Article

Sirovena Bouček (Pteromalidae: Pireninae), a new member of the fig wasp community associated with *Ficus microcarpa* (Moraceae)

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Abstract

Sirovena Bouček (Chalcidoidea: Pteromalidae, Pireninae) was known only from females of its type species, *S. stigma* Bouček, 1988. Males and host associations were both unknown. Both sexes of a second species of *Sirovena*, *S. costallifera* **sp. nov.**, reared from *Ficus microcarpa* L. (Moraceae) in Yunnan, China, are described based on morphological characters and mitochondrial COI sequences. The males of *S. costallifera* show sexual dimorphism in body coloration, structure of the antennae and notauli, and body sculpture. Reliable host records of Pireninae are Cecidomyiidae (Diptera). Because midges also parasitize some figs, we speculate that *S. costallifera* is a parasitoid of some cecidomyiid midge that attacks fig syconia. A key to differentiate the two species is provided.

Key words: taxonomy, molecular identification, sexual dimorphism

Introduction

Fig wasps that live within syconia of fig trees include pollinator species (Chalcidoidea: Agaonidae) and non-pollinating species of other chalcidoid families. The community of fig wasps associated with *Ficus microcarpa* L. has been well surveyed in China (Taiwan) (Chen *et al.* 1999), USA (Hawaii) (Beardsley 1998) and Brazil (Farache *et al.* 2009). During sorting of wasp specimens reared from *F. microcarpa* in Yunnan, China, we found a species that did not belong to any known genus of fig wasps associated with this fig. However, using the key to Australasian pteromalid genera by Bouček (1988), both females and males of the species keyed to *Sirovena* Bouček,1988 (Pteromalidae: Pireninae). Bouček based *Sirovena* on *S. stigma* Bouček, collected on Christmas Island in the south of Java, Indonesia, and known only from females. Males and host biology were unknown. Here we describe a second species of *Sirovena,* reared from *F. microcarpa* in Yunnan, China, and compare females with those of the type species, *S. stigma*. We also describe males of *Sirovena* for the first time and confirm subfamily placement using mitochondrial COI sequences. *Sirovena* shares the following morphological features with other genera of Pireninae: antennae inserted very low, close to clypeus; clypeus large with lower margin produced; mandibles weakly curved, with 4 teeth; notauli complete; propodeum not sculptured, lacking plicae; fore wing with marginal vein broaden and wedge-shaped, postmarginal vein absent, and stigmal vein short.

Material and methods

Morphological studies. In March, 2003, approximately 1–3 days before maturing, figs were collected from *F. microcarpa* in Yunnan province, China, and placed in fine-mesh bags (200×200 mm) that allowed the wasps to emerge naturally from the syconia. All emerged wasps were collected and preserved in 95% ethanol and stored at -20° for further morphological and molecular studies. Ethanol-preserved type specimens were subsequently dried

with an EMS 850 Critical Point Drier prior to mounting on small white points for further examination using a Nikon SMZ800 microscope. Photographs were obtained with a Nikon AZ100 microscope and figures edited with Adobe Photoshop CS3.Absolute measurements are used for body length, and relative measurements for all other dimensions. Comparison of females with those of *S. stigma* is based on examination of the holotype and four paratypes of *S. stigma* borrowed from The Natural History Museum, London.

Molecular identification. The total genomic DNA was extracted from one female and one male of *S. costallifera* using Easypure Genomic DNA Extraction kit (*TransGen Biotech*, Beijing, China). COI fragments were amplified for both samples by using the primers FWPTF1 (5'-CCTGGTTCTTTRATTGGTAATGATC-3') and Lep-R1 (5'-TAAACTTCTGGATGTCCAAAAA-3') with the PCR reaction reagents and programs as previously described by Li *et al.* (2010). The PCR products were sequenced directly, and the sequence from the male was submitted to the National Center for Biotechnology Information (NCBI) with the accession number JQ031787.

COI sequences of 10 other species of Chalcidoidea were downloaded from NCBI for comparison with *S. costallifera*. These included three species identified in NCBI only as "Hymenoptera sp.", which we identified as Pireninae based on associated photographs covered under the Creative Commons Attribution Non-Commercial ShareAlike that were sent to us by Dr. Julie Stahlhut, University of Guelph, Canada. The sequences were from unidentified species of *Philotrypesis* Förster and *Sycoscapter* Saunders (Pteromalidae: Sycoryctinae)*, Walkerella* Westwood (Pteromalidae: Otitesellinae), *Sycophila* Walker (Eurytomidae: Eurytominae), *Acophila* Ishii and *Sycobia* Walker (Eurytomidae: Epichrysomallinae), and *Ormyrus* Westwood (Ormyridae).

Sequences were aligned with Bioedit (Tippmann 2004) and a NJ tree was constructed by using Mega 5.0 (Kumar *et al.* 2008) with default parameters. Bootstrap analyses were based on 1000 replicates.

Systematics

Sirovena Bouček, 1988

Sirovena Bouček, 1988: 478. Type species: *Sirovena stigma* Bouček, by monotypy.

Diagnosis. Female head (Figs 7, 9) and thorax (Fig. 6) with finely engraved reticulation, propodeum and metasoma almost smooth; male body almost without reticulation (Fig. 15). Head subtrapezoidal in frontal view (Figs 2, 7, 13); occipital foramen situated near vertex; occiput very broadly excavated, without carina, but dorsally with sharp, arcuate edge (Fig. 6); toruli situated very low, adjacent to clypeus (Figs 2, 7, 13), with sharp longitudinal carina between toruli. Clypeus convex, setose, apical margin distinctly protruding (Figs 2, 7, 13), sides delimited by dorsally convergent grooves. Mandibles 4-toothed; maxillary palpus 2-segmented; labial palpus 1-segmented with long apical seta. Antennal formula 11253, but male without distinctly differentiated clava (Fig. 13). Pronotal collar shorter than neck; collar edge with several setae. Notauli complete. Axillae not advanced, wide apart (Figs 3, 6, 15). Propodeum (Figs 3, 15) almost smooth, without carinae; spiracles round, close to metanotal margin; without supracoxal flange; lateral pilosity consisting of a few thin hairs. Fore wing with marginal and stigmal veins forming wedge-shaped infuscate region, postmarginal vein absent (Figs 1, 5, 16). Legs fairly long; hind coxa inserted rather high (Figs 1, 7, 11). Gaster sessile; hypopygium of female rather large, extending near or beyond middle of gaster (Fig. 1, 5).

Key to females of *Sirovena*

1   Coxae white or light yellow (Fig. 1); clypeus testaceous, sides distinctly delimited by dorsally convergent grooves (Fig. 2); head wider than high (71:69) (Fig. 2); upper mesepimeron mostly smooth (Fig. 4) . . . . *Sirovena stigma* Bouček
-       Fore and mid coxae dark brown, hind coxae mostly white (Fig. 5); clypeus black, sides indistinctly delimited (Fig. 7); head higher than wide (73:70) (Fig. 7); upper mesepimeron with imbricate reticulation (Fig. 10) . . . . *Sirovena costallifera* Li, Xiao & Huang **sp. nov.**
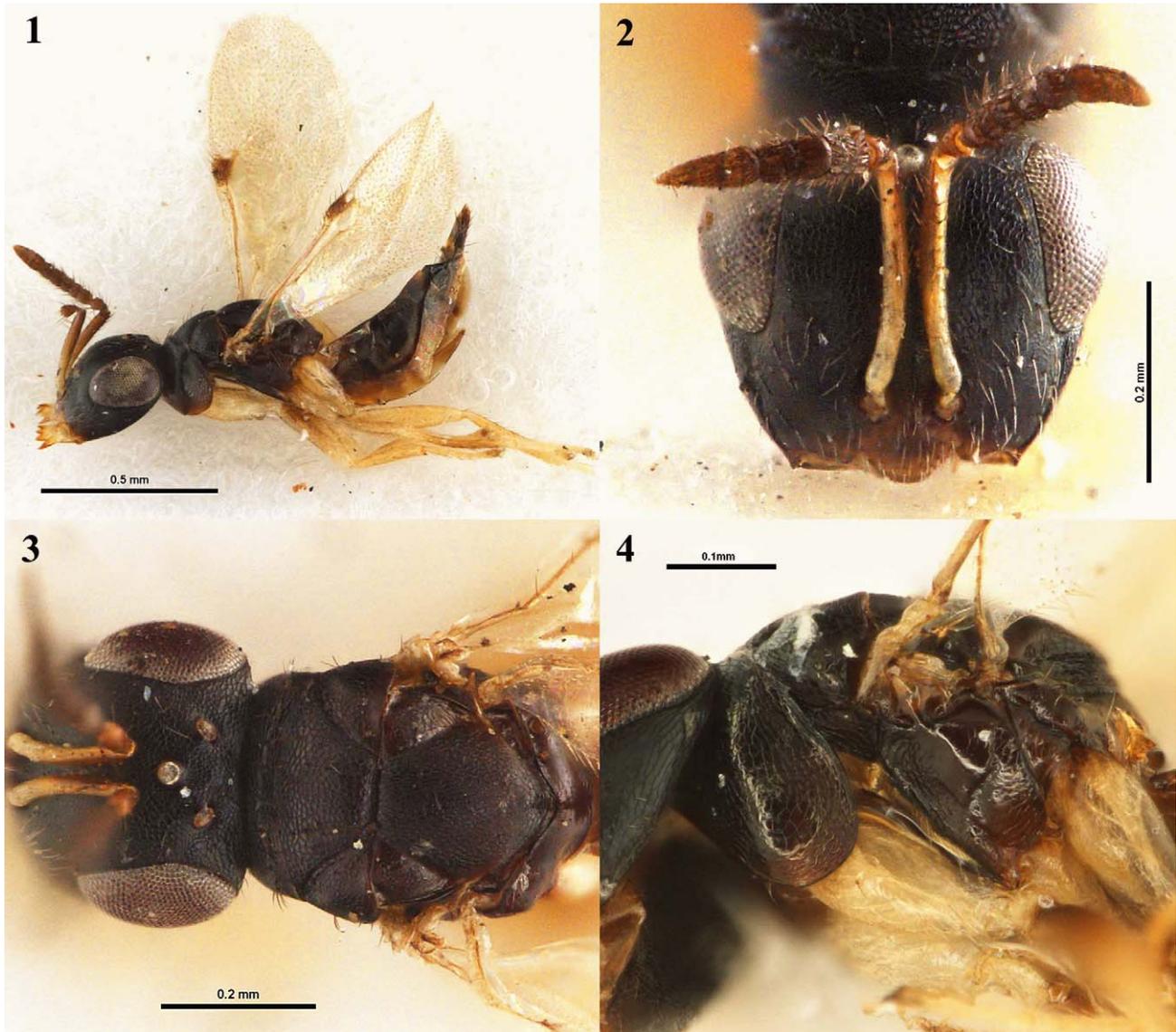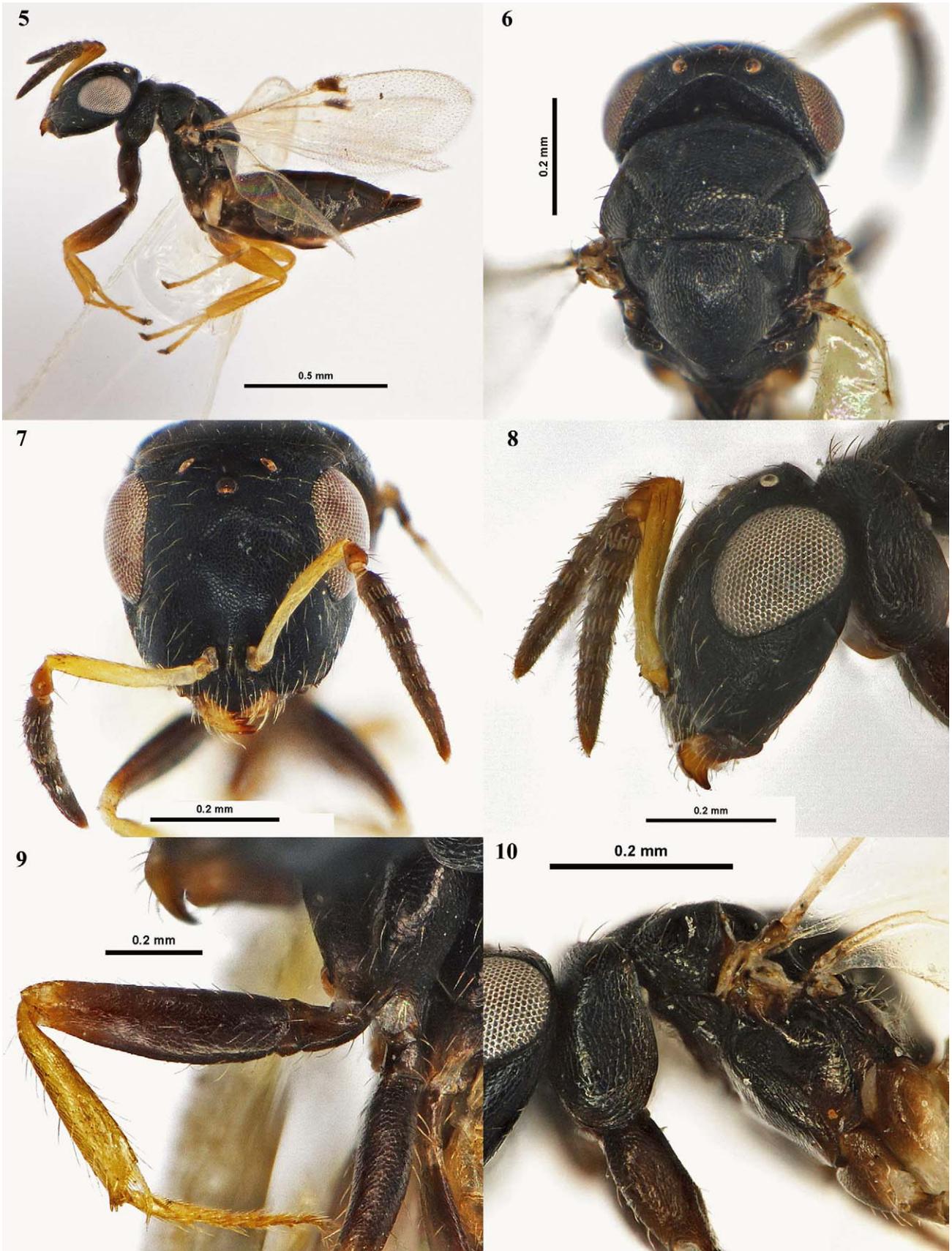
FIGURES 1–4. *Sirovena stigma* Bouček, female. 1, holotype, lateral habitus; 2, paratype, head, frontal view; 3, paratype, head and mesosoma, dorsal view; 4, paratype, mesosoma, lateral view.

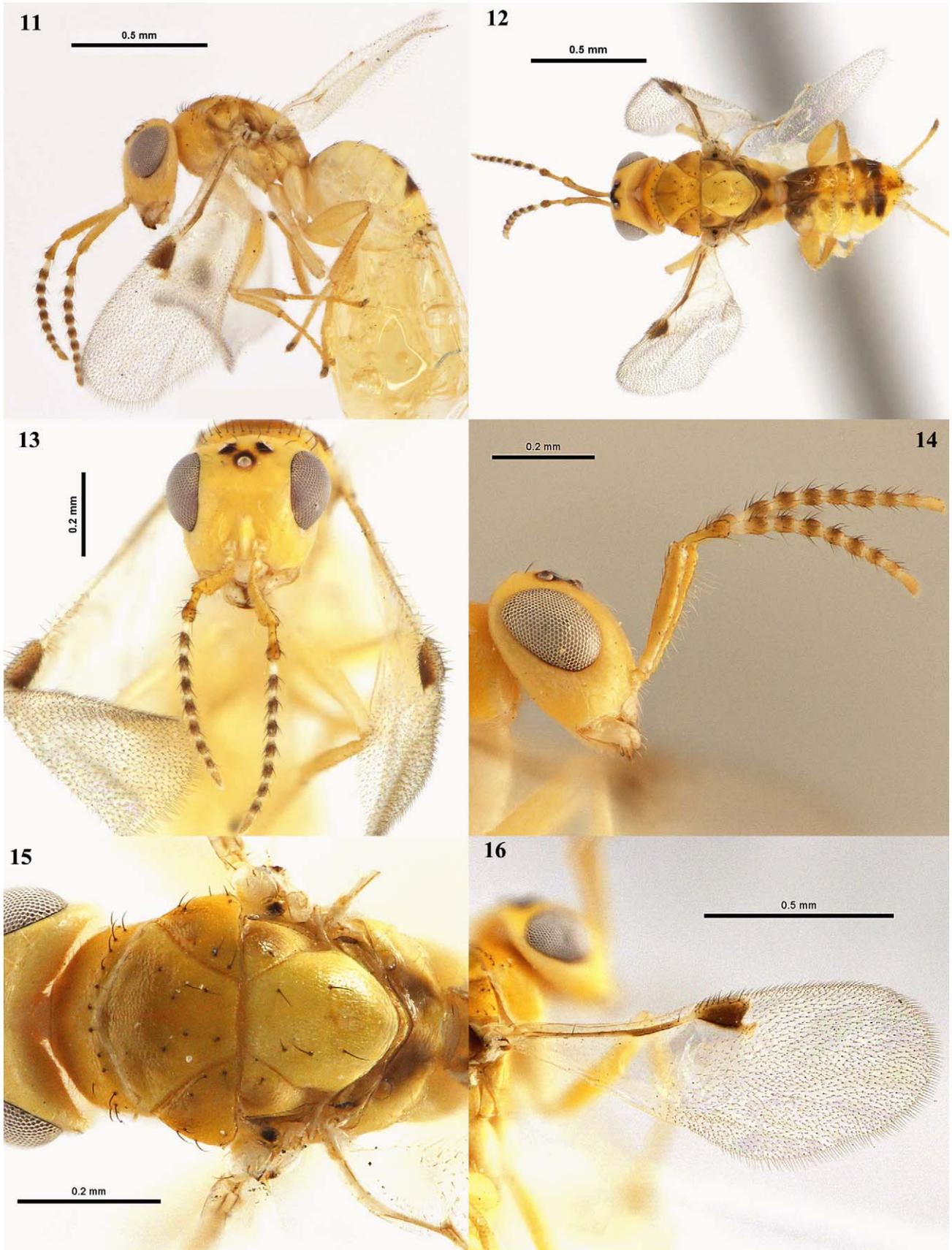## *Sirovena costallifera* sp. nov. Li, Xiao & Huang
(Figs 5–16)

Type material. Holotype ♀: CHINA, Xishuangbanna Tropical Botanical Garden, 20.iii.2003, W. Q. Zhen leg., ex *Ficus microcarpa*. Deposited in Institute of Zoology, Chinese Academy of Sciences. Paratypes: 6♀, 7♂, same data as holotype.

Description. Female (Figs 5–10). Body length 1.9–2.4mm. Head and mesosoma dark, black with faint metallic sheen; mandible yellow with teeth red; antenna with scape yellow, pedicel and anelli yellowish-brown, flagellum dark brown; fore and midcoxae and femora nearly black, lower part of hind coxae almost light yellow; hind femur and all tibiae and tarsi yellow; metasoma dark brown.

Head. In frontal view (Fig. 7) slightly higher than wide (73:70); head width and oral cavity width in ratio of 70:45; face and mandibles with white setae; scrobes indistinct; clypeus protruding, black, sides indistinctly delimited. Head in lateral view (Fig. 8) higher than long (27:17); malar sulcus complete; malar space, eye height and eye width in ratio of 3:5:4. POL longer than OOL (21:12). Antennal segments (Figs 7, 9) with long setae except anelli; scape claviform, shorter than pedicel+flagellum (35:50), approximately 8 times as long as wide and

FIGURES 5–10. *Sirovena costallifera* Li, Xiao & Huang, female. 5, holotype, lateral habitus; 6, paratype, head and mesosoma, dorsal view;7, paratype, head, frontal view; 8, holotype, head, lateral view; 9, paratype, fore leg; 10, holotype, mesosoma, lateral view.

FIGURES 11–16. *Sirovena costallifera* Li, Xiao & Huang, paratype, male. 11, lateral habitus; 12, dorsal habitus; 13, head and antennae, frontal view; 14, head and antennae, lateral view; 15, mesosoma, dorsal view; 16, fore wing.

5 times as long as pedicel; funicular segments compact, each slightly wider than long; clava as long as the last three funicular segments combined.

Mesosoma. Mesonotum (Fig. 6) with several irregular, outstanding setae. Length of pronotum, mesoscutum and scutellum approximately in ratio of 5:14:17; width of pronotum to mesoscutum in ratio of 4:5; pronotal collar edge with a line of setae; mesoscutum broader than long (35:19); notauli composed of irregular punctures. Scutellum longer than wide (73:70), subacuminate, with 5 or 6 setae. Upper and lower mesepimeron with imbricate reticulation. Fore wing (Fig. 5) length to width in ratio of 8:3; submarginal, marginal and stigmal veins in ratio of 80:15:17. All tarsi with abundant setae.

Metasoma. Longer than mesosoma (35:50); in dorsal view with seven tergites, the sixth longest; all tergites and ovipositor sheath with thin, evenly distributed setae. Ovipositor sheath moderately produced (Fig. 5).

Male (Figs 11–16). Body length 1.5–2.2mm. Body fragile and soft, meshlike coriaceous, mainly yellowish, sometimes with testaceous spot on propodeum, and on tergites (Figs 12, 15). Antenna with pedicel and flagellum, and wing veins brown. Head with long setae, thorax with black bristles. Structure mostly similar to female, but scrobes distinct, flagellar segments loose, clava not distinct, notauli as linear grooves, and proportions different: head in frontal view wider than high in ratio of 13:11; scape and pedicel length in ratio of 21:9; scape length to rest of antenna in ratio of 21:45; malar space to eye height in ratio of 23:13; length of pronotum, mesoscutum and scutellum approximately in ratio of 5:15:20; width of pronotum to mesoscutum in ratio of 3:4; and in lateral view metasoma approximately as long as mesosoma.

Etymology. The specific epithet is from the Latin word root "costallifer" (ridgy), referring to the sharp longitudinal carina present between the toruli, an important character of the genus *Sirovena.*

Sirovena stigma **Bouček**
(Figs 1–4)

Type material. Holotype ♀: Christmas Island, 1939–1940, C.A. Gibson-Hill leg. Deposited in The Natural History Museum, London. Paratypes: 4♀, same data as holotype.

Redescription. Female. Body length 1.5–2.1mm. Head and mesosoma brownish black, without greenish tinge; antenna brown; legs mostly light yellow; metasoma dark brown.

Head. In frontal view (Fig. 2) slightly wider than high (71:69); head width and oral cavity width in ratio of 93:50; clypeus protruding, testaceous, sides distinctly delimited by dorsally convergent grooves. Head in lateral view higher than long (40:27); malar sulcus complete; malar space, eye height and eye width in ratio of 10:22:15. POL longer than OOL (20:12) (Fig. 3). Antennal segments with setae except anelli; scape claviform, shorter than pedicel+flagellum (25:35), approximately 8 times as long as wide and 5 times as long as pedicel; funicular segments compact, each slightly wider than long; clava as long as the last three funicular segments combined.

Mesosoma. Mesonotum (Fig. 3) with several irregular, outstanding setae. Length of pronotum, mesoscutum and scutellum approximately in ratio of 2:7:12; width of pronotum to mesoscutum in ratio of 5:6; mesoscutum broader than long (36:14); scutellum longer than wide (95:90), with about 3 pairs of dark, outstanding hairs. Upper mesepimeron mostly glabrate, lower mesepimeron with imbricate reticulation (Fig. 4). Fore wing (Fig. 1) length to width in ratio of 8.0:3.4; submarginal, marginal and stigmal veins in ratio of 55:10:13.All tarsi with abundant setae.

Metasoma. Longer than mesosoma (40:55); in dorsal view with seven tergites, the sixth longest; all tergites and ovipositor sheath with thin, evenly distributed setae; hypopygium rather large, ending beyond middle of gaster. Ovipositor sheath apex upturned (Fig. 1).

Molecular analyses

Both the female and male of *S. costallifera* have the same 549bp *COI* sequences, confirming their conspecificity (Fig. 17). In the NJ tree (Fig. 17), *S. costallifera* clustered together with other genera in Pireninae (identified as Hymenoptera sp.) rather than other genera of pteromalid non-pollinating fig wasps, including *Sycoscapter, Walkerella* and *Philotrypesis*, and was more distantly placed relative to *Ormyrus* (Ormyridae) and *Sycophila, Acophila* and *Sycobia* (Eurytomidae).
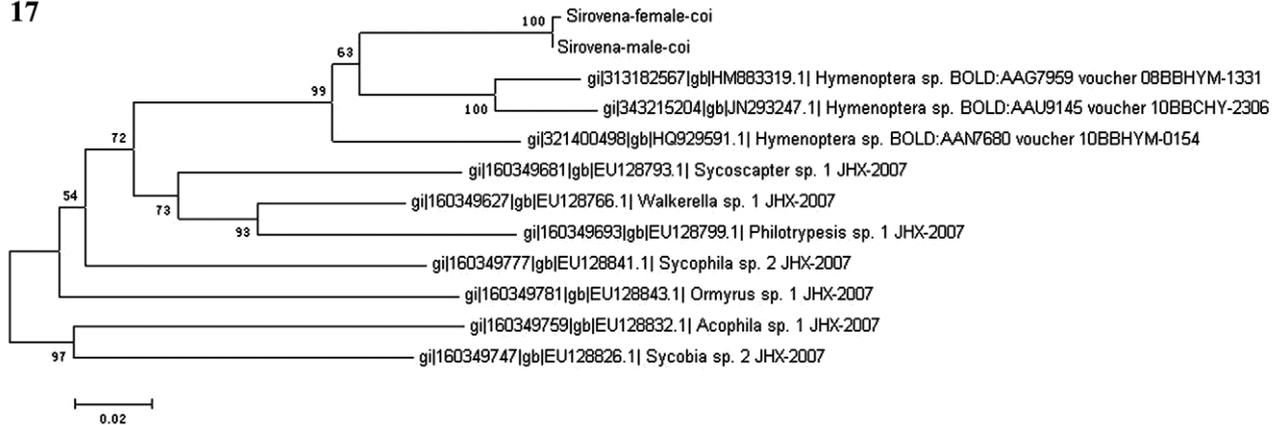
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FIGURE 17. NJ tree constructed with COI sequences from *Sirovena costallifera* and ten other unidentified species of Chalcidoidea obtained from the National Center for Biotechnology Information (accession numbers for the downloaded sequences given in figure).

Discussion

Speculated host. Association of chalcids with fig trees is well recorded (Cook & Rasplus 2003). For *Ficus microcarpa,* 27 species have been recorded, including 2 species of pollinating fig wasps and 25 species of non-pollinating wasps (Beardsley 1998, Chen *et al.* 1999, Farache *et al.* 2009, Noyes 2011). Non-pollinating fig wasps are divided into three functional groups: large gall-inducers and their parasitoids, small gall-inducers, and internal parasitoids (Cook &Rasplus 2003, Herre 1999, Kerdelhue *et al.* 2000, West *et al.* 1996).

Pireninae has a worldwide distribution (Bouček 1988, Noyes 2011), but little is known about host associations. The few reliable records suggest that members are parasitoids of Cecidomyiidae (Diptera). Some *Gastrancistrus* Westwood are parasitoids of cecidomyiid galls on various *Acacia*, *Melaleuca* and *Syncarpia*, and some European *Macroglenes* Westwood are parasitoids of cecidomyiids associated with Gramineae (Bouček 1988).

Although *S. costallifera* was not reared directly from a cecidomyiid in the fig, and there is no record of cecidomyiids within the syconium of *F. microcarpa*, we speculate that its host likely was a midge based on the following. First, the majority of the known hosts of Pireninae are cecidomyiids. Second, the midge larvae of an undescribed cecidomyiid genus develop inside fig ovules of *Ficus benjamina* (Bai *et al.* 2008, Meiswinkel *et al.* 2004, Miao *et al.* 2011*),* which is phylogenetically close to *F. microcarpa* (Jousselin *et al.* 2003, Rønsted *et al.* 2005). Third, some unidentified midges occasionally infest the syconia of *F. microcarpa* (Da-Wei Huang, pers. obs.).

Sirovena costallifera was found only in a batch of specimens from Yunnan in 2003, and has not been found among fig wasp communities in Hainan, Guangxi and Guangdong provinces. There is a male *Sirovena* reared from *F. microcarpa* from Hong Kong, May, 1961, D. S. Hill, in the collection of The Natural History Museum.

Sexual dimorphism. Most fig wasps show extreme sexual dimorphism— females are usually dark colored and have fully developed wings and compound eyes, whereas males are yellow and apterous or brachypterous (Cook &West 2005, Weiblen 2002). Sexual dimorphism in *S costallifera* is mainly limited to body color and sclerotization and flagellar structure. Females have a compact flagellum with a distinct clava, whereas males have the flagellar segments loose and the clava is not distinct. Females also have a raised-reticulate sculpture on the head and thorax and the notauli are composed of irregular areolates, whereas in males the reticulate sculpture on the head and thorax is almost obsolete and the notauli are linear grooves. Sexual dimorphism in other genera in Pireninae is less pronounced—*Gastrancistrus* females have 5 funicular segments and males 6 (Bouček 1988), whereas the main difference between females and males of *Macroglenes* is the shape of the funicular segments (Bouček 1993, Mitroiu 2010). Based on the above, we suggest that conditions within the fig syconia contributed to the more extreme sexual dimorphism of *S. costallifera* compared to other Pireninae.

Acknowledgements

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