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A new species of *Chimarra* from China (Trichoptera, Philopotamidae) with description of its larva

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Abstract

The adult male and larva of a new species in the genus *Chimarra* Stephens, *Chimarra paramonorum* n. sp., are described, diagnosed, and illustrated based on the specimens collected in Zhejiang Province, China. The larva and adult male were associated by mtCOI gene sequences. The male can be separated from *C. monorum* and *C. thienemanni* mainly by small preanal appendages and by wrinkled, hairless endotheca. The larva of the new species can be separated from other congeners by a combination of characters including the notch on the anterior margin of the frontoclypeus, the teeth of the mandibles, and the length of the seta-bearing process of each forecoxa. The larval description is the first for *Chimarra* in China.

Key words: caddisfly, larval-adult association, mtCOI, Zhejiang, China

Introduction

The nearly cosmopolitan genus *Chimarra* Stephens 1829 is the largest genus in the family Philopotamidae, with more than 879 described species (Morse 2018), distributed in all biogeographic regions except Antarctica (Johanson & Oláh 2012). Of four recognized subgenera, *Curgia* Walker 1860, *Chimarrita* Blahnik 1997, and *Otarrha* Blahnik 2002 are restricted to the New World (Blahnik & Holzenthal 2012), while the subgenus *Chimarra* is distributed worldwide. To date, 22 *Chimarra* species have been reported in China (Yang *et al*., 2016), mainly distributed in Oriental China.

Compared to the numerous known adult species described mainly based on male genitalia, the larvae of *Chimarra* have been poorly studied. Lloyd (1921) was probably the first person to describe larvae of the genus; he described two *Chimarra* larvae of North America (*C. aterrima* Hagen 1861 and *C. socia* Hagen 1861). Barnard (1934) briefly described and illustrated the South African larva of *C. ambulans* Barnard 1934. Ross (1944) keyed larvae of four *Chimarra* species from eastern North America, including those of the two described by Lloyd and another two [*C. obscura* (Walker 1852) and *C. feria* Ross 1941]. Hickin (1946) mentioned the asymmetrical larval clypeus of American species. Edwards & Arnold (1961) described the larva of *C. texana* Banks 1920 from the United States (as *C. betteni* Denning 1941). The larva of *C. marginata* Linnaeus 1767 was first described by Lepneva (1964), and was keyed by Waringer & Graf (2013). *Chimarra australica* Ulmer 1916 and *C. monticola* Kimmins 1953 (in Mosely & Kimmins, 1953) from Australia were described and keyed by Cartwright (1990), and then were re-keyed along with *C. uranka* Mosely 1953 (in Mosely & Kimmins 1953) and 14 other unspecific larvae by Cartwright (1997). Morse *et al*. (2017) keyed and illustrated larvae of seven *Chimarra* species from the USA, including above-mentioned species, *C. aterrima*, *C. feria*, *C. obscura*, and *C. socia*, and another three larvae of the genus, *C. florida* Ross 1944, *C. holzenthali* Lago & Harris 1987, possibly *C. augusta* Morse 1971, and an unspecified larva "*C*. sp. C." Kuhara (2017, 2018) described the larva of *Chimarra tsudai* Ross 1956 from Japan. In addition, many larvae of the genus were described and illustrated for unspecified species: One species from America by Wiggins (1977), one from Japan by Tanida (2005) and by Kuhara (2018), 12 from Vietnam by Hoang & Bae (2008), and the above-mentioned 14 from Australia by Cartwright (1997).

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In summary, larval stages of a total of 15 species along with those of 28 unspecified species of genus *Chimarra* have been described or illustrated in the world. Further investigation is required to clarify these unspecified larvae and to describe those of the remaining 837 or more species for which larvae remain unknown.

The first key to genera of Chinese Philopotamidae larvae was provided by Wiggins *et al*. (1994), but no larvae of Chinese species in the genus *Chimarra* have been described so far.

In this article, we report and describe the male of a new species of the genus *Chimarra*, *C. paramonorum* n. sp. with a description of its larva. This increases the total number of known Chinese *Chimarra* species to 23. The larva of the new species is described here, providing the first description for the larva of a Chinese species of this genus.

Material and methods

Sampling of specimens. Adults were captured in 95% ethanol using pan traps with 15-W ultraviolet light bulbs. Larvae were collected using a D-frame aquatic net. Specimens were then stored in 95% alcohol.

Morphological study. Larvae were examined with an Olympus SZX10 stereomicroscope and keyed to genera according to Morse's key (Morse *et al*. 2017). Photos of the larva were taken using a Nikon Eclipse 80i microscope and Nis-Element D® software (Version 3.22.14). A series of photos at different focal depths were taken, then stacked using Helicon Focus (Version 6.3.5) into an image with a greater depth of field. Other structures were traced in pencil using an Olympus SZX10 stereomicroscope equipped with a drawing tube. The original pencil drawings were inked digitally with Photoshop CS6 and a Wacom Intuos tablet-and-pen (CTL-671/KO-F) to produce illustrations. Plates were arranged using Adobe Photoshop®.

DNA analysis. Right hind legs (male) or the whole body (larvae) of nine individuals were taken for DNA extractions (Table 1). DNA extraction, fragment sequencing, and analysis followed the procedures of Xu *et al*. (2015). The PCR primers are listed in Table 1. The COI sequence of *Chimarra ammi* Malicky & Graf 2008 was downloaded from GenBank. Neighbor-joining (NJ) trees (Fig. 1) were constructed using Mega® 6.0 (Tamura *et al*. 2013). Larval specimens of *C. paramonorum* were associated with adult males of this species according to the criteria outlined by Zhou *et al.* (2007). COI sequences of male and larval *C. paramonorum* n. sp. and male *Dolophilodes secedens* Yang, Sun & Yang 2001 were uploaded to GenBank, the accession numbers are shown in Table 2. The COI sequence of another unspecified *Chimarra* sp. in this study will be uploaded to GenBank later when its identity is established.

TABLE 1. Polymerase chain reaction primers used to sequence mtCOI genes of Philopotamidae specimens.

Primer	Sequence (5' to 3')	Reference
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*.1994
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*.1994

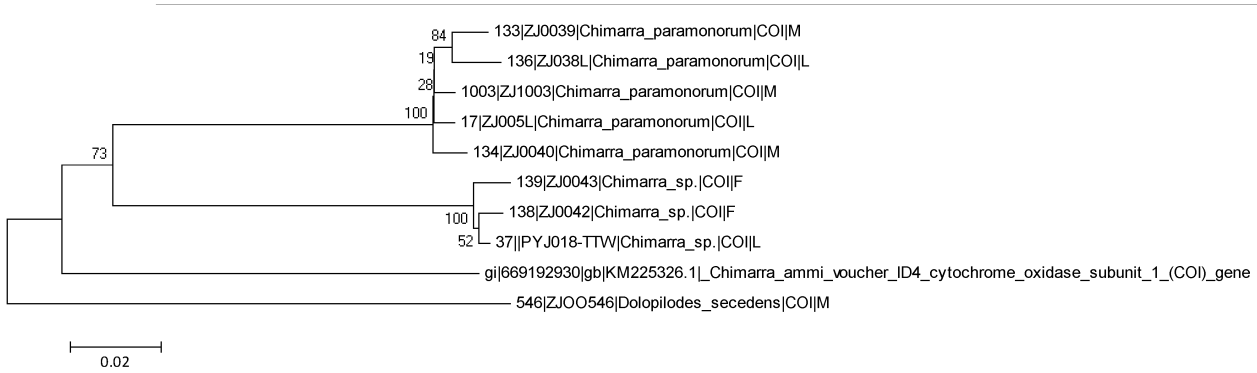

FIGURE 1. COI neighbor-joining diagram used to determine larva-male associations of Chinese Philopotamidae species. M = male; L = larva. Scale bar refers to branch length.

TABLE 2. Specimens used in larva-male associations of Philopotamidae.

Number	Sample ID	GenBank ID	Species	Life stage	Collection Site	Collection Date
546	ZJ00546	MG871357	*Dolophilodes secedens*	Male	Guang-shang, Lin'an, Zhe-jiang	21.Sep 2015
1003	ZJ01003	MG871356	*Chimarra parmonorum*	Male	Xin-dun-cun, Er-ba Town, Jiang-shan, Zhejiang	12.Aug 2016
133	ZJ0039	N/A	*Chimarra paramonorum*	Male	Xian-xi Town, Le Qing, Zhe-jiang	26.May 2017
134	ZJ0040	N/A	*Chimarra paramonorum*	Male	Xian-xi Town, Le Qing, Zhe-jiang	26.May 2017
136	ZJ038L	N/A	*Chimarra paramonorum*	Larva	Yao-chuan Town, Pan'an County, Zhe-jiang	27.Mar 2018
138	ZI0042	N/A	*Chimarra* sp.	Female	Xian-xi Town, Le Qing, Zhe-jiang	26.May 2017
139	ZJ0043	N/A	*Chimarra* sp.	Female	Xian-xi Town, Le Qing, Zhe-jiang	26.May 2017
17	ZJ005L	MG871355	*Chimarra paramonorum*	Larva	Xian-xi Town, Le Qing, Zhe-jiang	26.May 2017
37	PYJ0018	N/A	*Chimarra* sp.	Larva	Tai-tou-wan, Pujiang County, Zhe-jiang	30.Aug 2017
-	-	KM225326.1	*Chimarra ammi*			

Terminology. Terminology for male genitalia follows that of Blahnik *et al*. (2009), larval terminology follows that of Ross (1944) and Wiggins (1977). Type specimens have been deposited in the Insect Collection, Nanjing Agricultural University, Nanjing, Jiangsu Province, PR China (NJAU).

Results

Chimarra paramonorum new species

(Figs 2–4)

Diagnosis. The new species belongs to Ross' (1956) *C. digitata* Lineage or the *C. digitata* Species Group, closely related to *C. monorum* Chantaramongkol & Malicky 1989 from Thailand and *C. thienemanni* Ulmer 1951 from Java. *Chimarra paramonurum* n. sp. differs from *C. thienemanni* in the small preanal appendages and the narrow bases of the inferior appendages in lateral view. It can be separated from *C. monorum* readily by the overall shape of the lateral lobes of segment X in lateral view and the endotheca which is "wrinkled" rather than with dense hairs as in *C. monorum*. In addition, the apicoventral projection of the phallobase in *C. paramonurum* n. sp. is much longer than in the latter two species.

The larva of *C. paramonorum* n. sp. can be diagnosed from known larvae of other congeners by the following combination of characters: (1) The anterior margin of the frontoclypeus is somewhat rounded, with a small notch mesolaterally on the right side; (2) the left and right mandibles each have an inner margin with 6 and 5 subapical teeth, respectively, and (3) the forecoxae each have a much longer seta-bearing process.

Adult male. Length of each forewing 4.5 mm. Body fuscous; Head dark brown, antennae and palpi light brown. Thorax dark brown dorsally, light brown laterally and ventrally; legs light brown, with spurs dark brown; wings light brown. Abdomen brown.

Male genitalia. Segment IX (IX) in lateral view with upper portion longitudinally shorter than ventral portion, anteroventral margin produced and rounded anteriorly, posteroventral margin produced as large triangular ventral process (ve.pr.) (Fig. 2A); in dorsal view very short, forming transverse bridge, anterior margin shallowly concave, posterior margin somewhat fused with segment X (Fig. 2B); in ventral view ventral process elongate-triangular (Figs 2C). Preanal appendages (pr.ap.) small, setose, each elliptical in lateral view and elongate-elliptical in dorsal view (Figs 2A, 2B). Lateral lobes of segment X (la.lo.) in lateral view each with dorsolateral and posterolateral

angle produced into upper thumb-like process and lower digital process, with acute basoventral angle; in dorsal view upper thumb-like process with 2 sensilla (Figs 2A, 2C). Mesal lobes of segment X (me.lo) membranous, in lateral view elliptical, in dorsal view widely and deeply separated (Figs 2A, 2B). Inferior appendages (in.ap.) in lateral view with apices slightly extended beyond lower digitate processes of the lateral lobes of tergum X, each slightly S-shaped, with base about 2.5 times wider than apex, setose; in ventral view with base slightly wider than apex, curved mesad (Figs 2A, 2C). Phallobase (ph.) tubular, in lateral view basodorsally expanded, ventral apex obviously projecting, subacute; length of endotheca (en.) indiscernible (not everted), with one long endothecal spine (en.sp.); phallotremal sclerite (ph.sc.) composed of three short sclerites (Fig. 2D), basodorsal one irregular, basoventral one reniform, posterior one somewhat triangular.

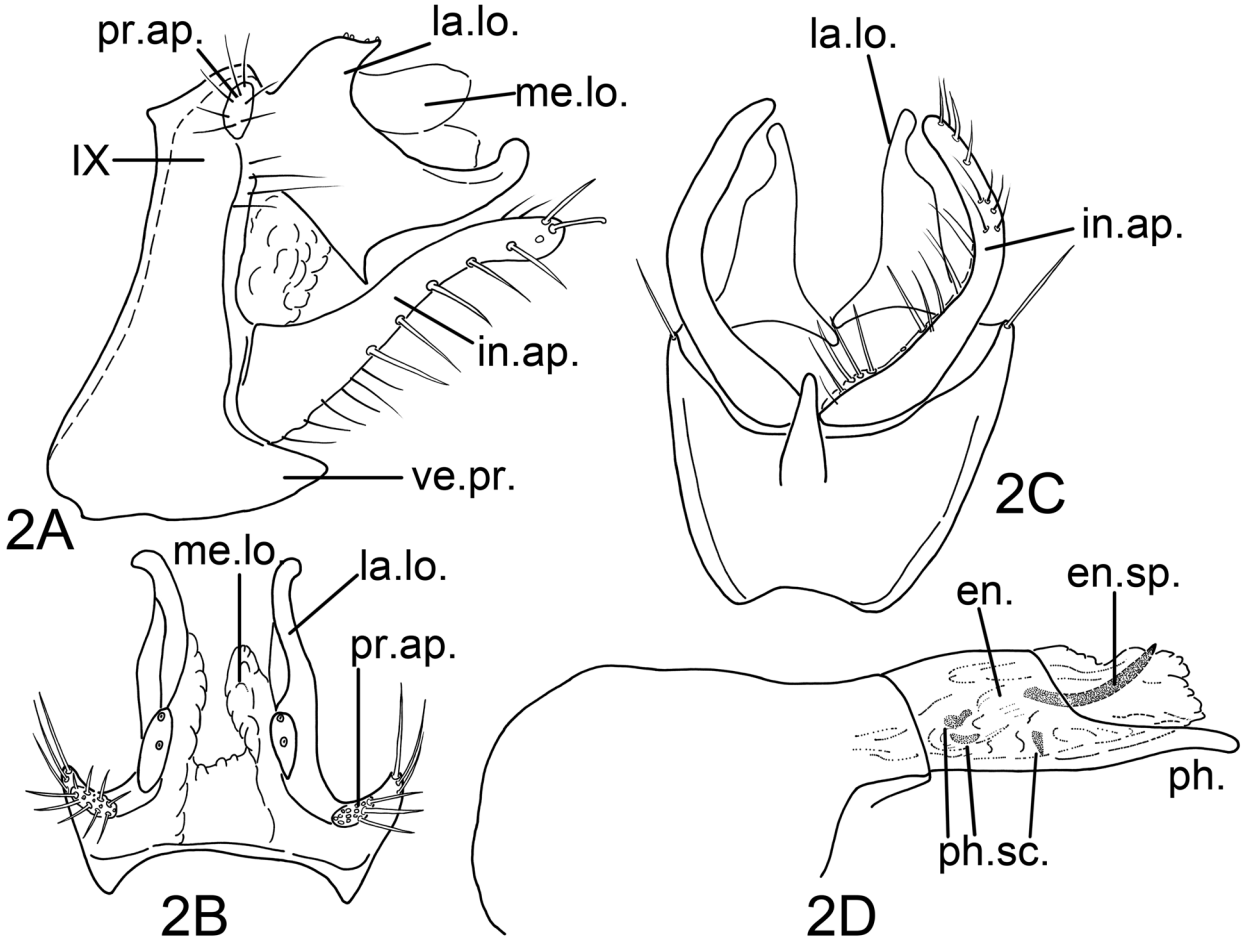

FIGURE 2. *Chimarra paramonorum* **n. sp.**, male genitalia. 2A, left lateral; 2B, dorsal; 2C. ventral; 2D, phallus, left lateral. en. = endotheca; en.sp. = endothecal spines; in.ap. = inferior appendages (paired); la.lo. = sclerotized lateral lobe of segment X (paired); me.lo. = membranous mesal lobe of segment X; IX = segment IX; ph. = phallotheca; ph.sc. = phallotremal sclerites; pr.ap. = preanal appendages (paired); ve.pr. = ventral process.

Larvae (5th instar) Body length 7–10 mm (n = 3), (in alcohol) generally white in colour with head, pronotum, legs, and anal claws yellowish brown.

Head. Head capsule (Figs 3B, 3C) with black spot at each posterolateral margin, elliptical in dorsal and lateral views, slightly narrower anteriorly, with smooth surface. Dorsal ecdysial suture 1/3 as long as frontoclypeus; frontoclypeus elongate-Omega-shaped, constricted at its posterior 1/3 (Figs 3B, 3D), anterior margin dark brown, asymmetrical, having broadly rounded projection on left side and narrower triangular one on right side, separated by single deep notch right of center, each projection with four setae, one long seta mesally and three short setae laterally. In lateral view (Fig. 3C), head capsule posterior margin slightly concave at middle, with above-mentioned black spot at concavity; frontoclypeus and mandibles separated widely to accommodate membranous labrum. In ventral view (Fig. 3F), both anterior and posterior margins acutely incised, with anterolateral angles each having

two long setae, pair of No. 18 setae located anterosubmesally near posterior apex of ventral apotome. Ventral apotome triangular, with anterior margin slightly concave. Eyes (Figs 3A, 3B) black, oval, each surrounded by paler yellow halo, distance from anterior margin of eye to base of mandible about 2 times eye diameter. Antennae (Fig. 3E) simple, each situated in small light area near base of mandible, consisting of two club-shaped spines accompanied by two short fine setae. Labrum (Fig. 3G) membranous, somewhat T-shaped in dorsal view; with anterior margin having brush of dense setae curved downwards, each anterolateral angle having tuft of long setae, and middle portion immediately behind brush of setae with 4 setae, two longer anterior ones separated widely and two shorter posterior ones near each other. Mandibles (Figs 3H1, 3H2, 3I1, 3I2) each with convex outer margin bearing two bristles; left mandible (Figs 3H1, 3I1) mesal margin with prominent projection near basal 1/3, and with 5 subapical teeth and larger acute tooth at apex; right mandible (Figs 3H2, 3I2) inner margin sinuate and bearing 4 subapical teeth and larger blunt tooth at apex. Submental sclerite (Fig. 3F) subrectangular, with two short setae at each anterolateral corner. Maxillary palpi (Fig. 3J) each 4-segmented and straight; palpifer inner margin having tuft of short incurved bristles; maxillary lobe (Fig. 3J) bearing several fine bristles on inner margin and small curved bristle on outer subapical margin.

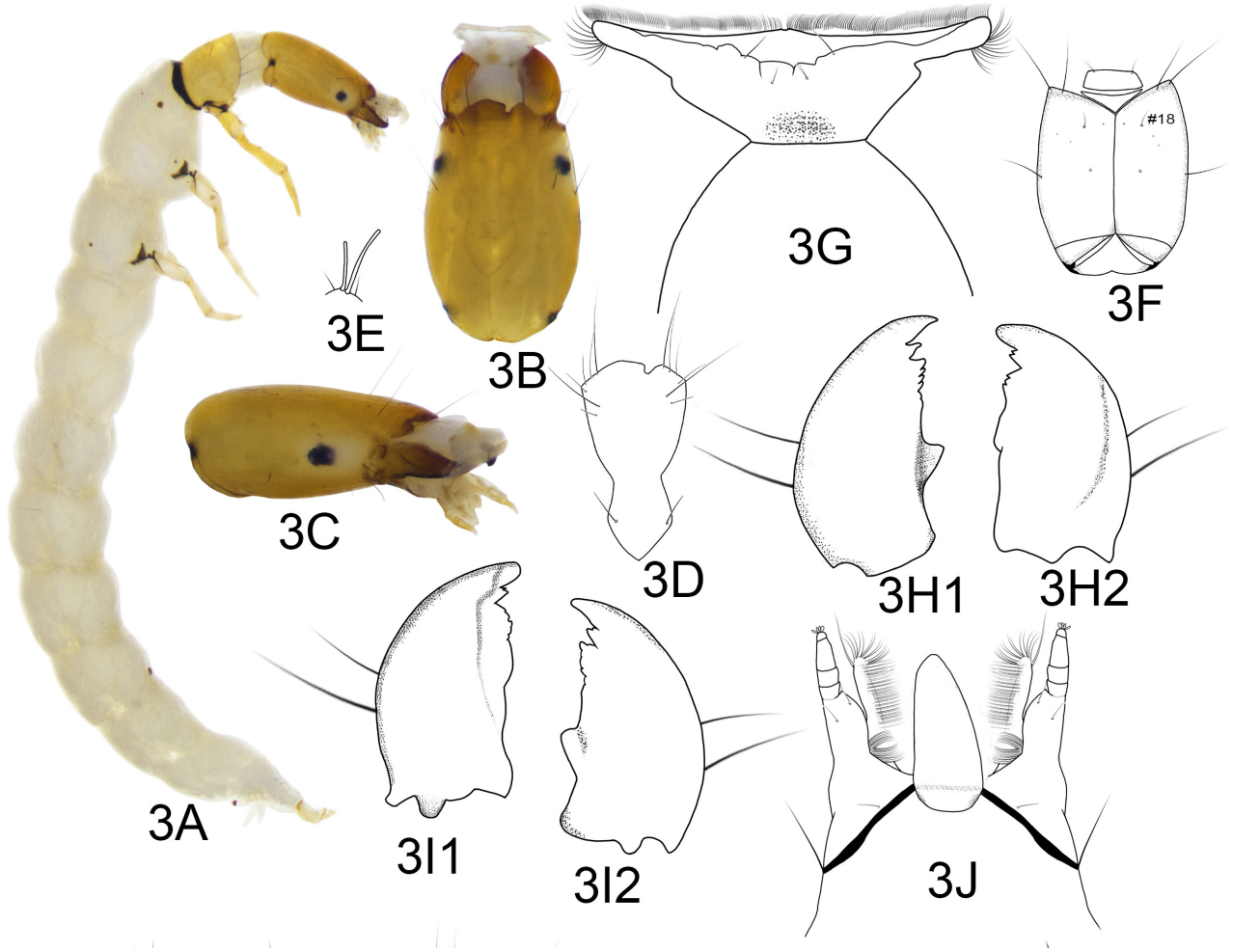

FIGURE 3. *Chimarra paramonorum* **n. sp.**, larva. 3A, habitus, right lateral; 3B, head, dorsal; 3C, head, right lateral; 3D, frontoclypeus, dorsal; 3E, right antenna, dorsal; 3F, head, ventral; 3G, labrum, dorsal; 3H1, left mandible, dorsal; 3H2, right mandible, dorsal; 3I1, right mandible, ventral; 3I2, left mandible, ventral; 3J, maxilla and labium, ventral.

Thorax. Pronotum (Figs 4A, 4B) subrectangular in dorsal view, slightly longer than wide, strongly sclerotized, with posterior and posterolateral borders black; with transverse depression across anterior 1/3 (also seen in Fig. 3A); anterolateral corners each with tuft of four setae, posterolateral margins each with two setae, and dorsal surfaces of each sclerite having three setae behind transverse depression, anterior seta much longer than two posterior setae. Foretrochantins (Fig. 4B) small, in dorsolateral view subrectangular. Meso- and metanota membranous.

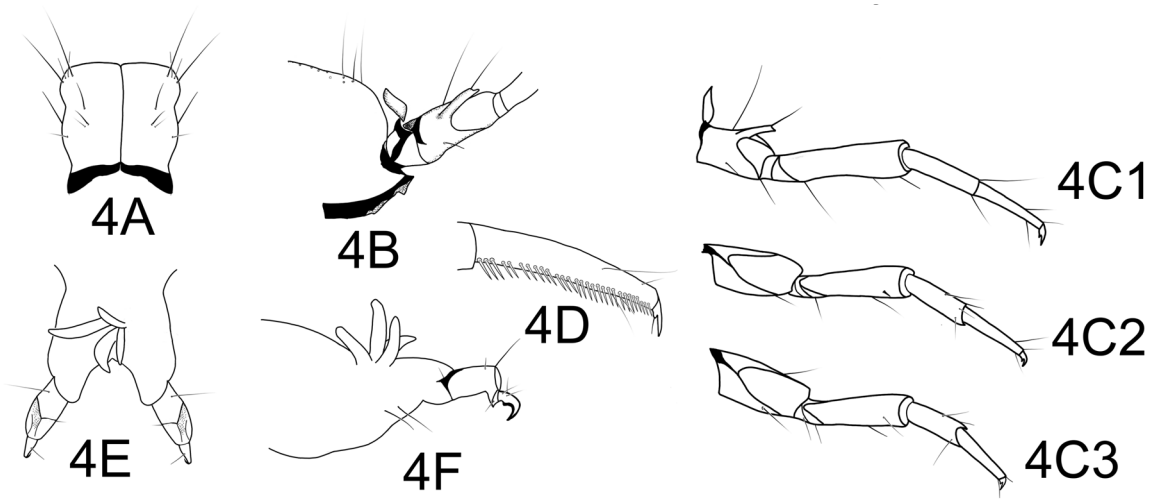

FIGURE 4. *Chimarra paramonorum* **n. sp.**, larva. 4A, pronotum, dorsal; 4B, right lateral edge of pronotum, right foretrochantin, and right forecoxa, right dorsolateral; 4C1, right foreleg, posterolateral; 4C2, right midleg, posterolateral; 4C3, right hind leg, posterolateral; 4D, terminal portion of tarsus and claw of right prothoracic leg, posterolateral; 4E, segment IX and prolegs, dorsal; 4F, left proleg, left lateral.

Legs. Legs (Fig. 4C) each with few bristles and several stronger curved yellow bristles on coxae, femora, and tibiae. Forelegs (Fig. 4C1) slightly longer and thicker than mid- and hind legs, and darker than mid- and hind legs; forecoxae short, subrectangular in posterior view, each with subapicodorsal seta-bearing process about half as long as coxa; trochanters each 2-segmented, first segment somewhat rectangular, second segment triangular; femora cylindrical, each with basal margin obliquely truncate, apex slightly thickened; tibiae slender, about half as wide as femora, and obviously shorter than femora; tarsi (Fig. 4D) cylindrical, each slightly tapering to apex, with row of short spine-like setae ventrally; claws (Fig. 4D) small but well developed, curved downwards, with conspicuous basal seta. Mid- and hind legs similar to forelegs in overall structure, except their coxae much longer and lacking seta-bearing processes, their femora, tibiae, and tarsi shorter; claws of mid- and hind legs also similar, about same size as those of forelegs.

Abdomen. Abdominal segments I–IX with sparse short setae. Lateral fringe absent; segments I–VIII without tracheal gills; segment IX in dorsal view with posterior margin concave, with four single apicodorsal anal gills (Figs 4E, 4F). Anal prolegs (Figs 4E, 4F) elongate and separated widely in dorsal view, lateral sclerites moderately sclerotized, apices truncate; ventral sole plate small, visible only in lateral view (Fig. 4F); anal claws (Fig. 4F) in lateral view with thick bases and sharp apices curved downwards at 90 degrees, each with three small basodorsal setae.

Holotype male, CHINA, Zhe-jiang Province, Jiang shan, Er-ba Town, Xin-dun-cun, 28.2996°N, 118.5359°E, Alt. 608.9 m, 12 Aug 2016, collected by Chang-hai Sun and Ji-hua Xu. **Paratypes**: 20 males, CHINA, Zhe-jiang Province, Le Qing, Xian-xi Town, Shang-bei-ge-cun, 29.26306°N, 120.9731°E, Alt. 229.3 m, 26 May 2017, collected by Yan-li Hu and Chang-hai Sun; 7 larvae, CHINA, Zhe-jiang Province, Le Qing, Xian-xi Town, Shang-bei-ge-cun, 29.26306°N, 120.9731°E, Alt. 229.3 m, 26 May 2017, collected by Yan-li Hu and Chang-hai Sun; 5 larvae, CHINA, Zhe-jiang Province, Jin hua, Yao-chuan Town, Niu-lan-ping, 29.1022°N, 120.5358°E, Alt. 251.79 m, 27 Mar 2018, collected by Yan-li Hu, Cong Wu, and Jin Gao.

Other materials: 25 males, CHINA, Zhe-jiang Province, Le Qing, Xian-xi Town, Shang-bei-ge-cun, 29.26306°N, 120.9731°E, Alt. 229.3 m, 26 May 2017, collected by Yan-li Hu and Chang-hai Sun.

Etymology. The specific epithet is from Latin prefix *para-*, meaning "similar to," and the congeneric species name *monorum*, referring to the similarity in the male genitalia of these two species.

Distribution. China (Zhe-jiang).

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