



A new species of *Polyplocia* Lestage from Malaysia with comments on the genus (Ephemeroptera, Euthyplociidae, Euthyplociinae)

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Abstract

Polyplocia nebulosa sp. nov. is described based on male and female imagos from Malaysia, Sabah State. Nymphs provisionally assigned to this species are also described. This species can be readily separated from *P. vitalisi* by the color pattern of the wings, with light brown longitudinal veins, narrow dark brown clouds on cross veins and margins of wings tinged with brown. The male genitalia are distinct from those of other species of *Polyplocia*: penes are broad, T-shaped, with large lateral projecting lobes, which are apically rounded, and with a small dorsolateral spine on each lobe; the styliger plate is short and not projected posteriorly. Eggs of the genus are described for the first time using scanning electron microscopy. Eggs are 265–267 μm in length, 170–186 μm in width, barrel-shaped, without polar caps or other attachment structures, with one visible micropyle and with chorion forming an irregular mesh with raised ridges (mesh between 3.8–8 μm). A key to male adults of *Polyplocia* is provided and additional records of *Polyplocia* from Thailand are given.

Key words: Borneo; egg scanning microscopy; identification key; Oriental Region; *Polyplocia nebulosa* sp. nov.; Sabah

Introduction

Euthyplociidae is a small mayfly family once regarded to belong to Polymitarcyidae (Lestage 1921, Demoulin 1952, Edmunds & Traver 1954, Demoulin 1958, McCafferty 1991, 2004). It is divided into two subfamilies, the Exeuthyplociinae and Euthyplociinae. The former is composed by only two genera and species and has a distribution restricted to continental Africa. The latter is the most diverse, being composed by five genera and 18 species spread throughout Central and South America, Madagascar and Asia. Imagos of the subfamily Euthyplociinae possess R_1 of hindwing rising from base of the wing and MP bifid. Nymphs are of the sprawling type, with foretibia cylindrical and forefemora at most slightly flattened and abdominal gills project laterally from the body (Gillies 1980). The Euthyplociinae are composed by *Campylocia* Needham & Murphy 1924, *Euthyplocia* Eaton 1871, *Mesoplocia* Demoulin 1952, *Polyplocia* Lestage 1921 and *Probosciodoplocia* Demoulin 1966b.

Nymphs of the Oriental genus *Polyplocia* can be identified by its long tusks with truncated apex, antennae almost the same length as tusks, foretibiae with short apical projection, foretarsi with apical projection and gills I with two lamellae. Imagos have a transverse pronotum, forewing with MA fork at same level as R_s fork, and at least one intercalary vein in the cubital field with its base connected to CuP, forceps with only one segment and three caudal filaments on abdomen (Demoulin 1952, 1966a). *Polyplocia* was established to house the species *P. vitalisi* Lestage 1921 based on one male imago from Tonkin (Lestage 1921). In 1939, Ulmer described two new species: *P. campylociella* Ulmer 1939, based on a female subimago and *P. crassinervis* Ulmer 1939, based on four male subimagos, both from North Borneo (now Malaysia, Sabah State). Demoulin (1952) reviewed the family providing new figures of *P. crassinervis* from male imagos collected in Sarawak. Later Demoulin (1953) described

the female imago, also from Sarawak, and synonymized the species based on wing venation: *P. campylociella* (= *P. crassinervis*). In spite of the synonymy, he later described a possible nymph under the name *P. ?crassinervis* from West Borneo (now Indonesia) (Demoulin 1966). The female subimago type of *P. campylociella* is in Museum für Naturkunde, Berlin, but not accessible. The male syntypes of *P. crassinervis* cannot be located and may be lost. Therefore, our concept of *P. campylociella* and its synonym depend on the material from Sarawak studied by Demoulin.

Nguyen & Bae (2003) described a new species *P. orientalis* Nguyen & Bae 2003 based on nymphs from Dak Lak, Vietnam, so at present the genus *Polyplocia* is composed of three valid species: *P. vitalisi*, *P. campylociella* and *P. orientalis*. In this paper a fourth species is described based on male and female imagos from Malaysia, Sabah State, which is distinct from the material described by Demoulin. This new species may actually belong to one of the species of Ulmer (1939) where type material is unknown. However, it seems preferable to give a clear description of one species without adding to further confusion of synonyms in Euthyplociidae (Gonçalves, in prep.). Based on study of other members of the family, structure of the chorion is useful for recognizing species and a description of the egg is included as a foundation for future study of the genus. A key to male imagos is provided. Additional *Polyplocia* material from Thailand was also studied, expanding the distribution records of the genus throughout Vietnam, Thailand and Malaysia (Lestage 1921; Lestage 1924; Ulmer 1939; Demoulin 1966a; Nguyen & Bae 2003; Kluge 2004).

Material and methods

Material was preserved in 80% ethanol, observed and photographed under a stereomicroscope with a digital camera coupled to the microscope. Photographic material was edited on Adobe Photoshop CS6, and drawings and plates were made on Adobe Illustrator CS6 from photos. Material is deposited at Florida A&M University (FAMU), Tallahassee, FL, U.S.A., and at Purdue University Entomological Research Collection (PERC), West Lafayette, Indiana, U.S.A. Scanning electron microscopy was performed at Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL, U.S.A. Eggs were not subjected to critical point drying prior to scanning but were air dried from 100% ethanol; terminology follows Koss (1974). Digital photos of the holotype of *Polyplocia vitalisi* and of specimens of *P. campylociella* identified by Demoulin were used for comparison. The aforementioned material is deposited at the Institut Royal des Sciences Naturelles de Belgique (IRSNB), Bruxelles, Belgium. A specimen of *P. orientalis* was also made available for this study and is housed at Coleção Entomológica Prof. José Alfredo Pinheiro Dutra (DZRJ), Rio de Janeiro, Brazil. The types of *P. crassinervis* could not be located.

Results

Polyplocia nebulosa sp. nov. Gonçalves & Peters

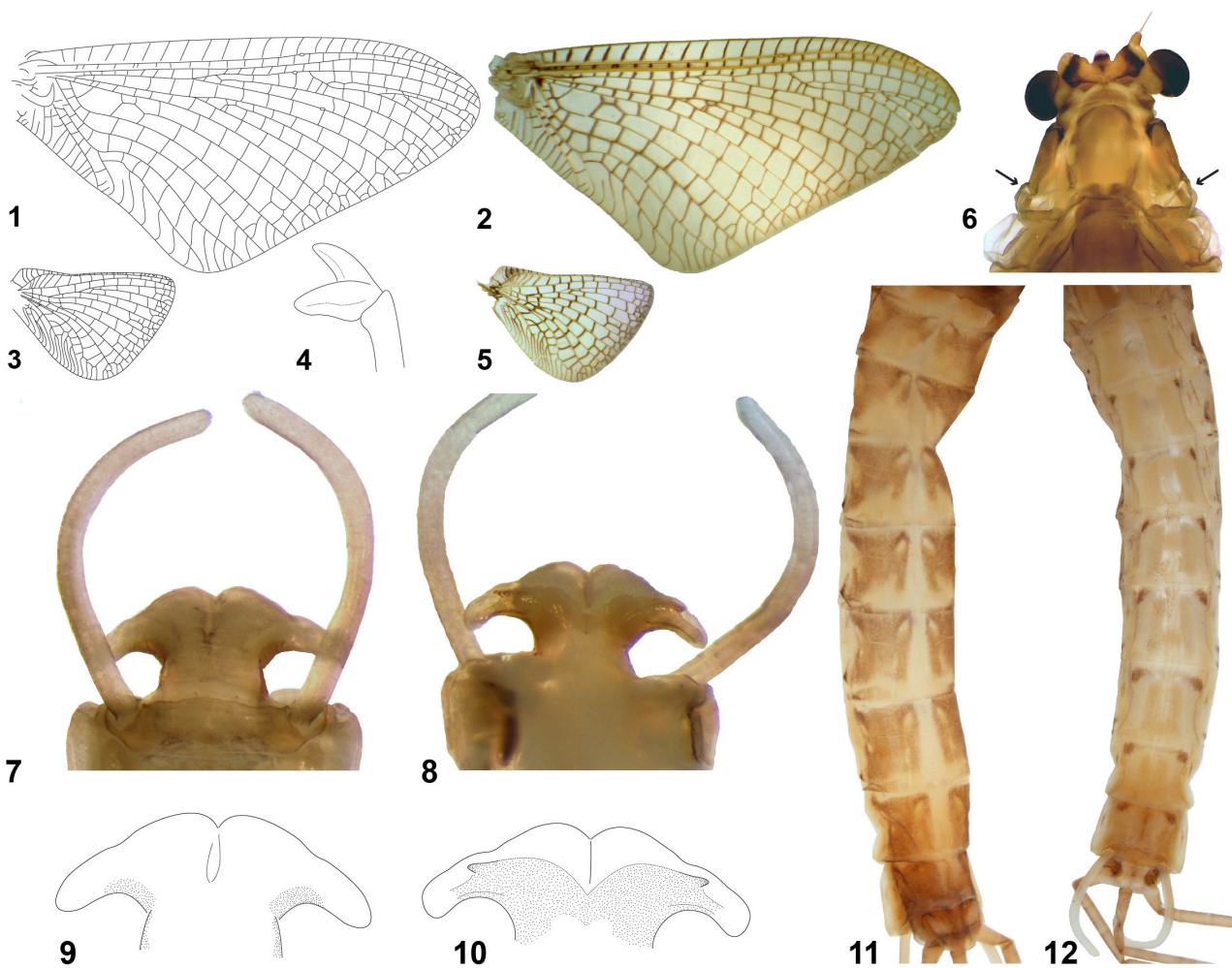
Diagnosis. 1) Wings with longitudinal veins light brown and cross veins brown, cross veins with narrow dark brown clouds and margins tinged with brown (Figs. 2 and 5); 2) Sterna II-IX with a pair of blackish brown anterolateral marks (Figs. 12); 3) Penes broad, T-shaped, fused, with medial groove extending from apex to half length of penes; large laterally projecting lobes apically rounded with a small dorsolateral spine; basal outer margin of each lobe sclerotized (Figs. 7–10); 4) Styliger plate short and straight, not projected posteriorly; 5) Eggs 265–267 μm in length and 170–186 μm in width, barrel shaped, without polar caps or other attachment structures, with one visible micropyle and chorion forming an irregular mesh with raised ridges, mesh size from 3.8–8 μm (Figs. 13–14).

Male Imago—Length (mm): Body = 13–17; Forewings = 13–16; Hind wings = 6–8.

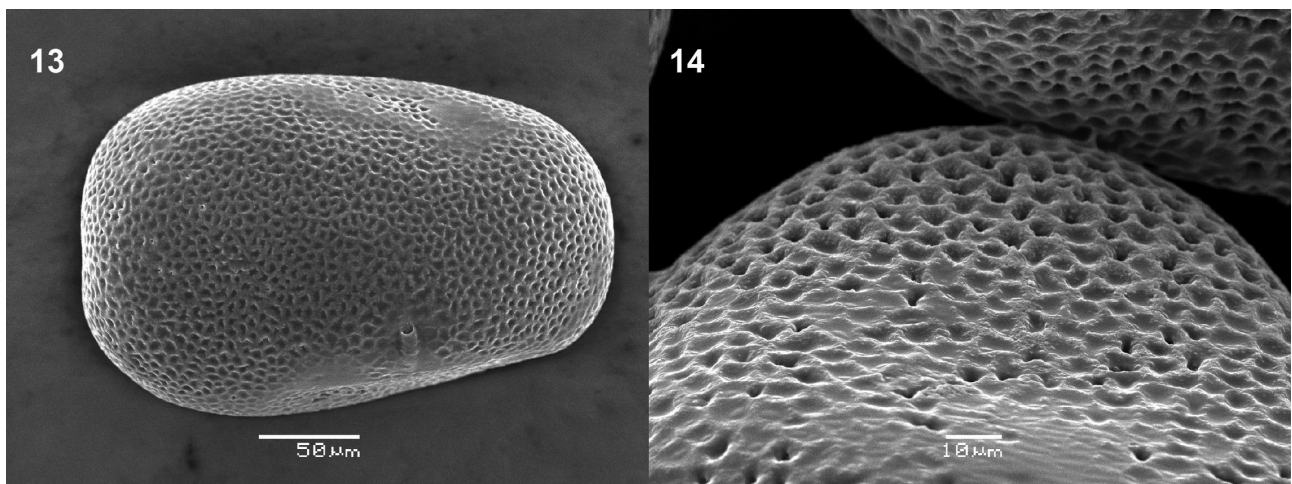
Head: Yellowish shaded with black between lateral ocelli; eyes black; ocelli yellowish white surrounded by black ring. Antennae covered by brown shade.

Thorax: Pronotum yellowish, translucent, shaded with black; yellowish brown laterally; about as long as wide, trapezoidal in shape with a membranous elevated ridge on posterior margin (Fig. 6). Meso- and metanota yellowish

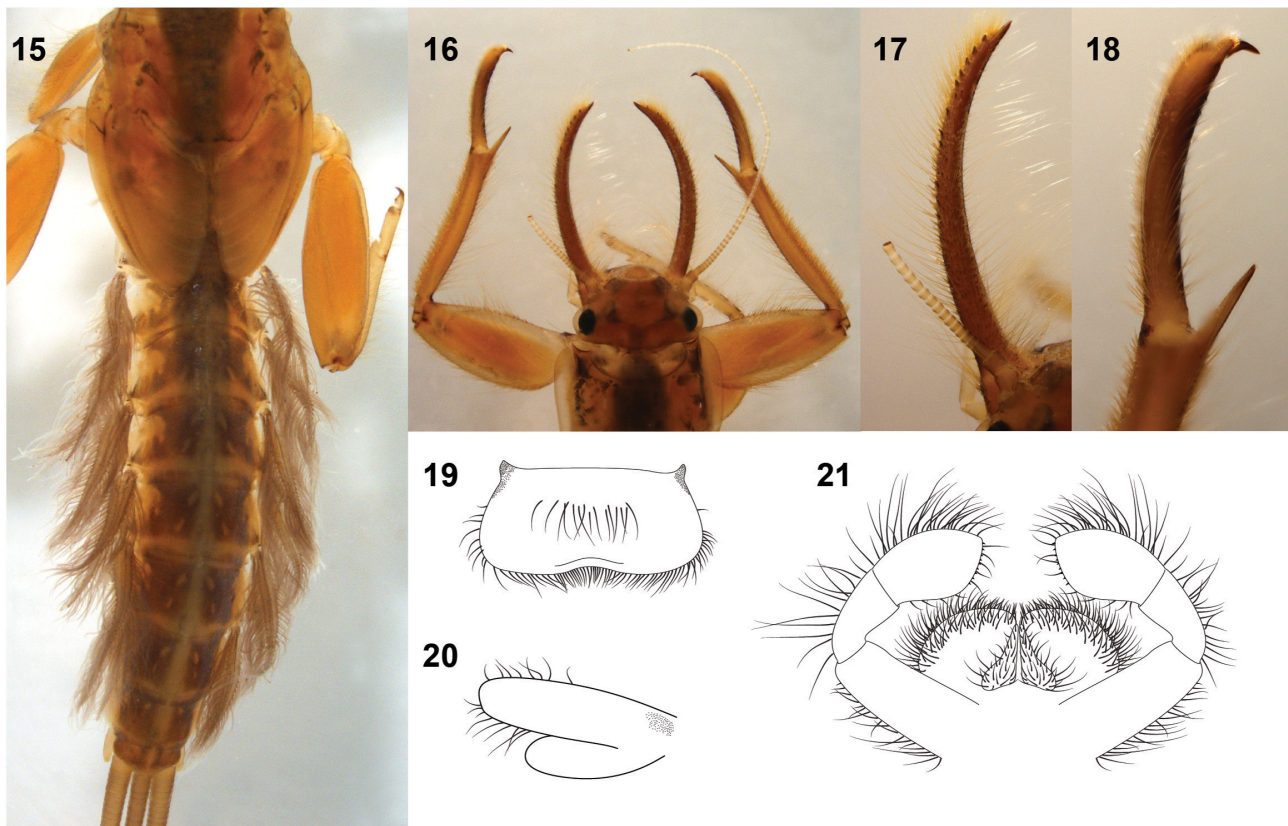
brown, parascutellum yellowish; posterior margin of metanotum shaded with black. Prosternum with median crest divided into two oblique ridges forming a wide triangle with the posterior margin.



FIGURES 1–12. Male of *Polyplocia nebulosa* sp. nov.: 1 and 2—Forewings; 3 and 5—Hind wings; 4—Tarsal claws of foreleg; 6—View of head and pronotum, arrows show elevated ridge; 7—Genitalia, ventral view; 8—Genitalia, dorsal view; 9—Detail of penes, ventral view; 10—Detail of penes, dorsal view; 11—Abdominal color pattern, dorsal; 12—Abdominal color pattern, ventral.



FIGURES 13–14. Eggs of subimago of *Polyplocia nebulosa* sp. nov.: 13—Overall appearance; 14—Detail of chorionic ultrasculpture.



FIGURES 15–21. Possible nymph of *Polyplocia nebulosa* sp. nov.: 15—Abdomen; 16—Head and prothorax; 17—Detail of tusks and distribution of spine-like setae; 18—Detail of foretibia; 19—Labrum, dorsal view; 20—Gill I; 21—Labium, ventral view.

Wings: Membrane translucent, longitudinal veins light brown, cross veins darker brown with narrow dark brown clouds; margins of wings tinged brown (Figs. 2 and 5). Vein C of forewings brown with small spines along entire length; fork of MA slightly distal to or on same level as fork of Rs; ICu reaching CuP, second parallel intercalary present (Figs. 1–2). Hind wings with C folding over Sc forming a groove to effect coupling with forewings; vein R1 rising from base of wing; MP forked; anal region with several long veinlets directed toward margin (Figs. 3 and 5).

Legs: Yellowish. Coxae of fore and median legs shaded with black, hind coxae sometimes with light black shading; apex of femora tinged with black, darker on forefemora. Forelegs with basal half of femora possessing spines on outer margin; apex of tibiae with black mark, tibiae covered with short spines. Tarsal claws similar, both somewhat pointed (Fig. 4).

Abdomen: Yellowish. Terga shaded with brown except for medial longitudinal line extending from terga I–X; a pair of pale, apical, submedial drop-shaped spots on terga II–IX, and paler more distal sublateral marks on terga II–X, pale marks large on terga II–V (Fig. 11). Sterna II–IX with a pair of anterolateral blackish brown marks (Fig. 12). Caudal filaments yellowish with light black shading, with short setae on terminal filament and shorter lateral setae on cerci. *Genitalia:* Styliger plate yellowish brown, short, straight, not projecting posteriorly (Fig. 7). Forceps yellowish, outer margin rugose with yellowish brown shading; distal portion with scattered short setae, particularly on apex (Figs. 7–9). Penes yellowish, T-shaped, broad, fused, with medial groove extending from apex to half length of penes; each penis lobe rounded apically and strongly curved laterally with a long, lateral projection and a small dorsolateral spine; basal outer margin of each lobe sclerotized (Figs. 7–10).

Variations: In some male imagos deposited at PERC, distomedial marks were not always visible on abdominal terga II–III. In others, these marks were indistinct and blended with the pale color of the posterior margin. Three males had deformed penes, with apical lobes collapsed; on these the genitalia had dried in such a way that the dorsal spines could be seen in ventral view.

Female Subimago—Length (mm): Body = about 18 (abdomen curved); Forewings = 18; Hind wings = 6.

Color pattern similar to male imago. Head with remnants of mandibular tusks. Posterior margin of pronotum not elevated; shading on abdomen darker. Fore and hind legs missing. Subanal plate short, truncate. Caudal filaments dark brown.

Female Imago—Length (mm): Body = about 18 (abdomen curved); Forewings = 17; Hind wings = 7.

Color pattern similar to male imago. Head without remnants of nymphal tusks. Posterior margin of pronotum not elevated. Wings as in male imago, with more long intercalaries on forewings: a pair between R_3/R_{4+5} , one between MA_1/IMA , a pair between MA_2/MP_1 as well as shorter intercalary between IMP/MP_2 . Forelegs missing. Brown shading on abdomen darker. Subanal plate short, truncate. Caudal filaments missing.

Variations: Presence of additional intercalary veins varied on the left and right wing on the single female described, evidence for intraspecific variation in this species. This may be true for the genus as a whole.

Eggs (described from female subimago)—Measurements (μm): Length = 265–267; Width = 170–186; Mesh = 3.8–8. Barrel-shaped, without polar caps or other attachment structures, one visible micropyle, chorion forming an irregular mesh with raised ridges (Figs. 13–14).

Possible Nymph—Length (mm): Female—Body = 19; Male—Body = 14. Nymphs are tentatively associated based on the color on abdominal sterna and shape of nymphal male genitalia.

Head: Brown with light black shading along lateral branches of epicranial suture, posterior to median ocellus and anterior to lateral ocelli, area between lateral ocelli slightly lighter. Clypeus projected anteriorly. Eyes black, ocelli white with inner margin black. Antennae with scape, pedicel and base of flagellum brown, flagellum yellow distally; antennae longer than mandibular tusks (Fig. 16).

Mouthparts: Labrum (Fig. 19): brown; lateral margins divergent, anterior margin with median shallow emargination; labrum with longitudinal sub-basal row of long setae, a median subapical row of short dense setae, and bordered with simple setae, setae dense on anterior margin. Mandibles (Figs. 16–17): yellowish brown, tusks brown and incisors and molae dark brown; tusks long, sickle shaped, apex truncate, length more than 2x that of head; several long slender setae on margins, rows of setae forming a filtering network on inner margin; dorsal surface covered with small spine-like setae; larger spine-like setae on distal half (Fig. 17); left mandible: 4 denticles on outer and 3 on inner incisors; prosthema short, truncate, broader apically; right mandible: 4 denticles on outer and 2 on inner incisors; prosthema absent. Maxillae: palpi with 3rd segment about 2.6x longer than 2nd segment, 3rd segment long, at least 6x longer than wide; palpi with many long setae, particularly on 2nd and 3rd segments. Labium (Fig. 21): glossae and paraglossae densely covered with setae ventrally; glossae drop shaped, ventral; paraglossae meet above glossae; palpi with long simple setae, mostly on outer margins; 3rd segment with several short strong setae dorsally, much broader and about 1.5 x longer than 2nd segment, acutely rounded apically. Hypopharynx: lingua cordiform with deep medial emargination distally; distal margin with short simple setae; superlingua with dense long setae on distal and inner lateral areas.

Thorax: Brown with black markings on pro- and mesonota. Pronotum with anterolateral corners projected beyond posterior border of eyes, not reaching half its length; lateral flanges pale, translucent and forming an acute apical projection subequal in size to anterolateral projections (Fig. 16). Metanotum yellowish brown.

Legs: Yellowish brown; tarsal claws without denticles. Fore and middle legs with a row of spine-like setae on inner and outer margins of femora; distoventral surface of hind femora with area covered by short setae. Foreleg (Fig. 16): tibia with apical projection short, less than half length of tarsus; margins of projection bordered with strong setae; tarsi with apical projection shorter than claw; apex of tarsi and its projection densely covered by short setae. Middle leg: apex of tibia with short projection; area around projection and inner margin of tarsi with dense concentration of short plumose setae; patello-tibial suture present.

Abdomen (Fig. 15): Yellow, heavily shaded with black, except for a medial longitudinal line, on terga I–X and paler submedial markings as in male. Sterna yellowish, with a pair of anterolateral black marks, one on each corner. Gills purplish gray, gills I bifurcated, vestigial, dorsal portion longer and wider than ventral portion (Fig. 20); gills II–VII long, longer than 1.5x length of subjacent abdominal segment, narrow, lanceolate, fringed; dorsal and ventral portions of same length. Cerci and median filament whitish yellow, shaded with black on base, paler toward apex; setae at annulations.

Etymology. *nebulosa* (latin) meaning “a cloud“ or “mist“ in allusion to the brown tinge on margins of wings.

Type material. Holotype: Malaysia, Sabah, Sungai Moyog, tributary 8 miles East of Penampang, 01.X.1978, G.F. Edmunds col., 1 male imago (FAMU)[approximate coordinates 5°55'N/116°09'E]; Paratypes: same data, 2 male imagos (FAMU) 11 male imagos (PERC); Malaysia, Sabah, Sungai Moyog, 3 miles East of Penampang,

29.IX.1978, G.F. Edmunds col., 1 female subimago (FAMU) [approximate coordinates 5°55'N/116°07'E]; same data, 1 female imago (PERC).

Additional material. *Polyplocia nebulosa* **sp. nov.** possible nymph: Malaysia, Sabah, Borneo stream 1 km S. of Poring Hot Springs, CL2023, 02.VIII.1985, J.T. & D.A. Polhemus col., 7 nymphs (PERC) [approximate coordinates 5°54'N/116°09'E]; Malaysia, Sabah, Borneo, Samalang River, 7 km S. of Ranau, CL2026, 03.VII.1985, J.T. & D.A. Polhemus col., 3 nymphs (PERC) [approximate coordinates 6°02'N/116°42'E]. *Polyplocia vitalisi* type: Tonkin, 08.IV.1920, Vitalis de Salvaza, 1 male imago (IRSNB). *Polyplocia campylociella*: Sarawak, 2 males, Lestage Collection (IRSNB); Sarawak, Mt. Penrissen, 3,000 ft, Dr. E. Mjöberg, 1 female, Lestage Collection (IRSNB). *Polyplocia orientalis*: Vietnam, Lam Dong Prov., Bao Loc, Da Mre stream, 450 (alt.), 22.III.2002, V.V. Nguyen & D.H. Hoang col., 1 nymph (DZRJ). *Polyplocia* spp.: Thailand, Phrae Province, Wieng Ko Sai National Park, Namtok Mae Koeng Luang, 350 m, 17°58'N / 99°35'E, L-295, 14.III.2002, R.W. Sites, Vitheepradit, Kirawanich, G. W. Courtney col., 3 female imagos, 1 female nymph (FAMU); Thailand, Phayao Province, Doi Luang National Park, Nam Tok Cham Pa Thong, 620 m, 19°13'N / 99°4'E, 17.III.2002, R.W. Sites, Vitheepradit, Kirawanich, G.W. Courtney col., 10 nymphs, both sexes (FAMU); Thailand, Nan Province, Doi Phu Ka National Park, Nam Tok Sila Phet, 400 m 19°05'N / 100°56'E, L-292, 13.III.2002, R.W. Sites, Vitheepradit, Kirawanich, G.W. Courtney col., 1 young nymph (FAMU); Thailand, Chiang Mai Province, Doi Suthep-Pui National Park, Huai Kaew above Monthathan, 800 m, 18°49'N / 98°55'E, 15.III.2002, G.W. Courtney col., 2 young nymphs (FAMU); Thailand, Chiang Rai Province, Doi Luang National Park, Namtok Pu Kaeng, 540 m, 19°26'N / 99°42'E, 17.III.2002, G.W. Courtney col., 5 nymphs, both sexes (FAMU);

Discussion

Polyplocia nebulosa **sp. nov.**, like *P. vitalisi*, does not possess a strongly projecting styliiger plate. Unlike *P. vitalisi*, the new species has fewer cross veins between C and Sc and the costal strip is translucent and not purplish (Figs. 1–3 and 5). Specimens are also larger in size: the male of *P. vitalisi* is 12 mm in length (Lestage 1921; Lestage 1924) and males of *P. nebulosa* **sp. nov.** range from 13–17 mm. The color and venation of *P. campylociella* are very similar to that of *P. nebulosa* **sp. nov.** though *P. campylociella* specimens possess more cross veins. The ventral anterolateral marks on the abdomen were described by Ulmer (1939) and Demoulin (1953) for *P. campylociella*, but are no longer visible on their specimens. The new species does not have a projecting styliiger plate as does *P. campylociella*. The size are also different, males of *P. campylociella* are 12–13 mm in length and females are 17–20 mm (Ulmer 1939; Demoulin 1952; Demoulin 1953).

The male genitalia of *P. nebulosa* **sp. nov.** differs markedly from the aforesaid species. In *P. vitalisi* and *P. campylociella* the penes are narrow, divided and V-shaped (Ulmer 1939; Demoulin 1952) but males of *P. nebulosa* **sp. nov.** have penes broad, fused, T-shaped and with large lateral projecting lobes (Figs. 7–10). There is also a small dorsal spine laterally directed on each penis lobe (Figs. 8 and 10); the apex of the forceps is rounded in the new species and pointed in *P. vitalisi* and *P. campylociella*. However, this could be due to desiccation of the pinned specimens, whereas material of *P. nebulosa* **sp. nov.** is preserved in alcohol. Whether the V-shaped penes of *P. vitalisi* and *P. campylociella* are also due to desiccation of the material is unknown. In *Campylocia* specimens, dried specimens often show alterations on penes morphology but never to such an extent. Additionally, the new species has spines extending the length of the costa of the forewings and on the base of the forefemora, which have not been described in any other species of *Polyplocia* and are not visible on available photographic material of *P. vitalisi* and *P. campylociella*. It is unclear if they are lacking on those species or not. The presence of small spines on forewings along the total length of C was also seen in female imagos from Thailand, although not so apparent.

Polyplocia orientalis, the third described species of the genus, is only known from nymphs. In addition to the possible nymphs of *P. nebulosa* **sp. nov.** described here, 19 unidentified nymphs of *Polyplocia* sp. from Thailand were examined and the genitalia of three male nymphs were studied. Unlike the possible nymphs of *P. nebulosa* **sp. nov.**, the specimens of *P. orientalis* and *Polyplocia* sp. did not possess paired black anterolateral marks on abdominal sterna. The genitalia of male nymphs of *Polyplocia* sp. from Thailand also had T-shaped penes as do nymphs of *P. nebulosa* **sp. nov.** These nymphs (18–20 mm in length) may be related to *P. orientalis* or belong to another species. In his book, Kluge (2004) also reports unidentified specimens of the genus *Polyplocia* from Thailand. There are not enough details in the description by Demoulin (1966a) to compare the nymph he studied

with the above specimens. Morphology of mouthparts (particularly the labium), apical spines and anterolateral projections of pronotum and projection of foretibia are consistent and may be good characters to identify *Polyplocia* nymphs.

Females from a different locality in Thailand were also available for this study: the specimens ranged from 19–22 mm in length, forelegs were missing and there were no eggs. These females showed darker brown veins, lacked clouds around crossveins and the wing membrane was entirely translucent. Furthermore, abdominal color was purplish and the terminal filaments were whitish-purplish at base. Because of the sexual dimorphism seen in imagoes of *Polyplocia* (Demoulin 1953), species identification of these females is difficult. The wing color resembles that of *P. vitalisi*—although lacking purplish color on C and Sc fields of forewing—but they could also be related to the unknown females of *P. orientalis* or to the *Polyplocia* sp. nymphs found at other localities in Thailand. A noteworthy feature of these females is that specimens have three long intercalary veins in the cubital field and not two as seen in other species of *Polyplocia* including *P. nebulosa* **sp. nov.** One of the females had one wing with four long intercalaries, suggesting the number of intercalaries may be as variable in *Polyplocia* as it is in *Campylocia* (Gonçalves, in prep.).

Key to male specimens of *Polyplocia*

1. Veins without dark clouds around cross veins and wing margins translucent; membrane of forewing colored only on C and Sc fields, purplish; abdominal sterna without markings *Polyplocia vitalisi*
- Veins with dark brown clouds around cross veins and margins of wings with brownish tinge; membrane of forewing without purplish color on C and Sc fields (Sc field may be a little darker); abdominal sterna with a pair of blackish marks on anterolateral margins 2
2. Styliger plate rounded and projecting posteriorly; penes narrow, divided, V-shaped, fused only near base; penes apparently without dorsal spine *P. campylociella*
- Styliger plate short and straight, not projected; penes broad, T-shaped, fused except at apex; each lobe of penes with small dorsal spine laterally directed *P. nebulosa* **sp. nov.**

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