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Ecto- and endo-parasitic monogeneans (Platyhelminthes) on cultured freshwater exotic fish species in the state of Morelos, South-Central Mexico

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Abstract

An extensive parasitological study of 365 freshwater exotic fish specimens belonging to 13 species of seven families (Cichlidae, Cyprinidae, Osphronemidae, Pangasidae, Poeciliidae, Characidae, and Loricariidae) collected from 31 Aquaculture Production Units (APU) from Central Mexico revealed the occurrence of 29 ecto- and endo-parasitic monogeneans found on gills and stomachs: *Cichlidogyrus sclerosus*, *C. thurstonae*, *C. tilapiae*, *Cichlidogyrus* sp. 1, *Cichlidogyrus* sp. 2, *Enterogyrus coronatus*, *E. malmbergi*, *Gussevia spiralocirra*, *Sciadicleithrum iphthimum*, *Sciadicleithrum* sp., *Scutogyrus longicornis* (all Dactylogyridae), *Gyrodactylus cichlidarum*, and *G. yacatli* (Gyrodactylidae) on *Oreochromis niloticus*, *Pterophyllum scalare* and *Hemichromis* sp. (Cichlidae); *Dactylogyrus baueri*, *D. formosus*, *D. intermedius*, *D. vastator*, *D. extensus*, *Dactylogyrus* sp. (all Dactylogyridae), and *G. kobayashii* on *Carassius auratus*, *Cyprinus carpio* and *Ctenopharyngodon idella* (Cyprinidae); *Trianchoratus acleithrium* and *T. trichogasterium* (Dactylogyridae) on *Trichogaster trichopterus* (Osphronemidae); *Thaparocleidus caecus*, *T. siamensis* (Dactylogyridae), and Dactylogyridae sp. on *Pangasianodon hypophthalmus* (Pangasidae); *G. poeciliae* on *Poecilia reticulata* (Poeciliidae); *Diaphorocleidus armillatus* (Dactylogyridae) on *Gymnocorymbus ternetzy* (Characidae); *Unilatus unilatus* (Dactylogyridae) and Gyrodactylidae sp. on *Hypostomus* sp. (Loricariidae). The paramount importance of the establishment of these monogeneans due to the importation/exportation of non-native ornamental and other exotic host fish species cultured for food in Mexico is briefly discussed. Quarantine is recommended for all transferred host species.

Keywords
characids, cichlids, cyprinids, fish introductions, loricariids, Monogenea, Morelos state, non-native ornamental fish, osphronemids, pangasids, parasites, poeciliids, quarantine, tilapia

Introduction

At a global level, increasing attention is being paid to generate useful ecological indicators that favor invasiveness and geographic range expansion by introduced species (Lavergne and Molofsky 2007, Blackburn and Ewen 2017). Conjointly, introductions of species are rising sharply because of increased trade, transport, travel, and tourism associated with globalization (IPPC Secretariat 2005). Within this context, trade of the non-native ornamental fish industry and/or fish farms for food production, has been the main cause of introductions of fish and their parasites around the world (Barroso de Magalhães and Jacobi 2013, Mendoza et al. 2015). Furthermore, the same industries pose a growing threat to native wildlife if non-native fishes are later released into the wild (see Mendoza-Franco et al. 2012). Culture of non-native ornamental and food fishes represents major activities in the state of Morelos (south-central Mexico) since these fishes are commercially distributed within and outside of Mexico in large quantities (Martínez et al. 2010).

Although non-native aquatic organisms are important to Morelos aquaculture and the economy of the state of Morelos, the aquaculture industry should be made aware of the considerable local, state, and national concern over the potential ecological or economic problems arising from non-native fish introductions and their parasites in natural environments (i.e., parasite transfer and/or fish competition with native species) (Barroso de Magalhães and Jacobi 2013). Recently, a total of 44 helminth species on introduced freshwater fishes were listed for Mexico, of which five are invasive species, i.e., *Cichlidogyrus sclerosus* Paperna & Thurston, 1969 *Dactylogyrus extensus* Mueller & Van Cleave, 1932 and *Gyrodactylus cichlidarum* (Paperna 1968) García-Vasquez & Hansen, 2007 (Monogenea); *Centrocestus formosanus* (Nishigori 1924) Price, 1932 (Digenea) and *Schyzocotyle acheilognathi* Yamaguti, 1934 (Cestoda), all of them introduced with their Asian and African hosts (Tapia Osorio et al. 2014). The present study was conducted to identify the most common ecto- and endo-parasitic monogeneans inhabiting commercially important ornamental and/or food fish species that have been imported into Mexico.

Materials and methods

Ornamental fish species were collected from 2010 to 2014 from different municipalities (Axochiapan, Ayala, Cuautla, Jiutepec, Jojutla, Tlaltizapan, Tlaquiltenango, Xochitepec, and Zacatepec) located in the state of Morelos. Live fish were examined thoroughly externally under a stereo-microscope before opening the visceral cavity.

Fish were sacrificed by puncturing the brain region and the gills of each fish were removed and placed in vials containing hot 4–5% formalin solution to fix any of the ectoparasites that might be present and labeled with data of each collection site. The internal cavity of each fish was exposed by an incision made along the venter from the anus to mouth. The entire alimentary canal was removed; the interior of the gut was thoroughly examined in situ, then placed in a Petri dish containing hot formalin solution 4–5%, where it was searched for monogeneans (Salgado-Maldonado et al. 2014). Subsequently, all monogeneans specimens were isolated and stained with Gomori's trichrome and mounted in Canada balsam. In addition, some specimens were mounted in a mixture of lactic-acid (LA) and glycerin- ammonium picrate (GAP) and then remounted in Canada balsam as permanent preparations (Mendoza-Franco et al. 2013). Parasite identifications were made using a Leica microscope DM2500 with Nomarski interference contrast and based on descriptions provided in the following references: García-Vásquez et al. 2007, 2015, Jogunoori et al. 2004, Kritsky et al. 1989, Lim 1996, Mendoza-Palmero et al. 2012, Pariselle and Euzet 1995, Yamaguti 1963. Reference specimens were deposited in the National Helminthological Collection of Mexico (**CNHE**). Prevalence (percent of hosts infected), mean abundance (mean number of parasites per examined fish), and intensity range for each monogenean species follows Bush et al. (1997). Host species and common names follow those in the FishBase (Froese and Pauly 2017).

Results

A total of 365 fish specimens of 13 species belonging to 7 families was examined for monogeneans: Cichlidae, Characidae, Cyprinidae, Loricariidae, Osphronemidae, Pangasidae, and Poeciliidae. Twenty-nine monogenean species infecting gills and/or stomachs were identified from hosts species of all families mentioned above from a total of 31 Aquaculture Production Units (APU) from different municipalities located in the state of Morelos (see Table 1 and Figure 1). The prevalence, mean abundance, and mean intensity of infections at each APU of individual species from different hosts are provided in Tables 2–4.

Discussion

Currently, 31 species of exotic monogeneans have been registered in the state of Morelos due to the introduction of their hosts that are cultured either for food or aquariums (present data; Caspeta-Mandujano et al. 2009). This current study on cultured exotic fish species revealed that cichlids (i.e., species of *Oreochromis*, *Hemichromis*, and *Pterophyllum*), harbored the highest number of monogeneans (14 species) followed by cyprinids with seven species of which *Dactylogyrus baueri* Gussev, 1955, *Dactylogyrus formosus* Kulwieć, 1927, *Dactylogyrus intermedius* Wegener, 1909, and

Table 1. Ecto- and endo-parasitic monogeneans (Platyhelminthes) on cultured exotic fish from several Aquaculture Production Units (APU) in the state of Morelos, South-Central Mexico.

Host species/Family	Monogeneans/CNHE	APU	Municipalities
Oreochromis niloticus (**Cichlidae**)	*Cichlidogyrus sclerosus* † /10743	Acuícola Jaloxtoc El Cifón 7 Hermanos La cascada Acuícola Ayala Maricultura Argos	Ayala Zacatepec Cuautla Tlaltizapan Ayala Zacatepec
	Cichlidogyrus thurstonae † /10744	La Cascada	Tlaltizapan
	Cichlidogyrus tilapiae † /10745	Acuícola Ayala	Ayala
		Maricultura Argos	Zacatepec
Oreochromis niloticus	**Cichlidogyrus* sp. 1 † /10746 **Cichlidogyrus* sp. 2 † /10747	Acuícola Ayala	Ayala
Hemichromis sp.	*Enterogyrus coronatus* ‡ /10748	Maleny	Zacatepec
Oreochromis niloticus	*Enterogyrus malmbergi* ‡ /10749-10750	Acuícola Ayala Adilene Marisol San Tilapia Acuícola de Jiutepec Pliego	Ayala Ayala Tlaltizapan Juitipec Ayala
Oreochromis sp.	*Enterogyrus malmbergi* ‡ /10751	San Tilapia La buena Fortuna	Tlaltizapan Jojutla
Oreochromis niloticus	*Gyrodactylus cichlidarum* £ /10756	Acuícola Jaloxtoc Centro Zacatepec	Ayala Zacatepec
	Gyrodactylus yacatli £ /10757	Centro Zacatepec	Zacatepec
Pterophyllum scalare	**Gussevia spiralocirra* † /10752	Jesús Madariaga	Zacatepec
	**Sciadicleithrum iphthimum* † /10753	El Chino	Juitepec
	**Sciadicleithrum* sp. † /10754	Olascoaga	Juitepec
Oreochromis niloticus	*Scutogyrus longicornis* † /10755	La Cascada	Tlaltizapan
Carassius auratus (**Cyprinidae**)	**Dactylogyrus baueri* † /10758 **Dactylogyrus formosus* † /10759 **Dactylogyrus intermedius* † /10760 *Dactylogyrus vastator* † /10761-10762	Centro de acopio La Perla El Invernadero Los Huajes Linda Vista Platanar Grupo Carsal	Tlaltizapan Ayala Ayala Ayala Ayala Ayala
	**Gyrodactylus kobayashii* £ /10765-10767	Linda Vista Los Huajes Grupo Carsal El Invernadero	Ayala Ayala Ayala Ayala
Cyprinus carpio	**Dactylogyrus extensus* † /10763	Ornapez	Ayala
Ctenopharyngodon idella	**Dactylogyrus* sp. † /10764	Centro Zacatepec	Zacatepec
Trichogaster trichopterus (**Osphronemidae**)	**Trianchoratus acleithrium* † /10768	Consorcio Lugo-Galeana Granja Acuícola Foras	Jiutepec Axochiapan
	**Trianchoratus trichogasterium* † /10769	Consorcio Lugo-Galeana	Jiutepec
Pangasianodon hypophthalmus (**Pangasidae**)	**Thaparocleidus caecus* † /10770 **Thaparocleidus siamensis* † /10771-10772	Betta Fish Betta Fish La buena Fortuna	Xochitepec Xochitepec Jojutla
	*Dactylogyridae sp. †	La buena Fortuna	Jojutla
Poecilia reticulata (**Poeciliidae**)	**Gyrodactylus poeciliae* £ /10773	Huertas de Cuatla Exopez Agua Fría	Ayala Tlaltizapan Tlaquiltenengo
Gymnocorymbus ternetzy (**Characidae**)	**Diaphorocleidus armillatus* † /10774-10775	Aquafish Tropipez	Zacatepec
Hypostomus sp. (**Loricariidae**)	*Gyrodactylidae sp. £ /10777	Consorcio Lugo-Galeana	Jiutepec
	**Unilatus unilatus* † /10776	Consorcio Lugo-Galeana	Jiutepec

* = new record in Mexico. Site of infection on host: † = gills lamellae; ‡ = stomach; £ = fins.

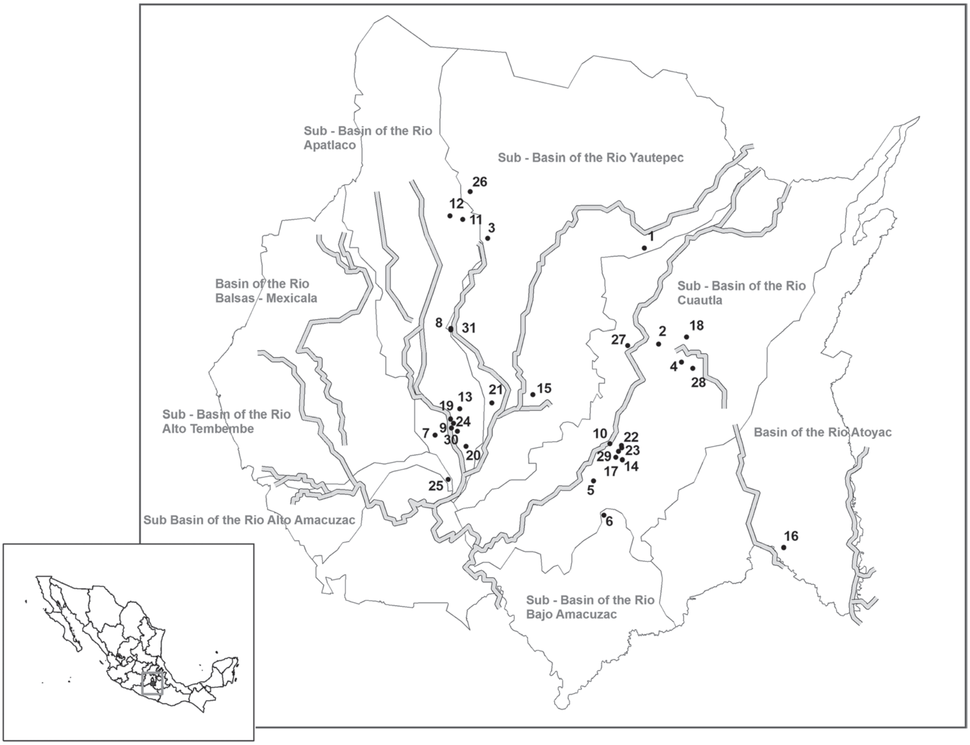

Figure 1. Map of the state of Morelos, Mexico showing position of each APU: **1** 7 Hermanos (18°51'49.82132"N; 98°58'01.20211"W **2** Acuícola Ayala (18°45'11.59525"N; 98°56'58.87989"W) **3** Acuícola de Jiutepec (18°52'29.84116"N; 99°09'24.49751"W) **4** Acuícola Jaloxtoc (18°43'56.72740"N; 98°55'20.14003"W) **5** Adilene Marisol (18°35'43.94208"N; 99°01'43.49419"W) **6** Agua Fría (18°33'22.41096"N; 99°00'57.44948"W) **7** Aquafish (18°38'53.20757"N; 99°13'13.80019"W) **8** Betta Fish (18°46'15.00012"N; 99°12'05.44263"W) **9** Centro Zacatepec (18°39'22.70079"N; 99°12'02.36030"W) **10** Centro de Acopio La Perla (18°38'18.23968"N; 99°00'32.15165"W) **11** Consorcio Lugo-Galeana (18°53'48.34681"N; 99°11'13.92251"W) **12** El Chino (18°54'03.35178"N; 99°12'10.27438"W) **13** El Cifón (18°40'42.68111"N; 99°11'26.16448"W) **14** El Invernadero (18°37'11.86468"N; 98°59'37.85120"W) **15** Exopez (18°41'41.78829"N; 99°06'07.81780"W) **16** Granja Acuícola Foras (18°31'07.09460"N; 98°47'54.39963"W); **17.** Grupo Carsal (18°37'21.23567"N; 99°00'05.49462"W) **18** Huertas de Cuatla (18°45'41.45252"N; 98°54'57.10516"W) **19** Jesús Madariaga (18°39'59.91903"N; 99°12'05.85187"W) **20** La Buena Fortuna (18°38'07.31312"N; 99°10'58.58424"W) **21** La Cascada (18°41'06.91860"N; 99°09'05.97650"W) **22** Linda Vista (18°38'11.27728"N; 98°59'41.36454"W) **23** Los Huajes (18°38'01.06064"N; 98°59'39.86312"W) **24** Maleny (18°39'43.43675"N; 99°11'52.86078"W) **25** Maricultura Argos (18°35'50.18775"N; 99°12'16.44262"W) **26** Olascoaga (18°55'43.39346"N; 99°10'40.92078"W) **27** Ornapez (18°45'06.02177"N; 98°59'14.37030"W) **28** Platanar (18°43'30.25259"N; 98°54'30.22690"W) **29** Pliego (18°37'45.93123"N; 98°59'53.99321"W) **30** San Tilapia (18°39'09.51796"N; 99°11'36.53955"W) **31** Tropipez (18°46'10.83544"N; 99°12'05.47184"W).

Table 2. Parameters of infection of monogeneans on cichlids (APU: Aquaculture Production Unit; P%: Prevalence; MA: mean abundance; RI: range of infection; MI: mean intensity; IH: infected hosts).

APU	Hosts	Monogeneans	Inds.	P%	MA	RI	MI	IH
Maleny	*Hemichromis* sp.	*Enterogyrus coronatus*	36	50	5.14	1–13	3.6	10/20
7 hermanos	*Oreochromis niloticus*	*Cichlidogyrus sclerosus*	12	57	1.71	2–4	3.0	4/7
Acuícola de Jiutepec	*Oreochromis niloticus*	*Enterogyrus malmbergi*	18	50	2.57	2–5	3.6	5/10
Acuícola Jaloxtoc	*Oreochromis niloticus*	*Gyrodactylus cichlidarum*	18	20	2.57	18	18	1/5
	Oreochromis niloticus	*Cichlidogyrus sclerosus*	13	100	2.60	1–7	2.6	5/5
Adilene Marisol	*Oreochromis niloticus*	*Enterogyrus malmbergi*	53	100	7.57	2–13	5.3	10/10
Centro Zacatepec	*Oreochromis niloticus*	*Gyrodactylus yacatli*	15	10	2.14	15	15	1/10
El Cifón	*Oreochromis niloticus*	*Cichlidogyrus sclerosus*	7	40	1.00	3–4	3.5	2/5
Acuícola Ayala	*Oreochromis niloticus*	*Cichlidogyrus tilapiae*	159	100	22.71	3–37	15.9	10/10
	Oreochromis niloticus	*Enterogyrus malmbergi*	6	50	0.86	1–2	1.2	5/10
	Oreochromis niloticus	*Enterogyrus malmbergi*	1	10	0.14	1	1.0	1/10
Pliego	*Oreochromis niloticus*	*Enterogyrus malmbergi*	2	25	0.29	2	2.0	1/4
San Tilapia	*Oreochromis niloticus*	*Enterogyrus malmbergi*	34	100	4.86	1–17	8.5	4/4
	Oreochromis sp.	*Enterogyrus malmbergi*	23	60	3.29	1–7	3.83	6/10
La Buena Fortuna	*Oreochromis* sp.	*Enterogyrus malmbergi*	76	76.9	10.86	2–19	7.6	10/13
Jesús Madariaga	*Pterophyllum scalare*	*Gussevia spiralocirra*	5	10	0.71	5	5.0	1/10
El Chino	*Pterophyllum scalare*	*Sciadicleithrum* spp.	6	83.3	1.00	1–2	1.2	5/6
Olascoaga	*Pterophyllum scalare*	*Sciadicleithrum* sp.	9	75	1.29	1–4	3.0	3/4

Table 3. Parameters of infection of monogeneans on hosts of the Cyprinidae (APU: Aquaculture Production Unit; P%: Prevalence; MA: mean abundance; RI: range of infection; MI: mean intensity; IH: infected hosts).

APU	Hosts	Monogeneans	Inds.	P%	MA	RI	MI	IH
Consorcio Lugo-Galeana	*Carassius auratus*	*Dactylogyrus* sp.	520	100	52.0	13–154	86.7	10/10
El invernadero	*Carassius auratus*	*Gyrodactylus kobayashii*	525	100	87.5	5–314	87.5	6/6
	Carassius auratus	*Dactylogyrus formosus*	1	17	0.17	1–8	1.0	1/6
Grupo Carsal	*Carassius auratus*	*Gyrodactylus kobayashii*	28	100	20	0.3–54	26.7	3/3
	Carassius auratus	*Dactylogyrus vastator*	5	33	1.7	5	5.0	1/3
Linda vista	*Carassius auratus*	*Gyrodactylus kobayashii*	12	20	1.2	2–10	6	2/10
	Carassius auratus	*Dactylogyrus vastator*	3	10	0.3	3	3.0	1/10
Los Huajes	*Carassius auratus*	*Dactylogyrus baueri*	1	20	0.2	1	1.0	5/5
	Carassius auratus	*Dactylogyrus* spp.	38	100	7.6	2–25	7.6	5/5
	Carassius auratus	*Gyrodactylus kobayashii*	102	100	20.4	2–58	20.4	5/5
Centro Zacatepec	*Ctenopharyngodon idella*	*Dactylogyrus* sp.	100	14	14.3	100	100.0	1/7
Ornapez	*Cyprinus carpio*	*Dactylogyrus extensus*	5	20	0.5	2–3	2.5	2/10

Table 4. Parameters of infection of monogeneans on characids, loricariids, osphronemids, pangasids, and poeciliids (APU: Aquaculture Production Unit; P%: Prevalence; MA: mean abundance; RI: range of infection; MI: mean intensity; IH: infected hosts).

APU	Host	Monogeneans	Inds.	P%	MA	RI	MI	IH
Aquafish	*Gymnocorymbus ternetzi*	*Diaphorocleidus armillatus*	131	100	13.1	2–24	13.1	10/10
Tropipez	*Gymnocorymbus ternetzi*	*Diaphorocleidus armillatus*	698	100	69.8	7–217	69.8	10/10
Consorcio Lugo-Galeana	*Hypostomus* sp.	*Unilatus unilatus*	15	60	1.5	1–11	2.5	6/10
	Hypostomus sp.	*Gyrodactylus* sp.	14	60	1.4	1–8	2.3	6/10
	Trichogaster trichopterus	*Trianchoratus* spp.	80	75	20	03–54	26.7	3/4
	Trichogaster trichopterus	*Trianchoratus trichogasterium*	250	80	25	16–61	31.3	8/10
Granja Acuícola Foras	*Trichogaster trichopterus*	*Trianchoratus trichogasterium*	564	90	56.4	1–262	62.7	9/10
Betta fish	*Pangasianodon hypophthalmus*	*Thaparocleidus* spp.	536	40	26.8	1–125	67.0	8/20
La Buena Fortuna	*Pangasianodon hypophthalmus*	*Thaparocleidus siamensis*	1000	100	200	130–300	200.0	5/5
	Pangasianodon hypophthalmus	Dactylogyridae sp.	10400	100	2080	1000–3000	1733.3	5/5
Exopez	*Poecilia reticulata*	*Gyrodactylus poeciliae*	4	33	0.67	2	2.0	2/6
Agua fría	*Poecilia reticulata*	*Gyrodactylus poeciliae*	75	90	7.5	1–37	8.3	9/10
Huertas de Cuautla	*Poecilia reticulata*	*Gyrodactylus poeciliae*	1	12.5	0.125	1	1.0	1/8

Gyrodactylus kobayashii Hukuda, 1940 are new geographical records in Mexico (see Tables 1 and 3). Despite the great number of parasitological studies on native and/or introduced species of Cichlidae in Mexico (Vidal-Martínez et al. 2001), studies on the parasite fauna of other exotic freshwater fishes, especially on their monogeneans, are relatively scarce. Exceptionally, there have been many reports of species of *Cichlidogyrus* on species of *Oreochromis* (often called tilapia) (see Kritsky et al. 1994, Jiménez-García et al. 2001). Even so, intensity of infection is comparatively high as well as the number of new records of these monogeneans, the latter which continues to grow each year (see Table 3, Mendoza-Franco et al. 2015b). In the present study, the angelfish *P. scalare* (Schultze) and *Hemichromis* sp. were studied for the first time and are shown to be parasitized with *G. spiralocirra* Kohn & Paperna, 1964, *S. iphthimum* Kritsky, Thatcher & Boeger, 1989, *Sciadicleithrum* sp. (new geographical records), and *E. coronatus* Pariselle, Lambert & Euzet, 1991.

Monogeneans usually exhibit high host specificity in comparison with other parasite groups, parasitizing a single or few closely related host species. The only zoogeographic range expansion of exotic monogeneans on native hosts is the discovery of species of *Cichlidogyrus* and *G. cichlidarum* from tilapia on native cichlids and poeciliids, respectively, in natural environments of Mexico (Jiménez-García et al. 2001, García-Vásquez et al. 2007, 2017). The present study revealed the highest intensity of infection with *G. cichlidarum* (identified as a tilapia pathogen by García-Vásquez et al. 2017) and *Cichlidogyrus* spp. on *Oreochromis* spp. (see Table 2). Therefore, preventing escape of these tilapia from culture systems due to their monogeneans' ability to infest and persist on other non- or related wild fish is urgently required. Another example of the persistence of monogeneans is seen with the dactylogyrid *Urocleidoides vaginoclaustrum* Jogunoori, Kritsky & Venkatanarasaiah 2004. This monogenean was originally described from fishes introduced to India via the aquarium trade. Its type host, the green swordtail *Xiphophorus hellerii* (Heckel) (Poeciliidae), is naturally distributed in southern Mexico and Central America, where the native profundulid *Profundulus labialis* (Günther) also hosts *U. vaginoclaustrum*. The problem is that *X. hellerii* has been artificially introduced along with *U. vaginoclaustrum* to other hydrological systems such as India and northern Mexico (Jogunoori et al. 2004, Mendoza-Palmero and Aguilar-Aguilar 2008, Mendoza-Franco et al. 2015a) from which other cyprinodontiform hosts could potentially become infected with this parasite. Additionally, in the present study the black tetra *G. ternetzi* (Boulenger) (Characidae) was studied for the first time and is revealed to be highly infested with *D. armillatus* Jogunoori, Kritsky & Venkatanarasaiah, 2004 (Dactylogyridae) (see Table 4). *Gymnocorymbus ternetzi* is native to South America and has been introduced via the aquarium trade to India and Mexico. Currently, there are nine species of *Diaphorocleidus* dispersed on native bryconid and characid (Characiformes) hosts in the neotropics (South and Central America) (Santos et al. 2018). The transfer and/or evidence of extensive cryptic speciation of other monogenean groups from exotic to native or vice versa on closely related hosts in Mexico remains unknown, but that potential exists.

Similarly to the introduced tilapia in Mexico, cyprinids (i.e., *C. idella*) are also widely distributed in the country including habitats located within areas protected for conservation (see Salgado Maldonado et al. 2014). These fishes were introduced to Central America (i.e., Mexico and Honduras) for aquaculture purposes from 1965-1980s (Salgado-Maldonado and Rubio-Godoy 2014, Salgado-Maldonado et al. 2015) and the presence of species of *Dactylogyrus* and *G. kobayashii* (see Table 1, 3) in Morelos might be originally related to these introductions. Poeciliids (known as guppies, mollies, platies, and swordtails) have been studied for ectoparasitic monogeneans in Mexico and mainly gyrodactylids have been reported on the skin and/or gills on these fishes (García-Vásquez et al. 2015). Currently, there are 11 gyrodactylid species described and/or reported from poeciliids. Only species of *Urocleidoides* (Dactylogyridae) have been reported on the gills of the poeciliids of the two-spot livebearers *Pseudoxiphophorus bimaculata* (Heckel), *X. hellerii*, and *Poeciliopsis retropinna* (Regan) from Mexico and Panama (Mendoza-Franco et al. 2015). In the present study, *G. poeciliae* Harris & Cable, 2000 was found for the first time on the guppy *Poecilia reticulata* Peters from Mexico (see Tables 1, 4). This monogenean species has been reported on *Poecilia caucana* (Steindachner) and *P. reticulata* from their natural ranges of distribution (Venezuela and Trinidad, respectively). Among all species of *Gyrodactylus* mentioned above, only *G. bullatarudis* Turnbull, 1956 and *G. turnbulli* Harris, 1986 have been reported on six poeciliid host species (*Gambusia* *holbrooki* Girard, *Poecilia sphenops* Valenciennes, *P. reticulata*, *P. bimaculata*, *Poeciliopsis* sp., and *X. hellerii*) from Mexico, Canada, Costa Rica, Peru, Trinidad, Australia, and Singapore (see García-Vásquez et al. 2015). Given the low host specificity of both gyrodactylid species and the invasive characteristic of poeciliids, the potential transfer of these gyrodactylids to native poeciliids and other ecologically-associated hosts in Mexico is high (see García-Vásquez et al. 2017, Mendoza-Franco et al. 2015).

The African tilapia (Cichlidae) and the Asian catfish (Pangasiidae) are both freshwater whitefish aquaculture species that potentially compete for similar markets. In fact, in 2013 Mexico was recognized as the second largest importer of pangasius fillet in the world (Martínez et al. 2016). No analysis concerning the environmental impact of the introduction of these latter fishes and their parasites from Vietnam into Mexican aquaculture and/or in wild habitats (Martínez et al. 2016) has been made. *Pangasianodon hypophthalmus* (Sauvage) was studied for the first time in the present study and it revealed to be parasitized with three monogenean species: *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) Lim 1996, *T. siamensis* (Lim 1990) Lim, 1996, and Dactylogyridae sp. (Table 4). Finally, Loricariids, otherwise known as plecos (species of *Hypostomus*) are very popular ornamental freshwater fish naturally found in tropical South America, Panama, and Costa Rica. In Mexico, *Hypostomus plecostomus* (L.) was introduced into the Balsas Basin (see geographic position in Figure 1) to control macrophytes and algae, and are now established in multiple water bodies (Ramírez-Morales and Ayala-Pérez 2009). The only report of a gill monogenean species on an introduced pleco to Mexico is that of *Heteropriapulus* sp. (Dactylogyridae) on the Amazon sailfin *Pterygoplichthys pardalis* Castelnau from the Reserva de la Biosfera Montes Azules (BRMA) in the state of Chiapas (Mendoza-Franco et al. 2012). The present study provides two new monogenean records for Mexico, Gyrodactylidae sp. and *Unilatus unilatus*, the latter belonging to the Dactylogyridae which was previously reported on the snow pleco *P. anisitsi* Eigenmann and Kennedy and on *Plecostomus* sp., from Brazil and Peru, respectively (Mendoza-Palmero et al. 2012).

The fish examined in the present study are ornamental and/or for food production that are commercialized in Mexico. Results clearly show that importation of these fish can carry several monogeneans, both ecto- and endo-parasitic species, which could infect other related fish in systems they invade. Therefore, determining the occurrence of parasitic species will help provide better aquaculture conditions and will help to solve some of the problems faced by fish farmers. In the literature, there are a number of reports dealing with the introduction of parasites by ornamental fish from which the consequences of parasite introduction can be detrimental to native fish. For example, epizootics that may lead to extensive mortality (i.e., *D. vastator* on cyprinids, see Cone 1999) as shown for several species of monogeneans introduced into farms or aquariums, and from there to natural populations (Bakke et al. 2002, 2007; García-Vásquez et al. 2017). In addition to the identification of invasive host fish species, it is recommended that all freshwater fish imported into the country for food (farmed) or ornamental purposes must comply, at least, with quarantine regulations.

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Searching for shelter in a ferruginous cave? A new species of *Pasipha* from a plateau in the Brazilian savanna (Platyhelminthes, Tricladida)

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Abstract

In a fauna survey in the eastern margin of Serra do Espinhaço Plateau, in an area belonging to the Brazilian savanna (Cerrado phytophysiognomy), a land flatworm was sampled in a ferruginous cave. Anatomical and histological analyses indicated that it belongs to a new species of the genus *Pasipha*, which is herein described. The new species shows an almost homogenous dark brown dorsal pigmentation, eyes spreading over the dorsal surface, a collar-shaped pharynx, and a prostatic vesicle with two portions separated by a canal. It differs from similar species mainly by anatomical and histological details of the ejaculatory duct, as well as male and female atria. The flatworm shows no troglomorphic traits and was collected once in the entrance zone of the cave. Hence, despite representing the first land flatworm species described from a Neotropical cave, we consider that its occurrence in the cave is probably occasional, using it as a shelter.

Keywords

Geoplaninae, land planarians, Neotropical region, taxonomy

Introduction

The genus *Pasipha* Ogren & Kawakatsu, 1990 encompasses 25 species, most of them known from southeast and southern Brazil (Carbayo et al. 2013; Leal-Zanchet et al. 2012; Amaral and Leal-Zanchet 2016; Negrete and Brusa 2016, 2017; Amaral et al. 2018). Most species, including the type-species, *Pasipha pasipha* (Marcus, 1951), were described from areas of dense ombrophilous forest of the states of São Paulo, Rio de Janeiro and Minas Gerais, in southeast Brazil (Riester 1938; Marcus 1951; E.M. Froehlich 1955). Other 10 species occur in areas of mixed ombrophilous forest and semideciduous or deciduous forests from southern Brazil and Argentina (Froehlich 1959; Leal-Zanchet et al. 2012; Amaral and Leal-Zanchet 2016; Negrete and Brusa 2016; Amaral et al. 2018), one of them also occurring in the Amazonian biome (Froehlich and Froehlich 1972).

In a recent fauna survey in the eastern margin of Serra do Espinhaço Plateau, belonging to the Brazilian savanna (Cerrado phytophysiognomy), southeastern Brazil, a flatworm with elongate body and parallel margins was collected in a ferruginous cave. This specimen was assigned to the genus *Pasipha* and is herein described as a new species.

Materials and methods

A single specimen was collected during the day by direct sampling in the entrance zone of a ferruginous cave (CSS-0004) in Conceição do Mato Dentro (18°55'02.2"S, 43°25'42.4"W), at an altitude of 931 m a.s.l., in the state of Minas Gerais, southeastern Brazil (Fig. 1). The land flatworm was fixed in 70% ethyl alcohol during field work. The preserved specimen was analysed regarding colour pattern, body shape, and dimensions and then photographed under a stereomicroscope. Methods described by Rossi et al. (2015) were used for histological processing of the material and analysis of external and internal characters. The material was sectioned at intervals of 6 μm and stained with Goldner's Masson or Haematoxylin and Eosin.

Type-material is deposited in the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo State, Brazil (**MZUSP**).

Abbreviations used in the figures:

cmc common muscle coat
cov common glandular ovovitelline duct
cs creeping sole
de dorsal epidermis
di dorsal insertion of pharynx
dm dorsal cutaneous musculature
dpv distal portion of prostatic vesicle
e eyes
ed ejaculatory duct
eg erythrophil secretion
fa female atrium
fc female canal
go gonoduct
h parasitic helminths
i intestine
lu pharyngeal lumen
ma male atrium
mo mouth

ms	median stripe	**sd**	sperm duct
n	nerve plate	**sg**	shell glands
om	outer musculature of pharynx	**sm**	sensory margin
ov	ovovitelline duct	**spm**	supra intestinal transversal muscles
pp	pharyngeal pouch	**t**	testes
ppv	proximal portion of prostatic vesicle	**v**	vitellaria
pv	prostatic vesicle	**vi**	ventral insertion of pharynx
r	rhabdites	**vm**	ventral cutaneous musculature
sbm	sub-intestinal transversal muscles		

Taxonomic description

Family Geoplanidae Stimpson, 1857
Subfamily Geoplaninae Stimpson, 1857
***Pasipha* Ogren & Kawakatsu, 1990**

***Pasipha ferrariaphila* sp. n.**
http://zoobank.org/FA4C4AA2-0064-40AA-848D-C76C7ADFC828

Type material. Holotype MZUSP PL 2141: leg. *Carste Ciência e Ambiente*, 16 January 2014, Conceição do Mato Dentro (18°55'02.2"S, 43°25'42.4"W; altitude 931 m a.s.l.), state of Minas Gerais (MG), Brazil – anterior tip: transverse sections on 8 slides; anterior region at the level of the ovaries: sagittal sections on 7 slides; pre-pharyngeal region in two fragments: transverse sections on 4 slides and sagittal sections on 6 slides; pharynx: sagittal sections on 5 slides; copulatory apparatus: sagittal sections on 8 slides.

Type-locality. Conceição do Mato Dentro, state of Minas Gerais (MG), Brasil.

Diagnosis. *Pasipha ferrariaphila* is characterised by almost homogeneous dorsal pigmentation pattern, eyes spreading over the dorsal surface, collar-shaped pharynx, prostatic vesicle with two portions separated by a canal, ejaculatory duct long and spacious, male and female atria separated by a constriction and female atrium spacious, long and with a strongly developed circular musculature in its proximal part, resembling a sphincter.

Description. Body elongate with parallel margins; anterior tip rounded and posterior tip pointed (Figs 2–3). After fixation, length of 22 mm, maximal width of 2 mm, and maximal height 0.7 mm. Mouth at 77% of body length and gonopore at 82% of body length.

Fixed specimen with dorsal surface covered by fine, almost homogeneous dark brown pigmentation over light brown ground colour, which is discernible under stereomicroscope on a thin, almost imperceptible median stripe (Fig. 3). Ventral surface pale yellow.

Eyes, initially monolobate (pigment cups of 15–25 µm) and disposed in an irregular row, surround anterior tip (Fig. 3). After that, some eyes become bilobed (pigment

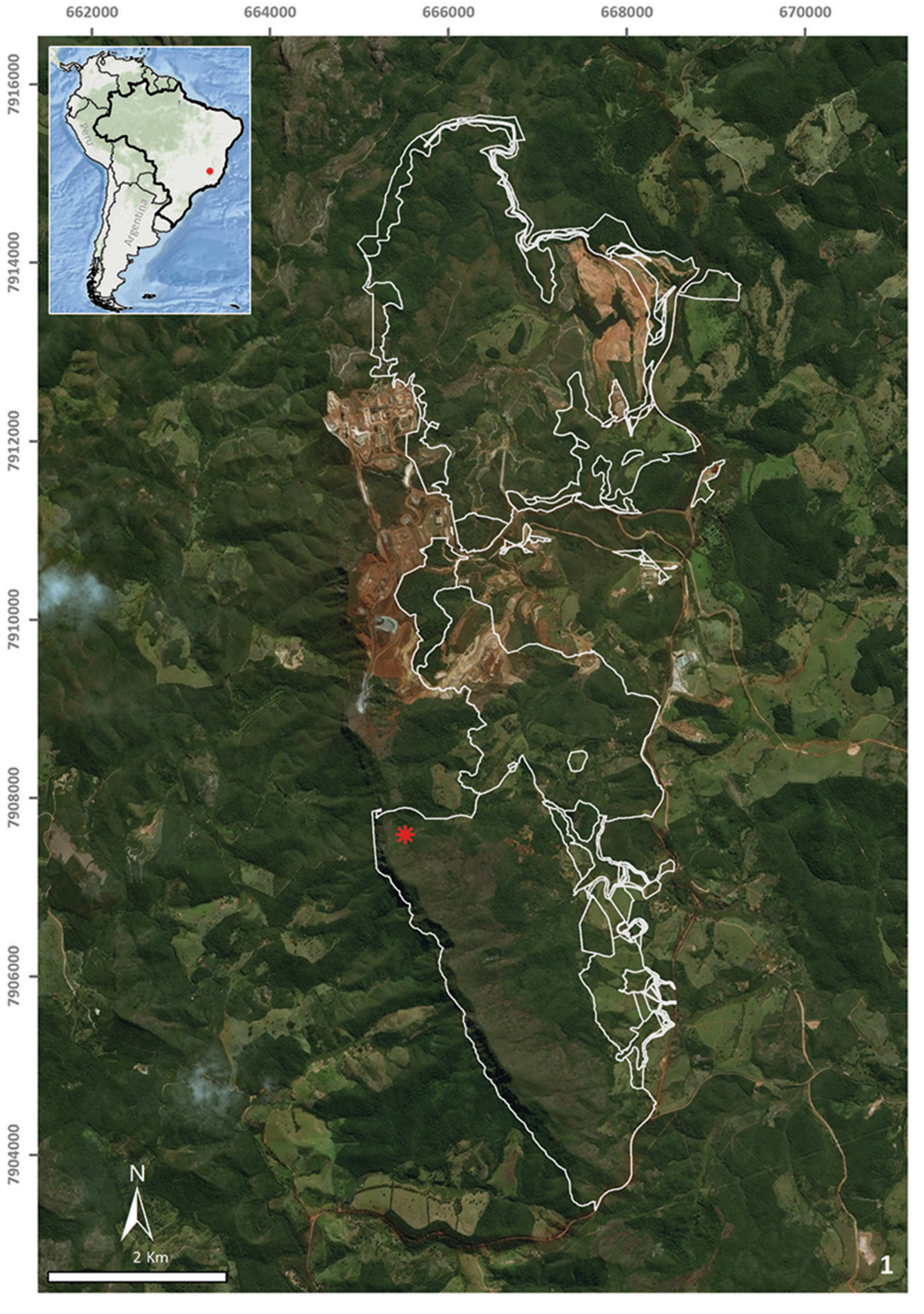
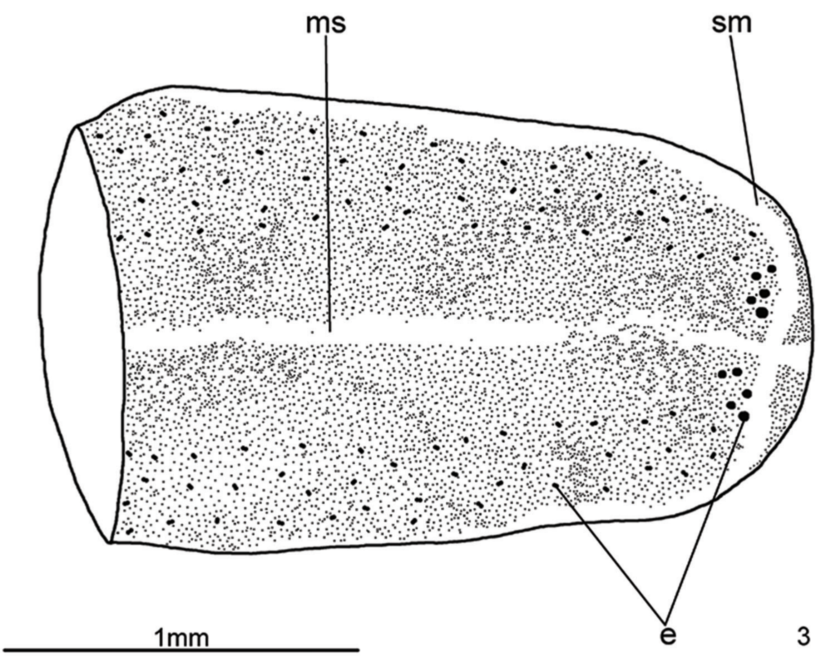


Figure 1. Location of the type locality of *Pasipha ferrariaphila* sp. n., represented by a ferruginous cave, Conceição do Mato Dentro, state of Minas Gerais, Brazil. The asterisk indicates the cave location; the outline indicates areas impacted by mining exploitation projects.



Figures 2–3. *Pasipha ferrariaphila* sp. n., holotype, dorsal view, **2** general view of preserved specimen **3** schematic drawing of the anterior region of body. The arrow indicates the anterior extremity. Eyes were drawn based on observations carried out under both stereomicroscope and microscope.

cups of 10–15 µm) and spread onto dorsal surface of body. Towards posterior end, eyes gradually becoming sparser.

Sensory pits, as simple invaginations (20–40 µm deep), contour anterior tip and occur ventro-marginally in a single row (Fig. 4) in approximately the anterior 1/6 of body. Creeping sole occupies almost whole body width.

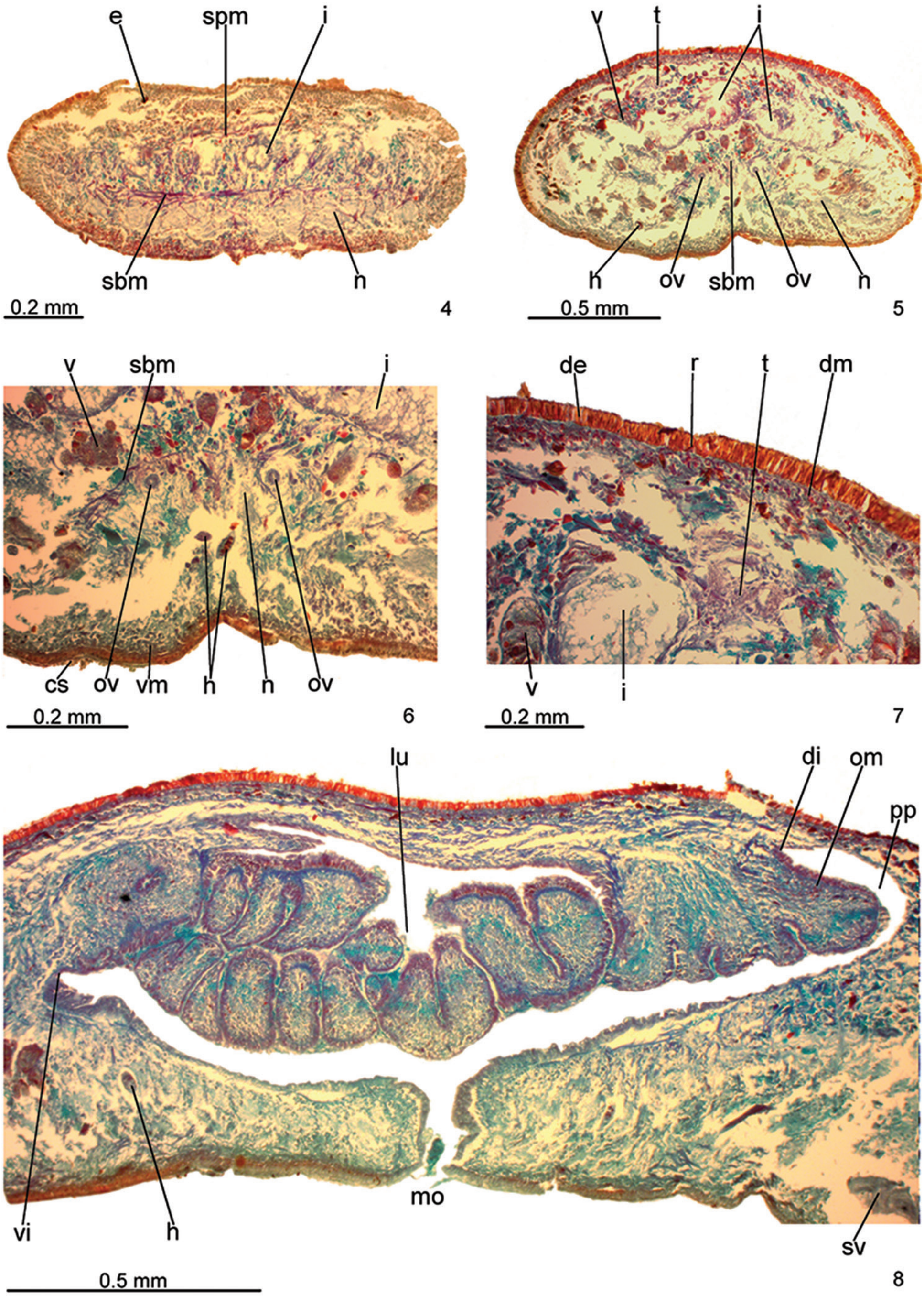
Four gland types discharge through dorsal epidermis and body margins of pre-pharyngeal region: abundant rhabditogen cells with xanthophil secretion (Figs 5–7), numerous erythrophil glands with coarse granular secretion of two types (with ovoid or rounded granules), and sparse cyanophil glands with amorphous secretion. Creeping sole receives three types of glands: cyanophil glands with amorphous secretion, rhabditogen cells with small rhabdites, as well as scarcer glands with rounded erythrophil granules. Glandular margin absent (Fig. 5). Glands discharging through anterior tip of body similar to those of pre-pharyngeal region.

Cutaneous musculature with usual three layers (circular, oblique, and longitudinal layers), longitudinal layer with small bundles (Figs 6–7). Mc:h 10%. Thickness of cutaneous musculature similar to that of epidermis. Ventral musculature (about 30 µm) two times thicker than dorsal musculature (about 15 µm) at sagittal plane in pre-pharyngeal region. Thickness of cutaneous musculature gradually diminishes towards anterior tip.

Mesenchymal musculature (Figs 6–7) poorly developed, mainly composed of three layers: (1) dorsal subcutaneous with oblique decussate fibres (about 2 fibres thick); (2) supra-intestinal transverse (about 2 fibres thick); (3) sub-intestinal transverse (about 3–4 fibres thick). Mesenchymal musculature thicker in cephalic region than in pre-pharyngeal region, especially sub-intestinal transverse layer (Fig. 4); thickness gradually diminishes towards anterior tip.

Pharynx collar-shaped, nearly 8% of body length, occupies almost all length of pharyngeal pouch. Pharyngeal dorsal insertion posteriorly shifted next to end of pharyngeal pouch. Mouth in median third of pharyngeal pouch (Fig. 8). Oesophagus absent.

Testes in two irregular rows on either side of body, located close to dorsal cutaneous musculature (Fig. 7). Testes begin at the same transversal level as ovaries, about 3.5 mm from anterior tip (16% of body length), and extend to near root of pharynx. Sperm ducts dorsal to ovovitelline ducts, laterally displaced, forming spermiducal vesicles laterally to pharynx. Behind pharynx, spermiducal vesicles well developed and sinuous, extending laterally to penis bulb. These vesicles recurve, ascend, and, subsequently, open through lateral walls of proximal portion of prostatic vesicle (Fig. 9). Large prostatic vesicle extrabulbar and not forked, close to pharyngeal pouch. This vesicle shows two portions united by a narrow canal: proximal portion oval-elongate, with a spacious lumen; distal portion globose with a narrower lumen (Figs 9–11). Ejaculatory duct sinuous, with irregular contour and ample lumen, arising from posterior region of prostatic vesicle and thereafter ascending to open into proximal portion of male atrium. Male atrium long with folded walls (Figs 9–10). Proximal region of male atrium, about anterior 1/4 of male atrium length, with narrower lumen (Figs 9–10; 12). Distal region of male atrium communicates with female atrium through a constriction (Figs 9–10).



Figures 4–8. *Pasipha ferrariaphila* sp. n., holotype. **4** anterior region, transverse section **5–7** pre-pharyngeal region, transverse sections **8** pharynx, sagittal section.

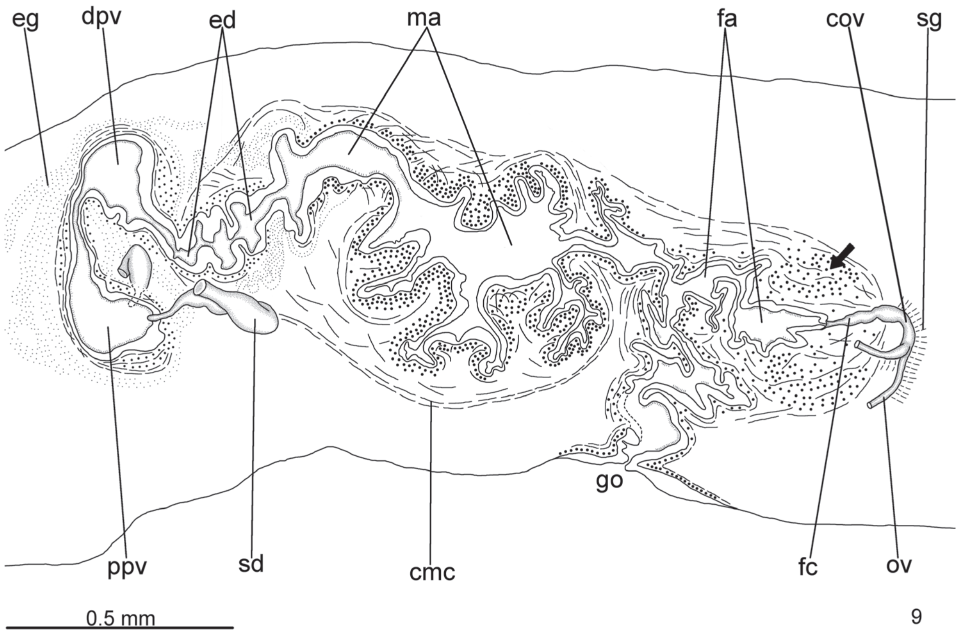
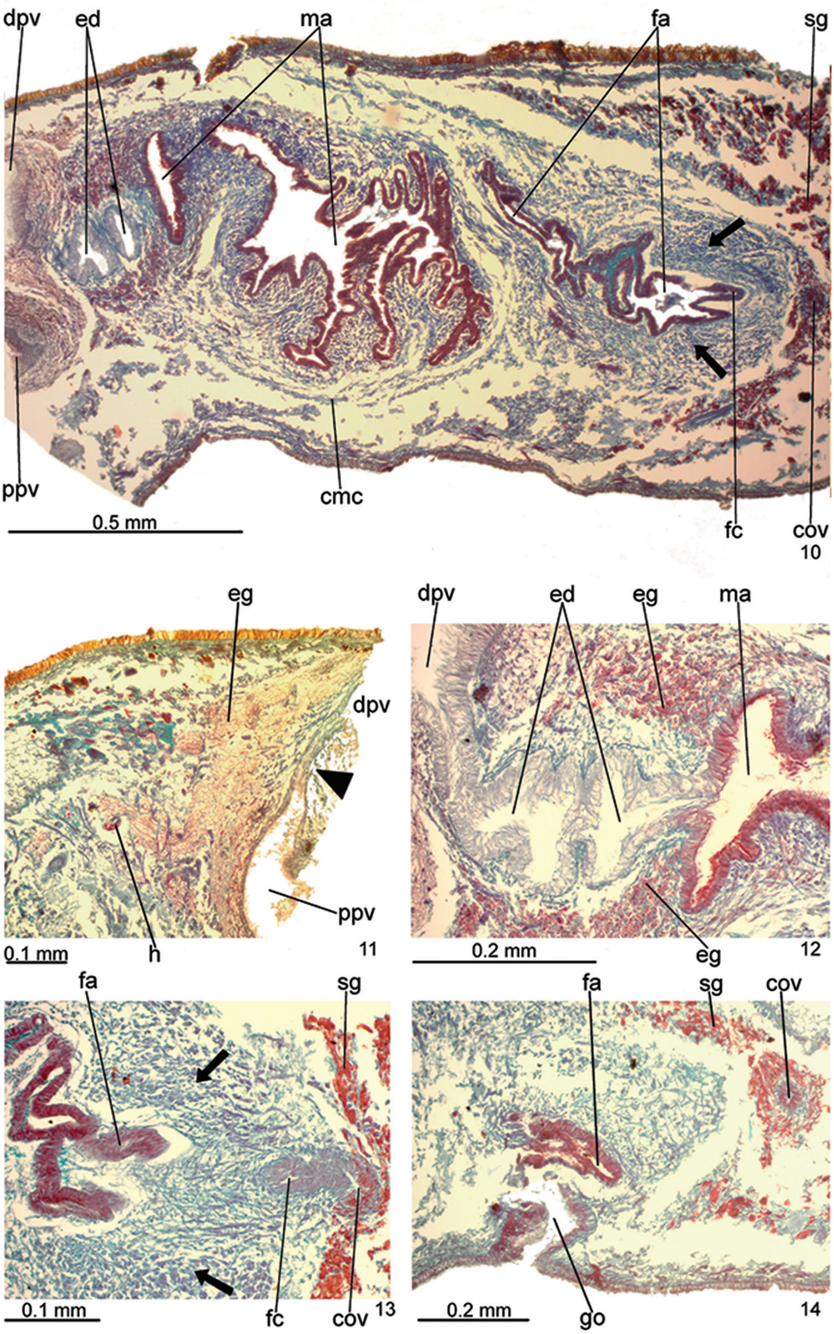


Figure 9. *Pasipha ferrariaphila* sp. n., holotype, sagittal composite reconstruction of copulatory apparatus. The arrow indicates the strong musculature in female atrium. Anterior to the left.

Epithelial lining of prostatic vesicle ciliated and columnar, receiving coarse granular erythrophil or probably mixed secretion (erythrophil core and a chromophobic peripheral part), more abundant in its proximal portion. Distal portion of prostatic vesicle receives numerous amorphous, slightly cyanophil secretions. Muscularis of prostatic vesicle thick (20–35 µm thick), constituted of interwoven longitudinal, circular and some oblique fibres (Fig. 11). Canal uniting both portions of prostatic vesicle receives few coarse granular erythrophil secretions. Ejaculatory duct lined with ciliated, columnar epithelium, receiving openings from finely granular, cyanophil glands. Muscle coat of ejaculatory duct relatively thick (about 20 µm), mainly constituted of circular fibres. Male atrium lined with non-ciliated and erythrophil epithelium in distal region, ciliated and cyanophil in proximal region. Glands of distal region of two types: with coarse granular, erythrophil secretion and with amorphous, cyanophil secretion, whereas proximal region receives a third type with finely granular, erythrophil secretion. Muscularis of male atrium thick (50–60 µm), mainly comprised of circular fibres followed by some longitudinal fibres, diminishing in thickness and number of fibres in proximal region (20 µm).

Vitelline follicles, situated between intestinal branches, well-developed (Figs 5–7). Ovaries oval-elongate, measuring about 0.3 mm in length; they are situated dorsally to ventral nerve plate, about 3.5 mm from anterior tip (16% of body length). Ovovitelline ducts emerge dorsally from median third of ovaries and run posteriorly, close to median plane, immediately above ventral nerve plate. Distal sections of ovovitelline ducts run medially lateral to female atrium, with a slight asymmetry, the left ovovitel-



Figures 10–14. *Pasipha ferrariaphila* sp. n., holotype, copulatory apparatus in sagittal sections. **10** general view **11** proximal region of prostatic vesicle **12** ejaculatory duct and proximal portion of male atrium **13** proximal portion of female atrium **14** gonoduct. Arrows indicate the strong musculature in female atrium; the arrow head indicates the canal separating proximal and distal regions of prostatic vesicle. Anterior to the left.

line duct contouring the atrial coat ventrally dislocated. They unite posteriorly to female atrium to form a C-shaped, ascending common glandular ovovitelline duct (Fig. 9). Female canal, horizontal, penetrates female muscle coat and opens into posterior-most part of female atrium. Female atrium ovoid with folded walls and narrow lumen (Figs 9–10; 13). Length of female atrium about 4/5 of male atrial length.

Female canal and atrium lined with columnar epithelium, sparsely ciliated in female canal. Glands of female atrium of two types: numerous glands with cyanophil, amorphous secretion and few glands with coarse granular erythrophil secretions. Female canal receives scant glands with coarse granular erythrophil secretion. Musculature of female atrium well developed, especially in proximal half (120 µm thick), composed mainly of circular fibres intermingled with some oblique fibres (Fig. 13).

Male and female atria with independent muscle coats (Figs 9–10), comprising longitudinal, oblique, and circular fibres. A constriction separates male and female atria (Figs 9–10). Gonoduct large and inclined backward at the sagittal plane (Figs 9; 14).

Ecology and distribution. *Pasipha ferrariaphila* is known only from its type locality. It was sampled in an area situated in the eastern margin of Serra do Espinhaço Plateau, in southeastern Brazil. The area is covered by Brazilian savanna on rocky outcrops, also known as rupestrian complexes (Rapini et al. 2008, Oliveira et al. 2018), which occur associated with quartzite, sandstone, and itabirite above 900 m of altitude along the Serra do Espinhaço (Giulietti et al. 2000). The sampling site is the entrance zone of a ferruginous cave, representing 80% of the cave area. The sampling place is a low cavity (1.6 m high) with an area of 37 m^2 and sandy soil covered by crushed ferruginous rocks. It is located in an area planned for mining activities, which is constituted by itabirite profoundly affected by such activities. Caves within iron formations are small and narrow, being formed by chemical, physical, and biological processes (Auler 2015). Ten samplings were conducted in the area between December 2010 and September 2014, but a single specimen of *P. ferrariaphila* was collected. Since this specimen shows no troglomorphic features and was collected only once in the entrance zone of the cave, we consider that its occurrence in the cave is probably occasional, using it as a temporary shelter.

Remarks. The holotype, directly fixed in 70% ethanol during field work, showed a coiled body with some artefacts (Fig. 2), such as loss of the epidermis in some body parts, numerous lacunae in the mesenchyme and ovaries. In spite of that, the anatomy and most histological aspects were relatively well preserved. Nevertheless, the specimen may have some distortion in its anatomical features. The flatworm was parasitised by helminths (Figs 6; 8; 11).

Comparative discussion. The new species herein described shows characteristics that match the diagnosis of the genus *Pasipha*, such as a body shape with parallel margins and prostatic vesicle with two portions receiving different secretions (Ogren and Kawakatsu 1990, Carbayo et al. 2013). *Pasipha ferrariaphila* also shows a folded male atrium and the female canal presenting a post-flex condition with ventral approach, i.e., the ovovitelline ducts join behind the female atrium and the female canal arises from posterior region of the female canal.

With eyes spreading over the dorsal surface of the body and a collar-shaped pharynx, *P. ferrariaphila* resembles five other species of *Pasipha*, namely *P. plana* (Schirch, 1929), *P. penhana* (Riester, 1938), *P. velutina* (Riester, 1938), *P. rosea* (E.M. Froehlich, 1955) and *P. hauseri* (Froehlich, 1959). Hence, we discuss *P. ferrariaphila* in relation to these five species in the following comparative discussion.

With respect to colour pattern, by having an almost homogeneous dorsal pattern with a thin, nearly imperceptible median stripe, *P. ferrariaphila* differs from the five species mentioned above. *Pasipha penhana* and *P. hauseri* show a quite distinct median stripe, *P. plana* and *P. rosea* a light median stripe and *P. velutina* a marbled appearance (Schirch 1929, Riester 1938, E.M. Froehlich 1955, Froehlich 1959).

Concerning internal anatomy, four of these species, namely *P. plana*, *P. penhana*, *P. velutina,* and *P. rosea*, present a not-forked prostatic vesicle with two distinct regions separated by a constriction or canal (Riester 1938, E.M. Froehlich 1955) similar to *P. ferrariaphila*. In contrast, *P. hauseri* stands apart in this group by having a forked proximal portion in its prostatic vesicle (Froehlich 1959). The new species shows a large and richly folded female atrium and, as usual in Geoplaninae, testes distributed pre-pharyngeally, differing from *P. plana*, which has a small, almost unfolded female atrium and testes almost reaching the level of the copulatory organs (E.M. Froehlich 1955). By having a horizontal female canal and an almost C-shaped common glandular ovovitelline duct, *P. ferrariaphila* differs from *P. rosea*, which shows a female canal with a C-shaped appearance and an almost horizontal common glandular ovovitelline duct (E.M. Froehlich 1955). In addition, the sperm ducts open anteriorly displaced into the proximal region of the prostatic vesicle of *P. rosea*, whereas in *P. ferrariaphila*, the openings of the sperm ducts occur into the posterior part of the proximal region of the prostatic vesicle. Froehlich (1955) describes the occurrence of a distinct circular musculature in the distal portion of the male atrium, similar to a sphincter, in both *P. plana* and *P. rosea*, which does not occur in *P. ferrariaphila*. In contrast, a strong circular musculature, resembling a sphincter, occurs in the proximal part of the female atrium of *P. ferrariaphila*.

Pasipha ferrariaphila shows a prostatic vesicle presenting pear-shaped proximal and distal regions of similar lengths with the distal part located above the proximal, differing from *P. penhana* and *P. velutina* (Riester 1938). In *P. penhana*, the prostatic vesicle is tubular-shaped with an elongate distal region (= *drüsiger Teil des Ductus ejaculatorius* according to Riester 1938) and a shorter proximal region (= *Ductus seminalis* according to Riester 1938). In *P. velutina*, both distal and proximal regions are tubular, giving an inverted U-shape to the prostatic vesicle (Riester 1938). In addition, besides the occurrence of a highly developed circular musculature in the proximal part of the female atrium, *P. ferrariaphila* differs from all species in this group by showing a longer and more spacious ejaculatory duct, as well as male and female atria separated by a constriction.

Etymology. The specific name is a composite of the Latin noun *ferraria* (iron mine) and a suffix from the Greek adjective *phílos* (affinity), referring to its sampling site.

Acknowledgements

We acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico (Nr. 306853/2015-9), Anglo American Brasil and Carste Ciência e Ambiente for financial support to this study. Carste Ciência e Ambiente is also acknowledged for samplings and information about the type locality, as well as for the map in Fig. 1. We thank G. Iturralde for the photo in Fig. 2 and the laboratory technicians R. Canello and L. Guterres for their help in section preparation, as well as M.Sc. E. Benya for an English review of the text. Dr. Hugh Jones (Natural History Museum, London, United Kingdom), Dr. Leigh Winsor (James Cook University, Townsville, Australia), and Dr. Lisandro Negrete (Universidad Nacional de La Plata, La Plata, Argentina) are gratefully acknowledged for their constructive comments on an early draft of the manuscript.

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RESEARCH ARTICLE

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Ten new species of the spider genus *Althepus* Thorell, 1898 from Southeast Asia (Araneae, Ochyroceratidae)

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Abstract

Spiders of the genus *Althepus* Thorell, 1898 are found throughout Southeast Asia, notable for their long walking legs. Ten new species are reported in this paper from China, Indonesia, Laos and Myanmar: *A. chengmenensis* Li & Li, **sp. n.** (♂♀), *A. cheni* Li & Li, **sp. n.** (♂♀), *A. gouci* Li & Li, **sp. n.** (♂♀), *A. hongguangi* Li & Li, **sp. n.** (♂♀), *A. phousalao* Li & Li, **sp. n.** (♂♀), *A. qianhuang* Li & Li, **sp. n.** (♂♀), *A. qingyuani* Li & Li, **sp. n.** (♀), *A. sepakuensis* Li & Li, **sp. n.** (♂♀), *A. xuae* Li & Li, **sp. n.** (♂♀) and *A. yizhuang* Li & Li, **sp. n.** (♂♀). These species were found in cave entrances and among tree-buttresses, indicating the spiders have a preference for dark and moist environments. All types are deposited in the Institute of Zoology, Chinese Academy of Sciences in Beijing, China (IZCAS).

Keywords

Biodiversity, endemism, Psilodercinae, taxonomy, tropical spiders

Introduction

The spider family Ochyroceratidae Fage, 1912, contains 20 genera and 216 species (World Spider Catalog 2018). They are small, web-spinning spiders, widely distributed in tropical regions worldwide. Among them, species of the genus *Althepus* Thorell,

* Both authors contributed equally to this work.

1898 build their maze-like, fine horizontal sheet webs 20–50 cm above the ground (Deeleman-Reinhold 1995). Before the current study, the genus *Althepus* contains 33 species, most of them confined to Indo-Burma and the Sunda Shelf Islands (World Spider Catalog 2018). Thorell (1898) described the type species, *A. pictus*, from Myanmar. Brignoli (1973) described two species, one from the Philippines and one from India. Deeleman-Reinhold (1985, 1995) described 13 species from Thailand, Borneo, and Indonesia. Wang and Li (2013) described one species from China. Li et al. (2014) described five species from Laos, Thailand, Myanmar, and Malaysia. Recently, Liu et al. (2017) described eleven species from Thailand.

In this paper, descriptions of ten new *Althepus* species are provided, based on specimens collected from China, Indonesia, Laos, and Myanmar. The genital organs of the males and females are described and images are provided.

Materials and methods

All spiders are preserved in a 95% ethanol solution. All types are deposited in the Institute of Zoology, Chinese Academy of Sciences in Beijing (IZCAS). Specimens were examined and measured using a Leica M205 C stereomicroscope. Further details were studied with an Olympus BX41 compound microscope. Photos were taken with an Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 stereomicroscope. The images were prepared using Helicon Focus 3.0 image stacking software and further processed with Adobe Photoshop. The map was generated in Arcview 3.3. Leg measurements are shown as total length (femur, patella, tibia, metatarsus, tarsus). Leg segments were measured from their retrolateral side. All measurements are given in millimetres (mm). Spider terminology follows that of Li et al. (2014) and Deeleman-Reinhold (1995).

Taxonomy

Family Ochyroceratidae Fage, 1912

Genus *Althepus* Thorell, 1898

Althepus: Thorell 1898: 271–378. Type species *Althepus pictus* Thorell, 1898 (by original designation), Myanmar.

Emended diagnosis. The genus *Althepus* belongs to the subfamily Psilodercinae and can be distinguished from other genera of Psilodercinae by the following combination of characters: cheliceral promargin with lamina and 1–2 teeth, retromargin with 1–2 small teeth; tarsus of male palp with lateral protrusion bearing a hook-shaped spine;

short bulb with embolus; and female internal genitalia often with paired spermathecae (Deeleman-Reinhold 1995).

Remarks. According to our observations, we use hook-shaped spine instead the "lanceolate apophysis" used by Deeleman-Reinhold (1995), and use lamina and 1–2 teeth instead the "3 teeth" used by Deeleman-Reinhold (1995).

Althepus chengmenensis Li & Li, sp. n.

http://zoobank.org/67BEA3D7-D377-45D8-8D9F-3A3D582CA643
Figs 1–2, 20–21

Types. Holotype: ♂, China, Yunnan Province, Baoshan City, Longyang District, Chengmen Cave, 24°55.691'N, 98°45.112'E, 2393 m a.s.l., 14.VII.2016, Y. Li and M. Xu. **Paratypes:** 1♂3♀, same data as holotype.

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

Diagnosis. *Althepus chengmenensis* Li & Li, sp. n. resembles *A. xuae* Li & Li, sp. n. in having a sigmoid conductor in males and curved, elongate spermathecae in females. Males can be distinguished from the latter species by the pleated margin of the conductor and by the three acuminate projections on the distal end of the conductor (Figure 1B); females can be distinguished by having longer spermathecae (versus shorter in *A. xuae* Li & Li, sp. n.) (Figure 2A). This new species can be distinguished from all the other known species of the genus by the bent middle part of the conductor (Figure 1A) and by the elongate spermathecae of similar lengths (Figure 2A).

Description. Male (holotype). Total length 3.44; carapace 1.14 length, 1.28 width; abdomen 1.95 length, 1.13 width. Carapace round, light yellow, with brown lateral margins and one wide, brown median band, the middle one wider than others (Figure 2C). Anterior margin of cephalic region distinctly elevated. Clypeus brown. Cheliceral promargin with one tooth, followed by a lamina, retromargin with two small teeth (Figure 20A), posterior surface of fang with 25 small denticles. Labium brown. Sternum yellow, with eight brown spots. Abdomen elongate, with complex patterns dorsally and ventrally. Legs brown, femur and tibia with white annulations (Figure 2C). Leg measurements: I 22.56 (5.26, 0.55, 6.15, 8.65, 1.95), II 15.48 (4.23, 0.52, 4.15, 5.10, 1.48), III missing, IV missing. Male palp (Figure 1A–D): tarsus with three slightly curved, serrated bristles at the top of cymbial protrusion (one of them was missing, Figure 1A), one curved spine and one twisted spine with the tip directed towards distally (Figure 1A); bulb yellow, ovate; embolus arising retrolatero-proximally from bulb, slightly sigmoid; conductor arising distally from bulb, sigmoid, with three acuminate projections distally (Figure 1B); embolus and conductor widely separated (distance almost equal to diameter of bulb).

Female (one of the paratypes). Total length 3.25; carapace 1.06 length, 1.28 width; abdomen 1.95 length, 1.13 width. Similar to male in colour and general features (Figure 2D–E) but smaller. Internal genitalia with two spermathecae on each side (Figure 2A). Leg measurements: I missing, II 10.82 (3.00, 0.47, 2.75, 3.30, 1.30), III 10.79 (2.95, 0.45, 2.80, 3.40, 1.19), IV 11.42 (3.20, 0.47, 3.15, 3.30, 1.30).

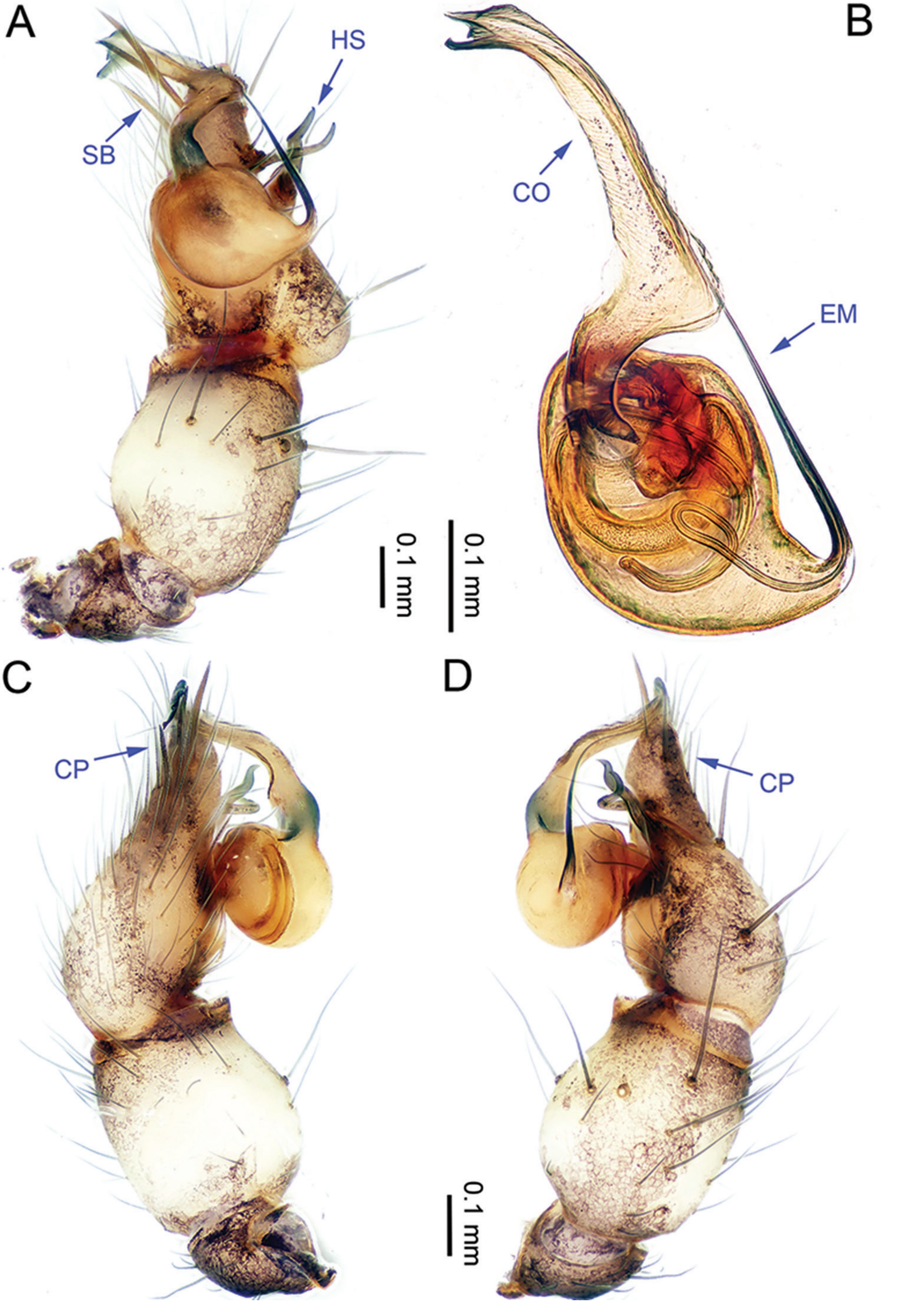

Figure 1. *Althepus chengmenensis* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, ventral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

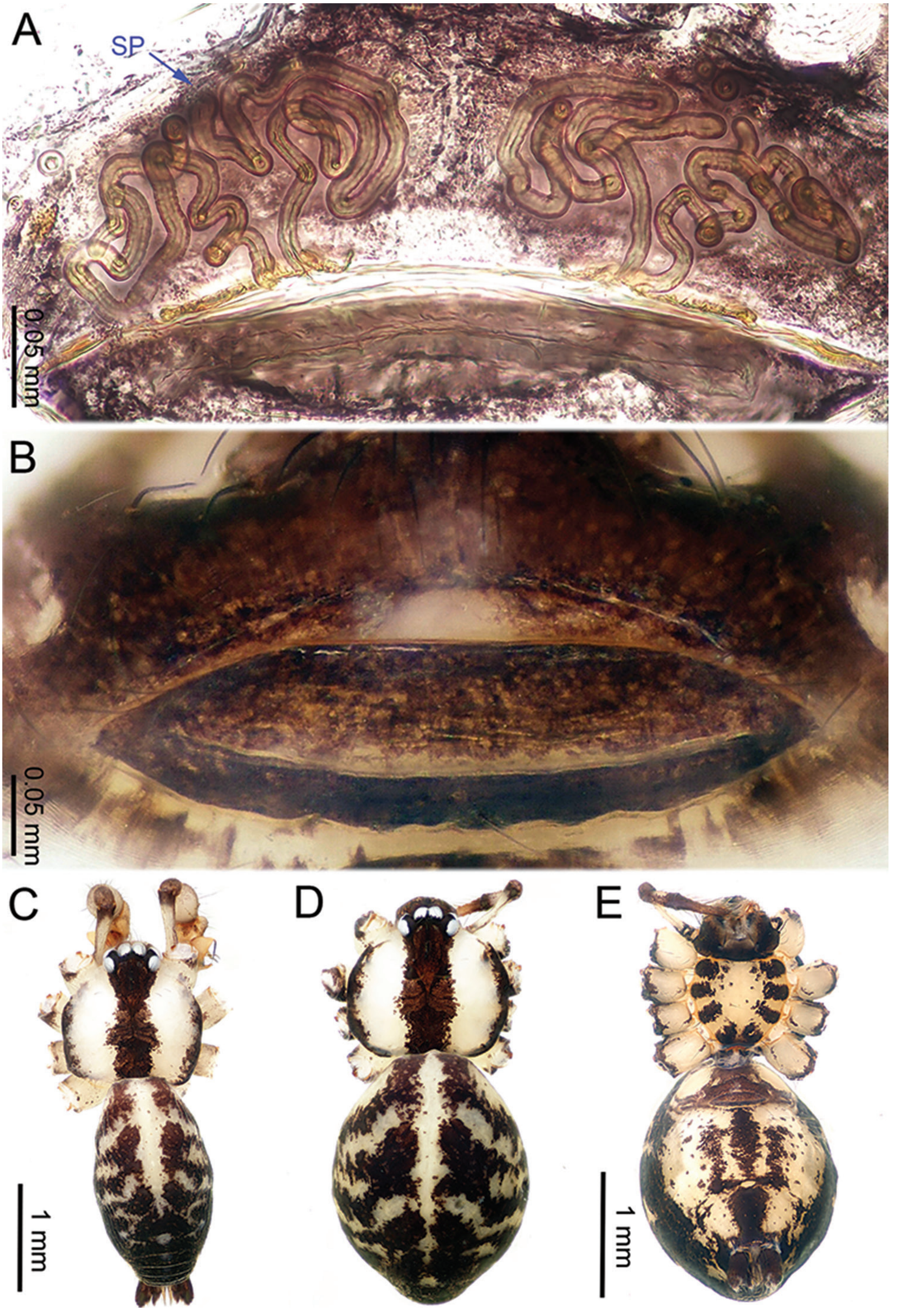

Figure 2. *Althepus chengmenensis* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

Variation. Males: carapace 1.14–1.38 length, 1.28–1.50 width, femur I 5.26–6.73 (the number of specimens = 2). Females: carapace 1.06–1.20 length, 1.13–1.28 width, femur I 2.88–3.91 (the number of specimens = 3).

Distribution. China. Known only from the type locality (Figure 21).

Natural history. Collected on rocks outside a cave at an altitude of 2393 m.

Althepus cheni Li & Li, sp. n.

http://zoobank.org/0A01B8A6-7DFB-4A55-9942-451C5DA62583

Figs 3–4, 20–21

Types. Holotype: ♂, Myanmar, Kadan Island, 12°29.113'N, 98°27.786'E, 3 m a.s.l., 27.X.2017, Z. Chen. **Paratypes:** 1♂3♀, same data as holotype.

Etymology. The specific epithet is a patronym in honour of Zhigang Chen who collected the types; noun (name) in genitive case.

Diagnosis. *Althepus cheni* Li & Li, sp. n. can be distinguished from all other known species of the genus by the large, curved, spine with tip directed distally of the palpal tarsus (Figure 3A) and by the needle-like projection on the distal end of the conductor in males (Figure 3A, B); females can be distinguished by a large membranous sac extending posteriorly and by two types of spermathecae: six short, curved spermathecae, and one globose spermatheca on a long stalk on each side (Figure 4A).

Description. Male (holotype). Total length 4.50; carapace 1.36 length, 1.52 width; abdomen 2.75 length, 1.40 width. Carapace round, light yellow, with brown margins and a narrow, brown median line behind ocular area (Figure 4C). Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20B), posterior surface of fang with 25 small denticles. Labium brown. Sternum yellow with a V-shaped pattern in the middle. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 4C). Legs brown, femur and tibia with white annulations. Leg measurements: I 46.53 (11.22, 0.63, 11.54, 19.49, 3.65), II 29.18 (7.76, 0.63, 7.37, 11.54, 1.88), III 18.59 (5.13, 0.60, 4.87, 6.41, 1.58), IV 25.91 (7.63, 0.63, 7.18, 8.72, 1.75). Male palp (Figure 3A–D): tarsus with three slightly curved, serrated bristles at the top of the cymbial protrusion (one of them was missing, Figure 3A) and one large, curved spine with tip directed distally; lateral protrusion small (Figure 3D); bulb yellow, ovate; embolus arising retrolatero-proximally from bulb, slightly sigmoid; conductor arising distally from bulb, slightly sigmoid, with a needle-like projection distally; embolus and conductor widely separated (distance less than diameter of bulb).

Female (one of the paratypes). Total length 4.35; carapace 1.25 length, 1.32 width; abdomen 2.50 length, 1.25 width. Similar to male in colour and general features (Figure 4D–E), but smaller. Internal genitalia with six short spermathecae, one globose spermatheca on a long stalk on each side, and a posterior sac (Figure 4A). Leg measurements: I 30.91 (7.37, 0.53, 7.82, 12.63, 2.56), II 19.88 (5.13, 0.52, 5.00, 7.31, 1.92), III 13.15 (3.60, 0.50, 3.25, 4.55, 1.25), IV 18.98 (5.51, 0.52, 5.13, 6.28, 1.54).

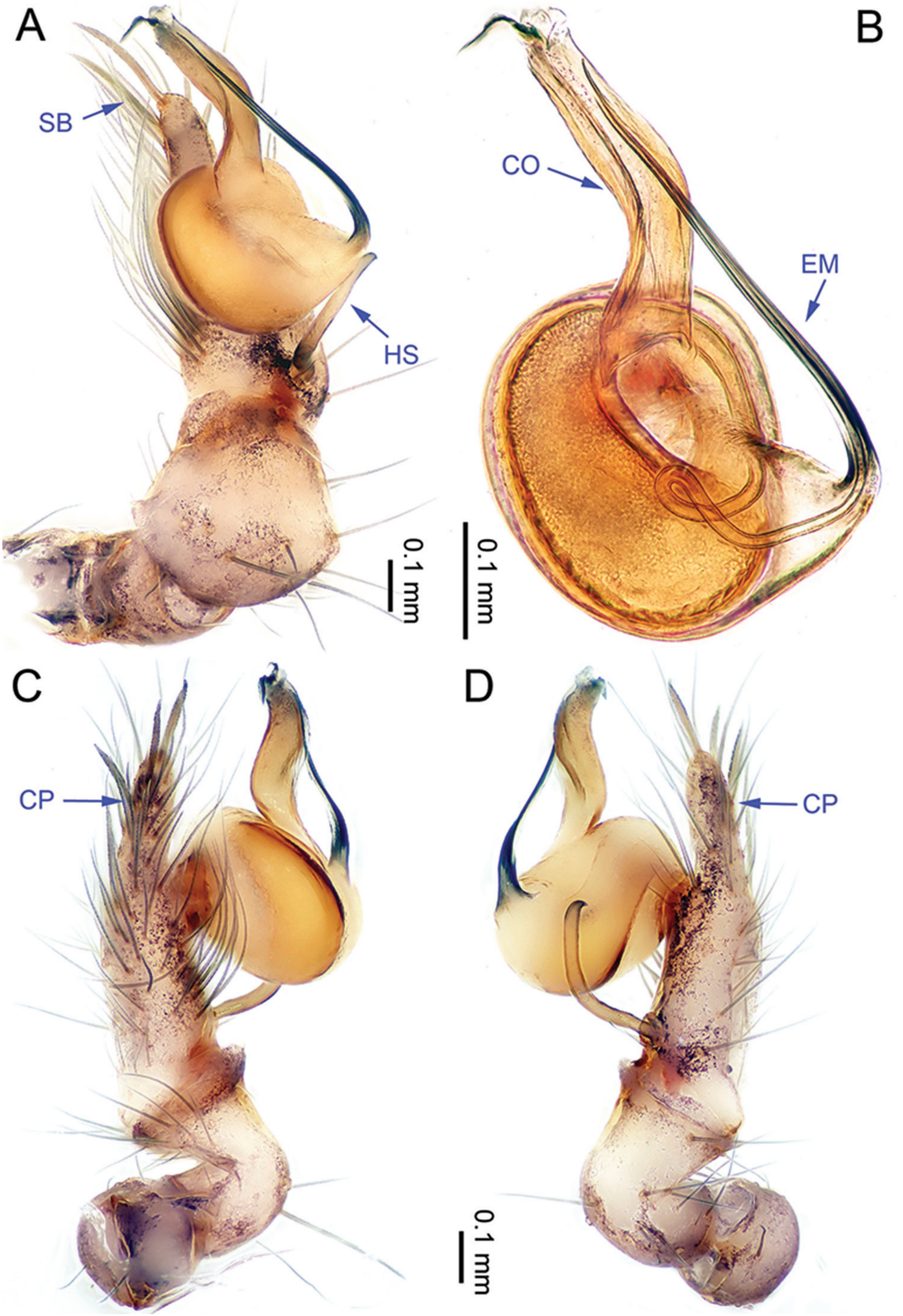

Figure 3. *Althepus cheni* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, ventral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

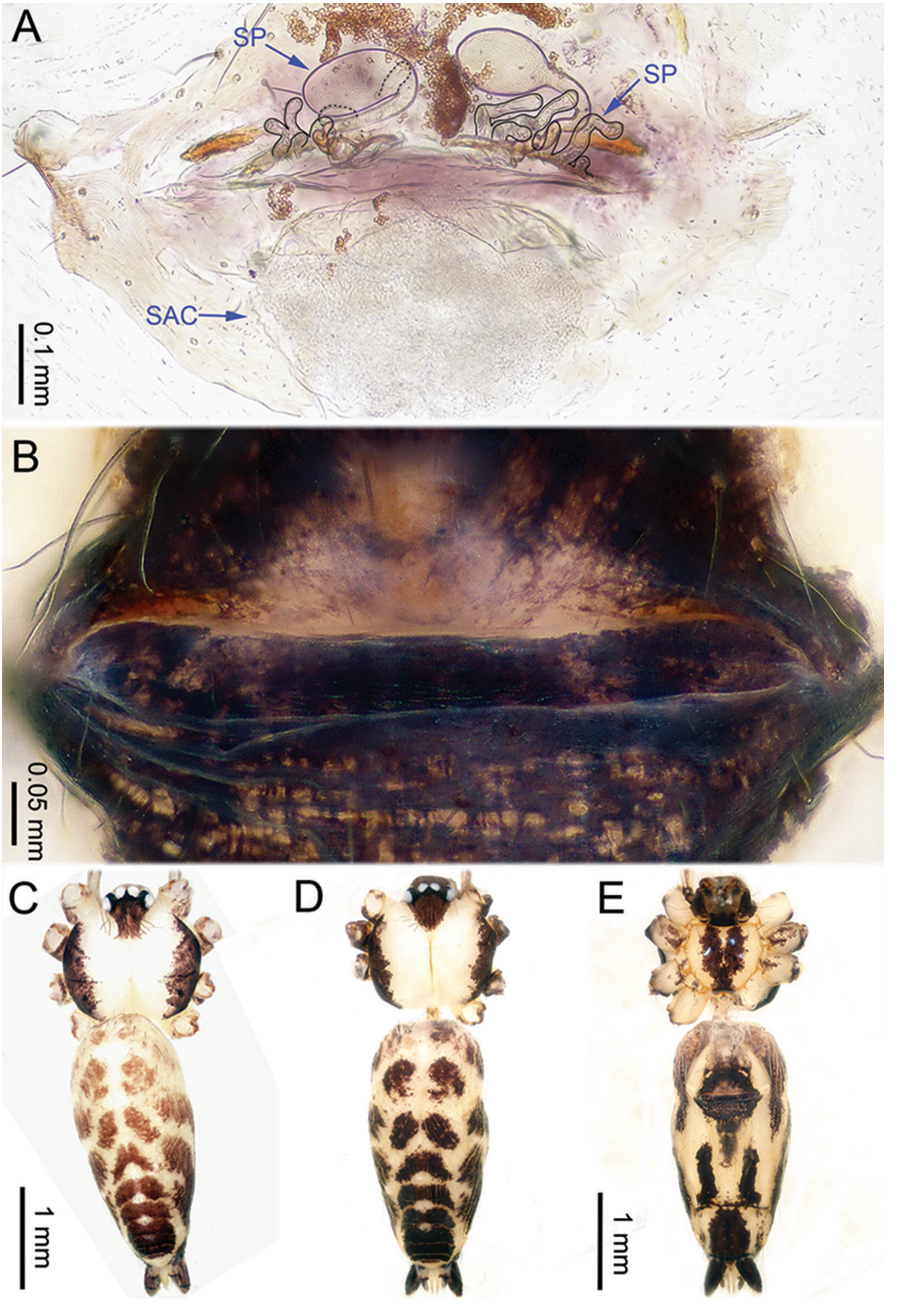

Figure 4. *Althepus cheni* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

Variation. Males: carapace 1.36–1.45 length, 1.50–1.52 width; femur I 11.22 (the number of specimens = 2; leg I lost in one specimen). Females: carapace 1.17–1.25 length, 1.26–1.38 width; femur I 7.37 (the number of specimens = 3).

Distribution. Myanmar. Known only from the type locality (Figure 21).

Natural history. Collected in a lowland evergreen broad-leaved forest at an altitude of 3 m.

Althepus gouci Li & Li, sp. n.

http://zoobank.org/831185ED-343E-43FB-9C24-C111CF892DBE

Figs 5–6, 20–21

Types. Holotype: ♂, Myanmar, Taninthayi Nature Reserve, 14°44.117'N, 98°11.554'E, 307 m a.s.l., 24.X.2017, Z. Chen. **Paratypes:** 2♀, same data as holotype.

Etymology. The specific name is derived from the Chinese pinyin 'gou ci', which means 'hooked spine', referring to the medially positioned hook-like projection on conductor (Figure 5); noun in apposition.

Diagnosis. *A. gouci* Li & Li, sp. n. can be distinguished from all other known species of the genus by the short embolus and by the hook-like projection on the widened conductor in males (Figure 5); females have two types of spermathecae: one spermatheca with 5–6 curved, long branches, and 5–6 short, thick spermathecae, on each side (two spermathecae with stalks on the left side and four spermathecae with stalks on the right side) (Figure 6A).

Description. Male (holotype). Total length 3.44; carapace 1.10 length, 1.15 width; abdomen 1.90 length, 0.88 width. Carapace round, yellow, with brown lateral margins and one wide, brown median band, the middle one wider than the others (Figure 6C). Anterior margin of cephalic region distinctly elevated. Clypeus light-brown. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20C), posterior surface of fang with 18 small denticles. Labium brown. Sternum yellow, with some small brown spots. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 6C). Legs all missing. Male palp (Figure 5A–D): tarsus with three slightly curved, serrated bristles at the top of the cymbial protrusion (one of them was missing, Figure 5A), and one hooked spine with the tip directed proximally (Figure 5D); bulb light yellow, ovate; embolus arising retrolatero-proximally from bulb, slightly sigmoid, distad; conductor arising distally from bulb, slightly sigmoid, distad, with wide base; embolus and conductor widely separated (distance less than diameter of bulb).

Female (one of the paratypes). Total length 3.80; carapace 1.10 length, 1.11 width; abdomen 2.30 length, 1.50 width. Similar to male in colour and general features (Figure 6D, E), but larger. Internal genitalia with two types of spermathecae on each side (Figure 6A). Leg measurements: I 24.61 (5.83, 0.43, 6.09, 10.26, 2.00), II missing, III 10.54 (3.00, 0.40, 2.70, 3.50, 0.94), IV - (4.232, 0.40, 4.17, 5.00, -).

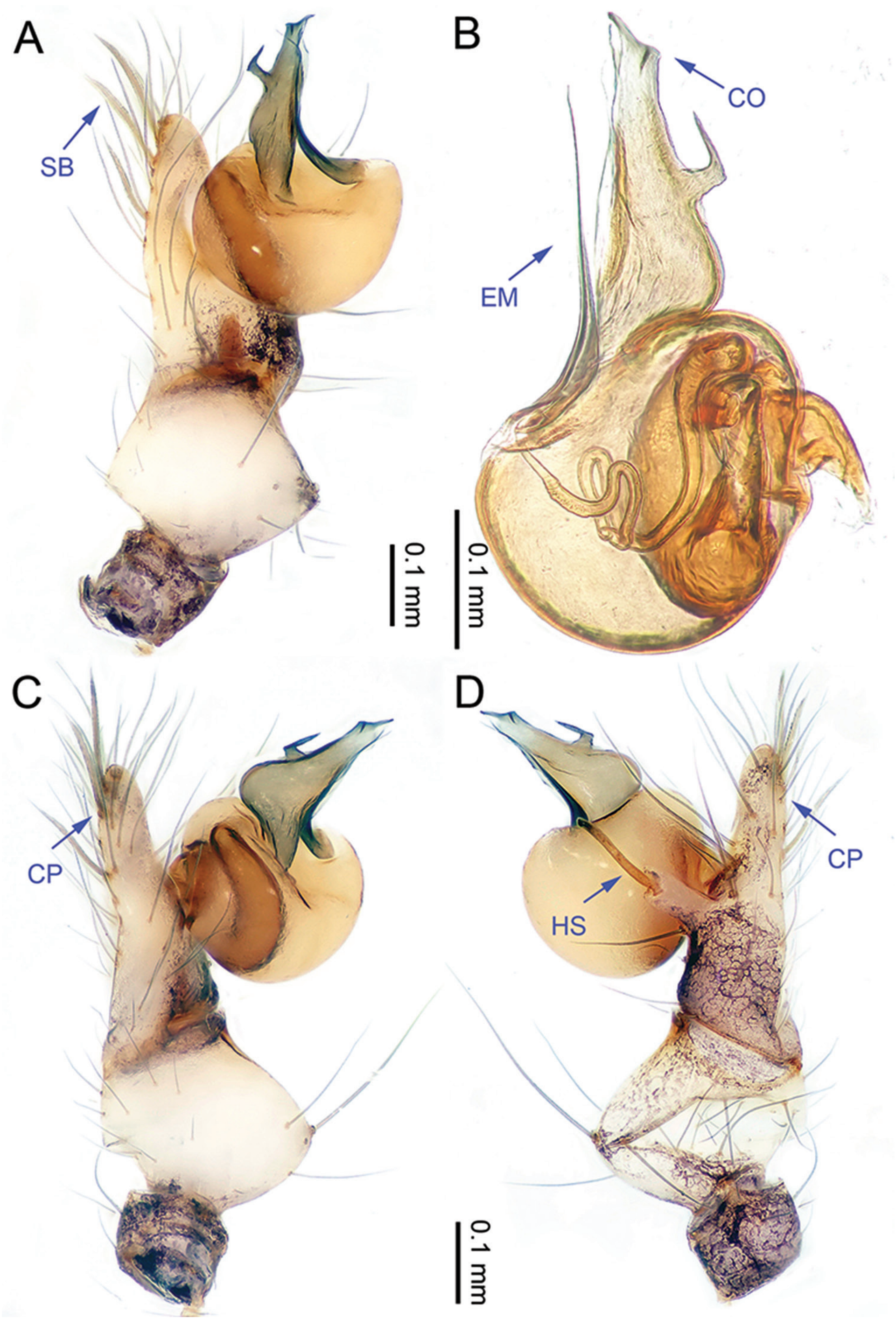

Figure 5. *Althepus gouci* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, retrolateral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

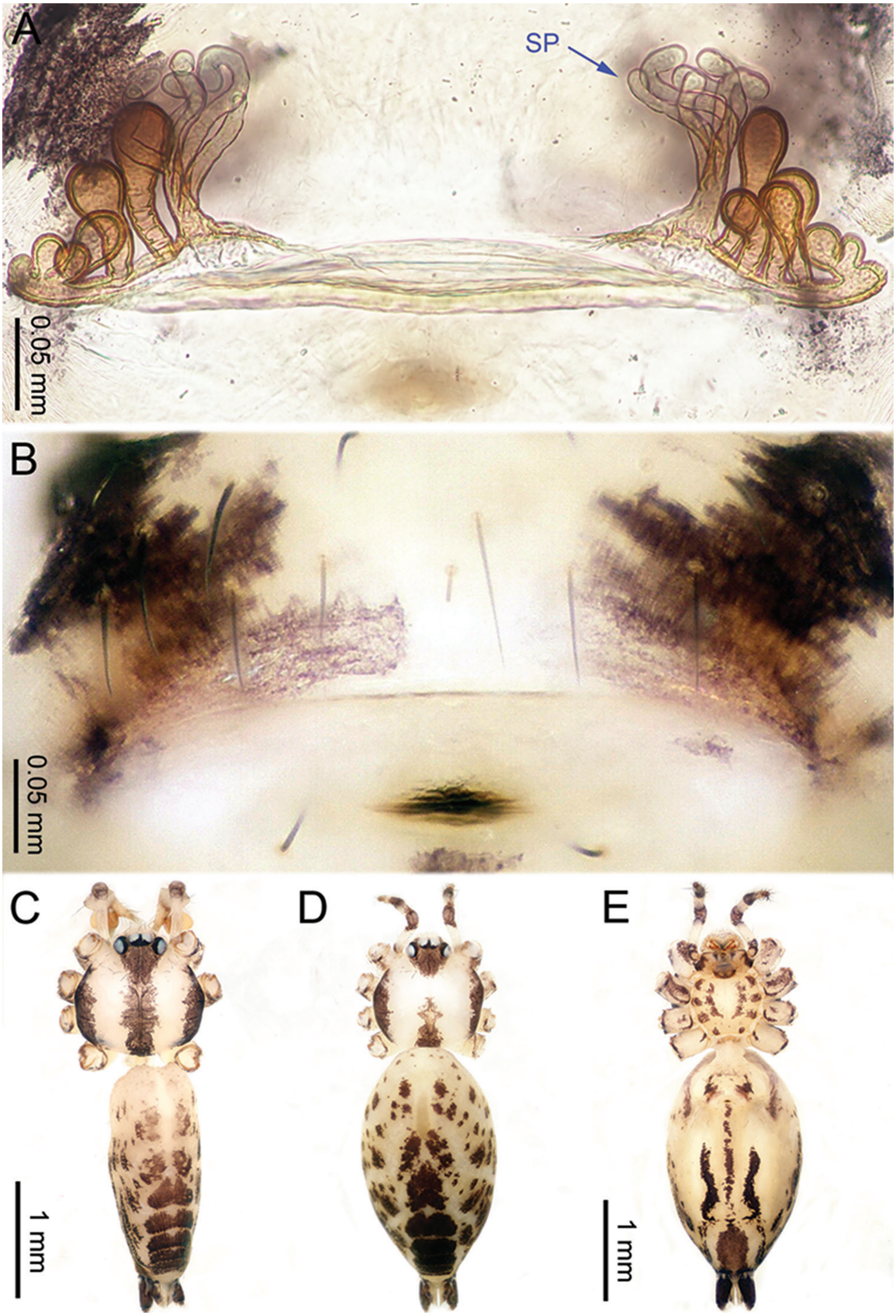

Figure 6. *Althepus gouci* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitali, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

Variation. Females: carapace 0.90–1.10 length, 1.02–1.11 width, femur I 5.83 (the number of specimens = 2; leg I lost in the other specimen).

Distribution. Myanmar. Known only from the type locality (Figure 21).

Natural history. Collected in a tropical evergreen forest at an altitude of 307 m.

Althepus hongguangi Li & Li, sp. n.

http://zoobank.org/C60BF344-587E-41B6-918E-34F1E450D22C
Figs 7–8, 20, 22

Types. Holotype: ♂, Indonesia, Sulawesi, Mountains in Palopo, 02°59.921'S, 120°08.565'E, 465 m a.s.l., 02.IX.2017, H. Liu and Z. Chen. **Paratypes:** 1♂2♀, same data as holotype.

Etymology. The specific epithet is a patronym in honour of Hongguang Liu who collected the types; noun (name) in genitive case.

Diagnosis. *Althepus hongguangi* Li & Li, sp. n. can be distinguished from all other known species of the genus by the wider basal area of the embolus in males (Figure 7B); and by three oblique, elongate spermathecae on each side in females (Figure 8A).

Description. Male (holotype). Total length 4.20; carapace 1.27 length, 1.27 width; abdomen 2.38 length, 1.15 width. Carapace round, yellow, with narrow, brown lateral margins and one wide, brown median band, the middle one wider than the others (Figure 8C). Anterior margin of cephalic region distinctly elevated. Clypeus brown. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth, posterior surface of fang with 24 small denticles (Figure 20D). Labium brown. Sternum brown, with a longitudinal yellow band in the middle. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 8C). Legs brown, femur and tibia with white annulations. Leg measurements: I - (11.47, -, -, -, -), II 29.26 (8.00 0.50 7.05, 11.60, 2.11), III missing, IV 24.94 (7.31, 0.51, 6.79, 8.65, 1.68). Male palp (Figure 7A–D): tarsus with three slightly curved, serrated bristles at the top of cymbial protrusion (Figure 7A), one hooked spine with tip directed proximally (Figure 7A); bulb yellow, ovate; embolus arising proximally from bulb, observably sigmoid, distad; conductor arising distally from bulb, oblique, distad; embolus and conductor widely separated (distance almost equal to half diameter of bulb).

Female (one of the paratypes). Total length 3.90; carapace 1.16 length, 1.20 width; abdomen 2.25 length, 1.17 width. Similar to male in colour and general features (Figure 8D–E), but smaller. Internal genitalia with three oblique, elongate spermathecae on each side, each side with a pore-plate at its base (Figure 8A). Leg measurements: I 39.41 (9.36, 0.50, 9.10, 16.92, 3.53), II 22.50 (5.77, 0.50, 5.71, 8.72, 1.80), III missing, IV 21.00 (6.02, 0.50, 5.78, 7.05, 1.65).

Variation. Males: carapace 1.25–1.27 length, 1.27–1.30 width, femur I 11.47–12.18 (the number of specimens = 2). Females: carapace 1.16–1.25 length, 1.20–1.41 width, femur I 9.36 (the number of specimens = 2; leg I lost in the other specimen).

Distribution. Indonesia. Known only from the type locality (Figure 22).

Natural history. Collected among tree buttresses at an altitude of 465 m.

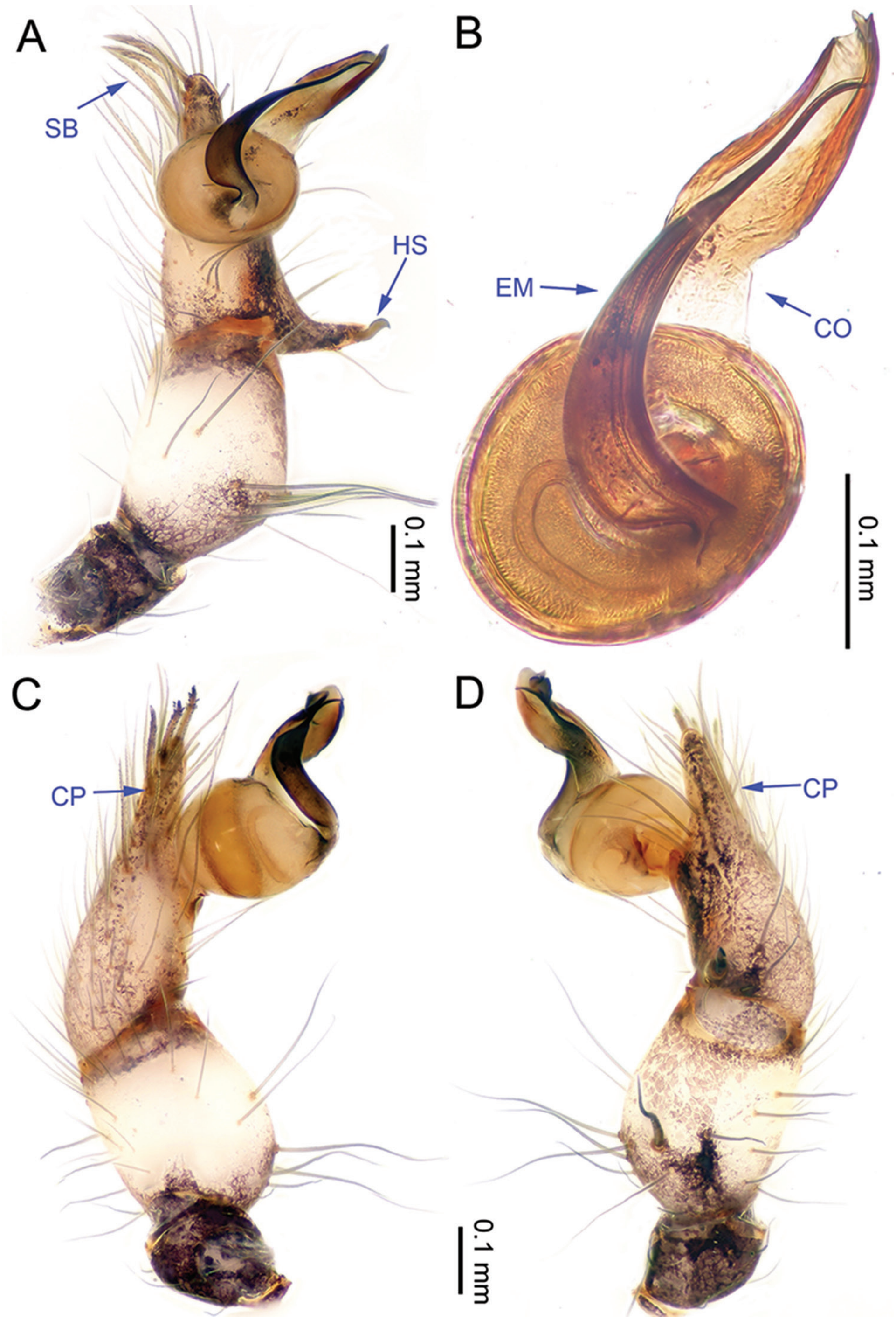

Figure 7. *Althepus hongguangi* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, ventral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

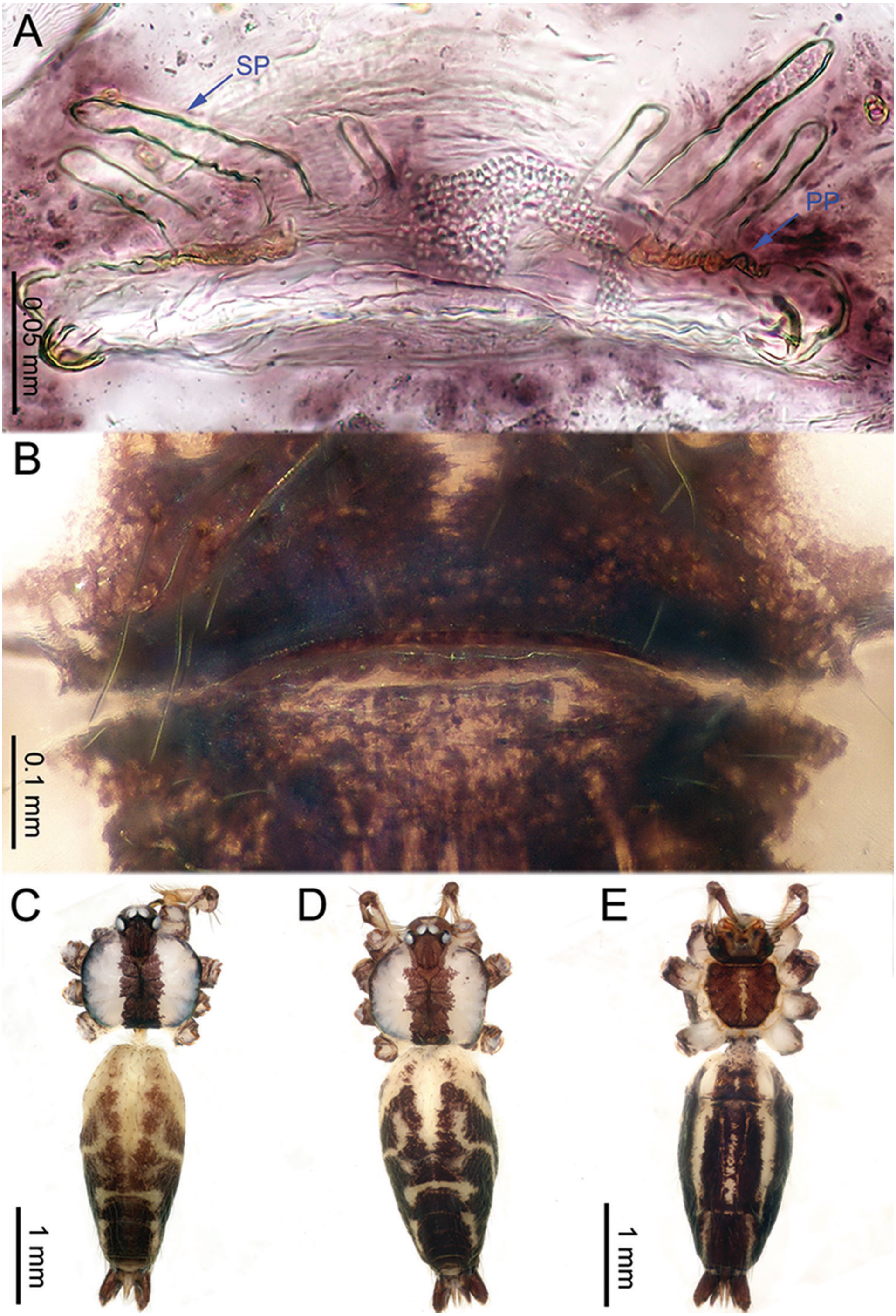

Figure 8. *Althepus hongguangi* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviations: SP spermatheca; PP pore plate.

Althepus phousalao **Li & Li, sp. n.**
http://zoobank.org/F4E8B6B1-8D7D-427B-BF79-20F8C17C7951
Figs 9–10, 20–21

Types. Holotype: ♂, Laos, Champasak Province, Pakse City, Phou Salao, 15°05.284'N, 105°48.671'E, 242 m a.s.l., 15.XI.2012, Z. Yao. **Paratype:** 1♀, same data as holotype.

Etymology. The specific epithet is a noun in apposition taken from the type locality Phou Salao, Laos.

Diagnosis. *Althepus phousalao* Li & Li, sp. n. resembles *A. leucosternum* Deeleman-Reinhold, 1995, in having a triangular distal end of the conductor and one retrolateral spine of cymbium in males, and one spermatheca on each side in females. Males can be distinguished by the longer conductor (versus shorter in *A. leucosternum*) (Figure 9B). Females can be distinguished by one thicker, longer spermatheca on each side (versus shorter in *A. leucosternum*) (Figure 10A), can be distinguished from all the other known species of the genus by the thick spermathecae (Figure 10A).

Description. Male (holotype). Total length -; carapace 1.09 length, 1.17 width; abdomen missing. Carapace round, yellow, with brown lateral margins and one wide, brown median band, the middle one wider than others (Figure 10C). Cheliceral promargin with two teeth, retromargin with two small teeth (Figure 20E), posterior surface of fang with 14 small denticles. Labium light brown. Sternum yellow, with some irregular brown spots. Legs brown, femur and tibia with white annulations. Leg measurements: I missing, II 19.31 (5.45, 0.44, 5.13, 6.79, 1.50), III 13.01 (3.75, 0.44, 3.40, 4.30, 1.12), IV missing. Male palp (Figure 9A–D): tarsus with three slightly curved, serrated bristles at the top of cymbial protrusion (two of them were missing, Figure 9D), one hooked spine with tip directed distally and one long spine retrolaterally (Figure 9A); bulb light yellow, ovate; embolus arising retrolatero-proximally from bulb, slender, slightly curved; conductor arising distally from bulb, observably sigmoid; embolus and conductor widely separated (distance less than diameter of bulb).

Female (paratype). Total length 3.20; carapace 0.94 length, 1.13 width; abdomen 1.72 length, 1.00 width. Similar to male in colour and general features of carapace (Figure 10D, E) but smaller. Abdomen elongate, with complex patterns dorsally and ventrally. Internal genitalia with one curved, elongate spermatheca on each side (Figure 10A). Leg measurements: I 26.18 (6.54, 0.48, 7.05, 10.51, 1.60), II missing, III 11.06 (3.25, 0.45, 2.75, 3.55, 1.06), IV 15.84 (4.55, 0.47, 4.36, 5.13, 1.33).

Distribution. Laos. Known only from the type locality (Figure 21).

Natural history. Collected in a pit of Phou Salao at an altitude of 242 m.

Remark. *Althepus phousalao* Li & Li, sp. n., was labelled as “sp. 23” in Li and Li (2018).

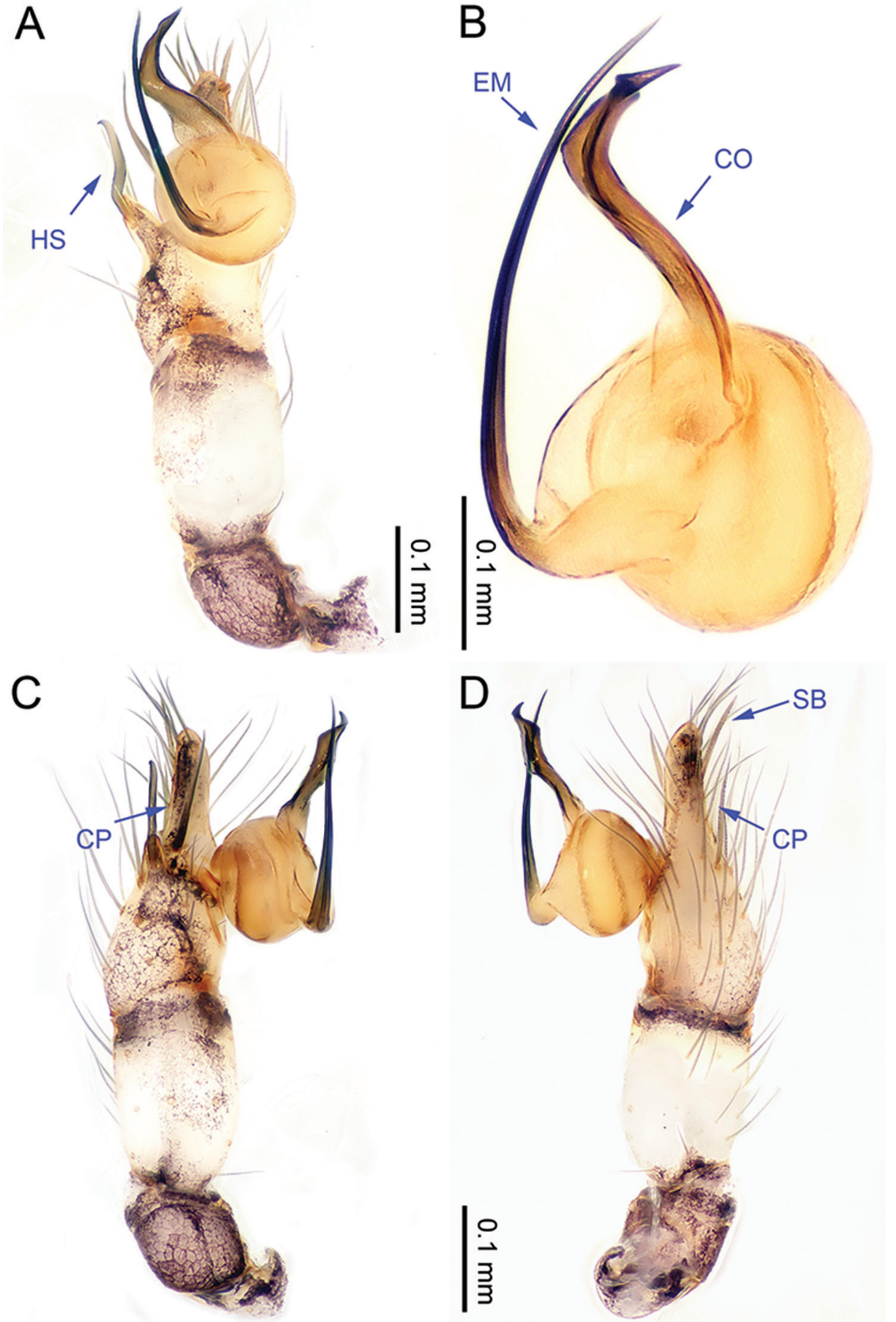

Figure 9. *Althepus phousalao* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, ventral view **C** Palp, retrolateral view **D** Palp, prolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

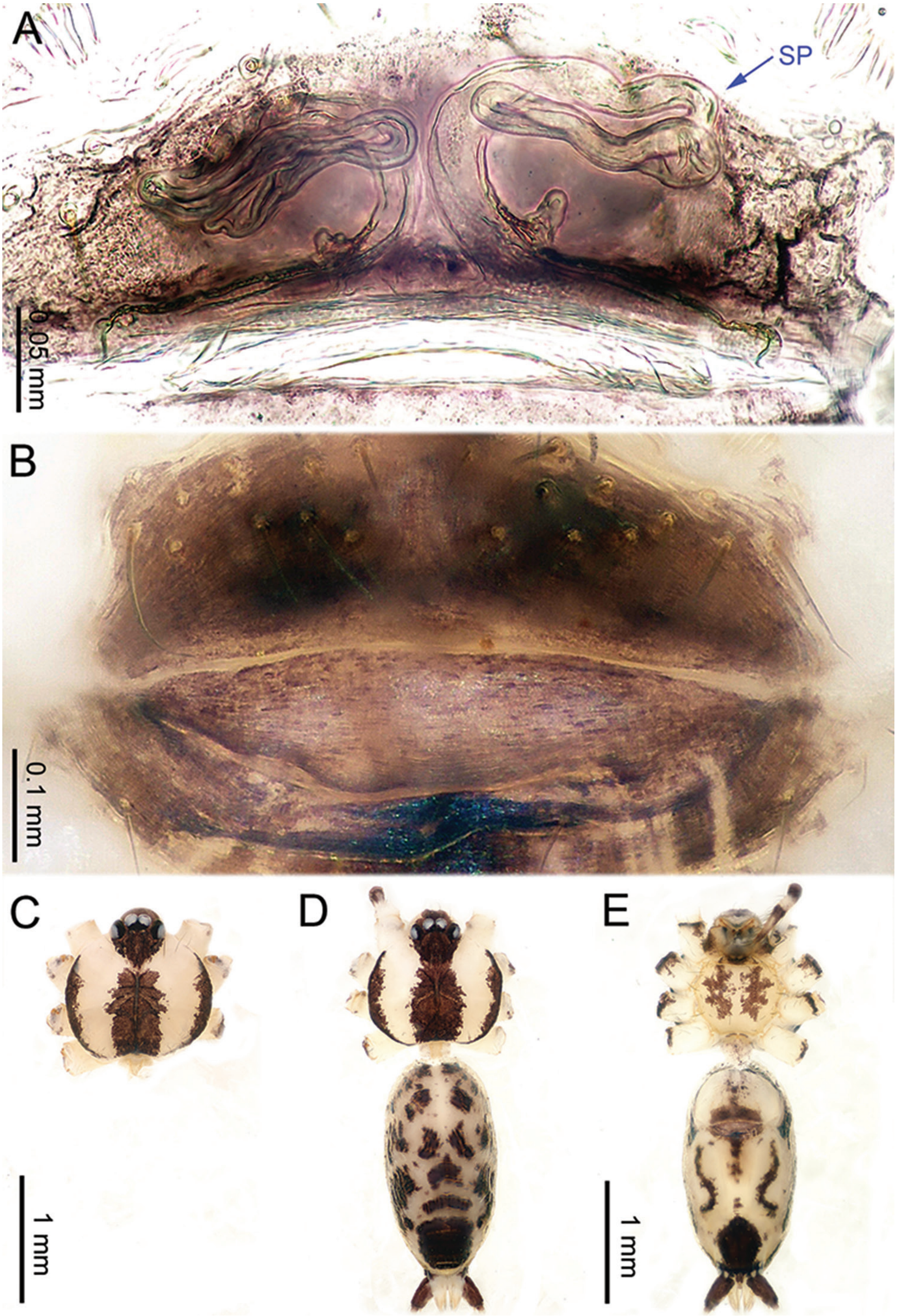

Figure 10. *Althepus phousalao* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

Althepus qianhuang **Li & Li, sp. n.**
http://zoobank.org/A20BFBA3-2F97-4A6C-A50C-E618569202A1
Figs 11–12, 20, 22

Types. Holotype: ♂, Indonesia, Jawa, Special District of Yogyakarta, Kulon Progo Town, Girimulyo, Jatimulyo Village, Gua (Cave) Kiskendo, 7°44.86'S, 110°07.87'E, 662 m a.s.l., 28.VIII.2014, Z. Yao and H. Zhao. **Paratypes:** 2♀, same data as holotype.

Etymology. The specific name is derived from the Chinese pinyin 'qian huang', which means 'pale yellow', referring to the pale yellow colour of ocular area (Figure 12C, D); adjective.

Diagnosis. *Althepus qianhuang* Li & Li, sp. n. can be distinguished from all other known species of the genus by the nearly parallel conductor and embolus in males (Figure 11); by a large membranous sac extending posteriorly and 1–2 small round spermatheca(e) on each side in the internal genitalia of females (Figure 12A).

Description. Male (holotype). Total length 4.49; carapace 1.55 length, 1.48 width; abdomen 2.80 length, 1.31 width. Carapace round, pale yellow, with brown lateral margins and one wide, brown median band, the middle one wider than the others (Figure 12C). Anterior margin of cephalic region distinctly elevated. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20F), posterior surface of fang with 21 small denticles. Labium brown. Sternum brown. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 12C). Legs brown, femur and tibia with white annulations. Leg measurements: I - (10.13, 0.63, 9.62, 17.31, -), II 25.30 (7.05, 0.63, 6.41, 9.75, 1.46), III 16.19 (4.65, 0.60, 3.92, 5.71, 1.31), IV missing. Male palp (Figure 11A–D): tarsus with one hooked spine with tip directed distally (Figure 11A); bristles at the top of the cymbial protrusion (Figure 11C) as in *A. hongguangi* Li & Li, sp. n.; bulb yellow, ovate; embolus arising distally from bulb, short, slightly curved; conductor arising distally from bulb, short, slightly curved; embolus and conductor slightly separated (distance less than diameter of bulb).

Female (one of the paratypes). Total length 4.87; carapace 1.39 length, 1.44 width; abdomen 2.96 length, 1.70 width. Similar to male in colour and general features (Figure 12D–E) but larger. Internal genitalia with 1–2 small round spermatheca(e) on each side and a large posterior sac (Figure 12A). Leg measurements: I missing, II missing, III 12.81 (3.68, 0.46, 3.28, 4.30, 1.09), IV missing.

Variation. Females: carapace 1.17–1.39 length, 1.27–1.44 width; leg I lost (the number of specimens = 2).

Distribution. Indonesia. Known only from the type locality (Figure 22).

Natural history. Collected at a cave entrance at an altitude of 662 m.

Remark. *Althepus qianhuang* Li & Li, sp. n., was labelled as "sp. 119" in the analysis of Li and Li (2018).

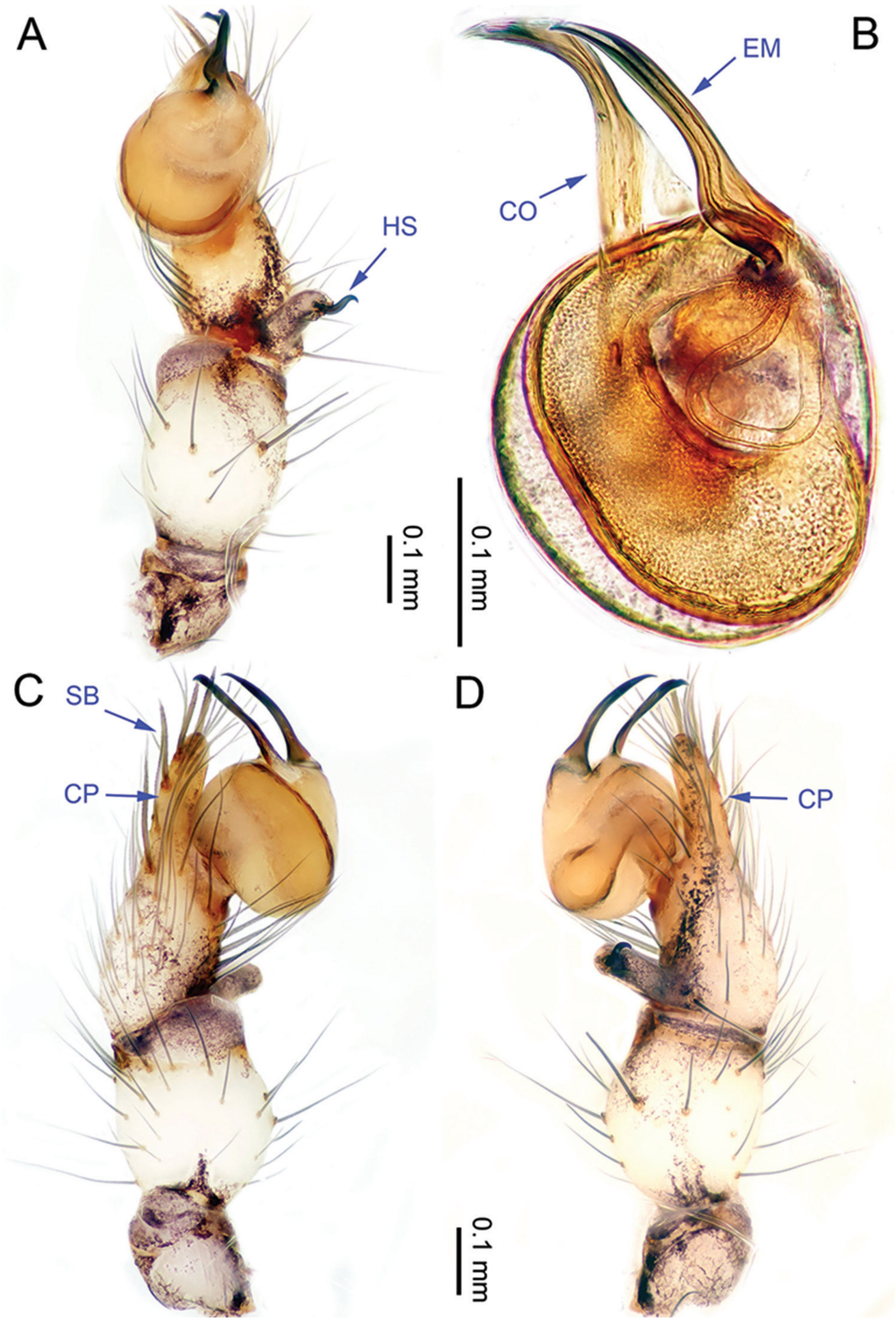

Figure 11. *Althepus qianhuang* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, prolateral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

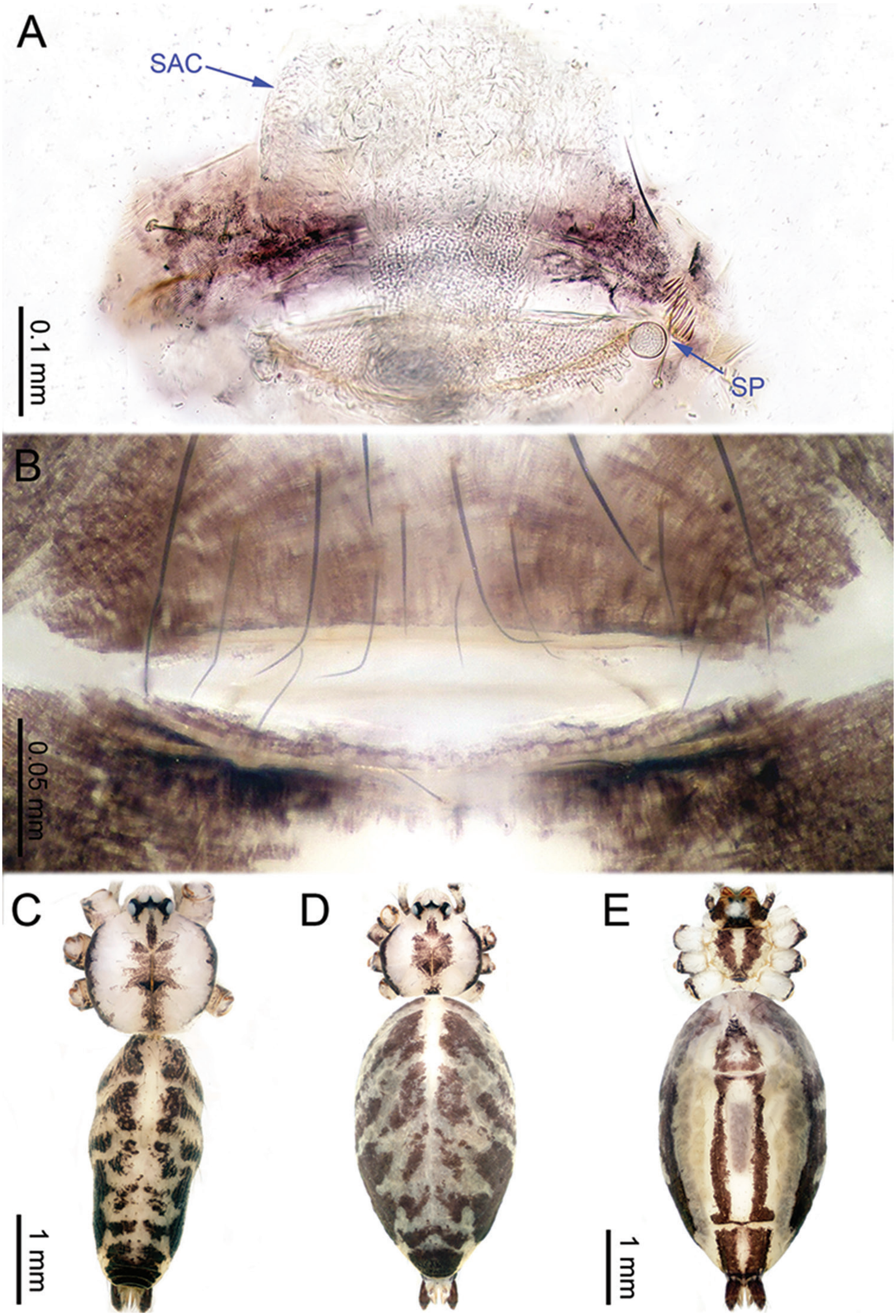

Figure 12. *Althepus qianhuang* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

Althepus qingyuani Li & Li, sp. n.

http://zoobank.org/5967A80C-65AC-46E1-81D0-AC1D1B50BBD0
Figs 13, 20–21

Types. Holotype: ♀, China, Yunnan Province, Lincang City, Yongde County, Xiaomengtong Village, Xiangquan Dam, Xianren Cave, 24°12.099'N, 99°18.607'E, 1499 m a.s.l., 02.VIII.2010, C. Wang, L. Lin and Q. Zhao. **Paratype:** 1♀, same data as holotype.

Etymology. The specific name is a patronym in honour of Dr. Qingyuan Zhao who collected the types; noun (name) in genitive case.

Diagnosis. *Althepus qingyuani* Li & Li, sp. n. can be distinguished from all other known species of the genus by 16 round spermathecae on curved stalks in the females (Figure 13A).

Description. Female (holotype). Total length 3.95; carapace 1.44 length, 1.58 width; abdomen 2.28 length, 1.33 width. Carapace round, yellow, with three longitudinal brown bands of similar widths (Figure 13D–E). Anterior margin of cephalic region distinctly elevated. Clypeus brown. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20G), posterior surface of fang with 23 small denticles. Labium brown. Sternum brown with a longitudinal yellow band. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 13D, E). Legs brown, femur and tibia with white annulations. Leg measurements: I missing, II 20.80 (5.67, 0.56, 5.26, 7.63, 1.78), III 14.11 (4.17, 0.56, 3.60, 4.45, 1.33), IV missing. Internal genitalia with 16 round spermathecae on curved stalks (Figure 13A).

Male. Unknown.

Variation. Females: carapace 1.17–1.44 length, 1.30–1.58 width; leg I lost (the number of specimens = 2).

Distribution. China. Known only from the type locality (Figure 21).

Natural history. Collected at a cave entrance at an altitude of 1499 m.

Remark. *Althepus qingyuani* Li & Li, sp. n., was labelled as "sp. 97" in the analysis of Li and Li (2018).

Althepus sepakuensis Li & Li, sp. n.

http://zoobank.org/598BEAF8-7E62-44FE-99F6-3479BFDAEE5F
Figs 14–15, 20, 22

Types. Holotype: ♂, Indonesia, East Kalimantan, Penajam, Paser Utara Town, Sepaku Village, on foot of Gunung Parung, 00°50.920'S, 116°46.284'E, 60 m a.s.l., 17.VIII.2014, H. Zhao and Z. Yao. **Paratype:** 1♀, same data as holotype.

Other material examined. 1♂, Indonesia, East Kalimantan, Penajam, Camp of International Timber Corporation of Indonesia, 01°05.291'S, 116°41.009'E, 64 m a.s.l., 17.VIII.2014, H. Zhao and Z. Yao.

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

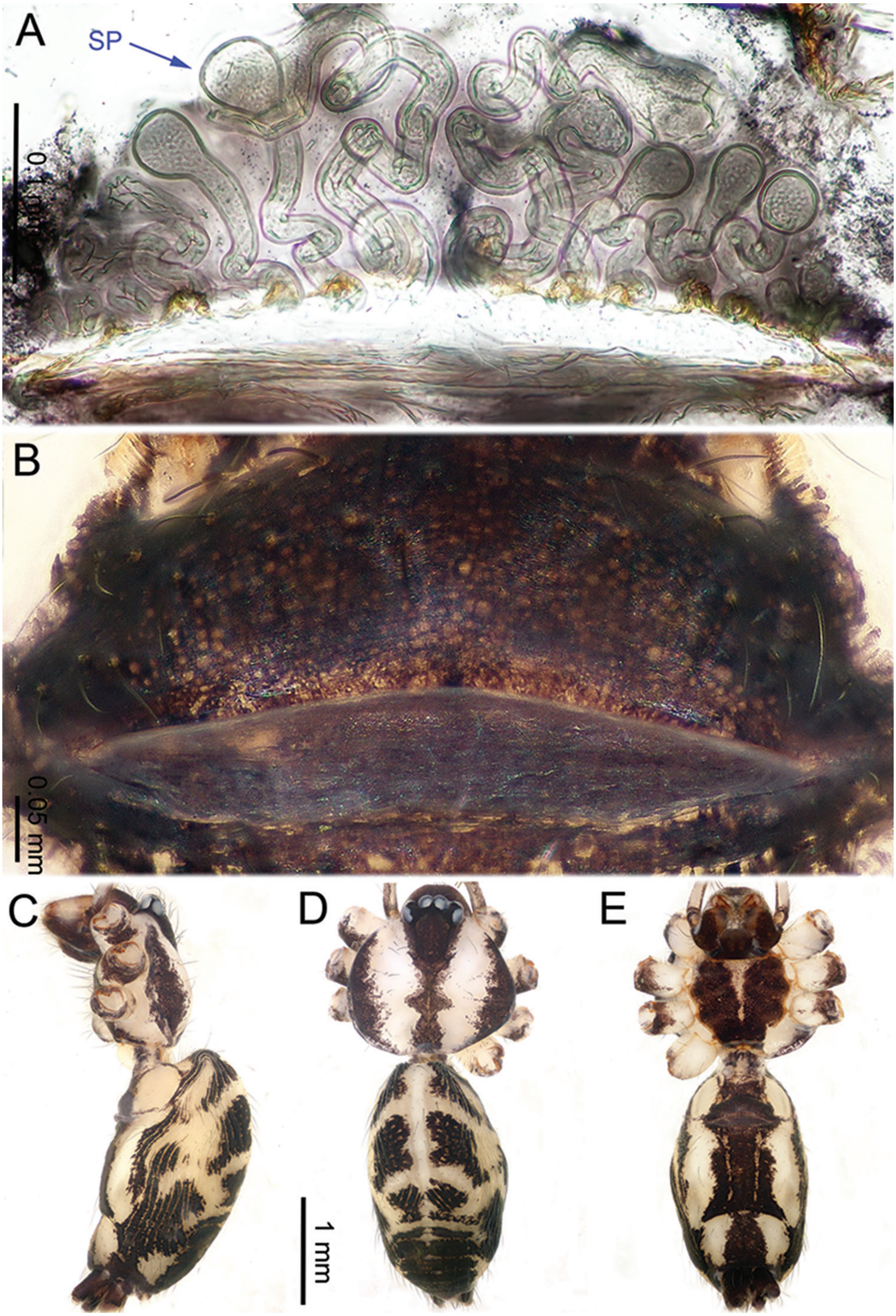

Figure 13. *Althepus qingyuani* Li & Li, sp. n., female holotype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Female habitus, retrolateral view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

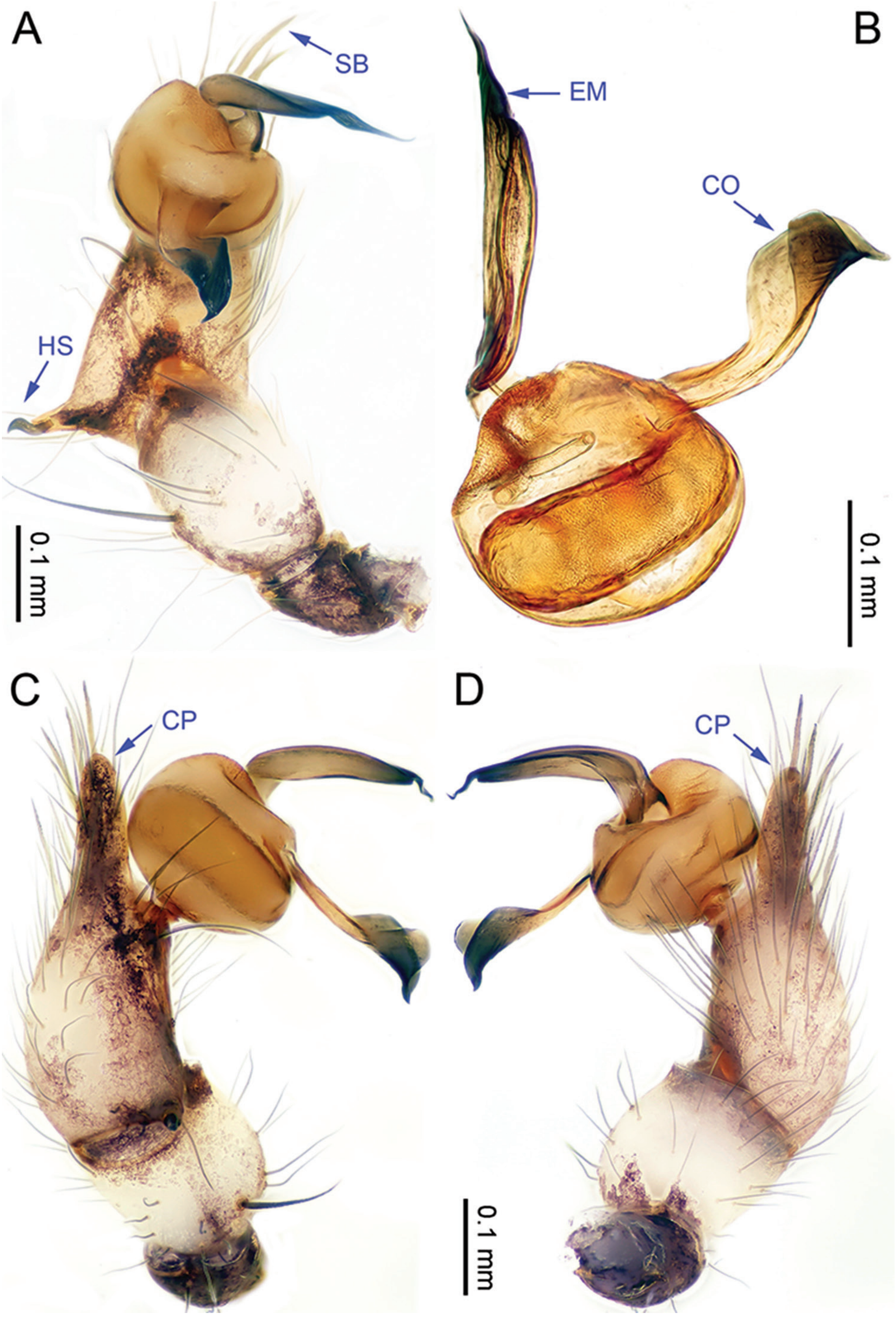

Figure 14. *Althepus sepakuensis* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, retrolateral view **C** Palp, retrolateral view **D** Palp, prolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

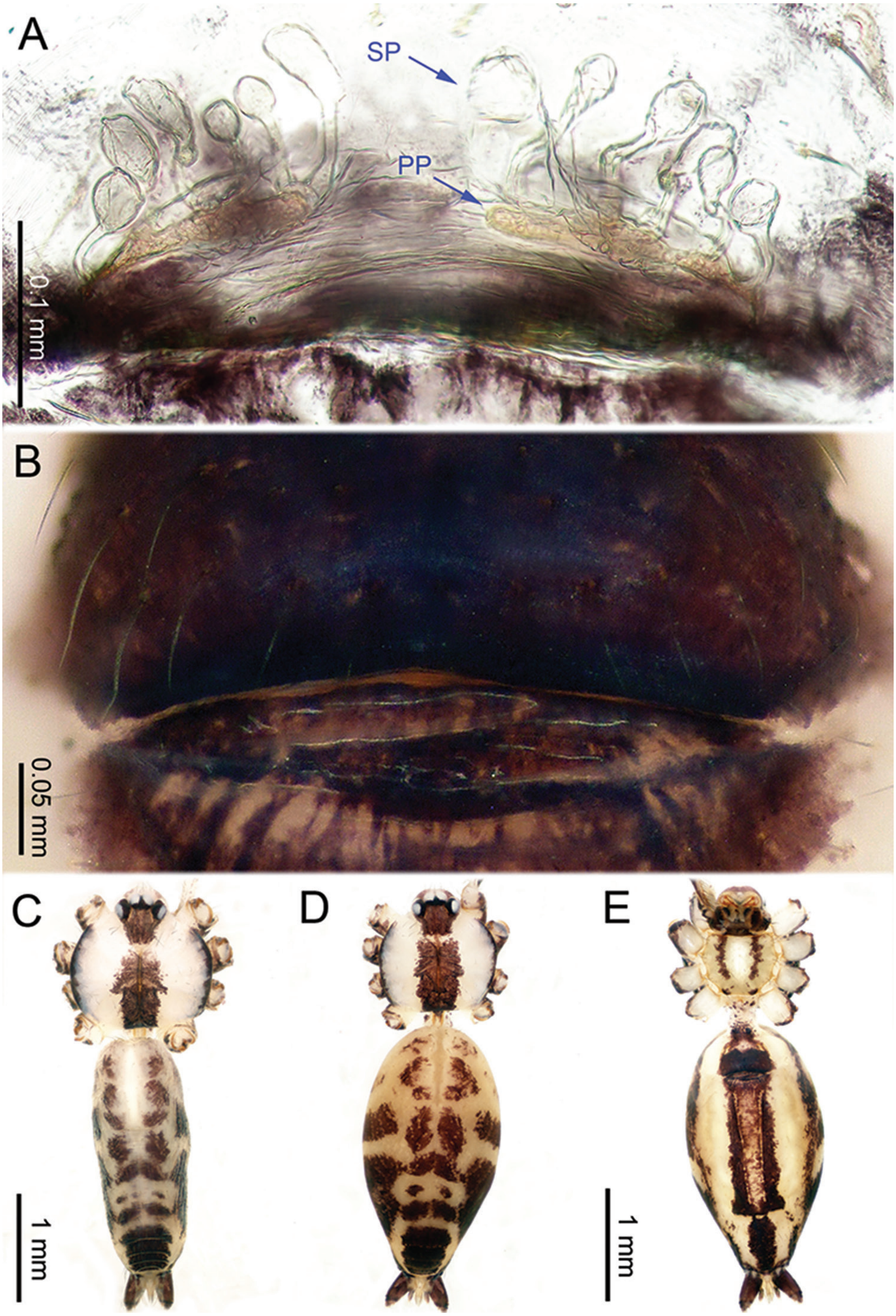

Figure 15. *Althepus sepakuensis* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviations: SP spermatheca; PP pore plate.

Diagnosis. Males of *A. sepakuensis* Li & Li, sp. n. can be easily distinguished from all other known species of the genus by the widened, laminar embolus with a distal acuminate end (Figure 14B); females, by the six round spermathecae on slender stalks on each side (Figure 15A).

Description. Male (holotype). Total length 4.23; carapace 1.33 length, 1.34 width; abdomen 2.25 length, 1.23 width. Carapace round, light yellow, with narrow, brown lateral margins and one wide, brown median band, the middle one wider than the others (Figure 15C). Anterior margin of cephalic region distinctly elevated. Clypeus brown. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20H), posterior surface of fang with 19 small denticles. Labium brown. Sternum yellow, with two longitudinal brown bands. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 15C). Legs brown, femur and tibia with white annulations. Leg measurements: I 37.7 (9.23, 0.53, 9.29, 15.77, 2.88), II 24.37 (6.35, 0.53, 6.03, 9.62, 1.84), III missing, IV 22.26 (6.41, 0.52, 5.90, 7.88, 1.55). Male palp (Figure 14A–D): tarsus with one hooked spine with tip directed distally (Figure 14A); bristles at the top of cymbial protrusion (Figure 14A) as *A. hongguangi* Li & Li, sp. n.; bulb yellow, ovate; embolus arising distally from bulb, wide, curved, distal part abruptly acute to acuminate; conductor arising proximally from bulb, wide, distal part incurved; embolus and conductor widely separated (distance less than diameter of bulb).

Female (paratype). Total length 4.00; carapace 1.05 length, 1.09 width; abdomen 2.34 length, 1.32 width. Similar to male in colour and general features (Figure 15D, E) but smaller. Six rounded spermathecae on slender stalks, adjoined to a large pore-plate on each side (Figure 15E). Leg measurements: I 28.24 (6.54, 0.43, 6.72, 11.67, 2.88), II 17.45 (4.49, 0.43, 4.30, 6.60, 1.63), III 11.58 (3.36, 0.40, 2.81, 3.88, 1.13), IV 16.31 (4.55, 0.43, 4.25, 5.64, 1.44).

Distribution. Indonesia. East Kalimantan, Penajam (Figure 22).

Natural history. Collected in a lowland tropical forest.

Remark. *Althepus sepakuensis* Li & Li, sp. n., was labelled as “sp. 131” in the analysis of Li and Li (2018).

Althepus xuae Li & Li, sp. n.

http://zoobank.org/E0535C6D-7C71-4AFB-9197-7A9851B4B145
Figs 16–17, 20–21

Types. Holotype: ♂, China, Yunnan Province, Nujiang of the Lisu Autonomous Prefecture, Lushui County, Nouth of Pianma Town, 26°01.513'N, 98°37.313'E, 2125 m a.s.l., 27.VI.2016, M. Xu and Y. Li. **Paratypes:** 1♂2♀, same data as holotype.

Other material examined. 1♂, China, Yunnan Province, Nujiang of the Lisu Autonomous Prefecture, Lushui County, Pianma Town, Fengxue Yakou 25°59.628'N, 98° 39.697'E, 2337 m a.s.l., 29.VI.2016, M. Xu and Y. Li.

Etymology. The specific epithet is a patronym in honour of Mingjie Xu who collected the types; noun (name) in genitive case.

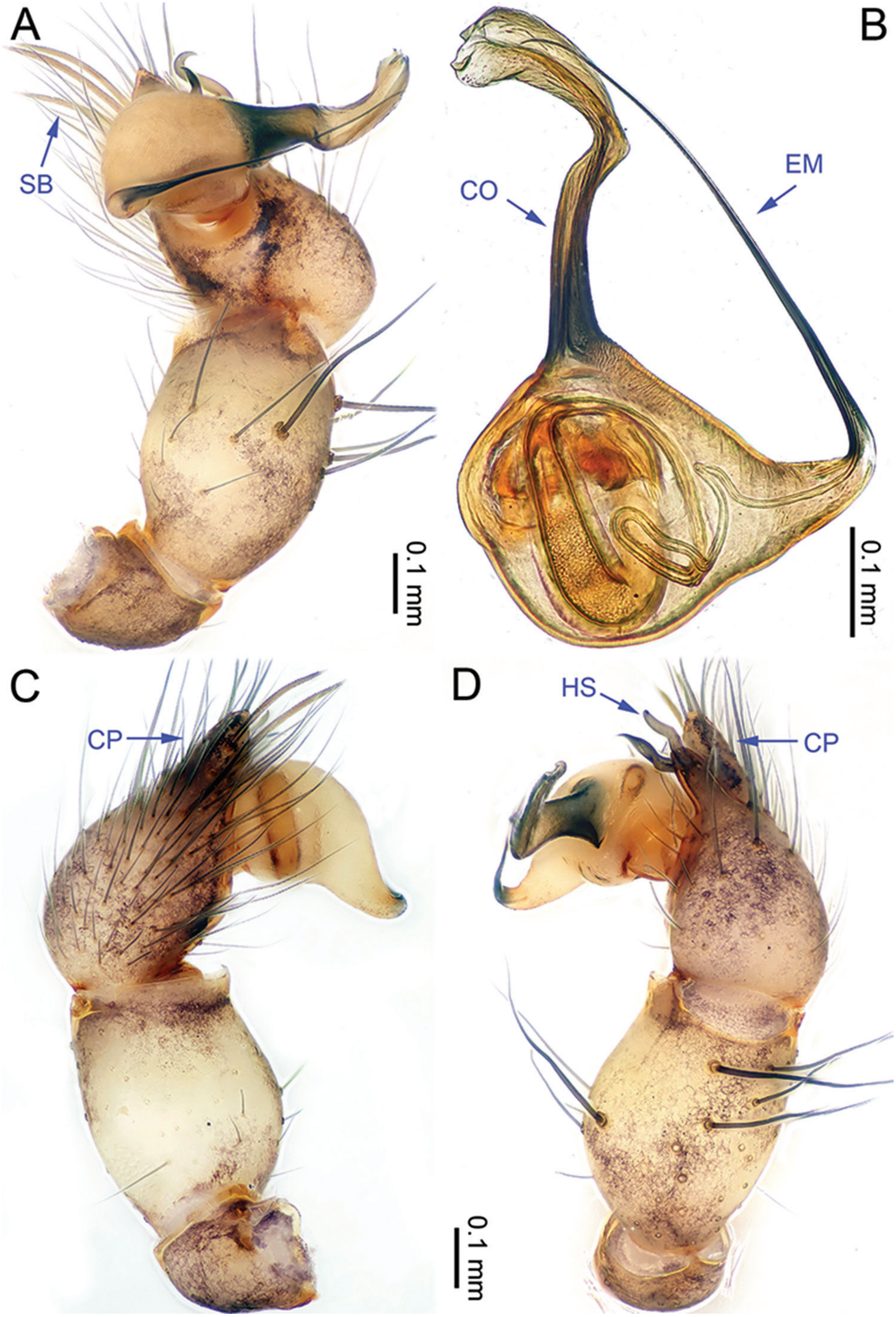

Figure 16. *Althepus xuae* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, prolateral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

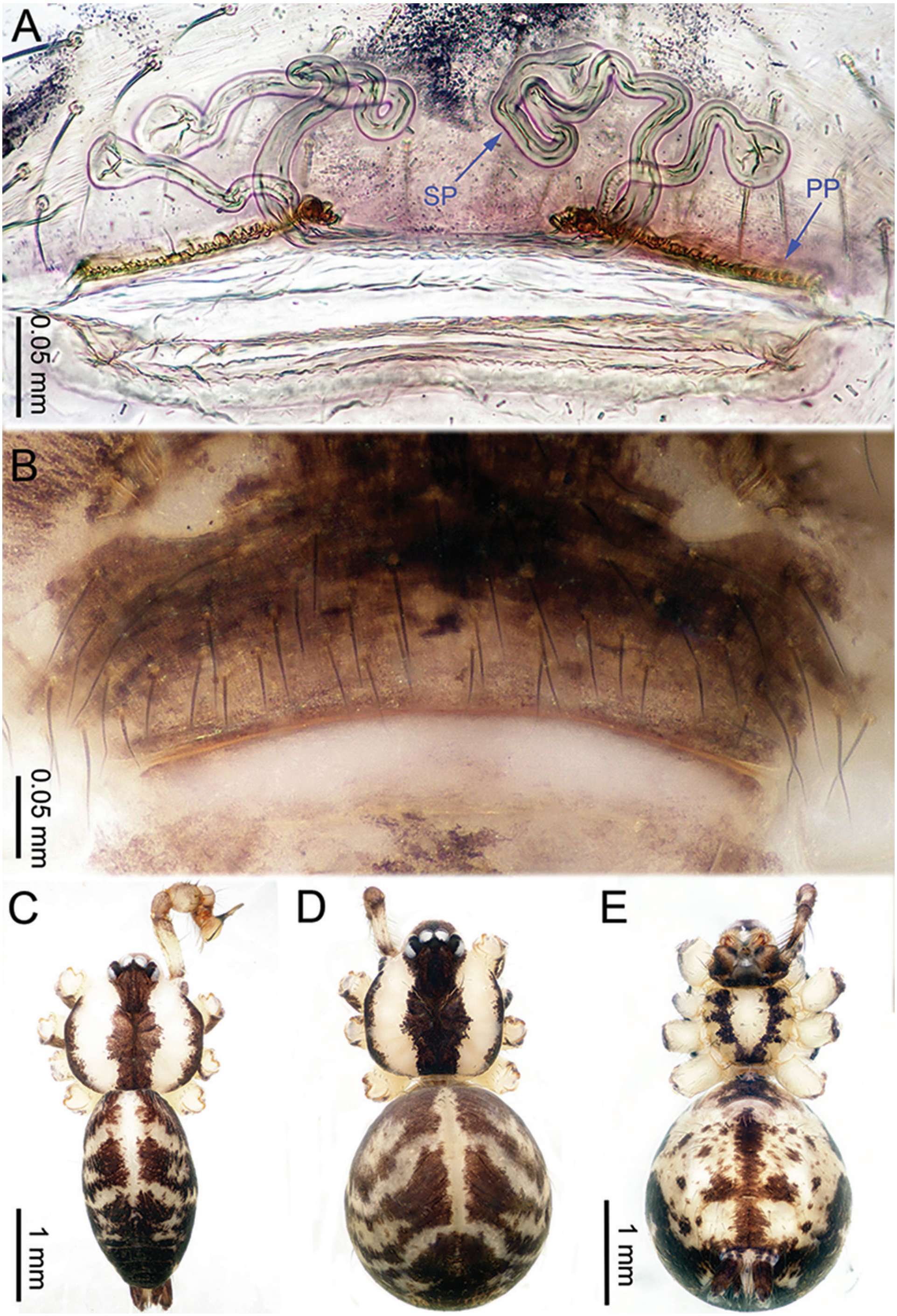

Figure 17. *Althepus xuae* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviations: SP spermatheca; PP pore plate.

Diagnosis. *Althepus xuae* Li & Li, sp. n. resembles *A. chengmenensis* Li & Li, sp. n. in having a sigmoid conductor in the males, and curved, elongate spermathecae in the females. Males can be distinguished from the latter species by the smooth margin and blunt distal part of the conductor (Figure 16B); females can be distinguished by the two shorter spermathecae on each side (versus longer in *A. chengmenensis* Li & Li, sp. n.) (Figs 2A, 17A), can be distinguished from all the other known species of the genus by the lateral spermathecae having a longer stalk than medial spermathecae (Figure 17A).

Description. Male (holotype). Total length 3.64; carapace 1.34 length, 1.40 width; abdomen 2.05 length, 1.24 width. Carapace round, yellow, with brown lateral margins and one wide, brown median band, the middle one wider than others. Anterior margin of cephalic region distinctly elevated (Figure 17C). Clypeus brown. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20I), posterior surface of fang with 27 small denticles. Labium brown. Sternum yellow, with two longitudinal brown bands. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 17C). Legs brown, femur and tibia with white annulations. Leg measurements: I missing, II 20.05 (5.32, 0.60, 5.13, 7.05, 1.75), III 12.82 (3.52, 0.59, 3.40, 4.00, 1.31), IV missing. Male palp (Figure 16A–D): tarsus with one retrolateral spine and one hooked spine with tip directed distally (Figure 16D); bristles at the top of cymbial protrusion (Figure 16A) as in *A. hongguangi* Li & Li, sp. n.; bulb yellow, ovate; embolus arising prolatero-proximally from bulb, slightly sigmoid; conductor arising retrolatero-distally from bulb, sigmoid; embolus and conductor widely separated (distance less than diameter of bulb).

Female (one of the paratypes). Total length 3.40; carapace 1.30 length, 1.20 width; abdomen 2.48 length, 1.85 width. Similar to male in colour and general features (Figure 17D–E), but smaller. Internal genitalia with two round spermathecae on long, slender stalks on each side and pores plate at the base (Figure 17A). Leg measurements: I missing, II missing, III 9.39 (2.64, 0.46, 2.40, 2.80, 1.09), IV 13.14 (3.80, 0.50, 3.52, 3.92, 1.40).

Variation. Males: carapace 1.33–1.34 length, 1.40–1.44 width, leg I lost (the number of specimens = 2). Females: carapace 1.03–1.30 length, 1.20–1.25 width, leg I lost (the number of specimens = 2).

Distribution. China. Yunnan Province (Figure 21).

Natural history. Collected by sieving leaf litter in dark and moist environments.

Althepus yizhuang Li & Li, sp. n.

http://zoobank.org/5647E1C8-8360-4333-A9A2-59806CACC6D0

Figs 18–20, 22

Types. Holotype: ♂, Indonesia, Sumatra, West Sumatra Province, Sijunjung, Padang Sibusuk Village, Bukit Ponggang Cave, 00°44.245'S, 100°50.330'E, 278 m a.s.l., 27.V.2014, Z. Yao. **Paratypes:** 1♂2♀, same data as holotype.

Etymology. The specific name is derived from the Chinese pinyin ‘yi zhuang’, which means ‘sigmoid’, referring to the sigmoid embolus (Figure 18); adjective.

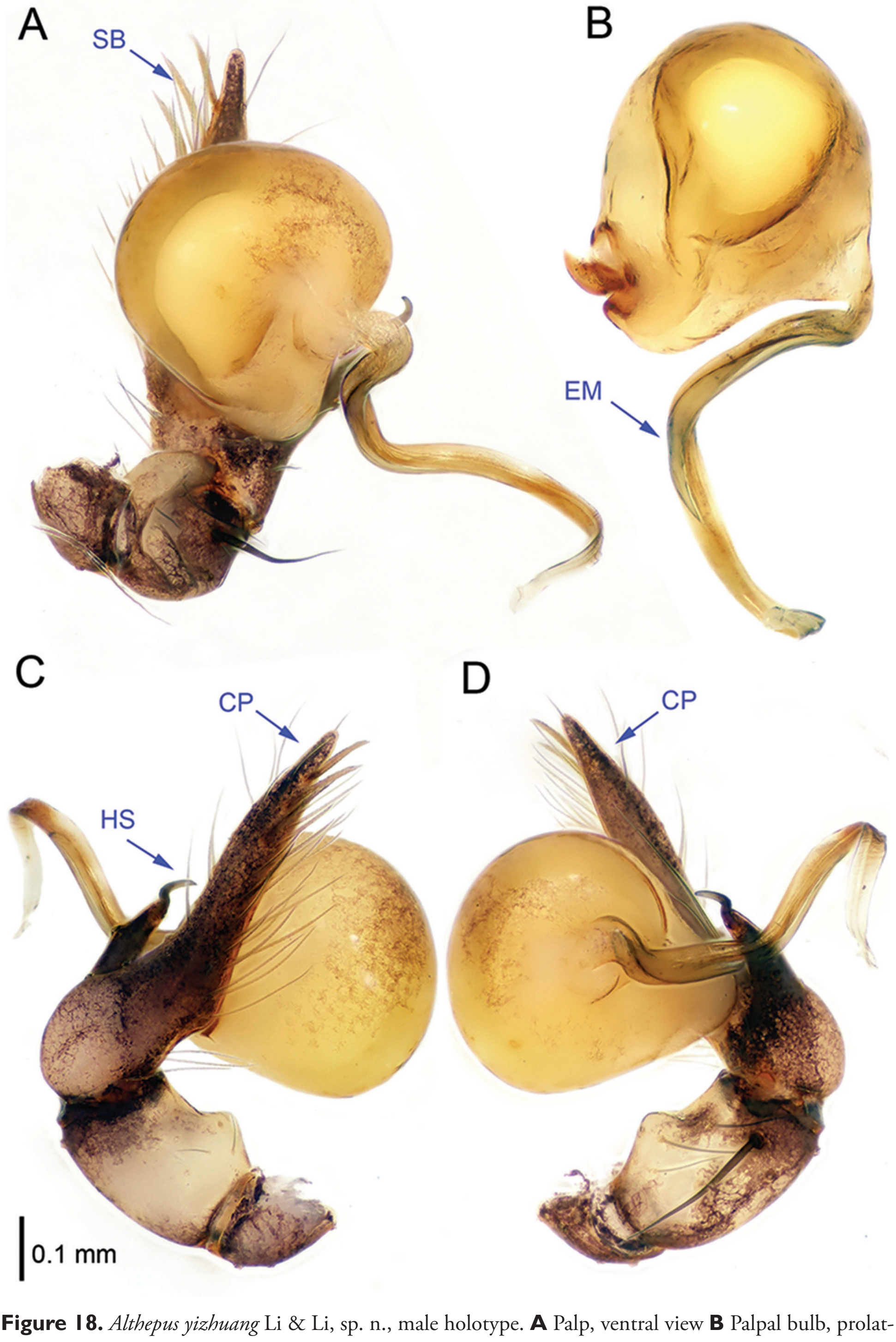

Figure 18. *Althepus yizhuang* Li & Li, sp. n., male holotype. **A** Palp, ventral view **B** Palpal bulb, prolateral view **C** Palp, prolateral view **D** Palp, retrolateral view. Abbreviations: CO conductor; EM embolus; CP cymbial protrusion; HS hook-shaped spine; SB serrated bristles.

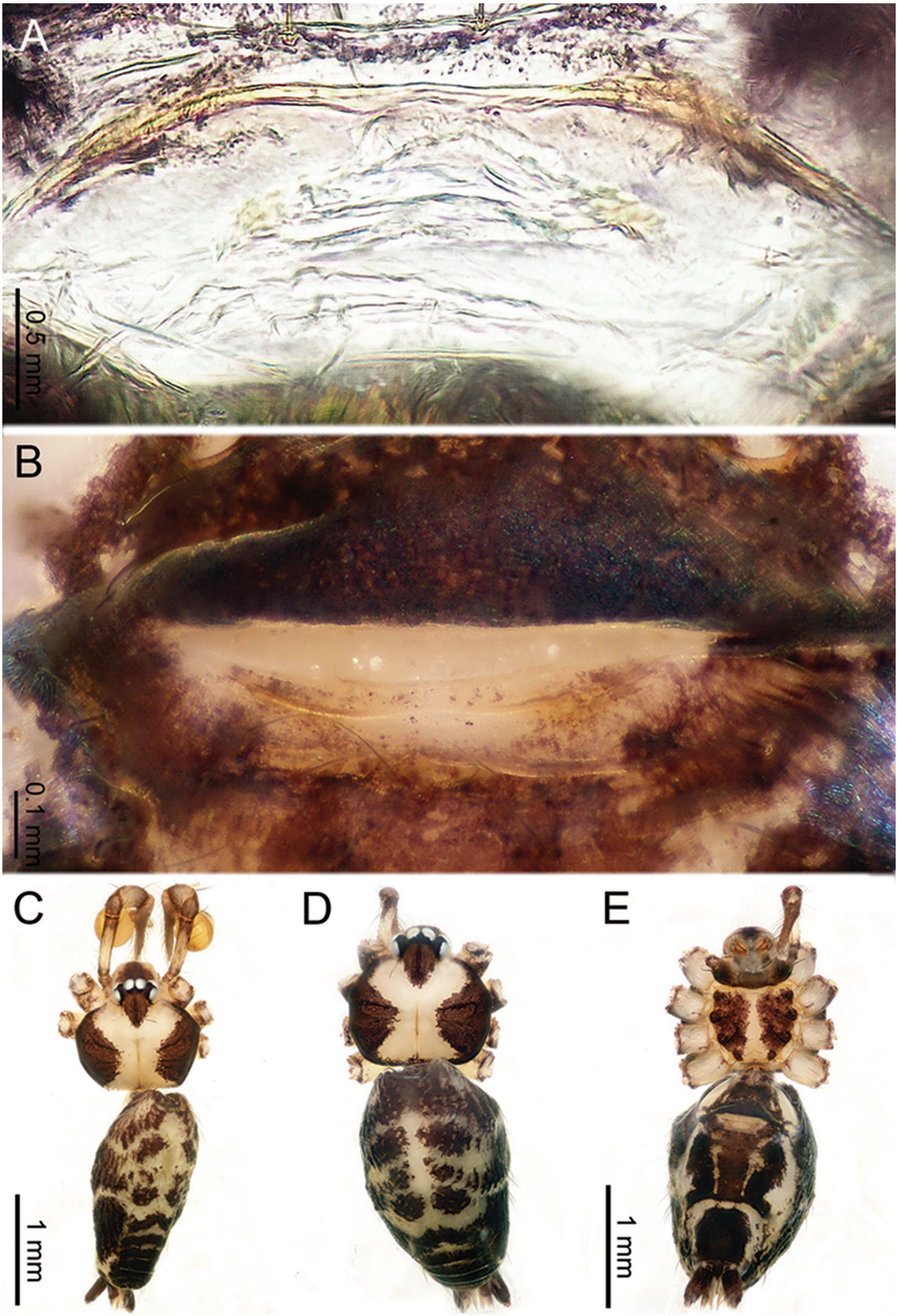

Figure 19. *Althepus yizhuang* Li & Li, sp. n., male holotype and female paratype. **A** Internal genitalia, dorsal view **B** Female epigastric furrow, ventral view **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Abbreviation: SP spermatheca.

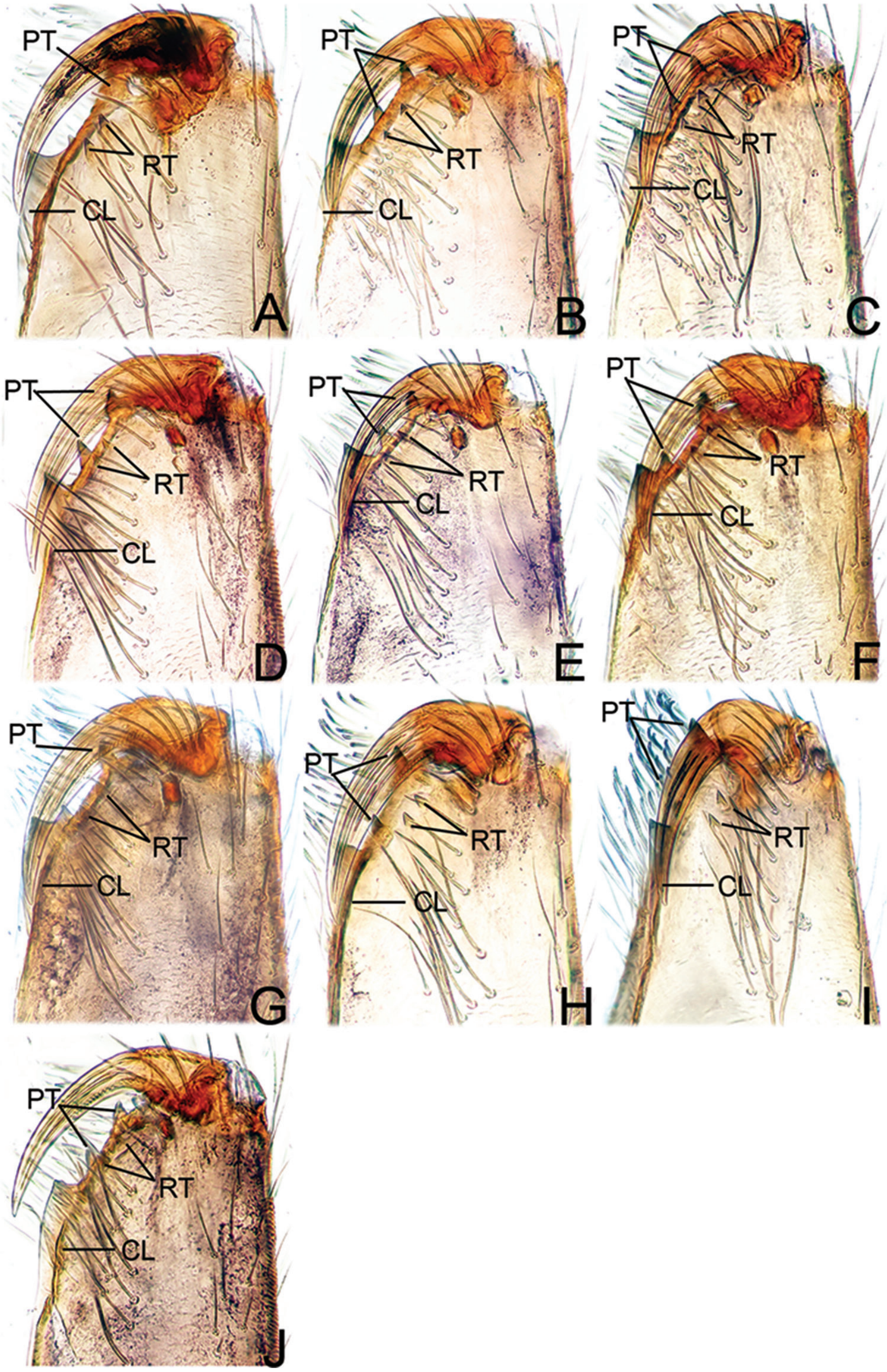

Figure 20. Cheliceral retromargin. **A** *Althepus chengmenensis* Li & Li, sp. n. **B** *A. cheni* Li & Li, sp. n. **C** *A. gouci* Li & Li, sp. n. **D** *A. hongguangi* Li & Li, sp. n., **E** *A. phousalao* Li & Li, sp. n. **F** *A. qianhuang* Li & Li, sp. n. **G** *A. qingyuani* Li & Li, sp. n. **H** *A. sepakuensis* Li & Li, sp. n. **I** *A. xuae* Li & Li, sp. n. **J** *A. yizhuang* Li & Li, sp. n. Abbreviations: PT promarginal teeth; RT retromarginal teeth; CL cheliceral lamina.

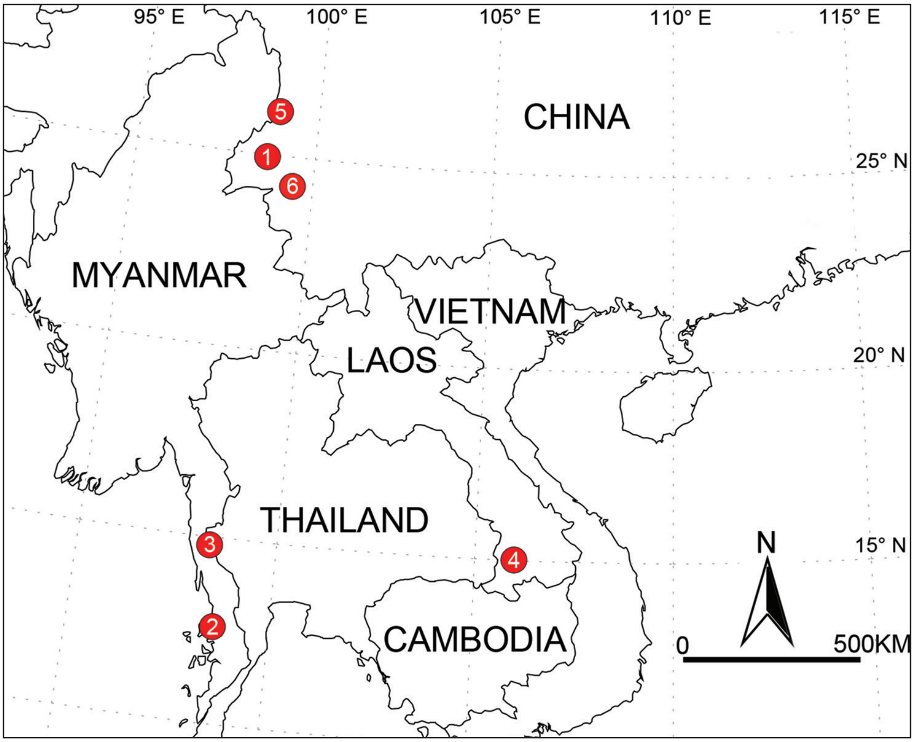

Figure 21. Known distribution of new *Althepus* species from Laos, Myanmar, and China. **1** *A. chengmenensis* Li & Li, sp. n. **2** *A. cheni* Li & Li, sp. n. **3** *A. gouci* Li & Li, sp. n. **4** *A. phousalao* Li & Li, sp. n. **5** *A. xuae* Li & Li, sp. n. **6** *A. qingyuani* Li & Li, sp. n..

Diagnosis. *Althepus yizhuang* Li & Li, sp. n. can be distinguished from all other known species of the genus by the remarkably long and sigmoid embolus as well as by the absence of a conductor in males (Figure 18). Females are distinguished by inconspicuous spermathecae (Figure 19A).

Description. Male (holotype). Total length 3.13; carapace 1.00 length, 1.14 width; abdomen 1.80 length, 0.94 width. Carapace round, yellow, with triangular brown margins and a narrow, brown median line behind ocular area (Figure 19C). Anterior margin of cephalic region distinctly elevated. Clypeus brown. Cheliceral promargin with two teeth, followed by a lamina, retromargin with two small teeth (Figure 20J), posterior surface of fang with 16 small denticles. Labium brown. Sternum brown, with a triangular yellow patch in the middle. Abdomen elongate, with complex patterns dorsally and ventrally (Figure 19C). Legs brown. Leg measurements: I 24.39 (5.83, 0.43, 6.22, 9.68, 2.23), II 14.81 (3.85, 0.41, 3.85, 5.45, 1.25), III 9.85 (2.81, 0.40, 2.50, 3.20, 0.94), IV 14.47 (4.17, 0.41, 3.91, 4.81, 1.17). Male palp (Figure 18A–D): tarsus with one hooked spine with tip directed proximally; cymbium slender (Figure 18C); bristles at the top of the cymbial protrusion (Figure 18A) as in *A. hongguangi* Li & Li, sp. n.; bulb bright yellow, ovate; embolus arising retrolatero-distally from bulb, bright yellow.

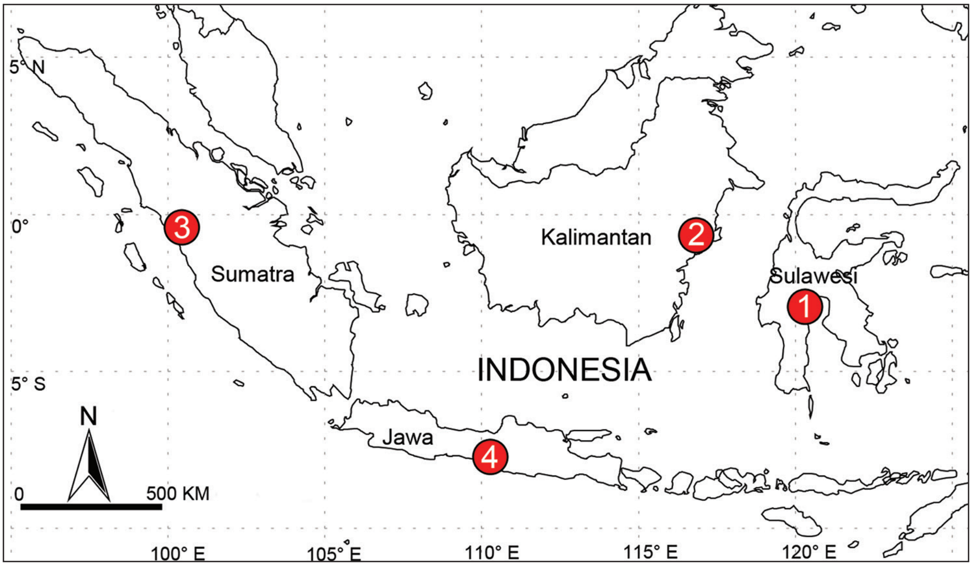

Figure 22. Known distribution of new *Althepus* species from Indonesia. **1** *A. hongguangi* Li & Li, sp. n. **2** *A. sepakuensis* Li & Li, sp. n. **3** *A. yizhuang* Li & Li, sp. n. **4** *A. qianhuang* Li & Li, sp. n..

Female (one of the paratypes). Total length 3.13; carapace 0.85 length, 0.95 width; abdomen 1.90 length, 1.17 width. Similar to male in colour, general features and body size (Figure 19D–E). Internal genitalia with inconspicuous spermathecae (Figure 19A). Leg measurements: I missing, II 11.57 (3.00, 0.35, 2.97, 4.00, 1.25), III 8.21 (2.34, 0.36, 2.10, 2.53, 0.88), IV 11.52 (3.20, 0.38, 3.20, 3.60, 1.14).

Variation. Males: carapace 1.00 length, 1.14–1.25 width; femur I 5.71–5.83 (holotype and paratypes with similar length).

Distribution. Indonesia. Known only from the type locality (Figure 22).

Natural history. Collected at a cave entrance at an altitude of 278 m.

Remark. *Althepus yizhuang* Li & Li, sp. n., was labelled as "sp. 84" in the analysis of Li and Li (2018).

Discussion

In addition to morphological studies, we used molecular data from our extensive sampling to test the monophyly of the genus *Althepus* and delimitate the species (Li and Li 2018). The molecular topologies inferred by two different approaches all supported *Althepus* as a monophyletic group. The species delimitation inferred by three different approaches supported the evolutionary independence of 54 distinct lineages. For details, see *Althepus* sp. 23, *Althepus* sp. 84, *Althepus* sp. 97, *Althepus* sp. 119, and *Althepus* sp. 131 in figure 1 and supplementary figures S1–S4 of Li and Li (2018).

In this paper, we describe seven new species in lowland habitats of southern Indo-Burma, Sunda shelf islands, and three new species in highlands of northern Indo-Burma. The genus appears to have a higher diversity in lowlands compared to highlands. Recent studies indicate that this may be due to the repeated isolation and reconnection of Southeast Asian landmasses caused by sea-level fluctuations (Liu et al. 2017, Li and Li 2018).

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RESEARCH ARTICLE

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Ultrastructure of spermatozoa in three cicada species from China (Hemiptera, Cicadomorpha, Cicadidae)

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Abstract

The ultrastructure of mature spermatozoa of three cicada species, *Subpsaltria yangi*, *Karenia caelatata,* and *Platypleura kaempferi*, was investigated using epifluorescence and transmission electron microscopies. This is the first investigation of the sperm ultrastructure of species in the subfamily Tibicininae and the tribe Sinosenini, represented by *S. yangi* and *K. caelatata*, respectively. The three species all produce two or three types of spermatozoa with various lengths, viz., polymegaly. The centriolar adjunct of spermatozoa in *S. yangi* shows a granular substructure, which is different from that of other cicada species, suggesting that spermatozoa in Tibicininae may have their own characteristics in comparison with other cicadas. The centriolar adjunct of spermatozoa of *K. caelatata* displays characteristics similar to that of the Cicadinae. Combined with other morphological characters, it is reasonable to remove *K. caelatata* and its allies (i.e., Sinosenini) from Cicadettinae to Cicadinae. The study of sperm ultrastructure, particularly in the species of Tibicininae and Sinosenini, expands the spermatological research of Cicadidae and provides more information for phylogenetic analysis of Cicadidae.

Keywords

Cicadoidea, Cicadomorpha, Hemiptera, Insecta, morphology, sperm

Introduction

As germ cells, sperm are evolving at the fastest speed and are among the most diverse cell types with the highest degree of variation in insect growth (Baccetti and Afzelius 1976, Jamieson 1987, Joly et al. 1989). Spermatological characteristics have been used for distinguishing taxa and for clarifying phylogenetic relationships of related taxa (Jamieson 1987, 1991, Jamieson et al. 1999, Lino-Neto and Dolder 2001, Zhang and Hua 2017). The sperm structure of insects, similar to those of other metazoans, is broadly the same, but each group has unique characteristics. Most of these generally tiny, motile spermatozoa are slender with a single flagellum, but some have more than one flagellum (Chawanji et al. 2005, 2006). The flagellum is the power source for sperm mobility (Ciolfi et al. 2016).

There are two aspects of insect sperm, the length and structure, together revealing any sperm polymorphism. The sperm length in Diptera extends across a great range (Joly et al. 1991, Joly et al. 1995). It has been reported that two types of nuclei of distinctly discrete lengths are produced in some species of Drosophilidae and Diopsidae (Diptera) (Snook et al. 1994, Pasini et al. 1996, Presgraves et al. 1999). Another aspect is nucleation. Males in butterflies and moths (Lepidoptera) can produce nucleated eupyrene sperm and non-nucleated apyrene sperm; the former has the ability to fertilize eggs, while the exact function of the latter is still uncertain (Katsuno 1977, Silberglied et al. 1984, Osanai et al. 1989, Friedländer 1997, Yamashiki and Kawamura 1997, Kubo-Irie et al. 1998, Watanabe and Bon'no 2001, Friedländer et al. 2005, Hayakawa 2007). There exists an extreme case in *Dahlbominus fuscipennis* (Hymenoptera, Eulophidae) which has at least five different types of spermatozoa with diverse appearances (Lee and Wilkes 1965). Although the function of polymorphic sperm in insects remains uncertain, some authors suggest that it may be related to sperm competition (Snook 1998, Swallow and Wilkinson 2002).

The family Cicadidae of the order Hemiptera includes approximately 3,000 extant species worldwide, and about 210 extant species distributed in China (Sanborn 2013, Chou et al. 1997). This family includes four subfamilies, i.e., Cicadinae, Cicadettinae, Tibicininae and Tettigomyiinae (Marshall et al. 2018). Up to the present, studies on the sperm ultrastructure of Cicadidae have addressed 13 species (Snook et al. 1994, Pasini et al. 1996, Presgraves et al. 1999, Kubo-Irie et al. 2003, Chawanji et al. 2005, 2006, Chawanji et al. 2007). It was found that *Graptopsaltria nigrofuscata* (Cicadinae) can produce two types of spermatozoa, but only the longer spermatozoa have fertility (Kubo-Irie et al. 2003). Chawanji et al. (2005) revealed polymegaly in spermatozoa of all four investigated species of African platypleurine cicadas of the subfamily Cicadinae. The sperm ultrastructure of five cicadas, currently belonging to Cicadettinae and Tettigomyiinae, respectively, has also been studied (Chawanji et al. 2006). Such scant information is applicable to phylogenetic study of Cicadidae from the point of view of spermatology. However, studies on sperm structure in Cicadidae are still insufficient, particularly for some taxa whose systematic status remains controversial, and for the subfamily Tibicininae, of which the sperm structure has never been investigated in any species.

Herein, the sperm ultrastructure of three cicada species were observed using both epifluorescence and transmission electron microscopies (TEM). The sperm ultrastructure of *Subpsaltria yangi* is the first detailed description of spermatozoa investigated in the subfamily Tibicininae. We also address the systematic placement of the genus *Karenia* based on a comparison of the sperm ultrastructure of this species and other species. In addition, coupled with previous studies, we discuss the similarities and differences in sperm ultrastructure among different subfamilies of Cicadidae, aiming to provide useful clues for taxonomic and phylogenetic studies of the Cicadoidea.

Materials and methods

Male adult cicadas were collected using a net. Their identities and detailed collecting information are shown in Table 1. The higher classification of Cicadidae follows that of Marshall et al. (2018).

Sample preparation and epifluorescence microscopy observation

Samples (at least five individuals of each species) were anesthetized with alcohol at a concentration of 75%, and dissected with a fine scalpel blade under a binocular microscope (Olympus SZX16, Olympus Corporation, Tokyo, Japan) to obtain the seminal vesicles from which spermatozoa were recovered. For measuring the sperm total length, sperm samples were placed in 1% bisbenzimidazole Hoechst 33258, a cell-permeable adenine–cytosine binding epifluorescent dye used to stain DNA (Sakaluk and O'Day 1984), for 1 min, then rinsed in three changes of 0.1 M phosphate buffered saline (PBS, pH 7.2). Spermatozoa were evenly spread on a microscope slide and covered with a coverslip. Slides were examined with an Olympus BX-51 epifluorescence microscope (Olympus Corporation, Tokyo, Japan) at a wavelength of 343 nm. Digital images of 50–100 spermatozoa were randomly captured from each species under the same microscope with an Olympus DP72 camera (Olympus Corporation, Tokyo, Japan). Sperm lengths were measured using the Olympus DP2-BSW software version 2.1.

Table 1. Taxonomic status and collecting information of three investigated species.

Species	Subfamily	Collecting sites	Collecting dates
Subpsaltria yangi Chen	Tibicininae	Helan Mountains, Ningxia, China	11–16 June 2016
Karenia caelatata Distant	Cicadinae	Ankang, Shaanxi, China	9–15 August 2016
Platypleura kaempferi (Fabricius)	Cicadinae	Yangling, Shaanxi, China	23 June–12 July 2016

Sample preparation and transmission electron microscopy observation

Sperm samples were fixed in 2.5% glutaraldehyde (0.1 M PBS, pH 7.2) for 12 h at 4 °C, and the materials were rinsed with 0.1 M phosphate buffered saline (PBS, pH 7.2), then fixed in 1% osmium tetroxide for 2 h at room temperature. Alcohol dehydration with a concentration gradient was performed after rinsing with the same PBS. Treated samples were then embedded in Epon 812 resin. A diamond knife was used to obtain ultrathin sections which were collected on 300 mesh copper grids before staining with uranyl acetate and lead citrate. Sections were examined and photographed with a HT7700 transmission electron microscope (HITACHI, Tokyo, Japan) at 80 kV.

Data analysis

The measured data were recorded using Microsoft Excel version 2010. Then an analysis of variance was conducted to verify the mathematically significant differences in sperm length between different sperm types within species using SPASS version 19. Correlation analysis between nucleus length and tail length was performed using R version 3.3.2. Measurements are reported as mean ± standard error.

Results

Epifluorescence Morphology

Mature spermatozoa of the three species are all linear with needle-like heads and long tails that tapered posteriorly (Fig. 1A–C). The heads of the spermatozoa are aggregated into bundles, with the thread-like tails scattered freely (Fig. 1D).

Sperm morphologies of the three species are similar, but the spermatozoa vary in length. Based on their remarkably different total length, spermatozoa within a species are divided into disparate types (Table 2). In *K. caelatata*, the spermatozoa can be divided into three types: long spermatozoa, medium spermatozoa, and short spermatozoa. The spermatozoa of the other two species, *P. kaempferi* and *S. yangi*, can be classified into two types: long spermatozoa and short spermatozoa. Within each species, the total lengths of different types of spermatozoa are significantly different ($P < 0.01$; one-way ANOVA). Among the long spermatozoa of these three species, the spermatozoa of *K. caelatata* is the longest (178.45 ± 10.82 μm); among the short spermatozoa, the spermatozoa of *S. yangi* is the shortest (64.36 ± 5.13 μm) (Table 2).

Additionally, the differences in total length of spermatozoa and the sizes of sperm nuclei and tails both within and between species are also significantly different. In *S. yangi*, the lengths of nuclei fall into two classes, and the lengths of tails present

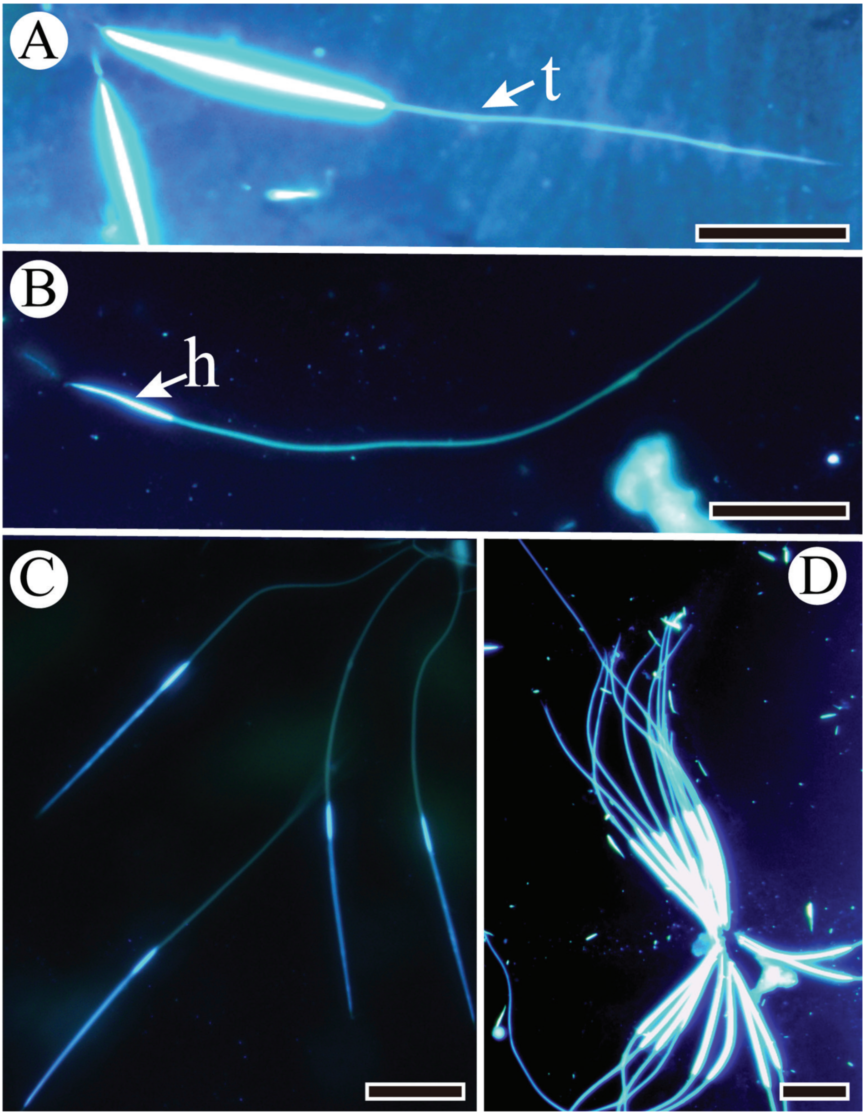

Figure 1. Epifluorescent microscope images of spermatozoa stained with Hoechst 33258. **A** Spermatozoon of *Subpsaltria yangi* with a head and a tail (t) **B** Spermatozoon of *Platypleura kaempferi* with a short head (h) and an elongated tail **C** Slender spermatozoa of *Karenia caelatata* with a head and a tail **D** Spermatozoa of *S. yangi* aggregated into bundles. Scale bars: 20 μm.

Table 2. Total sperm length (μm) (mean ± SE) of three cicada species.

Species	Length range	Length of long spermatozoa	Length of medium spermatozoa	Length of short spermatozoa	N
Subpsaltria yangi	55.82–110.58	105.90 ± 2.96	–	64.36 ± 5.13	74
Karenia caelatata	83.25–195.34	178.45 ± 10.82	117.13 ± 4.43	88.83 ± 2.15	99
Platypleura kaempferi	68.91–125.21	111.51 ± 9.46	–	89.35 ± 5.76	49

Table 3. Modal classes and correlation coefficients (r) of nucleus length (mean ± SE μm) vs tail length (mean ± SE μm) in the spermatozoa of three cicada species.

Species	Length of short nucleus	Length of long nucleus	Length of short tail	Length of median tail	Length of long tail	r	N
Subpsaltria yangi	16.79 ± 5.40	42.09 ± 4.03	31.57 ± 3.48	61.31 ± 8.56	102.67± 17.00	-0.24	74
Karenia caelatata	19.49 ± 4.75	47.19 ± 3.28	64.00 ± 5.30	–	122.88 ± 11.60	-0.53	99
Platypleura kaempferi	18.46 ± 2.80	32.85 ± 3.10	37.67 ± 4.83	56.75 ± 5.80	100.31 ± 8.28	-0.40	49

three classes. There is a weak correlation between the nucleus and tail lengths in *S. yangi* (Table 3). In *K. caelatata*, the lengths of nuclei fall into two classes; the lengths of tails fall into two clear classes. There is a moderately correlation between the nucleus and tail lengths in *K. caelatata* (Table 3). Sperm nuclei of *P. kaempferi* can be classified into two classes according to their length; the tail lengths fall into three classes. The nucleus length is moderately correlated to the tail length in this species (Table 3).

Ultrastructure

Subpsaltria yangi Chen, 1943

The head region that is embedded into a homogenous matrix consists of an acrosome and a nucleus, and the anterior section of nucleus intrudes into an invagination of the acrosome as shown in longitudinal section (Fig. 2A). The acrosomal contents are differentiated internally with tubular substructures (Fig. 2B–F). The acrosome has two processes with an extension on both sides of the anterior section of the nucleus, and gradually widens in diameter in cross-sections (Fig. 2D and E). The nucleus appears to have a cylindrical profile (Fig. 2A). The nucleus accommodates to the processes of the acrosome, and is bilaterally concave (Fig. 2D and E), finally becoming circular shape in a cross-section (Fig. 2F). There is a shallow invagination in the post-lateral part of the nucleus, where the granular centriolar adjunct is located in longitudinal sections (Fig. 3A, G). The centriolar adjunct is limited by the invagination of the nucleus, and its shape changes in different cross sections (Fig. 3B–E). The diameter of nucleus decreases towards the base of nucleus (Fig. 3B–F).

A centriole runs from the flat base of the nucleus, connecting the nucleus and the axoneme (Fig. 3A). The flagellum is formed by a 9 + 9 + 2 axoneme (i.e., nine accessory microtubules, nine double microtubules, and two central microtubules) flanked by a pair of equal mitochondrial derivatives with crystalline regions (Fig. 4B, C). The derivatives are composed of cristae arranged in an orderly array in longitudinal section (Fig. 4A). At the end of the tail, axonemal microtubules appear less well organized and disappeared gradually (Fig. 4D, E).

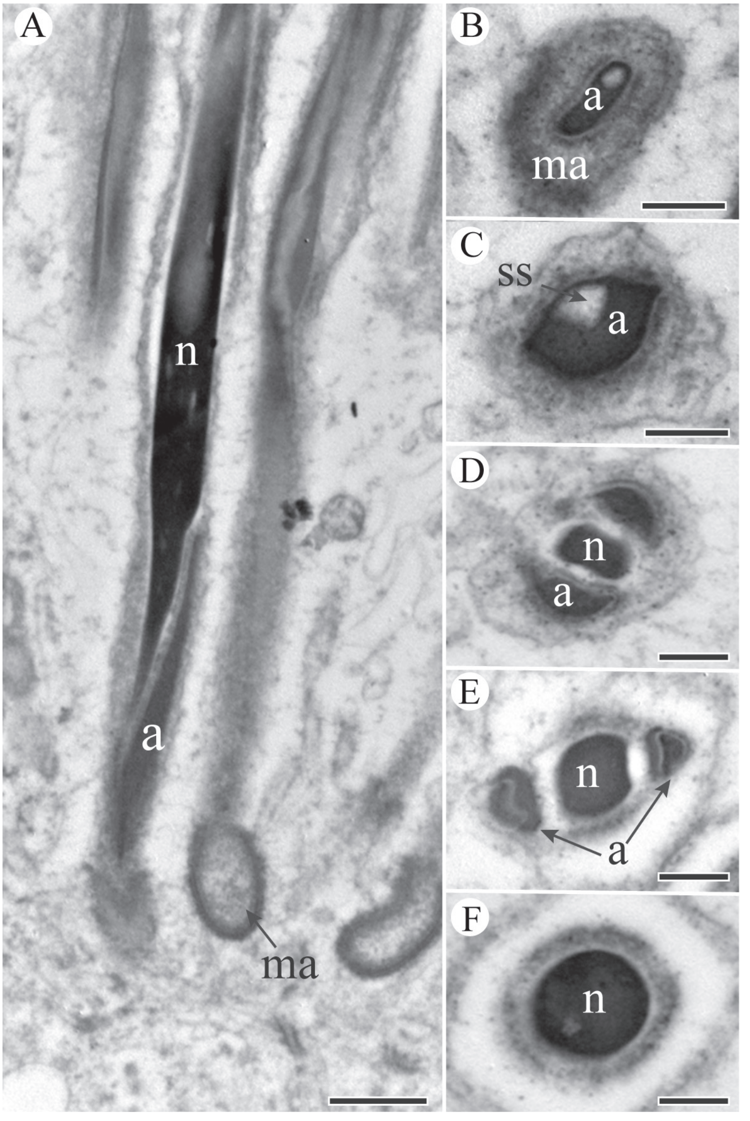

Figure 2. TEM micrographs of *S. yangi* sperm head region. **A** Longitudinal section of sperm head, showing the head region (including acrosome (a) and nucleus (n)) inserted into a homogenous matrix (ma) **B** Cross-section through the tip of acrosome (a), showing acrosome is surrounded by a homogenous matrix (ma) **C** Cross-section through the mid-acrosome (a), showing acrosome (a) and subacrosomal space (ss) **D** and **E** Cross-sections of base of acrosome (a), showing nucleus (n) and two acrosomal processes **F** Cross-section through circular nucleus (n). Scale bars: 500 nm (**A**), 200 nm (**B–F**).

Karenia caelatata Distant, 1890

Spermatozoa are all gathered together, with their conical acrosomes and part of the electron-dense nuclei inserted into a homogenous matrix forming a spermatodesm

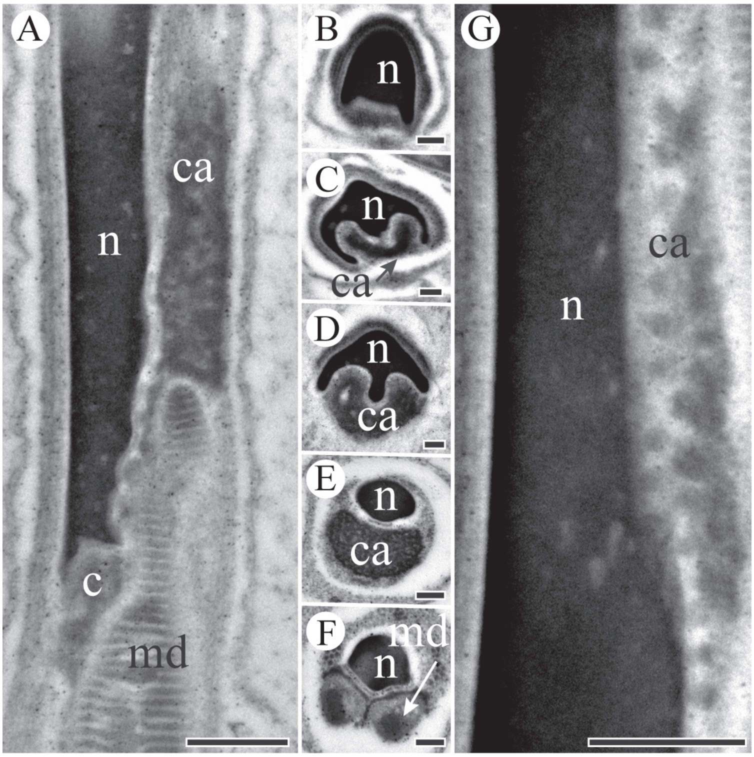

Figure 3. TEM micrographs of *S. yangi* sperm neck region. **A** Longitudinal section of the neck region, showing nucleus (n), centriole (c), granular centriolar adjunct (ca) and mitochondrial derivatives (md) **B** Cross-section anterior of the neck region, showing nucleus (n) **C** and **D** Cross-sections of the mid-neck region, showing nucleus (n) and centriolar adjunct (ca) **E** Cross-section through the terminal end of neck region, showing an eliptical nucleus (n) and a granular centriolar adjunct (ca) **F** Cross-section through the terminal end of neck region, showing a nucleus (n) and two mitochondrial derivatives (md) **G** Magnified longitudinal section of neck region, showing granular centriolar adjunct (ca) next to nucleus (n). Scale bars: 500 nm (**A, F, G**), 200 nm (**B–E**).

(Fig. 5A). In cross-section, the acrosome is conical, and a sub-acrosomal invagination is eccentric in position anteriorly (Fig. 5B). The acrosome forms two processes posteriorly, which flank the anterior part of nucleus (Fig. 5C–F). The acrosomal contents are not homogenous in appearance, which are filled with numerous tubular substructures as shown in cross sections (Fig. 5B–F). The diameters of the two tubular acrosomal processes increase towards the base of the acrosome, and the nucleus becomes mushroom-shaped in cross sections (Fig. 5B–G). The electron-dense centriolar adjunct forms a sheath shape and runs parallel to the posterior part of nucleus (Fig. 6A). The posterior segment of the nucleus develops a lateral invagination; the centriolar adjunct is confined within the invagination in cross sections (Fig. 6B–E).

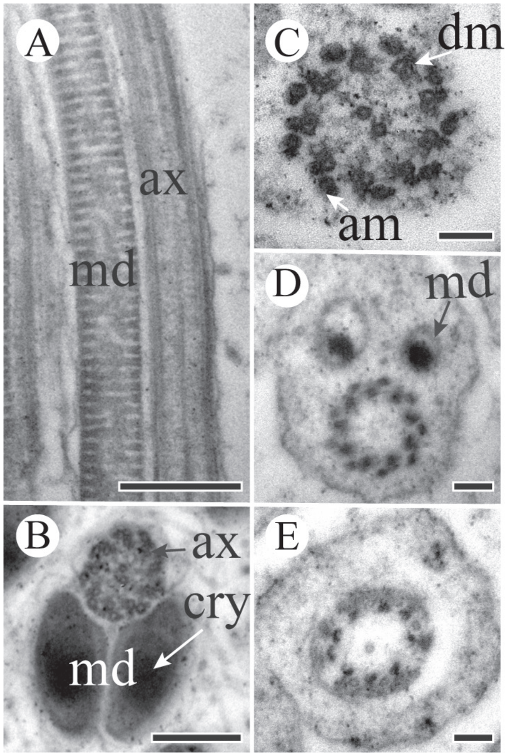

Figure 4. TEM micrographs of *S. yangi* sperm tail region. **A** Longitudinal section of sperm tail, showing axoneme (ax) and mitochondrial derivative (md) **B** Cross-section through the tail region, showing axoneme (ax) and mitochondrial derivatives (md) with crystalline region (cry) **C** Magnified cross-section of axoneme (ax), showing axoneme with a normal 9 + 9 + 2 arrangement of microtubules, i.e., 9 accessory microtubules (am), 9 double microtubules (dm) and 2 central microtubules **D** Cross-section of the terminal end of the sperm tail, showing paired mitochondrial derivatives (md) and axoneme (ax) with 9 accessory microtubules and 9 double microtubules left **E** Cross-section of the terminal end of the sperm tail, showing parts of microtubules of axoneme left. Scale bars: 500 nm (**A**), 200 nm (**B**), 100 nm (**C–E**).

The centriole is attached to the base of the nucleus (Fig. 6A).The flagellum is composed of an axoneme with a typical 9 + 9 + 2 microtubular pattern and a pair of mitochondria derivatives (Fig. 6F, G). The mitochondrial derivates are composed of numerous cristae, which can be seen from the longitudinal section of the tail (Fig. 6G). All of derivates with different diameters have a crystalline region in cross-section of tail (Fig. 6F).

Platypleura kaempferi (Fabricius, 1794)

Spermatozoa aggregate together with their heads inserted into a homogenous matrix to form a spermatodesm. The head region consists of an acrosome and a compact

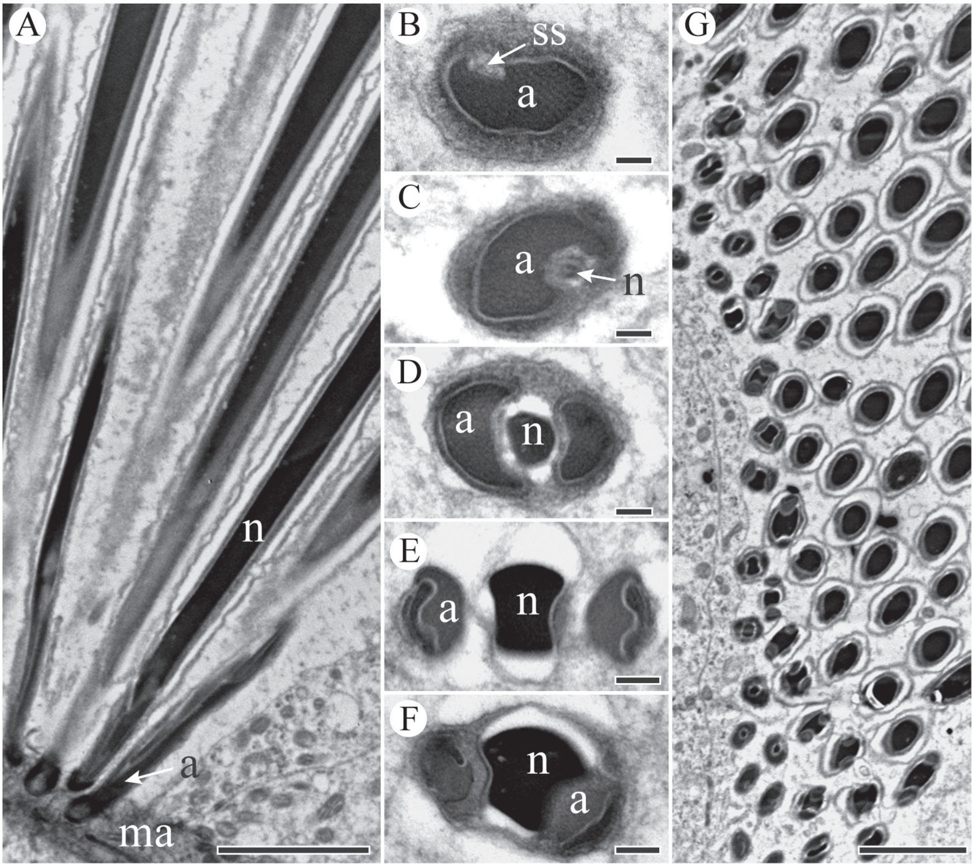

Figure 5. TEM micrographs of sperm head region of *K. caelatata.* **A** Longitudinal section of head region, showing the head region (including acrosome (a) and nucleus (n)) inserted into a homogenous matrix (ma) **B** Cross-section through the acrosome (a), showing the subacrosomal space (ss) located at an eccentric position of acrosome **C** Cross-section through the acrosome, showing the nucleus (n) located at an eccentric position of acrosome **D–F** Cross-sections through the posterior region of the acrosome (a), showing two acrosomal processes and the nucleus (n) **G** Lower magnification of cross-section through spermatodesmata, showing different transverse sections of spermatozoa. Scale bars: 2 µm (**A, G**), 100 nm (**B–F**).

and homogeneous nucleus (Figs 7A and 8A). The acrosomal contents have tubular substructures (Fig. 7B–G). The acrosome is laterally flattened, and an electron-lucent space (viz., subacrosomal space) lies in an eccentric position anteriorly (Fig. 7A). The acrosome gradually widens posteriorly, and forms two processes that flank the anterior part of the nucleus in cross-sections (Fig. 7C–G). The centriolar adjunct lies beneath the posterior of the nucleus and is parallel to it. The diameter and shape of the centriolar adjunct vary, adapting to the lateral invagination of the nucleus (Fig. 8E–G). With the extension of the nucleus, the diameter of nucleus gradually decreases (Fig.

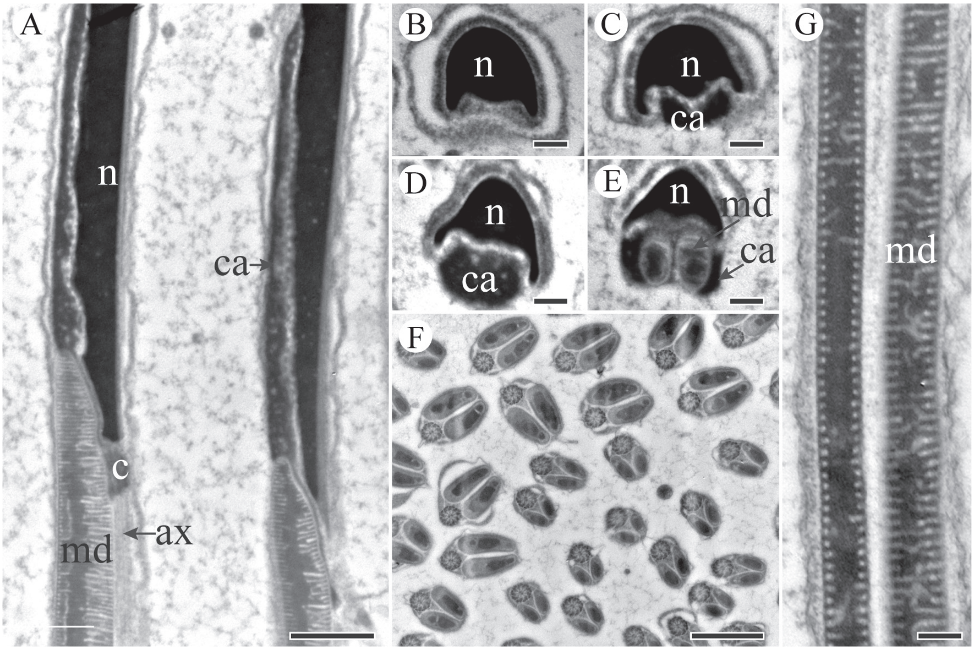

Figure 6. TEM sections through the neck and tail regions of the spermatozoa of *K. caelatata*. **A** Longitudinal section through the neck region showing nucleus (n), centriolar adjunct (ca), centriole (c), axoneme (ax) and mitochondrial derivatives (md) **B** Cross-section through the mid-neck region, showing one side of the nucleus forms two ridges. **C** and **D** Cross-sections through the posterior part of nucleus, showing centriolar adjunct (ca) flanked nucleus (n) **E** Cross-section of the base of the nucleus, showing triangular nucleus (n) and two mitochondrial derivatives (md) embedded into the material of the centriolar adjuncts (ca) **F** Cross-section through sperm tails, showing mitochondrial derivatives with distinct diameters **G** Longitudinal section of sperm tail, showing paired mitochondrial derivatives (md). Scale bars: 1 µm (**A, F**), 200 nm (**B–E, G**).

8C–H). The centriolar adjunct gradually vanishes where the mitochondrial derivatives emerge (Fig. 8H).

The centriole emerges from the base of the nucleus, connecting the nucleus and the axoneme (Fig. 8B). In the tail region, the paired mitochondrial derivatives are formed by cristae at the periphery, and a typical axonemal arrangement of 9 + 9 + 2 microtubules is present (Fig. 9B and C). Each derivative is positioned laterally to the axoneme and contains an elliptical crystalline region (Fig. 9C). Cross-sections through the end of the tail show a progressive loss of microtubules: the 9 accessory microtubules disappear first (Fig. 9D), followed by the two central microtubules (Fig. 9E). The axoneme extends to almost the end of the sperm tail (Fig. 9A).

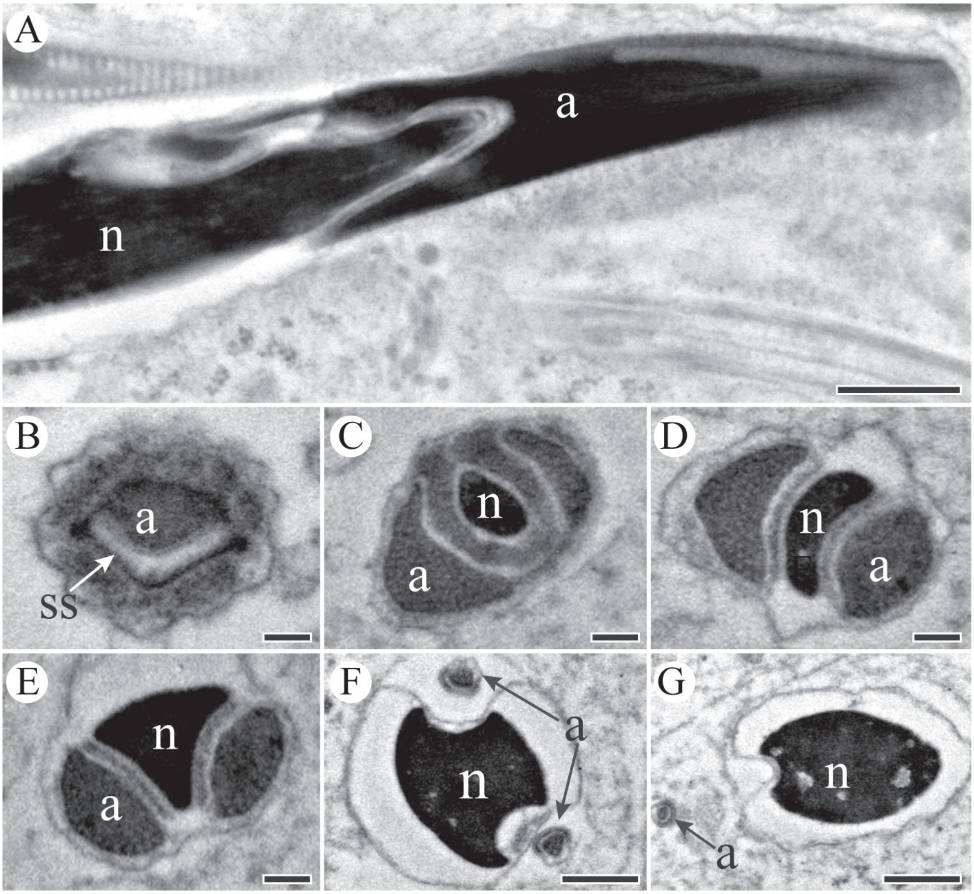

Figure 7. TEM micrographs of sperm head region of *P. kaempferi*. **A** Longitudinal section of sperm head, showing apex of acrosome (a), tapered nucleus (n) **B** Cross-section of the sperm head, showing acrosome (a) and subacrosomal space (ss) **C–F** Cross-sections of the sperm head, showing acrosome (a) and two acrosomal processes with numerous microtubules **G** Cross-section of the sperm head, showing nucleus (n) and an acrosomal process. Scale bars: 500 nm (**A**), 200 nm (**B–G**).

Discussion

In this study, a number of similarities are revealed in the mature spermatozoa of *S. yangi*, *K. caelatata* and *P. kaempferi*. The motile spermatozoa, all aggregated into bundles, intrude into a homogenous matrix to form a spermatodesm. The spermatozoa of each species can be divided into two or three types based on their total length, nucleus length, and tail lengths (viz., polymegaly). There is a conical acrosome with a subacrosomal space in an eccentric position, and the acrosome sits above the anterior part of the nucleus. The centriolar adjunct is located at the postero-lateral invagination of the nucleus and is parallel to it. In the tail region, two equal mitochondrial derivatives with electron-dense crystalline regions comprise cristae, which are arranged in

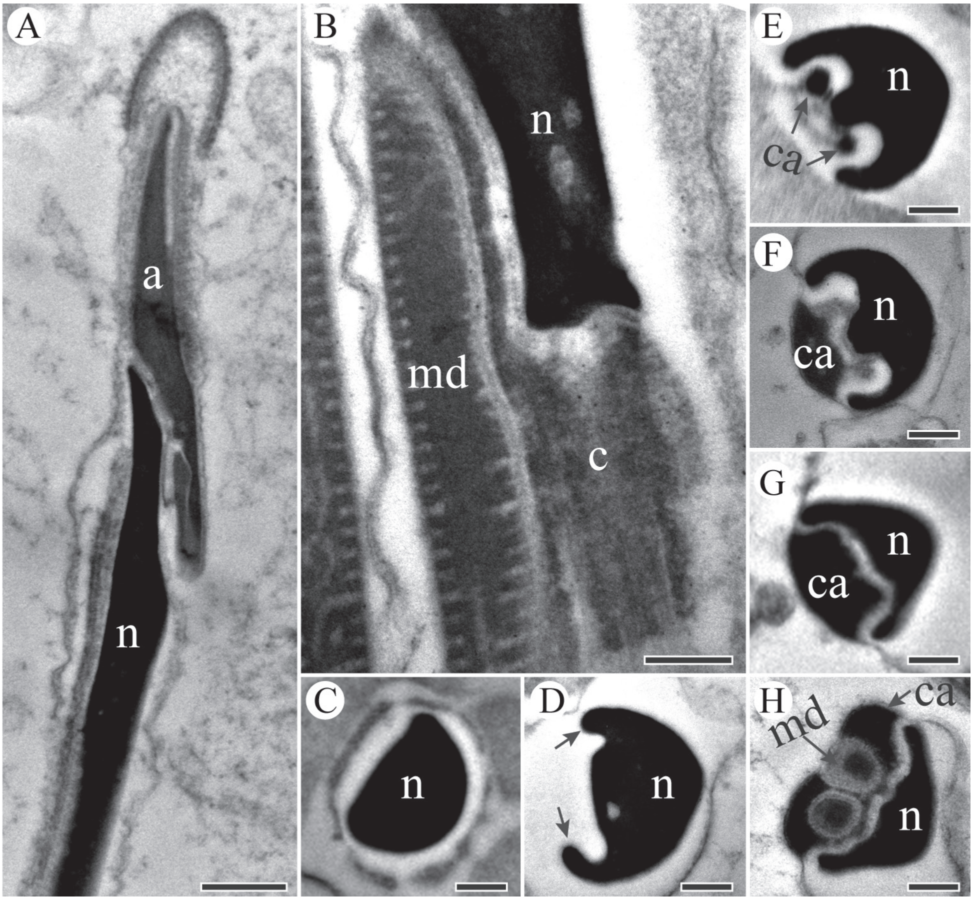

Figure 8. TEM micrographs of sperm neck region of *P. kaempferi*. **A** Longitudinal section showing head region, showing conical acrosome (a) and tapered nucleus (n) **B** Longitudinal section of nucleus-flagellum transition region, showing nucleus (n), mitochondrial derivative (md) and centriole (c) **C** Cross-section of nucleus (n) with a deltoid appearance **D** Cross-section through the mid-neck region, showing an invagination at one side of the nucleus (n) developing two ridges (arrowed) **E–G** Cross-sections through neck region, showing centriolar adjunct (ca) and nucleus (n). **H** Cross-section through the mid-neck region, showing nucleus (n), mitochondrial derivatives (md) and centriolar adjunct (ca). Scale bars: 500 nm (**A**), 200 nm (**B–H**).

an orderly array. A single axoneme displays a 9 + 9 + 2 microtubule arrangement. The mitochondrial derivatives and the axoneme both extend to almost the end of the tail. There is no accessory body in the sperm tail. These features are all likely common to spermatozoa of other investigated cicadas (e.g., Folliot and Maillet 1970, Kubo-Irie et al. 2003, Chawanji et al. 2005, 2006).

Although the sperm ultrastructures of these three cicada species have some similarities, the centriolar adjunct of *S. yangi* presents a different appearance, i.e., with a granular substructure. In many insects, the centriolar adjunct has been identified as derived from additional pericentriolar material (PCM) deposited beneath the nucleus

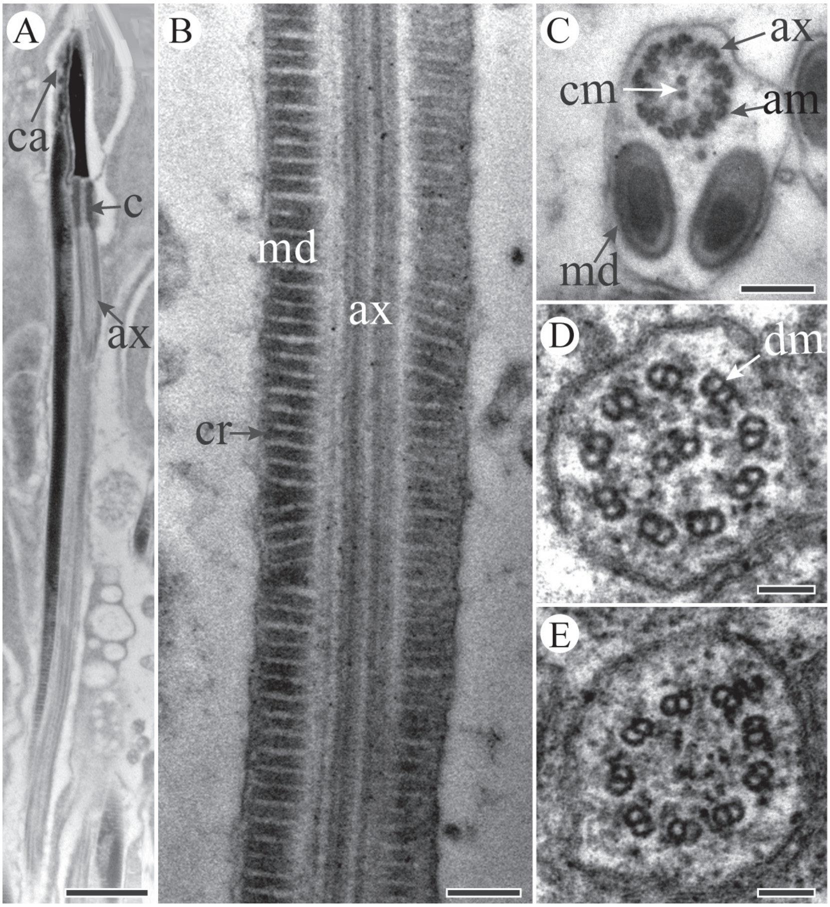

Figure 9. TEM micrographs of sperm tail region of *P. kaempferi*. **A** Longitudinal section through the neck and tail regions, showing nucleus (n), centriolar adjunct (ca), mitochondrial derivative (md), centriole (c) and axoneme (ax) **B** Higher magnification of longitudinal section of sperm tail, showing axoneme (ax) and mitochondrial derivatives with cristae (cr) **C** Cross-section of tail region, showing two mitochondrial derivatives (md) and a 9 + 9 + 2 microtubular pattern (i.e., 9 accessory microtubules (am), 9 double microtubules (dm), and two central microtubules (cm)) axoneme (ax) **D** Higher magnification of cross-section of axoneme (ax), showing 9 double microtubules (dm), and two central microtubules left **E** Higher magnification of cross-section of axoneme (ax) showing only 9 double microtubules (dm) remained. Scale bars: 1 μm (**A**), 200 nm (**B, C**), 100 nm (**D, E**).

at the end of spermiogenesis (Dallai et al. 2016a). The centriolar adjunct is apparently an apomorphy of Insecta (Dallai et al. 2016b). In Dicondylia of the Insecta the centriolar adjunct and the accessory bodies may be variably developed, and their size and shape are important characteristics for taxonomy (Dallai et al. 2016a). Cicadas are classified into two families, Cicadidae and Tettigarctidae (Moulds 2005, Marshall et al. 2018), with the former being divided into four subfamilies (Cicadinae, Cicadettinae, Tibicininae, and Tettigomyiinae) (Marshall et al. 2018). So far, the centriolar adjunct of spermatozoa in Cicadinae has been found to be composed of homogenous, moderately electron-dense material (Folliot and Maillet 1970, Kubo-Irie et al. 2003, Chawanji et al. 2005). In our present study, the structure of the centriolar adjunct in spermatozoa of *P. kaempferi* and *K. caelatata* is consistent with previous descriptions for the Cicadinae. In the Cicadettinae and Tettigomyiinae, the centriolar adjunct of the spermatozoa is lamellar (Chawanji et al. 2006). In our study, we found that the centriolar adjunct of *S. yangi* is granular, which is different from that of other cicada species, indicating that spermatozoa in Tibicininae may have their own characteristics in comparison with other cicadas. Chawanji et al. (2005) described vesicular-like structures associated with the centriolar adjunct in some sections of spermatozoa of the cicada *P. hirtipennis* (Germar, 1834), but the centriolar adjunct itself is not granular, which is different to the granular centriolar adjunct of *S. yangi*. This characteristic may add more information for spermatology of Tibicininae and, together with other results, may inform future studies on the phylogeny of Cicadoidea.

Cicadas of the genus *Karenia*, remarkably without timbals, are currently placed in the Cicadinae. This is the only genus of the tribe Sinosenini. The systematic placement of this tribe remains controversial (Boulard 1973, 1988, 2008, 2001, 2013, Chou et al. 1997, Moulds 2005, Wei et al. 2009, Marshall et al. 2018). Species of this group are restricted to southwestern China, Myanmar and Vietnam (Wei et al. 2009, Pham and Yang 2012). Moulds (2005) attributed *Karenia* to the subfamily Cicadettinae, but this genus and other probably related taxa were not included in his morphological phylogenetic analyses. Boulard (2008), Wei et al. (2009) and Pham and Yang (2012) followed Moulds (2005), attributing this genus into the Cicadettinae. However, Boulard (2013) and Marshall et al. (2018) put this genus in the Cicadinae. Morphologically, the metanotum of *Karenia* is distinctly concealed by the cruciform elevation at the dorsal midline, which is the same as species in Cicadinae, but is different from members of Cicadettinae whose metanotum is partly visible at dorsal midline (Moulds 2005, 2012). Furthermore, in Cicadettinae the uncus is duck-bill shaped and undeveloped, and the pair of claspers are well developed; while in Cicadinae the uncus is well developed with uncal lobes much swollen and elongated, and the claspers are usually degenerate or even disappeared (Moulds 2005, 2012). The strongly swollen uncus in *Karenia* is similar to that in the Cicadinae. In some species of Cicadettinae, the centriolar adjunct presents as a lamellate substructure (Chawanji et al. 2006). However, in our study, there is no such substructure in the centriolar adjunct of the spermatozoa of *K. caelatata*. Coupled with the above-mentioned morphological characters and the morphology of ovipositors (Zhong et al. 2017), antennae (Wang et al. 2018, in press) and Malpighian tubules (Li et al. 2015), our results confirm that it is reasonable to place this genus in Cicadinae, which is consistent with the results of Marshall et al. (2018) based on molecular data.

Spermatozoa possess more than one size type (viz., polymegaly), which has been described widely within the Insecta. For example, some species of vinegar flies (Diptera, Drosophilidae) and stalk-eyed flies (Diptera, Diopsidae) produce two discrete lengths of nucleated sperms (Snook et al. 1994, Pasini et al. 1996, Presgraves et al. 1999). In the Cicadomorpha, all of the 13 previously investigated species of Cicadoidea can produce more than one size type of spermatozoa reflected in nuclear length and total length within and between species (Kubo-Irie et al. 2003, Chawanji et al. 2005, 2006). In addition, a distinct correlation between nuclear length and total length was found in the spermatozoa of cicada *G. nigrofuscata* (Kubo-Irie et al. 2003). However, Chawanji et al. (2005, 2006) found the nucleus length and total length of spermatozoa have no significant correlations in their examined species. In our study, the three examined species also produce two or three distinct size types of spermatozoa, and there is a weak (in *S. yangi*) or modest (in *K. caelatata* and *P. kaempferi*) correlation between the nuclear length and tail length within a species (Table 3). In contrast, polymegaly of spermatozoa does not appear in the Cicadellidae and Cercopoidea within Cicadomorpha. The production of only one size type of spermatozoa has been revealed in investigated species of Membracoidea (Cruz-Landim and Kitajima 1972, Araújo et al. 2010, Zhang and Dai 2012, Su et al. 2014). Although the sperm length varies within individual males of *Locris transversa* (Cercopidae), this variation has no statistical significance, which was also observed in other two cercopids (Folliot and Maillet 1970). Hodgson et al. (2016) presumed that polymegaly may be an apomorphy of Cicadoidea within Cicadomorpha based on the study of *Locris transversa* (Cercopidae). Therefore, our results, coupled with other previously related studies, suggest that polymegaly is a narrow occurrence in the Cicadomorpha.

The results of our study provide more clues for further studies of classification and phylogeny of the Cicadoidea. There may also be some ultrastructural features that can be used as morphological evidence for the phylogeny of the Cicadomorpha.

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RESEARCH ARTICLE

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Two new species of the bamboo-feeding genus *Bambusicaliscelis* Chen & Zhang, 2011 from China (Hemiptera, Fulgoromorpha, Caliscelidae)

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Abstract

Two new species of the bamboo-feeding planthopper genus *Bambusicaliscelis* Chen & Zhang, 2011, *B. flavus* Chen & Gong, **sp. n.** and *B. guttatus* Chen & Gong, **sp. n.**, are described and illustrated from China. The generic characteristics are redefined and photographs of the new species are provided. A checklist and a key to species of *Bambusicaliscelis* are also given.

Keywords

Caliscelini, planthopper, taxonomy, bamboo, distribution

Introduction

The planthopper family Caliscelidae Amyot & Serville, 1843, including two subfamilies, five tribes, 76 genera, and more than 200 species (Bourgoin 2018), is a small group that widely distributed in the world. So far, in China, the taxa of the family contained four tribes (Caliscelini, Peltonotellini, Ommatidiotini and Augilini), 12 genera, and 29 species (Emeljanov 2008; Che et al. 2009, 2011; Chen et al. 2014; Meng et al. 2015). Two species of *Bambusicaliscelis* (*B. dentis* and *B. fanjingensis*), one species of

Pseudosymplanella (*P. nigrifasciata*), one species of *Augilodes* (*A. binghami*), three species of *Symplana* (*S. brevistrata*, *S. lii* and *S. longicephala*), and five species of *Symplanella* (*S. brevicephala*, *S. hainanensis*, *S. recurvata*, *S. unipuncta* and *S. zhongtua*) were found on bamboo from China (Che et al. 2009; Chen and Zhang 2011; Chen et al. 2014; Yang and Chen 2014). Unfortunately, no more other information on host plants is available except for *S. recurvata* collected on *Neosinocalamus* sp.

The planthopper genus *Bambusicaliscelis* was established by Chen and Zhang (2011) based on two species, *B. dentis* and *B. fanjingensis*, from China, and placed in the tribe Caliscelini of the subfamily Caliscelinae (Hemiptera: Fulgoroidea: Caliscelidae). The two species of *Bambusicaliscelis* are similar but can be easily distinguished from each other by their male genitalia.

In this paper, two new species, *Bambusicaliscelis flavus* sp. n. and *Bambusicaliscelis guttatus* sp. n., were collected from bamboo. Their descriptions and illustrations are given. The generic characteristics are redefined. A checklist and a key to species of *Bambusicaliscelis* are given.

Materials and methods

Terminology follows Chan and Yang (1994) and Chen and Zhang (2011). Dry specimens were used for the descriptions and illustrations. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. Measurements were given in millimeters; body length was measured from the apex of the head to tip of the abdomen in repose. The genital segments of the examined specimens were macerated in 10% NaOH, washed in water, and transferred to glycerin. Illustrations of the specimens were made with a Leica MZ 12.5 stereomicroscope. Photographs were taken with KEYENCE VHX-1000 system. Illustrations were scanned with CanoScan LiDE 200 and imported into Adobe Photoshop CS7 for labelling and plate composition.

The type specimens and material examined are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (**IEGU**).

Taxonomy

Bambusicaliscelis Chen & Zhang, 2011

Figs 1–24

Bambusicaliscelis Chen & Zhang, 2011: 95; Chen et al. 2014: 157.

Type species. *Bambusicaliscelis fanjingensis* Chen & Zhang, 2011, by original designation.

Diagnosis. General color yellowish brown to blackish brown. Vertex from apex to tip of abdomen with a pale longitudinal stripe along median line. Vertex with disc slightly concave, lateral margins subparallel, width at base wider than length in middle line. Frons rather broad, widest part under level of lower margin of eyes, length in median line longer than width; lateral margins distinctly carinate, from apex to level of lower margin of eyes subparallel then gradually incurved to frontoclypeal suture; median carina present, weak; submedian carinae arising from basal margin of frons, slightly divergent then convergent apically, not reaching to frontoclypeal suture; each lateral area between submedian carina and lateral carina with two rows include 12 small pustules. Postclypeus with median carina distinct, lateral carinate obscure. Rostrum reaching posterior trochanters. Pronotum broad transversely, 3-carinate, median carina weak, length in median line slightly shorter than vertex. Mesonotum 3-carinate, median carina weak, length in median line shorter than vertex and pronotum combined. Forewing with length slightly longer than width, anterior and posterior margins subparallel, apical margin subtruncate, veins obscure. Hindwing absent. Legs with fore and middle femora and tibiae normal. Hind tibiae with one spine at middle. Spinal formula of hind leg 6–3–2.

Male genitalia. Anal segment short, in dorsal view with length in middle line longer than broad at widest part. Pygofer in lateral view with ventral margin distinctly longer than dorsal margin, in posterior view long oval, with opening longer than broad. Aedeagus with phallobase tubular; phallus paired, slender and long, encircled in phallobase, tapering apically. Genital style broad, with a strong finger-like process apically arising from dorsal margin, directed basally.

Distribution. China (Guizhou, Yunnan, and Guangxi).

Host plant. Bamboo.

Checklist of species of *Bambusicaliscelis* Chen & Zhang

B. dentis Chen & Zhang, 2011; China (Guizhou).
B. fanjingensis Chen & Zhang, 2011; China (Guizhou).
B. flavus Chen & Gong, sp. n.; China (Yunnan).
B. guttatus Chen & Gong, sp. n.; China (Guangxi).

Key to species of genus *Bambusicaliscelis*

1	Vertex with anterior margin slightly convex (Figure 3); forewing yellow (Figs 1–2)	***B. flavus* sp. n.**
–	Vertex with anterior margin truncated; forewing yellowish brown to blackish brown	**2**
2	Phallus of male with 2–3 teeth-like processes (Chen and Zhang 2011: Figs 19–20)	***B. dentis***
–	Phallus of male without any teeth-like processes	**3**
3	Pygofer of male in posterior view ventral margin with medioventral process single (Chen and Zhang 2011: Figure 7)	***B. fanjingensis***
–	Pygofer of male in posterior view ventral margin with medioventral processes pair (Figure 21)	***B. guttatus* sp. n.**

Bambusicaliscelis flavus **Chen & Gong, sp. n.**

http://zoobank.org/2AE83E54-7C91-466C-AB23-654782A6FCEC
Figs 1–12

Measurements. Body length (from apex of vertex to tip of abdomen): male 4.2–4.3 mm (N = 2); forewing length: male 1.7–1.8 mm (N = 2).

Description. *Coloration.* Body mainly yellowish brown. The longitudinal stripe from apex of vertex to tip of abdomen pale yellow, abdomen blackish brown (Figs 1–2). Frons (Figure 4) brown with the small yellowish white pustules between lateral and submedian carinae. Clypeus, antennae and legs yellowish brown. Eyes brown. Pustules of pro- and mesonotum (Figure 3) yellowish white. Forewing (Figs 1–2, 6) yellow.

Head and thorax. Vertex with anterior margin slightly convex, width of vertex (Figure 3) including eyes 0.9 times narrower than pronotum. Vertex (Figure 3) with length in middle line 0.7 times than width at base. Frons (Figure 4) 1.1 times longer in middle line than widest part, submedian carinae slightly keeled; areas between submedian carinae and lateral carinae slightly depressed. Pronotum (Figure 3) shorter in middle line than vertex (1:1.3). Mesonotum (Figure 3) 0.8 times as long as vertex and pronotum together in middle line. Forewing (Figure 6) with length 1.1 times than broad at widest part, veins obscure.

Male genitalia. Anal segment in dorsal view (Figure 7) with length 1.5 times longer in middle line than widest part, lateral margins slight concave; in lateral view (Figure 8) dorsal margin slightly convex, broadening apically, to apical 1/2 widest, thence abruptly narrowed, ventral margin slightly concave. Pygofer in lateral view (Figure 8) with posterior margin sinuate; in posterior view (Figure 9) nearly oval, with length 1.9 times than widest part; in ventral view (Figure 11) with posterior margin slightly concave, anterior margin slightly convex, two lateral margins subparallel. Genital style in lateral view (Figure 10) with median portion broad, large, apical margin slightly concave, with length 1.7 times as long as widest part; in ventral view (Figure 11) pear-like. Aedeagus with phallobase relatively large, truncate; phallus (Figs 8, 12) tubular, slender and long, tapering apically, apical 1/2 beyond apical margin of phallobase, then apical 1/4 dorsally reflexed.

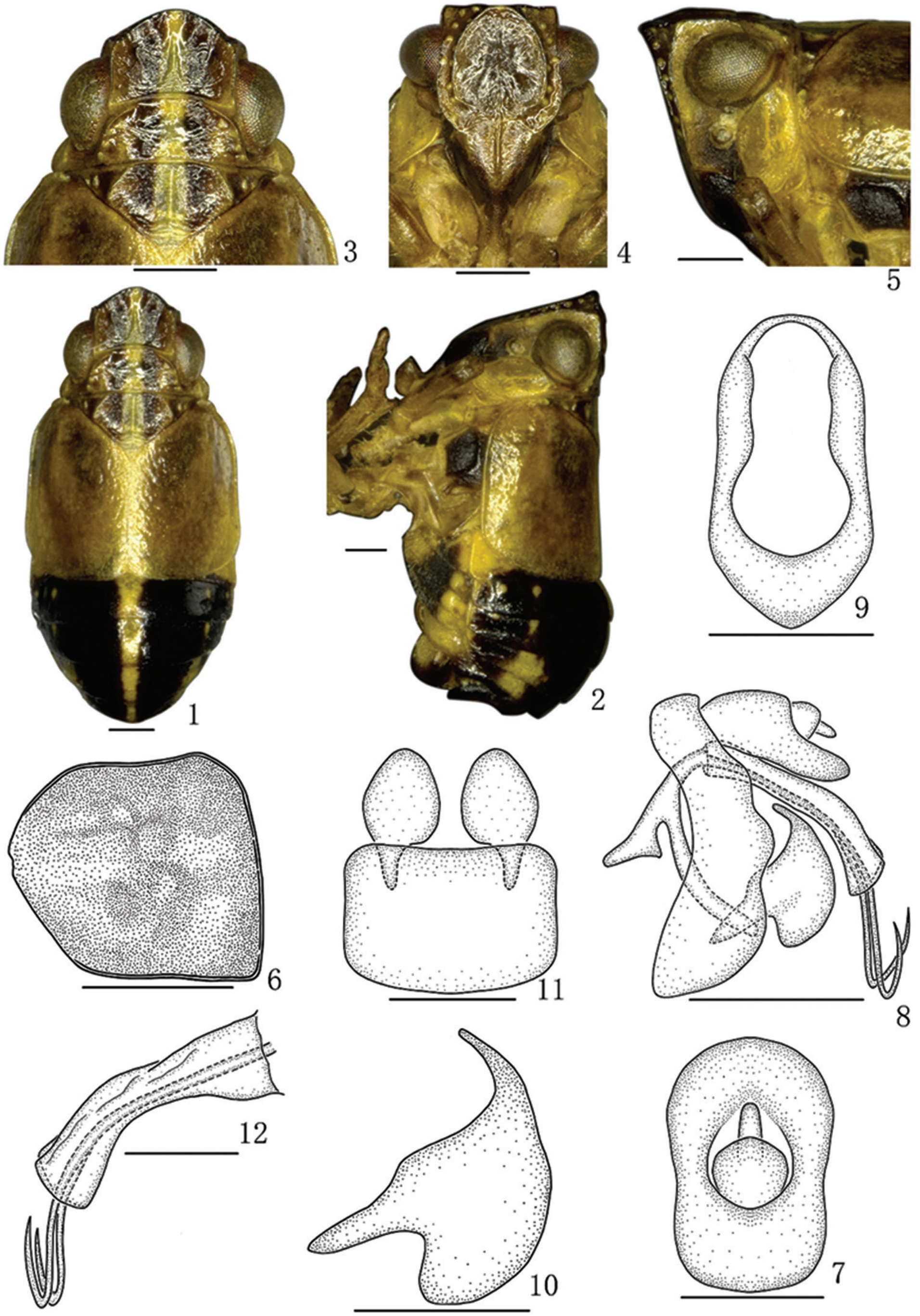
Type material. Holotype: ♂, **China:** Yunnan Province, Lushui County, Pianma Town (26°10'N, 98°38'E), 17 August 2008, Xiang-Sheng Chen; paratypes: ♂, data same as holotype.

Host plant. Bamboo.

Distribution. China (Yunnan).

Etymology. The specific name is derived from the Latin words "*flavus*" which refer to its forewing color.

Differential diagnosis. This new species is similar to *B. fanjingensis*, but differs in: 1) forewing yellow (dark brown in *fanjingensis*); 2) pygofer in posterior view, ventral margin without medioventral process (with a medioventral process in *fanjingensis*); 3)



Figures 1–12. *B. flavus* sp. n., male **1** Male habitus, dorsal view **2** Male habitus, lateral view **3** Head and thorax, dorsal view **4** Face **5** Head and thorax, lateral view **6** Forewing **7** Anal segment, dorsal view **8** Male genitalia, lateral view **9** Pygofer, posterior view **10** Genital Styles, lateral view **11** Pygofer and genital styles, ventral view **12** Aedeagus, lateral view. Scale bars: 0.5 mm (**1–5, 7, 10–12**), 1 mm (**6, 8–9**).

pygofer in lateral view with dorsal margin roundly convex and posterior margin sinuate (dorsal and posterior margin concave at middle in *fanjingensis*).

Bambusicaliscelis guttatus Chen & Gong, sp. n.

http://zoobank.org/89A104FC-DFF4-4AFD-BBB2-6A62C9CE79DB

Figs 13–24

Measurements. Body length (from apex of vertex to tip of abdomen): male 4.2 mm (N = 1); forewing length: male 1.7 mm (N = 1).

Description. *Coloration.* Body mainly yellowish brown to blackish brown. The longitudinal stripe from apex of vertex to tip of abdomen pale yellowish white (Figure 13). Frons (Figure 16) dark brown with the small pustules yellowish brown between lateral and submedian carinae. Clypeus brown. Eyes and antennae dark brown. Forewing (Figs 13–14,18) brown with one large yellowish white marking near apical margin. Legs brown.

Head and thorax. Vertex with anterior margin subtruncated, width of vertex (Figure 15) including eyes as long as pronotum. Vertex (Figure 15) with length in middle line 0.8 times than width at base. Frons (Figure 16) 1.3 times longer in middle line than widest part, submedian carinae slightly keeled, areas between submedian carinae and lateral carinae slightly depress. Pronotum (Figure 15) shorter in middle line than vertex (1:1.6). Mesonotum (Figure 15) 0.7 times as long as vertex and pronotum together in middle line. Forewing (Figure 18) with length 1.3 times than broad at widest part, veins obscure.

Male genitalia. Anal segment in dorsal view (Figure 19) with length 1.3 times longer in middle line than widest part, two lateral margins concave; in lateral view (Figure 20) dorsal margin slightly convex, the widest at apical 1/2, thence constricted, ventral margin slightly concave in the middle. Pygofer in lateral view (Figure 20) with posterior margin with upper half roundly convex, lower half truncated; in posterior view (Figure 21) nearly oval, with length 1.7 times as long as widest part; in ventral view (Figure 23) with posterior margin with two stout and short medioventral processes, anterior margin slightly convex, lateral margins subparallel. Genital style in lateral view (Figure 22) with basal 1/2 basally narrowing, median portion widest, apical margin slightly concave, with length 3.1 times as long as widest part, a strong finger-like process apically arising from dorsal margin, directed basad; in ventral view (Figure 23) long and narrow, with apex inward bent, nearly hook-like. Aedeagus with phallobase (Figs 20, 24) slender, long and tubular. Phallus (Figs 20, 24) tubular, much slender and longer, tapering apically, apical 1/2 beyond apical margin of phallobase, then apical 1/4 distinctly bent.

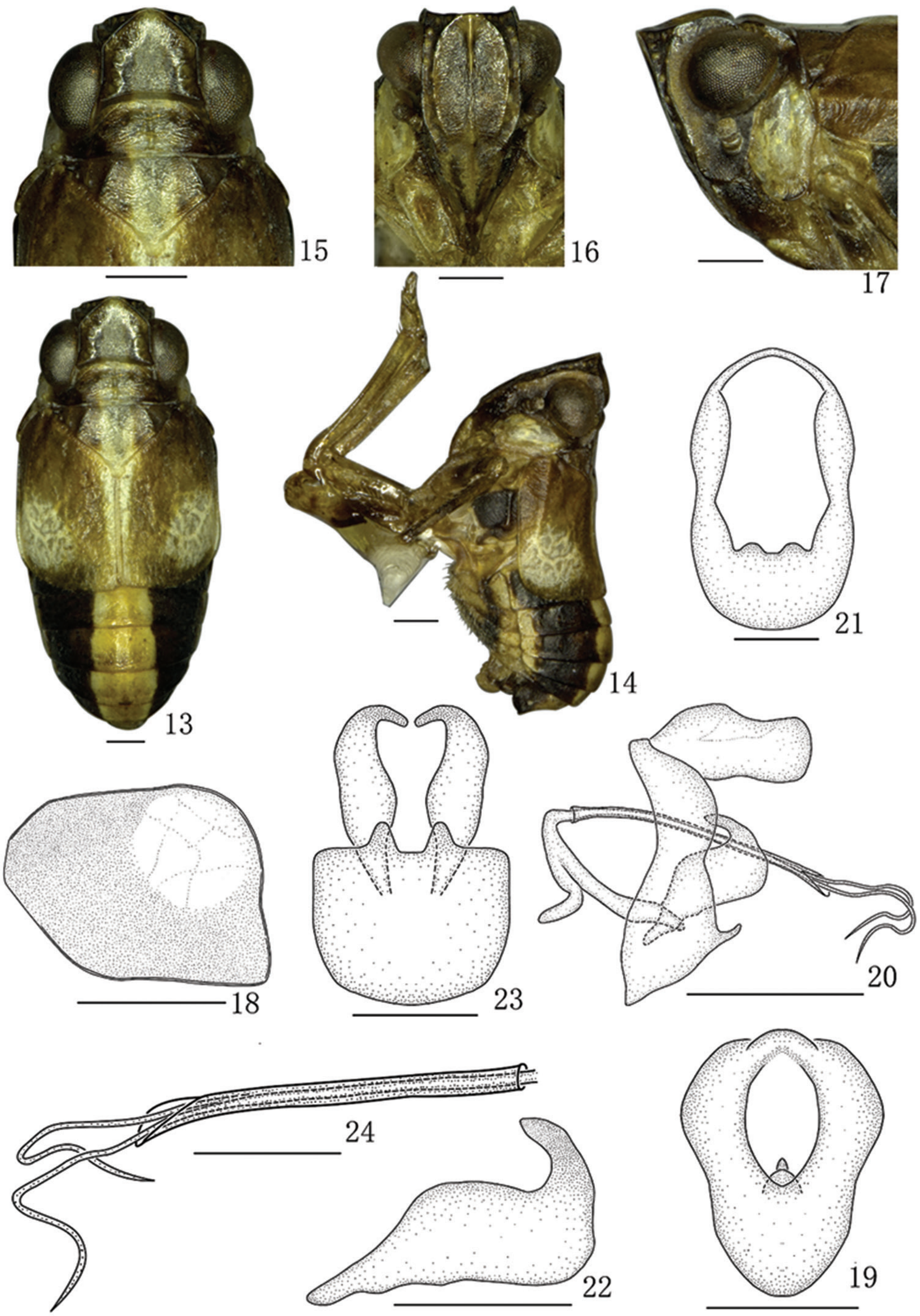
Type material. Holotype: ♂, **China:** Guangxi, Damingshan National Natural Reserve (23°54'N, 108°37'E), 10 August 2011, Zai-Hua Yang.

Host plant. Bamboo.

Distribution. China (Guangxi).

Etymology. The specific name is derived from the Latin words "*guttatus*" which refer to its forewing with a large yellowish white marking.

Differential diagnosis. *B. guttatus* sp. n. is similar to *B. fanjingensis*, but differs in: 1) forewing brown with one large yellowish white marking (blackish brown, without



Figures 13–24. *B. guttatus* sp. n., male **13** Male habitus, dorsal view **14** Male habitus, lateral view **15** Head and thorax, dorsal view **16** Face **17** Head and thorax, lateral view **18** Forewing **19** Anal segment, dorsal view **20** Male genitalia, lateral view **21** Pygofer, posterior view **22** Genital Styles, lateral view **23** Pygofer and genital styles, ventral view **24** Aedeagus, lateral view. Scale bars: 0.5 mm (**13–17, 19, 21–24**), 1 mm (**18, 20**).

any marking in *fanjingensis*); 2) pygofer in posterior view, ventral margin with two medioventral processes (medioventral process single in *fanjingensis*); 3) genital style in lateral view with dorsal process located apically, large and apical margin roundly convex (dorsal process located near apex, relatively slender and apex sharp in *fanjingensis*).

Discussion

The *Bambusicaliscelis* Chen & Zhang, 2011 and *Thaiscelis* Gnezdilov, 2015 are readily distinguished from other known genera of Caliscelini by carination of the frons (Figs 4, 16; Gnezdilov 2015: figs 6–7). The genus differs from *Thaiscelis* in general coloration being yellowish brown to blackish brown (dark brown or black in *Thaiscelis*); vertex with anterior margin truncate or roundly convex (anterior margin acutely angulate in *Thaiscelis*); each side of frons between lateral margin and submedian carina with two rows include 12 small pustules (eleven small pustules in *Thaiscelis*).

Bambusicaliscelis may be seen as one of the most primitive members of tribe Caliscelini according to its “closed-tube” type of phallobase (Figs 12, 24), which is possibly the primitive (ancestral) condition compared to the “open-tube” type of other Caliscelini (Gnezdilov and Bourgoin 2009: figs 63–65) and Peltonotellini (Emeljanov 2008: figs 2–3), which may be treated as a derived condition.

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RESEARCH ARTICLE

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A taxonomic study of *Muscidifurax* Girault & Sanders from China (Hymenoptera, Chalcidoidea, Pteromalidae)

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Abstract
Five species of *Muscidifurax* Girault & Sanders (Hymenoptera: Pteromalidae) are studied from mainland China, of which three new species, *M. similadanacus* Xiao & Zhou, **sp. n.**, *M. sinesensilla* Xiao & Zhou, **sp. n.**, *M. neoraptorellus* Xiao & Zhou, **sp. n**., and one newly recorded species, *M. adanacus* Doganlar, are reported. All species have been reared from pupae of *Musca domestica* Linnaeus. A key to Chinese *Muscidifurax* and illustrations of external features of the species are provided.

Keywords
China mainland, key, *Muscidifurax*, new species, Pteromalidae, taxonomy

Introduction

Muscidifurax was described by Girault and Sanders in 1910 to include *M. raptor* Girault and Sanders, parasitizing the common house fly (*Musca domestica* Linnaeus) from Illinois, USA. The genus can be recognized by the female antenna with one anellus and seven funicular segments (two anelli and six funicular segments in male), head protuberant at level of antennal toruli, marginal vein thickened in proximal half and progressively thinner in distal half. Since then, several researchers have studied the genus, including Graham (1969), Dzhanokmen (1978) and Bouček (1991). Kogan and Legner (1970) studied the genus and described four new species from Nearctic region. Doganlar (2007) described a new species of *Muscidifurax* which probably parasites *Fannia* sp. Thus, six valid species are described in the genus. All species are parasitoids of species of Calliphoridae and Muscidae (Diptera). Some species, such as *M. raptor*, were used in the biological control of the house fly (Legner 1971; Doganlar 2007). Until now, only one species, *Muscidifurax raptor* Girault and Sanders, has previously been recorded in China.

Materials and methods

All specimens were collected in the laboratory where they have been reared from pupae of house flies, and preserved in 75% ethanol. They were subsequently air-dried, point-mounted, and examined with a LEICA M10 stereomicroscope. Photographs were taken by using a Nikon Multizoom AZ100 system, and plates of illustrations were compiled using Adobe Photoshop® software. Five species have been identified, including three new species (*M. similadanacus* sp. n., *M. sinesensilla* sp. n., *M. neoraptorellus* sp. n.) and one newly recorded species (*M. adanacus* Doganlar). All type specimens are deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS).

Morphological terminology follows that of Graham (1969), Bouček (1988), and Gibson et al. (1997). All specimens were examined and identified based on the studies of Kogan and Legner (1970), Doganlar (2007) and Girault and Sanders (1910). Body length (i.e. the length of body excluding the ovipositor sheaths) is measured in millimeters (mm), other measurements are given as ratios.

Abbreviations of morphological terms used are:

Fu_n funicular segment number;
POL posterior ocellar distance;
OOL ocellocular distance;
Gt_n gastral tergite number.

Taxonomy

Key to species

1	Fore wing without marginal fringe and usually with reduced pilosity (Fig. 6); inner margins of eyes not angularly produced upwards near vertex	**2**
–	Fore wing with marginal fringe well developed, or at least with marginal fringe at posterior margin (Fig. 12); inner margins of eyes angularly produced upwards (small angle shape) near vertex (Figs 9, 14)	**4**

2 Second funicular segment without sensilla (Fig. 24); gaster 1.65× as long as broad, Gt_1 about 1/3 length of gaster; median area of propodeum without coarse rugae .... ***M. neoraptorellus* sp. n.**

– Second funicular segment with sensilla; gaster at least 1.9× as long as broad; Gt_1 about 1/4 length of gaster; median area of propodeum with weak or strong coarse rugae... **3**

3 Each funicular segment longer than broad; head width 1.25× head height; Fu_1 slightly longer than Fu_2; propodeum with distinct costula (Fig. 5).......... .... ***M. similadanacus* sp. n.**

– Fu_1-Fu_5 or Fu_1-Fu_6 longer than broad, Fu_7 quadrate; head width 1.17× head height; Fu_1 shorter or as long as Fu_2; propodeum without costula (Fig. 19)..***M. adanacus* Doganlar**

4 Antennal insertion under the lower ocular line, Fu_1 without sensilla (Fig. 10); head 1.82× as broad as long in dorsal view; propodeum without coarse rugae; gaster 1.8× as long as broad....................................... ***M. sinesensilla* sp. n.**

– Antennal insertion on the lower ocular line, Fu_1 with sensilla; head 2× as broad as long in dorsal view; propodeum with coarse rugae; gaster 2× as long broad .... ***M. raptor* Girault & Sanders**

Muscidifurax Girault & Sanders, 1910

Muscidifurax Girault & Sanders, 1910: 146.

Muscidifurax raptor Girault & Sanders, 1910: 146; original designation and monotypy. Kogan and Legner 1970: 1268–1290; Propp 1984: 705; Narendran et al. 2006: 29–34. [Type species.]

Smeagolia Hedqvist, 1973: 237. Type species: *Smeagolia perplexa* Hedqvist. Synonymized by Bouček 1991: 203.

Diagnosis. Body dark green, head, and mesosoma with distinctly white hairs, eye glabrous. Head wider than mesosoma, occipital carina strong. Antennal insertion placed on lower ocular line and face distinctly protuberant at antennal insertion; lower face receding almost horizontally. Antenna slender, formula 11173 in females, 11263 in males; lower margin of clypeus more or less incised medially, without median tooth. Pronotal collar margined; notauli incomplete; scutellum flattened; propodeum with median carina and complete plicae, nucha short but distinct. Marginal vein strongly thickened in proximal half (its lower margin distinctly sinuate) and progressively thinner in distal half. Gaster flattened dorsally, hind margin of Gt_1 trilobed.

Biology. Hosts include Calliphoridae (*Chrysomya* sp., *Phormia* sp.) and Muscidae (*Fannia canicularis*, *Fannia femoralis*, *Musca domestica*, *Stomoxys* sp. and *Stomoxys calcitrans*) (Noyes 2017).

Distribution. Palaearctic, Nearctic, Afrotropics, Neotropics, and Australasian regions (Noyes, 2017). China: Beijing, Shandong (Guo et al. 1997).

Muscidifurax similadanacus **Xiao & Zhou, sp. n.**
http://zoobank.org/24C8BB2B-9695-455A-AEB8-7F57EC21BB90
Figs 1–7

Diagnosis. Fore wing without marginal fringe; each funicular segment longer than broad; head width 1.25× head height; Fu_1 slightly longer than Fu_2; Fu_1 without sensilla; median area of propodeum with distinct costula; gaster 2.1× as long as broad, Gt_1 1/4 length of gaster.

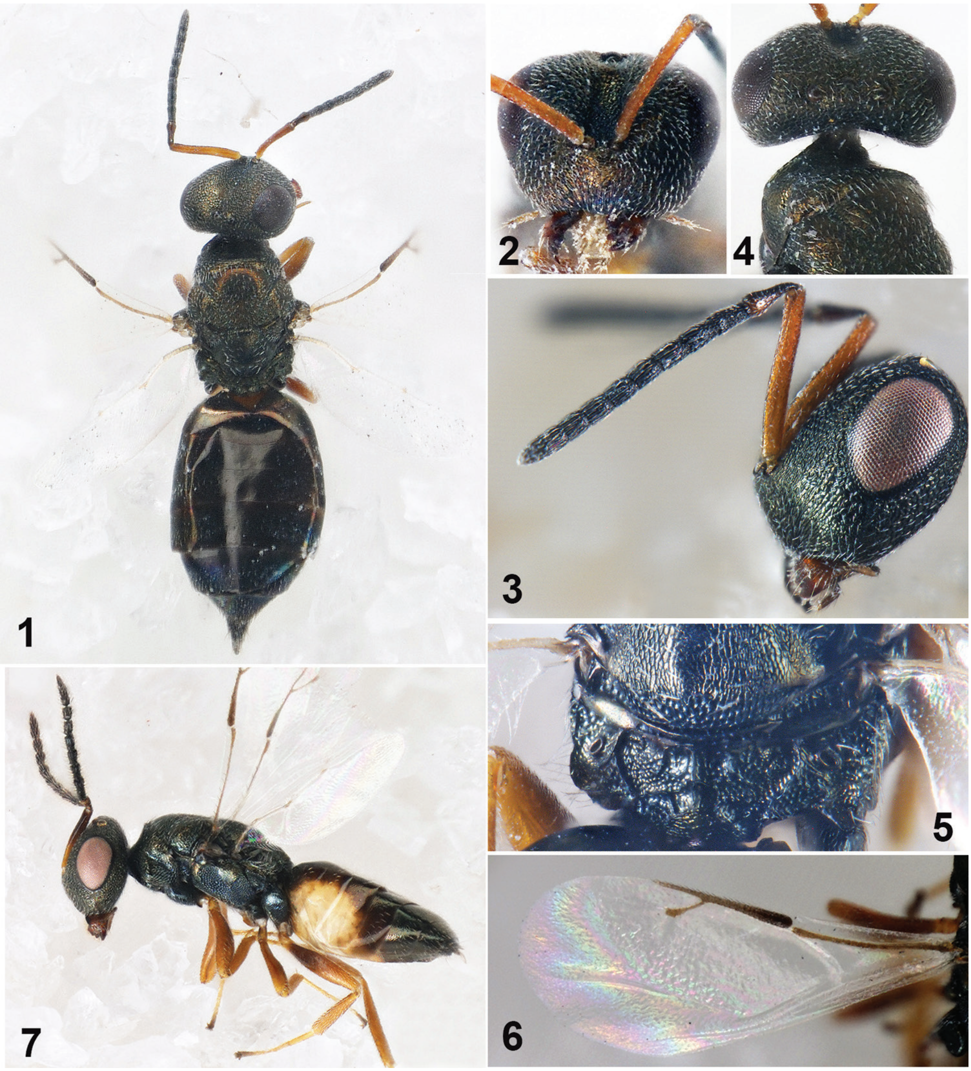
Description. Holotype. *Female*. 3.4 mm (Fig. 1). Head and mesosoma dark green, with metallic reflections and white hairs; gaster brown with yellow spot. Antennal scape brown, flagellum dark brown; legs yellow except coxae concolorous with body, femora and pretarsi brown; fore wings hyaline, venation brown except marginal vein dark brown.

Head in frontal view 1.25× as wide as high (Fig. 2); eyes with inner margins parallel, eye height 0.6× head height, eyes separated by 1.5× their height; antennal scrobes deep, reaching anterior ocellus. Antennal insertion on lower ocular line. Clypeal margin slightly protruded, straight; oral fossa 0.44× as wide as head; right mandible with four teeth, left mandible with three teeth. Head in lateral view with malar sulcus inconspicuous, eye height 1.74× malar space. Antennal scape length 1.44× eye height, exceeding vertex (Figs 2, 3); pedicel in lateral view 2.38× as long as broad; anellus transverse; Fu_1 1.8× as long as broad, slightly longer than Fu_2; each funicular segment with sensilla except Fu_1; clava not clavate, 2.25× as long as broad. Head in dorsal view (Fig. 4), 1.82× as wide as long; vertex convex; eye length 2.86× temple length; POL 1.33× OOL.

Head as broad as mesosoma. Mesosoma not distinctly convex, 2.13× as long as broad. Pronotum 0.85× as broad as mesoscutum, anteriorly margined, posterior band smooth. Mesoscutum 1.74× as broad as long, anterior half weakly reticulate and posterior half with deep reticulation; notauli incomplete, only distinct basally. Scutellum 1.18× as broad as long, frenal line absent; reticulation shallow. Propodeum (Fig. 5) medially 0.6× as long as scutellum, reticulation irregular on median area, with short irregular carinae; plicae distinct and complete, separated by 1.2× medial length of propodeum; median carina complete, costula distinct; nucha short; propodeal spiracles oval, 1.5× as long as broad. Fore wing 2.53× as long as broad, without marginal fringe (Fig. 6); basal vein and basal cell bare; upper surface of costal cell bare, lower surface with scattered setae; submarginal vein 1.75× marginal vein, marginal vein 1.8× postmarginal vein, postmarginal vein longer than stigmal vein (1.33×); stigmal vein slightly capitate.

Gaster spindle-shaped (Fig. 1) with apex pointed, 2.1× as long as broad, 1.49× as wide as mesosoma; Gt_1 covering 1/4 of gaster, each segment with hind margin entire except hind margin of Gt_1 trilobed.

Male. As female, with the following differences. Body length 3.0–3.5 mm (Fig. 7). Antennal insertion above the lower ocular line, each funicular segment longer than broad, with 3–4 rows setae.



Figures 1–7. *Muscidifurax similadanacus* sp. n., **1–6** female holotype **1** Body in dorsal view **2** Head in frontal view **3** Head in lateral view **4** Head in dorsal view **5** Propodeum 6 Fore wing **7** Male, Body in lateral view.

Variability. Females: body length 2.9–3.5 mm, others same as holotype. Males: body length 2.6–3.0 mm.

Remarks. This new species is similar to *M. raptor* and *M. sinesensilla* sp. n., but noticeably different by the absence of a marginal fringe on the fore wing. It is also very close with *M. adanacus* in having the fore wing without a fringe, but can be recognized with the characters listed in the key.

Material examined. Holotype. ♀, China: Xinjiang: Urumqi, 43.45°N 87.36°E, VII.2016, ex. Pupa of *Musca domestica*, leg. Hao-yuan Hu, IOZ(E)1812530

(2016-WJ-066). Paratypes. 7♂, IOZ(E)1812531-1812537 (2016-WJ-062), 7♀, IOZ(E)1812538-1812544(2016-WJ-066), same data to holotype.

Etymology. The name refers to the similarity of this species with *M. adanacus*, and is to be treated as an adjective.

Hosts. Pupa of *Musca domestica*.

Distribution. China (Xinjiang).

Muscidifurax sinesensilla Xiao & Zhou, sp. n.

http://zoobank.org/AAD25D3A-2FC7-4695-ACB0-208CBED8C1FD

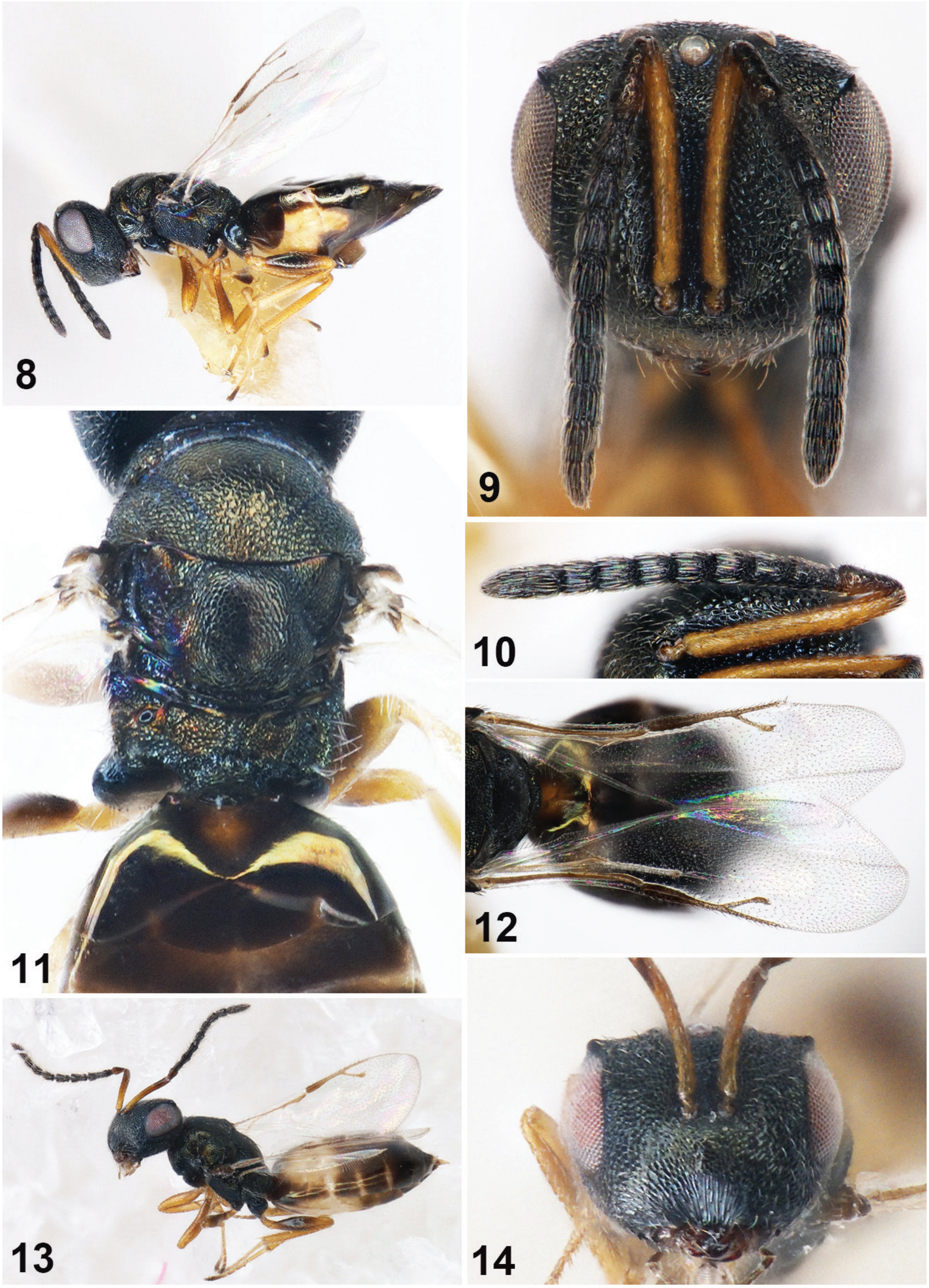
Figs 8–14

Diagnosis. Fore wing with marginal fringe; inner margins of eyes angularly produced upwards (small angle shape) near the vertex; Fu_1 without sensilla; head 1.82× as broad as long dorsally; propodeum without coarse rugae; gaster 1.8× as long as broad.

Description. Holotype. *Female*. 2.5 mm (Fig. 8). Head and mesosoma dark blue, with metallic reflections; gaster brown with yellow spot. Antennal scape yellowish brown, flagellum dark brown; legs yellow except coxae concolorous with body, and femora and pretarsi brown; fore wings hyaline, venation brown except marginal vein dark brown.

Head in frontal view 1.17× as wide as high; inner margins of eyes angularly produced upwards (small angle shape) near the vertex (Fig. 9); eye height 0.54× head height, eyes separated by 1.53× their height; antennal scrobes deep, not reaching anterior ocellus; reticulation in antennal scrobe smaller than that on parascrobe. Antennal insertion on lower ocular line, distance from upper margin of torulus to lower margin of anterior ocellus 1.78× distance from lower margin of torulus to lower margin of clypeus. Clypeus with longitudinal striation; clypeal margin slightly protruded, straight; oral fossa 0.5× as wide as head; right mandible with four teeth, left mandible with three teeth. Head in lateral view with malar sulcus conspicuous, eye height 1.25× malar space. Antennal scape length 1.34× eye height, reaching anterior ocellus, but not exceeding vertex; length of flagellum and pedicel combined longer than head width (1.2×); pedicel in lateral view 2× as long as broad; anellus transverse; Fu_1 1.67× as long as broad, slightly longer than Fu_2; each funicular segment with sensilla except Fu_1 (Fig. 10); clava not clavate, 2.35× as long as broad. Head in dorsal view, 1.82× as wide as long; vertex convex; eye length 2.55× temple length; POL 0.76× OOL.

Head 1.04× as broad as mesosoma. Mesosoma not distinctly convex, 1.41× as long as broad. Pronotum 0.74× as broad as mesoscutum, anteriorly margined, posterior band smooth and with a row of hairs. Mesoscutum 1.91× as broad as long; notauli incomplete, only distinct basally. Scutellum with reticulation shallow, frenal line absent. Propodeum (Fig. 11) medially 0.65× as long as scutellum, reticulation irregular; plicae complete, separated by 1.23× medial length of propodeum; median carina raised and complete; nucha short; propodeal spiracles oval, 1.5× as long as broad. Fore wing



Figures 8–14. *Muscidifurax sinesensilla* sp. n., **8–12** female holotype **8** Body in lateral view **9** Head in frontal view **10** Head in lateral view **11** Propodeum **12** Fore wing **13–14** Male **13** Body in lateral view **14** Head in frontal view.

2.35× as long as broad, with marginal fringe (Fig. 12); basal vein and basal cell bare; upper surface of costal cell hairy, lower surface with scattered setae; submarginal vein 1.32× marginal vein, marginal vein 1.82× postmarginal vein, postmarginal vein longer than stigmal vein (1.3×); stigmal vein straight, stigmal slightly capitate.

Gaster sessile, spindle-shaped with apex pointed, 1.8× as long as broad, 1.45× as wide as thorax; each segment with hind margin entire except hind margin of Gt_1 trilobed.

Male. As female, with the following differences. Body length 2.0 mm (Fig. 13). Antennal insertion above the lower ocular line (Fig. 14), Fu_1 0.44× as long as scape, each funicular segment longer than broad, with 3–4 rows of setae.

Variability. Females: body length 2.3–2.5 mm, others same as holotype. Males: body length 1.4–2.2 mm.

Remarks. This new species is very similar to *M. raptor* having fore wing with marginal fringe and inner margins of eyes angularly produced upwards near the vertex. It differs from *M. raptor* in having the first funicular segment without sensilla, propodeum without coarse rugae.

Material examined. Holotype. ♀, China: Xinjiang: Urumqi, 43.45°N 87.36°E, VII.2016, ex. Pupa of *Musca domestica*, leg. Hao-yuan Hu, IOZ(E)1812546 (2016-WJ-044). Paratypes. 7♂, IOZ(E)1812547-1812553(2016-WJ-045), 2♀, IOZ(E)1812554-1812555(2016-WJ-044), same data to holotype.

Etymology. The specific name is derived from the Latin *sine-* and *sensilla*, referencing the character of Fu_1 without sensilla. The name is to be treated as a noun in apposition.

Hosts. Pupa of *Musca domestica*.

Distribution. China (Xinjiang).

Muscidifurax adanacus Doganlar, 2007

Figs 15–21

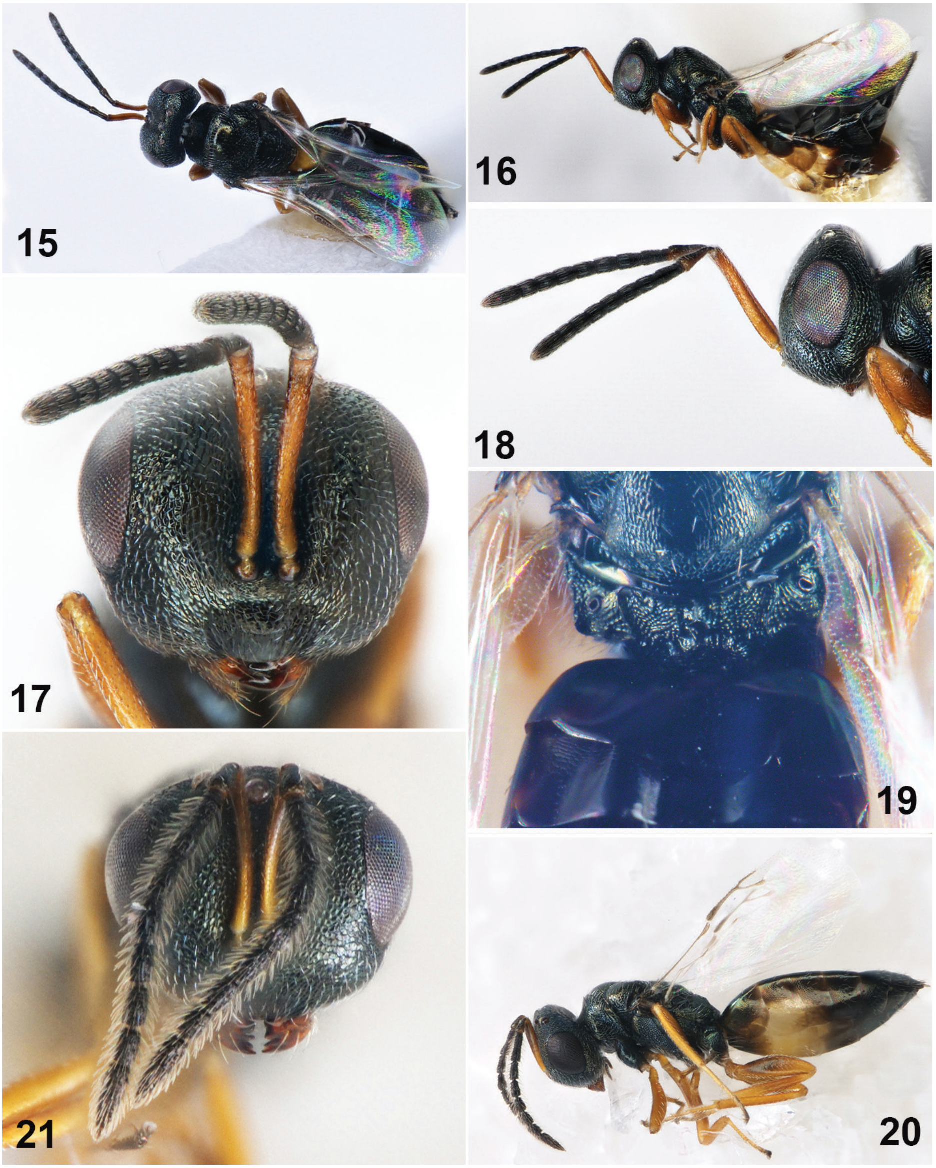
Muscidifurax adanacus Doganlar, 2007: 245–246. Holotype ♀, MKUT. Not examined.

Diagnosis. Antenna with scape longer than eye height (Figs 16, 17), exceeding vertex; each funicular segment longer than broad except Fu_7 subquadrate; Fu_1 without sensilla, longer than Fu_2 (Fig. 18); Fu_2 with sensilla. Propodeum with two slim median carinae, plicae present, nucha developed (Fig. 19); median area of propodeum with weakly or strong coarse rugae. Fore wing without marginal fringe, and with reduced pilosity. Gaster at least 1.9× as long as broad; Gt_1 about 1/4 length of gaster (Fig. 15). Male antennae with each funicular segment longer than broad, and with dense hairy (Figs 20, 21).

Material examined. China: 1♂ (2016-WJ-067), 4♀ (2016-WJ-004), Shandong: Jinan, 22.III.2016, reared from pupa of *Musca domestica* (captured on 27.II.2016), leg. Zhang-ze Hu.

Hosts. Pupa of *Musca domestica*.

Distribution. China (Shandong); Palearctic region (Turkey).



Figures 15–21. *Muscidifurax adanacus* Doganlar, 2007, **15–19** female **15** Body in dorsal view **16** Body in lateral view **17** Head in frontal view **18** Head in lateral view **19** Propodeum **20–21** Male **20** Body in lateral view **21** Head in frontal view.

Muscidifurax neoraptorellus **Xiao & Zhou, sp. n.**
http://zoobank.org/81DADF11-ADE6-45B4-A668-FFEBB95392A7
Figs 22–27

Diagnosis. Clypeus with longitudinal striation; clypeal margin not protruded; antenna with each funicular segment longer than broad, each funicular segment with sensilla except Fu_1 and Fu_2; median area of propodeum without coarse rugae; fore wing without marginal fringe, usually with reduced pilosity; gaster 1.65× as long as broad, Gt_1 1/3 length of gaster.

Description. Holotype. *Female*. 2.2 mm (Fig. 22). Head and mesosoma black, with blue metallic reflections; gaster dark brown with metallic reflections basally. Antennal scape brown, flagellum dark brown; legs brown except coxae concolorous with body; fore wings hyaline, venation brown except marginal vein dark brown.

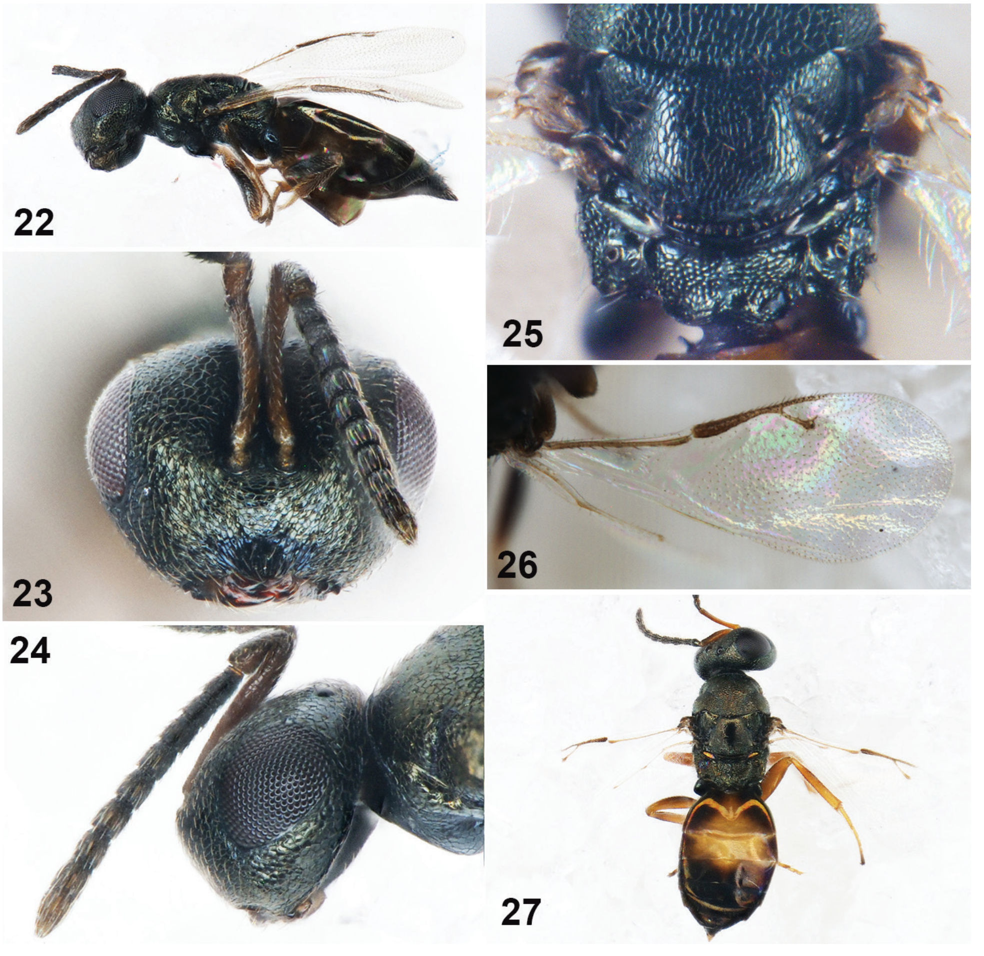
Head in frontal view 1.13× as wide as high (Fig. 23); eye height 0.54× head height, eyes separated by 1.63× their height; antennal scrobes deep, not reaching anterior ocellus; reticulation in antennal scrobe smaller than that on parascrobe. Antennal insertion on lower ocular line, distance from upper margin of torulus to lower margin of anterior ocellus 1.56× distance from lower margin of torulus to lower margin of clypeus. Clypeus with longitudinal striation; clypeal margin straight, not protruded; oral fossa 0.46× as wide as head; right mandible with four teeth, left mandible with three teeth. Head in lateral view (Fig. 24) with malar sulcus inconspicuous, eye height 1.24× malar space. Antennal scape length 1.33× as long as broad, reaching vertex; length of flagellum and pedicel combined longer than head width (1.28×); anellus transverse; each funicular segment longer than broad, Fu_1 1.38× as long as broad, as long as Fu_2; each funicular segment with sensilla except Fu_1 and Fu_2 (Fig. 24); clava not clavate, 2.67× as long as broad. Head in dorsal view, 1.75× as wide as long; vertex convex and with coarse reticulation; eye length 2.47× temple length; POL 0.75× OOL.

Head as broad as mesosoma. Mesosoma 1.33× as long as broad. Pronotum 0.83× as broad as mesoscutum, anteriorly margined, posterior band smooth and with a row of hairs. Mesoscutum 1.83× as broad as long; notauli only distinct basally. Scutellum with reticulation shallow, frenal line absent. Propodeum (Fig. 25) medially 0.8× as long as scutellum, reticulation irregular; plicae distinct and complete, separated by 1.44× medial length of propodeum; median carina raised and complete; nucha short, with coarse reticulation; propodeal spiracles oval. Fore wing 2.62× as long as broad, without marginal fringe (Fig. 26); basal vein and basal cell bare; submarginal vein 1.37× marginal vein, marginal vein 1.73× postmarginal vein, postmarginal vein longer than stigmal vein (1.32×); stigmal vein straight, stigmal slightly capitate.

Gaster sessile, spindle-shaped with apex pointed, 1.65× as long as broad, 1.14× as wide as mesosoma; each segment with hind margin entire except Gt_1 trilobed; Gt_1 covering 1/3 length of gaster.

Male. As female, with the following differences. Body length 2.5 mm. Antennal insertion above the lower ocular line, each funicular segment longer than broad; Fu_1 0.5× as long as scape, longer than other funicular segments, 2.46× as long as wide. Lateral panel of metanotum golden (Fig 27). Gaster dorsum yellow in median area.

Remarks. This new species is very close to *M. raptorellus*, but noticeably different from *M. raptorellus* in having the first and second funicular segments without sensilla



Figures 22–27. *Muscidifurax neoraptorellus* sp. n., **22–26** female holotype **22** Body in lateral view **23** Head in frontal view **24** Head and antenna in lateral view **25** Propodeum **26** Fore wing **27** Male, Body in dorsal view.

(only Fu_1 without sensilla in *M. raptorellus*), and the median area of propodeum without coarse rugae (with distinctly coarse rugae in *M. raptorellus*).

Material examined. Holotype. ♀, China: Shandong: Jinan, 36.40°N 117.00°E, 22.III.2016, reared from pupa of *Musca domestica* (captured on 27.II.2016), leg. Zhang-ze Hu, IOZ(E)1812557 (2016-WJ-002). Paratypes. 1♂, IOZ(E)1812559 (2016-WJ-005), 1♀, IOZ(E)1812558 (2016-WJ-002), same data as holotype.

Etymology. The species is intended to show similarities with *M. raptorellus*, hence the specific name is compound of '*neo*-' and '*raptorellus*'. It is to be treated as an adjective.

Hosts. Pupa of *Musca domestica*.

Distribution. China (Shandong).

Muscidifurax raptor Girault & Sanders, 1910

Muscidifurax raptor Girault & Sanders, 1910: 146; Doganlar 2007: 243–252.
Smeagolia perplexa Hedqvist, 1973: 237; Bouček, 1991: 203 (synonymy).

Diagnosis. Body black green. Head 2× as long as broad in dorsal view. Antennal scrobes deep, extending upwards and not reaching anterior ocellus; clypeus with shallowly longitudinal striation, lower margin slightly protruded. Antenna with each funicular segment longer than broad and with sensilla. Propodeum with plicae distinct and complete, median carina raised and complete; costula distinct. Fore wing with marginal fringe; stigmal vein straight, slightly capitate. Gaster 2× as long as broad, slightly broader than mesosoma width; Gt_1 covering 1/3 length of gaster.

Material examined. China: 1♂, 2♀, Shandong: Jinan, 22.III.2016, reared from pupa of *Musca domestica* (captured on 27.II.2016), leg. Zhang-ze Hu (2016-WJ-003); 1♀, Australia, N.S.W. Sydney, 10.I.1984, leg. R. Rilansow, det. B.R. Subba Rao, 1985.

Hosts. Pupa of *Musca domestica*.

Distribution. China (Beijing, Shandong) (Guo et al. 1997); Afrotropics, Australasian, Nearctic, Neotropics and Palearctic regions.

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RESEARCH ARTICLE

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Morphology and molecular genetics reveal two new *Leptobrachella* species in southern China (Anura, Megophryidae)

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Abstract

Based on morphological and phylogenetic analyses (16S rRNA mtDNA), two new species of the genus *Leptobrachella* are described from southern China, namely *L. yunkaiensis* Wang, Li, Lyu & Wang, **sp. n.** from Dawuling Forest Station of Guangdong Province and *L. wuhuangmontis* Wang, Yang & Wang, **sp. n.** from Mt. Wuhuang of Guangxi Province. To date, the genus *Leptobrachella* contains 68 species, among which 13 species are known from China. The descriptions of the two new species further emphasize that the species diversity of the genus *Leptobrachella* from China is still highly underestimated and requires further investigations.

Keywords

China, *Leptobrachella yunkaiensis* sp. n., *L. wuhuangmontis* sp. n., morphology, phylogenetic, species diversity

Introduction

The genus *Leptolalax* Dubois, 1983 within the family Megophryidae Bonaparte, 1850 was currently found to be non-monophyletic with *Leptobrachella* Smith, 1925, and was assigned as a junior synonym of *Leptobrachella* based on a large-scale molecular analysis (Chen et al. 2018).Their results also rejected the hypothesis that *Leptolalax* consists of two subgenera as proposed by Delorme et al. (2006) and Dubois et al. (2010). At present, the genus *Leptobrachella* contains sixty-six species, widely distributed from southern China west to northeastern India and Myanmar, through mainland Indochina to peninsular Malaysia and the island of Borneo (Frost 2017; Nguyen et al. 2018; Rowley et al. 2016, 2017; Yang et al. 2016; Yuan et al. 2017). They are commonly known as Asian leaf litter frogs. Currently, eleven species of this genus are known from China, i.e., *L. alpinus* from Yunnan and Guangxi provinces, *L. laui* from southern Guangdong including Hong Kong, *L. liui* from Fujian, Jiangxi, Guangdong, Guangxi, Hunan and Guizhou provinces, *L. oshanensis* from Gansu, Sichuan, Chongqing, Guizhou and Hubei provinces, *L.* cf. *pelodytoides* (which may represents a undescribed taxon), *L. purpura*, *L. tengchongensis*, *L. ventripuntatus,* and *L. yingjiangensis* from Yunnan Province, and *L. sungi* and *L. maoershanensis* from Guangxi Province (Sung et al. 2014; Yang et al. 2016; Yuan et al. 2017; Yang et al. 2018).

During field surveys in southern China from 2009 to 2016, a number of specimens were collected from Dawuling Forest Station of Guangdong Province and Mt. Wuhuang of Guangxi Province, respectively (Fig. 1), that can all be morphologically assigned to the genus *Leptobrachella*, based on the following characters: (1) comparatively small size, snout-vent length no overlap than 60.0 mm, (2) rounded finger tips, the presence of an elevated inner palmar tubercle not continuous to the thumb, (3) presence of macroglands on body including supra-axillary, pectoral, femoral and ventrolateral glands, (4) vomerine teeth absent, (5) tubercles on eyelids present, and (6) anterior tip of snout with whitish vertical bar (Dubois 1983; Matsui 1997, 2006; Lathrop et al. 1998; Delorme et al. 2006; Das et al. 2010). Subsequent molecular studies on 16S rRNA mtDNA sequences revealed that this collection represents two different undescribed species which can be distinguished from each other and from all other recognized congeners by a combination of morphological characters and molecular divergences; they are described herein as two new species.

Materials and methods

Sampling. For molecular analyses, a total of 65 samples (19 muscle tissues and 46 sequences downloaded from Genbank) from 29 species of the genus *Leptobrachella* were sequenced, in addition to two undescribed species from China, i.e., the population from Dawuling Forest Station of Guangdong Province and Mt. Wuhuang of Guangxi Province. Additionally, four sequences were downloaded from GenBank as the outgroups (see Table 1; *Pelobates syriacus*, *Pelobates varaldii*, *Leptobrachium* cf. *chapaense* and *Megophrys major*).

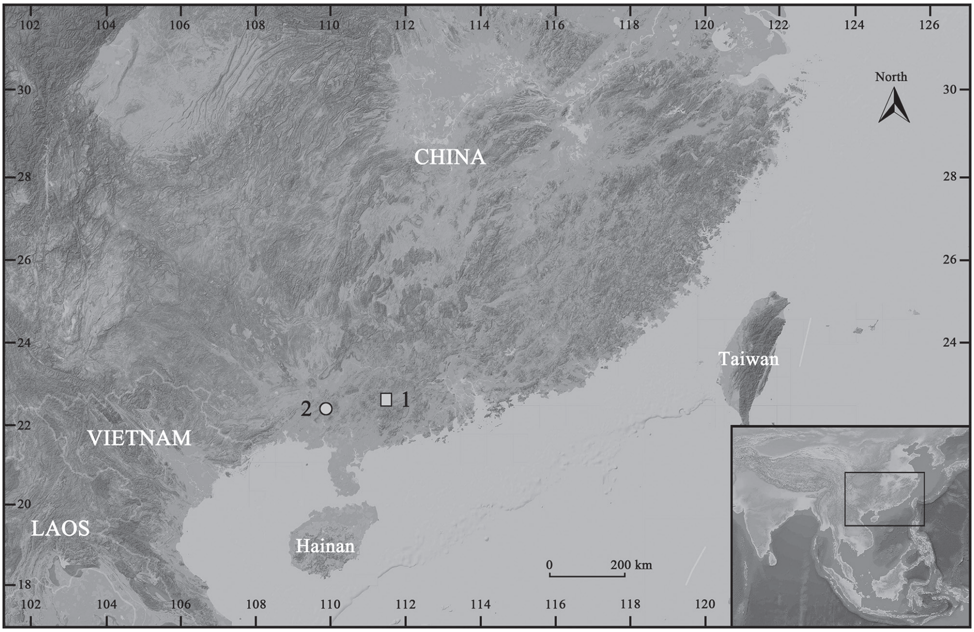

Figure 1. Collection localities of the two new *Leptobrachella* species: **1** the type locality of *Leptobrachella yunkaiensis* sp. n., Dawuling Forest Station in Guangdong Province **2** the type locality of *L. wuhuangmontis* sp. n., Mt. Wuhuang in Guangxi Province.

All specimens were previous to fixation in 10% buffered formalin and later transferred to 70% ethanol for preservation, and deposited at the Museum of Biology, Sun Yat-sen University (**SYS**) and Chengdu Institute of Biology, the Chinese Academy of Sciences (**CIB**), China; tissue samples were preserved in 95% ethanol for molecular studies.

DNA Extraction, PCR and sequencing. DNA was extracted from muscle tissue using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. The mitochondrial gene 16S ribosomal RNA gene (16S rRNA) from each sample was sequenced. Fragments of the genes were amplified using primer pairs L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3') for 16S rRNA (Simon et al. 1994). PCR amplifications were performed in a 20 μl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for five min; 35 cycles of denaturing at 95 °C for 40 s, annealing at 53 °C for 40 s and extending at 72 °C for one min, and a final extending step of 72 °C for 10 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer in Shanghai Majorbio Bio-pharm Technology Co., Ltd. All sequences have been deposited in GenBank (Table 1).

Phylogenetic analyses. Sequence alignments were first conducted using Clustal X 2.0 (Thompson et al. 1997), with default parameters and the alignment being checked

Table 1. Localities and voucher data for all specimens used in this study.

ID	Species	Locality	Voucher no.	GenBankNo.16SrRNA
1	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004663	MH605584
2	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004664 / CIB107272	MH605585
3	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004665	MH605586
4	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004666	MH605587
5	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004667	MH605588
6	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004668	MH605589
7	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004669	MH605590
8	*Leptobrachella yunkaiensis* sp. n.	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004690	MH605591
9	*Leptobrachella wuhuangmontis* sp. n.	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003485	MH605577
10	*Leptobrachella wuhuangmontis* sp. n.	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003486	MH605578
11	*Leptobrachella wuhuangmontis* sp. n.	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003487	MH605579
12	*Leptobrachella wuhuangmontis* sp. n.	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003499	MH605580
13	*Leptobrachella wuhuangmontis* sp. n.	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003500 / CIB107274	MH605581
14	*Leptobrachella wuhuangmontis* sp. n.	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003504	MH605582
15	*Leptobrachella aerea*	Vietnam: Quang Binh	RH60165	JN848437
16	*Leptobrachella applebyi*	Vietnam: Kon Tum	AMS R 173778	KR018108
17	*Leptobrachella applebyi*	Vietnam: Kon Tum	AMS R 173635	KU530189
18	*Leptobrachella bidoupensis*	Vietnam: Lam Dong	AMS R 173133	HQ902880
19	*Leptobrachella bidoupensis*	Vietnam: Lam Dong	NCSM 77321	HQ902883
20	*Leptobrachella bourreti*	Vietnam: Lao Cai	AMS R 177673	KR018124
21	*Leptobrachella eos*	Laos: Phongsaly	MNHN : 2004.0278	JN848450
22	*Leptobrachella firthi*	Vietnam: Kon Tum	AMS R 176524	JQ739206
23	*Leptobrachella fritinniens*	Malaysia: Borneo	KUHE55371	AB847557
24	*Leptobrachella gracilis*	Malaysia: Borneo	KUHE 55624	AB847560
25	*Leptobrachella hamidi*	Malaysia: Borneo	KUHE 17545	AB969286
26	*Leptobrachella heteropus*	Malaysia: Peninsula	KUHE 15487	AB530453
27	*Leptobrachella isos*	Vietnam: Gia Lai	VNMN A 2015.4 / AMS R 176480	KT824769
28	*Leptobrachella laui*	China: Tai Mo Shan, Hong Kong	SYS a002057	KM014546
29	*Leptobrachella laui*	China: San zhoutian, Shenzhen	SYSa002450	MH055904
30	*Leptobrachella laui*	China: Mt. Wutong, Shenzhen	SYS a003477	MH605576
31	*Leptobrachella liui*	China: Mt. Wuyi, Fujian	SYS a002478	MH605573
32	*Leptobrachella liui*	China: Mt. Wuyi, Fujian	SYS a002479	MH605574

ID	Species	Locality	Voucher no.	GenBankNo.16SrRNA
33	*Leptobrachella liui*	China: Mt. Wuyi, Fujian	SYS a001597	KM014547
34	*Leptobrachella liui*	China: Mt. Tongbo, Jiangxi	SYS a001702	KM014548
35	*Leptobrachella liui*	China: Mt. Daiyun, Fujian	SYS a001736	KM014550
36	*Leptobrachella liui*	China: Dongkeng Town, Jingning County, Zhejiang	SYSa002732	MH605575
37	*Leptobrachella liui*	China: Dongkeng Town, Jingning County, Zhejiang	SYSa002733	MH055909
38	*Leptobrachella marmorata*	Malaysia: Borneo	KUHE 53227	AB969289
39	*Leptobrachella maura*	Malaysia: Borneo	SP 21450	AB847559
40	*Leptobrachella maoershanensis*	China: Maoershan, Guangxi	KIZ 019386	KY986931
41	*Leptobrachella melica*	Cambodia: Ratanakiri	MVZ 258198	HM133600
42	*Leptobrachella minima*	Thailand: Chiangmai	/	JN848369
43	*Leptobrachella nyx*	Vietnam: Ha Giang	AMNH A 163810	DQ283381
44	*Leptobrachella oshanensis*	China: Sichuan	SYS a001830	KM014810
45	*Leptobrachella pallida*	Vietnam: Lam Dong	UNS 00511	KU530190
46	*Leptobrachella picta*	Malaysia: Borneo	UNIMAS 8705	KJ831295
47	*Leptobrachella pluvialis*	Vietnam: Lao Cai	MNHN:1999.5675	JN848391
48	*Leptobrachella pyrrhops*	Vietnam: Lam Dong	ZMMU A-5208	KP017575
49	*Leptobrachella pyrrhops*	Vietnam: Lam Dong	ZMMU A-4873 (ABV-00213)	KP017576
50	*Leptobrachella sabahmontana*	Malaysia: Borneo	BORNEENSIS 12632	AB847551
51	*Leptobrachella rowleyae*	Vietnam: Da Nang City, Son Tra	ITBCZ 4113	MG682549
52	*Leptobrachella rowleyae*	Vietnam: Da Nang City, Son Tra	ITBCZ 4114	MG682550
53	*Leptobrachella rowleyae*	Vietnam: Da Nang City, Son Tra	ITBCZ 2790	MG682551
54	*Leptobrachella rowleyae*	Vietnam: Da Nang City, Son Tra	ITBCZ 2783	MG682552
55	*Leptobrachella tengchongensis*	China: Tengchong County, Yunnan	SYS a004596	KU589208
56	*Leptobrachella tengchongensis*	China: Tengchong County, Yunnan	SYS a004598	KU589209
57	*Leptobrachella tengchongensis*	China: Tengchong County, Yunnan	SYS a004600	KU589210
58	*Leptobrachella ventripunctata*	Laos: Phongsaly	MNHN 2005.0116	JN848410
59	*Leptobrachella ventripunctata*	China: Zhushihe, Xishuangbanna, Yunnan	SYS a001768	KM014811
60	*Leptobrachella ventripunctata*	China: Zhushihe, Xishuangbanna, Yunnan	SYS a003957	MH605583
61	*Leptobrachella zhangyapingi*	Thailand: Chiang Mai	KJ-2013	JX069979
62	*Leptobrachium* cf. *chapaense*	Vietnam: Lao Cai	AMS R 171623	KR018126
63	*Pelobates syriacus*	/	MVZ 234658	AY236807
64	*Pelobates varaldii*	/	/	AY236808
65	*Megophrys major*	Vietnam: Kon Tum	AMS R 173870	KY476333

and manually revised, if necessary. Tested in Jmodeltest v2.1.2 (Darriba et al. 2012) with Akaike and Akaike information criteria, the best-fitting nucleotide substitution models are GTR + I + G. Phylogenetic trees were analyzed using maximum likelihood (ML) implemented in RaxmlGUI 1.3 (Silvestro and Michalak 2012), and Bayesian inference (BI) using MrBayes 3.2.4 (Ronquist et al. 2012). For ML analysis, the maximum likelihood tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 60% of bootstrap replicates were collapsed. For BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1000th iteration. The first 25% of samples were discarded as burn-in. Convergence of the markov Chain monte carlo simulations was assessed with PSRF ≤ 0.01 and ESS (effective sample size) value > 200 using Tracer v.1.4 (http://tree.bio.ed.ac.uk/software/tracer/). We also calculated pairwise sequence divergence based on uncorrected *p*-distance using MEGA 6.06 (Tamura et al. 2013).

Morphometrics. Measurements followed Fei et al. (2009) and Rowley et al. (2013), and were taken with digital calipers to the nearest 0.1 mm. These measurements were as follows:

SVL snout-vent length (from tip of snout to vent);
HDL head length (from tip of snout to rear of jaws);
HDW head width (head width of commissure of jaws);
SNT snout length (from tip of snout to anterior corner of eye);
EYE eye diameter (diameter of exposed portion of eyeball);
IOD interorbital distance (minimum distance between upper eyelids);
INDY internasal distance (distance between nares);
TMP tympanum diameter (horizontal diameter of tympanum);
TEY tympanum–eye distance (distance from anterior edge of tympanum to posterior corner of eye);
TIB tibia length (distance from knee to heel);
ML manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
LAHL length of lower arm and hand (distance from tip of the third finger to elbow);
PL pes length (distance from tip of fourth toe to proximal edge of the inner metatarsal tubercle);
HLL hindlimb length (distance from tip of fourth toe to vent).

Sex was determined by direct observation of calls in life, the presence of internal vocal sac openings, and the presence of eggs in abdomen through external inspection. Comparative morphological data of *Leptobrachella* species were obtained from examination of museum specimens (see Appendix 1) and from the references listed in Table 2. Due to the high likelihood of undiagnosed diversity within the genus (Rowley et al. 2016; Yang et al. 2016), where available, we relied on examination of topotypic material and/or original species descriptions.

Table 2. Obtained references of 66 known congeners of the genus *Leptobrachella*, respectively.

ID	*Leptobrachella* species	Literature obtained
1	*L. aereus* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010)	Rowley et al. 2010c
2	*L. alpinus* (Fei, Ye & Li, 1990)	Fei et al. 2009
3	*L. applebyi* (Rowley & Cao, 2009)	Rowley and Cao 2009
4	*L. arayai* (Matsui, 1997)	Matsui 1997
5	*L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
6	*L. baluensis* Smith, 1931	Dring 1983; Eto et al. 2016
7	*L. bidoupensis* (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
8	*L. botsfordi* (Rowley, Dau, & Nguyen, 2013)	Rowley et al. 2013
9	*L. bourreti* (Dubois, 1983)	Ohler et al. 2011
10	*L. brevicrus* Dring, 1983	Dring 1983; Eto et al. 2015
11	*L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010a
12	*L. dringi* (Dubois, 1987)	Inger et al. 1995; Matsui and Dehling 2012
13	*L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
14	*L. firthi* (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
15	*L. fritinniens* (Dehling & Matsui, 2013)	Dehling and Matsui 2013
16	*L. fuliginosa* (Matsui, 2006)	Matsui 2006
17	*L. gracilis* (Günther, 1872)	Günther 1872; Dehling 2012b
18	*L. hamidi* (Matsui, 1997)	Matsui 1997
19	*L. heteropus* (Boulenger, 1900)	Boulenger 1900
20	*L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015a
21	*L. itiokai* Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
22	*L. juliandringi* Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
23	*L. kajangensis* (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
24	*L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
25	*L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
26	*L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
27	*L. lateralis* (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
28	*L. laui* (Sung, Yang & Wang, 2014)	Sung et al. 2014
29	*L. liui* (Fei & Ye, 1990)	Fei et al. 2009; Sung et al. 2014
30	*L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
31	*L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
32	*L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
33	*L. marmorata* (Matsui, Zainudin & Nishikawa, 2014)	Matsui et al. 2014b
34	*L. maura* (Inger, Lakim, Biun & Yambun, 1997)	Inger et al. 1997
35	*L. melanoleuca* (Matsui, 2006)	Matsui 2006
36	*L. melica* (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010b
37	*L. minima* (Taylor, 1962)	Taylor 1962; Ohler et al. 2011
38	*L. mjobergi* Smith, 1925	Eto et al. 2015
39	*L. nahangensis* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
40	*L. natunae* (Günther, 1895)	Günther 1895
41	*L. nokrekensis* (Mathew & Sen, 2010)	Mathew and Sen 2010
42	*L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011

ID	*Leptobrachella* species	Literature obtained
43	*L. oshanensis* (Liu, 1950)	Fei et al. 2009
44	*L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
45	*L. palmata* Inger & Stuebing, 1992	Inger and Stuebing 1992
46	*L. parva* Dring, 1983	Dring 1983
47	*L. pelodytoides* (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011
48	*L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017
49	*L. pictua* (Malkmus, 1992)	Malkmus 1992
50	*L. platycephala* (Dehling, 2012)	Dehling 2012a
51	*L. pluvialis* (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000, 2011
52	*L. puhoatensis* (Rowley, Dau & Cao, 2017)	Rowley et al. 2016
53	*L. purpura* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
54	*L. pyrrhops* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
55	*L. rowleyae* (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
56	*L. sabahmontana* (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
57	*L. serasanae* Dring, 1983	Dring 1983
58	*L. sola* (Matsui, 2006)	Matsui 2006
59	*L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
60	*L. tadungensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
61	*L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
62	*L. tengchongensis* (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2016
63	*L. tuberosa* (Inger, Orlov & Darevsky, 1999)	Inger et al.1999
64	*L. ventripunctata* (Fei, Ye & Li, 1990)	Fei et al. 2009
65	*L. yingjiangensis* (Yang, Zeng & Wang)	Yang et al. 2018
66	*L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

Results

Bayesian inference (BI) and Maximum likelihood (ML) phylogenetic tree were constructed based on DNA sequences of the mitochondrial 16S gene with a total length of 476 bp. The two analyses resulted in essentially identical topologies (Fig. 2) with clustered the population of *Leptobrachella* from Dawuling Forest Station with *L. laui*, *L. liui*, and *L. maoershanensis* with very high node supporting values (1.00 in BI and 91% in ML) and represented a separately evolving lineage. Besides, the population from Mt. Wuhuang was a distinct separately evolving lineage with high node supporting values (1.00/100% in BI and ML). The smallest pairwise genetic divergences between the population from Dawuling Forest Station and all other species of the genus *Leptobrachella* for which comparable sequences were included was 6.0–6.7% (with *L. liui*), and between population from Mt. Wuhuang and all other species was 7.4% (with *L. aerea*) (Table 3). These values were significantly larger than observed pairwise genetic distances between recognized species

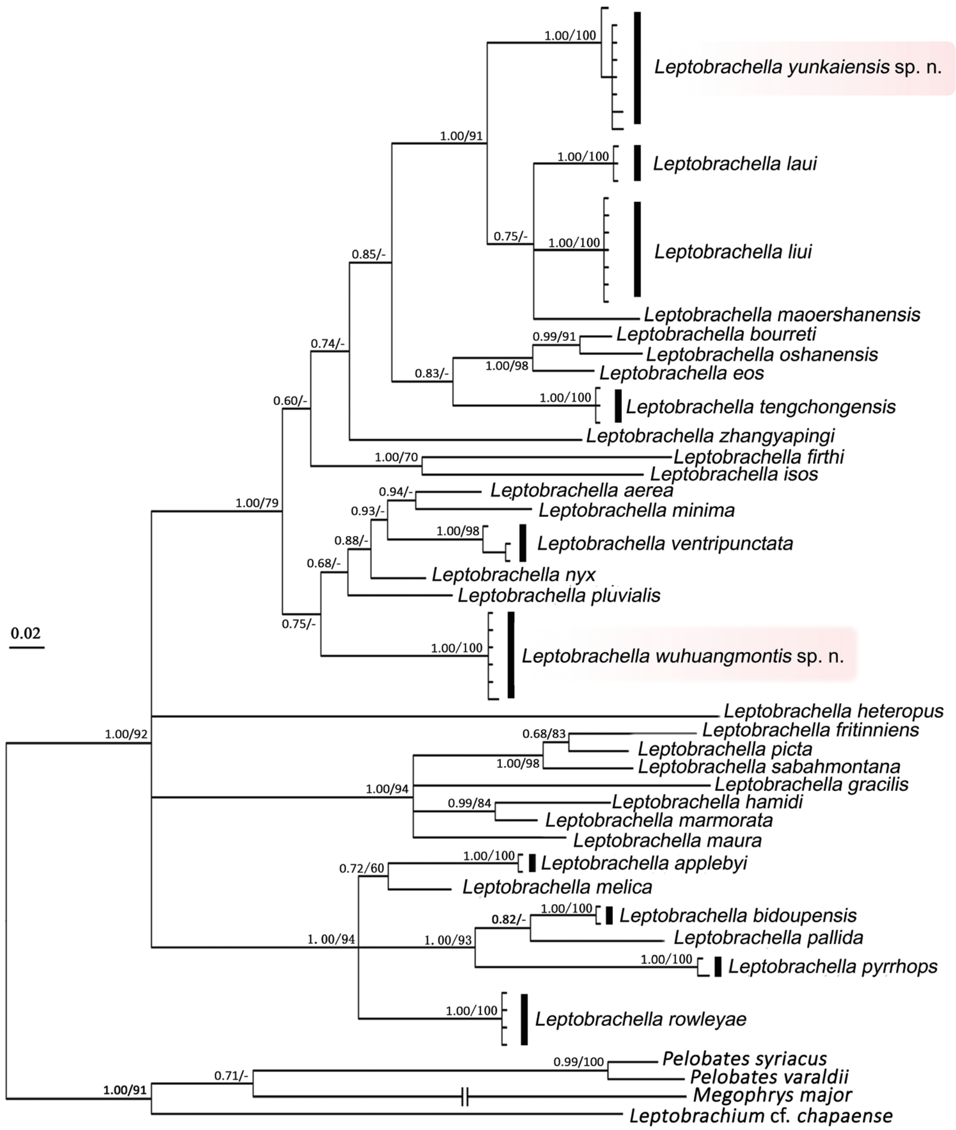

Figure 2. Bayesian inference tree derived from partial DNA sequences of the mitochondrial 16S r RNA gene. Numbers before slashes indicate Bayesian posterior probabilities (>60 retained) and numbers after slashes are bootstrap support for maximum likelihood (1000 replicates) analyses (>60 retained). The symbol "-" represents bootstrap value below 0.60/60%.

(*p*-distance = 2.6%, between *L. bourreti* and *L. oshanensis*). Given that the two populations both can be morphologically distinguished with each other, and from all known congeners, we herein describe these specimens as two new species, respectively.

Table 3. Uncorrected *p*-distances among *L.* species and outgroups based on 16S rRNA fragment (To be continued).

NO.	Species	1–8	9–14	15	16–17	18–19	20	21	22	23	24	25	26	27	28–30	31–37	38	39	40
1–8	*Leptobrachella yunkaiensis* sp. n.	0–0.3																	
9–14	*Leptobrachella wuhuangmontis* sp. n.	11.1–12.3	0–0.3																
15	*Leptobrachella aerea*	10.7–11.5	7.4	0															
16–17	*Leptobrachella applebyi*	15.4–15.9	13.8–14.2	14.5	0														
18–19	*Leptobrachella bidoupensis*	15.6–16.0	13.4–13.5	15.4	9.6	0													
20	*Leptobrachella bourreti*	8.1–8.9	10.3–10.7	10.3	14.3	17.2	0												
21	*Leptobrachella eos*	8.1–8.9	11.1–11.5	11.4	14.7	16.0	3.9	0											
22	*Leptobrachella firthi*	14.1–14.6	13.3–13.7	12.2	16.8	18.3	12.6	13.8	0										
23	*Leptobrachella fritinniens*	18.2–18.6	15.9–16.3	15.1	17.7	14.5	17.3	17.0	17.6	0									
24	*Leptobrachella gracilis*	20.3–20.8	19.9–20.4	18.1	16.4	18.7	19.5	20.8	22.2	13.0	0								
25	*Leptobrachella hamidi*	17.9–18.3	15.4–15.8	15.3	12.7	15.6	16.2	14.3	17.9	9.3	10.7	0							
26	*Leptobrachella heteropus*	20.1–21.0	16.6–17.7	17.5	15.5	17.4	20.5	21.4	22.4	19.6	20.8	17.3	0						
27	*Leptobrachella isos*	12.7–13.1	11.8–12.2	12.1	14.3	13.9	10.4	12.3	12.1	17.3	20.1	14.6	19.6	0					
28–30	*Leptobrachella laui*	6.3–6.7	12.5–12.9	10.7	16.1	17.9	8.8	8.8	13.4	18.0	19.4	15.8	20.8	13.3	0				
31–37	*Leptobrachella liui*	6.0–6.7	9.6	8.9	14.6	14.3	8.1	8.1	12.6	17.3	22.4	16.2	19.2	12.2	5.6	0			

NO.	Species	1–8	9–14	15	16–17	18–19	20	21	22	23	24	25	26	27	28–30	31–37	38	39	40
38	*Leptobrachella marmorata*	16.7–16.8	13.9–14.3	14.5	10.9	15.7	14.7	14.3	15.6	9.6	11.4	4.2	18.2	14.7	16.2	15.1	0		
39	*Leptobrachella maura*	17.5–17.9	15.8	14.5	12.6	15.5	15.8	15.8	18.0	11.1	11.5	8.8	18.6	14.7	17.4	17.0	8.4	0	
40	*Leptobrachella maoershanensis*	6.7–7.1	10.0	18.5	15.1	14.8	9.9	9.9	16.6	18.5	21.2	16.3	19.2	13.4	6.7	5.7	16.3	17.5	0
41	*Leptobrachella melica*	16.6–17.0	13.0	12.6	5.6	9.1	14.3	15.4	17.1	16.1	15.2	12.3	16.0	15.3	16.5	15.7	12.4	13.1	15.8
42	*Leptobrachella minima*	11.1–11.9	10.8–11.2	6.3	15.0	16.0	11.1	11.9	12.8	17.8	20.0	16.6	18.6	13.3	8.9	8.2	15.8	16.6	9.2
43	*Leptobrachella nyx*	9.3–10.0	7.7–8.1	4.9	13.7	14.3	9.2	10.0	10.8	15.6	19.8	15.0	16.6	12.2	8.9	7.1	14.3	15.4	8.9
44	*Leptobrachella oshanensis*	8.5–9.2	11.1–11.5	10.7	15.1	18.1	2.6	5.0	12.6	17.3	19.5	16.7	22.2	11.2	8.1	8.5	16.3	16.3	11.1
45	*Leptobrachella pallida*	16.0–16.5	14.7–15.1	15.8	10.4	5.3	17.6	15.6	18.8	14.4	16.9	14.8	19.0	16.6	16.2	15.2	14.5	14.7	15.6
46	*Leptobrachella picta*	18.4–18.9	16.9–17.4	15.8	14.5	15.7	17.1	17.2	15.9	6.0	11.9	10.3	19.1	17.6	17.1	16.4	8.9	10.7	17.6
47	*Leptobrachella pluvialis*	8.2–8.6	8.2–8.5	6.4	13.9	14.8	10.3	11.1	13.5	16.6	19.2	16.7	16.0	14.2	8.9	7.9	15.2	16.7	6.8
48–49	*Leptobrachella pyrrhops*	14.3–15.5	13.1–14.0	13.5–13.9	12.3–12.7	9.0–9.3	16.7–17.1	16.0–16.5	17.2–17.6	15.8–16.2	17.4–17.8	16.1–16.5	17.0–17.4	14.3–14.7	15.5–15.9	15.2–15.6	15.8–16.2	17.2–17.6	14.4–14.8
50	*Leptobrachella sabahmontana*	17.9–18.4	15.4–15.9	15.4	12.9	15.0	15.8	16.0	15.9	7.0	12.7	10.0	21.3	16.3	17.0	16.3	8.5	8.9	17.9
51–53	*Leptobrachella tengchongensis*	11.1–11.9	12.2–12.6	8.5	15.3	15.8	8.1	7.8	11.2	16.1	21.2	14.2	19.1	9.3	8.1	8.5	14.7	15.4	10.3
54–56	*Leptobrachella ventripunctata*	11.5–12.7	8.5–10.0	6.7–7.7	16.3–16.6	17.5–18.0	11.1–12.3	11.9–13.1	11.1–11.5	15.6–16.5	20.4–21.2	14.7–15.1	18.5	11.5–11.8	10.4–11.6	9.0–10.1	14.0	15.0–15.8	10.0–10.4
57	*Leptobrachella zhangyapingi*	12.5–12.9	13.3	10.6	15.4	16.2	11.0	10.3	13.5	18.9	22.4	18.3	20.5	12.0	10.3	10.6	16.8	16.8	12.1
58–61	*Leptobrachella rowleyae*	16.3–16.7	13.5–14.0	14.3	7.8	10.7	15.1	16.3	17.3	16.8	19.1	14.1	17.8	16.3	15.8	15.1	13.4	14.8	15.1

NO.	Species	1–8	9–14	15	16–17	18–19	20	21	22	23	24	25	26	27	28–30	31–37	38	39	40
62	*Leptobrachium* cf. *chapaense*	25.3–25.7	23.8–24.3	25.5	23.7	27.5	28.4	28.8	29.3	27.4	27.0	24.8	25.7	25.6	26.8	25.6	23.7	22.2	25.2
63	*Pelobates syriacus*	26.6–27.6	24.4–24.9	26.2	23.2	25.0	26.7	27.2	27.2	22.3	22.6	24.0	26.0	29.4	26.1	25.8	22.4	22.6	26.8
64	*Pelobates varaldii*	27.0–28.0	25.1–25.6	25.3	23.1	25.4	25.7	25.4	27.5	22.4	23.9	24.1	27.4	28.9	25.7	25.4	21.6	24.3	27.2
65	*Megophrys major*	28.8–29.9	26.4–27.0	27.6	25.6	28.8	25.8	26.2	30.9	28.1	25.9	25.1	27.3	27.4	29.4	27.6	25.2	23.1	28.2

Table 3. Continued.

NO.	Species	41	42	43	44	45	46	47	48–49	50	51–53	54–56	57	58–61	62	63	64	65
1–8	*Leptobrachella yunkaiensis* sp. n.																	
9–14	*Leptobrachella wuhuangmontis* sp. n.																	
15	*Leptobrachella aerea*																	
16–17	*Leptobrachella applebyi*																	
18–19	*Leptobrachella bidoupensis*																	
20	*Leptobrachella bourreti*																	
21	*Leptobrachella eos*																	
22	*Leptobrachella firthi*																	
23	*Leptobrachella fritinniens*																	
24	*Leptobrachella gracilis*																	
25	*Leptobrachella hamidi*																	
26	*Leptobrachella heteropus*																	
27	*Leptobrachella isos*																	
28–30	*Leptobrachella laui*																	

NO.	Species	41	42	43	44	45	46	47	48–49	50	51–53	54–56	57	58–61	62	63	64	65
31–37	*Leptobrachella liui*																	
38	*Leptobrachella marmorata*																	
39	*Leptobrachella maura*																	
40	*Leptobrachella maoershanensis*																	
41	*Leptobrachella melica*	0																
42	*Leptobrachella minima*	14.6	0															
43	*Leptobrachella nyx*	11.8	5.7	0														
44	*Leptobrachella oshanensis*	15.1	10.4	8.9	0													
45	*Leptobrachella pallida*	11.1	14.7	15.2	16.4	0												
46	*Leptobrachella picta*	14.4	16.5	14.7	17.6	15.3	0											
47	*Leptobrachella pluvialis*	14.2	7.8	6.4	11.1	14.8	15.7	0										
48–49	*Leptobrachella pyrrhops*	12.3–12.7	15.6–16.0	14.4–14.8	16.0–16.4	8.3–8.6	16.4–16.8	14.3–14.8	0.3									
50	*Leptobrachella sabahmontana*	12.9	15.7	15.1	15.9	13.3	5.0	16.9	15.0–15.4	00								
51–53	*Leptobrachella tengchongensis*	14.5	7.8	8.2	8.2	16.7	16.0	10.3	16.8–17.2	16.4	0							
54–56	*Leptobrachella ventripunctata*	15.5–15.9	6.8–7.9	5.0–6.0	11.1–12.3	17.6–18.1	15.5–16.4	8.2–8.6	15.2–15.6	14.8–15.6	9.7–10.8	0–0.1						
57	*Leptobrachella zhangyapingi*	17.0	11.4	9.5	11.1	17.5	18.1	10.3	16.7–17.1	18.5	9.2	11.1–11.5	0					
58–61	*Leptobrachella rowleyae*	6.3	14.7	12.7	15.9	10.8	15.5	13.5	11.2–11.6	13.9	15.0	16.1–16.5	17.1	0				
62	*Leptobrachium* cf. *chapaense*	26.1	25.5	25.7	27.5	26.2	25.9	25.1	23.8–24.3	27.5	26.3	24.4–24.8	29.1	25.9	0			
63	*Pelobates syriacus*	23.5	26.4	24.9	26.2	25.6	21.1	27.9	24.3–24.7	20.8	28.2	25.4–26.9	28.6	24.7	21.3	0		
64	*Pelobates varaldii*	23.5	27.4	24.0	25.7	25.8	21.2	27.8	25.5–26.0	21.6	28.1	24.5–25.9	27.2	25.0	23.1	3.6	0	
65	*Megophrys major*	27.1	30.5	28.0	26.6	30.3	27.6	28.2	29.0–29.5	27.2	26.0	26.2–27.7	31.3	29.2	27.9	24.3	22.4	0

Systematics

Leptobrachella yunkaiensis Wang, Li, Lyu & Wang, sp. n.

http://zoobank.org/CE563BA1-D6F5-40BE-ADEC-324190B239EA
Figures 3, 4C1–C3

Holotype. SYS a004665, adult male, collected on 15 April 2016 by Jian Wang (JW hereafter), Zhao-Chi Zeng (ZCZ hereafter), Ying-Yong Wang (YYW hereafter), Zu-Yao Liu (ZYL hereafter), Hai-Long He (HLH hereafter) and Zhi-Tong Lyu (ZTL hereafter) from Dawuling Forest Station (DWL hereafter) (22°16'32.9"N, 111°11'42.87"E; 1600 m a.s.l.), Maoming City, Guangdong Province, China.

Paratypes. Collectors and locality data of paratypes were the same as holotype: adult males, SYS a004664 / CIB107272, SYS a004666–4669 and an adult female SYS a004663, collected on 15 April 2016, the other adult female, SYS a004690, collected on 16 April 2017.

Diagnosis. (1) small size (SVL 25.9–29.3 mm in males, 34.0–35.3 mm in females), (2) dorsal skin shagreened with short skin ridges and raised warts, (3) iris bicolored, coppery orange on upper half and silver on lower half, (4) tympanum distinctly discernible, slightly concave, weakly black supratympanic line present, (5) dorsal surface yellowish-brown grounding, with distinct darker brown markings and rounded spots and scattered with irregular orange patches, (6) flanks with several dark blotches, (7) surface of belly pinkish, with distinct or indistinct light dark brown speckling, (8) supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible, (9) absence of webbing and presence of distinct lateral fringes on fingers, toes with rudimentary webbing and wide lateral fringes, (10) longitudinal ridges under toes not interrupted at the articulations, and (11) dense conical spines present on lateral and ventral surface of tarsus, surface of tibia-tarsal, inner-side surface of shank and surface around cloacal region.

Comparisons. Comparative morphological data of *Leptobrachella yunkaiensis* sp. n. with 66 recognized *Leptobrachella* species were obtained from examination of museum specimens (see Appendix 1) and from the references listed in Table 2. All comparative data were shown in Tables 4, 5.

Compared with the 24 known congeners of the genus *Leptobrachella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, *L. yunkaiensis* sp. n. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana* and *L. sola*, all of which lacking supra-axillary and ventrolateral glands; and by the significantly larger body size, SVL 25.9–29.3 mm in males, 34.0–35.3 mm in females, *L. yunkaiensis* sp. n. differs from the smaller *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males and 18.9–19.1 mm in females), *L. mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in male), *L. parva* (15.0–16.9 mm in males and 17.8 mm in female), L. palmata (14.4–16.8 mm in males), *L. serasanae* (16.9 mm in female) and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (15.0–16.0 mm in males).

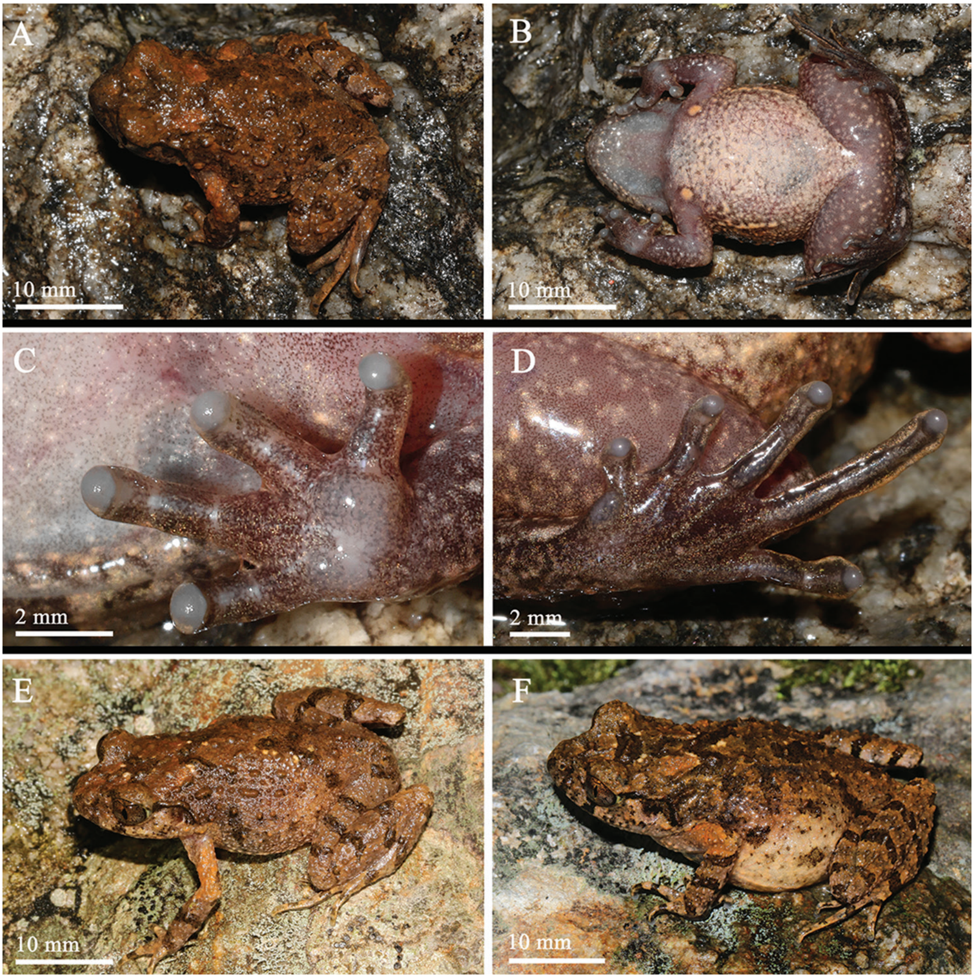

Figure 3. General aspect in life: **A–D** SYS a004665, the male holotype of *Leptobrachella yunkaiensis* sp. n. **E** SYS a004667, the male paratype **F** SYS a004690, the female paratype.

Leptobrachella yunkaiensis sp. n. is most similar to *L. laui* and *L. liui*, but it can be distinguished by the larger body sized, SVL 34.0–35.3 mm in females (vs. SVL 28.1 mm in a single female of *L. laui*; SVL 23.0–28.0 mm in females of *L. liui*), presence of short skin ridge and raised warts on dorsum (vs. absent in *L. laui*), black supratympanic line weak (vs. black supratympanic line distinct in *L. liui*), longitudinal ridges under toes not interrupted at the articulations (vs. interrupted in *L. liui*) (Fig. 4), belly pinkish with distinct or indistinct speckling (vs. belly creamy white with dark brown dusting on margins in *L. laui*; belly creamy white with dark brown spots on chest and margins in *L. liui*).

From the remaining 40 known congeners (Table 5), with SVL 25.9–29.3 mm in six males, SVL 34.0–35.3 mm in two females in *Leptobrachella yunkaiensis* sp. n., it can be distinguished from the larger *L. eos* (males 33.1–34.7 mm, female 40.7 mm), *L. nahangensis* (male 40.8 mm), *L. pyrrhops* (males 30.8–34.3 mm), *L. sungi* (males

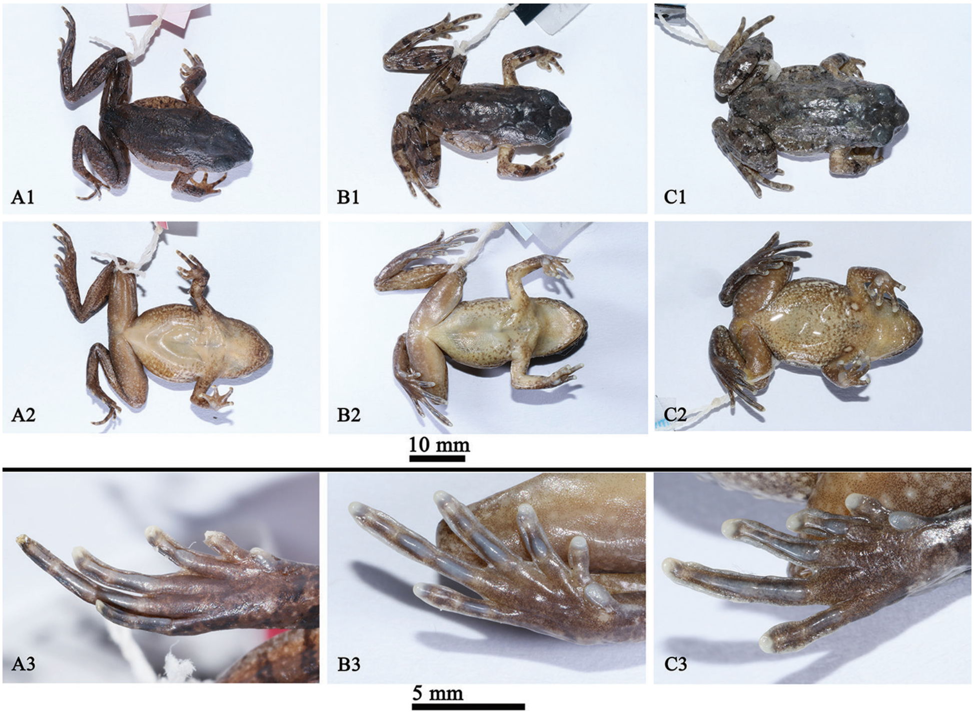

Figure 4. Specimens in preservative: **A1–A3** SYS a002957, the holotype of *Leptobrachella laui* **B1–B3** SYS a005925, the topotype of *L. liui* **C1–C3** SYS a004665, the holotype of *L. yunkaiensis* sp. n..

48.3–52.7 mm, females 56.7–58.9 mm) and *L. zhangyapingi* (males 45.8–52.5 mm), and the smaller *L. applebyi* (males 19.6–22.3 mm, females21.7–25.9 mm), *L. melica* (males 19.5–22.7 mm), and *L. pluvialis* (males 21.3–22.3 mm). By having wide fringes on toes, the new species differs from *L. applebyi*, *L. ardens*, *L. crocea*, *L. kalonensis*, *L. lateralis*, *L. maculosa*, *L. macrops*, *L. melica*, *L. minima*, *L. nahangensis*, *L. nyx*, *L. oshanensis*, *L. pallida*, *L. pluvialis*, *L. pyrrhops*, *L. rowleyae*, *L. tadungensis*, *L. tuberosa,* and *L. ventripunctata*, all of which have no lateral fringes on toes; *L. bidoupensis*, *L. bourreti*, *L. fuliginosa*, and *L. sungi*, all of which have weak lateral fringes on toes; *L. botsfordi*, *L. maoershanensis*, *L. pelodytoides*, *L. petrops*, *L. puhoatensis,* and *L. tengchongensis*, all of which have narrow lateral fringes on toes; *L. alpinus*, *L. firthi,* and *L. isos*, all of which have wide lateral fringes only in males. With rudimentary webbing on toes, the new species differs from *L. ardens*, *L. kalonensis*, *L. maculosa*, *L. oshanensis*, *L. pallida*, *L. petrops*, *L. rowleyae*, and *L. tadungensis*, all of which have no webbing on toes; *L. pelodytoides*, *L. sungi,* and *L. tamdil*, all of which have wide webbing on toes. By having black spots on flanks, the new species differs from *L. aerea*, *L. botsfordi*, *L. eos*, *L. firthi*, *L. isos*, *L. pallida*, *L. petrops*, *L. tuberosa,* and *L. zhangyapingi*, all of which have no black spots on flanks. With belly pink with distinct or indistinct speckling, the new species differs from *L. bourreti*, *L. eos*, *L. firthi*, *L. khasiorum*, *L. lateralis*, *L. minima*, *L. nahangensis,* and *L. nokrekensis*, all of which have creamy white belly

Table 4. Measurements (minimum–maximum (mean ± SD); in mm), and body proportions of *Leptobrachella yunkaiensis* sp. n. from Dawuling Forest Station.

Measurements	Males (n = 6)	Females (n = 2)
SVL	25.9–29.3 (27.6 ± 1.4)	34.0–35.3 (34.7 ± 0.9)
HDL	9.3–10.3 (9.9 ± 0.4)	12.2–12.6 (12.4 ± 0.2)
HDW	9.0–10.0 (9.7 ± 0.4)	12.0–12.2 (12.1 ± 0.1)
SNT	3.6–3.8 (3.7 ± 0.1)	4.4–4.7 (4.6 ± 0.2)
EYE	3.4–3.7 (3.6 ± 0.1)	3.8–3.9 (3.9 ± 0.1)
IOD	2.7–2.9 (2.8 ± 0.1)	3.0–3.2 (3.1 ± 0.1)
IND	2.5–2.8 (2.7 ± 0.1)	2.9–3.0 (3.0 ± 0.1)
TMP	1.5–1.7 (1.6 ± 0.1)	2.0
TEY	0.7–0.8 (0.8 ± 0.1)	1.0
TIB	12.2–12.8 (12.5 ± 0.2)	15.0–15.2 (15.1 ± 0.2)
ML	5.8–7.3 (6.9 ± 0.6)	7.4–7.8 (7.6 ± 0.2)
PL	10.8–12.4 (11.9 ± 0.6)	12.7–12.9 (12.8 ± 0.1)
LAHL	12.0–12.6 (12.3 ± 0.2)	14.7–15.0 (14.8 ± 0.2)
HLL	37.0–40.3 (38.7 ± 1.2)	47.0–49.5 (48.3 ± 1.8)
HDL/HDW	1.01–1.03 (1.02 ± 0.01)	1.02–1.03 (1.02 ± 0.01)
HDL/SVL	0.34–0.39 (0.36 ± 0.02)	0.36
SNT/HDL	0.36–0.41 (0.38 ± 0.02)	0.37
SNT/ED	1.03–1.06 (1.05 ± 0.02)	1.16–1.21 (1.18 ± 0.03)
EYE/TMP	2.12–2.40 (2.25 ± 0.13)	1.90–1.95 (1.93 ± 0.04)
TMP/EYE	0.42–0.47 (0.45 ± 0.03)	0.51–0.53 (0.52 ± 0.01)
TEY/TMP	0.47–0.53 (0.48 ± 0.03)	0.50
TIB/SVL	0.43–0.48 (0.45 ± 0.02)	0.43–0.44 (0.44 ± 0.01)
LAHL/SVL	0.43–0.47 (0.45 ± 0.02)	0.42–0.43 (0.43 ± 0.01)
HLL/SVL	1.33–1.51 (1.41 ± 0.06)	1.38–1.40 (1.39 ± 0.01)
TIB/HLL	0.31–0.33 (0.32 ± 0.01)	0.31–0.32 (0.32 ± 0.01)

without patterns; from *L. macrops*, which have greyish-violet with white speckling; from *L. purpura*, which have dull white belly with indistinct grey dusting; and from *L. yingjiangensis*, which have creamy white belly with dark brown flecks on chest and margins. By dorsal skin shagreened with short skin ridges and raised warts, the new species differs from *L. purpura*, *L. yingjiangensis* and *L. tengchongensis*, all of which have shagreened dorsal skin with small tubercles, and from *L. macrops*, which have no skin ridges dorsally.

Description of holotype. Adult male. Body size small, SVL in 28.7 mm. Head length slightly larger than head width, HDL/HDW 1.03; snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril equidistance between snout and eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, larger internarial distance; pineal ocellus absent; vertical pupil; snout length slightly larger than eye diameter, SNT/EYE 1.03; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye and larger than tympanum-eye distance, TMP/EYE 0.46 and TEY/TMP 0.47; weakly black supratympanic line

Table 5. Selected diagnostic characters for species described herein and species in the genus *Leptobrachella* occurring north of the Isthmus of Kra (modified from Rowley et al. 2017; Yuan et al. 2017).

Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
L. yunkaiensis sp. n.	25.9–29.3	Yes	Rudimentary	Wide	Belly pink with distinct or indistinct speckling	Shagreened with short skin ridges and raised warts
L. wuhuangmontis sp. n.	25.6–30.0	Yes	Rudimentary	Narrow	Greyish white mixed by tiny white and black dots	Rough, scattered with dense conical tubercles
L. aerea	25.1–28.9	No	Rudimentary	Wide	Near immaculate creamy white, brown specking on margins	Finely tuberculate
L. alpinus	24.0–26.4	Yes	Rudimentary	Wide in males	Creamy-white with dark spots	Relatively smooth, some with small warts
L. applebyi	19.6–22.3	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
L. ardens	21.3–24.7	Yes	No	No	Reddish brown with white speckling	Smooth- finely shagreened
L. bidoupensis	18.5–25.4	Yes	Rudimentary	Weak	Reddish brown with white speckling	Smooth
L. botsfordi	29.1–32.6	No	Rudimentary	Narrow	Reddish brown with white speckling	Shagreened
L. bourreti	28.0–36.2	Yes	Rudimentary	Weak	Creamy white	Relatively smooth, some with small warts
L. crocea	22.2–27.3	No	Rudimentary	No	Bright orange	Highly tuberculate
L. eos	33.1–34.7	No	Rudimentary	Wide	Creamy white	Shagreened
L. firthi	26.4–29.2	No	Rudimentary	Wide in males	Creamy white	Shagreened with fine tubercles
L. fuliginosa	28.2–30.0	Yes	Rudimentary	Weak	White with brown dusting	Nearly smooth, few tubercles
L. isos	23.7–27.9	No	Rudimentary	Wide in males	Creamy white with white dusting on margins	Mostly smooth, females more tuberculate
L. kalonensis	25.8–30.6	Yes	No	No	Pale, speckled brown	Smooth
L. khasiorum	24.5–27.3	Yes	Rudimentary	Wide	Creamy white	Isolated, scattered tubercles
L. lateralis	26.9–28.3	Yes	Rudimentary	No	Creamy white	Roughly granular
L. laui	24.8–26.7	Yes	Rudimentary	Wide	Creamy white with dark brown dusting on margins	Round granular tubercles
L. liui	23.0-28.7	Yes	Rudimentary	Wide	Creamy white with dark brown spots on chest and margins	Round granular tubercles with glandular folds
L. macrops	28.0–29.3	Yes	Rudimentary	No	Greyish-violet with white speckling	Roughly granular with larger tubercles
L. maculosa	24.2–26.6	Yes	No	No	Brown, less white speckling	Mostly smooth

Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
L. maoershanensis	25.2–30.4	Yes	Rudimentary	Narrow	Creamy white chest and belly with irregular black spots	Longitudinal folds
L. melica	19.5–22.7	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
L. minima	25.7–31.4	Yes	Rudimentary	No	Creamy white	Smooth
L. nahangensis	40.8	Yes	Rudimentary	No	Creamy white with light specking on throat and chest	Smooth
L. nokrekensis	26.0–33.0	Yes	Rudimentary	unknown	Creamy white	Tubercles and longitudinal folds
L. nyx	26.7–32.6	Yes	Rudimentary	No	Creamy white with white with brown margins	Rounded tubercles
L. oshanensis	26.6–30.7	Yes	No	No	Whitish with no markings or only small, light grey spots	Smooth with few glandular ridges
L. pallida	24.5–27.7	No	No	No	Reddish brown with white speckling	Tuberculate
L. pelodytoides	27.5–32.3	Yes	Wide	Narrow	Whitish	Small, smooth warts
L. petrops	23.6–27.6	No	No	Narrow	Immaculate creamy white	Highly tuberculate
L. pluvialis	21.3–22.3	Yes	Rudimentary	No	Dirty white with dark brown marbling	Smooth, flattened tubercles on flanks
L. puhoatensis	24.2–28.1	Yes	Rudimentary	Narrow	Reddish brown with white dusting	Longitudinal skin ridges
L. purpura	25.0–27.5	Yes	Rudimentary	Wide	Dull white with indistinct grey dusting	Shagreen with small tubercles
L. pyrrhops	30.8–34.3	Yes	Rudimentary	No	Reddish brown with white speckling	Slightly shagreened
L. rowleyae	23.4–25.4	Yes	No	No	Pinkish milk-white to light brown chest and belly with numerous white speckles	Smooth with numerous tiny tubercles
L. sungi	48.3–52.7	No or small	Wide	Weak	White	Granular
L. tadungensis	23.3–28.2	Yes	No	No	Reddish brown with white speckling	Smooth
L. tamdil	32.3	Yes	Wide	Wide	White	Weakly tuberculate
L. tengchongensis	23.9–26.0	Yes	Rudimentary	Narrow	White with dark brown blotches	Shagreened with small tubercles
L. tuberosa	24.4–29.5	No	Rudimentary	No	White with small grey spots/streaks	Highly tuberculate
L. ventripunctata	25.5–28.0	Yes	Rudimentary	No	Chest and belly with dark brown spots	Longitudinal skin ridges
L. yingjiangensis	25.7–27.6	Yes	Rudimentary	Wide	Creamy white with dark brown flecks on chest and margins	Shagreened with small tubercles
L. zhangyapingi	45.8–52.5	No	Rudimentary	Wide	Creamy-white with white with brown margins	Mostly smooth with distinct tubercles

present; vomerine teeth absent; vocal sac openings slit-like, located posterolaterally on floor of mouth in close proximity to the margins of the mandible; tongue deeply notched behind; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland; tubercles present on supratympanic ridge.

Tips of fingers rounded, slightly swollen; relative finger lengths I = II = IV < III; nuptial pad absent; subarticular tubercles absent; a large, rounded inner palmar tubercle distinctly separated from small, round outer palmar tubercle; absence of webbing and presence of distinct lateral fringes on fingers. Tips of toes like fingers; relative toe length I < II < V < III < IV; subarticular tubercles absent; distinct dermal ridges present under the 3rd to 5th toes; large, oval inner metatarsal tubercle present, outer metatarsal tubercle absent; toes webbing rudimentary; wide lateral fringes present on all toes. Tibia 43% of snout-vent length; tibiotarsal articulation reaches to middle of eye; heels just meeting each other when thighs are appressed at right angles with respect to body.

Skin on dorsum shagreened and scattered with fine, round tubercles; short skin ridges and raised warts on dorsum surface present; ventral skin smooth; pectoral gland and femoral gland large, oval; pectoral glands greater than tips of fingers and femoral glands; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary gland raised. Ventrolateral gland distinctly visible, forming an incomplete line. Dense conical spines on lateral and ventral surface of tarsus, surface of tibia-tarsal, inner-side surface of shank and surface around cloacal region present.

Measurements of holotype (in mm). SVL 28.7, HDL 10.3, HDW 10.0, SNT 3.8, EYE 3.7, IOD 2.9, IND 2.8, TMP 1.7, TEY 0.8, TIB 12.4, ML 7.2, PL 12.1, LAHL 12.3, HLL 38.3.

Coloration of holotype in life. Dorsal surface orange-brown with distinct dark brown blotches edged distinct light orange pigmentation. A dark brown triangular pattern between eyes, connected to the dark brown W-shaped marking between axillae. Tympanum black. Orange-brown tubercles present on dorsum of body and limb, those on flanks much distinct and dense; anterior upper lip with distinct blackish brown patches; transverse dark brown bars on dorsal surface of limbs; indistinct dark brown blotches on flanks from groin to axilla; elbow and upper arms without dark bars but with distinct coppery orange coloration; fingers and toes with indistinct dark brown blotches.

Surface of throat creamy white and scattered with small whitish dots; belly pinkish and scattered with small brown speckling; ventral surface of thighs pinkish and scattered with small light orange-brown spots. Supra-axillary coppery orange; femoral, pectoral and ventrolateral glands whitish orange. Iris bicolored, coppery orange on upper half and silver on lower half.

Coloration of holotype in preservative. Dorsum of body and hindlimbs dark brown while dorsum of forelimbs yellowish brown; transverse bars on limbs become more distinct, dark brown patterns, markings and spots on back become indistinct. Ventral surface of body yellowish brown, with brown marbling on sides and chest. Orange supra-axillary, femoral, pectoral and ventrolateral glands fade to greyish white (Fig. 4C1–C3).

Figure 5. The habitat of *Leptobrachella yunkaiensis* sp. n. in Dawuling Forest Station of Guangdong Province.

Sexual dimorphism. Females with a larger body size than males, SVL 34.0–35.3 mm (34.7 ± 0.9) (vs. SVL 25.9–29.3 mm (27.6 ± 1.4) in males); presence of a single vocal sac in males (vs. absent in females); dense conical spines on lateral and ventral surface of tarsus, surface of tibia-tarsal, inner-side surface of shank, surface of thighs and surface around cloacal region distinct in males, and barely visible in females.

Variations. All paratypes match the overall characters of the holotype except that: the heels just meeting each other when thighs are appressed at right angles with respect to body, tibiotarsal articulation reaches to middle of eye in holotype SYS a004665 (vs. tibiotarsal articulation reaches to anterior corner of eye in SYS a004666, reaches the posterior corner of eye in SYS a004669). Surface of belly scattered with distinctly dark brown speckling in holotype (vs. such speckling indistinct in female paratypes SYS a004663, 4690. Tympanum black in the holotype (vs. tympanum black grounding with orange speckling in SYS a004667–4668). Distinct black spots present on dorsum in the female paraype SYS a004690 (Fig. 3).

Etymology. The specific epithet, *yunkaiensis*, is in reference to the type locality, DWL of Guangdong, China located in the Yunkai Mountains Range. For the common name, we suggest "Yunkai Mountain's Leaf Litter Toad", and Chinese name "Yun Kai Zhang Tu Chan (云开掌突蟾)".

Distribution and habits. Currently, *Leptobrachella yunkaiensis* sp. n. is known only from its type locality DWL of Guangdong Province (Fig. 1). The new species was found along a clear-water rocky stream (ca. 2–3 m in width and ca. 20–30 cm in depth) and small nearby seeps in well-preserved montane evergreen broadleaf forest (1600 m a.s.l.) (Fig. 5). During April and June, males were found calling mainly hidden under leaf litter, and some were found calling perching on the rocks or under rocks by the side of the stream. Females collected on April bear pure white oocytes.

Leptobrachella wuhuangmontis **Wang, Yang & Wang, sp. n.**

http://zoobank.org/C87E92AA-081E-480B-839C-27CED127F6CA
Figures 6, 7

Holotype. SYS a003486, adult male, collected on 29 March 2015 by JW, ZTL, YYW and ZYL from Mt. Wuhuang (MWH hereafter) (22°08'30.77"N, 109°24'43.90"E; 500 m a.s.l.), Pubei County, Qinzhou City, Guangxi Province, China.

Paratypes. Adult males SYS a000578, 581 and an adult female SYS a000580, collected on 28 April 2009 by Jian-Huan Yang (JHY hereafter) and Run-Lin Li (RLL hereafter), adult males SYS a003487–3489, 3505–3506, SYS a003500 / CIB107274 and adult females SYS a003485, 3499, 3504,, collected from 29–30 March 2015 by JW, ZTL, YYW and ZYL, all from the same locality as the holotype.

Diagnosis. (1) small size (SVL 25.6–30.0 mm in males, 33.0–36.0 mm in females), (2) dorsal surface rough with skin ridges and dense conical tubercles, (3) iris bicolored, coppery yellow on upper half and silver on lower half, (4) tympanum distinctly discernible, slightly concave, dark brown, distinct black supratympanic line present, (5) dorsal surface greyish purple background with dark brown markings and scattered with orange-yellow blotches and white speckling, (6) distinct dark blotches on flanks, (7) ventral surface greyish white mixed by tiny white and black dots, (8) lateral fringes on fingers absent, (9) toes with narrow lateral fringes and rudimentary webbing, (10) longitudinal ridges under toes not interrupted at the articulations, and (11) dense conical spines on lateral and ventral surface of tarsus, dorsal surface of tibia-tarsal and surface of inner-side shank and surface around cloacal region.

Comparisons. Comparative morphological data of *Leptobrachella wuhuangmontis* sp. n. with the 66 recognized *Leptobrachella* species were obtained from examination of museum specimens (see Appendix 1) and from the references listed in Table 2. All comparative data were shown in Tables 4, 5, 6.

Compared with the 24 known congeners of the genus *Leptobrachella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, *L. wuhuangmontis* sp. n. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana,* and *L. sola*, all of which lacking supra-axillary and ventrolateral glands; and by the significantly larger body size, SVL 25.6–30.0 mm in males, 33.0–36.0 mm in females, *L. wuhuangmontis* sp. n. differs from the smaller *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males and 18.9–19.1 mm in females), *L. mjobergi* (15.7–19.0 mm in males), *L. natu-*

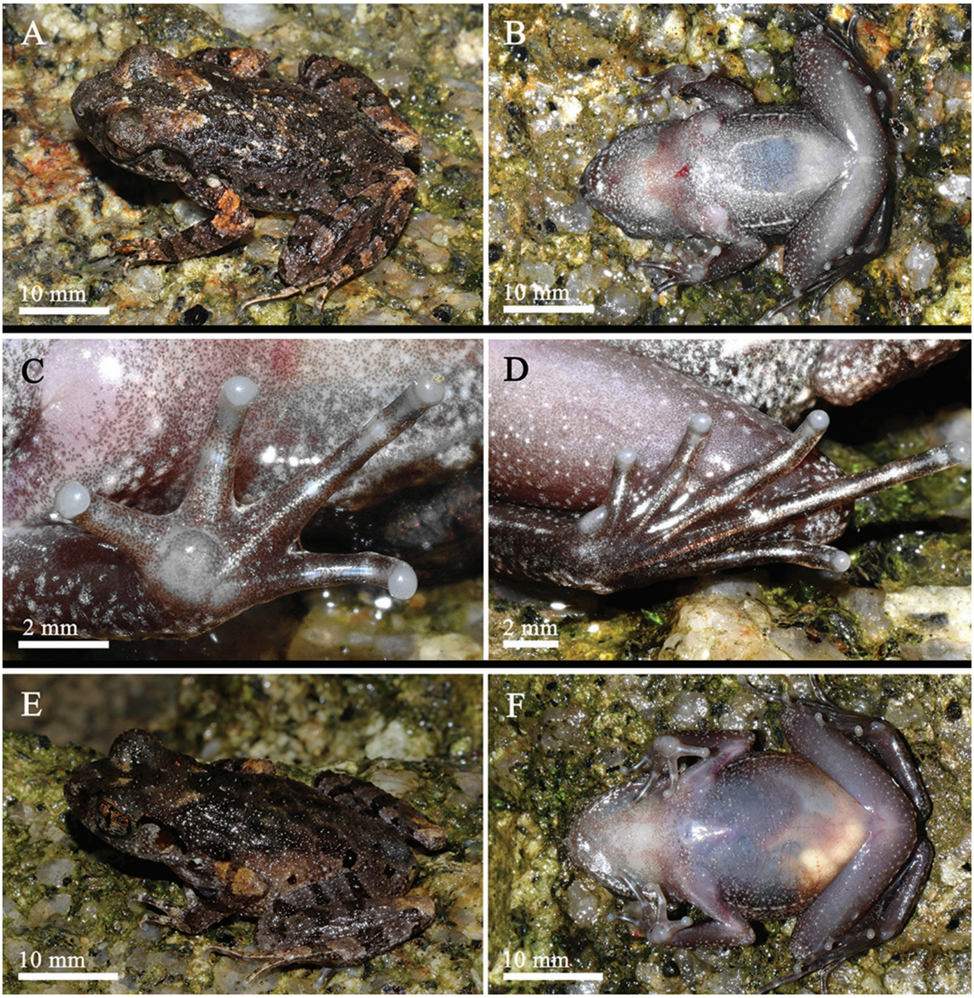

Figure 6. General aspect in life of SYS a003486 (**A–D**), the male holotype of *Leptobrachella wuhuangmontis* sp. n. and the female paratype SYS a003499 (**E, F**).

nae (17.6 mm in male), *L. parva* (15.0–16.9 mm in males and 17.8 mm in female), L. palmata (14.4–16.8 mm in males), *L. serasanae* (16.9 mm in female) and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (15.0–16.0 mm in males).

Leptobrachella wuhuangmontis sp. n. significantly differs from *L. yunkaiensns* sp. n. by a large genetic divergence (*p*=10.2–11.1%), lateral fringes on toes narrow (vs. wide), black supratympanic line distinct (vs. weak), dorsal surface of body rough and scattered with dense conical tubercles (vs. shagreened with short skin ridges and raised warts), belly greyish white mixed by tiny white and black dots (vs. belly pink with distinct or indistinct speckling).

From the rest 42 known congeners (Table 5), with SVL 25.6–30.0 mm in nine males and 33.0–36.0 mm in four females, *Leptobrachella wuhuangmontis* sp. n. differs

Table 6. Measurements (minimum–maximum (mean ± SD); in mm), and body proportions of *Leptobrachella wuhuangmontis* sp. n. from Mt. Wuhuang.

Measurements	Males (n = 9)	Females (n = 4)
SVL	25.6–30.0 (28.5 ±1.5)	33.0–36.0 (34.9 ± 1.4)
HDL	10.5–11.5 (10.9 ± 0.4)	12.4–12.6 (12.5 ± 0.1)
HDW	10.0–11.2 (10.5 ± 0.4)	12.1–12.3 (12.2 ± 0.1)
SNT	3.6–4.4 (4.1 ± 0. 2)	4.6–4.7 (4.6 ± 0.1)
EYE	3.5–4.4 (4.0 ± 0.3)	4.5–4.6 (4.6 ± 0.1)
IOD	2.8–3.0 (2.9 ± 0.1)	3.1–3.3 (3.2 ± 0.1)
IND	2.9–3.2 (3.1 ± 0.1)	3.2–3.4 (3.3 ± 0.1))
TMP	2.1–2.6 (2.4 ± 0.1)	2.6–2.8 (2.7 ± 0.1)
TEY	0.7–0.9 (0.8 ± 0.1)	0.8–0.9 (0.9 ± 0.1)
TIB	12.5–13.6 (13.3 ± 0.3)	15.0–16.3 (15.7 ± 0.5)
ML	7.0–8.0 (7.6 ± 0.3)	8.0–9.2 (8.5 ± 0.5)
PL	11.7–13.0 (12.5 ± 0.5)	13.9–14.8 (14.4 ± 0.4)
LAHL	14.2–16.0 (14.9 ± 0.6)	15.8–17.0 (16.4 ± 0.5)
HLL	38.8–44.9 (42.8 ± 1.9)	47.5–54.0 (51.2 ± 2.9)
HDL/HDW	1.03–1.06 (1.04 ± 0.01)	1.02–1.03 (1.03 ± 0.01)
HDL/SVL	0.36–0.41 (0.38 ± 0.02)	0.35–0.38 (0.36 ± 0.01)
SNT/HDL	0.34–0.40 (0.38 ± 0.02)	0.37 (0.37 ± 0)
SNT/ED	1.00–1.08 (1.03 ± 0.03)	1.00–1.02 (1.01 ± 0.01)
EYE/TMP	1.56–1.79 (1.68 ± 0.06)	1.64–1.73 (1.69 ± 0.04)
TMP/EYE	0.58–0.64 (0.60 ± 0.02)	0.58–0.61 (0.59 ± 0.01)
TEY/TMP	0.28–0.38 (0.33 ± 0.04)	0.30–0.33 (0.31 ± 0.02)
TIB/SVL	0.45–0.50 (0.47 ± 0.02)	0.44–0.47 (0.45 ± 0.01)
LAHL/SVL	0.50–0.55 (0.52 ± 0.02)	0.46–0.49 (0.47 ± 0.02)
HLL/SVL	1.45–1.54 (1.50 ± 0.03)	1.41–1.52 (1.47 ± 0.05)
TIB/HLL	0.29–0.33 (0.31 ± 0.01)	0.29–0.32 (0.31 ± 0.01)

from the larger *L. bourreti* (females 42.0–45.0 mm), *L. eos* (males 33.1–34.7 mm, female 40.7 mm), *L. lateralis* (female 36.6 mm), *L. nahangensis* (male 40.8 mm), *L. nyx* (females 37.0–41.0 mm), *L. sungi* (males 48.3–52.7 mm, females 56.7–58.9 mm), *L. tamdil* (male 32.3 mm) and *L. zhangyapingi* (males 45.8–52.5 mm); and from the smaller *L. aerea* (females 28.8–28.9 mm), *L. ardens* (female 24.5 mm), *L. alpinus* (females 32.1–32.5 mm in), *L. applebyi* (males 19.6–20.8 mm, female 21.7 mm), *L. bidoupensis* (males 18.5–25.4 mm), *L. botsfordi* (females 30.0–31.8 mm), *L. kalonensis* (females 28.9–30.6 mm), *L. laui* (female 28.1 mm), *L. liui* (females 23.0–28.0 mm), *L. maculosa* (female 27.0 mm), *L. maoershanensis* (female 29.1 mm), *L. melica* (males 19.5–22.7 mm), *L. oshanensis* (female 31.6 mm), *L. pluvialis* (males 21.0–22.0 mm), *L. puhoatensis* (females 27.3–31.5 mm), *L. rowleyae* (females 27.0–27.8 mm), *L. tadungensis* (female 32.1 mm), and *L. tengchongensis* (females 28.9–28.9 mm). Having head longer than wide in the new species (vs. head wider than long in *L. bourreti*, *L. khasiorum*, *L. lateralis* and *L. sungi*, and head width equal to or wider than long in *L. nokrekensis*). By having narrow fringes on toes, the new species differs from *L. applebyi*, *L. ardens*, *L. crocea*, *L.*

kalonensis, *L. lateralis*, *L. maculosa*, *L. macrops*, *L. melica*, *L. minima*, *L. nahangensis*, *L. nyx*, *L. oshanensis*, *L. pallida*, *L. pluvialis*, *L. pyrrhops*, *L. rowleyae*, *L. tadungensis*, *L. tuberosa,* and *L. ventripunctata*, all of which have no lateral fringes on toes; *L. bidoupensis*, *L. bourreti*, *L. fuliginosa,* and *L. sungi*, all of which have weak lateral fringes on toes; *L. alpinus*, *L. firthi* and *L. isos*, all of which have wide lateral fringes only in males; *L. aerea*, *L. eos*, *L. khasiorum*, *L. laui*, *L. liui*, *L. purpura*, *L. tamdil*, *L. yingjiangensis,* and *L. zhangyaping*, all of which have wide lateral fringes both in males and females. By having rudimentary webbing on toes, the new species differs from *L. ardens*, *L. kalonensis*, *L. maculosa*, *L. oshanensis*, *L. pallida*, *L. petrops*, *L. rowleyae,* and *L. tadungensis*, all of which have no webbing on toes; *L. pelodytoides*, *L. sungi,* and *L. tamdil*, all of which have wide webbing on toes. By having black spots on flanks, the new species differs from *L. aerea*, *L. botsfordi*, *L. eos*, *L. firthi*, *L. isos*, *L. pallida*, *L. petrops*, *L. tuberosa,* and *L. zhangyapingi*, all of which have no black spots on flanks. By having rough dorsal skin with skin ridges and dense conical tubercles, the new species differs from *L. applebyi*, *L. bidoupensis*, *L. kalonensis*, *L. melica*, *L. minima*, *L. nahangensis,* and *L. tadumgensis*, all of which have smooth dorsal skin, and from *L. purpura*, *L. tengchongensis,* and *L. yingjiangensis*, all of which have shagreened dorsal skin with small tubercles.

Description of holotype. Adult male. Body size small, SVL in 30.0 mm. Head slightly longer than wide, HDL/HDW 1.04; snout rounded in dorsal view, nostril rounded, closer to tip of snout than to eye; canthus rostralis distinct; lores slightly concave; eye large, diameter equal to snout length, in 4.3 mm; tympanum distinct, rounded and slightly concave, its diameter significantly shorter than eye, TMP/EYE 0.56; distinct black supratympanic line present; vomerine teeth absent; vocal sac opening slit-like; tongue deeply notched behind; supratympanic ridge distinct, running from eye towards supra-axillary gland with raised tubercles.

Tips of fingers rounded, slightly swollen; relative finger lengths I = II < IV < III; nuptial pad absent; subarticular tubercles absent; a large, rounded inner palmar tubercle distinctly separated from small, round outer palmar tubercle; finger webbing absent and lateral fringes absent. Tips of toes like fingers; relative toe length I < II < V < III < IV; subarticular tubercles absent; dermal ridges undeveloped but present under the 3rd to 5th toes; large, oval inner metatarsal tubercle present, outer metatarsal tubercle absent; toes webbing rudimentary; narrow lateral fringes present on all toes. Tibia 45% of snout-vent length; tibiotarsal articulation reaches to middle of eye; heels just meeting each other when thighs are appressed at right angles with respect to body.

Skin on dorsum body and limbs rough with skin ridges and dense conical tubercles, ventral skin smooth; pectoral gland and femoral gland large, oval, slightly elevated; femoral gland situated on posterovertral surface of thigh, closer to knee than to vent; supra-axillary gland raised. Ventrolateral gland distinct, forming an incomplete line. Dense conical spines present on surface of lateral and ventral tarsus, surface of tibia-tarsal, inner-side surface of shank and surface around cloacal region.

Measurements of holotype (in mm). SVL 30.0, HDL 10.9, HDW 10.5, SNT 4.3, EYE 4.3, IOD 2.9, IND 3.0, TMP 2.4, TEY 0.8, TIB 13.5, ML 7.8, PL 13.0, LAHL 15.4, HLL 44.9.

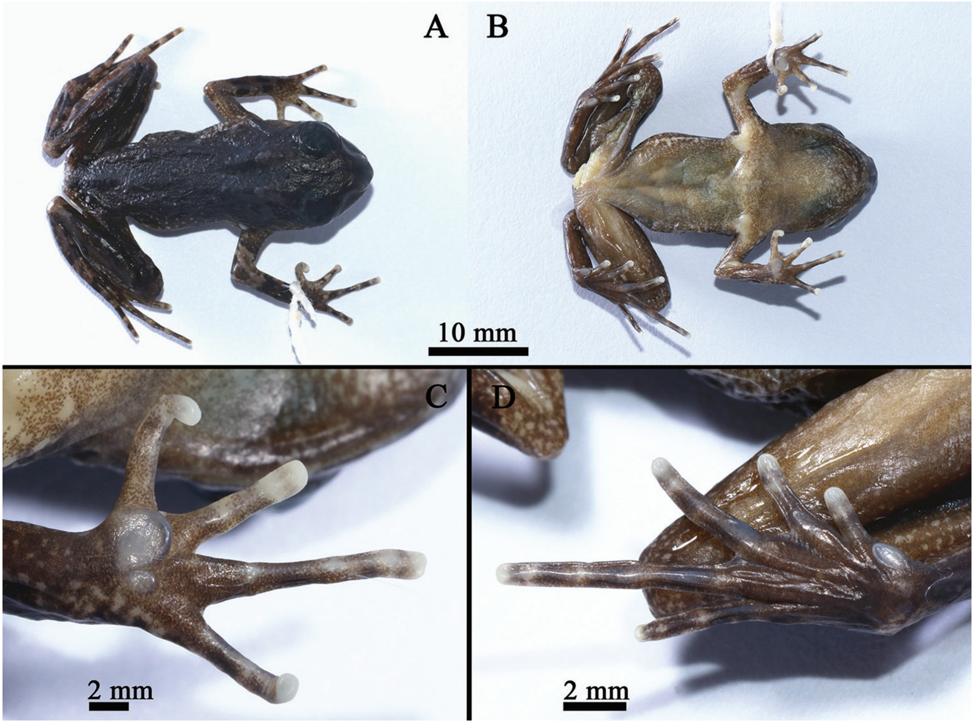

Figure 7. The holotype of *Leptobrachella wuhuangmontis* sp. n., SYS a003486 in preservative.

Coloration of holotype in life. Dorsal surface greyish purple with distinct dark brown markings and scattered with yellow blotches; distinct small white speckling present on edges of dark markings. A distinct dark brown triangle pattern between eyes, connected to the incomplete W-shaped dark brown marking between axillae. Tubercles on dorsum of body and limbs brown, those on lower flanks somewhat whitish; anterior upper lip with distinct blackish brown patches; transverse dark brown bars on dorsal surface of limbs; distinct dark brown blotches on flanks from groin to axilla; elbow and upper arms coppery orange and with distinct dark bars; fingers and toes with distinct dark brown blotches.

Ventral surface greyish-white mixed with tiny white and black dots. Supra-axillary, femoral, and ventrolateral glands white, pectoral gland greyish white as the color of ventral surface. Iris bicolored, coppery yellow on upper half and silver on lower half.

Coloration of holotype in preservative. Dorsal of body dark with greyish white dots on flanks, while dorsal of limbs dark brown, transverse bars on dorsal of forelimbs become more distinct, and indistinct on dorsal of hindlimbs, dark brown patterns, markings and spots on back become indistinct. Ventral surface light yellow with brown speckling. Supra-axillary, femoral, ventrolateral and pectoral glands light yellow (Fig. 7).

Sexual dimorphism. Females with a larger body size than males, SVL 33.0–36.0 mm (34.9 ± 1.4) (vs. SVL 25.6–30.0 mm (28.5 ±1.5) in males); presence of a single vocal sac in males (vs. absent in females); dense conical spines on lateral and ventral

Figure 8. The habitat of *Leptobrachella wuhuangmontis* sp. n. in Mt. Wuhuang of Guangxi Province.

surface of tarsus, surface of tibia-tarsal, inner-side surface of shank and surface around cloacal region distinct in males (vs. barely visible in females); pectoral gland and femoral gland large, oval, slightly elevated in males (vs. indistinct in females).

Variations. All paratypes match the overall characters of the holotype except that: tibiotarsal articulation reaches to posterior corner of eye in female paratypes SYS a003499, 3504 and reaches to anterior corner of eye in male paratypes SYS a003487 and SYSa 003500 / CIB 107274; pectoral gland large, oval, slightly elevated in all individuals in life, and become indistinct in preservation. Yellow blotches and white speckling present on dorsum in the holotype (vs. indistinct in the female paratype SYS a003499). Elbow and upper arms coppery orange and with distinct dark bars in the holotype (vs. elbow and upper arms light orange, dark bars indistinct in the male paratypes SYS a003488, 3505 and the female paratype SYS a003499) (Fig. 6).

Etymology. The specific epithet, *wuhuangmontis*, is in reference to the type locality, Mt. Wuhuang of Guangxi Province, China. For the common name, we suggest "Mt. Wuhuang's Leaf Litter Toad", and for the Chinese name "Wu Huang Shan Zhang Tu Chan (五皇山掌突蟾)".

Distribution and habits. Currently, *Leptobrachella wuhuangmontis* sp. n. is only known from its type locality MWH from Guangxi Province of China (Fig. 1). The new species was found along a clear-water rocky streams and small steep rocky streams in well-preserved montane evergreen broadleaf forest (500 m a.s.l.) (Fig. 8). During field surveys in March, males were found calling exposed on the rocks or hiding in the rock seams; gravid female collected on March and April bear pure white oocytes.

Discussion

Studies of the taxonomy and phylogeny of *Leptobrachella* are difficult to perform because of the morphological conservativeness and very similar characters (for example, the coloration and the texture of skin) in different environments, which may cause misidentifications (Ohler et al. 2010; Sung et al. 2014). With the evidence of both morphological and phylogenetic analyses, 15 cryptic species of the genus *Leptobrachella* have been discovered and described since 2010 (Frost 2017; Rowley et al. 2016, 2017; Yang et al. 2016; Yuan et al. 2017). With the description of *L. yunkaiensis* sp. n. and *L. wuhuangmontis* sp. n. based on an taxonomical approach, the number of the genus *Leptobrachella* herein is increased to 68, indicating the underestimated diversity.

During our examination, it was observed that the dense tiny conical spines present on the surface of the lateral and ventral aspects of the tarsus, surface of tibiatarsal, the inner surface of the shank and surface around cloacal region (distinct in males and barely visible in females) in the two new *Leptobrachella* species described in this study are also present in examined specimens of *L. alpinus*, *L. laui*, *L. liui*, and *L. tengchongensis* as well as in other cryptic taxa (Wang et al. unpublished data). Thus, this neglected morphological character may be common among congeners of the genus *Leptobrachella*, and further morphological studies are needed to study this in more detail.

Mt. Wuhuang of Guangxi Province in southern China is known for the extraordinarily high biodiversity, with some new national records discovered in recent years, for example, the national records of *Opisthotropis maculosa* and *Sphenomorphus tonkinensis* from Mt. Wuhuang were recorded (Wang et al. 2013; Yang et al. 2011). Except for the new species (*Leptobrachella yunkaiensis* sp. n.) described in this study, several new species of amphibians and reptiles have been discovered from Dawuling Forest Station during field surveys in the last two years (Wang et al. unpublished data; Wang et al. 2018; Lyu et al. 2018), which suggests a high herpetofaunal biodiversity of Dawuling Forest Station localized in western Guangdong Province, China. Recently, these areas have been subjected to tourism development; thus, conservation strategies and measures for references and enforcements are urgently needed.

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Appendix 1

Specimens examined

Leptobrachella alpinus (n = 6): China: Yunnan Province: Jingdong County: Mt. Wuliang: CIB 24353 (Holotype), CIB 24354; SYS a 003927.

Leptobrachella laui (n = 26): China: Hong Kong: SYS a002057 (Holotype), SYS a002058; China: Guangdong Province: Shenzhen City: SYSa 001505–1507, 1515–1521, 3471–3472, 5644–5645.

Leptobrachella liui (n = 18): China: Fujian Province: Mt. Wuyi: CIB 24355 (Holotype), CIB 24356, SYS a001571–1578, 1595–1599, 2478–2479, 5925–5826.

Leptobrachella tengchongensis (n = 6): China: Yunnan Province: Baoshan City: Mt. Gaoligong: SYS a004600 (Holotype), 4596–4599, 4601–4602.

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RESEARCH ARTICLE

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A critical evaluation of the exotic bird collection of the Šariš Museum in Bardejov, Slovakia

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Abstract

A collection of exotic birds deposited in the Šariš Museum in Bardejov (SMB), Slovakia, has not been evaluated critically since their deposition. We assessed the accuracy of identification of 465 bird specimens deposited in SMB with native distributions outside of Slovakia. Specimens belonged to 322 species of 82 families and 26 orders. Of the specimen represented, 34 belonged to species considered as 'near-threatened' (7.3%), 16 as 'vulnerable' (3.4%) and one as 'endangered' (0.2%). The SMB collection holds 10 of 28 extant Cuban endemic species and another 11 species endemic to the Caribbean archipelago. Even among birds that are relatively easy to identify, many specimens were identified incorrectly or species identification was missing. Of 465 specimens evaluated, 95 (20.4%) were identified incorrectly or were missing species identification, and another 79 (17%) were identified correctly, but their names have changed over time due to taxonomic shift, thus they required correction.

Keywords

Aves, biodiversity, museum, ornithological collections, species occurrence data

Introduction

Natural history collections have long served as a primary data source for addressing fundamental questions in systematics, biogeography, and conservation of organisms. Specimens in such collections represent an important source of documentation of present and past occurrences of species with each specimen being unique and irreplaceable (Winker et al. 1991, Wandeler et al. 2007, Ariño 2010, Kress 2014). Specimens provide a window into evolutionary processes in natural populations, enabling researchers to study evolution on timescales similar to those from long-term field studies or experiments in laboratories (Holmes et al. 2016). However, many natural history collections around the world are at risk in view of declining funding and expenses of adequate upkeep (Winker et al. 1991, 2010, Joseph 2011, Gardner et al. 2014, Krell and Wheeler 2014, Kemp 2015). Many collections, including the most renowned ones, still hold numerous specimens lacking identification or awaiting revision (Winker et al. 1991), to the extent that several bird species new to science are discovered each year (del Hoyo et al. 2013), often through the re-evaluation of museum specimens, mostly using new approaches such as genetic methods (Winker et al. 1991).

Natural ecosystems are currently changing at unprecedented rates owing to human activity, affecting both terrestrial and marine ecosystems (Vitousek et al. 1997, Ellis and Ramankutty 2008, Barnosky et al. 2011, Dirzo et al. 2014, Ceballos et al. 2015). For instance, roughly half of the world's terrestrial surface area has undergone conversion to grazed land or cultivated crops (Kareiva et al. 2007) and ~60% of the world's largest terrestrial herbivores are threatened with extinction owing to over-hunting, land-use change, and competition with livestock (Ripple et al. 2015). Although birds tend to be less threatened than other vertebrates (Hoffmann et al. 2010), several bird species have shown recent shifts in distribution and abundance as a result of human-induced environmental change (Thomas et al. 2004, Inger et al. 2015). Most vulnerable are species with small geographic distributions, particularly island species (e.g. Steadman 1995, Blackburn et al. 2004, Hoeck et al. 2011). However, not only rare species are endangered; for instance, over the last three decades, bird populations in Europe declined by ~20%, with many common species suffering steep declines (Kress 2014, Inger et al. 2015).

Museum collections include specimens collected in various places and times that provide important insights into long-term consequences of natural or anthropogenic environmental changes (Glenn et al. 1999, Godoy et al. 2004, Kuhn et al. 2013, Gardner 2014, Kress 2014, Mason and Unitt 2018). The importance of specimens has actually increased recently as specimen collection has been criticised increasingly, thus, obtaining of new voucher specimens is much complicated, often even stopped (Minteer et al. 2014, Waeber et al. 2017). Most museum-based studies are carried out in large, well-known collections as they are more accessible to scientific public (Mearns and Mearns 1998), however, thanks to technological development and public access to the internet, information from all natural history collections, including those smaller and / or less important ones, is becoming much more accessible (e.g. Navarro-Sigüenza et al. 2003, Graham et al. 2004, Peterson et al. 2016). This visibility increases the global importance of local museums and their collections, which were often unavailable to the wider scientific community (Monteiro et al. 2016, 2017).

One of the important European ornithological collections is held by the Natural History Department of the Šaris Museum in Bardejov (SMB), Slovakia (Roselaar 2003, Hromada et al. 2015). This collection was established by the prominent Slovak zoologist, Tibor Weisz, in 1956, with the majority of the bird specimens collected by the department's founder himself during 1956–1983 (Hromada et al. 2015). The collection is unique in several respects: besides building the general collection, the collector focused on several particular species, obtaining not only the largest series of some species in the world, but also systematically covering with his vouchers the long time period of four to five decades (Hromada et al. 2015). Compared to other collections (Mearns and Mearns 1998), data associated with bird collection in SMB are quite rich, including more measurements and notes, such as data on condition (general health), size of gonads, notes on colouration, and habitat and behaviour; specimens also include other associated voucher material (sternum, stomach, ecto- and endoparasites, egg clutches, etc.) (Tryjanowski et al. 2001, Hromada et al. 2003, Hromada and Klimovičová 2015, Hromada et al. 2015). The ornithological collection is focused predominantly on Slovak birds (5047 specimens of 251 species and 61 families; Hromada et al. 2015), but Weisz collected and maintained also collection of "exotic" species (not occurring in Slovakia). The majority of exotic specimens was collected by Weisz himself and taxidermist Vilém Borůvka (Hromada 2015) during a visit to Cuba (1968); others were received in exchange from foreign collectors (e.g., A. Kovács, Argentina; N. H. Gustafsson, Denmark). The bird collection was first catalogued by Weisz during his work at SMB; species were identified by his peer ornithologist Aristid Mošanský (Hromada 2015), but since that time no update or revision has been carried out.

Hence, our aim is a systematic and critical re-evaluation of the species identities of the exotic bird specimens in SMB collection, for several reasons. (1) In Weisz's time in SMB, it was problematic for Eastern bloc scientists to get good (if any) handbooks on birds, particularly for New World birds. (2) Recently, many bird clades have seen taxonomic revision, often resulting in splits of species and updates in nomenclature. Lack of recent and ongoing comprehensive taxonomic re-evaluation of specimens causes many specimens to have inaccurate names. (3) Despite the collection is held in small, local museum, it contains specimens and/or species important from global point of view, thus, it is crucial to make it publicly available. Therefore, we present here the results of a first detailed review of the exotic birds deposited in SMB, complementing a recent study dedicated to Slovak birds in SMB (Hromada et al. 2015).

Methods

Because the bulk of the exotic specimens were in exhibition, it was often not possible to base identification work on the in-hand specimens *per se*. Hence, we photographed all exotic bird specimens (i.e. those with distributions outside Slovakia) and data cards associated with data on identification, locality, date, sex, and catalogue number. We then visually inspected all photos and identified species using the online edition of "Handbook of the Birds of the World" (del Hoyo et al. 2017). If species identification was at all questionable, we asked experts (see acknowledgements) and members of online birding communities (e.g. http://www.birdforum.net) for help. All specimens with detailed locality given were georeferenced (see Suppl. material 1).

We were unable to find occurrence records for several specimens. To make information on these specimens as complete as possible, we added locality country and date for some based on our knowledge of when and where Weisz collected specimens. (1) Specimens marked as collected by T. Weisz (i.e. not received in exchange from other collectors) without given locality or date were associated with Cuba and the year 1968 (when Weisz and Borůvka visited Cuba) if their distributions included Cuba. We know of no other visit of Weisz to the Neotropical region nor any specimen exchanges with Cuban origins. (2) Specimens of species endemic to other countries were associated with that country, except for domesticated species (e.g., *Syrmaticus reevesii* (Gray, 1829)). (3) If date of collection was unknown but we had information on date of acquisition in SMB we used that date as in most cases the two dates were the same. The only exception was by 1977 when Weisz registered many old specimens; specimens registered in 1977 were thus left without a date if no date of collection was provided. However, if information on locality and / or date was not found in original documentation but added by us by aforementioned procedure, this was always clearly highlighted for each specimen in Suppl. material 1. Following identification, each species was then checked as to conservation status (IUCN Red List of Threatened Species, version 3.1; http://www.iucnredlist.org/static/categories_criteria_3_1) in the following categories: (1) Least Concern (LC), (2) Near Threatened (NT), (3) Vulnerable (VU), and (4) Endangered (EN).

Finally, we paid special attention to bird specimens originating in Cuba and the Caribbean region, especially those species endemic to this region, according to the online bird checklist, Avibase (http://avibase.bsc-eoc.org/), under "Handbook of the Birds of the World Alive" (del Hoyo et al. 2017) taxonomy. To address the importance of Cuban specimens deposited in SMB, we conducted searches on publicly accessible databases of global vertebrate biodiversity, such as VertNet (http://www.vertnet.org/) and Global Biodiversity Information Facility (GBIF; https://www.gbif.org/; we focused on number of preserved specimens only, excluding, for instance, human observations) (accessed on 25.10.2017).

Results

In total, SMB collection comprises 465 specimens (267 specimens are in public exhibition, 198 in scientific collection) of exotic bird species (for specimen details see Suppl. material 1). We were able to identify 454 specimens to species, in 11 specimens species identification remains questionable. Specimens identified belonged to 322 species, 82

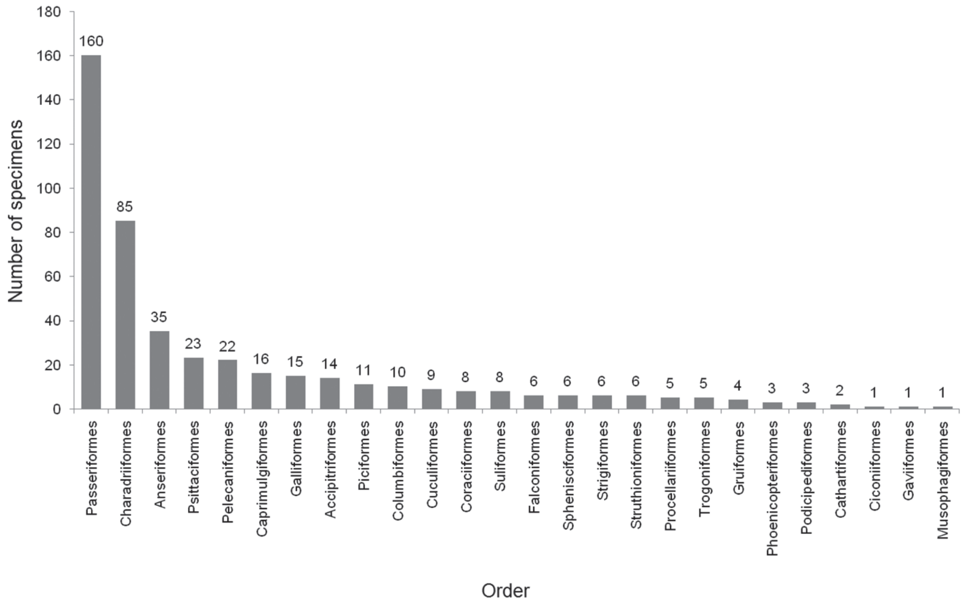

Figure 1. Total number of specimens per order represented in the exotic bird collection of the Šariš Museum in Bardejov, Slovakia.

families, and 26 orders. Most families were in the order Passeriformes (40.2%), followed by Charadriiformes (13.4%) and Pelecaniformes (4.9%). The most specimen-rich orders were Passeriformes (34.4%), Charadriiformes (18.3%), and Anseriformes (7.5%). Six orders (Cathartiformes, Ciconiiformes, Gaviiformes, Musophagiformes, Phoenicopteriformes and Podicipediformes) were represented by three or fewer specimens (Figure 1).

SMB holds a collection focused on the birds of Cuba and Caribbean archipelago. This collection holds 21 specimens that pertain to 10 of 28 extant Cuban endemic species and another 21 specimens corresponding to 11 species endemic to the Caribbean archipelago (Table 1).

Beside Cuba, specimens in the SMB exotic bird collection came from at least 22 countries. Most specimens with identified locality of collection at least on the country level came from Argentina (140), followed by Cuba (130), Denmark (including Greenland) (14), Germany (11), and Australia (8). Two exotic bird species often kept as pets were also included – *Melopsittacus undulatus* (Shaw, 1805) and *Serinus canaria* f. *domestica* (Linnaeus, 1758). We were not able to associate country of collection for 120 specimens (Figure 2). The SMB exotic bird collection covered years 1957–1981. Most specimens were collected in 1968, during the expedition to Cuba and 1970–1972 via exchanges with the Argentine collector, A. Kovács. We were unable to associate year of collection / acquisition year for 86 individuals (Figure 3).

The majority of SMB specimens belonged to species classified by the IUCN Red List as LC (414 specimens, 89%), followed by NT (34 specimens, 7.3%), VU (16 specimens, 3.4%) and EN (one specimen of *Oxyura leucocephala* (Scopoli, 1769), 0.2%).

Table 1. Bird species endemic to (a) Cuba and (b) the Caribbean region hold by the Šaris Museum in Bardejov, Slovakia, including number of specimens in public exhibition and the scientific collection and their conservation status according to the IUCN Red List. From online biodiversity databases, VertNet and GBIF, we obtained information on numbers each species deposited in other natural history collections worldwide.

Common name	Scientific name	Exhibition	Collection	VertNet†	GBIF†	IUCN Red List‡
(a) Cuban endemic species						
Cuban Black Hawk	*Buteogallus gundlachii*	1	0	5 (13)[1]	6 (26)	NT
Cuban Pygmy Owl	*Glaucidium siju*	1	0	205	258	LC
Cuban Trogon	*Priotelus temnurus*	1	1	253	336	LC
Cuban Tody	*Todus multicolor*	1	2	288	354	LC
Cuban Green Woodpecker	*Xiphidiopicus percussus*	1	0	252	340	LC
Cuban Gnatcatcher	*Polioptila lembeyei*	3	0	69	130	LC
Cuban Blackbird	*Ptiloxena atroviolacea*	1	1	7 (12)[2]	15 (169)	LC
Cuban Grassquit	*Phonipara canora*	2	0	0 (48)[3]	14 (346)	LC
Cuban Parakeet	*Psittacara euops*	0	1	7 (73)[4]	11 (128)	VU
Cuban Oriole	*Icterus melanopsis*	5	0	19 (188)[5]	30 (287)	LC
(b) Carribean endemic species						
Cuban Emerald	*Chlorostilbon ricordii*	1	2	349	420	LC
Cuban Lizard-cuckoo	*Coccyzus merlini*	1	1	28 (222)[5]	2 (380)	LC
West Indian Woodpecker	*Melanerpes superciliaris*	2	0	472	623	LC
Cuban Amazon	*Amazona leucocephala*	1	2	349	501	NT
Loggerhead Kingbird	*Tyrannus caudifasciatus*	1	1	614	914	LC
La Sagra's Flycatcher	*Myiarchus sagrae*	1	0	299 (14)[6]	437 (16)	LC
Cuban Pewee	*Contopus caribaeus*	0	1	351	527	LC
Cuban Crow	*Corvus nasicus*	1	0	81	138	LC
Red-Legged Thrush	*Turdus plumbeus*	1	1	548	1040	LC
Cuban Bullfinch	*Melopyrrha nigra*	3	0	224	325	LC
Greater Antillean Grackle	*Quiscalus niger*	1	0	804	1037	LC

†Number in brackets is a number of results from search using alternative species names: [1]*Buteogallus anthracinus*, [2]*Dives atroviolaceus*, both only with origin from Cuba; [3]*Tiaris canorus*; [4]*Aratinga euops*; [5]*Icterus dominicensis*; [5]*Saurothera merlini*; [6]*Myiarchus stolidus* with origin from Cuba
‡LC – Least concern, NT – Near threatened, VU – Vulnerable, EN – Endangered.

Out of 465 specimens, 291 (62.6%) were identified correctly, with scientific names that are still valid; another 69 (14.8%) specimens were correctly identified but names have changed over time. In 10 cases (2.2%), the species was treated as conspecific with other species, but current taxonomy recognised them as separate; in 21 (4.5%) specimens, the identification was incorrect; and 63 (13.6%) specimens had species identification missing at the time of our revision. In 10 cases (2.2%), species identification was previously missing, and we remain unsure of identification; in one case (0.2%), species identification was previously incorrect and we are not sure about the correct identification.

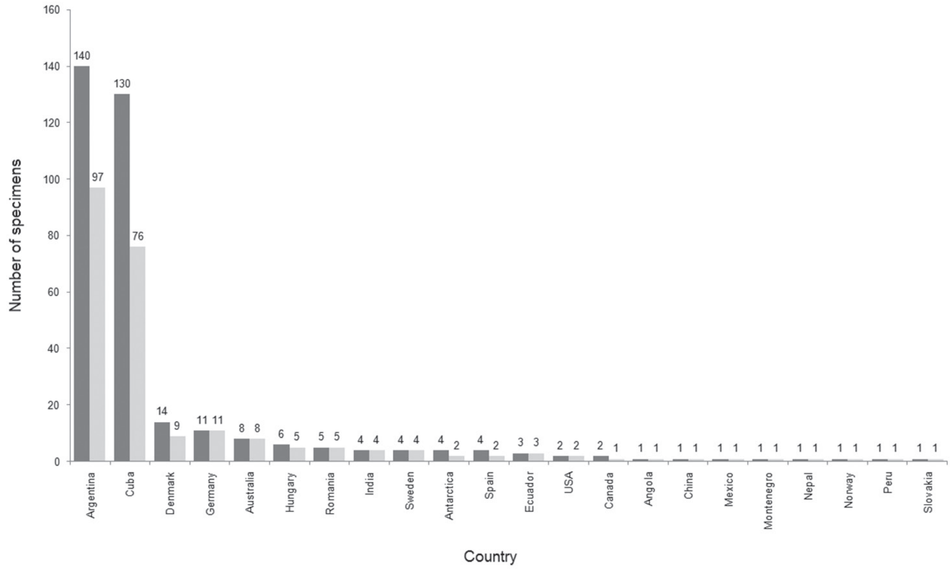

Figure 2. The geographic distribution of the Šariš Museum in Bardejov exotic bird collection with number of bird specimens (dark grey) and species (light grey) collected from each country.

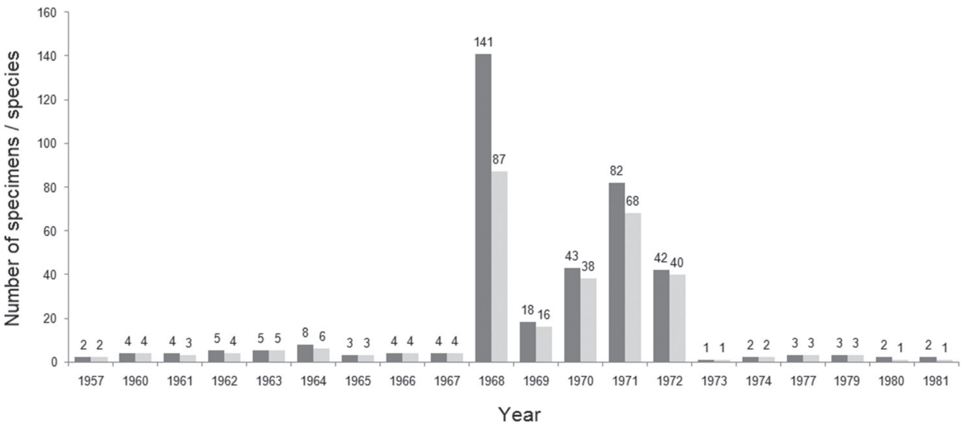

Figure 3. Temporal distribution of numbers of bird specimens (dark grey) and species (light grey) added to the Šariš Museum in Bardejov exotic bird collection during 1957–1981.

Discussion

Value of the SMB exotic bird collection

The Caribbean archipelago is known for high levels of species endemism, and forms part of a world hotspot region of endemism (Orme et al. 2005). Cuba is the largest island of the Caribbean region, with 28 out of 180 extant bird species endemic to this island. Many more endemic species vanished during subrecent to recent times owing to human activity (Milberg and Tyrberg 1993). Terrestrial ecosystems of Caribbean region are still under increasing pressure from human populations appropriating large portions of their distributional areas (Haberl et al. 2007). The SMB collection is thus valuable because it harbours one-third of all species endemic to Cuba, some of which are poorly represented in world collections, including one specimen of *Buteogallus gundlachii* (Cabanis 1855) for which only a very small number of specimens is registered on VertNet and GBIF (Table 1). Specimen data on rare species of Caribbean region from other collections were recently used, for instance, for species distribution modelling of charismatic and presumably extinct *Campephilus principalis* (Lammertink 1995, Gotelli et al. 2012).

The SMB exotic bird collection included 51 specimens of 33 species classified as near threatened or higher threat categories. We highlight a male specimen of the endangered species *Oxyura leucocephala*, which populations undergone fragmentation and rapid declines in recent decades, resulting in loss of genetic diversity (Muñoz-Fuentes et al. 2005). The specimen was collected in the breeding season (8. June) 1960 in Soltvadkert, Hungary. The last confirmed breeding of this species in Hungary was in 1961 (Green and Anstey 1992) and the breeding population of the species is now extinct there (Birdlife International 2017). From other examples, the collections included specimens of the vulnerable species *Phoenicoparrus andinus* (Philippi, 1854) and *Buteo ventralis* Gould, 1837 (both collected from Argentina), with only seven and 18 specimens, respectively, of these species from Argentina registered in GBIF (accessed on 2.11.2017).

We showed that many specimens in the SMB collection are rare and poorly represented in scientific collections in other museums. Hence, our results may help recognise the importance of the collection by responsible authorities and take actions that would provide adequate maintenance of the specimens in the collection. Despite the fact that many of the important specimens are on public display, we do not expect that this could affect their availability for scientific research because SMB is open to making the collection available to the scientific as well as broader community, e. g. in order to provide data to international databases. However, public display is a danger to specimens in several ways (e. g. damage by pests, fading, etc.). Fortunately, the museum regime currently provides protection to some extent to specimens placed in public exhibition because the direct sunlight in the exhibition is eliminated (no windows are present there) and the artificial light is switched on only when visitors are inside (number of visitors is low in general).

Moreover, specimens from the SMB exotic bird collection enabled description of several new species of obligate bird parasites, particularly quill mites (Acari: Syringophilidae), living within the feather calamus (Skoracki and Sikora 2002, 2004, Hromada and Klimovičová 2015). Probably most importantly, the first record of a parasitic quill mite from a palaeognath bird, *Eudromia elegans* Geoffroy Saint-Hilaire, 1832, named *Tinamiphilopsis elegans* Skoracki & Sikora, 2004, was described in 2004 as a new genus (Skoracki and Sikora 2004). Until then, quill mites were known only in neognath species (Skoracki and Sikora 2004).

Identification issues

Correct species identifications are essential in research, with key implications, for instance, for systematics, biogeography, and conservation (Johansson et al. 2013, 2018, Tritsch et al. 2017). Accuracy of identifications varies significantly between taxa, even when identified by experts; in some groups, such as insects, overall accuracy may be quite low (Austen et al. 2016). The situation is even more problematic in plants for which as many as half of plant specimens in tropical collections may have wrong identifications (Goodwin et al. 2015). In contrast, birds are relatively easy to identify (Panhuis et al. 2001, Edwards et al. 2005).

Nonetheless, we found that 95 specimens (20.4%) deposited in the SMB exotic bird collection were identified incorrectly or were missing species identification at the time of our revision. This gap can be attributed to limited access to identification literature at those times, mainly for scientists from the Eastern Bloc. Another 79 specimens (17%) had correct names, but they needed update. Altogether, then, more than one-third of specimens in the SMB exotic bird collection had names that were incorrect according current taxonomy and nomenclature.

One of the best examples that critical evaluation of accuracy of specimen names is important is the specimen of the endemic and near threatened Cuban species *Buteogallus gundlachii*, which was originally identified as *Buteo* (*Geranoaetus*) *albicaudatus* (Vieillot, 1816). Hence, even in collections of animals groups that are relatively easy to identify, accuracy of specimen names may be not as high as expected. Issues (i.e. missing, mistaken and outdated identifications) are expected to accumulate and concentrated in: (1) old collections without continuous evaluation of specimens; (2) local collections focused on local biota, but having some exotic voucher material; and (3) collections managed by amateurs or by experts of unbalanced expertise.

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Supplementary material 1

Table S1

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Data type: museum records

Explanation note: Exotic bird specimens held by the Šaris Museum in Bardejov, Slovakia, including their updated identification, previous identification provided by museum, family and order of bird specimen, conservation status according IUCN, country / region of specimen origin, locality of collection, acquisition number and year, inventory and notebook number, date of collection, and whether specimen is located in public exhibition or scientific collection. ? – information on locality and / or date was not found but we added this information for some specimens based on our knowledge of when and where Weisz collected specimens (for more details see method section).

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