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HpaII endonuclease distinguishes between two species in the *Anopheles funestus* group

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Abstract

The *Anopheles funestus* group consists of at least eight species that are currently identified mainly on morphological criteria. Until recently, only *An. funestus s.s.* was implicated in the transmission of malaria in Africa, but recent work in Tanzania has shown that *An. rivulorum* is also involved, albeit to a lesser degree than *An. funestus.* The constraints in the identification of the species and the need to clarify better their epidemiological role have led to the development of a PCR-RFLP method for the identification of two anthropophilic members of the group. Using PCR primers developed from the D3 region in the 28S ribosomal gene, amplified products were digested with the restriction endonuclease HpaII. This produced two distinct fragments on an agarose gel that could be used to separate *An. funestus* from *An. vaneedeni*. The technique needs to be tested on natural populations of these two species as well as on other members of the *An. funestus* group.

Keywords: *Anopheles funestus*, DNA identification, restriction enzyme endonuclease.

Introduction

Anopheles funestus Giles is widely distributed throughout tropical Africa and is one of the three major vectors of human malaria parasites on the continent. It belongs to a group of at least eight species that are difficult or impossible to distinguish morphologically in the adult stage (see Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). *Anopheles rivulorum* Leeson, also widespread, is the only other member of the group that has been implicated in malaria transmission, that being very recently in field-based studies in Tanzania (Wilkes *et al.,* 1996). *Anopheles vaneedeni* Gillies & Coetzee was experimentally infected with *Plasmodium falciparum* in the laboratory, but has never been implicated in transmission in nature, although it readily feeds on humans outdoors (De Meillon *et al.*, 1977). This species has been recorded only from South Africa. The rest of the *An. funestus* group are believed to be unimportant in the transmission of malaria parasites due mainly to their zoophilic feeding preferences, but because of the difficulty in identifying them morphologically, often confuse the picture (Gillies & De Meillon, 1968), much as did the *Anopheles gambiae* Giles complex before its resolution in the 1960s (Paterson, 1963; Coluzzi, 1984).

Morphologically, none of the members of the *An. funestus* group can be identified individually with absolute certainty on adult characteristics. Using immature characters, *An. rivulorum* and *An. confusus* Evans & Leeson can be identified on larval characteristics, whereas *An. leesoni* Evans can be identified on both egg and larval morphology (Gillies & De Meillon, 1968). Examination of these characters entails holding wild-caught blood-fed females for egg-laying and rearing of larvae to fourth instar. The latter is by no means simple, as larvae are difficult to maintain under standard insectary conditions. *Anopheles vaneedeni* and *An. parensis* Gillies are both identical to *An. funestus* in almost all respects. Where differences do occur, they are variable and necessitate rearing of family broods to achieve a reasonable probability of accuracy. The other members of the *An. funestus* group either do not occur in southern Africa or are extremely rare (Gillies & De Meillon, 1968).

Cytogenetic studies of the group have shown that *An. funestus, An. rivulorum, An. leesoni, An. parensis* and *An. confusus* each possess unique chromosome inversion rearrangements that can be used to identify them (Green, 1982). *Anopheles vaneedeni*, however, is homosequential, with *An. funestus* differing from it

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