Description of the sandfly species *Chinius samarensis* n. sp. (Psychodidae; Diptera) from the Philippines

Nicole Léger¹, Jérôme Depaquit², Frédérick Gay³

¹Reims University, France, ²Université de Reims Champagne-Ardenne, ANSES, EA4688 — USC Transmission vectorielle et épidémiosurveillance de maladies parasitaires (VECPAR), Faculté de Pharmacie, France, ³Laboratoire de Parasitologie-Mycologie and Pôle de Biologie Médicale, Faculté de Médecine et Université Pierre et Marie Curie, Paris, France

The authors have described *Chinius samarensis* n. sp. (Diptera; Psychodidae) from the Philippines (Island of Samar). This is the fourth known species belonging to the Asiatic genus *Chinius* Leng, 1987. Like the other *Chinius*, *C. samarensis* n. sp. is a cavernicolous species. The genital ducts of the male are four times shorter than the spermathecal ducts. However, the authors think that male and female belong to the same species due to their similar *cytochrome b* mt DNA sequences. A differential diagnosis with the other species belonging to the genus *Chinius* is provided.

Keywords: Phlebotomine sandflies, Chinius, New species, Description, Caves, Philippines, Cytochrome b mt DNA

Introduction

The genus *Chinius* was created by Leng for a cavernicolous sandfly, *Chinius junlianensis*, from Sichuan and Guizhou, China.¹ The female has been re-described by Depaquit *et al.*² Later on, two other species of the same genus: *C. barbazani* Depaquit, Léger & Beales, 2006 and *C. eunicegalatiae* Léger, Depaquit & Gay, 2010 have been described in the same type of habitat (caves) respectively in Thailand and Laos.^{3,4}

In this paper, we have described a fourth cavernicolous species belonging to the genus *Chinius*: *C. samarensis* from Samar Island (Philippines).

Material and Methods

Captures have been performed on 1 June and 2 June 2011 with CDC miniature incandescent white light traps without CO_2 in and at the entrance of a cave near Borongan (Basiad) (11°15′33″N, 125°09′47″E; alt. 0–12 m above sea level).

Collected sandflies (42 males and 53 females) were kept in 96% ethanol and mounted *in toto* for morphological study according to Abonnenc's⁵ method: 4–8 hours in 10% KOH solution followed by eight baths (20 minutes each) of water, then at least 1 hour in Marc–André solution. Females were directly mounted in this liquid with their spermathecae dissected, then re-mounted in chloral gum when possible, or in Canada balsam as described below. Some males and a few females were dehydrated in ethanol of growing concentrations (from 70% to absolute ethanol), then in beech creosote, and finally mounted in Canada balsam.

Moreover, two specimens (one male and one female) were prepared for both morphological and molecular studies. The head and genitalia of each sandfly were cut off in a drop of ethanol, cleared in boiling Marc–André solution, and mounted under a cover slip in chloral gum for identification. Genomic DNA was extracted from thorax and abdomen with the QIAmp DNA Mini Kit (Qiagen, Germany) as explained by Depaquit *et al.* (2004).⁶ *Cytochrome b* polymerase chain reactions were performed according to the primers N1N-PDR and C3B-PDR and protocols used by Esseghir *et al.* (1997).⁷ Direct sequencing of both DNA strands was performed using the primers used for DNA amplification.

Results

Description of the male of Chinius samarensis *n. sp.* (*Figs. 1 and 2*)

This species is small and very fragile (very few specimens had antennae). The head is difficult to mount dorso-ventrally. Consequently, we used several specimens for the description.

Head

Eye: with generally 18 facets.

Correspondence to: J Depaquit, EA4688 — USC Transmission vectorielle et épidémiosurveillance de maladies parasitaires (VECPAR), Faculté de Pharmacie, 51 rue Cognacq-Jay, 51096 Reims Cedex, France. Email: jerome.depaquit@univ-reims.fr



Figure 1 *Chinius samarensis* n. sp. male. (A) Pharynx and cibarium; (B) antennal segments III–V; (C) antennal segments X–XVI; (D) palp; (E) clypeus.

AIII=137 μ m; AIV=91 μ m; AV=93 μ m (measured on one specimen).

Ascoid formula (on one specimen): 2/III–X; 1/XI– XIV. Ascoids do not reach the next articulation. One papilla on antennal segments III, IV, V, two papillae on segments X, XII, XVI, three papillae on segments XII, XV, and four papillae on segments XII and XIV.

Palpal formula: 1-(2-4)-5-3. About 10 club-like Newstead's scales on the third segment.

Labrum (L)=90-100 µm

Clypeus: length=55–70 $\mu m;$ 3–4 setae on the dorsal side.

Cibarium: margins ornamented with tiny teeth. Absence of central teeth.

Pharynx: free of ornamentation, narrowed at its posterior part.

Thorax

Pleural setae absent

Wings rounded with a R2 (alpha) short= $150-170 \mu m$. Length= $1.400-1.500 \mu m$; width= $500-520 \mu m$; alpha: 140–180 μm ; beta: 240–260 μm ; gamma: 150–200 μm ; delta: 28–48 μm ; pi: 60–80 μm .

Abdomen and genitalia

Antero-lateral groups of about 50 trumpet-like structures on tergites 4 and 5, similar to those of other species of the genus *Chinius*.^{1,3}

Coxite measures about $145-170 \ \mu\text{m}$ in length and $70-90 \ \mu\text{m}$ in breadth, with the inner side covered with about 20 stout hairs.

Style measures $155-175 \mu m$ in length and about $30 \mu m$ in breadth with four nearly equidistant spines.



Figure 2 Chinius samarensis n. sp. male. (A) Wing; (B) 'trumpet glands' of the third abdominal; (C) aedeagus; (D) genitalia.

Paramere measures $150-170 \ \mu m$ in length, becoming gradually narrowed towards the apex, slightly curved, with a round extremity.

Surstyle is 100–130 µm long.

Aedeagus 57–70 μ m in length, strongly chitinized, large, with a special form (Fig. 2).

The lengths of the genital filaments $(250-320 \ \mu m)$ and genital pump $(185-215 \ \mu m)$ give a genital filament/pump ratio of about 1.4.

Description of the presumed female of Chinensis samarensis *n. sp. (Figs. 3 and 4)* **Head**

Eye with 18–20 facets.

AIII=125–160 μm; AIV=85–110 μm; AV=90– 120 μm.

Ascoid formula (on two preparations): 2/III-XV but some specimens showed only one ascoid on segments XIII–XIV. Ascoids slender, as long as the segment. Number of papillae similar to that observed in males, except one more (four) on the antennal segment XV.

Palpal formula: 1–2–3–5–4. About 10 club-shaped Newstead's scale on the third segment.

Labrum=120-130 µm.

Clypeus measures $80-100 \ \mu m$ in length with three to six setae on the dorsal side.

Cibarium: thin teeth arranged along two diagonally oriented lines located in the center of the cibarial chamber.

Pharynx: wide, narrowed at its posterior part which is covered with a few large crossing folds and with a few fringes of tiny scales.

Thorax

Pleural setae absent.

Wings rounded with an R2 (alpha) not very short= $230-270 \mu m$. Length= $1.750-1.950 \mu m$; width=



Figure 3 *Chinius samarensis* n. sp. female. (A) Pharynx and cibarium; (B) antennal segments III–V; (C) antennal segments X–XVI; (D) clypeus; (E) palp.

650–780 μm; alpha: 220–280 μm; beta: 250–320 μm; gamma: 200–240 μm; delta: 20–50 μm; pi: 120–160 μm.

Genitalia

Spermathecae are very long, tubular, and narrow with short individual ducts with no clear limit between each spermatheca and its individual duct.

The combined length of the individual duct and spermatheca is $100-130 \ \mu m$.

Common duct measures 850–1300 μm in length. It is thin and slightly pleated.

Furca thick, morphologically typical of the genus *Chinius*.

Molecular data

Sequences analyzed of partial *cytochrome b* obtained from a male and from a female (436 bp each) have been deposited in GenBank under accession numbers JX485975 and JX485976. They are exactly similar (100% homology).

Derivatio nominis

From the name of the Island of Samar where all our samples were obtained.

Depositories of Type Material

• Museum National d'Histoire Nature (Paris): holotype male, seven paratypes males, and four paratypes



Figure 4 Chinius samarensis n. sp. female. (A) Furca and spermathecae; (B) wing.

females, including an allotype mounted in choral gum (five slides);

- Natural History Museum (London): six paratype males and five paratype females (two slides);
- Bernice Pauahi Bishop Museum (Honolulu): three paratype males (one slide).

Discussion

We classified *C. samarensis* n. sp. in the genus *Chinius* because it possesses the morphological characters of the genus *Chinius*, as revised by Depaquit *et al.*:³

- absence of pleural setae;
- presence in the males of trumpet-like organs on abdominal tergites 4 and 5;
- interocular suture complete, very few setae on vertex and clypeus;
- short labrum;
- cibarium with several rows of fine teeth;

• broad and rounded wings with reduced or lacking R2; Moreover, males have

- trumpet-like organs on abdominal tergites 4 and 5;
- style with 4 spines.

And females have

- smooth spermathecae with a common duct;
- typical thick furca.

R2 is present as in the type species (absent in *C. barbazani* and *C. eunicegalatiae*) but longer in the female than in the male (same unequality observed in *C. junlianensis*). This state of character corroborates our opinion on the terminal synapomorphism of the character 'absence of R2' in Phlebotominae and more generally in Diptera.³

A question must be raised: the short genital filaments of the males are about four times shorter than the long spermathecal ducts of the female. For phlebotomine sandflies, the length of these genital organs is usually more or less similar. However, when they differ, the male genital filaments are usually longer than female spermathecal ducts, except that of *Sergentomyia clydei*.⁸ Consequently, we decided to describe the female as 'the presumed female of *C. samarensis* n. sp.' as we did previously for that of *C. eunicegalatiae.* However, the link between females and males was based on the following items: (1) they

were captured together, the same day, in the same trap; (2) both belong to the same genus, and we suppose, to the same species; and (3) the *cytochrome b* sequences obtained from a male and from a female are exactly similar.

As other *Chinius, C. samarensis* is a cave dweller associated with bats and reptiles. The states of characters 'small eyes with few facets' and 'short labrum' are probably adaptive ones.

The *Chinius* geographical repartition corresponds to the classical oriento-Australasian track used by many groups of insects. Note that the fossil species: *Palaeomyia burmitis* Poinar, 2004 from Burma, has a rounded wing showing a very short R2, looking like that of *C. junlianensis.*⁹

Differential Diagnosis of the Species Belonging to the Genus *Chinius*

- A males
- 1. No. R2 vein on the wing:
- 1.1 Insertion of the basal spine in the middle of the style: *C. barbazani*;
- 1.2 Insertion of the basal spine at the proximal third of the style: *C. eunicegalatiae*.
- 2. a short R2 vein on the wing:
- 2.1 genital filaments long (>1000 µm): C. junlianensis;
- 2.2 genital filaments short (<400 µm): C. samarensis.
- B females:
- 1. No. R2 vein on the wing:
- 1.1 Individual ducts/common duct=about 1: C. barbazani;
- 1.2 Individual ducts/common duct=about 0.35: *C. eunicegalatiae*.

- 2. a short R2 vein on the wing:
- 2.1 pharynx: enlarged posterior part with thick walls and armed surface: *C. junlianensis*;
- 2.2 pharynx: not enlarged, without ornaments: *C. samarensis.*

References

- 1 Leng YJ. A preliminary survey of phlebotomine sandflies in limestone caves of Sichuan and Guizhou Provinces, south-west China, with description and discussion of a primitive new genus *Chinius*. Ann Trop Med Parasitol. 1987;81:311–7.
- 2 Depaquit J, Léger N, Zhang LM, Leng YJ. Chinius junlianensis Leng, 1987 (Diptera: Psychodidae): new morphological data. Ann Trop Med Parasitol. 2007;101:181–4.
- 3 Depaquit J, Léger N, Beales P. *Chinius barbazani* n. sp. de Thailande (Diptera: Psychodidae). Parasite. 2006;13:151–8.
- 4 Léger N, Depaquit J, Gay F. *Chinius eunicegalatiae* n. sp. (Diptera; PSychodidae), a cavernicolous sandfly from Laos. Ann Trop Med Parasitol. 2010;104:595–600.
- 5 Abonnenc E. Les phlébotomes de la région éthiopienne (Diptera, Psychodidae). Entomol Méd Parasitol. 1972;55:1–239.
- 6 Depaquit J., Léger N., Ferté H. & Robert V. Les Phlébotomes de Madagascar (Diptera – Psychodidae). II - Description de la femelle de Phlebotomus (Anaphlebotomus) fertei Depaquit, Léger & Robert, 2002 ; description du mâle et redescription de la femelle de Phlebotomus (Anaphlebotomus) berentiensis (Léger & Rodhain, 1978) comb. nov. Parasite, 2004a, 11, 201-209.
- 7 Esseghir S., Ready P., Killick-Kendrick R. & Ben-Ismail R. Mitochondrial haplotypes and phylogeography of *Phlebotomus* vectors of *Leishmania major*. *Insect Molecular Biology*, 1997, 6, 211-225.
- 8 Ilango K, Lane RP. Coadaptation of male aedeagal filaments and female spermathecal ducts of the old world phlebotomine sand flies (Diptera: Psychodidae). J Med Entomol. 2000;37:653– 59.
- 9 Poinar G, Jr. *Paleomyia burmitis* (Diptera: Phlebotomidae) a new genus and species of Cretaceous sand flies with evidence of blood-sucking habits. Proc Entomol Soc Wash. 2004;106:598–605.