

Molecular Phylogenetics, Phylogenomics, and Phylogeography

A New Family of Stoneflies (Insecta: Plecoptera), Kathroperlidae, fam. n., with a Phylogenomic Analysis of the Paraperlinae (Plecoptera: Chloroperlidae)

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Abstract

Recent molecular analyses of transcriptome data from 94 species across 92 genera of North American Plecoptera identified the genus *Kathroperla* Banks, 1920 as sister group to Chloroperlidae + Perlodidae. Given that the genus *Kathroperla* has historically been included as a member of the family Chloroperlidae, this discovery indicated further investigation of the genus and the subfamily Paraperlinae was needed. Both transcriptome and genome sequencing datasets were generated from 32 species of the infraorder Systellognatha, including all described species of the Paraperlinae, to test the phylogenetic placement of these taxa. From these datasets, a large phylogenomic data matrix of 800 orthologous genes was produced, and multiple analyses were conducted, including both concatenated and coalescent analyses. Morphological comparisons were made among all Paraperlinae using light microscopy. All molecular results support a monophyletic *Kathroperla*, which is supported as sister taxon to the remaining Perloidea by five of six molecular analyses. Postocular head length is determined to be a distinct morphological character of this genus. Combined molecular and morphological evidence support the designation of Kathroperlidae, fam. n., as the seventeenth family of extant Plecoptera.

Key words: Kathroperlidae, *Kathroperla*, Plecoptera, Paraperlinae, new family

The Chloroperlidae [Okamoto, 1912](#) are typically small, delicate stoneflies (Plecoptera) with adult coloration ranging from pale yellow to bright green, though some species are of moderate size and/or with dark coloration ([Surdick 1985](#)). Adults are highly susceptible to desiccation and seldom travel far from the protective riparian vegetation of their emergence sites. Nymphs of most species utilize the interstices of coarse-bottomed streams and rivers, or shallow hyporheic habitat. A few species spend the majority of their life cycle as nymphs deep in alluvial aquifers up to one km or more from the shoreline ([Stanford and Ward 1993](#)). Chloroperlid

morphology is characterized by oval pronota, slender elongate bodies, and cerci shorter than abdominal length.

The Chloroperlidae have a Holarctic distribution, with 204 described extant species across 20 genera ([DeWalt et al. 2020](#)). Two chloroperlid subfamilies are recognized: Chloroperlinae [Okamoto, 1912](#) and Paraperlinae [Ricker, 1943](#), containing 194 and 10 described extant species, respectively ([DeWalt et al. 2020](#)). Three tribes comprise the Chloroperlinae: Alloperlini [Surdick, 1985](#), Chloroperlini [Okamoto, 1912](#), and Suwalliini [Surdick, 1985](#).

The genus *Kathroperla* Banks, 1920 (Plecoptera: Chloroperlidae) was proposed with the type species *K. perdita* Banks, 1920, described from a single female specimen from British Columbia, Canada. The genus remained monotypic until the description of *K. takhoma* Stark and Surdick, 1987 from Washington, USA. The genus was considered a Nearctic endemic until the description of *K. doma* Stark, 2010 from the Republic of Korea. A fourth species, *K. siskiyou* Stark and Kondratieff, 2015, was described from southern Oregon, USA.

Kathroperla (Fig. 1A), along with the genera *Paraperla* Banks, 1906 (Plecoptera: Chloroperlidae) (Fig. 1B) and *Utaperla* Ricker, 1952 (Plecoptera: Chloroperlidae) (Fig. 1C), is currently classified (Stark et al. 2020) within the Paraperlinae. Adults of the Paraperlinae are morphologically differentiated from the Chloroperlinae by an elongated head (Zwick 1973). Nymphs are distinguished by lacinia shape: quadrate in *Kathroperla* and triangulate in *Utaperla*, *Paraperla*, and Chloroperlinae (Stewart and Stark 2002). *Kathroperla* adults are distinguished among the genera of Paraperlinae by the presence of a well-developed vesicle on the ninth male sternite (Zwick 2000) and postocular elongation of the head (Baumann et al. 1977) (Fig. 2A). The tuberculate chorion of the ova has also been noted as distinct among the Chloroperlidae (Stark et al. 2015).

Adult morphological characters of *Kathroperla* are distinct among the stonefly families. The eyes are situated at the midpoint of an elongated head. Some Perlodidae Klapálek, 1909 and a few other chloroperlids have elongated heads, but the eyes are posterior to the midpoint. The anterodorsal margin of the epiproct of the male genitalia has a transverse orientation (Fig. 2B). This is contrasted with other taxa that have a button-like or longitudinal dorsal epiproct shape.

Chloroperlidae is one of three families in the superfamily Perloidea Latreille, 1802 (including Chloroperlidae, Perlidae Latreille, 1802, and Perlodidae) within the infraorder Systelognatha. Multiple maximum likelihood (ML) and multispecies coalescent (MSC) analyses performed by South et al. (2021) recovered a novel family-level phylogenetic relationship among the Perloidea: *Kathroperla* Banks (1920) was removed from the Chloroperlidae and placed as sister to Perlodidae + the remaining Chloroperlidae. One of the ML analyses recovered *Kathroperla* as sister to all Perloidea, similar to the relationship recovered by Terry (2003).

In this study, we propose a new stonefly family, Kathroperlidae, fam. n. The morphology of *Kathroperla* and all Paraperlinae

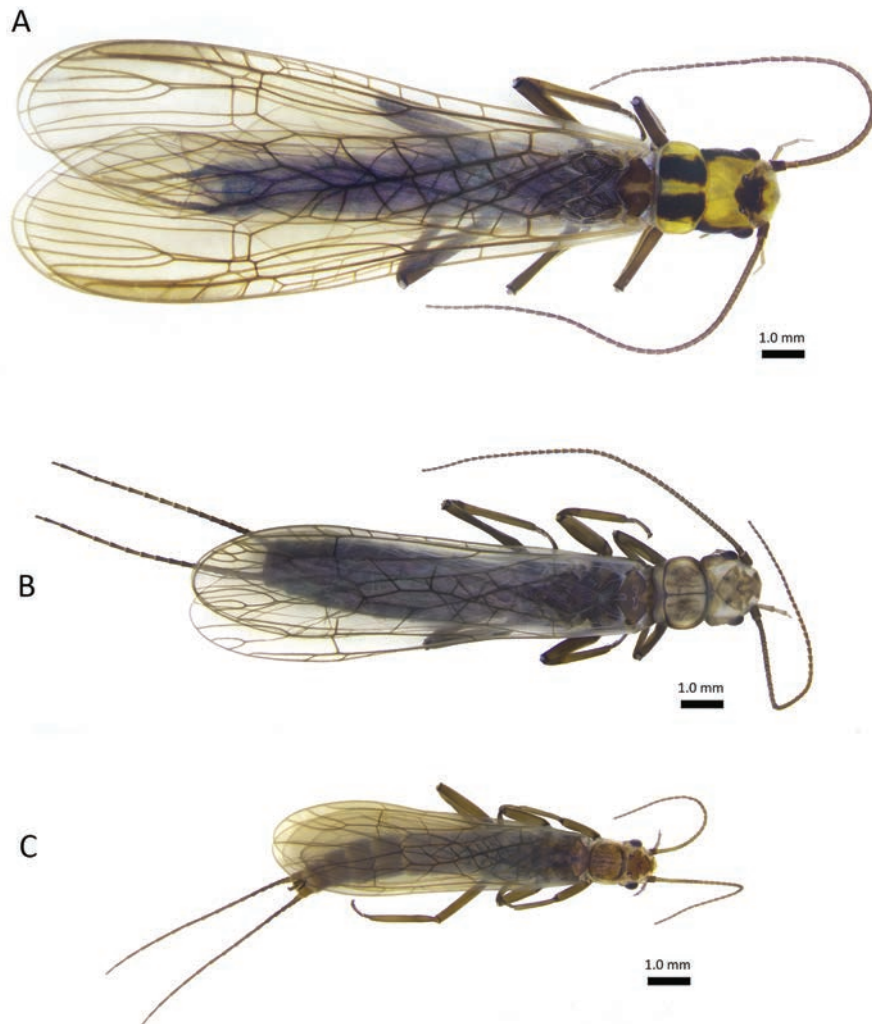


Fig. 1. Habitus of select Paraperlinae. (A) *Kathroperla takhoma*, male, Upper Willow Creek, California, USA (EJSC Ka_ta_01). (B) *Paraperla frontalis*, female, Sulphur Creek, California, USA (EJSC Pa_fr_02). (C) *Utaperla gaspesiana*, male, East Branch Delaware River, New York, USA (INHS Insect Collection 658549).

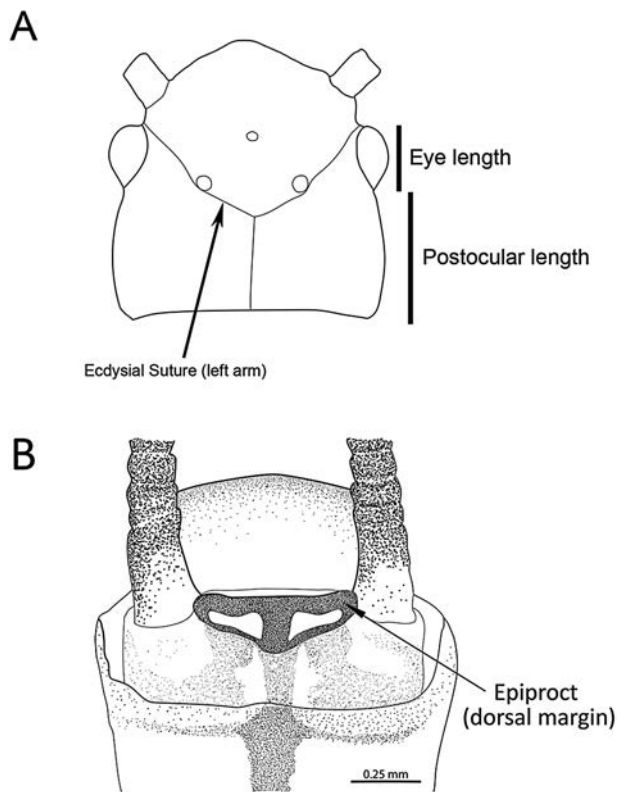


Fig. 2. *Kathroperla*, adult structures, dorsal. (A) *K. takhoma*, female, head, Boise Creek, California, USA (INHS Insect Collection 658554). (B) *K. perditia*, male, terminalia, Middle Fork Flathead River aquifer, Montana, USA (INHS Insect Collection 658497).

species is compared and new light microscopy images of key morphological features and color patterns provided. Phylogenetic trees from multiple molecular analyses of transcriptome and whole genomic data are presented for all species of the Paraperlinae and select outgroup taxa comprising the Systellognatha of North America.

Methods

Specimen Collection and Morphological Analyses

Adult specimens for all 10 described species of the Paraperlinae were obtained, including North American taxa collected by South et al. (2021) and newly acquired specimens from the western United States, Republic of Korea, and Russia (Table 1). Specimens were collected using a beating sheet, by handpicking, or by Malaise trapping, and subsequently preserved in 95% EtOH. Additional material was available from the Illinois Natural History Survey (INHS) Insect Collection in Champaign, Illinois (USA), the Colorado State University Collection (CSUC) in Fort Collins, Colorado (USA), and the personal collection of the Eric J. South Collection (EJSC).

Specimens were examined using a Nikon SMZ800 microscope and imaged with an Excelsis MPX-6C camera. Images were stacked and processed using Adobe Photoshop 2020. Complete intact single specimens were unavailable for *Kathroperla* and *Utaperla*. Therefore, detached structures from the most complete specimens were imaged separately and digitally stitched to their respective articulations. These modifications were performed to enhance the

following two habitus images: Fig. 1A, *K. takhoma* (left antenna stitched to scape; note: terminal segments of both cerci are missing); Fig. 1C, *U. gaspesiana* Harper & Roy, 1975 (right antenna stitched to scape; right metathoracic leg stitched to coxa; distal left cercus stitched to tenth cercal segment).

Molecular Study

Genomic DNA was extracted from seven Paraperlinae species [*K. doma*, *K. siskiyou*, *Paraperla wilsoni* Ricker, 1965, *Utaperla gaspesiana*, *U. lepnevae* (Zhiltzova, 1970), *U. orientalis* Nelson & Hanson, 1969, *U. sopladora*, Ricker, 1952] using the Qiagen Blood and Tissue extraction kit (Qiagen, Valencia, CA), following the manufacturer's protocol. DNA was extracted from excised thoracic tissue of the larger *Kathroperla* and *Paraperla* specimens and from the entire thorax of the smaller *Utaperla* specimens. Remaining unexcised tissue, including abdominal terminalia, was preserved in 95% EtOH and accessioned as specimen voucher material into the INHS Insect Collection or the CSUC. DNA samples were measured for concentration with Qiagen Probit and checked for quality with gel electrophoresis.

The seven genomic DNA samples were processed at the W. M. Keck Center for Comparative and Functional Genomics (Keck Center) at the University of Illinois at Urbana-Champaign, Illinois (UIUC). Shotgun genomic libraries were constructed via the Hyper Library construction kit from Kapa Biosystems (Wilmington, MA) and 150-bp paired-end reads were sequenced via an Illumina NovaSeq 6000 sequencer (Illumina, San Diego, CA). Raw reads were submitted to the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI). Raw reads received from the Keck Center were reviewed for quality using FASTQC (Andrews 2010). Trimming of raw reads was performed in two steps. First, bases with quality scores below 35 were removed using the fastq_quality_trimmer tool from the FASTX-Toolkit 0.0.14 (Gordon and Hannon 2010). Following this step, the FASTX-Toolkit tool fastx_trimmer was used to remove the first ten bases of each read to reduce noise.

Assembly of Target Orthologs

A targeted assembly method was used to avoid the computational requirements of full genome assembly and annotation. The software aTRAM (Automated Target Restricted Assembly Method) v2.3.3 (Allen et al. 2018) was used for this purpose, and requires a set of reference sequences for targeted assembly. To build this reference sequence set, a 'best ortholog' set for the chloroperlid taxa was generated from 10 chloroperlid taxa (seven Chloroperlinae; three Paraperlinae) that were included in the dataset generated in South et al. (2021). For each ortholog included in the original dataset, the longest sequence with the fewest ambiguities (undetermined X residues in the sequence) was selected from these ten species to serve as the reference sequence for that ortholog.

The aTRAM libraries for each taxon were constructed from the trimmed Illumina reads using the atram_preprocessor.py script. After library construction, assemblies were performed using the atram.py script with the abyss assembler option and 10 iterations. The atram_stitcher.py script was then used to obtain the final assembled sequences for downstream analyses. Amino acid sequences for the assembled nucleotide sequences were obtained using TransDecoder v5.5.0 (Haas and Papanicolaou 2018). In total, 1,445 orthologs were obtained following these procedures and were included in the alignment and trimming steps.

Table 1. Stonefly specimens used in phylogenetic analyses

Species	INHS/CSUC	Sex	Locality	Lat./Lon.	Ortho.
Chloroperlinae					
<i>Alloperla usa</i> Ricker, 1952	793088	♂	USA: VA	36.96919, -80.16414	738
<i>Haploperla brevis</i> (Banks, 1895)	658505	♀	USA: IN	39.30893, -86.72016	770
<i>Plumiperla diversa</i> (Frison, 1935)	658536	♂	USA: CO	40.57301, -105.86258	788
<i>Sasquaperla hoopa</i> Stark & Baumann, 2001	658483	♀	USA: CA	40.94097, -123.66079	755
<i>Suwallia pallidula</i> (Banks, 1904)	793178	♂	USA: CO	39.98273, -105.45035	763
<i>Sweltsa lamba</i> (Needham & Claassen, 1925)	658442	♂	USA: WY	41.37398, -106.25403	739
<i>Triznaka signata</i> (Banks, 1895)	658433	♂	USA: CO	40.59895, -105.08371	780
Paraperlinae					
<i>Kathroperla doma</i> Stark, 2010	658552	♂	KOR: Gangwon-do	37.41001, 128.53417	789
<i>Kathroperla perdita</i> Banks, 1920	658497	♂	USA: MT	48.46684, -113.81898	633
<i>Kathroperla siskiyou</i> Stark & Kondratieff, 2015	CSUC	♂	USA: OR	42.07864, -122.78843	791
<i>Kathroperla takhoma</i> Stark & Surdick, 1987	658478	♂	USA: CA	40.90095, -123.76742	682
<i>Paraperla frontalis</i> (Banks, 1902)	793097	♀	USA: CA	39.59619, -120.61041	706
<i>Paraperla wilsoni</i> Ricker, 1965	CSUC	♂	USA: MT	48.20782, -115.91459	798
<i>Utaperla gaspesiana</i> Harper & Roy, 1975	658543	♂	USA: NY	42.1242, -74.6726	799
<i>Utaperla lepnevae</i> (Zhiltzova, 1970)	658548	♂	RUS: Khabarovsk Region	49.44056, 136.55694	800
<i>Utaperla orientalis</i> Nelson & Hanson, 1969	658545	♂	RUS: Khabarovsk Region	49.44056, 136.55694	792
<i>Utaperla sopladora</i> Ricker, 1952	CSUC	♂	USA: MT	46.1302, -114.04882	777
Peltoperlidae					
<i>Peltoperla tarteri</i> Stark & Kondratieff, 1987	793136	♂	USA: VA	37.17601, -80.06274	762
<i>Sierraperla cora</i> (Needham & Smith, 1916)	658522	♂	USA: CA	41.32703, -122.32647	773
<i>Viehoerperla ada</i> (Needham & Smith, 1916)	658422	♂	USA: NC	35.07265, -83.60309	774
Perlidae					
<i>Acroneuria carolinensis</i> (Banks, 1905)	793036	♂	USA: OH	40.61294, -82.31696	687
<i>Anacroneuria wipukupa</i> Baumann & Olson, 1984	658439	♀	USA: AZ	34.28371, -111.06792	758
<i>Perlesta teaysia</i> Kirchner & Kondratieff, 1997	793170	♂	USA: VA	37.14702, -81.26314	726
<i>Agneta flavescens</i> (Walsh, 1862)	793083	♂	USA: VA	37.31615, -80.64498	621
<i>Claassenia sabulosa</i> (Banks, 1900)	658446	♂	USA: CO	40.62089, -105.13942	725
<i>Neoperla osage</i> Stark & Lentz, 1988	793104	♂	USA: AR	35.77562, -94.37487	727
Perlodidae					
<i>Isoperla quinquepunctata</i> (Banks, 1902)	658431	♂	USA: CO	40.59895, -105.08371	753
<i>Arcynopteryx dichroa</i> (McLachlan, 1872)	658526	♂	USA: MI	47.45269, -88.19545	788
<i>Diploperla robusta</i> Stark & Gaufin, 1974	658507	♂	USA: IN	39.30893, -86.72016	749
<i>Isogenoides zionensis</i> Hanson, 1949	793073	♂	USA: UT	40.08458, -111.35519	721
Pteronarcyidae					
<i>Pteronarcella badia</i> (Hagen, 1874)	793074	♂	USA: UT	40.08458, -111.35519	728
<i>Pteronarcys scotti</i> Ricker, 1952	793132	♂	USA: GA	34.68791, -84.20182	755

Ortho. = number of orthologs used in analysis.

Alignment, Trimming, and Taxon-Presence Filtering

Amino acid sequences for each ortholog were aligned individually using MAFFT v7.471 (Katoh et al. 2002, Katoh and Standley 2013) and trimmed using trimal v1.4.rev15 with the -gappout option (Capella-Gutiérrez et al. 2009). Nucleotide sequences were aligned on a per-ortholog basis with respect to the amino acid sequences using PAL2NAL (Suyama et al. 2006) and trimmed using the same settings. In order to reduce the potential effect of missing data while still retaining sufficient orthologs for gene tree analysis, both amino acid and nucleotide orthologs were filtered by species presence following trimming so that only orthologs containing at least 70% of species in Paraperlinae, at least one member of the genus *Kathroperla*, and at least half of all total species were retained. This filtering step resulted in a total of 800 orthologs being retained for maximum likelihood and gene tree analyses.

Maximum Likelihood (ML) Analyses

Amino acid and nucleotide alignments were concatenated using SEQUENCE MATRIX v. 1.8 (Vaidya et al. 2011). Because high variability in the third codon position was recovered in stonefly orthologs (see South et al. 2021, their Fig. S4) and has been

considered a potential source of systematic bias in phylogenomic analyses (Weisburg et al. 1989, Hasegawa and Hashimoto 1993, Collins et al. 2005, Simon et al. 2006), two datasets of concatenated nucleotide alignments were used for further downstream analyses: one with all three codon positions (hereafter complete nucleotide) and a second excluding the third codon position (hereafter nt12).

Maximum likelihood (ML) analyses were conducted on the concatenated amino acid, complete nucleotide, and nt12 alignments using IQ-TREE v. 2.1.1 (Nguyen et al. 2015) via the CIPRES Science Gateway v3.3 (Miller et al. 2012). Analyses were performed under the -m TEST option, which executes model selection followed by ML tree inference using the best-fit model identified by the Bayesian Information Criterion (BIC). The best-fit models selected by BIC were JTT + F + I + G4 for the concatenated amino acid alignment and GTR + F + I + G4 for the concatenated complete nucleotide and nt12 alignments. Branch support was assessed with 3,000 ultrafast bootstrap replicates (Minh et al. 2013, Hoang et al. 2018) under the -bnni option to reduce the risk of branch support overestimation.

The nucleotide partitioning schemes were obtained from the concatenated complete nucleotide and nt12 nexus files output from SequenceMatrix. ML analyses were performed on the partitioned

complete nucleotide and nt12 datasets via the CIPRES Science Gateway. Within IQ-TREE, a model search using the -TESTMERGE option was performed using MODELFINDER (Chernomor et al. 2016). Due to computational limitations, the relaxed hierarchical clustering algorithm (Lanfear et al. 2014) was used to limit partition merge examinations to the top 10% of partition merging schemes under the -rcluster option. The -Q partition option was used to allow each partition to have its own branch lengths to account for within-site evolutionary rate variation. Branch support was assessed by a generation of 3,000 ultrafast bootstrap replicates within partitions, reducing the risk of overestimating branch support by using the -bnni option. Trees were rooted with outgroup taxa from Peltoperlidae and Pteronarcyidae.

Multispecies Coalescent Analysis

Individual model tests and maximum likelihood analyses with 1,000 ultrafast bootstraps for all 800 orthologs were performed in IQ-TREE v1.6.9. After collapsing branches with less than 5% bootstrap support using TreeCollapserCL 4 (Hodcroft 2013), the concatenated gene tree sets were used as input for ASTRAL III v.5.15.1 (Zhang et al. 2018) under default settings for multispecies coalescent analysis (hereafter MSC). Because ASTRAL requires highly resolved gene trees to obtain accurate MSC species trees, and the fact that our focal group includes several closely related species unlikely to

differ at first and second codon positions, these analyses were only performed using all codon positions. Support values were generated as local posterior probabilities (Sayyari and Mirarab 2016).

Nomenclature

This paper and the nomenclatural act(s) it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:D78DEB28-9AD6-4424-9F2C-28BF26032F61.

Results

Taxonomy

Kathroperlidae South & DeWalt, fam. n.

Included genera: *Kathroperla* Banks, 1920

Zoobank LSID: urn:lsid:zoobank.org:act:5F9CAD65-BE49-4DEF-AD5F-1E315118A73F

Examination of *Kathroperla* specimens from several locations revealed intraspecific variation and morphological detail not provided in the original descriptions. Slight differences were observed

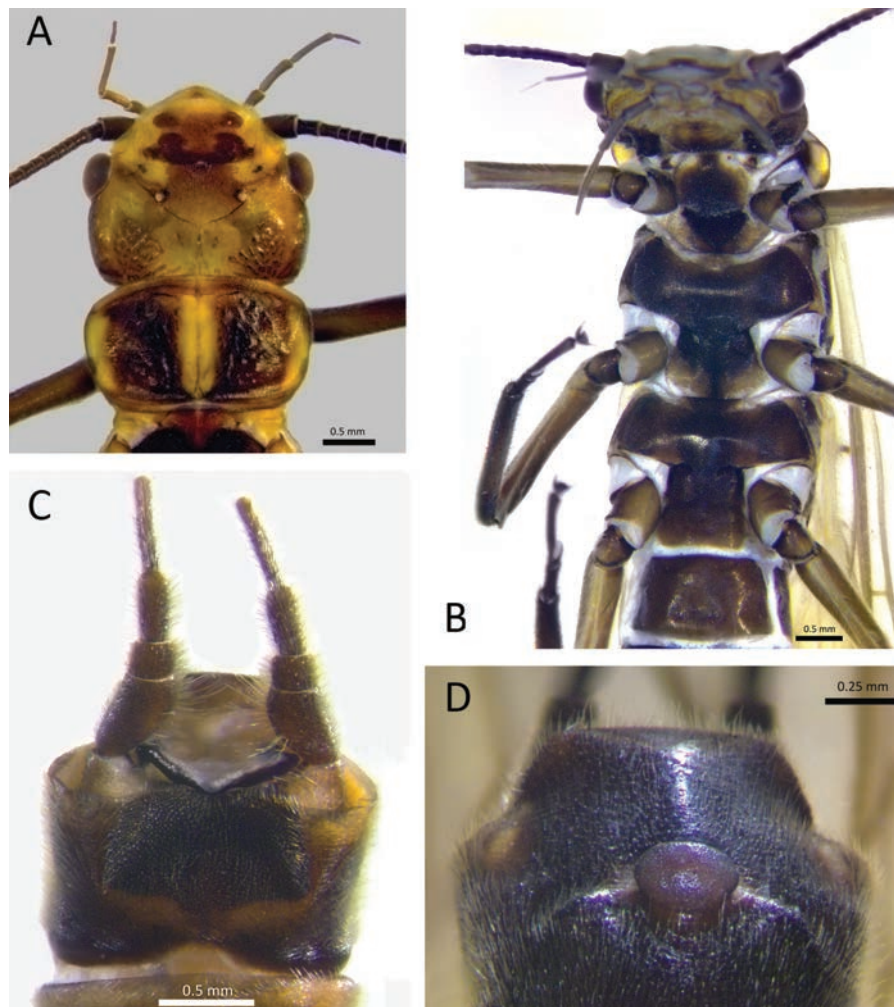


Fig. 3. *Kathroperla doma*, male, tributary of Yongtancheon, Gangwon-do, Republic of Korea (INHS 658552). (A) Head and pronotum. (B) Venter. (C) Terminalia, dorsal. (D) Vesicle.

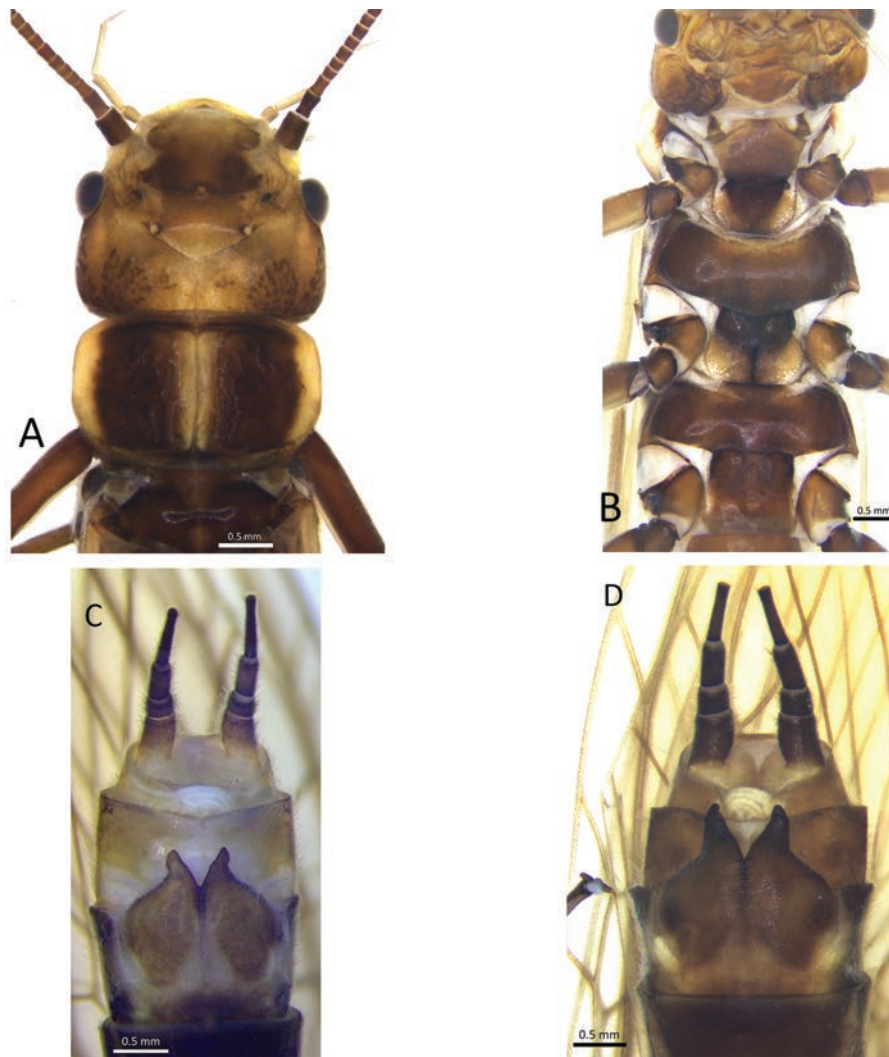


Fig. 4. *Kathroperla doma*, females. (A) Head and pronotum, Deokjeoncheon (stream), Gyeongsangnam-do, Republic of Korea (INHS Insect Collection 658551). (B) Venter. (C) Terminalia, ventral. (D) Terminalia, ventral, unnamed stream, Gangwon-do, Republic of Korea (INHS Insect Collection 658553).

in shape of the head, epiproct, and subgenital plate. Additionally, our first-time images of key structures display greater detail of coloration patterning than that shown in previous illustrations. Except for *K. siskiyou* paratypes, type specimens were not examined.

Kathroperla doma Stark

Figs. 3–4

Kathroperla doma Stark, 2010: 1. Holotype ♂ (National Museum of Natural History, Washington D. C., USA) Doma Pass, Dunjeon-ri, Sangchon-myeon, Yeongdong-gun, Chungcheongbuk-do, Republic of Korea

Distribution

Republic of Korea (Stark 2010).

Remarks

Male. The head is broad and subquadrate with slightly rounded lateral occipital margins (Fig. 3A). The postocular region is elongate and slightly less than two eye lengths from the posterior eye margin to the posterior head capsule margin, a proportional length less than that previously illustrated (Stark 2010, his Fig. 1, unspecified sex).

Head coloration shows diffuse dark pigmentation over the occiput and a dark quadrate patch covering the interocellar area which extends anteriorly to the frons, connecting with a darker transverse oblong patch and two anterior dark ovoid patches. This condition is contrasted with the more uniform head coloration pattern illustrated by Stark (2010, his Fig. 1). Anterior and posterior pronotal margins are darker than illustrated by Stark (2010, his Fig. 1). An image of the thoracic sterna is provided for the first time (Fig. 3B). Stark (2010) described the epiproct as butterfly-shaped with lateral margins curved mesially (his Figs. 2, 4). In contrast, Fig. 3C depicts a lateral margin that is not curled mesially, but is straight, the lateral margins expanding laterally and widening apically to form a wide V-shape. The imaged vesicle on the ninth sternum appears stalked (Fig. 3D), a feature not illustrated by Stark (2010, his Fig. 3).

Female. The head is similar to that of the male, but postocular length is approximately two eye lengths from posterior eye margin to the posterior head capsule margin (Fig. 4A). The pronotal margins (Fig. 4A) exhibit paler lateral pronotal margins than the male. The mesosternal suture anterior to parallel furcal pits is curved posteriorly (Fig. 4B). Both subgenital plates (Fig. 4C and D) show dark sclerotization of the lobes which is not illustrated by Stark (2010, his

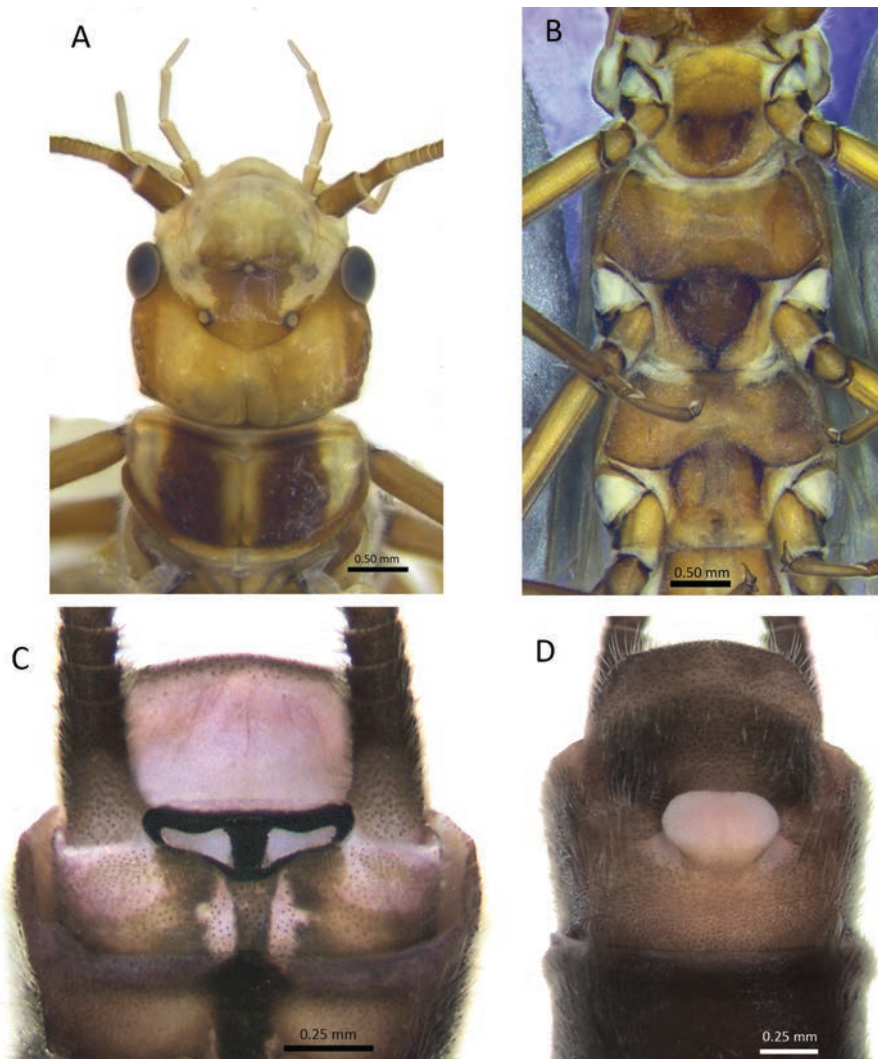


Fig. 5. *Kathroperla perdita*, males. (A) Head and pronotum, Rock Creek, Oregon, USA (INHS Plecoptera 13546). (B) Venter. (C) Terminalia, dorsal, Middle Fork Flathead River aquifer, Montana, USA (INHS Insect Collection 658497). (D) Vesicle.

Fig. 10). The specimen from Gyeongsangnam-do is a new province record (Fig. 4A–C).

Material Examined

Republic of Korea: Gangwon-do: Hoedong-ri, Jeongseon-eup, Jeongseon-gun, tributary of Yongtancheon, Gariwangsan Recreational Forest, 37.41001, 128.53417, 12.v–21.vi.2019, D.S. Ham, S.H. Park, ♂, ♀ (INHS Insect Collection 658552, 658553). *Gyeongsangnam-do:* (new province record) Samjeon-ri, Macheon-myeon, Hamyang-gun, Deokjeoncheon (stream), 35.35917, 127.64722, 8.v.–5.vi.2004, P. Tripotin, ♀ (INHS Insect Collection 658551).

Kathroperla perdita Banks

Figs. 5–6

Kathroperla perdita Banks, 1920: 315. Holotype ♀ (Museum of Comparative Zoology, Cambridge, Massachusetts, USA) Kaslo, British Columbia, Canada.

Kathroperla perdita: Needham & Claassen, 1925: 132. Description of male

Kathroperla perdita: Neave, 1934: 2. Description of nymph

Kathroperla perdita: Ricker, 1943: 141. Illustration of nymph

Kathroperla perdita: Illies, 1966: 426. Catalog

Kathroperla perdita: Zwick, 1973: 180. Catalog

Kathroperla perdita: Baumann et al., 1977: 186. Morphological diagnosis with illustrations

Kathroperla perdita: Stark & Surdick, 1987: 530. SEM image of egg

Kathroperla perdita: Stewart & Stark, 2002: 263. Description and illustration of nymph

Kathroperla perdita: Stark et al., 2015: 96. SEM image of epiproct

Distribution

Canada: AB, BC, YK. United States: AK, CA, ID, MT, NV, OR, WA (DeWalt et al. 2020).

Remarks

Male. The head (Fig. 5A) demonstrates ‘great length of the head behind the eyes’, as described by Banks (1920). However, our image shows that the lateral margins of the occiput are slightly rounded and not straight as that illustrated by Stark and Surdick (1987, their Fig. 9, unspecified sex). Our image of the thoracic

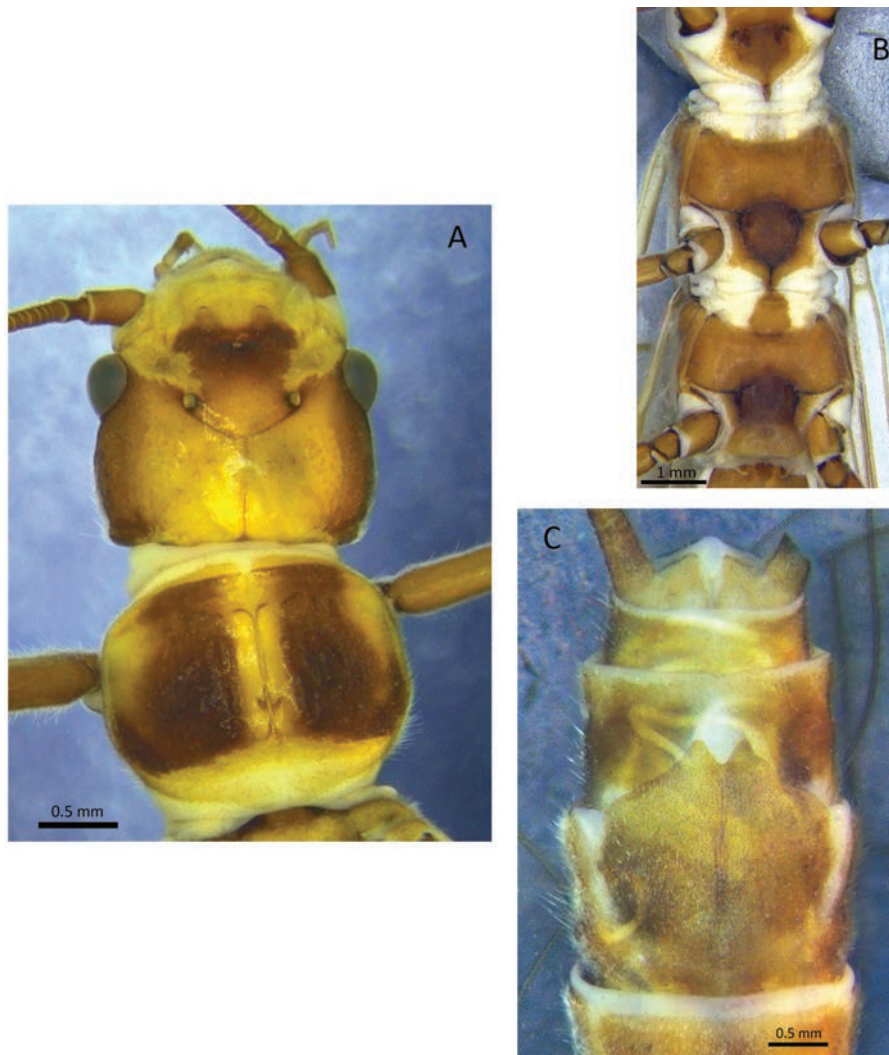


Fig. 6. *Kathroperla perditia*, female, Steven's Pass, Washington, USA (INHS Plecoptera 13539). (A) Head and pronotum. (B) Venter. (C) Terminalia, ventral.

sterna (Fig. 5B) shows the dark coloration of large sternal plates and prominent furcal pits. Terminalia display black pigmentation patterning of the epiproct (Fig. 5C) and a broad vesicle base (Fig. 5D), both character states described as species-specific by Stark et al. (2015).

Female. The head (Fig. 6A) exhibits similar pigment patterning as the male. The female mesosternal plate (Fig. 6B) is slightly more quadrate than the male. The posterior margin of the subgenital plate (Fig. 6C) shows a more pronounced median notch than previously illustrated (Needham and Claassen 1925, their Plate 23, Fig. 8).

Material Examined

United States: California: Butte Co., Butte Creek, 39.6 km NNE Paradise at Cherry Hill C. G., 40.10273, -121.49932, 26.v.2019, E.J. South, B.C. Kondratieff, J.B. Sandberg, ♀ (EJSC Ka_pe_01). *Montana:* Flathead Co., Middle Fork Flathead River aquifer, 12.3 km ESE West Glacier at well HA18, 400 m from the river, 48.46684, -113.81898, 1.vi.2018, W. Sigl, 2♂ (INHS Insect Collection 658497, 658498). *Oregon:* Benton Co., Rock Creek, 30.iii.1938, E. Yeaman, ♂ (INHS Plecoptera 13546). *Washington:* Chelan Co., Steven's Pass, Berne, 24.vi.1940, H.H. Ross, ♀ (INHS Plecoptera 13539); Snoqualmie Co.,

7.v.1932, G. Hoppe, ♀ (INHS Plecoptera 13548), same but Tokul Creek, 8.iv.1933, G. Hoppe, ♀ (INHS Plecoptera 13541)

Kathroperla siskiyou Stark & Kondratieff Figs. 7–8

Kathroperla siskiyou Stark et al., 2015: 96. Holotype ♀ (National Museum of Natural History, Washington D. C., USA) Split Rock Creek, Jackson County, Oregon, USA.

Distribution

United States: OR (Stark and Kondratieff 2015, DeWalt et al. 2020).

Remarks

Male. The head (Fig. 7A) shows two pigment spots lateral to the interocellar patch and one on the anterior frons, features not described or illustrated by Stark et al. (2015, their Fig. 15). The mesosternum image (Fig. 7B) shows the connection between the furcal pits and the spina, a character not mentioned or illustrated by Stark et al. (2015). Lateral margins of the epiproct (Fig. 7C) are directed slightly less caudad than shown in Stark et al. (2015, their Fig. 16). First images of ventral and dorsal views of the extruded aedeagus are provided (Fig. 7C and D).

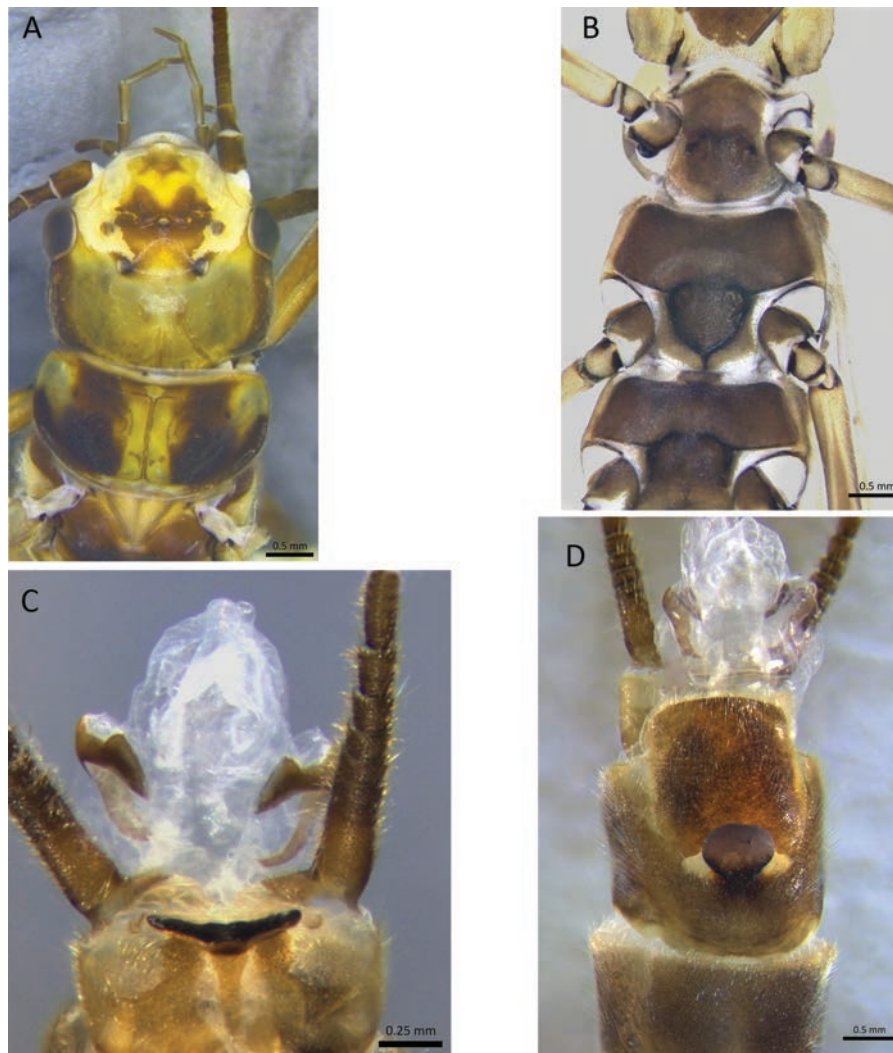


Fig. 7. *Kathroperla siskiyou*, male, McDonald Creek, Oregon, USA (CSUC). (A) Head and pronotum. (B) Venter. (C) Terminalia with extruded aedeagus, dorsal. (D) Terminalia with extruded aedeagus, ventral.

Female. The first images of *Kathroperla* wings are provided, showing numerous cross veins in the forewing (Fig. 8A) and four primary veins of the anal lobe in the hindwing (Fig. 8B). Similar to the male, the female head shows two pigment spots lateral to the interocellar pigment patch (Fig. 8C). The notched apex of the subgenital plate is bordered by projected rounded lobes (Fig. 8D), contrasted to the pointed projections illustrated by Stark et al. (2015, their Fig. 19).

Material Examined

United States: Paratypes, **Oregon:** Jackson Co., MacDonald Creek, 18.6 km S Talent at Wagner Gap Rd., 42.07864, -122.78843, 22.v.2014, B.C. Kondratieff, C.J. Verdone, ♂ (CSUC); Split Rock Creek, 19.3 km S Talent at Wagner Gap Rd., 42.0948, -122.77397, 22.v.2014, B.C. Kondratieff, B.P. Stark, J.B. Sandberg, C.J. Verdone, 2♀ (CSUC).

Kathroperla takhoma Stark & Surdick

Figs. 9–10

Kathroperla takhoma Stark & Surdick, 1987: 527. Holotype ♀ (National Museum of Natural History, Washington D. C., USA) Falls Creek, Mount Rainier National Park, Washington, USA.

Kathroperla takhoma Stark et al., 2015: 99. SEM and description of egg.

Distribution

United States: CA, WA (DeWalt et al. 2020).

Remarks

Male. Head (Fig. 9A) is more quadrate than illustrated by Stark and Surdick (1987, their Fig. 8). First images of dorsal, lateral, and ventral views of male terminalia are provided, showing the coloration and plate-like structure of the epiproct (Fig. 9B and C), and the vesicle (Fig. 9D) which displays slightly less convergent lateral margins than illustrated by Stark and Surdick (1987, their Fig. 2).

Female. Intraspecific variation in the pigmentation of the interocellar region and pronotum (Fig. 10A–C) and coloration of the mesosternum (Fig. 10D) are shown for the first time. The subgenital plate notch (Fig. 10E) is deeper than that illustrated by Stark and Surdick (1987, their Fig. 4).

Material Examined

United States: California: Humboldt Co., Upper Willow Creek, 12.3 km WSW Willow Creek at Hwy 299, 40.90095, -123.76742, 1.v.2018, J.J. Lee, 3♂ (INHS Insect Collection 658451, 658478,

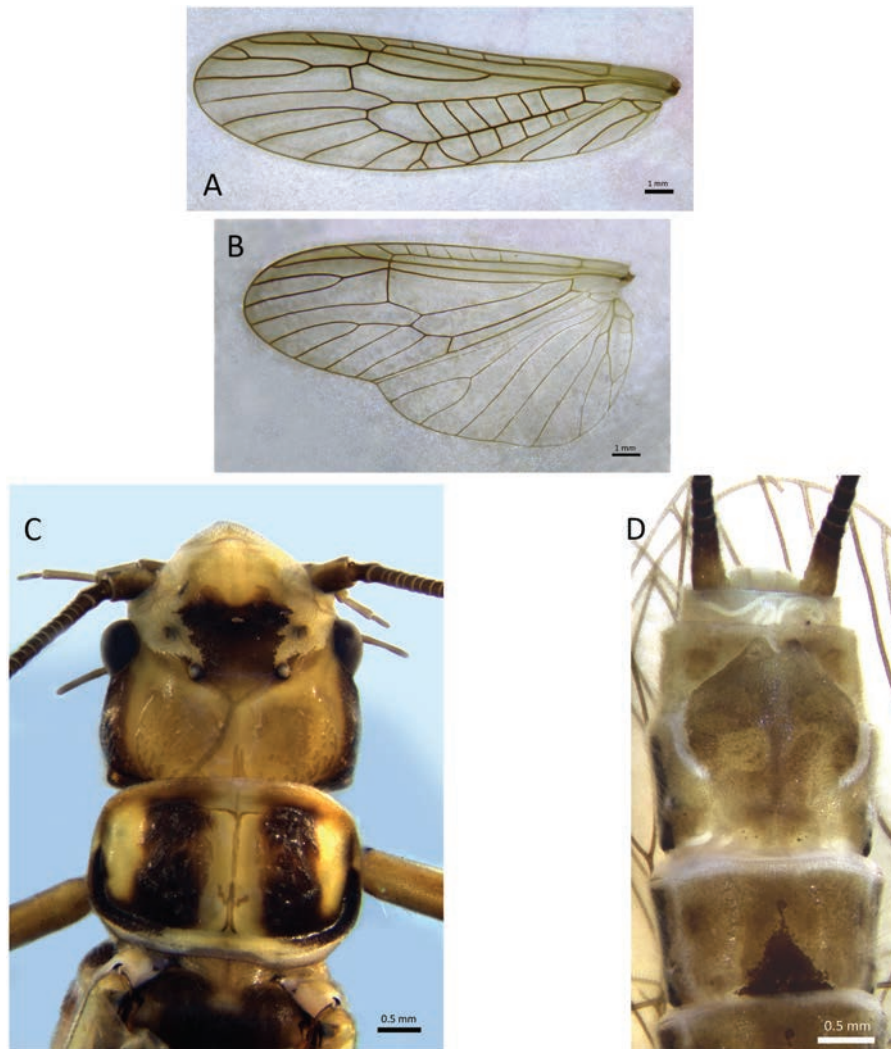


Fig. 8. *Kathroperla siskiyou*, female, Split Rock Creek, Oregon, USA (CSUC). (A) Left forewing. (B) Left hindwing. (C) Head and pronotum. (D) Terminalia, ventral.

EJSC Ka_ta_01), same but Boise Creek, 26.iv.2007, J.J. Lee, ♀ (INHS Insect Collection 658554), same but Red Mountain Creek, J.J. Lee, ♀ (INHS Insect Collection 658555). **Oregon:** Hood River Co., Casey Creek, Columbia Gorge, 27.v.2007, J.J. Lee, ♀ (INHS Insect Collection 658556).

Molecular Analyses

In total, 800 orthologous genes from 32 stonefly individuals were included in all analyses. The final concatenated amino acid alignment contained 353,872 positions, while the complete nucleotide and nt12 alignments contained 1,061,616 and 707,743 nucleotide positions, respectively (see Table 2 for more details). The amount of missing data (ambiguity/gap) was 26.7% for the nt, nt12, and amino acid alignments.

Maximum likelihood analyses of the concatenated complete nucleotide, nt12, partitioned nucleotide, and partitioned nt12 datasets generated trees showing *Kathroperla* as the monophyletic sister taxon to the remaining Perloidea with 100% bootstrap support (Figs. 11A and B and 12A and B). The ML analysis of the amino acid alignment showed 82% bootstrap support for a monophyletic *Kathroperla* as sister taxon to Chloroperlidae + Perlodidae (Fig. 13). Within *Kathroperla*, two topologies were recovered: 1) *K. doma* +

K. siskiyou as sister species to *K. perdita* + *K. takhoma* by the AA, nt12, and nt12 partitioned analyses and 2) *K. doma* as sister species to *K. siskiyou* + (*K. perdita* + *K. takhoma*) by the nt and nt partitioned analyses. The ML analyses of all concatenated datasets generated maximum support for a monophyletic Paraperlinae as sister to Chloroperlinae. Within the Paraperlinae, the *Utaperla* were supported as monophyletic in all ML analyses. Relationships between the *Utaperla* species varied among the datasets. However, a sister relationship between *U. gaspesiana* + *U. orientalis* and *U. lepnevae* + *U. sopladora* was supported by the AA, nt12, and nt12 partitioned analyses. A second topology, *U. lepnevae* as sister to *U. orientalis* + (*U. sopladora* + *U. gaspesiana*), was supported by the nt and nt partitioned analyses. *Paraperla frontalis* (Banks, 1902) was recovered as the earliest diverging lineage within the Paraperlinae clade by all five analyses.

Similar to the ML analyses, the MSC analysis recovered a monophyletic *Kathroperla* as sister to the remaining Perloidea and a monophyletic Paraperlinae as sister to Chloroperlinae (Fig. 14). Within the *Kathroperla* clade and dissimilar to the ML analyses, the MSC analysis recovered *K. doma* as sister to *K. takhoma* + (*K. perdita* + *K. siskiyou*). Within the Paraperlinae, the topology recovered for *Utaperla* was the same as the ML analyses of the nt and nt partitioned datasets. However, unlike the paraphyly recovered for

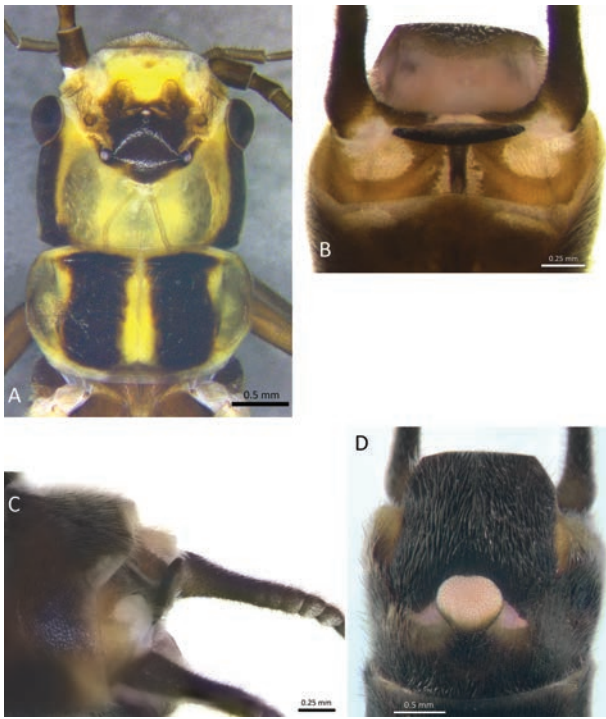


Fig. 9. *Kathroperla takhoma*, male, Upper Willow Creek, California, USA (INHS Insect Collection 658451). (A) Head and pronotum. (B) Terminalia, dorsal. (C) Terminalia, lateral. (D) Vesicle.

Paraperla in the ML analyses, *P. frontalis* and *P. wilsoni* were recovered as a monophyletic sister group to Chloroperlinae.

Discussion

Morphology

Several adult morphological characters separate *Kathroperla* from the Paraperlinae including head configuration, male terminalia, wing venation, female subgenital plate shape, and the connection of the mesosternal furcal pits to the spina (Zwick 2006, Stark et al. 2015). In *Kathroperla*, postocular head elongation is approximately two eye lengths from posterior margin of the eye to posterior head capsule margin, whereas this measurement is approximately one eye length or less in *Paraperla* (Fig. 15A and B) and *Utaperla* (Fig. 16A–D). For males, a transverse epiproct characterizes *Kathroperla* (Figs. 2B, 3C, 5C, 7C, and 9B and C), compared to an upturned supra-anal process in *Paraperla* (Fig. 17A–C) or upturned bifurcated supra-anal process in *Utaperla* (Fig. 18A–F). The tenth tergite is prominently cleft in *Paraperla* and *Utaperla*, but only partially cleft in *Kathroperla*. Additionally, a vesicle on the ninth sternite is present in *Kathroperla* (Figs. 3D, 5D, 7D, and 9D) but absent in *Paraperla* and *Utaperla*. Wing venation differentiates *Kathroperla* (Fig. 8A and B) from *Paraperla* and *Utaperla*, the hindwing of the latter two genera (*P. wilsoni*, Fig. 19B; *U. gaspesiana*, Fig. 19D) having fewer than four primary anal veins, which are present in *Kathroperla*, and fewer cross veins in the forewing (*P. wilsoni*, Fig. 19A; *U. gaspesiana*, Fig. 19C). Posterior emargination of the subgenital plate of *Kathroperla* forms a V-shape bordered by projected lobes (*K. doma*, Fig. 4C and D; *K. perdita*, Fig. 6C; *K. siskiyou*, Fig. 8D; *K. takhoma*, Fig. 10E). The Paraperlinae subgenital plates are either emarginate with broadly rounded lobes (*P. frontalis*, Fig.

20A), slightly emarginate (*P. wilsoni*, Fig. 20B; *U. sopladora*, Fig. 21D), or entire (*U. gaspesiana*, Fig. 21A; *U. lepnevae*, Fig. 21B; *U. orientalis*, Fig. 21C). The furcal pits of the mesosternum are connected posteriorly to the spina (Zwick 2006, his Fig. 10), whereas this connection is absent in *Paraperla* and *Utaperla* (Zwick 2006) (*Paraperla*, Fig. 22A and B; *Utaperla*, Fig. 23A–D).

As stated above, head configuration and epiproct shape differentiate *Kathroperla* from the Chloroperlidae. Elongated heads of *Kathroperla* adults and larvae have been recognized to be distinct from all other Chloroperlidae (Baumann et al. 1977). Although Zwick (2006) stated that head length is a poor diagnostic character mainly due to allometry, meaning early instar nymphs can be indistinguishable using only head shape. Head length was measured from the anterior ocellus to the rear of the head capsule. In this study, postocular head length of adults was determined using compound eye length. This measurement accounts for eye size (relatively small in *Kathroperla*) and the position of the eyes relative to the posterior head capsule margin. Examination of the taxonomic literature and all available specimens suggests that postocular elongation of approximately two eye lengths is distinct in *Kathroperla*. Other taxa, including a few perlodids and chloroperlids, have long heads. However, posterior elongation is usually less than one, or rarely one and a half eye lengths. The dorsal epiproct margin of *Kathroperla* is elongated and transverse, contrasted with the longitudinally elongated or button-shaped dorsal epiproct aspect of the Chloroperlinae, or the upturned supra-anal process of *Paraperla* and *Utaperla*.

Phylogeny

Transcriptome and genome sequencing datasets from stoneflies were successfully combined into a phylogenomic data matrix of over one million base pairs for all described species of Paraperlinae, as well as broad representation within Systellognatha. Molecular phylogenetic analyses performed in this study support evidence from previous morphology-based studies (Zwick 2000, 2006). Zwick (2006) identified apomorphic characters for adult and larval Paraperlinae (see Zwick 2006, Table 1) and transferred *Paraperla lepnevae* to *Utaperla*. This transfer is congruent with the monophyly recovered for *Utaperla* by all molecular analyses in this study. Zwick also noted the lack of unique apomorphies for *Paraperla*, suggesting possible paraphyly for the genus. This was supported in the present study by the nonmonophyly recovered for *Paraperla* in five of the six analyses. Unique apomorphies among the Paraperlinae for *Kathroperla* adults included presence of the ninth sternite vesicle, epiproct transformed into a short open bowl, forward median lobe of the basisternum, and forward curvature of the suture anterior to the furcal pits (Zwick 2006). However, the latter character no longer applies since the description of *K. doma* which displays a suture with a posterior curvature. Additionally, semiquadrate lacinia with no subterminal tooth was identified as a larval autapomorphy (Zwick 2006).

Kathroperla doma is distinct among the *Kathroperla*. Morphological distinctions include a broad subquadrate head, cerci with only four segments, a female subgenital plate with extended triangulate lobes, and a four-sided egg. Moreover, *K. doma* has an Asian distribution, contrasted to the Nearctic distribution of the other species. Additionally, three of the six molecular analyses in this study placed *K. doma* at the base of the *Kathroperla* clade. Further investigation is needed to determine the phylogenetic position of *K. doma*.

Molecular analyses from this study and previous studies (Terry 2003, South et al. 2021) separate *Kathroperla* from Chloroperlidae. However, the placement of the clade is unsettled. The highly supported position of *Kathroperla* as sister taxon to Perloidea in five

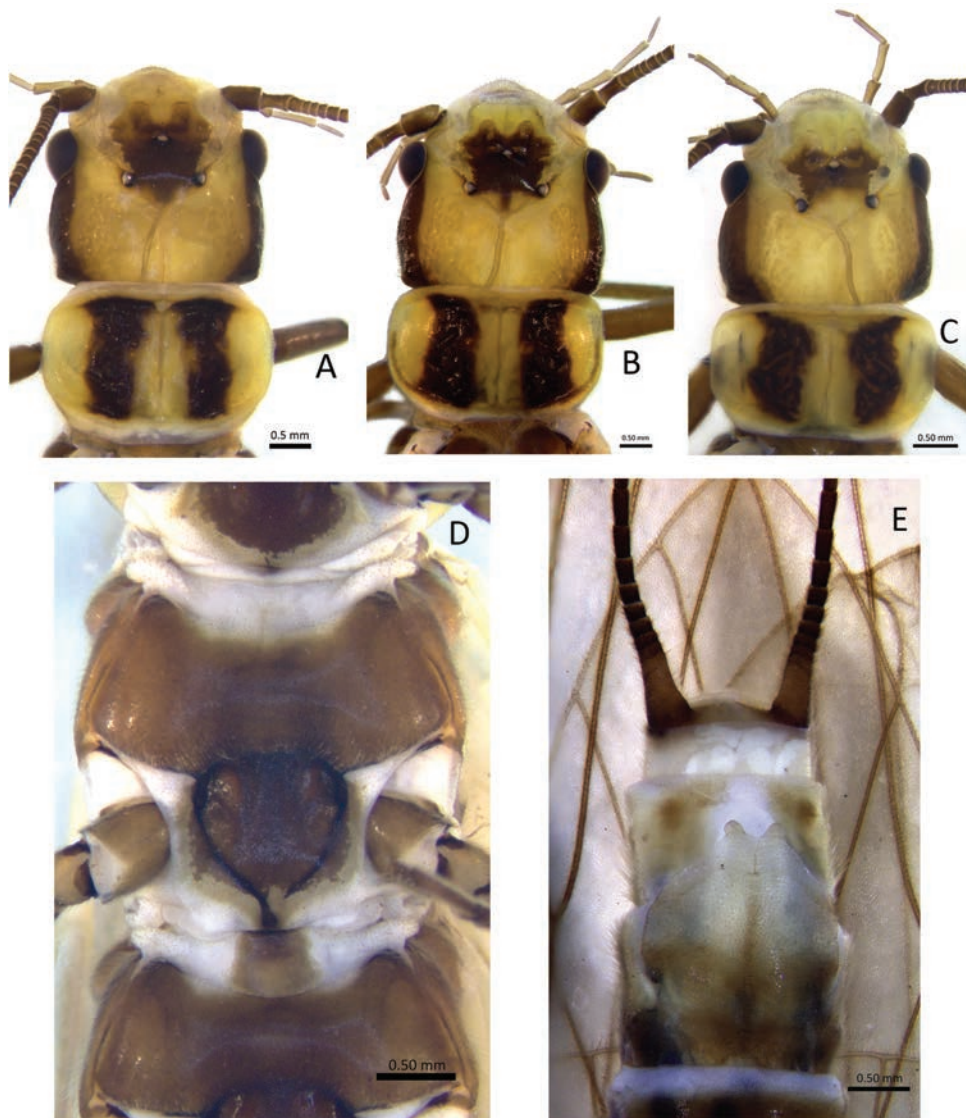


Fig. 10. *Kathroperla tahoma*, females. (A) Head and pronotum, Boise Creek, California, USA (INHS Insect Collection 658554). (B) Casey Creek, Oregon, USA (INHS Insect Collection 658556). (C) Red Mountain Creek, California, USA (INHS Insect Collection 658555). (D) Venter. (E) Terminalia, ventral.

Table 2. Summary counts and likelihood scores of the best trees obtained from maximum likelihood analysis using IQ-TREE

Dataset	Site total	Parsimony-informative sites	Singleton sites	Constant sites	Likelihood score of best tree
Concatenated amino acid	353,872	96,369	69,581	187,922	-3,793,979.5
Concatenated complete nucleotide	1,061,616	446,397	143,575	471,644	-10,460,540.5
Complete partitioned nucleotide					-10,100,860.6
Concatenated nt12	707,743	151,584	109,411	446,748	-4,089,834.7
Partitioned nt12					-3,828,928.6

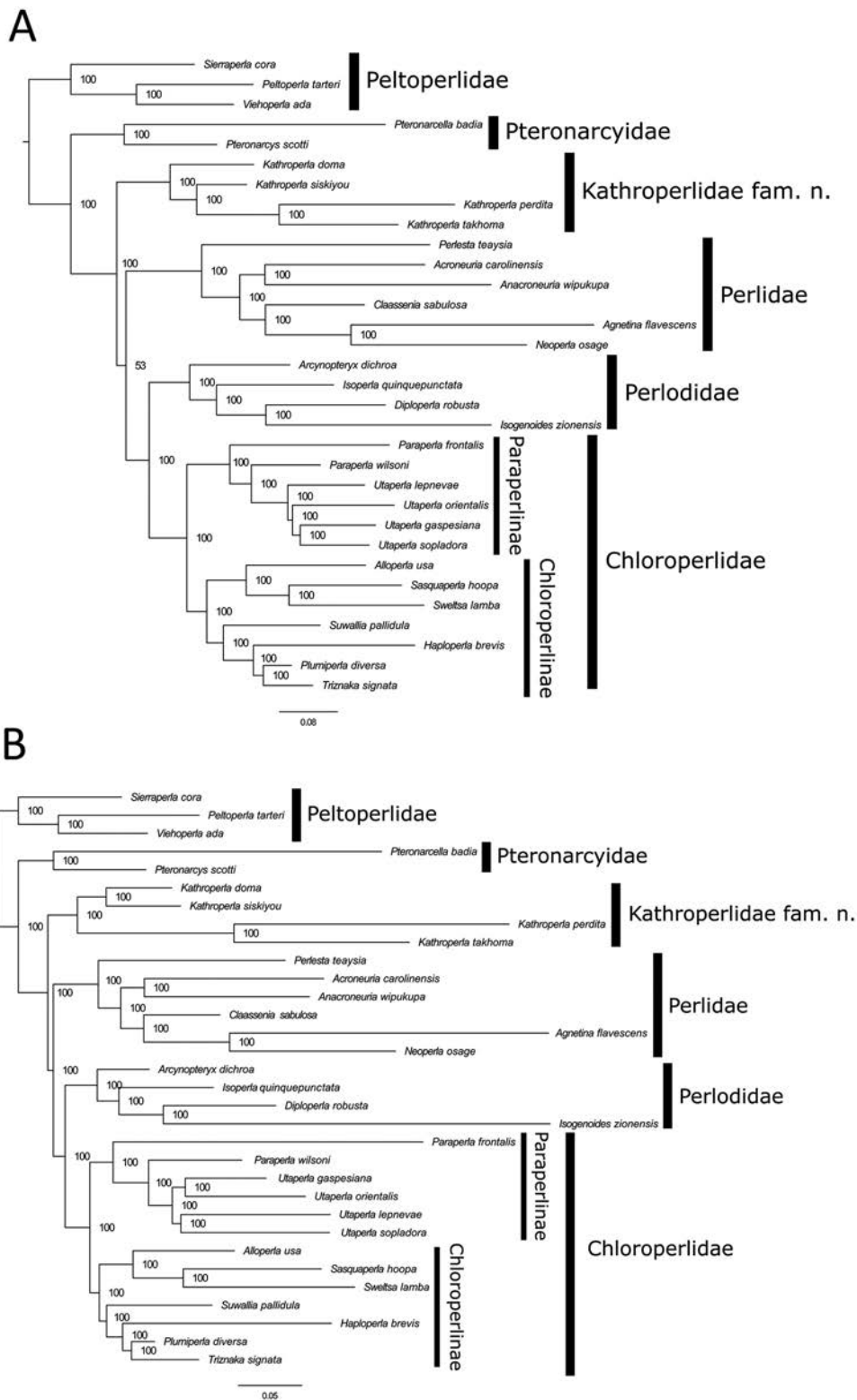


Fig. 12. Best tree for maximum likelihood analysis of partitioned datasets for (A) complete nucleotide and (B) third codon position removed.

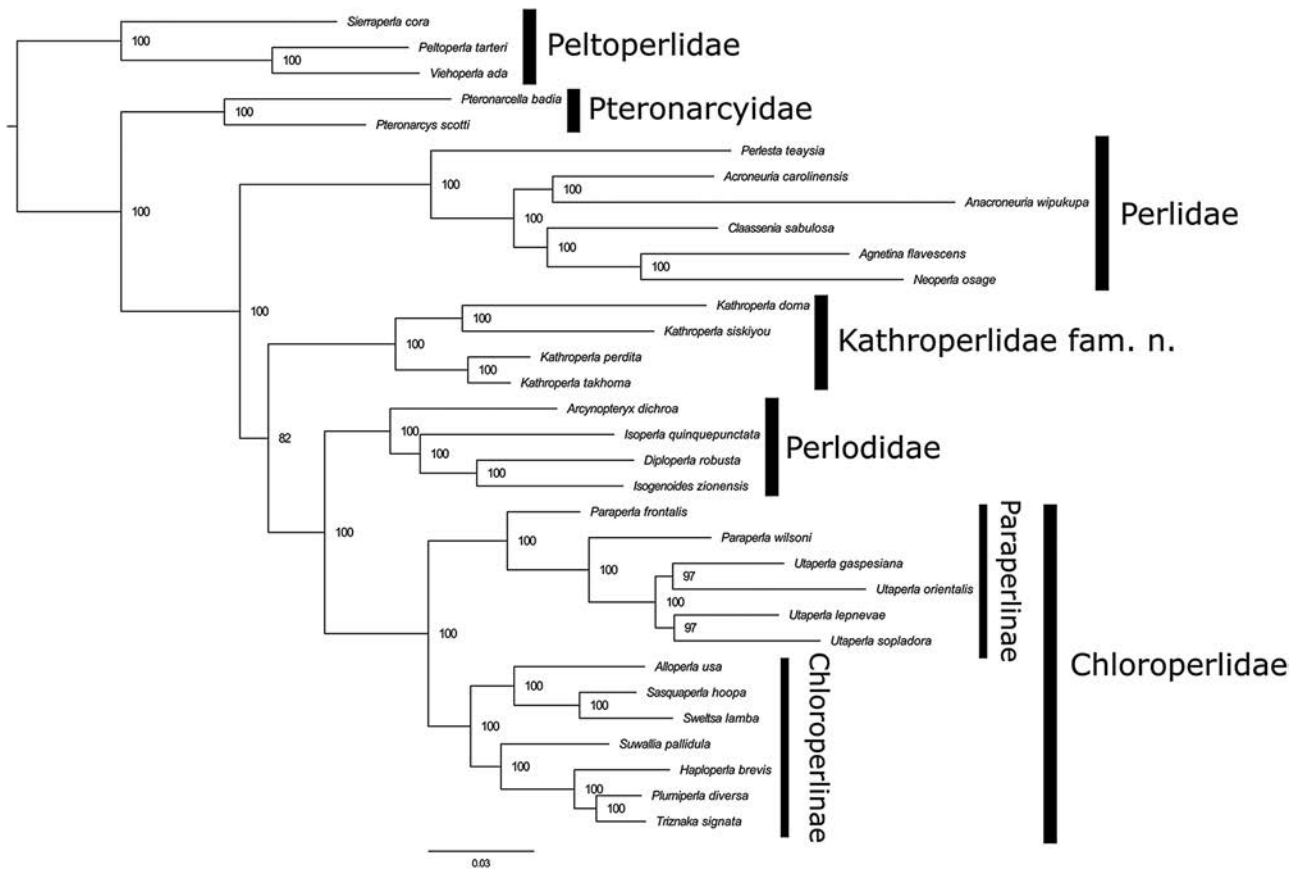


Fig. 13. Best tree for maximum likelihood analysis of amino acid alignment.

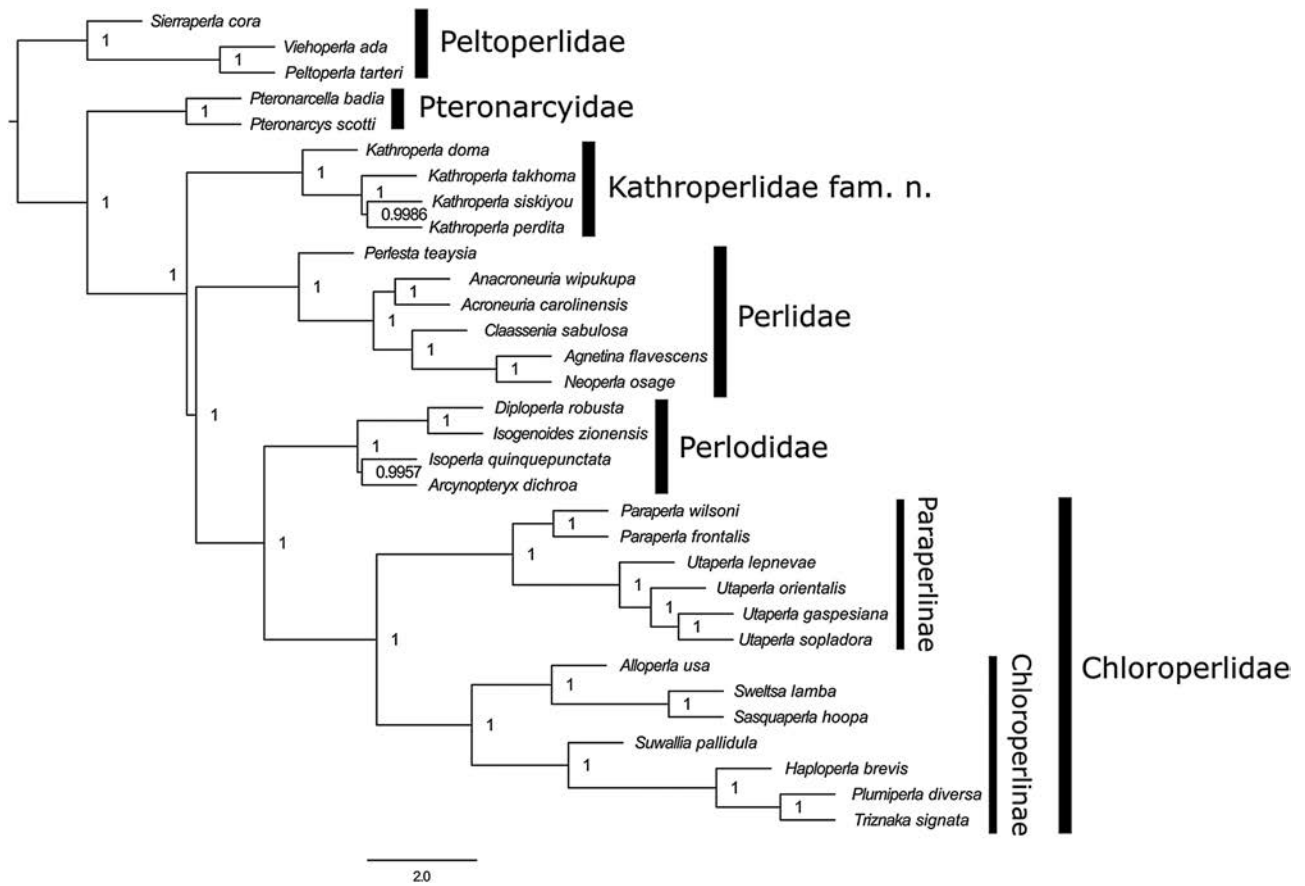


Fig. 14. Multispecies coalescent tree using ASTRAL.

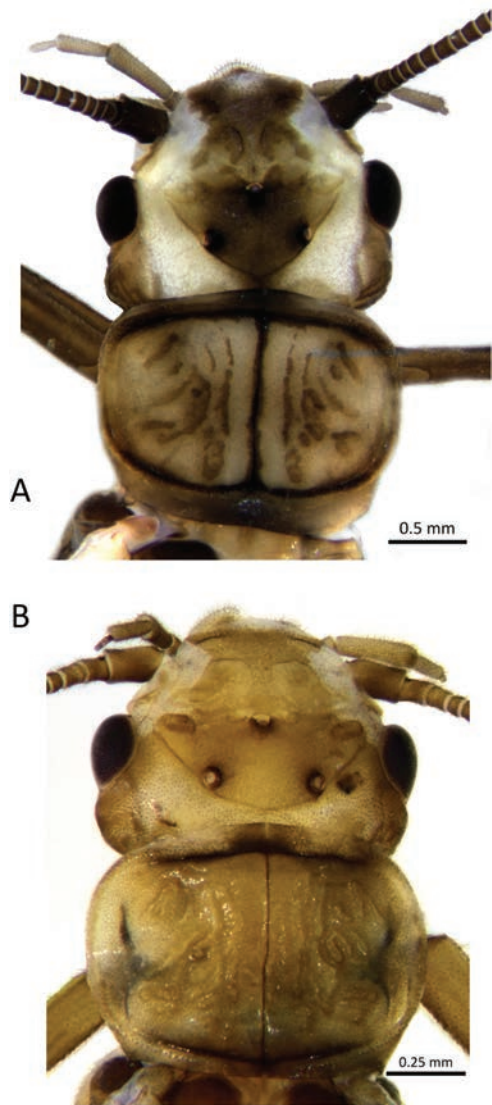


Fig. 15. *Paraperla*, head and pronotum. (A) *P. frontalis*, female, Sulphur Creek, California, USA (EJSC Pa_fr_02). (B) *P. wilsoni*, male, Ross Creek, Montana, USA (CSUC).

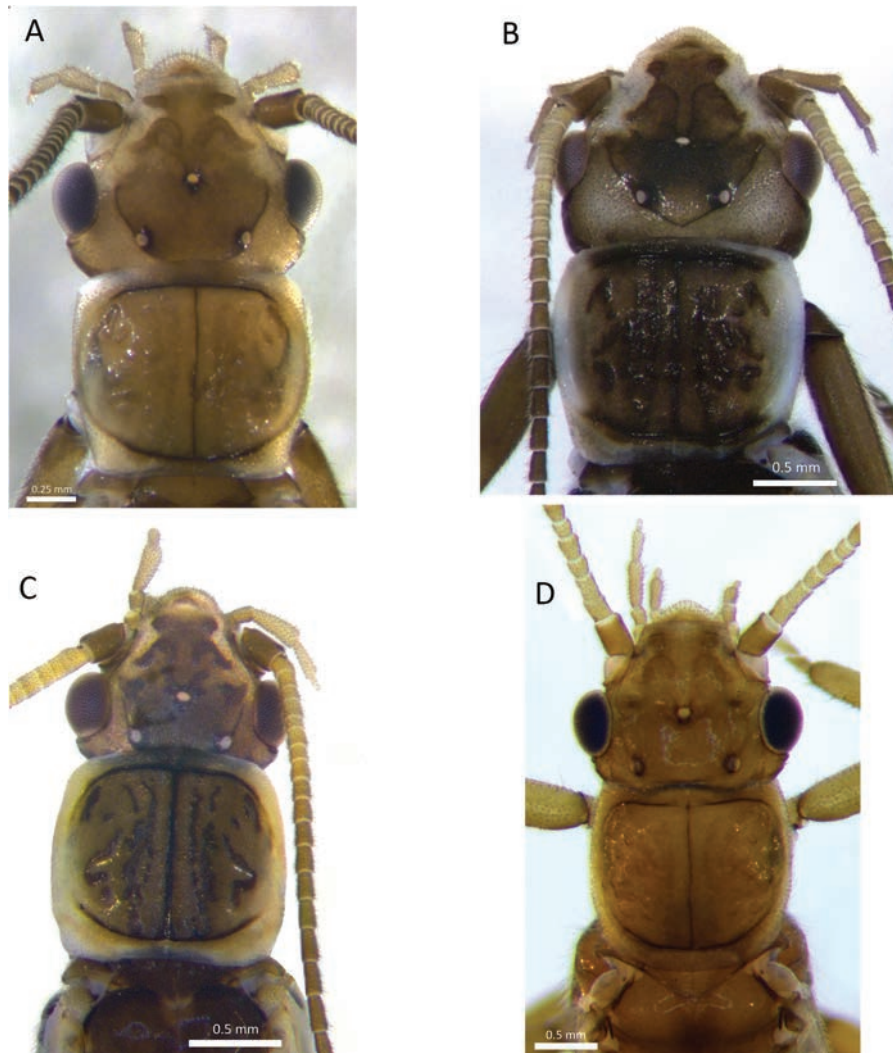


Fig. 16. *Utaperla*, head and pronotum. (A) *U. gaspesiana*, female, Lucas Creek, New York, USA (INHS Insect Collection 658544). (B) *U. lepnevae*, male, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658548). (C) *U. orientalis*, female, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658546). (D) *U. sopladora*, male, Bitterroot River, Montana, USA (CSUC).

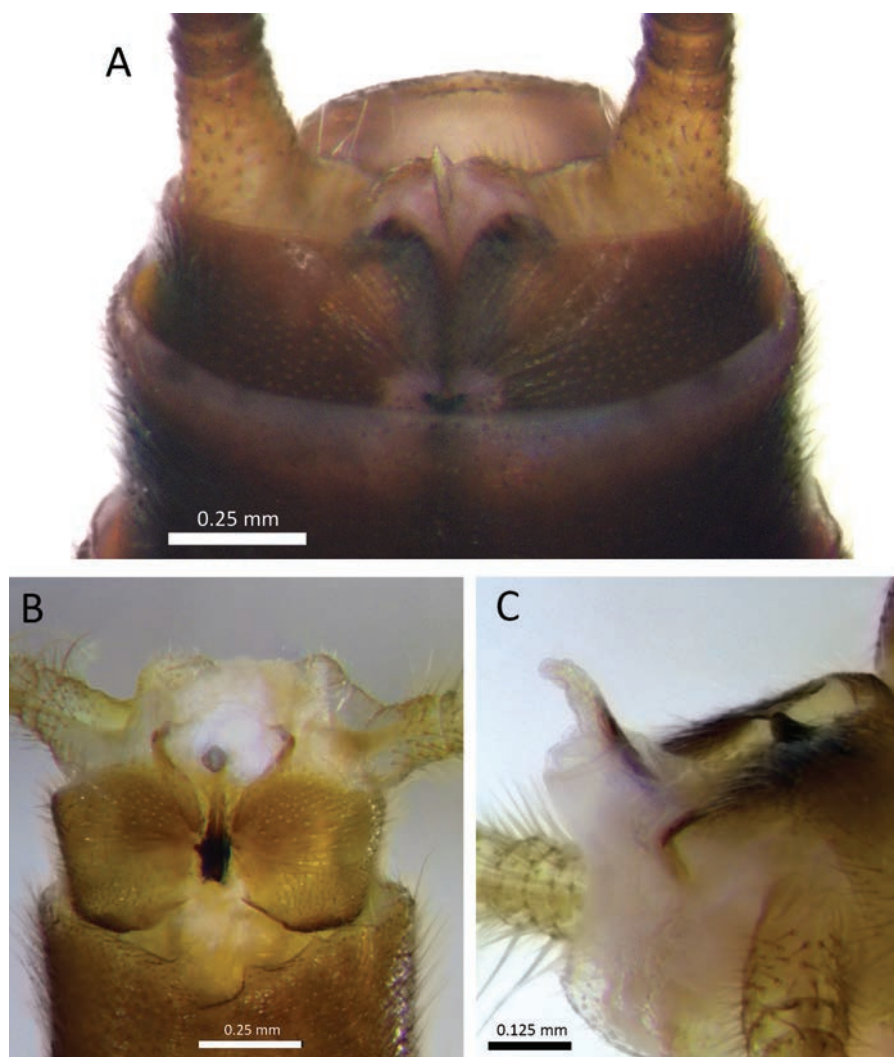


Fig. 17. *Paraperla*, male terminalia, dorsal. (A) *P. frontalis*, Sulphur Creek, California, USA (EJSC Pa_fr_01). (B) *P. wilsoni*, Ross Creek, Montana, USA (CSUC). (C) Lateral.

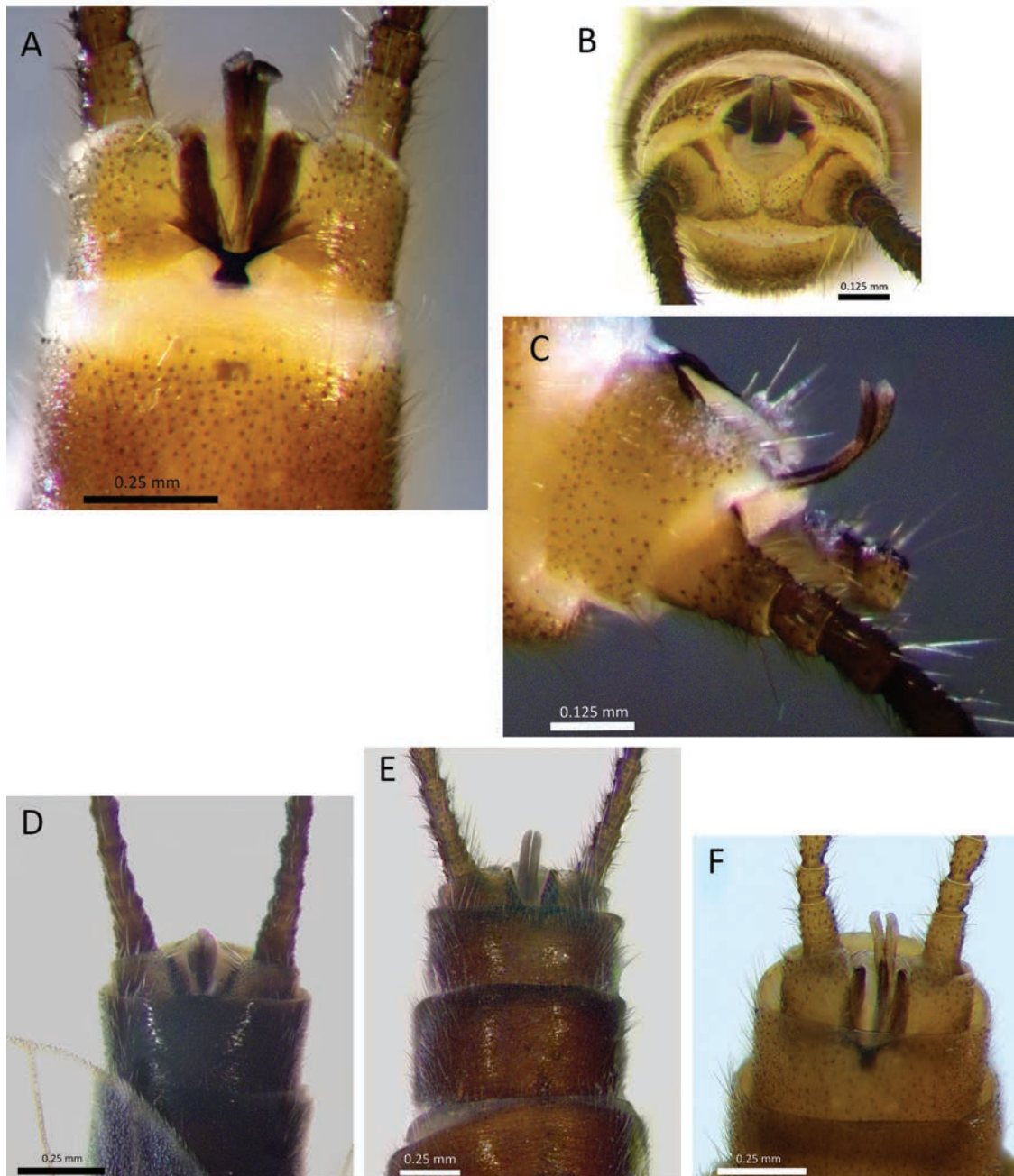


Fig. 18. *Utaperla gaspesiana*, male, terminalia, East Branch Delaware River, New York, USA (INHS Insect Collection 658543). (A) Dorsal. (B) Caudal. (C) Lateral. (D) Dorsal, *U. lepnevae*, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658548). (E) Dorsal, *U. orientalis*, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658545). (F) Dorsal, *U. sopladora*, Bitterroot River, Montana, USA (CSUC).

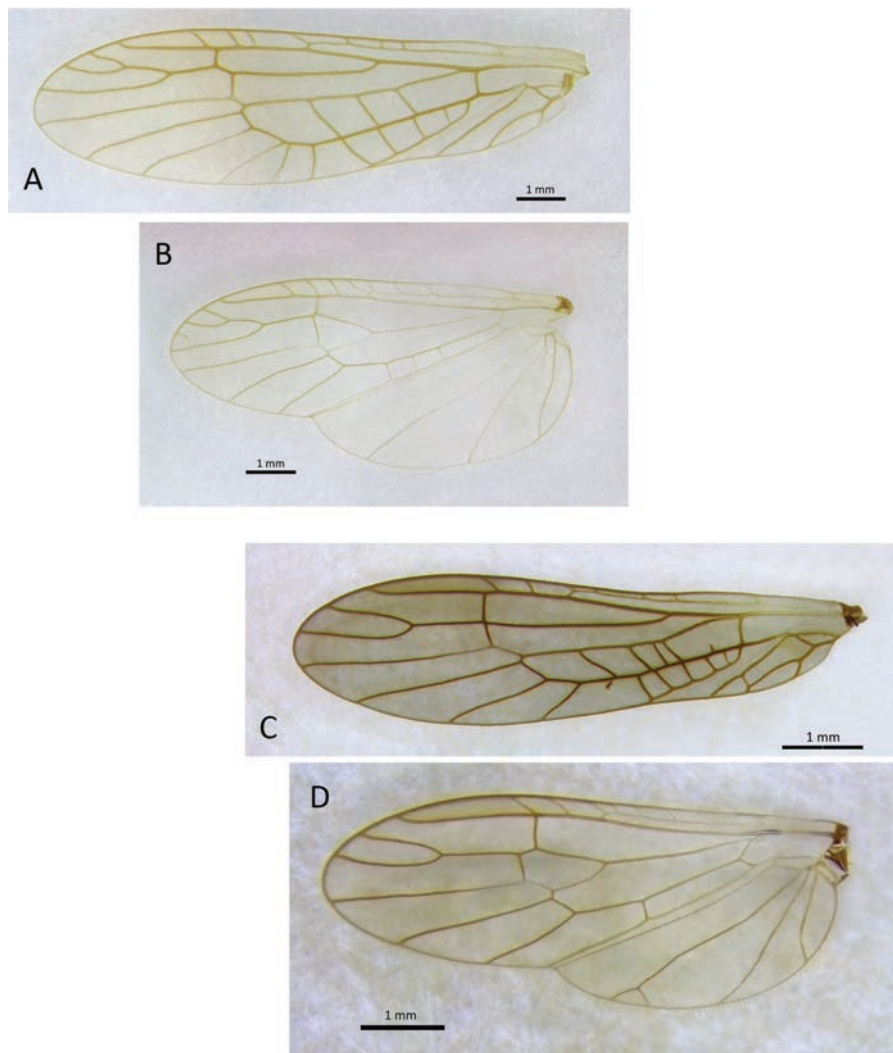


Fig. 19. *Paraperla* and *Utaperla*, wings. (A) *P. wilsoni*, male, left forewing, East Fork Six Mile Creek, Alaska, USA (INHS Insect Collection 487620). (B) Left hindwing. (C) *U. gaspesiana*, female, left forewing, Delaware River, New York, USA (INHS Insect Collection 658542). (D) Left hindwing.

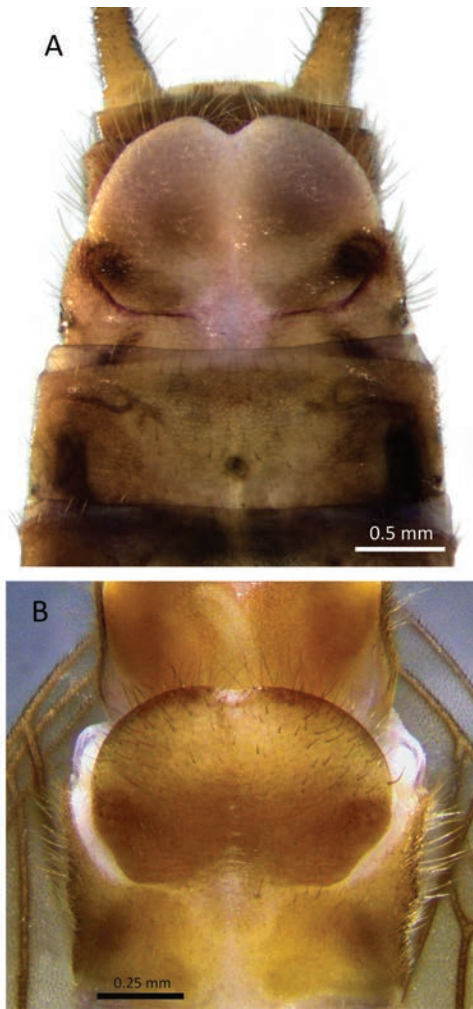


Fig. 20. *Paraperla*, female terminalia, ventral. (A) *P. frontalis*, Sulphur Creek, California, USA (EJSC Pa_fr_02). (B) *P. wilsoni*, Herman Creek, Oregon, USA (CSUC).

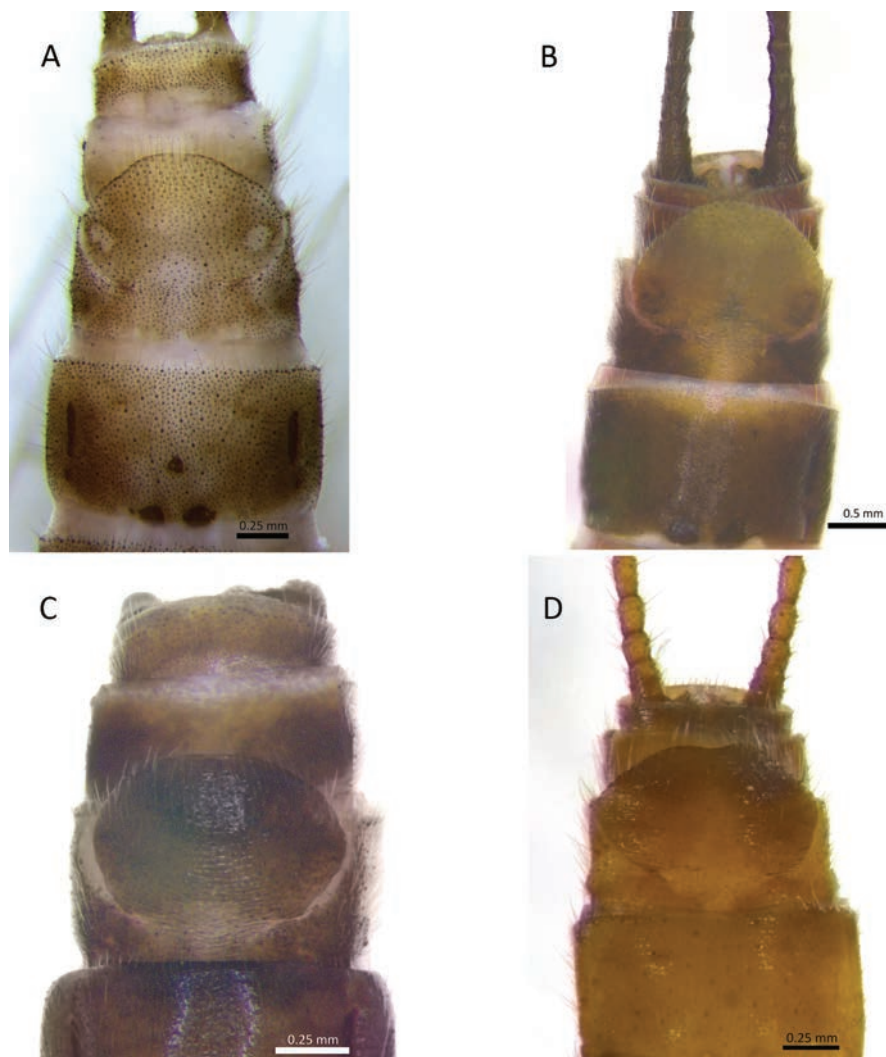


Fig. 21. *Utaperla*, female terminalia, ventral. (A) *U. gaspesiana*, Lucas Creek, New York, USA (INHS Insect Collection 658544). (B) *U. lepnevae*, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658547). (C) *U. orientalis*, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658546). (D) *U. sopladora*, St. Regis River, Montana, USA (CSUC).

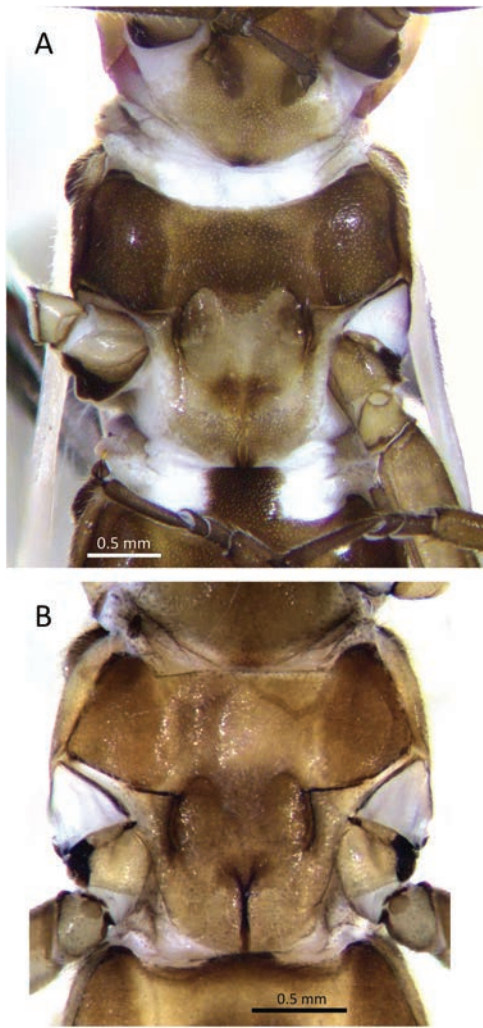


Fig. 22. *Paraperla*, venter. (A) *P. frontalis*, female, Sulphur Creek, California, USA (EJSC Pa_fr_02). (B) *P. wilsoni*, male, Ross Creek, Montana, USA (CSUC).

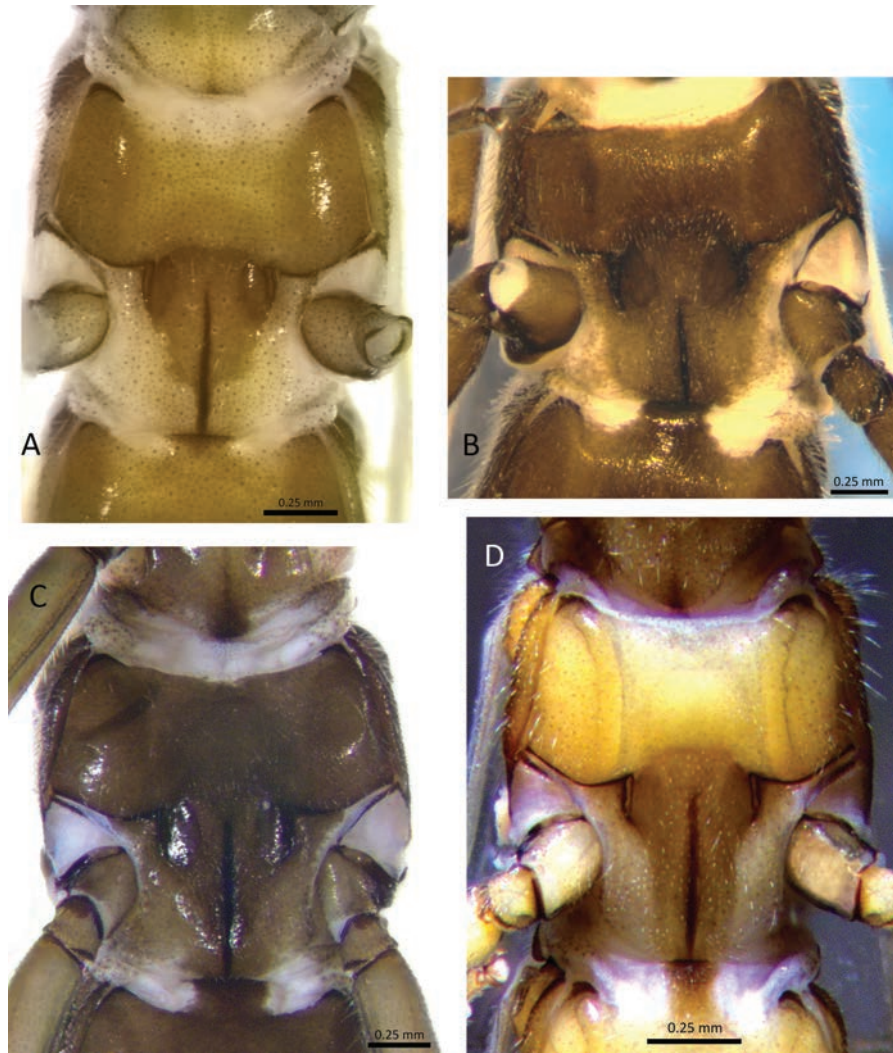


Fig. 23. *Utaperla*, venter. (A) *U. gaspesiana*, male, East Branch Delaware River, New York, USA (INHS Insect Collection 658549). (B) *U. lepnevae*, male, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658548). (C) *U. orientalis*, female, Anyui River, Khabarovsk Region, Russia (INHS Insect Collection 658546). (D) *U. sopladora*, male, Bitterroot River, Montana, USA (CSUC).

of six analyses from this study and the analysis from Terry's study (2003) is challenged by a highly supported placement of the clade as sister to Chloroperlidae + Perlodidae in four of the five analyses from South et al. (2021). The position of *Kathroperla* appears independent of tree construction methodology and dataset composition varying in inclusion/exclusion of the third nucleotide codon position. Differences in taxon sampling between this study and South et al. (2021) may account for these placement incongruencies, hence, further exploration is needed to fully resolve the phylogenetic position of *Kathroperla*.

Conclusions

Molecular and morphological evidence from this study support the designation of a seventeenth extant stonefly family, Kathroperlidae, fam. n. All molecular analyses show a monophyletic group, five of which recovered *Kathroperla* as sister group to the remaining Perloidea. Observed morphology shows postocular head length of *Kathroperla* as distinct among the Perloidea.

Material Examined of the Paraperlinae

Paraperla frontalis (Banks, 1902)

United States: California: Plumas Co., Sulphur Creek, 47.6 km W Cold Springs at CA-89, 39.70722, -120.53188, 25.v.2019, E.J. South, B.C. Kondratieff, 2♂, ♀ (INHS Insect Collection 658513, EJS Pa_fr_01, Pa_fr_02); Sierra Co., North Yuba River, 3.0 km SW Bassetts at CA-49, 39.59619, -120.61041, 21.v.2016, E.J. South, R.E. DeWalt, ♀ (INHS Insect Collection 793097).

Paraperla wilsoni Ricker, 1965

United States: Alaska: Kenai Peninsula, East Fork Six Mile Creek, 5 km N Hope turnoff at mile marker 59 AK 1 south, 16.v.2006, D.W. Webb, ♂ (INHS Insect Collection 487620). **Montana:** Lincoln Co., Ross Creek, Ross Creek Cedars Scenic Area, 48.20782, -115.91459, 27.iv.2008, R.S. Durfee, ♂ (CSUC). **Oregon:** Hood River Co., Herman Creek, near Oxbow Fish Hatchery, 14.v.2003, B.C. Kondratieff, R.W. Baumann, ♀ (CSUC).

Utaperla gaspesiana Harper & Roy, 1975

United States: New York: Delaware Co., East Branch Delaware River, Rt. 28, SW Margaretville, 42.1242, -74.6726, 27.v.2009, L.W. Myers, B.C. Kondratieff, ♂, ♀ (INHS Insect Collection 658543), same but 27.v.2010, L.W. Myers, B.C. Kondratieff, ♂, ♀ (INHS Insect Collection 658549, 658550); Ulster Co., Lucas Creek, E Manorville at Ralph Vedder Rd., 42.1408, -74.0331, 21.v.2008, L.W. Myers, B.C. Kondratieff, R.W. Baumann, ♀ (INHS Insect Collection 658544).

Utaperla lepnevae (Zhiltzova, 1970)

Russia: Khabarovsk Region, Anyui River, Anyui National Park, 6.iv.2019, N.M. Yavorskaya, ♂, ♀ (INHS Insect Collection 658548, 658547).

Utaperla orientalis Nelson & Hanson, 1969

Russia: Khabarovsk Region, Anyui River, Anyui National Park, 6.iv.2019, N.M. Yavorskaya, ♂, ♀ (INHS Insect Collection 658545, 658546).

Utaperla sopladora Ricker, 1952

United States: Montana: Mineral Co., St. Regis River, Haugan, exit 16, 47.38418, -115.39988, 16.vi.2006, R.S. Durfee, ♀ (CSUC); Ravalli Co., Bitterroot River, River Park, Hamilton, 46.24185, -114.16839, 9.vi.2009, R.S. Durfee, ♂ (CSUC), same but Little Sleeping Child Creek, Sleeping Child Rd., 46.13021, -114.04882, 13.vi.2020, B.C. Kondratieff, A. Mousa, 2♂ (CSUC).

Modified Key to Stark et al. (2015) for Species of *Kathroperla*

- 0) Adult cerci four-segmented (Figs. 3C and 4C and D); dark lateral pronotal bands cover more than 2/3 dorsal surface (Figs. 3A and 4A); head broad and subquadrate (Figs. 3A and 4A); egg four-sided (Stark 2010, his Fig. 8)..... *K. doma*
- 0') Adult cerci with more than four segments (> 12); dark lateral pronotal bands cover less than 2/3 dorsal surface; head less broad; egg circular in cross-section (Stark et al. 2015, his Figs. 1–6, 9–14, 20–25)..... 1
- 1) Lateral pronotal margin of adults usually entirely yellow; female subgenital plate usually reaching posterior margin of sternum 9 (Stark and Surdick 1987, their Fig. 4); egg collar absent and chorionic striations absent (Stark et al. 2015, their Figs. 20–25); dorsal margin of epiproct usually bicolored with a black anterior band adjacent to a dark brown band (Stark and Surdick 1987, their Fig. 1); basal male cercal segments about 3 times long as wide *K. takhoma*
- 1') Lateral pronotal margins of adults with black pigment extending at least a third of the margin length, but often completely dark (Stark et al. 2015, their Fig. 15); egg collar present, chorionic striations present although sometimes indistinct (Stark et al. 2015, their Figs. 1–6, 9–14); dorsal margin of epiproct narrow and black; basal male cercal segments about 2–2.5 times long as wide..... 2
- 2) Dorsolateral margin of male epiproct slanted conspicuously caudad (Stark et al. 2015, their Fig. 16); egg chorion covered with fine bead-like tubercles poorly organized into striations (Stark et al. 2015, their Figs. 9–14)..... *K. siskiyou*
- 2') Dorsal margin of male epiproct almost straight; egg chorion covered with thick tubercles organized into well-developed

longitudinal striations (Stark et al. 2015, their Figs. 1–6) *K. perdita*

Key to Families of Perloidea Adults

- 1) Thoracic remnant gill tufts present. Costal cross veins numerous..... Perlidae
- 1') Thoracic remnant gill tufts absent, though finger-like projections may be present. Costal cross veins less numerous, usually <10, present in basal half of wing 2
- 2) Postocular head length from posterior margin of eye to posterior margin of head capsule approximately two eye lengths Kathroperlidae, fam. n.
- 2') Postocular head length from posterior margin of eye to posterior margin of head capsule less than one and a half eye lengths, usually less than one eye length 3
- 3) Hindwing with less than four anal veins. Last segment of maxillary palps peg-like and small, connected asymmetrically atop the penultimate segment Chloroperlidae
- 3') Hindwing with more than four anal veins. Last segment of maxillary palp attached symmetrically to penultimate segment Perlodidae

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Author Contributions

EJS drafted the manuscript, provided specimens, extracted DNA, prepared all figures and illustrations, imaged all specimens, and obtained funding. RJS performed molecular analyses and edited the manuscript. RED contributed taxonomic expertise, edited the manuscript, and obtained funding. MAD provided molecular laboratory resources and edited the manuscript. KPJ provided guidance for molecular analysis and edited the manuscript. VAT, JJJ, RLM, JMH, YJB, and LWM provided specimens and reviewed the manuscript.

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