Morphological Differences Between Sibling Species of the Taxon *Anopheles subpictus* Grassi in India, with Notes on Relationships with Known Forms

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ABSTRACT. Females of *An. subpictus* Grassi laying two distinctive types of eggs have been distinguished in coastal villages near Pondicherry, and cytological evidence was earlier presented for considering these to be sibling species. These are provisionally designated species A and B. Morphological differences between the species are described, and a method of distinguishing between adult females using the ratio between palpal segments 5/4 is proposed. Species A resembles the description of *An. subpictus* from India, while in many particulars species B resembles *An. subpictus* from Malaysia. Further work is required to determine with certainty the identity of these forms.

In 1966, Reid raised *Anopheles indefinitus* Ludlow, until then a variety of *An. subpictus* Grassi, to full species status, and predicted that "*subpictus*" might prove to contain yet more species, on the analogy of *An. gambiae*, another member of the Pyretophorus series of subgenus *Cellia*. *An. subpictus* females laying two distinctive types of eggs have recently been distinguished in coastal villages near Pondicherry, and the difference has been associated with the presence, in one of the types, of a small fixed paracentric inversion on the X chromosome. Evidence has been presented for considering these forms to be sibling species, temporarily designated *Anopheles subpictus* species A and species B (Suguna 1982).

This paper presents further morphological differences between the two species, some of which can be used to distinguish between them in the field, and attempts to establish their identity in relation to other oriental forms.

MATERIAL AND METHOD. The material for this study came from coastal localities from Pondicherry to Cuddalore (20 kms to the south) and from inland localities around the Sathanur Dam in N. Arcot district of Tamil Nadu. Wild caught females were individually tubed and identified by the egg-batches laid by them. The identified females, as well as their progeny at the egg, larval and adult stages, were then examined. Palpal measurements were carried out on mounted heads of freshly killed females.

MORPHOLOGICAL DIFFERENCES. Based mainly on 86 females of species A identified by the egg batches laid by them, 33 larvae and 21 pupae; and 93 females and egg batches of species B, 29 larvae and 20 pupae. Specimens were collected between October 1980 and July 1981.

EGG. Species A. Length 0.586 ± S.D. 0.017 mm, 24-43 float ribs, av. 33, frill opaque, striated and equal to the float width, sometimes slightly wider or narrower. Float occupies 0.60 length of the egg and the deck is distinctly narrower in the middle. The bosses are positioned at the extremities of the deck, forming an interruption to the float at each end of the egg (Fig. 1A).

Species B. Length 0.508 ± S.D. 0.012 mm, 15-20 float ribs, av. 18. Frill transparent, striated and extending round the ends of the egg; slightly wider than float, in one case about twice as wide. Float occupies 0.50 of the length of the egg. Deck slightly narrowed in the middle, and bosses placed sub-terminally on the deck (Fig. 1B).

LARVAE. The larvae of the two forms are extremely close. Pigmentation of the head varies in both from three discrete spots to a bar or "cloud" of pigment. Species B larvae are in general more heavily pigmented, but this may be related to environmental conditions, as discussed later. In neither species has branching of the inner clypeal hairs been observed*, and in both the 1st abdominal palmate is weakly developed. Mesothoracic hair No. 1 has 17-22 branches in species A, and 21-31 in species B. Mesothoracic hair 4 has the form described and figured by Reid (1968) for *An. subpictus*. In species A it is 1-3 branched, with the third branch when present arising towards the apex of the hair. Three branched hairs are fairly common ("rare" in Malaysian specimens, Reid 1968). In species B mesothoracic hair 4 is 1-2 branched and relatively small and delicate compared with hairs 3 and 5. However, this character, depending on subjective judgement, is difficult to use on field material. Morphological differences between the larvae are summarized in Table 1.

PUPAE. In species A hair 6 on abdominal segment IV is simple, rarely 2 branched and nearly equal to the mid-dorsal length of tergite IV, whereas in species B it is 2-4, usually 3-branched and less than half the mid-dorsal length of the tergite. The paddle fringe hairs are clearly hooked at the tip in species B, but not in species A. In both these characters species A resembles *An. vagus* of Malaysia (Reid 1968) while species B resembles the Malaysian *An. subpictus*. In all other respects species A and species B are similar and do not differ in any important way from specimens from Malaysia (Reid 1968) and Guam (Darsie and Cagampang-Ramos 1972). Hair 5-I strong, 2-3 branched in species A and 2 branched in species B, with base of main stem as thick as 6-I. Hair 9-I long and simple. Hair 1-IV usually 3-4 branched but in some specimens of species A from Sathanur Dam this hair was 1-2 branched and as long as hairs 1-V to VII. Hair 3-VI simple, hair 2-VII 3-5 branched (1-3, Malaysian specimens) and 4-VIII simple.

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*Footnote: Var. *vadakadiensis* of Doraisamy (1963), in which the inner clypeal hairs of the larvae are bifid, does not seem to occur in this area, nor could it be found in a collection of larvae from the type locality on Rameswaram Island during a visit in 1979. Dr. Bruce Harrison points out that this form is not available, and has no official status according to the ICZN because it was published after 1960. The recognition of this name in Knight and Stone (1977) is therefore in error.

ADULTS. Females of species A are generally pale colored, with the dark margings on the pleura often incomplete, brown on a fawn background. Females of species B are often dark, with the markings on the pleurae picked out in sooty black on a grey-white background. The markings on the ventral aspect of the abdomen are very prominent (Fig. 1). However, pigmentation is not a stable character, and is influenced by environment. Wing markings are very variable. The prehumeral dark spot is undivided or divided and the humeral sometimes totally absent in both species, and in both "three spot" forms occur, in which the sector pale spot does not extend to the costal margin. An accessory sector dark mark may occasionally be present on one or both wings.

In species A the apical pale bond on the female palp is the same length as the subapical dark band. In species B some specimens are seen in which the apical pale band is longer than the subapical dark band, as in the Malaysian specimens described and figured by Reid (1968). However, this character cannot be used reliably to separate the species. The palpal ratio of Coluzzi (1964), i.e., the ratio between palpal segments 4 + 5/3, gave promising results. Species A showed values of 0.73 to 0.86, while in species B the range was 0.81 to 1.00. Much better separation was obtained by using the ratio of segments 5/4. As shown in Table 2, values for species A range from 0.42 to 0.59, while for B the corresponding values are 0.57 to 0.90. If specimens with values of 0.55 and below are considered to be species A, and those of 0.61 and above to be species B, reliable determinations can be obtained, with the loss of only a small proportion of individuals falling into the overlap range 0.56 - 0.60. These limits have been arbitrarily set, and a larger number of determinations should be carried out in each area where the identifications are to be made, as was done by Bryan (1980) in the Gambia for species of the *An. gambiae* complex.

BIONOMICS AND DISTRIBUTION. Species A breeds in fresh water and occurs in inland as well as coastal localities, while species B breeds in brackish water and has so far been recorded only on the coast, mainly in backwaters (Suguna 1982, and unpublished data, VCRC).

TAXONOMIC DISCUSSION. The discovery of two cytologically and morphologically distinct entities in the *An. subpictus* of coastal areas near Pondicherry poses problems of their identity in relation to other members of the Pyretophorus series occurring in the oriental region.

Neither the provisionally named species A nor species B resembles *An. sundaicus* in the morphology of the egg, adult, or in the characteristic forking of mesothoracic hair 4 of the larva.

As shown in Table 1 neither species resembles *An. indefinitus* at the larval, pupal or the adult stage. Bifid inner clypeal hairs have never been observed in the larva, the number of branches on the inner shoulder hair are fewer, and the first abdominal palmate is weakly developed and has fewer leaflets than in *An. indefinitus*. The egg of species B, however, resembles that of this species in having a transparent striated frill wider than the float. On the other hand the frill is rarely as wide as illustrated for *An. indefinitus* by Reid (1968) and the columellae on the deck are not noticeably coarse, nor are there as many float ribs as are found in this species.

An. subpictus species A seems to fit the description and illustrations given by Christophers (1933) for Indian *An. subpictus*. The egg has an opaque frill, interrupted at each end of the deck, but is slightly shorter and has fewer float ribs than in the description given by Christophers and Barraud (1931). Species B seems to resemble the Malaysian form in a number of respects. The egg is shorter, has a transparent frill which is continued around the ends of the egg and the number of float ribs is similar to that of the Java salt water egg (Walch and Walch - Sordrager 1936). The only difference is that the frill in the Indian species B is wider. At the larval stage also there are slight differences from species A in which species B resembles the Malaysian specimens - viz. the number of branches on mesothoracic hair 1 and the absence of a third branch on mesothoracic hair 4. The pupa has hook-tipped paddle fringe hairs, and hair 6 on abdominal segment IV has 2-4 branches. Finally adult females of species B often show the longer apical pale band on the palp which is characteristic of the Malaysian specimens. It is clear that there is in India a fresh water species of *An. subpictus* s.l. with a longer egg with many float ribs and an opaque frill, and a salt water species with a shorter egg, fewer float ribs and a transparent frill which appears in many respects to be close to the salt water *An. subpictus* of the South East Asian region. Long eggs with opaque frill reappear in the Philippines (see Table 1) but insufficient details are available to make any guesses about their significance (Urbino 1936).

The fact that the palpal ratio has been found to be useful in separating the two species offers an interesting parallel with the *An. gambiae* complex (Coluzzi 1964, Bryan 1980). In *An. subpictus* also the salt water member of the pair shows higher values of the palpal ratio than the fresh water member. Nevertheless, this is a stable character.

Pigmentation is definitely influenced by environment, darker adults of species B being raised from wild larvae in brackish habitats, than paler adults from their progeny raised in fresh water in the laboratory. Larvae from saline habitats often have heavily pigmented heads. Christophers (1933) noted that in larvae of *An. sundaicus*, a salt water species, and in those of salt water *An. subpictus* there was a band of pigment on the head rather than three distinct spots. Elsewhere the same author remarks of *An. sundaicus* "Its general colouration is also somewhat darker than in *An. subpictus* or *An. vagus*, and the lateral areas of the thorax more contrastingly dark as compared with the median area than in these species" (Christophers 1933).

Dr. J. A. Reid, who has kindly examined material in the British Museum for us, reports that the types of *An. rossii* and *An. error (Aldrichia error)*, both synonyms of *An. subpictus* and from Calcutta, the type locality, were very pale, with the prehumeral area of the costa almost entirely pale. Palpal ratios (5/4) were 0.56 and 0.53 for the left and right palps of *An. rossii* and 0.46 and 0.33 for *An. error*, all except the last measurement within the range of species A. On the other hand, two Malaysian specimens of *An. subpictus*, both fairly dark, did not resemble species B as regards palpal ratio, values of 0.34 and 0.31, 0.50 and 0.47 being obtained. It would be premature to come to any conclusions at this stage, since dry specimens, liable to shrinkage,

may not be directly comparable to the measurements reported in this paper which were all made on fresh mounted heads. Moreover, geographical variation in this character is almost certain to occur, as it does in the *An. gambiae* complex. Further studies on egg morphology and palpal ratios in the type locality, as well as in Malaysia are needed to determine the identity of the various forms.

VECTORIAL STATUS. *An. subpictus* was not generally believed to be a vector of malaria in India. Several long negative series of dissections of wild caught specimens were carried out in the past, but one sporozoite positive specimen was obtained (Russell, Rao and Jacob 1939). In recent years the species has been incriminated on circumstantial evidence as a vector in the Laccadive and Maldive Islands (Roy et al. 1978) and four sporozoite positive specimens have been obtained from coastal villages south of Pondicherry during an epidemic in the absence of any recognized vector species (Panicker et al. 1981). More recently Kulkarni (Pers. Comm.) has reported three sporozoite positive specimens from Madhya Pradesh. In Southeast Asia, where "*subpictus* is often a coastal species" (Reid 1968) it is known to be a minor vector of malaria, particularly on Sulawesi (van Hell 1952) and the south coast of Java (Sundararaman, Soeroto and Siran 1957). In this connection it is of interest that Roy (1943), who specifically mentions working with a salt-water form of *An. subpictus*, could get a high percentage of sporozoite positive specimens by experimental feedings on gametocyte carriers, while other workers have either been unable to obtain infections (Das, Reuben and Batra 1979) or have obtained low sporozoite rates (Russell and Mohan 1939). The question has now to be asked whether these relate to different species. Much more work is required in order to assess the distribution and vectorial potential of the two forms described here.

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*Not seen in the original.

Fig. 1. Captions

a - Egg of *An. subpictus* sp. A

b - Egg of *An. subpictus* sp. B

c - Palpal segments of sp. B

d - Palpal segments of sp. A

e - Ventral aspect of abdomen of sp. B

f - Mesothoracic hairs of larva, sp. A

g - Mesothoracic hairs of larva, sp. B

Note: Frills of eggs are striated throughout, but striations have been illustrated only on part of the frill.

Table 1. Comparison of morphology of *An. indefinitus* and various forms of *An. subpictus*, compiled from Christophers (1933), Reid (1968), Darsie and Cagampang-Ramos (1972) and present study.

	Malaysia, Java, Guam, Philippines	"India"	Pondicherry		Malaysia Java Guam	Philippines	
Species	*An. indefinitus*	*An. subpictus*	*An. subpictus*	*An. subpictus*	*An. subpictus*	*An. subpictus*	*An. subpictus*
Type	FW, 1	-	A(FW)	B(SW)	SW	2	3
Egg							
Length	-	0.66 mm	0.59 mm	0.50 mm	0.46 mm	-	-
Float/length	0.50	2/3	0.60	0.50	0.50	2/3	2/3
No. float ribs	18-24	30-40	24-43	15-20	-	-	-
	av. 21	-	av. 33	av. 18	av. 19	-	-
Frill	Transparent	Opaque	Opaque	Transparent	Transparent	Transparent	Opaque
Frill width	X2 float	= float	= float	> float	< float	< float	= float
Deck narrow in middle	No	No	Yes	Yes	Yes	No	Yes
Coarse columellae	Yes	-	No	No	No	-	-
Larva							
Inner clypeal	Often forked	-	Simple	Simple	Simple	-	-
Inner shoulder	11-21 br.	-	9-15 br.	8-13 br.	8-15 br.	-	-
Msthx. 1	23-50 br.	-	17-22 br.	21-31 br.	21-34 br.	-	-
Msthx. 4	1-2 br.	-	1-3 br.	1-2 br.	1-2, rarely 3	-	-
Abd. palm. I	6-17 br.	-	5-8 br.	5-9 br.	3-8 br.	-	-
	Strong		Weak	Weak	Weak		
Pupa							
Hair 6-IV	3-4 br.	-	1-2 br.	2-4 br.	3-4 br.	-	-
Hair 3-VI	3+ br.	-	1 br.	1 br.	1 br.	-	-
Paddle fringe hairs	Hooked	-	Not hooked	Hooked	Hooked	-	-
Adult. F.							
Subapical dark band of palp	1/3-1/2 of apical pale band	= apical pale band	= apical pale band	= to 2/3 of apical pale band	1/2-2/3 of apical pale band	-	-
Subapical pale band of palp	1/2 or more subapical dark	1/3 or less subapical dark	1/3 or less subapical dark	1/3 or less subapical dark	1/3 or less subapical dark	-	-

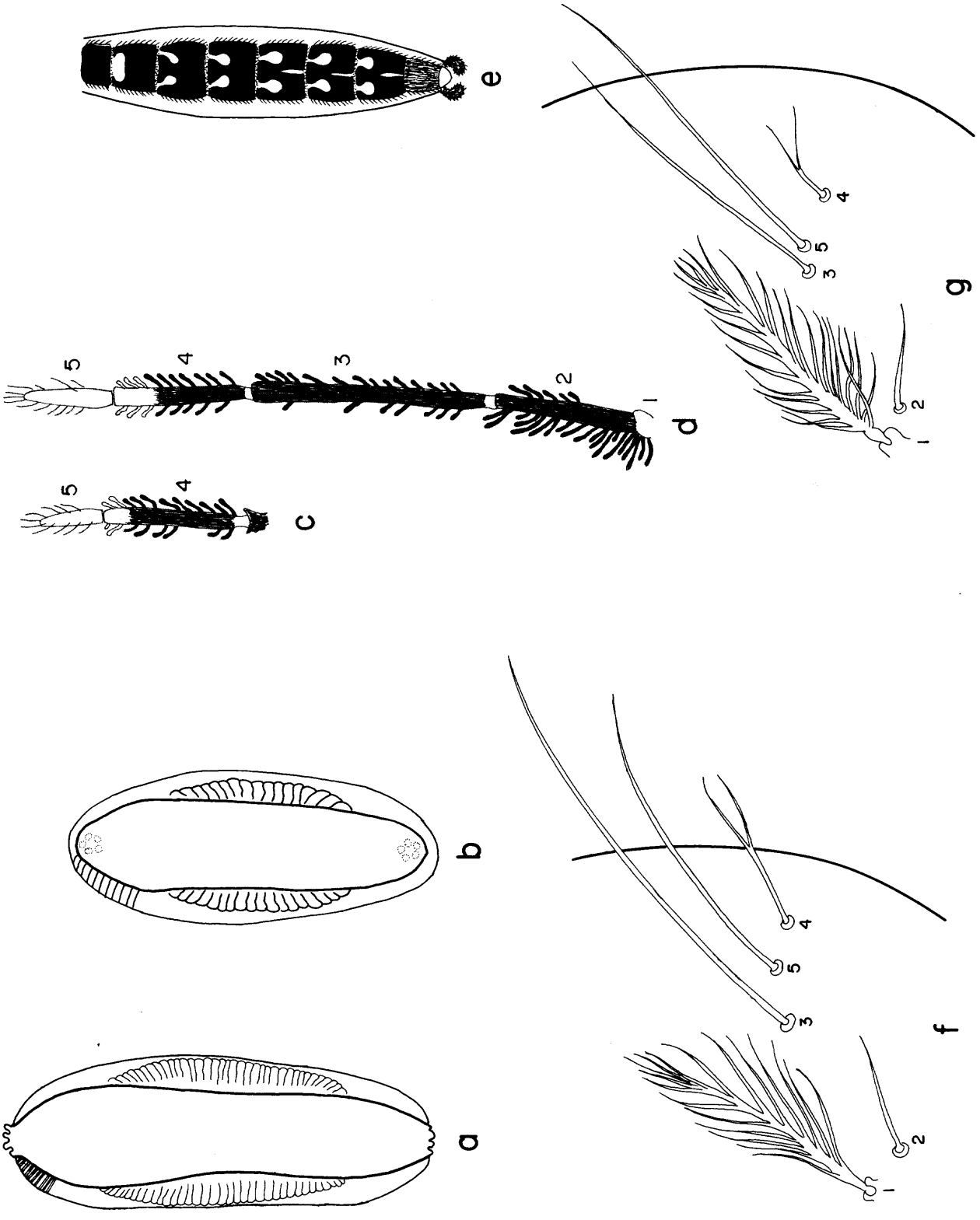
Table 2. Palpal ratios (5/4) of *An. subpictus* females identified by the egg batches laid by them.

Palpal ratio	No. observations		Palpal ratio	No. observations		Palpal ratio	No. observations	
	Sp. A	Sp. B		Sp. A	Sp. B		Sp. A	Sp. B
0.42	1	0	0.58	1	2	0.74	0	2
0.43	3	0	0.59	1	0	0.75	0	0
0.44	8	0	0.60	0	2	0.76	0	3
0.45	3	0	0.61	0	2	0.77	0	0
0.46	5	0	0.62	0	6	0.78	0	1
0.47	4	0	0.63	0	11	0.79	0	2
0.48	20	0	0.64	0	4	0.80	0	0
0.49	4	0	0.65	0	7	0.81	0	0
0.50	12	0	0.66	0	4	0.82	0	0
0.51	5	0	0.67	0	6	0.83	0	0
0.52	5	0	0.68	0	3	0.84	0	0
0.53	3	0	0.69	0	5	0.85	0	0
0.54	6	0	0.70	0	8	0.86	0	0
0.55	3	0	0.71	0	11	0.87	0	0
0.56	1	0	0.72	0	4	0.88	0	0
0.57	1	1	0.73	0	3	0.89	0	0
						0.90	0	1

Species A av. = 0.491 S.D. = 0.037 (n = 86)

Species B av. = 0.676 S.D. = 0.054 (n = 88)

FIGURE 1



An. subpictus