

# Considerations on the Species Complex of the Cortelezzii series (Diptera: Psychodidae) and Description of *Evandromyia chacuensis* sp. nov., a New Phlebotomine Species of the Chaco Region, Argentina

Enrique A. Szlag,<sup>1,2,3</sup> Juan R. Rosa,<sup>1</sup> Eunice A. B. Galati,<sup>4</sup> José D. Andrade Filho,<sup>5</sup> and Oscar D. Salomón<sup>2,3,6</sup>

<sup>1</sup>Instituto de Medicina Regional, Universidad Nacional del Nordeste, Av. Las Heras 727, Chaco, Argentina, <sup>2</sup>Instituto Nacional de Medicina Tropical, Ministerio de Salud, Misiones, Argentina, <sup>3</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CABA, Argentina, <sup>4</sup>Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, Av. Dr. Arnaldo 715, São Paulo, Brazil, <sup>5</sup>Coleção de Flebotomíneos, Centro de Referência Nacional e Internacional para Flebotomíneos, Instituto René Rachou, FIOCRUZ, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brazil, and <sup>6</sup>corresponding author, e-mail: [odanielsalomon@gmail.com](mailto:odanielsalomon@gmail.com)

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## Abstract

A new species of phlebotomine sand fly is described and illustrated based on male and female specimens collected in rural and semi-urban areas of the Chaco Province, Argentina. A separation of the Cortelezzii series in two species complexes is proposed, as is a species key for its identification. The morphological characters of the new species permit its inclusion in the Cortelezzii series of genus *Evandromyia*, subgenus *Barrettomyia* (Diptera: Psychodidae). The species is named *Evandromyia chacuensis* sp. nov. Szlag, Rosa, Galati, Andrade Filho & Salomón (Diptera: Psychodidae).

**Key words:** sand fly, phlebotomine, n. sp., new species, *Evandromyia*

Phlebotominae sand flies (Diptera: Psychodidae) are insects of medical and veterinary importance because some species are able to transmit various pathogens such as *Bartonella* spp., *Phlebovirus* spp., and protozoan parasites of the genus *Leishmania* to susceptible mammalian hosts. In the last 20 yr, numerous leishmaniasis outbreaks have been reported in the Chaco bioregion of Argentina (Salomón et al. 2008), with an increase of cases associated with periurban transmission. Of the 37 phlebotomine species recorded in Argentina (Szlag et al. 2016, Fuenzalida and Quintana 2017), 18 were found in the Chaco province. The genus *Evandromyia* Mangabeira, 1941 is one of the most frequent and widespread in the country. According to Galati (2003), this genus consists of three subgenera: *Evandromyia* s. str., *Aldamyia* Galati, 1995 and *Barrettomyia* Martins & Silva, 1968. Of the six species recorded in Argentina, *Evandromyia aldafalcaoae* (Santos, Andrade Filho & Honer 2001), *Evandromyia termitophila* (Martins, Falcão & Silva, 1964), and *Evandromyia evandroi* (Costa Lima & Antunes, 1936) are included in the *Aldamyia* subgenus. Three species, *Evandromyia cortelezzii* (Brèthes, 1923), *Evandromyia corumbaensis* (Galati, Nunes, Oshiro & Rego, 1989) and *Evandromyia sallesi* (Galvão & Coutinho, 1939) are included in the Cortelezzii series of subgenus *Barrettomyia*. All of

these species have been recorded in the Chaco province, with the exception of *Ev. evandroi*, which is restricted to Misiones Province.

The Cortelezzii series was previously formed by five species: a noncryptic species: *Evandromyia edwardsi* (Mangabeira, 1941), and four other cryptic species: *Evandromyia cortelezzii*, *Ev. sallesi*, *Ev. Corumbaensis*, and *Evandromyia spelunca* Carvalho, Brazil, Sanguinette & Andrade Filho, 2011. Males of these cryptic species can be distinguished using morphological characters, while females are difficult to differentiate. This is why females of these cryptic species were often identified not to species but as belonging to the ‘Cortelezzii complex’, a term used to differentiate them to *Ev. edwardsi* in the same series (Galati et al. 1989, Carvalho et al. 2011). Currently, the term Cortelezzii complex is no longer needed after the exclusion of *Ev. edwardsi* from the Cortelezzii series (Galati 2017). However, based on morphological characters discussed below we consider that the Cortelezzii series could be divided in two groups of cryptic species well differentiated, reconsidering the need for the informal category of complex to refer them.

It is important to note that females of the Cortelezzii series, collected in the dry area of Chaco Province, have been found with *Leishmania braziliensis* DNA (Rosa et al. 2012), underscoring the

importance of this species group in the region and the need for correct identification.

In the present paper, a new phlebotomine sand fly species from Chaco Province, Argentina, is described based on morphological characters of both sexes collected in rural and semi-urban areas of the province. A separation of the *Cortelezzii* series in two species complexes is proposed as is a species identification key based on Galati (2003).

## Materials and Methods

Collections were undertaken in known cutaneous leishmaniasis foci in two regions (Western Chaco and Eastern Chaco) of Chaco Province, between 2006 and 2013 (Rosa et al. 2010, Szelag et al. 2016). Captures were made monthly with mini CDC light traps (Sudia and Chamberlain 1962) installed 1.5 m above ground level from 7 pm to 7 am. In and around houses, the traps were placed indoors or on the veranda and in pens or animal resting places. Away from houses, they were placed in forest patches at least 100 m from habitations. In the Western Chaco region, captures were made from 2006 to 2008 for two consecutive nights each month in Fortín Arenales (S24°58'18"–W61°21'25"), approximately 40 km from the city of Misión Nueva Pompeya (capital of Comandante Brown Department). In the Eastern Chaco region, captures were carried out between 2010 and 2013 in the municipalities of Margarita Belén (S27°26'34"–W58°54'13"), Colonia Benítez (S27°19'16"–W58°59'53") and Resistencia (S27° 16'48"–W59° 1'11"). Specimens were cleared as described by Forattini (1973) and mounted in Canada balsam. Morphological structures were measured under a binocular microscope with a micrometer eyepiece (CX31RTSF; OLYMPUS CORP., Tokyo, Japan) and drawings were made using a camera lucida. Measurements (in micrometers) were made for the male holotype, the female allotype, and paratypes. The average, standard deviation, and numbers of paratype specimens examined are shown in parentheses after the holotype and allotype measurements. Measurements of some characters were not made, because some structures were lost during the preparation and mounting process. The nomenclature and abbreviations are based on Galati et al. (2017), and the classification scheme follows Galati (2003, 2017). The new species is described on the basis of 11 males and 12 females, caught at the sampling sites and, to ensure the female/male association, specimens were compared with second and third generations of a colony in the experimental insectaries of the Instituto de Medicina Regional, Universidad Nacional del Nordeste (IMR-UNNE). Further information of sampling sites and associated fauna could be found in Szelag et al. (2016).

## Other species

*Evandromyia corumbaensis*: BRAZIL—Mato Grosso do Sul state, Corumbá municipality (10 ♂, 10 ♀); *Evandromyia cortelezzii*: ARGENTINA—Chaco state, Resistencia municipality (10 ♂), Misión Nueva Pompeya municipality (10 ♂, 10 ♀); *Evandromyia sallesi*: ARGENTINA—Chaco state, Resistencia municipality (5 ♂), Margarita Belén municipality (10 ♂), Misión Nueva Pompeya municipality (10 ♂, 10 ♀); *Evandromyia spelunca*: BRAZIL: type locality—original description.

## Nomenclature

This paper and the nomenclatural act(s) it contains have been registered in Zoobank ([www.zoobank.org](http://www.zoobank.org)), the official register of the International Commission on Zoological Nomenclature. The LSID

(Life Science Identifier) number of the publication is urn:lsid:zoobank.org:pub:52D85D20-F27A-4967-9DDF-716E4FA14BE3

## *Evandromyia (Barrettomyia) chacuensis* Szelag, Rosa, Galati, Andrade Philo & Salomón sp. nov.

(Zoobank LSID: urn:lsid:zoobank.org:act:F6AC6450-843A-4518-BAAD-A5490630D292)

(Figs. 1–6)

### Male HOLOTYPE

Medium sized, general color light brown.

*Head* (Fig. 1A) length 363.6 (352; 7.5;  $n = 10$ ), width 283 (286; 8.3;  $n = 10$ ). Head length/head width ratio 1.29: 1 (1.23: 1; 0.04;  $n = 10$ ). Eye 136 (139; 6.2;  $n = 10$ ) long and 80 (80; 2.9;  $n = 10$ ) wide; eye length/head length 0.37: 1 (0.39: 1; 0.02;  $n = 10$ ). Clypeus 136 (129; 3.9;  $n = 10$ ) long and 90 (91; 2.4;  $n = 10$ ) wide; clypeus length/head length ratio 0.37: 1 (0.37: 1; 0.01;  $n = 10$ ). Interocular distance 133 (124; 9.0;  $n = 10$ ). Labrum-epipharynx (LE) 163 (167; 7.0;  $n = 10$ ). LE/head length 0.45: 1 (0.47: 1; 0.02;  $n = 10$ ). Labial suture forming a fork. First flagellomere with simple ascoids, internal one more basal than external one, flagellomeres II to XIII with simple and short ascoids, implanted at same level, not reaching the next flagellomere. Flagellomere lengths: FI 233 (232; 4.0;  $n = 10$ ); FII 118 (112; 3.6;  $n = 10$ ); FIII 115 (114; 4.3;  $n = 10$ ); FXIII 68 (64; 3.1;  $n = 10$ ); FXIV 68 (66; 4.0;  $n = 10$ ). Ratios: FI / LE 1.43:1 (1.39: 1; 0.06;  $n = 10$ ). FI / head length 0.64: 1 (0.66: 1; 0.02;  $n = 10$ ). FI, FII, FIII, FX, FXI, FXII, FXIII and FIVX with papillae. Palpal formula 1.2.4.3.5 (1.2.4.3.5;  $n = 10$ ). Palpal segment lengths: PI 25 (28; 2.1;  $n = 10$ ); PII 100 (101; 2.9;  $n = 10$ ); PIII 146 (139; 3.4;  $n = 10$ ); PIV

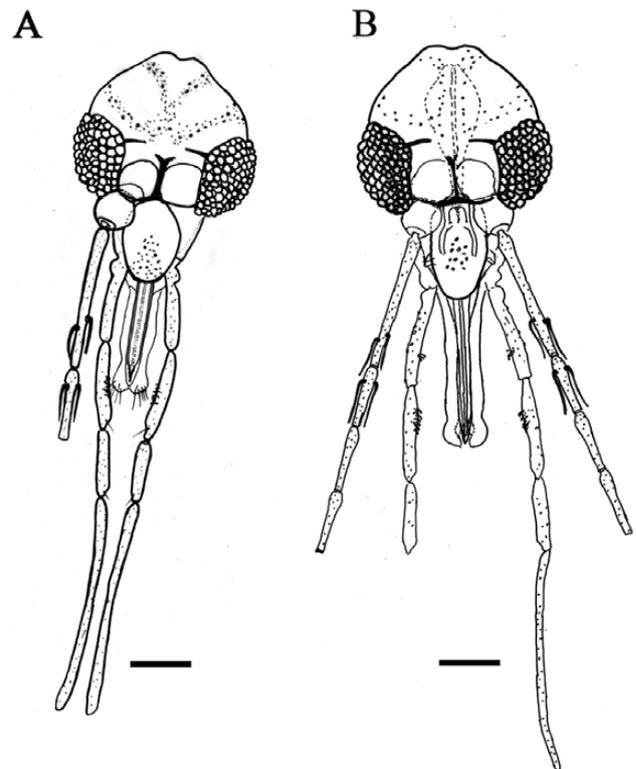


Fig. 1. *Evandromyia (Barrettomyia) chacuensis* sp. nov.: (A) male paratype head, frontal view and (B) female paratype head, frontal view. Bar = 100  $\mu$ m.

113 (105; 10.9;  $n = 10$ ); PV 356 (329; 32.4;  $n = 10$ ). Newstead's sensilla inserted medially on palpal segment III.

*Cervix.* Ventrocervical sensilla present.

*Thorax.* Proepimeral setae present 6-6 [(5-6;  $n = 4$ ) (7-6;  $n = 1$ ), (6-6;  $n = 2$ ), (6-4;  $n = 1$ ), (5-5;  $n = 1$ ), (4-3;  $n = 1$ )]; 11-16 upper anepisternal setae; setae on anterior katepisternum margin absent ( $n = 10$ ). Wing (Fig. 2A), 1,717 (1,679; 80.6;  $n = 10$ ) long and 465 (452; 10.4;  $n = 10$ ) at maximum width. Length/width ratio 3.69: 1 (3.71: 1; 0.06;  $n = 10$ ). Length of vein sections: *alpha* 313 (321; 21.7;  $n = 10$ ); *beta* 222 (198; 69.9;  $n = 10$ ); *gamma* 222 (237; 86.8;  $n = 10$ ); *delta* 91 (79; 33.6;  $n = 10$ ); *epsilon* 485 (506; 15.4;  $n = 10$ ); *pi* 141 (125; 45;  $n = 10$ ); R4 808 (820; 23.2;  $n = 10$ ); R5 1,111 (1,109; 6.4;  $n = 10$ ). Leg length: anterior, median and posterior, respectively: coxa 273 (266; 8.3;  $n = 10$ ), 273 (263; 11.7;  $n = 10$ ), 283 (265; 15.7;  $n = 10$ ); femur 657 (638; 18.9;  $n = 10$ ), 636 (623; 12.4;  $n = 9$ ) and 727 (724; 16.8;  $n = 9$ ); tibia 747 (700; 26.5;  $n = 10$ ), 808 (843; 29.9;  $n = 9$ ) and 1,061 (1,048; 31.9;  $n = 9$ ); tarsomere I 434 (422; 11.5;  $n = 10$ ), 505 (499; 15.2;  $n = 9$ ) and 606 (584; 14.1;  $n = 9$ ); tarsomeres II+III+IV+V 566 (566; 13.5;  $n = 10$ ), 596 (602; 17.6;  $n = 9$ ), and 616 (636; 13.4;  $n = 9$ ).

*Abdomen.* Presence of tergal papillae on central area of sixth and seventh tergites, without deciduous bristle scars between them. Terminalia (Fig. 3). Gonostylus 153 (152; 1.8;  $n = 10$ ) long, 38 (35; 3.0;  $n = 10$ ) maximum width, with four spines: one apical, one upper external inserted on tubercle in middle of segment, and two finer spines (lower external and internal) implanted almost at same level in basal third. Subterminal spine present. Gonocoxite 246 (245; 3.9;  $n = 10$ ) long and wide 93 (102; 5.9;  $n = 10$ ), with tuft containing three long, slender setae inserted on small unilobed tubercle, and six to eight shorter spine-like bristles inserted on globose upper area. Tuft surrounded by distinct hyaline area with small pigmented area on base. Paramere dorsal margin length 146 (143; 7.2;  $n = 10$ ); structure thicker at base, narrower in middle and wide at apex; dorsal margin of the apex convex, while ventral margin presents ventral projection with shape like that of a bird's head with beak pointing downward. Proximal limits of the dorsal and ventral expansions are almost at same level with former being a little bit more apical than

latter (Fig. 3). Paramere with longitudinal suture on apical half that divides dorsal and ventral margins; setae implanted almost along entire length. Epandrial lobe 351 (348; 9.2;  $n = 10$ ) long and 33 (33; 2.7;  $n = 10$ ) wide. Epandrial lobe/gonocoxite ratio 1.43:1 (1.42:1; 0.03;  $n = 10$ ). Parameral sheath conical and sclerotized. Aedeagal ducts (Fig. 4) 475 (472; 15.8;  $n = 10$ ) long and 4 (3; 1.03;  $n = 10$ ) wide, with simple tips. Length of sperm pump 158 (156; 2.6;  $n = 10$ ). Aedeagal ducts/sperm pump ratio 3:1 (3.02:1; 0.14;  $n = 10$ ).

## Female PARATYPES

Medium size, general color light brown.

*Head* (Fig. 1B) length 394 (404; 18.07;  $n = 11$ ), width 293 (300.25; 11.15;  $n = 11$ ). Head length/head width ratio 1.34: 1 (1.35; 0.07;  $n = 11$ ). Eye 141 (149.23; 7.81;  $n = 11$ ) long and 100 (91.27; 7.03;  $n = 11$ ) wide; eye length/head length 1.40: 1 (1.64; 1; 0.15;  $n = 11$ ). Clypeus 143 (149.46; 10.55;  $n = 11$ ) long and 93 (93.33; 2.46;  $n = 11$ ) wide; clypeus length/head length ratio 0.36: 1 (0.37: 1; 0.01;  $n = 11$ ). Interocular distance 138 (141.70; 4.81;  $n = 11$ ). Labrum-epipharynx (LE) 233 (232.97; 6.99;  $n = 11$ ). LE/head length 0.59: 1 (0.58: 1; 0.02;  $n = 11$ ). Labial suture forked. Flagellomeres with simple ascoids, in FI the internal one more basal than external one; on FII apex of ascoids reaches next segment. Flagellomere lengths: FI 221 (224.53; 10.67;  $n = 11$ ); FII 100 (104.05; 2.60;  $n = 11$ ); FIII 103 (104.96; 3.14;  $n = 11$ ); FXIII 68 (63.75; 2.42;  $n = 11$ ); FXIV 60 (56.22; 3.17;  $n = 11$ ). Ratios: FI/ LE 0.95: 1 (0.96: 1; 0.04;  $n = 11$ ); FI/ FIII 2.15: 1 (2.14: 1; 0.06;  $n = 11$ ). FI/head length 0.56: 1 (0.56: 1; 0.01;  $n = 11$ ). FI, FII, FIII, FX, FXI, FXII, FXIII and FIV with papilla. Palpal formula 1.4.2.3.5 (1.4.2.3.5;  $n = 10$ ) Palpal segment lengths: PI 33 (34.23; 2.03;  $n = 11$ ); PII 123 (122.08; 4.67;  $n = 11$ ); PIII 151 (154.62; 5.18;  $n = 10$ ); PIV 103 (109.94; 4.85;  $n = 10$ ); PV 341 (339.35; 14.29;  $n = 10$ ). Newstead's sensilla inserted medially on palpal segment II and III. Cibarium with four well-developed posterior (horizontal) teeth, equidistant, stilettiform, with broadened (not fused) base. Anterior (vertical) teeth present but not well-developed, slightly sclerotized and sometimes difficult to distinguish. Four anterior teeth arranged in single line forming arch on base of posterior teeth (Fig. 5). Lateral teeth evident. Sclerotized area conical and sclerotized arch incomplete. Pharynx unarmed. Lacinia of maxilla with 6-8 external teeth in single longitudinal row.

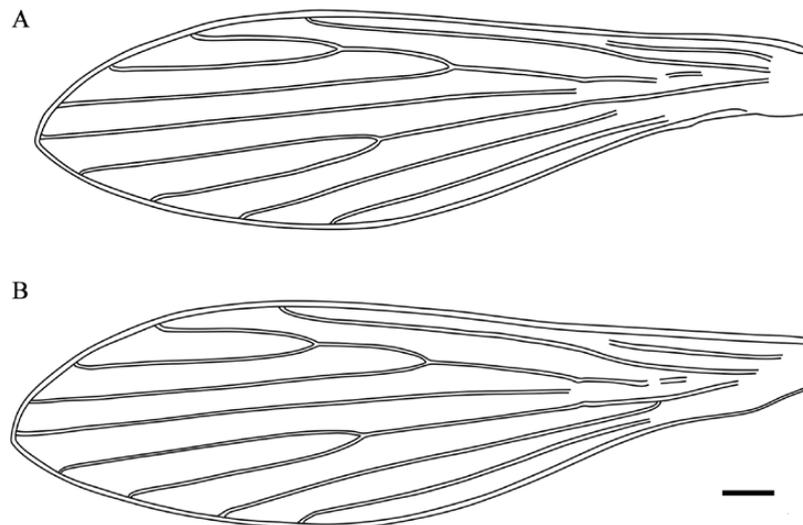


Fig. 2. *Evandromyia* (*Barrettomyia*) *chacuensis* sp. nov.: (A) male holotype wing and (B) female paratype wing. Bar = 100  $\mu$ m.



Fig. 3. *Evandromyia (Barrettomyia) chacuensis* sp. nov. male holotype: terminalia. Bar = 100  $\mu$ m.

*Cervix.* Ventrocervical sensilla present.

*Thorax.* proepimeral setae present, 4-4 [(4-4;  $n = 2$ ) (4-5;  $n = 1$ ), (5-5;  $n = 2$ ), (5-6;  $n = 4$ ), (6-6;  $n = 2$ )]; 13-19 upper anepisternal setae; setae on anterior katepisternum margin absent. Wing (Fig. 2B), 1,818 (1,798.81; 27.54;  $n = 10$ ) long and 505 (517.12; 33.26;  $n = 10$ ) at maximum width. Length/width ratio 3.6: 1 (3.49: 1; 0.23  $n = 10$ ). Length of the vein sections: *alpha* 333 (370.67; 41.52;  $n = 10$ ); *beta* 222 (244.42; 12.42;  $n = 10$ ); *gamma* 323 (289.87; 23.83;  $n = 10$ ); *delta* 81 (106.05; 15.24;  $n = 10$ ), *epsilon* 485 (526.21; 53.96;  $n = 10$ ); *pi* 141 (137.36; 12.78;  $n = 10$ ); R4 828 (879.71; 54.17;  $n = 10$ ); R5 1,040 (1,166; 74.58;  $n = 9$ ). Leg length, anterior, median and posterior, respectively: coxa 273 (291.98; 19.40;  $n = 11$ ), 273 (285.55; 16.34;  $n = 11$ ), 273 (286.47; 18.22;  $n = 11$ ); femur 667 (692.41; 32.77;  $n = 9$ ), 657 (685.68; 38.94;  $n = 9$ ), and 758 (789.82; 37.73;  $n = 10$ ); tibia 707 (726.08; 43.86;  $n = 9$ ), 859 (889.92; 45.01;  $n = 9$ ), and 1,061 (1,103.93; 51.73;  $n = 10$ ); tarsomere I 404 (435.42; 27.40;  $n = 9$ ), 485 (501.63; 30.30;  $n = 9$ ), and 576 (618.12; 38.33;  $n = 10$ ); tarsomeres II+III+IV+V 545 (587.06; 29.78;  $n = 8$ ), 616 (623.31; 19.96;  $n = 7$ ), and 687 (718.54; 41.84;  $n = 7$ ).

*Abdomen.* Spermathecae globular (Fig. 6), 13 (12.55; 1.12;  $n = 11$ ) long by 18 (19.17; 1.27;  $n = 11$ ) maximum width. Apical knob 10 (12.32; 0.76;  $n = 11$ ) long, with fine bristles in the apex. Sperm ducts smooth-walled, individual sperm duct length 228 (230.24; 8.10;  $n = 11$ ) width 3 (3.65; 0.38;  $n = 11$ ) pre-apical; common duct length 30 (27.84; 2.09  $n = 11$ ). Spermatheca width/individual sperm duct width (preapical) ratio: 6: 1 (5.25: 1; 0.60;  $n = 11$ ). Individual sperm duct length/common sperm duct length ratio: 7.58: 1 (8.32: 1; 0.76;

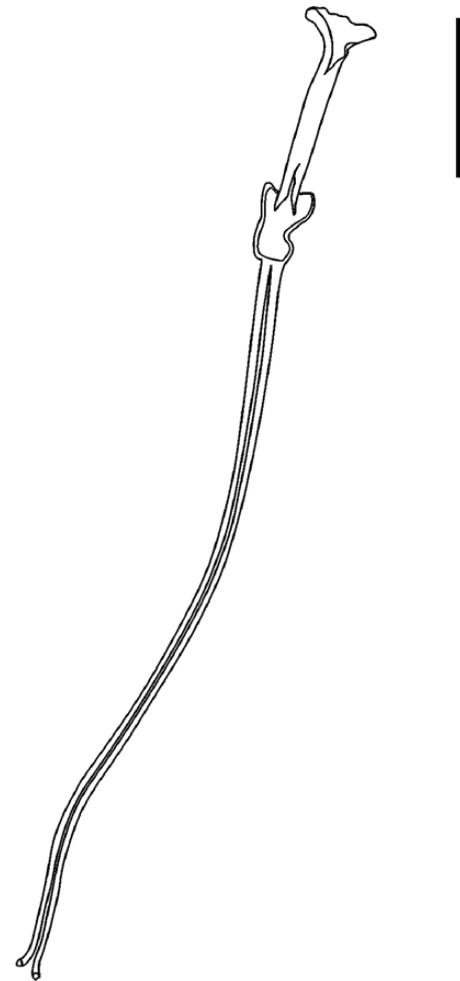


Fig. 4. *Evandromyia (Barrettomyia) chacuensis* sp. nov. male holotype: sperm pump and aedeagal ducts. Bar = 100  $\mu$ m.

$n = 11$ ). Individual sperm ducts length/rod height of genital fork 2.27: 1 (2.36; 1.70;  $n = 4$ ).

### Type Material

**HOLOTYPE:** 1  $\sigma$ , Misión Nueva Pompeya, CH, 15-X-2008, Rural area of Paraje Fortín Arenales, Szelag E.A. and Rosa J.R.. **PARATYPES:** 1  $\sigma$ , Colonia Benítez, CH, 03-XI-2010, Peridomicile in Rural Area, Szelag E.A. and Rosa J.R.; 1  $\sigma$ , Resistencia, CH, 09-IV-2012, Monte Alto periurban area, Szelag E.A. and Rosa J.R.; 1  $\sigma$ , Resistencia, CH, 17-I-2013, Monte Alto periurban area, Szelag E.A. and Rosa J.R.; 7  $\sigma$  and 12  $\varphi$  were obtained from the second and third generations of a colony in the experimental insectaries of the Instituto de Medicina Regional, Universidad Nacional del Nordeste (IMR-UNNE) to ensure the female/male association. The geographic origins of the colony and sample data are the same as those of the Holotype. The voucher specimens will be deposited in the Phlebotomine Sand fly Collection of the Instituto-Fundación Miguel Lillo (I-FML), San Miguel de Tucumán, Argentina.

*Type locality:* The holotype was collected in ARGENTINA, Chaco, Comandante Brown Department, Misión Nueva Pompeya (24°58'7.5"S–61°21'1.1"W), in the Chaco bioregion in the Dry Chaco subregion.

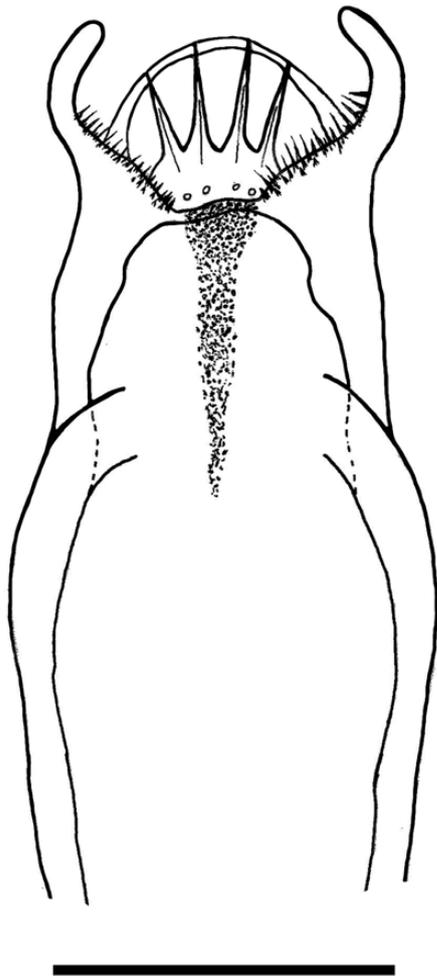


Fig. 5. *Evandromyia* (*Barrettomyia*) *chacuensis* sp. nov. female paratype: cibarium. Bar = 50  $\mu$ m.

### Distribution

This species was found in three municipalities of the Chaco province. Misión Nueva Pompeya located in the Dry Chaco bioregion north of the province, and Colonia Benítez and Resistencia in the Humid Chaco bioregion, South of the province.

### Etymology

The name *Evandromyia chacuensis* sp. nov. has been taken from the word ‘chacú’ used to name a hunting territory or the hunting technique in the native people’s language, from which the Hispanic name of the “Chaco” region is derived.

### Discussion

The morphological characters of the new species permit its inclusion in the Cortelezzii series of the genus *Evandromyia* (*Barrettomyia*). Currently, after the exclusion of *Ev. edwardsi*, this series consists of a group of four cryptic species: *Ev. corumbaensis*, *Ev. cortelezzii*, *Ev. sallesi* and *Ev. spelunca* (Galati 2017). Nevertheless, based on the description of the new species, and differences observed between the spermathecae, individual sperm duct, cibarium, aedeagal ducts and sperm pump, we considered that we can clearly separate the Cortelezzii series in two groups of species. One formed by

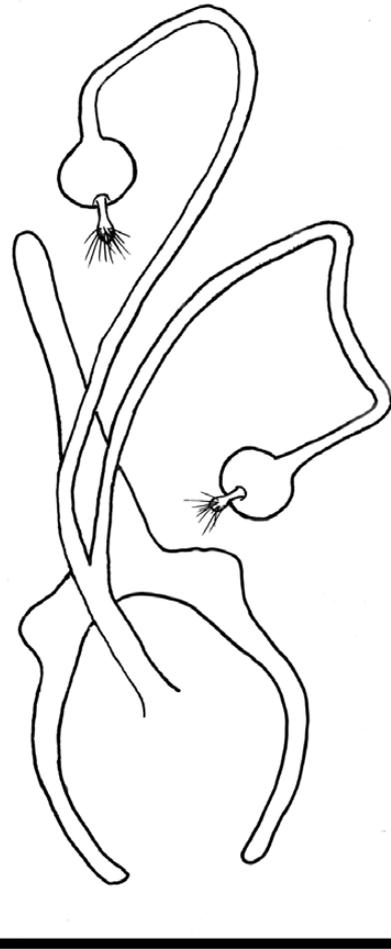


Fig. 6. *Evandromyia* (*Barrettomyia*) *chacuensis* sp. nov. female paratype: spermathecae. Bar = 100  $\mu$ m.

*Ev. cortelezzii* and *Ev. sallesi*, that we propose to continue calling Cortelezzii complex, the females presenting short individual sperm ducts of almost the same length as the height of the stem of the genital fork; the males being differentiated by the ratio between the length of the aedeagal ducts/sperm pump  $\leq 2.3$ . The other group, represented by *Ev. corumbaensis*, *Ev. spelunca*, and *Ev. chacuensis* sp. nov., we propose to name the Corumbaensis complex, with females presenting individual sperm ducts 2–2.5 times longer than the height of the stem of the genital fork, and males with the aedeagal ducts  $\geq 2.7$  times the length of the sperm pump.

As previously explained the new species presents more similarities to *Ev. corumbaensis* and *Ev. spelunca* but can be differentiated by the internal suture, curvature and shape of the paramere and by the gonocoxite tuft (Table 1). The shape of the paramere differentiates *Ev. chacuensis* sp. nov. and *Ev. corumbaensis* from *Ev. spelunca* (see Fig. 7B) by the dorsal curvature of the paramere, more evident in the two former species, resulting in a thinner neck in the middle of the segment (Fig. 7A). Also, in *Ev. corumbaensis*, *Ev. sallesi*, *Ev. cortelezzii*, and *Ev. chacuensis* sp. nov., the distal portion of the paramere ends in a clearly differentiated rounded head that suggests a bird’s head or a hood with a ventral protuberance, like a bird’s beak (Galati et al. 1989; Fig. 7C and D), while in *Ev. spelunca*, this rounded head is not so clearly differentiated because its dorsal margin extends up to the middle of the paramere, giving the head an ovoid shape (see Fig. 7B). Further, the shape and size of the paramere

**Table 1.** Morphological comparison between males and females of *Cortelezzii* series species

Cortelezzii series species	Male			Female		
	Paramere body	Paramere head	Gonocoxite tuft*	Individual spermathecal ducts	Spermathecae	Clypeus
<i>Ev. sallesi</i>	Robust (rectangular)	Hood-like structure	4 long bristles (2 + 2); 3–4 spiniform bristles	1–1.5 times longer than the stem height of the genital fork	3 times wider than the individual ducts	shorter or equal to the eye length
<i>Ev. cortelezzii</i>	Slender (dorsal margin concave)	Rounded head	5 long bristles (3 + 2); 3–5 spiniform bristles	1–1.5 times longer than the stem height of the genital fork	3 times wider than the individual ducts	shorter or equal to the eye length
<i>Ev. corumbaensis</i>	Slender (dorsal margin concave)	Rounded head	7 long bristles (4 + 3); 3–4 spiniform bristles	2–3 times longer than the stem height of the genital fork	6 times wider than the individual ducts	longer than the eye length
<i>Ev. chacuensis</i>	Slender (dorsal margin concave)	Rounded head	3 long bristles; 6–8 spiniform bristles	2–3 times longer than the stem height of the genital fork	6 times wider than the individual ducts	shorter or equal to the eye length
<i>Ev. spelunca</i>	Slender (dorsal margin concave)	Ovoid-shaped head	4 long bristles; 2 spiniform bristles	2–3 times longer than the stem height of the genital fork	6 times wider than the individual ducts	longer than the eye length

Gonocoxite tuft\*: the number of long bristles inserted in different tubercles is presented in parentheses.

differentiate them from *Ev. corumbaensis*, this last being longer and slenderer due to the pronounced dorsal curvature (Fig. 7A).

In the proposed new species, as in *Ev. spelunca*, the suture that divides the paramere is more central than in the other species and runs almost the entire length of the paramere. This suture is also evident in *Ev. cortelezzii* and *Ev. corumbaensis*, in the latter, however, being positioned more dorsally (Fig. 7).

*Evandromyia chacuensis* sp. nov. can be distinguished from *Ev. sallesi* and *Ev. cortelezzii* by the aedeagal ducts/sperm pump ratio, as previously described, but also by the shape of the paramere, the aspect of which is more similar to that of *Ev. cortelezzii*, and clearly different from the robust paramere of *Ev. sallesi* (Fig. 7).

Further, the more conspicuous character for the differentiation of these species is the gonocoxite tuft. The tufts present a small pigmented area on its base, and two different kinds of bristles: the longer slender and more conspicuous ones implanted in a tubercle that can be on one and the same lobe or on different lobes; and shorter less conspicuous spine-like bristles inserted on the upper side of the tuft. All the species of the *Cortelezzii* series present well differentiated tufts that vary in shape, number and implantation of the bristles (Table 1). In *Ev. chacuensis* sp. nov. (Fig. 3), the tuft is represented by 3 long, wide and well developed bristles implanted on a unilobed tubercle, and 6–8 short spine-like bristles implanted on a globose upper area of the tuft. In *Ev. corumbaensis* the tuft has 5–7 (most commonly 7) long, thin bristles implanted in two different lobes of the tubercle, and 3–4 (most commonly 4) spiniform bristles in a position slightly more apical than the other. Also, as previously explained, there are differences in the sperm pump and aedeagal ducts; in the species of the *Corumbaensis* complex the aedeagal ducts are thinner and longer and the ratio between the length of the aedeagal ducts/sperm pump 2.7 to 3.0 times greater, while in the *Cortelezzii* complex the ratio is  $\leq 2$ .

Distinction of the females of the other species of the *Corumbaensis* complex is more difficult and has not always been possible. However, the new species can be distinguished from the species of the *Cortelezzii* complex by the spermathecae and characteristics of their ducts, both individual and common ducts being noticeably longer in the *Corumbaensis* complex (Table 1). *Evandromyia chacuensis* sp. nov. females can also be differentiated

from *Corumbaensis* complex females by the eye/clypeus length ratio, the eye length in the former being equal or sub-equal to the clypeus as observed for the *Cortelezzii* complex, while *Corumbaensis* complex females have the clypeus longer than the eye (however, intra-species variations were observed). The characteristics of the cibarium were sufficient to distinguish the females of both complexes from each other but not between those of species of the same complex. The distance between the area of the implantation of the vertical teeth and the base of the cibarium arch is greater than the width of the middle of the segment in the *Corumbaensis* complex, while it is wider than longer in the *Cortelezzii* complex. Further, in the new species, the four vertical teeth are arranged in a single line in a less conspicuous arc than in *Ev. spelunca*. In addition, the vertical teeth are smaller and difficult to observe in most cases, while in the other species of the *Cortelezzii* series the teeth are bigger, more chitinous and easier to observe.

In the Chaco region of Argentina, all the species of the *Cortelezzii* series have been reported, with the exception of *Ev. spelunca*, probably due to the latter's caverniculous habitat preferences (Carvalho et al. 2011) as caves are rare in the Chaco landscape. However, reports of the distribution of *Ev. cortelezzii* and *Ev. sallesi* extend to the center of the country, in Cordoba Province (Visintin et al. 2016), suggesting that the *Cortelezzii* complex has a broader thermotolerance permitting colonization of southern provinces, while the *Corumbaensis* complex is restricted to the warmer environments in the north of the country. Further projects may focus on the phylogenetic status of this species group, supporting or disproving these morphological findings. Its sympatry in Argentina should be considered for proper identification in further studies.

### Identification Key for Males and Females of the *Evandromyia* (*Barretomyia*) *Cortelezzii* Series

#### Males

- 1 Aedeagal ducts/sperm pump ratio less than 2.3: 1.0 .....  
.....(*Cortelezzii* complex) 2
- Aedeagal ducts/sperm pump ratio 2.7–3.0: 1.0 .....  
.....(*Corumbaensis* complex) 3

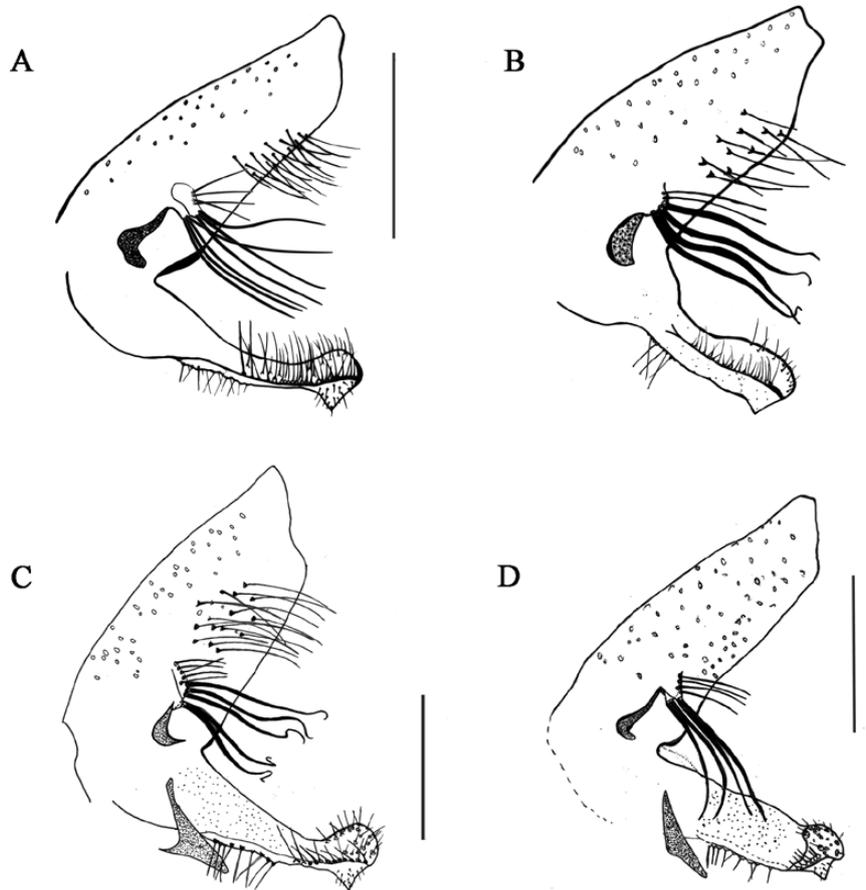


Fig. 7. Male terminalia of (A) *Ev. corumbaensis*, (B) *Ev. spelunca*, (C) *Ev. cortezzii* and (D) *Ev. sallesi*. Bar = 100  $\mu$ m.

2 (1) Paramere robust (Fig. 7D), rectangular from the base to the apical region, apically with a hood-like structure; gonocoxite tuft with four long bristles inserted on different lobes of the tubercle (2 + 2), and 3–4 upper short bristles..... *Ev. (Bar.) sallesi*

Paramere slender (Fig 7C), with a concavity on its dorsal margin, apically with no hood-like structure; gonocoxite tuft with five long bristles inserted on different lobes of the tubercle (3 + 2), and 3–5 upper short bristles..... *Ev. (Bar.) cortezzii*

3 (1) Clypeus longer than the eye length; gonocoxite basal tuft with 2–4 upper short spiniform bristles and 4–7 long basal conspicuous bristles inserted on a single-lobed tubercle .....4

Clypeus shorter or equivalent to the eye length; gonocoxite basal tuft with 6–8 upper short spiniform bristles and three long basal conspicuous bristles inserted on a single-lobed tubercle (Fig. 3).....*Ev. (Bar.) chacuensis* sp. nov.

4 (3) Gonocoxite basal tuft with 3–4 upper short spiniform bristles and 5–7 basal long conspicuous bristles inserted in two different lobes of the tubercle; paramere narrower in the middle of the segment ending in a rounded head (Fig. 7A) .....*Ev. (Bar.) corumbaensis*

Gonocoxite basal tuft with two upper short spiniform bristles and four basal long conspicuous bristles inserted

on a single-lobed tubercle; paramere less narrow in the middle of the segment ending in an ovoid-shaped head (Fig. 7B)..... *Ev. (Bar.) spelunca*

Females

1 Individual spermathecal ducts 1–1.5 times longer than the stem height of the genital fork; spermathecae ca. 3 times wider than the individual ducts (pre-apical).....(Cortezzii complex)

*Ev. (Bar.) cortezzii*  
*Ev. (Bar.) sallesi*

Individual spermathecal ducts 2–3 times longer than the stem height of the genital fork; spermathecae ca. 6 times wider than the individual ducts (pre-apical).....(Corumbaensis complex) 2

2 (1) Clypeus longer than the eye length.....  
..... *Ev. (Bar.) corumbaensis*

*Ev. (Bar.) spelunca*

Clypeus shorter than or equal to the eye length.....  
.....*Ev. (Bar.) chacuensis* sp. nov.

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