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Immature stages of *Culicoides innoxius* Sen & Das Gupta and pupa of *C. huffi* Causey (Diptera: Ceratopogonidae) from India

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Abstract

Both larval and pupal stages of *Culicoides innoxius* Sen & Das Gupta and pupal stages of *C. huffi* Causey are described and illustrated. All specimens were collected from the Lower Gangetic plains of West Bengal. Larvae of *C. innoxius* were obtained from rotten banana stem, while a single pupa of *C. huffi* was collected from an irrigation channel associated with the rice agroecosystem. A pictorial pupal key of the species of the genus reported from India is provided.

Key words: Taxonomy, Clavipalpis group, *Hoffmania*, immature, key, India

Introduction

Culicoides Latreille is one of the most species rich genera of the family Ceratopogonidae with approximately 1368 valid species, 32 subgenera, 38 species group, and 176 unplaced species described worldwide (Borkent & Dominiak 2020), and so far 76 species were reported from India (Chatterjee *et al.* 2020). Most female members of this genus are haematophagous and many species act as important vectors of pathogenic viruses, protozoans and filarial nematodes (Mellor *et al.* 2000; Borkent 2005). These midges are of great concern because they transmit bluetongue (BT), Akabane and other viruses that cause disease in sheep, cattle and wild ruminants (Kettle 1977; Linley *et al.* 1987). Immatures of this genus can be found in a variety of aquatic or semi aquatic habitats, including tree holes, ponds, marshes, streams, various muddy and saturated organic materials, damp or rotting vegetation, and manure (Kettle & Lawson 1952; Jamnback 1965; Borkent 2014; Saha *et al.* 2017; Shults & Borkent 2018). Immature stages of ceratopogonid midges including *Culicoides* are least studied. Only 3% of the larvae and 7% of the pupae are so far described worldwide from the total identified species of the genus (Borkent 2014). Immature stages of only nine species have been described from India. Seven species of *Culicoides* were reported from India which have putative role as a vector of BT virus (Prasad *et al.* 2009). *Culicoides* is a very complex group with many cryptic species, so that the species level identification became sometimes tough. In order to provide additional characters to identify cryptic species, the description of immature characteristics and their habitats may prove to be useful.

This paper aims to redescribe the pupal stage of two species, *Culicoides innoxius* Sen & Das Gupta, 1959 and *C. huffi* Causey, 1938 in light of modern terminologies with a pupal key to the species reported from India of the genus *Culicoides*.

Material and methods

The substrates with both larvae and pupae of *C. innoxius* Sen and Das Gupta, 1959 and only one pupa of *C. huffi* Causey, 1938 were obtained from their habitats. The immature stages were sorted under a stereozoom trinocular microscope

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(Olympus model SZX16, Japan) using a fine tipped brush (size 000). They were then reared in glass vials containing natural substratum plugged with non-absorbent cotton putting few drops of water and kept at room temperature (25°–34°C) till eclosion of midges. The newly emerged adults were kept alive 2–3 days for proper sclerotisation and pigmentation. Adult midges and pupal and larval exuviae preserved in 70% ethanol were then mounted on glass slides following Wirth & Marston (1968) method.

Morphology and terminology of pupae follow Borkent (2014), while that of larva follow Harsha *et al.* (2017). Measurements are in micrometres (µm), except total length of pupa, and length of pupal terminal process, which are in millimetres (mm). All measurements are presented as ranges when sample size was more than five, with the mean value in parentheses. The illustrations were produced using compound microscopes (Wild Leitz GMBH, Portugal) in combination with an attached camera lucida. The photographs were taken both with a trinocular compound microscope (Wild Leitz GMBH) and a Stereo zoom trinocular microscope (Olympus make, model SZX16, Japan).

Abbreviations of morphological terms used in the text and figures:

Pupa. DA - dorsal apotome; DAL - dorsal apotomal length; DAW - dorsal apotomal width; CL - clypeal/labral seta; O - ocular seta; AL - anterolaterals; AM - anteromarginals; D-n-T - dorsals; M-n-T - metathoracics; D-n-I - dorsal sensilla on first abdominal segment; L-n-I - lateral sensilla on first abdominal segment; D-n-IV - dorsal sensilla on fourth abdominal segment; L-n-IV - lateral sensilla on fourth abdominal segment; V-n-IV - ventral sensilla on fourth abdominal segment; D-n-IX - dorsal sensilla on ninth abdominal segment

Larva. HL - head length; HW - head width; ML – mandible length

Results

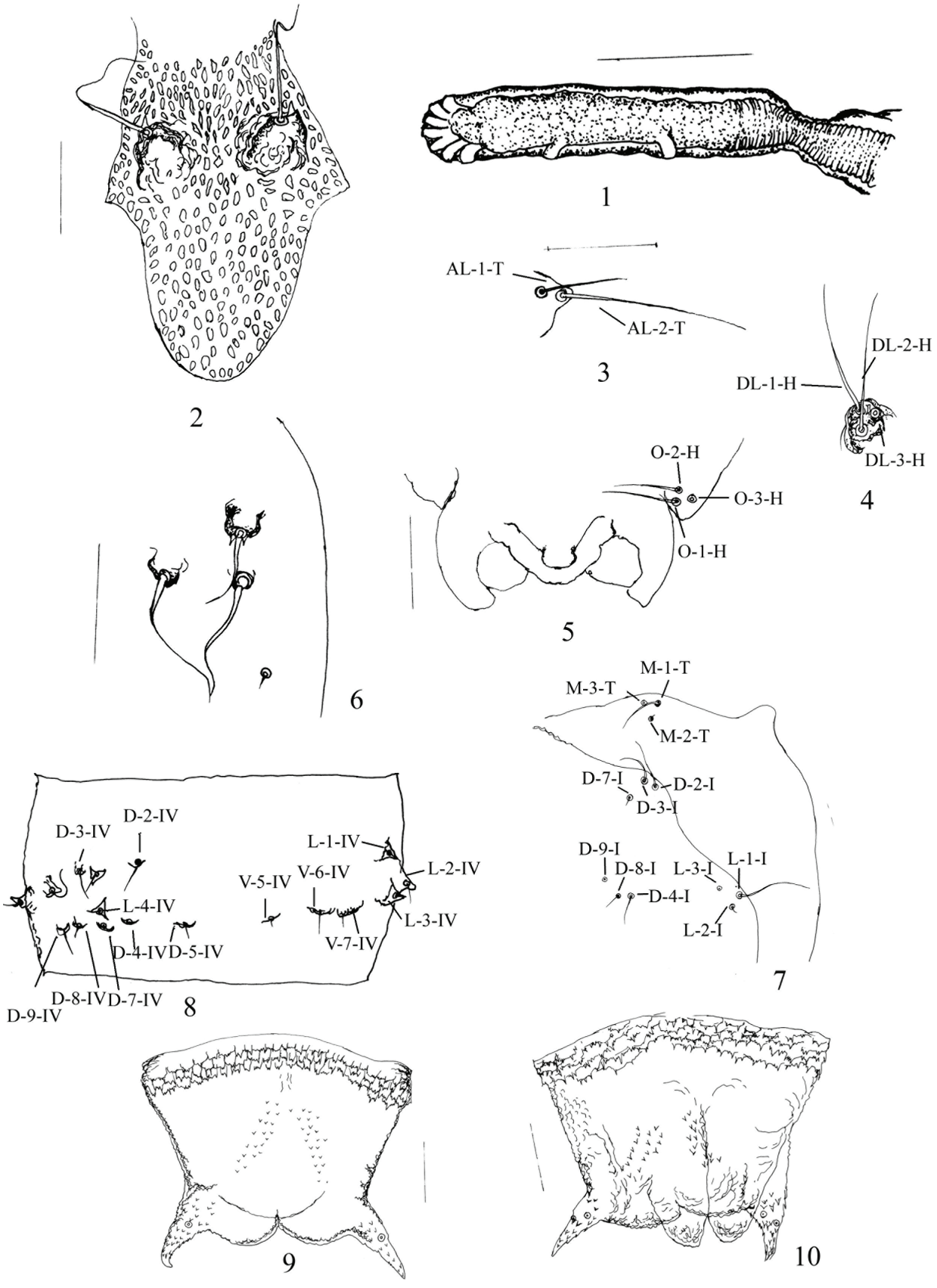
Subgenus *Hoffmania* Fox, 1948
Culicoides innoxius Sen & Das Gupta, 1959
(Figs. 1–10)

Material examined

Four males and 1 female with pupal exuviae (reared), India, West Bengal, South 24 parganas, Balibazar [22°08’88”N, 88°75’72” E], 16.IV.2019, rotten banana stem near cow shed, Coll. S. Chatterjee; one male with larval and pupal exuviae (reared), data same as before.

Description of pupa (n = 6)

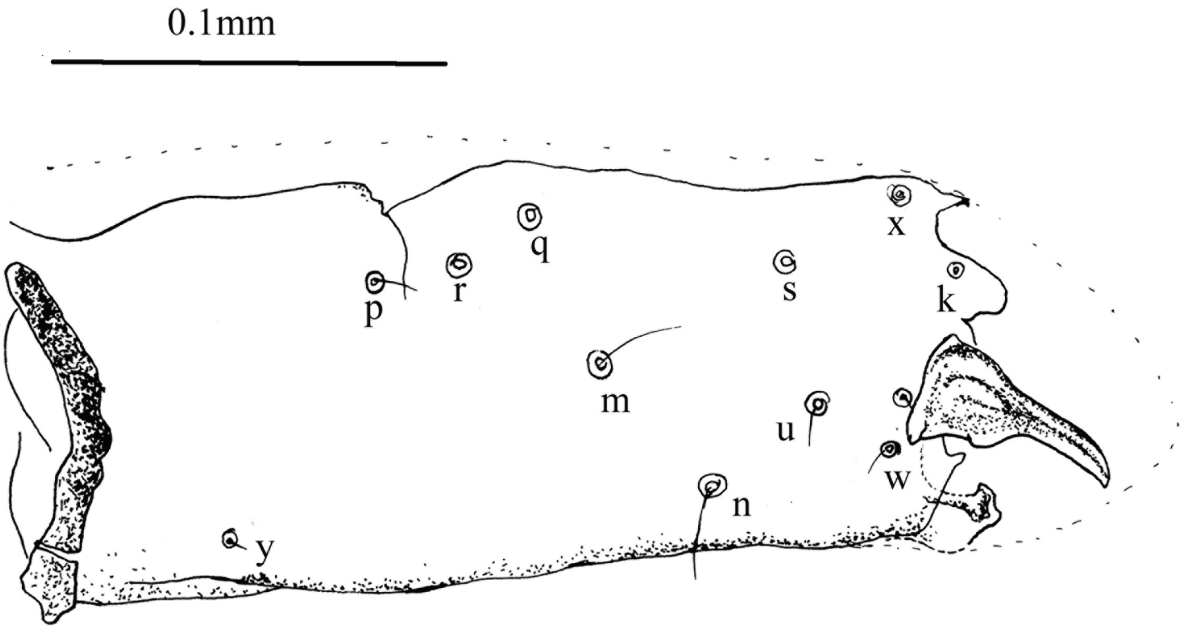
Total length 1.50–1.47 (1.48) mm; cephalothorax yellowish brown. Dorsal apotome (Fig. 2) covered by uniform spicules. DAL 218.50–232.30 (225.40) µm, DAW 142.60–147.20 (144.90) µm, DAW/DAL 0.64. Two DA; DA–1–H long, DA–2–H with spur. Respiratory organ (Fig. 1) 147.20–151.80 (149.50) µm long with 6–7 terminal and 2 lateral pores; scales restricted to proximal part. Pedicel length 66.70–69 (67.85) µm; pedicel: respiratory organ 0.45. Three DL (Fig. 4) setae present; DL-1-H and DL-2-H setae long and subequal in length, DL-3-H campaniform sensilla; CL absent; three oculars present (Fig. 5), O-1-H and O-3-H long setae, O–2–H campaniform sensilla. Five D setae present in thorax (Fig. 6); D-1-T, D-2-T and D-4-T long setae, D-5-T small seta. Two unequal AL (Fig. 3) setae present; AL-1-T two times longer than AL-2-T (Fig. 3). M-1-T with moderately long seta, M-2-T with small seta and M-3-T campaniform sensilla (Fig. 7). Tergite I (Fig. 7) with D-2-I and D-3-I with moderately long setae, D-7-I with small seta; D-4-I and D-8-I with moderately long setae, D-9-I campaniform sensilla; L-1-I long seta, L-3-I small seta and L-2-I campaniform sensilla. Fourth abdominal segment having the following types of tubercles (Fig. 8): L–1–IV, L–2–IV, L–3–IV, L–4–IV with spurs and setae. Three ventral setae with setal base; V–6–IV seta longest. D–5–IV, D–4–IV, D–8–IV, D–9–IV with setae, and D–7–IV without setae; terminal process 39.10–41.40 (40.25) µm long, D–5–IX and D–6–IX present at base of terminal processes (Figs. 9–10). Female pupa similar to male except usual sexual differences.



Figures 1–10. Pupa of *Culicoides innoxius* Sen & Das Gupta, 1959. **1**, Respiratory organ; **2,** Dorsal apotome and dorsal apotomals (DA); **3**, Anterolaterals (AL); **4,** Dorsolateral cephalic sensilla (DL); **5**, Oculars (O); **6**, Dorsal setae (D); **7**, Metathoracics and first abdominal segment; **8**, Fourth abdominal segment: left – dorsal view, right – ventral view; **9**, Segment 9 (female); **10**, Segment 9 (female). (Scale bar: 0.05 mm).

Description of damaged larval exuvium (n = 1)

Larval exuvium (Figs. 11–12) mostly damaged. Head capsule yellowish brown, heavily sclerotised. HL 195.5 µm, HW 87.5 µm, triangular (Figs. 11); mandible curved pointed apically, mandibular seta near base ML 43.7 µm. Epipharyx (Figs. 12) with moderately wide dorsal comb sclerite, teeth unequal in both length and width with slightly pointed tip.



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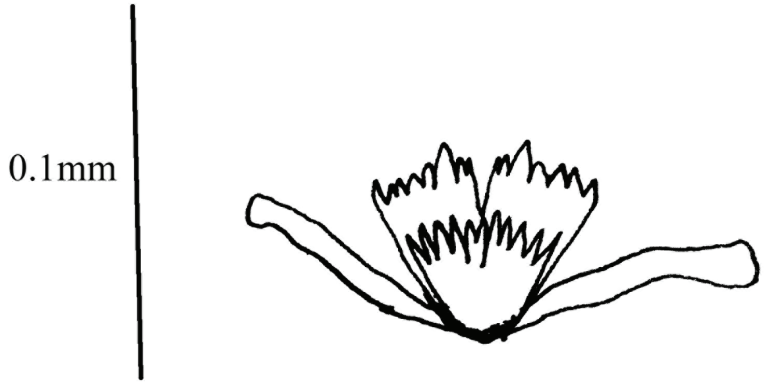


12

Figures 11–12. Larva of *Culicoides innoxius* Sen & Das Gupta, 1959. 11. Head capsule; 12. Epipharynx.

Remarks. The adults conform fully to the original description (Sen & Das Gupta, 1959). *Culicoides innoxius* is a member of the subgenus *Hoffmania* Fox (1948), and adults of this species are recognised by the following characteristics: mandible with 16–20 teeth, comparatively stouter third palpal segment, dark haltere colour, hind tibial comb with 5–6 spines, second one from spur longest; cell r_3 of the wing (Figs. 22) with roughly rounded distal pale spot present more or less equidistantly from vein M_1 and wing margin; ninth tergum rounded caudally without apparent apicolateral processes, the caudal margin with a small median lobe; aedeagus with basal arch extending to a fifth of total length, base of arch without strongly sclerotised anterior rim, no internal sclerotised peg distally, distal portion slender with spherical tip; parameres separate, each with moderately short and stout anterolateral arm; stem moderately stout, tapering gradually distally and curved ventrad, ending in a filamentous point with 5–6 minute fringing hairs. Although pupal stage of *Culicoides innoxius* was firstly reported from Sayaboury, Laos by Howarth (1985), it lacks comprehensive and illustrative taxonomic study. The pupa of this species shares some similarities with pupal features

of *C. peregrinus* Kieffer, 1910 (only immature of the subgenus *Hoffmania*, reported from India) like structure of dorsal apotome, shape of DL–1–H, DL–2–H, some 4th abdominal tubercles, and orientation of the transverse process but differs in dorsomedial tubercle (absent in *C. innoxius* but present in *C. peregrinus*), palpus tubercle (absent in *C. innoxius* but present in *C. peregrinus*) and D–4–IV (without spur in *C. innoxius*).

Distribution and bionomics. The species is distributed in Cambodia, Oriental China (Hainan), India, Indonesia, Laos, Malaysia, Sri Lanka and Thailand. Immature stages of the midges were obtained from rotten banana stem near a cowshed, adjacent to Sundarban Mangrove area, and reared in the laboratory. The specimens were collected at an altitude of 7.50 m above sea level.

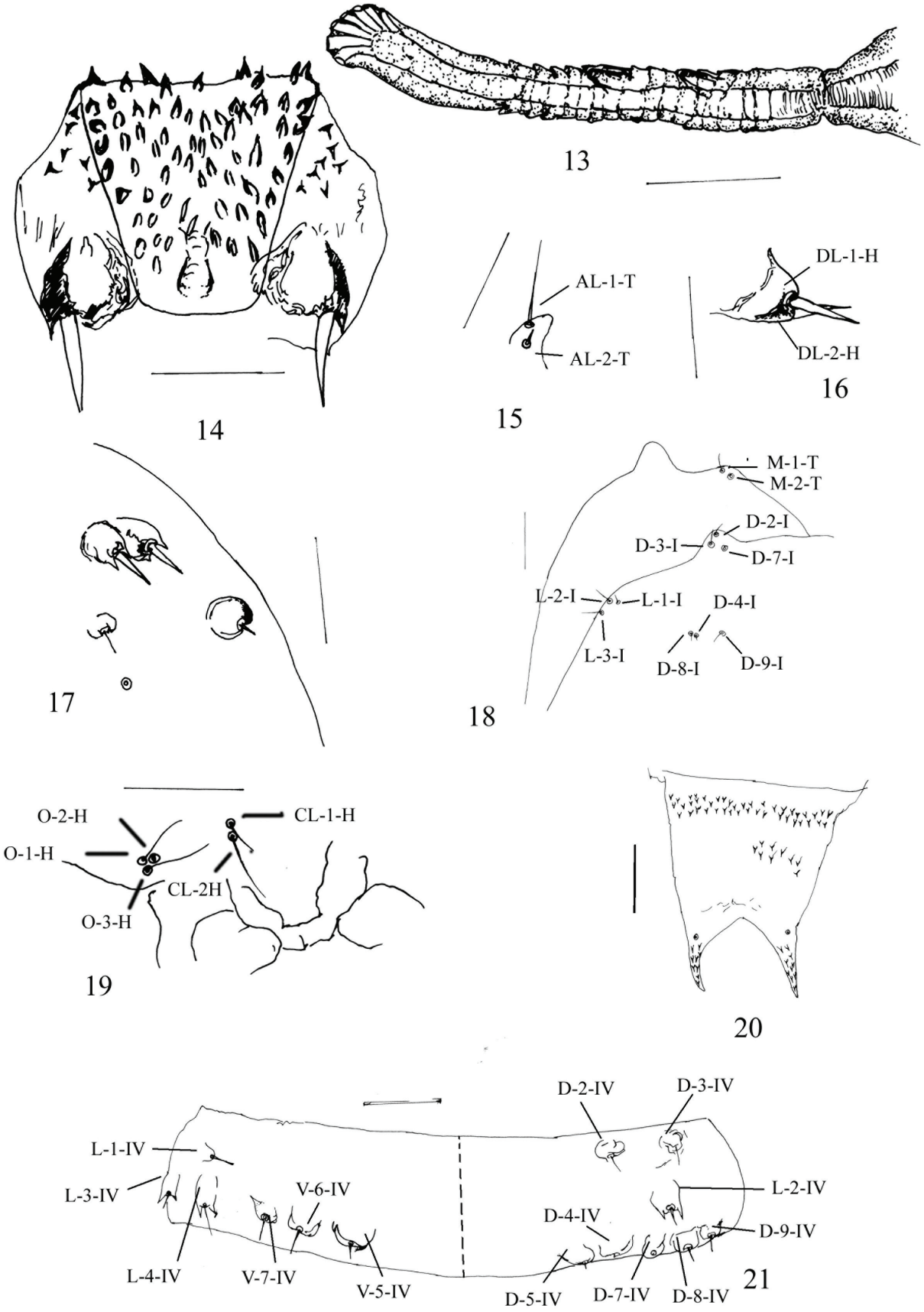
The *clavipalpis* species-group
***Culicoides huffi* Causey, 1938**
(Figs. 13–21)

Material examined
One female with pupal exuviae (reared), India, West Bengal, Murshidabad, Raghunathganj [22°08'88"N, 88°75'72" E], 19.IV.2018, irrigation channel associated with paddy field, Coll. S. Chatterjee.

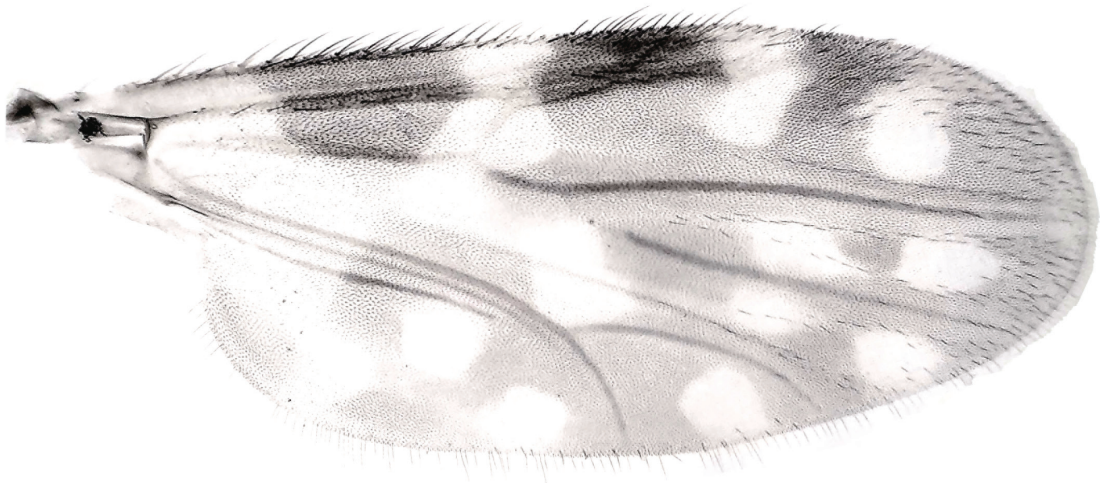
Description Pupa (n = 1)
Exuviae pale, yellowish brown. Total length 1.65 mm. Dorsal apotome as in figure 14. DAL 220.00 µm, DAW 131.00 µm. Disc surface of dorsal apotome with spines and spinules; DA-1-H moderately elongated, stout, pointed seta, DA-2-H sensillum present (Fig. 14). Respiratory organ (Fig. 13) 300 µm long, wider apically with 9 terminal, 3 lateral pores, middle portion covered by scales. Pedicel 36.60 µm long. Two CL (Fig. 19), CL-1-H seta long and thin, CL-2-H short and thin; three O (Fig. 19), O-3-H having longer seta than O-1-H, O-2-H campaniform sensilla. Two DL (Fig. 16). DL-1-H and DL-2-H, both are moderately long, stout, spur like and sub equal in size. Thorax with five D setae (Fig. 17); D-1-T and D-2-T stout and spur like, D-4-T thin, D-5-T short, D-3-T campaniform sensilla, Two AL (Fig. 15), AL-1-T long and thin, AL-2-T shorter and thin. Metathoracics (Fig. 18) M-1-T with 1 small seta and M-2-T campaniform sensilla. Abdomen pale, without scattered spinules, D-2-I, D-3-I with setae, D-7-I campaniform sensilla (Fig. 18), L-2-I, L-1-I and L-3-I with setae; D-4-I, D-8-I, D-9-I with setae. Fourth abdominal segment tubercles (Fig. 21) as follows: L-1-IV with seta, L-2-IV, L-3-IV, L-4-IV with spur and setae; V-5-IV and V-6-IV with small seta and bulbous base, V-7-IV with pointed spur and seta; all D-IV with small setae and bulbous base except D-7-IV with campaniform sensilla. Segment IX with (Fig. 20) band of spines at basal region both dorsally and ventrally; terminal process 50.60 µm long with pointed tip.

Remarks. The reared adult midge fully agrees with the description of Causey (1938). The pupal stage of *Culicoides huffi* was reported firstly from Sayaboury, Laos by Howarth (1985). Adults of *C. huffi* can be recognised by the following combination of characters: eyes bare, interocular space slightly wedge-shaped; hind tibial comb with 4 spines, the one nearest the spur longest, wing (Figs. 23) with 2nd radial cell in a very dark spot, small pale spot lying below poststigmatic pale spot crossing vein M_1, one small round distal pale spot present on cell r_3, cell m_1 with two pale spots, distal one is near but separated from the wing margin, cell m_2 with a pale spot immediately behind the medial fork and another one at the wing margin, anal cell with a small pale area at the basal region and one transverse medially constricted distal pale spot; spermathecae pyriform with very slender, elongated necks. The pupa of *C. huffi* shares some similarities with *C. similis* in structure of respiratory organ (both having 3 lateral pores and annulation), and structure of dorsal apotome but differs in the structure of anteromedial tubercle (stout and spine like in *C. similis* but thin in *C. huffi*) and some abdominal tubercles (ridge like in *C. similis* but spine like in *C. huffi*). It differs from *C. pseudosimilis* in structure of DL (DL-2-H three times longer in length in *C. huffi* than *C. pseudosimilis*), AL (AL-2-T of *C. pseudosimilis* almost 4 times longer than of *C. huffi*) and some abdominal tubercles (D-3-IV without seta in *C. pseudosimilis* but D-3-IV with seta in *C. huffi*).

Distribution and bionomics. The species is distributed in Brunei, Cambodia, India, Indonesia, Laos, Malaysia, Philippines, Singapore, Sri Lanka and Thailand. Only one pupa was collected from an irrigation channel along with the rice agroecosystem situated near River Ganges at an altitude of 31 m above sea level and reared in laboratory condition.



Figures 13–21. Pupa of *Culicoides huffi* Causey, 1938. **13,** Respiratory organ; **14,** Dorsal apotome and dorsal apotomals (DA); **15,** Anterolaterals (AL); **16,** Dorsolateral cephalic sensilla (DL); **17,** Dorsal setae (D); **18,** Metathoracics and first abdominal segment; **19,** Oculars (O) and clypeal labrals setae (CL); **20,** Segment 9; **21,** Fourth abdominal segment: left – ventral view, right –dorsal view. (Scale bar: 0.05 mm).



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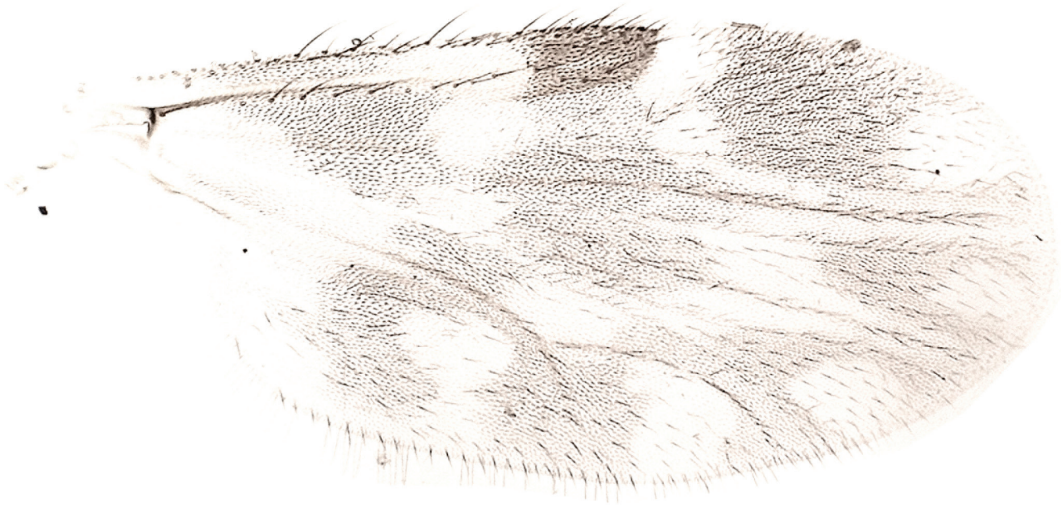

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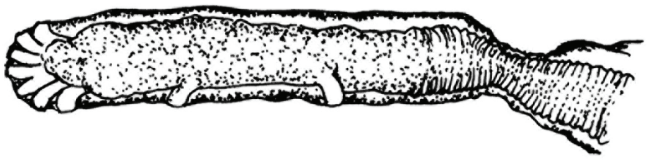
Figures 22–23. Wing of adult midges. 22. Wing of *Culicoides innoxius* Sen & Das Gupta, 1959; 23. Wing of *Culicoides huffi* Causey, 1938.

Pupal key to the species of the genus *Culicoides* Latreille from India

1. Presence of distinct annulations on respiratory organ (a).....2

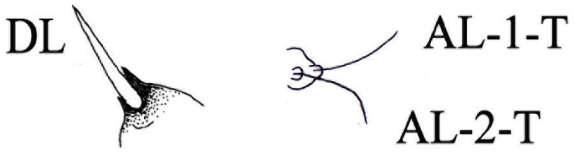
Respiratory organ of *C. huffi* Causey, 1938

- Absence of distinct annulations on respiratory organ 4



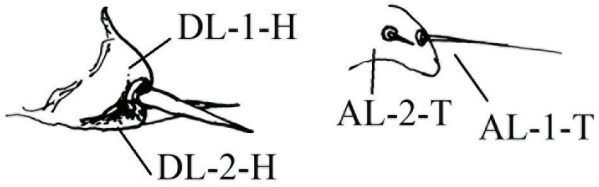
spiratory organ of *C. innoxius* Sen & Das Gupta, 1959

2. Incomplete annulations on respiratory organ; segment 9 with small patch of spinules or sometimes restricted in V shaped region (subgenus *Remmia* Glukhova).....*C. oxystoma* Kieffer, 1910
- Complete annulations on respiratory organ; segment 9 with scattered spinules or anterior band of spinules **(**subgenus *Diphaomyia* Vargas) 3
3. Presence of single stout DL; two subequal AL..... *C. pseudosimilis* Saha, Brahma & Hazra, 2017



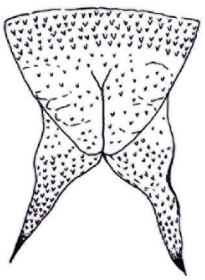
Dorsolateral cephalic sensillum and anterolaterals of *C. pseudosimilis* Saha, Brahma & Hazra, 2017

- Presence of two subequal stout DL; AL-2-T much smaller than AL-1-T ..... *C. huffi* Causey, 1938



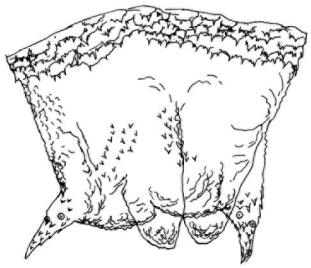
Dorsolateral cephalic sensilla and anterolaterals of *C. huffi* Causey, 1938

4. Terminal process as long as in respect of segment IX **(***shermani* species-group Wirth & Hubert).....5



Segment 9 of *C. selangorensis* Wirth & Hubert, 1989

- Length of terminal process almost ½ of segment IX **(**Subgenus *Hoffmania* Fox) 7

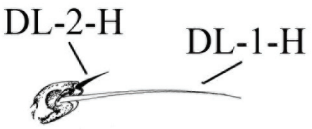


Segment 9 of *C. innoxius* Sen & Das Gupta, 1959

5. Presence of single small spur like DL ..... *C. selangorensis* Wirth & Hubert, 1989

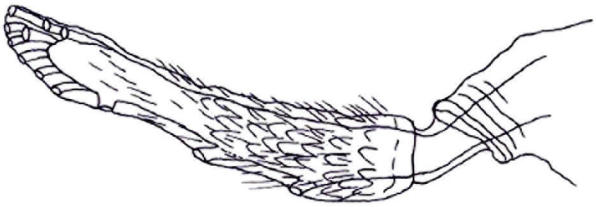
Dorsolateral cephalic sensillum of *C. selangorensis* Wirth & Hubert, 1989

- Presence of two DL, DL-1-H long and DL-2-H short.....6



Dorsolateral cephalic sensilla of *C. cornus* Chatterjee, Brahma & Hazra, 2020

6. Respiratory organ with 9 terminal pores appearing as a fan-like fashion and 3 lateral pores..... *C. dryadeus* Wirth & Hubert, 1972

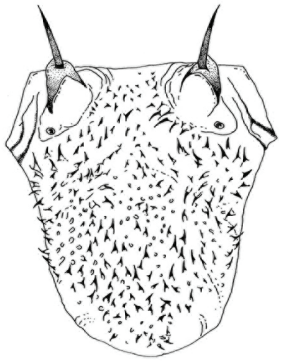


Respiratory organ of *C. dryadeus* Wirth and Hubert, 1972

- Respiratory organ with 5 terminal pores and 3 lateral pores..... *C. cornus* Chatterjee, Brahma & Hazra, 2020

Respiratory organ of *C. cornus* Chatterjee, Brahma & Hazra, 2020

7. Presence of stout, spine like DA-1-H on dorsal apotome; length of DA-1-H almost 1/3 of dosal apotome .... *C. obtusus* Chatterjee, Brahma & Hazra, 2020

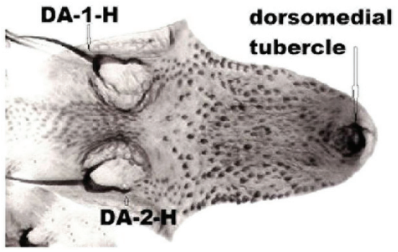


Dorsal apotome and dorsal apotomal sensilla of *C. obtusus* Chatterjee, Brahma & Hazra, 2020

- Presence of filamentous DA-1-H on dorsal apotome; length of DA-1-H almost ½ of dorsal apotome 8

Dorsal apotome and dorsal apotomal sensilla of *C. innoxius* Sen & Das Gupta, 1959

8. Presence of dorsomedial tubercle..... *C. peregrinus* Kieffer, 1910



Dorsomedial tubercle of *C. peregrinus* Kieffer, 1910
(Source: Harsha *et al.* 2017**)**

- Absence of dorsomedial tubercle ..... *C. innoxius* Sen & Das Gupta, 1959

Disclosure statement

The authors declare that there is no potential conflict of interests regarding the publication of this paper.

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