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Ablabesmyia sp. from Olchinger See, Germany. Photo: André Amaral, July 15th, 2024

CHIRONOMUS Journal of Chironomidae Research

The *CHIRONOMUS Journal of Chironomidae Research* is devoted to all aspects of chironomid research and serves as an up-to-date research journal and news bulletin for the Chironomidae research community. The journal is open access, and can be downloaded free from this website: http://www.ntnu.no/ojs/index.php/chironomus. The publisher is the NTNU Univeristy Museum at the Norwegian University of Science and Technology in Trondheim, Norway.

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Contributions to *CHIRONOMUS Journal of Chironomidae Research* should be submitted online through the online journal system: http://www.ntnu.no/ojs/index.php/chironomus following the author guidelines. There are no submission deadline for articles as they will be published continously upon acceptance. News should be submitted by December 1 for inclusion in the yearly issue.

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Front page layout: Chironomid in title from photograph by Steve Marshall, Graphic design by Kolbjørn Skarpnes, NTNU Information Division.

Front page photo: *Ablabesmyia* sp. from Olchinger See, Germany. Photo: André Amaral, July 15th, 2024

Editorial

Intrepid Explorers

Several wide-eyed, late elementary-aged students huddle around a large white tray, intently exploring the contents within. Their conversation consists of exclamations of "I found another one!", "What do you think this one is?", "Oooh, watch it move!", and "Can I use the magnifying glass now?!?" Several minutes later, the students reluctantly leave the tray behind, after receiving several prompts from their teacher that they must move on. As the group walks away, I hear one of the students proclaim, "That was the coolest thing ever!" Mission accomplished, I think to myself!

As part of one of the projects I am currently involved in, (shameless plug, check out *Bugs Below Zero* at: www.bugsbelowzero.com), this past year has brought more opportunity for me to engage with students, teachers, and families, sharing with them the world of aquatic insects. Whether exploring trays filled with insects hiding beneath leaves and organic debris, exploring the intricacies of a particular specimen under a microscope, or learning of unique adaptations, young learners typically leave with a look of excitement in their eyes. While most of these students probably will not go on to study insects, hopefully they will have a greater appreciation for them, and look for more opportunities to continue to explore the insect world on their own.

Many of us likely remember some of our first insect explorations. My introduction to aquatic insects was on what I recall to be a cold, blustery November afternoon when I was about twelve, tasked with collecting data for a school science fair project. Dressed in waders that were a few sizes too big, and dip-net in tow, I headed out with my Dad to a nearby stream – really, not much more than a ditch flanking a farm field. Despite cold hands and feet, it was exciting to search for and find the aquatic insects that accumulated in our net (many of which were probably chironomids!). That early exploration event led to several more years of science projects, and eventually graduate study.

Along with the title of "scientist," we should also consider ourselves as "explorers." Whether our time is spent exploring lakes or streams, exploring contents of samples, exploring morphological or molecular specimen attributes, exploring datasets, or exploring the literature, *exploring* is how we spend much of our time. Arguably, the continued desire to "explore" is what hooked so many of us.

This year's issue of *CHIRONOMUS* again highlights some excellent "explorations" made by our colleagues. These include descriptions of new species (Andersen et al. 2024) and previously undescribed life stages (Caldwell and Jacobsen 2024), redescriptions of previously known species (Li and Tang 2024, Martin 2024, Tang and Cranston 2024), and critical examination of differences between species (Martin and Epler 2024, Skála 2024).

We hope the year ahead brings a chance for you to continue exploring, as well as to share the excitement of those explorations with others.

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CHIRONOMUS Journal of Chironomidae Research No. 38, 2024: 4-13.

A NEW SPECIES OF *PETALOCLADIUS* SUBLETTE & WIRTH, 1972 (DIPTERA, CHIRONOMIDAE, ORTHOCLADIINAE) FROM THE DOMINICAN REPUBLIC, WITH AN EMENDED GENERIC DIAGNOSIS

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https://zoobank.org/CD4E0E2D-D6F2-419A-8858-45D01887944B

Abstract

Petalocladius dominiensis Andersen & Baranov sp. n. is described and figured based on an adult male collected in the Ebano Verde Scientific Reserve in the Dominican Republic. The new species can be separated from its only congener, *P. setosus* Sublette & Wirth, 1972 from Jamaica, as it is slightly smaller with about 115 dorsocentrals compared to 56 in *P. setosus.* The inferior volsella is broadly subtriangular, while more lingulate in *P. setosus,* and the gonostylus has a small, but distinct megaseta and a subapical brush of spine-like seta, which apparently lack in *P. setosus.* The generic diagnosis is emended including the new species, and the systematic position is briefly discussed.

Introduction

The genus *Petalocladius* was erected by Sublette and Wirth (1972) based on the male and female of *P. setosus* Sublette & Wirth, 1972. The species was collected at Hardwar Gap in Jamaica in February 1969 and the type material is housed in the Smithsonian National Museum of Natural History (USNM). The locality is situated at an altitude of about 1,200 m in the Blue Mountains in the central, eastern parts of Jamaica. There are apparently no later records of the species.

Phylogenetic position of this genus remains rather ambiguous, with Sæther (1977) noting possible close relations with *Abiskomiya* Edwards, 1937 and *Nasuticladius* Freeman, 1961, based primarily on the combination of the following synapomorphies: females with two seminal capsules; prominent inferior and superior volsella of the gonocoxite absent; female tergite IX is modified (notched, divided or reduced); female gonapophysis VIII without lateral lobe. Still, Sæther (1977) only tentatively placed the genus, due to the severe lack of known characters. He also noted that the study of the "exotic" genera, such as *Petalocladius* will be beneficial for better understanding of the ground pattern of Orthocladiinae and overall evolution of the group (Sæther 1977: 95).

During fieldwork in the Dominican Republic in December 2019, a single male of a new species of *Petalocladius* was collected at a stream in the Ebano Verde Scientific Reserve. The reserve is in the eastern region of the Cordillera Central, a mountain range with peaks above 1,500 m altitude. *Petalocladius dominiensis* Andersen & Baranov sp. n. is described and figured below, the generic diagnosis is emended, and the systematic position of the genus is briefly discussed based on Bayesian phylogenetic analysis and a-posteriori probability mapping (aka "Rouge plots") (Klopfstein and Spasojevic 2019).

Material and methods

The single male of *Petalocladius dominiensis* was collected in a light trap, preserved in ethanol, and later mounted in Euparal following the procedure outlined by Sæther (1969). The morphological nomenclature follows Sæther (1980).

The specimen was collected under the collection permit from the Ministro de Medio Ambiente y Recursos Naturales of the Dominican Republic for the project "Long peace of the Caribbean – have biota of the Dominican Republic really remained virtually unchanged for over 13 million years?" and was exported under export permit # VAPB-07404. The holotype is housed in the collection at the Department of Natural History, University Museum of Bergen, Norway (ZMBN).

The type specimen of *P. setosus* Sublette & Wirth, 1972 is housed in the United States National Museum (USNM). Photos of the holotype were kindly provided by David Pecor.

For the phylogenetic analysis we have used a morphological character matrix, with 46 taxa and 83 characters. Characters were sampled from larvae, pupae, and adult males and females. Fossils were of course underrepresented in terms of characters available for observation (see Supplementary file available at https://github.com/chironomus/Petalocladius/tree/main). The character matrix for the phylogenetic analysis was built using NEXUS Data Editor v.0.5.0 (Page 2001).

First, we conducted a Bayesian analysis of the morphological matrix alone in MrBayes 3.2.2. (Ronquist et al. 2012) using the Bayesian implementation of Lewis' Markov models (Lewis 2001). In Bayesian inference, two Markov chains were run simultaneously for 10,000,000 generations using a discrete Dirichlet distribution with equal state frequencies (Lewis 2001). Substitution model was set to "gamma" (Nylander et al. 2004). The first 25,0000 generations were discarded as a burn-in (number of MrBayes generations of the tree topology before the apparent stationary condition) (Nylander et al. 2004). Consensus trees showing all compatible groups and 50% compatible groups were computed in MrBayes. Ancestral character state analysis was conducted on the allcompat consensus tree based on morphology alone. Ancestral character state analysis was conducted using ancTresh in the phytools package (version 0.7-80) (Revell 2012). R code for this analysis, alongside the data is provided at https://github.com/chironomus/Petalocladius/tree/main.

To deal with the uncertainty of the positions of the genus on the tree, caused by the lack of knowledge of character states, we have applied Klopfstein and Spasojevic RoguePlots approach (Klopfstein and Spasojevic 2019). We have used a morphological allcompat tree from MrBayes (as described above) to place *Petalocladius* into the high posterior probability regions. RoguePlot for every species and accompanying R code are provided at https://github.com/chironomus/Petalocladius/tree/main.

Results

Petalocladius Sublette & Wirth, 1972

The genus *Petalocladius* was erected by Sublette and Wirth (1972: 1) based on the male and female of *P. setosus* Sublette & Wirth, 1972, and described in some detail. Below we give an emended diagnosis to the genus to include characters from the new species.

Included species:

P. setosus Sublette & Wirth, 1972: 2, Jamaica.

P. dominiensis Andersen & Baranov sp. n. Dominican Republic.

Emended generic diagnosis:

Small species, with wing lengths between 1.5 and 2.0 mm.

Antenna. With 13 flagellomeres, strongly setose, groove beginning at flagellomere 3; sensilla chaetica on flagellomeres 2, 3 and 13. Segment 13 slightly club-shaped apically, without strong subapical setae. Antennal ratio about 0.7.

Head. Eye reniform without dorsomedian extension, hairy. Temporal setae few, with inner verticals, outer verticals and postorbitals present. Clypeus trapezoid with 14–18 setae. Palp with five segments; with few subapical sensilla clavata on segment 3.

Thorax. Antepronotum well developed, separated medially by broad notch. Acrostichals short, decumbent, starting close to antepronotum. Dorsocentrals numerous, short, multiserial; alveolus of each seta surrounded by conspicuous pale area. Prealars uniserial. Scutellars numerous, multiserial, weak.

Wing. Membrane without setae, with moderate punctation. Anal lobe well developed, slightly produced. Costa moderately extended; R_{2+3} ending slightly beyond middle between R_1 and R_{4+5}; R_{4+5} ends proximal to M_{1+2} almost directly over M_{3+4}; Cu_1 moderately curved; FCu distal to RM; postcubitus ending well distal to FCu; anal vein ending at FCu. Brachiolum with 1 seta, R with few setae, remaining veins without setae. Squama with less than 10 setae.

Legs. Pseudospurs, sensilla chaetica and pulvilli absent.

Abdomen. Tergites sparsely covered with setae, concentrated in posterior band, most extensive on tergite VIII.

Hypopygium. Tergite IX short and broad, without anal point. Transverse sternapodeme arched, with weak oral projections. Phallapodeme with mesial apex forming a darkened, beak-like structure. Virga absent. Gonocoxite with well developed, triangular or lingulate inferior volsella. Gonostylus short, subrectangular with long, slightly curved heel; megaseta present or apparently absent.

Petalocladius dominiensis Andersen & Baranov sp. n.

Figs 1 A-D, Fig. 2, Figs 3 A-C

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Type material: Holotype ♂, slide mounted in Euparal. Dominican Republic, La Vega Province, Ebano Verde Scientific Reserve, 19°0’31”N 70°5’43”W, 1065 m asl., 08.12.2019, light trap, leg. Amelie Höcherl (ZMBN).

Etymology: Named after the Dominican Republic, using the latin suffix *-ensis* denoting country of origin.

Diagnostic characters

The new species is slightly smaller than *Petalocladius setosus,* with about 115 dorsocentrals compared to 56 in *P. setosus.* The inferior volsella is broadly subtriangular, while lingulate in *P. setosus,* and the gonostylus has a small but distinct megaseta and a subapical brush of spinelike seta, which seems to lack in *P. setosus.*

Description

Male (n = 1). Total length 2.94 mm. Wing length 1.62 mm. Total length / wing length 1.81. Wing length / length of profemur 2.64.

Coloration. Head, thorax and abdomen brown, legs light brown.

Antenna. AR 0.69. Ultimate flagellomere 296 µm long.

Head. Eye hairy. Temporal setae 6 including 2 inner verticals, 2 outer verticals and 2 postorbitals. Clypeus with 17 setae. Tentorium 139 µm long, 30 µm wide; stipes 109 µm long. Palp as in Fig. 1A; palp segment lengths (in µm): 39, 47, 78, 103, 131. Third palpomere with 2 sensilla clavata subapically, longest 13 µm long.

Thorax (Fig. 1B). Antepronotum with 4 ventrolateral setae. Scutum with 13 short, decumbent acrostichals in anterior half. With about 115 weak dorsocentrals in multiple rows, each sitting in circular, pale brown area. Prealars 8 in single line. Scutellum with about 32 weak setae.

Wing (Fig. 2). VR 1.16. Costal extension about 174 µm long. Brachiolum with 1 seta, R with 6 setae, remaining veins and cells bare. Squama with 7 setae. Sc and R_{2+3} could not be discerned, but wing apparently not fully hardened.

Legs. Spur of fore tibia 42 µm long, spurs of mid tibia 28 µm and 21 µm long, spurs of hind tibia 57 µm and 22 µm long. Width at apex of fore tibia 44 µm, of mid tibia 36 µm, of hind tibia 43 µm. Comb apparently with 6 setae, longest 33 µm long, shortest 21 µm long. Lengths and proportions of legs as in Table 1.

Hypopygium (Figs 1C, D, Figs 3 A-C). Tergite IX with 16 setae. Laterosternite IX with 6 setae. Transverse sternapodeme arched with weak oral projections, 117 µm long. Phallapodeme not measurable. Gonocoxite 267 µm long; densely setose, with some strong, wavy setae apico-laterally, longest about 110 µm long. Inferior volsella subtriangular, 51 µm wide basally, 68 µm long, with row of 7 strong spinelike setae along inner margin and additional weaker dorsal and ventral setae. Gonostylus 86 µm long, 66 µm wide, with brush of spine-like seta subapically, the longest slightly lanceolate and curved; megaseta small, 12 µm long, with denticles; heel 166 µm long, 16 µm wide medially, with rounded apex and few setae subapically, longest about 17 µm long. HR = 3.10; HV = 3.42.

Female and immatures. Unknown.

Habitat

The single male was collected in a light trap in the Ebano Verde Scientific Reserve in Cordillera Central. The trap was deployed on the bank of a small (4–6 m wide), rapid, river with rocky and stony substrate close to the Scientific Reserve rangers’ station (Fig. 4).

Table 1. Lengths (in µm) and proportions of leg segments in *Petalocladius dominiensis* Andersen & Baranov sp. n., male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
p_1	605	850	523	310	221	163	82	0.615	2.547	2.781	1.20
p_2	621	719	343	188	139	78	57	0.477	3.646	3.905	1.21
p_3	572	776	425	229	172	172	65	0.547	3.239	3.173	1.36

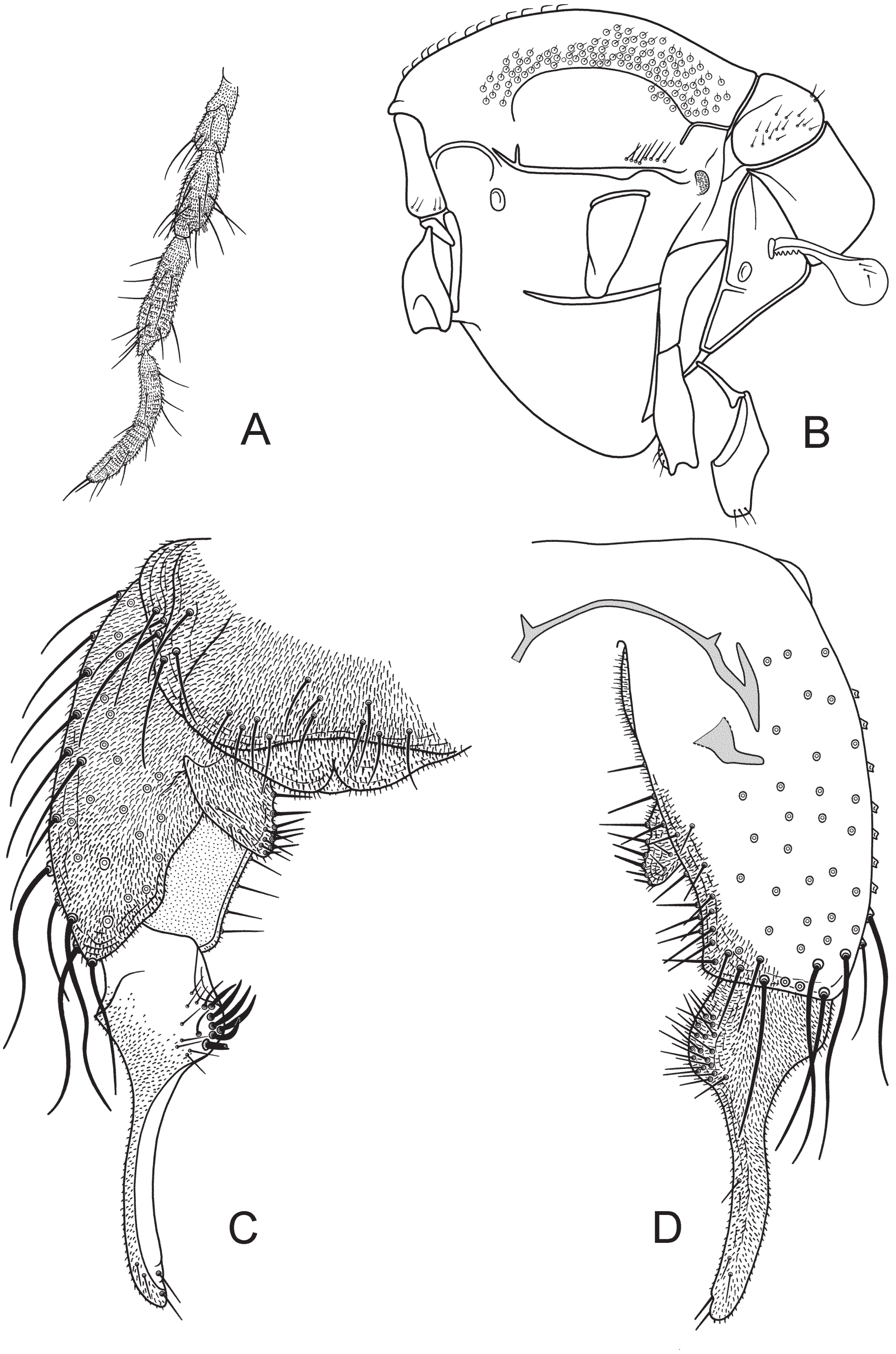

Figure 1. *Petalocladius dominiensis* Andersen & Baranov sp. n., male. A. Palp. B. Thorax. C. Hypopygium, dorsal view. D. Hypopygium, ventral view.

Figure 2. *Petalocladius dominiensis* Andersen & Baranov sp. n., male. Wing.

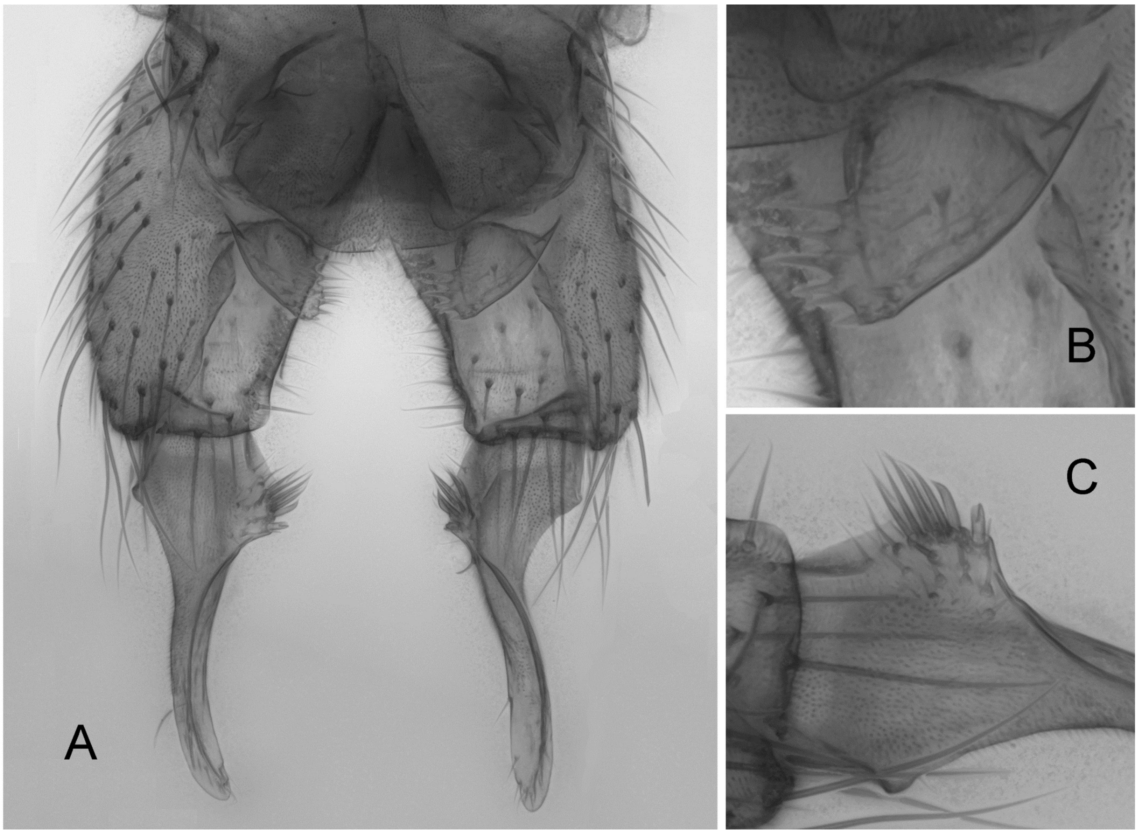

Figure 3. *Petalocladius dominiensis* Andersen & Baranov sp. n., male. A. Hypopygium, dorsal view. B. Inferior volsella. C. Apex of gonostylus, showing the megaseta.

Petalocladius setosus **Sublette & Wirth, 1972**

Figs 5 A-F

The male and female of *P. setosus* were described in detail by Sublette and Wirth (1972: 2). However, the species is rather sparsely figured by Sublette and Wirth (1972: figs 1-3). David Pecor has therefore kindly sent us photos of the head, thorax, wing and hypopygium of the male (Figs 5 A-F).

Systematics

Phylogenetic analysis using Bayesian inference led to *Petalocladius* being a sister group to the clade *Thalassosmittia* + *Mesosmittia* + *Pseudosmittia* + *Eretmoptera*, with a weak support of 0.16 (Fig. 6), common for morphology-only reconstructions, where some taxa are having many missing character states. In the RougePlot reconstruction, *Pet-*

Figure 4. The type locality of *Petalocladius dominiensis* Andersen & Baranov sp. n.

alocladius was recovered in >30% of trees next to *Abiskomyia* (Fig. 7).

Discussion

According to Sublette and Wirth (1972) *Petalocladius* most closely resembles *Diplocladius* Kieffer, 1908. As mentioned in the introduction, Sæther (1977) hypothesized close relationships with *Abiskomyia* and *Nasuticladius*. In a consensus tree produced in our Bayesian analysis neighboring search it falls out with *Thalassosmittia* Strenzke & Remmert, 1957 and *Eretmoptera* Kellog, 1900 (Figs 6, 7). A position close to *Thalassosmittia* and *Eretmoptera, Pseudosmittia* and *Mesosmittia* is notable. *Thalassosmittia* is a predominately marine genus with 11 named species distributed in the Afrotropical, Nearctic, Neotropical, and Palaearctic Regions (Ashe and O'Connor 2012). With few exceptions, the species are marine shore dwellers (Andersen et al. 2013). The only species described from the Neotropical Region, *T. amazonica* Andersen & Pinho, 2014, was, however, collected in the Amazon rainforest near Manaus (Andersen and Pinho 2014). *Eretmoptera* is a genus with two named species that are distributed in the Nearctic Region and in Antarctica. The adults are wingless. *Eretmoptera browni* Kellog, 1900 is distributed in California, while *E. murphyi* Schaeffer, 1914, was described from South Georgia and is later introduced to the Signy Island in the South Orkney Islands (Ashe and O'Connor 2012). The species is apparently parthenogenetic; the larva has a two-year life cycle and lives in damp moss and peat where they are thought to feed on decaying vegetation (Convey 1992, Cranston 1985).

However, when we have applied posterior position probability mapping ("Rouge plots"), *Petalocladius* was notably plotted in >30% of the cases next to *Abiskomyia* (Fig. 7), corroborating the hypothesis by Sæther (1977). While this was the most frequent position within the generated trees, it plots near *Eretmoptera* in the consensus tree due to cumulatively more frequent occurrence (ca. 40%) in or near this clade (Fig. 7). *Nasuticladius* position in relation to *Petalocladius*, remains highly uncertain, both due to the lack of knowledge of the immature stages of this genus and the probable paraphyletic nature of the "genus" (Hergstrom 1974).

Both *Petalocladius* species have been collected in mountain areas and there might be more species of the genus to be found in streams and rivers in the

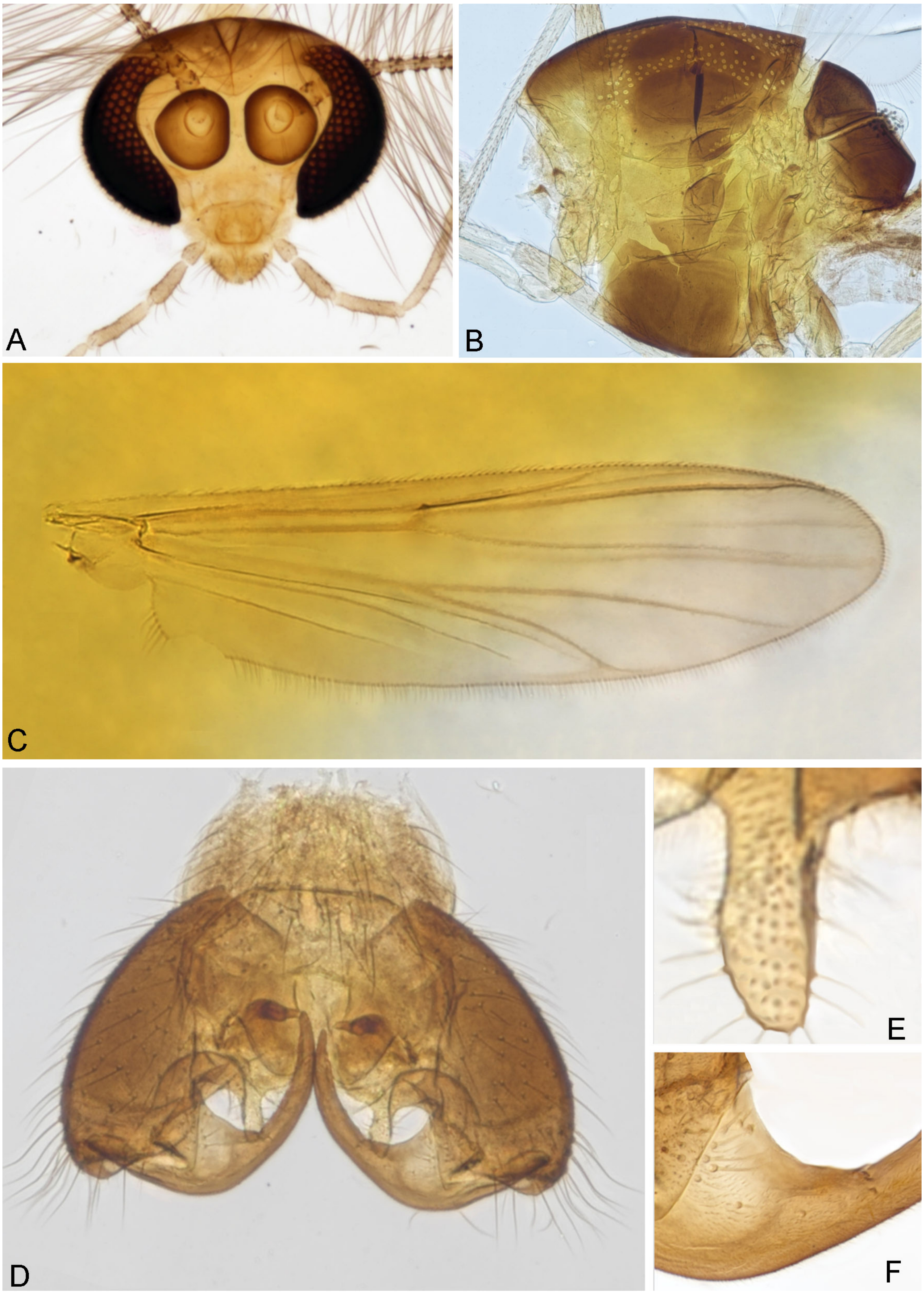

Figure 5. The male of *Petalocladius setosus* Sublette & Wirth, 1972. A. Head. B. Thorax. C. Wing. D. Hypopygium, dorsal view. E. Inferior volsella. F. Apex of gonostylus. Photos: David Pecor, Smithsonian Institution.

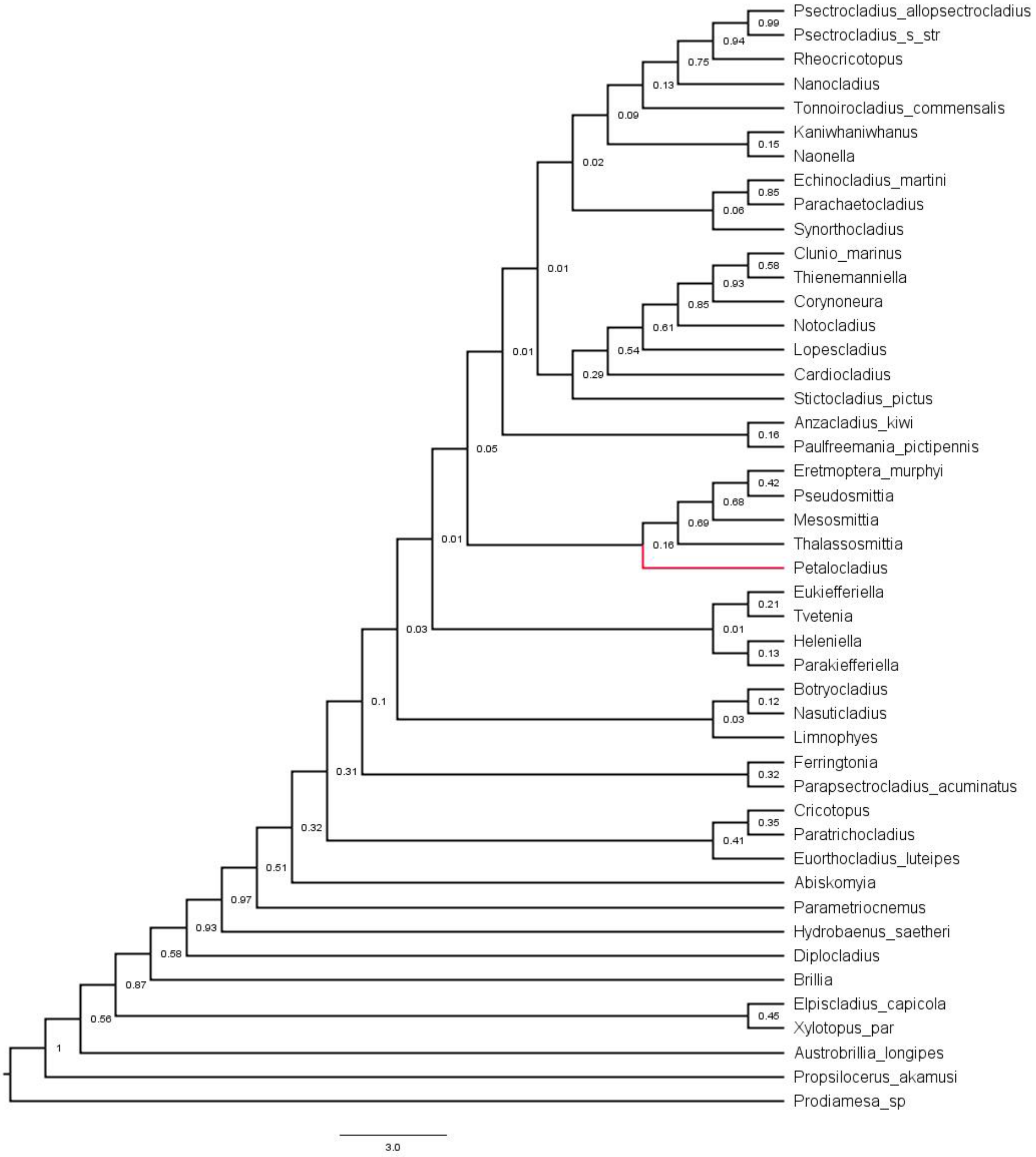

Figure 6. Position of *Petalocladius* (marked in red) in the phylogenetic tree of Orthocladiinae, reconstructed with Bayesian inference (all posterior probabilities of the nodes are displayed, regardless of the value). This is a majority rule consensus tree based on morphology only.

mountains on other islands in the Caribbean. The immatures remain unknown.

Overall, the discovery of *P. dominiensis* has provided the necessary impetus and additional characters required to improve the understanding the place of this enigmatic genus within Orthocladiinae.

Acknowledgements

We are indebted to David Pecor, United States National Museum (USNM) arthropod collections for kindly sending us photos of the holotype male *Petalocladius setosus*. We are also grateful to the Ministerio de Medio Ambiente y Recursos Naturales of the Dominican Republic for the permission to collect and export the specimen for the present study. The study was supported by the

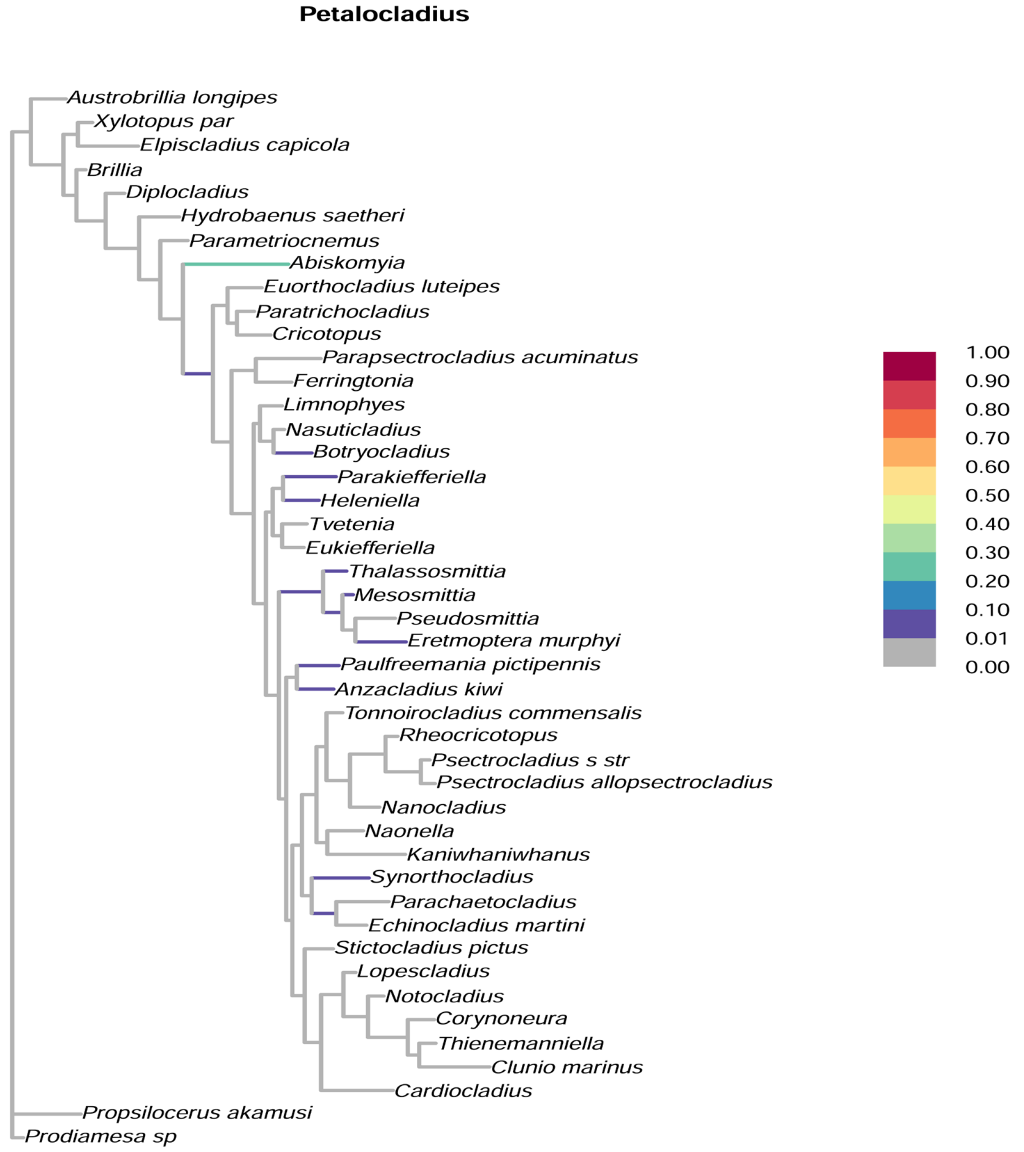

Figure 7. Rouge plot of the frequency of placement of *Petalocladius* on a Bayesian tree from Fig. 6, based on the frequency of occurrence of *Petalocladius* in a given node, based on 50001 most congruent trees from the Bayesian analysis of the genus's morphology.

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CLARIFICATION OF THE STATUS OF *CHIRONOMUS JAVANUS* KIEFFER, 1924 AND *C. VITELLINUS* FREEMAN, 1961

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Abstract

The status of the closely related species *Chironomus javanus* Kieffer, 1924 and *C. vitellinus* Freeman, 1961 has been largely confused and *C. vitellinus* even considered to be a junior synonym of a very widely distributed *C. javanus.* However, a comparison of the available mitochondrial COI barcode sequences reveals that there are two groups of sequences with consistent differences between them. Further, the geographic origins of these sequences indicates that *C. vitellinus* is much more widely distributed than *C. javanus.* This interpretation is confirmed from the details of various descriptions, with differences particularly in the male hypopygium and the larval premandible. *Chironomus vitellinus* is also found in the New World in Florida and Puerto Rico, indicating that the species is probably distributed through most of the tropical region. However, *C.* sp. "Florida" of Epler (2001), known only as a larva, and with a multi-toothed premandible has been determined to be a separate species.

Introduction

Current literature on *Chironomus javanus* suggests that it is a widespread species in tropical regions from Central Africa to the islands of Micronesia in the Pacific. On the other hand *C. vitellinus* is either not considered or regarded as a synonym of *C. javanus* (Chaudhuri et al. 1992, Martin 2022). A reassessment of the literature, along with additional specimens and, particularly, a comparison of the available DNA barcode sequences as advocated by Desalle et al. (2005), reveals that this picture is highly flawed. Rather, as will be outlined below, *C. javanus* and *C. vitellinus* are closely related species with relatively different distributions that apparently only overlap in a small area of India and the Malaysian Peninsula. It is not the purpose of this paper to re-describe these species but rather to provide the evidence relevant to confirming that they are distinct species and to correct the data on their respective distributions. The confusion seems to have arisen accidentally from Tokunaga (1964) who described the Micronesian populations of *C. vitellinus* as *C. javanus.* Even if Tokunaga was aware of Freeman's description of *C. vitellinus* just 2 years previously, he very likely would not have recognized that it was a different species as Freeman did not illustrate the tergite IX setae, which are the most easily recognizable character for distinguishing the two species. Subsequently, any specimens with the arrangement of TIX setae figured by Tokunaga were identified as *C. javanus.* It will be shown that this is not correct as the setation in the two species is quite different.

When these factors are taken into consideration, it becomes clear that most descriptions attributed to *C. javanus* in fact refer to *C. vitellinus* which is very broadly distributed, with individuals reported from Malawi, Africa to Japan, the Melanesian Islands of the Pacific Ocean and, as reported here, to Florida, U.S.A. and Puerto Rico. *C. javanus,* rather than being very broadly distributed, appears to have a restricted distribution in India and Peninsular Malaysia. The morphological, cytological and mitochondrial COI characters that separate these two species are outlined below.

Material and Methods

Material examined has included larvae, pupae, and adults from various localities including:

Chironomus javanus from Bukit Merah Agricultural Experimental Station (BMAES) Permatang Pauh, (Dr. S.A. Al-Sharmi) and Mendang, Penang (Warrin Ebau), with other information drawn from published works (Kieffer 1924, Johannsen 1932, and Chaudhuri et al. 1992).

Chironomus vitellinus from Mareeba, Sarina and Townsville in Queensland, Australia; a paratype male from Mafulu (Natural History Museum, London) and larvae from Sogeri, Central Province, Papua New Guinea (N.V. Dobrotworsky); Bukit Merah Agricultural Experimental Station (BMAES) Permatang Pauh, Penang (Dr. S.A. Al-Sharmi); Labasa, Vanua Levu and Nadi, Viti Levu, Fiji; Laboratory colony from Shizuoka, Shizuoka Prefecture, Honshu, Japan (Prof. H. Hashimoto); Blantyre, Malawi, Central Africa (Dr. A.J. McLachlan); Alachua, Charlotte and Wakulla Counties, Florida, U.S.A., and Puerto Rico (J.H. Epler); with other information from numerous published works, mostly as *C. javanus* (Freeman 1961, Tokunaga 1964, Hashimoto et al. 1981, Sasa and Hasegawa 1983, Hashimoto 1984, Cranston 2007, Al-Sharmi et al. 2012, Pramual et al. 2016).

Morphological terminology follows Sæther (1980), Webb and Scholl (1985) and Vallenduuk and Moller Pillot (1997). Abbreviations include JM – Jon Martin; JHE – John H. Epler; NA - North America.

Chromosome preparation techniques and the identification of chromosome arms are as previously described (e.g. Martin et al. 2006).

MtCOI sequence was obtained from ethanol/acetic acid fixed specimens (mostly larvae) for the conventional mitochondrial cytochrome c oxidase subunit I (COI) fragment using the Folmer et al. (1994) primers: LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). In some cases the condition of the specimens was such that the barcode region had to be amplified in two sections, using the reverse primer COI-Na-2 (5’- AGATAAAGGKGGATAAACWGTTCA-3’) for the 5’ section (Martin, 2023), and the forward primer of Carew et al. (2013) (5’-CCHCGAATAAATAATATAAGWTTYTG-3’) for the 3’ section, with PCR products sent to Macrogen Inc. Seoul, Republic of Korea for sequencing.

The resulting sequences were compared to sequences identified as *C. javanus* in the Barcode of Life Data Systems (BOLD) database (BIOUG21394-A08, BIOUG26140-B05, GMBCA5882-15 (all Bangladesh); ATISA8024-16, BIOUG-G04, MYP3811, MYA, MYA2 and MYA3, P_W02_132, P_W06_145 (all Malaysia), DIQTB182-10 (Australia), GMBCA5882-15, Mr-23 (China) and in GenBank (DQ648203 (Japan); KF408077 (India); KP462106 (Singapore); KT212984, KT212989, KM013379, KM013380, KM013385 (all Thailand); JF412082, JF412083, JF412084 and JF412085 (all Korea), JN298748 (Australia); MW201293 (China) along with sequences that have been lodged in GenBank as *C. vitellinus*: (OR367025 and OR486048 (both Malaysia); OR367026 (Papua New Guinea).

Results

An examination of the published figures of the male hypopygium of supposed *C. javanus* specimens subsequent to that of Johannsen (1932) shows that only those of the Indian specimens of Chaudhuri et al. (1992) are equivalent with a narrow tubular anal point and no patch of setae on tergite IX (TIX). All others are identical to that of the *C. javanus* of Tokunaga (1964) in having a small expansion at the end of the anal point (as in Freeman's original figure) and a patch of about 5-17 setae in individual pale patches on TIX which, although not shown in Freeman's original figure, were observed on the paratype male from Mafulu, Papua New Guinea (Fig. 1). This was the first clue that *C. vitellinus* was actually quite widely distributed.

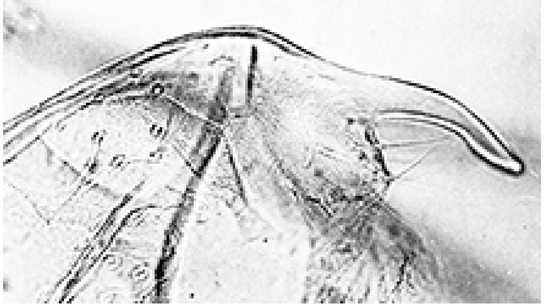

Figure 1. Terminal abdomen of paratype male from Papua New Guinea showing the TIX setae (at left) and narrow anal point.

There are also identification features for *C. javanus* in the larva: the central teeth of the mentum do not arise below the level of the other teeth as they do in *C. vitellinus* (Fig. 6) and the premandible which has multiple teeth and for which Peter Cranston (pers. comm.) determined that, if examined closely, there are 6 teeth in *C. javanus* and 7 teeth in *C. vitellinus.* Cranston (2007) illustrates a *C. vitellinus* premandible which clearly has 7 teeth but is labelled as *C. javanus* because at that stage he had not recognised the difference between the two species. Hashimoto (1984) claimed to have studied *C. javanus* but his figures indicate he actually had *C. vitellinus.* He states that the premandible has 6 teeth but does not provide a figure to confirm this count since the 7th tooth is small and easy to overlook if the premandible does not lie in an optimal orientation. However, the Central American samples available to us also have only 6 teeth, suggesting that there may be polymorphism for tooth number in *C. vitellinus.* One possibility is that the U.S. and Puerto Rico specimens actually arose by human transport from Japan – a situation recognised previously for a number of species in South America and southern U.S. (e.g. *C. striatipennis* Kieffer which is suggested to have originated in China or perhaps Japan – see Amora et al. 2015).

Dr. Al-Sharmi provided larvae and adults from his samples collected in rice paddies in Pulau Pinang, Malaysia. These samples contained both *C. vitellinus* (most common and the species described by Al-Sharmi et al., 2012) and *C. javanus.* It also included a small number of slide mounted adult males, most of which were *C. vitellinus,* but one rather distorted specimen fitted the description of *C. javanus* and agreed with the characters of adult males from previous descriptions except of Johannsen (1932) and Chaudhuri et al. (1992 in that there were two rows of long setae, an anterior one of 7 setae and a more posterior row of 6 setae (Fig. 2) on TIX. This specimen had simply been cleared and then mounted whole on a slide. This suggests that these setae may commonly be lost in preparation of specimens for slide mounting.

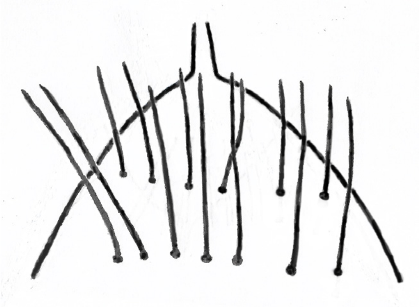
Figure 2. TIX of *Chironomus javanus* showing the long setae.

Barcode sequence was obtained from some larvae of this sample and produced some very informative results (discussed below). Consequently an analysis was made of the available sequence for *C. javanus* in the BOLD, where all samples were in the same Bin (BOLD:AAG6924) as our samples of *C. vitellinus,* or GenBank databases. The sequences were aligned so that the actual base sequence could be analysed as recommended by DeSalle et al. (2005). The samples could be separated into two groups which differed at 47 sites, 3 of which were polymorphic in *C. vitellinus* (Fig. 3), and mostly in the 5' region. The largest group (23 specimens) included my *C. vitellinus* samples, while the smaller group of 4 specimens included the *C. javanus* specimens from Malaysia along with two other Malaysian samples from the BOLD database. These results clarified that *C. vitellinus* was a distinct species and not a synonym of *C. javanus.*

Attempts to examine the polytene chromosomes of these species were only partly successful. Specimens of *C. vitellinus* from Australia reared in the laboratory at 20°C produced chromosomes from which it was possible to determine that there were four chromosomes with the thummi-chromosome arm combination (AB, CD, EF, G) with a nucleolus subterminal on arm G and a Balbiani ring about a third from the other end (Fig. 4). For *C. javanus* it was only possible to determine that there were four polytene chromosomes, possibly with the same arm combination as *C. vitellinus.*

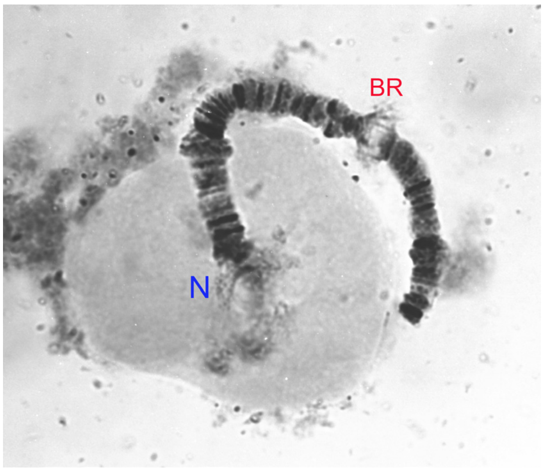

Figure 4. Arm G of *Chironomus vitellinus*: N- nucleolus; BR – Balbiani ring.

Discussion

Chironomus javanus and *C. vitellinus*

It is clear that all but three of the previous publications on *C. javanus* have in fact dealt with *C. vitellinus.* The problem appears to have arisen from the important publication on the Chironomidae of Micronesia by Tokunaga (1964), who may not have been aware of the recent description of *C. vitellinus* by Freeman (1962), as he does not cite this paper. However, since Freeman did not illustrate or mention the TIX setae in his description, Tokunaga as well as some other authors who did cite the Freeman paper, would not have immediately recognized it as a different species because only the small expansion of the end of the anal point of *C. vitellinus* is shown. Hashimoto et al. (1981) quote the paper but not in relation to *C. vitellinus* and Sasa and Hasagawa (1983) cite Freeman in their references but do not actually refer to it in the text. Al Shami et al. (2012) quote Johannsen (1932) and identify their specimens as *C. javanus.* However, they clearly state that the larvae had 7

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vitellinus   A T A T A A/G A C T C T T A/G A T T C C/T C T A T AT C T TC T C/T T T A TC  T T G C T A T T A G A T C
javanus   3' T A G A T  T    C T C T A C  C  G A A T  A   T A T A TC T A AT A  A  A A T AT A A A C A G C G G A T C T 5'
```

Figure 3. Alignment of the COI barcode base pairs that differ between *C. vitellinus* (above) and *C. javanus* (below).

teeth on the premandible and illustrate the patch of setae on TIX of the adult male, so have clearly actually re-described *C. vitellinus.* They apparently did not recognize the small number of true *C. javanus* in their samples but the adult sent to JM was so deformed they may not have been able to identify it. The larvae would not have appeared different if the premandibles were not lying in optimal orientation, and other recognized differences are minor differences in the relative sizes of the anal and ventral tubules (dorsal pair of anal tubules (AT) longer and ventral tubules (VT) of equal length in *C. vitellinus,* ventral pair of AT longer and thinner and anterior pair of VT longer with the two pairs of VT arising close together (Chaudhuri et al. 1992) in *C. javanus*; other characters may differ in mean values but with considerable overlap of individual ranges.

These results clearly indicate that the prevailing view that *C. javanus* is a widely distributed species and *C. vitellinus* a possible synonym is incorrect. Instead they are separate, but closely related species, with *C. vitellinus* widely distributed at least from Africa eastward to Micronesia and in new records reported here, southeastern North America and in Puerto Rico (Fig. 5), while *C. javanus* appears to be restricted to a small area around India and Malaysia, often in common with *C. vitellinus.* In this regard it can be noted that *C. daitocedius* Sasa and Suzuki (2001) is a synonym of *C. vitellinus,* not *C. javanus* as stated by Yamamoto and Yamamoto (2018).

Chironomus vitellinus has a short development time: Reyes-Maldonado et al. (2021) reared the larvae at 27^{O}C and found that adult males emerged after 10 days and females about 2 days later. In the wild, the species breeds in a number of different habitats – commonly in rice paddies (e.g. – in Asia (Al Sharmi et al., 2012), Australia and Papua New Guinea); in tsunami-affected coastal pools in Thailand (Cranston 2007); as well as artificial containers such as a 44 gallon drum (Papua New Guinea) or dark colored containers, bird baths and water troughs in Puerto Rico (Reyes-Maldonado et al.*,* 2021). The short development time would be advantageous in completing development before a temporary habitat dried out, as has also been reported for other species utilising such habitats (e.g. McLachlan and Cantrell, 1980; Nolte 1995).

There are some minor differences between specimens from different locations, probably reflecting times of separation and differential selection pressures, particularly in the Americas where there other species with similar ecology. The most notable difference is the coloration of the male abdomen, which is usually yellowish or greenish and lacking dark markings, while Florida and Puerto Rico specimens have a green abdomen with a light quadrilateral patch on tergites II-VI, with TVIII totally dark. In view of the suggestion that these populations may have derived from Japan, it may be noted that the Japanese specimens have a green abdomen, although lacking dark markings (Hashimoto 1984).

Chironomus sp. "Florida"

Epler (2001) keyed and illustrated an unusual *Chironomus* larva with a multitoothed premandible from Florida, calling it *Chironomus* sp. "Florida".

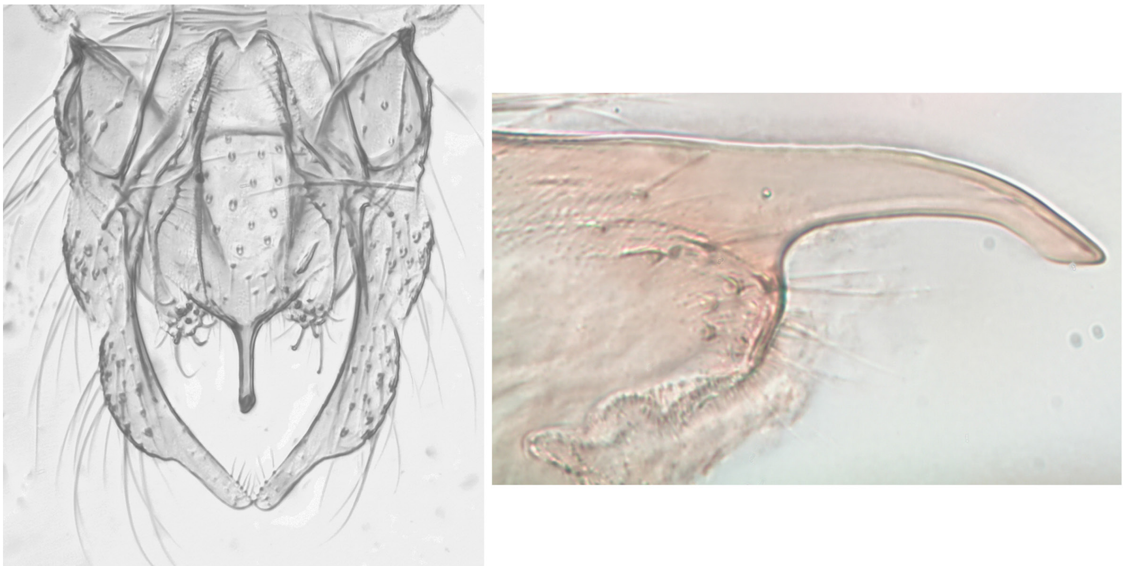

Figure 5. Male hypopygium (left) and anal point (right) of Florida specimen of *Chironomus vitellinus.*

When he reared *Chironomus* larvae from a birdbath at his house in northern Florida in 2013 and 2017, he noted that the larvae had multitoothed premandibles and thought he had reared *C.* sp. “Florida” larvae. However, lengthy correspondence between the authors resulted in an identification of *Chironomus vitellinus* for these birdbath larvae, as well as those reared from birdbaths and other outdoor containers by Bob Rutter in Port Charlotte, FL, Doug Strom in Gainesville, FL, and Alonzo Ramirez from several localities in Puerto Rico.

As well, in 2018, Alonzo Ramirez contacted JHE to identify the *Chironomus* he was breeding in a lab culture, collected from various sites in Puerto Rico. JHE examined larvae, pupae and adults and determined they were the same species he had reared from his birdbaths, but at this stage he still thought his birdbath larvae represented *C.* sp. “Florida”, and provided this incorrect identification that was subsequently used in Reyes-Maldonado et al. (2021). As noted above, it was after this that the identity of the birdbath larvae was revealed to be *C. vitellinus*.

Thus one mystery was cleared up – but what about *C.* sp. “Florida”? This taxon was initially known from the single larva described by Epler (2001) but was a bit different from the new birdbath larvae, perhaps due to a worn mentum and premandibles.

With the exception of the unusual mentum and premandible, *C.* sp. “Florida” is a rather typical *Chironomus* larva, including the presence of furrows at the base of the mandible. The single, apparently mature, larva in JHE’s collection is about 12 mm long, and has two pairs of long, straight ventral tubules and a pair of long lateral tubules, i.e. a melanotus-type larva (Proulx et al. 2013). No other life stages are known.

Larvae of *C. vitellinus* and *C.* sp. “Florida” are easily separated by the mentum and premandibles (Figs 6-7). *Chironomus vitellinus* has a typical *Chironomus* mentum with a trifid median tooth (Hashimoto 1984 and Fig. 6) arising lower than the lateral teeth; the mentum of *C.* sp. “Florida” has a simple rounded median tooth (all the teeth of the mentum are rounded) and arise at the same level as the other teeth (Fig. 7).

At first glance, the mentum of *C.* sp. “Florida” (Fig. 7) looks more like that of some *Polypedilum* (e.g. Fig. 10.58 of Pinder and Reiss (1983).

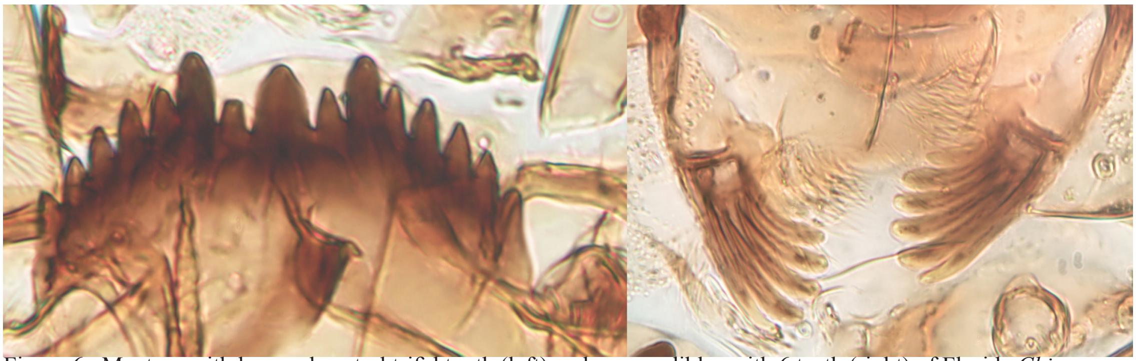

Figure 6. Mentum with lowered central trifid tooth (left) and premandibles with 6 teeth (right) of Florida *Chironomus vitellinus*.

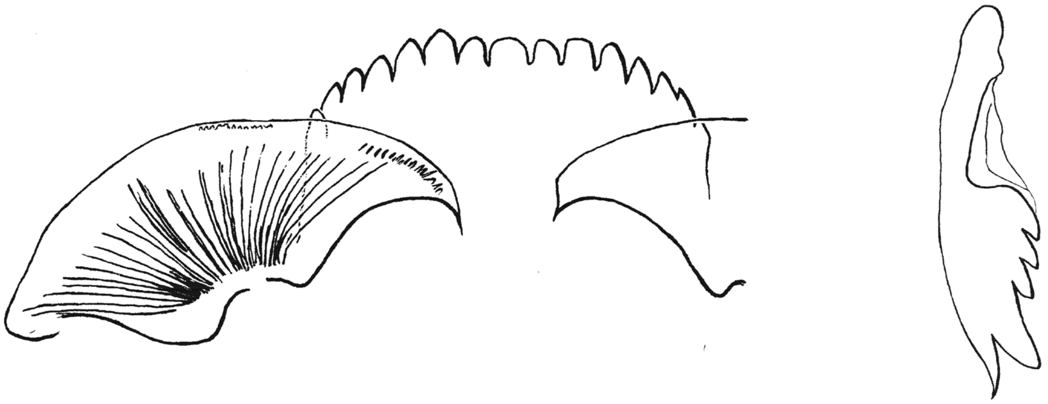

Figure 7. Mentum and ventromental plate (left) and premandible with 5 teeth (right) of *Chironomus* sp. “Florida”.

The premandible of *C. vitellinus* usually has 6 well defined teeth (Fig. 6) with a 7th tooth barely indicated in most populations, while the premandible of *C.* sp. “Florida” has 5 teeth (Fig. 7). The premandibular teeth of *C. vitellinus* are more widespread and fan-like in most mounts, with 6 long teeth, very similar to those of *Kiefferulus* Goetghebuer species; those of *C.* sp. “Florida” are shorter and wider, and quickly become progressively shorter (Fig. 7). An early instar larva of C. sp. “Florida” appears to have only 4 premandibular teeth.

The original *C.* sp. “Florida” larvae that Epler (2001) examined were collected in south Florida by Bob Rutter; he found larvae in burrows made in the water lily *Nuphar* Sm. (Nymphaeceae) by the aquatic larva of the noctuid moth *Bellura* Walker. More recently, Doug Strom (pers. comm.) looked at *Nuphar* from two Florida lakes and collected several larvae but was unsuccessful in rearing them. Perhaps the unusual mentum is an adaptation to life within aquatic moth larvae tunnels. The taxon remains an enigma.

Chironomus vitellinus and *C.* sp. “Florida” are not unique among *Chironomus* in having premandibles with multiple teeth as *C. okinawanus* Hasegawa & Sasa 1987 is widespread in China (Hongqu Tang, pers. comm.) and several species occur in South America (Reiss, 1974; Correia and Trivinho-Strixino, 2007). However these species are not conspecific with *C. vitellinus* or *C.* sp. “Florida” as they differ in adult and/or larval morphology, including the morphology and tooth number of the larval premandible.

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REDESCRIPTION OF MARINE *THALASSOSMITTIA NEMALIONE* (TOKUNAGA, 1936) (DIPTERA, CHIRONOMIDAE, ORTHOCLADIINAE) FROM THE EAST COAST OF CHINA

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Abstract

Thalassosmittia nemalione (Tokunaga, 1936) is redescribed based on a male from the east coast of China. This is the first record of marine *Thalassosmittia* from the coast of China.

Introduction

The genus *Thalassosmittia* was established by Strenzke and Remmert (1957) for *Camptocladius thalassophilus* Bequaert and Goetghebuer, 1914 from the North Atlantic coasts (Cranston et al. 1989). Thus far, this genus comprises ten species including eight marine species and two inland species (Tang et al. 2023). Most marine species were recorded in the North Pacific, with 2–3 members from the Atlantic Ocean and the Mediterranean Sea. Larval stages of marine species usually dwell in algal mats and barnacles of intertidal zones (Andersen et al. 2013, Gibson and Choong 2021).

T. nemalione (Tokunaga, 1936) was erected by Tokunaga (1936) based on Japanese material from Seto, Wakayama Prefecture. The author comprehensively described the male and female adults of this species; however, the details of some important diagnostic characters were ignored, such as the antenna, the genital sclerites and volsella of adult males. Although *T. nemalione* was established nearly 90 years ago, it has been rarely recorded in subsequent studies except for a few records listed in Yamamoto (2004), Kawai et al. (2011), and Yamamoto and Yamamoto (2014). One likely reason is that *T. nemalione* can easily be misidentified as a *Pseudosmittia* species due to the morphological similarity with species in this genus. To better distinguish the species, we here redescribe *T. nemalione* based on a single male collected on the East Coast of China.

Material and Methods

One adult male was collected over rock surfaces in the intertidal zone with a sweeping net. The specimen was dissected and mounted in Euparal on a microscopy slide. Morphological terminology and abbreviations follow Sæther (1980), except for the lobe posterior to tergite IX, which is termed proctiger according to the definition of the homologous structure in *Diamesa* (Hansen & Cook 1976). Digital photographs were taken under an Olympus CX41 compound microscope with phase-contrast optics, during which images were manipulated with Mshot™ software to automatically acquire the focused parts of exposures at different depths. The slide is deposited in the Department of Ecology, Jinan University, China (EJNU).

Results

Thalassosmittia nemalione (Tokunaga, 1936).

Spaniotoma (*Smittia*) *nemalione* Tokunaga, 1936: 305.

Thalassosmittia nemalione (Tokunaga, 1936), Yamamoto (2004: 102, list); Kawai et al. (2011: 251, record); Yamamoto & Yamamoto (2014: 302, list).

Material examined. CHINA. 1 male adult, Fujian Province, Pingtan County, Dalian Island, Yueju village, 25°39’58.72” N, 119°42’43.07” E, 02.v.2019, ltd. W. Han, (EJNU).

Diagnostic characters. *T. nemalione* can be separated from its congeners by the combination of the following characters: antenna with 8 flagellomeres, anal point pubescent, long-triangular, base with 4–5 long setae laterally; proctiger obviously; gonocoxite with spindle-shaped patch at the outer margin of subapex, virga composed of two apically bifid spines; gonostylus tapering towards apex; megaseta present.

Description

Male (n = 1). Total length 2.33 mm, abdomen 1.63 mm long. Wing length 1.33 mm.

Coloration. Generally brown to dark brown. Thoracic vittae light brown, with dorsocentrals arising from pale brown spots. Wings greyish brown. Legs generally light brown to brown, the two tibial spurs of middle and hind legs with contracting coloration, the shorter one is dark brown while the longer one is light brown.

Antenna (Fig. 1B). Eight flagellomeres, with distinct sensilla chaetica, plume reduced with sparsely distributed setae, terminal flagellomere tapered, apical seta absent. Length of flagellomeres (in µm): 45, 25, 30, 30, 33, 35, 35, 100–105. AR 0.40.

Head. Eye pubescent, without dorsomedian extension. Temporal setae 7, including 2 inner verticals, 2 outer verticals and 3 postorbitals. Clypeus with 6 setae bilaterally symmetrical. Palp 5-segmented, length (in µm) of segments 1–5: 25, 35, 55, 58, 70.

Thorax. Antepronotal lobe without seta. Acrostichals 10, dorsocentrals 6, prealars 2. Scutellum with 4 uniserial setae.

Wing (Fig. 1A). Wing membrane with fine granulation. R_{2+3} very close to R_{4+5}. R_{4+5} ending distal to end of M_{3+4}. Anal lobe present, weak. VR 1.20. Brachiolum with 1–2 setae, R with 7, R_1 with 3, and R_{4+5} with 8 setae. Costal extension 75 µm long. Squama bare.

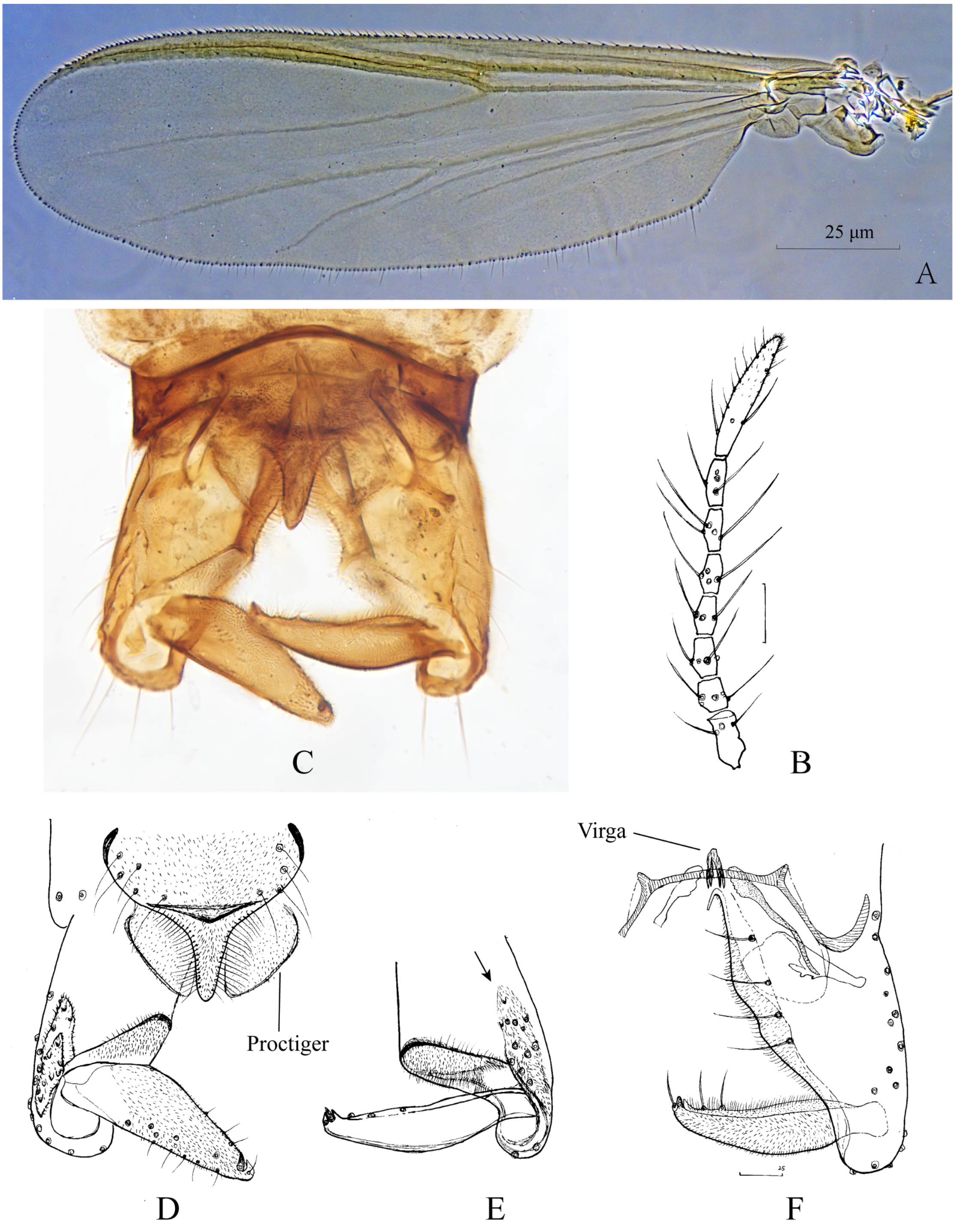

Figure 1. *Thalassosmittia nemalione* (Tokunaga, 1936), male. A, Wing; B, Antenna; C–E, hypopygium, dorsal; F, hypopygium, ventral. (Scale: B, 50 *µm* ; A, C–F, 25 µm).

Table 1. Length (in µm) and proportions of legs of *Thalassosmittia nemalione* (Tokunaga, 1936), male. (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	520	610	300	185	115	65	60	0.49	3.36	3.76
P_2	600	670	320	170	120	70	65	0.47	3.74	3.97
P_3	670	810	350	170	150	70	60	0.43	4.07	4.23

Legs. Foreleg with one slender tibial spur, 25 µm long; middle leg with two tibial spurs, 12.5 µm and 25 µm long; hind leg with two tibial spurs, 25 µm and 35 µm long, and a small tibial comb comprised of approximate 10 spines. Length (in µm) and proportions of legs as in table 1.

Hypopygium (Figs 1C–F). Tergite IX with 4–5 stout lateral setae on each side, anal tergite band absent. Anal point (Figs 1C–D) 55 µm long, broadly triangular at base with a conical apex, densely covered with microtrichia, bearing 20–25 long setae marginally. Proctiger obvious, flanked by anal point. Virga comprised of two slender spines, apically bifid. Gonocoxite 193 µm long; caudolateral surface bearing an oval patch with 75 µm long spindle-liked fenestra (Fig. 1E, arrow), bearing 9–11 long setae. Inferior volsella large lobe-like, projecting upward, with dense microtrichiae. Gonostylus 115 µm long, slightly swollen in basal 1/3, attenuated toward apex, with 3 strong setae at inner margin; megaseta small.

Discussion

The Chinese specimen mostly fits the original description of the Japanese type material, except for the shape of the inferior volsella. The inferior volsella of our specimen is tucked upward, while the lobe of the holotype projects to the inner margin of gonocoxite (Tokunaga, 1936: fig. 7). The difference is likely an artefact originating from the sliding-mounting process, actually representing the same structure in two different orientations.

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REDESCRIPTION OF ADULTS OF *KRIBIODORUM BELALONG* CRANSTON (DIPTERA, CHIRONOMIDAE, CHIRONOMINI) FROM GUNUNG MULU NATIONAL PARK, SARAWAK, MALAYSIA

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Abstract

The adult male of *Kribiodorum belalong* Cranston (Diptera: Chironomidae) was described only from a teneral adult specimen along with pupae. Certain key characters are unavailable or unmeasurable in the teneral form, e.g., wing dimensions, abdominal coloration pattern, and fine details of the hypopygium including the dorsal shape of the anal point. Here we redescribe and figure the adult male, and describe and illustrate the adult female of *Kribiodorum belalong* for the first time, both based on specimens recently collected in Gunung Mulu National Park, Sarawak, Malaysia. We provide some comments on the putative phylogenetic position of *Kribiodorum* Kieffer.

Introduction

Kribiodorum belalong Cranston was described based on a pharate male from Brunei (Borneo). However, the colours of teneral specimens such as this are imprecise, especially in the wing, legs and abdomen, which prevents comparison with congeners in these details. Furthermore, the anal point, which is important for species delimitation in this genus, was unavailable in dorsal view due to the mounting process. Therefore, *Kribiodorum belalong* is redescribed and figured from an adult male based on specimens collected recently in Sarawak, Malaysia. In addition, the adult female is described for the first time. The phylogenetic position of *Kribiodorum* Kieffer can now be discussed in more detail than previously.

Material and methods

Adults were collected by the UV-light traps at Lupar stream bank along the 'Botany loop' trail in Gunung Mulu National Park, Sarawak. Specimens were preserved in ethanol and dissected and slide-mounted in Euparal under an Olympus SZ61 stereomicroscope. Photographs were taken for each specimen under an Olympus BX53 compound microscope through a mounted camera-ToupView™. Digital photos of different focal planes were stacked using Helicon Focus version 7. Morphological terminology and abbreviations follow Sæther (1980). The colour is described based now on fresh specimens preserved in alcohol. The examined specimens are deposited in the following institutions: Department of Ecology, Jinan University, Guangzhou (EJNU), Australian National Insect Collection CSIRO, Canberra, Australia (ANIC) and Sarawak Forestry Corporation (SFC).

Taxonomy

Kribiodorum belalong Cranston, 2018

Kribiodorum belalong Cranston, 2018: 538 (pharate male and pupa).

Material examined. 2 females, slide mounted in Euparal, MALAYSIA: Sarawak, Gunung Mulu National Park, Lupar stream of Melinau River, 82 m.a.s.l., 04°2'15.7''N, 114°48'47.9''E, 12.vi.2023, light trap, H.Q. Tang (EJNU and SFC). 2 males as previous except 10.vii.2024 (EJNU and SFC). Holotype, 1 mature male pupa. BRUNEI: Temburong District, Kuala Belalong Field Study Centre, Sungai Belalong, 04°33'N 115°09'E, -.viii.1995 (Cranston) (ANIC).

Colour (Fig. 1A). Golden-yellow in general, with banded wing and dark ringed legs. Thorax shining yellow, slightly deeper in scutal vittae, sometimes close to background colour. Wing with two cross bands along the transect section, with a slender connection through the m cell, pale in the basal, middle and apical section. Leg darker subapically on femur and tibia of p_1 and p_3, of p_2 paler with dense grey setae on distal half femur and total length of Ti. Tarsus on p_1 brown, slight lighter in basal section of ta_1. In mid and hind legs, apical portion of ta_1 and ta_2, nearly whole length of ta_3, and full length of ta_4 and ta_5 dark brown. Abdomen light brown except brown AI and dark brown AVII–VIII, sometimes, a faint posterior brown band present in AII–VI. Hypopygium pale.

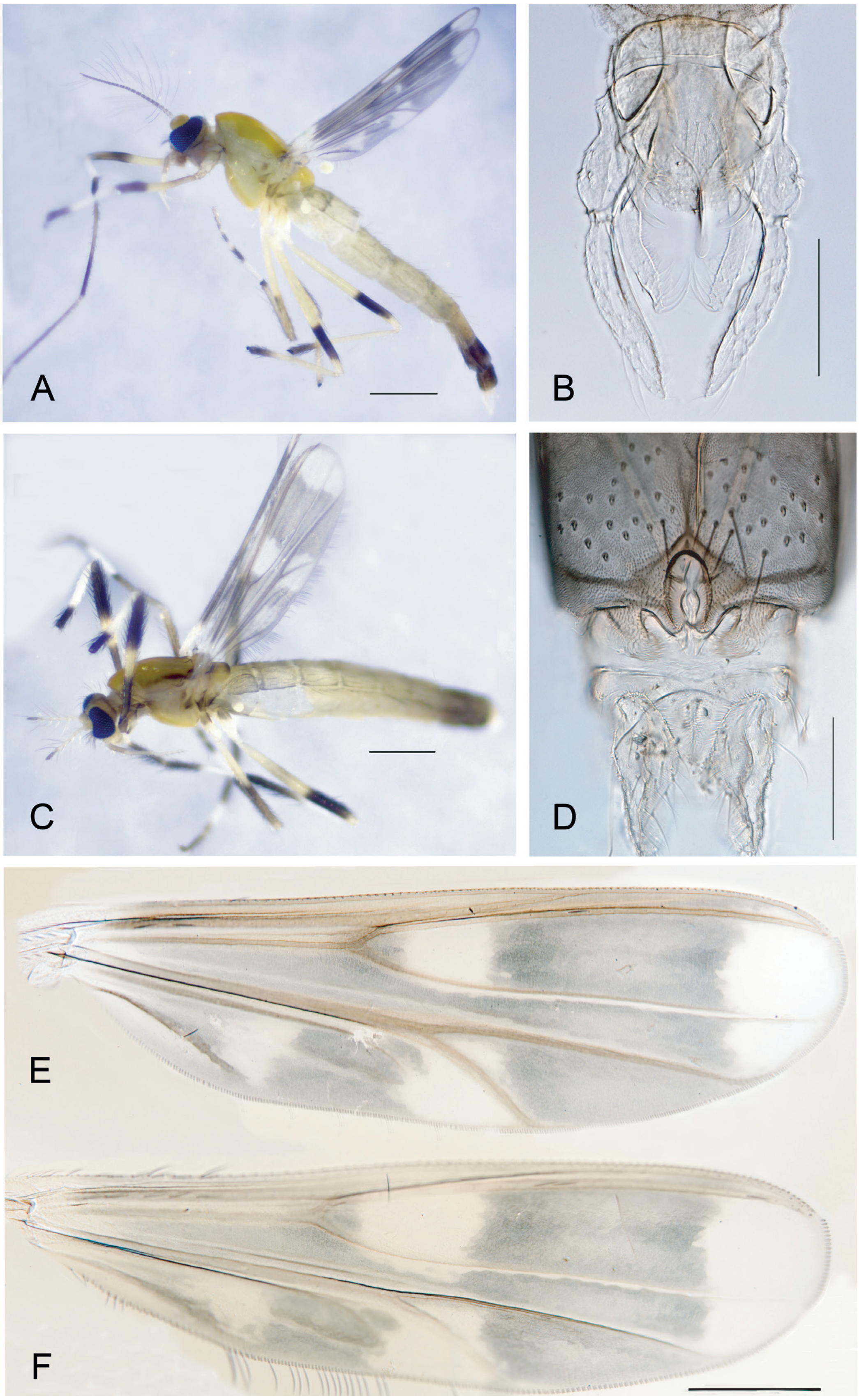

Figure 1. *Kribiodorum belalong*, adult. A. male habitus; B. male genitalia; C. female habitus; D. female genitalia; E. male wing; F. female wing (Scales: A, C, 500 µm; B, D, 100 µm; E–F, 250 µm).

Table 1. Lengths (µm) and proportions of legs of *Kribiodorum belalong* Cranston, male (n = 2–3). Data from holotype teneral (ten.) male (Cranston, 2018).

	fe	ti	ta$_1$	ta$_2$	ta$_3$	ta$_4$	ta$_5$	LR	BV	SV
P_1	740-850, 790	450-480, 460	1100	650	420	370	190	2.29	1.49	1.21
P_2	650-720, 680	400-430, 417	340	120	60	50	40	0.79	5.52	3.38
P_3	830-900, 867	660-700, 680	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
P_1 (ten.)	550	400	630	400	250	280	100	2.00	1.50	1.50
P_2 (ten.)	570	440	320	110	70	45	50	0.76	4.75	3.16
P_3 (ten)	600	610	320	240	110	190	110	0.53	2.00	3.78

Table 2. Lengths (µm) and proportions of legs of *Kribiodorum belalong* Cranston, female (n = 3).

	fe	ti	ta$_1$	ta$_2$	ta$_3$	ta$_4$	ta$_5$	LR	BV	SV
P_1	750-940, 837	420-520, 473	1020-1280, 1110	600-770, 660	380-500, 440	340-420, 381	160-210, 188	2.45-2.47, 2.46	1.44-1.47, 1.46	1.13-1.15, 1.14
P_2	600-750, 663	370-500, 427	310-400	85-120	50	35	40	0.80-0.84, 0.82	5.69-6.78, 6.31	3.09-3.13, 3.11
P_3	780-1000, 880	620-800, 693	460-500	260-270	240-245	170-180	80-90	0.75	2.53	3.04

Male (n = 2). Total length 3.03–3.25 mm. Wing length 1.35–1.40 mm.

Head. Flagellomere 1–12, 450–480 µm; flagellomere 13, 340–400 µm, apical seta 40–50 µm; AR 0.76–0.83. Lengths (µm) of Pm 1–5: 30–35; 20–30; 120–140; 120–145; 150–165, respectively. Temporals 5–6. Clypeus with 12–16 setae.

Thorax (Fig. 2A). Ac 2, restricted to the anterior of antepronotum lobe; Dc 5, including 1 humeral; Pa 3. Sct with 4–6 setae.

Wing (Figs 1A, C). Anal lobe reduced, VR 1.34–1.36. R with 12–15 setae; R_1 with 10–13 setae; R_{4+5} with 13–18 setae, other veins and cells bare.

Legs. Fore tibia with curved spur, 50–65 µm long. Most tarsi of legs broken, with only femur and tibia remaining. Lengths and proportions of legs as in Table 1.

Hypopygium (Figs 1B, 2B). Anal tergite bands weak, separate, medially with 10–16 anal setae, distally with 5–7 marginal setae each side. Anal point 40–50 µm long, spatulate distally, SVo 55–60 µm long, comprising setose pad-like base, bearing 4–5 long surface setae and digitus slightly curved inwardly, bare (Fig. 2B). MVo connected with SVo base, with 5–7 strong setae arising from distinct tubercles. Inferior volsella slender, distally bearing 4–6 long and strong setae. Gonocoxite 100–110 µm long. Gonostylus 110–125 µm long, may be with slightly swollen subapex. Hypopygium ratio 0.88–0.92.

Female (n = 2). Total length 2.95–3.50 mm, wing length 1.28–1.45 mm.

Colour (Fig. 1C). As male except lateral vittae are slightly brown in posterior section and last flagellomere is darker brown.

Head. Antenna with 6 flagellomeres, Fl 1-5 250–290, Fl 6 70–90 µm long, AR 0.28–0.31. Lengths (µm) of Pm 1–5: 30–35; 20–25; 120–150; 145–150; 170–175, respectively. Temporals 5–7. Clypeus with 18–26 setae.

Thorax. As male, Ac 2, Dc 5 with 1 in humeral position; Pa 3. Sct with 4 setae.

Wing. Pattern as in Fig. 1F. VR 1.30–1.34. Vein setation: R with 13–15, R_1 with 12–16 and R_{4+5} with 20–28.

Legs. Patterned as in male. Lengths and proportions of legs as in Table 2.

Genitalia (Figs 1D, 2C–D). Notum long, thin, 135–160 µm long, rami short, 30–35 µm long. Gonocoxapodemes gently curved, slightly fused medially, each enclosing setal patch, bearing 18–25 setae. Coxosternapodeme strongly sclerotized, weakly curved. Gonapophysis VIII (Figs 1D, 2C) comprising elongate dorsomesal lobe, microtrichiose, hyaline apico-medially, and distinct ventrolateral lobe, broad, microtrichiose with many simple medio-apically directed setae. Apodeme lobe small and lobe-like. Labia well developed, triangular apex, hyaline, without microtrichia. Gonocoxite IX small, with 3–4 setae. Tergite IX an undivided single plate, bearing 35–40 setae. Postgenital plate

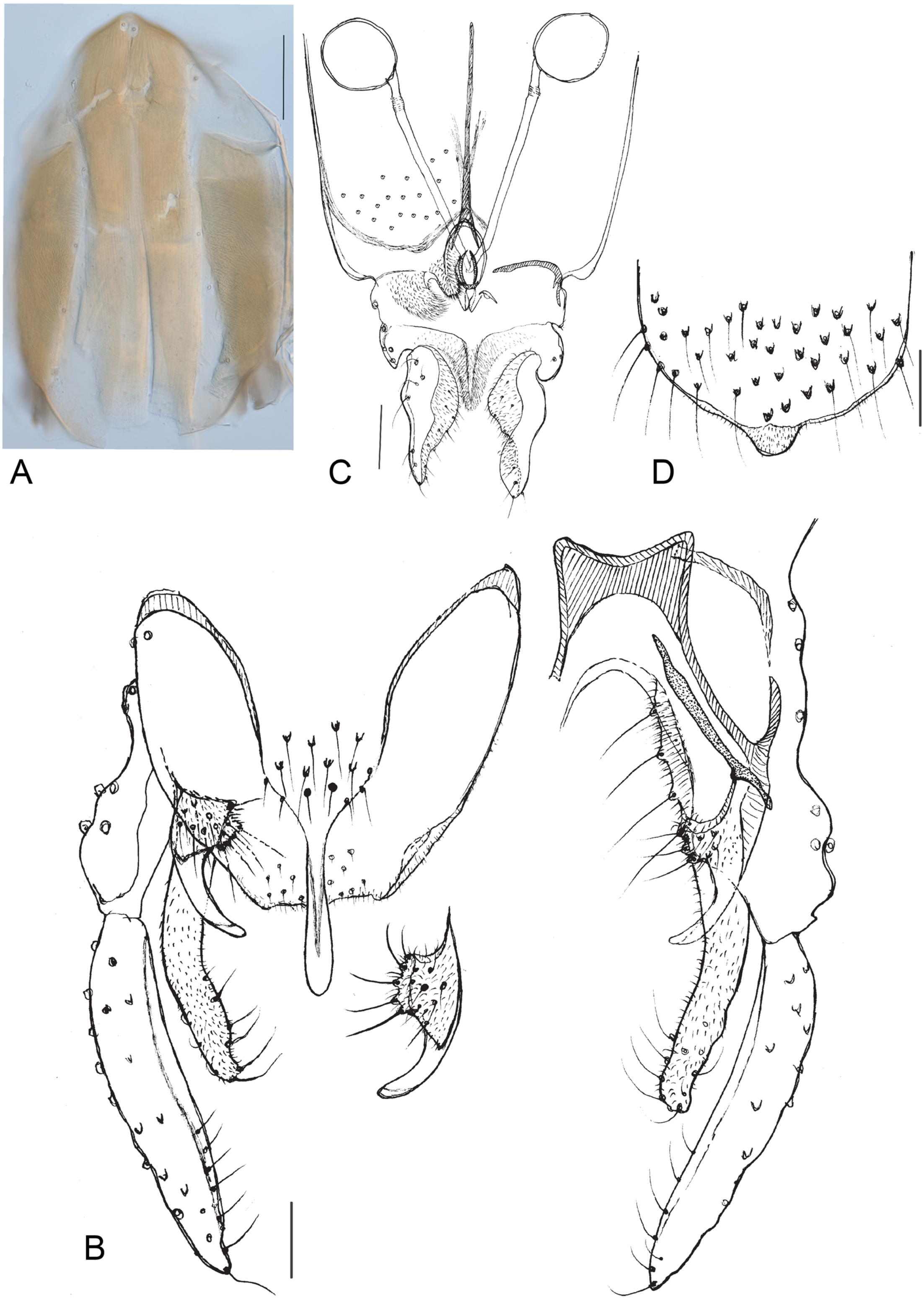

Figure 2. *Kribiodorum belalong*, adult. A. dorsal thorax; B. male hypopygium, left side dorsal, inset superior volsella, right side, semi-stylized ventral; C. female genitalia, ventral; D. female genitalia, tergite IX (Scales: A, 100 µm; B, 25 µm; C–D, 50 µm).

triangular, no clear boundary. Seminal capsule oval, 50–60 µm in diameter, with a wrinkled section near the neck. Cerci large, quadrate, 100–125 µm long by 45–50 µm wide in dorsal view.

Remarks. The combination of the wing pigment pattern and the form of the hypopygial gonocoxite lobes matches the teneral male holotype of *K. belalong* Cranston. There are no other candidate species in the region and the conspecificity of our material can be confirmed. Comparison of the described species of *Kribiodorum* shows that *K. belalong* resembles the Neotropical *Kribiodorum amazonicum* Dantas & Hamada in a similar (but not identical) hypopygium, and further, to the Nearctic species *Kribiodorum perpulchrum* (Mitchell). These species can be separated easily by wing and leg pigment patterns, and also by differing contours of the digitus of the superior volsella.

Systematics

When Reiss (1982) evaluated the systematic position of *Kribiodorum* (as *Stelechomyia*), he found no close Holarctic Chironomini genus that matched the character state combinations observed in *Kribiodorum.* However, Reiss (loc. cit.) noted that the characteristic wing and leg colour patterning, the long, curved fore tibial spur and a dilate subapex of the fore femora are shared with *Lauterborniella* Thienemann & Bause and *Zavreliella* Kieffer. However, the dramatically different immature stages induced Reiss to switch some undescribed Neotropical genera. If omitting the wing pattern and placing more weight on the male hypopygial morphology, especially of the superior volsella, *Kribiodorum* also resembles *Imparipecten* Freeman and *Nilodosis* Kieffer (Tang & Cranston, 2017; Fusari et al., 2018).

To date, six *Kribiodorum* species have been described (Cranston, 2018; Dantas & Hamada, 2021) but with no further discussion on systematic relationships. Ongoing phylogenetic studies of the tribe Chironomini, based on concatenated markers of five genes (Tang & Cranston, submitted), provides unequivocal support for *Kribiodorum* as sister to *Nilothauma* Kieffer (Bayesian posterior probability = 1). This surprising pairing is sister with highest support to a speciose cluster comprising *Imparipecten*, *Yaeprimus* + core *Chironomus* and relatives. *Zavreliella* and *Lauterborniella* are distant, refuting Reiss's (1982) suggestion of a close relationship.

The unexpected, yet maximally supported, sister relationship to *Nilothauma*, is warranted on morphology only in the pupal stage in which the thoracic horn, tergite spinulation and pattern of taeniae (LS – setae) are similar in both, but dramatic differences are evident in the morphology of males and larva. Perhaps a xylophagous and psammorheophilic life-style are distinct evolutionary pathways that shaped disparate morphologies in adult and larva stages.

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DESCRIPTION OF THE PUPA OF "DIAMESINAE GENUS P" DOUGHMAN, 1985 (DIPTERA: CHIRONOMIDAE: DIAMESINAE), WITH COMMENTS ON HABITAT, DISTRIBUTION AND FURTHER CHARACTERIZATION OF THE LARVA

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Abstract

The pupal stage of "Diamesinae Genus P" Doughman, 1985 is described from specimens collected in northwest Georgia, USA. The pupa is recognized as Diamesinae by dorso- central thoracic setation with no prealar setae and lack of hooklets on tergite II. Also, the anal lobe has apical short tubercles and 3 hooked macrosetae with very distinctive apices. However, leg sheath arrangement differs slightly from described Diamesini with fore and mid leg sheaths directed laterally at the wing apex and frontal setae are lacking, but for now this is considered variation within the tribe. The strikingly unusual larva is recognized as Diamesinae by the annulate third antennal segment as well as characteristics of the premento-hyphoryngeal complex. The larva fits within Diamesini in the most recent keys of Holarctic genera. Currently known distribution, habitat and additional larval morphological details are noted.

Introduction

An unusual and rarely collected Diamesinae larva was characterized by Doughman (1985) in a key to Nearctic Diamesini as "Genus P" from "Southeastern USA." This initial characterization included a single figure of the distinctive mentum, followed by records from sandy bottom Coastal Plain Province streams in Alabama, Georgia, Florida and North Carolina by Hudson et al. (1990) and Caldwell et al. (1997). For Florida and the southeastern USA, Epler (1992, 1995, 2001) and more recently Sæther and Andersen (2013) repeated these distribution records with further larval characterization.

During biomonitoring of a stream mitigation banking project (administered by the U.S. Army Corps of Engineers), larvae were discovered in February 2019 and December 2020 from multi-habitat samples collected in only one remote small, unnamed tributary stream, informally known for the project as "Main Creek." The site is a perennial second order stream in the Etowah River Basin, Dawson County, Georgia. Additional drift and aerial net sampling in February and March 2021 yielded pupal exuviae but no larvae, pupae, or adults. The drainage area at the sampling location is 2.85 km^2, with mean annual precipitation (1971-2000) of 1.76 m and range of 1.20 to 2.07 m. (Gotvald 2017). The stream is located in the Blue Ridge Physiographic Province and U.S. Environmental Protection Agency Blue Ridge Ecoregion, Southern Metasedimentary Mountains (66g), (Griffith et al. 2001b). The positive association of the pupa and larva was later confirmed in a Florida larva with some discernible developing key pharate pupal features.

Methods

Morphological terminology follows Sæther (1980) and Langton (1994a, 1994b, 2011). Counts and measurements were made following Soponis (1977) except left or right sides of pupal exuviae were utilized. Measurements are in units that are stated, and reported as the respective minimum, maximum and mean value when 3 or more specimens were measured. The number of specimens or structures measured or counted is given in parentheses (n). The letter L is sometimes used for larva(e) with Pex used for pupal exuviae (shed skins - all dissected). Project stream sampling followed Georgia Department of Natural Resources, Environmental Protection Division protocols (2007). Specimens were preserved in approximately 80-90% ethanol and directly mounted in CMC-10 or dehydrated in 95% ethanol and mounted in Canada balsam.

Results

"Diamesinae Genus P" Doughman, 1985

Material examined: USA: 1 L, Florida, Escambia County, Canoe Creek, 7-II-1966, leg. W. Beck; 1 L (w/ pupal characters), Perdido River, Barrineau Park Rd., 25-IV-2018, N30.6903°, W87.4404°, Sample #1987815, leg. N. Mulkey; 1 L, Calhoun County, Juniper Creek, Florida [Highway] 20, 4-XII-1969, leg. W. Beck; 1 L, Georgia, Dawson County, 8-II-2019, "Main Creek", Site 205, leg. Corblu Ecology Group, LLC; 4 L, same data except, 21-XII-2020; 15L, 15 Pex, same data except 17-II-2021, leg. B. A. Caldwell; 1 Pex, same data except 9-III-2021; 1 L, Harris County, [trib.] Mulberry Creek, 11-II-1981, leg. M. W. Heyn; 1 L, same data, except 14-I-1984. Specimens are in collections of the Florida Department of Environmental Protection (FLDEP), M. W. Heyn or the senior author.

Pupa (Pex, n = 6); small, in comparison to larva, generally about 3.5 – 4.8, 4.0 mm total length, light yellowish color, rather stiff and very buoyant.

Cephalothorax. Frontal apotome with two small, moderately rugose central areas, few facial creases, without frontal warts, cephalic tubercles, frontal setae or setal sockets. Wide area of prefrons covering labial sheaths projecting posteriorly, ending in a simple point. Thorax (Fig. 1a) mostly rugose anteriorly along middorsal suture with median and posterior areas less rugose with development as a sculptured/reticulate or wrinkled pattern. Antepronotum with two very thin median antepronotal setae; distance apart 36-52, 41 μm. Three thin precorneal setae present, with Pc_1 and Pc_2 being closest together and Pc_2 thickest of the three. Small socket-like structure close to Pc_1 and Pc_2. Thoracic horn absent. Dorsocentral setae Dc_1 and Dc_2 rather close together with Dc_1 seta generally longest; distant Sa seta usually most easily discernible. No prealar setae. Fore and mid leg sheaths in close contact, strongly curving laterally near apex of wing sheath, barely exceeding lateral margin (Fig. 1b). Apex of hind leg sheath curved medially, overlying tip of mid leg sheath just past lateral margin of wing sheath. Wing sheaths mostly smooth except for small area of fine wrinkles at anterior base joining thorax.

Figure 1. (a) Pupal thorax, right, lateral view.

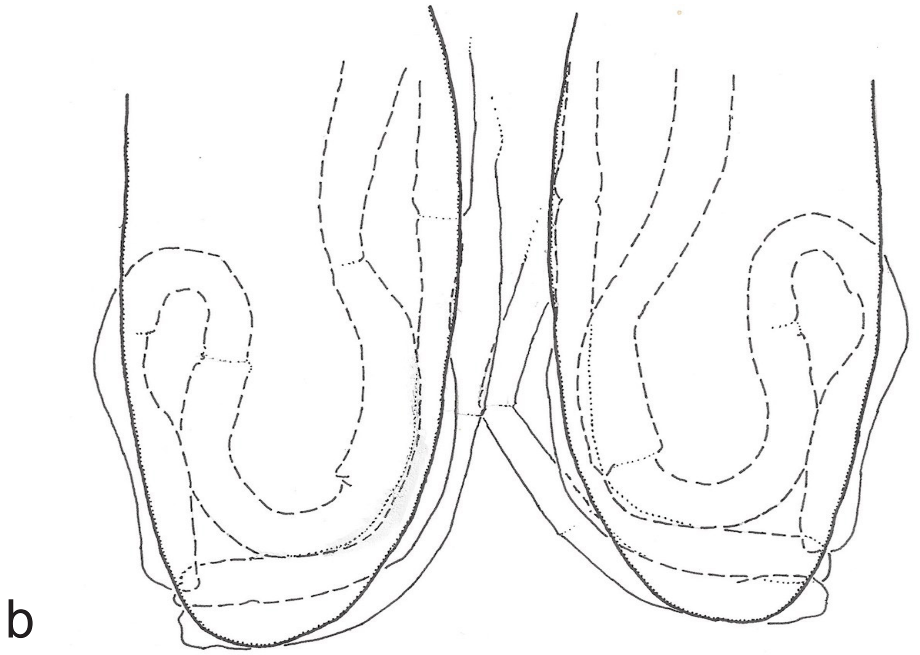

Figure 1 contd. (b) Pupal wing and leg sheaths.

Abdomen. Tergites I –VIII with fine shagreen, most sparse on TI and TVIII. Anterior shagreen points of other tergites merge into curved point rows. TVI and VII with median posterior area rounded and extended, with small, mostly rounded small points on TVI and short, sharp spines on TVII directed posteriorly. Sternite VIII with posterior band of anteriorly directed small spines in the male (Fig. 2a), band divided medially in the female (Fig. 2b). Faint lateral adhesion marks present on all eight abdominal segments. Tergite and sternite setae present, very thin and short but very difficult to discern or accurately count due to orientation or even possible loss. Number of O-setae discernable. All of the lateral setae on segments I-VII very thin, hair-like and short with 2 pair anterior and 2 pair posterior, each pair with one dorsal and one ventral seta. No lateral setae discernible on Segment VIII. Segment VIII with approximate third of posterior outer edge thickened and appearing at the corner as a projecting rough tubercle(s); in all specimens extending posteriorly with a very short knob-like shape. Male anal lobe (Fig. 3a) with posterior tip slightly darkened and extended as a short finger-like projection. Macrosetae (Fig. 3b) strongly hooked, curving medially, apically flared often with very, very slightly concave tips. Male genital sacs relatively straight, exceeding tip of anal lobe spur a short distance.

Larva (n = 10). Medium-sized larva (Fig. 4a), about 6-7, 6.25 mm long (n=4) with general body coloration slightly yellowish and other features as noted.

Head. Golden-tan with several brownish markings and distinct black occipital margin, often appearing widest in later instars (flattened mounts). Early instar larvae often with at least one additional very small lateral mentum tooth. Mandible in some specimens appearing rounded but slightly angled in apical third. Premandible (Fig. 4b) with large apical tooth, 3 much smaller inner teeth and an elongate, thin, apically pointed lateral spine appearing attached between base and apex. Labro-epipharyngeal complex with three "brushes" present but not strongly produced with the two groups of labral lamellae with several spinulae appearing very distinctive (Fig. 4b). Pecten epipharyngis appearing as three elongate pointed scales not readily separable from chaetae laterales (Fig. 4b).

Thorax and abdomen. Body with few scattered, thin, short setae present. Anterior parapods with numerous thin, gently curved claws as well as short strongly hooked, robust claws, some bifid and at least two very large, strongly hooked claws with as many as 3-4 inner teeth. Some very small, slightly curved, mostly simple claws also present basally. Each posterior parapod with a strong subbasal seta; claws robust, with at least two strongly hooked, others strongly curved. Procercus short, with robust basal/lateral seta and six rather short anal setae; four apically rounded anal tubules, about half as long as posterior proleg.

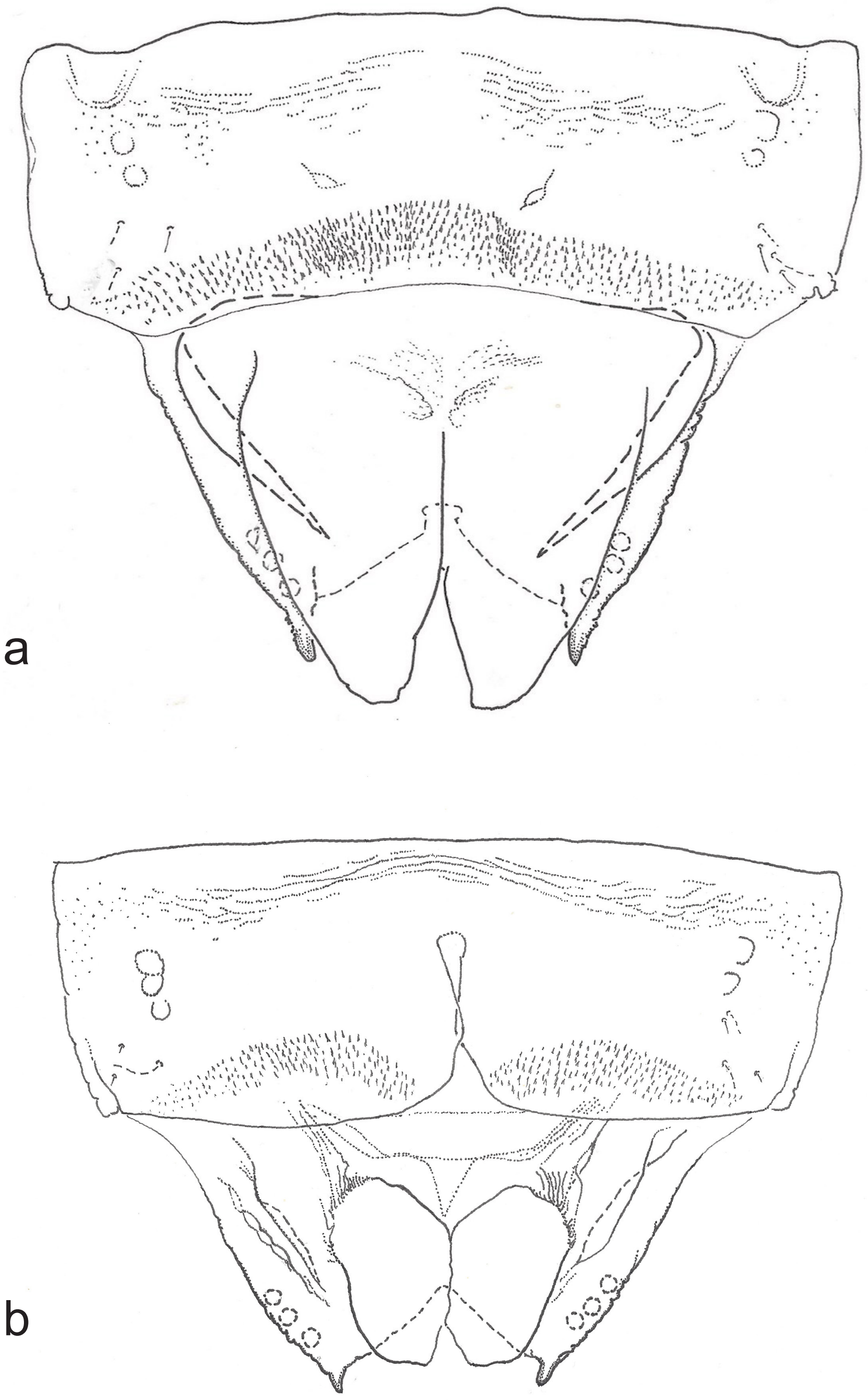

Figure 2. Pupal sternite VIII and anal lobe, macrosetae omitted (a) male; (b) female.

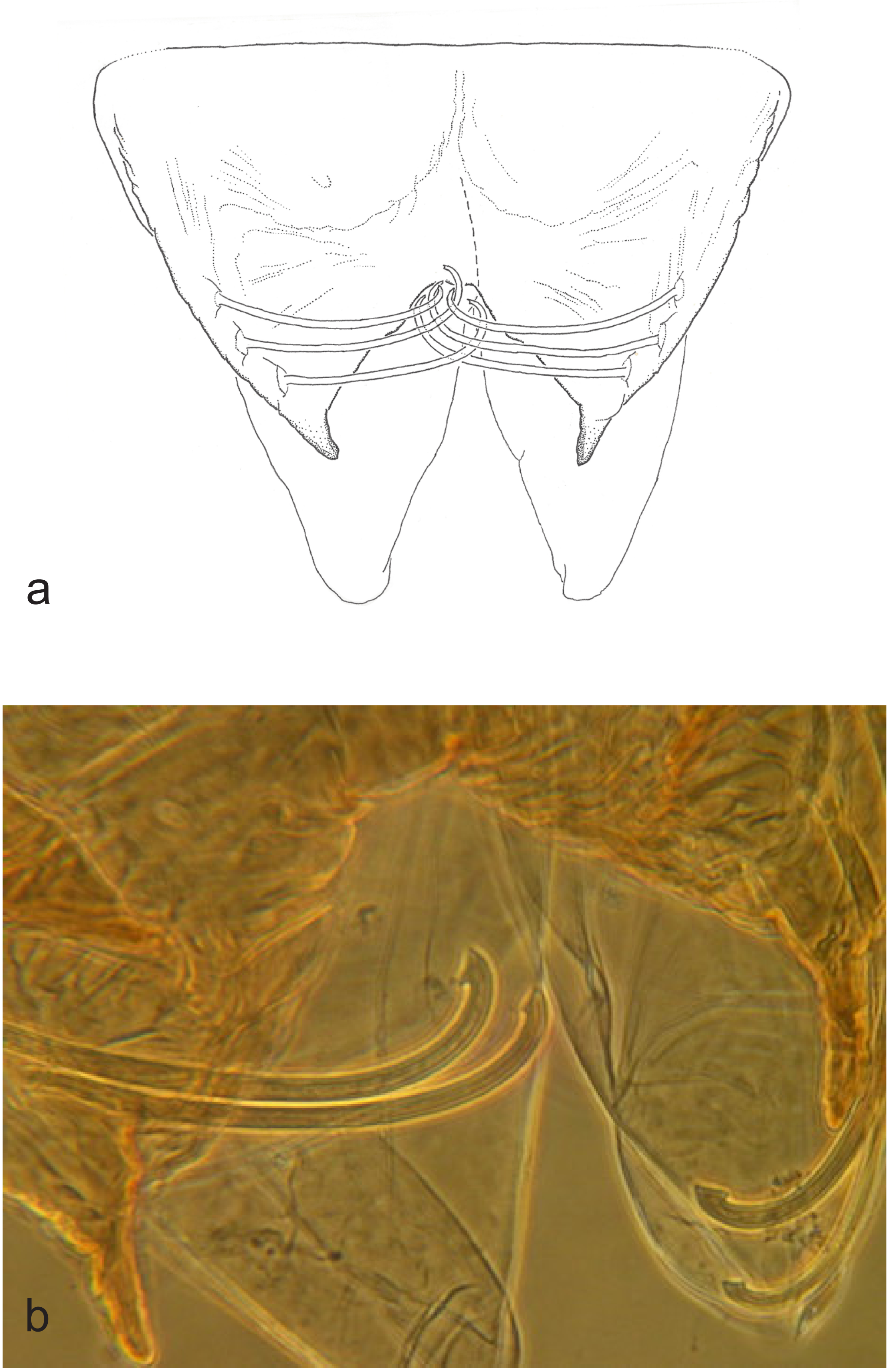

Figure 3. (a) Male pupal anal lobe, dorsal; (b) detail of macrosetae, note apices.

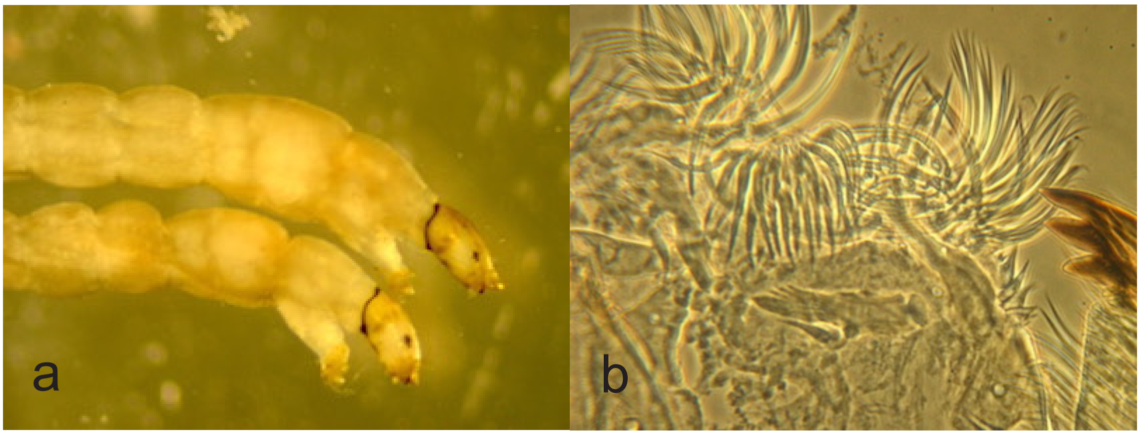

Figure 4. (a) Immature larvae, lateral head and thoracic area; (b) larval labro- epipharyngeal and palatum region, note premandible with sharp lateral spine near lower center.

Discussion

Important morphological features of the "Diamesinae Genus P" pupa include typical thoracic setation without any prealar setae, fine but sparse shagreen on tergites I and VIII, leg sheath arrangement, lack of hook row on tergite II, an anal lobe with macrosetae exhibiting rather uniquely expanded apices, sexual dimorphism in posterior spine row on sternite VIII and lack of thoracic horn. Although the hind leg sheath contacts the mid leg sheath, and fore and mid leg sheaths are oriented laterally at the wing apex, the arrangement is most similar to other Diamesini. However, frontal setae are lacking. Specimens key to couplet 5, Diamesini in Oliver (1986), with Holarctic Boreoheptagyiini (*Boreoheptagyia* Brundin) excluded. The larval ventromental plates show similarity to *Pagastia* Oliver and *Potthastia gaedi* group Meigen (Sæther and Andersen 2013) by entirely covering the mentum. The larval labral lamellae are somewhat similar to the long but simple ones of *Xenochironomus* Kieffer but are not on lobes.

"Diamesinae Genus P" larvae and pupae have unique morphological character states (or autapomorphies) within the Diamesini. One pupal character, no frontal setae, is shared with three other Diamesinae tribes - Harrisonini, Lobodiamesini and Heptagyiini. Recognition of a possible new genus and species awaits discovery of the adult male/female hopefully with molecular data. See Lin et al. (2022) on use of mitogenomes. Cranston and Krosch (2015) describe a similar situation concerning a formal description of the informal Podonominae taxon, Genus "Chile" Brundin, 1966 as *Podonomopsis* (*Araucanopsis*) *avelasse*, which was inadequately known as a Pex for over half a century.

The "Main Creek" sampling site can be characterized as forested with partly open canopy, including shrubs, grasses and herbaceous plants. Typical sampling reach morphology included about 65% riffles, 30% runs and 5% pools. Bottom substrate was generally a mix of boulders, cobble, gravel and sand, with sand constituting about 15% of the various components. No bedrock, silt or clay was noted. Thus, "Main Creek" is not a totally "sandy bottom" or even sand dominated stream as is found in the southeastern Coastal Plain Province, an area well known for the occurrence of "Diamesinae Genus P".

The preferred microhabitat of "Diamesinae Genus P" larvae appears to be in smaller streams to moderately wide rivers within relatively stable, sandy substrate. The Pex but no additional L from "Main Creek" were collected by drift net, especially downstream of short, relatively straight sandy stream reaches. Beck and Beck (1974) were unable to rear larvae, even to the initial pupal stage and lack of adults in recent Georgia sampling requires determining adult phenology and terrestrial preferences. Other sand dwelling and sand case making chironomid taxa collected in Main Creek include *Neostempellina reissi* Caldwell, *Stempellinella leptocelloides* (Webb) and *S. boltoni* Ekrem.

Collection data for Florida (M. W. Heyn, personal communication) documents "Diamesinae Genus P" larvae present in all months except June, July and October with one pre-pupal larva collected in late April. Beck and Beck (1974) and Beck (1977) incorrectly regarded Florida larvae as *Sympotthastia* Pagast, and being present only in winter months. Research of records in other southeastern states reveals few larvae mostly in late winter through early spring, which is typical for other Diamesinae life stages. See Table 1 for historical and updated distribution records. All Florida Department of Environmental Protection (FLDEP) historical and recent records are confined to the western panhandle area of the state (M. W. Heyn, personal communication, including specimens labeled as *Sympotthastia* (sensu Beck and Beck 1974).

Much research of historic and more current distribution records for other southeastern states is presented in Table 1. Information was gathered from the states and included incorporation of United States Environmental Protection Agency (USEPA) Level IV ecoregions and subregions (Griffith et al. 2001a, 2001b, 2002). The USEPA maps are very small scale and in certain instances, especially Georgia, a couple of collection sites are "borderline" and did not allow for more precise placement of these localities for now.

New distribution records include South Carolina presence of "Diamesinae Genus P" formally published for the first time. A new record other than Coastal Plain was found for Alabama, jointly shared with Florida (Perdido River). "Genus P" was found in a taxa list for Mississippi but apparently no specimens were retained (M. Chimahusky and A. Dossett, personal communication). This is the first formally published report of this taxon presence in Mississippi. No records were found for Tennessee although the standard chironomid larval midge key used does not include this taxon (D. Arnwine, personal communication). No other records are known for Tennessee from an additional source (W. Pennington, personal communication). The Kentucky "Master Macroinvertebrate Species List" (2015) does not include this Diamesinae taxon and knowledgeable sources in and outside of the state also knew of no records.

Table 1. Historical and updated distribution records of "Diamesinae Genus P" Doughman, 1985 for eight southeastern states including Physiographic Province and United State Environmental Agency (USEPA) ecoregions and subregions.

State	Physiographic province	USEPA ecoregion	USEPA subregion
Alabama	Coastal Plain	No other data	No other data
	Coastal Plain	Southeastern Plains (65)	Southern Pine Plains and Hills (65f)
Florida	Coastal Plain	Southeastern Plains (65)	Southern Pine Plains and Hills (65f)
		Southern Coastal Plain (75)	Gulf Coast Flatwoods (75a)
Georgia*	Coastal Plain	No other data	No other data
	Piedmont	Piedmont (45)	Pine Mountain Ridges (45h)
	Blue Ridge	Blue Ridge (66)	S Medisedimentary Mountains (66g)
Mississippi	Coastal Plain	In taxa list only	No other data
North Carolina	Coastal Plain	Southeastern Plains (65)	Sand Hills (65c)
	Piedmont	Piedmont (45)	Carolina Slate Belt (45c)
			N. Inner Piedmont (45e)
	Blue Ridge	Blue Ridge (66)	S Crystalline Ridges and Mountains (66d)
South Carolina	Coastal Plain	Middle Atlantic Coastal Plain (63)	Carolina Flatwoods (63h)
		S. E. Plains (65	Sand Hills (65c)
	Piedmont	Piedmont (45)	Carolina Slate Belt (45c)
	Blue Ridge	Blue Ridge (66)	S Crystalline Ridges and Mountains (66d)
Kentucky	No records in state database. No other records known.		
Tennessee	No records in state database. "Diamesinae Genus P" not included in currently used taxonomic keys. No other records known.		

*NOTE: The Georgia record for subregion (45h) is very near Southern Outer Piedmont (45b) and a record for subregion (66g) is very near Southern Inner Piedmont (45a). Larger scale Georgia Department of Transportation County maps were used in conjunction with a very small-scale color-coded USEPA ecoregion/subregion map of Georgia to assist in determining subregion placements.

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Two species of the genus *Nilotanypus* (Diptera: Chironomidae) in Europe

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Meigen (1804) described the female of *Nilotanypus dubius* (Meigen, 1804). *N. dubius* has been since reported as the only species of the genus *Nilotanypus* Kieffer, 1923 from Europe (Fittkau 1962, Fittkau and Roback 1983, Vallenduuk and Moller Pillot 2007, Cranston and Epler 2013, Bitušík and Hamerlík 2014). But Fittkau and Roback (1983) note that a second larval type is known from the western Palaearctic. Kownacki and Kownacka (1968) and Fittkau and Roback (1983) show claws on the larval posterior parapod of *N. dubius* with at most fine spinules, whereas Roback (1986) states that both *N. dubius* and *N. fimbriatus* have one claw of the posterior parapod strongly pectinate. Kownacki and Kownacka (1968) and Fittkau and Murray (1986) show pupa of *N. dubius* with posterior band of teeth on tergites, whereas Langton and Visser (2003) with posterior band of granules on tergites.

During the course of a survey of macroinvertebrates in Czechia (Skála 2011, Skála et al. 2019, Skála, unpublished records), pharate adults, pupae, pupal exuviae, prepupae and larvae of two different species of *Nilotanypus* were recorded. The pupa of the first morphotype, *Nilotanypus* sp. A has a transverse band of granules on posterior margin of tergites (n = 62; Fig. 1a), rarely with flat teeth up to 3 µm long. The larva has a comb of spines on one claw of posterior parapod (Fig. 2a). The antennal ratio of adult male is 0.50-0.88 (n = 7). The pupa of the second morphotype, *Nilotanypus* sp. B has a posterior band of teeth on tergites (the longest teeth 5-7 µm long; n = 9; Fig. 1b), and the larva has no comb but only fine spinules on larger claws of the posterior parapod (n = 24; Fig. 2b), male adult antennal ratio 0.53 (n = 1). No distinguishing characters were found in pharate adults, except a possible subtle difference in phallapodeme (Fig. 3). The small branchlet of phallapodeme is parallel with the main branch of phallapodeme in *Nilotanypus* sp. A (n=7), whereas it seems to be divergent in *N.* sp. B (n = 1 only). However not all characters were visible in pharate adults, especially the ones in legs or wings.

In central Bohemia (Czechia), *Nilotanypus* sp. A was commonly found in clean brooks, less frequently in rivers, at altitudes of 200-565 m a. s. l. (examples of localities were given by Skála 2011 under *N. dubius*). *Nilotanypus* sp. B was only found in the Brdy Highlands (max. 865 m a. s. l.) in three small forest brooks with high pH of 7.5-7.9 at altitudes 450-515 m a. s. l. (Skála et al. 2019, who reported it erroneously also from Mourový brook). In the Brdy Highlands, *Nilotanypus* sp. A was also found in five brooks at the same range of altitudes, but at somewhat lower pH of 6.2-7.1. In the Brdy Highlands, both species avoid acidic waters with pH less than 6.2.

Nilotanypus sp. B inhabits higher altitudes than *Nilotanypus* sp. A in Czechia. Kownacki and Kownacka (1968) describe larva of *Nilotanypus* sp. B from the Tatra Mountains, where they found *Nilotanypus* at altitudes 500-1400 m a. s. l. Thus, although *Nilotanypus dubius* has been regarded as a cold stenotherm species (Fittkau 1962), such description probably applies to *Nilotanypus* sp. B only.

Adults less distinguishable than larvae and pupae seem to be typical for the genus *Nilotanypus* (Cranston et al. 2022). The key to adult males by Cheng and Wang (2006) is not very useful due to use of unreliable characters (Cranston et al. 2022). Further study of the genus is necessary.

A brief neighbour joining analysis of the publicly available DNA barcodes of *Nilotanypus dubius* from Europe in BOLD (www.boldsystems.org, May 20, 2024) using MEGA 11 (Tamura et al. 2021) indicate a clear separation of two COI clusters (Fig. 4). Uncorrected pairwise distance between members of the two clusters are around 15 %. Thus, also available COI data indicate more than one species of *Nilotanypus* in Europe.

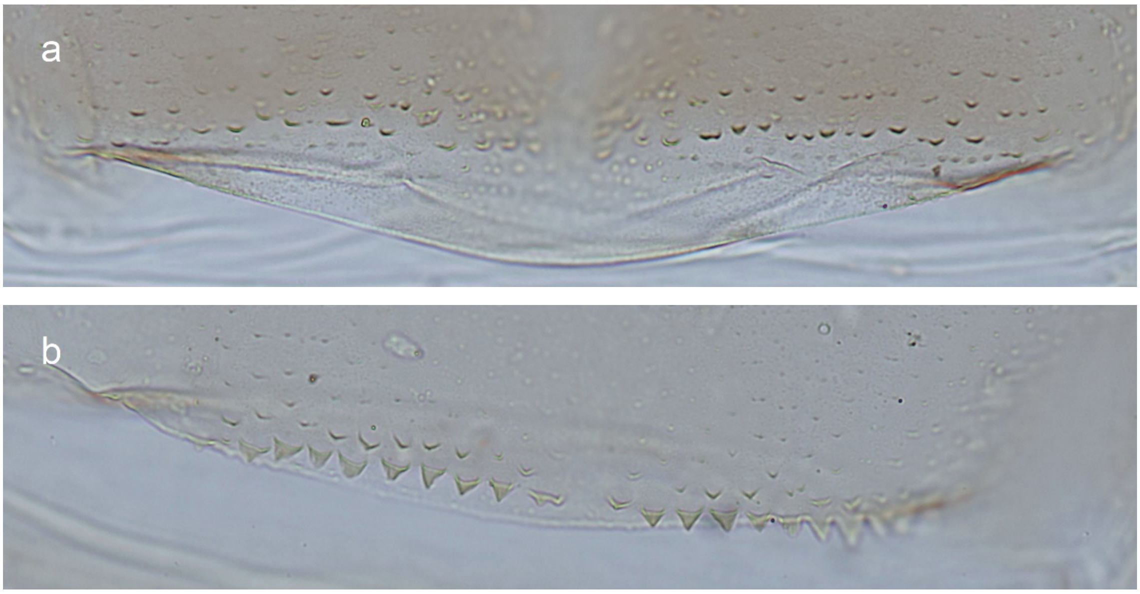

Figure 1. a-b. Pupa, tergite IV posterior margin. a. *Nilotanypus* sp. A; b. *Nilotanypus* sp. B.

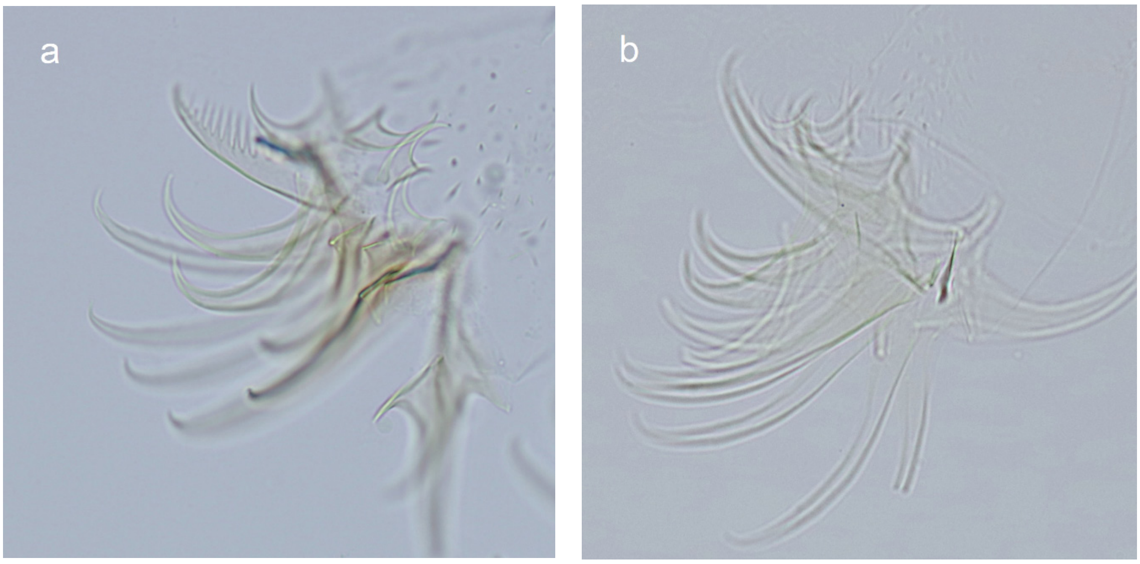

Figure 2. a-b. Larva, posterior parapod. a. *Nilotanypus* sp. A; b. *Nilotanypus* sp. B.

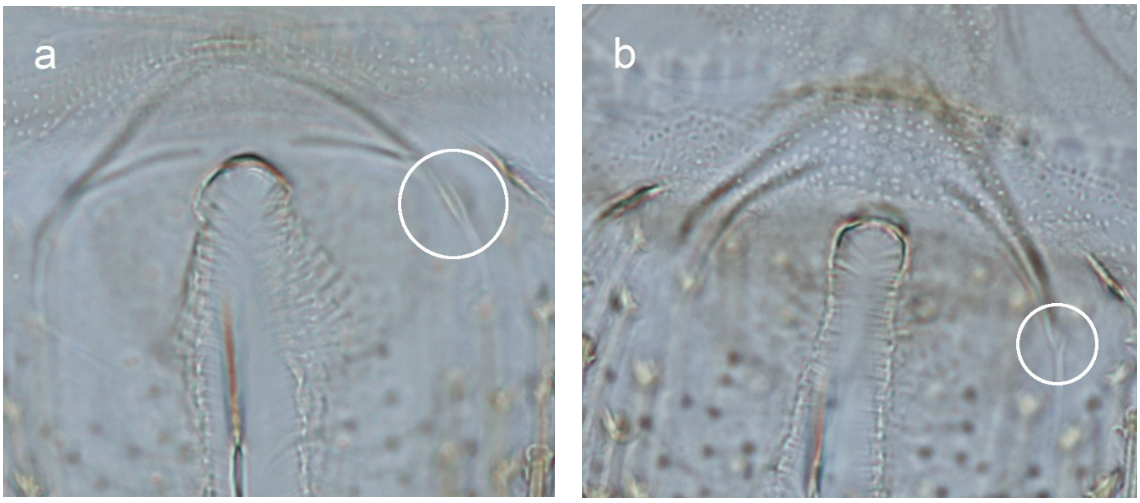

Figure 3. a-b. Male imago, apodemes of male hypopygium. a. *Nilotanypus* sp. A; b. *Nilotanypus* sp. B.

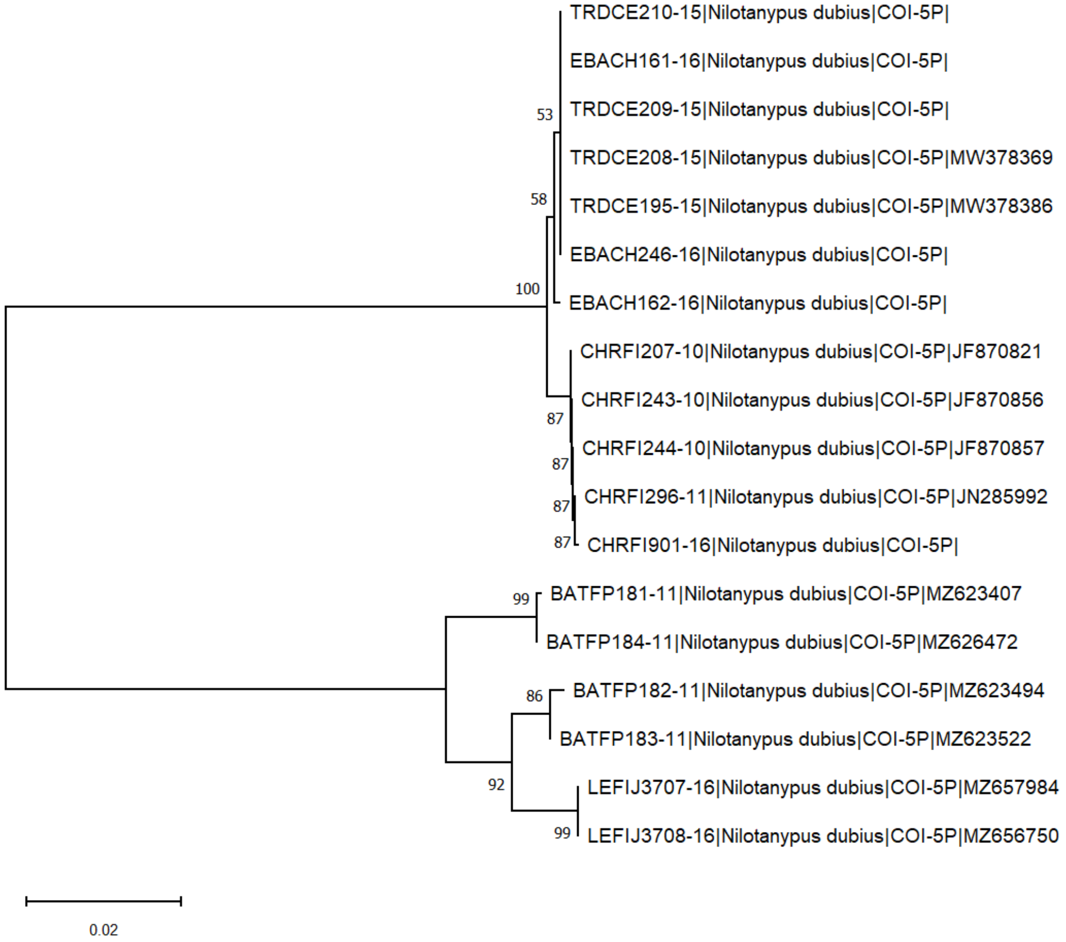

Figure 4. Neighbour joining tree with boostrap values on branches (500 replicates) generated by MEGA 11 (Tamura et al. 2021). Distances used were uncorrected p-distances, all codon positions were included. First alphanumeric code is BOLD Process ID, last alphanumeric code is GenBank accession.

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Redescription of the type specimen of *Chironomus oppositus* Skuse 1856

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Abstract

The type male of *Chironomus oppositus* Walker 1856 was redescribed and illustrated before the specimen was lost in transport back to the Natural History Museum London. The redescription enables the type to be associated with one of the currently recognised forms of *Chironomus oppositus.*

Introduction

Chironomus oppositus was described by Walker (1856) in Latin from a single male collected in "Van Diemen's Land" (i.e. Tasmania), Australia. This brief description was translated into English by Skuse (1889), who does not appear to have re-examined the type. The translation reads:

"Pale testaceous, green while living. Antennae brown. Thorax with three reddish stripes. Abdomen pubescent, green, with brown band on each segment. Legs pale green, long, slender, pubescent; tarsi brown towards the tip; fore tibia very much longer than the fore metatarsus. Wings limpid; veins whitish; discal transverse vein brown. Length of body 3 1/2 lines; of the wings 5 lines. *Hab.* – "Van Diemen's Land."

Freeman (1961) examined the type and considered it to be a synonym of *Chironomus alternans* Walker (1856). However rearing of specimens from larvae indicated that there were morphological differences as well as differences in the banding patterns of the larval polytene chromosomes (Martin 1969, 1971) indicating that there were several species included in Freeman's grouping of *C. alternans.* These could not be separated on the basis of existing descriptions particularly since the types of a number of the species were no longer available. However the type of *C. oppositus* was in the British Museum Natural Sciences (BMNS) who were kind enough to send it to me for further examination. I was able to record a more detailed description and take photographs and draw the terminalia. This more detailed description is given below because the type cannot be located in the BMNS and is assumed to have been lost on its return journey.

Material & Methods

The type male of *Chironomus oppositus* Walker, 1856 was obtained on loan from the Natural History Museum London (previously the British Museum Natural Sciences (BMNS)). The specimen was examined, details recorded, and reference photographs taken. These latter proved unsatisfactory for publication, but the details of the hypopygium were drawn using a WILD drawing tube. Unfortunately it appears that the specimen was lost in the mail on the return trip to the Museum. These measurements and figures are hence the only detailed record of the morphology of this specimen.

Terminology is according to Sæther 1980.

Results

Coloration: Antennae and palps brown; thorax greenish, stripes, sternopleuron and pronotum brown, halteres greenish. Abdomen greenish with saddle spots. Legs greenish, only hind tarsi present and darkened.

Thoracic setae: Acrostichal not obvious; dorsocentral about 10+; prealar 6, 7; supraalar 1; scutellum with a posterior row of about 10 setae, anteriorly with about 4 setae.

Wing with anterior veins hardly darker than posterior, crossvein only slightly darkened. R4+5 ends slightly proximal to M, at 0.08 of the distance between Cu1 and M. R2+3 evanescent at tip, ending 0.34 of the distance between the apex of R1 and R4+5. Anal ends distal to f-CU, at 0.61 of the distance between f-Cu and apex of Cu2. Anal lobe well developed, right angled.

Wing length 4.56 mm, width 0.96 mm; 4 SCf on stem vein, squama fully fringed..

Legs: Many tarsi missing, only one of the hind legs complete, so only the hind LR able to be calculated;

Available leg lengths (mm) and proportions in Table 1.

Table 1. Leg segment lengths and proportions.

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	F/T
PI	1580	1420	-	-	-	-	-	-	1.11
PII	1660	1560	-	-	-	-	-	-	1.06
PIII	1920	2020	1420	760	600	380	200	0.70	0.95

Abdominal segment TIX and hypopygium (Fig. 1): about 6 setae in individual pale areas near centre of TIX. SVo closest to D(e) of Strenzke (1959). IVo reaching only about half way along the anal point, with simple setae. Anal point long and slightly broadened at distal end, reaching about 2/3 of length of the gonostylus which is moderately broadened and narrows relatively gently over distal third.

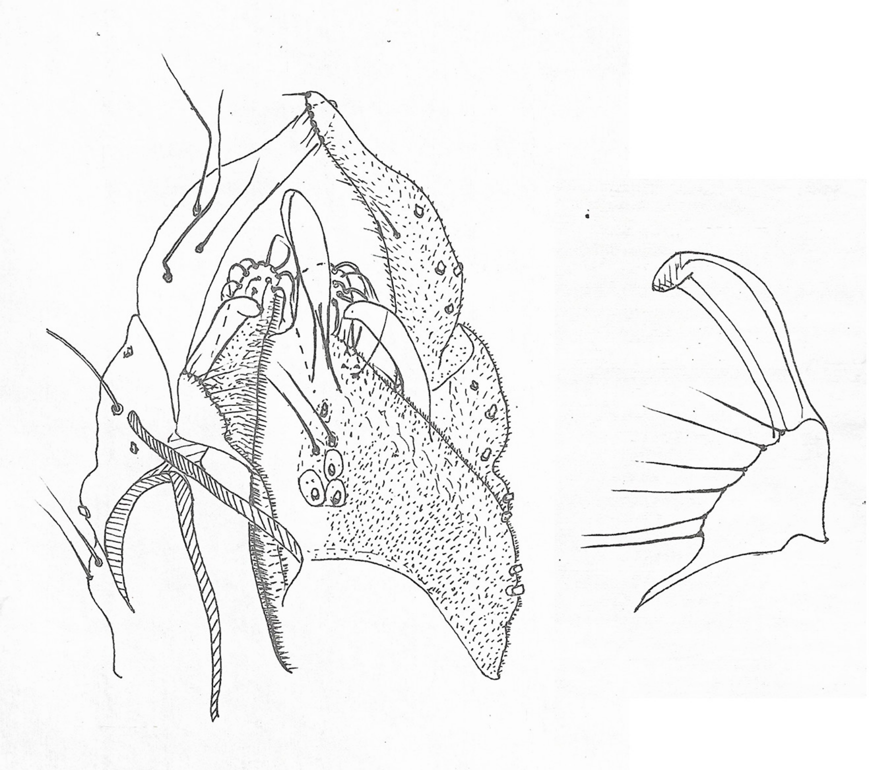

Figure 1. Male terminalia of *C. oppositus* type specimen. General view (left), superior volsella (right).

Discussion

This more extensive description of the holotype would have little value unless it can be correctly attributed to a currently recognised species. In this regard, the fact that the specimen comes from Tasmania is useful, as the Tasmanian fauna is reduced compared to the mainland fauna. Martin and Lee (1984) recognized four forms of *C. oppositus* (*f. oppositus, f. connori, f. whitei,* and *f. tyleri* (now considered a separate species)) mainly on the basis of the banding patterns of the larval polytene chromosomes, and a fifth form occurs in Western Australia. These forms were further defined by Martin (2011) and a further related species, *C.* 'tasmaniensis' with only three polytene chromosomes, is known from a single Tasmanian larva – so can be excluded from this discussion of the adult males.

All forms of *C. oppositus* are recorded from Tasmania but only forms *oppositus* and *connori* are common. Tasmanian researchers (Prof. Bill Jackson and Dr. Peter Tyler, both deceased) believed that Walker collected in the vicinity of the large central lakes, and only one form has been collected in that region, wing length range of 3.68-4.58 mm which covers the type specimen, so it has been designated as 'form oppositus'. This form tends to be more common in Tasmanian lakes (Martin and Lee (1981).

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