Zootaxa 2762: 37–48 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Description of *Chaetocladius longivirgatus* sp. n., with a review of *C. suecicus* (Kieffer) (Diptera: Chironomidae)

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Abstract

The male imago of *Chaetocladius longivirgatus* **sp. n.** is described and diagnosed against a refined characterization of the species understood as *C. suecicus* (Kieffer) since Edwards (1929). The two species are separated by both morphology and partial COI sequences (DNA barcodes). An additional single male specimen shows pairwise genetic distances to *C. suecicus* and *C. longivirgatus* **sp. n.** as high as those between the latter two species, thus may represent a third European member in this species group. Morphologically, however, that male is inseparable from those of *C. suecicus*.

Key words: new species, Orthocladiinae, taxonomy, morphology, DNA barcoding

Introduction

The immature stages of species in *Chaetocladius* Kieffer, 1911 are found in a wide variety of semiaquatic and truly aquatic habitats, mainly in alpine, arctic or subarctic areas of the Holarctic and Afrotropical regions (Cranston *et al.* 1989). More than 40 scientifically named species are currently recognized in the genus, 26 in Europe (Sæther & Spies 2004). However, *Chaetocladius* on the whole as well as a number of species within it have been in dire need of revision for a long time.

Freshwater research programs often generate a wealth of material, including previously undiscovered species, even in relatively well-investigated environments. Such a surveillance program was set up in the 1980s for the Atna River in the northeastern part of southern Norway that flows from about 1400 m a.s.l. in the Rondane Mountains to its discharge into the Glomma River at 338 m. Due to the survey's efforts to obtain long-term series of physical, chemical and biological data, much information on macroinvertebrates has been collected (Sandlund & Aagaard 2004). To obtain an impression of the faunal composition of that area, Malaise traps were set up at various altitudes from end of May to the beginning of October in 1986, and again during the same season in 2008. The adult chironomids have been processed comprehensively (Aagaard *et al.* 2004, Stur unpublished).

Through the first author's participation in the Atna River study, two adult chironomid morphotypes were discovered that clearly differ morphologically, but together fall within *Chaetocladius suecicus* (Kieffer in Thienemann & Kieffer, 1916) in the sense in which this name has been treated in the recent taxonomic literature. A review of specimens from various regions filed under *C. suecicus* in the ZSM collection showed both morphotypes to be present. Genetic analysis of Atna River specimens and one specimen from the Bavarian Alps in this complex, using partial COI sequences documented for DNA barcoding, even showed three haplotype groups. In the following, we therefore aim to characterize the three putative taxa, review usage of the name *Chaetocladius suecicus* (Kieffer), and propose a new scientific species name for the other clearly distinguishable morphotype.

Accepted by W. Giłka: 14 Dec. 2010; published: 10 Feb. 2011

Methods and terminology

The fresh material used in this study was collected in 2005 and 2008 by Malaise traps, and preserved in slightly dilute ethanol (~80–85%) to avoid breakage. One mid-leg was removed for DNA extraction and sequencing under a stereo microscope and shipped to the Canadian Centre for DNA Barcoding at the University of Guelph (CCDB, www.dnabarcoding.ca). The remainder of the specimens were macerated in KOH and slide mounted in Euparal. DNA extraction, PCR and sequencing followed standard protocols and primers (Folmer Primer Set LCO1490 and HCO2198 (Folmer *et al.* 1994)) at the CCDB and partial COI sequences with all meta-data are registered in the Barcode of Life Data Systems (Ratnasingham & Hebert 2007). GenBank accession numbers are listed under "material examined". Neighbour joining analyses were performed with the software Mega4 (Tamura *et al.* 2007) and utilized the Kimura 2-parameter substitution model in calculation of pairwise distances. In addition, slide mounted material of different collections was investigated.

Kimura-2-parameter distance is abbreviated as K2P, cytochrome c oxidase subunit I as COI. Morphological terminology and abbreviations generally follow Sæther (1980). Measurement results are given as ranges. Gonocoxite length was taken along the limb's longest axis (Fig. 8), which results in less orientation-dependent and slightly higher values than those measured according to Soponis (1977: fig. 19).

Abbreviations of collections: NHM = The Natural History Museum, London, UK; NHRS = Swedish Musem of Natural History, Stockholm, Sweden; PHL = private collection of Peter H. Langton, Londonderry, Northern Ireland; VM = Museum of Natural History and Archaeology, Norwegian University of Science and Technology, Trondheim, Norway; ZSM = Zoologische Staatssammlung München, Munich, Germany.

Individuals which have been genetically examined have an individual voucher ID.

We used a Leica DM6000 compound microscope fitted with a Leica DFC420 digital camera and connected to a personal computer with Leica Application Suite software including a Montage module to produce the photographic images in this paper. Images were made in bright field light setting.

Results

COI sequences. Partial COI gene sequences were obtained from 14 specimens morphologically fitting the description of *Chaetocladius suecicus* in the currently available literature. All specimens except one from the Bavarian Alps (Germany) were collected at the same site in the Rondane Mountains (Norway). Based on K2P-distances, the gene sequences clustered in three distinct haplotype groups (Table 1). The genetic distances within groups did not exceed 1.2 %, while those between groups were greater than 11.1 %. Qualitative comparison of aligned sequences revealed 41 unique base-calls for *Chaetocladius longivirgatus*, 34 for *Chaetocladius suecicus* and 32 for *Chaetocladius* sp. prope *suecicus*. Most of the nucleotide substitutions were in third position of the codon triplets, thus translating to the same amino acids. However, in three instances we observed unique amino acids for *Chaetocladius suecicus* compared to the other two species. No stop codons were present in the sequences, and Blast searches in GenBank did not indicate contaminants.

Species descriptions

Chaetocladius suecicus (Kieffer) sensu Edwards (1929)

(Figs. 1, 3, 6, 8, 9)

? *Dactylocladius suecicus* Kieffer, 1916: 517—Thienemann & Kieffer (1916), adult male and female. Taxonomic identity unclear; see Discussion section below.

Spaniotoma (Orthocladius) suecica (Kieffer)—Edwards (1929: 337, in part), adult male and female.

Chaetocladius suecicus (Kieffer) – Brundin (1947: 24), adult male; Lehmann (1971, in part), distribution, phenology; Langton & Visser (2003), pupa, distribution; Sæther & Spies (2004, in part), distribution; Langton & Pinder (2007, vol. 1: 86, 87; vol. 2: fig. 143B), adult male.

TABLE 1. Pairwise Kimura-2-parameter distances between COI sequences of specimens in the "*Chaetocladius suecicus* species group".

		Chaetocladius longivirgatus							*Chaetocladius suecicus*						sp.
		AT208	AT408	AT220	AT453	AT452	AT451	AT194	AT14	AT427	AT193	AT200	AT228	AT428	ES265
Chaetocladius longivirgatus	AT208	-													
	AT408	0.000	-												
	AT220	0.000	0.000	-											
	AT453	0.000	0.000	0.000	-										
	AT452	0.000	0.000	0.000	0.000	-									
	AT451	0.000	0.000	0.000	0.000	0.000	-								
	AT194	0.002	0.002	0.002	0.002	0.002	0.002	-							
Chaetocladius suecicus	AT14	0.127	0.127	0.127	0.127	0.126	0.127	0.128	-						
	AT427	0.128	0.128	0.128	0.128	0.129	0.127	0.128	0.008	-					
	AT193	0.129	0.129	0.129	0.129	0.131	0.129	0.127	0.012	0.006	-				
	AT200	0.129	0.129	0.129	0.129	0.128	0.128	0.129	0.006	0.005	0.008	-			
	AT228	0.129	0.129	0.129	0.129	0.131	0.128	0.129	0.006	0.002	0.004	0.003	-		
	AT428	0.134	0.134	0.134	0.134	0.136	0.134	0.129	0.007	0.005	0.008	0.007	0.003	-	
sp.	ES265	0.117	0.117	0.117	0.117	0.117	0.120	0.114	0.113	0.112	0.113	0.111	0.117	0.112	-

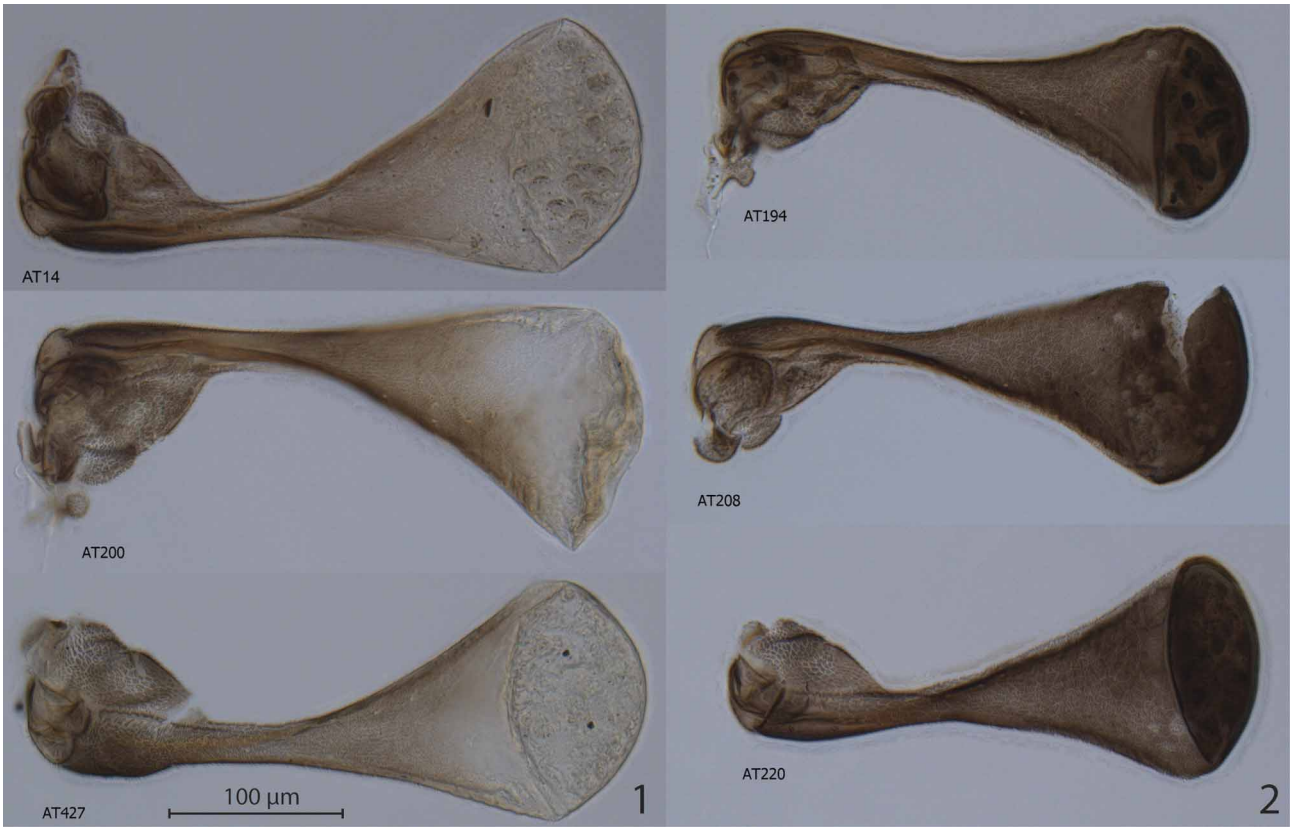
Material examined (listed alphabetically by country). GERMANY: 1 adult male (ZSM), Bavaria, Allgäu, Rappensee, 2000 m a.s.l., 25.vii.–08.viii.1977, leg. H. Mendl. — NORWAY, Oppland, Dovre, Rondane Nationalpark (all at VM): 1 adult male (genetic voucher AT14; Genbank accession number HQ712186), near Dørålseter, N 61.987°, E 09.789°, 1050 m, 30.v.2008, hand net, leg. E. Stur & T. Ekrem; 2 adult males (vouchers AT427, AT428; Genbank accession numbers HM421520 and HM421521 respectively) øvre Vidjedalsbekken, N 61.97168° E 09.83606°, 1280 m, Malaise trap, 30.vi.–07.vii.2008, leg. T. Hoffstad; 2 adult males (vouchers AT193, AT200; Genbank accession numbers HM421307 and HM421314 respectively), Vidjedalsbekken at Skranglehaugen, N 61.98186°, E 09.80454°, 1117 m, Malaise trap M1, 23.–30.vi.2008, leg. T. Hoffstad; 1 adult male (voucher AT228 Genbank accession number HM421340), further data as in preceding entry, except 07.–14.vii.2008. — SPAIN: 2 adult males (ZSM & VM), Prov. Castilla y León, Puerto de Leitariegos NW of Villablino, N 42.99°, W 06.41°, c. 1500 m, 04.vii.1988, leg. W. Schacht. — SWEDEN: 1 adult male (ZSM), Norbotten, Abisko, 13.x.1955, leg. E. J. Fittkau; 1 adult male (NHRS), northern Jämtland, Jormsjön, 8.x.1945, leg. A. Määr (Brundin coll. code "B.7"); 1 adult male, as preceding, except Stora Blåsjön, 23.–25.ix.1945 ("B1:6"); 1 adult male, as preceding, except 4.x.1945 ("B.12"). — UNITED KINGDOM: 1 pupal exuviae with pharate male inside (PHL), Scotland, Perthshire, "burn 14" (a small stream in montane heathland), 8.vii.1985, leg. J. Foster & S. Hogg; 1 adult male, as preceding, except Loch Lannoch, 5.iv.1991, leg. P.H. Langton; 2 adult males (NHM), Shropshire (=Salop), Snailbeach, 22.–28.vii.1920, leg. F.W. Edwards.

Diagnostic characters. The male imago is separable from other known species in the genus *Chaetocladius* by the following combination of characters: wing length 2.10–2.54 mm; AR higher than 1.25; eyes weakly pubescent; anteriormost acrostichals close to antepronotum; humeral pit ovoid, dark with several patches in one larger spot; LR_1 0.63–0.69; wing with setae on squama and on veins R, R_{1+2} and R_{4+5}; halters light-colored; median setae on tergite IX comparatively robust; virga shorter than half of anal point length, with 5–6 subequal spines arranged in a bundle; anal point not hyaline at base, parallel-sided, apically rounded, reaching about as far posterior as inferior volsella; inferior volsella double, ventrally covered with microtrichia; gonostylus without projections or significant expansions, with one distal macroseta; HR 2.27–2.56.

Male imago (n=6 unless otherwise stated). Wing length 2.43 (2.40–2.54) mm. Gonocoxite length/wing length 90 (81–95) (n=11). Coloration brownish black. Halters brown at base and light distally (Fig. 1).

Head. AR 1.45 (1.3–1.5) (n = 5). Terminal flagellomere 630 (600–680) µm long (n = 5). Temporal setae 9–12, including 2–4 inner verticals, 5–8 outer verticals and 1 postorbital. Clypeus 70–80 µm long, with 5–9 setae. Palpomere lengths (in µm): 30–40, 50–60, 155–165, 115–145, 200–230.

Thorax. Antepronotum with 5–7 setae. Acrostichals 17–20 (n=5), dorsocentrals 12–16 in one row, prealars 4–7. Scutellum with 10–11 setae in one transverse row.



FIGURES 1–2. Halter. 1: *Chaetocladius suecicus*. 2: *Chaetocladius longivirgatus*.

Wing (Fig. 3). Costa barely extended. VR 0.99–1.03. Anal lobe well developed, projecting, rightangled. Brachiolum with 1 seta; veins bare, except R with 15–18, R_1 with 0–3, R_{4+5} with 1–5 setae. Squama with 9–17 setae.

Legs. Spur of front tibia 74–85 µm long, spurs of middle tibia 25–33 µm (n=5) and 30–40 µm long, of hind tibia 23–30 µm (n=5) and 70–80 µm long. Lengths and proportions of leg segments as in Table 2.

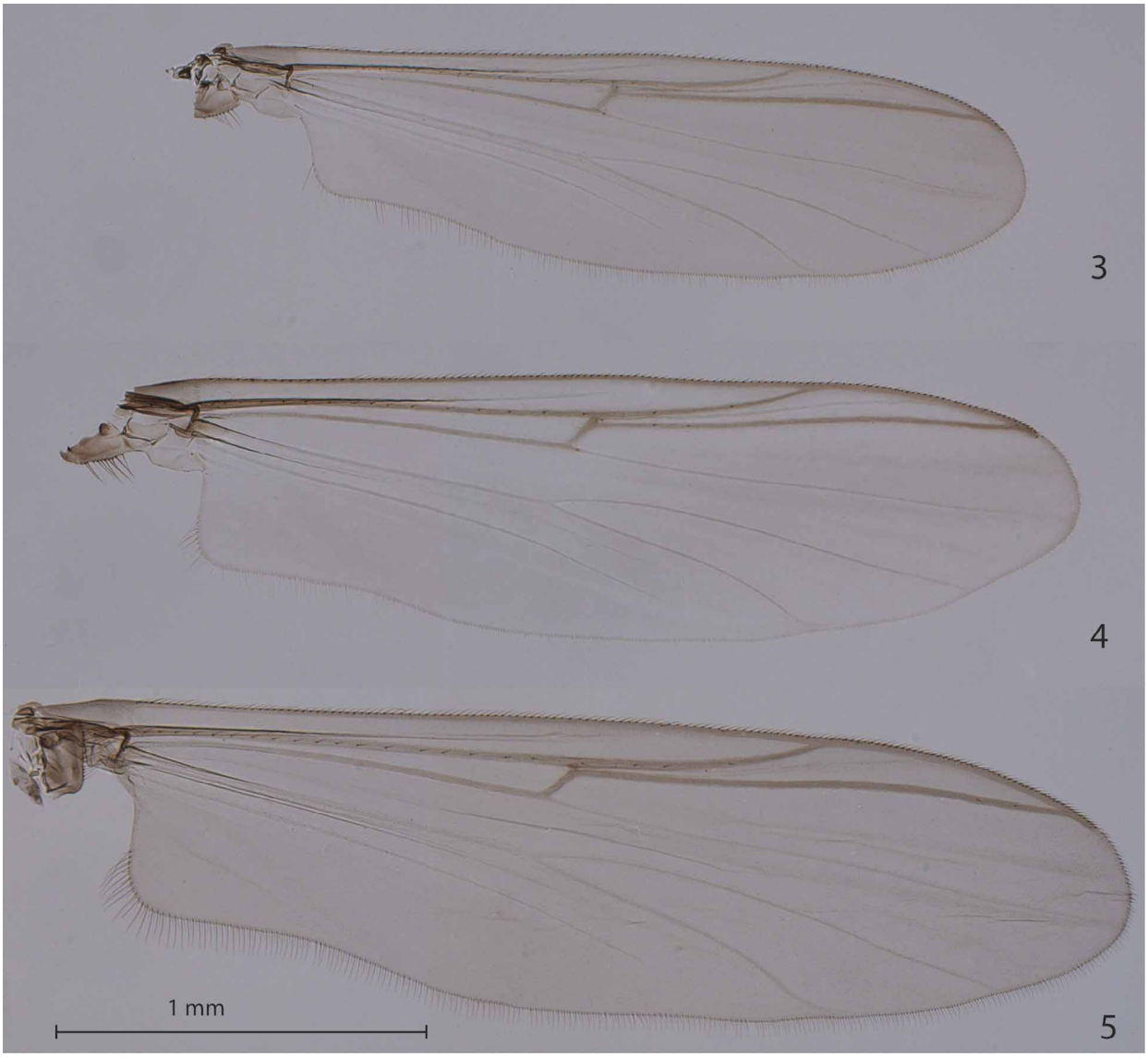
TABLE 2. Leg segment lengths (in µm) and proportions in adult males of *Chaetocladius suecicus* (n=6, except n=13 where marked with *).

	fe	ti	ta_1	ta_2	ta_3
p_1	900 (870–950)	1040 (840–1150)*	670 (580–740)*	390 (380–420)	280 (260–300)
p_2	940 (920–980)	980 (950–1020)	440 (420–460)	270 (260–290)	200 (190–210)
p_3	1070 (1040–1120)	1180 (1150–1240)	700 (660–730)	390 (380–400)	300 (280–330)

continued.

	ta_4	ta_5	LR	BV	SV	BR
p_1	170 (160–190)	110 (105–115)	0.65 (0.63–0.69)*	2.8 (2.72–2.84)	2.86 (2.80–2.96)	2.45 (1.77–2.67)
p_2	133 (130–140)	100	0.45 (0.43–0.47)	3.36 (3.31–3.40)	4.35 (4.20–4.51)	2.35 (2.00–2.70)
p_3	175 (160–190)	115 (110–120)	0.59 (0.57–0.62)	3.03 (2.97–3.13)	3.22 (3.01–3.36)	2.78 (2.27–3.20)

Hypopygium (Figs. 6, 8, 9). Tergite IX with 13–20 setae, laterosternite IX with 8–10 setae (n=5). Anal point 70 (65–75) µm long. Length ratio anal point/wing > 0.25. Phallapodeme 115 (108–125) µm long; transverse sternapodeme 113 (100–120) µm long. Virga 28 (25–30) µm long, with 5–6 subequal spines arranged in a bundle. Gonocoxite 223 (210–230) µm long. Gonostylus (Figs. 6, 9) 94 (90–97) µm long, simple, without projections or significant expansions; megaseta 14–17 µm long. HR 2.38 (2.30–2.56). Inferior volsella bilobed, ventrally covered with microtrichia.



FIGURES 3–5. Wing. 3: *Chaetocladius suecicus*. 4: *Chaetocladius longivirgatus*. 5: *Chaetocladius* sp. prope *suecicus*.

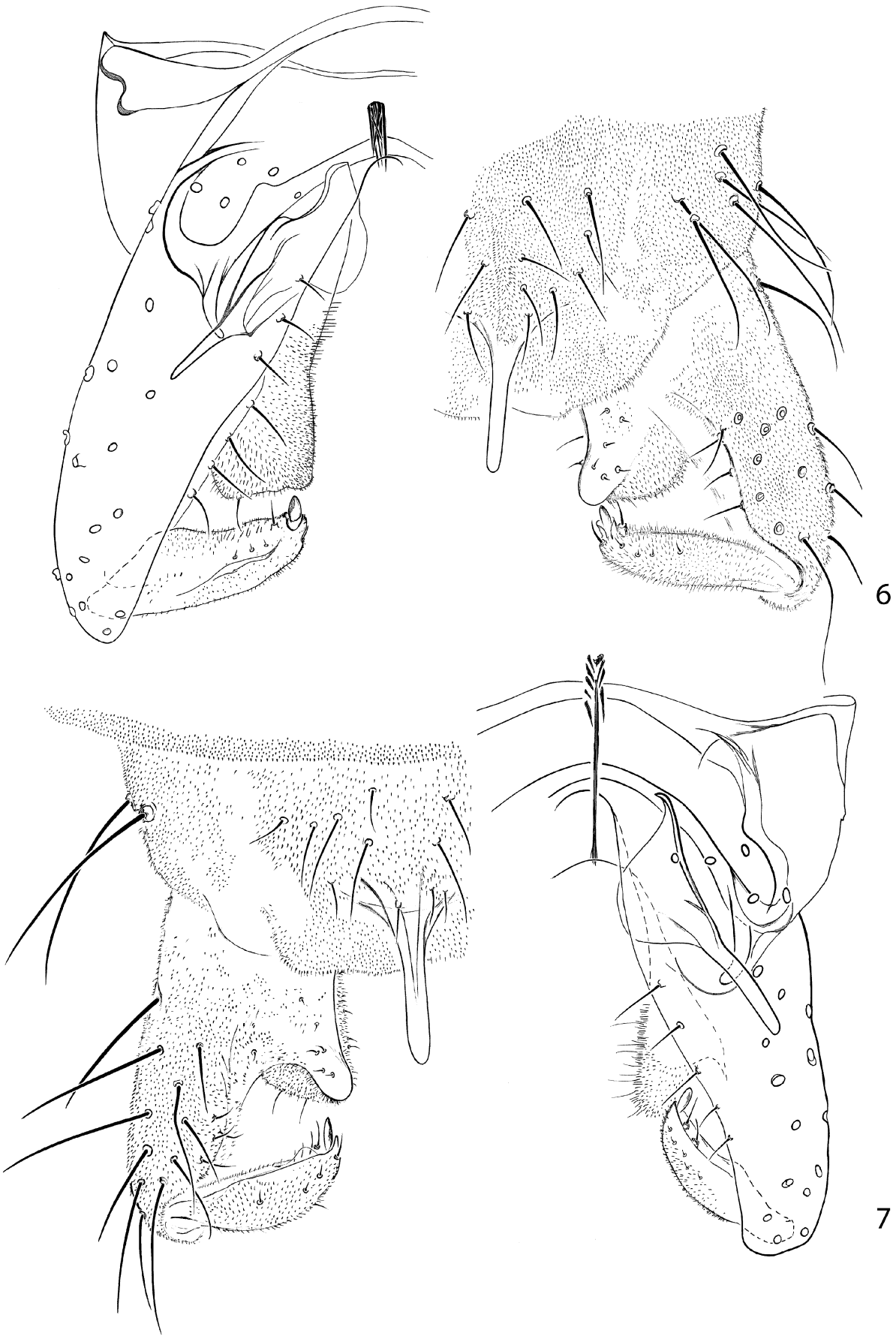
Pupa (n = 1). The single specimen available to us that is reliably identifiable on account of direct association with the adult stage is an exuviae containing a pharate male on which the features considered as diagnostic above could be evaluated, except for those of the wing. It keys to *C. suecicus* and agrees with all data in the corresponding description by Langton & Visser (2003), except that on abdominal segment IV the lateral seta 2 is c. 0.38 of segment IV length as described, but is 160 µm long, i.e. substantially longer than both, the maximum length of 135 µm given in the description and than setae ls 1 and ls 3. Thoracic horn length is 215 µm, wing sheath length c. 1.34 mm; the length of the dissected exuviae cannot be estimated, as the abdomen is strongly hyperextended in the mount.

Distribution. See Material examined. Earlier records (e.g., see Sæther & Spies 2004) should be revised with regard to the new species described below.

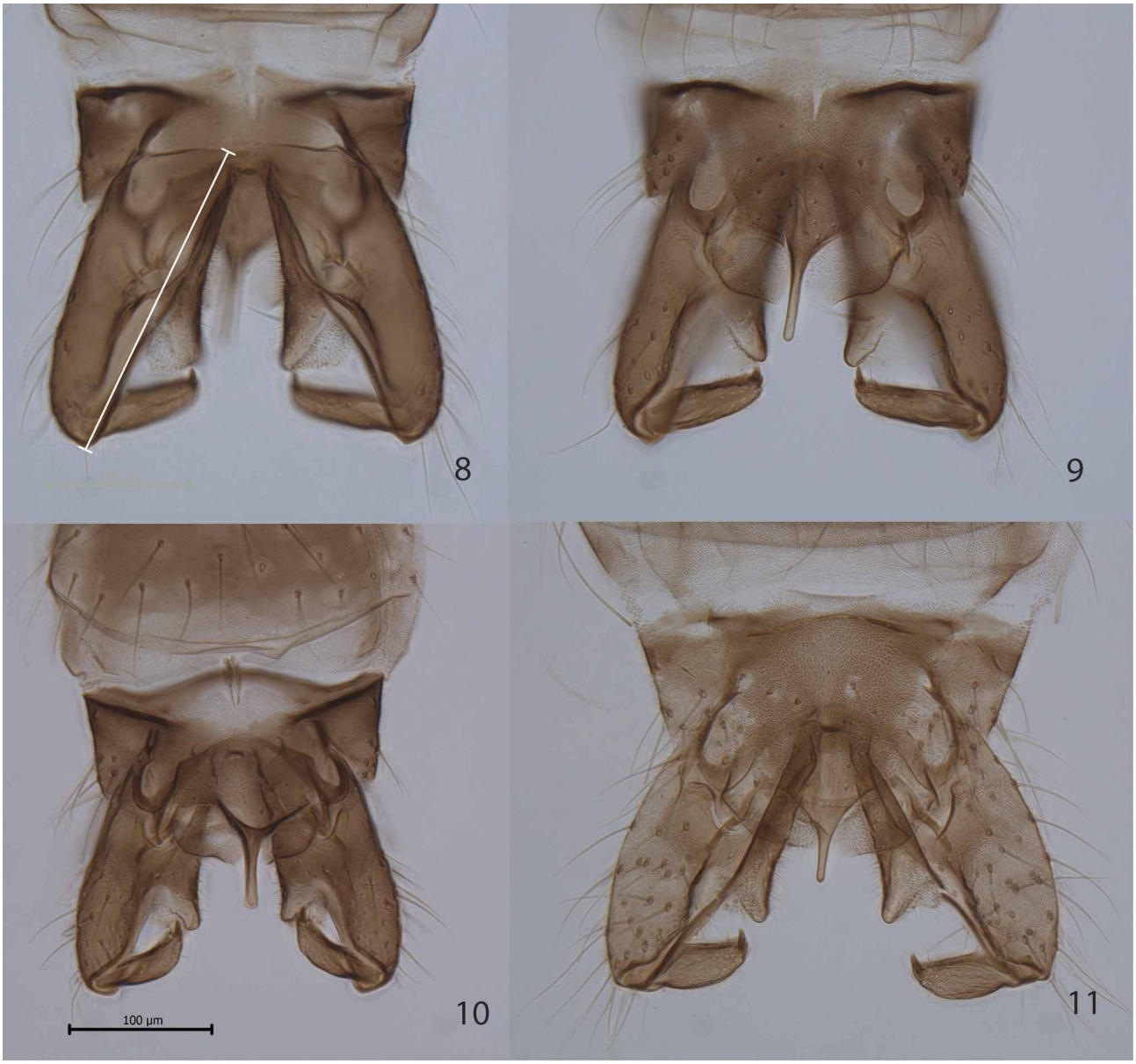
Chaetocladius sp. prope *suecicus* (Kieffer) sensu Edwards

(Figs. 5, 11)

Material examined. GERMANY (VM): 1 adult male (genetic voucher ES265; Genbank accession number HQ712185), Bavaria, Nationalpark Berchtesgaden, Schapbachquelle 360a, N 47.5823°, E 12.9581°, 1140 m a.s.l., 20.ix.–04.x.2005, leg. F. Eder.



FIGURES 6–7. Hypopygium. 6: *Chaetocladius suecicus*. 7: *Chaetocladius longivirgatus.*



FIGURES 8–11. Hypopygium. 8, 9: *Chaetocladius suecicus*. 10: *Chaetocladius longivirgatus*. 11: *Chaetocladius* sp. prope *suecicus*.

Diagnostic characters. The inspected morphological features of the male imago fit within the diagnosis given above for *Chaetocladius suecicus*. Minor differences would be the slightly higher leg ratios for all legs, lower numbers of acrostichal and prealar setae, and the shorter virga, even though body size (wing length) is higher than in *C. suecicus*.

Male imago (n = 1). Wing length 2.74 mm. Gonocoxite length/wing length 83. Coloration brownish black, scutellum lighter. Halters light at tips.

Head. AR 1.68. Terminal flagellomere 755 µm long. Temporal setae 12, including 3 inner verticals, 9 outer verticals and 0 postorbitals. Clypeus 125 µm long, with 6 setae. Palpomere lengths (in µm): 30, 70, 185, 160, 240.

Thorax. Antepronotum with 8 and 10 setae. Acrostichals 30, dorsocentrals 13 in one row, prealars 8. Scutellum with 11 setae in one transverse row.

Wing (Fig. 5). Costa barely extended. VR 1. Anal lobe projecting. Brachiolum with 1 seta; veins bare, except R with 18 or 19, R_1 with 4 or 6, and R_{4+5} with 2 setae. Squama with 13 setae.

Legs. Spur of front tibia 95 µm long, spurs of middle tibia both 37 µm long, of hind tibia 32 and 78 µm long. Lengths and proportions of leg segments as in Table 3.

TABLE 3. Leg segment lengths (in µm) and proportions in an adult male of *Chaetocladius* sp. prope *suecicus*.

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
p_1	1050	1250	840	480	350	200	120	0.67	2.73	2.74
p_2	1050	1120	550	320	240	160	110	0.49	3.28	3.95
p_3	1100	1330	860	470	360	210	120	0.65	2.84	2.83

Hypopygium (Fig. 11). 14 median setae on tergite IX, laterosternites IX with 8 and 9 setae. Anal point 60 µm long. Length ratio anal point/wing < 0.25. Phallapodeme 125 µm long; transverse sternapodeme 105 µm long. Virga 22 µm long, with subequal spines arranged in a bundle. Gonocoxite 227 µm long. Gonostylus (Fig. 11) 93 µm long, megaseta 15 µm long. HR 2.42. Inferior volsella ventrally covered with microtrichia.

Remarks. We refrain from proposing a new species name as long as the minor morphological differences listed in the diagnosis are not confirmed by more specimens and information on the other live stages. However, the large differences between the partial COI sequences (Table 1) suggest that the present specimen belongs to a species separate from *C. suecicus*.

Distribution. The only known specimen was collected in the Bavarian Alps.

Chaetocladius longivirgatus sp. n.

(Figs. 2, 4, 7, 10)

Chaetocladius suecicus (Kieffer) – Lehmann (1971), in part: distribution, phenology; Sæther & Spies (2004, in part), distribution.

Etymology. The specific epithet refers to the relatively long virga in the adult male genitalia; it is to be treated as adjectival for the purposes of nomenclature.

Holotype: adult male (deposited at VM; genetic voucher AT208; Genbank accession number HM421322), NORWAY, Oppland, Dovre, Rondane Nationalpark, Vidjedalsbekken at Skranglehaugen, N 61.98186°, E 0980454°, 1117 m a.s.l., Malaise trap M1, 23.–30.vi.2008, leg. T. Hoffstad.

Paratypes (listed alphabetically by country): GERMANY: 2 adult males (ZSM), Hessen, Rhön mountains E of Fulda, helocrene c. 40 m below peak of Wasserkuppe mountain ("Schluchtquelle" = locality "OC" in Lehmann 1971: 470; one of the spring sources of the Fulda river), 01.x.1953, leg. E. J. Fittkau (Fulda sample 615). — NORWAY (all at VM): 2 adult males (vouchers AT194, AT220; Genbank accession numbers HM421308 and HM421334 respectively), data as for holotype; 1 adult male (voucher AT451; Genbank accession number HM421543), as holotype, except N 61.98141°, E 09.80480°, 1119 m, Malaise trap M2, 14.–21.vi.2008, leg. T. Ekrem; 1 adult male (voucher AT452; Genbank accession number HM421544), as holotype, except N 61.98219°, E 09.80451°, 1115 m, Malaise trap M3, 30.vi.–07.vii.2008, leg. T. Hoffstad; 1 adult male (voucher AT453; Genbank accession number HM421545), as holotype, except N 61.98346°, E 09.80384°, 1105 m, Malaise trap M5, 23.–30.vi.2008, leg. T. Hoffstad; 1 adult male (voucher AT408; Genbank accession number HM421503), as holotype, except øvre Vidjedalsbekken, N 61.97168° E 09.83606°, 1280 m, Malaise trap, 23.–30.vi.2008, leg. T. Hoffstad; 2 adult males, as holotype, except Dørålseter, N 61.99347°, E 09.80343°, 1032 m, Malaise trap, 02.vii.1986 & 23.viii.1986, leg. K. Aagaard; 1 adult male, Møre og Romsdal, Rauma kommune, Raudstøl, N 62.31456º, E 08.096175º, 13.–20.v.1992, leg. K. Aagaard & O. Hanssen; 1 adult male, data as in preceding entry, except Fiva, N 62.51038º, E 07.762057º, 02.–12.v.1995.

Additional material examined. GERMANY: 1 adult male and 1 pupal exuviae (see the Discussion section) on a single slide (ZSM), Hessen, Rhön mountains E of Fulda, Fulda river upstream of Obernhausen, c. 1.5 km below its springs ("Erlenwald" = locality "1A" in Lehmann 1971: 470), 19.iii.1954, leg. E. J. Fittkau.

Diagnostic characters. The male imago can be separated from all other *Chaetocladius* species by the following combination of characters: Wing length 1.8–2.3 mm; AR 1.00–1.24; LR_1 0.54–0.66; eyes weakly pubescent; anteriormost acrostichals close to antepronotum; humeral pit ovoid, dark with several patches in one larger spot; wing with few setae on squama and on wing veins R and R_{1+2}, vein R_{4+5} bare (one specimen unilaterally with 1 seta); halters apically dark; median setae on tergite IX comparatively delicate; virga with spines about as long as

anal point and with additional short spines near base (Fig. 7); anal point not hyaline at base, parallel-sided, apically rounded, reaching about as far posterior as inferior volsella; inferior volsella double, with a ventral patch bare of microtrichia. Gonostylus without projections or significant expansions, with one distal macroseta. HR 2.6–2.8.

Male imago (n = 6 unless otherwise stated). Wing length 2.03 (1.84–2.26) mm (n=12). Gonocoxite length/wing length 93 (88–103) (n=11). Coloration brownish black. Halters dark brown at tips (Fig. 2).

Head. AR 1.13 (1.00–1.24) (n=11). Terminal flagellomere 490 (420–540) µm long. Temporal setae 10–14, including 2–5 inner verticals, 6–8 outer verticals and 1–3 postorbitals. Clypeus length 75–87 µm, with 4–7 setae. Palpomere lengths (in µm): 30–38, 50–58, 110–120, 90–100, 150–180.

Thorax. Antepronotum with 3–8 setae. Acrostichals 14–19, dorsocentrals 7–11 in one row, prealars 4–6. Scutellum with 4–8 setae in one transverse row.

Wing (Fig. 3). Costa barely extended. VR 1.01–1.05. Anal lobe less projecting than in *C. suecicus* (Fig. 4), not right-angled. Brachiolum with 1 seta; veins bare, except R with 8–14 and R_1 with 0–1 setae. Squama with 4–8 setae.

Legs. Spur of front tibia 58–72 µm (n=5) long, spurs of middle tibia 23–25 µm and 26–28 µm (n=4) long, of hind tibia 50–60 µm and 17–25 µm long. Lengths and proportions of leg segments as in Table 4.

TABLE 4. Leg segment lengths (in µm) and proportions in adult males of *Chaetocladius longivirgatus* (n=6, except n=11 where marked with #).

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	730 (670–790)	870 (780–940)#	530 (460–600)#	290 (280–320)	215 (190–240)	135 (120–150)
p_2	765 (700–820)	780 (710–830)	340 (320–360)	215 (190–230)	160 (140–180)	105 (90–120)
p_3	860 (780–920)	945 (840–1040)	550 (480–610)	290 (250–320)	240 (200–270)	135 (110–150)

continued.

	ta_5	LR	BV	SV	BR (n=5–6)
p_1	90 (80–100)	0.61 (0.54–0.66)#	2.82 (2.69–2.91)	3.18 (3.08–3.44)	1.91 (1.70–2.10)
p_2	90 (80–100)	0.43 (0.41–0.45)	3.30 (3.14–3.44)	4.57 (4.36–4.78)	2.01 (1.77–2.4)
p_3	90 (80–100)	0.58 (0.56–0.60)	3.10 (2.97–3.28)	3.29 (3.18–3.43)	2.8 (2.30–3.50)

Hypopygium (Figs. 7, 10). 14–24 median setae on tergite IX, laterosternite IX with 3–6 setae (n=5). Anal point 65 (62–68) µm long, parallel-sided and distally rounded. Length ratio anal point/wing > 0.25. Phallapodeme 104 (93–110) µm long; transverse sternapodeme 95 (88–100) µm long. Virga 80 (75–83) µm long, with up to 5 long, thin spines and 6–16 (n=10) shorter ones (Fig. 7). Gonocoxite 195 (188–202) µm long. Gonostylus 73 (68–77) µm long (Figs. 7, 10), megaseta 10–15 µm long. HR 2.69 (2.62–2.76). Inferior volsella ventrally square and dorsally tongue-shaped, ventrally without microtrichia in proximal half.

Pupa. See Discussion below.

Remarks. The long virga spines separate when the hypopygium is pressed under the cover slip.
Two other *Chaetocladius* species with dark halters are currently recognized in Europe (Edwards 1929, Goetghebuer 1942 in 1940–1950, Sæther & Spies 2004). Of these, *C. piger* (Goetghebuer, 1913) differs from the new species by the triangular anal point and square inferior volsella; *C. dissipatus* (Edwards, 1929) differs by the slender and delicate anal point and the much shorter virga.

Distribution. The new species is so far known only from a subboreal and an alpine mountain region in central and southern Germany (Rhön and Allgäu, respectively), and from the Rondane Mountains in Norway. *Chaetocladius longivirgatus* **sp. n.** has been found syntopically with *C. suecicus*.

Discussion

COI sequences. Partial COI gene sequences as DNA barcodes have proven to work well for species delimitation and identification of non-biting midges (Ekrem *et al.* 2007, 2010). In addition, they have been used to detect taxo-

nomically difficult species and species groups (e.g. Ekrem *et al.* 2010). In the present study, large barcode cluster divergence between specimens initially identified as *C. suecicus* prompted us to examine the morphology more closely and look for diagnostic characters that could warrant separate species status. Such discrepancies were found between *C. longivirgatus* and *C. suecicus*, but not for the third lineage only represented by one specimen, *Chaetocladius* sp. prope *suecicus*. Our results indicate a large gap between intraspecific and interspecific variation in COI sequences, but this gap probably will become smaller as more individuals from a wider geographical area are analyzed. Thus, 1.2 % K2P should not be regarded as the species limiting threshold for this group. Previous studies have shown that the intraspecific variation in Chironomidae can be as high as, but never greater than, 6–8 % (Ekrem *et al.* 2007, 2010, Sinclair & Gresens 2008). The alpine *Chaetocladius* sp. prope *suecicus* differs from both *C. suecicus* and *C. longivirgatus* by more than 11 %, which we think is a strong indication that this specimen belongs to a different species. However, a formal description with designation of a name-bearing type should include more material that allows evaluation of both, morphological and genetic variability.

Usage of the name *C. suecicus* (Kieffer). Kieffer (in Thienemann & Kieffer 1916) established the name *Dactylocladius suecicus* for at least, possibly no more than, one each adult male and female from Sweden, Scania, Pålsjö near Helsingborg, small spring stream flowing directly into the Öresund, 24.viii.1912, leg. A. Thienemann (sample code "Bäck Weiße"); for more on the locality, see op. cit.: 499. According to Thienemann documentation at ZSM, these adults had emerged from a mass rearing of locally common white larvae cultured on wet gravel or damp leaves (larvae did not survive extended submersion in water). The adult syntype series is missing and apparently has never been studied by anyone other than Kieffer. ZSM possesses one slide carrying a Thienemann label "Bäck Weiße / *Dactyloclad. / suecicus* K." along with three pupal exuviae of male sex. The available documentation does not allow recognizing any of these exuviae as part of a syntype, because no individual association with an adult male seen by Kieffer can be established (ICZN 1980).

Several features of the male mentioned in the original description appear incompatible with the morphotype currently identified as *C. suecicus*: at a body size of 3.5 mm, the AR was given as "slightly" higher than 1, the LR_1 as 0.67, and the inferior volsella was described and figured as having its dorsal and ventral lobes rather deeply separated ("with 2 ... lobes", op. cit.: 518; fig. 18). All three exuviae from the type sample key to *Chaetocladius perennis* (Meigen) in Langton & Visser (2003), and this identity looks possible also for the male as described by Kieffer.

Edwards (1929) used the name *Spaniotoma (Orthocladius)* "*suecica*, Kieff., 1916" for males and females from three northern English counties. He characterized the species by little more than body coloration and a rough sketch of the male hypopygium, and misrepresented the wing length as "4–5 mm" (diagnosis for all species in "Group A"). We have examined three males from that material (all from Snailbeach), and found two to correspond to the current species concept of *C. suecicus* (see Material examined), the third to be a misidentified *C. perennis* (Meigen) sensu Langton & Pinder (2007).

Goetghebuer's (1942 in Goetghebuer 1940–1950: 57, 58, 63) interpretation sub *Orthocladius (Chaetocladius) suecicus* appears to have followed that of Edwards. None of his Belgian specimens were examined for the present study.

Brundin (1947), referring to Edwards' and Goetghebuer's above-quoted treatments, reported many specimens of "*C. suecicus* Kieff." that had been caught by A. Määr around two "oligotrophic, oligohumose" lakes in the "upper coniferous forest region" of northern Sweden (op. cit.: 4). We have examined three males from both lakes (three different samples), all of which correspond to the current species concept of *C. suecicus* (see Material examined).

Langton & Visser (2003) and Langton & Pinder (2007) represent the most recent interpretations of the West Palaearctic pupal exuvial and British adult male morphotypes, respectively, of *C. suecicus* – linked by individual association of the two life stages. We have examined two relevant and informative specimens from the Langton collection that correspond to the current concept of the species (see Material examined).

Only comprehensive revision of *Chaetocladius* Kieffer, at least of all its western Palaearctic members, could clarify all relevant synonymies and embed the taxonomic identity of *C. suecicus* (Kieffer) within an optimal synthesis of both, the scientific evidence and considerations of nomenclatural stability and continuity of the historical data record. Since such revision was far beyond the reach of the present study, and no association of at least an adult male and pupal exuviae from near the type locality was available, we consider it as the most appropriate approach to refrain from any ad-hoc intervention such as the fixation of a neotype. Instead, we apply the long-standing, methodologically sound and nomenclaturally acceptable practice (see also Spies & Sæther 2004) of using

the species name in a way that facilitates access to its most influential published interpretations of the past at least 80 years, and reflects what is reproducible at this time, i.e. as *Chaetocladius suecicus* (Kieffer) sensu Edwards (1929).

Possible pupa of *C. longivirgatus* sp. n. t ZSM there is a slide carrying a fully emerged adult male of *C. longivirgatus* and a pupal exuviae. We interpret the male as a member of *C. longivirgatus* (for collecting data, see Additional material examined), but the exuviae is indistinguishable from *C. suecicus* (length values: exuviae 3.45 mm; thoracic horn 200 µm; wing sheath c. 1.10 mm; abdominal segment IV seta ls 2 150 µm, clearly longer than ls 1 and ls 3, c. 0.41 of segment IV length).

The available documentation on the sample does not allow conclusion whether the association between the two life-stage specimens is individual or merely circumstantial. Lehmann (1971: 492) reported all Fulda material sub *C. suecicus* known to him to have come from hand net or drift samples, none from any rearing. Moreover, while the exuviae is smaller than the Scottish one mentioned above under *C. suecicus*, the size relation of the corresponding adult males is the opposite (Fulda male with fore metatarsus 600 µm long, fore tibia 940 µm; LR_1 0.64; Scottish pharate male with fore ta1 580 µm long, fore ti 840 µm, LR_1 0.69.

Future associations will have to show whether or not the pupa of *C. longivirgatus* is distinguishable from that of *C. suecicus*.

Conclusions

The inclusion of DNA barcodes in species descriptions is clearly advantageous. Not only does it provide an additional set of diagnostic characters, but it also facilitates future identification and association of undescribed life stages (Ekrem *et al*. 2010). If name-bearing types are associated with DNA barcodes, it will increase the stability of species names and reduce inconsistency in future usage of species names. We thus recommend that the inclusion of species specific DNA sequences should become standard practice in the description of new species.

Acknowledgements

We thank Paul Hebert and the staff at the Biodiversity Institute of Ontario, University of Guelph, Canada, for sequencing of DNA barcodes and databasing; sequence analysis was enabled by support from Genome Canada through the Ontario Genomics Institute to Paul Hebert. Helmut Franz and Franz Eder (National Park Berchtesgarden) are thanked for support in the field and collecting. We are grateful to Torbjørn Ekrem (Trondheim) for comments on the manuscript. Peter Langton (Coleraine) kindly lent specimens from his collection; Erica McAlister (London), Niklas Jönsson and Yngve Brodin (both Stockholm) searched for and provided material and data from the Edwards (NHM) and Brundin (NHRS) collections, respectively. This study presents part of the results from a research project conducted by the first author together with Kaare Aagaard, Ola Diserud and Torbjørn Ekrem (all Trondheim). We thank two anonymous reviewers for their comments on the manuscript.

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