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Phaenopsectra flavipes with passengers. Photo: Aina Mærk Aspaas, NTNU University Museum

CHIRONOMUS Journal of Chironomidae Research

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Would you like to see your picture on the front page? Please send us your favourite midge photograph or drawing (*torbjorn.ekrem@ntnu.no*).

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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: *Phaenopsectra flavipes* with water mite larvae. Photo: Aina Mærk Aspaas, NTNU University Museum.

Editorial

We need chironomid symposia!

Time flies and another year has passed. This time, however, not without a Chironomidae Symposium! Many of us were looking forward to finally meet in Tsukuba last summer, maybe saving up a little to see some sights while visiting Japan. Unfortunately, a physical meeting became impossible due to travel restrictions, and the organizers faced a difficult choice: another delay or a digital conference?

I am glad the choice was to hold the symposium despite the many drawbacks of not meeting physically. It was good to see colleagues again (even if only on a screen) and to hear about exciting new research on Chironomidae. We need a venue to present and discuss our science, and if we can't meet in person, then online certainly is a decent alternative!

The symposium program was as diverse as it always is at our meetings, ranging from descriptions of new taxa, fossils and molecular systematics to ecology, physiology, genomics and the use or artificial intelligence to identify chironomid larva. The Honorary Thienemann Lecture was held by Valeria Lencioni on the topic of chironomids' responses to climate change, where she presented evidence of cold-, heat- and chemical tolerance in cold-adapted species. This was an interesting presentation, summarizing results and achievements in the field, with focus on work done by Lencioni and co-workers in the Italian Alps.

The symposium took the time to remember the chironomid workers that had passed since the last meeting. Two of them were very active participants in the symposia for decades, and Len Ferrington and Paddy Ashe were thoroughly missed and honored in several presentations. Read more about their achievements in this and last years' issue of CHIRONOMUS (Bouchard et al. 2021; Murray 2022).

The organizers did a fantastic job for the conference to run smoothly and there were few technical hiccups. With a program adjusted to the many different time zones of the participants, it was also possible for us on the other side of the planet to follow most of the meeting without overturning our days. Congratulations to Richard Cornette, Sachiko Shimura, Takahiro Kikawada, Kimio Hirabayashi, Natsuko Kondo and Kenzi Takamura from the Organizing Committee for a well-delivered symposium and wonderful job in keeping the community together. We look forward to the 22nd Chironomidae Symposium at the University of Niš, Serbia already in 2024, this time hopefully in person.

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PS! All presenters are invited to submit manuscripts to the Symposium Proceedings that will be published in this very journal with guest editors from the conference organizing committee. Contributions to the Special Issue can be submitted at any time through our website before the deadline on February 15, 2023.

References

Bouchard, R., Kranzfelder, P., and Anderson, A. 2021. Leonard C. Ferrington, Jr. (1948-2021): Chironomid cognoscente and modern-day Renaissance man. CHIRONOMUS Journal of Chironomidae Research 34: 51-66. https://doi.org/10.5324/cjcr.v0i34.4598

Murray, D.A. 2022. Patrick (Paddy) Ashe 18.03.1954 – 19.06.2022. - CHIRONOMUS Journal of Chironomidae Research 35: 60-62. https://doi.org/10.5324/cjcr.v0i35.5034

A HOME AT LAST! *CHANGANIA CHOUI* TSENG, 1965 BELONGS TO *THIENEMANNIELLA* KIEFFER, 1911 (DIPTERA: CHIRONOMIDAE: ORTHOCLADIINAE)

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Abstract

The midge *Changania choui* Tseng, 1965 (Insecta, Diptera, Nematocera), originally described in the family Cecidomyiidae and recently transferred to the Ceratopogonidae, is recognised as an adult female in the Chironomidae, subfamily Orthocladiinae. The type material is missing, and the published description and illustrations are limited. Although the genus name *Changania* Tseng, 1965 becomes a new junior synonym of *Thienemanniella* Kieffer, 1911, *Thienemanniella choui* (Tseng 1965), new combination, should be treated as a *nomen dubium*. A combination of two ratios calculated from wing measurements shows promise for taxonomic diagnostics in the grouping of genera around *Corynoneura* Winnertz.

Introduction

In a book on gall midges and other insects found as pests or visitors of wheat in China, Tseng (1965) described, figured and discussed *Changania choui* Tseng as a new genus and species in Cecidomyiidae (Diptera: Nematocera).

Tseng, Sheng [曾省; alternative transliterations: Ceng, Sheng or Zeng, Sheng] (1899-1968) was an agricultural entomologist who had received academic training in China and France; in 1957 he was transferred to Beijing to serve as a plant protection researcher at the Chinese Academy of Agricultural Sciences. Chou, Io [周尧; alternative transliteration: Zhou, Yao] (1912-2008) studied entomology in China and Italy, then founded one of the earliest entomological collections in his home country, where he became quite influential in both science and society.

The names *Changania* and *C. choui* have been mentioned very rarely in the literature. Eitschberger (1999: 362) incompletely referred to a 68-page book published in China (in the same year?) as "Six decades of glorious flowers in spring and solid fruits in autumn - In honour of the sixtieth anniversary of teaching activities of Prof. Chou Io". We have not seen this volume, but it reportedly includes a list of 43 patronyms for taxa named in Chou's honour. Jiao and Bu (2014) listed *Changania choui* in Cecidomyiidae, but indicated the identification as doubtful and added that the genus "may belong to Ceratopogonidae ... (Dr. Mathias Jaschhof, personal communication)" (*op. cit.*: 203). Gagné and Jaschhof (2021: 620) then excluded *Changania* from the Cecidomyiidae and suggested placement in Ceratopogonidae.

The latter re-assignment was questioned when one of us (AB) prepared updates and errata to a world catalog of the Ceratopogonidae (Borkent and Dominiak 2020). In September of 2021, a copy of Tseng's original description and figure was sent to PSC by AB accompanied by a suggestion that the species might be an orthoclad Chironomidae instead. Subsequent consultations soon led all present coauthors to concur that the taxon belongs to the grouping that includes *Corynoneura* Winnertz, 1846 and other genera. Consequently, we decided to settle *Changania* Tseng in its appropriate systematic home.

Material and methods

The sequence of present coauthors' names is in alphabetical order, except for the first author (designated by the others). It does not rank the respective individual contributions.

Upon our requests, material of *Changania choui* Tseng has been searched for at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing (the depository declared by Tseng 1965: 147), and at Northwestern A&F University, Xianyang (collection founded by Chou). Since

neither Tseng's slide nor any other specimen has been found, we interpret *Changania* and *C. choui* from their original presentation (Tseng 1965: 147-148).

Because the holotype's allocation to sex and the taxon's placement in contemporary systematics relies on Tseng's illustration (1965: fig 46; our Fig. 1D), we photographed (Fig. 1A-C) and/or measured representative female wings from the following material (all from China, in coll. H.-Q. Tang).

Corynoneura arctica Kieffer, 1923. 2♂, 2♀: Inner Mongolia, Hulunbuir City, Hailar River, 49°9.483', 119°45.150'; 31.vii.2016, leg. Feng, L.-H. 1♂, 1♀: Tibet, Lhasa City, Lalu wetland, 29°40.020', 91°05.870'; 05.vii.2014, leg. Liu, J.

Corynoneura medicina Fu, Sæther & Wang, 2009. 3♂, 5♀: Yunnan Prov., Yiliang County, Yangzong Town, Yangzong Lake wetland, 24°51.451', 103°0.073'; 15.vi.2016, leg. Tang, H.-Q.

Corynoneura yoshimurai Tokunaga, 1936. 1♂, 1♀: Guangdong Prov., Guangzhou City, Conghua District, Wenquan Town, 23°37.509', 113°38.107'; 16.i.2011, leg. Tang, H.-Q.

Onconeura togamijika (Sasa & Okazawa, 1992) [see Note 1]. 3♂, 3♀: Tibet, Medog County, Beibeng Town, the Third Bridge to Hanmi, 29°14.957', 95°08.762'; 11.viii.2015, leg. Tang, H.-Q.

Thienemanniella curva Fu, Fang & Wang, 2013 [see Note 2]. 1♂, 1♀: Guangdong Prov., Guangzhou City, Conghua District, Lyutian Town, Guifeng Mt., 23°48.036', 114°00.987'; 28.iii.2016, leg. Li, L.-M.

Thienemanniella majuscula (Edwards, 1924). 2♂, 4♀: Guangdong Prov., Guangzhou City, Conghua District, Liangkou Town, 23°43.087', 113°43.156'; 28.iii.2016, leg. Li, L.-M.

Notes

(1) *Onconeura togamijika* was described originally in *Thienemanniella*, but regarded as a new combination and first East Asian record of *Onconeura* Andersen & Sæther, 2005 by Li (2018), whose suggestion we follow here. (2) In the original publication (Fu *et al.* 2013) the species name was spelled in two ways, *Th.* 'curva' and *Th.* 'curvare', but in Fu *et al.* (2020) the original authors have fixed *Thienemanniella curva* as the correct spelling; see ICZN (1999) Article 24.2.4.

Identification

The species identifications of female specimens are based on respectively corresponding adult males linked by molecular sequences and/or by co-occurrence in the same sample; see Table 1. The BOLD data can be accessed as a dataset via http://dx.doi.org/10.5883/DS-CORY001.

Results

Original publication

Tseng (1965: 147-148) treated *Changania choui* as the fourth of five numbered taxa diagnosed in an 'Appendix. Sap-sucking insects similar to gall midges' (*op. cit.*: 137), subsection '(5) Examples of adults' (*op. cit.*: 144). All other taxa in this subsection continue to be considered as members of the Cecidomyiidae (*e.g.*, Jiao and Bu 2014). Likewise, all genus names other than *Changania* in

Table 1. References to individual molecular sequence data connected to the present study. Numbering of female specimens as in Tables 2-4.

Taxon	Specimen	BOLD Process ID	BOLD barcode index number (BIN)	GenBank accession number
Corynoneura arctica	female 3	JNU051-18	AAB0079	OM502160
Corynoneura arctica	male	JNU121-18	AAB0079	OM502166
Corynoneura medicina	female 5	JNU007-18	ADL1874	OM502158
Corynoneura medicina	male	JNU006-18	ADL1874	OM502159
Corynoneura yoshimurai	female	—	—	—
Corynoneura yoshimurai	male	JNU031-18	ADL1776	OM502163
Onconeura togamijika	female 1	JNU026-18	ADL0738	OM502165
Onconeura togamijika	male	JNU055-18	ADL0738	OM502162
Thienemanniella curva	female	—	—	—
Thienemanniella curva	male	JNU104-18	ADL1365	OM502161
Thienemanniella majuscula	female 1	JNU130-18	ADL1673	OM502167
Thienemanniella majuscula	male	JNU001-18	ADL1673	OM502164

Tseng's text as translated below refer to members of Cecidomyiidae (for details and current subfamily assignments see Gagné and Jaschhof 2021). The name "Memmieria" does not exist in zoological nomenclature. We interpret it as a lapsus for *Meunieria* Kieffer, which was still treated in Heteropezinae in all keys (Felt 1925, 1929; Mani 1946) referred to by Tseng (1965: 137).

The following translations attempt to stay as close to the Chinese texts as feasible, wherever this is considered as potentially critical. All terms given between square brackets are interpretations or comments inserted by the present authors.

4. Chang'an Chou Gall Midge (*Changania* Choui, Tseng)*[parenthesis with Latin lettering exactly as reproduced here]*. Among plenty of gall midge specimens kindly donated by professor Io Chou [Yao Zhou], Northwest Agricultural College, there is one (labelled Cec. 028) that is extremely small (body size in the slide mount 0.95 × 0.35 mm), the distribution of wing veins is very special, much as in *Leptosyna*, but the tarsus has 5 segments, the first segment is longer than the second, and the palp has 5 segments, different from *Epimyia* (palp with 3 segments), *Frirenia* (palp with 2 segments), *Lyptosyna[sic!, typographical error for Leptosyna]* (palp with 1 segment) and *Meinertomyia* (palp with 3 segments) in the subfamily Heteropezinae, and it is also different from *Neostenoptera*, *Memmieria[sic!, see the comment above this translation]*, *Miastor* and other genera. The third vein [R_{4+5}] does not reach the tip of the wing, the palp is with 5 segments, the wing carries spinules, hence a new genus and species is established, and the name Chang'an Chou Gall Midge (*Changania* Choui, Tseng, Gen. et Sp. Nov.) expresses the great pleasure and gratitude to Io Chou for his discoveries in Chang'an. The specimen is deposited in the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing. Its characteristics are as follows:

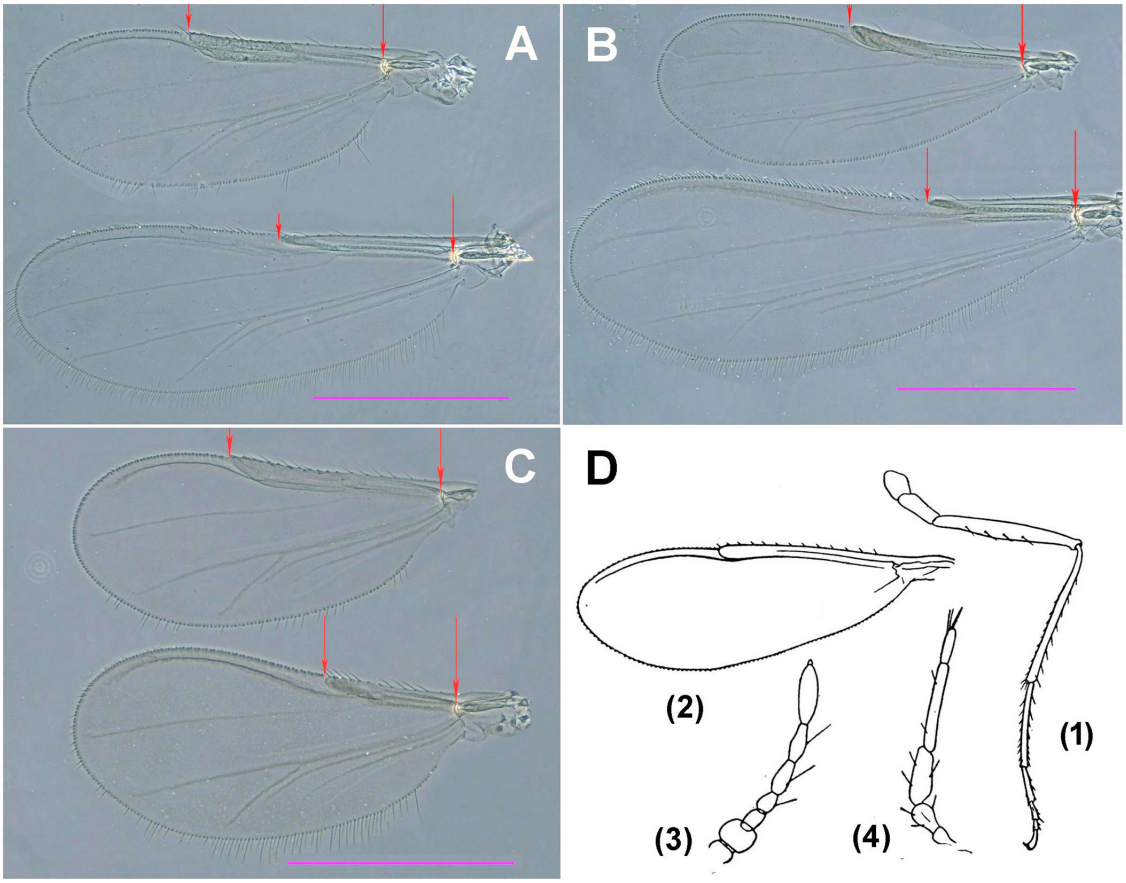

Figure 1. A-C. Photographs (by Tang, H.-Q.) of wings in the *Corynoneura* group (respective upper wing: female; lower: male; red arrows mark extent of clavus; scales 400 µm); for specimen data see Material and methods, and Table 2. D. Line-drawings modified from Tseng (1965). A. *Thienemanniella majuscula* (Edwards, 1924); B. *Corynoneura medicina* Fu, Sæther & Wang, 2009; C. *Onconeura togamijika* (Sasa & Okazawa, 1992); D. *Changania choui* Tseng, 1965. (1) leg, (2) wing, (3) antenna, (4) maxillary palp (caption texts translated from original fig. 46; subfigure positions re-arranged).

Tarsus with 5 segments, first segment longer than the other segments. Antennae without ring hairs [i.e., without distinct whorls of elongate setae], whole wing with only three longitudinal veins, and the third vein [R_{4+5}] is very close to the front edge (the fourth and fifth veins [M and Cu] are completely reduced, sometimes only remnant traces are vaguely seen), there are no transverse veins [RM, MCu], no scaly hairs on the wing surface, and the sparse clothing of spinules is more pronounced on the wing edge. The third longitudinal vein [R_{4+5}] ends before reaching the wing tip, but a small branch at about mid-length [of R_{4+5}] connects to the front edge, enclosing a long, narrow space (Cell)*[parenthesis printed in Latin letters]* beyond [that branch], which is dark brown in colour, different from its surroundings. The submarginal vein [subcosta] is very thin, extends between the anterior marginal vein [costa] and the third vein [R_{4+5}], and reaches only 1/3 of the length of the marginal vein. The palp is with 5 distinct segments. The antenna is 7-segmented, the second segment [pedicel] is enlarged and oblate, the remaining segments are oblong, the terminal segment is particularly elongate and with a flattened rod-shaped apex (fig. 46). *[end of translation]*

Morphological comparisons

The short, thickened and fused anterior wing veins (including R_{4+5}) form a 'clavus' (Fig. 1). This characteristic morphology implies a member of a grouping of genera in the subfamily Orthocladiinae that is represented in the known fauna of China by *Corynoneura* Winnertz, *Onconeura* Andersen & Sæther, 2005, and *Thienemanniella* Kieffer, 1911. A relative minority of published chironomid systems have allocated this grouping to a separate tribe, Corynoneurini, with support from molecular phylogenetic evidence (Cranston *et al*. 2011). At this time, however, the present authors consider tribal allocation amongst the diverse Orthocladiinae as unwarranted.

Two sources point to the holotype of *Changania choui* being female. The adult antenna described and figured by Tseng (1965: fig. 46, 3; our Fig. 1D, bottom left) comprises scape, pedicel and five flagellomeres, and although some few male orthoclads have female-like antennae (more commonly in some harsh environments, *e.g.* in marine and alpine fauna), in the grouping under consideration the male antenna comprises from 9 to 12 flagellomeres, and their structure differs from the female antennae.

Table 2. Wing lengths and proportions for females in the *Corynoneura* group sampled in China. Specimens (numbered within each species) in alphabetical order of genus and species names.

Taxon	Wing length [µm] (arculus to tip)	Wing width / wing length	Clavus length / wing length
Changania choui (Fig. 1D)	unknown	0.40	0.56
Corynoneura arctica 1, Inner Mongolia	1100	0.40	0.47
Corynoneura arctica 2, Inner Mongolia	1125	0.42	0.48
Corynoneura arctica 3, Tibet	1180	0.43	0.47
Corynoneura medicina 1	730	0.41	0.48
Corynoneura medicina 2	780	0.38	0.47
Corynoneura medicina 3	800	0.36	0.49
Corynoneura medicina 4	810	0.38	0.47
Corynoneura medicina 5 (Fig. 1B)	820	0.41	0.49
Corynoneura yoshimurai	720	0.40	0.42
Onconeura togamijika 1 (Fig. 1C)	630	0.46	0.57
Onconeura togamijika 2	700	0.46	0.56
Onconeura togamijika 3	850	0.47	0.59
Thienemanniella curva	750	0.44	0.59
Thienemanniella majuscula 1 (Fig. 1A)	800	0.44	0.55
Thienemanniella majuscula 2	800	0.43	0.55
Thienemanniella majuscula 3	930	0.43	0.54
Thienemanniella majuscula 4	940	0.41	0.55

A comparison with females from six species in the *Corynoneura* grouping of genera that are known and sampled from China allows further separation by reference to two ratios calculated from individual wing measurements (Table 2, columns 3 and 4 from left).

To test those ratios for possible dependence on adult body size, the same specimens are ranked by increasing wing length (second column from left) in Table 3. Column 1 shows some of the species sorted out near either end of the total size range in our sample, and others with their ranges overlapping. The ratio values in columns 3 and 4, respectively, evidently are not correlated to wing length.

However, when the table rows are reordered according to the ratios calculated from wing measurements (Table 4), a pattern independent of body size emerges that allows inference to be drawn on systematic relations at genus level.

Systematic deduction

As evident from Table 4, the relatively high clavus/wing length ratio shown by *Changania* rules out genus identity with *Corynoneura*, and the low relative wing width eliminates *Onconeura*. Consequently, we identify *Changania choui* as a member of *Thienemanniella*; for further explanation see the discussion below.

Taxonomic placements

Thienemanniella Kieffer, 1911
[for details see Ashe (1983)]
Changania Tseng, 1965: 147, ***syn. nov.***
Type species (by monotypy): *Changania choui* Tseng, 1965.

Thienemanniella choui (Tseng, 1965), ***comb. nov.***, *nomen dubium*.
Changania choui Tseng, 1965: 147, fig. 46.

Type material: Holotype female, on slide labelled 'Cec. 028', ex coll. I. Chou. – Although Tseng's (1965) text on the species does not include a term such as holotype, he did mention seeing one specimen only. His very detailed instructions on methods to study such midges (*op. cit.*: 137-139) may suggest that he had made the holotype slide himself, but he did not state so explicitly.

Type locality: CHINA, Shaanxi Province, Chang'an County (now Chang'an District, Xi'an City).

Table 3. Female specimens and their wing data from Table 2, resorted by ascending wing length, then (where necessary) alphabetically by taxon name.

Taxon	Wing length [µm] (arculus to tip)	Wing width / wing length	Clavus length / wing length
Changania choui (Fig. 1D)	unknown	0.40	0.56
Onconeura togamijika 1 (Fig. 1C)	630	0.46	0.57
Onconeura togamijika 2	700	0.46	0.56
Corynoneura yoshimurai	720	0.40	0.42
Corynoneura medicina 1	730	0.41	0.48
Thienemanniella curva	750	0.44	0.59
Corynoneura medicina 2	780	0.38	0.47
Corynoneura medicina 3	800	0.36	0.49
Thienemanniella majuscula 1 (Fig. 1A)	800	0.44	0.55
Thienemanniella majuscula 2	800	0.43	0.55
Corynoneura medicina 4	810	0.38	0.47
Corynoneura medicina 5 (Fig. 1B)	820	0.41	0.49
Onconeura togamijika 3	850	0.47	0.59
Thienemanniella majuscula 3	930	0.43	0.54
Thienemanniella majuscula 4	940	0.41	0.55
Corynoneura arctica 1, Inner Mongolia	1100	0.40	0.47
Corynoneura arctica 2, Inner Mongolia	1125	0.42	0.48
Corynoneura arctica 3, Tibet	1180	0.43	0.47

Table 4. Female specimens and their wing data from Table 2, resorted by descending relative clavus length, then (where necessary) by descending relative wing width.

Taxon	Wing length [µm] (arculus to tip)	Wing width / wing length	Clavus length / wing length
Onconeura togamijika 3	850	0.47	0.59
Thienemanniella curva	750	0.44	0.59
Onconeura togamijika 1 (Fig. 1C)	630	0.46	0.57
Onconeura togamijika 2	700	0.46	0.56
Changania choui (Fig. 1D)	unknown	0.40	0.56
Thienemanniella majuscula 1 (Fig. 1A)	800	0.44	0.55
Thienemanniella majuscula 2	800	0.43	0.55
Thienemanniella majuscula 4	940	0.41	0.55
Thienemanniella majuscula 3	930	0.43	0.54
Corynoneura medicina 5 (Fig. 1B)	820	0.41	0.49
Corynoneura medicina 3	800	0.36	0.49
Corynoneura arctica 2, Inner Mongolia	1125	0.42	0.48
Corynoneura medicina 1	730	0.41	0.48
Corynoneura arctica 3, Tibet	1180	0.43	0.47
Corynoneura arctica 1, Inner Mongolia	1100	0.40	0.47
Corynoneura medicina 4	810	0.38	0.47
Corynoneura medicina 2	780	0.38	0.47
Corynoneura yoshimurai	720	0.40	0.42

Discussion

Separation of *Thienemanniella* from *Corynoneura* can be made readily on the immature stages, although confident separation of adults has declined since Edwards' (1924, 1929) studies. In the following, we recall three pertinent statements in Edwards (1929: 365-367):

(1) Referring to *Corynoneura* in the broader sense, which included *C.* s. str. and *Thienemanniella* as subgenera: "R_1 and R_{4+5} entirely fused with one another and almost entirely so with the thickened costa, forming a "clavus" which extends less than half the wing-length in ♂ and about one-half to two-thirds of the wing-length in ♀; in the latter the clavus is thicker" (p. 365-366);

(2) In the diagnosis for *C.* (*Thienemanniella*): "Hind tibiae not swollen and without apical projection on inner side. Front trochanters keeled but evenly rounded above. Costa extending to about two-fifths of wing-length and nearly to FCu in ♂, beyond middle of wing and beyond FCu in ♀" (p. 366);

(3) In contrast, the diagnosis for *C.* (*Corynoneura*) begins: "Hind tibiae somewhat swollen at tip, obliquely truncate and with a conspicuous apical projection on inner side. Front trochanters with a more or less conspicuous flat dorsal expansion on apical half or more. Costa extending from scarcely one-third to about two-fifths of wing-length and ending far before FCu in ♂; to about middle of wing and not quite to FCu in ♀"(p. 367).

Tseng's (1965) low magnification illustration of the wing (see our Fig. 1D) lacks the faint posterior venation that requires good microscopy, yet together with his description is passable for comparison with Edwards' (1929) morphological differentiation of *Corynoneura* and *Thienemanniella*. Thus, the sexual dimorphism in wing shape, and the clavus strength and termination relative to the wing length (Fig. 1, quantified in Tables 2-4) conform best to a female of *Thienemanniella*.

Tseng (1965) did not specify which particular leg he described and illustrated, but the combination of unmodified tibial apex and relatively long trochanter (Fig. 1D) suggests a foreleg. The trochanter, shown lacking a keel, does not match expectations for this structure in a female of *Corynoneura*. With no description or illustrations of a hind leg, the posterior wing venation or the extent and length of microtrichia surrounding the facets of the eye, other potentially discriminatory features are unavailable. Few female adults in *Thienemanniella* or *Corynoneura* have been reported since Edwards

(1929). However, *Onconeura*, described from the Neotropics by Andersen and Sæther (2005), is now recognised as present in East Asia (Li 2018). The female wing in *Onconeura* is proportionally wider than many others in the genus grouping (Fig 1C and Table 4), significantly differing from the narrower *Changania* wing; thus, *Onconeura* can be eliminated from the present consideration. In addition, although the distinctiveness of adults of *Corynoneura* has been weakened especially due to recent Neotropical material (Wiedenbrug *et al.* 2013), *Changania* cannot be allocated to this genus either. The balance of available evidence indicates that *Changania* Tseng is congeneric with *Thienemanniella*, the latter now ranked as a genus (*e.g.*, Ashe 1983).

The species *Changania choui* Tseng from Shaanxi, China, cannot be associated with any named species of *Thienemanniella* from Asia (Makarchenko and Makarchenko 2006, Fu *et al.* 2010, Fu *et al.* 2013, Fu *et al.* 2020, Fang *et al.* 2021). Even if the material described by Tseng (1965) had included a male, it is unlikely that this could have improved the situation. With over 55 species globally, diversity is high and reared specimens clearly aid in discrimination (*e.g.*, Wiedenbrug *et al.* 2013). However, there is no information on immature stages for any of the species recently described from China. Thus, contemporary species discrimination still relies on features of the adult male such as ratios of flagellomeres, and subtle details of shapes in the hypopygium (genitalia), including the gonostylus and genitalic lobes (*e.g.*, Fu and Sæther 2012). Although these character states might not have been discernible to Tseng anyway, the female features discussed above evidently do locate *C. choui* within *Thienemanniella*. However, the missing holotype and inability to allocate to a described species mean that *C. choui* should be treated as a *nomen dubium*.

A larger-scale revision of morphology in the *Corynoneura* grouping of genera was beyond the scope of the present work, which focused on adult female specimens from China that were readily available and identifiable to species via reasonably sufficient links to respective males. Nevertheless, the sampling includes material from the Palaearctic and Oriental regions, and from lower and higher elevations. Thus, the authors hope that the wing features applied as taxonomic criteria here will prove useful also in future studies on such chironomids.

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Verifying Australian *Nilotanypus* Kieffer (Chironomidae) in a global perspective: molecular phylogenetic and temporal analyses, new species and emended generic diagnoses

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Abstract

Molecular data support two distinct species of *Nilotanypus* Kieffer (Chironomidae: Tanypodinae) in Australia, able to be differentiated on morphology in all stages. These are described as *Nilotanypus haplochelus* new species and *Nilotanypus ctenochelus* new species respectively. Morphological differentiation is clearer in the larva and pupa, with the adults less distinguishable, as seems typical in this genus. Both species are distributed widely across the Australian continent, yet seemingly absent from offshore islands and Tasmania. Lotic psammophily (sand-dwelling) is evident, with micro-sympatry at some tropical / subtropical locations. Addition of molecular data from non-Australian taxa shows that *N. ctenochelus* is sister to all other sampled in-group taxa, with *N. haplochelus* distant as sister to an undescribed species from oriental China. Review also of non-Australian species in all known stages requires modest revision of generic diagnoses, and, critically, recognition of *Pentaneura comata* Freeman, 1953 as synonymous with *Nilotanypus remotissimus* Kieffer, 1923 (new synonym), the type of the genus.

Introduction

In chironomid nomenclature the prefix *Nilo-* refers to the Sudanese White Nile where collections were made in European colonial times. Four genera named with this root belong to the tribe Chironomini, of which *Nilodosis* Kieffer, 1921 and *Nilothauma* Kieffer, 1921, are currently in use. Also based on this prefix is *Nilotanypus* Kieffer, 1923, named a century ago, in the subfamily Tanypodinae. Adult midges were collected on the Bahr al Jebel (White Nile), at Mongala (sic) = Mongalla, now in South Sudan. The type species *Nilotanypus remotissimus* Kieffer, 1923 is lost, but the description allowed Freeman to understand the taxon when describing *Pentaneura comata* Freeman, 1953 (elaborated in 1955) from southern Africa. Subsequently, Lehmann (1979) described the pupa from Zaire (as *Nilotanypus comatus*) and later Harrison (1991) included an associated larva from Ethiopia, and a linked female adult from Zimbabwe to the species concept.

Currently, 11 species are recorded and named worldwide, including two each from the Palaearctic, Nearctic, Afrotropical and Oriental regions, plus three species recently added from the Neotropics (Anderson & Pinho 2019, Shimbakuro *et al*. 2021). This is an underestimate, given barcoding DNA evidence of several cryptic Holarctic species, and two species described here as new from Australia.

Diagnoses of male and female adults, based on diminutive size, pubescent eye, and the foreshortened radial sector of the wing with vein R_{2+3} essentially absent, remain correct to this day. This robust concept allowed recognition of additional adult-based congeners and incorporation of immature stages (Fittkau 1962, Kownacki & Kownacka 1968, Fittkau & Roback 1983, Fittkau & Murray 1986, Roback 1986). Immature stages alone allowed recognition of diversity in Nepal (Roback & Coffman 1987) and southern India (Roback & Coffman 1989), although the taxa remained unnamed.

Nilotanypus was found first in Australia in seasonal monsoonal tropical streams in the Northern Territory and was discovered subsequently to be widespread across the mainland of the continent (Cranston 1996). Due to inadequate life history associations, the inferred presence of two species was not followed up at that time.

A survey of Australian Tanypodinae integrating morphological with molecular data (Krosch *et al*. 2017, Krosch *et al*. 2022), now with increased representation, confirms the two species of Australian *Nilotanypus*. Reconciliation with morphology allows description here of each species as new to science, assessed as endemic to Australia by wide regional comparisons.

Methods and materials

We used many collection techniques over the project duration (>40 years), including kick sampling and micro-sieving from repeatedly stirred sandy substrates and by interception of drift in flowing waters with 250–300 µm mesh nets. By intercepting drift, we sought immature stages including pharate adults. Light traps were used for adults at some locations. By preference a binocular microscope was used for initial field sorting. Specimens destined for DNA extraction and sequencing were isolated and preserved in 95–100% isopropanol. Following the rationale of Cranston *et al*. (2012), collections for greatest geographic and taxonomic diversity and recovery of DNA often were of larvae subsequently vouchered by their head capsules and posterior abdomen. Using non-destructive DNA extraction (Krosch & Cranston 2012), carcasses were retained for permanent vouchering on microscope slides using Euparal or occasionally Hoyer's mountant that clears well and from which vouchers can be remounted for permanence. Molecular vouchers (MV) are coded as in Table 1 and are preserved on slides in the Australian National Insect Collection, CSIRO, Canberra, Australia (ANIC). In addition to Australian material, we examined: (a) pharate material and pupal exuviae of *Nilotanypus comatus* (Freeman) from near the type-locality in the south-west of Western Cape Province, South Africa; (b) similar material from Belalong River, Brunei; and (c) males and immature stages from several localities in Palaearctic and Oriental China. On our behalf, Martin Spies examined Australian pharate material, pupal exuviae and a larva in the Zoologische Staatssammlung, Munich.

Morphological terminology largely follows Sæther (1980) with minor additions and emendations for larvae implemented by Cranston (2012) incorporating Kowalyk's (1985) valuable insights into the taxonomic value of the larval cephalic setation (Rieradevall & Brooks 2001). We prefer the terminology of Silva & Ferrington (2018) regarding the lumen of the thoracic horn as containing a respiratory atrium, without differentiating a horn sac from the horn chamber; thus, the atrium is treated as everything internally between the spiracle and the plastron (or in *Nilotanypus*, the aeropyle). We follow Roback (1986) in treating the distal ovoid structure of the horn apex in *Nilotanypus* as the corona (with small aeropyle) lacking any microsieve plastron. A row of tubercles on the pupal distal wing sheath of one species appears non-homologous with the 'pearl row' of Sæther (1980): we do not use the term pearl row here. In the adult male we use proctiger for the lobe posterior to tergite IX (Crampton 1942, van Emden & Hennig 1970), rather than 'anal point' which is best applied to a distinct projection on the dorsal tergal surface. The temporal setae form a curved uniserial row weakly segregated into inner and outer verticals. Furthermore, the substantial dorso–medial extension of the eye displaces some median setae to align dorsal – ventral, near the coronal suture and angled dorsally with the inner temporals. These correspond either to frontals (associated with the frons) or oculars (more associated with the eye): given their location, the term frontals is used as labelled, abbreviated as 'fr', in Fig. 1B. These setae can be sexually dimorphic and are easily damaged or lost; when intact the strength, relative length and number of these setae are potentially informative (Fig. 1A-D).

Extraction of DNA, PCR amplification, sequencing and analyses followed protocols of Cranston *et al*. (2012) and Krosch & Cranston (2012), using standard markers (*COI*, *28S*, *CAD* - Krosch & Cranston 2013; Krosch *et al*. 2011, 2015) and others derive from GenBank (Table 1). In total, sequence data was included for 23 *Nilotanypus* specimens from at least one locus and the concatenated multilocus alignment comprised 3427 nucleotides.

Sequences were concatenated and each locus partitioned individually. Phylogenies were inferred for single locus datasets and for a concatenated partitioned dataset. Bayesian phylogenetic inference was performed in MrBayes ver. 3.2.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), with the GTR model of sequence evolution applied to each partition individually and a gamma distribution of nucleotide frequencies incorporated. Runs were performed for 5 million generations and sampled every 1000 generations, with 25% of total samples removed as burn-in. Maximum likelihood (1000 bootstraps) reconstruction was performed using RAXML ver. 8.0.24 (Stamatakis 2006) under the GTRGAMMA model of sequence evolution. All analyses were conducted on the CIPRES Science Gateway High Performance Computing platform (http://www.phylo.org; Miller *et al*. 2010).

Table 1.

Species	Molecular voucher	Life stage	Country	Location	Collector	Genbank Accession				
						5P COI	3P COI	28S	CADI	CADIV
Nilotanypus dubius	Finnmark209	f	Norway	Finnmark, Lebesby, Eastorjavri	Ekrem	JF870856	MW378485	MW378399		
Nilotanypus dubius	Finnmark210	m	Norway	Finnmark, Lebesby, Eastorjavri	Ekrem	JF870857	MW378488	MW378402		
Nilotanypus dubius	TRD-Cer208	f	Norway	Sør-Trøndelag, Trondheim, Melhus	Stur et al.	MW378369				
Nilotanypus dubius	TRD-Cer195	f	Norway	Sør-Trøndelag Trondheim, Nildeva	Stur	MW378386	MW378555	MW378472		
Nilotanypus cf. *dubius*	BIOUG07413-G10	m	Germany	Bavaria, Niederbayern, National Park Bayerischer Wald	Sellmayer	GMGRF747-13				
Nilotanypus polycanthus	LX-03	m	China	Guangdong Prov., Guangzhou City, Zengtong District, up Lan Stream	H.Q. Tang	OM396932				
Nilotanypus haplochelus	KCU6	Pm	Australia	Queensland, Crater Lakes NP, Kauri Ck.	Krosch, Bryant, Cranston	MW283567		MW281148		
Nilotanypus haplochelus	RAV2.8	P	Australia	Queensland, Koombooloomba NP, Nitchaga Ck.	Krosch, Bryant	MW283573				
Nilotanypus haplochelus	ML4.6	Pm	Australia	Queensland, Mount Lewis NP, Churchill Ck.	Krosch, Bryant, Cranston	MW283568				
Nilotanypus haplochelus	RAV3.3	Pm	Australia	Queensland, Ravenshoe, The Millstream	Krosch, Bryant	MW283575		MW281149		
Nilotanypus haplochelus	RAV3.1	Pm	Australia	Queensland, Ravenshoe, The Millstream	Krosch, Bryant	MW283574				
Nilotanypus haplochelus	RAV2.7	Pf	Australia	Queensland, Koombooloomba NP, Nitchaga Ck.	Krosch, Bryant	MW283572				
Nilotanypus haplochelus	RAV2.6	Pf	Australia	Queensland, Koombooloomba NP, Nitchaga Ck.	Krosch, Bryant	MW283571				
Nilotanypus sp.	ZJ-44	m	China	Hainan Prov., Wanning City, Longgun Town, Heshun Country	H.Q. Tang	OM396933				
Nilotanypus fimbriatus	CHIR_CH303	m	Canada	Manitoba, Churchill, Goose Ck. Marina	Stur	MW378367	MW378534	MW378451		
Nilotanypus fimbriatus	CHIR_CH408	m	Canada	Manitoba, Churchill, Goose Ck. Marina	Ekrem, Stur	MW378346	MW378502	MW378417		
Nilotanypus fimbriatus	BIOUG01725-B12	L	Canada	Ontario, Algonquin Provincial Park	Martin, Zaheer	KR635238				
Nilotanypus fimbriatus	SEG15	m	USA	Wyoming, Teton County, Snake River	Gresens	JF870734	MW378503	MW378418	MW430063	
Nilotanypus sp.	CATP9.3.9	m	USA	California, Plumas Co., Sagehen Ck.	McLuen	MW283565	MW273990	MW281146		
Nilotanypus ctenochelus	RAV1.5	L	Australia	Queensland, Koombooloomba NP, Koombooloomba Ck.	Krosch, Bryant	MW283570				
Nilotanypus ctenochelus	RAV1.4	L	Australia	Queensland, Koombooloomba NP, Koombooloomba Ck.	Krosch, Bryant	MW283569				
Nilotanypus ctenochelus	FNQ16NIG15	L	Australia	Queensland, Herberton Range NP, Wondecla Ck.	Krosch, Bryant, Cranston	MW283566		MW281147		
Nilotanypus ctenochelus	AUNT.07	L	Australia	Northern Territory, Magela Ck.	Humphrey		HQ440944	HQ440781	HQ440315	HQ440480
Outgroups										
Ablabesmyia hilli	EACH1	P	Australia	Queensland, Crater Lakes NP, Lake Eacham	Krosch, Bryant, Cranston	MW283408	MW273940	MW281083	MW286137	MW320400
Australopelopia prionoptera	NSWDor17.1.1	L	Australia	New South Wales, Dorrigo NP, Sassafrass Ck.	Cranston	MW283493	MW273968	MW281114	MW286140	MW320402

We estimated the evolutionary tempo following the procedures of Krosch *et al*. (2017, 2022). A fossil-informed, divergence time estimate is provided for *Nilotanypus* (plus two outgroup Pentaneurini), with sampling reduced relative to that of Krosch *et al*. (2022) but expanded to include additional ingroup taxa. Calibration points used were the fossil *Nilotanypus prieuri* Doitteau & Nel (2007), assuredly assigned correctly, as a log-normal prior on the root height (offset = 45, mean = 50, stdev = 1.5), and a secondary normal prior calibration on the ingroup node using the estimated age for the *Nilotanypus* node in Krosch *et al*. (2022) (mean = 63.8, stdev = 7).

Abbreviations. ANIC, Australian National Insect Collection; AR, Antennal Ratio = length of terminal 2 flagellomeres, divided by sum of all preceding flagellomeres (in adult ♂) or terminal flagellomere, divided by sum of all preceding flagellomeres (in adult ♀); length of basal segment divided by summed lengths of segments 2–4 (larva); asl, above sea level (in metres); BV, 'Beinverhältnis': length of (Fe+Ti+Ta_1) / ΣTa_{2-5}; Ck, creek; Fl_{1-n}, combined lengths of antennal flagellomeres (1–12) (♂), 1-11 (♀); Fe, femur; L, larva; Le, larval exuviae; Le/Pe/♂(♀), reared adult male (female) with associated larval and pupal exuviae; LR_n, leg ratio = length Ta_1 / Ti; n, number measurements; Mt., mount; MV, molecular voucher; N.P., National Park; P_{1-3}, Leg(s) (1 = fore, 2 = mid, 3 = hind leg); P, pupa; Pe, pupal exuviae; R, river; S5, S7, S8, S9, S10, setae of cephalic area (larva); SSm, seta submentum (larva); SV, 'Schenkel-Schiene-Verhältnis' = summed lengths of (Fe+Ti) / Ta_1; $Ta_{(1-5)}$, tarsomere (1–5); Ti, tibia; VP, ventral pit of larval head; ZSM, Zoologische Staatssammlung München, Munich, Germany. If unstated, measurements are in µm.

Locations follow label data in the sequence Northern Territory, north to south, Queensland likewise, continuing clockwise to Western Australia, from south to north. Unless stated otherwise, the collector is the first author, Cranston. Square parentheses [ ] are used for comments and additional data such as locations for renamed cultural reasons.

Results

Descriptive taxonomy

Nilotanypus Kieffer, 1923

Type-species: *Nilotanypus remotissimus* Kieffer, 1923, by monotypy. = *Pentaneura comata* Freeman, 1953, **syn. nov.**

The identity of the genotype, *N. remotissimus* Kieffer, 1923, has been problematic. Freeman (1955: 34–35) could not find material matching the description by Kieffer of the wing as having surface hairs only at the tip (male) or sparse (female). Thus, essentially his concept for *Nilotanypus* (as a 'group' in *Pentaneura* (*Pentaneura*)) was based on *N. comatus* (Freeman, 1953), leaving open the possibility that *N. remotissimus* and *N. comatus* might prove to be synonyms.

The genus has been recognised subsequently as having densely setose wings in both sexes of all species. Since all other features of *N. remotissimus* described by Kieffer (1923), especially the hairy eyes and attenuated radial sector of the wing, matched his material, Freeman (1955) speculated that the wings of Kieffer's specimens may have been rubbed, but tempered this with "even then the hair pits should have been visible". Observations on the wings of pharate and teneral males of *N. comatus* (Freeman) confirm the macrotrichia (hairs) are dense, long, and dark, as in all examined congeners. The pits on rubbed wings are distinctive along the veins, but much less so on the membrane, being very small (about 1 µm diameter) and visible only with phase contrast optics at high magnification (> 400×). Under regular illumination and optics, the pits are not visible. Males of the Australian species have (a) macrotrichia on the wing membrane and veins are easily lost and may appear absent, (b) the last marginal macrotrichia to remain are distal, and (c) sockets (hair pits) may not be visible under regular illumination, even at high magnification.

Freeman calculated from Kieffer's description an AR of 0.3–0.4, notably lower than any values he obtained for his examined *N. comatus*. Problems include the segment or flagellomere count, as including the pedicel (as in a count of 15) distorts the calculated AR against a modern understanding of 14 flagellomeres, excluding the pedicel. Kieffer's estimate actually derived from "14^{e} seulement égal au tiers de 2–13 réunis, 15^{e} conique, à peine aussi long que le13^{e}" [14th only equal to one third of 2–13 combined, 15th conical, barely as long as 13th]. The pedicel was included as segment 1, as did Freeman who diagnosed 15 antennal segments for all males in the entire subfamily (Freeman 1955: 19). Inclusion or exclusion of the terminal 15th and inexactitude of 'one third' render doubtful Freeman's calculated value of 0.3 as too low. Furthermore, the accuracy of Freeman's own calculations is in doubt, appearing to derive from pinned dry specimens (Duncan Sivell, NHM, personal communication 2022). Thus, these values may not differentiate between *N. remotissimus* Kieffer and his *N. comatus*.

Actually, it is the value Freeman cited of 'about 1' for the upper end of the AR range in *N. comatus* that has not been verified subsequently, whereas his lower values of 0.4 and 0.6 have been confirmed. Lehmann (1979) redescribed *N. comatus* from Kivu, Zaire [=DRC], with the male 'Antenna 15 segmented; AR = 0.6'. Harrison (1991) also added description of the species from Zimbabwe and Ethiopia but did not emend previous measurements. Two pharate males from the Western Cape (South Africa) provide AR values of 0.4 and 0.53. Clearly in this widespread species (Ethiopia to the southernmost Cape) the absolute size of the adult male body varies as does the antennal ratio, and although no AR value as high as 1 (Freeman) has been observed since, it may derive in part from measurements of dry material by Freeman those of 0.4–0.6. Features suggestive of a second African species are the relative lengths of the gonostylar megaseta, the state of the L_3 seta on segment VII and the transverse spinule row on VIII in the pupa. Although the relative length of the megaseta is high (ratio to gonostylus length = 0.3–0.4), it is nearly impossible to determine as variable orientation of the gonostylus and megaseta prevents accuracy. Regarding the condition of the L_3 on VII all available material shows the seta is semitaeniate, and this does not distinguish two pupal types. Finally, the posterior margin on SVIII varies from quite robust, few very fine ones or absence of any such spinules. In female exuviae, the row(s) are separated medially by broad, spine-free area. Evidence of high variability derives from these variants as all occur in contemporaneous exuvial collections in similar streams of the western Cape.

The above indicates that *Nilotanypus remotissimus* Kieffer can be reconciled with *N. comatus* (Freeman). Uncertainty about the genotype would be resolved by synonymy, even in the absence of original type material for *N. remotissimus*. Given assurance that there is a single species of *Nilotanypus* in sub-Saharan Africa, we confidently assert conspecificity of *N. comatus* with *N. remotissimus* and propose the formal synonym here.

Generic diagnosis

The Australian fauna, comprising two species described below, unambiguously belong to Nilotanypus Kieffer in the tribe Pentaneurini of the subfamily Tanypodinae. Applicable previous diagnoses derive from: Fittkau 1962, Roback 1986 for all life stages; Murray & Fittkau 1989, Cheng & Wang 2006, Andersen & Pinho 2019 for adult males; Fittkau & Murray 1986, Roback & Coffman 1987, 1989 for pupae; Kownacki & Kownacka 1968; Fittkau & Roback 1983, Cranston & Epler 2013 for larvae.

We expand diagnoses from Australian material and elsewhere. Wavy setae on the apical antennal flagellomere (Murray & Fittkau 1989, fig. 5.27A) are not confirmed in any newly examined material (pharate, teneral or mature). The adult wing can be as short as 500 µm in the female, 750 µm in the male. No claw is spatulate in either sex. The variability of tarsal pseudospurs in number and location is greater than recognised previously. The posterior margin of the proctiger ('anal point') consistently is gently curved. In the male genitalia the gonostylus is gently to strongly curved and tapered, sometimes strongly from the midpoint to the megaseta, and may show or lack a subapical 'carina' or 'flange'. The female also is diagnosed by the wing venation, hairy eye and an isolated prescutellar seta; with 12 antennal flagellomeres, pedicel and scape with 4–5 setae; with unexceptional genitalia. In the pupa, the corona lacks any plastron and can extend to >70% of the horn length, and the atrium can vary from very narrow in basal half to broader throughout. A row of close-pressed small tubercles on the distal wing sheath is present in one species (Fig. 2C, 3A). The variation in posterior transverse row(s) of dark spinules is expanded concerning which segments have row / rows, the number and size of the component spinules, and some may even lack any differentiated spinules on any segment. In the larva, all posterior parapod claws can be simple, conventional, with external carina on some claws.

***Nilotanypus haplochelus* new species**

http://zoobank.org/3F39CDE1-B48A-4643-9D49-A09EF31A9A6D

Type material: *Holotype*, Australia: P♂, slide mounted in Euparal, Queensland, Mt. Lewis N.P., Mt. Lewis, Churchill Ck., 16°34'S 145°20'E, 6–7.iv.1997, leg. Cranston, ANIC. *Paratypes*, Australia: P♀, 6Pe (on 2 slides), as holotype; P♂, same except 8.x.2016, leg. Krosch, Bryant, Cranston, (MV) FNQ16ML4.6; 3Pe, same (non–MV).

Other material examined: AUSTRALIA: Northern Territory; 2Pe, Kakadu N.P., Magela floodplain, Stoned Billabong, 12°38'S 132°53'E, 11.iv.1989; L, Gulungul Billabong, Gulungul Ck., 12°39'S 132°53'E, 11.iv.1989; L, L(P), 10Pe, Djalkmara Billabong, 12°40'S 132°56'E, 10.iv.1989; 3L, Ranger, Magela Ck., 12°40'39"S 132°56'10"E, –.iv.2005, leg. Humphrey, (MV); ♂, Radon Springs, 12°45'S 130°47'E, 13–14.iv.1989; P♂, Nourlangie Ck., 12°49'S 132°45'E, 26.v.1988;

Pe, Litchfield N.P., Florence Falls Ck., 13°06'S 132°26'E, 29.vii.2014, legs. Krosch & Cranston; Pe, Koolpin Ck., 13°29S 132°35'E, 25.v.1988; Pe, Plum Tree Ck., 13°32'S 132°26'E, 25.v.1989; L, L(P), 3P♂, 3P♀, Rockhole Mine Ck., 13°30'S 132°30'E, 15.iv.1993, leg. Smith; P♂, [same slide as Pe, *N. haplochetus*], same except,13.v.1993; 7L, L(P), Kakadu N.P., Kambolgie Ck., 13°30'S 132°23'E, 6.ix.2017; L, Pe, 4♂, S. Alligator R., Gimbat spillway, Guratba [= Coronation Hill] 13°34'S 32°35'E, 19/20.iv.1989; 3♂, 2Pe, S. Alligator R., Guratba [= Coronation Hill], 13°35'S 132°36'E, 4/5.vi.1989. Queensland: Daintree N.P., Oliver Ck.,16°08'3''S 145°26'7''E, 9–10.ix.1997, leg. McKie; Pe, Cassowary House Ck., 1–2.x.2016, leg. Krosch & Cranston; 3Pe, Mossman, Rex Ck., 16°28'S 145°19'E 19–20.x.1998, legs. Dimitriadis & Cranston; 7Pe, same except 10–11.iv.1997; Pe, same except 17–18.xii.1987, leg. Cranston; Julatten, Kingfisher Lodge, Sandy Ck., 16°35'20"S 145°20'17"E, 6.x.2016 (to light); 6Pe, Shoteil Ck., 16°56'S 145°37'E, 9–10.ix.1997, leg. McKie; 2Pe, Clohesy R., 16°59'S 145°38'E, 7–8.ix.1997, leg. McKie; 2Pe, Mareeba, Davies Ck., above falls, 17°01'S 145°35'E, 11–12.iv.1997; Pe, same except 19–20.vi.1997; same except 27–28.viii.1997 [same slide includes Pe, *N. ctenochelus*]; Pe, 20 km E. Mareeba, Davies Creek N.P., [~17°01'S 145°35'E], drift, 14-15.vi.1993, legs. M & B. Baehr; det. M. Spies, 2022 (ZSM); P♂, Danbulla N.P., Kauri Ck., up from day–use area, 17°08'S 145°35'E, 9.ix.2018, leg. Krosch, (MV); 13Pe, Bartle Frere, Junction Ck., 17°16'S 145°55'E, 27–28.viii.1997; P♀, 3Pe, Koombooloomba N.P., Nitchaga Ck., 17°49'45"S 145°33'50"E, 12.x.2017, leg. Krosch & Bryant; P♀, Koombooloomba Ck., nr dam, 17°50'16"S 145°35'16"E, 12.x.2017, leg. Krosch & Bryant; 3P♂, 2P♀, Ravenshoe, The Millstream, Cemetery Rd., 17°36'50"S 145°28'40"E, 12.x.2016, leg. Krosch & Bryant, (MV); Pe, same except 17°36'51"S 145°28'39"E; 3Pe, Palmerston N.P., Tchooratippa Ck., 17°37'S 145°45'E, 8–9.iv.1997; Pe, Herberton, Carrington Falls Ck., 800 m a.s.l., 17°19'S 145°27'E, 9–10.iv.1997; 2Pe, nr Cardwell, 5–mile Ck., 18°19'S 146°03'E, 1– 4.iv.1997; Lawn Hill N.P., Indarri Falls, 18°42'S 138°29'E 16.v.1995; 2Pe [on slide with 5 Pe *N. ctenochelus*] Paluma, Birthday Ck., 18°59'S 146°10'E, 25–26.iii.1998; 2L, Camp Ck., 18°58'S 146°09'E, 21.ix.2008, leg. Krosch & Bryant; P♂, S. Paluma, unnamed Ck., 820 m a.s.l., 19°01'S 146°13'E, 25–26.iii.1998; Pe, Eungella N.P., Mt. Dalrymple track., Cattle Ck., 21°02'S 148°35'E, 950 m a.s.l., 22.iii.1998; Pe, Fitton Hatch Gorge, 200 m a.s.l., 21°05'S 148°37'E, 22.iii.1998; Pe, U. Brisbane R., Mount Stanley, 26°42'S 152°13'E, 19.i.1991; L(P), 3P♂, Bunya, n. Brisbane, Carter Court, South Pine R., 27°21'S 152°56'E, 21.iii.2013, 22 m a.s.l., leg. Krosch & Bryant; same except 5L, L(P), 21.x.2021; P♀, Mt. Barney N.P., Seidenspinner Rd, Mt. Barney Ck., 28°14'S 152°44'E, 21.iii.2013, 176 m a.s.l., leg. Krosch. New South Wales: P♂, U. Clarence R., Gaya–Dari, 28°44'S 152°47'E, 20.i.1991; Pe, Chaelundi S.F., Chandlers Ck., 30°2'22"S 152°29'26"E, 11.iv.1996; L, Bellinger R., 3 km W. Thora [~30°25'S 152°45'E], 1.xii.1990, leg. M. Baehr ["prep. F. Reiss, det. E. Stur"] examined by M. Spies, 2022 (ZSM); 2P♂, 1♀, New England, Cathedral Rock N.P., Sphagnum swamp drain, 30°26'42"S 152°16'.00"E, 13.iii.2017, (MV); P♀, Wollemi N.P., Newnes, Wolgan R., 33°13'16"S 150°13'22"E, 10.iii.2017; Pe, Morton N.P., Corang R., 35°15'S 150°06'E, 25.iv.1994; L, Brooman, Clyde R., 35°30'23"S 150°13'27"E, 10.ii.2009; Pe, Shoalhaven R., Hillview, 35°11'S 149°57'E 17.iii.1992; Pe, Warri Bridge, Shoalhaven R., 35°21'S 149°44'E, 31.iii.1991; Pe, same except 17.iii.1992; 2Pe, Currowan S.F., Cabbage Tree Creek, 35°34'S 150°02'E; Pe [same slide includes Pe *N. ctenochelus*] Brindabella, Goodradigbee R., 35°23'54''S 148°44'51''E, 4.i.2001; L., Captains Flat, Molonglo R., 35°35'S 149°28'E; Pe, Kosciuszko N.P., Yarrangobilly R., 35°39'S 149°28'E, 14–15.i.1991; P♂, 2Pe, S.E. Araluen, Deua R., 35°45'S 149°57'E, 29.iii.1990; 2Pe, Wallagaraugh Ck., 37°15'S 149°41'E, 13.i.1994; Pe, S.E. Cooma, Brown Mt., Rutherford Ck. [~36°36'S 149°47'E] 11.xi.1961 (Brundin), det. M. Spies, 2022 (ZSM).

Australian Capital Territory (ACT). 2L, Cotter R., 1.ii.1989. Victoria, Wodonga, Middle Ck., Kiewa Valley Highway, 36°10'S 146°56'E, 3.iv.1990, leg. Cook; P♀, U. Tambo R., 36°59'S 147°51'E, 8.iii.1990, leg. Hortle.

Western Australia: P♂, Hammersley Range N.P., Fortescue R., Crossing Pool, 21°34'22"S 117°05'02"E, 24.iv.1992, leg. Smith; 3Pe, Millstream Chichester N.P., Fortescue R., below Homestead, 21°33'S 117°03'E, 24–25.iv.1992; Pe, Circular Pool, Fortescue Falls, 21°28'S 118°33'E, 23–24.iv.1992; P♀, Richenda Gorge, 17°27'09"S, 125°26'07"E, 10.v.1995, leg. Smith); P♀, Kimberley, Upper Durack R., 16°52'33"S 127°11'43"E, 8.v.1995 (leg. Smith); Kimberley, King Edward R., 14°53'S 126°12'E, 5–6.v.1992.

Etymology: From Greek, *haplos* = simple, *chelus* = claw, recognising all larval posterior parapod claws are simple and none are comb-like.

Diagnostic characters. See below, under *Nilotanypus ctenochelus*.

Description

Male (n=12, including pharates). Total length 1.4–1.8 mm. Wing length 750–950 µm. Overall brown, legs paler, abdomen with slightly paler intersegments.

Antenna. With 14 flagellomeres, total length 492–560, terminal flagellomere 40-50 long, with angled apex, straight (not offset), separated indistinctly from penultimate (13th) flagellomere ~160–192, 4–5 × length of terminal flagellomere, apical 2 flagellomeres subequal to 5.5 (5–6) preceding segments. AR 0.49–0.57; terminal seta 50–70 long. Scape bare, pedicel with 2 setae.

Head. Eye (Fig. 1A) microtrichose, dorsomedial extension 8–9 ommatidia long, slightly tapered and angled, 3–4 ommatidia wide. Frontal setae 2, ~100 µm, 7–9 uniserial temporal setae, with slight gap separating 2 outer verticals (Fig. 1A). Clypeal setae 14–18. Palp (2–5) 25–38; 50–63; 100–110; 75–110.

Thorax (Figs 1E–G). With uniserial tuberculose mesonotal margin, smoothly curved with posteromedian projecting small sense organ (Fig. 1G); 2–4 lateral antepronotal setae; ~16–25 unevenly uni-biserial acrostichals; ~15–24 dorsocentrals, biserial anteriorly, uniserial from midpoint; separated posterior dorsocentral / prescutellar, 8–11 prealars in anterior and posterior clusters; 1 supraanal; scutellars with posterior-most row of 8 uniserial strong setae, with up to 20 shorter to much smaller setae anteriorly.

Wing (Fig. 1H). Hyaline, all veins pale, including crossveins, membrane and all veins densely setose; costa (C) extends to apex of R_{4+5}, strongly retracted from wing apex, and proximal to end of M_{3+4}; R_1 and R_{4+5} widely separated, R_{2+3} absent or at most, weakly indicated; R_{4+5} runs close to costa. Crossvein vertical. Brachiolum to crossvein 160–200, brachiolum to costa termination 500–670, costa terminal to wing tip 210–250. Squama with 16–20 uniserial setae,

Legs. Mensural: P_1 138-162, 90–118, 88–98, 28–38, 20–32, 25–35, 22–26, LR_1 0.70–0.86, BV_1 2.79–3.14, SV_1 2.86–3.02; P_2 165–230, 105–125, 155–178, 58–70, 40–52, 30–35, 30–33; LR_2 1.34–1.42, BV_2 1.3–1.7, SV_2 2.71–2.88; P_3 150–192, 125–172, 140–192, 75–88, 55–70, 38–43, 27–30; LR_3 1.07–1.28, BV_3 1.70–2.03, SV_s 2.02–2.53. Tibial spurs (Figs 1I, J) 1, 1, 1, each narrow, slightly curved, 30–40 long with basal fine divergent spines ('hairs'), without lateral comb-like teeth; tibial comb on P_3 comprising 7–8 curved spines (Fig. 1J) 25–30 long. One pseudospur (50 x 3) subapical on Ta_1 on P_1 on most specimens; a single specimen also has a shorter (20–25 x 2) pseudospur on Ta_3 and Ta_4; P_2 with pseudospur on Ta_3 and Ta_4 (missing on 50% specimens; if present, shorter, poorly differentiated); P_3 with no pseudospur. Claws simple, gently curved, distally rounded, with strong basal rounded lobe. Pulvilli absent.

Abdomen. Setae at least as long or longer than segment, in more or less anterior and median transverse rows, on tergum and sternum.

Hypopygium (Fig. 1K). Tergite IX posteriorly with 6 or 8 aligned long setae; proctiger rounded. Gonocoxite squat, externally bulging, 65–70 long, maximum width 38–50, microtrichose, laterally with extremely long posteriorly-directed setae, 250–330 long, filling pharate pupal genital sheaths, setose on dorsal and lateral surface, with slightly differentiated dorso-medial cluster of dense medially-directed fine setae, posteromedian dorsal surface with stronger medially-directed setae with strong tubercle bases that give appearance of a small lobe. Gonostylus 40–52 long, initially broadened (7–8) then tapering and gently curved to 3 wide apex; weakly microtrichose with 3–4 mid-length setae on outer surface, 3 on inner and 1 subterminal; without any carina; megaseta at subapex of gonostylus, slender (5–7 long, 1–1.5 wide), angled relative to direction of apical gonostylus (Fig. 1K). Gc:Gs ratio 1.66–1.88. Phallapodeme strong, sternapodeme shallow arched.

Female (n=4, pharate/teneral). Total length ~1.5–1.8 mm, wing length ~500–580 µm. Overall brown, abdomen with slightly paler intersegments.

Antenna. With 12 flagellomeres, total length 155–260, terminal 42–61, with tapered blunt apex; AR [0.20] 0.32–0.36; lacking differentiated terminal seta, cluster 40–50 long. Pedicel with 4 setae, scape with 3–4 setae.

Head (Fig. 1B). Eye microtrichose, dorsomedial extension tapered, of 4–6 ommatidia long. Frontal setae 3–4, 110 long, aligned dorso-ventral, contiguous (at right angles) with 7–8 long uniserial temporals. Clypeal setae 16–22, ~100 long. Palp (1–5) 21–38; 25–40; 40–55; 60–75; 66–135.

Thorax. With weakly tuberculose anterior margin and small posteromedian scutal sense pit (possibly absent in some). Setal pits (and likely setae) in each location (ac, dc, pa, scts) variable not bimodal in size, originating either from pale longitudinal band, or from paler circular areas: with 1–2 lateral

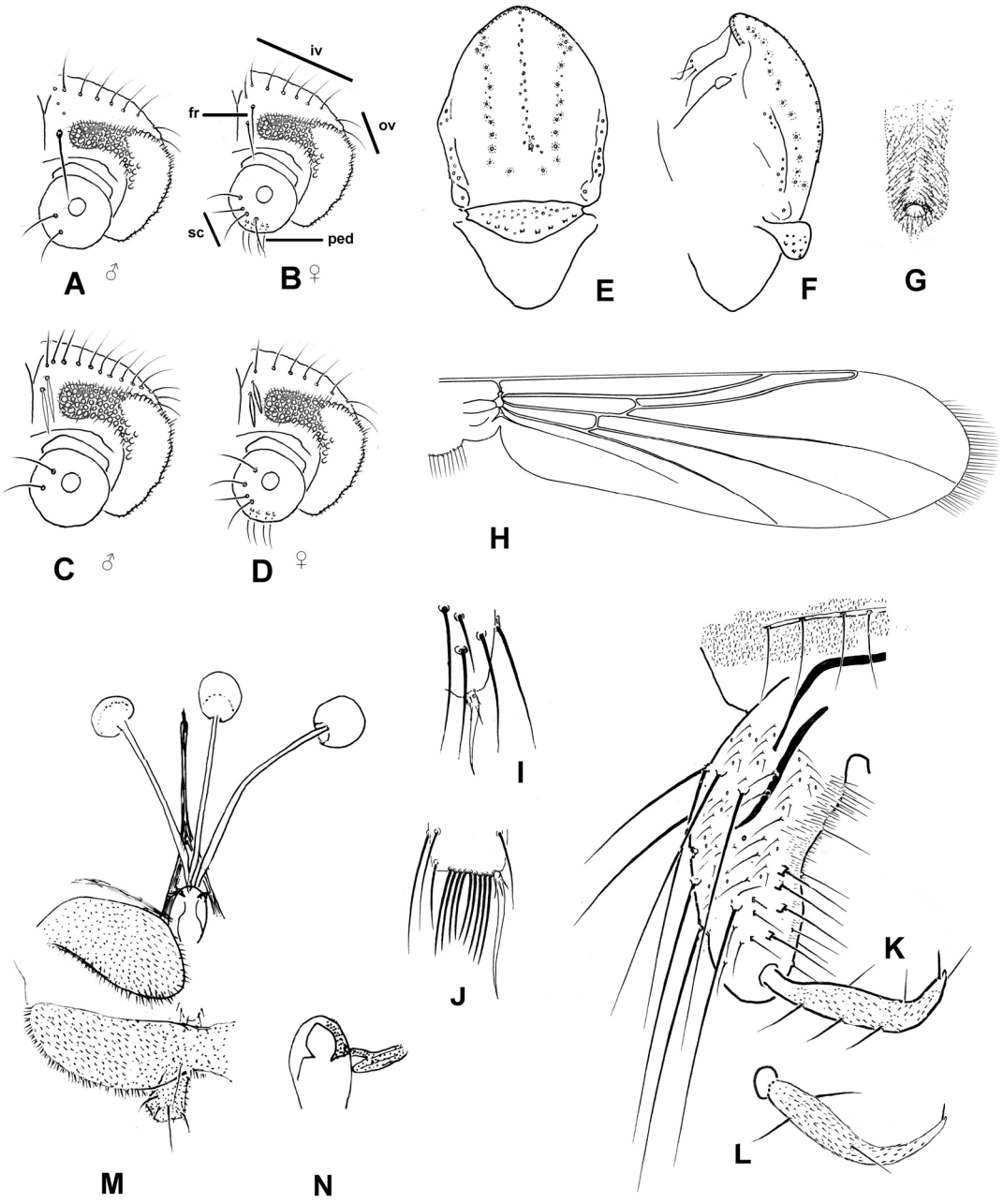

Figure 1. *Nilotanypus* Kieffer. Adult. A–D. Head, anterior view, right side, ♂, A, C. ♀, B, D.; E–F. Thorax, E. dorsal, F. lateral; G. Mid-dorsal sensory pit; H. Wing (male); I, J. Tibial apices, I. P1, J. P3; K. Male hypopygium; L. Gonostylus; M. Female genitalia left side only; N. Anterior vaginal cavity, detail. A–B, E–K, M–N. *N. haplochelus* sp. n.; C–D, L. *N. ctenochelus* sp.n. Abbreviations: fr–frontal setae, iv–inner vertical setae, ped–pedestal setae, ov–outer vertical setae, sc-scape setae. Fig. 1G after Roback, 1986.

antepronotal setae: 17–22 acrostichals +/- biserial throughout, with isolated posterior dorsocentral / prescutellar, 17–22 unevenly biserial dorsocentrals, 7 prealars separated into anterior 3–4 and posterior cluster of 2–3; 1 supra-anal; scutellum posteriorly with 8 uniserial strong setae, more anteriorly with up to 30 short, finer setae.

Wing. Apical marginal setae up to 80 µm. Squama with 8 uniserial setae.

Legs. No measurements calculable. Tibial spurs 1, 1, 1, and comb on P_3 apparently as in male. Claws simple, gently curved, distally rounded, with strong basal rounded lobe. Pulvilli absent.

Abdomen. Each tergite with 2 transverse bands (anterior and median) of strong setae and small lateral cluster.

Genitalia (Figs 1M, N). Gonocoxapodeme VIII indistinct. Gonapophysis VIII solitary simple microtrichose lobe covered only with short setae. Gonotergite IX weakly protruding, without setae. Coxosternapodeme strong, dark, curved. Notum thin, 40–45 long, subequal to seminal capsule, posterior part of rami 40–45 long. Three hyaline, globular, seminal capsules, 35–40 diameter, without distinct neck; spermathecal ducts 130–140 long, dilate prior to narrowing before common ending. Anterior vagina with short spine seemingly associated with mesal end of gonocoxapodeme VIII (Fig. 1N). Cerci squat, small, 20–25 by 15–18.´

Pupa (n=10). Small, total length 1.4–1.9 mm.

Cephalothorax. Thoracic horn (Fig. 2A), flattened-tubular, sparsely spinose, 120–140 long, 4–4.5 x as long as maximum breadth, with initially narrow atrium dilate distally to fill ~90% of lumen; ovoid corona 55–62 long. Thoracic comb uniserial row of ~9–12 apically rounded tubercles, 8–12 (longest) diminishing laterad. Basal lobe 25–32 wide, 25–30 high, domed. Thorax weakly granular at most; wing sheath smooth, nose shallow or absent.

Abdomen (Fig. 2D). Tergites with short tubercles (2–3 long) aligned in transverse rows of predominantly triplets on tergites, pleurae and sternites, absent from apophyses and scar marks. Tergite I with pigmented scar. Setation: 'O' setae on all tergal and sternal transverse apophyses except for VIII, 'D' setae seemingly short, 4 characteristically aligned anterior to posterior with 2 sensilla, 'L' setae 1–2 per segment, when 2, one dorsal, one ventral, none taeniate on VII; taeniate LS only on VIII, all 5 evenly distributed in posterior 60% of segment. Posterior SVIII with linear-aligned 21–30 subapical spinules, 4–6, essentially uniserial and continuous in male, multiserial, slightly shorter and medially interrupted in female. Anal lobe (Figs 2D, E) in both sexes 125–135 long, 140–155 wide, bare, smooth on outer or inner margin, terminating with recurved hyaline blunt hook; anal setae adhesive, with maximum breadth of AL_1 seta narrower than AL_2 (4–5 versus 11–15 wide). Genital sacs dimorphic, male tapering, 250–300, 2× anal lobe; in female bluntly rounded, 0.5× anal lobe length. Genital sacs basally spinulose in both sexes.

Larva (n=12). Total length 2.5–2.7 mm. Head capsule length 330–380, max. width 170–240, cephalic index 0.50–0.63. Pale yellow with mandible, ligula and occipital margin slightly darker yellow to mid-brown.

Head.

Antenna (Figs 2H, I). Basal segment 130–148, 2nd 41–46, 3rd and 4th 4–5 long; AR 2.9–3.5, ring organ flush, at 68–75% from base; style and Lauterborn organ ~4 long; blade and accessory blade subequal to flagellum (Fig. 2H); antenna / mandible ratio 3.8–4.1.

Mandible (Fig. 2J). 47–52 long, seta subdentalis arising on strong distal molar projection ('tooth'), proximal to rounded inner tooth.

Ligula (Fig. 2K). 42–48 long, 2.5 × as long as apical width, narrowed in middle; with 5 teeth, central tooth slightly broader and extending beyond outer teeth. Muscle attachment area weak. Paraligulae bifid, 32–36 long slender; 2/5 length of ligula; outer point at least 2× as long as inner. Pecten hypopharyngis (Fig. 2K) with 5-6 teeth, innermost tooth largest and directed antero-medially, remainder subequal and directed anteriorly.

Maxillary palp (Fig. 2L). 27–35 long, ring organ large ~70% from base, longest component of apical crown 14–16 long.

Mentum and M appendage. Dorsomentum without teeth, a sclerotized complex each side of base of M appendage, connected by ridges to ventromentum and ventral region of premento-hypopharyngeal complex, from which labial vesicles arise apically; dorsally with anteriorly directed tooth on each side. Ventromentum separated from M appendage by a fold. Pseudoradula finely and uniformly granulose, broadened near base.

Submentum / anterior gula (Fig. 2M). Straight with weak transverse 'creases' of paler cuticle. V9, V10, VP near longitudinally aligned, SSm posteriorly retracted; dorsal pit (DP) present, S7 well separated from S8, S5 retracted posterior to S8 (Fig. 2F).

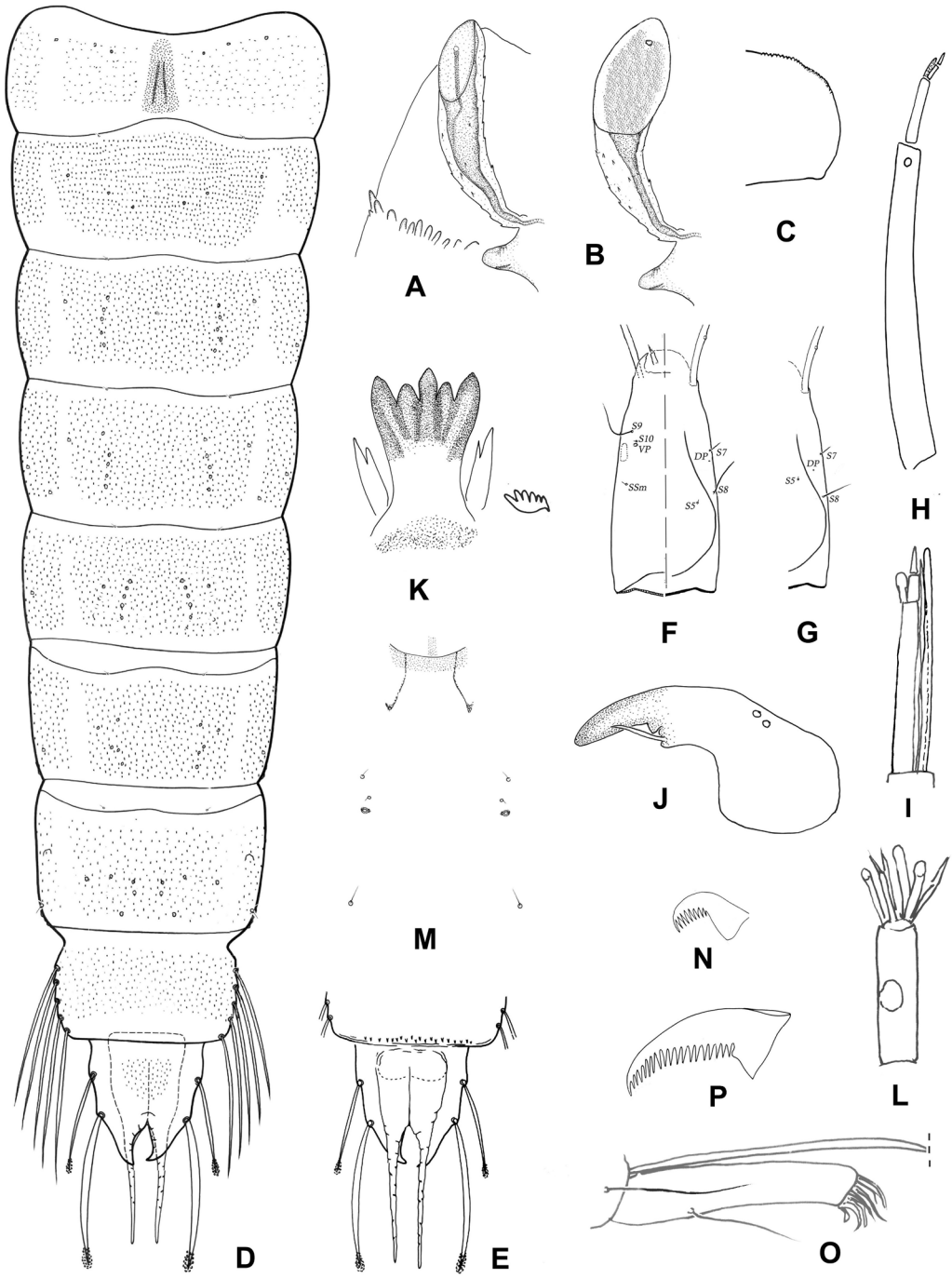

Figure 2. *Nilotanypus* Kieffer. Pupa. A, B. Thoracic horn; C. Wing sheath; D–F. Abdomen, male); D. dorsal, E. ventral. Larva. F. Head capsule, left side ventral, right side dorsal; G. Dorsal head capsule; H. Antenna; I. Antennal apex, detail; J. Mandible; K. Ligula, paraligula; l. Maxilla; M. Submentum; N. Anterior parapod small comb claw; O. Posterior body; P. Posterior parapod comb claw. A, D–F, H–O. *N. haplochelus* sp. n.; B, C, G, P. *N. ctenochelus* sp. n. Abbreviations: S5 – S10 – cephalic setae, DP – dorsal pit, VP – ventral pit.

Abdomen. Anterior parapods 170–200 long, fused from base, divided only subapically (90% of length). Each claw cluster comprising many simple claws, up to 30 long, mostly simple, some with hyaline outer including 4-6 short combs amongst simple basal spinules. Posterior abdomen (Fig. 2O) with parapod 250–275 long, near midpoint bearing isolated ventral 100 long spine; claws pale, variable in size and shape, several with hyaline carina, none pectinate lacking even fine spinules on inner margin. Procercus hyaline anterior, darker posteriorly, 26–35 long, 9–12 wide, with short darkened spur at the base; near midpoint bearing procercal seta 10–12 long, apically with 7 anal setae length 210–240. Supraanal seta strong, 220 long. Anal tubules narrow, tapered, elongate, at least as long as posterior parapods (>250), but difficult to measure precisely.

3rd instar. Head capsule 200 long, 125 wide, ratio 62%, antenna 1 70, 2–4 30, AR 2.3. Mandible length 32. Ligula length 32. Cephalic seta S5 relatively more anterior than in 4th instar.

***Nilotanypus ctenochelus* new species**

http://zoobank.org/139262E4-37D0-4487-BC82-093A9A05FBD7

Type material: *Holotype*: Australia, P♂, slide mounted in Euparal, Queensland, Paluma, Birthday Ck., 18°59'S 146°10'E, 650 m a.s.l., 25–26. iii.1989, leg. Cranston, deposited ANIC. *Paratypes*, P♂, 2P♀, on same slide as holotype, same data.

Other material examined: Australia, Northern Territory. Kakadu N.P., Pe, Djalkmara Billabong, 12°40'S 132°56'E, 10.iv.1989; 9Pe, Rockhole Mine Ck., 13°30'S 132°30'E, 1.iv.1993, 8.v.1993, leg. Smith; same except P♂, P♀, [on same slide as Pe, *N. haplochelus*] 13.v.1993; Pe, Koolpin Ck., 13°29'S 132°35'E, 15–16.v.1992. Queensland, Daintree N.P., Pe, Noah Ck., 16°08'28"S 145°25'37"E, 2–3.x.2016, leg. Krosch & Cranston; 3Pe, Oliver Ck.,16°08'S 145°26'E, 9–10.ix.1998; Mt Windsor N.P., 16°15'11"S 145°2'24"E, 6.x.2016, leg. Krosch, Bryant & Cranston; Pe, Mt. Lewis N.P., Windmill Ck., 8–9.ix.1997, leg. McKie; Pe, nr Mareeba, Davies Ck., 17°01'S 145°35'E, 27–28.viii.1997 [same slide includes Pe, *N. haplochelus*]; L, Mt. Hypipamee N.P., Wondecla Ck. [=Nigger Ck.,] 17°27'S 145°29'E, 11.x.2016, leg. Krosch & Cranston; (MV) FNQ16NIG15; L, same except 29.viii.2012, leg. Cranston; Pe, Tully Gorge N.P., Pixies Ck., 2–3.ix.1997, 17°47'S 145°41'E, leg. McKie; Pe, Palmerston N.P., Learmouth Ck., 650 m a.s.l., 17°35'S 145°42'E, 8–9.iv.1997, 3L, Koombooloomba N.P., Koombooloomba Ck., nr dam, 17°50'16"S 145°35'16'E, 12.x.2018, leg. Krosch & Bryant; (MV) FNQ16RAV1.4, 1.5; 2Pe, Yuccabine Ck., 18°11'07"S 145°46'00"E, 9.vi.1997, leg. McKie; 2Pe, Yuccabine Ck., 10.vi.1997, leg. McKie; 2P♂, 2P♀, Paluma, Birthday Ck., 18°59'S 146°10'E, 650 m a.s.l., 25–26. iii.1989; 3L, same except 1.x.2009, leg. Krosch; L, same except 31.viii.2005, leg. Cranston; 2L, Camp Ck., 18°58'S 146°09'E, 21.ix.2008, leg. Krosch & Bryant; L, Cooloola N.P., Franki's Gulch, 26°03'S 153°04'E, 6.iv.1996; 3L, Tamborine Mt., Cedar Ck., 27°54'S 153°11'E, 26.ix.1989. New South Wales. 2L, Bungonia, Bungonia Falls, 34°47'S 150°00'E, 11.xi.1988; 2Pe, Currowan S.F., Cabbage Tree Ck., 35°34'S 150°02'E; 2Pe [same slide includes 1Pe *N. haplochelus*] Brindabella, Goodradigbee R., 35°23'54"S 148°44'51"E, 4.i.2001; 7 Pe, above Captains Flat, Molonglo R., 35°35'S 149°28'E, 6.iii.1993; Pe, nr. Mongarlowe, Mongarlowe R., 35°24'S 149°57'E, 17.iii.1993; L., Kosciuszko N.P., Leather Barrel Ck., 36°31'S 148°11'E, 4.xii.2010. Victoria, Pe, Buckland R., 36°48'S 146°51'E, 1.vii.1991, leg. Cook; 2L, Tambo R., south branch, 12.xii.1990, 36°59'S 147°51'E, leg. Hortle.

Etymology: From Greek, *cteno* = comb, *chelus* = claw, recognising the comb-like larval posterior parapod claw.

Diagnostic characters

The two new Australian species described here conform in all stages to *Nilotanypus*, with additional features noted above in an expanded generic diagnosis. Male adults may be separable by the tarsal pseudospurs: *N. haplochelus* sp. n. has a subapical pseudospur on fore tarsomere on the foreleg (P_1), whereas *N. ctenochelus* sp. n, lacks pseudospurs on Ta_1 of P_1. Midleg pseudospurs may distinguish but confirmation based on teneral specimens is unsafe. The gonostylus of the male genitalia can separate: *N. ctenochelus* sp. n. has few (2–3) setae and tapers to thin distal part (Fig. 1L) in contrast to the more setose (7) *N. haplochelus* sp. n. with conventional taper to broader distal part (Fig. 1K).

The two frontal setae in the female *N. ctenochelus* sp. n. are diagnostically stout (Fig. 1D), in contrast to the conventional narrower frontal setae of *N. haplochelus* sp. n. (Fig. 1B). The spermathecal ducts are of even width in *N. ctenochelus* sp. n., but have a dilate section in *N. haplochelus* sp. n., and seminal vesicles are small with a neck in *N. ctenochelus* sp. n. but in *N. haplochelus* sp. n. are larger and lack a neck.

The described pupae of *Nilotanypus* especially from Roback (1986) and Roback & Coffman (1987, 1989) show subtle differentiation with variation in the thoracic horn and in the strength of abdominal armament. Separation of the Australian species depends upon the (unique) row of tubercles on the distal wing sheath of *N. ctenochelus* sp.n. (Figs 2C, 3A). The two Australian species may be separable also on the thoracic horn: in *N. ctenochelus* sp.n. the atrium is very narrow in the basal 1/3 and expanded from near the midpoint (Fig. 2B) whereas in *N. haplochelus* sp. the atrium broadens nearer the base (Fig. 2A).

The larvae of the two species of Australian *Nilotanypus* are differentiated by the posterior parapod in *N. ctenochelus* sp. n. having a long comb-toothed claw (Fig. 2P, 3B) that is lacking in *N. haplochelus* sp.n. – hence the species epithets. Other differences include dense-packed short comb-teeth claws (Fig. 2N) on the anterior parapod of *N. ctenochelus* compared to few simple spinules in claws of *N. haplochelus*; and the mid-tooth of the ligula tending to be wider and to protrude further in *N. haplochelus* (Fig. 2K). The location of the dorsal cephalic seta S_5 relative to the dorsal pore and lateral cephalic setae S_7 and S_8 may inform (Fig. 2F, G). Although the head capsule of *N. haplochelus* is narrower (cephalic ratio ~0.5) compared to *N. ctenochelus* (~0.6–0.7), the ratio varies with slide preparation. Otherwise, all mensural features ranges encompass both larval types.

Description

Male (n=1–3, all teneral). Total length ~1.3 mm, wing length 750–800 µm. Overall brown throughout, legs slightly paler, abdomen with slightly paler intersegments.

Antenna. With 14 flagellomeres, total length 487, terminal flagellomere 40, separate but not offset from penultimate (13th) flagellomere 122, 3× length of terminal flagellomere, apical 2 flagellomeres subequal to 6.5 (6–7) preceding segments. AR 0.50; terminal seta 45–50 long. Pedicel with 1–2 setae, scape without setae.

Head (Fig. 1C). Eye hairy with dorsomedial extension of 6 ommatidia long. Frontal setae 2, thin, at right angle to 10 uniserial temporal setae, all arising from paler field. Clypeal setae 15. Palp (2–5) 25, 47, 70, 100.

Thorax with uniserial tuberculose anterior margin, curved with posteromedian projecting small sense organ (half size of adjacent setal sockets); with 2–3 lateral antepronotal setae; ~17 unevenly uni-/biserial acrostichals; ~16–20 dorsocentrals, humeral cluster disorganised becoming uniserial in pale areas; isolated prescutellar, 10–12 prealars comprising anterior cluster of 4, posterior prealars disorganised; 1 supra-anal; scutellars with posteriormost row of 8 uniserial strong setae, with shorter to much smaller setae anteriorly numbering up to 22.

Wings hyaline, veins pale, membrane and veins densely setose, submarginal apical setae dense, strong, 100–120 long. Venation as in *N. haplochelus*. Squama with 16–20 uniserial setae,

Legs. Mensural: P_1 250–255, 212–225, 178, 75, 63, 52, 50; LR_1 0.83, BV_1 2.68, SV_1 2.62; P_2 320–350, 210–275, 245, 110, 90, 60, 55 LR_2 1.18, BV_2 2.53, SV_2 2.27; P_3 290–295, 200, –, –, –, –, –; spurs 1, 1, 1, each narrow, slightly bent, 30–40 long with basal fine spines ('hairs'), without lateral comb teeth; tibial comb on P_3 comprising slightly

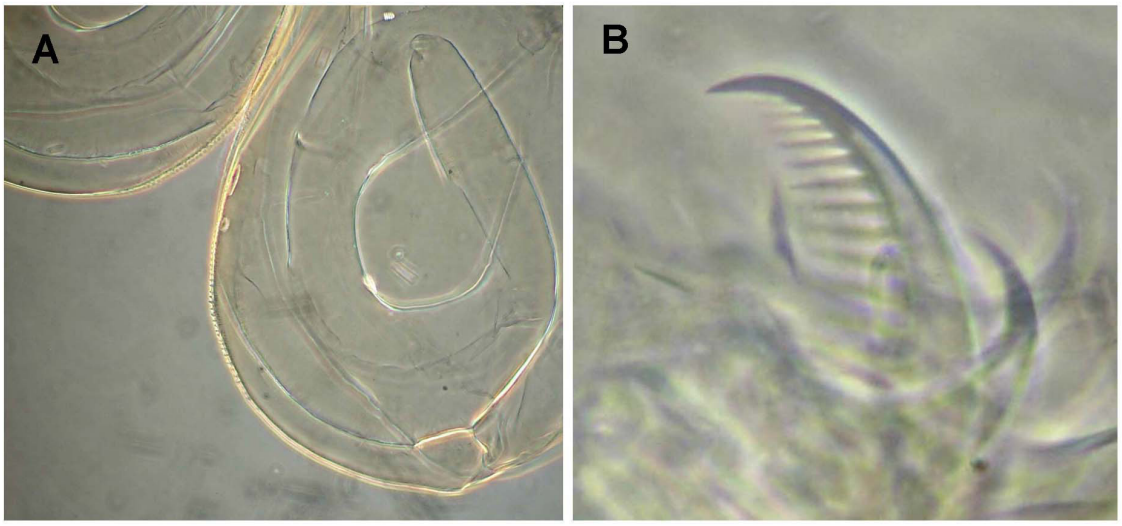

Figure 3. *Nilotanypus ctenochelus* sp. n. A. Wing sheath, tubercle row; B. Larval posterior parapod, comb claw.

curved spines ~30 long. Pseudospurs 30–35 long, 2.5 wide, 2 on subapex of ta_2 of midleg (P_2), 3 slightly longer on subapex of ta_3; seemingly absent on other legs. Claws simple, gently curved, distally rounded, with strong basal rounded lobe. Pulvilli absent.

Abdomen setose with setae as long as an abdominal segment, organised into partial anterior median and lateral bands, both tergal and sternal.

Hypopygium. Tergite IX posteriorly with 6 or 8 aligned long setae; proctiger gently curved (median hyaline). Gonocoxite cylindrical, 55–60 long, maximum width 40, microtrichose, with dense dorso-laterally originating setae >300 long, filling pharate pupal genital sheaths, antero-median inner surface with 4–5 medially-directed setae arising from strong tubercle bases, not coalesced to appear as a lobe. Gonostylus (Fig. 1I) 38–42 long, microtrichose, broadest near base, curved from midpoint distally tapering to narrow apex, with 2 fine outer setae, 1 internally, none adjacent to slender megaseta (1 wide, 6–7 long), continuing direction of apical gonostylus, Gc:Gs ratio 1.3–1.88. Phallapodeme strong, sternapodeme shallow arched.

Female (n=3, pharate/teneral). Total length ~2 mm, wing length ~550–650 µm. Overall brown.

Antenna with 12 flagellomeres, total length 287, ultimate ~76–78, with blunt apex; AR 0.32–0.37; terminal seta 100 long. Pedicel with 4–5 setae, scape with 4-5.

Head (Fig. 1D). Eye hairy with dorsomedial parallel-sided extension of 6–7 ommatidia. With 2 stout lanceolate frontal setae 40 long, aligned anteroposterior, separated from 8 slender uniserial temporals, all arising from paler field. Clypeal setae 20-23, ~100 long. Palp (1–5) 30, 25; 38; 50; 75.

Thorax. With tuberculose anterior margin, without posteromedian scutal sense pit. Setal pits (and setae) in each location (ac, dc, pa, scts) bimodal, all originating from pale areas of cuticle: 2–3 lateral antepronotal setae; ~22 acrostichals +/-biserial throughout, with isolated posterior dorsocentral prescutellar, 14–15 unevenly biserial dorsocentrals; 9–10 prealars separated into anterior 2–3 and posterior cluster; 1 supra-anal; scutellum posteriorly with 8 uniserial strong setae, more anteriorly with shorter / finer setae numbering up to 30.

Wings. Apparently as in male. Apical marginal setae up to 80 long. Squama with 17–19 uniserial setae.

Legs. Mensural: P_1 225–250, 220–230, 150, 75, 70, 55, 75, LR 0.67, SV 2.96–3.20, BV 2.18; spur 30; P_2 325–375, 225–238, –, –, –, –; spur 38–40; P_3 325–350, 300, –, –, –, –; spur 40. Tibial spurs 1, 1, 1, fine, straight, 30–40 long with basal fine spines ('hairs'), without lateral teeth; tibial comb on P_3 comprising 4-5 straight spines, longest 25. Paired proximate pseudospurs 38–42 long, 2.5 wide, subapical of ta_1 of foreleg (P_1), no others detected. Claws simple, gently curved, distally rounded, with strong basal rounded lobe. Pulvilli absent.

Abdomen. Moderately dense setae more or less aligned in anterior and median transverse rows.

Genitalia: Gonocoxapodeme VIII weak. Gonapophysis VIII simple microtrichose lobe with short setae throughout. Gonotergite IX weakly protruding, without setae. Coxosternapodeme strong, dark, curved. Notum thin, short (40–50 long) 2× seminal capsule length, posterior part of rami 45–50 long. Three globular seminal capsules, 25–28 diameter, with distinct neck; spermathecal ducts 120–125 long, of overall even width, bare, ending uncertain. Gonocoxapodeme VIII forming continuous arc across anterior vaginal chamber. Cerci squat, small, 20–25 × 15–18.

Pupa (n=10). Small, total length 2.0–2.7 mm.

Cephalothorax. Thoracic horn (Fig. 2B) flattened-tubular, spinulose, 130–175 long, 3–3.5 × maximum breadth, with narrow poorly-defined atrium expanded only distally (beyond 50%), with ovoid corona 75–90 long (ratio 48–51%). Thoracic comb uniserial row of 12–15 apically rounded, tubercles, 12–16 (longest) diminishing in size laterad. Basal lobe 32–50 wide, 25–30 high. resembling sharkfin. Thorax microtuberculose anteriorly and close to mid-dorsal ecdysial line. Wing sheath apicodistally with row of c. 20 small marginal tubercles aligned on anterior distal sheath (Fig. 2C, 3A), nose shallow to strong.

Abdomen. Armament as in *N. haplochelus*, except reduced on anterior segments to very fine scattered spinules, more microtuberculose on caudal tergites and all pleurae. Setation apparently as in *N. haplochelus* including L setae fine, short on VII; on VIII the 5 taeniate LS are distributed across caudal 70% of segment. SVIII posteriorly with subapical spinules, 3–4 long, numbering >50 spinules, unibiserial, continuous in male; multiserial, shorter and medially interrupted in female. Anal lobe in both sexes, 175–205 long, 170–190 wide, bare, without spinules on either margin, terminating with inwardly curved hyaline blunt hook; anal setae adhesive, with greatest width of anterior (AL_1) seta much narrower than broad posterior (AL_2) seta (width 5–8 versus 20–25). Genital sacs sexu-

ally dimorphic, of male tapering, > 400 long, >2× anal lobe; in female bluntly rounded, 0.4× anal lobe length. Bases of genital sacs microspinulose more so in male.

Larva (n=3–4). Head length 380–440, width 190–240, cephalic index ~0.51–0.60. Yellow with mandible, ligula and occipital margin mid-brown.

Antenna: basal segment 140–155 long, segment 2 30–36, segment 3 ~ 5–6, segment 4 ~4 long; style and Lauterborn organ ~5 long; blade and accessory blade subequal to flagellum; AR 2.95–3.4; Ring organ slightly protruding at ~55–70% from base. Antenna / mandible ratio 4.2–4.8.

Mandible. 40–47 long, seta subdentalis on well-developed distal molar projection ('tooth'), proximal to distinct, rounded inner tooth.

Maxillary palp. 21–23 long, ring organ faint ~60% from base, longest component of apical crown 16-20 long.

Ligula. 35–39 long, 3–3.5 × as long as apical width, 'waisted', with 5 teeth, near straight with central tooth extending only slightly beyond outer teeth. Paraligula squat, bifid, 16 long, Pecten hypopharyngis with 6–8 teeth, innermost largest and directed antero-medially, remainder subequal / narrower points directed anteriorly.

Submentum / anterior gula. Ventrally V_9, V_{10}, VP, SSm as in *N. haplochelus* (Fig. 2F, left). Dorsally with S_7 well separated from S_8 with dorsal pit (DP) near midway, but closer to ecdysial line, S5 anterior to DP (Fig. 2G).

Abdomen. Anterior parapod with many small pectinate spinules (Fig. 2N) proximal to conventional claws. Posterior parapod 175–300 long, ventrally with slender spine, 130–185 long, inserted at 1/3 from base; solitary pectinate claw, 50–55 long with 16–21 internal teeth (Fig. 2P, 3B), amongst otherwise simple claws. Procercus slightly darkened posteriorly, paler anteriorly, length 42–50, width 12–16, bearing 7 anal setae length 300–400. Supra-anal seta strong, 200–250 long. Anal tubules narrow, tapering, hyaline, up to 400 long, often damaged.

Comments

Morphological and taxonomic issues

Roback's (1986) treatment of the *Nilotanypus* of the eastern United States is an authoritative guide to the genus as known at that time. At least some life stages were described in detail. Roback's statement concerning 'remarkable uniformity' of morphology is confirmed, but an unusual feature appears to have been missed by subsequent authors. Roback noted and illustrated a scutal "sense" pit (Roback 1986: figs 1, 2, 5; Fig. 1G) on the posterior scutum, nearly aligned with an isolated prescutellar setae between the posterior ends of acrostichal and dorsocentral rows. Although unmentioned by Murray & Fittkau (1989), Cheng & Wang (2006) or Andersen & Pinho (2019), this could be a potentially significant synapomorphy unobserved in any other Pentaneurini. The minute feature requires oil immersion optics (×1000) on a dorsal view of the thorax and in lateral view may be indistinguishable from the socket of a regular but lost acrostichal seta.

All stages of this genus are small and dissected parts may be orientated differently on the slide mount, such as the lateral thorax, tergite IX and proctiger, and the gonostylus. Some inconsistent or erroneous adult character states have appeared in diagnoses, species discrimination and keys. Thus, the location and number of tarsomere pseudospurs (Cheng & Wang 2006) cited onward (Andersen & Pinto 2019, Shimbakuro *et al*. 2021) have been considered significant. However, pseudospurs can be lost easily by abrasion and seemingly in their absence cannot be recognised by setal pits becuase the sockets resemble those of regular setae. Pseudospurs remain visible and are not abraded on legs of the pharate adult and, although difficult to interpret, a true count can be made. Significant variation including differences between the same leg on opposite side of the body are revealed, confirming what is seen in series of males from the same light trap. The character may be unreliable and should be treated with caution. Also of doubtful utility is the proctiger (termed anal point elsewhere), the hyaline extension of TIX purportedly informative in shape, yet highly susceptible to differential pressure on the coverslip. Viewing this structure with Nomarski optical interference and phase contrast microscopy (x1000, oil immersion) shows the structure always is a gently rounded lobe, finely microtrichose with the hyaline central area that lacks microtrichia. It is easily distorted producing alternative descriptions (e.g., conical, quadrate) by some authors.

Additionally, in Cheng & Wang's (2006) key an Australian species was included as "*Nilotanypus parvus* (Freeman)" but this clearly belongs to *Zavrelimyia* (*Paramerina*). No evidence was provided for its novel generic placement in *Nilotanypus* and was not stated as a new combination. In the same couplet of the key the species *Nilotanypus minutus* (Tokunaga, 1937) appears, seemingly possessing two transverse marks

in the basal one-fourth of the wing, yet this species has no pattern (Tokunaga 1937), or at most, faint brown background (Hiromi Niitsuma, Shizuoka, personal communication, 2022). Indeed, no known species of *Nilotanypus* has such patterned wings in either sex. Just possibly Cheng & Wang (2006) mistook the (correct, plain) wing of Tokunaga (1937: fig. 40) for the next on the Plate (fig. 41), a patterned wing of *Pentaneura maculipennis* Zetterstedt, 1838 (=*Rheopelopia*), although the couplet description does not exactly conform.

Part of the problem in this naive species discrimination is the sole use of the male adult, in small numbers or across limited geographical populations. It is evident that understanding of variation by large samples, inclusion of immature stages and increasing evidence from DNA are required to interpret. These issues, and others, mean that the 'new' species described and the key provided in Cheng & Wang (2006) do not represent the diversity in China, and the 'global' key to males is unacceptable. Unfortunately, these errors in keys were repeated by Shimabukuro *et al.* (2021) although this did not affect the judgment of their new species with madicolous habitat.

Taxon comparisons

Prior indications of the existence of two species of *Nilotanypus* in Australia are supported here by molecular analyses (Fig. 4) from a subset described from elsewhere. However, we cannot simply assume endemicity for each Australian species of Chironomidae, as evidenced, for example, by *Polypedilum* anticus Johannsen, 1932, distributed from Australia through China to Japan (Tang & Cranston 2019). Outside Australia, we consulted the record by Zavřel (1933) of unnamed larvae (as sp. 'Neuer Typus') from Sumatra collected by the Thienemann Sunda-Expedition. This evidently belongs to *Nilotanypus*, but material was not found in ZSM (Martin Spies, ZSM, personal communication. 2022) or the Brno collection of Zavřel material. As it is undescribed it is of no further significance here.

South-east Asian material in ANIC (Brunei and Thailand especially) was examined: as reported by Cranston (2004) the genus was abundant especially in Sungai Belalong, Brunei. A sole species is represented by a pharate male, several pharate females and many pupal exuviae that differ from Australian taxa including in the very extensive corona (c. 50–75% of horn), stronger spinosity of the pupal posterior tergites and sternites, and in the male adult by antennal ratio and robust, bent but not tapering, gonostylus. We compared our material to pupal forms described from Nepal and South India (Roback & Coffman 1987, Roback & Coffman 1989) and can eliminate these from consideration due to thoracic horn morphology and tergal spinulation that lie outside morphological variation seen in Australia.

Further comparisons with non-Australian taxa developed from barcode sequences from males of two species from China, involving use of a key to adult males from Cheng and Wang (2006). One species appeared to be that described as *Nilotanypus polycanthus* Cheng and Wang, 2006 that differed from both Australian taxa on male morphology and barcode but possibly is synonymous with *Nilotanypus minutus*. Based also on barcode, an undescribed species from Hainan Island was postulated as sister to the Australian *N. haplochelus*. The sampled male differs from its putative Australian sister taxon significantly including the strength and arrangement of the frontal setae, and in the stout gonostylus with a clear subapical flange (carina). In potentially 4 Asian species of *Nilotanypus* the following character states were examined: in the adult male, 3–4 inner verticals comprising mostly 2 setae plus 1–2 common (simple) setae; in the anterior section dorsocentral setae in irregular 2–3 rows but strictly uniserial throughout in 1 species; the apical contour of gonostylus variable but with expanded subapex in *N. polycanthus*. In the pupa (male) the pattern of posterior spinulations of TII-VI varies, either with no clear pattern / small spinules or with distinct pebble-like marbled extended spines, and in the number and length of SVIII spines informatively cluster respectively as <15, >20, of lengths >10 µm or <8 µm. In the larva, the relative position of cephalic S5/S8 setae can be informative: thus *N. polycanthus* (and *N. minutus*) resembles *N. ctenochelus* (Fig. 2G), but the gap between S10-VP is larger, in a looser cluster. Differences in the posterior parapod toothed claw(s) also may be useful: in *N. 'polycanthus'* the apical tooth is clearly longer and wide-gapped from smaller inner teeth than in *N. ctenochelus* (Fig. 2P).

Distribution and Ecology

The immature stages of the two morphologies seem to not segregate into preferred aquatic habitats. Apparently, all lotic habitats are used excepting the most polluted and the ephemeral. At some locations they are sympatric and co-temporal as pupal exuviae (Queensland: Davies Ck.; Northern Territory, Djalkmara Billabong and Rockhole Mine Creek). Although quite abundant in warmer running waters in the northern 2/3 of the continent,

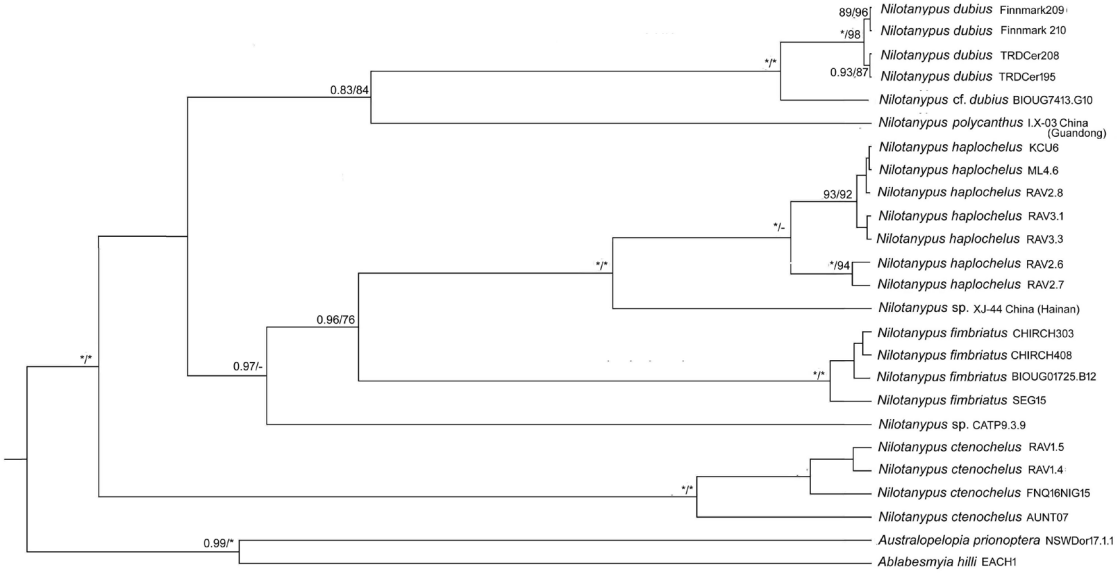

Figure 4. Phylogenetic tree from Bayesian inference for *Nilotanypus* Kieffer and outgroups (Table 1) based on concatenated gene fragments. Posterior probabilities (PP) and Bootstrap support (BS from Maximum Likelihood analysis) are indicated above branches only for nodes with PP > 0.95 or BS > 70. Maximal supported nodes are indicated with an asterisk. A dash (–) for either PP or BS indicates a value below the threshold for support; unlabelled nodes lack support under both criteria.

neither species has been found on offshore islands including Tasmania. Larvae have been considered to be psammophilous (sand-dwelling) and undoubtedly this is substantially correct. Preference for such an unstable substrate may explain why full life stage associations have been elusive because all psammophiles are difficult to rear. Most survive for a lengthy duration in individual rearing vials but eventually die in the 4th instar with some failing as pharate pupae and with no successful emergence. Drift netting shows the genus also may be abundant in cobble-bedded fast-flowing streams lacking the extensive sand accumulations of larger rivers. Given the richness of sampling, it remains a mystery why full associations and more extensive DNA evidence have not been available across all stages.

In the manipulation study in Rockhole Mine Creek, larvae of *Nilotanypus* showed a strong negative response to the addition of acidic mine drainage into the creek and a strong positive response to alleviation of the pollutant by relocation of the adit (Smith & Cranston 1995; fig. 6).

Recently Shimbakuro *et al*. (2021) extended the larval ecology to the madicolous (hygropetric) habitat in Amazonian Brazil and clearly psammophily is no longer the universal habitat preference.

As noted above, the genus prefers warmer latitudes in Australia, and it seems the same preference is exhibited in the neotropics. Although known in meso-America, *Nilotanypus* is reported in South America from Brazil alone, and neither from Patagonia, elsewhere in Argentina (Augusto Siri, CONICET, personal communication 2022) nor from any other Andean country.

Evolutionary tempo of Nilotanypus

Although the monophyly of *Nilotanypus* is irrefutable (Krosch *et al*. 2022), its sister group remains elusive despite recent studies (e.g. Silva & Ekrem 2016, Krosch *et al*. 2017, 2022). Some analyses show *Ablabesmyia* (and adjacent relatives) to be close, with *Australopelopia* (and related genera) at one node removed, albeit without support. We enforced these taxa as outgroups prior to analysis, with no claim as to their relationships.

The two new species of Australian *Nilotanypus* are not each other's sister taxa but are distant in our molecular -based analysis (Fig. 4). With robust support, *N. ctenochelus* is sister to all other sampled congeners, whereas *N. haplochelus* is shallower in the phylogeny and robustly sister to an undescribed species from oriental China (Hainan). Lacking Neotropical material, we cannot assess how Australian species relate to those described from Brazil by Andersen & Pinho (2019) and Shimabukuro *et al*. (2021), which would allow testing inference of Gondwanan vicariance. Absence from New Zealand, and the tropical / subtropical distribution in the neotropics (as in Australia) rejects a cool Gondwanan pattern. That the

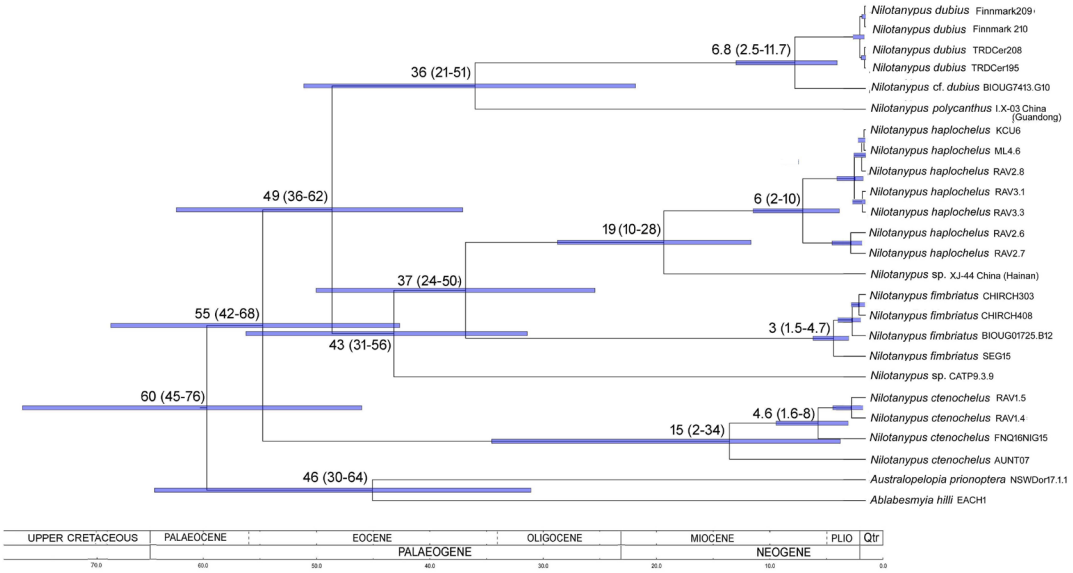

Figure 5. BEAST chronogram from a data set corresponding with Table 1. Values at nodes are time to most recent common ancestor (tmrca) with HPD (95% Highest Posterior Density) intervals in parentheses. The time scale is in millions of years before present.

African species is widespread likely eliminates participation in any austral radiation.

The sister grouping of *N. haplochelus* and the undescribed species from oriental China (Hainan) suggests a relationship between north Australian and oriental Chinese taxa dating to the early Miocene (Fig. 5). At this time the Australian plate, including New Guinea, was making its journey towards Asia, allowing faunal interchange as proposed similarly for *Skusella* and *Conochironomus* in the Chironomini (Cranston 2016, Cranston & Tang 2018, Tang 2018). The presence of pupal exuviae of *Nilotanypus* in Brunei (island of Borneo) and Thailand provides evidence of biogeographic continuity, as does species diversity in China (Cheng & Wang 2006). However, in the absence of reared material further speculation is unwarranted. The pair of taxa is sister sequentially to *N. fimbriatus* (Walker, 1828) (N. America) and then an undetermined species from California, suggesting faunal interchange between the Nearctic and China/Australia in the mid-late Eocene.

Diversification in Australia within *N. haplochelus* took place in the late Miocene/Pliocene, originating somewhat earlier within *N. ctenochelus* with early separation of a monsoonal (Northern Territory) clade from a tropical north Queensland cluster. Although our sampling limits speculation, the radiation within our two sampled Holarctic taxa (*N. dubius* (Meigen, 1804) and *N. fimbriatus*) is congruence around the mid-late Miocene (Fig. 5). However, both these northern hemisphere species concepts include molecular diversity with several BINS in BOLD reflecting cryptic speciation, precluding assessment of the tempo.

Conclusions

Interpreting species segregates is challenging especially in taxa with limited informative morphological variation, even as complete life stages become available. For *Nilotanypus*, subtle characters cannot be understood as species delimiting without guidance from molecular data and vice-versa. Problems include difficulty in associating life stages even of widespread species, and prior descriptions that lack truly diagnostic or even accurate and comprehensive descriptions. With molecular evidence in Australia for two species, we have described and illustrated these in all life stages. By locating them in a wider molecular-based phylogeny for the genus, we show that they are not each other's closest relatives. Collections from outside Australia allow us to understand the genus better, notably that the genotype *N. remotissimus* Kieffer is widespread in Africa under the junior synonym *N. comatus* proposed here, allowing stability in the generic concept. Evidently even 'well known' morphologically-defined species of the northern hemisphere are composites and despite the pioneering work of Roback (1986) intensive study such as ours in Australia still is required to reconcile non-traditional morphology with molecular taxonomy. This project illustrates that isolated descriptions of inadequately described and inaccu-

rately keyed isolated adult males cannot advance our understanding of chironomid biodiversity.

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Two new species of *Monopelopia* Fittkau, 1962 from forests in India along with a key to adult males of Oriental and Palearctic species (Diptera: Chironomidae)

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https://zoobank.org/B1D3FF9C-12C6-493F-8D39-CCF4D70BAE2F

Abstract

Two new species of *Monopelopia* Fittkau, 1962 are described and illustrated from the Oriental region based on adult males and immature stages. *Monopelopia* (*Monopelopia*) *recta* sp. n. and *Monopelopia* (*Monopelopia*) *obscurata* sp. n. are described from India and a DNA barcode of *M. recta* is compared with congeneric sequences in NCBI GenBank. Additionally, a key to the adult males of genus *Monopelopia* reported from the Oriental and Palearctic regions is given.

Introduction

The genus *Monopelopia,* belonging to the tribe Pentaneurini, was erected by Fittkau (1962). The genus is divided into two subgenera *Cantopelopia* Roback, 1971 and *Monopelopia* s.str. (Cranston and Epler 2013; Silva and Ekrem 2016). According to the world catalogue of Chironomidae (Ashe and O'Connor 2009), this genus includes 11 species. Later, six new species from the Neotropical region were described by Oliveira et al. (2010) and Dantas and Hamada (2017) and two from the Oriental region (Paul et al. 2014; Duan et al. 2021). So far, a total of 19 species (15 belonging to *Monopelopia* s.str. and 4 species within *Cantopelopia*) have been described, of which four are from the Oriental region.

The present study includes description of two new species of the genus *Monopelopia* from India. A key to the adult male *Monopelopia* reported from the Oriental and Palearctic regions is also given.

Materials and methods

Adult midge specimens were caught using open light trap and preserved in 70% ethanol. To facilitate association, larvae and pupae were reared individually in glass vials containing water and a small amount of habitat substrate (Epler 1995). Emerged specimens and immature skins were preserved in 70% ethanol. Specimens were slide mounted in Canada Balsam following the technique of Wirth and Marston (1968). The general terminology follows Sæther (1980). All specimens examined are now retained in the collection of insects in the Entomology Division, Department of Zoology, The University of Burdwan, West Bengal, India and will be deposited in the National Zoological Collections (NZCI), Kolkata.

Thorax and one set of legs from one of the collected specimens were processed for DNA extraction using Qiagen DNeasy Blood and tissue kit. The extracted DNA was amplified using cytochrome c oxidase subunit I (COX I) universal primers LCO 1490 and HCO 2198 (Folmer *et al.* 1994) following the protocol of Lin et al. 2018. The amplified products were visualised by 1% agarose gel electrophoresis. The amplified products were outsourced for bidirectional Sanger's sequencing. The obtained sequence, trace files and other details were uploaded to the NCBI GenBank. MEGA X (Kumar et al. 2018), was used to calculate pairwise 2-Parameter (K2P) distances among the fifteen most similar sequences obtained through a BLAST search on NCBI GenBank. The K2P substitution model, 1000 bootstrap replicates, and pairwise deletion option for missing data were used to build the neighbor-joining tree in MEGA X.

Selected abbreviations are: BV – Beinverhältnisse (combined length of femur, tibia and tarsomere 1/ combined length of tarsomeres 2 to 5), SV – Schenkel–Schiene–Verhältnis (length of femur and tibia/ length of tarsomere 1), OR – Oriental region, PA – Palaearctic region, NE – Nearctic region.

Results

***Monopelopia* (*Monopelopia*) *recta* sp. n.**

https://zoobank.org/3E037AAF-2981-42A3-A04C-959B52B4752C

Type Material. Holotype male, labelled '*Monopelopia recta* sp. n. Mondal, Mukherjee and Hazra., India, West Bengal, Matha (23.11, 86.06),

03.VII.2019, Coll. D. Mondal'. Paratypes 3 males, same data as holotype. GenBank accession number: MW168820.

Diagnosis. AR 1.78–1.81 (1.81); squamal setae 15–17 (17); wing with macrotrichia on the distal portion; abdominal tergites I–V with broad anterior bands, T VI–VIII brown; 4–5 stout setae on second palpomere; fore, mid, and hind tibial spurs each with 3 lateral teeth, hind tibial comb with 8 setae; anal point short and conical; gonocoxite bearing with 3 strong dorsomedial setae in a uniform row; T IX with 2 dorsolateral setae on each side.

Etymology. The name '*recta*' is of Latin origin meaning 'straight' referring to inner side of gonocoxite bearing 3 strong basal setae in a straight row.

Description

Male imago (n = 4). Total length 1.92–1.99 (1.98) mm.

Colouration. Head brown. Antenna pale brown, maxillary palp light brown. Thorax dark, vittae pale, antepronotum dark, wing membrane pale, cross vein dark brown, legs pale brown, T I–V of abdomen (Fig. 1D), with dark anterior bands, T VI–VIII brown. Hypopygium brown, megaseta dark.

Head. Eyes with dorsomedial extension 62–66 (62) µm. Antenna with strong preapical seta (Fig. 1A); number of flagellomeres 14, AR 1.78–1.81 (1.81). Temporal setae uniserial, 10–12, postorbitals 2–3. Clypeus with 17–19 (18) setae. Length of palpomeres I–V (µm): 22–27 (23): 28–35 (30): 98–104 (102): 100–108 (104): 116–128 (119); second palpomere with 4–5 long pale setae. CA 0.66–0.70 (0.70). CP 1.29–1.34 (1.32).

Thorax. Scutal tubercle absent. Antepronotum with 3–4 (4) lateral setae; acrostichals 26–28 (26), irregularly biserial; humerals 8; dorsocentrals 15–18 (15) on each side, uniserial in middle and biserial distally; prealars 5–6; scutellars 9–10 (9).

Wing (Fig. 1B). Wing length from arculus 1.15–1.18 (1.18) mm, width 0.39–0.42 (0.40) mm, L/W 2.89–2.98 (2.95). Total length/WL 1.67–1.72 (1.68). WL/ length of forefemur 2.12–2.19 (2.15). Wing membrane with macrotrichia on distal portion; squama with 15–17 (17) setae; brachiolum with 2 setae; vein lengths (µm): C 1045–1055 (1050), Sc 568–577 (575), R_1 445–453 (450), R_{2+3} absent, R_{4+5} 646–652 (650), M_{1+2} 794–807 (800); anal lobe well developed, angular; CR 0.86–0.89 (0.89); VR 0.86–0.88 (0.88).

Legs (Fig. 1C). Fore tibial spur 43–48 (46) µm long bearing 3 lateral teeth; spurs of mid tibia 47–52 (52) µm long bearing 3 lateral teeth; spurs of hind tibia 58–65 (64) µm long, with 3 lateral teeth [not visible in Fig. 1C]. Hind tibial comb with 8 setae. Lengths and proportions of leg segments as in table 1.

Abdomen. T IX with 2 dorsolateral setae on each side (Fig. 1E). Abdominal banding pattern as in Fig. 1D.

Hypopygium (Fig. 1E). Anal point short and conical. Gonocoxite cylindrical, 138–145 (140) µm long, 67–70 (69) µm wide, 2.02 × as long as broad, 3-setal row. Gonostylus simple, slightly curved inwardly, 60–84 (72) µm long, basal width 26–28 (28) µm, Gs/Gc 0.67. Megaseta 9–11 (11) µm long. Phallapodeme 19–22 (21) µm long. HR 1.50–1.72 (1.69); HV 2.70–2.86 (2.83).

Remarks

Some distinguishing male characters of *Monopelopia* (*Monopelopia*) *recta* sp. n. are compared with eight morphologically similar species in Table 2.

The submitted sequences have shown 8.6 % divergence with the closest sequences in GenBank of NCBI (Fig. 2).

Distribution and bionomics. *Monopelopia recta* is so far known only from India. Matha is a dense forested area with deciduous vegetation occupying the eastern fringes of the Chota Nagpur plateau. There are small streams within the forests and trees with tree holes containing water at the time of col-

Table 1. Lengths (µm) and proportions of leg segments of *Monopelopia* (*M.*) *recta* sp. n. Mean values in parentheses.

	fe	ti	ta_1	ta_2	ta_4	ta_5	LR	BV	SV
P_1	520–550 (550)	432–470 (450)	415–440 (425)	260–290 (280)	140–170 (152)	90–120 (102)	0.90–0.95 (0.94)	1.87–1.95 (1.93)	2.06–2.15 (2.13)
P_2	650–688 (675)	456–480 (475)	500–536 (525)	280–310 (300)	152–186 (175)	115–137 (125)	1.02–1.13 (1.11)	1.92–2.02 (2.03)	3.79–3.88 (3.83)
P_3	552–586 (575)	510–545 (525)	–	–	–	–	–	–	–

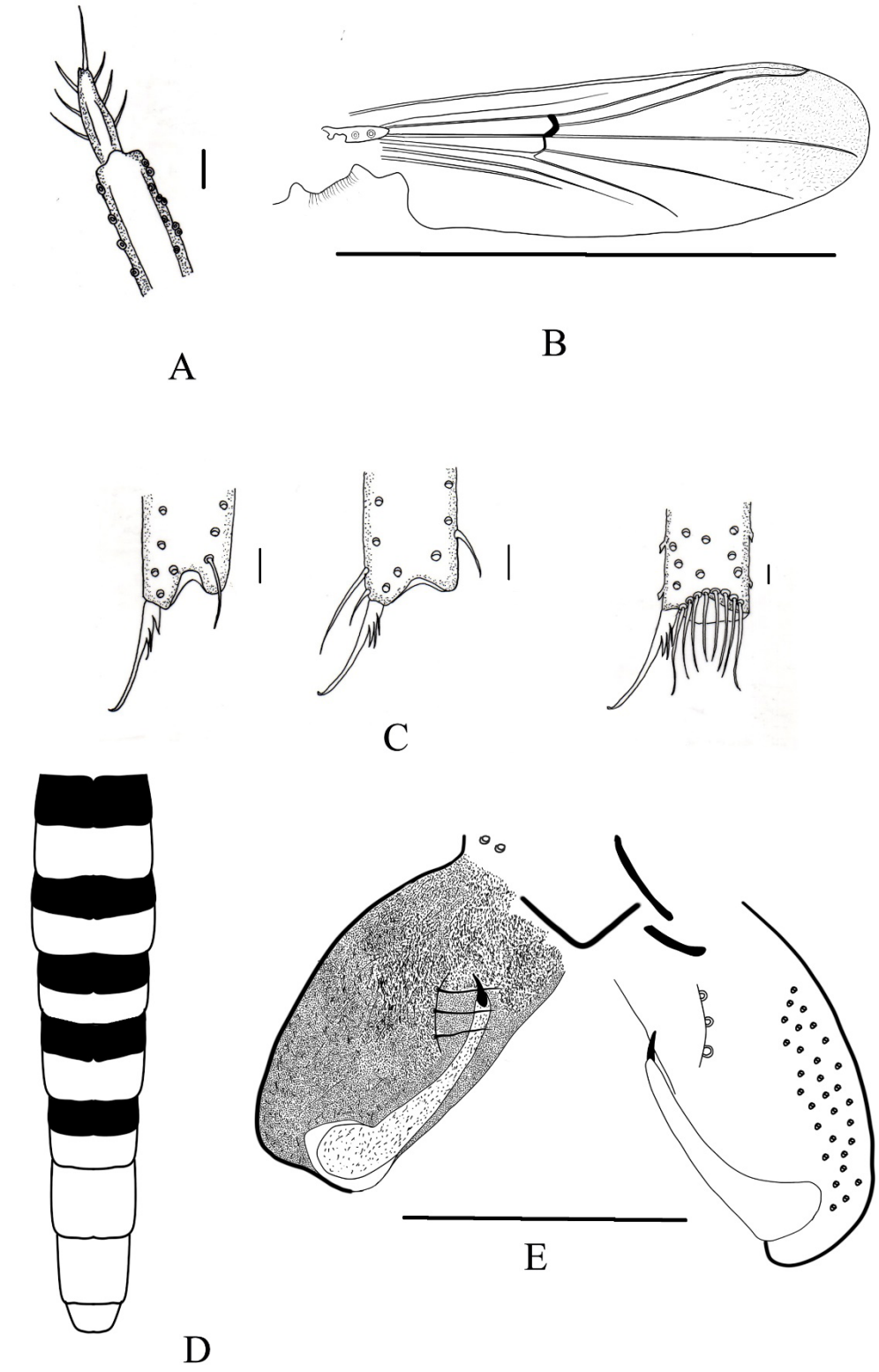

Figure 1. Male of *Monopelopia* (*Monopelopia*) *recta* sp. n. A, ultimate antennal flagellomere, scale: 30 μm; B, wing, scale: 1 mm; C, tibial spurs, scale: 10 μm; D, abdomen showing dark bands; E, hypopygium, scale: 100 μm.

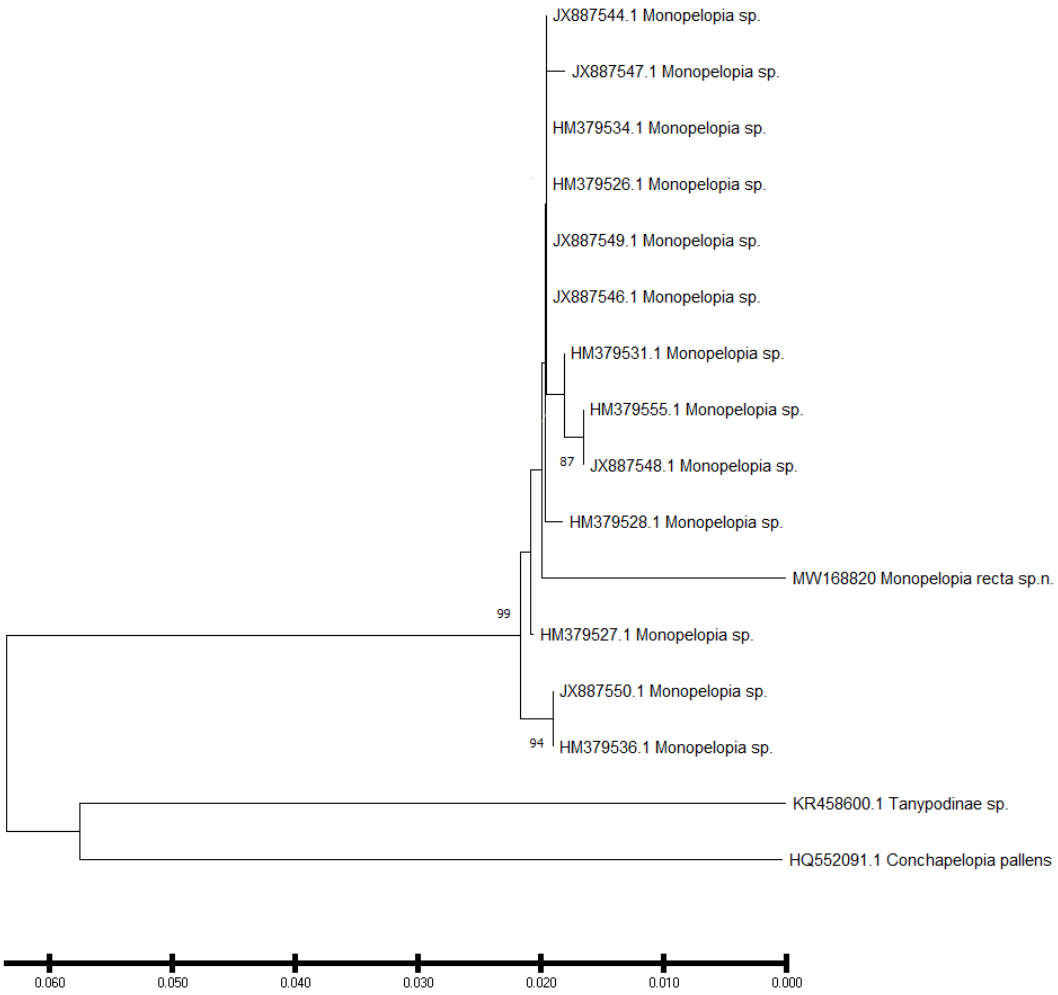

Figure 2. Neighbor-Joining tree based on the COX1 gene sequences of *Monopelopia recta* sp. n. and thirteen most similar sequences from NCBI GenBank. Scale: K2P genetic distance.

lection. The species was collected from light traps set from dusk to dawn using incandescent bulb.

***Monopelopia* (*Monopelopia*) *obscurata* sp. n.**

https://zoobank.org/0A5C24CA-B5A8-489A-AC6A-B5C3F69C385D

Type Material. Holotype male with larval and pupal exuviae (reared), labelled as '*Monopelopia obscurata* sp. n. Mondal, Mukherjee and Hazra, India, West Bengal, Suntaley khola (27.01, 88.78), 03.VII.2019, Coll. D. Mondal'.

Diagnosis. The new species can be separated from other members of the subgenus *Monopelopia* by having the following combination of characters: Male. AR 1.46; wing uniformly covered with dense macrotrichia; darkened r-m cross vein; hind tibial comb with 6 setae; T IX with 2 dorsolateral setae on each side. Pupa. Thoracic horn without acute apical projection, plastron plate occupying distal fifth of thoracic horn, L/W 6.1. Larva. Pecten hypopharyngis with 4 teeth, posterior parapod with one darkened strongly curved claw with 5 inner teeth.

Etymology. The name '*obscurata*' is of Latin origin meaning 'darkened' referring to darkened cross-vein, to be treated as adjective.

Description

Male imago (n = 1). Total length 1.62 mm. Wing length from arculus 1.16 mm, width 0.33 mm, L/W 3.5. Total length / WL 1.39. WL / Length of forefemur 2.44.

Colouration. Head brown. Antenna pale brown, maxillary palp light brown. Thorax brown, vittae dark, antepronotum dark, wings uniform pale except, dark brown cross vein, legs pale brown, abdomen entirely pale brown. Hypopygium brown.

Head. Eyes bare, dorsomedian extension 73.6 µm. Apical seta of antenna (Fig. 3B) 34.5 µm, AR 1.46. Temporal setae 9, uniserial. Clypeus with 28 setae. Length of palpomeres I-V (µm): 27.6: 34.5: 110.4: 115: 128.8. CA 0.66. CP 0.97.

Table 2. Comparison of male adult *Monopelopia* (*M.*) *recta* sp. n. and *Monopelopia* (*M.*) *obscurata* sp. n. with eight morphologically similar species.

	AR	LR_1	Setae in Ti_3 comb	Wing membrane	Squamal setae	Dorsolateral setae on TIX	Median setal row on Gc	Megaseta (μm)	HR	Anal point
M. (*M.*) *iara*	1.13	0.97	9	Sparsely covered with macrotrichia	14	2 each side	Absent	11	1.31	Conical
M. (*M.*) *tenuicalcar*	1.16	0.80	6	Densely macrotrichiose	—	1 each side	Absent	12	1.52	Conical
M. (*M.*) *caraguata*	1.05	0.95	5	Macrotrichiose	13	4 each side	Absent	10	1.58	Long
M. (*M.*) *macunaima*	0.87	0.82	9	Few macrotrichiae over entire wing	12	3 each side	Absent	12	1.44	Broad based, conical
M. (*M.*) *edentata*	0.91	0.88	—	Few macrotrichiae over entire wing	12	4 each side	Absent	13	1.35	Broad based, conical
M. (*M.*) *mongpuense*	1.12	1.08	8	Macrotrichiose progressively denser toward the apex	18	3 each side	Absent	16	1.44	Short, straight
M. (*M.*) *adeliae*	1.17	0.75	7	Sparsely covered with macrotrichia	21	3 each side	Absent	14	1.35	Conical
M. (*M.*) *obscurata* sp. n.	1.46	1.04	6	Densely macrotrichiose	14	2 each side	Absent	13.8	2.0	Broad based, conical
M. (*M.*) *recta* sp. n.	1.81	0.94	8	Macrotrichiose distally	17	2 per side	Present	11	1.69	Short, conical

Thorax. Scutal tubercle and pit absent. Antepronotum with 2 lateral setae; acrostichals 32, irregularly biserial; dorsocentrals 19 each side, biserial anteriorly and uniserial posteriorly; prealars 5; scutellars 9.

Wing (Fig. 3A). Wing membrane with dense macrotrichia; squama with 14 setae; brachiolum with 2 setae; vein lengths (µm): C 980, Sc 475, R_1 375, R_{4+5} 550, M_{1+2} 700, R_{4+5} ending long before M_{1+2}, anal lobe round, poorly developed; CR 0.84; VR 0.86.

Legs. Tibial spurs as in Fig. 3C. Ti I spur 39.1 µm long; Ti II spur 41.4 µm long; Ti III spur 52.9 µm long; hind tibial comb with 6 setae. Length (µm) and proportions of leg segments as in Table 3.

Abdomen. T IX with 2 dorsolateral setae on each side.

Hypopygium (Fig. 3D). Anal point conical in shape with broad base. Gonocoxite 135 µm long, 51 µm wide, L/W 2.64. Gonostylus simple, curved inwardly, 64.4 µm long, basal width 18.4 µm, Gs/Gc 0.75. Megaseta 13.8 µm long. Phallapodeme 48.3 µm long; HR 2; HV 2.53.

Pupa (n = 1)

Colouration. Exuviae pale yellow without apparent pattern.

Total length. 2.58 mm.

Cephalothorax. Frontal apotome triangular. Wing sheath 968 µm long. Thoracic horn (Fig. 4A) tubular, 285 µm long, 46.7 µm broad without apical spine, surface with scattered broad–based spinules, ThR 6.1, plastron plate egg-shaped, 142 µm long, 84 µm wide occupying 0.38 length of horn; respiratory atrium tubular, about a third of the width of Th, walls thick with narrow duct-like lumen, basal lobe reduced. Dc_1 112 µm long, Dc_2 111 µm long and Sa 86 µm long.

Abdomen (Figs. 4b–c). Scar on tergite I 128 µm long, elongate and without pigmentation. Tergites I–VIII without shagreen, 4 LS setae on tergite VII located at 0.27, 0.47, 0.62 and 0.91 respectively from anterior margin; tergite VIII with 5 LS setae located 0.36, 0.50, 0.73, 0.87 and 0.98 respectively from anterior margin. Anal lobe 320 µm long, 265 µm wide; L/W 1.2, outer margin with 6 spinules, male genital sacs 351 µm long, 187 µm wide, not extending beyond apices of anal lobes, G/F 1.09, L/W 2.70.

Fourth instar larva (n = 1)

Total length 3.2 mm.

Colouration. Pale yellow.

Head. Cephalic index 0.49. Antenna (Fig. 5A). AR 3.54; length of antennal segments I–IV (µm): 253, 59.8, 4.6, 6.9; ring organ situated 0.54 from base; blade 55 µm long, accessory blade 51 µm long. Mandible (Fig 5B.). 69 µm long; apical tooth 23 µm long, basal tooth 16.1 µm long; A1/MD 3.67. Maxilla (Fig 5C.). Basal segment 32.2 µm long; ring organ situated 0.46 from base. Mentum and M appendage (Fig. 5D). Two small dorsomental teeth reduced, 4 µm long, on each side of base. Pseudoradula 69 µm long with distally coarser granulation. Ligula (Fig. 5E). 54 µm long, with 5 subequal teeth forming slightly concave margin; paraligula 34.5 µm long, bifid. Pecten hypopharyngis with 4 teeth.

Cephalic chaetotaxy (Fig. 5F). Dorsal seta. S7 and S8 closely placed each other and along with S5 formed acute angle. Ventral seta. VP and SSm directly medial; S_{10} further anterolateral; S_9 even further anteromedial.

Body. Anal tubules cylindrical, 94.3 µm long, 25.3 µm wide; supra-anal setae 264.5 µm long. Procercus 88 µm long and 33 µm wide with 7 apical setae. Length of sub basal setae of posterior parapod 128 µm. total number of setae 4; 2 long claws each with 4 and 2 inner teeth, short claw one with 2 inner teeth and another strongly curved claw with 4 inner teeth (Fig. 5G).

Table 3. Length (µm) and proportions of leg segments of *Monopelopia* (*M.*) *obscurata* sp. n.

	Fe	Ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV
P_1	475	382	400	232	158	98	76	1.04	2.22	3.69
P_2	600	410	501	256	195	110	88	1.22	2.32	4.33
P_3	512	450	–	–	–	–	–	–	–	–

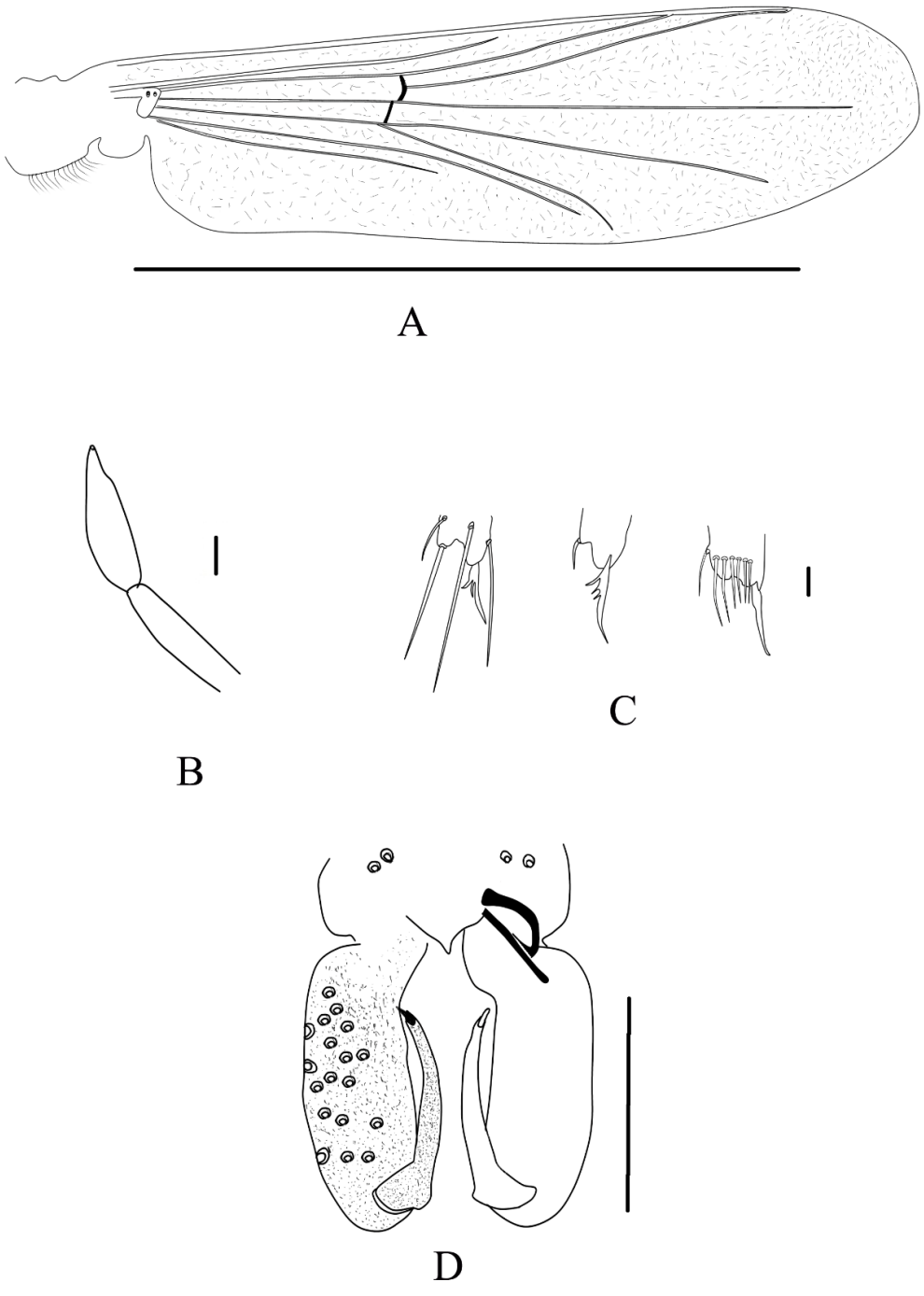

Figure 3. Male of *Monopelopia* (*Monopelopia*) *obscurata* sp. n. A, wing, scale: 1 mm; B, ultimate flagellomere, scale: 30 µm; C, tibial spurs, scale: 10 µm; D, hypopygium, scale: 100 µm.

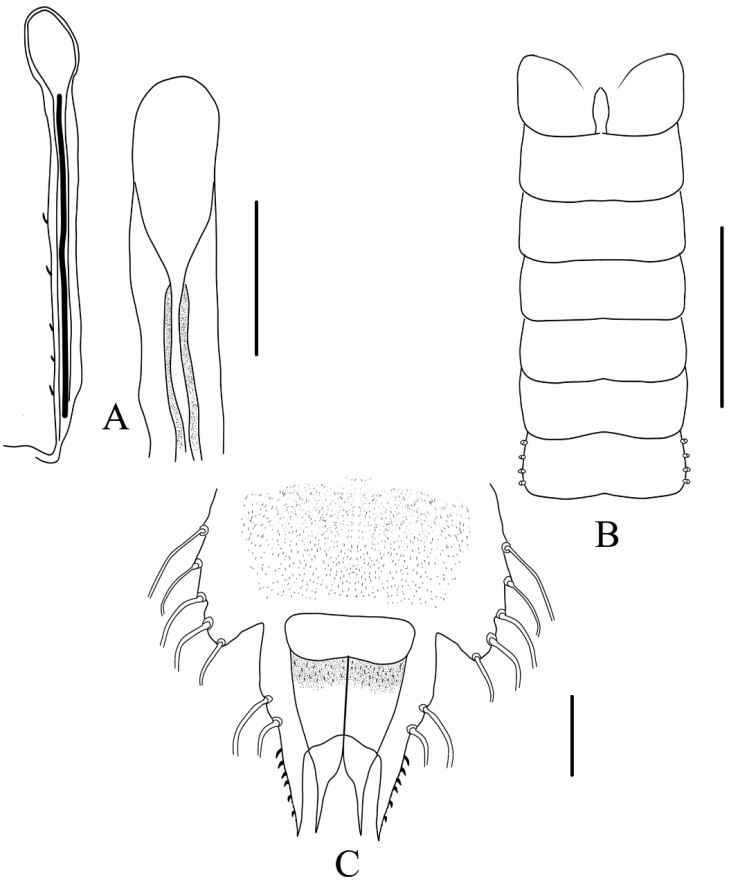

Figure 4. Pupal exuvia of *Monopelopia* (*Monopelopia*) *obscurata* sp. n. A, thoracic horn, scale: 100 µm; B, abdominal TI-TVII, scale: 1 mm; C, tergite VIII and anal lobe, scale: 100 µm.

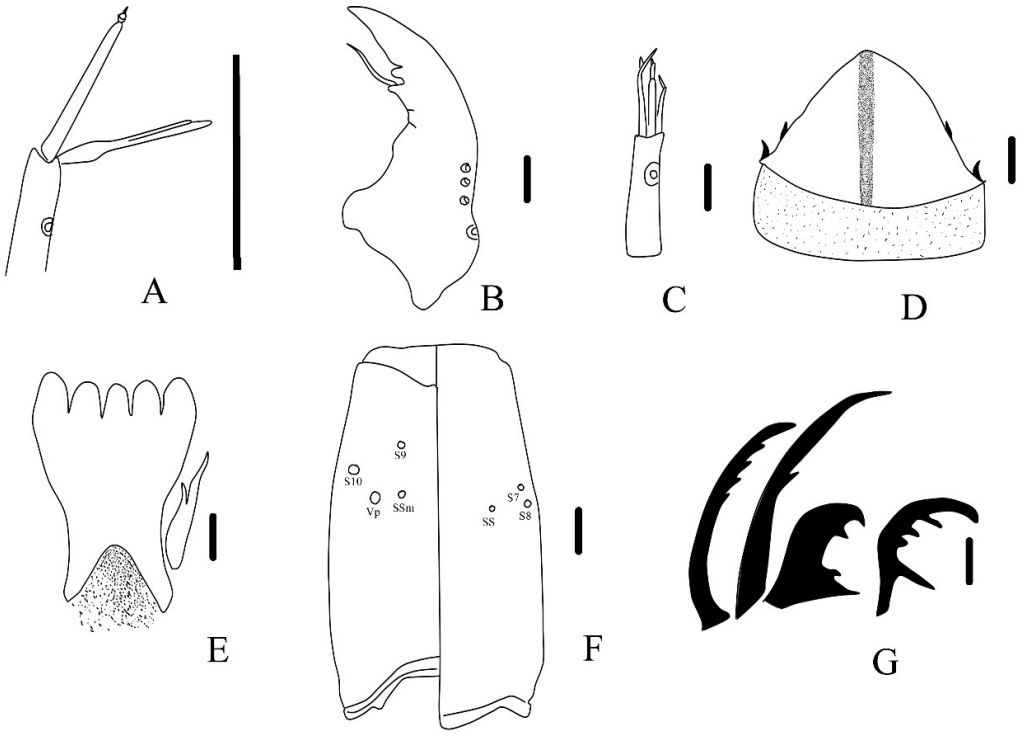

Figure 5. Larva of *Monopelopia obscurata* sp. n. A, antenna, distal end, scale: 100 µm; B, mandible, scale: 10 µm; C, maxillary palp, scale: 10 µm; D, mentum and m appendage, scale: 10 µm; E, ligula and paraligula, scale: 10 µm; F, cephalic chaetotaxy; G, claws of posterior parapod, scale: 10 µm.

Table 4. Comparison of *Monopelopia (M.) obscurata* sp. n. immature characters with four other species.

	Thoracic horn	ThR	Shagreen on pupal exuviae	Location of LS-VII	Loc. LSV-VIII	Male genital sac	Head- CI	AR	A1/ MD	PP claws
M. (*M.*) *macunaima*	Large robust	11.2	TII with patches of 2-5 spinules	0.30: 0.38: 0.53: 0.84	0.25: 0.5: 0.62: 0.75: 0.87	Not extending	0.71	2.26	2.10	Smaller claws indistinct, larger claws serrated
M. (*M.*) *mongpuense*	Tubular without spine	4.02	Dense in TII	0.29: 0.42: 0.57: 0.84	0.27: 0.49: 0.69: 0.85: 0.96	Not extending	0.53	3.26	2.79	4, heavily sclerotised claw deeply curved with 4 teeth on inner margin
M. (*M.*) *adeliae*	Large and robust	5	Absent from TI-TVIII	0.30: 0.46: 0.61: 0.92	0.42: 0.57: 0.71: 0.78: 0.92	Not extending	0.70	2.73	2.08	4, heavily sclerotised claw deeply curved with 2 teeth on inner margin
M. (*M.*) *edentata*	Elongated slender with spine	5.8	Absent from TI-TVIII	0.23: 0.38: 0.76: 0.92	0.31: 0.43: 0.56: 0.72: 0.95	Extending beyond anal lobe	0.70	2.40	1.82	4, pale
M. (*M.*) *obscurata* sp. n.	Slender elongated without spine	6.1	Absent from TI-TVIII	0.27: 0.47: 0.62: 0.91	0.36: 0.50: 0.73: 0.87: 0.98	Reaching the tip of anal lobe	0.49	3.54	3.67	4 dark claws, one strongly curved with 3 inner teeth

Remarks

A comparison among *M. mongpuense*, *M. recta*, *M. adeliae*, *M. macunaima*, *M. edentata* and *M. obscurata* sp. n, is given in Table 2 and Table 4.

Distribution and bionomics. *M. obscurata* is so far known only from India.

Suntaleykhola is a dense forested area with temperate climate, occupying the eastern fringes of the Himalayan foothills. The larva was collected from a marshy area at the bank of a small stream.

Key to males of *Monopelopia* Fittkau from the Oriental and Palaearctic Regions

1. Mid and hind legs with single tibial spur (subgenus ***Monopelopia*** Fittkau) 2

– Mid and hind legs with two tibial spurs (OR: Oriental China) .... ***M.* (*Cantopelopia*) *zhengi* Lin, 2021**

2 (1). R_{2+3} present 3

– R_{2+3} absent or faintly indicated 4

3 (2). Macrotrichia cover entire wing surface (OR: Indonesia) .... ***M.* (*M.*) *divergens* (Johannsen, 1932)**

– Macrotrichia cover distal third wing surface only (OR: India) ..... ***M.* (*M.*) *mongpuense* Paul, Hazra and Mazumdar, 2014**

4 (2) AR < 1.2; r-m crossvein pale (PA: Europe; NE: Canada, U.S.A.) .... ***M.* (*M.*) *tenuicalcar* (Kieffer, 1918)**

– AR > 1.2; r-m crossvein darkened 5

5 (4) AR 1.5; wing surface entirely covered with macrotrichia; abdomen without dark bands (OR: India) ..... ***M.* (*M.*) *obscurata* sp. n.**

– AR 1.8; wing surface with macrotrichia covering distal third; abdomen with dark bands (OR: India) ..... ***M.* (*M.*) *recta* sp. n.**

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A case of phoresis of midges on Zygoptera

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Several midges live phoretically on different invertebrates, as was described by Thienemann in his book *Chironomus* (Thienemann 1974) and may be found in many papers published since. For example, I found some time ago a *Podonomus* living on the gastropod *Chilina dombeiana* (Prat et al. 2004). This is a classic topic in midge studies; I found the first revision of the topic in White et al. (1980). Phoresis between Chironomidae and Odonata was one of the most common phoretic associations. Last year, two colleagues working on Odonata sent me several larvae of midges living on Zygoptera larvae. In the first sample (Fig. 1), tubes of *Rheotanytarsus* larvae were attached to *Calopteryx virgo meridionalis*. Larvae of the midge inside the tubes were very small. In the second sample examined, cases were simple tubes, and the midges were from the genus *Paratanytarsus*, and were present on *Calopteryx haemorrhoidalis* and *Calopteryx xanthostoma* (R. Martin pers. comm.). Both samples are from the River Tordera, in Catalonia (NE Spain). More details of the sites and the number of larvae found, and the instar of the Odonata may be found in Martin and Maynou (2021). Although phoresis between Chironomidae and Odonata has been frequently described over many years, the picture of this occurrence may be of interest to people working on midges.

Figure 1. Cases of *Rheotanytarsus* sp. on *Calopteryx virgo meridionalis* larvae. Photo: Ricard Martin.

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The *Chironomus* species studied by Letha Karunakaran in Singapore, with a review of the status of selected South-East Asian *Chironomus*

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Abstract

In the 1960s Letha Karunakaran studied the chironomid fauna of Singapore but faced a lack of sufficiently detailed descriptions to enable identification of her material with any certainty. She recognized seven species of *Chironomus* (s.s) but sent me fixed larval material of only four of these which she tentatively identified as *C. apicatus* Johannsen 1932, *C. costatus* Johannsen 1932, *C. javanus* Kieffer 1924, and *C. stupidus* Johannsen 1932. She sent fixed larvae to me for confirmation of her identifications, but died before I was able to determine accurate identities from morphology alone. With additional comparative material, along with polytene chromosome banding patterns and DNA barcode sequence from the mitochondrial COI gene, the species have been identified as a form of *C. flaviplumus* (auct, not Tokunaga)(here called *C. flaviplumus* Type B), *C. circumdatus* Kieffer 1916, probably *C. striatipennis* Kieffer 1910, and *Kiefferulus barbatitarsis* (Kieffer 1911), respectively. The identification of one species as a form of *C. flaviplumus* required an assessment of the present state of knowledge of this species where the name has been applied to at least five different species. Determination of a valid name for this species is not currently possible. The confusion of species identification is an indication that there are a number of closely related species which constitute a "*C. flaviplumus* group".

Introduction

Letha Karunakaran worked on Chironomidae in Singapore from the 1960s to the early 1970s, when she tragically died in a fire that took her life and consumed her collection. When Letha began her studies, essentially the only taxonomic descriptions of Malaysian midges were those of Johannsen (1932), from which Letha concluded that four of the species she considered to belong in the genus *Chironomus* were *C. apicatus* Johannsen 1932, *C. costatus* Johannsen 1932, *C. javanus* Kieffer 1924 and *C. stupidus* Johannsen 1932 (transferred to *Stictotendipes* Lenz, 1937 by Sublette and Sublette 1973) and placed in *Nilodorum* Kieffer, 1921 by Alfred and Michael (1990), a resemblance noted by Johannsen (op.cit.), but considered to be *Kiefferulus barbatitarsis* (Kieffer 1911) by Cranston (2002). Her studies were included in her unpublished Ph.D. Thesis (1969) and in the report of nematode parasitism in an adult identified as *C. costatus* (Karunakaran 1966). In her thesis she also included *C. striatipennis* Kieffer 1910, correctly identified (see below), and *C. bicoloris* Tokunaga 1964, which may be an undescribed species.

In the hope that I might be able to confirm these identifications from cytological analysis of the polytene chromosomes of the larvae, she sent me samples of four of her species. At that time there was no information on the cytology of southeast Asian species, and they could not be identified morphologically. The slides and the fixed larvae (in 3:1 ethanol/acetic acid fixative) remained in my collection until the group of Prof. Rudolph Meier in Singapore began identifying the local chironomids by barcoding and contacted me to see if I knew the identity of the Karunakaran specimens. It was only at this time that I learned that all Letha's specimens had been lost and the few larvae that she had sent to me were probably all that remained. With this in mind, I began to study the material again.

Material & Methods

The samples comprised 3 larvae of presumed "*C. apicatus*", 7 larvae of "*C. costatus*"; 3 larvae of "C. *javanus*" and 1 specimen of *C. stupidus*. Specimens for comparison were available from India, Indonesia, Japan, Malaysia, Singapore and Thailand. Morphological and cytological analyses were by the usual methods (Martin et al. 2006). Where appropriate, the larval body was mounted on the same slide as the chromosome squash. A couple of Karunakaran's specimens were able to be barcoded for the conventional mitochondrial cytochrome c oxidase subunit I (*COI*) fragment (Hebert et al. 2003) using the Folmer et al. (1994) primers: LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). However, the condition of the larvae was such that

the barcode region had to be amplified in two sections, using the unpublished reverse primer COI-Na-2 (5'- AGATAAAGGKGGATAAACWGTTCA-3') for the 5' section, and the forward primer of Carew et al. (2013) (5'- CCHCGAATAAATAATATAAGWTTYTG-3') for the 3' section, with PCR products sent to Macrogen Incorporated. Seoul, Republic of Korea for sequencing. The resulting sequences were compared to sequences in BOLD, including those mined from GenBank and sequences from my material that have been lodged in GenBank: the *C. costatus* sequence is ON406921 and the *C. apicatus* sequence is ON406926. Other sequences are GenBank accessions ON406917-920 and ON406921-928 for *C. flaviplumus* Type B, and AF192215, KT212957-976 for *C. circumdatus* Kieffer 1916. Sequences identified as *C. incertipenis* Chaudhuri and Das 1996, or *C. ramosus* Chaudhuri *et al.* 1992 were obtained from the BOLD database and from GenBank (KY835558, KY846714, MN934105-MN934321).

The slide mounted specimens will be lodged in the Lee Kong Chian Natural History Museum

(National Museum of Singapore). Morphological abbreviations follow Sæther (1980), and for some larval characters from Vallenduuk and Moller Pillot (1997).

Results and Discussion

These studies have indicated that some of Letha's identifications were correct: An identification of *Kiefferulus barbatitarsis* was consistent with the subsequent synonymising of *C. stupidus* with that species (Cranston 2002). This species was readily recognised as a species of *Kiefferulus* by the presence of only one pair of ventral tubules, the sclerites of the dorsal head and the long narrow ventromentum. Amplification of DNA was unsuccessful, but the characters of the head matched those of *K. barbatitarsis* in Figs. 45 and 47 and the key of Cranston (2007). The immature stages were described by Chaudhuri and Ghosh (1986). The present specimen has a darkened posterior half of the gula.

The specimens noted as *C. costatus* proved, on the basis of the polytene chromosome banding patterns and the BARCODE sequence, to be *C. circumdatus.* This species has been well characterized for morphology (Martin and Saxena, 2009), polytene chromosome cytology (Alfred and Michael, 1990, Kumar and Gupta 1990, Pramual et al. 2009) and by mt*COI* barcoding (Pramual et al. 2016), and the *C. costatus COI* sequence had better than 90% homology, so the identification was quite simple. However, the actual identity of *C. costatus* has not been clarified and it seems likely that more than one species was included under this name since Lenz (1937) lists four larval types for the species.

The identity of the specimens called *C. apicatus* is not simple and reflects the general state of uncertainty over the identity of *Chironomus* species of Southeast Asia. *C. apicatus* was initially described as a variety of *C. costatus* (Johannsen, 1932), but the barcode results suggest it is not so closely related to that species. Rather, the mt*COI* sequence corresponds to those in a BOLD bin where most specimens are identified as *C. flaviplumus.* However, specimens identified as *C. flaviplumus* also occur in three other BOLD bins, indicating that the current concept of this species encompasses a number of species in the "*C. flaviplumus*-group". This group would also include other species such as *C. yoshimatsui* Martin and Sublette (1972) (one of the species incorrectly identified as *C. flaviplumus* in the BOLD database), *C. circumdatus, C. incertipenis* Chaudhuri and Das (1996), *C. ramosus* (Chaudhuri et al., 1992) and the Japanese concept of "*Chironomus samoensis*" (e.g. Kikuchi and Sasa, 1990).

C. flaviplumus was originally described by Tokunaga (1940) from Saga, Kyoto, Japan, but Sasa (1978) states that the description was very brief and not illustrated. Sasa (1978) redescribed the species from Japanese material, but not from the type locality. He lists the important features as a foreleg ratio of 1.6-1.8 and a relatively long anterior Ta5 which is about 0.35-0.4 of the length of the anterior Ti. However, in a later paper, Sasa and Hasegawa (1983) give a much broader range of values (including Ta5/Ti values of only 0.25) which could suggest that they had material of more than one species. Such a conclusion is supported by *COI* sequences attributed to *C. flaviplumus* from Japan being in two different BOLD bins. One is recorded only from Japan (called Type A), while the other is broadly distributed through Japan, Malaysia, Singapore, Thailand, India, Pakistan and also Israel (called Type B). Since both types occur in Japan, it cannot be determined with certainty which is *C. flaviplumus sensu* Tokunaga (1940), although Type A better fits the few known characters from Tokunaga's original description.

The situation is further complicated in that material of Type B from Pakistan is mostly listed as *C. incertipenis* and some Indian material as *C. ramosus* Chaudhuri, Das and Sublette (1992). I have a number of specimens of Type B from various locations, confirmed by the *COI* barcode sequence. A detailed comparison of

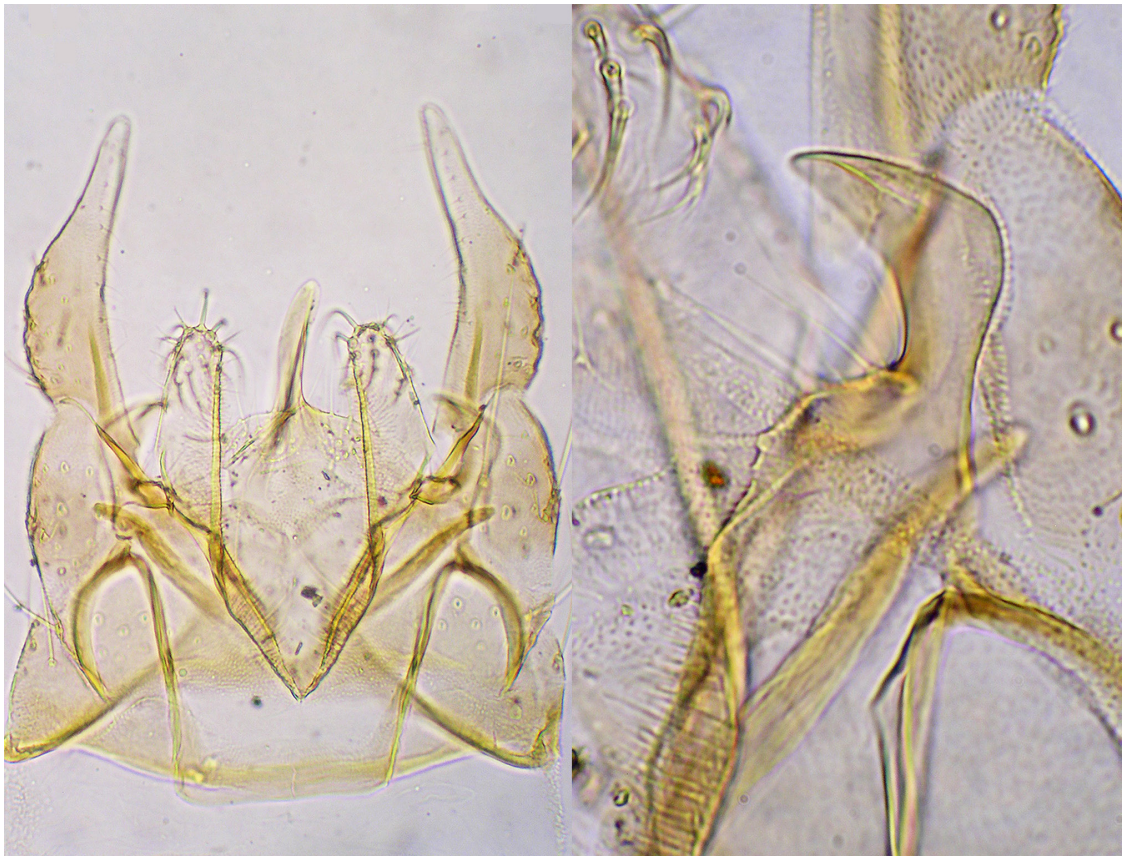

Figure 1. Male hypopygium of *Chironomus flaviplumus*-Type B. Note that the anal point is not black and not sharply turned-down, and that the SVo is beaked and not gently curved.

the *COI* sequences under these three names showed there were the same nine polymorphic bases (i.e. 1.2% variation) regardless of the name applied. Thus, there is no indication of multiple species in this material.

The adult males among these specimens have an AR of 2.94 (2.84-3.05) and an anterior LR of 1.65 (1.59-1.75), so any species to be considered for the name of Type B should also have similar values. *C. incertipenis* was created as a new name for *Chironomus niger* Chaudhuri, Das and Sublette (1992) since that name was preoccupied. While the AR and LR of *C. incertipenis* are within the range for Type B, the critical character that led Das *et al*. to originally call it *C. niger,* was the dark, sharply downturned anal point. Specimens of *C. flaviplumus* Type B do not have this dark anal point (Fig. 1), but a more usual yellow-brown one. As well, the SVo of *C. incertipenis* is described as gently curved, while that of *C. flaviplumus* Type B is strongly curved and beaked (Fig. 1). Therefore, an association of the name *C. incertipenis* with this taxon is unlikely, as noted by Pramual *et al.* (2016), although the types should be re-examined to confirm the accuracy of the original description of this species.

In the case of the name *C. ramosus*, as used by Laviad-Shirit et al. (2020) and Sela et al. (2021), the original description of the adult male indicates that it is a smaller species, with an AR of 3.86 (3.73-3.94) and an LR of 1.4 (Chaudhuri et al., 1992), outside the range of values for Type B. As well, the polytene chromosomes show some significant differences, notably that the nucleolus is on arm B (Nath and Godbole 1997), while in *C. flaviplumus* Type B it is on arm F, near the centromere (Martin, 2022). Therefore this name is not applicable to this species. Currently there is no obvious name for this taxon but the descriptions of many Oriental *Chironomus* species do not include the critical characters, so it is not appropriate to describe it as a new species until existing names, particularly *C. incertipenis,* can be ruled out.

The third type (Type C) was initially known only from *COI* sequence in GenBank (KP902730 & -31 from China and KT213029-038 from Thailand). However, in BOLD they have 99.5% homology to a sequence called ChironomidaeGC sp. 7 from Queensland, Australia. Other specimens from Australia indicate that these are not *C. flaviplumus* but a related species with the manuscript name of *"C. orientalis"* (Martin 2022).

With that explanation, we return to the question of the identity of the other two species sent by Letha Karunakaran. Her *C. apicatus* does not appear to fit the description of this species since LR1 is lower (1.59-1.75) c.f. 1.85 in *C. apicatus*, and the larvae of *C. apicatus* are found in salt ponds and a pool at 29°C and pH 2.83 (Lenz 1937). However, it can be easily placed as *C. flaviplumus* Type B on the basis of larval morphology, cytology and *COI* sequence.

The remaining species in the material sent to me was labelled as *C. javanus.* The original description by Kieffer (1925) other than being a greenish species is not definitive, but the redescription by Johannsen (1932) is likely correct. The Lenz (1937) description of the larva states only that it is "plumosus" type. Chaudhuri *et al*. (1992) listed *C. vitellinus* Freeman 1961 as a synonym, which is likely correct as the larvae characteristically have a premandible with 6 or 7 teeth and specimens identified with this premandible type have been recorded as *C. javanus* from Micronesia (Tokunaga 1964), through northern Australia (Freeman's original description of *C. vitellinus*), Singapore, Malaysia (Al-Shami et al. 2012), India (Chaudhuri et al. 1992) and to Malawi in Africa (larvae sent to me by A. McLachlan).

However, the larvae from Letha had the more usual two-toothed premandible of *Chironomus*, so do not fit the usual concept of *C. javanus.* The chromosomes were of very poor quality and the larvae were slide mounted before DNA sequencing was available. Therefore, while it seems that the material she sent was not *C. javanus* Kieffer, an accurate identification is not easy. More to the point, the morphology of the larvae do not fit that provided in her thesis – that description and the accompanying figures are much more like *C. javanus* but do not mention the premandibles, probably because the multitoothed nature in *C. javanus* was not recorded at that time. One possible explanation for the difference is that she accidentally sent larvae of one of her other species (*C. striatipennis* Kieffer 1910 or *C. bicoloris* Tokunaga 1964). She notes, for example that the anterior pair of ventral tubules are longer (true of *C. javanus*), while in the larvae I received the posterior pair of ventral tubules are longer – which is the situation in *C. striatipennis* and *C. bicoloris.* The darkened gula head coloration and other larval characters (e.g. mentum of Ty II, see below) strongly suggest it is *C. striatipennis* rather than *C. bicoloris*.

Further to the identity of these last two species: *C. striatipennis* should be easily recognizable by the patterned wing, but whether they were the more common Type 1 or the rarer Type 2 (Pramual *et al.* 2016) can currently only be determined from DNA analysis. *C. bicoloris* was described only on the basis of adults. I have a small number of reared specimens from northern Australia which fit Tokunaga's (1964) description of *C. bicoloris*. The two pupae have one and two spines (Fig. 2) on the spurs which are not spine-like as illustrated by Karunakaran (1969). Her illustration of the larval mentum is also slightly different - it is Type II of Vallenduuk and Moller Pillot (1997), i.e. 4th lateral tooth reduced to the height of the 5th lateral, while in the Australian larva is Type I (Fig. 2) i.e. 4th lateral in line with other lateral teeth. As well, *C. bicoloris* has not been identified elsewhere in south east Asia, so it is possible that Karunakaran's material was an undescribed species.

In summary: Letha Karunakaran did quite a commendable job in the identification of her *Chironomus* specimens given the difficulty even today of identifying many species and that she was largely working without specialist assistance. I have been able to confirm that her identification of *C. stupidus,* and quite possibly *C. striatipennis* and *C. javanus,* were correct. DNA sequence confirmed that her *C. costatus* was the well-known *C. circumdatus* and that her *C. apicatus* was Type B of the extensive but not well defined "*C. flaviplumus* group". If nothing else this analysis highlights the difficulties involved in trying to accurately identify the *Chironomus* species of southeast Asia on the basis of morphology and, even where DNA barcode data is available, the sequence may have been attributed to an incorrect species in the BOLD database.

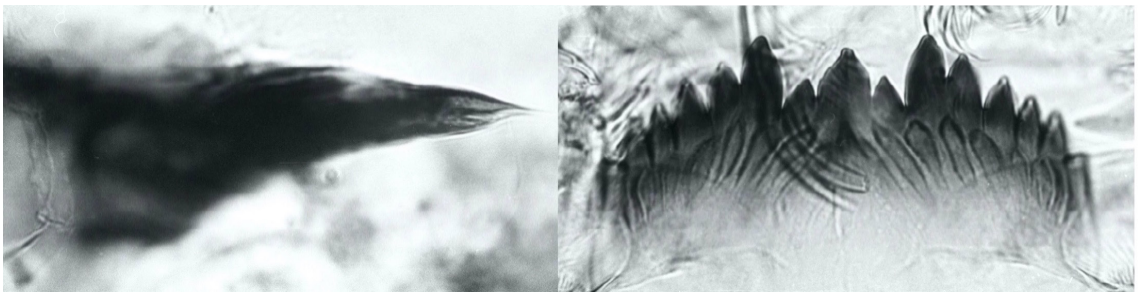

Figure 2. Pupal spur (left) and mentum (right) of *C. bicoloris.*

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Where the rare species hide: a new record of *Parachironomus monochromus* (van der Wulp, 1874) for Slovakia from artificial urban waterbodies

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Abstract

Parachironomus monochromus (van der Wulp, 1874) was recorded for the first time in Slovakia in an urban pond and a city fountain along with a total of 27 other chironomid taxa recorded both as larvae and pupal exuviae. Our finding emphasizes the role of urban waterbodies as habitats for rare species and for maintaining and documenting aquatic biodiversity in cities.

Introduction

Urban waterbodies are common but usually neglected habitats by limnologists (Davies et al. 2009). Knowledge on the exact number and distribution of urban ponds in cities on a global scale as well as contribution of these waterbodies to biodiversity remains fragmentary (Hassall et al. 2016). Even though there is a general pattern of biodiversity decline from rural areas to the urban core (McKinney 2008), previous studies have shown that ponds within urban areas can provide considerable biodiversity habitat (Hassall and Anderson 2015). Even city fountains, as extremely simple temporal aquatic habitats, can support high diversity and unusual communities (for review see Čerba and Hamerlík 2022). To emphasize the importance of small urban waterbodies and how they harbour unknown diversity, here we present a new record of *Parachironomus monochromus* for Slovakia from an urban pond and a city fountain.

Material and methods

The study sites are located in the city of Banská Bystrica, Slovakia, central Europe. With about 80 thousand inhabitants, it is the sixth most populous city in Slovakia. The pond is located in the suburbs, in the garden of the Matej Bel University and is surrounded by a meadow and scattered apple trees (Fig. 1, left). It harbours a population of Koi carp (*Cyprinus carpio haematopterus* Martens, 1876) and aquatic plants (genera *Myriophyllum, Nymphaea* and *Typha*) fill up most of its water volume.

The fountain is located in the historic centre of the city on the main square surrounded by historic buildings and impermeable stone pavement (Fig. 1, right). Basic characteristics of the sites are presented in Table 1.

Chironomid pupal exuviae (CPET, Wilson and Ruse 2005) were collected from the water surface using a circular net (mesh size 0.5 mm) from the end of April to the end of October in weekly (pond) or biweekly (fountain) intervals. Larvae collected accidentally in the drift samples were also processed in the study. The

Figure 1. View of the urban pond (left) and city fountain (right) in Banská Bystrica, where *Parachironomus monochromus* exuviae were collected. Photo: S Bartóková and L Hamerlík.

Table 1. Basic characteristics of the study sites.

		Pond	Fountain
Latitude		48°44´29.03”N	48°44’07.1”N
Longitude		19°07´26.30”E	19°08’42.5”E
Altitude (m a.s.l.)		401	357
Max depth (cm)		100	60
Area (m^2)		15	62
Temperature (°C)	mean	15.8	17.4
	max.	22.5	22.9
	min.	5.4	12.3
pH	mean	7.7	8.5
	max.	8.6	8.9
	min.	6.4	6.8
Conductivity (mS cm^{-1})	mean	259	531
	max.	343	664
	min.	45	412
No. of chironomid taxa		18	16

material collected was preserved with 4% formaldehyde and transferred to the laboratory where organisms were hand sorted. Pupal exuviae were mounted on permanent slides and identified using a compound microscope (400 × magnification) with a reference to Langton and Visser (2003). The material is deposited at the Department of Biology and Ecology, Matej Bel University, Banská Bystrica, Slovakia.

Results and discussion

The surveyed urban waterbodies harbored 16 to 18 chironomid species (see Table 2). In the pond, Chironomini, such as *Chironomus* spp*., Dicrotendipes lobiger* and *Paratanytarsus laccophilus* dominated, while in the fountain orthoclads, especially *Psectrocladius limbatellus* and *Cricotopus sylvestris* prevailed.

An important discovery was the documentation of *Parachironomus monochromus* (van der Wulp, 1874) which is the first record of this species in Slovakia. A total of 19 pupal exuviae of *P. monochromus* were recorded in an urban pond between 12 May and 7 September, 2021. Additionally, 4 pupal exuviae were collected in a city fountain on 27 August, 2021.

Parachironomus monochromus belongs to the *P. arcuatus* group sensu Moller Pillot (1984). *P. monochromus* has Palaearctic distribution and occurs in the majority of European countries (Sæther and Spies, 2013). The species has been recorded mainly from small waterbodies, such as pools and ditches, however few records are known from oligotrophic to eutrophic lakes and flowing waters (Moller Pillot 2013 and references therein). In terms of pH, Moller Pillot (2013) suggest the species is mostly known from waterbodies with generally high pH, with only one record from a pool with pH between 5 and 6.

Table 2. List of chironomid taxa found in the surveyed urban waterbodies. PE = pupal exuviae, L = larvae.

Taxon/ Site	Urban pond		City fountain	
	PE	L	PE	L
Tanypodinae				
Monopelopia tenuicalcar (Kieffer, 1915)	-	+	-	-
Macropelopia nebulosa (Meigen, 1804)	-	-	+	-
Procladius (*Holotanypus*) *choreus* (Meigen, 1804)	+	-	+	-
Zavrelimyia sp.	-	+	-	-

Taxon/ Site	Urban pond		City fountain	
	PE	L	PE	L
Orthocladiinae				
Acricotopus lucens (Zetterstedt, 1850)	+	+	+	-
Corynoneura scutellata gr.	-	+	-	-
Cricotopus (*Isocladius*) *ornatus* (Meigen, 1818)	-	-	+	-
Cricotopus (*Isocladius*) *reversus* Hirvenoja, 1973/ *intersectus* (Staeger, 1839)	+	-	+	-
Cricotopus (*Isocladius*) *sylvestris* (Fabricius, 1794)	-	-	+	-
Cricotopus (*Isocladius*) *trifasciatus* (Meigen, 1813)	+	-	-	-
Eukiefferiella coerulescens (Kieffer, 1926)	-	-	+	-
Limnophyes sp.	-	+	-	-
Orthocladius (*Eudactylocladius*) *fuscimanus* (Kieffer and Thienemann, 1908)	+	-	+	-
Paracricotopus niger (Kieffer, 1913)	-	-	+	-
Paratrichocladius rufiventris (Meigen, 1830)	-	-	+	-
Psectrocladius (*Psectrocladius*) *limbatellus* (Holmgren, 1869)	+	+	+	-
Chironominae				
Chironomus spp.	+	+	+	-
Dicrotendipes lobiger (Kieffer, 1921)	+	+	-	-
Dicrotendipes modestus (Say, 1823)	+	-	-	-
Glyptotendipes (*Glyptotendipes*) cf. scirpi (Edwards, 1929)	-	+	-	-
Parachironomus monochromus (Wulp, 1858)	+	-	+	-
Polypedilum cf. *nubeculosum* (Meigen, 1804)	-	-	-	+
Polypedilum nubifer (Skuse, 1889)	-	-	-	+
Polypedilum (*Pentapedilum*) cf. *uncinatum* (Goetghebuer, 1921)	+	-	-	-
Micropsectra lindrothi (Goetghebuer, 1931)	-	-	+	-
Paratanytarsus laccophilus (Edwards, 1929)	+	-	-	-
Paratanytarsus bituberculatus (Edwards, 1929)	+	-	-	-
Tanytarsus mendax (Kieffer, 1925)	-	-	+	-

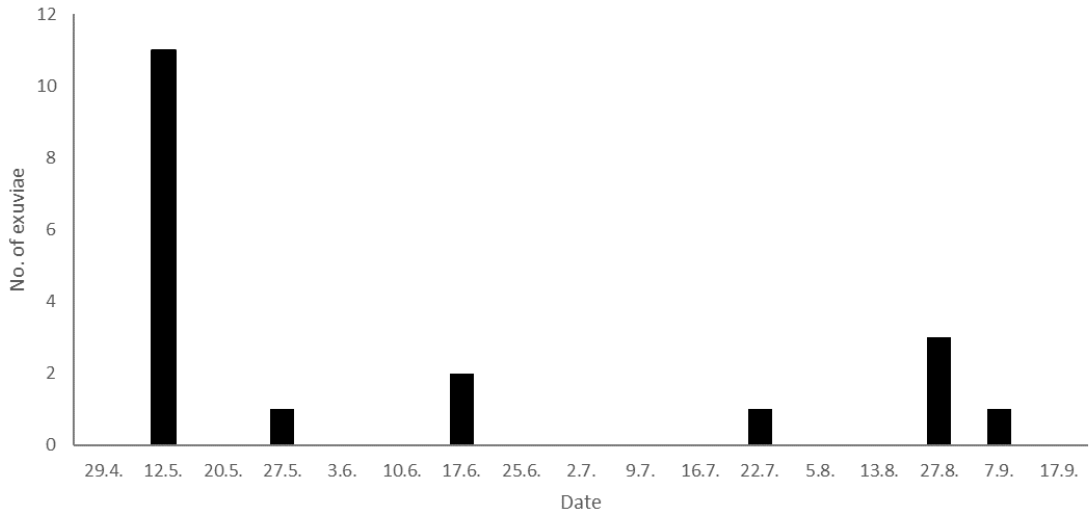

Figure 2. Number of *Parachironomus monochromus* exuviae recorded in the urban pond during the study period. Dates on x-axis refer to sampling dates. Dates after 17. 9. 2021 are not shown due to the absence of the species in the samples.

In the urban pond, pupal exuviae were recorded on several occasions between May and September with the highest numbers collected in May (Fig. 2). In the fountain, the species was collected at the end of August. This emergence pattern is in accordance with references in Moller Pillot (2013) reporting emergence of *P. monochromus* adults from April to September. In some cases, however, the highest density was documented in the summer, while in the case of our urban pond, summer emergence was scarce.

All in all, the documentation of a new species from two different urban water bodies within the same city - in a country with relatively well-studied chironomid fauna - emphasizes the importance of urban ecosystems as valuable habitats for not only aquatic biota in general, but also for rare species. This stresses the significance of including these habitats in more intense ecological research.

Acknowledgements

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Nematodes infest winter-active chironomids in Minnesota trout streams

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Abstract

We present preliminary findings of the effects of nematode infestation on winter-active chironomid biology, and how short-term temperature spikes could affect host-parasite interactions. Results are limited but indicate nematodes may infest winter-active chironomid communities and significantly affect host chironomid biology. Further research on winter-active insects should include investigations into nematode parasitism to better understand how climate change will affect chironomid survival at the population and community level.

Introduction

Winter-active chironomids are abundant in temperate regions, and are especially common in groundwater-fed streams in the Midwestern US (Bouchard and Ferrington 2009). These chironomids are key members of winter food webs in aquatic and terrestrial ecosystems, and are important food sources for brown trout in Minnesota trout streams (Anderson et al. 2016). Winter warming from climate change will likely have detrimental effects on winter-active chironomids because of their extreme cold-adaptation (Anderson et al. 2022).

Many nematodes are obligate parasites of arthropods (Kiontke and Fitch 2013). Research on parasitic nematodes has focused on the biological control of pestiferous insects (e.g., Edmunds et al. 2017) or the developmental and morphological effects on the host insect (e.g., Bhattacharya et al. 2014). However, little research has explored the ecological consequences of nematode parasitism in chironomid populations.

We collected cold-adapted chironomids for analyses of longevity and reproduction in the winter of 2021. Incidentally, we discovered many chironomids were parasitized by nematodes. This note reports our limited findings on how nematodes affect winter-active chironomids to encourage further work on this topic.

Materials and Methods

Chironomidae collections and temperature treatments

Chironomidae were collected from two groundwater-fed streams in 2021: Ike's Creek in Bloomington, MN (21 February 2021) and Pickwick Creek in Winona County, MN (4 January 2021) following protocol from Ferrington et al. (2010). The adult midges were placed in a 6°C incubator, a temperature consistent with previous studies on chironomid longevity (e.g., Anderson et al. 2022). The chironomids were split into three equal-sized treatment groups to investigate potential impacts of winter temperature spikes on longevity and reproduction. The control group was kept at constant 6°C and treatment groups were exposed to 22°C for 24 or 48hrs before returning to 6°C. One group of midges from Ike's Creek was also exposed to constant 22°C due to larger sample sizes than Pickwick Creek. Individual chironomids were inspected daily to record longevity, reproduction, and nematode emergence. Dead midges were preserved in >70% ethanol for taxonomic identification. *Diamesa* Meigen, 1835 identifications were made using Hansen and Cook (1976). Male Orthocladiinae were identified using Oliver and Dillon (1989) and female Orthocladiinae were identified using Sæther (1977).

Statistical analysis

All statistical analyses were conducted with RStudio (v.1.4.1717, R Core Team 2021), and figures were produced with packages *ggplot2* (Wickham 2016), *survival* (Therneau 2020), and *survminer* (Kassambara et al. 2021). Only Ike's Creek Orthocladiinae females were used in statistical analyses because of small numbers of Orthocladiinae males and parasitized chironomids from Pickwick Creek. Differences in survivorship due to temperature treatments and nematode parasitism were assessed with boxplots and Kaplan-Meier analyses. Non-parametric tests were performed to test differences in boxplots because of small sample sizes. Log-rank tests were used to analyze Kaplan-Meier curves.

Results and Discussion

Chironomidae collections summary

The composition of chironomids varied by stream (Table 1). The majority of chironomids collected from Pickwick Creek were *Diamesa* sp. with few other species present, whereas the majority of chironomids collected from Ike's Creek were in the subfamily Orthocladiinae (Table 1). The longevities of Orthocladiinae and Diamesinae were significantly different across all temperature treatments and both streams (Wilcoxon, p=0.019). The longevities of male and female *Diamesa* sp. were not significantly different across streams (Wilcoxon, p=0.081). Conversely, the longevities of male and female Orthocladiinae were significantly different (Wilcoxon, p=0.002), with females living longer than males.

Table 1. Summary of total collected chironomid taxa, including number of chironomids and number and percent of parasitized chironomids in each taxon.

	Stream					
	Ike's Creek			Pickwick Creek		
Taxon	N°	N° Parasitized	% Parasitized	N°	N° Parasitized	% Parasitized
Total Orthocladiinae	179	22	12.3%	2	0	0
Males	67	2	3.0%	2	0	0
Females	112	20	17.9%	0	0	0
Total *Diamesa*	36	0	0	295	8	2.7%
Total males	22	0	0	221	0	0
D. mendotae	11	0	0	218	0	0
D. nivoriunda	11	0	0	3	0	0
Females	14	0	0	74	8	10.8%
Total	215	22	10.2%	297	8	2.7%

NOTE.– *Diamesa* females could only be identified to genus using available keys. Two additional chironomids were collected from Pickwick Creek: one Chironominae specimen and one chironomid specimen too decomposed to identify.

Nematode parasitism summary

In Pickwick Creek, there were 20 nematodes found only in female *Diamesa* (Table 1). Most parasitized *Diamesa* sp. were parasitized by two nematodes, with up to four nematodes in a single midge. Conversely, in Ike's Creek, there were 43 nematodes found only in Orthocladiinae, with the majority of parasitized Orthocladiinae being female (Table 1). The parasitism rate was significantly different between male and female Orthocladiinae from Ike's Creek (Fisher's Test, p=0.004). The majority of parasitized Orthocladiinae were parasitized by one nematode, with a few having two or three nematodes, and one midge with eight nematodes.

Nematode parasitism altered chironomid biology

Surprisingly, the presence of nematodes significantly increased female Orthocladiinae longevity from Ike's Creek in our constant 6°C, 22°C/24hr, and 22°C/48hr treatment groups (Fig. 1a). A small increase in longevity was observed in parasitized females in the constant 22°C group, but this difference was not significant (Fig. 1a). We found similar increases in longevity in parasitized females compared to non-parasitized females using a log-rank test and Kaplan-Meier survival analysis, but this increase was only significant in constant 6°C individuals (Fig. 1b). Parasitized female Orthocladiinae appeared to have greater survivorship early in life, but maximum survivorship did not surpass that of non-parasitized females (Fig. 1b).

We did not statistically analyze parasitized *Diamesa* sp. longevity from Pickwick Creek because sample sizes were small. There were no nematodes that emerged from *Diamesa* sp. at constant 6°C. However, we found mean longevities of parasitized *Diamesa* were 9.2 and 5.7 days in the 22°C/24hr and 22°C/48hr groups, compared to 12.3 and 10.7 days of non-parasitized *Diamesa* sp., respectively. Future studies could determine whether this decrease in longevity with parasitism is significant, as it contradicts our findings from parasitized Ike's Creek Orthocladiinae.

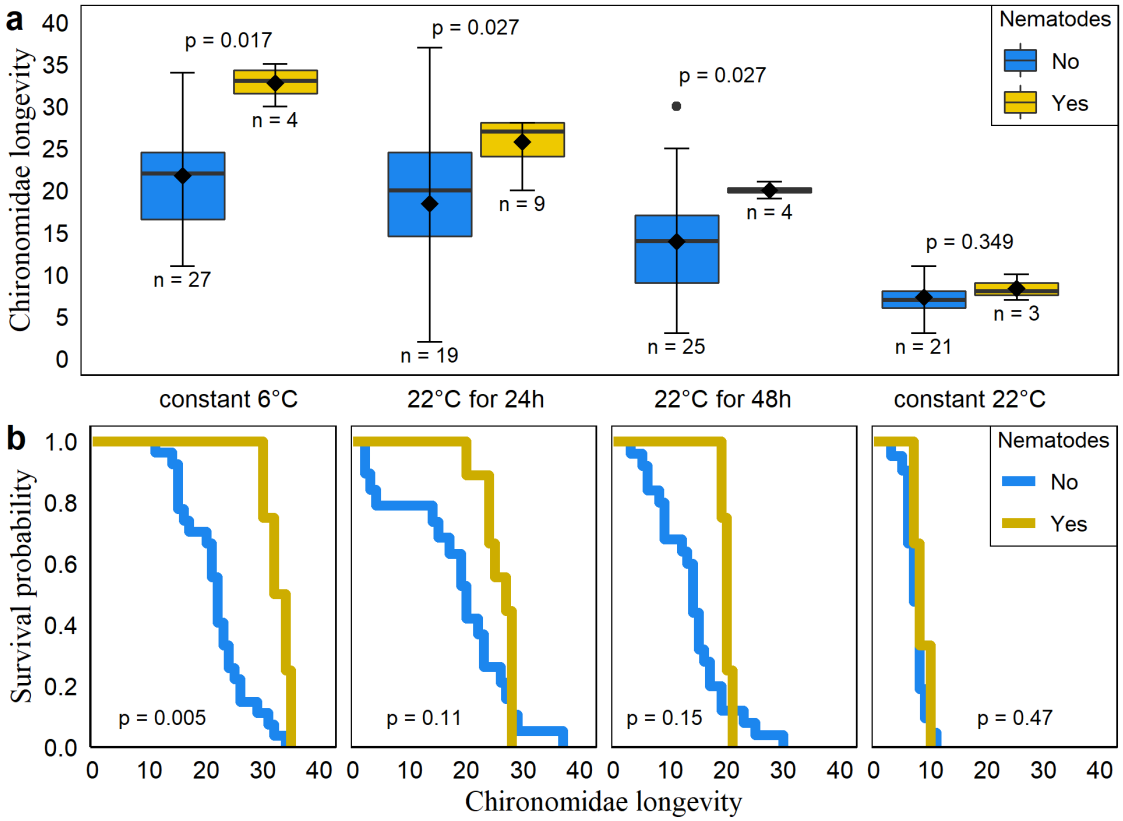

Figure 1. Female Orthocladiinae longevity and survival probability with and without nematode parasitism in Ike's Creek. Longevity defined as days lived post collection. (a) Box and whisker boundaries signify the maximum, 75th percentile, median, 25th percentile, and minimum longevity values. Black diamonds (◆) indicate mean longevity and dots (•) indicate outliers. Wilcoxon tests with Benjamini & Hochberg multiple test corrections were used to determine statistical differences between parasitized and non-parasitized individuals. (b) Kaplan-Meier survivorship curves indicate the proportion of individuals alive on a given day. Log-Rank tests were used to determine statistical differences between parasitized and non-parasitized individuals.

Conclusion

Our findings are preliminary due to small sample sizes and a lack of nematode identifications. However, we demonstrate nematodes are present in winter-active chironomid populations in Minnesota and nematode parasitism affects chironomid longevity. Further research is needed to determine how nematodes affect winter-active chironomid populations, and in turn, how climate change may disrupt these community dynamics.

Acknowledgements

We are grateful for the late Len Ferrington's guidance and mentorship during this project, and we thank the many members of the Chironomidae Research Group for their support, especially Bruce Vondracek for his comments on this manuscript. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR) under grant M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 03i awarded to Len Ferrington.

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The 21st International Symposium on Chironomidae – 2022 online

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After its original announcement in Chironomus No 32 (Cornette 2019), the 21st International Symposium on Chironomidae has been postponed to summer 2022 due to the COVID-19 pandemic (Cornette 2021). At that time, the symposium was still planned as an in-person meeting in Tsukuba, Japan. However, the arise of the Omicron variant led Japan to close its borders and, in such conditions, we had no other choice but to organize this symposium online.

The 21st International Symposium on Chironomidae is now expected to be held online, between the 4th and the 7th of July 2022. The symposium website is now open to public at the following link:

https://kinki-convention.jp/isc2022/

Please mark your calendars and visit our site for registration and abstract submission (we welcome oral and poster presentations as well).

Five years after last symposium in Trento, Italy, we hope that this meeting will be the occasion of fruitful exchanges on the recent progresses in chironomid research. We expect different sessions focusing on various topics such as systematics, ecology, biomonitoring or recent advances in Chironomidae genomics and molecular biology. The big challenge will be to organize a taxonomy workshop as convivial as possible within the framework of an online symposium. Please check the announcement section of the website for recent updates about the symposium.

We look forward to seeing all of you at the 21st International Symposium on Chironomidae!

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BOOK: Chironomidae of Central America: An Illustrated Guide to Larval Subfossils

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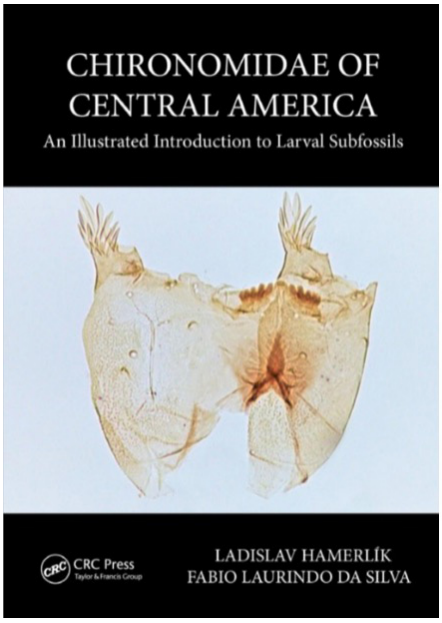
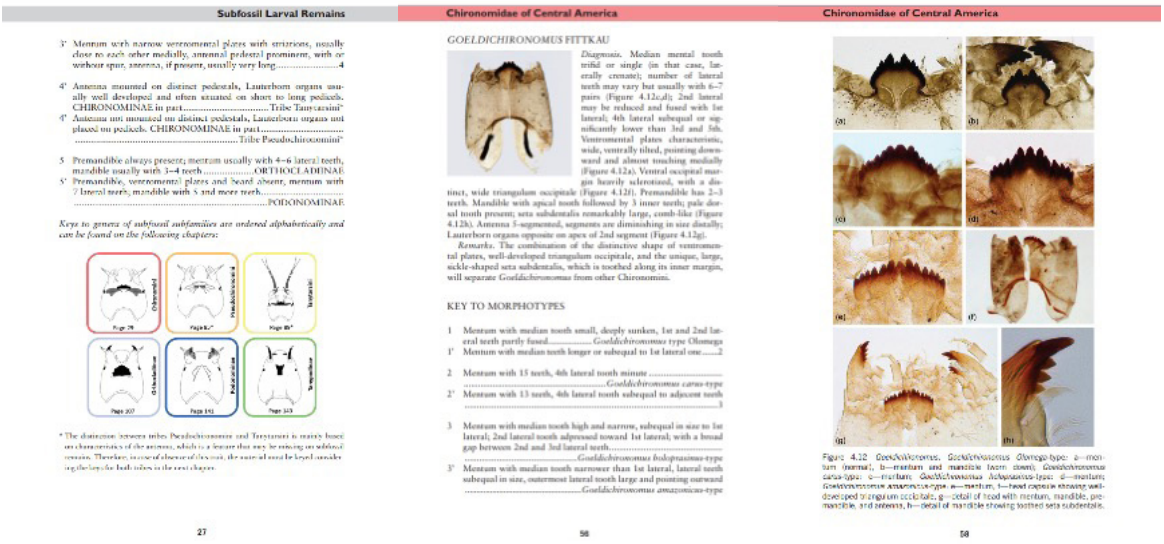
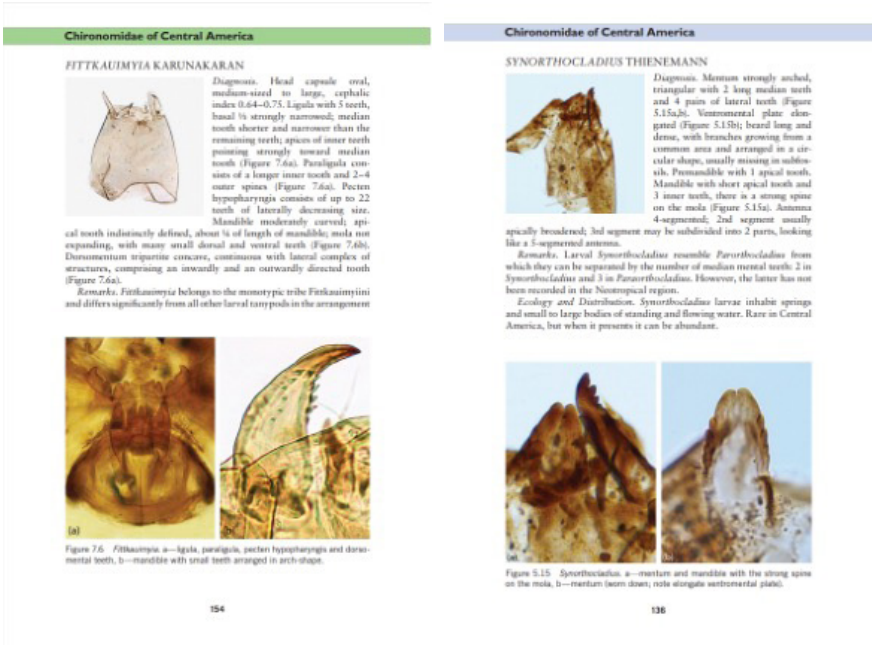
The illustrated introduction to Central American Chironomidae offers extensive photography material, as well as detailed morphological and ecological description of chironomid subfossils found in Central American lake sediments. The book uniquely provides two identification keys: one for living larvae occurring (or potentially to be found) in Central America, and one for the recorded subfossil remains, using limited morphological characters.



Paleolimnological investigations using chironomid remains have undergone a resurgence of interest, and this taxonomic guide will aid the thorough analysis of the diversity and distribution of the taxa encountered to date in Central America. On 189 pages, the book contains almost 300 original photographs of 64 genera (and more than 100 morphotypes), of which about a third are endemic to the Neotropical region, and absent in Brooks et al (2007). Plates are included for each taxon with



Sample of pages from the book Chironomidae of Central America: An Illustrated Guide to Larval Subfossils.



Sample of pages from the book Chironomidae of Central America: An Illustrated Guide to Larval Subfossils.

generic characters. Keys to morphotypes are provided, if applicable, along with their specific diagnostic characters, distribution and ecology.

Authored by a (paleo)limnologist and a taxonomist, this guide draws on a thorough taxonomical knowledge of the region's recent chironomid fauna. It uses a paleolimnological approach to transmit this information to morphotypes that can be linked with ecology and used to reconstruct the past development of nature. The guide is primarily addressed to researchers working with both subfossil and recent larvae not only in Central America, but in the whole Neotropical region. Moreover, the guide will also be of interest to non-academic professionals working on applied research and biomonitoring of lakes, providing a comprehensive reference for aquatic ecologists, palaeolimnologists, students and researchers.

The book can be purchased here: https://www.routledge.com/Chironomidae-of-Central-America-An-Illustrated-Introduction-To-Larval-Subfossils/Hamerlik-da-Silva/p/book/9780367076061

Patrick (Paddy) Ashe 18.03.1954 – 19.06.2022

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I first met Paddy Ashe 49 years ago when, as a 19 year old student, he enrolled in the Faculty of Science in University College Dublin in September 1973 at the time when Carmel F. Humphries (who had studied with August Thienemann in Plön) was Professor and head of the Department of Zoology and I was a member of the academic staff. During the second year of his studies Paddy often consulted me about course matters. He had a particular interest in zoology with an inquiring mind, seeking information additional to course content and frequently asking about my own specialist area of research in freshwater ecology and insect taxonomy. That was when he first heard the term *Chironomidae*.

Paddy Ashe 2016. Photo: Declan Murray.

Paddy achieved a high standard in his second year examinations and easily qualified to enrol in the honours zoology degree course for a further two years. On entering year 3 of the 4-year BSc. Honours Zoology degree course in September 1975, Paddy approached me and expressed a particular interest in joining my research group. At that time we were investigating the freshwater insect fauna in southwest Ireland, where the Limnology Research Unit of the Zoology Department was coordinating a major multi disciplinary project on the rivers and lakes of the Killarney Valley. On discussing various options, Paddy indicated that he would particularly like to be involved in our ongoing studies on the chironomid fauna of the River Flesk. He knew that we had discovered several interesting species, including the second record in Europe of *Buchonomyia thienemanni*, as well as several undescribed pupal morphotypes that included exuviae of *Eurycnemus crassipes* and other Orthocladiinae of taxonomic interest. He joined my small research group and this introduction to ecological studies on chironomids in 1976 was the beginning of his lifelong taxonomic interest in non-biting midges.

He prepared an undergraduate thesis based on material collected in April, July, September and December 1976 and in April 1977, during field trips to the site on the River Flesk where *B. thienemanni* had been found. He graduated in September 1977 with a BSc Honours degree in Zoology. After graduation, Paddy asked if I would "take him on" as a PhD candidate. I readily agreed and he immediately commenced his PhD studies on a more detailed investigation of the ecology and taxonomy of the Chironomidae of the River Flesk. This was largely based on specimens regularly collected by drift nets in 1978 and 1979, at several locations on the river from its source to its point of entry to Lough Leane, at Killarney. During this period Paddy was increasingly absorbed by the multiple taxonomic and nomenclatural issues in the Chironomidae and he acquired an exceptional knowledge of details, and interpretation, of the Zoological Code. Consequently the scope of his doctorate research was expanded into two parts; Part I giving an account of his studies in the River Flesk, and Part II to be a literature-based study to clarify and resolve taxonomic issues in the Chironomidae at generic and subgeneric level by compiling an up to date Catalogue of chironomid genera and subgenera of the world. He submitted his thesis in November 1982. The external examiner of the thesis reported "*Part I is a very valuable faunistic work and Part II is of very high importance for Chironomidae research in general…..since a long time we have needed such a catalogue of Chironomid genera – everybody in the world dealing with aquatic insects will be thankful of the basic contribution to make systematics in Chironomidae more comprehensible"*. Paddy was conferred with the degree of PhD in Spring 1983. The catalogue, Part II of his thesis, was published as Supplement No. 17 of *Entomologica scandinavica.*

Paddy Ashe (center in dark sweater) at the banquet in Dunguaire Castle, Kinvarra, County Galway during the excursion following the Dublin Chironomidae Symposium, 1979. Upper row from left: NN (not chironomidologist), Endre Willassen, NN (not chironomidologist), Gail Grodhaus, H. Ryser, I. Evaldsson, B. Krebs, Godtfred Halvorsen, Paddy Ashe, A. Kovacs, Ernst Josef Fittkau, Helen Roback, Lars Brundin, Umberto Ferrarrese, Claus Lindegaard, Henk Moller-Pillot. Lower row from left: Friedrich Reiss, Colette Dowling, Dave Rosenberg, Ole Sæther, Bernhard Lindeberg, Georgy Devai, Selwyn Roback, Freddie Murray. Photo: Declan Murray.

Irish Biogeographical Society meeting in Kennedy's Pub, Lincoln Place Dublin, 2016. James P. O'Connor (Editor), Declan Murray(Executive), Paddy Ashe (Chairman), John Walsh (Treasurer), Mark Holmes (Executive). The display case in the background contains publications of the Society. Photo by Freddie Murray (Executive).

After graduation Paddy held a temporary post-doctoral position in Trinity College Dublin. He went on to participate in the Royal Entomological Society of London sponsored expedition "*Project Wallace*" in 1985 and, immediately after attending the Chironomidae Symposium in Bergen in 1985, he travelled to Indonesia spending almost 6 months researching the diversity of the chironomid fauna in the rainforest of Sulawesi. On his return to Ireland he had a temporary research period in the Zoology Department of University College Galway and simultaneously established himself as a freelance entomologist. He commenced consultancy work for commercial businesses and State Institutes, including the Irish Forestry Service that regularly called on his expertise, until he became unwell in November 2021. Throughout his working career as a freelance consultant entomologist Paddy was never distracted from his main interest and remained a very active researcher on Chironomidae. He maintained lasting contact with similarly minded colleagues throughout the world and continued to publish - with numerous contributions on taxonomy, phylogeny, zoogeography and systematics.

At Bodensee 1997. Friedrich Reiss, Samantha Hughes, Declan A. Murray, Paddy Ashe, Peter H. Langton. Photo: Freddie Murray.

Paddy assisted in organisation of the 7th chironomid symposium in Dublin in 1979 and was an enthusiastic and active participant, as a scientist and socially, at subsequent symposia in Talahassee, 1982; Bergen, 1985; Debrecen, 1988; Amsterdam, 1991; Canberra, 1994; Freiburg, 1997; Madeira, 2006; Nankai 2009; Trondheim, 2011, Ĉescké Budějovice, 2014 and Trento, 2017. He merited the international reputation he achieved through cooperation with his colleagues in Ireland and in Australia, France, Germany, Norway and the USA. Paddy has co-authored more than 100 publications from his international cooperation. We are indebted to him for these significant contributions, sadly abruptly cut off. The situation regarding the unfinished Volumes 3 and 4 of the World Catalogue is being resolved. Please see the Chironomidae Bibliography for an overview of Paddy's publications on Chironomidae.

Before the pandemic era in 2020 Paddy and I, frequently also joined by our friend and colleague Jim (J.P.) O'Connor, would meet regularly every Friday, either in the National Museum of Ireland, Natural History, or (and!) in a Dublin pub, to share opinions on our multiple ongoing "projects" and discuss, or argue about, taxonomic issues.

Paddy became unwell in November 2021 and following a long illness in hospital he died on the morning of June 19 2022. Paddy has left a notable legacy, as a person, a researcher and in what he achieved. Paddy Ashe has been a good friend and colleague. He is greatly missed.