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The larvae of Chinese Hydropsychidae (Insecta: Trichoptera), Part II: *Potamyia chinensis* and *Cheumatopsyche trifascia*

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Abstract

The larvae of Chinese caddisflies *Potamyia chinensis* and *Cheumatopsyche trifascia* were successfully associated with identifiable adults using independent DNA markers, mitochondrial COI barcodes and nuclear ribosomal 28S D2 genes. A total of 49 specimens collected in China were employed in the molecular analyses. The two markers were congruent on species boundaries for 11 distinctive haplogroups, while D2 failed in differentiating two closely related species. A brief summary for larval studies of both genera is given, followed by an introduction to the generic morphological characteristics, and detailed morphological descriptions and illustrations for the two successfully associated species. The larva of *P. chinensis* is re-described here based on Chinese materials, following the previous larval description for *P. echigoensis*, which was recently synonymized with *P. chinensis*.

Key words: caddisfly, life-stage association, China, DNA barcoding, COI, 28S D2

Introduction

Molecular taxonomy uses nucleotide sequences to facilitate species delineation and identification. DNA-based methods are especially helpful in associating unknown life-stages to identifiable specimens, which, for Trichoptera, are typically name-bearing males (Zhou *et al*. 2007). Such an endeavor has benefited significantly from large molecular biodiversity registration initiatives, such as the Barcode of Life project (Hebert *et al*. 2003), where standard DNA reference databases are being built for various groups of organisms across the world. In particular, significant effort has aimed at constructing a comprehensive DNA barcode library for the world caddisfly species (Insecta: Trichoptera) (Zhou *et al*. 2016). This dataset has not only allowed for accelerated faunistic surveys of global Trichoptera diversity (Zhou *et al*. 2009, 2010, 2011; Pauls *et al*. 2010; Morinière *et al*. 2017; Erasmus *et al*. 2018), but has also enabled larval taxonomy for regional caddisfly communities (Geraci *et al*. 2011; Ruiter *et al*. 2013; Xu *et al*. 2015, 2018; Xie *et al*. 2017; Hjalmarsson *et al*. 2018; Hu *et al*. 2018, 2020; Ge *et al*. 2020).

The present study represents the second installment of a series of molecular-association and morphological-description works on the Chinese Hydropsychidae larvae. The first publication (Zhou 2009) provided larval descriptions for three hydropsychid species from three genera, *Arctopsyche*, *Parapsyche*, and *Diplectrona*. Among these, *Parapsyche* sp. A was described as an unnamed larval form, which shared identical COI and D2 sequences with an undescribed male specimen. Later that year, adult males bearing the same morphological characters, which were collected at the same site on the same day by Xin Zhou, were described as a new species *Parapsyche beijingensis* Sun & Morse 2009 (in Sun *et al*. 2009) in an independently study (Sun *et al*. 2009). Therefore, the *Parapsyche* sp. A larva described by Zhou (2009) should be assigned to *P. beijingensis*. This incident demonstrates that species description can be accelerated by DNA evidence as distinct life-stages can now be described independently and linked by shared species-specific DNA markers.

In this study, we use the same method to associate larvae of two Chinese Hydropsychidae species in different genera, *Potamyia* and *Cheumatopsyche*, and describe them.

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Materials and methods

The template by Zhou (2009) is followed, first providing independent molecular phylogenetic trees built from two independent markers, i.e., mitochondrial COI barcode sequences and the nuclear ribosomal 28S D2 region. Species boundaries were delineated based on the topology and positions of identifiable adult males on both phylograms. Larval associations were then inferred from these phylograms when larval sequences were either identical to those derived from male adults, or falling within species boundaries delineated by the latter, following criteria proposed by Zhou *et al*. (2007).

Larvae and adults of Chinese hydropsychids used in this study were collected in China during 2001–2005 (Table 1 and Appendix 1). Detailed collection and preservation protocols are identical to those described by Zhou (2009). Briefly, larval specimens were primarily collected by kick-nets or hand-picking and preserved in 95% ethanol, while adult specimens were collected using light traps and also preserved in 95% ethanol. Judging by the size of the specimens, *Potamyia* and *Cheumatopsyche* larvae studied in this work are late instars and most likely the last instar, with consistent morphology among multiple individuals examined in the field and in the laboratory (except for *Potamyia*, where only a single specimen was collected). However, definite identification of the larval stage cannot be made due to limited knowledge on the Chinese larval fauna.

A proportion of the specimen (abdominal segments III–VI of larvae with guts removed, and one hind leg of each adult male) was used for genomic DNA extraction. Standard DNA extraction and PCR programs were applied (Zhou *et al*. 2007, Zhou 2009). All COI barcodes and D2 sequences were publicly available in GenBank (Accession Numbers are provided in Table 1) and in the Barcode of Life System (Project "Larvae of Chinese Hydropsychidae Part II"). D2 sequences were aligned manually according to secondary structure before uploading to BOLD (Zhou *et al*. 2007).

We employed a Maximum-Likelihood-based method to construct both phylogenies. Both COI and D2 trees were constructed using IQ-TREE (v2.1.1, Minh *et al*. 2020), with branch supports assessed by ultrafast bootstrap (Hoang *et al*. 2017). The best-fit substitution model was calculated by ModelFinder (Kalyaanamoorthy *et al*. 2017) integrated in IQ-TREE, which identified the TIM2+F+I+G4 model for COI and TIM+F+G4 for D2, respectively, according to Bayesian information criterion scores (BIC).

Finally, larval descriptions are made for those species with successful associations. Larval morphologies were examined and illustrated using a Nikon SMZ-U Zoom 1:10 compound microscope and camera lucida.

Species boundaries and larval association

Two *Potamyia* species, *P. chinensis* (HPGP1, Figs 1a, 1b) and *P. chekiangensis* (HPGP3, Fig. 1a) were identified to described species based on male morphology. In addition, two morphospecies that were close to *P. chekiangensis* were assigned provisional names *P. chekiangensis* A (HPGP4, Figs 1a, 1b) and *P*. XZ sp. CN1 (HPGP2, Fig. 1a). COI and D2 results were congruent on most species boundaries, except that D2 failed to differentiate *P*. XZ sp. CN1 from *P. chekiangensis* (HPGP2+3, Fig. 1b), which was likely caused by the relatively slow divergence with the ribosomal DNA.

Within *Cheumatopsyche*, nine species or morphospecies were recognized by morphology and by both COI and D2 markers (HPGP 5-13, Figs 1a, 1b).

Based on these species boundaries, the larvae of *P. chinensis* and *C. trifascia* were successfully associated with male adults.

GENUS *Potamyia* Banks 1900

A total of 10 *Potamyia* species have been recorded from China (Yang *et al.* 2016). Of these, three species were collected during 2004 and 2005 from southern China. In the present study, the larva of *P. chinensis* (Ulmer 1915) is associated with adults using independent DNA sequences (Table 1; Fig. 1), which enables the first report on larval morphology for the species distributed in China. The low association number of species in the genus is mainly due to the fact that *Potamyia* are mostly inhabitants of large rivers, such as the North American species *P. flava* (Hagen) (Wiggins 1996), which presents an obvious challenge for larval collection. Only one larval specimen was collected during the course of this study.

TABLE 1. Specimens used in larval-adult association and GenBank accession numbers.

BOLD Sample ID	Sex/life stage	Taxon	GenBank Accession (COI)	GenBank Accession (D2)	**Locality**
CNCAD_0003	larva	*Cheumatopsyche* sp. 8	KX105568	MW128399	Guangdong Prov., Nan-kun-shan
CNCAD_0004	larva	*Cheumatopsyche trifascia*	KX104817	MW128411	Guangdong Prov., Nan-kun-shan
CNCAD_0005	larva	*Cheumatopsyche* sp. 8	KX106411	MW128400	Guangdong Prov., Nan-kun-shan
CNCAD_0006	larva	*Cheumatopsyche trifascia*	KX103841	MW128417	Guangdong Prov., Nan-kun-shan
CNCAD_0012	male	*Cheumatopsyche trifascia*	KX106691	MW128415	Guangdong Prov., Nan-kun-shan
CNCAD_0015	male	*Cheumatopsyche trifascia*	KX105560	MW128404	Guangdong Prov., Nan-kun-shan
CNCAD_0016	larva	*Cheumatopsyche* nr. *infascia*	KX106960	MW128398	Guangdong Prov., Nan-ling
CNCAD_0017	male	*Cheumatopsyche trifascia*	KX102807	MW128403	Guangdong Prov., Nan-ling
CNCAD_0018	male	*Cheumatopsyche trifascia*	KX103254	MW128421	Guangdong Prov., Nan-ling
CNCAD_0033	male	*Cheumatopsyche trifascia*	KX102642	MW128401	Guangdong Prov., Nan-ling
CNCAD_0041	male	*Cheumatopsyche trifascia*	KX104281	MW128424	Guangdong Prov., Da-dong-shan
CNCAD_0042	male	*Cheumatopsyche trifascia*	KX104240	MW128419	Guangdong Prov., Da-dong-shan
CNCAD_0046	larva	*Cheumatopsyche trifascia*	KX106005	MW128406	Guangdong Prov., Che-ba-ling
CNCAD_0047	larva	*Cheumatopsyche trifascia*	KX103903	MW128408	Guangdong Prov., Che-ba-ling
CNCAD_0048	larva	*Cheumatopsyche trifascia*	KX103686	MW128418	Guangdong Prov., Che-ba-ling
CNCAD_0051	male	*Cheumatopsyche dubitans*	KX103737	MW128391	Guangdong Prov., Che-ba-ling
CNCAD_0052	male	*Cheumatopsyche longiclasper*	KX103952	MW128395	Guangdong Prov., Che-ba-ling
CNCAD_0053	male	*Cheumatopsyche dubitans*	KX104039	MW128390	Guangdong Prov., Che-ba-ling
CNCAD_0054	male	*Cheumatopsyche longiclasper*	KX105192	MW128396	Guangdong Prov., Che-ba-ling
CNCAD_0081	male	*Cheumatopsyche trifascia*	KX106615	MW128405	Guangdong Prov., Luo-fu-shan
CNCAD_0089	male	*Cheumatopsyche trifascia*	KX105005	MW128423	Guangxi Prov., Shi-wan-da-shan
CNCAD_0090	male	*Cheumatopsyche guadunica*	KX104807	MW128393	Guangxi Prov., Cen-wang-lao-shan
CNCAD_0094	male	*Cheumatopsyche guadunica*	KX103620	MW128394	Guangxi Prov., Cen-wang-lao-shan
CNCAD_0098	male	*Potamyia chekiangensis* A	KX105759	MW128430	Guangxi Prov., Cen-wang-lao-shan
CNCAD_0102	male	*Potamyia chekiangensis* A	HM102283	MW128431	Guangxi Prov., Cen-wang-lao-shan
CNCAD_0103	male	*Cheumatopsyche* XZ sp. CN8	KX104356	MW128427	Guangxi Prov., Cen-wang-lao-shan
CNCAD_0119	male	*Cheumatopsyche trifascia*	KX104851	MW128409	Guangdong Prov., Qi-mu-zhang
CNCAD_0126	male	*Potamyia chinensis*	KX102935	MW128434	Guangxi Prov., Nan-pan-jiang

.....continued on the next page

TABLE 1. (Continued)

BOLD Sample ID	Sex/life stage	Taxon	GenBank Accession (COI)	GenBank Accession (D2)	**Locality**
CNCAD_0128	male	*Potamyia chekiangensis*	KX106900	MW128429	Guangxi Prov., Mo-li
CNCAD_0134	male	*Cheumatopsyche* nr. *infascia*	KX103383	MW128397	Guangxi Prov., Yang-shuo
CNCAD_0136	male	*Cheumatopsyche guadunica*	KX105980	MW128392	Guangxi Prov., Cen-wang-lao-shan
CNCAD_0185	larva	*Potamyia chinensis*	KX104242	MW128432	Guangxi Prov., Mo-li
CNCAD_0204	male	*Potamyia* XZ sp. CN1	KX104739	MW128437	Jiangxi Prov., Lei-gu-ling
CNCAD_0205	male	*Potamyia chinensis*	KX107456	MW128433	Sichuan Prov., Tian-quan
CNCAD_0206	male	*Potamyia chinensis*	KX106954	MW128436	Sichuan Prov., Ma-bian
CNCAD_0214	male	*Potamyia* XZ sp. CN1	KX107406	MW128438	Jiangxi Prov., Lei-gu-ling
CNCAD_0215	male	*Potamyia chekiangensis*	KX104463	MW128428	Jiangxi Prov., Li-tou-jian
CNCAD_0284	male	*Potamyia chinensis*	KX104413	MW128435	Jiangxi Prov., Li-tou-jian
CNCAD_CU01	larva	*Cheumatopsyche trifascia*	KX104864	MW128416	Guangdong Prov., Nan-ling
CNCAD_CU03	larva	*Cheumatopsyche trifascia*	KX103414	MW128410	Guangdong Prov., Nan-ling
CNCAD_CU04	larva	*Cheumatopsyche trifascia*	KX105228	MW128420	Guangdong Prov., Nan-kun-shan
CNCAD_CU05	male	*Cheumatopsyche* XZ sp. CN4	KX103452	MW128426	Sichuan Prov., Feng-tong-zhai
CNCAD_CU06	male	*Cheumatopsyche trifascia*	KX103028	MW128407	Guangdong Prov., Nan-ling
CNCAD_CU07	male	*Cheumatopsyche trifascia*	KX105687	MW128414	Guangdong Prov., Nan-ling
CNCAD_CU08	male	*Cheumatopsyche trifascia*	KX107449	MW128402	Guangdong Prov., Nan-ling
CNCAD_CU09	male	*Cheumatopsyche trifascia*	KX107172	MW128413	Guangdong Prov., Nan-ling
CNCAD_CU10	male	*Cheumatopsyche trifascia*	KX106526	MW128422	Guangdong Prov., Nan-ling
CNCAD_CU11	male	*Cheumatopsyche trifascia*	KX105097	MW128412	Guangdong Prov., Nan-ling
CNCAD_PT01	male	*Cheumatopsyche* XZ sp. CN1	KX103917	MW128425	Guangdong Prov., Nan-ling

The species associated here, *P. chinensis* (Ulmer 1915) is a senior synonym of *Hydropsyche echigoensis* Tsuda 1949 (Oláh *et al*. 2006, 2008) and *Cheumatopsyche tienmuiaca* Schmid 1965 (Tian *et al.* 1996; Oláh *et al*. 2006, 2008). The larva of *P. chinensis* has been described from Japan (Tanida 2005); we provide here a redescription of the larva of the species based on our Chinese material.

Generic characteristics of the genus. Although only one Chinese larval specimen is available at the time of the study, descriptions and illustrations are available for *P. flava* from North America (Ross 1944, 1959; Wiggins 1996) and for *P. chinensis* from Japan (Tanida 2005). A few characteristics seem to be consistently diagnostic for the genus: Front margin of frontoclypeal apotome complete and symmetrical; primary setae and some secondary setae on head long and thin; front margin of submentum cleft, anterior borders of two small submental lobes oblique and straight; anterior border of anterior ventral apotome short and protrusive in middle (not obvious in *P. chinensis* described from Japan); anterior ventral apotome shorter than sub-mentum; foretrochantin not forked at least in some individuals [a series of intermediates ranging from unforked to forked have been observed in *P. flava* (Wiggins 1996)]; prosternal plate large, followed by pair of small posterior prosternal sclerites; pair of ventral tracheal gills present on abdominal segment VII, each with single gill stem.

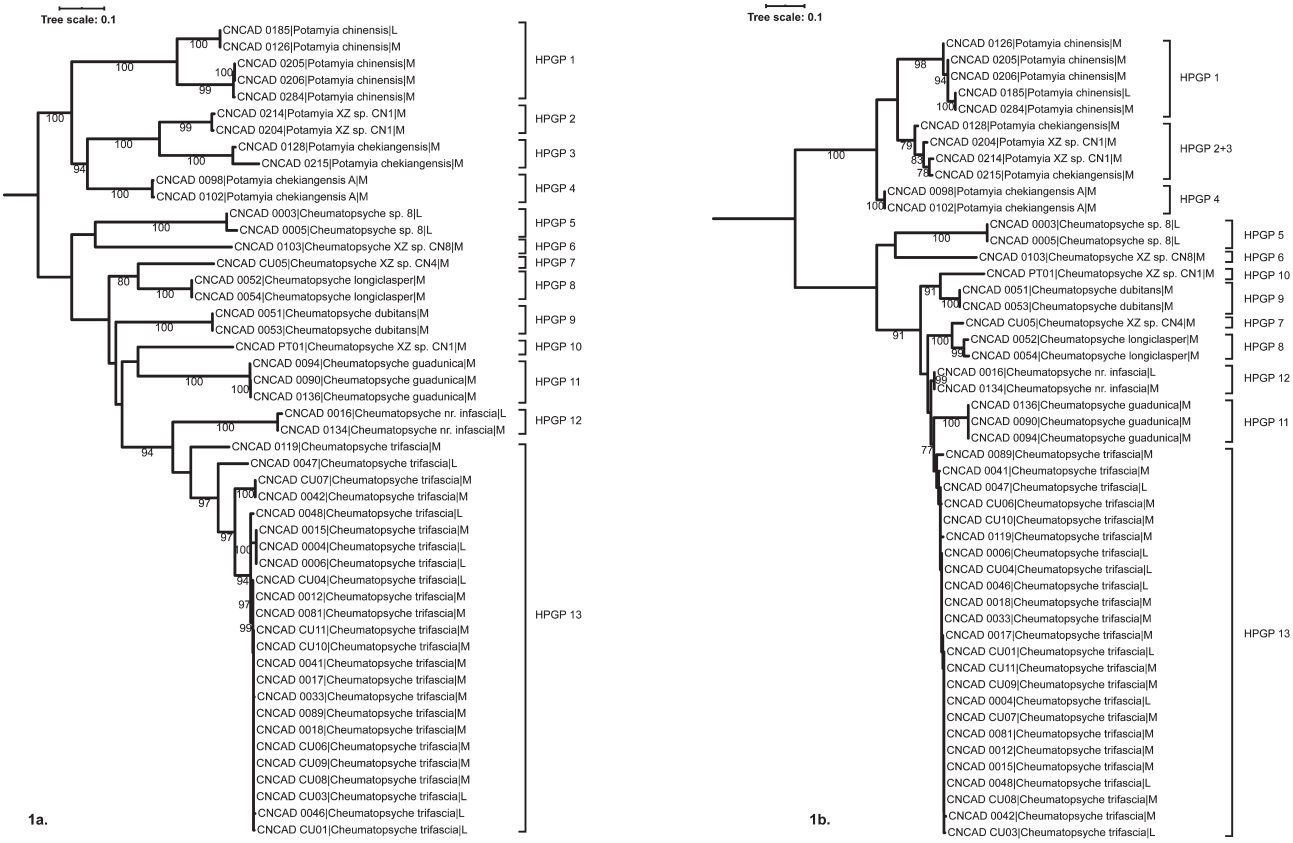

FIGURE 1. Larval-adult association of Chinese *Potamyia* and *Cheumatopsyche* species. 1a, COI Maximum Likelihood phylogram; 1b, D2 Maximum Likelihood phylogram. L = larva; M = adult male. Bootstraps values are shown below stems; values less than 75 are not shown in the phylograms.

Potamyia chinensis (Ulmer 1915)

(Figs 2a–2j)

HEAD. Head sub-quadrate, lateral sides of head parallel with each other, 1.0 mm long, 0.8 mm wide. Anterior margin of frontoclypeal apotome straight, crenulate, with series of tiny tooth-like structures. Lateral sides of frontoclypeal apotome narrower at eye level. Mediotransversal fold of frontoclypeal apotome not prominent (Fig. 2a). Anterior margin of frontoclypeus pigmented, reddish brown, both anterolateral corners brownish. Rest of frontoclypeal apotome mostly light brownish, with four pale yellowish markings: anterior marking situated on middle of anterior margin, large (almost one-fourth as long as entire length of frontoclypeus, and one-third as wide as width

of front margin), more or less rectangular; two lateral markings small, sub-triangular, each with tentorial pit situated on short posterior border; posterior marking situated on middle of frontoclypeus, rounded. Parietal sclerite on each side mostly pale yellowish; area along frontoclypeal suture and anterior half of coronal suture darker; this darker area extended ventrolaterad behind eye, not reaching ventral side of head (Fig. 2c). Posterodorsal margins of parietal sclerites bearing transverse brownish thin stripes.

Primary setae very long; secondary setae on anterolateral corners of frontoclypeus longer than those on rest of dorsal head. All secondary setae relatively long, fine, tapered, bearing prominent sockets, brownish (on dark background) or yellowish (on pale background). Frontoclypeus evenly distributed with fine secondary setae.

Ventral side of head mostly pale yellow; striae on middle half of each stridulating file darker; pair of stridulating files connected mesally by transverse dark band anterior of setae #18 (Fig. 2b). Anterior ventral apotome darker, sub-triangular, shorter than one-fourth of ventral ecdysial line, anterior margin mostly concave, slightly protrusive in middle; posterior ventral apotome minute, triangular. Submentum trapezoidal, lateral sides slightly concave in middle; posterior border curved; anterior margin cleft, middle incision narrow and straight, anterior borders of lateral lobes oblique, shorter mesally than laterally. Lateral region of each parietal sclerite bearing long, tapered, clear secondary setae on anterior half.

THORAX. Thoracic nota yellowish, without prominent markings, densely covered by long, tapered, *hair-like* secondary setae. Anterior border of each notum bearing dense series of long, tapered setae.

Seta 22 on anterolateral corner of each side of pronotum prominent, long and thick (Fig. 2d, 2e). Posterior border of pronotum with thin, brownish line; lateral borders black.

Prosternal plate large, followed posterolaterally by pair of small, sub-triangular, posterior prosternal sclerites (Fig. 2f).

Foretrochantin finger-like, not forked, yellowish, bearing few thick, long, black bristles on dorsal and lateral surfaces. Apex of foretrochantin slightly curved upward, pointing cephalad (Fig. 2g).

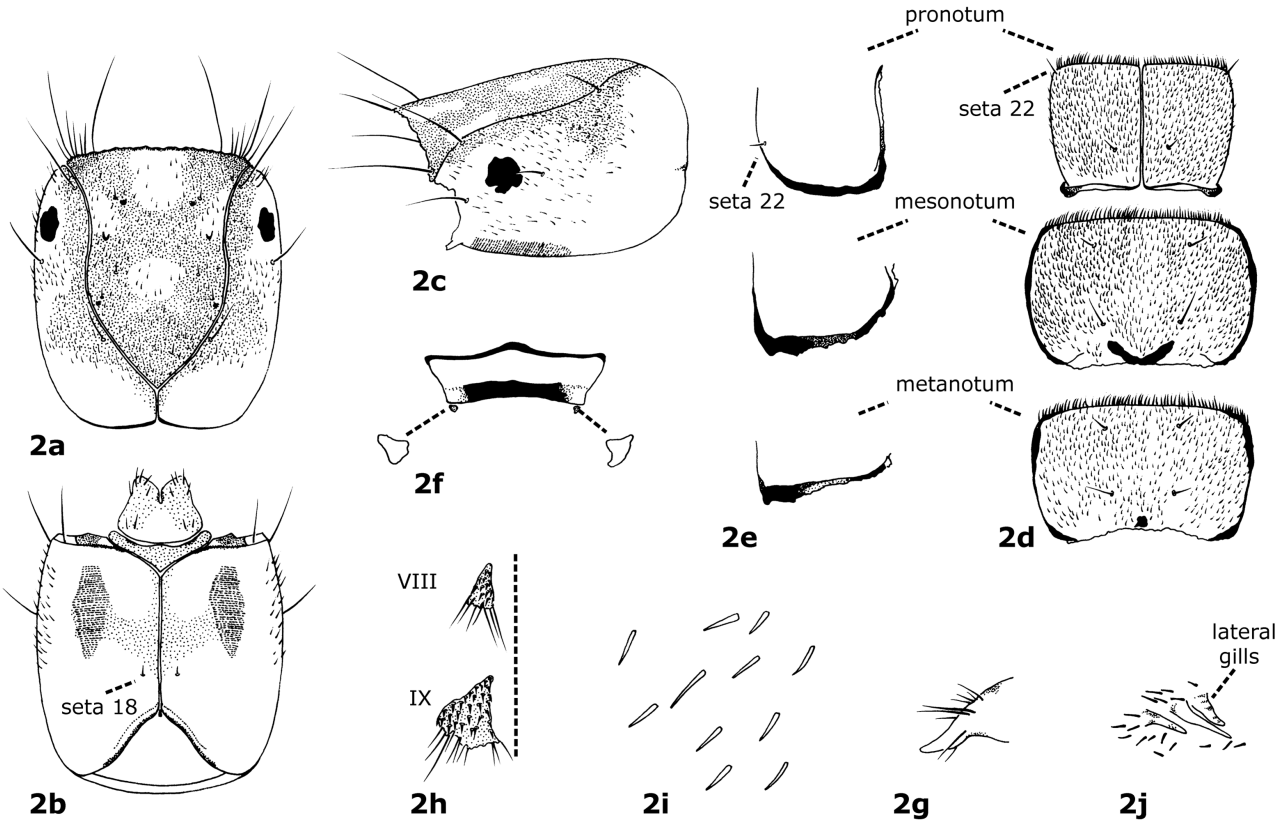

FIGURE 2. *Potamyia chinensis* (Ulmer 1915) larva. 2a, head, dorsal; 2b, head, ventral; 2c, head, left lateral; 2d, pro-, meso-, and metanota, dorsal; 2e, pro-, meso-, and metanota, left lateral; 2f, prosternal plate and posterior prosternal sclerites (latter also shown further enlarged), ventral; 2g, left foretrochantin, left lateral; 2h, right ventral sclerites on sterna VIII and IX, ventral; 2i, hairs on tergum of abdominal segment II; 2j, lateral gills (pleural gills) on left side of abdominal segment V, left lateral.

Hair-like setae on meso- and metanota longer and thicker than those on pronotum. Middle third of lateral border of mesonotum paler, brownish, whereas rest of lateral border black. Posterior border of mesonotum with curved, black marking on middle half; this mark with no lateral pieces.

Anterior fourth of lateral border of metanotum black, thicker; remainder of lateral border with thin dark brownish line. Posterior border of metanotum with small, round black mark on middle. Secondary setae sparser than those on mesonotum.

Mesosternum with one pair of ventral tracheal gills; metasternum with two pairs of ventral tracheal gills (Table 2). All thoracic gills single-stemmed.

ABDOMEN. Abdominal terga and pleura covered by only one type of secondary setae: each moderately long, fine, with apex slightly enlarged and more or less flattened (Fig. 2i). Density of secondary setae somewhat sparse, usually separated by distance greater than lengths of setae.

Lateral gills on abdominal pleura simple, finger-like, long. Abdominal segments I, II, VIII, and IX with no lateral gills; segment III with one pair of lateral gill; segments V–VI with three pairs of lateral gills; VII with two pairs of lateral gills, posterior gills small, short and conical (Fig. 2j; Table 2).

Abdominal ventral gills of two typical hydropsychid types: single-stemmed gills and bifid-stemmed gills, stalks of all gills not segmented. Single-stemmed gills situated anteromesad of bifid gills on segments II–VI. Abdominal segment I with two pairs of single-stemmed gills (ventrolateral and ventromesal), situated very closely; segments II–VI each with one single-stemmed gill and one bifid gill on each side; segment VII with one pair of single-stemmed gills (Table 2).

Ventral sternites on abdominal VIII small, triangular, with light yellowish pigmentation. Anterior part of each sternite VIII with tapered, short, thick, golden setae; posterior border with long, thick, dark brownish bristles, pointing posterad. Ventral sternites IX large, sub-triangular, each with posterior border very wide, mesal border straight; light brownish. Golden setae on anterior part of each sternite IX longer than anterior setae on sternites VIII; posterior border with long, thick, brownish bristles, pointing posterad. Posteromesal corner of each sternite IX with seta-less area (Fig. 2h).

Diagnosis. The color pattern on the head of *Potamyia chinensis* somewhat resembles that of *Hydropsyche simulata* (Xu *et al*. 2018). But these two species can be easily differentiated based on other characters, such as the shape of the anterior ventral apotome, the shape and size of posterior prosternal sclerites, the shape of the foretrochantins, etc.

TABLE 2. Number and arrangement of gills in the mature larva of *Potamyia chinensis* (Ulmer 1915)[1]

	Thorax		Abdominal segments								
	Mesothorax	Metathorax	I	II	III	IV	V	VI	VII	VIII	IX
Lateral (pleural)	-	-	-	-	1[a]	3[a]	3[a]	3[a]	2[a]	-	-
Ventrolateral	-	1[c]	1[c]	1[b]	1[b]	1[b]	1[b]	1[b]	-	-	-
Ventromedial	1[c]	1[c]	1[c]	1[c]	1[c]	1[c]	1[c]	1[c]	1[c]	-	-

[1] Numbers in the table represent the number of gills present on one side of a particular body segment. Gill types: a = simple, finger-like gill; b = bifid-stemmed gill; c = single-stemmed gill.

GENUS *Cheumatopsyche* Wallengren 1891

Cheumatopsyche larvae are typically small and may lack conspicuous diagnostic characters among species. Some general larval characters considered useful in previous studies (e.g., Statzner 1984) include the shape and number of notches on the front margin of the frontoclypeus; the shape of the foretrochantins, especially the shape of the dorsal branch; the shape of the submentum; the morphology of primary seta 3 on the frontoclypeus; the shape of the posterior prosternal sclerites; characters of the secondary setae, and the morphology of the stridulating files. In addition, Schefter and Wiggins (1987) promoted the use of secondary setae located on the head, thoracic nota and abdominal segments in diagnoses of *Cheumatopsyche* larvae.

A total of 29 *Cheumatopsyche* species have been recorded from China (Yang *et al*. 2016). While only the larva of *C. ventricosa* was illustrated (Dudgeon 1999), the larval stage of *C. spinosa* was readily recognized in a life history study (Dudgeon 1997). In the present work, seven Chinese *Cheumatopsyche* species were studied for possible

larval-adult associations using DNA sequences, among which one species (*C. trifascia*) was successfully associated following Zhou *et al.* (2007) (Table 1; Fig. 1). Meanwhile, the other six species remained unassociated because the identification of adults was in progress, or the larvae (HPGPs 6–11, Figs, 1a, 1b) or adult males (HPGP 5, Figs. 1a, 1b) were not available to this study. However, both D2 and COI fragments appear able to provide enough nucleotide variation among *Cheumatopsyche* species to enable future associations. A single larva (CNCAD_0016) had identical D2 sequence with an adult male (CNCAD_0134), which was tentatively identified as *C*. nr. *infascia* (HPGP 12, Fig 1b). However, this larva was not considered successfully associated because its COI sequence showed differences from that of the adult, therefore violating the stringent criteria proposed by Zhou *et al*. (2007). The larvae of *Cheumatopsyche* are among the most abundant caddisfly larvae in many stream systems. Yet, the difficulties in identifying species of both adults and larvae make them one of the most challenging groups in freshwater ecological research and biomonitoring projects. The DNA association approach used in this work could play an important role in future larval-adult association and species identification for the genus.

Generic characteristics of the genus. Larva generally small; head and thoracic nota often lacking prominent marks. Head bearing dense, short, tapered *hair-like* setae, no *blunt-setae*. Anterior margin of frontoclypeal apotome with or without median notch (usually with a notch). Mediotransversal fold of frontoclypeus and tentorial pits not pronounced. Anterior ventral apotome subtriangular. Posterior ventral apotome minute, triangular. Submentum trapezoidal with anterior margin cleft. Seta 22 on anterolateral corners of pronotum prominent, long. Prosternal plate large, followed posterolaterally by pair of posterior sclerites usually minute. [The North American species *C. etrona* Ross 1941 has large posterior sclerites (Wiggins 1996)]. Foretrochantins forked. Metasternum with three ventral gills: single ventromesal tracheal gill in addition to pair of coxal (lateral) gills—based on *C. trifascia* and African species *C. thomasseti* (Ulmer). [But see Scott (1983) for number of metasternal gills in *Cheumatopsyche*.] No *scale-hairs* on abdominal segments. Abdominal segment VII with or without ventral gills.

Cheumatopsyche trifascia Li 1988 (in Li & Dudgeon 1988)

(Figs 3a–3g)

HEAD. Head oval, 0.7 mm long, maximum width at middle of head 0.6 mm. Anterior margin of frontoclypeal apotome with large emargination in middle, entire margin crenulate, bearing series of small, rounded flanges. Lateral sides of frontoclypeus slightly narrower at eye level. Mediotransversal fold and tentorial pits of frontoclypeus not pronounced (Fig. 3a).

In dorsal aspect, dorsum of head mostly orange yellow, lacking pronounced markings. Anterior fourth of frontoclypeal apotome reddish brown, areas on posterior parietal sclerites along posterior third of frontoclypeal sutures slightly darkener. Muscle scars not pronounced but visible.

Secondary hairs on dorsum and sides of head tapered, blackish, often slightly curved. Hairs on anterior fourth of frontoclypeal apotome sparse; those on sides of head generally thicker than those on frontoclypeus. Posterior sixth of dorsum of head seta-less.

Ventral side of head slightly constricted at posterior two-fifths. Anterior ventral apotome sub-triangular, with anterior border concave, both subanterolateral corners angled; posterior ventral apotome minute, triangular; both anterior and posterior ventral apotomes dark brownish. Submentum trapezoidal, lateral borders suddenly narrower at midlength; posterior border convex; anterior border cleft, height of median incision about half as long as submentum (Fig. 3b). Ventral head mostly yellowish orange, striae of stridulating ridges not darker. Anterior half of ventrolateral areas of both parietal sclerites bearing tapered, clear, yellowish secondary setae.

THORAX. Thoracic nota generally yellowish to brownish, lacking distinct color patterns; all nota densely covered with long, tapered, dark *hair-like* setae; anterior borders all bearing dense row of long, curved, tapered hairs.

Pronotum subdivided longitudinally along mid-dorsal ecdysial line. Seta 22 at anterolateral corner of each side of pronotum prominent, long, thick. Posterior border of pronotum appearing black near posterolateral corners only, median portion not thicker, generally light brownish in dorsal aspect (Figs 3c, 3d).

Prosternal plate large, constricted laterally at posterior third, transverse anterior border and majority of transverse posterior border black. Posterior prosternal sclerites minute, each undivided (Fig. 3e).

Foretrochantins forked, each with dorsal branch seta-less, ventral branch setose. Dorsal branch shorter than ventral branch, basal end of dorsal branch about half as wide as that of ventral branch (Fig. 3f).

Posterior border of mesonotum with large, V-shaped, black marking on middle third with no lateral pieces. Secondary setae on mesonotum more or less curved.

Posterior border of metanotum with small, sub-triangular, black marking on middle ninth. Anterior and posterior ends of lateral borders black, middle half of each lateral border paler, brownish. Posterolateral corners of lateral borders each with small notch (Fig. 3d).

Thoracic ventral tracheal gills simple, each with single-stemmed, thick gill stalk and two to three gill filaments. Mesosternum with pair of submesal gills. Metasternum with pair of ventrolateral gills (coxal gills) and one single median gill situated on mesal line (Table 3).

ABDOMEN. Abdominal segments bearing sparse, fine, dark, tapered hairs on pleura and sterna. Secondary setae on abdominal terga clear, transparent, scarcely visible. No *scale hairs* or *club hairs* present on abdominal segments.

Lateral gills (pleural gills) simple, finger-like, often clear and transparent. Abdominal segment III with one pair of lateral gills; segment IV with two; segments V and VI each with three; segment VII with one or two pairs of lateral gills (Table 3).

Abdominal ventral gills of two typical hydropsychid types: single-stemmed gills and bifid-stemmed gills, stalks of all gills not segmented. Single-stemmed gills situated anteromesal to bifid gills on segments II–VI. Gill stalks or branches of bifid gill stalks thick and long, each stalk or each branch of bifid stalk bearing two to three gill filaments at apex. Abdominal sternum I with two pairs of single-stemmed gills (ventrolateral and ventromesal), situated very closely; sterna II–VI each with one submesal pair of single-stemmed gills and one sublateral pair of bifid gills; no ventral gills on segment VII (Table 3).

Ventral sternites on abdominal VIII small, triangular, with light yellowish pigmentation. Anterior part of each sternite VIII with tapered, short, thick, golden setae; posterior border with long, thick, brownish bristles, pointing posterad. Ventral sternites IX larger, sub-triangular, mesal borders straight, light yellowish. Golden setae on anterior part of sternites IX longer than those on sternites VIII; each with posterior border bearing long, thick, brownish bristles, pointing posterad. Posteromesal corner of each sternite IX with seta-less area and small notch (Fig. 3g).

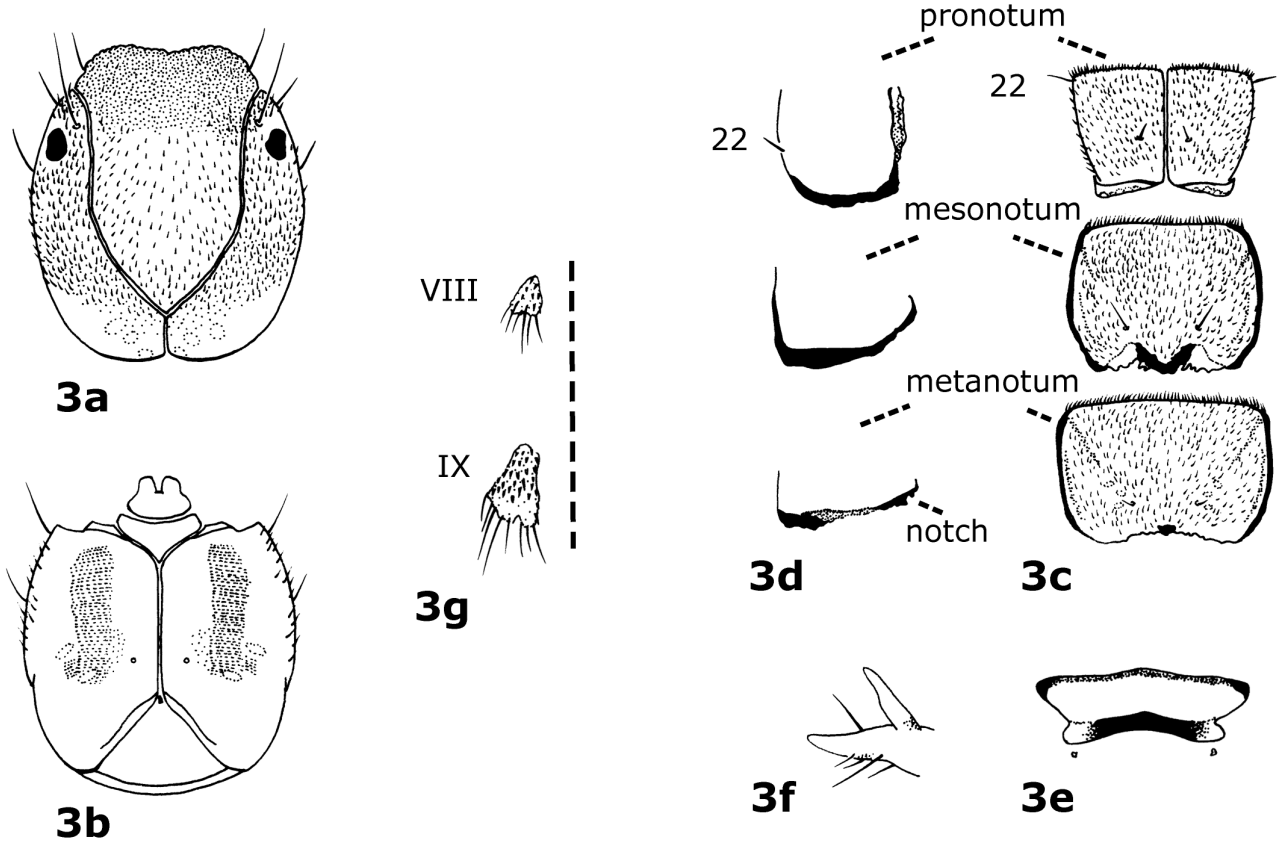

FIGURE 3. *Cheumatopsyche trifascia* Li 1988, larva. 3a, head, dorsal; 3b, head, ventral; 3c, pro-, meso-, and metanota, dorsal; 3d, pro-, meso-, and metanota, left lateral; 3e, prosternal plate and posterior prosternal sclerites, ventral; 3f, left foretrochantin, left lateral; 3g, right ventral sternites on sterna VIII and IX, ventral.

TABLE 3. Number and arrangement of gills in the mature larva of *Cheumatopsyche trifascia* Li 1998[1]

	Thorax		Abdominal segments								
	Mesothorax	Metathorax	I	II	III	IV	V	VI	VII	VIII	IX
Lateral (pleural)	-	-	-	-	1[a]	2[a]	3[a]	3[a]	1[a]-2[a]	-	-
Ventrolateral	-	1[c]	1[c]	1[b]	1[b]	1[b]	1[b]	1[b]	-	-	-
Ventromedial	1[c]	1[c**]	1[c]	1[c]	1[c]	1[c]	1[c]	1[c]	-	-	-

[1] Numbers in the table represent the number of gills present on one side of a particular body segment. Gill types: a = simple, transparent, finger-like gill; b = bifid-stemmed gill; c = single-stemmed gill.
**Note: The metathorax possesses only a single ventromedial gill on the middle of the metasternum.

Author contributions

XZ conducted field collections, molecular work and larval description. AZ carried out phylogenetic analyses using COI and D2, prepared the phylogenetic figure, and was responsible for data curation and submission. XZ wrote the manuscript and both authors proofed the paper.

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