



## ***Conochironomus* (Diptera: Chironomidae) in Asia: new and redescribed species and vouchers issues**

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### **Abstract**

The presence of the Afro-Australian genus *Conochironomus* Freeman, 1961 (Diptera: Chironomidae) in Asia has been recognised only informally. An unpublished thesis included *Conochironomus* from Singapore, and the genus has been keyed from Malaysia without named species. Here, the Sumatran *Conochironomus tobaterdecimus* (Kikuchi & Sasa, 1980) **comb. n.** is recorded from Singapore and Thailand. The species is transferred from *Sumatendipes* Kikuchi & Sasa, 1980, rendering the latter a junior synonym (**syn. n.**) of *Conochironomus* Freeman. *Conochironomus nuengthai* **sp. n.** and *Conochironomus sawngthai* **sp. n.** are described as new to science, based on adult males from Chiang Mai, Thailand. All species conform to existing generic diagnoses for all life stages, with features from male and female genitalia, pupal cephalic tubercles and posterolateral ‘spurs’ of tergite VIII providing evidence for species distinction. Some larvae are linked to *C. tobaterdecimus* through molecular barcoding. Variation in other larvae, which clearly belong to *Conochironomus* and are common throughout Thailand, means that they cannot be segregated to species. Larval habitats include pools in river beds, urban storage reservoirs, drains with moderately high nutrient loadings, and peat swamps. *Endochironomus effusus* Dutta, 1994 from north-eastern India may be a congener but may differ in adult morphology, thereby precluding formal new combination until discrepancies can be reconciled. Many problems with vouchers taxonomic and molecular material are identified that need to be rectified in the future.

**Key words.** Chironominae, new species, immature stages, south-east Asia

### **Introduction**

In revising the Afro-Australian genus *Conochironomus* Freeman, 1961 (Diptera: Chironomidae), Cranston & Hare (1995) expanded the concept to include an additional 3 Afrotropical species for which the immature stages were known only for the type, *C. acutistilus* (Freeman, 1955), and 4 Australian species for which the immature stages of 3 were described. Shortly after publication, Dr Frieder Reiss of the Zoologische Staatssammlung, Munich (ZSM), Germany, mailed the author several slides of adult midges from Thailand that he believed to belong to *Conochironomus*. Based on contemporary published information Cranston & Hare (1995) had stated that the genus was not known from Asia. However, in an unpublished thesis of very limited availability Karunakaran (1969) had recognised *Conochironomus* from Singapore, treating it as a subgenus of *Chironomus* Meigen and informally describing and illustrating all stages of a ‘new’ species. Unfortunately Karunakaran’s valuable material appears to have been destroyed (P. Murphy, National University of Singapore, *pers. comm.*); furthermore, her thesis does not constitute a valid publication (ICZN 1999: Article 8.1). Subsequently, reviews of the Chironomidae of Malaysia and Singapore (Cranston 2004) and Thailand (Cranston 2007) included the genus. In both publications *Sumatendipes* Kikuchi and Sasa (1990) was mentioned as a junior synonym to *Conochironomus*, but supporting evidence was not provided. In a survey of waterbodies impacted by a tsunami in south-western Thailand, Cranston (2007) keyed and illustrated larval *Conochironomus* from two locations, in the first formal report of the genus from the region, and repeated the synonymy of Kikuchi & Sasa’s *Sumatendipes* to *Conochironomus*. Recent surveys of midges in Singapore (*e.g.* Cranston *et al.* 2013) showed that *Conochironomus* larvae occur in several standing water bodies, but are never abundant. Similar urban lakes and reservoirs in Kuala Lumpur, Malaysia, also support *Conochironomus* larvae (Min 2014). Associations of larvae and adults using molecular techniques allow an

understanding of the regional diversity of the genus. Thus, it is timely to formalise the taxonomy of Oriental species in the genus *Conochironomus*.

## Methods

Collecting predominantly involved kick nets and disturbance of lotic and lentic marginal habitats to seek larvae and pupae. Pupae, exuviae and drowned adults were sought also by exposing drift nets with a 300µm mesh to intersect flowing water surfaces. Adults were swept from marginal vegetation beside aquatic sites. Collections were field-sorted by preference with a dissector microscope. Rearings were not attempted. Material for molecular study was collected into isopropanol (propan-1-ol), maintained cold in the dark. Specimens were vouchered by retention of all body parts not sacrificed for molecular extraction (such as a specimen's mid-abdomen), or by retaining the complete carcasses of pupae and adults post-tissue lysis for DNA extraction (Krosch & Cranston 2012).

Specimens required slide preparation using standard procedures (Cranston 2000). Identifications of larvae, pupae and adults were made using a compound microscope and a wide range of identification tools. For DNA sequences the barcoding section of mitochondrial gene *COI* was sought to associate un-reared larvae with adults, and to associate morphologically determined adults (*e.g.* Krosch *et al.* 2015).

**Morphology.** Morphological terminology follows Sæther (1980), Cranston (1995; Cranston *et al.* (1989). Interpretation of the median volsella follows Pinho *et al.* (2013). Morphological measurements, unless otherwise stated, are in µm rounded to the nearest 5 µm, except when measurement at maximum magnification (oil immersion, x1000) provided accuracy to +/- 1 µm.

**Images.** Adult 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 from slide mounts were obtained using a Leica™ DMRX compound microscope with Nomarski™ interference optics, and compiled with an Automontage™ system that allows automated retention of focused parts of a sequence of exposures at different focal depths. Post image-capture manipulations were made in Adobe® Photoshop™. Ink drawings were made by hand using a drawing tube, then scanned for editing. Redundancy in photographic and line image content is intended to accommodate users' opinions on the differential value and inclusion in either type of illustrations (Yuchen *et al.* 2013).

**Abbreviations.** Abbreviations of morphological terms and organisation names: 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); b.l., Body length (necessarily an approximation); dc, dorsocentral setae (count); gs.l., gonostylus length; gs.R., gonostylus ratio (maximum length / maximum width); h.l., head capsule length (L); L, larva; l.apn, lateral anteprenotal setae; LR<sub>1</sub>, Leg Ratio of adult foreleg = tarsomere 1 length / tibia length; MV, molecular voucher; n, number of specimens measured; NHM, The Natural History Museum, London, UK; N.P., National Park; P(e), Pupa (exuviae); pa, prealar setae (count); Prov., province (Thailand); R., river; sct, scutellar setae (count); sq, setae on squama of wing (count); sR<sub>1</sub>, sR<sub>2+3</sub>, sR<sub>4+5</sub>, setae on wing veins R<sub>1</sub>, R<sub>2+3</sub>, R<sub>4+5</sub>, respectively (count); T, tergite; TMSI, Tropical Marine Science Institute, National University of Singapore; w.l., wing length (arculus to apex); ZSM, Zoologische Staatssammlung München (Munich, Germany).

## Taxonomy

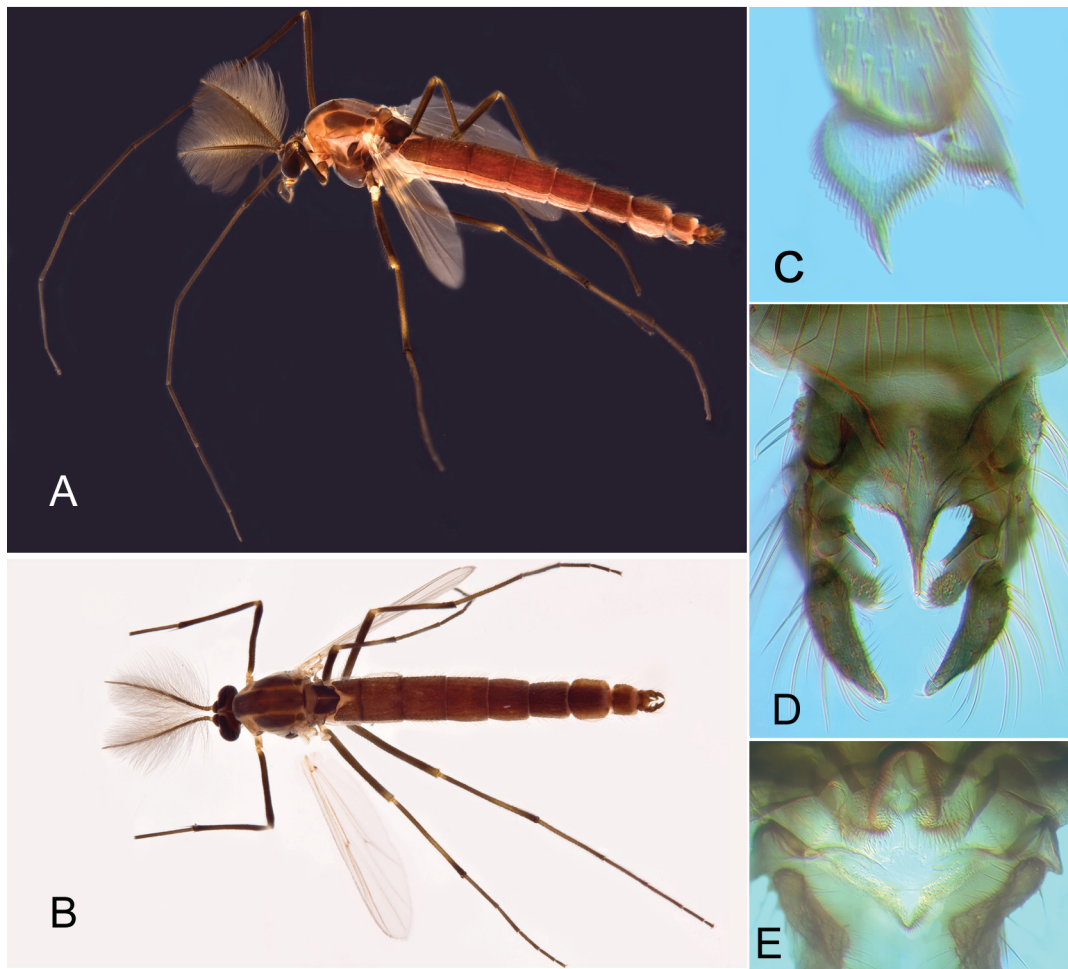
### *Conochironomus* Freeman

*Conochironomus* Freeman, 1961 (Freeman 1961: 701, Cranston & Hare 1995).

*Sumatendipes* Kikuchi & Sasa, 1990 (Kikuchi & Sasa 1990: 313). Type-species *Sumatendipes tobaterdecimus* Kikuchi & Sasa, 1990, by monotypy. **Syn. n.**

**Type-species:** *Endochironomus acutistilus* Freeman, 1955: 288, by original designation (Freeman 1955).

**Other included species:** *C. australiensis* Cranston & Hare, 1995; *C. avicula* (Freeman, 1955); *C. cervus* Cranston & Hare, 1995; *C. cygnus* Cranston & Hare, 1995; *C. deemingi* Cranston & Hare, 1995; *C. kakadu* Cranston & Hare, 1995; *C. nuengthai* **sp. n.**; *C. sawngthai* **sp. n.**; *C. tobaterdecimus* (Kikuchi & Sasa, 1990).



**FIGURE 1.** *Conochironomus tobaterdecimus* (Kikuchi & Sasa). A, B. Male habitus in dark-field and bright-field illumination (B lacking fore tarsomeres); C. Apex of mid tibia; D. Male genitalia, dorsal view; E. Part of female genitalia, ventral view.

***Conochironomus nuengthai* sp. n.**

(Figs. 2A; 3A, B)

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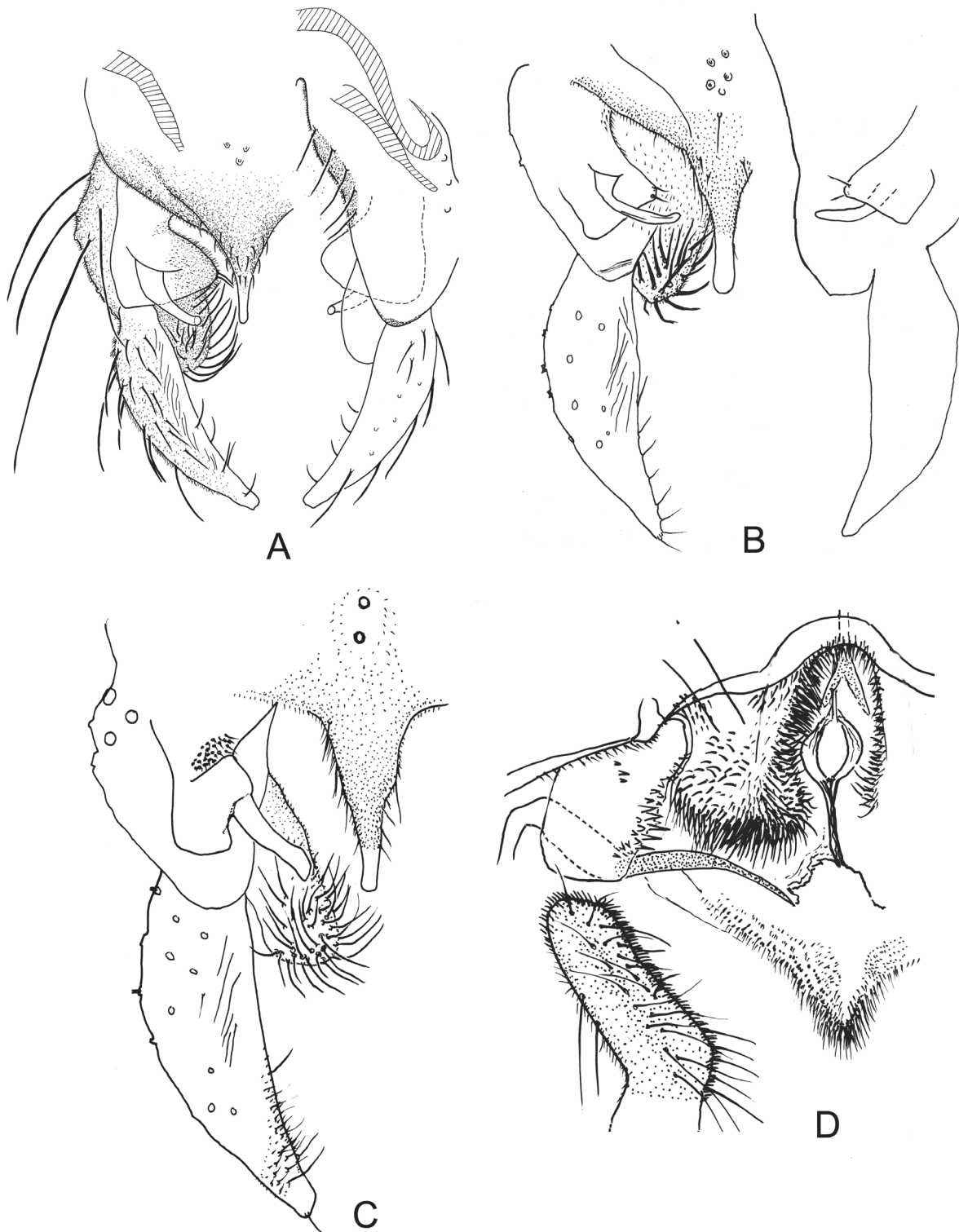
**Type-material. Holotype:** ♂, slide mounted, uncleared, in Euparal (by Reiss), at ZSM; THAILAND: Chang Mai, Zoo, 19–26.ii.1990 (Malicky). **Paratype** ♂ as holotype, except 2–9.iv.1990.

**Description.** MALE (n = 2). Body apparently uniformly brown without delimited thoracic vittae (from specimens mounted uncleared into Euparal. All tibiae paler in distal third; tarsomeres missing. Mensural features as in Table 1. Genitalia (Figs. 2A; 3A, B) with few median anal tergite setae far anterior to broad base of anal point, which tapers evenly to end near level of inferior volsellae apices. Superior volsella characteristic, somewhat cuneate with gently convex inner and posterior margins, microtrichiose across median 1/3 (both dorsal and ventral); inner rounded apex with 2 stronger, medially directed setae (perhaps absent in some specimens); bare digitiform projection arising dorsally near middle of volsella, curving, then narrowing beyond posterior margin of volsella, ending prior to median apex of volsella. Small tubercle near base of gonocoxite. Median volsella absent. Inferior volsella substantially fused to medial margin of gonocoxite, with recurved simple setae, none directed posteriorly. Gonostylus with strong creases on inner surface (Fig. 3B).

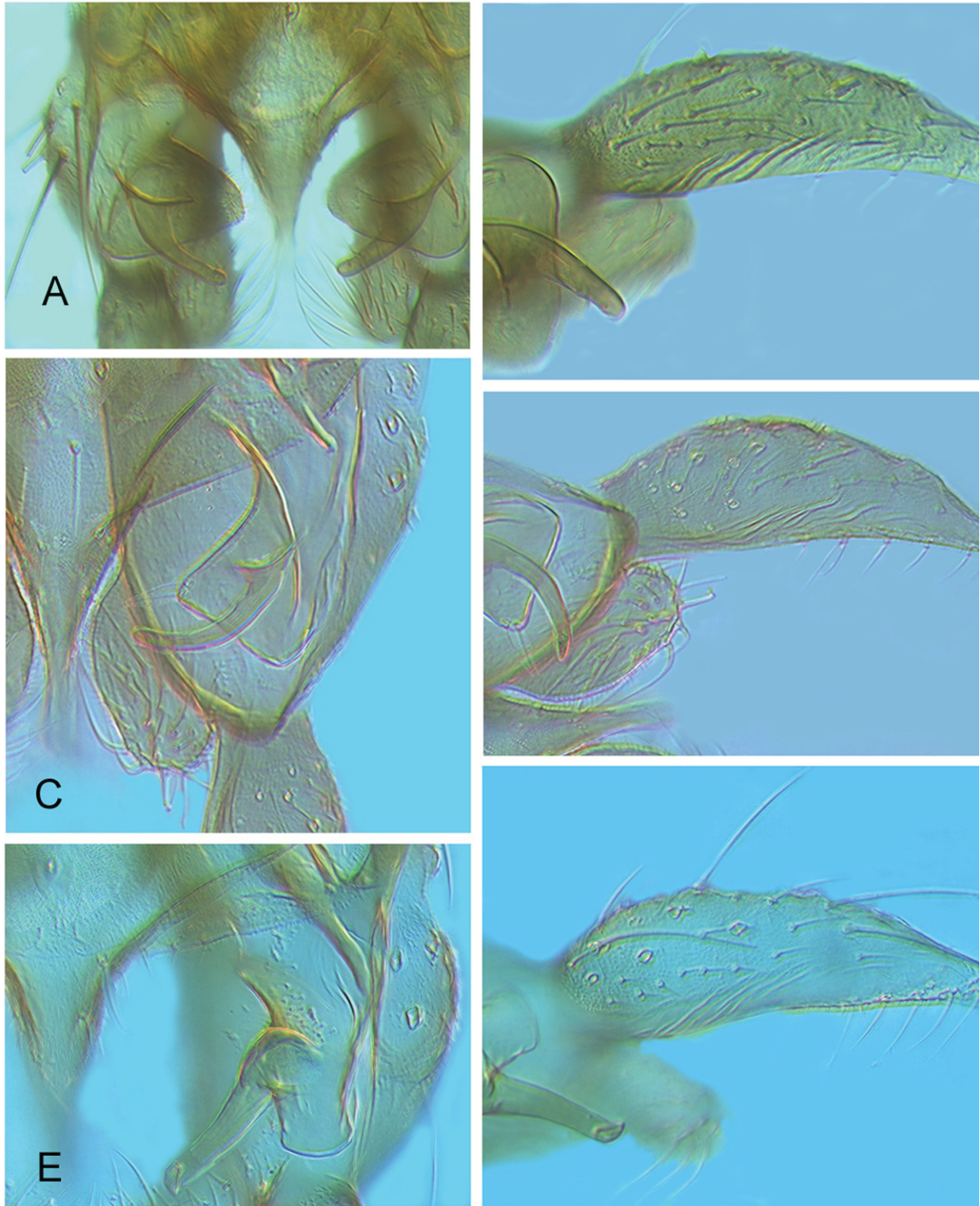
FEMALE, PUPA, LARVA unknown.

**Etymology.** Derived from the Thai words *nueng* and *thai*, meaning the ‘first’ Thai species. This combination of a numbering and a location term follows a style used by the group of M. Sasa in naming *C. tobaterdecimus* (see below) and many other species. However, this approach to naming is not generally recommended. Epithet is to be treated as noun in apposition for the purposes of nomenclature.

**Remarks.** Both specimens are somewhat damaged, lacking antennae and with no leg complete. Each tibial comb is typical for the genus, with a protruding central spur (Cranston & Hare 1995: fig. 1f). Subtle differences, especially in the shape of the superior volsellae (Fig. 3A versus Fig. 3C), led F. Reiss (pers. comm. c. 1996) to differentiate this species from the one below. I concur.



**FIGURE 2.** *Conochironomus* spp., adult genitalia. A. *C. nuengthai* sp. n., Male, left side dorsal, right side ventral; B. *C. sawngthai* sp. n., Male, left side dorsal, right side ventral stylised; C. *C. tobaterdecimus* (Kikuchi & Sasa), Male, dorsal, and superior volsella, ventral (inset, detail); D. *C. tobaterdecimus* (Kikuchi & Sasa), Female genitalia, (partial, ventral).



**FIGURE 3.** *Conochironomus* spp., parts of male genitalia. A, C, E. Anal point and superior volsella; B, D, F Gonostylus. A, B. *C. nuengthai* sp. n.; C, D. *C. sawngthai* sp. n.; E, F. *C. tobaterdecimus* (Kikuchi & Sasa).

**Conochironomus sawngthai sp. n.**

(Figs. 2B; 3C, D)

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**Type-material. Holotype:** ♂, slide mounted, uncleared, in Euparal (by Reiss), at ZSM; THAILAND: Chang Mai, Zoo, 26.iii-5.iii.1990 (Malicky). **Paratype:** ♂ genitalia only, as holotype except 23–30.v.1990.

**Description.** MALE (n = 1–2).

Body apparently uniformly brown with poorly delimited thoracic vittae (specimen mounted uncleared, in Euparal). Tibiae slightly paler in distal third, tarsomeres missing. Mensural features as in Table 1. Genitalia (Figs. 2B; 3C, D) as compared to *C. neungthai sp. n.* with more median anal tergite setae anterior to broad base of anal point, which is parallel sided and slightly spatulate apically. Superior volsella more compressed, parallelogram-shaped, with slightly concave inner and posterior margins, microtrichia restricted to narrow medio-basal area, inner point with 2 strong setae at apex; digitiform bare projection arising near middle of volsella, curving medially and more strongly narrowed beyond posterior margin of volsella, projection longer, ending medial to apex of volsella. Median volsella not evident in any form. Inferior volsella extensively fused to medial margin of gonocoxite, with swollen free apex densely covered with thick, recurved, simple setae, none of them directed posteriorly. Gonostylus broader than in *C. neungthai sp. n.*, tapering, with more but weaker inner creases (Fig. 3D), apex slightly bifid due to swollen base of subapical seta.

FEMALE, PUPA, LARVA unknown.

**Etymology.** Derived from the Thai words *sǎwng* and *thai*, meaning the ‘second’ Thai species. To be treated as noun in apposition for the purposes of nomenclature.

**Remarks.** The two slide mounted specimens comprise one damaged and incomplete adult male, and an isolated hypopygium on a second slide. On one tibia, likely that of a midleg, the comb has a central spur that is very short relative to that on the other comb. This species subtly differs from *C. nuengthai sp. n.* notably in the shape of the superior volsellae (Fig. 3C versus Fig. 3A).

**TABLE 1.** Adult male measurements.

	n=	b.l.	w.l.	A.R.	sR	sR <sub>1</sub>	sR <sub>4+5</sub>	sq	ac
<i>C. tobaterdecimus</i> holotype (after Kikuchi & Sasa)*	1	6.5	2.8	2.6	n/a	n/a	n/a	8	0
<i>C. tobaterdecimus</i> (Singapore)	1–2	6.5–6.9	2.5–2.7	2.4	23–26	22–23	0–1	11	0
<i>C. "togunagai"</i> Karunakaran* (unavailable name)	?	3.2	2.8	2.5	n/a	n/a	n/a	n/a	n/a
<i>E. effusus</i> (Dutta <i>et al.</i> )*	9	3.5–4.0	1.8–2.0	2.6	32	28	22–24	10–12	2
<i>C. neungthai sp.n.</i>	1–2	6.0–6.2	2.8–2.9	n/a	27–33	23–27	17	6–9	0
<i>C. sawngthai sp.n.</i>	1	6	2.4	2.0	30	16	14	4	0

continued.

	dc	pa	l.apn	sct	LR <sub>1</sub>	TIX setae	gs.l.	gs.R.
<i>C. tobaterdecimus</i> holotype (after Kikuchi & Sasa)*	8	3	n/a	6	n/a	?0	220	3.1
<i>C. tobaterdecimus</i> (Singapore)	10–11	3	4–6	5–7	n/a	3–6	205–210	3.3
<i>C. "togunagai"</i> Karunakaran* (unavailable name)	7	n/a	n/a	n/a	1.4	n/a	n/a	3.0
<i>E. effusus</i> (Dutta <i>et al.</i> )*	9–10	3–4	n/a	8	1.4	n/a	n/a	3.2
<i>C. neungthai sp.n.</i>	9–10	3	3–5	9–12	n/a	3–5	200–215	3.6–3.8
<i>C. sawngthai sp.n.</i>	9–10	3	6	9	n/a	6	170–175	3.0–3.2

\* from literature, including measurements from figures.

n/a: not available (not stated in description, or damaged)

## ***Conochironomus tobaterdecimus* (Kikuchi & Sasa)**

(Figs. 1A–E; 2C,D; 3E,F; 4A,C; 5A–C, D, F)

*Sumatendipes tobaterdecimus* Kikuchi & Sasa, 1990 (Kikuchi & Sasa 1990: 313).

*Chironomus* (*Conochironomus*) "tokunagai" of Karunakaran (1969), unavailable name (ICZN 1999: Article 8a).

*Conochironomus tobaterdecimus* (Kikuchi & Sasa, 1990). **Comb. n.** (merely inferred in Cranston 2004: 715).

**Material examined.** All slide mounted in Euparal; SINGAPORE: ♂, Bukit Timah N.P., Jungle Falls, 1°21'21"N 103°48'26"E, 12.iii.2009 (Cranston); ♂, Bedok Reservoir, Floating deck A, 1°20'N 103°55'E 13.iv.2013, emergence trap, CP379 (TMSI team) (GenBank KU507300); ♀, Upper Seletar Reservoir, forest area, 1°24'10"N 103°48'27"E, emergence trap, 16.vii.2013, CP459 (TMSI team) (association by barcode, GenBank 507304); 4L, THAILAND: Roi Et Prov., Chaturaphak Phiman District, Nong Lad, 15°53'36"N 103°32'54"E, 1.iii.2012 (Simwisat) (association by barcode).

**Putative immature material.** Pupae. Pe, SINGAPORE: Bedok Reservoir, NE shore, 1°20'47"N 103°55'31"E, 23.ii.2012 (Ang); Larvae. L, SINGAPORE: Central Catchment, Nee Soon Swamp, 1°23'N 103°48'E, 13.iii.2009 (Cranston); L, same as preceding except 27.ii.2012 (MV NS27-2-12#1).

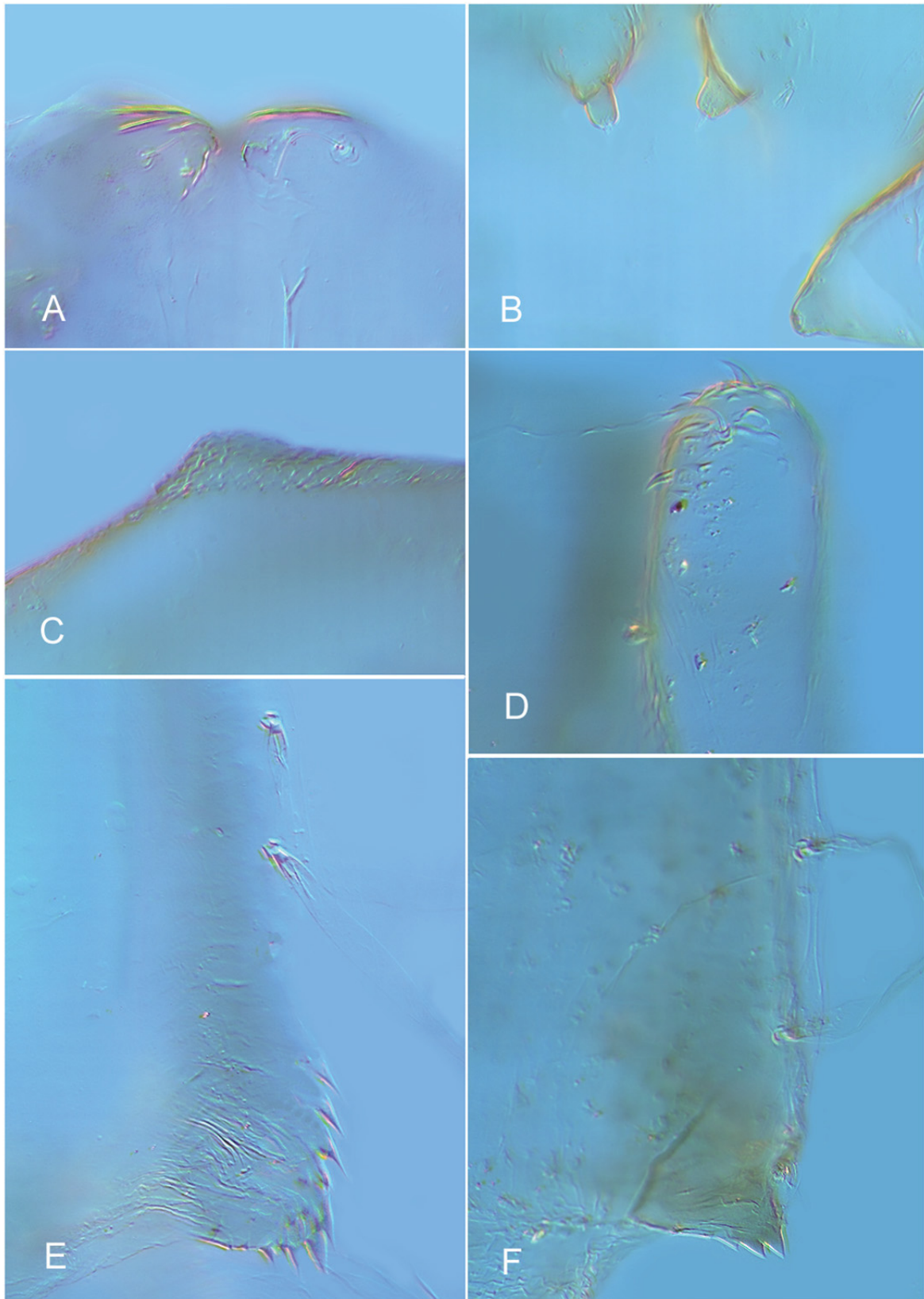
**Redescription** (partial; additional material substantially conforms to previous descriptions of all stages in Karunakaran 1969, sub *Chironomus* (*Chironomus*) *tokunagai*; of male in Kikuchi & Sasa 1990, sub *Sumatendipes tobaterdecimus*).

**MALE** (Figs. 1A–D; 2C; 3E, F) (n = 1–2). Body dark brown with slightly darker delimited thoracic vittae, and paler pronotum, trochanters, femoral apices and distal sections of tibiae (Fig. 1A, B). Mensural features as in Table 1. Genitalia (Figs. 1D; 2C; 3E, F) with few anal tergite setae located in mid-tergite anterior to base of elongate anal point flanked with lateral setae; anal point tapering to narrower parallel-sided medial section, narrowly rounded at apex. Superior volsella structurally complex (Figs. 2C, 3E), basal lobe bearing strong microtrichia on median (inner) contour, otherwise smooth; sinuous digitiform projection arises from broad base dorsally on basal lobe, initially dorsally directed, then narrowed and curved medio-posteriorly, terminating in up-turned, rounded tip; Basal lobe without strong setae, digitiform projection bare. Median volsella absent. Inferior volsella basally fused to medial margin of gonocoxite, with swollen free apex densely covered with thick, recurved, simple setae, none directed posteriorly. Gonostylus tapering, with weak inner creases (Fig. 3F), terminating in rounded apex.

**FEMALE** (Figs. 1E; 2D) (n = 1). Body length c 5.4 mm. Antenna: flagellomeres 1–4 500 µm, terminal (5<sup>th</sup>) 300 µm; AR 0.6. Thoracic setation: ac absent, dc 7, pa 3, sct 6. Wing length 3.0 mm, numbers of setae on wing veins R 34, R<sub>1</sub> 34, R<sub>4+5</sub> 40, on sq 14. Genitalia (Figs. 1E; 2D). Notum thin, long, extending full length of segment, flared posteriorly prior to short rami. Seminal capsules oval, abruptly darkened in distal 1/3 to base of very short neck; spermathecal ducts straight, broad, ending separately. Gonapophysis VIII in ventral view (Figs. 1E, 2D) clearly divided into large, quadrate, densely chaetose dorsomesal lobe and slightly smaller, rectangular ventrolateral lobe bearing spine-like chaetae on its median submargin, otherwise essentially bare. Cerci elongate rectangular in dorsal view.

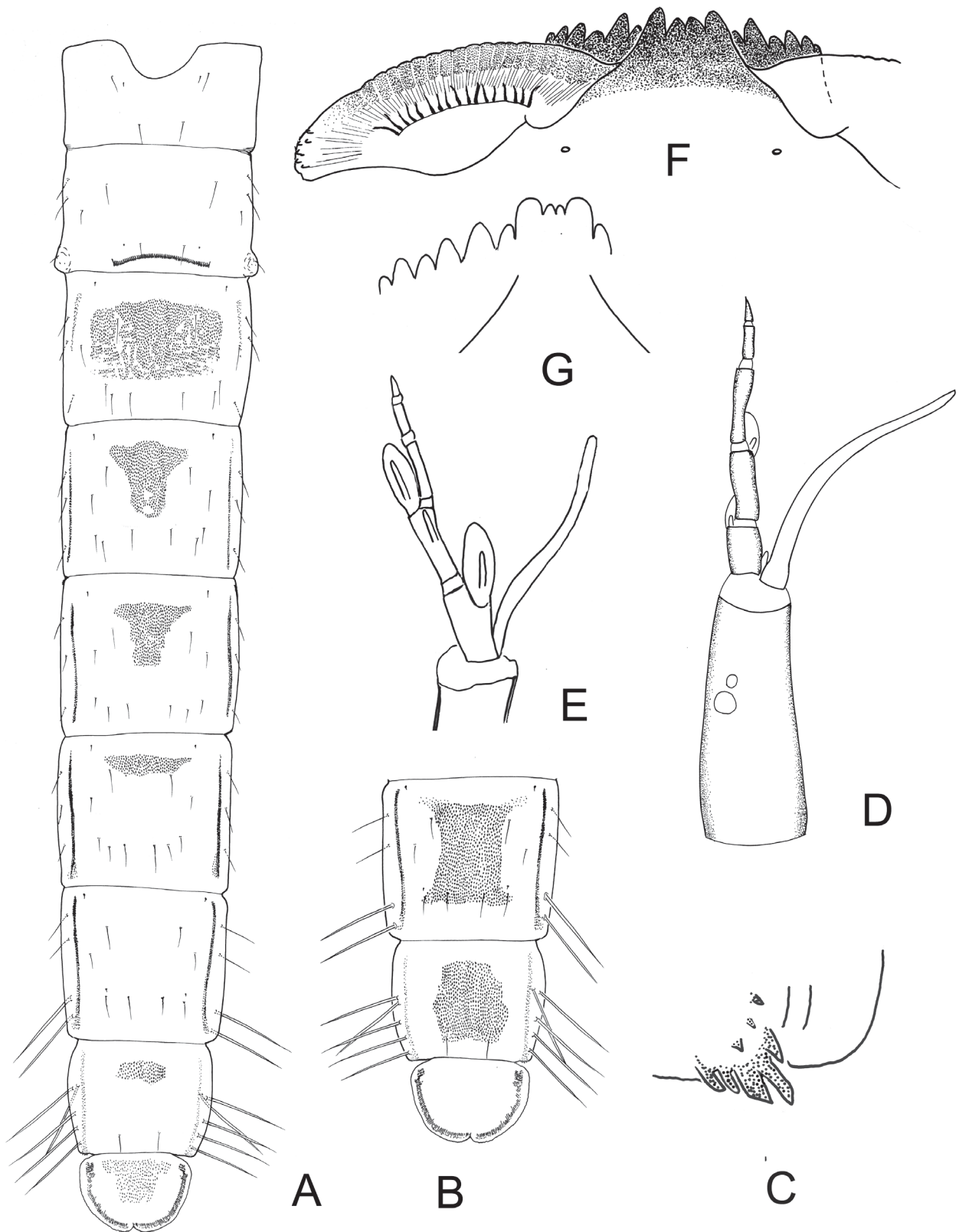
**PUPA** (Figs. 4A,C; 5A–C), based on tentatively associated exuviae (n = 1). Length c. 8 mm, pale, with brownish margins to thoracic appendages, abdominal segment apophyses indistinct.

**Cephalothorax.** Cephalic tubercle (Fig. 4A) squat, 12 µm high, with hyaline but strong frontal seta, 50µm. Pedicel sheath with one inner tubercle. Anteprenotum dorsally tuberculose, with 1 hyaline dorsal seta; l.apn not visible. Dorsal region of scutum weakly creased, non-rugose; scutal tubercle with tuberculose surface (Fig. 4C). Thoracic horn hyaline; number of branches not detectable in slide preparation; tracheal bundle simple, ovate. No prealar tubercle.

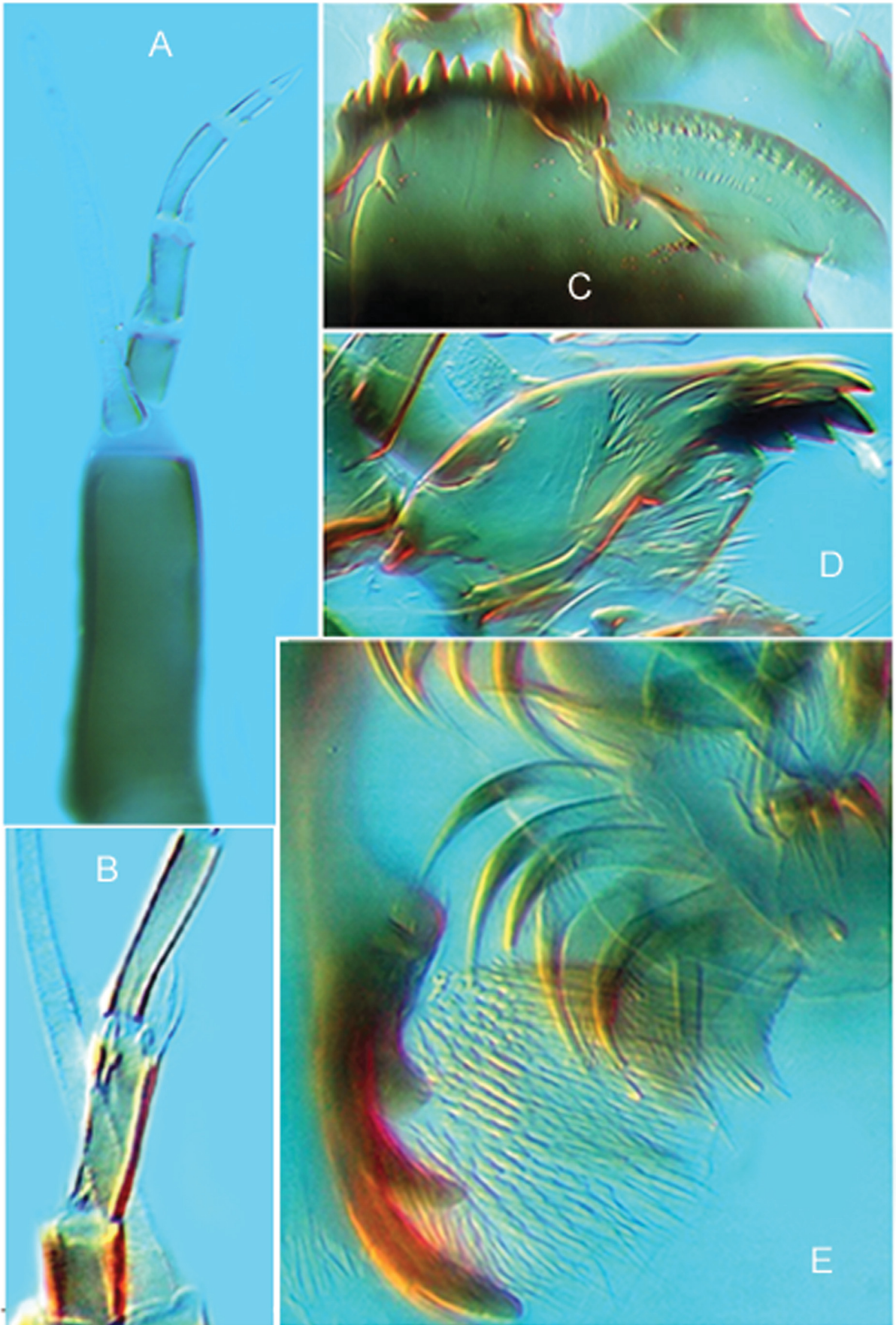


**FIGURE 4.** *Conochironomus* spp., pupa. A, B. Cephalic tubercles; C. Scutal tubercle; D. Antepronotum; E, F. Posterolateral corner of abdominal segment VIII, ventral). A, C. *C. tobaterdecimus* (Kikuchi & Sasa); B, D, F. *C. 'Thai'* sp. indet.; E. *C. 'Nee Soon'* sp. indet.





**FIGURE 5.** *Conochironomus* spp., immature stages. A–C. *C. tobaterdecimus* (Kikuchi & Sasa), pupal abdomen (anal lobe taeniae omitted): A. Dorsal view; B. Segments VII–IX, ventral; C. Posterolateral corner of VIII, ventral. D–G. *Conochironomus* spp., Larvae. D. Antenna, *C. tobaterdecimus*; E. Antenna, *C. 'Mae Ping' sp. indet.*; F. Mentum and ventromental plate, *C. tobaterdecimus*; G. Mentum (part), *C. 'Mae Ping' sp. indet.*



**FIGURE 6.** *Conochironomus* spec. indet., unassociated larvae. A. Antenna, whole; B. Antenna, mid-section; C. Mentum and ventromental plate; D. Mandible; E. Labrum-epipharynx, premandible.

Abdomen. Tergal armament as in Fig. 5A. Segments I and II without spinules. Hook row comprising 55 hooks in uniserial row, extending c. 60% of width of tergite II. Tergite III with wide and deep area of spinules, this area smaller and ending more anteriorly on T IV and V, T VI with anterior transverse band only, T VII without spinules; T VIII with antero-medial transverse patch, T IX with wide patch of spinules. Conjunctives bare. Most sternites bare, S VII and VIII with large quadrate area of spinules (Fig. 5B). Caudolateral corner of segment VIII ventrally with 4–5 transversely aligned, basally fused, straight spines (Fig. 5C). Pedes spurii B strong on segment II, absent on III. Pedes spurii A (vortices) absent. Segments V–VIII with 0, 0, 2, 5 taeniate lateral setae. Anal lobe dorsally with broad spinulose area (Fig. 5A, B) with multiseriate fringe of c. 100 taeniae (not shown), without dorsal seta.

LARVA (Fig. 5, D, F; 6A–E) (n = 4). Conforms to generic diagnosis (Cranston & Hare 1995) and closely resembles Australian *C. australiensis*. Body length c. 7–9 mm.

Head capsule with dark postoccipital margin; most of postmentum and posterior 1/3 of head darkened, anteriorly paler yellow-brown. Eye double, with larger spot exactly dorsal to smaller ventral spot. Body red, claws golden to golden brown (posterior). Head capsule length c. 490–530, postmentum length 164–180. Dorsal head and frontoclypeal apotome as in Cranston & Hare (1995: fig. 7h); Antenna (Fig. 5D; Cranston & Hare 1995: fig. 7e) with segment lengths (base to apex): 68–75; 13–15; 18–22; 8–12; 5–7. AR 0.9–1.2; Lauterborn organs large, alternate, 8–9 long; style slender, 10 long; blade 78–82 long, extending to apical segment or slightly beyond. Mandible (identical to Fig. 6D; Cranston & Hare 1995: fig. 7f) 155–165, with somewhat darkened outer tooth as long as dark apical tooth; two dark inner teeth; mola includes small darkened distal area close to base of long, simple seta subdentalis. Labrum (as in Cranston & Hare 1995: fig. 7g) with SI setae arising from common, fused bases (illustrated but not stated in Cranston & Hare 1995); SI and SII finely plumose; pecten epipharyngis of 3 separated scales, each with 2–3 blunt teeth; premandible 87–92, with 3 well-developed teeth and small basal 4th tooth. Mentum (Fig. 5F; Cranston & Hare 1995: fig. 7a–d) total width 130–152, with 4 median (ventromental) teeth, varying in relative height of median pair of teeth (1 specimen has only 3 teeth; Pramual *et al.* 2016: fig. 4), and varying in pigment intensity from yellow-brown to as dark as lateral (dorsomental) teeth. Ventromental plate (Fig. 5F) 62–65 apart medially, single plate 162–178 long, with characteristic ultrastructure (Cranston & Hare 1995: fig. 7b) and variably wavy anterior margin.

Abdomen. Anterior parapod claws simple, forming dense cluster. Procercus and supraanal setae pale-mid brown.

**Remarks.** Type material of *Sumatendipes tobaterdecimus* was not examined. Recognition, including membership of *Conochironomus*, is based on the description and drawings of Kikuchi & Sasa (1990), plus images of the holotype male genitalia available at <http://www.type.kahaku.go.jp/TypeDB> (species name misspelled 'tobaterdecimus'). The anal point of the type appears to differ in shape from Singaporean males, but this may arise from the poor preparation and distortion of the specimen (as too often in material from Sasa's studies).

Identical DNA barcoding *COI* sequences allow association of an adult male and female from Singapore (above). Larvae of *Conochironomus* collected from Nee Soon (Singapore) provided no DNA capable of allowing further association; thus, larvae (and pupae) are associated only putatively with *C. tobaterdecimus*. Later five larval specimens (CP461 from Bedok reservoir; CP1136, 1137, 1716, 1245 from Upper Seletar Reservoir) yielded barcodes identical to *C. tobaterdecimus* (GenBank accessions KU507299, KU507301-3, KU507305, respectively) but have not been examined by the author. Two larvae sequenced for barcode *COI* by Pramual *et al.* (2016, GenBank codes KT213039 and KT213040) are less than 1.5% different from adult *C. tobaterdecimus* from Singapore. This value lies well within the values of 4–5% taken to reflect species differences in *Tanytarsus* (Lin *et al.* 2015) and found appropriate in *Cricotopus* (Krosch *et al.* 2015); this boundary may have applicability across all Chironomidae. Unfortunately vouchers were not retained (P. Pramual, pers. comm. 2015) and morphology had to be derived from photographs of menta. However, further specimens from the same locality have been provided for morphometrics and barcoding, confirming association with *C. tobaterdecimus* from Singapore.

## Unassociated immature stages

### *Conochironomus* pupa, 'Thai' sp. indet. (Fig. 4B, D, F).

Pe; THAILAND: Nan Prov., Mae Charim N.P., Nam Wa R., 18°36'N 100°59'E 335 m. asl., 13.iii.2002, L-293 (Cranston).

This unassociated pupa clearly belongs to *Conochironomus*. It differs from the likely pupa of *C.*

*tobaterdecimus* (above) and ‘Nee Soon’ below, and may belong to *C. nuengthai* or *C. sawngthai*. Differences include the stronger cephalic tubercles and frontal setae (Fig. 4B), the spinose antepnotum which shows even some small hooklets (Fig. 4D), and perhaps the posterolateral corner (spur) of abdominal segment VIII (Fig. 4F).

***Conochironomus* pupa, ‘Nee Soon’ sp. indet. (Fig. 4E).**

P(♀); SINGAPORE: Central Catchment, Nee Soon Swamp, 1°23'N 103°48'E, 13.iii.2009 (Cranston).

This unassociated pupa differs from the putative *C. tobaterdecimus* and *C. ‘Thai’* above in: cephalic tubercles scarcely developed, frontal setae short, dorsal thorax and scutal tubercle strongly rugulose, tergites VII and VIII densely and broadly spinulose, sternites with much reduced spinulation (relative to Fig. 5B), hooklets of row on II smaller and the row occupying only 30% of segment width, pedes spurii B scarcely developed, and posterolateral corner of VIII (Fig. 4E) with more numerous spines that extend around the segment corner from postero-lateral to fully posterior.

***Conochironomus* larva, ‘Mae Ping’ sp. (Fig. 5E, G).**

L; THAILAND: Lamphum Prov., Mae Ping N.P., Nam Tok Koh Luang, 17°34'N 98°49'E, 6.iii.2002, L-266, TH73 (Courtney).

Head capsule length c. 380, postmentum length 147. Antenna (Fig. 5E) with segment lengths (base to apex): 50, 18, 19, 14, 8.5, 5; AR 0.77; Lauterborn organs alternate, large, 20–21 long; origin of basal Lauterborn organ broadens apex of segment 2 (Fig. 5E). Style slender, 9; blade 59 long, not extending to apex of antenna. Mandible 125, with outer tooth as dark as apical tooth; two dark inner teeth; mola has three spines, one close to base of seta subdentalis, two on inner margin. Labrum with SI setae arising from common base; SI, SII plumose; pecten epipharyngis with 4, 2, 4 blunt teeth in 3 closely approximated scales; premandible with 2 strong apical teeth but orientation precludes count of inner teeth; with very strong and long beard. Mentum (Fig. 5G); total width 95, with cluster of 4 median (ventromental) teeth much smaller and retracted median pair of teeth; all teeth brown. Ventromental plates 21 apart medially, single plate 132 long, anterior margin smooth.

Abdomen. Anterior parapod claws pale golden, simple, forming dense cluster. Procercus and apical setae yellow. Posterior parapod claws golden brown. Anal papillae 350, each with two obvious constrictions.

This larva is differentiated from that of *C. tobaterdecimus* on antennal features, *i.e.* the Lauterborn organs (Fig. 5E) being much larger (20 versus 8–9 µm) and with the basal Lauterborn organ placed subapically, the 2<sup>nd</sup> antennal segment being subequal in length to the 3<sup>rd</sup> from the lower Antennal Ratio (0.78 versus 0.9–1.0) and the blade not reaching the terminal antennal segment. The reduced size of the median pair of mental teeth may differentiate also, but variation in dorsomental teeth proportions seems high.

***Conochironomus* larvae, Thailand**

*Stictochironomus* sp. sensu Sriariyanuwath *et al.* (2015).

Locations listed North to South; 1 larva at each location unless stated otherwise. All elevations in metres above sea level (GPS determined).

THAILAND: Chiang Mai Prov., Fang Horticulture Exp. Farm, 19°57'N 99°09'E, 17.iii.2002 (Sites); Chiang Rai. Prov., Doi Laung N.P., 19°26'N 99°41'E, 26.v.2002 (CMU team); same as preceding, except: NT Poo Kaeng, 19°26'N 99°42'E, 540 m., 24.vii.2002 (Courtney); Chang Mai Prov., Doi Suthep-Pui N.P., Huai Kaew, 18°49'N 98°55'E, 5.iii.2002 (Cranston); same, except Palad waterfall, 18°47'N 98°58'E, 713 m., 19.x.2004, (Vitthepradit, Prommi, Laudee); Chiang Mai, Doi Inthanon NP., Nam Mae Klang, Eco-lodge, 18°32'N 98°32'E, 1000 m., 6.x.2002 (Thamsenanupap); same, except Siriphum lower waterfall, 18°32'N 98°31'E, 1380 m., 2.ii.2002 (Vitthepradit, Kirawanchi); same, except stream from Hua Sai Luang W.F., 18°31'N 98°27'E, 3.iii.2002 (Vitthepradit, Kirawanchi); same, except Mae Klang @ Ban Khun Klang, 18°31'N 98°31'E, 1296 m., 18.v.2004 (DuCharme); 3L, same, except Mae Klang @ Ban Mae Pan Noi, 18°31'N 98°25'E, 750 m., 17.ii.2003 (Thamsenanupap); Phrae Prov., Heuy Rong, 18°26'N 100°26'E, 426 m., 14.v.2004 (DuCharme); 2L, same, except Weng Ko Sai N.P., lower NT Mae Kueng Luang, 17°58'N 99°35'E, 400 m., 27.v.2003 (CMU team); same, except TH75, 22.vii.2002 (Courtney); same, except 23.iv.2002 (CMU team); Lamphun Prov., Mae Ping N.P., Namtok Koh Leung, 17°34'N 99°49'E., 7.iii.2002 (Courtney); Sakhon Nakorn Prov., Huey Yai waterfall, 17°01'N 103°59'E, 22.iv. 2004 (DuCharme); Phitsanulok Prov., Phu Hin Rong Kla N.P., Namtok Rongkla, 16°59'N 101°00'E, 1190

m., 11.iii.2002, (CMU team); 3L, Phu Hin Rongkla N.P., Waterwheel Falls, 16°59'N 101°00'E, 1280m., 9–10.iv.2002 (Cranston); Chalyaphum Prov., Tad Tone N.P., Tad Tone, 15°58'N 102°02'E, 210 m., 29.iv.2004, (DuCharme), same, except L-650 (Vitheepradit) Molecular voucher (MV) TH59; 2L, Nakhon Ratchasima Prov., Khao Yai N.P., Haew Narok Falls, 14°17'N 101°23'E, 400 m., L-601 (Sites & Vitheepradit); Sra Keaw Prov., Pang Sida N.P., Klong Huey Nam Yen, 14°02'N 102°15'E, 218 m., 16.iv.2004 (DuCharme); same, except 3L, Nam Tone waterfall 14°02'N 102°02'E, 108 m., 15.iv.2004 (DuCharme).

**Previous molecular voucher.** 3<sup>rd</sup> instar L; THAILAND. Chalyaphum Prov., Tad Tone N.P., Tad Tone, 15°58'N 102°02'E, 29.iv.2004, 210 m asl. L-650 (Vitheepradit) (MV) TH59. GenBank# HQ440724 (28S), HQ440888 (*COI*), HQ440577 (18S), HQ440433 (CADIV), HQ440261 (CAD1) (Cranston *et al.*, 2012).

This 3<sup>rd</sup> instar larva provided the molecular basis for placement of *Conochironomus* in the phylogeny of Chironomidae (Cranston *et al.* 2012). Even if features were to be found that differentiate species as mature larvae, this 3<sup>rd</sup> instar could not be compared to any final instar. Furthermore, Cranston *et al.* (2012) used primers 's2183' and 'a3014' after Simon *et al.* (1994) for the mitochondrial protein-coding gene Cytochrome *c* oxidase subunit I (*COI*), whereas other regional barcoding studies amplify a different fragment of the gene using universal invertebrate barcoding *COI* primers LCO1490 and HCO2198 (Folmer *et al.* 1994), as in Pramual *et al.* (2016).

### Definition and recognition of *Conochironomus*

Distinctive adult males provided the basis for recognition, originating with Freeman's (1961) observation that some African species of *Endochironomus* were aberrant. Since erection of the genus (Freeman 1961), the adult male continues to provide strong evidence for generic distinction. As stated by Cranston & Hare (1995), amongst taxa in which the male has 13 flagellomeres, lacks acrostichal setae and there is no spur on the anterior tibial apex, the conical shape of the tibial spurs is uniquely diagnostic. In Cranston *et al.* (1989), *Conochironomus* males possessing median volsellae would key to *Paratendipes*; those without median volsellae key to *Stictochironomus*. Microtrichia extending onto the anal point (Fig. 2A–C) are distinctive. Sæther (1977: 160) keyed female *Conochironomus* with a diagnostic combination of rounded anterior tibial apical scale, conical mid and hind tibial combs each with spur, six flagellomeres, lack of acrostichals, and gonapophysis VIII with ventrolateral lobe smaller than dorsomesal lobe. The latter observation reflects a higher level of variation in female genitalia in some taxa than documented by Sæther (1977), as exemplified in closely related *Polypedilum* species (Cranston *et al.* 2016).

Regionally, pupal *Conochironomus* can be recognised by having few (6–8) branches to the thoracic horn, no pedes spurii A, and an unusual, perhaps unique organisation of lateral setae, with the LS fine and short on segments V and VI and anteriorly on VII, and with taeniate LS<sub>3,4</sub> on VII and all LS<sub>1–5</sub> on VIII. The posterolateral corner of VIII ('comb') in *Conochironomus* with few to several small teeth is somewhat distinctive and varies specifically.

Larval *Conochironomus* appear well-characterised by a six-segmented antenna with Lauterborn organs in alternate apical positions on the second and third segments, and by the distinctive median mentum with four (ventromental) teeth that protrude relative to the lateral, dorsomental components. In the Holarctic key (Epler *et al.* 2013) the separation suggested in couplet 9 based on colour intensity of mandibular and mental teeth may not always work in practice, at least outside the Holarctic where there is greater diversity of the corresponding taxa. The arrangement of the median mental teeth in *Conochironomus* and the relative length of the antennal flagellum resemble the conditions in some *Paratendipes* but this genus differs in the pecten epipharyngis that comprises only 3 simple teeth. In *Stictochironomus*, the Australian *S. fluviatilis* (Skuse) and *S. illawara* Freeman fail to conform to the Holarctic diagnosis (Cranston 1996), and regionally *Imparipecten* Freeman and Afro-Australian *Skusella* Freeman also must be considered (Cranston 1996). Perhaps the only consistent feature differentiating larval *Conochironomus* is the shape of the 3<sup>rd</sup> antennal segment, which is narrow basally alongside the basal Lauterborn organ, and more flared apically. There is an indication of this shape also on the 4<sup>th</sup> antennal segment beside the apical Lauterborn organ.

## Key to adult males of Asian *Conochironomus*

1. Wing vein  $R_{4+5}$  with, at most, 1 subapical seta. . . . . *C. tobaterdecimus* (Kikuchi & Sasa)
- Wing vein  $R_{4+5}$  with >10 setae. . . . . 2
2. Humeral area with 1–2 isolated setae (anterior dorsocentrals). Hypopygium as in Fig. 2B, 3C,D . . . . . *C. sawngthai* sp. n.
- Humeral area bare. Hypopygium differs (e.g. Fig. 2A, 3A,B) . . . . . 3
3. Superior volsella base more-or-less rectangular, with digitiform projection crossing median margin and directed posteromesally . . . . . '*E.* *effusus* Dutta
- Superior volsella base ovo-cuneate with gently convex inner and posterior margins, with digitiform projection crossing posterior margin, curved mesally. . . . . *C. nuengthai* sp. n.

## *Endochironomus effusus* (Dutta)

*Endochironomus effusus* Dutta in Dutta *et al.* (1994: 256).

The description and placement of the adult male of *Endochironomus effusus* Dutta in Dutta *et al.* (1994) is based on an inadequate generic concept and does not conform to the diagnosis for *Endochironomus* Kieffer males (Cranston *et al.* 1989). The list of features justifying placement of *E. effusus* in *Endochironomus* does not do so. Features that negate the generic allocation (derived from the description and communication with P.K. Chaudhuri) include that that anteprenotal lobes meet dorsally and do not project forward, the rounded foretibial scale lacks any spur, the tibial combs are conical and strong, the inferior volsella is relatively short, stout and appressed along the inner gonocoxite, the superior volsella has a digitiform process arising from a broad, transverse base, and the gonostylus is fused inflexibly to the gonocoxite. Dutta *et al.* (1994) appeared unaware that *Endochironomus acutistilus* Freeman, to which they recognised affinity, had been placed by Freeman as the type of his genus *Conochironomus* (Freeman 1961). Although resembling *Conochironomus* in described features and additional ones examined by P.K. Chaudhuri (emeritus, Burdwan University, pers. comm. 29 November 2015), *E. effusus* differs from *Conochironomus*, e.g. in the purported presence of 2 acrostichals and lack of lateral anteprenotals. The hypopygium (Dutta *et al.* 1994: fig. 2d) closely resembles that of *C. tobaterdecimus*. Following re-examination of the sole remaining paratype of *Endochironomus effusus* by P.K. Chaudhuri (pers. comm., 2015, mensural features are similar to those in *C. tobaterdecimus*, excepting 'numerous setae' on wing vein  $R_{4+5}$ , purported presence of acrostichals and lateral anteprenotals. However, the specimen is poorly mounted (P.K. Chaudhuri, pers. comm), making it impossible to assess all features required to confirm generic placement in *Conochironomus* and it is premature to propose a new generic combination before suitable material becomes available for evaluation.

Locations for *E. effusus* at 27°N in the Duar foothills of the Himalaya in West Bengal, are at elevations up to 450 m. a.s.l., which is subtropical-temperate compared to the more tropical habitats of *Conochironomus* in Africa and Australia. However, the most northerly sites at which definitive *Conochironomus* larvae have been found in Thailand, at about 19–20°N, are at elevations above 600 m a.s.l. and also are cool subtropical to warm temperate.

## Discussion

*Conochironomus* was understood previously to be Afro-Australian in distribution, but is reported here formally from south-east Asia with recognition of a widespread species, *Conochironomus tobaterdecimus*, known in all life stages and two new species based on adults alone. The ecology and distribution of larvae indicates the genus is regionally widespread in standing waters, including in the dry season. For example, molecular material from eastern Thailand (Pramual *et al.* 2016) came from a 50 m wide, 25 cm deep pool, with water conductivity 380  $\mu$ S/cm and pH 7.8. In the Phong River, also in eastern Thailand, the genus was assessed as 'tolerant' by Sriariyanuwath *et al.* (2015, sub *Stictochironomus* spp.). Most sites for which data are available from the wider Thai survey appear similar in that they are dominated by riverine pools in the dry season. Two larvae found in a post-tsunami survey of south-western Thailand (Cranston 2007) came from a dilute, unimpacted, non-peaty pool (conductivity 205  $\mu$ /cm, dissolved solids 102 ppm), and from another in a recovering tsunami-impacted site (505  $\mu$ /cm, 259 ppm). *Conochironomus* occurs in two moderately enriched reservoirs in Singapore, Seletar and Bedok, and in urban water reservoirs in Malaysia. *Conochironomus* larvae have been found also in a peaty pool at Nee Soon, Singapore, but

these may not be conspecific with those from the reservoirs, given the differences between pupae from the respective sites.

Most information presented here might have been published two decades ago: the hiatus was due to an unmet hope that regional life history studies might eventuate to reveal the identity of the immature stages. Traditionally, as in the northern hemisphere and Australasia, this has come from individual larval rearings, or from life stage associations of pharate stages found in drift (e.g. Brundin 1966, Cranston 2015). DNA barcoding now allows association of unreared larvae by sequence identity with a known (named) adult, as exemplified here for *C. tobaterdecimus*. However, this technique often requires additional collection of adults, and does not generally provide association of the pupa. The latter stage has high value in systematics and descriptive taxonomy (Brundin 1966, Krosch & Cranston 2012) and biomonitoring (Raunio *et al.* 2007), and it can provide barcode DNA (Krosch & Cranston 2012, Kranzfelder *et al.* 2015). Fortunately, it is likely that the development of quick extraction methods (Kranzfelder *et al.* 2016), Direct PCR (Wong *et al.* 2014), and cheap NGS barcoding (Meier *et al.* 2015) will rapidly increase the number of species with matched life history stages.

The welcome growth in regional aquatic biological monitoring (e.g. Sriariyanuwath *et al.* 2015) relies upon the accuracy of an inventory of immature taxa encountered, which requires that adult Chironomidae be identified reliably and that the inventory is reasonably complete. This requirement is as true for taxonomic morphological voucher material as it is for molecular (barcode) libraries (Ekrem *et al.* 2007). There are no excuses for non-retention and loss of type and voucher taxonomic specimens. No impediments exist to proper vouchering of the actual specimen used in DNA barcoding (Krosch & Cranston 2012) or in direct PCR (Wong *et al.* 2014). The present study has been constrained by pervasive problems with specimens, including loss of all material from a thesis (largely unpublished but very valuable nonetheless), loss of all but one specimen from a type-series for a published description, and failure to retain any vouchers for a published molecular analysis. Even when slides have been retained, the quality can be so poor that they are essentially useless for taxonomy. It has been argued that modern high quality images can substitute for specimens, and this can be true when a scientist is involved who knows the salient features needed for identification. However, a salutary tale of ‘experts’ failing to photograph taxonomically important features (Cranston & Bickel 2015) suggests that unless many ‘seemingly unnecessary’ illustrations (Yuchen *et al.* 2013) are compiled, images alone will not substitute for properly made specimen vouchers placed in a proper long-term repository.

The critique above of poor contemporary practice in chironomid systematics should not be seen as relating to this group only, although issues relating to the requirement for full life history data for these holometabolous insects are of prime importance. Neither should this be seen as a critique of behaviour in the region under study: inadequate or absent vouchering issues are global.

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