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

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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 meas-ures 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). *Culicoide*s midges have public health importance as vectors of viruses, nematodes and protozoans. 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 21.8ºC 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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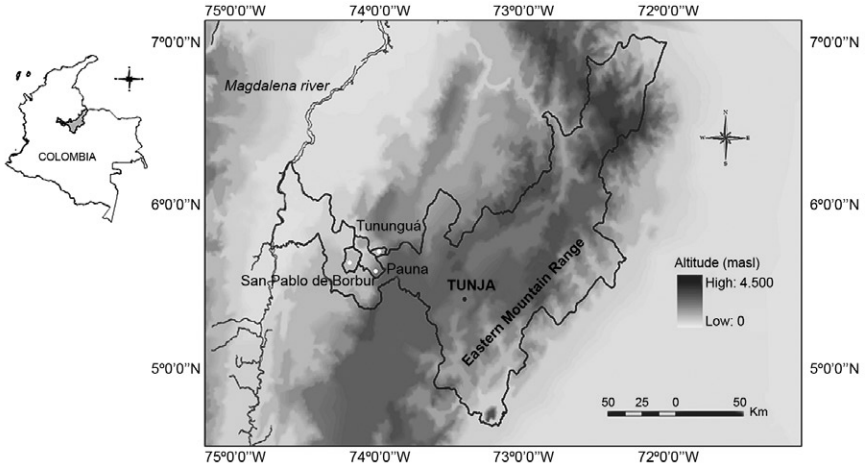

Fig. 1: map representing Colombia, Boyacá and the studied municipalities.

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) peridomiciliary muddy substrate formed by springs of water and constant rainwater accumulation, (ii) peridomiciliary muddy substrate formed by leakage of the shower and washbasin, (iii) soil of the peridomicile: 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 peridomicile was defined as the area immediately surrounding a dwelling to a distance of 50 m, beyond which the extradomicile 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 m^2 of peridomiciliary muddy substrate of 12 dwellings was inspected in each of the first two microhabitats, as well as 3 m^2 (3 dwellings) of peridomiciliary semi-humid soil, 5 m^2 (5 dwellings) of stream bank (4 m^2 in the peridomicile and 1 m^2 in the extradomicile) and 5 m^2 (five dwellings) of river bank from the extradomicile. 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 m^2, 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.

Fig. 2: emergence trap. a: black plastic cylinder: 26.5 cm high and 30 cm diameter; b: black cloth in form of truncated cone 46 cm high, which permits teneral insects to move toward the collecting recipient; c: transparent collecting container: 12 cm high, 6 cm diameter with a concentric cylindrical tube: 9 cm high, 3 cm diameter, it was impregnated with castor oil to trap insects.

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/6 m^2 (25.8 larvae/m^2) 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 Cuepar, 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/m^2 belonging to five different species were collected, of which *C. pachymerus* was the most abundant (35 males/m^2, 30 females/m^2), followed by *Culicoides iriartei* Fox (17 males/m^2, 16 females/m^2). Only a few individuals of *Culicoides debilipalpis* Lutz (1 male/m^2, 5 females/m^2), *Culicoides leoni* Barbosa (1 male/m^2 male, 1 female/m^2) and *Culicoides insignis* Lutz (1 female/m^2) 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. pachymerus* 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. pachymerus* was established as the most abundant species (99.3% of 3,389 *s*pecimens collected) and few specimens of *C. debilipalpis* and *C. leoni* were collected. *C. iriartei* has not been previously reported in the area in collections with human bait; however, in our emergence traps, *C. iriartei* comprised 30.8% of caught specimens.

Domiciliation of *Culicoides* species is a consequence of man-made environmental modifications, especially the clearing of forest for pastureland and construction of dwellings. These activities probably favoured the development of *C. pachymerus*, either by providing more breeding sites, as occurred with *Culicoides paraensis* in Brazil (Tesh 1994) and/or by eliminating the natural pathogens that may have controlled populations of this species in breeding sites similar to those found in forests. In addition, the huge increase in the availability of human sources of blood probably favoured the increase in number of *C. pachymerus* and converted it into an intolerable peridomiciliary biting nuisance.

The number of larvae of *Culicoides* spp recovered from muddy substrates of the peridomicile formed by springs of water and rainwater accumulation (25.8/m^2) is relatively low compared with the number of adults collected in the region by Santamaría et al. (2008). We cannot discount the possibility that the substrates in which *Culicoides* larvae were not found in our study might eventually serve as breeding sites for *Culicoides* species in the west of Boyacá, as the flotation technique is not 100% effective and may fail to detect their presence. Since the neotropical distribution of *Culicoides* immatures is wide, it is also important to inspect other substrates, such as banana stalks and empty cacao pods, that have been demonstrated as breeding sites for other *Culicoides* species (Hoch et al. 1986, Mercer et al. 2003), even though, in the study area, they are only cultivated by household consumption.

In conclusion, in this preliminary study, the mud formed around houses by springs of water and the constant accumulation of rainwater was identified for the first time as a breeding microhabitat of *C. pachymerus* and other midge species in western Boyacá. This information could be applied to the design of integrated control measures that use environmental management to eliminate potential *C. pachymerus* breeding sites.

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