

ENTOMOLOGY

Addition of species to the hematophagous biting midges of *Forcipomyia* Meigen (Diptera: Ceratopogonidae) from West Bengal, India

Gouri Sankar Pal, Niladri Hazra

Entomology Research Unit, Department of Zoology, University of Burdwan, West Bengal, India

Correspondence: Niladri Hazra, Entomology Research Unit, Department of Zoology, The University of Burdwan, Burdwan, West Bengal, 713104, India
E-mail: nhazra@zoo.buruniv.ac.in

Key words: Biting midges; hematophagous; *Forcipomyia*; *Lasiohelea*; DNA barcoding.

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Abstract

Two new species and a new record of blood sucking biting midges of the subgenus *Lasiohelea* Kieffer, 1921 of the genus *Forcipomyia* Meigen, 1818 are described after the morphological and molecular data. The new species, *Forcipomyia (Lasiohelea) peditata* and *F. (L.) falcata* were fetched from the Sub-Himalayan region, and *F. (L.) parvitas* (Liu and Yu, 1996) from both the Gangetic plain and western plateau regions of West Bengal. The DNA barcoding of Mitochondrial COX I gene has also been used as molecular evidence.

Introduction

The genus *Forcipomyia* Meigen, 1818 is the one of the hyper diverse and as well as well-studied groups. The Members of the genus are present in almost all zoogeographical regions except Antarctica, but many more species still wait for proper identifications. There are 1142 extant worldwide species under 36 different subgenera (Borkent & Dominiak, 2020) contributing only 72 species from India with the species of the subgenus *Lasiohelea* Kieffer, 1921 are reported as hematophagous of vertebrates and only one from India (Borkent and Dominiak, 2020; Glukhova, 1989; Paul *et al.*, 2014; Maheshwari, 2003; Saha *et al.*, 2009). The species of the subgenus *Lasiohelea* are nasty biter to human in daytime, show allergic reaction as well (Chen *et al.*, 2005; Yeh and Chuang, 1996) and are possible vectors of livestock diseases (Lee *et al.*, 1963). Since India is one of the richest megadiverse countries, many more species of this subgenus are expected to come to light of hematophagy nature.

Materials and methods

Specimens were collected by using 8W UV light trap and open light trap with (8W) and normal filament blub (60 W). The specimens were sorted under Stereo zoom trinocular microscope (Olympus Model, SZX16, Japan), treated with mixture of Phenol-Alcohol (50:50) and slide mounted after Wirth and Marston (1968). Morphology and terminology of the adult follow Borkent *et al.* (2009) with measurements in micrometer (µm) except length and width of wing in millimeters (mm). The illustrations are drawn in a compound microscope (Wild Leitz GMBH, Portugal)

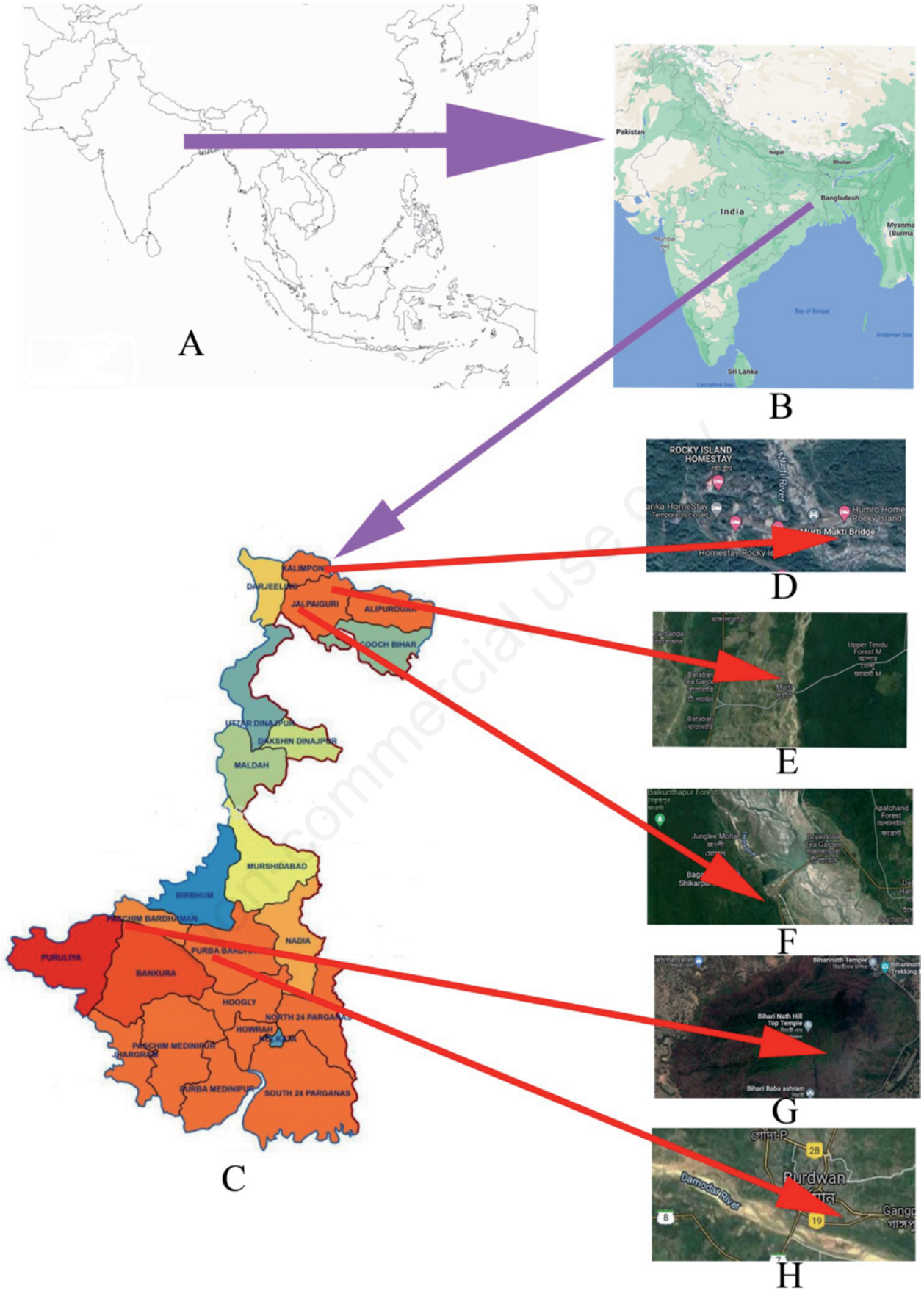


Figure 1. A) Map of South-East Asia; B) Map of India; C) Map of West Bengal. D-H) collection sites: D, Rocky Island (Samsing); E, Uttarakhand (Chalsa); F, Gajoldoba; G, Biharinath Hills; H, Burdwan.

in combination with a Camera Lucida. The photographs were prepared with a fluorescence microscope in bright field (Lecica DM 1000) and trinocular compound microscope (Wild Leitz GMBH, Portugal). The location of insect collecting sites depicted via Google Map (Figure 1D-H). Types currently retained at Entomological collection of Department of Zoology, University of Burdwan (India) will be later deposited at the National Zoological collections, Zoological Survey of India, Kolkata (NZCI).

Extraction of DNA was done by non-destructive method of Harrup (2018) for molecular identification which were amplified with the help of universal primers LCO1490 and HCO2198 of Cytochrome oxidase I (COX I) (Folmer *et al.*, 1994). The samples were examined by gel electrophoresis technique and outsourced for Sanger's sequencing ultimately got successfully uploaded in GenBank, NCBI.

Abbreviations with their full forms. AR – Antennal ratio, PR – Palpal ratio, TR – Tarsal ratio, WL – Wing length, WW – Wing width, CR – Costal ratio, HTC – Hind Tibial Comb.

Results

Forcipomyia (Lasiohelea) falcata sp. nov. (Figure 2A-N)

lsid:zoobank.org:act:

04D841DD-FB3E-4ADC-9E47-D52BAF276F42

GenBank Accession Number: OM632705

TYPE MATERIAL. Holotype ♂, India, West Bengal, Jalpaiguri, Gajoldoba (26°74'78.57"N, 88°57'45.46"E), 14.XI.2021, Col. G.S. Pal. Paratypes: 8♂♂ and 3♀♀, data same as holotype.

DIAGNOSIS. Male. Palpus with 8-10 protruding capitate sensilla on sensory pit of palpomere III; apical portion of aedeagus crescent like. Female with clypeal setae 14; cibarium with 28-30 spines; mandible with 15-16 teeth, and large and rounded spermatheca
ETYMOLOGY. The specific name '*falcata*' derives of from the crescent shaped aedeagal plates.

Male (n=10)

Head. Dark brown. Eyes bare, contiguous. Antenna (Figure 2A) brown, flagellomeres X-XIII longer; length ratio of flagellomeres (I-XII): 20-22 (21): 12: 12-13 (12.5): 12-13 (12.5): 12-13 (12.5): 12-13 (12.5): 12-13 (13.5): 13-14 (13.5): 26-28 (27): 31-33 (32): 29-31 (30): 30-33 (31.5). AR 1.20-1.28 (1.24). Palpus (Figure 2B) dark brown; length ratio of palpomeres (I-V): 7-8 (7.5): 10-12 (11): 13-14 (13.5): 9-10 (9.5): 12-13 (12.5); PR 1.85-2.00 (1.92). Wing. WL 0.75-0.82 (0.785), WW 0.25-0.28 (0.27), CR 0.53-0.57 (0.55). Wing covered with microtrichia, 1st radial cell obliterated, 2nd radial cell longer opening narrowly (Figure 2C).

Leg. Pale brown without any banding pattern, Length ratio of tarsal segments as TR_I 1.80-1.84 (1.82), TR_{II} 1.51-1.57 (1.54), TR_{III} 1.54-1.60 (1.57). HTC with 7 spines and a spur (Figure 2D). Abdomen. Dark brown.

Genitalia (Figure 2E-G). Sternite IX length 67-75 (71) × width 112-122 (117), almost 1.6 times wider than length, tergite IX as wide as sternite IX, length 87-93 (90) × width 112-125 (118.5), almost 1.3 times wider than length with well-developed apicolateral process bearing tuft of setae. Gonocoxite dark brown 80-90 (85) × 37-43 (40) with highly sclerotised basal part, inner margin with short stout setae. Gonostylus 80-85 (82.5) as long as gonocoxite, almost straight. Parameres fused forming bell-shaped structure. Aedeagus bipartite, with two triangular sclerotized plates, basal arms of aedeagus highly sclerotized, upwardly directed from base and horizontally projected, base wider gradually tapered towards

apex subapical part each with small projection and apex with slightly curved appearing crescent in shape (Figure 2G).

Female (n=3)

Similar to male in overall structure and general body color with some sex differences. Head. Dark brown. Eye bare and contiguous. Antenna (Figure 2H) with darker flagellomeres IX-XIII longer, terminal one bearing nipple like apical papilla; length ratio of flagellomeres (I-XIII): 13-14 (13.5): 7-8 (7.5): 7-8 (7.5): 7-8 (7.5): 8: 8: 8: 10: 21-23 (22): 24-26 (25): 24-25 (24.5): 24-25 (24.5): 30-32 (31); AR 1.75-1.87 (1.81). Cibarium (Figure 2I) with 28-30 "comb teeth" like spines. Palpus (Figure 2J) brown, Palpomere III largest, barrel shaped with sensory pit containing protruding capitate sensilla; length ratio of palpomeres (I-V): 6: 9-10 (9.5): 12-13 (12.5): 6-7 (6.5): 10; PR 1.44-1.50 (1.47).

Wing. WL 0.67, WW 0.29, CR 0.59. Wing more or less covered with microtrichia; 1st radial cell obliterated and 2nd narrowly open (Figure 2K).

Leg. Brown without banding pattern. Length ratio of tarsal segments as TR_I 2.11, TR_{II} 1.68, TR_{III} 2.27. HTC with 7 spines and a spur (Figure 2L).

Abdomen (Figure 2M,N). Brown in color. Spermatheca (Figure 2M) one, large and round measuring about 50 × 52.5 μm, without any neck but with a large opening.

DISCUSSION. The proposed new species is similar to *Forcipomyia (L.) borneoensis* Yu and Wirth, 1997 in bare eyes differs from the latter in lower PR, WL and WW. Further in *F. (L.) borneoensis* apical part of aedeagus is pennant like while it is being crescent like in the new species. The bare eyes, absence of spines in cibarium, structure of aedeagus in male, and spermatheca of female of the new species shows similarity with those of *Forcipomyia (L.) paenedentula* Debenham, 1983 but presence of 28-30 spines of cibarium, capitate sensilla in palpomere III, lower WL, and other structures of female are different from each other. Similarly, the species, *F. (L.) townsvillensis* (Taylor, 1918) of Australia bears affinities with the present new species in bare eyes, shape of the aedeagus and size of spermatheca but spines in cibarium, high PR and distinctively larger wing suggest its proposal of the new species.

DISTRIBUTION: India, West Bengal.

Forcipomyia (Lasiohelea) peditata sp. nov. (Figure 3A-N)

lsid:zoobank.org:act:

FB478223-A2C6-4405-BD75-FDEBDB5DAE63

GenBank Accession Number: OM632704

TYPE MATERIAL. Holotype ♂, India, West Bengal, Jalpaiguri, Uttar Dhupjhora (26°84'16.16"N, 88°82'77.05"E), 06.IX.2021, Col. G.S. Pal.

PARATYPES. 4♂♂ and 3♀♀, data same as holotype. 2♂♂ and 1♀, India, West Bengal, Kalimpong, Samsing, Rocky Island (27°00'82.3"N, 88°80'29.4"E), 08.XI.2021, Col. G. S. Pal.

DIAGNOSIS. Male. Aedeagus with shoe like apex, with arms little directed upward, and the base of subapical plate encircled by a "V" shaped sclerotized structure.

Female. Cibarium with 6 cibarial spines arranged in a row, mandible with 19-21 teeth, palpal segment III with 15-18 protruding capitate sensilla with irregular sensory pits.

ETYMOLOGY. The specific name '*peditata*' is derived from 'shoe-like' apex of the aedeagus.

Male (n=7)

Head. Dark brown. Eyes bare, contiguous. Antenna (Figure 3A) brown to dark brown, flagellomeres I, X-XIII longer, ultimate flagellomere with apical papilla; length ratios of flagellomeres (I-XIII): 22-23 (22.5): 13-14 (13.5): 13-14 (13.5): 13-14 (13.5): 12-

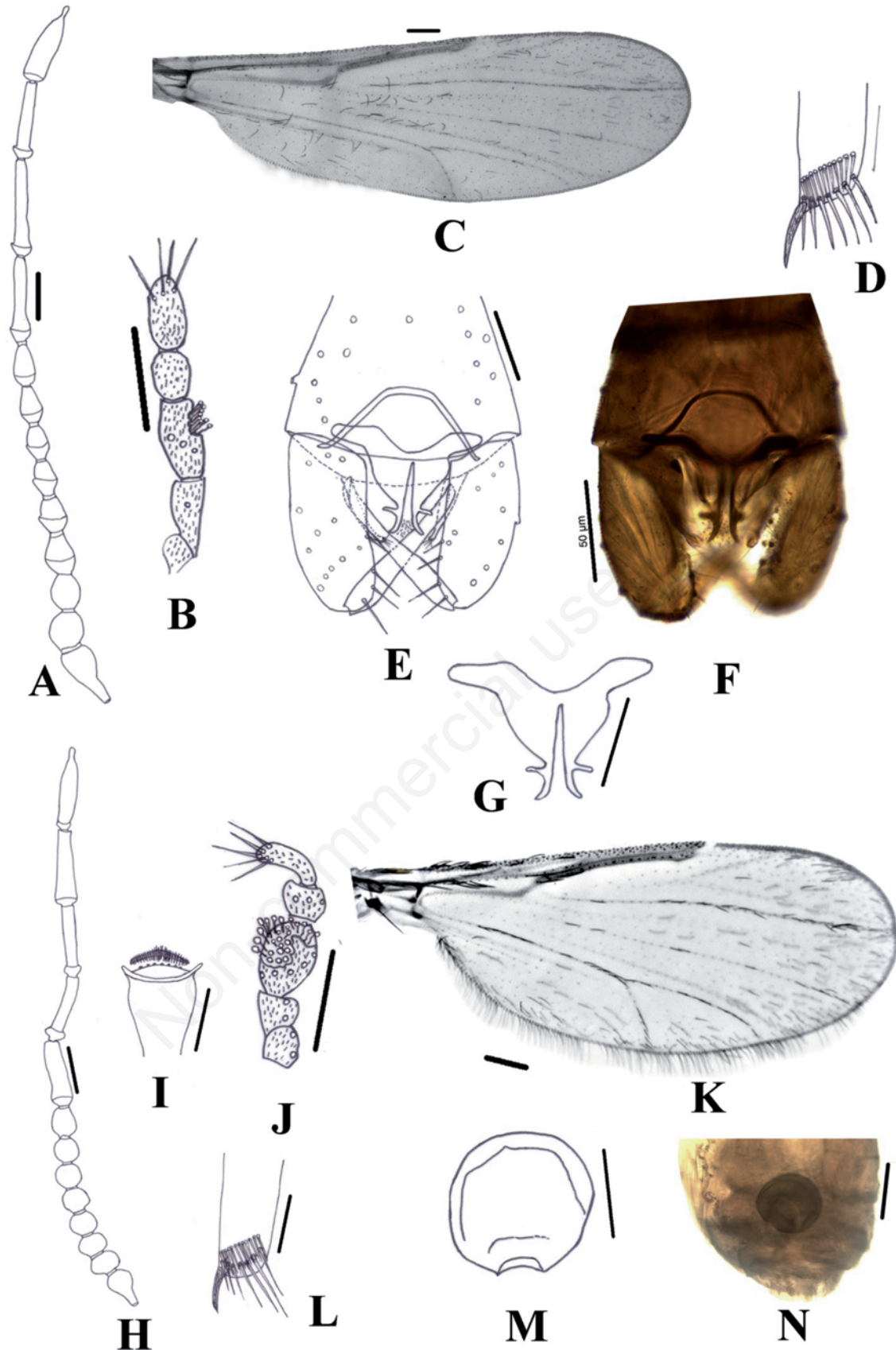


Figure 2. *Forcipomyia (Lasiohelea) falcata* sp. nov. A-G) Male: A, Flagellum; B, Maxillary palpus; C, Photograph of wing; D, Hind tibial comb (HTC); E, Illustration of male genitalia; F, Photograph of male genitalia; G, Aedeagus. H-N) Female: H, Flagellum; I, Cibarium; J, Maxillary palpus; K, Photograph of wing; L, Hind tibial comb (HTC); M, Illustration of spermatheca; N Photograph of spermatheca (Scale bar 0.05 mm).

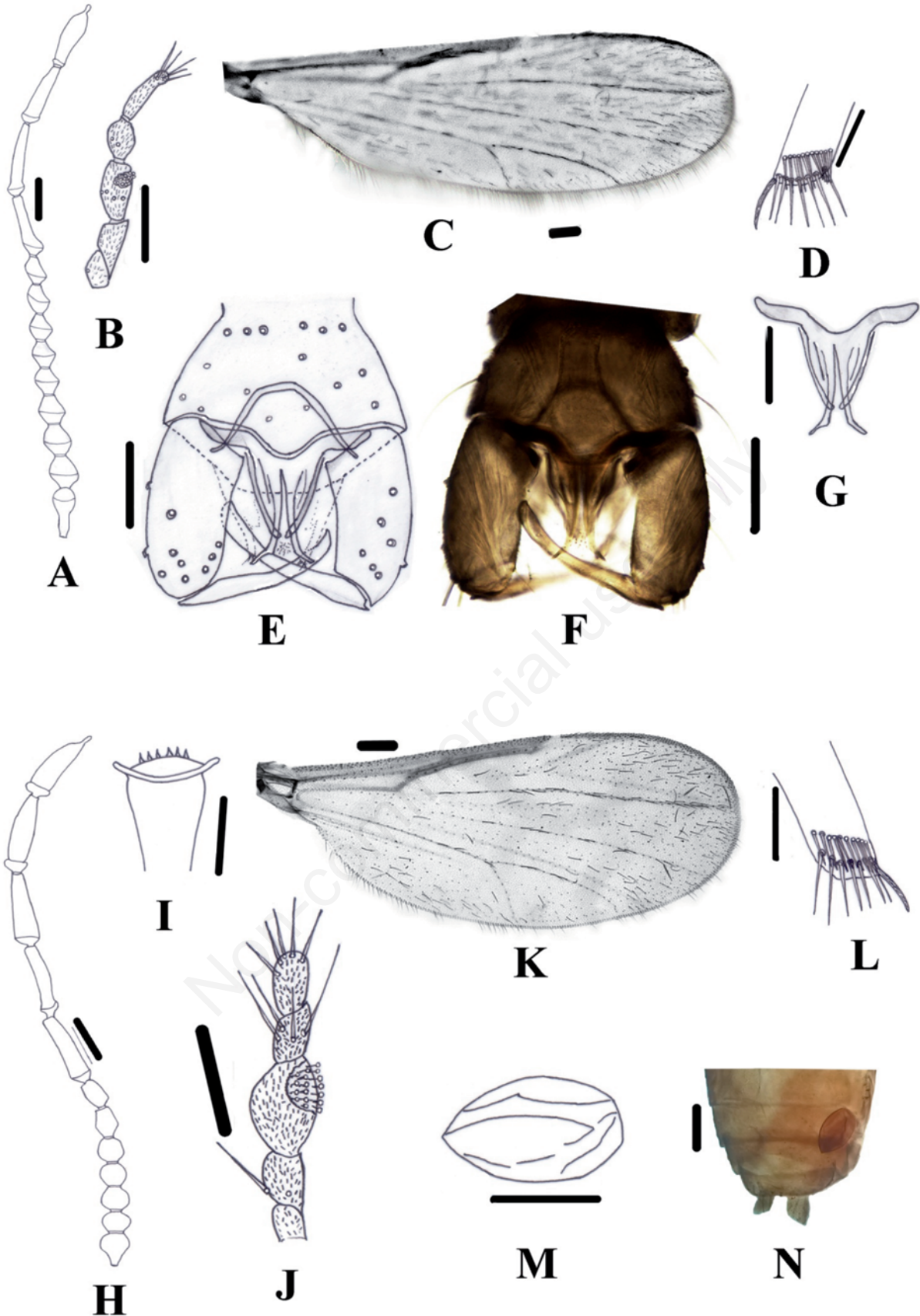


Figure 3. *Forcipomyia (Lasiohelea) peditata* sp. nov. A-G) Male: A, Flagellum; B, Maxillary palpus; C, Photograph of wing; D, Hind tibial comb (HTC); E, Illustration of male genitalia; F, Photograph of male genitalia; G, Aedeagus. H-N) Female: H, Flagellum; I, Cibarium; J, Maxillary palpus; K, Photograph of wing; L, Hind tibial comb (HTC); M, Illustration of spermatheca; N, Photograph of spermatheca (Scale bar 0.05 mm).

13 (12.5): 12-13 (12.5): 12-13 (12.5): 12-13 (12.5): 13-14 (13.5): 26-29 (27.5): 30-32 (31): 27-28 (27.5): 31-33 (32); AR: 1.13-1.19 (1.16). Cibarium without spines. Palpus (Figure 3B) pale brown, palpomere III as long as palpomere V barrel shaped with irregular apical sensory pit length ratios of palpomeres (I-V): 6-7 (6.5): 11-12 (11.5): 12-13 (12.5): 7-8 (7.5): 11-13 (12); PR 1.71-1.85 (1.78). Thorax. Uniformly dark brown.

Wing. WL 0.75-0.78 (0.76), WW 0.26-0.28 (0.27), CR 0.55-0.57 (0.56). Wing more or less covered with microtrichia, 1st radial cells obliterated, 2nd one open and longer (Figure 3C).

Leg. Pale brown without any banding pattern and scales; tarsal claws equal, curved, empodium well developed. HTC with 7 spines and a spur (Figure 3D). Length of tarsal segments as TR_I 1.85-1.91 (1.88), TR_{II} 1.61-1.67 (1.64), and TR_{III} 1.54-1.65 (1.60). Abdomen. Dark brown. Genitalia (Figure 3E-G): Sternite IX 87-93 (90) × 112-125 (118.5), almost 1.7 times wider than length. Tergite IX 92-97 (94.5) long × 125-130 (127.5), almost 1.3 times wider than length with long apicolateral process bearing 5 apical setae. Gonocoxite dark brown, 83-87 (85) × 39-42 (41), basal part intensely sclerotized. Gonostylus as long as gonocoxite, slightly curved from middle to apex. Parameres fused, bell shaped. Aedeagus of two triangular plate like sclerites, fused at base and arms well sclerotized, aedeagal plates encircled by a "V" shaped structure from its basal to subapical portion, apex of aedeagus shoe shaped (Figure 3G).

Female (n=4)

Similar to male with usual sex differences. Flagellomeres (Figure 3H) X-XIII largest, terminal one with apical papilla; length ratio of flagellomeres (I-XIII): 12-15 (13.5): 8: 8: 8-9 (8.5): 9: 9-10 (9.5): 9-11 (10): 10-11 (10.5): 23-25 (24): 25-26 (25.5): 25-27 (26): 26: 36-38 (37); AR 1.63-1.91 (1.77). Cibarium (Figure 3I) with 6 spines, arranged in a single row. Palpus (Figure 3J) brown, palpomere III largest and oval to barrel shaped with a well-developed sensory pit at middle containing 15-18 protruding capitate sensilla; length ratio of palpomeres (I-V): 5-6 (5.5): 8-9 (8.5): 12-13 (12.5): 7-9 (8): 8-11 (9.5); PR 1.33-1.50 (1.42). Mandible with 19-21 teeth.

Wing. WL 0.69-0.74 (0.715), WW 0.30-0.31 (0.305), and CR 0.62-0.65. First radial cells obsolete, 2nd one narrowly open and longer than the first covered with macrotrichia being denser in costa and proximity of wing margin (Figure 3K).

Leg. TR_I 2-2.03, TR_{II} 1.82-1.83, TR_{III} 1.83-1.96 (1.89). HTC with 7 spines and a spur (Figure 3L).

Abdomen (Figure 3M, N). Dark brown, spermatheca (Figure 3M) one, large, heavily sclerotized measuring about 65×38 μm with wide opening.

DISCUSSION. The new species is linked with *F. (L.) plumosa* Debenham, 1983 due to some similarities with the structure of aedeagus, but bare eyes and narrower subapical part of the new species differ from each other. The female of *F. (L.) plumosa* possesses 8 spines whereas the new species has 6 spines. The only Indian species, *F. (L.) manasi* Maheshwari, 2003 though poorly described shows significant differences with the new one in having 10 clypeal setae while 14 in the new species. Similarly, second HTC of the new species bears 14 spines but the spines are 10 in the known Indian species. The present species resembles also Chinese species *F. (L.) phototropia* (Yu and Zhang, 1982) in wing and bare eyes but significant difference lies in PR. The palpus of female *F. (L.) phototropia* is without sensory pit on palpomere III but the scattered capitate sensilla in the sensory pit makes the difference from the above one. The new taxon possesses only 6 thorn like spines in the cibarium while 23 spines of cibarium in the species of China making different from each other. Another species of China, *F. (L.)*

propria Chan and LeRoux, 1970 in wing and to some extent in aedeagus but they differ in the clypeal setae, and structure of basal arms of the aedeagus.

DISTRIBUTION: India, West Bengal.

Forcipomyia (Lasiohelea) parvitas (Liu and Yu, 1996) (Figure 4)
Forcipomyia (Lasiohelea) parvitas (Liu and Yu, 1996): Borkent and Dominiak, 2020: 85

Lasiohelea parvitas Liu and Yu, 1996: 49

GenBank Accession Number: OM632702

MATERIAL EXAMINED. 2♂♂, India, West Bengal, Purba Bardhaman, Burdwan (23°22'46.9"N, 87°85'91"E), 12.III.2021, Coll. N. Hazra; 4♂♂ India, West Bengal, Bankura, Saltora, Biharinath Hill (23°58'04.90"N, 86°94'84.45"E), 06.X.2021, Coll. G. S. Pal.

DIAGNOSIS: Aedeagus bipartite of two triangular plates fused at base, apices outwardly directed terminating in a sharp point

Male (n=6)

More or less similar like *Forcipomyia (Lasiohelea) parvitas* (Liu and Yu, 1996) with the additional descriptions as Head dark brown. Eyes bare and contiguous. Antenna pale brown, except pedicel, length ratio of flagellomeres (I-XIII): 22-24 (23): 12-15 (13.5): 12-14 (13): 13-14 (13.5): 13-14 (13.5): 13-14 (13.5): 13-14 (13.5): 14-15 (14.5): 13-15 (14): 28-30 (29): 32-35 (33.5): 26-29 (27.5): 30-33 (31.5), AR: 1.14-1.17 (1.15). Cibarium without any spines; length ratio of palpomeres (I-V) 7-8 (7.5): 10-11 (10.5): 13-14 (13.5): 11-13 (12); PR 1.75-2.00 (1.87). WL 0.90-0.86 (0.88), WW 0.27-0.29 (0.28), CR 0.52-0.54 (0.53). Genitalia (Figure 4) honey brown. Sternite IX 75-85 (80) × 125-133 (129), almost 1.6 times as broader than the length, tergite IX 75-85 (80) × 125-133 (129) as wide as sternite IX. Gonocoxite brown, more or less bean shaped 92-95 (93.5) × 42-45 (43.5), more than twice of its greatest



Figure 4. Photograph of male genitalia of *Forcipomyia (Lasiohelea) parvitas* Liu & Yu (Scale bar 0.05 mm).

width, basal part slightly sclerotized. Gonostylus as long as gonocoxite. Parameres fused to form inverted 'V' shaped structure. Aedeagus bipartite of two triangular plates fused basally, apices outwardly directed terminating in a sharp point.

DISTRIBUTION. China (Sichuan) and India (West Bengal).

REMARKS. Overall morphological parameters are similar except sub apical region of aedeagus is narrower than that of the Chinese species.

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