Morphotaxonomical Studies to Identify the Member of the *Anopheles Subpictus* Grassi (Diptera: Culicidae) Species Complex in Villages of District Mewat Haryana State, India

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Abstract

Anopheles subpictus complex is to include four sibling species provisionally designated as A, B, C and D. Species B is the only species restricted to costal salt water habitats, while species A, C and D generally breed in fresh waters. Present studies in District Mewat, Haryana have revealed the sympatricity of species A, C and D based on the reported distinct morphotaxonomical identification characters in different life stages of the mosquito .Studies on field collected adult mosquitoes and iso-female progeny did not show variation in the prevalence of sibling species and also showed a possibility of use of a single reported morphological character in a given life stage for the identification of the members of this complex.

KEYWORDS: *Anopheles subpictus*, sibling species, morphological character, identification.

Introduction

Anopheles subpictus Grassi is very widely distributed and the most abundant anopheline in this part of the subcontinent and is prevalent throughout except very high altitudes (Rao 1984) .*An. subpictus* was reported to be a complex of two sibling species(Suguna 1982, Reuben,and Suguna 1983), provisionally designated as species A and B breeding in fresh and salt water respectively. Species A was reported to be a non-vector (Das et al. 1979) and species B was reported with natural infection of malaria in Indonesia and Malaysia (Reid 1968) and in some coastal villages of Pondicherry in Southeast India (Panicker et al 1981). Later, two more species were reported in this complex (Suguna et al. 1994) based on morphological and cytotaxonomical description. Presently *An. subpictus* is reported to be a complex of four sibling species provisionally designated as species A, B, C and D (Suguna et al. 1994).The present study envisaged the results of the morphotaxonomic studies undertaken to establish the distribution of members of *An. subpictus* complex in villages of district Mewat, Haryana state Northern India.

MATERIALS AND METHOD

Adults and larvae of *An. subpictus* were collected from villages- Chilawali, Gundvas, Karmchandpur, Jaisinghpur and Ujina district Mewat. A total of six collections from different localities of each village were made in the months of August, September and October 2012 and 2013. In these villages *An.subpictus* breeding was found in borrow pits, ground pools in open land and paddy fields. Indoor resting adult female anophelines were collected from cattle sheds and human dwelling with the help of aspirator and

torchlight (Anonymous1975). Collected mosquitoes were brought to the laboratory in cloth cages covered with a wet towel. The field collected adult anopheles were identified to species based on morphological characters standard using identification key (Wattal and Kalra 1967). *An.subpictus* were separated in to unfed, full fed, semi gravid and gravid mosquitoes based on its abdominal condition (Anonymous1975) and were placed in separate cages. Semi gravid and gravid mosquitoes were separated and held individually in 100ml plastic bowls with little amount of water and nylon net fastened on the rim to collect single female progeny for the identification to species based on stage (eggs, larvae, pupae and adults) specific distinct morphotaxonomical characteristics (Suguna et al. 1994) .The remaining mosquito was identified to sibling species based on the distinct ornamentation of proboscis and palpi.

Morphotaxonomical studies:

These include observation of number of ridges on eggs and other larval, pupal and adult characters. For egg morphology, 20-35eggs were examined from iso-female progeny in each month. The number of ridges on eggs surface was counted under the microscope 25 late third or early fourth instar larvae and an equal number of pupae and adults were examined from each line in each month. Larval mesothoracic setae -4m, pupal setae 6,7and 9 of I^{st} abdominal segment and adult proboscis and palpi were examined for the distinct characters and ornamentation.

The stage specific morphotaxonomical characteristics used for the identification are as follows:

Number of ridge on egg surface (range): Species A- 35(31-36), Species B- 18(16-29), Specie C - 27(25-29) and Species D - 22(21-24).

Larval mesothoracic seta -4M: Species A-bifurcate and Species C and D- trifurcate (in C, the branches were joined closer than in Species D).

Pupal set a 7-I: Species A- simple and setae 6,7,9 were equal (in C, 7-I two branched and about half of 6-I and 9-I, species D, three branched and about half of 6-I and 9-I.

Adult palpal and proboscis is characters: Species A- apical band longer than sub apical dark band , species C and D- apical band and sub apical dark band equal (in C palpi was shorter than proboscis).

Statistical Analysis:

Per cent prevalence of sibling species as found from the identifications made of the populations derived from iso-female progeny and of the field collected female *An. subpictus* was subjected to one-way analysis of variance (ANOVA) to find statistical differences .The per cent prevalence was arcsine transformed for the analysis.

Results

During the year 2012 total of 56 iso-female progeny *An. Subpictus* were identified to sibling species in August and the per cent prevalence of species A, C and D was 32.1,

42.8 and 25 respectively. During the same period 126 female adult mosquitoes were identified to sibling species based on the distinct ornamentation on palpai and proboscis. The proportional prevalence of sibling species A, C and D from the field collected female adult populations were 32.5, 42.0 and 25.3. No significant difference was found in the distribution of sibling species in identification of these two populations ($p>0.05$).

During the month of September the per cent prevalence of the sibling species A,C and D identified from the iso-female progeny was 36.8, 36.8 and 26.3 respectively (n=38) and from the field collected female populations were 37.6,35.6 and 25.3 respectively (n=101) and October the percent prevalence of species A, C and D from the iso-female progeny were 30.4, 34.7 and 34.7 respectively (n=46) and from the field collected female were 30.5, 34.7 and 34.7 respectively (n=95). Similar to the observed distribution in August, September and October 2012 there was no significant difference in the distribution of sibling species in September and October 2012 between the two populations i.e. iso-female progeny and field collected populations($p>0.05$) .

During the year 2013 in August the percent prevalence of species A, C and D from iso-female progeny were 30.9, 43.6 and 25.4 respectively (n=55) and during the same period the field collected female were 30.9, 43.3 and 25.6 respectively (n=113). During the month of September the percent prevalence of species A, C and D from iso-female progeny were 37.5, 32.5 and 30.0 respectively (n=40) and the same period field collected female were 37.0, 33.0 and 30.0 respectively (n=100) and October the percent prevalence of species A, C and D from the iso-female progeny were 31.1, 33.3 and 35.5 respectively (n=45) and the same month from the field collected female were 30.1, 32.3 and 34.4 respectively (n=93).

Similar to the observed distribution in August, September and October 2012 there was no significant difference in the distribution of sibling species in August, September and October 2013 between two populations from iso-female progeny and from field collected populations ($p>0.05$). These results have unequivocally established the sympatricity of species A, C and D and no difference in the distribution of the species from identification of the mosquitoes derived from iso-female progeny and field populations of female *An. subpictus*.

Discussion

In the present study the distribution of the species was in accordance with the earlier reported distribution in with fresh water habitats (Suguna et al. 1994) where species A, C and D are reported to be prevalent in the Mewat district of Haryana state. The distribution pattern studies in two different years and month were more or less uniform. The observed morphological characters of larva (meso-thoracic seta-4M), pupa (seta 6, 7, 9-I) and female adult (palpai and proboscis) were in quite agreement with the characteristics mentioned in earlier identification (Suguna et al. 1994) except for some variations in number of egg ridge. In the present study, the mean ridge number in species A was 32(range -29-33), species C-27(25-28) and species D-23(22-26)and was in broad agreement of the earlier reported observations(Suguna et al 1994) .The reported mean ridge number of species A was 35(range 31-36),species C-27(25-39) and of species D-22(21-24) (Suguna et al 1994) . A study in Delhi indicated field populations of

An.subpctus to comprise of species A based on the cytotaxonomical examination but the egg ridge number was in the range of 21-30(Subbarao et al. 1988) while in species A described from different ecotypes of fresh water in states of Maharashtra and Pondicherry was in range of 32-39 (Suguna 1982) and 35(Suguna et al. 1994).

Reports of utility of morphological characters for the identification of members of sibling species complex already exist. Use of egg morphology variation was found to differentiate few or all the members of the species complexes namely. *An. maculipennis* Meigin complex (White 1977), *An.gamiae* (Colluzi 1964, White 1985) and *An.punctulatus* Donitz complex (Bryan 1974). Mesothoracic seta 4M was used to distinguish *An. subpictus* and *An. indefinetus* (Ludlow) from *An. sundaicus* (Reid 1966).There were reports on the utility of pupal characters to distinguish four species namely *An. subpictus, An. vagus, An. indefinetus* and *An. Sundaicus*(Reid 1966) and adult palpai characters to distinguish the members of *An. gambiae* complex(Colluzi 1964) .

Broadly the species were found to breed in two different breeding habitats namely species A, C and D in fresh waters and species B in salt waters i.e. in coastal areas (Suguna et al. 1994) and was implicated in the transmission of the human malaria (Reuben and Suguna 1983). However, there are a few reports on the involvement of fresh water species in the transmission of malaria(Kulkarni 1983) and experimental infection in laboratory in *An. subpictus* strain from District Ghaziabad, Utter Pradesh fed on *Plasmodium vivax* infected blood(Nanda et al 1987) .

The two populations derived from the iso-female progeny and the field collected female *An. subpictus* mosquitoes did not differ in the distribution pattern of the sibling species in different months during the two years of the study. Agreement of identification with different distinct morphological characters in different stages of the individual members picked randomly for the identifications from the populations further simplifies the technique of identification of the members of this species complex. Thus a single identification character in a given life stage of *An. subpictus* can be used for the identification of sibling species. More studies need to be carried out in other geographical areas to validate this technique for the identification of members of sibling species complex of *An. subpictus* and understand the role of the members in the transmission of malaria. No studies exist on the extent of influence of this non vector species (which are generally prevalent in high densities) in the transmission of malaria. Their role in the transmission cannot be completely negated specially in view of some reports on their potential to transmit malaria in certain specialized ecotypes (Amersainghe et al 1992).

Acknowledgements

Authors are thankful to M/s. V.P.Singh and Mangal Sen for the maintenance of mosquito colonies in the insectary and Mr. Rajender Singh for secretarial assistance in the preparation of the manuscript.

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Table 1: percent distribution of *An. subpictus* species A, C and D in different months in the years 2012-2013 in District Mewat, Haryana based on morphological character egg, larva, pupa and adult in isofemale line progeny and of the field collected female *An.subpictus* mosquitoes.

Period in months	**Iso-female progeny** Species complex			**Field collected female adult** Species complex			
	A (%)	**C (%)**	**D (%)**	**A (%)**	**C (%)**	**D (%)**	P value
August, 2012	18 (32.1)	24 (42.8)	14 (25.0)	41 (32.5)	53 (42.0)	32 (25.3)	0.05
September,2012	14 (36.6)	14 (36.8)	10 (26.3)	38 (37.6)	36 (35.6)	27 (26.7)	0.01
October,2012	14 (30.4)	16 (34.7)	16 (34.7)	29 (30.5)	33 (34.7)	33 (34.7)	0.001
August, 2013	17 (30.9)	24 (43.6)	14 (25.4)	35 (30.9)	49 (43.3)	29 (25.6)	0.06
September,2013	15 (37.5)	13 (32.5)	12 (30.0)	37 (37.0)	33 (33.0)	30 (30.0)	0.004
October,2013	14 (31.1)	15 (33.3)	16 (35.5)	28 (30.1)	30 (32.3)	32 (34.4)	0.001