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Two new species of *Conostigmus* (Hymenoptera: Megaspilidae) from Yintiaoling National Nature Reserve, China

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Abstract

Two new species, *Conostigmus asperatus* Wang and Zhu **sp. nov.** and *Conostigmus longus* Wang and Zhu **sp. nov.** (Hymenoptera: Megaspilidae), are described and figured. A key to the species of *Conostigmus* from China is provided. The barcode region of the mitochondrial COI gene for the new species was sequenced. Based on genetic distance analysis, the application of COI sequences in species delimitation of *Conostigmus* was discussed.

Key words: Ceraphronoidea, morphology, new species, taxonomy, Yintiaoling National Nature Reserve

Introduction

Megaspilidae (Hymenoptera: Ceraphronoidea) is a relatively small family of parasitoid wasps that has been largely overlooked in modern taxonomic studies, despite being frequently collected and having a global distribution (Johnson and Musetti, 2004). The family is easily recognized and separated from other families of Hymenoptera by the following traits: tibiae of all legs with two apical spurs, fore wing usually with large pterostigma, fore wing R-rs crossvein curved, and mesoscutum commonly with three longitudinal furrows or rarely reduced to a narrow sclerite (Alekseev and Radchenko, 2001; Mikó and Deans, 2009; Pezzini *et al*., 2019).

With more than 170 extant species, *Conostigmus* Dahlbom, 1858 is the most species-rich genus of Megaspilidae (Trietsch *et al*., 2020). The genus can be recognized by the following combined characters: male flagellomeres cylindrical, first female flagellomere less than 1.5 times as long as the pedicel; the ocular ocellar length (OOL) equal to or longer than posterior ocellar length (POL) (Dessart, 1972; Fergusson, 1980; Alekseev and Radchenko, 2001; Macedo and Kawada, 2013; Mikó *et al*., 2016, 2018). It is distributed widely in all geographical realms, especially in the Palaearctic region, but only eight species have been recorded from China (Dessert, 1997; Cui *et al*., 2023; Qian *et al*., 2024; Wang *et al*., 2024).

Yintiaoling National Nature Reserve (YNNR) is located in northeast Chongqing City of China, whose average elevation is 1900 m (Cai *et al*., 2023). Yintiaoling National Nature Reserve is the only primary forest with rich species diversity in Chongqing, but the *Conostigmus* fauna has not been recorded. In this paper, we describe and

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illustrate two new species from YNNR (*C. asperatus* Wang and Zhu **sp. nov.** and *C. longus* Wang and Zhu **sp. nov.**). Based on the COI gene, we reconstructed the phylogenetic tree of *Conostigmus* using maximum likelihood (ML) and Bayesian inference (BI), as a way to discuss the validity of molecular species definitions on the basis of morphological identifications, and to lay the foundation for future phylogenetic analyses. We also provide a key to Chinese *Conostigmus* to aid identification efforts.

Materials and methods

Specimen collection and identification

Specimens of the two new species described in this study were obtained by sweeping vegetation with sweep nets and preserving the specimens in absolute ethanol. All examined specimens are deposited in Auhui Normal University (AHNU), Anhui, China.

The specimens were card mounted on point-card tips. To prepare male genitalia for study, the apical metasomal segments were removed from specimens and placed in 35% NaOH solution, heated in a water bath at 100°C for 8 mins and then transferred to a droplet of glycerin on a concavity slide. Dissections were performed in glycerin using #5 forceps and #2 insect pins. Genitalia were stored in glycerin after dissection. Genitalic terminology follows the Hymenoptera Anatomy Ontology (Yoder *et al*., 2010).

Description and pictures

Abbreviations and morphological terms follow Mikó and Deans (2009), Trietsch *et al*. (2020) and Bijoy and Rajmohana (2021). All measurements are in micrometres, except for body length (excluding antennae), which is in millimetres. Photographs were taken with a Leica M205A stereomicroscope and a Leica DFC-500 digital camera, with extended focusing software. Plates were created using Adobe Photoshop CS3.

DNA extraction and barcoding

Non-destructive DNA extraction from the entire specimen was performed using the TIANamp Genomic DNA Kit. The barcode region of mitochondrial cytochrome oxidase subunit 1 (COI) was amplified using the LCO1490/HCO2198 primers (Folmer *et al*., 1994). Amplification of mitochondrial COI was conducted in a 25 μL PCR reaction with 2 μL DNA template, 8.5 μL ddH_2O, 12.5 μL master mix, 2 μL LCO1490/HCO2198 primers, cycler conditions set as follows: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 1 min, and then a final extension at 72°C for 5 min. All COI sequences generated in this study were uploaded to GenBank (Table 1).

TABLE 1. Taxa used in the phylogenetic analyses including barcode sequence length and GenBank accession numbers.

Species	Length (bp)	Family, subfamily	GenBank accession number
Ceraphron thomsoni	392	Ceraphronidae	GBMNA39216-19
Aphanogmus fijiensis	510	Ceraphronidae	GMBCB2052-15
Conostigmus abdominalis	603	Megaspilidae	NOMEG012-20
Conostigmus rugiceps	619	Megaspilidae	NOMEG187-21
Conostigmus speculiger	304	Megaspilidae	NOMEG384-21
Conostigmus crassicornis	619	Megaspilidae	NOMEG471-21
Conostigmus bipunctatus	587	Megaspilidae	NOMEG520-22
Conostigmus asperatus 1	627	Megaspilidae	OR797510
Conostigmus asperatus 2	627	Megaspilidae	OR797511
Conostigmus asperatus 3	627	Megaspilidae	OR797512
Conostigmus longus 1	627	Megaspilidae	OR797513
Conostigmus longus 2	627	Megaspilidae	OR797514

Using *Aphanogmus fijiensis* Ferriere, 1933 (Ceraphronidae) and *Ceraphron thomsoni* Dalla Torre, 1890 (Ceraphronidae) as outgroups, the maximum likelihood (ML) and Bayesian inference (BI) trees were reconstructed using 5 *Conostigmus* COI sequences from the Barcode of Life database (http://www.boldsystems.org/) and 5 sequences newly generated in this study. Sequence alignment and pairwise corrected genetic distances were

performed in MEGA-X. ML trees were reconstructed using the IQ-TREE web server (Trifinopoulos *et al*., 2016), an ultrafast bootstrap (UFB) (Minh *et al.*, 2013) of 1000 replications and the SH-aLRT test (Guindon *et al*., 2010) were used to assess branch supports. The BI tree was reconstructed using MrBayes in Phylosuit v1.2.3 (Zhang *et al*., 2020; Xiang *et al*., 2023). Trees were sampled every 1000 generations (Huelsenbeck, 2012). FigTree v.1.3.1 (Rambaut, 2011) was used to view the resulting trees. The trees were beautified with Adobe Photoshop CS3.

Results

Molecular analysis

The present study generated 5 COI sequences with an average of 627 bp. Including the sequences of *C. asperatus* **sp. nov.** and *C. longus* **sp. nov.**, the 12 studied COI sequences belong to 9 species. The pairwise corrected genetic distances were shown in Table 2. The intraspecific distances of the COI sequences generally were below 1%, indicating that molecular species delimitation is consistent with the identifications based on morphology. While the interspecific distances range between 17.14% and 37.50%. Each species was strongly supported on the ML and BI trees (Fig. 1).

TABLE 2 . Pairwise genetic distances of COI between nine species of Megaspilidae.

	1	2	3	4	5	6	7	8	9	10	11	12
Ce. thomsoni												
A. fijiensis	0.1714											
Co. abdominalis	0.3210	0.2831										
Co. rugiceps	0.2889	0.3244	0.2565									
Co. speculiger	0.3043	0.3573	0.2868	0.0133								
Co. crassicornis	0.3372	0.2982	0.2135	0.2280	0.2302							
Co. bipunctatus	0.3248	0.2750	0.2057	0.2274	0.2398	0.2095						
Co. asperatus 1	0.3750	0.3303	0.2555	0.2570	0.2528	0.2615	0.2316					
Co. asperatus 2	0.3750	0.3303	0.2555	0.2570	0.2528	0.2615	0.2316	0.0000				
Co. asperatus 3	0.3750	0.3303	0.2555	0.2570	0.2528	0.2615	0.2316	0.0000	0.0000			
Co. longus 1	0.3007	0.2462	0.1734	0.2418	0.2349	0.2072	0.1755	0.2083	0.2083	0.2083		
Co. longus 2	0.2967	0.2413	0.1760	0.2398	0.2349	0.2052	0.1714	0.2111	0.2111	0.2111	0.0017	

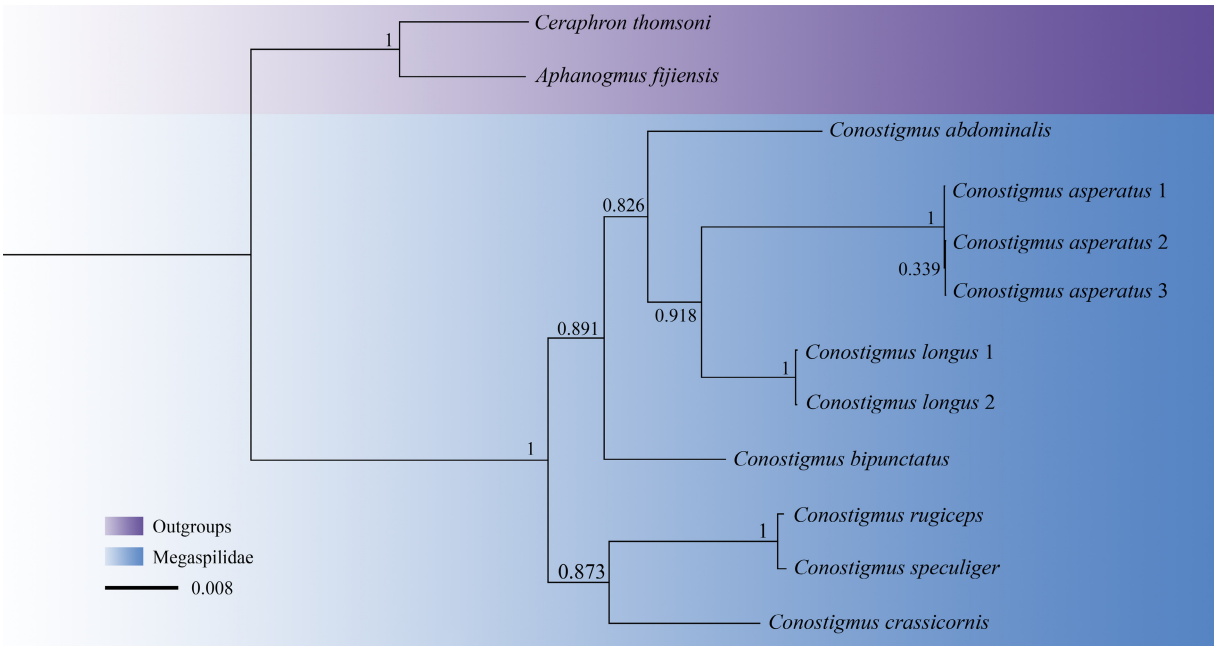

FIGURE 1. Bayesian inference (BI) analysis of *Conostigmus* based on the barcode region ofmitochondrial cytochrome oxidase 1. ML bootstrap values and Bayesian posteriorprobabilities are indicated at internal nodes.

Taxonomy

Key to the species of *Conostigmus* from China (male)

1.	F1 length longer than scape (F1 length vs. scape length: 1.2–1.4)	***C. longus* Wang and Zhu sp. nov.**
-	F1 length shorter than scape or equal to scape (scape length vs. F1 length: less than 1.5)	2
2.	Mesosoma more than 2 times longer than width	***C. ampullaceus***
-	Mesosoma at most 1.5 times longer than width	3
3.	Anteromedian projection of the metanoto-propodeo-metapecto-mesopectal complex absent	4
-	Anteromedian projection of the metanoto-propodeo-metapecto-mesopectal complex present	6
4.	Facial pit present	***C. villosus***
-	Facial pit absent	5
5.	Harpe spatulate or spoon-shaped and longer than the gonostipes in lateral view	***C. abdominalis***
-	Harpe clubbed and shorter than the gonostipes in lateral view	***C. nankunensis***
6.	Facial pit present.	7
-	Facial pit absent	***C. quadripetalus***
7.	Facial sulcus present.	***C. asperats* Wang and Zhu sp. nov.**
-	Facial sulcus absent	8
8.	Basal gastral carinae reaching 1/3 of syntergum length.	***C. electrinus***
-	Basal gastral carinae reaching 1/4 of syntergum length	9
9.	Body length less than 2 mm; posterior end of notauli always adjacent to median mesoscutal sulcus.	***C. acutus***
-	Body length more than 2 mm; posterior end of notauli never adjacent to median mesoscutal sulcus.	***C. xui***

Conostigmus asperatus Wang and Zhu sp. nov.

Figures 2, 3

Diagnosis. Males of this species can be distinguished from other *Conostigmus* by the following combination of characters: head coarse with pubescence; facial sulcus, preoccipital lunula and postocellar carina present; preoccipital furrow ending posterior to anterior ocellus; intertorular carina present; median process of intertorular carina acute, with the process extending across the intertorular area to dorsal margin of clypeus; sternaulus present and exceeding 2/3 of mesopleuron length at level of sternaulus; harpe shorter than gonostipes.

Material examined. ***Holotype***: male **China:** Chongqing, Wuxi, Yintiaoling National Nature Reserve, Sweep nets, 31°27′42.23″N, 109°56′15.29″E, 25.IX.2022, De-cai Lu SCAU 3045200 (deposited in AHNU); *Paratypes*: 2• males **China:** Chongqing, Wuxi, Yintiaoling National Nature Reserve, Sweep nets, 31°27′42.23″N, 109°56′15.29″E, 15.IX.2022, De-cai Lu SCAU 3045201; SCAU 3045218 (deposited in AHNU).

Description.

Male: Body length: 1.7–2.0 mm (N = 3).

Coloration (Fig. 2). Color hue pattern: head and mesosoma black; metasoma dark brown; base of legs dark brown, rest of legs brown; Mandibles reddish brown and palps yellow; scape and pedicel yellow, F1–F9 brown; pterostigma, costal vein, radial vein and marginal fringes of wings brown; body pubescence brown; male genitalia brownish yellow. Color intensity pattern: head and mesosoma darker than metasoma; hind legs darker than fore and mid legs.

Head (Fig. 2C, E). Head width, dorsal view: slightly wider than mesosoma (about 1.1× wider than mesosoma). Head width vs. head height: HW vs. HH = 1.3–1.4. Head height vs. eye height: HH vs. EHf = 1.7–1.8. Head height vs. head length: HH vs. HL = 1.4–1.5. Head width vs. interorbital space: HW vs. IOS = 1.7. Lateral ocellar length: ocular ocellar length: LOL vs. OOL = 0.3–0.4. Lateral ocellar length: posterior ocellar length: LOL vs. POL= 0.6–0.7. Ocular ocellar length: posterior ocellar length: OOL vs. POL= 1.4–1.8. Head shape (anterior view): circular or triangular, coarse with pubescence. Eyes large and bare. Ocellar foveae distinct and width equal to ocellus diameter. Postocellar carina present. Preoccipital lunula: present. Preoccipital carina: present. Preoccipital furrow distinct and crenulate. Preoccipital furrow anterior end: preoccipital furrow ending posterior to anterior ocellus. Facial sulcus present and complete, extending from intertorular carina to anterior ocellus. Occipital carina complete and crenulate. Intertorular carina present. Median process on intertorular carina shape: acute. Median process of intertorular carina structure: process extend across intertorular area to dorsal margin of clypeus.

Antennae (Fig. 2F). Scape length vs. pedicel length: 3.2–4.0. Scape length vs. F1 length: 0.9–1.0. F1 length vs. pedicel length: 3.5–4.0. Longest male flagellomere: F1. F1 length vs. F2 length: 1.2–1.3. F5 length almost equal to

F6. F2 length almost equal to F9. Length of pubescence on flagellomere vs. flagellomere width: flagellomeres width about twice than pubescence length.

Mesosoma (Fig. 2B, C). Mesosoma slightly narrow (1.2× longer than wide) (Length/width/height = 591/485/501 μm). AscW/PscW = 0.6–0.7. Pronotum not elongate. Mesoscutum 2.4× wider than long (Length/width = 200/485 μm). Transscutal articulation evident, dividing mesonotum into two parts: mesoscutum and scutellar-axillar complex. Notaulus: present and complete. Notaulus posterior end: adjacent to transscutal articulation, posterior end of notaulus not curve and not adjacent to median mesoscutal sulcus. Median mesoscutal sulcus: present and complete. Median mesoscutal sulcus posterior end: adjacent to transscutal articulation. Scutoscutellar sulcus count: present. Scutoscutellar sulcus structure: scutoscutellar sulcus angled medially, foveolate. Mesoscutellum 1.4× longer than wide, limited by a u-shaped carina. Sternaulus count: present. Sternaulus shape: elongate and exceeding 2/3 of mesopleuron length at level of sternaulus. Mesopleural sulcus shape: with slight curvature medially. Lateral propodeal carina shape: inverted "Y". Anteromedian projection of the metanoto-propodeo-metapecto-mesopectal complex present.

FIGURE 2. *Conostigmus asperatus* Wang and Zhu **sp. nov.**, male, holotype. **A.** lateral habitus. **B.** head and mesosoma, lateral view. **C.** mesosoma, dorsal view. **D.** metasoma, dorsal view. **E.** head, anterior view. **F.** antennae. **G.** metasoma, lateral view.

Wings (Fig. 2A). Forewing length 1.4 mm, translucent. Forewing macropterous with apex extending past petiole. Forewing darkly pigmented, translucent, less melanized proximally. Pterostigma semi-elliptical, length vs. width: 2.0–2.1. Radius (288 μm), a little curved in the middle, longer (1.5×) than pterostigma. Hind wing without vein.

Metasoma (Fig. 2D, G). Metasoma 1.9× longer than wide (Length/width/height = 787/415/315 μm). Transverse carina on petiole shape: concave. Syntergum smooth, wider than long. Gastral carinae count: 3. Gastral carinae length vs. syntergum length: gastral carinae length reaching 1/3 of syntergum length. Syntergal translucent patch count: present. Syntergal translucent patch shape: transverse and irregularly cylindric. Rest of tergites smooth, but with sparse hairs on both sides.

Male genitalia (Fig. 3). Proximodorsal notch of cupula shape: arched. Harpe shorter than gonostipes. Lateral setae of harpe present and oriented distally. Harpe shape: simple and not bilobed. Harpe orientation: medial. Distal margin of harpe narrowed. Parossiculus count or parossiculus and gonostipes fusion: present and parossiculi not fused with the gonostipes. Gonossiculus and gonossiculus spines present. Number of spines on gonossiculus: 3. Penisvalva curved proximally.

Females. Unknown.

Distribution. China (Chongqing).

Etymology. The species name is a Latin masculine adjective meaning “roughly”, indicating the rough surface sculpture of the male head.

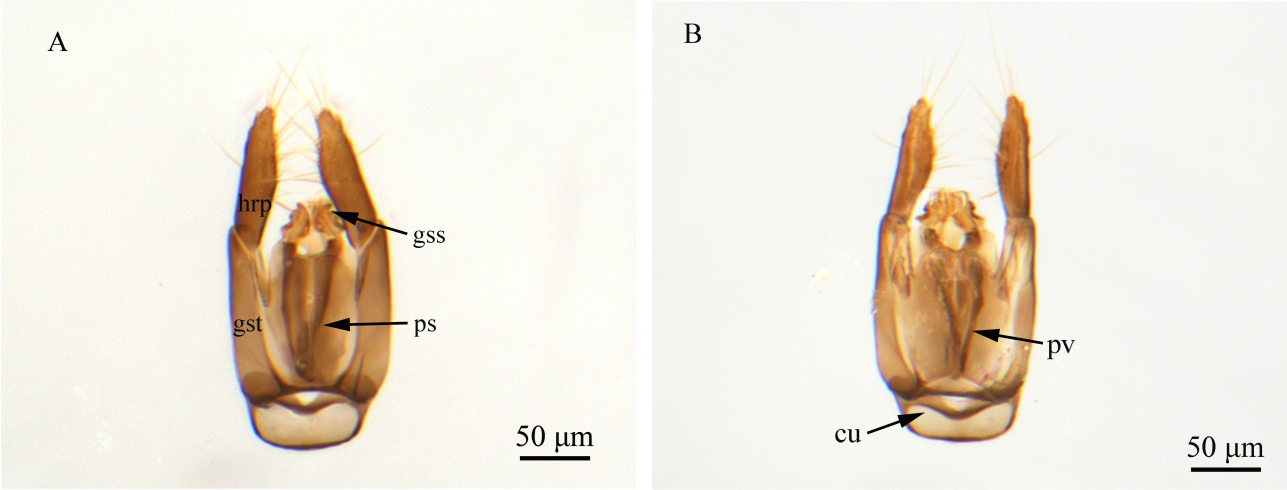

FIGURE 3. *Conostigmus asperatus* Wang and Zhu **sp. nov.**, male, holotype, genitalia. **A.** dorsal view. **B.** ventral view (ps, parossiculus; gst, gonostipes; gss, gonossiculus; hrp, harpe; cu, cupula; pv, penisvalva).

Conostigmus longus Wang and Zhu sp. nov.

Figures 4, 5

Diagnosis. Males of this species can be distinguished from other *Conostigmus* by the following combination of characters: F1 is longer than scape (F1 length vs. scape length: 1.2–1.4). Facial pit present. Intertorular carina present and straight. Preoccipital furrow present and ending posterior to ocellar triangle. Wings macropterous and almost equal to body length. Sternaulus present and exceeding 1/2 of mesopleuron length. Harpe shorter than gonostipes. Distal margin of harpe shrinking to an acute angle.

Material examined. ***Holotype***: male **China:** Chongqing, Wuxi, Yintiaoling National Nature Reserve, Watchtower, Sweep nets, 31°27′42.23″N, 109°56′15.29″E, 25.IX.2022, De-cai Lu SCAU 3045217 (deposited in AHNU); *Paratypes*: 1• male **China:** Chongqing, Wuxi, Yintiaoling National Nature Reserve, Watchtower, Sweep nets, 31°27′42.23″N, 109°56′15.29″E, 15.IX.2022, De-cai Lu SCAU 3045216 (deposited in AHNU).

Description.

Male: Body length: 1.67–1.75 mm (N = 2).

Coloration. Color hue pattern: head and mesosoma black; metasoma dark brown; ventral part of scape and pedicel tawny; F1–F9 black; mandibles reddish brown; maxillary palp yellow; fore legs yellow, middle and hind

legs yellow to dark brown; pterostigma, costal vein, radial vein and marginal fringes of wings pale brown; body pubescence brown; harpe dark brown to yellow, rest of male genitalia brown. Color intensity pattern: F1–F9 lighter than head and mesosoma; maxillary palp lighter than fore legs; ventral part of male genitalia darker than dorsal.

Head (Fig. 4C, E) Head width, dorsal view: slightly wider than mesosoma (about 1.2× wider than mesosoma). Head width vs. head height: HW vs. HH = 1.3–1.7. Head height vs. eye height: HH vs. EHf = 1.5–1.9. Head height vs. head length: HH vs. HL = 1.0–1.2. Head width vs. interorbital space: HW vs. IOS = 1.7–1.8. Lateral ocellar length: ocular ocellar length: LOL vs. OOL = 0.3–0.4. Lateral ocellar length: posterior ocellar length: LOL vs. POL = 0.5–0.7. Ocular ocellar length: posterior ocellar length: OOL vs. POL= 1.5–1.7. Head shape (anterior view): circular or triangular. Eyes large and hairy. Preoccipital lunula: present. Preoccipital carina: present. Preoccipital furrow: present. Preoccipital furrow anterior end: preoccipital furrow ending posterior to ocellar triangle. Occipital carina: present. Occipital carina structure: complete and crenulate. Intertorular carina: present. Intertorular area: present. Intertorular carina shape: straight. Median region of intertorular area shape: flat. Facial sulcus absent. Facial pit present. Ocellar foveae distinct, and ocellar foveae width equal to ocellus diameter. Maxillary palp divided into 4 palpal segments.

Antennae (Fig. 4F). Scape length vs. pedicel length: 4.0–4.1. F1 length vs. scape length: 1.2–1.4. F1 length vs. pedicel length: 4.9–5.5. Longest male flagellomere: F1. F1 length vs. F2 length: 1.2–1.4. F3 length almost equal to F4. F7 length almost equal to F8. Length of pubescence on flagellomere vs. flagellomere width: flagellomeres width about twice than pubescence length.

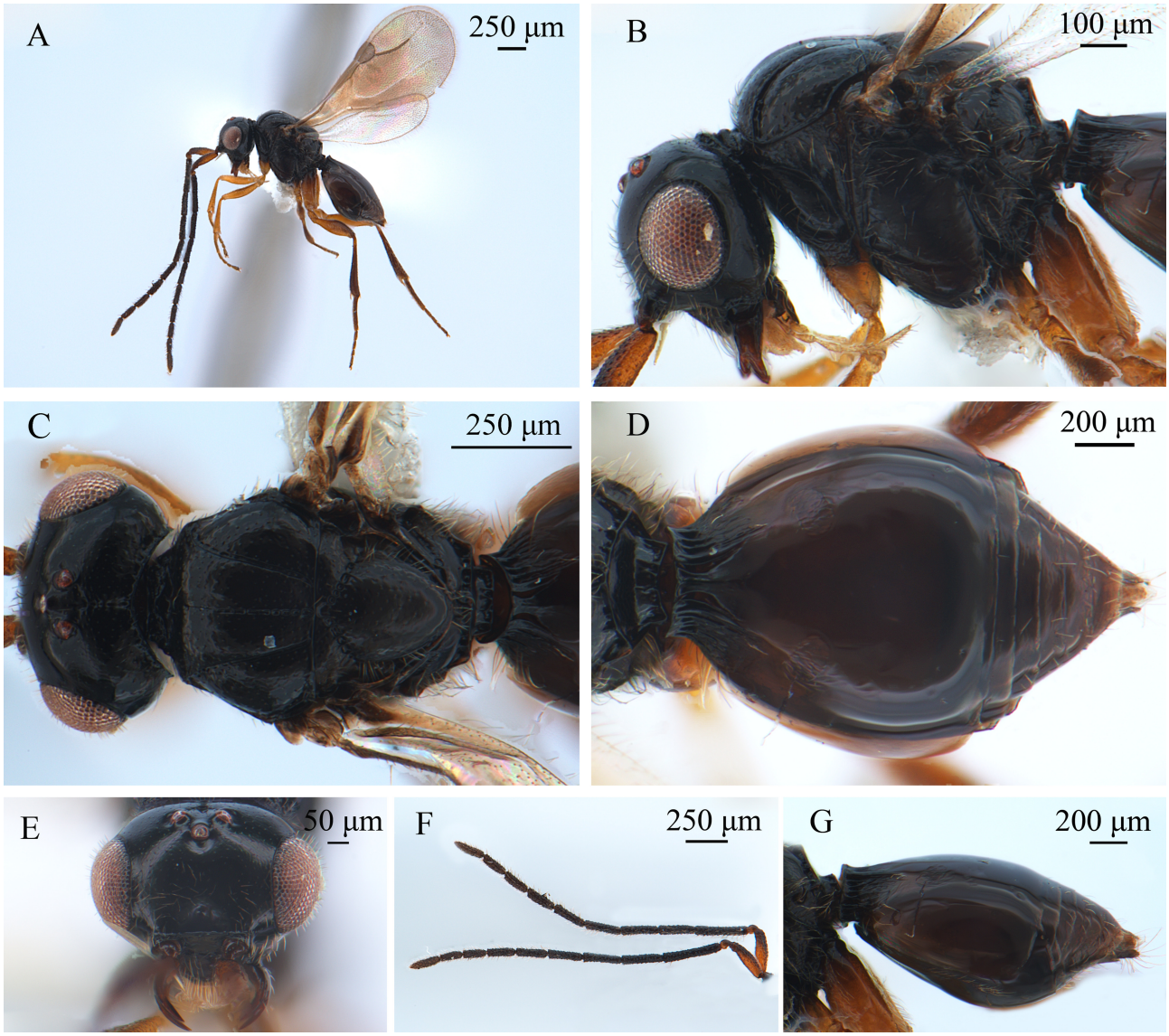

FIGURE 4. *Conostigmus longus* Wang and Zhu **sp. nov.**, male, holotype. **A.** lateral habitus. **B.** head and mesosoma, lateral view. **C.** head and mesosoma, dorsal view. **D.** metasoma, dorsal view. **E.** head, anterior view. **F.** antennae. **G.** metasoma, lateral view.

Mesosoma (Fig. 4B, C). Mesosoma slightly narrow (1.2× longer than wide) (Length/width/height = 674/500/560 μm). AscW/PscW = 0.8–0.9. Pronotum not elongate. Mesoscutum 1.6× wider than long (Length/width = 300/500 μm). Notaulus count: present and complete. Notaulus posterior end: adjacent to transscutal articulation. Median mesoscutal sulcus: present. Median mesoscutal sulcus posterior end: adjacent to transscutal articulation. Scutoscutellar sulcus: present. Scutoscutellar sulcus structure: foveolate. Mesoscutellum 1.5× longer than wide, not limited by a U-shaped carina. Sternaulus: present and exceeding 1/2 of mesopleuron length. Mesopleural sulcus shape: straight. Mesopleural fovea count: present and adjacent to mesopleural sulcus. Metapleuron shape: trapezoidal. Lateral propodeal carina shape: inverted "Y". Anteromedian projection of the metanoto-propodeo-metapecto-mesopectal complex count: absent.

Wings (Fig. 4A). Forewing length 1.78 mm, macropterous with almost equal to body length, Forewing darkly pigmented, translucent. Pterostigma semi-elliptical, length vs. width: 1.8–2.7. Radius (357 μm), a little curved in the middle, longer (1.8×) than pterostigma. Pigmentless narrow strip (forewing) length reaching 1/2 of forewing length. Hindwing without vein.

Metasoma (Fig. 4D, G). Metasoma 1.5× longer than wide (Length/width/height = 716/492/385 μm). Transverse carina on petiole shape: concave. Syntergum smooth, longer than wide. Gastral carinae present and less than 1/3 of syntergum length. Syntergal translucent patch count: present. Syntergal translucent patch shape: transverse and rod-shaped. Rest of tergites smooth, but with sparse hairs.

Male genitalia (Fig. 5). Proximodorsal notch of cupula shape: arched. Harpe shape: simple and not bilobed. Distal margin of harpe shape: shrinking to an acute angle. Harpe orientation: medial. Harpe shorter than gonostipes. Lateral setae of harpe count: present. Lateral setae of harpe orientation: oriented distally. Dense patch of setae on the distoventral edge of the harpe: present. Parossiculus or parossiculus and gonostipes fusion: present and parossiculi not fused with the gonostipes. Gonossiculus and gonossiculus spines present. Number of spines on gonossiculus: 3. Penisvalva curved proximally.

Females. Unknown.

Distribution. China (Chongqing).

Etymology. The species name is a Latin masculine adjective meaning "long", indicating a long first flagellomere.

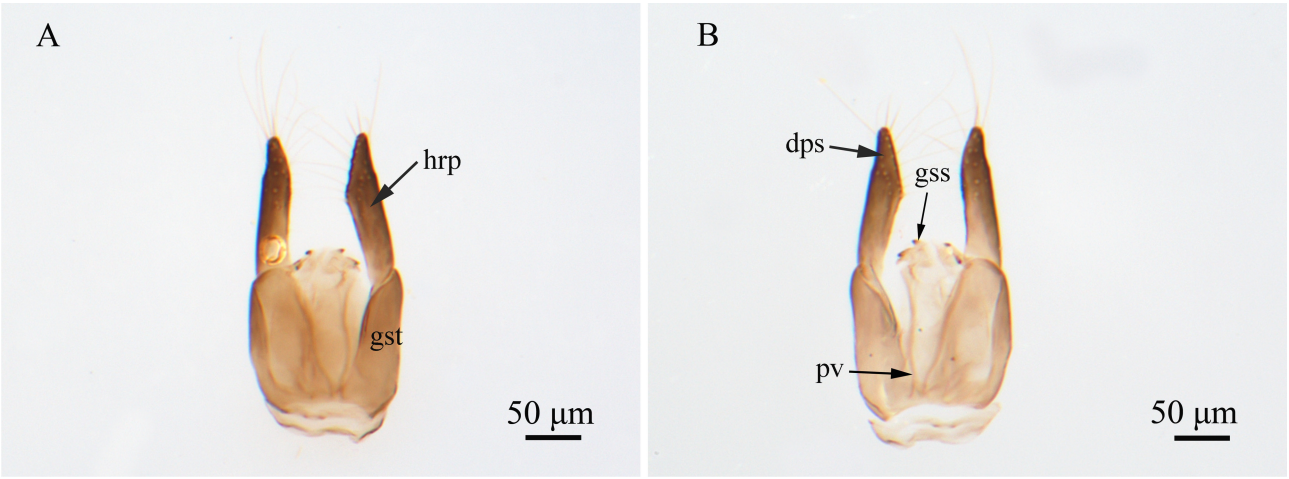

FIGURE 5. *Conostigmus longus* Wang and Zhu **sp. nov.**, male, holotype, genitalia. **A.** ventral view. **B.** dorsal view (gst, gonostipes; hrp, harpe; gss, gonossiculus; pv, penisvalva; dps, dense patch of setae on the distoventral edge of the harpe).

Discussion

In this study, we described two new species of *Conostigmus*: *C. asperatus* Wang and Zhu **sp. nov.** and *C. longus* Wang and Zhu **sp. nov.**, which enriches the species number of *Conostigmus* to ten in China. *Conostigmus asperatus* **sp. nov.** is morphologically similar to *C. abdominalis* in having the facial sulcus, but they are distinguishable by the sternaulus length. The length of the sternaulus of *C. asperatus* **sp. nov.** exceeds 2/3 of mesopleuron length at the level of the sternaulus (1/2 in *C. abdominalis*). The length of the first flagellomere in the male of *C. longus* **sp.**

nov. is greater than the combined length of scape and pedicel, which has never been found in other *Conostigmus* species.

Megaspilidae are relatively monotonous and strongly sexually dimorphic (Vasilita *et al*., 2022), so it is important to use molecular tools to accelerate species identification and male-female matching. In our previous article on *Dendrocerus* species, we successfully matched male and female individuals of the same species using the 28S rDNA gene (Li *et al*., 2023), validating the effectiveness of 28S rDNA in species identification. In this paper, we further explored species delimitation using COI sequences, and the results were consistent with morphological identification, but we were not able to address the male and female matches due to the lack of female specimens. In the future, we will verify the validity of DNA barcoding for sex matching of Megaspilidae by obtaining COI genes from additional specimens of both genders.

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