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The genus *Leucophenga* (Diptera, Drosophilidae), part I: the *abbreviata* species group from the Oriental region with morphological and molecular evidence

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Abstract

A new species group, the *abbreviata* group is established within the genus *Leucophenga* based on one known and three new species, all of which are endemic to the Oriental region: *L. abbreviata* (de Meijere, 1911), *L. brevivena* **sp. nov.**, *L. sujuanae* **sp. nov.** and *L. zhenfangae* **sp. nov.** A key to four species of the *abbreviata* group and the DNA barcoding are provided. Twenty-three mtDNA *COI* sequences belonging to the above species are analyzed; the molecular data are used as interactive evidence to evaluate the species boundaries defined by the morphological data.

Key words: DNA barcoding, *Leucophenga abbreviata* species group, new species, Oriental region

Intrduction

A total 203 species of the genus *Leucophenga* Mik, 1886 (Diptera: Drosophilidae) have been reported from the world, 69 spp. from the Afrotropical region, 40 spp. from the Australasian region, eight spp. from the Nearctic region, 21 spp. from the Neotropical region, 75 spp. from the Oriental region and 25 spp. from the Palearctic region (Brake & Bächli 2008); and approximately 142 species of these are orgainzed in the following ten species groups established (Bächli 1971; Okada 1990; Bächli *et al*. 2002): the *argentata* group (6 spp.), the *cuthbertsoni* group (2 spp.), the *flaviseta* group (4 spp.), the *flavopuncta* group (9 spp.), the *maculata* group (22 spp.), the *mutabilis* group (37 spp.), the *ornata* group (25 spp.), the *proxima* group (17 spp.), the *sorii* group (3 spp.) and the *subpollinosa* group (18 spp.) (Bächli 2012), the rest can not be placed to any of the above species groups.

In the present study, three new species from southern China and Nepal are described; they are morphologically similar to *Leucophenga abbreviata* (de Meijere, 1911) in wing M_1 vein distally abbreviated, not reaching wing margin (Fig. 2), which is unique in the genus. Thus, a new species group is established here, the *abbreviata* group, based on one known and three new species. Two Afrotropical species: *Leucophenga apicifera* (Adams, 1905) and *L. subvittata* Duda, 1939, share wing M_1 vein being distally abbreviated (Bächli 1971; Fig. 38, t, u), but the postocellar seta are longer than the inner vertical seta distinctly at least in *L. apicifera* (Bächli 1971; Fig. 17, k), while the postocellar seta are as long as the inner vertical seta in all the Oriental species; we provisionally exclude the two Afrotropical species in the *abbreviata* group to avoid further confusion.

It's widely accepted that molecular data have the potential to facilitate both the identification of known species and the discovery of new ones. DNA barcoding, initially proposed by Hebert *et al.* (2003a), is the use of a standardized segment of the genome for rapid species identification. The 5' end region of the mitochondrial cytochrome *c* oxidase I (*COI*) gene is recommended as the universal and standard barcoding marker for species identification (Hebert *et al.* 2004a; Ward *et al.* 2005; Ratnasingham & Hebert 2007). Various methods for DNA barcoding analysis are available and these include genetic distances, phylogenies and character attribute evaluation (Hebert *et al.* 2003b; DeSalle *et al.* 2005; Rach *et al.* 2008; Yassin *et al.* 2010; Reid *et al.* 2011; Zou *et al.* 2011). In this research, we analyze twenty-three barcode sequences of *COI* gene belonging to the one known and three new species in order to evaluate these species hypotheses.

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Material and methods

Morphological study. All specimens examined were collected by sweeping on tussocks and tree trunks along streams in forest. The type specimens are deposited in Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China (KIZ) and Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU). We followed Zhang & Toda (1992) and Chen & Toda (2001) for the definitions of measurements, indices and abbreviations.

Molecular study. Twenty-three specimens of the *abbreviata* group (twelve samples of *L. abbreviata*, one sample of *L. brevivena*, seven samples of *L. sujuanae* and three samples of *L. zhenfangae*) were analyzed for molecular work in this study and the localities where they were collected are listed in Table 1.

TABLE 1. Sampling and Genbank accession numbers for the *COI* sequences employed.

Taxa	Localities	Accession numbers of *COI*
L. abbreviata–GD1	Dinghushan, Zhaoqing, Guangdong, China	JX235946
L. abbreviata–GD2	Huolushan, Guangzhou, Guangdong, China	JX235945
L. abbreviata–GD3	Huolushan, Guangzhou, Guangdong, China	JX235944
L. abbreviata–GD4	Huolushan, Guangzhou, Guangdong, China	JX235943
L. abbreviata–GD5	Dinghushan, Zhaoqing, Guangdong, China	JX235939
L. abbreviata–GX	Bapeng, Fusui, Guangxi, China	JX235936
L. abbreviata–HN	Jianfengling, Ledong, Hainan, China	JX235937
L. abbreviata (♀)–TW1	Maolin, Gaoxiong, Taiwan, China	JX235953
L. abbreviata–TW2	Taidong, Taiwan, China	KC424636
L. abbreviata–YN1	Menglun, Mengla, Yunnan, China	JX235951
L. abbreviata–YN2	Menglun, Mengla, Yunnan, China	JX235933
L. abbreviata–YN3	Zhengxing, Jinggu, Yunnan, China	JX235942
L. brevivena	Menglun, Mengla, Yunnan, China	JX235938
L. sujuanae–YN1	Zhengxing, Jinggu, Yunnan, China	JX235935
L. sujuanae–YN2	Yixiang, Puer, Yunnan, China	JX235950
L. sujuanae–YN3	Menglun, Mengla, Yunnan, China	JX235934
L. sujuanae–YN4	Hesong, Menghai, Yunnan, China	JX235947
L. sujuanae–YN5	Hesong, Menghai, Yunnan, China	JX235948
L. sujuanae–YN6	Hesong, Menghai, Yunnan, China	JX235941
L. sujuanae–YN7	Muyiji Park, Ximeng, Yunnan, China	JX235952
L. zhenfangae–YN1	Yixiang, Puer, , Yunnan, China	JX235949
L. zhenfangae–YN2	Hesong, Menghai, Yunnan, China	JX235940
L. zhenfangae–Nepal	Beni, Dhawalagini, Nepal	JX235954

DNA Extraction and sequencing. For each species, total DNA was extracted using Tiangen® DNA extract kit following the manufacture's protocols. The 5' end region of *COI* gene sequences were PCR-amplified and then sequenced following the methods in He *et al.* (2009), using the primers *COI*-F1: CGCCTAAACTTCAGCCACTT (He *et al*. 2009) and HCO2198: TAAACTTCAGGGTGACCAAAAAATCA (Folmer *et al.* 1994). All sequences generated in this study were submitted to GenBank. The accession numbers are given in Table 1.

Sequences were aligned using the ClustalW (Thompson *et al.* 1994) method implemented in MEGA 5.0 (Tamura *et al.* 2011) with default options. MEGA 5.0 was also used to calculate sequence divergences and to create a neighbor-joining (NJ) tree based on the Kimura-2-parameter (K2P) model which was recommended by Hebert *et al.* (2003a). The "barcoding gap" was estimated as the difference between the maximal intraspecific distance and

minimal interspecific distances (Meyer & Paulay 2005; Meier *et al.* 2008). The character-based analysis was implemented using the Characteristic Attribute Organization System (CAOS) (Sarkar *et al.* 2008), a method that defines "DNA diagnostics", i.e. character states shared by members of a given taxon and simultaneously absent from comparable groups. The guide tree from the NJ analysis was incorporated into a nexus file containing *COI* sequence data in MacClade v4.06 (Maddision & Maddision 2000). Next, the nexus file was exported to the CAOS software online program (Sarkar *et al.* 2002, 2008; Bergmann *et al.* 2009; http://boli.uvm.edu/caos-workbench) in order to define for each nominal species the set of its *COI* diagnostic nucleotides.

TABLE 2. Combinations of diagnostic nucleotides for *L. abbreviata*, *L. brevivena*, *L. sujuanae* and *L. zhenfangae*.

	15	42	51	70	72	90	93	96	99	103	105	129	132	144	171	174	201	208	213	223	231	234	243
L. abbreviata	T	T	G	C	T	A	T	C	A	C	T	C	A	T	A	T	A	C	T	C	T	T	T
L. brevivena	T	T	G	T	A	T	T	A	A	C	T	T	A	C	A	T	T	C	T	T	C	A	C
L. sujuanae	A	T	A	C	T	A	T	C	A	C	T	T	A	A	G	A	A	C	A	T	A	T	C
L. zhenfangae	T	A	T	C	T	T	C	T	T	T	A	T	T	C	A	A	A	T	A	T	A	T	T

TABLE 2. (Continued)

	246	267	270	277	285	288	294	303	306	309	312	315	333	336	339	345	354	357	369	378	384	387	393
L. abbreviata	T	T	C	T	T	C	A	A	C	A	T	G	A	A	T	T	T	T	T	T	T	T	T
L. brevivena	C	A	T	T	T	T	T	G	T	A	A	A	A	A	A	T	A	T	T	T	A	T	A
L. sujuanae	T	T	T	C	T	T	T	A	C	T	A	G	G	T	T	A	A	C	A	C	A	A	A
L. zhenfangae	T	T	T	C	A	T	T	G	C	A	T	G	A	A	T	A	T	T	A	T	G	T	A

TABLE 2. (Continued)

	397	402	412	418	420	423	429	432	444	450	462	468	480	483	484	489	492	496	498	504	510	511	525
L. abbreviata	T	T	C	T	A	A	T	T	G	A	A	A	A	T	A	T	C	T	A	A	C	C	A
L. brevivena	T	T	C	T	A	C	T	T	A	A	T	T	A	A	A	T	T	T	A	T	T	T	T
L. sujuanae	T	T	C	C	A	T	T	T	A	A	A	A	A	T	A	T	T	T	A	A	T	T	A
L. zhenfangae	C	A	T	C	T	T	C	C	G	T	A	A	T	T	T	A	T	C	T	A	T	T	T

TABLE 2. (Continued)

	528	537	540	544	546	550	552	553	573	579	588	591	599	604	617	618	624	627	630	633	639	642
L. abbreviata	A	T	A	T	A	C	A	T	T	A	G	T	C	T	A	A	C	C	G	T	T	A
L. brevivena	A	T	A	T	A	T	A	T	C	A	A	A	T	A	T	T	T	C	T	A	G	T
L. sujuanae	T	T	T	T	A	T	A	T	T	T	A	T	C	T	A	T	T	T	T	A	A	A
L. zhenfangae	A	A	T	C	T	T	T	C	T	T	A	T	T	A	A	T	T	C	A	T	T	A

Molecular result

Prior to analysis the aligned sequences were edited to fragments of 678 base pairs, and no insertions/deletions or

codon stops were found as expected in the functional sequences. The intraspecific distances of *L. abbreviata*, *L. sujuanae* and *L. zhenfangae* ranged from 0 to 0.59%, 0 to 2.70%, and 0.59% to 1.34%, respectively. It was impossible to measure the intraspecific distance of *L. breviena* because only a single specimen was applied in our study. The interspecific distances ranged from 8.36% of 11.39%. The differences between the maximal intraspecific distance (K2P) and minimal interspecific distances of *L. abbreviata*, *L. sujuanae* and *L. zhenfangae* were 7%, 5% and 8%, respectively (Fig. 1).

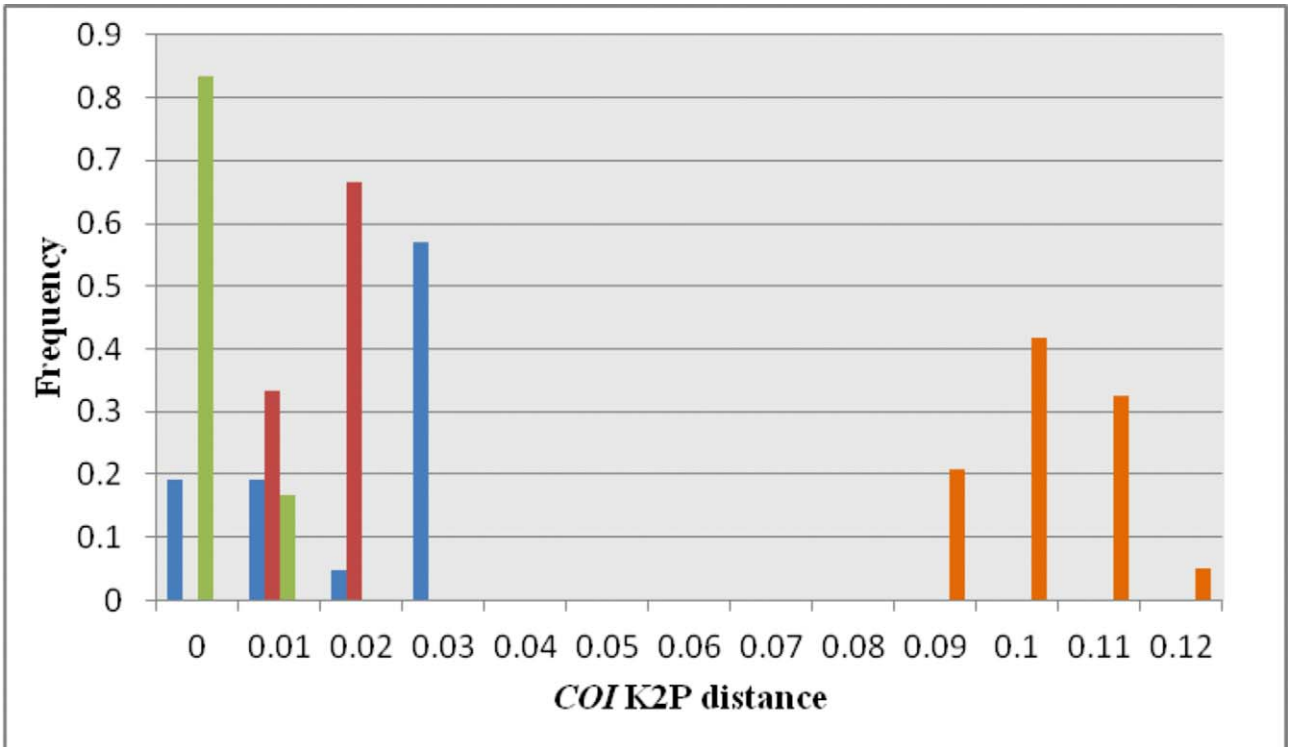

FIGURE 1. Distribution of the intraspecific genetic variabilities of *L. abbreviata* (in green), *L. sujuanaee* (in blue), *L. zhenfangae* (in red) and the interspecific genetic variabilities (in orange).

For the NJ tree-based analysis, all individuals of a species were recovered as a monophyletic lineage (Fig. 2) with strong support (bootstrap values = 100). For the character-based analysis, 91 diagnostic sites were found in the *COI* gene region for these four species of the *abbreviata* group (Table 2). All species revealed a unique combination of character states at 91 nucleotide positions.

Systematic account

Leucophenga abbreviata species group

Diagnosis. M_1 distally abbreviated, not reaching wing margin (Fig. 3A, 3D, 3G, 3J).

Description. Male and female: Eyes red to brownish red. Ocellar triangle dark brown to black, with a pair of setae above ocellar setae. Postocellar seta usually small. Frons brown, narrow, nearly parallel, with a few minute setulae medially. All orbital setae large; proclinate and anterior reclinate orbital setae very close together, separated by distance less than 1/2 of that between anterior reclinate and posterior reclinate. Pedicel yellow; first flagellomere brownish; arista long plumose. Face mostly yellow; facial carina undeveloped. Clypeus yellow. Palpus brownish yellow, slender in both sexes. Vibrissa prominent; other orals small. Gena and postgena narrow. Mesonotum yellow to brown, not pollinose. Postpronotal lobe with 2–4 long setae and a few of shorter setae. Acrostichal setulae in ca. 12–14 irregular rows. Prescutellar setae large. Katepisternum yellowish, with small setae medially, and 2 large ones anteriorly and posteriorly, respectively. Subscutellum swollen. Basal medial-cubital crossvein absent. Wing costal vein between R_{2+3} and R_{4+5} distally with more than 5, 6 peg-like spinules on ventral surface; R_{2+3} sometimes curved to costa at tip. Halter mostly yellowish white. Legs mostly yellowish. Abdominal tergites variable in the color and

pattern. Male terminalia: Epandrium usually with sparse pubescence and several setae around posterodorsal to ventral margins; apodeme usually developed (Figs 5–8A). Surstylus broad, flat, nearly entirely pubescent, with several setae on outer and inner surface (Figs 5–8A). Cercus separated from epandrium, with several setae, lacking pubescence (Figs 5–8A). Hypandrium (gonopod in Bächli *et al*. 2004) anteriorly fused to aedeagal apodeme, laterally broad, usually with paramedian setae subbasally. Gonopods (dorsal arch in Bächli *et al*. 2004) fused with each other, forming slightly triangular plate, anterioventrally with curved, median rod. Paramere (outer paraphysis in Bächli *et al*. 2004) contiguous to arm of aedeagal apodeme basally, lacking pubescence (Figs 5–8B). Aedeagus glabrous (Figs 5–8C); basal bridges contiguous to median rod of gonopod; apodeme with a pair of arms each contiguous to base of paramere.

In the following descriptions of each species, only characters that depart from the upper universal characters are provided for brevity.

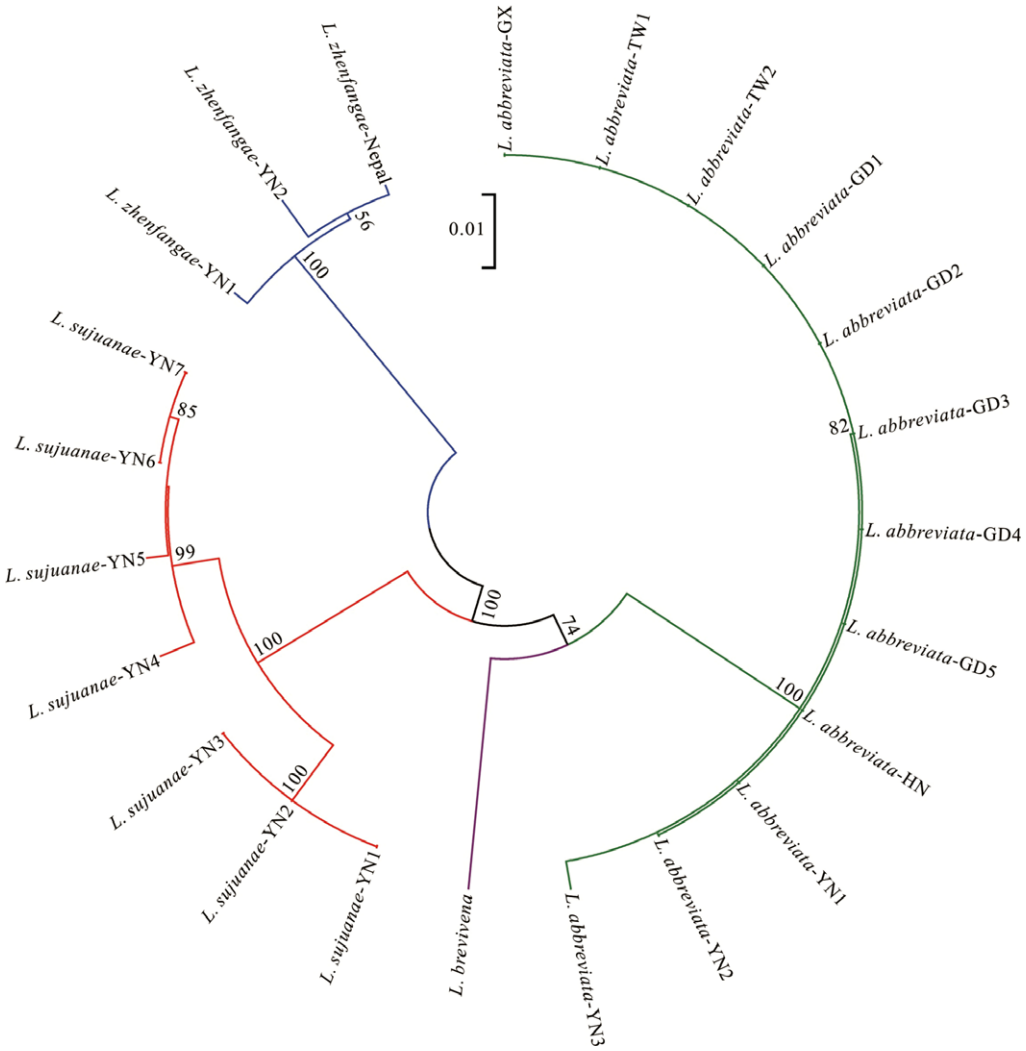

FIGURE 2. Neighbor-joining tree of the *abbreviata* group inferred from the *COI* sequences. Bootstrap values generated from 1000 pseudoreplicates are given next to the nodes, and only the values above 50% are shown.

Leucophenga abbreviata **(de Meijere, 1911)**

(Figs 3A, 3B, 3C, 4A, 5)

Drosophila abbreviata de Meijere, 1911: 400.
Drosomyiella abbreviata. Hendel, 1914: 113.
Leucophenga (*Leucophenga*) *abbreviata*. Duda, 1924: 185.
Leucophenga abbreviata. Okada, 1966: 18.

Diagnosis. This species is similar to *L. sujuanae* **sp. nov.** in the patterns of abdominal tergites (Fig. 4A), but can be differentiated from it by having the black bands of abdominal second to fifth tergites continuous on posterior margins (Fig. 4A), the pleura with a curved, brown longitudinal stripe above (running from base of foreleg to base of halter), but sometimes variable in length (Fig. 3C), the aedeagus round apically (Fig. 5C).

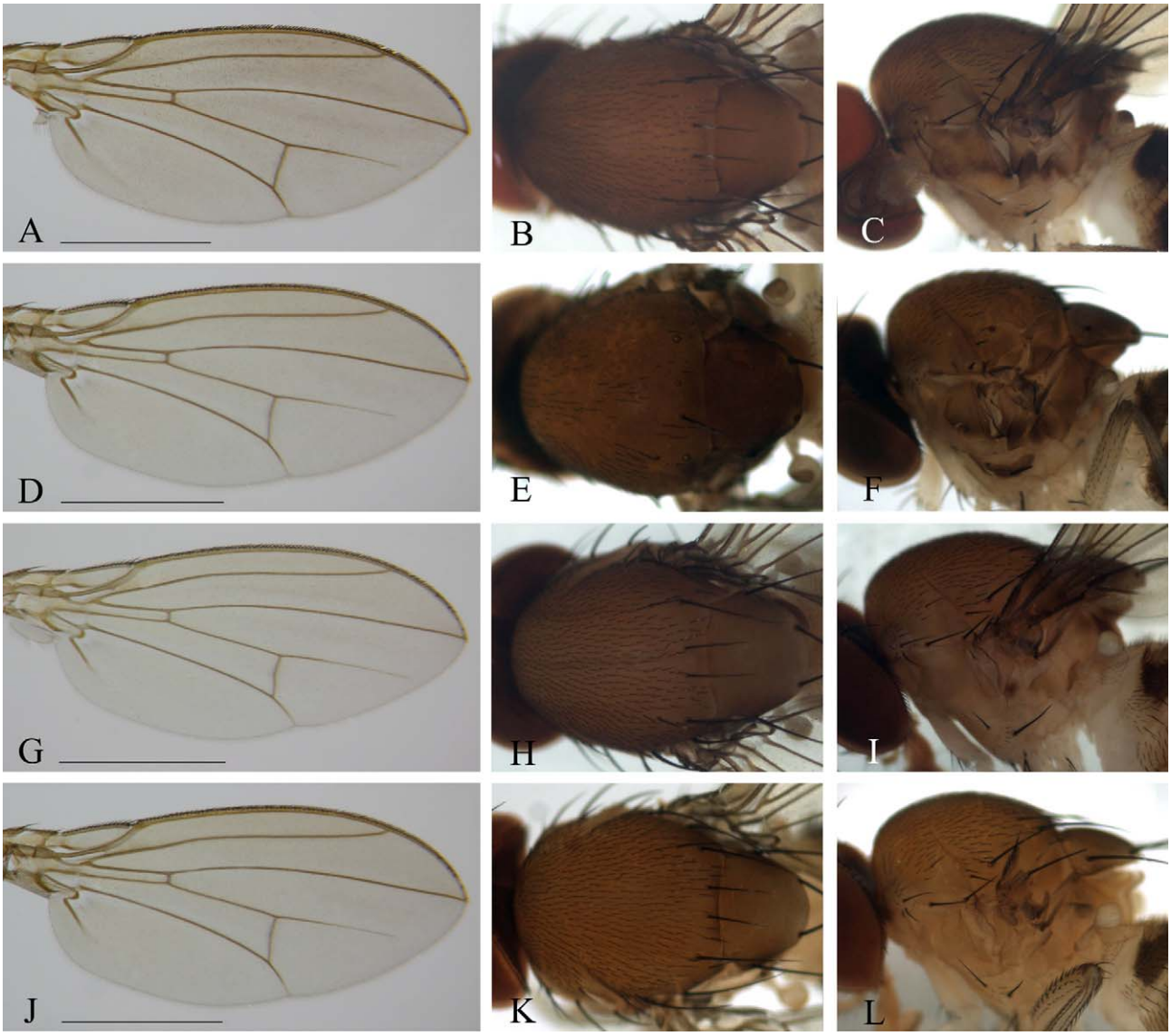

FIGURE 3. Wing, mesonotum, scutellum and pleura of male: A, B, C. *Leucophenga abbreviata* (de Meijere, 1911); D, E, F. *Leucophenga brevivena* **sp. nov.**; G, H, I. *Leucophenga sujuanae* **sp. nov.**; J, K, L. *Leucophenga zhenfangae* **sp. nov.**

Description. Mesonotum and scutellum mostly brownish yellow (Fig. 3B). Postpronotal lobe yellow. Katepisternum yellowish, pale below (Fig. 3C). Halter white basally, grayish brown apically (Fig. 3C). Abdominal fifth tergite sometimes nearly entirely black in some Taiwan samples. Male terminalia: Epandrium with sparse pubescence and ca. 8 setae near posterodorsal to ventral margins per side; apodeme developed (Fig. 5A). Hypandrium with a few minute sensilla subbasolaterally (Fig. 5B). Paramere slender, with a few minute sensilla distally (Fig. 5B).

Measurements. BL = 3.02–3.82 mm in 6♂, 2.96–3.40 mm in 5♀, ThL = 1.42–1.52 mm in ♂, 1.40–1.48 mm in ♀, WL = 2.60–3.11 mm in ♂, 2.48–3.00 mm in ♀, WW = 1.16–1.47 mm in ♂, 1.08–1.24 mm in ♀, arb = 5–7/3–5, avd = 0.79–1.00, adf = 2.50–2.80, flw = 2.00–2.27, FW/HW = 0.30–0.32, ch/o = 0.05 (0.05–0.06), prorb = 0.50–0.72, rcorb = 0.48–0.78, vb = 0.40–0.55, dcl = 0.50–0.56, presctl = 0.37–0.39, sctl = 1.47–1.52, sterno = 0.60–1.00,

orbito = 1.80–2.33), dcp = 0.23–0.25, sctlp = 0.67– 0.71, C = 1.88–2.66, 4c = 0.92–1.16, 4v = 1.25–1.71, 5x = 0.83–1.00, M = 0.21–0.44, C3F = 0.67–0.88.

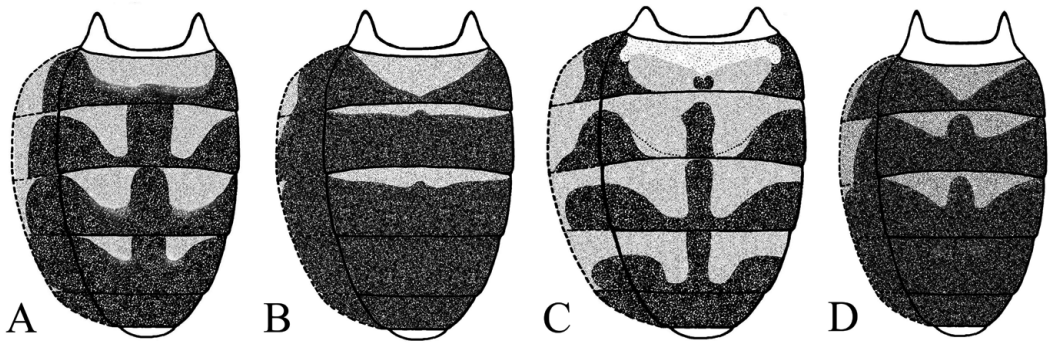

FIGURE 4. Patterns of abdominal tergites of male: A. *Leucophenga abbreviata* (de Meijere, 1911); B. *Leucophenga brevivena* **sp. nov.**; C. *Leucophenga sujuanae* **sp. nov.**; D. *Leucophenga zhenfangae* **sp. nov.**

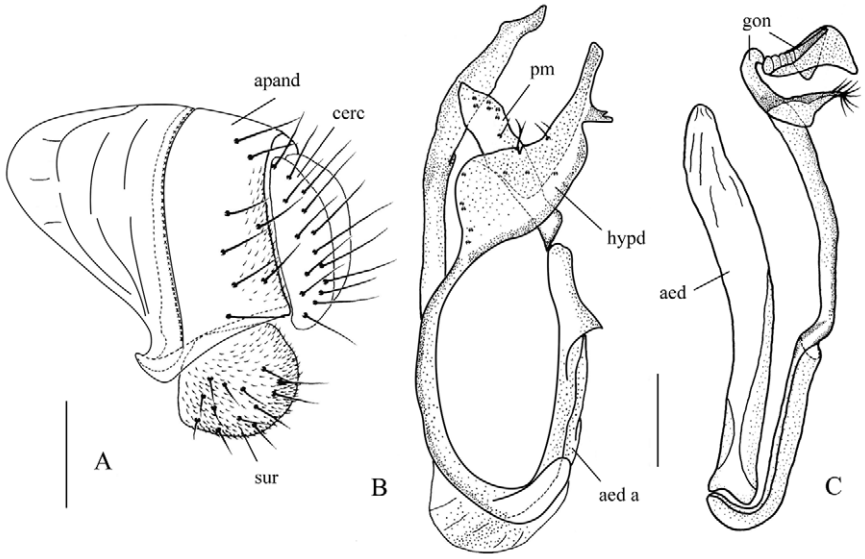

FIGURE 5. *Leucophenga abbreviata* (de Meijere, 1911), male terminalia. A. Epandrium (epan), cercus (cer) and surstylus (sur) (lateral view); B. Hypandrium (hypd), paramere (pm) and aedeagal apodeme (aed a) (lateral view); C. Aedeagus (aed) and gonopods (gon) (lateral view). Scale lines = 0.1 mm.

Specimen examined. CHINA: 1♀ (SCAU, No. 122273), Maolin, Gaoxiong, Taiwan, alt. 300m, 3.vi.2011, XY Liu; 8♂1♀ (SCAU, Nos. 122300–122308), Xiushan, Nantou, Taiwan, 23°46′N, 120°45′E, alt. 350m, 18.x.2012, swept from tree trunks and tussock, HW Chen, JJ Gao; 1♂1♀ (SCAU, Nos 122309–10), Zhiben, Taidong, Taiwan, 23°10′N, 121°03′E, 30.x.2012, alt. 340m, swept from tree trunk, HW Chen; 14♂12♀ (SCAU, Nos. 122274–122299), Huolushan, Guangzhou, Guangdong, 24°50′N, 123°52′E, alt. 230m, 19.iii.2005, 5.iv.2005, JJ Jiang, MF Xu; 8♂13♀ (SCAU, Nos 122311–31), Dinghushan, Zhaoqing, Guangdong, 24.xi.2004, 15.iv.2005, HL Cao, HW Chen, MF Xu; 1♂ (SCAU, No. 122334), Jianfengling, Ledong, Hainan, 18°41′N, 108°52′E, alt. 940m, 24.iv.2007, T Li; 1♂1♀ (SCAU, Nos 122332, 33), Bapeng, Fusui, Guangxi, 22°05′N, 107°32′E, alt. 220m, 18.viii.2004, HW Chen; 2♂6♀ (SCAU, Nos 122357–64), Zhengxing, Jinggu, Yunnan, 22°49′N, 100°02′E, alt. 1100m, 24.vii.2009, L Wang, L Wu; 9♂15♀ (4♂4♀ in KIZ; 5♂11♀ in SCAU, Nos 122335–50), Menglun, Mengla, Yunnan, 21°41′N, 101°25′E, alt. 700m, 12.ix.2002, 24–26.xii.2003, 17.iv.2007, 12.iv.2010, 27, 28.ix.2011, HW Chen, JJ Gao, YR Su, L Wang, L Wu; 1♂5♀ (SCAU, Nos 122351–56), Wangtianshu, Mengla, Yunnan, 21°28′N, 101°38′E, alt. 600m, 13.iv.2010, JJ Gao, YR Su.

Distribution. China (Taiwan, Guangdong, Hainan, Guangxi, Yunnan), Myanmar, Nepal, India (Orissa), Sri Lanka, Malaysia, Singapore, Indonesia (Sumatra, Java).

***Leucophenga brevivena* sp. nov.**
(Figs 3D, 3E, 3F, 4B, 6)

Diagnosis. This species is similar to *L. zhengfangae* in the patterns of abdominal tergites (Fig. 4B), but can be differentiated from it by having the mesonotum mostly yellowish brown and the scutellum brown (Fig. 3E), the pleura with a dark brown longitudinal stripe above (Fig. 3F), the katepisternum dark brown above, yellow below (Fig. 3F), the aedeagus acute apically (Fig. 6C).

Description. Halter white basally, brownish distally (Fig. 3F). Abdominal tergites brownish; second yellow medially and a small patch laterally; third and fourth each with narrow bands on anterior margins and a small patch laterally (Fig. 4B). Male terminalia: Epandrium with pubescence except for anterior margin and ca. 10 setae near posterodorsal to ventral margins per side; apodeme developed (Fig. 6A). Hypandrium with a few minute, sensilla subbasolaterally (Fig. 6B). Paramere slender, with a few minute sensilla distally (Fig. 6B).

Measurements. BL = 3.60 mm in the holotype (range in 2♂ and 2♀ paratypes: 3.08–3.80 mm in ♂, 3.20–3.40 mm in ♀), ThL = 1.60 mm (1.40–1.80 mm in ♂, 1.28–1.60 mm in ♀), WL = 2.88 mm (2.16–3.08 mm in ♂, 2.68 mm in ♀), WW = 1.36 mm (1.00–1.40 mm in ♂, 1.08–1.24 mm in ♀), arb = 7/4 (7–8/4–6), avd = 0.77 (0.67–0.83), adf = 2.60 (2.20–2.40), flw = 2.20 (2.00–2.20), FW/HW = 0.29 (0.27–0.31), ch/o = 0.06 (0.05–0.06), prorb = 0.67 (0.59–0.81), rcorb = 0.72 (0.65–0.79), vb = 0.63 (0.42–0.67), dc1 = 0.48 (0.44–0.52), presct1 = 0.58 (0.47–0.55), sct1 = 0.90 (0.90–1.29), sterno = 0.79 (0.82–0.92), orbito = 1.20 (1.17–1.75), dcp = 0.52 (0.32–0.67), sct1p = 0.77 (0.73–1.25), C = 2.33 (1.60–2.37), 4c = 0.94 (0.77–1.00), 4v = 1.06 (1.04–1.29), 5x = 0.67 (0.56–1.00), ac = 3.25 (3.25–5.33), M = 0.31 (0.16–0.33), C3F = 0.67 (0.56–0.68).

Type material. Holotype ♂ (SCAU, No. 122365), CHINA: Menglun, Mengla, Yunnan, alt. 700m, 12.ix.2002, HW Chen. Paratypes: 2♂2♀ (SCAU, Nos 122366–69), same data as holotype.

Etymology. A combination of the Latin words: *brevis* (= short) and *vena* (= vein), referring to the M_1 not reaching wing margin.

Distribution. China (Yunnan).

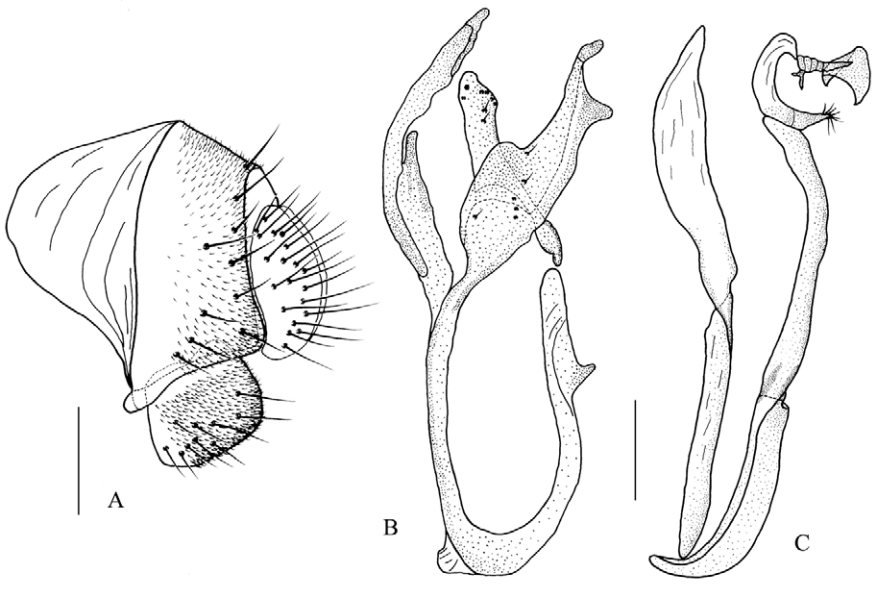

FIGURE 6. *Leucophenga brevivena* **sp. nov.**, male terminalia. A. Epandrium, cercus and surstylus; B. Hypandrium, paramere and aedeagal apodeme; C. Aedeagus and gonopods. Scale lines = 0.1 mm.

***Leucophenga sujuanae* sp. nov.**
(Figs 3G, 3H, 3I, 4C, 7)

Diagnosis. This species is similar to *L. abbreviata* in the patterns of abdominal tergites (Fig. 4C), but can be differentiated from it by having the black bands of abdominal second and third tergites discontinuous on posterior

margins at least, sometimes also occur on fourth and fifth tergites (Fig. 4C), the pleura brownish yellow above, yellow below, sometimes with a small brown patch below base of wing (Fig. 3J), the aedeagus acute apically (Fig. 7C).

Description. Mesonotum mostly yellow (Fig. 3H). Scutellum mostly yellow, slightly brownish laterally, pale at tip (Fig. 3H). Halter mostly white (Fig. 3I). Male terminalia: Epandrium with sparse pubescence and ca. 9 setae near posterodorsal to ventral margins per side; apodeme slightly developed (Fig. 7A). Hypandrium with 1 long sensilla and ca. 4 sensilla subbasolaterally (Fig. 7B). Paramere slender, with a few minute sensilla distally (Fig. 7B).

Measurements. BL = 3.60 mm in the holotype (range in 5♂ and 5♀ paratypes: 2.72–4.00 mm in ♂, 2.60–4.15 mm in ♀), ThL = 1.68 mm (1.28–1.96 mm in ♂, 1.24–2.00 mm in ♀), WL = 2.92 mm (2.40–3.40 mm in ♂, 2.32–3.50 mm in ♀), WW = 1.44 mm (1.24–1.60 mm in ♂, 1.08–1.60 mm in ♀), arb = 8/4 (6–8/3–5), avd = 0.80 (0.56–1.18), adf = 2.00 (1.57–2.57), flw = 2.80 (1.71–2.80), FW/HW = 0.25 (0.18–0.30), ch/o = 0.06 (0.06–0.07), prorb = 0.78 (0.45–0.67), rcorb = 0.50 (0.44–0.70), vb = 0.43 (0.44–0.75), dc1 = 0.38 (0.29–0.58), presct1 = 0.58 (0.50–0.75), sct1 = 1.37 (0.78–1.70), sterno = 0.75 (0.58–1.00), orbito = 1.29 (1.13–2.67), dcp = 0.25 (0.25–0.52), sct1p = 1.00 (0.63–1.36), C = 2.33 (2.27–3.52), 4c = 0.83 (0.56–1.07), 4v = 1.11 (1.13–1.35), 5x = 0.54 (0.60–1.00), M = 0.19 (0.22–0.36), C3F = 0.83 (0.67–0.81).

Type material. Holotype ♂ (SCAU, No. 122370), CHINA: Zhengxing, Jinggu, Puer, Yunnan, alt. 1100m, 24.vii.2009, L Wang. Paratypes: CHINA: 36♂31♀ (SCAU, Nos 122371–122437), same data as holotype; 14♂23♀ (KIZ), Yixiang, Puer, Yunnan, 22°47′N, 101°02′E, alt. 1200m, 6.xii.2000, 18, 19.ix.2007, 2.x.2011, HW Chen, JJ Gao; 90♂98♀ (SCAU, Nos 122438–625), Ximeng, Puer, Yunnan, 22°37′N, 099°35′E, alt. 1100m, 31.iii–4.iv.2011, JM Lu, ZF Shao, YR Su, SJ Yan; 19♂25♀ (SCAU, Nos 122626–69), Menglun, Mengla, Yunnan, alt. 700m, 17.iv.2007, 12.iv.2010, HW Chen, JJ Gao, YR Su, L Wang, L Wu; 2♂6♀ (SCAU, Nos 122670–77), Wangtianshu, Mengla, Yunnan, 21°28′N, 101°38′E, alt. 670m, 25.iv.2007, HW Chen, JJ Gao; 10♂11♀ (SCAU, Nos 122678–98), Hesong, Menghai, Yunnan, 21°28′N, 101°38′E, alt. 1900m, 16,17.iv.2010, 2.iv.2011, 8–11.iv.2011, 17.vi.2011, 6.v.2012, HW Chen, JJ Gao, ZF Shao, YR Su, SJ Yan.

Etymology. Patronym of the collector Ms. Sujuan Yan (SCAU).

Distribution. China (Yunnan).

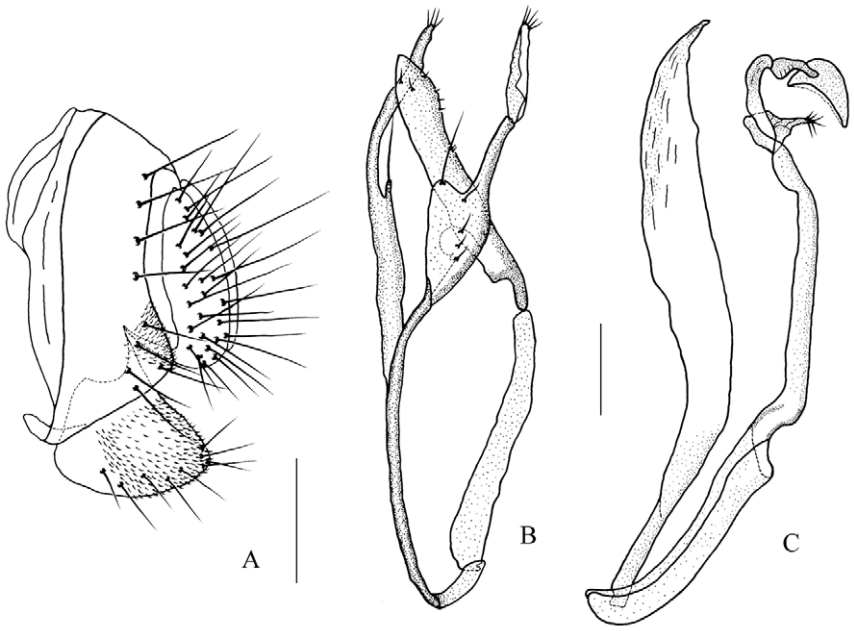

FIGURE 7. *Leucophenga sujuanae* **sp. nov.**, male terminalia. A. Epandrium, cercus and surstylus; B. Hypandrium, paramere and aedeagal apodeme; C. Aedeagus and gonopods. Scale lines = 0.1 mm.

***Leucophenga zhenfangae* sp. nov.**
(Figs 3J, 3K, 3L, 4D, 8)

Diagnosis. This species is similar to *L. brevivena* **sp. nov.** in the patterns of abdominal tergites (Fig. 4D), but can be differentiated from it by having the mesonotum mostly yellow, the scutellum grayish yellow (Fig. 3K), the pleura yellow, slightly pale below (Fig. 3L), the aedeagus bifurcated from base to dorsal 1/3, round apically (Fig. 8C).

Description. Halter grayish yellow (Fig. 3L). Abdominal tergites brown; second yellow medially and along lateral margins; third and fourth each with distinct W-shaped patches medially (Fig. 4D). Male terminalia: Epandrium with pubescence except for anterior margin and ca. 10 setae near posterodorsal to ventral margins per side; apodeme developed (Fig. 8A). Hypandrium with 2 minute, sensilla subbasolaterally (Fig. 8B). Paramere slightly broadened, with a few minute sensilla distally (Fig. 8B).

Measurements. BL = 3.51 mm in the holotype (range in 5♂ and 1♀ paratypes: 3.25–3.47 mm in ♂, 2.67 mm in ♀), ThL = 1.64 mm (1.47–1.78 mm in ♂, 1.25 mm in ♀), WL = 2.80 mm (2.58–2.98 mm in ♂, 2.40 mm in ♀), WW = 1.24 mm (1.11–1.25 mm in ♂, 0.98 mm in ♀), arb = 9/4 (6–8/3–4), avd = 0.89 (0.71–1.00), adf = 3.00 (1.75–3.00), flw = 2.67 (2.25–3.33), FW/HW = 0.29 (0.29–0.40), ch/o = 0.07 (0.06–0.08), prorb = 0.59 (0.58–0.91), rcorb = 0.65 (0.56–0.75), vb = 0.33 (0.33–0.50), dc1 = 0.38 (0.38–0.50), presct1 = 0.50 (0.42–0.70), sct1 = 1.50 (0.79–1.67), sterno = 0.70 (0.71–0.83), orbito = 2.00 (1.50–2.33), dcp = 0.31 (0.21–0.30), sct1p = 1.14 (1.14–1.50), C = 2.12 (2.00–2.35), 4c = 1.06 (0.89–1.33), 4v = 1.38 (1.18–1.67), 5x = 0.71 (0.71–1.00), M = 0.31 (0.29–0.42), C3F = 0.76 (0.73–0.81).

Type material. Holotype ♂ (SCAU, No. 122698), Yixiang, Puer, Yunnan, alt. 1200m, 2.x.2011, HW Chen. Paratypes: 3♂1♀ (122699–702), same data as holotype; 1♂ (SCAU, No. 122703), Ximeng, Puer, Yunnan, alt. 1100m, ZF Shao; 1♂ (SCAU, No. 122704), Hesong, Menghai, Xishuangbanna, alt. 1900m, 9.iv.2011, ZF Shao. NEPAL: 1♂ (SCAU, No. 122705), Beni, Dhawalagini, 27°42′N, 85°19′E, alt. 820m, 17.xii.2011, XS Chen.

Etymology. Patronym of the collector Ms. Zhenfang Shao (SCAU).

Distribution. China (Yunnan), Nepal (Beni).

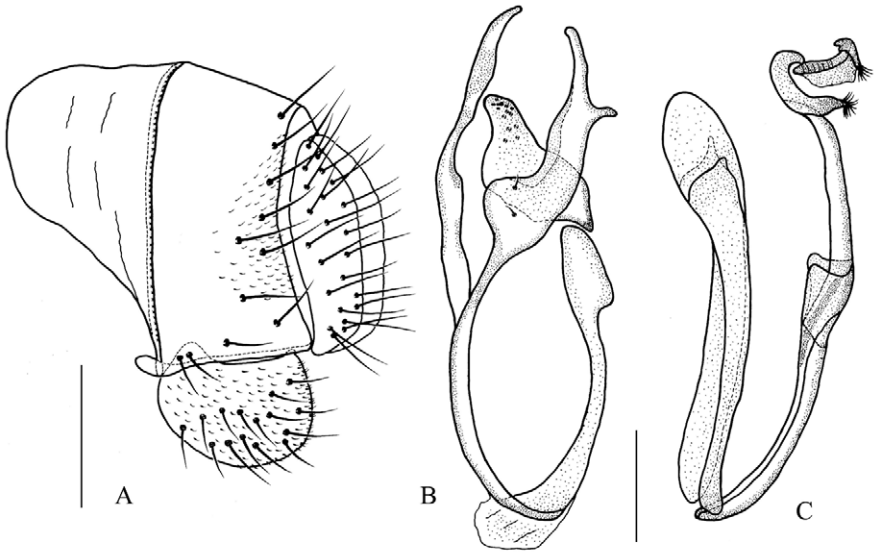

FIGURE 8. *Leucophenga zhenfangae* **sp. nov.**, male terminalia. A. Epandrium, cercus and surstylus; B. Hypandrium, paramere and aedeagal apodeme; C. Aedeagus and gonopods. Scale lines = 0.1 mm.

A key to the Oriental species of the *abbreviata* group

1. M_1 distally abbreviated, not reaching wing margin . . . . . *abbreviata* group 2
- M_1 reaching wing margin . . . . . other *Leucophenga* species
2. Abdominal fifth tergite brown, with yellow patches 3
- Abdominal fifth tergite entirely brownish to brown 4

3. Dorsal yellow patches of abdominal second and third tergites not reaching posterior margins (Fig. 4A); pleura with curved, brown stripe above (Fig. 3C); aedeagus round apically (Fig. 5C) . . . . . *L. abbreviata*
- Dorsal yellow patches of abdominal second and third tergites reaching posterior margins (Fig. 4C); pleura brownish yellow above (Fig. 3I); aedeagus acute apically (Fig. 7C) . . . . . *L. sujuanae* **sp. nov.**
4. Mesonotum mostly yellowish brown (Fig. 3E); scutellum brown (Fig. 3E); the pleura with dark brown longitudinal stripe above (Fig. 3F), the katepisternum dark brown above, yellow below (Fig. 3F); aedeagus acute apically (Fig. 6C) . . . . . *L. brevivena* **sp. nov.**
- Mesonotum mostly yellow (Fig. 3K); scutellum grayish yellow (Fig. 3K); pleura yellow above, slightly pale below (Fig. 3L); aedeagus round apically (Fig. 8C). . . . . *L. zhenfangae* **sp. nov.**

Discussion

The integration of morphological and DNA-based approaches has revealed an effective way to improve species discovery and description (Dayrat 2005; Lumley & Sperling 2010; Padial & De La Riva 2010; Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). In the present study, 23 specimens of the *abbreviata* group are determined into 4 species (including 1 previously described and 3 new species) based on morphology. The information of the molecular data is employed as interactive evidence to test these species hypotheses.

DNA barcoding is a useful tool for species identification. Recently, tree-based and character-based methods have been used for DNA barcoding research (Rach *et al.* 2008; Damm *et al.* 2010; Yassin *et al.* 2010; Zou *et al.* 2011; Xia *et al.* 2012). In this case study, the level of divergence among same species was about 3 to 10 times higher than intraspecific genetic distance and the distribution of divergences at intraspecies and interspecies scales doesn't overlap, notwithstanding the genetic divergences between and within species do not conform to the "10× rule" threshold (Hebert *et al.* 2004b). However, a universal similarity cut-off value for species determination will simply not exist, because of the broad overlap of interspecific and intraspecific distances (Goldstein *et al.* 2000). Several authors (e.g., Hebert *et al.* 2004a; Burns *et al.* 2004) also proposed an important notion of a "barcoding gap", a distance gap between intraspecific and interspecific sequences for species identification (Meyer & Paulay 2005; Meier *et al.* 2006; Meier *et al.* 2008). In the *abbreviata* group, intraspecific and interspecific genetic divergences fall into separate intervals. An obvious "barcoding gap" was found in the *COI* sequence. Species recognition always closely links with the concept of a taxon being monophyletic (Mishler & Robert 1987; Hull & Michael 1998; Funk & Omland 2003). Our results showed that each lineage forms distinct clusters, and therefore the four *abbreviata* group species can be identified by monophyletic criterion on the *COI* NJ tree. As the tree-based method resolved, the character-based method could discriminate among *L. abbreviata*, *L. brevivena*, *L. sujuanae* and *L. zhenfangae*. Thus all methods of DNA barcoding can distinguish the four *abbreviata* group species in this study. This mtDNA differentiate between all species-level taxa that could also be defined by morphological differences.

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References

Bächli, G. (1971) *Leucophenga* und *Paraleucophenga* (Diptera, Brachycera), fam. Drosophilidae. *In*: Mission GF de Witte en collaboration avec W Adam, *Exploration du Parc national de l'Upemba*. Institut des parcs nationaux du congo et du Rwanda, Bruxelles, pp 1–192 (in German).

Bächli, G. (2012) The database on Taxonomy of Drosophilidae: http://taxodros.uzh.ch/ (accessed 20 July 2012).

Bächli, G., Vilela, C.R., Andersson, S. & Saura, A. (2004) *The Drosophilidae* (*Diptera*) *of Fennoscandia and Denmark. In*: *Fauna Entomologica Scandinavica. Volume* 39, Brill, Leiden, New York, pp. 362.

Bächli, G., Vilela, C.R. & Haring, E. (2002) Four new species of West Palaearctic Drosophilidae (Diptera). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, 75, 304.

Bergmann, T., Hadrys, H., Breves, G. & Schierwater, B. (2009) Character-based DNA barcoding: a superior tool for species

classification. *Berl Munch Tierarztl Wochenschr*, 122, 446–450.
Brake, I. & Bächli, G. (2008) Drosophilidae (Diptera). *World catalogue of Insects*. Volume 9. Apollo Books, Stenstrup: 412 pp.
Burns, J.M., Janzen, D.H., Hajibabaei, M., Hallwachs, W. & Hebert, P.D.N. (2007) DNA barcodes of closely related (but morphologically and ecologically distinct) species of butterflies (Hesperiidae) can differ by only one to three nucleotides. *Journal of the Lepidopterists Society*, 61, 138–153.
Chen, H.W. & Toda, M.J. (2001) A revision of the Asian and European species in the subgenus *Amiota* Loew (Diptera, Drosophilidae) and establishment of species-groups based on phylogenetic analysis. *Journal of Natural History*, 35, 1517–1563.
http://dx.doi.org/10.1080/002229301317067665
Damm, S., Schierwater, B. & Hadrys, H. (2010) An integrative approach to species discovery in odonates: from character-based DNA barcoding to ecology. *Molecular Ecology*, 19, 3881–3893.
http://dx.doi.org/10.1111/j.1365-294X.2010.04720.x
Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society*, 85, 407–415.
http://dx.doi.org/10.1111/j.1095-8312.2005.00503.x
de Meijere, J.C.H. (1911) Studien uber südostasiatische Dipteren. *Tijdschrift voor entomologie*, 54, 258–432.
DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 360, 1905–1916.
http://dx.doi.org/10.1098/rstb.2005.1722
Duda, O. (1924) Beitrag zur Systematik der Drosophiliden unter besonderer Berücksichtigung der paläarktischen und orientalischen Arten (Dipteren). *Archiv Mecklenburgischer Naturforscher*, 90A, 172–234.
Funk, D.J. & Omland, K.E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematics*, 34, 397–423.
http://dx.doi.org/10.1146/annurev.ecolsys.34.011802.132421
Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
Goldstein, P.Z., DeSalle, R., Amato, G. & Vogler, A.P. (2000) Conservation genetics at the species boundary. *Conservation Biology*, 14, 120–131.
http://dx.doi.org/10.1046/j.1523-1739.2000.98122.x
He, X.F., Gao, J.J., Cao, H.Z., Zhang, X.L. & Chen, H.W. (2009) Taxonomy and molecular phylogeny of the *Phortica hani* species complex (Diptera, Drosophilidae). *Zoological Journal of the Linnean Society*, 157, 359–372.
http://dx.doi.org/10.1111/j.1096-3642.2009.00516.x
Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003a) Biological identification through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, S313–S321.
http://dx.doi.org/10.1098/rspb.2002.2218
Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004a) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817.
http://dx.doi.org/10.1073/pnas.0406166101
Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, S96–S99.
http://dx.doi.org/10.1098/rsbl.2003.0025
Hebert, P.D.N, Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. (2004b) Identification of birds through DNA barcodes. *PLoS Biology*, 2, 1657–1663.
http://dx.doi.org/10.1371/journal.pbio.0020312
Hendel, F. (1914) Acalypterate Musciden (Diptera) III. *Supplementa Entomologica*, 3, 90–117.
Hull, D.L. & Michael, R. (1998) *The Philosophy of Biology*. Oxford; New York: Oxford University Press, p310.
Lumley, L.M. & Sperling, F.A.H. (2010) Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). *Systematic Entomology*, 35, 416–428.
http://dx.doi.org/10.1111/j.1365-3113.2009.00514.x
Maddison, D.R. & Maddison, W.P. (2000) *MacClade 4: Analysis of phylogeny and character evolution*. Version 4.0. Sinauer Associates, Sunderland, Massachusetts.
Meier, R., Kwong, S., Vaidya, G. & Ng, P.K.L. (2006) DNA Barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Sysematic Biology*, 55, 715–728.
http://dx.doi.org/10.1080/10635150600969864
Meier, R., Zhang, G. & Ali, F. (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the ‘‘barcoding gap” and leads to misidentification. *Systematic Biology*, 57, 809–813.
http://dx.doi.org/10.1080/10635150802406343
Meyer, C.P. & Paulay, G. (2005) DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology*, 3, 2229–2238.
http://dx.doi.org/10.1371/journal.pbio.0030422

Mik, J. (1886) Dipterologische miscellen. *Wiener entomologische Zeitung*, 5, 317–318.
Mishler, B. D. & Robert, N. B. (1987) Individuality, Pluralism, and the Phylogenetic Species Concept. *Biology and Philosophy*, 2, 397–414.
http://dx.doi.org/10.1007/BF00127698
Okada, T. (1966) Diptera from Nepal, Cryptochaetidae, Diastatidae and Drosophilidae. *Bulletin of the British Museum (Natural History)*, 6 (Suppl.), 1–129.
Okada, T. (1990) *Leucophenga maculata* species group (Diptera, Drosophilidae) of the Palearctic and Oriental regions. *Japanese Journal of Entomology*, 58, 555–562.
Padial, J.M. & De La Riva, I. (2010) A response to recent proposals for integrative taxonomy. *Biological Journal of the Linnaean Society*, 101, 747–756.
http://dx.doi.org/10.1111/j.1095-8312.2010.01528.x
Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16.
http://dx.doi.org/10.1186/1742-9994-7-16
Rach, J., DeSalle, R., Sarkar, I.N., Schierwater, B. & Hadrys, H. (2008) Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 275, 237–247.
http://dx.doi.org/10.1098/rspb.2007.1290
Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: the barcode of life data system (http://www. barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364.
http://dx.doi.org/10.1111/j.1471-8286.2007.01678.x
Reid, B.N., Le, M., McCord, W.P., Iverson, J.B., Georges, A., Bergmann, T., Amato, G., DeSalle, R. & Naro-Maciel, E. (2011) Comparing and combining distance-based and character-based approaches for barcoding Turtles. *Molecular Ecology Resources*, 11, 955–967.
Sarkar, I.N., Thornton, J.W., Planet, P.J., Figurski, D.H., Schierwater, B. & DeSalle, R. (2002) An automated phylogenetic key for classifying homeoboxes. *Molecular Phylogenetics and Evolution*, 24, 388–399.
http://dx.doi.org/10.1111/j.1755-0998.2011.03032.x
Sarkar, I.N., Planet, P.J., DeSalle, R. (2008) CAOS software for use in characterbased DNA barcoding. *Molecular Ecology Resources*, 8, 1256–1259.
http://dx.doi.org/10.1111/j.1755-0998.2008.02235.x
Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R.H. (2010) Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology*, 55, 421–438.
http://dx.doi.org/10.1146/annurev-ento-112408-085432
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28, 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121
Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
http://dx.doi.org/10.1093/nar/22.22.4673
Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360, 1847–1857.
http://dx.doi.org/10.1098/rstb.2005.1716
Xia, Y., Gu, H.F., Peng, R., Chen, Q., Zheng, Y.C., Murphy, R.W. & Zeng, X.M. (2012) *COI* is better than 16S rRNA for DNA barcoding Asiatic salamanders (Amphibia: Caudata: Hynobiidae). *Molecular Ecology Resources*, 12, 48–56.
http://dx.doi.org/10.1111/j.1755-0998.2011.03055.x
Yassin, A., Markow, T.A., Narechania, A., O'Grad, P.M. & DeSalle, R. (2010) The genus *Drosophila* as a model for testing tree- and character-based methods of species identification using DNA barcoding. *Molecular Phylogenetics and Evolution*, 57, 509–517.
http://dx.doi.org/10.1016/j.ympev.2010.08.020
Zhang, W.X. & Toda, M.J. (1992) A new species-subgroup of the *Drosophila immigrans* species-group (Diptera, Drosophilidae), with description of two new species from China and revision of taxonomic terminology. *Japanese Journal of Entomology*, 60, 839–850.
Zou, S., Li Q., Kong, L., Yu, H. & Zheng, X. (2011) Comparing the usefulness of distance, monophyly and character-based DNA barcoding methods in species identification: a case study of Neogastropoda. *PLoS ONE*, 6, e26619.
http://dx.doi.org/10.1371/journal.pone.0026619