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Description of the aberrant *Leptopilina lasallei* n. sp., with an updated phylogeny of *Leptopilina* Förster (Hymenoptera: Figitidae: Eucoilinae)

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ABSTRACT

In the search for native Asian parasitoids of *Drosophila suzukii*, the notorious spotted-wing *Drosophila* (SWD), an odd new species of Eucoilinae was discovered. *Leptopilina lasallei* **sp. nov**. is herein described and diagnosed relative to other eucoilines associated with drosophilid hosts. Morphologically, *L. lasallei* is somewhat aberrant within *Leptopilina*; phylogenetically, *L. lasallei* is sister group to the core *Leptopilina*. In the process of investigating *L. lasallei*, a *de novo* molecular phylogeny of *Leptopilina* was generated and is included here. The integrated approach used for the characterisation of *L. lasallei*, and the resulting phylogeny of *Leptopilina*, produced data useful to select parasitoid species for SWD biological control.

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Species of *Leptopilina* (Hymenoptera: Figitidae: Eucoilinae), parastioids of drosophilid flies (Diptera), have been studied in laboratory settings for decades. These wasps are easily cultivated into lab strains, which are studied in captivity for research ranging from host resistance (e.g. Vass and Nappi 2000; Lee et al. 2009) to host-finding cues (e.g. Van Alphen et al. 1991). Understanding the taxonomy and evolutionary history of *Leptopilina* species has been the focus of research since Nordlander's (1980) groundbreaking work on the genus. Since then, several new species have been described in the genus (Novković et al. 2011; Wachi et al. 2015; Lue et al. 2016), and several phylogenies have been published (Schilthuizen et al. 1998; Allemand et al. 2002; Novković et al. 2011; Wachi et al. 2015). Collectively, we know more about the systematics of this genus than most other Eucoilinae, and this has benefitted efforts to locate parasitoids of the notorious spotted-wing *Drosophila*, *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) (SWD).

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Several species of *Leptopilina* have been implicated as natural enemies of SWD. This pest fly is established in all soft-fruit growing regions of the United States, as well as Mexico, Canada, parts of Central and South America, and Europe. Spotted-wing *Drosophila* has a wide host range in its native home (Asia) and invaded countries where it feeds on numerous soft fruits (e.g. strawberry, caneberry, blueberry and cherry), using a serrated ovipositor to tear open and lay several eggs within each fruit; the eggs hatch and the developing larvae feed within the fruit with little to no external evidence of damage. Since 2008, when SWD was first recognised as a major pest in North America, hundreds of millions of dollars in production loss due to SWD (as well as to mitigation of SWD) occurred in California alone (Farnsworth et al. 2017). Including the East Coast fruit-growing region of the United States, as well as soft-fruit production in Canada, Mexico and Europe, could triple that number.

The search for effective natural enemies of this pest fly has been the focus of a great deal of research in recent years (Daane et al. 2016; Guerrieri et al. 2016; Nomano et al. 2017; Girod et al. 2018; Giorgini et al. 2019). This exploration in Asia for natural enemies of SWD has identified 5–7 species of *Leptopilina* and *Ganaspis* (Figitidae), *Asobara* (Braconidae) (larval parasitoids) and *Pachycrepoides* (Pteromalidae) and *Trichopria* (Diapriidae) (pupal parasitoids) (see Giorgini et al. 2019).

In the pursuit of these natural enemies, an unidentified figitid wasp was recovered in consistent quantities from banana-baited traps in Yunnan Province, China, totalling 11% of all collected parasitoids and about 30% of all collected figitids (referred to as 'new genus' in Giorgini et al. 2019); the same species was reared from *Myrica rubra* infested with *Drosophila suzukii* and *D. pulcrhella* (referred to as '*Leptopilina* sp.' in Girod et al. 2018). The species did not match any description in Lin (1988) or Lee and Choi (1993); the species initially looked similar to *Leptopilina decemflagella* Lue and Buffington, but lacked a number of characters present in that species. Additional *Leptopilina* material has been examined and compared to this new taxon from the Taiwanese Agriculture Research Institute (TARI, Taichung, Taiwan; major depository of the Lin Collection; Lin, 1988) and the Bernice P. Bishop Museum (BPBM, Honolulu, HI; major depository of the Maa and Yoshimoto cynipoid collections (Yoshimoto, 1962; Yoshimoto and Yasumatsu, 1965)), as well as further comparison with specimens housed at the National Museum of Natural History, Smithsonian Institution, Washington DC (USNM); however, none of these collections had specimens conspecific with this unknown wasp.

The combination of morphological and molecular data clarified the identity of this new species discovered in Yunnan Province, China, which is herein described as *Leptopilina lasallei* Buffington and Guerrieri, sp. nov. While pursuing the phylogenetic placement of *L. lasallei*, we generated an updated phylogeny of *Leptopilina* and discuss the relationships therein.

Materials and methods

Field collections

Surveys for *Drosophila* parasitoids were conducted between 2013 and 2016 in different locations of Yunnan Province, China (see material examined for details), using banana-baited traps placed in natural vegetation or cultivated fields, or collections of berries from

natural vegetation (see Giorgini et al. 2019). Banana-baited traps were made from plastic food boxes (10 × 15 × 30 cm) with 0.5-cm holes along the side for ventilation and provisioned with sliced sections of banana for fruit fly egg deposition (developing into fresh larvae available for parasitisation). At each of the sampled sites, 4–11 traps were placed in a linear transect at distances of ~100 m from each other. After 7 days, traps were collected and transferred to a laboratory (Yunnan Academy of Agricultural Science), where the ventilation holes were covered with organdie and the traps were held at 25 ± 3°C, 65 ± 5% relative humidity, 12L:12D photoperiod and observed daily for fly or parasitoid emergence. Field-collected fruits were placed into aerated boxes under the same conditions as described above and observed daily for parasitoid emergence. Emerged parasitoids were collected and immediately killed in 95% ethanol and preserved at −20°C until identification.

Integrated characterisation of insects

An integrated approach was followed to describe the new species by combining morphological and molecular diagnostic data.

Morphological examination and description

The diagnosis, description and morphological terms are derived from Lue et al. (2016). Specimens used in this study were dry mounted (using a vacuum dryer; Gates and Buffington 2011) for long-term preservation and examined in the Hymenoptera Unit at USNM. Morphological structures of insects were observed using a Leica M205 c binocular stereomicroscope with fluorescent light sources. Diagnostic characters for each species were illustrated using a scanning electron microscope (SEM; Hitachi®™ TM3000) and a Macropod®™ multiple-focus imaging system. For SEM images, vacuum-dried samples were mounted to adhesive SEM stubs and sputter-coated with gold-palladium for a 240-s interval resulting in 25–30 nm of gold-palladium alloy (using a Cressington®™ 108 Autosputtercoater). Zerene Stacker®™ was used to make composite images from image stacks generated by the Macropod.

Molecular characterisation

Newly field-collected wasps were sequenced for the mitochondrial barcoding cytochrome oxidase subunit 1 (COI) gene region, the ribosomal 28S-D2 region, and the Internal Transcribed Spacer 2 (ITS2) region.

DNA was extracted using a non-destructive whole-specimen extraction Chelex-proteinase K protocol (e.g. Guerrieri et al. 2016). Polymerase chain reactions (PCR) were performed in 20 µL volumes containing 4 µL of 1X GoTaq buffer (Promega Corp., Madison, Wisconsin, USA), 1.6 µL dNTP (2.5 mM each), 1 µL of forward and reverse primer (10 µM each), 0.4 µL GoTaq G2 DNA Polymerase (Promega) (5 u/µL) and 2 µL template DNA. Amplifications were achieved using a Bio-Rad Mycycler thermocycler (Bio-Rad, Hercules, California, USA) programmed for 1 min at 94°C, followed by 40 cycles of 30 s at 94°C, 90 s at 48°C and 60 s at 72°C, and a final step of 7 min at 72°C. Amplification of the mitochondrial COI gene was performed using one of the following primer combinations: LCO and HCO (Folmer et al. 1994) or

LepF1 and LepR1 (Hebert et al. 2004). The thermocycler was set at 94°C for 1 min, followed by 40 cycles at 94°C for 30 s, 48°C for 90 s and 72°C for 60 s, and at 72°C for 7 min as the final step.

Amplification of the 28S-D2 ribosomal gene region was performed using the primer combination D2 F and D2Ra (Campbell et al. 2000). The thermocycler was set at 94°C for 3 min, followed by 35 cycles at 94°C for 45 s, 55°C for 45 s and 72°C for 45 s, and at 72°C for 7 min as the final step.

Amplification of the ITS2 region was performed using the primer combination ITS2 F and ITS2revb (Stouthamer et al. 1999). The thermocycler was set at 94°C for 1 min, followed by 35 cycles at 94°C for 45 s, 55°C for 60 s and 72°C for 60 s, and at 72°C for 7 min as the final step.

PCR products were visualised after electrophoresis on 1% agarose gel stained with Gel Red™ (Biotium Inc, Fremont, California, USA) to confirm the amplification. Fragments obtained were sequenced in both sense and antisense directions by adopting EZ-seq standard service (Macrogen Inc., Seoul, South Korea). The chromatograms obtained were viewed and edited in Chromas v. 2.6.4 (Technelysium, South Brisbane, Queensland, Australia). Protein-coding of the COI gene region was checked by translating the sequences into amino acids, and no evidence for the presence of pseudogenes (i.e. no stop codons or frame shifts) was detected. All sequences were deposited in GenBank under the accession numbers reported in Table 1, and parasitoid wasps were vouchered at the USNM. Other taxa used in this study were previously sequenced by our group at the USNM (see Table 1 for details) following the protocols reported in Lue et al. (2016).

Phylogenetic reconstruction

Sequences not generated *de novo* in this study were taken from Lue et al. (2016), and previous studies where vouchers of sequenced individuals could be examined (Table 1). Alignment of concatenated COI, 28S-D2 and ITS2 sequenced regions were examined in Mesquite 3.5 (Maddison and Maddison 2019) and verified by eye for errors. The 28S D2 fragment was compared to the Buffington et al. (2007) structural alignment. As these species are all within Eucoilini *sensu* Forshage (2008), alignment was uncontroversial. The resulting concatenated matrix of COI, 28S-D2 and ITS2 was exported from Mesquite for Mr. Bayes 3.2, applying the GTR+I + G rate matrix for each data partition (COI divided into three partitions, one for each position) and running 15 million generations with a burn-in of 25%; explanation and justification of these protocols are provided in Buffington et al. (2007). The resulting tree was visualised in FigTree 1.3.1, and the out-group (*Trybliographa*) was assigned; the final tree figure was generated using Adobe Illustrator.

Table 1. GenBank accession numbers of taxa reported for the first time in this paper (identified with the code dsz) and of taxa already sequenced in previous works.

Species	Specimen identification code	Country	GenBank accession number			Reference
			COI	D2	ITS2	
Leptopilina lasallei	dsz102	Yunnam, China	MK268784	MK259996		This paper
Leptopilina lasallei	dsz095	Yunnan, China	MK268789	MK259991	MK937816	This paper
Leptopilina lasallei	dsz104	Yunnan, China	MK268790	MK259998	MK937817	This paper
Leptopilina lasallei	dsz184	Yunnan, China	MK268791	MK260003	MK937818	This paper
Leptopilina orientalis	strain G544-2	Madagascar			AY124562	Allemand et al. 2002
Leptopilina orientalis	strain G504-2	France			AY124563	Allemand et al. 2002
Leptopilina boulardi	dsz035	California, USA	MK268792	MK259987		This paper
Leptopilina boulardi	dsz034	California, USA	MK268798	MK259986		This paper
Leptopilina boulardi	dsz028	California, USA	MK268793	MK920206		This paper
Leptopilina boulardi	dsz030	California, USA	MK268794	MK259982		This paper
Leptopilina boulardi	dsz031	California, USA	MK268795	MK259983		This paper
Leptopilina boulardi	dsz032	California, USA	MK268796	MK259984		This paper
Leptopilina boulardi	dsz033	California, USA	MK268797	MK259985		This paper
Leptopilina boulardi	strain LbFr	France	JQ808437			Kacsoh & Schlenke 2012
Leptopilina maia	USNMENT00917769	USA	KY077431			Lue et al. 2016
Leptopilina maia	USNMENT01022751	USA	KY077436			Lue et al. 2016
Leptopilina maia	BIOUG08597-F08	USA	KR878814			Hebert et al. 2016
Leptopilina clavipes	USNMENT01022205	USA	KY077407			Lue et al. 2016
Leptopilina clavipes	strain LbNet	Holland	JQ808441			Kacsoh & Schlenke 2012
Leptopilina clavipes	USNMENT01022819	USA	KY077406			Lue et al. 2016
Leptopilina clavipes	USNMENT01022296	USA	KY077408			Lue et al. 2016
Leptopilina leipsi	USNMENT00917775	USA	KY077424			Lue et al. 2016
Leptopilina leipsi	USNMENT01022612	USA	KY077422			Lue et al. 2016
Leptopilina leipsi	USNMENT01022355	USA	KY077421			Lue et al. 2016
Leptopilina leipsi	USNMENT01022504	USA	KY077423			Lue et al. 2016
Leptopilina tokionensis	sp.TK1	Japan	AB624302		AB894846	Wachi et al. 2015
Leptopilina guineaensis	strain Lg500	Guinea	JQ808442			Kacsoh & Schlenke 2012
Leptopilina guineaensi	strain Lg510	Guinea	JQ808443			Kacsoh & Schlenke 2012
Leptopilina guineaensi	strain G510	South Africa			AY124559	Allemand et al. 2002
Leptopilina ryukyuensis	strain IR	Japan	AB546869		AB546893	Novković et al. 2011
Leptopilina ryukyuensis	strainAM	Japan	AB546871		AB546895	Novković et al. 2011
Leptopilina ryukyuensis	strainNH	Japan	AB546870		AB546894	Novković et al. 2011
Leptopilina ryukyuensis	IRIOMOTE 11	Japan	AB583586		AB583715	Novković et al. 2011
Leptopilina ryukyuensis	TAIPEI 8	Taipei	AB583606		AB583724	Novković et al. 2011

(*Continued*)

Table 1. (Continued).

Species	Specimen identification code	Country	GenBank accession number			Reference
			COI	D2	ITS2	
Leptopilina japonica	dsz094	Yunnan, China	MK268802	MK259990	MK937819	This paper
Leptopilina japonica	dsz100	Yunnan, China	MK268803	MK259994	MK937820	This paper
Leptopilina japonica	dsz036	Yunnan, China	MK268799	MK259988	MK937821	This paper
Leptopilina victoriae	Bogor 13	Indonesia	AB583619		AB583736	Novković et al. 2011
Leptopilina victoriae	USNMENT01197517	USA	KY077427			Lue et al. 2016
Leptopilina victoriae	Borgor 10	Indonesia	AB583616		AB583733	Novković et al. 2011
Leptopilina victoriae	Borgor 3	Indonesia	AB583609		AB583727	Novković et al. 2011
Leptopilina victoriae	strain KK	Malaysia	AB546872		AB546891	Novković et al. 2011
Leptopilina victoriae	strain BG	Indonesia	AB546873		AB546892	Novković et al. 2011
Leptopilina heterotoma	Iriomote h	Japan	AB583626		AB583743	Novković et al. 2011
Leptopilina pacifica	strainBGp	Indonesia	AB583624		AB583741	Novković et al. 2011
Leptopilina pacifica	Borgor 22	Indonesia	AB583622		AB583739	Novković et al. 2011
Leptopilina pacifica	strain Irp	Japan	AB583623		AB583740	Novković et al. 2011
Leptopilina tsushimaensis	strain TS1	Japan	AB894842			Wachi et al. 2015
Leptopilina decemflagella	dsz096	Yunnan, China	MK268773	MK259992	MK937822	This paper
Leptopilina decemflagella	dsz098	Yunnan, China	MK268776	MK259993	MK937823	This paper
Leptopilina decemflagella	dsz103	Yunnan, China	MK268775	MK259997	MK937824	This paper
Leptopilina decemflagella	dsz106	Yunnan, China	MK268777	MK259999	MK937825	This paper
Leptopilina decemflagella	dsz107	Yunnan, China	MK268778	MK260000	MK937826	This paper
Leptopilina decemflagella	dsz111	Yunnan, China	MK268774	MK260002	MK937827	This paper
Leptopilina decemflagella	USNMENT00917585	USA	KY077409			Lue et al. 2016
Leptopilina decemflagella	USNMENT00927662	USA	KY077410			Lue et al. 2016
Leptopilina freyae	strain G519	Benin			AY124560	Allemand et al. 2002
Leptopilina freyae	strain G449	Gambia			AY124561	Allemand et al. 2002
Trybliographa sp.	strain AAW8614	Canada			KR404907	Hebert et al. 2013
Trybliographa sp.	BIOUG17475-D01	Canada			KR805542	Hebert et al. 2016
Trybliographa sp.	BIOUG04242-B02	Canada			KR808151	Hebert et al. 2016
Trybliographa	BIOUG16088-A02	Canada			KR892096	Hebert et al. 2016

Table 2. Summary of diagnostic characters for separating *L. lasallei* from other eucoilines of similar habitus and habitat.

	Genus			
Character	*Leptopilina lasallei*	*Other Leptopilina*	*Ganaspis*	*Hexacola*
Female petiole	As deep as long	As deep as or deeper than long	As deep as long	As deep as long
Setal band at base of female metasoma	Complete	Interrupted dorsally or largely incomplete	Complete	Complete
Posteroventral corner of female metapleuron	Glabrous	Glabrous	Setose	Setose
Number of flagellomeres in female antenna	10	10 or 11	11	11
Length of male antenna F2	As long as F1	As long or longer than F1	Shorter than F1	Shorter than F1
Dorsal surface of scutellum	Striate	Striate to rugulose	Rugulose	Striate

Results

Morphological description

***Leptopilina lasallei* Buffington and Guerrieri sp. nov.**

(Figures 1–4)

Diagnosis

Female. Setal band complete at base of metasoma (Figures 1b and Figure 2e); dorsal surface of scutellum anteriorly striate, posteriorly foveate (Figure 2f); posteroventral corner of metapleuron glabrous (Figure 2e); antenna with 10 flagellomeres (Figure 2c); petiole as long as wide (Figure 2e). Male similar to female except setal band incomplete at base of metasoma (Figure 3a); antenna with 13 flagellomeres (Figure 3a), F1 the same size as F2, not distinctly excavated laterally (Figure 3b). See Table 2 for a summary of characters.

Description

Female. Holotype length 1.4 mm.

General. Body overall very smooth, glabrous, lacking sculpture except on scutellum (Figures 1 and 3). Head, mesosoma, metasoma dark brown, wings hyaline, legs honey yellow.

Head. In anterior view, ovate (Figure 2a). Head glabrous with very sparse setae scattered on face, clypeus and mandibles. Lateral margin of occiput smooth. Ratio of length of gena (I, Figure 2a) to length of compound eye (II, Figure 2b) 1 to 3. Gena smooth. Lateral margin of occiput evenly rounded, not well defined. Occiput smooth. Ratio of maximum diameter of a lateral ocellus (III, Figure 2b) to shortest distance between lateral ocelli (IV, Figure 2b) 1:2. Posterior margin of anterior ocellus clearly separated from anterior margins of posterior ocelli. Ratio of vertical distance between inner margin of antennal foramen and ventral margin of clypeus (V, FIgure 2a) to vertical distance between anterior ocellus and antennal rim (VI, Figure 2a) 1:4. Median keel absent. Vertical carina adjacent to ventral margin of

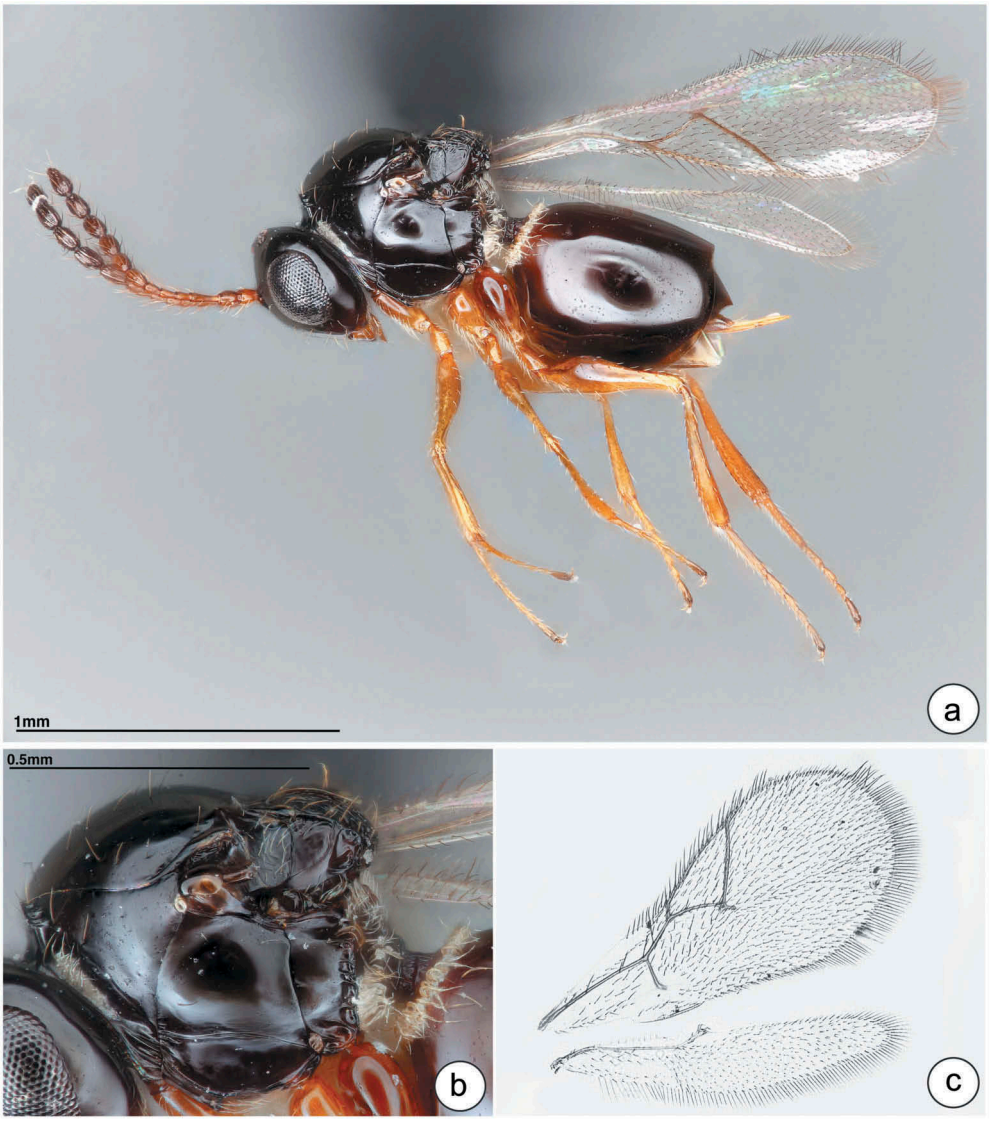

Figure 1. Holotype of *Leptopilina lasallei* sp. nov. (USNMENT00896641). a. Lateral habitus. b. Close-up of mesosoma. c. Left fore and hind wings.

antennal socket present, minute. Facial sculpture absent, surface smooth. Facial impression absent, face flat. Antennal scrobe absent. Anterior tentorial pits small (VII, Figure 2a). Vertical delineations on lower face absent. Ventral clypeal margin laterally, close to anterior mandibular articulation, straight. Ventral clypeal margin medially with 6 setae. Clypeus smooth with gently curved ventral margin. Malar space adjacent to anterior articulation of mandible evenly rounded, smooth. Malar sulcus (VIII, Figure 2a) present, simple. Ratio of distance between compound eye and posterior mandibular articulation to distance between posterior ocellus and compound eye 1:1. Compound eyes, in dorsal view, not distinctly protruding from the surface of the head (Figure 2). Short, sparse setae

on eyes (Figure 2). Orbital furrows absent. Lateral frontal carina of face absent. Dorsal and posterior aspects of vertex smooth. Hair punctures on lateral aspect of vertex absent. Posterior surface of head deeply impressed around postocciput.

Labial-maxillary complex. Apical segment of maxillary palp with pubescence, consisting of 1 long erect seta. Apical seta on apical segment of maxillary palp longer than twice length of second longest apical seta. Maxillary palp composed of 4 segments. Last 2 segments of maxillary palp (in normal repose) straight. Apical segment of maxillary palp 2X longer than preceding segment.

Antenna. Terminal flagellomere with 3 basiconic sensillae. Basiconic sensillae present on F5–F9. Articulation between flagellomeres in antenna moniliform, segments distinctly separated by narrow neck-like articulation. Antenna composed of 10 flagellomeres (Figure 2c); F1 2.5X longer than F2. Flagellomeres cylindrical, distinctly widened towards apex, clavate. Placoidal sensilla present on F5–F10 (Figure 2c).

Mesosoma. Macrosculpture on lateral surface of pronotum absent dorsally and laterally (Figure 1). Anteroventral inflection of pronotum narrow. Pubescence on lateral surface of pronotum present in pronotal trough. Anterior flange of pronotal plate distinctly protruding anteriorly, transversely strigate (Figure 2). Ridges extending posteriorly from lateral margin of pronotal plate distinct but short, not extending to the dorsal margin of pronotum. Lateral pronotal carina absent. Crest of pronotal plate absent. Dorsal margin of pronotal plate (in anterior view) spatulate. Submedian pronotal depressions open laterally, deep (Figure 2). Lateral margin of pronotal plate defined all the way to the dorsal margin of the pronotum. Width of pronotal plate narrow, not nearly as wide as head.

Mesoscutal surface convex, evenly curved (Figure 1). Sculpture on mesoscutum absent, entire surface smooth, shiny, with sparse long hairs. Notauli absent. Median mesoscutal carina, anterior admedial lines and median mesoscutal impression all absent. Parascutal carina nearly straight.

Mesopleuron entirely smooth (Figure 1). Subpleuron entirely smooth, glabrous. Lower pleuron entirely smooth, glabrous. Epicnemial carina absent. Lateroventral mesopleural carina present, marking abrupt change in slope of mesopectus. Mesopleural triangle absent. Subalar pit present, located under subalar area, not easily observed. Speculum absent. Mesopleural carina present, complete, composed of one complete, uninterrupted carina. Anterior end of mesopleural carina inserting above notch in anterior margin of mesopleuron.

Dorsal surface of scutellum distinctly striate on anterior 2/3, posterior 1/3 foveate (Figure 2). Circumscutellar carina present, complete, delimiting dorsal and ventral halves of scutellum. Posterior margin of axillula marked by distinct ledge, axillula distinctly impressed. Lateroventral margin of scutellum posterior to axillula smooth. Dorso-posterior part of scutellum rounded. Transverse median carina on scutellar plate absent. Scutellar plate, in dorsal view, exposing more than half of scutellum. Scutellar fovea present, 2, distinctly margined posteriorly, smooth on bottom. Longitudinal scutellar carinae absent. Single longitudinal carina separating scutellar foveae present, short, ending at posterior margin of foveae. Posterolateral margin of scutellum rounded. Lateral bar smooth, narrow.

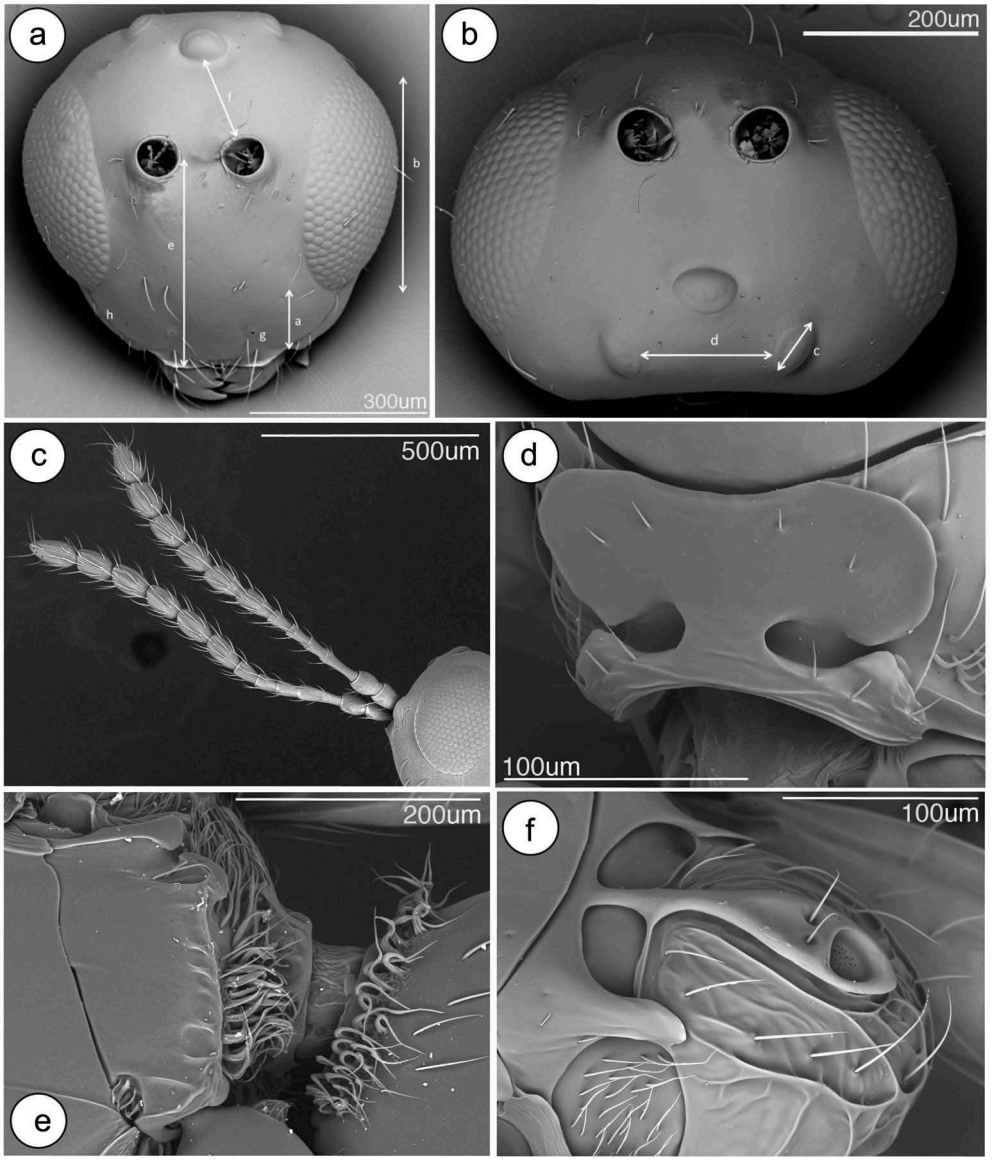

Figure 2. Scanning electron micrographs of the paratypes of *Leptopilina lasallei* sp. nov. (USNMENT01525765). a. Head, anterior view; I, distance from ventral margin of eye to posterior margin of malar space; II, height of eye; V, distance between inner rim of torulus to posterior clypeal margin; VI; distance between anterior ocellus and posterior rim of torulus; VII, tentorial pit; VIII, malar furrow. b. Head, dorsal view; III, width of lateral ocellus; IV, distance between lateral occeli. c. Female antenna, lateral view. d. Anterior aspect of pronotal plate, male. e. Close-up lateral aspect of metapleuron, propodeum, petiole and anterior margin of metasoma. f. Male scutellum, dorso-lateral view.

Metapleural-propodeal complex. Posterior impression of metepimeron absent (Figures 2 and 2e). Metapectal cavity anterodorsal to metacoxal base present, well defined. Anterior margin of metapectal-propodeal complex meeting mesopleuron at same level at point

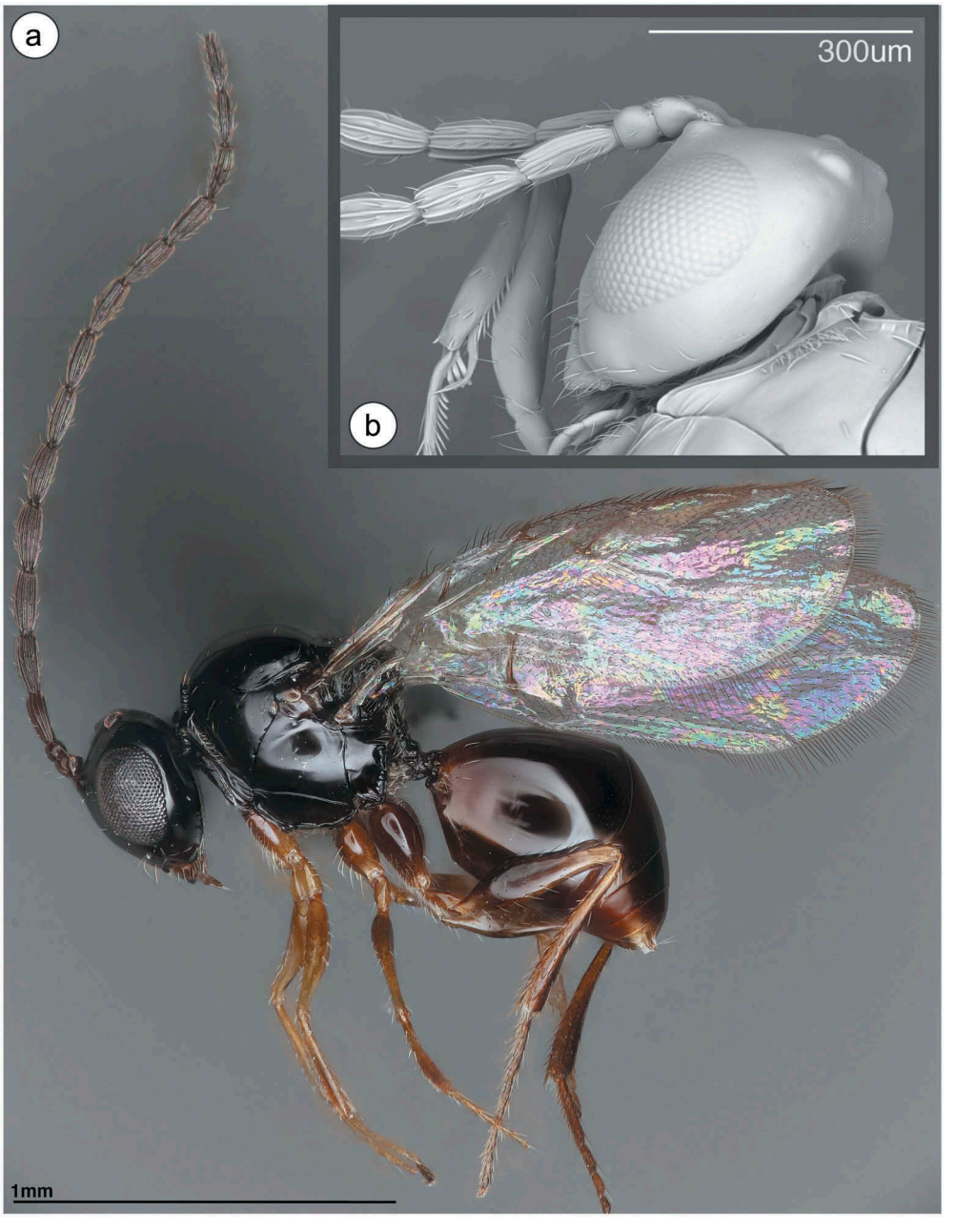

Figure 3. Male paratype. a. lateral habitus. b. Scanning electron micrograph of head and basal segments of antennae.

corresponding to anterior end of metapleural carina. Posteroventral corner of metapleuron (in lateral view) not extended posteriorly, glabrous. Anterior impression of metepimeron absent. Posterior margin of metepimeron distinct, separating metepimeron from propodeum. Subalar area broadened anteriorly, narrowed posteriorly. Prespiracular process present, blunt, lobe-

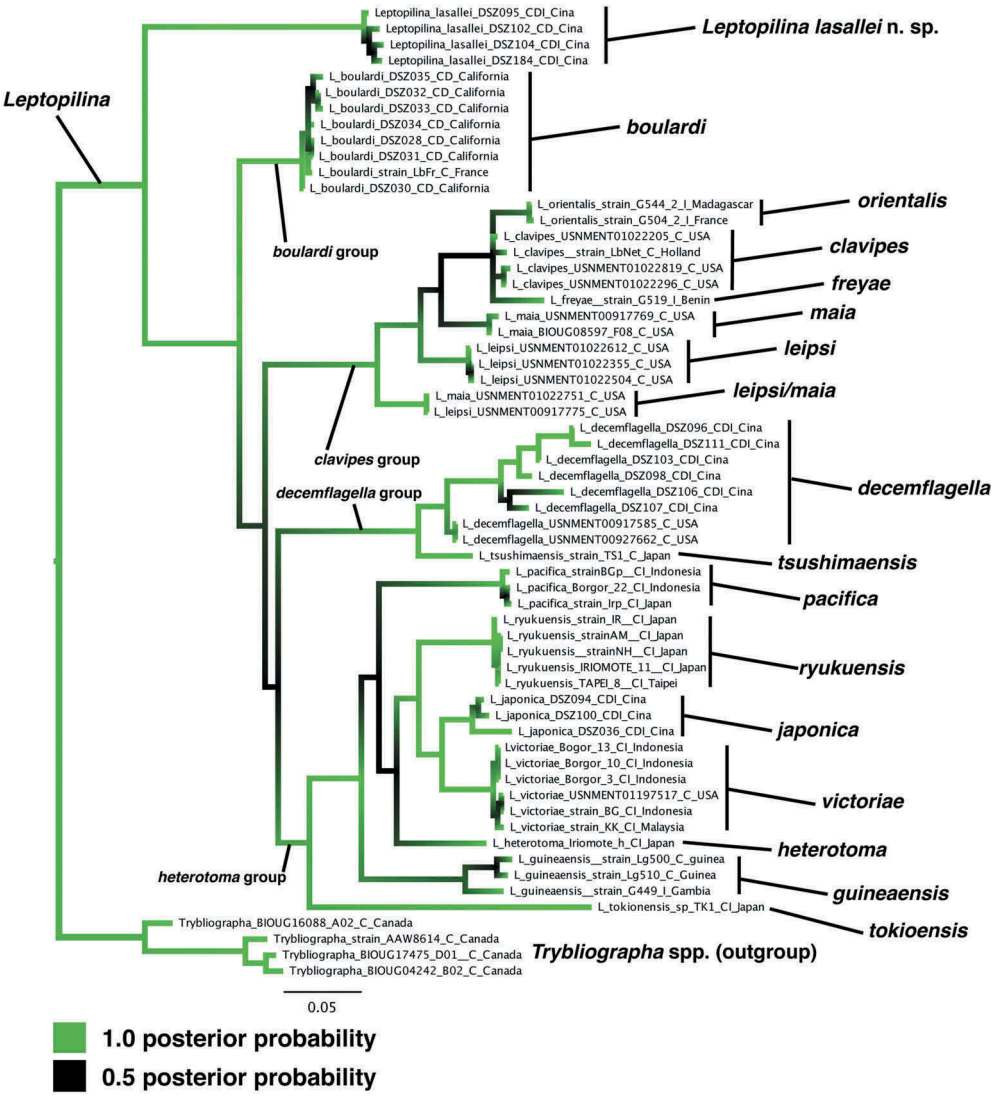

Figure 4. Phylogeny of *Leptopilina* based on Bayesian analysis of concatenated nucleotide sequences of Cytochrome Oxidase I (COI) gene 'barcode' region, 28S D2 and ITS2 regions. Taxa denoted by 'DSZ' are newly sequenced for this study; other number/letter designations are those of Genbank sequences. See Table 1 for GenBank accession numbers and references.

like, rough. Dorsellum absent. Anterior impression of metepisternum present. Pubescence consisting of few hairs on posterior part of metepisternum, dense hair on propodeum.

Propodeal spurs absent. Lateral propodeal carinae present, not reaching scutellum, lyre-shaped, stout. Ventral end of lateral propodeal carina reaching nucha, carinae separated from each other. Inter-propodeal carina space lightly setose, underlying surface smooth. Petiolar rim of uniform width along entire circumference. Petiolar foramen removed from metacoxae, directed posteriorly. Horizontal carina running anteriorly

from lateral propodeal carina present medially, effaced laterally. Calyptra, in lateral view, rounded; in posterior view, elongate. Propodeum neck-like, drawn out posteriorly.

Legs. Pubescence posterolaterally on metacoxa, present, small, rounded, with adjacent sparse pubescence (Figure 1). Microsculpture on hind coxa absent. Longitudinal ridge on the posterior surface of metatibia absent. Metafemoral tooth present, elongate, with adjacent serrate ridge posteriorly. Ratio of first metatarsal segment to remaining 4 segments 2.1:1.

Wings. Wing vein M absent (Figure 3). Pubescence of fore wing present, long, dense on most of surface. Apical margin of fore wing rounded; Rs+M of fore wing defined but nebulous at point of origin from basal vein at posterior third; mesal end of Rs+M vein situated closer to anterior margin of wing, directed towards middle of basalis; vein R1 forming marginal cell completely; basal abscissa of R1 as broad as adjacent wing veins. Colouration of wing absent, entire wing hyaline. Marginal cell of fore wing membranous, similar to other wing cells. Areolet absent. Hair fringe along apical margin of fore wing present, of medium length.

Metasoma. Petiole about as long as wide (Figures 2). Surface of petiole longitudinally costate, ventral keel absent. Posterior part of petiole not abruptly widened. Ventral and lateral parts of petiolar rim broad. Setal band at base of tergum 3 present, uninterrupted dorsally and ventrally (Figures 1 and 2). Tergum 3 indistinct, fused with syntergum. Posterior margin of tergum 4 evenly rounded. Sternum 3 encompassed by syntergum. Sculpture on metasomal terga absent (Figure 1). Syntergum present with terga 3 to 5 fused, ventral margin rounded. Peglike setae on T6–T7 absent. Postero-ventral cavities of female metasoma T7 present, glabrous save for few, long setae. Female postero-ventral margin of T6–T7 straight, parallel. Terebrum and hypopygium (in lateral view) straight, pointing posteriorly. Ovipositor clip present.

Male: Similar to female except for antenna with 13 flagellomeres (Figure 2) with unmodified F2 (Figure 2), absence of setal band at base of tergum 3 (Figure 2). Metasoma, posteriorly, directly ventrally, somewhat truncate.

Variation. Body size ranges from 1.2 to 1.5 mm. Overall body colouration varies slightly from dark brown to nearly black; dorsal surface of scutellum can range from slightly striate to deeply striate, foveate at posterior end of scutellum; very faint setal tracks present on the mesoscutum of some specimens, absent in others.

Etymology. Named in honour of the late Dr John La Salle. John's dedication to Hymenoptera research, and biodiversity research in general, will be greatly missed. We hope this honorific helps to keep his memory alive for years to come.

Material examined. **Holotype:.** ♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m above sea level (asl), 20 July 2015, from banana trap, EGWY124 (Wang Yan) USNMENT00896641; left fore and hind wings mounted on slide USNMENT01525760.

Paratypes: 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, October 2014, from banana trap, EGWY36 (Wang Yan) USNMENT00896646; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, October 2014, from banana trap, EGWY41 (Wang Yan) USNMENT00896639; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 May 2014, from banana trap, EGWY87 (Wang Yan) USNMENT00896644; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 July 2015, from banana trap, EGWY103 (Wang Yan) SEM USNMENT01525765; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 July 2015, from banana trap, EGWY135 (Wang Yan) USNMENT00896648; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 July 2015, from banana trap, EGWY137 (Wang Yan) USNMENT00896633; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 July 2015, from banana trap, EGWY129, DS095 (Wang Yan) USNMENT01525764; 1♂, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 July 2015, from banana trap, EG15CZ6R BT-26, DSZ102 (Wang Yan) USNMENT01525761; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697°E, 2209 m asl, 20 July 2015, from banana trap, EG15CZ6R-BT, DS194 (Wang Yan) USNMENT01525762; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.176593°N, 102.794697° E, 2209 m asl, 20 May 2015, from banana trap, EGWY119 (Wang Yan) USNMENT00896626; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.1178°N, 102.4842°E, 23 June 2015, from banana trap, EG15CZ6RBT-19 (E. Guerrieri, M. Giorgini, K. Hoelmer) USNMENT00896625; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.112378 °N, 102.481742°E, 23 June 2015, from banana trap, EG15CZ6RBT-27 (Wang Yan) USNMENT00896628; 1♀, CHINA, Kunming, Xiao He Research Farm, Pan Long District 25.112378°N, 102.481742°E, 23 June 2015, from banana trap, EGCZ6RBTR19-4 (Wang Yan) USNMENT00896627; 1♀, CHINA, Kunming, Kunming Botanical Gardens, Pan Long District, 25.145348°N, 102.741543°E, 1958 m asl, 12 July 2015, from blackberry fruits, EG15CZ5-6 (Wang Yan) USNMENT00896638; 1♂, CHINA, Kunming, Kunming Botanical Gardens, Pan Long District, 25.145348°N, 102.741543°E, 1958 m asl, 12 July 2015, from blackberry fruits, EG15CZ5-23, DSZ 184 (Wang Yan) USNMENT01525763; 1♀, CHINA, Kunming, Kunming Botanical Gardens, Pan Long District, 25.145348°N, 102.741543°E, 1958 m asl, 12 July 2015, from blackberry fruits, EG15CZ5-32 (Wang Yan) USNMENT00896620; 1♀, CHINA, Kunming, Kunming Botanical Gardens, Pan Long District, 25.145348°N, 102.741543°E, 1958 m asl, 12 July 2015, from blackberry fruits, EG15CZ5-34 (Wang Yan) USNMENT00896649; 1♀, CHINA, Kunming, Cheng Jiang County, Long Jie Zuo Suo Cun, 24.711991°N, 102.870912° E, 2053 m asl, 24 July 2013, from banana trap, EGCZ1BTR2-2 (E. Guerrieri, M. Giorgini, K. Hoelmer) USNMENT00896624; 1♀, CHINA, Kunming, Cheng Jiang County, Long Jie Zuo Suo Cun, 24.711991°N, 102.870912°E, 2053 m asl, 24 July 2013, from banana trap, EGCZ1BTR2-3 (E. Guerrieri, M. Giorgini, K. Hoelmer) USNMENT00896647; 1♀, CHINA, Kunming, Cheng Jiang County, Fu Xian Lake, 24.506364°N, 102.860508°E, 1759 m asl, 24 July 2013, from banana trap, EGCZ2BTR4-2 (E. Guerrieri, M. Giorgini, K. Hoelmer) USNMENT00896635; 4♀, 6♂, CHINA, Yunnan Prov., Fumin, 25.1475°N, 102.5289°E, ex *D. suzukii* or *D. pulchrella* on *Myrica rubra*, 7 February 2016, Jinping Zhang (CABI2) USNMENT01525940-USNMENT01525949; 1♀, 3♂, CHINA, Yunnan Prov., Kunming, West

Mountain, 25.1475°N, 102.5289°E, ex *D. suzukii* on *Myrica rubra*, 7.II.2016, Fang Huan/Wu Hao (CABI3) USNMENT01525936-USNMENT01525939.

Biology. Reared from *Drosophila suzukii* or *D. pulchrella* on *Myrica rubra* (CABI specimens). Other specimens were collected in the wild with banana-baited traps from May to October, with the majority in July (USNMENT00896649 emerged from blackberries). Banana traps were set up in wild vegetation in natural reserves (Kunming, Cheng Jiang County, Long Jie Zuo Suo Cun), in natural habitats surrounding orchards (Kunming, Xiao He Research Farm, Pan Long District), in blueberry crops (Kunming, Cheng Jiang County, Fu Xian Lake), and in a botanical garden (Kunming Botanical Gardens, Pan Long District).

Comments. *Leptopilina lasallei* possesses some unusual character states for members of *Leptopilina*. Using van Noort et al. (2015), male *L. lasallei* runs to *Leptopilina*; a female specimen may also run to *Leptopilina* after some hesitation, but an often relied upon character, the 'broken' or 'interrupted' metasoma hairy ring character state, is not present. Instead, the hairy ring is complete in the females of *L. lasallei*, and this may add some confusion to diagnosing this taxon. In fact, this dimorphism is stronger than in most Eucoilinae, and interestingly, the pattern is inverted compared to other *Leptopilina* where the hairy ring of the female is more reduced than that of the male. This switch in dimorphism begs for clarifying observations of the behaviour of live specimens and the physiology of this trait.

Leptopilina is one of the better studied genera of Eucoilinae, with laboratory strains genetically and behaviourally studied, and a relatively large number of species that have been described or redescribed in modern times. Nevertheless, a large portion of worldwide diversity is still unaccounted for.

Leptopilina often gets confused with other small drosophilid parasitoids such as *Ganaspis* (Ganaspini) and *Hexacola* (Ganaspini) but is easily recognised by the characters that reveal its belonging in Eucoilini rather than Ganaspini: F2 in male antennae equally or more modified (curved/excavated/elongated) than F1, glabrous and more or less oblique posteroventral corner of metapleuron. Furthermore, *Leptopilina* are characterised by a well-developed petiolar rim. The setal bands ('hairy ring') of the base of the metasoma are often reduced to varying extents. In a few species, female flagellomere number is reduced to 10. Most but not all species have a high, convex scutellum. These additional characters commonly occur among the genera of Ganaspini but distinguish *Leptopilina* from most of its closer relatives within the Eucoilini.

Within *Leptopilina*, two species that *L. lasallei* can be confused with are *L. decemflagella* and *L. tsushimaensis*, as females in both of these species have 10 flagellomeres in their antennae (Lue et al., 2016). However, *L. decemflagella* and *L. tsushimaensis* females both have incomplete hair rings at the base of their metasoma. The striate dorsal surface of the scutellum in *L. lasallei* is shared with *L. freyae* and *L. boulardi* (Allemand et al., 2002); again, the latter two have a metasomal hairy ring in the female which is more or less strongly reduced (incomplete or even absent); male *L. lasallei* have the F1 and F2 of the antennae equal in size, whereas in in *L. freyae* and *L. boulardi*, F2 is clearly longer than F1. Another useful character for distinguishing *L. lasallei* from *L. freyae* and *L. boulardi*, and from *L. orientalis* which is similar too, is the shape of the propodeal carinae: in *L. lasallei*, these are heavily sclerotised and thick, overall lyre-shaped;

in the other species of *Leptopilina* that are overall similar looking, the propodeal carinae are finer, less massive and parallel sided.

Phylogenetic reconstruction

Bayesian analysis of the concatenated COI, 28S D2 and ITS2 data set produced a tree that highly supports the sister-group relationship of *L. lasallei* and the remaining species of *Leptopilina* (Figure 12). Five highly supported species groups were identified within the *Leptopilina* clade. While the *lasallei* and *boulardi* groups each have a single species in the current analysis, the *clavipes*, *decemflagella* and *heterotoma* groups include species from different continents.

Discussion

The new species clearly ended up as the sister group of the other *Leptopilina* species included, constituting the vast majority of the better known species. Thus, an argument could be made to either erect a new genus for *lasallei* or to somewhat expand the concept of *Leptopilina*. Since it is uncertain where many less-known species of *Leptopilina* as well as many undescribed species would end up in this phylogeny, it has been deemed best to pursue with caution. Thus, we recommend waiting to erect new genera until more species have been accounted for, especially to the extent they are intermediate between typical *Leptopilina* and related genera such as *Linaspis* and *Maacynips*, genera which are very poorly known at this stage, and in which most species remain undescribed (Forshage and Buffington, pers. obsv.).

The COI, 28S D2 and ITS2 molecular data sets recovered *L. lasallei* as sister group to *Leptopilina*. Clearly, more molecular data beyond these three gene fragments are needed to infer evolutionary trends; however, the phylogeny presented here is the most comprehensive in terms of the taxon sampling of *Leptopilina* since Allemand et al. (2002) and Novković et al. (2011). Allemand et al. (2002) suggested species groups for African *Leptopilina*, and in the data presented here, these groups appear to be supported. The *lasallei* and *boulardi* groups are each represented in our study by a single species only, but are both clearly distinct lineages from the rest of *Leptopilina* species (Figure 4), and sister groups to the remaining *Leptopilina*. Our data suggest expanding the *heterotoma* group to include additional Asian species, namely *L. ryukuensis*, *L japonica* and *L. pacifica*. Two new species groups emerged from our concatenated data set (Figure 4): the *clavipes* group (*L. clavipes*, *L. leipsi*, *L. maia*, *L. freyae* and *L. orientalis*) and the *decemflagella* group (*L. decemflagella* and *L. tsushimaiensis*). The *clavipes* group is not clearly delineated, and this was already discussed in Lue et al. (2016). It should be noted that Allemand et al. (2002) recovered *L. freyae* and *L. orientalis* in the *boulardi* group; we speculate that our expanded data set in terms of taxon sampling is responsible for this difference.

The *decemflagella* group shows a remarkable amount of sequence divergence (Figure 4). In fact, this is the most divergent among eucoilines sampled here, and certainly warrants future investigation. The need for further work in *decemflagella* is underscored by the fact that the sister group is *tsushimaensis*. Additional data may suggest these two names be synonymised, but at the present, we prefer to keep them as distinct species.

The groups of Allemand et al. (2002) and our study (presented here) largely reflect the topologies presented in Novković et al. (2011), although the taxon sampling of the latter was restricted to *heterotoma*-group species from Japan. Groups I–V from Novković et al. (2011) are contained within our *heterotoma* group of species, with a key difference in the placement of *L. pacifica*. In our study, *L. pacifica* is sister group to *L. heterotoma* plus the remaining species; in Novković et al. (2011), *L. heterotoma* comprises Group V and is sister group to the remaining *Leptopilina*. Some differences between our study and that of Novković et al. (2011) are our more robust taxon sampling, a more suitable out-group taxon and differences in tree-building methods [Bayesian analysis in our case; neighbour joining in Novković et al. (2011)].

Morphological and molecular data produced in this work, other than adding new information on taxonomy of drosophilid parasitoids, may help biological control practitioners to identify and select potential biocontrol agents. In terms of future directions, we are currently analysing ultra-conserved element phylogenomic data (Blaimer et al. 2016) for both Cynipoidea and Hymenoptera as a whole (in prep.). The goal is to evaluate these data for cryptic species and discrimination of closely related species in parasitoids. Currently, data on bees (Blaimer et al. 2016; Bossert et al. 2019) indicate that these data are suitable for species discrimination, and we plan to have diagnostic data for both *Leptopilina* and *Ganaspis* species in the near future, a fundamental step for their use in biocontrol programmes against destructive pest species.

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Author contributions

Conceived the project: EG, MB
Wrote the initial manuscript: MB, EG
Edited and revised the manuscript thereafter: MG, MF, MB
Sampled and sorted parasitoids in China: EG, MG
Conducted the taxonomic work: MB, EG, MF, CL
Carried out molecular characterisation and phylogenetic analysis: MB, MG, PC, GF, AD
All authors read and approved the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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