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CYTOTAXONOMIC EVIDENCE FOR THE PRESENCE OF *ANOPHELES NIVIPES* IN INDIA

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ABSTRACT. *Anopheles philippinensis* mosquitoes were collected from 5 states in India: Assam, Meghalaya, Arunachal Pradesh, Manipur, and Nagaland. Half-gravid females were examined for variations in wing venation using the presector dark mark on vein I and polytene chromosomes derived from ovarian nurse cells. Polytene chromosomes were examined for diagnostic inversions, t on chromosome arm 2 and l on arm 5. Based on wing characteristics, both *An. philippinensis* and *An. nivipes* were identified. Polytene chromosome examinations revealed that all specimens from these 2 populations had 2t; 5l inversion genotype, a diagnostic character for *An. nivipes*. The wing character was not diagnostic; therefore, it was concluded that all the specimens examined were actually *An. nivipes* and not *An. philippinensis*. Further, the X chromosome was of $x+^{b}$ type, that is, the standard arrangement with reference to the inversion b, reported in the *An. nivipes* population in Thailand. This is the 1st report that unequivocally establishes the occurrence of *An. nivipes* in India and also shows that the adult wing character is not reliable in distinguishing *An. philippinensis* from *An. nivipes*, as has been observed in Thailand.

KEY WORDS Cytotaxonomy, *Anopheles nivipes*, India

INTRODUCTION

Anopheles philippinensis Ludlow and *Anopheles nivipes* (Theobald) belong to the *Annularis* group of mosquitoes in the Neocellia series. These 2 species are morphologically very similar and the only diagnostic character in adults is the presector dark mark on wing vein 1. In *An. philippinensis* this dark mark does not reach as far back as the distal end of the humeral dark mark on the costa, whereas in *An. nivipes* the presector dark mark either reaches or overlaps the humeral dark mark on the costa. Reid (1967) reported that this wing character is not equivocal and suggested that no overlap is found if morphologic characteristics of the paddle at the pupal stage are used. Klein et al. (1982) established laboratory colonies of *An. philippinensis* and *An. nivipes* from the progeny identified at the pupal stage. Subsequent studies revealed genetic incompatibility in the form of hybrid mortality at the egg, larval, and pupal stages and hybrid sterility in the surviving males among the F_1 progeny of the reciprocal crosses of *An. philippinensis* and *An. nivipes* (Klein et al. 1984). These observations confirmed the decision of Reid (1967) to raise *An. nivipes* from synonym to species level.

Green et al. (1985) examined polytene chromosomes of *An. nivipes* and *An. philippinensis* laboratory colonies established by Klein et al. (1982) and identified 2 autosomal inversions, t on chromosome arm 2 and l on arm 5, that could be used to distinguish these 2 species at the adult stage. Ovarian polytene chromosome photomaps of *An. philippinensis* and the breakpoints of these inversions are shown in Green et al. (1985).

Anopheles philippinensis has been reported from several places in India and has also been incriminated as a vector of malaria (Rao 1984). For the 1st time Nagpal and Sharma (1987) reported *An. nivipes* along with *An. philippiensis* from Assam and Meghalaya states in India based on the wing character. Therefore, we examined populations known as *An. philippinensis* from 5 northeastern states of India for wing characters and ovarian polytene chromosomes.

MATERIALS AND METHODS

Mosquito surveys and collections were carried out in 1987, 1988, and 1991 in districts Bhelapur (27°30′N, 92°94′E) of Arunachal Pradesh State, Kamrup (27°29′N, 94°58′E) of Assam State, Umbling (25°54′N, 91°56′E) of Meghalaya State, Imphal (24°44′N, 93°58′E) of Manipur State, and Dimapur (26°27′N, 94°95′E) of Nagaland State. Adult mosquitoes were collected resting in cattle sheds between 2000 and 2200 h. In the areas surveyed, *Anopheles annularis* van der Wulp is expected to be found. Because *An. annularis* cannot be distinguished from *An. philippinensis* by the naked eye, no efforts were made to sort them out immediately after collection. Ovaries were removed from half-gravid females and placed individually in vials containing modified Carnoy's fixative (1:3 glacial acetic acid : methanol) and the carcasses were kept in microfuge tubes and given corresponding numbers. Care was taken not to damage wings while removing the ovary. Each adult mosquito wing was examined under a dissection microscope. Morphologic characters were used to identify the species.

Anopheles annularis was also found in the collections and this species was distinguished from *An. nivipes* and *An. philippinensis* by examining wing vein 5, which is mainly dark with a dark spot at the point of bifurcation in *An. annularis*. The iden-

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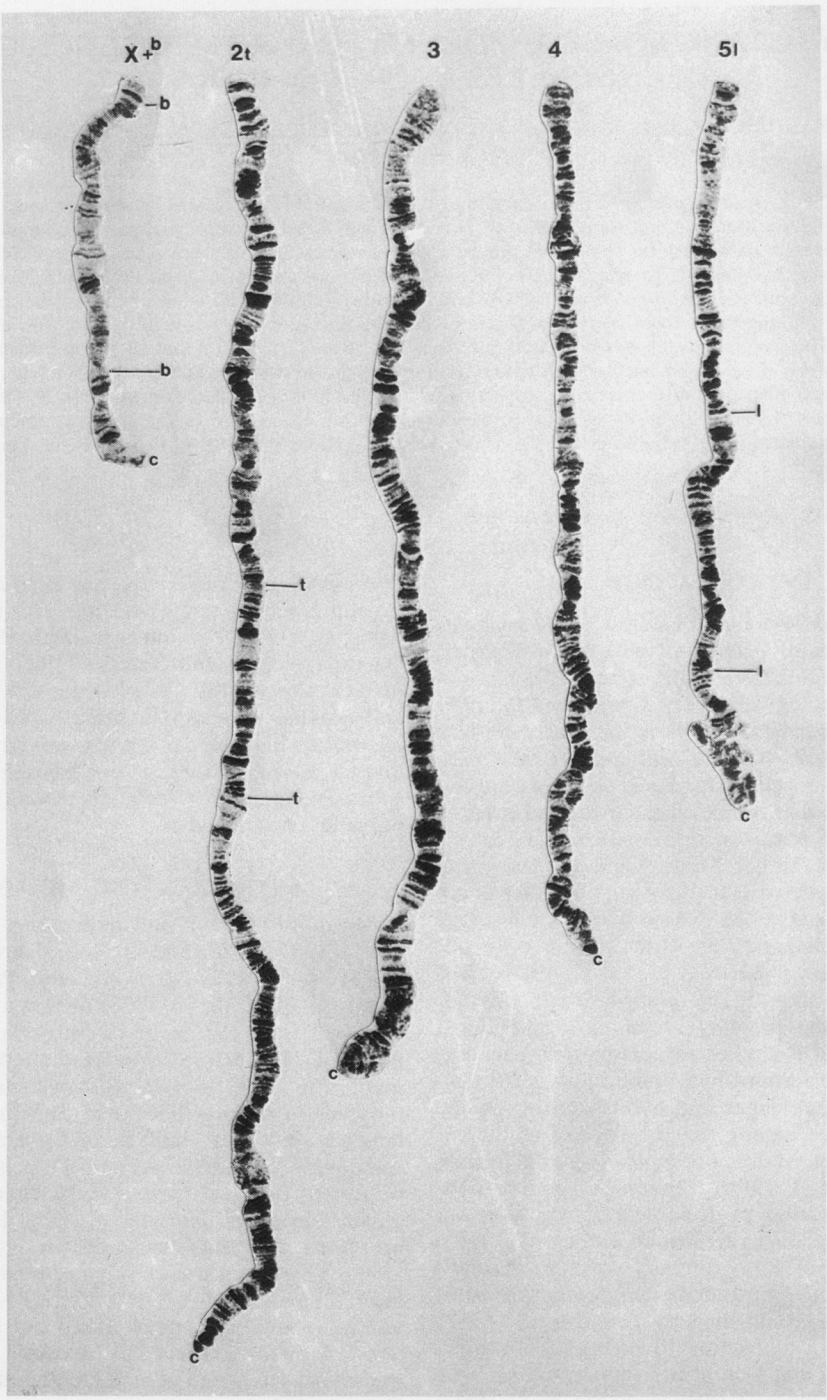
X +b
2t
3
4
5l
b
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t
t
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Table 1. Results of morphologic and cytotaxonomic identifications of *Anopheles philippinensis* and *Anopheles nivipes.*

Date of collection	State, district	Identification by wing character[1]		Cytotaxonomic identification 2t; 5l as *An. nivipes*[2]
		An. p.	*An. n.*	
March 1987	Assam, Kamrup	0	25	25
	Meghalaya, Umbling	0	1	1
November, 1987	Assam, Kamrup	40	24	57(7)
	Arunachal Pradesh, Bhelapur	14	0	8(6)
	Manipur, Imphal	2	4	7[3](1)
	Nagaland, Dimapur	1	1	1(1)
May 1988	Assam, Kamrup	21	97	121[3]

[1] *An. p., Anopheles philippiensis*; *An. n., Anopheles nivipes.*

[2] Number in parenthesis are those that could not be identified cytologically.

[3] A few specimens could be identified cytologically but could not be identified morphologically; hence the number is more than the morphologically identified specimens.

tification of these specimens was further confirmed as *An. annularis* by examining polytene chromosome preparations made from corresponding ovaries following the map of Green et al. (1985). *Anopheles annularis* specimens were removed from further analysis. Remaining specimens were differentiated to *An. philippiensis* and *An. nivipes* by the relationship between the presector dark mark on vein I and the humeral dark mark on the costa.

Chromosome plates were made from fixed ovaries following the procedure of Green and Hunt (1980). The diagnostic inversion genotypes used for the species identification were from Green et al. (1985): *An. philippinensis,* $2+^{t}$; $5+^{l}$ and *An. nivipes,* 2t; 5l.

The paddle of pupae from 1 isofemale line of the November 1987 collection and the June 6, 1991, collection from Assam State were examined for fringe teeth and the length of paddle hair. The adults from these isofemale lines were also examined for diagnostic wing character and inversions in ovarian polytene chromosomes.

RESULTS

Data on adult wings and ovarian polytene chromosomes of the specimens are given in Table 1. Following the relationship observed between the presector dark mark of vein I and the humeral dark mark on the costa, both *An. philippinensis* and *An. nivipes* were identified in all the collections (Table 1). The banding pattern of the X chromosome and of the chromosome arms 3 and 4 was similar to that reported for *An. philippinensis* (Green et al. 1985), whereas that of chromosome arms 2 and 5 differed by inversions t on 2 and l on 5. A photomap of polytene chromosomes of *An. nivipes* prepared from the ovarian nurse cells is shown in Fig. 1.

In the 7 isofemale lines examined from Assam, fringe teeth on the paddle of the pupae were prominent and >10 in number, occupying a large refractile border. The length of paddle hair was ¼ that of the paddle. In the wings of adult females from these isofemale lines, the presector dark mark was found either reaching or overlapping the humeral dark mark on the costa. Only 3 isofemale lines could be identified for inversion genotype and all were of 2t; 5l type.

DISCUSSION

Cytotaxonomic identification of all females examined from Assam, Arunachal Pradesh, Manipur, Meghalaya, and Nagaland states were of 2t; 5l inversion genotype, indicating that they were *An. nivipes.* These results do not correlate well with the wing character, which has indicated the prevalence of both *An. philippinensis* and *An. nivipes* in all the areas. We report that all the specimens we examined were actually *An. nivipes* and that no *An. philippinensis* were found among them. Green et al. (1985) further reported 2 allopatric populations among *An. nivipes* in Thailand. These populations had alternate arrangements with reference to b inversion on the X chromosome but because they were not found in sympatry, no taxonomic status was given. The populations we have examined were of the standard type, that is, $X+^{b}$, the type that was found in northern and southern Thailand.

Analysis of the results of the present study indicates that *An. philippinensis* and *An. nivipes* can be accurately identified at the adult stage by examining ovarian polytene chromosomes and at the

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Fig. 1. Photomap of the ovarian nurse cell polytene chromosomes of *Anopheles nivipes.* The break points for inversions b, t, and l on chromosome arms X, 2, and 5, respectively, are marked. C indicates the centromeric end of each chromosome arm.

pupal stage by examining the fringe teeth and the length of paddle hair, and that wing character cannot be relied upon for Indian populations, as has been pointed out by Reid (1967) for Thailand populations. Although only *An. nivipes* was observed in this study, we do not rule out the possibility of the presence of *An. philippinensis* in these areas. Cytotaxonomic examination of a larger sample representing different geographical areas is required to firmly establish the presence or absence of *An. philippinensis* in India.

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REFERENCES CITED

Green CA, Harrison BA, Klein T, Baimai V. 1985. Cladistic analysis of polytene chromosome rearrangements in anopheline mosquitoes of subgenus *Cellia,* series Neocellia. *Can J Genet Cytol* 27:123–133.

Green CA, Hunt RH. 1980. Interpretation of variation in ovarian polytene chromosomes of *Anopheles funestus* Giles, *A. parensis gillies* and *A. aruni*? *Genetica* 51: 187–195.

Klein TA, Harrison BA, Baimai V, Phunkitchar V. 1984. Hybridization evidence supporting separate species status for *Anopheles nivipes* and *Anopheles philippinensis. Mosq News* 44:466–470.

Klein TA, Harrison BA, Inlao I, Boonyakanist P. 1982. Colonization of Thailand strains of *Anopheles nivipes* and *Anopheles philippinensis. Mosq News* 42:374–380.

Nagpal BN, Sharma VP. 1987. Survey of mosquito fauna of northeastern region of India. *Indian J Malariol* 24: 143–149.

Rao TR. 1984. *The anophelines of India* Delhi, India: Malaria Research Centre (ICMR).

Reid JA. 1967. Two forms of *Anopheles philippinensis* in Malaya. *J Med Entomol* 4:175–179.