Breeding sites of Culicoides pachymerus Lutz in the Magdalena River basin, Colombia

María Cristina Carrasquilla1,2,*, Felipe Guhl2, Yaneth Zipa4, Cristina Ferro1, Raúl Hernando Pardo1, Olga Lucía Cabrera1, Erika Santamaría1

1Laboratorio de Entomología, Instituto Nacional de Salud, Avenida calle 26 51-20, Zona 6 CAN, Bogotá, Colombia 2Centro de Investigaciones en Microbiología y Parasitología Tropical, Universidad de los Andes, Bogotá, Colombia 3Grupo de Entomología y Enfermedades Transmitidas por Vectores, Universidad de La Salle, Bogotá, Colombia 4Secretaría de Salud de Boyacá, Boyacá, Colombia

The breeding sites of Culicoides pachymerus are described for the first time in western Boyacá Province, Colombia, where this species is a public health problem. In addition to being a nuisance due to its enormous density and its high biting rates, C. pachymerus cause dermatological problems in the human population. Analysis of microhabitats by the sugar flotation technique and the use of emergence traps allowed us to recover 155 larvae of Culicoides spp and 65 adults of C. pachymerus from peridomiciliary muddy substrates formed by springs of water and constant rainwater accumulation. These important findings could aid in the design of integrated control measures against this pest.

Key words: Ceratopogonidae - breeding sites - midges - Colombia

Bloodsucking midges of the genus Culicoides Latreille belong to the family Ceratopogonidae. These insects have a long evolutionary history, with fossil records dating back 90 million years; at this time, they were already feeding on vertebrate blood (Borkent 2005). Culicoides midges have public health importance as vectors of viruses, nematodes and protozoa. In addition, their intensive biting is a significant nuisance and can cause dermatological problems when the insects are very abundant (Blanton & Wirth 1979, Borkent 2005). According to Borkent (2009), about 1,300 species of this genus have been described worldwide, of which 114 have been recorded in Colombia (Spinelli et al. 2009). Some of these species have been reported to cause problems in the Colombian Andes due to their high biting rates; Culicoides puracensis Wirth & Lee was reported as a pest in the uplands (páramo) of Puracé by Wirth and Lee (1967) and Culicoides pachymerus has been reported as a public health problem in western Boyacá since 1987 (Villareal 1998). Santamaría et al. (2008) reported peridomiciliary biting rates of up to 51.8 females/person/5 min for the latter region. As in other members of the family Ceratopogonidae, the life cycle of Culicoides midges consists of an egg stage, four larval instar stages, a pupal stage and an adult stage. The ecology and population dynamics of the larvae are key to understanding their patterns of abundance and the relationship of these patterns to environmental factors such as climate and habitat. Furthermore, control of the immature stages may play a significant part in pest and vector control. However, little is known about the microhabitats in which Culicoides larvae develop and immature stages have been described for only 238 (Borkent 2005) of the currently known species (Borkent 2009). Although the breeding sites of Culicoides are extremely variable, all are aquatic or semi-aquatic, including damp or wet decomposing vegetation, wet leaf packs, manure, many different types of phytotelmata, tree holes, swamps, ponds, lakes, streams and river margins, mangrove swamps, bogs and salt marshes (Borkent 2005).

Despite the relatively wide neotropical distribution of C. pachymerus, which is found in Brazil, Colombia, Ecuador, Venezuela, Guatemala, El Salvador and Panamá (Forattini 1957, Wirth et al. 1988), the preferred breeding sites of this species have not been determined. The aim of the present study was to identify the microhabitats in which immatures of C. pachymerus occur.

Sampling was carried out in three scattered rural dwelling areas (veredas) in the foothills of the eastern Cordillera of the Andes, which surrounds the central Magdalena River valley in Boyacá department: vereda Chizo Cuepar in the municipality of San Pablo de Borbur, vereda Santa Rosa in the municipality of Tunungua and vereda Topo Grande in the municipality of Pauna (Fig. 1). These rural dwelling areas were selected to be in municipalities with the highest biting rates of C. pachymerus according to a previous study (Santamaría et al. 2008). The predominant Holdridge life zones of the study area are tropical rain forest (bh-T) and humid premontane forest (bmh-PM). The mean temperature of the study area is 21.8°C and the mean annual precipitation is 2,247 mm. The precipitation pattern is bimodal, with peaks in rainfall occurring during April and October and the altitude is between 439-831 masl.

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+ Corresponding author: mccarrasquilla@yahoo.es
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The study was carried out in two stages. The first stage took place from October-November 2006 and in February, March and May 2007. During this stage, flotation in saturated sugar solution (Uslu & Dik 2006) was used to inspect the most frequent microhabitats in the study area, based on the microhabitats described for other Culicoides species worldwide (Borkent 2005). The following microhabitats were investigated: (i) peridomestic muddy substrate formed by springs of water and constant rainwater accumulation, (ii) peridomestic muddy substrate formed by leakage of the shower and washbasin, (iii) soil of the peridomestic: semi-humid substrate, not flooded and without a muddy appearance, (iv) stream bank: rocky and flooded substrate of three streams of the region (La Piaché, Aguas Negras, La Chana) and (v) river bank: sandy substrate and overflow of the Minero River. The peridomestic was defined as the area immediately surrounding a dwelling to a distance of 50 m, beyond which the extradomestic occurs. In each microhabitat, parcels of 30 cm x 30 cm x 5 cm (length x width x depth) were sampled and placed in a container and a saturated sugar solution was then added (Uslu & Dik 2006). Immature Ceratopogonidae forms that were visible to the naked eye (i.e., those at least 1.3 mm long) were recovered using dissecting needles and preserved in 70% ethanol. Finally, immature individuals were counted and identified in the laboratory as Culicoides spp using the keys and descriptions of Kettle and Lawson (1952), Blanton and Wirth (1979) and Borkent and Spinelli (2007). An area of 6 m² of peridomestic muddy substrate of 12 dwellings was inspected in each of the first two microhabitats, as well as 3 m² (3 dwellings) of peridomestic semi-humid soil, 5 m² (5 dwellings) of stream bank (4 m² in the peridomestic and 1 m² in the extradomestic) and 5 m² (five dwellings) of river bank from the extradomestic. The second stage of the study was done to confirm the presence of C. pachymerus in the microhabitats that tested positive for Culicoides larvae. In this part of the study, 14 emergence traps (Fig. 2), which occupied a total surface area of approximately 1 m², were positioned by taking into account the results of the first stage; thus, traps were only placed over muddy substrates surrounding two dwellings from which the Culicoides larvae were collected using the flotation technique.

In the dwelling from which the most Culicoides larvae were collected, 10 emergence traps were placed and in the dwelling with the second highest number of collected larvae, four emergence traps were placed. This second stage was carried out from December 2007-January 2008. Traps were inspected for the presence of C. pachymerus every 24 h for 20 consecutive days. The
presumably teneral adult insects caught were removed gently with a fine paintbrush, rinsed in 2% detergent solution and preserved in 70% ethanol. Specimens were counted by sex and identified in the laboratory using the keys of Wirth and Blanton (1959) and Spinelli and Wirth (1985). Immatures were found only in the muddy substrate of the peridomicile, in the microhabitat formed by springs of water and constant rainwater accumulation; 155 larvae/m² (25.8 larvae/m²) of Culicoides spp were recovered from this microhabitat. These larvae were distributed among three of the 12 dwellings sampled, with most (136 larvae) recovered around a single dwelling of the vereda Chizo Cueva, San Pablo de Borbur in October 2006. In the dwelling with the second highest number of collected larvae, located in the same vereda, 17 larvae were recovered in May 2007. Only two larvae were found around the third dwelling (in March 2007), located in Santa Rosa, Tunungua. During the second stage of the study, 107 teneral Culicoides adults/m² belonging to five different species were collected, of which C. pachyrurus was the most abundant (35 males/m², 30 females/m²), followed by Culicoides iriartei Fox (17 males/m², 16 females/m²). Only a few individuals of Culicoides debilipalpis Lutz (1 male/m², 5 females/m²), Culicoides leoni Barbosa (1 male/m² male, 1 female/m²) and Culicoides insignis Lutz (1 female/m²) were found.

These findings reveal that in western Boyacá, muddy substrate formed by springs of water and the constant accumulation of rainwater is a breeding site for C. pachyrurus and other Culicoides species (C. iriartei, C. debilipalpis, C. leoni and C. insignis).

The combination of the flotation technique (Uslu & Dik 2006) with emergence traps was very important because the former allowed us to identify the preferred microhabitats of the Culicoides genus and the latter focused the sampling effort in only the positive sites to confirm the species found there. The species could not be confirmed using only the flotation technique because the immature stages of most of the species of the area have not been described. We performed some breeding assays with larvae captured in the field in an attempt to obtain adults to confirm identification, but these were fruitless. Breeding assays were also done with wild females, but only first instar larvae were obtained.

Our results are consistent with those of Santamaría et al. (2008) based on samples using human bait. In that study, C. pachyrurus was established as the most abundant species (99.3% of 3,389 specimens collected) and few specimens of C. debilipalpis and C. leoni were collected. C. iriartei has not been previously reported in the area in number of Culicoides spp larvae, to Marco Fidel Suárez, for his constant support of fieldwork, to the inhabitants of Pauna, San Pablo de Borbur and Tunungua, to Johan Gómez, for his help with insect sampling, and to Bruce Alexander, for his help in the english version of the manuscript.

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