This is the accepted manuscript of an article published online by Taylor & Francis in *Systematics and Biodiversity* on 29-Aug-2024, available at https://doi.org/10.1080/14772000.2024.2383214

Cox1-based phylogeny of Eastern Palearctic *Drunella* (Ephemeroptera: Ephemerellidae), description of new species, and redescription of *D. cryptomeria* (Imanishi)

running title: Molecular phylogeny and morphological description of *Drunella* species

Kei Wakimura[1,6], Shin-ichi Ishiwata[2], Minami Hamamura[3], Yasuhiro Takemon[4,7], Kazumi Tanida[3,5], Koji Inai[1], Mikio Kato[1,3]*

[1] Biology Laboratory, Faculty of Liberal Arts, Sciences and Global Education, Osaka Metropolitan University Nakamozu Campus, 1-1 Gakuencho, Naka-ku, Sakai 599-8531, Japan. [2] Department of Applied Chemistry, Kanagawa Institute of Technology, 1030 Shimoogino, Atsugi 243-0292, Japan. [3] Department of Biology, Graduate School of Science, Osaka Metropolitan University Nakamozu Campus, 1-1 Gakuencho, Naka-ku, Sakai 599-8531, Japan. [4] Disaster Prevention Research Institute, Kyoto University, Gokasho, Uji 611-0011, Japan. [5] Osaka Museum of Natural History, Nagai-Park, Higashi-Sumiyoshi-ku, Osaka 546-0034, Japan. [6] Present address: Faculty of Pharmacy, Osaka Ohtani University, 3-11-1 Nishikiori-kita, Tondabayashi 584-8540, Japan. [7] Present address: Biology Laboratory, Faculty of Liberal Arts, Sciences and Global Education, Osaka Metropolitan University Nakamozu Campus, 1-1 Gakuencho, Naka-ku, Sakai 599-8531, Japan.

*Corresponding author: Mikio Kato Email: mkato@omu.ac.jp

Abstract

The diversity of a mayfly genus, *Drunella* Needham (Ephemeroptera: Ephemerellidae), was examined using relationships of the barcode region of the cytochrome *c* oxidase subunit I (*cox1*) gene. Two novel species, *Drunella campicola* Ishiwata sp. nov. and *Drunella parvicarnivora* Ishiwata sp. nov. were described and *Drunella cryptomeria* was redescribed. A *cox1* dendrogram based on the maximum likelihood method consisted of ten clusters of DNA sequences sampled in Japan, which were roughly divided into three groups. The first group comprised *D. cryptomeria* and the two newly described species (*D. campicola* and *D. parvicarnivora*), the second group included *D. basalis* and *D. ishiyamana*, and the third group comprised *D. trispina*, *D. triacantha, D. kohnoi*, and *D. sachalinensis*. A cryptic lineage of *D. trispina* was observed and the distance between the two *D. trispina* clades is comparable to that between *D. triacantha* and each of the two *D. trispina* clades. The inheritance of the mitochondrial genome in these species is yet unclear, and therefore needs to be studied further. In the present study, we were able to confirm the distinct lineages based on *cox1* and aided in the understanding of the taxonomy of the genus *Drunella* with descriptions of two new species.

Key words: *cox1*, cryptic lineage, DNA barcoding, DNA taxonomy, *Drunella*, Ephemeroptera, Japan.

Introduction

The discrimination of highly diverse aquatic insects has been greatly advanced by DNA-based taxonomy (Ge et al., 2021; Hebert et al., 2003; Morinière et al., 2017; Suh et al., 2019). DNA barcoding using a partial coding region of mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) has been used to characterize mayflies in North America, and the efficacy of species identification has been evident (Ball et al., 2005; Webb et al., 2012). As for aquatic insects in Japan, information on a reference collections (mainly mayflies) is available online (http://www.b.s.osakafu-u.ac.jp/~mkato/J-amir_home.htm; Wakimura et al., 2016, 2020).

The members of the genus *Drunella* Needham 1905 are known in Asia and North America (Allen, 1980; Jacobus & McCafferty, 2008). They tend to be conspicuous and common in clear and rapid streams and occur in groups of large numbers of individuals in Japan (Ishiwata and Inada, 1996). *Drunella* was studied previously as a species group by McDunnough (1931a, b) and Traver (1932), and as a subgenus of *Ephemerella* Walsh (Needham, 1927). Allen (1980) and Tshernova (1972) elevated it to generic rank and Allen (1980) proposed five subgenera. Subsequently, these subgenera have been synonymized with the genus *Drunella* by Jacobus & McCafferty (2008). Additionally, Ishiwata (1987) reviewed the generic characters of Japanese Ephemerellidae based on the concepts of Allen & Edmunds (1962) and recorded eight species and a subspecies from Japan under the genus *Drunella*; *basalis*, *cryptomeria*, *ishiyamana* (= *yoshinoensis*), *kohnoi*, *sachalinensis* (= *bifurcata*), *trispina*, and *triacantha*. Afterward, seven species (*basalis*, *cryptomeria*, *ishiyamana*, *kohnoi*, *sachalinensis*, *trispina*, and *triacantha*) were recorded from Japan in the genus *Drunella*, as *D. yoshinoensis* was a junior synonym of *D. ishiyamana*, and *D. bifurcata* was a junior synonym of *D. sachalinensis* (Ishiwata, 2001, 2018b; Ishiwata & Takemon, 2005a).

DNA taxonomy of the genus *Drunella* in Japan and eastern Asia had been investigated by Jo & Tojo (2019), and they found two lineages (a major lineage and a cryptic lineage) in *D. basalis* (Imanishi, 1937) and *D. ishiyamana* Matsumura, 1931, respectively. Independently of their work, it

was preliminarily noted that *D. trispina* (Uéno, 1928), which was collected in two localities, were divided into two distinct lineages (Wakimura et al., 2020). One of which (a lineage of *D. trispina*) is shared with a minor clade of *D. basalis* that was analysed by Jo & Tojo (2019).

According to Wakimura et al. (2020), two undescribed groups of a spiny crawler mayfly genus, *Drunella* Needham, had clustered in a DNA-based neighbour-joining phylogenetic tree, both of which are closely related to *Drunella cryptomeria* (Imanishi, 1937). Consequently, to resolve the complicated DNA taxonomy of the genus *Drunella* in Japan, we have collected specimens more extensively and conducted molecular phylogenetic analysis of this taxon. In the course of this work, we have morphologically described two new species, *D. campicola* Ishiwata sp. nov. and *D. parvicarnivora* Ishiwata sp. nov. We also re-described *D. cryptomeria* (Imanishi, 1937).

Materials and methods

Collection of specimens for DNA analyses

Nymphs were collected in a riverbed. Imagoes were caught using an aerial net. Specimens for DNA analyses were stored at 4 °C and maintained alive until processing (for a maximum of 2 d) or preserved in 80% ethanol (for long-term storage), and those for morphological analysis were stored in 75% ethanol at ambient temperature. The sampling information for the specimens that were used for DNA analysis is summarized in Table 1. Some of the mature nymphs were reared until emergence in the laboratory for morphological analyses.

DNA analyses

Total genomic DNA was extracted from the whole body (for very small specimens), muscles (wing or leg), or larval haemolymph. Dissected tissues were treated with proteinase K (Promega, Madison, WI, USA) and extracted using phenol, and then genomic DNA fractions were precipitated with ethanol, as previously described (Wakimura et al., 2020). *Cox1* was used as the DNA barcode

(Hebert et al., 2003), and the target for sequencing was amplified by polymerase chain reaction (PCR) using the primer set described by Folmer et al. (1994) and Sensizyme HotStart Taq Premix (RBC Bioscience, New Taipei City, Taiwan) as previously described (Wakimura et al., 2016, 2020). The amplified products were sequenced using a BigDye Terminator v3.1 and an ABI3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The sequence data were deposited in the International Nucleotide Sequence Database (INSD). The accession numbers of the sequence data obtained in the present study as well as those used for the phylogenetic analysis are listed in Tables 1 and 2, respectively.

Sequence comparison and phylogenetic analysis of *cox1*

The sequence data were aligned using the ClustalW algorithm in the BioEdit software package ver. 7.2.5 (Hall, 1999), and phylogenetic relationships were estimated using PhyML 3.0 (Guindon et al., 2010) with the Smart Model Selection option (Lefort et al., 2017). The tree was visualized using FigTree ver. 1.4.4 (Rambaut, 2018). A 606 bp homologous region of the *cox1* gene (ranging from position 53–658 of MK774360) was used for sequence comparison and phylogenetic analysis. We sequenced 88 DNA samples that were collected for this study in Japan and included 102 sequences of *Drunella* (sampled from Japan, Korea, and Russia) that were retrieved from the INSD. The phylogenetic relationships among the total 190 DNA sequences were examined.

Morphological analyses

Imagoes, subimagoes, nymphs, and eggs were included in the morphological analysis. All the specimens for morphological analyses were preserved and examined in 75% ethanol. Fully developed eggs were obtained from preserved mature female nymphs, female subimagoes, or female imagoes. Scanning electron microscopy analysis was performed as described previously (Ishiwata & Fujitani 2018). Morphological nomenclature in this work follows that of Edmunds et al. (1976) and

Bauernfeind & Soldán (2012). Egg terminology follows Koss (1968), Koss & Edmunds (1974), Ubero-Pascal & Puig (2009), and Ishiwata & Fujitani (2018). The terms for thoracic morphology used in this study follow Kluge (2004). The type series in this study are deposited in the collections of the Museum of Nature and Human Activities, Hyogo, Japan (MNHAH). The other specimens used in this study are deposited in the authors' collections.

Abbreviations used in the Description section

For sexes and stages: ♂, male imago; ♀, female imago; ♂s, male subimago; ♀s, female subimago; n, nymph. For collection depositories: MNHAH, Museum of Nature and Human Activities, Hyogo, Japan; KM, the Kyoto University Museum, Kyoto, Japan; no indication, author's collection.

Taxonomy of the genus *Drunella* Needham, 1905 in Japan

Drunella Needham, 1905 (Type species: *Ephemerella grandis* Eaton, 1884), monotypy.

Eatonella Needham, 1927 (Type species: *Ephemerella doddsii* Needham, 1927). Synonymized by Jacobus & McCafferty (2008).

Myllonella Allen, 1980 (Type species: *Ephemerella coloradensis* Dodds). Synonymized by Jacobus & McCafferty (2008).

Tribrochella Allen, 1980 (Type species: *Ephemerella trispina* Uéno). Synonymized by Jacobus & McCafferty (2008).

Unirhachella Allen, 1980 (Type species: *Ephemerella tuberculata* Morgan). Synonymized by Jacobus & McCafferty (2008).

Diagnosis

The genus *Drunella* in Japan can be characterized in the adult male stage by: (1) the terminal segment of the genital forceps is two to three times as long as broad, (2) the long second segment of the genital forceps is more or less distinctly bowed, and (3) the penes are without lateral tubercles,

lobes, or dorsal or ventral spines. The nymphal stage is characterized by: (1) a flat body (especially the femora), (2) protrusions in front of the head (except for some), (3) a transverse field of bristles on clypeus, (4) tubercles usually present on the ventral margin of the fore femora, (5) a tibial projection with a row of small teeth (in part), and (6) the tarsal claws strongly bowed, usually having only a few denticles. The egg is characterized by: (1) an oval form with a polar cap, (2) a smooth chorion, without reticulations, covered with fine rectangular maculae (only visible at high magnification under scanning electron microscope), and (3) an attachment structure with a single polar cap and a multithread-folded with terminal fibre cluster (MTF) (Ubero-Pascal & Puig, 2009). In contrast, chorion is covered with reticulations and 1 to 4 tubercles in each reticulation appear in *Cincticostella* Allen and *Ephacerella* Paclt, while no tubercle in *Torleya* Lestage and *Teleganopsis* Ulmer. The surface of chorion in *Ephemerella* Walsh is rough and pitted, with or without reticulations (Ishiwata & Fujitani, 2018).

Note

Ishiwata (1987) provided the characters of Japanese *Drunella* referring to the concept proposed by Allen & Edmunds (1962) for adults and nymphs. Kluge (2004) had proposed the diagnostic characters for the nymphs. In the present study, the diagnoses described for the adult and nymphal stages follow the literatures (Allen & Edmunds, 1962; Ishiwata 1987, Kluge 2004). The egg characters were described based on Jacobus & McCafferty (2008) and Ishiwata & Fujitani (2018). Jacobus & McCafferty (2008) stated that, none of the polyspecific subgenera indicated by Allen (1980) were recovered as monophyletic, and they synonymized four nominal subgenera with *Drunella* (*sensu stricto*). Ogden et al. (2009) considered the genus *Drunella* to be monophyletic. Bauernfeind & Soldan (2012) took a more reserved approach towards the genus level taxonomy of Ephemerellidae and emphasized need for more study.

Results and discussion

DNA taxonomy of the genus *Drunella* in Japan

Sequence comparisons of *cox1* have been successful in distinguishing individual mayfly specimens as DNA barcodes (Ball et al. 2005) and multiple species simultaneously (metabarcoding) (Inai et al. 2020), although the DNA-based phylogenetic trees were occasionally not consistent with the morphological taxonomy, owing to the highly variable nature of *cox1* (Wakimura et al., 2016). A maximum likelihood *cox1* phylogeny in the genus *Drunella* was estimated as shown in Fig. 1. When the phylogenetic tree is rooted by *Ephemella strigata* (Ephemeridae), the genus *Drunella* is monophyletic under the family Ephemerellidae.

The *D. cryptomeria* cluster was surrounded by two clusters of yet undescribed groups that are designated as *Drunella* sp. 1 (named *Drunella campicola* sp. nov. in this study) and sp. 2 (named *Drunella parvicarnivora* sp. nov. in this study) in Fig. 1. The nymph of *Drunella* sp. 2 had been reported to be morphologically identical to *D. cryptomeria* but lacking a pair of tubercles on the vertex of head (Hatta & Ishiwata, 1990; Ishiwata, 2000). *D. cryptomeria* and the two proposed new species showed around 88% sequence similarity. Since 99% of the data pairs of the intrageneric-interspecific comparison in Ephemeroptera showed less than 94.2% sequence similarity and the median sequence similarity for intraspecific comparisons was 98.2% (Inai et al., 2020), *D. cryptomeria* and the two undescribed groups are suggested to belong to different species. The morphological description of these two new species and re-description of *D. cryptomeria* were performed (see the Description sections below). The *cox1* sequences of *D. lepnevae* obtained by Jo & Tojo (2019) have conformed to a distinct cluster away from *D. cryptomeria*, as suggested by Jacobus & McCafferty (2008).

Jo & Tojo (2019) have reported that two lineages of *D. ishiyamana* were found, with the cluster of *D. latipes* interposed between the two lineages. Note that *D. latipes* has been considered to be a synonym of *D. ishiyamana* (Ishiwata, 2018b; Jacobus & McCafferty, 2004). In this study, one specimen (sequence ID: 2017-208; a young nymph specimen) was clustered with the clades of *D.*

ishiyamana species group (Fig. 1), suggesting the presence of another cryptic lineage of *D. ishiyamana*. Extensive morphological and genetic investigations using matured nymph and adult specimens would reveal the classification of this species complex (Jo & Tojo, 2019). Also, two distinct lineages of *D. basalis* were reported (Jo & Tojo, 2019); one (major clade) is neighbouring to the *D. ishiyamana* group, and the other (minor clade) is shared with *D. trispina*. As for *D. trispina*, two lineages were suggested based on a preliminary analysis of a small number of specimens collected from Nara and Okayama Prefectures (Wakimura et al., 2020). In the present analyses, we have examined the specimens that were identified morphologically as *D. trispina*, and two distinct lineages were confirmed (Fig. 1). One clade of *D. trispina* (clade I in Fig. 1) comprised specimens collected from Kyoto, Nara, and Wakayama Prefectures, and those examined by Jo & Tojo (2019). The second clade (clade II in Fig. 1) of *D. trispina*, which is shared with *D. basalis* reported by Jo & Tojo (2019), comprised specimens from Kanagawa, Kyoto, and Okayama Prefectures. Sequence similarity between these two clades (I and II) of *D. trispina* was approximately 88% and it was much less than the median similarity score for the intraspecific comparison in Ephemeroptera *cox1* (98.2%, as mentioned above). The subpopulations of *D. trispina* in Kyoto Prefecture, where both clades are found, require more extensive examination to elucidate the inheritance of the mitochondrial genome in this species group. The examination of additional nuclear genes in a full phylogenetic framework would assist in species delimitation for this genus.

D. sachalinensis and *D. kohnoi* are closely related as evidenced by the *cox1* sequences, but are morphologically distinguishable (Ishiwata et al., 2018).

Description of *Drunella campicola* Ishiwata, sp. nov.

(Figs. 2.1–2.18)

[Japanese name: Sato-togemadarakagerou]

Male imago

Length (N = 10): Body, 9.2–11.5 mm; forewing, 11.0–12.9 mm; hind wing, 2.7–3.2 mm; caudal filaments, 9.0–13.0 mm.

Head: Upper part of compound eye dark brown, lower part black.

Thorax: Pronotum chocolate brown to blackish brown. Basisternum of prosternum yellowish brown, with longitudinal carinae; carinae converging anteriorly (maximum width between lateral margins of carina more than twice minimum width; Fig. 2.1, upper arrow); furcasternum chocolate brown to blackish brown. Mesonotum (Fig. 1.2) chocolate brown to blackish brown; lateroparapsidal sutures (LPs) not terminating at medioparapsidal sutures (MPs); scutellum without posterior prolongation and with a pair of membranous posterior lamellae. Mesosternum chocolate brown; basisternum parallel or slightly narrowed posteriorly; furcasternal protuberances parallel (Fig. 2.1, lower arrow).

Forelegs: Femora dark brown to black; tibiae dark brown to black, about twice as long as femora; tarsi light gray, and ranked in order of 2nd, 3rd, 4th, 5th, and 1st tarsus, based on their lengths.

Middle and hind legs: Femora dark brown; tibiae dark brown to black; tarsi yellowish brown, more than half length of tibia.

Forewings (Fig. 2.3): Hyaline; stigmatic area opaque; primary longitudinal veins yellowish brown basally, blackish brown apically; crossvein brown.

Hind wings: Hyaline; costal projection rounded (Fig. 2.4, arrow).

Abdomen: Terga reddish brown to dark brown, with a transverse paler band along each posterior segment. Penes (Fig. 2.5) slightly expanded apico-laterally, with a V-shaped apical median cleft, shaft slightly swollen medially; second segment of genital forceps sharply angled inward and with subapical constriction; terminal segment more than twice as long as broad. Caudal filaments brown basally, pale apically, with brown annulation; terminal filament slightly longer than cerci.

Female imago

Length (N = 8): Body, 9.0–11.0 mm; forewing, 11.2–14.5 mm; hind wing, 2.6–4.0 mm; caudal filaments, 9.0–13.9 mm.

Other features are similar to male imago except for the usual sexual differences and the following characters:

Thorax: Width of prosternal carinae slightly greater than those of male. Mesosternum, basisternum nearly quadrate; furcasternal protuberances wider than those of male. Fore tibiae relatively short, only about 1.2 times as long as fore femora.

Abdomen: Apex of sternum 9 truncate and often with shallowly rounded median emargination.

Male subimago

Characteristics are similar to male imago except for the duller general colouration and the following characters:

Head: Upper part of compound eye reddish brown, lower part black.

Thorax: Pronotum chocolate brown to black. Mesonotum chocolate brown without membranous tubercle at junction of mesonotal suture (MNs); pigmented sclerotization on MPs indistinct; scutellum without long posterior prolongation and with a pair of membranous posterior lamellae. Mesosternum, basisternum nearly rectangle.

Forewings: Brown; longitudinal vein, crossvein and intercalary black.

Hind wings: Whitish brown basally, brown apically; vein light brown.

Abdomen: Abdominal terga light brown. Caudal filaments subequal to, or slightly shorter than body length.

Female subimago

As in male subimago except for the usual sexual differences and the following characters:

Thorax: Width of prosternal carinae slightly greater than those of male. Width of mesobasisternum subequal to length; furcasternal protuberances wider than those of male.

Abdomen: Apex of sternum 9 truncate and often with shallowly rounded median emargination.

Mature nymph (Fig. 2.6)

Length (N = 16): Body, 8.5–12.0 mm; caudal filaments, 8.3–10.7 mm.

Colouration: General colour uniformly brown, with transverse whitish bands on thorax and abdomen.

Head (Fig. 2.7): Vertex dark brown without tubercles, with lateral genal projections and a broad frontal shelf with short lateral projections; without occipital tubercles (frontal ocellus somewhat protuberant). Clypeus with a transverse field of bristles projecting forwards (Fig. 2.8, arrow). Labrum (Fig. 2.9) with broad, shallow anteromedian emargination. Mandibles (Figs. 2.10–2.11) well-developed, with incisive canines; molar surface of left mandible (Fig. 2.10) not parallel to its outer margin (narrower apically).

Hypopharynx (Fig. 2.12): Lingua rounded; superlingua rounded, not truncated with a row of hair along anterior margin. Maxillae (Fig. 2.13) not widened apically, with apical canines and apical setae, in contrast to genus *Cincticostella*, where maxillae are not truncated and without a field of long setae (Ishiwata, 2003); maxillary palpi three-segmented, moderately developed, more than 3/4 as long as galea-lacina.

Labium (Fig. 2.14): Glossae wide (about half as long as width of paragolossa), rounded apically, lacking apical projection; paraglossae rounded apically; labial palpi three-segmented; submentum widened basally.

Thorax: prothorax brown (sometimes light gray) without tubercles, mesothorax and metathorax brown without tubercles, with dark spots.

Forelegs (Fig. 2.15): Femora yellowish brown, covered with granules, with a transverse ridge basally, two brownish transverse bands (slightly paler in middle), and large and small tubercles on ventral (leading) edge (Fig. 2.15); tibiae with brown band medially, with apical projection (Fig. 2.15, arrow) 1/4 length of tarsi; tarsi with two brownish bands basally and apically; tarsal claws sharply bowed inward, with a denticle basally (Fig. 2.16).

Middle legs: Femora yellowish brown with two brownish transverse bands, basal band darker than subapical band; tibiae with brown band medially; tarsi with two brown bands basally and apically; tarsal claws sharply bowed inward, with a denticle basally.

Hind legs: Colouration of tibiae and tarsi similar to those of middle legs; small spines along outer margin, without spines along inner margin; tarsal claws sharply bowed inward, with a denticle basally.

Abdomen: Terga 1–8 brown to light brown (terga 6–7 sometimes chocolate brown); terga 9–10 chocolate brown; terga 4–9 with a pair of submedian tubercles; these tubercles are small on segments 4–5, often barely discernible; terga 3–7 with lamellate, imbricated gills; terga 4–9 with distinct postero-lateral projections. Abdominal sterna brown. Caudal filaments (Fig. 2.6) less than 1/2–2/3 as long as body length, with spines at apex of each segment and with heavy intersegmental setae (2–3 times as long as segment); terminal filament slightly longer than cerci.

Egg (Figs. 2.17–2.18)

Size (N = 30): Long axis length with polar cap, 153 μm; short axis length, 96 μm.

Oval, with a polar cap; chorion smooth, covered with fine and rectangular maculae (length, ca 2.5 μm; width, ca 1.0 μm), with MTF and micropyles (Fig. 2.18); micropyle tagenoform type, with chorion sperm guide (csg); chorion sperm guide without micropylar rim; micropylar canal subtle, indistinct in some specimens.

Diagnosis

Male imagoes are distinguishable from other Japanese *Drunella* species by the shape of the penes, having a broad V-shaped emargination. Female imagoes, subimagoes of both sexes, and eggs of this species are indistinguishable from other Japanese *Drunella* species. The nymphs of this species can be distinguished from all other Japanese *Drunella* species by lateral genal projections. There are certain Nearctic *Drunella* species that have nymphal genae produced into wide flanges or antero-

lateral projections (Allen & Edmunds, 1962). In the Japanese *Drunella* species, *D. campicola* is the only species that has nymphal genae produced into lateral projections, however.

Type Material

Holotype: female nymph with eggs (in alcohol), labelled, Seiryu Bridge, Kiyotake River, Kiyotake-machi, 31 51' 20.8" N 131 23' 34.3" E, 23.III.1988, **Miyazaki Prefecture**, Kyushu Island, Japan, determined by S. Ishiwata and deposited in MNHAH (MNHAH B2-446671).

Paratypes: Two female nymphs with eggs (MNHAH B2-446672, MNHAH B2-446673), same data and depository as holotype.

Other material examined: [Kyushu Island]: **Fukuoka Prefecture**: 1n, 21.IV.1988, Tsukushino-shi, Chikugo Riv., Homan Riv., collected by T. Nozaki; 1n, 15.IV.2012, Asakura-shi, Chikugo Riv., Erizeki, 33°21'47.6"N 130°39'55.4"E, collected by S. Ishiwata; 2n, 16.IV.2012, Yame-shi, Hoshino Riv., Yoriguchi Bridge, 33°11'41.1"N 130°42'50.1"E, collected by S. Ishiwata; 1n, 16.IV.2012, Yame-shi, Yabe Riv., Yabe, 33°11'41.1"N 130°42'50.1"E, collected by S. Ishiwata; 2n, 15.IV.2012, Asakura-shi, Myoken Riv., Sakaai, collected by S. Ishiwata. **Saga Prefecture**: 2n, 15.IV.2012, Nabeshima-machi, Kakihisa, Tafuse Riv., Sakura Bridge, 33°17'30.9"N 130°16'48.4"E, collected by S. Ishiwata. **Miyazaki Prefecture**: 1n, 21.IV.1988, Kiyotake-machi, Kiyotake Riv., Seiryu Bridge, 31°51'20.8"N 131°23'34.3"E, collected by S. Ishiwata; 6n, 5.II.1988, ibid., Y. Kuroki; 23n, 23.III.1988, ibid, collected by S. Ishiwata; 3♂, 2♀, 1♂s, 7♀s, 23.III.1988 (reared from nymph. 4.IV.–7.V.1988), ibid, collected by S. Ishiwata.

Distribution

Japan (Kyushu Island: Fukuoka, Miyazaki and Saga Prefectures)

Biology

The nymphs of *D. campicola* are common in shallow and gently flowing waters of downstream rivers (third-order streams). Although they inhabit diverse types of river bottoms, they are most commonly found among gravel bottoms. The life cycle of *D. campicola* is univoltine-fast, and all

adults emerge from April to mid-May. The appearance of nymphs and adults from April to June suggests that a long egg diapause occurs from summer to winter. The nuptial flight of this species has never been observed.

Etymology

The specific epithet '*campicola*' derives from nymphal habitat of this species, 'campus' and 'colo' which mean 'plain' and 'habitat', respectively.

Description of *Drunella parvicarnivora* Ishiwata, sp. nov.

(Figs. 3.1–3.11)

[Japanese name: Mukobu-togemadarakagerou]

Drunella sp., Hatta & Ishiwata (1990), (nymph, faunal list) [Honshu Island: Aichi Prefecture].

Drunella sp., Ishiwata et al. (1991), (nymph, faunal list) [Shikoku Island: Kochi Prefecture, Tokushima Prefecture].

Male imago

General colour brown with light lateral stripes. Body slender with long caudal filaments.

Length (N = 33): Body, 5.0–6.5 mm; fore wing, 6.2–6.5 mm; hind wing, 1.5–1.8 mm; caudal filaments, 10.0–12.0 mm.

Head: Colour chocolate brown; upper part of compound eye light brown, lower part black.

Thorax: Pronotum chocolate brown to blackish brown. Basisternum of prosternum yellowish brown to blackish brown, with longitudinal carinae; carinae slightly converging anteriorly (maximum width between lateral margins of carina less than twice minimum width; Fig. 3.1, upper arrow); furcasternum yellowish brown to blackish brown. Mesonotum (similar to Fig. 2.2) chocolate brown to blackish brown; LPs not terminating at MPs; scutellum without posterior prolongation and with a pair of membranous posterior lamellae. Mesosternum chocolate brown; basisternum slightly narrowed posteriorly; furcasternal protuberances parallel (Fig. 3.1, lower arrow).

Forelegs: Brown, sometimes with a pale spot; fore tibiae about twice as long as fore femora; fore tarsi ranked in order of 2nd, 3rd, 4th, 5th, and 1st tarsus based on their lengths.

Middle and hind legs: Femur with two dark spots basely and sub-apically; tarsi more than half length of tibia.

Forewings: hyaline; stigmatic area opaque; longitudinal veins brown basally, hyaline apically; intercalary and crossvein pale.

Hind wings: hyaline; costal projection pointed (Fig. 3.2, arrow).

Abdomen: Abdominal segments 1–3 dark brown, 4–7 translucent, 8–10 dark brown to black. Terga dark brown to black, with transverse paler band along each posterior segment. Sterna pale. Apical lateral margin of penes not expanded (Fig. 3.3), sides nearly parallel; second segment of genital forceps is slightly distinctly bowed, but not strongly bowed inwards with a deep constriction as in *D. basalis* (Imanishi, 1937, p. 321, pl. 23, fig. 1); the terminal segment of genital forceps two to three times as long as broad. Caudal filaments brown basally, pale apically, with brown annulation; terminal filament slightly longer than cerci.

Female imago

Length (N = 10): Body, 5.9–6.0 mm; forewing, 5.2–7.0 mm; hind wing, 1.2–2.0 mm; caudal filaments, 5.0–6.2 mm.

Other features are similar to male imago except for the usual sexual differences and the following characters:

Thorax: Width of prosternal carinae slightly greater than those of male. Mesobasisternum nearly quadrate; furcasternal protuberances wider than those of male. Fore tibiae relatively short, only about 1.2 times as long as fore femora.

Abdomen: Abdominal segments 1–3 dark brown, 4–7 yellowish brown, 8–10 dark brown to black. Terga dark brown to black, with transverse paler band along each posterior segment. Sterna pale. Apex of sternum 9 slightly elongate, not truncate.

Male subimago

Characteristics are similar to male imago except for the duller general colouration and the following characters:

Head: Upper part of compound eye reddish brown, lower part black.

Thorax: Pronotum chocolate brown to black. Mesonotum chocolate brown without membranous tubercle at junction of MNs; pigmented sclerotization on MPs indistinct; scutellum without long posterior prolongation and with a pair of membranous posterior lamellae. Wings gray; vein dark; intercalary and crossvein infuscated.

Abdomen: Abdominal segments 4–7 light brown. Caudal filaments subequal to, or slightly shorter than body length.

Female subimago

Characteristics are similar to male subimago except for the usual sexual differences and the following characters:

Thorax: Width of prosternal carinae slightly greater than those of male. Width of mesobasisternum subequal to length; furcasternal protuberances wider than those of male.

Abdomen: Apex of sternum 9 slightly elongate, not truncate.

Mature nymph

Length (N = 10): Body, 5.2–6.2 mm; caudal filaments, 2.0–3.2 mm.

Colouration: General colour yellowish to brown with variable dark brown markings, and with a transverse blackish band on thorax, legs, and abdomen.

Head: Vertex dark brown without tubercles. Genae rounded. Clypeus (similar to Fig. 2.8) with a transverse field of bristles projecting forwards. Labrum (similar to Fig. 1.9) with broad, shallow anteromedian emargination. Mandibles (Figs. 3.5–3.6) with narrow incisors on right mandible; molar surface of left mandible not parallel to its outer margin.

Hypopharynx (Fig. 3.7): Lingua rounded; superlingua rounded, with a row of hairs along anterior margin. Maxillae (Fig. 3.8) not widened apically, with apical canines, without an apical tuft of setae, and without a cuticular tooth on medio-anterior edge of galea-lacinia (not as in genus *Cincticostella*); maxillary palpi three-segmented, moderately developed, more than 3/4 as long as galea-lacina.

Labium (Fig. 3.9): Glossae wide (approximately 1/2 times as long as width of paragolossa), rounded apically, lacking apical projection; paraglossae rounded apically; labial palpi three-segmented; submentum widened basally.

Thorax: Prothorax dark brown without tubercles; mesothorax dark brown with variable dark brown markings, with a black band posteriorly (posterior margin of forewing pads); metathorax dark brown.

Forelegs: Femora yellowish, covered with granules, with two brownish transverse bands (basal bands ridged), and around 15 small tubercles on ventral (leading) edge; tibiae brown medially and without apical tibial projection; tarsi light gray basally; tarsal claws (similar to Fig. 2.16) sharply bowed inward, with a denticle basally.

Middle legs: Femora yellowish with two brown transverse bands, basal band darker than subapical band; tibiae with a brown band medially; tarsi with two brown bands basally and apically; tarsal claws sharply bowed inward, with a denticle basally.

Hind legs: Colouration of tibiae and tarsi similar to those of middle legs; small spines along outer margin, without spines along inner margin; tarsal claws sharply bowed inward, with a denticle basally.

Abdomen: Terga 1–8 yellowish brown (tergum 7 sometimes chocolate brown posteriorly); terga 9–10 chocolate brown, terga 1–8 yellowish brown; terga 3–9 with a pair of submedian tubercles; these tubercles are small on segments 3–4, often discernible; terga 3–7 with lamellate, imbricated gills; terga 4–9 with distinct postero-lateral projections. Abdominal sterna brown. Caudal filaments less

than 1/2–2/3 as long as body length, with brown annulation at apex of each segment, and with short hair-like setae (setae length less than each segment); terminal filament slightly longer than cerci.

Egg (Figs. 3.10–3.11)

Size (N = 30): Long axis length with polar cap, 154 μm; short axis length, 92 μm.

Oval, with a polar cap; chorion smooth, covered with fine and rectangular maculae (length, ca 3.0 μm; width, ca 1.0 μm), with MTF and without rim; micropylar canal subtle, indistinct in some specimens.

Diagnosis

Imagoes, and subimagoes of both sexes and eggs of this species are indistinguishable from that of *D. cryptomeria*. The nymphs of this species can be distinguished from *D. cryptomeria* by not having tubercles on the vertex of the head, and by three-segmented maxillary palpi. See also the 'Note' below.

Type Material

Holotype: nymph (in alcohol), labelled, Nikko-shi, Ojika Riv., 36°57'51.2"N 139°41'29.2"E, **Tochigi Prefecture**, Honshu Island, Japan, 7.VI.2022, determined by S. Ishiwata and deposited in MNHAH (MNHAH B2-446674).

Paratypes: 4 nymphs (in alcohol; MNHAH B2-446675, 446676, 446677, and 446678), same data and depository as holotype.

Other material examined: **Hokkaido**:1n, 28.VI.1993, Atsuta-mura, Atsuta Riv., collected by T. Ito; 7n, 29.V.1996, Tomakomai-shi, Horonai Riv., collected by H. Miyake; 3n, 21.VII.1987, Tomakomai-shi, Yufutsu Riv., Tomakomai-enshurin, collected by R. Kuranishi; 5n, 6.VII.1992, Chitose-shi, Rankoshi Riv., Rankoshi, collected by T. Ito & Y. Nagayasu; 7n, 9.VII.1985, Nakagawa-machi, Teshio Riv., Tomiwa, collected by S. Ishiwata; 1n, 9.VII.1985, Asahi-machi, Nisama Riv., Daininisama Bridge, 44°05'29.7"N 142°45'47.9"E, collected by S. Ishiwata; 1♀, 12.VII.2012, Sapporo-shi, Toyohira Riv., Misumai, 42°57'38.1"N 141°15'26.6"E, collected by S. Ishiwata.

[Honshu Island]: **Aomori Prefecture**: 4n, 16.V.1987, Shiura-mura, Imaizumi Riv., Imaizumi, collected by S. Sasaki; 1n, 15.V.1987, Imabetsu-machi, Yomouchi Riv., collected by S. Sasaki. **Iwate Prefecture**: 3n, 21.VI.1993, Daito-cho, Satetsu Riv., Orikaeshi Bridge, collected by S. Ishiwata; 1n, 21.VI.1993, Tono-shi, Hayase Riv., Kamigou Bridge, collected by S. Ishiwata; 3n, 21.VI.1993, Miyako-shi, Nagasawa Riv., Shinden Bridge, 39°37'39.8"N 141°54'59.2"E, collected by S. Ishiwata. **Ibaraki Prefecture**: 1n, 8.VI.1987, Daigo-machi, Kuji Riv., collected by S. Ishiwata. **Tochigi Prefecture**: 1n, 7.VI.2022, Nikko-shi, Ojika Riv., 36°57'51.2"N 139°41'29.2"E, collected by S. Ishiwata; **Saitama Prefecture**: 2n, 22.IV.1994, Hannou-shi, Iruma Riv., Ichinose Bridge, 35°52'09.8"N 139°15'39.7"E, collected by S. Ishiwata; 5n, 22.IV.1994, Hidaka-shi, Koma Riv., Kinchaku, collected by S. Ishiwata. **Tokyo**: 6n, 8.VI.1991, Ome-shi, Kamagafuchi, collected by T. Yamasaki. **Kanagawa Prefecture**: 2n, 23.VI.1988, Sagamihara-shi, Midori-ku, Hayato Riv., 35°30'27.9"N 139°10'00.3"E, collected by S. Ishiwata; 1n, 15.VI.1989, Yamakita-machi, Sakawa Riv., Shiraishizawa, 35°29'26.2"N 139°03'32.6"E, SI; 2n, 23.VI.1988, Kiyokawa-mura, Nakatsu Riv., Sakaigawa, collected by S. Ishiwata; 1♂s, 27.VI.1997, Isehara-shi, Hinata Riv., Hinatayakushi, 35°26'17.5"N 139°15'09.0"E, collected by S. Ishiwata; 5♂, 5♀, sampling by light traps, 30.V.1993, Kiyokawa-mura, Yatarou Riv., Gongen Bridge, 35°28'24.0"N 139°16'13.0"E, collected by S. Ishiwata. **Niigata Prefecture**: 8n, 2.VI.1986, Asahi-mura, Nagatsu Riv., collected by T. Nozaki. **Shizuoka Prefecture**: 1n, 2.VI.1989, Shimizu-shi, Okitsu Riv., collected by S. Ishiwata. **Shiga Prefecture**: 1n, 9.VI.2019, Moriyama-shi, Meta Riv., Moriyama-machi, 35°03'46.7"N 135°59'02.7"E, collected by M. Uenishi. **Kyoto Prefecture**: 1♂s, 2♀, 7.VI.1987, Kyoto-shi, Saihouzi Riv., collected by S. Ishiwata. **Osaka Prefecture**: 4n, 21.V.2022, Takatsuki-shi, Akutagawa Riv., Hara, 34°55'14.4"N 135°36'10.1"E, collected by T. Fujitani. **Wakayama Prefecture**: 4n, 24.IV.2021, Kudoyama-machi, Nyu Riv., 34°16'43.0"N 135°36'28.0"E, collected by Y. Takemon; 8n, 21.V.2022, Hashimoto-shi, Kitamata Riv., Hikotani, 34°15'56.5"N 135°38'35.9"E, collected by M. Kato & K. Wakimura; 13n, 29.V.2022, ibid. [Shikoku Island]: **Tokushima Prefecture**: 10n,

25.V.1984, Handa-machi, Handa Riv., Banzai, collected by H. Inubuse. [Kyushu Island]: **Fukuoka Prefecture**: 1n, 13.V.1986, Yoshii-machi, Kose Riv., Enjuji Riv., collected by S. Ishiwata; 1n, 13.V.1986, Tanushimaru-machi, Kose Riv., collected by T. Nozaki; 1n, 13.V.1986, Fukuoka-shi, Hacho Riv., Hacho Bridge, 33°29'43.4"N 130°17'46.9"E, collected by T. Nozaki. **Kumamoto Prefecture**: 1n, 12.V.1986, Izumi-son, Kuriki Riv., collected by T. Nozaki.

Note

Currently, nine species of the genus *Drunella* have been recognized in Japan. Of these species, the nymphs of *D. parvicarnivora* and *D. cryptomeria*, are distinguishable from other Japanese *Drunella* nymphs based on the following characteristics: The frontal shelf of the head has no projections; the fore femora have no tibial projections; and the caudal filaments have no long, hair-like setae. As for adults, the typical characteristics of both species are a costal projection on the hind wing, and long caudal filaments (about 1.5–2 times as body length). Most of the other characters are similar to those of the other species in the genus *Drunella*. Of these characteristics mentioned above, having costal process on hind wing in adult stage is not a morphological feature of this genus that has been conventionally pointed out (Kluge, 2004, p.296, as the plesiomorpies of *Ephemerella*/fg.2: *Attenella* Edmunds*, Drunella, Timpanoga* Needham and etc.; Bauernfeind & Soldán, 2012: p.461). Therefore, further research is also needed on Japanese *Drunella* species.

Distribution

Japan (Hokkaido, Honshu, Shikoku, and Kyushu: except for Okinawa).

Biology

In Kanagawa Prefecture, *Drunella parvicarnivora* is restricted from piedmont to mountain streams (Ishiwata, 2000, 2005, 2018a). The nymphs of *D. parvicarnivora* are rather common in the shallow and gently flowing waters of second- and third-order streams. Although they inhabit very diverse types of river bottoms, they are most commonly found among gravel and sandy bottoms, as is the habitat for *D. cryptomeria*. *D. parvicarnivora* has never been collected among leaves, debris, or silt.

The life cycle of this species is univoltine-fast, and all adults emerge in June. Since nymphs and adults appear only from April to June, a long egg diapause is considered to occur from summer to winter.

Etymology

The specific epithet is derived from the Latin ‘parvus’ and ‘carnivora’ which mean ‘small’ and ‘carnivorous’, respectively, as S. Ishiwata had observed that the nymphs captured the chironomid larva to predate.

Redescription of *Drunella cryptomeria* (Imanishi, 1937)

(Figs. 4.1–4.11)

[Japanese name: Futakobu-madarakagerou]

Ephemerella cryptomeria Imanishi, 1937.

Ephemerella (*Drunella*) *cryptomeria*; Edmunds (1959).

Drunella cryptomeria; Ishiwata, 2001. checklist [Japan (Honshu Island)]; Ishiwata, 2002. nymph, faunal list [Japan (Honshu Island: Kanagawa Prefecture)]; Jacobus & McCafferty, 2004. male, nymph [Japan, Korea, Mongolia]; Ishiwata, 2005. male, nymph, female subimago [Japan (Honshu Island)]; Ishiwata & Takemon, 2005b. male, nymph, key [Japan (Honshu Island)]; Enkhtaivan & Soldán, 2008. faunal list [Mongolia]; Zhou, 2013. checklist [China]; Maruyama & Hanada, 2016. male, female, male subimago, female subimago [Japan (Honshu Island: Kyoto Prefecture)]; Wakimura et al., 2016, 2020. DNA data [Japan (Honshu Island: Kanagawa Prefecture)]; Ishiwata et al., 2018. male, nymph, key [Japan (Honshu Island)]; Ishiwata, 2018a. faunal list [Japan (Honshu Island)].

Ephemerella (*Drunella*) *bicornis* Gose, 1980. Type series: unknown. Type locality: unknown. Type depository: unknown. Synonymized by Ishiwata (2001).

Drunella bicornis; Ishiwata, 1987; Yamasaki, 1986 [Japan (Honshu Island: Tokyo)]; Ishiwata, 2000 [Japan (Honshu Island: Kanagawa Prefecture)].

Ephemerella ‘sp. (*trispina* group); Tanaka, 1966 [Japan (Honshu Island: Tochigi)].

Distribution

Japan (Honshu, Kyushu: except for Okinawa), China, Korea, and Mongolia. The continental distribution of this species has been reported by Ishiwata (2018b) and Jacobus & McCafferty (2004) but needs to be investigated further.

Male imago

Characteristics identified in the present study are described below in addition to Imanishi (1937).

General colour brown with light lateral stripes. Body slender with long caudal filaments.

Length (N = 5): Body, 4.5–6.2 mm; forewing, 5.8–6.6 mm; hind wing, 1.0–1.7 mm; caudal filaments, 11.0–12.3 mm.

Head: Colour chocolate brown; upper part of compound eye light brown, lower part black.

Thorax: Pronotum chocolate brown to blackish brown. Basisternum of prosternum yellowish brown to blackish brown, with longitudinal carinae; carinae slightly converging anteriorly (maximum width between lateral margins of carina less than twice minimum width; Fig. 4.1); furcasternum yellowish brown to blackish brown. Mesonotum (similar to Fig. 2.2) chocolate brown to blackish brown; LPs not terminating at MPs; scutellum without posterior prolongation and with a pair of membranous posterior lamellae. Mesosternum (similar to Fig. 2.1) chocolate brown; basisternum narrowed anteriorly; furcasternal protuberances parallel.

Middle and hind legs: Tarsi is more than half length of tibiae.

Hind wings: Hyaline; costal projection pointed (similar to Fig. 3.2).

Abdomen: Apical lateral margin of penes not expanded (Fig. 4.2), sides nearly parallel; second segment of genital forceps is slightly distinctly bowed, but not strongly bowed inwards with a deep

562 constriction as in *D. basalis* (Imanishi, 1937, p. 321, pl. 23, fig. 1); terminal segment of genital
563 forceps two to three times as long as broad.
564 Female imago
565 Length (N = 10): Body, 5.2–6.0 mm; forewing, 6.2–6.5 mm; hind wing, 1.4–1.6 mm; caudal
566 filaments, 5.0–6.2 mm.
567 Characteristics are similar to male imago except for the usual sexual differences and the following
568 features:
569 Thorax: Width of prosternal carinae slightly greater than those of male. Mesobasisternum nearly
570 quadrate; furcasternal protuberances wider than those of male. Fore tibiae relatively short,
571 approximately 1.0–1.2 times as long as fore femora. Abdomen: Apex of sternum 9 slightly elongate,
572 not truncate.
573 Male subimago
574 Characteristics are similar to male imago except for the duller general colouration and the following
575 characters:
576 Head: Upper part of compound eye reddish brown, lower part black.
577 Thorax: Pronotum chocolate brown to black. Mesonotum chocolate brown without a membranous
578 tubercle at junction of MNs; pigmented sclerotization on MPs indistinct; scutellum without long
579 posterior prolongation and with a pair of membranous posterior lamellae. Forewings gray; hind
580 wings more whitish than forewings, costal processes pointed. Vein dark gray.
581 Abdomen: Abdominal terga 1–3 and 9–10 chocolate brown, terga 4–8 yellow brown. Caudal
582 filaments subequal to, or slightly longer than body length.
583 Female subimago
584 Characteristics are similar to male subimago except for the usual sexual differences and the
585 following characters:

Thorax: Width of prosternal carinae slightly greater than those of male. Width of mesobasisternum subequal to the length; furcasternal protuberances wider than those of male.

Abdomen: Apex of sternum 9 slightly elongate, not truncate.

Mature nymph

Length (N = 20): Body, 5.0–6.2 mm; caudal filaments, 4.3–6.5 mm.

Colouration: General colour light brown to whitish brown, with a transverse blackish band on thorax, legs, and abdomen (Fig. 4.3).

Head: Vertex black to chocolate brown, with a pair of tubercles (Figs. 4.4–4.5); genae rounded. Clypeus with a transverse field of bristles projecting forwards (Fig. 4.4, arrow). Labrum (similar to Fig. 2.9) with broad, shallow anteromedian emargination. Mandibles (Figs. 4.6–4.7) with narrow incisors on right mandible; molar surface of left mandible not parallel to its outer margin.

Hypopharynx (similar to Fig. 3.7): Lingua rounded; superlingua with a row of hairs along anterior margin. Maxillae (Fig. 4.8) not widened apically, with apical canines, without an apical tuft of setae, and without a cuticular tooth on medio-anterior edge of galea-lacinia (not as in genus *Cincticostella*); maxillary palpi two-segmented, not developed, less than a 1/3 as long as galea-lacina.

Labium (similar to Fig. 3.9): Glossae wide (about half as long as width of paragolossa), rounded apically, lacking apical projection; paraglossae rounded apically; labial palpi three-segmented; submentum widened basally.

Thorax: Prothorax dark gray, sometimes whitish to light gray with a pair of blunt tubercles (Fig. 4.5). Mesothorax and metathorax dark brown to black.

Forelegs (Fig. 4.9): Femora whitish with a slightly light gray band subapically, a band of transverse granular processes basally, and 10–15 small tubercles on ventral (leading) edge; tibiae light gray medially, and without apical tibial projection; tarsi light gray basally; tarsal claws (similar to Fig. 2.16) sharply bowed inward, with a denticle basally.

Middle legs: Femora whitish, with dark gray at basal half; tibiae light gray medially; tarsi light gray basally; tarsal claws sharply bowed inward, with a denticle.

Hind legs: Colouration of tibiae and tarsi similar to those of middle legs; small spines along outer margin, without spines along inner margin; tarsal claws sharply bowed inward, with a denticle basally.

Abdomen: Terga 1–3 and 8–10 chocolate brown, terga 4–7 yellowish brown; terga 2–9 with a pair of submedian tubercles; these tubercles are small, often barely discernible; terga 3–7 with lamellate, imbricated gills; terga 5–9 with distinct postero-lateral projections. Abdominal sterna brown. Caudal filaments whitish brown to white, about 2/3–3/4 as long as body length, with pale annulation at apex of each segment, and lacking long, hair-like setae (length of setae less than each segment); terminal filament slightly longer than cerci.

Egg (Figs. 4.10–4.11)

Size (N = 30): Long axis length with polar cap, 153 μm; short axis length, 96 μm.

Oval, with a polar cap; chorion smooth, covered with fine and rectangular maculae (length, ca 2.5 μm; width, ca 1.0 μm), with MTF; micropyles tagenoform type; sperm guide weakly defined, without rim; micropylar canal subtle, indistinct in some specimens.

Diagnosis

D. cryptomeria is indistinguishable from *D. parvicarnivora* based on the morphology of imaginal and subimaginal specimens of both sexes as well as characters of eggs. The nymph of this species is discriminated from *D. parvicarnivora* on the basis of a pair of tubercles on the vertex of head and two-segmented maxillary palpi.

Type Material

Holotype: male. Type locality: Kyoto Prefecture (Kibune), Honshu Island, Japan. Type depository: KM. No type material examined [Holotype not found].

Other material examined: [Honshu Island]: **Aomori Prefecture**: 1n, 21.VI.1993, Mogita-mura, Amida Riv., collected by S. Sasaki. **Iwate Prefecture**: 5n, 21.VI.1993, Rikuzentakada-shi, Yahagi Riv., Koguroyama, collected by S. Ishiwata. **Fukushima Prefecture**: 3n, 16.V.1988, Koriyama-shi, Abukuma Riv., Ishimushiro Riv., collected by S. Ishiwata; 2n, 15.V.1988, Aizuwakamatsu-shi, Yu Riv., Higashiyama Dam, collected by S. Ishiwata. **Tochigi Prefecture**: 1n, 6.VI.1987, Nikko-shi, Yu Riv., Uraminotaki, 36°45'14.4"N 39°33'35.3"E, collected by S. Ishiwata. **Kanagawa Prefecture**: 10n, 28.V.2015, Atsugi-shi, Yatarou-zawa, 35°27'33.0"N 139°15'52.7"E, collected by S. Ishiwata; 1♂, 29.VI.1988, Hadano-shi, Kaname Riv., Genjirou-sawa, 35°26'28.5"N 139°10'03.0"E, collected by S. Ishiwata; 5n, 30.V.1988, ibid.; 3♂, 4♀ (reared from nymph, 10.VII.1988), ibid.; 1♂s, 14.VI.1996, Hadano-shi, Harutake-sawa, Minoge, 35°25'00.2"N 139°13'49.9"E, collected by S. Ishiwata; 1♂s, 30.V.1988, Yamakita-machi, Sakawa Riv., Shiraishi-zawa, 35°29'26.2"N 139°03'32.6"E, collected by S. Ishiwata; 2♂, 2♀ (reared from nymph, 10.VII.1988), ibid.; 1♂s, 1♀s (reared from nymph, 30.VI.1988), ibid.; 1n, 18.VI.1981, Yamakita-machi, Sakawa Riv., Shiraishi-zawa, 35°29'26.4"N 139°03'32.8"E, collected by S. Ishiwata; 1s♂1s♀ (reared from nymph, 30.VI.1988), 29.VI.1988, ibid.; 2♀, 29.VI.2022, sampling by light traps, Yamakita-machi, Sakawa-Riv., Tanasawa-bashi, 35°15'52.8"N 135°46'45.1"E, collected by S. Ishiwata. **Fukui Prefecture**: 5n, 23.V.2016, Ono-shi, Kamihanbara, Kuzuryu Riv., Kuzuryu-dam, 35°53'37.8"N 136°47'02.5"E, collected by N. Honda. **Yamanashi Prefecture**: 1n, 11.VIII.1986, Koshu-shi, Hi Riv., 35°43'30.0"N 138°50'34.3"E, collected by T. Nozaki. **Shiga Prefecture**: 1n, 9.VI.2019, Moriyama-shi, Meta Riv., 35°03'46.7"N 135°59'02.7"E, collected by M. Uenishi. **Kyoto Prefecture**: 1n, 19.V.1988, Kyoto-shi, Kibune Riv., Azo-dani, collected by Y. Takemon; 3n, 23.V.2021, Kyoto-shi, Kuramakibune-machi, Kibune Riv., Azo-dani, 35°08'00.0"N 135°45'50.0"E, collected by Y. Takemon & M. Kato; 1n, 21.V.2022, Kyoto-shi, Nomi-cho, Nomi Riv., 35°15'52.8"N 135°46'45.1"E, collected by T. Fujitani. [Kyushu Island]: **Oita Prefecture**: 1n, 22.III.1989, Yufuin-machi, Oita Riv., Yunohira, 33°11'30.4"N 131°19'31.6"E, collected by S. Ishiwata.

Note

Although Imanishi (1937) stated that the length of the tarsi is less than half of the tibiae in the hind legs of male imagoes (tibia:tarsus ≈ 5:2), the tarsi of the specimens examined in this study were longer than half of the tibiae. Moreover, Imanishi (1937) stated that there was a brownish stain at the base of the forewings, but this character was indistinct in some specimens.

Imanishi (1937) described *E. cryptomeria* based on the male imagoes, and the nymph had remained undescribed. Gose (1980) described *E. bicornis* based on the nymphs and synonymized *E. yoshinoensis* under *E. cryptomeria*. Ishiwata (2001) concluded that *E. bicornis* and *E. cryptomeria* were synonymous, based on the investigation of fresh materials obtained by rearing of *E. bicornis* nymphs collected from the type locality of *E. cryptomeria* (Kyoto, Kibune River). Ishiwata (2001) also revealed that, *E. yoshinoensis*, which was synonymized with *E. cryptomeria* by Gose (1980), should instead be a synonym of *D. ishiyamana*. Tiunova and Belov (1984) synonymized *D. latipes* under *D. cryptomeria*, following the incorrect concept of *D. cryptomeria* (Gose, 1980). Jacobus and McCafferty (2004) placed *E. latipes* as a synonym of *D. ishiyamana* in light of the concepts of Ishiwata (2001), based on the material examined. See also, the notes of Ishiwata (2001) and Ishiwata & Takemon (2005a) under the species names *D. cryptomeria* and *D. ishiyamana*, respectively.

The synonymy of *D. cryptomeria* and morphologically similar species, *D. lepnevae*, *D. longipes*, and *D. fuso* (=*Drunella fusongensi*s Su and Gui, 1995), has extensively been discussed. Jacobus & McCafferty (2008) proposed that *D. lepnevae* and *D. cryptomeria* are different species and synonymized *D. longipes*, *D. bicornis*, and *D. fuso* under *D. lepnevae*. We also consider that *D. cryptomeria* and *D. lepnevae* are distinct species but *D. bicornis* to be a synonym of *D. cryptomeria* as mentioned above. These species, *D. cryptomeria* (= *D. bicornis*), *D. lepnevae*, and *D. longipes* shared the feature of paired occipital tubercles. The nymphs of *D. cryptomeria* (=*D. bicornis*) are distinguishable from the others by its small body length (approximately 5mm in length: 5.0–6.2 mm), and by the two-segmented maxillae palpi. *D. fuso* was described based on adults collected in

China (Jilin Province) (Su and Gui, 1995). We have not examined any specimen of *D. fuso*, and the original description by Su & Gui (1995) was insufficient as it did not give taxonomic characters to distinguish *D. fuso* from the other known Asian species. The male imagoes of both *D. cryptomeria* and *D. parvicarnivora* are smaller (4.5-6.5 mm in length), than those of above-mentioned species (over 9.0 mm in length). Morphological characters in the adults are variable, and the reliable taxonomic characters may appear only in the nymphal stages.

Biology

In Kanagawa Prefecture, *D. cryptomeria* is restricted to the mountain streams (Ishiwata 2018a). The nymphs of *D. cryptomeria* are rather common in the shallow and gently flowing waters of first- and second-order streams. Although they inhabit diverse types of river bottoms, they are most commonly found among gravel and sandy bottoms, as is the case for *D. parvicarnivora*, and they have never been collected among leaves, debris, or silt. The life cycle of *D. cryptomeria* is univoltine-fast, and all adults emerge from June to July. The appearance of very early instar nymphs in April suggests that a long egg diapause occurs from summer to winter. Mating flights usually take place over water at heights ranging from 150–200 cm to as high as the tops of the trees that border the stream. Flights occur from midafternoon to evening on sunny days.

Acknowledgements

We are pleased to acknowledge the help of those who have found the original literature that we needed namely Kluge N. J. (Department of Entomology, Biological Faculty, Saint-Petersburg State University, Saint-Petersburg Russia) and Tiunova T. (Federal Scientific Centre of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok Russia). We thank the collectors listed in the text who kindly gave us the specimens used in this study.

This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 17K07541 to MK.

Disclosure statement

The authors have no competing interests to disclose.

References

Allen, R. K. (1980). Geographic distribution and reclassification of the subfamily Ephemerellinae (Ephemeroptera: Ephemerellidae). In J. F. Flannagan, & K. E. Marshall (Eds.), *Advances in Ephemeroptera biology. Proceedings of the Third International Conference on Ephemeroptera* (pp. 71–91). Plenum Press, New York.

Allen, R. K., & Edmunds, G. F. (1962). A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae) V. The subgenus *Drunella* in North America. *Miscellaneous Publications of the Entomological Society of America*, *3*(5), 147–179.

Ball, S. L., Hebert, P. D. N., Burian, S. K., & Webb, J. M. (2005). Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society*, *24*(3), 508–524. https://doi.org/10.1899/04-142.1

Bauernfeind, E., & Soldán, T. (2012). *The mayflies of Europe (Ephemeroptera)*. Apollo Books, Ollerup.

Edmunds, G. F. (1959). Subgeneric groups within the mayfly genus *Ephemerella* (Ephemeroptera: Ephemerellidae). *Annals of the Entomological Society of America*, *52*(5), 543–547. https://doi.org/10.1093/aesa/52.5.543

Edmunds, G. F., Jensen, S. L., & Berner, L. (1976). *The mayflies of North and Central America*. University of Minnesota Press, Minneapolis.

Enkhtaivan, S., & Soldán, T. (2008). Mayflies of the Lake Hovsgol region, Mongolia. In F. R. Hauer, J. A. Stanford, & R. L. Newell (Eds.), *International advances in the ecology, zoogeography and systematics of mayflies and stoneflies* (pp. 103–115). University of California Press, Berkeley.

734 Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification
735 of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates.
736 *Molecular Marine Biology and Biotechnology*, *3*(5), 294–299.
737 Ge, Y., Xia, C., Wang, J., Zhang, X., Ma, X., & Zhou, Q. (2021). The efficacy of DNA barcoding in
738 the classification, genetic differentiation, and biodiversity assessment of benthic
739 macroinvertebrates. *Ecology and Evolution*, *11*, 5669–5681. https://doi.org/10.1002/ece3.7470
740 Gose, K. (1980). The mayflies of Japanese. (8) Key to families, genera and species. *Aquabiology*
741 (*Kaiyo to seibutsu*), *9*, 286–288. (in Japanese)
742 Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New
743 algorithms and methods to estimate maximum-likelihood phylogenies: assessing the
744 performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321.
745 https://doi.org/10.1093/sysbio/syq010
746 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis
747 program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.
748 Hatta, K., & Ishiwata, S. (1990). Fauna of aquatic insects in the major rivers of the Tokai District,
749 central Japan (III). Ephemerellidae (Ephemeroptera) in the major rivers of the Tokai District.
750 *The Journal of Nagoya Women's University*, *36*, 167–178. (in Japanese)
751 http://id.nii.ac.jp/1103/00002120/
752 Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications
753 through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological*
754 *Sciences*, *270*, 313–321. https://doi.org/10.1098/rspb.2002.2218
755 Imanishi, K. (1937). Mayflies from Japanese torrents VII. Notes on the genus *Ephemerella*.
756 *Annotationes Zoologicae Japonenses*, *16*(4), 321–329. https://dl.ndl.go.jp/pid/10853520
757 Inai, K., Wakimura, K., & Kato, M. (2020). Pairwise sequence comparison data of the DNA barcodes
758 of aquatic insects. *Data in Brief*, *32*, 106284. https://doi.org/10.1016/j.dib.2020.106284

Ishiwata, S. (1987). Structure and keys of the family Ephemerellidae (1). Structure and keys to genera from the family Ephemerellidae. *Aquatic Organisms in Kanagawa Prefecture*, *9*, 27–34. (in Japanese)

Ishiwata, S. (2000). Notes on mayflies in Kanagawa Prefecture. *Natural History Report of Kanagawa*, *21*, 73–82. (in Japanese)

Ishiwata, S. (2001). A checklist of Japanese Ephemeroptera. In Y. J. Bae (Ed.), *The 21st century and aquatic entomology in East Asia. Proceedings of 1st Symposium of Aquatic Entomology Societies of East Asia* (pp. 55–84). The Korean Society of Aquatic Entomology, Seoul.

Ishiwata, S. (2002). Mayflies of Kanagawa Prefecture, Japan. *Kanagawa-Chûhô Odawara*, *138*, 1–46. (in Japanese)

Ishiwata, S. (2003). A revision of the genus *Cincticostella* (Insecta: Ephemeroptera: Ephemerellidae) from Japan. *Species Diversity*, *8*(4), 311–346. https://cir.nii.ac.jp/crid/1390001204457821696

Ishiwata, S. (2005). Ephemeroptera. In *Insect larvae of Japan* (pp. 10–19). Gakken, Tokyo. (in Japanese)

Ishiwata, S. (2018a). Ephemeroptera. In Kanagawa Entomologists' Association (Ed.), *Insect fauna of Kanagawa* (pp. 26–44). Kanagawa Entomologists' Association, Odawara. (in Japanese)

Ishiwata, S. (2018b). An annotated catalogue of Japanese Ephemeroptera. Revised edition. *Kanagawa Institute of Technology, Division for Environmental Chemistry Research Report*, *7*, Supplement 1, 1–103.

Ishiwata, S., & Fujitani, T. (2018). Eggs of Japanese Ephemeroptera. *Kanagawa Institute of Technology, Division for Environmental Chemistry Research Report*, *7*, Supplement 2, 1–63.

Ishiwata, S., Furuya, Y., Kuwata, K., & Horiuchi, Y. (1991). Ephemerellidae (Ephemeroptera) in the major rivers of Shikoku District. *Bulletin of the Biological Society of Kagawa*, *18*, 19–31. (in Japanese) http://id.nii.ac.jp/1731/00000947/

Ishiwata, S., & Inada, K. (1996). Mayflies common in Japan. *Biology of Inland Waters (Rikusuiseibutsugakuho), 11*, 36–44. (in Japanese)

Ishiwata, S., & Takemon, Y. (2005a). Checklist of Japanese names of Japanese mayflies with notes on nomenclature. *Japanese Journal of Limnology*, *66*(1), 11–35. (in Japanese) https://doi.org/10.3739/rikusui.66.11

Ishiwata, S., & Takemon, Y. (2005b). Ephemeroptera. In T. Kawai, & K. Tanida (Eds.), *Aquatic insects of Japan: Manual with keys and illustrations* (pp. 31–128). Tokai University Press, Hadano. (in Japanese)

Ishiwata, S., Takemon, Y., & Fujitani, T. (2018). Ephemeroptera. In T. Kawai, & K. Tanida (Eds.), *Aquatic insects of Japan: Manual with keys and illustrations* (2nd ed.) (pp. 47–149, pl. 1–16). Tokai University Press, Hiratsuka. (in Japanese)

Jacobus, L. M., & McCafferty, W. P. (2004). Revisionary contributions to the genus *Drunella* (Ephemeroptera: Ephemerellidae). *Journal of the New York Entomological Society*, *112*(2), 127–147.

Jacobus, L. M., & McCafferty, W. P. (2008). Revision of Ephemerellidae genera (Ephemeroptera). *Transactions of the American Entomological Society, 134*(1), 185–274. https://www.jstor.org/stable/25078993

Jo, J., & Tojo, K. (2019). Molecular analyses of the genus *Drunella* (Ephemeroptera: Ephemerellidae) in the East Asian region. *Limnology*, *20*(3), 243–254. https://doi.org/10.1007/s10201-019-00573-3

Kluge, N. (2004). *The phylogenetic system of Ephemeroptera*. Kluwer Academic Publishers, Dordrecht.

Koss, R. W. (1968). Morphology and taxonomic use of Ephemeroptera eggs. *Annals of the Entomological Society of America, 61*, 696–721. https://doi.org/10.1093/aesa/61.3.696

Koss, R. W., & Edmunds, G. F. (1974). Ephemeroptera eggs and their contribution to phylogenetic studies of the order. *Zoological Journal of the Linnean Society*, *55*(4), 267–349. https://doi.org/10.1111/j.1096-3642.1974.tb01648.x

Lefort, V., Longueville, J.-E., & Gascuel, O. (2017). SMS: smart model selection in PhyML. *Molecular Biology and Evolution*, *34*, 2422–2424. https://doi.org/10.1093/molbev/msx149

Maruyama, H. & Hanada, S. (Eds). (2016). *A field guide to Japanese aquatic insects: Adults of mayflies, stoneflies and caddisflies*. Zenkoku Noson Kyoiku Kyokai Co., Ltd., Tokyo. (in Japanese)

McDunnough, J. (1931a). The bicolor group of the genus *Ephemerella* with particular reference to the nymphal stages (Ephemeroptera). *The Canadian Entomologist*, *63*(2), 30–42, 61–68.

McDunnough, J. (1931b). The eastern North American species of the genus *Ephemerella* and their nymphs (Ephemeroptera). *The Canadian Entomologist*, *63*(9), 187–197, 201–216.

Morinière, J., Hendrich, L., Balke, M., Beermann, A. J., König, T., Hess, S. K., Müller, R., Leese, F., Hebert, P. D. N., Hausmann, A., Schubart, C. D., & Haszprunar, G. (2017). A DNA barcode library for Germany's mayflies, stoneflies and caddisflies (Ephemeroptera, Plecoptera and Trichoptera). *Molecular Ecology Resources*, *17*, 1293–1307. https://doi.org/10.1111/1755-0998.12683

Needham, J. G. (1927). The Rocky Mountain species of the mayfly genus *Ephemerella*. *Annals of the Entomological Society of America*, *20*, 107–117. https://doi.org/10.1093/aesa/20.1.107

Ogden, T. H., Osborne, J. T., Jacobus, L. M., & Whiting, M. F. (2009). Combined molecular and morphological phylogeny of Ephemerellinae (Ephemerellidae: Ephemeroptera), with remarks about classification. *Zootaxa*, *1991*(1), 28–42. https://doi.org/10.11646/zootaxa.1991.1.2

Rambaut, A. (2018). Computer program distributed by the author, https://github.com/rambaut/figtree/releases [accessed 20 January 2022]

Su, C., & Gui, H. (1995). The first record of the genus *Drunella* in China with description of a new species (Ephemeroptera: Ephemerellidae). *Acta Zootaxonomica Sinica*, *20*(4), 451–454.

Suh, K. I., Hwang, J. M., Bae, Y. J., & Kang, J. H. (2019). Comprehensive DNA barcodes for species identification and discovery of cryptic diversity in mayfly larvae from South Korea: Implications for freshwater ecosystem biomonitoring. *Entomological Research*, *49*, 46–54. https://doi.org/10.1111/1748-5967.12334.

Tanaka, H. (1966). Ecological studies on aquatic insects in upper reaches of the Kinu-gawa River, Tochigi Prefecture, Japan. *Bulletin of the Freshwater Fisheries Research Laboratory*, *15*, 123–146.

Traver, J. R. (1932). Mayflies of North Carolina. *Journal of the Elisha Mitchell Scientific Society*, *47*(1), 85–161, *47*(2), 163–236.

Tiunova, T. M., & Belov, V. V. (1984). On the taxonomy of mayflies (Ephemeroptera, Ephemerellidae) in the south of the Soviet Far East. In I. M. Levanidova, E. A. Makartshenko, & A. Y. Sementshenko (Eds.), *Biology of freshwaters of Far EastUSSR* (pp. 74–77). Akademiya Nauk SSSR, Dal'nevostochnyi Nauchnyi Tsentr, Vladivostok. (in Russian)

Tshernova, O. A. (1972). Some new species of mayflies from Asia (Ephemeroptera, Heptageniidae, Ephemerellidae). *Entomologichekoye Obozrenie*, *51*, 604–614. (in Russian)

Ubero-Pascal, N., & Puig, M. A. (2009). New type of egg attachment structure in Ephemeroptera and comparative analysis of chorion structure morphology in three species of Ephemerellidae. *Acta Zoologica*, *90*(1), 87–98. https://doi.org/10.1111/j.1463-6395.2008.00367.x

Wakimura, K., Takemon, Y., Ishiwata, S., Tanida, K., Abbas, E. M., Inai, K., Taira, A., Tanaka, A., & Kato, M. (2020). A reference collection of Japanese aquatic macroinvertebrates. *Ecological Genetics and Genomics*, *17*, 100065. https://doi.org/10.1016/j.egg.2020.100065

Wakimura, K., Takemon, Y., Takayanagi, A., Ishiwata, S., Watanabe, K., Tanida, K., Shimizu, N., & Kato, M. (2016). Characterization of genes for histone H3, 18S rRNA, and cytochrome oxidase

subunit I of East Asian mayflies (Ephemeroptera). *DNA Barcodes, 4*(1), 1–25. https://access.portico.org/stable?au=phx133j8rrk

Webb, J. M., Jacobus, L. M., Funk, D. H., Zhou, X., Kondratieff, B., Geraci, C. J., DeWalt, R. E., Baird, D. J., Richard, B., Phillips, I., & Hebert, P. D. N. (2012). A DNA barcode library for North American Ephemeroptera: progress and prospects. *PLos One*, *7*, e38063.

Yamasaki, T. (1986). Ephemeroptera of the Tama River system. In R. Ishikawa (Ed.) *Distribution analysis of less migratory insects in Tamagawa area* (pp. 79–120). Tokyu Foundation for the Better Environment, Tokyo. (in Japanese) https://foundation.tokyu.co.jp/environment/wp-content/uploads/2011/04/b72cfec801650b2f53f631bfddce43f3.pdf

Zhou, C. (2013). A species list of Chinese mayflies (Insecta: Ephemeroptera). In K. Tojo, K. Tanida, & T. Nozaki (Eds.) Proceedings of the 1st Symposium of the Benthological Society of Asia. *Biology of Inland Waters*, Supplement 2, 167–225.

869 **Legends for Figures**

870 Fig. 1. Maximum likelihood *cox1* phylogenetic tree of *Drunella*.

871 The phylogenetic tree is rooted by the outgroup species *Ephemera strigata* (MN961293). The boot-
872 strap reproducibility of the tree topology is indicated at the respective nodes (scores > 50% are
873 shown). The leaves that are labelled with the year and number (part of the specimen identifications)
874 were sequenced for this study, and those labelled with the International Nucleotide Sequence Data-
875 base (INSD) accession number were retrieved from the INSD. The INSD accession numbers and
876 sampling information for the DNA sequences that were sequenced for this study are listed in Table 1.
877 *Drunella latipes* is considered to be a synonym of *D. ishiyamana* (as mentioned in the text).

878

879 Fig. 2. *Drunella campicola* Ishiwata, sp. nov.

880 1–5, male imago: 1, pro- and mesosterna; 2, mesonota; 3, forewing; 4, hind wing; 5, male genitalia
881 (dorsal view). 6–16, nymph: 6, mature nymph (dorsal view); 7, head (dorsal view); 8, head (frontal
882 view); 9, labrum; 10, left mandible; 11, right mandible; 12, hypopharynx; 13, maxilla; 14, labium;
883 15, foreleg (dorsal view); 16, claw. 17, 18, eggs: 17, general view; 18, chorion. Abbreviations for
884 imaginal thorax; BSl, probasisternum; BS2, mesobasisternum; FSl, profurcasternum; FS2,
885 mesofurcasternum; LPs, lateroparapsidal suture; MLs, median longitudinal suture; MNs, mesonotal
886 suture; MPs, medioparapsidal suture. For eggs; csg, chorion sperm guide; mc, micropylar canal; mo,
887 micropylar opening; MTF, multithread-folded with terminal fibre cluster; pc, polar cap.

888

889

Fig. 3. *Drunella parvicarnivora* Ishiwata, sp. nov.

1–3, male imago: 1, pro- and mesosterna; 2, hind wing; 3, male genitalia (dorsal view). 4–9, nymph: 4, mature nymph (dorsal view); 5, left mandible; 6, right mandible; 7, hypopharynx; 8, maxilla; 9, labium. 10, 11, eggs: 10, general view; 11, chorion. For abbreviations, see the legend of Figure 2.

Fig. 4. *Drunella cryptomeria* (Imanishi, 1937).

1, 2, male imago: 1, prosterna; 2, male genitalia (dorsal view; Ishiwata & Takemon, 2005b; fig. 13.6). 3–9, nymph: 3, mature nymph (dorsal view); 4, head (frontal view); 5, head and thorax (lateral view; Ishiwata & Takemon, 2005b; fig. 12.6); 6, left mandible; 7, right mandible; 8, maxilla; 9, foreleg (dorsal view). 10, 11, eggs: 10, general view; 11, chorion. For abbreviations, see the legend of Figure 2.

Table 1. List of *Drunella* specimens analysed.

Species	Sequence ID (Laboratory ID)	INSD accession number	Developmental stage/ sex	Locality	Sampling date year/month/day
Drunella cryptomeria	2016-027	MH260770	nymph	Japan: Kanagawa	2015/5/28
	2016-029	MH260771	nymph	Japan: Kanagawa	2015/5/28
	2016-030	MH260772	nymph	Japan: Kanagawa	2015/5/28
	2016-031	MZ820945	nymph	Japan: Kanagawa	2015/5/28
	2016-034	MZ820946	nymph	Japan: Kanagawa	2015/5/28
	2016-036	MZ820947	nymph	Japan: Kanagawa	2015/5/28
	2021-232	MZ820904	nymph	Japan: Kyoto	2021/5/23
	2021-233	MZ820918	nymph	Japan: Kyoto	2021/5/23
	2021-234	MZ820955	nymph	Japan: Kyoto	2021/5/23
Drunella campicola sp.nov.	2012-096	KF563055	nymph	missing data	missing data
	2012-179	KF563048	nymph	Japan: Fukuoka	2012/4/15
	2012-180	KF563049	nymph	Japan: Fukuoka	2012/4/15
	2012-181	KF563050	nymph	Japan: Fukuoka	2012/4/15
	2012-182	MZ820938	nymph	Japan: Fukuoka	2012/4/15
Drunella parvicarnivora sp. nov.	2011-166	OK501169	nymph	Japan: Wakayama	2011/5/15
	2016-090	MH260779	nymph	Japan: Kanagawa	2015/6/25
	2016-092	MH260780	nymph	Japan: Kanagawa	2015/6/25
	2016-093	MH260781	nymph	Japan: Kanagawa	2015/6/25
	2016-097	MZ820905	nymph	Japan: Kanagawa	2015/6/25
	2016-107	MZ820906	nymph	Japan: Kanagawa	2015/6/25
	2016-108	MZ820907	nymph	Japan: Kanagawa	2015/6/25
	2021-063	OK501170	nymph	Japan: Wakayama	2021/4/24
	2021-064	OK501171	nymph	Japan: Wakayama	2021/4/24
	2021-065	OK501172	nymph	Japan: Wakayama	2021/4/24
	2021-066	OK501173	nymph	Japan: Wakayama	2021/4/24
	2021-067	OK501174	nymph	Japan: Wakayama	2021/4/24
Drunella basalis	2012-001	KF563033	nymph	Japan: Kanagawa	missing data
	2012-002	KF563034	nymph	Japan: Kanagawa	missing data
	2012-005	KF563035	nymph	Japan: Kanagawa	missing data
	2012-006	KF563036	nymph	Japan: Kanagawa	missing data
	2012-007	KF563037	nymph	Japan: Kanagawa	missing data
	2012-100	KF563042	nymph	Japan: Fukuoka	2012/4/16
	2012-101	KF563043	nymph	Japan: Fukuoka	2012/4/16
	2012-160	KF563044	nymph	Japan: Fukuoka	2012/4/16
	2012-161	KF563045	nymph	Japan: Fukuoka	2012/4/16
	2012-164	KF563046	nymph	Japan: Fukuoka	2012/4/16
	2013-241	MZ820936	subimago/ female	Japan: Wakayama	2013/4/28
	2013-274	MZ820937	subimago/ male	Japan: Wakayama	2013/4/28
	2017-097	MK774308	nymph	Japan: Nara	2017/3/18
	2021-156	MZ820916	nymph	Japan: Kyoto	2021/4/25
	2021-157	MZ820923	nymph	Japan: Kyoto	2021/4/25
	2021-158	MZ820903	nymph	Japan: Kyoto	2021/4/25
Drunella ishiyamana	2017-165	MK774329	nymph	Japan: Nara	2017/3/18
	2017-166	MK774330	nymph	Japan: Nara	2017/3/18
	2017-176	MK774333	nymph	Japan: Nara	2017/4/22
	2017-177	MK774334	nymph	Japan: Nara	2017/4/22
	2017-178	MK774345	nymph	Japan: Nara	2017/4/22
	2017-179	MK774346	nymph	Japan: Nara	2017/4/22
	2017-180	MK774347	nymph	Japan: Nara	2017/4/22
	2017-181	MK774348	nymph	Japan: Nara	2017/4/22
	2017-182	MK774349	nymph	Japan: Nara	2017/4/22
	2017-183	MK774350	nymph	Japan: Nara	2017/4/22
	2017-184	MK774351	nymph	Japan: Nara	2017/4/22
	2017-185	MK774352	nymph	Japan: Nara	2017/4/22
	2017-203	MK774362	nymph	Japan: Nara	2017/4/22
	2021-182	OK501191	nymph	Japan: Wakayama	2021/4/24
	2021-184	OK501193	nymph	Japan: Wakayama	2021/4/24
Drunella sachalinensis	2012-054	KF563040	nymph	Japan: Kanagawa	missing data
	2012-055	KF563041	nymph	Japan: Kanagawa	missing data
	2016-040	MZ820924	nymph	Japan: Kanagawa	2015/5/28
	2016-043	MZ820939	nymph	Japan: Kanagawa	2015/5/28
	2021-154	MZ820914	nymph	Japan: Kyoto	2021/4/25
	2021-155	MZ820915	nymph	Japan: Kyoto	2021/4/25
Drunella kohnoi	2017-102	MK774396	nymph	Japan: Nara	2017/3/18
	2017-103	MK774397	nymph	Japan: Nara	2017/3/18
	2017-104	MK774398	nymph	Japan: Nara	2017/3/19
	2017-106	MK774401	nymph	Japan: Nara	2017/3/20
	2017-196	MK774399	nymph	Japan: Nara	2017/4/22
	2017-197	MK774400	nymph	Japan: Nara	2017/4/22
	2021-030	MZ820902	nymph	Japan: Wakayama	2021/4/11
Drunella trispina	2016-037	MZ820948	nymph	Japan: Kanagawa	2015/5/28
	2016-038	MZ820949	nymph	Japan: Kanagawa	2015/5/28
	2016-039	MZ820950	nymph	Japan: Kanagawa	2015/5/28
	2017-164	MK774328	nymph	Japan: Nara	2017/3/18
	2017-192	MK774359	nymph	Japan: Nara	2017/4/22
	2017-193	MK774360	nymph	Japan: Nara	2017/4/22
	2017-194	MK774361	nymph	Japan: Nara	2017/4/22
	2017-213	MK774382	nymph	Japan: Okayama	2017/6/10
	2017-214	MK774385	nymph	Japan: Okayama	2017/6/10
	2021-015	MZ820900	nymph	Japan: Wakayama	2021/4/11
	2021-018	MZ820901	nymph	Japan: Wakayama	2021/4/11
	2021-095	MZ820917	nymph	Japan: Kyoto	2021/4/25
	2021-164	OK501176	nymph	Japan: Wakayama	2021/4/24
	2021-167	OK501178	nymph	Japan: Kyoto	2021/4/25
	2021-168	OK501179	nymph	Japan: Kyoto	2021/4/25
	2021-177	OK501189	nymph	Japan: Kyoto	2021/4/25
	2021-178	OK501190	nymph	Japan: Kyoto	2021/4/25
Drunella sp.	2017-208	MK774366	nymph	Japan: Nara	2017/4/22

Table 2. List of DNA sequences retrieved from INSD.

Taxon	Sequence ID (Laboratory ID)	INSD accession number	Locality	Reference
Drunella sp.	n.a.	LC461325	Russia:Primorskii, ShkotovskiyRayon	Jo and Tojo (2019)
	n.a.	LC461326	Russia:Primorskii, ShkotovskiyRayon	Jo and Tojo (2019)
	n.a.	LC461327	Russia:Primorskii, ShkotovskiyRayon	Jo and Tojo (2019)
D. basalis	n.a.	LC461328	Japan:Hokkaido, Chitose	Jo and Tojo (2019)
	n.a.	LC461329	Japan:Hokkaido, Isoya	Jo and Tojo (2019)
	n.a.	LC461330	Japan:Yamagata, Yamagata	Jo and Tojo (2019)
	n.a.	LC461331	Japan:Yamanashi, Nirasaki	Jo and Tojo (2019)
	n.a.	LC461332	Japan:Mie, Taiki	Jo and Tojo (2019)
	n.a.	LC461333	Japan:Yamagata, Yamagata	Jo and Tojo (2019)
	n.a.	LC461334	Japan:Iwate, Hanamaki	Jo and Tojo (2019)
	n.a.	LC461335	Japan:Iwate, Hanamaki	Jo and Tojo (2019)
	n.a.	LC461336	Japan:Akita, Yuzawa	Jo and Tojo (2019)
	n.a.	LC461337	Japan:Miyagi, Kami	Jo and Tojo (2019)
	n.a.	LC461338	Japan:Miyagi, Kawasaki	Jo and Tojo (2019)
	n.a.	LC461339	Japan:Miyagi, Shiroisi	Jo and Tojo (2019)
	n.a.	LC461340	Japan:Niigata, Sado	Jo and Tojo (2019)
	n.a.	LC461341	Japan:Fukushima, Aizubange	Jo and Tojo (2019)
	n.a.	LC461342	Japan:Fukushima, Iwaki	Jo and Tojo (2019)
	n.a.	LC461343	Japan:Gunma, Midori	Jo and Tojo (2019)
	n.a.	LC461344	Japan:Tokyo, Hino	Jo and Tojo (2019)
	n.a.	LC461345	Japan:Tokyo, Hino	Jo and Tojo (2019)
	n.a.	LC461346	Japan:Nagano, Shinano	Jo and Tojo (2019)
	n.a.	LC461347	Japan:Nagano, Nagano	Jo and Tojo (2019)
	n.a.	LC461348	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461349	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461350	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461351	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461352	Japan:Toyama, Toyama	Jo and Tojo (2019)
	n.a.	LC461353	Japan:Nagano, Ueda	Jo and Tojo (2019)
	n.a.	LC461354	Japan:Nagano, Ueda	Jo and Tojo (2019)
	n.a.	LC461355	Japan:Nagano, Chikuma	Jo and Tojo (2019)
	n.a.	LC461356	Japan:Nagano, Koumi	Jo and Tojo (2019)
	n.a.	LC461357	Japan:Tochigi, Shioya	Jo and Tojo (2019)
	n.a.	LC461358	Japan:Gifu, Takayama	Jo and Tojo (2019)
	n.a.	LC461359	Japan:Mie, Tsu	Jo and Tojo (2019)
	n.a.	LC461360	Japan:Shiga, Nagahama	Jo and Tojo (2019)
	n.a.	LC461361	Japan:Shiga, Nagahama	Jo and Tojo (2019)
	n.a.	LC461362	Japan:Shiga, Nagahama	Jo and Tojo (2019)
	n.a.	LC461363	Japan:Shiga, Nagahama	Jo and Tojo (2019)
	n.a.	LC461364	Japan:Shiga, Nagahama	Jo and Tojo (2019)
	n.a.	LC461365	Japan:Shiga, Otsu	Jo and Tojo (2019)
	n.a.	LC461366	Japan:Kyoto, Uji	Jo and Tojo (2019)
	n.a.	LC461367	Japan:Nara, Tenkawa	Jo and Tojo (2019)
	n.a.	LC461368	Japan:Osaka, Kaizuka	Jo and Tojo (2019)
	n.a.	LC461369	Japan:Hyogo, Asago	Jo and Tojo (2019)
	n.a.	LC461370	Japan:Hyogo, Sayo	Jo and Tojo (2019)
	n.a.	LC461371	Japan:Okayama, Maniwa	Jo and Tojo (2019)
	n.a.	LC461372	Japan:Okayama, Maniwa	Jo and Tojo (2019)
	n.a.	LC461373	Japan:Hiroshima, Otake	Jo and Tojo (2019)
	n.a.	LC461374	Japan:Kagawa, Takamatsu	Jo and Tojo (2019)
	n.a.	LC461375	Japan:Miyazaki, Shiiba	Jo and Tojo (2019)
D. sachalinensis	n.a.	LC461376	Japan:Nagano, Iida	Jo and Tojo (2019)
	n.a.	LC461377	Japan:Okayama, Okayama	Jo and Tojo (2019)
	n.a.	LC461378	Japan:Nagano, Nagano	Jo and Tojo (2019)
	n.a.	LC461379	Japan:Nagano, Azumino	Jo and Tojo (2019)
	n.a.	LC461380	Japan:Niigata, Sado	Jo and Tojo (2019)
	n.a.	LC461381	Japan:Hokkaido, Bibai	Jo and Tojo (2019)
	n.a.	LC461382	Japan:Hokkaido, Horokanai	Jo and Tojo (2019)
	n.a.	LC461383	Japan:Tottori, Tottori	Jo and Tojo (2019)
	n.a.	LC461384	Japan:Nagano, Iida	Jo and Tojo (2019)
	n.a.	LC461385	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461386	Japan:Nagano, Nagano	Jo and Tojo (2019)
	n.a.	LC461387	Japan:Nagano, Nagano	Jo and Tojo (2019)
	n.a.	LC461388	Japan:Tottori, Chizu	Jo and Tojo (2019)
	n.a.	LC461389	Japan:Hokkaido, Horokanai	Jo and Tojo (2019)
	n.a.	LC461390	Japan:Hokkaido, Bibai	Jo and Tojo (2019)
	n.a.	LC461391	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461392	Japan:Hokkaido, Bibai	Jo and Tojo (2019)
	n.a.	LC461393	Japan:Hokkaido, Chitose	Jo and Tojo (2019)
D. trispina	n.a.	LC461394	Japan:Nagano, Shiojiri	Jo and Tojo (2019)
	n.a.	LC461395	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
D. kohnoi	n.a.	LC461396	Japan:Okayama, Maniwa	Jo and Tojo (2019)
	n.a.	LC461397	Japan:Hiroshima, Hiroshima	Jo and Tojo (2019)
D. lepnevae	n.a.	LC461398	South Korea:Gangwondo, Injegun	Jo and Tojo (2019)
	n.a.	LC461399	South Korea:Gangwondo, Injegun	Jo and Tojo (2019)
D. latipes [#1]	n.a.	LC461400	South Korea:Gyeongsangnamdo, Sancheonggun	Jo and Tojo (2019)
	n.a.	LC461401	South Korea:Gyeongsangnamdo, Sancheonggun	Jo and Tojo (2019)
	n.a.	LC461402	South Korea:Gyeongsangnamdo, Sancheonggun	Jo and Tojo (2019)
	n.a.	LC461403	South Korea:Gyeongsangnamdo, Sancheonggun	Jo and Tojo (2019)
	n.a.	LC461404	South Korea:Gangwondo, Pyeongchang	Jo and Tojo (2019)
	n.a.	LC461405	South Korea:Gangwondo, Pyeongchang	Jo and Tojo (2019)
D. triacantha	n.a.	LC461406	South Korea:Gangwondo, Jeogeseongun	Jo and Tojo (2019)
	n.a.	LC461407	South Korea:Gangwondo, Jeogeseongun	Jo and Tojo (2019)
	n.a.	LC461408	South Korea:Gyeongsangnamdo, Miryangsi	Jo and Tojo (2019)
	n.a.	LC461409	South Korea:Gyeongsangbukdo, Yeongcheonsi	Jo and Tojo (2019)
D. ishiyamana	n.a.	LC461410	Japan:Okayama, Maniwa	Jo and Tojo (2019)
	n.a.	LC461411	Japan:Okayama, Okayama	Jo and Tojo (2019)
	n.a.	LC461412	Japan:Yamaguchi, Iwakuni	Jo and Tojo (2019)
	n.a.	LC461413	Japan:Tottori, Kurayoshi	Jo and Tojo (2019)
	n.a.	LC461414	Japan:Niigata, Iwafune	Jo and Tojo (2019)
	n.a.	LC461415	Japan:Niigata, Iwafune	Jo and Tojo (2019)
	n.a.	LC461416	Japan:Tottori, Kurayoshi	Jo and Tojo (2019)
	n.a.	LC461417	Japan:Okayama, Maniwa	Jo and Tojo (2019)
	n.a.	LC461418	Japan:Nara, Gojo	Jo and Tojo (2019)
	n.a.	LC461419	Japan:Nara, Gojo	Jo and Tojo (2019)
	n.a.	LC461420	Japan:Nagano, Matsumoto	Jo and Tojo (2019)
	n.a.	LC461421	Japan:Tottori, Houki	Jo and Tojo (2019)
	n.a.	LC461422	Japan:Hokkaido, Kamikawa	Jo and Tojo (2019)
	n.a.	LC461423	Japan:Yamanashi, Kai	Jo and Tojo (2019)
	n.a.	LC461424	Japan:Yamanashi, Kai	Jo and Tojo (2019)
	n.a.	LC461425	Japan:Shizuoka, Izu	Jo and Tojo (2019)
(outgroup)				
Ephemera strigata	2013-025	MN961293	Japan:Nara, Yoshino	Wakimura et al. (2016)
Ephemerella atagosana	2012-033	KF563038	Japan:Shiga, Takashima	Wakimura et al. (2016)
Ephemerella imanishii [#2]	2016-010	MH260767	Japan:Kanagawa, Atsugi	Wakimura et al. (2020)
Ephemerella notata	2014-023	KP970724	Japan:Kyoto, Uji	Wakimura et al. (2016)
Serratella setigera	2011-605	JQ655113	Japan:Kanagawa, Ashigarakami	Wakimura et al. (2016)

[#1] *D. latipes* is a synonym of *D. ishiyamana* as mentioned in the text and no longer valid. This name appears yet in INSD, however.

[#2] *Ephemerella imanishii* was synonymized with *Serratella occiprens* by Jacobus & McCafferty (2008). This name appears yet in INSD, however.

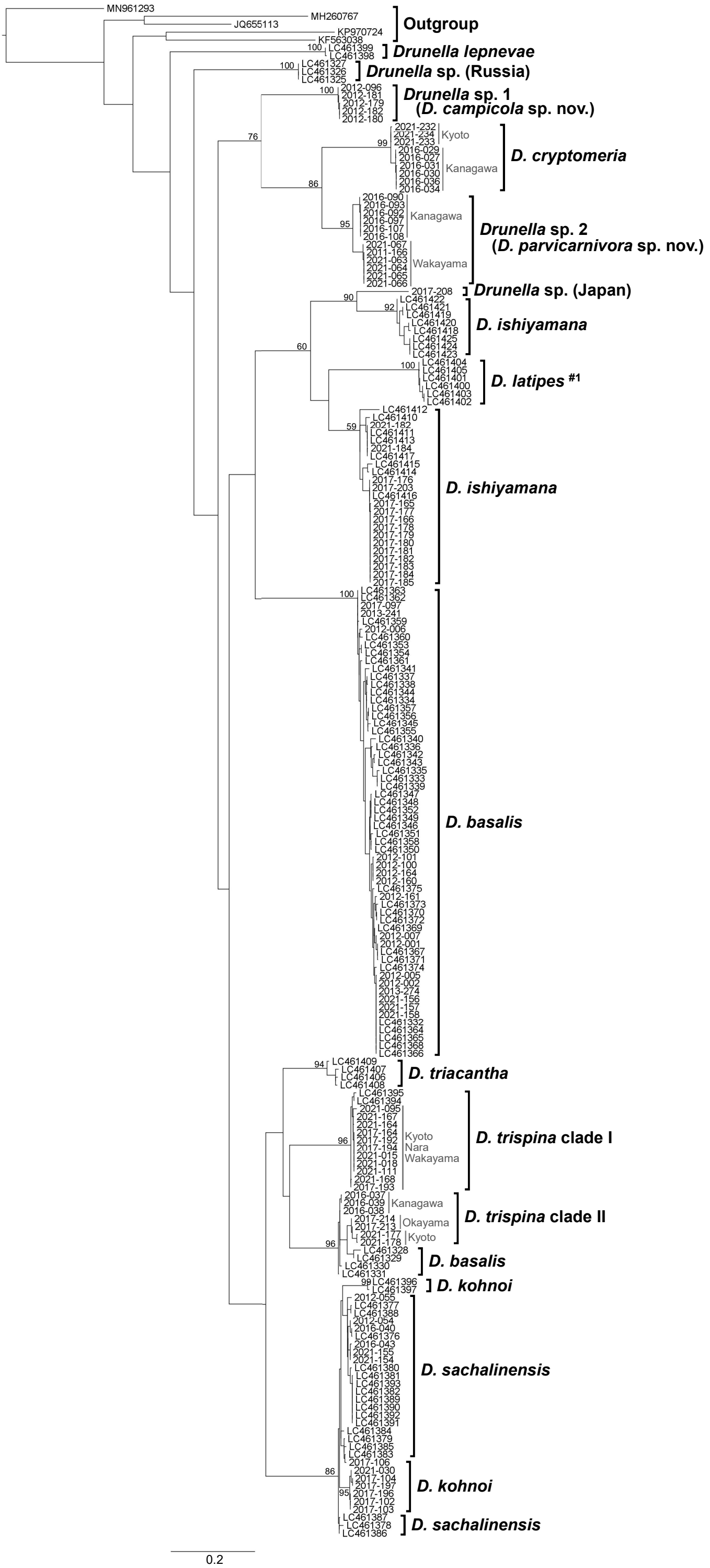
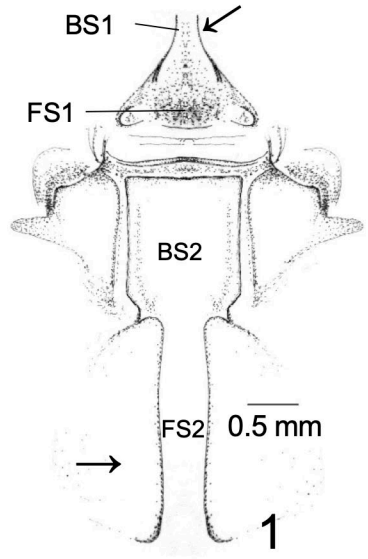
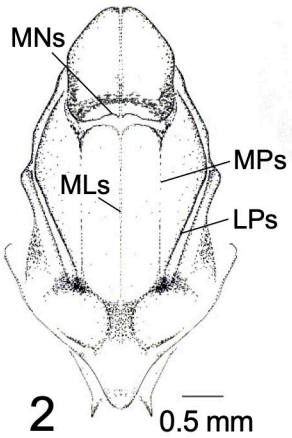

MN961293
MH260767
JQ655113
KP970724
KF563038
Outgroup
100
LC461399
LC461398
Drunella lepnevae
100
LC461327
LC461326
LC461325
Drunella sp. (Russia)
100
2012-096
2012-181
2012-179
2012-182
2012-180
Drunella sp. 1
(D. campicola sp. nov.)
76
2021-232
2021-234
2021-233
Kyoto
99
2016-029
2016-027
2016-031
2016-030
2016-036
2016-034
Kanagawa
D. cryptomeria
86
2016-090
2016-093
2016-092
2016-097
2016-107
2016-108
Kanagawa
95
2021-067
2011-166
2021-063
2021-064
2021-065
2021-066
Wakayama
Drunella sp. 2
(D. parvicarnivora sp. nov.)
2017-208
Drunella sp. (Japan)
90
LC461422
92
LC461421
LC461419
LC461420
LC461418
LC461425
LC461424
LC461423
D. ishiyamana
60
100
LC461404
LC461405
LC461401
LC461400
LC461403
LC461402
D. latipes #1
LC461412
LC461410
59
2021-182
LC461411
LC461413
2021-184
LC461417
LC461415
LC461414
2017-176
2017-203
LC461416
2017-165
2017-177
2017-166
2017-178
2017-179
2017-180
2017-181
2017-182
2017-183
2017-184
2017-185
D. ishiyamana
100
LC461363
LC461362
2017-097
2013-241
LC461359
2012-006
LC461360
LC461353
LC461354
LC461361
LC461341
LC461337
LC461338
LC461344
LC461334
LC461357
LC461356
LC461345
LC461355
LC461340
LC461336
LC461342
LC461343
LC461335
LC461333
LC461339
LC461347
LC461348
LC461352
LC461349
LC461346
LC461351
LC461358
LC461350
2012-101
2012-100
2012-164
2012-160
LC461375
2012-161
LC461373
LC461370
LC461372
LC461369
2012-007
2012-001
LC461367
LC461371
LC461374
2012-005
2012-002
2013-274
2021-156
2021-157
2021-158
LC461332
LC461364
LC461365
LC461368
LC461366
D. basalis
94
LC461409
LC461407
LC461406
LC461408
D. triacantha
LC461395
LC461394
2021-095
2021-167
2021-164
2017-164
96
2017-192
2017-194
2021-015
2021-018
2021-111
2021-168
2017-193
Kyoto
Nara
Wakayama
D. trispina clade I
2016-037
2016-039
2016-038
Kanagawa
2017-214
2017-213
Okayama
2021-177
2021-178
Kyoto
96
D. trispina clade II
LC461328
LC461329
LC461330
LC461331
D. basalis
99
LC461396
LC461397
D. kohnoi
2012-055
LC461377
LC461388
2012-054
2016-040
LC461376
2016-043
2021-155
2021-154
LC461380
LC461381
LC461393
LC461382
LC461389
LC461390
LC461392
LC461391
LC461384
LC461379
LC461385
LC461383
D. sachalinensis
2017-106
86
2021-030
2017-104
2017-197
95
2017-196
2017-102
2017-103
D. kohnoi
LC461387
LC461378
LC461386
D. sachalinensis
0.2

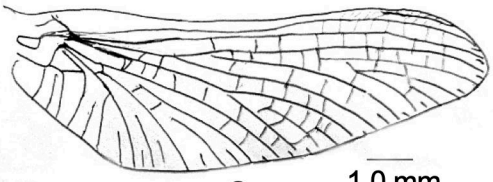
Fig. 2



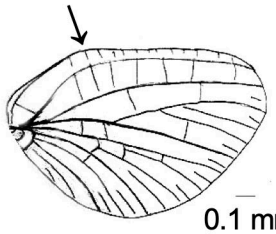
1



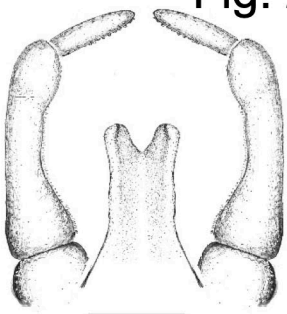
2



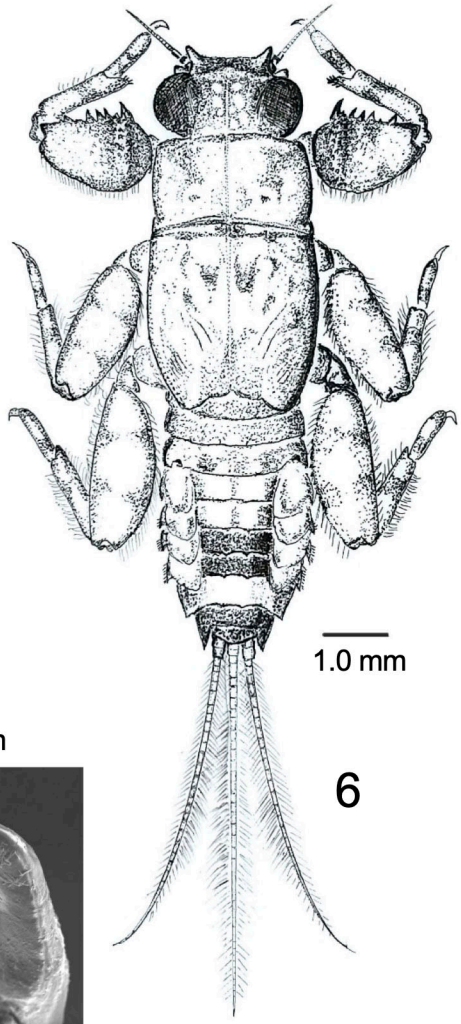
3



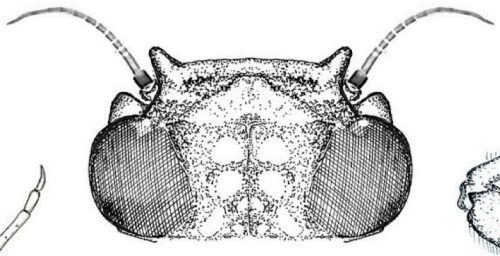
4



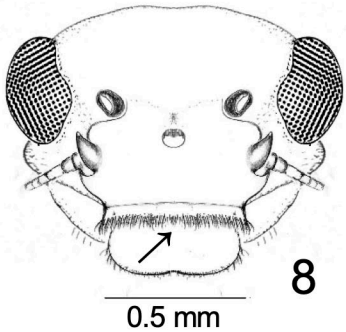
5



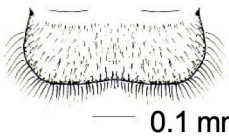
6



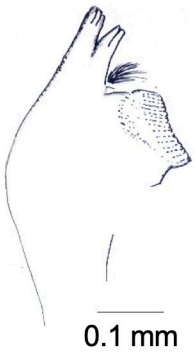
7



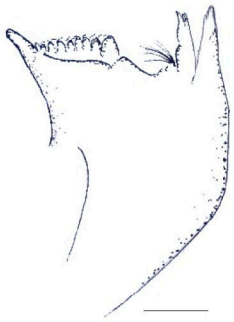
8



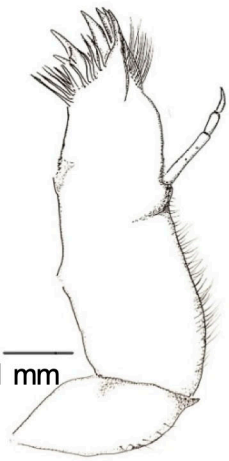
9



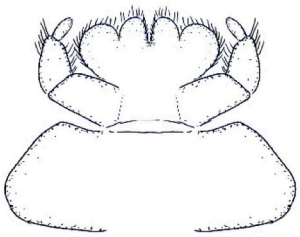
10



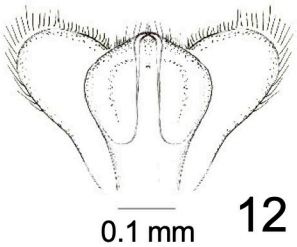
11



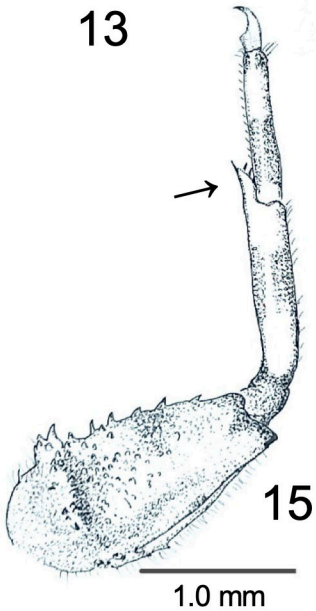
13



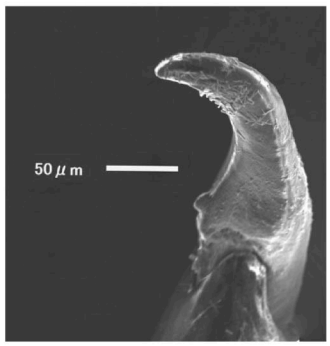
14



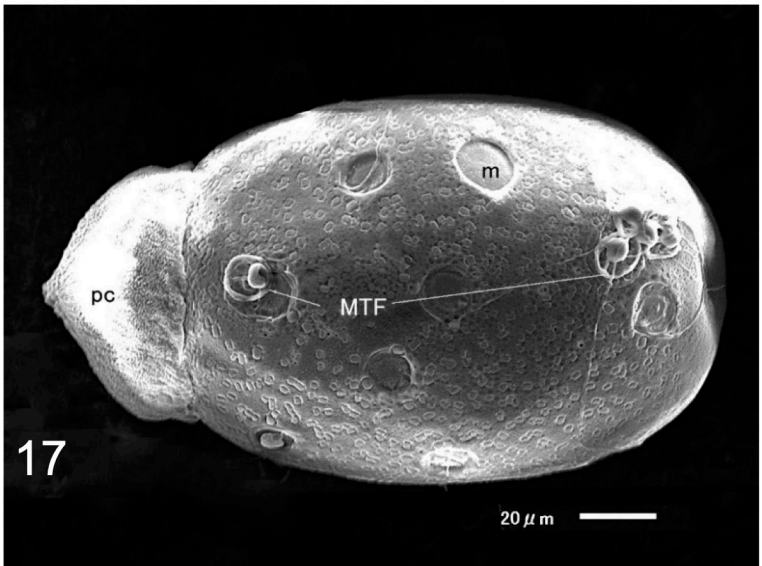
12



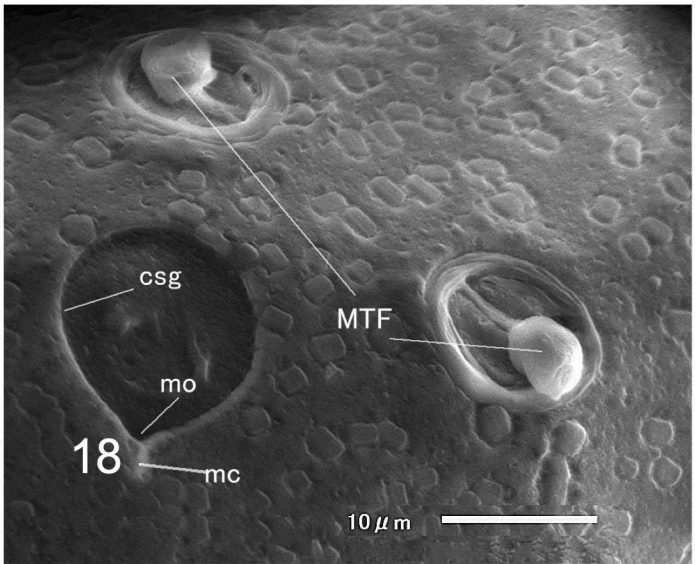
15



16

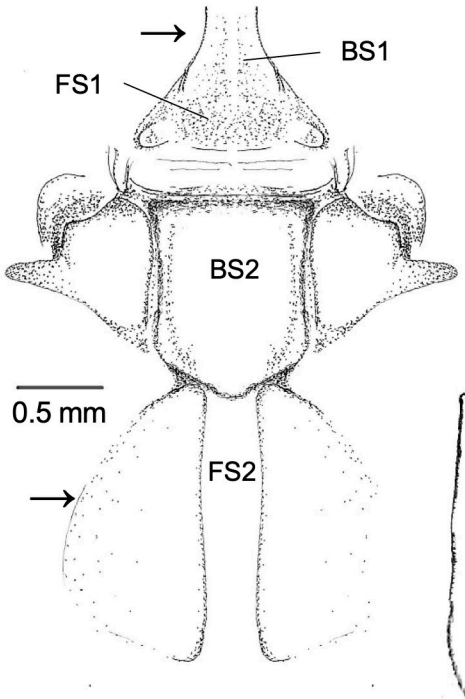


17

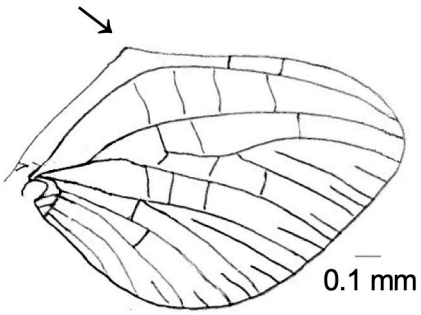


18

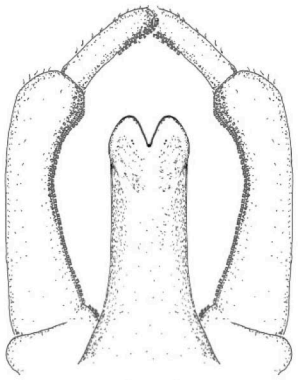
Fig. 3



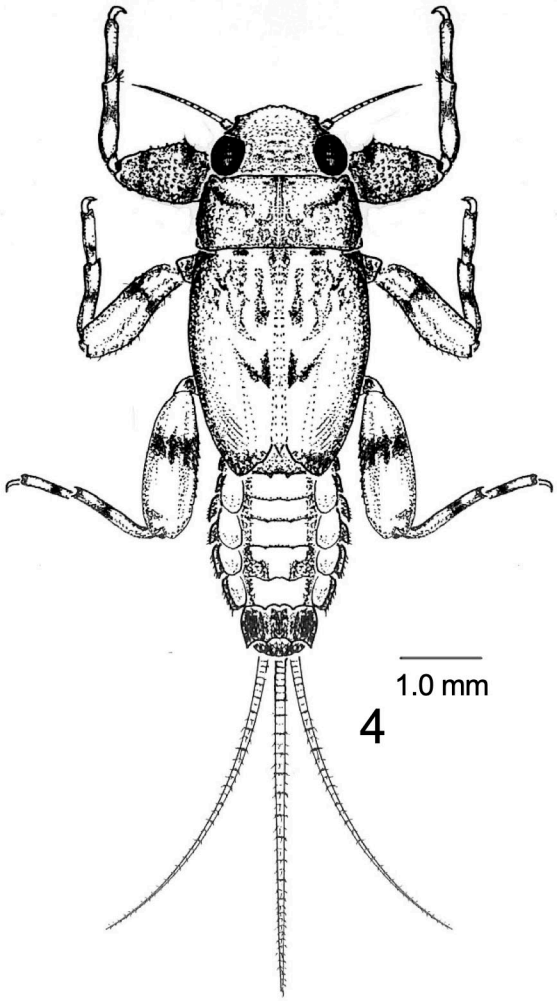
1



2

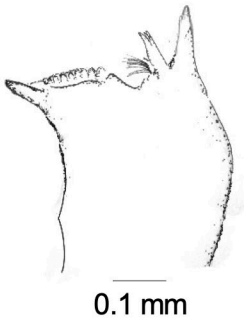


3

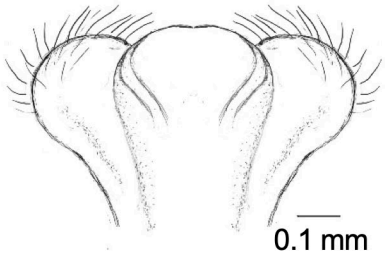


4

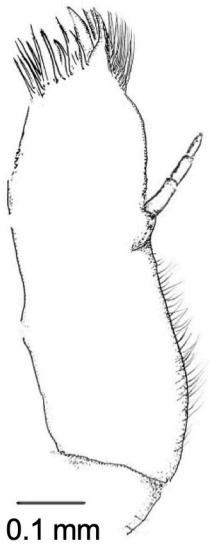
5



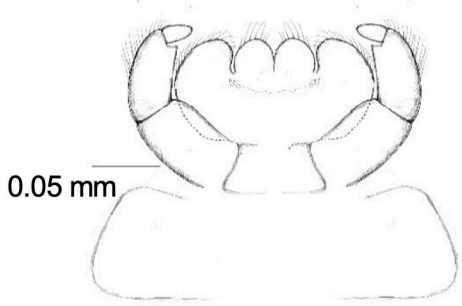
6



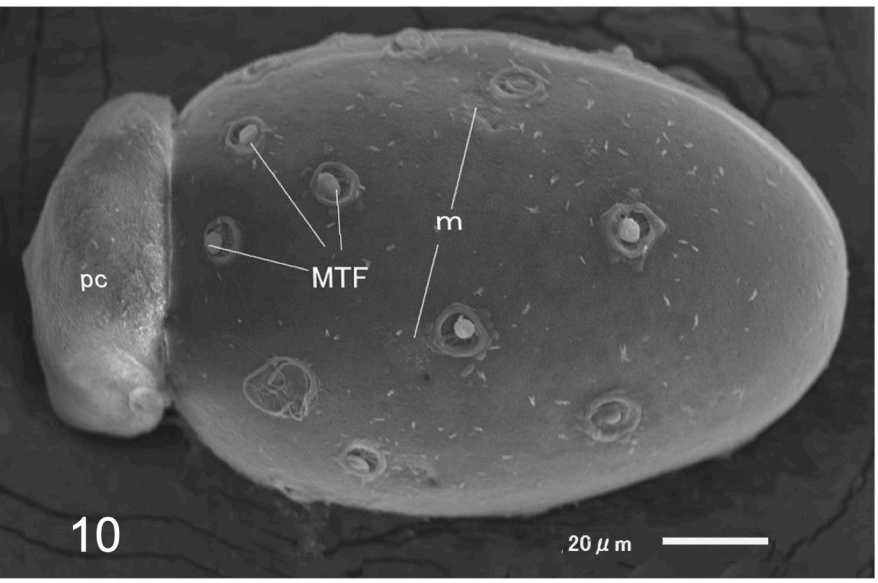
7



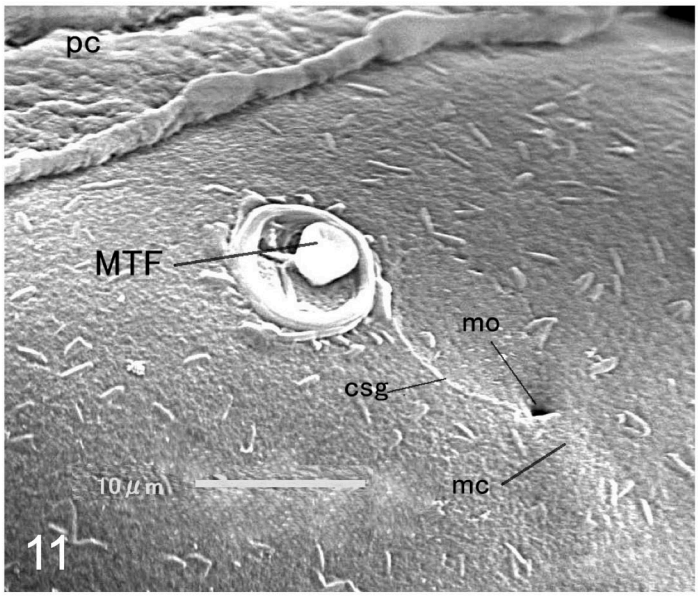
8



9

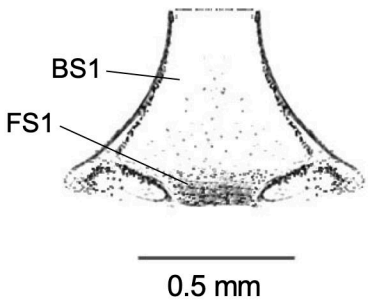


10



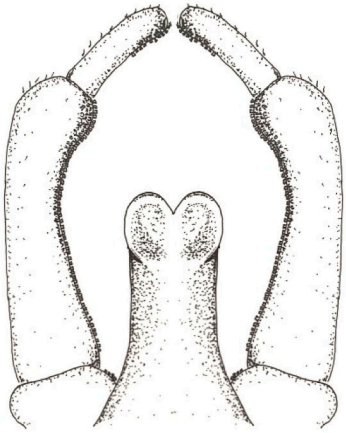
11

Fig. 4



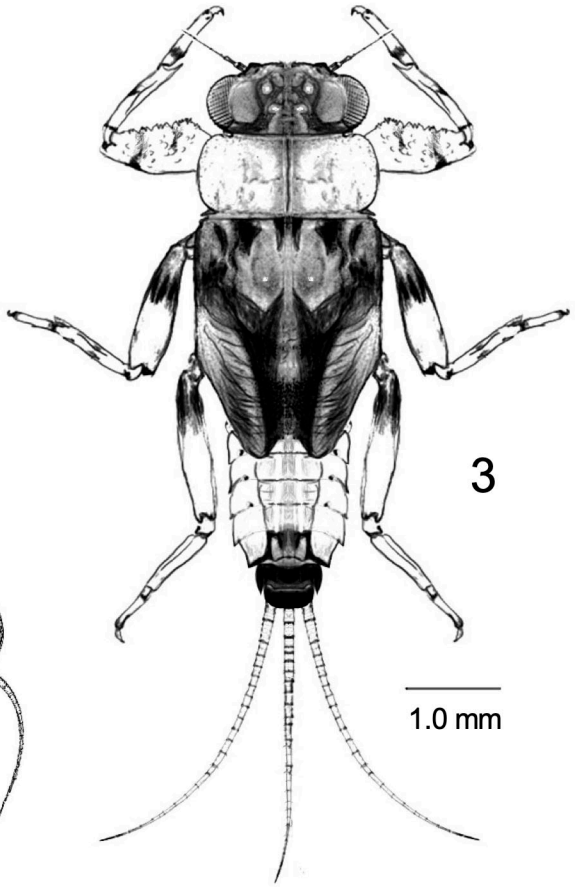
0.5 mm

1



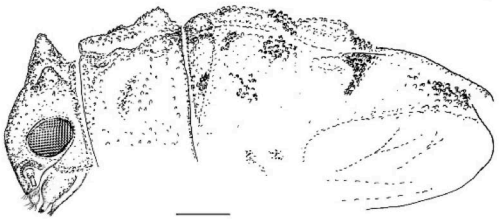
0.1 mm

2



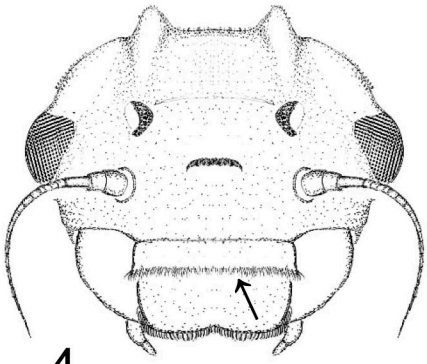
1.0 mm

3



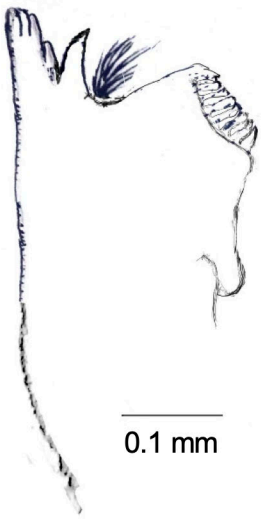
0.5 mm

5



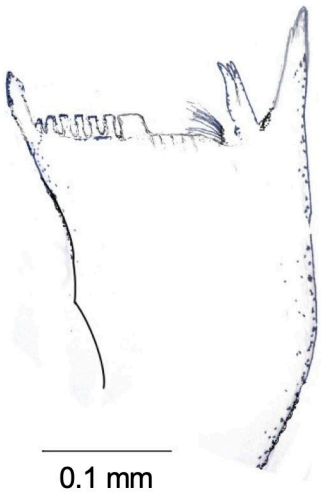
4

0.5 mm



0.1 mm

6

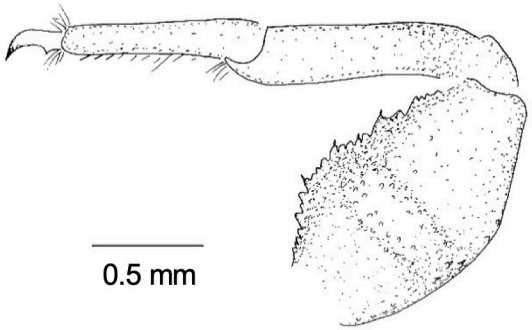


0.1 mm

7

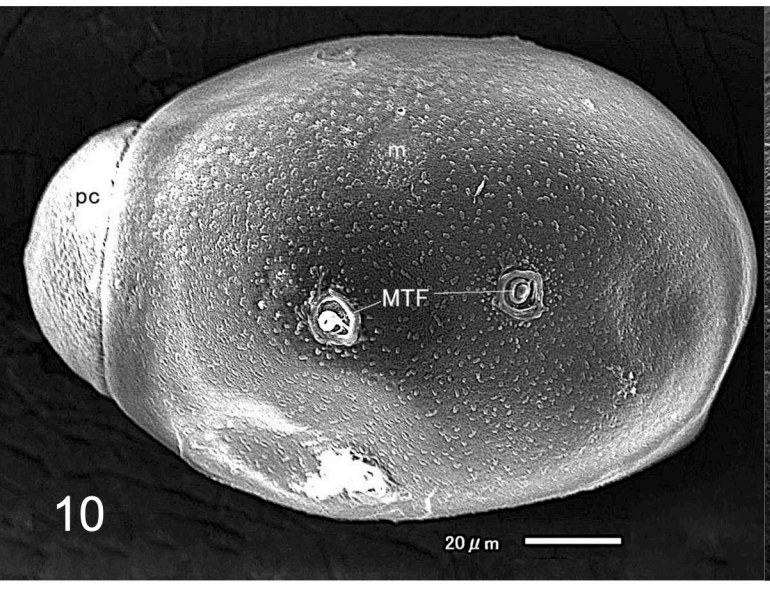
0.1 mm

8

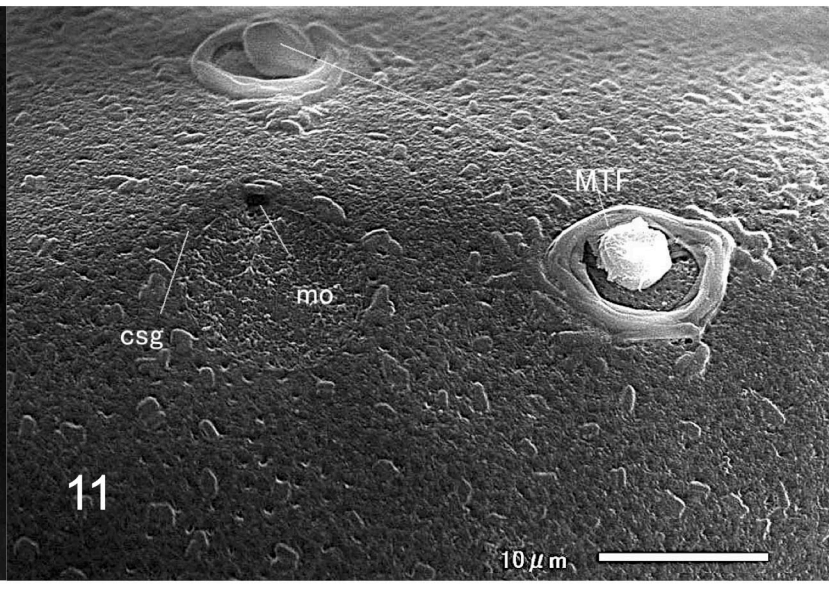


0.5 mm

9



10



11