



First record of hygropetric species in the genus *Paegniodes* Eaton, 1881 (Ephemeroptera: Heptageniidae) with description of a new species from China

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Abstract

Paegniodes oligopilus sp. nov., a first hygropetric species in the genus *Paegniodes* Eaton, 1881 found from madicolous habitat, is described based on larvae from Southwest China. The new species is characterized by the posterior margin of 1st segment bearing a row of 13–15 stout setae; mesonotum and abdominal tergites II–IX having a reddish brown median stripe; swimming setae of cerci and median caudal filament are vestigial. The morphological affinities and feeding habits with other known species are discussed.

Key words: Mayfly, nymph, taxonomy, new species, madicolous, Southwest China

Introduction

The genus *Paegniodes* Eaton, 1881 is a small group of the family Heptageniidae, with only three species recognized in the world, i. e., *Paegniodes cupulatus* (Eaton, 1871) from China (Eaton 1871, 1881; Hsu 1936; Dudgeon 1999, Ma *et al.* 2018), *P. dao* Nguyen & Bae, 2004 from Vietnam (Nguyen & Bae 2004) and *P. sapanensis* Boonsoong, Sartori & Auychinda, 2021 from Thailand (Boonsoong *et al.* 2021). The distribution of the genus *Paegniodes* is currently restricted to tropical and subtropical areas of the Oriental Region. Type species *P. cupulatus* is a very widespread species in China and mainly distribute in the following provinces or regions based on the authors' collection records, e.g., Sichuan, Guizhou, Hubei, Hunan, Jiangxi, Zhejiang, Fujian, Guangxi, Guangdong and Hong Kong. Its larvae are active crawlers but not good at swimming, and they are usually found in moderately rapid to swift montane shaded streams where they occur gravel and sand substrates overlain by cobbles. The larvae of *P. dao* and *P. sapanensis* also occur in the habitats similar to that of *P. cupulatus* (Nguyen & Bae 2004, Boonsoong *et al.* 2021). However, during our surveys on mayflies in Southwest China, an undescribed species of *Paegniodes* is accidentally discovered to cling on vertical rocky outcrop where thin layers of water run over rocky surfaces (Figs 1–3), which is the first record of hygropetric species in the genus *Paegniodes*. In general, most, if not all, heptageniid larvae present rheophilous adaptations, their flattened body with scutiform head and sprawled legs make their lives better adapted to faster water current, thus they are most typically found in lotic habitats (e.g. runs, riffles and rapids) though some heptageniid species can also be found in lentic habitat. However heptageniid species dwelled in hygropetric habitat have received very little attention, because such habitats have so far been largely overlooked. To date, only *Bleptus fasciatus* Eaton, 1885 and *B. michinokuensis* Tojo & Miyairi, 2021 were reported as hygropetric species exclusively and they live on rock surface of seepage zones or habitat that are kept minute flow by splash water of waterfall (Miyairi & Tojo 2007, Tojo *et al.* 2021). *Atopopus tarsalis* Eaton, 1881, though not belonging to hygropetric species, have a peculiar behavior that moved away from the water temporarily, they can actively climb surface of boulder for scraping periphyton and stay out of the water for up 20 minutes, and then return into water before dehydration (Sartori *et al.* 2007). In the present paper, we describe this new *Paegniodes* species inhabited in madicolous habitat.

Materials and methods

The collection site is located at the Huagaoxi National Nature Reserve, Sichuan, China, which is famous for the largest areas of tree ferns (*Alsophila spinulosa*) known as “living fossil” in China and the Danxia landforms. The specimens were collected by picking larvae directly from the seeping rock surface with forceps, and then placed them into vials containing 90% ethanol in the field. Some specimens were dissected under the stereomicroscope and were mounted on slides with Hoyer’s solution for examination under the microscope. Photographs were taken using a Canon EOS 5D Mark IV camera with MP-E 65 mm macro lens and the microscope with a digital camera attached. The holotype (mature male larva) and paratypes are deposited in the Insect Collection, South China Agricultural University (SCAU), Guangzhou, China.

Results

Paegniodes oligopilus sp. nov.

(Figs 1–31, 39)



FIGURES 1–3. Habitat of *Paegniodes oligopilus* sp. nov. in the Huagaoxi National Nature Reserve, Sichuan, China. 1–2: general view of habitat; 3: detail of the microhabitat.

Material examined. Holotype: male mature larva (in ethanol, deposited in SCAU), CHINA, Sichuan Province, Xuyong County, Shuiwei Town, Huagaoxi National Nature Reserve, 20.v.2012, coll. Weifang Shi. **Paratypes** (in ethanol): 10 larvae and 2 mature larvae on mounted slides same data as holotype.

Diagnosis. Larva: Body length 9.0–12.0mm. Coloration yellowish brown with a reddish brown median stripe on mesonotum and abdominal tergites II–IX. Antennae short, about half of head width. Maxillary palpi 2-segmented, with a row of 13–15 stout setae on posterior margin of 1st segment. Posterolateral projections of abdominal segments weakly developed. Gills present on abdominal segments I–VII; dorsal lamellae of gill I much smaller than those on other segments, approximately 1/3 as long as fibrillar portion. Cerci subequal to median caudal filament, swimming setae of cerci and median caudal filament rudimentary.

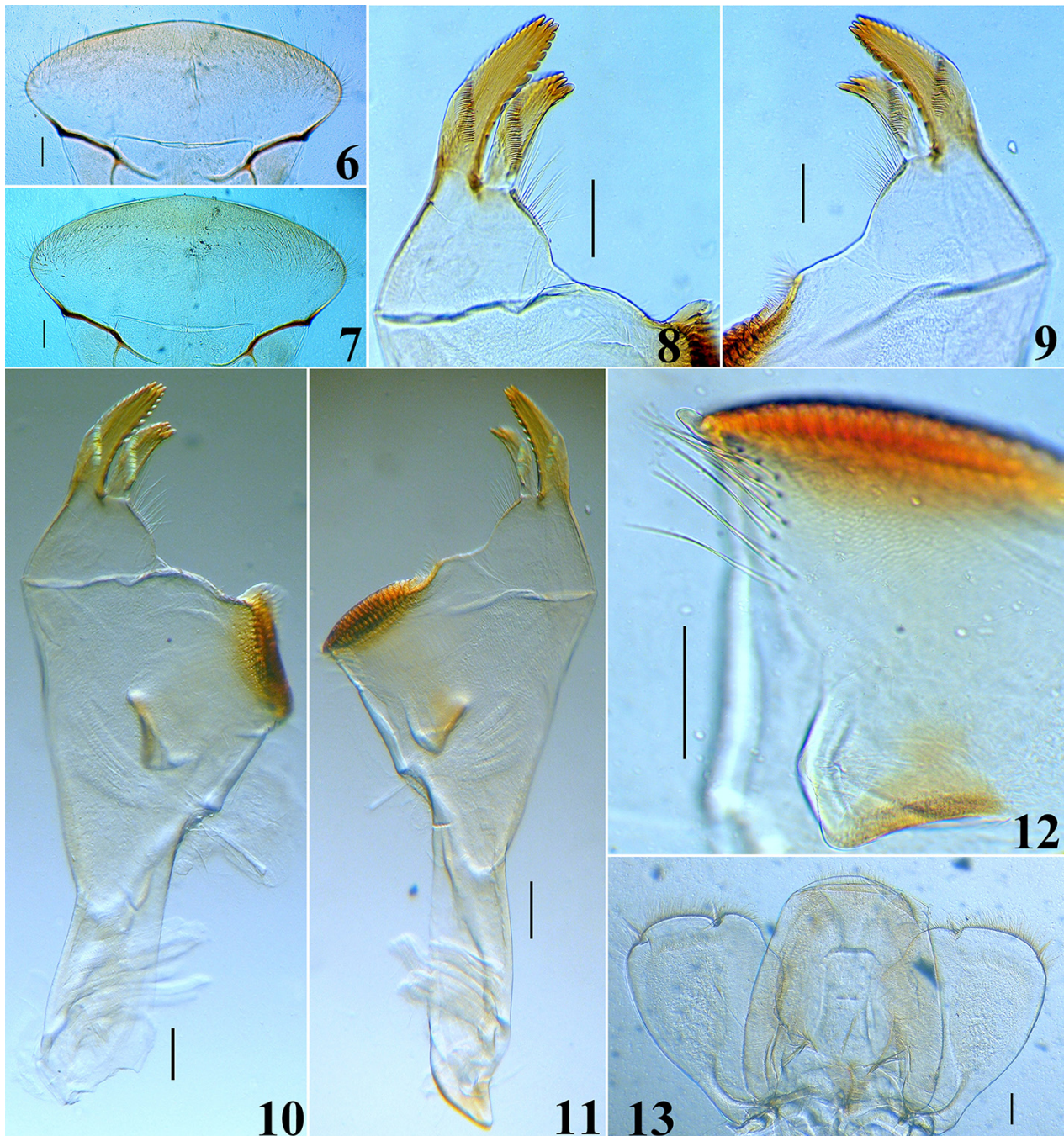
Description. Mature larva (in ethanol) (Figs 4, 5). Body length 10.5 (9.0–12.0) mm; cerci subequal to median caudal filament, approximately 0.7 times of body length. General body coloration (deposited in ethanol) yellowish brown with a reddish brown median stripe on mesonotum and abdominal tergites II–IX.



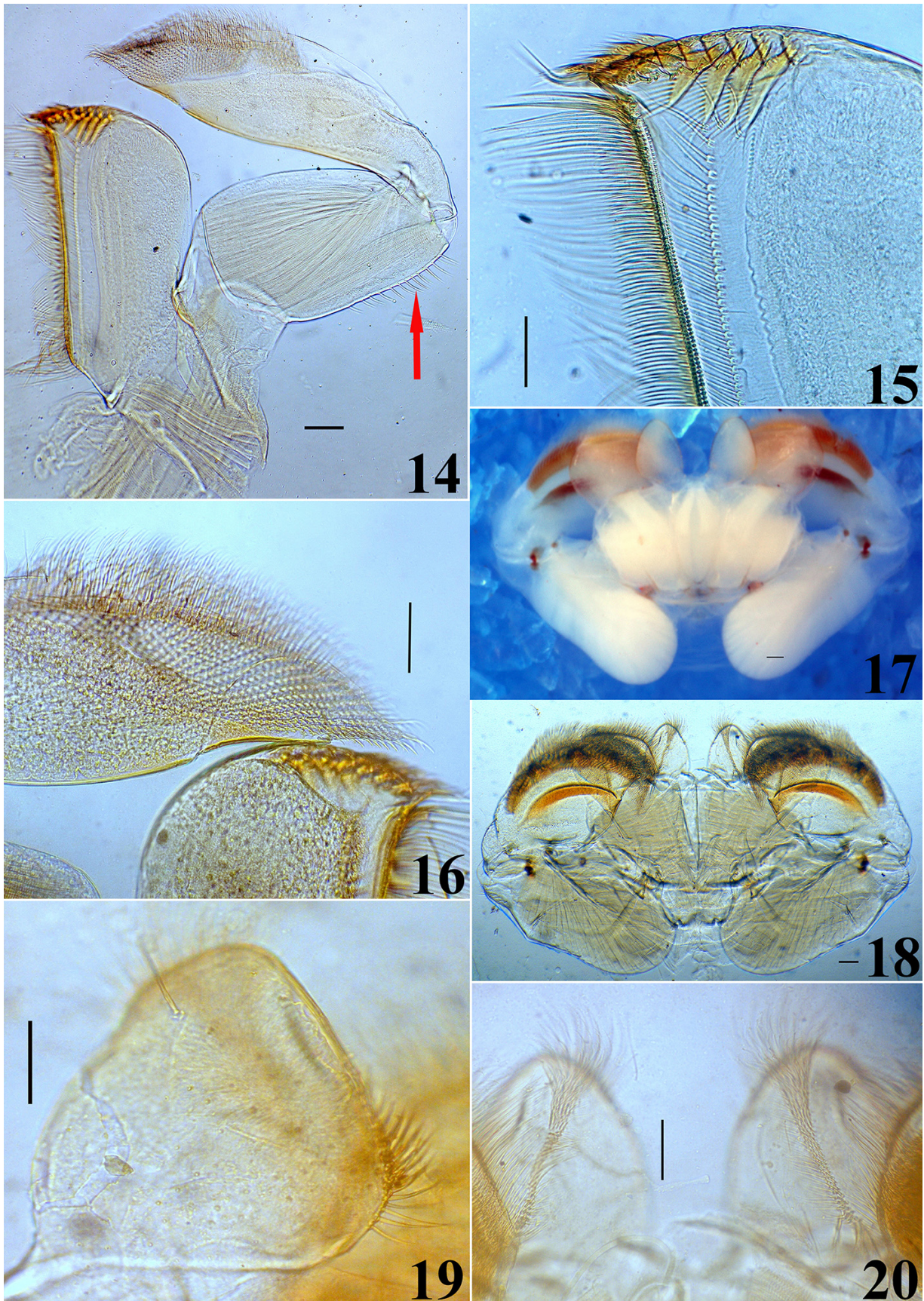
FIGURES 4–5. Larval habitus of *Paegniodes oligopilus* sp. nov. (Scale bars = 1mm). 4: dorsal view; 5: ventral view.

Head. Head capsule yellowish brown, oval-shaped without any setae or hairs on margin, approximately 2.0 mm in length, 2.5–2.8 mm in width. Compound eyes and ocelli black, front ocellus ovoid, lateral ocelli kidney-shape. Antenna yellowish except scape with dark brown ring apically; antennal length approximately 0.5× of head width. Labrum pale brown and small, approximately 1/3 as wide as head; anterior margin with shallow median emargination in normal condition (the emargination faintly on slide-mounted as Figures 6, 7); dorsal surface covered with scattered hair-like fine setae and long, simple setae subanteriorly, ventral surface with tufts of dense and long setae along anterolateral margin. Outer incisor of left mandible (Figs 8, 10) with dentate margins and three teeth at apex; inner incisor with three teeth at apex, with dentate margin outwardly and smooth margin inwardly (Fig. 8); incisors with row of comb-like fine teeth on basal half; below inner incisor with one row of 19–23 bristles decreasing in length (Fig. 8); right mandible (Figs 9, 11) similar to left one in structure, outer incisor (Fig. 9) with dentate margins and three teeth at apex; inner incisor with two teeth apically, outer margin with dentate and inner margin smooth, a bunch of spine-like setae on inner margin of mola and a thumb-like denticle (Fig. 12) at apex of mola; below inner incisor with one row of about 20 bristles. Lingua of hypopharynx (Fig. 13) nearly rectangle, slightly longer than superlingua, covered with abundant setae anteriorly; superlingua inverted pear-shaped, slightly concave

on inner margin near base, with 1–2 emarginations on anterior margin covered with simple, fine setae. Maxilla nearly rectangle (Fig. 14), approximately 2.0× as long as wide, with one row of approximately eight comb-shaped pectinate spines on crown of galea-lacinia, medial ones bearing 8–10 teeth (Fig. 15), apex of maxilla with three slender canines (not forming a trinity of canines) and two dentisetae, inner margin with row of long setae, ventral surface with one straight row of submarginal setae parallel to inner margin; maxillary palpi 2-segmented, but suture between initial 2nd and 3rd segments clearly visible (Fig. 16), 2nd segment lanceolate, approximately 1.5× length of basal segment; anterior margin of basal segment smooth without setae but posterior margin with 13–15 stout setae (Fig. 14). Labium (Figs 17, 18) with moderately separated glossae; glossae pyramidal, ventral face with one long seta subapically and row of short, stout setae posterolaterally (Fig. 19), dorsal face with a longitudinal row of fine dense setae medially (Fig. 20); paraglossae oval, with dense setae dorsally; terminal segment of palpi subequal to basal segment in length, with rows of dense hair-like setae ventrally.



FIGURES 6–13. *Paegniodes oligopilus* sp. nov. (Scale bars = 0.1mm). 6: labrum (dorsal view); 7: labrum (ventral view); 8: enlarged left mandible (partial); 9: enlarged right mandible (partial); 10: left mandible; 11: right mandible; 12: partial mola of right mandible (show thumb-like denticle at apex); 13: hypopharynx.



FIGURES 14–20. *Paegniodes oligopilus* sp. nov. (Scale bars = 0.1mm). 14: maxilla; 15: enlarged crown of galea-lacinia; 16: partial maxillary palp (show 3th segment); 17: labium (ventral view); 18: labium (dorsal view); 19: glossa (ventral view); 20: glossae (dorsal view).

Thorax. Pronotum yellowish brown, weakly expanded laterally, posterolateral margins not clearly demarcated to mesonotum. Mesonotum with a longitudinal brownish stripe medially. All legs yellowish brown; supracoxal spurs present and nearly rectangle, the spur of foreleg slightly shorter than those of middle and hind legs. Femur of foreleg (Fig. 21) with regular row of long, stout and simple setae along anterior (outer) margin, many platy setae with slightly divergent margins and rounded apex on dorsal surface (Fig. 23), ventral surface almost smooth with a few hair-like setae sparsely. Tibia devoid of patella-tibial suture, with sparse short and thin setae along outer margin, inner margin with row of approximately 10 stubby and blunt setae directed toward apex; tarsal claw slightly hooked, bearing one submedian tooth and 2–3 apical teeth. Middle and hind legs similar to foreleg in structure, except presence of patella-tibial suture and apex of tibia with a cluster of setae (Fig. 27).

Abdomen. Abdominal tergites generally yellowish brown, tergites I–IX each with a transverse reddish brown band on posterior margin, tergites II–IX each with a longitudinal reddish brown stripe medially, but the stripe on IX only extended to anterior half (Fig. 4); posterior margin of tergites with weakly developed spines of different size and shape; posterolateral projections weakly developed, with apical blunt and sclerotized weakly (Fig. 24). Gills present on abdominal segments I–VII; dorsal lamellae of gill I much smaller than those on other segments, approximately 1/3 as long as fibrillar portion (Fig. 22); gills II–VII similar in shape (Figs 25–26, 29), lamellae much longer than fibrillar portion, tracheation visible. Cerci subequal to median caudal filament in length; inner of cerci with a few short hair-like swimming setae on intersegment (Figs 28, 30); basal portion of median caudal filament without setae (Fig. 28), with about 1–2 hair-like thin setae every 2 segments on intersegment of both margins in the median and distal portions (Fig. 31).

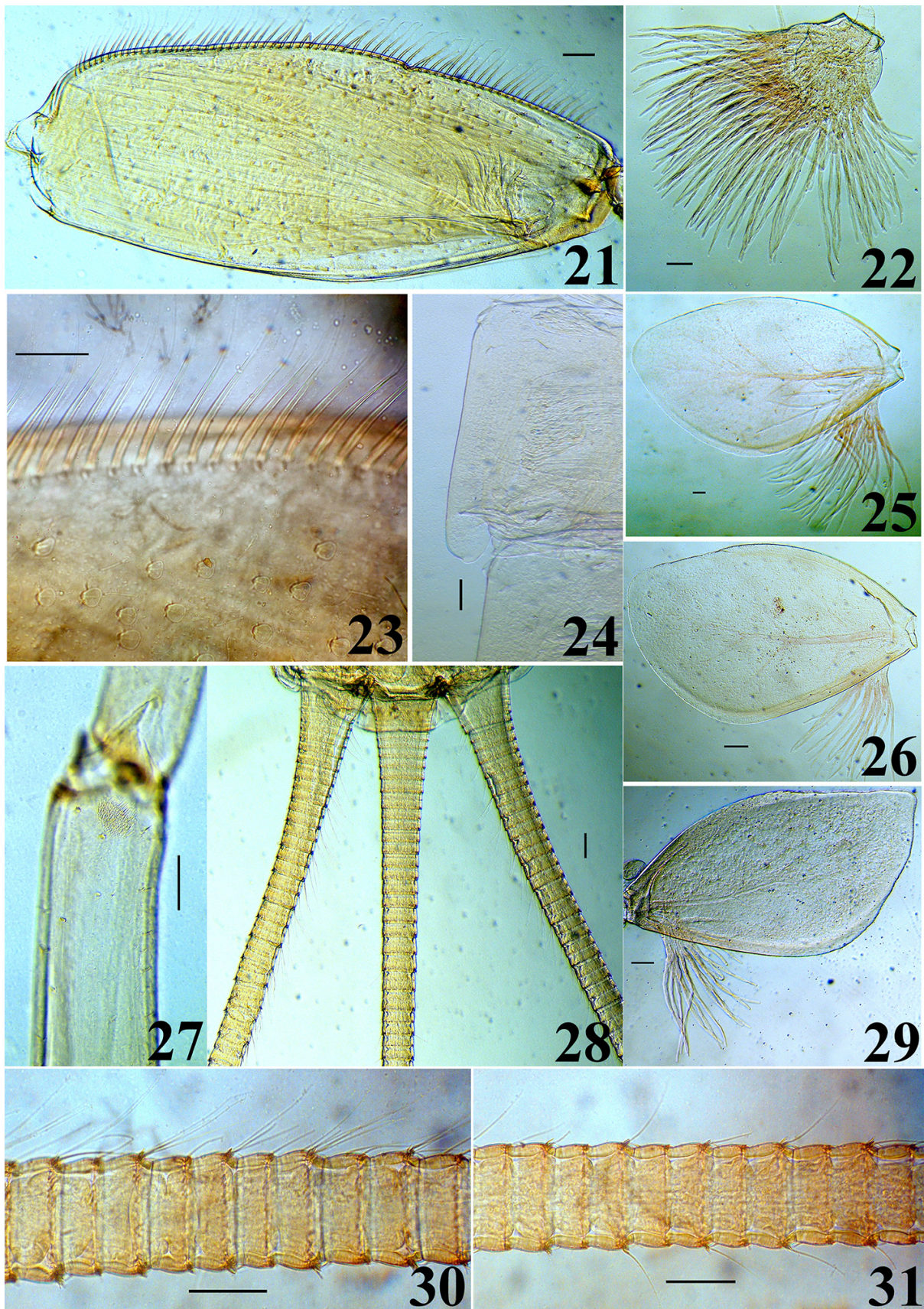
Alate stage: Unknown.

Etymology. The specific epithet is an arbitrary combination of “*oligo*” (from Greek, meaning less) and “*pilus*” (from Latin, meaning hairs), in reference to the swimming setae of cerci and median caudal filament of the new species are vestigial and much less and shorter than those of its congeners.

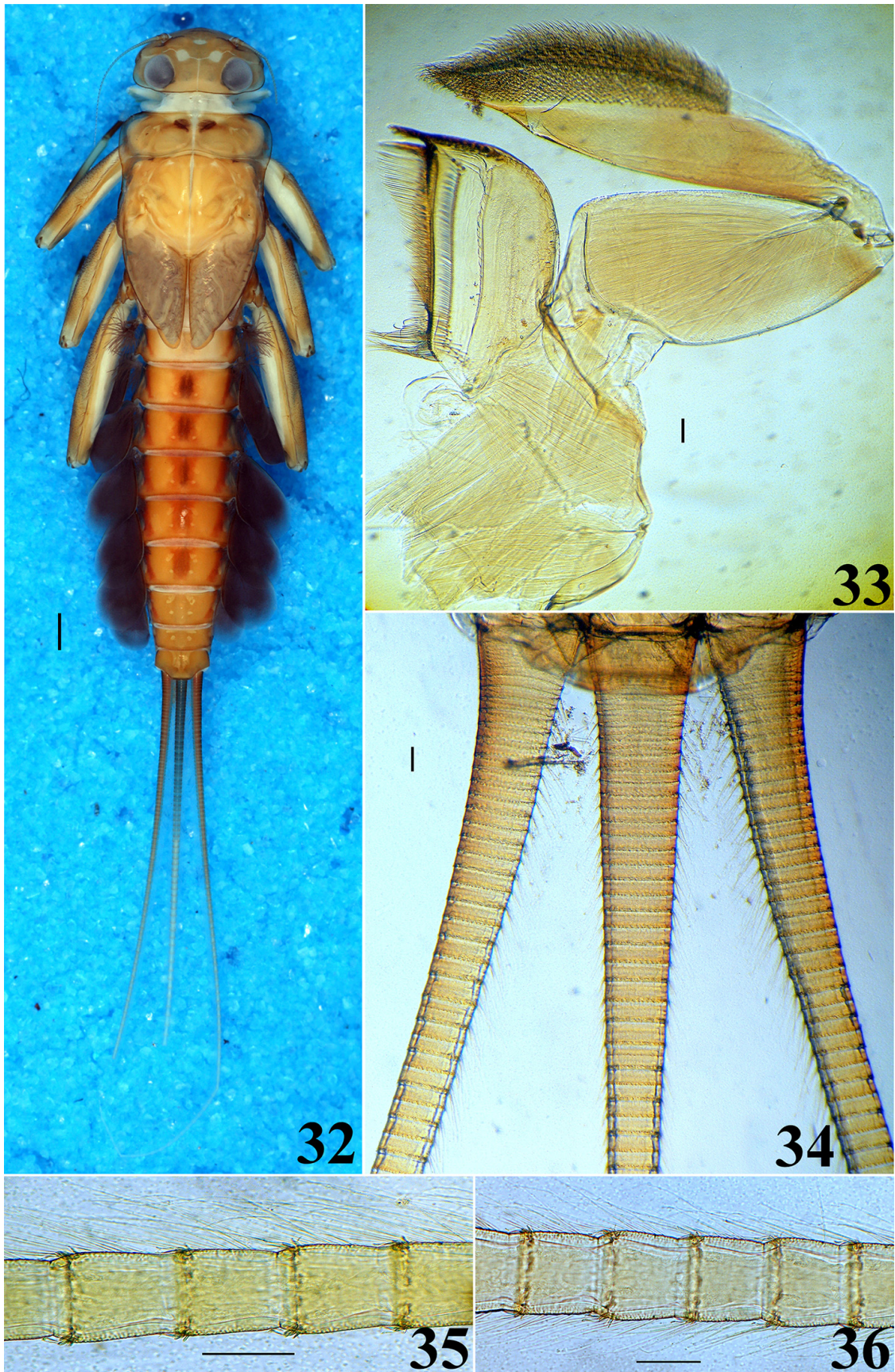
Distribution. China (Sichuan).

Larval habitat and feeding habit. The larvae are found from seeping rocky surface of vertical rocky outcrop (Figs 1–3) where is located at side of footpath on steep hillside, and the seepage water is temporarily flowed down into the adjacent (about 20 meters away) stream in raining season. To understand the feeding habit of this new hygropetric *Paegniodes* species, the gut contents of the two larvae, along with two larvae of *P. cupulatus* for comparing, were analyzed. The results showed that all two guts of the new species were similar in content; they predominantly contained mineral material and FPOM (fine particulate organic matter) (Fig. 40), which suggest that the larvae of *Paegniodes oligopilus* sp. nov. are filter-feeders. While the gut contents of *P. cupulatus* larvae contained mineral material, sediment particulates and considerable organic detritus (Fig. 41), including plant epidermal tissues, intact filamentous algae and recognizable insect remains etc., and only a few periphyton were found in the gut. Microhabitat distribution and gut contents suggest that larvae of *P. cupulatus* larvae are opportunistic collectors (gatherers).

Discussion. The mature larvae of new species can be easily distinguished from other members of the genus *Paegniodes* by the following diagnostic characters: (1) posterior margin of basal segment in maxillary palpi bearing 13–15 stout setae (Fig. 14), which is somewhat similar to *P. dao*, but the latter bearing rows of densely hair-like thin setae on both margins of basal segment (Nguyen & Bae 2004: Fig 1D) and the mandibles also having such dense hairlike setal field on the outer margin; while those in *P. cupulatus* (Fig. 33) and *P. sapanensis* are glabrous; (2) abdominal tergites II–IX each with a longitudinal reddish brown stripe medially (Fig. 5) and without any oblique stripes laterally. However, in *P. cupulatus* (Fig. 32) and *P. sapanensis* (Boonsoong *et al.* 2021: Fig. 6A), other than having a median stripe present on abdominal tergites II–VII, tergites II–VIII also bearing a pair of oblique stripes laterally; although the larva of *P. dao* was not mentioned having such stripes, each abdominal segment of its subimago possessing the stripes similar to those found in the adults of *P. cupulatus* (Nguyen & Bae 2004). In immature larvae of *P. cupulatus*, based on our long experience of collecting and field observation, the body color is usually uniform with brownish green, it is only in the final instars that such stripes become apparent, which suggest that the last instar larvae of *P. dao* probably have similar stripes; (3) posterolateral projections on abdominal segments are weakly developed, apical blunt and weak sclerotized (Fig. 24) (vs. the projections are clearly long and acute in *P. cupulatus* and *P. sapanensis*); (4) swimming setae on cerci and median caudal filament are rudimentary and only present on intersegment (Figs 30, 31), which suggest that these caudal filaments lost swimming function in order to adapt the hygropetric habitat. However, in *P. cupulatus* and *P. sapanensis*, the swimming setae on caudal filaments



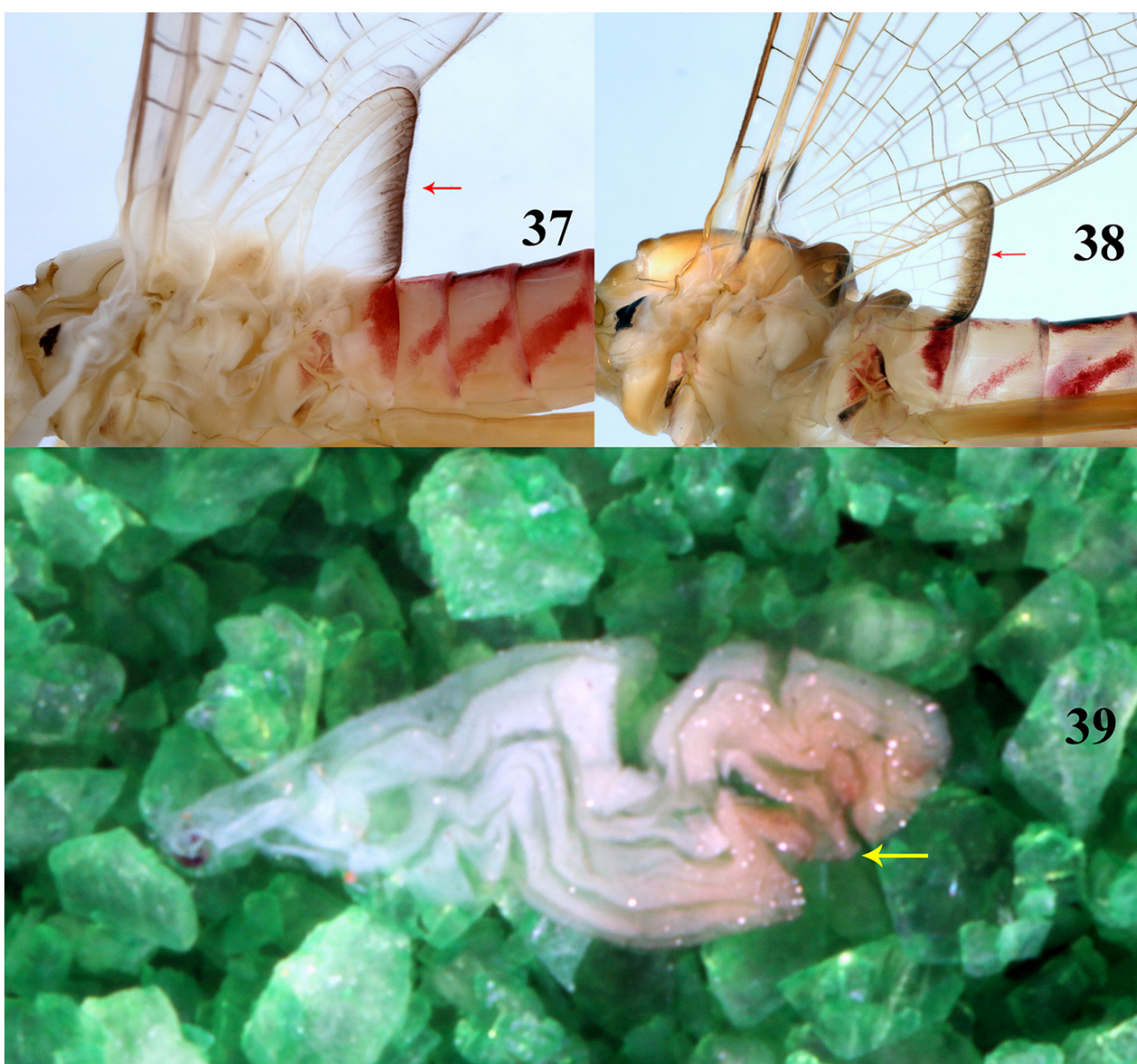
FIGURES 21–31. *Paegniodes oligopilus* sp. nov. (Scale bars = 0.1mm). 21: femur of foreleg; 22: gill I; 23: partial anterior margin of fore femur; 24: posterolateral projection of abdominal segment VI; 25: gill III; 26: gill VI; 27: apex of tibia on middle leg; 28: base of cerci and median caudal filament; 29: gill VII; 30: middle portion of cerci; 31: middle portion of median caudal filament.



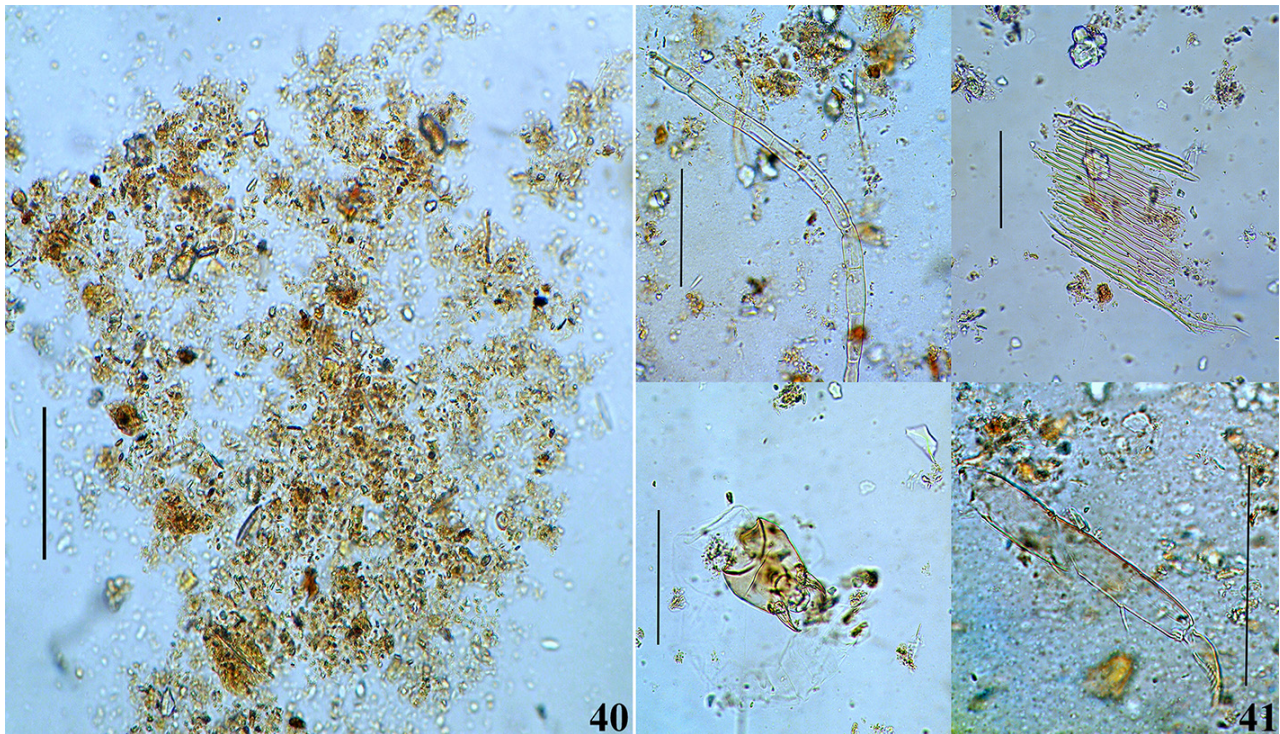
FIGURES 32–36. *Paegniodes cupulatus* (Eaton, 1871). 32: larval habitus of *Paegniodes cupulatus* (Scale bar = 1mm); 33: maxilla; 34: base of cerci and median caudal filament; 35: middle portion of cerci; 36: middle portion of median caudal filament (Scale bars of Figs 33–36 = 0.1mm).

are well developed from proximal to terminal, in addition to the intersegment, both lateral margins (median caudal filament) or inner margin (cerci) of each segment bearing long and strong setae (Figs 34–36); (5) body size of mature larvae is much smaller than other species of the genus, for example, the body length of *P. cupulatus* is about twice as long as new species, which may be related to their different feeding habits as mentioned above. In addition, compared with its congeners, the antenna of this new species is very short, being only half of the head width (Fig. 4), which may be an adaptation to the hygropetric habitat. The subimago or imago of *P. cupulatus* is characterized by hind wings with a pronounced brown band at outer margin (Figs 37–38). This characteristic is also shared with *P. sapanensis* (Boonsoong *et al.* 2021). Although the winged stage materials of the new species are not available in this study, we selected a final instar larva that is ready to molt to subimago to examine if the new species has the same unusual hind wing. After peeling off the epidermis of hind wing pad, it is clearly showed that the outer margin of crumpled hind wing (Fig. 39) stained with raw-umber brown, which indicated that, in alate stage, the hind wing of the new species also has the brown band at outer margin.

The hygropetric heptageniid species seem not uncommon because we also collected two larval specimens of *Epeorus* (*Epeorus*) sp. which cohabitate with *Paegniodes oligopilus* **sp. nov.** on the same rocky surfaces. Due to insufficient specimens, not until to collect more materials including adults will we describe this *Epeorus* species in the near future.



FIGURES 37–39. 37: subimago hind wing of *Paegniodes cupulatus*; 38: imago hind wing of *Paegniodes cupulatus*; 39: hind wing pad (after removing epidermis) of final instar larva of *Paegniodes oligopilus* **sp. nov.**



FIGURES 40–41. (Scale bars = 0.1mm). 40: partial gut contents of *Paegniodes oligopilus* sp. nov.; 41: partial gut contents of *Paegniodes cupulatus* (Eaton, 1871).

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