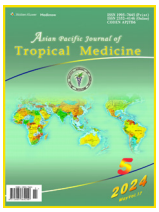

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# Morphological and molecular description of a new species of sandfly, *Sergentomyia (Neophlebotomus) ashwanii* sp. nov. (Diptera: Psychodidae) from Western Ghats, India

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## ABSTRACT

**Objective:** To report a new species of sandfly, *Sergentomyia (Neophlebotomus) ashwanii* sp. nov. (Diptera: Psychodidae) from Western Ghats, India.

**Methods:** A systematic sandfly survey was conducted in the Thrissur and Kollam districts of Kerala, India using mechanical aspirators, light and sticky traps, both indoor and outdoor habitats, for a period of one year. Deoxyribonucleic acid barcoding of samples was performed targeting mitochondrial cytochrome oxidase I (*COI*) gene and sequence generated was subjected to phylogenetic analysis.

**Results:** *Sergentomyia (Neophlebotomus) ashwanii*, a new sandfly species is recorded and described in this communication. A single row of 10-12 pointed teeth in the cibarium with 4-6 small denticles or fore-teeth are the key characteristics that is distinctive from other members of the subgenus *Neophlebotomus*. Mitochondrial COI barcode followed by phylogenetic analysis of the nucleotide sequence confirms that specimens of the species belong to the same taxonomic group while the genetic distance (14.2%) with the congeners established it to be a different species.

**Conclusions:** The Western Ghats' being an important biodiversity hotspot and has dearth of systematic entomological surveys on sandflies. The current study tried to fill the void and also report a new sandfly species.

**KEYWORDS:** *Sergentomyia (Neophlebotomus) ashwanii*; COI barcode; Western Ghats; Phlebotomine sandflies

## 1. Introduction

Phlebotomine sandflies (Diptera: Psychodidae) are small, hematophagous insects feeding on different vertebrate hosts reliant on species[1]. These insects have a wide distribution in tropical and sub-tropical regions, including some parts of temperate regions. The distribution of these flies is greatly dependent on confined environmental factors such as humidity, temperature, rainfall, habitat and host availability, soil type *etc.*[2]. About 1 000 species of sandflies have been reported so far from all around the globe[3], of which approximately 10% (including both New and Old world species) have been proven to be involved in transmission of

**Significance**

The Western Ghats' being an important hotspot for biodiversity, lacks systematic surveys on sandflies. The present study aimed to enrich knowledge on the sandfly fauna of two districts of Kerala, India. During this entomological survey, a new species of sandfly was recorded, and its morphological and molecular descriptions are discussed in this communication. This study makes an effort to fill the gap in systematic surveys and reports a new species.

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various pathogens and parasites such as *Bartonella bacilliformis*, Chandipura virus and *Leishmania etc.*[1]. Among these the protozoan parasite-*Leishmania* contributes for about 1 million new cases of leishmaniasis annually[4].

Leishmaniasis is one of the neglected tropical diseases and is endemic in many countries like India, Brazil, Sudan, Bangladesh, Ethiopia and Nepal for many centuries[4]. In Indian sub-continent Western Ghats have emerged to be an endemic belt for visceral leishmaniasis and cutaneous leishmaniasis in the last few decades (2003-2020), with more than 30 new case reports[5]. The dense forests of Western Ghats are biologically rich and bio-geographically unique with an account of more than 30% of all mammal, fish, bird, and plant species found in India[6] including around 350 globally endangered spices[7]. Considering the fact that the Western Ghats is one of the world's hottest hotspots of the biological diversity[7], many animal and plant species remain yet to be discovered in this region.

From India, a series of taxonomic research on sandflies have been published[8–10]. In 1978 and 1987 sandfly fauna of the Oriental Regions, including India was thoroughly researched by Lewis[11,12]. Subsequently, sandfly distribution in several areas of southern India was documented by Lewis[11], Kaul[13,14], Ilango[15], Ilango[16,17], Renganathan and Purushothaman[18], Srinivasan and Jambulingam[19,20] and Srinivasan[21,22]. However, in a recent review article on faunal richness of sandflies in India has reported that still many species of sandflies endure unreported and ungrouped in the country[23]. We undertook a systematic entomological survey on sandflies in the Thrissur and Kollam districts of Kerala, India. During the survey a novel species of sand fly was encountered which is described here as *Sergentomyia (S.) (Neophlebotomus) ashwanii* sp. nov.

## 2. Materials and methods

### *2.1. Study area*

The study was conducted in the Kadaar tribal settlement (10°22'05.5"N 76°26'32.8"E) of Thrissur (Panchayat: Mattathoor; Taluk: Mukundapuram) and Kani tribal settlement (8°56'23.5"N 77°1'48.1"E) of Kollam (Panchayat: Kulathupuzha; Taluk: Pathanapuram) districts in Kerala for a period of one year, January 2022-December 2022 (Figure 1).

These tribal hamlets are strewn in the mountain forest range of Western Ghats of southern India. Locally known as the Sahyadri, this hilly region of Kerala is actually situated on the Deccan Plateau's edge, which divides the plateau from a confined coastline alongside of the Arabian Sea. The Ghats are covered with a wide variety of vegetation, including grasslands, dry and humid deciduous woods, evergreen and semi-evergreen forests, scrub jungles, and more. Coconut, rubber, pepper, jackfruit and teak plantation within these forests are some of the major source of livelihood for these tribes. The region's heritage has been preserved and protected due to the region's complex topography and heavy rains. In-addition to this, the forest under these ranges have been categorised under reserve forest and are being guarded by forest rangers deputed by Kerala Government.

The tribes inhabiting in these ranges are now living in dwellings made of concrete blocks under an initiative by Kerala Government for development of tribal population. Since these tribes rely on the forest for their source of income they move to interior parts of the forest. Hence, the house abandoned by these tribes with moisture and humidity due to rain provides favourable microhabitat for sandfly breeding. Within these settlements houses are located at 100-200 meters apart with around a total of 70-80 houses. Sandfly collection were conducted using standard methods like mechanical aspirators, light and sticky traps both in indoors (cattle sheds and human dwellings) and outdoors (rodent burrows, tree holes, termite mounts *etc.*). Resting collections employing mechanical aspirator was made during morning hours *i.e.*, 09:00 h and 12:00 h, whereas, trap collections were made from evening 18:00 h (preceding day) to 08:00 h (following day).

Sandfly specimens were transported to Indian Council of Medical Research-Vector Control Research Centre, Field Station at Kottayam and were preserved in 70% ethanol. The samples were dissected under stereo-microscope (Weswox Optik-SZM-100) and mounted in Hoyer's medium on microscopic slides. The sandfly specimens were identified to species by examining under Zeiss binocular microscope (Primostar 3, Carl Zeiss Suzhou Co., Ltd.,) following standard keys[11,12,24]. A few specimens did not match to the characters of the reported species in the keys. Taxonomic features of these specimens (both female and male) are distinct from the congeners sandfly species (sub genus *Neophlebotomus*); such as *Sergentomyia (Neophlebotomus) monticola*, *S. (Neophlebotomus) malabarica* and *S. (Neophlebotomus) gemmea*, which have been already described[11]. The morphometric analysis of the new sandfly specimens was carried out using Zeiss binocular microscope aided with a micrometre. All measurements were recorded in millimetre (mm). Photographs of unique identifying features of female and male specimens were taken using camera mounted over the same microscope. A holotype female, allotype male, and 9 paratypes female and male samples were used for morphometric analysis. Terminologies of the characteristics for description were adopted from Lewis[11,12]. Nomenclature was adopted following the guidelines given by the International Code of Zoological Nomenclature (ICZN)[25]. Measurements (mm) of *S. (Neophlebotomus) ashwanii* sp. nov. (holotype female and allotype male) are described below (Table 1).

DNA barcoding of both male and female samples were performed

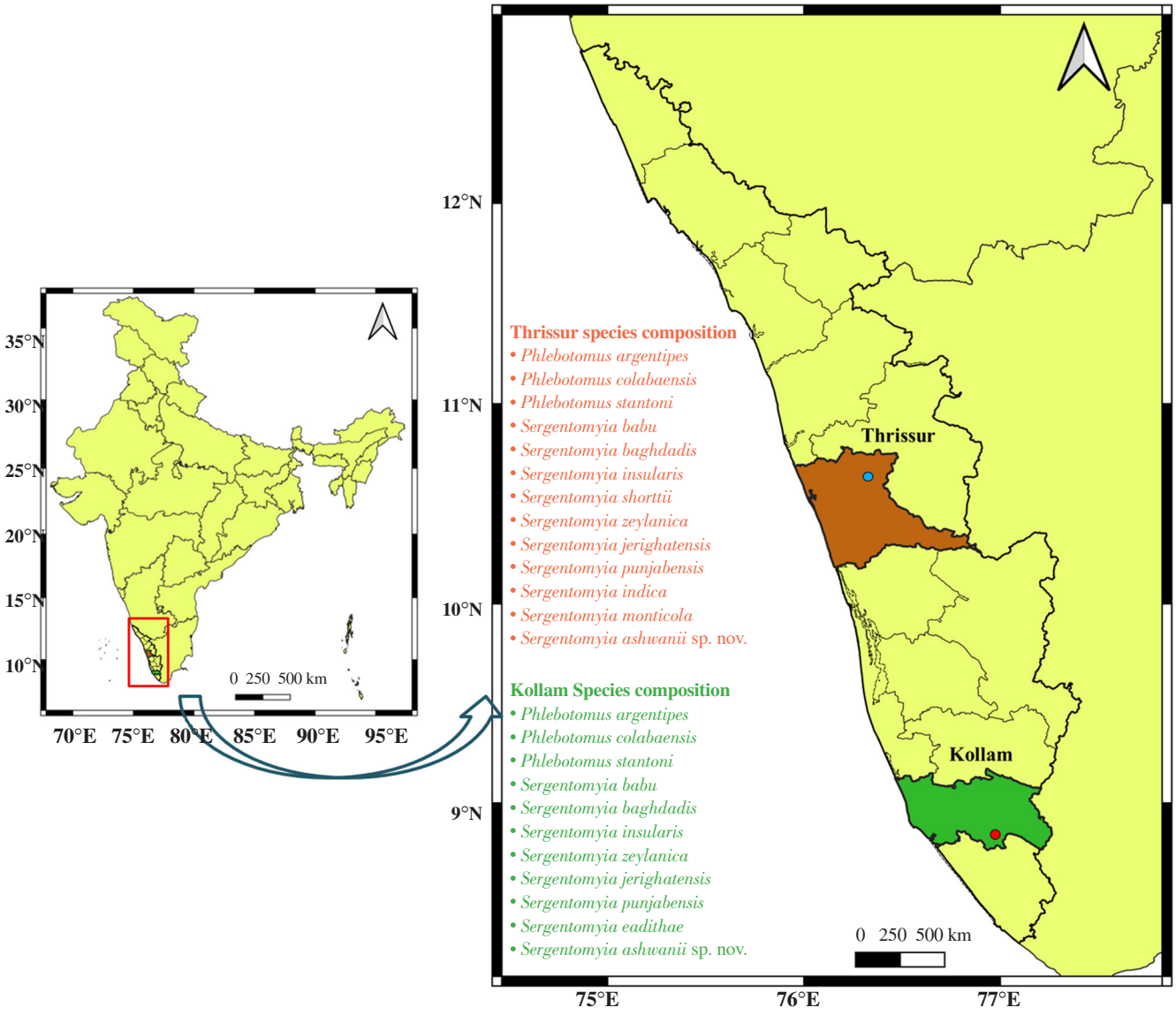


**Figure 1.** Study area in Thrissur and Kollam districts of Kerala, India along with their species composition.

targeting mitochondrial cytochrome oxidase I (*COI*) gene (~720 bp). The gene was amplified following the protocol described by Kumar *et al.* 2012[26]. The nucleotide sequences generated were deposited in GenBank. For phylogenetic analysis, the sequences were blasted with nucleotide repository (GenBank) and the sequences were aligned using MEGA 7.0 using Kimura 2.0 parameter to estimate the genetic distance.

## 3. Results

### *3.1. Diagnosis of Sergentomyia (Neophlebotomus) ashwanii sp. nov.*

The features of recumbent hairs on abdominal tergites (2-6) found in both the sexes are specific to the genus *Sergentomyia*. Cibarial teeth are parallel, often equal but not very narrow. Antenna Ⅰ is longer than Ⅰ + Ⅱ. Spermathecae are thin-walled capsule and with cross-striations in female. Aedeagus is slender with blunt tip and paramere with hairy tubercule on the ventral side in male. These taxonomic characters confirm the inclusion of the species into subgenus *Neophlebotomus* of genus *Sergentomyia*. Cibarium had a row of 10-12 hind teeth in both the sexes. In females, the hind teeth are arranged in a single row, tapering to fine points. In males teeth are not very distinct. In addition to the hind teeth, both sexes bear 4-6 small denticles or fore-teeth (distinct in female, while indistinct in male). These features are distinctive in holotype, allotype and paratype female and male *S. (Neophlebotomus) ashwanii* sp. nov.

### *3.2. Female*

Holotype female: General colour of the specimen is homogeneous

**Table 1.** Morphometric parameters of female *Sergentomyia (Neophlebotomus) ashwanii* sp. nov. (in mm).

| Morphometric parameters | Female (*n*=10) | | | | Male (*n*=10) | | | |
|---|---|---|---|---|---|---|---|---|
| | Max | Min | Mean | SD | Max | Min | Mean | SD |
| **Main characters** | | | | | | | | |
| Body length | 1.88 | 1.80 | 1.86 | 0.02 | 1.75 | 1.68 | 1.71 | 0.03 |
| Head length | 0.36 | 0.33 | 0.35 | 0.01 | 0.34 | 0.30 | 0.31 | 0.01 |
| Head width | 0.43 | 0.38 | 0.40 | 0.01 | 0.34 | 0.31 | 0.33 | 0.01 |
| Interocular distance | 0.20 | 0.16 | 0.18 | 0.01 | 0.16 | 0.15 | 0.16 | 0.01 |
| Labarum | 0.17 | 0.15 | 0.16 | 0.01 | 0.15 | 0.13 | 0.14 | 0.01 |
| No. of maxillary ventral teeth | - | - | 17.00 | - | - | - | - | - |
| No. of maxillary lateral teeth | - | - | 7.00 | - | - | - | - | - |
| Palpomere length P1 | 0.09 | 0.07 | 0.08 | 0.01 | 0.07 | 0.07 | 0.07 | 0.00 |
| Palpomere length P2 | 0.12 | 0.10 | 0.11 | 0.01 | 0.10 | 0.10 | 0.10 | 0.00 |
| Palpomere length P3 | 0.14 | 0.11 | 0.13 | 0.01 | 0.12 | 0.10 | 0.11 | 0.01 |
| Palpomere length P4 | 0.15 | 0.13 | 0.14 | 0.01 | 0.13 | 0.11 | 0.13 | 0.01 |
| Palpomere length P5 | 0.34 | 0.24 | 0.31 | 0.02 | 0.28 | 0.26 | 0.27 | 0.01 |
| Antenna Ⅰ (A Ⅰ) | 0.24 | 0.21 | 0.23 | 0.01 | 0.25 | 0.15 | 0.24 | 0.03 |
| Antenna Ⅱ (A Ⅱ) | 0.11 | 0.09 | 0.10 | 0.01 | 0.11 | 0.11 | 0.11 | 0.00 |
| Antenna Ⅲ (A Ⅲ) | 0.11 | 0.09 | 0.10 | 0.01 | 0.11 | 0.11 | 0.11 | 0.00 |
| Sensilla chaetica in A Ⅱ | 0.03 | 0.03 | 0.03 | 0.00 | 0.03 | 0.03 | 0.03 | 0.00 |
| No. of teeth in cibarium | - | - | 10-12 | - | - | - | 10-12 | - |
| Pharynx length | 0.16 | 0.13 | 0.14 | 0.01 | 0.15 | 0.12 | 0.13 | 0.01 |
| Pharynx width | 0.06 | 0.05 | 0.05 | 0.01 | 0.05 | 0.04 | 0.04 | 0.01 |
| Pharyngeal armature (depth/length) | 0.03 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.02 | 0.01 |
| Wing length | 1.63 | 1.45 | 1.54 | 0.05 | 1.50 | 1.28 | 1.40 | 0.08 |
| Wing width | 0.45 | 0.35 | 0.41 | 0.03 | 0.30 | 0.25 | 0.27 | 0.02 |
| **Principal vein length** | | | | | | | | |
| Alpha | 0.35 | 0.28 | 0.30 | 0.02 | 0.28 | 0.25 | 0.26 | 0.01 |
| Beta | 0.35 | 0.28 | 0.32 | 0.02 | 0.30 | 0.25 | 0.28 | 0.02 |
| Gamma | 0.40 | 0.20 | 0.28 | 0.05 | 0.25 | 0.20 | 0.23 | 0.02 |
| Delta | 0.18 | 0.13 | 0.16 | 0.02 | 0.13 | 0.10 | 0.11 | 0.01 |
| Radius 5 length | 1.23 | 1.08 | 1.14 | 0.04 | 1.05 | 1.00 | 1.02 | 0.02 |
| **Fore leg** | | | | | | | | |
| Coxa | 0.38 | 0.25 | 0.29 | 0.04 | 0.28 | 0.25 | 0.26 | 0.01 |
| Trochanter | 0.08 | 0.07 | 0.07 | 0.01 | 0.07 | 0.07 | 0.07 | 0.00 |
| Femur | 0.80 | 0.53 | 0.61 | 0.08 | 0.55 | 0.50 | 0.53 | 0.02 |
| Tibia | 0.62 | 0.50 | 0.56 | 0.04 | 0.54 | 0.50 | 0.52 | 0.01 |
| **Tarsomeres** | | | | | | | | |
| T1 | 0.45 | 0.28 | 0.33 | 0.06 | 0.38 | 0.30 | 0.34 | 0.03 |
| T2 | 0.25 | 0.15 | 0.19 | 0.03 | 0.25 | 0.18 | 0.21 | 0.03 |
| T3 | 0.15 | 0.10 | 0.13 | 0.01 | 0.15 | 0.10 | 0.13 | 0.02 |
| T4 | 0.15 | 0.10 | 0.12 | 0.01 | 0.13 | 0.10 | 0.12 | 0.01 |
| T5 | 0.08 | 0.08 | 0.08 | 0.00 | 0.08 | 0.08 | 0.08 | 0.00 |
| Spermatheca length | 0.63 | 0.55 | 0.58 | 0.02 | - | - | - | - |
| Spermatheca width | 0.25 | 0.23 | 0.24 | 0.01 | - | - | | - |
| Spematheca duct length | Not clear | | | | - | - | - | - |
| Cerci length | 0.16 | 0.10 | 0.12 | 0.02 | - | - | - | - |
| Genital pump length | - | - | - | - | 0.10 | 0.10 | 0.10 | 0.00 |
| Genital filament length | - | - | - | - | 0.31 | 0.29 | 0.30 | 0.01 |
| Genital filament/genital pump ratio | - | - | - | - | - | - | 3.02 | - |
| Coxite length | - | - | - | - | 0.20 | 0.18 | 0.19 | 0.01 |
| Style length | - | - | - | - | 0.09 | 0.08 | 0.09 | 0.00 |
| Style spine length | - | - | - | - | 0.09 | 0.08 | 0.09 | 0.00 |
| Length between terminal and sub-terminal spines | - | - | - | - | 0.02 | 0.01 | 0.01 | 0.00 |
| Location of accessory spine from sub-terminal spine | - | - | - | - | Below the sub-terminal spine | | | |

N: number of specimen; Max: maximum; Min: minimum; SD: standard deviation; -: not applicable; Since almost all value for the measured specimen were same, SD calculated was less than 0.01 and we have mentioned those SD values as 0.00 in the table.

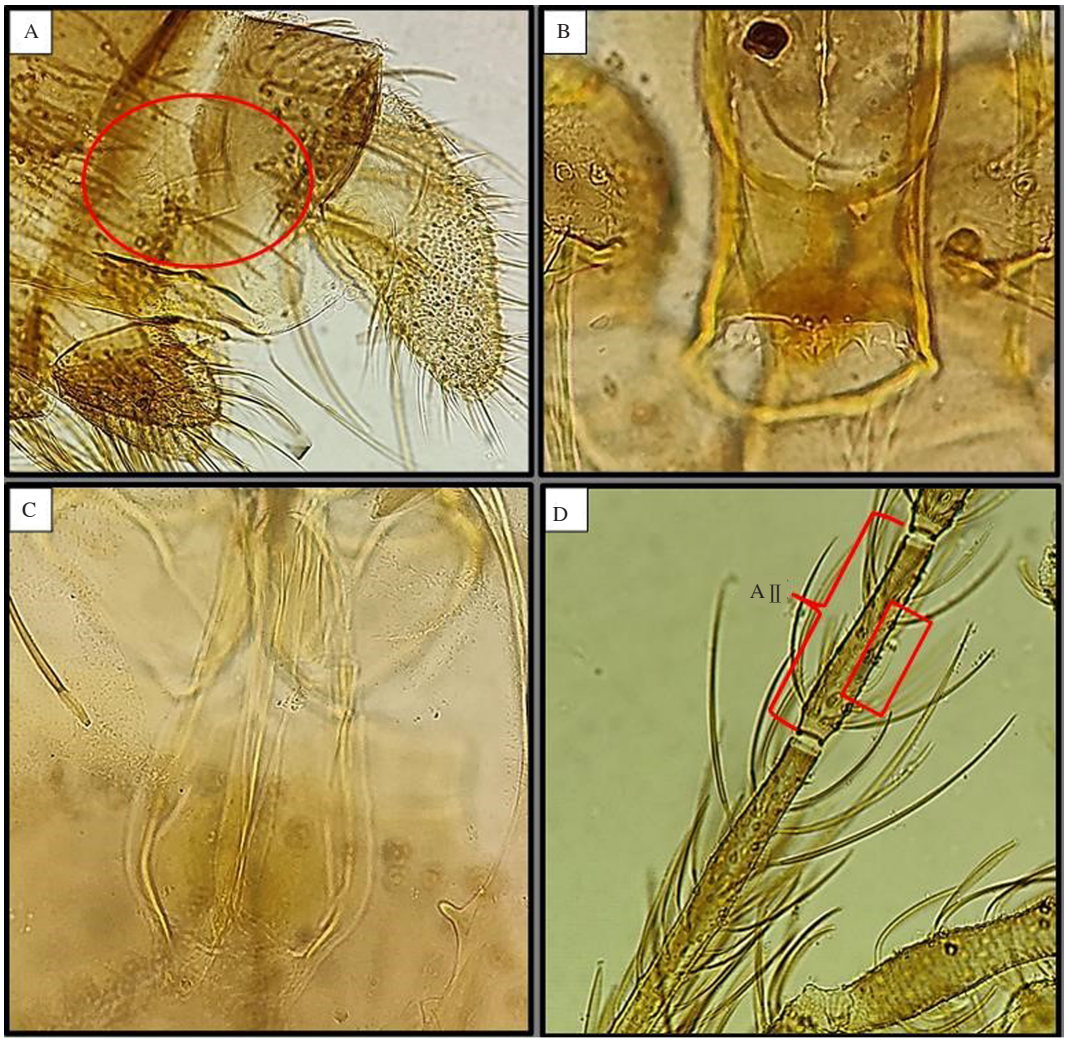


**Figure 2.** Female *Sergentomyia (Neophlebotomus) ashwanii* sp. nov.. A: spermathecae; B: Cibarium; C: Pharynx; D: Antenna with ascoid.

light brown. Body size 1.86 mm. Head: length 0.35, width 0.40. Interocular distance 0.18. Number of maxillary ventral teeth: 17; lateral teeth: 7. Palps: formula 5, 4, 3, 2, 1. Palpomere measurements: P1 0.08, P2 0.11, P3 0.13, P4 0.14 and P5 0.31. Antenna: A Ⅰ 0.23, A Ⅱ 0.10, A Ⅲ 0.10 (A Ⅲ > A Ⅰ + A Ⅱ). Antennal formula: each antenna (A Ⅰ-A Ⅺ) has two ascoids, one on either side. Ascoid length in A Ⅱ: 0.03. Cibarium: the ventral plate bears cibarial teeth (hind teeth) and denticles (fore-teeth), dorsal plate with light golden brown coloured pigment patch, which is funnel shaped. No dorsal process. Cibarium bears 10-12 varying teeth. All the teeth are arranged in a single row, tapering to fine points. There are 4-6 small denticles in the ventral plate, arranged in a row, adjacent to cibarial teeth. Pharynx: nearly slender shaped, unarmed with minute spicules, length 0.14, width 0.05, pharyngeal armature depth 0.02. Wings: length 1.54, width 0.41. Length of principal vein sections: alpha 0.30, beta 0.32, gamma 0.28, delta 0.16, R5 length 1.14. Fore leg: coxa 0.29, trochanter 0.07, femur 0.61, tibia 0.56, tarsomeres: T1 0.36, T2 0.20, T3 0.13, T4 0.12, T5 0.08. Genitalia: Spermathecae: thin-walled capsule with cross-striations. Length 0.58, width 0.24. Spermatheca with secretary cells at the distal end, narrow individual spermathecal duct, length (not clear). Common spermathecal duct: not evidently visible. Cerci (simple) length 0.12 (Figure 2).

### *3.3. Male*

Allotype male: Same colour as the female specimen. Body size 1.71 mm. Head: length 0.31, width 0.33. Interocular distance 0.33. Complete interocular suture. Palps formula: 5, 4, 3, 2, 1. Palpomeres: P1 0.07, P2 0.10, P3 0.11, P4 0.13 and P5 0.28. Antenna: A Ⅰ 0.24, A Ⅱ 0.11, A5 0.11. (A Ⅰ > A Ⅱ + A Ⅲ). Antennal formula: each antenna (A Ⅰ-A Ⅺ) has two ascoids, one on either side. Shape and size of ascoids (*i.e.*, Ascoid length in A Ⅱ: 0.03) similar to that noted in female. Cibarium: Cibarium bears faint 10-12 teeth in the ventral plate. The teeth are parallel but irregular. Denticles present, but are not distinct. Pigment patch present and funnel shaped (indistinct in comparison of female). Pharynx: nearly slender in shape, gradually

narrowing towards anterior part, length 0.13, width 0.04, pharyngeal armature depth 0.02. Wings: length 1.40, width 0.27. Length of principal veins: alpha 0.26, beta 0.28, gamma 0.23, delta 0.11, R5 length 1.02. Fore leg: coxa 0.26, trochanter 0.07, femur 0.53, tibia 0.52, tarsomeres: T1 0.34, T2 0.21, T3 0.13, T4 0.12, T5 0.08. Genitalia: genital-pump 0.10, length: genital filament 0.30 with striation on it, Genital filament/genital pump ratio-3.02. Aedeagus: slender with blunt tip. Paramere is hooked. Coxite: length 0.19. Style: length 0.09. Style with two terminal and two sub-terminal spines: length 0.09. The distance between terminal and sub-terminal spine: 0.01. Style present with an accessory spine. Accessory spine is located below the sub-terminal spine.

### 3.4. Variability

The morphometric characteristics indicated that the holotype, allotype and paratypes female and male were similar (Table 1). Both holotype female, allotype male were collected from the same habitat. Both sexes of *S. (Neophlebotomus) ashwanii* sp. nov. showed resemblances in the taxonomic characteristic, indicating their association. Although the structure of the cibarial teeth is varying, the number of hind teeth was 10-12 in both the sexes (Figure 2). Moreover, both male and female bears a single row of teeth in the ventral plate of the cibarium. In addition to this, DNA barcode sequences of the collected specimens showed an overall negligible genetic distance (K2P), thus suggesting a single taxonomic group. On the other hand the genetic distance with the other congeners was 14% (Figure 3).

### 3.5. Type materials

*S. (Neophlebotomus) ashwanii* constitute of 5% of the total species composition. Paratype female & male were obtained from the Kadaar tribe of Sasthampoovam tribal settlements, taluka-Mukundapuram, Panchayat-Mattathoor, District-Thrissur, Kerala, India: (10°22'05.5"N 76°26'32.8"E, altitude=262 feet). Voucher specimens, comprising both holotype female and allotype male were mounted separately on microscopic glass slides and serially numbered with details on place of collection, date of collection and type of habitat and were deposited at the museum, ICMR-Vector Control Research Centre, Puducherry-605006, India. Besides, each paratype male and female were also deposited in the National Museum, Zoological Survey of India, Alipur, Kolkata, India.

### 3.6. Etymology

Sandfly specimens of the new species were collected initially from a Kadaar tribal settlement of Sasthampoovam (Mukundapuram taluk) and later from other settlements. The new species *S. (Neophlebotomus) ashwanii* is named after Dr. Ashwani Kumar (Director, ICMR-VCRC, Puducherry) acknowledging his contribution in the field of public health entomology.

## 4. Discussion

The distribution of sandflies from the rain forests of Western Ghats in India were abridged and updated by Lewis[11,12]. The present systematic entomological survey which was impelled in the Kani and Kadaar tribes of Cheukara (Kollam) and Sasthampoovam (Thrissur) settlements respectively. These areas were selected due to their epidemiological relevance *i.e.*, case reports of leishmaniasis. The area provides favourable macro and microhabitat *i.e.*, rainfall, organic rich soil and variety of host *etc.*, proliferation and abundance of sandflies all around the year. Additionally, this investigation provides data on record of a new sandfly species from these tribal settlements.

There are several genera of sandflies, of which genus *Sergentomyia* Franca & Parrot comprised 277 species from the Old World[22]. Subgenus *Neophlebotomus*, being one of the biggest groups under the genus *Sergentomyia* includes a total of 24 species from the Oriental region[11,22]. *S. (Neophlebotomus) verghese*, kurandamalai, kottamala and cherukara[13,14] and *S. (Neophlebotomus) nilamburensis*[27] are few of the species [subgenus *S.* (*Neophlebotomus*)] which was recorded from the country. Later, this was revised to 23 species in 1978 by D.J. Lewis in his monograph titled "The phlebotomine sanflies (Diptera: Psychodidae) of the Oriental region" with nine species records from India *i.e.*, *S. (Neophlebotomus) arboris*, *chakravarti*, *dhandai, hodgsoni hodgsoni, iyengari, linearis, malabarica, purii* and *zeylanica*[11]. Further, 6 more new species under this subgenera were described from southern part of India, which were *S. (Neophlebotomus) verghese*, *kurandamalai, kottamal* and *cherukara*[13,14], *S. (Neophlebotomus) nilamburensis*[28], and *S. monticola*[22]. In this continuation, a new species, *S. (Neophlebotomus) ashwanii* sp. nov. is described through this communication.

While following the standard taxonomic keys, the female specimens of *S. (Neophlebotomus) ashwanii* sp. nov. were found to be very close to the congeners' female, such as *S. (Neophlebotomus) malabarica*, *S. (Neophlebotomus) gemmea* and *S. (Neophlebotomus) monticola*[11,22]. However, based on some peculiar taxonomic characteristics the new species specimens were separated. In *S. (Neophlebotomus) malabarica*, the female has about eight hind teeth in a nearly straight row with distinct pigment patch (pear shaped) and pharynx is almost unarmed. In *S. (Neophlebotomus) gemmea*, the cibarium has with ten hind teeth with broad bases tapering abruptly to fine points, with one row of eight very large fore teeth, two rows of small teeth in front of them, and a patch of small fore teeth at each side; pigment patch pale; arch strong. Pharynx with liner and

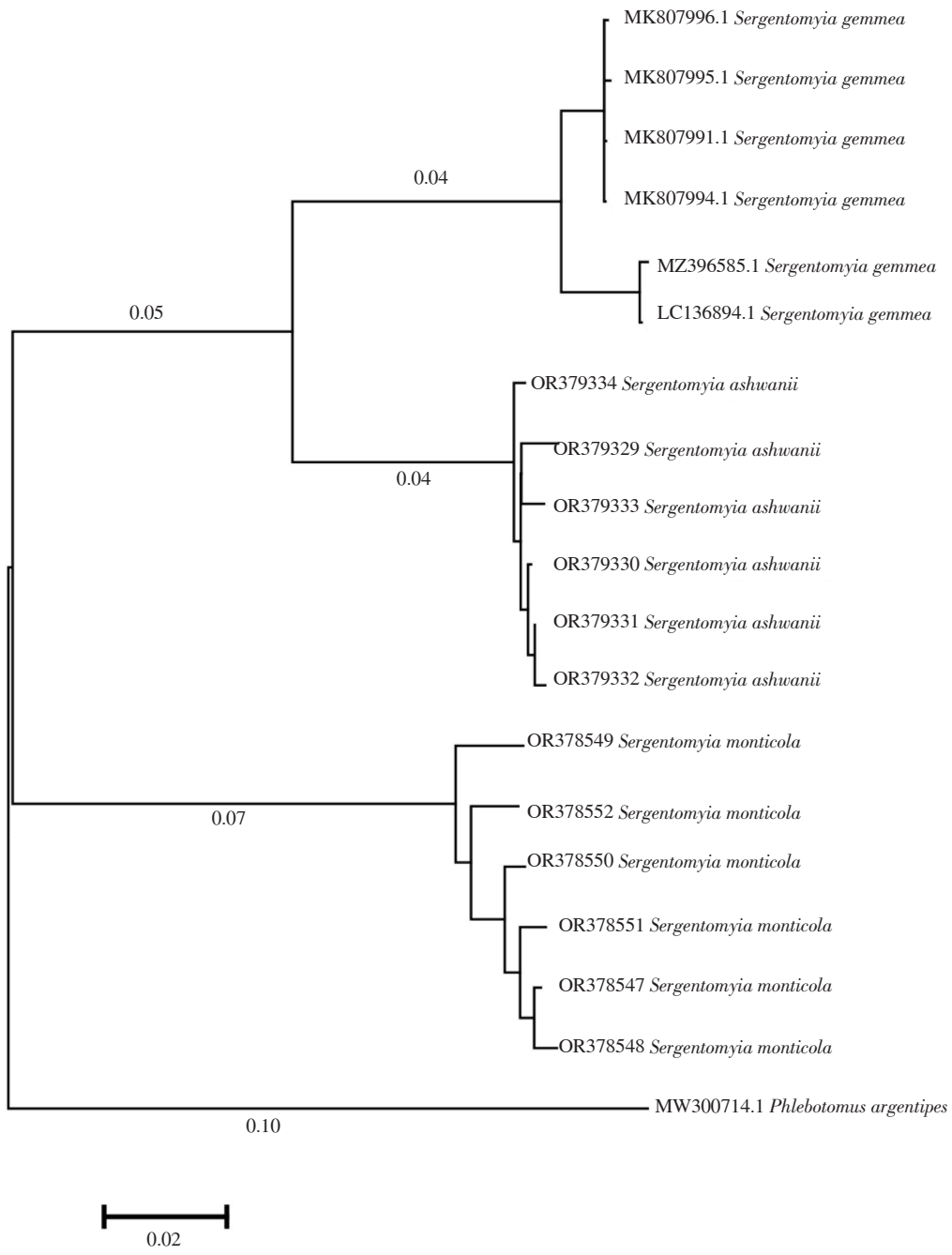


**Figure 3**. Phylogenetic tree of mitochondrial COI sequences for species of *Sergentomyia (Neophlebotomus) ashwanii* sp. nov. along with *S. (Neophlebotomus) gemmea* and monticola; outgroup; *Phlebotomus argentipes*.

with finely speculates ridges[11]. In *S. (Neophlebotomus) monticola*, female has 10 cibarial teeth and arranged in a single row parallel to one another, with broad bases, tapering abruptly to fine points and almost equal in shape and size. There are 3-4 denticles (fore-teeth) in the ventral plate, arranged in a single row, contiguous to cibarial teeth and dorsal plate with pigment patch (golden brown colour), which is dome shaped. Pharynx, almost slender and unarmed with minute spicules. Besides, all these species were lacking small, light golden coloured 4-6 denticles in a single row in the cibarium. On the other hand, *S. (Neophlebotomus) ashwanii* sp. nov. female's cibarium bears hind teeth (cibarial teeth; 10-12) and fore teeth (denticles; 4-6) in the ventral plate. Whereas, dorsal plate has a light golden brown coloured pigment patch, which is funnel shaped. Pharynx is nearly slender in shape, gradually narrowing towards anterior part. Cibarium does not possess dorsal process.

On the contrary, closely associated male specimen, which are *S. (Neophlebotomus) malabarica*, *S. (Neophlebotomus) gemmea* and *S. (Neophlebotomus) monticola* have similar spine's structural arrangement in the style to that of *S. (Neophlebotomus) ashwanii* sp. nov.[11]. In *S. (Neophlebotomus) malabarica* the cibarium is armed with ten strewn hind teeth and no fore teeth[11]. In *S. (Neophlebotomus) gemmea*, the cibarium has about 6 irregular hind teeth and about twenty irregular denticles of which a few posterior ones are marginally larger than the others; pigment patch indefinite. In *S. (Neophlebotomus) monticola*, *cibarium* has ten parallel but irregularly distributed hind teeth and in-distinct fore teeth or denticles. Whereas, in *S. (Neophlebotomus) ashwanii* sp. nov., cibarium bears faint 10-12 teeth in the ventral plate which are parallel but irregular. Fore-teeth are present, but are not distinct. Pigment patch present and funnel shaped (indistinct in comparison of female). Further DNA barcoding followed by phylogenetic analysis confirmed the association between specimens of *S. (Neophlebotomus) ashwanii* sp. nov. with a very minor genetic distance, on the other hand the genetic distance is of 14.2% with the congener species. The population genetics parameter also confirmed a very high genetic diversity ($H_{ST}$=0.915) and negligible gene flow ($N_m$=0.02). Hence, based on these taxonomic differences and molecular analysis it evidently suggests that *S. (Neophlebotomus) ashwanii* sp. nov. being a divergent from the other already reported and described species under this subgenera *S. (Neophlebotomus)*. This species were mainly collected from indoor human dwellings, but it's any role in disease transmission is yet to be explored. The Western Ghats' being an important hotspot of biodiversity and has lack of entomological surveys on sandflies. The current study tried to fill this void and also report a new sandfly species.

Since the specimen for *S. (Neophlebotomus) ashwanii* sp. nov. was collected from human settlements, its infection assessment (plausible role in disease transmission) was not carried out. Moreover, this species was only recorded in two districts of Kerala state in this report. Therefore, its distribution in other districts of Kerala and neighboring states needs to be estimated in future studies.

## Conflict of interest statement

Authors hereby declared that no conflict of interest is involved in this study.

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## Authors' contributions

PS conceptualized the study, analysed the data and corrected the manuscript, HKS analysed the data and wrote the manuscript, JM, AT and AKP collected and identified the specimen, ST carried out the molecular studies of the samples.

## Data availability

All data generated or analysed during this study are included in this published article.

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## Publisher's note



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