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A Description of the Second species of the Family Dipteromimidae (Insecta, Ephemeroptera), and Genetic Relationship of Two Dipteromimid Mayflies Inferred from Mitochondrial 16S rRNA Gene Sequences

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ABSTRACT—A new mayfly species, *Dipteromimus flavipterus* sp. nov., of the family Dipteromimidae is described on the basis of specimens of males, females and mature nymphs collected from Ofunato, Iwate Prefecture, Japan. This new *Dipteromimus* species is characterized by a large body size, a yellowish body color (especially the frontal border of the forewings), short penis lobes, a slight elongation backwards of the female 7th abdominal sternum, and a rounded backward projection of the female 9th abdominal sternum, etc., in comparison with another known dipteromimid mayfly *Dipteromimus tipuliformis* McLachlan, 1875. Full descriptions of males, females, mature nymphs and eggs of this new species have been provided. We also examined and discussed the genetic relationship of two dipteromimid mayflies to settle the taxonomic status, inferred from the partially sequenced large mitochondrial ribosomal subunit (16S rRNA) genes. Consequently, phenetic and molecular phylogenetic analyses agreed in terms of clustering.

Key words: taxonomy, morphology, phylogeny, phylogeography, histology

INTRODUCTION

The family Dipteromimidae is the smallest group of mayflies, with only one sepciese described, *Dipteromimus tipuliformis* McLachlan, 1875 (cf. McLachlan, 1875; Kluge *et al.*, 1995; Ishiwata, 2001). *D. tipuliformis* is strictly endemic to Japan, but is distributed widely in Honshu (except for the Tohoku District), Shikoku and Kyushu Islands, and Amami-oshima Island (an island of the Ryukyu Islands). This family is very important for understanding the ground plan or evolution of Ephemeroptera, due to its basal positioning in the ephemeropteran phylogeny.

Recently, we obtained some specimens of probably a new species belonging to this family from the Tohoku District: Ofunato, Iwate Prefecture. On the basis of these specimens, full descriptions of adults, mature nymphs and eggs have been provided. The keys to species of dipteromimid mayflies are also presented. The holotype, the allotype and a paratype are deposited as the type series specimens in the collections of the Natural History Museum and Institute, Chiba (CBM-ZI 94276-94278). Other specimens are part of the private collection of the senior author (KT).

In addition to morphological and taxonomic descriptions, we also examined the genetic relationship of two dipteromimid mayflies inferred from variations in about 380 base positions (375-381 bp.) of the large mitochondrial ribosomal subunit (16S rRNA) gene sequence.

MATERIALS AND METHODS

Materials

As for the newly described species in this paper, nymphs were collected in the side pools of a small brook “Sakamoto-zawa” in Ofunato (Iwate Prefecture, Japan; Table 1, Fig. 1) at June 13, 2002. Some of the nymphs were fixed with 70% ethanol, the others were incubated in a water tank with many fallen leaves collected in the field, at about 10–13°C, to obtain adults. Some of the emerged adults were fixed with 70% ethanol, others were fixed with pure

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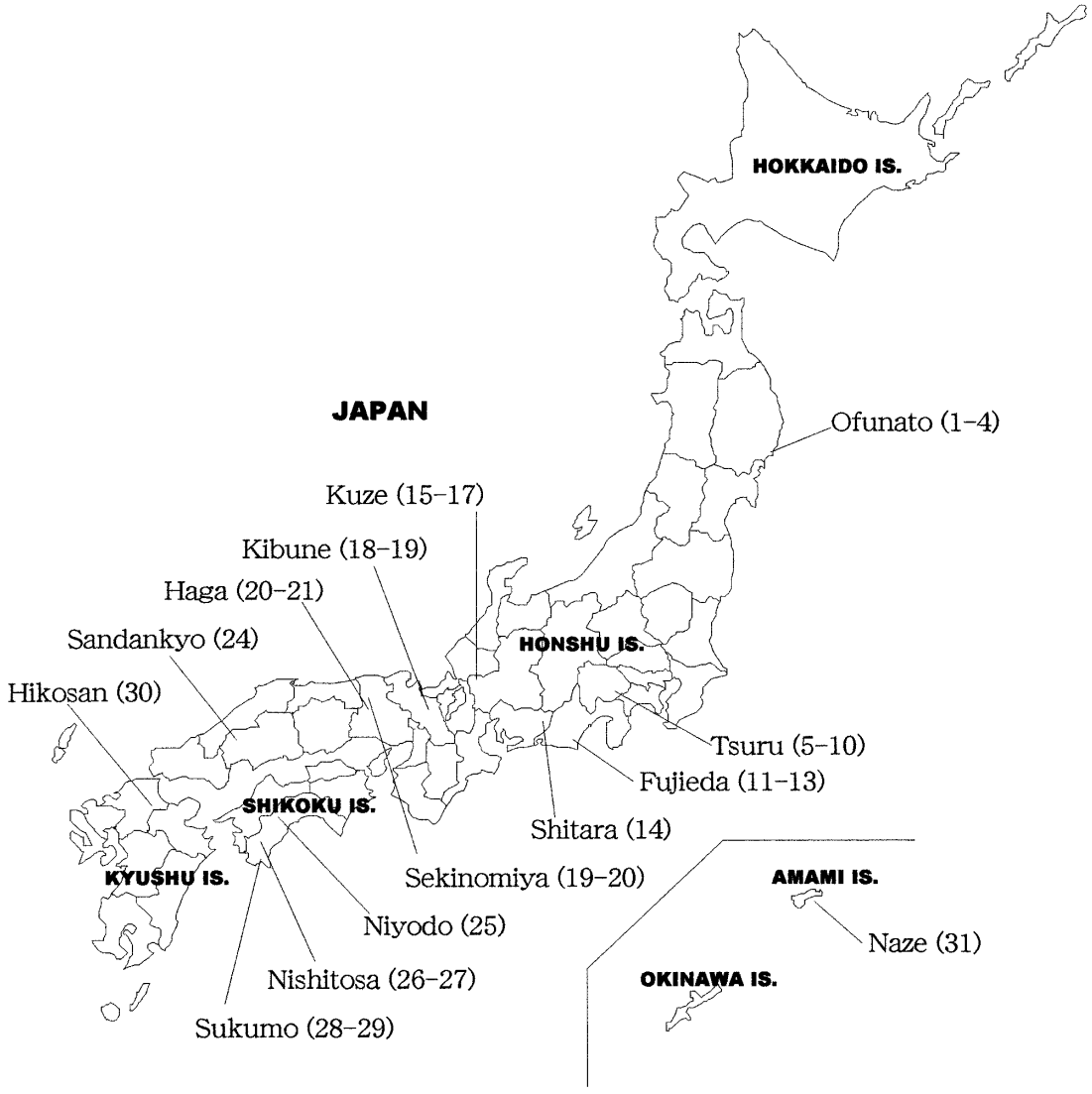


Fig. 1. Sampling localities of dipteromimid mayflies, *Dipteromimus flavipterus* sp. nov. (1–4) and *D. tipuliformis* (5–31). Refer to Table 1 for details of the sampling localities and sample numbers.

ethanol for molecular examination.

Twenty-seven individuals of *Dipteromimus tipuliformis* from 13 localities including Honshu, Shikoku, Kyushu Islands and Amami-oshima Island were used for analyzing genetic relationships. Sampling localities of the mayflies are shown on the map (Fig. 1), and sample numbers are described in the sample list (Table 1). Nymphs and adults of the species, and two nymphs of *Ameletus montanus* Imanishi and a nymph of *Siphlonurus binotatus* (Eaton) of the related families Ameletidae and Siphlonuridae, respectively, were also fixed with pure ethanol, and used for molecular phylogenetical analyses as outgroups (Table 1): these three mayfly groups Dipteromimidae, Ameletidae and Siphlonuridae have been gathered in the family "Siphlonuridae (s. lat.)" until recently (e. g., Gose, 1985).

Morphology

Specimens were examined under a dissecting microscope (SZH10; Olympus, Tokyo) and a transmission microscope (BHP; Olympus, Tokyo). The drawings were made with the aid of a drawing tube mounted on the microscope. The eggs were obtained in the laboratory from females, and incubated in water at 13°C (±0.5°C). They were fixed with alcoholic Bouin's fluid (saturated alcoholic solution of picric acid : formalin : acetic acid=15 : 5 : 1) at room temperature for 24 hr. The fixed eggs were processed into methacrylate resin Thechnovit 7100 (Kulzer, Wehreim) sections of 2 μm thickness, in accordance with Machida *et al.* (1994a, b), and Tojo and Machida (1997, 1998, 2003). Sections were stained with Mayer's acid hemalum, eosin and fast green FCF.

For scanning electron microscopy (SEM), the fixed eggs were sonicated for a few seconds with an ultrasonic cleaner, dehydrated in a graded ethanol series, and then transferred to acetone. The eggs were dried in a critical-point drier, coated with gold, and observed under a microscope (SM300; Topcon, Tokyo), in accordance with Tojo and Machida (2003). The adhesive layer (specialized attachment apparatus) was resolved with 10% antiformin (sodium hypochlorite solution) to show the chorion and micropyles.

DNA analyses by sequencing of the mitochondrial 16S rRNA region

DNA was extracted from the specimens fixed with pure ethanol, and purified using the DNeasy® Tissue Kit (QIAGEN, Hilden). The 16S rRNA genes were amplified by a PCR method using as the forward primer, 5'-TTACGCTGTTATCCCTAA-3', and the reverse

Table 1. Specimens of dipteromimid and related mayflies used mitochondrial 16S rRNA analyses

Sample no.	Family	Genus	Species	Locality				Accession no.
					Prefecture	Latitude	Longitude	
	Dipteromimidae							
1–4		*Dipteromimus*	*flavipterus sp. nov.*	Ofunato (Sakamoto-zawa)	Iwate	39°07'40" N,	141°38'18" E	AB110236–110239
5–8		*Dipteromimus*	*tipuliformis*	Tsuru (O-sawa)	Yamanashi	34°30'54" N,	138°52'27" E	AB110240–110243
9		*D.*	*tipuliformis*	Tsuru (O-sawa)	Yamanashi	34°30'54" N,	138°52'27" E	AB110244
10		*D.*	*tipuliformis*	Tsuru (O-sawa)	Yamanashi	34°30'54" N,	138°52'27" E	AB110245
11–12		*D.*	*tipuliformis*	Fujieda (Utouge)	Shizuoka	34°58'52" N,	138°11'47" E	AB110246–110247
13		*D.*	*tipuliformis*	Fujieda (Utouge)	Shizuoka	34°58'52" N,	138°11'47" E	AB110248
14		*D.*	*tipuliformis*	Shitara (Kawamukai)	Aichi	35°06'56" N,	137°33'06" E	AB110249
15		*D.*	*tipuliformis*	Kuze (Ozu)	Gifu	35°37'04" N,	136°32'33" E	AB110250
16		*D.*	*tipuliformis*	Kuze (Ozu)	Gifu	35°37'04" N,	136°32'33" E	AB110251
17		*D.*	*tipuliformis*	Kuze (Ozu)	Gifu	35°37'04" N,	136°32'33" E	AB110252
18–19		*D.*	*tipuliformis*	Kyoto (Kibune)	Kyoto	35°08'08" N,	135°46'12" E	AB110253–110254
20		*D.*	*tipuliformis*	Haga (Onzui)	Hyogo	35°14'32" N,	134°31'42" E	AB110255
21		*D.*	*tipuliformis*	Haga (Onzui)	Hyogo	35°14'32" N,	134°31'42" E	AB110256
22–23		*D.*	*tipuliformis*	Sekinomiya (Yagi-gawa)	Hyogo	35°21'17" N,	134°39'09" E	AB110257–110258
24		*D.*	*tipuliformis*	Togouchi (Sandankyo)	Hiroshima	34°38'44" N,	132°10'25" E	AB110259
25		*D.*	*tipuliformis*	Niyodo (Torigata)	Kochi	33°28'23" N,	133°04'35" E	AB110260
26		*D.*	*tipuliformis*	Nishitosa (Shimanto-gawa)	Kochi	33°26'11" N,	133°05'15" E	AB110261
27		*D.*	*tipuliformis*	Nishitosa (Shimanto-gawa)	Kochi	33°26'11" N,	133°05'15" E	AB110262
28–29		*D.*	*tipuliformis*	Sukumo (Nakasuji-gawa)	Kochi	32°55'13" N,	132°49'23" E	AB110263–110264
30		*D.*	*tipuliformis*	Soeda (Hikosan)	Fukuoka	33°28'01" N,	130°54'25" E	AB110265
31		*D.*	*tipuliformis*	Naze (Amami)	Kagoshima	28°21'05" N,	129°28'16" E	AB110266
	Ameletidae							
32		*Ameletus*	*montanus*	Enzan (Hi-kawa)	Yamanashi	35°42'33" N,	138°49'39" E	AB110267
33		*A.*	*montanus*	Enzan (Hi-kawa)	Yamanashi	35°42'33" N,	138°49'39" E	AB110268
	Siphlonuridae							
34		*Siphlonurus*	*binotatus*	Akiruno (Aki-gawa)	Tokyo	35°43'08" N,	139°16'38" E	AB110269

primer, 5'-CGCCTGTTTATCAAAAACAT-3'. PCR products were purified with a Microcon® Kit (MILLIPORE, Massachusetts). The purified DNA was sequenced directly by an automated method using the DYEnamic™ ET Terminator Cycle Sequencing Kit (Amersham Biosciences, New Jersey) on an automated sequencer (ABI PRISM 377 Genetic Analyzer; Perkin Elmer/Applied Biosystems, California). The sequence data of 31 samples from 14 populations, and of three samples of two related groups as outgroups, have been submitted to the DNA Data Bank of Japan (DDBJ database), and their accession numbers are given in Table 1. Aligned data can be provided on request.

Phylogenetic analyses were performed by the neighbor-joining (NJ) method (Saitou and Nei, 1987), implemented using PHYLIP version 3.57 (Felsenstein, 1995). The NJ analyses employed matrices of genetic distances generated using Kimura's two-parameter method (Kimura, 1980), and confidences of branches were assessed by 1,000 bootstrap resamplings.

SYSTEMATICS

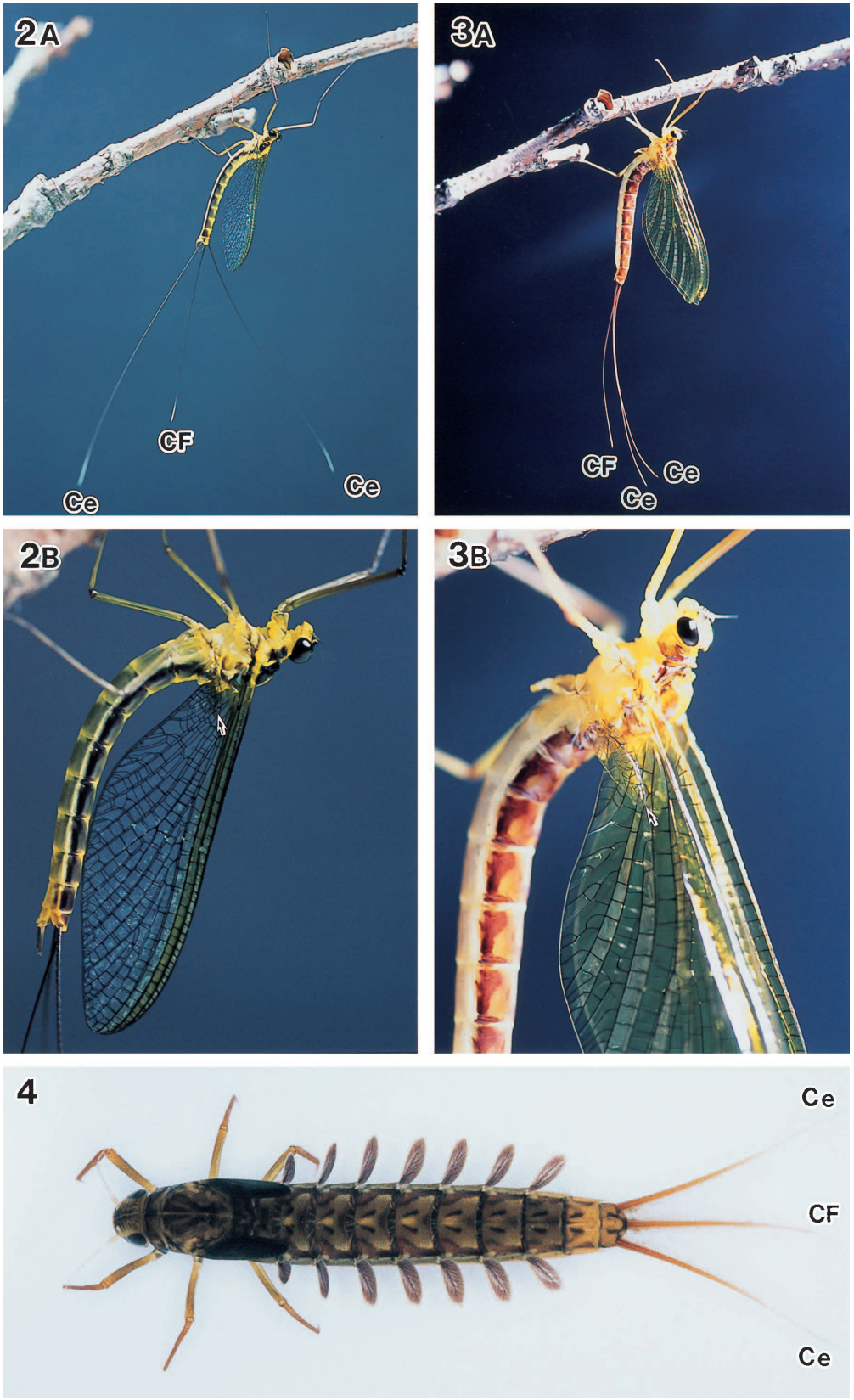
Description

Family Dipteromimidae McLachlan, 1875
Genus *Dipteromimus* McLachlan, 1875
Dipteromimus flavipterus sp. nov.
[Japanese name: Kiiro-gagambo-kagerou]

Adults

Male (Figs. 2, 5–7, 8A, 9–10 and 13)

Measurements (N=2): Body 13.8, 16.5 mm (antennae, cerci and caudal filament [=terminal filament or median filament] omitted); antenna 1.6–1.7 mm; cerci 31.5–33.6 mm; caudal filament (median filament) 23.0, 23.1 mm; fore wings 15.1–15.4 mm. *Head*: Penes light yellow, area between three ocelli black; compound eyes black, clearly divided



Figs. 2–4. *Dipteromimus flavipterus* sp. nov. 2: Male (A), and enlargement (B). An arrow shows the vestigial hind wing. 3: Female (A), and enlargement (B). The arrow is the same as in Fig. 2B. 4: Mature (ultimate instar) nymph. Ce, cercus; CF, caudal filament.

transversely into an upper part with large facets and a lower part with small facets; ocelli translucent, with black basal area (Fig. 2A, B). Antennae milky white, unicolorous; scapus and pedicellus short (scapus : pedicellus ≒ 1 : 3); flagellum conspicuously long, moderately tapered distally, about 2.5 times as long as scapus + pedicellus (Fig. 7). Mouthparts extremely minute. *Thorax*: Penes light yellow, with paired dark brownish lines on pro-, meso- and metanotum (Fig. 2A, B; cf. Fig. 5). Fore wings hyaline with yellowish transparent frontal border, hind wings strickingly degenerated (arrow in Fig. 2B); all veins brownish (Fig. 2A, B). Fore legs light yellow, and elongated as long as body length (Fig. 2A, B); femur and tibia long, about 10 times the length of coxa or trochanter; tarsus five-segmented, much long, about twice the length of femur or tibia (Fig. 8A); 1st tarsomere not fused to tibia; middle and hind legs light yellow, about 2/3 the length of fore legs, tarsus five-segmented, but the 1st tarsomere fused to tibia (Fig. 2A, B; Fig. 8A vs. Figs. 9A and 10); pretarsus of all legs with paired claws of the same shape (Figs. 8A, 9 and 10). *Abdomen*: Tergum broadly light yellow with paired dark brownish lines (Fig. 2A, B). Sternum light yellow (Fig. 2A, cf. Fig. 2B). Genital forceps brownish, four-segmented (Figs. 2B and 13). Penis lobes short, about 1/4 the length of genital forceps (Fig. 13). Cerci dark brownish with white tip, well developed about twice the length of body (Fig. 2A). Caudal filament also dark brownish with white tip, and also well developed, although a little shorter than cerci (Fig. 2A).

Female (Figs. 3, 8B and 11)

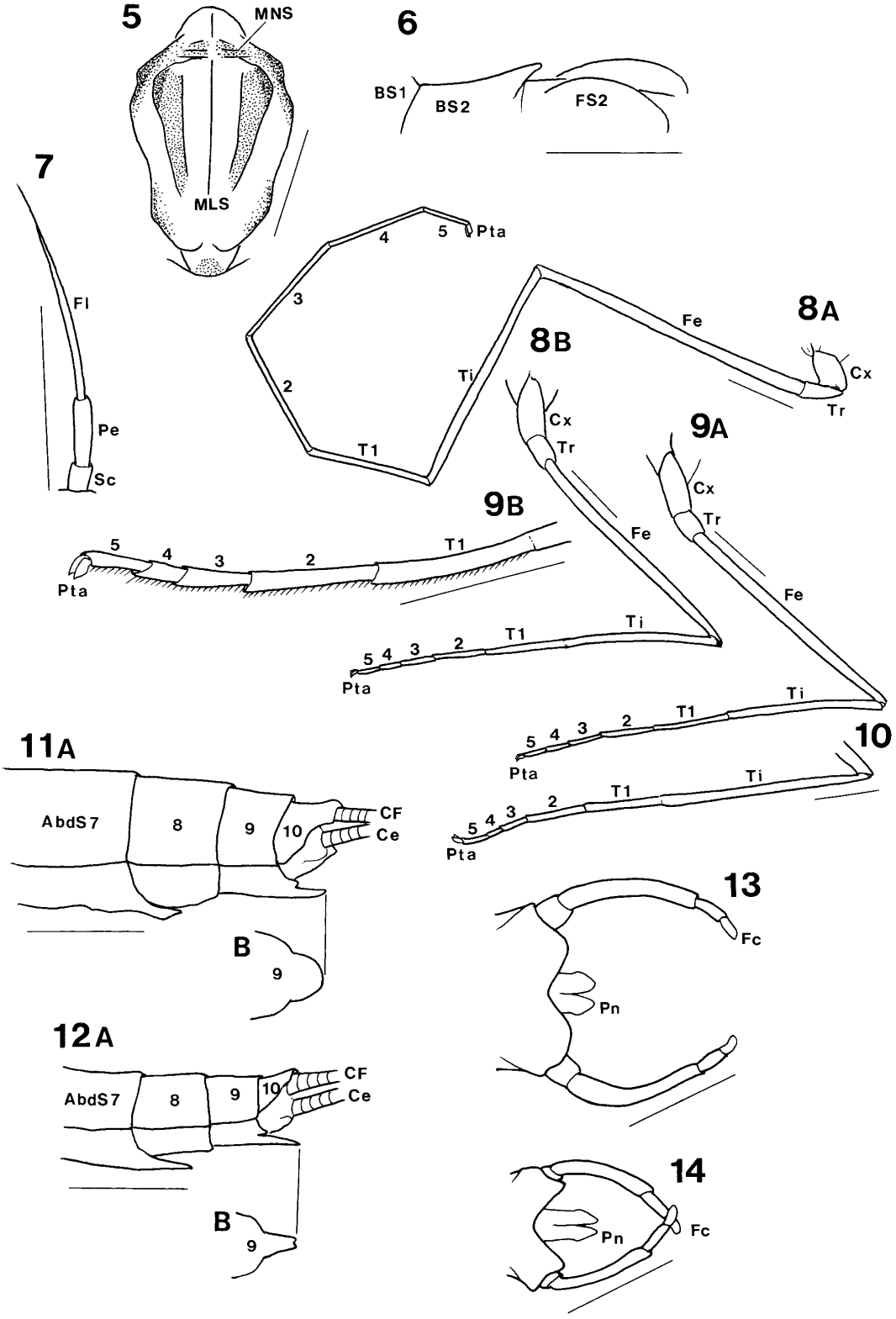
Measurements (N=4): Body 17.2–23.5 mm; antennae 1.5–1.7 mm; cerci 23.4–28.0 mm; caudal filament 21.1–23.1 mm; fore wings 16.5–20.1 mm. *Head*: Penes light yellow, and area between three ocelli black; compound eyes black, somewhat smaller than those of male, undivided transversely into two parts; color pattern of compound eyes and ocelli similar to those of male (Fig. 3A, B; cf. Fig. 2A, B). Antennal structure and color pattern similar to those of male (Fig. 3A, B; cf. Fig. 2A, B). Mouthparts extremely minute. *Thorax*: Penes light yellow with paired light brownish lines on pro-, meso- and metanotum (Fig. 3A, B). Structure and color pattern of wings similar to those of male, although fore wings slightly larger than those of male (Fig. 3A, B vs. Fig. 2A, B). Fore legs much shorter than those of male, the 1st tarsomere fused to tibia (five-segmented tarsus visible) such as middle and hind legs of male (Fig. 8B); pretarsus of all legs with paired claws of the same shape (cf. Fig. 9B). *Abdomen*: Tergum broadly light brown, because paired brownish lines almost fused on dorsal side, sternum light yellow as in male (Fig. 3A, B). Seventh and 9th sterna posteriorly elongated (Fig. 11A), the elongation of 9th sternum roundish (Fig. 11B). Structure and color pattern of cerci and caudal filament similar to those of male, although somewhat shorter; caudal filament a little shorter than cerci (Fig. 3A).

Mature (ultimate instar) nymph (Figs. 4, 15–22)

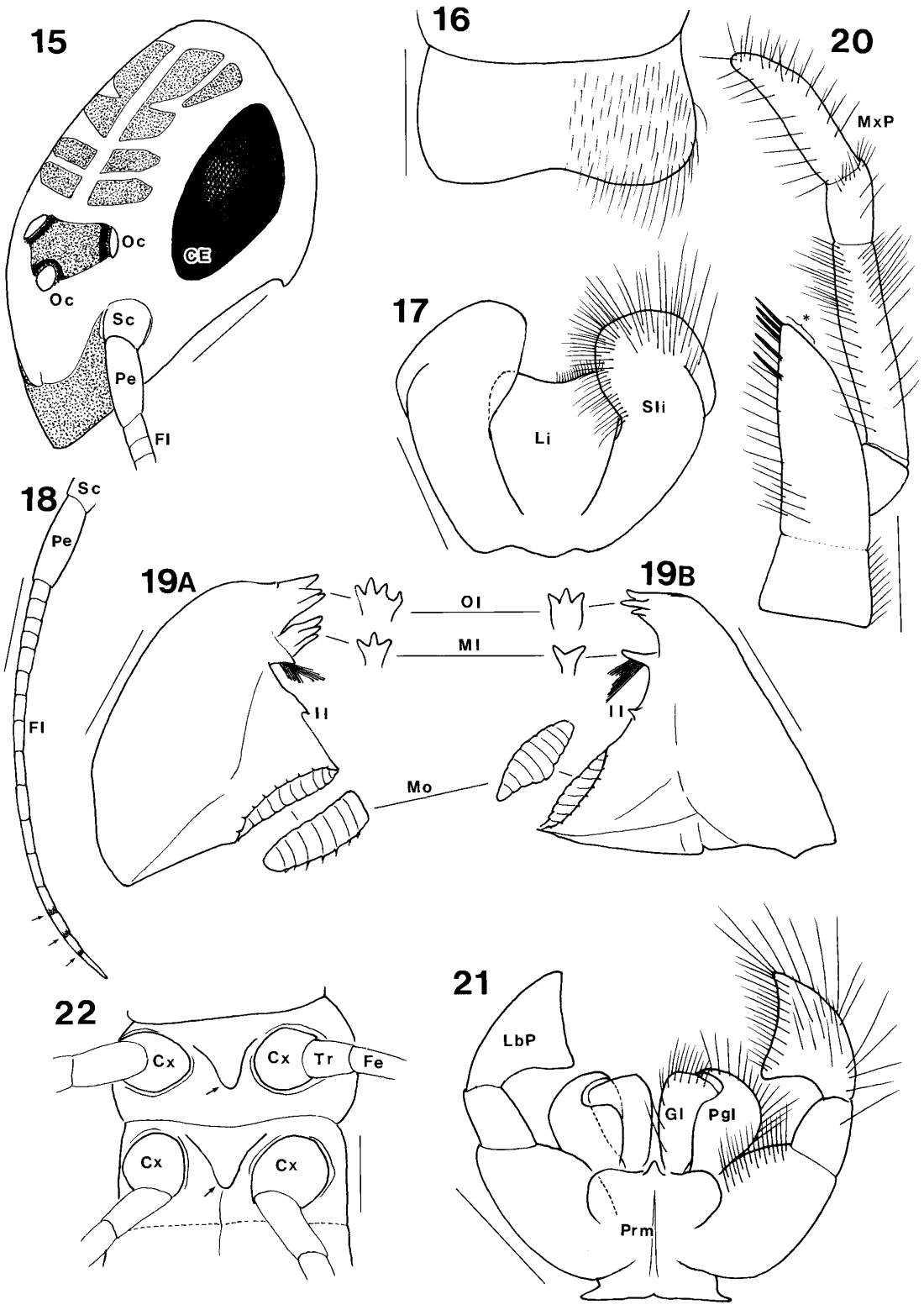
Head: Light yellow with dark brownish lateral stripes, area between three ocelli black; compound eyes black; ocelli translucent, with black basal area (Figs. 4 and 15). Antennae milky white, unicolorous, but three annulates of distal part of flagellum with dark brownish annulated pattern (arrows in Fig. 18); scapus and pedicellus short (scapus : pedicellus ≒ 1 : 3); flagellum conspicuously long, moderately tapered distally, about 4 times as long as scapus + pedicellus (Figs. 4 and 18). Labrum about twice as broad as long, oblong-shaped with slightly dented anterocentrally; its frontal surface and anterior margin with dense long hairs (Fig. 16). Hypopharyngeal lingua rectangular-shaped with moderately convex anterolateral margins, anterior margin of the lingua with numerous tiny hairs; superlinguae rounded with dense marginal hairs (Fig. 17). Outer and middle incisor of mandible equal in length, the former somewhat wider than the latter; outer incisor with pointed three (left) or four (right) teeth, middle one with pointed two (left) or three (right) teeth and brush-like hair bundle; inner incisor minute; molar part of mandible with especially long and laterally denticulate chitinous projections (Fig. 19). Maxillary endite pointed, undifferentiated into galea and lacinia parts; distal part of maxillary endite without comb-like bristles (asterisk in Fig. 20); maxillary palps four-segmented (Fig. 20). Paraglossa, and anterior and medial margins of glossa with numerous hairs; labial palps strong, three-segmented, with numerous hairs (Fig. 21). *Thorax*: Pronotum brown, about 5 times as broad as long. Mesonotum yellowish brown with darker smudges, with black wing pads (wing buds). Metanotum yellowish brown, with minute black wing pads (Fig. 4). Thoracic sterna milky white; meso- and metasterna centrally with upheaval structures (arrows in Fig. 22). Legs light yellow, unicolorous, covered with sparse short hairs (Fig. 4). Claws on pretarsi slender, as long as 1/3 of tarsi, without denticles. *Abdomen*: Penes light yellow; 1st to 9th abdominal terga with paired laterally brownish bands and paired dark brown cylindric spots, moderately spread out posteriorly; posteromedian region of the 1st to 8th abdominal terga with fan-shaped dark brownish pattern, and median region of the 6th to 9th abdominal terga with narrow brownish longitudinal line (Fig. 4). Abdominal sterna uniformly milky white, unicolorous without any markings. The 1st to 7th abdominal segments with a pair of leaf-like gill plates; the 1st pair of those somewhat smaller than those of other segments (Fig. 4). Cerci long, about half length of body, light yellow with white tip, furnished with rows of hairs only on inner (medial) side (Fig. 4); caudal filament as long as cerci, light yellow with white tip, fernished with rows of long hairs on lateral sides (Fig. 4).

Egg (Figs. 23–26)

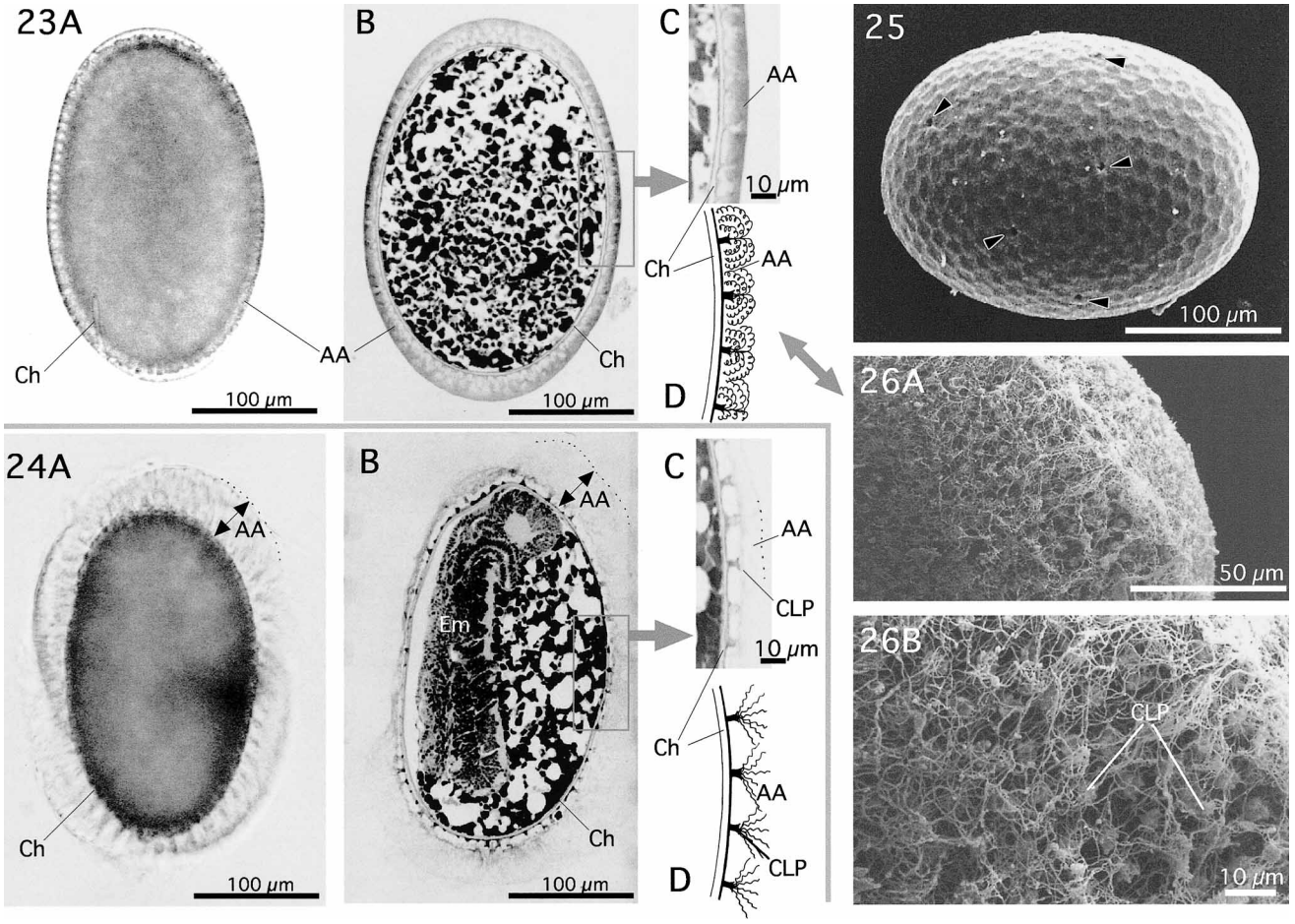
Egg pale yellowish translucent, ellipsoidal about 240 μm in length and about 160 μm in width; chorion about 3 μm in thickness (Fig. 23A, B; cf. Fig. 25). Egg with unique, specialized attachment apparatus consisting of chimney-like



Figs. 5–11, 13. *Dipteromimus flavipterus* sp. nov. **Figs. 12, 14.** *Dipteromimus tipuliformis*. 5: Dorsal view of male mesonotum. 6: Ventrolateral view of male pro- and mesosternum. 7: Male left antenna. 8: Male (A) and female (B) left fore legs. 9: Male left middle leg (A), and enlargement of tarsus and pretarsus part (B). 10: Male left hind leg. 11, 12: Lateral view of posterior part of female abdomen (A), and ventral view of 9th abdominal sternum (B). 13, 14: Ventral view of male genitalia. AbdS7-10, 7th to 10th abdominal segments; BS1-2, basisterna of pro- and mesothorax; Ce, cercus; CF, caudal filament; Cx, coxa; Fc, forceps; Fe, femur; Fl, flagellum; FS2, furcasternum of mesothorax; MLS, median longitudinal sutures; MNS, mesonotal suture; Pe, pedicellus; Pn, penis lobes; Pta, pretarsus; Sc, scapus; T1-5, 1st to 5th tarsomeres; Ti, tibia; Tr, trochanter. Scales=1 mm.



Figs. 15–22. *Dipteromimus flavipterus* sp. nov., mature (ultimate instar) nymph. 15: Anterolateral view of head capsule. 16: Dorsal view of labrum. 17: Ventral view of hypopharynx. 18: Lateral view of left antenna. 19: Ventral view of right (A) and left (B) mandibles. 20: Ventral view of maxilla. Bristles are lacking at the distal area of endite (asterisk). 21: Vental view of labium. 22: Ventral view of meso- and metasternum. Arrows show upheaval structures, which are ones of diagnostic characters of dipteromimid mayflies (cf. Ishiwata and Kobayashi, 2003). CE, compound eye; Cx, coxa; Fe, femur; Fl, flagellum; Gl, glossa; Il, inner incisor; LbP, labial palp; Li, lingua; Ml, middle incisor; Mo, molar; MxP, maxillary palp; Oc, ocellus; Ol, outer incisor; Pe, pedicellus; Pgl, paraglossa; Prm, prementum; Sc, scapus; Sli, superlingua; Tr, trochanter. Scales=0.5 mm.



Figs. 23–26. *Dipteromimus flavipterus* sp. nov., egg. 23: A newly laid living egg (A) and a section (B). Enlargement of the circumference of chorion of B (C), and diagrammatic representation (D). 24: A living egg about 5-hr-old (A), and a section about 10-day-old (B). Enlargement of circumerence of chorion of B (C), and diagrammatic representation (D). 25: SEM of a newly laid egg, without attachment apparatus. Attachment apparatus or adhesive layer completely removed to show the chorion and micropylar openings (arrowheads). 26: SEM of a newly laid egg, with the unique, specialized attachment apparatus (A), and an enlargement, showing the densely coiled fine filamentous threads (B). AA, attachment apparatus; Ch, chorion; CLP, chimney-like projection. Scales are shown in the respective figures.

projections and densely coiled fine filamentous thread from the projections (Figs. 23B–D, 26A, B; at some hr after oviposition, the fine thread-like filaments spread in all directions [Fig. 24A–D], and then, the egg may attach to materials on the riverbed). Chorion with some micropyles (arrowheads in Fig. 25).

Etymology

The prefix "flavi" of the name *flavipterus* is derived from Latin "flavus (=yellow)", derived from one of the diagnostic characters of this mayfly, the ending "pterus" is from Latin "ptera (=wing)".

Distribution

This mayfly is collected from riverhead areas, in some side pools, of a small brook "Sakamoto-zawa" (39°47'40" N, 141°38'18" E, about 350 m above sea level [alt.]) and Takou-gawa River (39°10'04" N, 141°43'20" E, about 500 m alt.) (branches of Sakari-gawa River), Ofunato, Iwate Prefecture. In these localities, ultimate nymphs are found in summer (from June to August). We have no other information on distribution at present.

Type series and other materials examined

The specimens of type series: holotype (male), allotype (female) and a paratype (nymph), are preserved with 70% ethanol and deposited in the collections of the Natural History Museum and Institute, Chiba (CMB-ZI).

HOLOTYPE: One male adult (imago); Sakamoto-zawa (a branch of Sakari-gawa River), Ofunato, Iwate Prefecture, Honshu, Japan; 13-VI-2002 (emergence at 14-VII-2002 and molt to adult at 15-VII-2002 in laboratory; fixation at 15-VII-2002); K.Tojo, K. Matsukawa and T. Tsutsumi leg. (CMB-ZI 94276). ***ALLOTYPE***: One female adult (imago) with eggs; same data as holotype for locality and collectors; 13-VI-2002 (emergence and molt to adult at 28-VII-2002 in laboratory; fixation at 29-VII-2002). (CMB-ZI 94277). ***PARATYPE***: One ultimate instar nymph; same data as holotype for locality and collectors; 13-VI-2002 (fixation at 13-VI-2002). (CMB-ZI 94278). ***OTHER MATERIALS***: one male,

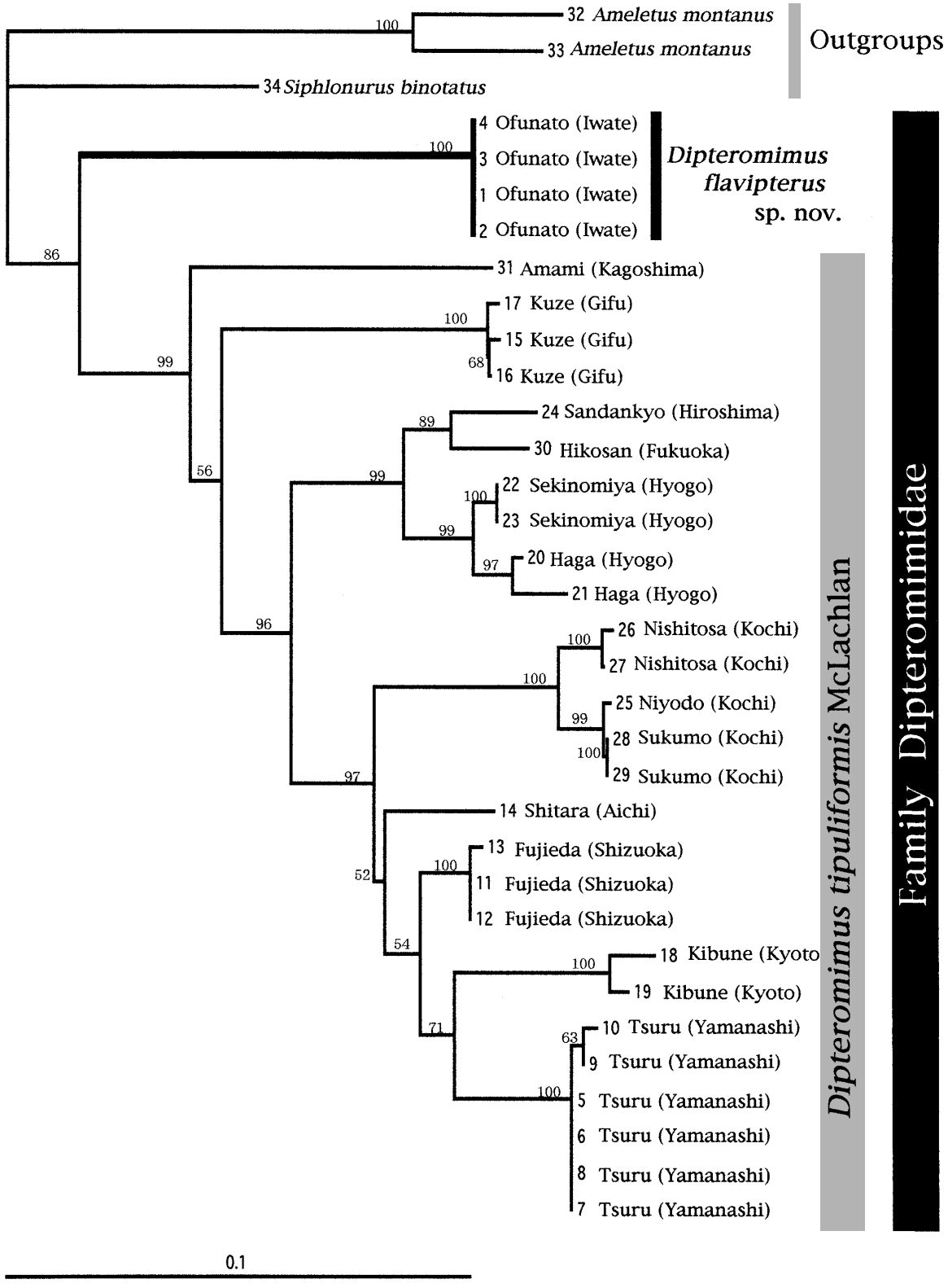


Fig. 27. Neighbor-joining dendrogram (mitochondrial 16S rRNA) of 31 specimens from 14 populations of dipteromimid species (Dipteromimidae), *Dipteromimus flavipterus* sp. nov. (sample no. 1–4) and *D. tipuliformis* (no. 5–31), based on Kimura's (1980) genetic distance matrix, with two related mayflies *Ameletus montanus* (Ameletidae; no. 32, 33) and *Siphlonurus binotatus* (Siphlonuridae; no. 34) as outgroups. Bootstrap values for 1,000 replicates are indicated at major nodes. Information or sample localities and populations information are given in Table 1 and Fig. 1.

six females and seven nymphs (same data as holotype for locality and collectors) are preserved with 70% ethanol and deposited in the personal collection of the senior author (KT).

Keys to the species of dipteromimid mayflies

Keys to the species of dipteromimid mayflies are as follows. For eggs, however, we have not found any characters to discriminate between *Dipteromimus flavipterus* sp. nov. and *D. tipuliformis*.

Keys to the species for males

1. Yellowish body, frontal border of fore wings yellowish transparent (Fig. 2A, B). Penis lobes short, about 1/4 the length of genital forceps (Fig. 13). Caudal filament a little shorter than cerci (Fig. 2A). Cerci and caudal filament dark brownish with white tip (Fig. 2A)..... *D. flavipterus* sp. nov.
2. Creamly white body, non-yellowish (colorless transparent) frontal margin of fore wings. Penis lobes long, about 1/3 the length of genital forceps (Fig. 14). Caudal filament as long as cerci. Cerci and caudal filament uniformly dark brownish. ..... *D. tipuliformis*

Keys to the species for females

1. Yellowish body, with broadly light brownish abdominal turgum. Frontal margin of fore wings yellowish transparent (Fig. 3A, B). Backward elongation of 7th abdominal sternum shorter than half length of 8th abdominal sternum (Fig. 11A). Backward elongation of 9th abdominal sternum rounded (Fig. 11A, B). Caudal filament a little shorter than cerci (Fig. 2B). Cerci and caudal filament dark brownish with white tip (Fig. 2B)..... *D. flavipterus* sp. nov.
2. Creamly white body, abdominal turgum with paired dark brownish lines on each segment. Frontal border of fore wings colorless transparent, without yellowish frontal border. Backward elongation of 7th abdominal sternum longer than half length of 8th abdominal segment (Fig. 12A). Backward elongation of 9th abdominal sternum pointed (Fig. 12A, B). Caudal filament as long as cerci. Cerci and caudal filament uniformly dark brownish ..... *D. tipuliformis*

A key to the species for nymphs

1. With brownish median narrow lines on the 6th to 9th abdominal turgum..... *D. flavipterus* sp. nov.
2. Without median lines on the 6th to 9th abdominal turgum. ..... *D. tipuliformis*

DNA analyses by sequencing of the mitochondrial 16S rRNA region

Four individuals of *Dipteromimus flavipterus* sp. nov. from a small brook "Sakamoto-zawa", Ofunato (Iwate Prefecture), and 27 individuals from 13 localities of *D. tipuliformis* were used for examining genetic relationships, with the related mayflies *Ameletus montanus* (family Ameletidae) and *Siphlonurus binotatus* (family Siphlonuridae) as outgroups (Table 1, cf. Fig. 1).

The neighbor-joining (NJ) dendrogram derived from Kimura's (1980) distance matrix from aligned sequences is shown in Fig. 27. The monophyly of the dipteromimid mayflies (family Dipteromimidae) could be strongly supported (bootstrap proportion [BP]=86%). As for the ingroup, although *D. tipuliformis* has high intraspecific variations and some local populations are distinguished on the basis of genetic differentiation (the subclusters were well separated geographically), the monophyly of *Dipteromimus tipliformis* was also strongly supported (BP=99%). That is, the ingroup portion of this dendrogram was divided into two major clusters: *D. flavipterus* sp. nov. from Ofunato and a monophyletic cluster of *D. tipliformis* from Honshu, Shikoku and Kyushu Islands, and Amami-oshima Island.

DISCUSSION

A second species of dipteromimid mayfly was collected from Ofunato (Iwate Prefecture), and described in detail, under the name *Dipteromimus flavipterus* sp. nov. This is the first discovery of a dipteromimid mayfly from the Tohoku District. The species described in this paper, is characterized as belonging to the dipteromimid mayfly group based on the following morphological points. For adults, extremely degenerated hind wings (Figs. 2B and 3B); comparatively well developed legs (Figs. 2A, B, 3A, B); pretarsus of legs with paired claws of the same shape (Figs. 8–10); well developed caudal filament (Figs. 2A and 3A); line of mesonotal suture (MNS in Fig. 5) and that of median longitudinal suture (MLS in Fig. 5) cross perpendicularly (Kluge *et al.*, 1995); upheaval postal margin of basisternum (BS2 in Fig. 6); simple penis lobes (Fig. 13; cf. Fig. 14), female 7th abdominal sternum elongated posteriorly (Fig. 11A; cf. Fig. 12A)(Ishiwata, 2001; Ishiwata and Kobayashi, 2003), etc. For nymphs, maxilla with small pointed maxillary endite (galea-lacinia) of which distal part is without comb-like bristles (asterisk in Fig. 20), and with developed palp (Uéno, 1931; Kluge *et al.*, 1995); upheaval structures on median parts of thoracic meso- and metasternum (arrows in Fig. 22)(Ishiwata, 2001); a pair of single leaf-like gill plates on the lateral sides of the 1st to 7th abdominal segments (Fig. 4), etc. Eggs of the newly described mayfly with unique specialized attachment structures closely resemble those of a dipteromimid mayfly *Dipteromimus tipuliformis*, and the egg structures are hardly distinguishable. Consequently, it is proper that the new mayfly belongs to the genus *Dipteromimus*, and family Dipteromimidae.

On the other hand, in the dipteromimid ingroup, *D. flavipterus* sp. nov. is characterized by many characters as mentioned above (see description in the paragragh of "Description" or "Keys to the species of dipteromimid mayflies"), in comparison with *D. tipuliformis* which is the only one described species in the group .

In addition to the morphology, in the analyses of genetic relationships of dipteromimid mayflies inferred from the partially sequenced mitochondrial 16S rRNA genes, it was also

supported that *D. flavipterus* sp. nov. is a sister group of *D. tipuliformis*. That is, the phenetic and molecular phylogenetic analyses agreed in terms of clustering.

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