# **Neotropical Entomology**

ISSN: 1519-566X

journal homepage: www.scielo.br/ne



## **PUBLIC HEALTH**

Description of the Female of *Evandromyia rupicola* (Martins, Godoy & Silva) with a Review of the *rupicola* Series (Diptera: Psychodidae: Phlebotominae)

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#### Kevwords

Distribution, identification key, taxonomy

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Edited by Neusa Hamada – INPA

Received 30 November 2010 and accepted 25 January 2011

## Abstract

The *rupicola* series was proposed initially for *Evandromyia rupicola* (Martins *et al*) and *Evandromyia correalimai* (Martins *et al*), and recently extended with the inclusion of *Evandromyia gaucha* Andrade-Filho *et al* and *Evandromyia grimaldii* Andrade-Filho *et al*. The female of *E. rupicola* is here described and illustrated for the first time and its male is redescribed and drawn on the basis of specimens captured in forest on the coast of the state of São Paulo, Brazil. The head and genitalia of both sexes of *E. correalimai* are also illustrated. The distinctive traits among females of the four species and of males of *E. rupicola*, *E. correalimai* and *E. grimaldii*, and the distribution range of these species are commented.

## Introduction

The notification of cases of leishmaniasis at the Centro de Vigilância Epidemiológica da Secretaria Estadual de Saúde de São Paulo in the municipalities of the northern coast of this state, has led the Superintendência de Controle de Endemias do Estado de São Paulo (SUCEN) to undertake captures of sandflies in these areas for the identification of the probable sites of infection (Condino 2007). Several specimens of *Evandromyia rupicola* (Martins *et al* 1962), including females, which had not been previously described, were found among the phlebotomines captured.

Lewis *et al* (1977) proposed the *rupicola* species-group in the genus *Lutzomyia* França, which was later included by Galati (1995, 2003a) as the *rupicola* series in the genus *Evandromyia* Mangabeira, subgenus *Evandromyia s. str. Evandromyia* is included in the subtribe Lutzomyiina, which species present the ventro-cervical sensilla, and the papilla on the flagellomere III; flagellomere I with the

external ascoid implanted more apical than the internal one; palpomere II longer than or as long as the palpomere IV; Newstead spines on the palpomere III implanted on its middle or beyond this and pharynx without spines (Galati 1995, 2003a).

The *Evandromyia* genus is characterized by having males with the genitalia presenting gonocoxites with a compact and basal tuft of setae; parameres simple or branched; lateral lobes with tapered apex and the females presenting the ratio between the lengths: clypeus/head greater than 1/3 and eyes/head smaller than 1/2. The genitalia of males of the *rupicola* series have gonostyles with the two external spines implanted at the apex of a single tubercle, while the spermathecae of females are cross striated and the common sperm duct exceeds half the length of the stem of the genital furca.

The *rupicola* series, originally composed of *E. rupicola* and *Evandromyia correalimai* (Martins *et al* 1970), has recently been expanded (Andrade Filho *et al* 2009) with the addition of *Evandromyia gaucha* Andrade-Filho *et al* 

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and Evandromyia grimaldii Andrade-Filho et al.

Prior to Galati's classification (1995), E. rupicola and E. correalimai were included in the genus Lutzomyia França by the phlebotomine taxonomists. Forattini (1973) included them in the subgenus Coromyia Barretto and suggested that E. correalimai could be a geographical variant of *E. rupicola*. In this case, the former species would then be considered a junior synonym of the latter. Young & Duncan (1994) also adopted the species-group rupicola. Martins et al (1978) considered both to be isolated species and agreed with Forattini (1973) that E. correalimai could be a geographical variant of E. rupicola and concluded that this question would only be solved with the description of the female of the latter species. They also declared that these two species could constitute a distinct species-group only after the validation of E. correalimai. Mayrink et al (1979) reported five males and six females of *E. rupicola* in Caratinga municipality, state of Minas Gerais, but the females were not described (Young & Duncan 1994).

This study consists of a review of the *rupicola* series with the description of the female and redescription of the male of *E. rupicola*, as well as of some characteristics of the male and female of *E. correalimai*, which might help in the differentiation from other species of the series. The objective also includes an update of the geographical distribution of the four species of the *rupicola* series.

## **Material and Methods**

The specimens were macerated following Forattini (1973), mounted on microscope slides in NC medium (Cerqueira 1943), measured under a microscope and drawn with an Olympus® camera lucida. All measurements are given in micrometers. The nomenclature adopted is that of Galati (2003a) and the characters are described according to Galati (2003b). The specimens have been deposited in the entomological collection of the Faculdade de Saúde Pública da Universidade de São Paulo (FSP/USP).

#### Specimens examined

Evandromyia rupicola. Brazil, state of São Paulo: Ubatuba municipality, Camburi district (forest) 19 27-28 Jun 1998; Ilha Bela municipality, Castelhanos district: (domicile) 19 14-15 Jan 1998, 29 23-24 Apr 1998 and 1-2 Nov 1998 (forest) 13 17-18 Apr 1998. All the specimens were captured with a CDC light trap installed from 18.00 to 06.00 h by the SUCEN (Taubaté Section) field team.

## Description of Evandromyia rupicola (Figs 1-16)

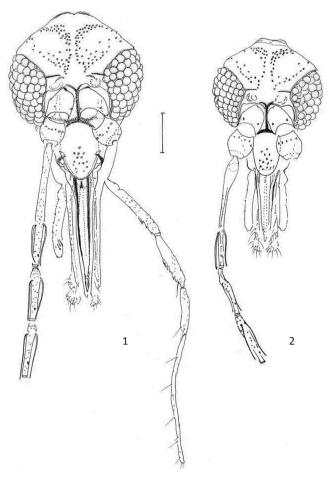
#### Female

Head (Fig 1) 398.8  $\pm 15.3$  long, 312.0  $\pm$  2.8 wide (n = 4), ratio between length/width 1.28:1.0  $\pm$  0.04 (n = 4). Clypeus  $128.1 \pm 4.3$  (n = 4) long; ratio clypeus length/ head length  $0.32:1.0 \pm 0.01$  (n = 4). Eye  $170.8 \pm 6.3$  long (n = 3); eye length/head length  $0.43:1.0 \pm 0.001$  (n = 3). Interocular distance  $135.0 \pm 20.1$  (n = 4). Labrumepipharynx (LE)  $260.6 \pm 10.5$  (n = 4); ratio: LE/head length  $0.65:1.0 \pm 0.008$  (n = 4). Antenna: antennomere length: AIII 310 (n = 1); AIV 135.0 (n = 1); AV 137.5 (n = 1); the last flagellomeres were lost; AIII with the external ascoid implanted slightly more apically than the internal one; AIV with the ascoid apex going beyond the apex of the segment (Fig 3); AV with papilla (Fig 4); ratios: AIII/head length 0.74:1.0; AIII/LE 1.13:1.0 (n = 1). Palpus: palpomere length: I 35.0  $\pm$  3.06 (n = 4); II 143.3  $\pm$  1.44 (n = 3); III 148.3  $\pm$  3.8 (n = 3); IV 112.5  $\pm$  2.5 (n = 3); V 298.8  $\pm$  15.9 (n = 2). Palpal formula 1.4.2.3.5 (n = 2) and 1.4.(2.3) (n = 1); palpomere II with two or three Newstead's spines; palpomere III with the Newstead's spines situated between its middle and the apical third (Fig 5). Cibarium (Fig 8) with two pairs of equidistant and needle-like horizontal posterior teeth; the anterior teeth being very small, vertically positioned in relation to the lumen, arranged in one or two irregular transversal rows, and also laterally grouped; the sclerotized area is clearly evident and funnel-shaped; sclerotized arch complete. The pharynx has atrophied spines on its apical quarter. Hypopharynx (Fig 6) has ca. 18 well-delimited short teeth situated apicolaterally. Lacinia of the maxilla (Fig 7) presents 4-5 very short external teeth, difficult to observe, arranged in a single row, and ca. 35 internal teeth. Labial sutures form a fork.

Ventro-cervical sensilla present.

Thorax 610  $\pm$  37.4 long; n = 4; Mesonotum 556  $\pm$  40.7 long. Presence of 1-2 (n = 4) proepimeral setae and 22-32 (n = 4) anepisternal superior setae; setae on the anterior region of the katepisternum absent. Wing (Fig 9) (n = 4): 2,075  $\pm$  122.8 long, 677.5  $\pm$  25.0 wide; R<sub>5</sub> 1,220.0  $\pm$  87.6; alpha 622.5  $\pm$  47.9; beta 205  $\pm$  5.8; gamma 213.7  $\pm$  28.7; delta 227.5  $\pm$  22.2; pi 60  $\pm$  11.5; ratio: length/width 3.06:1.0  $\pm$  0.13; Legs (n = 1), respectively anterior, median e posterior: coxa 310, 300, 320; femur 800, 780, 840; tibia 940, 1070, 1300; tarsomere I 610, 650, 700, and tarsomeres II+III+IV+V 710, 720, 780.

Abdomen 1,730  $\pm$  183.85 (n = 4) long. Tergite VIII with 2-3 setae, or absent (n = 4), on both sides (Fig 11). Spermathecae (Fig 12): 22  $\pm$  1.2 long by 11  $\pm$  2.4 (n = 4) maximum width, with two basal and one apical ring, with a striated region between them (sometimes the



Figs 1-2 Evandromyia rupicola, head, frontal view. 1)  $\cite{}$ ; 2)  $\cite{}$ 3. Bar = 100  $\cite{}$  µm.

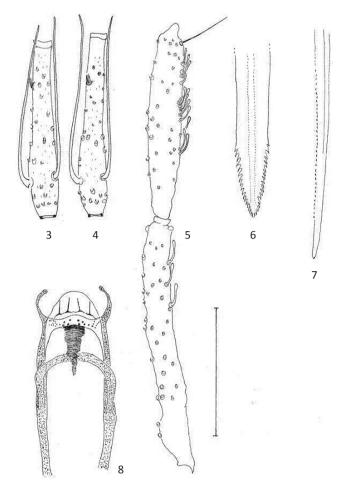
spermathecae are completely covered by a membrane - Fig 13); the transition to the individual sperm ducts is clear. These ducts are smooth, membranous, measuring  $64\pm7.4$  in length by  $14\pm6.6$  (n = 4) at maximum width; common sperm duct is also smooth and membranous,  $60\pm6.3$  (n = 4) long by  $15\pm2.5$  (n = 3) wide. Cercus  $133\pm15.5$  (n = 4) long, with oblong apex.

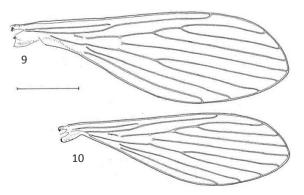
## Male

Head (Fig 2) 340 long, 270 wide; clypeus 106 long, ratio clypeus length/head length 0.31:1.0; eyes 150 long; ratio eye length/head length 0.44:1.0, interocular distance 115. Labrum-epipharynx (LE) 170. Palpus: palpomere length: I 30, II 125; the last three were lost. Antenna: antennomere length: AIII 278, AIV 125, AV 122, AXV and AXVI were lost. AIII with the external ascoid implanted slightly more apically than the internal one; AIV with the ascoid apex close to the apex of the segment; AV with papilla; ratio: AIII/LE 1.63:1.0. Labial sutures form a fork.

Ventro-cervical sensilla present.

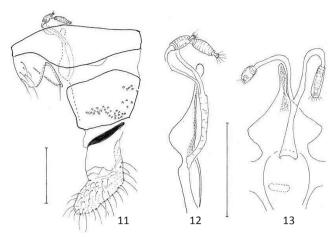
Thorax. 480 long, mesonotum 440 long. Presence of only



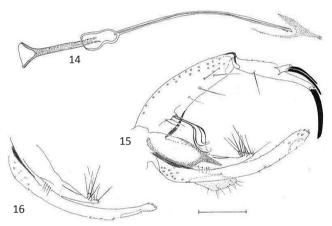


Figs 9-10 Evandromyia rupicola, wings. 9)  $\cite{2}$ ; 10)  $\cite{3}$ . Bar = 500  $\mbox{\mbox{$\mu$m}}$ .

one proepimeral seta and 26 upper anepisternal setae. Setae on the anterior margin of the katepisternum absent. Wing (Fig 10): 1,870 long, 580 wide; ratio length/width 3.22:1.0;  $R_{\rm S}$  1,110; alpha 520; beta 180; gamma 190; delta 170; pi 100. Legs were lost.



Figs 11-13 *Evandromyia rupicola*  $\circlearrowleft$ . 11) terminalia, lateral view; 12, genital fork and spermathecae, lateral view; 13, genital fork and spermathecae, ventral view of another specimen. Bar = 100  $\mu$ m.

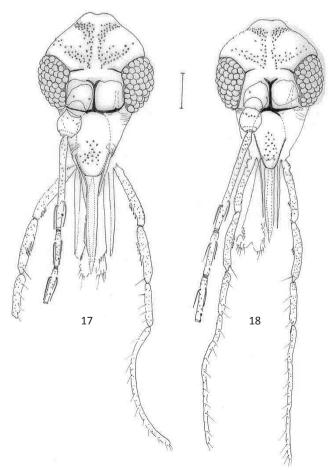


Figs 14-16 *Evandromyia rupicola*  $\circlearrowleft$ , lateral view. 14) aedeagus, genital pump and ducts; 15) genitalia; 16) view of the other paramere and lateral lobe. Bar = 100  $\mu$ m.

Abdomen 1,415 long; presence of tergal papillae from tergite V to VII. Terminalia (Figs 15, 16). Gonocoxite 250 long by 65 wide (in the middle) with a sclerotized longitudinal band on its basal internal face and a tuft with 3-4 long bristles implanted in a narrow, distinct tubercle; there are 1-2 isolated setae externally and more basal to the tubercle. Gonostyle 175 long, with one apical spine and absence of the pre-apical seta; the two external spines implanted in the apex of a single tubercle, the inferior one being shorter and thinner than the superior, the internal spine is setiform and situated close to the basal quarter. Paramere (dorsal margin 123 long and ventral 223) relatively wide with a narrowing in the middle. It presents a protuberance in the pre-apical region of the ventral margin, and a small tubercle with 8-9 setae distributed all over its surface in the pre-apical region of the dorsal margin, one of them being longer than the others, and 4-5 setae in the region between this tubercle and the apex of the paramere, one of them clearly longer than the others, as well as some very short setae. Aedeagus simple, with an acute apex. Lateral lobes 332 long, 22.5 wide (in the middle) and with a preapical constriction. Genital pump 207 long; piston 163 long; genital filaments with simple apex, 365 long or 1.77x the length of the pump (Fig 14).

The association of specimens from different sexes to *E. rupicola* was based on the similarity of the extra-genital characteristics: wings, body coloring, large number of upper anepisternal setae, as well as on female characteristics that permit its inclusion in the *rupicola* series. The only male found in the area belonged to *E. rupicola*.

The drawings of the head of a female (Fig 17) and a male (Fig 18) of *E. correalimai*, and their genitalia (Figs 19-21) are here presented because these structures were not sufficiently illustrated in the original description of this species. New illustrations for *E. gaucha*, which is known only by the female, and *E. grimaldii*, known by male and female, were considered unnecessary as both species were described on the basis of many morphological characteristics.



Figs 17-18 Evandromyia correalimai, head, frontal view. 17)  $\bigcirc$ ; 18)  $\bigcirc$ . Bar = 100  $\mu$ m.

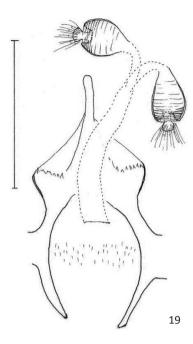
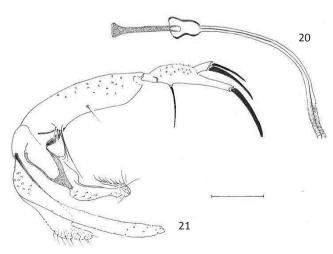


Fig 19 Evandromyia correalimai ♀, genital fork and spermathecae, ventral view. Bar = 100 um.

#### Distribution of the rupicola series

Evandromyia correalimai. SÃO PAULO state – Taquarituba and Ribeirão Grande municipalities (Forattini et al 1976, Galati et al 2010), PARANÁ state (Apucarana, Arapongas, Borrazópolis, Bandeirantes, Curitiba, Doutor Camargo, Jacarezinho, Jussara, Londrina and Marumbi municipalities (Martins et al 1970, 1978, Gomes & Galati 1977, Aguiar et al 1989, Luz et al 2000, Massafera et al 2005, Dias-Sversutti et al 2007, Silva et al 2008) and RIO GRANDE DO SUL (Derrubadas and Tenente Portela municipalities) (Dias et al 1997, Silva & Grunewald 1999). Evandromyia rupicola: MINAS GERAIS - Caratinga municipality (Mayrink et al 1979), RIO DE JANEIRO state – Magé, Petrópolis, Resende/Itatiaia municipalities (Martins et al 1962, 1978, Aguiar et al 1985, Afonso et



Figs 20-21 *Evandromyia correalimai*  $\delta$ , lateral view. 20) genital pump and ducts; 21) genitalia. Bar = 100  $\mu$ m.

al 2007) and SÃO PAULO state – Ilhabela and Ubatuba municipalities (Condino 2007). Evandromyia gaucha: RIO GRANDE DO SUL state - Caçapava do Sul municipality (Andrade Filho et al 2007). Evandromyia grimaldii: ESPÍRITO SANTO state - Cariacica municipality (Andrade Filho et al 2009).

#### Discussion

Evandromyia correalimai has been previously referred to as a possible geographical variant of E. rupicola (Forattini 1973, Martins et al 1978). However, we clearly demonstrate that these two species can be distinguished by several characteristics in both sexes. The clypeus of the male and female of *E. rupicola* (Figs 1, 2) is approximately 2/3rds the length of the eye, while the clypeus of E. correalimai (Figs 17, 18) is longer than the eye (1.15:1.0 in the female and 1.08:1.00 in the male). The spermathecae of E. rupicola (Figs 12, 13) also differ from those of E. correalimai (Fig 19). Spermathecae of E. rupicola are at least 25 µm wide and the individual sperm ducts are shorter than the common sperm duct, while the maximum width of the spermathecae in E. rupicola is 11  $\mu$ m  $\pm$  2.4  $\mu$ m (n = 4), and the individual and common sperm ducts are practically equal.

The male terminalia of both species may also be distinguished by several aspects: E. rupicola (Fig 15) presents the internal spine of the gonostyle atrophied whereas it is highly developed in *E. correalimai* (Fig 21). The apical lobe of the paramere is shorter and turned towards the gonocoxite in E. rupicola (Fig 16) while it is turned towards the base of the genitalia in *E. correalimai* (Fig 21). The genital pump and filaments of E. rupicola (Fig 14) are longer (207 μm and 365 μm, respectively) than those of E. correalimai (Fig 20) (ca. 158 µm; 335 μm), and the diameter of the pavilion of the piston and the filaments of *E. correalimai* are wider (ca. 50 µm and 10 um) than those of *E. rupicola* (30 μm and 5 μm). Further, E. correalimai presents tergal papillae from tergite IV to VII and *E. rupicola* from tergite V to VII; the tergal papillae being more conspicuous in the former species.

The male genitalia of *E. rupicola* are very similar to those of *E. grimaldii*, but *E. grimaldii* has longer genital filaments (480-518  $\mu$ m) than *E. rupicola* (364-371  $\mu$ m) (Andrade Filho *et al* 2009).

The clearly narrower apical half (bottlenecked shape) of the spermathecae of *E. grimaldii* differentiates it from the three other species. The terminal knob as long as the spermatheca in *E. gaucha* distinguishes it from *E. rupicola* and *E. correalimai*.

The identification keys presented in here help in distinguishing the three species based on their known males and the four species based on their known females.

#### Males

- 2. Genital filaments length *ca*. 370 μm ...... *E. rupicola* Genital filaments length *ca*. 500 μm ..... *E. grimaldii*

#### **Females**

- 2(1). Width of the apical half of the spermatheca equivalent to 1/3 of basal half (bottlenecked shape) ...... *E. grimaldii* Spermatheca slightly narrower in its apical half ...... 3

So far, we have information that the rupicola series is restricted to Brazil and is limited to the Atlantic forest domain. Evandromyia correalimai is the species with the widest known distribution, including interior areas of the Southeastern (Forattini et al 1976, Galati et al 2010) and Southern regions (Martins et al 1970, 1978, Gomes & Galati 1977, Aguiar et al 1989, Dias et al 1997, Silva & Grunewald 1999, Luz et al 2000, Massafera et al 2005, Dias-Sversutti et al 2007, Silva et al 2008). Evandromyia rupicola is restricted to the Southeastern region, in the mountainous region of eastern Minas Gerais state (Mayrink et al 1979), Rio de Janeiro state (Martins et al 1962, 1978, Aguiar et al 1985, Afonso et al 2007) and in the coastal area of São Paulo state (Condino 2007). Evandromyia gaucha and E. grimaldii, known only from their type-localities, the southeastern region of the state of Rio Grande do Sul and the coastal range in the southeast of the Espírito Santo state, respectively (Andrade et al 2007, 2009).

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