

## Research article

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# SEM morphology and courtship rituals of a new species of *Rhamphomyia* (Diptera: Empididae: Empidinae) from the Kashmir Himalayas (India)

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**Abstract.** *Rhamphomyia bhagati* Barták, Akbar, Kanturski, Wachkoo & Maqbool sp. nov. (Diptera: Empididae) is described and illustrated based on male and female specimens. The discovery marks the first record of the genus *Rhamphomyia* from the Kashmir Valley. Scanning Electron Microscopy (SEM) analysis was carried out to elucidate the general morphology and sensilla of the male and female specimens. The species is most prevalent during April and early May. The male provides female with a nutritious prey, as a courtship gift through a series of rituals discussed herewith.

**Keywords.** Empididae, *Rhamphomyia*, courtship, Kashmir valley, India, new species.

## INTRODUCTION

Empidinae (Diptera: Empididae) is a subfamily of dipteran flies with over 3,000 described species and many still undescribed (Thompson 2005; Yang et al. 2007; Pape et al. 2011). These flies occur worldwide with majorities found in the Holarctic region, exhibit enormous structural diversity and inhabit a broad range of biotopes (Sinclair & Cumming 2006; Moulton & Wiegmann 2007). These are commonly known as balloon or dance flies, which characterizes their elaborate mating displays, aerial swarming, nuptial gift transfers, and other courtship rituals (Cumming 1994).

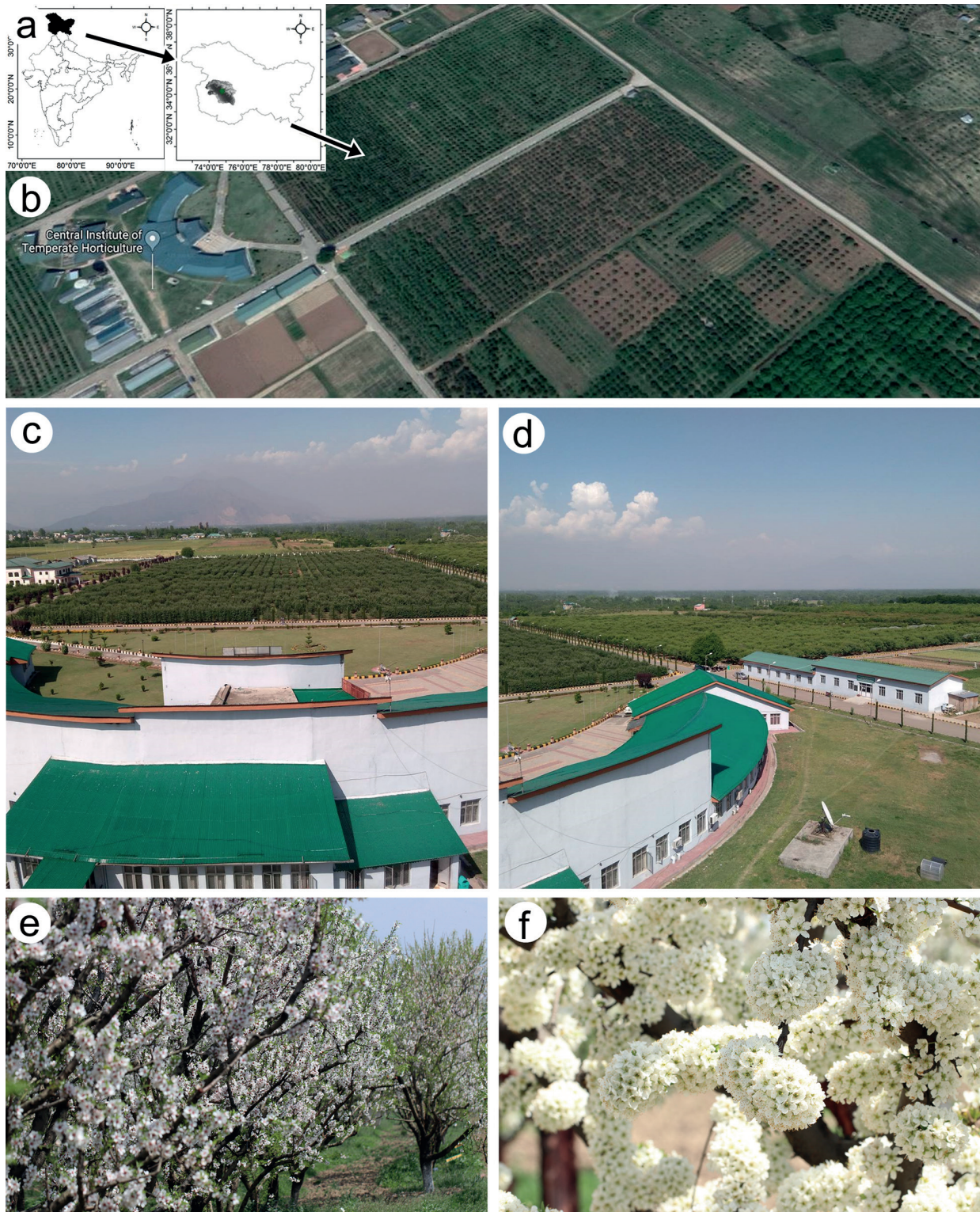
The subfamily is divided into two tribes (i.e., Empidini and Hilarini) centered on three megadiverse genera: *Empis* Linnaeus, 1758, *Rhamphomyia* Meigen, 1822 (tribe Empidini), and *Hilara* Meigen, 1822 (tribe Hilarini), and around 27 other smaller genera (Watts et al. 2016). Large gaps in the taxonomic knowledge of the subfamily remain for most regions of the world, especially in the

Southern Hemisphere (Sinclair & Cumming 2006). Limited literature is also available on the Indian fauna. Apart from some old works by Walker (1849), Bigot (1889), Bezzi (1904), Brunetti (1913, 1917, 1920) and Collin (1960), no recent pertinent literature is available. Of 57 species of Indian Empididae, the subfamily Empidinae in India currently comprises about 20 species belonging to four genera (Brunetti 1920; Collin 1960; Alfred et al. 1998; Mitra et al. 2015); however, like other Indian flies these have not been the subject of a dedicated study and the true number of species is still unknown (Shah et al. 2014; Wachkoo et al. 2017).

The megadiverse genus *Rhamphomyia* is the most speciose of dance flies, currently represented by almost 610 known species globally (Barták & Kubík 2012; Saigusa 2012; Barták et al. 2014; Rhodén & Wahlberg 2020), and with many unpublished records, likely to increase to about 1,500 species (Sinclair et al. 2019). These flies thrive in mountainous regions with an enormous radiation in the Holarctic region and are well represented in the arctic en-

vironment as well (Sinclair et al. 2019; Shamshev et al. 2020). These flies are also an ideal taxon for site quality assessment studies (Grootaert 2004). Several Palaearc-

tic species groups of *Rhamphomyia* have been revised (Barták 1982, 2003; Barták 2007, 2014; Barták & Danielson 2007; Barták & Kubík 2008a, b, c, 2009, 2010,



**Fig. 1.** Study area. **a–d.** Aerial view of Central Institute of Temperate Horticulture. **e–f.** Full blossom of fruit trees.



2012, 2015). The genus *Rhamphomyia*, however, still awaits global taxonomic revision with the dubious status of some of the proposed subgenera (Barták 1982; Chvála 1994). Little attention has been paid to Southeast Asian species and our knowledge of the Indian Himalayan Region fauna is also very poor. Only three species, *R. griseonigra* Brunetti, 1913, *R. himalayana* Brunetti, 1913 and *R. unifasciata* Brunetti, 1913 have been previously reported from Indian Himalayas (Brunetti 1913, 1920) and it is highly likely that many more *Rhamphomyia* species await discovery (Barták & Kubík 2012). Herein a new species belonging to *Rhamphomyia* (*Pararhamphomyia*) is described from the Kashmir valley. This also marks the first record of the genus from the region. The species placement within the subgenus (*Pararhamphomyia*) is followed after Collin (1961) and Barták & Sinclair (2003). This subgenus is characterized by the prosternum and mostly propleura without setae, biserial acrostichals, incomplete anal vein, long setose labela and usually a distinct basal costal seta. The species is described and differential diagnoses with closely related species are provided. Notes on courtship rituals of the new species are also provided, along with SEM analysis to elucidate morphological details. Male and female flies are frequent flower visitors, observed feeding on nectar all day long from flowers belonging to genera *Pyrus*, *Prunus*, and *Cydonia*.

## MATERIAL AND METHODS

### Material and taxonomy

Specimens were collected by hand picking method from orchards of the Central Institute of Temperate Horticulture (CITH) located in Kashmir Valley. CITH is situated at 33.59° N, 74.50° E with an altitude of 1,640 m a.s.l. (Fig. 1), in the Palaearctic portion of India on the northern fringe of the Western Himalayas (Akbar et al. 2018, 2020; Wachkoo & Akbar 2019). Behavioral patterns were observed similarly as in previous works (Kessel 1955; LeBas et al. 2004; Daugeron & Grootaert 2005; Wilkinson & Johns 2005). The field photographs were obtained using a Canon 80D DSLR fitted with 100 mm macro lens. Five mating couples were observed, thrice a month for three successive months (April–June) from 2015 to 2018 for their courtship rituals. The pairs, formed in air and descend from the swarm, were observed after they commence copulation. The timing of copulation was counted once the pair settled on nearby vegetation till copulation was terminated and the pair separated. Upon separation, these were caught and their body measurements taken. Statistical analysis was carried out with Statistical Analysis Software (SAS).

Taxonomic analyses were conducted using an Olympus SZX16 stereo zoom microscope. For digital imag-

es, ProgRes0 CapturePro ver. 2.8.0. evolution digital camera was used on the same microscope with Combine ZP-Montage software. Later, images were cleaned with Adobe Photoshop CS6. Genitalia together with 2–3 pre-genital segments were removed and macerated in potassium hydroxide solution (10%) in small vials, for 1–2 hours, treated with 8% acetic acid and dissected in glycerine. The morphological terms used here follow Sinclair and Cumming (2006). All body measurements (including body and setae length) were taken from dry specimens (therefore the actual length may differ) by means of an ocular micrometer on the above Olympus microscope.

Description pattern and measurements adopted here follow Barták & Kubík (2015). Length of antennal segments = length of scape:pedicel:postpedicel:stylus (in 0.01 mm). Male body length was measured from antennal base to the tip of last abdominal segment (without the genitalia) and female body length from the base of antennae to the tip of the cerci. Wing measurements:  $M_2/d$  = length of vein  $M_2$ :greatest length of discal medial cell (discal cell);  $CuA_1$  ratio = length of apical: preapical sections of vein  $CuA_1$ ;  $lw/ww$  = greatest length of the wing (from basicosta to apex):greatest width of the wing. Length of frons is measured from front margin of anterior ocellus to antennal base. Holotype and paratypes are deposited in the Central Institute of Temperate Horticulture, Srinagar, India (CITH). Two paratypes are deposited in CULSP (Czech University of Life Sciences Prague), two in University of Silesia, Katowice (DZUS) and two will be deposited in Natural History Museum, London, UK (BMNH).

### Scanning electron microscopy

Specimens for SEM analyses were preserved in 70% ethanol for several days. From ethanol, the specimens were transferred into 6% phosphotungstic acid (PTA) solution in 70% ethanol for 24 hours. Dehydration was provided by ethanol series of 80, 90, 96% and two changes in absolute ethanol for 10 minutes each. Some of the dehydrated specimens were treated with chloroform for 24 h. Dehydrated and cleaned specimens were dried using the Leica EM CPD 300 automated critical point dryer (Leica Microsystems, Vienna, Austria). Dry samples were mounted on aluminum stubs with double-sided adhesive carbon tape and sputter-coated with 30 nm layer of chromium in a Quorum 150 T ES Plus sputter coater (Quorum Technologies Ltd, Laughton, East Sussex, UK). The specimens were imaged by the Hitachi SU8010 field emission scanning electron microscope FESEM (Hitachi High-Technologies Corporation, Tokyo, Japan) at 5 and 7 kV accelerating voltage with a secondary electron detector (ESD) in the SEM laboratory of the Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice (Katowice, Poland).

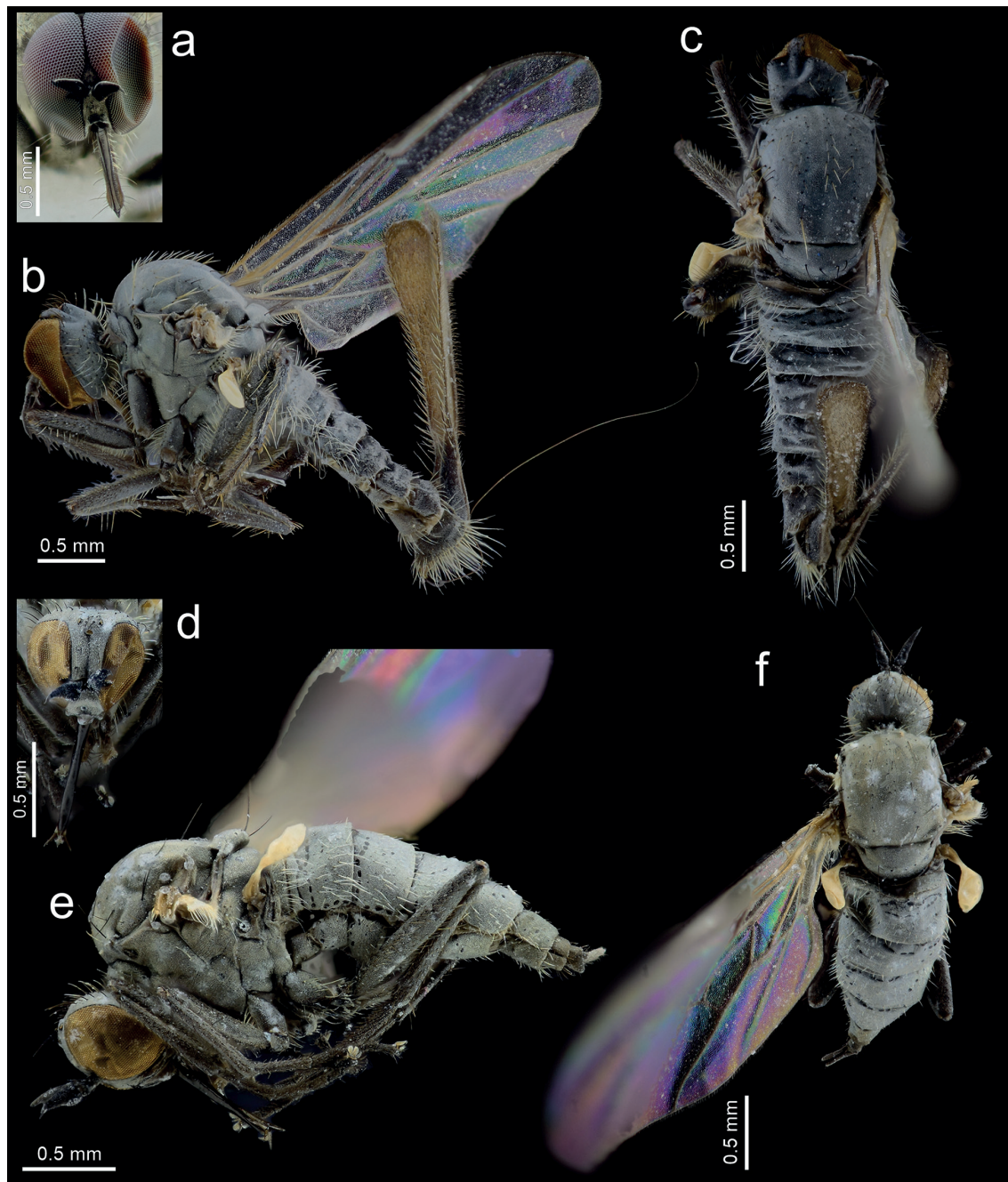
## RESULTS

*Rhamphomyia* (*Pararhamphomyia*) *bhagati* sp. nov.  
(Figs 2–9)

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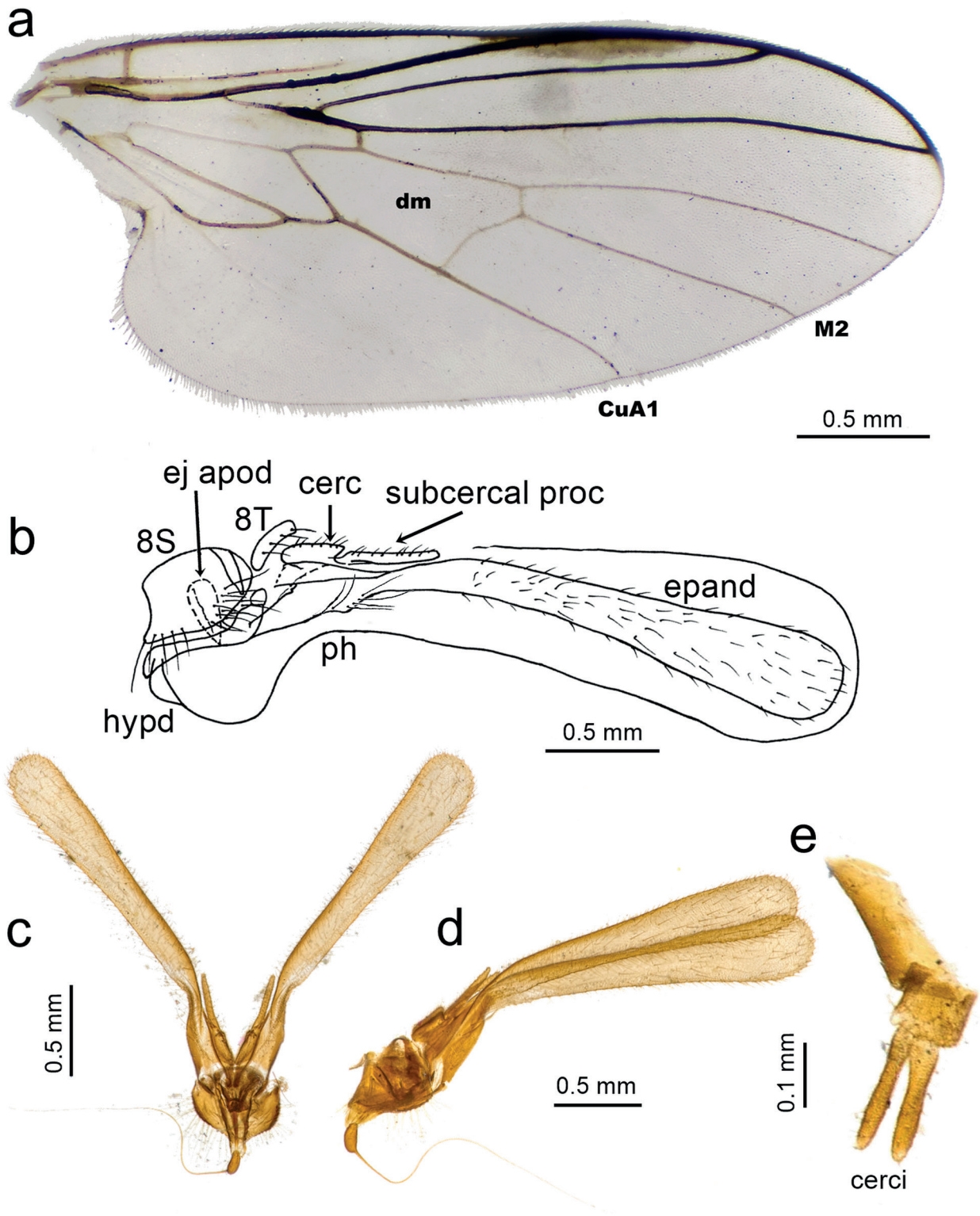
**Diagnosis.** Light grey microtrichose species of *Rhamphomyia* (*Pararhamphomyia*) with brown legs, both black and pale setae on the body, palpus black, acrostichals

narrowly biserial, dorsocentrals irregularly biserial and slightly longer than acrostichals, both mostly whitish yellow, often with some dark setae intermixed, axillary angle  $90^\circ$ , anal vein incomplete, basal costal seta long, cercus elongate, subcercal process long and thin, epan-drium extremely elongate with broadly rounded tip, phal-lus long, filamentous with short basal swelling followed by S-shaped arc.



**Fig. 2.** *Rhamphomyia bhagati* sp. nov. **a–c.** Male. **d–f.** Female. **a, d.** Head, frontal view. **b, e.** Habitus, lateral view. **c, f.** Habitus, dorsal view.





**Fig. 3.** *Rhamphomyia bhagati* sp. nov. **a.** Wing venation with labeled discal cell (dm), cubitus (CuA1) and medial veins (M2), (note: costal seta broken). **b–d.** Male genitalia, cercus (cerc), ejaculatory apodeme (ej apod), epandrium (epand), hypandrium (hypd), phallus (ph), subcercal process (subcercal proc), sternite 8 (8S), tergite 8 (8T). **e.** Female genitalia cerci.

### Material examined

**Holotype.** ♂, INDIA: Kashmir: Srinagar: Central Institute of Temperate Horticulture, 34.0094°N 74.7984°E, 1640m.a.s.l., 11 April 2015 (CITH).

**Paratypes.** Same collection data as for holotype, except: 11 April 2015 (4 ♂♂, 2 ♀♀, CITH; 1 ♂, 1 ♀, CUL-SP), 16 May 2015 (2 ♂♂, 1 ♀, CITH; 1 ♂, 1 ♀, DZUS), 28 May 2016 (1 ♂, 1 ♀, CITH), 09 June 2017 (4 ♂♂, 1 ♀, CITH; 1 ♂, 1 ♀, BMNH), 11 June 2018 (5 ♂♂, 5 ♀♀, CITH), 07 May 2019 (15 ♂♂, 11 ♀♀, CITH), 09 May 2019 (21 ♂♂, 14 ♀♀, CITH), Shahid Ali Akbar leg.

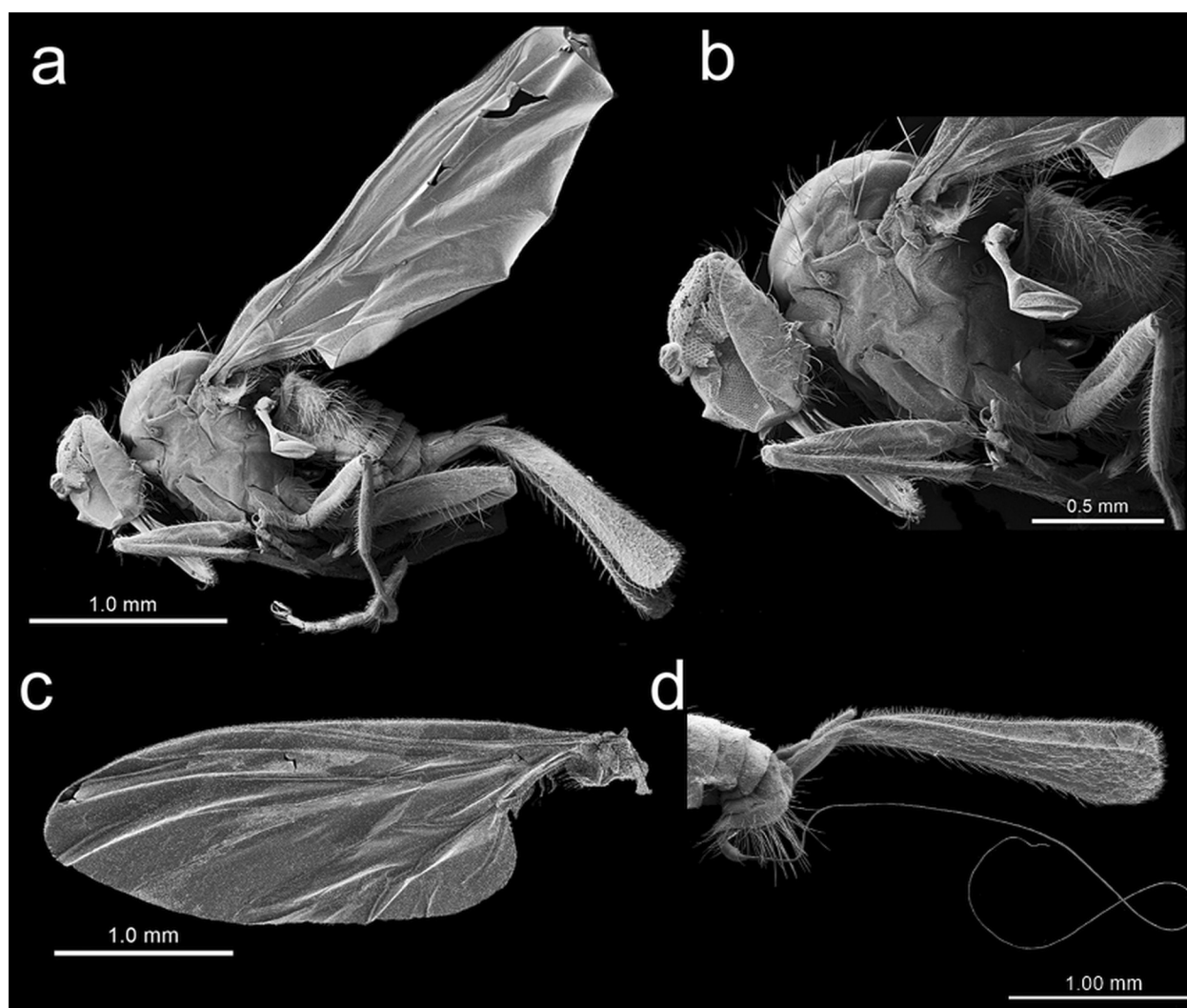
**Distribution.** India: Western Himalaya, Kashmir.

**Dates of occurrence.** April to June.

### Description

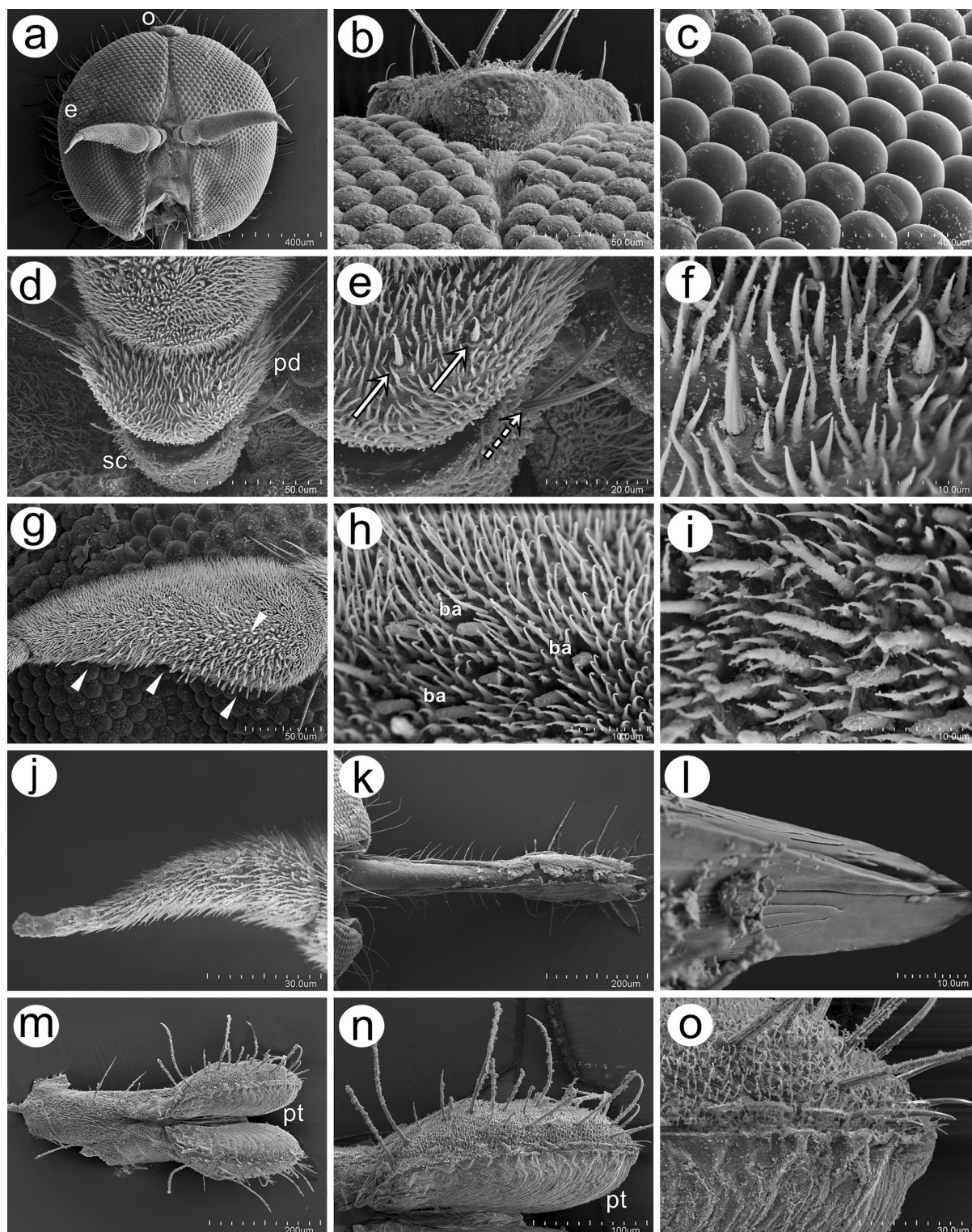
**Male** (Figs 2a–c; 3a–d; 4–8; 9a–i)

**Head.** Light grey, microtrichose; eyes holoptic, meet along median dorsal line, facets in dorsal half of eye considerably enlarged (Fig. 2a); frons confined as small triangle above antennae, without setulae. Frons blackish brown, 0.15–0.18 mm long. Ocellar triangle prominent, microtrichose; ocellar setae black, fine, approximately 0.26–0.27 mm long. Occiput sparsely covered with setae subequally long as ocellars on dorsal part, colour variable, mostly black to brown, often intermixed with white setae; lower part of occiput mostly with pale and somewhat shorter setae; postocular row incomplete, irregular or absent on lower half. Face approx. 0.12–0.14 mm wide, 0.15–0.16 mm long, without setae; microtrichose except



**Fig. 4.** Scanning electron microscopy (SEM) showing general morphology of the male of *Rhamphomyia bhagati* sp. nov. **a.** Lateral view. **b.** Head and thorax lateral view. **c.** Wing. **d.** Genitalia.



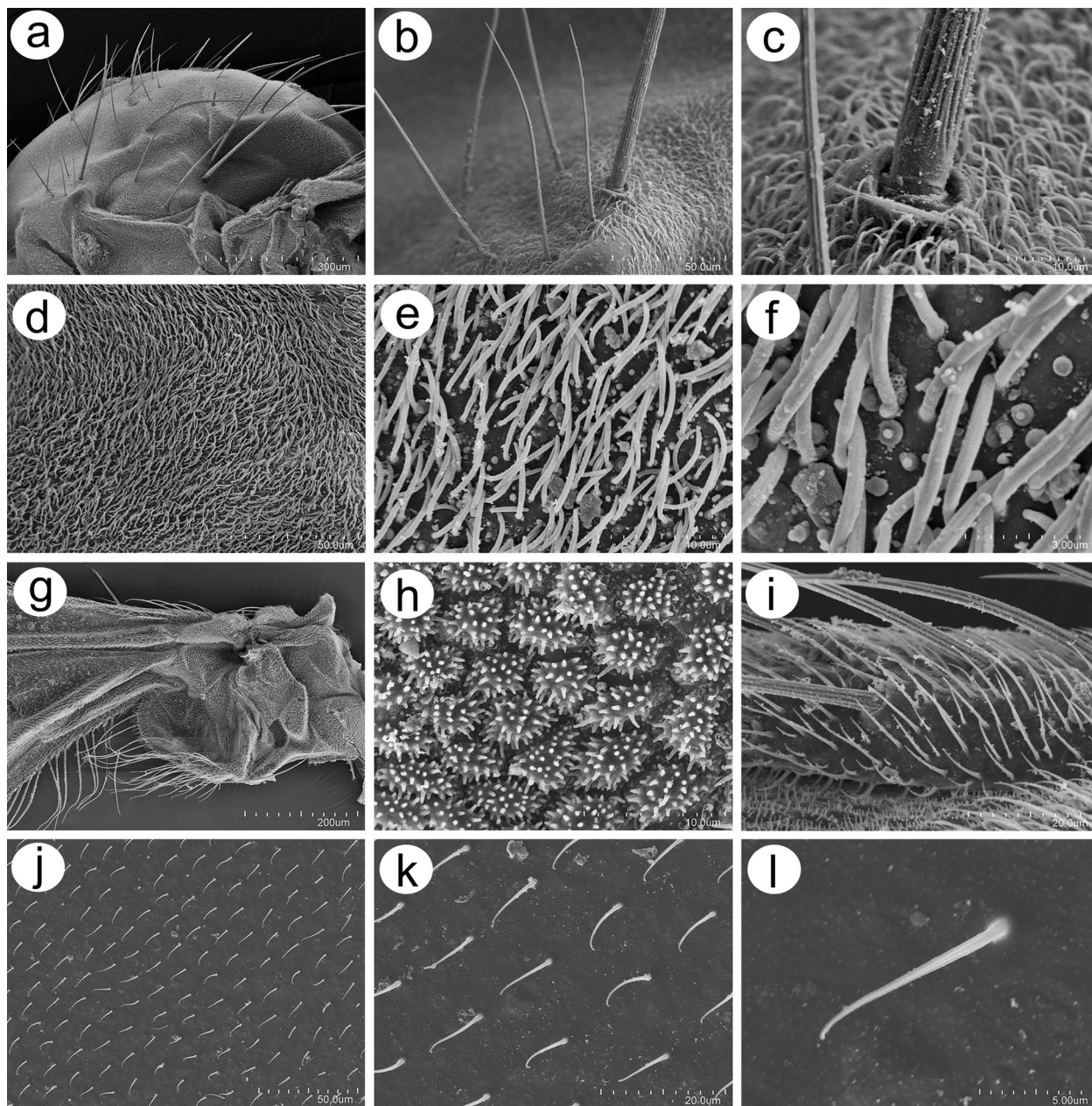


**Fig. 5.** SEM of *Rhamphomyia bhagati* sp. nov. head, antennae and mouth parts. **a.** Holoptic eyes (e) with enlarged facets along dorsal half and ocelli (o). **b.** Fine structure of the ocelli. **c.** Fine structure of the ommatidia. **d.** Scape (sc), and pedicel (pd) with sclerotic microtrichia. **e.** Structure of type III trichoid sensilla (dotted arrows) and type IV trichoid sensilla (solid arrows). **f.** Fine structure of the type IV trichoid sensilla sockets. **g.** Postpedicel with the area of basiconic sensilla (arrow heads) on the ventral side. **h.** Postpedicel microtrichia and basiconic sensilla (ba). **i.** Fine structure of the basiconic sensilla covered by wax layer. **j.** Stylus. **k.** Labrum. **l.** Fine structure of the labrum apical end. **m.** Labium. **n–o.** Fine structure of the ventral side of the labellum with visible pseudotracheae (pt).



extreme lower margin. Clypeus microtrichose, gena narrow and microtrichose. Palpus black and short, with several long setae (up to 0.20 mm). Labrum black, shiny, distinctly longer than head height; labellum with rather long setae. Antenna black, both basal segments short setose (Fig. 2a); length of antennal segments (scape:pedicel:postpedicel:stylus) = 0.06–0.09 mm:0.06–0.8 mm:0.27–0.31 mm:0.09–0.11 mm.

**Thorax.** Black, light grey microtrichose with two narrow brownish stripes between dorsocentrals and acrostichals (Fig. 2b–c). Most thoracic setae pale, posterior dorsocentrals, postalar and scutellars black; acrostichals and dorsocentrals mostly whitish yellow, often with some dark setae intermixed. Chaetotaxy: antepronotum with a few short setae in middle; proepisternum with several fine setulae; prosternum and propleura bare; acrosti-



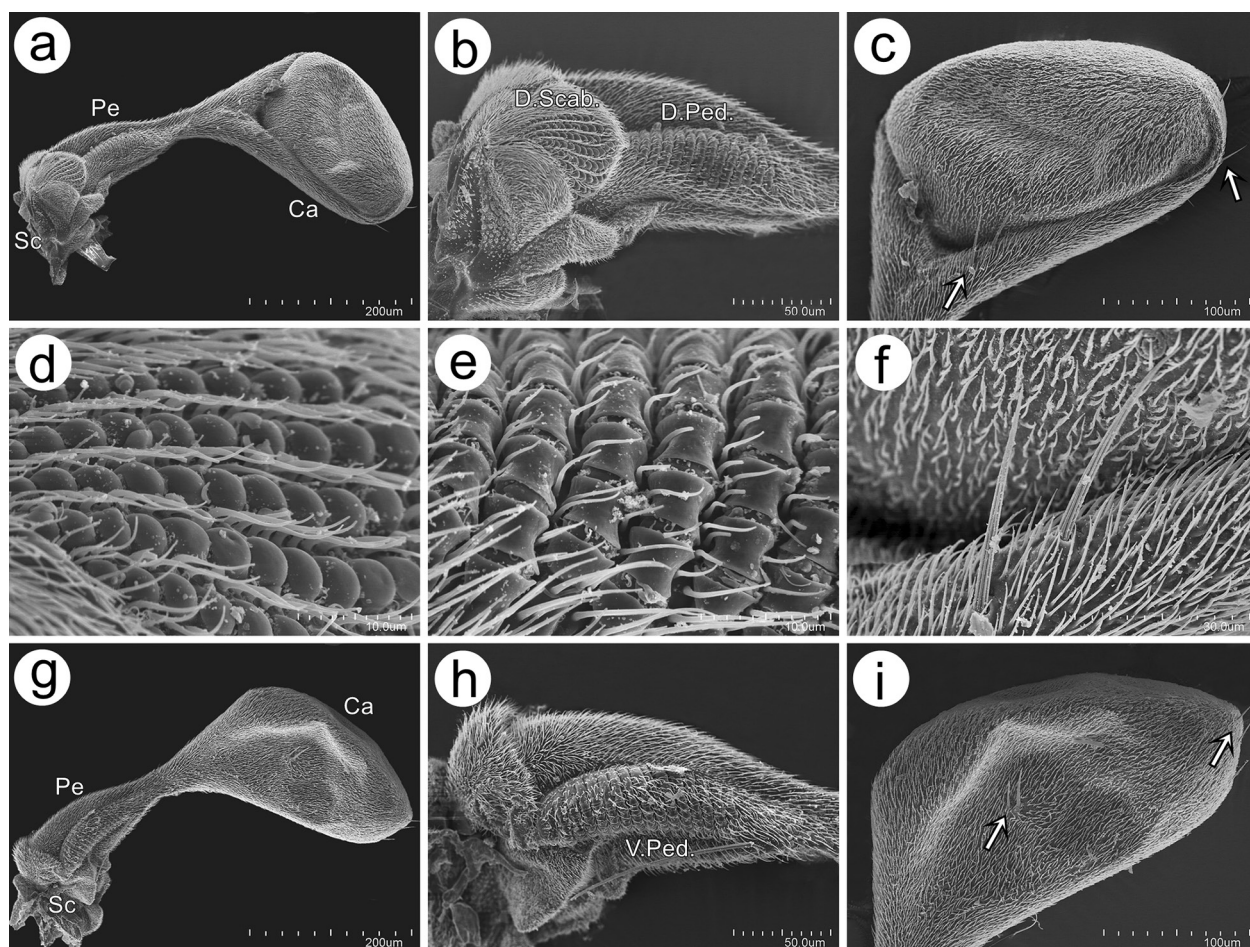
**Fig. 6.** SEM of *Rhamphomyia bhagati* sp. nov. thorax and wings. **a.** Long and rigid setae (trichoid and chaetic sensilla) on thorax. **b.** Structure of trichoid sensilla. **c.** Fine structure of socket and basal part of chaetic sensillum. **d.** General view of thoracic microtrichia. **e–f.** Fine structure of thoracic cuticle and microtrichia with waxy secretion. **g.** Wing articulation and base. **h.** Pillow-like structures on the articulation part of the wing. **i.** Chaetic sensilla on the wing edges. **j.** Wing membrane with linearly arranged microtrichia. **k–l.** Fine structure of the wing microtrichia.



chals narrowly biserial (almost uniserial anteriorly) and few in number (5–7 in one row), about 0.10–0.12 mm long; dorsocentrals irregularly biserial, slightly longer than acrostichals, ending in 2–3 long black prescutellar pairs; one presutural intra-alar (intrahumeral), one presutural supra-alar (posthumeral) and additional 5–8 setae between dorsocentrals and presutural supra-alar seta; one long postpronotal seta and several much shorter setulae; 2–3 notopleural setae and 1–3 rather long but fine setae on anterior part of notopleuron; two pairs of black scutellars.

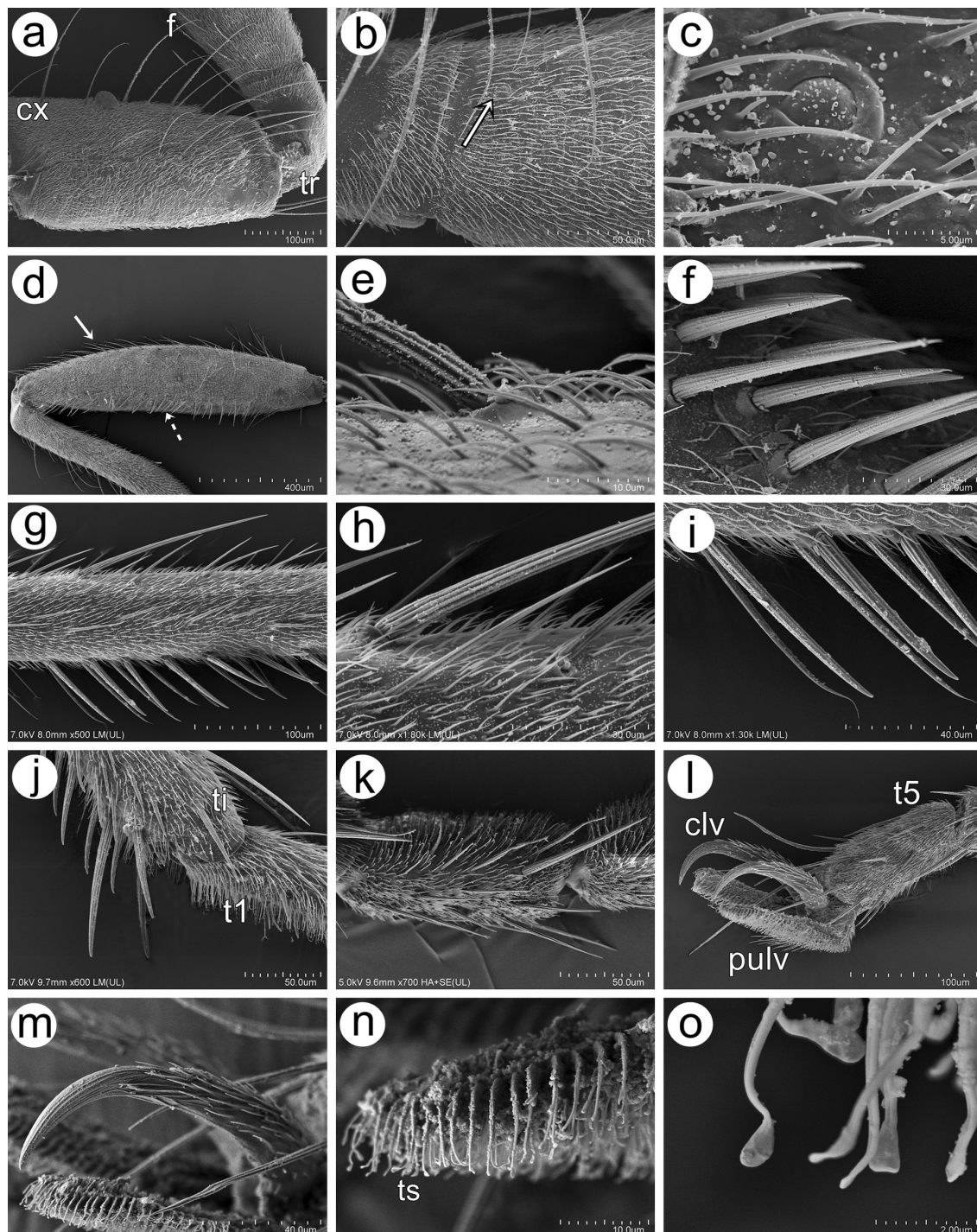
**Legs.** Brown, light grey microtrichose, with both whitish and brown setae (Fig. 2b). Fore femur with rows of setae ventrally slightly shorter than femur depth, dorsal setae much shorter. Fore tibia with very short setulae ventrally, with irregularly arranged setae dorsally subequally long as tibia depth. Mid femur with irregularly arranged setae anteroventrally, slightly shorter than femur depth,

more ventral and thicker proximally and finer and more anteroventral distally; posteroventral setae much longer, on apical third longer than femur depth. Mid tibia with rather dense fine setae anteriorly and anteroventrally; posteroventral setae slightly thicker, more regularly arranged, all at most as long as tibia depth; dorsally with short setae; in some specimens with poorly distinct 1–2 anterodorsal setae. Hind femur distinctly thickened and flattened, with irregularly arranged rather dense and thick pale antero- and posteroventral setae on distal two-thirds, slightly shorter than femur depth; dorsal setae rather long, especially on proximal part of femur, otherwise shorter and finer setose. Hind tibia distinctly curved, shortened and thickened (almost as in *R. gibba* (Fallén, 1816)), short setose ventrally, with 4–5 rather long posterodorsal setae (up to 0.20–0.23 mm long). Tarsi of all legs narrow and long, short setose, with apical circlelets of



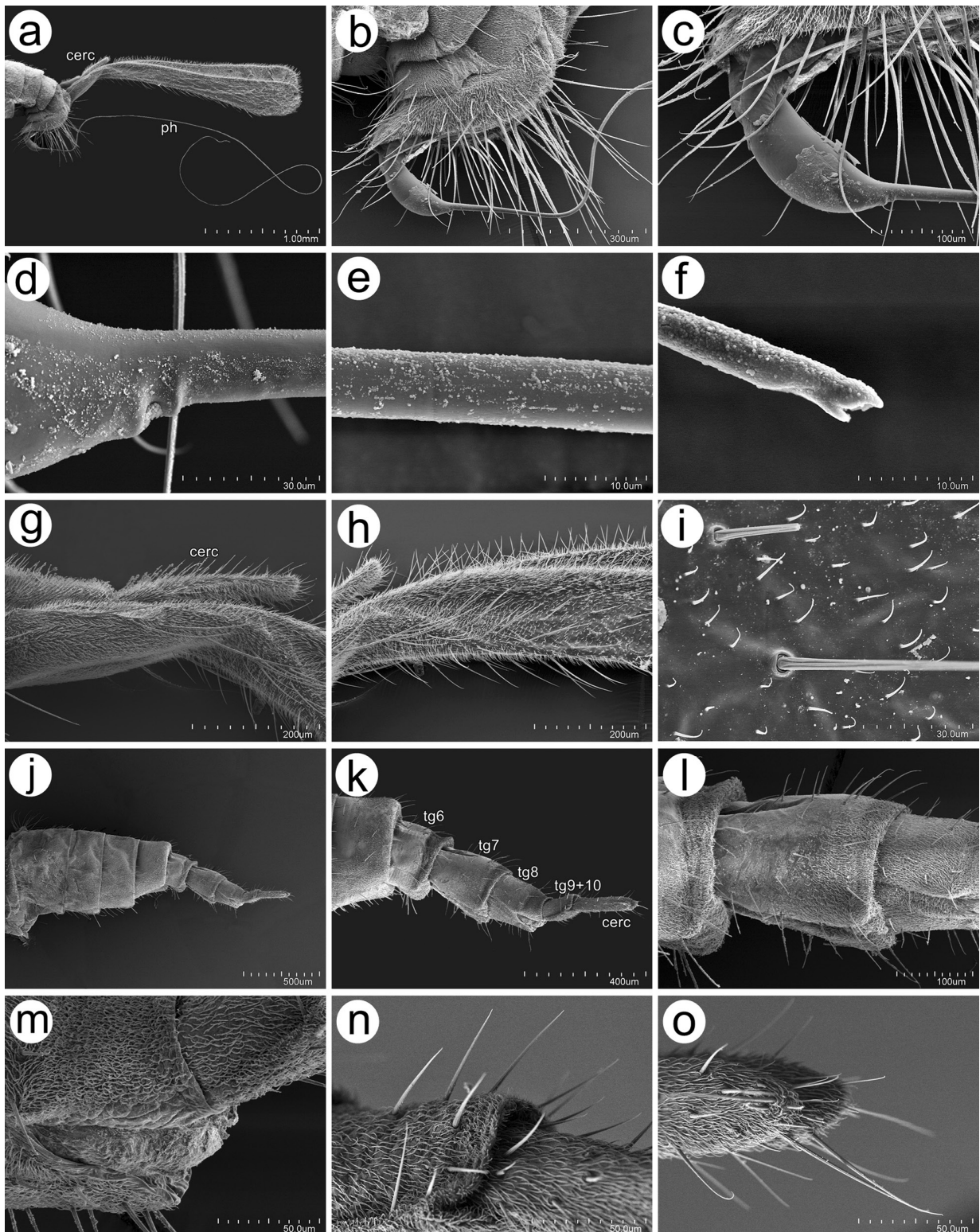
**Fig. 7.** SEM of *Rhamphomyia bhagati* sp. nov. halter. **a.** General view of the dorsal side of the halter with robust scabellum (Sc), pedicellus (Pe) and oval capitellum (Ca). **b.** Sensilla on the dorsal scabellum (D.Scab.) and dorsal pedicellus (D.Ped.). **c.** Capitellum with single trichoid sensilla (arrows). **d.** Fine structure of the basal plate sensilla. **e.** Fine structure of the flanking sensilla. **f.** Fine structure of the trichoid sensilla. **g.** General view of the ventral side of halter. **h.** Flanking sensilla on the ventral pedicellus (V.Ped.). **i.** Single trichoid sensilla on the capitellum.





**Fig. 8.** SEM of *Rhamphomyia bhagati* sp. nov. legs. **a.** Coxa (cx) with long trichoid sensilla, trochanter (tr) femur (f) with much shorter ones **b.** Single campaniform sensillum (arrow) on the proximal part of femur. **c.** Fine structure of the campaniform sensillum. **d.** Fine trichoid sensilla on the dorsal side (solid arrow) and thick and rigid chaetic sensilla (dotted arrow) on the ventral side of femur. **e.** Fine structure of the socket and basal part of trichoid sensillum on dorsal femur. **f.** Fine structure of chaetic sensilla on ventral femur. **g.** Part of tibia with trichoid and chaetic sensilla. **h.** Fine structure of dorsal tibiae with trichoid and chaetic sensilla. **i.** Fine structure of two kinds of chaetic sensilla with thick and very fine apices on ventral tibia. **j.** Chaetic sensilla on the distal part of tibia (ti) and first tarsomere (t1). **k.** Trichoid and chaetic sensilla on the third tarsomere. **l.** Trichoid sensilla on the fifth tarsomere (t5), curved claws (clv) and pulvilli (pulv). **m.** Fine structure of claw. **n.** Fine structure of the tentent setae (ts). **o.** Fine structure of the tenent setae terminal plates.





**Fig. 9.** SEM of *Rhamphomyia bhagati* sp. nov. male and female genitalia. **a.** General view of male genitalia with labelled phallus (ph) and cerci (cerc). **b.** Long and rigid chaetic sensilla on the genitalia. **c.** Basal part of the phallus. **d–f.** Fine structure of different parts of the phallus. **g.** Trichoid sensilla on the cerci (cerc). **h.** Trichoid and chaetic sensilla on the epandrium. **i.** Surface of the epandrium and fine structure of trichoid sensilla sockets. **j.** Female abdomen. **k.** Chaetotaxy of the terminal segments of female abdomen tergites 6–10 (tg6–tg10) and cerci (cerc). **l.** Fine structure of seventh segment of abdomen. **m.** Genital pore. **n–o.** Chaetic sensilla on the last abdominal segment and cerci.

setae and long claws. Posteroapical comb of hind tibia with 1 long seta.

**Wing.** Clear, veins brownish-black to yellowish brown, pterostigma brown, anal vein incomplete (apparent as depigmented vein in basal two-thirds); anal lobe well developed with axillary angle right angled (Fig. 3a); basal costal seta rather long, black. Halter yellow with brown stem, calypter yellow with whitish fringes (Fig. 2b–c).

**Abdomen.** Black, very light grey microtrichose, covered with whitish yellow setae; epandrium with black setae and setulae (Fig. 2b–c). Lateral marginal setae on tergites 2–4 subequally long as their segments, dorsal setae and setae on segments 5–7 much shorter, sternites with long, yellowish white setae, sternite 1 without setae, sternite 8 with very long and dense setae (dorsally up to 0.35 mm long). Segments 6–7 as simple unmodified structures. Segment 8 with tergite and sternite separated; tergite 8 simple, somewhat C shaped viewed laterally, sternite 8 simple, enlarged, subrectangular in lateral view. Terminalia as in Fig. 3b–d. Hypandrium short, lustrous, without setae; epandrium extremely elongated (apical part about 1.7 mm long), apex broadly rounded; cercus elongate, lower cercus or subcercal process long and thin, as an extension of the cercus; phallus filamentous, with short basal swelling followed by S-shaped arc and then broadly arching around epandrium exceeding lamellae (total length of very thin hair-like phallus up to 5 mm).

**Length.** Body about 4.0–4.6 mm long, wing 3.6–4.0 mm.

#### Female (Figs 2d–f; 3e, 9j–o)

Similar to male, but body setae much shorter and darker; legs simple and short setose. Eyes dichoptic with all facets almost equal in size (Fig. 2d). Frons approximately 0.15–0.18 mm, broad with 3–5 black or pale, rather long setae on sides. Labrum black, labella with rather long setae. Thoracic chaetotaxy: acrostichals and dorsocentrals mostly black, dorsocentrals distinctly longer (Fig. 2e–f). Both fore and mid femora short setose, longest (distal) posteroventrals shorter than femur depth. Both fore and mid tibiae very short setose, without prominent setae. Hind femur with only a few anteroventral setae on distal half, slightly shorter than femur depth, otherwise very short setose (including very short posteroventrals). Hind tibia with several antero- and posterodorsal setae as long as tibia diameter. Terminalia as in Fig. 3e. Length: body 3–3.5 mm; wing 3.4–3.6 mm.

**Etymology.** The species is named in the honour of Professor Ramesh Chander Bhagat.

**Remarks.** The species described above is allied to *Pararhamphomyia* with extremely elongated epandrium and very long and extremely thin, hair-like phallus, present (but not in such an extreme form) as in *R. (P.) tenuiterfilata* Becker, 1900 or *R. (P.) longestylata* Frey,

1916. However, the former species has the mesoscutum disc shiny and the latter has all body setae black. The new species shares some affinities with *R. himalayana*, but differs in having the abdomen with long pale setae; labrum distinctly longer than head height; wing clear with pterostigma brown; thorax with a few long and pale setae, whilst *R. himalayana* has the abdomen with only short blackish setose; labrum as long as head height; wing pale brownish without distinct pterostigma and thorax setae short and blackish. The male terminalia of the new species share some similarities with *R. macrura* Loew, 1871. However, the two species can be differentiated by combination of the following characters: *R. macrura* is entirely black setose species with dark halter and peculiar comb of ventral setae on mid basitarsus. Moreover, male terminalia also differs slightly: basal part is bent anteriorly and epandrial lamellae are much narrower, whilst the new species has yellow halter, mid basitarsus without peculiar setae, basal part of terminalia not bent anteriorly and the epandrial lamellae are broader.

#### Notes on SEM morphology of *Rhamphomyia bhagati* sp. nov.

The male is generally similar to the female, except for the normal sexual dimorphism. The general morphology of the male specimen with the ratios of body appendages can be observed on Fig. 4.

#### Head, antennae and mouthparts

The ommatidia of the compound eye are tightly and regular deployed 20–25 µm, with dimorphism of the ommatidia in the upper and the lower half (Fig. 5a–c). The ocelli are rounded, slightly convex, 31–47 µm, with slightly developed sclerotic rim on the edges (Fig. 5b). All antennomeres are deeply covered by numerous short and fine sclerotic microtrichia without sockets and different number of sensilla with visible sockets (Fig. 5d). Lateral sides of the scape and pedicel bear quite long, thick, rigid and deeply ribbed type III trichoid sensilla with pointed apices (Fig. 5e), whereas the pedicel bears additionally much shorter type IV trichoid sensilla with very fine and pointed apices (Fig. 5f). Type III trichoid sensilla on the scape are 22–25 µm long whereas those on the pedicel are 30–60 µm long. Type IV trichoid sensilla on the scape are only 5–6 µm long. The postpedicel is the larger segment with expanded and convex ventral basal part (Fig. 5g). The whole surface of this segment is densely covered by numerous short and fine microtrichia and its ventral side bears numerous basiconic sensilla (most probably type I) (Fig. 5g–h). The sensilla are rather regular, lengthwise located. They are 5–9 µm long, mostly covered by wax layer but on the raw sensilla porous surface can be seen (Fig. 5i). The stylus is densely covered by numerous microtrichia, its very apical part (mechanoreceptor) is raw and seems to be porous (Fig. 5j). Labrum is covered by a



wax layer with pointed epipharyngeal blades (Fig. 5k–l). The labium is densely covered by numerous microtrichia, with thick long setae (chaetic sensilla), especially on the dorsal side of labellum (Fig. 5m–n) and well-visible pseudotracheae (Fig. 5o).

#### *Thorax, wings and legs*

The thorax is densely covered by numerous, thin, short and pointed microtrichia. They are slightly curved, have rounded apices and a lot of wax secretion can be noted between them. The dorsal side of thorax is covered by thin, fine and pointed trichoid sensilla and very long, thick, rigid and pointed chaetic sensilla. Both kinds of sensilla are furthermore ribbed and the chaetic sensilla arise from large, protuberant sockets (Fig. 6a–f). The wing has a distinct wax layer, covered by numerous microtrichia (wing membrane) and chaetic sensilla, especially on wing edges and wing articulation (Fig. 6g, i–l). The surface of the basal part of the wing and wing articulation is densely covered by small pillow-shaped structures with short and conical projections (Fig. 6h). The chaetic sensilla are thick and ribbed as on other parts of the thorax (Fig. 6i). The microtrichia are deployed regularly, are fine, almost pointed and also slightly ribbed especially near the basal half (Fig. 6j–l). The halter is covered by numerous microtrichia with well-visible sensilla plates on the scabellum and pedicellus and a large oval capitellum (Fig. 7a–c). Dorsal scabellus is characterized by well-visible and densely arranged spherical and protuberant basal plate sensilla (Fig. 7d), which also have a linear orientation similar to flanking sensilla on the dorsal pedicel stem (Fig. 7e). The capitellum, besides short, fine and pointed microtrichia, bears rather single trichoid and campaniform sensilla (Fig. 7f). The ventral side of the halter is rather similar but only on the ventral pedicel,

linearly arranged flanking sensilla are visible and trichoid sensilla on the capitellum (Fig. 7g–i).

The legs are densely covered by numerous short, fine and pointed microtrichia and mechanoreceptors. Many long, fine and pointed trichoid sensilla are found on coxae (Fig. 8a). On the basal inner side of femora a single campaniform sensillum can be found. The campaniform sensilla is about 5.5–6.5 µm in diameter with quite protuberant inner part and without visible pore (Fig. 8b–c). The femora bear besides microtrichia, fine and pointed trichoid sensilla on the dorsal side and rigid and pointed chaetic sensilla (Fig. 8d). Both kinds of sensilla are ribbed (but the trichoid sensilla are deeper ribbed) and are characterized by well-developed and protuberant sockets (Fig. 8e–f). Chaetic and trichoid sensilla are numerous on the tibiae where they are located rather regularly (Fig. 8g), having the same morphology (Fig. 8h) with the exception that some chaetic sensilla on the ventral side have elongated and very fine apices (Fig. 8i). Distal parts of tibiae and tarsomeres are characterized by larger numbers of chaetic sensilla, while distal part of the last tarsomere bears more trichoid sensilla (Fig. 8j–l). Claws with proximal halves covered by pointed microtrichia. The distal halves are curved and ribbed (Fig. 8m). Ventral side of the pulvilli are characterized by numerous and regularly arranged tenent setae with flat capitate terminal plates (Fig. 8n–o).

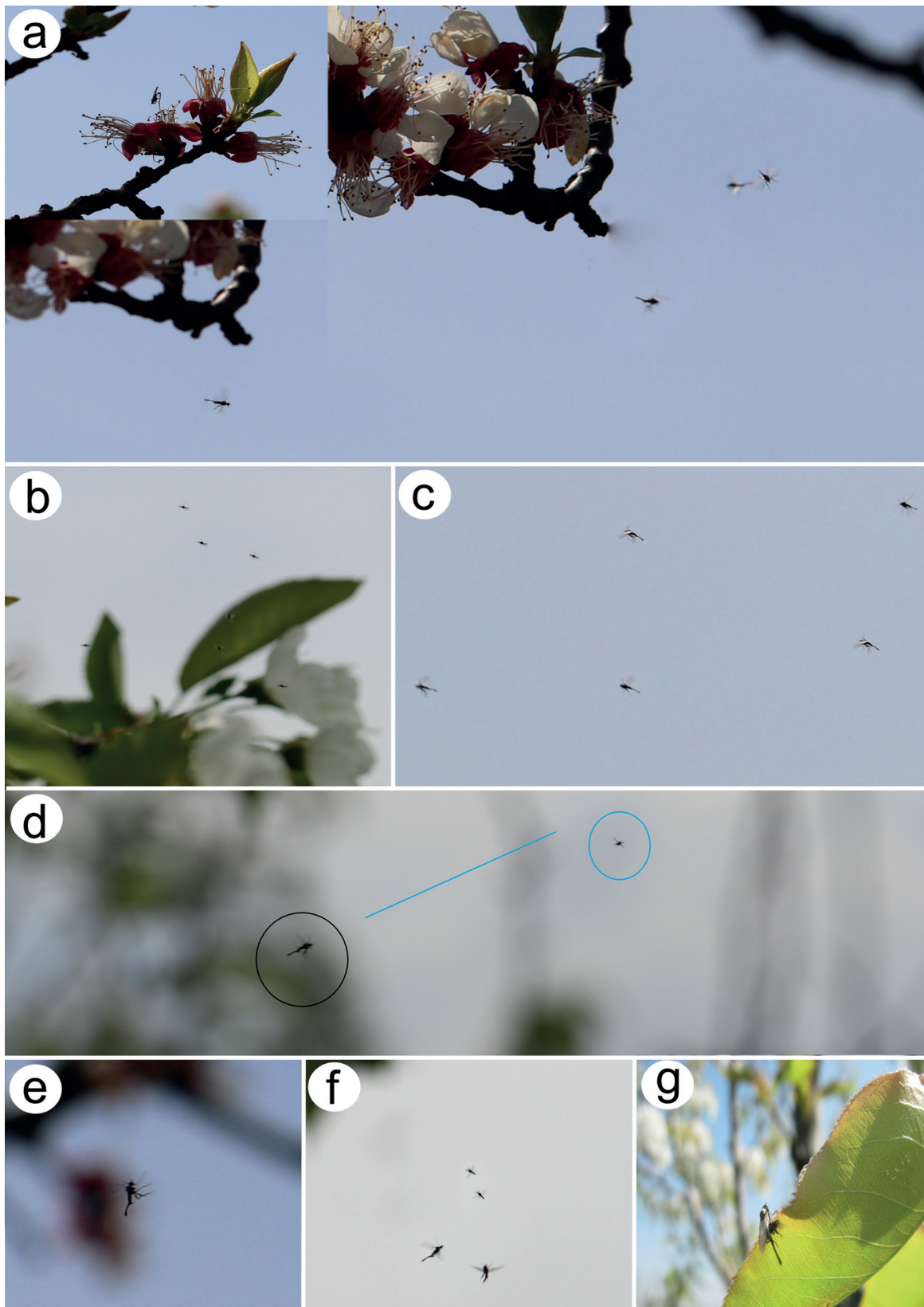
#### *Male and female terminalia*

The terminal part of the male abdomen is densely covered by very long, rigid and pointed chaetic sensilla (Fig. 9a–b). The basal part of the phallus is very smooth, enlarged, with an evident indentation on the ventral side (Fig. 9c–d). The rest of the phallus is also smooth but often covered by waxy secretions, especially on the apex which is double pointed (Fig. 9e–f). The cercus and ep-

**Table 1.** Descriptive analysis of observed courtship characters.

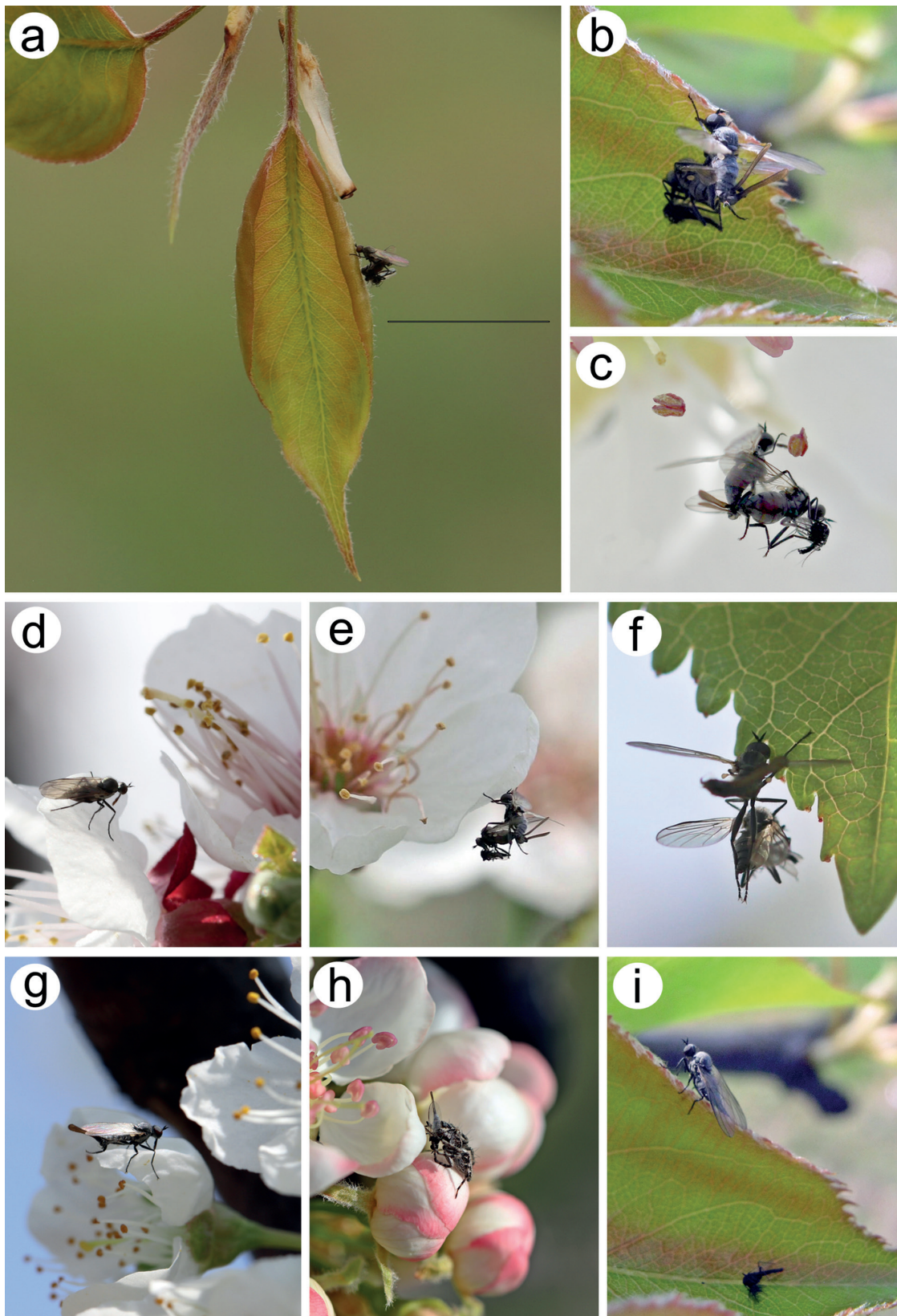
Character	ANOVA test results						
	Source of Variation						
	between groups	SS	df	MS	F	P-value	F crit
	within groups						
LH	between groups	318.94	2	159.47	2.47	0.08	3.06
	within groups	8521.46	132	64.55			
ML	between groups	0.42	2	0.21	1.62	0.20	3.06
	within groups	17.46	132	0.13			
FL	between groups	0.95	2	0.47	1.61	0.20	3.06
	within groups	38.92	132	0.29			
DC	between groups	1000.84	2	500.42	0.98	0.37	3.07
	within groups	60760.34	120	506.33			

Abbreviations: LH = Lek height; ML = Male length; FL = Female length; DC = Duration of copulation



**Fig. 10.** Courtship rituals of *Rhamphomyia bhagati* sp. nov. **a.** Male occupy terminal tips prior to aerial flights for raids. **b.** Aerial nematoceran swarms. **c.** Aerial hunting male swarms. **d–f.** Aerial raids on male nematocerans. **g.** Male with captured prey.





**Fig. 11.** Courtship rituals of *Rhamphomyia bhagati* sp. nov. **a.** Tangled male and female land on the leaf. **b–c.** Male and female in copulation. **f, i.** Female terminates copulation, and female with leftover prey. **e, h.** Male terminates copulation and predated by spider. **d, g.** Male and female resumes flower visiting.

**Table 2.** Correlation among factors affecting duration of copulation.

Correlation table (n = 572)		
	DC	LH
FL	-0.04	-0.06
ML	0.08	-0.07
LH	-0.04	1

andrium are characterized by numerous microtrichia, trichoid and long chaetic sensilla (Fig. 9g–i). The female terminal segments (besides the very last ones and cerci), especially the tergites appear with less microtrichia as they are partially retracted into the proximal segment (Fig. 9j–l), while the ventral sides and cerci are characterized by more chaetic sensilla (Fig. 9m–o).

### Courtship rituals

The male and female flies are frequent flower visitors, without showing any strong territoriality. These are observed visiting flowers from April to June with increase in incidence towards the middle half of June. Most of the major temperate fruit crops from the region are visited by these insects. However, it seems that the white floral varieties of almond, apple, cherry, pear, plum, and quince plants are preferred over pink floral varieties of apricot, peach and nectarine. Apart from being flower visitors, these flies are seen forming courtship lek swarms during different phases of the day. On cloudy, cooler days with suitable conditions, lek swarms are more frequently seen compared with hot days. Most of the lek sites orient in open areas in an otherwise complete canopy (Fig. 10a). The aerial swarms were usually formed at  $74.83 \pm 8.12$  cm from the ground with lower and higher heights of 60 and 95 cm respectively. These flies usually do not form swarms above 100 cm, although may attain individual flight heights of about 300–500 cm. Before the formation of mating swarms, males raid small nematocerans (Fig. 10b–f) and carry this prey as nuptial gifts to the lek swarms, where other males also display their captured raid to the nearby hovering females. All males capture small male chironomid midges of a particular species, and no polymorphism in gift giving was observed. The males after capture of midges may settle on nearby vegetation and reorient the prey, before entering the swarm (Fig. 10g) or they may directly enter the lek after capturing the prey. The prey presentation to the female by male occurs in the air. The male displays captured prey by holding it in between the hind legs and hovering near females in air. Females approach the males in the mid-air for accepting the prey and to mate. Coupling takes place abruptly and as the female accepts the gift, a male gets a better hold of the female in the air with his mid legs.

Soon the tangled couple with prey settles along the underside of a leaf with the help of the forelegs of the male (Fig. 11a). The couple rearranges with the female getting a proper hold of the prey and starting to consume it, while the male gets hold of the leaf with its forelegs and orients for the genital union (Fig. 11b–c). It was observed that the extremely elongate phallus helps detain an initially wilting female until the ejaculate is transferred. The mean duration of copulation (seconds) was  $137.27 \pm 23.57$ , ranging from 87–190. Upon completion the male terminate copulation by releasing its hold of the vegetation. This makes the pair separate either on downward descent or on impact with the ground. The mated pair is frequently also disturbed by other insects. In these cases mainly the female terminates the copulation, by beating her wings and it may stay with the prey for some time (Fig. 11f–i). If mating was interrupted in an earlier stage, the male keeps hold of the prey and re-enters the lek (Fig. 11e); sometimes interruption to mating is caused by predation of either male or female by spiders, robber flies and other predator insects (Fig. 11h). Excluding these episodic behavioral events, both male and female suck nectar to fulfill most of their dietary requirements (Fig. 11d, g).

The correlation coefficient exhibited a non-significant negative relationship of female body length with both duration of copulation and lek height from the ground (Tables 1–2). Females with higher body sizes usually take at a lower lek height and have lower duration of copulation, as against females of lower body size who exhibit a higher duration of copulation and show a higher lek height from the ground. Males with larger body size exhibited a higher duration of copulation ( $r = 0.08$ ,  $p > 0.05$ ), however, with lek height, a non-significant negative relationship was recorded ( $r = -0.07$ ,  $p > 0.05$ ). A non-significant negative relationship also existed between duration of copulation and lek height ( $r = -0.04$ ,  $p > 0.05$ ), suggesting that at lower heights the duration of copulation was higher and vice-versa. This may be explained by environmental factors like wind and gravity, which makes the attachment to the leaves continuously challenging for the compromised couples. However, the statistical significance of the various observations exhibited that except for the lek height, none of the other variables were statistically significant at 95% probability and require further understanding.

### Conclusion

Several hypotheses have tried to correlate diversification rate and traits presumed to intensify sexual selection. The typical empidine suite of mating behaviors is of particular interest for understanding such proposed hypotheses and processes of speciation. During the present study, observations were made on some of the courtship rituals performed by the new species. Whether some of the



exhibited dimorphic sexual structures have any impact to facilitate speciation and scale relationships among conspecific members, needs to be explored further. By using SEM, a more precise picture of the body surface of these insects was revealed, which in future can facilitate and clarify function of various parts of the body and even assist in character identification and misidentification aspects in their systematics.

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