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ANOPHELES CRYPTICUS, NEW SPECIES FROM SOUTH AFRICA IS DISTINGUISHED FROM *ANOPHELES COUSTANI* (DIPTERA: CULICIDAE)

MAUREEN COETZEE

Medical Entomology, Department of Tropical Diseases,
School of Pathology of the South African Institute for Medical Research
and the University of the Witwatersrand,
P.O. Box 1038, Johannesburg 2000, South Africa

ABSTRACT. The two genetic species currently called *Anopheles coustani* species A and B are named *Anopheles* (*Anopheles*) *coustani* Laveran and *Anopheles* (*Anopheles*) *crypticus* n. sp., respectively. A neotype for *An. coustani* is designated from chromosomally identified material from Madagascar, the original type locality. Polymorphic inversions and differences between *An. crypticus* and *An. coustani* are shown on a chromosomal map compiled from the neotype family.

INTRODUCTION

Species of the subgenus *Anopheles* in Africa have been shown to be of negligible importance in the transmission of human malaria parasites (Gillies and De Meillon 1968). However, *Anopheles coustani* Laveran, which is widespread and abundant over much of the continent, readily feeds on humans outdoors (Coetzee 1983) and may play a role in the transmission of disease pathogens.

Coetzee (1983) published evidence, based on polytene chromosomes and cross-mating characteristics, for the existence of two biological species within the taxon *Anopheles coustani* in southern Africa. While morphological differences were also described, neither Coetzee (1983) nor Gillies and Coetzee (1987) made the taxonomic decisions regarding naming of the species. This was due to the small sample size of species B and the lack of information about which species occurs at the type locality.

Anopheles coustani was described by Laveran (1900) from specimens sent to him by Dr. Coustan of Montpellier, collected by Dr. Rasamimanana in Madagascan swamps, exact locality unknown. Around the same time, De Grandpré and De Charmoy (1901 *nec* 1900) described *Anopheles mauritianus* from Mauritius and Réunion. Dye (1902) compared specimens from both collections and concluded that the two species were very similar, if not identical. Senevet (1932), after some considerable detective work, established that the name *An. coustani* had priority over *An. mauritianus* and synonymized the latter with the former after reexamining the specimens that Dye had examined. These decisions were based on adult female characters.

There has been some confusion in the literature over the type locality of *An. coustani*. Laveran (1900) clearly stated that the specimens were from Madagascar but did not stipulate the town or region. Dye (1902) and Christophers (1924), however, added the town of Tananarive (Antananarivo). Grjebine (1966), in his monograph on the anophelines of Madagascar, listed it simply as "Madagascar, unknown swamps." It is puzzling, therefore, that Evans (1938), two subsequent major taxonomic revisions by De Meillon (1947) and Gillies and De Meillon (1968), and two catalogs of the Culicidae by Knight and Stone (1977) and White (1980) recorded the type locality as "Réunion." The evidence suggests that an error occurred in Evans'

monograph and has been perpetuated. Curiously, the 1959 catalog of the mosquitoes of the world by Stone, Knight, and Starcke listed the type locality correctly as Madagascar, but this was changed in the later edition.

A collection of live mosquitoes was received recently from Madagascar and amongst them were specimens of "*An. coustani.*" These were compared with the descriptions of the morphology and chromosomes in Coetzee (1983). The present paper designates a neotype for *An. coustani* species A as the nominal species, provides a chromosomal map of the neotype family, and formally names species B as a new species.

MATERIALS AND METHODS

Specimens of *An. coustani* were collected biting humans at the forest station of Ampijoroa in the Ankarafantsika hills, 300 km northwest of Antananarivo, Madagascar (16° 15′ S, 46° 50′ E), on June 23, 1993. Egg batches were obtained from the wild females and progeny were reared in the laboratory. Adults correlated with immature pelts were kept for morphological study, and chromosome preparations were made from fourth-stage larvae (Coetzee 1983). Four families were obtained, and one (coded MAD 31) was chosen as the neotype family (15♀, 11♂, 24 larvae, 26 pupae). The photographs of chromosomes used for the map (Fig. 1) came from this family. Other families include MAD 39: 5♀, 6♂, 11 larvae, 11 pupae; MAD 46: 8♀, 2♂, 10 larvae, 12 pupae; and MAD 52: 4♀, 8♂, 12 larvae, 12 pupae. Additional chromosomally identified material of species A from South Africa also was collected: Transvaal, Onderstepoort, 10 larvae, 13 pupae, ♀ collected in a CO_2-baited net, March 10, 1991, R.H. Hunt.

Additional specimens of species B were received from Rondevlei, The Wilderness, Cape Province, collected inside houses, May 3, 1991, I. Russell. They were treated as above, and one chromosomally identified family was obtained (6♀, 5♂, 9 larvae, 16 pupae).

TAXONOMIC TREATMENT

Anopheles (*Anopheles*) *coustani* Laveran

1900. *Anopheles coustani* Laveran, 1900:109.
1901. *Anopheles mauritianus* De Granpré and De Charmoy, 1900 (1901):8.
1901. *Anopheles paludis* var. *similis* Theobald, 1901:129.

Adult. As described in Laveran (1900), Grjebine (1966), and Gillies and De Meillon (1968).

Pupa. Full setal counts for *An. coustani* from various localities were given in Coetzee and Newberry (1980). Coetzee (1983) separated species A and B using the sum of the branches of setae 6-I,II and 9-I. The range of branching on these pupal setae, not given in Coetzee (1983), was 1–2, 1–3, and 1–3, respectively. Coetzee (1983) recorded the sums of these branches ranging from 6 to 12 (n = 293) for species A and 13 or more for species B (n = 25). Examination of 61 pupae from Madagascar showed all 3 setae with 1–2 branches (except for 1 specimen, MAD 31.4, which had seta 6-I with 3 branches on the left-hand side and simple on the right, sum = 8 branches). The sums of these setae ranged from 6 to 8, with most specimens having a sum of 6 branches.

Larva. Full setal counts for *An. coustani* from various localities were given in Coetzee and Newberry (1980). Coetzee (1983) separated families of species A and B using the mean number of branches on seta 9-V per family, i.e., species A with a mean of 12.9 or less and species B with a mean of 13.0 or more. The range of branching, not given by Coetzee (1983), was 8–14 for species A. Examination of 40 Madagascan larvae showed this seta to have a range of 8–13 branches with an average of 9.9.

Egg. Gillies and De Meillon (1968) described and illustrated the egg of *An. coustani* without a continuous deck opening on the dorsal surface. Coetzee and Newberry (1980) noted that the eggs of *An. coustani* are variable with both open and closed decks seen in egg batches from single females. Scanning

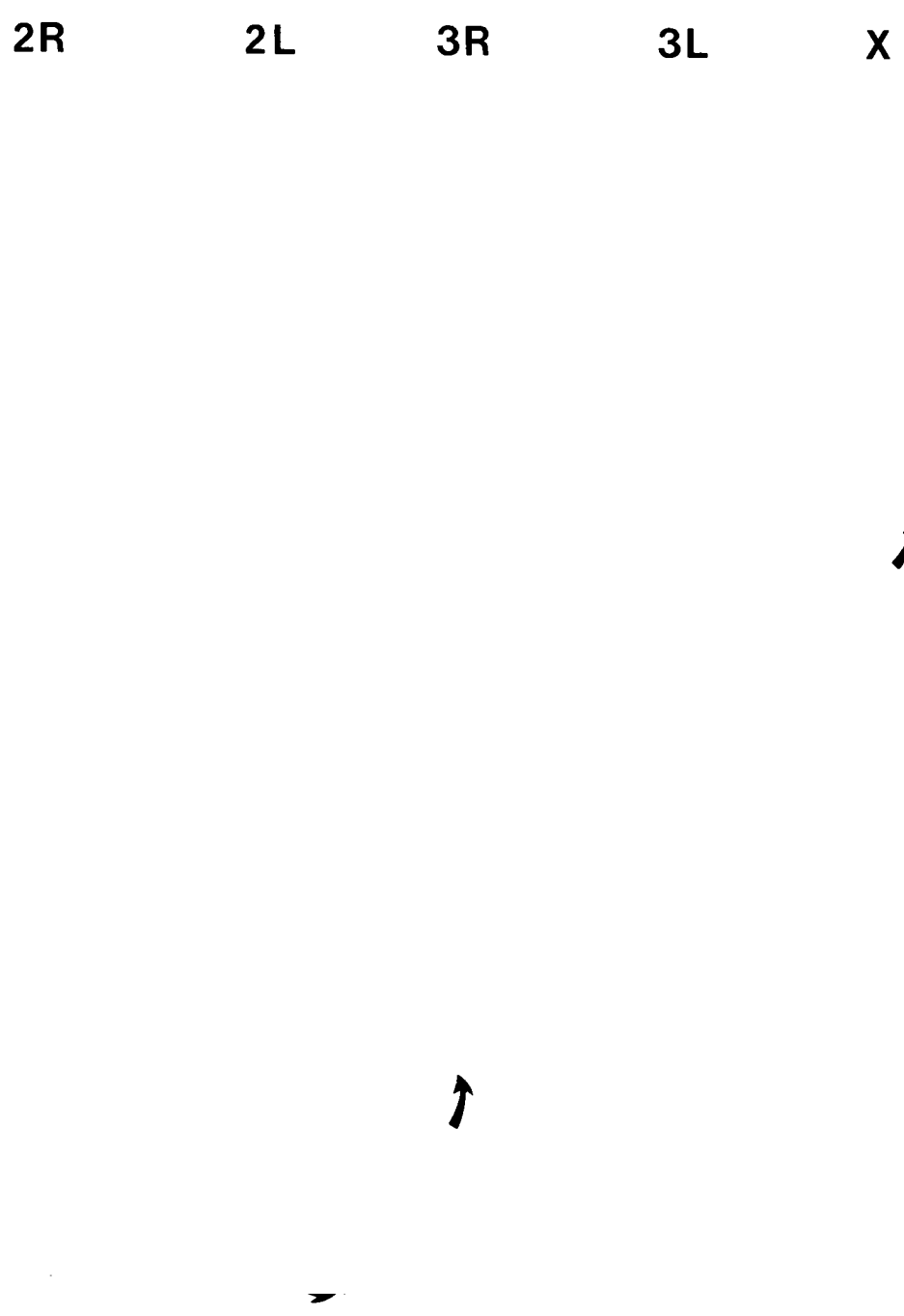

Fig. 1. Chromosomal map of the neotype family of *Anopheles coustani* Laveran. Inversions 2Ra, 3Rc, and 3Rd are polymorphic in *An. coustani* and 2La homozygous in South African populations. Xa is the fixed inversion difference between *An. coustani* and *An. crypticus,* while 3Rab is polymorphic in *An. crypticus.* The arrows indicate the centromeres.

electron studies of eggs of both species A and B showed no difference between the species (Coetzee 1983) with both having continuous openings along the decks (M. Coetzee, personal observation). Three of the 4 families from Madagascar had the egg ornamentation with continuous deck openings. The 4th family (MAD 52) had eggs that did not have a distinct, narrow deck opening. This opening tended to be wavy, sometimes approaching the edge of the floats, with the demarcation between the opening and the rest of the exochorion indistinct in part. No other morphological or chromosomal differences were found between this family and the other 3.

Chromosomes. Preparations of salivary gland polytene chromosomes from 4th-stage larvae produced chromosomes of varying quality. Figure 1 is a composite made from photographs taken of preparations from MAD 31. The chromosome arm nomenclature follows the arbitrary format of Frizzi and Holstein (1956). When homologies between the chromosome arms of *An. coustani* and other anopheline species have been established, perhaps by using *in situ* hybridization techniques (A. Cornel, personal communication), formal designations will be made. Most of the 2R and 3R arms were heterozygous for inversions close to the teleomeres (2Ra and 3Rd). Homozygotes of 3Rd were seen only in poor quality preparations; therefore the heterozygote is shown in Fig. 1. The inversion notations follow from Coetzee (1983). Inversion 2La appeared to be fixed, and 3Rc was polymorphic in South African populations of *An. coustani.*

Type material. *Neotype* ♀ (MAD 31.5) with associated larval and pupal pelts mounted on separate slide: MADAGASCAR. Ankarafantsika, Ampijoroa forest station, 23.vi.1993, S. Laventure. Deposited in the collections of the South African Institute for Medical Research (SAIM). The neotype is designated from amongst the progeny of a single female, the remainder of which are deposited as follows: 4♂, 6♀ (MAD 31.1–11) (SAIM); 4♂, 4♀ (MAD 31.12–16, MAD 31.20–22) (BMNH); 3♂, 4♀ (MAD 31.17–19, MAD 31.23–26) (USNM). The chromosomes of this family were used to produce the map in Fig. 1.

Remarks. In accordance with the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1985), it is considered that a special case exists here for the designation of a neotype for the following reasons.

The Catalog of Mosquitoes of the World (Knight and Stone 1977) records the holotype as being deposited in the Laboratoire de Parasitologie et Mycologie, Faculte de Medicine, University of Paris, France. On writing to this depository, I received a reply from Prof. F. Rodhain, head of the Unité d'Ecologie des Systèmes Vectoriels, Institut Pasteur, Paris, informing me that all entomological collections in the University of Paris had been transferred to his laboratory. He also informed me that he could find no trace of the type specimens of *An. coustani* and that they should be considered lost. This is in accord with Grjebine (1966), who states that the type specimen is "probably lost."

Furthermore, because the nominal species *An. coustani* has been shown to comprise more than one species based on genetical evidence of the polytene chromosomes and cross-mating studies, it is considered appropriate that a neotype be designated that is correlated with the inversion arrangements seen in specimens from Madagascar.

Because the original type specimens of *An. coustani* are lost, it is not possible to establish whether Christophers's (1924) designation of Tananarive was correct, and the original type locality therefore remains as unlocated swamps on Madagascar. However, under the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1985), the type locality becomes that where the neotype was collected.

Distribution. Examination of the material listed below showed that *An. coustani* is widespread, occurring in Africa, Madagascar, Mauritius, and Réunion, with all specimens except those from Fish Hoek in the Cape

Province, South Africa, having pupal setae 6-I,II and 9-I with 1–2 branches each and larval seta 9-V with 8–13 branches.

Material examined. In addition to the above chromosomally identified material and those specimens noted in Coetzee (1983), morphological characters of the larva and pupa of the following samples were examined: ABYSSINIA. Bahr Dar, 5 larvae, G. Covell, 7.xii.1952 (BMNH). CONGO. Exhopo, 1 larva, I. Vincke, 1941; Jadotville, 1 larva, 1 pupa, I. Vincke, 30.x.1941; Lufira, 1 larva, I. Vincke, 1941 (SAIM); Elizabethville, 2 pupae, 20.iii.1947; Mpoka, 1 pupa, P. Carnevale, 24.xi.1975 (BMNH). KENYA. Muheza, 4 larvae, 2 pupae, M.T. Gillies, v.1954 (BMNH). MAURITIUS. Gallets River, 1 larva, F. Starmuhlner, 1.v.1974; locality not mentioned, 6 larvae, M.E. McGregor, no date (BMNH). RÉUNION. Riv. St. Sutanne "Cascades de Niagara," 1 larva, F. Starmuhlner, 12.iv.1974 (BMNH). SOUTH AFRICA. *Cape Province*: Fishhoek, 2 larvae, 4 pupae, B. de Meillon, i.1934; *Natal*: Durban, 2 pupae, v.1927; *Transvaal*: Machai Pan, Kruger National Park, 15 larvae, 27 pupae, ♀♀ collected in CO_2-baited net, A. Cornel, 7.iii.1991; Tzaneen, 1 larva, 1 pupa, B. de Meillon, 6.vii.1932; Politsi, 2 larvae, 2 pupae, B. de Meillon, 8.iv.1932 (SAIM). UGANDA. Jinja, 1 larva, 2 pupae, E.G. Gibbins, 16.x.1930 (BMNH). ZIMBABWE. Ace of Spades, Harare, 2 larvae, 2 pupae, C. Meeser, 9.vi.1941; Brocks Stream, Bindura, 2 pupae, C. Meeser, 4.vi.1941 (SAIM).

Anopheles (*Anopheles*) *crypticus*, new species

Anopheles coustani sp. B of Coetzee 1983: 137.

Diagnosis. The X chromosome differs from *An. coustani* by a single fixed inversion, Xa (Fig. 1). Pupal setae 6-I,II and 9-I with the sum of the branches being 13 or more gives an estimated 86.2% accurate identification.

Adult. Indistinguishable from *An. coustani* (Coetzee 1983).

Pupa. Setae 6-I,II and 9-I with 1–5, 1–7, and 1–4 branches, respectively. The sum of the number of branches ranged from 7 to 29 (n = 29) with an average sum of 17.2. No other differences between *An. crypticus* and *An. coustani* were observed.

Larva. The only difference observed between *An. crypticus* and *An. coustani* was in the number of branches of abdominal seta 9-V. This character was not absolute, and overlap was observed. Coetzee (1983) gave a mean value per family of 12.9 and less for *An. coustani* (species A) and 13.0 and more for *An. crypticus* (species B). The range of branching, not given by Coetzee (1983), for *An. crypticus* from Johannesburg and Benoni was 12–17. The family recently obtained from the Cape Province, however, had a range of 11–14 branches and a mean value of 12.2 for 9 larvae, placing it well within the range of *An. coustani.*

Egg. Indistinguishable from *An. coustani.*

Chromosomes. Differ from *An. coustani* by fixed inversion Xa and polymorphic inversions 3Rab (Coetzee 1983).

Type material. *Holotype* ♀ (25.7c), SOUTH AFRICA. Transvaal, Johannesburg, Northcliff, 26° 05′ S, 28° 05′ E, 7.iii.1980, R.H. Hunt, collected inside a house (SAIM). *Paratypes,* same data as holotype, 2♂, 2♀ (25.4c, 25.6c with male genitalia mounted, 25.9c, 25.10c with male genitalia mounted) (SAIM); 2♂, 2♀ (25.8c, 25.11c, 25.12c, 25.13c) (BMNH); and 2♂, 2♀ (25.1c, 25.2c, 25.3c, 25.5c) (USNM). All type specimens are the progeny of a single female that was identified chromosomally.

Etymology. Named from the term "cryptic" which is used commonly in anopheline literature to indicate species that are virtually indistinguishable morphologically.

Distribution. Known only from South Africa, on the Transvaal highveld (Johannesburg and Benoni) and from the Cape Province. Six specimens collected by B. de Meillon at Fish Hoek in the Cape Province in 1934 showed 2 pupae having setae 6-I,II and 9-I with 2–3, 3–4, and 2 branches, respectively, identifying them as *An. crypticus.* The re-

maining 2 pupae had these setae with 1–2 branches and could be either species. The 2 larvae had seta 9-V with 13–14 branches, typical of *An. crypticus*.

DISCUSSION

Evans (1938:65) gave a detailed illustration of the abdomen of the larva of ". . . *A. coustani* Lav. type form." Seta 9-V in the drawing appears to have eight branches, which correlates with the larva of *An. coustani* as described by Coetzee (1983) and with *An. coustani* as designated here. Examination of museum collections has indicated that this form is widespread in Africa and the associated Indian Ocean islands. In deciding on a name for "species B," the current synonyms of *An. coustani* were considered. Unfortunately, both *An. mauritianus* and *An. paludis* var. *similis* were described only from adults. Furthermore, Theobald (1901) headed his description of the variety as "*A. paludis* var. *similis* = *A. mauritianus* de Grandpré and De Charmoy," indicating that he thought they were the same. Examination of available larval and pupal material from Mauritius and Zimbabwe (Mashonaland), the type localities of the above synonyms, showed typical *An. coustani* characters. It was decided, therefore, to describe "species B" as new.

Specific conditions are laid down in the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1985) for the designation of a neotype. I believe that these conditions have been met in full in the case of *An. coustani* and that exceptional circumstances exist to warrant the designation [Art. 75 (b) (ii)]. The present publication complies in all respects with the qualifying conditions laid down in Article 75 (d).

The chromosomes examined from Madagascan families of *An. coustani* and presented in Fig. 1 differ from South African *An. coustani* by a fixed inversion on arm 2L (2La) and a floating inversion on arm 3R (3Rd). Floating inversion 3Rc was found only in South African populations. Both populations share the floating inversion 2Ra and have homosequential X chromosomes and 3L arms. The differences between the two populations may prove to be species-specific markers, but only a study of sympatric populations will provide conclusive evidence for this. Examination of more material may show fixed inversion 2La to be polymorphic and floating inversions 3Rc and 3Rd distributed more evenly between the populations. Cross-mating studies would provide information on genetic compatibility. Until further evidence is presented to the contrary, South African and Madagascan populations of *An. coustani* are considered to be the same species.

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