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A first report of *Anopheles funestus* sibling species in western Kenya highlands

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Abstract

Understanding disease vector composition is of priority in designing effective disease control programs. In integrated vector control management, understanding of disease vector species among species complexes simplifies priorities for effective control tools selection. This study identified members of the *Anopheles funestus* complex sampled in western Kenya from 2002 to 2011 from different breeding sites. Larval sampling was carried out using the standard dipper (350ml) in larval habitats in western Kenya highlands from January 2002 to December 2012. The morphologically identified *Anopheles funestus* larvae were preserved in absolute ethanol for molecular identification using polymerase chain reaction (PCR). Among the 184 identified specimens of *Anopheles funestus* sampled, only 76 specimens were clearly identified after DNA amplification and PCR. Among these, 25 (32.9%) were *An. funestus s.s*, 22 (28.9%) *An. leesoni*, 9 (11.8%) *An. rivulorum* and 20 (26.3%) were *An. vaneedeni*. None was identified as *An. parensis*. This study has demonstrated the existence of the siblings species of *An. funestus* complex in western Kenya highlands. However, there is need for further studies to evaluate the dynamics of the adults and sporozoite infectivity rates throughout the region based on these findings.

1.0. Introduction

In sub Saharan Africa malaria transmission is potentially vectored by *Anopheles gambiae* s.s. Giles, *An. arabiensis* Patton (Coetzee, *et al.*, 2000) and *An. funestus* Giles (Gillies and

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Authors' contribution

EJK conceived and designed the study, carried out data analysis and results interpretation. EJK, LK and SM drafted the manuscript. LK, MCL, SM, AKG, GY and EJK revised the manuscript. All authors approved the final version of the manuscript before submission.

Competing interest

Authors declare to have no competing interest.

Coetzee, 1987). Members of each complex group are difficult to distinguish morphologically. Both *An. gambiae* and *An. funestus* complex groups have been found to be potential vectors of malaria parasites in western Kenya highlands (Zhou, *et al.*, 2004), with *An. gambiae* s.s. being the primary vector while *An.funestus* s.l is considered a secondary vector (Atieli, *et al.*, 2011, Kweka, *et al.*, 2011, Zhou, et al., 2004).

Anopheles funestus Giles complex consist of nine species that are distributed throughout Africa, these are *An. parensis* Gillies, *An. aruni* Sobti, *An. confusus* Evans and Leeson, *An. funestus, An. vaneedeni* Gillies and Coetzee, *An. rivulorum* Leeson, *An. fuscivenosus* Leeson, *An.leesoni* Evans, and *An. brucei* Service (Gillies and Coetzee, 1987). Apart from the morphological similarities among these sibling species their biology and vectorial competency is different. Except *An. funestus* s.s., other sibling species are zoophilic. *An. funestus* is closely associated with human dwellings hence it plays a critical role in malaria transmission. Both in Tanzania and Bukina Faso, *An. funestus* population have been found to increase at the end of the rainy season (Costantini, *et al.*, 1999, Dabire, *et al.*, 2007, Kweka, *et al.*, 2008) hence are suggested to extend malaria transmission during the dry season. *An. rivulorum* has been found in Kenya to be an important malaria vector (Kawada, *et al.*, 2012) and in Tanzania by Wilkes and others (Wilkes, *et al.*, 1996). *An. rivulorum* has been found to have close association with human feeding preference of 40% in Nigeria (Awolola, *et al.*, 2005) while in Kenya, incredibly higher biting and sporozoites rates in this species have been recorded (Kawada, et al., 2012). *An. vaneedeni* feeds readily on human outdoors but have been found to be infected with *P. falciparum* only under laboratory conditions and hence its efficiency in malaria transmission is questionable in natural settings (De Meillon, *et al.*, 1977). Kamau and others in Kenya demonstrated that *An. parensis* resting indoors in human houses had low human blood index and were not infected with circumsporozoite protein (Kamau, *et al.*, 2003, Kamau, *et al.*, 2003). Other studies conducted elsewhere in Africa have not found *An. parensis* infected with sporozoites (Awolola, et al., 2005, Hargreaves, *et al.*, 2000). In ecological studies in sub Saharan Africa, *An. funestus* occurs with other species and overlap with other three members of the group; *An. rivulorum; An. leesoni* and *An. parensis* (Awolola, et al., 2005, Kamau, et al., 2003, Temu, *et al.*, 2007). In designing a cost-effective vector control tool it is important to understand the available vector species composition, biology and insecticide resistance status (Coetzee, et al., 2000, Hunt, *et al.*, 2010, Hunt, *et al.*, 2011).

In most studies conducted in western Kenya, the identification of *An. funestus* has been done based on morphological features (Zhou, et al., 2004). However, the modern molecular identification technique for *An. funestus* sibling species developed by Koekemoer and others (Koekemoer, *et al.*, 2002) have made it possible for molecular differentiation of *An. funestus* complex members. This method identifies five species of *An. funestus* (*An. vaneeden, An. parensis, An. leesoni An. rivulorum* and *An. funestus*) which are most common with a minimum amount of DNA from any part and life stage of the mosquito. This reliable species identification method has increased the precision of effective control method selection and implementation for *An. funestus* sibling species. It has given better understanding of feeding, resting and host seeking behaviors which have given the best insight in vector control tool selection.

In order to clearly understand malaria vector ecology in western Kenya highlands, this study investigated the composition of *An. funestus* sibling species for larvae specimens collected from January, 2002 to December, 2011 to determine their species abundance.

2.0. Material and Method

2.1. Sampling and morphological identification

Mosquito larval specimens were collected from January 2002 to December, 2011 using standard 350 mL dipper (BioQuip Products, Inc. California, USA) from larval habitats. This study sites was conducted at Emakhanga, Iguhu, Emutete and Mbale (Figure 1). Four habitat types were considered during this study: drainage ditches, swamps, abandoned goldmines and hoof prints. All larvae were first identified morphologically as *An. funestus* complex using morphological keys by Gillies and Coetzee (Gillies and Coetzee, 1987) and preserved in absolute ethanol (98.7% purity) for species identification using polymerase chain reaction (PCR).

2.2. Molecular identification of sibling species of *An. funestus* complex

Mosquito specimens belonging to the *An. funestus* group were analysed by a multiplex PCR assay protocol developed by Koekemoer and others (Koekemoer, et al., 2002). In each case, DNA was extracted from whole larvae body, amplified and the PCR product of unknown/ known??? specimens together with positive controls (*An. funestus, An. rivulorum* and *An. leesoni*) were separated on a 2.5% agarose gel stained with ethidium bromide and visualized on a UV transilluminator.

3.0. Results

A total of 52,043 *An. funestus* larvae were sampled throughout the study duration. Among these; 31,812 (61.1%) were sampled from swamps, 12,319 (23.7%) in drainage ditches, 7912(15.2%) in abandoned goldmines and no *An.funestus* were sampled from hoof prints. Due to constraints of time and resources only 184 specimen were selected for PCR identification from the preserved specimens. These specimens were picked from different habitats singly during the study period. Among the 184 specimens of *Anopheles funestus* sibling species identified by polymerase chain, 76 had clear PCR results, 2 with unclear results and 106 had no DNA amplification. There are two plausible reasons for the PCR amplification failure in the 106 specimens. 1) Degradation of DNA in the larval samples occasioned by long storage period. 2) Poor storage since the samples which did not give the DNA amplification were those collected before 2008. The DNA might have degraded due to storage techniques not been good enough. Among the 76 specimens with clear DNA amplification and PCR results, 25 (32.9%) *An. funestus s.s*, 22 (28.9%) *An. leesoni*, 9(11.8%) *An. rivulorum* and 20 (26.3%) were *An. vaneedeni*. None was identified as *An. parensis* (Figure 2).

4.0. Discussion

This study has documented the presence of *An. funestus s.s, An. leesoni, An. rivulorum* and *An. vaneedeni* in western Kenya highlands. However, absence of *An. parensis* was noted. Among the specimens tested, 106 (56.7%) of the specimens identified morphologically as *An. funestus* could not be identified by PCR possibly due to poor preservation method and hence protein degradation.

In western Kenya highlands, malaria control programmes thus needs to consider the biology of all species of *Anopheles* vectors involved in malaria transmission. Most of the information available currently at this study site is on *An. gambiae* s.l. (Kweka, et al., 2011, Zhou, et al., 2004). Due to availability of molecular tools identification of *An. funestus* has been made easier for choice of effective control tool and implementation. The four species of *An. funestus* identified in the current study site have been reported at the coastal regions of Kenya and Tanzania (Kamau, et al., 2003, Temu, et al., 2007) and west Africa (Awolola,

et al., 2005, Coetzee and Fontenille, 2004) suggesting that, these species share the same ecological niche. The presence of these four species in aquatic stages revealed that, the adults of these species are available and might contribute to malaria transmission as it has been observed with *An. rivulorum* and *An. funestus* s.s. in western Kenya (Kawada, et al., 2012, Zhou, et al., 2004).

Among those four identified *An. funestus* sibling species, *An. rivulorum* has been shown to have malaria parasites circumsporozoite protein in lowland areas of western Kenya (Kawada, et al., 2012). With the current findings, there is need to further investigate and ascertain the biology of each species in western Kenya and any relevant active role in malaria transmission. These results should be taken as a basis for further studies to enhance the design of effective control programme of malaria vectors in this malaria epidemic prone area.

5.0. Conclusion

The findings of this study indicate that malaria vectors other than *An. gambiae* s.l. are found in the study area. Because of the presence of *An. funestus* s.s. and *An. rivulorum* the role played by each of these species in malaria transmission should be investigated further in western Kenya highlands.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *Anopheles funestus* sibling species characterization was conducted for specimen from western
- The present members were *An. funestus s.s, An. leesoni, An. rivulorum* and *An. vaneedeni*.
- *An. funestus* s.s (32.9%) was the dominant member among others in identified samples.

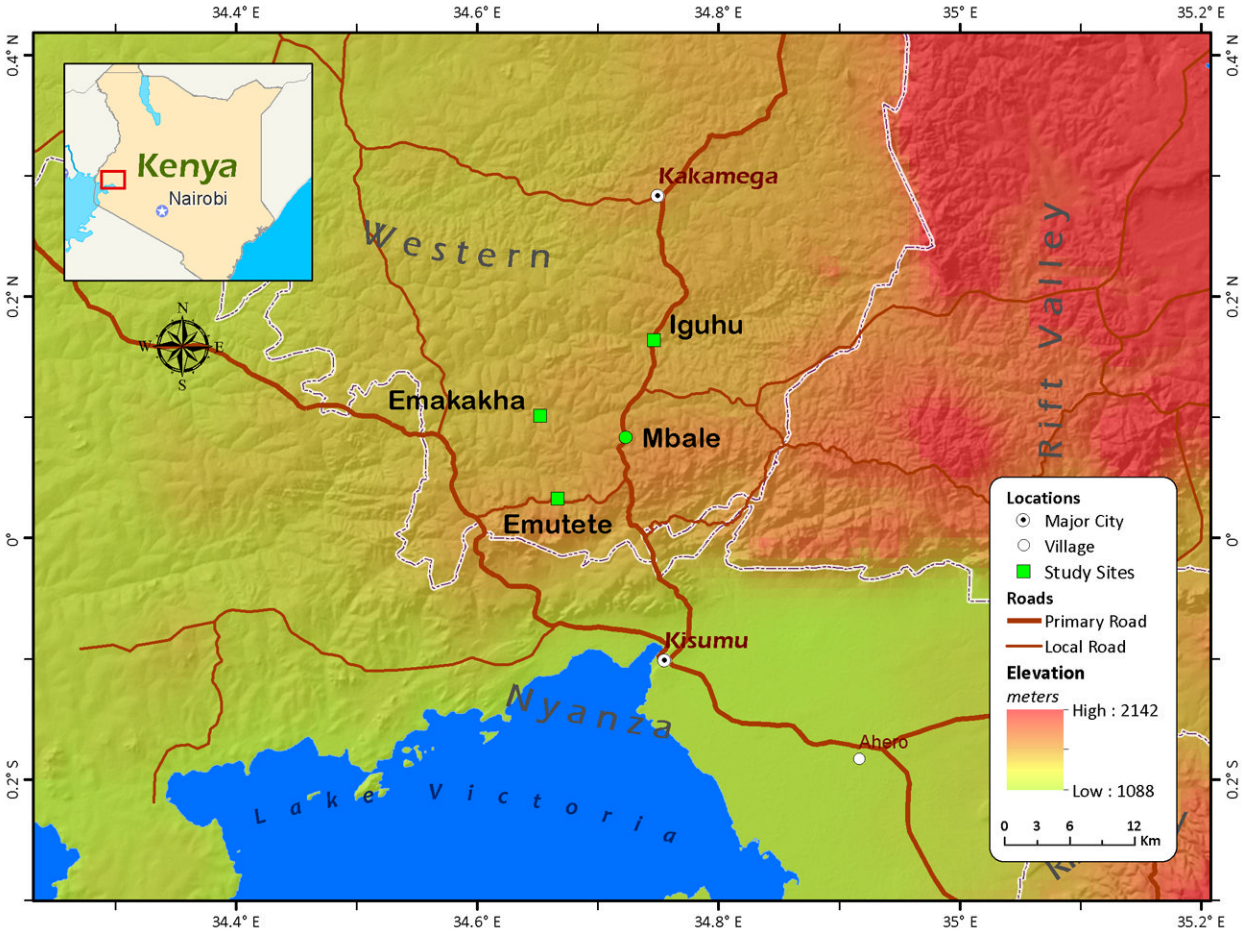

Figure 1.
A map showing study sites in western Kenya highlands

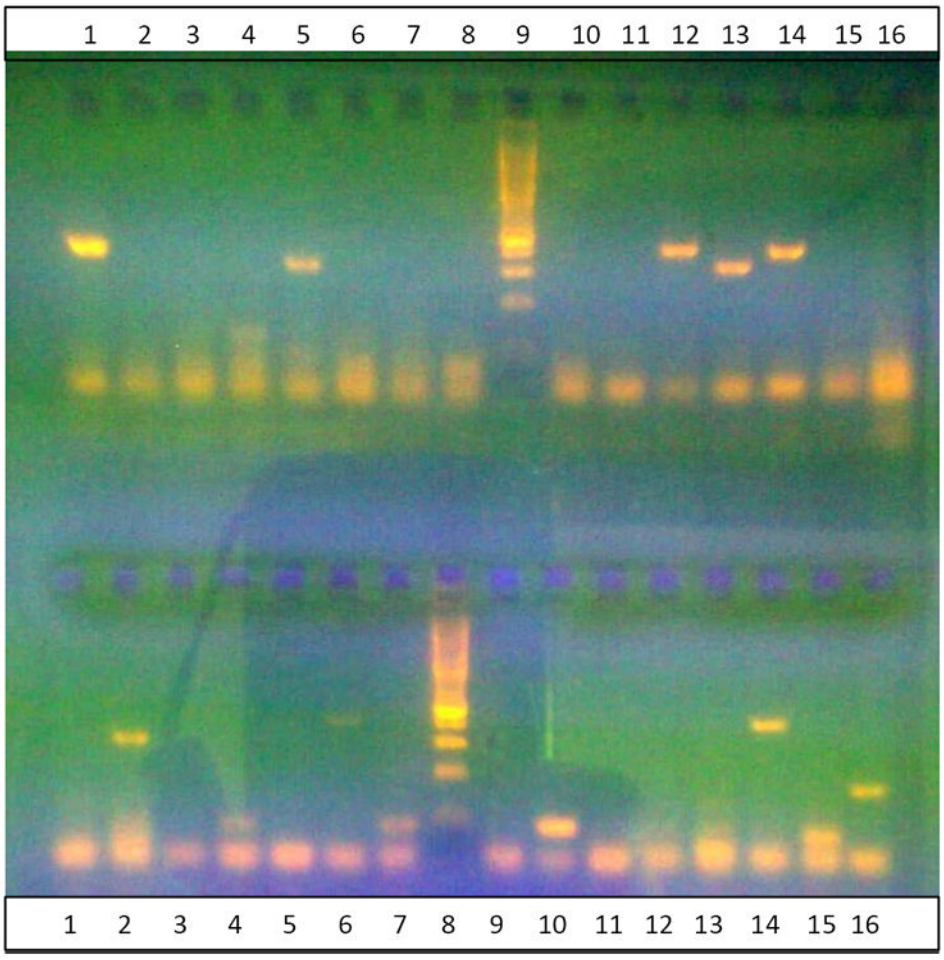

Figure 2.

The PCR results for identified sibling species of *Anopheles funestus* results

Gel showing amplified fragments using the species-specific PCR method of Koekemoer et al. 2002. The DNA size ladder is in Lane 9 in the top row and Lane 8 in the bottom row; the bright band is 600bp. Top row; *An. vaneedeni* in Lane 1: *An. Rivulorum* in Lanes 5 & 13: *An. funestus* in Lanes 12 & 14. Bottom row; *An. rivulorum* in Lane 2; *An. leesoni* in Lanes 4, 7, 10,15&16; *An. funestus* in Lane 14.