



The imaginal and nymphal morphology of *Gilliesia pulchra* Zhou, 2004 and its contribution to understanding generic phylogeny (Ephemeroptera: Leptophlebiidae)

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Abstract

The nymphs of the leptophlebiid mayfly *Gilliesia pulchra* Zhou, 2004 have not been reported before, which inhibits deeper understanding of the taxonomy and phylogenetic position of this species and its genus. In 2022, we reared nymphs from southwestern China to the adult stage. All stages are described and photographed here. Nymphs of this species have single deeply-forked gills, somewhat elongated maxillary palpi and two setal tufts on the lingua. Its imaginal characteristics, including wings and penes, are quite similar to *G. hindustanica* (Gillies, 1951), the type species of the genus. Comparatively, the third species of the genus, *G. ratchaburiensis* Boonsoong & Sartori, 2015, seems less similar, both in adults and nymphs. Generally, features of *G. pulchra* nymphs link the two genera *Gilliesia* Peters & Edmunds, 1970 and *Habrophlebiodes* Ulmer, 1919 together, while features of the nymphs of *G. ratchaburiensis* bridge the genera *Gilliesia* and *Dipterophlebiodes* Demoulin, 1954.

Key words: nymph, character, *Gilliesia*, phylogeny, China

Introduction

The species *Gilliesia pulchra* Zhou, 2004 was originally established upon imaginal materials. Before that, the genus contained a single species, *Gilliesia hindustanica* (Gillies, 1951), which also was described only from adults. That means the nymphal stage of the genus *Gilliesia* Peters & Edmunds, 1970 was not known up to then, although one of us (CFZ) made a short oral report on this issue during the 2012 International Conference on Ephemeroptera in Japan.

Boonsoong & Sartori (2015) described a third species, *Gilliesia ratchaburiensis* Boonsoong & Sartori, 2015 based on both imaginal and nymphal stages, throwing some new light on this relatively rare genus. However, the male of *G. ratchaburiensis* has laterally extended sharp penes, while the other two previous species males have elongated, laterally and ventrally bent penial tubes. In addition, the base of MP₂ of this species is connected to MP₁ with a crossvein, while the other two species have an independent MP₂, the latter being the key point given by Peters & Edmunds (1970) to define the genus *Gilliesia*. In the nymphal stage, moreover, the gills of this species, *G. ratchaburiensis*, do not have any trachea branches nor marginal setae. Those characteristics, along with its concave leading margin of imaginal hindwings, show the species *G. ratchaburiensis* is greatly different to other two congeners (see details in Results section, below). So, more information on the genus, especially for the two earlier known species, will provide additional evidence to test the status of the species *G. ratchaburiensis* and the generic concept of the *Gilliesia*.

Based on imaginal characters, Peters & Edmunds (1970) placed the genus *Gilliesia* next to *Habrophlebiodes* Ulmer, 1919 and *Dipterophlebiodes* Demoulin, 1954 in the subfamily Leptophlebiinae. Boonsoong & Sartori (2015) showed clearly that their nymphs are also generally similar to these. But besides the points mentioned above, some other differences can be found in nymphs: the mandibles of *G. ratchaburiensis* without any lateral setae and the claw of it with one row of about ten spines only, two characters neither genus *Habrophlebiodes* nor *Dipterophlebiodes* have. So details of the nymphs of the other species will also contribute to understanding the phylogenetic position of the genus *Gilliesia*.

Material and methods

In the summer of 2022, some leptophlebiid nymphs with single fork gills were found and collected by hand net from a little stream in Yunnan province, southwestern China. Some of them were moved into a plastic tray and reared in the motel where we stayed during our field work. Two males and two females were reared to the adult stage. These were the same as some other adults we collected from a wall near the stream. We compared adult material to the types of *G. pulchra*, and concluded they are fresh materials of this species, based on a morphological species concept. Given that this is the first discovery of the nymphs of this species, we describe them below, photograph the imagoes and discuss the related issues of this species and its genus.

Some digital photos were taken by Sony A7RII (Interchangeable Lens Digital Camera), and some of them were taken under Nikon Eclipse 50i (Microscope) and Mshot MZ81 (Stereo microscope). Final plates were prepared and improved with Adobe Photoshop CC.

Eggs were dissected from female imagoes. All SEM (scanning electronic microscope) samples were prepared with a standard protocol: fixed in 4% glutaraldehyde for 5–8 hours, rinsed with PBS (physiological saline) 2–3 times (10–15 minutes each), dehydrated in concentration gradient acetone (30%, 50%, 70%, 80%, 90%, 100%, 10 to 15 minutes each), and coated with gold film in a vacuum.

Other than eggs, all materials are stored in ethanol (more than 80%). All specimens used in this study are deposited in the Mayfly Collection, College of Life Sciences, Nanjing Normal University (NNU).

Abbreviations: c: canine, dd: distal dentiseta, pd: proximal dentiseta, ps: pectinate submarginal seta.

Results

Gilliesia pulchra Zhou, 2004

Material examined: 2 male imagoes and 2 female imagoes (reared from nymphs), 2 female subimagoes, 21 nymphs, Bang-Gun-Jian Mountain, De-Hong City, Yunnan Province, China, 97°50'52"E, 24°23'13"N, 1800 m alt., 28-VI-2022, leg. Xuhongyi Zheng, Pengxu Mu; 2 male imagoes and 1 female imago, Nan-Zhu forestry center, Chi-Shui City, Guizhou Province, China, 105.42°E, 28.34°N, 9-VI-1995, leg. Changhai Sun, Beixin Wang.

Holotype: male imago, Jing-Fu-Shan Mountain, Huang-Cao-Ping, Chongqing municipality, China, 107.10°E, 29.00°N, 1200 m alt., 3-IX-2000, leg. Chuangren Li, Changfa Zhou.

Descriptions

Mature male nymph (in alcohol, Figs 1–3, 7): Body length 6.0–7.0 mm, terminal filament 8.0–8.5 mm, cerci 6.0–6.5 mm. Body yellowish to amber, nota and tergites with brown spots or markings, ventral body pale to cream (Figs 1A–B, 1D–E). Head amber to yellowish brown, with three distinct pale areas surrounding ocelli respectively; antennae about 3.0× width of head (Figs 1A, 1G); hypognathous (Figs 1A, 1D, 1G).

Mouthparts. Labrum: length ca. 2.0× width; anteromedian margin smoothly concave; dorsal surface scattered with relatively long hair-like setae; row of additional submarginal setae along emargination of anterior margin, latter with row of spine-like setae, progressively longer from middle to lateral (Fig. 7B). Ventral surface with two tufts of short hair-like setae near emargination (Fig. 2A). Lateral margins of labrum sclerotized, brown to dark (Fig. 2A). **Mandibles:** left mandible lateral surface with sparse hair-like setae, dorsal surface with shorter hair-like setae, ventral surface with row of hair-like setae in middle (Figs 2D, H); outer incisor divided into four denticles, inner incisor with two apical denticles, prostheca composed of distinct spur and tuft of spines (Figs 2D–E). Right mandible similar to left one in setal pattern but with additional tuft of hair-like mesal setae near mola (Fig. 2F); outer incisor with three apical denticles and inner one with two; spur of prostheca almost invisible compared to left one (Figs 2F–G). **Hypopharynx:** superlinguae with slightly concave lateral margins, apex with setal tufts (Fig. 2B); lingua nearly square in shape and anterior margin concave, with two small tufts of hair-like setae (Fig. 2I). **Maxilla:** with sparse hair-like setae on lateral margin; crown with tuft of dense hair-like setae, 8 pectinate setae near galealacinia (Figs 2J–K, 7A); segment I and III of maxillary palpi equal in length, ca. 1.4× segment II, both segment

I and III with relatively long hair-like setae on surface, those of apical segment III longer than others; segment II with apical hair-like setae on inner margin only (Fig. 2K). **Labium:** paraglossae expanded laterally; glossae slightly elongated; surfaces of both with dense hair-like setae; segment II of labial palpi subequal in length to segment III, combined length of them subequal to segment I; all of them with hair-like setae on surface; segment III cone-like, with numerous bristles on inner margin and setae on surface. Mentum and submentum with very sparse and tiny hair-like setae on ventral surface (Fig. 2C).

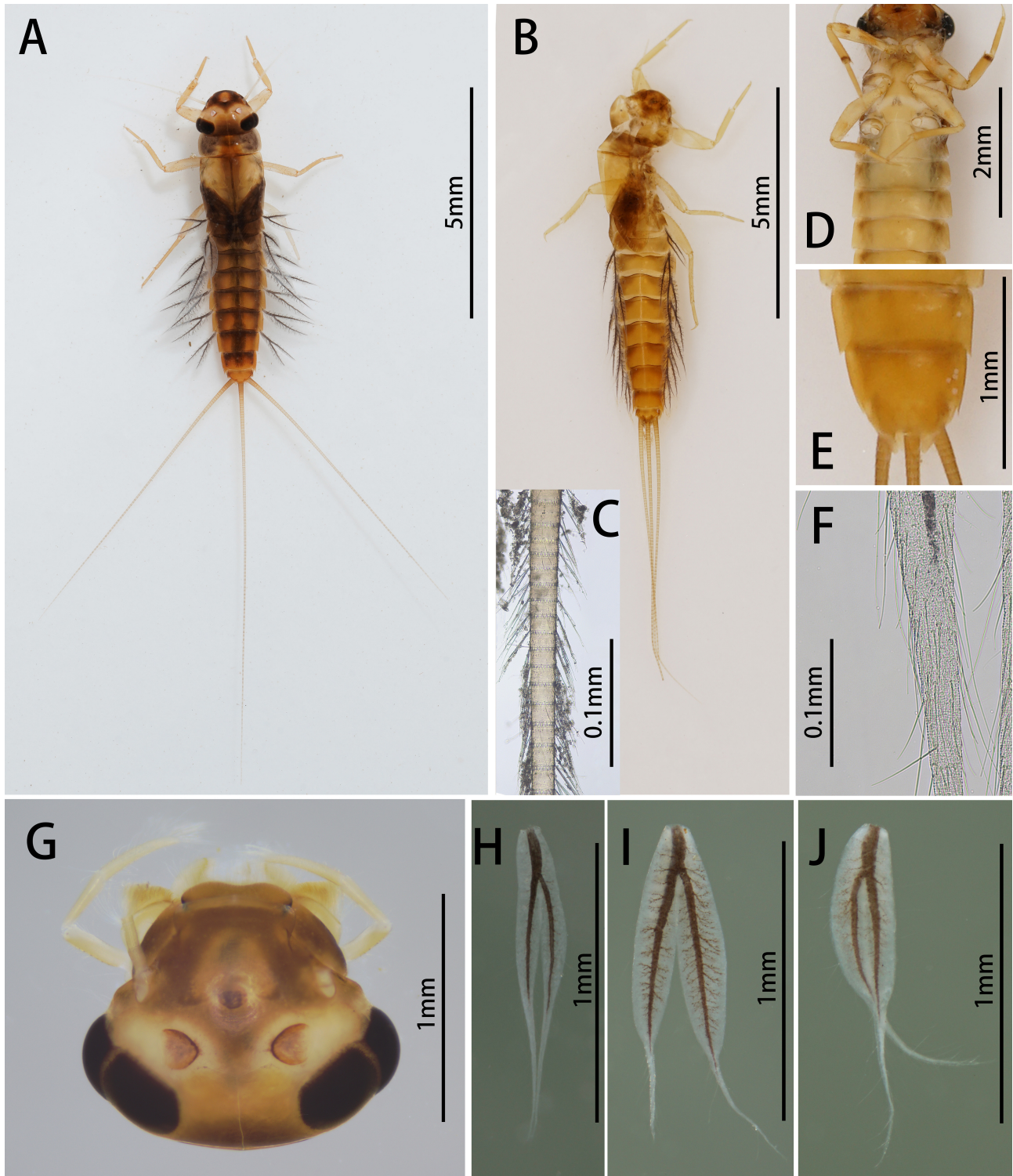


FIGURE 1. Nymphal structures of *Gilliesia pulchra* (digital photos): A. Habitus; B. Exuviate of Nymph (dorsal view); C. Terminal filament enlarged; D. Thorax and sterna I-IV (ventral view); E. Sterna VIII-X (ventral view); F. Gill I enlarged (showing marginal setae); G. Head (dorsal view); H. Gill I; I. Gill IV; J. Gill VII.

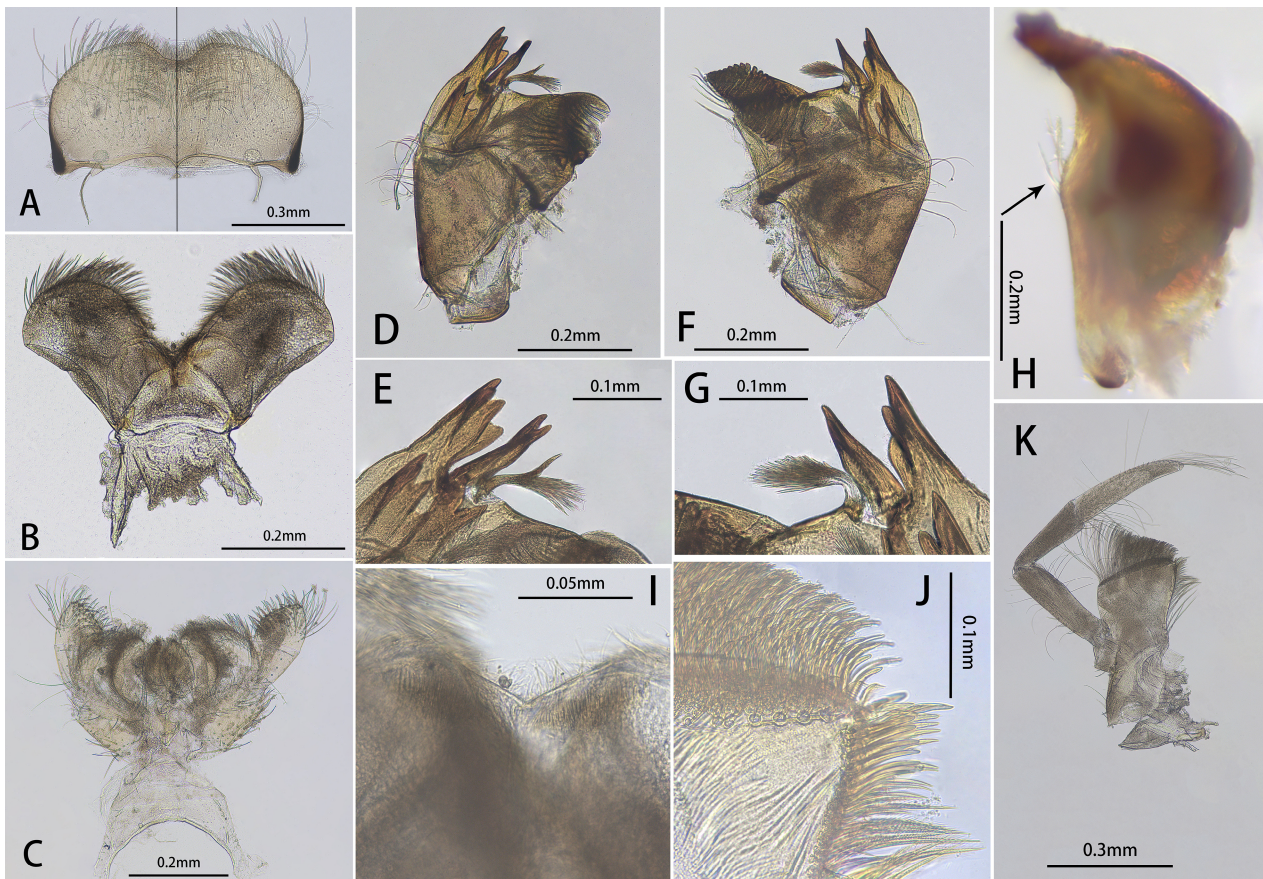


FIGURE 2. Mouthparts of *Gilliesia pulchra* (digital photos): A. Labrum (left: dorsal view, right: ventral view); B. Hypopharynx (ventral view); C. Labium (ventral view); D. Left mandible (dorsal view); E. Incisors of left mandible; F. Right mandible (dorsal view); G. Incisors of right mandible; H. Setae on ventral mandible (shown in black arrow); I. Anterior lingua enlarged (showing the setal tuft); J. Canines and dentisetae of maxilla; K. Right maxilla (ventral view).

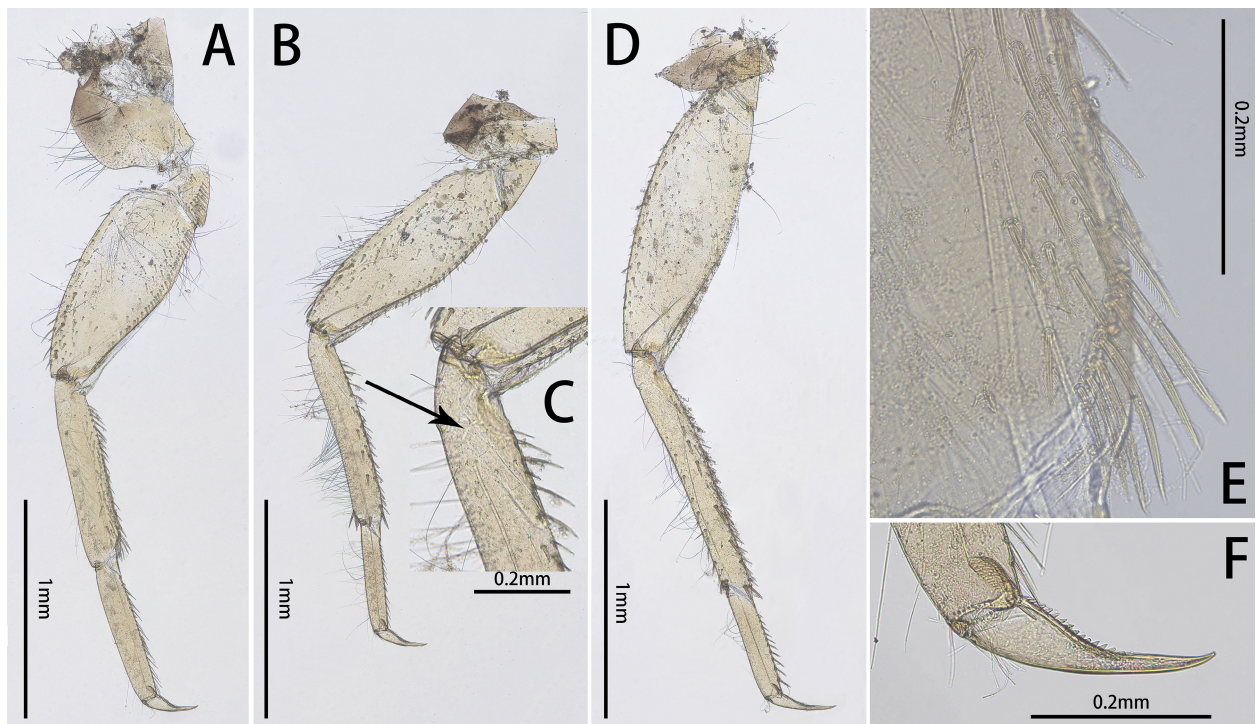


FIGURE 3. Nymphal legs of *Gilliesia pulchra* exuviae (digital photos): A. Foreleg; B. Midleg; C. Patellar-tibial fusion line of Midleg; D. Hindleg; E. Inner apex of foretibia; F. Foreleg claw.

Legs: forecoxae very close to one another on sternum, midcoxae separation wider than forecoxae, hindcoxae separation wider than middle ones (Fig. 1D); fore- and midcoxae larger than hindcoxae, all of them with hair-like setae (Figs 3A–D); fore- and mid-trochanters each with row of spine-like bristles on inner margin (Figs 3A–B). All legs yellowish to cream, forecoxa amber to brown, forefemur with subapical brown spot (Fig. 1D); all legs with hair-like setae on outer margin and few on inner basal margin of femora (Figs 3A–D); stout bristles on inner and outer margins of femora but those on hindleg much shorter (Figs 3A–D); all tibiae with pectinate setae on inner margin (Fig. 3E); all tarsi with short and acute setae on inner margin (Figs 3A–D); patellar-tibial fusion lines present on mid- and hindlegs (Figs 3B–D); tarsal claws of legs alike, each with two rows of denticle-like teeth, one row with 15–18 teeth, the other with 5–8 tiny ones (Fig. 7C); apices of claws elongated into bent spine-like shape (Fig. 3F). Ratio of femora: tibiae: tarsi of forelegs = 1.56: 1.36: 1.00, that of midlegs = 2.28: 1.88: 1.00, and hindlegs = 2.30: 2.16: 1.00 (Figs 3A–D).

Each abdominal tergite with pair of brownish spots on anterolateral corners and dark line on posterior margin (longitudinal brown stripes of adults visible in mature individuals) (Figs 1A–B). Posterolateral expansions on abdominal segments VIII and IX, those on segment VIII much smaller than segment IX (Fig. 1E). **Gills:** gills I–VII alike, forked at 1/5 distance from base with numerous tiny hair-like setae on margin, especially on apex (Figs 1F, 1H–J); all gills with branched tracheae, more distinct in gill IV (Figs 1H–J). **Caudal filaments:** with verticillate stout spine-like setae on articulations, both lateral sides of articulation with tuft of hair-like setae (Fig. 1C).

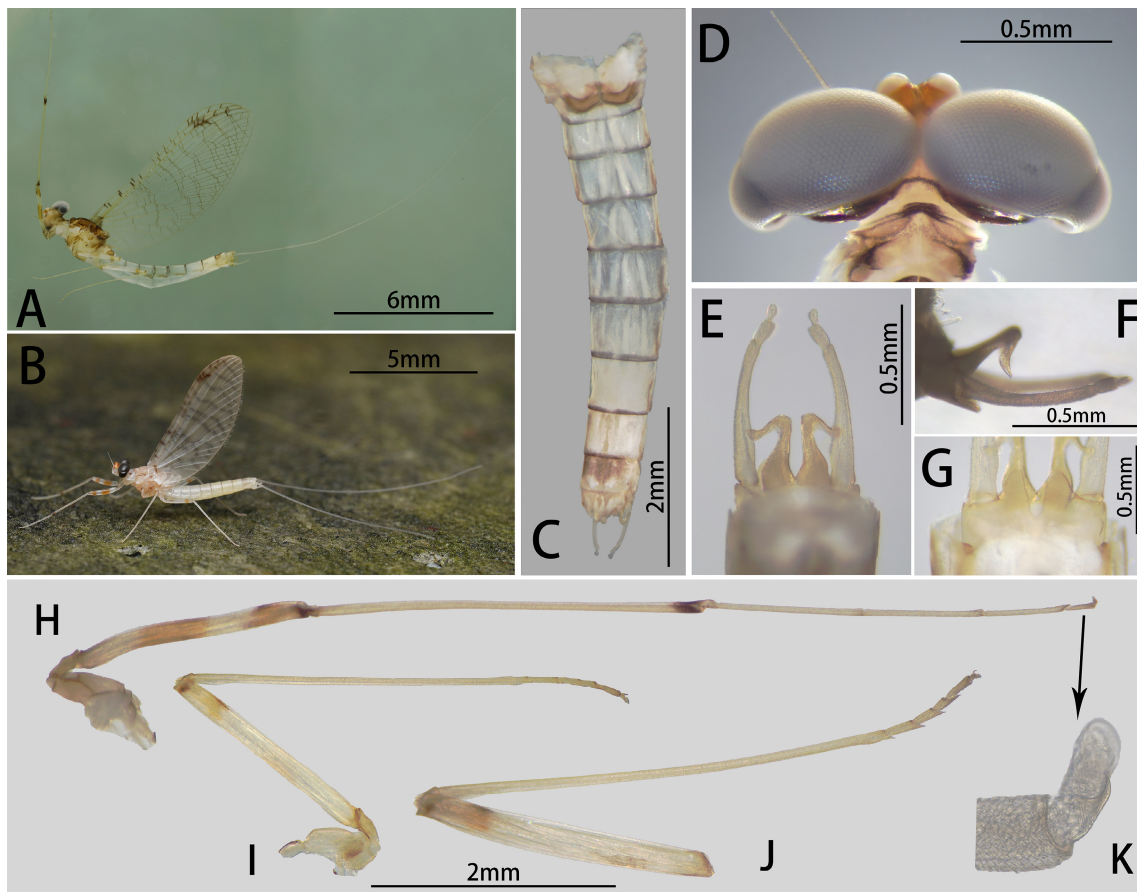


FIGURE 4. Male imaginal structures of *Gilliesia pulchra* (digital photos): A. Imago; B. Subimago; C. Abdomen (dorsal view); D. Head (dorsal view); E. Genitalia (dorsal view); F. Genitalia (lateral view); G. Subgenital plate; H. Foreleg; I. Midleg; J. Hindleg; K. Claw.

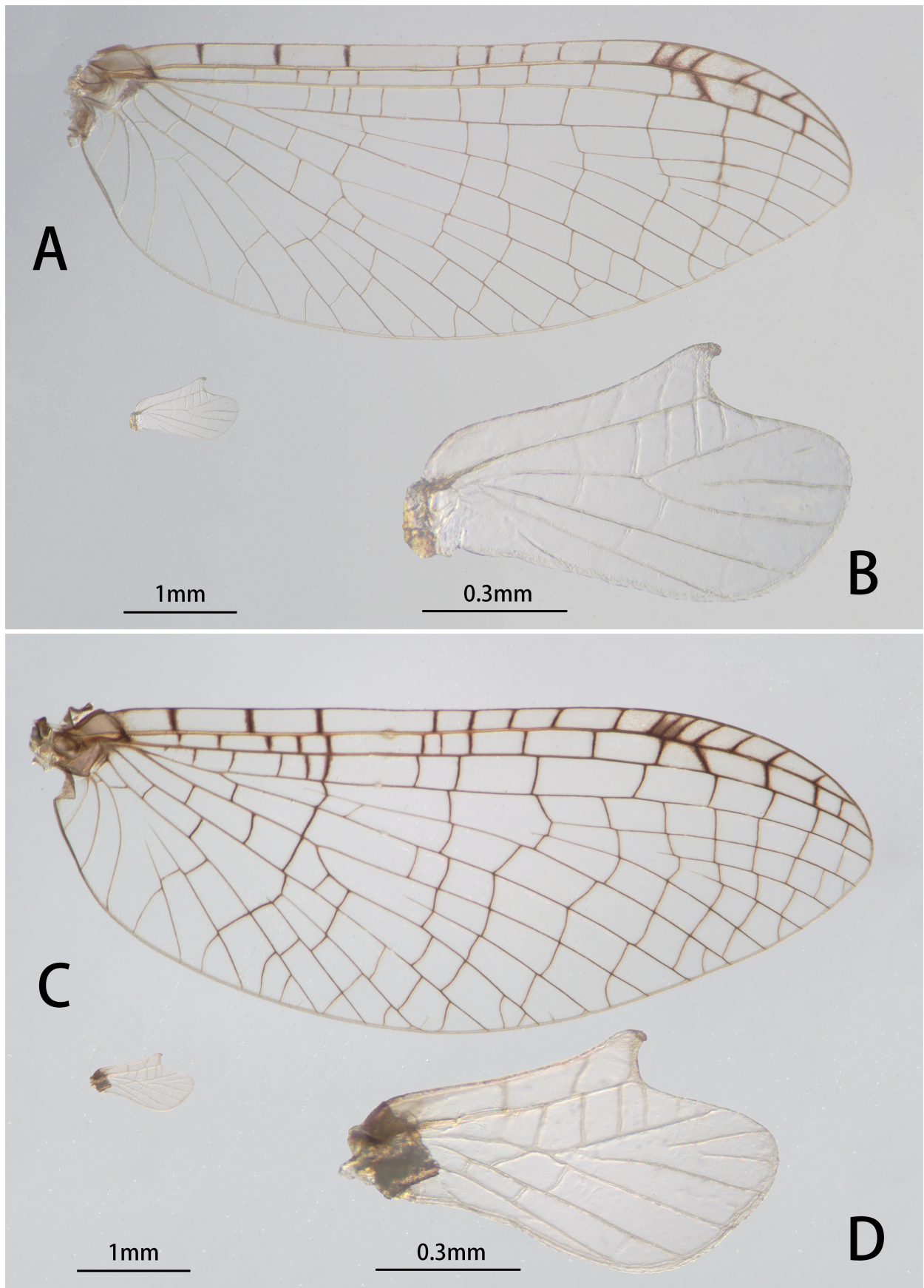


FIGURE 5. Wings of *Gilliesia pulchra* (digital photos): A. Forewing and hindwing of male; B. Male hindwing enlarged; C. Forewing and hindwing of female; D. Female hindwing enlarged.

Male imago (see Zhou, 2004) main characters include: Compound eyes almost contiguous (distance between them shorter than half of one ocellus) (Fig. 4D); forewings transparent, veins amber to brown, crossveins in costal and subcostal portions washed with brown to dark pigments, base of MP_2 unconnected to MP_1 (Fig. 5A); hindwings with somewhat hooked costal projection, leading margins slightly concave, three visible longitudinal veins behind MA (Fig. 5B). All femora with two marked bands at middle and apex respectively, but those of mid- and hindlegs much paler than forefemora; joints between femora, tibiae and tarsi of forelegs brown to dark (Figs 4H–J); two claws dissimilar, one blunt, one with hooked apex (Fig. 4K); ratio of femora: tibiae: tarsi of forelegs = 1.0: 1.6: 1.7, those of mid- and hindlegs = 1.0: 1.5: 0.4, and ratio of foretarsal segments from I to V = 1.0: 13.0: 10.0: 7.0: 2.0 (Figs 4H–J). Tergites of abdomen each with pair of lateral brown stripes and dark posterior margin; besides, tergites I–VII pale, tergites VIII–X reddish to brown, especially tergite IX (Figs 4A, 4C). Subgenital plate with deep median cleft; segment I of forceps ca. $3.5\times$ combined length of segments II–III; penial tubes tapered, turning and extending laterally and ventrally (Figs 4E–G).

Male subimago: Body length 6.5 mm; forewing 6.6 mm. General coloration slightly paler than male imago. Wings semi-hyaline, marginal setae clear. Other characters resemble male imago (Fig. 4B).

Female imago: Darker than male, brown to deep brown; each tergite with median brown to dark longitudinal stripe narrowed posteriorly and pair of anteromedian pale dots in this dark stripe; midline of abdomen usually pale (Figs 6A, C). Compound eyes located posterolaterally on head, distance between them about $3.0\times$ eye diameter (Fig. 6D). Venation of wings similar to male but crossveins more distinct (Figs 5C–D). Sternites VII–VIII with straight posterior margins; sternite IX with wide V-shaped notch (Figs 6E–F).

Female subimago: Body length 6.8 mm; forewing 7.0 mm. Body much paler than female imago. Wings semi-hyaline, with distinct pigments on all crossveins. Other characters resemble female imago (Fig. 6B).

Egg: Oval, covered with numerous small tubercles, subequal in size; each tubercle with several small protuberances (Figs 7D–F).

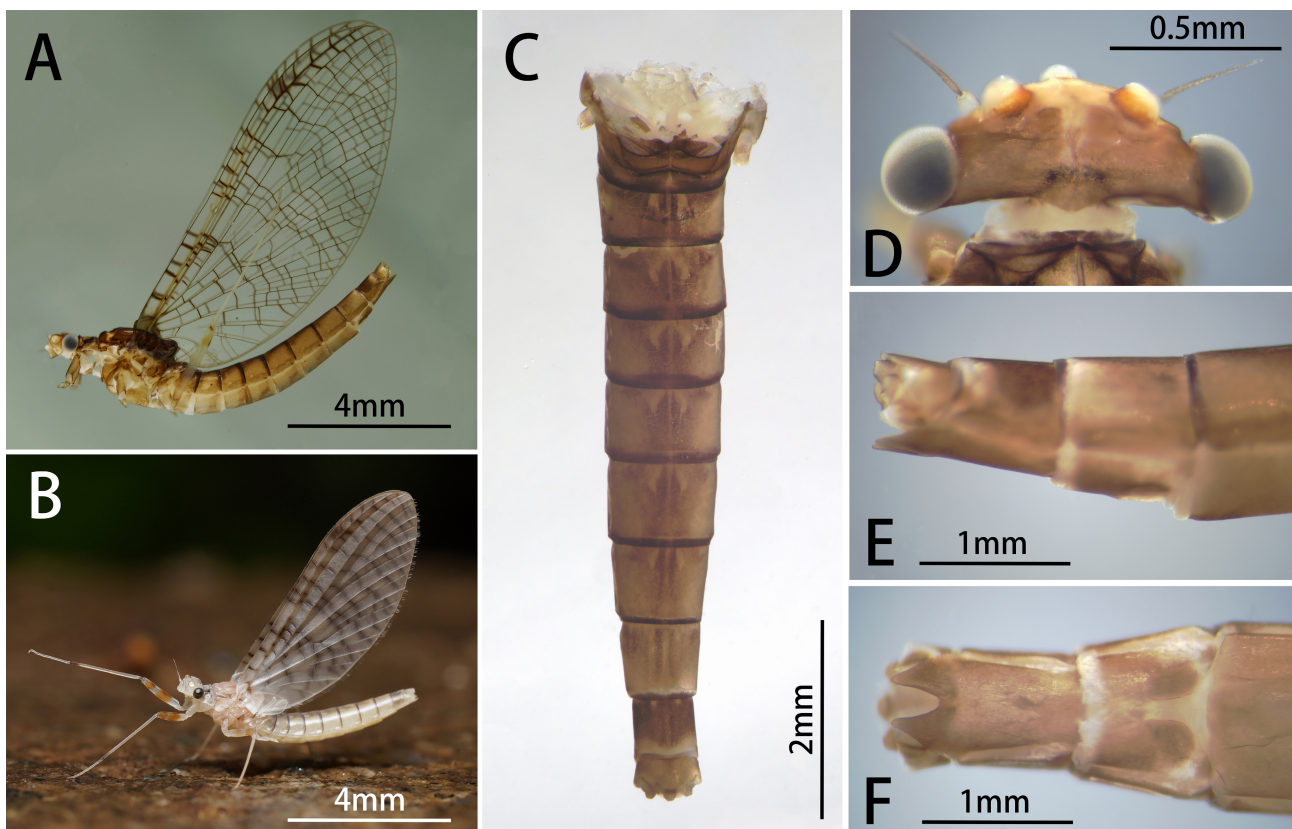


FIGURE 6. Female imaginal structures of *Gilliesia pulchra* (digital photos): A. Imago; B. Subimago; C. Abdomen (dorsal view); D. Head (dorsal view); E. Terminal abdomen (lateral view); F. Terminal abdomen (ventral view).

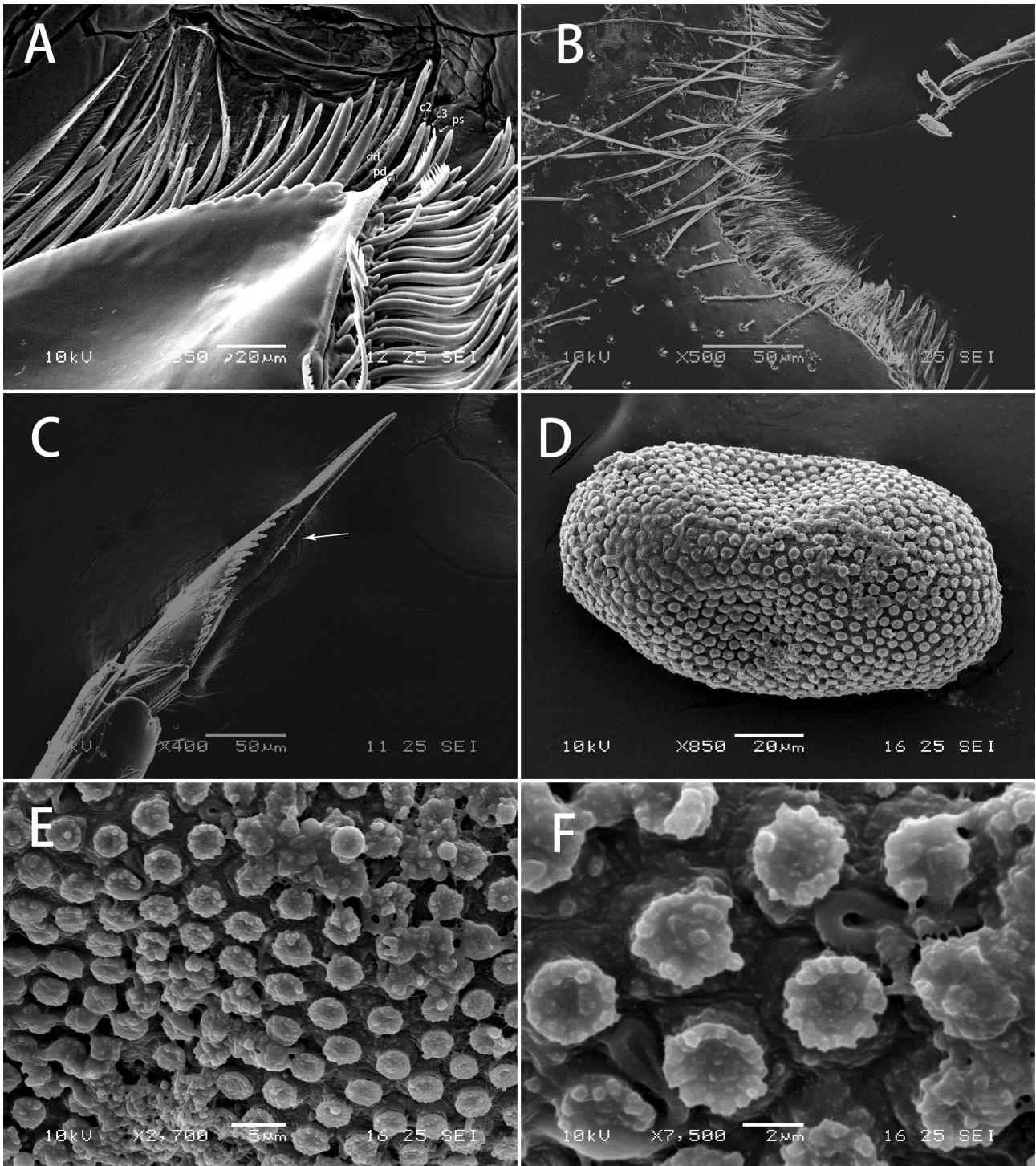


FIGURE 7. SEM photos: A. Details of maxilla; B. Anterior emargination of labium (dorsal view); C. Nymphal foreleg claw (arrow indicates the second row of spines); D. Egg; F. Egg surface enlarged; G. Tubercles enlarged.

Diagnoses

In the genus, the nymph of *Gilliesia pulchra* can be easily distinguished from *G. ratchaburiensis* by the following characters: 1) the presence of hair-like setae on the lateral margin of both left and right mandibles (Figs 2D, 2F), this character does not present in the nymphs of *G. ratchaburiensis*; 2) setae covering whole maxillary crown while outer portion of *G. ratchaburiensis* crown without setae (Fig. 2K); 3) the claws slightly slimmer than counterparts of the latter species and with more and an additional row of denticles (15–18) (Fig. 7C); 4) main tracheae of gills

with clear branches (Figs 1H–J); 5) the gills with marginal fine setae (Fig. 1F), while *G. ratchaburiensis* with neither branch nor seta on its gills; 6) the coloration of abdominal tergites: the species *G. ratchaburiensis* nearly uniform brown to dark, with small anteromedian pale spots only, while *G. pulchra* has larger pale portions (Figs 1A–B); 7) posterolateral expansions on abdominal segment 8–9 (Fig. 1E). In contrast, the nymph of *G. ratchaburiensis* with posterolateral projections on tergite IX only. 8) the eggs with more and denser tubercles, each tubercle with more distinct protuberances than *G. ratchaburiensis* (Figs 7F–G).

In male adults, the species *G. pulchra* has a pointed apex of penis (Figs 4E–F), which is different from the other two congeners. The species *G. ratchaburiensis* has sharp lateral lobes of the penis, while *G. hindustanica* has an expended apex of the same structure.

All three above species have colorful forewings and shortened hindwings in adults. Their bodies are usually a beautiful yellowish to amber color. The nymphs of *G. pulchra* and *G. ratchaburiensis* both have elongated maxillary palpi and single forked gills. The hypopharynxes are similar to each other in shape (superlinguae do not expand laterally, lingua with two setal tufts).

Discussion

Among the three species in the genus *Gilliesia*, *G. pulchra* and *G. hindustanica* are more similar to each other in both genitalia and wings: their penes bent laterally and ventrally, MA and MP forked sub-equally from wingbase to margin, base of MP_2 detached from MP_1 , hindwing with almost straight costal margin and three longitudinal veins behind MA. However, the third species, *G. ratchaburiensis*, is different from them in all those characters: each penis is turned laterally only, MP forked slightly more basally than MA, base of MP_2 attached to MP_1 with a crossvein in forewing, hindwing with a greatly concave leading margin, only two longitudinal veins behind MA. So, based upon characteristics of its forewings and genitalia, this species seems more similar to the genus *Dipterophlebiodes* than its two congeners. Likely, the nymphs of the species *G. ratchaburiensis* is also alike to the genus *Dipterophlebiodes* in similar gills (without tracheae branch) and mandibles (without lateral hair-like setae). Moreover, the nymphs of *G. ratchaburiensis* have extended posterolateral projections of tergite IX, which is similar to *Dipterophlebiodes*, rather than *G. pulchra*, the latter of which has expanded posterolateral processes on tergites VIII–IX. In short, except that the adults of the species *G. ratchaburiensis* has hindwings, it can be seen as a member of *Dipterophlebiodes* or a new genus, based on its nymphs with setal tufts on lingua of hypopharynx and with submarginal setae on labrum.

Peters and Edmunds (1970) grouped the three genera *Dipterophlebiodes*, *Gilliesia* and *Habrophlebiodes* into a common clade, regarding the former two genera as closer to one another. On one hand, the newly found nymphs of *G. pulchra* do have same key characters they applied to this issue, like the submarginal setae row on the labrum, which supports grouping those three genera together. Imaginal characters (such as only two intercalaries between CuA and CuP) also support this grouping. On the other hand, the nymphs of *G. pulchra* clearly show that both known nymphs (*G. ratchaburiensis* and *G. pulchra*) have similar setal tufts on the hypopharynx, canines of maxillae, and labrum, which are also found in *Habrophlebiodes* (Li, 2013) but not in *Dipterophlebiodes* (Peters, 1972). So, the sister-group relationship between *Dipterophlebiodes* and *Gilliesia* needs more research or new data, such as from the males of *Dipterophlebiodes*, to test it. Related to this observation, Kluge (1994) regarded some synapomorphies indicated by Peters & Edmunds (1970) as plesiomorphies, which implied that higher level study of the family Leptophlebiidae is necessary to solve this case. Based on the nymphs of *G. pulchra* described in the present paper, we assume the genus *Gilliesia* is closer to *Habrophlebiodes* than *Dipterophlebiodes* because they share similar mouthparts and abdominal tergites VIII–IX.

Both nymphs and imagoes of *G. pulchra* were collected in or near small streams (width less than 1.0 m), just as reported by Boonsoong & Sartori (2015) for the species *G. ratchaburiensis*. Interestingly, both species were caught near bamboo forest. Their yellowish body may be a camouflage, similar to the yellow leaves of bamboo, both in water and on land.

Acknowledgments

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