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First instar nymphs of two peltoperlid stoneflies (Insecta, Plecoptera, Peltoperlidae)

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Abstract

The first instar nymphs of two peltoperlid stoneflies, i.e., *Microperla brevicauda* Kawai, 1958 of Microperlinae and *Yoraperla uenoi* (Kohno, 1946) of Peltoperlinae, were examined and described. Additionally, the phylogeny and groundplan of the first instar nymphs of Peltoperlidae and Plecoptera were considered. The first instar nymphs of *M. brevicauda* have a slender body with a prognathous head of typical shape; they represent a groundplan in Plecoptera. On the other hand, the first instar nymphs of *Y. uenoi* have a broad, cockroach-like body with an orthognathous and shortened head, the latter being regarded as a potential autapomorphy of Peltoperlinae. Such differences in body shape between the subfamilies are speculated to arise from heterochrony. The three-segmented cerci of *Y. uenoi* are characteristic to Systellognatha, whereas the four-segmented cerci of *M. brevicauda* were independently acquired within Microperlinae. The structure and distribution pattern of chloride cells in the first instar nymphs of Plecoptera were also discussed. The presence of coniform chloride cells is a potential groundplan of Arctoperlaria. One to two pairs of chloride cells are distributed on the first nine abdominal segments of *M. brevicauda*; this represents a groundplan character of Systellognatha. On the other hand, one to four pairs of chloride cells are found on the second to ninth abdominal segments of *Y. uenoi*; this distribution pattern may be an apomorphic groundplan of Peltoperlinae.

Key Words

Arctoperlaria, Systellognatha, Microperlinae, Peltoperlinae, phylogeny, chloride cell

Introduction

Plecoptera, commonly known as stoneflies, are a hemimetabolous, neopteran order containing approximately 3,700 described species with a worldwide distribution on all continents except Antarctica (e.g., Zwick 1973; Fochetti and Tierno de Figueroa 2008; DeWalt and Ower 2019). In terms of phylogenetic position, recent morphological, embryological, and molecular evidence supports the placement of Plecoptera in the monophyletic group Polyneoptera (e.g., Ishiwata et al. 2011; Yoshizawa 2011; Mashimo et al. 2014; Misof et al. 2014; Wipfler et al. 2015, 2019; Song et al. 2016; Mtow and Machida 2018). The relationships between family group taxa within Plecoptera, i.e., 16 families, are widely accepted based on the two-suborder concept (e.g., Zwick 2000; Beutel et al. 2014; McCulloch et al. 2016; Ding et al. 2019). The suborder Arctoperlaria mainly occurs in the Northern Hemisphere, comprising 12 families within two subgroups, Euholognatha and Systellognatha, which each contain six families, i.e., Scopuridae, Taeniopterygidae, Capniidae, Leuctridae, Nemouridae, and Notonemouridae of Euholognatha, and Pteronarcyidae, Styloperlidae, Peltoperlidae, Perlidae, Chloroperlidae, and Perlodidae of Systellognatha. In contrast, the suborder Antarctoperlaria is found only in the Southern Hemisphere and contains four families, i.e., Eustheniidae, Diamphipnoidae, Austroperlidae, and Gripopterygidae.

Peltoperlidae is a systellognathan family present in North America and East Asia; it contains almost 70 described species (DeWalt and Ower 2019) and is comprised of two subfamilies, Microperlinae and Peltoperlinae

(Uchida and Isobe 1989; Zwick 2000). The monophyly of Peltoperlidae and each subfamily is supported by both morphological (e.g., Zwick 1973, 2000; Uchida and Isobe 1989) and molecular phylogenetic evidence (Cao et al. 2019). However, despite extensive research having been conducted by multiple stonefly researchers, the systematic position of Peltoperlidae in Plecoptera remains to be clarified. Groups that have been proposed as sister group candidates are Pteronarcyidae (Mtow and Machida 2018), Styloperlidae (e.g., Uchida and Isobe 1989; Zwick 2000; Wang et al. 2017, 2019; Shen and Du 2020; Zhao et al. 2020), Perlidae (e.g., Chen and Du 2017b; 2018; Ding et al. 2019), Austroperlidae + Scopuridae (Illies 1965), Pteronarcyidae + Styloperlidae (Ding et al. 2019), Styloperlidae + Perlidae (Chen and Du 2017a), Chloroperlidae + Perlidae (Ding et al. 2019), Perloidea (= Perlidae + Chloroperlidae + Perlodidae) (Zwick 1973, 1980; Nelson 1984; Thomas et al. 2000), Pteronarcyidae + Perloidea (e.g., Ricker 1952; Terry 2004; Kjer et al. 2006; Shen and Du 2019; South et al. 2021), (Pteronarcyidae + Chloroperlidae) + (Styloperlidae + Perlidae) (Chen and Du 2017a), (Pteronarcyidae + Styloperlidae) + Perloidea (Chen et al. 2018), (Taeniopterygidae + (Nemouridae + Notonemouridae)) + (Leuctridae + Capniidae) (Ricker 1950), ((Eustheniidae + Diamphipnoidae) + Austroperlidae) + (Gripopterygidae + (Capniidae + (Scopuridae + (Nemouridae + Notonemouridae)))) (Terry and Whiting 2005), and (Pteronarcyidae + Perloidea) + ((Taeniopterygidae + Leuctridae) + ((Scopuridae + Notonemouridae) + (Capniidae + Antarctoperlaria))) (Kjer et al. 2006).

It has previously been suggested that studies of Plecoptera first instar nymphs could be a potential source of phylogenetic information that could contribute to clarifying phylogenetic relationships (Harper 1979; Sephton and Hynes 1982). To date, the data collected in this area has been fragmentary and a detailed study has yet to be conducted. In addition, while the taxonomy and morphology of adults, older nymphs, and egg structures from Peltoperlidae have been studied extensively (e.g., Stark and Stewart 1981; Uchida and Isobe 1988, 1989; Stark and Nelson 1994; Stark and Sivec 2000, 2007; Stark et al. 2015; Chen 2020), information on peltoperlid hatchlings is entirely lacking.

Given this background, in the present study we examined and described, for the first time, the first instar nymphs of two Japanese peltoperlids, i.e., *Microperla brevicauda* Kawai, 1958 (Kawai 1958) (Fig. 1A) of Microperlinae and *Yoraperla uenoi* (Kohno, 1946) (Kohno 1946) (Fig. 1B, C) of Peltoperlinae, as two representative species. We compared the data obtained to that from previous studies on other plecopterans with the aim of discussing the groundplan and phylogeny of Peltoperlidae within Plecoptera as well as reconstructing the groundplan of their first instar nymphs.

Methods

Females of *Microperla brevicauda* and *Yoraperla uenoi* were collected from Japan• Nara, Higashi yoshino, a tributary of the Shigo river; alt. 420 m; around 34°22.67'N, 136°01.80'E; 13 Apr. 2016, and Japan• Nagano, Ueda, Kara-sawa stream; alt. 1220 m; around 36°31.19'N, 138°20.26'E; 12 Jul. 2019, respectively. They were kept separately at 12°C in plastic cases (68 mm × 39 mm × 15 mm) containing tissue paper and fed on Mitani Mushi-jelly, i.e., commercial food for insects (Fig. 1C). The first instar nymphs were obtained from eggs deposited and incubated in plastic cases (36 mm × 36 mm × 14 mm) filled with water at 12°C. These were then fixed with either Bouin's fixative (saturated picric acid aqueous solution : formalin : acetic acid = 15 : 5 : 1) or Kahle's fixative (ethyl alcohol : formalin : acetic acid : distilled water = 15 : 6 : 2 : 30) for 24 h and stored in 80% ethyl alcohol at room temperature. The following measurements were taken from the fixed nymphs: (1) body length (from the top of head to the tip of abdomen), (2) antennal length, (3) length and (4) width of head, (5) length and (6) width of pronotum, (7) pronotum width/body length ratio, (8) abdominal width, and (9) cercus length.

To observe chloride cells, some fixed specimens were stained with Mayer's acid haemalum for 1 h, mounted in distilled water, examined using an Olympus BX43 biological microscope, and finally photographed with a Pentax K-70 camera. Other fixed specimens were dehydrated in a graded ethanol series, immersed in acetone, and embedded in a Kulzer Technovit 7100 methacrylate resin in accordance with the protocol described by Machida et al. (1994). Serial, semi-thin sections at a thickness of 2 μm were cut using a Leica RM2235 semi-thin microtome equipped with a Leica TC-65 tungsten carbide knife. Sections were then stained with Mayer's acid haemalum for 1 h, 1% eosin Y for 1 h, and 1% fast green FCF 100% ethanol solution for 1 min, before being observed under the Olympus BX43 biological microscope and photographed with the Pentax K-70 camera.

For scanning electron microscopy, the fixed specimens were dehydrated in a graded ethanol series, naturally dried with HMDS (1,1,1,3,3,3-Hexamethyldisilazane) as described by Faull and Williams (2016), mounted on a stab, and then observed under a Hitachi TM-1000 scanning electron microscope at 15 kV without coating. Some mounted specimens were examined under the Olympus BX43 biological microscope and photographed with the Pentax K-70 camera.

The specimens examined in the present study have been deposited in the collection of the Faculty of Symbiotic Systems Science, Fukushima University.

Results

The present study follows the view of Matsuda (1976) on the abdominal segmentation on Plecoptera, i.e., that the sternum of the first abdominal segment is greatly reduced or absent. The definition of chloride cells, which are divided morphologically into four types, i.e., caviform, coniform, bulbiform, and floriform, follows that of Wichard et al. (1999).

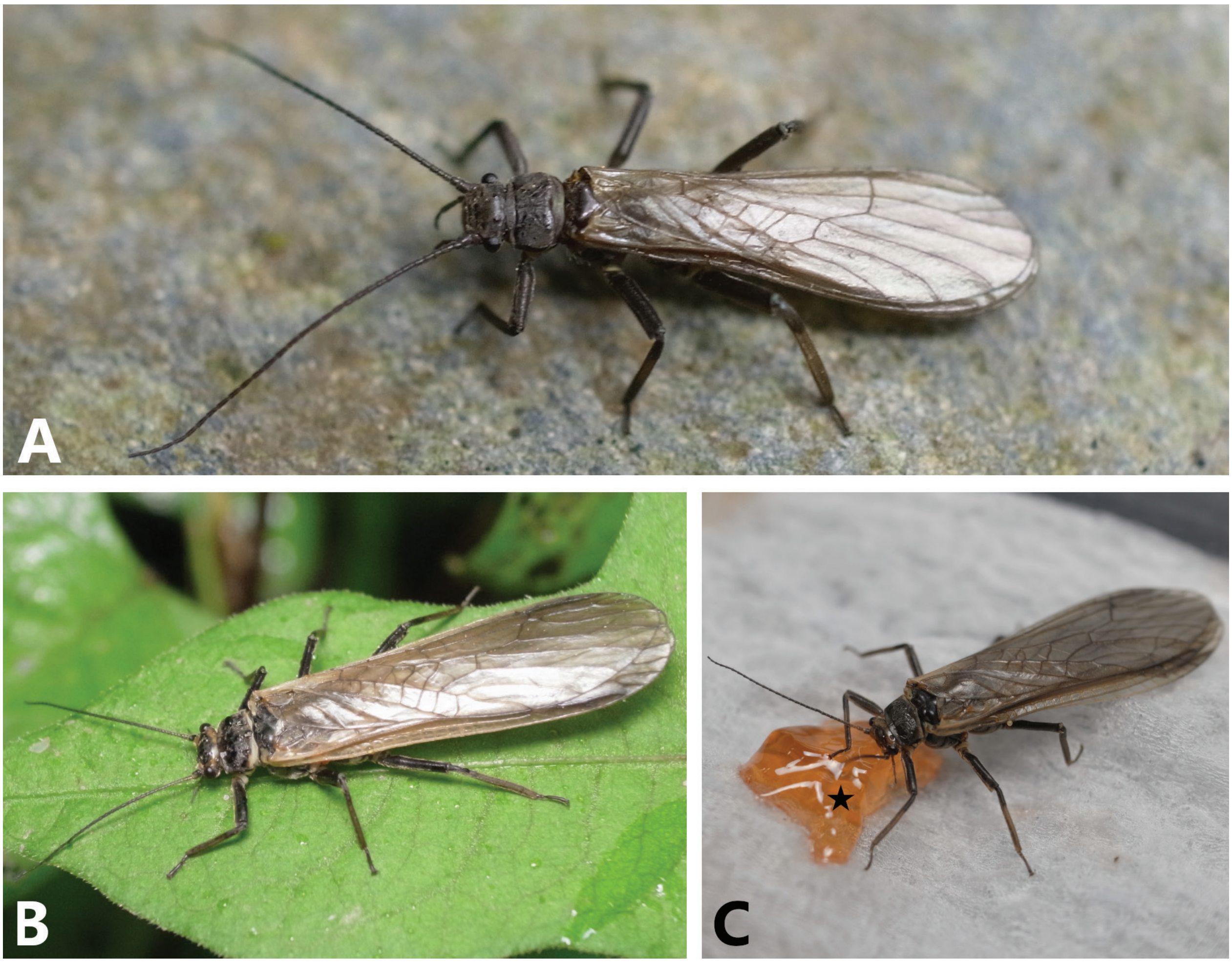

Figure 1. Adults of *Microperla brevicauda* and *Yoraperla uenoi*. **A.** *M. brevicauda*; **B.** *Y. uenoi*; **C.** *Y. uenoi* eating a commercial food (star) in a plastic case.

Table 1. Measurements of the fixed specimens of first instar nymphs of *Microperla brevicauda* and *Yoraperla uenoi*.

	Microperla brevicauda	*Yoraperla uenoi*
Specimens examined	5	5
Body length (µm)	623.5 ± 12.3	574.1 ± 6.0
Antennal length (µm)	402.3 ± 53.5	282.3 ± 32.2
Head length (µm)	115.3 ± 10.9	96.5 ± 6.0
Head width (µm)	180.0 ± 2.9	217.6 ± 8.3
Pronotum length (µm)	72.9 ± 6.0	82.4 ± 6.4
Pronotum width (µm)	171.8 ± 4.4	236.5 ± 12.0
Pronotum width / Body length	0.27 ± 0.01	0.41 ± 0.02
Abdominal width (um)	114.1 ± 2.9	156.47 ± 2.9
Cercus length (µm)	195.3 ± 14.6	160.0 ± 6.9

Measurements of the first instar nymphs of *Microperla brevicauda* and *Yoraperla uenoi* are shown in Table 1.

Microperla brevicauda Kawai, 1958

Figs 2A–D, 4A, B, D, E

Description. Body slender, uniformly white, sparsely covered by long and short fine setae, without gill and ocelli (Fig. 2A, B). Head prognathous, subtriangular (Fig. 2A, B). Antenna nine-segmented, longer than two-thirds of body length (Fig. 2A, B; Table 1). Compound eye reddish-black with four ommatidia. Labrum nearly semicircular, covering part of mandible (Fig. 2C). Maxillary coxopodites divided into distal cardo and proximal stipes (Fig. 2C); maxillary palp and endites of maxilla and lateral galea well developed, but mesal lacinia hardly visible externally (Fig. 2C). Labial coxopodites divided into proximal postmentum and distal prementum, but hardly recognizable in fixed specimen (Fig. 2C); labial palp and endites of labium, lateral paraglossa, and mesal glossa well developed (Fig. 2C). Pronotum rectangular, almost same width as the head, and wider than the abdomen (Fig. 2A; Table 1). Lateral margin of mesonotum and metanotum less developed (Fig. 2A). Thoracic appendage consisting of coxa, trochanter, femur, tibia, tarsus with three tarsomeres, and pretarsus with ungues (Fig. 2D); tibia longer than femur (Fig. 2D). Abdominal segments with a row of long fine setae along posterior margin of each tergum (Figs 2A, 4A). Coniform chloride cells approximately 10 µm in diameter and 1 µm in height distributed on lateral side of first nine abdominal segments; one pair on first, eighth, and ninth targa; two pairs on second to seventh segments, i.e., one pair on each of second to seventh terga and sterna (Fig. 4A, B, D, E). Less sclerotized supraanal lobe on hind margin of tenth tergum (Fig. 2A). Cerci four-segmented, with a crown of long and short fine setae on

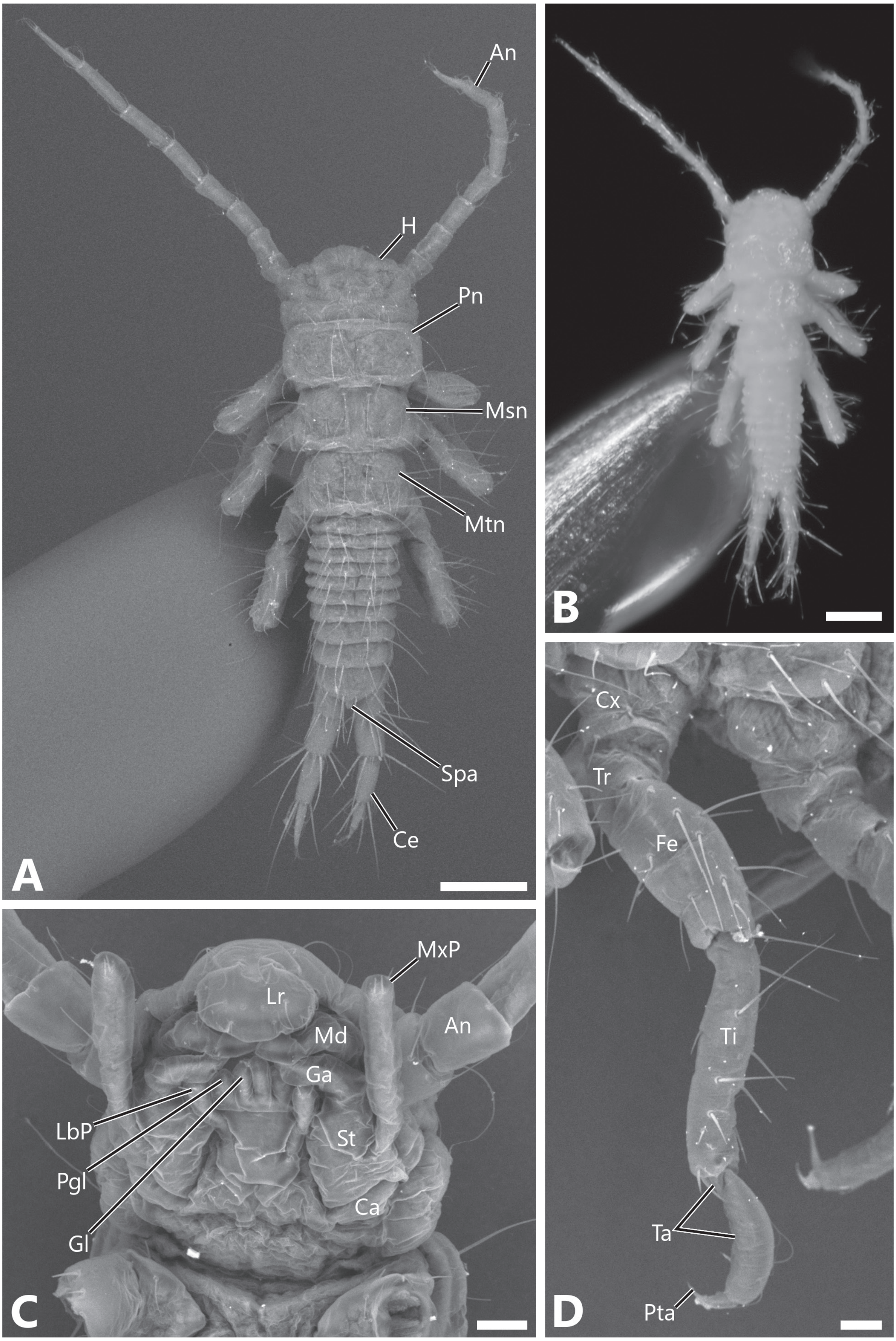

Figure 2. First instar nymphs of *Microperla brevicauda*. **A.** Habitus, dorsal view, scanning electron microscopy (SEM); **B.** Habitus, dorsal view, same specimen as in (**A**), light microscopy; **C.** Mouth parts, ventral view, SEM; **D.** Middle leg, SEM. Abbreviations: An, antenna; Ca, cardo; Ce, cercus; Cx, coxa; Fe, femur; Ga, galea; Gl, glossa; H, head; LbP, labial palp; Lr, labrum; Md, mandible; Msn, mesonotum; Mtn, metanotum; Pgl, paraglossa; Pta, pretarsus; Spa, supraanal lobe; St, stipes; Ta, tarsus; Ti, tibia; Tr, trochanter. Scale bars: 100 µm (**A, B**); 20 µm (**C, D**).

posterior margin of first three segments; short fine setae at apex of fourth segment (Fig. 2A).

Yoraperla uenoi (Kohno, 1946)

Figs 3A–D, 4C, F, G

Description. Body broad and slightly cockroach-like, uniformly white, covered by brownish, long and short, stout setae, without gill and ocelli (Fig. 3A, B). Head orthognathous, trapezoidal, and highly shortened (Fig. 3A). Antenna nine-segmented, same as half of body length (Fig. 3A, B; Table 1). Compound eye reddish-black but ommatidia inconspicuous in fixed specimens; according to Mtow and Machida (2018), four ommatidia formed in full-grown embryos of *Y. uenoi*. Labrum slightly trapezoidal, covering part of mandible (Fig. 3C). Mandible well-developed with teeth at its apex (Fig. 3C). Maxillary coxopodites divided into distal cardo and proximal stipes, but latter hardly visible from ventral view (Fig. 3C); maxillary palp and endites of maxilla, lateral galea, and mesal lacinia well developed (Fig. 3C); teeth formed at tip of lacinia (Fig. 3C). Labial coxopodites divided into proximal postmentum and distal prementum, but hardly recognizable in fixed specimen (Fig. 3C); labial palp and endites of labium, lateral paraglossa, and mesal glossa well developed (Fig. 3C). Pronotum rectangular with corners slightly rounded, slightly wider than head and wider than abdomen (Fig. 3A; Table 1). Mesonotum and metanotum trapezoidal, slightly widening posteriorly (Fig. 3A). Thoracic appendage consisting of coxa, trochanter, femur, tibia, tarsus with three tarsomeres, and pretarsus with ungues (Fig. 3D); tibia almost identical in length to femur (Fig. 3D); two claws can be recognized from ventral view (data not shown). Abdominal segments with a row of long and short stout setae along posterior margin of each tergum, except first to third terga, covered by metanotum (Figs 3A, 4F); first terga without setae, second and third tergum with setae barely visible in section (data not shown). Coniform chloride cells approximately 10 µm in diameter and 1.5 µm in height distributed on posterior margin of second to ninth abdominal segments; one pair on second sternum; three pairs on third sternum; four pairs on fourth to seventh sterna; three or four pairs on eighth sternum; two pairs on ninth sternum (Fig. 4C, F, G). Cerci three-segmented, with a crown of long and short stout setae on posterior margin of first two segments; short stout setae on apex of third segment (Fig. 3A).

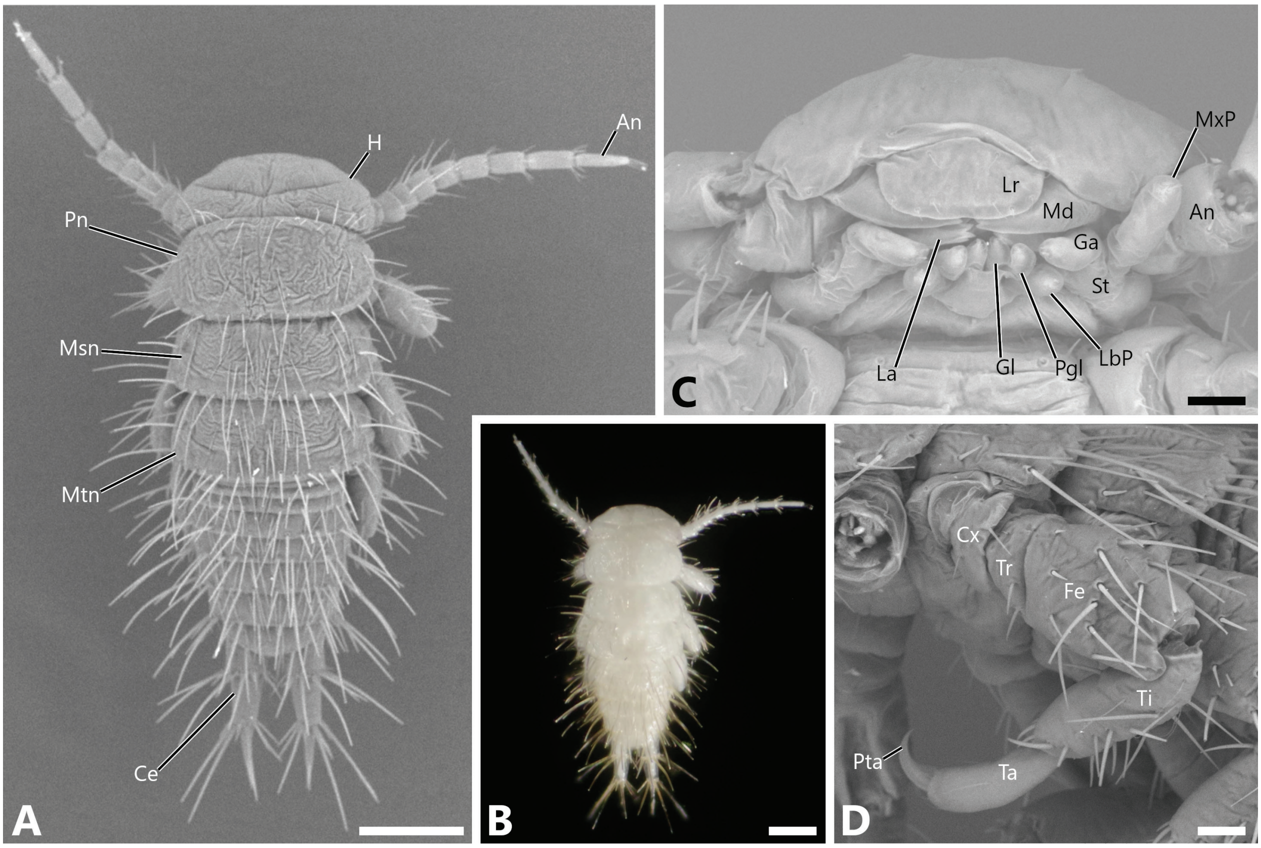

Figure 3. First instar nymphs of *Yoraperla uenoi*. **A.** Habitus, dorsal view, scanning electron microscopy (SEM); **B.** Habitus, dorsal view, same specimen as in (**A**), light microscopy; **C.** Mouth parts, ventral view, SEM, right side of maxillary pulp artificially lacking; **D.** Middle leg, SEM. Abbreviations: An, antenna; Ce, cercus; Cx, coxa; Fe, femur; Ga, galea; Gl, glossa; H, head; La, lacinia; LbP, labial palp; Lr, labrum; Md, mandible; Msn, mesonotum; Mtn, metanotum; Pgl, paraglossa; Pta, pretarsus; St, stipes; Ta, tarsus; Ti, tibia; Tr, trochanter. Scale bars: 100 µm (**A, B**); 20 µm (**C, D**).

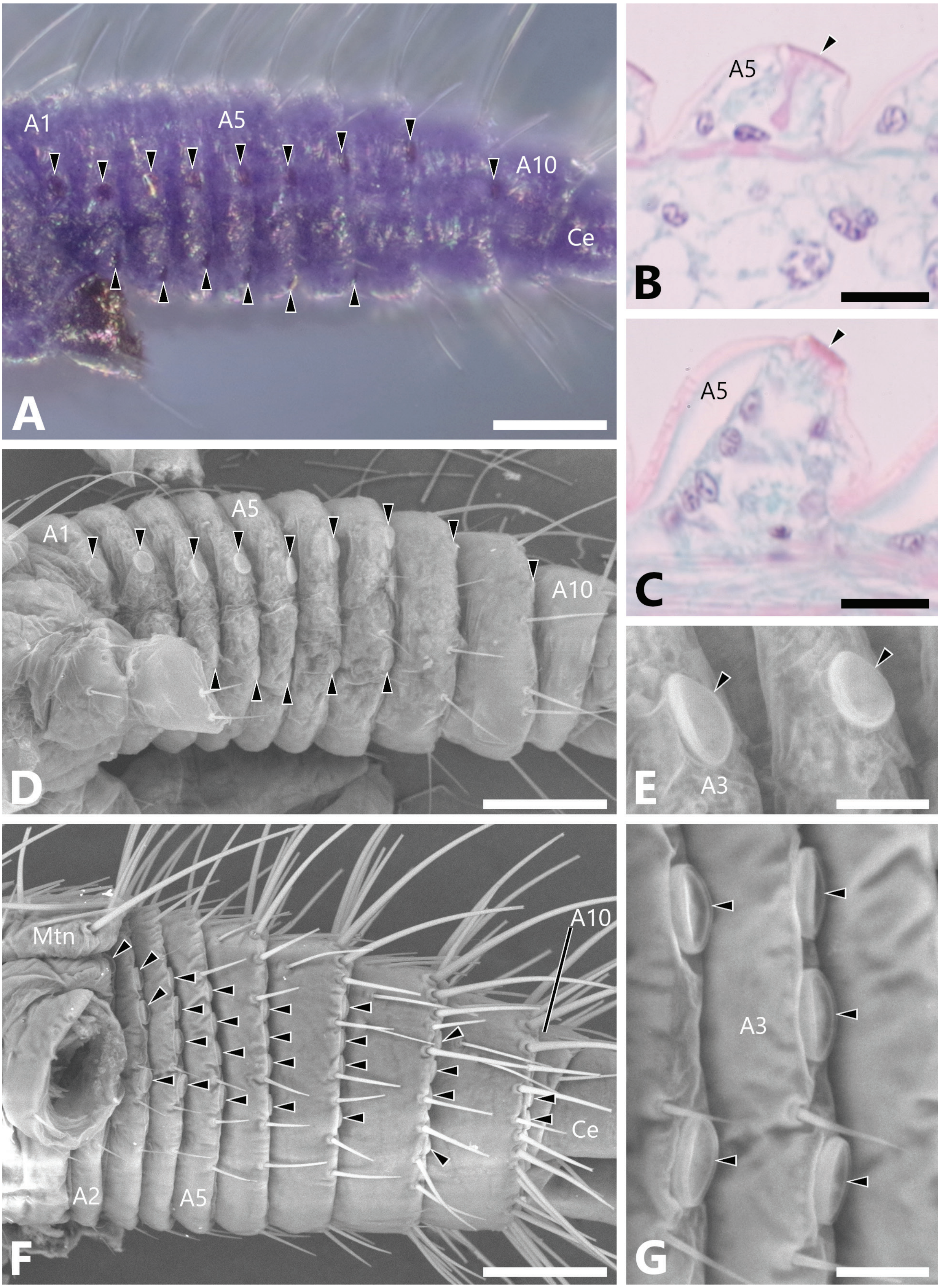

Figure 4. Chloride cells of first instar nymphs of *Microperla brevicauda* and *Yoraperla uenoi*, anterior to the left. **A.** Abdomen of *M. brevicauda*, lateral view, stained with Mayer's acid haemalum; **B, C.** Horizontal sections of fifth abdominal segment of *M. brevicauda* (**B**) and *Y. uenoi* (**C**); **D, E.** Abdomen of *M. brevicauda*, lateral view, scanning electron microscopy (SEM), all abdominal segments (**D**) and enlargement of chloride cells (**E**); **F, G.** Abdomen of *Y. uenoi*, ventrolateral view, SEM, all abdominal segments (**F**) and enlargement of chloride cells (**G**). Arrowheads show the chloride cells. Abbreviations: A1, 2, 3, 5, and 10: first, second, third, fifth and tenth abdominal segments, respectively; Ce, cercus; Mtn, metanotum. Scale bars: 50 µm (**A, D, F**); 10 µm (**B, C, E, G**).

Discussion

The first instar nymphs of *Microperla brevicauda* of Microperlinae can be characterized by a slender body and typical head, i.e., being prognathous and subtriangular in shape. These features are predominant in Plecoptera, i.e., Antarctoperlaria: Eustheniidae (Helson 1935; Sephton and Hynes 1982), Austroperlidae (Sephton and Hynes 1982), and Gripopterygidae (Sephton and Hynes 1982); Arctoperlaria: Euholognatha, Scopuridae (Komatsu 1956; Mtow 2019), Taeniopterygidae (e.g., Berthélemy 1979; Harper 1979), Leuctridae (e.g., Harper 1979; Snellen and Stewart 1979a), Capniidae (Harper 1979), Nemouridae (e.g., Harper 1979), and Notonemouridae (Sephton and Hynes 1982); Systellognatha: Pteronarcyidae (Miller 1939), Perlidae (e.g., Harper 1979; Kishimoto and Ando 1985), Chloroperlidae (Harper 1979), and Perlodidae (Berthélemy 1979; Harper 1979). Therefore, we conclude that these characters represent a groundplan of Plecoptera. In contrast, the first instar nymph of *Yoraperla uenoi* of Peltoperlinae can be characterized by a broad, slightly cockroach-like body and modified head, i.e., being orthognathous, shortened, and trapezoidal in shape. The head shape is unique to Peltoperlinae within Plecoptera and could be a potential autapomorphy of this group, which is consistent with the understanding of Zwick (2000), i.e., that a modified head shape such as this may be an apomorphic groundplan of Peltoperlinae.

Notably, the cockroach-like body shape, which is regarded as an autapomorphy of Peltoperlidae (Zwick 1973, 2000; Uchida and Isobe 1989), was found in the first instar nymphs of Peltoperlinae but did not appear in those of Microperlinae. Given that the older nymphs of *M. brevicauda* have much broader, cockroach-like bodies (e.g., Shimizu et al. 2005) and that the full-grown embryos of *Y. uenoi* acquire the configuration of the first instar nymphs (Mtow and Machida 2018), such differences in the body shape of peltoperlid first instar nymphs could be interpreted as a result of heterochrony. In other words, morphogenesis of the cockroach-like body shape may commence by the later embryonic period in Peltoperlinae but occur only during the postembryonic stages in Microperlinae. Further detailed examination of the embryonic and postembryonic development of Peltoperlidae will be required to broaden our knowledge of nymphal shape morphogenesis in this family.

The present study revealed that the first instar nymphs of *M. brevicauda* and *Y. uenoi* have four-segmented and three-segmented cerci, respectively. In Systellognatha, three-segmented cerci are found predominantly in Pteronarcyidae (Miller 1939), Perlidae (e.g., Khoo 1964; Harper 1979; Kishimoto and Ando 1985), Chloroperlidae (Khoo 1964; Harper 1979), and Perlodidae (e.g., Khoo 1964; Harper 1979) with two exceptional cases, which have four cercal segments: *Perlesta placida* (Hagen) of Perlidae (Snellen and Stewart 1979b) and *Hydroperla crosbyi* (Needham & Claassen) of Perlodidae (Oberndorfer and Stewart 1977). Therefore, the sharing of three-segmented cerci, which are found in *Y. uenoi* of Peltoperlinae, might be characteristic to Systellognatha, whereas four cercal segments, such as those found in *M. brevicauda*, were most likely acquired independently within Microperlinae.

The chloride cells, which are known to have osmoregulatory functions (e.g., Wichard et al. 1999), of Antarctoperlaria nymphs are floriform type only (e.g., Zwick 1973), even in the first nymphal stage (Sephton and Hynes 1982), which is regarded as an apomorphic groundplan of Antarctoperlaria (e.g., Zwick 1973, 2000). On the other hand, the presence of three other types of chloride cells, i.e., caviform, coniform, and bulbiform, has been observed in arctoperlarian nymphs (e.g., Wichard et al. 1999; Tamura and Kishimoto 2010), but only the coniform type has been observed in hatchlings of Arctoperlaria (Berthélemy 1979; Kishimoto and Ando 1985) with the exception of euholognathan Notonemouridae, which has bulbiform type (Sephton and Hynes 1982). In the present study, the first instar nymphs of *M. brevicauda* and *Y. uenoi* had chloride cells of coniform type. Thus, the type of chloride cells found in *M. brevicauda* and *Y. uenoi* is apparently comparable to those found in euholognathan *Brachyptera braueri* Klapálek of Taeniopterygidae (Berthélemy 1979), as well as systellognathan *Kamimuria tibialis* (Pictet) of Perlidae (Kishimoto and Ando 1985) and *Perlodes microcephalus* (Pictet) of Perlodidae (Berthélemy 1979). Therefore, the coniform type of chloride cells may be regarded as a potential groundplan of Arctoperlaria. The bulbiform type of chloride cells found in Notonemouridae might be due to a secondary modification from the coniform type, because as Wichard et al. (1999) pointed out, the coniform type is regarded as the basic type of chloride cells.

In the present study, we also distinguished two distribution types of chloride cells in Peltoperlidae: (1) the first type, in which one to two pairs of chloride cells are distributed on the first nine abdominal segments, is found in *M. brevicauda* of Microperlinae; (2) the second type, in which one to four pairs of chloride cells are distributed on the second to eighth abdominal segments, is found in *Y. uenoi* of Peltoperlinae. Additional examination of chloride cells will be required to cover more lineages of Plecoptera in detail. However, given the distribution of the chloride cells on the abdomen of first instar nymphs, it may be meaningful that the first type has also been observed in Perlidae (Kishimoto 1983; Kishimoto and Ando 1985) and Perlodidae (Berthélemy 1979). Therefore, we could postulate the following scenario: (1) the first type is a groundplan character in Systellognatha, and (2) this groundplan feature was inherited by Microperlinae in Peltoperlidae; whereas, (3) the second type was acquired by Peltoperlinae as an apomorphic groundplan.

Conclusion

In the present study, we (1) examined and described the first instar nymphs of Peltoperlidae for the first time, (2) reconstructed the groundplan of first instar nymphs from Peltoperlidae and Plecoptera, and (3) demonstrated that data collected from first instar nymphs could provide a new basis for discussion and reconstruction of the groundplan and phylogeny of Plecoptera. To improve understanding of the plecopteran groundplan and phylogeny further, more detailed studies of first instar nymphs must be conducted; these should consider all major lineages of Plecoptera, especially the antarctoperlarian Diamphipnoidae and arctoperlarian Styloperlidae, on which information is entirely lacking.

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