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► **To cite this version:**

Fano José Randrianambinintsoa, Vincent Robert, Antoine Blavier, Nicole Léger, Jérôme Depaquit. Two new phlebotomine sandfly species (Diptera: Psychodidae) from the Highlands of Madagascar. *Acta Tropica*, 2020, 202, pp.105260. 10.1016/j.actatropica.2019.105260 . hal-03079874

**HAL Id: hal-03079874**

**<https://hal.univ-reims.fr/hal-03079874v1>**

Submitted on 21 Dec 2021

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## **Article Title**

# **Two new Phlebotomine sandfly species (Diptera: Psychodidae) from the Highlands of Madagascar**

## **Authors**

Fano José Randrianambinintsoa<sup>1</sup>, Vincent Robert<sup>2</sup>, Antoine Blavier<sup>1</sup>, Nicole Léger<sup>1</sup>,  
and Jérôme Depaquit<sup>1,3</sup>

## **Affiliations**

1. Université de Reims Champagne-Ardenne, Faculté de Pharmacie,  
ANSES, SFR Cap Santé, EA7510 ESCAPE – USC VECPAR,  
51 rue Cognacq-Jay, 51096 Reims CEDEX, France
2. MIVEGEC Unit, IRD, CNRS, Univ Montpellier, 911 avenue Agropolis, BP 64501,  
34394 Montpellier CEDEX 5, France
3. Laboratoire de Parasitologie-Mycologie, CHU de Reims, 51100 Reims, France

## **Corresponding author**

Fano José RANDRIANAMBININTSOA

Phone: (+33) 3 26 91 37 23

E-mail : fanoujo@yahoo.fr

## Highlights

- First record of Phlebotomine sandflies from the highlands of Madagascar.
- They belong to two new species for science.
- Their spermathecae shows an original sclerification at the base of their bodies.
- They are considered as ungrouped *Sergentomyia* waiting the revision of this genus.

## Abstract

The Malagasy phlebotomine sandfly fauna includes 17 species: five belong to the genus *Phlebotomus*, ten to the genus *Sergentomyia*, and two to the genus *Grassomyia*. The current article describes *Sergentomyia brunhesi* sp. nov. and *Sergentomyia vistellei* sp. nov. from the Malagasy highlands. Females were described morphologically from specimens collected at Ranomafana – Ifanadiana and Andringitra. Partial molecular sequences of cyt b and 28S rDNA were retrieved for *Se. vistellei* sp. nov. Waiting new data, we consider these species as ungrouped *Sergentomyia*. Two new species of *Sergentomyia* are recorded at higher altitudes in Madagascar. Knowledge of local biodiversity is increasing. New investigations have to be carried out to describe males, to understand their bionomics, and to identify other potential new species at higher altitudes.

## Key words

*Sergentomyia*, new taxa, morphological and molecular taxonomy, Andringitra, Ranomafana-Ifanadiana

## **1. Introduction**

Apart from a few records of sandflies in the 1930's (Le Gac, 1937; Raynal and Le Gac, 1937) and then in the 1970's (Léger and Rodhain, 1978), the 21<sup>st</sup> century has provided most of the knowledge related to Malagasy phlebotomine sandflies.

The sandfly fauna of Madagascar currently includes 17 species: five belong to the genus *Phlebotomus*, 10 to the genus *Sergentomyia*, and two to the genus *Grassomyia* (Blavier et al., 2019).

Most of these sandflies are associated to microendemic biotopes, i.e., karstic stones called *tsingys* (Ankarana, Namoroka and Bemaraha (Blavier et al., 2019; Depaquit et al., 2008; Depaquit et al., 2002) found in caves (Anjohibe, Anjohikely and Anjohikinakina) (Randrianambinintsoa et al., 2014) in forests (Berenty and Mikea) (Depaquit et al., 2004b; Randrianambinintsoa and Depaquit, 2013; Randrianambinintsoa et al., 2013) and sometimes in man-made environments (Antsiranana) (Le Gac, 1937; Raynal and Le Gac, 1937). All these locations are found at low altitudes (under 200 m above sea level). To date, only one population of *Ph. fertei* (initially identified as *Se. berentiensis*) has been recorded at a relatively high altitude in Andasibe/Périnet (at 950 m a.s.l.) (Clerc et al., 1982; Depaquit et al., 2004b).

The present paper provides a description of two new species of *Sergentomyia* caught at 800 m – 1,400 m above sea level.

## **2. Materials and methods**

### **2.1. Sandfly sampling**

Sampling was carried out during field works focused on the mosquito fauna:

- In Ranomafana, district of Ifanadiana, region of Vatovavy-Fitovinany (province of Fianarantsoa), at an altitude of 1,380 m above sea level, on April 23<sup>rd</sup>, 2000. Caught in the daytime (10.30 a.m.), by hand, as it had just been spotted on a traveler's tree (*Ravenala madagascariensis*) (21°15' 33.56" S.; 47° 26' 01.07" E.).
- In Andringitra National Park, district of Ambalavao, region of Matsiatra Ambony (province of Fianarantsoa), in a small grassy clearing (5 m diameter) at an altitude of 820 m above sea level, on November 29<sup>th</sup>, 2002, 40 meters from the confluence of the Sahanivoraky and Sahavatohy rivers. Caught using CDC miniature light traps (22°13'40" S.; 47° 0' 13" E.).

## **2.2. Morphological analysis**

Sandflies were stored in 96% ethanol. They were mounted *in toto* i.e soft tissues were lysed in a bath of KOH 10% (12 hours), then washed 4 times in distilled water, cleared in Marc-André solution (12 hours), and mounted individually between microscope slide and cover slip in Canada Balsam after dehydration in successive alcohol baths (70%, 90%, 96%); or processed individually to allow for molecular biology analysis. The head, thorax and genitalia were cut off in a drop of ethanol and mounted in chloral gum directly after the Marc-André step. The abdomen of each specimen was dried and stored in a vial at –20°C before DNA extraction.

Visual analysis of the specimens was performed by means of a BX61 microscope (Olympus, Japan). Measurements and counts were made using Stream Motion software (Olympus, Japan) and a video camera connected to the microscope.

Drawings were made using a *camera lucida*.

The terms chosen for the description are the most recent ones for phlebotomine sandflies (Galati et al., 2017).

### **2.3. Molecular analysis**

Genomic DNA was extracted from the first segments of the abdomen of the sandfly using a QIAmp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions, except that we crushed the tissues with a piston pellet (Treff, Switzerland), and used an elution volume of 50 to 200 µl (Depaquit, Léger, Ferté, Robert, 2004a).

Fragments of cytochrome b (cyt b) and of the D1 - D2 28S rDNA fragments were amplified by PCR using the following primers: C3B-PDR: 5'-

CAYATTCAACCGAATGATA-3' and N1N-PDR: 5'-

GGTAYWTTGCCTCGAWTTCGWTATGA-3' for cyt b, and C1': 5'-

ACCCGCTGAATTTAAGCAT-3' and D2: 5'-TCCGTGTTTCAAGACGGG-3' for 28S rDNA (Esseghir et al. 1997, Depaquit et al., 1998).

Amplicons were analyzed by electrophoresis in 1.5% agarose gel containing Gel Green at a concentration of 0.005% V/V. Direct sequencing in both directions was performed using the primers used for DNA amplification. Sequence correction was performed using the Pregap and Gap software tools included in the Staden Package (Bonfield and Staden, 1996).

Consensus sequences were aligned using the Clustal W algorithm (Thompson et al., 1994) from the BioEdit 4.8.10 sequence editor (Hall, 1999), and corrected manually.

The sequences were compared with all the sequences available in Genbank related to the cyt b and D1-D2 sequences of sandflies from Madagascar.

Sequence data were analyzed using MEGA7 (Kumar et al., 2016) based on maximum likelihood. The maximum likelihood trees were constructed using the substitution model HKY85. All the positions containing gaps and missing data were removed from the analyses.

### 3. Results

We caught only two specimens: one female at Ranomafana and one at Andringitra.

These specimens belonged to two different species, were new ones, and are described below as *Se. brunhesi* sp. nov. and *Se. vistellei* sp. nov., respectively.

We successfully sequenced the cyt b and D1-D2 sequences of the specimen from Andringitra. Unfortunately, the specimen from Ranomafana was mounted *in toto* and no molecular data was available for it. The phylogenetic trees indicating the position of *Se. vistellei* sp. nov. are shown in figures 1 and 2.

#### ***Sergentomyia brunhesi* Léger, Randrianambinintsoa and Depaquit sp. nov.**

**(Figs. 3 – 4)**

Genus: *Sergentomyia* França and Parrot 1920

*Authorship* – Note that the authors of the new taxon are different from the authors of this paper; Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature.

#### **Description**

One female specimen was examined. The male is unknown.

*Head* (Fig. 3A): Occiput with two narrow lines of well individualized setae. On the line above the eyes, two greater insertions of setae on each side. Clypeus 150  $\mu\text{m}$  long, 60  $\mu\text{m}$  wide, with *ca.* 30 randomly distributed setae. Eyes 197/95  $\mu\text{m}$  with *ca.* 100 facets. Interantennal suture complete. Interocular suture not reaching the interantennal suture. Flagellomeres (Fig. 3B and table 1): Flagellomere 1 longer than  $f_2+f_3$ . Absence of ascoids on  $f_1$ . Ascoidal formula: 2/ $f_2$ - $f_{13}$  (including one atrophied ascoid on  $f_2$ ), as detailed in table 1. Palpi (Fig 4A – 4B): Palpi showing a very wide P3. Palpal formula: 1, 2, 3, 4, 5. Presence of many Newstead's sensilla on P3 implanted basally (Fig. 4B). Absence of Newstead's sensilla on P2. Presence of one distal spiniform seta on P3 and of five spiniform setae on P4. Labrum-epipharynx 258  $\mu\text{m}$  long (Fig. 3C). Hypopharynx without apical teeth on each side of the salivary canal (Fig. 3F). Maxillary lacinia with *ca.* 36 distal big internal teeth and *ca.* 9-10 external ones (Fig. 3E). Many microtrichia on the median part of the external side (Fig. 3E). Labium with an open labial suture (Fig 3G). Cibarium (Fig. 6C) with 72 clear and palissadic teeth organized along a horizontal line. The pigment patch is pale, triangular. Well-armed pharynx on its posterior part (Fig. 6C). Cervix: Presence of two cervical and two ventro-cervical sensilla on each side.

*Thorax*: Sclerites brown. Absence of post-alar setae. Absence of paratergital setae. Absence of proepimeral setae. Absence of upper anepisternal setae. Absence of lower anepisternal setae. Absence of anepimeral setae. Absence of metepisternal setae. Absence of metepimeral setae. Presence of setae on the anterior region of the katepisternum. Absence of a suture between the metepisternum and the katepimeron. Metafurca mounted laterally. Whether the vertical arms are joined by a membrane or not remains to be determined. Wing (Fig 4E): Length: 1,627  $\mu\text{m}$ ; width:



489  $\mu\text{m}$ .  $r_5 = 1,331 \mu\text{m}$ ; alpha ( $=r_2$ ) = 302  $\mu\text{m}$ ; beta ( $=r_2+3$ ) = 382  $\mu\text{m}$ ; gamma ( $=r_2+3+4$ ) = 341  $\mu\text{m}$ ; delta = 126  $\mu\text{m}$ ; pi = 231  $\mu\text{m}$ ; epsilon ( $=r_3$ ) = 462  $\mu\text{m}$ ; theta ( $=r_4$ ) = 848  $\mu\text{m}$ . Legs: Segment length: Anterior leg: coxa = 346  $\mu\text{m}$ ; femur = 668  $\mu\text{m}$ ; tibia = 609  $\mu\text{m}$ ; tarsomere i = 306  $\mu\text{m}$ ; sum of tii, tiii, tiv, tv = 494  $\mu\text{m}$ . Median leg: coxa = 235  $\mu\text{m}$ ; femur = 680  $\mu\text{m}$ ; tibia = 765  $\mu\text{m}$ ; tarsomere i = 383  $\mu\text{m}$ ; sum of tii, tiii, tiv, tv = 565  $\mu\text{m}$ . Posterior leg: coxa = 333  $\mu\text{m}$ ; femur = 743  $\mu\text{m}$ ; tibia = 991  $\mu\text{m}$ ; tarsomere i = 461  $\mu\text{m}$ ; sum of tii, tiii, tiv, tv = 621  $\mu\text{m}$ . Absence of spines on the metafemur. One verticil of one spine in the middle and on the distal part of the metatarsomere.

*Abdomen*: Setae randomly distributed on tergites ii–v. Tergite VIII with 16 setae on each side. Tergite IX without any protuberance. Cerci 147  $\mu\text{m}$  long. Setae not observed on sternite X.

*Genitalia* (Fig. 4D): Smooth spermathecae with rather thick walls. Their proximal part is slightly sclerified. The body is unsegmented but covered with a few transverse parallel folds giving it a striated appearance. Terminal knob slightly embedded in the distal part of the body, carrying 10 canaliculi in a wide collar. No common spermathecal duct observed, but the mounting did not allow for their observation *in toto*. The genital furca has wide and well-developed lateral arms and a stem widening towards the apex.

*Type-locality* – Ranomafana Ifanadiana.

*Type data and depository* – holotype female collected in the daytime (10.30 a.m.) by hand on a traveler's tree (*Ravenala madagascariensis*) in Ranomafana, district of Ifanadiana, region of Vatovavy-Fitovinany (province of Fianarantsoa), at an altitude of

1,380 m above sea level, on April 23<sup>rd</sup>, 2000. The holotype was deposited in the Entomology department of the *Muséum National d'Histoire Naturelle*, Paris, France.

*Distribution* – *Se. brunhesi* sp. nov. has only been recorded in its type-locality.

*Bionomics* - No data.

*Etymology* - In honour to our colleague Jacques Brunhes, researcher of the ORSTOM and IRD who caught this specimen, and in recognition of his contribution to medical entomology in Madagascar.

***Sergentomyia vistellei* Depaquit, Randrianambinintsoa and Léger sp. nov. (Figs 5-6)**

Genus: *Sergentomyia* França and Parrot 1920

Authorship: Note that the authors of the new taxon are different from the authors of this paper; Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature.

*Description*

One female specimen was examined. The male is unknown.

The measurements indicated below are those related to the holotype. Some organs were broken when the insect was caught, stored or mounted, so they were not observed.

*Head* (Fig. 5A): Occiput with two narrow, well individualized lines of setae. On the line above the eyes, two bigger insertions of setae on each side. Clypeus 109  $\mu\text{m}$  long, 84  $\mu\text{m}$  wide, with setae randomly distributed in its middle part. Eyes 150/100  $\mu\text{m}$ , well developed, composed of more than 120 facets. Interantennal and interocular sutures complete. Flagellomeres (Fig. 6A) broken after f11; f1 shorter than f2+f3. Two ascoids on flagellomeres f2 to f11. No ascoid on f1. The internal ascoid is implanted a little more proximally than the external one. One terminal papilla on f1 and f2; No papilla on f3. One median papilla on flagellomeres f4 to f9 and on f11. Two papillae on f10. No simple seta observed on flagellomeres f1 to f4; one on f5; Four on f6 and more on the following segments. Palpi broken after p2. Absence of Newstead's sensilla or spiniform seta on p2. Labrum-epipharynx (Fig. 5B) 151  $\mu\text{m}$  long.

Hypopharynx (Fig. 5B) difficult to observe, appearing smooth at the top, toothless. Mandibles (Fig. 5B) with *ca.* 25 little teeth. Maxillary lacinia (Fig. 5B) with *ca.* 20 internal teeth not reaching the top of the lacinia and 8 large external teeth at the top. Labium (Fig. 5B) exhibiting an open labial suture. Cibarium (Fig. 5C) with a straight horizontal line of *ca.* 29 posterior long, thin, curved and pointed teeth oriented randomly. The most lateral ones are smaller than the others. The anterior 30 dot-like teeth are implanted along two lines that sometimes overlap. The biggest one is located in the central part of the line, and well individualized from the others. Sclerotized area (=pigment patch) wide and flattened. Pharynx (Fig. 5C) armed in its distal part with long teeth oriented backwards, implanted randomly. Cervix: Two cervical sensilla on each side. Vento-cervical sensilla not observed.

*Thorax:* 590  $\mu\text{m}$  long. Dark sclerites. Post-alar setae on the mesonotum not observed. Paratergital setae on the pleural sclerites not observed. Absence of proepimeral setae. Absence of upper anepisternal setae. Absence of lower anepisternal setae. Absence of anepimeral setae. Absence of metepisternal setae. Metepimeral setae not observed. Setae on the anterior region of the katepisternum not observed. Suture between the metepisternum and the katepimeron not observed. Metafurca not observed. Wing and legs not observed.

*Abdomen:* Setae on tergites ii–v not observed. Presence of 12 setae on tergite VIII. Absence of a protuberance on tergite IX. Setae not observed on sternite X.

*Genitalia* (Fig. 6C): Elongated spermathecae, thin-walled, smooth, and easy to fold. The basal part is slightly pigmented and probably with sclerified walls. Two collars

around the terminal knob. The central one is short and rounded at the top whereas the external one is *ca.* four times higher, and appears to be pointed at the top. The terminal knob carries less than 10 canaliculi. Furca with a long stem showing a widening. Genital chamber with grouped spines. Distal part of the individual ducts of the spermathecae without any sclerotization or striation. Basal part of the spermathecal ducts not observable. Cerci 139/53  $\mu\text{m}$

*Type-locality* – Andringitra, district of Ambalavao, region of Matsiatra Ambony (province of Fianarantsoa), in a small grassy clearing (5 m diameter) at an altitude of 820 m. a.s.l., on November 29<sup>th</sup>, 2002, 40 meters from the confluence of the Sahanivoraky and Sahavatohy rivers.

*Type data and depository* – the female holotype (MADA97) was deposited in the *Muséum National d'Histoire Naturelle*, Paris, France.

*Bionomics* – No data

*Genbank accession numbers* of *Se. vistellei* n. sp. holotype: MK465181 (cyt b mt DNA) and MK452287 (D1-D2 28S rDNA).

*Etymology* – The species *Se. vistellei* sp. nov. is dedicated to the memory of our colleague Richard Vistelle, former Dean of the Faculty of Pharmacy and former President of the Université de Reims Champagne-Ardenne.

#### **4. Discussion**

The first phlebotomine sandflies of Madagascar were reported in 1930's, when *Grassomyia squamipleuris* Newstead, 1912 was caught in the north of the country (Raynal and Le Gac, 1937). The species was observed in the nests of mud daubers (Le Gac, 1937). With the passing of time, taking into account that all other species

are endemic to Madagascar, we agree with other authors who think that this identification could be doubtful, but the specimens seem to have been lost (Abonnenc, 1969; Brygoo, 1973). The second record was that of *Grassomyia madagascariensis* Abonnenc, in 1969. From a taxonomic point of view, based on the setae of the thorax, we consider *Grassomyia* as a genus, following Abonnenc and Léger (1976).

Before the 1960's, the Malagasy fauna had already been reported to be poor in phlebotomine sandflies (Brygoo, 1973). At the end of the 1970's, *Phlebotomus berentiensis* (Léger and Rodhain, 1978) was described as the first anthropophilic sandfly of the island initially described in the genus *Sergentomyia* (Léger and Rodhain, 1978; Léger et al., 1979).

Since the 2000's, many new taxa have been described from several locations in Madagascar, all of them at low altitudes (Depaquit et al., 2004b; Depaquit et al., 2002; Depaquit et al., 2004, 2007, 2008; Léger et al., 2005; Randrianambinintsoa and Depaquit, 2013; Randrianambinintsoa et al., 2013, 2014).

Andasibe/Périnet, a highland located 800-1,000 m a.s.l. where *Ph. fertei* was recorded, was long considered as the sole spot harboring Malagasy sandflies at higher altitudes. The records of *Se. vistellei* sp. nov. and *Se. brunhesi* sp. nov. from Andringitra and Ranomafana, respectively, unveil new locations where sandflies can be caught at higher altitudes.

*Se. brunhesi* was caught in the daytime, when it jumped. However, we cannot say more about its bionomics.

The sclerification of the basal part of the spermathecal bodies of *Se. vistellei* sp. nov. and *Se. brunhesi* sp. nov. is an original character. In addition to Malagasy

*Vattieromyia*, it was found in the American sandflies of the genus *Pressatia* Mangabeira and in two Australian ungrouped species: *Se. pugifera* Lewis and Dyce and *Se. standfasti* Lewis and Dyce. Nevertheless, the structure of the spermathecae of *Se. vistellei* sp. nov. and *Se. brunhesi* sp. nov. is very original. Spermathecae are the most determining character to define subgenera within *Sergentomyia*. We think both *Se. vistellei* sp. nov. and *Se. brunhesi* sp. nov. could belong to a new subgenus. However, taking into account their scarcity, we suggest to consider them as ungrouped *Sergentomyia* waiting the analysis of new specimens. Moreover, we also noted the absence of ascoids on the first flagellomere, which seems to be a common trait among the *Sergentomyia* of Madagascar.

*Se. vistellei* sp. nov. and *Se. brunhesi* sp. nov. differ by their cibarium. The cibarium of *Se. brunhesi* sp. nov. includes more than 70 palissadic teeth and no vertical teeth (figure 4C), whereas the cibarium of *Se. vistellei* sp. nov. (figure 5C) includes less than 30 long, randomly oriented cibarial teeth, and also vertical teeth, including a big, central, well isolated one.

### Identification keys of female Malagasy *Sergentomyia*

- |   |   |                   |
|---|---|-------------------|
| 1 | Spermathecae opium poppy capsule-like       | <i>Grassomyia</i> |
|   | Spermathecae not opium poppy capsule-like   | 2                 |
| 2 | Body of the spermathecae sclerified basally | 3                 |
|   | Body of the spermathecae not sclerified     | 6                 |



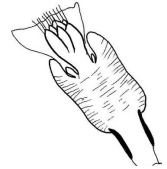
3 Thin-walled and smooth spermathecae with a slightly pigmented basal part with sclerified walls

5



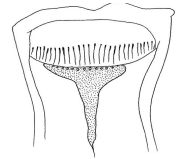
Spermathecae narrower in their median part

4 *Se. (Vattieromyia)*



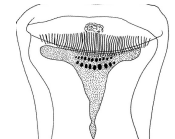
4 Cibarial teeth straight; a row of small, non-refractive dot-like vertical teeth

*Se. sclerosiphon*



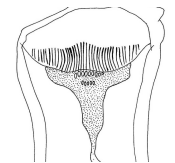
Median cibarial teeth curved; two rows of big, refractive vertical teeth

*Se. anka*



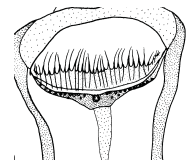
Cibarium armed with dorsoventrally arched teeth, laterally surmounted by a median ring

*Se. namo*



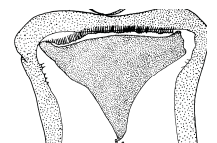
5 Cibarium with less than 30 long cibarial teeth oriented randomly; vertical teeth including a big, central, well isolated one

*Se. vistellei* sp. nov.



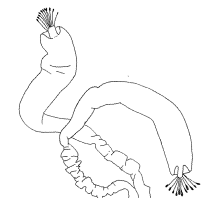
Cibarium with more than 70 palissadic teeth. Absence of vertical teeth.

*Se. brunhesi* sp. nov.



6 Smooth spermathecae

*S. majungaensis*



Spermathecae different

7



7

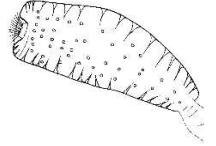
Fully segmented spermathecae; strong pharyngeal armature

8 *Se. (Trouilletomyia)*



Partially segmented spermathecae; inconspicuous pharyngeal armature

9 *Se. (Rondanomyia)*



8

Cibarial armature with ca. 15 well developed pointed teeth, oriented backward, along a curved line

*S. boironis*



Cibarial armature with inconspicuous teeth or denticles

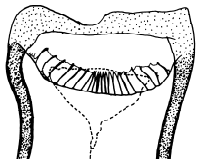
*S. huberti*



9

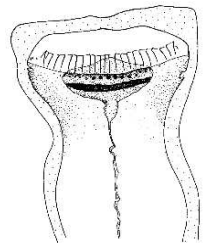
Absence of cibarial vertical teeth

*S. goodmani*



One row of vertical teeth

*Se. ozbeli*



Molecular analyses showed that *Se. vistellei* sp. nov. was well individualized and well supported on the tree regarding the cyt b and D1-D2 sequences (Figs 1, 2), although its phylogenetic position did not seem to be clearly defined due to incongruent results between the markers. The two specimens of *Grassomyia* included in the tree belong to distinct species, new for science. They will be described in a future revision of the systematics of this genus. Their positions based on D1-D2 sequences (figure 2) could be an artefact linked to the limited number of mutation and their too important weight on the topology of the tree. The too limited number of mutations also explains the unresolved position of the subgenus *Rondanomyia* species *Se. ozbeli* and *Se. goodmani*.

These two newly described species of *Sergentomyia* were each caught once. New investigations have to be carried out to describe males, to understand their biology (e.g., host preferences) and to identify other potential new species at higher altitudes. A few decades ago, phlebotomine sandflies were considered as scarce in Madagascar (Brygoo, 1973).

The prevailing dogma was that only species of the genus *Phlebotomus* could transmit *Leishmania* to humans in the Old World (Killick-Kendrick, 1990). However, the role of the genus *Sergentomyia*, which has been ignored too long, cannot be neglected (Maia Depaquit, 2016). Many *Sergentomyia* species feed on reptiles, but some of them feed on mammals (Senghor et al., 2016). We do not know if Malagasy sandflies could be vectors of *Leishmania*. We know that *Leishmania* can be transmitted by new vectors (i.e. Old World *L. infantum* by New World *Lu. longipalpis*), therefore some *Leishmania* could also be transmitted locally by sandflies differing from their usual

vectors (Galvis-Ovallos et al., 2017). Testing the vectorial competence of Malagasy sandflies for *Leishmania* seems to be an interesting challenge for future research.

**Acknowledgements:** The authors thank Jacques Brunhes, a researcher of the *Institut de Recherche pour le Développement*; who collected the specimen of *Se. brunhesi* sp. nov., and pay a tribute to the memory of Donadieu Randrianambinina, technician of the Entomology laboratory of the *Institut Pasteur de Madagascar*, who collected the specimen of *Se. vistellei* sp. nov.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Author's contribution:** FJR and JD wrote the manuscript with contributions from VR, AB and NL; FJR, AB and JD conducted molecular work and data analysis. All authors read and approved the final manuscript.

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**Fig. 1:** Maximum likelihood tree obtained from partial cyt b sequences based on the HKY85 model after rooting on *Phlebotomus* spp. The tree with the highest log likelihood (-4,862.21) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured as the numbers of substitutions *per* site. The analysis involved 126 nucleotide sequences. All sequences available in Genbank have been included in the study and the total number of specimens per branch is indicated in brackets.

**Fig. 2:** Maximum likelihood tree obtained from partial 28S rDNA sequences (D1 and D2 domains) based on the HKY85 model after rooting on *Phlebotomus fertei*. The tree with the highest log likelihood (-1,979.25) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured as the numbers of substitutions *per* site. The analysis involved 126 nucleotide sequences. All sequences available in Genbank have been included in the study and the total number of specimens per branch is indicated in brackets.

**Fig. 3:** *Sergentomyia brunhesi* sp. nov. female: A: head; B: flagellomeres 1 – 3 and 11 – 14; C: labrum-epipharynx; D: mandible; E: maxillary lacinia; F: hypopharynx, and G: labial furca.

**Fig. 4:** *Sergentomyia brunhesi* sp. nov. female: A: palpi 1 – 5; B: third palpal segment; C: pharynx and cibarium; D: spermathecae and genital furca; E: wing.

**Fig. 5:** *Sergentomyia vistellei* sp. nov. female: A: head; B: mouth part: labrum-epipharynx, hypopharynx, mandible, maxilla, and labium with furca; C: pharynx and cibarium.

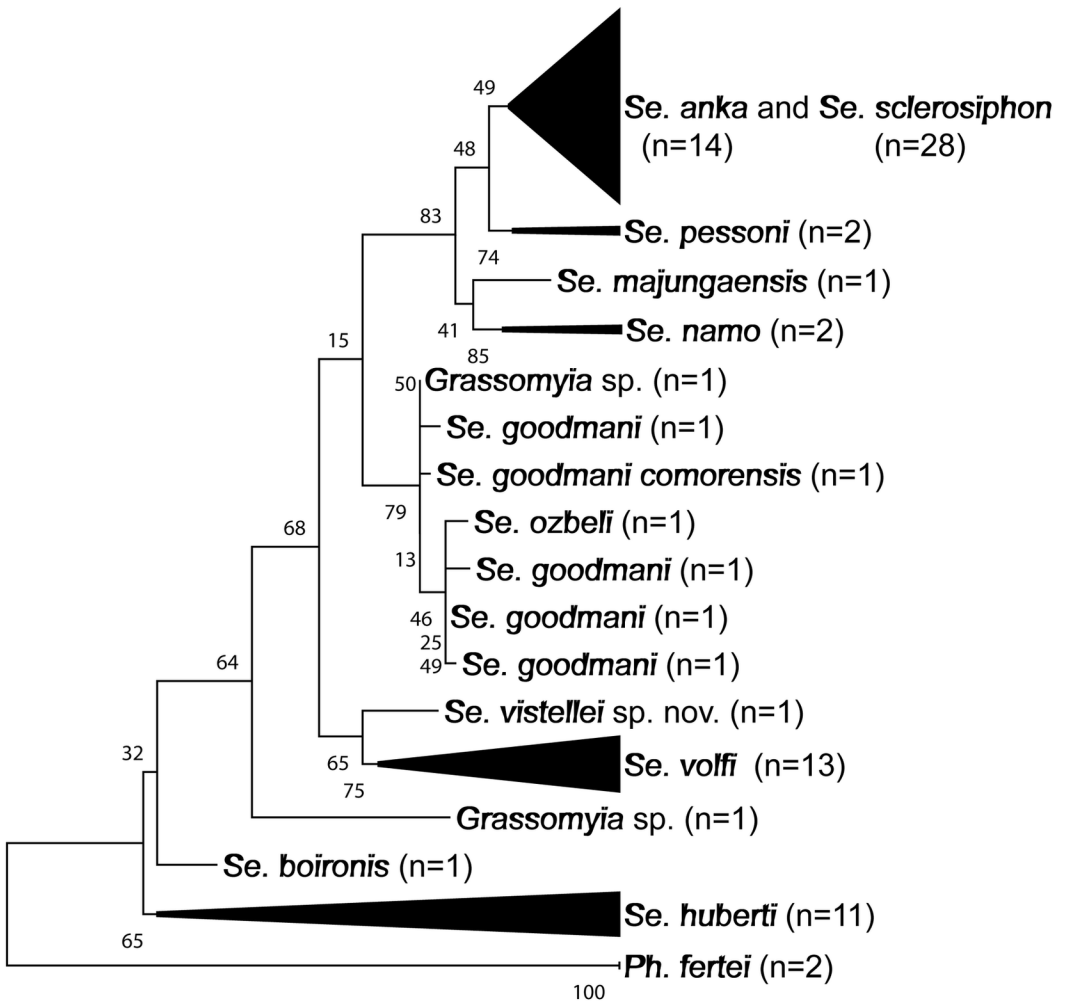
**Fig. 6:** *Sergentomyia vistellei* sp. nov. female: A: flagellomeres 1 – 11; B: cercus; C: spermathecae and genital furca.

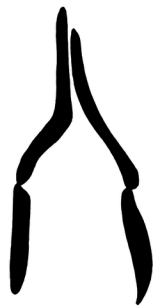
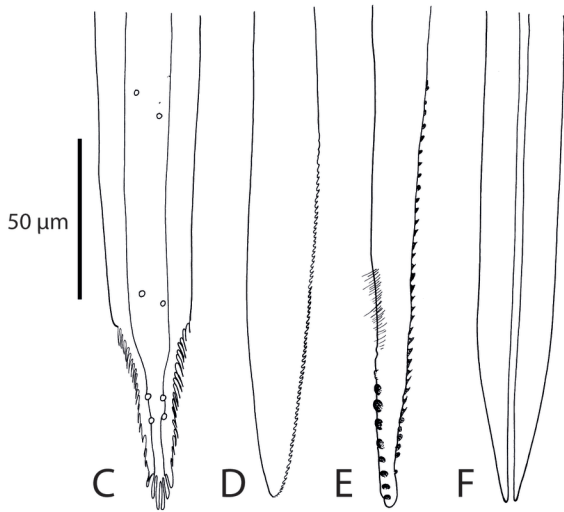
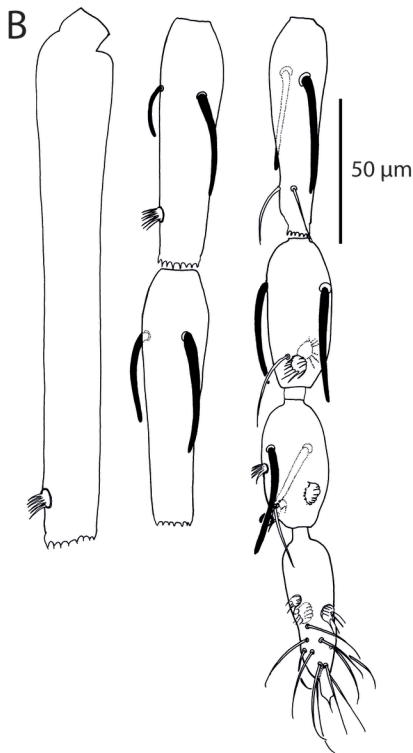
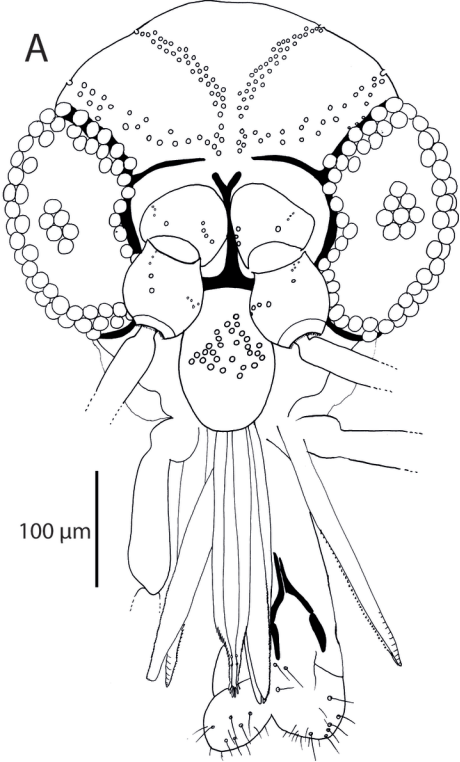


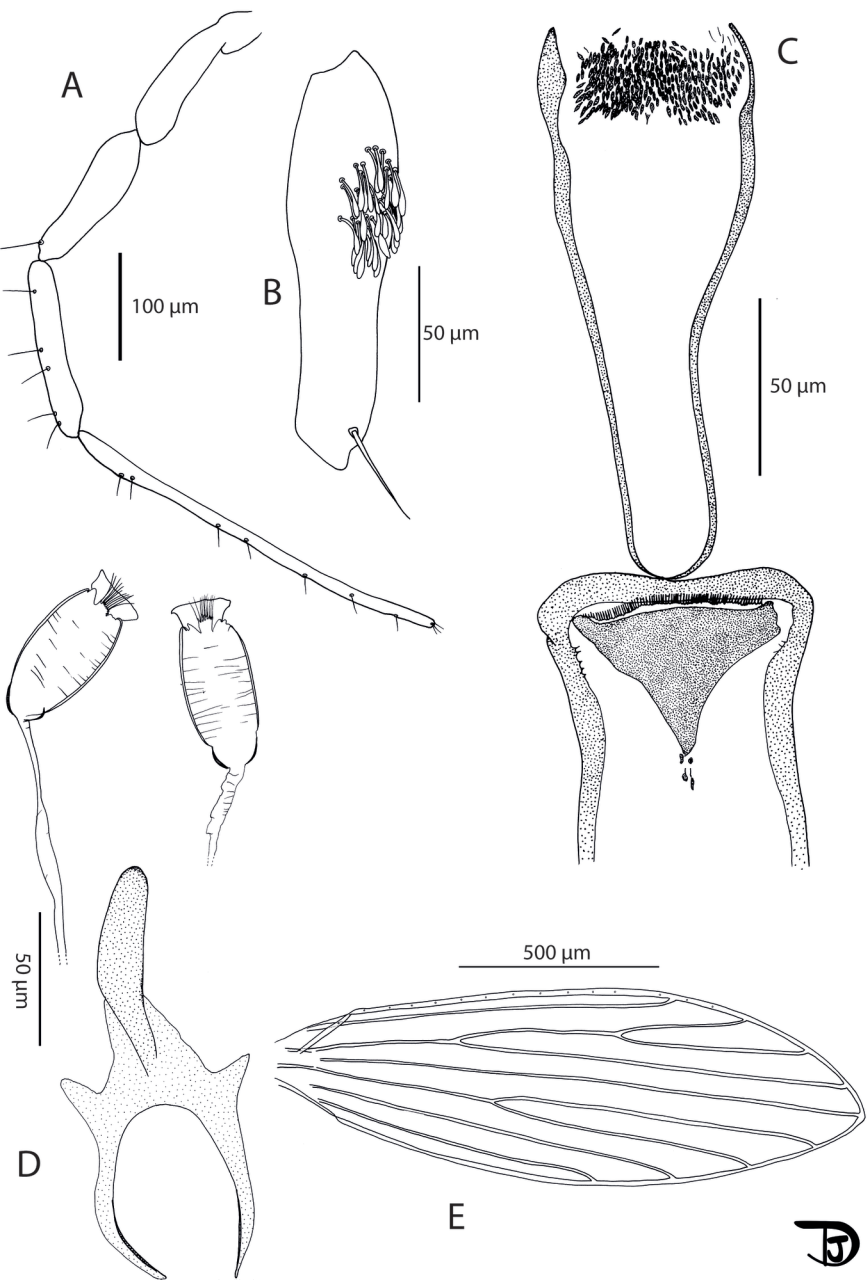
**Table 1. Numbers of ascoids, sensillae and simple setae on the flagellomeres of female *Se. brunhesi* sp. nov.**

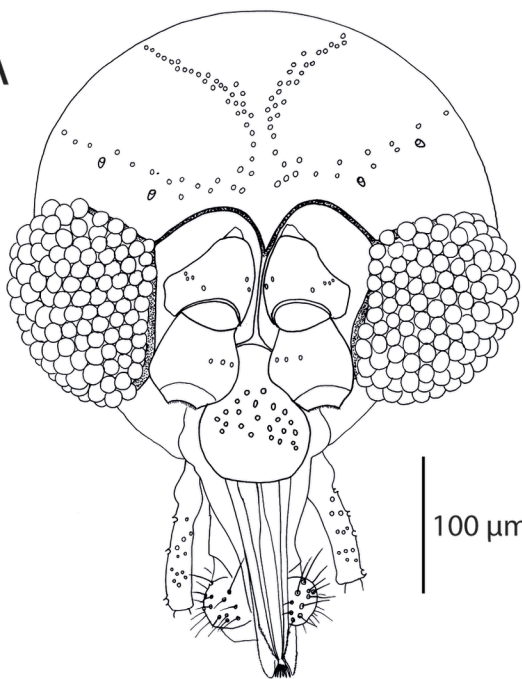
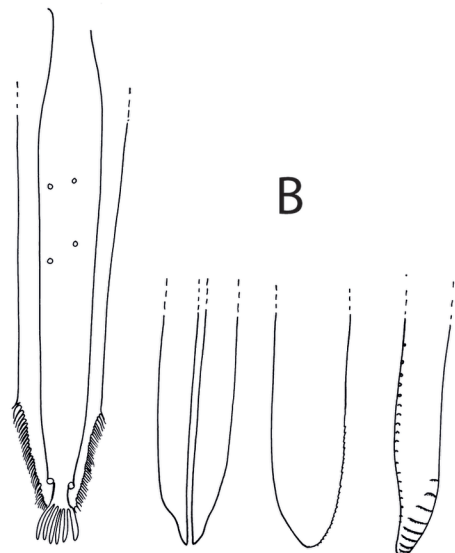
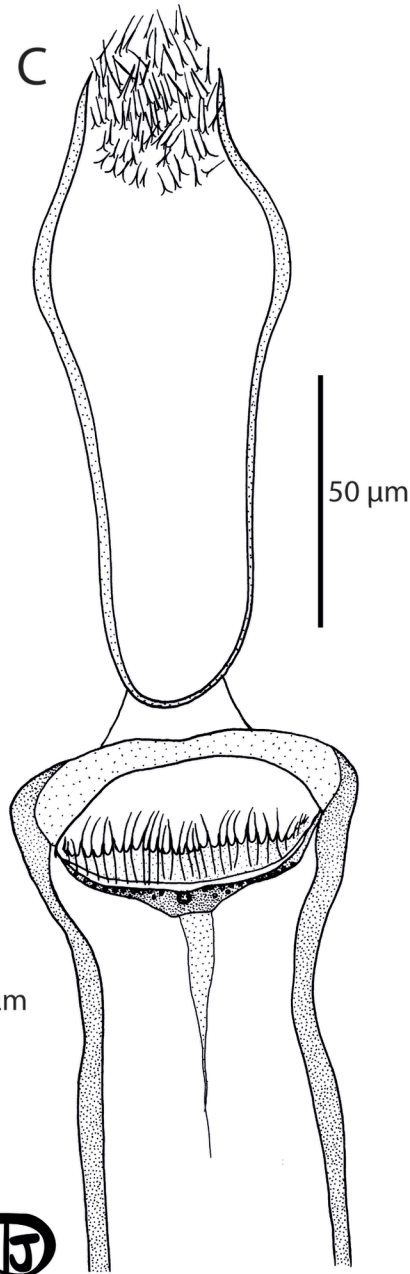
Flagellomeres	Ascoids	Sensilla	Simple setae
1	0	1	0
2	1+1 atrophied	1	0
3	2	0	0
4	2	0	2
5	2	0	2
6	2	0	2
7	2	0	1
8	2	0	1
9	2	0	3
10	2	0	2
11	2	0	3
12	2	2	1
13	2	4	1
14	0	3	11

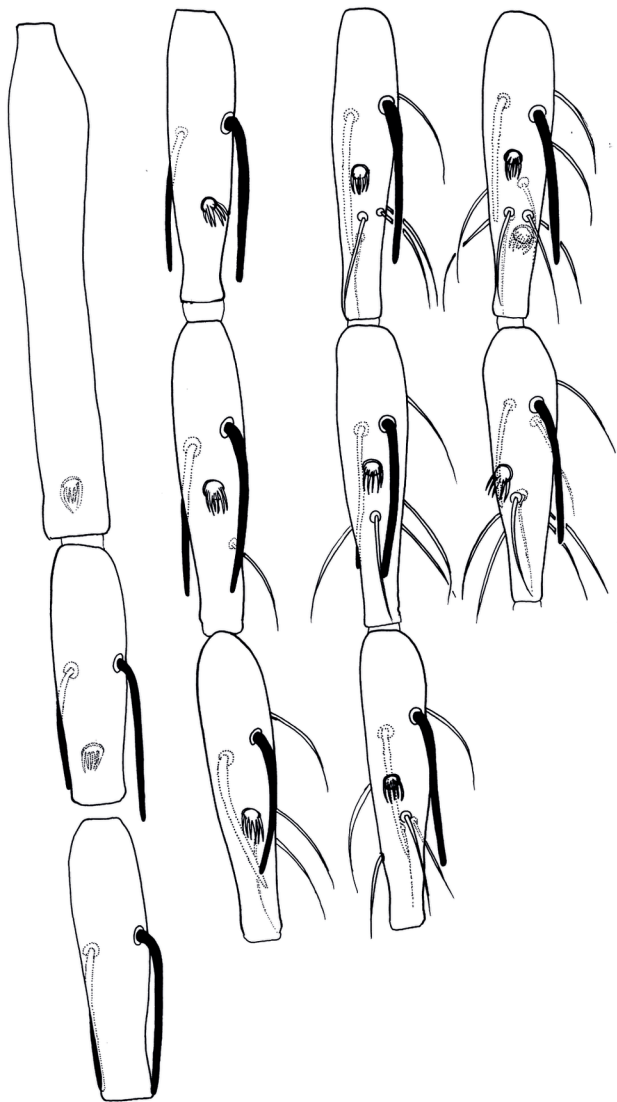
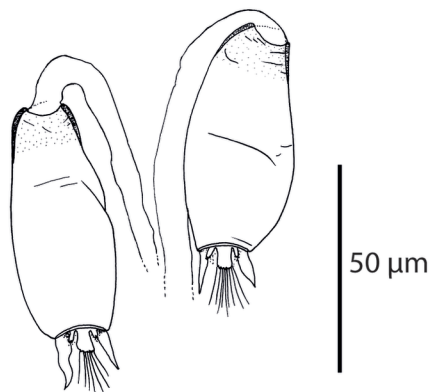
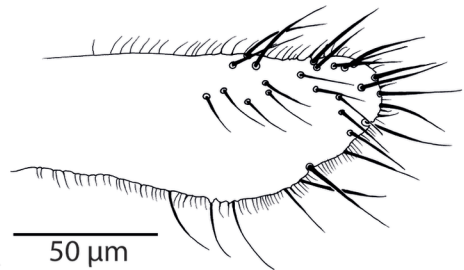
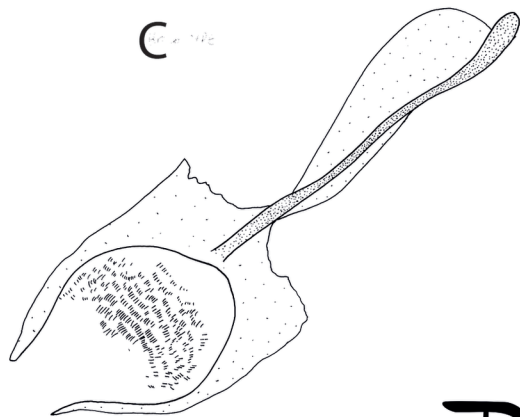








**A****B****C****D**

**A****B****C**

## Two new Phlebotomine sandfly species (Diptera: Psychodidae) from the Highlands of Madagascar

Fano José Randrianambinintsoa<sup>1</sup>, Vincent Robert<sup>2</sup>, Antoine Blavier<sup>1</sup>, Nicole Léger<sup>1</sup>, and Jérôme Depaquit<sup>1,3</sup>

1. Université de Reims Champagne-Ardenne, Faculté de Pharmacie,  
ANSES, SFR Cap Santé, EA7510 ESCAPE – USC VECPAR,  
51 rue Cognacq-Jay, 51096 Reims CEDEX, France

2. MIVEGEC Unit, IRD, CNRS, Univ Montpellier, 911 avenue Agropolis, BP 64501,  
34394 Montpellier CEDEX 5, France

3. Laboratoire de Parasitologie-Mycologie, CHU de Reims, 51100 Reims, France

**Summary:** Phase contrast microphotography of the cibarium of *Sergentomyia vistellei* Depaquit, Randrianambinintsoa & Léger sp. nov.

