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Feeding and indoor resting behaviour of the mosquito *Anopheles longipalpis* in an area of hyperendemic malaria transmission in southern Zambia

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Abstract

Anopheles longipalpis (Theobald) (Diptera: Culicidae) is a predominantly zoophilic mosquito that has not been implicated in malaria transmission. However, this species was collected indoors with *An. funestus* s.l. in southern Zambia, where transmission of *Plasmodium falciparum* is hyperendemic, and we initially misidentified it morphologically and molecularly as *An. funestus* s.l. The indoor resting density and blood-feeding behaviour of *An. longipalpis* were investigated during the 2004 – 05 and 2005 – 06 transmission seasons in Mufwafwi village in southern Zambia. Numbers of endophilic *An. longipalpis* increased towards the end of the rainy season. Although specimens were collected during human landing catches, the feeding behaviour of *An. longipalpis* was significantly biased towards cattle (88.7%), with other bloodmeals originating from dogs, goats and chickens. None of the 177 specimens of *An. longipalpis* were infected with *P. falciparum*. These data are consistent with existing reports that *An. longipalpis* is not involved in malaria transmission. However, more extensive sampling is necessary. Importantly, the correct identification of *An. longipalpis* is crucial for malaria control programmes in areas where *An. funestus* s.l. and *An. longipalpis* exist sympatrically so that scarce resources are not wasted on the control of a non-vector.

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Keywords

Anopheles funestus; *Anopheles longipalpis*; endophily; exophagy; foraging ratio; zoophily; Zambia

Introduction

Anopheles longipalpis (Theobald) is a predominantly zoophilic mosquito with a known range that extends along the eastern half of the African continent from Ethiopia to South Africa (Gillies & De Meillon, 1968; White, 1972). Although this species has not been implicated in malaria transmission, it shares morphological, behavioural and geographical similarities with one of the primary vectors of *Plasmodium falciparum* in Africa, *Anopheles funestus* Giles.

Morphologically, adult *An. longipalpis* is identified by maxillary palps approximately three to four times longer than the head, and pale basal and apical bands spanning the hind tarsal joints (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). However, this species can be mistaken for *An. funestus* due to morphological similarities in wing venation, palpal banding patterns and small body size. Additionally, tattered or missing legs on many field specimens make identification difficult or impossible. The morphological confusion between larval and male specimens of *An. longipalpis* and *An. funestus* s.l. has also been noted in the literature (Ingram & De Meillon, 1927).

Bloodmeal analyses of *An. longipalpis* from indoor and outdoor collections have demonstrated anthropophagic encounters in this species. Ingram & De Meillon (1927) and Symes (1931) documented *An. longipalpis* resting inside human sleeping huts and tents. Smith (1955) reported that three of seven *An. longipalpis* collected indoors on Ukara Island in Lake Victoria contained human bloodmeals, and one of 31 specimens collected outdoors was positive for human blood. Smith (1961) later documented *An. longipalpis* biting humans outdoors in Tanzania, which was corroborated by the finding that the bloodmeals in eight of 11 *An. longipalpis* collected in Ethiopia originated from humans (Adugna & Petros, 1996). Given these anthropophilic encounters, it is plausible that *An. longipalpis* may be a secondary vector of *P. falciparum* in areas of sufficiently high endemicity and densities of this species. *Anopheles rivulorum* Leeson, a zoophilic member of the *An. funestus* complex, has been incriminated in malaria transmission in Tanzania (Wilkes *et al.*, 1996).

The Southern Province of Zambia experiences hyperendemic transmission of *P. falciparum* by both *An. arabiensis* Patton and *An. funestus* s.s. (Larkin & Thuma, 1991; Biemba *et al.*, 2000; National Malaria Control Centre, unpublished data). In Mufwafwi village, approximately 30 km north-west of Choma, *An. longipalpis* were observed in sympatry with *An. arabiensis* and *An. funestus* during preliminary mosquito sampling efforts carried out during March and May 2004 (unpublished data). Initially, samples of *An. longipalpis* were identified as *An. funestus* based on morphology and the fact that they were collected in an engorged state from inside houses where humans slept. This led us to further investigate the possibility that *An. longipalpis* was a vector of *P. falciparum* in Mufwafwi village. Here we demonstrate how *An. longipalpis* can be misidentified molecularly as a hybrid member of

the *An. funestus* species complex, and present supporting data on the feeding and resting behaviour of this species in southern Zambia.

Materials and methods

Mosquito collection and handling

Blood-engorged specimens of *An. longipalpis*, *An. arabiensis*, and *An. funestus* s.l. were collected by pyrethrum spray catch, human landing catch and aspiration in Mufwafwi village in the Macha region of the Southern Province of Zambia (16°46'85" S, 26°94'09" E). Pyrethrum spray catches were executed with locally purchased DOOM Super[®] (Adcock Ingram Ltd, Bryanston, South Africa) combination synthetic pyrethroid (D-phenothrin 0.92 g/kg, prallethrin 0.4 g/kg, and imiprothrin 0.25 g/kg), and were used to calculate the average number *An. longipalpis* per house. Pyrethrum spray catches were performed in 15 representative houses in Mufwafwi village in the early (November 2004, 2005), middle (January 2005, 2006) and late (March 2005, 2006) rainy season as part of a larger, regional entomological survey of malaria vectors. The 15 houses selected varied throughout the course of the season. To address whether or not *An. longipalpis* attempted to feed on humans indoors, human landing catches were performed in April 2005 in two houses where the highest densities of *An. longipalpis* had been repeatedly observed during the 2004 – 05 season. Pyrethrum spray catches were completed the following morning in these two houses to collect any additional resting mosquitoes. All specimens were individually placed in 1.5-mL tubes containing silica gel desiccant and cotton immediately following collection and stored at ambient temperature. Mosquitoes were morphologically identified according to Gillies & De Meillon (1968) and Gillies & Coetzee (1987).

Foraging ratios

Numbers of people, cattle, goats, dogs, cats and pigs per household were obtained from each family to determine mosquito feeding preferences relative to the local availability of different mammal species (Hess *et al.*, 1968). The proportion of all surveyed potential hosts represented by each mammal was calculated to obtain the percentage of bloodmeals expected to be taken from each species based on relative host availability. The significance of differences between observed and expected bloodmeals from engorged *An. longipalpis* was analysed using chi-square tests. The one mixed cow + dog bloodmeal was treated as two separate bloodmeals in the calculations.

DNA preparation

Prior to homogenization, dry field specimens were rehydrated at room temperature in 20 µL dH₂O for 20 min. DNA was extracted using a modified salt procedure as described previously (Kent & Norris, 2005). The relative quality of all DNA extractions was evaluated by polymerase chain reaction (PCR) amplification of a fragment of the mitochondrial NADPH (nicotinamide adenine dinucleotide phosphate reduced) dehydrogenase subunit 4 (ND4) using arthropod-specific primers (Simon *et al.*, 1994; Kent & Norris, 2005). Abdomens were extracted separately from the head/thorax of each mosquito to maximize the concentration of mammalian DNA in extractions of engorged abdomens and to ensure

any parasite DNA amplified from mosquito head/thorax originated from a disseminated infection.

Polymerase chain reaction

All specimens were molecularly confirmed to be *An. longipalpis* or *An. funestus s.l.* by PCR (Koekemoer *et al.*, 2002). Positive control specimens were obtained from the following locations: *An. funestus s.s.* : Vector Control Reference Unit, National Institute for Communicable Diseases, Johannesburg, South Africa; *An. parensis* Gillies: KwaZulu Natal, South Africa; *An. vaneedeni* Gillies and Coetzee: Tzaneen, Limpopo Province, South Africa; *An. lesoni* Evans: Macha, Zambia. Bloodmeals of all engorged *An. longipalpis* collected by pyrethrum spray catch and aspiration were identified by PCR (Kent & Norris, 2005) using 2 (μL of the abdomen DNA extraction as a template. Bloodmeals unidentifiable by this method were tested with avian- and mammal-specific primer sets reported by Ngo & Kramer (2003) to determine whether any of these bloodmeals originated from birds or mammals not included in the Kent & Norris (2005) PCR diagnostic. A total of 37 DNA extractions from whole specimens collected during preliminary sampling in Mufwafwi village in March 2004 plus 140 head/thorax DNA extractions from the May 2004 – March 2006 collections were screened for *P. falciparum* by a *Plasmodium*-specific nesting (Snounou *et al.*, 1993). All DNA amplifications were completed on an MJ Research® PTC-200 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.) and visualized on ethidium bromide-stained 2% agarose gels. All gels were run with a GeneRuler 100-bp DNA ladder (Fermentas Life Science, Hanover, MD, U.S.A.).

Results

Species identification

We observed blood-engorged *An. longipalpis* resting indoors in a village in southern Zambia, where transmission of *P. falciparum* is hyperendemic. In this study, *An. longipalpis* was initially identified both morphologically and molecularly as a member of the *An. funestus* species complex, which might have led to confusion over the identification and relative abundance of malaria vectors vs. zoophilic anophelines in this region. Both the 587-bp *An. vaneedeni* -specific product and the 252-bp *An. parensis* -specific product were amplified from *An. longipalpis* using the Koekemoer *et al.* (2002) PCR diagnostic. The results indicated that the *An. longipalpis* samples appeared to be a species hybrid between *An. vaneedeni* and *An. parensis* (Fig.1). This misidentification is presumably due to sequence similarity between *An. longipalpis* and the *An. funestus* complex in the rDNA internal transcribed spacer region 2. The *An. vaneedeni* and *An. parensis* diagnostic fragments were specifically amplified from *An. longipalpis* when the annealing temperature was raised from the suggested temperature of 45 °C to 55 °C, and when starting template concentrations were diluted 100- to 1000-fold from 380 ng (mean for 1 (μL of a genomic DNA extraction). Amplification of both fragments at this wide range of template concentrations and more stringent annealing temperatures demonstrated that primer annealing is sensitive and specific to this non-target species and thus cannot be remedied by simply altering PCR conditions. However, the Koekemoer *et al.* (2002) PCR diagnostic may be used to confirm the molecular identity of *An. longipalpis* from collections as long as the

results do not lead to the misinterpreting of *An. longipalpis* as a species hybrid between *An. vaneedeni* and *An. parensis*.

Seasonal indoor resting densities

Eighty-two *An. longipalpis* were collected by pyrethrum spray catch during preliminary sampling in March and May 2004. An additional 14 *An. longipalpis* were collected by spray catch during the 2004 – 05 season and 13 during the 2005 – 06 season. Supplementary aspiration and human landing catch collections during the study period increased the total sample size to 177 *An. longipalpis*. Mosquito densities were low throughout 2005 and 2006 due to severe drought conditions throughout southern Zambia. Still, indoor resting densities of *An. longipalpis* increased towards the end of the rainy season in Mufwafwi village for both the 2004–05 and 2005–06 transmission seasons. Densities increased from zero in November 2004 to an average of 0.1 *An. longipalpis* per house in January 2005, to 1.0 per house in March 2005. During the 2005–06 season, the indoor resting density of *An. longipalpis* increased from zero in November 2005 to an average of 0.06 per house in January 2006 and 0.70 per house in March 2006. In Mufwafwi village during preliminary spray catch sampling in March and May 2004 there was an average of three and two *An. longipalpis* per house, respectively. Indoor resting behaviour has also been reported for *An. parensis* and *An. lesoni*, both zoophilic members of the *An. funestus* complex (Kamau *et al.*, 2003; Kent & Norris, 2005). Therefore, it is crucial for malaria research and control programmes located in areas where *An. funestus s.l* and *An. longipalpis* exist sympatrically to be aware of the potential for these zoophilic species to be collected indoors together with *An. funestus s.s.*

Blood-feeding behaviour

A total of 244 domestic animals and 87 people were enumerated in Mufwafwi village. Each household comprised on average 13 people, 4.5 cows, 7.4 goats, 1.9 dogs, 0 pigs and 0.6 cats. The number of bloodmeals observed and expected to originate from these hosts is given in Table 1. Of 70 *An. longipalpis* bloodmeals analysed, 53 (74%) were successfully identified with the Kent & Norris (2005) PCR diagnostic. Mosquitoes imbibed a significantly greater proportion of bloodmeals than expected from cattle and fewer bloodmeals than expected from goats and humans ($\chi^2 = 131.43$, d.f. = 5, $P < 0.001$). Cattle comprised only 23% of the potential hosts but accounted for 88.7% of identifiable bloodmeals. Of the remainder, 3.8% fed on goats and 7.6% fed on dogs. None of the identified bloodmeals were human. Three of the 17 unidentified bloodmeals were classified as avian using avian-specific primers (Ngo & Kramer, 2003). The remaining 14 abdomens contained too little vertebrate DNA for the host to be positively identified.

Consideration of foraging ratios added perspective to the bloodmeal identification results by demonstrating that the distribution of bloodmeals observed among different hosts did not always reflect host availability (i.e. dogs), but rather a specific host preference (i.e. cattle).

Although none of the bloodmeals from indoor-resting *An. longipalpis* were human, human landing catches performed in April 2005 confirmed previous reports that *An. longipalpis* will attempt to bite people indoors (Ingram & De Meillon, 1928; Smith, 1955; Adugna &

Petros, 1996). Two houses were selected for human landing catches. In the first house, four *An. longipalpis* were collected from human baits between 20.00 hours and 24.00 hours, and an additional two specimens between 03.00 hours and 06.00 hours. Spray catches the following morning produced two additional *An. longipalpis*: one gravid and one that had fed on cow blood. In the second house, eight *An. longipalpis* were collected by spray catch the morning following the human landing catches, including one gravid female, six that had fed on cattle and one that had a mixed bloodmeal (cow + goat). Each of these specimens was morphologically identified and PCR-confirmed as *An. longipalpis*.

Plasmodium falciparum was not detected in any of the 177 *An. longipalpis* collected from Mufwafwi village between March 2004 and March 2006. From these data we were unable to conclude that *An. longipalpis* plays a role in malaria transmission; more extensive sampling is necessary. Severe drought conditions during 2004 – 05 resulted in small mosquito collections throughout the entire study period. Numbers of *An. arabi-ensis* and *An. funestus* s.s. were also nominal and did not fully rebound by the conclusion of the second year, despite plentiful rains during 2005 – 06. Therefore, the chance of detecting sporozoite-positive *An. longipalpis* in so few samples was exceptionally slim, and the true vectorial potential of this species could not be evaluated adequately. The confirmed attraction of *An. longipalpis* to bite humans indoors leaves open the possibility that this species could become incidentally infected with *P. falciparum* in hyperendemic areas. The strong feeding bias towards domestic mammals would weaken its vectorial capacity and potential contribution to malaria transmission, although nothing is known about the other factors that affect vectorial capacity for this species, such as longevity. However, the observations presented here illustrate the importance of obtaining accurate and reliable species identifications. Failure to confirm the identity of morphologically similar species could result in the overestimation of the abundance of true malaria vectors, misinterpretation of which species are actually present in a study/control area, and misdirection of resources towards the control of a zoophilic species.

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References

- Aduana N, Petros B. Determination of the human blood index of some anopheline mosquitoes by using ELISA. *Ethiopian Medical Journal*. 1996; 34:1–10. [PubMed: 8674495]
- Biemba G, Dolmans D, Thuma PE, Weiss G, Gordeuk VR. Severe anaemia in Zambian children with *Plasmodium falciparum* malaria. *Tropical Medicine and International Health*. 2000; 5:9–16. [PubMed: 10672200]
- Gillies, MT.; Coetzee, M. A Supplement to the Anophelinae of Africa South of the Sahara. South African Institute for Medical Research; Johannesburg: 1987. p. 1-143.

- Gillies, MT.; De Meillon, B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). 2nd. South African Institute for Medical Research; Johannesburg: 1968. p. 1-343.
- Hess AD, Hayes RO, Tempelis CH. The use of forage ratio technique in mosquito host preference studies. *Mosquito News*. 1968; 28:386–389.
- Ingram, A.; De Meillon, B. A Mosquito Survey of Certain Parts of South Africa, with Special Reference to the Carriers of Malaria and their Control, Part I. South African Institute for Medical Research; Johannesburg: 1927. p. 1-81.
- Ingram, A.; De Meillon, B. A Mosquito Survey of Certain Parts of South Africa, with Special Reference to the Carriers of Malaria and their Control, Part II. South African Institute for Medical Research; Johannesburg: 1928. p. 83-170.
- Kamau L, Koekemoer LL, Hunt RH, Coetzee M. *Anopheles parensis*: the main member of the *Anopheles funestus* species group found resting inside human dwellings in Mwea area of central Kenya toward the end of the rainy season. *Journal of the American Mosquito Control Association*. 2003; 19:130–133. [PubMed: 12825663]
- Kent RJ, Norris DE. Identification of mammalian blood-meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. *American Journal of Tropical Medicine and Hygiene*. 2005; 73:336–342. [PubMed: 16103600]
- Koekemoer LL, Kamau L, Hunt RH, Coetzee M. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene*. 2002; 6:804–811. [PubMed: 12224596]
- Larkin GL, Thuma PE. Congenital malaria in a hyperendemic area. *American Journal of Tropical Medicine and Hygiene*. 1991; 45:587–592. [PubMed: 1951868]
- Ngo KA, Kramer LD. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. *Journal of Medical Entomology*. 2003; 40:215–222. [PubMed: 12693851]
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*. 1994; 87:651–701.
- Smith A. The transmission of Bancroftian filariasis on Ukara island, Tanganyika. I. A geographical and ecological description of the island with an annotated list of mosquitoes and other arthropods of medical importance. *Bulletin of Entomological Research*. 1955; 46:419–436.
- Smith A. Observations on the man-biting habits of some mosquitoes in the South Pare area of Tanganyika. *East Africa Medical Journal*. 1961; 38:246.
- Snounou G, Viriyakoso S, Zhu XP, et al. High sensitivity of detection of human malaria parasites by use of nested polymerase chain reaction. *Molecular and Biochemical Parasitology*. 1993; 61:315–320. [PubMed: 8264734]
- Symes, CB. Descriptions of Fourth Stage Larvae of Certain Anophelines in East Africa, with Brief Notes on Breeding, Distribution and Economic Importance in Kenya. *Records of the Medical Research Laboratory*; Nairobi: 1931. p. 1-78.
- White GB. Confirmation that *Anopheles longipalpis* (Theobald) and *Anopheles confusus* Evans and Leeson occur in Ethiopia. *Mosquito Systematics*. 1972; 4:131–132.
- Wilkes TJ, Matola YG, Charlwood JD. *Anopheles rivulorum*, a vector of human malaria in Africa. *Medical and Veterinary Entomology*. 1996; 10:108–110. [PubMed: 8834753]

Fig. 1.

Ethidium bromide-stained agarose gel illustrating the misidentification of *An. longipalpis* as a hybrid between *An. vaneedeni* and *An. parensis* in the *An. funestus* complex diagnostic polymerase chain reaction (Koekemoer *et al.*, 2002). Lane 1: *An. funestus* s.s.; lane 2: *An. vaneedeni*; lane 3: *An. parensis*; lane 4: *An. lesoni*; lanes 5–9: *An. longipalpis*; lane 10: negative control. Outer lanes contain a 100-bp DNA ladder.

Table 1

Numbers of observed and expected mammalian bloodmeals based on relative host availability in Mufwafwi village. Bloodmeals were identified by polymerase chain reaction from engorged *An. longi-palpis* in Mufwafwi village; $n = 53$.

Host	Number of observed bloodmeals	Number of expected bloodmeals	χ^2
Cow	47	12.19	99.40
Goat	2	20.14	16.34
Dog	4	5.30	0.32
Human	0	13.78	13.78
Pig	0	0.00	0.00