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The exact morphology of *Oligoneuriella pallida* (Hagen, 1855) from China (Ephemeroptera: Oligoneuriidae)

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Abstract

The brushlegged mayfly *Oligoneuriella pallida* (Hagen, 1855) has been reported from a series of countries by quite several different researchers, which also caused some confusions and inconsistence of morphological descriptions, and led to mis-identification of Chinese *Oligoneuriella* species historically. Here the Chinese oligoneuriid specimens are compared to European *Oligoneuriella* species morphologically and molecularly. The results show China harbors one species *O. pallida*. It can be diagnosed by nymphal long oval lamellae of gills I, fimbriate part of gill I longer than lamellae, subequal tarsi and tibiae of mid- and hindlegs, and all femora without hair-like setae on outer margins. In adults, the femora and abdomen of this species have grey to purple pigmented markings, well-developed titillators and subgenital plate. Remarkably, the Chinese *O. pallida* also shows some differences to European populations, like longer filamental part of gills I and spine-like setae on abdominal sterna of nymphs, and with pigments on femora and nearly straight posterior margin of subgenital plate in male adults. It has 6.15% K2P distance of COI gene to European sequence. The detailed description in the present study not only show the exact features of the species *O. pallida* but also provide good photos for further classification on Asian brushlegged mayflies, which is still lack of study and clarification.

Key words: brushlegged mayflies, identification, diagnosis, insect, Palearctic

Introduction

The family Oligoneuriidae has ten extant genera around 67 species worldwide and three genera 23 species in Palearctic region (Barber-James *et al*. 2008; Bauernfeind & Soldán 2012; Sroka *et al*. 2019; Massariol *et al*. 2019). However, historically only one species *Oligoneuriella rhenana* (Imhoff, 1852) was reported from China (Uéno 1941). No more specific recent research on this Chinese taxon was found except two lists (Zhou 2013; Zhou *et al*. 2015).

In history, the species *Oligoneuriella rhenana* was found in Europe (Bauernfeind & Soldán 2012), China (Uéno 1941) and Japan (Ishiwata & Takemon 2005; Gose 1979; Ishiwata 2001). But Tshernova *et al*. (1986), Kluge (1997) and Bauernfeind & Soldán (2012) regarded this species in western Europe only, it is the species *Oligoneuriella pallida* (Hagen, 1855) distributing trans-palearctically. Specifically, it has been found in Europe (Bauernfeind & Soldán 2012), Far East of Russia (Tshernova *et al*. 1986; Kluge 1997), Mongolia (Soldán *et al*. 2008) and Turkey (Kazanci & Türkmen 2016). So both Japanese and Chinese *Oligoneuriella* species under the name "*Oligoneuriella rhenana*" need confirming study.

The exact morphology of *Oligoneuriella pallida* is still unclear. Bauernfeind & Soldán (2012) stated clearly that its "gill 1 slightly smaller than gill 4, filamental part of equal length as lamella". However, Kluge (1997) keyed it as "lamella of tergalia of 1st pair normally developed, as large as lamellae of tergaliae 2–7". Sroka *et al*. (2015) listed the same lamellae "markedly smaller" than others. Besides, in the drawing pictures provided by Sowa (1961) (as O. *mikulskii*) and Ikonomov (1962) (as O. *poecile*), the gills I of this species has oval dorsal lamella and it is shorter than or subequal to filamental part. But in the photo of Chovet & Lécureuil (2001), the lamella of gills I is long oval, and is longer than gills II and its lamella is longer than its filaments.

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In addition, Sroka *et al*. (2015) used some new characters (like setae on mouthparts, gills and legs) to delineate the *Oligoneuriella* species, which provides a format and chance to identify Chinese *Oligoneuriella* species and to clarify the species *O. pallida*. In this study, we check all *Oligoneuriella* specimens in our collection validate their status and show its real characters with pictures.

Materials

Material examined. **CHINA:**10 nymphs, Ili second Bridge, Ili River, Xinjiang Uygur Autonomous Region (N 43°52.595", E 81°18.211", alt. 683 m), 2024-VI-4–6, De-Wen Gong, Xu-Hong-Yi Zheng leg.; 2 female adults, Yunke county, Heilongjiang Province, 2007-VIII-22, Xie Hui & Shilei Wang leg.; 1 female adult, Ermu river, Muhe county, Heilongjiang Province, 2024-VII-16, Xin-He Qiang, Ning-Ning Wang, Yu-Xian Sun leg.; 1 male adult, Laocao river, Muhe county, Heilongjiang Province, N 52.833392°, E 122.591656°, 480 m alt., 2024-VII-19, Xin-He Qiang, Ning-Ning Wang, Yu-Xian Sun leg.; 20 nymphs, Yimin River, Helaer city, Inner Mongolia Autonomous Region, 2007-VIII–VI, Xie Hui & Shilei Wang & Changfa Zhou leg.; 100 male and 100 female adults, 60 nymphs, same as the former, N 49.105077°, E 119.755963°, 615 m alt., 2024-VII-20–23, Xin-He Qiang, Ning-Ning Wang, Yu-Xian Sun leg.

Methods

Collecting: nymphs were collected with hand screen, and adults were attracted by collecting light, some of them were reared from collected mature nymphs (placed in plastic trays with appropriate water, supplied air with a pump used in golden fish cultivating in house).

Observing: photographs of the specimens were taken by a digital camera (Canon 90D) and subsequently observed and photographed under a stereomicroscope (MShot) at different magnifications for fine structures, including mouthparts, legs, gills. Some digital photos were taken by Sony A7RIV (Interchangeable Lens Digital Camera) with Laowa FF 25mm F2.8 Ultra Macro (Macro Lens), and some of them were taken under Nikon Eclipse 50i (Microscope) with MShot MDX10 (Image System). Final plates were edited with Adobe Photoshop 2022.

Molecular comparing: the DNA extraction and amplification followed the process by Zheng & Zhou (2021). The sequences used in this study are listed in table 1. The sequences were aligned with the software Clustal W and COI distances are calculated by MEGA 11. The genetic distances are measured by Kimura-2-Parameter (K2P).

All specimens were preserved in 85% alcohol and deposited in the Mayfly Collection at Nanjing Normal University, Jiangsu Province, China.

TABLE 1. The *Oligoneuriella* COI sequences used in this study.

Species	GenBank Accession number or Bold Process ID	Source	Location
O. sp. (1)	XJDQD856-18	Bold Systems	China (Xinjiang)
O. sp. (2)	XJDQD857-18	Bold Systems	China (Xinjiang)
O. pallida (1)	PV257815	This study	China (Heilongjiang)
O. pallida (2)	PV257832	This study	China (Heilongjiang)
O. pallida (3)	KU609047.1	GenBank	Hungary
O. rhenana (1)	PP403731.1	GenBank	Slovakia
O. rhenana (2)	PP403677.1	GenBank	Slovakia
O. orontensis (1)	MN958844.1	GenBank	Israel
O. orontensis (2)	MN958842.1	GenBank	Israel

Result

Oligoneuriella pallida (Hagen, 1855)

Oligoneuria rhenana var. *pallida* Hagen, 1855: 268 (one male and one female adult, from Hungary).

Oligoneuria pallida: Eaton, 1871: 56 (male, female, from Hungary) (synonymized to *Oligoneuria rhenana* Imhoff, 1852 by Eaton 1883: 31 and Jacobson & Bianchi 1905: 872 by mistake); Szilády 1912: 55 (from Romania)(restated and transferred to genus *Oligoneuriella* as *Oligoneuriella pallida* by Sowa 1973: 660; male was designated as lectotype and female as paralectotype by Mol 1984: 126).

Oligoneuriella rhenana: Ujhelyi 1966: 204 (Hungary) (*nec Oligoneuria rhenana* Imhoff 1852: 177) (mis-identification, corrected by Kovács *et al*. 1999: 350).

Oligoneuriella yugoslauica Ikonomov, 1958: 858 (nomen nudum)(synonymized by Mol 1984: 126) (Yugoslavia).

Oligoneuriella poecile Ikonomov, 1960: 44; 1962: 69 (nymph, Yugoslavia, nomen nudum) (synonymized by Mol 1984: 126).

Oligoneuriella mikulskii Sowa, 1961: 287 (nymph, Poland); Keffermüller 1978: 101 (male and female, Poland) (synonymized by Mol 1984: 126).

Oligoneuriella mongolica Soldán et Landa, 1977: 14 (male and female, Mongolia) (synonymized by Kluge 2004: 141 but without reason).

Oligoneuriella pallida: Tshernova *et al*. 1986: 136 (key, Russia); Chovet & Lécureuil 2001: 125 (nymph, France); Beketov 2007: 387 (south-west Siberia, Russia); Tiunova 2008:185 (Amur River, Russia); Kovács *et al*. 2008: 124 (Lithuania); Kluge 2009:121 (East Siberia, Mongolia); Tiunova & Bazova 2010: 327 (Selenga River, Russia and Mongolia); Bauernfeind & Soldán 2012: 233 (Europe); Sroka *et al*. 2015: 329 (comparison); Zhou *et al*. 2015: 141 (China); Kazanci & Türkmen 2016: 100 (Turkey); Salur *et al*. 2016: 82 (Turkey); Sroka *et al*. 2019: 102 (COI comparison).

Oligoneuriella rhenana: Uéno 1941: 19 (male) (*nec Oligoneuria rhenana* Imhoff 1852: 177) **(first record from China)**; Zhou 2013: 200 (list); Zhou *et al*. 2015: 250 (list) (Mis-identification).

Distribution: China (Heilongjiang, Xinjiang); Middle East, Northern Palearctic of Asia, Europe.

Description

Nymph (in alcohol): body length 10.0–13.0 mm (female, Fig. 1A), 7.0–10.0 mm (male, Fig. 1B); cerci 3.5–4.0 mm, terminal filament 3.0–3.5 mm (Figs 1A–B). Body generally yellowish brown to reddish brown, male usually slightly paler than female; head and thorax washed with dark brown markings and stripes; middle line and laterally expanded plate of terga without color, other place of terga brown but those pigments usually not on posterior margins of each terga; in addition, abdominal terga with two pale longitudinal sub-median lines on brown portion of terga, this making each tergum with four brown markings, each marking with pale dot, two median markings with dots in the middle, two lateral markings with dots at anterior corner, but those pale dots unclear on terga VII–X or VIII–X (Figs 1A–B). Caudal filaments pale to shallow gray (Figs 1A–B).

Head: Capsule expanded anteriorly and laterally, female larger than male (Figs 2A–D); anterior margin of capsule with dense and long hair-like setae, orientated anteriorly (Figs 2A–D); vertex usually with three brown markings, two near posterior margin, one between two eyes (Figs 2A, 2C). Anterior half of mesal margin of compound eyes nearly straight, posterior half convex (Figs 2A, 2C). Outline of male eyes circular in dorsal view (Fig. 2A), oval in lateral view (Fig. 2B); shortest distance between two eyes slightly shorter than diameter of one eye, ratio nearly 1.0:1.1 (Fig. 2A); lateral margin of eye exceeding head margin in dorsal view (Fig. 2A). Mesal margin of female eyes convex (Fig. 2C), outline of eye long-oval in both dorsal and ventral view (Figs 2C–D); shortest distance between two eyes slightly larger than diameter of one eye, ratio of them near 1.2:1.0; lateral margin of eye not exceeding head margin in dorsal view (Fig. 2C). Antennae without color, pedicel nearly twice as long as scape in dorsal view; tiny hair-like setae on scape, pedicel and articulations of flagella (Figs 2A–D).

Mouthparts: Both dorsal and ventral surfaces of labrum with dense and long hair-like setae, especially area near anterior margin except median portion, those setae on dorsal surface longer than ventral setae; anterolateral corner of labrum expanded greatly, making lateral margin almost straight but anterior margin convex; posterolateral corner sclerotized and dark (Fig. 3A). Two mandibles nearly same and symmetric, molar much stronger than incisor; both of them with hair-like setae at middle of outer margin (Figs 3D–E); outer and inner incisors with three teeth apically, apex of prostheca divided into a tuft of spines (Figs 3C–E). Crown of maxillae covered with long hair-like setae, besides mesal setal line, an additional setal line on ventral surface of galea-lacinia (Fig. 3G); maxillary palpi covered with dense yellowish hair-like setae, those setae on ventral surface much denser than dorsal ones, setae subequal in length; segment II of maxillary palpi crescent in shape, ca. 6.0x long about segment I (Fig. 3G). Gill tuft

between maxillae and head with well developed filaments, longer than maxillae (Fig. 3G). Lingua and superlinguae of hypopharynx covered with hair-like setae too but those of superlinguae much longer and denser than others; lingua heart-shaped, superlinguae with concave lateral margin and extended inwards forming a lobe (Fig. 3B). Labium with greatly expanded paraglossae, completely covering labial palpi and glossae (Fig. 3H); two paraglossae hinged together at basal half; ventral surface of paraglossae with very tiny hair-like setae, its margins with spine-like or spur-like setae; dorsal surface of paraglossae with a heart-shaped chamber at base, other place covered with dense hair-like setae and those setae lining up forming regular setal lines (Fig. 3H). Glossae in chamber of paraglossae, surface with hair-like setae too (Fig. 3H). Segment II of maxillary palpi ca. 1.6x segment I in length (Figs 3F, 3H); both of them with a ridge on dorsal surface, making their cross-section triangular in shape (Fig. 3F); outer margin of segment I with hair-like setae (ca. 3.0x longer than others), inner margin with one or two similar setae (Fig. 3F); on its dorsal surface, some spine-like setae between ridge and inner margin (Figs 3F, 3H). Ventral surface of segment II with setal line, just like those of paraglossae; on dorsal surface, dense hair-like setae between ridge and inner margin, both ridge and outer margin with spine-like setae at basal half (Figs 3F, 3H).

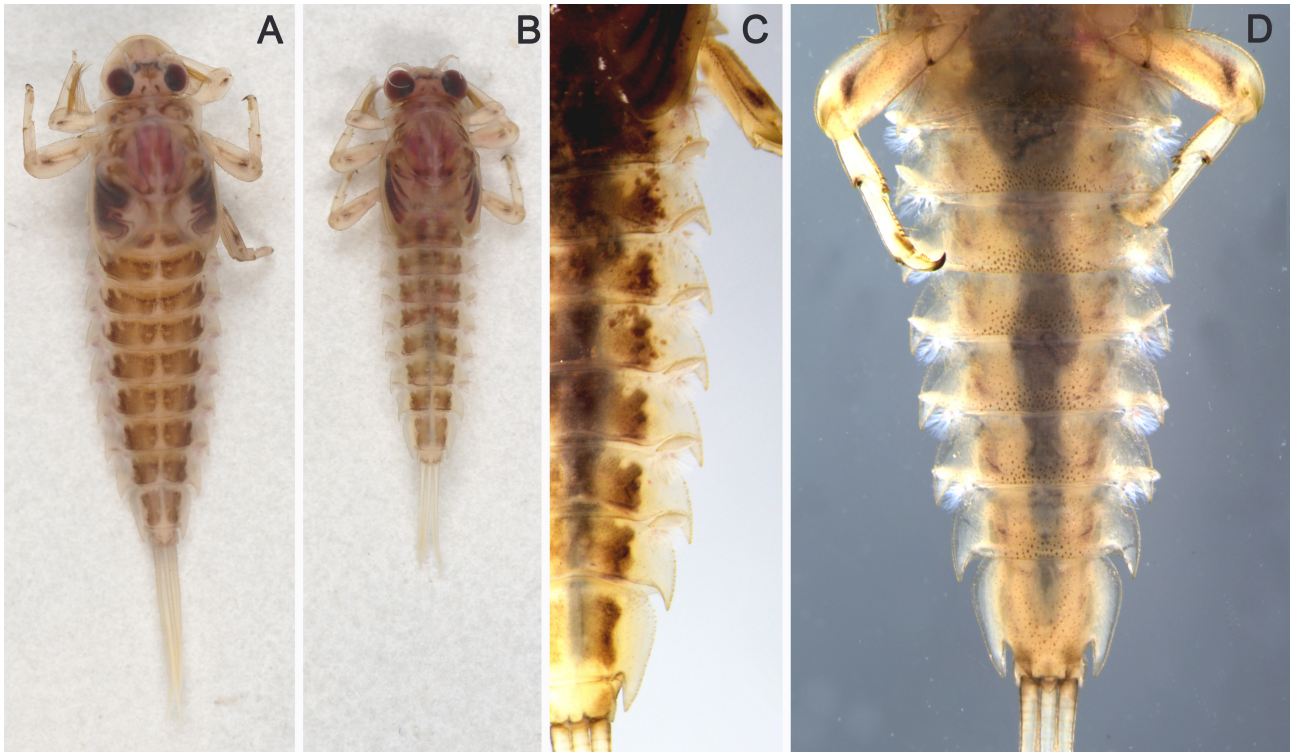

FIGURE 1. Nymphal habitus and abdomen of *Oligoneuriella pallida* from China: **A**. female nymph (dorsal view); **B**. male nymph (dorsal view); **C**. left half of abdominal terga (dorsal view); **D**. abdominal sterna (ventral view).

Thorax: nota with irregular brown markings, generally, lateral margins darker than other parts (Figs 1A–B); lateral margin of pronotum expanded slightly, forming anterolateral lobes and lateral plates (Figs 1A–B, 2A, 2C). Anterolateral corner of mesonotum expanded too, forming a very small lobe (Figs 2A, 2C). Thoracic pleura of three segments expanded into two distinct lobes (supracoxal projections, Figs 2A, 2C), dorsal plates of coxae expanded into lobes too, those lobes of prothorax smaller than others. **Forelegs**: surface and apical margin of coxae with spine-like setae; surface of trochanter smooth; inner margin of femora with a circle of long hair-like setae, dorsal surface and outer margin with spine-like setae, middle portion with more and denser setae than others; femora with a subapical dark dot in dorsal; ventral surface of femora with spine-like setae too but sparser than dorsal one. Tibiae with expanded and convex base, long hair-like setae almost on whole inner margin except apical portion, only spine-like setae on there; both dorsal and ventral surfaces of tibiae with a longitudinal line of spine-like setae, those dorsal setae positioned almost to proximal base of femora but leaving 1/5–1/6 portion empty, instead, several spine-like setae on outer margin here. Those setae on ventral surface almost to base of femora but several proximal ones near outer margin too; apex of tibiae forming a finger-like projection. Inner and apical margin of tarsi with spine-like setae; length ratio of femora: tibiae: tarsi=5.5: 7.5: 2.0 (Fig. 3E); claw with a hooked and deep reddish-brown apex

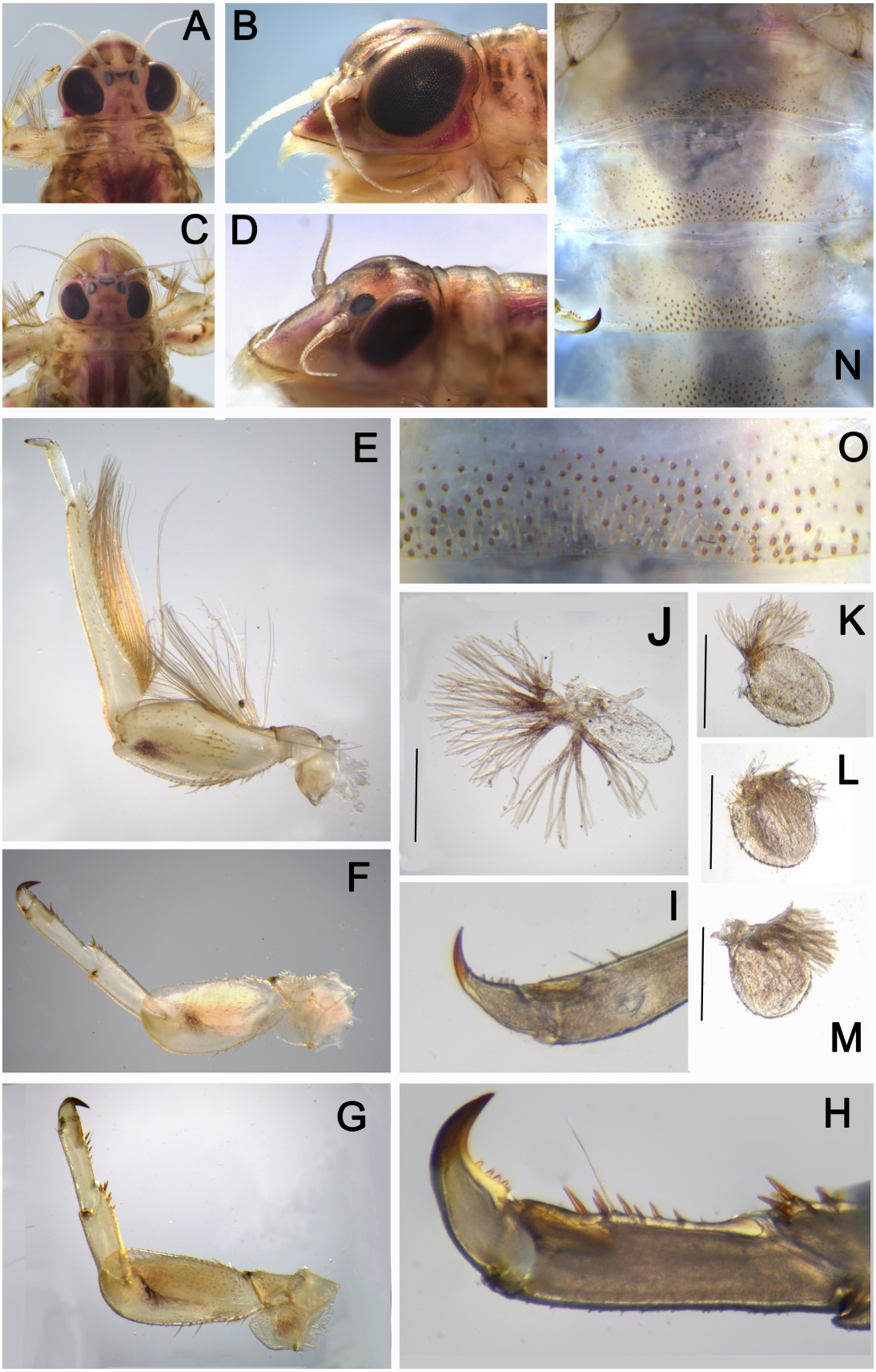

FIGURE 2. Nymphal structures of *Oligoneuriella pallida* from China: **A**. male head (dorsal view); **B**. male head (lateral view); **C**. female head (dorsal view); **D**. female head (lateral view); **E**. foreleg (dorsal view); **F**. Midleg (dorsal view); **G**. hindleg (dorsal view); **H**. hindclaw; **I**. foreclaw; **J**. gills I; **K**. gills II; **L**. gills III; **M**. gills IV; **N**. sterna I–IV of male (ventral view); **O**. sterna II of male (enlarged, showing the details of setae (ventral view)(scale bar of gills = 0.5mm).

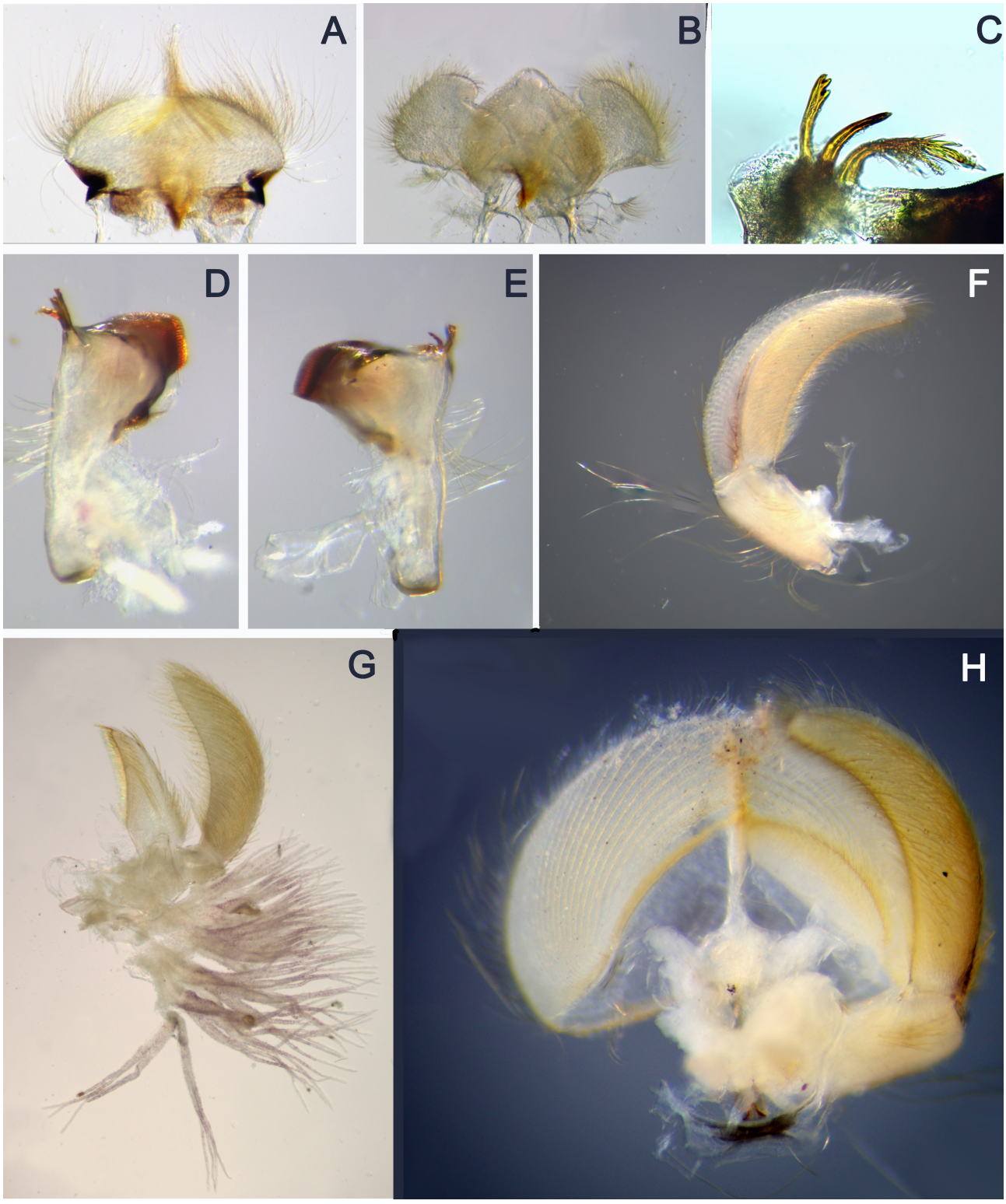

FIGURE 3. Nymphal mouthparts of *Oligoneuriella pallida* from China: **A**. labrum (dorsal view); **B**. hypopharynx (dorsal view); **C**. incisor and prostheca of mandible; **D**. left mandible (dorsal view); **E**. right mandible (dorsal view); **F**. labial palpi (dorsal view); **G**. maxilla (dorsal view); **H**. Labium (dorsal view, left palpi and glossae moved).

and five denticles at base (Fig. 3I). **Midlegs**: all parts covered with spine-like setae but without any hair-like setae except one on inner margin of tarsi; setal pattern of femora similar to forefemora, setae near apex of tibiae and tarsi larger and stronger than others, claw with five denticles too; length ratio of femora: tibiae: tarsi =13.0:6.0:8.0 (Fig. 3F). **Hindlegs**: similar to hindlegs, length ratio of femora: tibiae: tarsi =10.5:4.0:6.0 (Figs 3G–H).

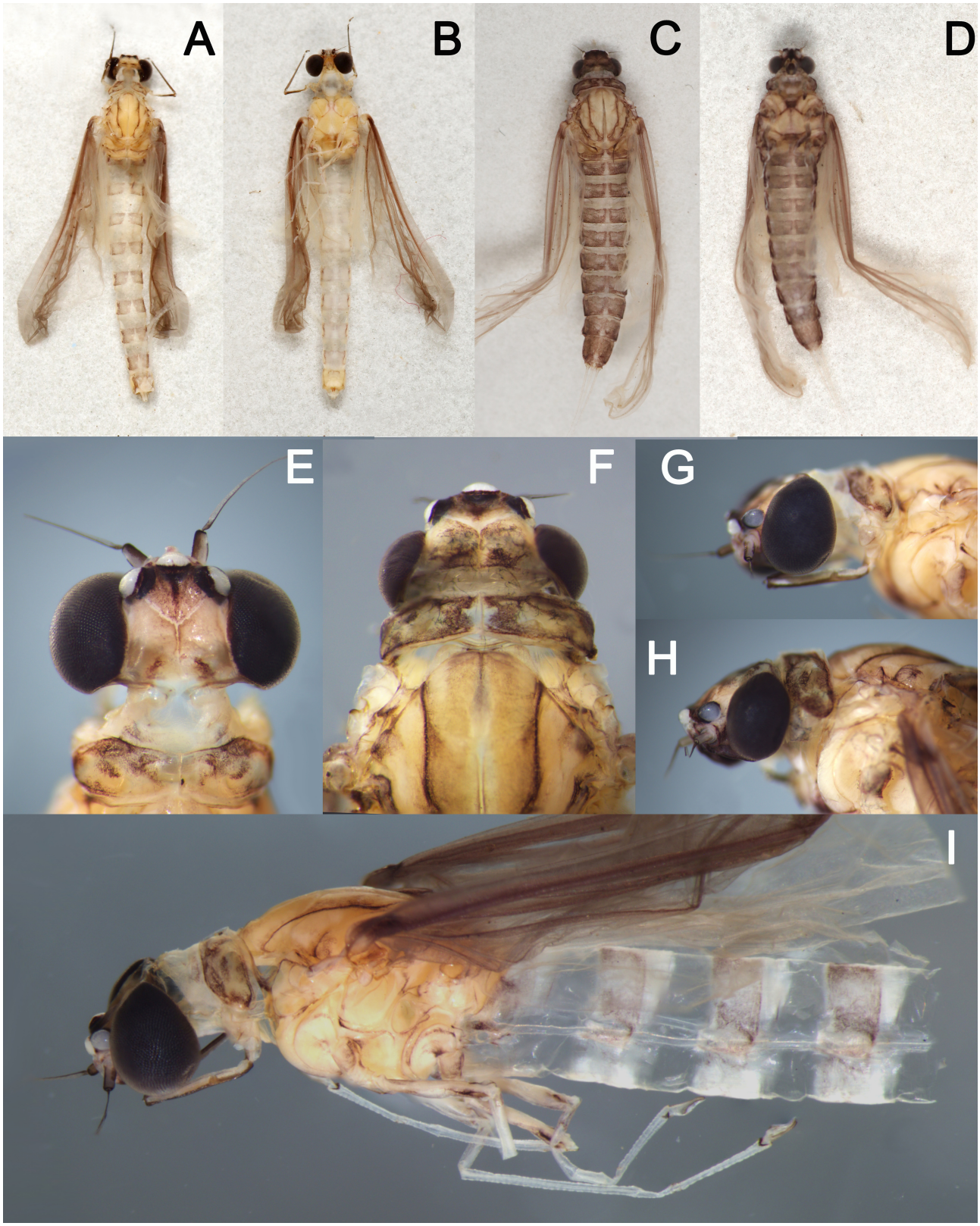

FIGURE 4. Imaginal structures of *Oligoneuriella pallida* from China: **A**. male habitus (dorsal view); **B**. male habitus (ventral view); **C**. female habitus (dorsal view); **D**. female habitus (ventral view); **E**. male head (dorsal view); **F**. female head (dorsal view); **G**. male head (lateral view); **H**. female head (lateral view); **I**. anterior half of male (showing color pattern of abdomen and legs).

Abdomen: terga and sterna covered with tiny scale-like setae, those setae on sterna concentrated and enlarged at median portion near posterior margin (Figs 1D, 2N); most of those setae with golden to reddish golden color, length subequal to width, but some of them longer than others, length at least 2x width, especially some sterna II and III (Fig. 2O). Posterolateral corners of terga expanded into sharp projections; those projections larger progressively from anterior to posterior; projections of tergum I distinct, projections of terga II–VII with convex outer margins, oriented outwards to body longitudinal axis, ca. 1/3 length of each segment; projection of tergum VIII with almost straight outer margin and parallel to body axis; projections of tergum IX oriented inwards slightly (Figs 1C–D). Gills I with long-oval shaped dorsal lamella and well-developed ventral fimbriate filaments, filaments distinctly longer than lamella; both lamella and filaments with purple pigments; lamella with a ventral chamber and its dorsal surface with tiny spine-like setae (Fig. 2J). Gills II–VII similar to each other although gills IV–V slightly larger than others; dorsal lamellae of them slightly shorter or subequal to ventral filaments, surfaces of lamellae with spine-like setae and circular outline (Figs 1C, 2K–M). Subanal plate with distinct U-shaped concave posterior margin, making it like with two sharp posterior lobes (Fig. 1D). Cerci with dense hair-like setae on mesal margins from base to apex but terminal filament with setae on both margins; the latter slightly shorter than cerci.

Male adult (in alcohol): body length 10.0–11.0 mm, caudal filaments 5.0–6.0 mm, terminal filament subequal to cerci. Body general pale with grey to reddish brown pigments (Figs 4A–B).

Head: vertex grey, with two brown dots or short stripes near occiput (Fig. 4E); compound eyes dark, on lateral margin of head, nearly oval in dorsal view (Fig. 4E); shortest distance between two eyes: largest width of eye in dorsal view 5.5:4.5; in lateral view, outline of eye oval too but dorsal end slightly narrower than ventral end (Fig. 4G). Antennae with pale scape but grey to deep brown pedicel and flagella (Fig. 4E).

Thorax: pronotum and metanotum washed with irregular-shaped grey pigments, sutures and margins of mesonotum grey to dark (Figs 4A, 4I); mesonotum with a pair of thread-like projections on posterior margin. Forelegs with grey to deep brown femora, tibiae and claw, other parts pale (Fig. 4I). Length ratio of femora: tibiae: tarsi 1.0:1.5:0.25; claw without regular shape. Mid- and hindlegs similar, both of them longer than foreleg, pale but with grey joints and claw, femora usually grey and with a subapical dark dots (Fig. 4I); length ratio of femora: tibiae: tarsi 1.0:1.5:0.5. Wings usually grey, semi-hyaline, folded together, without regular shape (Figs 4A–B). When unfolded, its subcostal brace usually dark; Sc and R_1 very close, apical half of them almost fused together; Rs, MA and MP forked at very base, Rs1 and IRs, Rs2 and MA_1, MA_2 and MP_1, MP_2 and CuA_1, and CuA_2 and CuP running very closely or fused together; crossveins only visible at anterior half and base of wing (Fig. 5A). Hindwing also deformed, with very weak veins (Fig. 5B).

Abdomen: anterior 2/3 to 4/5 of each tergum grey to reddish brown but midline pale, margins of those pigmented portion darker than median area (Figs 4A, 4I); posterolateral angles presents sharp projections, those of tergum IX larger than others and curved inwards (Fig. 5C). Sterna usually pale, without clear markings but lateral margins of them grey to brown (Fig. 4B).

Genitalia: Gonopods 3–4 segmented, basal segments distinctly curved inwards near apex (Fig. 5C). Subgenital plate expanded into broad lobe posteriorly but its posterior margin nearly straight (Fig. 5E). Penis lobes less than half the length of basal segment of gonopods (Fig. 5C); outer margins of penes convex, outer lobe of penis bent inwards slightly, with a slightly expanded apex; inner lobe of penis distinctly shorter than outer lobe, with almost straight posterior margin; its width ca. 2.x outer lobe; gonoduct in penis distinct (Fig. 5D). Medioventral projection of lateral penis lobes (so-called titillator) curved posteriorly at right angle, margins sclerotized heavily and serrated into a line of denticles (Figs 5C, 5F). Caudal filament pale.

Female adult (in alcohol): body length 7.0–9.5 mm, caudal filaments 2.5–3.0 mm (Figs 4C–D). Similar to male but usually greyer or darker, pale brown to purplish brown. Compound eyes semi-oval in dorsal view but oval in lateral view (Figs 4F, 4H). Shortest distance between two eyes: largest width of eye in dorsal view 7.0:2.0 (Fig. 4F). All legs deformed, without regular shape, tibiae and tarsi much narrower than femora, visible parts of legs grey (Fig. 4D).

Diagnosis

The nymph of this species *Oligoneuriella pallida* can be identified by its long oval lamellae of gills I (Fig. 2J), those lamellae subequal to (from slightly longer to slightly shorter) all lamellae of remaining gills; well developed

filamental part of gills (from subequal to slightly longer than dorsal lamellae of gills, Fig. 2J); subequal size of gills II–VII, oval in shape (Figs 2K–M); subequal length of tibiae and tarsi of mid- and hindlegs (Figs 2F–G), relatively short posterolateral projections of abdominal terga (1/3x length of the respective segment, Figs 1C–D). In addition, all outer margins of femora of this species without any hair-like setae (Figs 2E–G), abdominal sterna usually with short scale-like setae only (Figs 2N, 2O).

Male imago of this species have conspicuous convex posterior margin of subgenital plate (Figs 5C–E), slightly convex to parallel lateral margins of penes and inward curved apex of outer penal lobes (Fig. 5D). Additionally, it has clear pigments on dorsal body, especially on terga (Figs 4A, 4I).

Female imago can be recognized by its clear markings on abdomen (Figs 4C–D).

Genetic distance

The genetic distance (K2P) of those sequences in table 1 are list in Table 2. From this table, we can see: (1) all Chinese *Oligoneuriella* specimens are same species, the most distance between them is 1.06%; (2) the Chinese *Oligoneuriella* species is the *O. pallida* but not the *O. rhenana* because it is 6% to the former but more than 15% to the latter, the recommended data of COI distance between mayfly species is 18% (Ball *et al*. 2005); (3) the Chinese *Oligoneuriella pallida* has diverged from European population for some distance, they have at least 6.15% COI distance. The final point is also reflected in the morphology (see below).

The phylogenetic tree including Chinese *Oligoneuriella* species has been reconstructed by Sroka *et al*. (2019). Our new COI sequences are nearly equal to those Chinese data in the study.

TABLE 2. Genetic distance of *Oligoneuriella* species based on COI genes (K2P, %).

	O. sp. (1)	*O*. sp. (2)	*O. pallida* (1)	*O. pallida* (2)	*O. pallida* (3)	*O. rhenana* (1)	*O. rhenana* (2)	*O. orontensis* (1)
O. sp. (1) (China)								
O. sp. (2) (China)	0.45							
O. pallida (1) (China)	1.05	0.91						
O. pallida (2) (China)	1.06	0.91	0					
O. pallida (3) (Hungary)	6.85	6.85	6.32	6.15				
O. rhenana (1)	15.03	15.03	15.00	14.83	15.92			
O. rhenana (2)	15.03	15.04	15.00	14.83	15.92	0		
O. orontensis (1)	19.05	19.06	19.05	19.09	17.68	20.11	20.11	
O. orontensis (2)	21.07	21.08	20.22	20.25	18.92	20.82	20.82	0.66

Discussion

Comparison to close congeners of *Oligoneuriella pallida*

Some missing characters of *O. pallida* in Table 1 of Sroka *et al*. (2015) are shown clearly here. The nymphs of this species have dense setae on basal paraglossae, they lined up in rows; setae on distal segment I of labial palps are hair-like, not numerous; all legs have no hair-like setae on outer margins. Other characters mentioned in the Sroka *et al*. (2015) are consistent with our descriptions here.

Sowa (1961), Soldán & Landa (1977) and Bauernfeind & Soldán (2012) grouped *O. pallida* and *O. keffermuellerae* Sowa, 1973 together because they have no hair-like setae on the outer margin of the femora. The differences between these two species have been presented by Bauernfeind & Soldán (2012) and Sroka *et al*. (2015). The nymphs of the species *O. keffermuellerae* have very short and expanded lamellae of gills I; the males of it have much longer titillators than *O. pallida*.

Two species *Oligoneuriella rhenana* and *Oligoneuriella pallida* have broader distribution than other congeners. Those two species can be divided by their gills I (lamellae of *O. rhenana* are also long-oval but much larger than

those of gills II–VII and its filamental part of gills I while all lamellae of *O. pallida* are subequal, filaments of gills slightly longer than lamellae, Figs 2J–M), legs (mid- and hindtarsi much shorter than tibiae in *O. rhenana* but they are subequal in *O. pallida*, Figs 2F–G) and posterolateral spines on abdominal segments (longer in *O. rhenana* but short in *O. pallida*). Besides those, the nymphs of *O. rhenana* have setae on the outer margin of forefemora, which are missing in *O. pallida*.

In male imago, they can be separated easily by their genitalia. The subgenital plate of *O. rhenana* is shorter but broader than that of *O. pallida*, and its penal apex is longer and more parallel than that of *O. pallida*.

Both nymphs and imago of *O. rhenana* are paler than *O. pallida* and without clear color on abdominal terga. In contrast, the *O. pallida* has reddish brown to dark pigments on terga, usually forming two pairs washed patches on each tergum (Figs 1A–B, 4A–D).

Variations of Chinese *O. pallida*

During the identification, a series of differences are found between Chinese materials and European descriptions on *Oligoneuriella pallida* provided by Bauernfeind & Soldán (2012). In nymphs, (1) the fimbriate part of gills I of Chinese specimens are larger than European *O. pallida*, clearly longer than the dorsal lamella (Fig. 2J). In the book of Bauernfeind & Soldán (2012), the gills are reading "filamental part of equal length as lamella"; (2) the lamellae of gills I are slightly larger than counterparts of gills II–VII in Chinese materials (Figs 2J–M), while those lamellae of European *O. pallida* are subequal or shorter than others; (3) the dorsal setal arrangement on forelegs (not on whole tibiae in Chinese species as in Fig. 2E, the dorsal proximal base without setae but in whole tibiae in European specimens); (4) abdominal sterna of Chinese specimens sometimes have longer scale-like setae (Fig. 2O), but those setae are totally missing in European *O. pallida*. In adults, (5) Chinese *O. pallida* has clear markings on legs and abdomen (Fig. 4I), but European *O. pallida* is pale, without any markings on legs at least; (6) lateral projection of penis of Chinese materials is longer than European *O. pallida;* (7) the posterior margin of subgenital plate is almost straight in Chinese *O. pallida* (Fig. 5E) while convex in European *O. pallida.*

The most possibility is that the Asian Palearctic species is the *O. mongolica*, but this species has been synonymized to *O. pallida* by Kluge (2004).

Despite of above mentioned, the Chinese *Oligoneuriella* species is still regarded as *O. pallida* here because: (1) the low COI distance (about 6% only between two populations); (2) the morphological similarity of those two populations; (3) the *Oligoneuriella* species of Mongolia and the Russian Far East have been recognized as *O. pallida* by Tshernova *et al*. (1986) and Kluge (2004). We do not want to make more confusion here; (4) we do not have any abroad *Oligoneuriella* specimens to compare.

Distribution of *O. pallida*

Based on present research and figures of Gose (1979) and Ishiwata & Takemon (2005), the Japanese "*O. rhenana*" should be the *O. pallida* too because it has perfect similar morphology to our Chinese specimens.

The species *O. rhenana* has been found in Europe (from France, Hungary, Romania, Yugoslavia, Poland to European Russia), Asia (from Turkey, Mongolia, southwest to the Far East of Russia, China, Japan, and see above citation). The two populations in our collections (Ili River and Heilongjiang River) are the exact same species morphologically and molecularly. That information shows this species distributes widely, but its geographic distribution may be disjunct.

There are three genera and nine species of Oligoneuriid species in Europe, but only one species, *O. pallida*, is found in the eastern Palearctic. Hopefully, the more intensive collection in this vast region will disclose more species. In contrast, a related family Isonychiidae in the taxa Heptagenioidea has about ten species in the eastern Palearctic but one species in the western part. The real reason for it needs more work to explore.

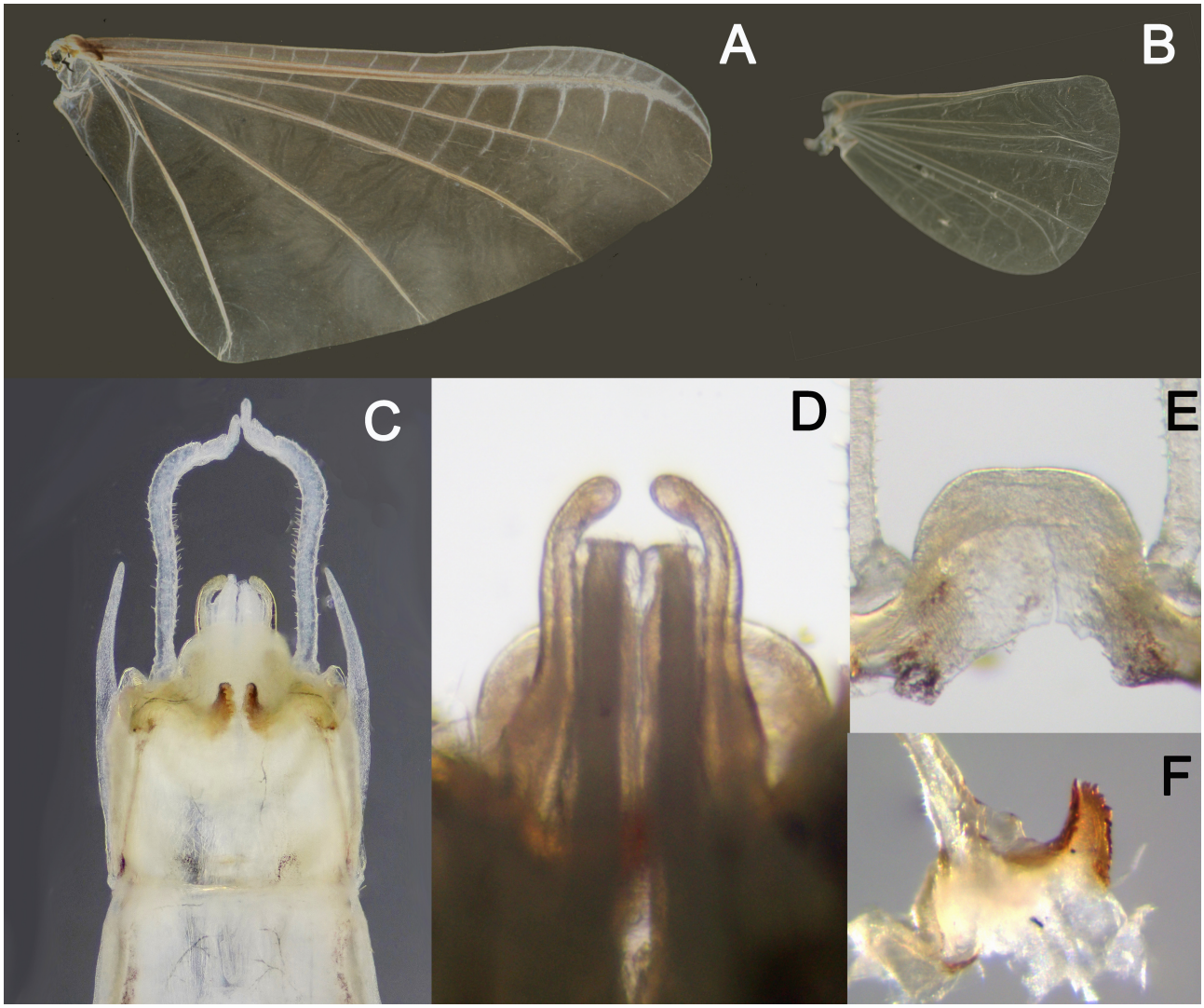

FIGURE 5. Male imaginal structures of *Oligoneuriella pallida* from China: **A**. forewing; **B**. hindwing; **C**. genitalia (ventral view); **D**. penes (dorsal view); **E**. subgenital plate (ventral view); **F**. titillator (dorsal view).

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