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Zavrelia parapentatoma (Chironomidae: Diptera), a curious new species from North America, revealed by molecular methods

ARMIN NAMAYANDEH[1,*], PATRICK L. HUDSON[2], EDRIS GHADERI[3,4] & WENDYLEE STOTT[5]
[1]*Department of Environmental and Life Sciences, Trent University, 1600 West Bank Drive, Peterborough, ON, Canada.*
arminnamayandeh@trentu.ca; a.namayan@taxanama.com; https://orcid.org/0000-0003-2136-0497
[2]*5597 New Meadow Drive, Ypsilanti, MI 48197.*
patrick_hudson@sbcglobal.net; https://orcid.org/0000-0002-7646-443X
[3]*Department of Fisheries Sciences, Faculty of Natural Resources, University of Kurdistan, Sanandaj, Iran.*
ed.ghaderi@uok.ac.ir; https://orcid.org/0000-0002-9875-7735
[4]*Department of Fisheries and Aquatic Ecology, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.*
[5]*Department of Fisheries and Oceans, Arctic and Aquatic Research Division, 501 University Cr., Winnipeg, MB, Canada.*
Wendylee.Stott@dfo-mpo.gc.ca; https://orcid.org/0000-0002-5252-4901
[*]*Corresponding author. arminnamayandeh@trentu.ca; a.namayan@taxanama.com*

Abstract

In this study, we describe *Zavrelia parapentatoma* **sp. nov.** based on specimens collected near a cattail marsh in Michigan, USA. At first glance, the new species resembles *Zavrelia pentatoma* Kieffer & Bause, 1913 closely. However, a detailed morphological and molecular assessment revealed the differences. Morphologically the two species can be separated mainly based on the shape and pattern of long spinulae between long anal crests of adult males and setal patterns on the pupal abdomen. Larvae of both species also have some subtle morphological differences that are discussed in this study. Our molecular analysis of cytochrome oxidase (COI) genes and methods for delimiting species further supported the presence of a new species. In this study, we describe all the life stages, provide life history observations for this new species, and provide keys to adult males and immatures of the genus *Zavrelia*.

Key words: Chironomidae, *Zavrelia*, new species, molecular, North America, cattail marsh

Introduction

The genus *Zavrelia* of the tribe Tanytarsini, subtribe Zavreliina is represented by 11 species in the northern hemisphere (Ekrem & Stur 2009; Lin & Wang 2017). Kieffer, Thienemann and Bause erected this genus in Bause (1913) based on *Zavrelia pentatoma* Kieffer & Bause, 1913. *Z. pentatoma* is considered a Palearctic species with widespread distribution only throughout Europe (Ekrem & Stur 2009, Giłka 2008). Giłka (2008) discussed the variability of morphological characteristics of adult males from different populations of *Z. pentatoma* in Poland and Finland. This variability of characteristics is also found in widely distributed populations of other species of *Zavrelia,* hinting at the possible existence of several cryptic species within this genus (Lin & Wang 2017).

In this study, we collected the adult male of a curious species that, at first glance, looks very similar to *Z. pentatoma*. These adults were sampled near a cattail marsh in Michigan, USA, with a habitat similar to that of *Z. pentatoma* which includes peat bogs, moor ponds, and moor lakes rich in humic acids and relatively low pH (Brundin 1949). *Z. pentatoma* is likely adapted to temporary, oxygen-poor shallow water habitats (Brundin 1949), and so is the new species.

We here describe a new species *Zavrelia parapentatoma* based on all its life stages and provide keys to adult males and immatures of the genus *Zavrelia*. Additionally, we have provided life history observations for the new species. The characteristics of the shape and pattern of long spinulae between long anal crests of adult males, setal patterns on the pupal abdomen, shape, and the size of Lauterborn organ of larval antenna, along with the placement

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of ventromental plates relative to mental teeth separates the new species from closely related *Z. pentatoma*. Further, molecular analysis of cytochrome oxidase (COI) genes and methods for delimiting species supported the presence of a new species.

Material and methods

Study area

Our collection site was in a 0.022 km^2 cattail marsh in the Erwin Stuki Preserve (Degrees. Minutes 42.1368, -83.9808) southeastern Michigan, which is part of Washtenaw County's Natural Areas Preservation Program. The marsh is about 400 m from the Raisin River and is a residual natural marsh, one of the hundreds that were originally been found in the area and were quickly drained with ditches or tile.

Sampling collection and preparation and imagery

We collected the adult specimens of *Z. parapentatoma* with a sweep net and immatures from the boardwalk along the marsh using a plastic cup. We preserved the specimens in 70% ethanol. The type and voucher specimens of adults and immatures were mounted on microscope slides following a procedure outlined in Namayandeh & Hudson (2022). We produced the image of specimens using a Diagnostic Instruments Inc. Spot 5.1 camera mounted on an Olympus BX51 compound scope. Morphological terminology and measurements follow those of Sæther (1977, 1980). The following additional abbreviations are from Ekrem & Stur (2009): AAR—the ratio of larval antennal pedestal/antennal segment 1 length; AHR—the ratio of larval antennal pedestal/head length; MVR—mentum/ventromental plate width ratio. In the taxonomy section, values after the comma denote the mean. All type specimens of the studied Chironomidae are deposited at the Albert J. Cook Arthropod Research Collection (ARC), Michigan State University.

Ekrem & Stur (2009) and Lin & Wang (2017*)* have provided keys for adults and immature stages. Therefore, in this study, we modified their keys to include the new species.

Molecular analysis

We extracted the genomic DNA from the tissues of two adult males (thorax or legs) using the Qiagen DNA Blood and Tissue Kit (Qiagen, Inc., Germantown, MD). We amplified a 685 base pair fragment of the cytochrome c oxidase subunit 1 (COI) using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). DNA amplification was carried out in 20 µl reactions using GoTaq DNA polymerase (Promega Co., Madison, WI), 1X manufacturer's buffer, 10mM dNTP mix, 10mM of each primer, and 200 to 250ng template DNA. We performed the amplification cycles using an initial denaturation step of 95°C for five minutes, then followed by 34 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for one minute, and one final extension at 72°C for three minutes. Excess primers were removed, and Taq polymerase was deactivated using ExoSAP-IT (Life Technologies, Waltham MA), and amplicons were shipped to Michigan State University's RTSF Genomics core (https://rtsf.natsci.msu.edu/genomics/) for bidirectional sequencing using BigDye 3.1 termination chemistry (Life Technologies, Waltham MA). Reverse and forward sequences were assembled and edited using Bioedit 7.2.5 (Hall 1999). All new sequences were submitted to the GenBank (OQ301917 and OQ301918) and BOLD databases (ERSTU001-23 and ERSTU002-23).

We used seven other sequences from the genus *Zavrelia* and ten from *Stempellinella* in addition to the two sequences obtained in this study. The list of sequences, codes, GenBank, or BOLD accessions is provided in Supplementary file 1-Tables S1. We obtained the phylogenetic trees based on three methods Neighbour-Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI). The NJ phylogenetic tree was made using Kimura's 2-parameter (K2P) model in MEGA X with 10,000 bootstrap replications (Kumar *et al.* 2018). To construct the tree using ML and BI methods, sequences were aligned using Clustal X version 2.1 software (Larkin *et al.* 2007). We determined the best model for nucleotide substitution using jModelTest 2.1.3 (Darriba & Posada 2014) and chose the best model using the Akaike Information Criterion (AIC). We constructed the ML trees using RAxML-HPC BlackBox (8.2.12) software (Stamatakis 2014) in the CIPRES Scientific Gateway v.3.3 XSED (Miller *et al.* 2010) and with 10,000 Bootstrap repeats. We constructed the BI trees using MrBayes 3.2.7 software (Huelsenbeck & Ronquist 2001). We performed Markov chains with 1000000 generations (as burn-in %25) and determined

stationarity by examining traces of the likelihood using graphics in Tracer v. 1.4 (Drummond & Rambaut 2007). Trees constructed in ML and BI models were visualized in FigTree v. 1.4.2 (Rambaut 2014).

We obtained the phylogenetic distances using Kimura-2-parameter (K2P) model (Kimura 1980) in MEGA X (Kumar *et al.* 2018). We first determined the intraspecific K2P distance between the two sequences of *Zavrelia* from Michigan and the intraspecific K2P distance between sequences of *Z. pentatoma* available in BOLD and NCBI. We also obtained the distance between these two aforementioned groups. Because there are no other sequences of *Zavrelia* besides *Z. pentatoma*, we used its closest genus *Stempellinella* Brundin, 1947 and determined the intraspecific and interspecific K2P distances between four species in this genus, and in comparison, to *Z. pentatoma* sequences in BOLD and NCBI.

To determine the limits of "molecular species" we used four species delimitation methods, three based on distance and one based on topology. The distance-based methods were Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.* 2012) (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html), Assemble Species by Automatic Partitioning method (ASPA) (Puillandre *et al.* 2021), (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html), and K2P distances which we considered 5% as a threshold value. For the topology method, we used Multiple-rate Bayesian Poisson Tree Process (mPTP) (https://mptp.h-its.org). We ran ABGD with P min = 0.001, P max = 0.1, and a gap width of 1.21, all for a total of 10 steps setting to calculate the barcode gap in the distribution of pairwise differences. We ran ASAP with Kimura (K80) ts/tv = 2.0 setting. We ran mPTP analysis using the obtained topology in MrBayes as input and the default settings.

Results

Molecular analysis

All three analyses, NJ, ML, and BI, produced the same tree topology. The two Michigan specimens of *Z. parapentatoma* clustered with a sequence identified as *Stempellinella* sp., accession number KR659992.1, from Ontario, Canada (Hebert *et al.* 2016). This places this sequence in *Z. parapentatoma* (Fig. 1). The phylogenetic trees show the presence of three separate clades (with support of 100), which we named Z1 (*Z. parapentatoma* sequences from Nearctic), Z2 (*Z. pentatoma* from Sweden and Germany), and Z3 (*Z. pentatoma* from Finland). These indicate the presence of three possible separate species, which means the European *Z. pentatoma* belongs to two separate species, and the Nearctic specimens also belong to a separate species (Figure 1).

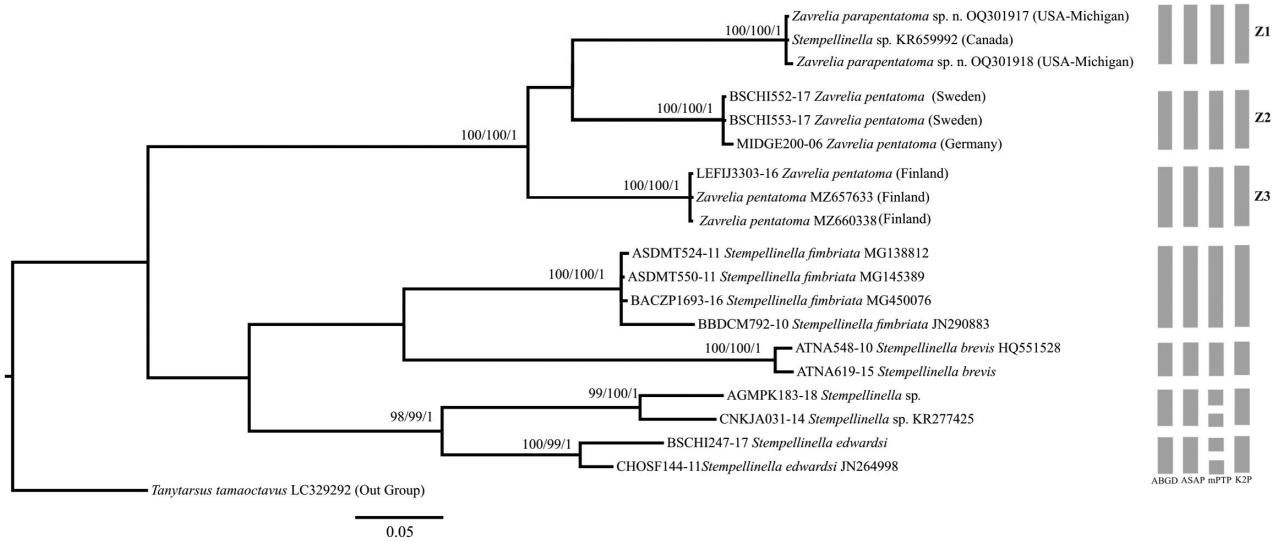

FIGURE 1. Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) trees of the genera *Zavrelia* Kieffer & Bause, 1913, *Stempellinella* Brundin, 1947, and one outgroup *Tanytarsus tamaoctavus* Sasa, 1980 inferred from the COI nucleotide sequence data (658 bp). Numbers on branches represent the bootstrap value for Neighbor-Joining (NJ) and Maximum Likelihood (ML) (10000 replicates) and posterior probabilities for BI, respectively. The gray bars on the right are clusters estimate using four molecular species delimitation methods: ABGD, ASAP, mPTP, and K2P (species delimitation based on the K2P distance new species threshold value 5% for sequences used).

The intraspecific K2P distance calculated for three groups of *Zavrelia*, Z1-Z3, are 0.00%, 0.20%, and 0.00%, respectively, with an average of 0.07%. The average intraspecific K2P distance within true *Stempellinella* species (i.e., not including KR659992) was 2.45%. Interspecific K2P distances between Z1 with Z2 and Z3 were 9.28% and 10.32%, respectively and between Z2 and Z3 8.65%. The average Interspecific K2P distance was 9.42% between Z1-Z3. The average interspecific K2P distance for true *Stempellinella* species was 13.06% (Supplementary file 1-Tables S2).

Using our sequence data and obtained sequences data from other studies, the analyses used to show the limit of species indicated agreement across both distance and topology methods suggesting *Z. pentatoma* contains two species and *Z. parapentatoma* is a separate species (Figs. 1–2). The mPTP result indicates that sequences of *Stempellinella* used in this study belong to 6 separate species, whereas ABGD and ASPA only indicate four. The higher interspecific differences among *Stempellinella* species can partially explain this result. Using ABGD, we saw a gap between the highest intraspecific K2P distance (0.05 or 5%) and the lowest interspecific K2P distance (0.08 or 8%). This gap (i.e., for sequences used) suggests that if the distance between two sequences is less than 5%, the sequences belong to the same species, and if it is more than 8%, the sequences belong to two different species (Fig. 2). The ASPA result indicates the same results as those of ABGD (Supplementary file 1-Figure S1).

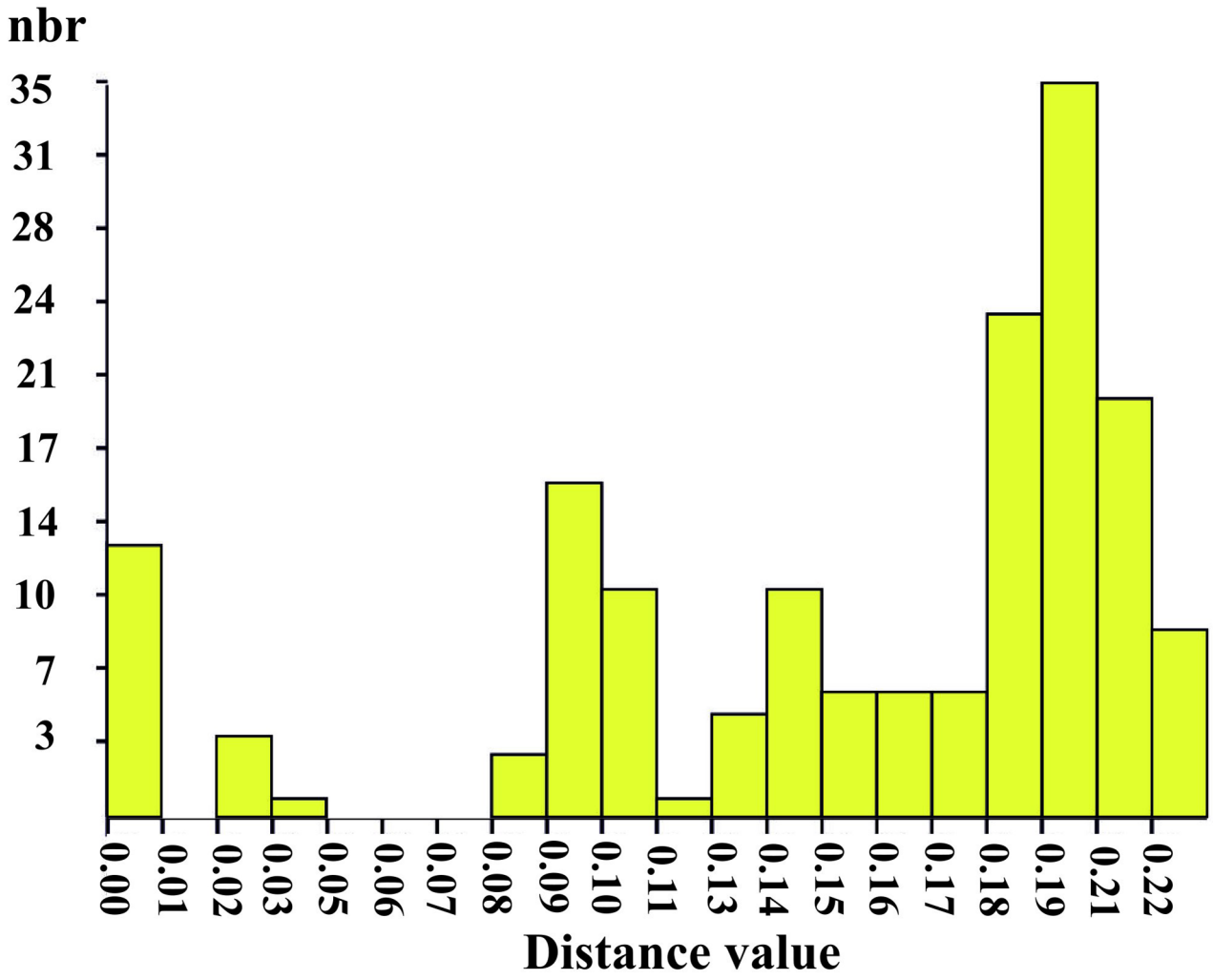

FIGURE 2. Histogram of genetic distance estimates from ABGD (Automatic Barcode Gap Discovery) for a partition analysis of 19 cytochrome oxidase subunit1 sequences of the *Zavrelia* and *Stempellinella* species. nbr = number of runs.

To delimit the morphologically close or indistinguishable species, the molecular methods provide more unbiased alternatives (Anderson *et al.* 2013; Carew *et al.* 2011; Giłka, *et al.* 2018; Sinclair & Greens 2008; Song *et al.* 2017). Recent studies have successfully demonstrated the Chironomidae species' delimitation using tree-based and distance-based molecular methods; however, the threshold of distance-based methods for the species between the chironomid genera varies. For instance, Lin *et al.* (2015) set the threshold for *Tanytarsus* species at 4–5%, Giłka, *et al.* (2018)

set the threshold for *Tanytarsus mendax* group at 1–6%, Song *et al.* (2017) set the threshold for *Polypedilum* species at 5–8%, and Song *et al.* (2022) set the threshold for *Stenochironomus* species at 4–6%. Although in the absence of a more comprehensive DNA library for *Zavrelia* species, it is still too early to set a threshold for the species of this genus, the gap of 8% obtained in this study, based on the distance-based methods (i.e., ABGD and ASAP), is large enough (i.e., larger than previous studies) to support delimitation of *Z. parapentatoma* from that of *Z. pentatoma*.

Taxonomy

Zavrelia parapentatoma sp. nov.

Figs. 3–4

LSID: urn:lsid:zoobank.org:act:3AEFFA97-108E-493C-91A4-7CCCE57A5E91

Type material. Holotype male; USA, Michigan, Washtenaw Co., Ervin-Stucki Preserve; 42.136730°, -83.981339°; 8.vii.2021; leg. Patrick Hudson; deposited at ARC. Paratypes: 3 males, 1 female; same as holotype. 1 male, 1 female; USA, Michigan, Washtenaw Co., Ervin-Stucki Preserve; 42.136730°, -83.981339°; 8.v.2022; leg. Patrick Hudson; deposited at ARC. 5 pupae, 5 larvae; USA, Michigan, Washtenaw Co., Ervin-Stucki Preserve; 42.136730°, -83.981339°; 8.vi.2022; leg. Patrick Hudson; deposited at ARC.

Diagnostic characters. *Zavrelia parapentatoma* can be separated from other *Zavrelia* species by the following combination of characters: Adult male with wing length ~1.3 mm, 4–5 longer than broad; AR 1.19; frontal tubercle minute, ~4 µm long; anal point with numerous (35) long spinulae (at least 8 times as long as wide) placed between long anal crest and flexing with their pointed ends directed anteriorly; 8 long median tergite setae placed on 1–3 light roundish fields at mid tergite IX; median volsella short, stout, with simple and subulate lamellae. Adult female with AR 0.23–0.28; temporal setae 8; frontal tubercle small, ~6 µm long; sternite VIII with 30 setae; notum including rami 131–170, 151 µm long; coxosternapodeme with developed anterior and posterior lobes. Pupa with cephalic tubercle weak and short; the median patch of microspinules much more prominent on tergites II–V but not well-extended to the lateral edge; those in II-IV appear divided with only a few microspinules in between; tergite II with small anterior patches of lateral shagreens; lateral shagreens prominent and extend along the lateral edge of tergites III–VI; segment VIII with a simple or bifid posterolateral spur; anal lobe seta taeniate. Larva with AR 0.93–1.23; AAR 0.64–0.77, 0.70; AHR 0.31–0.36, 0.30; ventromental plates medially reaching the border of the second and third lateral teeth of mentum; MVR 1.00–1.18, 1.11.

Etymology. The new species is named after its similarity to *Z. pentatoma*. The prefix "*para*" is Latin, meaning "near" or "close".

Description. Male (n = 5).

Total length 1.7–1.9, 1.8 mm. Wing 1.3 mm long and 0. 3 mm wide.

Coloration of the mounted specimen. Head, thorax, and tergites brown. Legs and sternites yellowish brown. Wing and halters greyish-brown.

Head. Plume well-developed; antenna with 10 flagellomeres, ultimate flagellomere with 2 sensilla clavata (Fig. 3A); AR 1.12–1.29, 1.19. Frontal tubercle minute, 4 µm long. Temporal setae with only 2 inner verticals. Tentorium 99–110, 105 µm long (Fig. 3B). Clypeus squared about 82 µm long and 89 µm wide, with 10 setae 78–94 µm long. Third palpomere with 1 sensillum clavatum; lengths of palpomeres (in µm): 46–70, 53; 30–42, 38; 93–116, 105; 76–88, 83; 92–106, 97.

Thorax chaetotaxy. Ac 12; Dc 9; Pa 1; Scts 4–5.

Wing (Fig. 3C). Wing 4–5 times longer than wide. R with 17–20 setae, R_1 with 6–7, R_{4+5} with 18–19 setae. VR 1.5. Wing cell setation mainly confined to the wing apex.

Legs. Fore leg tibia with 40 µm long spur; mid and hind legs tibiae with well-separated combs; mid leg tibial combs 12 µm long, and spurs 13–16 µm long; hind leg tibia with combs 9–11 µm long and spurs 13–17 µm long. Lengths and ratios of leg segments as in Table 1.

TABLE 1. Male leg lengths (μm) and proportions of *Zavrelia parapentatoma* **sp. nov.**

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
p_1	493	340	442	266	203	122	74	1.30	1.9	1.9
$\mathbf{p_2}$	481	375	232	128	104	69	55	0.62	3.1	3.7
$\mathbf{p_3}$	596	486	296	170	150	88	60	0.61	2.9	3.7

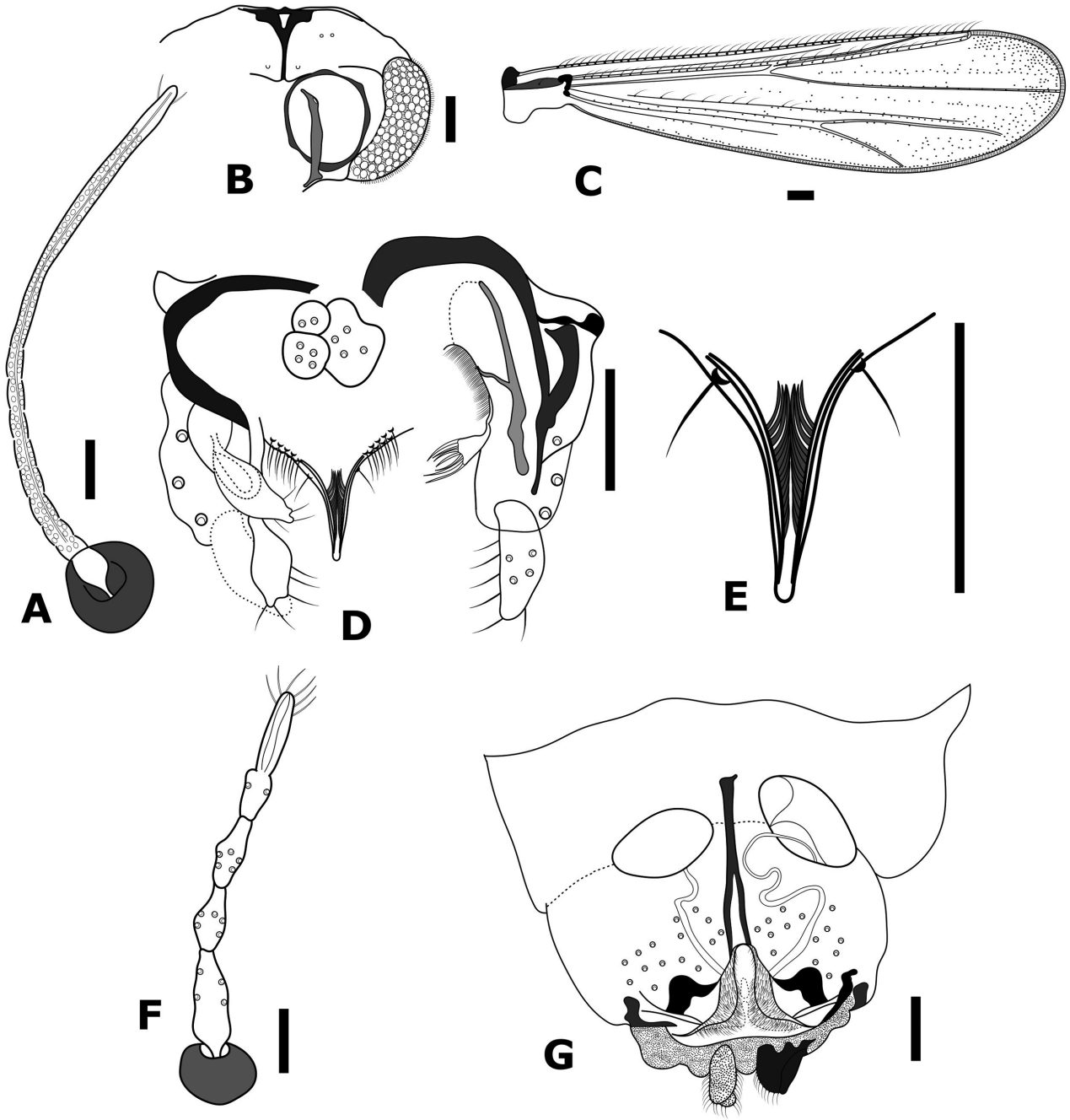

FIGURE 3. *Zavrelia parapentatoma* **sp. nov.**, male (A-E), female (F-G). A. antenna; B. head; C. wing; D. hypopygium, dorsal and ventral view; E. anal point; F. antenna; G. genitalia. dorsal view. Scale bars represent 50 μm.

Hypopygium (Fig. 3D–E). Tergite IX 103–129, 114 μm long and 60–86 μm wide, with 8 median setae on 1–3 light roundish fields, and 20 apical setae. Anal point 42–58, 50 μm long and 20–24, 22 μm wide (maximum); with 2 lateral setae close to the base; well-developed crests present; 33–37, 35 very long spinulae present between crests of anal point (Fig. 3D–E). Gonocoxite 64–70, 67 μm long; gonostylus 47–54, 49 μm long; HR 1.30–1.5, 1.4. Superior volsella 42–45 μm long, digitiform, medially directed, with 3 dorsal and 3 median setae on setiger; median volsella

stout with cluster of simple and subulate, lamella, stem 16–19, 17 µm long; inferior volsella club-shaped, 37–52, 49 µm long with several distal setae, dorsal surface without microtrichia. HV 3.6.

Female (n = 2).

Total length 1.8–1.9 mm. Wing 1.3 mm long and 0.32 mm wide.

The coloration of the mounted specimen. Same as male.

Head. Antennae with 5 flagellomere (Fig. 3F) with ultimate flagellomere 59–61 µm long, AR 0.23–0.28, 0.26. Temporal setae 8. Frontal tubercle small, 6 µm long. Tentorium 107–116, 112 µm long. Clypeus with 12 setae, setae 77 µm long. Lengths of palpomeres (in µm): 40–49, 44; 27–37, 32; 71–80, 76; 62–63; 94.

Thorax chaetotaxy. Ac 8; Dc 7; Pa 1; Scts 6.

Wing. Wing L/W 3.8–4.0. Brachiolum with 2 setae; R with 19 setae, R4+5 with 24 setae, R1 with 7 setae. Wing membrane setation same as in male. VR 1.5–1.6.

Legs. Foreleg tibial spur with 23 µm long; mid and hind legs tibiae with well-separated combs; mid leg tibial spurs 16 µm long; hind leg tibial spurs 9–11 µm long. Lengths and ratios of leg segments as in Table 2.

TABLE 2. Female leg lengths (µm) and proportions of *Zavrelia parapentatoma* **sp. nov.**

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	**LR**	**BV**	**SV**
$\mathbf{p_1}$	487	366	501	245	181	113	70	1.37	2.2	1.7
$\mathbf{p_2}$	555	466	271	118	116	60	52	0.58	3.7	3.8
$\mathbf{p_3}$	627	557	326	167	136	80	67	0.59	3.4	3.6

Genitalia (Fig. 3G). Tergite IX 138–163, 151 µm long. Sternite VIII with 30 setae. Gonocoxite with 1 seta. Notum including rami 131–170, 151 µm long, notum alone 83–102, 92 µm long. Seminal capsule 56 µm in diameter (n = 1). Coxosternapodeme with developed anterior and posterior lobes. Cercus 49 µm long and 30 µm wide.

Pupa (n = 5).

Total length 2.2–2.3 mm; abdomen 1.6–1.7 mm long.

Colouration of the pupal exuviae. Pale brown. Lateral margins of abdominal segment VIII much darker.

Cephalothorax: Cephalic tubercle weak and short, 18–29, 22 µm. Taeniate frontal seta 135–149, 142 µm long (Fig. 4A). Pedicel sheath tubercle absent. Thoracic horn 248–428, 349 µm long, 18–28, 22 µm wide, with few spines in mid-section (Fig. 4B); precorneals taeniate, $Pc_{1–3}$ 136, 183, 196 µm long (n = 1). Wing sheath 699–754, 725 µm long and 177–195, 187 µm wide.

Abdomen (Fig. 4C): Tergite I bare. Tergites II–IX with median patch of microspinules. Median patch of microspinules much more prominent on tergites II–V and not well extended to the lateral edge; those in II–IV appear divided in the middle with only a few microspinules; the median patch of microspinules on tergites VI–IX reducing gradually and well-divided. Pleura II with small anterior patches of lateral shagreens; lateral shagreens prominent and extend along the lateral edge of pleura III–VI. No visible lateral shagreens on pleura VII–IX. Tergite II with pedes spuri B and posterior hook row of 50–60, 55 hooks, 120–155, 137 µm wide. Sternites bare. Segment II–III with 3 simple lateral setae; segment IV with 2 simple and 1 taeniate lateral setae; segment V–VII with 4 taeniate lateral setae; segment VIII with 3 taeniate lateral setae and a simple to bifid posterolateral spur, spur 29–35, 32 µm long. Anal lobe 108–123, 116 µm long and 61–83, 74 µm wide; genital sac 163–190 µm long and 50–69 µm wide; anal lobes with fring of 16–17 taeniate setae of 73–112, 90 µm long; anal lobe seta taeniate 77–81, 79 µm long.

Larva (n = 5).

Total length 2.9–3.3, 3.0 mm, case 2.9 mm long.

Coloration. Head capsule brown, mentum, inner teeth of mandibles, and occipital region darker. Abdomen yellowish brown.

Head: HL/HW 0.84–1.05, 0.9. AR 0.93–1.23, 1.1; antennal pedestal 55–64, 57 µm long with well developed, 29–31, 30 µm long spur; antennal segment lengths (in µm): 73–88, 82; 32–40, 37; 18–22, 19; 11–15, 13; 7–8; third segment inserted subapically on segment two; AAR 0.64–0.77, 0.70; AHR 0.31–0.36, 0.30; antennal blade 113–114 µm long; Lauterborn organs 17–26, 22 µm long (Fig. 4D). Labral SI comb-like, SII plumose, SIII simple. S3 simple, 86–104, 95 µm long. Premandible 49–53, 51 µm long; quartered and with a well-developed brush (Fig. 4E). Mandible 82–88, 84 µm long, with 1 subapical, 1 apical, and 3 inner teeth; setae subdentalis 48–56, 51 µm long, reaching well-beyond apical tooth; setae interna with 3 blades of well-branched spines (Fig. 4F). Mentum with one median tooth and six lateral teeth; first lateral teeth slightly lower than second and third; ventromental plates medially reaching the border of the second and third lateral teeth of mentum (Fig. 4G); MVR 1.00–1.18, 1.11.

Body: Posterior parapod 86–102, 94 µm long and 124–133, 130 µm wide, with 16 simple claws. Procercus 23–29, 24 µm long and 23–29, 26 µm wide; each procercus with 4 apical setae, 2 shorter 372–404, 380 µm long, and the 2 longer 502–679, 617 µm long; subapical seta 163–188, 171 µm long; supranal seta 237–255, 248 µm long. 4 conical anal tubules present, 73–92, 82 µm long.

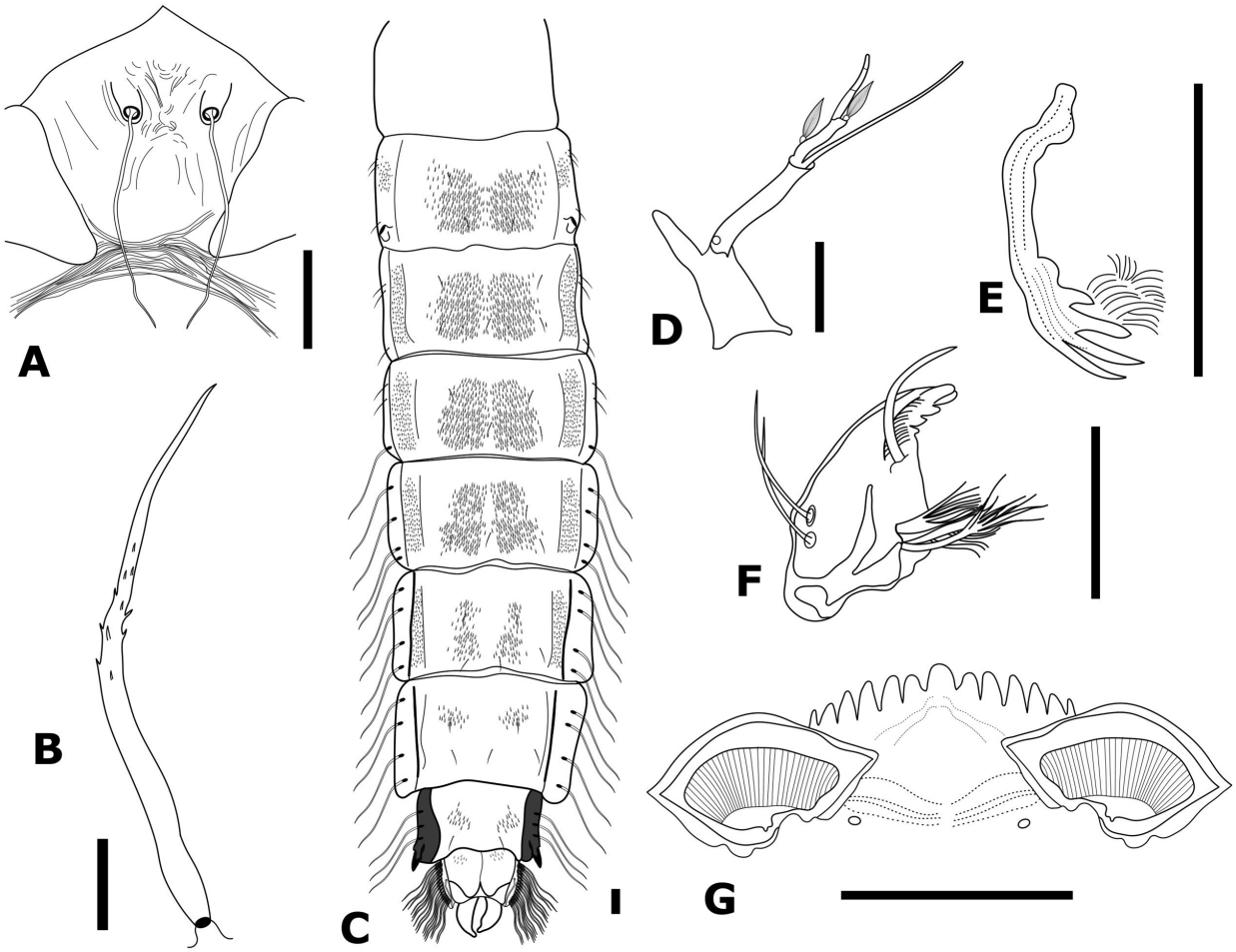

FIGURE 4. *Zavrelia parapentatoma* **sp. nov.**, pupa (A-C), larva (D-G). A. frontal apotome; B. thoracic horn; C. abdomen; D. antenna; E. premandible; F. mandible; G. mentum. Scale bars represent 50 µm.

Taxonomic remarks. The *Z. parapentatoma* and *Z. pentatoma* are close species and probably form a sister group. The morphological characters that separate the two species are, at the most, very subtle. There are many overlapping sizes and ratios between both species, which makes the distinction between the two species difficult (Table 3). For adult males, the only distinguishing character is the spinulae of the anal point, which in *Z. parapentatoma* are much longer (at least 8 times as long as wide) compared to *Z. pentatoma* (no more than 4 times as long as wide). Females of both species are hard to separate; the range of notum and ramus length combined may be slightly different between the two species, with *Z. parapentatoma* being in the higher range. The wing venarum ratio of adults of both species also differs slightly, with *Z. parapentatoma* having the higher ratio. The pupae of both species show the most significant difference among life stages. *Z. parapentatoma* has median patches of microspinules on tergites II–V less extended, and those in II–IV appear divided in the middle with only a few microspinules. Additionally, the anal lobe seta is prominent and taeniate in *Z. parapentatoma*. *Z. pentatoma* has a well-extended and joined patch of microspinules on tergites II–V, and the anal lobe seta is simple. Few characters could separate the larva of *Z. parapentatoma* from that of Z. *pentatoma* including the extent of the ventromental plates, size of Lauterborn organs, length of the shortest anal setae, and length of the anal tubules. In *Z. parapentatoma* ventromental plates medially reach the border of the second and third lateral teeth of the mentum, whereas in *Z. pentatoma* hardly reach the third lateral teeth. *Z. parapentatoma* has a slightly longer and narrower Lauterborn organ, and its shortest-anal setae and anal tubules are also longer than those of *Z. pentatoma*.

TABLE 3. Comparison of characters of *Zavrelia parapentatoma* **sp. nov.** with *Zavrelia pentatoma* Kieffer & Bause, 1913. L = Length; No. = Number; TL = Total Length. All measurements are in µm unless otherwise indicated.

Parameters	***Z. pentatoma***	***Z. parapentatoma***	Parameters	***Z. pentatoma***	***Z. parapentatoma***
Male TL (mm)	1.7–1.9, 1.8	1.7–1.9, 1.8	HR	1.30–1.46, 1.35	1.30–1.50, 1.40
Female TL (mm)	1.6	1.7–1.8, 1.8	Female Notum + Ramus L	132	131–170, 151
Male AR	0.96–1.36, 1.21	1.12–1.29, 1.19	Female Notum L	105	83–102, 92
Female AR	0.28	0.23–0.28, 0.26	Female Seminal capsule L	63	56
Male Frontal Tubercele L	2.0–3.0	4.0	Pupa TL	2.6–2.4	2.2–2.3
Female Frontal Tubercele L	—	6.0	Frontal tubercle L	15.00	18–29, 22
Male No. Temporal setae	3.0	2.0	Frontal setae L	120–150, 141	135–149, 142
Female No. Temporal setae	—	8.0	Thoracic horn L	380–435, 397	248–428, 349
Male No. Achrostichals	10–16, 14	12	Tergite II No. hook row	51–58, 55	50–60, 55
Female No. Achrostichals		8.0	Larva TL (mm)	2.3	2.9–3.3, 3
Male Wing L	1.19–1.46, 1.35	1.3	Larva Case L (mm)	3	2.9
FemaleWing L	1.28	1.3	Larva AR	1.1–1.27, 1.21	0.93–1.23, 1.1
Male VR	1.29–1.40	1.5	Larva AAR	0.7–0.82	0.64–0.77, 0.70
Female VR	1.30	1.5–1.6	Larva Lauterborn organ L	15	17–26, 22
Male LR_1	1.3–1.5, 1.4	1.3	Larva MVR	1.0–1.18, 1.1	1.0–1.18, 1.1
Female LR_1	1.30	1.37	Larva posterior parapod L	—	86–102, 94
Male LR_2	0.53–0.59, 0.56	0.62	Larva procercus L	30	23–29,24
Female LR_2	0.53–0.59, 0.56	0.58	Larva short anal setae L	120	372–404, 380
Male LR_3	0.55–0.59, 0.58	0.61	Larva long anal setae L	640	502–679, 617
Female LR_3	0.55–0.59, 0.58	0.59	Larva anal tubules L	30	73–92, 82

Key to adult males of *Zavrelia*

1. Anal point densely covered with strong spinulae 2
- Anal point bare, with microtrichia or short spinules only 3
2. Anal point spinulae of varying size and shapes but never very long, ≤ 4 × as long as wide (Ekrem and Stur, 2009: Fig. 8E; Giłka 2008: Fig. 4) . . . . . *Z. pentatoma* Kieffer & Bause (Palearctic)*
- Anal point spinulae very long, ≥ 8 × as long as wide (Fig. 3D–E) . . . . . *Z. parapentatoma* **sp. n.** (Nearctic)*
3. Setiger of superior volsella with conspicuous constriction in apical 1/3 4
- Setiger of superior volsella without conspicuous constriction in apical 1/3 6
4. Setiger of superior volsella with pointed apex (Ekrem and Stur, 2009: Fig. 5E). . . . . *Z. clinovolsella* Guo & Wang (Oriental)
- Setiger of superior volsella with somewhat rectangular apex 5
5. Anal point with only a few microtrichia in between crests; distinct microtrichia-free areas on anal tergite around the base of anal point (Ekrem and Stur, 2009: Fig. 10E) . . . . . *Z. sinica* Ekrem & Stur (Palearctic)
- Anal point with numerous microtrichia in between crests; microtrichia present all around the base of anal point (Zorina 2008: Figs. 13–14) . . . . . *Z. pseudopentatoma* Zorina (Palearctic)
6. Anal point with small spinules in between crests. 7
- Anal point bare, or with microtrichia in between crests at most 9
7. AR close to 0.6 . . . . . *Zavrelia casasi* Ekrem & Stur
- AR > 0.7 8
8. AR 1.23; wing length about 1.50 mm; LR_1 about1.45; laterosternite with one seta; anal point with small spinules scattered between the entire length of anal crests (Ekrem & Stur 2009: Fig. 12E). . . . . *Z. tusimatijea* (Sasa & Suzuki, 1999) (Palearctic)
- AR 0.91–1.08; wing length 0.88–1.04 mm; LR1 about 2.09; laterosternite without seta; anal point with small spinules scattered between anal crests at anal point base only (Guo and Wang, 2007: Fig. 3; Lin & Wang 2017: Figs. 8–9). . . . . *Z. bragremia* Guo & Wang (Oriental)
9. Anal point bare 10
- Anal point with microtrichia in between crests 11
10. AR 1.00–1.18; LR_1 1.36–1.46; superior volsella with pointed apex (Zorina, 2008: Figs. 1–2). . . . *Z. elenae* Zorina (Palearctic)
- AR 0.45; LR1 1.96; superior volsella with rounded apex (Kobayashi 2014: Fig. 7). . . . . *Z. simantoneoa* (Sasa, Suzuki & Sakai, 1998) (Palearctic)
11. Wing length 1.40 mm; AR close to 0.75 . . . . . *Z. hudsoni* Ekrem & Stur (Nearctic)
- Wing length close to 1.00 mm; AR close to 0.90 . . . . . *Z. aristata* Ekrem & Stur (Nearctic)

Key to the known pupae of *Zavrelia*

1. The anterior of thorax and frontal apotome rugose. Anterior dorsocentral taeniate and longer than the remaining dorsocentrals (Ekrem & Stur 2009: Fig. 4A, B). . . . . *Z. casasi* Ekrem &Stur
- The anterior of thorax and frontal apotome smooth. Anterior dorsocentral simple, shorter, or longer than the remaining dorsocentrals. 2
2. Frontal tubercle absent to weakly developed 3
- Frontal tubercle well-developed. 4
3. The median patches of microspinules on tergites II–V well-extended, and those of II–IV not divided in the middle (Ekrem & Stur 2009: Fig. 9C). Anal lobe seta is simple. . . . . *Z. pentatoma* Kieffer & Bause
- The median patches of microspinules on tergites II–V not well-extended, and those of II–IV appear divided in the middle with only a few micospinules (Fig. 5C). Anal lobe seta is prominent and taeniate . . . . . *Z. parapentatoma* **sp. nov.**
4. Segment V of the abdomen with 4 taeniate lateral setae 5
- Segment V of the abdomen with 3 taeniate lateral setae 6
5. Tergites V–VI with large parallel rectangular patches of microspinules that could be connected anteriorly. Pleura II–III without shagreens (Ekrem & Stur 2009: Fig. 2C). . . . . *Z. aristate* Ekrem & Stur
- Tergites V–VI with continous patches of microspinules. Pleura II–III with shagreens (Zorina, 2008: Fig. 6) . . . . . *Z. elenae* Zorina
6. Tubercle of prealar not developed . . . . . *Z. hudsoni* Ekrem & Stur
- Tubercle of prealar moderately to well-developed. 7
7. Segment III of the abdomen with semi-taeniate lateral setae. Thoracic horn < 300 μm long (Ekrem & Stur 2009: Figs. 11B–C) . . . . . *Z. sinica* Ekrem & Stur
- Segment III of the abdomen with simple lateral setae. Thoracic horn > 330 μm long (Zorina, 2008: Figs. 16, 18). . . . . *Z. pseudopentatoma* Zorina

Key to the known larvae of *Zavrelia*

1. AAR ≥ 1.2 2
- AAR ≤ 1 3
2. Inner margin of mandible with two spines. First antennal segment 66 μm long. Antennal blade < 100 μm long (Ekrem & Stur 2009: Figs. 11D, G) . . . . . *Z. sinica* Ekrem & Stur
- Inner margin of mandible smooth. The first antennal segment 55 μm long. Antennal blade > 100 μm long . . . . . *Z. hudsoni* Ekrem & Stur
3. Antennal pedestal ≥ 80 μm long. 4
- Antennal pedestal < 80 μm long. 5
4. Inner margin of mandible with one spine. Lauterborn organs prominent with second Lauterborn organ ≥ 30 μm long (Zorina, 2008: Figs. 8, 11) . . . . . *Z. elenae* Zorina
- Inner margin of mandible with two spines. Lauterborn organs less prominent with second Lauterborn organ < 30 μm long (Zorina, 2008: Figs. 21, 23) . . . . . *Z. pseudopentatoma* Zorina
5. First antennal segment 65–70 μm long. Antennal blade 125–150 μm long; AAR about 1.0 (Ekrem & Stur 2009: Fig. 2D) . . . . . *Z. aristate* Ekrem & Stur
- First antennal segment > 70 μm long. Antennal blade < 120 μm long; AAR < 1.0. 6
6. Ventromental plates extend to the third lateral teeth of the mentum. Lauterborn organ 15 μm long (Ekrem & Stur 2009: Figs. 2D–E). The shortest anal setae, close to 120 μm long . . . . . *Z. pentatoma* Kieffer & Bause
- Ventromental plates extend to the border of second and third lateral teeth of the mentum. Lauterborn organ 17–26 μm long (Figs.) The shortest anal setae, close to 380 μm long. . . . . *Z. parapentatoma* **sp. nov.**

Life history observations

Patrick L. Hudson (PLH) made the following observations on the life history and ecology of this species. PLH collected the adults from a swarm on July 8, 202l, in an opening between some cattail stands with a sweep net along the boardwalk at Ervin-Stucki Preserve. The wooden boardwalk that bisects the marsh and is bordered by cattails (Typha) interspersed with reed canary grass (*Phalaris arundinacea* L.). The emergent vegetation adjacent to the boardwalk is trimmed near the water line during the growing season allowing the persistence of aquatic floating vegetation (Figs. 5A–B). PLH collected additional adults in early August, mid-May, and June 2022. Some first collections were mounted and morphologically determined to be a species near *Z. pentatoma*. PLH began an effort during several visits to the site in 2021 and into the early spring of 2022 to collect the immature stages by sorting through debris on the surface of the water, material collected from the water column, and the bottom substrate in a white pan. PLH found no larvae or pupae, and he wondered if the adults came from the nearby river. Eventually, in early June of 2022, in an attempt to collect some pupal exuviae floating on the surface, PLH dipped two paper cups into the water's surface and filled them half full in open areas next to matted vegetation and debris. Sorting this live material under a microscope revealed numerous live larvae, in their cases, crawling around.

Due to the larvae's relatively small size (2–3 mm) and the texture of the cases, they can easily be confused with debris. Consistent observation of debris collected from the floating vegetative mats in the following weeks allowed the recognition of the cases at the bottom of the cup. Several collected prepupae larvae were placed in a small sauce dish. They eventually crawled to the edge of the dish and attached their cases to the meniscus, where the water met the edge of the dish (Figs. 5C–D). They stayed there for several days until they began to emerge, which allowed for the collection of pupal exuviae. These observations suggest that the fourth instars probably attach to the floating vegetational mats on the water surface to prepare for the transition to an adult. The absence of first and second instars during these sampling efforts suggests they may live in a different habitat. Sampling the vegetational mat a week after emergence also produced no early instars.

From the collection of the third and fourth instars, the following measurements were produced. The third instars have a head capsule width range of 112–128 μm with a mean of 122 μm (n = 11). The fourth instars have a head capsule width range of 160–213 μm (n = 17) with a mean of 176 μm. The pre-pupae length of the fourth instars ranges from 3.19–3.50 mm long (n = 5). The measurement of the third and fourth instars' larval cases was as follows: the third instars ranged from 1.24 to 1.92 mm (n = 10), and the fourth instars ranged from 2.17 to 3.47 mm (n = 29). The third instar larvae were observed to be able to reverse their direction within the case. The ratio of the case length to mid-width is 6, with about 26% tapering from the head to the tail. During pupation, the larger end of the case is partially sealed with approximately a 135 μm opening (Fig. 5E) and a narrow end with a 73 μm opening (Fig. 5F).

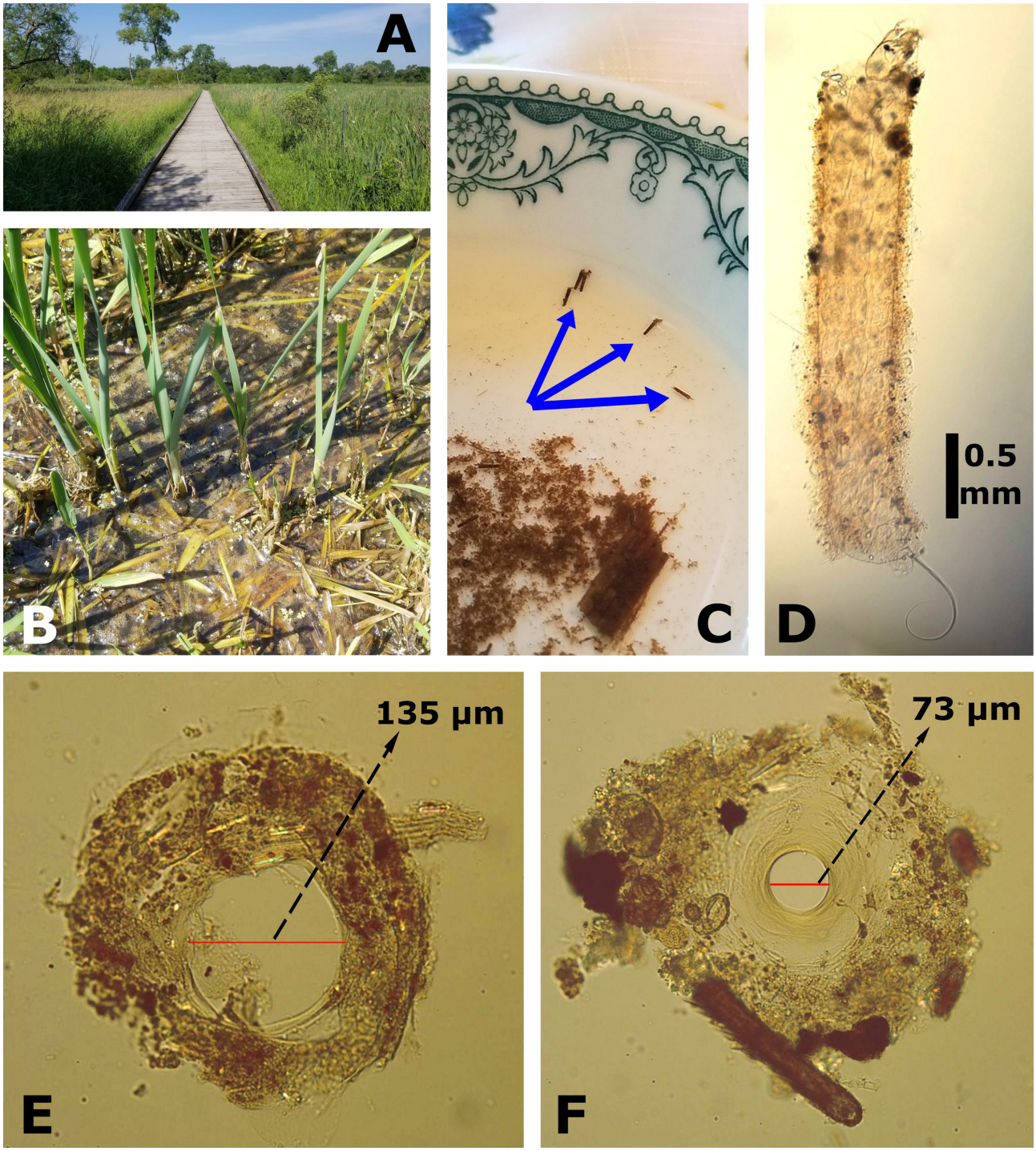

FIGURE 5. *Zavrelia parapentatoma* **sp. nov.** location and habitat of larva (A–B), mature larva in the case (C–D), prepupa case openings (E–F). A. Ervin-Stucki Preserve in Washtenaw County, Southeast Michigan, looking towards the Raisin River; B. Detrital material along the edge of the boardwalk; C. prepupae larvae that crawled to the edge of the dish (arrows); D. mature larva in the case; E. larger opening of the case; F. smaller opening of the case.

Based on the consistent collection of immatures and adults, it is inferred that the species has up to four generations per year in this marsh. The overwintering cohort emerged in mid-May, and other emergence periods in the middle part of June, July, and August. This seems reasonable considering their small size and the rich environment they inhabit. Cohort size differences were also observed, with males averaging 2.1 mm (n = 3) in length in May compared to an early July emergence, averaging 1.6 mm (n = 5).

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References

Anderson, A.M., Stur, E. & Ekrem, T. (2013) Molecular and morphological methods reveal cryptic diversity and three new species of Nearctic *Micropsectra* (Diptera: Chironomidae). *Freshwater Science*, 32, 892–921. https://doi.org/10.1899/12-026.1

Bause, E. (1913) Preprint of Bause E. (1914) Die metamorphose der gattung *Tanytarsus* und einiger verwandter Tendipedidenarten. *Archiv für Hydrobiologie Supplement*, 2, 1–139.

Brundin, L. (1949) *Chironomiden und andere Bodentiere der südschwedischen Urgebirgsseen: ein Beitrag zur Kenntnis der bodenfaunistischen Charakterzüge schwedischer oligotropher Seen*. Institute of Freshwater Research, Drottningholm, Lund, 915 pp.

Carew, M.E., Pettigrove, V., Cox, R.L. & Hoffmann, A.A. (2007) DNA identification of urban Tanytarsini chironomids (Diptera: Chironomidae). *Journal of the North American Benthological Society*, 26, 587–600. https://doi.org/10.1899/06-120.1

Darriba, D. & Posada, D. (2014) jModelTest 2.0. Manual Version 0. 1.1. Available from: https://github.com/ddarriba/jmodeltest2

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology*, 7, 1–8. https://doi.org/10.1186/1471-2148-7-214

Ekrem, T. & Stur, E. (2009) A review of the genus *Zavrelia* (Diptera: Chironomidae). *European Journal of Entomology*, 106, 119. https://doi.org/10.14411/eje.2009.016

Folmer, R.H.A., Nilges, M., Folkers, P.J.M., Konings, R.N.H. & Hilbers, C.W. (1994) A model of the complex between single-stranded DNA and the single-stranded DNA binding protein encoded by gene V of filamentous bacteriophage M13. *Journal of molecular biology*, 240, 341–357. https://doi.org/10.1006/jmbi.1994.1449

Giłka, W. (2008) An intraspecific morphological variability of *Zavrelia pentatoma* Kieffer (Diptera: Chironomidae). *Dipteron, Bulletin of the Dipterological Section of the Polish Entomological Society*, 24, 11–15.

Giłka, W., Paasivirta, L., Gadawski, P. & Grabowski, M. (2018) Morphology and molecules say: *Tanytarsus latens*, sp. nov. from Finland (Diptera: Chironomidae). *Zootaxa*, 4471 (3), 569–579. https://doi.org/10.11646/zootaxa.4471.3.8

Hall, T.A. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hebert, P.D., Ratnasingham, S., Zakharov, E.V., Telfer, A.C., Levesque-Beaudin, V., Milton, M.A., Pedersen, S., Jannetta, P. & DeWaard, J.R. (2016) Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150333. https://doi.org/10.1098/rstb.2015.0333

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16,111–120. https://doi.org/10.1007/BF01731581

Kobayashi, T. (2014) A redescription of *Zavrelia simantoneoa* (Sasa, Suzuki and Sakai, 1998) comb. nov. *CHIRONOMUS Journal of Chironomidae Research*, 27, 41–44. h ttps://doi.org/10.5324/cjcr.v0i27.1699

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and `Evolution*, 35, 1547–1549. https://doi.org/10.1093/molbev/msy096

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A. & Lopez, R. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948.

https://doi.org/10.1093/bioinformatics/btm404
Lin, X., Stur, E. & Ekrem, T. (2015) Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. *PLoS ONE*, 10, e0138993.
https://doi.org/10.1371/journal.pone.0138993
Lin, X.L. & Wang, X.H. (2017) A redescription of *Zavrelia bragremia* Guo & Wang, 2007 (Diptera: Chironomidae). *CHIRONOMUS Journal of Chironomidae Research*, 30, 67–71.
https://doi.org/10.5324/cjcr.v0i30.2345
Miller, M.A., Pfeiffer, W. & Schwartz, T. (2012) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. *In*: Stewart, C. (Ed.), *Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond*, 2012, pp. 1–8.
Namayandeh, A. & Hudson, P.L. (2022) Two new Orthocladiinae (Diptera: Chironomidae) collected in overlooked places of Midwest USA. *Zootaxa*, 5099 (1), 120–128.
https://doi.org/10.11646/zootaxa.5099.1.5
Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular ecology*, 21, 1864–1877.
https://doi.org/10.1111/j.1365-294X.2011.05239.x
Puillandre, N., Brouillet, S. & Achaz, G. (2021) ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, 21, 609–620.
https://doi.org/10.1111/1755-0998.13281
Rambaut, A. (2014) FigTree. Version 1.4.2. Software. Institute of Evolutionary Biology, Univ. Edinburgh. Available from: http://tree.bio.ed.ac.uk/software/figtree/ (accessed 14 February 2023)
Sæther, O.A. (1977) Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the Fisheries Research Board of Canada*, 197, 1–220.
Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Diptera, Chiromodiae). *Entomologica Scandinavica Supplement*, 14, 1–51.
Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30 (9), 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033
Sinclair, C.S. & Gresens, S.E. (2008) Discrimination of *Cricotopus* species (Diptera: Chironomidae) by DNA barcoding. *Bulletin of Entomological Research*, 98, 555–563.
https://doi.org/10.1017/S0007485308005865
Song, C., Lin, X.-L., Wang, Q. & Wang, X.-H. (2018) DNA barcodes successfully delimit morphospecies in a superdiverse insect genus. *Zoologica Scripta*, 47, 311–324.
https://doi.org/10.1111/zsc.12284
Song, C., Zhu, B.-Q., Moubayed-Breil, J., Lei, T. & Qi, X. (2022) Taxonomic study on the genus *Stenochironomus* Kieffer from the Baishanzu Nature Reserve, China (Diptera, Chironomidae). *ZooKeys*, 1104, 93–113.
https://doi.org/10.3897/zookeys.1104.81403
Zorina, O.V. (2008) Russian *Zavrelia* Kieffer (Diptera: Chironomidae), with the description of two new species. *Zootaxa*, 1845 (1), 60–68.
https://doi.org/10.11646/zootaxa.1845.1.4