Horizontal and vertical distribution of mosquitoes (Diptera: Culicidae) in the rainforest of Maromizaha, Madagascar: implications for pathogen transmission to humans and animals

Luciano M Tantely,1* Fano J Randrianambinintsoa,2 Frederike Woog,3 Manou R Raharinirina,1 Jonah Ratsimbazafy,4 Sébastien Boyer5 and Romain Girod1

1Medical Entomology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar.
2ANSES, EA7510, ESCAPE - USC ANSES, transmission vectorielle et épidémiosurveillance de maladies parasitaires (VECPAR), University of Reims Champagne-Ardenne, Reims, France.
3Ornithology section, State Museum of Natural History Stuttgart, Stuttgart, Germany.
4Department of Paleontology and Biological Anthropology, University of Antananarivo, Antananarivo, Madagascar.
5Medical Entomology Platform, Institut Pasteur du Cambodge, Phnom Penh, Cambodia.

Abstract
The horizontal and vertical distribution of mosquito species was studied in Maromizaha rainforest, Madagascar. In November 2014, horizontal distribution was studied using light traps that were placed in four habitat types, ranging from degraded forest to primary rainforest. In November to December 2016, vertical distribution was studied using light traps placed near to the ground and elevated near the canopy. From the horizontal survey, 476 adults were collected: Uranotaenia (52.10%) and Culex (35.2%) were the most abundant genera. The highest numbers of mosquitoes were observed in the rainforest edge habitat, while the highest mosquito diversity was observed within primary rainforest. From the vertical survey, 276 adults were collected: Culex was the most abundant genus and represented 75.70% of adults caught. Mosquitoes were more abundant in traps placed near the ground than those near the canopy. The highest species diversity was observed near the canopy. The higher abundance of mosquitoes at the forest edge agrees with the role of this zone as an ecotone between intact rainforest and the nearest human habitation. Increased mosquito abundance in lower strata might heighten their contact with particular vertebrate hosts. The presence of vectors in different habitats and in varying elevations likely influences the risk of pathogen transmission between animals and humans in this rainforest.

Key words Culicidae, distribution, diversity, pathogen transmission, rainforest.

INTRODUCTION

Many forested ecosystems of Madagascar have been fragmented as a result of agricultural activity and domestic animal breeding (Gamba et al. 2013; Klanderud et al. 2010; Newsome & Hassel 2014). In these forested areas and neighbouring habitats, circulation of arthropod-borne pathogens has been observed by isolation of arboviruses and heamoparasitic parasites from human and wild animal blood, as well as from mosquitoes (Fontenille 1989; Savage et al. 2009; Schmid et al. 2017). Moreover, a significantly higher prevalence of arthropod-borne pathogens was observed in those forest-dwelling vertebrate fauna capable of occupying other habitat types as compared to vertebrate fauna that solely lived in forested areas (Fontenille 1989; Savage et al. 2009).

Little knowledge about the different mechanisms by which forest conversion and alteration contribute to the emergence of infectious diseases is available from Madagascar. Although not well studied in Madagascar, forest fragmentation impacting the distribution of mosquito vector species (Obsomer et al. 2007; Patz et al. 2004; Wilcox & Ellis 2006) is plausible based on studies from forested areas in other countries.

Among 237 mosquito species known from Madagascar, 64 species (27.0%) have medical or veterinary importance and are known to occur in forested areas (Brunhes et al. 2017; Fontenille 1989; Grebine 1966; Schmid et al. 2017; Tantely et al. 2013, 2016). In Madagascar, there have been a few studies of the vector community’s importance as a cause of pathogen heterogeneity and emergence from forested areas (Fontenille 1989; Savage et al. 2009; Schmid et al. 2017; Schmid et al. 2017; Tantely et al. 2013). However, these studies did not provide any information on the horizontal and vertical distribution of Culicid fauna and the potential influences on pathogen maintenance and emergence from forested areas, as underlined in other countries (Despommier et al. 2006).

Our current study has been performed in the Maromizaha forest and aimed to assess the effect of habitat type, from intact forest to degraded areas, and the effect of stratification of vegetation formation, on the richness, distribution and abundance of mosquito species. Maromizaha forest was chosen according to the following criteria: (1) this area is undergoing deforestation and fragmentation (Woog et al. 2006), (2) mosquito species collected in this forest have been previously found to be naturally infected with heamoparasitic parasites (Schmid et al. 2017) and (3) four...
arboviruses genera were detected and isolated from mosquitoes caught in the neighbouring Andasibe rainforest (ex-Périnet) (Fontenille 1989). The implications of variation in mosquito diversity, distribution and abundance on the risk of pathogen transmission to humans and animals are discussed.

MATERIALS AND METHODS

Study site

Maromizaha forest (18°56′49″ S, 48°27′33″ E), comprising 1,880 ha, is located 2 km east of the Andasibe forest (ex-Périnet) and belongs to the municipality of Andasibe, district of Moramanga and province of Toamasina (Fig. 1). Its vegetation formation is tri-stratified (Andrianandrasana 2011; Andrianandrasana et al. 2018). The lower stratum is up to 3 m high and characterised by the dominance of Acanthaceae and Poaceae families, tree ferns (Cyathea spp.) and epiphytic plants (Fucus spp, Orchids, mosses, lichens and lianes). The middle stratum is ranging from 3 to 6 m above ground and consists of young and sciaphilic trees such as Myrtaceae (Eugenia spp., Syzygium spp., Dyspis spp) and Liliaceae (Dracaena spp.). The upper stratum is above 6 m where Clusiaceae (Symphonia spp), Lauraceae (Ocotea spp.), Monimiaceae (Tambourissa spp) and Pandanaceae (Pandanus spp) predominate (Andrianandrasana 2011, Andrianandrasana et al. 2018).

Fig. 1. Location of the four study sites and mosquito sampling sites: (a) map showing the locations of the Anorana forest, Andasibe forest and Maromizaha forest, the locations of four habitats where horizontal and vertical studies were carried out in November 2014 and November–December 2016. (b) On the left side of the figure are shown the location of the 12 light traps in the four study sites during the horizontal study. (Satellite images extracted from Google Earth pro. July 11, 2017, December 05, 2018), and on the right side are the locations of the eight light traps placed in lower strata and upper strata of trees located on the stream (A) in lower slope (B), on upper slope (C) and on the ridge (D).
Mosquito adult stage sampling

From November 17 to 21, 2014, a survey of the horizontal distribution of mosquito vectors was performed in four different habitats at sites named “Quarry,” “Former banana field,” (FBF) “Col” and “Camp 2.” These habitats form a forest fragmentation gradient from the degraded area to the primary rainforest (Table 1 and Fig. 1). In each habitat, adult mosquitoes were collected using 12 CDC light traps (BioQuip products, Inc, Rancho Dominguez, USA), distributed along three sampling lines that were named “valley-floor,” “slope,” and “ridge” (four traps per sampling line). These sampling lines were chosen because they were the same as previously used to capture understory birds using mist nets in previous bird studies (Woog et al. 2006). For the horizontal survey, each trap was placed between just above ground level to a maximum height of 1 m. There were four consecutive nights of capture with one night of capture per habitat. The traps were operated from 18:00 to 06:00. The total sampling effort was 12 light traps × 12 h × 1 night × 4 habitats, corresponding to a total of 576 trap hours.

Survey of the vertical distribution of mosquitoes was conducted in two of the four habitats (“FBF” and “Camp 2”) between November 28 to December 2, 2016 (Table 1 and Fig. 1). There were four consecutive nights of capture with two nights of capture per habitat. Adult mosquitoes were collected using eight CDC light traps. In each habitat, traps were installed on four trees located on the valley floor, on the lower slope, on the upper slope and on the ridge. On each tree, one trap was fixed in lower strata, just above ground level to a maximum height of 1 m, and one in upper strata, at a height of 10 to 15 m, as close to the canopy layer as possible. Each night, traps were operated from 16:00 to 07:00. The total sampling effort was 8 traps × 15 h × 2 nights × 2 habitats, totalling 480 traps hours. Mosquito attraction to light traps was not supplemented by either carbon dioxide (CO2) or octenol.

Mosquito larval stage sampling

From November 28 to December 2, 2016, mosquito larvae were sampled in different larval habitats types such as leaf axils of plants, tree holes, dead leaves, streams, rock holes, cement lined pits and hoof prints using a flexible plastic hose and a siphoning technique (Tantely 2013) and via dipping method with a white tray (Robert et al. 2002).

Species identification processing

Adult mosquitoes were anaesthetised with chloroform vapour and morphologically identified using the dichotomous keys of Ravaonjanahary (1978) for Aedes Meigen, Grjebine (1966) for Anopheles Meigen, Doucet (1951) for Coquillettidia Dyar, Edwards (1941) for Culex Linnaeus, Brunhes & Hervy (1995) for Orthopodomyia Theobald, Brunhes et al. (2011) for Aedeomyia Theobald and da Cunha Ramos & Brunhes (2004) for Uranotaenia Lynch Arribálzaga. For larvae, collected larvae were separated using a pipette in the field and stored in 70% ethanol before later processing. In the laboratory, ethanol-stored larvae were mounted on microscope slides in Euparal® slide-mounting reagent, and the mosquito genus was morphologically identified using the dichotomous keys of Harbach (1985).

Statistical analysis

Statistical analysis of data obtained from horizontal and vertical distribution studies were performed in R version 2.10.1 (R Foundation for Statistical Computing, http://www.r-project.org). The effect of habitats and sampling lines for the horizontal study and the effect of location of trees and trap elevation on mosquito abundance for the vertical study were evaluated using one-way (1 W) analysis of variance (ANOVA). Two-way (2 W) ANOVA was used to determine the effect of interaction between factors:

Table 1 Description of the four habitats where mosquito collections were carried out in November 2014 and November–December 2016, forest of Maromizaha, district of Moramanga, Madagascar. FBF: Former banana field

| Habitat | Coordinates     | Altitude | Description of landscape                                                                 | Presence of humans                                                                                     |
|---------|----------------|----------|-----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Quarry  | 18°57′44.00″S, 48°27′10.57″E | 1118 m   | Located at 400 m far from the primary forest border. Deforested, dry habitat, surrounded by some slash-and-burn agriculture (so-called “Tavy”) and non-native plant species | A stone pit with the highest level of disturbance (quarry nearby)                                         |
| FBF     | 18°58′13.93″S, 48°27′53.54″E | 1008 m   | Located almost at the primary forest edge, landscape consists of secondary forest characterised by Verbenaceae and Rosaceae and primary forest. Agricultural “island” surrounded by primary forest, within area secondary growth | Regular intermediate level of disturbance by farmers, camping sites, frequently visited by tourists, students and researchers guided by farmers |
| Col     | 18°58′14.02″S, 48°27′53.37″E | 1050 m   | Mountain saddle and river valley, within the primary forest, 500 m from the forest edge, intermediate logging in the past. | Moderate human disturbance (tourists), located at the crossroads of many trails leading to different points of the forest |
| Camp 2  | 18°58′29.24″S, 48°27′53.34″E | 1057 m   | Primary Dragon Tree forest, wet valley, some logging in the past (large trees missing), moderate disturbance within the primary forest 1000 m far from the forest edge | Camping sites, visited by tourists, students and researchers guided by farmers                             |
habitat/sampling line for the horizontal study and location of trees/trap elevation for the vertical study for the vertical study on mosquito abundance.

Simpson and Shannon indices were calculated to estimate mosquito species richness and the combined effects of mosquito species richness and relative abundance. Both indices indicate that any site with greater Shannon and Simpson indices have a higher number of species present, with the individuals in the community being distributed more equitably among these species.

For avoiding observed species richness bias depending on sites and traps and for extrapolating species richness in each habitat and elevation, non-parametric estimators Chao1 (Colwell & Coddington 1994) and abundance-based coverage estimators (ACE) (Magurran 2004) were calculated. Both estimators are complementary and calculate the “true” number of species in each habitats and elevation. Change in community composition in four habitats, at lower and upper strata, was assessed using Detrended Correspondence Analysis (DCA, vegan function “decorana”).

RESULTS
Diversity and abundance of mosquitoes
A total of 752 adult mosquitoes, including 476 from the horizontal survey and 276 from the vertical survey, were collected overall (Table 2). They belonged to nine genera and at least to 23 species, corresponding to $S_{\text{Chao1}} = 25.00$ and $S_{\text{ACE}} = 25.50$ as true estimated species richness. The most prevalent genus overall was Culex, which accounted for 59.45% of the collection and included six Culex species, with Culex pipiens Linnæus (50.1%) being the predominant species. The genus Uranotaenia accounted for 34.7% and included three species, with Uranotaenia alboabdominalis Theobald as the predominant

Table 2  Adult mosquitoes collected by CDC light trap in November 2014 and November–December 2016 in the Maromizaha rainforest, district of Moramanga, Madagascar. Former banana field

| Species            | Horizontal | Vertical | Total | Percentage |
|--------------------|------------|----------|-------|------------|
|                    | Camp 2 | Col | FBF | Quarry | Canopy | Ground |       |           |
| Aedes              |         |       |     |        |        |        |       |           |
| circumhaleolus†    | 5      | 3    | 1   | 0      | 0      | 1      | 10    | 1.33      |
| argenteopunctatus‡| 0      | 0    | 0   | 0      | 1      | 0      | 1     | 0.33      |
| Anopheles          |         |       |     |        |        |        |       |           |
| gambiae†‡          | 0      | 0    | 0   | 3      | 0      | 0      | 3     | 0.40      |
| lacani†            | 1      | 0    | 0   | 0      | 0      | 0      | 1     | 0.13      |
| mascarensis‡       | 0      | 0    | 1   | 1      | 0      | 0      | 2     | 0.27      |
| coustani†‡         | 0      | 0    | 0   | 0      | 3      | 1      | 4     | 0.53      |
| funestus†          | 0      | 0    | 0   | 0      | 0      | 1      | 1     | 0.13      |
| Copallitididae†    | 0      | 3    | 0   | 1      | 0      | 0      | 4     | 0.53      |
| Culex              |         |       |     |        |        |        |       |           |
| annulioris†‡       | 0      | 0    | 0   | 5      | 0      | 0      | 5     | 0.66      |
| antennatus†‡       | 1      | 2    | 0   | 0      | 17     | 9      | 29    | 3.86      |
| decens†‡           | 5      | 3    | 1   | 3      | 1      | 0      | 13    | 1.73      |
| giganteus†‡        | 1      | 0    | 5   | 2      | 5      | 3      | 16    | 2.13      |
| pipiens†‡          | 45     | 46   | 45  | 32     | 85     | 124    | 377   | 50.13     |
| quinquefasciatus‡† | 0      | 0    | 0   | 0      | 1      | 0      | 1     | 0.13      |
| sp.                | 3      | 0    | 1   | 1      | 0      | 1      | 6     | 0.80      |
| Ficalbia†          |         |       |     |        |        |        |       |           |
| sp.                | 0      | 0    | 0   | 0      | 2      | 2      | 4     | 0.53      |
| Hodgesia           |         |       |     |        |        |        |       |           |
| sp.                | 0      | 0    | 0   | 0      | 0      | 2      | 2     | 0.27      |
| Latzia             |         |       |     |        |        |        |       |           |
| tigripes†‡         | 0      | 3    | 0   | 0      | 2      | 2      | 7     | 0.97      |
| Orthopodomyia      |         |       |     |        |        |        |       |           |
| milloti†‡          | 0      | 1    | 2   | 0      | 0      | 0      | 3     | 0.40      |
| sp.                | 1      | 1    | 0   | 0      | 0      | 0      | 2     | 0.27      |
| Uranotaenia        |         |       |     |        |        |        |       |           |
| alboabdominalis†   | 1      | 41   | 7   | 0      | 0      | 0      | 49    | 6.52      |
| anopheloides†      | 0      | 1    | 3   | 0      | 0      | 0      | 4     | 0.53      |
| neireti‡           | 1      | 1    | 4   | 0      | 0      | 1      | 7     | 0.93      |
| sp.                | 44     | 27   | 118 | 0      | 3      | 9      | 201   | 26.73     |
| Total              | 108    | 132  | 188 | 48     | 120    | 156    | 752   | 100       |

Distribution by species, habitat types (horizontal study) and trapping height in trees (vertical study). FBF: Former banana field
†Species collected 40 years ago in Andasibe forest (Fontenille 1989).
‡Species collected in Anorana rainforest (Tantely et al. 2013). Anopheles mascarensis and Uranotaenia anopheloides are endemic to Madagascar and to the Comoros archipelago. Anopheles lacani, Coquillettidia granddierii and Orthopodomyia milloti are endemic to Madagascar (Tantely et al. 2016).
identified species. An undescribed species of *Uranotaenia* was the most frequently captured and accounted for 26.7% of the total caught. The remainder (5.85%) consisted of the genera *Aedes* (two species), *Anopheles* (five species), *Ficalbia* (one undescribed species), *Hodgesia* (one undescribed species), *Lutzia* (one species) and *Orthopodomyia* (one species).

**Horizontal distribution**

In the horizontal survey, at least 15 mosquito species belonging to seven genera were collected (Table 2). The genus *Uranotaenia* accounted for 52.10% (*n* = 248/476) of mosquitoes caught and included three species. One undescribed *Uranotaenia* species accounted for 39.70% (189/476) of mosquitoes caught. At least five species of the genus *Culex* together represented 42.22% (201/476), with the highly abundant species *Culex pipiens* accounting for 35.29% (168/476). Species of the genera *Aedes*, *Anopheles*, *Coquillettidia*, *Lutzia* (Tanaka) and *Orthopodomyia* were represented by seven species and accounted for 5.67% (27/476) of all mosquitoes caught. Only species of *Anopheles*, *Culex* and *Coquillettidia* genera were caught at the Quarry site.

The number of mosquitoes caught differed significantly between habitat types (1 W ANOVA, *F* = 3.584, d.f. = 3, *P* < 0.05). The highest number of collected mosquitoes was obtained in FBF site, followed by Col, Camp 2 and Quarry sites (Table 2 and Fig. 2a). No significant difference in mosquito abundance was observed between valley floor, slope and ridge (1 W ANOVA, *F* = 1.030, d.f. = 2, *P* = 0.3577). Significant effect of interaction between habitats and sampling line on mosquito abundance were observed (2 W ANOVA, *F* = 3.651, d.f. = 6, *P* < 0.05).

As shown in Panel A of Table 3, the highest Shannon and Simpson indices were observed at the Col site, indicating that a greater number of species were collected and mosquitoes were distributed more equitably among mosquito species in this habitat, followed by Camp 2, FBF and Quarry sites. However, the *S*<sub>Chao1</sub> and *S*<subACE</sub> estimated that the highest number of species would be present in the intact forest (Camp 2), and the number of species decreased from Camp 2 site to Quarry site (Table 3, Panel A). The DCA ordination indicated that the community composition was significantly different across the four habitat types, with the community from the three forest habitats being strongly separated from the Quarry site on the first axis (Fig. 3a). Only *Culex pipiens* and *Culex decens* Theobald were commonly found in the four habitats.

**Vertical distribution**

A total of 276 adult mosquitoes belonging to seven genera and at least 13 species were caught during the vertical survey. The dominant species was *Culex pipiens*, which accounted for
75.70% of adult mosquitoes caught, followed by *Culex antennatus* Becker, which represented 9.4%. The remaining 14.8% of the total caught consisted of 11 other species.

When each tree was considered as one unit, no significant difference of mosquito abundance was observed between sampling position (valley floor, lower slope, upper slope and ridge (1 W ANOVA, $F = 0.752$, d.f. = 3, $P = 0.5326$)). Traps installed near the ground collected more mosquitoes than traps installed near the canopy (1 W ANOVA, d.f. = 1, $F = 4.322$, $P < 0.05$) (Fig. 2b). No evidence of interaction between location of trees or trap elevation and mosquito abundance was observed (2 W ANOVA, $F = 0.858$, d.f. = 3, $P = 0.4767$).

Shannon and Simpson indices were higher in the upper elevation than near the ground (Table 3, Panel B), indicating that a greater diversity of species was collected in the upper strata where mosquitoes were distributed more equitably. Moreover, $S_{\text{Chao1}}$ and $S_{\text{ACE}}$ indicated that the estimated number of mosquito species in the upper strata was twice as high than near the ground (Table 3, Panel B). The DCA ordination indicated that the mosquito community in trees located near the stream and lower slope (in the upper stratum and near the ground) were separated from the trees located in the upper slope and ridge on the second axis (Fig. 3b). *C. pipiens* was the only species commonly found in abundance in the eight traps.

| Table 3 | Estimated species richness and diversity of mosquito communities collected in four distinct habitats and at lower and upper strata of the rainforest of Maromizaha, district of Moramanga, Madagascar |
|-----------------------------------------------|-----------------------------------------------|
| Species no. | Shannon indices | Simpson indices | $S_{\text{Chao1}}$ | $S_{\text{ACE}}$ |
|----------------|----------------|----------------|----------------|----------------|
| Panel A: Four distinct habitats |
| Quarry | 7 | 1.15 | 0.51 | 7.50 | 8.20 |
| FBF | 10 | 1.13 | 0.54 | 11.50 | 12.37 |
| Col | 11 | 1.57 | 0.73 | 12.50 | 12.71 |
| Cp2 | 9 | 1.24 | 0.63 | 19.00 | 20.00 |
| Panel B: Lower vs. upper strata |
| Upper FBF | 9 | 1.02 | 0.43 | 14.00 | 18.24 |
| Lower FBF | 7 | 0.76 | 0.32 | 7.25 | 9.27 |
| Upper Cp2 | 8 | 1.09 | 0.52 | 13.00 | 23.00 |
| Lower Cp2 | 8 | 0.88 | 0.37 | 8.75 | 12.28 |

$S_{\text{Chao1}}$ and $S_{\text{ACE}}$ are the estimated number of species. Cp2, Camp 2 site; FBF, former banana field site.

75.70% of adult mosquitoes caught, followed by *Culex antennatus* Becker, which represented 9.4%. The remaining 14.8% of the total caught consisted of 11 other species. When each tree was considered as one unit, no significant difference of mosquito abundance was observed between sampling position (valley floor, lower slope, upper slope and ridge (1 W ANOVA, $F = 0.752$, d.f. = 3, $P = 0.5326$)). Traps installed near the ground collected more mosquitoes than traps installed near the canopy (1 W ANOVA, d.f. = 1, $F = 4.322$, $P < 0.05$) (Fig. 2b). No evidence of interaction between location of trees or trap elevation and mosquito abundance was observed (2 W ANOVA, $F = 0.858$, d.f. = 3, $P = 0.4767$).

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**Larval habitats**

A total of 118 larvae, belonging to five genera, were collected, and five types of larval habitat were identified (Table 4). *Culex* larvae were frequently collected and found in artificial breeding sites (cement-lined pit) in association with *Uranotaenia*, *Lutzia* and *Aedes*. The leaf axils of Aceraceae were found to be colonised only by *Uranotaenia*, while hoof prints in the ground were colonised only by *Culex*. Leaf axils of Pandanaceae were found to be colonised by the *Culex*, *Uranotaenia* and *Mimomyia* Theobald genera.

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Fig. 3. Detrended correspondence analysis (DCA) (a) between the four habitats types and (b) at lower and upper strata. The two axes represent linear summaries of the variation in the species numbers and areas. Ae.ar, *Aedes argenteopunctatus*; Ae.ci, *Aedes circumluteolus*; An.co, *Anopheles costanti*; An.fu, *Anopheles funestus*; An.ga, *Anopheles gambiense*; An.Ia, *Anopheles lacani*; An.ma, *Anopheles mascarensis*; Col, Col; Cp2, Camp 2; Cx.at, *Culex antennatus*; Cx.an, *Culex annulioris*; Cx.de, *Culex decens*; Cx.gi, *Culex giganteus*; Cx.pi, *Culex pipiens*; Cx.qu, *Culex quinquefasciatus*; Cq.gr, *Coquillettidia grandidieri*; FBF, Former banana field; Fi.sp, *Ficalbia* sp.; Ho.sp, *Hodgesia* sp.; L, Lower slope; lo-S, lower strata; Lt.ti, *Lutzia tigripes*; Lt.sp, *Lutzia* sp.; Or.mi, Orthopodomyia milloti; R, ridge; U, upper slope; up-S, upper strata; Ur.an, *Uranotaenia anopheloides*; Ur.al, *Uranotaenia alboabdominalis*; Ur.ne, *Uranotaenia neireti*; Ur.sp, *Uranotaenia* sp.; V, Valley.

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Table 4  Types of mosquito larval habitats found in Maromizaha forest in November–December 2016

| Breeding sites       | Mosquito genera found |
|----------------------|-----------------------|
|                      | Culex | Uranotaenia | Lutzia | Mimomyia | Aedes |
| Cement-lined pit     | 42    | 1           | 7      | 0        | 2     |
| Stream               | 0     | 2           | 0      | 2        | 0     |
| Hoof prints          | 12    | 0           | 0      | 0        | 0     |
| Leaf axis of Araceae | 0     | 21          | 0      | 0        | 0     |
| Leaf axis of Pandanaceae | 12    | 14          | 0      | 3        | 0     |

Larval stages were mainly collected in rocky interstices.

DISCUSSION

Understanding the occurrence and circulation of mosquito-borne pathogens in locales like the Maromizaha forest and its surrounding area (Fontenille 1989; Savage et al. 2009; Schmid et al. 2017; Tantely et al. 2013) requires knowledge on mosquito diversity including variation in distribution and abundance across spatial axes. As to diversity, to the list of 23 species collected in this study can be added one non-identified species of Mimomyia collected at larval stage (Table 4) and Eretmapodites quinquemittatus Theobald collected biting on human during diurnal activities. These observations are consistent with the presence of 25 species as estimated by Chao1 and ACE, which have already proven useful to estimate the true species richness of mosquito communities (Brant et al. 2016). Of the 51 species belonging to Aedeamyia, Mansonina and Toxorhynchites genera found in the neighboring forest of Andasibe (ex-Périnet) (Fontenille 1989), we did not find 34 species. Furthermore, this study is the first to report Hodgesia Theobald genus in this region. The last collection of specimens of this genus dated from 1984 in the south-east coastal area of Madagascar (Fontenille 1989). These differences might be due to the various sampling methods used by Fontenille (1989), in contrast to the single trapping method we used in this study. For example, mosquitoes from the Aedes genus were rarely collected in this study, but were the most dominant mosquitoes collected via human landing catch in the Andasibe forest (99.21% of total number collected) – a technique not used in this study (Fontenille 1989).

However, the present results were more similar to those obtained from the Anorana forest where the same sampling method (light traps) were used (Fig. 1) (Tantely et al. 2013). Eighteen of the 29 species reported from the Anorana forest (Tantely et al. 2013) were collected in this study (Table 2). In the Anorana forest, highest mosquito abundance and diversity were reported at the beginning of the rainy season (November), justifying the choice of November to perform this present study. In the Anorana forest, mosquito abundance decreased during the rainy season and flushing of mosquito breeding sites was considered as its cause (Tantely 2013). This observation corroborates the low density of species of the Uranotaenia genus in 2016. Higher abundance of Culex pipiens was already reported in other sites of the Central Highland, highlighting its role as forest habitat indicator (Tantely et al. 2013, 2016).

Although not documented in the literature, different wind flow patterns within forested areas as compared to open areas (Haddow & Corbet 1961; Swanson & Adler 2010) likely affects the dispersion plume of released CO2. To avoid having a differing radius of attraction for traps in different habitat types, no mosquito attractant (CO2) was thereby added to the light traps. Trapping without using mosquito attractant and by targeting crepuscular and nocturnal mosquitoes allows comparing our results to those of a few previous studies in forested area of Madagascar using the same sampling methods (Fontenille 1989; Tantely et al. 2013). However, one limitation of this approach is that our results cannot be compared with results from numerous other studies that used attractant and have been performed in others countries (Junglen et al. 2009; Swanson & Adler 2010). In some cases, CO2-baiting of light traps was shown to increase capture of mosquitoes (Mboera & Takken 1997; Russell 2004). However, Sriwichai et al. (2015) demonstrated a variation of mosquito capture rate with CO2-baited light traps and reported no significant difference between CO2-baited and unbaited traps during the rainy and dry seasons in Thailand. Moreover, the use of CDC light traps generally is warranted by their suitability for studying mosquito abundance and diversity as evidenced by data obtained in the Anorana forest (Tantely et al. 2013) and in other sub-Saharan African countries (Amusan et al. 2005; Meyrowitsch et al. 2011).

In the Maromizaha forest, two patterns of the effect of forest fragmentation on mosquito communities were observed. The first one suggests that mosquito species diversity varied across the four habitats and increased from the open area to the primary forest (Table 3, Panel A). The second shows that mosquito abundance varied across the four habitats with higher mosquito abundance in the FBF site. Rainfall, vegetation, gradients of human presence, temperature and humidity were considered to impact mosquito diversity and abundance (Junglen et al. 2009; Meyer Steiger et al. 2012). Although not tested in this study, the potential effect of rainfall and vegetation could be summarised as follows: foliage cover, absent in the Quarry site and might protect and maintain created larval habitats in the forest against raindrops that are known to kill and eject immature stages out of their habitats (Paaijmans et al. 2007). The presence of Ficalbia, Lutzia, Orthopodomyia, Uranotaenia species in the forest habitats was expected because its aquatic stages grow mainly in phytotelmata and natural terrestrial habitat (Brunes & Hervy 1995; da Cunha Ramos & Brunhes 2004; Tantely 2013; Tantely et al. 2016). The remaining genera are exotic and their presence within the forest and in degraded habitat was expected even if these larval stages can exploit natural and artificial terrestrial breeding sites (Fontenille 1989; Tantely et al. 2016).

Studies describing mosquito distributions in forested areas have repeatedly reported higher abundances of mosquito along forest edges than in other habitats (Junglen et al. 2009; Meyer Steiger et al. 2012). The occurrence of shared mosquito communities both inside and outside the forest, in the case of Culex and Uranotaenia genera (Fig. 2a), already reported in the forest edge (Despommier et al. 2006; Lothrop et al. 2002; Meyer Steiger et al. 2016) could explain this abundance pattern. If the edge zone was defined as the area of forest adjacent to the non-forest land but covered with 80% foliage (Weathers et al. 2011), the
The presence of these mosquito species and the occurrence of arthropod-borne pathogens in the Maromizaha rainforest and neighbouring forest areas represent a risk for humans and animals due to their potential role as pathogen vectors (Fontenille 1989; Tantely et al. 2012, 2013, 2016; Schmid et al. 2016). Degraded biotopes such as FBFB exhibiting higher mosquito abundance could act as ecotones defined as areas where prevalence of pathogen circulation is higher (Desponnier et al. 2006). However, forest-dwelling vertebrate fauna capable of exploiting other habitat types were found to display a significantly higher prevalence of infection as compared to forest-dependent vertebrate fauna (Savage et al. 2009). The role of C. pipiens as a bridge vector connecting hosts located outside, at the forest-edge, or within the rainforest (Tantely et al. 2013) is consistent with this hypothesis. Increased populations of mosquito vectors in lower strata might increase their contact with human and wild hosts (Savage et al. 1999), considering that Maromizaha forest has been developed for ecotourism, involving villagers and others for the purposes of research activities and student training (Gamba et al. 2013; Newsome & Hassel 2014).

Conversely, high mosquito diversity in upper strata might limit pathogen transmission due to the dilution effect (Dobson et al. 2006). The presence of Eretmapodites quinquevittatus, a degraded areas indicator species (Tantely et al. 2016), in Maromizaha highlights the deforestation and fragmentation of this rainforest as already reported (Woog et al. 2006). Continuous disruption of this forest may enhance local vector-borne disease outbreaks due to ecological changes as highlighted in previous works in other countries (Patz et al. 2004).

In conclusion, a high abundance of mosquitoes was observed at the edge of the Maromizaha rainforest, which could act as an ecotone between intact rainforest to the nearest human habitation or vice versa. The role of C. pipiens as a potential bridge vector was highlighted. The presence of mosquito vectors in different elevation of forest areas could impact pathogen transmission between vertebrate populations, including human, in the Maromizaha forest. As a result, continued disruption of this forest may enhance local vector-borne disease outbreaks.

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