

# *Chaetocladius (Amblycladius) britae* Säwedal, 1976 (Diptera, Chironomidae, Orthoclaadiinae) representing a little-known subgenus from the High-North, discovered in Norway and DNA barcoded for the first time

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New records of *Chaetocladius britae* Säwedal, 1976 are presented, representing the first records of the species in Norway. Twenty-four male specimens were caught in a Malaise trap set in a tiny ravine with an underground spring brook with associated tiny pools of standing water connected to the meandering River Nakkelva at Breivikeidet in Troms, northern Norway. The poorly known species was originally described from Lule Lappmark in Sweden, based on a single male only, and has subsequently been recorded only three times in northern Finland. Following recent reviews of the subgenus *Amblycladius* Kieffer, 1923 *Chaetocladius* (s. str.) *britae* is transferred to *Amblycladius* **new combination** mainly due to its large hypopygium and complex gonostylus. The subgenus *Amblycladius* is then represented with five species, four of which are confined to northernmost Europe, and a fifth species, described from Far East Russia, that was recently added to the subgenus. The DNA barcodes of both the species *Chaetocladius (Amblycladius) britae* and the subgenus *Amblycladius* are presented for the first time, and the closest DNA barcode matches of unidentified materials on BOLD indicate that the subgenus may be present also in North America and China.

Key words: Diptera, Chironomidae, Orthoclaadiinae, *Chaetocladius*, *Amblycladius*, new combination, Norway, new country records, DNA barcoding.

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## Introduction

The genus *Chaetocladius* Kieffer, 1911 is a species rich genus of the subfamily Ortho-

cladiinae distributed in the Nearctic, Palearctic, Afrotropical and Oriental regions. Among Holarctic genera, *Chaetocladius* can be recognized on the prominent projecting denticles

on the tibial spurs and the coarse punctuation on the wing membrane. According to Andersen *et al.* (2013) the larvae of *Chaetocladius* are found in wet leaves, among plants, and in mud in springs, wells, streams, ditches, ponds and permanent or temporary pools. Most species can be characterized as semi-aquatic, but a few are truly aquatic.

Two subgenera are recognized, *Chaetocladius* s. str. and *Amblycladius* Kieffer, 1923 (Ash & O'Connor 2012). Kieffer (1923) erected *Amblycladius* as a separate genus based on *A. subplumosus* Kieffer, 1923 from Novaya Zemlya due to its reduced pulvilli and the unique structure of the gonostylus. Later *Amblycladius* was placed as a subgenus of *Chaetocladius* (see Cranston *et al.* 1989). The genus *Arctosmittia* Zelentsov, 2006 was described based on *A. biserorovi* Zelentsov, 2006 from Novaya Zemlya (Zelentsov 2006). However, the species was placed as a synonym of *C. (A.) subplumosus* by Krasheninnikov & Prizhiboro (2022). A second species of *Amblycladius*, *C. (A.) franzjosephiensis* Krasheninnikov in Krasheninnikov & Gavrilo, 2013 was described from Franz Josef Land by Krasheninnikov & Gavrilo (2013). Recently Makarchenko & Yavorskaya (2023) transferred a third species, *C. (s. str.) amurensis* Makarchenko & Makarchenko, 2006 described by Makarchenko & Makarchenko (2006) from the Amur River basin in the Russian Far East to *Chaetocladius* (*Amblycladius*).

Below we transfer *Chaetocladius* (s. str.) *britae* Säwedel, 1976 to *Chaetocladius* (*Amblycladius*) **new combination**. The species was described based on a single male from Messaure in Swedish Lapland by Säwedel (1976). Subsequently, the species has been recorded three times from northernmost Finland (Tuiskunen & Lindeberg 1986, Paasivirta 2014, Finnish Biodiversity Information Facility 2024), but has hitherto never been recorded from Norway. Below the male is redescribed and illustrated based on the new material from northern Norway and the DNA barcodes of three males are presented.

### Study area, material and methods

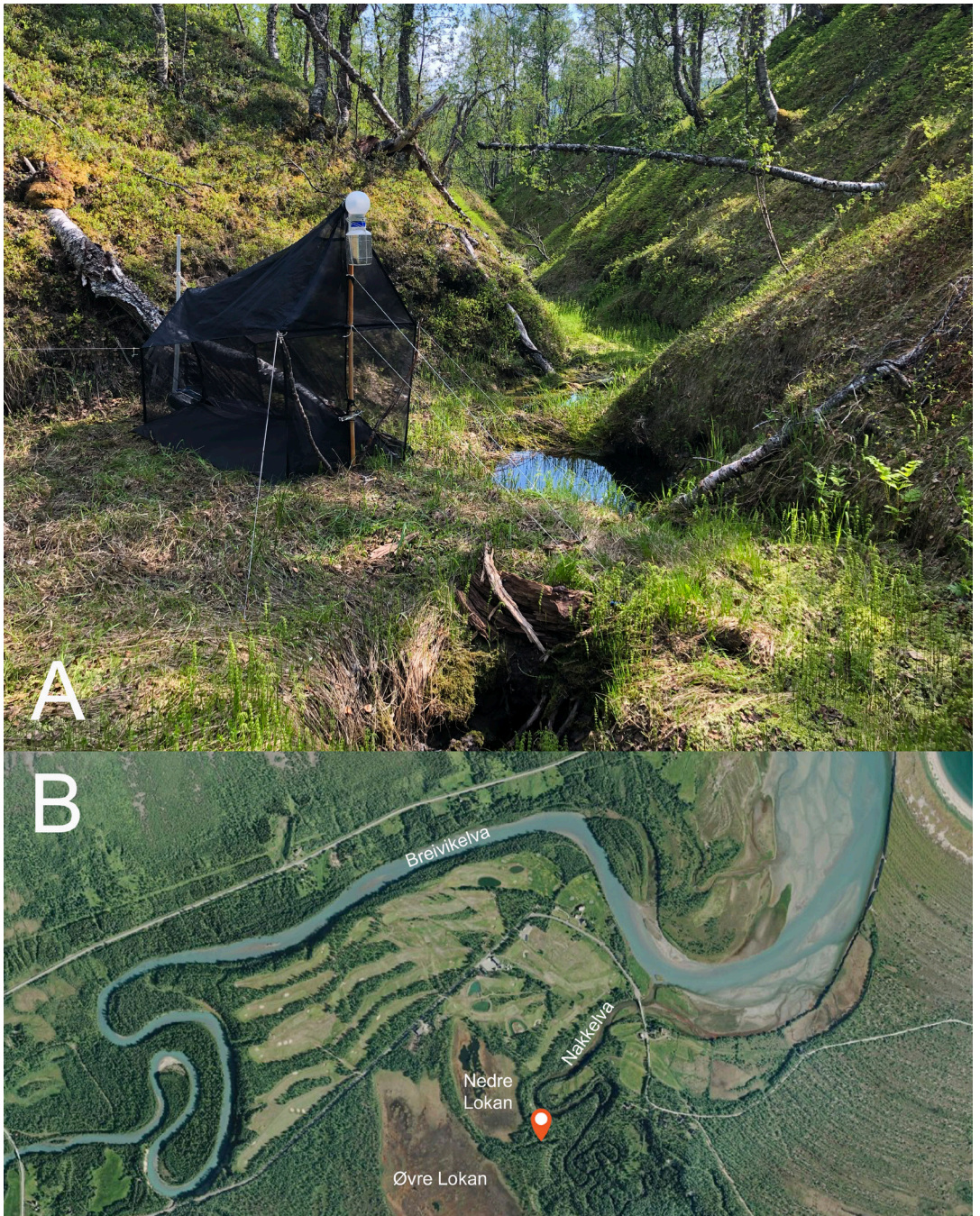
The specimens studied were collected in a

Malaise trap placed in a tiny ravine at Breivikeidet in Troms, Norway, 69.6528261°N 19.6158729°E (Figure 1A). The trap was placed directly over a cold, largely underground running spring brook with associated tiny pools of standing water. The underground spring brook is draining water from two large bogs at Øvre and Nedre Lokan, and connects downstream to second order meanders of River Nakkelva that is running into the larger River Breivikelva just before the river mouth (Figure 1B). The forest surrounding the ravine is dominated by birch (*Betula pubescens*) with some *Salix* spp. along the mires and grey alder (*Alnus incana*) along the riverbed further downstream. The ground in the area is dominated by postglacial marine sediments where a rich network of meanders has dug into older sediments (Evison 2012) creating a characteristic landscape with high diversity of soil types (Arnesen & Sommersel 2013). The marine sediments are dominated by clay that affects the drainage from the surrounding mountains by channeling water into springs and several cold springs are found on the south side of Breivikeidet (Arnesen & Sommersel 2013).

The studied material was preserved in 80% ethanol. Five males were mounted on slides in Euparal following the procedure outlined by Sæther (1969). The morphological nomenclature follows Sæther (1980). Measurements are given as ranges followed by the mean.

A Leica K5C microscope camera mounted on a Leica M205C stereomicroscope was used to capture images of the whole insect, the wing and detached terminalia. Stacked images were processed by use of Helicon Focus software and edited into a collage image by use of Adobe Photoshop. Enhanced focus was obtained by Topaz Sharpen AI (Artificial Intelligence) software. The male terminalia of a representative specimen were detached, cleared in hot lactic acid by short pulse-heating in a microwave oven, before being transferred to glycerol in excavated slides for microscope imaging. The dissection of the terminalia for imaging of details of its parts is partly a destructive procedure resulting in fragmented specimens, but all parts are preserved and stored in glycerol in sealed microtubes together with the rest of the specimen.





**FIGURE 1.** **A.** The Malaise trap was placed in a tiny ravine at Breivikeidet in Troms, Norway, photographed on June 20, 2023, the day the trap was set up. Note the pool next to the trap which is connected to an underground spring brook. Photo: J. Kjærandsen. **B.** Aerial photo of Breivikeidet [© Kartverket] showing the two large bogs at Øvre and Nedre Lokan connected via the underground spring brook in the ravine downstream to second order meanders of the river Nakkkelva that is running into the larger river Breivikelva just before the river mouth. The mark shows the exact collecting site at 69.6528261°N 19.6158729°E.



The thorax of three males were used for non-destructive DNA extraction carried out at the Biodiversitetslaboratorium (BDL), University Museum of Bergen. The resulting DNA barcodes are uploaded to BOLD.

## Results

### Taxonomy

*Chaetocladus britae* was described by Säwedal (1976) from northern Sweden and the species is placed in subgenus *Chaetocladus* (s. str.) (see Ashe & O'Connor 2012). However, as pointed out by Makarchenko & Yavorskaya (2023) the species should be placed in subgenus *Amblycladius* as it has hypopygial structures typical for representatives of that subgenus. According to Cranston et al. (1989) *Amblycladius* differs from other *Chaetocladus* species in the following characters: gonostylus double, with several lobes; without crista dorsalis and megaseta; inferior volsella reduced; virga very weakly sclerotized or possible absent. Krasheninnikov & Prizhiboro (2022) gave an updated, extended diagnosis for the subgenus, stating e.g. that the megaseta is well developed.

Säwedal (1976) described *C. britae* based on a single male. The original description is rather short, and several important characters are not mentioned. Below *C. britae* is placed in the subgenus *Amblycladius* and the male is redescribed based on five specimens from Breivikeidet. Measurements or counts given by Säwedal (1976) are placed in brackets.

### *Chaetocladus (Amblypygus) britae* Säwedal, 1976 (Figures 2, 3)

**Material studied.** NORWAY: Troms (TRY), Nedre Lokan, Breivikeidet, 69.6528261°N 19.6158729°E, 24♂♂, 27 August–26 November 2023, Malaise trap, leg. J. Kjærandsen, (TMU-JKJ-COL-002219). Three of the males (TSZD-JKJ-118409, TSZD-JKJ-118410, TSZD-JKJ-118411) were DNA barcoded and then slide mounted and deposited in the entomological collections at the University Museum of Bergen. A fourth male (TSZD-JKJ-116242) was

submitted to BOLD (results pending), kept in 80% ethanol with the rest of the materials (TSZD-JKJ-118415–118432), and these are deposited in the entomological collections at the Tromsø University Museum.

**Redescription. Adult male** (n = 4–5). Total length 3.46–3.99, 3.73 mm. Wing length 2.14–2.72, 2.41 [2.45] mm. Total length / wing length 1.51–1.73, 1.59. Wing length / length of profemur 2.00–2.49, 2.20.

**Colouration.** Dark brown to blackish brown (Figure 2A, B). Wing hyaline (Figure 2C).

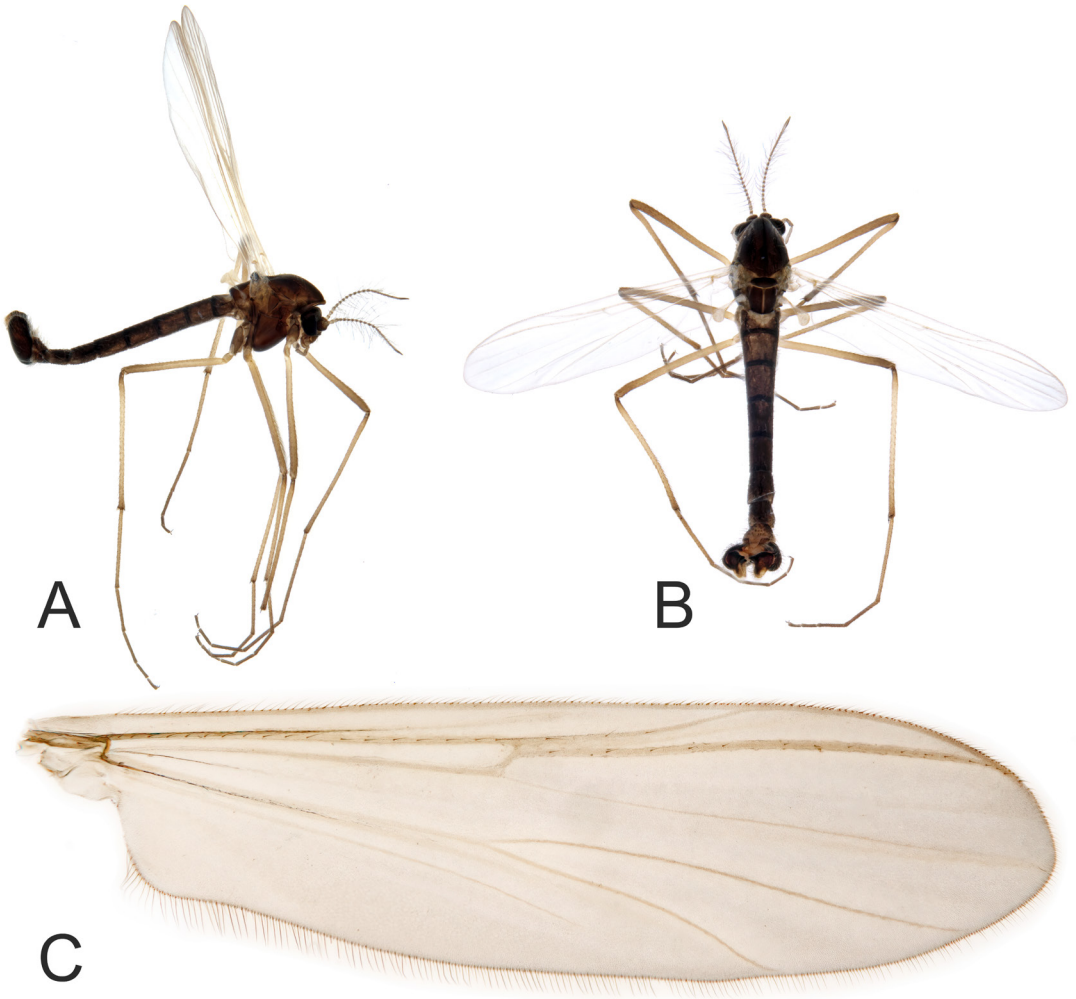
**Antenna.** With 13 flagellomeres, plume reduced. Antennal Ratio (AR) = 0.68–0.85, 0.76 [0.75]. Terminal flagellomere slightly clubbed, 328–460, 397 µm long, with 46–58, 51 mm long subapical seta. Groove starts on flagellomere 2; with sensilla chaetica on flagellomeres 2–5 and 13.

**Head.** Eye faintly pubescent. Temporal setae 13–16, 15 [16], consisting of 6–8, 7 inner verticals, in double row, 3–6, 5 outer verticals in single to double row, and 3–4, 4 postorbitals in single row. Clypeus with 10–13, 11 [10] setae. Tentorium 145–182, 160 µm long, 22–30, 26 µm wide. Stipes 172–193, 180 µm long, 12–17, 14 µm wide. Palpomere lengths (in µm): 37–49, 43; 57–72, 63 [57]; 133–170, 148 [131]; 96–115, 108 [95]; 164–191, 176 [186]. Third palpomere with 7–9, 8 sensilla clavata subapically, longest about 20 µm long.

**Thorax.** Anteprenotum with 6–8, 7 ventrolateral setae. Acrostichals 11–13, 12, weak, beginning close to anteprenotum; dorsocentrals 9–13, 12 [10] in single row; prealars 3–4, 4 [3]. Scutellum with 5–7, 6 [9] setae in single row.

**Wing** (Figure 2C). Wing Ratio (VR) = 0.89–0.97, 0.95. Costal extension 97–117, 108 µm long. Brachiolium with 1 [1] seta; R with 15–20, 17 [16]; R<sub>1</sub> with 3–5, 4 [5]; R<sub>4+5</sub> with 12–15, 14 [15] setae; other veins and membrane bare. Anal lobe well developed, membrane with coarse punctuation. Squama with 7–10, 9 [8] setae.

**Legs.** Spur of fore tibia 52–61, 56 µm long; spurs of mid tibia 26–33, 29 µm and 35–39, 36 µm long; spurs of hind tibia 28–39, 32 µm and 50–67, 59 µm long. Width at apex of fore tibia 48–61, 52 µm; of mid tibia 46–55, 51 µm; of

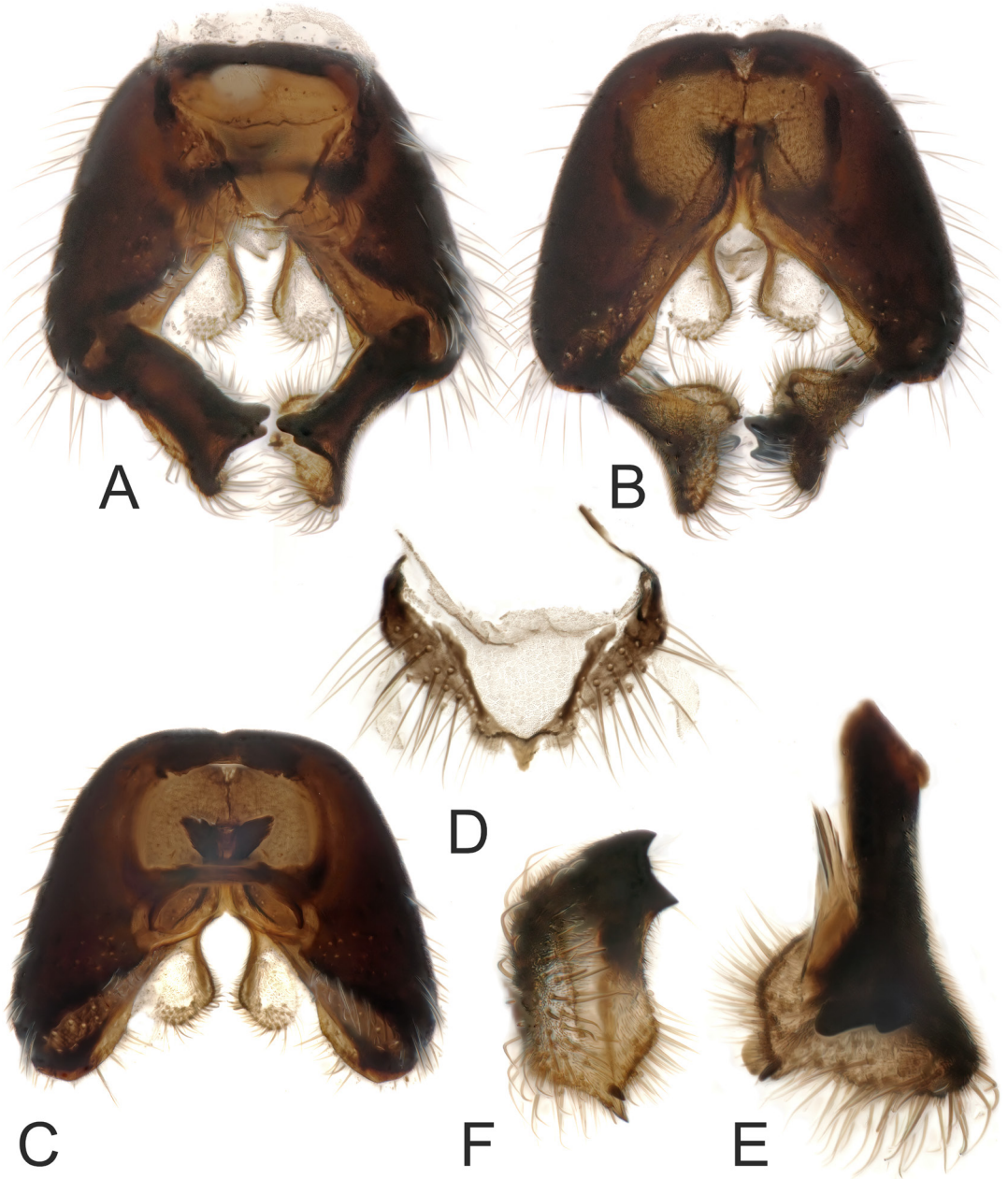


**FIGURE 2.** *Chaetocladius (Amblycladius) britae* Säwedal, 1976, male (TSZD-JKJ-116242, Tromsø University Museum). **A.** Habitus, lateral view. **B.** Habitus, dorsal view. **C.** Wing. Photos: J. Kjærandsen.

hind tibia 52–68, 61  $\mu\text{m}$ . Comb on hind leg with 15–19, 16 setae, longest setae 50–55, 52  $\mu\text{m}$  long, shortest 19–26, 22  $\mu\text{m}$  long. Mid- and hind leg with pseudospurs on first and second tarsomere. Lengths and proportions of legs as in Table 1.

*Hypopygium* (Figure 3A–F). Tergite IX with nearly straight posterior margin, with small, median, rounded projection above anal point and lateral corners, with 10–14, 12 setae to each side of the anal point. Anal point (Figure 3D), small, pale, tapering to rounded apex, 35–48, 41  $\mu\text{m}$  long, 22–28, 26  $\mu\text{m}$  wide at base. Laterosternite

IX closely connected to tergite IX, with 12–15, 14 long setae. Phallapodeme 96–117, 105  $\mu\text{m}$  long. Transverse sternapodeme nearly straight, 92–103, 98  $\mu\text{m}$  long, with weak oral projections. Virga (Figure 3C), subtriangular, 107–133, 118  $\mu\text{m}$  long, 117–123, 121  $\mu\text{m}$  wide orally, apparently consisting of two plates dorsally separated by deep V-shaped oral incision, Gonocoxite 440–512, 475  $\mu\text{m}$  long. Inferior volsella rounded with median margin ridge-like, 108–120, 113  $\mu\text{m}$  long, 64–76, 71  $\mu\text{m}$  wide medially, with microtricha and setae. Gonostylus as in Figure 3E–F, 232–256, 242  $\mu\text{m}$



**FIGURE 3.** *Chaetocladius (Amblycladius) britae* Säwedal, 1976, male (TSZD-JKJ-116242, Tromsø University Museum). **A.** Terminalia, dorsal view. **B.** Terminalia, ventral view. **C.** Terminalia, dorsal view with tergal parts removed showing the virgae and parts of the apodemes. **D.** Tergite IX and laterosternite IX detached showing the anal point, **E.** Gonostylus enlarged, dorsal view. **F.** Gonostylus enlarged, caudal view. Photos: J. Kjerandsen.

long; megaseta 21–22, 22  $\mu\text{m}$  long, crista dorsalis rounded, 23–28, 26  $\mu\text{m}$  long, 32–37, 34  $\mu\text{m}$  wide at base. Hypopygium Ratio (HR) = 1.90–2.03,

1.96. Hypopygium Value (HV) = 1.52–1.57, 1.53.

**Female and immatures.** Unknown.

**Remarks.** *Chaetocladius (A.) britae*

**TABLE 1.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Chaetocladius (Amblycladius) britae* Säwedal, 1976, male. LR = Leg Ratio, BV = “Bein-Verhältnisse”, SV = “Schenkel-Scheine-Verhältnisse”, BR = Bristle Ratio.

	$P_1$	$P_2$	$P_3$
<b>Fe</b>	1051–1257, 1145	1030–1277, 1137	1195–1442, 1298
<b>Ti</b>	1154–1442, 1290	1.051–1298, 1149	1174–1504, 1318
<b>ta<sub>1</sub></b>	783–968, 853	474–556, 507	701–845, 750
<b>ta<sub>2</sub></b>	391–494, 441	247–330, 284	350–453, 399
<b>ta<sub>3</sub></b>	268–350, 301	185–227, 210	288–350, 313
<b>ta<sub>4</sub></b>	165–185, 169	124–165, 144	144–185, 161
<b>ta<sub>5</sub></b>	113–134, 122	103–124, 115	124–144, 132
<b>LR</b>	0.645–0.678, 0.661 [0.66]	0.429–0.455, 0.442 [0.45]	0.556–0.596, 0.570 [0.56]
<b>BV</b>	3.150–3.237, 3.187	3.462–3.875, 3.714	3.265–3.388, 3.349
<b>SV</b>	2.634–2.925, 2.816	4.391–4.625, 4.509	3.382–3.571, 3.488
<b>BR</b>	2.09–2.53, 2.29	2.30–2.62, 2.46	2.72–3.00, 2.84

groups with *C. (A.) amurensis* Makarchenko & Makarchenko in having a well-developed megaseta and a small, rounded crista dorsalis. Makarchenko & Yavorskaya (2023: fig. 4) state that the virga in *C. (A.) amurensis* consists of 3–4 setae. However, in their fig. 5 they stipple a subtriangular structure quite similar to the structure considered to represent the virga in *C. (A.) britae*.

### DNA barcodes

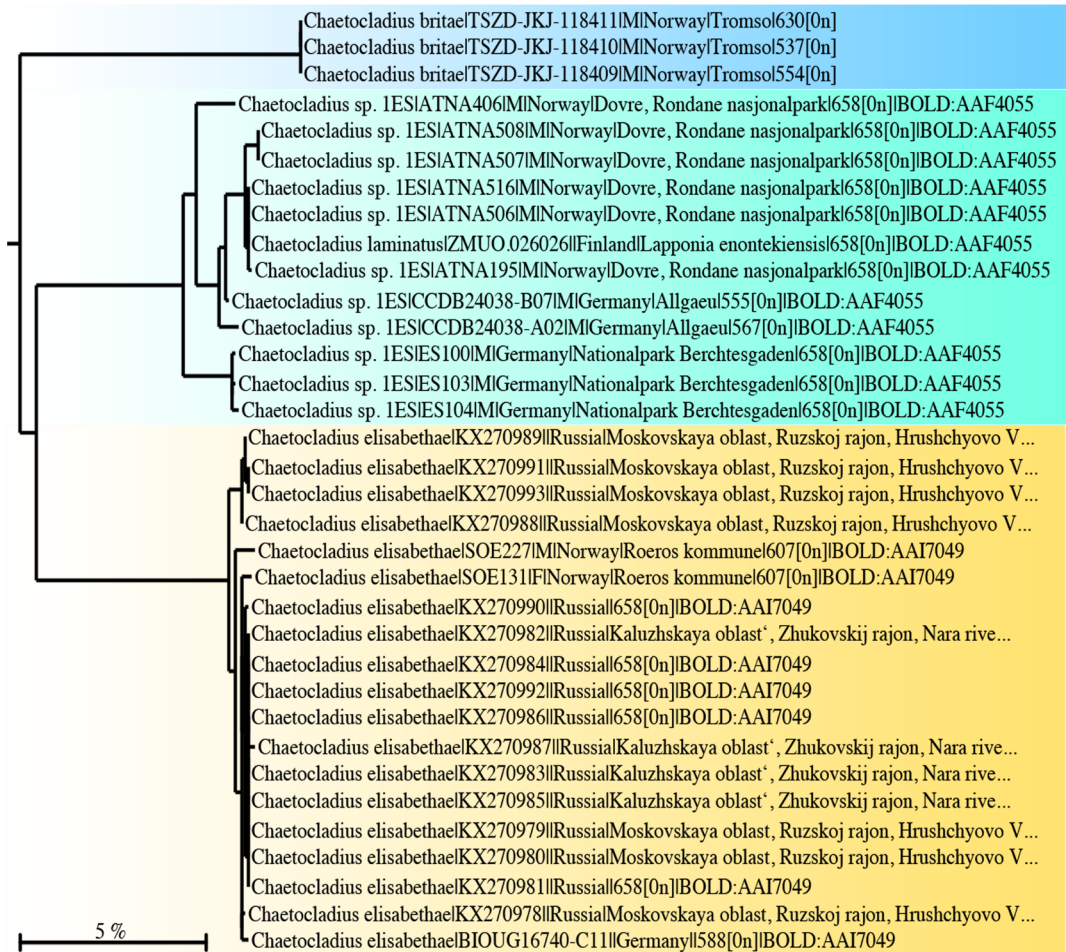
The DNA barcodes of both the species *Chaetocladius (Amblycladius) britae* and the subgenus *Amblycladius* are presented for the first time. The fresh barcodes of three males of *C. (A.) britae* (TSZD-JKJ-118409, TSZD-JKJ-118410, TSZD-JKJ-118411) were uploaded to BOLD and are uniquely assigned to the Barcode Index Number (BIN) BOLD:AGA1535. Among 770 public records of the genus *Chaetocladius* on BOLD, *C. (A.) britae* comes closest to *Chaetocladius* (s. str.) *elisabethae* Makarchenko, Makarchenko, Semenchenko & Palatov, 2018 and an unnamed species (*Chaetocladius* sp1ES), both with representatives from Norway (Figure 4). These three species have an average distance of 12% (10.25% to 14.50%). However, the closest DNA barcode matches to *C. (A.) britae* on BOLD are found at around 8% distance, all consisting of unreleased and unidentified

Chironomid materials, indicating that the subgenus *Amblycladius* may also be present in Canada (Alberta, Newfoundland, Labrador, and Yukon), USA (California) and China (Sichuan, Tibet).

### Discussion

Representatives of *Chaetocladius (Amblycladius)* are poorly known. Three of the five described species are known from singleton holotypes only, two from Novaya Zemlya and one from Franz Josef Land. The fourth species is known with a few specimens confined to the Amur River basin of Far East Russia. Adding *C. (A.) britae* to this subgenus widens the western distribution range to include Nordic boreal forests of High-North Europe. The DNA barcodes presented further indicates that the subgenus may be represented also in North America and in China. All species of the subgenus seem to be cold adapted and confined to either high latitude or high-altitude habitats. The collecting period in autumn and the site with tiny pools connected to an underground spring brook with cold water conforms with the records from Finland stemming from late summer at localities connected with cold water and spring brooks (Lauri Paasivirta *pers. comm.*). The type locality for the species at Messaure in





**FIGURE 4.** Subsection of ID-tree (Kimura-2-distance) obtained from BOLD with 34 public sequences and BIN assignments for the three fresh sequences of *Chaetocladius* (*Amblycladius*) *britae* Sæwedal, 1976 and its two closest species clusters among 770 public records of the genus *Chaetocladius* on BOLD. The scalebar representing 5% genetic distance reveals that there is considerable genetic distance (10.25% to 14.50%) in the barcode marker between *C. (A.) britae* and its closest DNA barcoded species within *Chaetocladius*.

Lule Lappmark (Sweden) is situated at 66.41°N 20.22°E and marks the southernmost known locality for the species. We are not aware of any spring brooks with cold water there but the likely collecting site Kaltisbäcken Nature Reserve near Messaure is a renowned destination for entomologists with a very rich fauna of insects. The reserve includes a large stretch of wet, old-growth riverine forest of predominantly Norway spruce (*Picea abies*) and aspen (*Populus tremula*), with rich soil and understorey vegetation and surrounded by drier and younger coniferous (*Picea abies*, *Pinus sylvestris*) forest (Kjærandsen

& Jaschhof 2019).

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