

# A NEW SPECIES OF *SYMBIOCLADIUS* KIEFFER, 1925 (DIPTERA: CHIRONOMIDAE: ORTHOCLADIINAE) FROM THE EASTERN PALAEARCTIC

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## ABSTRACT

The adult male, pupa, and fourth instar larva of *Symbiocladius* (s. s.) *villosus* Makarchenko et Makarchenko, sp. n. from the Russian Far East and Japan are described and figured. Adult male of a new species separated from all known species of subgenus by presence of a pair rod-shaped chitinized structures which situated above apical parts of phallapodemes, and is closely related to the Palearctic species *S.* (s. s.) *rhithrogenae* and the Nearctic *S.* (s. s.) *chattahoocheensis*. *S.* (s. s.) *villosus* sp. n. can be distinguished from the first species by shape of gonostylus which expands from the base to the middle and with longer and more numerous setae. From *S.* (s. s.) *chattahoocheensis* a new species can be separated by roundish-triangular dorsal part of inferior volsellae and presence of 2 megasetae on gonostylus.

Keywords: Diptera, Chironomidae, *Symbiocladius*, new species, East Asia.

## Introduction

Prior to the present study, the genus *Symbiocladius* Kieffer, 1925 included 3 species in the subgenus *Symbiocladius sensu stricto* – *S.* (S.) *chattahoocheensis* Caldwell (U.S.A.), *S.* (S.) *equitans* (Claassen) (U.S.A., Canada), and *S.* (S.) *rhithrogenae* (Zavřel) (Palearctic region) – and 3 species in the subgenus *Acletius* Roback – *S.* (A.) *aurifodinae* Hynes (Australia), *S.* (A.) *renatae* Spies (Argentina, Chile), and *S.* (A.) *wygodzinskyi* Roback (Argentina, Chile) (Ashe and O'Connor 2012). The larvae of species in this genus occur ectoparasitically on mayfly nymphs, where they live, feed and pupate between or under the wing pads.

The type species, *S.* (S.) *rhithrogenae*, was thought to be distributed all across the Palearctic region, from mainland Spain and France through central and eastern Europe (Sæther and Spies 2013) to the Russian Far East (Makarchenko and Makarchenko 2006) and Japan (Hayashi and Kobayashi 2000). However, a comparison of European material with East Asian specimens has indicated to us that in the Far East there is a previously undetected species that is closely related to but different from the other three members of *Symbiocladius* s. s.

Below we provide a description of the adult male, pupa, and fourth instar larva of *Symbiocladius* (s. s.) *villosus* sp. n. from the Russian Far East and Japan.

## Materials and Methods

Most of the material was collected in Primorye and Khabarovsk Territories, Amur and Magadan regions and

on Sakhalin Island of the Russian Far East. Rearing of adult males from the larva and pupa, and determination of mayfly nymphs inhabited by *S.* (s. s.) *villosus* sp. n., was carried out by Dr. T. M. Tiunova. Material of *S.* (s. s.) *villosus* sp. n. from Japan was collected and reared by Dr. T. Ueno, and made available to us by Dr. T. Kobayashi. For comparison with European material we used specimens of *S.* (s. s.) *rhithrogenae* (mature pupae and larval skins) collected with mayflies in the Chornohora Mountains of Ukraine by M. Kłonowska-Olejnik and R. J. Godunko; this material was used previously in the paper by Giřka et al. (2007).

The associations between larva, pupa and adult male of *S.* (s. s.) *villosus* sp. n. were obtained by individual rearing of live larvae to mature pupae in their natural habitats, and of pupae to adults. For some populations we used pharate adult males extracted from their pupal exuviae.

Larvae and pupae were collected from nymphs belonging to 9 host species of Ephemeroptera, family Heptageniidae – *Cinygma lyriformis* McDunnough, *Ecdyonurus aspersus* Kluge, *Ec. joernensis* Bengtsson, *Epeorus ninae* Kluge, *Ep. pellucidus* (Brodsky), *Heptagenia flava* (Rostock), *Rhithrogena japonica* Ueno, *Rh. lepnevae* Brodsky, and *Rh. sibirica* Brodsky (Table 1).

The material was fixed in 70% ethanol and later mounted on slides, following the procedure outlined by Makarchenko (1985). The morphological terminology follows that of Sæther (1980).

The holotype and paratypes of the new species are deposited in the Institute of Biology and Soil Sciences, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (IBSS FEB RAS).

**Table 1** Mayfly hosts of *Symbiocladius* (s. s.) *villosus* sp. n. in Far Eastern regions.

Host species	Locality				
	Sakhalin Region	Primorye Territory	Khabarovsk Territory	Magadan Region	Japan, Honshu
<i>Cinygma lyriformis</i> McDunnough 1933	+	–	–	–	–
<i>Ecdyonurus aspersus</i> Kluge 1980	–	+	–	–	–
<i>Ec. joernensis</i> Bengtsson 1909	–	+	+	–	–
<i>Epeorus ninae</i> Kluge 1995	+	–	–	–	–
<i>Ep. pellucidus</i> (Brodsky 1930)	+	+	+	–	–
<i>Heptagenia flava</i> (Rostock 1878)	–	+	–	–	–
<i>Rhithrogena japonica</i> Ueno 1928	–	–	–	–	+
<i>Rh. lepnevae</i> Brodsky 1930	–	+	+	–	–
<i>Rh. sibirica</i> Brodsky 1930	–	+	+	+	–

***Symbiocladius villosus* Makarchenko et Makarchenko, sp. n.**

**Etymology:** The species epithet, *villosus* (Latin for hairy, woolly), refers to the pubescence on the gonostylus.

**Material:** Holotype: adult male, reared from larva, Russian Far East, Primorye Territory, Bol. Ussurka River, Ussuri River basin, 20.VI. 1990, leg. T. Tiunova.

**Paratypes. RUSSIAN FAR EAST, Primorye Territory:** 7 larvae, Frolovka River, 22.X. 1988, leg. E. Makarchenko; 1 pupal exuvia, 1 larval skin, same data as holotype, 20.VI. 1990, leg. T. Tiunova; 1 pupa with larval skin, 2 larvae, same data as holotype, except 18.VI. 1990, leg. T. Tiunova; 1 pupa with larval skin, Ussuri River, 21.VII. 1991, 1 mature pupa, 20.IX. 1991, 1 male, 1 pupal exuvia, 1.VIII. 1992, leg. T. Tiunova; 2 larvae, Bikin River, 1.VIII. 1995, leg. T. Tiunova; 1 male, Samarga River, 5.VIII. 2005. **Khabarovsk Territory:** 3 males, Bureya River, 4.VII. 1994, leg. E. Makarchenko; 4 pupae, 4 larvae, Khoidur River, 31.VII. 1996, leg. T. Tiunova; 3 males, same data except 17.VII. 2003, leg. V. Teslenko; 1 male, Niman River, 15.VII., 2003, leg. T. Tiunova; 1 male with pupal exuvia and larval skin, 1 female with pupal exuvia, 1 pupa with larval skin. Gur River, 1.VIII. 1996, leg. T. Tiunova; 1 pupa, 3 larvae, Okhota River, 6–8.VIII. 1988, leg. T. Tiunova; 1 male with pupal exuvia, Maya River, about 1 km below Nel'kan Village, 5–8.VIII. 1999, leg. T. Tiunova; 2 males, Anyui River, 28.VII. 1996, leg. T. Arefina. **Amur Region:** 1 pupa, Amur River, 28–29.VIII. 2004, leg. T. Tiunova. **Magadan Region:** 2 males, Chelomdzha River, 5.VIII. 2001, 1 male, 3.IX. 2009 leg. S. Kocharina; 1 male, same data, 5.VIII. 2002, leg. E. Khamenkova; 2 larvae, Ola River, 21.VII. 2013, 1 male, 4.VIII. 2013, leg. E. Khamenkova. **Sakhalin Island:** 1 male, Liu-

toga River in region of Vysokoie Village, 25–26.VII. 2001, leg. E. Makarchenko; 1 larva, Avgustovka River, 24.VII. 2003, leg. E. Makarchenko; 2 larvae, Leonidovka River, 25.VII. 2002, leg. V. Teslenko; 1 pupa, Uanga River, 20.VIII. 2001. **JAPAN:** 2 males, reared from larvae, 2 pupal exuviae, 2 larval skins, Yagi River, Sekinomiya, Yofu City, Hyogo Prefecture, 21.V.–27.V. 2007, leg. T. Ueno.

**Adult male** (n = 6)

Total length 3.0–4.5 mm. Wing length 2.08–3.12 mm. Total length/wing length 1.21–1.53. Total coloration brown, wings grayish.

**Head:** Eyes slightly pubescent or bare. Temporal setae (from one side) including only 0–7 postorbitals. Clypeus with 6–13 setae. AR 1.12–2.17. Palps usually with 3 palpomeres, but 2nd and 3rd palpomeres sometimes fused; in the first case lengths ( $\mu\text{m}$ ) of palpomeres 1–3: 48–84, 52–108, 40–100, in the second case lengths ( $\mu\text{m}$ ) of palpomeres 1–2: 68, 116.

**Thorax:** Each anteprenotal lobe with 3–7 lateral setae. Acrostichals absent, dorsocentrals 4–14, prealars 4–7. Scutellum usually without setae, only one male with 3 setae on one side.

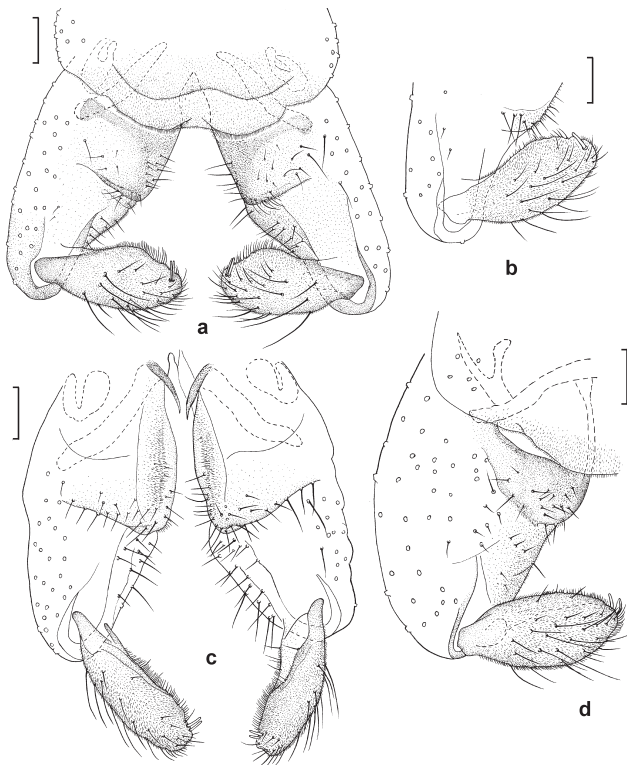
**Wing:** R, R<sub>1</sub> and R<sub>4+5</sub> without setae. Costal extension very short. Anal lobe roundish, protruding. Squama with 25–50 setae. Apex of R<sub>4+5</sub> distal of apex M<sub>3+4</sub>; Cu1 straight.

**Legs:** BR<sub>1</sub> 1.7–2.0, BR<sub>2</sub> 3.4–3.9, BR<sub>3</sub> 3.0–4.25. Front tibia with 1 spur 44–48  $\mu\text{m}$  long. Middle tibia with 1–2 spurs 36–40  $\mu\text{m}$  long and 24–36  $\mu\text{m}$  long. Hind tibia with 1–2 spurs 72–80  $\mu\text{m}$  and 28–36  $\mu\text{m}$  long. Hind tibia without comb but with apical group of 12–13 spinules. Lengths and proportions of leg segments as in Table 2.

**Table 2** Lengths (in  $\mu\text{m}$ ) and proportions of leg segments in *Symbiocladius* (s. s.) *villosus* sp. n., male (n = 6).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
<b>P<sub>1</sub></b>	704–976	896–1248	720–944	400–704	320–551	160–184	135–144	0.60–0.76	2.02–2.27	2.26–2.56
<b>P<sub>2</sub></b>	800–1184	848–1264	432–672	320–512	216–336	112–176	112–144	0.46–0.53	2.57–2.71	3.64–3.93
<b>P<sub>3</sub></b>	816–1232	976–1328	464–784	352–528	288–384	128–160	112–144	0.46–0.59	2.66–2.84	3.26–3.86

Hypopygium (Figs. 1a–d): Tergite IX without setae and anal point. Laterosternite IX with 7–11 setae. Transverse sternapodeme reduced. Virga absent but above apical parts of phallapodemes a pair of rod-shaped chitinized structures are situated (Fig. 1c). Gonocoxite 312–380  $\mu\text{m}$  long, with large roundish-triangular dorsal lobe covered by some setae and with ventral rounded protrusion of inner edge in middle part. Gonostylus 140–180  $\mu\text{m}$  long, with numerous setae and, from the base to the middle, with 2 light-colored megasetae 12–24  $\mu\text{m}$  long and about 4  $\mu\text{m}$  wide. From numerous specimens in our material only one male from Bol. Ussurka River had gonostylus with 1 megaseta.



**Fig. 1** Male adult of *Symbiocladius* (s. str.) *villosus* sp. n. a, d, hypopygium in dorsal view; b, gonostylus; c, hypopygium in dorsal view without tergite IX (extracted from mature pupa). Scale bars 50  $\mu\text{m}$ .

#### Pupa (n = 4)

Total length 3.3–4.5 mm.

Cephalothorax: Light brown, with darker spots in the wing sheath area. Frontal apotome smooth, without setae or tubercles. Medial edge of antennal pedicel sheath with a spine. Thoracic horn and precorneals absent. Anteprenotal area with 5 weak setae (Fig. 2c). Dorsocentrals hair-like, 8–20  $\mu\text{m}$  long; distance between Dc1 and Dc<sub>2</sub> 12–60  $\mu\text{m}$ ; distance between Dc2 and Dc3 26–66  $\mu\text{m}$ ; distance between Dc3 and Dc4 12–70  $\mu\text{m}$ .

Abdomen: Tergites light brown. Tergites I–II without shagreen. Tergites III–V with two bands of spinules in posterior part, spinules in the more posterior band with apices directed forward (Fig. 2d). Tergites VI–VIII with one band of spinules in posterior part, their apices pointing rearward; anterior halves of tergites with very weak

and fine shagreen, more intensive on tergite VIII. Segment I with 2–3 pairs of lateral setae. Segments II–VII with 3 pairs of lateral setae situated in middle part of lateral edge. Segment VIII with 1–2 pairs of lateral setae. PSA, PSB and apophyses absent. Anal lobe 224–328  $\mu\text{m}$  long, without fringe and anal macrosetae but with 2–3 pairs of very short setae. Male genital sac overreaching anal lobe by 144–172  $\mu\text{m}$  (Fig. 2e).

#### Fourth instar larva (n=5)

Total length 2.8–4.8 mm.

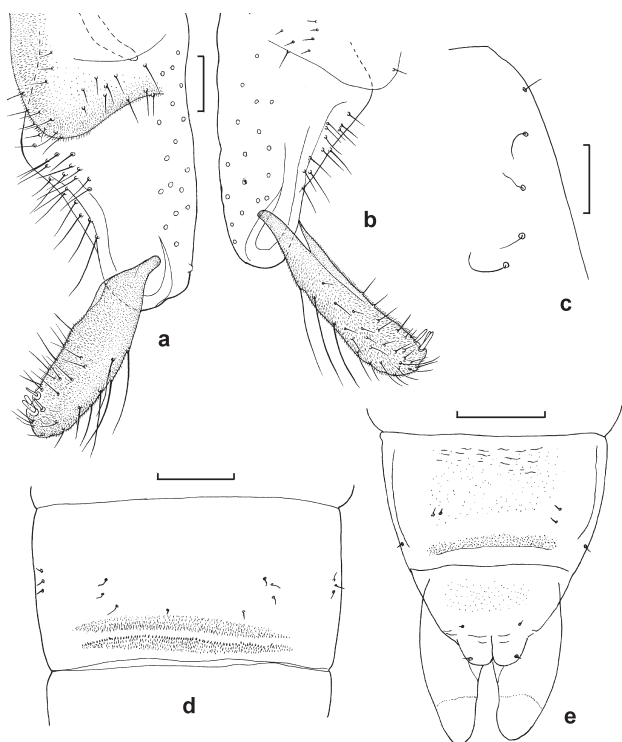
Head: Head capsule 160–300  $\mu\text{m}$  long and 180–250  $\mu\text{m}$  wide; light yellow, postoccipital margin brown, interrupted in two places. Labral setae simple and very weak (Fig. 3a); premandible almost completely reduced. Antenna with 5 segments; total length 15–20  $\mu\text{m}$ ; basal segment length 5.0–6.7  $\mu\text{m}$ ; AR 0.50–0.66; antennal seta shorter than segments 2–5 combined (Fig. 3f). Mandible 38–54  $\mu\text{m}$  long, brownish, with strong apical tooth, 1 large basal inner tooth and 3 thin and spine-like inner teeth, sometimes one spine-like tooth is poorly visible; seta subdentalis and seta interna absent (Figs. 3d,e). Middle part of mentum concave, without median teeth, only with lateral convexities and 4 pairs of lateral teeth which sometimes invisible because situated under ventromental plates; setae submenti bifurcate (Figs. 3b,c).

Abdomen: Procercus absent and 5–7 setae sit on body in as a beam. Supraanal setae 100–120  $\mu\text{m}$  long. Posterior parapods short and separated, on top with a hook arranged in a circle; number of hooks 27–33.

#### Diagnosis

Adult male of *S.* (s. s.) *villosus* sp. n. is separated from all known species of subgenus by presence of a pair rod-shaped chitinized structures which situated above apical parts of phallapodemes (Fig. 1c). A new species is most closely related to the Palearctic species *S.* (s. s.) *rhithrogenae* and the Nearctic *S.* (s. s.) *chattahoocheensis* and can be distinguished from the first species by shape of gonostylus which expands from the base to the middle and with longer and more numerous setae; in *S.* (s. s.) *rhithrogenae* gonostylus with shorter and not so numerous setae and more straight and narrow, without expansion in the middle part (Figs. 2a,b). Shape of gonostylus of *S.* (s. s.) *chattahoocheensis* almost the same as *S.* (s. s.) *villosus* sp. n. if to compare hypopygium of both species extracted from mature pupae, but shape of inferior volsellae of these species and number of megasetae on gonostylus are different, namely a new species with roundish-triangular dorsal part of inferior volsellae and 2 megasetae, *S.* (s. s.) *chattahoocheensis* with roundish dorsal part of inferior volsellae and 1 megaseta. It should be noted that the transverse sternapodeme in species listed above is reduced. Data on this feature in *S.* (S.) *equitans* absent. The pupa of *S.* (s. s.) *villosus* sp. n. is close related to *S.* (s. s.) *rhithrogenae* and cannot be distinguished from latter. Both these

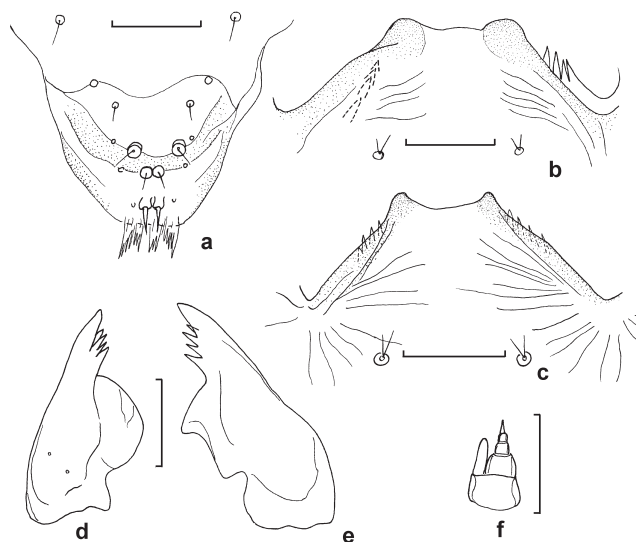
species have tergites III–V with two bands of spinules in posterior part. But they are well differ from pupae of *S. (s. s.) chattahoocheensis* and *S. (s. s.) equitans*, which have two transverse bands of spinules on tergites IV–V (Claassen 1922; Caldwell 1984). To do this carry out additional research of pupae listed species. The larvae of all known species of *Symbiocladius* are not so well studied for a good comparison of their features, but nevertheless larvae of a new species may be separated from those of *S. (s. s.) rhithrogenae* by following characters: length of head capsule is 160–300 µm, width – 180–250 µm; mandible with 3 inner teeth; mentum with 4 pairs of lateral teeth. According to Schmid (1993) and our data larvae of *S. (s. s.) rhithrogenae* with head capsule 275–339 µm long and 284–351 µm wide; mandible with 4 inner teeth; mentum with 5 pairs of lateral teeth.



**Fig. 2** Male adult of *Symbiocladius (s. str.) rhithrogenae* (Zavřel) (a–b), and pupa of *S. (s. str.) villosus* sp. n. (c–e). a–b, gonocoxite and gonostylus; c, setae of anteprenotal area; d, tergite V; e, tergites VIII–IX and anal lobes. Scale bars: Figs. 5–7 – 50 µm; Figs. 8–9 – 200 µm.

**Remarks**

In this paper, we did not plan the revision of all species the *Symbiocladius* s. s. and amend or add of some characters in subgeneric diagnosis, but in the process of studying we noticed a discrepancy between some features of adults, pupae and larvae as a new species and previously known from the data given in the diagnosis of the genus, are published in the “Chironomidae of the Holarctic region” (Cranston et al. 1983, 1989; Coffman et al. 1986; Andersen et al. 2013). Work on the preparation of a diagnosis for subgenera and genus can be done and should be done in the future only after the taxonomic revision of both subgenera of *Symbiocladius*.



**Fig. 3** Fourth instar larva of *Symbiocladius (s. str.) villosus* sp. n. a, labrum; b–c, mentum; d–e, mandible; f, antenna. Scale bars 20 µm.

Some authors interpreted the large basal inner tooth on the larval mandible as a seta subdentalis (e.g., Andersen et al. 2013: 260). However, in *Symbiocladius* the structure in question here does not show anything like the typical base from which true setae always arise. Therefore, we support the position of Caldwell (1984), who did not interpret the large basal inner tooth as a seta subdentalis.



**Fig. 4** Distribution of *Symbiocladius (s. str.) villosus* sp. n. in the Far East. Black circles denote the collecting locations.

**Table 3** Comparison of adult males of *Symbiocladius* (s. s.) *villosus* sp. n. from different regions in the Far East.

Characters	Bol. Ussurka River (n = 3)	Gur River (n = 1)	Rivers: Ljutoga, Bureya Anjui, Niman (n = 4)	Japan (n = 2)
Total length, mm	4.2–4.5	4.1	3.2–4.0	3.0–3.2
Wing length, mm	3.0–3.12	2.52	2.08–2.84	2.44–2.48
AR	1.23–1.38	1.12	1.34–2.17	1.17
Palpomeres 1–3 length, $\mu\text{m}$	76–84 : 80–108 : 96–100	68 : 76 : 76	48–72 : 52–116 : 40–76	68 : 68 : 60
Postorbitals	0–3	6–7	0–3	0
Dorsocentrals	4–14	12	5–7	5–6
Prealars	5–10	7	5–7	4
Scutellars	0	0	0–3	0
LR <sub>1</sub>	0.72–0.76	0.69	0.70–0.71	0.60–0.65
LR <sub>2</sub>	0.49–0.53	0.48	0.49–0.51	0.46–0.50
LR <sub>3</sub>	0.55–0.59	0.54	0.48–0.51	0.46–0.50
Number of spurs on hind tibia	1–2	2	1–2	2
Gonocoxite length, $\mu\text{m}$	364–380	324	244–320	312
Gonostylus length, $\mu\text{m}$	180	160	128–148	140
Number of megasetae	1–2	2	2	2
HR	2.02–2.11	2.0	1.90–2.16	2.22
HV	2.33–2.50	2.56	2.35–2.7	2.14–2.28

Adult males from different regions in the Far East are slightly different from each other. For example, specimens from Bol. Ussurka River have the greatest body lengths (4.2–4.5 mm), whereas the smallest males (3.0–3.2 mm length) were registered from Japan. Variation in other features is shown in Table 3.

#### Distribution and biology

*S.* (s. s.) *villosus* sp.n. is known from many regions of the Russian Far East and from Japan (Fig. 4). All mayfly nymphs from which larvae and pupae of *S.* (s. s.) *villosus* sp. n. were collected belong to the family Heptageniidae (Table 1).

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