type locality; a small, spring-fed stream. Snails were found on small stones and pieces of wood.

Conservation status. *Pyrgulopsis ojaiensis* was found in moderate abundance in Sisar Creek both in 2000 and 2015. This creek runs alongside a frequently used road (between Ojai and Santa Paula) in a populated area and has been considerably impacted by anthropogenic activities.

***Pyrgulopsis torrida* Hershler, Liu, Babbitt, Kellogg & Howard, sp. n.**

<http://zoobank.org/2FBB4B8B-32C2-4308-AB78-C8454A1B8ED1>

Figs 3C, 6

Pyrgulopsis stearnsiana.—Hershler and Liu 2010 (in part).

Types. Holotype, SBMNH 74238, Little Sycamore Canyon, creek 3.2 km up flow from Hwy 1, Ventura County, California, 34.07509°N, 118.95508°W, 11/11/1961, W. B. Miller. Paratypes, SBMNH 460492 (ca. 200 dried shells), from same lot.

Referred material. California. *Ventura County*: SBMNH 74236, *USNM 1120443, *ibid*, 9/9/1956, 10/21/2008, respectively.

Diagnosis. A medium-sized congener (maximum shell height, 2.8 mm) having an ovate-conic shell. Distinguished from *P. stearnsiana* by its shorter penial filament and larger terminal gland.

Description. Shell (Fig. 6A–B, Table 5) ovate-conic, whorls 4.00. Teleoconch whorls medium convex, narrowly shouldered. Aperture ovate, slightly angled above; parietal lip complete, nearly straight, narrowly adnate adapically or slightly disjunct,

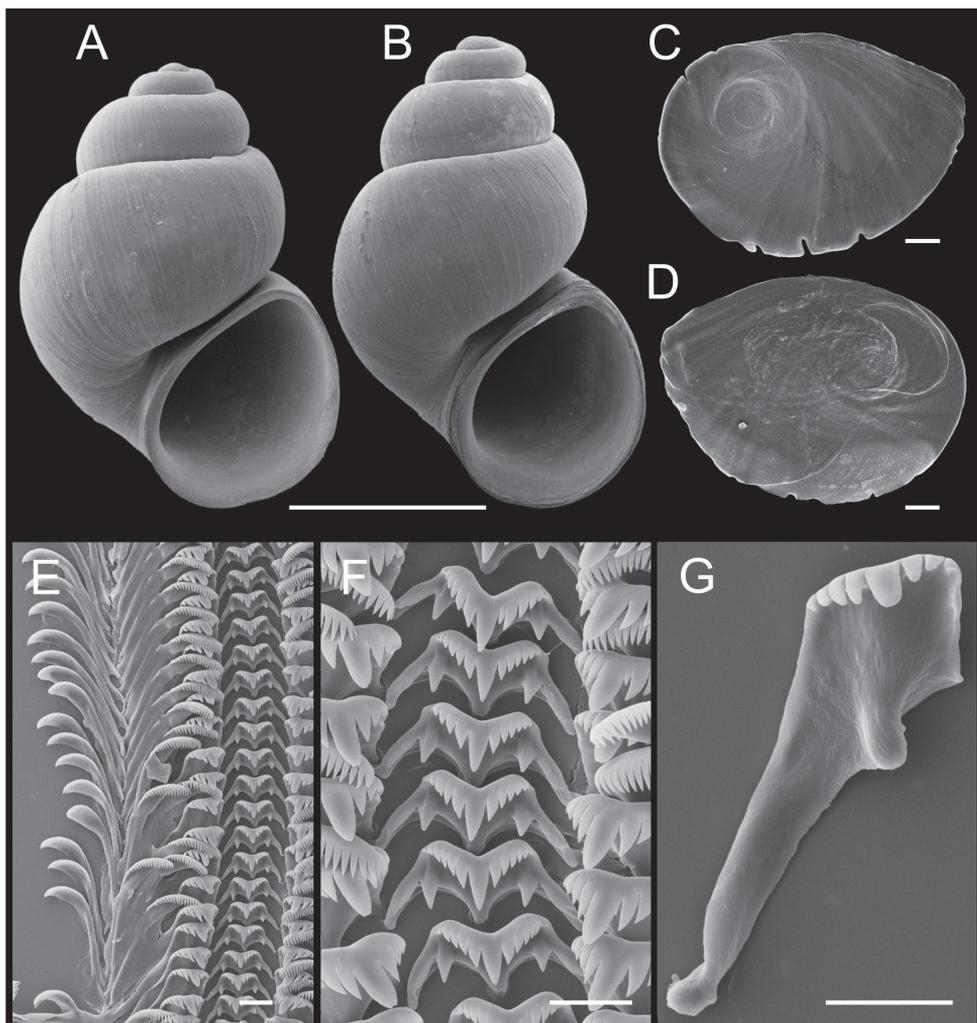


Figure 6. Shells, opercula and radula, *P. torrida* sp. n. **A** Holotype, SBMNH 74238 **B** Shell, SBMNH 460492 **C, D** Opercula (outer, inner sides), SBMNH 460492 **E** Portion of radular ribbon, SBMNH 460492 **F** Central teeth, SBMNH 460492 **G** Lateral teeth, SBMNH 460492. Scale bars: **A–B**=1.0 mm; **C–D**=100 μ m; **E–G**=10 μ m.

thin or slightly thickened; umbilicus small. Outer lip thin, orthocone. Teleoconch smooth or sculptured with weak spiral striae.

Operculum (Fig. 6C–D) as for genus; portion of attachment scar margin slightly thickened on inner side. Radula (Fig. 6E–G) as for genus; dorsal edge of central teeth concave, lateral cusps three–six, basal cusps one to (rarely) two. Lateral teeth having two–four cusps on inner and three–five cusps on outer side. Inner marginal teeth with 19–24 cusps, outer marginal teeth with 21–27 cusps. Radula data are from SBMNH 460492.

Table 5. Shell parameters for *P. torrida*. Measurements are in mm.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
Holotype, SBMNH 74238										
	4.00	2.511	1.72	1.96	1.45	1.21	1.04	0.68	0.79	0.48
SBMNH 460492 (n=17)										
Mean	3.94	2.53	1.71	1.98	1.44	1.19	1.06	0.68	0.78	0.47
S.D.	0.17	0.10	0.10	0.07	0.07	0.06	0.05	0.04	0.03	0.03
Range	3.75– 4.25	2.33– 2.69	1.56– 1.92	1.87– 2.13	1.34– 1.59	1.07– 1.29	0.97– 1.16	0.61– 0.73	0.73– 0.82	0.42– 0.51

Penis (Fig. 3C) small, filament weakly pigmented or pale, filament short, narrow, horizontal, weakly tapering; lobe small, rectangular, horizontal; terminal gland fairly large, narrow, overlapping dorsal and ventral edges of lobe. Penial data are from USNM 1120443 (2 specimens).

Etymology. The species name is an adjective derived from the New Latin *torridus*, meaning dry or parched, and refers to the recent desiccation of the stream in Little Sycamore Canyon. We propose “Little Sycamore pyrg” as the common name for this species.

Distribution. Endemic to the type locality, a small, shallow stream which runs for about 1.6 km. Snails were collected from the mud bottoms of a series of small puddle-like pools along the middle section of the stream.

Conservation status. *Pyrgulopsis torrida* was found only rarely in the Little Sycamore Canyon creek in 2000. The entirely length of the canyon was dry when re-visited in 2015, suggesting that this population may now be extirpated.

Discussion

Taxonomic status of *Pyrgulopsis stearnsiana*

As is often the case with animal species (Funk and Omland 2003; Ross 2014), *P. stearnsiana*, as newly circumscribed herein, was resolved as a paraphyletic assemblage based on mtDNA evidence (Fig. 2; also see Liu et al. 2003, figs 2–3; Hershler and Liu 2010, fig. 2). The two congeners that are nested within the *P. stearnsiana* clade—*P. diablensis* and *P. giulianii*—are somewhat distinct genetically (differing from *P. stearnsiana sensu stricto* by 1.5% and 1.9% for COI and 2.0% and 2.3% for NDI; Tables 1–2) and are further differentiated by their penes: *P. diablensis* has a small penial gland (Hershler 1995, fig. 3B; observed in 29/30 specimens from USNM 883791), and *P. giulianii* has both a ventral gland (30/30 specimens, USNM 874141) and a gland on the dorsal penis proximal to the base of the filament (28/30 specimens, USNM 874141) (Hershler and Pratt 1990, fig. 3). The distinction between *P. stearnsiana* and *P. diablensis* is somewhat blurred as a small penial gland (diagnostic of the latter) was detected at a very low frequency in the

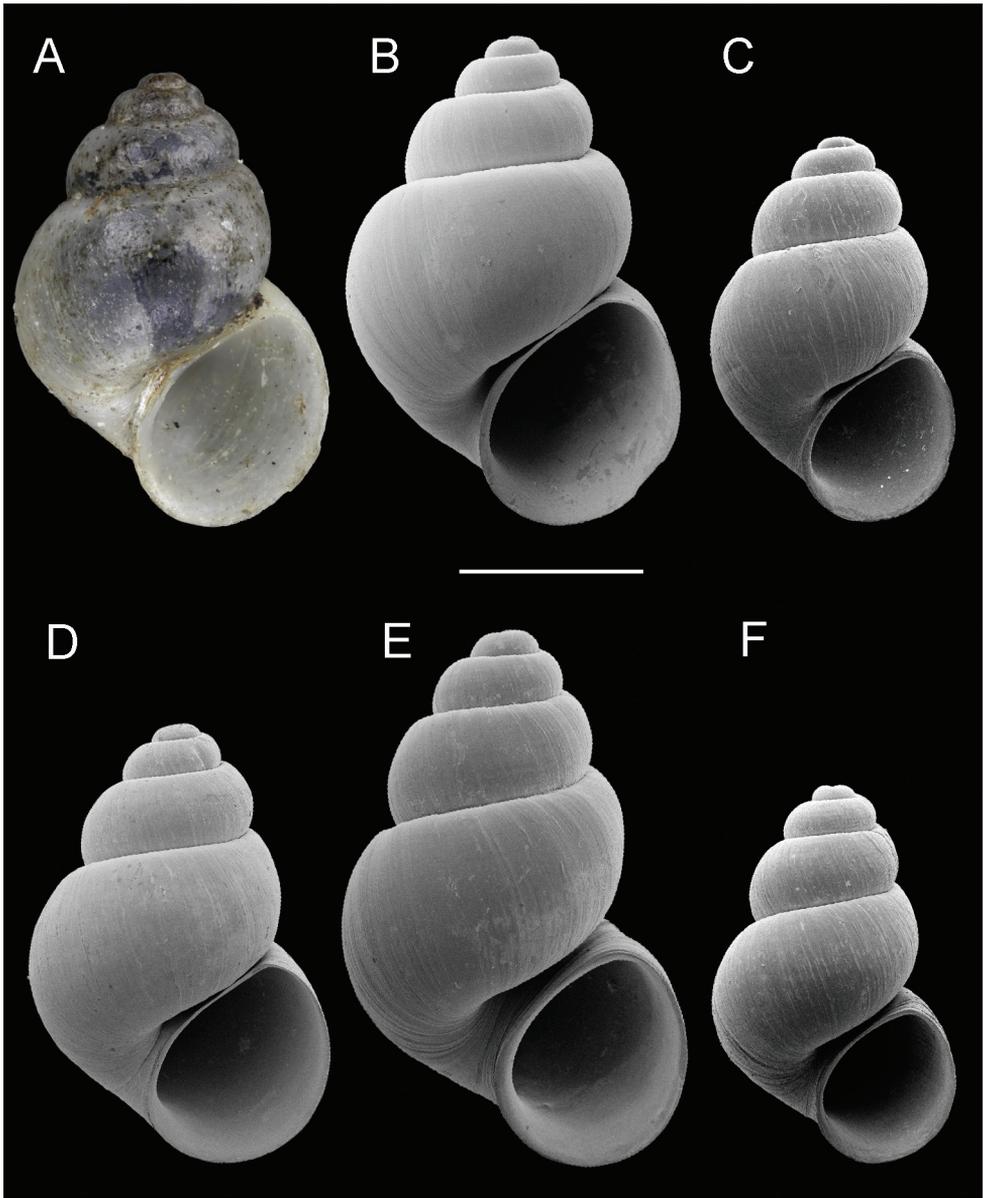


Figure 7. Shells, *P. stearnsiana*. **A** Lectotype, ANSP 27961 **B** USNM 1291731 **C** USNM 894756 **D** USNM 1287760 **E** USNM 1287759 **F** USNM 1252041. Scale bar: 1.0 mm.

P. stearnsiana material that we examined during the course of this study (12/156 specimens from seven populations; BellMNH 20811, BellMNH 20814, BellMNH 20932, USNM 874181, USNM 905251, USNM 1152039, USNM 1252041). Nonetheless, we do not see a compelling basis for treating the entire *stearnsiana* clade as a single species (and thus “avoiding” paraphyly), especially given the very clear morphological distinction

between *P. stearnsiana* and *P. giulianii*. Our molecular data suggest that a more appropriate action may be to further split *P. stearnsiana* taxonomically. The sequence divergence within *P. stearnsiana* was fairly large—2.0 +/- 0.3% for COI (Table 1) and 2.4 +/- 0.4% for NDI (Table 2)—and five small subclades of these snails were well supported. Furthermore, *P. stearnsiana* is morphologically variable, especially in shell size and shape (Fig. 7A–F), and shape of the penial filament and terminal gland (Fig. 3D–F). However, we do not have a sufficiently robust dataset at this time to confidently tease apart this cryptic diversity, although we anticipate being able to do so when data from a rapidly evolving molecular marker (such as microsatellites) become available. Thus, for the time being, we recognize only three species in the *P. stearnsiana* clade (*P. diablensis*, *P. giulianii*, *P. stearnsiana*) while acknowledging that the paraphyly of *P. stearnsiana* is probably an artifact of incompletely resolved taxonomy.

Conservation considerations

Springsnails are a current focus of conservation attention in many parts of the West owing to the threats posed by groundwater pumping, surface water diversions, and other anthropogenic activities (Hershler et al. 2014b). All three of the new species described herein are narrowly distributed (note that the precise limits of these geographic ranges are uncertain) and for this reason alone should be placed on conservation watch lists. Although the three species were extant in 2000, only one of them (*P. ojaiensis*) was found in 2015. The habitats of *P. lindae* and *P. torrida* were severely impacted by the recent (2012–2015), extreme California drought (Robeson 2015) and it appears likely that the single known population of the latter species has been extirpated. Field surveys are needed to determine (1) whether *P. torrida* may have re-populated the creek in Little Sycamore Canyon and whether there are other, previously unknown populations in the southern California coastal drainage; and (2) to similarly assess whether *P. lindae* is extant in San Domingo Creek and Salvada Gulch. The remaining habitats of the three species may require protective measures to ensure their persistence; we note in this context that all of the known localities for these snails are on private land.

During the course of our fieldwork we also found that quite a few populations of *P. stearnsiana* have recently (post-1960) become extirpated, including, for example, those in Palo Seco Creek, San Leandro Creek, and Russellman Park Spring in the San Francisco Bay area. In most of these cases the previously inhabited spring or stream is now dry. Most of the extant populations of *P. stearnsiana* live in small water bodies (springs or streams) that have been variously impacted by anthropogenic activities (e.g., flow diversions, recreational use, livestock grazing). *Pyrgulopsis stearnsiana* is currently ranked as imperiled (G2) by NatureServe (2015), threatened by the American Fisheries Society (Johnson et al. 2013), and as a species of Least Concern by the IUCN (Cordeiro and Perez 2011). We recommend that the conservation rankings of *P. stearnsiana* be updated to reflect the recent spate of population extirpations and the highly modified condition of the remaining habitats of this snail.

Acknowledgments

Linda Lee Crisostomo, Pat Jones, David Lindberg, Thomas Melia and Tim Sullivan assisted with fieldwork. Jim Avant, Rick Duffy and Don Lampe kindly identified springs and facilitated access to San Francisco Public Utilities Commission watershed lands. Jeremy Lukins (SFPUC) shared pertinent GIS shapefiles, and Maggie Ditmore and Chris Higgins obtained useful information on the Salvada Gulch locality. Yolanda Villacampa prepared the scanning electron micrographs, Karolyn Darrow photographed the lectotype of *P. stearnsiana*, and Freya Goetz prepared the anatomical drawings and figures. This project was supported, in part, by awards (to RH) from the San Francisco Public Utilities Commission (Contract CS-108), and The Nature Conservancy (Contract 06132013-1651). We thank reviewers Art Bogan, Gonzalo Collado, and Alejandro Varela-Romero for their helpful comments on the submitted manuscript.

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Supplementary material 1

Specimen codes, number of sequenced specimens, locality details, and GenBank accession numbers

Authors: Robert Hershler, Hsiu-Ping Liu, Caitlin Babbitt, Michael G. Kellogg, Jeanette K. Howard

Data type: species data

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Supplementary material 2

Specimens of *Pyrgulopsis stearnsiana* sensu stricto that were examined as part of this study

Authors: Robert Hershler, Hsiu-Ping Liu, Caitlin Babbitt, Michael G. Kellogg, Jeanette K. Howard

Data type: specimens data

Explanation note: All material is from California.

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Cheiracanthium ilicis sp. n. (Araneae, Eutichuridae), a novel spider species associated with Holm Oaks (*Quercus ilex*)

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Abstract

We describe a novel species *Cheiracanthium ilicis* sp. n. (Araneae, Eutichuridae) collected in the province of Toledo (Central Spain). It was found during a systematic sampling campaign carried out in an agricultural landscape with isolated Holm oaks *Quercus ilex* and small forest patches. Its morphology and affinities with other species of the genus are discussed. Furthermore, one mitochondrial gene was sequenced to confirm species membership and its differentiation from other *Cheiracanthium* species. The molecular phylogenies based on mitochondrial and nuclear genes showed a close relationship of *C. ilicis* sp. n. with *C. inclusum* and *C. mildei*, with which it also shares morphological similarities. Nonetheless, the sparse sampling of the phylogeny, due to the low number of sequences available, impedes drawing any definitive conclusion about these relationships; it is first necessary to perform an extensive review of the genus worldwide and more thorough phylogenies. *C. ilicis* sp. n. also shares certain ecological and phenological characteristics with *C. inclusum* and *C. mildei*. Like them, *C. ilicis* sp. n. is an obligate tree dweller that prefers a tree canopy habitat and reproduces primarily in late spring and summer. From a conservation perspective, the present study suggests the need to preserve isolated trees in agricultural landscapes. They are not only the refuge of common forest organisms but also of novel species yet to be discovered.

Keywords

Cheiracanthium ilicis sp. n., DNA taxonomy, Iberian Peninsula, isolated trees, molecular phylogeny

Introduction

Cheiracanthium C. L. Koch, 1939 is the only genus of the family Eutichuridae Lehtinen, 1967 in Europe. This genus was transferred from the family Clubionidae Wagner, 1887 to Miturgidae Simon, 1886 (Ramírez et al. 1997) and, more recently, to the family Eutichuridae Lehtinen, 1967 (Ramírez 2014). The debate on the taxonomical status of this genus of spiders still remains open, since Wunderlich (2012) revalidated the genus *Chiracanthops* Mello-Leitão, 1942, which would include some of the species currently ascribed to *Cheiracanthium*. The spiders of this latter genus would be characterized by certain structures of the external sexual organs: male pedipalp apophysis and female copulatory ducts (Wunderlich, 2012).

Cheiracanthium has worldwide distribution and is only absent from the polar regions. Of the 209 known species of this genus in the world (World Spider Catalog 2016), 29 have been found in Europe, 14 of which on the Iberian Peninsula (Morano et al. 2014). With regard to the Iberian Peninsula, Urones (1987) provided the first extensive report on this genus including data on taxonomy, biology, habitat and geographical distribution of the 12 species cited up to that time. Later, Piñol et al. (2010) and Wunderlich (2012) increased this list by adding two new species observed in the Ibero-Balearic region.

Spiders of this genus are swift hunters on woody or herbaceous plants, and their dense claw tufts help them to crawl along inclined surfaces. Their body colours usually range from yellow to greenish, with orange and brownish tones in some species. *Cheiracanthium* spiders belong to the group known as “sac spiders” because they spin small silk bags which shelter these nocturnal hunters during the day. These bags are quite conspicuous, as often the spiders build them on top of tall grass shoots and so they are easily seen in wet meadows, on crops and besides paths.

In this article we describe a novel species of *Cheiracanthium* found during a sampling campaign carried out in Central Spain. Different habitats were periodically sampled in an agricultural landscape with isolated oaks and forest fragments interspersed between crop fields and grasslands. In addition to its morphological description we sequenced two genes (mitochondrial and nuclear) to build a molecular phylogeny and assess its status with respect to those species of the genus for which molecular data were available in GenBank. Finally, we provide data on habitat selection and phenology recorded during a one-year long systematic sampling.

Material and methods

Study area

We carried out the spider sampling in the village of Huecas, in the province of Toledo, Central Spain (40.02°N, 4.22°W; altitude 581 m.a.s.l.). The climate is dry Mediterranean, with hot summers in which temperatures may reach 40°C and scarce precipi-



Picture 1. Isolated Holm oak *Quercus ilex* in the study area.

tation (365 l/m^2) concentrated in spring and autumn. The study area extends over 9 km^2 of flat agricultural landscape with isolated Holm oaks *Quercus ilex* and forest plots interspersed within a matrix of grasslands and cereal fields (Picture 1; see Bonal et al. 2012 for a detailed description). Tree density in the forest plots ranges from 20 to 50 trees per ha, whereas the distance between isolated oaks ranges from 40 metres to more than two kilometres.

Sampling procedure

In 2013 we conducted a systematic sampling, collecting spiders once a month from January to December. We sampled four different habitats: tree branches, tree trunks, grasses and soil. We then randomly selected 23 Holm oaks (isolated ones and within a forest patch). Tree spiders were collected by shaking the branches, and beating the canopy of each tree six times in each cardinal direction. We placed a white sheet below the branches and immediately collected all of the spiders falling onto it. Trunk traps consisted of a mosquito net attached to the tree trunk with an inverted cone with a closed bottle on the top. They covered the trunks partially and trapped the spiders that climbed the trunks and eventually walked into the net. At 10–15 meters from each study tree we set up a pair pit-fall traps separated by 10 metres. These traps were located in grasslands and consisted of a cone through which ground-dwelling spiders fell into a bottle filled with 90% ethanol and 10% glycerine to preserve the specimens. The traps were protected from direct sunlight by a small plastic roof to prevent alcohol evaporation. Lastly, grass spiders were sampled using a sweeping net along two 10-m long transects on both

sides of the straight line joining the two pitfall traps. All specimens were preserved in 96% alcohol for further anatomical and molecular analyses and placed individually in Eppendorf tubes with all the information on the collection date and habitat.

Taxonomical analyses

The spiders were inspected under a Meiji EMZ-5 estereomicroscope. Drawings were made and photos were taken of specimens and their copulatory organs using a Canon EOS 350D camera connected to the estereomicroscope. All of the specimens were separated by age (adults and immatures) and sexed whenever the development of the sexual organs so permitted. In some females, the epigyne was removed, cleaned and mounted on slides for further analysis of the internal anatomy. In the case of males, only one palp was extracted for a detailed study. The epigyne and palp removed were placed in microvials within the Eppendorf tubes of the corresponding specimens. The individuals were deposited in the collection of the Museo Nacional de Ciencias Naturales (National Museum of Natural Sciences) (CSIC), Madrid, Spain (MNCN collection of non-insect arthropods; EMH collection Eduardo Morano Hernández).

In the present study the opisthosoma length has been measured without the spinnerets and the pedicel. The total leg length (femur, patella, tibia, metatarsus, tarsus) and the leg spination pattern follow the model of Davies (1994). The spination of the legs maintains the femur, patella, tibia, metatarsus, tarsus order. First, all the spines of the prolateral surface of each segment are counted, then the dorsal ones, the retrolaterals and, lastly, the ventral spines. Hence, the resulting number is usually a four-digit one. If the spination model differs between the right and left segments the number of spines in the right one is shown within brackets. All the measurements are given in millimetres.

Abbreviations: Eyes: ALE – Anterior lateral eye(s). AME – Anterior median eye(s). PLE – Posterior lateral eye(s). PME – Posterior median eye(s). imm – immatures. CS – cymbial spur; C – conductor; TA – tegular apophysis.

Molecular analyses

To confirm the species identity of the specimens classified as *Cheiracanthium ilicis* sp. n. the DNA of three individuals was extracted following the salt extraction protocol (Aljanabi and Martínez 1997). For each individual we amplified a fragment (627 bp long) of the mitochondrial gene cytochrome oxidase I (cox1) using the universal primers pair LCO11490 / HCO12198 commonly used in DNA barcoding (Folmer et al. 1994). Sequence chromatograms were assembled and edited using Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI, USA). These sequences were pooled with the cox1 sequences of *Cheiracanthium* identified to the species level available in GenBank. (Accession codes JN817218.1, JN817219.1, JN018131.1, KP975945.1, KP657470.1). We compared the intra-specific genetic divergence among the three specimens of *C. ilicis* sp. n., and

of this species with the rest of *Cheiracanthium* cox1 sequences downloaded from GenBank. Genetic divergence was calculated by dividing the number of different nucleotides by the total number of compared nucleotides (uncorrected genetic distance).

To further assess the phylogenetic relationships between *C. ilicis* sp. n. and the rest of the species of the genus, we concatenated the mtDNA matrix (cox1) with sequences of the 28SrRNA nuclear ribosomal gene available in GenBank for specimens identified to the species level (four spp.) (Accession codes JN817007.1, JN817008.1, JN018345.1, KM225049.1). We obtained the 28S sequenced of one individual of *C. ilicis* sp.n. using the primer pair (28S a: GACCTGCCTTGAAACACGGA; 28S b: TCGGAAGGAAC-CAGCTTACTA) (Whiting et al. 1997). The 28S matrix, including the GenBank sequences and the new sequence of *C. ilicis* sp.n. (GenBank accession code KX272625), was aligned using MUSCLE (Edgard 2004). The aligned 28SrRNA data matrix was combined with the cox1 for a final concatenated data matrix 915 bp long.

Before concatenating the two genes for the phylogenetic reconstruction, two gene trees (one for cox1 and another for 28SrRNA) were built to assess any significant incongruence that could prevent concatenation. In all cases (combined phylogeny and separate gene trees) Bayesian inference analyses were used as implemented in Mr Bayes 3.2 software (Ronquist et al. 2012). The nucleotide substitution models needed for the Bayesian analyses were calculated for each gene using jModelTest 0.1.1 (Posada 2008).

Both in the combined phylogeny and in the gene trees, the sequences downloaded from Genbank of the closely related genus *Clubiona lena* were included as outgroup. The parameters in all Bayesian inference analyses were set up to two parallel runs of 2 million generations each conducted using one cold and two incrementally heated Markov chains (L=0.2), sampling every 1,000 steps. The standard convergence diagnostics implemented in MrBayes and the average standard deviation of the split frequencies were checked to deduce that the Markov chain had reached stationarity. After 500,000 generations, the average standard deviation of the split frequencies stabilised in values close to zero (0.001) and the phylogenetic trees were summarised using the all-compatible consensus command with 25% burn-in.

Statistical analyses

We used a Chi-square analysis to assess whether the percentage of *C. ilicis* sp. n. individuals captured from tree branches, trunks, grass and soil differed with respect to the proportion of the whole sample (including all species of spiders) captured at each of those habitats. The differences in habitat distribution between adults and immatures of this novel species were also calculated by means of a Chi-square test. The same type of analysis was used to test the phenological differences among life stages. To do so, we divided the year into four quarters starting in January and determined whether the number of immatures and adults differed over these periods.

We investigated whether the characteristics of the oaks had any effect on the number of individuals trapped. More specifically, we used a GLM (Generalised Linear

Model, Poisson distribution, Logistic link function) in which the number of *C. ilicis* sp. n. individuals collected at each oak was the dependent variable and the size (canopy surface in m²) of the tree was the independent one. The number of individuals collected could be spatially autocorrelated (i. e. it could be more similar among trees nearby). Hence, we performed an additional partial Mantel test (using distance matrixes) to calculate the correlation between the number of *C. ilicis* sp. n. individuals and canopy surface while checking the effect of the distance between trees. Generalised Linear Models were carried out in R (R Development Core Team, 2012). Mantel tests were performed as implemented in the R package `ecodist` (Goslee and Urban 2007). For the rest of statistical analyses we used Statistica 7.0 (StatSoft, Inc Tulsa, OK, USA).

Results

A total of 6048 spiders were collected throughout the whole sampling campaign. During the examination of the specimens a novel species of *Cheiracanthium* was found. It was the only species of the genus *Cheiracanthium* found in the study area and a total of 179 individuals were collected: 162 immatures and 17 adults (six males and 11 females).

Taxonomy

Cheiracanthium ilicis sp. n.

<http://zoobank.org/F86C27CA-700B-4906-B66E-B68D56693D6D>

Holotype. The holotype is a male collected in Spain: Huecas (Toledo), 581 metres above sea level (40.029915°N, 4.226789°W) by E. Morano et al. on 27 May 2013. The specimen is deposited in the collection of Arachnids of the Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain with the following reference MNCN 20.02/17491.

Paratypes. 2 males and 3 females: same data as the holotype (males: MNCN 20.02/17494 and MNCN 20.02/17496; females: MNCN 20.02/17492; MNCN 20.02/17493 and MNCN 20.02/17495).

Other specimens examined. Collected in the same village as the holotype but on a different date the following specimens have been studied and deposited in Eduardo Morano's personal collection: 30 Jan 2013, 6 imm (branches); 26 Feb 2013, 2 imm (branches); 21 Mar 2013, 15 imm (branches) and 1 imm (trunk); 26 Apr 2013, 8 imm (branches) and 3 imm (trunk); 27 May 2013, 4 ♀, 3 ♂, 1 imm (branches); 25 Jun 2013, 3 ♀ (branches) and 1 imm (grass); 19 Jul 2013, 1 ♀, 31 imm (branches) and 2 imm (grass); 23 Aug 2013, 14 imm (branches); 27 Sep 2013, 15 imm (branches) and 5 imm (trunk); 22 Oct 2013, 29 imm (branches) and 9 imm (trunk); 28 Nov 2013, 8 imm (branches) and 10 imm (trunk); 19 Dec 2013, 2 imm (branches).

Etymology. Most of the individuals of this novel species were collected from Holm oaks *Quercus ilex*. The Latin name of this oak species (*ilex*) in its singular geni-

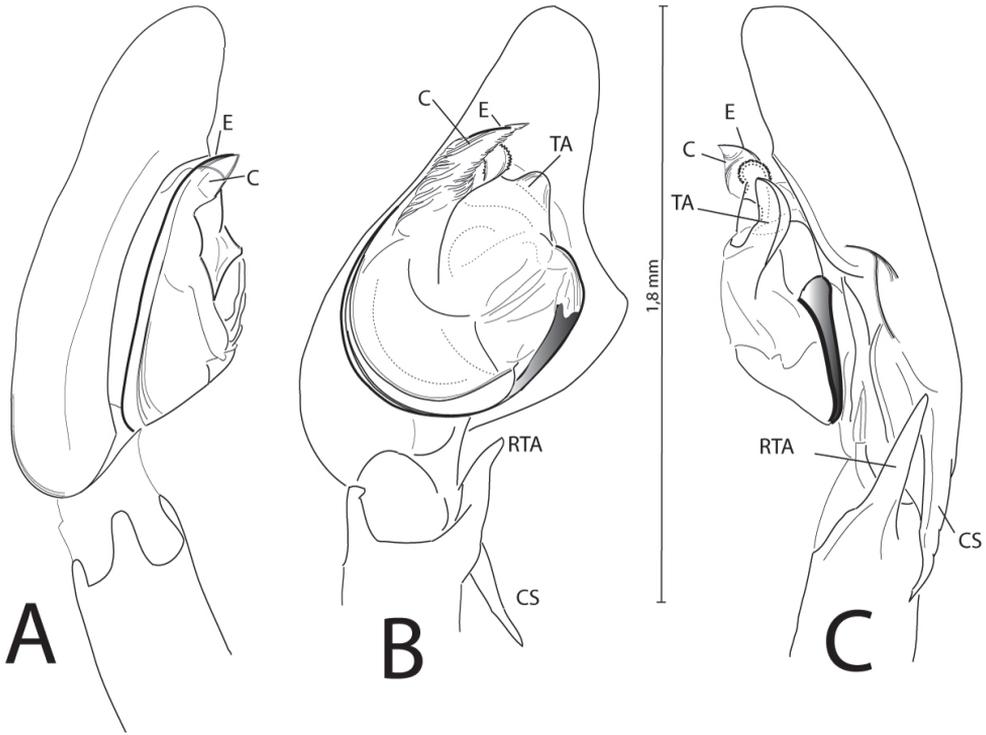


Figure 1. Palp of *Cheiracanthium ilicis* sp. n. In prolateral view (A), ventral (B) and retrolateral (C). Abbreviations: C (conductor); CS (cymbial spur); E (embolus); RTA (retrolateral tibial apophysis); TA (tegular apophysis).

tive form (*ilicis*) has been thus used to name this spider so as to link it to the main habitat it occupies.

Diagnosis. *Cheiracanthium ilicis* sp. n. closely resembles *C. inclusum* and *C. mildei* but can be distinguished by the structure and morphology of the copulatory organs of both sexes. The palps of *C. ilicis* sp. n. males have a characteristic bifurcated tegular apophysis (TA; Fig. 1). Females exhibit a wide central septum that separates the copulatory openings, which are laterally opened in both depressions, and a very characteristic loop in the copulatory duct (Fig. 2).

Description. MALE (HOLOTYPE). All measurements are given in millimetres. Medium size. Total length: 9.4; dorsal shield length: 4.1; anterior dorsal shield width: 1.9; opisthosoma length: 5.0; opisthosoma width: 3.1. Eye diameter. AME: 0.150; ALE: 0.175, PME: 0.200, PLE: 0.225. Distance between eyes: AME – AME: 0.350, AME – ALE: 0.425, PME – PME: 0.225, PME – PLE: 0.275, AME – PME: 0.225, ALE – PLE: 0.05; height from clypeus to AME: 0.1; height from clypeus to ALE: 0.1.

Prosoma. Yellowish, the ocular region is darker. The immatures show a homogeneous pale green colour, changing to yellow as they reach sexual maturity (see Pictures 2 and 3). The dorsal shield has an oval shape, is slightly raised in the ocular region and



Picture 2. *Cheiracanthium ilicis* sp. n. adult male.



Picture 3. *Cheiracanthium ilicis* sp. n. immature

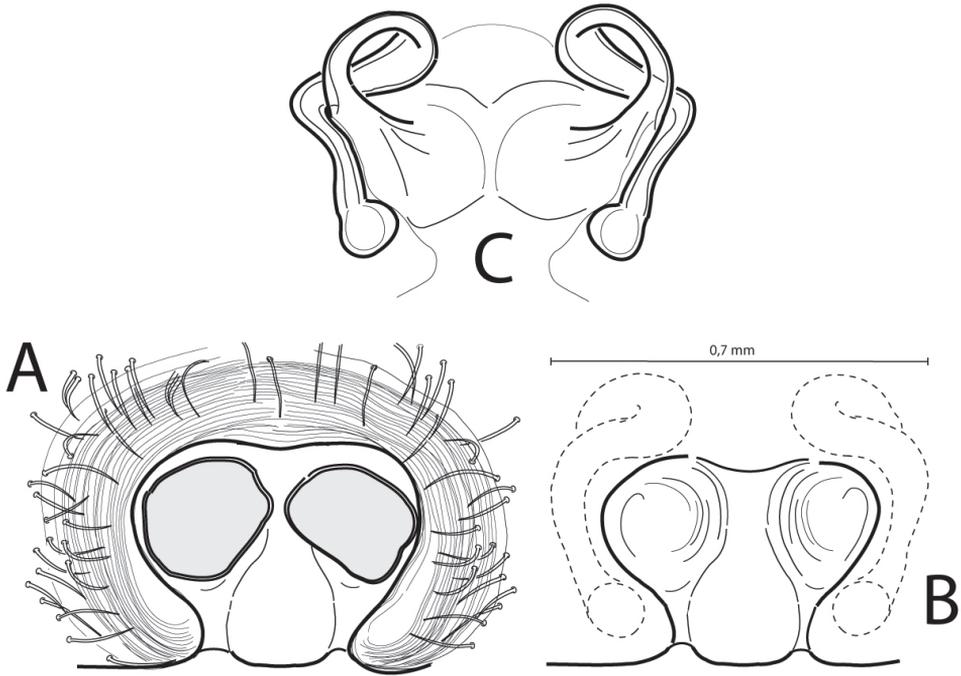


Figure 2. Epigyne and vulva de *Cheiracanthium ilicis* sp. n. Epigyne ventral view (A) with the copulatory openings closed; epigyne in ventral view (B) and vulva, in dorsal view (C).

has a scarcely marked thoracic furrow. The eyes are of similar size and placed in two transversal rows of four eyes each: the posterior row is longer than the anterior one; the anterior row is straight and the posterior one slightly curved; the eyes in the middle of the anterior row are less distant from each other than from the lateral ones. In the posterior row the eyes are almost regularly spaced, in such a way that the distance between the two posterior medium eyes is smaller than with those in the anterior medium eyes. The lateral eyes are very close together. The clypeus is narrower than the diameter of the AME. The labium is elongated and with a blunt end; the maxillas elongated and laterally cleaved; both structures have a light brown-orange colour with the apical ends paler, whitish. The sternum is triangular and orangish. The chelicera are dark brown-orange with a small basal condyle on its external surface. The basal segment does not have any modification and has few teeth on its margins. The promargin has two teeth, the superior one being larger; the retromargin has a decreasing series of three teeth, the first one being larger than the rest. Visualising these teeth is difficult due to the dense brush of hairs that covers them.

Legs. Yellowish and relatively long, ordered according to their length in the following way, 1:4:2:3. The trochanters show a marked external notch. The metatarsus and tarsus have scopulae on the distal portion and the latter have dense tarsal tufts.

Spinination (Table 1). The palp is completely spineless as are the leg patellas and tarsus. Usually, the specimens examined have shown two pairs of femoral lateral spines

Table 1. Morphological measurements of *Cheiracanthium ilicis* sp. n. holotype. All measurements are given in millimetres.

holotype ♂			
leg	segment	long.	spines
palps	Femur	1.7	0
	Patella	0.6	0
	Tibia	1.1	0
	Cymbium	1.2 without or 1.8 with apophysis	0
	total	4.6-5.2	-
I	Femur	4.8	2020
	Patella	1.9	0
	Tibia	5.9	0009(00010)
	Metatarsus	5.7	0003
	Tarsus	1.9	0
	total	20.2	-
II	Femur	3.9	2020(2010)
	Patella	1.6	0
	Tibia	4.0	0004
	Metatarsus	4.0	0004
	Tarsus	1.1	0
	total	14.6	-
III	Femur	3.1	2020
	Patella	1.1	0
	Tibia	2.4	1011(1012)
	Metatarsus	3.2	3033
	Tarsus	1.0	0
	total	10.8	-
IV	Femur	4.1	2020
	Patella	1.5	0
	Tibia	3.8	2031(3032)
	Metatarsus	4.7	3036
	Tarsus	1.2	0
	total	15.3	-

leg formula: I>VI>II>III

and three pairs of lateral spines in the metatarsus III and IV. There is variability, for instance, in the tibia I, which can bear from nine to 11 ventral spines.

Opisthosoma: Elongated, oval and slightly covered with pubescence, without erect antero-dorsal hairs. It has a uniform creamy colour all over its surface and its dorsum shows just a superficial heart mark, which is creamy or light green in adults and immatures respectively (see Pictures 2 and 3). The spinnerets are formed by two segments. The posterior spinnerets are longer than the contiguous anterior ones and are cone-shaped.

Male palps (Fig. 1). Most segments are light yellow with only the tarsus being brownish in colour; the tibia shows a curved retrolateral apophysis (RTA) and, in lateral view, it is inclined outwards; the tarsus is longer than the patella and the tibia taken together. The cymbium is elongated with retrolateral dilation, bearing a spur (CS) projected towards the tibia close to its retrolateral apophysis. The embolus is filiform, long and black; it starts on the retrolateral flank, surrounds the base and ends lying on the conductor (C) at the distal end of the palp. This conductor is membranous and with a pointed end. The apex hides the anterior branch of the tegular apophysis (TA). This inner branch is much more sclerotised and has the shape of a dentated disc perpendicularly oriented with respect to the external branch; the external branch of the tegular apophysis is laminar and is spoon-shaped.

FEMALE (Paratypes, n=3). All measurements are given in millimetres. Medium size (ranges and means within brackets). Total length: 8.8–9.7 (9.13); prosoma length:

3.7–4.2 (4.00); prosoma width: 2.9–3.3 (3.06); opisthosoma length: 4.7–6.0 (5.23); opisthosoma width: 3.8–4.9 (4.30). Eyes diameter. AME: 0.200; ALE: 0.175, PME: 0.200, PLE: 0.175; distance between eyes: AME–AME: 0.300, AME–ALE: 0.325, PME–PME: 0.350, PME–PLE: 0.375, AME–PME: 0.200, ALE–PLE: 0.075, height from clypeus to AME: 0.175, height from clypeus to ALE.

In general, the appearance and colouration of males and females is similar, although the latter are larger and with shorter legs than the slender males. Compared to males, the female dorsal shield is much wider. Eye arrangement is similar in both sexes, but female eyes are a slightly larger. The margins of the chelicera bear 3 teeth (the middle one being larger) in the promargin and two teeth in the retromargin, where the first one is larger. As in the case of males, visualisation is difficult due to the dense brush of hair covering them.

Spination (Table 2). Female palp has a single, simple, straight claw. In the females examined the variability found in the spination patterns is greater than that of the males. In general, the most frequent pattern repeated has been the presence of a single spine on either side of tibiae III and IV. Other than this, the spine arrangement is very variable among individuals.

Epigyne (Fig. 2A, B). It is an oval-shaped plate, wider than longer, with a rim resulting from the protrusion of the spermathecae and copulatory ducts. The copulatory openings are placed laterally, protected by the rim, and in the middle zone of a spherical funnel-shaped depression. In some females these depressions were covered by a dark substance that had to be removed in order to examine the morphology of the epigyne (Fig. 2A) (Dondal and Redner 1982). In the centre of the plate a septum separates each of these two depressions. In some specimens, the transparency allows the observation of the lateral section of the copulatory ducts, and also the spermathecae located in the posterior zone of the epigynal rim (Fig. 2B).

Vulva (Fig. 2C). The copulatory ducts start in each depression and go backwards, where they are ventrally curved forming a loop and going towards the spermathecae located behind. These spermathecae are small, almost circular and separated.

Phylogenetic relationships

The sequence of the cytochrome oxidase I gene was identical in the three individuals of *C. ilicis* sp. n. (GenBank Accession code KX272624). The divergence with respect to the closest species (*C. mildei*) was 7.5% and 11.4% with respect to *C. inclusum*.

The two gene trees (cox1 and 28SrRNA) showed congruent topologies, what allowed the concatenation of the sequences of both genes. Sequences of both genes were available for all species with the exception of 28SrRNA for *C. inclusum*. The Bayesian phylogeny combining both genes (Fig. 3) retrieved a clade with a highly supported node (PP=1) that included four species (*C. punctorium*, *C. inclusum*, *C. mildei* and the new *C. ilicis* sp. n.); within this group, *C. mildei* and *C. ilicis* sp. n. were sister groups (PP=0.86) and *C. inclusum* was sister to the *C. mildei*-*C. ilicis* sp. n. clade. *C. mildei*-*C. ilicis* sp. n. (Fig. 3) were also sister-species in the two gene trees.

Table 2. Morphological measurements of *Cheiracanthium ilicis* sp. n. paratypes. All measurements are given in millimetres.

legs	segment	paratype ♂		paratype ♀	
		long.	spines	long.	spines
palps	Femur	1.7–1.8	0	1.2–1.5	0
	Patella	0.5–0.6	0	0.4–0.6	0
	Tibia	1.0–1.1	0	0.8–0.9	0
	Cymbium/Tarsus	1.0–1.2 without or 1.5–1.8 with apophysis	0	1.2–1.3	0
	total	4.2–5.3	-	3.7–4.2	-
I	Femur	4.8–5.6	2020	3.8–4.3	1000(0000)-2020
	Patella	1.7–2.0	0	1.5–1.9	0
	Tibia	5.3–6.3	0009–00010(00011)	3.2–4.0	0001(0000)-0003(0002)-1001
	Metatarsus	5.3–6.5	0004–0005	3.5–4.4	0002(0001)-0005(0004)
	Tarsus	1.9–1.9	0	1.2–1.5	0
	total	19.0–22.3	-	13.2–16.1	-
II	Femur	3.7–4.1	2010(2020)-2020	2.8–3.5	1000
	Patella	1.4–1.7	0	1.0–1.5	0
	Tibia	3.4–4.3	0004(0005)-2004	2.7–2.9	0000–1000(0000)
	Metatarsus	3.7–4.7	0003(0004)-1005	2.5–3.2	0004(0003)-0005(0004)
	Tarsus	1.1–1.3	0	0.8–1.0	0
	total	13.3–16.1	-	9.9–12.1	-
III	Femur	2.8–3.2	2020	2.2–2.8	1010
	Patella	1.1–1.5	0	1.1–1.3	0
	Tibia	2.1–2.5	1010–2031(2021)	1.6–1.9	1010
	Metatarsus	2.8–3.5	3034–3035(3036)	2.0–2.3	1024(2024)-2025(2034)
	Tarsus	0.9–1.1	0	0.7–0.8	0
	total	9.7–11.8	-	7.6–9.1	-
IV	Femur	4.0–4.3	2020	3.2–3.6	1000(1010)
	Patella	1.5–1.8	0	1.3–1.7	0
	Tibia	3.2–4.0	2011(2020)- 2032(2022)	2.7–3.0	1010
	Metatarsus	4.3–5.5	3035(3036)- 3036(3037)	3.1–3.8	2027–2037(2038)-3027
	Tarsus	1.0–1.2	0	0.9–1.1	0
	total	14.0–16.8	-	11.3–13.1	-
leg formula		I>IV>II>III	-	I>IV>II>III	-

Habitat distribution and phenology

The novel species of *Cheiracanthium* was not randomly distributed in the four habitats sampled ($\chi^2=98.59$; $df=3$; $P<0.0001$). Most of the individuals were collected from Holm oak branches (82.68%) and trunks (15.64%); only three (1.67%) from grass and none from pit-fall traps. All adults were captured from the tree branches but the

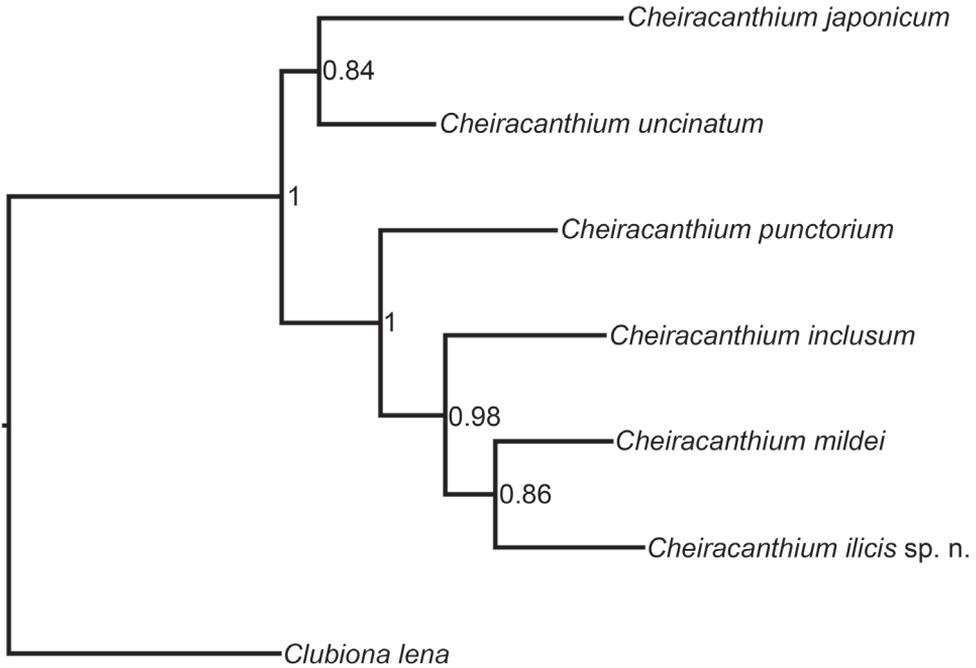


Figure 3. DNA phylogeny of one mitochondrial (cox1) and one nuclear (28S) genes showing the phylogenetic position of *Cheiracanthium ilicis* sp. n. within its genus. Tree topology was inferred using maximum likelihood (GTR + I + Gamma substitution model) and Bayesian inference.

habitat distribution differences between adults and immatures were not statistically significant ($\text{Chi}=4.02$; $\text{df}=2$; $\text{P}=0.13$). There was a positive relationship between the number of individuals collected from each tree and the surface of its canopy (Fig. 4; Estimate=0.026170; $\text{Z}=7.894$; $\text{P}<0.001$). The positive effects of tree size on *Cheiracanthium ilicis* sp. n. numbers were independent of the spatial distribution of the trees. The Mantel test demonstrated that the number of individuals collected was not spatially autocorrelated ($\text{R}=-0.004$; $\text{P}=0.41$) and the positive relationship between canopy surface and *Cheiracanthium ilicis* sp. n. numbers remained significant after checking the Euclidean spatial distance between trees ($\text{R}=0.30$; $\text{P}<0.01$).

We collected individuals of *Cheiracanthium ilicis* sp. n. all year round, but the numbers were lower in the winter months (Fig. 5). There were significant differences between age classes ($\text{Chi}=170.79$; $\text{df}=3$; $\text{p}<0.0001$), as almost all adults were collected only in the second quarter of the year (spring-early summer).

Discussion

We describe a novel species of *Cheiracanthium* that can be easily diagnosed based on male and female genitalia from other species in the genus. The amount of genetic di-

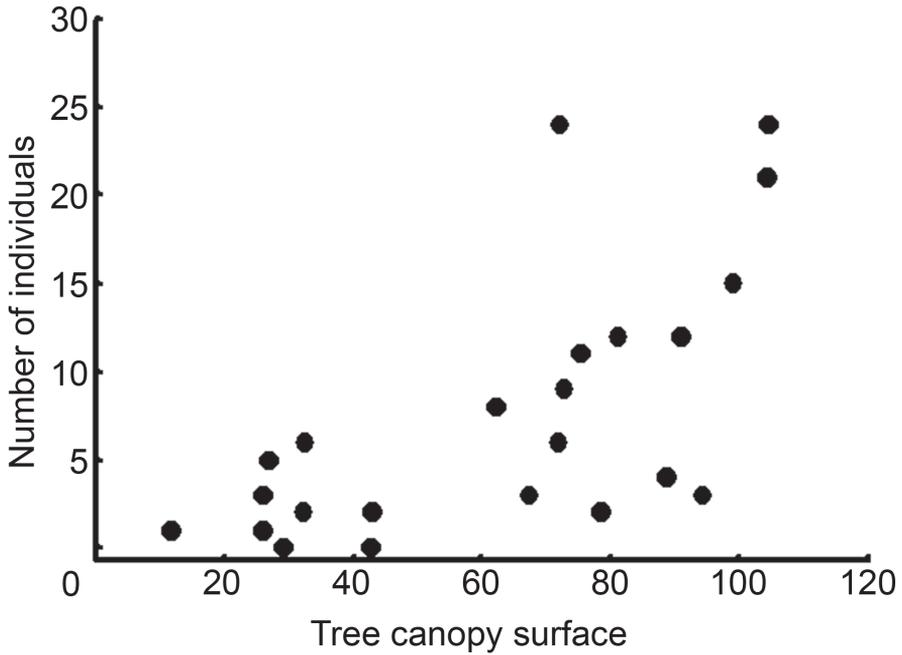


Figure 4. Relationship between the number of individuals collected and the tree size (canopy surface in m²).

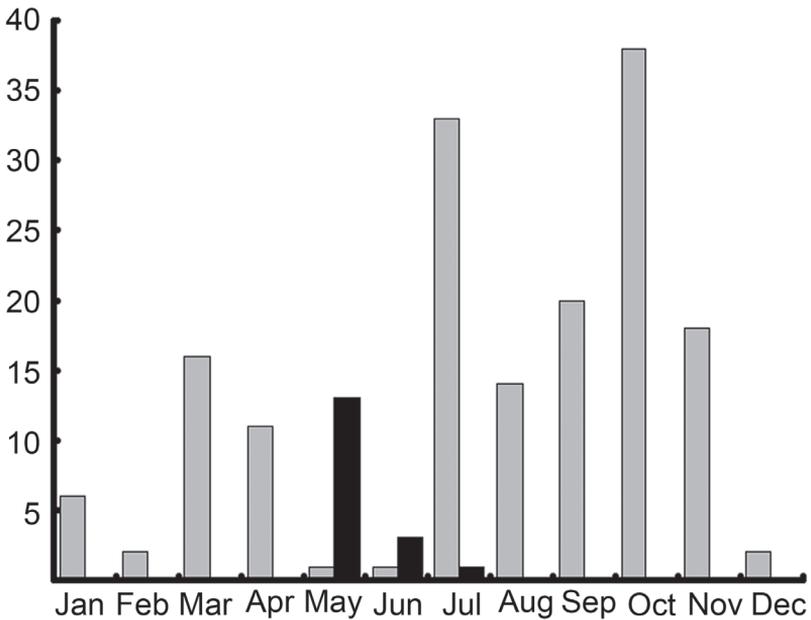


Figure 5. Number of immatures (grey bars) and adults (black bars) *Cheiracanthium ilicis* sp. n. collected throughout the year.

vergence in DNA barcode sequence (cox1) (7 to 11 % from closest relatives) provides further support for its distinctiveness.

Morphologically, *Cheiracanthium ilicis* sp. n. resembles *C. inclusum* and *C. mildei*. Like them, *C. ilicis* sp. n. males have a pedipalp with a relatively stout cymbial apophysis, a strongly sclerotized, stout median apophysis and no tegular apophysis. Also, in these three species the embolus is on the retrolateral margin of the bulbus and females have relatively stout copulatory ducts that do not encircle the two pairs of receptacula seminis on the vulva. *C. ilicis* sp. n. shares other characteristics with these species, such as a weakly developed or absent thoracic fissure and basal cheliceral articles that, at least in *C. mildei*, are smaller in females and not pigmented in the distal half. Male chelicera do not have modified basal articles and are powerful and more elongated than in females, especially in *C. inclusum* and less in *C. mildei*. In *C. mildei* male pedipalps have an additional dorsal tibial apophysis. In turn, in *C. mildei* females the copulatory ducts are placed in the middle of a strongly sclerotised epigyne. The phylogenetic analysis of the concatenated cox1+28S data matrix also supports the close relationship of the new species with *C. mildei* and *C. inclusum*. The two last species are included in *Chiracanthops*, a genus recently resurrected by Wunderlich (2012), which may suggest that the new species actually belongs to the genus *Chiracanthops*. Unfortunately, the sparse sampling of our phylogeny, only six species out of the 209 known species worldwide, deters us to draw any definitive conclusion about these relationships.

The somatic traits of this species are a combination of characteristics common to several afro-tropical species—such as *C. aculeatum* Simon, 1884, *C. denisi* Caporiacco, 1939 and *C. furculatum* Karsch, 1879, and species with a wide geographic distribution *C. inclusum* (Hentz, 1847) (New World, Africa and Reunion) (Lotz 2007a; Bayer 2014; World Spider Catalog 2015). *Cheiracanthium ilicis* sp. n. presents the tegular apophysis with a configuration similar to *C. denisi*, although the rest of the bulb differs in the cymbial spur and the retrolateral tibial apophysis. In the case of the epigyne, it resembles those of *C. furculatum* and *C. inclusum*, yet it differs in the wide septum that separates the depressions where the copulatory openings are located and in the way these are oriented antero-dorsally in the latter; whereas in *C. ilicis* sp. n. it is lateral and mid-positioned. By contrast, the shape of the vulva is more similar to *C. furculatum* and even to *C. mildei* L. Koch, 1864, as in both cases the copulatory ducts form a loop analogous to that found in *C. ilicis* sp. n.

The new species is sympatric with *Cheiracanthium mildei*, which is native to Southern Europe (Bryant 1951) and could thus co-exist with it. The information about the distribution of *C. mildei* on the Iberian Peninsula is recent and scarce. It is present in the north (provinces of Guipúzcoa) (Castro 2003; Castro 2009) and on the Mediterranean coast (province of Valencia) (Barrientos et al. 2010). There is not available information for the centre and the northwest of Iberia (Morano et al. 2014). *C. mildei* has been found to co-exist with *C. inclusum* in North America, where *C. mildei* is an invasive species (Hogg and Daane 2011). However, *C. ilicis* sp. n. was the only species of the genus found in our study area.

We agree with Bayer (2014) regarding the need to perform an extensive review of the *Cheiracanthium* genus for Europe, the Mediterranean Basin, Africa and the Middle East. For example, the type material of some species like *C. salsicola* Simon, 1932 was insufficiently described and, unfortunately, it seems to be lost, as we could not find it even after trying to do so. There is a need to study the intraspecific variability as well as to characterise lesser known species and their affinities; the discovery of this novel species in the Iberian Peninsula supports this claim. Furthermore, it would be worthwhile to sequence the genes commonly used for DNA barcoding, such as the mitochondrial *cox1*, in the existing species and in all the potentially novel ones. There are DNA sequences available in Gen-Bank for very few species of this genus; only if this number increases will we be able to build more thorough phylogenies that can establish reliable phylogenetic relationships among species.

The spiders of this genus occupy different habitats and can be found in grasslands, under stones, on shrubs and trees, etc (Dondale and Redner, 1982; Urones, 1988; Lotz, 2007a; Nentwig et al. 2016). In the case of *C. ilicis* sp. n. it has a preference for tree canopies, where most of the individuals were collected. Closely related *C. inclusum* and *C. mildei* have also been found in woody habitats (Corrigan and Bennett 1987; Hogg and Daane 2011), in which adults reproduce. We have found silk sacks in the empty acorn cups that remained attached to the oak shoots after the acorns are dropped. Only immatures were collected on the tree trunks, where they are likely to find shelter, and just three in the grasslands, probably immatures dispersing between trees. In fact, the dispersal abilities of species like *C. inclusum* by excreting a long silk thread that is carried by the wind (ballooning) are well known (Peck et al. 1970). The good dispersal abilities of these spiders may also explain the lack of a spatial autocorrelation among trees in the number of spiders collected.

Based on Ysnel and Canard (1986) *C. ilicis* sp. n. would be a spring stenocorus species, characterized by a short biological cycle with a brief adult presence in late spring and early summer. As in the case of *C. inclusum* in temperate North America (Peck et al. 1970), then was when we collected adults and reproduction takes place. After that, samples are dominated by juveniles and later mostly by subadults during winter and early spring. This is because the species is probably annual like other species of the genus; adults mate in early summer and then die in winter. The number of individuals and reproducing adults collected from each tree was closely related to size (canopy surface). Foliage biomass is greater in large trees and positively correlated with the abundance of herbivore insects on which spiders prey (Halaj et al. 1998). Caterpillar samplings in our study site have indeed shown that their numbers are higher in large Holm oaks (unpublished data). Also, large trees are older and the probability of tree colonisation in fragmented landscapes increases with time (Floren et al. 2011).

Cheiracanthium spp. spiders are nocturnal hunters and all of the individuals that we collected during the day by branch beating were quite inactive. They are very effective predators, which probably explains their success as invaders in areas outside their distribution range (Hogg et al. 2010). These spiders feed on Lepidoptera eggs, caterpillars, leafhoppers, leafminers and other herbivorous insects, some of them being insects that feed on

cultivars of economic interest (e. g. vineyards, apple tree orchards). In fact, some studies have highlighted the potential role of these spiders in pest control (Corrigan and Bennett 1987; Hogg and Daane 2011). In the case of *C. ilicis* sp. n. inhabiting Holm oaks *Quercus ilex*, the main tree species of man-made savannahs used for livestock rearing (so called *dehesas* and *montados* in Spain and Portugal, respectively), this role has to be taken into account. Lastly, the present study supports many others that emphasise the importance of isolated trees in agricultural landscapes (Manning et al. 2006). They not only harbour common forest organisms but are also refuges for species yet to be discovered.

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First description of the male of *Draconarius jiangyongensis* (Peng et al., 1996) (Araneae, Agelenidae)

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Abstract

The male of *Draconarius jiangyongensis* (Peng, Gong & Kim, 1996) is described for the first time from Xinning County, Hunan Province, China. Morphological descriptions and illustrations of both sexes of this species are given in this study. The placement of this species in *Draconarius* is doubted.

Keywords

China, Hunan, spider, Coelotinae, *Coelotes*, Langshan Mountain

Introduction

The spider genus *Draconarius* Ovtchinnikov, 1999 is distributed in Central and East Asia and has a high level of species diversity with 244 species described to date (World Spider Catalog 2016, Wang 2016). A total of 151 *Draconarius* species has been reported from China, but more than half of these species are described from only the male or female (World Spider Catalog 2016).

Draconarius jiangyongensis (Peng, Gong & Kim, 1996) was first described as a member of the genus *Coelotes* Blackwall, 1840 based on six female specimens from Jiangyong County, Hunan Province, China (Peng et al. 1996). Wang (2003) transferred this species to the genus *Draconarius*. *Draconarius jiangyongensis* was illustrated by Song et al. (1999), and redescribed by Wang (2003) and Yin et al. (2012), but only based on the females from the type locality.

During the expedition to Langshan National Geopark in November of 2014, ten females and eleven males were identified to be *D. jiangyongensis* based on comparison with the type specimens. The female is redescribed here and the male is described for the first time in the present study.

Material and methods

Specimens were examined with an Olympus SZX16 stereomicroscope and an Olympus BX53 compound microscope. Photos were taken with a Canon PowerShot G12 digital camera mounted on an Olympus BX53 compound microscope. Both the male palp and the female epigyne were examined and illustrated after being dissected from the spider bodies. All specimens examined in this study are deposited in the College of Life Sciences, Hunan Normal University (HNU).

All measurements are given in millimeters. Eye diameters are taken at the widest point. Leg measurements are given as: total length (femur, patella + tibia, metatarsus, tarsus). Abbreviations used in the text are as follows:

AME	anterior median eyes;
ALE	anterior lateral eyes;
MOA	median ocular area;
PME	posterior median eyes;
PLE	posterior lateral eyes.

Taxonomy

Family Agelenidae C. L. Koch, 1837

Genus *Draconarius* Ovtchinnikov, 1999

***Draconarius jiangyongensis* (Peng, Gong & Kim, 1996)**

Figs 1–4

Coelotes jiangyongensis Peng et al. 1996: 19, figs 7–9 (description and illustration of ♀);

Song et al. 1999: 376, figs 220J–K (♀ figures reproduced from Peng et al. 1996).

Draconarius jiangyongensis: Wang 2003: 536, figs 36A–B, 96B (transferred from *Coelotes*);

Yin et al. 2012: 1010, figs 521a–c (redescription and illustration of ♀).

Type material examined. Holotype, 1♀ (HNU), Jiangyong County, **Hunan Province, China**, 1 October 1991, Liansu Gong leg.; paratypes, 4♀ (HNU), same data as holotype.

Additional material examined. **Hunan Province**, Xining County, Langshan National Geopark: 2♀ (HNU), Tianyixiang (26°21.218'N, 110°48.246'E, 590m), 21.11.2014; 3♀, 2♂ (HNU), same locality as above (26°21.447'N, 110°48.190'E,

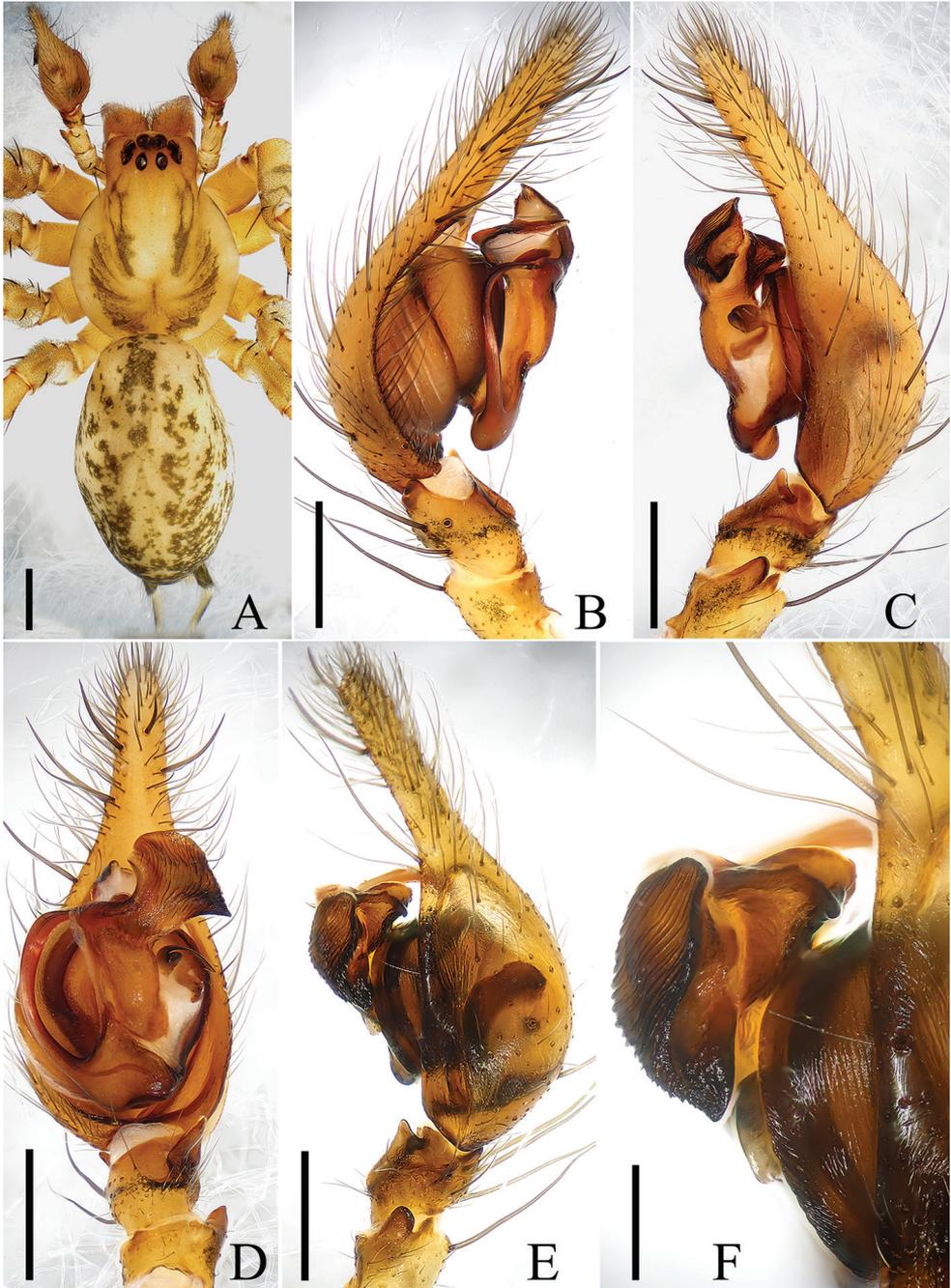


Figure 1. Male of *Draconarius jiangyongensis*. **A** Habitus, dorsal view **B** Left palp, prolateral view **C** Ditto, retrolateral view **D** Ditto, ventral view **E** Ditto (after maceration), retrolateral view **F** Conductor (after maceration), retrolateral view. Scales: **A** = 1 mm; **B–D, E** = 0.5 mm; **F** = 0.2 mm.



Figure 2. Female of *Draconarius jiangyongensis*. **A** Habitus, dorsal view **B** Epigyne, ventral view **C** Vulva, dorsal view. Scales: **A** = 1 mm; **B–C** = 0.2 mm.

560–640m), 22.11.2014; 1♀, 1♂ (HNU), Cave Feiliandong (26°21.447'N, 110°47.921'E, 400m), 23.11.2014; 1♀ (HNU), Bajiaozhai (26°16.354'N, 110°44.308'E, 820m), 24.11.2014; 5♀, 8♂ (HNU), Peak Lajiaofeng (26°23.135'N, 110°48.464'E, 400–640m), 27.11.2014. All specimens are collected by hand picking by Haiqiang Yin, Cheng Wang, Bing Zhou, Jiahui Gan and Yuhui Gong.

Diagnosis. Female of *D. jiangyongensis* can be distinguished from other *Draconarius* by the presence of a vase-shaped septum of epigyne (Figs 2B, 3E), the anteriorly originating and laterally extending copulatory ducts, and the spermathecae widely separated basally and contiguous distally (Figs 2B, C, 3E, F). The male of *D. jiangyongensis* is similar to *D. yadongensis* (Hu & Li, 1987) in having a simple conductor, an embolus arising at approximately 10 o'clock (left palp) and the short cymbial furrow (Figs 1C, D, 3B, C), but can be distinguished from the latter by the shape of the conductor (the conductor axe-shaped, with a wrinkly surface in *D. jiangyongensis*, but narrow with a sharp end tip and broad dorsal edge in *D. yadongensis*) (Figs 1D, F, 3B, D).

Description. Male. Total length 8.7. Carapace 4.1 long, 3.1 wide; opisthosoma 4.5 long, 2.9 wide. Clypeus height 0.15. Cephalic part much elevated from the tho-

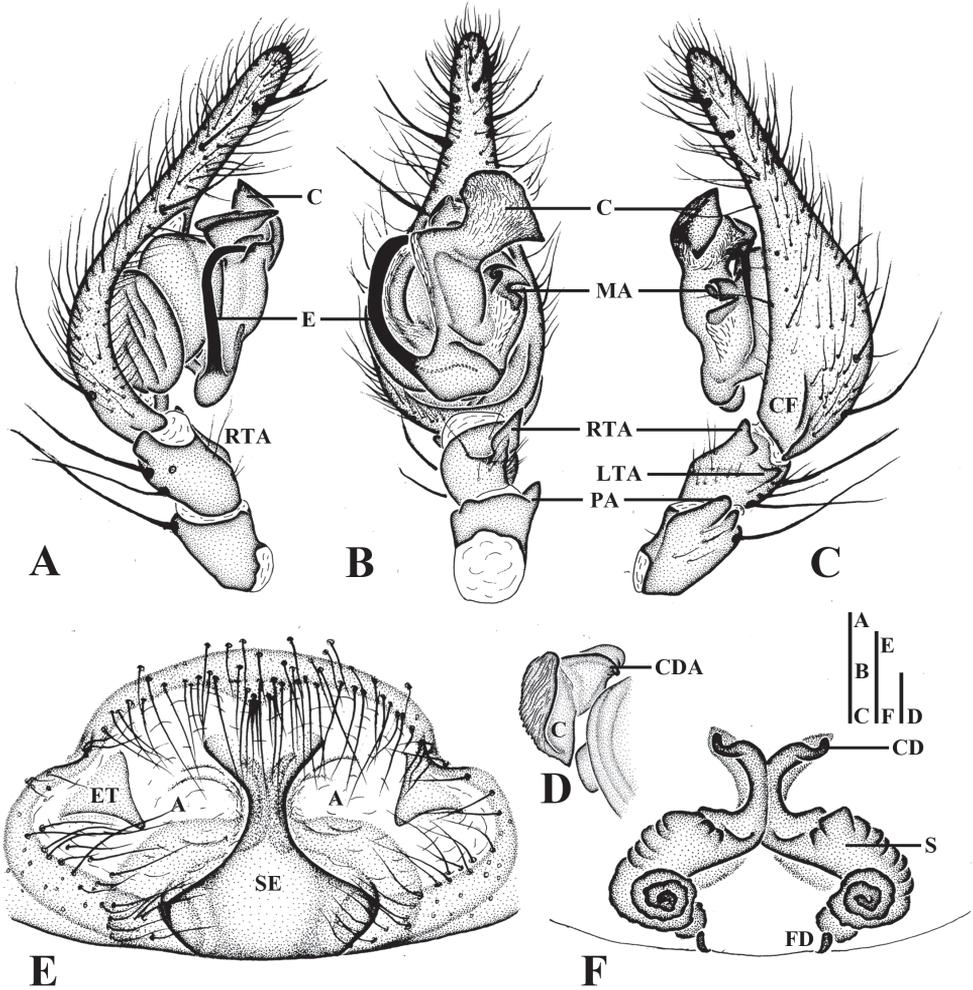


Figure 3. *Draconarius jiangyongensis*. **A-D:** **A** Male left palp, prolateral view **B** Ditto, ventral view **C** Ditto, retrolateral view **D** Conductor (after maceration), retrolateral view **E, F** Female: **E** Epigyne, ventral view **F** Vulva, dorsal view. Abbreviations: A — atrium; C — conductor; CD — copulatory duct; CDA — dorsal conductor apophysis; CF — cymbial furrow; E — embolus; ET — epigynal teeth; FD — fertilization duct; LTA — lateral tibial apophysis; MA — median apophysis; PA — patellar apophysis; RTA — retroventral tibial apophysis; SE — septum; S — spermathecae. Scales: **A-C** = 0.5 mm; **D-F** = 0.2 mm.

racic region. Cervical and radial grooves greyish-black (Fig. 1A). Eye sizes and inter-distances: ALE 0.18, AME 0.20, PLE 0.16, PME 0.16; ALE-AME 0.04, AME-AME 0.06, PLE-PME 0.08, PME-PME 0.16; MOA 0.54 long, anterior width 0.58, posterior width 0.70 (Fig. 1A). Labium reddish-brown, 0.6 long, 0.6 wide. Sternum brown, slightly longer than wide (2.3 long, 1.9 wide). Chelicerae with three promarginal and four retromarginal teeth. Leg measurements: I 18.0 (4.5, 6.0, 4.9, 2.6), II 15.8 (4.2,

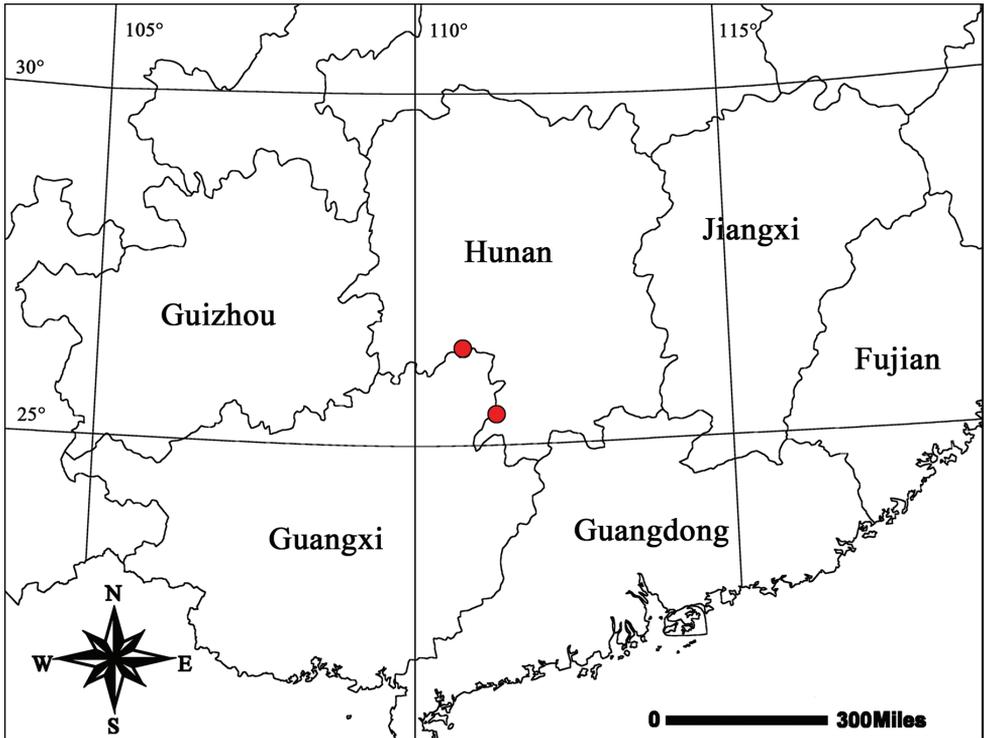


Figure 4. Distribution records of *Draconarius jiangyongensis*.

5.3, 4.2, 2.1), III 14.0 (3.7, 4.3, 4.0, 2.0), IV 17.8 (4.5, 5.7, 5.4, 2.2). Opisthosoma with dorsal pattern composed of several chevrons patterns (Fig. 1A).

Male palp (Figs 1B–F, 3A–D): femur nearly $3/4$ length of cymbium; patellar apophysis large; retroventral tibial apophysis moderately long, about $2/3$ length of tibia; lateral tibial apophysis small, widely separated from retrolateral tibial apophysis; cymbial furrow short, less than $1/3$ length of cymbium; conductor broad, axe-shaped, with a wrinkly surface; dorsal conductor apophysis large; median apophysis large, with a sharp end in ventral view; embolus long and flat, arising at approximately 10 o'clock and encircling for about 180 degrees around bulb.

Female. Total length 8.60. Carapace 4.0 long, 2.9 wide; opisthosoma 4.6 long, 3.1 wide. Clypeus height 0.14. Eye sizes and interdistances: ALE 0.2, AME 0.22, PLE 0.18, PME 0.18; ALE-AME 0.04, AME-AME 0.08, PLE-PME 0.10, PME-PME 0.20; MOA 0.52 long, anterior width 0.58, posterior width 0.66. Labium greyish brown, 0.60 long, 0.50 wide. Sternum brown, slightly longer than wide (2.10 long, 1.80 wide). Leg measurements: I 14.3 (3.8, 5.0, 3.5, 2.0), II 12.2 (3.5, 4.2, 3.0, 1.5), III 10.8 (3.0, 3.6, 2.9, 1.3), IV 14.1 (4.0, 4.8, 3.5, 1.8). Promarginal and retromarginal teeth of chelicera and the dorsal pattern of opisthosoma are the same as male (Fig. 2A).

Epigyne (Figs 2B, C, 3E, F): teeth triangular, large and thin, located anterolaterally; septum large, with the base much wider than the stem; atrium divided into two parts by septum; the bases of spermathecae highly convoluted and separated about two times their diameter from each other, and the distal ends of spermathecae contiguous; copulatory ducts short, anteriorly situated and laterally extending.

Remark. The *Draconarius* and *Coelotes* are two most species-rich genera in the Coelotinae, with 244 and 183 species described to date, respectively. Most of those species were described based on only the male or female. As a result, some might be incorrectly placed. This species described here is more likely to be a member of the genus *Coelotes* than *Draconarius* based on the following combination of characters: the large epigynal teeth, the atrium (atrium divided into two parts by septum) and short copulatory ducts in the female; the large patellar apophysis, the short and prolaterally originating embolus and the short cymbial furrow (less than 1/3 length of the cymbium) in the male. It differs from *Coelotes atropos* (Walckenaer, 1830) by the presence of septum. It also differs from the type species and many other species of *Draconarius* (for example, *D. guizhouensis* (Peng, Li & Huang, 2002), *D. latellai* Marusik & Ballarin, 2011 and so on) by the number of cheliceral teeth (this species with three promarginal and four retromarginal teeth while the type species and many other species of *Draconarius* have three promarginal and two retromarginal teeth.)

Distribution. China (Hunan).

Acknowledgements

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Nine new species of the spider genus *Pireneitega* Kishida, 1955 (Agelenidae, Coelotinae) from Xinjiang, China

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Abstract

Nine new *Pireneitega* species collected from Xinjiang, China are described as new to science: *P. burqinensis* sp. n. (♂♀), *P. fuyunensis* sp. n. (♂♀), *P. gongliuensis* sp. n. (♂♀), *P. huochengensis* sp. n. (♂♀), *P. lini* sp. n. (♀), *P. liui* sp. n. (♂♀), *P. wensuensis* sp. n. (♂), *P. wui* sp. n. (♂) and *P. yaoi* sp. n. (♀). DNA barcodes were obtained for all these species for future use.

Keywords

Taxonomy, description, diagnosis, Central Asia, *Paracoelotes*

Introduction

The spider genus *Pireneitega* was established by Kishida (1955). Its type species is *Amaurobius roscidus* C.L. Koch, 1843 from Germany, considered to be a junior synonym of *P. segestrifformis* (Dufour, 1820). *Pireneitega* was for a long time regarded as a *nomen nudum* until Wang and Jäger (2007) found reasons to revalidate this name and to make *Paracoelotes* Brignoli, 1982 its junior synonym. Currently, there are twenty-one valid *Pireneitega* species, distributed widely from the Iberian Peninsula to Japan and Sakhalin; eleven of them are known from East Asia, six are known from Central Asia, and other four from Europe (Li and Lin 2015, World Spider Catalog 2016). This paper provides descriptions of nine new *Pireneitega* species collected from Xinjiang in northwestern China.

Material and methods

Specimens were examined with a Leica M205C stereomicroscope. Images were captured with an Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 dissecting microscope. Epigynes and male palps were examined after dissection from the spiders' bodies. The epigyne was cleared by boiling it in a 10% KOH solution before taking photos of the vulva.

All measurements were obtained using a Leica M205C stereomicroscope and are given in millimeters. Leg measurements are given as: Total length (femur, patella + tibia, metatarsus, tarsus). Only structures (palp and legs) of the left side of the body are described and measured. The terminology used in the text and the figure legends follows Wang (2002). Abbreviations used in this paper and in the figure legends are: A = epigynal atrium; ALE = anterior lateral eye; AME = anterior median eye; AME-ALE = distance between AME and ALE; AME-AME = distance between AME and AME; ALE-PLE = distance between ALE and PLE; CD = copulatory duct; CF = cymbial furrow; CO = conductor; E = embolus; EB = embolic base; ET = epigynal tooth; FD = fertilization duct; H = epigynal hood; MA = median apophysis; PA = patellar apophysis; PLE = posterior lateral eye; PME = posterior median eye; PME-PLE = distance between PME and PLE; PME-PME = distance between PME and PME; R = receptacle; RTA = retroventral tibial apophysis; ST = subtegulum; T = tegulum; TC = tip of conductor.

DNA barcodes were obtained for future use. A partial fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced for nine new species and one old species using Primers LCO1490-oono (5'-CWACAAAYCA-TARRGATATTGG-3') (Folmer et al. 1994; Miller et al. 2010) and HCO2198-zz (5'-TAAACTTCCAGGTGACCAAAAAATCA-3') (Folmer et al. 1994; Chen et al. 2015). For additional information on extraction, amplification, and sequencing procedures, see Zhao et al. 2013. All sequences were deposited in GenBank and the accession numbers are provided in Table 1.

All of the specimens (including molecular vouchers) are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing, China.

Table 1. Voucher specimen information.

Species	GenBank accession number	Sequence length	Collection localities
<i>Pireneitega burqinensis</i> sp. n.	KX011867	630bp	China: Xinjiang: Burqin
<i>Pireneitega fuyunensis</i> sp. n.	KX011859	630bp	China: Xinjiang: Fuyun
<i>Pireneitega gongliuensis</i> sp. n.	KX011862	630bp	China: Xinjiang: Gongliu
<i>Pireneitega huochengensis</i> sp. n.	KX011861	630bp	China: Xinjiang: Huocheng
<i>Pireneitega lini</i> sp. n.	KX011865	630bp	China: Xinjiang: Akto
<i>Pireneitega liui</i> sp. n.	KX011860	630bp	China: Xinjiang: Xinyuan
<i>Pireneitega tianchiensis</i> (Wang, Yin, Peng & Xie, 1990)	KX011858	630bp	China: Xinjiang: Changji
<i>Pireneitega wensuensis</i> sp. n.	KX011864	630bp	China: Xinjiang: Wensu
<i>Pireneitega wui</i> sp. n.	KX011866	630bp	China: Xinjiang: Kizilsu
<i>Pireneitega yaoli</i> sp. n.	KX011863	630bp	China: Xinjiang: Hoboksar

Taxonomy

Family Agelenidae C.L. Koch, 1837

Subfamily Coelotinae F.O. P.-Cambridge, 1893

Genus *Pireneitega* Kishida, 1955

Pireneitega Kishida, 1955: 21. Type species *Amaurobius roscidus* C.L. Koch, 1843 (= *P. segestriformis* Dufour, 1820) from Germany; Wang and Jäger 2007: 46 (synonymized 2 genera).

Paracoelotes Brignoli, 1982: 348. Type species *Coelotes armeniacus* Brignoli, 1978 from Turkey; Wang 2002: 112.

Diagnosis. The chelicerae of all *Pireneitega* have 3 promarginal and 3 retromarginal teeth; other coelotines usually have 2 or 4 retromarginal teeth. The females of this genus can be separated from other coelotines by the widely separated long epigynal teeth, the large epigynal atrium with weakly sclerotized septum, and broad copulatory ducts (Fig. 6A–B); other coelotines usually have a small epigynal atrium, the short epigynal teeth and narrow copulatory ducts. The males of this genus can be distinguished from other coelotines by with an elongated and flattened conductor which is usually twisted into a circle horizontally or vertically and a large median apophysis (Fig. 1A–C); other coelotines usually have a broad or short conductor and a reduced or indistinct median apophysis.

Description. Described in Wang (2002, sub *Paracoelotes*).

Composition. Twenty-one *Pireneitega* species are known from Germany, Italy, Spain, France, Turkey, Georgia, Azerbaijan, Uzbekistan, Tajikistan, Russia, South Korea, Japan and China (World Spider Catalog 2016). Eleven *Pireneitega* species were known from China before the current study, including *P. involuta* (Wang et al., 1990) (♂♀), *P. liansui* (Bao & Yin, 2004) (♀), *P. luctuosa* (L. Koch, 1878) (♂♀), *P. luniformis* (Zhu & Wang, 1994) (♂♀), *P. neglecta* (Hu, 2001) (♀), *P. spinivulva* (Simon, 1880) (♂♀), *P. taishanensis* (Wang et al., 1990) (♂♀), *P. taiwanensis* Wang & Ono, 1998 (♂♀), *P. tianchiensis* (♂♀), *P. triglochinata* (Zhu & Wang, 1991) (♂♀), and *P. xinping* Zhang, Zhu & Song, 2002 (♂♀).

***Pireneitega burqinensis* Zhao & Li, sp. n.**

<http://zoobank.org/403E6B13-C543-4EE1-9387-6CB2F9F9005A>

Figs 1–2, 17

Type material. **Holotype** ♂: China: Xinjiang, Ili Kazakh Autonomous Prefecture, Altay Prefecture: Burqin County, on the way from Jiadenyu to Hemu Village, birch forest, N48°31'08", E87°11'13", 1469 m, 23.VII.2013, Z. Yao and Z. Zhao. **Paratype:** 1♀, same data as holotype.

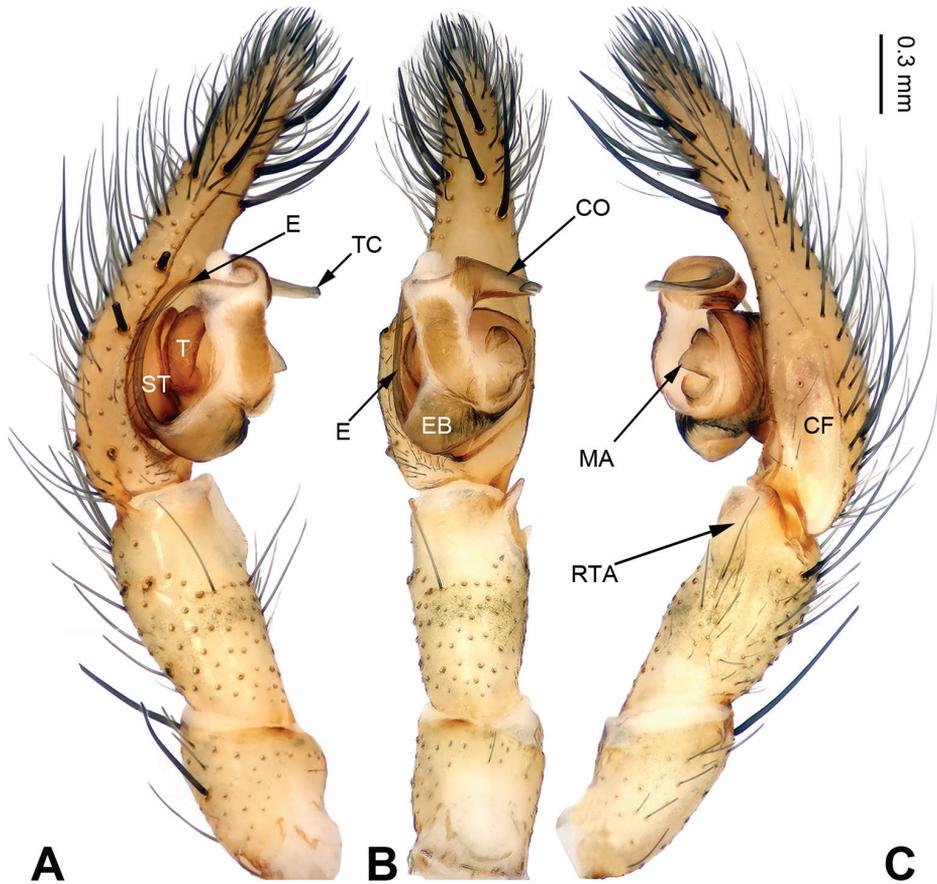


Figure 1. Left palp of *Pireneitega burqinensis* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A, B, C**.

Other material studied. *Pireneitega tianchiensis*: 1♀1♂ (Figs 12–13): China: Xinjiang, Changji Hui Autonomous Prefecture: Fukang City, Crater Lake Scenic Spot (in Chinese: Tianchi), N43°54'05", E88°07'29", 1878 m, 16.VII.2013, Z. Yao and Z. Zhao.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male can be distinguished from all other *Pireneitega* species, except *P. tianchiensis*, by having narrow tibia and tarsus. From *P. tianchiensis*, it can be distinguished by the nearly trapezoidal embolic base (while *P. tianchiensis* has the nearly fingernail-shaped embolic base) (cf. Figs 1 and 12; Wang et al. 1990: figs 81–83). The female can be distinguished from all other *Pireneitega* species, except *P. tianchiensis*, by having the weakly sclerotized tip of septum and the closely spaced copulatory opening. From *P. tianchiensis*, it can be distinguished by the sharply narrowed epigynal teeth (while in *P. tianchiensis* the epigynal teeth are broad and nearly horn-shaped) (cf. Figs 2A–B and 13A–B; Wang et al. 1990: figs 84–85).

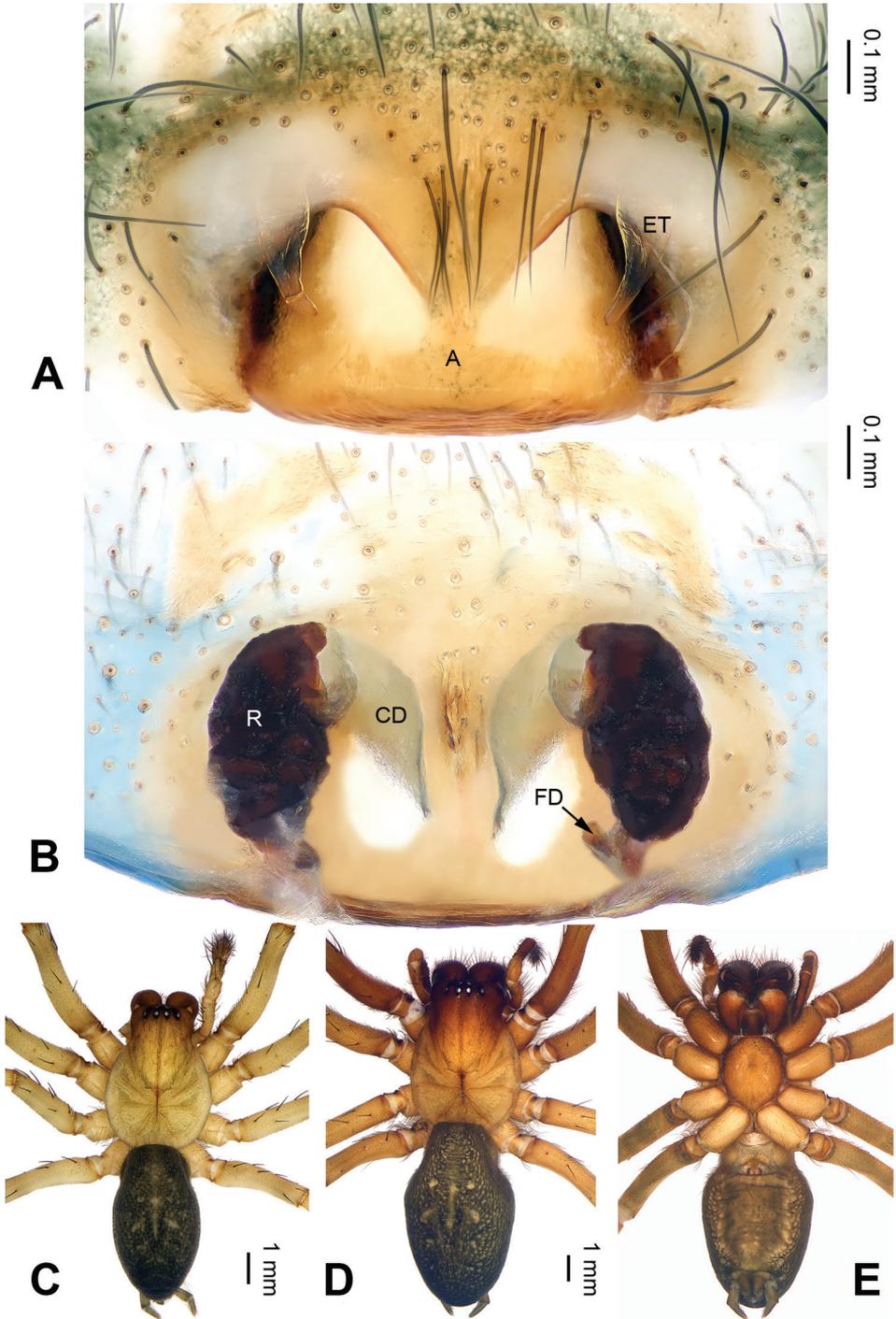


Figure 2. *Pireneitega burqinensis* sp. n., female paratype and male holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D, E**.

Description. Male (holotype): Total length 12.25. Carapace 5.25 long, 4.25 wide. Abdomen 7.00 long, 4.00 wide. Eye sizes and interdistances: AME 0.27, ALE 0.28, PME 0.23, PLE 0.23; AME-AME 0.10, AME-ALE 0.06, PME-PME 0.17, PME-PLE 0.23. Leg measurements: I: 18.40 (5.50, 6.25, 4.50, 2.15); II: 17.25 (5.00, 6.00, 4.25, 2.00); III: 16.15 (4.75, 5.15, 4.25, 2.00); IV: 20.15 (5.75, 6.50, 5.75, 2.15). Carapace greenish, the radial grooves indistinct, with black lateral margins. Abdomen blackish, with yellow spots. Palp: patellar apophysis absent; tibia short, about 1/2 length of cymbium; RTA short, about 1/3 of tibial length, without pointed tip, extended beyond the tibia; cymbial furrow short, about 1/3 length of cymbium; conductor long, nearly hook-shaped, with one loop; median apophysis broad and nearly triangular; embolus with broad and nearly trapezoidal base, beginning at 6:30 o'clock position (Fig. 1A–C).

Female (paratype): Total length 9.50. Carapace 4.50 long, 3.60 wide. Abdomen 5.00 long, 3.00 wide. Eye sizes and interdistances: AME 0.20, ALE 0.25, PME 0.18, PLE 0.18; AME-AME 0.10, AME-ALE 0.05, PME-PME 0.15, PME-PLE 0.23. Leg measurements: I: 17.90 (5.00, 6.00, 4.75, 2.15); II: 17.00 (5.00, 5.50, 4.50, 2.00); III: 16.00 (4.75, 5.00, 4.50, 1.75); IV: 19.75 (5.50, 6.00, 6.00, 2.25). Carapace reddish, with brown lateral margins. Abdomen blackish, with yellow sigilla. Epigyne: epigynal teeth light-colored and hyaline, about 0.5 times as long as epigynal atrium, located in anterior part of epigynal atrium; septum about 0.6 times as long as wide, nearly triangular; atrium about 1.2 times as long as wide, with weakly sclerotized posterior margin and nearly triangular, about two times as long as septum, subequal to the width of septum; receptacles about two times as long as wide, located in the posterior part of epigyne; copulatory opening indistinct; hoods indistinct (Fig. 2A–B).

Distribution. Known only from the type locality (Fig. 17).

***Pireneitega fuyunensis* Zhao & Li, sp. n.**

<http://zoobank.org/21C8277B-74D3-4C6D-9E4F-0F8C35E9DDDD4>

Figs 3–4, 17

Type material. Holotype ♂: China: Xinjiang, Ili Kazakh Autonomous Prefecture, Altay Prefecture: Fuyun County, Ocoa Sea Breeze, Erqis Grand Canyon, on the way from the Carla Chale Waterfall to the Shenzhong Mountain, N47°19'28", E90°01'51", 1355 m, 19.VII.2013, J. Liu, K. Meng, Z. Yao, and Z. Zhao. **Paratypes:** 4♀1♂, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male can be distinguished from all other *Pireneitega* species, except *P. burqinensis* sp. n. and *P. tianchiensis*, by having a hook-shaped conductor, and can be distinguished from these two species by the small and narrow median apophysis (the broad and nearly fins-shaped apophysis in *P. burqinensis* sp. n. and *P. tianchiensis*)

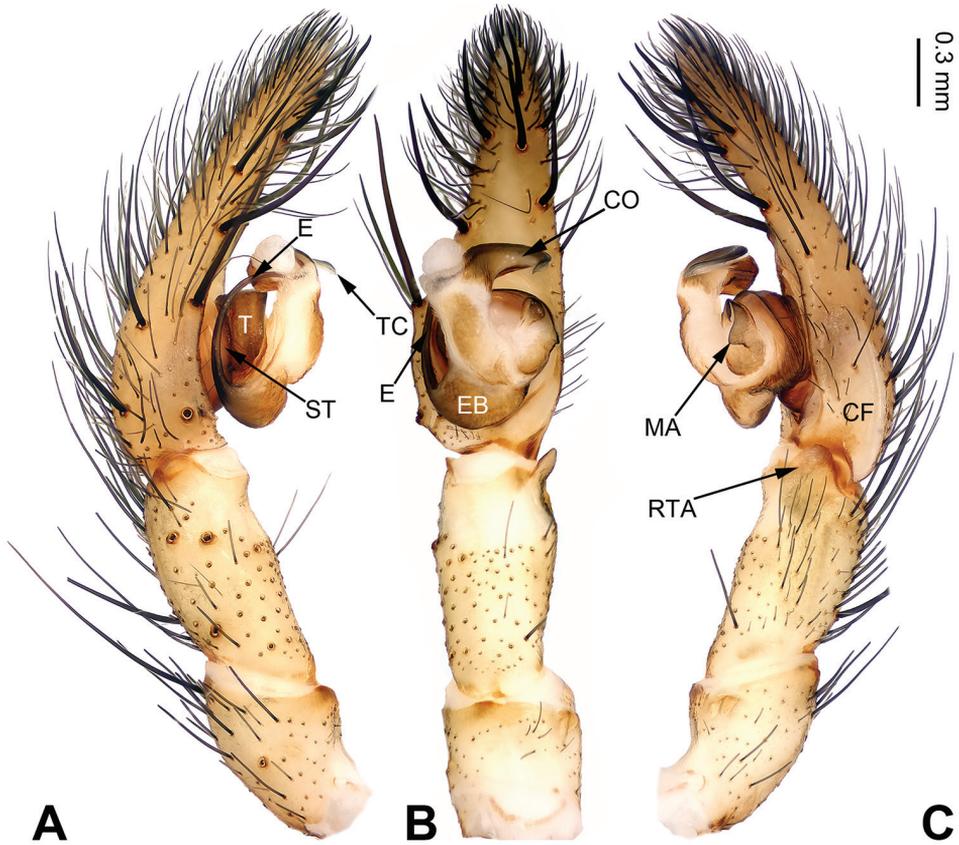


Figure 3. Left palp of *Pireneitega fuyunensis* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A, B, C**.

(cf. Figs 1, 3 and 12; Wang et al.1990: figs 81–83). The female can be distinguished from all other *Pireneitega* species, except *P. burqinensis* sp. n. and *P. tianchiensis*, by having short receptacles and the large epigynal atrium, and can be distinguished from these two species by the receptacles, about 1.5 times as long as wide (about two times longer than wide in *P. burqinensis* sp. n. and about 1.2 times in *P. tianchiensis*) (cf. Figs 2A–B, 4A–B and 13A–B; Wang et al. 1990: figs 84–85).

Description. Male (holotype): Total length 12.18. Carapace 5.45 long, 4.75 wide. Abdomen 6.73 long, 3.50 wide. Eye sizes and interdistances: AME 0.26, ALE 0.30, PME 0.25, PLE 0.25; AME-AME 0.08, AME-ALE 0.10, PME-PME 0.20, PME-PL 0.20. Leg measurements: I: 22.63 (6.25, 7.69, 6.09, 2.60); II: 22.15 (6.20, 7.45, 6.00, 2.50); III: 20.75 (5.50, 7.00, 6.00, 2.25); IV: 25.67 (7.05, 8.33, 7.69, 2.60). Carapace beige, the radial grooves indistinct. Abdomen blackish, with gray herringbone pattern. Palp: patellar apophysis absent; tibia long, about 1/2 length of the cymbium; RTA short, about 1/4 of tibial length; cymbial furrow short, about 1/4

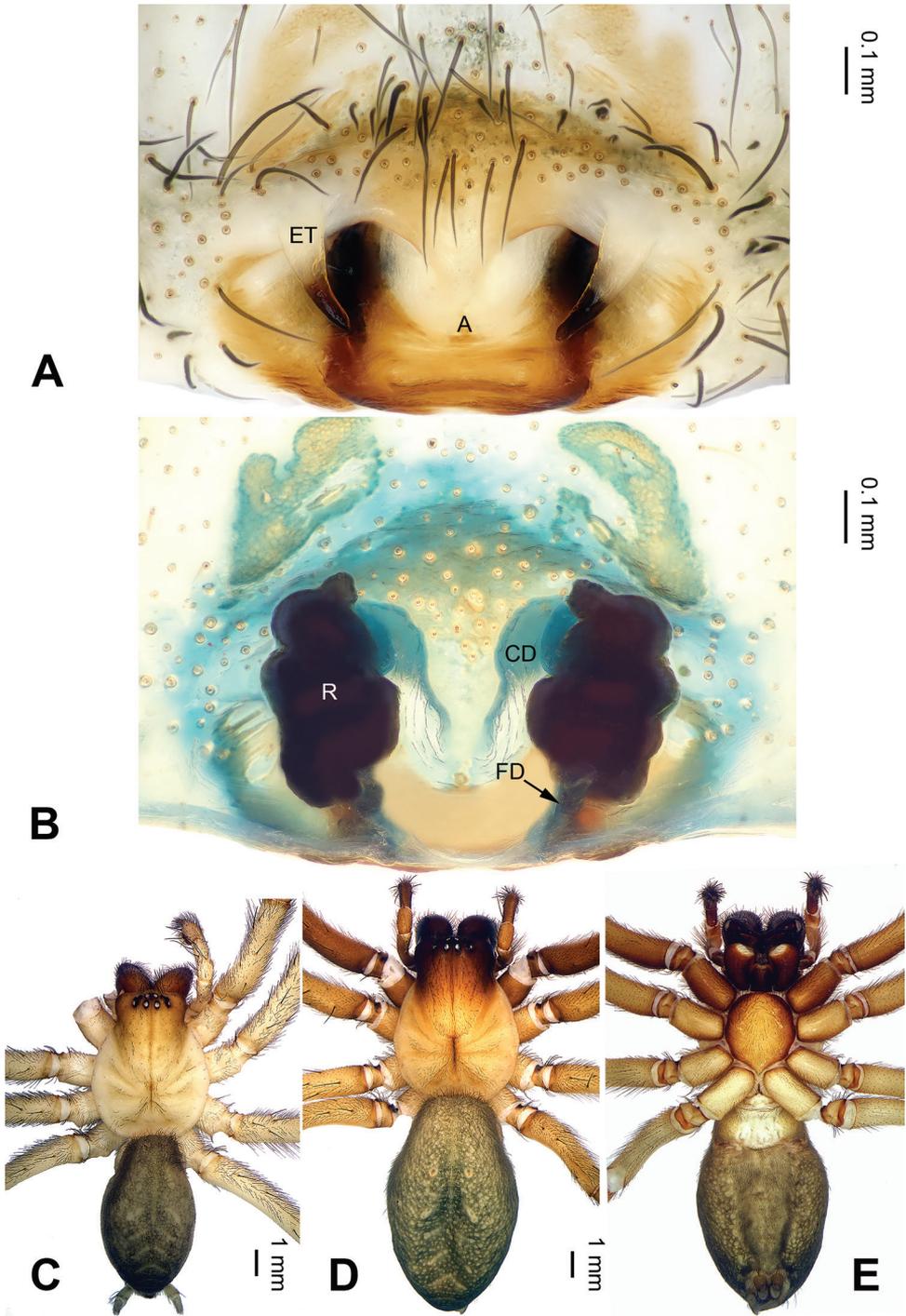


Figure 4. *Pireneitega fuyunensis* sp. n., female paratype and male holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D**, **E**.

length of cymbium; conductor short, with tapering apex, with one loop; embolus with nearly tongue-shaped base, beginning at 7:00 o'clock position (Fig. 3A–C).

Female (one of paratypes): Total length 13.80. Carapace 5.25 long, 4.50 wide. Abdomen 8.55 long, 4.75 wide. Eye sizes and interdistances: AME 0.25, ALE 0.30, PME 0.24, PLE 0.24; AME-AME 0.10, AME-ALE 0.10, PME-PME 0.20, PME-PLE 0.28. Leg measurements: I: 18.75 (5.50, 6.50, 4.50, 2.25); II: 18.00 (5.25, 6.25, 4.50, 2.00); III: 16.98 (4.99, 5.74, 4.50, 1.75); IV: 21.60 (6.10, 7.25, 6.03, 2.22). Carapace yellowish, with brown lateral margins. Abdomen khaki, with yellow sigilla and herringbone pattern. Epigyne: epigynal teeth long and thin, about 0.8 times as long as epigynal atrium; septum about 0.5 times as long as wide, with the weakly sclerotized tip, nearly triangular; epigynal atrium about 1.5 times as long as wide, with well delimited posterior margin, about two times as long as septum, about 0.7 times as wide as septum; receptacles about 1.5 times as long as wide; copulatory opening distinct; hoods indistinct (Fig. 4A–B).

Distribution. Known only from the type locality (Fig. 17).

***Pireneitega gongliuensis* Zhao & Li, sp. n.**

<http://zoobank.org/31392283-8A5E-4011-9EEA-D84A3CB4D54B>

Figs 5–6, 17

Type material. Holotype ♂: China: Xinjiang, Ili Kazakh Autonomous Prefecture: Gongliu County, N43°22'23", E81°51'45", 1515 m, 9.VIII.2013, J. Liu, K. Meng, Z. Yao, and Z. Zhao. **Paratypes:** 2♀2♂, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male can be distinguished from all other *Pireneitega* species, except *P. involuta* and *P. xinping*, by having a broad conductor and thick patellar apophysis, and can be distinguished from these two species by the tapering tip of conductor (the rounded tip of conductor in *P. involuta* and *P. xinping*) (cf. Fig. 5; Wang et al. 1990: figs 13–15; Zhang et al. 2002: figs 9–10). The female can be distinguished from all other *Pireneitega* species, except for *P. xinping*, by having large copulatory ducts, and can be distinguished from this species by the short and thick epigynal teeth, about 0.5 times as long as epigynal atrium (the long and narrow epigynal teeth in *P. xinping*, subequal to the length of epigynal atrium) (cf. Fig. 6A–B; Zhang et al. 2002: figs 7–8).

Description. Male (holotype): Total length 13.27. Carapace 6.09 long, 4.49 wide. Abdomen 7.18 long, 3.21 wide. Eye sizes and interdistances: AME 0.20, ALE 0.20, PME 0.23, PLE 0.22; AME-AME 0.08, AME-ALE 0.08, PME-PME 0.13, PME-PLE 0.25. Leg measurements: I: 19.87 (5.14, 6.73, 5.00, 3.00); II: 18.50 (5.00, 6.00, 4.75, 2.75); III: 16.70 (4.60, 5.10, 4.50, 2.50); IV: 21.59 (5.45, 6.73, 6.41, 3.00). Carapace yellow. Abdomen gray, with nearly chevrons-shaped stripes. Palp: patellar apophysis long, more than half of the tibia; tibia short, about 1/4 of tarsus; RTA subequal to the tibial length; cymbial furrow long, more than half of cymbium;

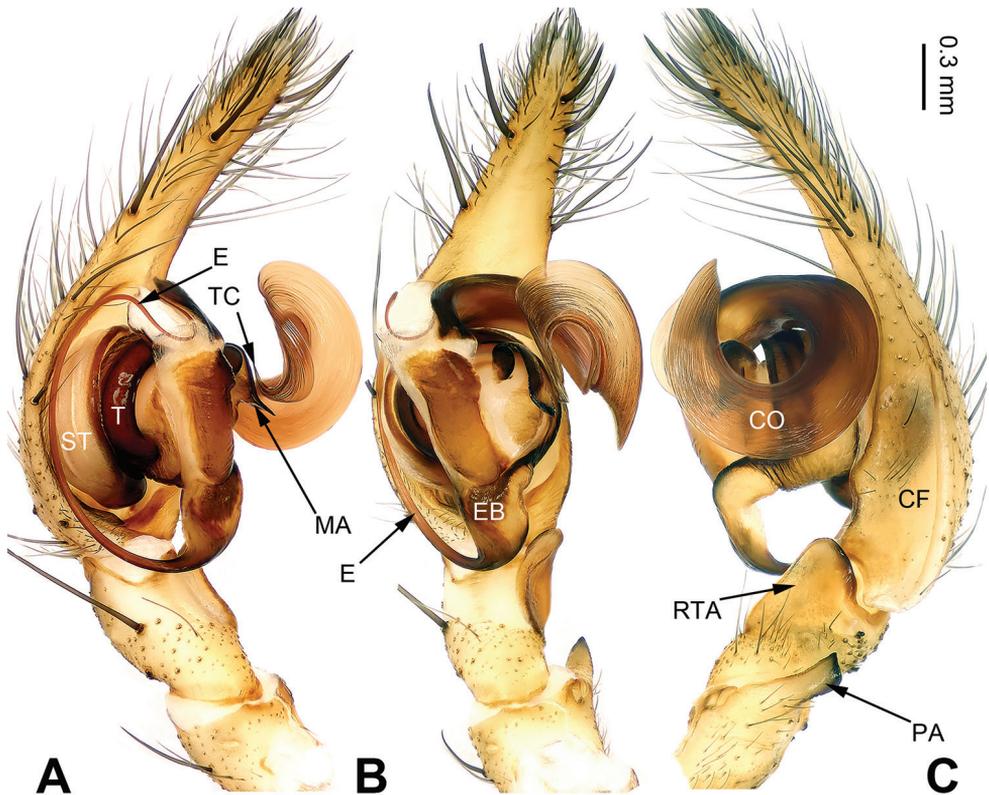


Figure 5. Left palp of *Pireneitega gongliuensis* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A, B, C**.

conductor broad and long, with two loops; embolus with broad base, beginning at 5:30 o'clock position (Fig. 5A–C).

Female (one of paratypes): Total length 12.18. Carapace 5.13 long, 3.80 wide. Abdomen 7.05 long, 3.50 wide. Eye sizes and interdistances: AME 0.20, ALE 0.25, PME 0.21, PLE 0.22; AME-AME 0.10, AME-ALE 0.07, PME-PME 0.15, PME-PLE 0.20. Leg measurements: I: 15.28 (4.50, 5.26, 3.40, 2.12); II: 14.91 (4.40, 5.13, 3.33, 2.05); III: 12.99 (3.72, 4.17, 3.30, 1.80); IV: 17.96 (4.75, 5.96, 5.00, 2.25). Carapace yellowish. Abdomen gray, with green spots. Epigyne: epigynal teeth about 0.5 times as long as atrium, light-colored; septum about 0.5 times as long as wide; atrium large, the length subequal to the width, with weakly sclerotized posterior margin, about 1.3 times as long as septum, about 0.6 times as long as septum; receptacles about 1.5 times as long as wide, almost covered by copulatory ducts; copulatory opening distinct; hoods distinct (Fig. 6A–B).

Distribution. Known only from the type locality (Fig. 17).

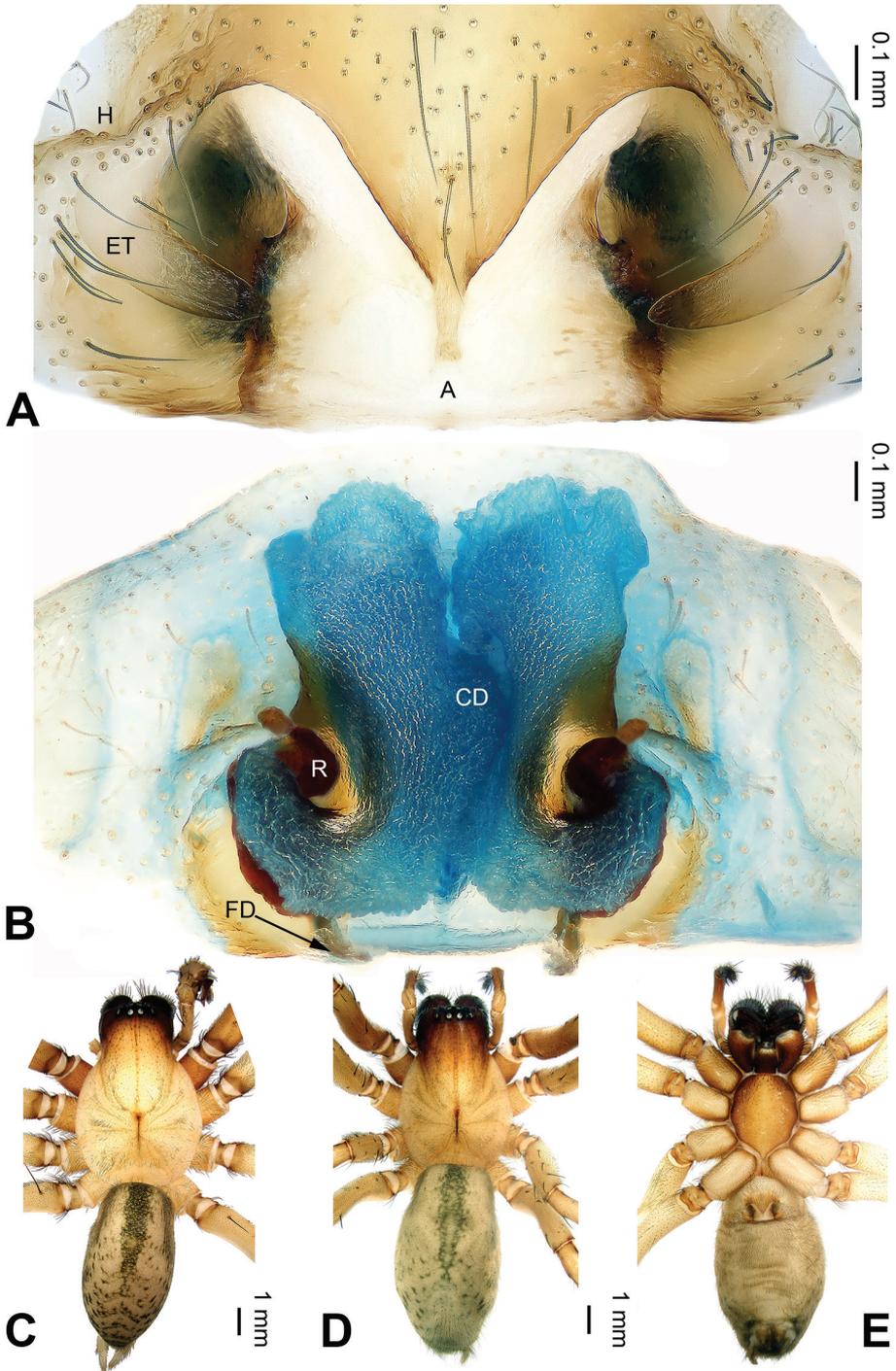


Figure 6. *Pireneitega gongliuensis* sp. n., female paratype and male holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D, E**.

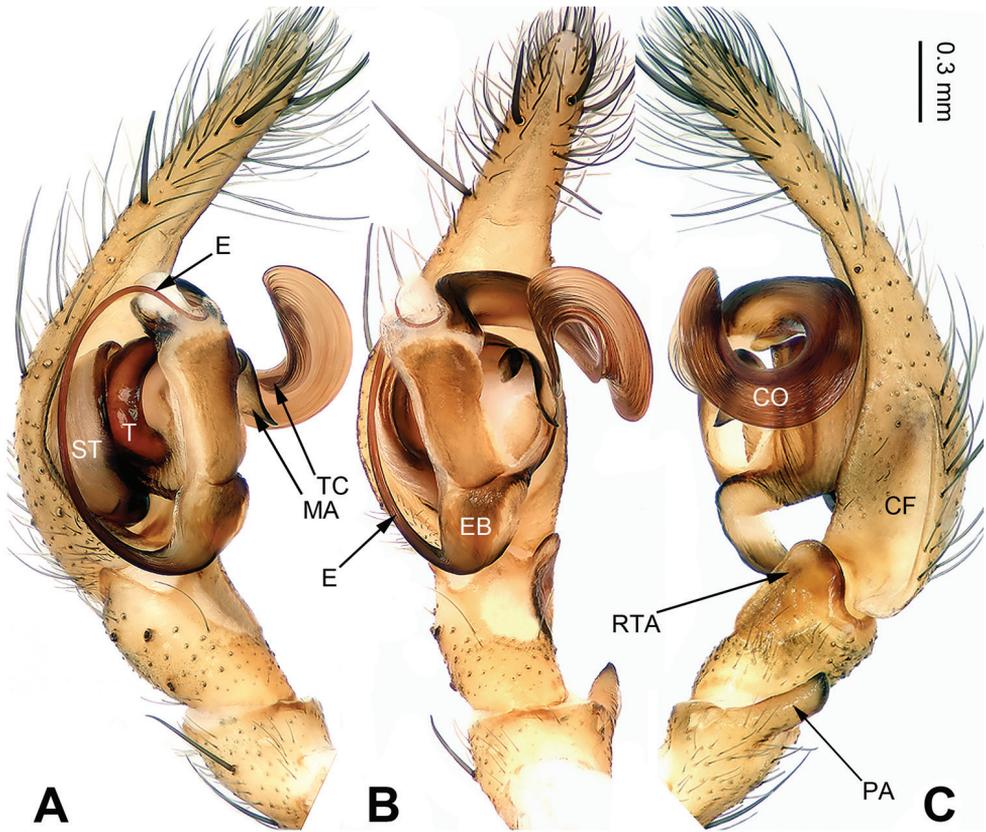


Figure 7. Left palp of *Pireneitega huochengensis* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A, B, C**.

***Pireneitega huochengensis* Zhao & Li, sp. n.**

<http://zoobank.org/C537A7D3-8C52-4740-A699-0C104A51640C>

Figs 7–8, 17

Type material. Holotype ♂: China: Xinjiang, Ili Kazakh Autonomous Prefecture: Huocheng County, Sarbulak town, N44°13'14", E81°10'13", 987 m, 4.VIII.2013, J. Liu, K. Meng, Z. Yao, and Z. Zhao. **Paratypes:** 3♀3♂, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male can be distinguished from all other *Pireneitega* species, except *P. gongliuensis* sp. n., *P. involuta* and *P. xinping*, by having a broad conductor and thick patellar apophysis, and can be distinguished from these species by the blunt tip of patellar apophysis (the tapering tip in *P. involuta* and *P. xinping*, the nearly rectangular in *P. gongliuensis* sp. n.) (cf. Figs 5 and 7; Wang et al. 1990: figs 13–15; Zhang et al. 2002: figs 9–10). The female can be distinguished from all other *Pireneitega* species, except for *P. gongliuensis* sp. n., by having long and broad epigynal teeth, and can be

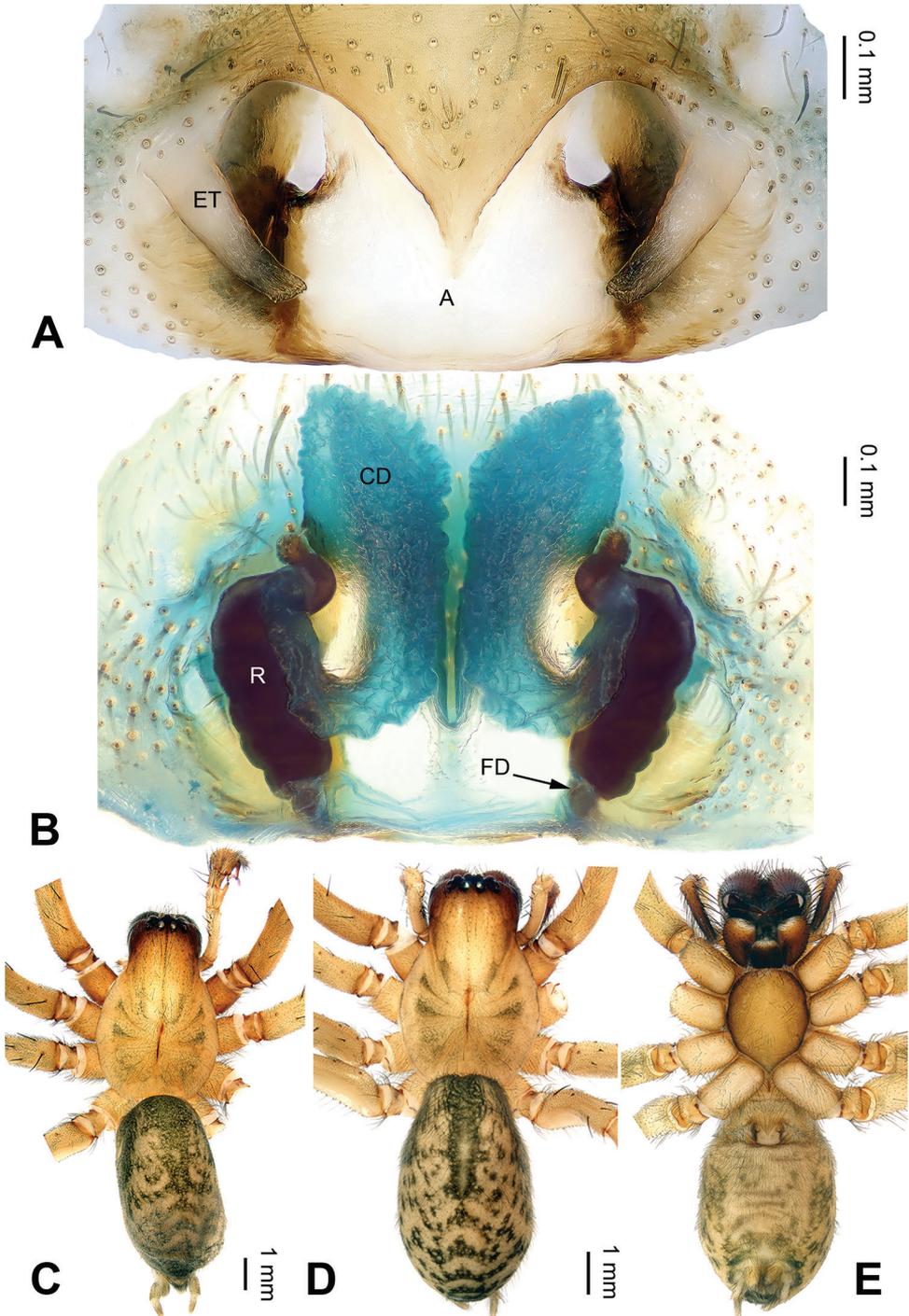


Figure 8. *Pireneitega huochengensis* sp. n., female paratype and male holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D**, **E**.

distinguished from this species by the blunt tip of epigynal teeth (the tapering tip in *P. gongliuensis* sp. n.) (cf. Figs 6A–B and 8A–B).

Description. Male (holotype): Total length 11.54. Carapace 5.13 long, 3.60 wide. Abdomen 6.41 long, 3.00 wide. Eye sizes and interdistances: AME 0.20, ALE 0.24, PME 0.24, PLE 0.24; AME-AME 0.12, AME-ALE 0.06, PME-PME 0.14, PME-PLE 0.16. Leg measurements: I: 15.15 (4.00, 5.20, 3.75, 2.20); II: 14.15 (3.75, 4.75, 3.65, 2.00); III: 13.30 (3.50, 4.25, 3.75, 1.80); IV: 17.50 (4.75, 5.40, 5.10, 2.25). Carapace yellow. Abdomen black, with 2 types of spots, one group yellow, and another transversal spots. Palp: patellar apophysis long, subequal to the tibial length; tibia short, about 1/4 of the cymbium length; RTA long, subequal to the tibial length; cymbial furrow short, about 1/3 length of cymbium; conductor broad, with broad apex, with two loops; embolus with broad base, beginning at 6:00 o'clock position (Fig. 7A–C).

Female (one of paratypes): Total length 9.94. Carapace 4.49 long, 3.05 wide. Abdomen 5.45 long, 2.90 wide. Eye sizes and interdistances: AME 0.18, ALE 0.23, PME 0.24, PLE 0.30; AME-AME 0.10, AME-ALE 0.05, PME-PME 0.15, PME-PLE 0.10. Leg measurements: I: 11.25 (3.25, 4.00, 2.50, 1.50); II: 10.30 (3.00, 3.50, 2.50, 1.30); III: 9.70 (2.75, 3.00, 2.65, 1.30); IV: 13.75 (3.75, 4.25, 4.00, 1.75). Carapace yellow, with brown lateral margins. Abdomen yellow, with black and nearly chevrons-shaped stripes. Epigyne: epigynal teeth thick, about 0.7 times as long as epigynal atrium; septum with the well delimited tip, about 0.3 times as long as wide; atrium with well delimited posterior margin, the length subequal to the width, about 2.3 times as long as septum, about 0.8 times as wide as septum; receptacles long, about 2.7 times as long as wide; copulatory opening distinct; hoods indistinct (Fig. 8A–B).

Distribution. Known only from the type locality (Fig. 17).

***Pireneitega lini* Zhao & Li, sp. n.**

<http://zoobank.org/8269EC62-A11C-4CAC-A2E9-A8D1CAC6B1A3>

Figs 9, 17

Type material. Holotype ♀: China: Xinjiang, Kizilsu Kirghiz Autonomous Prefecture: Akto County, N38°57'31", E75°30'16", 1833 m, 2.VIII.2014, Y. Lin.

Etymology. The specific name is a patronym in honor of the collector Yucheng Lin; noun (name) in genitive case.

Diagnosis. The female can be distinguished from all other *Pireneitega* species, except for *P. luniformis*, by having spiral receptacles, and can be distinguished from this species by the narrow and straight epigynal teeth (the broad and bent epigynal teeth in *P. luniformis*) (cf. Fig. 9A–B; Zhu and Wang 1994: figs 5–6).

Description. Female (holotype): Total length 13.82. Carapace 5.49 long, 4.50 wide. Abdomen 8.33 long, 4.50 wide. Eye sizes and interdistances: AME 0.25, ALE 0.30, PME 0.25, PLE 0.24; AME-AME 0.10, AME-ALE 0.08, PME-PME 0.19,

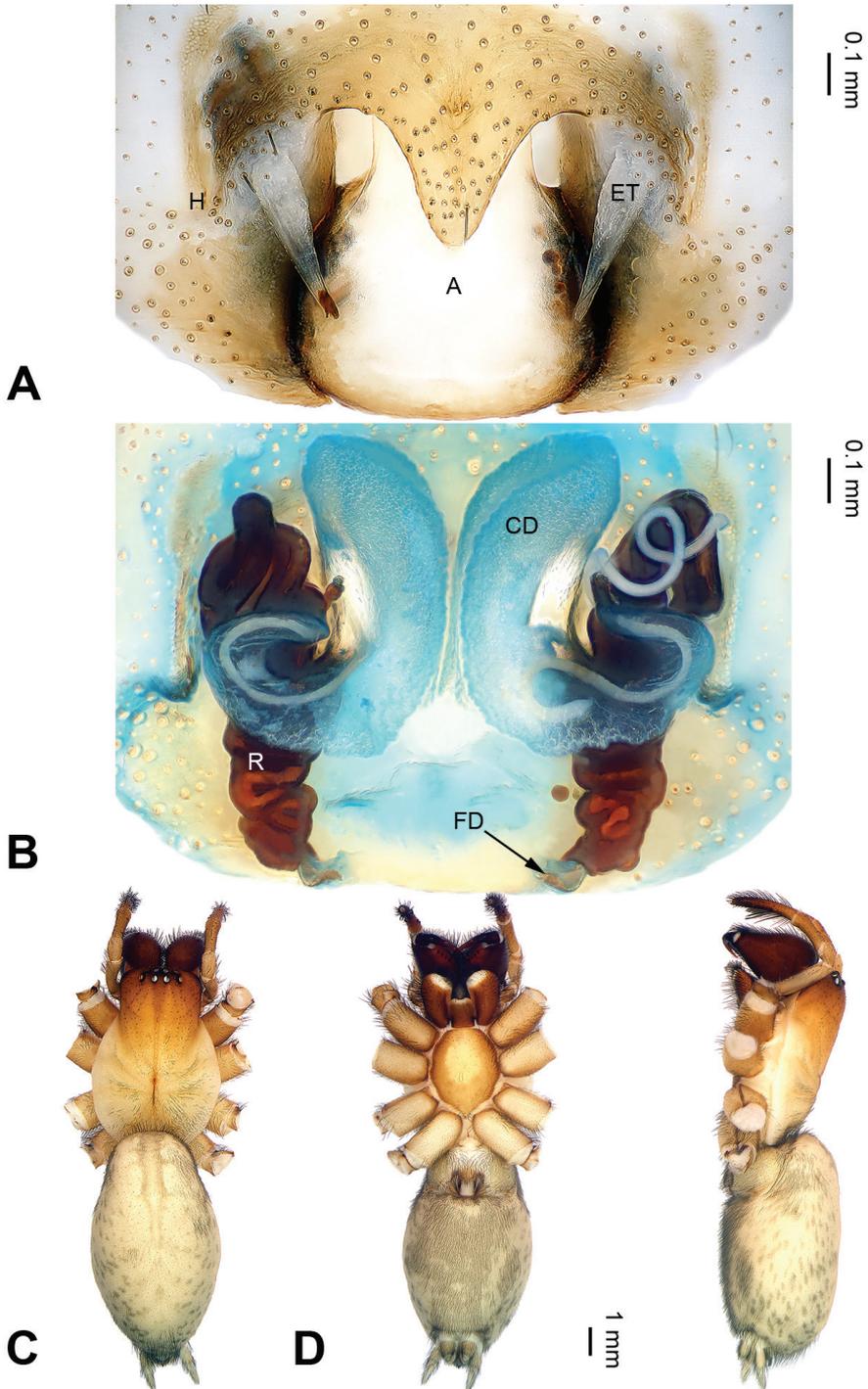


Figure 9. *Pireneitega lini* sp. n., female holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Female habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **C, D, E**.

PME-PLE 0.25. Leg measurements: I: 18.14 (5.00, 6.41, 4.17 2.56); II: 16.99 (4.81, 5.77, 4.17, 2.24); III: 16.69 (4.49, 5.45, 4.50, 2.25); IV: 21.68 (5.78, 6.73, 6.41, 2.76). Carapace yellow. Abdomen beige, with gray spots. Epigyne: epigynal teeth long and narrow, about 0.7 times as long as epigynal atrium; septum with the well delimited tip, about 0.6 times as long as wide; epigynal atrium with well delimited posterior margin, about two times as long as wide, about 2.5 times as long as septum, about 0.7 times as wide as septum; receptacles long, about three times as long as wide, mightily spiral; copulatory opening distinct; hoods distinct (Fig. 9A–B).

Distribution. Known only from the type locality (Fig. 17).

***Pireneitega liui* Zhao & Li, sp. n.**

<http://zoobank.org/A633D7BA-526B-4393-BFD9-DDFE370EC052>

Figs 10–11, 17

Type material. Holotype ♂: China: Xinjiang, Ili Kazakh Autonomous Prefecture: Xinyuan County, N43°21'58", E84°21'34", 2010 m, 12.VIII.2013, J. Liu. **Paratype:** 1♀, same data as holotype.

Etymology. The specific name is a patronym in honor of the collector Jincheng Liu; noun (name) in genitive case.

Diagnosis. The male can be easily distinguished from all the other *Pireneitega* species, except *P. luniformis*, by having a long and narrow conductor, and can be distinguished from this species by the blunt tip of the patellar apophysis (the tapering tip of conductor and the patellar apophysis in *P. luniformis*) (cf. Fig. 10; Zhu and Wang 1994: figs 7–8). The female can be distinguished from all other *Pireneitega* species, except *P. major* (Kroneberg, 1875) by having the nearly trapezoidal epigynal atrium, and can be distinguished from this species by the abrupt tip of epigynal teeth (the pointed tip of teeth in *P. major*) (cf. Fig. 11A–B; Kroneberg 1875: fig. 6).

Description. Male (holotype): Total length 8.25. Carapace 3.75 long, 2.85 wide. Abdomen 4.50 long, 2.50 wide. Eye sizes and interdistances: AME 0.18, ALE 0.25, PME 0.18, PLE 0.18; AME-AME 0.08, AME-ALE 0.05, PME-PME 0.10, PME-PLE 0.18. Leg measurements: I: 12.55 (3.50, 4.25, 2.80, 2.00); II: 11.75 (3.25, 3.75, 3.00, 1.75); III: 11.00 (3.00, 3.50, 3.00, 1.50); IV: 15.00 (4.00, 4.50, 4.50, 2.00). Carapace yellow, the radial grooves indistinct, with black lateral margins. Abdomen brown, with yellow herringbone pattern. Palp: patellar apophysis thick, with the blunt tip; RTA long, subequal to the length of tibia, without pointed tip; cymbial furrow short, about 1/3 length of cymbium; conductor broad and curving, with blunt apex; median apophysis small, with pointed tip; embolus with broad base, beginning at 6:00 o'clock position (Fig. 10A–C).

Female (paratype): Total length 8.75. Carapace 4.00 long, 3.25 wide. Abdomen 4.75 long, 3.00 wide. Eye sizes and interdistances: AME 0.18, ALE 0.25, PME 0.20, PLE 0.20; AME-AME 0.05, AME-ALE 0.08, PME-PME 0.15, PME-PLE 0.23. Leg

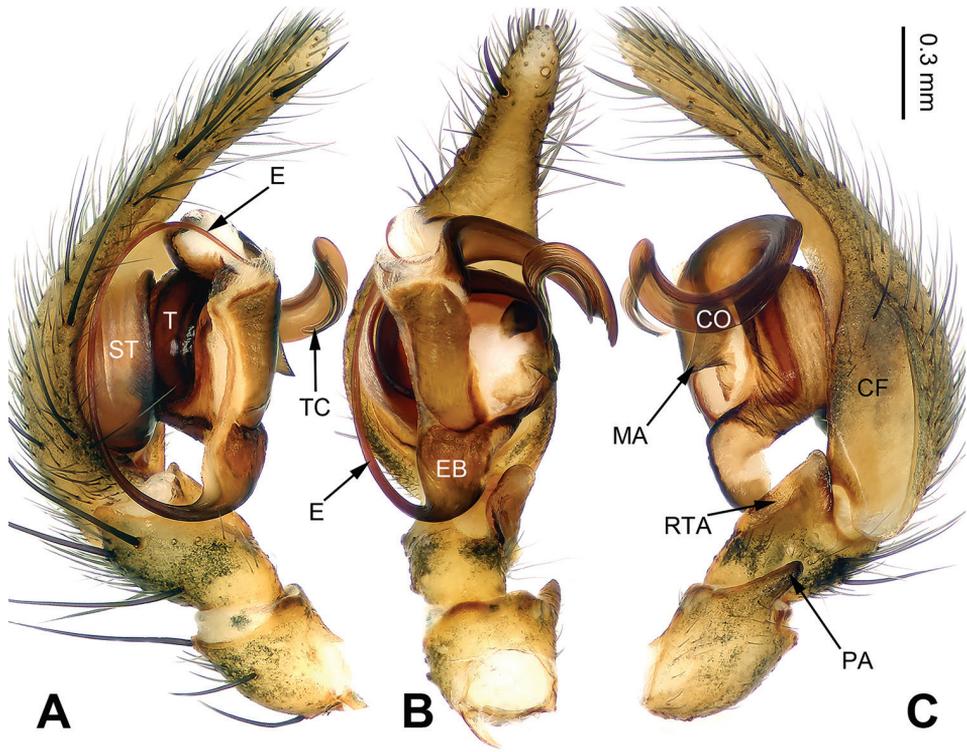


Figure 10. Left palp of *Pireneitega liui* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retro-lateral view. Scale bar: equal for **A, B, C**.

measurements: I: 11.40 (3.25, 4.00, 2.65, 1.50); II: 10.90 (3.25, 3.65, 2.50, 1.50); III: 10.25 (3.00, 3.25, 2.50, 1.50); IV: 13.00 (3.75, 4.25, 3.50, 1.50). Carapace yellow, with black lateral margins. Abdomen blackish, with yellow herringbone pattern. Epigyne: epigynal teeth broad and blunt, about 0.5 times as long as atrium; septum with the well delimited tip, about 0.5 times as long as wide; atrium with well delimited posterior margin, about 0.7 times as long as wide, about two times as long as septum, about 0.7 times as wide as septum; receptacles long, about 1.7 times as long as wide; copulatory opening distinct; hoods distinct (Fig. 11A–B).

Distribution. Known only from the type locality (Fig. 17).

***Pireneitega wensuensis* Zhao & Li, sp. n.**

<http://zoobank.org/0CFFC8E7-622A-4D9B-9F05-21D001F4B1AC>

Figs 14, 17

Type material. **Holotype** ♂: China: Xinjiang, Aksu Prefecture, Wensu County, Bozidun Kirgiz Village, N41°44'37", E80°43'05", 1991 m, 22.VII.2014, J. Wu.

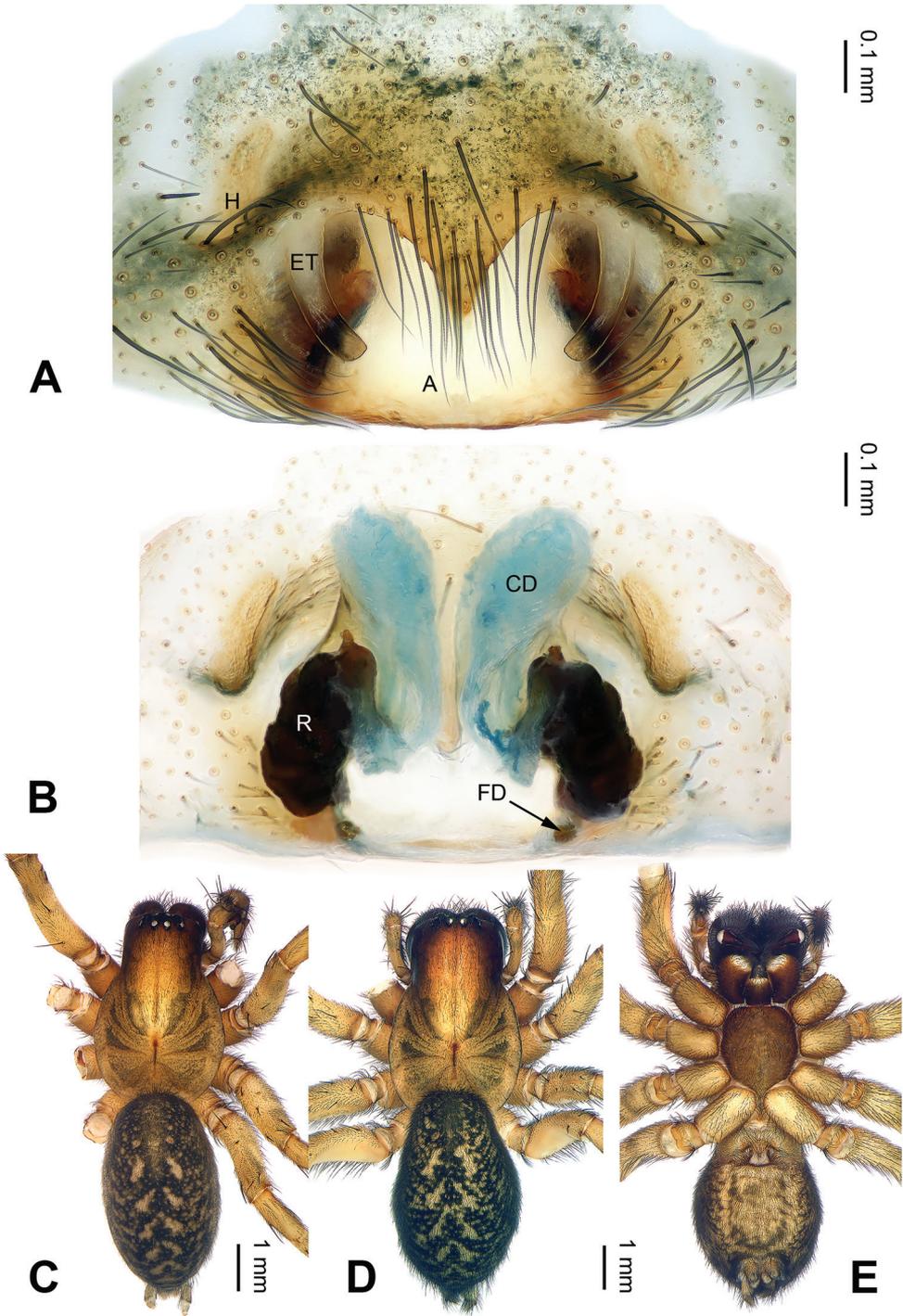


Figure 11. *Pireneitega liui* sp. n., female paratype and male holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D**, **E**.

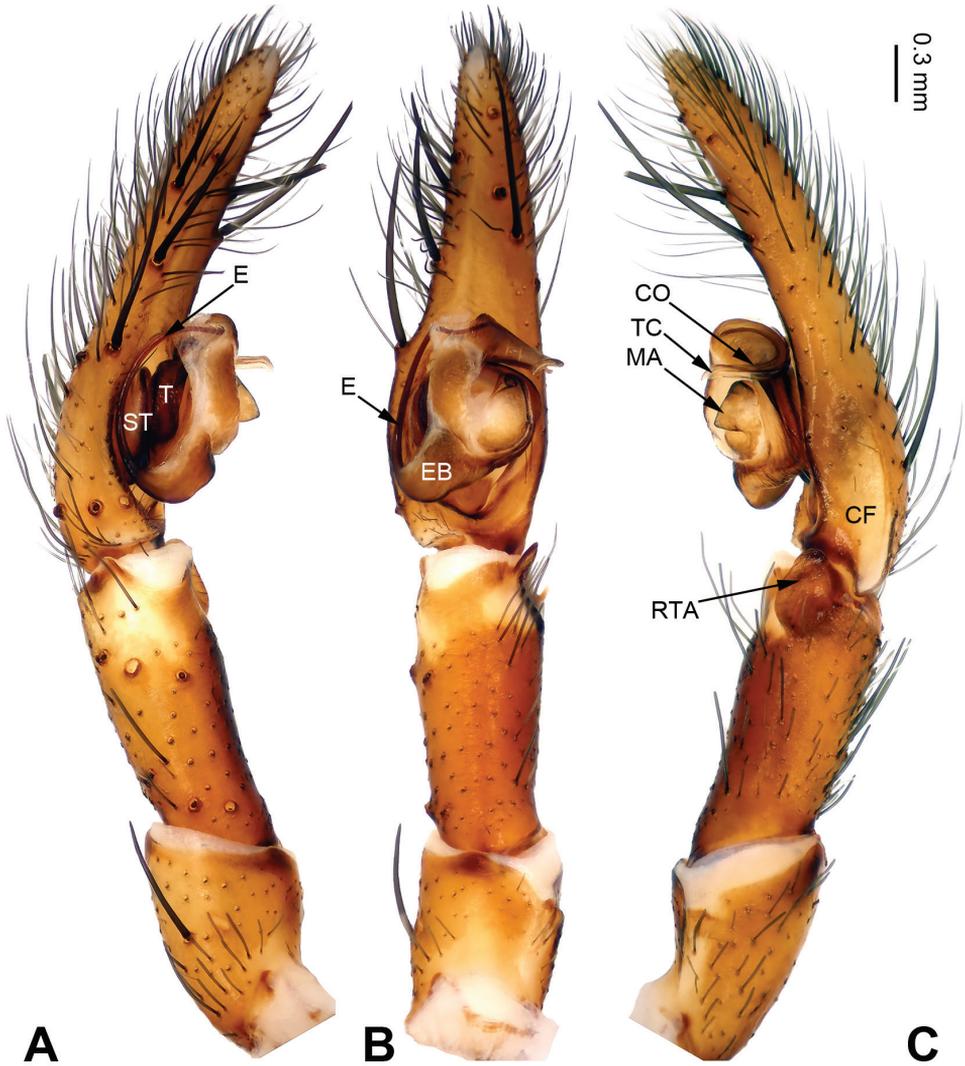


Figure 12. Left palp of *Pireneitega tianchiensis*, male from Crater Lake. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A, B, C**.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male can be distinguished from all other *Pireneitega* species, except *P. burqinensis* sp. n., *P. fuyunensis* sp. n. and *P. tianchiensis*, by having a hook-shaped conductor and the small bulb, and can be distinguished from these species by the long tibia, subequal to the length of cymbium (the short tibia, about 1/2 length of cymbium, in *P. burqinensis* sp. n., *P. fuyunensis* sp. n. and *P. tianchiensis*) (cf. Figs 1, 3, 12 and 14; Wang et al. 1990: figs 81–83).

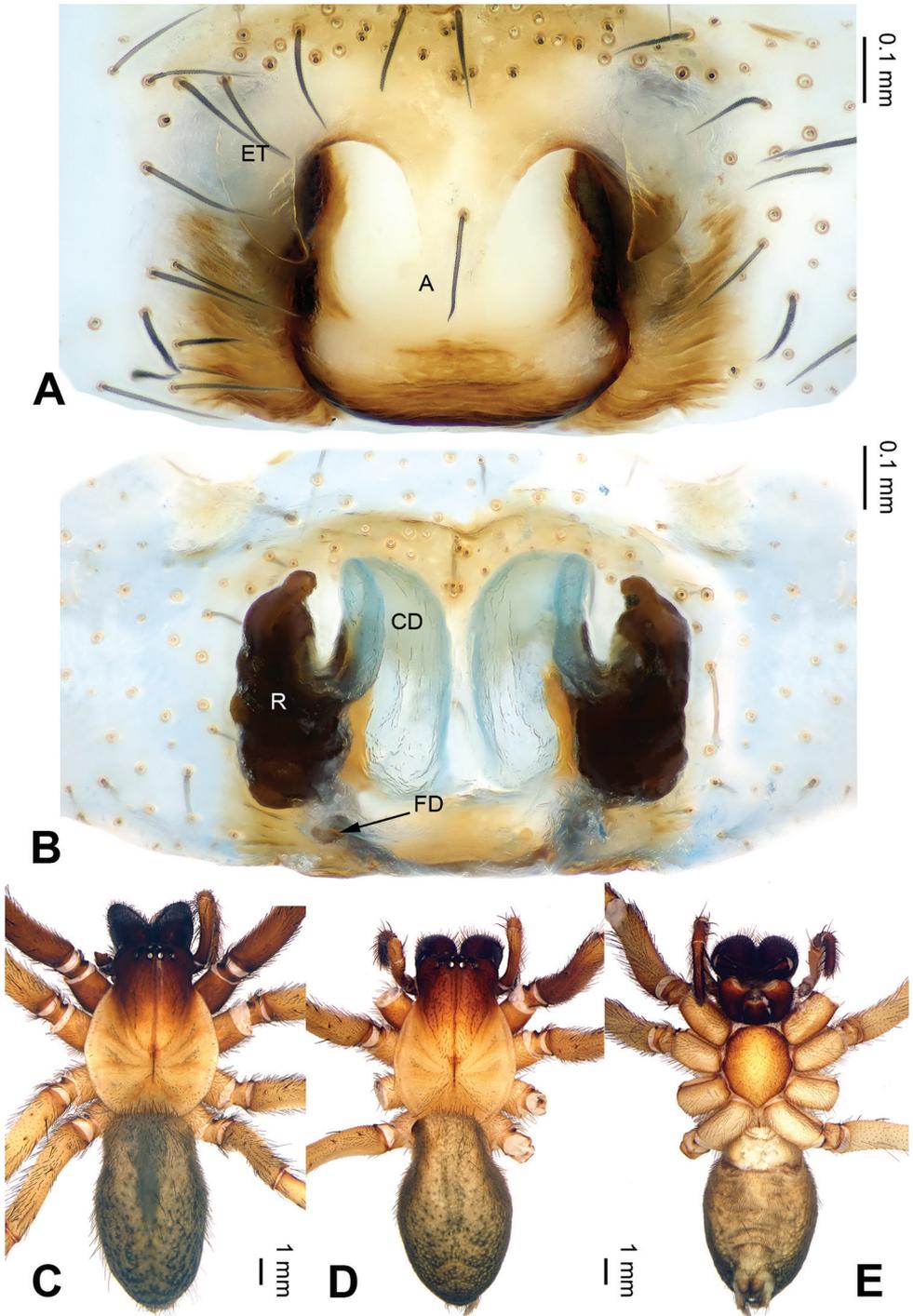


Figure 13. *Pireneitega tianchiensis*, specimens from Crater Lake. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D**, **E**.

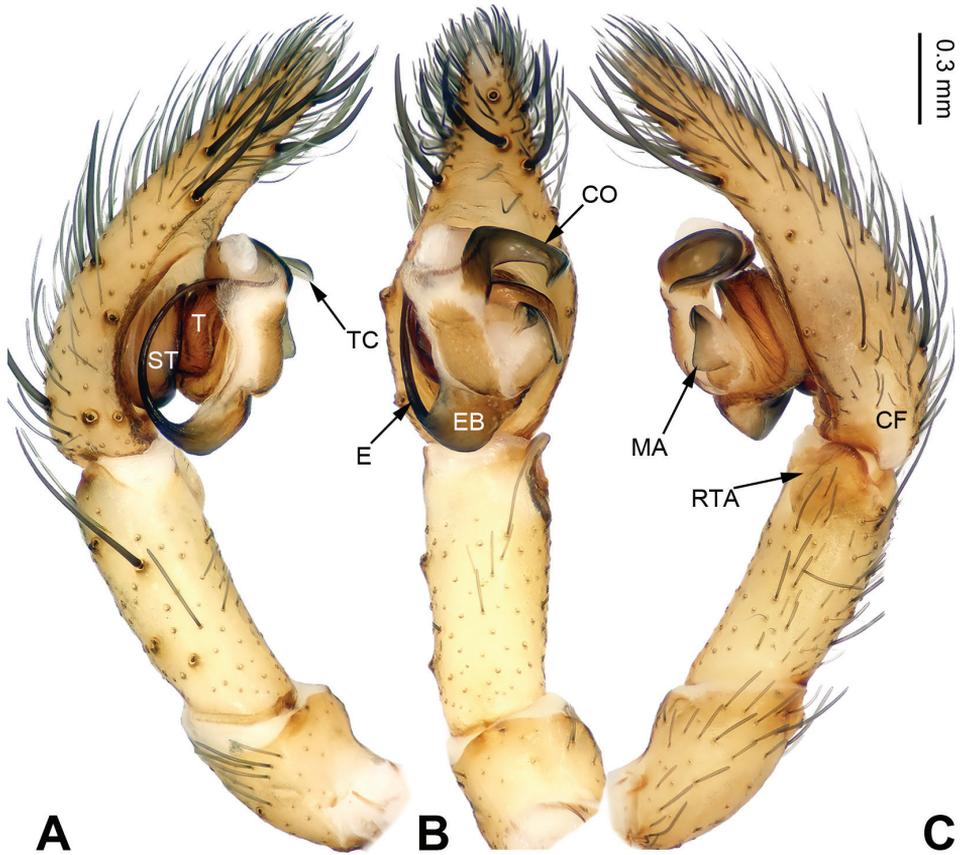


Figure 14. Left palp of *Pireneitega wensuensis* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A, B, C**.

Description. Male (holotype): Total length 11.75. Carapace 4.50 long, 3.75 wide. Abdomen 7.25 long, 4.25 wide. Eye sizes and interdistances: AME 0.20, ALE 0.30, PME 0.25, PLE 0.25; AME-AME 0.05, AME-ALE 0.05, PME-PME 0.13, PME-PLE 0.13. Leg measurements: I: 16.75 (5.00, 5.75, 3.75, 2.25); II: 15.75 (4.50, 5.50, 3.75, 2.00); III: 15.25 (4.50, 5.00, 3.75, 2.00); IV: 19.75 (5.50, 6.25, 5.50, 2.50). Carapace yellow, the radial grooves indistinct, with black lateral margins. Abdomen blackish, with yellow spots. Palp: patellar apophysis absent; palpal tibia long; RTA short, about 1/4 of tibial length; cymbial furrow short, about 1/5 length of cymbium; conductor short, with blunt apex; median apophysis broad and nearly the flipper-shaped; embolus with broad and nearly square base, beginning at 6:30 o'clock position (Fig. 14A–C).

Distribution. Known only from the type locality (Fig. 17).

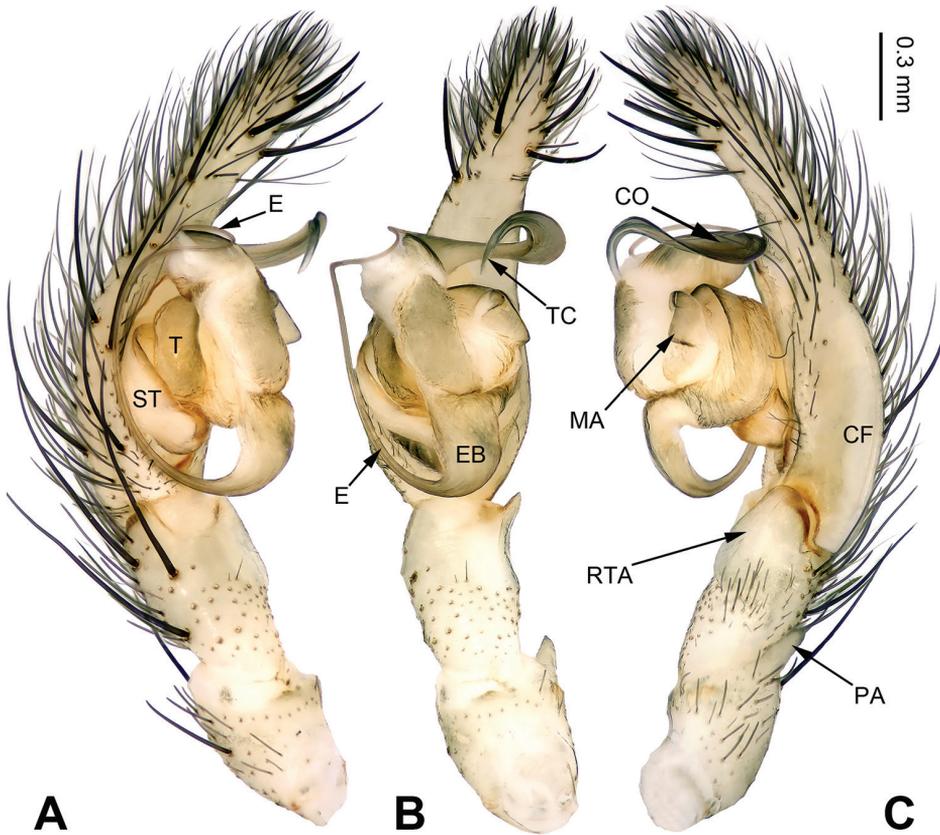


Figure 15. Left palp of *Pireneitega wui* sp. n., male holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **C**.

***Pireneitega wui* Zhao & Li, sp. n.**

<http://zoobank.org/D015D5F3-5D22-4545-8C81-FFFA6FE956FC>

Figs 15, 17

Type material. Holotype ♂: China: Xinjiang, Kizilsu Kyrgyz Autonomous Prefecture, Akqi County, N40°47'32", E78°15'48", 3020 m, 25.VII.2014, J. Wu.

Etymology. The specific name is a patronym in honor of the collector Jiānglāng Wu; noun (name) in genitive case.

Diagnosis. The male can be distinguished from all other *Pireneitega* species, except *P. armeniaca* by having bended and narrow conductor, and can be distinguished from this species by the blunt tip of median apophysis (the tapering tip of median apophysis in *P. armeniaca*) (cf. Fig. 15; Brignoli 1978: figs 117–121).

Description. Male (holotype): Total length 8.98. Carapace 3.85 long, 2.75 wide. Abdomen 5.13 long, 3.00 wide. Eye sizes and interdistances: AME 0.18,

ALE 0.23, PME 0.20, PLE 0.20; AME-AME 0.05, AME-ALE 0.05, PME-PME 0.10, PME-PLE 0.10. Leg measurements: I: 14.75 (4.25, 5.25, 3.25, 2.00); II: 13.00 (3.50, 4.50, 3.00, 2.00); III: 12.25 (3.50, 4.00, 3.25, 1.50); IV: 17.50 (5.00, 5.25, 5.00, 2.25). Carapace yellow. Abdomen beige, with gray spots. Palp: patellar apophysis thick; palpal tibia short, about 1/3 of cymbial length; RTA short, about 1/3 of tibial length; cymbial furrow short, about 1/3 length of cymbium; conductor narrow, with the tapering tip; median apophysis broad and nearly triangular; embolus with broad and nearly trapezoidal base, beginning at 7:00 o'clock position (Fig. 15A–C).

Distribution. Known only from the type locality (Fig. 17).

***Pireneitega yaoi* Zhao & Li, sp. n.**

<http://zoobank.org/9DFF584F-38EA-4FC3-AED2-176969E0D309>

Figs 16, 17

Type material. Holotype ♀: China: Xinjiang, Ili Kazakh Autonomous Prefecture: Tacheng (Tarbagatay) Prefecture, Hoboksar Mongol Autonomous County, Songshugou, N46°59'21", E85°57'20", 1858 m, 29.VII.2013, Z. Yao and Z. Zhao. **Paratype:** 1♀, same data as holotype.

Etymology. The specific name is a patronym in honor of the collector Zhiyuan Yao; noun (name) in genitive case.

Diagnosis. The female can be distinguished from all other *Pireneitega* species, except *P. burqinensis* sp. n., *P. fuyunensis* sp. n. and *P. tianchiensis*, by having the weakly sclerotized tip of septum. It can be distinguished from these three species by the nearly rectangular epigynal atrium (while *P. burqinensis* sp. n. has a nearly triangular atrium; and *P. tianchiensis* and *P. fuyunensis* sp. n., large and nearly square-shaped atrium) (cf. Figs 2A–B, 4A–B, 6A–B and 16A–B; Wang et al. 1990: figs 84–85).

Description. Female (holotype): Total length 12.25. Carapace 5.00 long, 4.25 wide. Abdomen 7.25 long, 4.50 wide. Eye sizes and interdistances: AME 0.23, ALE 0.27, PME 0.23, PLE 0.25; AME-AME 0.12, AME-ALE 0.08, PME-PME 0.13, PME-PLE 0.25. Leg measurements: I: 16.05 (4.90, 5.35, 3.75, 2.05); II: 15.55 (4.75, 5.05, 3.75, 2.00); III: 14.75 (4.50, 4.75, 3.50, 2.00); IV: 17.20 (5.00, 6.05, 4.05, 2.10). Carapace yellow, with black lateral margins. Abdomen greyish white. Epigyne: teeth long, subequal to the length of epigynal atrium; septum with weakly sclerotized posterior margin, about 0.3 times as long as wide; atrium with well delimited posterior margin, about 0.8 times as long as wide, about 1.7 times as long as septum, about 0.7 times as wide as septum; receptacles long, about 1.3 times as long as wide; copulatory opening indistinct; hoods indistinct (Fig. 16A–B).

Distribution. Known only from the type locality (Fig. 17).

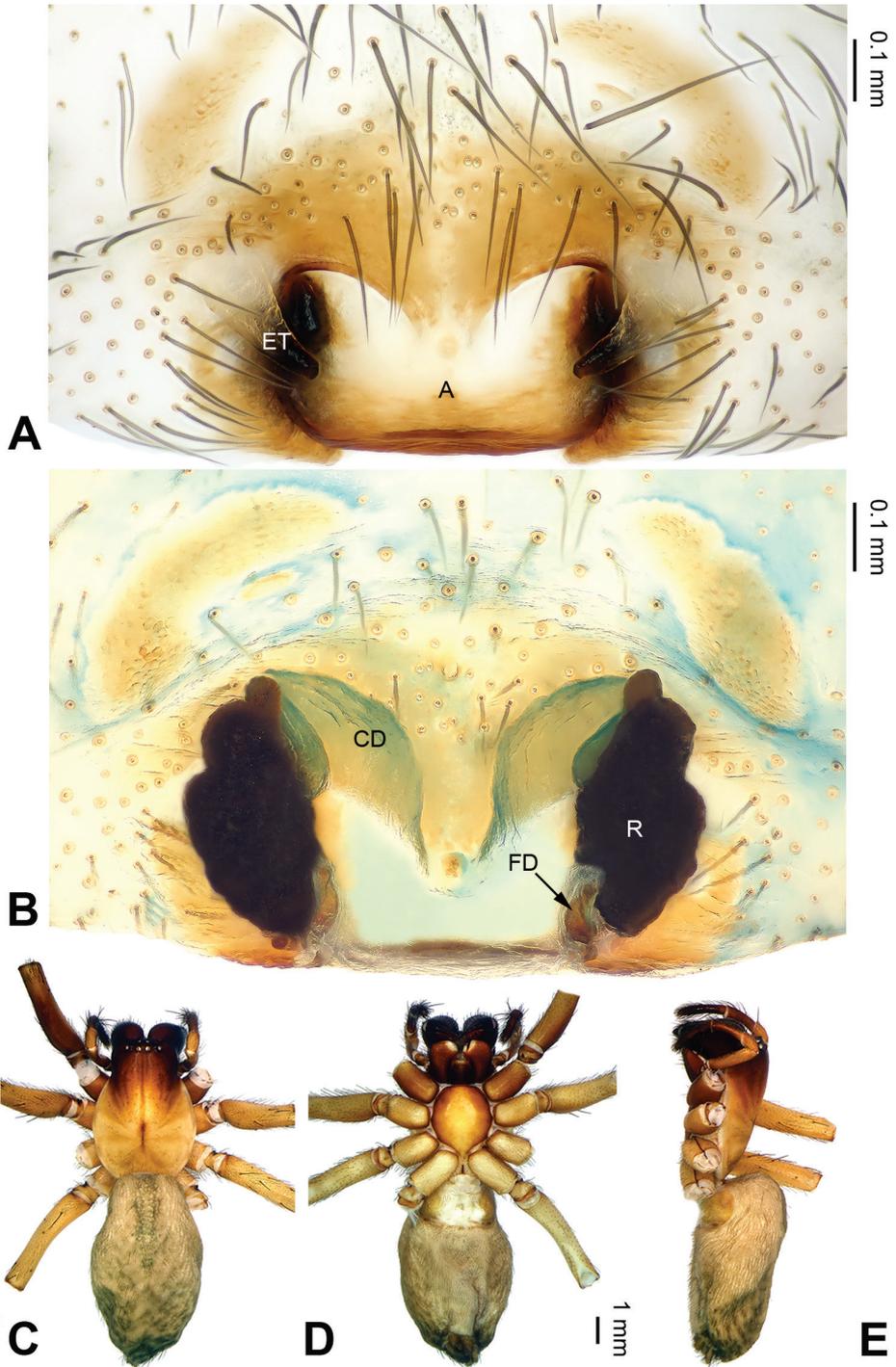


Figure 16. *Pireneitega yaoi* sp. n., female holotype. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Female habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **C, D, E**.

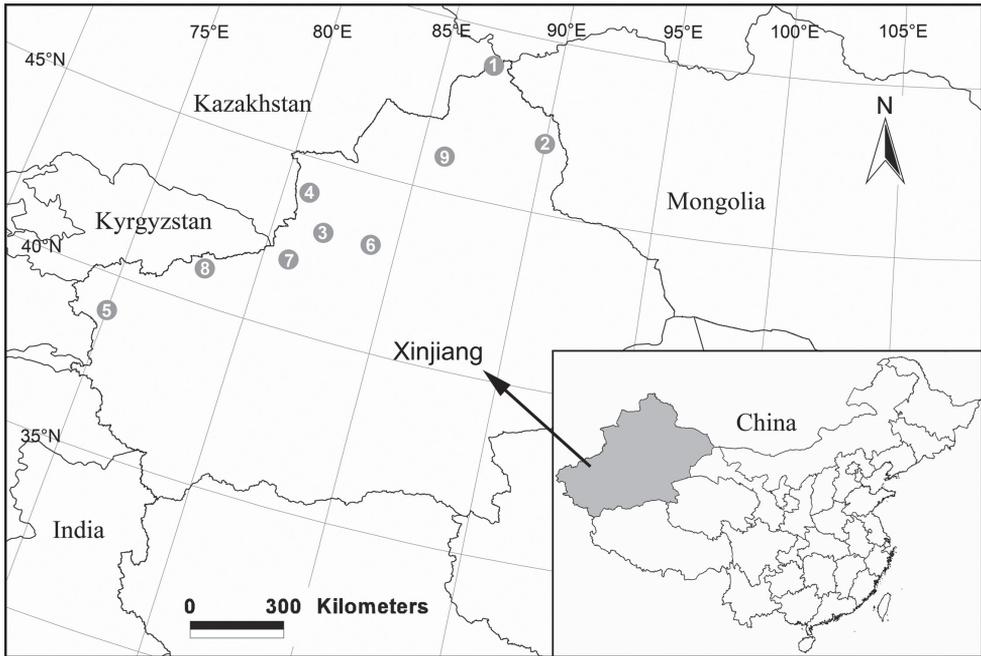


Figure 17. Localities of new *Pireneitega* species from Xinjiang, China. **1** *P. burqinensis* sp. n. **2** *P. fuyunensis* sp. n. **3** *P. gongliuensis* sp. n. **4** *P. huochengensis* sp. n. **5** *P. lini* sp. n. **6** *P. liui* sp. n. **7** *P. wensuensis* sp. n. **8** *P. wui* sp. n. **9** *P. yaoi* sp. n.

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A further study of the spider genus *Notiocoelotes* (Araneae, Agelenidae) from Hainan Island, China

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Abstract

Two new *Notiocoelotes* species, *N. maoganensis* sp. n. (♂♀) and *N. qiongzhongensis* sp. n. (♂♀) are described from Hainan Island, China. In addition, the female of *N. membranaceus* Liu & Li, 2010 is described for the first time. DNA barcodes of three species treated in this paper were obtained for future use.

Keywords

Taxonomy, Coelotinae, description, diagnosis, Southeast Asia

Introduction

The spider genus *Notiocoelotes* was established by Wang et al. (2008) for one coelotine species from Hainan Island, China: *Coelotes palinitropus* Zhu & Wang, 1994. Additionally, Wang et al. (2008) described three new species: *N. laosensis* Wang, Xu & Li, 2008, *N. lingulatus* Wang, Xu & Li, 2008, *N. vietnamensis* Wang, Xu & Li, 2008, and transferred *Iwogumoa sparus* Dankittipakul, Chami-Kranon & Wang, 2005 to *Notiocoelotes*. Currently, eleven species of *Notiocoelotes* are known (World Spider Catalog 2016), six of which are restricted to Hainan, China. This paper provides the descriptions of two new *Notiocoelotes* species and a redescription of *N. membranaceus*.

Material and methods

Specimens were examined with a LEICA M205C stereomicroscope. Images were captured with an Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 dissecting microscope. Epigynes and male palps were examined after dissection from the spiders' bodies.

All measurements were obtained using a LEICA M205C stereomicroscope and are given in millimeters. Leg measurements are shown as: Total length (femur, patella + tibia, metatarsus, tarsus). Only structures (palp and legs) of the left body side were described and measured. The terminology used in the text and the figure legends follows Wang (2002). Abbreviations used in this paper and in the figure legends: A = epigynal atrium; ALE = anterior lateral eye; AME = anterior median eye; AME-ALE = distance between AME and ALE; AME-AME = distance between AME and AME; ALE-PLE = distance between ALE and PLE; CD = copulatory duct; CF = cymbial furrow; CL = conductor lamella; CO = conductor; E = embolus; EB = embolic base; ES = epigynal scape; FD = fertilization duct; LTA = lateral tibial apophysis; MA = median apophysis; PLE = posterior lateral eye; PME = posterior median eye; PME-PLE = distance between PME and PLE; PME-PME = distance between PME and PME; R = receptacle; RTA = retroventral tibial apophysis; ST = subtegulum; T = tegulum.

DNA barcodes were obtained for future use. A partial fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced for *N. maoganensis* sp. n., *N. membranaceus* and *N. qiongzhongensis* sp. n. using primers LCO1490-ooon (5'-CWACAAAYCATARRGATATTGG-3') (Folmer et al. 1994; Miller et al. 2010) and HCO2198-zz (5'-TAAACTTCCAGGTGACCAAAAATCA-3') (Folmer et al. 1994; Chen et al. 2015). For additional information on extraction, amplification, and sequencing procedures, see Zhao et al. 2013. All sequences were deposited in GenBank and the accession numbers are provided in Table 1.

All specimens (including molecular vouchers) are deposited in the Institute of Zoology, Chinese Academy of Sciences in Beijing (IZCAS).

Table 1. Voucher specimen information.

Species	GenBank accession number	Sequence length	Collection localities
<i>Notiocoelotes maoganensis</i> sp. n.	KU886075	657 bp	Baoting County, Hainan, China
<i>Notiocoelotes membranaceus</i>	KU886076	666 bp	Qiongzhong County, Hainan, China
<i>Notiocoelotes qiongzhongensis</i> sp. n.	KU886074	666 bp	Qiongzhong County, Hainan, China

Taxonomy

Family Agelenidae C.L. Koch, 1837

Subfamily Coelotinae F.O.P.-Cambridge, 1893

Genus *Notiocoelotes* Wang, Xu & Li, 2008

Notiocoelotes Wang et al, 2008: 11. Type species *Coelotes palinitropus* Zhu & Wang, 1994, from Hainan Island, China.

Diagnosis. The chelicerae of all *Notiocoelotes* have 3 promarginal and 2 retromarginal teeth, while other coelotines usually have 3 or 4 retromarginal teeth. Females of this genus can be separated from other coelotines by the absence of epigynal teeth and the presence of a tongue-shaped epigynal scape (Fig. 2A–B); other coelotines usually have long and broad epigynal teeth. Males can be distinguished from other coelotines by the absence of a patellar apophysis, the presence of a large and strongly bifurcated lateral tibial apophysis and the reduced or invisible median apophysis (Fig. 1); other coelotines usually have a thick patellar apophysis and the special shaped median apophysis.

Composition. Thirteen *Notiocoelotes* species are currently known: *N. laosensis* (♀) from Laos; *N. parvitriangulus* Liu, Li & Pham, 2010 (♀), *N. pseudovietnamensis* Liu, Li & Pham, 2010 (♂♀) and *N. vietnamensis* (♂♀) from Vietnam; *N. sparus* (♂) from Thailand; *N. lingulatus* (♀), *N. membranaceus* (♂), *N. orbiculatus* Liu & Li, 2010 (♂♀), *N. palinitropus* (♂♀), *N. pseudolingulatus* Liu & Li, 2010 (♂♀), and *N. spirellus* Liu & Li, 2010 (♂♀) from Hainan, China (World Spider Catalog 2016), and two new species described in this paper: *N. maoganensis* sp. n. (♂♀), *N. qiongzhongensis* sp. n. (♂♀) from Hainan.

***Notiocoelotes maoganensis* Zhao & Li, sp. n.**

<http://zoobank.org/77DC1620-6C90-4167-BA47-CE30A39BF135>

Figs 1–2, 7

Type material. Holotype ♂: China: Hainan: Baoting County: Maogan Village, Xi-ananshilin Cave, N18°35'52", E109°25'37", 616 m, 26.VI.2014, F. Li & X. Wang.

Paratype: 1♀, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of *N. maoganensis* sp. n. can be easily distinguished from all other *Notiocoelotes* species, except *N. palinitropus*, by having a semi-circular conductor. From *N. palinitropus*, the male of the new species can be distinguished by the short cymbial furrow about 1/3 of cymbial length (while *N. palinitropus* male has a long cymbial furrow, about 0.5 times as long as cymbial length) (cf. Fig. 1A–C; Zhu and Wang 1994: figs 19–21). The female of *N. maoganensis* sp. n. can be easily distinguished from all the other *Notiocoelotes* species, except *N. palinitropus*, by the

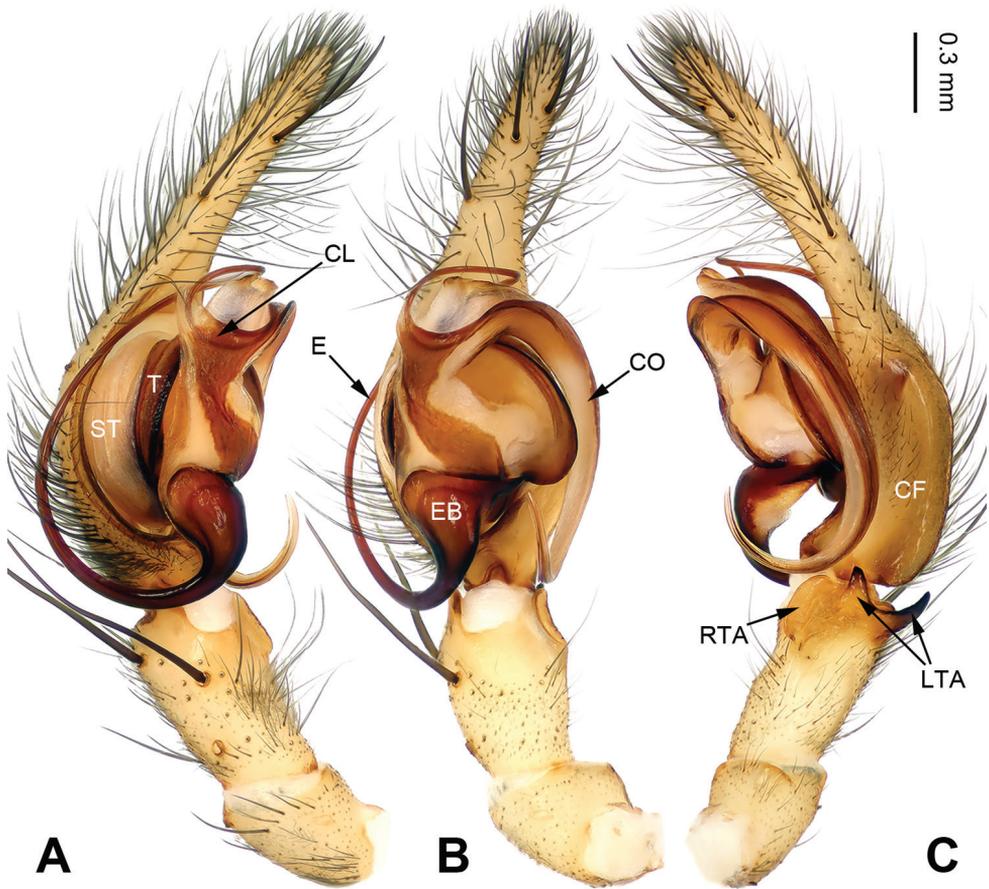


Figure 1. Left palp of *Noticoelotes maoganensis* sp. n., holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. CF = cymbial furrow; CL = conductor lamella; CO = conductor; E = embolus; EB = embolic base; LTA = lateral tibial apophysis; RTA = retroventral tibial apophysis; ST = subtegulum; T = regulum. Scale bar: Equal for **A**, **B** and **C**.

almost rectangular atrium. From *N. palinitropus*, the female of the new species can be distinguished by a broad atrium, about two times as long as wide (while *N. palinitropus* female has a narrow atrium, about three times as long as wide) (cf. Fig. 2A–B; Liu and Li 2010: fig. 9B).

Description. Male (holotype): Total length 8.60. Carapace 4.75 long, 3.50 wide. Abdomen 3.85 long, 2.75 wide. Eye sizes and interdistances: AME 0.20, ALE 0.23, PME 0.25, PLE 0.25; AME-AME 0.08, AME-ALE 0.02, PME-PME 0.08, PME-PLE 0.10. Leg measurements: I: 21.15 (5.75, 6.00, 5.50, 3.90); II: 18.00 (5.50, 5.25, 4.50, 2.75); III: 17.00 (5.00, 5.00, 4.75, 2.25); IV: 22.35 (6.25, 6.70, 6.50, 2.90). Carapace yellowish, the radial grooves indistinct, with the nearly lip-shaped dark pattern, sternum yellowish, about almond-shaped. Abdomen brownish, with yellow and transversal spots, nearly oval-shaped. Legs yellowish, with black annulations. Palp: tibia long,

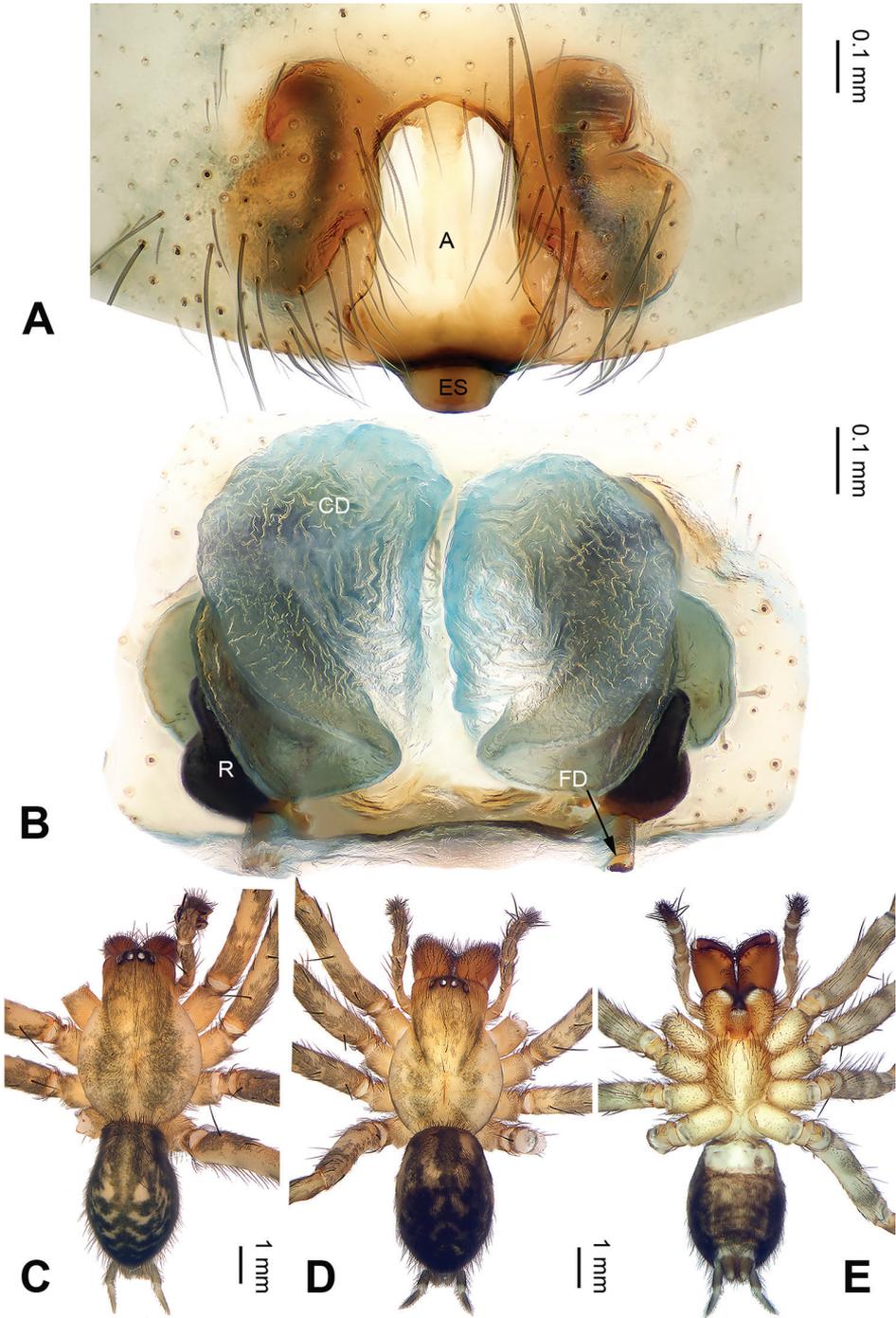


Figure 2. Epigyne and habitus of *Notiocoelotes maoganensis* sp. n. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. A = epigynal atrium; CD = copulatory duct; ES = epigynal scape; FD = fertilization duct; R = receptacle. Scale bars: Equal for **D** and **E**.

about 1/3 of cymbial length; RTA small, 1/3 of tibial length; LTA divided into two parts, most crescent-shaped and about 1/2 length of RTA; conductor long, with one loop; embolus beginning at 6:30 o'clock position, with the triangular base, about 1/3 width of tibia (Fig. 1A–C).

Female (paratype): Total length 8.75. Carapace 4.25 long, 3.50 wide. Abdomen 4.50 long, 3.00 wide. Eye sizes and interdistances: AME 0.20, ALE 0.28, PME 0.25, PLE 0.25; AME-AME 0.05, AME-ALE 0.01, PME-PME 0.08, PME-PLE 0.10. Leg measurements: I: 16.50 (4.75, 5.00, 4.00, 2.75); II: 14.25 (4.25, 4.50, 3.50, 2.00); III: 13.10 (4.00, 4.10, 3.25, 1.75); IV: 17.65 (5.15, 5.75, 4.50, 2.25). Carapace beige, with grey lateral margins; sternum nearly almond-shaped, light brown, with wide yellow median band. Abdomen grey-brown, nearly oval-shaped, with beige herringbone pattern. Legs yellowish, with black annulations. Epigyne: atrium elongated, with distinct septum, about two times as long as wide, posterior broaden; copulatory ducts covering anterior parts of receptacles, about 1.2 times as long as wide; receptacles narrow, about 2.5 times as long as wide; copulatory openings distinct (Fig. 2A–B).

Distribution. Known only from the type locality (Fig. 7).

Notiocoelotes membranaceus Liu & Li, 2010

Figs 3–4, 7

Notiocoelotes membranaceus Liu & Li, 2010: 33, figs 2A–C, 3A–D (♂).

Type material. Holotype ♂: China: Hainan: Qiongzong County: Mt. Limushan Nature Reserve, 13 August 2007, S. Li, C. Wang, L. Lin & J. Xu leg.

Other material examined. 3♀3♂: China: Hainan: Qiongzong County: Mt. Limushan Nature Reserve, Binlang Lake, N19°11'59", E109°43'45", 576 m, 4.XII.2015, X. Zhang & Z. Chen; 2♀, China: Hainan: Qiongzong County: Mt. Limushan, N19°10'52", E109°45'19", 962 m, 2.V.2011, Y. Zhou.

Diagnosis. The female of *N. membranaceus* can be distinguished from all the other *Notiocoelotes*, except *N. orbiculatus*, by the almost oval atrium. From *N. orbiculatus*, the new species can be distinguished by the egg-shaped receptacles (while *N. orbiculatus* has globular and widely separated receptacles) (cf. Fig. 4A–B; Liu and Li 2010: fig. 7B).

Description. Male: described in detail by Liu and Li (2010: fig. 2A–C) (Fig. 3A–C).

Female: Total length 4.75. Carapace 2.50 long, 1.75 wide. Abdomen 2.25 long, 1.75 wide. Eye sizes and interdistances: AME 0.08, ALE 0.15, PME 0.15, PLE 0.15; AME-AME 0.03, AME-ALE 0.01, PME-PME 0.05, PME-PLE 0.06. Leg measurements: I: 7.00 (2.05, 2.25, 1.60, 1.10); II: 6.10 (1.85, 1.90, 1.55, 0.80); III: 5.50 (1.70, 1.75, 1.30, 0.75); IV: 7.50 (2.25, 2.30, 2.00, 0.95). Carapace yellowish, with black dark lateral margins; sternum yellow, margins darker than median part. Abdomen yellowish-brown, with black and nearly chevrons-shaped stripes, nearly pineapple-shaped. Legs yellowish, with black annulations. Epigyne: atrium semicircular, about 1.5 times as long as wide, with distinct septum;

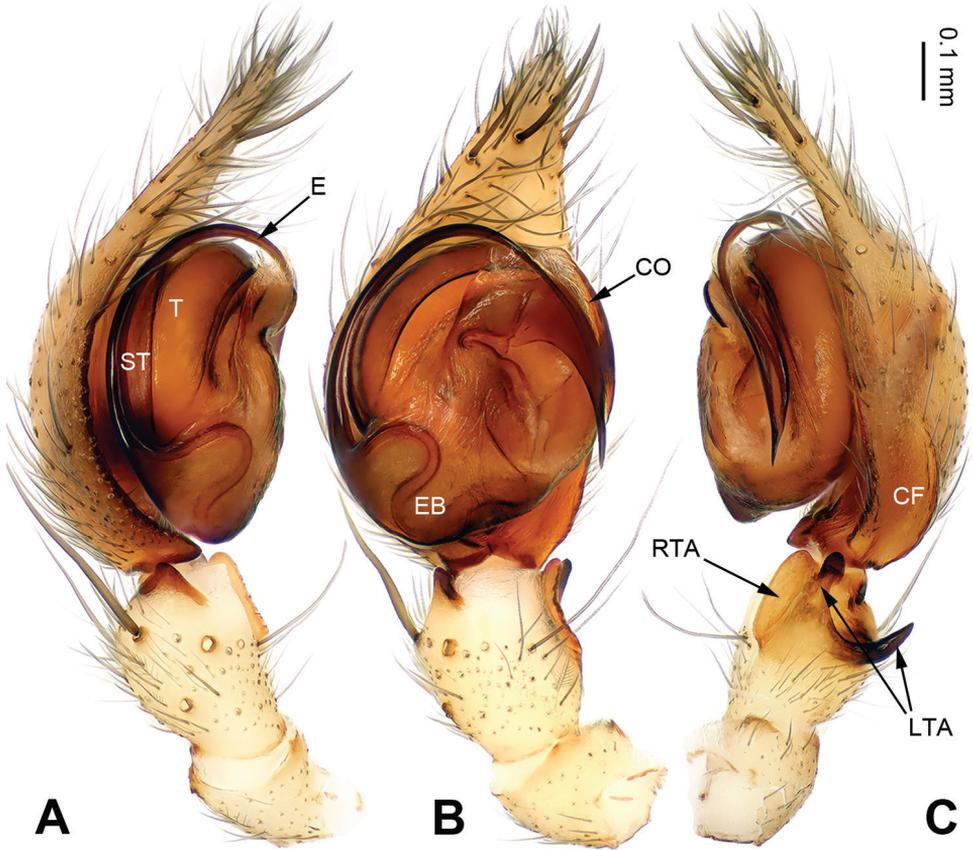


Figure 3. Left palp of *Notiocoelotes membranaceus*, specimen from Hainan. **A** Prolateral view **B** Ventral view **C** Retrolateral view. CF = cymbial furrow; CO = conductor; E = embolus; EB = embolic base; LTA = lateral tibial apophysis; RTA = retroventral tibial apophysis; ST = subtegulum; T = tegulum. Scale bar: Equal for **A**, **B** and **C**.

epigynal scape wide; copulatory ducts long, about 0.9 times as long as receptacles, well sclerotized; receptacles long, about 1.5 times as long as wide; copulatory openings distinct (Fig. 4A–B).

Distribution. China (Hainan) (Fig. 7).

Remarks. Female of this species is described for the first time. Although the shape of the palp and epigyne of *N. membranaceus* are a little different from those of the type species of the genus *Notiocoelotes*, the taxonomic placement of this species is supported by the following two features. First, according to the molecular data (our COI sequences, unpublished), *N. membranaceus* is closely related to *N. orbiculatus* and *N. qiongzhongensis* sp. n. Second, the male of *N. membranaceus* has a strongly bifurcated lateral tibial apophysis, characteristic for the males of all *Notiocoelotes* species; the female of *N. membranaceus* has a tongue-shaped epigynal scape, characteristic for the females of all *Notiocoelotes* species.

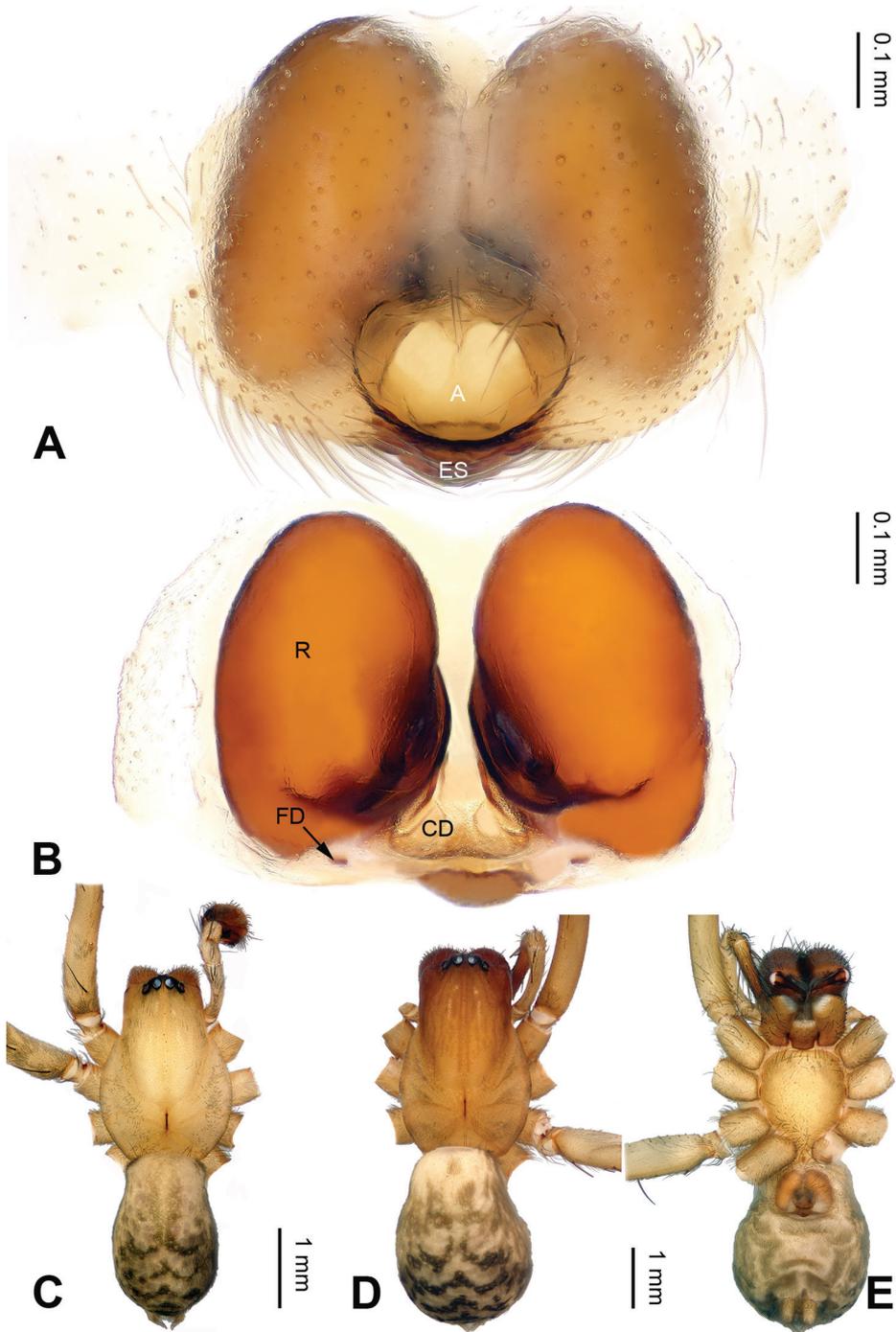


Figure 4. Epigyne and habitus of *Notiocoelotes membranaceus*, specimens from Hainan. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. A = epigynal atrium; CD = copulatory duct; ES = epigynal scape; FD = fertilization duct; R = receptacle. Scale bars: Equal for **D** and **E**.

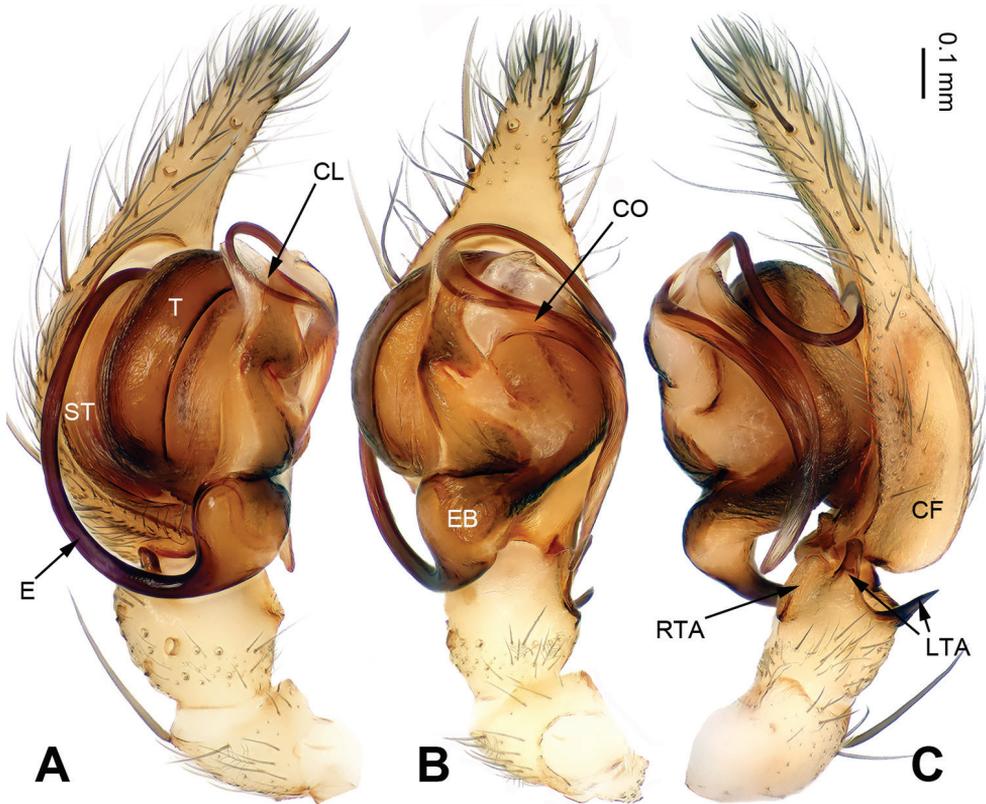


Figure 5. Left palp of *Notiocoelotes qiongzhongensis* sp. n., holotype. **A** Prolateral view **B** Ventral view **C** Retrolateral view. CF = cymbial furrow; CL = conductor lamella; CO = conductor; E = embolus; EB = embolic base; LTA = lateral tibial apophysis; RTA = retroventral tibial apophysis; ST = subtegulum; T = tegulum. Scale bar: Equal for **A**, **B** and **C**.

***Notiocoelotes qiongzhongensis* Zhao & Li, sp. n.**

<http://zoobank.org/1AE669ED-AA8C-4EF0-A883-D0E0984D1F55>

Figs 5–6, 7

Type material. Holotype ♂: China: Hainan: Qiongzhong County: Mt. Limushan, Pine forest, N19°10'53", E109°45'20", 537 m, 2.XII.2015, X. Zhang & Z. Chen. **Paratypes:** 2♀3♂, same data as holotype; 1♀, same area, N19°10'55", E109°45'17", 637 m, 3.V.2011, Y. Zhou.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of *N. qiongzhongensis*, sp. n. can be distinguished from all of the other *Notiocoelotes* species, except *N. pseudolingulatus* and *N. sparus*, by having posteriorly extended conductor and cymbial furrow almost half of cymbial length. From the latter two species, it can be distinguished by the semicircular conductor apex (while *N. pseudolingulatus* has a blunt apex, and *N. sparus* has an acute apex) (cf. Fig. 5A–C; Liu and Li 2010: figs 10–11; Dankittipakul et al. 2005: figs 1–3). The female of *N.*

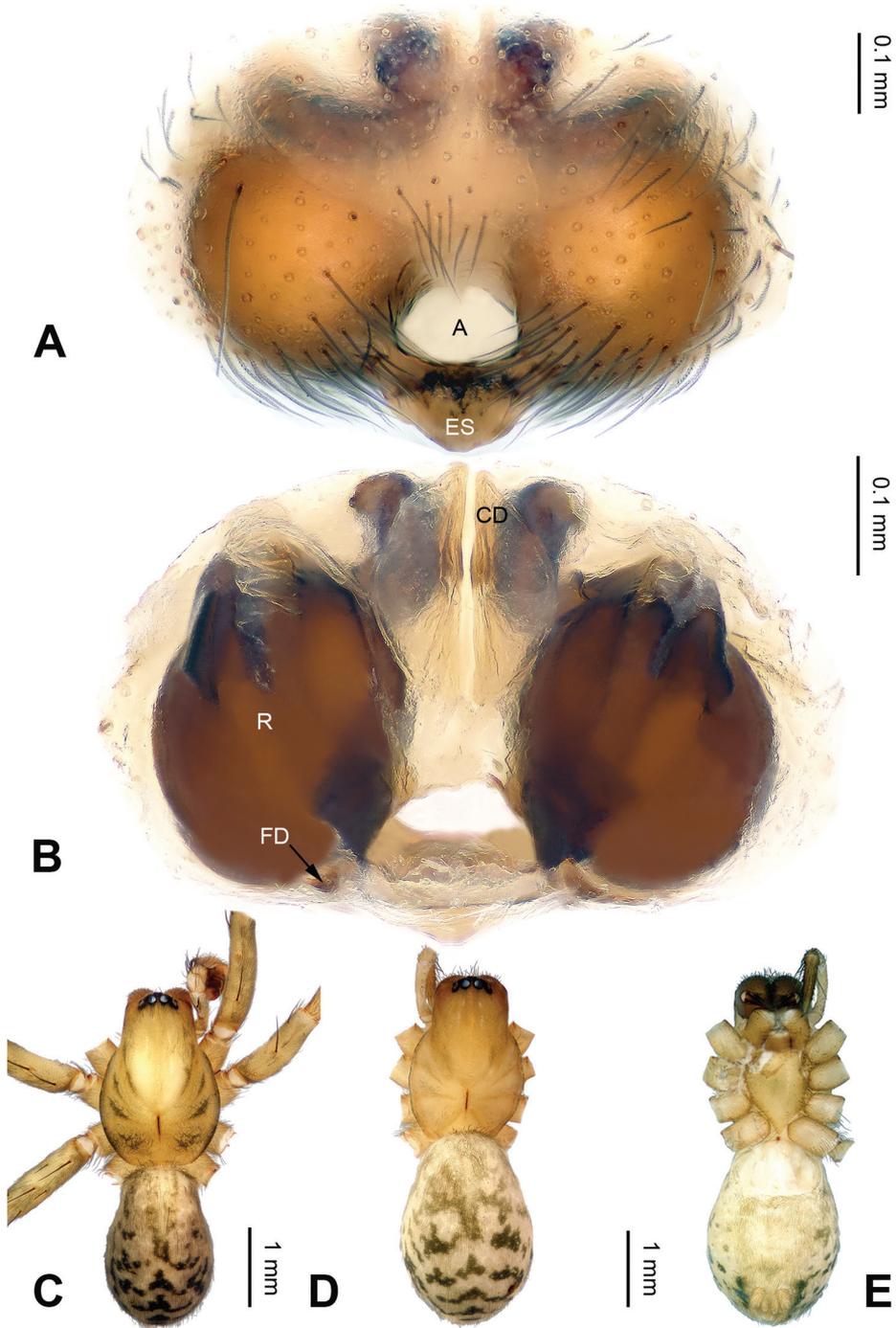


Figure 6. Epigyne and habitus of *Notiocoelotes qiongzhongensis* sp. n. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. A = epigynal atrium; CD = copulatory duct; ES = epigynal scape; FD = fertilization duct; R = receptacle. Scale bars: Equal for **D** and **E**.

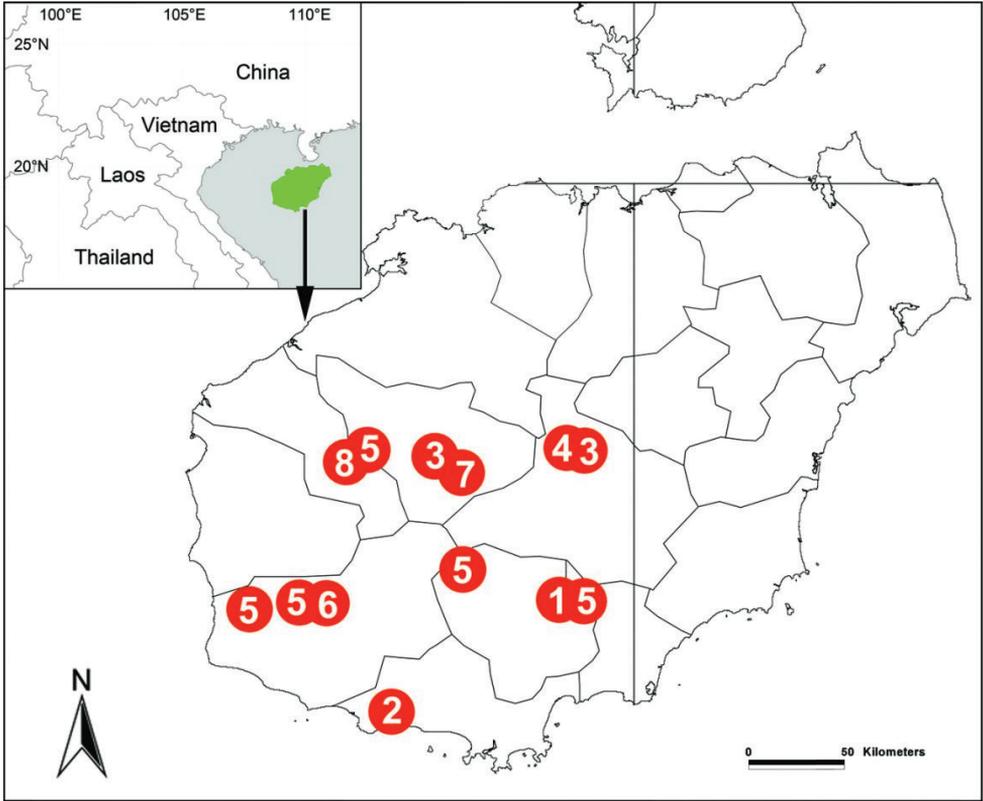


Figure 7. Localities of *Notiocoelotes* species from Hainan. **1** *N. lingulatus* **2** *N. maoganensis* sp. n. **3** *N. membranaceus* **4** *N. orbiculatus* **5** *N. palinitropus* **6** *N. pseudolingulatus* **7** *N. qiongzhongensis* sp. n. **8** *N. spirellus*.

qiongzhongensis, sp. n. can be distinguished from all *Notiocoelotes*, except *N. orbiculatus* and *N. parvitriangulus*, by the rounded receptacles. It can be distinguished from *N. orbiculatus* by the head of receptacles situated on anterior part of receptacles (while the head of receptacles is situated on posterior part of receptacles in *N. orbiculatus*); and it can be distinguished from *N. parvitriangulus* by the nearly square-shaped atrium (while *N. parvitriangulus* has a triangular atrium) (cf. Fig. 6A–B; Liu and Li 2010: fig. 7B; Liu et al. 2010: fig. 78A).

Description. Male (holotype): Total length 4.20. Carapace 2.15 long, 1.60 wide. Abdomen 2.05 long, 1.50 wide. Eye sizes and interdistances: AME 0.05, ALE 0.12, PME 0.15, PLE 0.12; AME-AME 0.04, AME-ALE 0.01, PME-PME 0.04, PME-PLE 0.03. Leg measurements: I: 6.65 (1.95, 2.15, 1.55, 1.00); II: 5.60 (1.75, 1.80, 1.25, 0.80); III: 5.05 (1.50, 1.55, 1.25, 0.75); IV: 7.05 (2.00, 2.25, 1.90, 0.90). Carapace yellowish, with the black and broad radial grooves, with black lateral margins. Abdomen grey, with black spots, nearly eggplant-shaped. Legs yellowish, with black annulations. Palp: tibia short, about 1/4 of cymbial length; RTA about half of tibial length;

LTA divided into two parts, almost hook-shaped and subequal the length of RTA; conductor nearly arc-shaped, about 1.5 times as long as tegulum, with two loops; embolus beginning at 7:00 o'clock position, with a nearly chestnut-shaped base, about 1/2 width of tibia (Fig. 5A–C).

Female (one of paratypes): Total length 4.50. Carapace 2.00 long, 1.50 wide. Abdomen 2.50 long, 1.75 wide. Eye sizes and interdistances: AME 0.04, ALE 0.11, PME 0.13, PLE 0.15; AME-AME 0.03, AME-ALE 0.09, PME-PME 0.03, PME-PLE 0.02. Leg measurements: I: 5.15 (1.50, 1.75, 1.15, 0.75); II: 4.50 (1.35, 1.50, 0.95, 0.70); III: 4.05 (1.25, 1.30, 0.90, 0.60); IV: 5.85 (1.75, 1.85, 1.50, 0.75). Carapace yellowish; sternum flavescent. Abdomen beige, with black and wavy stripes, nearly egg-shaped. Legs yellowish, with black annulations. Epigyne: atrium small, almost square-shaped (width=length); receptacles oval, separated by less than 1/2 of their width, about 1.2 times as long as wide; copulatory ducts folded, with two parts, one membranous and another heavily sclerotized cylindrical, almost covered by receptacles; copulatory openings indistinct (Fig. 6A–B).

Distribution. Known only from the type localities (Fig. 7).

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Kankuamo, a new theraphosid genus from Colombia (Araneae, Mygalomorphae), with a new type of urticating setae and divergent male genitalia

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Abstract

A new monotypic Theraphosidae genus, *Kankuamo* Perafán, Galvis & Pérez-Miles, **gen. n.**, is described from Colombia, with a new type of urticating setae. These setae differ from others principally by having a small distal oval patch of lanceolate reversed barbs. Males of *Kankuamo* **gen. n.** additionally differ by having a palpal bulb organ very divergent from all known species, with many conspicuous keels dispersed across the median tegulum to the tip, mostly with serrated edges. Females differ by having spermathecae with a single notched receptacle, with two granulated lobes and several irregular sclerotized longitudinal striations. The new urticating setae, type VII, is characterized, illustrated and its releasing mechanism is discussed. It is hypothesized that these setae are the first in Theraphosinae subfamily whose release mechanism is by direct contact. *Kankuamo* **gen. n.** is described and illustrated on the basis of the type species *Kankuamo marquezii* Perafán, Galvis & Gutiérrez, **sp. n.**, and their remarkable characteristics, morphological affinities and cladistic relationship are analyzed.

Keywords

New species, Sierra Nevada de Santa Marta, Theraphosinae phylogeny, urticating setae type VII

Introduction

Theraphosidae Thorell, 1869 is the most speciose of the Mygalomorphae with more than 130 genera and 980 species (World Spider Catalogue 2015), mainly distributed in the tropical and subtropical regions, and currently divided into 11 subfamilies (Guadanucci 2014). This family comprises large sized and setose spiders commonly known as tarantulas in the New World. A unique morphological characteristic of most New World theraphosids is the presence of defensive urticating setae (Cooke et al. 1972). This defense mechanism is found in roughly 540 of the 600 theraphosid Neotropical species (Bertani and Guadanucci 2013). Representatives of all known species of the subfamily Theraphosinae, as well as species of the Aviculariinae genera *Avicularia* Lamarck, 1818, *Ephobopus* Simon, 1892, *Iridopelma* Pocock, 1901, *Pachistopelma* Pocock, 1901, and *Typhochlaena* C.L. Koch, 1850 have urticating setae. The arboreal tarantulas *Tapinauchenius* Ausserer, 1871 and *Psalmopoeus* Pocock, 1895, and several ‘Ischnocolinae’ genera are the only New World theraphosids that lack any urticating setae (Bertani and Guadanucci 2013).

The morphological characteristics of urticating setae have long been used in taxonomy and systematics of Theraphosidae, being useful as a set of characters for differentiation of subfamilies and genera as shown in phylogenetic analysis (Cooke et al. 1972, Raven 1985, Pérez-Miles et al. 1996, Pérez-Miles 2002, Perafán 2010, Bertani and Guadanucci 2013). Six different types of urticating setae have been described based on their morphology, ornamentation, length and releasing mechanism; two types are known to Aviculariinae (II and V), and another four in Theraphosinae (I, III, IV and VI) (Cooke et al. 1972, Marshall and Uetz 1990, Pérez-Miles 1998) (see Bertani and Guadanucci 2013, Figure 1). Excepting type V which occurs on the distal prolateral surface of the palpal femora (Marshall and Uetz 1990, Foelix et al. 2009), all other types are found on the dorsum of the abdomen.

During study of Colombian tarantulas, we discovered specimens from Sierra Nevada de Santa Marta, Colombia, with a different type of urticating setae which did not fit with any known types. These setae mainly differ by having a small distal patch of reversed lanceolate barbs (regarding the main barbs; *sensu* Cooke et al. 1972) near the penetrating tip, and small main barbs that extend along the whole seta from the apex. Additionally, males present a palpal bulb remarkably different from all known Theraphosidae, with a large number of conspicuous keels on tegulum and embolus. The morphology and arrangement of these keels do not easily fit with the palpal bulb homologies proposed by Bertani (2000) for the Theraphosinae. However, this taxon shares the main general characteristics of the Theraphosinae subfamily, extended subtegulum, keels on palpal bulb, and urticating setae (Raven 1985, Pérez-Miles et al. 1996). Females differ from other Theraphosinae genera by having spermathecae with a single notched receptacle with two granulated lobes and several irregular sclerotized longitudinal striations.

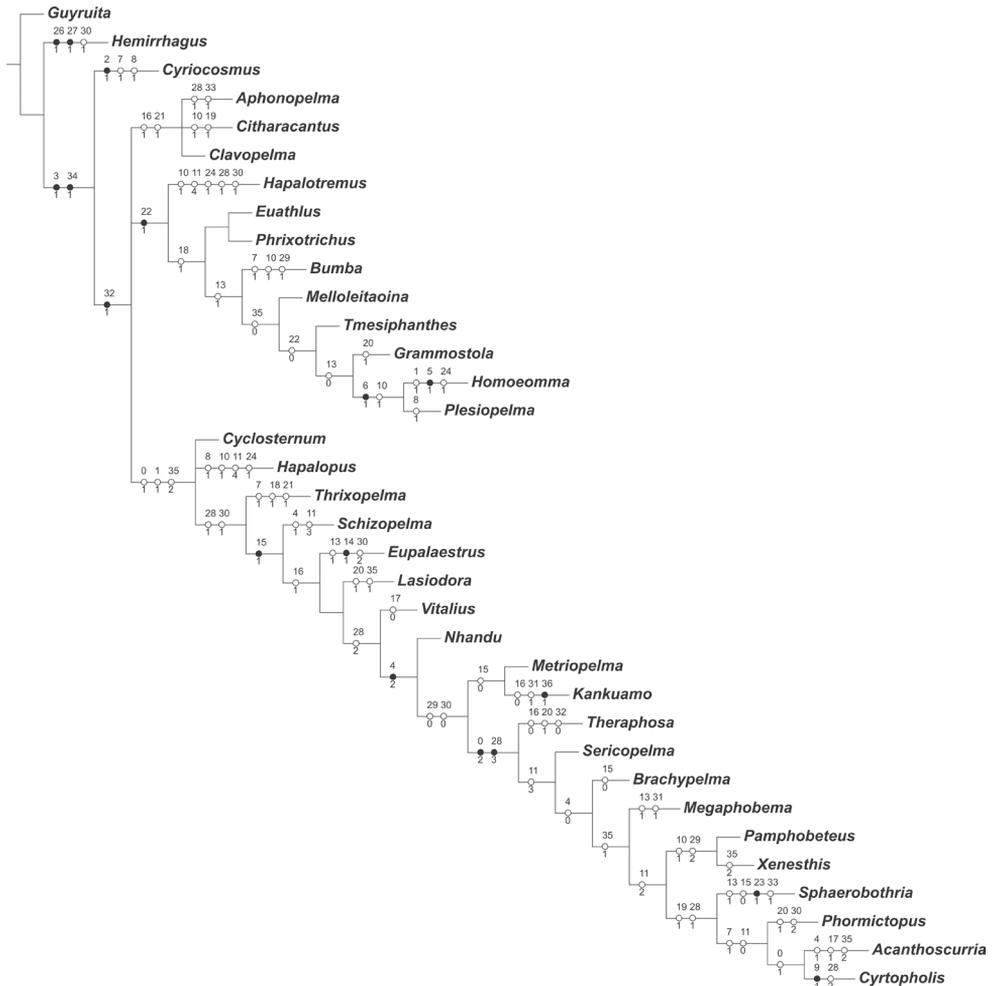


Figure 1. Preferred phylogeny of Theraphosinae. Strict consensus of three trees (maximum parsimony, heuristic search and implied weighting $k = 8$, $L = 127$; $Ci = 36$; $Ri = 64$). Black and white circles represent homologous and homoplastic characters, respectively.

Based on its unique combination of characters, we propose the new monotypic Theraphosinae genus *Kankuamo* Perafán, Galvis & Pérez-Miles, gen. n., which is here diagnosed, described and illustrated on the basis of the type species *Kankuamo marquezii* Perafán, Galvis and Gutiérrez sp. n. Morphological aspects are discussed and its phylogenetic relationship are analyzed based on a Theraphosinae cladistic re-analysis presented in this paper. Considering the size and morphology of the urticating setae in *Kankuamo* gen. n., we propose them as a novel type, here naming them as type VII urticating setae. These setae are described and illustrated, and their releasing mechanism is discussed.

Material and methods

Urticating setae terminology follows Cooke et al. (1972) and Bertani and Guadanucci (2013). Male palpal organ keel terminology follows Bertani (2000). Number and disposition of spines are enumerated from the anterior third to the posterior third, modified from Petrunkevitch (1925). Spination was recorded from the right-side limbs. All measurements were taken using an ocular micrometer and are given in millimeters (mm). Leg and palp measurements were taken on the dorsal side, along the central axis of the left-side limbs. The genital structures were removed for examination: either male left palpal bulb, or female spermathecae. Urticating setae from different regions of the abdomen were removed and examined under optical microscope and scanning electron microscope (SEM). Photographs were taken with an Infinity Lite camera adapted to the stereoscope lens (Nikon SMZ-10), SEM with JEOL JSM-5900 LV. The geographic coordinates are taken by GPS, Datum WGS84. The distribution map was produced using DIVA-GIS version 7.5.0 (<http://www.diva-gis.org/>). The type material is stored in 70% ethanol, and deposited in the Instituto de Ciencias Naturales Arachnological Collection (ICN-Ar), at the Universidad Nacional de Colombia, Bogotá, Colombia.

Abbreviations are listed below:

AcK	accessory keels;
AK	apical keel;
ALE	anterior lateral eyes;
AME	anterior median eyes;
ap	apical;
D	dorsal;
DKs	dorsal keels;
ICN	Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia;
k	concavity constant;
OQ	ocular quadrangle (including lateral eyes);
P	prolateral;
PIK	prolateral inferior keel;
PME	posterior median eyes;
PMS	posterior median spinnerets;
PLE	posterior lateral eyes;
PLS	posterior lateral spinnerets;
PSK	prolateral superior keel;
SAK	subapical keel;
SpAcK	supra-accessory keels;
R	retrolateral;
V	ventral.

Cladistic analysis. Cladistic analysis was based on the previous matrix of Theraphosinae genera used by Pérez-Miles et al. 1996 and Pérez-Miles 2000 with some modifications. This matrix was completed as far as possible, including material examined and literature, complemented with the new evidences of the palpal bulb homology proposed by Bertani (2000, 2001), and the characters used on the matrix of Perafán and Pérez-Miles (2014). The original matrix from Pérez-Miles et al. 1996 was modified on characters referred to palpal bulb keels (characters 3 and 4) and replaced with those proposed by Bertani (2000, 2001), Perafán and Pérez-Miles (2014). Character 13 related to the spermathecae shape was also amended. Furthermore, the hypothetical outgroup used on the analysis previous (Pérez-Miles et al 1996, Pérez-Miles 2000) was modified by a Schimatothelinae taxon *Guyruita* Guadanucci et al., 2007 (Guadanucci 2014) and the terminal *Pseudotheraphosa* Tinter, 1991 was eliminated because it's a junior synonym of *Theraphosa* Thorell, 1869 (Bertani 2001).

A data matrix composed of 37 morphological characters and 35 genera has been constructed (Table 1). The cladistics analysis was carried out in TNT version 1.1 (Goloboff et al. 2008), under maximum parsimony. The characters were polarized according to the out-group criterion (Watrous and Wheeler 1981), and all characters treated unordered, with other settings as in Pérez-Miles et al. (1996). A heuristic search was used with 15 addition sequences and tree-bisection reconnection processes, with and without implied weighting (Goloboff 1993) under different concavity values ($k = 3-12$). Character optimization was performed in Winclada 1.00.08 (Nixon 1999-2002) and characters discussed below are those that are unambiguously optimized.

Data set. Characters used in the cladistic analysis. The data matrix is listed in Table 1.

Pérez-Miles et al. 1996, Pérez-Miles 2000 Characters: (0) Apical region of palpal bulb: subcylindrical = 0; subconical = 1; concave-convex = 2. (1) Relative width of sclerites II+III of bulb: narrow (less than 10% of length) = 0; wide = 1. (2) Paraembolic apophysis: absent = 0; present = 1. (3) Subtegulum: not extended = 0; large extended = 1. (4) Male tibial apophysis (leg I): double = 0; one = 1; absent = 2. (5) Digitiform apophysis of bulb: absent = 0; present = 1. (6) Metatarsus I of male: without basal process = 0; with basal process = 1. (7) Male palpal tibia: without retrolateral process = 0; with retrolateral process = 1. (8) Male palpal tibia: without retrolateral cluster of spines = 0; with retrolateral cluster of spines. (9) Male palpal tibia: without prolateral process = 0; with prolateral process = 1. (10) Flexion of metatarsus I on males: on outer side of tibial spurs = 0; between tibial spurs = 1. (11) Spermathecae (modified character): two separated longitudinal seminal receptacles = 0; two separated transversal seminal receptacles = 1; two seminal receptacles widely fused = 2; single semicircular receptacle = 3; single oval receptacle = 4. (12) Spermathecae: multilobular in each side = 0; unilobular at least in each side = 1. (13) Femur III: not incrassate = 0; incrassate = 1. (14) Tibia IV: not incrassate = 0; incrassate = 1. (15) Femur IV: without retrolateral scopula = 0; with retrolateral scopula = 1. (16) Urticating setae type I: absent = 0; present = 1. (17) Urticating setae type III: absent = 0; present = 1. (18) Urticating setae type IV: absent = 0; present = 1. (19) Trochanteral stridulatory setae: absent = 0; present = 1.

Table 1. Character matrix used in cladistic analysis of Theraphosinae genera. (?) inapplicable, unknown or doubtful.

	0	1	2	3	4	5	6	7	8	9	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	3	3						
<i>Guyruita</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Acanthoscurria</i>	1	1	0	1	1	0	0	1	0	0	?	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	0	1	0	1	2	0				
<i>Aphonopelma</i>	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	?	1	0	0					
<i>Brachypelma</i>	2	1	0	1	0	0	0	0	0	0	0	3	1	0	0	0	1	1	0	0	0	0	0	0	0	0	3	0	0	0	1	0	1	2	0			
<i>Bumba</i>	0	0	0	1	0	0	0	1	0	0	1	0	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	?	0	1	1	0	0				
<i>Citharacantus</i>	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0	0	?	0	1	0	1	1	0				
<i>Clavopelma</i>	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1	0	?	?	?	?	?	?	0				
<i>Cyclosternum</i>	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0				
<i>Cyriocosmus</i>	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0				
<i>Cirtopholis</i>	1	1	0	1	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	1	0	?	0	0	0	0	0	2	0	0	0	1	0	1	0			
<i>Euathlus</i>	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	1	0	1	1	0			
<i>Eupalaestrus</i>	1	1	0	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	2	0	1	0	1	2	0			
<i>Grammostola</i>	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0			
<i>Hapalopus</i>	1	1	?	1	0	0	0	1	0	1	4	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	2	0	0			
<i>Hapalotremus</i>	0	0	0	1	0	0	0	0	0	1	4	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	1	0	1	0			
<i>Hemirrhagus</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	?	0	1	1	0	0	1	0	0	1	0		
<i>Homoeomma</i>	0	1	0	1	0	1	1	0	0	?	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	?	1	0	0			
<i>Lasiadora</i>	1	1	0	1	0	0	0	0	0	0	2	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	0		
<i>Megaphobema</i>	2	1	0	1	0	0	0	0	0	0	3	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	3	1	0	1	1	0	1	1	0		
<i>Melloleitainia</i>	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0		
<i>Metriopelma</i>	1	1	0	1	2	0	0	0	0	?	?	1	?	0	0	1	?	0	0	0	0	0	0	0	0	0	0	?	?	0	0	1	0	1	2	0		
<i>Nhandu</i>	1	1	0	1	2	0	0	0	0	0	2	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	2	2	1	0	1	0	1	2	0		
<i>Pamphobeteus</i>	2	1	0	1	0	0	0	0	0	0	1	2	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	3	2	0	0	?	0	1	1	0		
<i>Phrixotrichus</i>	0	0	0	1	0	0	0	0	0	0	1	?	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	0		
<i>Phormictopus</i>	2	1	0	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0	2	0	1	0	1	1	0		
<i>Plesiopelma</i>	0	0	0	1	0	0	1	0	?	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0		
<i>Schizopelma</i>	1	1	0	1	1	0	0	0	0	?	3	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	?	?	?	0	1	0	1	?	0		
<i>Sericopelma</i>	2	1	0	1	2	0	0	0	0	?	3	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	3	0	0	?	?	1	2	0	0		
<i>Sphaerobothria</i>	2	1	0	1	0	0	0	0	0	0	2	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	1	1	0	0		
<i>Theraphosa</i>	2	1	0	1	2	0	0	0	0	?	2	1	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	3	0	0	0	0	0	1	2	0	0	
<i>Thrixopelma</i>	1	1	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	2	0
<i>Tmesiphantes</i>	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	
<i>Vitalius</i>	1	1	0	1	0	0	0	0	0	0	2	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	1	0	1	0	1	2	0
<i>Xenesthis</i>	2	1	0	1	0	0	0	0	0	1	2	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	1	0	1	0	1	2	0
<i>Kankuamo</i>	1	1	0	1	2	0	0	0	0	?	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1	0	1	2	1	0

(20) coxal stridulatory setae: absent = 0; present = 1. (21) Coxal spinules: absent = 0; present = 1. (22) Labial cuspules: numerous (more than 15) = 0; few or none = 1. (23) Fovea: normal = 0; with spheroid process = 1. (24) Metatarsus I of males: normal = 0; strongly curved = 1. (25) urticating hairs on prolateral palpal femur: absent = 0;

present = 1. (26) Urticating setae type VI: absent = 0; present = 1. (27) Coxae: normal = 1; retrolaterally extend = 1.

Bertani 2000, 2001 Characters: (28) Apical keel: absent = 0; small = 1; intermediated = 2; very long = 3. (29) Retrolateral keel: absent = 0; present, not pronounced = 1; present, pronounced = 2. (30) Subapical keel: absent = 0; present, not serrated = 1; present, serrated = 2. (31) Prolateral accesoty keel, under the prolateral inferior keel: absent = 0; present = 1. (32) Prolateral inferior keel: absent = 0; present = 1. (33) Denticulate row in the PIK: absent = 0; present = 1. (34) Prolateral superior keel: absent = 0; presente = 1.

Perafán and Pérez-Miles 2014 Character: (35) Embolus direction: directed ventrolaterally = 0; directed retrolaterally = 1; straight = 2.

(36) Urticating setae type VII: absent = 0; present = 1.

Results and discussion

Kankuamo Perafán, Galvis & Pérez-Miles, gen. n.

<http://zoobank.org/622CB9E5-59D1-4E45-A5CE-21F5248CF9EF>

Type species. *Kankuamo marquezzi* Perafán, Galvis & Gutiérrez, sp. n.

Diagnosis. Differs from all previously known genera of Theraphosidae by having a distinct type of urticating setae (Fig. 2, see description below), mainly characterized by having a small distal patch of lanceolated barbs arranged in reversed direction, regarding the main barbs, oriented with their tips towards the penetration tip (Fig. 2B). Male differs additionally from other genera by having a curved sub-conical palpal bulb with many conspicuous keels distributed throughout the majority of the subtegulum and embolus, especially developed on the dorsal and prolateral faces, most of them with serrated edges (Fig. 3E–3I). PSK, AcK, PIK, AK and SAK present (*sensu* Bertani 2000), additionally dorsal keels (DKs) and supra-accessory keels (SpAcK). Tibial apophysis on leg I absent (Fig. 3D). Females differ by having spermathecae with a single notched receptacle, with two granulated lobes, and several irregular sclerotized longitudinal striations (Fig. 3D).

Etymology. *Kankuamo* is a noun in apposition and refers to the indigenous people of the Chibcha family from the Caribbean region of Colombia, which inhabits the eastern slope of the Sierra Nevada de Santa Marta, whose language and culture are at endangered. *Kankuamo* gender is neuter.

Description. See description of the type species.

Distribution. Only known from its type locality, Vereda San Lorenzo, Corregimiento Minca, Santa Marta, Magdalena, Colombia, 11.1 N, -74.05 W (Fig. 7).

Discussion. Morphology. *Kankuamo* gen. n. clearly presents the diagnostic characters of the subfamily Theraphosinae (Theraphosidae): male palpal bulb with large and extended subtegulum, and numerous developed keels; legs spinose, with normal scopulae on tarsi and presence of abdominal urticating setae (Figs 2–6). However, this

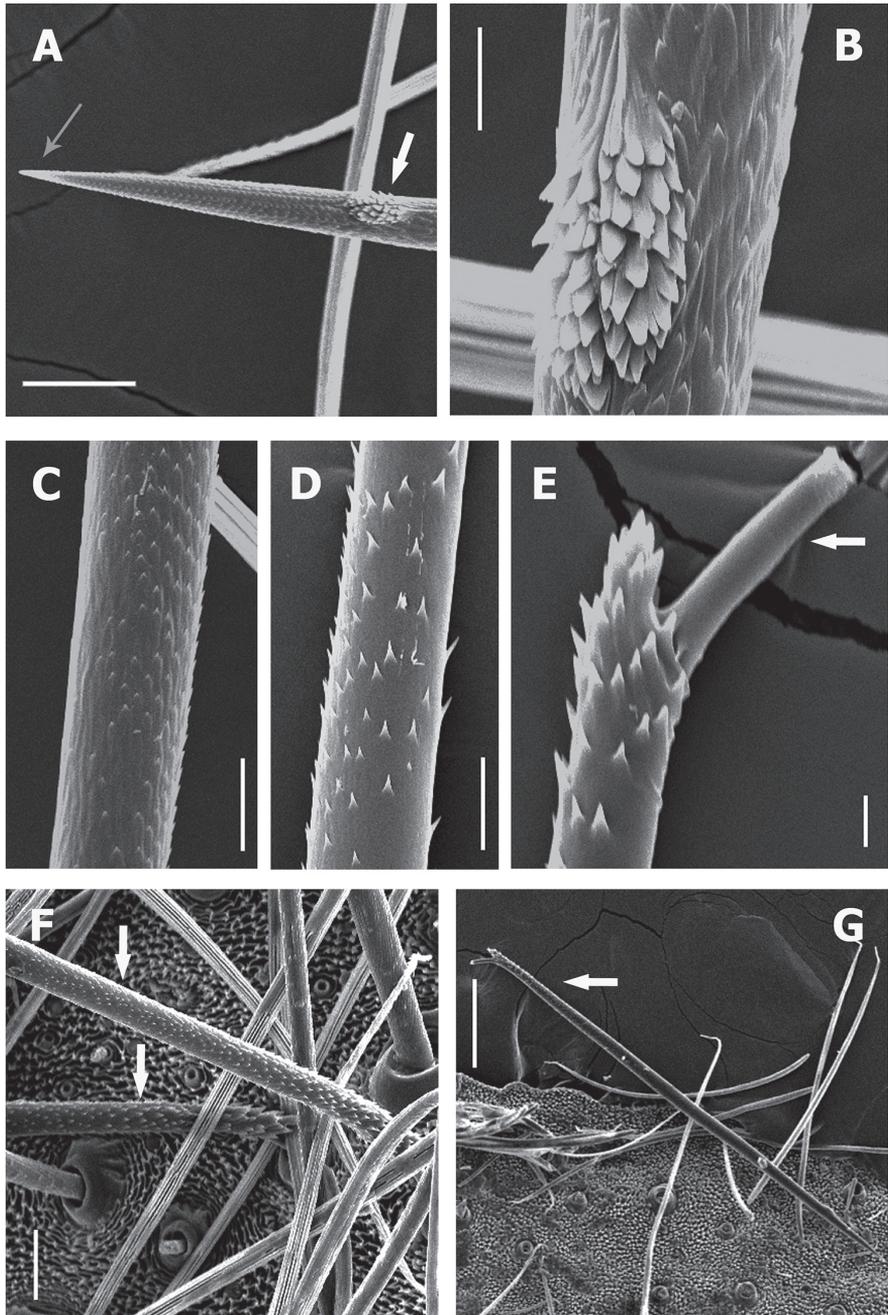


Figure 2. *Kankuamo* gen. n., urticating setae type VII. **A** Distal apex, white arrow indicates patch of lanceolated reversed barbs, grey arrow indicates penetrating tip **B** patch of lanceolated reversed barbs **C** main barbs on distal area **D** main barbs on medial area **E** basal end and detail of main barbs on basal area, white arrow indicates the attachment stalk with the abdomen **F–G** abdomen, dorsal surface, showing setae attachment points, white arrow indicates urticating setae. Scale bars: **A, F** = 50 μm ; **B, E** = 10 μm ; **C, D** = 20 μm ; **E** = 200 μm .

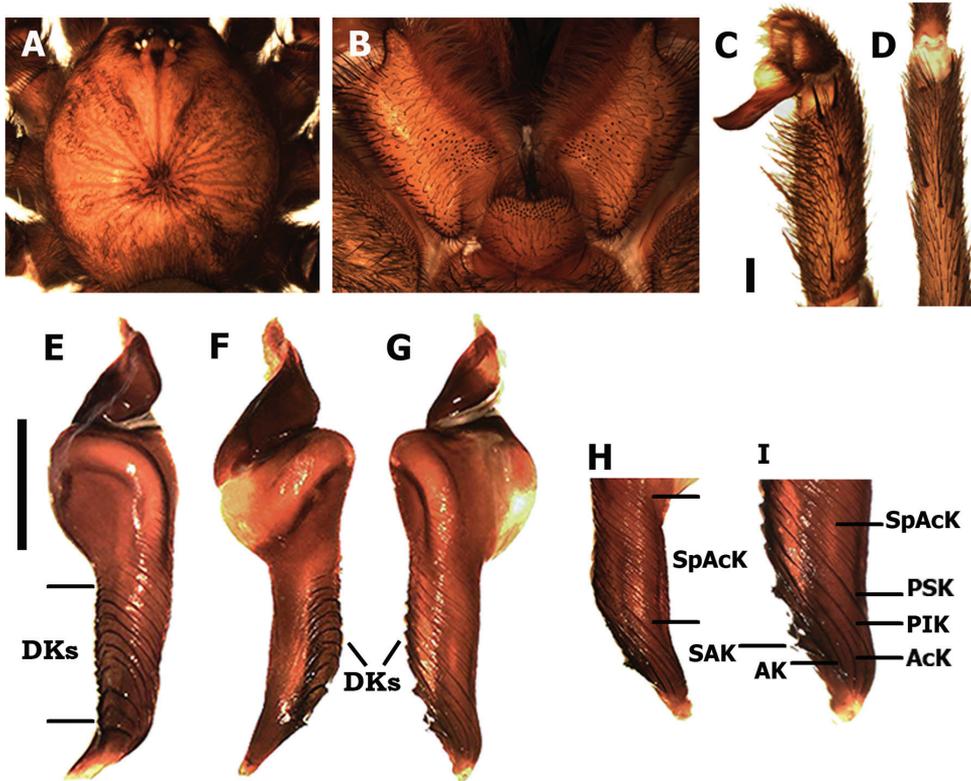


Figure 3. *Kankuamo marquezii* gen. n., sp. n., male. **A** Cephalothorax **B** labium and maxillae **C** right palpal bulb, ventro-prolateral view **D** tibia I in ventral view, showing absence of apophysis **E-I** left palpal bulb: **E** dorsal view **F** retrolateral view **G** prolateral view **H-I** detail of apex. AcK = accessory keels, AK = apical keel, DKs = dorsal keels, PIK = prolateral inferior keel, PSK = prolateral superior keel, SAK = sub-apical keel, SpAcK = supra-accessory keels. Scale bars: **C**, **D** and **E**, **F**, **G** = 1 mm.

new genus differs dramatically from all previously known genera by their novel urticating setae type and the detailed morphology of male palpal bulb, making it difficult to establish their phylogenetic affinities. The general shape of the palpal bulb resembles approximately those of the genus *Ami* Pérez-Miles, 2008 (mainly *A. bladesi* Pérez-Miles, Gabriel and Gallon, 2008 and *A. weinmanni* Pérez-Miles, 2008), but with the subtegulum more elongated in *Kankuamo* gen. n. (Fig. 3G). However, the transverse arrangement of bulb keels is also superficially similar to those of some *Acanthoscurria* Ausserer, 1871, or to the ring shaped keel of *Hapalopus* Ausserer, 1875. Numerous distal keels are also known in other genera such as *Lasiadora* C.L. Koch, 1850, *Nhandu* Lucas, 1983 and *Vitalius* Lucas, Silva and Bertani, 1993, but the higher number of keels in *Kankuamo* gen. n. easily distinguish it from all those and other remaining genera in Theraphosinae. Furthermore, the high number of keels make difficult to establish homology with those of the family as Bertani (2000) suggested. Considering the extension and positional similarity we tentatively propose the presence of PSK,

AcK, PIK, AK, and SAK, and we also propose the name supra-accessory keels (SpAcK) for those placed on proximal subtegulum (Fig. 3H, 3I) and dorsal keels (DKs) for those placed principally on dorsal face (Fig. 3E–3G). The absence of tibial apophysis is shared with *Agnostopelma* Pérez-Miles and Weinmann, 2010; *Aguapanela* Perafán, Cifuentes and Estrada, 2015; *Metriopelma* Becker, 1878; *Nhandu*; *Sericopelma* Ausserer, 1875 and *Theraphosa* Thorell, 1870.

The spermathecae of *Kankuamo* gen. n. are similar to those of Theraphosinae genera with only one entire receptacle (e.g. *Brachypelma* Simon, 1891, *Megaphobema* Pocock, 1901, *Mygalarachne* Ausserer 1871, *Sericopelma* Ausserer, 1875, *Theraphosa* Thorell, 1870), but notched and longitudinally striated (Fig. 5D).

Accordingly, *Kankuamo* gen. n. clearly differs from all genera of Theraphosidae known by the urticating setae type and male palpal bulb characters.

Cladistic relationship. A search using equal weights found 171 most parsimonious trees and the strict consensus of these did not provide any resolution. Search with implied weighting and different concavity indices ($k = 3$ to 12) found between 3 and 10 shortest trees. The strict consensus of each of these trees recovered different topologies, but between $k = 8$ and $k = 12$ the topology of strict consensus did not vary, for this reason we have selected this topology to test *Kankuamo* gen. n. affinities (Fig. 1). It is noted that this new phylogeny analysis of Theraphosinae is an incomplete analysis including only half of the Theraphosinae genera and relatively few characters.

Kankuamo gen. n. was resolved as the sister group of *Metriopelma*, supported by the character “femur IV without retrolateral scopula”. Both genera also share the lack of the apophysis on males and a spermathecae with seminal receptacles completely fused. *Kankuamo* gen. n. presents a reversion to state (0) on character 16 “absence the urticating setae type I”, a parallelism of the state (1) on character 31 “presence the accessory keels under prolateral inferior keel” and an autapomorphic character “presence of urticating setae type VII”.

Kankuamo gen. n. and *Metriopelma* were resolved as sister genera within the largest clade of our selected topology. The relationship of *Kankuamo* gen. n. + *Metriopelma* as the sister group of the clade (*Theraphosa* (*Sericopelma* (*Brachypelma* (*Megaphobema* (*Xenesthis* *Pamphobeteus*) (*Sphaerobothria* (*Phormictopus* (*Cyrtopholis* *Acanthoscurria*)))))) is supported by “absence of retrolateral keel” and “absence of sub-apical keel”, with some homoplasies. The *Theraphosa* clade is supported by two synapomorphies “apical region of palpal bulb with concave-convex aspect” and “apical keel very long”.

***Kankuamo marquezii* Perafán, Galvis & Gutiérrez, sp. n.**

<http://zoobank.org/7EF097DE-5147-4995-985C-35504F164C61>

Figs 3–6 and Table 2

Type material. Holotype male from Colombia, Magdalena, Santa Marta, Corregimiento Minca, Sector San Lorenzo, 2200m above sea level, 11.11 N, -74.058 W,

30-Aug-2014, *leg.* W. Galvis and J. Moreno (ICN-Ar 7983). Allotype female, same data as the holotype (ICN-Ar 7983). Paratypes: one female, same data as the holotype (ICN-Ar 7984); one male from the same locality as the holotype, 11.1 N, 74.05 W, 9-10-Sept-2014, *leg.* Miguel Gutierrez (ICN-Ar 7985).

Etymology. The specific epithet is a noun in genitive in honor to Gabriel García Márquez (Aracataca, Colombia, 1927 - Mexico D.F., Mexico, 2014), who was a renowned Colombian writer, considered one of the most significant authors of the 20th century, and awarded the 1982 Nobel Prize in Literature for “One hundred years of solitude”.

Diagnosis. See diagnosis of the genus.

Description. *Male* (holotype ICN-Ar 7982) (Figs 3 and 4). Total length, not including chelicerae or spinnerets 27; including chelicerae 30. Carapace length 12, width 11. Abdomen length 14. PLS with three segments, distal digitiform, basal length 2, medial 1.3, apical 1.6. PMS well developed mono-segmented, length 1.3. Anterior eye row slightly procurved, posterior slightly recurved. Eyes sizes and interdistances: AME 0.43, ALE 0.56, PME 0.4, PLE 0.43, AME-AME 0.36, AME-ALE 0.13, ALE-ALE 1.26, PME-PME 0.93, PME-PLE 0.06, PLE-PLE 1.46, AME-PME 0.16, ALE-PLE 0.1. OQ elevated sub-rectangular, length 2.1, width 1.33, clypeus 0.33. Fovea transverse deep, straight, width 2.16. Cephalic area slightly raised, thoracic striations slightly conspicuous (Fig. 3A). Basal segments of chelicerae with 9 well-developed teeth on furrow promargin and 19/21 (left/right) small teeth on the proximal area of furrow, intercheliceral tumescence absent. Labium trapezoidal (Fig. 3B), length 1.46, width 2.16, with 56 cuspules. Maxillae sub-rectangular (Fig. 3B), with 56/78 (left/right) cuspules restricted on the proximal prolateral angle. Labio-sternal junction narrow in the middle with two lateral nodules. Sternum length 4.7, width 4.6, with 3 pairs of sigilla; oval, smaller pair anterior, larger pair posterior, anterior pair half distanced at half their diameter from margin, posterior pairs distanced less than 1/3 of their diameter. Superior tarsal claws with teeth on proximal half: I 4 teeth; II-IV 5 teeth. Tarsal scopulae: I-IV scopulated with distal rhomboidal group of conical setae (as Figs 5E and 5F); I and II entire; III and IV divided by a medial stripe of longer conical setae, wider in IV. Metatarsal scopulae extent: I and II scopulate on distal half; III distal 1/4; IV apically, little scopulate. Stridulatory setae absent. Type VII urticating setae present (see description), located on dorsal patch of the abdomen. Metatarsus I straight. Tibia I without apophysis (Fig. 3D). Palpal tibia with spines on ventral and prolateral faces (Fig. 3C). Cymbium bilobed. Palpal bulb sub-conical (Fig. 3E–3I), curved, with a wide membranous area between the subtegulum and tegulum. Distal sclerites of palpal bulb with many conspicuous keels distributed throughout most tegulum and embolus. Eleven large semicircular keels on the dorsal faces (DKs), most of them with serrated edge (Fig. 3E). Presence of PSK, AcK, PIK, AK, SAK, and approximately 14 smaller keels present on the prolateral face of proximal subtegulum (SpAcK) (Fig. 3G–3I). Colour (in alcohol): Cephalothorax and legs light brown with black setae. OQ with black stains and surrounded by black setae, cephalothorax with black stripes. Abdomen brown with golden setae. Iridescent scopulae and claw tuft.



Figure 4. *Kankuamo marquezii* gen. n., sp. n., male, dorsal view of *habitus*. Scale bar = 1 cm.

Spination (proximal to distal): Femur: palp: 0V, 0D, 0-0-1P, 0R; I: 0V, 1-1-0D, 0-1-1P, 0-1-2R; II: 0V, 1-1-0D, 1-1-3P, 1-5-2R; III 0V, 1-1-0D, 0-2-1P, 0-2-3R; IV: 0V, 3-2-0D, 1-2-1P, 0-1-2R. Patella: palp: 0V, 0D, 0-2-0P, 0R; I: 0-2-2V, 0D, 0-2-0P, 0R; II: 0-0-2V, 0D, 0-2-0P, 0R; III: 0V, 0D, 0-3-0P, 0-1-0R; IV: 0V, 0D, 0-3-0P, 0-1-0R. Tibia: palp: 2-1-2V, 0D, 2-2-2P, 0R; I: 4-6-2apV, 0D, 0-2-2P, 0-1-1R; II: 3-5-2apV, 0D, 0-1-1P, 0-2-0R; III: 3-3-2apV, 0D, 2-2-1P, 2-2-1R; IV: 3-3-2-1-2apV, 0D, 2-2-2P, 1-2-2-2apR. Metatarsus: I: 2-3-1V, 0D, 0-1-1apP, 0-1-1apR; II: 3-3-1V, 0D, 0-1-1-1apP, 0-1-1apR; III: 4-4-2-1apV, 0-0-2D, 2-2-1-1apP, 1-2-1-1apR; IV: 5-5-3-1apV, 0-0-2D, 2-3-1-1apP, 2-2-1-1apR. Tarsus: palp and legs: 0. Legs and palpal segments lengths in Table 1.

Female (allotype ICN-Ar 7983) (Figs 5 and 6). Total length, not including chelicerae or spinnerets 39; including chelicerae 44.5. Carapace length 17.1, width 16.3. Abdomen length 17.4. PLS with three segments, distal digitiform, basal length 2.25, medial 1.25, apical 2.45. PMS well developed mono-segmentated, length 1.9. Anterior eye row slightly procurved, posterior recurved. Eyes sizes and interdistances: AME 0.5, ALE 0.5, PME 0.53, PLE 0.66, AME-AME 0.5, AME-ALE 0.33, ALE-ALE 2, PME-PME 1.3, PME-PLE 0.06, PLE-PLE 1.83, AME-PME 0.16, ALE-PLE 0.33. OQ elevated sub-rectangular, length 1.93, width 2.83, clypeus 0.65. Fovea transverse deep, straight, width 3. Cephalic area slightly raised, thoracic striations conspicuous with black setae (Fig. 5A). Basal segments of chelicerae with 10 well-developed teeth on furrow promargin and 21/17 (left/right) small teeth on the proximal area of furrow, intercheliceral tumescence absent. Labium trapezoidal (Fig. 5B), length 2.27, width

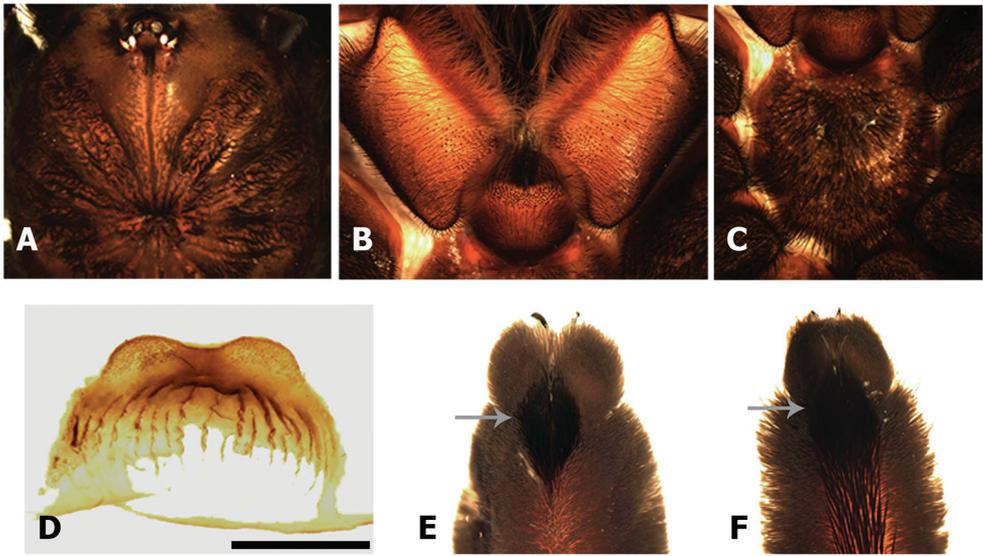


Figure 5. *Kankuamo marquezii* gen. n., sp. n., female. **A** Cephalothorax **B** labium and maxillae ventral **C** sternum **D** spermathecae, dorsal view **E–F** legs tarsi in ventral view **E** tarsus I **F** tarsus IV, arrow indicates distal rhomboidal group of conical setae. Scale bar = 1 mm.

3.06, with 99 cuspules. Maxillae sub-rectangular (Fig. 5B), with 159/164 (left/right) cuspules restricted on the proximal prolateral angle. Labio-sternal junction narrow in the middle with two lateral nodules. Sternum (Fig. 5C) length 7.4, width 7, with three pairs of oval sigilla; posterior sigilla the largest, all of them separated from the margin by approximately their diameter. Superior tarsal claws with teeth on proximal half; palp and leg I, three teeth, smaller in palp; II four teeth; III–IV five teeth. Tarsal scopulae: palp and I–IV scopulated with distal rhomboidal group of conical setae (Fig. 5E–5F); palp and legs I–II entire; III and IV divided by a medial stripe of longer conical setae, wider in IV. Metatarsal scopulae extent: I scopulate on distal 2/3; II distal half; III distal 1/3; IV scopula absent. Stridulatory setae absent. Type VII urticating setae present (see description), located on dorsal patch of the abdomen. Spermathecae with two short wide rounded and granulated seminal receptacles, fused widely on a sub-rectangular wide basal plate, with several irregular sclerotized striations perpendicular to the basal edge (Fig. 5D). Color (in alcohol): darker than male. Iridescent scopulae and claw tuft.

Spination (proximal to distal): Femur: palp: 0V, 0-1-0D, 0-0-3P, 0-0-1R; I: 0V, 0-1-0D, 0-0-3P, 0R; II: 0V, 0-2-0D, 0-0-3P, 0-1-0R; III: 0V, 0D, 0-2-1P, 1-3-1R; IV: 0V, 1-0-0D, 0-0-3P, 0-0-1R. Patella: palp: 0V, 0D, 0-2-0P, 0R; I: 0V, 0D, 0-1-0P, 0R; II: 0V, 0D, 0-2-0P, 0R; III: 0V, 0D, 0-2-0P, 0R; IV: 0V, 0D, 0-1-0P, 0R. Tibia: palp: 1-4-4V, 0D, 0-3-0P, 0-1-0R; I: 0-1-2V, 0D, 1-1-0P, 0R; II: 0-2-2V, 0D, 0-2-0P, 0R; III: 3-3-3V, 0D, 2-1-2P, 1-2-1R; IV: 2-2-2V, 0D, 2-2-1P, 2-2-1R. Metatarsus: I: 1-5-1V, 0D, 0-1-1P, 0R; II: 1-5-1V, 0D, 0-0-1P, 0R; III: 4-3-5V, 0-0-2D, 2-2-2P, 1-3-1R; IV: 5-4-5V, 0-0-2D, 3-3-2P, 1-2-2R. Legs and palpal segments lengths in Table 1.



Figure 6. *Kankuamo marquezii* gen. n., sp. n., female, dorsal view of *habitus*. Scale bar = 1 cm.

Distribution. See distribution of the genus (Fig. 7).

Natural history. *Kankuamo marquezii* sp. n. inhabits Cuchilla San Lorenzo from Sierra Nevada de Santa Marta National Natural Park. Cuchilla San Lorenzo is located in its northwestern flank, in a gradient of altitude from 2000–2300 meters above sea level, in life zone of lower montane wet forest (Espinal and Montenegro 1963). The Sierra Nevada de Santa Marta is an isolated mountain range separated from the Andes chain. The locations where the specimens were collected are covered mainly by shrubby plants of the families *Arecaceae* and *Chrysobalanaceae* (Cuadrado-Peña 2005), and inhabited by the snakes genus *Atractus* and frogs of the genera *Atelopus*, and the endemic species from Sierra Nevada de Santa Marta *Ikakogi tayrona* and *Geobatrachus walkeri* (MG personal observations).

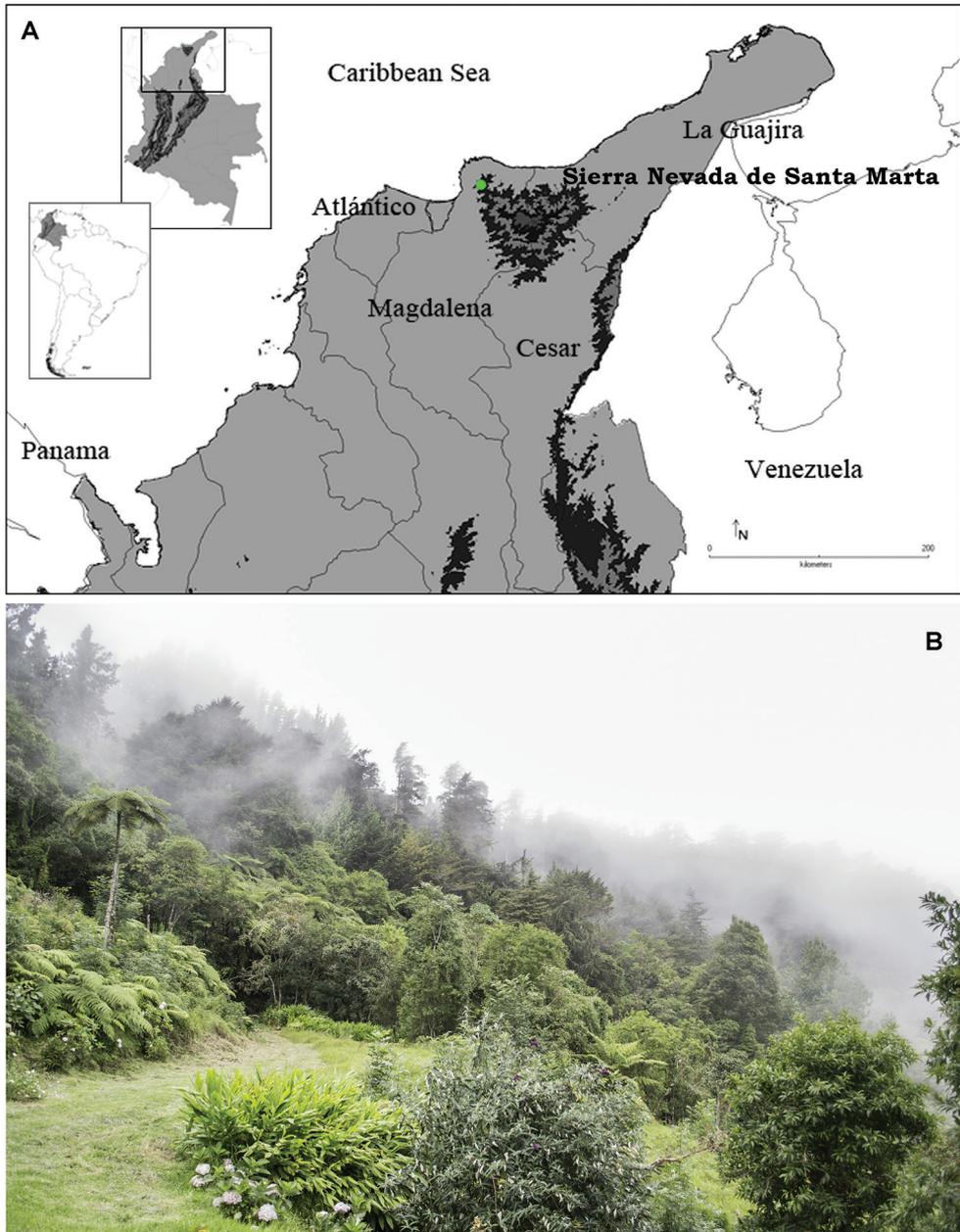


Figure 7. **A** Map of northern Colombia showing the distribution of *Kankuamo marquezii* gen. n., sp. n. **B** habitat of *Kankuamo marquezii*, Cuchilla San Lorenzo, Sierra Nevada de Santa Marta.

Table 2. Length of legs and palp segments in millimeters of holotype male/allotype female *Kankuamo marquezzi* gen. n., sp. n.

Segments	Palp	I	II	III	IV
Femur	6.2 / 8.0	9.0 / 10.8	8.5 / 10.2	8.4 / 9.7	9.5 / 11.5
Patella	2.8 / 4.5	4.5 / 6.0	3.9 / 5.7	3.5 / 5.0	3.8 / 5.4
Tibia	5.8 / 6.0	7.4 / 8.0	6.9 / 7.6	6.3 / 7.0	8.0 / 9.3
Metatarsus	-	7.0 / 8.0	7.0 / 7.5	7.8 / 8.6	10.9 / 12.0
Tarsus	2.1 / 5.4	4.3 / 4.5	4.5 / 4.5	4.2 / 4.4	4.5 / 4.5
Total	16.9 / 23.9	32.2 / 37.3	30.8 / 35.5	30.2 / 34.7	36.7 / 42.7

Description of urticating setae type VII. (Fig. 2)

Morphology. Urticating setae differ from body covering setae by the insertion feature through a stalk (types I, II, III and IV) or attached into a specialized socket (types V and VI) that facilitates detachment, plus presence of a penetrating acute tip, and barbs or scales that aid embedding them into targets (Cooke et al. 1972, Bertani and Guadanucci 2013).

Urticating setae type VII are located in a dorsal wide area of the abdomen intermixed with the covering setae, and attached to the cuticle by a thinner stalk, to facilitate their release (Fig. 2G). Setae length is $1122 \pm 40 \mu\text{m}$, and width is $33 \pm 3 \mu\text{m}$, length/width ratio 34 ($n=10$), with a very sharp penetrating tip on the distal apex, opposite to the stalk (Fig. 2A). The stalk of the setae is larger than in the other urticating types (Fig. 2E), approximately length $45 \pm 3 \mu\text{m}$, and width $10 \pm 1 \mu\text{m}$. The main shaft is straight, having small barbs (main barbs) that extend along the whole setae (Fig. 2C, D), plus a small oval patch of lanceolated reversed barbs near the penetrating tip (Fig. 2A, B).

Main barbs are subtriangular denticles not homogeneous in size and density, longer on the basal region (Fig. 2E) and densest on the distal (Fig. 2A), and oriented with the acute extreme toward the basis of the setae. Main barbs length on distal area less than $1 \mu\text{m}$ (Fig. 2A, C), medial area around $3 \mu\text{m}$ (Fig. 2D), and on basal area of $9 \pm 2 \mu\text{m}$ (Fig. 2E).

The patch of lanceolate barbs its located sub-apically at a distance approximately of $160 \mu\text{m}$ from the tip to the patch centre (Fig. 2A, 2B). Patch approximately $30 \mu\text{m}$ length and width $15 \mu\text{m}$, with around 50 lanceolate barbs. These are arranged in reversed direction, regarding the main barbs (*sensu* Cooke et al. 1972), oriented with the acute extreme toward the penetration tip. They are longer, broader and less acute than the main barbs (Fig. 2B); with each lanceolate barb of length $5 \pm 1 \mu\text{m}$ and width $2.5 \pm 0.5 \mu\text{m}$.

Discussion. The newly characterized type VII urticating setae resemble the type II found in Aviculariinae (Cooke et al. 1972, Bertani and Marques 1996, Bertani and Guadanucci 2013), but the main differences from those are: 1) The presence of a sub-apical oval patch of lanceolated reversed barbs, 2) The penetration tip is apical (proximal in type II), 3) The main barbs are oriented towards the base of the setae (oriented towards the apex on type II), 4) The proximal end of the setae is covered by larger main barbs, and 5) Usually, the stalk remains attached to the seta (the stalk remains attached to the body in type II).

The similarities of the morphology and size of setae type VII with type II Aviculariinae (see Cooke et al. 1972, figs 11–12, 20–21) suggest a releasing mechanism by direct contact, as indicated for *Avicularia* (excepting *A. versicolor*, see Bertani et al. 2003), *Iridopelma*, *Pachistopelma* and *Typhochlaena* (Bertani 2012). These tarantulas direct the abdomen toward the stimulus and transfer the urticating setae when the abdomen of the spider touches against the target (Bertani and Marques 1996). Contrary, in Theraphosinae, the various types of urticating setae are dislodged by friction of the hind legs against the dorsum of the abdomen, and air-transported (Cooke et al. 1972).

Bertani and Marques (1996) proposed that the differences of the shape, size and thickness between Theraphosinae and Aviculariinae urticating setae could explain the two releasing mechanisms. The morphological characteristics of all then known urticating hair types in Theraphosinae, particularly their light weight and aerodynamics, would allow them to float through the air. They suggest that short and/or thin setae with a ratio of 100:1 or 200:1 are able to float through the air. Bertani et al. (2003) compared length/width ratio between urticating setae type II and III and concluded that all airborne setae (including *A. versicolor* type II) are narrow, mean width ranging from 6 to 7 μm , and length/width ratio ranging from 98.3 to 208.3. In contrast, for urticating setae released by direct contact with the target, the width was greater, ranging from 15 to 22 μm and the length/width ratio of 37.3–46.8 (see Bertani et al. 2003, Table 1). Here, the newly described urticating setae type VII width is 33 ± 3 μm and length/width ratio 34, which therefore represents the thickest setae known until now, and with the lowest known length/width ratio, aspects that together reinforce the hypothesis that their releasing mechanism is by direct contact.

Furthermore, the larger size, broader shape and often dispersed arrangement of many of the main barbs of Theraphosinae urticating setae (types I, III, VI, VI) involved in their urticating effects, presumably often contribute their ability to float in the air. Conversely, in both urticating setae types II and VII, which presumably do not float by air, the main barbs are only residual denticles, being much smaller than those of other types with known air dispersal.

The differences in the position of the penetrating tip between type VII and II also suggest a different penetration mechanism. On first contact with the target, the urticating setae type II pivots on its stalk so the apical end moves away from the target, while the basal penetration tip (which is actually adjacent to the stalk) instead lodges into the target as the stalk releases (see Bertani and Marques 1996, fig. 3). By contrast, the apical penetrating tip of the urticating setae type VII (at the opposite end to the stalk) is able to penetrate directly into the target, at the first contact with the object.

Experimentally, the possible mechanism of action of the urticating setae type VII was observed while handling specimens in alcohol. These urticating setae easily perforated the skin of human fingers perpendicularly. The dorsum of the tarantula's abdomen was touched intentionally, and on further examination of the affected fingers with a stereoscope microscope, many of these setae were found embedded in the skin (Fig. 8). These setae were firmly fixed into the skin but none penetrated more than a

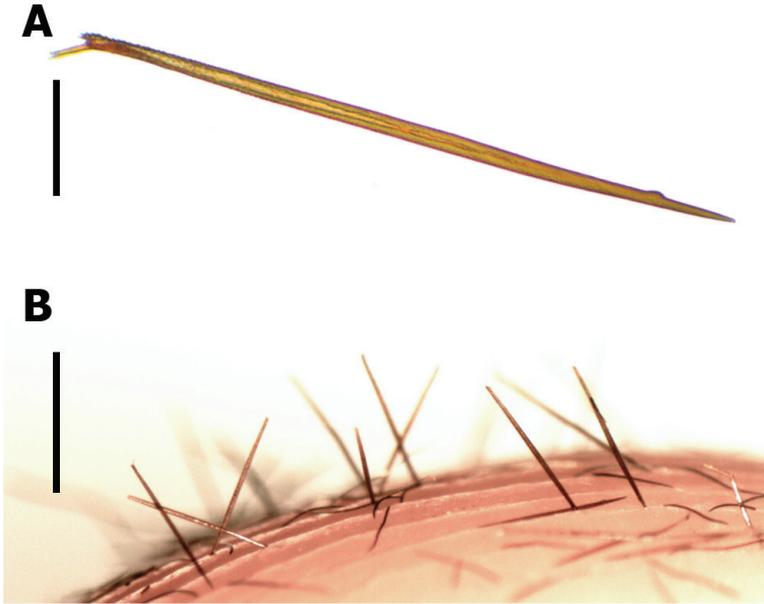


Figure 8. Urticating setae type VII **A** setae viewed in optical microscope **B** urticating setae embedded into the finger skin. Scale bars: **A** = 200 μm ; **B** = 1 mm.

third of their length. When we attempted to remove them from the skin, these setae were easily broken at their distal end. Based on our observations together, our assumption is that the patch of the lanceolate reverse barbs in the distal area can serve as a breakpoint into the skin, ensuring that the apex of the seta remains within the tissues.

Another aspect to consider is that all previously known species with contact urticating setae have leg spines absent or reduced (Cooke et al. 1972, Bertani et al. 2003). *Kankuamo* gen. n. therefore represents a remarkable exception to this suggested character association because, in contrast, they do have legs with some spines, mainly conspicuous on metatarsi and tarsi of hind-leg pair IV. Leg spines in other theraphosids have been functionally associated with releasing of airborne urticating setae by facilitating rubbing, with the exception of *A. versicolor* which use claw tufts for rubbing (Cooke et al. 1972, Bertani and Marques 1996, Bertani and Guadanucci 2013). One possible interpretation is the plesiomorphic retention of contact setae could reflect a close phylogenetic relationship between Aviculariinae and Theraphosinae, as proposed by Pérez-Miles et al. (1996). In this scenario, the additional development of posterior leg spines (such as found in *Kankuamo* gen. n.) could have facilitated the evolutionary transformation of contact setae into derived airborne setae, which are now widespread among Theraphosinae. However, if contact setae are interpreted as derived, the posterior leg spines present in *Kankuamo* gen. n. could be regarded as a plesiomorphic retention.

Conclusions

Kankuamo gen. n. fits the diagnostic characters of Theraphosinae, but also shows a very divergent palpal bulb morphology and the presence of a new abdominal urticating setae type. These setae are unique, and here are proposed to be the only contact released urticating setae yet known within the Theraphosinae, although this release mechanism was previously well known only for Aviculariinae. Also, the supernumerary keels on the male palpal bulb clearly distinguish it from all known theraphosid species. *Kankuamo* gen. n. was resolved as the sister group of *Metriopelma* on our preferred phylogeny of Theraphosinae.

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The first record of the millipede genus *Streptogonopus* Attems, 1914 from Vietnam, with description of a new species (Diplopoda, Polydesmida, Paradoxosomatidae)

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Abstract

This paper describes a new species of the millipede genus *Streptogonopus* Attems, 1914, *S. montanus* **sp. n.** from Vietnam, the first record of *Streptogonopus* in Vietnam. The new species is characterised by the solenophore completely sheathing the solenomere, both coiled twice, and the solenophore with a small spine at its middle. The species was found at ca. 1,800–2,100 m on Ngoc Linh Mountain. This first record for the genus in the Indochina peninsula has expanded its distributional range to the easternmost part of Southeast Asian mainland. An identification key to *Streptogonopus* species is also provided.

Keywords

Millipede, Paradoxosomatidae, first record, new species, Vietnam

Introduction

The genus *Streptogonopus* Attems, 1914 was created for three species, *Strongylosoma contortipes* Attems, 1898, *Strongylosoma jerdani* Pocock, 1892 and *Strongylosoma phipsoni* Pocock, 1892 (Attems 1914). Later, Attems (1929) specified the diagnostic characters for the genus *Streptogonopus*, and synonymised *Streptogonopus contortipes* (Attems, 1898)

under *Streptogonopus phipsoni* (Pocock, 1892). Attems (1936) added a new species, *Streptogonopus nitens* Attems, 1936 from Bombay Presidency, India. Soon after that, Attems (1937) revised the genus, and listed three species, *S. phipsoni*, *S. jerdani* and *S. nitens* in his major revision of the family Strongylosomidae.

Jeekel (1956) referred *Strongylosoma neglectum* Silvestri, 1895 to this genus, and described another new species, *Streptogonopus aethiopicus* Jeekel, 1956 from Eritrea. He later synonymised *S. aethiopicus* with *S. neglectus* (Jeekel 2004). Golovatch (2000) reported the first occurrence of the genus in Thailand with a new species, *S. degerboelae* Golovatch, 2000. Another species, *S. jeekeli* Golovatch, 2009, was described from China, but recently re-assigned to the genus *Hedinomorpha* Verhoeff, 1933 (Golovatch 2009, 2013). Shelley (2014) and Golovatch (2015) recently reported the occurrence of the species, *S. phipsoni*, from Pakistan, Bangladesh and Nepal.

Currently, the genus *Streptogonopus* Attems, 1914 comprises only five valid species: *S. neglectus* from Eritrea; *S. phipsoni* from India, Pakistan, Bangladesh and Nepal; *S. jerdani*, *S. nitens*, both from India; *S. degerboelae*, from Thailand. This work provides the first record of the genus in Vietnam with the description of a new species.

Materials and methods

Material examined was collected from the Ngoc Linh Mountain, the second highest mountain in Vietnam (ca. 2,600 m a.s.l.). All material was preserved in ethanol 80% and is housed in the Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Gonopods were removed for morphological examination. Only the left gonopod of holotype was coated with gold for scanning electron microscopy (SEM) using an ABT 32 scanning electron microscope. Line drawings were made using an Olympus microscope SZX10. Digital images were taken using a camera *Infinity3 Lumenera* attached to a Leica M205C stereomicroscope and stacked using the software *I-Solutions*.

Taxonomy

Family Paradoxosomatidae Daday, 1889

Tribe Xanthodesmini Jeekel, 1968

Genus *Streptogonopus* Attems, 1914

Streptogonopus Attems, 1914: 219; Attems 1929: 271; Attems 1931: 113; Attems 1936: 215–216; Attems 1937: 146–147; Jeekel 1956: 76; Jeekel 1968: 84, 111; Golovatch 2000: 218; Jeekel 2004: 15–16; Golovatch 2009: 4; Nguyen and Sierwald 2013: 1315.

Type species. *Strongylosoma contortipes* Attems, 1898, by original designation.

Diagnosis. Jeekel (2004) stated that the genus can be recognised by the paraterga being small or completely reduced; the gonopod femorite erect, narrow at the base and widening abruptly; demarcation between femorite and postfemoral region present; the solenomere and solenophore twisted one or two times; and the solenomere completely sheathed by solenophore.

Remarks. The genus can be separated into two small groups based on the absence or presence of paraterga. The former group contains *S. jerdani* and *S. degerboelae*, the latter comprises *S. neglectus*, *S. phipsoni*, and *S. nitens*.

***Streptogonopus montanus* sp. n.**

<http://zoobank.org/DD5ECD19-5548-4BF2-964D-E59BB69ADFE1>

Figs 1–3

Material examined. *Holotype*: 1 male (**IEBR-Myr 131H**) Vietnam, Kon Tum Prov., Ngoc Linh Mts. (107°58'30"E; 15°04'09"N), primary forest, 1,900 m a.s.l., pitfall traps, 29 March – 4 April 2006, leg. Nguyen A.D.

Paratypes: 1 female (**IEBR-Myr 131P**) same data as holotype; 1 male (**IEBR-Myr 145**) same locality, but 1,900–2,100 m a.s.l., 21 March–9 April 2006; 2 males, 1 juvenile (**IEBR-Myr 132**) same locality, but 1,800 m a.s.l., pitfall traps, 20–26 March 2006, all leg. Nguyen A.D.

Diagnosis. This species is recognised by the gonopod femorite grooved mesally, slightly curved and expanded distally, without processes; solenophore and solenomere coiled twice, equal in length. Solenophore with a small spine at ½ its length.

Etymology. “*montanus*”, an adjective to emphasise that the species has been found in a mountainous region.

Description. Length 26.7–28.2 mm (male) and 30 mm (female). Width of mid-body pro- and metazonae 2.3–2.5 mm (male), 2.8 mm (female) and 2.7–2.9 mm (male), 3.1 mm (female), respectively.

Coloration (Fig. 1A–B, D): body generally castaneous or reddish brown, except legs, antenna and sterna somewhat brownish yellow.

Head subequal to collum in width; clypeolabrum modestly setose. Epicranial suture distinct. Antennae slender and long, reaching the end of segment 4 when stretched posteriorly. Antennomere 2=3=4=5=6>1>7 in length.

Collum (Fig. 1B) somewhat narrower than segment 2 in width. Surface dull and weakly wrinkled, with three rows of 4+4, 4+4 and 2+2 setae. Transverse sulcus absent. Axial line thin and evident. Paraterga modestly developed, subtriangular with broadly rounded anterior corner.

In width, segment 3<4<2=5–16, thereafter gradually tapering towards telson (Figs 1B, 1D). Prozonae shagreened. Metaterga dull, somewhat rugose with short longitudinal wrinkles, and with a row of 3+3 setae near anterior margin and a row of 3(4)+3(4) small knobs near posterior margin. Transverse sulcus starting present on metatergum 5, rather broad, not reaching base of paraterga, neither striolate nor beaded at bottom.

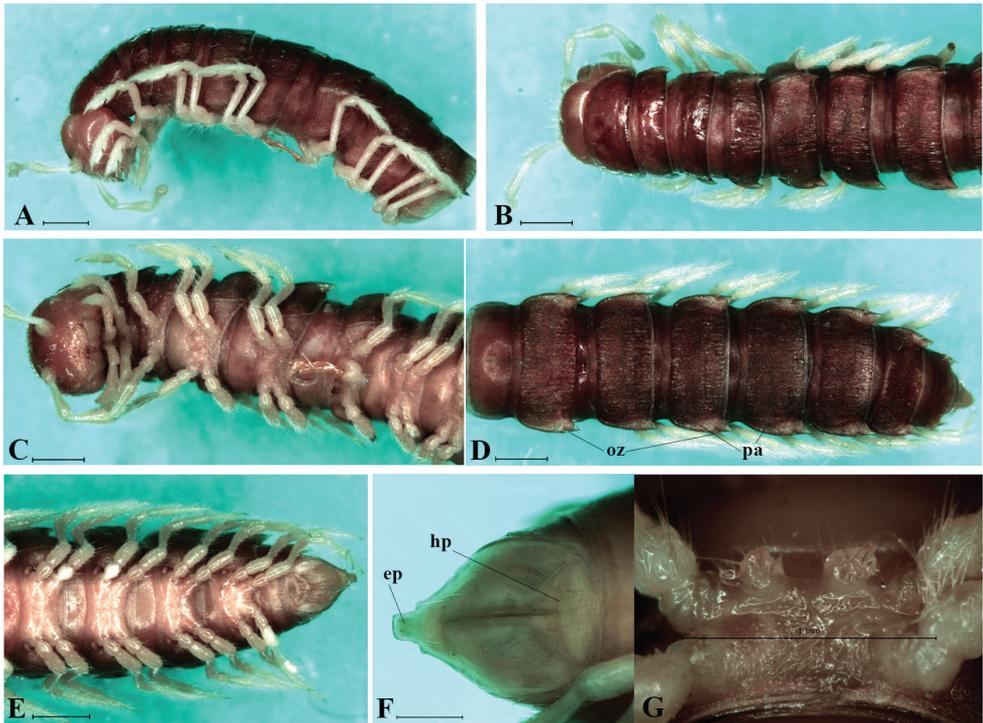


Figure 1. *Streptogonopus montanus* sp. n., holotype; anterior segments, lateral view (A), dorsal view (B), ventral view (C); posterior segments, dorsal view (D), ventral view (E); telson, ventral view (F); sternal processes between coxae of legpair 4, subventral view (G). Scale bar = 1 mm. For abbreviations see text.

Constriction between pro- and metazonae wide, striolate at bottom. Pleura with dense microgranulation. Pleurosternal carinae well developed on segments 2–7, reduced as a small caudal denticle on segments 8–14, then missing on subsequent segments.

Paraterga (**pa**) (Fig. 1A–B, D) modestly developed, wing-shaped, set lower than metatergal surface. Caudolateral corner pointed, spiniform on caudal segments. Paraterga surpassing posterior contour of metaterga, but not reaching next metaterga. Calleses very small, somewhat wanting on poreless paraterga, with a long seta laterally. Ozopores (**oz**) located near caudolateral corner of paraterga 5, 7, 9–10, 12–13, 15–19.

Epiproct (**ep**) (Fig. 1D, E–F) long, but broadly truncated, with four spinnerets at tip. Hypoproct (**hp**) roundly triangular, with two separated, distolateral, setiferous knobs.

Sterna (Figs 1C, 1E) moderately setose, without modifications except sternum 5 with two independent setiferous projections between coxae of legpair 4 (Fig. 1G).

Legs (Figs 1A–E) thin and slender, about 1.3 times as long as midbody height. Prefemora not swollen. Femora without modification. Tarsal brushes present only on pre-gonopodal legs.

Gonopods relatively simple (Figs 2–3). Coxite long, subcylindrical; distoventral part sparsely setose. Prefemorite (**pref**) densely setose, separated laterally from femorite by transverse sulcus. Femorite (**fe**) grooved mesally, slightly curved and expanded distad,

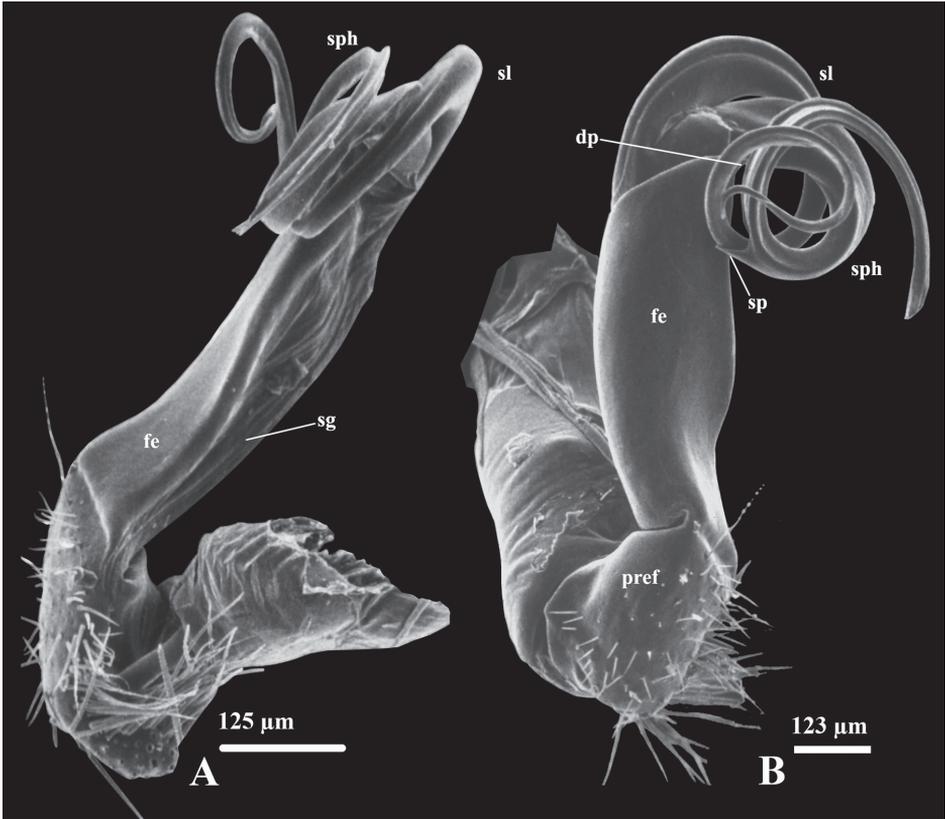


Figure 2. *Streptogonopus montanus* sp. n., holotype, left gonopod, mesal view **(A)**, subventral view **(B)**. For abbreviations see text.

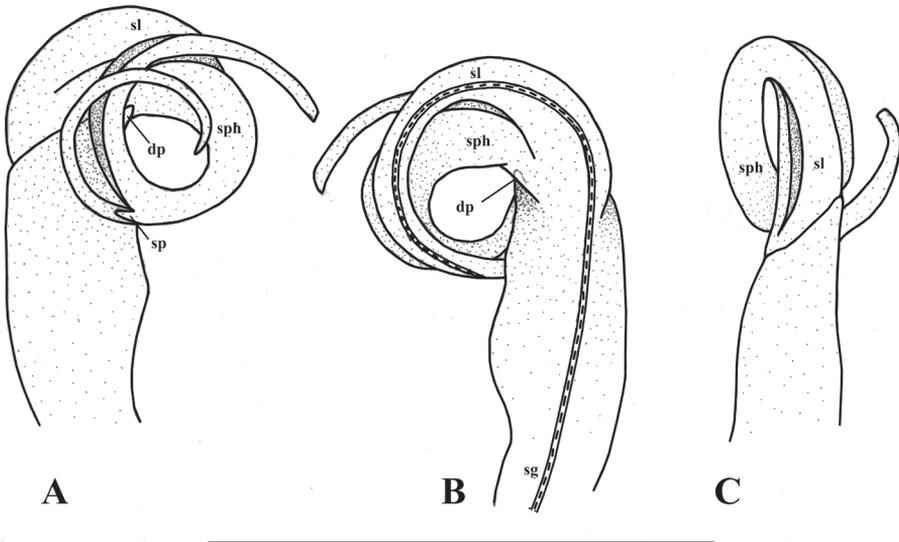


Figure 3. *Streptogonopus montanus* sp. n., holotype, left gonopod, ventral view **(A)**, dorsal view **(B)**, lateral view **(C)**. Scale bar = 1 mm. For abbreviations see text.

with distolateral processes (**dp**); demarcated laterally from the postfemoral region (= solenophore) by an oblique sulcus. Solenophore (**sph**) and solenomere (**sl**) coiled more or less twice, equal in length. Solenophore with a small spine (**sp**) at half its length. Seminal groove (**sg**) running mesodorsad entirely mesally on femorite before entering the flagelliform solenomere.

Remarks. This new species could be assigned to the second group characterised by modestly developed paraterga. However, it differs from three its congeners, *S. neglectus*, *S. phipsoni*, and *S. nitens* in solenomere and solenophore being equal in length and strongly coiled twice and the solenophore having a small spine at its middle.

Key to species of the genus *Streptogonopus* Attems, 1914

(based on the keys compiled by Jeekel (1956) and Attems (1936))

- 1 Paraterga strongly reduced, even totally missing.....2
- Paraterga poorly to modestly developed3
- 2 Body coloration yellowish. Body surface wrinkled and dull. India ... *S. jerdani*
- Body coloration marble brown. Body surface smooth and somewhat shining. Thailand *S. degerboelae*
- 3 Midbody width large, more than 2 mm. Asia.....4
- Midbody width small, less than 2 mm. Africa (Eritrea) *S. neglectus*
- 4 Solenophore and solenomere strongly coiled twice; solenophore with a small spine at its middle. Vietnam *S. montanus* sp. n.
- Solenophore and solenomere not coiled twice; solenophore without a small spine at its middle. South Asia5
- 5 Pleurosternal carinae caudally sharp and dentiform..... *S. phipsoni*
- Pleurosternal carinae caudally rounded and lobiform *S. nitens*

Discussion

The genus is known to occur in Eritrea, India, Pakistan, Bangladesh, Nepal and Thailand (Nguyen and Sierwald 2013; Shelley 2014; Golovatch 2015). The discovery of a new species in Vietnam has expanded the distribution range of the genus *Streptogonopus* to the easternmost part of the Southeast Asian mainland. However, there is still a gap in the distribution with no records reported from Myanmar, Laos and Cambodia (Nguyen and Sierwald 2013; Likhitrakarn et al. 2014, 2015).

The new species was found at a high elevation (ca. 1,800–2,100 m a.s.l.) on Ngoc Linh Mountain (Kon Tum Province), the second highest mountain, in Vietnam and is thus unlikely to be an introduction due to human activities. The relatively few records for the genus indicates that there are likely to be more *Streptogonopus* species awaiting discovery in Indochina and its adjacent regions.

Being located in the Burmese-Indochinese Biodiversity Hotspot (Sterling et al. 2006), Vietnam is known to have a rich fauna including millipedes. The discovery of the millipede genus *Streptogonopus* in Vietnam indicates that the Vietnamese millipede fauna may be richer at genus level than previously suspected.

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Description of a new species of *Pseudomegischus* van Achterberg from China (Hymenoptera, Stephanidae)

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Abstract

A new species of the genus *Pseudomegischus* van Achterberg, *Pseudomegischus yunnanensis* sp. n., is described and illustrated from Yunnan Province, China. This is the second species of the genus reported from China. A modified section of the identification key to species of *Pseudomegischus* is added to include the new species.

Keywords

Stephanidae, *Pseudomegischus*, new species, Oriental Region, China

Introduction

The genus *Pseudomegischus* van Achterberg, 2002 is a small group of parasitic wasps in the family Stephanidae (Hymenoptera), with only five described species worldwide (Tan et al. 2015). The genus seems to have an Indo-Australian distribution (van Achterberg 2002; Hong et al. 2011; Tan et al. 2015). According to Tan et al. (2015), the potential hosts of the genus include Cerambycidae (Coleoptera) and/or Siricidae (Hymenoptera). Here we report the second species of the genus from the Oriental part of China. We modify the key published by Tan et al. (2015) to include the new species.

Material and methods

Descriptions of the species have been made under an Olympus SZ61, with lighting achieved through a 27W fluorescent lamp. Photographic images were produced by a digital microscope (VHX-2000c, KEYENCE, Osaka, Japan), and plates were finished with ACDSee 10.0 and Photoshop CS 8.0.1, mostly to adjust the size and background.

Morphological nomenclature follows van Achterberg (2002) and the identification key is modified from the key to species of the genus *Pseudomegischus* in Tan et al. (2015).

Type material is deposited in the Shanghai Entomological Museum, Shanghai, China (SEMC) (Curator: Dr. Hai-sheng Ying).

Taxonomy

Pseudomegischus van Achterberg, 2002

Pseudomegischus van Achterberg, 2002: 169; Aguiar 2004: 73–74 (list of literature); Hong et al. 2011: 7; Tan et al. 2015: 104. Type species (by original designation): *Stephanus sulcifrons* Schletterer, 1889.

Pseudomegischus yunnanensis Chen & Xu, sp. n.

<http://zoobank.org/D24C3295-179F-4732-8A0E-D2D8E0CEB32D>

Figs 1–15

Material examined. Holotype, ♀ (SEMC), CHINA: Yunnan, Xishuangbanna, Menglun, 1000 m, 3.VI.2009, No. 34001533.

Etymology. Named after the province of the type locality.

Diagnosis. Frons coarsely obliquely rugose near anterior coronal tooth, transversely rugose ventrally (Fig. 1); vertex transversely carinate antero-medially, becoming smooth posteriorly (Fig. 3); middle part of pronotum transversely rugose anteriorly, largely



Figures 1–4. *Pseudomegischus yunnanensis* sp. n., holotype, female. **1** Head frontal **2** head lateral **3** head dorsal **4** pronotum and mesonotum dorsal.

smooth and without distinct carina posteriorly (Fig. 4); scutellum smooth medially, foveolate laterally (Figs 4, 7); propodeum coarsely and densely foveolate, without distinct smooth interspaces (Fig. 7); first tergite largely transversely rugose (Fig. 13).

In the key to species of the genus *Pseudomegischus* by Tan et al. (2015), the new species can be included by replacing couplet 3 as follows:

- 3 Vein 1-M of fore wing $4.8\text{--}5.4 \times$ as long as vein 1-SR; hind femur with distinct third medium-sized tooth behind large posterior tooth; length of ovipositor sheath about $2.1 \times$ fore wing..... *P. sulcifrons* (Schletterer, 1889)
- Vein 1-M of fore wing about $3 \times$ as long as vein 1-SR (Fig. 12); hind femur without distinct third medium-sized tooth behind large posterior tooth (Fig. 10); length of ovipositor sheath $2.3\text{--}2.5 \times$ fore wing..... **4**

- 4 Hind basitarsus orange-brown, distinctly contrasting with blackish hind tibia; temple without distinctly differentiated ivory streak; hind femur moderately widened medially in lateral view...*P. celebensis* van Achterberg, 2002
- Hind basitarsus largely blackish-brown, not distinctly contrasting with blackish hind tibia (Figs 10, 11); pale or yellow streak of temple well differentiated (Fig. 2); hind femur strongly widened medially in lateral view 5
- 5 Propodeum densely irregularly rugose; first tergite largely smooth except irregular rugae basally and some oblique and fine striae after middle of first tergite *P. notiochinensis* Tan & van Achterberg, 2015
- Propodeum coarsely and densely foveolate, without distinct smooth interspaces (Fig. 7); first tergite largely transversely rugose (Fig. 13) *P. yunnanensis* sp. n.

Description. Holotype. *Female*. Body length 21.2 mm; fore wing length 9.6 mm.

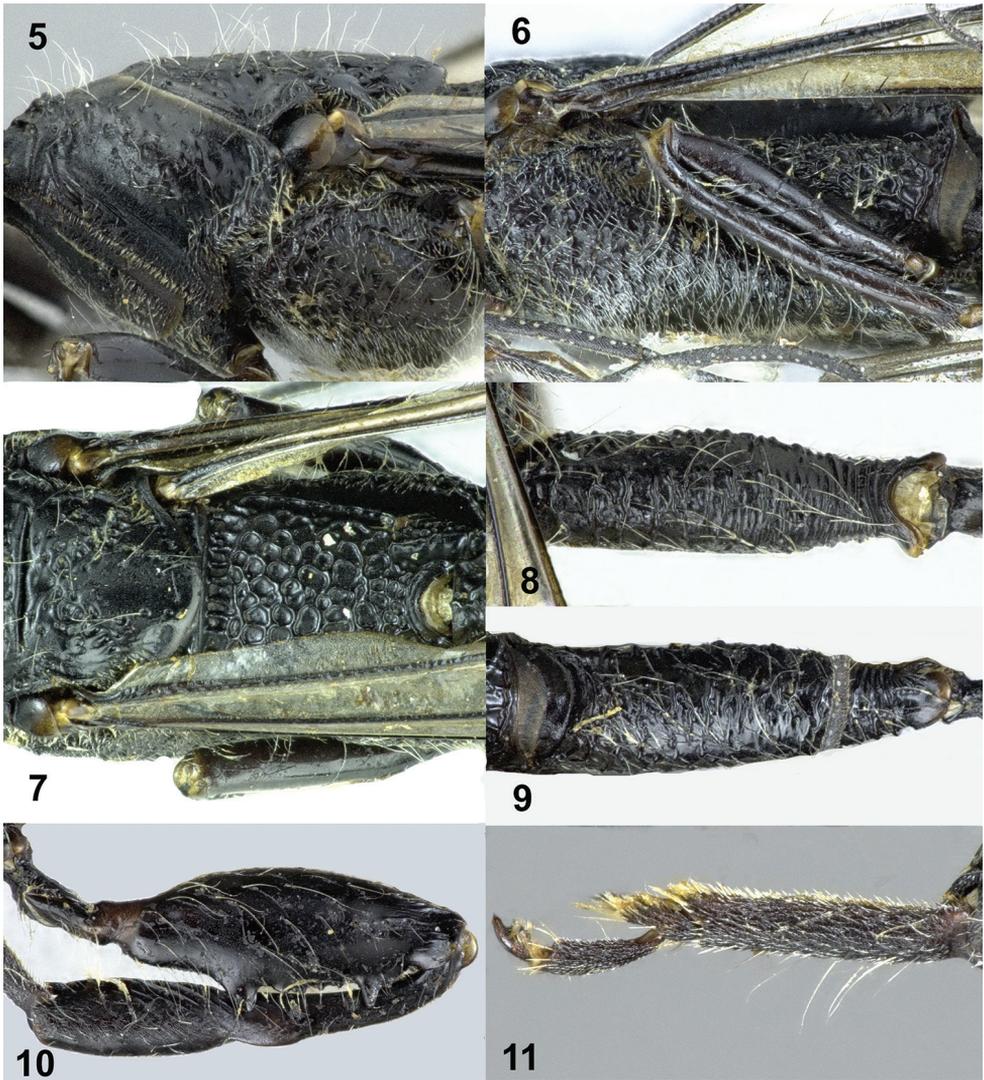
Colour. Black, except: frons yellowish-brown; temple brown with yellow streak along eye, streak becoming widened near vertex; base of mandible yellow; palpi, vertex, scape, pedicel, tegula and base of femora dark brown; veins and pterostigma largely dark brown, but base of pterostigma ivory; wing membrane slightly brownish; ovipositor sheath blackish apically (Fig. 15).

Head. Antenna with 41 segments; frons coarsely obliquely rugose near anterior coronal tooth, transversely rugose ventrally (Fig. 1); three anterior coronal teeth large and acute, both posterior ones arcuate and lamelliform, with two small lobe-shaped carinae on each side in front of both posterior ocelli; vertex transversely carinate antero-medially, becoming smooth posteriorly (Fig. 3); temple non-angulate, smooth and shiny (Fig. 2).

Mesosoma. Neck short and robust, transversely rugose, neck at much lower level than middle part of pronotum (Fig. 4); middle part of pronotum transversely rugose anteriorly, largely smooth and without a distinct carina posteriorly; propleuron largely coriaceous with sparse small punctures, shiny and densely setose (Fig. 5); mesonotum sparsely and irregularly foveolate and area between foveae smooth; notauli and median groove distinct; scutellum smooth medially, foveolate laterally; axillae irregularly and rather densely foveolate; mesopleuron distinctly convex, convex part coarsely foveolate-rugose and covered with long whitish setae and dense short setae; metapleuron coarsely foveolate (Fig. 6); propodeum coarsely and densely foveolate, without distinct smooth interspaces (Fig. 7).

Wings. Fore wing (Fig. 12): vein 1-M $3.1 \times$ as long as vein 1-SR and curved; vein r ends slightly behind level of apex of pterostigma; first subdiscal cell robust, $3.2 \times$ as long as its maximum width, vein cu-a entirely pigmented.

Legs. Hind coxa robust, without tubercle dorsally, transversely and densely rugose (Figs 8, 9); hind femur widened, smooth and with long whitish setae, ventrally with two large acute teeth (the anterior one larger than posterior one) and four denticles in between (Fig. 10); hind tibia $1.2 \times$ as long as hind femur, basal narrow part of hind tibia about $1.2 \times$ as long as widened part, widened part ventrally distinctly obliquely



Figures 5–11. *Pseudomegischus yunnanensis* sp. n., holotype, female. **5** Propleuron and mesopleuron lateral **6** mesopleuron, metapleuron and propodeum lateral **7** scutellum and propodeum dorsal **8** hind coxa dorsal **9** hind coxa lateral **10** hind femur and tibia lateral **11** hind tarsus lateral.

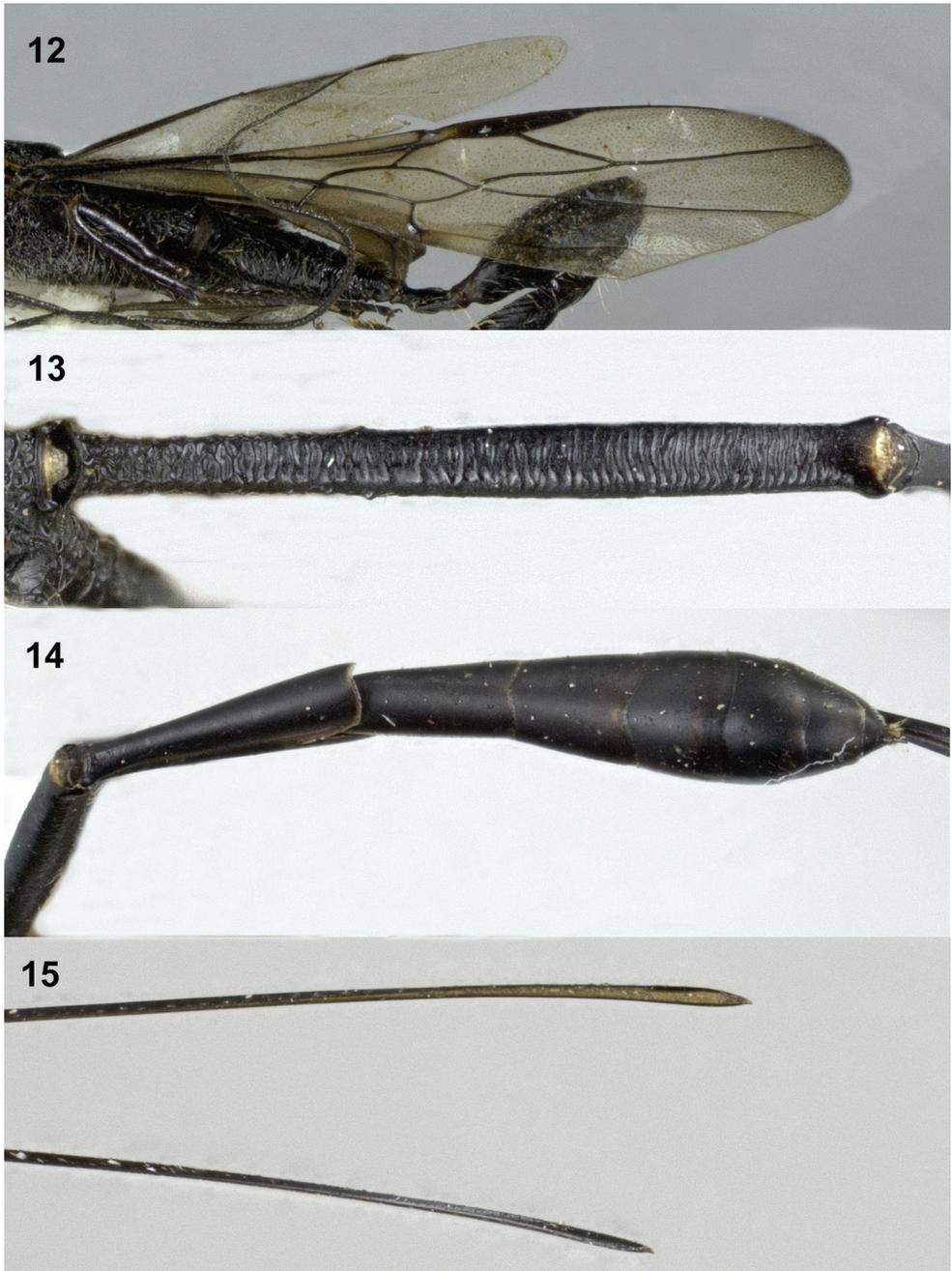
carinate (Fig. 10); hind basitarsus subparallel-sided, length of hind basitarsus $4.8 \times$ as long as wide medially and $3.8 \times$ as long as second tarsal segment (Fig. 11).

Metasoma. First tergite $9.0 \times$ as long as its maximum width, $1.6 \times$ as long as second tergite, cylindrical, largely transversely rugose (Fig. 13); remainder of tergites smooth and shiny (Fig. 14); length of ovipositor sheath $2.5 \times$ length of fore wing.

Male. Unknown.

Distribution. Oriental: China (Yunnan).

Biology. Collected in June. Host not known.



Figures 12–15. *Pseudomegischus yunnanensis* sp. n., holotype, female. **12** Wings **13** first tergite dorsal **14** metasoma dorsal **15** apex of ovipositor sheaths.

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A review of the Japanese species of the family Tischeriidae (Lepidoptera)

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Abstract

This paper provides taxonomic and biological data on one new and one newly recorded species of *Coptotriche* Walsingham and one new and one newly recorded species of *Tischeria* Zeller from Japan. *Coptotriche symplocosella* Kobayashi & Hirowatari, **sp. n.** (host *Symplocos lucida*, Symplocaceae), and *Tischeria kumatai* Sato, Kobayashi & Hirowatari, **sp. n.** (host *Tilia japonica*, Malvaceae) are described. The pupal morphology of *C. symplocosella* is illustrated with scanning electron micrographs. *Coptotriche minuta* Diškus & Stonis, 2014 and *Tischeria relictana* Ermolaev, 1986 are newly recorded from Japan. The female, hostplants (*Carpinus*, *Corylus*, and *Ostrya* species), and immature stages of *C. minuta* and the adult features, female, and hostplants (*Betula* species) of *T. relictana* are described with photographs and drawings for the first time. Mine types and characters of Japanese Tischeriidae are reviewed with photographs.

Keywords

Betula, *Carpinus*, *Corylus sieboldiana*, genitalia, leafminer, mine, *Ostrya*, taxonomy

Introduction

The Tischeriidae is a lepidopteran family comprising some of the smallest moths, with a wing expanse of only 5–11 mm. Tischeriid adults are rather similar to one another in appearance, with a brown or blackish gray vestiture. The family can be distinguished from other families by a frontal tuft projecting over a triangular face smoothly covered with scales, numerous, long and recurved, cilia-like sensilla trichodea (see Davis 1986 and van Nieukerken and Dop 1987) on the male antenna, in the male genitalia by a strongly narrowed phallus, usually bifurcate or with apical spines, and in the female genitalia with four to five pairs of apophyses (Puplesis and Diškus 2003). Puplesis and Diškus (2003) recognized three genera: *Tischeria* Zeller, 1839, *Coptotriche* Walsingham, 1890, and *Astrotischeria* Puplesis & Diškus, 2003. Until now, 115 tischeriid species have been described globally. Nearly eleven plant families have been reported as their hosts, among which Rosaceae, Fagaceae and Asteraceae are major groups (Puplesis and Diškus 2003; Stonis et al. 2014). Several species of Tischeriidae have recently been added to the Eastern Palaearctic fauna, including two species from China (Huang and Tan 2009) and two new species from the Russian Far East (Stonis et al. 2014).

In Japan, two genera and seven species of Tischeriidae have been described to date (Sato 2011; see checklist below). In addition, some unnamed species have also been collected (Oku 2003, Sato 2011). Among them, according to Sato (2011), two are unidentified *Tischeria* species associated with *Tilia* (Malvaceae) and *Betula* (Betulaceae) respectively, and two are unidentified *Coptotriche* species associated with *Carpinus* (Betulaceae) and *Quercus* (Fagaceae) respectively.

In this paper, we taxonomically review the Japanese species of the family Tischeriidae, resolving the identity of three of these unidentified species with descriptions of two new species and two newly recorded species. For the two *Coptotriche* species, larval and/or pupal stages are also described. Eight Japanese species were reared, and their mine types and characters are reviewed with photographs.

Materials and methods

Adults were collected with light traps and leaves with mining larvae and cocoons were sampled from March to November in 2008 to 2015 in locations shown in Table 1. Adult specimens are preserved in the Osaka Prefecture University (OPU) and Tokushima Prefectural Museum (TKPM). Immatures in leaves were reared in plastic cups (420 ml: 129 mm in top diameter and 60 mm in depth) containing wet cotton at 20±5 °C under a photoperiod of 13–16L : 8–12D in the laboratory. In addition, specimens collected by Dr H. Kuroko in OPU, those collected by Dr T. Kumata in Hokkaido University Museum (HUM), and collections of the third author (Hirano) were examined.

Photographs of leaf mines were taken primarily in the field using an OLYMPUS μ 1060 digital camera. Some leafmines were scanned using an EPSON GT7400. Some pupae were dried and sputter-coated with a 60 : 40 mixture of gold-palladium for examination with

Table 1. Study sites of Tischeriidae species.

Locality	Prefecture	Island	Longitude and latitude	Altitude (m)	Figure number
Sai-ko, Fuji-Kawaguchiko	Yamanashi	Honshu	35°29'58"N, 138°39'32"E	930	
Soni, Uda	Nara	Honshu	34°30'N, 136°07'E	400–1000	1A–E
Mt. Wasamata, Kamikitayama	Nara	Honshu	34°13'05"N, 135°58'58"E	1150	
Mt. Kumoso, Kamiyama	Tokushima	Shikoku	33°54'43.4"N, 134°17'22.7"E	1123	Hirowatari et al. (2011): 1A, B
Adachi Park, Kokura	Fukuoka	Kyushu	33°51'56"N, 130°54'21"E	80–150	1F
Mikata, Tsushima Is.	Nagasaki	Kyushu	34°17'10"N, 129°16'20"E	20–30	1G–I

a scanning electron microscope (SEM). SEM photographs were taken using HITACHI SU1510 with a lanthanum hexaboride (LaB6) source at an accelerating voltage of 15 kV. For preparation of the male and female genitalia, the abdomen was removed and boiled for 3–4 min in 10% aqueous KOH. They were stained with acetocarmine.

Terms for genitalia, in principle, follow Stonis et al. (2014). The term “prela” introduced by Braun (1972) is used to designate the two or three paired rod-like or plate-like apophyses that extend from the inner side of the 8th and 9th sternites. The term “antrum” is employed to indicate the strongly thickened, differently shaped walls of the vestibulum following Puplesis and Diškus (2003). Scientific names of plants follow the Missouri Botanical Garden *Tropicos* database (2015).

A checklist of the Japanese species of the family Tischeriidae

I. Genus *Tischeria* Zeller, 1839

1. *Tischeria naraensis* Sato, 1993

Distribution: Japan: Honshu (Kinki region).

Hostplants: *Quercus acutissima* and *Q. variabilis*, Fagaceae.

2. *Tischeria quercifolia* Kuroko, 1982

Distribution: Japan: Hokkaido, Honshu, Shikoku, Kyushu.

Hostplants: *Quercus acutissima*, *Q. crispula*, *Q. dentata*, and *Q. serrata*, Fagaceae.

3. *Tischeria decidua siorkionla* Kozlov, 1986

Distribution: Japan: Hokkaido, Honshu, Kyushu (Tsushima Is.); the Russian Far East.

Hostplants: *Quercus acutissima*, *Q. crispula*, *Q. dentata*, *Q. serrata*, and *Q. variabilis*, Fagaceae.

4. *Tischeria kumatai* Sato, Kobayashi & Hirowatari, sp. n.

Distribution: Japan: Hokkaido, Honshu (Nagano).

Hostplants: *Tilia japonica*, Malvaceae.

5. *Tischeria relictana* Ermolaev, 1986

Distribution: Japan: Hokkaido, Honshu, Shikoku; the Russian Far East.

Hostplants: *Betula ermanii* and *B. grossa*, Betulaceae.

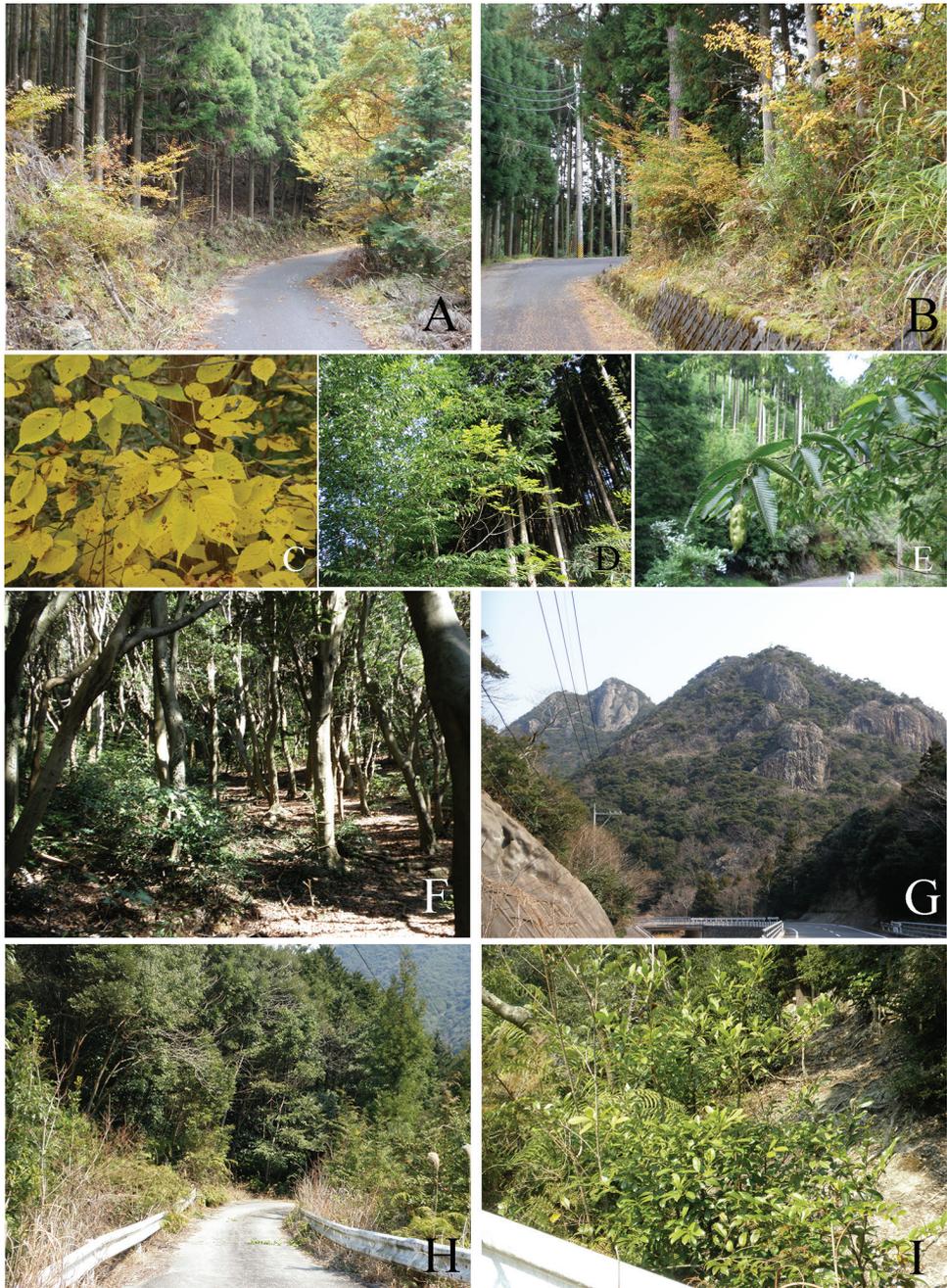


Figure 1. Habitats and hostplants of *Coptotriche* species. **A–E** *C. minuta* Diškus & Stonis, 2014, Soni, Nara Prefecture **F–I** *C. symplacosella* sp. n. **A** Habitat, Mitsuiyatani, Konagao, 710 m **B** Habitat, Nagano, 600 m **C** Leaves of *Carpinus laxiflora* at Nagano **D** Branches of *C. japonica* at Kumawata, Konagao **E** Leaves and fruits of *C. japonica* at Kumawata **F** Type locality, Adachi Park, Kokura, Fukuoka Prefecture **G** Habitat, Jyozan, Mitsushima, Tsushima Is., Nagasaki Pref. **H** Habitat and host plants, *Symplocos lucida* **I** *Symplocos lucida* tree.

II. Genus *Coptotriche* Walsingham, 1890

6. *Coptotriche angusticollella* (Duponchel, 1843)
Distribution: Japan: Hokkaido, Honshu; Europe; Tunisia; Turkey; Caucasus; Turkmenistan; South Korea; the Russian Far East.
Hostplants: *Rosa multiflora*, *R. wichuraiana*, *Rosa* spp., Rosaceae.
7. *Coptotriche heinemanni* (Wocke, 1871)
Distribution: Japan: Honshu, Shikoku, Kyushu; Europe; Tunisia; South Korea; Russia.
Hostplants: *Agrimonia pilosa* var. *japonica*, *Geum japonicum*, *Rubus crataegifolius*, *R. microphyllus*, *R. leucodermis* and *R. palmatus* var. *palmatus*, Rosaceae.
8. *Coptotriche japoniella* Puplesis & Diškus, 2003
Distribution: Japan; China.
Hostplants: *Eurya emarginata* and *E. japonica*, Theaceae.
9. *Coptotriche szoecsi* (Kasy, 1961)
Distribution: Japan*; Europe.
Hostplants: *Sanguisorba officinalis*, Rosaceae.
* Hokkaido: subsp. *szoecsi*; Honshu: subsp. *japonica* (Kuroko, 1982).
10. *Coptotriche minuta* Diškus & Stonis, 2014
Distribution: Japan: Honshu, Shikoku, Kyushu; the Russian Far East.
Hostplants: *Carpinus cordata*, *C. japonica*, *C. laxiflora*, *C. tschonoskii*, *Corylus sieboldiana* and *Ostrya japonica*, Betulaceae.
11. *Coptotriche symplocosella* Kobayashi & Hirowatari, sp. n.
Distribution: Japan: Kyushu.
Hostplants: *Symplocos lucida*, Symplocaceae.

Taxonomy***Coptotriche minuta* Diškus & Stonis, 2014**

Figs 2A–C, 3A–D, 7, 8

Coptotriche minuta Diškus & Stonis, 2014: 143–144, figs 5–10.*Coptotriche* sp.: Sato 2011: 128, 559, figs II-14.3A, B.**Type locality.** Russia: the Russian Far East (Primorskiy Territory).**Material examined.** 38 (17♂ 17♀ 4 exs).Host *Carpinus cordata*: 1♀, Oshirakawa, Azumi, Matsumoto, Nagano Pref., 29.vii.1990, N. Hirano leg., 7.vii.1990(larva), (genitalia slide no. OPU-SK568)Host *C. japonica*: 1♂ 1♀, Oshirakawa, Azumi, Matsumoto, Nagano Pref., 26&29. iv.1991, N. Hirano leg., 27.x.1990(larva), SK563, 564; [Soni, Uda, Nara Pref., S. Kobayashi leg.]: 1♀, Konagao, 18.vii.2012em., 14.vii.2012(larva); 1♀, Kameyama, Taroji, 23.vii.2011em., 24.vi.2011(larva). 3♂, Kabuto-dake climb point, 9&16. ix.2010em., 17.x.2010(larva), SK406.

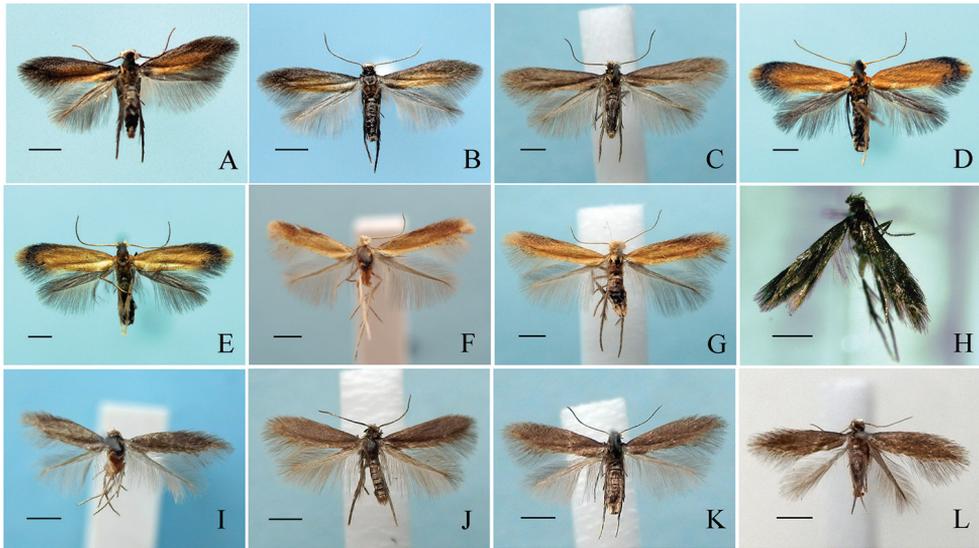


Figure 2. Adults of Tischeriidae species from Japan. **A** *Coptotriche minuta* Diškus & Stonis, 2014, male (Nara Prefecture) **B** Female (Nara Pref.) **C** Male, overwintering form (Nagano Pref.) **D** *C. symplacosella* sp. n., holotype male **E** Paratype female. **F** *Tischeria kumatai* sp. n., holotype male **G** Paratype female (Nagano Pref.) **H** *T. relictana* Ermolaev, 1986, male (Tokushima Pref.), hostplant unknown **I** Female (Hokkaido), hostplant: *B. ermanii* **J** Male (Nagano Pref.), hostplant: *Betula grossa* **K** Female, same hostplant **L** Female (Nara Pref.), hostplant unknown. Scale bar: 1 mm.

Host *C. laxiflora*, S. Kobayashi leg.: 1♂, Mitsuiyatani, Konagao, Soni, Uda, Nara Pref., 16.ii.2011em., 14.ix.2011(larva), SK407; 1♂ 1♀, Ohshirakawa, Nagawa, Matsumoto, Nagano Pref., 7.viii.2012em., 3.viii.2012(larva).

Host *C. tschonoskii*: 1♂, Kiso-Hukusima, Nagano Pref., 27.iv.1976em., T. Kumata leg., Rearing code: Kumata 1520, Genitalia slide no. HS-G54, deposited in HUM (Sato, 2011: fig.II-14.3A); 1 ex, Tawamine, Konagao, Soni, Uda, Nara Pref., 18.viii.2015em., S. Kobayashi leg., 15.viii.2015(pupa); Ehime Pref.: 3♂ 4♀, Matsuyama, 23.iv.1965, H. Kuroko leg.; 1♀, Nametoko nr. Uwazima, 1.v.1981em., T. Kumata leg., K2279, HS-G57 (HUM) (Sato, 2011: fig.II-14.3B); [Hikoson, Fukuoka Pref., H. Kuroko leg.]: 1♀ 1 ex, 2.v.&22.vii.1954; 2♀ 1 ex, 1&4.v.1957; 1♀, 10.viii.1957.

Host *Corylus sieboldiana*: 1♂, Saiko-nishi, Fuji-Kawaguchiko, Yamanashi Pref., 16.viii.2011em., S. Kobayashi leg., 6.viii.2011(larva), SK408. 1♀, Mt. Kuroiwa, Nagano Pref., 6.iv.1987em., H. Kuroko leg., 16.x.1986(larva).

Host *Ostrya japonica*: 1♂, Sapporo, Hokkaido, 21.vii.1959, T. Kumata leg.

Host unknown: 5♂ 2♀ 1 ex, Mt. Wasamata, Nishihara, Kamikitayama, Nara Pref., 23&24.viii.2011, collected by light trap (L.T.), T. Hirowatari, K. Ikeuchi, Y.-S. Bae & S. Kobayashi leg., SK570.

Diagnosis. See original description.

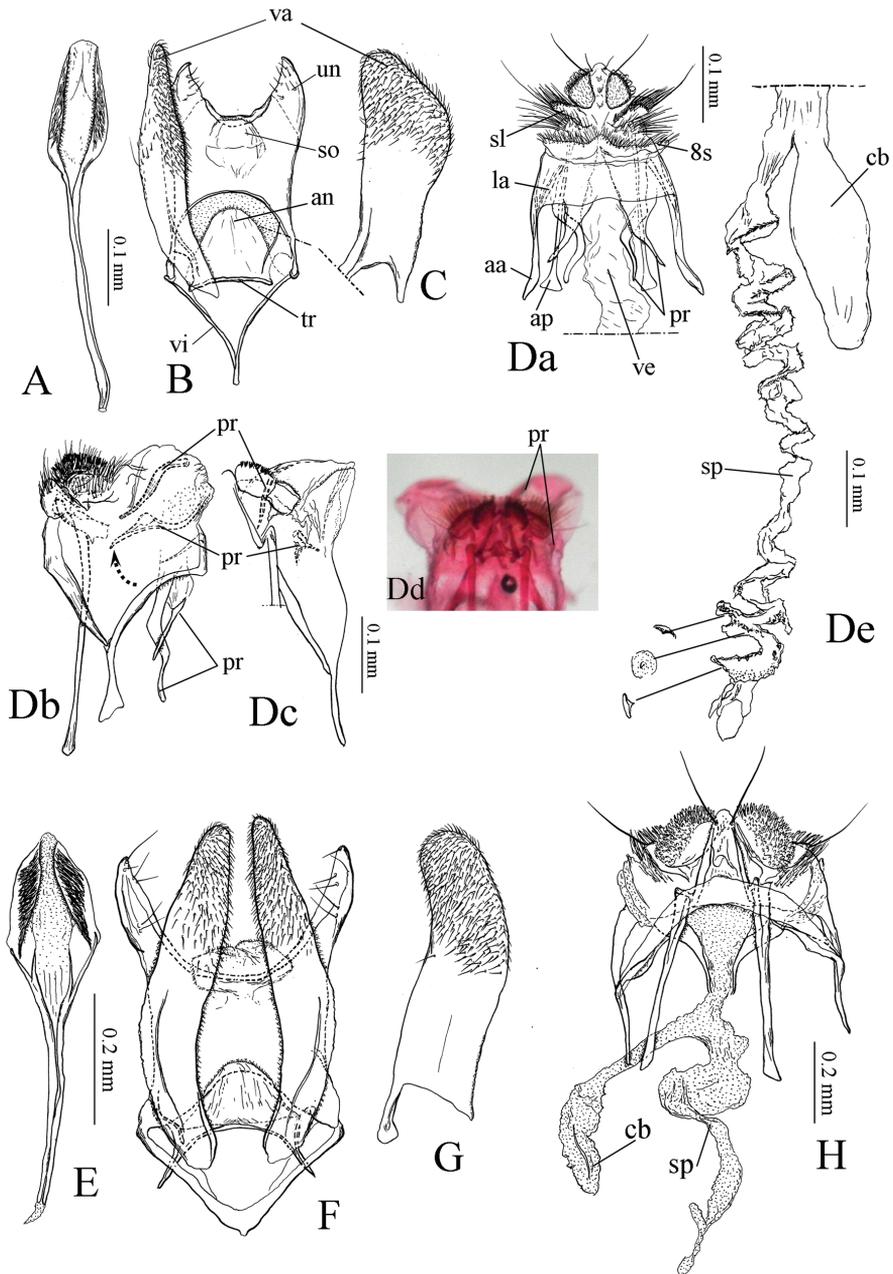


Figure 3. Genitalia of *Coptotriche minuta* and *C. symlocosella*. **A–D:** *C. minuta*. **E–H:** *C. symlocosella*. **A, E** Phallus, ventral view **B, F** Male genitalia, ventral view **C, G** Right valva, inner view **D, H** Female genitalia, ventral view **Db, Dc, Dd** Same, separated two pairs of prela towards posterior part **Db** Lateral view **Dcd** ventral view **De** Corpus bursae and ductus spermatheca. Abbreviations: aa: apophysis anterioris; an: anellus; ap: apophysis posterioris; cb: corpus bursae; la: lateral arm of 8th tergite; pr: prela; sl: setose lobe on 9th tergite; so: socius; sp: spermatheca; tr: transtilla; un: uncus; va: valva; vi: vinculum; ve: vestibulum; 8s: 8th sternite.

Additional description. Adult (Fig. 2A–C). Male and female. Wing expanse 6.6–8.8 mm in Japanese specimens, 8.6, 8.8 mm in hibernating generation, and 6.6–7.7 mm in summer and autumn generations. Forewing (Fig. 2A–C) pale to dark ochreous, especially blackish brown in overwintering form (Fig. 2C). Japanese specimens of summer and autumn generations have more distinctly grayish brown scales along the costal margin and apical part of the forewing than the type series collected in July and August.

Male genitalia (Fig. 3A–C) (4 preparations examined).

Female genitalia (Fig. 3D) (3 preparations examined). Similar to *C. japoniella*, but different in having a long ductus spermathecae with convolutions; anterior part with minute spines (Fig. 3De).

Distribution. Russia: the Russian Far East (Primorskiy Territory) (Stonis et al., 2014); Japan: Honshu (Nagano, Yamanashi and Nara Prefectures), Shikoku (Ehime Prefecture), Kyushu (Fukuoka Prefecture).

Host plants. *Carpinus cordata* Blume, *C. japonica* Blume, *C. laxiflora* (Siebold & Zucc.) Blume, *C. tschonoskii* Maxim., *Corylus sieboldiana* Blume and *Ostrya japonica* Sarg. (Betulaceae).

Biology (Figs 7–8). The larvae were observed from June to October and hibernated in the final larval stage in Nara Prefecture. The first to second larval instars form a short linear mine towards the leaf edge (Fig. 8F, H). Later instar larvae fold the leaf edge down, forming a blotch mine, then widening it into the surrounding area (Fig. 8C, D); there are usually one to two mines per leaf (Figs 7, 8A, B, G). Frass is ejected through holes in the mine (Fig. 8H). Late and final instar larvae are about 4.0–5.5 mm long and pale yellowish green in coloration (Fig. 8E, I). The folded mines in the leaf edge are 10–20 mm in length, and the late blotch mines are 2–6 mm in width and 7–15 mm (Fig. 7B, C, E) or 20–46 mm (Fig. 7A, D) in length and ochreous in coloration. Pupation takes place within the mine.

Remarks. Two pairs of prelae were observed to expand caudally and form a hump-shape in the female genitalia of some specimens (Fig. 3Db–d).

The folded, leaf-edge mines of this species resemble at first sight those of foreign congeneric species feeding on Fagaceae and Rosaceae, e.g. *Coptotriche citrinipennella* (Clemens, 1859), *C. gaunacella* (Duponchel, 1843), *C. crataegifoliae* (Braun, 1972) and *C. agrimoniella* (Braun, 1972). However, larvae of *C. citrinipennella* form more tightly folded and narrow mines (Braun, 1972), while other species form more expanded mines. Fully expanded mines of *C. minuta* are easily distinguishable from those of the other Japanese *Coptotriche* species as shown in Fig. 13, although all of them are irregular blotch mines lined with a few folds. The mine of *C. minuta* is most similar to that of *C. angusticollella*, but the fold of *C. minuta* is obviously smaller than that of *C. angusticollella*. A mine of *C. minuta* may look like a pupal shelter of *Roeslerstammia pronubella* ([Denis & Schiffermüller], 1775), Roeslerstammiidae, which utilizes the same hostplant, *Carpinus laxiflora* (Hirawatari et al. 2012, figs 7, 8).

***Coptotriche symplocosella* Kobayashi & Hirowatari, sp. n.**

<http://zoobank.org/C82BE915-F1FF-4FDC-8265-8C7AE249F647>

Figs 2D–E, 3E–H, 9–11

Material examined. 47(11♂ 9♀ 27 exs)

Type material. Holotype ♂, JAPAN: Kyushu: Adachi Park, Kokura, Fukuoka Pref., 9.iv.2012em., host *Symplocos lucida*, 20.iii.2012(larva), S. Kobayashi leg. Paratypes 10♂ 9♀, Mikata, Mitsushima, Tsushima, Nagasaki Pref., 25.iv.–6.v.2012em., S. Kobayashi leg., host *S. lucida*, 27.iii.2012 (larva), SK402–405.

Other material. 20 exs, same data as paratypes.

Pupae. 7 exs, Mikata, Mitsushima, Tsushima, Nagasaki Pref., 27.iv.2012, S. Kobayashi leg., host *S. lucida*, 27.iii.2012(larva).

Diagnosis. The color of the scaling is very similar to that of many other *Coptotriche* species; the new species differs from other members of the genus in the combination of the rather long uncus (Fig. 3F), and the gently curved slender valva (Fig. 3G) in the male genitalia, and the very small corpus bursae (Fig. 3H) in the female genitalia.

Adult (Fig. 2D–E). Wing expanse 8.7 mm; forewing length 4.0 mm in holotype, 7.3–9.2 mm in paratypes (8.0 mm on average for 21 specimens). Head: palpi cream white to ochereous; frons smooth, blackish brown; vertex tuft blackish brown centrally, frontal and lateral tufts brown; collar brown to grayish black apically, comprised of slender lamellar scales; antenna minimally 2/3 length of forewing, brown to golden. Thorax: anterior part black, posterior part grayish black. Forewing brown to ochereous with blackish scales densely covering apex, and tipping termen and tornus; termen with brown scales. Cilia and hindwing blackish gray. Legs pale ochereous. Abdomen: black; anal tuft grayish ochereous.

Male genitalia (Fig. 3E–G) (2 preparations examined). Uncus with oblong claw-shaped lateral lobes. Socii membranous. Tegumen broad and rather long. Valva slender, gently curved inwards (Fig. 3G). Transtilla present. Anellus membranous, indistinct. Vinculum with rather short triangular ventral plate, with rounded anterior part. Phallus tulip-shaped, slender with broad ended apical part (Fig. 3E).

Female genitalia (Fig. 3H) (2 preparations examined). Similar to *C. japoniella* and *C. bifurcula*, except corpus bursae very small, slender, with two narrow signa with minute spines, and a short slender ductus spermathecae.

Pupa. (Fig. 11) (3 preparations examined). Brown to dark brown, 4.4–5.1 mm in length. Vertex (Fig. 11A–B) smooth, with a pair of short setae laterally (Fig. 11C). Dorsum A2–A7 with a pair of long setae, and a concentration of very small spines (Fig. 11E). Dorsum A8–A10 (Fig. 11G, H) with a pair of long dorsal spines and a pair of long lateral spines; A10 (Fig. 11F–H) furcated with a pair of short acute processes from caudal apex, rolled on the dorsal side.

Distribution. Japan: Kyushu (Fukuoka and Nagasaki (Tsushima Is.) Prefectures).

Host plants. *Symplocos lucida* (Thunb.) Siebold & Zucc. (Symplocaceae).

Etymology. The specific epithet, *symplocosella*, refers to the genus of the hostplant, *Symplocos*.

Biology (Figs 9–10). Because many young larvae were observed in leaf mines in March, the species seems to overwinter in the larval stage. The larvae mine leaves of an evergreen tree, *Symplocos lucida*, forming an elongate full-depth blotch mine beginning with a slender, linear shape (Fig. 9A, E), and gradually expanding as they feed and grow (Fig. 9B, F, G); about ~3 cm in length, white to dark yellow; the older mines turn brown in coloration (Fig. 9C). There were usually 1–3 mines per leaf (Fig. 9G). The larva ejects frass through circular holes (Fig. 10). From shed larval head capsules in the mine, we estimate that the species has six larval instars (Figs 9I, 10). The semifinal and final instar larvae are 6.0–7.0 mm long and pale green in coloration. Head capsule widths are 1st instar: 0.21 mm, 2nd: 0.25 mm, 3rd: 0.30 mm, 4th: 0.40 mm. The mature larva lines the mine with silk, so that the upper surface of the mine shows a few folds (Fig. 9C); a pupal cocoon is situated at the end of the mine.

Remarks. The pupal characters of the new species are similar to those of other *Coptotriche* species, but the new species has rather short caudal processes.

***Tischeria kumatai* Sato, Kobayashi & Hirowatari, sp. n.**

<http://zoobank.org/BDB99F16-F635-487C-AF81-D32A8C546829>

Figs 2F–G, 4

Tischeria sp.: Sato 2011: 559

Material examined. 6 (3♂ 3♀)

Type material. Holotype ♂, JAPAN: Hokkaido: Teine, 14.v.1959, host *Tilia japonica*, T. Kumata leg. (genitalia slide no. OPU-SK486). Paratypes Host. *Tilia japonica*: 1♂ 1♀, same locality and data of holotype, SK485; 1♀, Mt. Maruyama, Sapporo, Hokkaido, 2.v.2007em., H. Sato leg., 7.ix.2006(larva); 1♀, Oshirakawa, Azumi, Nagano Pref., 31.v.1992em., N. Hirano leg., 7.ix.1991, SK567. Host *Tilia* sp.: 1♂, Mt. Maruyama, Sapporo, Hokkaido, 24.iii.2007em., H. Sato leg., 7.ix.2006(larva).

Diagnosis. The color of the scaling of this new species has little or no diagnostic value. However, the female genitalia exhibit good diagnostic characters, especially the thickened plate-like vestibulum (antrum) (Fig. 4I). Among *Tischeria* species having a similarly unusual antrum (e.g. *T. ptarmica* Meyrick (see van Nieuwerkerken 2010) and *T. zestica* Meyrick (see Puplesis and Diškus 2003), the new species is most similar to *T. unca* Diškus & Stonis from the Russian Far East (feeding on *Quercus*), but is recognizable by the slender posterior plate of the antrum and the lack of spines in the corpus bursae in the female genitalia, and the long spiral shaped juxta (Fig. 4E–F) and the valva with a very slender basal half (Fig. 4A) in the male genitalia. The South African species, *T. antilope* Puplesis, Diškus & Mey (female unknown) also has a similarly shaped juxta and valva, but differs from the new species by the narrow ventral plate of the vinculum, the longer valva and the lack of a pair of short lateral processes on the juxta (Puplesis and Diškus 2003, figs 586, 589). A Far Eastern Russian species, *T. puplesisi* Kozlov (female unknown), differs from the new species by the broader valva and short, stout juxta (Kozlov 1986, fig. 2).

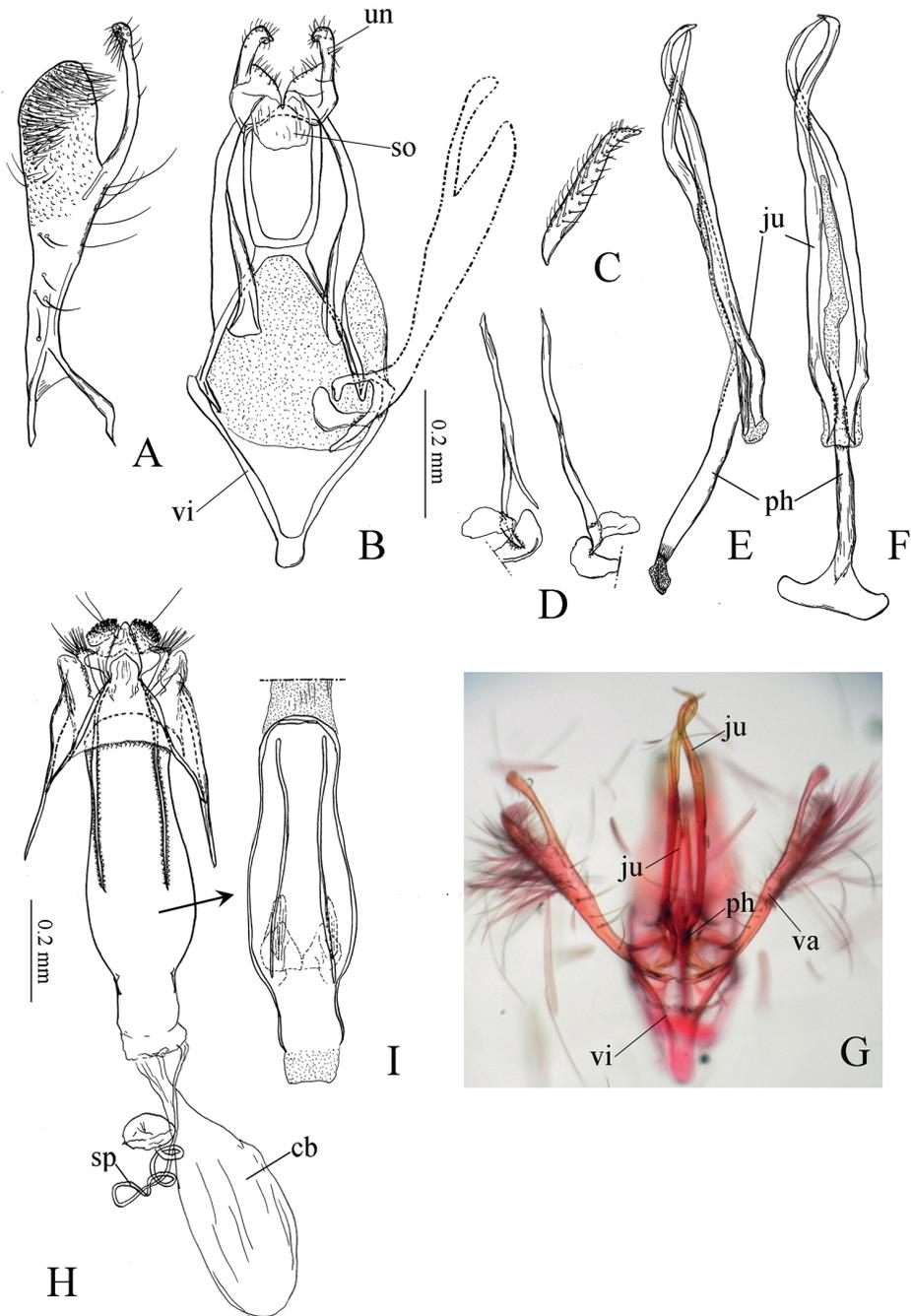


Figure 4. Genitalia of *Tischeria kumatai*. **A** Left valva, inner view **B** Male genitalia with phallus, juxta and left valva removed, ventral view **C** Left lateral lobe of uncus, lateral view **D** Juxta, ventral view **E** Phallus and juxta, lateral view **F** Same, ventral view **G** Valvae, phallus, juxta and vinculum, ventral view **H** Female genitalia, distal part, ventral view **I** Antrum, ventral view **J** Corpus bursae, ventral view. Abbreviations: ju: juxta; ph: phallus; so: socius; un: uncus; va: valva; vi: vinculum.

Adult (Fig. 2F–G). Wing expanse 7.3 mm; forewing length 3.5 mm in holotype, 7.4 and 8.4 mm (3.3 and 3.9 mm) in paratypes. Head: palpi cream white; face smooth, cream white; vertex tuft white mixed with slender pale ocherous lamellar scales; antenna slightly longer than or equal to half of forewing, basally pale ocherous, apically ocherous to brown. Thorax pale ocherous. Forewing pale ocherous with scattered brown scales on the costal half towards the apical area. Cilia and hindwing blackish gray. Legs white to pale ocherous. Abdomen: brown; anal tuft grayish ocherous.

Male genitalia (Fig. 4A–G) (1 preparation examined). Uncus with very slender lateral lobes (Fig. 4C). Socii membranous. Tegumen narrow, marginally reinforced with a pair of slightly inwardly curved arms (Fig. 4B). Valva long, basally very slender, apically rounded, distally covered with fine setae and with a long slender dorsal process (Fig. 4A). Ventral plate of vinculum long and narrow, rounded anteriorly. Juxta very long, comprising two pairs of spiral curved processes (Fig. 4D–F); one pair connecting to the middle of the phallus, equal to the length of valva, apically forming a spiral shape (Fig. 4E–F); the other pair more slender, half the length of the valva, basally slightly curved laterally (Fig. 4D). Phallus slender, distinctly broadened at basal end, forming a pale slender membranous structure from the middle to apex.

Female genitalia (Fig. 4H–I) (2 preparations examined). Antrum (Fig. 4I) strongly sclerotized, plate-like, slightly broadened medially, with a pair of pale spatulate plates at 1/3 of antrum and a pair of very slender long processes. Ductus spermathecae membranous and slender, with 3–4 coils. Corpus bursae small and smooth (Fig. 4H).

Distribution. Japan: Hokkaido, Honshu (Nagano Prefecture).

Host plants. *Tilia japonica* (Miq.) Simonk. (Malvaceae).

Etymology. The specific epithet, *kumatai*, is dedicated to Dr Tosio Kumata, who is one of the great Lepidoptera taxonomists and collected the holotype and some of the paratypes.

Biology. The larvae form dark gray blotch mines on the leaf edge which are very similar to the folded mines of *Coptotriche minuta* on *Carpinus japonica*. *Tischeria kumatai* is common in Nagano Prefecture.

***Tischeria relictana* Ermolaev, 1986**

Figs 2H–L, 5

Tischeria relictana Ermolaev, 1986: 6–8, fig. 1

Tischeria sp.: Sato 2011: 127, 559, fig. II-14.2H.

Tischeria sp.: Hirowatari et al. 2015: 26.

Type locality. Russia: the Russian Far East (Sakhalin).

Material examined. 6(3♂ 3♀)

Host *Betula ermanii*: 1♂ 1♀, Sapporo, Hokkaido, 1.v.1959, T. Kumata leg., HK387♂, SK487♀.

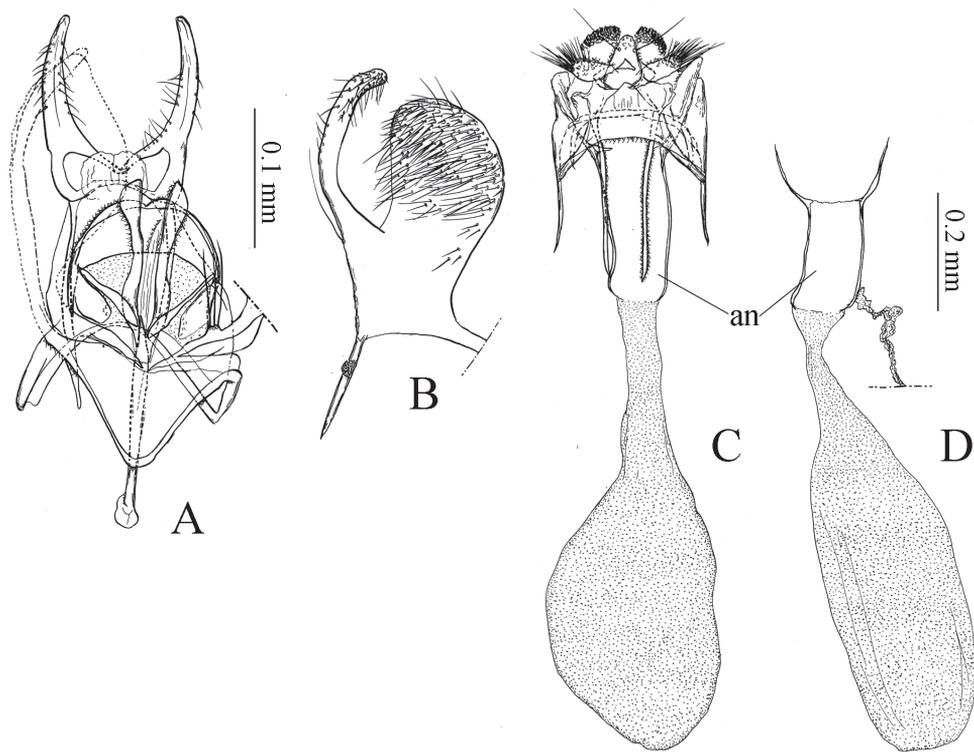


Figure 5. Genitalia of *Tischeria relictana*. **A** Male genitalia, ventral view, with right valva removed **B** Right valva, inner view **C** Female genitalia, ventral view (hostplants: *Betula grossa*) **D** Antrum, spermatheca and corpus bursae (hostplants unknown). Abbreviations: an: antrum.

Host *Betula grossa*, N. Hirano leg.: 1♂, Oshirakawa, Azumi, Matsumoto, Nagano Pref., 25.iv.1990em., 23.x.1989(larva), SK565; 1♀, same locality, 20.v.2004em., 11.x.2003(larva), SK566.

Host unknown: 1♀, Mt. Wasamata, Nishihara, Kamikitayama, Nara Pref., 23.viii.2011(L.T.), T. Hirowatari, K. Ikeuchi, Y.-S. Bae & S. Kobayashi leg., SK569. 1♂, Kumosa-yama, Kamiyama, Tokushima Pref., 22.viii.2010(L.T.), K. Yamada, T. Hirowatari, K. Ikeuchi, S. Kobayashi and K. Akita leg., SK463, deposited in TKPM.

Diagnosis. *Tischeria relictana* resembles *Coptotriche* species associated with Rosaceae in that the wings and thorax are covered with gray scales. However, this species can be regarded as a member of *Tischeria* by the presence of a developed juxta in the male genitalia. Although having divided valvae as well as some other congeneric species (e.g., *T. zestica* Meyrick and *T. martinkrugi* Puplesis & Diškus), *T. relictana* clearly differs from the others in the double juxta with anteriorly semicircular sclerotized diaphragma (Fig. 5A). A Far Eastern Russian species, *T. sichotensis* Ermolaev, has female genitalia similar in shape to *T. relictana*, but the former is separated from the latter by the presence of two acute lateral lobes of the antrum and the short spine-like pectinations in the caudal part of the corpus bursae (Stonis et al. 2014, figs 42, 43).

Additional description. Adult (Fig. 2H–L). Male and female. Wing expanse 6.1–7.1 mm; forewing length 3.0–3.2 mm in Japanese specimens. Head: palpi blackish brown; face smooth, blackish brown; vertex tuft blackish brown; antenna blackish brown, equal to half of forewing in length. Thorax black. Forewing blackish brown to black. Cilia and hindwing blackish gray. Legs blackish brown. Abdomen blackish brown; anal tuft grayish ochereous.

Male genitalia (Fig. 5A, B) (2 preparations examined). Uncus with long and very slender lateral lobes. Socii membranous. Tegumen strongly sclerotized marginally with a pair of slightly inwardly curved frames. Valva (Fig. 5B) broad, covered distally with fine setae, and having a long, slender, dorsal process. Ventral plate of vinculum narrow, triangular. Juxta short, comprising two pairs of processes, one pair connecting to the middle of the phallus, half the length of the valva, narrow medially, broadened apically; the other pair needle-shaped, 1/4 length of valva, slightly broadened basally. Transtilla absent. Diaphragma anteriorly sclerotized, a semicircular plate, folding round the phallus and contacting the needle-shaped part of the juxta ventrally. Phallus (Fig. 5A) slender, distinctly broadened at basal end, forming a pale slender membranous structure from the middle to apex.

Female genitalia (Fig. 5C, D) (3 preparations examined). Similar to *T. sparmanniae* and *T. zestica*, but differs in having short apophyses anteriores and posteriores, a slender ductus bursae and the corpus bursae without spines.

Distribution. Russia: the Russian Far East (Sakhalin) (Ermolaev 1986); Japan: Hokkaido, Honshu (Nagano and Nara Prefectures), Shikoku (Tokushima Prefecture).

Host plants. *Betula ermanii* Cham., *B. grossa* Siebold & Zucc. (Betulaceae).

Biology. The detailed biology of this species is unknown. The larvae mine leaves of *Betula* spp., according to label data of adult specimens.

Remarks. We collected a female adult of this species at a light trap on Mt. Wasamata, Nara Prefecture, where we also collected tischeriid mines on *Betula grossa* (Fig. 12). The larvae formed an ochereous to dark gray oblong mine, similar to that of *Coptotriche minuta*, on the leaf edge or along the leaf vein. Unfortunately, adults did not emerge. We also collected a male adult of this species in a light trap in a deciduous broadleaf forest where *Betula grossa* grows, on Mt. Kumoso, Tokushima Prefecture. C. Doorenweerd (pers. comm. E.J. van Nieuwerkerken) collected a pupa in Hokkaido from a folded leafmine at the leaf edge on *Betula* of which the DNA barcode groups with other species of *Tischeria* and not *Coptotriche*. Judging from these data, the mines on *Betula* can be considered to have been made by *T. relictana*.

Tischeria decidua siorkionla Kozlov, 1986

Fig. 6

Tischeria decidua Wocke, 1876: 41–43; Sato 1993: 552–553, fig. 4.

Tischeria decidua siorkionla Kozlov, 1986: 25; Stonis et al. 2014: 148–151, figs 25–35.

Type locality. Russia: the Russian Far East (Primorskiy Territory).

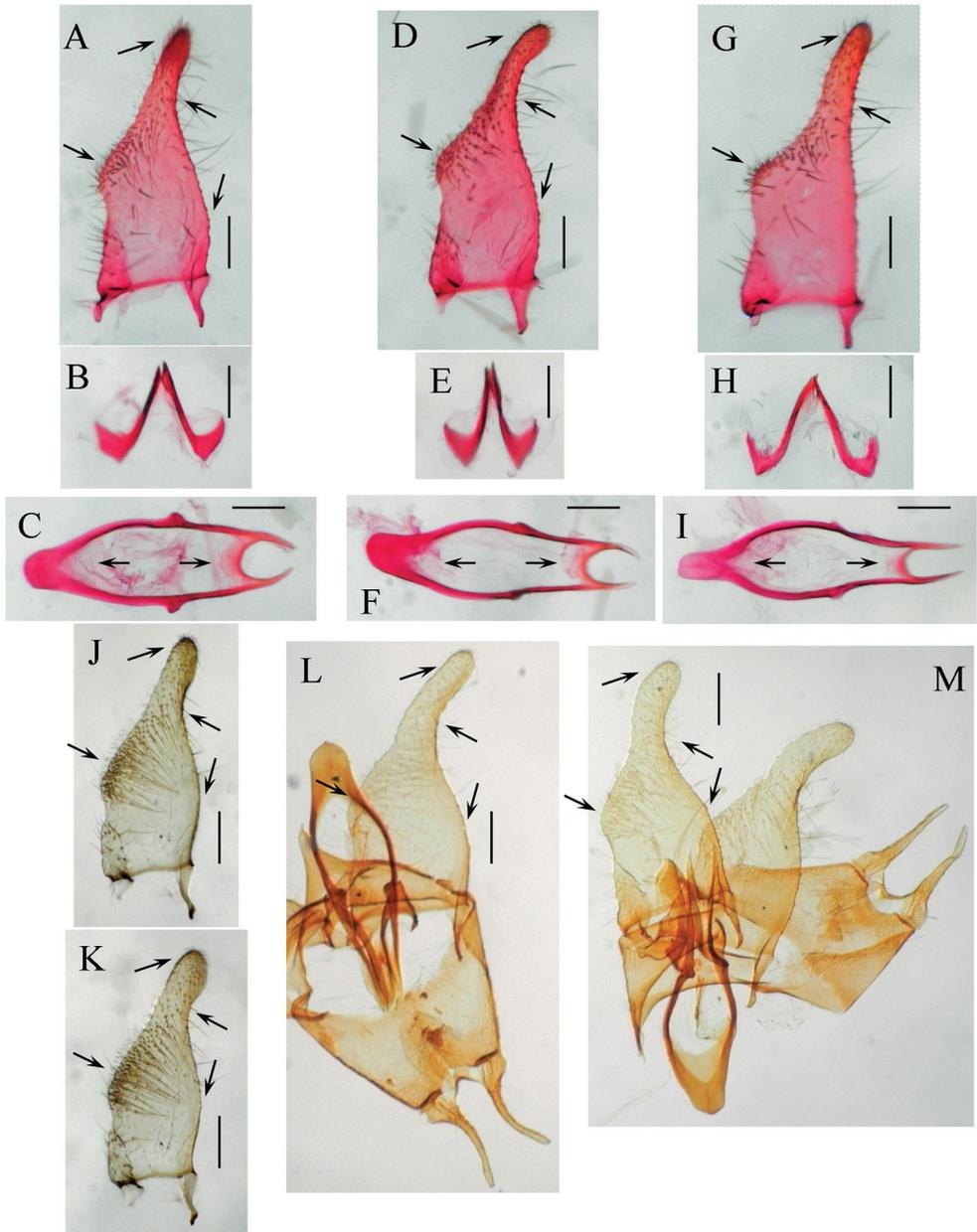


Figure 6. Male genitalia of Japanese specimens of *Tischeria decidua siorkionla*. **A, D, G, J, K** Right valva, outer view **B, E, H** Juxta, ventral view **C, F, I** Phallus, ventral view **L, M** Preparation by Dr H. Kuroko **A-F, J, L** Host *Quercus acutissima* **G-I** Host *Q. crispula* **K** Host unknown **A-C** Osaka Pref., genitalia slide no. SK596 **D-F** Nagano Pref., SK594 **G-I** Nagano Pref., SK595 **J** Nara Pref., SK555 **K** Nara Pref., SK214 **L** Ishikawa Pref., HK705 **M** Same data, HK706. Scale bar 100 μ m.

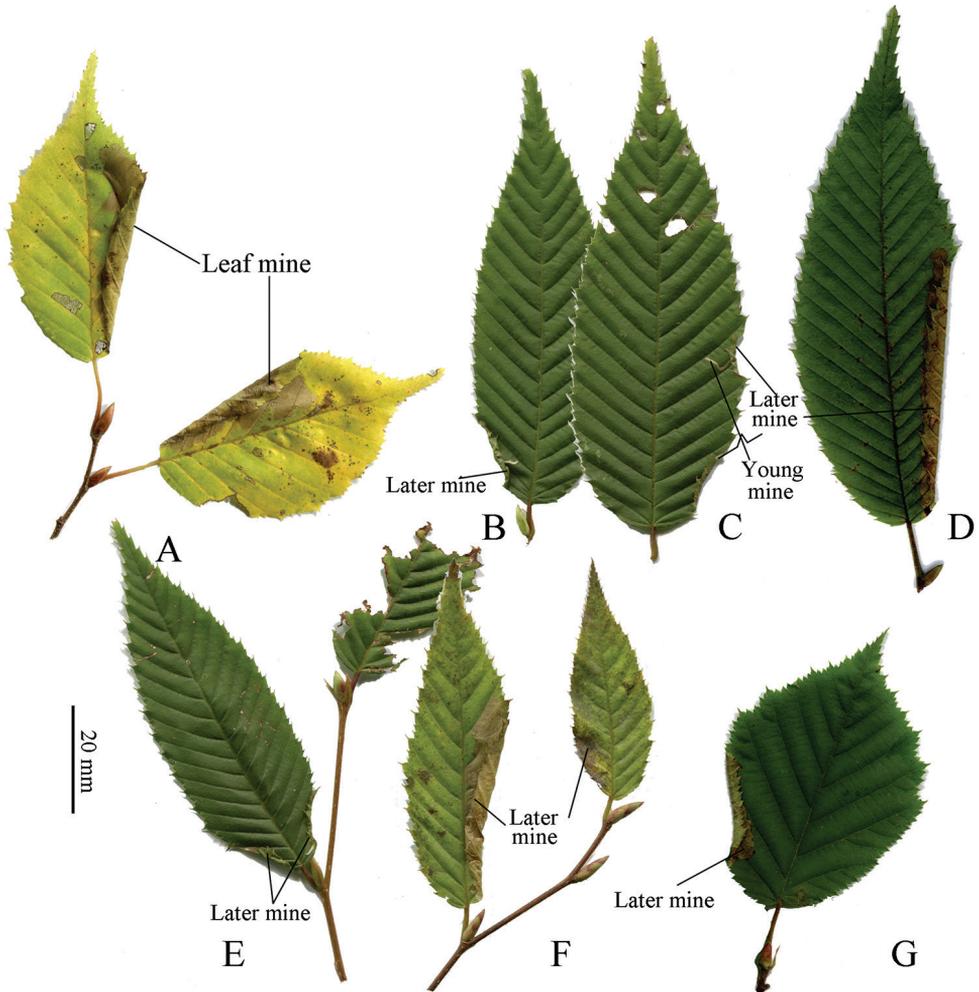


Figure 7. Mines of *Coptotriche minuta* on its hostplants. **A** *Carpinus laxiflora* **B–F** *C. japonica* **G** *Corylus sieboldiana*.

Material examined. 29(18♂ 11♀)

Host *Quercus acutissima*: 2♂ 3♀, Komaga-take-SA, Komagane, Nagano Pref., 12–15.viii.2010em., S. Kobayashi leg., 1.viii.2010(larva), SK554, 594; 9♂ 8♀, Tsubata-cho, Ishikawa Pref., ix.1983em., I. Togashi leg., viii.1983, HK705, 706; 1♂, Awara-cho, Fukui Pref., 25.viii.1987em., HK948; 1♂, Kazura, Soni, Uda, Nara Pref., 31.vii.2013em., S. Kobayashi leg., 13.vii.2013(larva), SK555; 2♂, Iwawaki-san, Osaka Pref., 23.v.1980em., H. Kuroko leg., 3.xi.1979.

Host *Quercus crispula*: 2♂, Minodo, Nagano Pref., 13.v.1980em., H. Kuroko leg., SK595.

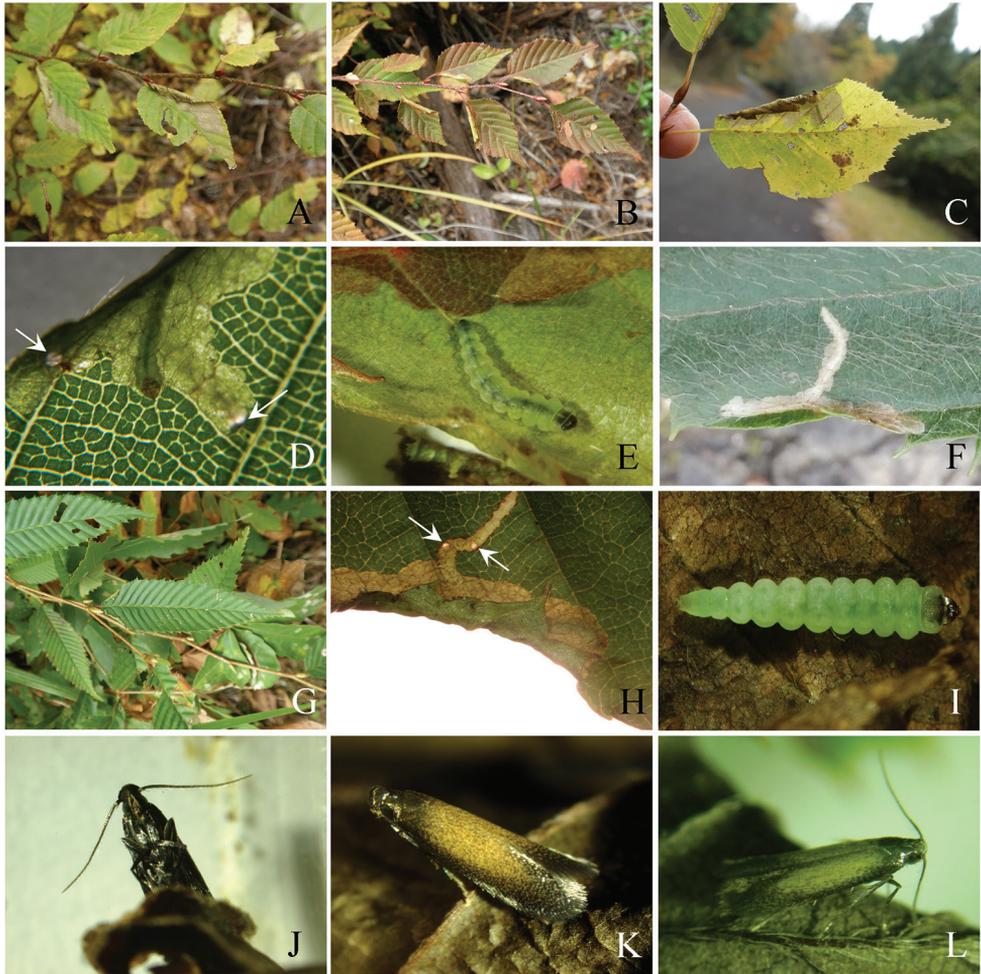


Figure 8. Biology of *Coptotriche minuta* and its hostplants. **A–E** *Carpinus laxiflora* **F** *C. tschonoskii* **G–J** *C. japonica* **L** *Corylus sieboldiana* **A–C, G** Blotch mines and branches of hostplant **D** Mine by later instar larva **E** Later instar larva **F, H** Young mines **I** Later instar larva in winter **J** Resting posture of the adult, ventral view **K** Same, dorsal view **L** Same, lateral view. Arrows show holes for ejecting frass.

Host unknown: 1♂, Ohdaigahara, Kamikitayama, Yoshino, Nara Pref., 20.vii.2009(L.T.), T. Hirowatari, K. Ikeuchi, S. Kobayashi, K. Akita, A. Inotsuka & T. Yoshida leg., SK214.

Diagnosis. See Stonis et al. (2014).

Male genitalia (Fig. 6). (9 preparations examined). See Sato (1993, 4A–E) and Stonis et al. (2014, fig. 26–29, 31, 33, 35).

Female genitalia See Sato (1993, fig. 4F).

Distribution. Russia: the Russian Far East (Primorskiy Territory); Japan: Hokkaido, Honshu, Kyushu (Tsushima Is.).

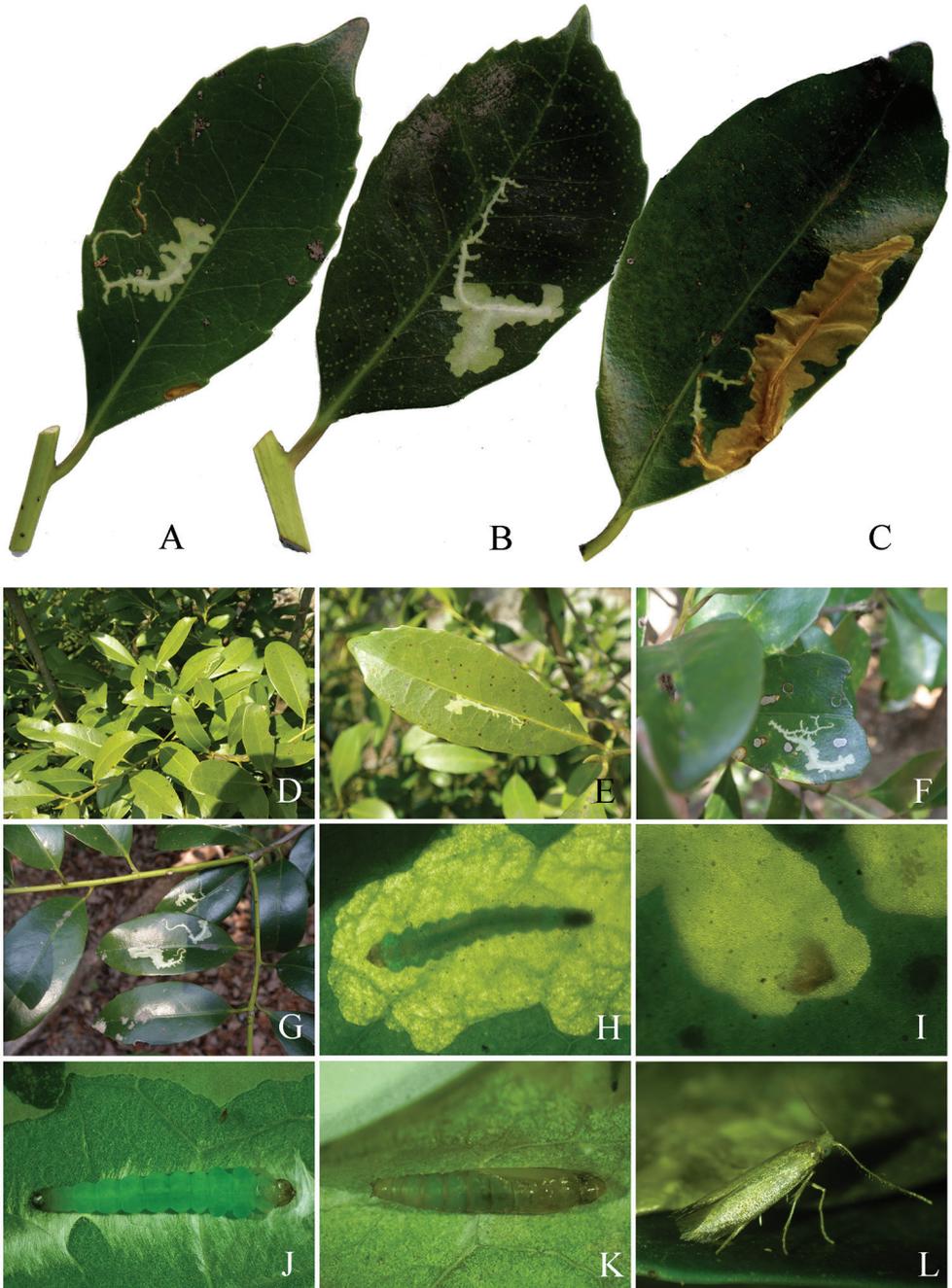


Figure 9. Biology of *Coptotriche symplocosella* and its hostplant, *Symplocos lucida*. **A, E** Young mines **B, F–G** Later mines **C** Old mine **D** Young mines and shoots of *Symplocos lucida* **H** Later instar larva **I** Head capsule within mine **J** Final instar larva, dorsal view **K** Pupa, dorsal view **L** Resting posture of adult, lateral view.

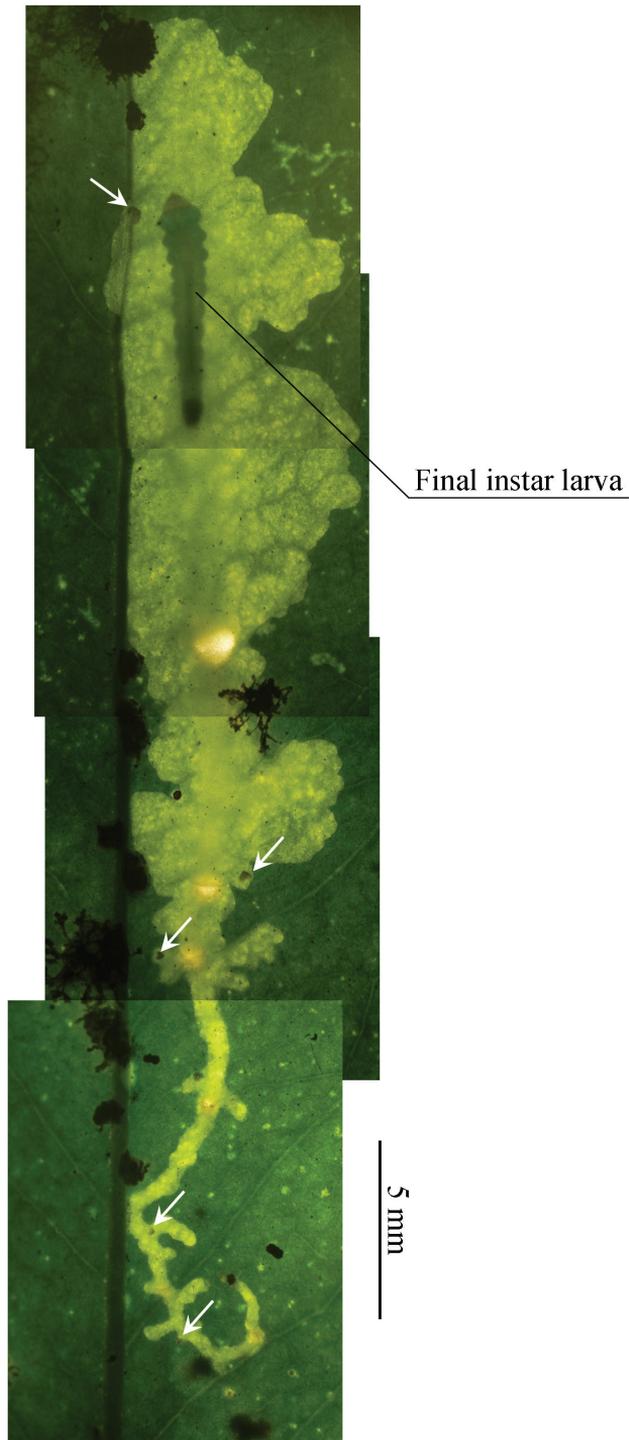


Figure 10. Immature stage of *Coptotriche symlocosella* and its hostplant, *Symlocos lucida*. Arrows show head capsules.

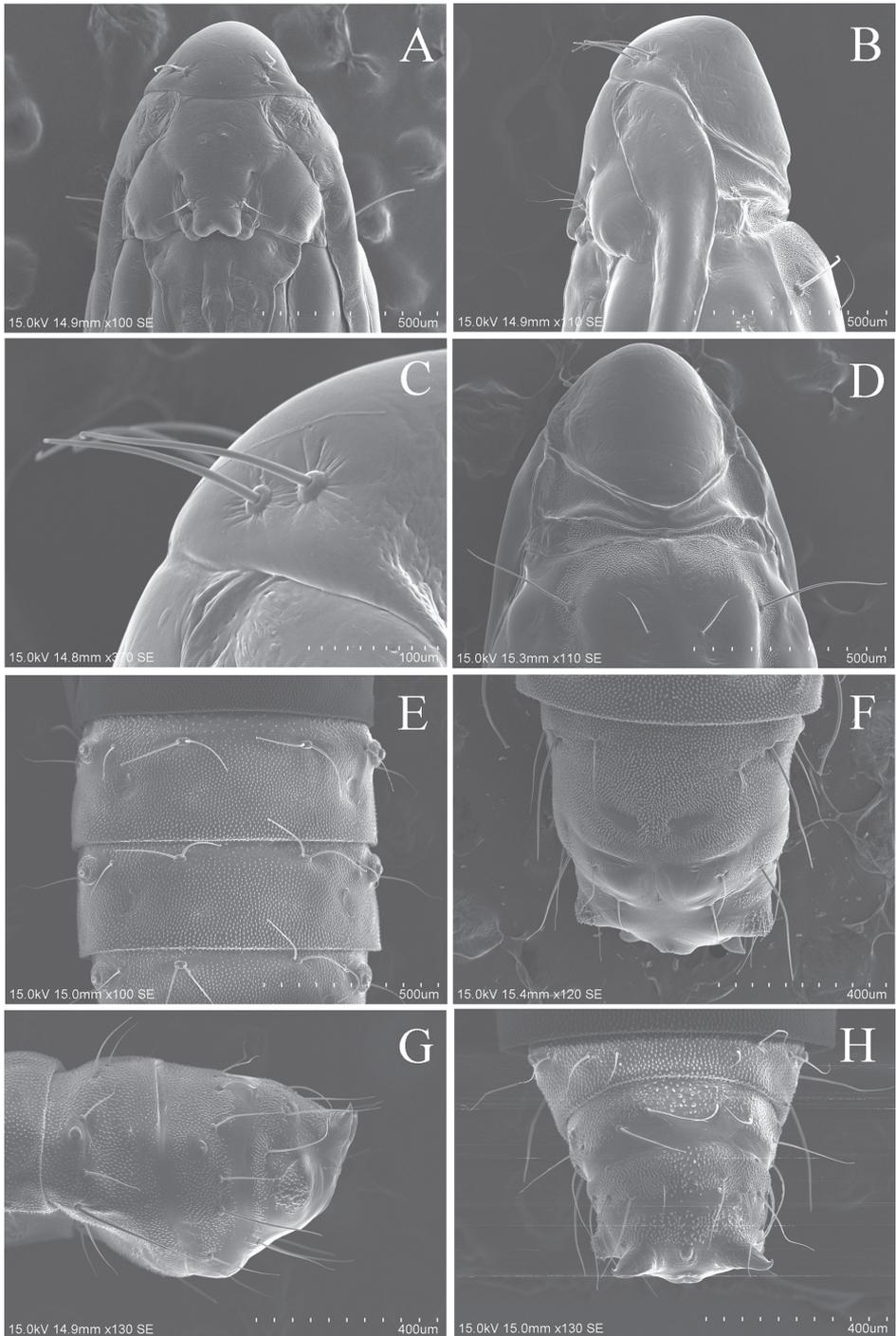


Figure 11. Pupa of *Coptotriche symplocosella*. **A** Head, ventral view **B** Lateral view **C** Frontal setae, lateral view **D** Head, dorsal view **E** Setae of abdominal tergum **F** A8–A10, ventral view **G** Lateral view **H** Dorsal view.

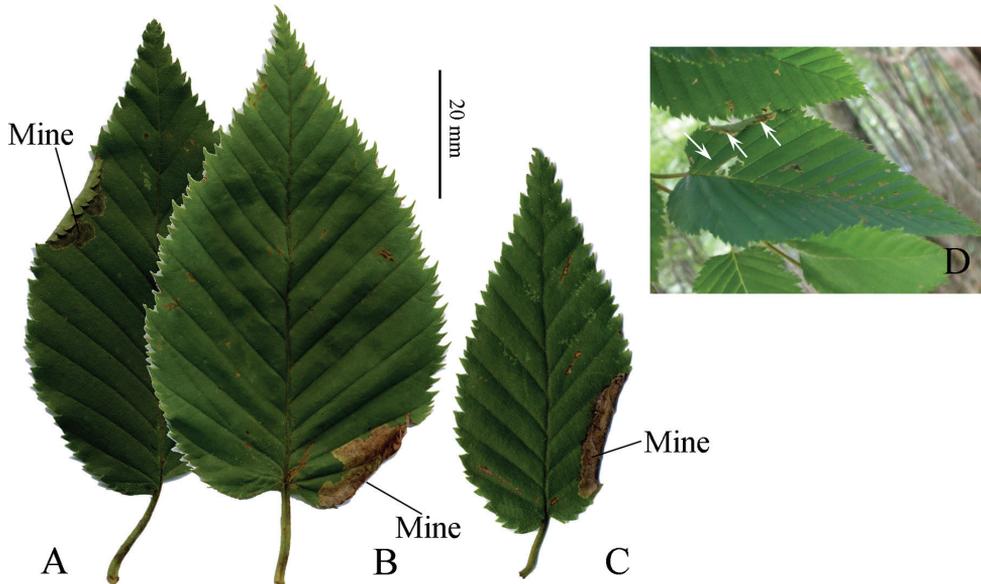


Figure 12. Mines of Tischeriidae species, possibly *Tischeria relictana*, and its hostplant, *Betula grossa*. **A–C** Folded mines **D** Folded mine and blotch mine among leaf veins. See remarks of *T. relictana*.

Host plants. *Quercus acutissima* Carruth., *Q. crispula* Blume, *Q. dentata* Thunb., *Q. serrata* Thunb., and *Q. variabilis* Blume, Fagaceae in Japan (Sato 1993). *Q. mongolica* Fisch. ex Ledeb. and *Q. serrata* in Russia (Kozlov 1986; Stonis et al. 2014).

Biology. (Fig. 13-3). See Sato (1993) and Stonis et al. (2014).

Remarks. In Japan, this species had been treated as '*T. decidua* Wocke' until the East Asiatic subspecies *T. decidua siorkionla* was described by Kozlov (1986). Stonis et al. (2014) reported that Japanese representatives of *T. decidua* belonged to the subspecies *siorkionla* Kozlov. In the Japanese specimens we studied, the apex of the valva is broader (present study: 50–65 μm ; Stonis et al. (2014): 65 μm), but other characters were considered to lie within the range of individual variation, e.g., some specimens have a rather prominent median bulge and sinuous inner margin of the valva (Fig. 6A, D, G, L; Sato 1993, 4D), i.e. more similar to that of the nominotypical European subspecies; others have a rather longer but less prominent median bulge and nearly straight inner margin of the valva (Fig. 6J, K, M; Sato 1993, fig. 4A, E), i.e. more similar to that of *T. d. siorkionla*. The chitinized basal part of the phallus tends to be less developed and the transverse bar is shorter in Japanese material than in the nominotypical European subspecies (Fig. 6C, F, I), as shown by Stonis et al. (2014). In conclusion, we treat the Japanese representatives as *T. decidua siorkionla* following Stonis et al. (2014) on the basis of the broader apex of the valva and the less developed basal part of the phallus (Fig. 6).

Genus	<i>Tischeria</i>	<i>Coptotriche</i>
Mining type	Irregular or oval blotch mine	Linear to irregular blotch mine
Species	1. <i>naraensis</i> Sato 2. <i>quercifolia</i> Kuroko 3. <i>decidua storkionla</i> Kozlov 4. <i>kumatai</i> sp. n.* 5. <i>relictana</i> Ermolaev* *Mine not examined.	6. <i>angusticollella</i> (Duponchel) 7. <i>heinemanni</i> (Wocke) 8. <i>japoniella</i> Puplesis & Diskus 9. <i>szoecsi</i> (Kasy)† 10. <i>minuta</i> Diškus & Stonis 11. <i>symplocosella</i> sp. n. †Mine not examined. Similar to other Rosaceae feeding sp. (Kuroko, 1982)

Figure 13. Mine characters of Japanese Tischeriidae. Hostplants: **1, 3a** *Quercus acutissima* **2, 3b** *Q. serrata* **3c** *Q. crispula* **6** *Rosa multiflora* **7** *Rubus palmatus* var. *palmatus* **8a** *Eurya japonica* **8b** *E. emarginata* **10** *Carpinus laxiflora* **11** *Symplocos lucida*. Mine characters of *Tischeria* species follow Sato (1993).

Discussion

In the present study, a total of eleven Tischeriidae species are recognized from Japan, not including an unidentified *Tischeria* species which occurs on evergreen *Quercus* (recorded by Sato 2011), of which nine of them were reared by us. It is revealed that the previously unknown hostplants for *Coptotriche minuta* were *Carpinus* spp., *Corylus sieboldiana* and *Ostrya japonica*, Betulaceae, while those for *Tischeria relictana* were *Betula* spp, Betulaceae. *Coptotriche symplocosella* and *C. japoniella* utilize evergreen plants of Ericales as hosts and hibernate in the larval stage. Oishi and Sato (2009) reviewed the voltinism and leaf type of hostplants of the Tischeriidae; *C. japoniella* has a univoltine life cycle and long larval period, and overwinters as 5th instar. The seasonal development of *C. symplocosella* was not examined, but it has a similar hibernating form and larval mine as *C. japoniella*. Other Japanese species including *Tischeria kumatai* and *C. minuta* probably have a bivoltine life cycle and hibernate as mature larva within the cocoon. The overwintering generation of *C. minuta* has color morphs of forewings from ochreous to black and a larger body size than other generations. According to Kuroko (1982), *Coptotriche szoecsi japonica* has a brighter color and smaller body size compared with the nominotypical subspecies *szoecsi*. The subspecies *japonica* was described from specimens collected in May and July. The polytypic concept of *C. szoecsi* could not be confirmed, because there existed no opportunity to examine additional material.

Figures 12 and 13 provide a pictorial key to the leafmines of Japanese Tischeriidae. Mines of *Coptotriche* species are distinguished from those of blotch miners of other lepidopteran families by the ejection of frass through holes and the folds of the fully expanded mine; e.g., *C. japoniella* (Fig. 13(8ab)) is distinguished by these characters from the *Eurya* blotch miner, *Lyonetia euryella* Kuroko, 1964. According to Sato (1993), Japanese *Tischeria* mines are distinguished mainly by colors and patterns of the surface and cocoon nidus (Fig. 13(1–3)).

As regards the morphology of the female genitalia, Puplesis and Diškus (2003) pointed out that the corpus bursae and ductus spermathecae have great value in generic and species diagnoses. *Coptotriche* species were often distinguished from one another by the size of the corpus bursae and the length of the ductus spermathecae. Virgin female adults of five Japanese *Coptotriche* species obtained from rearing in the laboratory were examined, not including *C. szoecsi* (Kasy). The size of the corpus bursae and the length of the ductus spermathecae differed from one another as indicated in the species descriptions, e.g., the corpus bursae of *C. angusticollella* was larger than in the other four species.

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