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Identification of *Anopheles* species in Sud Kivu, Democratic Republic of Congo, using molecular tools

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Abstract

The mosquito fauna of the Democratic Republic of Congo remains understudied, including that of the province of Sud Kivu. To improve understanding of species presenting Sud Kivu, adult mosquitoes were collected from houses and larvae were collected from standing water at altitudes between 1627 and 1875m above sea level. Morphological and molecular methods were used to identify the species of *Anopheles* collected. Six species were found, including several primary and potential secondary malaria vectors. Further work is needed to characterize mosquito populations in Sud Kivu, as well as to improve methods for identifying *Anopheles* in general.

Keywords

Sud Kivu; Democratic Republic of Congo; *Anopheles* ; CO1; ITS2

Introduction

Malaria is one of the most important diseases in the Democratic Republic of Congo (DRC), but the distributions of *Anopheles* mosquitoes in the country are poorly understood. Morphological identification is the main method used for identifying mosquito species and PCR can also be used for the detection of sibling species within known species complexes.[1]

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Supplementary data

Supplementary data are available at Transactions online (http://trstmh.oxfordjournals.org/).

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Furthermore, CO1 or ITS2 regions can be used to discriminate species and recent work in neighboring countries detected unknown species of *Anopheles* mosquitoes using these methods. These studies suggest that there are likely to be unknown species of *Anopheles* in the DRC.[2,3]

Sud Kivu Province is in eastern DRC with a low malaria prevalence (12% rapid diagnostic test positivity in children under 5 years of age, compared with 31% nationally).[4] The province is ecologically unique, with mountains and proximity to Lake Kivu and Lake Tanganyika. For these reasons, and because the recent reports describing species of *Anopheles* collected in this area have not used molecular methods for species identification, we applied such methods to describe *Anopheles* species diversity in this area.

Materials and methods

Mosquitoes were collected between 25 September and 5 October, 2015, in villages near Katana, in Sud Kivu, including Lwiro (−2.244097, 28.815232), Maziba (−2.232256, 28.796143), Buloli (−2.247485, 28.827531), and Camp Matete (−2.252844, 28.815062) (Figure 1). These sites are at altitudes of between 1627 and 1875 m.

Adult mosquitoes were collected from inside 15 houses in indoor resting collections using mouth aspirators, after receiving consent from homeowners. Mosquitoes were transferred to tubes blocked with cotton wool for transport to the laboratory. Larvae were collected from bodies of standing water, including ditches and fish ponds, using larval dippers and ladles. Larvae were kept in containers and were transported to the laboratory for rearing.

Adult mosquitoes were identified under a stereomicroscope using standard identification keys.[5] Specimens morphologically identified as *An. gambiae* or *An. funestus* were tested using the appropriate PCR to identify sibling species within these complexes.[1] Specimens identified as other species were initially screened using these PCRs, and the ITS2 or CO1 sequence were used to confirm the identifications.[2]

Results and discussion

In total, 306 mosquitoes were successfully identified (Table 1, Supplementary data). Six species were collected, including *Anopheles gambiae* subsp. (129), *Anopheles arabiensis* (1), *Anopheles funestus* (13), *Anopheles maculipalpis* (1), *Anopheles theileri* (6), and the species reported as *An.* 1 BSL 2014/Species A(156).[2,3] Sequences generated in this study are available in GenBank, under the following accession numbers: *An. maculipalpis* (MH384978), *An.* 1 BSL-2014 (MH378772, MH384962–384969, MH384971–384977, MH392206), *An. funestus* (MH384970), and *An. theileri* (MH378771).

Anopheles gambiae subsp. and *An. funestus* subsp. were collected as both larvae and adults. *An. gambiae* subsp. was collected at altitudes up to 1847m elevation, which has been previously reported in this area.[6] *Anopheles arabiensis* was collected as a single larva in a fish pond at 1677 m. *Anopheles funestus* subsp. was collected in a pond at 1627m above sea level, the lowest elevation sampled, and in a house at 1875m above sea level, the highest elevation that was sampled.

The most abundant species sampled was the species identified as *An*. 1 BSL 2014 previously reported from Zambia[2] and the Kenyan Highlands.[3] The larvae for this species were collected from two similar sites in Buloli. The first was a shaded ditch in the middle of a bean field, and the larvae were found amongst the vegetation that floated on the surface of the water. The second was a ditch in a cassava field, also covered with vegetation. Adult mosquitoes were collected indoors, in Buloli, Camp Matete and Maziba.

An. maculipalpis was collected in a house at 1660m elevation. This species has been previously reported from elsewhere in DRC, but we were not able to find previous records of this species from Sud Kivu. *Anopheles maculipalpis* larvae are commonly found in small foul pools in drying swamps and in rice-fields,[5] both of which were present in the study area.

Anopheles theileri was only collected from houses (no larval samples were collected). Houses where *An. theileri* were collected were all between 1651 and 1860m elevation. While mosquitoes collected in this study were not tested for presence of *Plasmodium* parasites, *Plasmodium*-positive head and thoraces of *An. theileri* were previously found in eastern Zambia.[2] Larvae of *Anopheles theileri* are commonly found on shady margins of streams, where they may wriggle up the sides of vegetation.[4]

Using PCR and sequencing to identify mosquito species was important in this study to allow identification of sibling species within the *An. gambiae* and *An. funestus* complexes. It was also important to identify unknown species, which may be existing species without registered ITS2 or CO1 sequences, or undescribed species. Further association of species with ITS2 and CO1 sequences (and retention of voucher specimens) should be a priority.

Further research in Sud Kivu should be conducted to better understand the vectors involved in malaria transmission, as well as to understand the geographic ranges of known species along altitudinal transects. The understanding of malaria transmission at different altitudes may allow for a better targeting of control interventions, as has been done in East Africa[7] and Madagascar[8].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Map of study area, showing sites for larval collection (Lwiro and Buloli) and mouth aspirators catches (other sites), which were conducted in the area indicated by the inset within Sud Kivu.

Table 1.

Identification of 306 *Anopheles* by morphology and PCR, nucleotide sequence comparison of the second internal transcribed spacer (ITS2) and partial sequences of the cytochrome c oxidase subunit I (COI). Mosquitoes were collected in larval habitats and as adults resting inside houses of four villages near Katana, Sud Kivu, DRC. September 25–October 5, 2015

			Method of identification		
Village	**Species**	**Altitude (m)**	**Morphology/PCR**	**ITS2**	**CO1**
Buloli	*An. funestus*	1627–1659	6		
	An. sp. 1 BSL-2014	1627–1659		3	22
	An. maculipalpis	1660			1
	An. theileri/*An. sp.* 9 BSL-2014	1651		1	
Camp Matete	*An. gambiae* s.s.	1665–1677	22		
Lwiro	*An. arabiensis*	1677	1		
	An. gambiae s.s.	1676–1683	36		
Maziba	*An. funestus* s.s.	1847–1875	7		1
	An. gambiae s.s.	1847	57		
	An. sp. 1 BSL-2014	1737–1875		4	140
	An. theileri/*An. sp.* 9 BSL-2014	1737–1860		4	1