



Cryptic species in the nuisance midge *Polypedilum nubifer* (Skuse) (Diptera: Chironomidae) and the status of *Tripedilum* Kieffer

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Abstract

Polypedilum nubifer (Skuse, 1889), originally described from Australia, is an apparently widespread species of Chironomidae (Diptera) that can attain nuisance densities in some eutrophic water bodies. Appropriate management depends upon the identity and ability to distinguish from potential cryptic taxa. A morphological study of larvae, pupae and adults of both sexes confirmed *P. nubifer* as widely distributed and frequently abundant, but also revealed two previously cryptic species of limited distribution in northern Australia. These species are described as new and illustrated in all stages here. *Polypedilum quasimubifer* Cranston **sp. n.** is described from north-west Queensland, Australia and also from Thailand and Singapore. *Polypedilum paranubifer* Cranston **sp. n.** is known only from retention ponds of a uranium mine in Northern Territory, Australia. Unusual morphological features of *P. nubifer* including alternate Lauterborn organs on the larval antenna, cephalic tubules on the pupa and frontal tubercles on the adult head are present in both new species as well. Newly slide-mounted types of *Polypedilum pelostolum* Kieffer, 1912 (lectotype designated here) confirm synonymy to *Chironomus nubifer* Skuse, 1889, examined also as newly-slide mounted types. Reviewed plus new evidence does not support recognition of *Tripedilum* Kieffer, 1921 as a separate taxon; therefore, *Tripedilum* is returned to junior synonymy with *Polypedilum* s. str.

Key words: Chironominae, taxonomy, new species, synonymy, immature stages

Introduction

Polypedilum Kieffer, 1912 (Diptera: Chironomidae) is distributed nearly globally and includes some 500 described species. Classification within the genus has been problematic for a long time; taxonomic and nomenclatural issues begin with the identity and name of the type species. A type designation by Kieffer (1913) was followed for decades but then found to be invalid by Ashe (1981), because the species designated in Kieffer (1913) was ineligible for type fixation as it was not one of the two species first included in *Polypedilum* by Kieffer (1912). From these latter two species, *Polypedilum pelostolum* Kieffer, 1912 and *P. ceylanicum* Kieffer, 1912, Ashe (1981) selected *P. pelostolum* (from Taiwan) as the type for *Polypedilum* Kieffer. Ashe acknowledged that *P. pelostolum* was regarded as a junior synonym of the Australian *Polypedilum nubifer* Skuse, 1889, but did not discuss that the morphology of this species was considered as atypical in the genus. The latter view led to nomenclatural instability when Sæther *et al.* (2010) proposed subgeneric reclassification in *Polypedilum* and stated (on p. 11) that "a case will be made to the ICZN for rejecting the type designation of Ashe and maintaining the type designation of Kieffer

(1913).” However, no such application for ICZN decision has been submitted; thus, the corresponding proposals in Sæther *et al.* (2010) are invalid, and the situation remains as fixed in Ashe (1981). Recently, the concern that *P. nubifer* might lie outside the ‘core *Polypedilum*’ was alleviated when a phylogenetic position for *P. nubifer* embedded within a monophyletic *Polypedilum* was proposed by Cranston *et al.* (2011), based on diverse molecular sampling.

Traditionally, most species delimitations in Chironomidae have relied on the morphology of the adult male, especially on the genitalia (hypopygium) in combination with a range of morphometrics, including counts and ratios. Taxon diagnoses are often aided by the immature stages (which are of environmental significance); individual associations of the larva, pupa and adult are essential in modern descriptive taxonomy (Cranston, 2000). For the present study, standard and unusual morphology of all stages of *P. nubifer* and similar species has been examined. Notably, features of larvae and pupae have been compared to reared associated adult males of *P. nubifer* of Australian provenance, validated as conspecific by comparison with (unreared) type specimens.

Polypedilum nubifer is encountered often in regional studies of chironomid midges, and has contributed to nuisance problems (Tabaru *et al.*, 1987; Trayler *et al.*, 1994; Cranston, 2007; Cranston *et al.*, 2013). During recent work in Australia some forms have been found that resemble *P. nubifer* in at least one life stage. For example, a species reared from northern Australia, and subsequently from Singapore, conforms to *P. nubifer* in the larval antennal structure previously believed to be unique in *Polypedilum* (e.g. Cranston, 1996; Jacobsen & Perry, 2007). However, this new form is of smaller body size than seen in any population of *P. nubifer*, and the males differ in having a dorsolateral seta on the superior volsella of the hypopygium. Other specimens from the Northern Territory, Australia, resemble *P. nubifer* in the larval antenna, pupal morphology and the adult wing pattern, but the male differs in having a strong seta on the superior volsella and in lacking setae on wing vein R₄₊₅. In the present paper, new species recognised in a partially iterative procedure by the first author are described in all available life stages.

The taxonomic identity and systematic placement of *P. nubifer* have implications at the genus-group level. Sæther *et al.* (2010) proposed to revive *Tripedilum* Kieffer, 1921a as a subgenus to receive *P. nubifer* and two Afrotropical species, *P. fuscipenne* Kieffer, 1921b and *P. lobiferum* Freeman, 1954. In doing so, they followed Freeman’s (1958) synonymies listed for *P. nubifer* and *P. fuscipenne*, respectively. Here we review this underlying evidence and evaluate the justification for *Tripedilum* as a separate taxon. We conclude with a sceptical review of the recent subgeneric classification in *Polypedilum*.

Methods and material

Collections were made throughout Australia, subsequently in Thailand and more recently Singapore, using standard techniques with aquatic and sweep nets and light traps. Rearings were undertaken especially from individual egg masses in Australia (by J. Martin); the material is now slide-mounted, curated and housed in the Australian National Insect Collection (ANIC), CSIRO, Canberra, Australia, and has been included here for morphometrics.

In a related molecular study (W. H. Wong, Singapore, pers. comm.) new specimens were obtained from the field and/or laboratory rearings (South Australia—field; Western Australia—rearing; New South Wales—rearing from egg masses). Attempts to obtain specimens from other recorded localities for *P. nubifer*, including Florida and Southern France, were not successful. Specimens resembling *P. nubifer* were collected in Singapore particularly from Pandan (1°18’50”N, 103°44’30”E) and Upper Seletar (1°24’04”N, 103°48’14”E) reservoirs (Cranston *et al.*, 2013). Live specimens were preserved in 70% ethanol or isopropanol (adults) or in 99% ethanol or isopropanol (larvae). Those recognised on gross morphology as resembling *P. nubifer* were subjected to DNA extraction and barcoding (Wong *et al.*, 2014). Post-extraction carcasses have been vouchered, slide mounted and preserved in ANIC, the Raffles Museum of Biodiversity Research (RMBR) (now Lee Kong Chian Natural History Museum), University of Singapore, The Natural History Museum (NHM=BMNH), London, England and in the Senckenberg Deutsches Entomologisches Institut (SDE), Müncheberg, Germany (SDEI).

The morphological study used specimens microscope-slide mounted in Euparal, dissected under multiple coverslips according to standard procedures. Relevant pre-existing materials from the past 3 decades, held in ANIC, was enhanced by inclusion of vouchered specimens post-DNA extraction. Although McKie & Cranston

(2005) and Gresens *et al.* (2012) challenge the value of weakly interpreted mensural features and morphometric ratios, conventional standard discriminatory measurements were made using a calibrated graticule (at magnifications of x400 or x1000) or with software using video camera images. Illustrations were prepared from line drawings made with a camera lucida attached to an Olympus BH2 compound microscope or from digital images made with Automontage™ image stacking attached to a Leica® DMRX compound microscope with Nomarski® interference optics. Images were manipulated subsequently in Adobe™ Photoshop.

Larval features of potential diagnostic significance included the fine detail of the distinctive antenna, the shape of the mental teeth, structure of the ventromental plate, and presence and extent of postmentum pigment (Cranston, 2007: figs 52, 55; Cranston *et al.*, 2013: figs 17–25). Pupal exuviae were compared to reared Australian material and Cranston *et al.* (2013: figs 12–15). Adult morphology was compared to all type specimens, reared Australian material and to Cranston *et al.* (2013: figs 12–15). Specimens and descriptions were sought for species names treated as synonyms of *P. nubifer* following Freeman (1961), and of *P. fuscipenne* Kieffer, 1921 and synonyms following Freeman (1958).

Additional emphasis was given to structures on the adult 5th (terminal) tarsomere, namely to the pulvilli and empodium. As indicated in the genus name—‘poly’ and ‘pedilum’—these structures are multiple; that is, each pulvillus is characteristically subdivided. Even with perfect lighting and appropriate background the structures cannot always be interpreted under a dissector microscope due to the fine detail often being obscured. Conclusive evaluation of these structures requires perfect orientation of the apical segments of a leg mounted under a coverslip, displayed symmetrically, ventral side upwards. Lateral orientation (as usual in conventionally slide-mounted specimens) rarely allows accurate interpretation. Details visible only under x400 magnification and with correct orientation are required to understand the structural variation in the pulvilli and empodium.

Unless stated otherwise, morphological measurements are given in μm rounded to the nearest 5 μm , except when they were made at maximum magnification (x1000, oil immersion), which provides accuracy to $\pm 1 \mu\text{m}$. Abbreviations: ac, acrostichal setae (count); ant (1-5), antennal segment lengths (L); AR, Antennal Ratio (length of terminal flagellomere divided by combined length of preceding flagellomeres) (adult); length of basal segment divided by combined length of all other segments (larva); bl, blade length (L); BMNH, British Museum, Natural History, London, UK; dc, dorsocentral setae (count); h.l., head length (L); L, larva; L., Lake; Le, larval exuviae; Le/Pe/♂(♀), reared adult male (female) with associated larval and pupal exuviae; LR₁, Leg Ratio of adult foreleg (leg 1): tarsomere 1 length : tibia length; m.l., mentum length (L); md.l., mandible length (L); MV, molecular voucher; n, number of specimens measured; NHM, The Natural History, London, UK; om.l., occipital margin length (L); P, Pupa; pa, prealar setae (count); Pe, pupal exuviae; pm.l., postmentum length (L); R., river; R₁, R₂₊₃, R₄₊₅, setal counts on wing veins R₁, R₂₊₃, R₄₊₅; sct, scutellar setae (count), sq, squamal setae (count); TIX, adult male tergite IX setal count; vmp.l.(w).(R).(s.), ventromental plate length(width)(ratio l:w)(separation) (L); w.l., wing length (arculus to apex).

Results

Australian specimens belong to three morphotypes. The dominant one, sampled across the continent in all stages, shows morphological variation (for example in intensity of pigment, shape of the hypopygium and of larval antenna) as expected in any widely distributed and extensively sampled taxon (Tables 1, 2). Setal counts and morphometrics of the types of *P. nubifer* and *P. pelostolum* now mounted on microscope slides lie within this morphospace. All adults have small but distinct frontal tubercles, contrary to Freeman (1961), and pupal sternite IV has strong pedes spurii A (vortices) contrary to Sæther *et al.* (2010). All Australian specimens sampled for a parallel molecular study (W. H. Wong, pers. comm.) belong to a single cluster, with less than 1% variation in the CO1 barcode across the continent (35° of longitude, 10° of latitude). We infer that these belong to ‘true’ *P. nubifer* (Skuse), following type material described from the Sydney region, New South Wales, Australia.

A second morphotype reared only from uranium mine retention ponds in Northern Territory, Australia, falls within the morphospace of ‘typical’ Australian *P. nubifer*. However, in all male specimens the superior volsella is less elongate and has a distinct subapical seta. Other differences in all stages are assessed under a newly described species *Polypedilum paramubifer* **sp. n.** below.

TABLE 1. Mensural features of *Polypedilum* spp. adults. n/a = not visible; n/s not stated (by author).

males	n=	w.l.	A.R.	R	RI	R4+5	ac	dc	pa	sct	sq	LRI	TIX
<i>nubifer</i> (types)	2	2.3-2.7	2.9-3.1	23-25	17-21	25-34	15-19	22-26	6-8	19	11	1.3-1.45	13-15
<i>pelostolum</i> (types)	3	2.3-2.5	2.4-2.8	23-28	18-21	19-24	11-22	19-34	7-9	15-18	9-24	1.42	13-20
<i>nubifer</i> (Australia)	>10	2.1-2.9	2.3-3.0	20-24	17-21	20-27	15-20	15-31	7-25	16-23	12-27	1.3-1.5	7-16
<i>nubifer</i> (Japan)	8	2.5-2.6	2.4-2.8	11-15	17-30	16-24	11-24	17-33	7-9	10-16	11-14	1.44-1.5	10-11
<i>nubifer</i> (Japan, Sasa lit.)	16	2.6-3.5	2.3-2.8	n/s	n/s	n/s	n/s	c 30	6-8	c 25	>10	1.4-1.6	n/s
<i>nubifer</i> (Israel)	1-2	2.4	2.3-2.5	n/a	19-23	n/a	11-14	20-27	5-8	12	31	1.4	9-11
<i>nubifer</i> (Singapore)	8	2.4-2.6	2.2-2.3	14-28	15-20	14-20	12-18	21-26	5-8	14-18	10-15	1.37-1.49	11-15
<i>paramubifer</i>	10	2.6-2.7	2.5-2.7	12-19	4-8	0	22-31	15-16	8-9	8-11	10-13	1.24-1.41	12-17
<i>quasinubifer</i> (Singapore)	10	1.2-1.6	1.6-1.9	18-21	13-21	29-42	8-12	9-15	3-4	6-9	5-9	1.9-2.2	
<i>quasinubifer</i> (Australia)	2	1.65-1.7	1.83	16-19	13-14	20-23	12-13	14-15	4-5	11-15	5-7	1.9	5-7
females	n=	w.l.	A.R.	R	RI	R4+5	ac	dc	pa	sct	sq	LRI	
<i>nubifer</i> (types)	3	2.5-2.7	0.33-0.39	25	21	25	16-24	38-51	8-12	24-37	14-20	1.5	
<i>pelostolum</i> (types)	2	2.6-2.9	0.38-0.39	26-27	24-26	40-41	28-29	29-38	6-8	23-32	13-16	1.4	
<i>nubifer</i> (Australia)	>10	2.3-3.0	0.35-0.38	20-23	18-23	29-41	15-29	29-43	6-24	25-43	14-20	1.3-1.5	
<i>nubifer</i> (Japan, Sasa lit.)	15	2.0-3.1	0.32	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	1.4-1.5	
<i>nubifer</i> (Singapore)	5	1.8-1.9	0.4-0.5	21-24	20-26	32-41	18-13	30-36	5-10	18-22	11-12	1.5	
<i>quasinubifer</i> (Singapore)	3	0.8-0.9	0.43	20-22	20-21	29-42	10-12	17-21	3-4	9-13	7	2	
<i>quasinubifer</i> (Australia)	1	0.9	0.37	17	24	32	15	24	4	18	7-8	1.9	

n/a not visible, n/s not stated (by author)

TABLE 2. Mensural features of *Polypedium* spp. larvae. n/a = not visible; n/s = not stated (by author).

	n=	h.l.	pm.l.	om.l.	m.l.	vmp.l.	vmp.w.	vmp.R.	vmp.S.
<i>nubifer</i> (Australia)	10	475-550	190-235	18-25	122-150	145-165	50-65	2.5-2.8	30-35
<i>nubifer</i> (Hawai'i)	3	420-450	165-185	15-17	120-132	132-140	47-52	2.6-2.8	32-36
<i>nubifer</i> (Singapore)	10	360-465	180-205	10-22	125-140	137-155	52-60	2.5-2.8	30-36
<i>nubifer</i> (Japan, Sasa lit.)	n/s	n/s	n/s	n/s	140	152	60	2.5	n/s
<i>nubifer</i> (Israel)	6	n/a	145-162	16-25	117-160	140-152	55-60	2.5-2.8	28-35
<i>paramubifer</i>	5	430-500	185-205	10-20	128-132	140-150	53-58	2.5-2.8	28-35
<i>quasinubifer</i> (Singapore)	8	295-360	150-170	12-20	93-100	95-100	37-43	2.3-2.7	30-35
<i>quasinubifer</i> (Australia)	1	330	152	10	105	130	42	3	17

Continued.

	n=	md.l.	ant 1	ant 2+3	ant 4	ant 5	AR	bl
<i>nubifer</i> (Australia)	10	140-175	62-82	35-43	11-15	5-6	1.1-1.4	42-52
<i>nubifer</i> (Hawai'i)	3	125-150	62-68	37-40	11-13	5-7	1.13-1.18	40-52
<i>nubifer</i> (Singapore)	10	135-160	62-75	35-42	12-17	5-7	1.05-1.42	35-55
<i>nubifer</i> (Japan, Sasa lit.)	n/s	n/s	77	39	13	5	1.2	48
<i>nubifer</i> (Israel)	6	150-160	71-80	35-46	13-15	5-6	0.85-1.4	33-43
<i>paramubifer</i>	5	138-155	73-75	32-38	15-16	5	1.2-1.3	25-40
<i>quasinubifer</i> (Singapore)	8	112-122	45-52	25-31	12-13	5	0.9-1.1	40-52
<i>quasinubifer</i> (Australia)	1	125	55	28	12	6	1.2	30

A third morphotype reared from northern Queensland, Australia shares many features with *P. nubifer* including the distinctive larval antenna. Differences in each life stage are assessed under a newly described species *Polypedilum quas nubifer* sp. n. below.

The morphology of the pulvilli and empodium has been reported to differ between *Tripedilum* Kieffer and other members of *Polypedilum*. In all species we have examined each pulvillus is divided in two parts. An outer, ventralmost branch varies in size and density of branchlets, and its distal extent ranges from around the mid-point of the claw to the apex of the latter. Dorsal to this, but also ventral to the claw, a second, narrower inner branch consists of a simple base and a low to modest number of branchlets, and terminates usually slightly beyond the outer branch. Medially, arising posterior to the base of the pulvilli, is a single empodium that may be simple or pulvilliform with variably dense branching, and usually extends beyond the pulvilli.

For Australian specimens molecular data are available only for ‘true’ *P. nubifer*. The two newly-differentiated species are based on morphology alone. Outside of Australia two clusters are revealed by barcoding (W. H. Wong, pers. comm.). Specimens from Hawai’i, Israel, Japan and Singapore that are identical morphologically with Australian *P. nubifer* (Tables 1, 2) differ in the barcodes by 4% at the most. This value is within the range of intra-specific variation amongst well-sampled Chironomidae (e.g. Ekrem *et al.*, 2010; Silva *et al.*, 2013; Silva & Wiedenbrug, 2014; Stur & Ekrem, 2015; Krosch *et al.*, 2015) and lies near the suggested average 4–5% threshold between species proposed by Lin *et al.* (2015) for *Tanytarsus* Wulp. The identity of the Australian and Israeli material is further supported by an analysis of the banding patterns of the polytene chromosomes by Porter & Martin (1977), where 5 of the 7 chromosome arms share common banding patterns. The relationship of the banding patterns in the other arms could not be determined with certainty due to the relatively poor quality of those patterns in the Israeli sample. A genetically more diverse form of *P. nubifer* exists outside of Australia. Another, differentiated at a level of about 12%–14% is smaller in body size, distinguishable in all stages, and seems to be identical in morphology to *P. quas nubifer*.

Taxonomy

The species discussed and described here conform to generic diagnoses for *Polypedilum* in the larvae (Pinder & Reiss, 1983; Epler *et al.*, 2013), pupae (Pinder & Reiss, 1986), adult males (Cranston *et al.*, 1989) and females (Sæther, 1979). However, not all these diagnoses incorporated the ‘anomalous’ *P. nubifer*. The distinct and alternate Lauterborn organs on a partially divided larval 2nd antennal segment, the strong cephalic tubercles on the pupa and presence of frontal tubercles on the adults, and the absence of a distinct subapical ‘waist’ on the adult male abdomen extend ‘core’ diagnoses, and added to the suspicion that *P. nubifer* might be misplaced in *Polypedilum*. Molecular evidence (Cranston *et al.*, 2011; W. H. Wong, pers. comm.) substantiates assignment of *P. nubifer* within *Polypedilum*; thus, those morphological features must be included in the respective generic diagnoses.

Polypedilum nubifer (Skuse)

(Figs 1B, E, H; 2A, D; 3A, B, D; 4A, C,E; 5A)

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Chironomus nubifer Skuse, 1889 (Skuse, 1889, description of both adult sexes).

Chironomus (Polypedilum) octoguttatus Tokunaga, 1936; Sasa (1979, description of all stages).

Remaining synonymy as in Freeman (1961), Sasa & Sublette (1980), Kobayashi & Endo (2008), Cranston *et al.* (2013).

Type material. Lectotype ♂, of *Chironomus nubifer*, designated by Freeman (1961), slide mounted in Euparal (by PSC), [AUSTRALIA: New South Wales] Berowra, F.A.A. Skuse (ANIC).

Other material. Paralectotypes 1♂, 3♀♀, slide mounted in Euparal (by PSC), [New South Wales] Wheeny (*sic!* = Wheeny), Jan. [F.A.A. Skuse] (ANIC).

Lectotype ♂ of *Polypedilum pelostolum* Kieffer, hereby designated, slide mounted in Euparal (by PSC), label data verbatim ‘Tainan / Formosa / H. Sauter (print), XI, 09’ (ink), ‘Kieffer det’ (print), ‘~~COTYPUS~~’, ‘paratypus’ (print), ‘DEI Müncheberg / Dip-00019’.

Paralectotypes (only those examined on slide mounts in Euparal (PSC)), 1♀, same slide as Lectotype; same data except 'DEI Müncheberg / Dip-00018'; 1♂, same data as Lectotype except 'DEI Müncheberg / Dip-00009'; 1♂, 'Formosa, Sauter', 'Tainan 902IV', 'pelostolum Kieff. [ink] det. Kieffer', 'Purchd. from Budapest Mus. B.M. 1922-72' 'BMNH (E) 1240747'; 1♀, same, except BMNH (E) 1240748'.

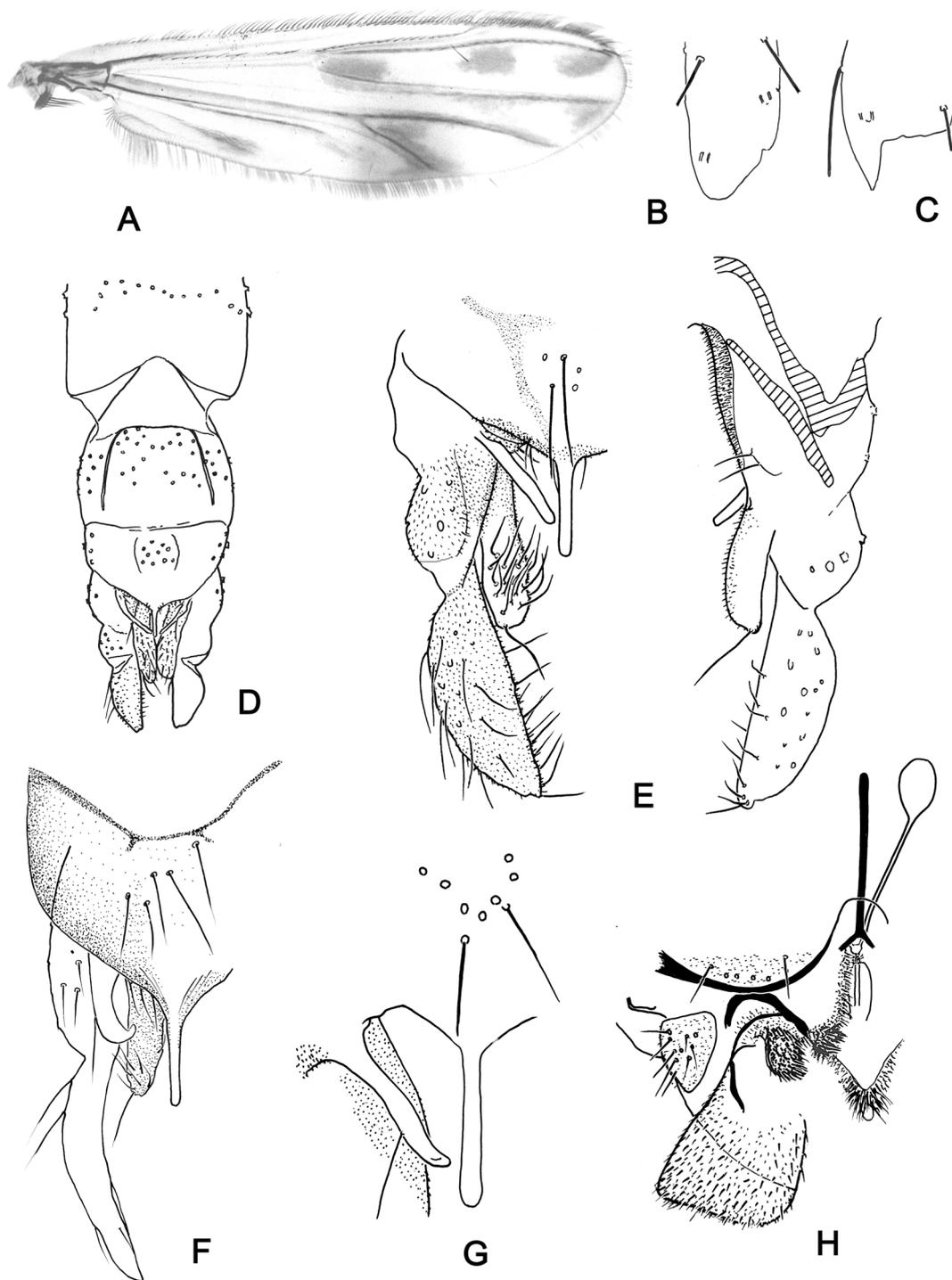


FIGURE 1. *Polypedilum* spp., adults. A. Wing of female *P. quasinubifer* sp. n. B, C. Apex of fore tibia of: B. *P. nubifer* (Skuse), C. *P. quasinubifer*. D. Posterior abdomen of *P. paranubifer* sp. n. E–G. Male genitalia of: E. *P. nubifer* (left: dorsal, right: stylised ventral plus apodemes in segment IX); F. *P. quasinubifer* (dorsal, left side only); G. *P. paranubifer* (T IX dorsomedial setae, anal point, superior volsella). H. Female genitalia of *P. nubifer* (ventral, parts of some paired structures omitted).

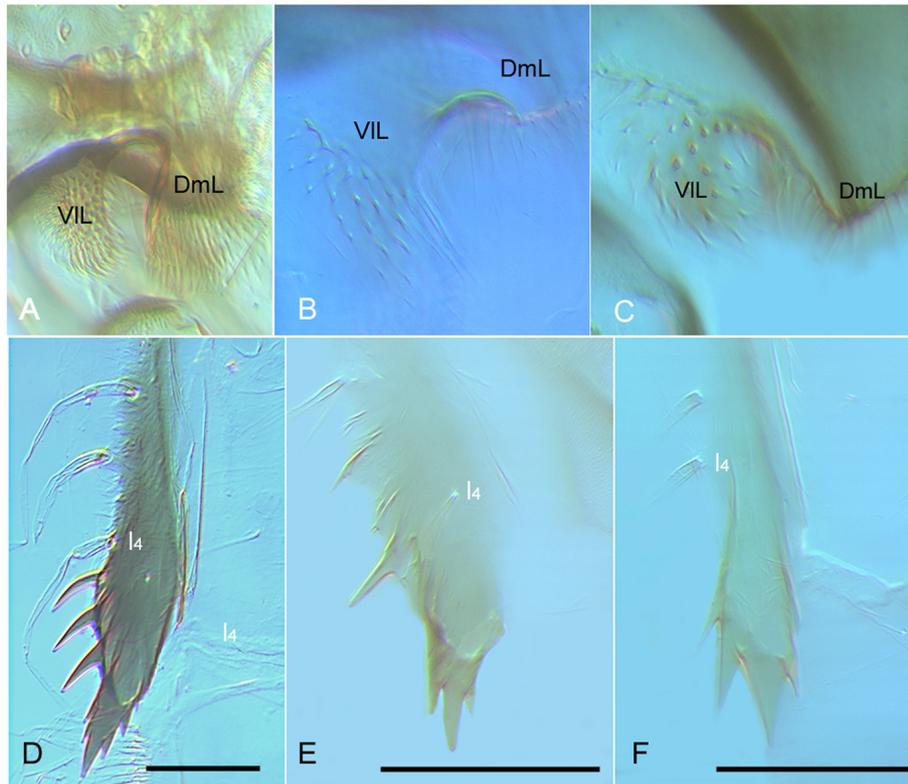


FIGURE 2. *Polypedilum* spp. A–C. Female genitalia, gonapophysis VIII of: A. *P. nubifer* (Skuse); B. *P. paranubifer*; C. *P. quasimubifer*; DmL—dorsomesal lobe, VIL—ventrolateral lobe. D–F. Pupae, posterolateral corner of abdominal segment VIII (ventral) of: D. *P. nubifer*; E. *P. paranubifer*; F. *P. quasimubifer*; l_4 —4th (posterior-most) lateral seta. Scale bars = 100 μ m.

Additional material, examined for mensural (Table 1) and molecular vouchering.

AUSTRALIA: Victoria, Werribee, ‘Metropolitan Farm’, 23.iv.1970 (*Martin*), many σ^7 $\text{reared from individual egg masses}$ (ANIC); New South Wales, Yanco Agricultural Institute, reared *ex egg masses from rice fields* (*Stevens*) (ANIC) (L, MV); South Australia, Bolivar lagoon 32, 2.v.2013 (*Hincks*) (ANIC-MV) (Larvae); Western Australia, Cockburn City, Yangebup Lake, .i.2014 (*Harris*) (ANIC-MV) (Larvae); Northern Territory, Palm Valley, 24°03’N 132°42’E, 29.v.1992 (*Cranston*), reared from egg mass (*Martin*); Pe, Ranger Uranium Mine Retention Pond 1, 31.v.1988 (*Cranston*)(ANIC).

SINGAPORE: Pandan Reservoir, 1°19’7”N 103°44’35”E, multiple dates from .xi.2012 and .v.2014 (*NUS team*) (ANIC, BMNH, most to RMBR) (all life stages, MV).

JAPAN: Okinawa: Yonaguni Is., Iriomote Is., Miyako Is., Okinawa Is, Kume Is; Kagoshima: Amami Is., Tanegashima Is.; Miyazaki: Nichinan Coast; Yamaguchi, R. Oze; Hiroshima: R. Gonogawa, R. Ashida, R. Nuta; Shiga: L. Biwa; Gifu: R. Nagara (K. Kawai) (ANIC), 1 σ^7 , slide mounted, from each location.

ISRAEL: Bief Netufa, Biq’at Valley area, 32°46’N 35°15’E, 15.ix.1968 (*J. Kugler*) (ANIC), 2 σ^7 , $\text{reared from individual egg masses}$, L(cytology); Kazorea Waste Pond (L, MV); Elroi Spring, Kiryat Tivon, 32°43’N 35°7’E 20.viii.2005 (*L. Meltzer*) (ANIC) L; Kfar Yehoshua, 32°41’N 35°9’E 19.vi.2007 (*N. Meltzer*) (ANIC) L (MV). HAWAII: Kealia Pond, Kihei, Maui, 20°47’06” 156°27’56”, 8.xi.1999 (*M. Nishimoto*) (L, MV).

Description. Adults (Figs 1B,E, H, 2A, 5A). For mensural features of specimens of both sexes in the type series and various geographic populations, see Table 1. Descriptions by Sasa (1979) and Sasa & Sublette (1980) are not contradicted. Frontal tubercles digitiform, 10–30 μ m long. Fore tibial apex a rounded scale without spine (Fig. 1B). Pulvillus (Fig. 5A) divided in 2 parts; outer densely plumose but not forming ‘pad’, not extending to tip of claw; inner part longer and multiple branched; empodium narrow, weakly plumose and extending slightly beyond inner pulvillus branch. Tergite IX with ovoid pale setiferous area (TIX, Table 1). Male genitalia as in Fig. 1E. Anal point parallel-sided. Gonocoxite bulging outward, broadly connected to gonostylus by pale membranous area. Gonostylus broad, distally with weak to strongly bilobed apex. Superior volsella narrow and sinuous, its shape varying according to aspect, apex upcurved; no seta at mid-length.

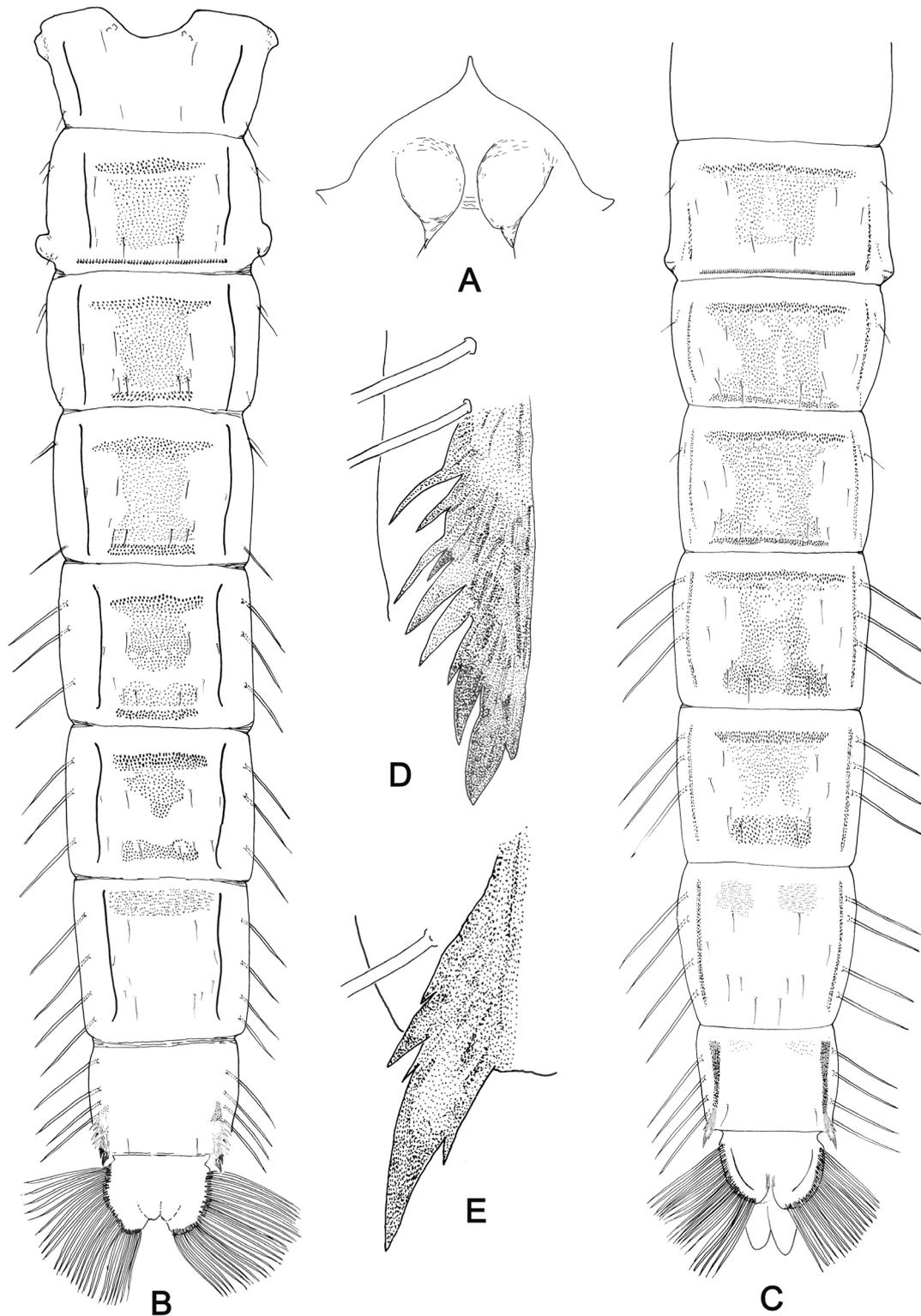


FIGURE 3. *Polypedilum* spp., pupae. A. Cephalic tubercles, frontal setae of *P. nubifer* (Skuse). B, C. Abdomen (dorsal) of: B. *P. nubifer*, C. *P. quasimubifer* **sp. n.** D, E. Posterolateral corner of abdominal segment VIII (ventral) of: D. *P. nubifer*; E. *P. paramubifer* **sp. n.**

The female genitalia (of a paralectotype) are illustrated for the first time in detail (Figs 1H, 2A) to allow comparison to congeners. Seminal capsules located near anterior end of notum, pale, ovoid, c. 100 x 70–80 μm ; seminal ducts short, straight, leading directly to separate openings. Cerci rounded rectangular, 85–100 μm long x

60–70 wide. Gonapophysis VIII lobes (Fig. 2A): apodeme lobe not visible, dorsomesal lobe (DmL) strongly developed, almost rectangular on inner contour, densely setose with apical setae curved mesad; ventrolateral lobe (VIL) c. 60–70 μm long, densely setose with apical setae directed more posteriad than mesad. VIL extended more posteriorly than DmL, but both ‘large’. Postgenital plate with distinct, strongly setose projection.

Pupa (Figs 2D, 3A, B, D). Exuvial length 5.5–7.5 mm. Pale yellow with golden-brown highlights (spinules, hooklets, cephalic tubercles, wing and antenna sheath margins, apophyses), and medium to dark brown caudolateral ‘comb’. Cephalic tubercles (Fig. 3A) triangular, 55–72 μm long and about equally wide at base, subapically bearing 25–32 μm long frontal seta inserted on tubercle just subapically. Thoracic horn highly plumose, arising from golden, oval basal ring. Thorax near dorsal midline with small tubercles aligned in uni-biserial row. Abdominal tergite spine/spinule pattern and setation as in Fig. 3B. Hook row on segment II c. 60–65% of tergite width, with c. 76–88 hooklets. Caudolateral ‘comb’ on VIII (Figs 2D, 3D) extends from L_4 setal base on broad dark apophysis, with dominant (apical-most) spine and several subsidiary spines extending along strongly darkened submargin. Anal lobe fringe unevenly bi-serial, especially posteriorly, with 44–75 taeniae, without dorsal seta.

Larva (Fig. 4A, C, E). For mensural features see Table 2. Head golden yellow, with dark, broad occipital margin (Table 2, oml) and usually substantially dark brown to black postmentum. Antenna (Fig. 4A) with alternate Lauterborn organs on a segment that might be either a more or less divided 2nd segment; blade extending no further than apex of this segment. Mentum (Fig. 4C) with paired median teeth, and 7 pairs of laterals of which 1st are very small, 2nd as high as medians, and 6th higher than 4th, 5th and 7th laterals. Microsculpture of ventromental plate (Fig. 4E): with dense striae, terminating anteriorly on outer (lateralmost) corner of plate in elongate lamellae without hooks or lobes. Body conventional for the genus.

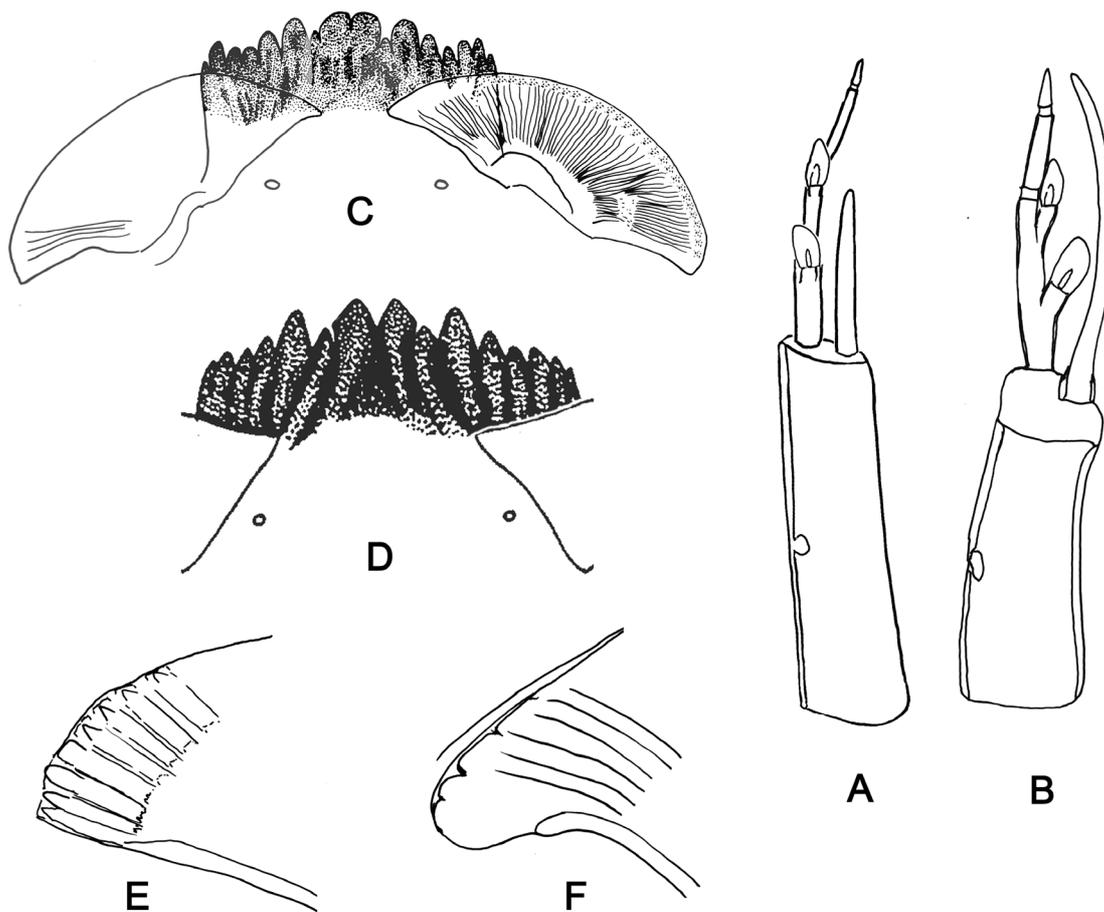


FIGURE 4. *Polypedilum* spp., larvae. A, B. Antenna of: A. *P. nubifer* (Skuse), B. *P. quasinubifer* sp. n. C, D. Mentum of: C. *P. nubifer*, D. *P. quasinubifer*. E, F. Lateral end of ventromental plate of: E. *P. nubifer*, F. *P. quasinubifer*.

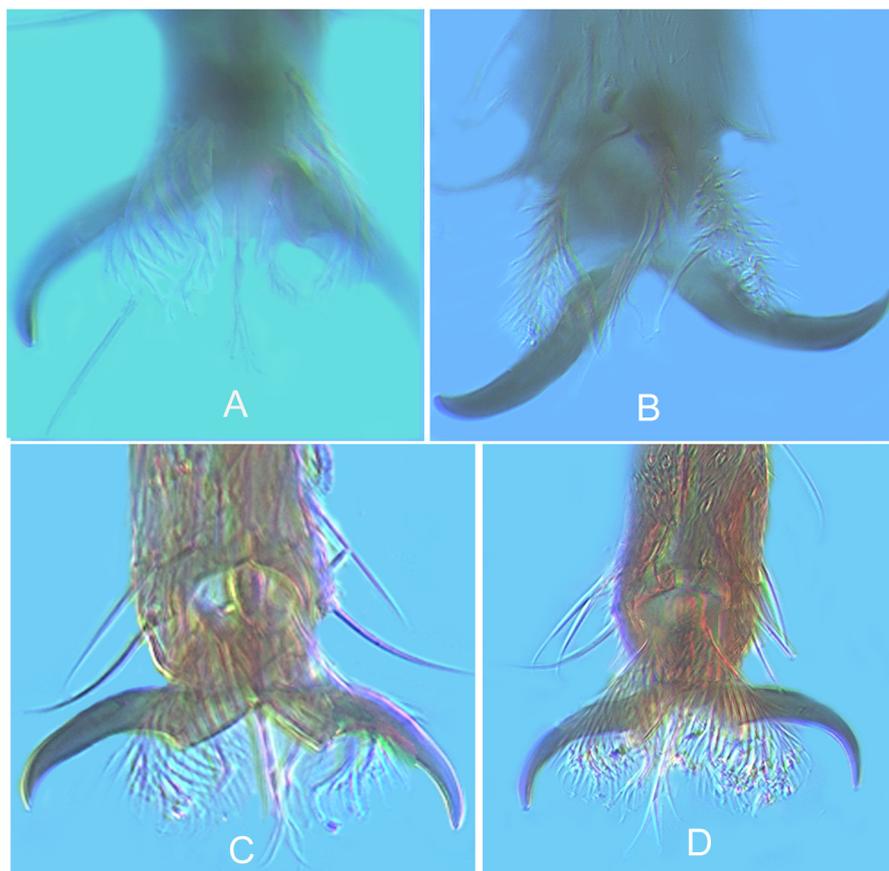


FIGURE 5. *Polypedilum* spp., adult pulvilli: of A. *P. nubifer* (Skuse), B. *P. quas nubifer* sp. n. C. *P. longiforceps* Kieffer, D. *P. lobiferum* Freeman.

***Polypedilum paranubifer* Cranston sp. n.**

(Figs 1D, G; 2B, 2E, 3E)

urn:lsid:zoobank.org:act:063C158B-3D62-4A56-9F52-AD5F84204456

Type material. Holotype: Le/P♂, AUSTRALIA: Northern Territory, Ranger Uranium mine, retention pond 2, 12°41'S 132°55'E, 31.v.1988 (*Cranston*) (ANIC).

Paratypes: Le/P♀, as holotype, except retention pond 1, 15.v.1992; 4♂, as holotype (ANIC); 11♂, Ranger Uranium mine, Retention pond 4, light trap, .v.1988 (*Wells & Suter*) (ANIC, 2 to BMNH).

Other material examined: 4L, Northern Territory, Ranger Uranium mine, Retention Pond 1, 31.v.1988 (*Cranston*) (ANIC).

Description as for *P. nubifer*, except as follows.

Adults (Figs 1D, G, 2B; female from pharate only). For mensural features see Table 1. Both sexes differ from *P. nubifer* in non-overlapping ranges of fewer setae on wing veins R and R₁ and in the complete absence of setae on R₄₊₅. Tergite VIII anteriorly appearing tapered to connection with T VII (Fig. 1D). Male genitalia (Fig. 1G) with gonocoxite weakly bulging, connected to gonostylus by pale membranous area. Dorsolateral seta present on a superior volsella otherwise similar in shape to that in *P. nubifer*. Gonostylus broad without bilobed apex, distally rounded. Female genitalia: gonapophysis VIII with dorsolateral lobe scarcely developed; ventrolateral lobe well-developed, 55 µm long, highly setose, with apical setae directed mesad (Fig. 2B). Cerci 100 x 100 µm, rounded rectangular.

Pupa (Figs 2E, 3E). Exuviae 5.4–5.8 mm long, pale with yellowish highlights and yellow-brown posterolateral comb on abdominal segment VIII. Cephalic tubercles conical, 50 µm high and 100 µm wide at base, bearing 35–45 µm long frontal seta inserted subapically on narrowed apical spine or 'nipple' of tubercle. Hook row on II c. 58–

64% of tergite width, with c. 44–45 hooklets. Spinule pattern apparently as in *P. nubifer* (Fig. 3B). Caudolateral ‘comb’ on VIII ((Figs 2E, 3E) with 2–3 stronger spines, none dominant; 2–5 smaller subsidiary spines, not extending to L₄ setal base. Anal lobe fringe uneven, uniserial, with 47–50 taeniae, without dorsal seta.

Larva. All measurements and ratios fall within the corresponding ranges for *P. nubifer* (see Table 2). Microsculpture of ventromental plates as in *P. nubifer*.

Notes. Adult males of *P. paranubifer* consistently differ from *P. nubifer* in the presence of a dorsolateral seta on the superior volsella. By itself, this difference could be seen as no more than population level variation, but it is accompanied by a suite of mensural differences in wing setation (Table 1). The pupa seems identical to *P. nubifer* except for the smaller size and slightly different structure of the ‘comb’ on the posterolateral corner of segment VIII. The latter, however, varies even within ‘true’ *P. nubifer*, thus probably is unreliable for discrimination. Likewise, the larvae of *P. paranubifer* cannot be distinguished from those of *P. nubifer* on morphology. Unreared larvae from retention pond 1, although seemingly identical with those associated by rearing with *P. paranubifer* adults, cannot be included in the type series since one pupal exuviae clearly belonging to *P. nubifer* was found also from the same pond system.

***Polypedilum quas nubifer* Cranston sp. n.**

(Figs 1A, C, F; 2C, F; 3C; 4B, D, F; 5B)

urn:lsid:zoobank.org:act:70DEF8F7-C4A2-445B-8550-B8417B29393F

Type material. Holotype, Le/Pe/♂, AUSTRALIA: Queensland, Lawn Hill National Park, Musselbrook, Amphitheatre, 18°21’S 138°09’E, 13.v.1995 (*Cranston*) (ANIC).

Paratypes, Le/Pe/♂, Le/Pe/♀, as Holotype; Pe, Queensland, Lawn Hill N.P., Musselbrook, Border Pool, 18°36’53’’S 137°59’25’’E, 14.v.1995 (*Cranston*) (ANIC).

Other material examined. SINGAPORE: Upper Seletar Reservoir, 1°24’10’’N 103°48’27’’E, 29.vii.2012, 16.vii.2013, 8.x.2013 (*NUS team*) (all life stages); Bedok Reservoir, 1°20’47’’N 103°55’31’’E, 11.ix.2013, (*NUS team*) (all life stages) (some of each stage to ANIC, remainder to RMBR—MV).

THAILAND: single L from each of: Chiang Mai, Amphur Doi Saket, Nam Kuang, 19°00’N 99°17’E, 15.v.2004, 554 m., L-689 (*Vitheepradit & Prommi*); Mae Kuang, 19°00’N 99°17’E, 15.v.2004 (*DuCharme*) Kalasin, Amphur Khao Wong, Tad Tong Waterfall, 16°45’N 104°07’E, 204 m., TH62 (*Vitheepradit*); Petchabun, Khao Khor N.P., stream from Tamtip Waterfall, 16°39.594’N 101°07.901’E, 182 m., 11.v.2004, L-678, TH5 (*Vitheepradit & Prommi*); Chaiyaphum, Phu Kiew WS, Lam Num Prom, 16°27’N 101°39’E, 589 m., 2.v.2004 (*DuCharme*); Prachin Buri, Khao Yai N.P., Kaeng Wang Thong, 14°10.077’N 101°43.420’E (*Sites & Vitheepradit*) (all ANIC).

Description. Adults (Figs 1A, C, F, 2C, 3B). For mensural features see Table 1. Frontal tubercles very small, 8–10 µm long. Foretibial apex triangularly tapering without apical spine (Fig. 1C). Pulvillus (Fig. 5B) divided in 2 parts; outer plumose but not forming ‘pad’, extending to mid-claw; inner part simple, unbranched, subequal in length to outer; empodium sparsely plumose, extending beyond pulvillus apex. Wing pattern very faint on (teneral) type specimens, variably to strongly developed on those from Singapore (Fig. 1A). Male T VIII waisted (tapering anteriorly); TIX with cluster of setae not lying within delimited paler area; anal point narrow. Male genitalia (Fig. 1F) with conventional gonocoxite and slender gonostylus tapering to blunt apex. Superior volsella curved, shorter and stouter than in *P. nubifer*, bearing dorsolateral seta inserted at about 2/3 length of volsella. Inferior volsella slender, with apical seta arising from weakly bilobed apex, scarcely longer than any other in sparse cluster of setae. Female genitalia: gonocoxite IX with 7–11 setae. Lobes of gonapophysis VIII (Fig. 2C): ventrolateral lobe squat (15–20 µm) bearing short setae; dorsomedial lobe no more than a rounded microtrichiose contour without setae, c. 90 x 125 µm, rounded rectangular.

Pupa (Figs 2F, 3C). Exuviae 5.0–5.5 mm long, pale with yellow highlights and yellow-brown comb on abdominal segment VIII. Cephalic tubercles triangular, 25–35 µm long and about equally wide at base, bearing 40–55 µm long frontal seta inserted basally on tubercle. Thoracic horn plumose, arising from oval, golden basal ring. Thorax mid-dorsally with dense cluster of small tubercles. Abdominal tergite spine/spinule pattern and setation as in Fig. 3C. Hook row on segment II c. 45–50% of tergite width, with c. 30–42 hooklets. Caudolateral spur on VIII (Figs 2F, 3E) well-developed, with dominant (apical-most) spine and few significantly smaller subsidiary spines not extending to L₄ setal base. Anal lobe fringe evenly uniserial, with 23–30 taeniae, without dorsal seta.

Larva (Fig. 4B, D, F). For mensural features see Table 2. Head yellow, with pale postmentum and narrow dark occipital margin; head total length, postmentum, mentum (Fig. 4D) and mandible c. 30–40% shorter than in *P. nubifer*. Antennal segment lengths shorter but ratios similar: Antennal blade relatively longer, reaching antennal apex (Fig. 4B). Microsculpture of ventromental plate (Fig. 4F): with sparser striae, terminating anteriorly on outer (lateralmost) corner of plate in 3–4 shallow lobes.

Notes. *Polypedilum quasinubifer* is smaller in all measurements (e.g. wing length c. 40% of *P. nubifer/paranubifer*), has fewer setae, and differs significantly in ratios, notably the fore leg ratio of 1.9–2.2 (in both sexes). The pupa is smaller and paler than those of *P. nubifer/paranubifer*, with smaller cephalic tubercles but longer frontal setae arising nearer the tubercle base. The thorax has a dorsal cluster of small tubercles (rather than aligned rows of larger tubercles). The hook row is narrower (no more than 50% of tergite width) and with fewer hooklets. The 'comb' on segment VIII is paler, smaller, with a more dominant major spine and fewer subsidiary spines. The anal lobe has fewer taeniae (<30) in a uniform uniserial row.

Further material examined pertaining to *Tripedilum* Kieffer

Polypedilum (Polypedilum) longiforceps Kieffer

(Fig. 5C)

Polypedilum longiforceps Kieffer, 1921; Kieffer (1921b); Freeman (1958).

Polypedilum fuscipenne Kieffer, 1921; Kieffer (1921b), synonymised by Freeman (1958), invalid junior homonym of *P. fuscipenne* (Meigen, 1818).

Tripedilum armatifrons Kieffer, 1921; Kieffer (1921c), syn. Freeman (1958).

Microtendipes longiventris Kieffer, 1922; Kieffer (1922), syn. Freeman (1958).

Material examined. 1♂, 'A–E. Sudan, Yirol, xii.1954, E.T.M. Reid, BM 1954-153', 'BMNH (E) 1240737' '*Polypedilum fuscipenne* Kieffer, Euparal slide P.S. Cranston July 2015'; 1♂, 'Gold Coast, Nangodi, 8.x.1954, G. Crisp, BM 1954-675', 'BMNH (E) 1240739' '*Polypedilum fuscipenne* Kieffer, Euparal slide P.S. Cranston July 2015'

1♀, 'Congo belge, Maka Lualaba, 23.i.1939, H. Brédo', 'R. Mus. Hist. Nat. Belg. I.G. 12.290' 'BMNH (E) 1240742' '*Polypedilum fuscipenne* Kieffer, Euparal slide P.S. Cranston July 2015'.

Notes. The species is readily recognised from Freeman's key and description. The adult frontal tubercles are 30 µm long; the fore tibial apex carries a darkened triangular spine without any rounded base; the superior volsella is narrow, apically upcurved, and without a distal seta. The pulvillus (Fig. 5C) is divided in 2 parts; outer broad and plumose forming thin 'pad', extending to near tip of claw; inner part sinuous, multiple branched, subequal to outer; empodium strong, apically plumose and extending beyond pulvillus.

Polypedilum (? Tripedilum) lobiferum Freeman

(Fig. 5D)

Polypedilum lobiferum Freeman, 1954; Freeman (1954, 1958).

Material examined. 1♂, 1♀, [South Africa, Eastern Province] 'Port Elizabeth, 31st Aug. 1936' [*de Meillon*] 'BMNH (E) 1240743/4' '*Polypedilum lobiferum* Freeman / Euparal slide PS Cranston, July 2015'.

Notes. The species is readily recognised from Freeman's key and description. Adult frontal tubercles 25 µm wide at base, 25–30 µm long; fore tibial scale curved from base to small asymmetric point; superior volsella stubby, straight and without any seta.

Pulvillus (Fig. 5D) divided in 2 parts; outer densely plumose forming large broad 'pad', extending to tip of claw; inner part sinuous, highly branched, subequal in length to outer; empodium strong, plumose and extending beyond pulvillus and tip of claw.

Discussion

The correct valid name for *Chironomus nubifer* Skuse, 1889. After submission of the text on Chironomidae for a regional catalogue of the Diptera (Cranston & Martin, 1989), the entry for the species name *nubifer* was emended to *nubiferum*, presumably to match the neuter gender of the genus name *Polypedilum*. Although this change was followed subsequently in the Australian regional catalogue (Bugledich & Cranston, 1999), it should be rejected as an unjustified emendation. The International Code of Zoological Nomenclature Article 32.2.2 reads: "Where the author of a species-group name did not indicate whether he or she regarded it as a noun or as an adjective, and where it may be regarded as either and the evidence of usage is not decisive, it is to be treated as a noun in apposition to the name of its genus (the original spelling is to be retained, with gender ending unchanged)" (ICZN, 1999). The name *Chironomus nubifer* was published by Skuse (1889) without explanation or evidence that would qualify the epithet as adjectival for the purposes of nomenclature. Thus, the epithet must be treated as a noun in apposition that remains unchanged, regardless of the gender of any genus name with which it may be combined.

Consequences for the definition of *Polypedilum*. Some of the purportedly aberrant morphology previously thought to occur in *Polypedilum nubifer* only is shared now by *P. paranubifer* Cranston **sp. n.** and *P. quasinubifer* Cranston **sp. n.** The female genitalia in *P. nubifer*, described and illustrated here for the first time, conform to those seen in other species of *Polypedilum* and strongly resemble the genitalia in *P. nubeculosum* (Meigen) in the sense of Sæther (1969). Compared to these two species, *P. paranubifer* and *P. quasinubifer* show markedly different relative development of the lobes of gonapophysis VIII (Fig. 2A–C). Evidently, closely related species can differ in the female genitalia, and the morphology of the latter may poorly reflect relationships at this level. Concerning the males of *P. paranubifer* and *P. quasinubifer*, a dorsolateral seta is present on the distal projection of the superior volsella, and tergite VIII tapers anteriorly; both these features narrow the gap between *P. nubifer* and other members of *Polypedilum*. Contrary to Sæther *et al.* (2010), strong pedes spurii A are present on pupal sternite IV removing a previously assumed difference in this stage. However, the strong frontal setae remain distinctive for the species grouping. The phylogenetic position of *P. nubifer*, deeply embedded within the *Polypedilum* tree as proposed by Cranston *et al.* (2011) based on molecular evidence, further implies that the species is less divergent than has been asserted.

Ashe (1981) has validly fixed *P. pelostolum* Kieffer (from Taiwan) as the type species of *Polypedilum*: this species name had been considered as a synonym of *P. nubifer* by Freeman (1961) but based on unmounted material only. We have examined new microscope mounts of male and female *P. pelostolum* using syntype specimens seen by Freeman. All adult morphology, including mensural features (Table 1), falls within the ranges known from other material of *P. nubifer*, especially close to values for the Australian type specimens. We have no doubt that Freeman's (1961) synonymy of *P. pelostolum* and *P. nubifer* is correct. The taxonomic consequences of *P. nubifer* as the nomenclatural type of *Polypedilum* are not problematic, since on molecular evidence the species (and *P. quasinubifer* Cranston **sp. n.**; W. H. Wong, pers. comm.) clearly lies within *Polypedilum*.

The status of *Tripedilum* Kieffer. *Tripedilum* Kieffer, 1921a first appeared in a key, thus providing a diagnosis for the genus although no names of included species were given. Since the genus name was established prior to 1931, ICZN Code Article 67.2.2 (ICZN, 1999) applies, and the first subsequently and expressly included nominal species are deemed to be the only originally included nominal species. The first included species is *Tripedilum armatifrons* Kieffer, 1921c, which was based on females from Kribi (Cameroon). As the only nominal species mentioned in the genus by Kieffer (1921c), *T. armatifrons* is the type of *Tripedilum* by monotypy (Freeman, 1958; Ashe, 1983). Subsequently, *Tripedilum* or its members were mentioned rarely. Freeman (1958) revised the African fauna and synonymised *T. armatifrons* with both *Polypedilum longiforceps* Kieffer, 1921b and *P. fuscipenne* Kieffer, 1921b, although he was not certain that he was interpreting *P. fuscipenne* correctly ("seems to have been described from a dark female"; Freeman, 1958: 288). Freeman (*loc. cit.*) also synonymised under *P. fuscipenne* the name *Microtendipes longiventris* Kieffer, 1922. He had been unable to find original type material for any of these four species names, but relied on Kieffer's (1921b, 1922) drawings of male hypopygia (*P. longiforceps*, *M. longiventris*), and on subsequent associations of such males with simultaneously collected females that fell within his species concept of *P. fuscipenne*. Freeman (1958: 288) noted correctly that *P. fuscipenne* had been published two days earlier than *T. armatifrons* (see also Ashe & Spies, 2011: 11), but he did not notice that the name *Polypedilum fuscipenne* Kieffer is invalid as a junior secondary homonym of *Polypedilum fuscipenne* (Meigen, 1818), which was established originally in the genus *Chironomus* but has been treated as a valid name in

Polypedilum. Freeman (1958) discussed the difficulty of evaluating the pulvilli, and reported mistakes that Kieffer had made in such evaluations. Indeed Kieffer's diagnoses of *P. fuscipenne* and *P. longiforceps* do not mention the pulvilli because he gave them in keys including *Polypedilum* species exclusively, and thus implied that all shared the pulvillus condition with which he had diagnosed this genus. On this evidence Freeman (1958: 268) treated *Tripedilum* as a new synonym of *Polypedilum* based on examination of material detailed above.

Tripedilum Kieffer was not used as valid again until Sæther *et al.* (2010) proposed a reclassification in *Polypedilum* that involved *Tripedilum* as the name for a new subgenus. Included were the type-species under the name *P. fuscipenne* Kieffer (following Freeman's synonymy, *i.e.*, also overlooking the homonymy), *P. nubifer*, and another Afrotropical species, *P. lobiferum* Freeman. Diagnostic features cited for the adult male were the possession of distinct frontal tubercles and the lack of any outer (distolateral) seta on the superior volsella. The projection of the superior volsella was seen as very long relative to the short base, and the gonostylus as wide with a broad, blunt apex. Sæther *et al.* (2010) characterised *P. fuscipenne* and *P. lobiferum* as having a spur on the fore-tibial scale, in contrast to the rounded scale in *P. nubifer*. Although diagnoses were given for all stages of *Tripedilum*, in reality those for the immature stages were based on *P. nubifer* only, as there is no evidence on the immature stages of the Afrotropical species. Moreover, although the three species share the lack of a distal seta on the superior volsella, the genitalia otherwise are quite diverse, *e.g.* concerning the anal point, inferior volsella and gonostylus shape (see Freeman, 1958).

The species diagnoses for *P. fuscipenne* and *P. longiforceps* (both given in keys in Kieffer 1921b and Kieffer 1922, respectively) and the delimitation of *Tripedilum* (in Kieffer, 1921a) provide no substantial distinguishing details, and the relevant key couplets in Kieffer (1921b, 1922) even lack contrast. In agreement with Freeman (1958), our morphological examinations, including of the structures at the apex of the 5th tarsomere have shown nothing that would stand out from normal variation across *Polypedilum*, and thus no basis exists for taxonomic segregation. In summary, the accuracy and generality of the diagnoses for *Tripedilum* in Sæther *et al.* (2010) are doubtful, there is little evidence that the species which these authors included in *Tripedilum* form a clade, and no reason is apparent for excluding those three species from *Polypedilum* (*s. str.*).

The status of other subgenera in *Polypedilum*. Following the rearrangement of subgenera in *Polypedilum* by Sæther *et al.* (2010), challenges to their concepts, diagnoses and the resulting taxonomic implications have been published. Concerning their revised concept of *Tripedilum* the present different interpretation is the first such dissent expressed in a publication. However, Zhang *et al.* (2015) noted that in certain *Polypedilum* species the 'outer seta' of the superior volsella can be absent on both body sides, or present on one side only, which undermines the diagnostic value of this feature. Earlier, *Polypedilum maculipennatum* Yamamoto, Yamamoto & Hirowatari had been described as lacking a dorsolateral seta on the superior volsella, but Yamamoto *et al.* (2012a) made no comment on *Tripedilum*. This was likely due to their new species lacking a frontal tubercle and having a triangular fore tibial scale with smoothly rounded apex, thus not conforming to *Tripedilum* in these features. Examination of the character state matrix in Sæther *et al.* (2010: pp. 33–34, Appendix 1) shows that the distribution of the states 'superior volsella seta absent / polymorphic' is not limited to the species in their subgenus *Tripedilum*. Furthermore, in some cases these states conflict with the condition scored in the following matrix column; for example, *Asheum beckae* is scored with the seta present and absent (char. 26: '0' = seta present; char. 27: '3' = seta absent). These inconsistencies add to the confused and inadequate differentiation of *Tripedilum* and other subgenera in Sæther *et al.* (2010), and render suspect the already weakly supported phylogenetic conclusions.

Concerning the subgenus *Pentapedilum*, diagnosed largely by the microtrichiose wing, doubts of monophyly have long existed due to the paucity of any other distinguishing features, especially in the immature life stages, and to the likelihood that the wing setosity is plesiomorphic. In a molecular study using a single gene, Kawai *et al.* (2012) found *Pentapedilum* to be paraphyletic, as does an unpublished estimate (W. H. Wong pers. comm., 2014). Bringing morphology to bear, Yamamoto *et al.* (2012b) suggested that at least those species with wing microtrichia only at the wing apex should be removed from *Pentapedilum*, but it is uncertain if this 'solves' the problem of non-monophyly.

In discussing their inability to place subgenerically a new species of *Polypedilum* (*P. notabile* Yamamoto, Yamamoto & Hirowatari) these authors provide an insightful survey of the highly variable shapes of the superior volsellae across several subgenera, stating "... it appears that a tendency of continual variation is present throughout each subgenus. These might destabilize some positions of the subgenera defined by the features of the superior volsella in the genus *Polypedilum*. Further study might serve to elucidate the taxonomic and phylogenetic

significance of the superior volsella” (Yamamoto *et al.*, 2012a: p. 83). Most recently, Yamamoto & Yamamoto (2015) challenged the concept of subgenus *Probolum* Andersen & Sæther, 2010 (in Sæther *et al.*, 2010) based on reinterpretation of the morphology of the superior volsella in *Polypedilum simantokeleum* Sasa, Suzuki et Sakai (Sasa *et al.*, 1998).

Evidently difficulties with the delimitation of subordinate groups within *Polypedilum* extend well beyond *Tripedilum*, so much so that the value of the entire subgenus scheme in Sæther *et al.* (2010) is highly questionable.

Morphology of the larval ventromental plate. Yamamoto & Yamamoto (2015) draw attention to and illustrate (fig. 11) a ‘posterior lobe’ on the ventromental plate in *Polypedilum nubifer*. Whereas Sæther *et al.* (2010) had described the plate as lacking such a lobe, Yamamoto & Yamamoto (2015) assert that in *P. nubifer* the mentum and ventromental plates, being double-walled and strongly sclerotized, form a sclerotized ridge due to invagination into the cranial cavity, and that this constitutes a fundamental morphological structure that is observable in virtually all larval Chironominae. Examination of many larval *Polypedilum* by the present first author, including *P. nubifer* and the species described here as new, shows that this ventromental plate structure might be better termed an apophysis (an internalised exoskeletal insertion or apodeme) rather than a ‘lobe’. Apparently it is always present, although the depth of sclerotization, visibility through cuticular pigmentation, and perhaps its orientation relative to a compressed larval head, make interpretation somewhat subjective, and may explain why most published descriptions have omitted this structure.

Conclusions

The traditional concept of *Polypedilum nubifer* sensu lato is found to include two new species from Australia, one of which occurs also in south-east Asia. These bridge the previously perceived morphological disparity between *P. nubifer* and ‘core’ *Polypedilum*, as does the (so far limited) molecular evidence. *Polypedilum nubifer* is confirmed as the valid name for the synonym *P. pelostolum*, the type species of *Polypedilum* Kieffer. This study illustrates how single egg mass rearings, barcodes from diagnosed conspecifics and examination of type series all contribute to understanding the true morphological variability within and among cryptic species.

Placement of *P. nubifer* in a subgenus *Tripedilum* by Sæther *et al.* (2010) is based on incorrect and/or confused nomenclatural argumentation and lacks sufficient morphological justification. Specifically, (1) any proposal to change the type species for *Polypedilum* is invalid as long as no corresponding application for ICZN decision has been submitted; (2) the genus and subgenus that contains the type species of *Polypedilum*, *P. nubifer*, must be called *Polypedilum* and *P. (Polypedilum)* respectively and therefore (3) *Tripedilum* could be revalidated only if it excludes *P. nubifer* or any junior synonym of it (for example, *Tripedilum* could contain *P. lobiferum* only, or *P. lobiferum* plus *P. longiforceps* (jun. syn. *P. fuscipenne* Kieffer), if the latter were found not to be synonymous with *P. nubifer*). Returning *Tripedilum* to its status as a junior synonym of *P. (Polypedilum)* removes any problem seen by Sæther *et al.* (2010) concerning the type species of *Polypedilum* and, thus, all necessity for an application to the ICZN.

The publication by Sæther *et al.* (2010), especially as concerns its review of the status, concepts and delimitation of subgroups within *Polypedilum*, cannot be recommended for use, except with extreme caution, on account of errors of nomenclature, incomplete and sometimes erroneous descriptions, faults with character state scoring and the weakly supported morphological phylogenetic hypothesis. Many groups perceived in that work lack morphological credibility across all stages and conflict even with the so-far modest molecular sequence analyses. We concur with Japanese colleagues in questioning any wide applicability for the study.

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