Mosquito (Diptera: Culicidae) grouping based on larval habitat characteristics in high mountain ecosystems of Antioquia, Colombia

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ABSTRACT: Information about mosquito ecology in the high mountain ecosystems of the Neotropical region is sparse. In general, few genera and species have been reported in these ecosystems and there is no information available on habitats and the mosquitoes occupying them. In the present study, specimens collected from NW Colombia in HME were grouped using larval habitat data via an Operational Taxonomic Unit (OTU) determination. A total of 719 mosquitoes was analyzed belonging to 44 OTUs. The analysis considered habitat features and clustered the specimens into six groups from A-F. Five of these included species from different genera, suggesting common habitat requirements. Group E with four genera, seven subgenera, and six species occupied the highest areas (above 3,000 m), whereas three groups (B, D, F) were detected at lower altitudes (1,960-2,002 m). Bromeliads were the most common larval habitat, with 47% (335/719) of the specimens; five genera, six subgenera, and eight species were identified and classified into 66% (29/44) of the OTUs. This work showed some similarities to the habitat requirements and provides a grouping system that constitutes an important baseline for the classification of mosquito fauna from high mountain ecosystems according to altitude and larval habitat. ***Journal of Vector Ecology* 43 (1): 71-79. 2018.**

Keyword Index: High mountain ecosystems, mosquitoes, Colombia, Operational Taxonomic Units, habitat, ecology.

INTRODUCTION

According to the current classification system, the family Culicidae includes two subfamilies, 11 tribes, 112 genera, and 3,554 species worldwide. The subfamily Anophelinae has three genera and the subfamily Culicinae has 109 (Harbach 2017). Several species inhabit high mountain ecosystems (HME) but they have not been well studied. In Colombia, an annotated list of the mosquitoes collected above 2,000 m reported a total of 35 species representing 11 genera and 15 subgenera (Rosero-García et al. 2017). However, mosquito fauna and their ecology in HME require more research, because no information exists on their larval habitats and the altitude ranges where they can be found (Berlin 1969, Arnell 1976). Frequently, mosquito use of natural water containers (e.g., bromeliads) as larval habitats, has been noted in these mosquitoes. However, artificial water containers derived from anthropic activities, such as agriculture, livestock, and ecotourism, may also be used (Githeko et al. 2000, FAO 2013). For example, *Aedes* (*Stegomyia*) *aegypti* was collected in artificial water containers at 2,150 m in Puebla City, Mexico (Lozano-Fuentes et al. 2012), and more recently in plastic ovitraps at 2,302 m in Bello municipality within the Antioquia Department (ANT), NW Colombia (Ruiz-López et al. 2016). Also, in Jardín municipality (ANT), nine species of the tribe Sabethini were collected in bromeliads and leaf axils of *Xanthosoma* in a forest environment between 2,245-2,515 m, whereas other Sabethini species were found on a different *Xanthosoma* sp. in an urban setting at 2,002 m in Jericó municipality (ANT), Colombia (Suaza-Vasco et al. 2015).

Mosquito larval habitats have specific characteristics that determine fauna composition. However, some characteristics of the habitats have contributed to understanding the occurrence of different species on a habitat (Pemola Devi and Jauhari 2007). This is important to identify potential and suitable habitats for the application of control methods (Almirón and Brewer 1996, Pemola Devi and Jauhari 2007). Although most authors refer to an Operational Taxonomic Unit (OTU) when delimiting a taxa by purely phenetic or heuristic means, an OTU may or may not correspond to a species in the strict sense. In studies including mosquito species, OTUs based on the similarity of their habitats helped to identify common patterns for species groups (Almirón and Brewer 1996, Pemola Devi and Jauhari 2007, Stein et al. 2011, Vanlalruia et al. 2014). Furthermore, the findings of species of different genera in the same habitat suggests similarity of habitat requirements (Almirón and Brewer 1996).

In Colombia, studies have explored the insect fauna of HME (Amat 1991, Amat and Vargas 1991, Morales-Castaño and Amat 2012, Chaves-Forero et al. 2015), but few have been focused on mosquitoes in those ecosystems (Suaza-Vasco et al. 2015, Rosero-García et al. 2017). Therefore, little is known about the mosquito fauna, their larval habitats, and the altitudinal range in these ecosystems. The current study is the first attempt to recognize larval habitats for mosquitoes of NW Colombian HMEs and group them based on OTUs grouping system. The information we obtained increased the knowledge of larval habitat requirements for species collected in HME of the Department of Antioquia.

MATERIALS AND METHODS

Study area and description of sampling sites

Antioquia is the second largest Department in Colombia, located in the NW, with an area of 63,612 km^2 and altitudes ranging from sea level to 3,969 m. Mosquitoes were collected from 2013-2016, including a minimum of two field collections per year. The collection sites were the municipalities of Belmira (06° 36′ 18″ N, 75° 39′ 57″ W), Jardín (05° 35′ 03″ N, 75° 46′ 02″ W), and Jericó (05° 47′ 18″ N, 75° 47′ 26″ W), located in HME between 1,960-3,098 m (Figure 1). The mean temperatures were 16° C in Belmira and 19° C in both Jardín and Jericó. Mean annual rainfall for the three municipalities ranged between 2,140 and 2,457 mm. Each locality was selected based upon available information about mosquito habitats and ecosystem types, presence of agriculture, and livestock activities including coffee plantations, protected or natural reserve areas determined more recently by local authorities, or ecotourism activities such as walks through natural parks. Sampling to recognize larval habitats was performed according to the methodology of rapid inventories of biodiversity (Ward and Larivière 2004). Morphological revision and identification of specimens were previously performed once the category was identified (Rosero-García et al. 2017).

Ecological characters for OTU determination

An OTU was composed of the group of species found in the same larval habitat. Specifically, the following characteristics were considered to form an OTU: a) type of larval habitat: animal water trough, laundry pond, wasted tire, bamboo stump, bromeliad, palm-leaf, rock pool, or leaf base of a *Xanthosoma*; b) nature of larval habitat: natural or artificial; c) water permanence (Stein et al. 2011): permanent (water present throughout the study), semi-permanent (water present for over one month in larval habitat), temporal (water present for less than a week in a habitat); d) sunlight exposure: sunlight, partial shade, complete shade; e) Holdridge vegetation classification (Espinal 1964): very humid premontane forest (bmh-PM), lower montane very humid forest (bmh-MB), or montane rain forest (bp-M) (Figure 1); f) land cover type: bamboo forest, forest edge, primary or secondary forest, pastureland, semi-urban; and g) altitude: meters above sea level (m).

Data analysis

The analysis was conducted according to Almirón and Brewer (1996), Stein et al. (2011), and Ramirez et al. (2016) with some modifications. These included: 1) once a data matrix was manually defined, the similarity between all possible pairs was calculated using the simple matching coefficient-S_{SM} (Sokal and Sneath 1963); 2) the resulting S_{SM} coefficients were used to construct a similarity matrix consisting of 44 rows and 44 columns, in Microsoft® Excel v. 2016; and 3) the Dice coefficient was applied to construct an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendogram, using DendroUPGMA (Garcia-Vallvé et al. 1999) and newick.js (Huerta-Cepas et al. 2016).

RESULTS

A total of 719 specimens belonging to five genera and nine subgenera were analyzed, and of these, 51% (364/719) were identified to species level (Table 1). Many of the specimens collected have not been well studied in Colombia or elsewhere, thus there were taxonomical difficulties to determine their specific status. In some cases, even with reared adults, the best we could do was to genus, subgenus, or species groups. A total of 44 OTUs were defined according to ecological characteristics of mosquito habitats.

The most common larval habitats were bromeliads, with 335 individuals within 29 OTUs including five genera, six subgenera, two species groups, and eight species (Figure 3). The more representative genera and subgenera found in bromeliads were *Aedes* (*Howardina*) and *Culex* (*Culex*). Bromeliads as habitats were found between 2,009 and 3,098 m (Table 1). Other natural phytotelmata containers used by mosquitoes in the HMEs studied were bamboo stumps, with 44 specimens. Six OTUs and only *Trichoprosopon* spp. were identified between 2,002-2,044 m. In addition, a leaf base of *Xanthosoma* sp. contained 18 immatures and four OTUs, including specimens of the genera *Culex* and *Trichoprosopon*. This plant was found between 2,002 to 2,230 m (Figure 2).

According to the association coefficients among OTUs, six groups (A-F) were distinguished in the UPGMA dendogram. Most of the habitat characteristics were shared among groups, but variations of larval habitat type and altitude allowed them to be separated (Figure 3). In general, many of the specimens have not been well studied and there are no specialists to correctly classify them to the species level. However, Group A, *Culex* (*Culex*) *coronator,* was the only species collected in an animal water trough at 2,092 m and *Aedes* (*Ochlerotatus*) Scapularis Group were detected exclusively in a rock pool at 2,100 m; both species were collected only in the Jardin municipality (Table 2). This group was clearly separated from the other five groups (Figure 3). Group B contained six OTUs including *Trichoprosopon andinum*, *Trichoprosopon pallidiventer* s.l., and *Trichoprosopon* spp., that were collected in bamboo stumps at Jardín and Jericó municipalities, between 2,002-2,044 m (Table 2). Group C species were collected only in bromeliads in partial shade, between 2,009-2,905 m in Belmira, Jardín, and Jericó municipalities. This group included *Aedes* (*Howardina*) aff. *osornoi*, *Aedes* (*How.*) Eleanorae Group spp., *Aedes* (*How.*) *leei*, *Aedes* (*How.*) *osornoi*, *Aedes* (*How.*) *spinosus*, *Aedes* (*How.*) spp., *Culex* (*Cux.*) *quinquefasciatus*, *Culex* (*Cux.*) sp4-sp7, *Culex* (*Microculex*) *imitator*, *Culex* (*Mcx.*) sp6, *Trichoprosopon* spp., and *Wyeomyia* (*Nunezia*) spp. (Table 2). In group D, *Culex* spp. and *Trichoprosopon* spp. were collected in leaf axils of *Xanthosoma* in the Jardín (2,100-2,230 m) and Jericó (2,002-2,009) municipalities (Table 2). The single *Culex* sp. was collected in Jericó at 2,009 m, sharing larval habitat with *Trichoprosopon* spp. in leaf axils of *Xanthosoma*.

Group E included *Ae.* (*How.*) aff. *osornoi*, *Ae.* (*How.*) *brevis*, *Ae.* (*How.*) Allotecnon Group, *Ae.* (*How.*) *osornoi*, *Aedes* (*How.*) spp., *Anopheles* (*Kerteszia*) spp., *Culex* (*Anoedioporpa*) aff. *conservator*, *Culex* (*Carrollia*) *bihaicola*, *Cx. quinquefasciatus*, *Culex* (*Mcx.*) sp6-sp8, *Culex* (*Mcx.*) spp., *Culex* spp., and *Wyeomyia* (*Nuz.*) sp. (Table 2). Some of these groups and species are considered to be

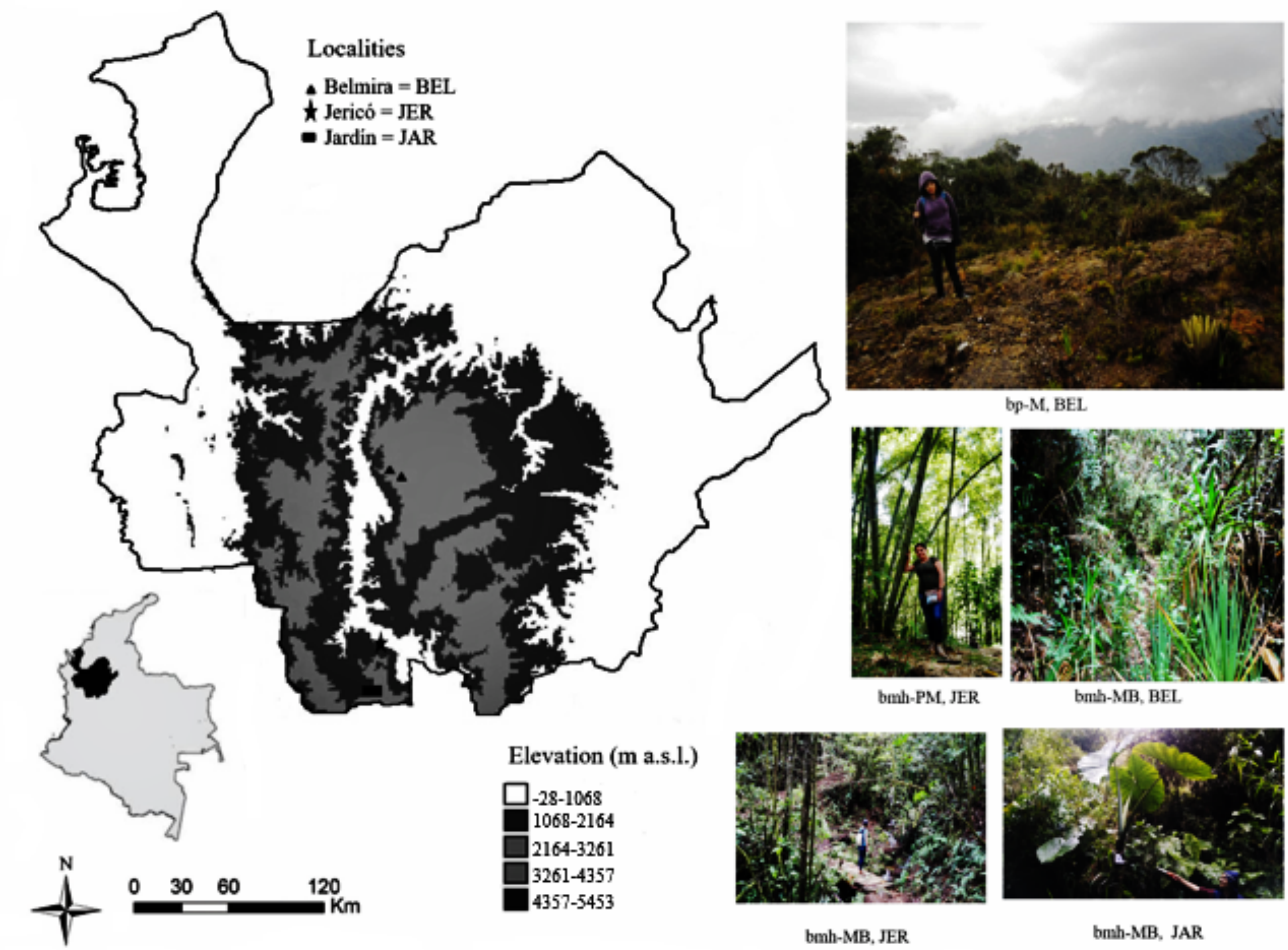

Figure 1. Collection sites (BEL = Belmira, JAR = Jardín, JER = Jericó) and Holdridge life zones for mosquito records in high mountain ecosystems from Antioquia, Colombia. Very Humid Pre-Montane Forest (bmh-PM), Lower Montane very humid forest (bmh-MB), Montane rain forest (bp-M).

bromeliad specialists (Marques and Forattini 2008, Marques et al. 2012, Chaves et al. 2016). *Culex bihaicola* was only collected in a palm leaf at 2,526 m in the Jardín municipality. Finally, group F was clearly separated from the other groups and included *Aedes* (*Stegomyia*) *aegypti*, *Aedes* (*Stg.*) sp., *Cx. quinquefasciatus,* and *Culex* sp. They shared environmental conditions and were found in artificial containers in Jericó at 1,960 m, *Ae. aegypti*, *Cx. quinquefasciatus,* and *Culex* sp. on wasted tires, and *Ae. aegypti* in a laundry pond (Table 2).

DISCUSSION

Habits and ecological characteristics can vary widely among mosquito species, but due to the lack of studies in Colombian HMEs, there are few references that can be used for comparative purposes. The present study was undertaken to provide some baseline information to help fill the gap. Our results indicate that specimens of groups A and F are separated from the other groups and each one was collected at only one locality, Jardín and Jericó, respectively. Both municipalities are characterized as coffee growing areas and pastures, located in the forest with bromeliads and bamboo commonly present. The species found in these habitats coincide with previous ones reported by these municipalities (Suaza-Vasco et al. 2013, 2015). Specimens of groups B, D, and E were collected at two municipalities with different altitudinal ranges, and only mosquitoes of group C were collected from the three municipalities. Moreover, group B showed the highest homogeneity of habitat characteristics compared with other groups (A, C-F). In this case the type of larval habitat, bamboo stump, influences the grouping, whereas geographical distribution was not relevant for the formation of the group (Almirón and Brewer 1996).

In Jardín municipality, a rock pool constituted a larval habitat for specimens of *Aedes* (*Och.*) Scapularis Group. Some arbovirus vectors, for example, *Aedes* (*Ochlerotatus*) *japonicas,* is often found associated with rock pools (Armistead et al. 2008). Other species reported from this larval habitat include *Culex* (*Culex*) *pipiens*, *Culex* (*Cux.*) *restuans*, *Anopheles* (*Anopheles*) *punctipennis*, *Anopheles* (*Ano.*) *quadrimaculatus,* and *Orthopodomyia signifera* (Armistead et al. 2012, Kaufman and Fonseca 2014), but none of

Table 1. Numbers of mosquitoes collected in high mountain ecosystems.

Genus / species (Number of specimens - larval habitat*)	Altitude (m)	Municipality	Geographic coordinates
Ae. (*Stg.*) *aegypti* (17WT, 3LP), *Ae.* (*Stg.*) sp. (18LP, 12WT), *Cx.* (*Cx.*) *quinquefasciatus* (4WT), *Culex* sp. (91WT)	1,960	Jericó	05°47'28"N, 75°47'09"W
Trichoprosopon sp. (3XA, 3BS)	2,002	Jericó	05°47'18"N, 75°47'26"W
Culex sp. (1XA), *Trichoprosopon* sp. (11BS, 3BR, 11XA)	2,009	Jericó	05°47'17"N, 75°47'27"W
Trichoprosopon sp. (9BS), *Wyeomyia* (*Nunezia*) sp. (51BR)	2,018	Jericó	05°47'50"N, 75°46'49"W
Trichoprosopon pallidiventer s.l. (2BS), *Trichoprosopon* sp. (6BS)	2,025	Jericó	05°35'06"N, 75°47'39"W
Trichoprosopon sp. (7BS)	2,032	Jericó	05°35'06"N, 75°47'39"W
Trichoprosopon andinum (3BS), *Trichoprosopon* sp. (3BS)	2,044	Jericó	05°35'06"N, 75°47'39"W
Ae. (*How.*) Allotecnon Group (1BR), *Ae.* (*How.*) *osornoi* (2BR), *Ae.* (*How.*) spp. (4BR), *Cx.* (*And.*) aff. *conservator* (1BR), *Culex* (*Cux.*) *coronator* (15AWT), *Cx. quinquefasciatus* (11BR), *Culex* sp. (6BR), *Wy.* (*Nuz.*) sp. (3BR)	2,092	Jardín	05°37'18"N, 75°49'35"W
Ae. (*Ochlerotatus*) Scapularis Group (152RP), *Culex* (*Microculex*) (1XA), *Culex* sp. (1XA)	2,100	Jardín	05°35'03"N, 75°46'02"W
Cx. (*Cux.*) *quinquefasciatus* (38BR), *Trichoprosopon* spp. (1XA)	2,230	Jardín	05°35'51"N, 75°47'40"W
Aedes (*How.*) *leei* (3BR), *Aedes* (*How.*) spp. (43BR), *Culex* (*Cux.*) sp4.-sp7. (4BR)	2,251	Jardín	05°36'49"N, 75°48'57"W
Cx. quinquefasciatus (32BR), *Cx.* (*Microculex*) sp6. (11BR)	2,261	Jardín	05°35'52"N, 75°47'40"W
Aedes (*How.*) spp. (5BR), *Cx. quinquefasciatus* (3BR), *Cx.* (*Mcx.*) sp6. (3BR), *Wy.* (*Nuz.*) spp. (5BR)	2,317	Jardín	05°35'13"N, 75°46'18"W
Aedes (*How.*) spp. (1BR)	2,376	Jardín	05°35'03"N, 75°46'02"W
Ae. (*How.*) *osornoi* (1BR), *Ae.* (*How.*) spp. (3BR), *Culex* sp. (1BR) *Cx.* (*Mcx.*) sp6.-sp8. (3BR), *Cx.* spp. (1BR), *Wy.* (*Nuz.*) spp. (1BR)	2,403	Jardín	05°37'59"N, 75°49'48"W
Aedes (*How.*) spp. (5BR)	2,412	Jardín	05°37'59"N, 75°49'48"W
Ae. (*How.*) *brevis* (2BR), *Ae.* (*How.*) spp. (5BR), *Cx.* (*Mcx.*) spp. (22BR)	2,481	Jardín	05°35'03"N, 75°46'02"W
Aedes (*How.*) *osornoi* (2BR), *Anopheles* (*Kerteszia*) spp. (6BR)	2,521	Jardín	05°35'03"N, 75°46'02"W
Culex (*Carrollia*) *bihaicola* (10PL), *Wy.* (*Nuz.*) sp. (3BR)	2,526	Jardín	05°35'13"N, 75°46'18"W
Ae. (*How.*) spp. (4BR), *Ae.* (*How.*) *spinosus* (1BR), *Ae.* (*How.*) *osornoi* (16BR), *Ae.* (*How.*) Eleanorae Group (6BR)	2,734	Belmira	06°37'22"N, 75°39'19"W
Ae. (*How.*) spp. (1BR), *Ae.* (*How.*) aff. *osornoi* (1BR), *Ae.* (*How.*) *osornoi* (4BR), *Ae.* (*How.*) Eleanorae Group (1BR), *Culex* (*Mcx.*) *imitator* (1BR), *Culex* sp. (1BR)	2,862	Belmira	06°37'10"N, 75°39'26"W
Aedes (*How.*) sp. (1, BR), *Aedes* (*How.*) aff. *osornoi* (1, BR)	2,905	Belmira	06°37'10"N, 75°39'26"W
Aedes (*How.*) *osornoi* (2, BR)	3,000	Belmira	06°38'40"N, 75°42'23"W
Ae. (*How.*) *osornoi* (16, BR), *Ae.* (*How.*) sp. (3, BR), *Ae.* (*How.*) aff. *osornoi* (7, BR)	3,098	Belmira	06°38'48"N, 75°42'35"W

*AW: animal water trough, LP: laundry pond, WT: wasted tire, BS: bamboo stump, BR: bromeliad, PL: palm-leaf, RP: rock pool, XA: *Xanthosoma* spp. leaf base.

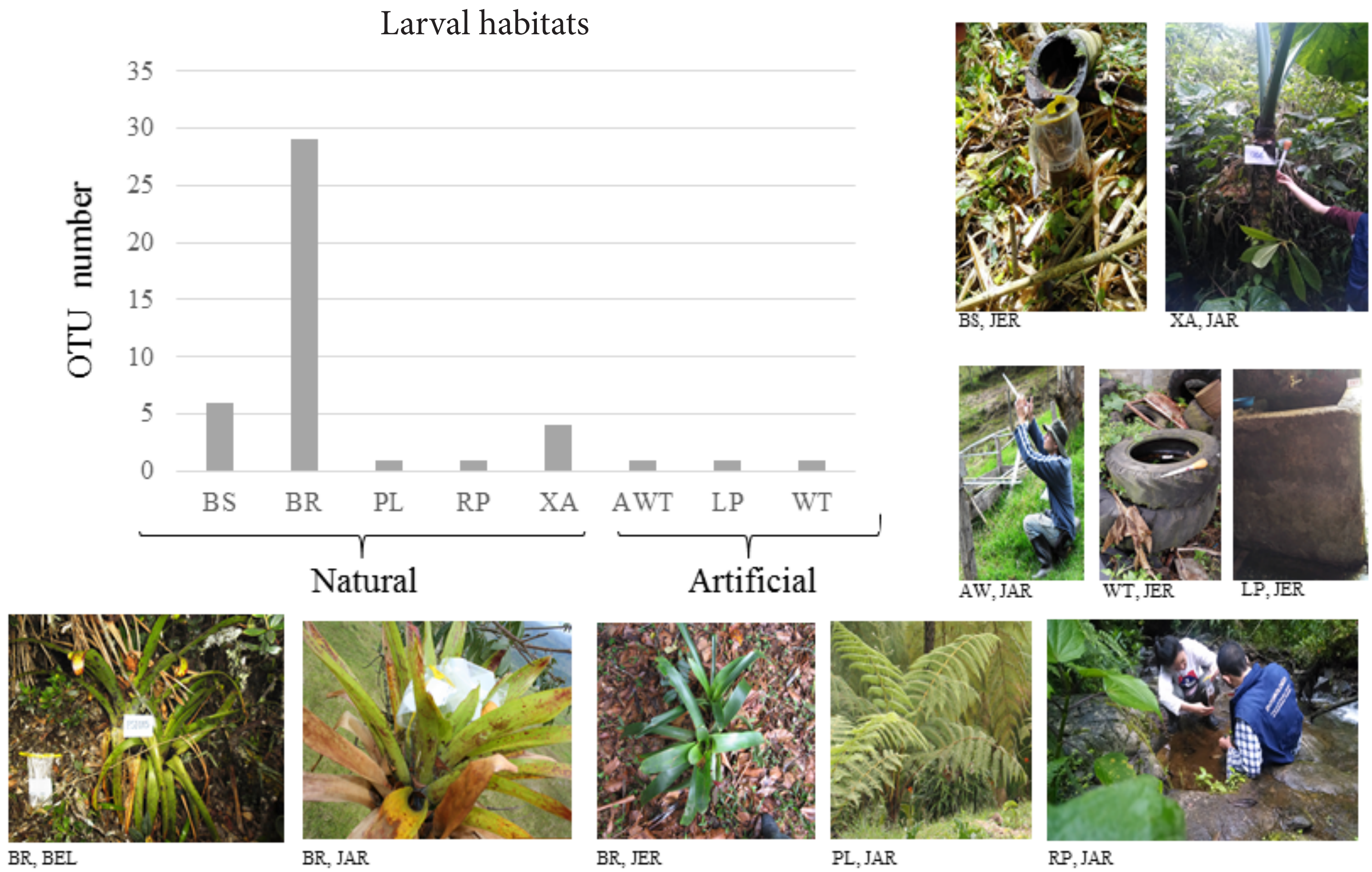

Figure 2. Operational Taxonomic Unit (OTU) number and illustrations of each breeding site. Natural: BS: bamboo stump, BR: bromeliad, PL: palm-leaf, RP: rock pool and XA: Xanthosoma sp. leaf base. Artificial: AWT: animal water trough, LP: laundry pond, WT: wasted tire.

these species were found in our study in the OTU of RP belonging to group A. Perhaps specimens of *Aedes* (*Och.*) Scapularis Group found in Jardin do not share habitat requirements with the other species collected in rock pools above 2,000 m in this locality. Future studies should be conducted to evaluate this and the species presence and composition in this very common larval habitat in this locality.

Bromeliads were the primary larval habitats for mosquitoes collected in HME of the three municipalities, suggesting a similarity of habitat requirement for the mosquito communities inhabiting these ecosystems. Approximately 200 species of Culicidae have been recorded in bromeliads (Frank and Lounibos 2009). Among these, *Aedes* (*How.*) spp., *Anopheles* (*Ker.*) spp., *Culex* (*Mcx.*) spp., and *Wyeomyia* (*Nuz.*) are considered bromeliad specialists (Berlin 1969; Lounibos et al. 2003, Navarro et al. 2007, Chaves et al. 2016). The only exception in *Anopheles* (*Ker.*) is *Anopheles* (*Ker.*) *bambusicolus* (Komp, 1937), that uses bamboo internodes as larval habitat (Zavortink 1973, Chaves et al. 2016). Because bromeliads have specific characteristics that determine mosquito faunal composition, the OTU grouping system permitted cluster bromeliad specialist species. Based on our results, the detection of one species at one bromeliad can predict other species likely to be found in such a habitat. Regarding the *Kerteszia* subgenus (*Anopheles* genus), this group is present in the Jardin municipality inhabiting bromeliads, being more diverse than it was thought (Suaza-Vasco et al. 2013). This group deserves more attention and the study of its presence and peculiarities in HME will be interesting, facilitating a better understanding of its distribution and taxonomic variation.

The larval habitat of phytotelmata represented 55% of 719 records in this study that agree with results of previous studies in similar ecosystems in other countries. For example, in Venezuela, above 2,000 m, *An.* (*Ker.*) spp., *Cx.* (*Mcx.*) spp., *Wy.* (*Nuz.*) spp., and *Runchomyia* spp. were associated with phytotelmata and represented 60% of 9,607 records (Navarro et al. 2010). Surprisingly, in our study larvae of *Cx. quinquefasciatus* were found in phytotelmata (bromeliads). This was unexpected result because this species is usually restricted to human urban environments and the altitudinal distribution limit is determined by human occupation (Navarro et al. 2010). Some *Cx. quinquefasciatus* larvae have been also found in exotic bromeliads in south Florida (O'Meara et al. 2003). Moreover, larvae of this species were collected in bromeliads in semi-urban and rural localities in Tucuman province, NW Argentina (Stein et al. 2013). The presence of *Cx. quinquefasciatus* in bromeliads is interesting to study in a public health context because this species is cosmopolitan, highly opportunistic, and a vector of important pathogens such as West Nile virus (Eastwood et al. 2011) and St. Louis encephalitis virus

Table 2. Groups and OTUs distribution in three municipalities of Antioquia.

Group	OTUs	Characteristics of larval habitat: Type	Nature	WP[1]	Sunlight exposure	Holdridge life zone[2]	Land cover type	Altitude (m)	Municipality
A	AWT	Animal water trough	Artificial	Temporary	Sunlight	bmh-MB	Secondary forest	2,092	Jardín
A	RP	Rock pool	Natural	Semi-permanent	Sunlight	bmh-MB	Secondary forest	2,100	Jardín
B	BS4, BS5	Bamboo stump	Natural	Permanent	Partial shade	bmh-MB	Bamboo	2,025-2,032	Jardín
B	BS1-BS3, BS6	Bamboo stump	Natural	Permanent	Partial shade	bmh-MB	Bamboo	2,002-2,044	Jericó
C	BR10, BR19	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Forest edge	2,734-2,862	Belmira
C	BR20	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Forest edge	2,734-2,862	Belmira
C	BR21	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Paddock	2,905	Belmira
C	BR3-BR6	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Forest edge	2,230-2,376	Jardín
C	BR12-BR14	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Forest edge	2,230-2,376	Jardín
C	BR27	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Forest edge	2,230-2,376	Jardín
C	BR24, BR25	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Secondary forest	2,009-2,018	Jericó
D	XA3	*Xanthosoma* spp.	Natural	Permanent	Partial shade	bmh-MB	Secondary forest	2,100	Jardín
D	XA4	*Xanthosoma* spp.	Natural	Permanent	Partial shade	bmh-MB	Forest edge	2,230	Jardín
D	XA1, XA2	*Xanthosoma* spp.	Natural	Permanent	Partial shade	bmh-MB	Secondary forest	2,002-2,009	Jericó
E	BR9	Bromeliad	Natural	Permanent	Complete shade	bmh-MB	Interior forest	2,862	Belmira
E	BR22-BR23	Bromeliad	Natural	Permanent	Complete shade	bp-M	Interior forest	3,000-3,098	Belmira
E	BR1, BR2,	Bromeliad	Natural	Permanent	Complete shade	bmh-MB	Interior forest	2,092-2,526	Jardín
E	BR7, BR8,	Bromeliad	Natural	Permanent	Complete shade	bmh-MB	Interior forest	2,092-2,526	Jardín
E	BR15-BR18,	Bromeliad	Natural	Permanent	Complete shade	bmh-MB	Interior forest	2,092-2,526	Jardín
E	BR28, BR29	Bromeliad	Natural	Permanent	Complete shade	bmh-MB	Interior forest	2,092-2,526	Jardín
E	BR15	Bromeliad	Natural	Permanent	Partial shade	bmh-MB	Interior forest	2,403	Jardín
E	BR2, BR11,	Bromeliad	Natural	Permanent	Sunlight	bmh-MB	Interior forest	2,092	Jardín
E	BR26	Bromeliad	Natural	Permanent	Sunlight	bmh-MB	Interior forest	2,092	Jardín
E	PL	Palm-leaf	Natural	Permanent	Sunlight	bmh-MB	Interior forest	2,526	Jardín
F	LP	Laundry pond	Artificial	Temporary	Complete shade	bmh-PM	Semi-urban	1,960	Jericó
F	WT	Used tired	Artificial	Temporary	Sunlight	bmh-PM	Semi-urban	1,960	Jericó

1: water permanence, 2: bmh-MB: Lower Montane very humid forest, bp-M: Montane rain forest, bmh-PM: Very humid pre-Montane forest.

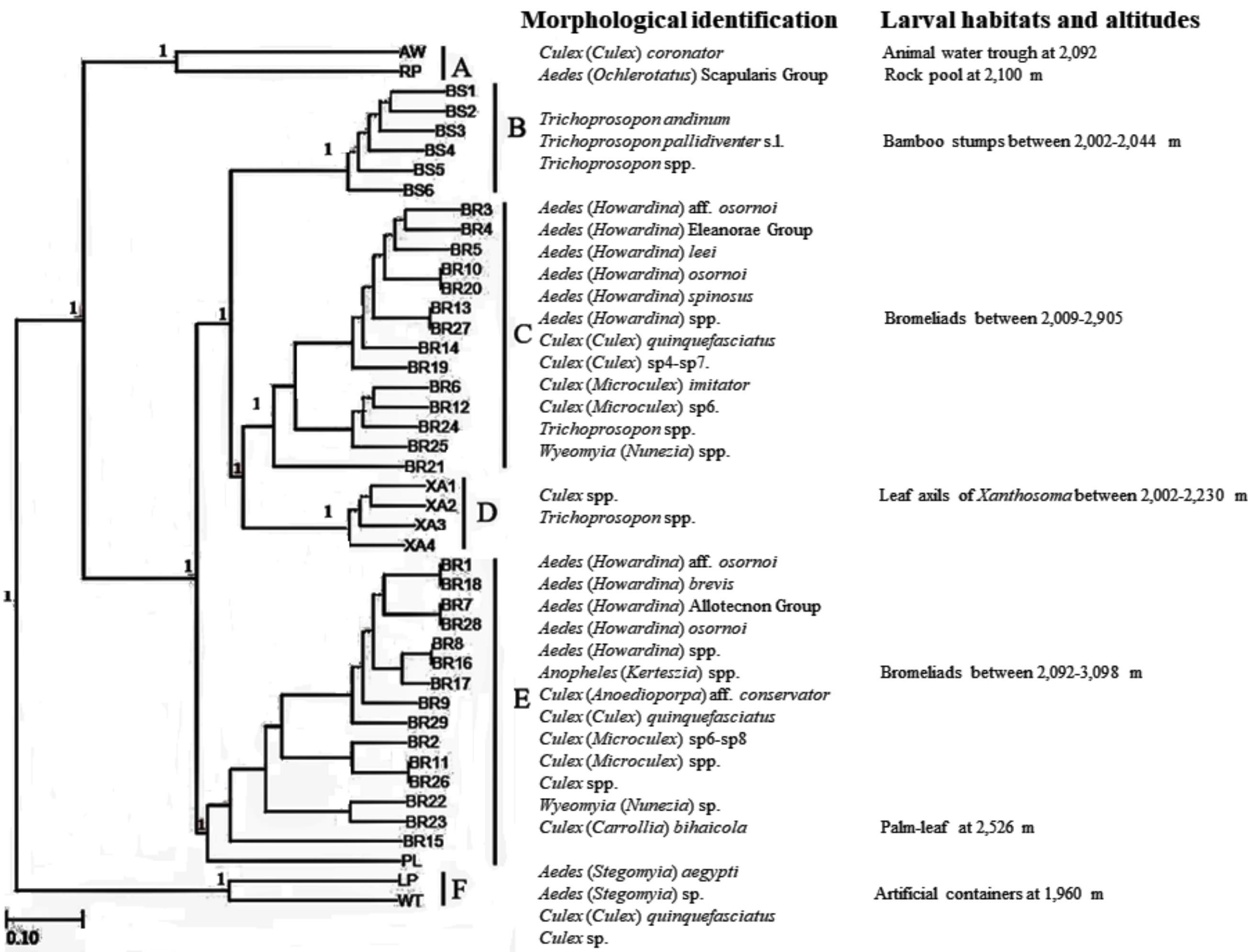

Figure 3. Dendogram of 44 OTUs of the larval habitats clustered by the UPGMA method.

(Diaz et al. 2013).

In the present study, *Ae. aegypti* larvae or pupae were not detected in any OTU grouping by natural water containers, but they were found on OTUs including wasted tires and in a laundry pond at 1,960 m in Jericó municipality. This is probably because this species is highly adapted to human environments, commonly found in artificial water containers (Forattini 2002). Generally, *Ae. aegypti* in Colombia has been detected in urban areas below 1,800 m (Padilla et al. 2012, Olano 2016). Nevertheless, the altitudinal range of this species (1,960 to 2,302 m) suggests adaptation to lower temperatures at higher elevations (Rosero-García et al. 2017). Monitoring of this species at high altitude is important and necessary. *Culex* sp. was also found in waste tires in the Jericó municipality at 1,960 m.

Although *Ae. aegypti* and *Cx. quinquefasciatus* were not collected at altitudes exceeding 2,000 m Jericó, both species were found to share similar habitat requirements in artificial water containers, which is a common measure of high biological adaptability (Stein et al. 2002). It has been suggested that only minor changes in environmental conditions would be required to promote species movement into highlands (Wu et al. 2016). Therefore, studies of this type, combining taxonomy and ecology should be periodically performed to understand the ecological characteristics that determine the possible extent of species invasion and adaptation at altitudes of 1,960 m or higher in HME. *Culex coronator* and *Aedes* (*Och.*) Scapularis Group were not found in plants in any of the three municipalities. Care must be taken with this species, as it was the only one found in an OTU formed for an animal water trough at 2,092 m, because in Brazil, St. Louis encephalitis virus was isolated from this species (Vasconcelos et al. 1991), and it is known as a competent vector of West Nile virus in Florida (Alto et al. 2014). Given the epidemiological importance of these mosquito species, we recommend the establishment of appropriate local vector surveillance.

In summary, in the present study, OTUs defined by ecological characteristics allowed the definition of six mosquito species groups. Five of them included species from various genera, suggesting that they share similar habitat/ecological requirements (Almirón and Brewer 1996, Bueno-Marí and Jiménez-Peydró 2011). To our knowledge, this is the first study in Colombia that provides information on the ecological aspects of mosquitoes from high mountain ecosystems. This, together with a taxonomic study, will provide a better baseline for knowing and describing mosquito fauna from these ecosystems.

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