Description of the Alpine *Micropsectra oberaarensis* sp. n. with taxonomic comments on the *attenuata* group (Diptera: Chironomidae)

Elisabeth Stur & Torbjørn Ekrem

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A hitherto unknown species in the *Micropsectra attenuata* group, *Micropsectra oberaarensis* sp. n., has recently been collected from high elevation in the Berner Alps (Switzerland). The male and pupa of the new species, and the previously unknown larva of the closely related *Micropsectra seguyi* Casas & Laville, are here described and diagnosed. These two species, *Micropsectra attenuata* Reiss and *Micropsectra auvergnensis* Reiss are morphologically similar as adult males, but can easily be separated in their pupal life stage. Partial COI gene sequences indicate sufficient interspecific variation to separate the morphologically two most similar species, *M. oberaarensis* and *M. seguyi*. *Micropsectra davigra* Giłka & Abramczuk is presented as a new junior synonym to *M. pharetrophora* Fittkau & Reiss and a key to males of all eight West Palaearctic species in the *attenuata* group is given. The diagnostic characters separating the genera *Parapsectra* and *Krenopsectra* from the *attenuata* group are briefly discussed.

E. Stur & T. Ekrem, Museum of Natural History and Archaeology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway; Emails: Elisabeth.Stur@vm.ntnu.no, Torbjorn.Ekrem@vm.ntnu.no

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1. Introduction

Species of the genus *Micropsectra* Kieffer are distributed throughout the Holarctic region and the immature stages are found in all kinds of freshwater habitats. Many of the species appear to have specific habitat requirements, and are thus well suited as biological indicators in assessments and biomonitoring of freshwater ecosystems.

Systematically, most European *Micropsectra* species can be placed in one of four described species groups. The *atrofasciata* group was recently revised by Stur and Ekrem (2006), while the remaining three species groups were reviewed some 25–40 years ago: the *attenuata* group (Reiss 1969), the *notescens* group (Säwedal 1976) and the *recurvata* group (Säwedal 1981).

The *attenuata* species group originally contained five species: *Micropsectra attenuata* Reiss, 1969, *M. auvergnensis* Reiss, 1969, *M. bodanica* Reiss, 1969, *M. clastrieri* Reiss, 1969, and *M. styriaca* Reiss, 1969. *Micropsectra styriaca* was, based on pupal morphology, later transferred to the genus *Parapsectra* Reiss (Reiss 1982), and three more European species were added to the group in the 1990's and in 2006: *M. seguyi* Casas & Laville, 1990, *M. pharetrophora*

Fittkau & Reiss, 1998 and *M. davigra* Giłka & Abramczuk, 2006. Giłka and Abramczuk (2006) also placed the Nepal *M. repentina* Reiss, 1971 in the *attenuata* group and discussed group-diagnostic characters of the adult males. Including the new species described below and regarding *M. davigra* as a junior synonym of *M. pharetrophora*, the *attenuata* group now contains eight West Palaearctic *Micropsectra* species. The immature stages of *M. repentina* remain unknown to science, thus, its placement in the *attenuata* group is only based on morphological characters in the adult male.

Several species in the *attenuata* group are typically associated with the mountain ranges in Central Europe, and some of them have only been found in cold water springs or at high elevations. In this study we describe the pupa and adult male of *M. oberaarensis*, a new species in the *attenuata* group, and the previously unknown larva of *M. seguyi*. The new species was discovered during field work at high altitude in the Swiss Alps.

2. Material and methods

The examined material (except for type material of *M. seguyi*) was found in benthos, drift or emergence samples from two different localities in the European Alps. Live larvae and pupae were placed separately in small water-filled containers for rearing, and the emerged adults and associated larval and pupal skins were fixed on ethanol. Non-sequenced specimens were macerated in KOH and mounted in Euparal on microscope slides. For the remaining specimens, DNA was extracted using Qiagen DNeasy tissue extraction kit before mounting.

The terminology and abbreviations follow Sæther (1980), the term "setiger" is used for the elevated, setae bearing part of the superior volsella. The following additional abbreviations are used: AAR = Larval antennal pedestal/antennal segment 1 ratio; AHR = Larval antennal pedestal/head length ratio; LOR = Lauterborn organ/length of larval antennal segment 3–5 ratio; MVR = Mentum/ventromental plate width ratio; UAS = Departamento de Biología Vegetal y Ecología, Universidad de Almería, Spain; VM = Museum of Natural History and Archaeology, Norwegian University of Science and Technology, Trondheim, Norway; ZSM = Zoologische Staatssammlung München, Munich, Germany.

PCR amplification and sequencing of partial cytochrome oxidase subunit I (COI) gene sequences were carried out with the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR was performed with a step-up program including 5 cycles with annealing temperature of 45°C and 35 cycles with annealing temperatures of 51°C. Forward and reverse sequences were assembled and edited using BioEdit 7.0.1 (Hall 1999). Kimura 2-parameter (K2P) distances were computed with PAUP* 4.10b (Swofford 1998). Sequences are available in GenBank under the accession numbers AM398687, AM398688, AM398689, AM 398714, AM398715, AM398727, AM398728, AM398734 and AM398735.

3. Results

3.1. *Micropsectra oberaarensis* sp. n.

Holotype: adult male with associated pupal exuviae (VM No. To336), Switzerland, Berner Oberland, Grimselpass, Oberaar dam, drift in stream at Berghaus N46°32.932' E08°16.684', 2350 m a.s.l., 14.VII.2005, T. Ekrem. Paratypes: 11 pupal exuviae, all data as for holotype.

3.1.1. Etymology

The species epithet 'oberaarensis' refers to the name of the type locality Oberaar in the Berner Alps.

3.1.2. Diagnostic characters

Micropsectra oberaarensis can be separated from other species in *Micropsectra* by the following combination of characters. Adult male with low AR (0.36); anal point strong and elongate; superior volsella posteromedially directed, setiger broad at base, gradually tapered to a pointing apex; digitus small, triangular point; median volsella long, slightly S-shaped, almost reaching apex of inferior volsella, spoon-shaped lamellae

Table 1. Lengths (in µm) and ratios of leg segments.

	Femur	Tibia	Tarsus 1	Tarsus 2	Tarsus 3	Tarsus 4	Tarsus 5	LR	BV	SV
p1	950	740	1,010	500	370	280	130	1.36	2.11	1.67
p2	920	840	450	250	190	130	85	0.54	3.37	3.91
p3	1,050	1,050	690	420	320	200	110	0.66	2.66	3.05

densely set on apical 1/3; inferior volsella abruptly bent medially and without obvious dorsomedian swelling. Pupa with shagreen on abdominal pleurae II–III; tergites IV–V with well sclerotized, strong, medially directed denticles in longitudinal lateral bands and large posterior patches of shagreen; less than 25 filamentous setae in anal fringe.

3.1.3. Description

Adult male (n = 1). Colouration: Body with greenish ground colour, slightly darker scutal stripes, postnotum, preepisternum, legs and antenna. Eyes brown.

Head. Antenna with 13 flagellomeres, AR 0.36, pedicel c. 75 µm long, terminal flagellomere 250 µm long, longest antennal seta 850 µm long. Head width 390 µm, distance between eyes 175 µm. Frontal tubercles present as minute knobs; temporal setae in 2 rows, 3 inner verticals, 3 outer verticals, 8 postorbitals. Lengths of palpomeres (in µm): 30, 30, 135, 130, 175; palpomere 3 with 6 sensilla clavata in subapical pit. Clypeus 65 µm long with 16 setae; cibarial pump 50 µm wide; tentorium 125 µm long, 35 µm wide.

Wing. Subcosta and media bare, all other veins and major cells with macrotrichia. Brachiolum with 2 setae; squama bare.

Legs. Pulvilli absent. Fore tibia with small scale, 25 µm long spur; mid tibial comb 15 µm long; hind tibial comb 20 µm long. Sensilla chaetica absent from all tarsi. Lengths and ratios of leg segments in Table 1.

Hypopygium (Fig. 1a). Anal tergite with separate, posteriorly directed anal tergite bands; 4 median tergite setae placed on an elevated hump anterior to anal point base; 12 ventral apical setae. Anal point 50 µm long, 25 µm wide at base, 10 µm wide at apex; with well developed, slightly curved anal crests; apex with transverse apical margin; knob between crests absent; relatively small microtrichia-free area at base of anal point. Superior volsella posteromedially directed, setiger broad at base, gradually tapered to pointing apex, with 6–7 dorsal and 2 median setae, 1 strong median seta on stem; superior volsella otherwise bare. Digitus small, triangular point. Median volsella 125 µm long, slightly S-shaped, almost reaching apex of inferior volsella, numerous simple lamella along median and ventral margins, spoon-shaped lamella densely set on distal 1/3 dorsomedially. Distal end of inferior volsella abruptly medially bent, without obvious dorsomedian swelling, bearing several median and distal setae. Inner margin of gonocoxite with 4 strong setae.

Pupa (n = 10 unless otherwise noted). Coloration: pupal exuviae brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker brown. Length 4.0–4.4 mm long, abdomen 3.1–3.5 mm long.

Cephalothorax (Figs. 1b–c). Cephalic tubercle weak, low mound; frontal seta 60–80, 74 µm long; pedicel sheath tubercle absent. Thoracic horn 300–350, 322 µm long, 35–65, 47 µm wide, with numerous 100–125, 113 µm long chaetae along most of ventral margin; precorneals arranged in a slight triangular pattern, the two anteriormost setae situated close together, anterior precorneal 65–100, 82 µm long; median precorneal 70–80, 74 µm long; posterior precorneal 90–130, 106 µm long; 1 median antepronotal 60–130, 96 µm long; 2 lateral antepronotals (1 seta 60–75, 64 µm long, 1 sensillum basiconicum); 2 pairs of dorsocentrals, Dc_1 50–90, 65 µm long; Dc_2 45–60, 55 µm long; Dc_3 70–95, 88 (7) µm long; Dc_4 55–75, 65 (9) µm long, setae in posterior pair stronger than in anterior pair. Prealar tubercle weak; nose of wing sheath weak hump.

Abdomen (Figs. 1d–e). TII almost covered by shagreen, except for one posteromedian oval patch; pedes spurii B on segment II present; hook

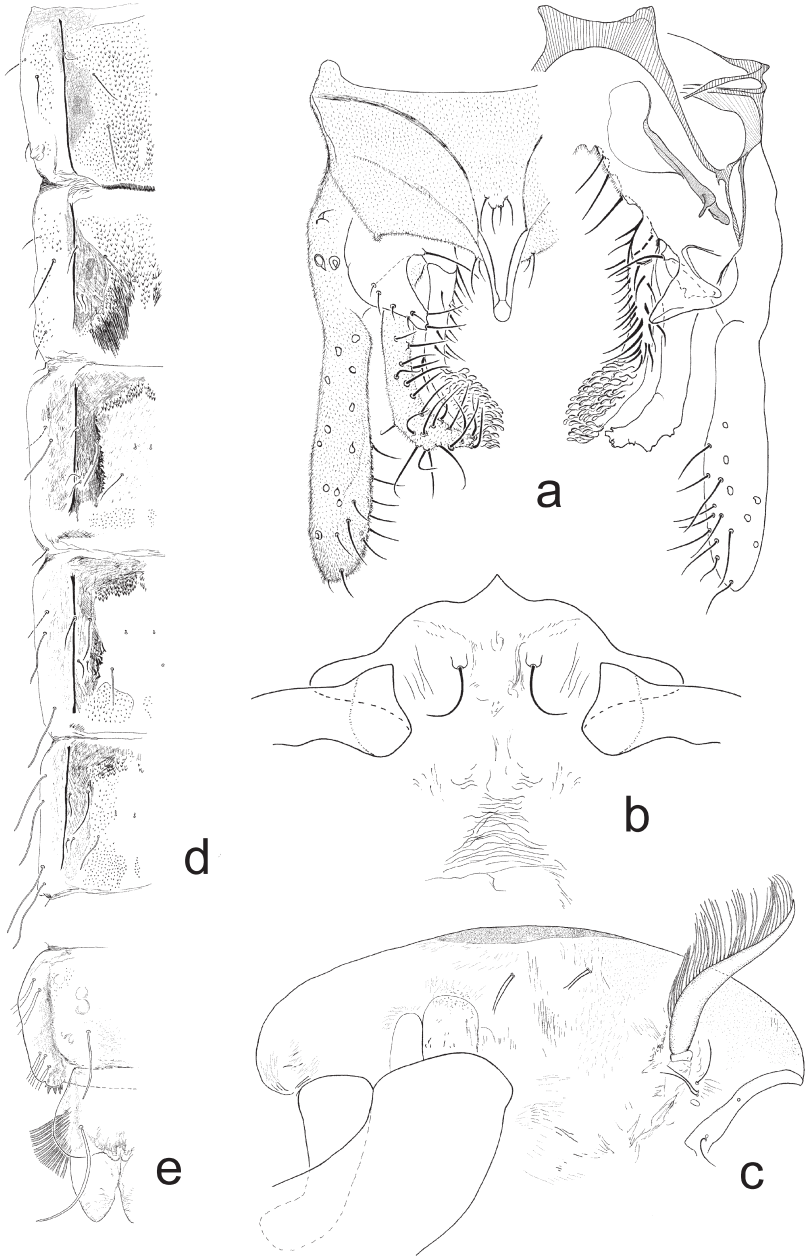

Fig. 1. *Micropsectra oberaarensis* sp. n. – a. Adult male hypopygium. – b. Pupal frontal apotome. – c. Pupal thorax. – d. Pupal abdominal tergites II–VI. – e. Pupal abdominal tergites VIII–IX.

row 160–210, 186 µm long, about half as long as segment width, with 74–85, 80 hooks; pleura on segment II with shagreen. Spines of TIII in large, laterally curved patches, shagreen extensively distributed lateral, anterior to and in between anterior part of spine patches; pleura with anterior and posterior shagreen. Armament on TIV–V consisting of spinules in anterior, oval patches and longitudinal, lateral sclerotized bands with strong, short, medially directed denticles; shagreen present in oval patches posterior to lateral bands; pleurae without shagreen. TVI with shagreen and points in π-shaped patch. Patches TVII similar to those on TVI, except much weaker and consisting only of shagreen. TVIII with small anterolateral, oval patches of shagreen. Setation: Segment I with 3 D, 3 V and 0 L setae; segment II with 2–4 D, 4 V, 3 L; segment III with 5 D, 4 V, 3

L; segment IV with 5 D, 3–4 V, 3 L; segment V with 5 D, 4 V, 2–3 L, 0–1 lateral filamentous setae; segment VI with 5 D, 4 V, 2 L, 2 lateral filamentous setae; segment VII with 4–5 D, 4 V, 2 L, 2 lateral filamentous; segment VIII with 1 dorsal and 1 ventral semi-filamentous seta, 5 lateral filamentous setae; anal tergite with 1 dorsal filamentous seta. Two pairs of small sensorial setae medially on TII–VI; 1 pair of O-setae present anteriorly on both tergites and sternites II–VII. Anal lobe with lateral margins abruptly curved posteriorly, fringe with 16–21, 18 about 400 µm long filamentous setae in 1 row. Posterolateral comb of segment VIII 25–40, 33 µm wide, usually with 5 marginal teeth. Male genital sheaths 275–300 µm long, female genital sheaths 130–150 µm long.

Larva unknown.

3.2. *Micropsectra seguyi* Casas & Laville

Micropsectra seguyi Casas & Laville, 1990: 421. Holotype male (pharate adult) (UAS) Spain, Sierra Nevada, Rio Monachil (M1), 2100 m a.s.l., 24.VI.1986. 2 paratypes (pupal exuviae) 30.V. 1987, otherwise as holotype. 1 paratype (pupal exuviae), 28.IV.1987, sample site as holotype except (M-3) red mano fuente [all examined].

Additional material examined: 6 adult males (ZSM) Germany, Bavaria, Berchtesgaden National Park, Schapbach spring, N47°34.967' E12°57.483', 1140 m a.s.l., 14.VI.1996, 21.VI. 1996, 26.VII.1996, I. Schrankel; 1 adult male (VM) & 2 pupae (1 ZSM, 1 VM) with associated larval exuviae, locality as previous, 17.VII.2000, 1.VIII.2000, 28.VI.2001, E. Stur & S. Wiedenbrug; 2 adult males (VM, To333, To334) 27.V. – 14.VI.2005, F. Eder, locality as previous specimens.

3.2.1. Diagnostic characters

Micropsectra seguyi can be separated from other *Micropsectra* species by the following combination of characters. Adult male with AR c. 0.6; anal point well developed, triangular, with blunt apex; superior volsella posteromedially directed, setiger broad at base, gradually tapered to a pointing apex; digitus small, triangular point; median volsella long, S-shaped, almost reaching apex of inferior volsella, large, spoon-shaped lamellae densely set on apical 1/3; inferior volsella slightly bent medially, with broad, rounded apex and without obvious dorsomedian swelling. Pupa without spines on abdominal tergites IV–V; abdominal tegite IV with semi-circular denticulate and well sclerotized protrusion in the lateral pigment bands; abdominal tergite V with a few points only in the lateral bands; few shagreen points in small posterior patches in abdominal tergite IV; 22–37 filamentous setae in anal fringe. Larva without spur on antennal pedestal; AR very high (over 2.0); LOR > 5; mentum with 11 teeth; ventromental plates more curved laterally, with rather coarse striation.

3.2.2. Description

Adult male and pupa described by Casas & Laville (1990).

Larva (n = 2 unless otherwise noted). Colouration: Head capsule brownish. Total length not measurable.

Head (Figs. 2a–e). Head capsule 300–325 µm long, 250–275 µm wide; antennal pedestal 75–80 µm long without spur; length of antennal segments (in µm): 135–155, 55(1), 7 (1), 7 (1), 5 (1); AR 2.09 (1); antennal segments 1–2 well sclerotized, segments 3–5 pigmented, antennal seta placed at about 2/3 length of antennal segment 1, apparently minute c. 10 µm long; antennal blade present, length not visible in mount; Lauterborn organs small, on 100 µm long pedestals; LOR 5.52; AHR 0.25; AAR 0.52–0.56. SII plumose; chaetae pectinate; some chaetulae pectinate; labral lamella with 20 teeth; premandible (Fig. 2b) with 2 robust teeth, inner tooth broader, well developed brush. S3 simple. Mandible (Fig. 2c) 113–120 µm long, 55–68 µm wide, with three inner teeth, one apical and one dorsal tooth, pecten mandibularis slightly convex, seta subdentalis 50 µm long extending beyond apical mandibular tooth; two spines closely set on mola. Mentum (Fig. 2d) with 11 teeth, 87–88 µm wide, middle 3 teeth somewhat paler than rest, middle tooth without lateral notches; ventromental plates somewhat stronger curved laterally, 92–95 µm wide with fairly coarse striation along whole length; MVR 0.92–0.95. Postoccipital plate (Fig. 2e) well developed, semicircular.

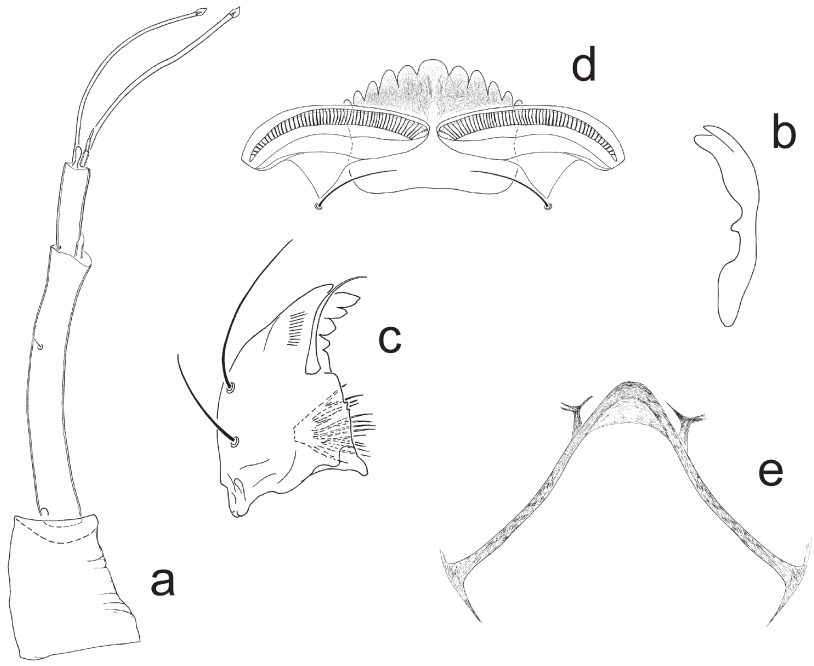

Fig. 2. *Micropsectra seguyi* Casas & Laville, larva. – a. Antenna. – b. Premandible. – c. Mandible. – d. Mentum. – e. Postoccipital rim and plate.

Body. Anterior parapods with long, simple spines; hind parapods with c. 30 simple hooks in two rows; L2 simple; anal tubules not observable; supraanal seta c. 100 µm long; procercus with 1 short and 1 long dorsal seta, with 4 short (250 µm long) and 4 long (450 µm long) anal setae.

3.3. Genetic variation in mitochondrial DNA sequences

The genetic K2P distances between partial cox1 sequences of nine specimens and four species in the *attenuata* group show that interspecific variation greatly exceeds intraspecific variation (Table 2). The minimum interspecific variation among the analysed species is almost 7.3% (average = 14.6%) while the maximum intraspecific variation is close to 1.1% (average = 0.7%).

4. Key to male imagines of West Palaearctic species of the *Micropsectra attenuata* species group

Males of *Krenopsectra*, *Parapsectra* and the *Micropsectra attenuata* group can be difficult to separate as adult males (Cranston *et al*. 1989).

Table 2. Pairwise Kimura-2-parameter distances between COI sequences of specimens in the *attenuata* species group.

	To109	To131	To302	To11	To12	To333	To334	To336	To349
M. attenuata To109	–								
M. attenuata To131	0.01081	–							
M. attenuata To302	0.01081	0.00306	–						
M. pharetrophora To11	0.15898	0.16096	0.16292	–					
M. pharetrophora To12	0.15306	0.15501	0.15696	0.00461	–				
M. seguyi To333	0.15454	0.15842	0.16234	0.14518	0.14496	–			
M. seguyi To334	0.15648	0.16038	0.16431	0.14708	0.14686	0.00153	–		
M. oberaarensis To336	0.16431	0.16038	0.16431	0.15859	0.15842	0.07284	0.07458	–	
M. oberaarensis To349	0.16038	0.15648	0.16038	0.15470	0.15454	0.07284	0.07458	0.00306	–

However, the stem of the median volsella in *Krenopsectra* is always long and distinctly club-formed with broad, sickle-shaped, medially curved lamellae, a combination of characters which is never encountered in *Parapsectra* or *Micropsectra*. Good diagnostic characters for males of *Parapsectra* are harder to find, thus species from this genus and the *attenuata* group are included in the below key. Immature stages of the genus *Micropsectra* are more readily separable from those of *Parapsectra* and *Krenopsectra* (Pinder & Reiss 1983, 1986), although the larvae of *Micropsectra pharetrophora* resembles those of *Krenopsectra* in having a dorsal hump on segment 11 (Fittkau & Reiss 1998).

1. Wing length less than 3 mm; AR lower than 1.0; mid and hind tibial combs without spurs; superior volsella triangular, tapered to a pointed apex; digitus strongly reduced or absent; median volsella long, stem reaching beyond posterior margin of superior volsella *Parapsectra* part., *attenuata* species group 2
– Character combination not as above *Parapsectra* part., other species groups in *Micropsectra*, not keyed.
2. Anal point apex sharply pointed, acute; median volsella slightly thicker in distal end, straight or slightly laterally curved, bearing numerous (c. 35–45) spoon-shaped lamellae 3
– Anal point apex blunt or concave or slightly pointed; median volsella long, thin, usually tapered towards tip, straight or medially curved; usually bearing comparatively few, spoon-shaped or spatulate lamellae; if numerous spoon-shaped lamellae, then anal point blunt and median volsella medially curved 4
3. Crests of anal point ending at half length of point; anal tergite with well developed lateral teeth; superior volsella with 3 apical setae on median margin *M. repentina*
– Crests of anal point ending close to apex of point; anal tergite without or very reduced lateral teeth; superior volsella with 2 apical seta on median margin *M. pharetrophora*
4. Anal point with concave apex; median volsella more or less straight 5
– Anal point with convex, blunt or pointed apex; stem of median volsella S-shaped with apex medially directed 6
5. Small digitus present; spoon-shaped lamellae placed distally on median margin of long, thin median volsella; distal inner margin of gonostylus oblique towards apex *M. auvergnensis*
– Digitus absent; large spatulate lamellae placed distally on comparatively short median volsella; gonostylus evenly tapered distally *Parapsectra styriaca*
6. Stem of median volsella < 55 µm long, reaching only slightly beyond distal margin of superior volsella 7
– Stem of median volsella > 60 µm long, reaching well beyond distal margin of superior volsella, almost to apex of inferior volsella 8
7. Green; small, wing length 1.3 mm; LR_1 c. 1.7; anal point apex pointed *M. clastrieri*
– Green with brown mesonotal stripes; comparatively larger, 1.7–2.0 mm; LR_1 c. 1.4; anal point apex blunt *M. bodanica*
8. Median volsella with distal, large spatulate lamellae that are medially directed *M. attenuata*
– Median volsella with distal spoon-shaped lamellae 9
9. AR >0.6; anal tergite without lateral teeth *M. seguyi*
– AR < 0.4; anal tergite with small lateral teeth *M. oberaarensis*

5. Discussion

Micropsectra oberaarensis is similar to *M. attenuata, M. auvergnensis* and *M. seguyi* in the adult male stage, but can be separated from these by several characters: From *M. attenuata* by the lower AR and more numerous and smaller spoon-shaped lamellae of the median volsella, from *M. auvergnensis* by the lower AR, S-shaped median volsella and abruptly bent inferior volsella, and from *M. seguyi* by the lower AR (0.36 opposed to > 0.6) and by the presence of a small lateral tooth on the anal tergite. The pupa of *M. oberaarensis* will key to *M. auvergnensis* in the key to west Palaearctic pupae (Stur & Ekrem 2006) due to the presence of shagreen on tergites II–III, but it is

more similar to the pupa of *M. seguyi* (Casas & Laville 1990, figs. 5–6). However, the stronger sclerotization and stronger armament of tergites IV–V and fewer filamentous setae in the anal fringe will separate *M. oberaarensis* from this species. The larva of *M. seguyi* is included in Stur and Ekrem's (2006) key to West Palaearctic *Micropsectra* larvae, and can easily be separated from all other known *Micropsectra* larvae by the absence of a spur on the antennal pedestal, high AR and high LOR.

It has recently been demonstrated that partial COI gene sequences, so called DNA barcodes, can be valuable tools to separate and identify species in Chironomidae (Ekrem *et al*. 2007), and by utilising the gap between intraspecific and interspecific variation in genetic distances, DNA barcoding has been proposed as a standardized method for species identification (e.g. Hebert *et al*. 2003). Although the sample size in our study is too small to fully understand the COI variation among all species in the *attenuata* group, the 'barcode gap' in genetic distances between *M. attenuata, M. pharetrophora, M. oberaarensis* and *M. seguyi* (Table 2) supports our morphological data that these taxa are separate species.

Giłka and Abramczuk (2006) were unaware of Fittkau and Reiss' (1998) description of *M. pharetrophora* when they presented *M. davigra* as a species new to science (Giłka pers. comm.). The two species have no notable morphological differences in the adult male (Giłka pers. comm.) and the immatures of *M. davigra* are unknown. We therefore consider *M. davigra* to be a new junior synonym of *M. pharetrophora*. Another species suspected to be synonym to *M. pharetrophora* is the morphologically similar *Micropsectra shinaensis* (Tokunaga, 1940). The type material of *M. shinaensis* apparently is lost, but we were able to examine the specimen described by Sasa in 1988 (specimen no. 128:31 in the Sasa collection) and can confirm that this specimen fits Tokunaga's (1940) description in having numerous microtrichia among the setae on the setiger of the superior volsella (Sasa 1988, plate 4, fig. A5–6; Tokunaga 1940, fig. 84). *Micropsectra pharetrophora* also has microtrichia on the superior volsella, but these are lining only the base of the superior volsella (Fittkau & Reiss 1998, own observation). This character will separate males of *M. shinaensis* from those of *M. pharetrophora* and the two geographically disjunct species are here therefore not regarded as synonyms.

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