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Two new species of the *Neoperla* (*Formosita*) *lushana*-group (Plecoptera: Perlidae) from China, supported by morphological and mitochondrial *COI* data

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Abstract. The *Neoperla* (*Formosita*) *lushana*-group is revised using an integrative taxonomic approach combining morphological and molecular evidence. Two new species of this group are described and illustrated: *Neoperla foping* Wang, Wang & Li sp. nov. from Shaanxi Province and *Neoperla huangi* Wang, Wang & Li sp. nov. from Hunan Province. Diagnostic features are compared with those of related congeners. DNA barcode sequences (*COI*) were generated for both species, and their species boundaries were supported by morphological differentiation, phylogenetic analyses, and interspecific genetic distances. Phylogenetic reconstruction further corroborated the monophyly and distinctiveness of the two new species within the *N. (F.) lushana*-group.

Keywords. Perlinae, *Neoperla*, *N. foping* sp. nov., *N. huangi* sp. nov., integrative taxonomy.

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Introduction

The perlid genus *Neoperla* Needham, 1905 (Plecoptera Burmeister, 1839: Perlidae Latreille, 1802) comprises approximately 385 described species worldwide (DeWalt *et al.* 2026), with up to 122 known species in China (Mo *et al.* 2025; Wang *et al.* 2026). Zwick (2023) formally divided the *Neoperla montivaga*-group into two distinct subgenera, *Neoperla* (*Borneella*) Zwick, 2023 and *Neoperla* (*Formosita*) Klapálek, 1914. The latter was further recognized and diagnosed into five

species groups: *Neoperla* (*Formosita*) *anjiensis*-group, *Neoperla* (*Formosita*) *diehli*-group, *Neoperla* (*Formosita*) *fasciata*-group, *Neoperla* (*Formosita*) *lushana*-group, and *Neoperla* (*Formosita*) *sinensis*-group. The *N. (F.) lushana*-group is characterized by a tubular, broadly straight penis, bearing paired spiny lobes or a single divided lobe ventrally near the apex, with the surface uniformly covered by spinules or spines. This group exhibits a broad distribution, encompassing the Himalayas, the Russian Far East, Korea, and Japan (Zwick 2023) (Table 1). The *Neoperla* (*Formosita*) *lushana*-group, established within the revised framework of the *N. montivaga*-group (Zwick 2023), represents a taxonomically dynamic assemblage whose circumscription has undergone significant refinement in recent years. Since Zwick's (2023) initial circumscription of the *N. (F.) lushana*-group, including 32 species, taxonomic revisions have progressively refined its composition. Subsequent revisions revealed critical complexities: Chen *et al.* (2024) removed two species and updated the checklist of the *N. (F.) lushana*-group. (e.g., *Neoperla flagellata* Li & Murányi, 2012, syn.; *Neoperla guangxiensis* Du & Sivec, 2004), while Mo *et al.* (2024) substantially expanded the group's composition to 36 species via incorporation of previously overlooked lineages and description of novel diversity. Zeng *et al.* (2025) transferred *Neoperla lahu* Stark, 1983 to the group, and Wang *et al.* (2026) further enriched its diversity by describing an additional species.

Despite continuous taxonomic efforts and expanding geographic sampling across East Asia, the *N. (F.) lushana*-group continues to reveal cryptic diversity among morphologically conserved lineages, where subtle diagnostic characters necessitate integrative approaches for reliable species delimitation. In this context, the present study describes two new species from Shaanxi and Hunan provinces: *Neoperla foping* Wang, Wang & Li sp. nov. and *Neoperla huangi* Wang, Wang & Li sp. nov., which are morphologically similar to related congeners, yet diagnosable by consistent minor characters and unambiguously supported by *COI* barcoding. Additionally, the study clarifies their phylogenetic placement within the group. By providing refined diagnostic criteria and molecular validation for these closely related taxa, this work strengthens species identification protocols within the *N. (F.) lushana*-group and offers a methodological reference for resolving cryptic diversity in East Asian *Neoperla*.

Material and methods

Sample collection and morphological study

All adults were collected by handpicking, sweeping, or light trapping and preserved in 95% ethanol. The male terminalia were cleared in 10% NaOH. The types are deposited in the Natural History Museum of China (NHMC) and the Insect Collection of Henan Institute of Science and Technology, Xinxiang (HIST). Specimens for molecular analysis were stored at -20°C. Specimens were examined with a Leica S8AP0 dissecting microscope and the color photographs were made with the aid of a Leica M205 FCA dissecting microscope mounted with a digital camera. The terminology of the genitalia follows Zwick (2023) and Zwick & Zwick (2023).

DNA extraction, amplification, and sequencing

Mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences were obtained for all specimens and deposited in the National Center for Biotechnology Information (NCBI); accession numbers are listed in Table 2. Phylogenetic analyses included both newly generated sequences and homologous sequences retrieved from GenBank.

DNA extraction

Genomic DNA was extracted from 13 specimens using an alkaline lysis protocol (HotSHOT method; Truett *et al.* 2000). Briefly, each sample was incubated in 10 µL of lysis buffer at 95°C for 25 min and subsequently neutralized with an equal volume of neutralization buffer. For the remaining 5 specimens, total DNA was extracted from leg or thoracic tissues using the TIANamp Genomic DNA Kit (Tiangen Biotechnology, Beijing, China), following the manufacturer's protocol.

Table 1. World list of the *Neoperla (Formosita) lushana*-group.

Species	Distribution
<i>N. adamantea</i> Murányi & Li, 2015	Korean Peninsula
<i>N. aestiva</i> Uchida, 1990	Japan
<i>N. baishuijiangensis</i> Du, 2005	China
<i>N. caii</i> Li, Wang & Wang, 2017	China
<i>N. coralliata</i> Uchida, 1990	Japan
<i>N. coreensis</i> Ra, Kim, Kang & Ham, 1994	China, Korean Peninsula
<i>N. costalis</i> (Klapálek, 1913)	China
<i>N. dashahena</i> Du, 2005	China
<i>N. donglaili</i> Li & Li, 2024	China
<i>N. emeishana</i> Li, Wang & Wang, 2017	China
<i>N. foping</i> Wang, Wang & Li sp. nov.	China
<i>N. geniculata</i> (Pictet, 1841)	Japan
<i>N. geniculatella</i> Okamoto, 1912	Japan
<i>N. goguryeo</i> Murányi & Li, 2015	Korean Peninsula
<i>N. hatakeyamae</i> Okamoto, 1912	China, Japan
<i>N. henana</i> Li, Wu & Zhang, 2011	China
<i>N. huangi</i> Wang, Wang & Li sp. nov.	China
<i>N. klapaleki</i> Banks, 1937	China
<i>N. lahu</i> Stark, 1983	China
<i>N. latamaculata</i> Du, 2005	China
<i>N. lushana</i> Wu, 1935	China, India
<i>N. magisterchoui</i> Du, 2000	China
<i>N. nebulosa</i> Stark & Sivec, 2008	Vietnam
<i>N. niponensis</i> (McLachlan, 1875)	Japan
<i>N. pianma</i> Wang, Wang & Li, 2026	China
<i>N. pluvia</i> Uchida, 1991	Japan
<i>N. qinlingensis</i> Du, 2005	China
<i>N. quadrata</i> Wu & Claassen, 1934	China, India, Korean Peninsula
<i>N. signatalis</i> Banks, 1937	China
<i>N. spinaloba</i> Stark & Sivec, 2008	Vietnam
<i>N. taibaina</i> Du, 2005	China
<i>N. taihorinensis</i> (Klapálek, 1913)	China
<i>N. ussurica</i> Sivec & Zhiltzova, 1996	Korean Peninsula, Russia
<i>N. vallis</i> Uchida, 1990	Japan
<i>N. verna</i> Uchida, 1990	Japan
<i>N. xuansongae</i> Li & Li, 2013	China
<i>N. yanlii</i> Li & Wang, 2014	China
<i>N. yaoshana</i> Li, Wang & Lu, 2011	China
<i>N. yiliangensis</i> Mo, Li & Murányi, 2024	China
<i>N. zhiltzovae</i> Teslenko, 2012	Russian Far East

PCR amplification and sequencing

The mitochondrial *COI* barcode region was amplified for all 18 samples, using different primer sets and workflows depending on the extraction and sequencing method. Genomic DNA of 13 specimens was extracted with 10 µL lysis buffer per specimen following the HotSHOT protocol (*N. huangshana* Huo, Du & Zhu, 2021, *N. sinensis* Chu, 1928, *N. baishuijiangensis* Du, 2005, *N. caii* Li, Wang & Wang, 2017, *N. foping* sp. nov., and *N. magisterchoui* Du, 2000). PCR amplification of the *COI* gene was performed using the dual-indexed primer set and thermal cycling conditions described by Zhou *et al.* (2025): F-primer (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') and R-primer (5'-TTTCAACAAATCATAAAGATATTGG-3'). The 19 µL reaction mixture contained 10 µL 2 × Es Taq MasterMix, 3 µL DNA template, and 1 µL each of forward and reverse primers (10 µM) and 5 µL ultrapure water. PCR amplification conditions were as follows: 95°C for 5 min; 35 cycles of 94°C for 1 min, 45°C for 2 min, 72°C for 1 min; and a final extension at 72°C for 5 min. PCR products were purified using SPRIselect beads (Beckman Coulter), and libraries were prepared using the SQK-LSK114 Ligation Sequencing Kit (Oxford Nanopore Technologies) in combination with the NEB#E7546S Ultra II End Repair Ligation Module (New England Biolabs Ltd., Beijing, China) and NEB#E6056S Quick Ligation modules (New England Biolabs). Sequencing was conducted on a MinION Mk1B device with R10.4.1 flow cells. Demultiplexing was performed using ONTbarcode (Srivathsan *et al.* 2021).

For the five specimens extracted using the TIANamp Genomic DNA Kit (Tiangen Biotech) (*N. bilineata* Wu & Claassen, 1934, *N. huangi* sp. nov., *N. quadrata* Wu & Claassen, 1934 and *N. xuansongae* Li & Li, 2013), *COI* amplification was carried out with the universal primers LCO1490 (5'-GGTCWACWAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCWGGRTGWCAAARAATCA-3') (Folmer *et al.* 1994). PCR reactions were performed in 25 µL volumes containing 14.5 µL of ultrapure water, 5 µL of Taq buffer, 1 µL of each primer (0.01 µM), 2 µL of each dNTP (0.05 mM), 0.5 µL of Easy Taq polymerase, and 1 µL of DNA template, under the following conditions: 95°C for 30 s; 40 cycles of 95°C for 30 s, 40–60°C for 50 s, 65°C for 60 s; and 65°C for 10 min. Amplification success was verified by electrophoresis on 1% agarose gels. Successful amplicons were purified and bidirectionally sequenced using the Sanger method at Sangon Biotech (Shanghai, China).

Molecular data analysis

Four sequences were downloaded from NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>): the outgroup taxa *Capnia vidua* Klapálek, 1904 (MT482878) and *Kyphopteryx dorsalis* Kimmins, 1947 (MW553915), along with two ingroup sequences of *Neoperla yiliangensis* Mo, Li & Murányi, 2024 (PP920375, PP920376). Phylogenetic relationships were inferred using maximum likelihood (ML) as implemented in IQ-TREE ver. 3.0.1 (Wong *et al.* 2026). The best-fit substitution model for the *COI* dataset was selected using ModelFinder (Kalyaanamoorthy *et al.* 2017). Branch support was assessed with 1000 bootstrap replicates in ML. The final ML tree was visualized and annotated in iTOL (<https://itol.embl.de/>, Letunic and Bork 2021). Genetic divergence among DNA barcode sequences was quantified as pairwise distances under the Kimura 2-parameter (K2P) model (Kimura, 1980), using MEGA ver. 11 (Tamura *et al.* 2021).

Results

Molecular phylogeny

We newly generated 18 *COI* sequences of *Neoperla*. All sequences comprised the full 658 bp barcode region and were deposited in the NCBI system (Table 2). Maximum likelihood (ML) analyses yielded highly congruent phylogenetic topologies. Both new species formed well-supported monophyletic clades (Fig. 1), providing robust support for their taxonomic distinctiveness. Moreover, interspecific genetic distances (3.13%–22.18%) between the two new species and their congeners were higher than their respective intraspecific maxima (0.16% for *N. foping* sp. nov. and 1.30 % for *N. huangi* sp. nov.; Table 3), supporting the effectiveness of *COI* as a DNA barcode for species discrimination.

Table 2. List of specimens sequenced in this study used for phylogenetic analysis. Species studied, isolate numbers, location and GenBank accessions.

Species	Voucher ID	Sex	Province	Accession
<i>Neoperla bilineata</i>	PC144	♂	Guangxi	PX504991
<i>Neoperla huangshana</i>	HISTSY0486	♂	Anhui	PX504989
<i>Neoperla huangshana</i>	HISTSY0487	♂	Anhui	PX504990
<i>Neoperla sinensis</i>	HISTSY0341	♂	Fujian	PX504987
<i>Neoperla sinensis</i>	HISTSY0344	♂	Fujian	PX504988
<i>Neoperla baishuijiangensis</i>	HISTSY0406	♂	Shaanxi	PX504994
<i>Neoperla baishuijiangensis</i>	HISTSY0408	♀	Shaanxi	PX504995
<i>Neoperla baishuijiangensis</i>	HISTSY0407	♀	Shaanxi	PX504996
<i>Neoperla caii</i>	HISTSY0348	♀	Sichuan	PX504997
<i>Neoperla caii</i>	HISTSY0349	♂	Sichuan	PX504998
<i>Neoperla foping</i> sp. nov.	HISTSY0365	♂	Shaanxi	PX505001
<i>Neoperla foping</i> sp. nov.	HISTSY0366	♂	Shaanxi	PX505003
<i>Neoperla huangi</i> sp. nov.	C90	♂	Hunan	PX505005
<i>Neoperla huangi</i> sp. nov.	C91	♀	Hunan	PX505004
<i>Neoperla magisterchoui</i>	HISTSY0235	♂	Shaanxi	PX504999
<i>Neoperla magisterchoui</i>	HISTSY0237	♂	Shaanxi	PX505000
<i>Neoperla quadrata</i>	C93	♂	Yunnan	PX585047
<i>Neoperla xuansongae</i>	C65	♂	Guangxi	PX585046

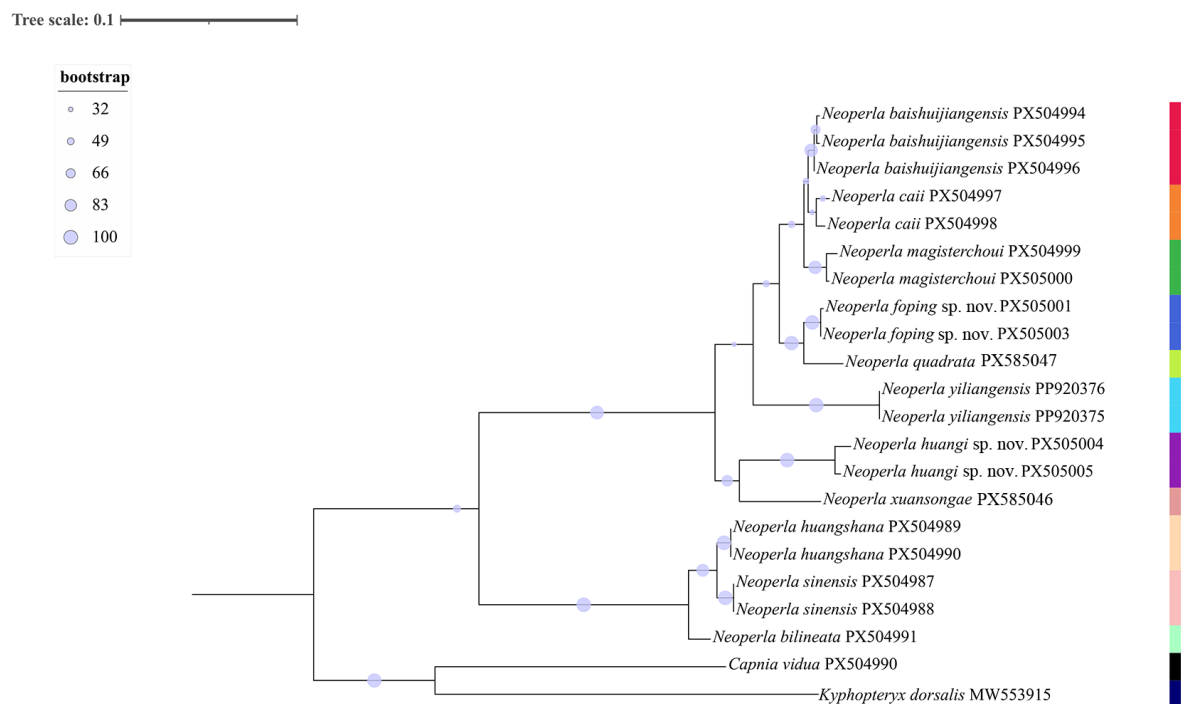


Fig. 1. Phylogenetic relationships among sequenced specimens based on Maximum Likelihood analysis. The scale bar indicates the number of substitutions per site.

Table 3. Genetic distances among all sequenced specimens. Values in bold indicate the minimum (3.13%) and maximum (22.18%) interspecific genetic distances, and the intraspecific genetic distances of the two new species (0.16% in *N. foping* Wang, Wang & Li sp. nov. and 1.30% in *N. huangi* Wang, Wang & Li sp. nov.).

	C93	C65	PX50 4994	PX50 4995	PX50 4996	PX50 4991	PX50 4997	PX50 4998	PX50 5001	PX50 5003	PX50 5004	PX50 5005	PX50 4989	PX50 4990	PX50 4999	PX50 5000	PX50 4987	PX50 4988
C93	\																	
C65	10.75	\																
PX504994	5.73	10.53	\															
PX504995	6.09	10.53	0.32	\														
PX504996	5.73	10.14	0.32	0.32	\													
PX504991	19.92	20.58	21.07	21.07	20.61	\												
PX504997	6.44	11.30	1.96	1.96	1.63	20.17	\											
PX504998	6.45	10.53	1.63	1.63	1.30	20.15	1.30	\										
PX505001	3.30	10.14	4.84	4.84	4.49	20.33	5.01	5.01	\									
PX505003	3.13	9.94	4.66	4.66	4.31	20.10	4.83	4.84	0.16	\								
PX505004	11.87	10.18	10.86	10.86	10.47	21.92	11.23	10.47	10.66	10.47	\							
PX505005	11.89	9.42	11.26	11.26	10.87	22.18	11.64	10.48	11.07	10.87	1.30	\						
PX504989	19.68	20.56	21.27	21.27	20.81	3.48	20.38	20.81	19.63	19.41	20.97	21.68	\					
PX504990	19.68	20.56	21.27	21.27	20.81	3.48	20.38	20.81	19.63	19.41	20.97	21.68	0.00	\				
PX504999	5.37	10.12	2.97	2.97	2.63	21.04	3.30	3.31	5.18	5.01	11.63	11.64	21.01	21.01	\			
PX505000	5.37	10.12	2.46	2.46	2.12	19.95	2.79	2.80	4.83	4.66	11.23	11.25	20.15	20.15	0.81	\		
PX504987	19.70	20.13	21.76	21.76	21.30	3.66	20.86	20.84	20.10	19.88	21.92	21.71	1.79	1.79	21.50	20.63	\	
PX504988	19.70	20.13	21.76	21.76	21.30	3.66	20.86	20.84	20.10	19.88	21.92	21.71	1.79	1.79	21.50	20.63	0.00	\

Taxonomy

Class Insecta Linnaeus, 1758
Order Plecoptera Burmeister, 1839
Family Perlidae Latreille, 1802
Subfamily Perlinae Latreille, 1802
Tribe Neoperlini Enderlein, 1909

Genus *Neoperla* Needham, 1905

Neoperla Needham, 1905: 108. Type species: *Perla occipitalis* Pictet, 1841

Diagnosis

Neoperla is unequivocally distinguished from all other Perlidae genera by the anterior portion of the hemitergal lobe distinctly slender and process-like, and tergite 7 of the male genitalia modified in structure, together with paired lateral sclerotized knobs projecting from the penial base.

Neoperla foping Wang, Wang & Li sp. nov.

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Figs 2A, 3–5

Diagnosis

Head with a brown triangular spot on the anterior frons and an oval dark brown spot over the ocelli; hemitergal lobe very short; penis tube shorter than endophallus, endophallus with one prominent spine patch subapically and 2–3 longitudinal ventral rows of large spines.

Etymology

The specific epithet ‘foping’ is derived from Foping National Nature Reserve (Shaanxi Province, China), the type locality. The name is used as a noun in apposition and is gender-neutral.

Material examined

Holotype

CHINA • ♂; Shaanxi Province, Hanzhong City, Foping National Nature Reserve, Liangfengya; 33°41'46" N, 107°54'52" E; alt. 1555 m; 12 Jul. 2023; light trap; Weihai Li, Yao Zhang and Gang Yao leg.; GenBank no.: PX505003; HIST, PLE035.

Paratypes

CHINA • 1 ♂; same data as for holotype; GenBank no.: PX505001; NHMC • 9 ♀♀; same locality as for holotype; 28 Jul. 2026; Zhishan Cao leg.; NHMC • 6 ♀♀; same data as for preceding; HIST.

Description

Male

ADULT HABITUS (Figs 2A, 3A). Forewing length ca 16.7–17.2 mm, hindwing length ca 14.1–14.8 mm (N=2). Head mostly light brown, with a smaller brown triangular spot on anterior frons and a dark elliptical spot over the ocelli; M-line pale; antennae dark brown (Figs 2A, 3A). Distance between ocelli nearly as wide as the diameter of one ocellus. Pronotum brown, median area with a darker stripe and scattered rugosities (Figs 2A, 3A). Wing membrane clear, light gray, with veins brown (Fig. 2A). Legs mostly brownish, with femur-tibia joint and tarsi dark brown. Abdomen brown, sclerites of terminal segments 7–10 darker.

TERMINALIA (Fig. 3B–D). Tergum 7 with a subquadrate process on posterior margin, covered with several sensilla basiconica. Tergum 8 with an upcurved tongue-shaped process medially, with several small spines apically (Fig. 3B–C). Tergum 9 with few sensilla on posterior margin. Hemitergal processes of tergum 10 strongly sclerotized, very short, finger-like, and upcurved at mid-length (Fig. 3B–C).

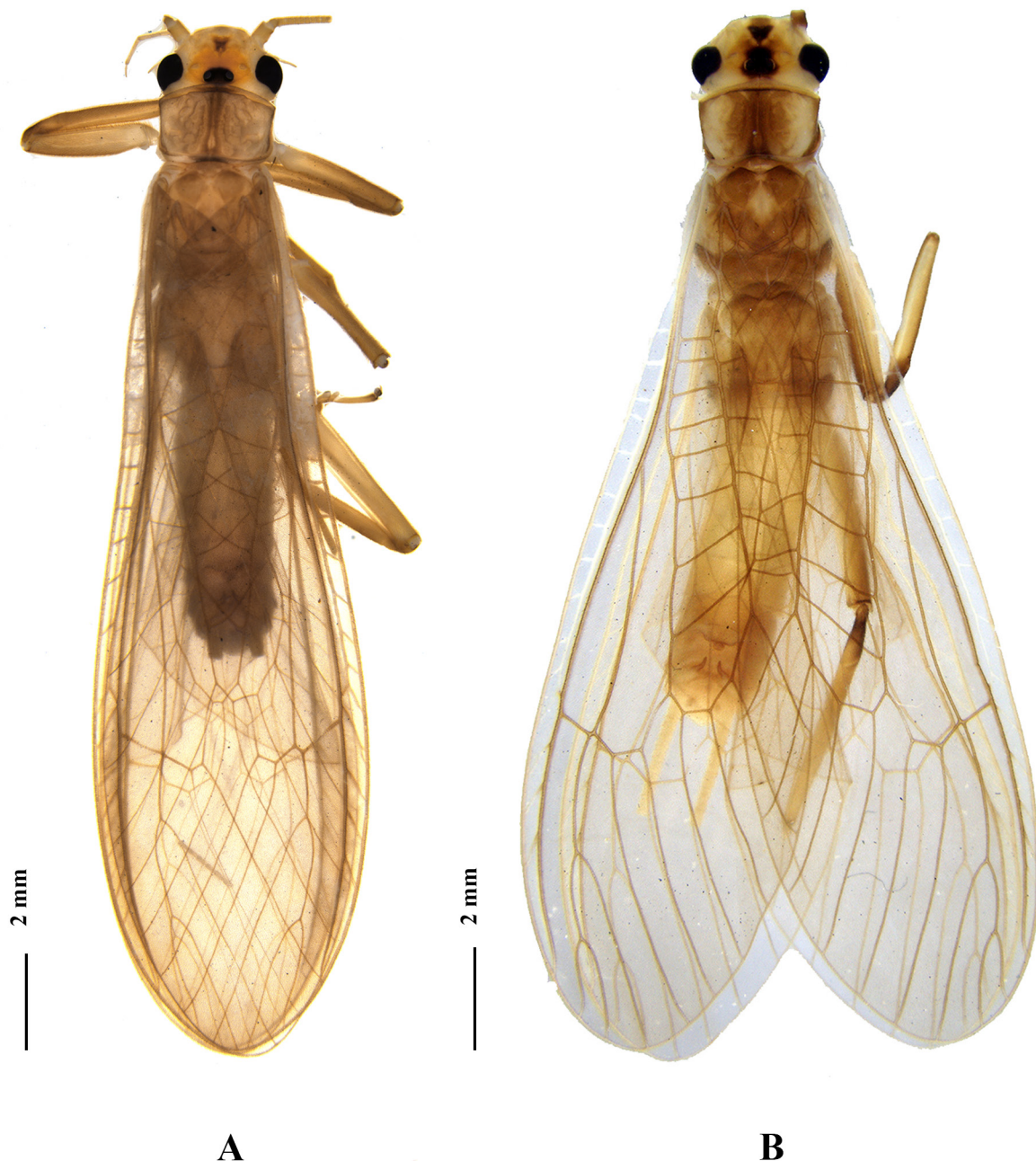


Fig. 2. Habitus, ♂. **A.** *Neoperla foping* Wang, Wang & Li sp. nov., holotype (HIST, PLE035). **B.** *Neoperla huangi* Wang, Wang & Li sp. nov., holotype (HIST, PLE036).

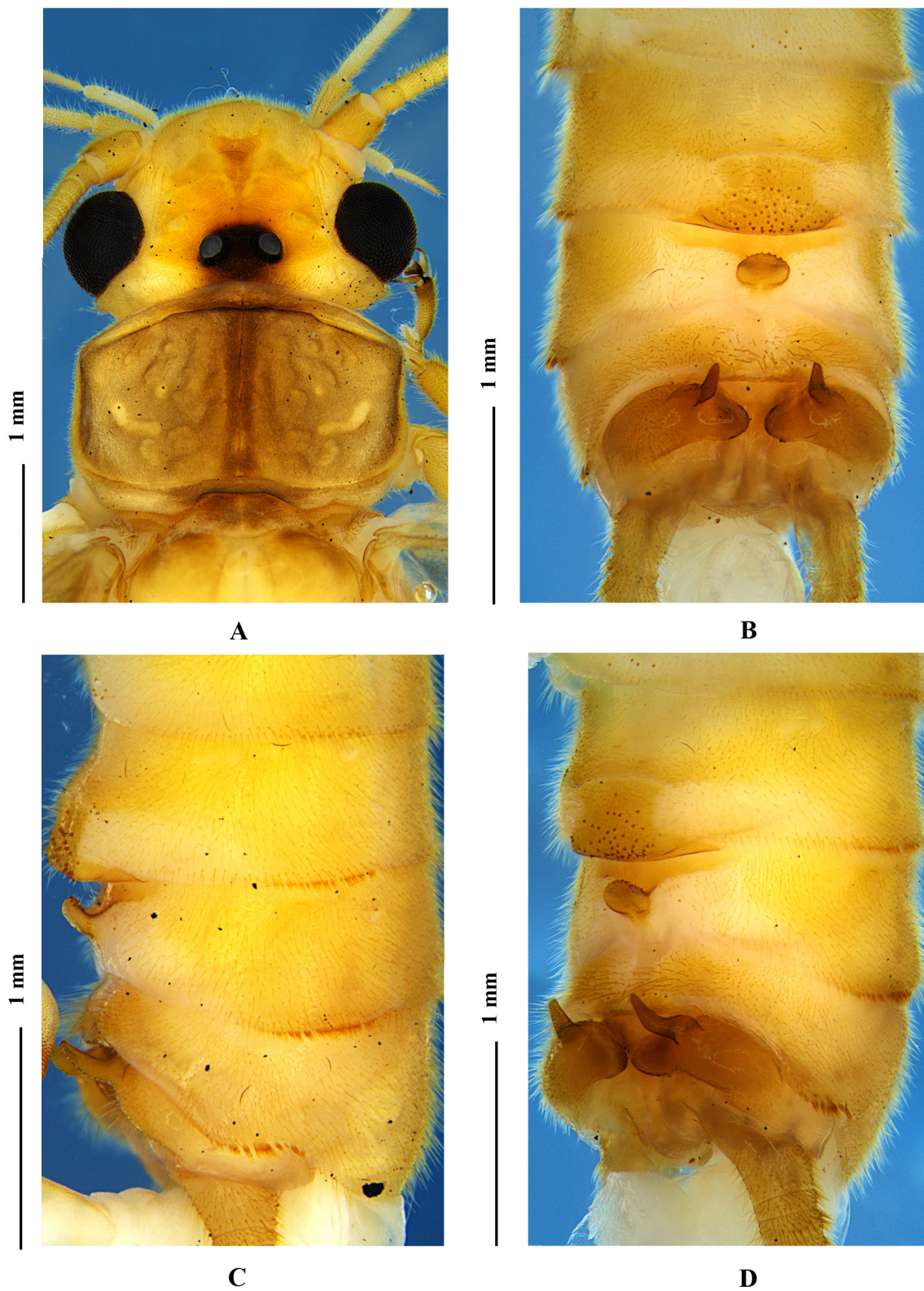


Fig. 3. *Neoperla foping* Wang, Wang & Li sp. nov. ♂, holotype (HIST, PLE035). **A.** Head and pronotum, dorsal view. **B.** Terminalia, dorsal view. **C.** Terminalia, lateral view. **D.** Terminalia, dorsolateral view.

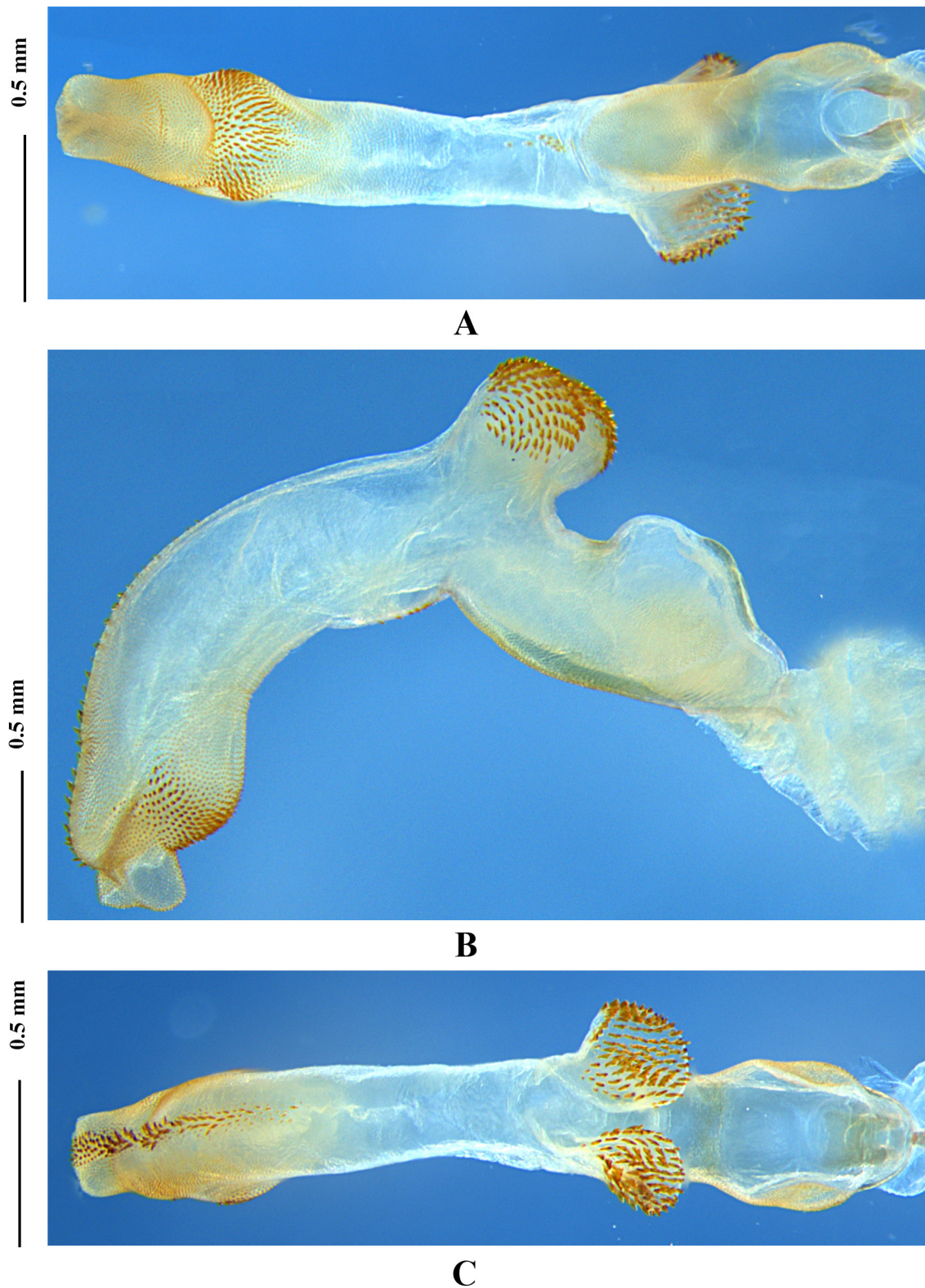


Fig. 4. *Neoperla foping* Wang, Wang & Li sp. nov. ♂, holotype (HIST, PLE035). **A.** Penis, dorsal view. **B.** Penis, lateral view. **C.** Penis, ventral view.

PENIS (Fig. 4). Penis tube plump and lightly sclerotized, distal ventral surface with a pair of large spinous lobes; penis tube surface almost bare (Fig. 4A–C). Endophallus about $1.5 \times$ as long as tube, distinctly curved dorsad, with a short and gradually enlarged apex (Fig. 4B). Endophallus armatures comprise spines of various sizes, with the largest spines concentrated on the ventral surface, forming two or three longitudinal rows of spines extending from the middle to the apex (Fig. 4B–C); dorsal surface with a plump spinous patch near the apex and a small spine patch consisting of 9–10 spines basally (Fig. 4A–B).

Female

ADULT HABITUS (Fig. 5). Forewing length ca 17.9–18.6 mm, hindwing length 15.4–16.2 mm (N=5). General color pattern similar to males (Fig. 5A).

TERMINALIA (Fig. 5B). Posterior margin of sternum 8 slightly produced, posteromedial part forming a small subrectangular subgenital plate. The sclerites large, median cleft with base fused. The vagina membranous, with slightly sclerotized folds (Fig. 5C).

Egg

Unknown.

Distribution

China: Shaanxi Province. Presently only known from Foping National Nature Reserve.

Remarks

The new species seems similar to *N. quadrata* Wu & Claassen, 1934, sharing a similar head pattern and a plump spinous patch on the dorsal surface of the endophallus subapically. However, *N. foping* sp. nov. differs from *N. quadrata* in having an enlarged tip (vs gradually tapering to triangular tip, see Murányi *et al.* 2015: fig. 35). In addition, the endophallus of *N. foping* sp. nov. is about $1.5 \times$ as long as the penis tube (vs subequal to *N. quadrata*, see Murányi *et al.* 2015: figs 34–37). The short and gradually enlarged apex of the endophallus in *N. foping* distinguishes it from all other congeners in the *N. (F.) lushana*-group.

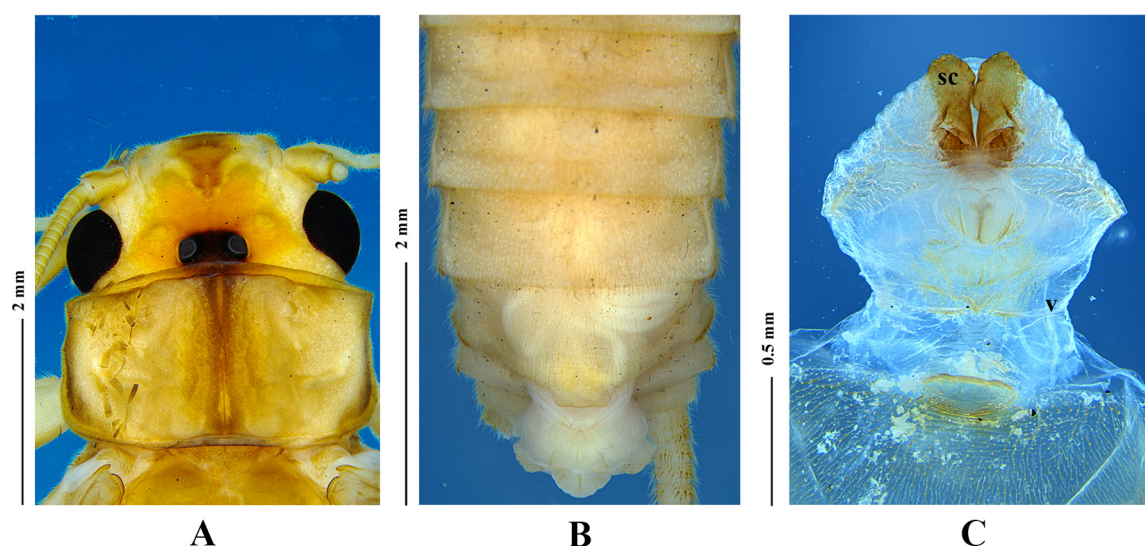


Fig. 5. *Neoperla foping* Wang, Wang & Li sp. nov. ♀, paratype (NHMC). **A.** Head and pronotum, dorsal view. **B.** Terminalia, ventral view. **C.** Vagina, ventral view. Abbreviations: sc = sclerite; v = vagina.

Neoperla huangi Wang, Wang & Li sp. nov.

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Figs 2B, 6–8

Diagnosis

Head with a dark triangular spot on the anterior frons and a dark brown quadrangular spot over the ocelli; endophallus with one prominent spine patch subapically on the dorsal surface, a pair of small patches consisting of seven or eight spines subapically and two or three longitudinal ventral rows of large spines on ventral surface.

Etymology

The specific epithet '*huangi*' is a patronym honoring Dr Huang [Guo-hua Huang], collector of the holotype.

Material examined

Holotype

CHINA • ♂; Hunan Province, Zhangjiajie City, Sangzhi County, Badagongshan Town, Doupengshan Village, Mts Doupeng management station; 29°40'44" N, 109°43'50" E; alt. 1785 m; 24–26 Jun. 2020; Guohua Huang, Chengqing Liao and Min Deng leg.; GenBank no.: PX505005; HIST, PLE036.

Paratypes

CHINA • 3 ♀♀; same data as for holotype; GenBank no.: PX505004; NHMC.

Description

Holotype male

ADULT HABITUS (Figs 2B, 6A). Forewing length 13.0 mm, hindwing length 11.4 mm. General body color light brown. Head mostly yellowish-brown, with a dark quadrangular spot over the ocelli and a dark triangular spot anteromedially on frons; M-line pale; antennae dark brown (Figs 2B, 6A). Distance between ocelli almost as wide as the diameter of the ocellus. Pronotum brown, median area with darker stripe and scattered lateral rugosities; wing membrane brown with darker veins. Legs mostly brownish, with femur-tibia joint dark brown (Fig. 2B). Scutum and scutellum of meso- and metathoracic nota dark brown, background lighter. Abdomen brown, with terminal segments darker (Fig. 2B).

TERMINALIA (Fig. 6B–D). Tergum 7 with a subquadrate process on posterior margin, covered with many sensilla basiconica (Fig. 6B). Tergum 8 with an upcurved tongue-shaped process, with several small spines distally. Tergum 9 without sensilla patches (Fig. 6B–C). Hemitergal processes of tergum 10 strongly sclerotized, short, and slightly bent ventrally at the mid-length (Fig. 6B–D).

PENIS (Fig. 7). Penis tube plump, with a sclerotized dorsal surface and a pair of spinous apical projections on the ventral surface (Fig. 7). Endophallus approximately 2 × as long as penis tube, curved dorsad at mid-length, with the distal half nearly parallel-sided in lateral view (Fig. 7A–B). Endophallus armatures bear spines of various sizes, with a plump spinous patch on the dorsal surface near apex (Fig. 7A–B); ventral surface with a pair of paramedian spinous patches near the apex, and 2–3 rows of median spines extending from basal 1/3 to apex (Fig. 7B–C).

Female

ADULT HABITUS (Fig. 8). Forewing length 18.4 mm, hindwing length 16.2 mm. General color pattern similar to that of males (Fig. 8A).

TERMINALIA (Fig. 8B–C). Posterior margin of sternum 8 slightly produced, posteromedial part forming a small triangular subgenital plate. The vagina hook-shaped and membranous, spermathecal stalk slender (Fig. 8B–C). Vagina with curved sclerites on the posterior margin. Curved spermathecal stalk (SSt) with numerous longitudinal folds along the anterior margin, densely covered with brown scales. The spermathecal duct (sd) slender. The spermatheca (sa) sausage-shaped, constricted medially, with pale internal spicules and an accessory gland.

Egg

Egg oval, ca 0.45–0.46 mm long and 0.34–0.38 mm wide (Fig. 8D). Collar and anchor absent. Chorion uniformly thin and seemingly smooth. Micropyles invisible (Fig. 8D).

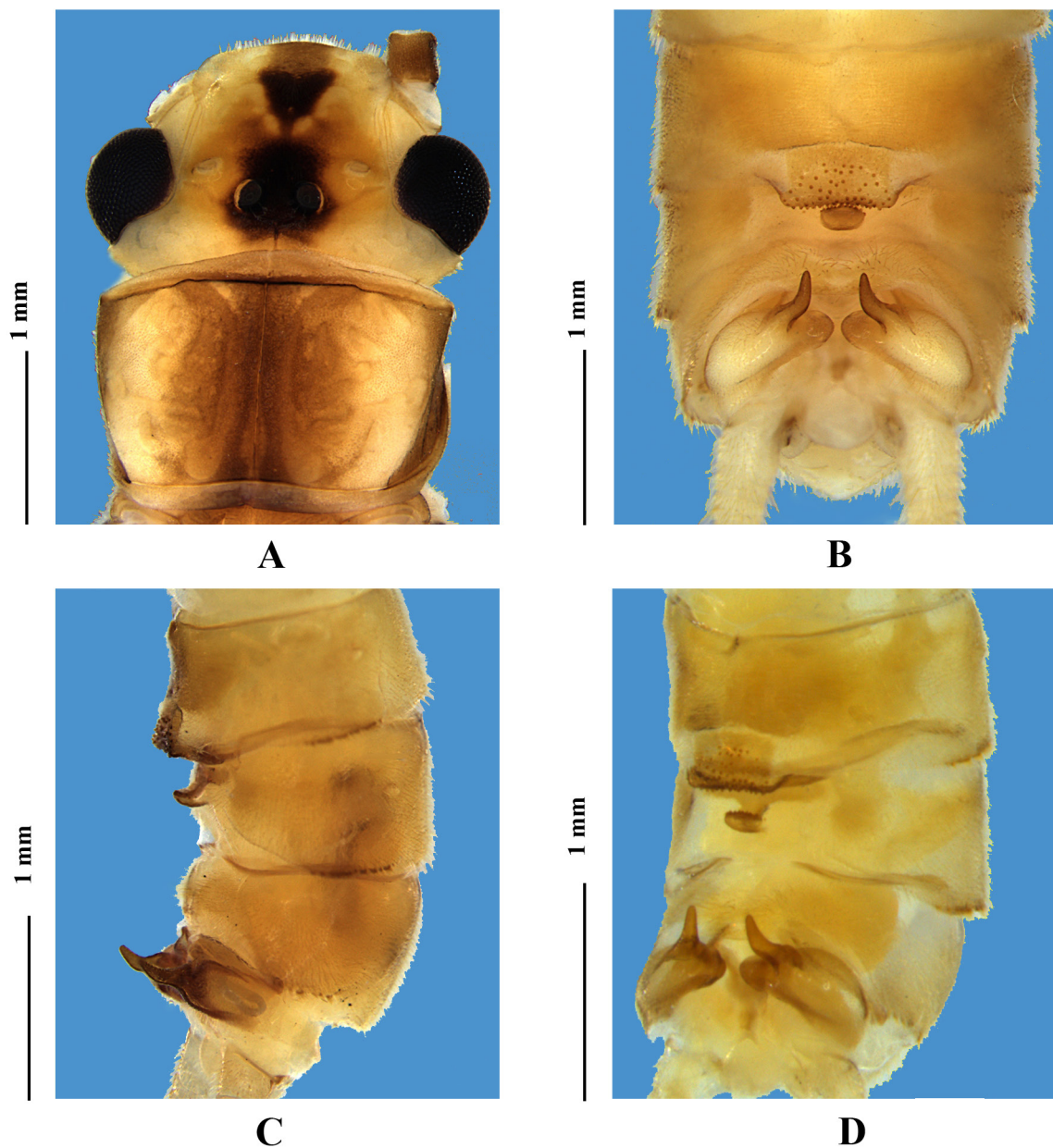


Fig. 6. *Neoperla huangi* Wang, Wang & Li sp. nov. ♂, holotype (HIST, PLE036). **A.** Head and pronotum, dorsal view. **B.** Terminalia, dorsal view. **C.** Terminalia, lateral view. **D.** Terminalia, dorsolateral view.

Distribution

China: Hunan Province. Presently only known from Doupengshan Village.

Remarks

The new species resembles *N. xuansongae* in sharing a similar head pattern and a pair of ventral spines near the apex of the endophallus. However, *N. huangi* sp. nov. differs from *N. xuansongae* by the absence of a small basal spinulose patch on the dorsal surface of the endophallus (vs patch present basally, see Qin *et al.* 2013: fig. 8), and by having a longitudinal ventral spinulose patch extending from the apex to $\frac{2}{3}$ of the endophallus (vs median patch extending from apex to about $\frac{1}{3}$ apically, see Li *et al.* 2013: fig. 8). The endophallus of *N. huangi* lacks any additional projections and has a nearly parallel-sided distal half, which distinguishes it from all other congeners in the *N. (F.) lushana*-group.

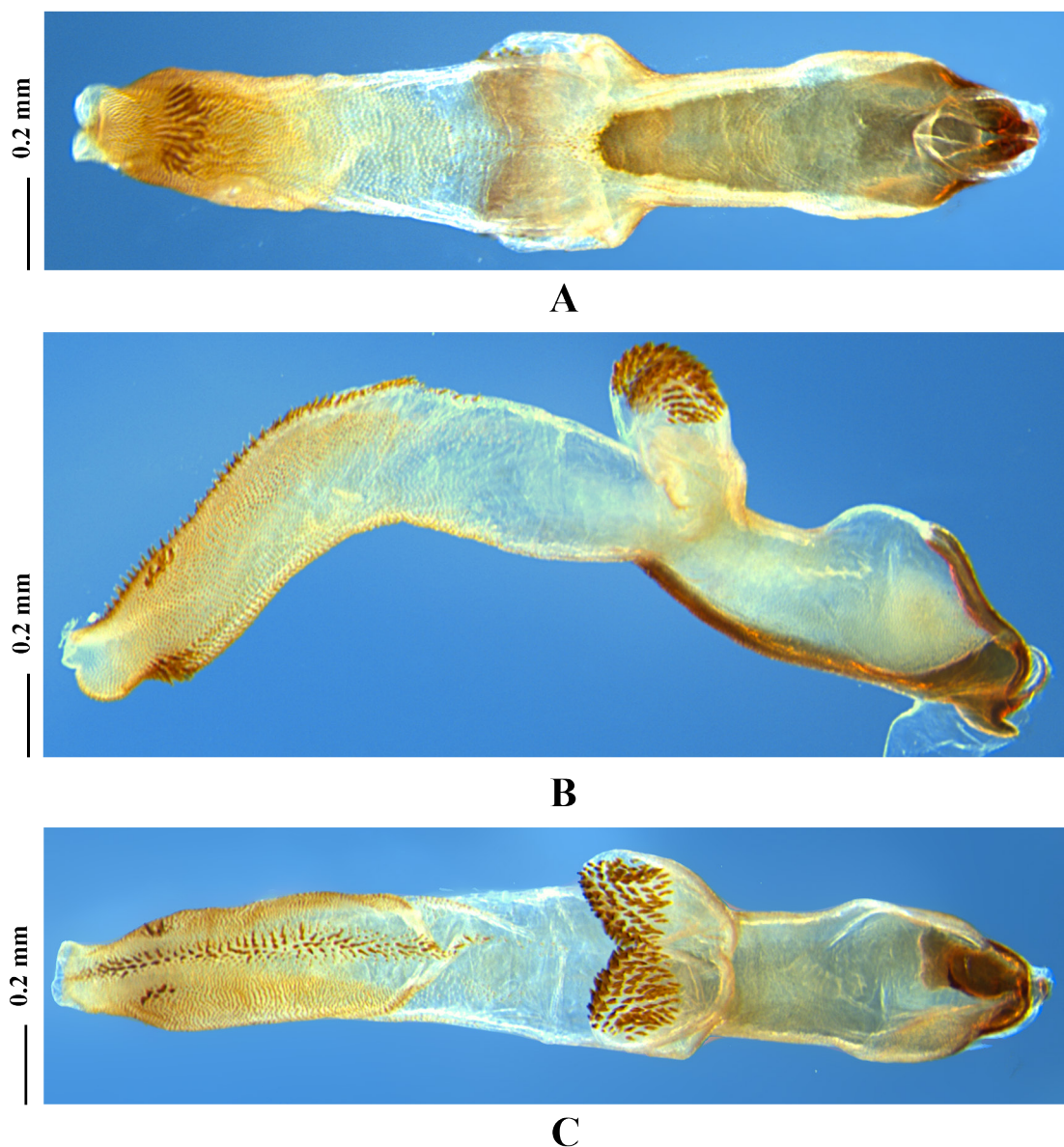


Fig. 7. *Neoperla huangi* Wang, Wang & Li sp. nov. ♂, holotype (HIST, PLE036). A. Penis, dorsal view. B. Penis, lateral view. C. Penis, ventral view.

Discussion

Neoperla is the most species-rich genus in the subfamily Perlinae Latreille, 1802 (Plecoptera: Perlidae), exhibiting a broad distribution across the Holarctic and Oriental realms. Its range encompasses East and Southeast Asia, South Asia, parts of Europe, western North America, and eastern and southern Africa (Zwick 2023). Since its establishment, the taxonomy of *Neoperla* has undergone successive refinements through the contributions of numerous authors. The nomenclatural foundation was laid by Banks (1892), Needham (1905), and Klapálek (1907, 1913); subsequent morphological revisions by Wu (1935, 1938, 1962, 1973), Claassen (1940), Zwick (1973), and Sivec *et al.* (1988) substantially shaped the current delimitation and internal classification of the genus. More recently, integrative approaches combining molecular phylogenetics with morphological data, particularly those of Chen *et al.* (2020), Mo *et al.* (2022, 2024), Zwick (2023), and Zwick & Zwick (2023), have provided robust support for a clade-based framework, resulting in the recognition of several well-defined species groups within *Neoperla*.

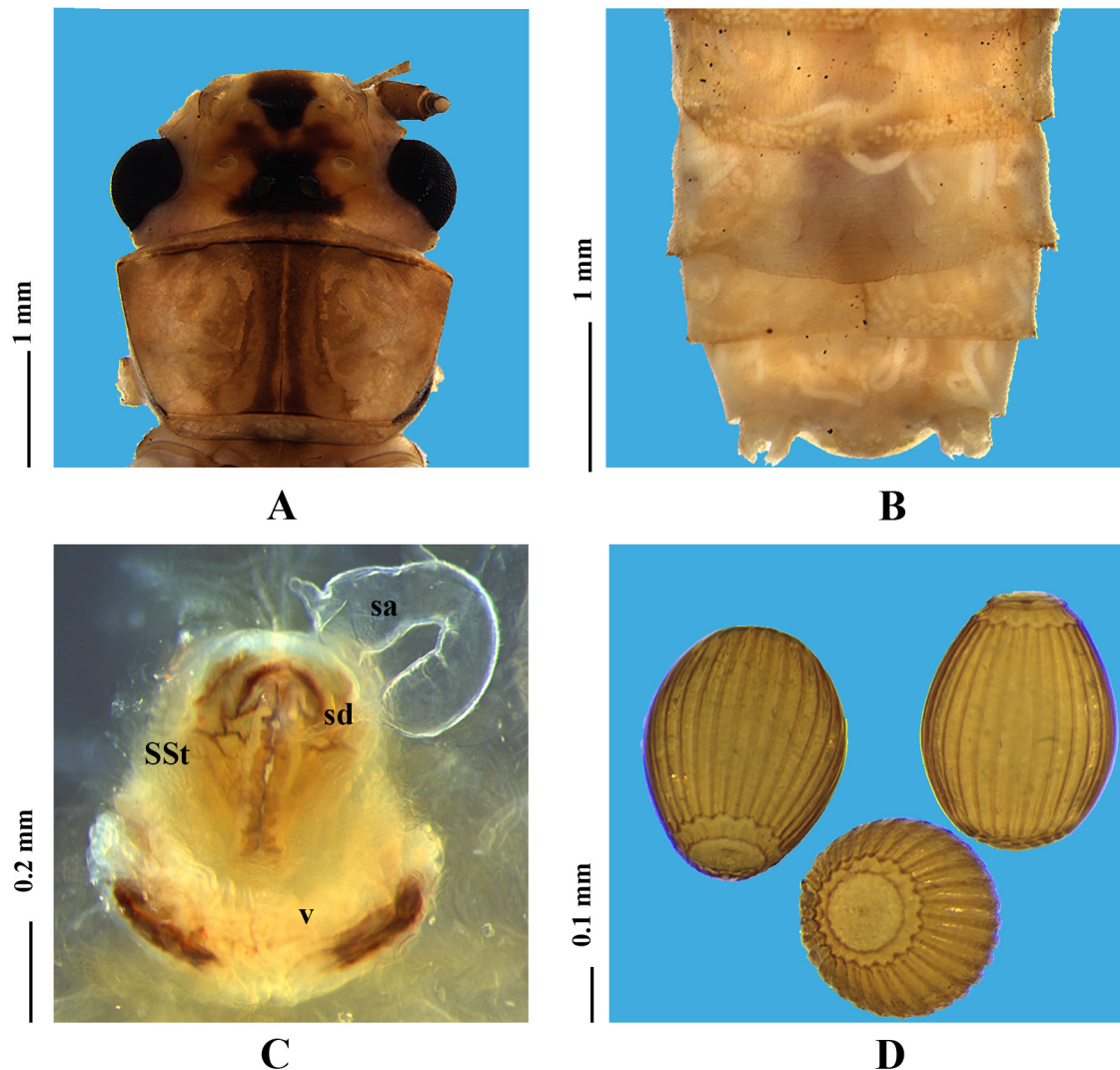


Fig. 8. *Neoperla huangi* Wang, Wang & Li sp. nov. ♀, paratype (NHMC). **A.** Head and pronotum, dorsal view. **B.** Terminalia, ventral view. **C.** Vagina, ventral view. **D.** Eggs. Abbreviations: sa = spermatheca; sd = spermathecal duct; SSSt = spermathecal stalk; v = vagina.

The *Neoperla* (*Formosita*) *lushana*-group is characterized by elongate terminal filaments, a slender epiproct, a pair of quadrangular processes on the posterior margin of tergum 7, and a distinctive pair of membranous lobes on the ventral surface of the penis. The *lushana*-group was recognized by Zwick & Sivec (1980) and recently assigned to the *N. (Formosita)* subgenus by Zwick (2023).

In this study, both morphological diagnostics and molecular phylogenetic analyses based on the mitochondrial *COI* gene strongly support the validity of two newly described species, *N. foping* sp. nov. and *N. huangi* sp. nov. Furthermore, our phylogenetic reconstruction including representative taxa from the *N. (F.) sinensis*-group, reveals that *N. bilineata* consistently clusters within a highly supported monophyletic clade comprising *N. sinensis* and its close relatives. This result is congruent with findings reported by Li & Yan (2021), reinforcing its robustness. Notably, this placement conflicts with the previous assignment of *N. bilineata* to the *N. (F.) lushana*-group by Zwick (2023). Given the strong molecular and combined evidence, we herein transfer *N. bilineata* from the *N. (F.) lushana*-group to the *N. (F.) sinensis*-group. This reclassification improves the monophyly and diagnostic coherence of both groups and highlights the importance of integrating molecular data to resolve taxonomic ambiguities in morphologically conservative lineages.

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Data availability

The mitochondrial *COI* sequences generated in this study have been deposited in GenBank.

Author contribution

Conceptualization: YYW, WHL. Data curation: YYW. Funding acquisition: WHL. Methodology: WHL, GQW. Validation: WHL, DM. Writing – original draft: YYW, WHL. Writing – review & editing: WHL, GQW, DM.

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