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The genera *Luzonimyia* and *Pararhinoleucophenga* from China (Diptera: Drosophilidae), with DNA barcoding information

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Abstract

Four new species are described from Yunnan, China, which belong to two different genera within the subfamily Steganinae: *Luzonimyia hirsutina* sp. nov., *Luzonimyia setocauda* sp. nov., *Pararhinoleucophenga amnicola* sp. nov. and *Pararhinoleucophenga sylvatica* sp. nov. The DNA sequences and GenBank accession numbers of the mitochondrial *COI* gene among Chinese species are provided.

Key words: Barcoding, *COI* gene, Drosophilid, East Asia, Fauna, taxonomy

Introduction

Luzonimyia Malloch, 1926 and *Pararhinoleucophenga* Duda, 1924 are all smaller genera in the subfamily Steganinae (Brake & Bächli 2008). The genus *Luzonimyia* includes six species (Cao & Chen 2008): 1 sp. each from Australia and the Philippines, and 2 spp. each from southern China and Africa. The genus *Pararhinoleucophenga* is endemic to the Oriental region, and includes nine species: 1 sp. from Indonesia (Java, Sumatra), 1 sp. from India and Sri Lanka, and 7 spp. from southern China (Cao & Chen 2009).

In this paper, four new species are described from Yunnan, China: 2 spp. of *Luzonimyia* and 2 spp. of *Pararhinoleucophenga* species. In addition, a total of 16 DNA sequences and GenBank accession numbers of the mitochondrial *COI* gene among ten Chinese species are provided (Table 1).

Material and methods

Materials and morphological terminology. All specimens examined were collected by sweeping on tree trunks or tussocks near streams in the forest, preserved in 75% ethanol, and then dried and pinned after morphological examination and identification. The specimens are deposited in 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.

DNA extraction and sequencing. A total of 16 samples of 10 species were investigated for molecular work (Table 1); their total DNA was extracted from the abdominal tissue of samples after dissection of the genitalia, using the TIANGENT™ DNA extraction kit following the recommended protocol. The *COI* fragments were amplified using the cycle protocol as in Zhao *et al.* (2009). The PCR/sequencing primer pairs are *COI*-F1 (5'–ATCGCCTAAACTTCAGCCAC–3', Wang *et al.* 2006), HCO2198 (5'–TAAACTTCAGGGTGACCAAAAATCA–3', Folmer *et al.* 1994), *COI*-F3 (5'–CGCGACAATGRYTATTTTCTAC–3', Li *et al.* 2013) and *COI*-R3 (5'–CCATTGCACTAATCTGCCATA–3', Li *et al.* 2013).

TABLE 1. Details of the Chinese samples used in the DNA analysis of of the *COI* gene, with BOLD process ID and GenBank accession numbers.

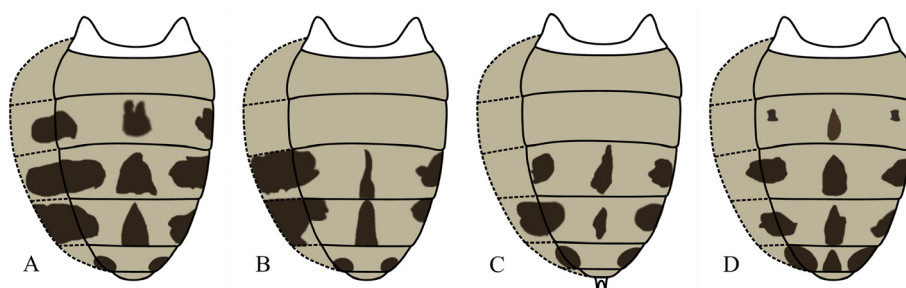
Genus	Species	Sex	Location	BOLD process ID	GenBank accession number
<i>Luzonimyia</i>	<i>flavipedes</i> Cao & Chen, 2007	♀	Menglun, Mengla, Yunnan	BDORL004-14	KJ841738
	<i>stictogaster</i> Cao & Chen, 2007-1	♀	Jizushan, Binchuan, Yunnan	BDORL005-14	KJ841741
	<i>stictogaster</i> Cao & Chen, 2007-2	♂	Kuankuoshui, Suiyang, Guizhou	BDORL006-14	KJ841742
	<i>hirsutina</i> sp. nov.	♂	Menglun, Mengla, Yunnan	BDORL007-14	KJ841739
	<i>setocauda</i> sp. nov.	♀	Menglun, Mengla, Yunnan	BDORL008-14	KJ841740
<i>Pararhinoleuc ophenga</i>	<i>alafumosa</i> Cao & Chen, 2009	♂	Mt. Qinling, Foping, Shanxi	BDORL009-14	KJ841744
	<i>meichiensis</i> (Chen & Toda, 1994)	♂	Nanling, Shaoguan, Guangdong	BDORL011-14	KJ841746
	<i>setifrons</i> Cao & Chen, 2009	♂	Ailaoshan, Jingdong, Yunnan	BDORL012-14	KJ841747
	<i>setipes</i> Cao & Chen, 2009-1	♂	Wuliangshan, Jingdong, Yunnan	BDORL013-14	KJ841748
	<i>setipes</i> Cao & Chen, 2009-2	♂	Wuliangshan, Jingdong, Yunnan	BDORL015-14	KJ841750
	<i>setipes</i> Cao & Chen, 2009-3	♂	Baihualing, Baoshan, Yunnan	BDORL014-14	KJ841749
	<i>annicola</i> sp. nov.	♂	Guanlei, Mengla, Yunnan	BDORL016-14	KJ841745
	<i>sylvatica</i> sp. nov. -1	♂	Caiyanghe, Puer, Yunnan	BDORL017-14	KJ841753
	<i>sylvatica</i> sp. nov. -2	♀	Hesong, Menghai, Yunnan	BDORL018-14	KJ841752
	<i>sylvatica</i> sp. nov. -3	♂	Yixiang, Pier, Yunnan	BDORL019-14	KJ841754
<i>sylvatica</i> sp. nov. -4	♂	Guanlei, Mengla, Yunnan	BDORL020-14	KJ841751	

Taxonomic descriptions

Genus *Luzonimyia* Malloch

Luzonimyia Malloch, 1926: 491; Cao & Chen, 2008: 251. Type species: *Luzonimyia nigropuncta* Malloch, 1926.

Diagnosis (Cao & Chen 2008). Frons lacking interfrontal setulae; ocellar setae very weak or absent; proclinate orbital setae shorter than reclinate ones; R_{4+5} and M_1 distally somewhat divergent; epandrium and surstylus entirely fused (Figs. 2A, 3A); paramere absent (Figs. 2C, D, 3C, D).



FIGURES 1. Color patterns of male abdominal tergites: A. *Luzonimyia hirsutina* **sp. nov.**; B. *L. setocauda* **sp. nov.**; C. *L. flavipedes* Cao & Chen, 2009; D. *L. stictogaster* Cao & Chen, 2009.

***Luzonimyia hirsutina* sp. nov.**

(Figs. 1A, 2)

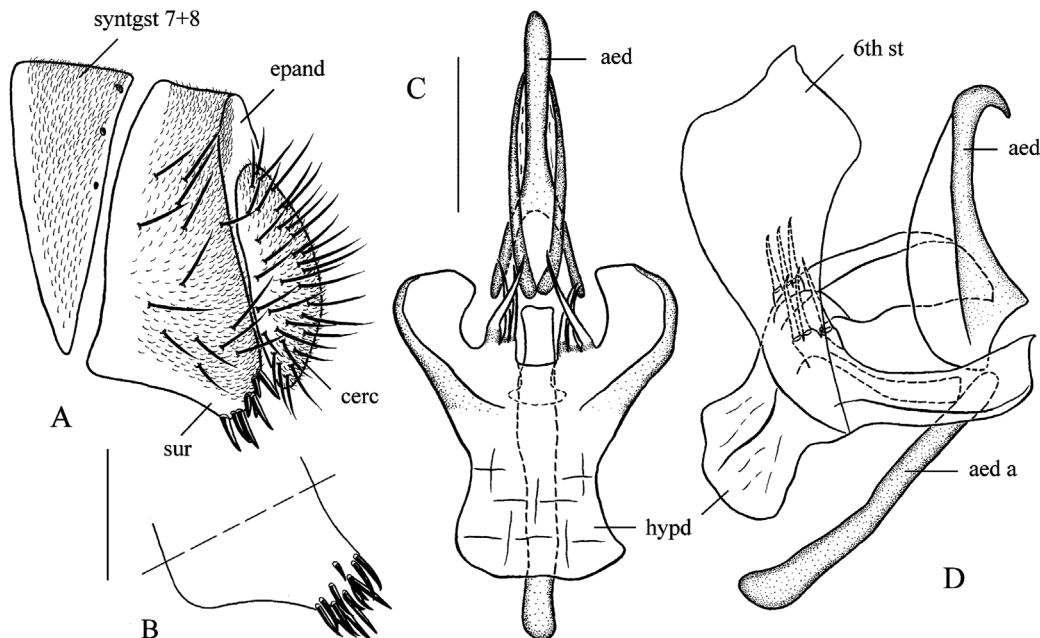
Diagnosis. This species resembles *L. stictogaster* Cao & Chen, 2008 from Yunnan Province, China in the male terminalia, but can be distinguished by the abdominal pattern (Fig. 1A, 1D); the aedeagus thin apically in lateral view (Fig. 2C); surstylus with ca. 13 strong setae on inner surface (Fig. 2A).

Description. Only characters that depart from the general description given by Cao & Chen (2008) for this genus are provided for brevity. Male: Postvertical setae long, ca. 0.4 times as long as inner vertical setae, standing at vertical ridge. Ocellar setae small, slightly longer than half length of postvertical seta, outside triangle made by ocelli. Frons narrow, black, with dense grey pollinosity. Pedicel and first flagellomere brownish; arista short plumose distally, with pubescence basally. Face, clypeus, gena, postgena and occiput slightly glossy black. Gena narrow and linear. Palpus large, black, lobe-like. Thorax black with dense grey pollinosity. Scutellar black, yellow on tip; subscutellum swollen in lateral view. Legs yellow except for third to fifth tarsomeres brown. Fore femur without long setae on posterior surface; mid femur with 1 row of 4–5 strong setae on anterior surface. Abdominal tergites yellow, with black patches on third to sixth (Fig. 1A). Sternites yellow except for fifth black on posterior 1/3; sixth sternite developed (Fig. 2C). Male terminalia: Syntergosternite 7+8 (McAlpine 1981; misjudged as sixth tergite in Cao & Chen 2008) very small, narrowed on lateral margins (Fig. 2A). Epandrium with ca. 14 setae per side (Fig. 2A). Cercus black, nearly oval (Fig. 2A). Hypandrium with 3 pairs of paramedian setae (Fig. 2B, C).

Measurements. BL = 3.07 mm in holotype (3.17 mm in 1♂ paratype), THL = 1.67 mm (1.53 mm), WL = 3.20 mm (3.16 mm), WW = 1.20 mm (1.20 mm), arb = 11/9 (12/8), avd = 0.80 (0.80), adf = 0.50 (0.43), flw = 1.25 (1.37), FW/HW = 0.30 (0.30), ch/o = 0.05 (0.07), pror = 0.57 (0.52), rcorb = 0.81 (0.86), vb = 0.41 (0.46), dcl = 0.63 (0.60), presctl = 0.61 (0.62), sctl = 1.38 (damaged), sterno = 1.24 (1.18), orbito = 2.00 (1.44), dcp = 0.39 (0.29), sctlp = 0.95 (1.00), C = 5.33 (5.50), 4c = 0.46 (0.48), 4v = 1.27 (1.28), 5x = 0.89 (0.94), ac = 0.82 (0.85), M = 0.29 (0.38), C3F = 0.17 (0.17).

Type specimens. Holotype ♂ (SCAU, No. 124316), CHINA: Menglun, Mengla, Yunnan, alt. 680 m, 10.iv.2010, tree trunk, L Wu. Paratype: 1♂ (SCAU, No. 124317), same data as holotype.

Etymology. From the Latin word: hirsutus, referring to the cercus with dense setae.



FIGURES 2. *Luzonimyia hirsutina* sp. nov., male terminalia: A. Syntergosternite 7+8 (syntgst 7+8), epandrium (epand), cercus (cerc) and surstylus (sur) (lateral view); B. surstylus (inner view); C. sixth sternite (6th st), hypandrium (hypd), aedeagus (aed) and aedeagal apodeme (aed a) (ventral view); D. ditto (lateral view). Scale bars = 0.1 mm.

***Luzonimyia setocauda* sp. nov.**

(Figs 1B, 3)

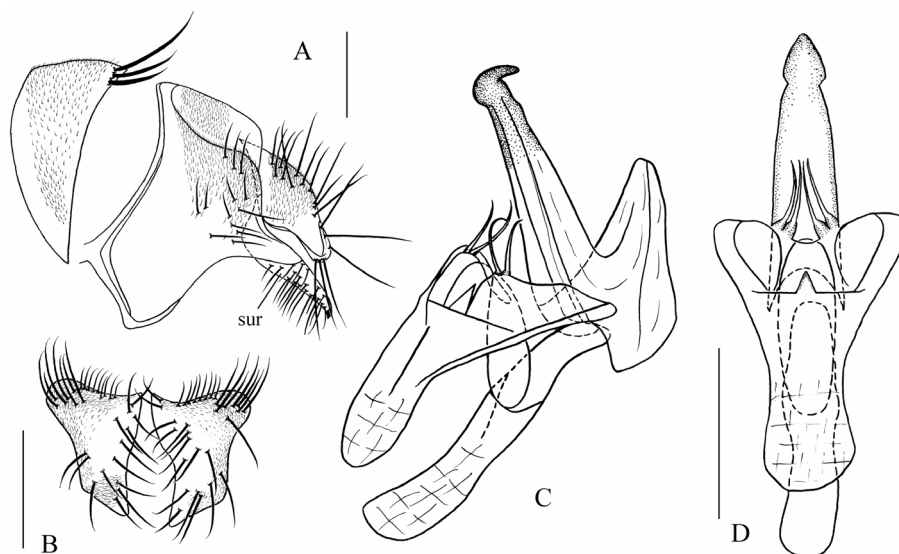
Diagnosis. This species differs from other species of this genus in having the following characters: syntergosternite 7+8 with 3 pairs of thick, long setae medially (Fig. 3A); epandrium deeply constricted more than one-half width mid-dorsally, strongly protruded mid-posteriorly (Fig. 3A); cercus broadened dorsoally in posterior view (Fig. 3B); surstylus slender, with 1 strong seta apically (Fig. 3A).

Description. Characters common to *L. hirsutina* sp. nov. are not referred to in the following description. Male: Abdominal tergites yellow with black patches on fourth to sixth (Fig. 1B); all sternites yellow except black fifth. Female: Abdominal tergites yellow with black patches on fourth and fifth as in male, sixth tergite black with yellow, longitudinal stripe medially; all sternites yellow except black sixth. Male terminalia: Epandrium with 13 setae per side and pubescence dorsally (Fig. 3A). Hypandrium with 2 pairs of paramedian setae (Fig. 3C, D). Aedeagus strongly sclerotized (Fig. 3C, D).

Measurements. BL = 3.23 mm in holotype (range in 2♀ paratypes: 2.86–3.64 mm), THL = 1.53 mm (1.30–1.43 mm), WL = 2.62 mm (3.11–3.33 mm), WW = 1.07 mm (1.03–1.30 mm), arb = 9/8 (10/7–8), avd = 0.67 (0.60–0.67), adf = 0.50 (0.44–0.56), flw = 1.88 (2.00–2.22), FW/HW = 0.30 (0.28–0.30), ch/o = 0.08 (0.10–0.13), prorb = 0.52 (0.39–0.42), rcorb = 0.87 (0.91–0.92), vb = 0.57 (0.49–0.61), dcl = 0.60 (0.56–0.60), presctl = 0.68 (0.64–0.69), sctl = 1.11 (0.87), sterno = 1.15 (1.32), orbito = 1.30 (1.56–1.60), dcp = 0.40 (0.35–0.42), sctlp = 1.06 (1.00–1.05), C = 6.44 (6.09–6.20), 4c = 0.33 (0.32–0.35), 4v = 0.96 (0.84), 5x = 0.65 (0.53), ac = 0.68 (0.65–0.78), M = 0.22 (0.15–0.17), C3F = 0.24 (0.20–0.29).

Type specimens. Holotype ♂ (SCAU, No. 123293), CHINA: Menglun, Mengla, Yunnan alt. 680 m, 11.iv.2010, tree trunk, RY Su. Paratypes: 2♀ (SCAU, No. 123294, 95), same data as holotype.

Etymology. A combination of the Latin words: seta + cauda, referring to the abdominal 6th tergite with 3 pairs of thick, long setae medially.



FIGURES 3. *Luzonimyia stictogaster* sp. nov., male terminalia: A. Syntergosternite 7+8, epandrium, cercus and surstylus (lateral view); B. cerci (posterior view); C. hypandrium, aedeagus and aedeagal apodeme (lateral view); D. ditto (ventral view). Scale bars = 0.1 mm.

Genus *Pararhinoleucophenga* Duda

Pararhinoleucophenga Duda 1924: 185; Okada 1988: 618; Cao & Chen, 2009: 127. Type species: *Drosophila maura* de Meijere, 1911.

Diagnosis (Cao & Chen 2009). Facial carina broadly prominent below; 2nd and 3rd vibrissa slightly shorter than 1st; costal vein with 2–4 heavily sclerotized hooked peg-like spines on ventral surface between R_{2+3} and R_{4+5} ; M_1

weakly curved to R₄₊₅ distally; hypandrium laterally with 1 pair of large, vertical flaps bearing minute setulae (Figs. 4C, D, 5C, D); and cercus mostly with ventral processes (Figs. 5A, B).

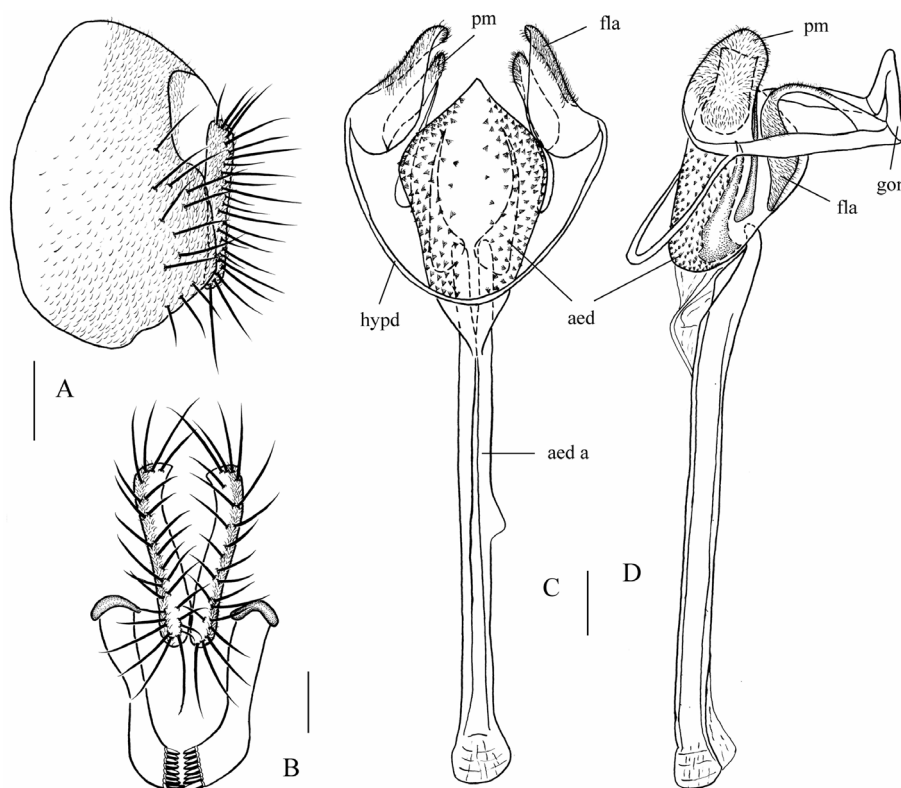
Remarks. The two new species to be described below belong to the *maura* species group, sharing the following characters: R₄₊₅ with 5–6 setae on basal section, C3F ≈ 1; epandrium with small setae near anteroventral corners except for *P. amnicola* sp. nov. (Fig. 5A); paramere with pubescence (Figs. 4C, D, 5C, D); aedeagus finely serrated (Figs. 4C, D, 5C, D).

***Pararhinoleucophenga amnicola* sp. nov.**

(Fig. 4)

Diagnosis. This species is related to *P. maura* (de Meijere, 1911) from Java and Sumatra, Indonesia in the male terminalia, but can be distinguished by the epandrium with several setae near posterior margin only (Fig. 4A); cercus with short setae (Fig. 4A, B); aedeagus acute apically, lacking lobe-like processes ventrobasally (Fig. 4C).

Description. Only characters that depart from the universal description given by Cao & Chen (2009) for this group are provided for brevity. Male. Eye brownish red. Pedicel and first flagellomere brownish. Face brown laterally, dark brown medially; facial carina black. Palpus dark brown. Thorax brownish yellow, with unapparent, dark brown longitudinal stripes. Katepisternum dark brown, with 1 row of setae below katepisternal setae. Scutellum brownish yellow. Legs mostly brown; mid femur with 2 rows of strong setae on anterior surface. First to fourth abdominal tergites yellow except for black bands on posterior margins of second to fourth tergites; fifth and sixth tergites nearly entirely black. Abdominal sternites dark brown, longer than broad. Male terminalia: Surstylus with 8 prenisetae on inner margin of distal part (Fig. 4B). Cercus lacking ventral process (Fig. 4B). Hypandrium posterolaterally without pubescence; vertical flap (fla) of hypandrium with dense pubescence (Fig. 4C, D). Posteromedian plate of gonopod very narrow, arcuate (Fig. 4D). Paramere basally rod-like, distally flap-like and pubescent (Fig. 4C, D). Aedeagus robust, with dense, fine serrations: apodeme slender (Fig. 4C, D).



FIGURES 4. *Pararhinoleucophenga amnicola* sp. nov., male terminalia: A. Epandrium and cercus (lateral view); B. surstyli and cerci (posterior view); C. hypandrium (hypd), vertical flaps of hypandrium (fla), gonopods (gon), parameres (pm), aedeagus (aed), and aedeagal apodeme (aed a) (ventral view); D. ditto (lateral view). Scale bars = 0.1 mm.

Measurements. BL = 3.69 mm, THL = 1.70 mm, WL = 3.29 mm, WW = 1.33 mm, arb = 5/4, avd = 0.67, adf = 1.71, flw = 2.29, FW/HW = 0.33, ch/o = 0.08, prorb = 0.88, rcorb = 0.92, vb = 0.82, dcl = damaged, presctl = damaged, sctl = damaged, sterno = 0.85, orbito = 2.67, dcp = 0.25, sctlp = 0.95, C = 3.29, 4c = 0.65, 4v = 1.65, 5x = 1.39, ac = 2.47, M = 0.50, C3F = 1.00

Type specimen. Holotype ♂ (SCAU, No. 124318), CHINA: Guanlei, Mengla, Yunnan, 670m, 21°42'N, 101°16'E, 14.xi.2012, ex. tussock, JJ Gao.

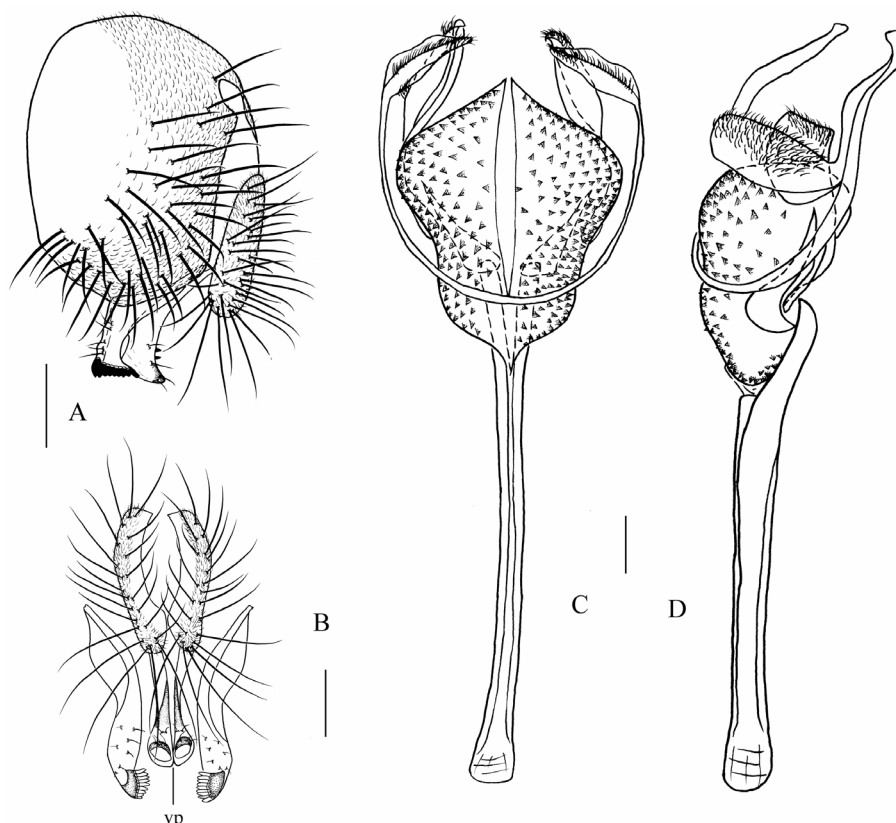
Etymology. From the Latin word: amnicola, referring to the fly dwelling near stream.

***Pararhinoleucophenga sylvatica* sp. nov.**

(Fig. 5)

Diagnosis. This species resembles *P. brunnea* Cao & Chen, 2009 from India and Sri Lanka in the male terminalia, but can be distinguished by the following characters: thorax yellow, slightly gray on anepimeron; epandrium with dense setae near posterior and ventral margins (Fig. 5A); aedeagus bifurcated medially (Fig. 5C). In *brunnea*: epandrium with ca. 10 small setae near anteroventral corner and ca. 15 setae near posterior to ventral margin per side (fig. 17 in Cao & Chen, 2009); aedeagus with pubescence, trefoil-like apically (fig. 19 in Cao & Chen, 2009).

Description. Characters common to *L. brunnea* are not referred to in the following description. Male and female. Thorax yellow to yellowish brown, with dark patches; katapisternun yellow to dark brown, with 2 rows of setae below katapisternal setae. Scutellum yellow to brown.



FIGURES 5. *Pararhinoleucophenga sylvatica* sp. nov., male terminalia: A. Epandrium, surstylus, and cercus; B. surstyli, cerci and ventral processes of cercus (vp); C. hypandrium, vertical flaps of hypandrium, parameres, aedeagus and aedeagal apodeme. Scale bars = 0.1 mm.

Measurements. BL = 3.80 mm in holotype (range in 4♂ and 1♀ paratype: 3.27–4.04 mm in ♂, 4.13 mm in ♀), THL = 1.87 mm (1.37–2.03 mm in ♂, 2.13 mm in ♀), WL = 3.91 mm (3.07–3.73 mm in ♂, 4.00 mm in ♀), WW = 1.50 mm (1.23–1.40 mm in ♂, 1.60 mm in ♀), arb = 5/4 (4–5/4), avd = 0.69 (0.62–0.89), adf = 1.44 (1.13–1.86), flw = 1.75 (1.33–2.00), FW/HW = 0.28 (0.26–0.31), ch/o = 0.10 (0.07–0.10), prorb = 0.96 (0.92–1.05), rcorb =

0.88 (0.88–0.95), vb = 0.53 (0.62–0.91), dcl = 0.42 (0.39–0.50), presctl = 0.23 (0.55–0.82), sctl = damaged (1.09–1.20), sterno = 0.77 (0.73–0.87), orbito = 2.00 (2.00–2.33), dcp = 0.27 (0.22–0.33), sctlp = 1.17 (1.00–1.19), C = 3.26 (3.38–3.78), 4c = 0.61 (0.56–0.67), 4v = 1.35 (1.45–1.59), 5x = 1.56 (1.63–1.87), ac = 2.25 (2.00–2.38), M = 0.49 (0.49–0.58), C3F = 1.00 (1.00)

Type specimens. Holotype ♂ (SCAU, No. 124320), CHINA: Yixiang, Puer, Yunnan, 27°12' N, 100°03' E, alt. 1400m, 18.xi.2012, ex. tussock, JJ Gao. Paratypes: 2♂ (SCAU, Nos. 124321, 22), same data as holotype; 1♂ (SCAU, No. 124323), Caiyanghe, Puer, Yunnan, 22°36' N, 101°07' E, alt. 1200m, 27.vii.2009, fallen logs, L Wang; 1♀ (SCAU, No. 124324), Hesong, Menghai, Yunnan, 21°49' N, 100°06' E, alt. 1900m, 17.iv.2010, ex. fallen logs, L Wang; 1♂ (SCAU, No. 124319), CHINA: Guanlei, Mengla, Yunnan, 14.xi.2012, ex. tussock, JJ Gao.

Etymology. From the Latin word: *sylvaticus*, referring to the fly dwelling in forest.

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