

## Two new species of *Bryophaenocladus* Thienemann (Diptera: Chironomidae: Orthoclaadiinae) from Darjeeling and Kolkata, West Bengal, India with a note on biology of montane species

Dipak Kumar Som\*, Tuhar Mukherjee, Torpita Das and Rima Chakraborty

Postgraduate Department of Zoology  
Maulana Azad College, Kolkata

### Abstract

Two new species of *Bryophaenocladus* are proposed and described from West Bengal, India. *Bryophaenocladus kolkataensis* sp.nov. is reported as first Orthoclad from metropolitan areas of Kolkata while *B. pollexus* sp.nov. is reported from the montane regions of Eastern Himalayas biodiversity hotspot. The two species can be separated from other species of *Bryophaenocladus* by the presence of unique inferior volsella and gonostylus. A brief note on the biology of *B. pollexus* sp. nov. with special reference to its habitat preference, larval behaviour and adult swarming has been incorporated. DNA barcode of *B. kolkataensis* sp. n. is also given. A taxonomic key to the adult males of 14 Oriental species of the genus *Bryophaenocladus* is also provided.

**Keywords:** Chironomidae, Orthoclaadiinae, *Bryophaenocladus*, new species, chironomid biology

### Introduction

The genus *Bryophaenocladus* was erected by Thienemann in 1934 for the species *Orthocladus muscicola* Kieffer, 1906. The genus can be recognized by strong and decumbent acrostichals beginning close to antepnotum; wing membrane bare, with coarse punctation visible at  $\times 40$  magnification; tibial spurs strongly developed, with well-developed lateral denticles; tergite IX distinctive, with strongly pigmented, semi-circular band running around posterior margin; anal point large, semi-circular to triangular; virga consisting of simple spines; gonostylus often with a distinctly broadened, strong megaseta (Cranston *et al.* 1989). However, there are significant exceptions to all the generic characters (Du *et al.* 2011). Up to date 14 species have been reported from the Oriental realm, among which only three species have been described from India (Hazra *et al.* 2016, Kong *et al.* 2021).

In this study two new species are described along with a key to the adult males from the Oriental region.

### Material and Methods

#### Sample collection and preparation

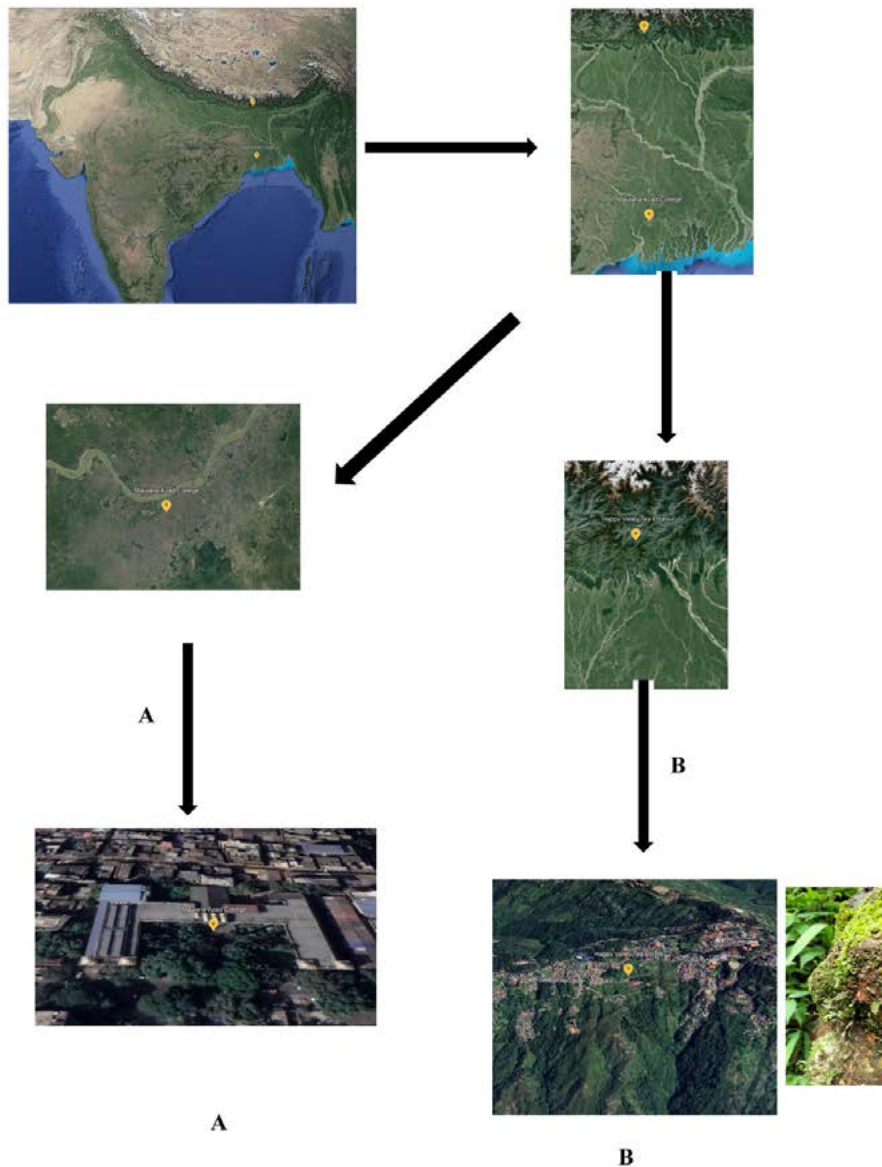
Adult midges were collected using pan traps and sweep nets from Maulana Azad College Garden, Kolkata (22° 33'N 88° 19'E; 9.14 m). Montane specimens were collected near the habitat of immature midges in happy valley region (27°3'N 88°15'E, 2100 m) of Darjeeling Himalaya (Figure1). Midge specimens were mounted on glass slides following Wirth and Marston's Phenol Balsam technique (1968). The material examined is temporarily retained in the collection of insects in the Entomology Division, Department of Zoology, Maulana Azad College, Kolkata, West Bengal, India and has deposited to the National Zoological Collections (NZC), Kolkata.

## Morphology and Terminology

Morphology and terminology of larvae, pupal exuviae and adults follow Sæther, 1980. The abbreviations used are: IV – Inner Verticals, OV – Outer Verticals, Po – Post orbitals, AR – Antenna ratio, VR – Veneral ratio, CR – Costal ratio, Fe – Femur, Ti – tibia, Ta – tarsomere, LR – Leg ratio, BV – Beinverhältnisse, SV – Schenkel–Schiene–Verhältnis, BR – Bristle ratio, HR – Hypopygium ratio, HV – Hypopygium value.

## Molecular methods

Thorax and one set of legs from one of the collected specimens were processed and outsourced for DNA extraction, amplification using cytochrome c oxidase subunit I (COI) universal primers LCO 1490 and HCO 2198 (Folmer *et al.* 1994) following Lin *et al.* (2018). The amplified products were sequenced by bidirectional Sanger's sequencing. The obtained sequence, trace files and other details were uploaded to the NCBI GenBank.



**Figure 1. Sites of midge collection (A–B)**  
A, Maulana Azad College, Kolkata; B, Happy Valley tea Estate, Darjeeling.  
(Image source: Google Earth).

## Taxonomy

### *Bryophaenocladus kolkataensis* sp. nov.

**Etymology** *Bryophaenocladus kolkataensis* sp.nov. is named after type locality.

**Diagnosis.** *Bryophaenocladus kolkataensis* sp.nov. can be recognized by the combination of AR 1.6; gonostylus subapically expanded with well-developed crista; anal point conical.

**Material examined.** Holotype male: India, West Bengal, Kolkata, Maulana Azad College Garden 22° 33'N 88° 19'E, 9.14 m, 28.vii.2019 Coll. Torpita Das, Regn. No. MAC/DKS 011; paratypes 1 male, data same as holotype Regn. No. MAC/DKS 012.

**Genbank accession number.** OQ749605

## Description

### Male imago (n=2)

Total length 1.62-1.67 mm. Wing length 1.47-1.51 mm. Total length/wing length 1.82-1.84. Wing length/length of profemur 2.51-2.53

**Colouration** Thorax pale, abdomen uniform, legs pale

**Head** AR 1.6. Ultimate flagellomere 336-364  $\mu$ m long. Temporal setae 8 (IV 3, OV4, PO 1) clypeus with 8 setae; tentorium 168  $\mu$ m long, 28  $\mu$ m wide; palpomere lengths ( $\mu$ m): 36:53:103:110:112; L: 5th/3rd 1.087. Third palpomere without apical digit form projection.

**Wing** (Figure 2A) Anal lobe not developed; Coarse punctation easily visible at  $\times 40$  magnification. VR 1.1; Costa extension 52  $\mu$ m long; R with 0 setae; R<sub>1</sub> with 0 setae; brachiolaris 1 Squama with 3-4 setae.

**Legs** Fore tibial spur 56  $\mu$ m long; mid tibial spurs 58  $\mu$ m and 27  $\mu$ m long; hind tibial spurs 62  $\mu$ m and 37  $\mu$ m long; Hind tibial comb with 12 spines. Lengths ( $\mu$ m) and proportions of legs as in Table 1.

**Table 1.** Lengths and proportions of leg segments of *Bryophaenocladus kolkataensis* sp.nov., male (n=2).

	Fe	Ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	518	672								
P <sub>2</sub>	588	630	196	112	98	84	84	0.31	3.74	6.21
P <sub>3</sub>	700	560	322	168	140	70	70	0.57	3.53	3.91

**Thorax** (Figure 2B) Anteprenotum with 2 lateral setae; dorsocentrals 8; acrostichals 3-4 prealars 5; scutellars 3-4, uniserial.

**Hypopygium** (Figure 2C) Anal point triangular, 64  $\mu$ m long, 28  $\mu$ m wide; Anal point length/width: 2.3. Tergite IX with 17-18 setae; laterosternite IX with 6 setae; phallapodeme 120  $\mu$ m long; transverse sternapodeme 98  $\mu$ m long; Gonocoxite subtriangular 168  $\mu$ m long; Gonostylus subapically expanded with prominent crista dorsalis, 96  $\mu$ m long; Megaseta 28  $\mu$ m long. Inferior volsella narrow, elongated, slightly expanded distally. Virga absent. HR 1.75. HV 2.99.

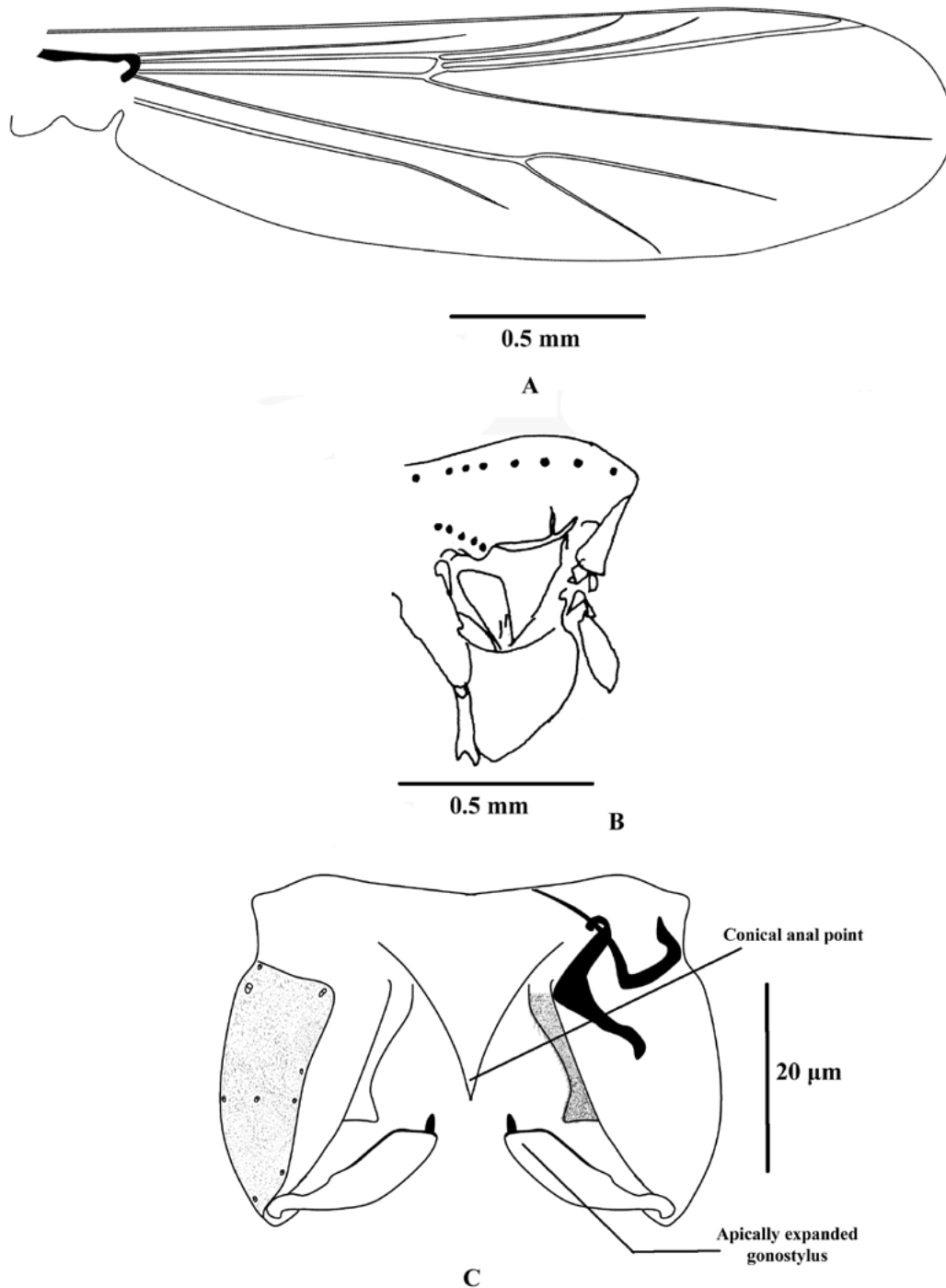
**Remarks.** The species is similar to *B. longipenis* Ghosh and Chaudhuri, 1984 in the shape of inferior volsella and AR. However, the shape of anal point, and of gonocoxite are quite different. The new species is similar to *B. manifestus*

Ghosh and Chaudhuri, 1984 in the shape of gonostylus, but the anal point of *B. manifestus* is much shorter than in *B. kolkataensis* sp. nov. Inferior volsella of *B. manifestus* is more expanded distally when compared with the proposed new species. The COI sequences of *B. kolkataensis* sp. nov. can be differentiated from other sequenced species by more than ~8% divergence. Among all the sequences available at NCBI GenBank, the barcode sequence of the proposed new species showed least divergence with *Bryophaenocladus huadingensis* with accession number MG301870.

**Ecology.** The species have been recorded from a small patch of shrub vegetation at Maulana Azad College Garden from Kolkata. Large swarms occur over a tree branch as marker at a height of about 160 cm. Swarming took during the afternoon when weather conditions are hot and humid with temperature ranging from 35°C to 38°C and relative humidity

ranged between 87 and 91. The adults of this species are often observed to visit flowers of *Ocimum tenuiflorum* during the daytime and may be considered as one of the potential

candidates engaged in pollination activities (Tiusanen *et al.* 2016). However, further study is required in this respect.



**Figure 2. Adult of *Bryophaenocladius kolkataensis* sp.nov.:  
A: Wing; B: Thorax; C: Hypopygium**

***Bryophaenocladius pollexus* sp.nov.**

**Etymology** *Bryophaenocladius pollexus* sp. nov. is named due to thumb like inferior volsella.

**Diagnosis:** *Bryophaenocladius pollexus* sp. nov. can be recognized by the combination of thumb-like inferior volsella with 5 strong setae and parallel sided gonostylus.

**Material examined.** Holotype male: India, West Bengal, Darjeeling, Happy Valley 27°3'N 88°15'E, 2100 m, 10.vi.2002 Coll. D.K. Som, Regn. No. MAC/ DKS 021; paratypes 2 larvae, 2 pupae, 4 males data same as holotype Regn. No. MAC/DKS 022.

**Description****Male imago (n=5)**

Total length 1.8-2.1 mm. Wing length 1.6-1.7 mm. Total length/wing length 1.1-1.2 Wing length/length of profemur 2.72

	Fe	Ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
P <sub>1</sub>	628-642	712-726	468-482	296-320	211-223	134	72	0.30-0.31	4.33-4.91	2.86-2.93
P <sub>2</sub>	557-571	623-635	187-201	103-112	86-88	84-92	84-90	0.25-0.28	5.03-5.21	6.31-6.43
P <sub>3</sub>	689-695	588-602	322-328	173-178	138-143	68-82	72	0.54	4.21-4.33	3.96-4.01

**Thorax** (Figure 3B) Anteprenotum well developed with 3 lateral setae; dorsocentrals 9, acrostichals 2 decumbent, uniserial, beginning near anteprenotum; prealars 4; scutellars 3 uniserial.

**Hypopygium** (Figure 3C) Anal point triangular, 64 µm long, 32 µm wide; Anal point length/width 2.0 Tergite IX with 26 setae; laterosernite IX with 4 setae; phallapodeme 128-

**Colouration** Thorax with lateral and median vittae; abdomen, legs pale

**Head** AR 1.23-1.33. Ultimate flagellomere 431-432 long. Temporal setae 8-11 (IV 4-5; OV 5-7; PO 0-1); clypeus with 6-8 setae; tentorium 106-112 long, 30-44, wide; palpomere lengths (µm): 28; 44-52; 220-234; 144-148; 243-255. L: 5th/3rd 1.08-1.10.

**Wing** (Figure 3A) Anal lobe not developed; Coarse punctuation easily visible at ×40 magnification; Costa extension 95-155 long; R with 7 setae; R<sub>1</sub>, R<sub>4+5</sub> with 0 setae; R<sub>2+3</sub> ends ¼ of distance between R<sub>1</sub> and R<sub>4+5</sub>; brachiolaris 2; Squama bare; VR 1.25; CR 1.16.

**Legs** Fore tibial spur 36-40 long; mid tibial spurs 28 and 28 long; hind tibial spurs 36 and 38 long; Hind tibial comb with 10 spines. Lengths (µm) and proportions of legs as in Table 2

**Table 2.** Lengths and proportions of leg segments of *Bryophaenocladius pollexus* sp. nov., male (n=5).

130 long; transverse sternapodeme 133 µm long; lateral sternapodeme 67 µm long; Gonocoxite cylindrical 256 µm long; Gonostylus parallel sided with prominent crista dorsalis, 149 µm long; Crista reduced; Megaseta 21-24 µm long. Inferior volsella thumb-like, with 5 strong setae. Virga 8-9 long. Hypopygium ratio (HR) 1.61-1.63. Hypopygium value (HV) 2.20

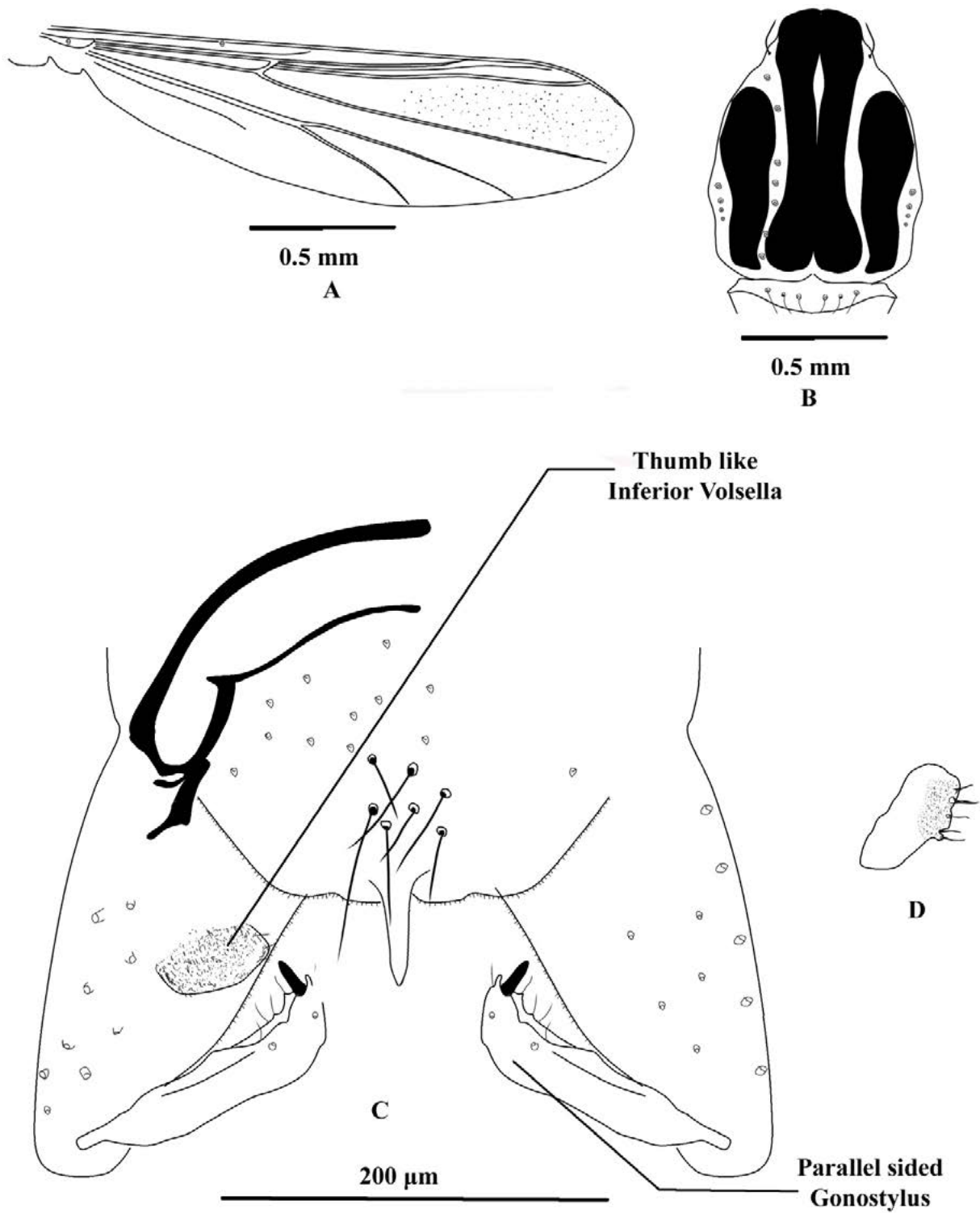


Figure 3. Adult of *Bryophaenocladius pollexus* sp.nov.:  
A: Wing; B: Thorax; C: Hypopygium; D: Inferior volsella

**Pupa (n= 6)**

Total length of exuviae: 2.93. Exuviae transparent, light brown.

*Cephalothorax* (Figure 4A): Frontal apotome as in figure; Antennal sheath 700 long in male and 400 long in female.

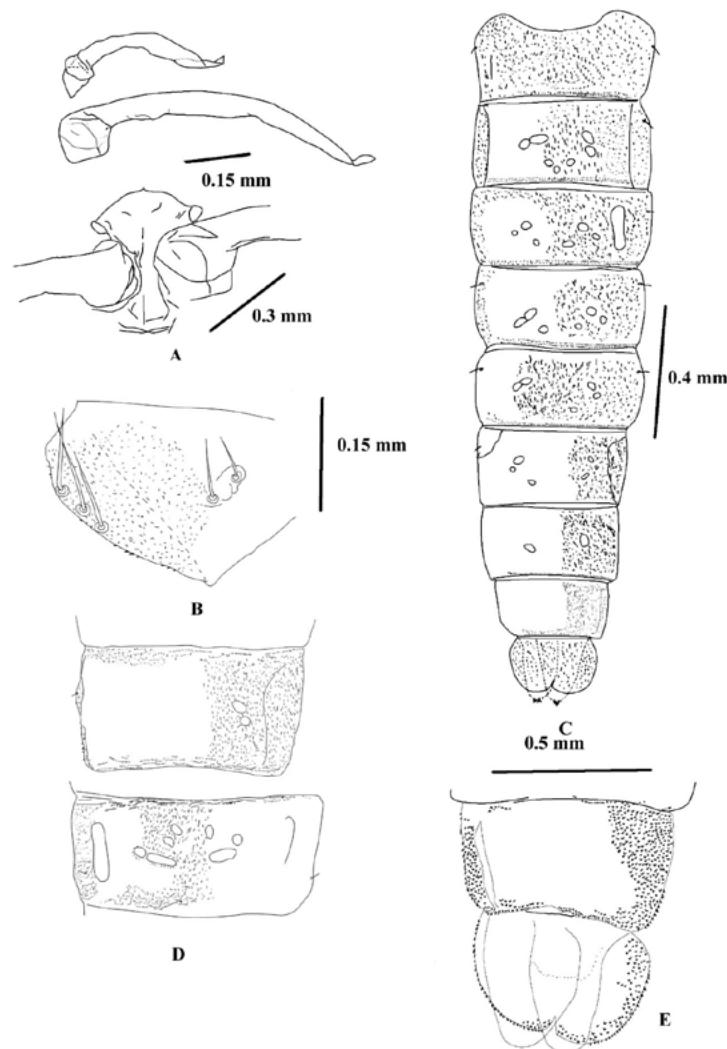
*Thorax* (Figure 4B) Thoracic horn absent; Frontal setae each of 50 long; Anterior and posterior precorneal setae each 70-80 long, spine-like, median precorneal seta absent; median anteprenotals three, spine-like, each about 54 long; lateral anteprenotals 2; Dorsocentrals 28-30 long; distance between  $Dc_1$  and  $Dc_2$  36-45, between  $Dc_2$  and  $Dc_3$  24-28, between  $Dc_3$  and  $Dc_4$  34-36; wing sheath 0.08 to 0.09 mm long, basal part 0.07 long.

*Abdomen* (Figure 4C) Tergites entirely covered by strong shagreen spinules, shagreen spinules distributed from Tergite I to IX. Number of dorsal and lateral setae present from tergites I to VIII are given in Table 3.

*Anal lobe* (Figure 4D) 200-300 long, shagreen spinules stronger and marginal spinules small spine-like; Genital sac overreaches anal lobe by 50; anal lobe without fringe and macrosetae.

**Table 3.** Setae present on segments I-VIII

	I	II	III	IV	V	VI	VII	VIII
Dorsal	0	0	0	0	0	0	0	0
Lateral	1	1	1	1	1	1	1	1



**Figure 4.** Pupa of *Bryophaenocladus pollexus* sp. nov. (Dorsal view): A: Frontal Apotome, Wing sheath and antennal sheath; B: Anterior part of Thorax; C: Pupal exuviae; D: Tergite VII; E: Tergite VIII and anal lobe

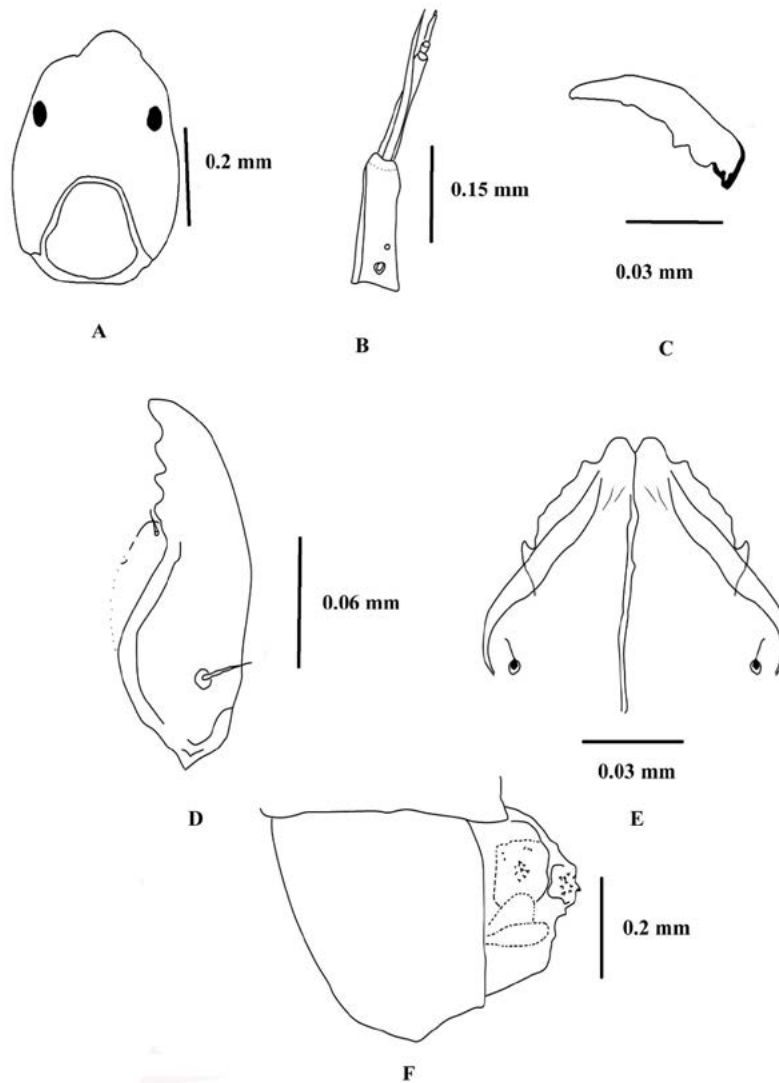
**Fourth instar larva (n= 2)**

Body colour golden yellow; Body length 3.2 – 4.0 mm; Ventral Head capsule length 0.35 – 0.4 mm.

*Head* (Figure 5). *Antenna* (Figure 5B), Length ratio of antennal segments (I – V): 18.75 : 16.5 : 2.25 : 3.75 : 1.5. AR 0.78. Basal antennal segment 0.12 mm wide with ring organ 0.015 mm in diameter; distance from base to ring organ 0.019 mm. Blade 0.24 mm long, nearly equal to flagellum; Lauterborn organ 0.008 mm long; *Premandible* (Figure 5C), 0.065 mm long, with 2 teeth; *Mandible* (Figure 5D) 0.158 mm long, seta subdentalis sharp spine-like, 0.008 mm long, seta interna with 2 branches; *Mentum* (Figure 5E)

width 0.064 mm with dome-shaped 2 median teeth, total width 0.018 mm, and 4 pairs of lateral teeth; *ventromental plate* 0.008 mm wide, slightly extends beyond outer lateral tooth on flattened mentum; setae submenti 0.012 mm long. *Abdomen* (Figure 5F): Abdominal segment VIII and posterior parapods bearing segment are flexed at right angle to the rest of the body; posterior parapods separate with minute simple claws; anal tubules not measurable; Procercus absent, seta absent at the end of the body.

*Remarks:* The proposed new species is similar to *B. nodosus* in the shape of the morphology. However, the shape of gonostylus, HR of *B. nodosus* (2.28) is much higher than the proposed new species.



**Figure 5.** Larva of *Bryophaenocladius pollexus* sp.nov.: A: Head Capsule; B: Antenna; C: Premandible; D: Mandible; E: Mentum; F: Abdomen



**Ecology:** Very little is known of the biology of *Bryophaenocladus pollexus* sp. nov. Larvae and pupae of this species are terrestrial in their habitat. Immature are found to live on solid substrata within mat of rhizoids of certain bryophytes and leafy liverworts (*Porella* sp.). This kind of microhabitat holds some humid soil-like particles (Pinder 1995). They are also found on the surface of woody stem of tea plants *Camellia sinensis*. Optimum temperature, sunlight intensity and humid environment probably play important role for their abundance in the montane regions like Darjeeling Himalaya (Armitage *et al.* 1995). Larvae are golden in colour. The abdominal parts of live pupae have

golden coloured abdomen while the cephalothorax is light brown coloured. The larvae, when come out of rhizoid mat, do not crawl over the vegetative structure of bryophytes, instead show characteristic jumping movement. After imaginal moult, pupal exuviae are found attached to the vegetative surface of bryophytes. The emergence pattern of *B. pollexus* sp. nov. shows a definite peak during the months of September and October. Adults are day-light swarmer with low height swarming occurs near their habitat indicating ground as visual swarming marker. They are found to swarm over the substratum during sunny weather probably indicating sun light as one of the important swarming cues.

#### Key to the adult males Oriental species of the genus *Bryophaenocladus*

1. Squama bare -----2  
- Squama with setae -----6
- 2(1). Crista absents -----3  
-Crista present -----4
- 3(2). Costal extension absent; AR>1.0 (India) ----- *B. manifestus* Ghosh and Chaudhuri, 1984  
- Costal extension present; AR<1.0 (China) ----- *B. parictericus* Lin, Qi and Wang, 2012
- 4(2). Anal point absent (Japan) ----- *B. iriopequesus* (Sasa and Suzuki, 2000)  
- Anal point present -----5
- 5(4). Gonostylus parallel sided; inferior volsella thumb-like (India) ----- ***B. pollexus* sp.nov.**  
- Gonostylus wide at apex; inferior volsella inconspicuous (China) ----- *B. parimberbus* Du and Wang, 2010
- 6(1). Squama with more than 5 setae -----7  
- Squama with less than 5 setae -----9
- 7(6). Gonostylus curved; inferior volsella knob-like (India) ----- *B. nodosus* Hazra and Das, 2011  
- Gonostylus not curved; inferior volsella elongated ----- 8
- 8(7). Anal point pointed; pseudospurs present on tarsomeres 1 and 2 of mid and hind legs (China) -----  
*B. mucronatus* Lin, Qi and Wang, 2012  
- Anal point blunt; pseudospurs absent (India)-----*B. longipenis* Ghosh and Chaudhuri, 1984
- 9(6). Gonostylus curved; inferior volsella hyaline (China)----- *B. huadingensis* Kong, Wang and Lin, 2021  
- Gonostylus not curved; inferior volsella not hyaline ----- 10
- 10(9). Gonostylus wide at apex ----- 11  
- Gonostylus apically expanded (China) -----*B. xinglongensis* Du and Wang, 2010
- 11(10). Gonostylus acutely hooked apically; HR<1.5(Japan, Russia) ----- *B. akiensis* (Sasa, Shimomura and Matsuo, 1991)  
- Gonostylus not apically hooked; HR>1.5 ----- 12

- 12(11). *Gonostylus triangularis*; gonocoxite lobe with strong setae (China, Canary Islands) -----  
*B. cuneiformis* Armitage, 1987  
- *Gonostylus* not triangular; gonocoxite lobe absent ----- 13
- 13(12). Crista well developed; veins R, R<sub>1</sub> bare (India) ----- ***B. kolkataensis* sp.nov.**  
- Crista not well developed; veins R, R<sub>1</sub> with setae (China) ----- *B. wufengensis* Du and Wang, 2010

## Acknowledgement

The authors are thankful to the Principal, Maulana Azad College, Kolkata and Head, Post graduate Department of Zoology, Maulana Azad College, West Bengal, India for rendering laboratory and library facilities. The authors are thankful to Dr. Martin Spies, Zoologische Staatssammlung

München, Germany and Dr. Xiaolong Lin, Associate Professor, Engineering Research Center of Environmental DNA and Ecological Water Health Assessment, Shanghai Ocean University, China and Professor P. K. Chaudhuri for proving literature pertaining to the study.

## References

- Armitage, D.D. 1987. A new species of the genus *Bryophaenocladius* Thienemann, (Diptera: Chironomidae) from Tenerife Canary Islands. *Aquatic Insects*, 9(1) : 33-38.
- Armitage, P., Cranston, P.S. and Pinder, L.C.V. 1995. The Chironomidae. The biology and ecology of non-biting midges. *Chapman & Hall, London*, 572 pp.
- Cranston, P.S., Oliver, D.R. and Saether, O.A. 1989: The adult males of Orthoclaadiinae (Diptera, Chironomidae) of the Holarctic region — keys and diagnoses. — *Entomologica Scandinavica* 34 (Supplement): 165–352.
- Du, J., Sæther, O.A. and Wang, X.H. 2011. Redescriptions of species of *Bryophaenocladius* Thienemann, 1934 (Diptera: Chironomidae) described by Brundin (1947). *Zootaxa*, 2743: 40–48.
- Du, J. and Wang, X. 2010. Three new species of *Bryophaenocladius* Thienemann, from Oriental China, with inconspicuous inferior volsella (Diptera: Chironomidae). *Acta Zootaxonomica Sinica*, 35: 750-755.
- Folmer, O., Black, M., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294–299.
- Ghosh, M. and Chaudhuri, P.K. 1984. Indian species of the genus *Bryophaenocladius* Thienemann. *Journal of the Bengal Natural History Society*, 2(1): 27-33.
- Hazra, N. and Das, N. 2011. A new species of *Bryophaenocladius* Thienemann, 1934 (Diptera: Chironomidae) from Darjeeling Himalayas, India. *International Journal of Dipterological Research.*, 22(3): 139-143.
- Hazra, N., Niitsuma, H. and Chaudhuri, P.K. 2016. Checklist of Chironomid midges (Diptera: Chironomidae) of the Oriental Region. *Zoological Survey of India*, New Alipore, India, pp. 1–206.
- Kieffer, J.J. 1906. Description d'une genre nouveau et de quelques espèces nouvelles de Diptères de l'Amérique du Sud. *Annales de la Société scientifique de Bruxelles*, 30: 349-358.
- Kong, F.Q., Wang, X.H. and Lin, X.L. 2021. *Bryophaenocladius huadingensis* (Diptera: Orthoclaadiinae), a new species from China *Annales Zoologici Fennici*, 58: 1-3.
- Lin, X., Qi, X. and Wang, X. 2012. Two new species of *Bryophaenocladius* Thienemann, 1934 (Diptera, Chironomidae) from China. *ZooKeys*, 208: 51-60.
- Lin, X., Stur, E. and Ekrem, T. 2018. DNA barcodes and morphology reveal unrecognized species in Chironomidae (Diptera). *Insect Systematics & Evolution*, 49(4): 329–398.
- Pinder, L.C.V. 1995. The habitats of chironomid larvae. Springer Netherlands, 107–135.

- Sæther, O.A. 1980. Glossary of chironomid morphology terminology (Diptera: Chironomidae). *Entomologica Scandinavica, Supplement, 14*: 1–51.
- Sasa, M. and Suzuki, H. 2000. Studies on the chironomid species collected on Ishigaki and Iriomote Islands, southwestern Japan. *Tropical Medicine, 42*(2): 1-37.
- Sasa, M., Shimomura, H. and Matsuo, Y. 1991. Description of three chironomid species collected in Hiroshima Prefecture, Japan (Diptera, Chironomidae). *Japanese Journal of Sanitary Zoology, 42*(4) : 281- 287.
- Thienemann, A. 1934. Die Tierwelt der tropischen pflanzengewasser. *Archiv für Hydrobiologie, Supplement, 13*: 1-91.
- Tiusanen, M., Hebert, P.D., Schmidt, N.M. and Roslin, T. 2016. One fly to rule them all-muscid flies are the key pollinators in the Arctic. *Proceedings of the Royal Society B: Biological Sciences, 283*(1839) : 20161271.
- Wirth, W.W. and Marston, N. 1968. A method for mounting small insects on microscope slides in Canada Balsam. *Annals of the Entomological Society of America, 61*: 783–784.

---