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Description of *Anopheles gabonensis*, a new species potentially involved in rodent malaria transmission in Gabon, Central Africa

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ABSTRACT

The genus *Anopheles* includes mosquito vectors of human malaria and arboviruses. In sub-Saharan Africa, the anopheline fauna is rich of nearly 150 species, few of which are anthropophilic and capable of transmitting pathogens to humans. Some of the remaining species are found in forests far from human environments and are vectors of wildlife pathogens. The diversity and the biology of these species have yet to be fully described. As a contribution to furthering knowledge of sylvan Anophelinae, using morphological and molecular tools we describe a new *Anopheles* species collected in Gabon (Central Africa), which we have named *Anopheles gabonensis* n. sp. We also molecularly screened this species to detect infections by *Plasmodium* parasites. The results showed the species to have been infected by *Plasmodium vinckei*, a rodent parasite. We discuss the role of *An. gabonensis* n. sp. in the transmission of *P. vinckei* in the rainforest areas of Central Africa and its potential to transfer pathogens to humans.

1. Introduction

The genus *Anopheles* is widely known because it includes mosquito vectors of human malarial parasites present throughout the world (Sinka et al., 2010). In the Afrotropical Region, the number of formally recognized *Anopheles* species currently stands at 147 (Hervy et al., 1998; Harbach, 2014; WRBU, 2014), but fewer than 10 are considered major human malaria vectors and of epidemiological interest (Manguin et al., 2008). The remaining species play either a secondary role or no role at all in human malaria transmission in Africa. However, *Anopheles* species are of medical interest due to their role in the transmission of lymphatic filariasis (i.e. *Wuchereria bancrofti*), particularly in western and Central Africa (Ughasi et al., 2012), and of several arboviruses affecting humans in Africa, such as O'nyong-nyong (Vanlandingham et al., 2005) and Rift Valley fever viruses (Ratovonjato et al., 2011). In addition, some of the anopheline species in Africa transmit other non-human *Plasmodium*, zoonotic cycles that are commonly overlooked (Bray and Garnham, 1964).

Most of the studies concerning the taxonomy, biology and genetics of *Anopheles* fauna in Africa have focused primarily on anthropophilic species and the characterization of zoophilic species is therefore far from complete. In particular, there is a significant scientific gap when it comes to vectors of pathogens circulating in sylvatic wildlife. For example, little is known about the anopheline mosquitoes involved in simian malaria transmission despite considerable interest in deciphering the evolution and diversity of *Plasmodium* parasites of African apes and in achieving a better understanding of the mechanisms by which parasites transfer between apes and humans (Prugnolle et al., 2013). Only two species, *Anopheles moucheti* and *Anopheles vinckei*, have so far been found to be infected by ape *Plasmodium* (Paupy et al., 2013). Similarly, only one species, *Anopheles millecampsi,* has proven to be involved in the natural transmission cycle of rodent malarial parasites found in Africa (Bafort, 1969; Bray and Garnham, 1964). These parasites, *P. berghei*, *P. chabaudi*, *P. vinckei* and *P. yoelii*, were discovered in central and western Africa in the 1940s (reviewed in Killick-Kendrick, 1978). They are of great scientific interest because they have been extensively used in laboratory as animal models to study the biology of *Plasmodium* (Stephens et al., 2012). Due mainly to insufficient knowledge of natural vectors, these models were implemented using "artificial" vector species (e.g. *Anopheles stephensi*, *Anopheles gambiae*), which are easy to breed in insectary

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conditions but certainly inadequate in terms of reproducing the interactions between parasites and their vectors in the natural environment (Boëte, 2005; Cohuet et al., 2006).

To further knowledge of sylvan Anophelinae and understand the natural transmission of rodent malarial parasites, we describe here a new species of *Anopheles*, collected in Gabon, naturally infected by *Plasmodium vinckei lentum*, a rodent *Plasmodium* species. We discuss its potential role in the transmission of rodent malaria and the transfer of zoonotic pathogens to humans.

2. Materials and methods

2.1. Study sites and mosquito sampling

We carried out a longitudinal survey of sylvan *Anopheles* from October 2012 to September 2013 (Fig. 1). Mosquitoes were collected inside two wildlife reserves, La Lékédi, a private nature park near Bakoumba (Haut Ogooué Province), and the Mikongo research station in La Lopé National Park (Ogooué Ivindo Province). The landscapes of the study sites are forest-savannah mosaic in Bakoumba and equatorial forest in Mikongo. *Anopheles* mosquitoes were captured monthly over five nights using CDC light traps placed in several sites in the forest between 17:00 and 7:00. The annual sampling efforts were 744 traps in Bakoumba and 648 in Mikongo. The mosquitoes collected underwent morphological identification with reference to standard morphological features and identification keys (Gillies and Coetzee, 1987). None of the 42 specimens collected, respectively 39 females in Bakoumba and 3 in Mikongo, could be assigned to an already known species. Unknown specimens underwent further detailed morphological analysis. The CDC light traps are known to cause potential morphological damage sometimes leading to mis-identification of mosquitoes sampled. For this reason we selected only well preserved specimens to undertake the morphological analysis. The remaining specimens were stored in liquid nitrogen, sent to the CIRMF and kept at −80 °C until processing for molecular analyses.

2.2. Morphological analysis of mosquitoes

Several morphological characteristics in accordance with the usual *Anopheles* taxonomic features were observed under a Leica M80 binocular. For specimens with well preserved wings, these were removed and mounted on microscope slides under cover slips. Pharyngeal armatures were dissected and extracted from the head and immediately mounted in Euparal after several successive baths: 2 h in 10% potassium hydroxide, 2 h in distilled water, 10 h in a Marc-André solution (Abonnenc, 1972), 10 h in distilled water, 20 min in 70% ethanol, 20 min in 90% ethanol, 20 min in 100% ethanol and 10 h in a beech wood solution. They were then observed under a DM 2000 Leica microscope.

2.3. Molecular analyses

2.3.1. PCR amplification and DNA sequencing

DNA was isolated and purified from whole mosquitoes (minus the wings for specimens analyzed for wing variations) using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions, and used as a template for amplifying a portion of the Cytochrome Oxydase Subunit II (COII) of mitochondrial DNA. The PCR amplifications were done using a GeneAmp 9700 thermal

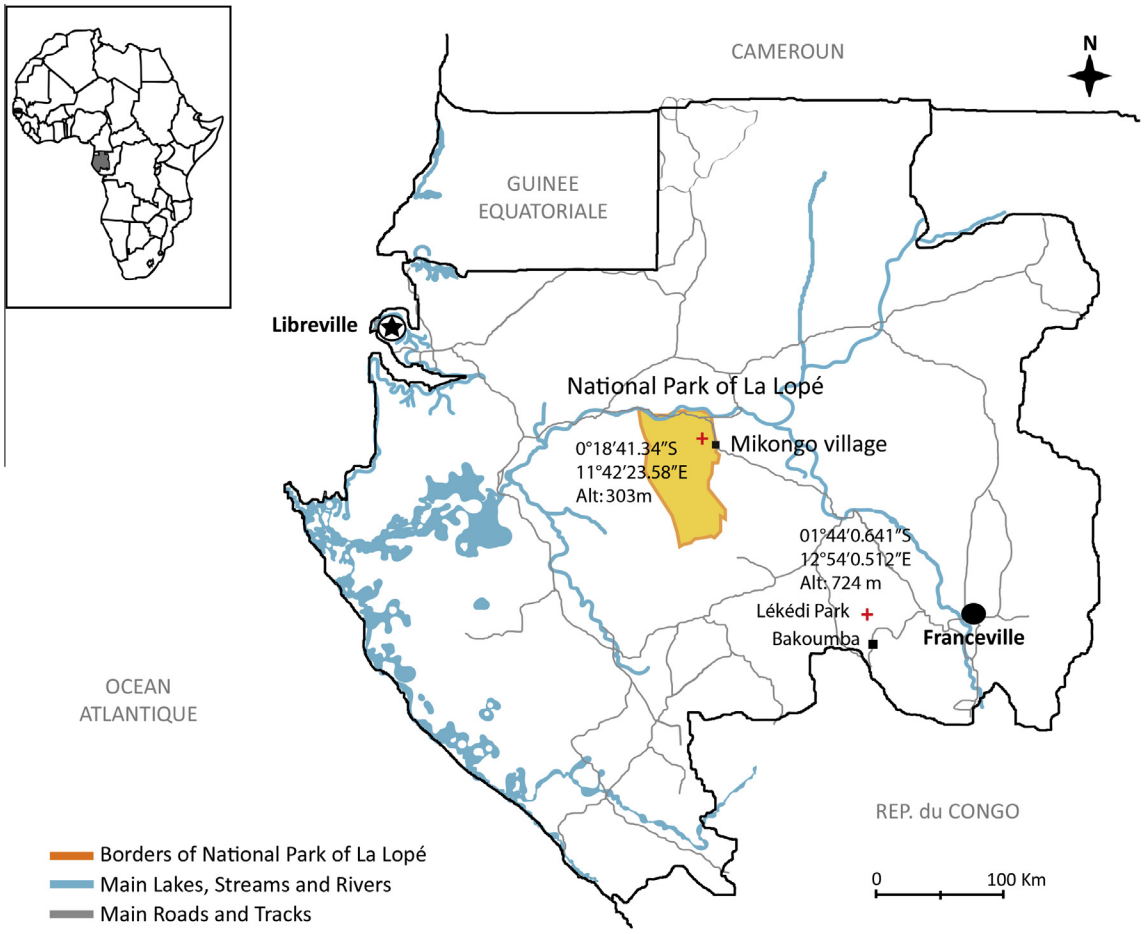

Fig. 1. Location of mosquito collection sites in Gabon.