CHIRONOMUS Journal of Chironomidae Research No. 35, 2022: 12-31. Current Research.

VERIFYING AUSTRALIAN *NILOTANYPUS* KIEFFER (CHIRONOMIDAE) IN A GLOBAL PERSPECTIVE: MOLECULAR PHYLOGENETIC AND TEMPORAL ANALYSES, NEW SPECIES AND EMENDED GENERIC DIAGNOSES

Peter S. Cranston[1*], Matt N. Krosch[2] & Hongqu Tang[3]

[1]*Evolution, Ecology & Genetics, Research School of Biology, Australian National University, Canberra, A.C.T. 2601, Australia. E-mail: pscranston@gmail.com*
[2]*Forensic Services Group, Queensland Police Service, Brisbane, QLD 4000, Australia.*
E-mail: krosch.mattn@police.qld.gov.au
[3]*Life Science and Technology College, Jinan University, Guangzhou 510632, China.*
E-mail: townningt@gmail.com
[*]*Corresponding author*

http://zoobank.org/94BBD58A-85FD-4E52-807E-97D27636437C

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 lognormal 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 semi-taeniate, 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 supra-anal; 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

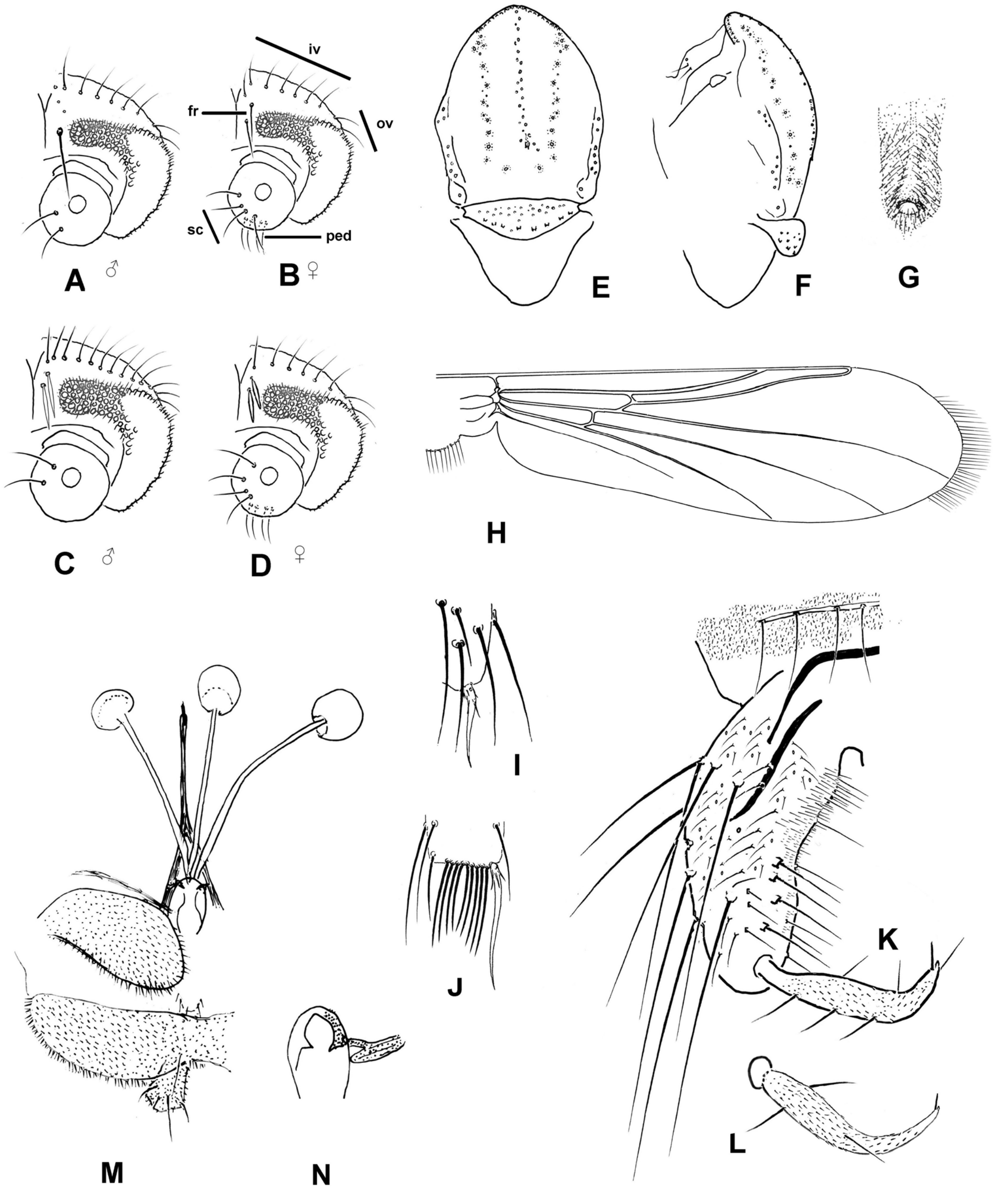

Figure 1. *Nilotanypus* Kieffer. Adult. A–D. Head, anterior view, right side, ♂, A, C. ♀, B, D.; E–F. Thorax, E. dorsal, F. lateral; G. Mid-dorsal sensory pit; H. Wing (male); I, J. Tibial apices, I. P1, J. P3; K. Male hypopygium; L. Gonostylus; M. Female genitalia left side only; N. Anterior vaginal 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).

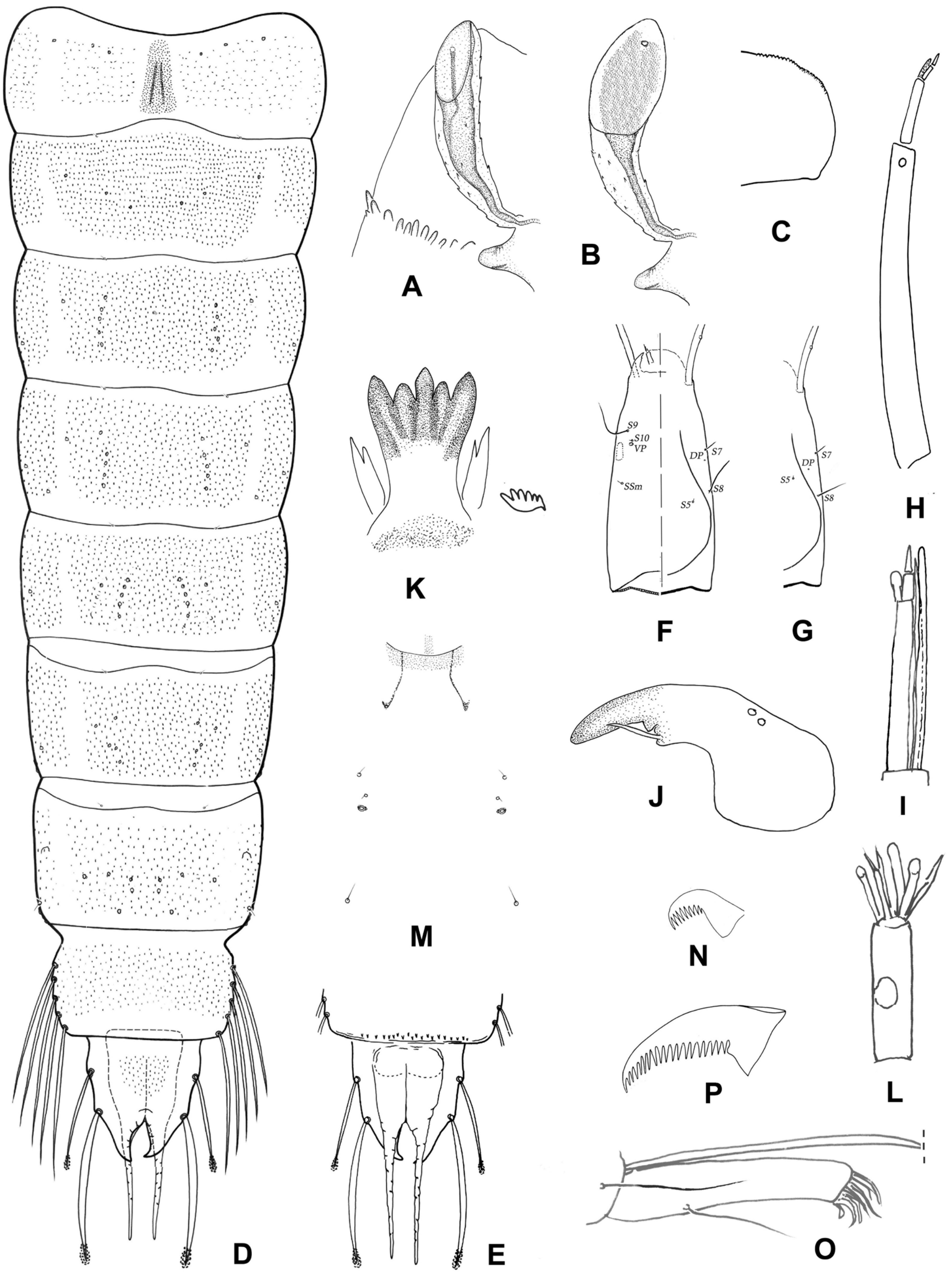

Figure 2. *Nilotanypus* Kieffer. Pupa. A, B. Thoracic horn; C. Wing sheath; D–F. Abdomen, male); D. dorsal, E. ventral. Larva. F. Head capsule, left side ventral, right side dorsal; G. Dorsal head capsule; H. Antenna; I. Antennal apex, detail; J. Mandible; K. Ligula, paraligula; l. Maxilla; M. Submentum; 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 antero-posterior, 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 shark-fin. Thorax microtuberculose anteriorly and close to mid-dorsal ecdysial line. Wing sheath apico-distally 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, uniserial, 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 sexually 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,

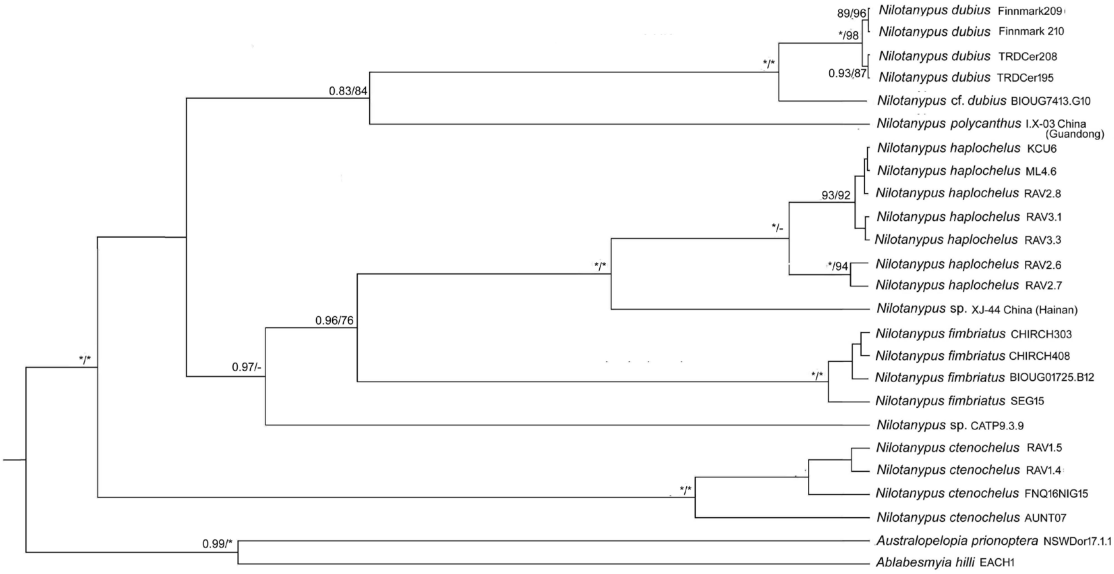

Figure 4. Phylogenetic tree from Bayesian inference for *Nilotanypus* Kieffer and outgroups (Table 1) based on concatenated gene fragments. Posterior probabilities (PP) and Bootstrap support (BS from Maximum Likelihood analysis) are indicated above branches only for nodes with PP > 0.95 or BS > 70. Maximal 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

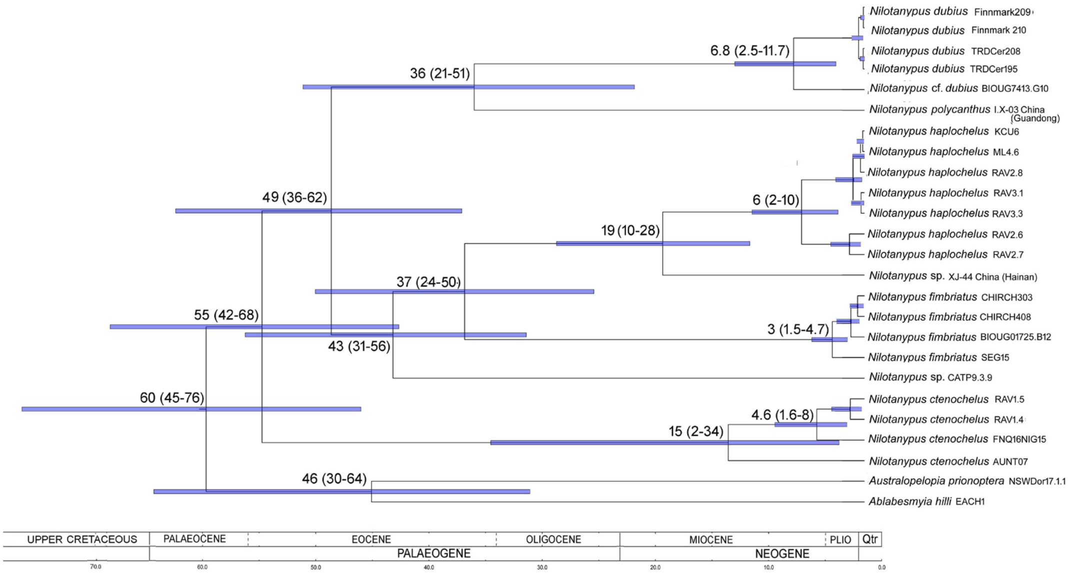

Figure 5. BEAST chronogram from a data set corresponding with Table 1. Values at nodes are time to most recent common ancestor (tmrca) with HPD (95% Highest Posterior Density) intervals in parentheses. The time scale is in millions of years before present.

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

Acknowledgements

Recent collections were supported by the Australian Biological Resources Study (ABRS) Grant RF216-36 “Systematics and biogeography of the Australian Tanypodinae (Diptera: Chironomidae): placing Australian diversity in a global context”. For earlier collections there are many sources to acknowledge and we appreciate the personnel of diverse state, territory and federal agencies for permission to sample in their estate throughout the long gestation of this research.

As ever, we are grateful to Martin Spies (ZSM) for examining specimens and discussing morphology and nomenclature. Our erudite colleague Robert Hoare (New Zealand Arthropod Collection, Auckland) provided linguistic guidance and Duncan Sivell, NHM examined their collection of *N. comatus*. For discussion of *Nilotanypus* in Japan, we thank Hiromi Niitsuma, and also acknowledge Augusto Siri who verified South American distributions.

References

Andersen, T. and Pinho, L.C. 2019. Two new species of *Nilotanypus* Kieffer, 1923 (Diptera, Chironomidae, Tanypodinae) from Brazil. *Norwegian Journal of Entomology* 66:11–18.

Cheng, M. and Wang, X. 2006. *Nilotanypus* Kieffer from China (Diptera: Chironomidae: Tanypodinae). *Zootaxa* 1193: 49–53. https://doi.org/10.11646/zootaxa.1193.1.3

Crampton, G.C. 1942. The external morphology of the Diptera. In Eds. Crampton G. C, Curran C. H., Alexander C. P. and Friend R. B. *Guide to the insects of Connecticut. Part VI. The Diptera or true flies of Connecticut. First fascicle: External morphology; key to families; Tanyderidae, Ptychopteridae, Trichoceridae, Anisopodidae, Tipulidae.* State Geological and Natural History Survey, Hartford, pp. 10–65.

Cranston, P.S. 1991. Immature Chironomidae of the Alligator Rivers Region. 269 pp. Open File Report 82. Supervising Scientist for the Alligator Rivers Region.

Cranston P.S. 1996. *Identification guide to the Chironomidae of New South Wales*. In B. Atkins (Ed.), AWT Identification Guide Number 1, p. 376. Australian Water Technologies Pty Ltd., West Ryde, NSW.

Cranston, P. S., 2004. Chironomidae. In Eds: Yule, C.M. and H.S. Yong. *The Freshwater Invertebrates of Malaysia and Singapore,* Academy of Sciences, Malaysia. pp. 711–735.

Cranston, P.S. 2012. Some proposed emendations to larval morphology terminology. *Chironomus - Journal of Chironomidae Research* 25: 35–38. https://doi.org/10.5324/cjcr.v0i25.1540

Cranston, P.S. 2016. *Conochironomus* (Diptera: Chironomidae) in Asia: new and redescribed species and vouchering issues. *Zootaxa* 4109: 315–331. https://doi.org/10.11646/zootaxa.4109.3.3

Cranston, P.S. and Epler, J. 2013. The larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region – Keys and diagnoses. *Chironomidae of the Holarctic Region: Keys and Diagnoses. Part 1. Larvae*, *Insect Systematics and Evolution*, Vol. 66 (ed. T. Andersen, P.S. Cranston and J.H. Epler), pp. 39–136. Scandinavian Entomology, Lund, Sweden.

Cranston, P.S. and Tang, H.Q. 2018. *Skusella* Freeman (Diptera: Chironomidae): new species, immature stages from Africa, Asia and Australia, and expanded distributions. *Zootaxa* 4450: 41-65. https://doi.org/10.11646/zootaxa.4450.1.3

Cranston, P.S., Hardy, N.B., Morse, G.E. 2012. A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology* 37: 172-188.

Doitteau, G. and Nel, A. 2007. Chironomid midges from early Eocene amber of France (Diptera: Chironomidae). *Zootaxa* 1404: 1-66.

Fittkau, E.J. 1962. Die Tanypodinae (Diptera: Chironomidae). (Die Tribus Anatopynyiini, Macropelopiini und Pentaneurini). *Abhandlungen zur Larvalsystematik der Insekten* 6: 1–453.

Fittkau E.J. and Murray, D.A. 1986. The pupae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region. Keys and diagnoses. *Entomologica scandinavica Supplement* 28: 31–113.

Fittkau E.J. and Roback S.S. 1983. The larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region. Keys and diagnoses. *Entomologica scandinavica Supplement* 19: 33–110.

Freeman, P. 1953. Chironomidae (Diptera) from Western Cape Province 1. *Proceedings of the Royal Entomological Society of London, Series B*. 22: 127–135.

Freeman, P. 1955. A study of the Chironomidae (Diptera) of Africa south of the Sahara. Part I.

Bulletin of the British Museum (Natural History), Entomology 4: 1–67.

Harrison, A.D. 1991. Chironomidae from Ethiopia. Part 1. Tanypodinae (Insecta, Diptera). *Spixiana* 14: 45–69.

Huelsenbeck, J. P., and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kieffer, J.J. 1921 Chironomides de l'Afrique équatoriale (1re partie). *Annales de la Société entomologique de France* 90: 1–56, pls. 1–2.

Kieffer, J.J. 1923. Chironomides de I'Afrique Equatoriale (3iéme partie). *Annales de la Societé entomologique de France* 92: 149–204.

Kowalyk, H.E. 1985. The larval cephalic setae in the Tanypodinae (Diptera: Chironomidae) and their importance in generic determinations. - *Canadian Entomologist* 117: 67–106. https://doi.org/10.4039/Ent11767-1

Kownacki, A. and Kownacka, M. 1968. Die Larve des *Nilotanypus dubius* (Meigen) 1804 (Diptera, Chironomidae*). Acta hydrobiologica, Kraków* 10: 343-347.

Krosch, M.N. and Cranston, P.S. 2012. Non-destructive DNA extraction, including of fragile pupal exuviae, extends analysable collections and enhances vouchering. *Chironomus Newsletter on Chironomidae Research* 25: 22–27. https://doi.org/10.5324/cjcr.v0i25.1532

Krosch, M.N. and Cranston, P.S. 2013. Not drowning, (hand)waving? Molecular phylogenetics, biogeography and evolutionary tempo of the 'gondwanan' midge *Stictocladius* Edwards (Diptera: Chironomidae). *Molecular Phylogenetics and Evolution* 68: 595-603. https://doi.org/10.1016/j.ympev.2013.04.006

Krosch, M.N., Baker, A.M., Mather, P.B., Cranston, P.S. 2011. Systematics and biogeography of the Gondwanan Orthocladiinae (Diptera: Chironomidae). *Molecular Phylogenetics and Evolution* 59: 458-468. https://doi.org/10.1016/j.ympev.2011.03.003

Krosch, M.N., Cranston, P.S., Baker, A.M. and Vink, S. 2015. Molecular data extend Australian *Cricotopus* midge (Chironomidae) species diversity, and provide a phylogenetic hypothesis for biogeography and freshwater monitoring. *Zoological Journal of the Linnean Society* 175: 496-509. https://doi.org/10.1111/zoj.12284

Krosch, M.N., Cranston, P.S., Bryant, L., Strutt, F. and McCluen, S. 2017. Towards a dated molecular phylogeny of the Tanypodinae (Chironomidae, Diptera). *Invertebrate Systematics* 31: 302–316. https://doi.org/10.1071/IS16046

Krosch, M.N., Silva, F.L., Ekrem, T., Baker, A.M., Bryant, L.M., Stur, E. and Cranston, P.S. 2022. A new molecular phylogeny for the Tanypodinae (Diptera: Chironomidae) places the Australian diversity in a global context. *Molecular Phylogeny and Evolution* 166*:* 107324. https://doi.org/10.1016/j.ympev.2021.107324

Lehmann, J. 1979. Chironomidae (Diptera) aus Fließgewässern Zentralafrikas (Systematik, Ökologie, Verbreitung und Produktionsbiologie). Teil I: Kivu-Gebiet, Ostzaire. *Spixiana Supplement* 3: 1–144.

Miller, M. A., Pfeiffer, W., and Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 'Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA'. pp. 1–8.

Murray, D.A. and Fittkau, E.J. 1989. The adult males of Tanypodinae (Diptera: Chironomidae) of the Holarctic region. Keys and diagnoses. Part 3. Adult males. *Entomologica Scandinavica Supplement* 34: 37–123.

Rieradevall, M. and Brooks, S.J. 2001. An identification guide to subfossil Tanypodinae larvae (Insecta: Diptera: Chironomidae) based on cephalic setation. *Journal of Paleolimnology*, 25: 81–99.

Roback, S.S. 1986. The immature chironomids of the eastern United States VIII. Pentaneurini - genus *Nilotanypus*, with the description of a new species from Kansas. *Proceedings of the Academy of Natural Sciences of Philadelphia* 138: 443–465.

Roback, S.S. and Coffman, W.P. 1987. Results of the Nepal Alpine zone research project, Chironomidae (Diptera). *Proceedings of the Academy of Natural Sciences of Philadelphia* 139: 87–158.

Roback, S.S. and Coffman, W.P. 1989. Tanypodinae Pupae from Southern India (Diptera: Chironomidae). *Proceedings of the Academy of Natural Sciences of Philadelphia* 141: 85–113.

Ronquist, F., and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sæther O.A.1980. Glossary of chironomid morphology terminology (Diptera: Chironomidae). *Entomologica scandinavica, Supplement* 14:1–51.

Shimbakuruo, E.M., Dantas, G.P.S. and Lamas, C.J.E. 2021. A new madicolous species of *Nilotanypus* (Diptera: Chironomidae) from Amazon region, northwestern Brazil. *Zootaxa* 4948: 151– 438. https://doi.org/10.11646/zootaxa4948.3.7

Silva, F.L. and Ferrington L.C. 2018. Systematics of the new world genus *Pentaneura* Phillip (Diptera: Chironomidae: Tanypodinae): Historical review, new species and phylogeny. *Zoologischer Anzeiger* 274: 60-89. https://doi.org/10.1016/j.jcz.2017.11.010

Silva, F.L. and Ekrem, T. 2016. Phylogenetic relationships of nonbiting midges in the subfamily Tanypodinae (Diptera: Chironomidae) inferred from morphology. *Systematic Entomology* 41: 73–92. https://doi.org/10.1111/syen.12141

Smith, M. and Cranston, P.S.1995. “Recovery” of an acid mine-waste impacted tropical stream – the chironomid story. In Ed. Cranston P.S. *Chironomids: from Genes to Ecosystems*. CSIRO, Melbourne, pp. 161–73.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa, and mixed models. *Bioinformatics* 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Tang, H. 2018. *Conochironomus* Freeman, 1961 (Diptera: Chironomidae) newly recorded in China, with description of a new species. *The Pan-Pacific Entomologist* 94: 167-180.

Tang, H. and Cranston, P.S. 2019. Phytophagy in a *Polypedilum* (Diptera: Chironomidae) species new to Australia and Japan: taxonomy and expanded Asian distribution. *Austral Entomology* 59: 102–118. https://doi.org/10.1111/aen.12428

Tokunaga, M. 1937. Chironomidae from Japan (Diptera), IX. Tanypodinae and Diamesinae. *Philippine Journal of Science* 62: 21–65 + 4 plates.

Van Emden, F. and Hennig, W. 1970. Diptera. In Ed. Tuxen, S.L. *Taxonomist's glossary of genitalia in insects*. 2nd ed., 395 pp. Copenhagen, Munksgaard, pp. 130–140.

Zavřel, J. 1933. Larven und Puppen der Tanypodinen von Sumatra und Java. *Archives für Hydrobiologie. Supplement* 11: 604-624.

Article submitted 21. February 2022, accepted by Torbjørn Ekrem 4. May 2022, published 27. June 2022.