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Description of a New Species of *Lichtwardtia* Enderlein, 1912 with a New Record of *Dolichopus* Latreille, 1797 from India Based on Morphological and Molecular Evidence (Diptera: Dolichopodidae)

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ABSTRACT

One new species *Lichtwardtia bengalensis* Chowdhury, Kar, Naskar & Banerjee sp. nov. of the subfamily Dolichopodinae Latreille, 1809 is described from India and *Dolichopus howjingleei* Olejníèek, 2002 is newly reported from Darjeeling, West Bengal and Dihang-Dibang Biosphere reserve, Arunachal Pradesh, India. The new species is distinctly distinguishable from other *Lichtwardtia* Enderlein, 1912 with respect to their wing and male genital structures. *Dolichopus howjingleei* is re-described on the basis of Indian specimens. In addition, the DNA analyses of the species are provided. The updated key to the genus *Lichtwardtia* Enderlein, 1912 from India is also provided.

Keywords: Long legged flies, description, re-description, barcoding, key.

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INTRODUCTION

The Dolichopodinae Latreille, 1809 is distinguished from other subfamilies of long legged flies (Dolichopodidae) by dorsal stylus but rarely apical, post pedicel usually slightly enlarged, scape usually with dorsal hairs, head slightly depressed behind the vertex, epypharyngeal armature with serrated denticulation, with four prongs, thorax narrow, acrostichals biseriate, posterior slope of mesonotum not flattened, upper proepisternum with some hairs, M_{1+2} usually unbranched, femora with preapical setae, hypopygium usually very large (Grichanov, 2004).

The genera *Lichtwardtia* Enderlein, 1912 and *Dolichopus* Latreille, 1797 belongs to Dolichopodinae. *Lichtwardtia* is easily distinguished from the other genera by its almost angular broken, zigzag-shaped bend in vein M_{1+2}, with long hairs on arista like style (Grichanov, 2020) and extremely different male genitalia structure. *Dolichopus* is separable from other Dolichopodinae by, stylus with short hairs all over, M_{1+2} weakly or strongly bent anteriorly with or without rudimentary M_1 or M_2 as a stub vein but not in zigzag form, vein R_{4+5} and distal part of M_1 converging or subparallel (Grichanov & Brooks, 2017), cercus with generally large fingerlike marginal digitations. *Lichtwardtia* is highly diverse in the Oriental region as well as Afrotropical region (Tang, Yang, & Grootaert, 2018) with 41 species known from the World (Grichanov, 2023) but *Dolichopus* is found in the Holarctic and Oriental region with about 650 species worldwide (Grichanov, 2016). From India's perspective only four species of *Lichtwardtia* have been recorded: *L. hirsutiseta* (De Meijere, 1916); *L. dentalis* Zhang, Masunaga & Yang, 2009; *L. ziczac* (Wiedemann, 1824) and *L. singaporensis* Grootaert & Tang, 2009 and only three species of *Dolichopus* have been reported: *D. exsul* Aldrich, 1922, *D. indicus* Parent,1934 and *D. saphirus* Becker,1922 so far.

In this paper, one new species is added to the genus *Lichtwardtia* and one new record is added to the genus *Dolichopus* from India. DNA sequencing has been carried out for both the species. The phylogenetic analysis of the *Dolichopus howjingleei* Olejníèek, 2002 is provided for the first time in this paper with the re-description. In addition, a key to the species of *Lichtwardtia* from India has been provided.

MATERIALS AND METHODS

The type specimens of *L. bengalensis* sp. nov. were collected by net sweeping technique and yellow pan trap from Bethuadahari Wildlife Sanctuary, West Bengal and specimens of *D. howjingleei* were collected by net sweeping technique and yellow pan trap from Darjeeling, West Bengal and Dihang-Dibang biosphere reserve, Arunachal Pradesh, India. All the collected specimens were preserved in 70% ethanol. Then the specimens were pinned, stretched and examined further under the Leica EZ4 HD Stereo zoom microscope and the photographs were taken using the Leica M205A Stereo zoom microscope.

Morphological terminologies were used following Cumming & Wood, 2017 and Grichanov & Brooks, 2017. For the taxonomic study and identification standard protocol of Olejníček, 2002; Grichanov, 2004; Zhang, Masunaga, & Yang, 2009; Grichanov, 2016; Tang, et al., 2018; Grichanov, 2019, 2020, 2023 were used.

The genomic DNA of specimens was extracted using the QiaAmp DNA extraction kit (Qiagen) by the manufacturer's instructions to barcode the new species and the new record. A Qubit fluorometer (Life Technologies, USA) was utilized to measure the quantity of DNA (Kar, et al*.,* 2024). Amplification of about 700 base pairs from the 5' end of the mitochondrial COI gene was performed utilizing 4µl of genomic DNA employing primers forward LCO-1490 (F) (GGT CAA CAA ATC ATA AAG ATA TTG G) and reverse HCO-2198 (R) (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer, et al*.,* 1994). The PCR amplified products were purified with the QIAquick Gel Extraction Kit (Qiagen, Germany) following the manufacturer's instructions. About 15 ng of the purified PCR products were employed for sequencing. Sequencing was performed using approximately 15 ng of the purified PCR product, according to Banerjee, et al., 2015 and Ghosh, et al*.,* 2022. The resulting forward and reverse COI sequences were examined using the MEGAX software for sequence analysis, and consensus sequences were obtained after verifying and editing the stop, insertion, and deletion codons (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). All sequences were matched to identical reported sequences in the NCBI database utilizing the BLAST (Basic Local Alignment Search Tool) (https://blast.ncbi.nlm.nih.gov) algorithm (Chakraborty, et al*.,* 2019; Samerjai, et al., 2019). Using the similarity search result performed through the NCBI BLASTn and the BOLD (Barcode of Life Data system) search IDs our sequences were uploaded to the GenBank library, and unique accession numbers were issued to each one. Tree-based identification approach based on the Neighbour-Joining (NJ) algorithm was utilized to construct two trees with 1000 bootstrap replicates in MEGAX software to further distinguish the specimens under discussion via its DNA barcode (Kar, et al., 2024). Figtree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used for editing the phylogenetic trees. *Scathophaga stercoraria* Linnaeus, 1758, a member of the subfamily Scathophagidae, was chosen as the outgroup (KM630704) following the findings of Bernasconi, Pollet, & Ward, 2007.

The following abbreviations are used: **dc**- dorsocentral bristles; **sc**- scutellar bristles; **acr**- acrostichal bristles; **R_1**- first radial vein; **M_1**- first median vein; **dm-m** - discal medial-medial cross vein; **LI**- fore legs; **LII**- mid legs; **LIII**- hind legs; **Cer**- Cercus; **Ph**- phallus; **Hyp**- hypandrium; **Den**- denticles.

RESULTS

Family Dolichopodidae

Subfamily Dolichopodinae

Lichtwardtia Enderlein, 1912

Lichtwardtia Enderlein, 1912: 406. Type species: *Lichtwardtia formosana* Enderlein, 1912, original designation.

Vaalimyia Curran, 1926: 398. Type species: *Vaalimyia violacea* Curran, 1926 [= *Dolichopus angularis* Macquart, 1842], original designation.

Diagnosis. Head including vertex metallic green, the latter flat, ocellar tubercle distinct, scape bears short dorsal bristles, pedicel shorter, arista-like stylus two segmented bearing feather like long hairs. Eyes dichoptic. Scutellum with two pairs of scutellar bristles, basal pair weak. Hind femur thicker,tarsomere I bearing one strong dorsal bristle and shorter than tarsomere II. Vein M_{1+2} likely to be broken into anteroproximal and posterodistal stump veins, vein R_{4+5} and distal part of M_1 virtually parallel.

Its closest ally is *Dolichopus* Latreille, 1797 but readily distinguished by vein R_{4+5} and distal part of M_1 converging or subparallel (Grichanov & Brooks 2017).

Lichtwardtia bengalensis Chowdhury, Kar, Naskar & Banerjee sp. nov. (Fig. 1-5)

Type Material. HOLOTYPE: 1♂, INDIA, West Bengal, Nadia, Bethuadahari Wildlife Sanctuary, 23.5978°N, 88.3927°E, 31 m, 8.xii.2021, K. Rajmohana.

PARATYPE: 2♂♂, 3♀♀, Same as the holotype.

ZOOBANK REGISTRATION ID: urn:lsid:zoobank.org:pub:2A7F93D6-3A15-443D-81BB-027C818314D6

Holotype and paratypes are deposited in the National Zoological Collection (NZC) of Zoological Survey of India, Kolkata.

Etymology. The species name *bengalensis* (from the state of West Bengal) refers to the type locality of the new species.

Diagnosis. **Male**. Antennal scape and pedicel yellowish, postpedicel dark, arista-like style feathery; wing clear, faintly brownish tinged, no thickening of costa where R_1 joins the costa; fore and hind coxae entirely yellow, mid coxa with a rectangular brown spot anteriorly but posteriorly yellow; cercus pale, margin dark, distinctly digitated and bearing five strong blunt tipped marginal bristles, three strong bristles present along the lower margin of the dark band of cercus; hypandrium simple, without any projection, phallus pointed at the apex and ventrally with at least twenty black denticles.

Description. **Male**. Body length- 2.8 mm (Approx.), Wing length- 3.0 mm (Approx.).

Head. (Fig. 1) Metallic violet and green with thick grey pollinosity; face slightly raised, frons violet, grey pollinose, face violet with grey pollinosity, almost parallel-sided; hairs and bristles on head black but lower post-ocular weak and pale; antennal scape and pedicel yellowish, post-pedicel dark brown, almost triangular, apex blunt, arista-like stylus black, nearly as long as head width, feather like with long pubescence; proboscis light brown, bare; palpus yellowish with a short black hairs.

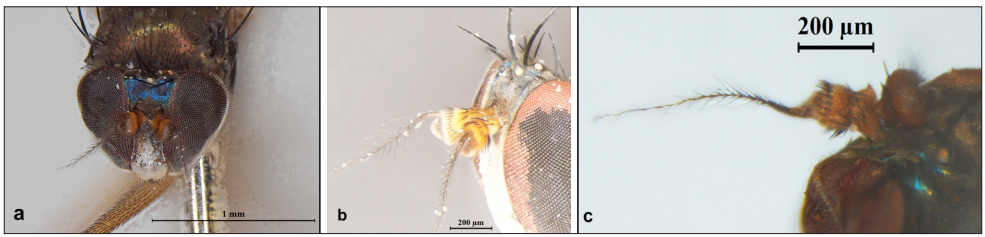

Figure 1. *L. bengalensis*. sp. nov. ♂. a) Head (Frontal view); b) Head (Lateral view); c) Antennae.

Thorax. (Fig. 2) Dark green with grey pollinosity, bristles and hairs on thorax black, five pairs of strong dc, ten pairs of acr; scutellum with two strong bristles and 2 short lateral hairs.

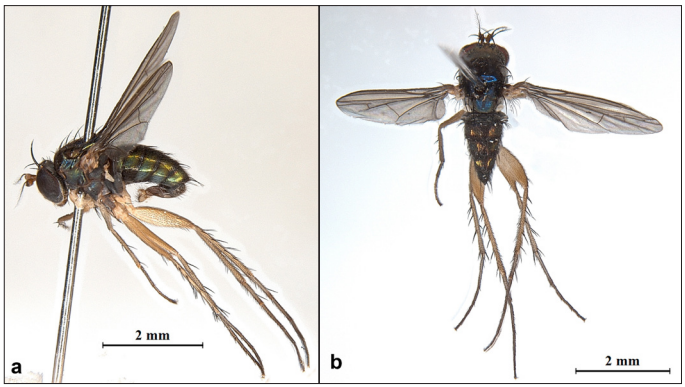

Figure 2. *L. bengalensis.* sp. nov., ♂. a) Lateral habitus (Paratype); b) Dorsal habitus (Holotype).

Legs. (Fig. 3) Brownish yellow but mid coxa with a dark brown rectangular spot anteriorly; fore coxa with three subapical bristles dorsally, mid coxa with a row of bristles anterodorsally of which one strong subapically, hind coxa with one bristle subapically at posterodorsal side; fore femur bare, devoid of bristles, mid femur with one anterodorsal bristle present subapically and hind femur with one anterodorsal bristle present subapically; fore tibia with a row of anterodorsal bristles and a posteroventral bristle at the middle, mid tibia with a row of three bristles anteriorly and one bristle anterodorsally, hind tibia with two rows of dorsal bristles and two ventral bristles; tarsal segments with no bristles.

Relative lengths of the femur, tibia and five tarsomeres of each leg of the type specimen are as follows:

LI- 1.070/0.891/0.533/0.206/0.164/0.115/0.114

LII- 1.259/1.142/0.686/0.379/0.209/0.195/0.166

LIII- 1.198/1.260/0.567/0.582/0.402/0.259/0.235.

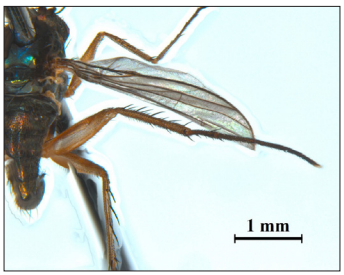

Figure 3. *L. bengalensis*. sp. nov. ♂. Hind leg.

Wings. (Fig. 4) Nearly clear, faintly brownish tinged with no thickening of costa where vein R_1 joins the costa, M_1 with one short sub vein, cross vein dm-m straight; lower calypter yellowish with black hairs at the periphery; halteres yellow.

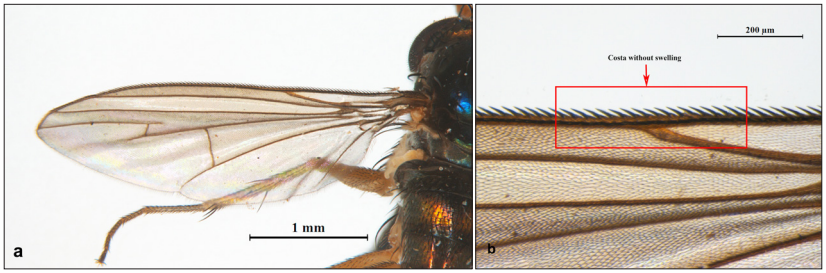

Figure 4. *L. bengalensis.* sp. nov., ♂. a) Wing; b) Wing with simple costal vein.

Abdomen. Metallic green with whitish pollinose spots, black hairs and bristles present on abdomen.

Male genitalia. (Fig. 5) Epandrium 1.5 times longer than wide, swollen basally, tapering distally, concave ventrally, slightly projected dorsally; hypandrium basoventral relatively broad, reaching about more than half of the epandrium, without teeth; phallus thin and long with at least 20 tinny black denticles on the half of the ventral part; surstylus bilobed; cercus with five blunt tipped bristles present on digitation of apical half and three strong bristles present along the lower margin of the dark band of cercus ; postgonite long, half as long as cercus, forked at distal half with curved and pointed apex.

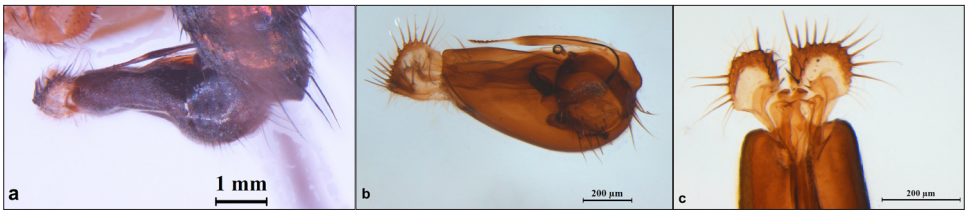

Figure 5. *L. bengalensis.* sp. nov. ♂. a) Lateral view of genitalia (Before dissection); b) Lateral view of genitalia (After dissection and macerated); c) Ventral view of genitalia.

Barcoding. (Fig. 6) One male (Accession no: PP582340) and one female paratype (Accession no: PP587643) of our species were subjected to DNA barcoding; both specimens showed 100% similarity, indicating that the sexes are appropriately related. There were no full matches in the databases. '*Lichtwardtia nodulata*' (Singapore) is the uploaded sequence that is closest and most similar to our sequences, with 97.61% similarity. *Sybistroma* Meigen, *Hercostomus* Loew, *Dolichopus* Latreille, *Pelastoneurus* Loew, and *Paraclius* Loew genera differ distinctly from our species in the phylogenetic tree that was constructed (Fig. 6). Both our specimens are clustered with those of the genus *Lichtwardtia*, but they also clearly differ from those of the existing *Lichtwardtia* species available in NCBI and BOLD, indicating that it is a new species.

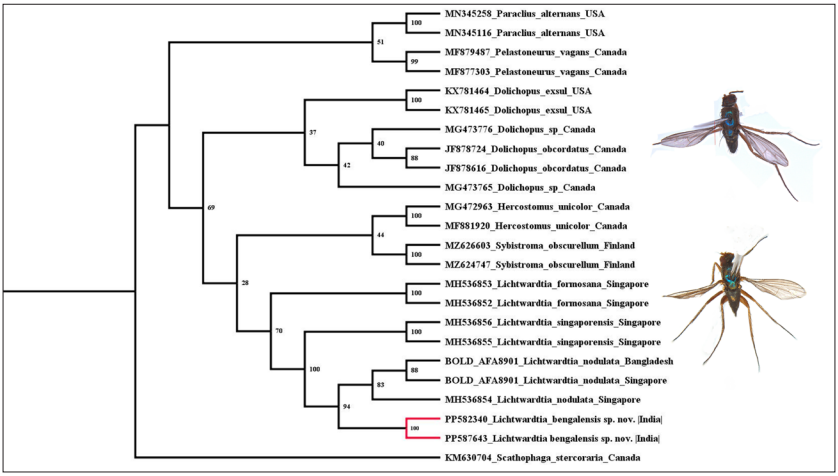

Figure 6. *L. bengalensis*. sp. nov. ♂. The phylogenetic tree displays our specimen grouped with *Lichtwardtia* individuals, while *Sybistroma, Hercostomus, Dolichopus, Pelastoneurus* and *Paraclius* exhibit clear differentiation from our specimen. Even though our specimen is grouped with those of the genus *Lichtwardtia*, there is clear differentiation of species within the group. The numbers beside the branches indicates the bootstrap values. Red coloured branch denote our new species within the phylogenetic tree.

Distribution. India [West Bengal].

Genus *Dolichopus* Latreille, 1797

Dolichopus Latreille, 1797: 159. Type species: *Musca ungulata* Linnaeus, 1758 (design. Latreille, 1810), original designation.

Diagnosis. Head with vertex metallic green, antennal scape and pedicel yellowish, post pedicel dark brown, stylus with short hairs all over, wing with dark brown veins, M_{1+2} weakly or strongly bent anteriorly with or without rudimentary M_2 as a stub vein, vein R_{4+5} and distal part of M_1 converging or subparallel (Grichanov & Brooks, 2017), cercus with generally large fingerlike marginal digitations.

Dolichopus howjingleei Olejníèek, 2002 (Fig. 7-10)

Materials examined. 2♂, INDIA: West Bengal: Darjeeling, Upper Sittong, 26.9175°N, 88.3751°E, 122 m, 23.ii.2023, S. Singh; 1♂, INDIA, West Bengal, Darjeeling, Upper Sittong, 26.9175°N, 88.3751°E, 122 m, 23.ii.2023, S. Singh; 5♂, INDIA, Arunachal Pradesh, Dihang-Dibang Biosphere Reserve Upper Siang, Yingkiong, 28.6325°N, 95.0275°E, 385 m, 28.ii.2024, D. Mondal.

Identified specimens are deposited in the National Zoological Collection (NZC) of Zoological Survey of India.

Diagnosis. Male. Antennal scape and pedicel yellowish, post pedicel dark brown, stylus black with short hairs all over, wing clear with dark brown veins and a distinct swelling at costa where R_1 meets the costa, M_1 with very short stub vein, cercus with 5 digitations at dorsoapical half, each digitation with 2-3 strong bristles along the length, phallus with big prominent teeth dorsally just before the phallus comes out from hypandrium and two rows of dorsal denticles just before the dorsal teeth.

Re-description. Male. Body length- 6.87 mm (Approx.), Wing length- 4.202 mm (Approx.).

Head. (Fig. 7) Almost spherical, vertex not excavated; forehead metallic green with light grey pollinosity, vertex with a pair of strong bristles, postvertical bristles small, proclinate; face narrower at the middle with thick silver pollinosity; upper postocular bristles black but lowers pale. Antennae (Fig. 7c) dark brown mainly, scape and pedicle yellow but dorsally brown, scape with dorsal black hairs, post-pedicle brownish with yellow base, rounded apically, dorsal arista-like stylus black with short hairs; proboscis and palpi dark yellow, latter with black hairs; eye with short pubescence.

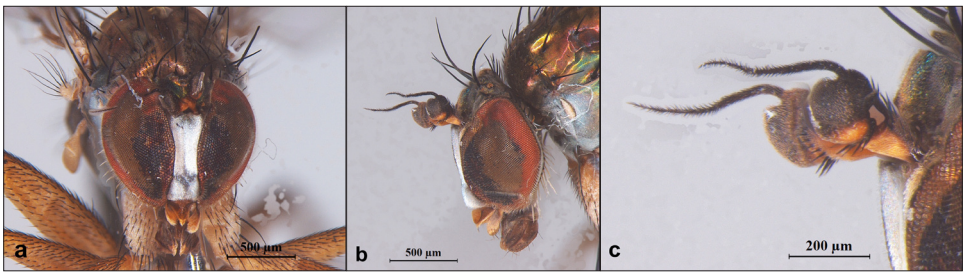

Figure 7. *D. howjinglei*. ♂. a) Head (Frontal view); b) Head (Lateral view); c) Antennae.

Thorax. (Fig. 8a) Metallic green with light grey pollinosity. 6 pairs of strong dc, ac black and short in two rows, scutellum with one pair of strong median scutellars and one pair of hair like lateral scutellars.

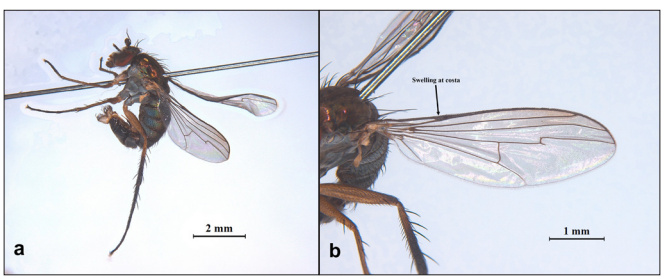

Figure 8. *D. howjinglei*. ♂. a) Lateral Habitus; b) Wing.

Abdomen. Metallic greenish-purple, silver pollinosity at the sides of the abdomen.

Legs. Mainly yellow, fore coxa yellow with grey spot at base and with 5 black bristles apically; femur and tibia yellow, tibia with 2 dorsal, 3 posterodorsal and 1 anteroventral bristles; basitarsus yellow but apex black, other tarsomeres black. Mid coxa with a row of black bristles dorsally; femur and tibia yellow, femur with 1 strong anterior preapical, tibia with 2 rows of 3-4 dorsal and 1 anteroventral bristles; tarsomeres black from the apex of the basitarsus. Hind coxa blackish bare, femur yellow, blackish dorsoapically with one anterior preapical seta; tibia blackish on apical half with 4 anterodorsal, 1 dorsal, 4 posterodorsal bristles and 1 anteroventral bristle at 4/5th; tarsomeres black, basitarsus with 2 anterodorsals.

Relative lengths of the femur, tibia and five tarsomeres of each leg of the type specimen are as follows:

LI- 0.925/1.072/0.329/0.180/0.165/0.189/0.152

LII- 0.540/1.194/0.749/0.449/0.301/0.236/0.203

LIII- 1.137/1.745/1.282/0.465/0.468/0.332/0.262.

Wing (Fig. 8b). Hyaline, costa distinctly swollen where R_1 meets costa; M_1 with very short stub vein, dm-m slightly curved posteriorly; halters yellow; calypter with long black hairs and some pale fine hairs.

Male genitalia (Fig. 9). Epandrium dark brown, about 2 times longer than high; hypandrium dark brown, basoventral, reaching about 2/3rd of the epandrium without any projection. Phallus, surstylus and cercus very pale, border of the cercus brown; surstylus with 4 strong but short setae at apex; phallus thin and long, reaching nearly to apex of epandrium with big prominent teeth dorsally just before the phallus comes out from hypandrium, phallus also has two rows of dorsal denticles at the edges of phallus just before the dorsal teeth; cercus almost rounded, narrower at base with 5 digitations at apical half, each digitation with 2-3 strong bristles along the length, cercus also with 2 strong bristles beside the digitations and fringes of black hairs on the apical position.

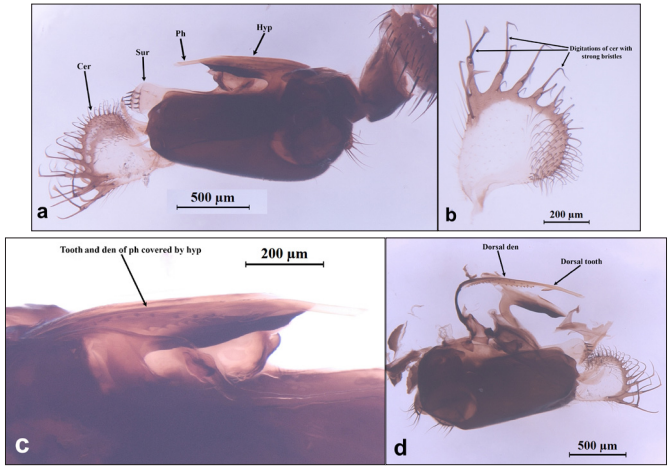

Figure 9. *D. howjinglei*. ♂. a) Lateral view of genitalia; b) Cercus; c) Phallus and hypandrium; d) Exposed phallus with ventral tooth and denticles.

Distribution. India [Arunachal Pradesh, West Bengal]

Global distribution. Oriental: Northern Taiwan.

Barcoding (Fig. 10). The sequences were instantly matched to the barcodes of every species in the NCBI database using the best-match technique. Two males (Accession numbers: PQ621871 and PQ621872) of our species were subjected to DNA barcoding. There were no full matches in the databases. '*Dolichopus bigeniculatus*' (FJ808380, Singapore) is the uploaded sequence closest match to our sequences, with 95% similarity. Other *Dolichopus* species have been distinctly differentiated from *Dolichopus howjingleei* in the constructed phylogenetic tree. Our species differ distinctly from other genera like *Licthtwardtia*, *Thinophilus*, and *Hydrophorus*, as seen

in the Neighbour-Joining (NJ) phylogenetic tree. Both our specimens are clustered with those of the genus *Dolichopus*. Still, they differ from the existing *Dolichopus* species available in NCBI, indicating that this species is barcoded for the first time and is a new record from India.

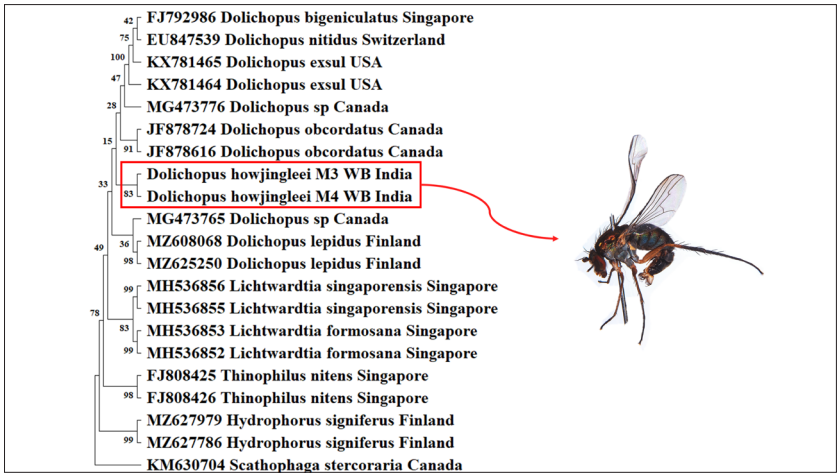

Figure 10. The phylogenetic tree displays our specimen grouped with *Dolichopus* individuals. The number beside the branches indicates the bootstrap values. Red coloured branch denotes our new record of species (*D. howjinglei*) from India within the phylogenetic tree.

Remarks. Some specimens of the Indian *D. howjingleei* are varying in their wing venation. Some have small stub vein in M_1 but others are lacking this character.

Key to males of Indian *Lichtwardtia* (after Grichanov, 2023)

1. Costa with a swelling on or before R_1 joining the costa……2

- Costa without swelling on or before R_1 joining the costa……..……3

2. Costa with swelling far before the R_1 joining the costa…….. …… *L. hirsutiseta* (De Meijere, 1916).

- Costa with swelling at the point where the R_1 joining the costa…… ……..…… *L. dentalis* Zhang, Masunaga & Yang, 2009.

3. Phallus thin with 6 dorsolateral teeth…… *L. ziczac* (Wiedemann, 1824).

- Phallus thin but without any dorsolateral teeth……..……4

4. Phallus with five big clear and sharp black denticles ventrally, with one blunt denticle hidden in hypandrium……..*L. singaporensis* Grootaert & Tang, 2018.

- Phallus with at least twenty black denticles covering the ventral half, no denticles hidden under the hypandrium; Body length 2.8 mm..... ....*L. bengalensis* Chowdhury, Kar, Naskar & Banerjee sp. nov.

DISCUSSION

In this paper, one new species is added to the genus *Lichtwardtia* which takes the number of *Lichtwardtia* species from India to five and one new record is added to the genus *Dolichopus* which takes the number of *Dolichopus* species from India to **four**. The genus *Lichtwardtia* is reported for the first time from the state of West Bengal. The genus *Dolichopus* is reported from the first time from the state of West Bengal and Arunachal Pradesh.

Lichtwardtia bengalensis sp. nov. is closely resembled to *L. semakau* having a simple costal vein without any distinct swelling where R_1 meets the costa (a few specimens have slightly swollen costa which may be negligible) but differ from it in the cercus having 5 strong blunt tipped marginal bristles and three strong bristles present along the lower margin of the dark band of cercus (Fig. 5b, c). The new species is closely related to *Lichtwardtia cambodiensis* Tang & Grootaert, 2018 but differ from its costal vein and structure of male genitalia. *L. cambodiensis* has distinct swollen costal vein but the new species has simple costal vein. On the other hand, *L. cambodiensis* has weakly digitated cercus with thin marginal bristles and phallus has two rows of denticles but the new species has strongly digitated cercus with strong marginal bristles and phallus has not double rows of denticles. The new species is also completely distinct from the other Indian *Lichtwardtia. L. hirsutiseta* and *L. dentalis* differ from *L. bengalensis* sp. nov. by having a swollen costa. Whereas *L. ziczac* and *L. singaporensis* differ from it by the structures of their male genitalia.

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DISCLOSURE STATEMENT

There is no potential conflict of interest reported by the authors.

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