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THE NUISANCE MIDGES (DIPTERA: CHIRONOMIDAE) OF SINGAPORE'S PANDAN AND BEDOK RESERVOIRS

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ABSTRACT. — The two species of chironomid midges that are known to be involved in mass swarming on the shores of Singapore's Pandan and Bedok reservoirs are described or redescribed. All life stages are illustrated to allow identification. *Polypedilum nubifer* (Skuse), the predominant nuisance midge of Pandan reservoir, is globally known as a coloniser of new and unstable aquatic habitats. Its biology and methods of control are understood. In contrast, *Tanytarsus oscillans* Johannsen is the predominant nuisance midge at Bedok reservoir. This species is restricted to Asia from India to southern Japan and its immature stages were unknown previously. Its nuisance status (restricted to Bedok Reservoir, Singapore) is of recent origin. Means of separating these species from similar chironomid species in the region are provided. We conclude with some observations on the chironomid fauna of standing waters in Singapore.

KEY WORDS. — Chironomidae, nuisance, Singapore reservoirs, mass emergence, *Tanytarsus*, *Polypedilum*, control

INTRODUCTION

Non-biting midges (Diptera: Chironomidae) are ubiquitous and often abundant insects encountered everywhere, including even the Antarctic (Cranston, 1994a). The perception that adult flying midges can cause nuisance to humans is widespread, but it is rather subjective. The immense swarms of small midges belonging to the species *Cladotanytarsus lewisi* Freeman that emerge seasonally from the White and Blue Niles (especially in Khartoum and Omdurman) undeniably cause nuisance (Cranston et al., 1983a). Their large numbers and small size means they trouble local residents along the banks of the Nile, and the midges cause inhalant allergy both when freshly emerged and subsequently, when houses are cleaned (Cranston et al., 1983b; Cranston, 1994c). The equally impressive mass emergences from East African lakes are surely also nuisances but this status is tempered by the use of 'lake flies' (Chironomidae plus Chaoboridae [phantom midges], MacDonald, 1956) as human food called 'kungu' cake (Gullan, 1995).

The regular emergence of numerous adult midges from nutrient-enriched (eutrophic) aquatic habitats, such as urban lakes, water-storage reservoirs, rice fields and sewage treatment plants undoubtedly cause nuisance when humans come into close contact with the insects (e.g., via housing, recreation; McHugh et al., 1988; Ali, 1994). Consideration also must be given to the differing reaction thresholds of urban populations that are divorced from natural conditions and are more likely to complain when exposed to natural phenomena such as midge swarms. It is known that they do so at much lower exposure than more rural people who are used to a higher level of interactions with insects (McHugh et al., 1988; Lin & Quek, 2011).

In Singapore, informal data suggests that the water storage reservoirs in the Central Catchment Area have generated swarms of adult Chironomidae over at least the past half century (P. Murphy and D. H. Colless pers. comm.; P. S. Cranston, pers. obs.). However with limited numbers of Singaporeans accessing the area surrounding these reservoirs and little close habitation, this was of low concern. It was not until the completion of the construction of Pandan Reservoir in the southwestern Area in the late 1970s that numerous swarming midges started to impact residents. Subsequently, Bedok Reservoir in the East (completed 1986) more recently developed massive seasonal swarms of midges causing nuisance to local residents, who brought the problem to the attention of politicians and the media (Lin & Quek, 2011). Most reports concern midges of the genera *Tanytarsus* Kieffer and *Polypedilum* Kieffer, as is often the case elsewhere in the world.

Chironomidae have aquatic immature stages and the vast majority of the over 7,000 species described globally lay eggs at the water surface. The larvae develop in bottom and marginal substrates of aquatic ecosystems, in both running and standing waters. Most feed on diatoms and/or algae and/or cyanobacteria. All pass through four larval instars, with a growth increment between each that accords with the expectation of Dyar's law (Gullan & Cranston, 2010). As in all holometabolous insects (Gullan & Cranston, 2010), the final larval instar completes the immature stage growth and then develops into a quiescent pupa. In this stage the larval tissues are reorganised into the adult midge, which emerges at the water surface from the pupa which rises to the surface to eclose. Thus the life history involves several morphologically and ecologically distinct stages—the feeding larva, the transformational pupa and the free-flying adults of both sexes. All stages are used in making identifications and in reconstructing the phylogenetic relationships of Chironomidae.

MATERIAL AND METHODS

Collections. — Adult midges were collected close to Pandan, Bedok, and Upper Seletar reservoirs and in adjacent apartments during nuisance outbreaks by sweep netting and aspiration of individuals from margins and cement walls, and netting from the surface of reservoirs. Pupal exuviae and a few pupae, from which adults had not eclosed, were skimmed from the water surface on the lee shore (Wright & Cranston, 2000). Larvae were sought using a range of techniques—a benthic grab sampler, sweeping with a fine-mesh pond net at the margins, and from rearing.

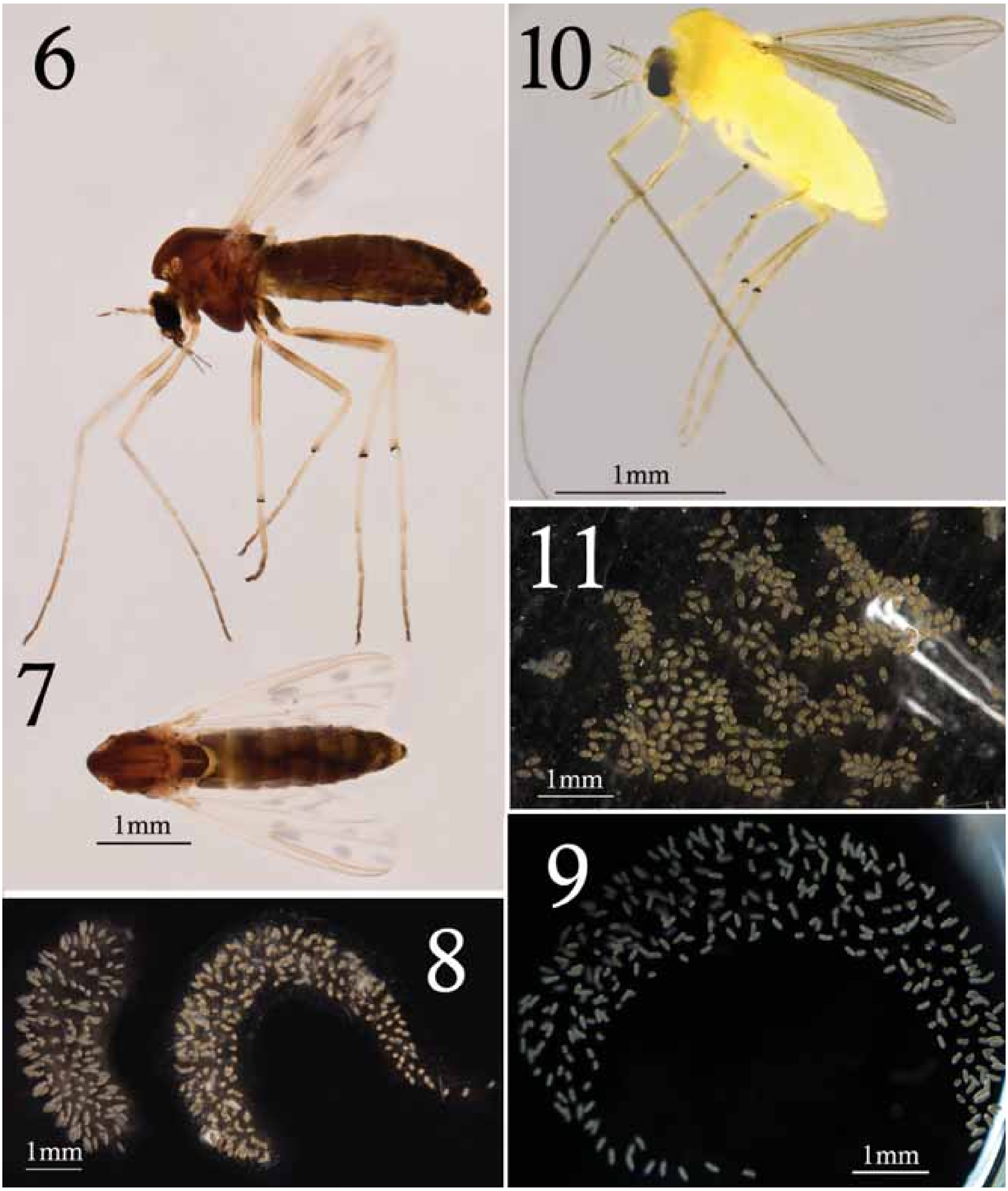
Adults of abundant midges were collected in the evening at Bedok or Pandan Reservoir using a mechanical aspirator, transferred into a 30-cm cube cage, and provided with cotton soaked in sugar solution enriched with Vitamin B complex. Next morning females were transferred individually into plastic vials containing 2 ml of settled water and checked daily. Egg masses confirmed subsequently as belonging to *Polypedilum nubifer* (Figs. 8, 9) were pipetted individually into a plastic cup containing 25 ml of aged water, incubated at a room temperature of 27°C, and checked under a microscope daily for hatching. Eggs identified subsequently as those of *Tanytarsus oscillans* (Fig. 11) were laid singly and were left to incubate in the plastic vials. Larval *P. nubifer* were transferred into separate 25 × 30 cm plastic rearing trays filled with 2L of aged water, and those of *T. oscillans* into separate 13 × 13 cm plastic containers filled with 500 ml of aged water. Finely blended and pre-washed ANF Adult Formula™ dog food and Azoo Premium Peat Moss™ were added as food and substrate respectively. Trays were covered securely with cloth and kept at 27±1°C and 10:14 (L:D) photoperiod. Fresh food was added every 3rd or 4th day, and water changed before feeding, when larvae were visible to the eye (at ca. 11–14 days after setup). Larvae were reared until emergence with adults collected from under the cloth cover and on insides of the tray. Post-oviposition females were preserved in 70% ethanol.

All morphospecies, including all stages of *P. nubifer* and *T. oscillans* were photographed in as complete (undamaged) condition as possible. Subsequently a small and disposable section of the abdomen (from larva or adult) was dissected off and used for DNA extraction and sequencing. Small larvae and some specimens of adults were extracted whole, with preparation of the complete individual following Krosch & Cranston (2012). Most specimens required slide preparation using standard procedures (Cranston, 2000). Identifications of larvae, pupae and adults were made using compound microscope optics and a wide range of identification tools. For DNA sequences we used the barcoding section of mitochondrial gene cytochrome oxidase 1 (*CO1*) to associate wild-caught larvae with mature stages, and to associate morphologically-determined vouchers of adults (Krosch & Cranston, 2012).

DNA extractions and sequences. — Genomic DNA of specimens from Bedok, Pandan, and Upper Seletar Reservoirs was extracted using Qiagen DNeasy Blood and Tissue Kit

Figs. 1–5. Midges as nuisance in Singapore. *Polypedilum nubifer* male: 1, habitus, lateral; 2, habitus, dorsal; 3, abdomen, dorsal; 4, hypopygium, dorsal; 5, anal point, superior volsella. Abbreviations: apt, anal point; gc, gonocoxite; gs, gonostylus; iv, inferior volsella; sv, superior volsella.

with some modifications to the manufacturer's protocol as described in Krosch & Cranston (2012). We amplified the DNA barcoding region which consists of a 658-bp fragment of the cytochrome oxidase c subunit I (*COI*) using the well-known general invertebrate primers LCO 1490 – 5' GGT CAA CAA ATC ATA AAG ATA TTG G 3' and HCO 2198 – 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3' (Folmer et al., 1994). PCR amplifications were carried out using ExTaq (TaKaRa™). The cycling conditions started with a temperature of 95°C for DNA denaturation for 30 seconds, followed by 30 seconds of annealing at 51°C, and 1 minute of extension at 72°C. The replication cycle was repeated 40 times. Amplified DNA products were purified using SureClean™ (Bioline) according to the manufacturer's protocol. Purified products were sequenced using an ABI 3100 Avant DNA Sequencer. All chromatograms were analysed



Figs. 6–11. Midges as nuisance in Singapore. *Polypedilum nubifer* female: 6, habitus, lateral, 7, habitus dorsal. *P. nubifer* eggs: 8, Egg masses; 9, solitary egg mass. *Tanytarsus oscillans*: 10, female habitus lateral; 11, eggs.

and edited using Sequencher™ 4.6. Edited sequences were translated into amino acid sequence to ensure there were no stop-codons, and then exported in Fasta format for alignment in MAFFT version 7 (default options; Katoh et al., 2005). Aligned sequences were analysed using SpeciesIdentifier ver. 1.7.9 (Meier et al., 2006) through objective clustering at 1%, 2%, 3% and 4% thresholds as discussed in Meier (2008). Objective clusters consist of a collection of sequences for which each has at least one other sequence below the threshold; this also means that the most distant sequences may have distances above the threshold. Note that the range of thresholds employed here covers the empirically observed distances between most species (Meier et al., 2008) and that we used uncorrected distances given that the use of Kimura-2P cannot be justified (Srivathsan & Meier, 2011). The sequences for this study are available from GenBank (KF273986 – KF273995, KF273997, KF274000 – KF274002, KF274005, KF274006 and KF274008).

Images. — Habitus photographs were taken with a Visionary Digital™ BK Plus Lab System at different focal lengths and compiled into a fully focused image using Helicon Focus Pro™. Higher magnification images were obtained using a Leica™ DMRX compound microscope with Nomarski™ interference optics. Photographs were taken with an Automontage™ system, allowing automated retention of focused parts of a sequence of exposures at different focal depths. All post image-capture manipulations were made in Adobe® Photoshop™.

Identifications. — Making genus-level identifications in the Chironomidae is now relatively straightforward using a range of identification keys (see below) and including reference to original descriptions (Skuse, 1989; Johannsen, 1932) and redescriptions. Although historically a schism existed with immature stages (larvae and pupae) differently arranged to the adults (reviewed by Cranston, 1994b), reconciliation followed collaborative Holarctic keys (Wiederholm, 1983, larvae; 1986, pupae; 1989, adult males). Although these guides focused on the Holarctic (northern hemisphere) midges, they provided a framework for a wider region including much of Asia. The regional key to the larval Chironomidae of Malaysia and Singapore (Cranston, 2004) provides expanded keys and short comments on the regional genera.

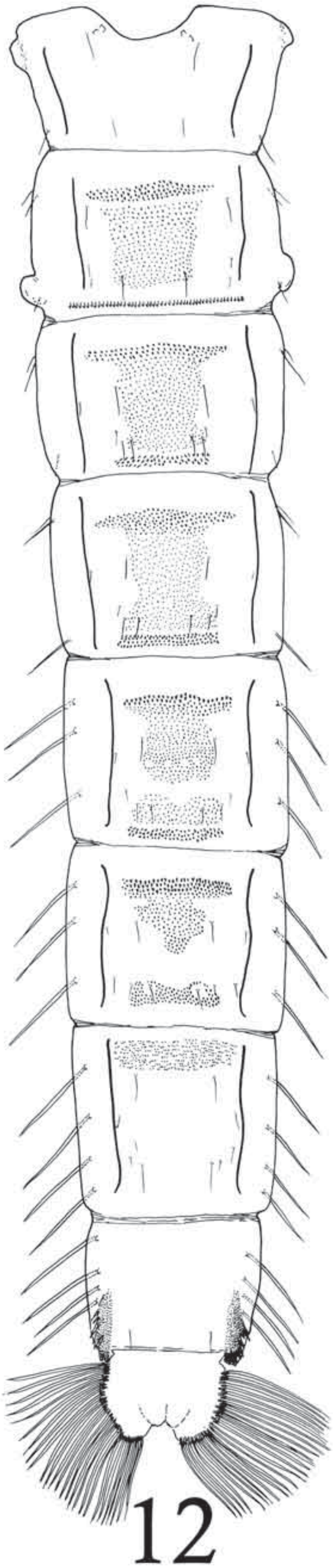
Problems are evident with species-level determination. Existing descriptions and illustrations often are inadequate, numerous names exist for common but widespread species, and different names continue to be used for the same species. Early scientists were unaware of the widespread distributions of many species, perhaps notably those that we now know to cause nuisance. Reconciliation requires detailed examination of all previous specimens used in descriptive taxonomy, yet these 'types' often are badly preserved or even unavailable for study. Many studies involve only adults, usually the males but sometimes the female alone which is difficult to recognise and place into modern morphological species. The larva usually is unknown or poorly known, and even if so, descriptions are usually superficial. The pupa, which provides a wealth of species-level identification features, was rarely included in any description. In comparison with the reconciled generic features mentioned above, harmonisation of the species-level nomenclature and identification is less advanced. The two dominant chironomid species causing nuisance in Singapore exemplify many of these problems.

Abbreviations. Descriptions: L = larva, P = pupa, Pe = pupal exuviae, P♀ = pharate female pupa, P♂ = pharate male pupa. All measures are in μm if not otherwise stated. Institutions: ANIC, Australian National Insect Collection, Canberra, Australia; BMNH, British Museum (Natural History), London, UK; NUS, National University of Singapore, Singapore; ZRC, Zoological Reference Collection, Raffles Museum of Biodiversity Research, Singapore; TMSI, Tropical Marine Science Institute, National University of Singapore, Singapore.

RESULTS

The sequences aligned gap-free in MAFFT and we used these data and rearings to associate life stages. Seventeen sequences pertained to the predominant nuisance species at Pandan and Bedok Reservoirs. Three (1 adult, 2 larvae) were for the nuisance species of Pandan, *Polypedilum nubifer* (Skuse, 1889), which was identified based on adult and larval morphology. All DNA sequences obtained for *P. nubifer* were identical. The nuisance species of Bedok was identified as *Tanytarsus oscillans* (Johannsen, 1932) based on the adult male morphology. Fourteen sequences (10 adult, 4 larval) revealed six haplotypes with pairwise distances ranging from 0–1.475% between the three reservoirs. Note that while this level of intraspecific variability would be considered high in most insect species (Whitworth et al., 2007; Meier et al., 2008; Renaud et al., 2012), such large distances are not uncommon in barcoding studies of Chironomidae (Ekrem et al., 2007, 2010; Sinclair & Gresens, 2008; Krosch et al., 2012). Each sequence for *T. oscillans* had at least one other sequence with a distance of <1% so that the cluster analysis was stable from 1–4%. To test whether the observed differences are due to sequencing error, we assessed the relative position of the changes by aligning the *T. oscillans* sequences to the COI of *Drosophila melanogaster* (GenBank accession number U37541.1). The 12 variable sites are distributed across the full length of the sequence (positions: 181, 271, 281, 282, 316, 373, 412, 478, 487, 559, 628, 631). All except one change (position 282) are synonymous (amino acid position 94; Methonine => Threonine). Searches of public databases using the COI-sequence of *T. oscillans* revealed only one species with >88% similarity: *Tanytarsus kiseogi* Ree & Jeong 2010, matched at 92%.

Polypedilum nubifer *(Skuse)*. — This species was described from New South Wales, Australia, based on male and female adults, by Skuse (1889), in *Chironomus*; Freeman (1961) transferred it to *Polypedilum*. Freeman's revision of adult Australian Chironomidae revealed a complex nomenclatural history for this species. Despite the prolific Abbé J. J. Kieffer (1906) being aware of the Australian species, apparently he described the same species as new to science four times in



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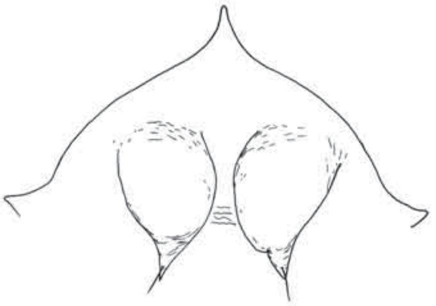

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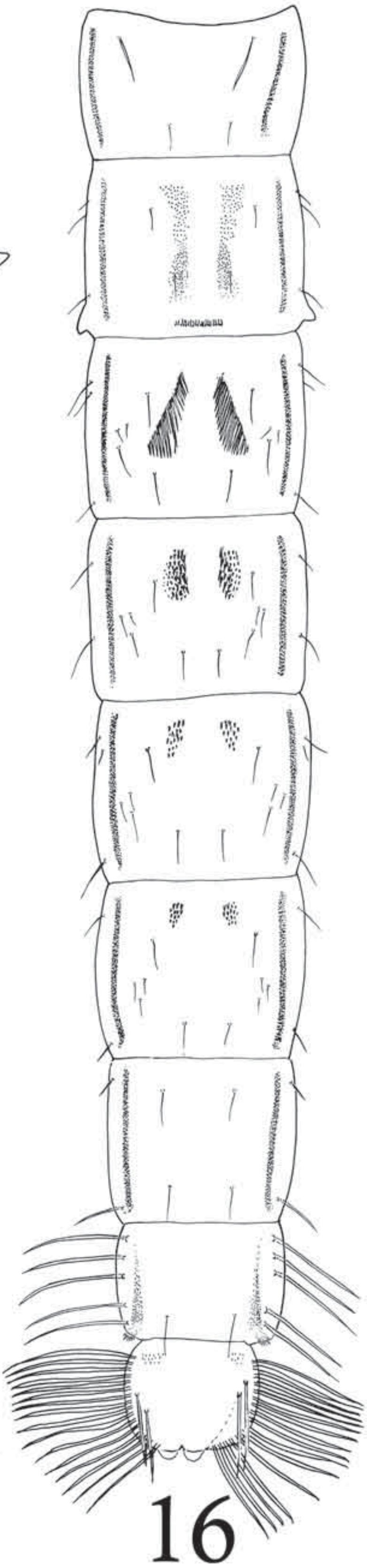

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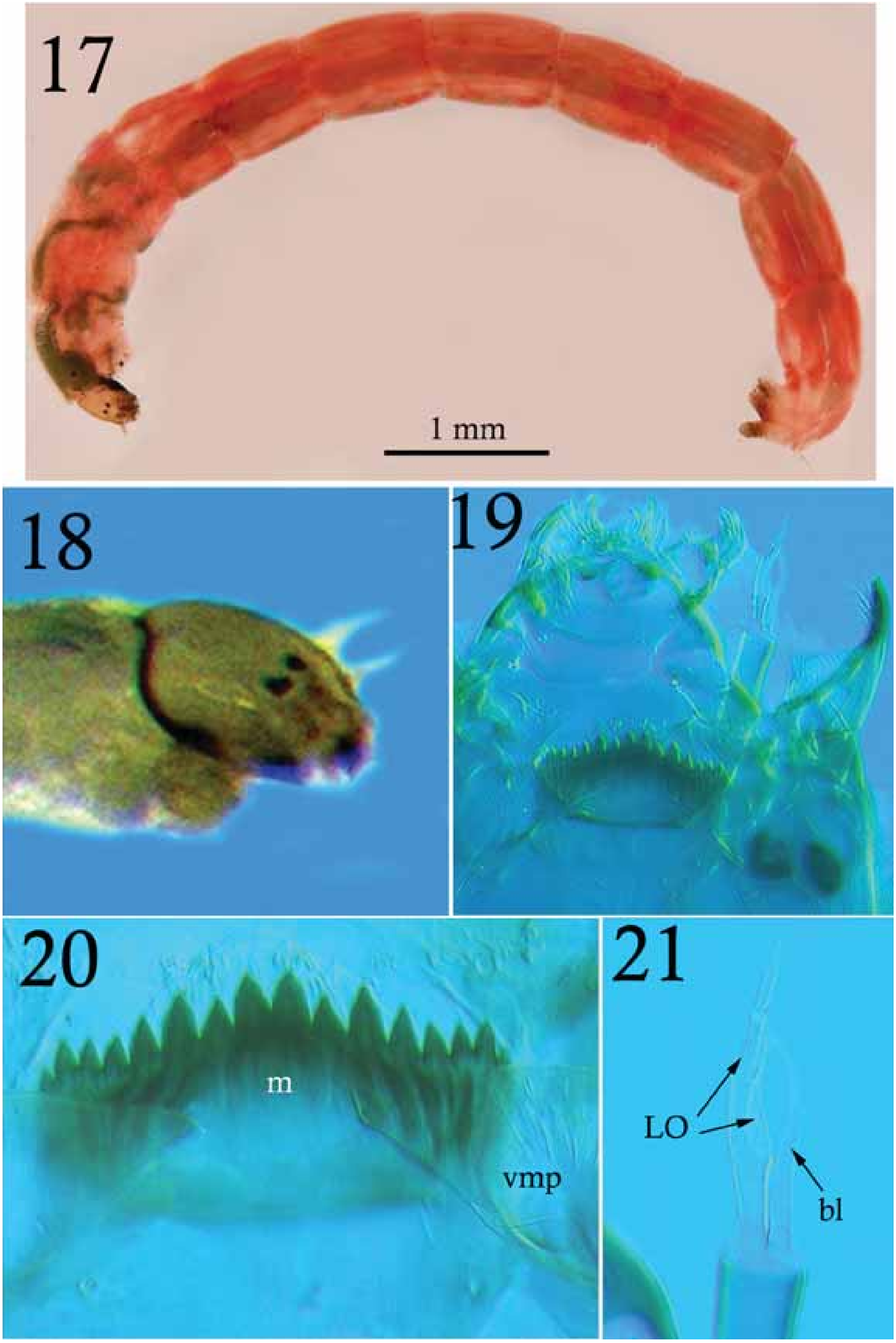
Figs. 12–16. Midges as nuisance in Singapore. *Polypedilum nubifer* pupa: 12, dorsal tergites; 13, frons; 14, base of thoracic horn, 15, posterolateral of tergite VIII. *Tanytarsus oscillans* pupa: 16, dorsal tergites.

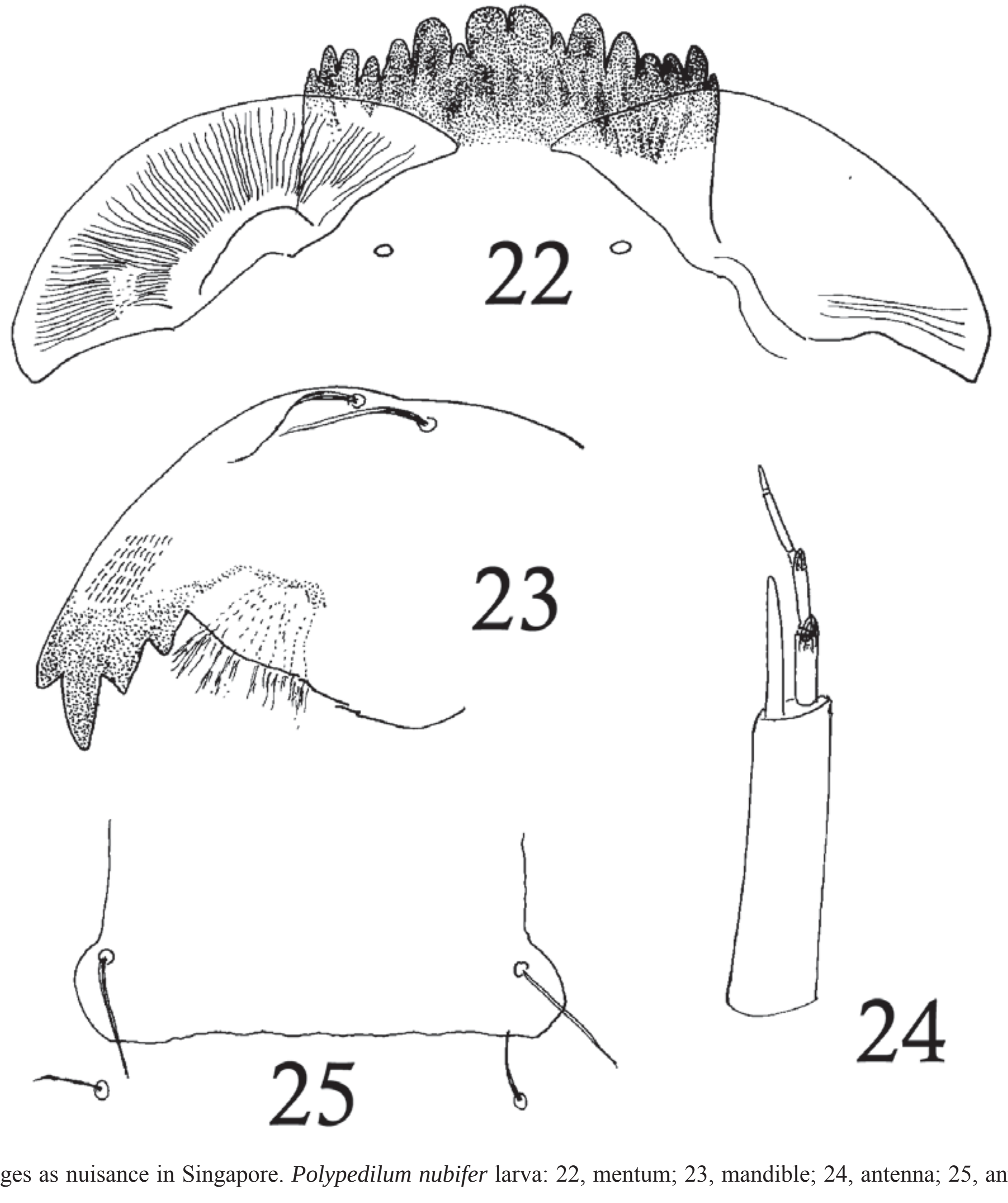


Figs. 17–21. Midges as nuisance in Singapore. *Polypedilum nubifer* larva: 17, habitus; 18, lateral head; 19, ventral head; 20, mentum; 21, antenna. Abbreviations: bl, blade; LO, Lauterborn organ; m, mentum; vmp, ventromental plate.

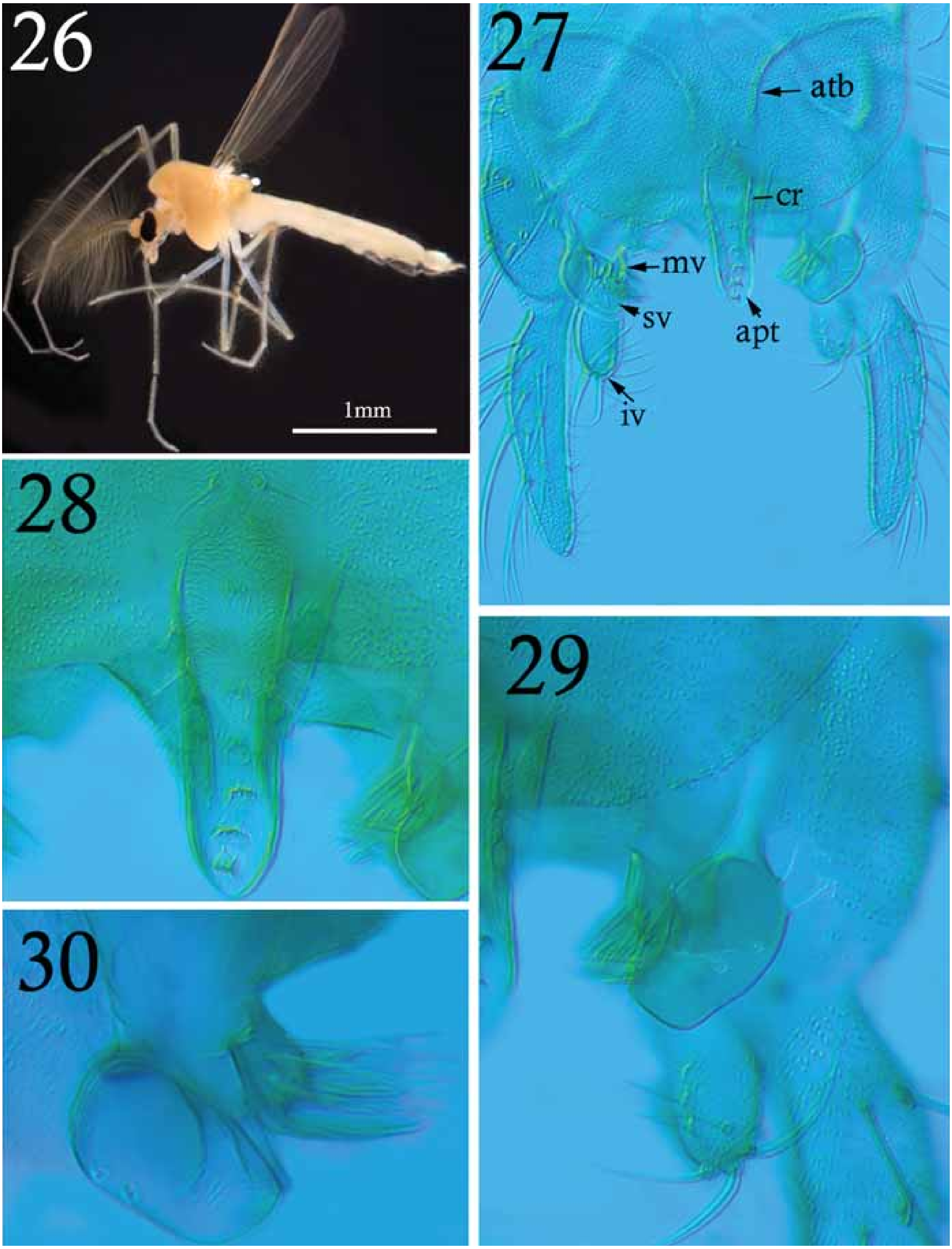
the next 6 years, and again in 1925 (Freeman, 1961; Sasa & Sublette, 1980). Subsequently, Johannsen (1932) described *Chironomus* (*Polypedilum*) *albiceps*, followed in 1934 by Goetgebuer who described *P. pruinosum* as new, and two years later Tokunaga (1936) joined the list in describing *Chironomus* (*Polypedilum*) *octoguttatus* as new to science: all are synonyms of *P. nubifer*.

Synonyms of *nubifer* due to Kieffer's publications are *tripartitus* (Suez Canal), *ceylanicus* (Colombo, Ceylon = Sri Lanka), *pelostolum* (Formosa = Taiwan), and *pharao* (Maadi, Egypt). Subsequent synonyms come from specimens described from Bali (*albiceps* Johannsen), Basra, Iraq (*pruinosum* Goetghbuer), Seto Prefecture, Japan (*octoguttatum* Tokunaga). The species evidently was widespread at least a century ago, and perhaps prior, and was reported under many names until 1961 when Freeman established the correct nomenclature for this distinctive species.

Although authors have described the adults of *P. nubifer* as distinctive in the adult (e.g., Sasa & Sublette, 1980; Sasa, 1979 [as *P. octoguttatum*]), the patterned wing with spots and clouds (Figs. 1, 2, 5, 6) is shared to a greater or lesser degree by many congeners, some of which are sympatric in the Old World range. Other stated distinguishing features concerning the anal point and superior volsella of the genitalia, and pale legs with long fore-tarsal beard, but these are scarcely discriminatory either. Comments accompanying SEM photographs and cursory descriptions of the pupa and larva by Sasa & Sublette (1980) are equally uninformative concerning the purported resemblances to other congeners (i.e., *Polypedilum*, all subgenera). In reality *Polypedilum nubifer* differs enough from all congeners, in each life stage, that serious doubts have been raised concerning its relationships with implications from its type-status in the genus (Sæther et al., 2010). Apparently significant features include the lack of a lateral seta on the superior volsella and lack of a singularly long apical seta on the apex of the inferior volsella, in the pupa the strength of the cephalic tubercles bearing the frontal setae, and in the larva the unique second antennal segment bearing alternate Lauterborn organs on a variably subdivided segment. Notwithstanding the variation



Figs. 22–25. Midges as nuisance in Singapore. *Polypedilum nubifer* larva: 22, mentum; 23, mandible; 24, antenna; 25, anterior frons.

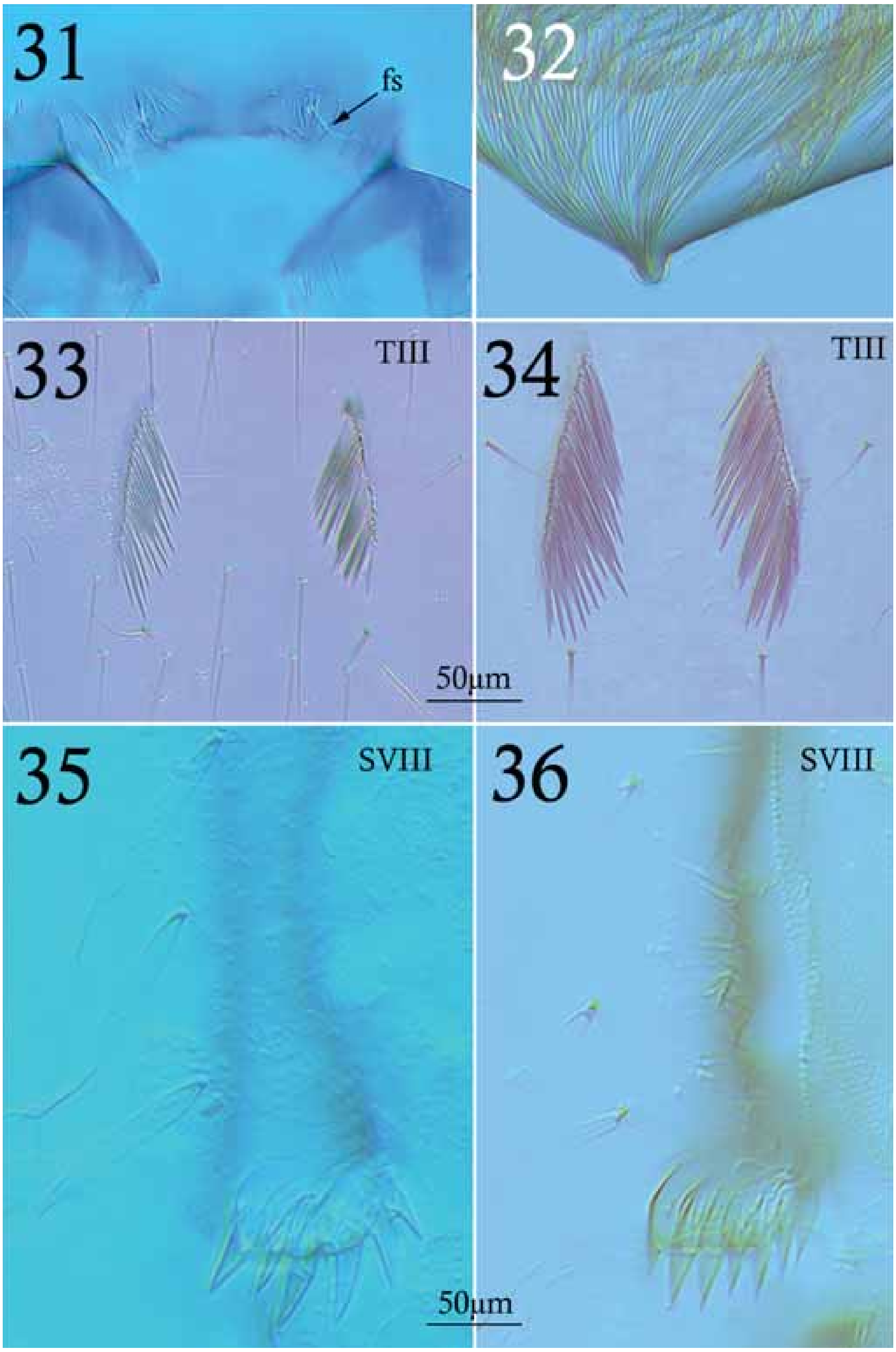


Figs. 26–30. Midges as nuisance in Singapore. *Tanytarsus oscillans* male: 26, habitus; 27, hypopygium, dorsal; 28, anal point, dorsal, detail; 29, superior volsella, dorsal, detail; 30, superior and median volsella, dorsal, detail. Abbreviations: apt, anal point; atb, anal tergite band; cr, anal crest; gc, gonocoxite; gs, gonostylus; iv, inferior volsella; mv, median volsella; sv, superior volsella.

in the heterogenous *Polypedilum* group, and the suggestion that *nubifer* 'probably belongs to *Stictochironomus'* (e.g., Pinder & Reiss, 1986), molecular data confirms its placement within *Polypedilum* (Cranston et al., 2011).

Tanytarsus oscillans *Johannsen*. — This species was described from male adult specimens collected by the Thienemann 'Sunda-expedition'. Specimens of all Chironomidae collected by Thienemann and his colleagues in Sumatra and Java were disseminated to colleagues for description, sometimes involving separation of reared immature stages from their adults. Johannsen, based in the United States, described the adult Chironominae (Johannsen, 1932), and Lenz (1937) cursorily described some few immature stages for the reared species in this subfamily.

Fortunately Ekrem's (2002) revision of the South and East Asian *Tanytarsus* not only allows identification of



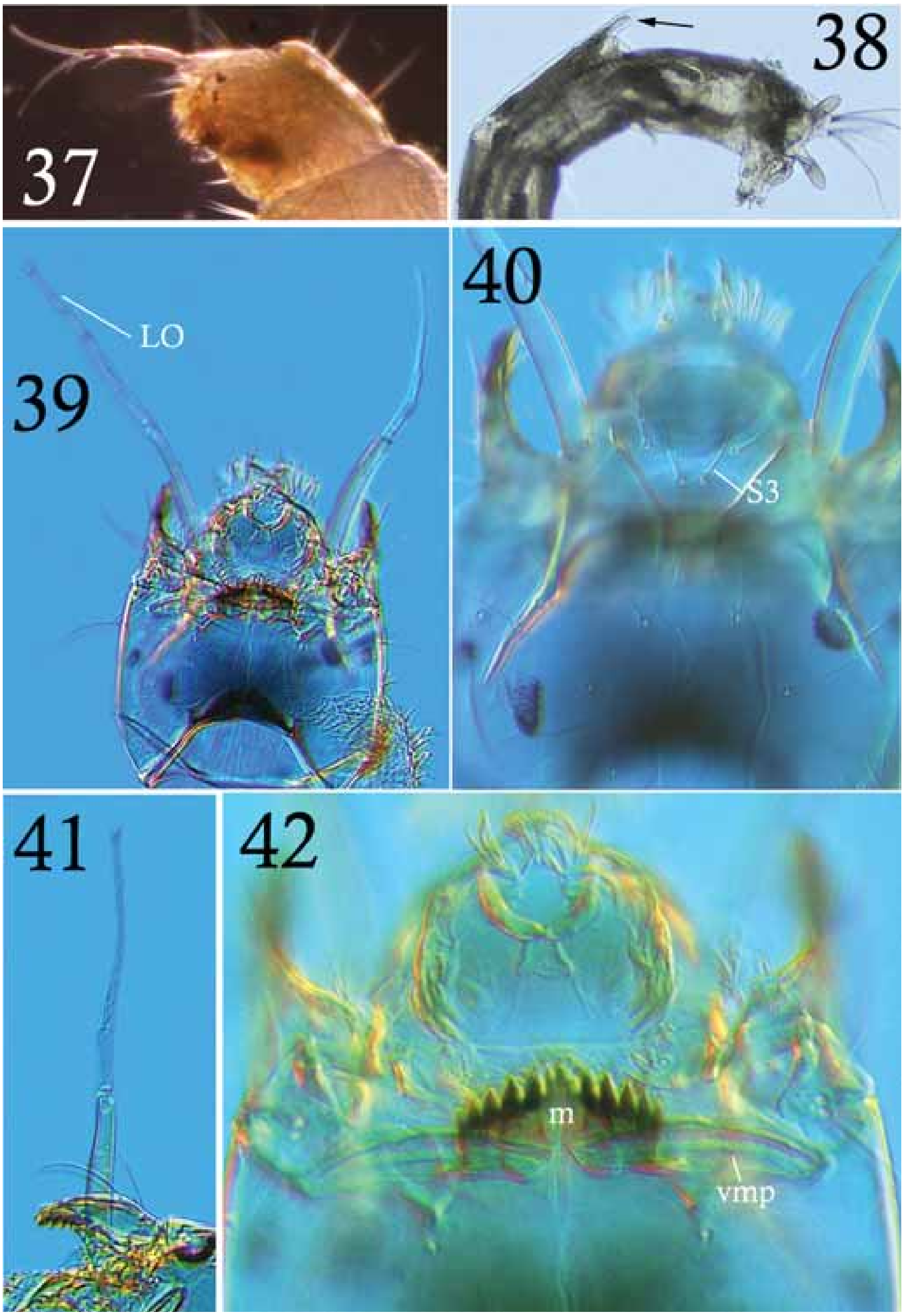
Figs. 31–36. Midges as nuisance in Singapore. *Tanytarsus oscillans* pupa: 31, frons; 32, nose of wing sheath; 33, tergite III of female; 34, tergite III of male; 35, posterolateral tergite VIII of female; 36, posterolateral tergite VIII of male. Abbreviations: fr, frontal setae; S, sternite (VIII=8th); T, tergite (III=3rd).

the males of the Bedok midge as *T. oscillans*, but verifies that the species was poorly known previously and the immature stages were unknown. Although little recognised, Ekrem (loc. cit.) established that the species also has been redescribed a number of times—as *Tanytarsus cultellus* by Chaudhuri & Datta (in Datta et al., 1992) from West Bengal, India, and as *Tanytarsus sibafegeus* Sasa et al. (1999) from Shibayamagatata Lake, Ishikawa Prefecture, Japan. These are the only published reports of the species to date.

DESCRIPTIVE TAXONOMY

Polypedilum nubifer (Skuse)

Material examined. — (Singapore material only cited). Many larvae, some pupae from Pandan, U. Pierce, Bedok, Poyan Reservoirs, Oct.2008, coll. NUS team including from colonisation experiments. Deposited ANIC, ZRC. **Molecular vouchers**: “Pnub”, Singapore, Pandan reservoir, between points 4 and 5, 6 Oct.2012, coll. Ang; CP28, 30, larvae, Pandan reservoir, between points 4 and 5, 18 Jul.2012, coll. Ang; ZRC.



Figs. 37–42. Midges as nuisance in Singapore. *Tanytarsus oscillans* larva: 37, lateral head, 38, posterior abdomen; 39, dorsal head; 40, dorsal view of head, antennal bases, S3 setae; 41, antenna; 42, mentum. Abbreviations: LO, Lauterborn organ; S3, cephalic seta; m, mentum; vmp, ventromental plate.

Description. — *Polypedilum nubifer* has been described and illustrated adequately in all stages by, amongst others, Freeman (1961), Sasa & Sublette (1980), Chattopadhyay et al. (1988), Cranston & Judd (1989), and Cranston (2000, 2007).

To assist in recognition we provide images including habitus photographs of all stages: adult male (Figs. 1–5), adult female (Figs. 6, 7), egg masses (Figs. 8, 9), pupae (Figs. 12–15), and larvae (Figs. 17–25).

A feature requiring consideration, and perhaps further study, is the apparent variation in the larval antenna. Variously described as 4, 5 or 6 segmented, the delimitation of segments is unclear. The Lauterborn organs, alternate and opposite on segment 2 (unique in the genus) can be considered apical on the two parts of a divided second segment, but the distinctness of division into 2 varies and can be difficult to verify. Equally, the number of segments distal to the apical Lauterborn organ seems to be three, but in some specimen preparations appears to be only two. Scanning electron microscopy by Sasa & Sublette (1980) helps, despite the external surface of the segment and the hyaline membrane between not differing in their reflection of electrons. Clearly from their fig. 27 there are 3 post apical Lauterborn organ segments, and the preceding segment (the 2nd) has a break near the midpoint that should represent at least a weakened area in mid-segment.

Tanytarsus oscillans Johannsen

Material examined. — (All slide mounted). **Malaysia**: Kuala Lumpur, Muzium Negara, pool in grounds, 2 males, 1 Oct.1976, coll. Cranston (BMNH). **Singapore**: Poyan Reservoir, 1°22'33"N, 103°39.16"E, 1L, Oct.2008 (NUS colonisation experiment); Lower Seletar Reservoir, 1°25'27"N, 103°51.29"E, 9 males, 19 Mar.2009, coll. Cranston; Singapore, Bedok Reservoir, 1°20′47″N, 103°55.31"E, 6 males, 2Pe, 9 Jan.2011, coll. Ang; same location, 6Pe, 23 Feb.2012, coll. Ang; same location and date, 3 P♂, 1Pe, coll. Cranston; same location, ex-rearing, 5L, 1L(P), 1Le(P), 5P♀, 2P♂, 31 Oct.2012, coll. Woodford. Deposited ANIC, ZRC. **Molecular vouchers** (all coll. TMSI, unless stated): CP5, ♂, Pandan reservoir, between points 4 and 5, 4 Jul.2012; CP8, ♂, Bedok Reservoir, Floating deck A, 2 Jul.2012; CP14, ♂, Upper Seletar Reservoir, Fishing area, 15 Aug.2012; CP54, ♂, Bedok Reservoir, Floating deck A, 23 Oct.2012; CP58, 62, 65, 66, 177, ♂♂, Upper Seletar Reservoir, Fishing area, 12 Sep.2012; CGBED1, ♂, Bedok, 'Waterfront Key' condominium balcony, 1 Dec.2012, coll. Gerald Lui; CP115, 121, 128, 130, Larvae, Upper Seletar Reservoir, Fishing area, 26 Sep.2012; Deposited ZRC.

Description. — Adult. The adult male was described in detail, accompanied by line drawings of genitalic features, by Ekrem (2002). We provide photographs of the male habitus (Fig. 26), genitalia (Figs. 27–30) and female (Fig. 10).

Pupa (n=10) (all measurements in µm, unless stated).
Body length 3.0–3.3 mm, cephalothorax length 1.0–1.3 mm. Cephalic area without tubercle or warts, frontal seta (Fig. 31) 35–38. Thoracic horn hyaline, straight and tapering with sparse bristle-like short setae, 300–335 long. Nose (Fig. 32) well developed, visibly containing many fringe setae of the pharate adult wing.

Abdomen (Fig. 16) with D and V setae normal for the genus, none taeniate or especially strongly developed: L-setae of T (tergites) II–VI non-taeniate, VII with 3 nontaeniate and 1 (rarely 2) taeniate L-setae, VIII with 5 taeniate L-setae. TI bare; TII with elongate spinule bands extending from somewhat transverse area for tergite length, with bare median area; TIII with band of long spines commencing more anterior than site of D1 seta, anteriorly without fine spinules; TIV with anterior patches of mixed short spines and spinules, located alongside D1 seta; TV & TVI with anterior patches of fine spinules; TVII and TVIII without spinules. Hook row on posterior of TII with 30–35 hooks extending 15–18% of segment width. Posterolateral 'comb' of segment VIII with 5–7 larger marginal spines and 9–12 submarginal smaller spinules (Figs.35, 36). Anal lobe with 27–31 taeniae and 2 dorsal setae. Genital sacs of males extend slightly beyond anal lobe apex, those of females shorter.

Pupal exuviae sexually dimorphic with long spines of TII in female shorter, fewer and paler (cf. Figs. 33 and 34, same scale), and with fewer stronger spines in posterolateral comb (cf. Figs. 35 and 36, same scale).

Larva (all measurements in µm unless stated):
Body length 2.0–2.25 mm, body pale green in 4th instar.
Head capsule. Length 260–280, light golden with pale occipital margin except pale brown ventrally, as is postmentum; mental and mandibular teeth golden brown (Figs. 37, 39). Cephalic setae S3 simple (Fig. 40). Antennal pedicel rounded, lacking spur. Antenna plus Lauterborn organ stems 99–102% length of head (Fig. 39). Antennal segment lengths 104–120, 37–45, 13–17, 7–8, 1–1.5. Antenna ratio 1.6–1.8. Lauterborn organ stems 120–130, with organs scarcely wider than stem, 8–10 long. Second antennal segment with incomplete pigmentation: basal sclerotised portion 50–60% of total length (some specimens with unpigmented 2nd antennal segment including asymmetry between sides). Mentum (Fig. 42) 67–70 wide, brown, with paler median triple tooth; ventromental plates 68–78 wide.

Body. Anterior parapods claws all yellow, simple, fine. Thoracic setation simple. Each abdominal segment bearing a bifid, plumose seta, 210–225 long, in the l_4 position on the postero-lateral of the segment. Abdominal segment 7 with paired lateral 'tubules' of length 25–30 (Fig. 38). Procercus bearing 7–8 anal setae of maximum length 350–360. Anal tubules short, squat, 100–120 long (Fig. 39). Posterior parapod claws all simple and robust, golden-yellow.

Comments. — The adult male of *T. oscillans* is recognised by the features stated by Ekrem (2012) mainly deriving from the genitalia in which the anal tergite bands are separate and nearly reach the anal point crests, with 2 median setae and small microtrichiose area medially; anal point with 4–6 large spinulae between crests with extensive microtrichiae between crests; oval superior volsella, finger-like digitus extending to near or slightly beyond apex of superior volsella and median volsella short, with foliate and setose lamellae orientated medially. The closest species (92%) in barcoding databases, *Tanytarsus kiseogi* described from Korea by Ree & Jeong (2010), is based on the adult alone. The uniform yellow thorax and Antennal Ratio of 1.0–1.2 are shared. The male hypopygium of *T. kiseogi*, as described and drawn, differs from *T. oscillans* only subtly including in the 'pinched' superior volsella and shorter digitus. The presence of microtrichiae between the crests of the anal point cannot be determined. Adults were collected beside a river but the immature stages to verify the habitat, and assist in taxonomy remain unknown.

The pupa of *T. oscillans* is distinctive by virtue of the pattern of tergal spines and spinules patterns. The pupa of *T. oscillans* resembles, and coexists in standing waters in Singapore with, *T. formosanus* Kieffer but can be distinguished by the near bare thoracic horn, minute frontal setae, different patterns on tergites including the continuous but sparser longitudinal spinule rows on TII, spines of TII commencing more anteriorly (rather than mid-segment), without spinule patches anterior to longitudinal spine rows on TIV. From the Australian pupal type 'B2' distinguished by the very short frontal setae, narrower hook row and reduced spinulation on TII, few spinules anterior to the long rows of spines on T IV, the fewer spines in patches on T IV and V, the location and number of taeniate setae on segment VII.

The larva possesses tubules which can be seen at low magnification (Fig. 38) on the lateral penultimate abdominal segment. Such structures are reported previously in larvae of this genus only from *Tanytarsus dibranchius* Kieffer in Zavřel (Spies, 1998) from western Europe, and by Dejoux (1968) for *T. nigrocinctus* Freeman from Chad. This latter taxon is now treated as a junior synonym of the more widespread (including Malaysia and Singapore) *Tanytarsus formosanus* Kieffer; however, larval tubules have not been reported since from regional specimens, and the significance in identification is unclear. Whatever, *T. formosanus* differs amongst other features in the antenna, with Lauterborn organs no more distal than the antennal apex. Many regional (Australasian) larval *Tanytarsus* have antennae + Lauterborn organs as long (or even longer) than those of *T. oscillans*, and thus the feature, although distinctive, is not characteristic.

Variation includes sexual dimorphism of the pupa—both in strength and intensity of pigment of the spines on TIII (Figs. 33, 34) and the number and size of the spines of the posterolateral 'comb' of segment VIII (Figs. 35, 36). Such sexual dimorphism, known in some other Tanytarsiini, must be taken into account in segregating pupal exuviae collected in routine surveillance/monitoring.

BIOLOGY AND NUISANCE OF *P. NUBIFER* AND *T. OSCILLANS*

Rearing revealed that for both of these species egg-laying usually occurred overnight, one to two days after the isolines were set up. The egg laying rate was 62% (62 out of 100) for *P. nubifer* after two days and 96% (47 out of 49) for *T. oscillans* after one day. Fertile *P. nubifer* eggs hatched within two days; fertile *T. oscillans* eggs hatched within one and a half days. The minimum egg-to-adult period required for *P. nubifer* was 18 days; *T. oscillans* larvae took at least 24 days to reach adulthood. Larvae of *T. oscillans* (Figs. 37, 38) were red in second and third instar, started turning green in fourth and pupal instars.

These development times fall well within the known time for Chironomidae from oviposition to adult. For example, a species the size of *T. oscillans* can develop to adult in less than 7 days (Nolte, 1995). Small, fecund, rapidly developing midges in warm waters can build up numbers extremely rapidly when environmental conditions allow. The region has many species that fit this category, mostly in the tribe Tanytarsiini, including several species of *Tanytarsus* and *Cladotanytarsus*. *T. oscillans* appears to be a native species to the region, currently quite widespread in Singapore's reservoirs, and with a propensity to occur in many types of standing waters without reaching nuisance numbers. Evidently some change occurred in Bedok in 2010–2011, and 2011–12 that allowed, or encouraged, an outbreak of *T. oscillans*. Exactly what conditions led to the outbreak remain to be identified, and clearly monitoring of numbers of immature stages against seasonal physicochemical factors must be continued. In addition, the particular dietary requirements of the larvae should be studied.

In contrast *P. nubifer* seems largely to be associated with substantial larval densities, either by out-competing other species, or simply being able to thrive in disturbed, new, or unstable, nutrient-rich, standing waters. The adults disperse widely, colonise readily, and the species is becoming cosmotropical. It has been present in Singaporean inland waters at least since the 1960s when reported by Karunakaran (as *Polypedilum albiceps*) in her unpublished thesis (Karunakaran, 1969). The only record was from her site #41 (Seletar) but it is noteworthy that her study concerned taxonomy of the Singapore midges with no special regard for nuisance midges or targeting of the larger reservoirs. Studies centered on the drains and ditches close to the university, which were sites for the *Chironomus* species that she studied in most detail. Unfortunately the valuable collection of specimens on which Karunakaran's studies were based cannot be located, and we cannot confirm that her concept of *P. albiceps* was what we consider *P. nubifer*, or if any of her species of *Tanytarsus* might have belonged to *T. oscillans*.

The options for the control of *P. nubifer* are better understood than those for *T. oscillans*, since the former species has been studied widely through parts of its range. Always the first measures suggested are physical barriers, for example, in the form of vegetational buffer zones between source and residents. A small proportion of flying adults breach effective barriers, but high rise apartments close to reservoirs present a particularly challenging problem that is not amenable to simple dispersal barriers. Elsewhere chemical control has been studied. As detailed in Traylor et al. (1994) and in several 'grey' literature reports, a juvenile hormone mimic "pyriproxyfen" can be effective when it is strategically applied. Such chemicals prevent metamorphosis especially to the adult moult. Authors including Pont et al. (1999) showed that when tested in treated enclosures application of bacterial insecticide (Bti) effectively reduced chironomid larval densities but in Pont et al.'s study (1999) the authors also found an undesirable side effect of Bti: it enhanced the relative abundance of *P. nubifer*. Obviously, further research is needed to test and optimise the different control measures in a Singaporean context.

Although recent reports of nuisance midges in Singapore can be ascribed to *P. nubifer* and *T. oscillans*, ongoing study of the biodiversity of standing waters has revealed other species whose biology suggests potential past or future problems. There are many species of *Cladotanytarsus*, a genus of small, fecund midges that includes nuisance species elsewhere including the Sudan. In many Singapore reservoirs *Tanytarsus formosanus*, a congener of *T. oscillans*, occurs in substantial numbers, and attains high larval densities especially in regional rice fields and ponds. Several species of *Kiefferulus* have the potential to become dominant and productive in saline-impacted habitats, including full (but non-marine) sea-water (Cranston, 2007) and all are potential nuisance species.

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LITERATURE CITED

Ali, A., 1994. Nuisance, economic impact and possibilities for control. In: Armitage, P. D., P. S. Cranston & L. C. V. Pinder (eds.), *The Chironomidae: The Biology and Ecology of Non-biting Midges*. Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras. Pp. 339–364.

Chattopadhyay, S., T. Dutta & P. K. Chaudhuri, 1988. Morphology and biology of *Polypedilum nubifer* (Skuse) from India (Diptera: Chironomidae). *Journal of the Bengal Natural History Society*, **7**: 29–41.

Cranston, P. S., 1994a. Introduction. In: Armitage, P. D., P. S. Cranston & L. C. V. Pinder (eds.), *The Chironomidae: Biology and Ecology of Non-biting Midges*. Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras. Pp. 1–7.

Cranston, P. S., 1994b. Systematics. In: Armitage, P. D., P. S. Cranston & L. C. V. Pinder (eds.), *The Chironomidae: Biology and Ecology of Non-biting Midges*. Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras. Pp. 31–61.

Cranston, P. S., 1994c. Medical significance. In: Armitage, P. D., P. S. Cranston & L. C. V. Pinder (eds.), *The Chironomidae: Biology and Ecology of Non-biting Midges*. Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras. Pp. 365–84.

Cranston, P. S., 2000. *Electronic Guide to The Chironomidae of Australia.* http://169.237.77.3/chiropage/index.html. (Accessed Dec.2012).

Cranston, P. S., 2004. Chironomidae. In: Yule, C. M. & H. S. Yong (eds.), *The Freshwater Invertebrates of Malaysia and Singapore.* Academy of Sciences, Malaysia. Pp. 711–735.

Cranston, P. S., 2007. The Chironomidae larvae associated with the tsunami-impacted waterbodies of the coastal plain of south-western Thailand. *Bulletin of the Raffles Museum*, **55**: 231–244.

Cranston, P. S., R. D. Tee, M. O. Gad El Rab & A. B. Kay, 1983a. Hypersensitivity to the 'green nimitti' midge (*Cladotanytarsus lewisi*, Diptera, Chironomidae), and other Chironomidae in the Sudan and Egypt. *Annals of Tropical Medicine and Parasitology*, **77**: 527–33.

Cranston, P. S., R. D. Tee, P. F. Credland & A. B. Kay, 1983b. Chironomid haemoglobins: Their detection and role in allergy to midges in the Sudan and elsewhere. *Memoirs of the Entomological Society of America*, **34**: 71–87.

Cranston, P. S. & D. D. Judd, 1989. Diptera: Fam. Chironomidae of the Arabian Peninsula. *Fauna Saudi Arabia*, **10**: 236–89.

Cranston, P. S., N. B. Hardy & G. E. Morse, 2011. A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*, **37**: 172–188.

Datta, T., A. Majumdar & P. K. Chaudhuri, 1992. Taxonomic studies on *Tanytarsus* v.d. Wulp (Diptera: Chironomidae) in India. *Oriental Insects*, **26**: 39–66.

Dejoux, C., 1968. Contribution à l'étude des premiers états des Chironomides du Tchad (première note). *Hydrobiologia*, **31**: 449–464.

Ekrem, T., 2002. Review of selected South- and East Asian *Tanytarsus* v. d. Wulp (Diptera: Chironomidae). *Hydrobiologia*, **474**: 1–39.

Ekrem, T., E. Willassen & E. Stur, 2007. A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution*, **43**: 530–542.

Ekrem, T., E. Stur & P. D. N. Hebert, 2010. Females do count: Documenting Chironomidae (Diptera) species diversity using DNA barcoding. *Organisms, Diversity and Evolution*, **10**: 397–408.

Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.

Goetghebuer, M., 1934. Zur Erforschung des Persischen Golfes (Beitrag No 15). Ceratopogonidae et Chironomidae. *Arbeiten iiber morphologische und taxonomische Entomologie aus Berlin*, **1**: 36–39.

Gullan, P. J., 1995. Letter concerning kungu. *Food Insects Newsletter*, **8**(2): 6.

Gullan, P. J. & P. S. Cranston, 2010. *The Insect: An Outline of Entomology. 4th ed*. Wiley-Blackwell, Chichester. 584 pp.

Freeman, P., 1961. The Chironomidae of Australia. *Australian Journal of Zoology*, **9**: 611–737.

Johannsen, O. A., 1932. Chironominae of the Malayan Subregion of the Dutch East Indies. *Archiv für Hydrobiologie, Supplement*, **11**: 503–552.

Karunakaran, S., 1969. *Studies on the Bionomics and Taxonomy of Singapore Chironomidae*. PhD Thesis. Department of Zoology, University of Singapore, Singapore. iv + 404 pp.

Katoh, K., K.-i. Kuma, H. Toh & T. Miyata. 2005. MAFFT version 5: Improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, **33**: 511–518.

Kieffer, J. J., 1906. Diptera fam. Chironomidae. In: Wytsman, P. (ed.), *Genera Insectorum. Volume 44*. 78 pp.

Kieffer, J. J., 1910. L'étude sur les Chironomides des Indes Orientales, avec description de quelques nouvelles espèces d'Egypte. *Memoirs of the Indian Museum*, **2**: 181–242.

Kieffer, J. J., 1911. Descriptions de nouveaux Chironomides de l'Indian Museum de Calcutta. *Records of the Indian Museum*, **6**: 113–177.

Krosch, M. N. & P. S. Cranston, 2012. Non-destructive DNA extraction from Chironomidae, including of fragile pupal exuviae, extends analysable collections and enhances vouchering. *Chironomus*, **25**: 25–27.

Krosch, M. N., A. M. Baker, P. B. Mather & P. S. Cranston, 2012. Comparison of intraspecific genetic structure among related chironomids (Diptera) from New Zealand and Patagonia: Disparity between potential and realized dispersal. *Freshwater Science*, **31**: 1105–1120.

Lenz, F., 1937. Chironomariae aus Niederlandisch-Indien. Larven und Puppen. *Archiv für Hydrobiologie, Supplement*, **15**: 1–29.

Lin, Y. J. & R. F. Quek, 2011. Observations on mass emergence of chironomids (Diptera: Chironomidae) in Bedok, Singapore with notes on human–chironomid interactions. *Nature in Singapore*, **4**: 339–347.

MacDonald, W. W., 1956. Observations on the biology of chaoborids and chironomids in Lake Victoria and on the feeding habits of the 'elephant-snout fish' (*Mormyrus kannume* Forsk.). *Journal of Animal Ecology*, **25**: 36–53.

McHugh, S. M., P. F. Credland, R. D. Tee & P. S. Cranston, 1988. Evidence of allergic hypersensitivity to chironomid midges in an English village community. *Clinical Allergy*, **18**: 275–285.

Meier, R., S. Kwong, G. Vaidya & P. K. L. Ng, 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology*, **55**: 715–728.

Meier, R., G. Zhang & F. Ali, 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the ''barcoding gap'' and leads to misidentification. *Systematic Biology*, **57**: 809–813.

Nolte, U., 1995. From egg to imago in less than seven days: *Apedilum elachistum* (Chironomidae). In: Cranston, P. S. (ed.), *Chironomids: from Genes to Ecosystems*. CSIRO, Melbourne. Pp. 225–32.

Pinder, L .C .V. & F. Reiss, 1986. Chironomidae. *Entomologica Scandinavica, Supplement*, **28**: 299–456.

Pont, D., E. Franquet & J. N. Tourenq, 1999. Impact of different *Bacillus thuringiensis* variety *israelensis* treatments on a chironomid (Diptera: Chironomidae) community in a temporary marsh. *Journal of Economic Entomology*, **92**: 266–272.

Ree, H. I. & K. Y. Jeong, 2010. Fauna of non-biting midges (Diptera, Chironomidae) from Soyang River in Chuncheon-si, Gangwon-do, Korea. *Korean Journal of Systematic Zoology*, **26**: 115–140**.**

Renaud, A. K., J. Savage & S. J. Adamowicz, 2012. DNA barcoding of Northern Neartic Muscidae (Diptera) reveals high correspondence between morphological and molecular species limits. *BMC Ecology*, **12**: 24

Sæther, O. A., T. Andersen, L. C. Pinho & H. F. Mendes, 2010. The problems with *Polypedilum* Kieffer (Diptera: Chironomidae), with the description of *Probolum* subgen.n. *Zootaxa*, **2497**: 1–36.

Sasa, M., 1979. A morphological study of the adults and immature stages of 20 Japanese species of the family Chironomidae (Diptera). *Research Reports of the National Institute of Environmental Studies*, **7**: 1–151.

Sasa, M., & J. E. Sublette, 1980. Synonymy, distribution, and morphological notes on *Polypedilum* (s.s.) *nubifer* (Skuse) (Diptera: Chironomidae). *Japanese Journal of Sanitary Zoology*, **31**: 93–102.

Sasa, M., M. Sumita & H. Suzuki, 1999. The chironomid species collected with light traps at the side of Shibayamagata Lake, Ishikawa Prefecture. *Tropical Medicine*, **41**: 181–196.

Sinclair, C. S. & S. E. Gresens, 2008. Discrimination of *Cricotopus* species (Diptera: Chironomidae) by DNA barcoding. *Bulletin of entomological research*, **98**: 555–563.

Skuse, F. A. A., 1889. Diptera of Australia. Part VI. The Chironomidae. *Proceedings of the Linnean Society of New South Wales*, **2**: 215–311.

Spies, M., 1998. Resolution of *Tanytarsus mendax* Kieffer, 1925, *T. dibranchius* Kieffer in Zavřel, 1926, and associated names (Diptera: Chironomidae). *Studia dipterologica*, **5**: 195–210.

Srivathsan, A. & R. Meier, 2011. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA "barcoding literature". *Cladistics*, **28**: 190–194.

Tokunaga, M., 1936. New species and new variety of the genus *Chironomus* Meigen. *Philippine Journal of Science*, **40**: 71–84.

Traylor, K. M., A. M. Pinder & J. A. Davis, 1994. Evaluation of the juvenile hormone mimic pyriproxyfen (S-31183) against nuisance chironomids (Diptera: Chironomidae), with particular emphasis on *Polypedilum nubifer* (Skuse). *Journal of the Australian Entomological Society*, **33**: 127–130.

Whitworth, T. L., R. D. Dawson, H. Magalon & E. Baudry, 2007. DNA barcoding cannot reliably identify species of the blowfly genus *Protocalliphora* (Diptera: Calliphoridae). *Proceedings of the Royal Society B*, **274**: 1731–1739.

Wiederholm, T. (ed.), 1983. Chironomidae of the Holarctic region. Keys and Diagnoses. Part 1. Larvae. *Entomologica Scandinavica, Supplement*, **19**: 1–457.

Wiederholm, T. (ed.), 1986. Chironomidae of the Holarctic region. Keys and Diagnoses. Part 2. Pupae. *Entomologica Scandinavica, Supplement*, **28**: 1–482.

Wiederholm, T. (ed.), 1989. Chironomidae of the Holarctic region. Keys and Diagnoses. Part 3. Adult. *Entomologica Scandinavica, Supplement*, **34**: 1–538.

Wright, I. & P. S. Cranston, 2000. Are Australian lakes different? Chironomid and chaoborid exuviae from Lake McKenzie, a coastal temperate dune lake. *Internationale Vereinigung für Theoretische und Angewandte Limnologie Verhandlungen*, **27**: 303–8.