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Molecular and morphological methods reveal cryptic diversity and three new species of Nearctic *Micropsectra* (Diptera:Chironomidae)

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Abstract. We used an integrative-taxonomy approach to help resolve taxonomic issues within the genus *Micropsectra* (Diptera:Chironomidae). We used partial cytochrome *c* oxidase subunit I (COI) and nuclear carbamylphosphate synthetase (CAD) sequences and morphological data to provide a framework for better understanding of North American species in this group of nonbiting midges. As part of our results, we describe 3 new species: *Micropsectra neoappendica*, n. sp., *Micropsectra penicillata*, n. sp., and *Micropsectra subletteorum*, n. sp., and 1 species new to the north-central USA, *Micropsectra xantha* (Roback). Two of the species, *M. neoappendica* n. sp. and *M. subletteorum* n. sp., initially appeared to be morphologically identical to species known from the Palearctic. However, molecular data indicated that they were genetically distinct, and reexamination of adult and pupal morphology revealed slight but consistent diagnostic differences. The implications of using species-level identifications for cryptic-species complexes in biological monitoring and conservation management are briefly discussed with reference to our findings. Our results emphasize the importance of using molecular tools in conjunction with traditional morphological techniques when studying Chironomidae diversity, especially when relying on diagnoses from other geographic regions.

Key words: Tanytarsini, *subletteorum*, *penicillata*, *neoappendica*, *xantha*, descriptions, key, phylogeny, cryptic species, integrative taxonomy, DNA taxonomy, mitochondrial COI, CAD, Nearctic.

Chironomidae taxonomy and systematics for several genera are arguably more advanced in the Palearctic region than in the Nearctic. Therefore, keys and species descriptions used for many Nearctic Chironomidae often are drawn from Palearctic literature. A common experience among users of taxonomic keys for many aquatic invertebrates is to end with a name that fits the specimen in question, but to find slight discrepancies when comparing species descriptions and the specimen. These discrepancies may or may not indicate that the specimens are heterospecific. Many transAtlantic species do bear a strong resemblance to one another, but it is often unknown whether Nearctic and Palearctic counterparts are truly conspecific (Epler 2001).

The fact that many species descriptions are based primarily on morphology of the adult male, often

without incorporating other life-stages, adds to this problem, especially because closely related species can exhibit strong similarities in this life stage. For example, Townes (1945) originally synonymized the Nearctic *Polypedilum* (*Polypedilum*) *flavum* (Johannsen, 1905) with the Palearctic species *Polypedilum* (*Polypedilum*) *convictum* (Walker, 1856) because of morphological similarity of the adult males. However, the immature stages are quite different (Epler 2001). This example emphasizes the importance of including multiple life stages in a description whenever possible.

Molecular techniques, such as deoxyribonucleic acid (DNA) barcoding, may provide alternative means for differentiating species. These techniques are especially useful for separating morphologically cryptic species (Sinclair and Gresens 2008, Pauls et al. 2010, Carew et al. 2011, Takano et al. 2011) and associating multiple life stages (Carew et al. 2005, Willassen 2005, Ekrem et al. 2007, 2010a, Zhou et al. 2007, Stur and Ekrem 2011). DNA barcoding is an

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invaluable tool that aids in species identification and evaluation of whether morphologically similar trans-Atlantic species are truly conspecific (Sinclair and Gresens 2008).

One of the most species-rich genera within Chironomidae is *Micropsectra* Kieffer, 1909, which comprises ≥ 140 named species known from the Holarctic region and a few species known from areas outside the Holarctic (see Stur and Ekrem 2006). Some *Micropsectra* are found in oligotrophic or mesotrophic lakes, but most species in the genus occur in lentic areas of springs and streams (Säwedäl 1982). For example, Stur and Wiedenbrug (2006) found high abundance and diversity of *Micropsectra* species in 2 groundwater spring communities in Berchtesgaden National Park, Germany. The new species described below come from similar spring-fed stream habitats. These specific habitat preferences make the genus quite important in biological monitoring studies (Stur and Ekrem 2006), but habitat preferences are most informative when used at the species level. For example, the rapid bioassessment protocol published by Barbour et al. (1999) lists tolerance values for many Nearctic Chironomidae genera and species, partitioned by region. The values assigned to the genus *Micropsectra* differ significantly by region, and range from 1.4 to 7 on a 10-point scale, where 0 indicates extreme sensitivity. Species-level sensitivities for the *Micropsectra* in our study were not presented by Barbour et al. (1999). Unfortunately, not enough information is currently available for Nearctic species in the genus to provide an accurate assignment of pollution tolerance of the genus or to assign tolerance values to species within *Micropsectra*. Further knowledge of habitat preference and identification tools for the genus will help resolve this problem.

Presently, species within *Micropsectra* are divided into 4 species groups, including *atrofasciata*, *attenuata*, *notescens*, and *recurvata* groups. However, these groups are based primarily on European fauna and have to be reconsidered as more species from other parts of the world are described. Moreover, all recent revisions of the genus *Micropsectra* have been confined primarily to major species groups recognized from the Palearctic (Reiss 1969a, Säwedäl 1976, 1981, 1982, Stur and Ekrem 2006, 2008, Ekrem et al. 2010b). Some *Micropsectra* species are thought to be Holarctic in distribution, but close examination of some Palearctic and Nearctic populations can reveal genetic and morphological differences large enough to be regarded as diagnostic at the species level.

We used a combination of morphological and molecular approaches to help refine species-group classifications within *Micropsectra* and began an

examination of morphological and molecular differences between some cryptic species within the genus. We attempted to provide a framework for a better understanding of North American *Micropsectra* and, subsequently, the genus as a whole. We used sequences from mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear carbamylphosphate synthetase (CAD) genes to assess whether morphologically similar specimens from Nearctic and Palearctic populations are conspecifics with a trans-Atlantic distribution or whether sufficient genetic variation exists to classify these specimens as distinct species. We also used the resulting CAD phylogenies to discuss whether morphological traits can be used independently to define species groups within *Micropsectra*.

Materials and Methods

Sampling and data collection

Larval *Micropsectra* specimens were obtained from the benthos of 5 streams in Minnesota, USA, during late winter/early spring 2010. All specimens were held in constant-temperature incubators at 5°C to approximate stream temperatures and allowed to develop to adulthood in mass rearings, resulting in a cumulative total of 99 adult *Micropsectra* with associated pupal exuviae. Rearing chambers were typically checked twice per day to ensure that emerging adults were associated with the appropriate exuviae. Upon emergence, adults and associated exuviae were stored in individual vials and preserved in 95% ethanol. Unfortunately in nearly all cases, larval exuviae could not be retrieved. The antennae, head, wings, legs, and hypopygium were dissected. The head and hypopygium were cleared in 10% KOH and slide mounted in Euparal® with the pupal exuviae, legs, wings, and antennae, for species identification. The thorax and abdomen were slide-mounted after DNA extraction (described below). On the few occasions where >1 fly of the same sex emerged between observations, the adults and exuviae were stored in the same vial until dissection. Adults were mounted on individual slides as described above, but both exuviae were mounted on a single, separate slide. All specimens were identified and separated into 4 morphotypes. A subset of 46 specimens collected from Minnesota streams was selected for molecular analyses (Table S1; available online from: <http://dx.doi.org/10.1899/12-020.1.s1>). Specimens were selected to include representatives from each *Micropsectra* morphotype. In addition, 1 *Tanytarsus* specimen was sequenced to include among the outgroup species.

The full COI data set consisted of 291 individuals, including 5 outgroup species and 44 *Micropsectra* species (31% of known *Micropsectra* species). Of these, 4 species (43 individuals) were collected in Minnesota: *Micropsectra neoappendica* n. sp., *Micropsectra penicillata* n. sp., *Micropsectra subletteorum* n. sp., and *Micropsectra xantha* (Roback, 1955) (Table S1). The remaining sequences were acquired from GenBank, and included 3 taxa of *Tanytarsus* and 2 taxa of *Paratanytarsus*, which served as outgroups (Table S1). A 2nd reduced COI data set that conformed to the CAD data set (described below) also was generated and analyzed. The CAD data set consisted of 82 individuals from 37 species (33 *Micropsectra* and 4 outgroup taxa; 27% of known *Micropsectra* species). Twelve individuals were from the above-named *Micropsectra* taxa. The remaining sequences were acquired from GenBank, and included 2 *Paratanytarsus* and 2 *Tanytarsus* species that served as outgroups. Voucher and sequence information for all specimens collected are also accessible in BOLD project “Nearctic *Micropsectra* (NAMIC)” and the BOLD data set “Holarctic *Micropsectra* (DS-HOLMIC)” (<http://boldsystems.org>). Remaining DNA extracts are stored at the Museum of Natural History and Archaeology, Norwegian University of Science and Technology (VM). A detailed record of all taxa, including collection localities and GenBank accession numbers, is available in Table S1.

DNA extraction, amplification, and sequencing

A GeneMole[®] instrument (Mole Genetics AS, Lysaker, Norway) was used to extract DNA from most specimens, following standard protocol for the MoleStrips DNA Tissue kit. DNA was extracted from remaining specimens using protocol for the Qiagen DNeasy tissue extraction kit (Qiagen, Oslo, Norway), with the exception that less elution buffer (150 µL) than recommended was used because of small specimen sizes. Polymerase chain reaction (PCR) amplification and sequencing was carried out on partial COI and CAD genes using the primer pairs and PCR programs described in table 1 of Ekrem et al. (2010b). We used HotStar Taq (Qiagen) when amplifying COI and TaKaRa HS ExTaq (Takara Bio Inc., Otsu, Shiga, Japan) for amplification of CAD. PCR products were purified using ExoSAP-IT[®] (Affymetrix Inc., Cleveland, Ohio) and sent to an external sequencing service (Eurofins MWG Operon, Ebersberg, Germany). All gene products were sequenced bidirectionally, and forward and reverse sequences were aligned and manually edited using Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan). Conflicting or ambiguous base calls were initially

given the appropriate International Union of Biochemistry ambiguity symbol. Initial ambiguities were checked after alignment and corrected, if possible, with reference to trace files. Consensus sequences were assembled and initially aligned in BioEdit (version 7.0.5.3; Hall 1999) for inspection. Introns in CAD sequences were recognized by the guanine thymine-adenine guanine (GT-AG) rule (Rogers and Wall 1980) and eliminated from the alignment because these areas exhibited extreme variation among species. All CAD sequences had intron placement at position 725 and no indication was seen of paralogous gene copies. CAD exons were aligned by their amino acids in MEGA5 (Tamura et al. 2011; available from: www.megasoftware.net) using the Clustal-W algorithm with the BLOSUM weight matrix and gap opening costs of 10 and gap extension costs of 0.1 and 0.2 in pairwise and multiple alignments, respectively. Alignment of COI sequences was trivial because no indels were observed within the sequences.

Tree-based species delimitation and phylogenetic analyses

Phylogenetic analysis of the initial full COI data set was inferred with neighbor joining (NJ) and maximum likelihood (ML) methods in MEGA5. The Kimura 2-parameter (K2P) substitution model was used for the NJ analysis to infer evolutionary distances because it has been a standard substitution model in other barcode studies, thus allowing comparisons among studies. Although this method is commonplace in barcoding studies, recent authors have questioned the strict use of the K2P model (Srivathsan and Meier 2012). This concern prompted us to include ML analyses with 500 bootstrap replicates using the General Time Reversible model with a discrete Gamma distribution and invariable sites (GTR+G+I), which was the most appropriate nucleotide substitution model for our data set based on the Bayesian (*BIC*) and corrected Akaike Information Criterion (*AIC_c*) in the ML model selection feature of MEGA5.

The phylogenetic relationships among species in *Micropsectra* were further explored using CAD because of its greater ability to reconstruct lower-level phylogenies in Diptera (Winterton et al. 2007, Winkler et al. 2009, Ekrem et al. 2010b). The best-fit model of substitution was identified using a ML criterion in MEGA5. *BIC* indicated that the Tamura 3-parameter model with a discrete Gamma distribution and invariant sites (T92+G+I) was the most appropriate model, whereas *AIC_c* indicated that the GTR+G+I model best fit our CAD data set. Therefore, we used the GTR+G+I model for all ML and Bayesian analyses

because the T92 model is not incorporated in RAxML (version 7.4.2; Stamatakis 2006) or MrBayes (version 3.1.2; Huelsenbeck and Ronquist 2001; <http://mrbayes.sourceforge.net/>). The best-fit partition scheme was found using PartitionFinder (version 1.0.1; Lanfear et al. 2012) and contained 2 partitions in the nucleotide sequences: 3rd position and 1st+2nd position. Multiple tree-building methods were used to assess whether phylogenetic results were robust to different analytical assumptions. ML analysis with 500 bootstrap replicates was run on the partitioned data set in RAxML using the software *raxmlgui* (Silvestro 2012). Bayesian analysis (BA) was done on the partitioned data set with MrBayes. For BA, we specified flat prior probabilities and ran 2 parallel analyses, each with 4 chains, for 5 million generations, sampling every 1000th generation and using a 10% burn-in. We examined the trace files with Tracer 1.5 (Rambaut and Drummond 2008; <http://tree.bio.ed.ac.uk/software/tracer/>) to verify that Markov Chain Monte Carlo (MCMC) convergence had occurred and that the estimated sample size (ESS) had reached an acceptable level before summarizing the data from the sampled trees in a consensus. Trees created by BA were imported and modified for presentation in MEGA5. A reduced COI data set, corresponding to the same individuals analyzed for CAD, also was analyzed phylogenetically with ML using the GTR+G+I substitution model and 500 bootstrap replicates in MEGA5. Last, a concatenated data set containing COI and CAD sequences was constructed and analyzed with both RAxML and MrBayes with the following 5 partitions identified by PartitionFinder: COI 1st position, COI 2nd position, COI 3rd position, CAD 1st+2nd position, CAD 3rd position.

Species descriptions and specimen deposition

Most of the morphological terminology and abbreviations used here follow Sæther (1980), with the following exceptions: *taeniae*, introduced by Langton (1994), is used to describe the flattened setae found on the pupal exuviae and the term *setiger* (Spies 1998) describes the setae-bearing area of the superior volsella on the male hypopygium. The following abbreviations also are used: P♂ = associated pupal exuviae and adult male; P♀ = associated pupal exuviae and adult female; L_{hc}P♂ = associated larval head capsule, pupal exuviae, and adult male. Measurement techniques follow those outlined by Sponis (1977), with the exception of the gonocoxite length, which was measured along the limb's longest access, as described by Stur and Spies (2011). Lengths of the genital volsellae were measured along their median margin, and the anal point was measured from the

anterior ends of the anal crests to the tip of the anal point. Mensural data are presented as ranges, followed by the mean and number of observed specimens.

Holotypes and most paratypes are deposited in the University of Minnesota Insect Collection in St Paul, Minnesota, USA (UMSP). Additional paratypes are deposited in VM. We also examined type material and other reference material of closely related taxa from the following collections (abbreviations correspond to those used in text): The Natural History Museum, London, UK (BMNH); Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (CNC); private collection of Peter H. Langton, Londonderry, Northern Ireland (PHL); UMSP; VM; Natural History Collections, Bergen Museum, University of Bergen, Norway (ZMBN); and Zoologische Staatssammlung München, Munich, Germany (ZSM).

Results

Tree-based species delimitation

All Nearctic specimens collected for our study formed distinct monophyletic groups at the species level in analyses of the COI, CAD, and the concatenated COI/CAD data, and distinctly separated the 4 Nearctic *Micropsectra* from morphologically similar Palearctic counterparts (Figs 1, 2, Figs S2, S3; available online from: <http://dx.doi.org/10.1899/12-026.1.s2> and <http://dx.doi.org/10.1899/12-026.1.s3>). This result is particularly notable for 2 of the Nearctic species, *M. neoappendica* n. sp. and *M. subletteorum* n. sp., which are very similar morphologically to species known from the Palearctic. *Micropsectra subletteorum* n. sp. is closely allied morphologically with many species within the *notescens* species group. Males of *M. neoappendica* n. sp. fit the species description of the Palearctic *Micropsectra appendica* Stur and Ekrem, 2006 almost perfectly. Only after divergence patterns were discovered with molecular analyses of COI and CAD markers, did subsequent examination reveal subtle but corroborating morphological characters that can be used to differentiate *M. neoappendica* and *M. appendica* (see Species Description below).

NJ and ML analysis yielded very similar results for the analysis of our COI data. Other than minor differences in bootstrap support values, no notable differences were found, and all species clusters were well resolved in both analyses. However, because of recent contention regarding the use of K2P and strictly NJ approaches (Srivathsan and Meier 2012), the results based on the ML analysis are the focus of our discussion. COI ML analyses of the full and the

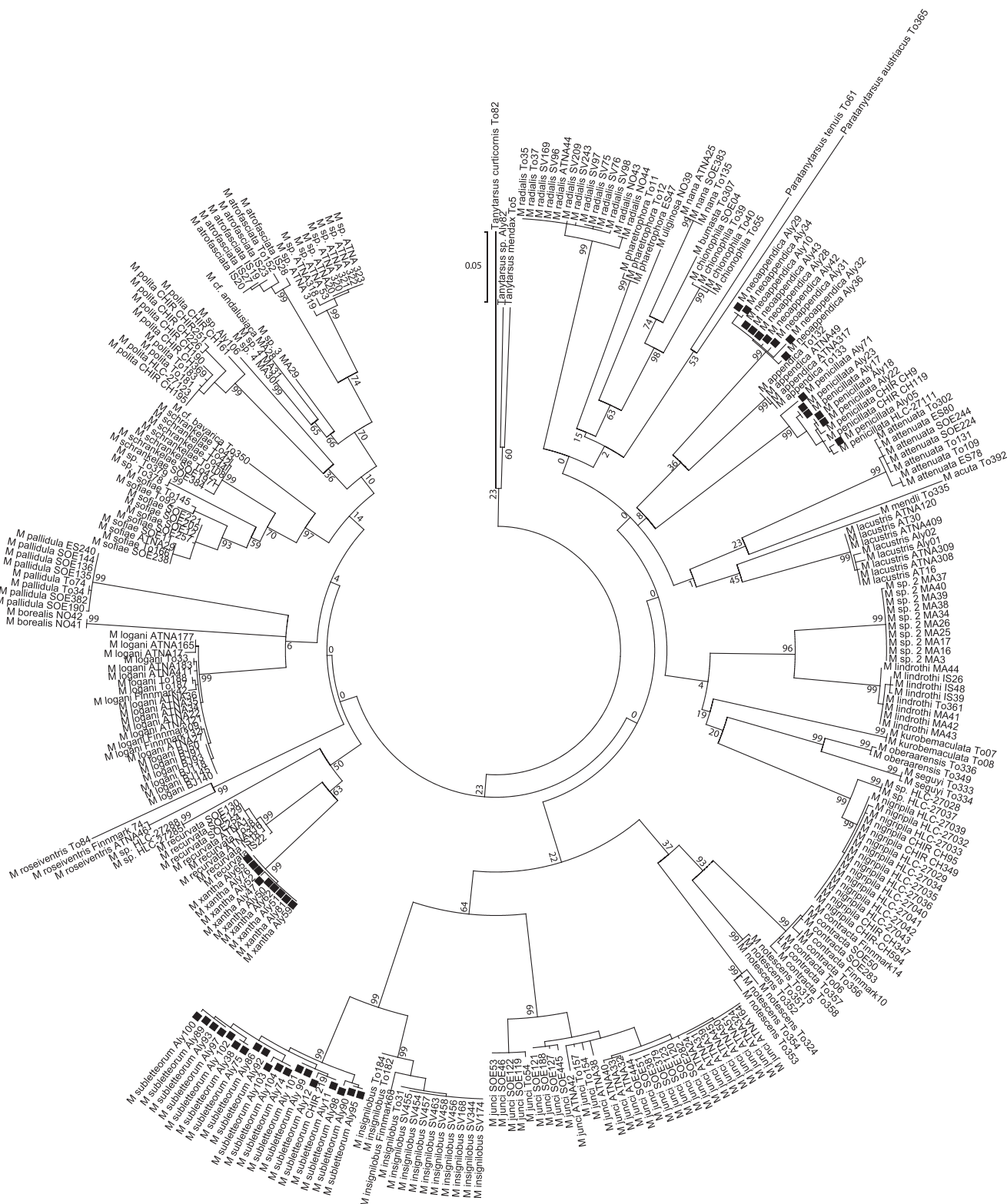


FIG. 1. Full mitochondrial cytochrome *c* oxidase subunit I (COI) maximum likelihood (ML) phylogeny of 291 individuals from 49 species. Taxa denoted with ■ were collected in Minnesota and are described/redescribed in our study. Numbers on branches indicate ML bootstrap support values. Values within highly supported (>90%) species clades were removed for ease of interpretation. Low bootstrap support values are included for the higher-level relationships to indicate the inability of COI

reduced data sets formed strongly supported species clusters. All bootstrap support values for species clusters in the analysis of the full data set were >90% (Fig. 1). A similar trend was seen in the reduced COI ML and NJ/K2P analyses, with all species clusters with bootstrap support > 90%, except *Micropsectra sofiae* Stur and Ekrem, 2006 (bootstrap support = 65%).

Phylogenetic analyses

CAD phylogenies from ML and BA yielded similar species-group patterns, with no notable differences other than support values for certain clades (Fig. 2). One such difference was found in the support for the *Micropsectra kurobema*–*Micropsectra lindrothi* clade. BA indicated strong support for this clade and placed these 2 species together (posterior probability [pp] = 95%) and as sister to the remaining species within the *notescens* group (pp = 99%), whereas ML analysis indicated much weaker support for these placements (bootstrap support values <50%). We choose to leave these 2 species as members of the *notescens* group at this point, but recognize that as more species are included in analyses, a more appropriate placement may exist for *M. kurobema*–*M. lindrothi* (Sasa and Okazawa, 1992) and *M. lindrothi* Goetghebuer, 1931. Another difference between BA and ML analysis was in the placement of *Micropsectra roseiventris* (Kieffer, 1909). BA placed this species as sister to the remaining in-group taxa (Fig. 2), with a pp = 0.66. ML analysis, on the other hand, weakly indicated that this taxon is sister to *Paratanytarsus* (bootstrap support = 0.44). Further analysis of the relationship between *Paratanytarsus* and *Micropsectra* is beyond the scope of our study, but warrants future research.

Analysis of the concatenated COI/CAD data yielded results that were similar to the CAD phylogeny with a few discrepancies (Fig. S2). Most notable is the placement of the *lacustris* group within the phylogeny. The CAD phylogeny depicts this group as sister to the *atrofasciata* group, whereas the combined COI/CAD phylogeny indicates this group is sister to the *atrofasciata*, *notescens*, and *recurvata* groups.

Analyses yielded strongly supported *atrofasciata* and *notescens* species groups and a paraphyletic *recurvata* group, results that correspond well with

conclusions of Ekrem et al. (2010b) (Fig. 2). *Micropsectra lacustris* Sæwæd, 1975, here designated as a member of the newly re-erected *lacustris* group was previously considered a member of the *recurvata* group. We could not confirm the finding of a paraphyletic *attenuata* group found by Ekrem et al. (2010b) because CAD sequences are not currently available for *Micropsectra pharetophora* Fittkau and Reiss, 1999, the specimen accounting for paraphyly in this group. Results for the species described in our study show *M. neoappendica* n. sp. as sister to *M. appendica* in the *atrofasciata* group, *M. penicillata* n. sp. as sister to *M. lacustris* in the *lacustris* group, *M. subletteorum* n. sp. as sister to *M. insignilobus* Kieffer, 1924 in the *notescens* group, and *M. xantha* as sister to *M. recurvata* Goetghebuer, 1928 in the *recurvata* group (Fig. 2).

Description of Species

Micropsectra neoappendica Anderson, Stur & Ekrem, new species (Figs 3, 4A)

Type material

Holotype.—P♂. **USA, Minnesota:** Cook County, Fiddle Creek, Forest Rd. 152/Lima Grade, stream access ~100 m NW of South Brule River bridge near edge of road, 47°56'35"N; 90°26'17"W, 505 m, larva collected 11.iv.2010, A. M. Anderson (UMSP, on slide; VM, DNA extract; voucher ID: ALY29).

Paratypes.—8 P♂, 11 P♀. All data as for holotype (7 P♂, 10 P♀ UMSP; 1 P♂, 1 P♀ VM), voucher ID and GenBank accession codes in Table S1.

Diagnostic characters.—The following combination of characters separates this species from others currently in the *atrofasciata* group: Adult male. AR ~1.27, LR₁ ~1.52. Anal point long with high, slightly curved crests, with long, rounded point. Elevated hump anterior to base of anal point; hump typically with small rounded point at tip. Superior volsella roundish. Digitus short, thin, and triangular. Median volsella club-shaped with numerous spoon-shaped lamellae on distal 1/3; length of median volsellae ~123 µm. Pupa. Total length ~4.6 mm. Thoracic horn relatively short, ~306 µm with chaetae on distal 2/3. Distance between anterior and posterior dorsocentral setae pairs approximately equal. Nose of wing sheath moderate to strong. Patches of TIV

←

sequences to resolve relationships between species and species groups clearly, particularly when compared to nuclear carbamylphosphate synthetase (CAD) sequences (Fig. 2). The scale bar indicates branch lengths as number of substitutions per site corrected by the General Time Reversible model with a discrete Gamma distribution and invariable sites model (GTR+G+I).

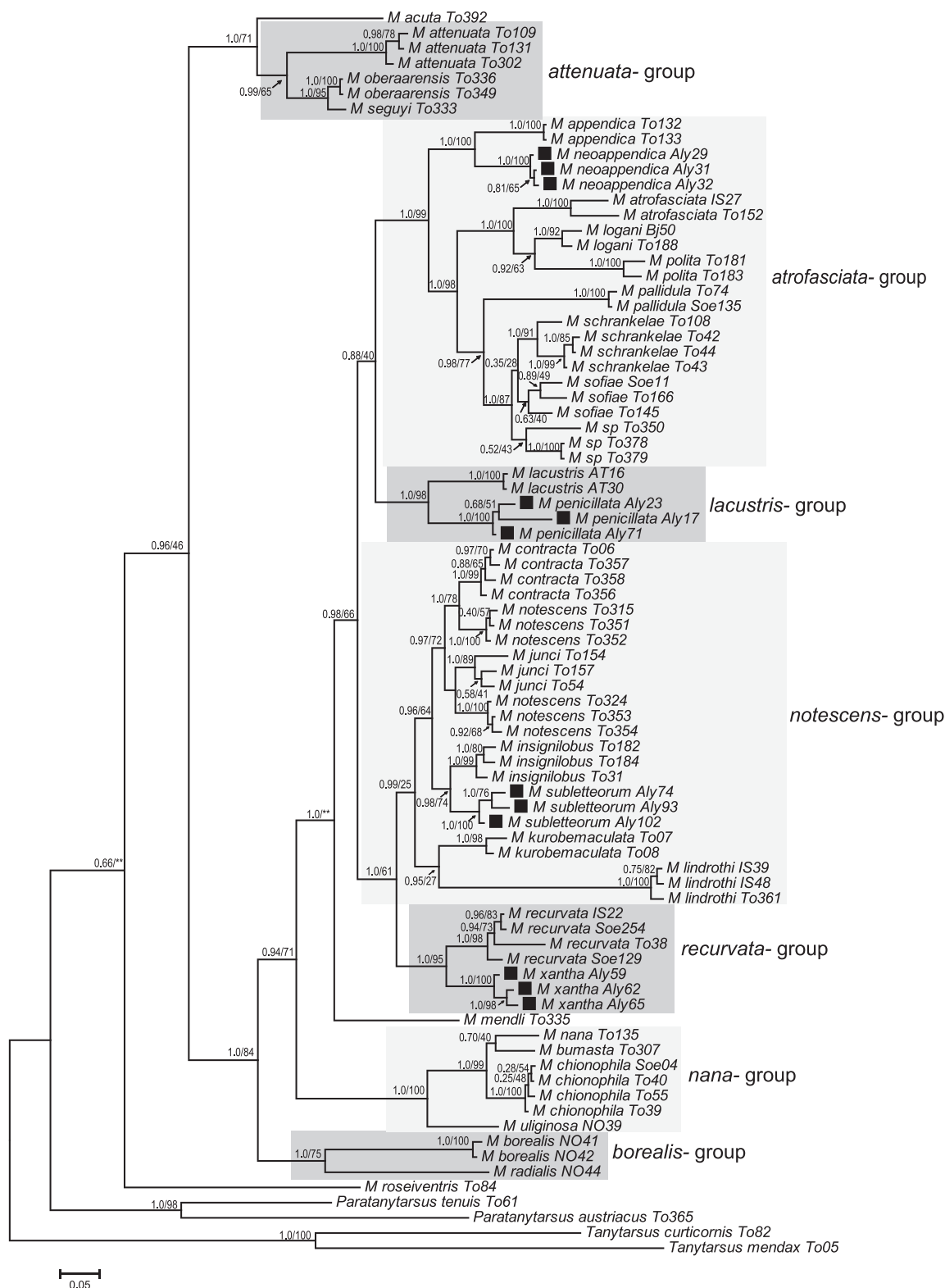


FIG. 2. Carbamylphosphate synthetase (CAD) phylogeny of 82 individuals from 37 species, based on Bayesian (BA) and maximum likelihood (ML) analysis. Taxa denoted with ■ were collected in Minnesota and are described/redescribed in our study. Shaded boxes enclose current or proposed species groups. Values on branches indicate BA posterior probabilities and ML bootstrap support values, respectively. The scale bar indicates branch lengths as number of substitutions per site corrected by the

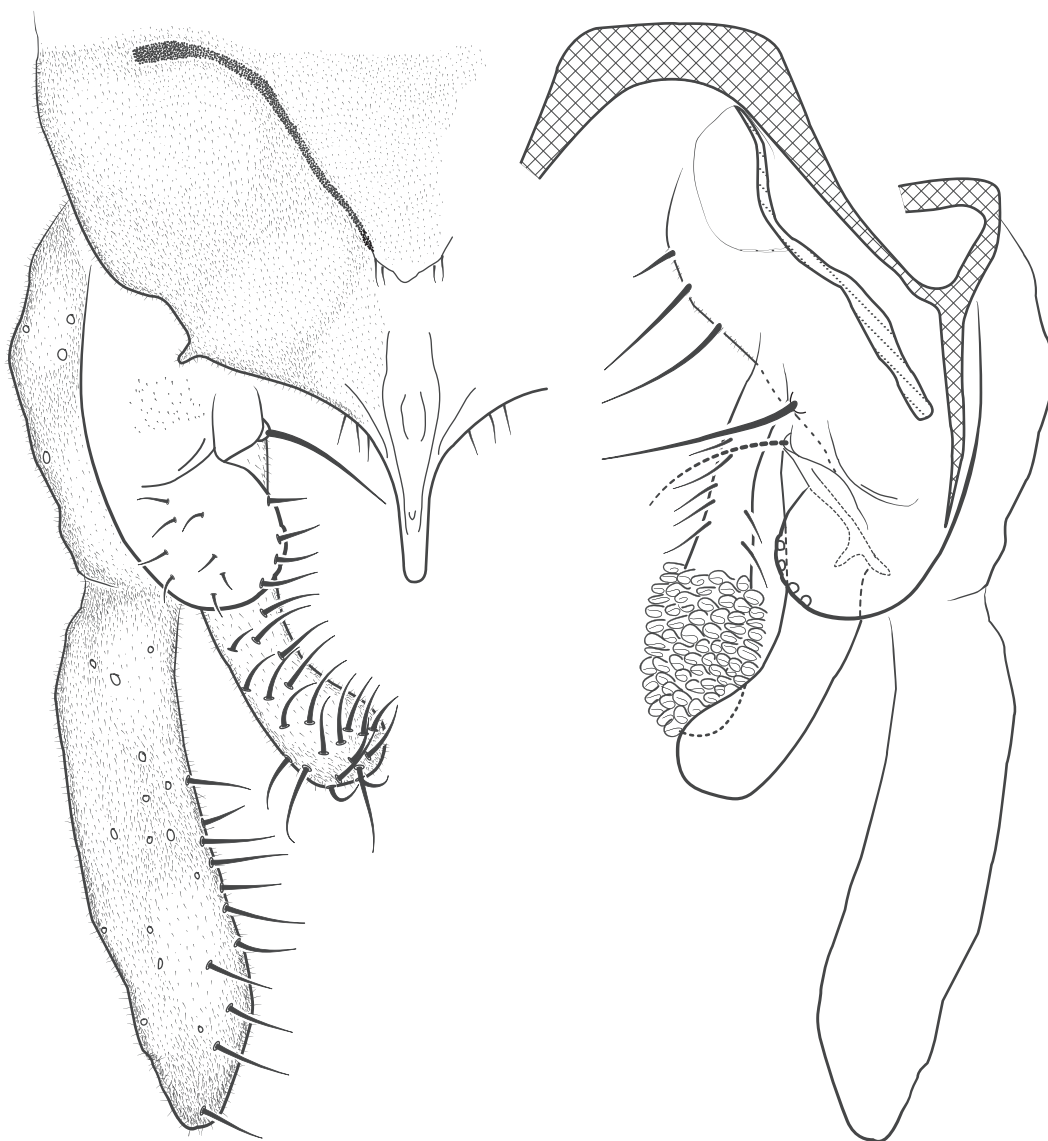


FIG. 3. *Micropsectra neoappendica* n. sp., male hypopygium.

consisting of long longitudinal spines extending nearly to the posterior end of tergite. Patches of TV consisting of spinules and points in a similar pattern to TIV. Anal fringe with ~30 setae.

Etymology.—The species epithet *neoappendica* refers to the striking similarity that this Nearctic species has with its putative sister species in the Palearctic, *Micropsectra appendica*.

Description

Adult male.—Measurements and ratios in Table 1.

Coloration.—Thorax with light green to light brown ground color, dark brown scutal stripes, preepisternum, postnotum, and median anepisternum; scutellum and halteres greenish to light brown; abdomen green to light brown; head with dark brown antennal

←

General Time Reversible model with a discrete Gamma distribution and invariable sites model. ** indicates discrepancies between the BA and ML analysis (e.g., *Micropsectra roseiventris* was sister to remaining in-group taxa in BA, but sister to *Paratanytarsus* taxa in ML).

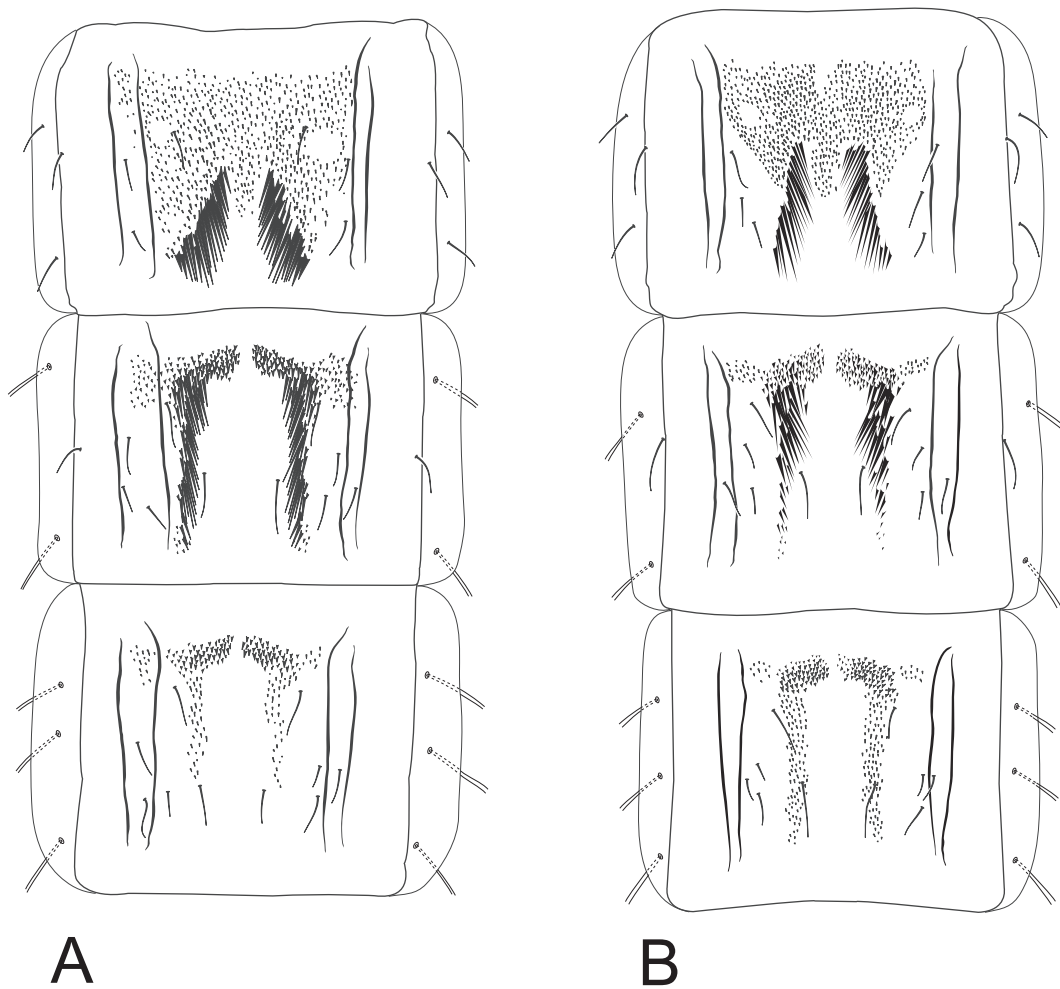


FIG. 4. Pupal abdominal segments III-V. A.—*Micropsectra neoappendica*, n. sp. B.—*Micropsectra appendica*.

pedicel, black eyes, and light brown palps; legs light brown.

Head.—Antenna with 13 flagellomeres. Frontal tubercles present (8–11, 9 μ m), temporal setae in 1 row; palpomere 3 with 2 sensilla clavata in subapical pit.

Wing.—Subcosta and media bare, brachiolum with 2–4 setae, squama bare.

Legs.—Pulvilli present, nearly $\frac{1}{2}$ as long as claws. Fore tibia with spur (17–25, 22 μ m); mid and hind tibial combs \sim 15 μ m long; middle tarsomere Ta₁ with 4–5 sensilla chaetica.

Hypopygium (Fig. 3).—Anal tergite with separate, posteriorly directed anal tergite bands; 4–5, 4.3 median tergite setae situated on an elevated hump anterior to anal point base; 9–11, 9.5 ventral apical setae. Anal point long with high, slightly curved anal crests, apex long and rounded; large knob present between crests; microtrichia-free area at base, except

lateral of anal point. Superior volsella round with 6–8, 7 dorsal setae and 3–4 median setae on setiger, 1 strong setae on stem; microtrichia fields located dorsally on stem and ventrally on setiger. Digitus small, thin, triangular, not extending past margin of superior volsella. Median volsella club-shaped, comparatively long and thick, with setiform and spoon-shaped lamellae; spoon-shaped lamellae on distal $\frac{1}{3}$; stem nearly reaching apex of inferior volsella. Inferior volsella without dorsoapical or dorsomedian swellings, numerous setae at tip. Inner margin of gonocoxite with 3–4 strong setae.

Pupa.—Measurements given in Table 2.

Coloration.—Pupal exuviae brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker brown.

Cephalothorax.—Cephalic tubercles present, small, conical; pedicel sheath tubercle weakly developed.

TABLE 1. Measurements and ratios for adult males of *Micropsectra* species. Lengths are in μm unless indicated. Data are presented as ranges, followed by the mean and the number of specimens observed in parentheses. VR = venarum ratio, AR = antennal ratio, LR = leg ratio, BV = “Beinverhältnisse”, BR = bristle ratio, SV = “Schenkel-Schiene-Verhältnis”, HR = hypopygium ratio. Morphological terminology and abbreviations follow Sæther (1980).

Measurement	<i>M. neoappendica</i>	<i>M. penicillata</i>	<i>M. xantha</i>	<i>M. subletteorum</i>
Wing				
Wing length (mm)	2.39–2.56, 2.46 (6)	2.14–2.44, 2.27 (8)	2.21–2.98, 2.61 (7)	2.77–3.27, 3.03 (8)
Wing width (mm)	0.59–0.62, 0.61 (4)	0.53–0.65, 0.59 (8)	0.57–0.76, 0.65 (6)	0.70–0.85, 0.77 (7)
VR	1.09–1.13, 1.11 (4)	1.05–1.20, 1.10 (8)	1.03–1.13, 1.08 (6)	1.03–1.07, 1.05 (8)
Head				
Terminal lagellomere length	650–705, 681 (5)	590–720, 649 (4)	640–820, 753 (5)	805–930, 858 (6)
AR	1.20–1.37, 1.27 (5)	1.13–1.38, 1.29 (4)	1.16–1.37, 1.29 (5)	1.47–1.70, 1.58 (6)
Head width	450–490, 466 (4)	405–510, 443 (6)	440–540, 492 (7)	500–675, 592 (7)
Distance between eyes	195–240, 226 (5)	150–200, 171 (7)	160–230, 199 (5)	210–260, 234 (7)
Palpomere lengths	45–58, 50 (6); 45–55, 49 (6); 145–158, 153 (6); 130–150, 141 (6); 233–255, 246 (4)	30–50, 42 (7); 40–50, 44 (7); 133–170, 142 (6); 110–150, 127 (6); 170–290, 231 (5)	46–75, 55 (7); 51–75, 63 (6); 153–213, 186 (6); 123–185, 160 (6); 295–320, 304 (4)	36–78, 54 (7); 43–68, 57 (7); 168–213, 195 (7); 168–190, 178 (7); 270–345, 293 (6)
Cephalic tubercle length	8–11, 9 (6)	5–13, 8 (6)	3–5, 4 (3)	5–10, 7 (7)
No. inner verticals	5–6, 5 (4)	4–5, 5 (7)	4–5, 5 (6)	7–9, 7 (7)
No. outer verticals	3–4, 3 (4)	3–4, 4 (7)	4–5, 4 (6)	4–5, 5 (6)
No. post orbitals	1–1, 1 (5)	1–2, 2 (7)	2–3, 3 (4)	2–2, 2 (6)
Legs				
LR ₁	1.50–1.55, 1.52 (6)	1.42–1.55, 1.49 (7)	1.56–1.74, 1.66 (6)	1.52–1.54, 1.53 (5)
BR ₁	3–5, 4 (5)	3–5, 4 (7)	4–4, 4 (5)	3–5, 4 (5)
BV ₁	1.85–1.89, 1.87 (4)	1.89–2.04, 1.98 (7)	1.81–1.88, 1.84 (6)	1.75–1.87, 1.80 (5)
SV ₁	1.54–1.60, 1.57 (6)	1.53–1.71, 1.61 (7)	1.43–1.52, 1.47 (6)	1.53–1.59, 1.56 (5)
LR ₂	0.55–0.59, 0.57 (6)	0.53–0.57, 0.55 (7)	0.58–0.70, 0.62 (5)	0.56–0.61, 0.59 (5)
BR ₂	5–7, 5 (6)	3–6, 5 (7)	3–5, 4 (3)	4–7, 5 (5)
LR ₃	0.68–0.75, 0.71 (5)	0.65–0.68, 0.67 (4)	0.68–0.71, 0.70 (6)	0.68–0.71, 0.70 (5)
BR ₃	5–7, 6 (5)	5–6, 5 (4)	4–6, 4 (6)	5–7, 6 (5)
Thorax				
No. dorsocentrals	7–11, 10 (6)	9–14, 11 (6)	10–12, 11 (6)	9–14, 11 (6)
No. acrosticals	14–18, 16 (6)	11–13, 12 (6)	11–17, 15 (5)	7–14, 12 (4)
No. prealars	2–3, 3 (6)	2–3, 3 (6)	2–3, 3 (5)	2–4, 3 (5)
No. scutellars	6–8, 7 (6)	7–8, 8 (5)	6–8, 7 (6)	8–11, 10 (6)
No. halterals	6–7, 7 (6)	4–5, 5 (6)	5–11, 7 (5)	7–12, 9 (5)
Hypopygium				
Tergite IX length	163–205, 183 (6)	145–173, 159 (6)	195–248, 228 (6)	205–229, 218 (7)
Gonostylus length	150–170, 163 (6)	143–175, 157 (8)	163–203, 184 (7)	193–223, 210 (7)
Gonocoxite length	165–185, 174 (6)	146–170, 159 (8)	180–200, 193 (6)	180–220, 203 (7)
HR	1.01–1.14, 1.07 (6)	0.93–1.08, 1.01 (8)	0.99–1.10, 1.03 (6)	0.90–1.02, 0.97 (7)
Anal point length	63–74, 68 (6)	53–60, 58 (5)	65–75, 70 (6)	61–74, 68 (7)
No. median setae	4–5, 4 (6)	3–3, 3 (5)	2–4, 3 (7)	3–7, 5 (8)
No. apical setae	9–11, 10 (6)	7–8, 7 (5)	8–10, 9 (6)	8–12, 10 (7)
Median volsella length	120–125, 123 (6)	103–138, 122 (8)	83–114, 96 (6)	68–85, 74 (7)
Median volsella lammellae length	10–16, 13 (6)	20–38, 26 (8)	26–43, 34 (7)	18–31, 24 (7)
Inferior volsella length	125–148, 134 (6)	105–135, 121 (8)	124–148, 135 (7)	135–165, 149 (7)
No. superior volsella dorsal setae	6–8, 7 (6)	4–6, 5 (7)	5–7, 6 (6)	4–7, 6 (8)
No. superior volsella median setae	3–4, 4 (6)	2–2, 2 (8)	2–2, 2 (7)	2–3, 2 (8)

TABLE 2. Measurements of pupal exuviae and pupal structures of *Micropsectra* species. Lengths in μm unless indicated. Data are presented as ranges, followed by the mean with the number of specimens observed in parenthesis. DC = dorsocentral setae. Morphological terminology and abbreviations used here follow Sæther (1980), except taeniae (Langton 1994), is used to describe the flattened setae found on the pupal exuviae.

Measurement	<i>M. neoappendica</i>	<i>M. penicillata</i>	<i>M. xantha</i>	<i>M. subletteorum</i>
Total length (mm)	4.34–4.81, 4.63 (6)	3.89–4.68, 4.31 (6)	4.77–5.91, 5.28 (6)	5.05–5.65, 5.39 (6)
Abdomen length (mm)	3.20–3.67, 3.47 (7)	2.87–3.52, 3.19 (6)	3.58–4.42, 3.99 (4)	3.70–4.31, 4.08 (6)
Cephalothorax				
Frontal setae length	50–123, 80 (6)	95–130, 111 (5)	130–198, 161 (3)	78–180, 132 (6)
Cephalic tubercle length	25–30, 27 (6)	20–35, 26 (6)	25–33, 28 (3)	30–51, 38 (6)
Thoracic horn length	265–395, 306 (6)	303–375, 336 (6)	378–553, 507 (3)	350–580, 450 (6)
Thoracic horn width	20–40, 28 (6)	23–48, 38 (6)	28–58, 44 (3)	48–65, 57 (5)
Thoracic horn chaetae length	148–173, 157 (6)	118–158, 135 (6)	160–233, 189 (3)	175–223, 198 (5)
Anterior precorneal length	73–115, 85 (6)	55–85, 71 (5)	78–125, 99 (3)	80–150, 113 (6)
Median precorneal length	80–133, 100 (6)	55–115, 74 (6)	110–146, 122 (3)	80–148, 110 (6)
Posterior precorneal length	130–173, 146 (6)	128–190, 153 (6)	130–203, 164 (4)	148–243, 194 (6)
Median anteprenotal length	80–95, 87 (5)	85–118, 98 (6)	78–130, 109 (3)	95–153, 115 (4)
Lateral anteprenotal length	68–80, 73 (6)	78–105, 93 (5)	68–130, 90 (4)	58–83, 68 (5)
DC ₁	55–78, 70 (6)	70–105, 85 (5)	53–85, 70 (4)	63–115, 96 (5)
DC ₂	38–45, 42 (4)	48–73, 56 (5)	43–75, 50 (2)	45–60, 53 (6)
DC ₃	55–100, 85 (6)	85–118, 99 (5)	70–108, 86 (4)	80–145, 112 (6)
DC ₄	65–85, 73 (6)	78–113, 95 (5)	70–100, 80 (4)	75–140, 106 (6)
Abdomen				
Hook row width	240–285, 254 (6)	258–315, 278 (5)	270–410, 350 (4)	375–425, 401 (6)
Spine/spinule patch TIII	200–235, 218 (7)	148–190, 165 (6)	170–270, 220 (4)	200–255, 223 (6)
Spine/spinule patch TIV	283–338, 313 (7)	285–325, 308 (6)	300–383, 350 (4)	320–420, 355 (6)
Spine/spinule patch TV	195–268, 236 (7)	190–280, 225 (6)	295–380, 339 (4)	310–410, 348 (6)
Spine patch VI	193–230, 205 (5)	145–175, 164 (4)	255–350, 308 (4)	310–385, 339 (6)
Spine length TIII	73–85, 78 (5)	48–60, 56 (3)	50–53, 51 (2)	50–50, 50 (1)
Spine length TIV	65–85, 76 (4)	53–55, 54 (3)	15–16, 16 (2)	20–20, 20 (2)
Spine length TV	9–13, 11 (5)	13–18, 15 (3)	13–15, 14 (2)	15–19, 17 (2)
Spine length TVI	4–5, 4 (4)	4–4, 4 (3)	3–5, 4 (2)	4–6, 5 (2)
No. apical teeth on comb	3–5, 5 (6)	3–6, 4 (5)	4–6, 5 (4)	5–6, 6 (6)
No. taeniae in anal fringe	27–33, 30 (7)	23–31, 28 (6)	35–48, 43 (4)	40–47, 44 (6)

Thoracic horn relatively short with numerous long chaetae on distal $\frac{2}{3}$; precorneals arranged in triangular pattern, the 2 anterior-most setae situated closer to each other than to the 3rd; 1 median and 2 lateral anteprenotals (1 seta, 1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior and posterior pair approximately equidistant from each other. Coarse granulation dorsally on anterior $\frac{1}{2}$ of thorax. Prealar tubercle absent; nose of wing sheath moderate to strong.

Abdomen (Fig. 4A).—TII almost covered by shagreen except for 2 mediolateral and 1 posteromedian oval patches; pedes spurii B on segment II obvious; hook row width $\sim\frac{1}{2}$ as long as segment width. Spines of TIII in large, laterally curved patches in posterior $\frac{1}{2}$ of tergite, shagreen extensively distributed lateral and anterior to spine patches and in between. Patches on TIV consisting of spinules in anterior, oval patches with spines heavily distributed in longitudinal, lateral extensions; shagreen present lateral to the anterior patches. Patches on TV similar in shape to those in TIV, however distinctly shorter and consisting only of

spinules and points. Patches on TVI similar in shape to those of TIV and TV, but somewhat shorter than patches of TV and consisting only of shagreen. Tergite TVII bare or with weak anterolateral shagreen patches. TVIII with weak anterior shagreen patches. Segment I with 3 D, 1 V, and 2 L setae; segment II with 3 D, 4 V, and 3 L; segment III with 5 D, 4 V, and 3 L; segment IV with 5 D, 4 V, 1 L, 2 lateral taeniae; segment V with 5 D, 4 V, 3 lateral taeniae; segment VI with 5 D, 4 V, 4 lateral taeniae; segment VII with 5 D, 4 V, 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, 5 lateral taeniae; anal tergite with 1 dorsal taenia. Two pairs of small sensorial setae medially on TII–VII; 1 pair of O-setae present anteriorly on segments II–VII. Anal lobe with 27–33, 30 long taeniae in 1 row. Posterolateral comb of segment VIII narrow with 3–5 apical teeth, median tooth often longer than the rest.

Taxonomic remarks

Adult males of *M. neoappendica* are quite similar morphologically to *M. appendica*, and thus, fit well in

the *atrofasciata* group. Two characters that help to differentiate these 2 species include the comparatively shorter median volsella of *M. neoappendica* (120–125, 122.5 μm than the 130–144, 137 μm reported in Stur and Ekrem 2006) and the shape of the elevated hump anterior to the anal point base (Fig. 3). In the specimens we have examined, this hump appears less curved in *M. neoappendica* and typically exhibits a small rounded point or hump at the tip of the elevated region. Setae extending from the gonocoxite and the seta on the stem of the superior volsella appear to be somewhat stronger than those on *M. appendica* (Fig. 3). The 2 species are best differentiated in the pupal stage. Pupal exuviae of *M. neoappendica* can be separated by the placement of the dorsocentrals, where the distance between anterior and posterior pairs is approximately equal, as opposed to having a much greater separation of the anterior pair ($\sim 4\times$ greater) than the posterior pair as seen in *M. appendica*. In addition, the thickness of DC₃ and DC₄ is similar for *M. neoappendica*, whereas 1 thick and 1 thin setae are noted for *M. appendica*. The nose of the wing sheath of *M. neoappendica* is typically quite strong, whereas it is generally weak in *M. appendica*. Spine patches of TIV and TV further differentiate the species, with long longitudinal spines on TIV of *M. neoappendica* extending nearly to the posterior end of the tergite and spinule/point patches of TV distinctly shorter than spine patch of TIV, with patch tapering off $\sim \frac{2}{3}$ the length of the tergite (Fig. 4A). Conversely, longitudinal spines of TIV of *M. appendica* typically taper off approximately at the mid-point of the tergite, with much shorter spines and spinules extending to the posterior end of the tergite; the spine/spinule patch of TV is subequal in size and pattern to TIV patches (Fig. 4B). Last, pupae of *M. neoappendica* seem to have fewer anal lobe taeniae than *M. appendica* (27–33, 30 as compared to 35–42, 38 as described in Stur and Ekrem 2006). Males of *M. neoappendica* are also quite similar to *Micropsectra tusimalemea* Sasa and Suzuki, 1999, but can be separated by the differentiating characters for *M. appendica* described in Stur and Ekrem (2006).

Ecology and distribution.—*Micropsectra neoappendica* is known from a shallow stretch of a small coldwater trout stream in northeastern Minnesota, USA.

***Micropsectra penicillata* Anderson, Stur & Ekrem,
new species
(Fig. 5A–H)**

Type material

Holotype.—P♂, **USA, Minnesota:** Cook County, South Brule River, Forest Rd. 152/Lima Grade, 15 m

upstream of bridge, 47°56'35"N; 90°26'17"W, 499 m, larvae collected 11.iv.2010, A. M. Anderson, (UMSP, on slide; VM, DNA extract; voucher ID: ALY71).

Paratypes.—11 P♂, 7 P♀. All data as for holotype, voucher IDs and GenBank accession in Table S1; 1♂, **USA, Colorado:** Lake Co., East Fork Arkansas River, 3041 m, 9–30.viii.1984, J. S. Herrmann, originally determined by J. Sublette as *M. recurvata*, (UMSP); 3♂, **USA, Michigan:** Alcona Co., Mikado, Huron National Forest, White Cedar Swamp, Pine River Campground, 8.v.1983, P. Hudson, (UMSP); 1 P♂, **USA, South Carolina:** Oconee Co., Horse Pasture River, 17.iii.1979, P. L. Hudson, (UMSP); 1♂, **USA, South Carolina:** Oconee Co., small stream near Devils Fork Landing, Jocassee Reservoir, 19.ii.1982, P. L. Hudson, (UMSP); 1 P♂, **USA, Tennessee:** Sevier Co., Gatlinburg, LeConte Creek, 4.v.1980, P. L. Hudson (UMSP); 1♂, **Canada, Alberta:** Hotchkiss, 16.viii.1961, F. L. Kessel-Light; 2♂, **Canada, Manitoba:** Churchill, Ramsey Creek, 58°43'50"N; 93°46'48"W, 13 m; adult collected with malaise trap over brook, 23–24.viii.2006, T. Ekrem and E. Stur, (VM, voucher IDs CHIR_CH9, CHIR_CH119); 4♂, **Canada, Ontario:** Caledon Hills, Woodrow Farm Stream, Trap 4, 29.viii–5.ix.1980, (UMSP).

Diagnostic characters.—The following combination of characters separates this species from all other *Micropsectra* species: Adult male. AR ~ 1.3 . LR₁ ~ 1.5 . Anal point slender with pointed tip. Superior volsella finger-shaped. Digitus absent. Stem of median volsella long and s-shaped, with numerous narrow, leaf-like lamellae, often held tightly together as in a fine-tipped painting brush. Inferior volsella tapered towards apex and laterally curved, with ~ 6 –8 distal setae (Fig. 5A). Pupa. Total length ~ 4.33 mm. Thoracic horn relatively short, ~ 336 μm with numerous chaetae on distal $\frac{2}{3}$ (Fig. 5B). Anterior pair of dorsocentrals placed further from each other than posterior pair (Fig. 5F). Long, strong, longitudinal spines on TIV; spinules and points on TV, similar in shape to TIV (Fig. 5B). 23–31, 28 anal fringe setae (Fig. 5H).

Etymology.—The species epithet *penicillata* (brush-like), refers to the median volsella in the male hypopygium, which has the appearance of a painter's brush or a small tail.

Description

Adult male.—Measurements and ratios in Table 1.

Coloration.—Thorax with light green to light brown ground color, dark brown scutal stripes, preepisternum, postnotum, and median anepisternum; scutellum and halteres greenish to light brown; abdomen green to light brown; head with dark brown antennal

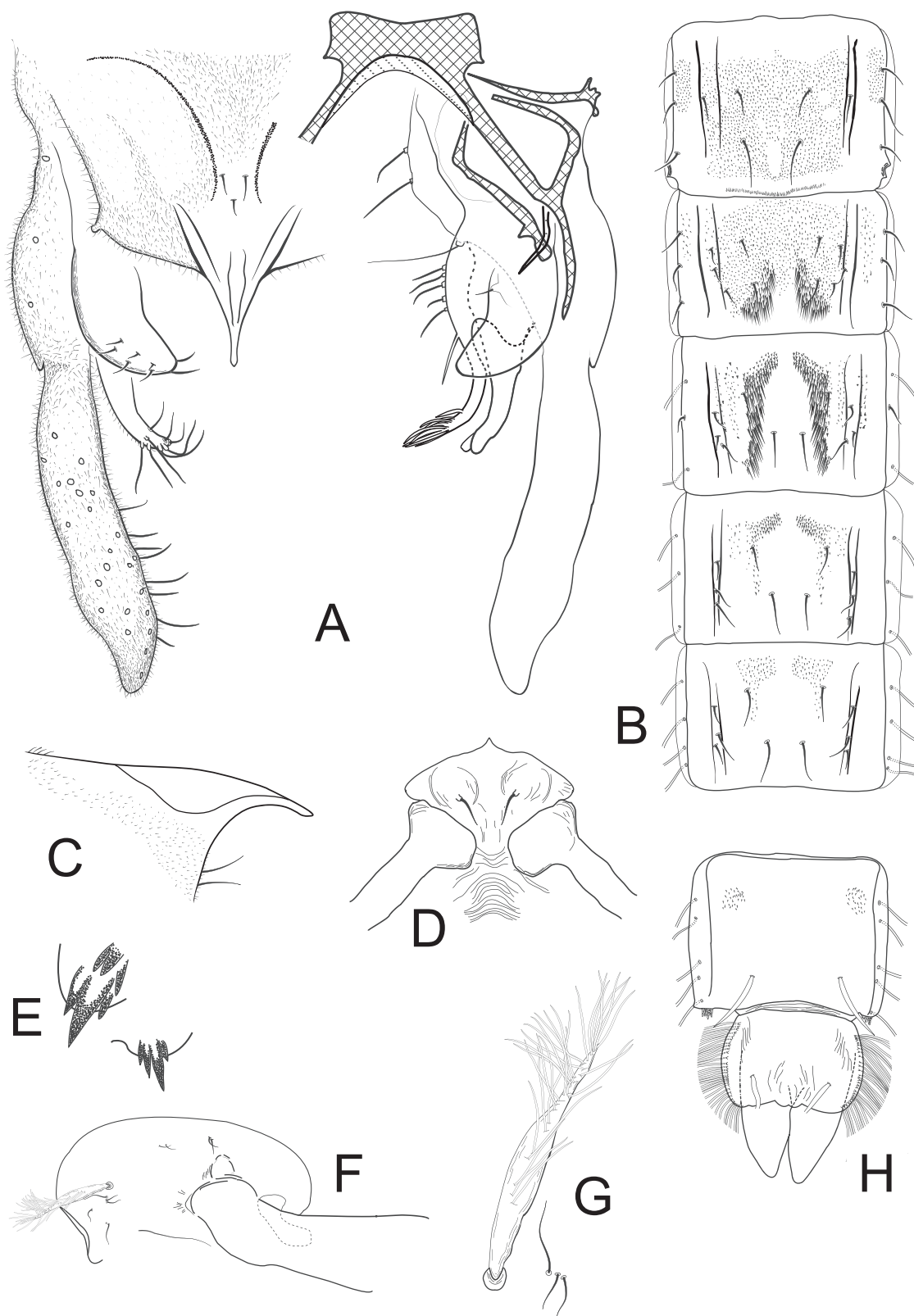


FIG. 5. *Micropsectra penicillata* n. sp., male. A.—Hypopygium. B.—Pupal abdominal segments II–VI, dorsal view. C.—Anal point, lateral view. D.—Pupal frontal apotome. E.—Posterolateral combs of pupal segment VIII. F.—Pupal thorax. G.—Pupal thoracic horn. H.—Pupal abdominal segments VIII–IX.

pedicel, black eyes, and light brown palps; legs light brown.

Head.—Antenna with 13 flagellomeres. Frontal tubercles present, 5–12, 8 μ m; temporal setae in 1 row; palpomere 3 with 2 sensilla clavata in subapical pit.

Wing.—Subcosta and media bare, brachiolium with 3 setae, squama bare.

Legs.—Pulvilli present, reaching half length of claws. Fore tibia with spur 20–35, 28 μ m long, middle and hind tibial combs 12–22, 18 μ m and 12–22, 19 μ m, respectively. Middle tarsomere Ta₁ with 2–4, 3 sensilla chaetica.

Hypopygium (Fig. 5A).—Anal tergite with tergal bands separate, posteriorly directed, not reaching crests of anal point; 3 median tergite setae anterior to anal point base; 7–8 ventral apical setae. Anal point with long, curved anal crests; apex of anal point long, slender with tip pointed; small knob between crests present; superior volsella finger-shaped; setiger with rounded apex and bent in median direction, 4–6, 5 dorsal and 2 median setae on setiger, 1 strong seta on stem; stem of superior volsella lacking field of microtrichia; digitus absent; median volsella long, s-shaped, with numerous narrow, leaf-like lamellae extending from distal end, often held tightly together; tip reaching far beyond apex of superior volsella. Inferior volsella narrowing toward distal end, with ~6–8 distal setae, tip seemingly bifid and curved medially. Inner margin of gonocoxite with 3–4 strong setae.

Pupa.—Measurements in Table 2.

Coloration.—Pupal exuviae brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker; large pigment-free area dorsally in posterior half of thorax.

Cephalothorax.—Cephalic tubercles present, comparatively small, cone-shaped (Fig. 5D); pedicel sheath tubercle weakly developed; thoracic horn fairly short with numerous chaetae on distal $\frac{3}{4}$, chaetae ~ $\frac{1}{3}$ to $\frac{1}{2}$ length of thoracic horn (Fig. 5G); precorneals arranged nearly in a line, with 1 setae offset, the 2 anterior-most setae situated closer to each other than the 3rd (Fig. 5G); 1 median anteprenotal, 2 lateral anteprenotals (1 sensillum basiconicum); 2 pairs of dorsocentrals (DCs), anterior pair placed further from each other than posterior pair and with thin setae as compared to the thicker setae of posterior pair, DC₁ longer than DC₂, DC₃ and DC₄ typically nearly equal in length (Fig. 5F). Some granulation present along median suture line of thorax. Prealar tubercle absent; nose of wing sheath weakly developed.

Abdomen.—TI bare. TII almost covered by shagreen except 2 lateral round patches and 1 posteromedian oval patch; pedes spurii B on TII obvious; hook row

~ $\frac{1}{2}$ as long as segment width. Spines of TIII in large, laterally curved patches in posterior $\frac{1}{2}$ of tergite; shagreen extensively distributed lateral and anterior to spine patches with numerous points between patches. Patches of TIV consisting of spinules in anterior, oval, slightly diagonal patches with long spines in longitudinal, lateral extensions, with a slight lateral, posterior curve; few points present laterally of longitudinal spines. Patches of TV similar in shape to TIV, somewhat shorter, and consisting only of spinules and points. Patches of TVI consisting only of shagreen, similar to patches TIV and TV in shape, but often much shorter (Fig. 5B). Tergite TVII and TVIII with small, anterior, oval shagreen patches, with patches of TVIII somewhat larger and more distinct than TVII in some specimens. Segment I with 3 D and 1 V setae; segment II with 3 D, 5 V, 3 L; segment III with 5 D, 4 V, 3 L; segment IV with 5 D, 4 V, 1 L, 2 lateral taeniae; segment V with 5 D, 4 V, 3 lateral taeniae; segment VI with 5 D, 4 V, 4 lateral taeniae; segment VII with 5 D, 4 V, 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, 5 lateral taeniae; anal tergite with 1 dorsal taenia (Fig. 5B, H). Two pairs of small sensorial setae medially on TII–VII; 1 pair of O-setae present anteriorly on sternites II–VII. Anal lobe with evenly convex lateral margins, fringe with long taeniae in 1 row (Fig. 5H). Posterolateral comb of segment VIII with 3–6, 4 apical teeth longer than rest (Fig. 5E).

Taxonomic remarks

Micropsectra penicillata exhibits morphological characteristics that tentatively place this species as a member of the Palearctic *recurvata* group (sensu S  wed  l 1981), with the exception of the lack of a digitus, an atypical character for the current *recurvata* group. Specimens from this species have been collected from various localities throughout North America but have never been formally described. The Sublette collection at UMSP contains adult male specimens of this species from various localities including 1 adult male from Colorado, USA, that was previously identified as *M. recurvata* because of morphological similarities. Several others from the collection were left undetermined. Adult males can be differentiated from *M. recurvata* by the absence of the digitus in *M. penicillata*, and appearance of the median volsella lamellae, which lack the spoon-shaped appearance of many *Micropsectra* species, including *M. recurvata*, and instead appear individually as thin leaves that are often held tightly together in a cluster, giving an appearance similar to a thin, pointed, round paintbrush or pointed tail (Fig. 5A), showing most

morphological similarity to *M. lacustris* (see discussion below). Pupae of *M. penicillata* differ from *M. recurvata* by the presence of long longitudinal spines on tergite IV (Fig. 5B) as opposed to a short spine patch in *M. recurvata*. Males of *M. penicillata* also share morphological similarities with *M. lacustris*, and phylogenetic analyses based on CAD nuclear gene sequences indicate a sister relationship between these 2 species (Fig. 2). *Micropsectra penicillata* can be differentiated from *M. lacustris* based on a distally tapered and laterally curved inferior volsella, the absence of a digitus, and a comparatively longer median volsella (102–137, 122 μm compared to 74–79 μm reported by Säwedäl (1975) for *M. lacustris*). The median volsella lamellar setae of *M. penicillata* are often held together more tightly than those of *M. lacustris*, which tend to appear more fanned. Pupae of the 2 species are differentiated based on the pattern of tergite armament; specifically, *M. penicillata* does not exhibit long longitudinal spines on TV (Fig. 5B) as seen in *M. lacustris* (see fig. 6 by Säwedäl 1975). Further, Säwedäl indicates that *M. lacustris* has 2 L and 2 LS setae on TIV, whereas *M. penicillata* has 1 L and 2 LS setae.

Ecology and distribution.—*Micropsectra penicillata* is currently known from stream localities from Colorado eastward in the USA and from Manitoba and Ontario, Canada.

***Micropsectra subletteorum* Anderson, Stur & Ekrem, new species
(Fig. 6A–G)**

Type material

Holotype.—P♂, **USA, Minnesota:** Washington Co., Valley Creek, Belwin Conservancy, 44°55'09"N; 92°47'54"W, 218 m, larva collected 3.iii.2010, A. M. Anderson, (UMSP, on slide; VM, DNA extract; voucher ID: ALY95).

Paratypes.—10 P♂, 4 P♀. All data as for holotype, voucher ID and GenBank accession codes in Table S1 (9 P♂ UMSP, 1 P♂ VM); 6♂, **USA, Minnesota:** Goodhue Co., Trout Brook, 44°32'97"N; 92°48'07"W, 229 m, adults collected 30.iii.2008, R. W. Bouchard. (UMSP, on slide; VM, DNA extracts); 6 P♀, Cook Co., South Brule River, Forest Rd. 152/Lima Grade, 15 m upstream of bridge, 47°56'35"N; 90°26'17"W, 499 m, larvae collected 11.iv.2010, A. M. Anderson, (UMSP, on slide; VM, DNA extracts); 1 P♀, Cook Co., Fiddle Creek, Forest Rd. 152/Lima Grade, stream access ~100 m NW of South Brule River bridge near edge of road, 47°56'35"N; 90°26'17"W, 505 m, larva collected 11.iv.2010, A. M. Anderson (VM); 1♂, **USA, Ohio:** Ottawa Co., Lake Erie, Put-in-Bay, Gibraltar Island,

17.vi.1980, P. L. Hudson (UMSP); 1♂, **USA, Wisconsin:** Burnett Co., Spring Brook, 11 mi E, 4 mi S of Siren, WI; 45°43'N; 92°09'W, 17.iv.1966, Dean Hansen, (UMSP LR11-64); 1♂ Burnett Co., Spring Brook, 11 mi E, 4 mi S of Siren, WI, 45°43'N; 92°09'W, 16.iv.1966, Dean Hansen (UMSP, S73-183); 1♂, **Canada, Manitoba:** Churchill, Ramsey Creek, 58°43'50"N; 93°46'48"W, 13 m, adult collected with malaise trap, 23–24.viii.2006, T. Ekrem and E. Stur (VM, voucher ID: CHIR-CH219); 1♂, **Canada, Ontario:** Renfrew Co., White Lake, 23.vi.1968, R. D. MacDonald (CNC).

Diagnostic characters.—The following combination of characters separates this species from other *Micropsectra* species: Adult male. AR ~1.6, LR₁ ~1.53. Anal point long and slender, with pointed tip. Superior volsella finger-shaped, field of microtrichia on stem absent. Digitus slender with slight curve, often extending past median margin of superior volsella. Median volsella long, parallel-sided with medially directed spoon-shaped lamellae. Inferior volsella with dorsoapical swelling. Gonostylus relatively narrow and tapering distally. Pupa. Total length ~5.4 mm. Thoracic horn relatively short, ~450 μm long with numerous long chaetae on distal $\frac{5}{6}$; chaetae length ~ $\frac{1}{3}$ to $\frac{1}{2}$ length of horn. Abdominal segment IV with 1 L setae and 2 lateral taeniae. Patches of TIV and TV consisting of spinules in anterior, oval patches, with shagreen lateral and posterior. Anal lobe with ~44 setae.

Etymology.—This species is named in honor of Jim and Mary Sublette in recognition of their contribution to Chironomidae taxonomy and systematics and the large number of Nearctic *Micropsectra* specimens they collected and prepared over the years. Many of these specimens provided a wealth of information for our research and are referenced throughout our paper.

Description

Adult male.—Measurements and ratios in Table 1.

Coloration.—Thorax dark brown with yellowish to light brown halteres; abdomen dark brown; head with dark brown antennal pedicel, black eyes, and light brown palps; legs dark brown.

Head.—Antennae with 13 flagellomeres. Frontal tubercles small 5–10 μm ; temporal setae in 1 row; palpomere 3 with 2–3 sensilla clavata in subapical pit.

Wing.—Macrotrichia on entire wing except for most basal part and fields between c and r₄₊₅. Subcosta and media bare, distal $\frac{1}{3}$ of cubitus and distal $\frac{1}{2}$ of post cubitus bare; brachiolum with 3 setae, squama bare.

Legs.—Pulvilli present, ~ $\frac{1}{3}$ as long as claws. Fore tibia with 22–38, 30 μm spur, mid and hind tibial combs, respectively 17–25, 21 and 17–25, 22 μm long; middle tarsomere Ta₁ with 3–5, 4 sensilla chaetica.

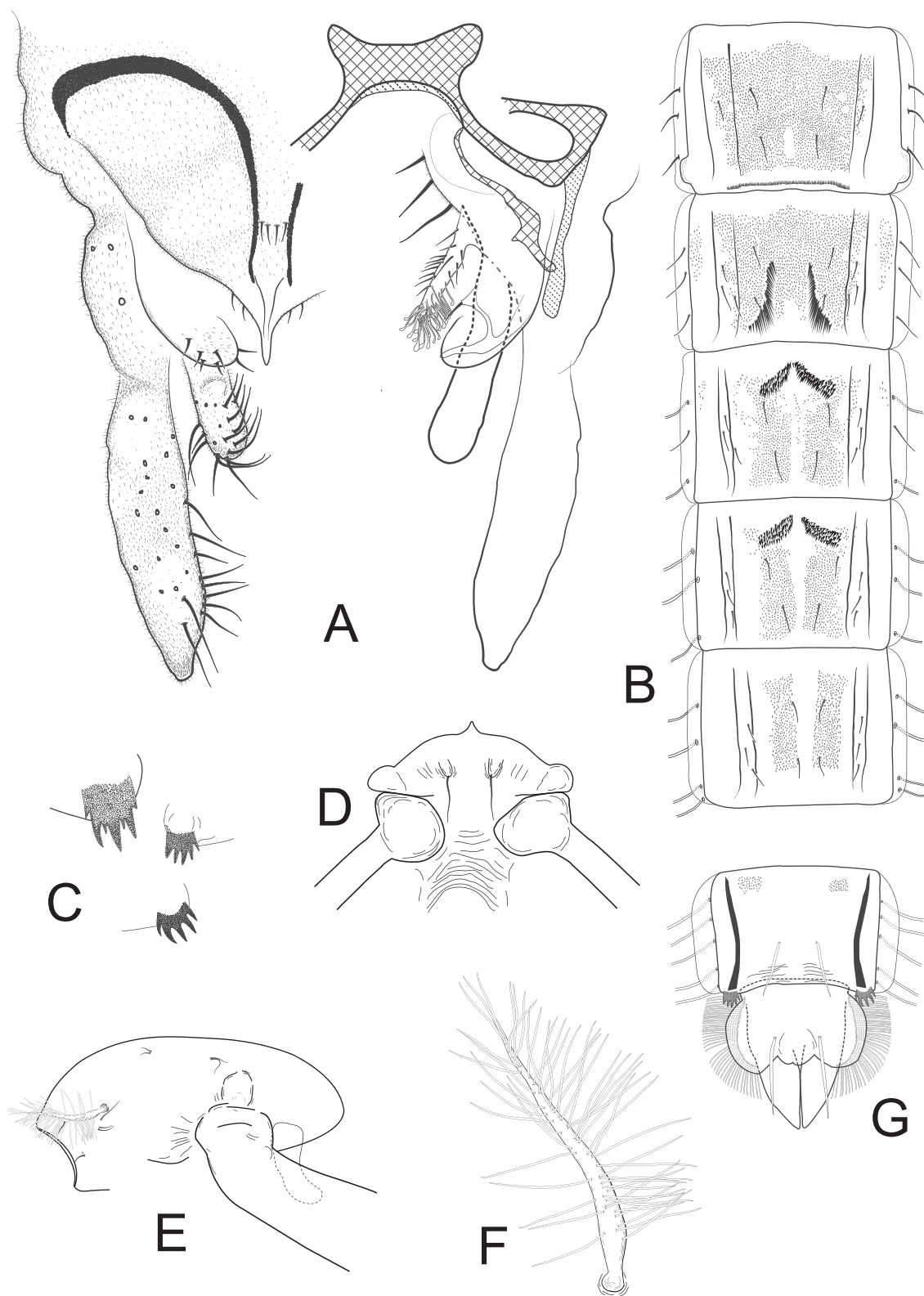


FIG. 6. *Micropsectra subletteorum* n. sp., male. A.—Hypopygium. B.—Pupal abdominal segments II–VI, dorsal view. C.—Posterolateral combs of pupal segment VIII. D.—Pupal frontal apotome. E.—Pupal thorax. F.—Pupal thoracic horn. G.—Pupal abdominal segments VIII–IX.

Hypopygium (Fig. 6A).—Anal tergite with tergal bands separate, posteriorly directed, reaching crests of anal point; 3–7, 5 median tergite setae anterior to anal point base; anal tergite without hump in front of anal point crests; 8–12, 10 ventral apical setae. Anal point with long, slightly curved anal crests; apex long, slender, and pointed; knob between crests absent; microtrichia between crests absent; microtrichia surrounding base of anal point. Setiger of superior volsella with 4–7, 5 dorsal and 2 median setae, 1 strong seta on stem; dorsal microtrichia on stem absent, field of microtrichia present ventrally on setiger, anterior to base of digitus. Digitus finger-like and slender with slight curve, often reaching past median margin of superior volsella. Median volsella long, parallel sided, with caudally directed setiform and spoon-shaped lamellae, on distal $\frac{1}{3}$ – $\frac{1}{4}$; tip generally not extending beyond superior volsella. Inferior volsella with moderately developed median, dorsoapical swelling and bearing numerous distal setae. Inner margin of gonocoxite with 3 strong setae.

Pupa.—Measurements in Table 2.

Coloration.—Pupal exuviae light brownish with dark brown apodemes; cephalothorax, TVIII and anal lobe darker; large pigment-free area dorsally in posterior $\frac{1}{2}$ of thorax.

Cephalothorax.—Cephalic tubercles present, cone-shaped, 30–51, 38 μm ; pedicel sheath tubercle absent (Fig. 6D). Thoracic horn 350–580, 450 μm long with numerous comparatively long chaetae on distal $\frac{5}{6}$; precorneals arranged in triangular pattern, the 2 anterior-most setae situated closer to each other than the 3rd, posterior precorneals longer than the other 2 (Fig. 6E); 1 median and 2 lateral anteprenotals (1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior pair shorter and weaker than posterior pair, DC₁ longer than DC₂, DC₃ and DC₄ of similar length. Some granulation present along median suture line. Prealar tubercle present, roundish; nose of wing sheath weak to absent.

Abdomen (Fig. 6B, G).—TI bare. TII covered by shagreen except for 1 posteromedian oval patch and 2 small mediolateral patches; pedes spurii B on TII obvious; hook row $>\frac{1}{2}$ as long as segment width. Spines of TIII in large, laterally curved patches in posterior $\frac{1}{2}$ – $\frac{2}{3}$ of tergite, shagreen extensively distributed lateral and anterior to spine patches, also covering much of the region between patches. Patches of TIV consisting of anterior, ovoid patches; shagreen present lateral and posterior to spine patches. Spine patches and shagreenation of TV similar to TIV. TVI consisting only of shagreen, similar to shagreenation in TIV and TV, except shagreen does not extend laterally in the posterior region as in TIV and TV. TVII

with weak shagreenation in a pattern resembling that of TVI. TVIII with lateral oval shagreen patches. Segment I with 3 D and 1 V setae; segment II with 3 D, 4 V, and 3 L; segment III with 5 D, 4 V, and 3 L; segment IV with 5 D, 4 V, 1 L, and 2 lateral taeniae; segment V with 5 D, 4 V, and 3 lateral taeniae; segment VI with 5 D, 4 V, and 4 lateral taeniae; segment VII with 5 D, 4 V, and 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, and 5 lateral taeniae; anal tergite with 1 dorsal taenia. One pair of O-setae present anteriolaterally on sternites II–VII. Anal lobe with long taeniae in 1 row on lateral margins (Fig. 6G). Posterolateral comb of segment VIII with 5–6 marginal teeth (Fig. 6C).

Taxonomic remarks

Micropsectra subletteorum is closely allied with many species in the *notescens* group, particularly *M. apposita* (Walker, 1856), *Micropsectra brundini* Sawedal, 1979, *Micropsectra contracta* Reiss, 1965, *Micropsectra insignilobus*, *Micropsectra junci* (Meigen, 1818), *Micropsectra lindebergi* Sawedal, 1976, *Micropsectra nigripila* (Johannsen, 1905), and *Micropsectra notescens* (Walker, 1856). The primary characteristic distinguishing adult males of *M. subletteorum* from the aforementioned species is that *M. subletteorum* lacks a field of microtrichia on the basal part of the superior volsella and lateral teeth on the anal tergite (Fig. 6A). The following characteristics also are useful for distinguishing males of *M. subletteorum* from *M. brundini*, *M. insignilobus*, and *M. lindebergi*: a comparatively higher LR₁ and stronger anal point crests (see tables 1–3 in Sawedal 1979). The gonostylus of *M. subletteorum* also is gradually narrowing towards the distal end (Fig. 6A) and the AR is lower than seen in the 3 aforementioned species. Sawedal (1979) indicates that the low number of sensilla chaetica of *M. brundini* (0–2) also may help differentiate *M. brundini* from other *notescens* group species. However, we have observed specimens of *M. brundini* from the PHL collection that have up to 4 sensilla chaetica, indicating this character may not be reliable. *Micropsectra subletteorum* can be further differentiated from *M. insignilobus* by having a comparatively longer median volsella (68–85 μm compared to 40–59 μm). *Micropsectra subletteorum* differs from *M. junci* and *M. nigripila* by having a comparatively longer digitus that often extends to or past the margin of the superior volsella (Fig. 6A). The digitus of *M. subletteorum* also exhibits more curvature than that of *M. notescens*, *M. contracta*, and *M. apposita*. *Micropsectra subletteorum* is further differentiated from *M. notescens* by lacking the ball-shaped swelling at the tip of the inferior volsella

(see fig. 10 by Säwedal 1976) and having a longer median volsella, 67–85, 74 compared to 38–64 μm (Säwedal 1976). The short, blunt-tipped anal point of *M. nigripila* also differentiates males of the 2 species, and the rounded tip of the superior volsella (Fig. 6A) sets *M. subletteorum* apart from the more triangular superior volsella of *M. contracta* and *M. apposita*.

Pupae of *M. subletteorum* can be easily differentiated from *M. contracta* and *M. apposita* by the absence of semi-long longitudinal spines posterior to the oval spinule patch on TIV (Fig. 6B). The species differs from *M. insignilobus* and *M. lindebergi* by exhibiting 2 LS and 1 L setae on TIV rather than 3 LS setae. The thoracic horn of *M. subletteorum* also is shorter than in these 2 species, *M. nigripila*, and the parthenogenetic *Micropsectra silvesterae* Langton, 1998, ($\sim 450 \mu\text{m}$ as compared to 680 μm for *M. insignilobus* and *M. lindebergi*; 624–947, 825 μm for *M. nigripila* (Oliver and Dillon 1994); and 440–835, 672 μm for *M. silvesterae*; Säwedal 1976, Oliver and Dillon 1994, Langton 1998). Further, thoracic horn setae start basally for both outer and inner lateral margins, and filament length is $\frac{1}{3}$ – $\frac{1}{2}$ the length of the thoracic horn as compared to $\frac{1}{5}$ – $\frac{1}{6}$ for *M. insignilobus* and *M. lindebergi* (see Säwedal 1976); the particularly long thoracic horn with short chaetae of both *M. nigripila* and *M. silvesterae* also serves to differentiate *M. subletteorum* from these species (cf. fig. 10 by Oliver and Dillon 1994, fig. 2C by Langton 1998). *Micropsectra subletteorum* also can be distinguished from *M. silvesterae* by the presence of only 1 median antepnotal seta as compared to 2 in *M. silvesterae* (Langton 1998). *Micropsectra subletteorum* differs from pupae of *M. junci* by lacking the finger-like prealar tubercle in front of the wing sheath. *Micropsectra subletteorum* can be further differentiated from *M. insignilobus* and *M. junci* in that it has 40–47, 44 anal lobe filaments, as compared to 53–60 in *M. insignilobus* and 62–73 in *M. junci*. Pupae of *M. subletteorum* are perhaps most similar to *M. notescens*, and few reliable characters have been found to separate the 2. The thoracic horn length and setae arrangement is perhaps the best distinguishing character at present, with the thoracic horn of *M. subletteorum* typically shorter than *M. notescens*. Säwedal (1976) reported the average length of the thoracic horn of *M. notescens* as 660 μm , but we have seen specimens with thoracic horn length as low as 525 μm , which falls within the range we report for *M. subletteorum*. Thoracic horn setae appear to begin slightly lower on the inner lateral margin for *M. subletteorum*, covering $\frac{4}{5}$ to $\frac{5}{6}$ as compared to $\frac{2}{3}$ for *M. notescens*.

Ecology and distribution.—This species is common in small groundwater-dominated trout streams throughout

Minnesota and has been found in other lotic and lentic localities in the eastern half of the USA and Canada.

***Micropsectra xantha* (Roback, 1955)
(Figs 7A–G, 8A, B)**

Calopsectra (*Micropsectra*) *xantha* Roback, 1955: 4

Type material

Neotype (here designated).—P σ , USA, Minnesota: Goodhue Co., Cold Spring Brook, spring pool off of County Rd. 68, $\sim 1 \text{ km N}$ of Hwy 60, 44°17'46"N; 92°25'35"W, 256 m, larvae collected 25.iv.2010, A. M. Anderson (UMSP, on slide; VM, DNA extract; voucher ID: ALY52).

Additional material examined.—13 P σ , 17 P ϕ , 1 L_{hc}P ϕ . All data as for neotype, voucher IDs and GenBank accession codes in Table S1 (12 P σ , 1 P ϕ UMSP; 13 P σ , 1 P ϕ VM); 3 σ , 1 P σ , USA, New York: Cattaraugus Co., Allegany State Park, adult collected with light trap 28.v–3.vi.1963, W. W. Wirth, (UMSP, 573–676). 1 σ , USA, Virginia: Fairfax Co., Falls Church, Holmes Run, adult collected with light trap 31.v.1961, W. W. Wirth, (UMSP); 7 σ , USA, Wisconsin: Burnett Co., Spring Brook, 11 mi E, 4 mi S of Siren, WI, 45°43'N; 92°09'W, adults collected with sweep net 13.x.1967, Dean Hansen (UMSP); 3 σ , Burnett Co., Spring Brook, 11 mi E, 4 mi S of Siren, WI, 45°43'N; 92°09'W, adults collected with light trap 7.vi.1968, Dean Hansen (UMSP); 1 σ , Canada, Quebec: Lewis Co., 31.v.1929, (UMSP).

Diagnostic characters.—The following combination of characters separates this species from other species of *Micropsectra*: Adult male. AR ~ 1.3 , LR₁ ~ 1.6 . Anal point long and slender, with pointed apex. Superior volsella finger-shaped. Digitus long and narrow, extending beyond median margin of superior volsella. Median volsella moderately long and s-shaped, with numerous long, medially directed spoon-shaped lamellae. Inferior volsella swollen at tip, with ~ 10 strong setae (Fig. 7A). Pupae. Total length $\sim 5.3 \text{ mm}$. Thoracic horn relatively long, $\sim 507 \mu\text{m}$ with numerous chaetae covering distal $\frac{5}{6}$; thoracic horn chaetae $\sim \frac{1}{3}$ length of horn (Fig. 7F). Abdominal segment IV with 3 lateral taeniae. Patches of TIV and TV consisting of spinules in anterior, oval patches, with shagreen lateral and posterior (Fig. 7B). Anal fringe with ~ 43 setae (Fig. 7G).

Etymology.—Roback (1955) does not include information regarding the name choice for this species in his original description, however, the Greek term xanthos means yellow and was probably given in reference to the coloration of this species.

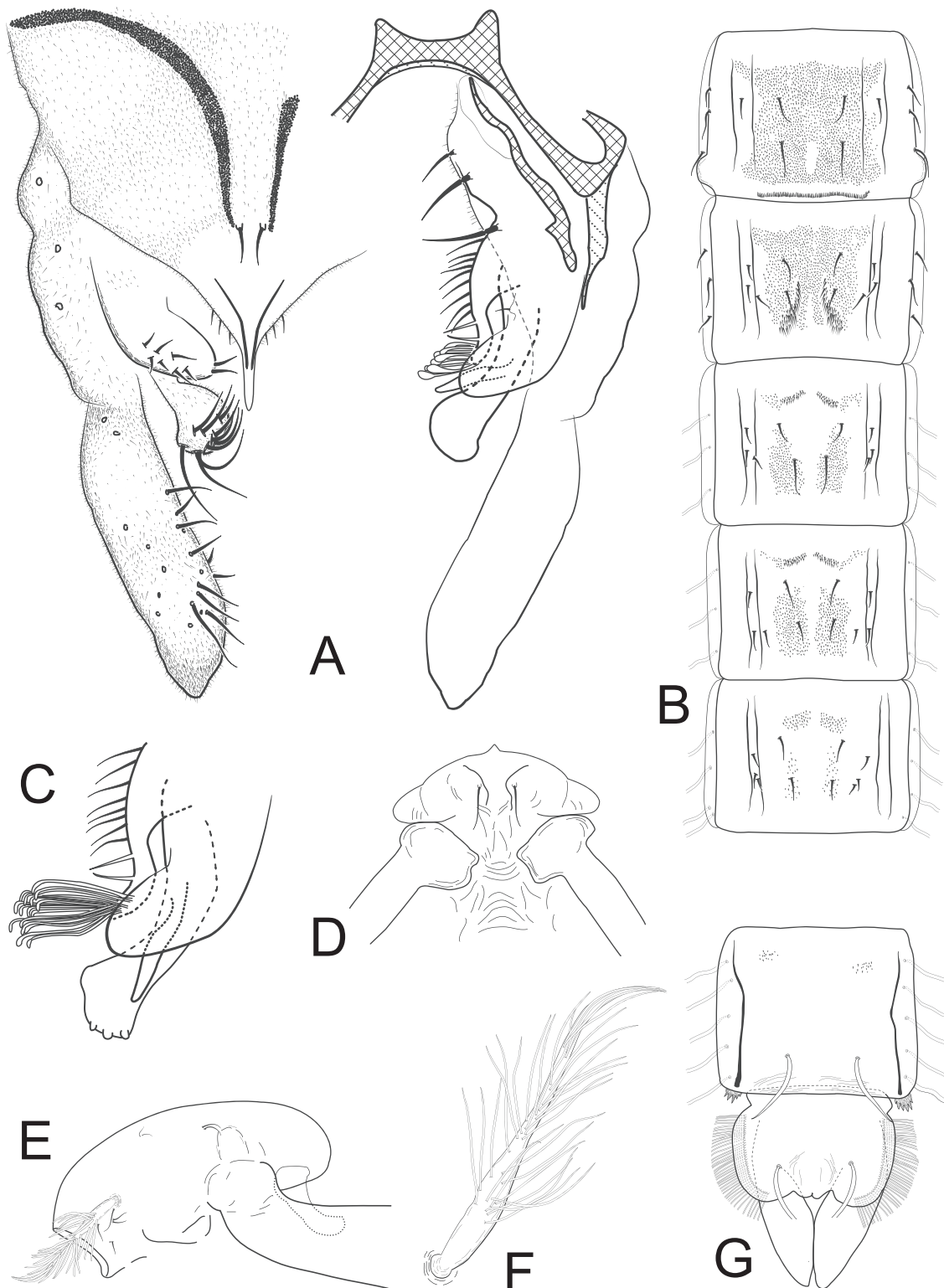


FIG. 7. *Micropsectra xantha*, male. A.—Hypopygium. B.—Pupal abdominal segments II–VI, dorsal view. C.—Superior, median, and inferior volsellae. D.—Pupal frontal apotome. E.—Pupal thorax. F.—Pupal thoracic horn. G.—Pupal abdominal segments VIII–IX.

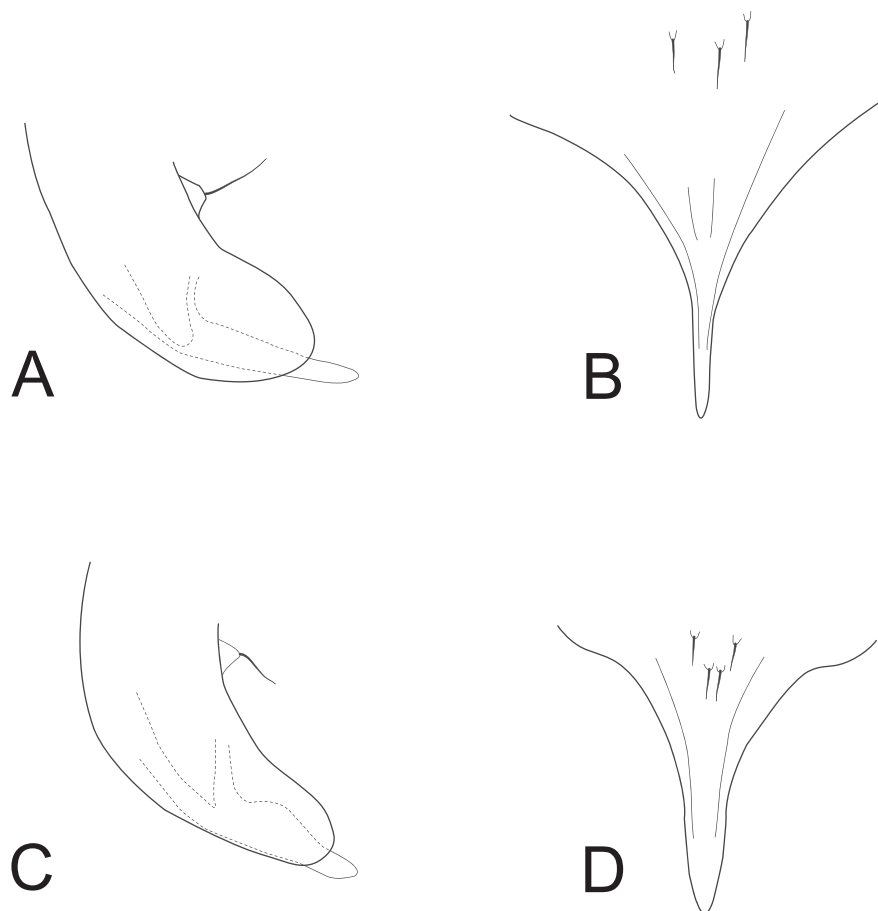


FIG. 8. A.—Superior volsella, *Micropsectra xantha*. B.—Anal point, *M. xantha*. C.—Superior volsella, *Micropsectra connexa*. D.—Anal point, *M. connexa*.

Description

Adult male.—Measurements and ratios in Table 1.

Coloration.—Ground color of head, thorax, abdomen, and legs light yellow or green; antennal pedicel, scutal stripes, preepisternum, postnotum, and median anepisternum darker yellow; eyes black.

Head.—Antenna with 13 flagellomeres. Frontal tubercles present as small dots (3–5, 4 μ m); temporal setae in 1 row; palpomere 3 with 2–3 sensilla clavata in subapical pit.

Wing.—Subcosta and media bare, brachiolium with 2 setae, squama bare.

Legs.—Pulvilli present, nearly $\frac{1}{2}$ the length of claws. Fore tibia with 28–38, 33 μ m spur; middle and hind tibial combs 15–24 and 15–27 μ m respectively; middle tarsomere Ta₁ with 3–4 sensilla chaetica.

Hypopygium (Fig. 7A).—Anal tergite with tergite bands separate, posteriorly directed, not reaching crests of anal point; 2–4, 3 median tergite setae on slightly elevated ridge; 8–10, 9 ventral apical setae.

Anal point with long, pointed apex; anal crests slightly curved; knob between anal crests absent; large microtrichia-free area around base. Setiger of superior volsella with 5–7, 6 dorsal setae and 2 median setae; 1 seta on stem; a small field of dorsal microtrichia on stem of superior volsella, and a large field ventrally on setiger. Digitus long and narrow, reaching well beyond the median margin of the superior volsella. Median volsella long and s-shaped, long, medially directed setiform and spoon-shaped lamellae, ~10–15 numerous spoon-shaped lamellae extending from tip. Inferior volsella greatly swollen at tip, appearing somewhat ball-shaped and bearing ~10 strong setae. Inner margin of gonocoxite with 2–3 strong setae.

Pupa.—Measurements given in Table 2.

Coloration.—Pupal exuviae light brownish with darker brown apodemes; cephalothorax, TVIII, and anal lobe darker brown.

Cephalothorax.—Cephalic tubercles present, small, conical; pedicel sheath tubercle well developed (Fig. 7D). Thoracic horn fairly long with numerous

chaetae covering distal $\frac{5}{6}$; thoracic horn chaetae $\sim \frac{1}{3}$ length of thoracic horn (Fig. 7F); precorneals arranged nearly in a line, with 1 seta somewhat offset, and the 2 anterior-most setae situated closer to each other than the 3rd, anterior precorneal typically shorter than the other 2; 1 median anteprenotal, 2 lateral anteprenotals (1 sensillum basiconicum); 2 pairs of dorsocentrals, anterior pair often shorter than posterior pair. Some granulation present along median suture line of thorax. Prealar tubercle present, roundish; nose of wing sheath weak to moderate.

Abdomen (Fig. 7 B, G).—TI bare. TII almost covered by shagreen except for 1 posteromedian oval patch; pedes spurii B on TII obvious; hook row $> \frac{1}{2}$ as long as segment width. Spines of TIII in laterally curved patches in posterior $\frac{1}{2}$ of tergite, shagreen extensively distributed lateral and anterior to spine patches, and covering most of the area between patches. Patches of TIV consisting of spinules in anterior, oval patches; shagreen lateral and posterior to patches. TV patches and shagreenation similar to TIV. Patches of TVI consisting only of shagreen; shagreen in anterior region of tergite somewhat larger than those in mid and posterior region, resembling shape of spinule patches in TIV and TV. TVII with shagreen in anterior region. TVIII with small anterolateral shagreen patches. Segment I with 3 D, 1 V setae; segment II with 3 D, 4 V, 3 L; segment III with 5 D, 4 V, 3 L; segment IV with 5 D, 4 V, 3 lateral taeniae; segment V with 5 D, 4 V, 3 lateral taeniae; segment VI with 5 D, 4 V, and 4 lateral taeniae; segment VII with 5 D, 4 V, and 4 lateral taeniae; segment VIII with 1 dorsal taenia, 1 ventral taenia, and 5 lateral taeniae; anal tergite with 1 dorsal taenia. One pair of O-setae anteriolaterally on sternites II–VII. Anal lobe with fringe of laterally curved taeniae in 1 row. Posterolateral comb of segment VIII with 4–6 teeth longer than the rest.

Taxonomic remarks.—The holotype could not be located at the Academy of Natural Sciences Natural History Museum in Philadelphia, USA (ANSP) (J. Weintraub, ANSP, personal communication). However, as noted by Säwedäl (1981), the hypopygium of the holotype was poorly preserved. In addition, as discussed below, males of *Micropsectra xantha* are very similar morphologically to *Micropsectra connexa* (Kieffer, 1906). There are no known paratypes of *M. xantha* and, therefore, to stabilize future taxonomy of the species, we find it necessary to designate a neotype for *M. xantha*.

As noted by Säwedäl (1982), the adult male of *M. xantha* is very similar to *M. connexa* (see additional nomenclature notes on this species by Stur and Ekrem 2006) and warrants placement in the Palearctic *recurvata* group. We have examined the male holotype

of *M. connexa*, located in BMNH, and confirm that the most reliable difference between *M. xantha* and *M. connexa* is in the anal point. The anal point of *M. xantha* is slender and parallel-sided toward the tip, whereas in *M. connexa*, the anal point is broader and cone-shaped toward the tip (Fig. 8B, D). In addition, the anal point crest is stronger in *M. xantha* than in *M. connexa*. We also have noted differences in the superior volsella (Fig. 8A, C). *Micropsectra xantha* appears to have a broader superior volsella with a long, narrow digitus that is generally $< \frac{1}{3}$ the width of the superior volsella. The superior volsella of *M. connexa* is comparatively narrower with a wider digitus that is $\frac{1}{2}$ – $\frac{1}{3}$ the width of the superior volsella. Last, the seta on the stem of the superior volsella appears more basal on *M. connexa* than *M. xantha*.

Stur and Ekrem (2006) place *M. connexa* with the *attenuata* group. Here, we refute this placement, and instead find a more appropriate placement in the *recurvata* group, together with *M. xantha*, based on the combination of a long and s-shaped median volsella, long anal point (Fig. 8D), and a digitus that extends past the margin of the finger-shaped superior volsella (Fig. 8C). We attempted to amplify both standard and short (130 base pair [bp]) COI-fragments from specimens of *M. connexa* found in the Sublette collection (UMSP; all originally labeled as *M. xantha*) to provide additional evidence that *M. xantha* and *M. connexa* are truly separate species. However, we were not successful in obtaining a usable sequence, probably because of the age (> 40 y) of the specimens available to us. *Micropsectra xantha* is also quite similar to *M. digitata* Reiss, 1971, however the digitus in *M. digitata* is missing and the anal point is wider and more rounded at the tip.

Ecology and distribution.—The original holotype was collected from a small, cold spring west of Concord, Massachusetts, USA (Roback 1955). In the original description, Roback mentions the water temperature of the spring varied from 7–10°C, with a mean temperature of 9°C. The neotype is known from a southeastern Minnesota spring with very similar thermal characteristics. Specimens are also known from various other localities in the eastern $\frac{1}{2}$ of the USA and Canada.

Discussion

New Nearctic Micropsectra and cryptic diversity

This small survey of only 5 Minnesota streams, each visited on only 1 sampling occasion, yielded 3 new species of a single chironomid genus, a clear indication that many Nearctic chironomid species are left to be discovered and described. These new

species more than double the number of *Micropsectra* previously known from Minnesota with 24 species now known from the Nearctic. Without the aid of molecular techniques or examination of multiple life stages, 2 of the species described in this work, *M. neoappendica* and *M. subletteorum*, probably would have gone unnoticed if identified using existing Palearctic keys and species descriptions, thereby satisfying the definition of cryptic species by Bickford et al. (2007). This fact is particularly important for *M. neoappendica*, which, to date, has been found only in 2 neighboring, pristine streams in northeastern Minnesota. The 3 other species described/redescribed in our work have been collected by the authors at multiple Nearctic localities or were found in the large set of *Micropsectra* material collected and curated by Jim Sublette, now at UMSP. To contrast—and to point out potential habitat differences between the *appendica/neoappendica* cryptic species complex (the Palearctic counterpart of *M. neoappendica*)—*M. appendica* is described as having a wide geographical distribution, with collections made from various types of ponds, rivers, brooks, streams, and springs (Stur and Ekrem 2006). This broad distribution suggests different habitat preferences between the 2 species and potential differences in pollution sensitivity. Habitat partitioning by cryptic species, such as suggested here, is not uncommon and is known for various cryptic invertebrates (e.g., Tarjuelo et al. 2001, Folino-Rorem et al. 2009) and vertebrates (e.g., Davidson-Watts et al. 2006). Therefore, considering that cryptic species may exhibit different responses to environmental conditions, use of a single species, rather than a cryptic species-complex, will allow much more accurate biological monitoring and is important for appropriate and accurate conservation and management programs (Bickford et al. 2007).

Molecular species delimitation and phylogeny

As pointed out by Stur and Spies (2011), including species-specific DNA sequences as a standard component of new species descriptions has clear advantages that extend beyond the ability of recognizing cryptic diversity. These advantages include improved consistency in appropriate use of species names (this paper, Stur and Spies 2011), association of multiple or unknown life stages (Zhou et al. 2007, Ekrem et al. 2010a, Pauls et al. 2010, Stur and Ekrem 2011), and an additional set of characters that can be used to identify and establish relationships among species.

Our results emphasize that COI is an effective tool for species delimitation and recognition of cryptic species, but we choose not to discuss relationships

among species based on this gene. COI has a weak phylogenetic signal and is unsuitable for inference of species relationships in the Chironomidae (Ekrem et al. 2007, 2010b). The COI trees generated in our analyses did not correspond well with our knowledge of *Micropsectra* phylogeny and many of the relationships between species had low (<50%) bootstrap support (Figs 1, S3). Furthermore, in the COI phylogenies of both the full (Fig. 1) and reduced (Fig. S3) data sets, the 2 outgroup *Paratanytarsus* species were nested within the *Micropsectra*, rendering this genus paraphyletic. Thus, we recognize the power of COI for species delimitation and that it can be quite effective at identifying higher relationships in certain taxa, but we have chosen not to focus on interpreting results from our COI or combined COI/CAD data set to avoid misinterpretations of the data with regard to phylogenetic relationships. Based on results of our analyses and those of others (Winterton et al. 2007, Winkler et al. 2009, Ekrem et al. 2010b), we think that CAD is most reliable for reconstruction and interpretation of phylogenetic relationships for the Chironomidae and some other dipteran families. Results based on our combined COI/CAD data did largely conform to our CAD-only results (Figs 2, S2). The few discrepancies that exist between the 2 methods of analysis probably are the result of conflicting phylogenetic signals from COI that partially mask more reliable species-group hypotheses based on the CAD marker alone. Consequently, all discussion of phylogenetic relationships is based on the nuclear CAD gene, shown by others to have a strong phylogenetic signal for estimating genus-level relationships within Chironomidae (Ekrem et al. 2010b).

Revision and expansion of Micropsectra species groups

Palearctic species of *Micropsectra* have traditionally been subdivided into species groups to aid in identification, species revisions, and classification. Recent authors have questioned the appropriateness and accuracy of these groups (Gilka 2001, Gilka and Paasivirta 2008, Ekrem et al. 2010b). Present molecular and morphological evidence shows a well supported *atrofasciata* group (e.g., Ekrem et al. 2010b, Stur and Ekrem 2006), but lines of evidence supporting monophyletic *attenuata*, *recurvata*, and *notescens* groups are less distinct (e.g., Gilka 2001, Gilka and Abramczuk 2006, Gilka and Paasivirta 2008, Ekrem et al. 2010b), particularly considering the recent synonymy of *Krenopsectra* and *Parapsectra* with the *Micropsectra* (Ekrem et al. 2010b). Additional problems with the groups become apparent when attempting to place Nearctic species in the Palearctic-derived classification system.

Most Nearctic *Micropsectra* have not yet been described (Reiss 1995, our study). Many species awaiting description and some that have been described do not conform to the current species-group classification, which is based primarily on the adult male (e.g., *Micropsectra spinigera* Reiss, 1995, *Micropsectra borealis* (Kieffer, 1922), *Micropsectra radialis* Goetghebuer, 1939, and the parthenogenetic species *Micropsectra sedna* Oliver, 1976, and *M. silvesterae*). This situation highlights the need for a major revision of the genus and expanded species-group system.

In our study and in the study by Ekrem et al. (2010b), molecular evidence indicated a paraphyletic *recurvata* group, with former *recurvata* group members *M. recurvata* and *M. lacustris* falling into genetically distinct clades (Figs 2, S2; figs 3, 4 by Ekrem et al. 2010b). If chironomid taxonomists choose to retain current species groups, perhaps S wed l's original classification of a separate *lacustris* group (S wed l 1975) was more appropriate than his later placement of *M. lacustris* in the *recurvata* group (S wed l 1981). Molecular evidence from our CAD data set suggests placement of *M. penicillata* as sister species to *M. lacustris*. *Micropsectra penicillata* shares similarities with the *recurvata* group, as pointed out by preliminary classification of the undescribed species by J. Sublette in his personal collection (now part of UMSP), but distinct differences, which we pointed out in the taxonomic remarks for the species (see above) also exist. These morphological differences, combined with molecular evidence (Figs 2, S2), indicate an alternative grouping for the species.

A further drawback of the existing species-group classification is that it is based primarily on the morphological characteristics of the adult male, and it was founded before molecular data were commonly used in systematics. The current species-group classification does not extend to immature stages (Pinder and Reiss 1986). Most chironomid descriptions historically have been based on the adult male, but descriptions of immature life stages are becoming increasingly important, particularly for purposes of biological monitoring and studies of species diversity (e.g., Ekrem et al. 2010a, Zhou et al. 2010).

Chironomid pupal exuviae are a simple and effective method for assessing species diversity (e.g., Ferrington et al. 1991, Calle-Martinez and Casas 2006, Raunio et al. 2007, Ruse 2011), particularly since descriptions of pupal exuviae have become more common and readily available. We think that this life stage may provide important features that, combined with characters of the adult male and molecular data, may help resolve future species-group concepts. For example, when examining Fig. 2 (our study) and fig. 3

by Ekrem et al. (2010b), the exuviae of all members of the *atrofasciata* group that have described exuviae have long longitudinal spine patches on abdominal tergite IV. Conversely, exuviae known for the *notescens* group, with the exception of *M. contracta* and *M. apposita*, have short anterior patches consisting of spinules and points. *Micropsectra contracta* and *M. apposita* have semilong spines (none long enough to be synonymous with spine-type from the *atrofasciata* group) extending partway down tergite IV. Members of a reduced *recurvata* group, including *M. recurvata* and *M. xantha*, also fit the exuviae generalization of the *notescens* group, forming a *recurvata*–*notescens* clade. Adult male morphology clearly separates *recurvata* group members from the *notescens* group members, but we have not yet identified pupal characters that can be used to separate them. Furthermore, if the *lacustris* group is re-established with *M. penicillata* as a member, this group fits better with a combined *lacustris*–*atrofasciata* clade (Fig. 2). As with the *recurvata*–*notescens* clade, members of the *lacustris*–*atrofasciata* clade have similar spine patch patterns on the exuviae, with known species in this clade exhibiting long longitudinal spine patches on tergite IV. Adult males separate the *lacustris* group from the *atrofasciata* group, but no definite pupal characters distinguish the groups at this point. The results of our analysis of the concatenated data placed the *lacustris* group as sister to the *atrofasciata*, *notescens*, and *recurvata* groups (Fig. S2) rather than to the *atrofasciata* group. Placement of this group should become clearer as more species and markers are added to the data set. However, most importantly, all species groups we propose are strongly supported in both CAD and combined COI/CAD analyses.

To expand our ideas further and to express the need for a revision and expansion of current species groups, *M. radialis* and *M. borealis* form a separate group in our phylogeny (Fig. 2) and that of Ekrem et al. (2010b). Moreover, the morphologies of the adult male and pupa are markedly different from those of other *Micropsectra* species mentioned here. Thus, these species do not conform to any of the current species groups or the proposed system described above. S wed l (1981) mentioned constructing a *borealis* group, based on redescription of *M. borealis* (S wed l and Willassen 1980), but apparently, no subsequent treatment of the group was completed. S wed l and Willassen (1980) suggested that *M. borealis* and *M. radialis* form a monophyletic clade with the *atrofasciata* group based largely on similarities of the superior volsella. We dispute placement of these species in the *atrofasciata* group. Instead, we propose erecting the *borealis* group, which currently includes *M. borealis*

2. Setiger of superior volsella more or less circular (Fig. 3), sometimes with straight median margin 3
- 2'. Setiger of superior volsella triangular or finger-like (Fig. 5A), never with straight median margin 6
3. Mid and hind tibiae each with one obvious spur; digitus digitiform, extending well beyond median margin of superior volsella (fig. 2 by Säwedal and Willassen 1980) ... *M. borealis* (Kieffer, 1922)
- 3'. Mid and hind tibiae without spurs; digitus digitiform or triangular, occasionally pointed, sometimes extending slightly beyond median margin of superior volsella (Fig. 3; fig. 12A, B by Stur and Ekrem 2006) 4
4. Median volsella large, thick, club-shaped; knob between crests of anal point present; digitus short, never reaching margin of median volsella (Fig. 3) *M. neoappendica* n. sp.
- 4'. Median volsella small, thin, never club-shaped; knob between crests of anal point absent; digitus extending to or beyond margin of median volsella 5
5. Apex of median volsella not extending to or beyond that of inferior volsella (fig. 12A by Stur and Ekrem 2006) *M. logani* (Johannsen, 1928)
- 5'. Apex of median volsella reaches to or beyond that of inferior volsella (fig. 7 by Oliver and Dillon 1994) *M. polita* (Malloch, 1919)
6. Median volsella with leaf-like lamellae, sometimes held tightly together (Fig. 5A; fig. 8 by Säwedal 1976) or large and spatulate (fig. 3 by Reiss 1969a) 7
- 6'. Median volsella with spoon-shaped lamellae (Figs 6A, 7A, C) 9
7. Lamellae of median volsella large and spatulate; superior volsella triangular; digitus reduced, never reaching margin of median volsella (fig. 3 by Reiss 1969a) *M. attenuata* Reiss, 1969
- 7'. Lamellae of median volsella leaf-like; superior volsella finger-shaped; digitus either absent or extending to or past margin of superior volsella 8
8. Lamellae of median volsella often held tightly together, appearing brush- or tail-like; digitus absent; median volsella long (103–138 µm) (Fig. 5A) *M. penicillata* n. sp.
- 8'. Lamellae of median volsella not as above; digitus long, often extending to or past margin of superior volsella; median volsella short (23–31 µm) (fig. 8 by Säwedal 1976) *M. lindrothi* Goetghebuer, 1931
9. Median volsella long and sinuous or s-shaped (Figs 5A, 7A) 10
- 9'. Median volsella of varying length, more-or-less straight and parallel sided (Fig. 6A) 15
10. Median volsella nearly as wide as inferior volsella; inferior volsella strongly curved medially, gradually narrowing to tip (fig. 1 in Oliver and Dillon 1994) *M. dives* (Johannsen, 1905)
- 10'. Median volsella never as wide as inferior volsella; inferior volsella may or may not curve medially or narrow to tip 11
11. Inferior volsella either very slender or swollen at tip, appearing somewhat ball-shaped 12
- 11'. Inferior volsella of normal width 14
12. Inferior volsella very slender, distal part bent medially (fig. 3 by Reiss 1974) *M. recurvata* Goetghebuer, 1928
- 12'. Inferior volsella swollen at tip, appearing somewhat ball-shaped (Fig. 7A) 13
13. Anal point slender and parallel-sided towards tip (Fig. 8B); superior volsella broad with long, narrow digitus; digitus < 1/3 width of superior volsella (Fig. 8A) *M. xantha* (Roback, 1955)
- 13'. Anal point relatively broad and cone-shaped toward tip (Fig. 8D); superior volsella comparatively narrow; digitus > 1/3 width of superior volsella (Fig. 8C) *M. connexa* (Kieffer, 1906)
14. Superior volsella finger-like (fig. 1 by Säwedal 1981); LR₁ = 1.38–1.40; AR = 1.57–1.73 (Säwedal 1981) *M. tori* Säwedal, 1981
- 14'. Superior volsella comparatively triangular (fig. 2 by Oliver and Dillon 1994); LR₁ = 1.50–1.69; AR = 0.73–1.46 *M. geminata* Oliver and Dillon, 1994
15. Digitus short, rarely reaching margin of superior volsella (fig. 11 by Reiss 1969b, figs 22, 24 by Säwedal 1976); if digitus reaches close to margin of superior volsella, gonostylus is thick and stout and anal tergite with large lateral tooth or inferior volsella is enlarged at tip 16
- 15'. Digitus long, extending to margin of superior volsella or slightly beyond 17
16. Anal point short, triangular with blunt tip; inferior volsella enlarged at tip; digitus not extending beyond margin of superior volsella (fig. 7 by Oliver and Dillon 1994). *M. nigripila* (Johannsen, 1905)
- 16'. Anal point long or acute; inferior volsella not enlarged at tip 17
17. Median volsella of medium length (57–78 µm); AR = 1.04–1.49; gonostylus thick and stout, narrowing towards tip (fig. 24 by Säwedal 1976); anal tergite with lateral tooth *M. junci* (Meigen, 1818)
- 17'. Median volsella short (28–36 µm); AR = 0.50–0.73; gonostylus not thick and stout, but may

- narrow towards tip; anal tergite without lateral tooth *M. nana* (Meigen, 1818)
18. Superior volsella triangular (fig. 17 by Säwedäl 1976); anal tergite with small lateral tooth; middle tarsomere Ta₁ with 5–7 sensilla chaetica. *M. apposita* (Walker, 1856)
- 18'. Superior volsella finger-shaped (Fig. 5A; fig. 2 by Säwedäl 1976), more-or-less parallel at base; anal tergite with or without lateral tooth; middle tarsomere Ta₁ with ≤5 sensilla chaetica 19
- 19a. Gonostylus narrowing toward tip (Fig. 6A); LR = 1.52–1.54; anal tergite without lateral tooth; superior volsella lacking field of microtrichia on basal part; AR = 1.47–1.70; digitus often extending to or past margin of superior volsella; median volsella 68–85 µm *M. subletteorum* n. sp.
- 19b. Gonostylus not narrowing toward tip (fig. 2 by Säwedäl 1976); LR = 1.34–1.51; anal tergite with small lateral tooth; few microtrichia on basal part of superior volsella; AR = 1.47–1.83; digitus extending to or past margin of superior volsella; median volsella = 40–59 µm *M. insignilobus* Kieffer, 1924
- 19c. Gonostylus not narrowing toward tip (fig. 2 by Säwedäl 1976); LR = 1.37–1.41; anal tergite with small-to-medium lateral tooth; large group of microtrichia on basal part of superior volsella; AR = 1.75–2.14; digitus extending to or past margin of superior volsella; median volsella = 63–78 µm *M. brundini* Säwedäl, 1979

Key to Known Nearctic *Micropsectra* Pupae and Pupal Exuviae

This key adopts several couplets given by Säwedäl (1976), Langton (1991), or Stur and Ekrem (2006), but several species and diagnostic characters have been added. Pupae of *Micropsectra connexa*, *M. spinigera*, and *M. tori* are unknown and, therefore, are not included in the key. We have elected not to arrange the key by species group at this time because many of the species included in the key either are not known in multiple life stages or are not available for inclusion in molecular analyses. Morphological terminology and abbreviations follow Sæther (1980).

1. Abdominal segments II–V each with 4–7 lateral setae + taeniae (fig. 3 by Säwedäl and Willassen 1980) *M. borealis* (Kieffer, 1922)
- 1'. Abdominal segments II–V each with 3 lateral setae + taeniae (Fig. 5B) 2
2. Tergite II with shagreen in 2 triangular patches that are not connected anteriorly *M. sedna* Oliver, 1976
- 2'. Tergite II extensively covered with shagreen, leaving only a median and usually lateral rounded patches free of shagreen (Fig. 5B) 3
3. Tergites III–VI with similarly paired, anterior point patches (fig. 10.54E by Pinder and Reiss 1986) *M. nana* (Meigen, 1818)
- 3'. Tergite III with posterior spine patches (Fig. 4A, B) 4
4. TIV with long longitudinal spine patches extending from anterior spinule patches (Fig. 3A, B) 5
- 4'. TIV with only short anterior patches consisting of spinules and points (Fig. 6B) 9
5. Longitudinal spine patches of TIV extend ~½ the length of the tergite; a row of fine shagreen extends posteriorly from each spine patch (fig. 20 by Säwedäl 1976) *M. apposita* (Walker, 1856)
- 5'. Longitudinal spine patches of TIV extend well over halfway down length of tergite; shagreenation not extending posteriorly from spine patches 6
6. TIII with 1 lateral setae and 2 lateral taeniae. *M. logani* (Johannsen, 1928)
- 6'. TIII with 3 lateral setae; no taeniae 7
7. Longitudinal patches of TV consisting of shagreen, points, spinules, and often spines; spines, if present, similar in size to those of TIV; total patch length subequal to that of TIV (fig. 11 by Oliver and Dillon 1994) *M. polita* (Malloch, 1919)
- 7'. Longitudinal patches of TV consisting only of spinules and points; length of patches less than those of TIV (Figs 4A, 5B) 8
8. Distance between anterior and posterior pairs of dorsocentrals is approximately equal; nose of wing sheath moderate to strong. *M. neoappendica* n. sp.
- 8'. Distance between anterior pair of dorsocentrals 2–3× greater than that of posterior pair and anterior pair thinner than posterior pair (Fig. 5F); nose of wing sheath weak to moderate *M. penicillata* n. sp.
9. Prealar tubercle with finger or nose-like projection (fig. 5 by Reiss 1969a, fig. 25 by Säwedäl 1976) 10
- 9'. Prealar tubercle without finger-like projection ... 11
10. Anal lobe with 23–31 lateral taeniae; tergites IV and V almost entirely covered with shagreen and spinules; the spinules of the anterior transverse patches similar in size, but more dense and darker than elsewhere on tergites (fig. 7 by Reiss 1969a) *M. attenuata* Reiss, 1969
- 10'. Anal lobe with 46–52 (Langton 1991) or 62–73 (Säwedäl 1976) lateral taeniae; tergites IV and V with distinct patches of spines or spinules that

- are stronger than surrounding shagreen (fig. 16 by Säwedal 1976) *M. junci* (Meigen, 1818)
11. Tergite VI with a single, crescent-shaped anterior point patch; pleura without shagreen (fig. 5 by Oliver and Dillon 1994) *M. dives* (Johannsen, 1905)
- 11'. Tergite VI with paired anterior point patches or only with paired longitudinal shagreenation; pleura with or without shagreen 12
12. Tergite IV with 3 lateral taeniae 13
- 12'. Tergite IV with 1 or 2 lateral taeniae 16
13. Shagreen absent between spine patches of TIII (fig. 146J by Langton 1991) *M. recurvata* Goetghebuer, 1928
- 13'. Shagreen present between spine patches of TIII (Fig. 6B) 14
14. Tergite III with spinules ($<40\ \mu\text{m}$) in paired, comparatively longitudinal patches, approximately equal in size to those on TIV and TV, the greater part of the spinule patch anterior to seta D5 (fig. 148C by Langton 1991); pedicel sheath tubercle weakly developed *M. insignilobus* Kieffer, 1924 and *M. brundini* Säwedal, 1979
- 14'. Tergite III with spines ($>40\ \mu\text{m}$) or spinules in paired patches that curve in lateral direction posteriorly, if spinules ($<40\ \mu\text{m}$) present, they are noticeably larger than those on TIV and TV; the greater part of spine patch posterior to seta D5 or approximately equally distributed above and below seta D5 (Fig. 6B; fig. 148I by Langton 1991); pedicel sheath tubercle usually well developed 15
15. Anal lobe with >43 lateral taeniae (48–70, Langton 1991; 43–56, Säwedal 1976); thoracic horn $>450\ \mu\text{m}$ (495–710 μm , Langton 1991; 450 μm , Säwedal 1976) *M. lindrothi* Goetghebuer, 1931
- 15'. Anal lobe with <48 taeniae; thoracic horn 378–553 μm ; moderate nose on wing sheath *M. xantha* (Roback, 1955)
16. Thoracic horn setae covering apical $\frac{2}{3}$ and diminishing in length towards tip; setae short, less than $\frac{1}{5}$ of horn length (fig. 10 by Oliver and Dillon 1994; fig. 2C by Langton 1998) 17
- 16'. Thoracic horn setae covering apical $\frac{5}{6}$ and not diminishing significantly in length toward tip; setae long, $\sim\frac{1}{3}$ of horn length (Fig. 6F; fig. 4 by Oliver and Dillon 1994) 18
17. Cephalothorax with 2 median anteprenotal setae; wing sheath without nose; anal lobe with 48–66 taeniae *M. silvesterae* Langton, 1998
- 17'. Cephalothorax with 1 median anteprenotal seta; wing sheath with small nose; anal lobe with 22–52 taeniae *M. nigripila* (Johannsen, 1905)
18. Tergite VI with paired anterior point patches (fig. 3 by Oliver and Dillon 1994); anal lobe with 31–36 taeniae *M. geminata* Oliver and Dillon, 1994
- 18'. Tergite VI consisting only of shagreen (Fig. 6B); anal lobe with 40–47 taeniae *M. subletteorum* n. sp.

Taxonomic note: *Micropsectra brundini* was described from oligotrophic lakes in Greenland (Säwedal 1979) and is morphologically similar to *M. lindebergi* and *M. insignilobus* in both the adult male and pupal stages; females of this species are not yet known. The type material of this species could not be located at ZSBS (M. Spies, ZSBS, personal communication). We attempted unsuccessfully to locate type material of this specimen at various other museums. However, we have examined material identified as *M. brundini* in the collections of Peter Langton, Claus Lindegaard, and the Sublette material at UMSP and confirm that this species is morphologically very similar to *M. lindebergi* and *M. insignilobus*. Values for the AR seem to be the most consistent and obvious differences used to separate *M. brundini* from *M. lindebergi* (1.75–2.14 and 1.31–1.58, respectively), and a longer median volsella aids in differentiation from *M. insignilobus* (63–78 vs 40–59 μm). Säwedal (1979) lists other characters that may aid in differentiation, but overlap between these species, particularly *M. brundini* and *M. insignilobus*, is considerable. Resolution of these taxonomic issues is beyond the scope of our paper, but we think sampling of localities known to produce *M. brundini* and subsequent molecular analyses of fresh specimens could help assess relationships among these species.

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