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Molecular identification of *Anopheles gambiae sensu stricto* Giles (formerly *Anopheles gambiae* Savannah Form) in Kamuli District, Uganda

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Anopheles gambiae sensu stricto Giles (formerly *A. gambiae* S molecular form), the largely anthropophilic species, is reportedly the most important malaria vector in Uganda among the *A. gambiae* complex species. Indoor and outdoor human-biting mosquitoes were caught for four consecutive nights in each of 48 households in Kamuli district using human-baited bed net traps for subsequent identification of the principal *Anopheles* sibling species responsible for transmitting malaria. Sibling species under the *A. gambiae* complex were characterized by polymerase chain reaction using species specific single nucleotide polymorphism (SNPs) in the intergenic spacer region (IGS) with primers specific for *A. gambiae s.s., Anopheles arabiensis, Anopheles melas, Anopheles merus* and *Anopheles quadriannulatus*. Molecular forms of the *A. gambiae s.s.* were further discriminated using primers specific for Mopti and Savannah forms. Out of 300 *A. gambiae s.l.* amplified, 98% (n= 294) were *A. gambiae s.s.* Out of 142 *A. gambiae s.s.* samples analyzed for molecular forms, 78.9% (n=112) were identified as *A. gambiae s.s.* Giles *(A. gambiae* Savannah (S) form, while the other 21.1% were not identifiable. the presence of *A. gambiae s.s. Giles* in Kamuli was also reported. Considering the anthropophilic, endophagic and endophilic behavior of *A. gambiae s.s.* (and of the molecularly similar *A. gambiae s.s.* Giles), the combined use of insecticide-treated nets (ITNs), indoor residual spraying, larval source management and improved house design in the context of integrated vector management, may be the appropriate vector control strategies in the area. There is also need for regular monitoring of the vector species composition, distribution and behavior for proper planning of appropriate vector control interventions in the future.

Key words: Sibling species, molecular forms, *Anopheles gambiae* complex, anthropophily, IPM.

INTRODUCTION

Malaria is endemic in Uganda and is the leading cause of illness especially among young children (Echodu et al.,

2010; Lanier, 2012). The 2011 World Health Statistics showed that Uganda's malaria mortality rate of 103 per

100,000 was more than seven fold that of Kenya (12/100,000), 18% more than that of Tanzania and 9% more than that of sub-Saharan Africa (Ministry of Health, Uganda, 2011). In Eastern Uganda, malaria is endemic, with perennial and high levels of transmission, despite the widespread distribution of insecticide-treated nets (ITNs), in addition to other anti-malarial interventions in the region (Helinski et al., 2015; Ojuka et al., 2015). In Kamuli district too, malaria has remained the leading cause of morbidity and mortality in all age groups with 38.4% of all diagnosed patients being malaria cases. The district is one of the few districts in Uganda with a good number of Non-Government Organizations (E.g. Plan International, Christian Children's Fund, etc) that have intervened with large supplies of free ITNs since the late 1990s to curb the high malaria challenge. Nevertheless, malaria has remained a very big challenge (Kamuli District Health Sector Strategic Plan, 2005/06-2010; Kamuli District Health Status Reports, 2005/06-2009/10, Unpublished). Members of *Anopheles gambiae* complex, known for being one of the most efficient vectors of human malaria in the world (Cohuet et al., 2003; Mayagaya et al., 2009), are the most common vectors in most parts of Uganda (Echodu et al., 2010). *A. gambiae sensu stricto*, one of the recognized sibling species of the complex and known for being the most anthropophilic malaria vector in Africa (Toure' et al., 1994) is the most important vector of human malaria parasites in Uganda (MoH, Uganda, 2005; Echodu et al., 2010).

The major vector control interventions like the rapid scale-up of ITNs and IRS have been deployed without a detailed understanding of the species composition, distribution and behaviour dynamics of the local vectors. This may complicate impact monitoring (Coetzee et al., 2000).

The principal mosquito species under the *A. gambiae* complex responsible for transmission of malaria parasites in Kamuli district were not yet known, while the molecular forms of *A. gambiae s.s.* that occurred in this area and Uganda as a whole, that is, *Anopheles coluzzii* and *A. gambiae s.s.* Giles (formerly "*A. gambiae* molecular "M" and S" forms, respectively) (Coetzee et al., 2013; Sawadogo et al., 2013), were also not yet known. Perhaps there was a particular species/molecular form responsible for the transmission.

The knowledge of which form, *Anopheles coluzzii*, *A. gambiae s.s.* Giles, or both of them occur in Uganda will have epidemiological implications in terms of vectorial capacity, distribution range and susceptibility to the currently available vector control interventions. The two molecular forms, *A. coluzzii* and *A. gambiae s.s.* Giles have high vectorial capacity but differ in their susceptibility to pyrethroids (Lehmann and Diabate,

2008), the preferred class of insecticide for treatment of long lasting bed nets (Harris et al., 2013). Thus, identification of the sibling species and molecular forms using molecular methods can have important implications in subsequent planning and implementation of the most appropriate vector control measures (Coetzee et al., 2000; Fanello et al., 2002; Koekemoer et al., 2002).

This study was therefore aimed at establishing the principal *Anopheles* mosquito sibling species and molecular forms within the *A. gambiae* complex responsible for transmission of malaria parasites in Kamuli District, Uganda, as part of a major study to investigate the effect of long-term use of insecticide-treated bed nets on the biting behaviour and vectorial capacity of *A. gambiae s.l.* and *A. funestus* group in this part of the country.

MATERIALS AND METHODS

Study area and mosquito sampling

The study was conducted in Kamuli district in Eastern Uganda, located at (01° 05'N 33° 15' E), 68 km North of the source of River Nile. Kamuli district was chosen because several villages were well supplied with Insecticide Treated Bed Nets (ITNs) that were given to the population by NGOs in an effort to supplement government efforts to control malaria among particularly vulnerable groups– pregnant mothers, children under five years and the People Living with HIV/AIDS.

Mosquitoes were collected from forty-eight households randomly selected from ten villages with five villages in Kamuli Town Council and Nabwigulu Sub County where ITNs had been in use for over five years, with 69% of the households having at least one ITN. These were the intervention villages. The other five villages were located in Bugaya and Buyende sub counties where ITNs had not been in use before the entomological survey and comprised the non-intervention zone. These were in the North East of Kamuli Town Council, and well over twenty kilometers away from the intervention zone.

Both intervention and non-intervention zones were in the same climatic and ecological zone (NEMA, 2007) and were surrounded by a variety of vegetation types including swamps, crop fields and grazing lands. Therefore, at the time of entomological sampling, ITN use was taken to be the only unique factor between the two study zones.

From December 2009 to November 2010, hourly indoor biting mosquitoes were collected from 19:00 to 07:00 h for four consecutive nights per month by a two-person team of trained catchers using bed net traps (Okello et al., 2006). The bed net trap was made by making a 3 x 3 inch hole on each of the sides of an untreated bed net, making a total of 4 to 6 holes on the net. The catcher sat under the bed net trap which gave him some protection which is denied when the human-landing catch method is used. This method was preferred to the CDC light trap (used initially) which was more costly to run overnight, requiring replacement of batteries after a few days. Outdoor human biting catches were carried out concurrently using the same method at the same household ten meters away (Okello et al., 2006).

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People living in a room were protected with an untreated net each, and as hungry mosquitoes persisted in their attempts to look for a blood meal, they got near the human-baited trap and were caught (Lines et al., 1991) by the human bait (collector) using an aspirator and a torch (Okello et al., 2006). It was assumed that the mosquitoes that entered a trap during any hour were those actively seeking hosts and, in most cases, would bite human hosts in the same hour and room/house if the bed net trap was absent (Maxwell et al., 1998).

The indoor and outdoor human-biting fractions of the *Anopheles* mosquitoes were determined and recorded throughout the whole sampling period for both intervention and non-intervention zones. Each hourly catch were separately placed in a disposable polystyrene container pre-labeled with date, time and location of capture and taken to the laboratory for identification of mosquitoes collected (Curtis et al., 1998). Mosquitoes were kept alive by providing them with a 10% sugar solution to feed on through a cotton wick (Styer et al., 2007).

Morphological identification

Each hourly catch of the human-biting fractions of the mosquito population were identified morphologically using a simplified key adopted from Gillies and Coetzee (1987), while the morphological identifications were confirmed by an Entomologist at the Vector Control Division, Ministry of Health, Uganda.

Extraction of DNA, PCR amplification and species identification

To confirm and improve on the accuracy of the morphological identification, PCR techniques were used to separate the *A. gambiae complex* samples. A total of 300 mosquito samples (150 samples from each of the two zones) were characterized into sibling species under the *gambiae* complex using species specific single nucleotide polymorphism (SNPs) in the intergenic spacer region (IGS) using primers specific for *A. gambiae sensu stricto, Anopheles arabiensis*, *Anopheles melas Anopheles merus* and *Anopheles quadriannulatus* (Scott et al., 1993; Mbogo et al., 1996; Curtis et al., 1998). Same proportions of the samples caught indoors and outdoors were analyzed (n = 75) by PCR. At least fifty samples were randomly selected from each of the 1st, 2nd and 3rd (last) of the night collections in both intervention and non-intervention zones. In cases where samples caught in any third of the night were less than 50, all the samples were taken for characterization.

All the members of the *A. gambiae* complex were discriminated by the SNP based PCR with primers that bind to the SNP sites utilizing Intentional Mismatch Primers (IMPs) within the intergenic spacer region, producing unique bands for each of the species and sub-forms (Wilkins et al., 2006).

One to two legs or wings of a single adult mosquito (as DNA source) were placed in 22.0 µl of PCR reaction mixture. This mixture contained the following: 14.5 µl of double distilled water, 5.0 µl of 5X High Fusion reaction buffer (100 mM Tris-HCl pH 8.3, 500 mM $MgCl_2$), 0.5 µl of 10 mM dNTPs,1.5 µl of UN, ME, AR, GA and QD primers (synthesized by Inqaba Biotechnical Industries, Pretoria, South Africa) and 0.5 µl of Phusion ™ DNA polymerase. Finally, 3.0 µl of double distilled water was added to make the total volume 25.0 µl and the reaction mixture was agitated a few times [(MgCl_2, Buffer, Phusion ™ DNA polymerase and dNTPs (supplied by Celtic Molecular Diagnostics (Pty) Ltd, South Africa)].

Negative controls containing PCR reaction mixtures without DNA were added to each PCR experiment. The PCR reaction conditions were as follows: Initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and final auto extension at 72°C for 5 min. A volume of 10.0 µl of the PCR product was mixed with 3.0 µl of ficoll dye (50% sucrose, 0.05 M EDTA pH 7, 0.1% bromophenol blue, 10% ficoll powder) and loaded on 2% agarose gel stained with 12.0 µl ethidium bromide (10 mg/100 ml) (Cat. No. 15585-011, Gibco BRL, UK), submerged in 1XTAE buffer and electrophoresed at 100 V for one hour. 5.0 µl of molecular marker (Gene Ruler TM DNA ladder Mix, Cat. No. SM0331) was loaded on the first well of the agarose gel, followed by the wild samples in the next wells, positive control in the second last well and the negative control in the last well.

The DNA fragments were visualized under ultra violet (UV) light and the size of the products was confirmed using the molecular ladder. Molecular forms of the *A. gambiae sensu stricto* were further discriminated by PCR using primers specific for *A. coluzzii* (formerly *A. gambiae* Mopti or 'M' form) and *A. gambiae s.s.* Giles (formerly *A. gambiae* Savannah or 'S' form), that is, M_3, M_5, S_3 and S_5, using the same PCR protocol as described above (Scott et al., 1993) using the following cycling conditions: Initial denaturation at 98°C for 3 min, 39 cycles of denaturation at 98°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and final auto extension at 72°C for 7 min. Samples were run on a 2% agarose gel for visualization using standard markers.

Specificity of the *A. gambiae sensu lato* primers was checked by using three samples of *A. gambiae s.s.* positive controls. A sample known to contain trypanosomes was also included in checking the specificity of the primers. The *A. gambiae s.s.* DNA positive controls were obtained from the Uganda Virus Research Institute's Entomology Laboratory in Entebbe, while characterization of molecular forms was confirmed using positive controls in Benin's Ministry of Health Entomology laboratory.

Ethical issues

Prior to start of the study, approval was obtained from the Uganda National Council for Science and Technology and Health Research Ethics Committee (Reference Number: HS 263).

Household owners, village and district authorities were sensitized prior to the study and their permission was obtained, while the privacy and psycho-social needs of the individual participants and household members were highly protected. Catchers were selected from the local community to facilitate acceptance from residents. Informed consent was obtained from each catcher.

The catchers were trained to collect landing mosquitoes prior to blood feeding to minimize the risk of malaria transmission. They were given anti-malarial drugs as this geographical area has high transmission of *Plasmodium falciparum* with resistance to anti-malarial drugs (Dr. Lopita Micah, Pers. Communication). At least two bed nets (LLNS) were donated to each participating household following the study.

Statistical analysis

Comparison of the indoor and outdoor human biting catches of the *A. gambiae* complex and *A. funestus* group of mosquitoes for the whole sampling period between the intervention and non-intervention zones was done using the Kruskal-Wallis rank sum test of the R-Statistics software, version 2.15.0 (2012.03.30) (R Development Core Team, 2002), given their non-normal distribution.

RESULTS

Morphological identifications

Over 70% of the *Anopheles* species caught were A.

Table 1. Numbers of female *Anopheles* mosquitoes caught indoors and outdoors in both non-intervention and intervention zones over a 12-month sampling period.

Mosquito group	Non-intervention zone		Intervention zone		Total	%
	Indoor	Outdoor	Indoor	Outdoor		
Anopheles gambiae s.l	853	1079	299	346	2,577	73.2
Anopheles funestus s.l.	453	411	39	39	942	*26.8*
Total	1,306	1,490	338	385	3,519	

Other mosquito species caught but not included in this table were: *A. mouch*eti (n > 500), *Culex* species (n> 1,840) *and Aedes aegypti* (n > 150).

Table 2. Polymerase chain reaction (PCR) primers and sizes of the amplified products for species within *A. gambiae* complex (Scott et al., 1993).

Primers	Primer sequence (5' to 3')	Temperature (°C)	Identified species	Size of the PCR product (bp)
UN(F)	[GTG TGC CCC TTC CTC GAT GT]	58.3		
GA(R)	[CTG GTT TGG TCG GCA CGT TT]	59.3	*A. gambiae s.s.*	390
ME (R)	[TGA CCA ACC CAC TCC CTT GA]	57.2		
AR (R)	[AAG TGT CCT TCT CCA TCC TA]	47.4		
QD (R)	[CAG ACC AAG ATG GTT AGT AT]	42.7		

TM = Melting temperature; bp = base pairs. UN primer anneals to the same position of the rDNA of all the five species, GA anneals specifically to *An gambiae*, ME anneals to both *A. merus* and *A. melas*, AR anneals to *A. arabiensis* and QD anneals to *A. quadriannulatus.* F= Forward orientation; R= reverse orientation.

gambiae s.l, and 26.8% were *A. funestus* group. Other mosquitoes caught included *A. moucheti*, *Culex* and *Aedes* species. Based on morphological identification, there was approximately four times more *Anopheles* spp. caught in the non-intervention as compared to the intervention zone (2,796 and 723 anophelines in the respective zones (Table 1); Chi-squared = 159.894, df = 1, *P* < 0.001). For both zones, *A. gambiae s.l.* catches exceeded those of *A. funestus* (Chi-squared = 86.662, df = 1, p < 0.001), this trend being greater in the intervention zone. Outdoor biting apparently exceeded the indoor biting catches although with no statistically significant difference (Chi-squared = 0.227, df = 1, p > 0.05). Detailed biting behavior and seasonal abundances of the *Anopheles* mosquitoes were discussed in another earlier study (Kabbale et al., 2013).

PCR amplification and identification of species and molecular forms

Fifty percent (50%) of the indoor and outdoor catches from both intervention and non-intervention were amplified by PCR. A total of 300 samples of *A. gambiae s.l.* was amplified of which 98% (294 out of 300) were all identified as *A. gambiae sensu stricto* (Table 2 and Figure 1). The identity of the remaining 2% of the *A. gambiae s.l.* could not be established. Out of the 294 identified samples, 145 (49.3%) and 149 (50.7%) were

from intervention and non-intervention zones, respectively. Approximately 50% of the identified *A. gambiae s.s.* samples were caught from indoors and outdoors in both intervention and non-intervention zones.

Out of 142 *A. gambiae s.s.* samples analyzed for molecular forms (50%, n= 71, from each zone), 78.9% (112 out of 142) were identified as *A. gambiae* s.s. Giles (Formerly Savannah (S) form) (Table 3 and Figure 2). The remaining 21.1% (30 out 142 *A. gambiae s.s.*) could not be identified. The results therefore showed that *A. gambiae s.s.* Giles exists in Kamuli district, and probably other parts of the country.

DISCUSSION

Morphological identifications and *Anopheles* species abundance

The difference in relative proportions of *A. gambiae s.l.* and *A. funestus* (89.2 and 10.8% respectively) in the intervention zone was observed to be much higher than the difference in the relative proportions in the non-intervention zone (69.1 and 30.9% for *A. gambiae s.l.* and *A. funestus* group, respectively. The lower mosquito abundance in the intervention zone is probably suggestive of effectiveness of the vector control intervention (ITNs/ LLINs) under use in this zone as compared to the non-intervention zone without treated bed nets. However, this
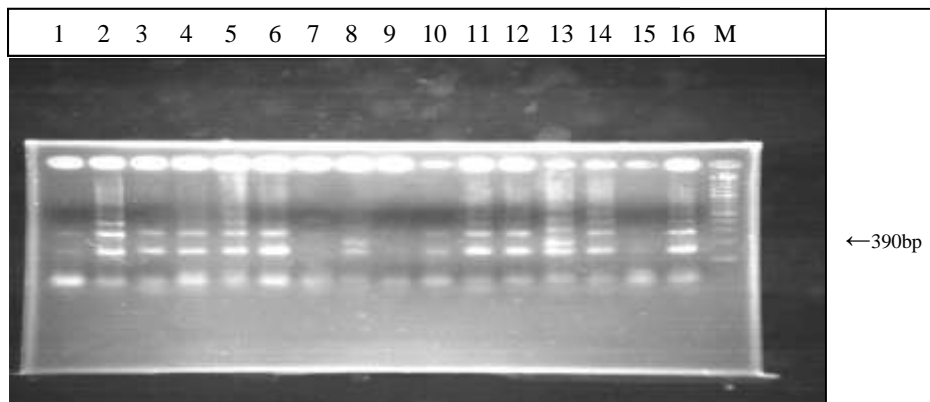
Figure 1. PCR-amplified fragments for identification of *Anopheles gambiae* complex species:
Lane 1: Negative control, Lane 2: *A. gambiae s.s.* positive control, Lanes 3-16: *A. gambiae
s.s.*, Lane M: 100-basepair, DNA size marker ladder.

Table 3. Polymerase chain reaction (PCR) primers and sizes of the amplified products for molecular forms within the *Anopheles gambiae
sensu stricto* (Scott et al., 1993; Wilkins et al., 2006).

Primers primer sequence (5' to 3')	Temperature (°C)	Identified forms	Size of the PCR product (bp)
M5 (F) [CTT GGT CTG GAG ACC GTT CCa TA]	59.5		
M3 (R) [GAC ACG TCA ACT AAG TCA ACA CAT tAC]	58.2		
S5 (F) [GCC CCT TCC TCG ATG Ga GC]	61.5		
S3 (R) [CAA CCG GCC CAA ACG GcT T]	59.4	S (*A. gambiae s.s.* Giles)	335

TM = Melting temperature; bp = base pairs; M3, M5, S3 and S5: Specific primers for the identification of molecular forms under *A. gambiae sensu
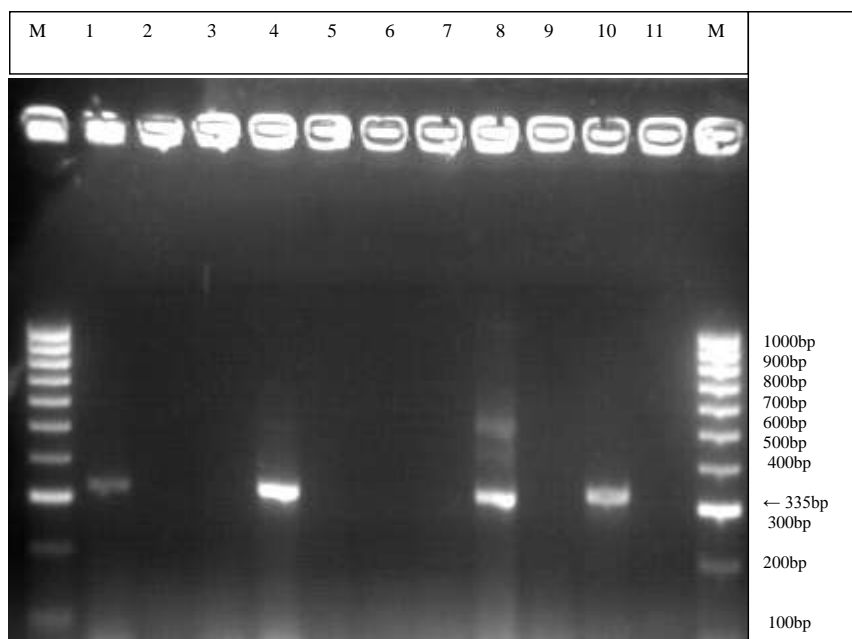stricto.*

Figure 2. PCR amplified fragments using M3, M5, S3, and S5 Specific primers for the
identification of molecular forms under *Anopheles gambiae sensu stricto.* Lanes M
and M, 100-base pair DNA size marker ladder, lanes 1, 4, 8 and 10: *A. gambiae s.s.*
Giles (formerly molecular form S).

could not rule out the fact that there could be other prevailing ecological or human behavioural factors in the intervention zone.

The relatively higher proportion of the *A. funestus* group in the non-intervention zone could be attributed to the presence of more permanent water for breeding provided by a larger swamp (Nabigaga) in this locality. *A. funestus* group is known to breed all year round and prefer permanent, stagnant water bodies such as shores of rivers and creeks, swamps or fish ponds for breeding, while *A. gambiae* complex breed in temporary/man-made water bodies e.g. pools, puddles or brick pits, fields, construction sites, hoof prints or even tyre tracks (Kabbale et al., 2013).

The presence of other mosquito species particularly *culex* species and *Aedes aegypti* poses a threat of other emerging and re-emerging parasitic and viral infections (Rozendaal, 1997).

Polymerase chain reactions

The *A. gambiae s.s.* positive control showed 2 to 3 bands. This could not be regarded as a hybrid of *A. gambiae s.s.* and *A. arabiensis* or *A. gambiae s.s./A. arabiensis/An. melas,* nor could it have been a result of contaminations, but possibly due to unsuitability of one of the cycling conditions, or probably due to incomplete digestion of DNA by Taq polymerase. Therefore, all field samples that showed the same pattern of bands on the electrophoresis gel were regarded as *A. gambiae s.s.* positive samples. The challenge is the identity of the band below the 390 base pair band. This may call for later sequencing of the unidentified band and further characterization.

Molecular identifications and relevance to malaria epidemiology and control

In the present paper, based on molecular data, the presence of *A. gambiae s.s.* as the sole sibling species under the *A. gambiae* complex in Kamuli district, Uganda was shown. This is a species known for its highly anthropophilic, endophagic and endophilic behavior (Rozendaal, 1997; MoH, Uganda, 1999b).

These results are consistent with previous reports in Uganda that revealed the presence of *A. gambaie s.l.*, (and *A. funestus)* as the main vectors responsible for transmission of human malaria parasites in the different regions of the country (MoH, Uganda, 2005) and the entire sub-Saharan Africa region (Cohuet et al., 2003; Mayagaya et al., 2009).

According to Ministry of Health, Uganda studies (Okello et al., 2006,), *A. gambiae s.s.* (33%) and *A. arabiensis* (39.5%) under *A. gambiae* complex and *A. funestus* (49%) under the *A. funestus* group were identified in Jinja

area, which is located only 62 km away from Kamuli district, having the same ecological characteristics suitable for breeding of these species of *Anopheles* mosquitoes. However, some efforts to identify chromosomal or molecular forms of *A. gambiae s.s.* prevalent in Uganda did not yield results (Vector Control Division, Ministry of Health, Uganda, 2006-Un published report).

In the present paper, further discrimination of molecular forms under the *A. gambiae sensu stricto* revealed the presence of *A. gambiae s.s.* Giles in the study area. Therefore, it is reported here, the presence of *A. gambiae s.s.*Giles in Kamuli district.

These findings are consistent with an earlier report that *A. gambiae s.s.* Giles (formerly Savannah (S) form) is the most common and widespread in sub-Saharan Africa. This finding is also consistent with the ecological requirements for the *A. gambiae s.s.* Giles. This form is highly diverse and breeds in a wide variety of small, rain dependent habitats (Cuamba et al., 2006; Coetzee et al., 2013) including hoof prints and rice paddies. Such habitats are very predominant in the study area and most parts of Uganda, resulting from the increased economic activities for example cultivation for agricultural activities and constructions (MoH, Uganda, 2006). The *A. coluzzii* (formerly the mopti (M) form) is believed to breed in dry season and arid areas typical of the drier Northern savannah and Sahel zone of Senegal, many parts of West Africa and the Sudan (Toure et al., 1994).

The finding, however, may not completely rule out the possibility of co-existence of the two molecular forms (*A. gambiae s.s.* Giles and *A. coluzzii*), as was exceptionally reported in Kanyemba, the Zambezi valley, Zimbabwe (Masendu et al., 2004). Further analysis of more samples of *A. gambiae s.s.* mosquitoes sampled from different parts of Uganda need to be carried out to establish whether or not the *A. coluzzii* which is believed to be restricted to West Africa (Cuamba et al., 2006), contrary to other reports (Masendu et al., 2004), does exist. A fine population genetics analysis of *A. gambiae s.s.* samples using molecular markers may reveal presence of *A. coluzzii* in East Africa and suggest migration patterns (active or passive) of the mosquito populations (Samb et al., 2012; Coletta-Filho et al., 2011). This will enable mapping the distribution of the chromosomal form(s) of *A. gambiae s.s.* in the different ecological zones in the country.

The two molecular forms, *A. gambiae s.s.* Giles and *A. coluzzii* differ in ecological preference and their susceptibility to pyrethroids (Lehmann and Diabate, 2008), the preferred class of insecticide for treatment of long lasting bed nets (Harris et al., 2013). Therefore, findings of the study may provide evidence-based guidance in the planning and implementation of the most appropriate vector control interventions (Coetzee et al., 2000), and monitoring insecticide resistance (Masendu et al., 2004), all geared towards malaria control and possibly

elimination (Moiroux et al., 2013).

Conclusion and recommendations

This study identified the principal sibling species under *A. gambiae* complex and the molecular form under *A. gambiae sensu stricto* that occur in Kamuli district, Uganda. Since *A. gambiae s.s.* Giles (formerly, the savannah form) identified in the study area molecularly belongs to *A. gambiae* sensu *stricto,* known for its highly anthropophilic, endophagic and endophilic behaviour, the use of long lasting insecticide treated nets, indoor residual spraying may be the appropriate vector control strategies in this part of the country. Additionally, considering the abundance of *Anopheles* breeding sites, larval source management, where appropriate and environmental hygiene strategies, and improved house design to reduce the indoor and outdoor human biting densities may be promoted in this area in the context of integrated vector management strategy. There is also need for regular monitoring of the vector species composition, distribution and behavior for proper planning of appropriate vector control interventions in the future.

Conflict of interest

The authors declare that they have no conflict of interest.

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