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# FIRST RECORD OF THE GENUS *CONOCHIRONOMUS* FREEMAN (DIPTERA: CHIRONOMIDAE) WITH INTEGRATIVE TAXONOMY OF *C. TOBATERDECIMUS* (KIKUCHI & SASA) FROM INDIA

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**ABSTRACT**

**The genus *Conochironomus* Freeman is recorded for the first time from India. The known species *C. tobaterdecimus* (Kikuchi and Sasa, 1990) is redescribed and illustrated. DNA barcoding of *C. tobaterdecimus* is also done. The molecular barcoding infers us that species have a wide range of distribution. *Endochironomus effusus* Dutta, Majumdar and Chaudhuri, 1994 is proposed here as junior synonym of *C. tobaterdecimus*. A pictorial key and a tentative phylogenetic relationship of the described species are also given based on known males.**

**Key words:** *Conochironomus*, key, cytochrome oxidase I, genetic distance, molecular barcoding, new record, Oriental region, phylogeny, consensus tree, consistency index, retention index, synonymy

The genus *Conochironomus* Freeman, 1961, one of the least speciose genera in the subfamily Chironominae, is recorded from India for the first time. Kikuchi and Sasa (1990) established a genus *Sumatendipes* based on the type species *Sumatendipes tobaterdecimus* from Indonesia. During the revision of Chironomidae of Malaysia and Singapore (Cranston, 2004), and Thailand (Cranston, 2007), he synonymised the genus *Sumatendipes* Kikuchi and Sasa, 1990 with the genus *Conochironomus* Freeman, 1961. So *Sumatendipes* is a junior synonym of the genus *Conochironomus* (Cranston, 2016). The genus *Conochironomus* comprises 11 species; *C. acutistilus* (Freeman, 1955a), *C. avicula* (Freeman, 1955a), and *C. deemingi* Cranston and Hare, 1995 from Afrotropics; *C. australiensis* Cranston and Hare, 1995, *C. kakadu* Cranston and Hare, 1995, *C. cygnus* Cranston and Hare, 1995, and *C. cervus* Cranston and Hare, 1995 (only pupa) from Australasia; *C. nuengthai* Cranston, 2016, *C. sawngthai* Cranston, 2016, *C. tobaterdecimus* (Kikuchi and Sasa, 1990), and *C. jat* Tang, 2018 from the Oriental region. According to Dutta et al. (1994), *Endochironomus effusus* bears numerous setae on $R_{4+5}$ and acrostichals and lacks lateral antepronotals, by which it can be differentiated from *C. tobaterdecimus* (Cranston, 2016). Upon careful examination of one paratype, we found that *Endochironomus effusus* Dutta, Majumdar and Chaudhuri (1994) is a junior synonym of *C. tobaterdecimus* as the former one does not possess any acrostichal and setae on $R_{4+5}$. The present study includes redescription and molecular barcoding of *C. tobaterdecimus*, along with a key and tentative phylogenetic relationship of described species based on male and synonymisation of *Endochironomus effusus*.

## MATERIALS AND METHODS

The specimens collected using white CFL light of 8W, were sorted for identification. Some specimens were preserved in 100% ethanol for DNA extraction; thoracic muscle and one set of legs were outsourced for DNA extraction, and sequence of Cytochrome C oxidase I gene were uploaded in NCBI and some specimens were washed with 70% alcohol and slide-mounted with the phenol-balsam technique of Wirth and Marston (1968). The general terminology follows Sæther (1980). The number of specimens measured is indicated by 'n'. Measurements of structural parts are made in micrometre (µm) except the body length, wing length, and antennal length in millimetre (mm) with the ranges followed by mean. The abbreviations in the text are as follows: IV-Inner Verticals, OV-Outer Verticals, Po-Post orbitals, CA-Head-Antennal ratio, AR-Antennal ratio, VR-Veneral ratio, CR-Costal ratio, RM-Cross vein between Radius and Media, R-Radius vein, Fe-Femur, Ti-Tibia, Ta-Tarsomere, LR-Leg ratio, BV-Beinverhältnisse, SV-Schenkel-Schiene-Verhältnis,

TBR-Tree bisection and reconnection, HR-Hypopygium ratio, HV-Hypopygium value, BUENTD-Burdwan University Entomology Division, RI-Retention Index, CI-Consistency Index, NCBI- National Center for Biotechnology Information.

Morphological cladistics. Selection of outgroup- The genera *Microtendipes umbrosus* Freeman, 1955b, *Zavreliella marmorata* (Wulp, 1859) and *Endochironomus ampliceps* Dutta, 1994 were considered as outgroup for the morphological cladistics analysis of 10 species of the genus *Conochironomus* Freeman. Selection of morphological characters and its character states- The cladistics is based on 22 discrete characters (17 binary states, 5 multiple states) of adult male, including 11 characters of male hypopygium. The characters of larvae and pupae are not considered in the cladistics study as only few species have so far been described with their immature stages. The scoring of characters was based on available literature as follows-

Adult male: 1. Body length: (0) less than 6 mm, (1) greater than 6 mm; 2. Wing length: (0) 3 mm, (1) greater than 3 mm; 3. AR: (0) 2, (1) greater than 2; 4. Scutellars: (0) 8 or greater than 8, (1) less than 8; 5. DC: (0) 10 or less than 10, (1) greater than 10; 6. Prealars: (0) less than 5, (1) greater than 5; 7. Setae on R: (0) less than 30, (1) greater than 30; 8. Setae on $R_1$: (0) less than 20, (1) greater than 20; 9. Setae on $R_{4+5}$: (0) many, (1) few, (2) absent; 10. Squamal setae: (0) absent, (1) less than 10, (2) greater than 10; 11. HV: (0) greater than 3, (1) less than 3; 12. Median setae on tergite IX: (0) present, (1) absent; 13. Crease on gonostylus: (0) absent, (1) weak, (2) strong; 14. Superior volsella: (0) simple, (1) bifid; 15. Shape of superior volsella: (0) digitiform, (1) globular, (2) rectangular, (3) cuneate or boot; 16. Digitus: (0) absent, (1) present; 17. Microtrichia on digitus: (0) absent, (1) present; 18. Median volsella: (0) present, (1) absent; 19. Disposition of Microtrichia on superior volsella: (0) medially, (1) dorso-ventrally; 20. Fusion of inferior volsella with gonocoxite: (0) not fused, (1) basally fused, (2) extensively fused; 21. Frontal tubercles: (0) present, (1) absent; and 22. Acrostichals: (0) present, (1) absent.

The character matrix was compiled using Mesquite version 3.2 (Maddison and Maddison, 2017). The data matrix containing 22 discrete characters and 13 taxa (Appendix I), was analysed with TnT version 1.5 (Goloboff and Catalano, 2016). Data matrix used for the cladistic analysis (0= Plesiomorphic character state; 1, 2 & 3 = Apomormhic character states;?= Unknown data)- *Microtendipes umbrosus*. 0000100102?00000000010; *Zavreliella marmorata*. 00000000000000000000; *Endochironomus ampliceps*. 00101000011000000010000; *Conochironomus acutistilus*. 01010000220/10102100?211; *Conochironomus avicula*. 11001011020011010 1?111; *Conochironomus deemingi*. 10010101010010200 1?111; *Conochironomus australiensis*. 1000/10/1000210020210 0?211; *Conochironomus kakadu*. 00010000/12100201101?111; *Conochironomus cygnus*. 10010/10002201101110?111; *Conochironomus nuengthai*. 0000000/1101?02031001111; *Conochironomus swangthai*. 00100000001?01031000211; *Conochironomus tobaterdecimus*. 1101001011101011000111; and *Conochironomus jat*. 0000000101102031002 1?1

Traditional search was used with 100 replications and 10 trees were saved per replication. Implied weighting (K=3.00000) was used. The search has returned two trees. The strict consensus tree was used for cladistics. The tree was viewed with Winclad (Nixon, 1999-2002). DNA barcoding of COI gene and genetic distance of COI sequences were done as follows- The preserved adult specimens were sent to Acne Progen Biotech (India) Pvt. Ltd. for extraction of DNA and sequencing of cytochrome oxidase I using universal primers (forward primer LCO 1490 and reverse primer HCO 2198) (Folmer et al., 1994). A sequence length 539 base pair of mitochondrial COI region was obtained and uploaded to NCBI. The sequence (OK179068) was matched against all barcode sequences named as *Sumatendipes tobaterdecimus* in BLAST. The sequence similarities of the taxon between different geographical locations were also noted. Pairwise genetic distances among COI sequences from India, Singapore and Thailand were done using MEGA X (Kumar et al., 2018).

## RESULTS AND DISCUSSION

### A. Redescription

***Conochironomus tobaterdecimus* (Kikuchi and Sasa, 1990)** (Figs. 1 A-D)

*Sumatendipes tobaterdecimus* Kikuchi and Sasa, 1990

*Endochironomus effusus* Dutta, Majumdar and Chaudhuri, 1994: 253 syn. n.

GenBank Accession Number. OK179068

**Material examined:** Five males, labelled

'*Conochironomus tobaterdecimus* (Kikuchi and Sasa, 1990), India, West Bengal, Alipurduar, Falakata (26.88° N, 88.79° E), 26.V.2019, open light trap, Coll. K. K. Sow'. Two males, labelled '*Conochironomus tobaterdecimus* (Kikuchi and Sasa, 1990), India, West Bengal, Birbhum, Illambazar (23.62° N, 87.54° E), 21.iii.2022, open light trap, Coll. P. Hui'. One male, labelled '*Endochironomus effusus* Dutta, Majumdar and Chaudhuri, India, West Bengal, Gorubathan (26.95° N, 88.69° E), 22.10.1984, Coll. T. K. Datta'.

**Supplementary description of male (n=8)-** Total length 6.12-6.5, 6.31 mm. Wing length 2.8-3.25, 3.02 mm. Costal length 2.65-3.00, 2.82 mm. Antennal length 1.6-1.62, 1.61 mm. Colouration. Thorax brown in colour with prominent thoracic vittae. Apices of fore femora and fore tibiae light brown, apices of tarsomeres I-IV of hind and mid legs dark, tarsomere V of mid and hind legs fully dark in colour. Abdomen light olive green with black marking at apical end. Apices of gonostylus darker than rest.

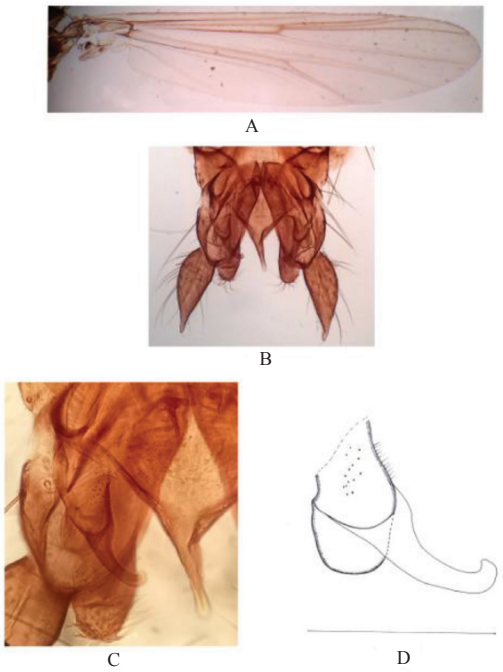


Fig. 1. A-D. *Conochironomus tobaterdecimus* (Kikuchi and Sasa, 1990). A. Wing, B. Hypopygium, C. An enlarged part of hypopygium showing anal point, superior and inferior volsellae, D. Superior volsella. (Scale bar 0.01 mm).

Head. Head width 750-760, 755 µm. Temporal setae 8-9 (IV 0, OV 5-6, Po 2-3). Clypeal setae 13-15. Frontal tubercles absent. AR 2.3-2.5, 2.4; ultimate flagellomere 1150-1165 µm long. Palpomere lengths (µm) (I-V): 55: 65-70, 67.5: 170-175, 172.5: 210: 300. CA 0.47-0.49, 0.48. Thorax. Scutum without tubercles. Acrostichals absent, dorsocentrals 8, scutellum with 3-4 setae. Others not countable. Wing (Fig. 1A). VR 1.1-1.15, 1.125. R with 33-36 setae, $R_1$ 4-6, $R_{4+5}$ 1. Brachiolum with 2 setae. Squama fringed with 12-14 setae. Anal lobe well developed. Legs. Fore tibia with a rounded scale and 3 subapical setae. Mid legs with 2 tibial spurs, 27.6-30, 28.8 µm and 23 µm long, comb with 34-38 teeth. Hind leg with 2 tibial spurs, 27.6-30, 28.8 µm and 23 µm long, comb with 40-42 teeth. Lengths and proportions of leg segments shown in Table 1. Abdomen Tergite IX with 15-21 median setae. Anal tergite band V shaped. Hypopygium (Figs. 1B-D). Anal point 87-90, 88.5 µm long, 36.8-39, 39.9 µm wide at base. Anal point widest basally, tapering with rounded apex with 4-5 lateral setae on each side. Superior volsella comprising of a basal globular lobe, 71-74 µm long, covered with microtrichia in basal part and large digitiform bare projection with apically inverted tip, 46-50 µm long. Inferior volsella basally fused with medial margin of gonocoxite, 92-94.5, 93.25 µm long, 23 µm wide with apically and subapically numerous long recurved setae. Gonocoxite 207-218, 212.5 µm long. Gonostylus 190-195.5, 192.75 µm long, with weak creases medially and on inner margin, tapered apically and pointed at apex bearing 1 short seta. HR 1.06-1.11, 1.08. HV 2.95-2.98, 2.96.

Table 1. Lengths (µm) and proportions of leg segments in *C. tobaterdecimus*

| | Fe | Ti | $Ta_1$ | $Ta_2$ | $Ta_3$ | $Ta_4$ | $Ta_5$ | LR | BV | SV |
|---|---|---|---|---|---|---|---|---|---|---|
| $P_1$ | 1750-1800, 1775 | 1250-1300, 1275 | — | — | — | — | — | — | — | — |
| $P_2$ | 1500-1550, 1525 | 1425 | 700 | 400 | 350 | 250 | 150 | 0.49 | 3.15-3.19, 3.17 | 4.17-4.25 |
| $P_3$ | 1725-1750, 1712 | 1500-1550, 1525 | 1100 | 800 | 550 | 350 | 175 | 0.71-0.73, 0.72 | 2.30-2.34, 2.32 | 2.93-3, 2.96 |

**Distribution:** Oriental region: Indonesia, Singapore, Thailand and India (Kerala, West Bengal).

**Female**. Unknown.

**Remarks:** After careful examination of the specimens, it fully corroborates with *C. tobaterdecimus* with the following combination of characters: presence of globular base of superior volsella and bare digitus. The genus *Conochironomus* Freeman is reported for the first time from India. Indian specimens have 14-19 anal tergite setae while it is few in number in the specimens described by Cranston, 2016. The type specimen of *Endochironomus effusus* Dutta, Majumdar and Chaudhuri, 1994 is found to have identical superior volsella bearing globular base and bare digitus with *C. tobaterdecimus*. After examining one paratype specimen of *E. effusus*, it is also inferred that the said species share roughly similar morphometric values with *C. tobaterdecimus*.

Distribution of the species clearly denotes it is an Indomalayan fauna. The species is supposed to have extended its range through the Assam gateway.

**B. Key to the species of *Conochironomus* Freeman (male) (after Cranston and Hare, 1995; and Cranston, 2016)**

1. Median volsella present ..... (2)
- Median volsella absent ..... (4)

2(1). Superior volsella without digitus, with broad base (Afrotropics: Nigeria) ..... .....*C. deemingi* Cranston and Hare, 1995
- Superior volsella with digitus..... (3)

3(2). Bifid superior volsella with short basal digitus, narrow base (Afrotropics: Cameroon, Madagascar, Nigeria, Senegal, Congo) ..... .....*C. avicula* Freeman, 1961
- Simple superior volsella with bare digitus, globular base (Australasia: Australia) ..... .....*C. kakadu* Cranston and Hare, 1995

4(1). $R_{4+5}$ bare ..... (5)
- $R_{4+5}$ with few to many setae ..... (7)

5(4). Superior volsella globular; tergite IX without setae (Australasia: Australia) ..... .....*C. cygnus* Cranston and Hare, 1995
- Superior volsella rectangular; tergite IX with setae .....(6)

6(5). Media-dorsal surface of gonostylus microtrichiose and setose, with weak creases (Afrotropics: Uganda, Zimbabwe, Nigeria, Congo (Zaire), Sierra Leone, Burkina Faso (Upper Volta), Rwanda) ..... ..... *C. acutistilus* Freeman, 1961
- Medio-dorsal surface of gonostylus bare, with prominent creases (Australasia: Australia) ..... .....*C. australiensis* Cranston and Hare, 1995

7(4). Base of superior volsella globular; thoracic vittae strongly present (Orient: India, Thailand, Singapore, Indonesia) ..... .....*C. tobaterdecimus* (Kikuchi & Sasa, 1990)
- Base of superior volsella cuneate shaped; thoracic vittae poorly developed to absent ..... 8

8(7). Gonostylus with weak creases; inferior volsella extensively fused with gonocoxite (Orient: Thailand) ..... ..... *C. swangthai* Cranston, 2016
- Gonostylus with strong creases; inferior volsella basally fused with gonocoxite ..... (9)

9(8). Superior volsella microtrichiose across medial 1/3 on both dorsal and ventral surface (Orient: Thailand) ..... .....*C. nuengthai* Cranston, 2016
- Superior volsella microtrichiose restricted only on ventral surface (Orient: China) ..... ..... *C. jat* Tang, 2018

Morphological cladistic analysis revealed that the consensus tree has consistency index (CI) 0.56 and retention index (RI) 0.56. *Conochironomus cervus* Cranston and Hare, 1995 is not included in the cladistics study as it is known only from pupal exuviae. The genus *Conochironomus* has been recovered as a monophyletic group based on the presence of crease of gonostylus and absence of acrostichals. The tree contains 2 clades: clade A contains *C. swangthai*, *C. nuengthai* and *C. jat*

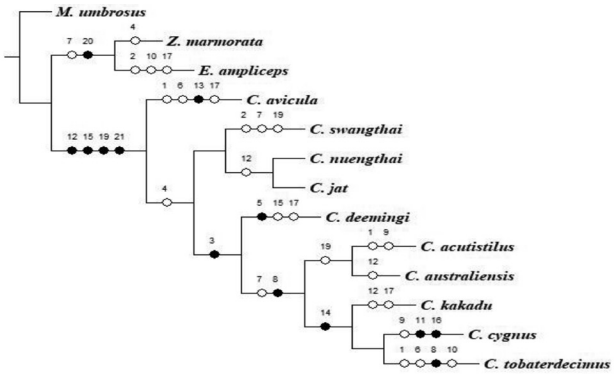


Fig. 2. Cladistic relationship among species of the genus *Conochironomus* Freeman worldwide (CI 0.56, RI 0.56)

Table 2. Information of COI sequences

| Genera | Similarity (%) | Status | GenBank Accession |
|---|---|---|---|
| *Sumatendipes* | 99.26 | published | MK645547 |
| *Sumatendipes* | 97.03 | published | KU507304 |
| *Sumatendipes* | 97.03 | published | KU507305 |
| *Conochironomus* | 96.84 | published | KT213040 |
| *Conochironomus* | 96.29 | published | KT213039 |
| *Conochironomus* | 96.94 | published | KU507300 |

Table 3. Pairwise genetic distances using maximum likelihood based on CO1 sequences

| | KT213040 (Thailand) | MK645547 (Kerala, India) | OK179068 (West Bengal, India) | KU507304 (Singapore) |
|---|---|---|---|---|
| KT213040 (Thailand) | | | | |
| MK645547 (Kerala, India) | 0.02953 | | | |
| Ok179068 (West Bengal, India) | 0.03821 | 0.0075 | | |
| KU507304 (Singapore) | 0.01902 | 0.02176 | 0.03045 | |

(the latter two species are seeming to be alike; humeral pit is present in *C. nuengthai* while this is absent in *C. jat* (Tang et al., 2018)). The clade B comprises of 2 subclades based on the number of setae on $R_{4+5}$. *Conochironomus acutistilus* and *C. australiensis* form a subclade B1 while subclade B2 is represented by *C. kakadu*, *C. Cygnus* and *C. tobaterdecimus* (all species bearing similar shaped superior volsella). Presence of few setae on $R_{4+5}$ is an autapomorphy for *C. tobaterdecimus* (Fig. 2).

Genetic distance analysis of mtCOI between the sequences OK179068 (India, West Bengal) and MK645547 (India, Kerala) shows 99% similarity. These confirms that the sequences publicly available from Kerala, India are that of *C. tobaterdecimus*. There is 97% similarity among OK179068, KU507304 and KU507305; latter two sequences uploaded from Singapore as *Sumatendipes tobaterdecimus*. The sequences of larvae KT213040 and KT213039 (*Conochironomus* sp.) from Thailand have 95% similarity with the uploaded sequence OK179068 (Table 2). The pairwise genetic distances (Table 3) among the sequences from India, Singapore and Thailand were done to infer the relationship of *C. tobaterdecimus* from different zoogeographical regions. The Indian specimens are much alike to *C. tobaterdecimus* from Singapore. The integrative taxonomy in our study indicates that COI-based DNA barcode data are useful and effective for identification of Chironomid midges.

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**AUTHOR CONTRIBUTION STATEMENT**

BM and NH conceived and designed research. BM and PH measured morphometrics. BM, PH and NH analysed data. BM wrote the manuscript. All authors read and approved the manuscript.

**CONFLICT OF INTEREST**

No conflict of interest.

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