Variation and Vector Status in Anopheles barbirostris

Ъy

J.A. Reid¹, B.A. Harrison² and Soeroto Atmosoedjono³

ABSTRACT. A comparison of series of specimens of the filaria and malariatransmitting forms of *Anopheles (Ano.)* barbirostris Van der Wulp, from Sulawesi and Flores, with specimens of the non-vector forms from Java, peninsular Malaysia and Thailand, shows that the 2 forms are conspecific and supports the synonymy of the subspecific name *innominata* Stoker and Koesoemawinangoen, 1949 (Knight and Stone 1977). This conclusion agrees with that of Lien et al. (1977).

A pupal character has been found which appears to be correlated with vector status.

Introduction

The discovery of a new filarial parasite of man in the island of Timor, E. Indonesia, by David and Edeson (1965), led to investigations which incriminated An. barbirostris as the vector of the Timor filaria (Partono et al. 1976). This revived the question of the true identity of this vector form of barbirostris which Reid (1962, 1968) had been unable to settle.

In May 1975, D.T. Dennis of the U.S. Naval Medical Research Unit at Jakarta, wrote to G.B. White at the British Museum (Natural History) asking about this question, and Dennis and Atmosoedjono sent fresh material to Reid for study early in 1976. The material consisted of a series of pinned adults with their individually associated last larval and pupal skins in alcohol from Flores and Sulawesi. They also sent live eggs by air from Java to G. Davidson at the Ross Institute, London School of Hygiene and Tropical Medicine, who reared them and preserved samples of last larval and pupal skins and pinned some adults.

During correspondence between Reid and Harrison in 1976 it emerged that Harrison had already studied specimens of *barbirostris* from Sulawesi collected

¹43, The Orchard, North Holmwood, Dorking, Surrey, England. RH5 4JT

²Department of Medical Entomology, USA Medical Component, AFRIMS, APO San Francisco 96346. Supported in part by Research Contract No. DAMD-17-74-C-4086 from the U.S. Army Medical Research and Development Command, Office of the Surgeon General, Fort Dietrick, MD 21701.

³U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco 96356. Supported through funds provided by the Naval Medical Research and Development Command, Navy Department, for Work Unit MF 51.524-009-0080.

Reprints should be requested from the second or third addresses.

by R.T. Collins of the World Health Organization. He had concluded on morphological grounds that the specimens belonged to *barbirostris* and not to a distinct species or subspecies. Reid agreed with this conclusion from his own studies, and wrote to Dennis to this effect on 28 April 1976. He explained that he and Harrison thought the vector forms of *barbirostris* in Flores and Sulawesi should be considered only local variants of *barbirostris*, differing more in biology than morphology from the non-vector forms of Java and penninsular Malaysia. The separate morphological studies of Harrison and Reid have been combined in the present paper.

Specimens and Methods

Specimens. Details of the specimens examined in the present studies are given below. Unless shown otherwise they are in the British Museum (Natural History), London (= BM). Other abbreviations are LSHTM = London School of Hygiene and Tropical Medicine; MEP = Medical Entomology Project, Smithsonian Institution, Washington, D.C., and WHO = World Health Organization.

SULAWESI

Studied by Harrison. South Sulawesi Province, Luwu Regency, Wotu District, Lambarese Pamona village (approx. 2°,38'S, 120°,40'E); 1974, 6?, 6ð all with skins except 1?, R.T. Collins WHO.

Central Sulawesi Province, Donggala Regency, Dolo District, Bobo village (01°,05'S,119°,52'E); 1974, 8°, 4° all with skins, except 1° (no skins) and 2° with only larval skins, R.T. Collins WHO.

Studied by Reid. Bobo village as above; 1975, 359, 4& all with skins except 19 (no skins) and 19 with only pupal skin, S. Atmosoedjono and D.T. Dennis. Donggala Regency as above, Parigi District, Sumbersari village; 20. iv. 72, 39, S. Atmosoedjono and C.T. O'Connor WHO. South Sulawesi, Makassar (= Ujung Pandang), 1931, Buddie, LSHTM.

FLORES

North coast, Sikka Regency, Talibura District, Hengga village (8°,24'S, 122°,37'E): Nov. 1975, 149, 28 all with skins except 19, S. Atmosoedjono and D.T. Dennis.

JAVA

Central Java, Semarang, April 1976, 14 batches of eggs from 14 wild-caught 9 were sent to G. Davidson LSHTM, by S. Atmosoedjono and C.T. O'Connor, and from these, pupal and larval skins in alcohol from 6 of the batches reared, plus 6 pinned 9 were given to J. Reid by G. Davison; Reid mounted 27 larval and 24 pupal skins. West Java, Buitenzorg (= Bogor), Feb., 1933, 1 9, R.W. Paine. Bandoeng, 700m, 12.v. 1940, 19, J. Olthof.

PENINSULAR MALAYSIA

Perak State, 1900, 19, Dr. M.J. Wright. Selangor State, Kuala Lumpur Town, 18.xi.1903, 19, own bungalow, and 19 without date or locality (probably ex Kuala Lumpur), Dr. G.F. Leicester. Malay States (?Selangor), BM acc. no. 1920-454, 4? with larval skins, Dr. H.P. Hacker. Malay Peninsula (?Selangor), viii and ix 1931, 6? with pupal and larval skins, E.P. Hodgkin. The following all with pupal and larval skins: Pahang State, Kuantan Town, Telok Siseh 19.vii. 1957, 2?, 1&; Selangor State, Kuala Lumpur, Sentul Pasar, 10.vii 1952, 2?, 1&; Selangor, Puchong, 8.vii.1958, 2?; Kuala Lumpur, Ulu Klang Road, Oct. 1956, 1?, 1&; Kelantan State, Kuala Krai, 1959, 1?, 1&; J.A. Reid.

THAILAND

Chiangmai Province, vii-xi, 1959, 3?, 28 with skins except 1?, E.I. Coher and P.F. Beales WHO.

BURMA

Rangoon, xii 1962-ii 1963, 109 with skins, plus pupal skins of several others including 43, P.F. Mattingly attached WHO.

CAMBODIA

Snoul, Kbal Trach, v.58, 19, Snoul, 10.iii.58,39; Kampot, Po Phum Twea, 18.vi.58, 29, W. Büttiker and P.F. Beales WHO.

VIETNAM

Tonkin, 1931, 7º, 2 whole larvae, C. Toumanoff.

In addition to the above recently examined specimens, data for *barbiro-stris* from peninsular Malaysia, especially those for pupal and larval setae, are from Reid (1962, 1968) and include some figures from the unpublished Tables (see 1962, p. 4), which are in the General Library (Biological Records) of the BM. Additional data on *barbirostris* from Thailand, especially the pupal setae, are from Harrison and Scanlon (1975).

Methods. Essentially, the method used by Lien et al. (1977), Harrison, and by Reid, has been to identify their specimens using the keys and descriptions of Reid (1968) and Harrison and Scanlon (1975).

Harrison and Reid, having larger numbers of specimens with associated skins than Lien and his colleagues, were able to carry their examinations further. In particular, they looked for adult specimens corresponding to the vector form of Celebes (Sulawesi) described in a preliminary note by Venhuis (1939). Venhuis compared the vector form from Sulawesi with the non-vector form from Java (the type locality of *barbirostris*) and found a slight difference in the banding of the hindtarsi. In specimens from Sulawesi the pale band on the 3rd hindtarsomere was usually confined to the apex and only crossed the joint onto the base of tarsomere 4 in 8 percent of his specimens, whereas in Javan specimens the pale band was present on the base of tarsomere 4 in about 75 percent. Venhuis does not state the number of specimens examined. Harrison and Reid also compared the pupal and larval setal characters with those of Malayan *barbirostris* as published by Reid (1962, 1968). Additionally, Reid was able to extend this comparison to include the specimens already listed from Flores, Java and mainland Asia. He made measurements of the 3rd hindtarsal pale bands of females and looked for any correlations between this and other characters, and between any of these characters and vector status. The tarsal pale bands were all measured using the same Wild stereoscopic microscope with camera lucida attachment.

Results

The results of using the keys and descriptions on the specimens from Sulawesi and Flores showed that all appeared to be *barbirostris* Van der Wulp and not any other known member of the *barbirostris* group. In particular the key pupal and larval characters agreed well for *barbirostris* (Table I and II).

Key pupal and larval setal characters. Harrison and Scanlon (1975) examined hundreds of pupal and larval skins of barbirostris and campestris Reid from Thailand, and numbers of those of barbirostris from elsewhere in Southeast Asia. They found the pupal characters the most reliable and easiest to use. There was scarcely any overlap between barbirostris and campestris from Thailand in the number of branches on the pair of abdominal pupal setae 2, VI. This sum ranged from 6-17 in 775 pupae of barbirostris (Malaya, Reid 9-16), compared to 17-58 in 248 campestris (Malaya, Reid, 17-38). It will be seen from Table I that the range in Sulawesi specimens of barbirostris was a little higher, 8-21, but still only slightly overlapping that for campestris, which is not yet known outside mainland Asia. The figures for the Flores and Javan specimens, 10-13 and 9-17, are within the ranges for Malayan and Thai specimens.

For the larvae, the key character for *barbirostris*, the sums of 13,III -5,VII, agree well (Table II) for specimens from Sulawesi 0-10, Java 0-8 and peninsular Malaysia 0-10, and differ sharply from those for *campestris* 9-23. The figures for the Flores specimens 9-13, are somewhat intermediate between the 2 species. The reason appears to be that the Flores specimens have a lower than average number of branches on the setal pair 5,VII (8-12 versus 10-15 for Sulawesi and Malaysia), combined with a higher than average number of branches on the pair 13,III (17-24 versus 14-24 Sulawesi and 10-24 Malaysia). But the latter range, 17-24, is considerably lower than that for *campestris*, 21-39, and the range for the sum of the pairs 13,III + III at 41-52 agrees well with the specimens from Sulawesi, 40-51 and Malaysia, 29-52, which all show only small overlap with the equivalent range for *campestris*, 48-81.

Pupal seta 9, II. An unusual variation of this seta, which is normally a small stout spine, was found in one third (5/15) of the pupae from Flores. These 5 pupae had one or both of the setae long and setose (Fig. 1), a character only found otherwise in Oriental anophelines in the related bancroftii species group where it appears to be a group character [see Baisas, 1935, An. pseudobarbirostris Ludlow; Peters, 1962, Fig. 2, An. bancroftii (Giles)]. Table I

Pupae. Ranges in the sum of branches on pairs and combinations of pairs of setae for Figures in () are An. barbirostris and An. campestris from different places.

the numbers examined.

Species	Place		Setal _F	airs and c	ombinations o	f pairs
4		2,111	2, VI	2, VII	2, VI + VII	2, III + VI + VII
<i>harbirostris</i>	Sulawesi					
	Harrison (19)	11-18	8-18	7-16	19-31	32-47
	Reid (20)	13-20	10-21	9-20	22-41	36–58
	Total (39)	11-20	8-21	7-20	19-41	32-58
	- Flores (11)	12-17	10-13	10-13 (10)	20-25 (10)	32-41 (10)
	Java (21)	12-21 (20)	9-17	10-17	20-31	33-52 (20)
	Peninsular Malaysia* (20)	11-19	9-16	10-18	19-34	33-48
	Thailand**(775		6-17			
compestris	Peninsular Malaysia* (20)		17-38	17-34	35-70	
	Thailand**(248		17-58			
* From Reid (19	(62); the number	s examine	d are fro	m the unpul	lished Tables	s (see p. 5)

.

** From Harrison and Scanlon (1975)

Table II

Larvae. Ranges in the sum of branches on pairs and combinations of pairs of setae for An. barbinostris

and An. campestris from different places. Figures in () are numbers examined.

ner es	Place				etal pai	rs and co	mbinatic	ns of p	airs		
		5,I	5, III	5 , VII	13 , I	13 , II	13,111	13, IV	13, II + III	5,III + 13,IV	13, III - 5, VII
barbirostris	Sulawesi	-									
	Harrison				16-25 (17)					13-30 (21)	1-10 (19)
	Reid (10)	6-9	13-17 (11)	10-15	14-27	21-27	14-24	8-15	40-51	21–28	0-10
	Total				14-27 (27)					13-30 (31)	0-10 (29)
	Flores	7-10 (14)	13-17 (13)	8-12 (14)	17-24 (13)	21-29 (14)	17-24 (13)	7-15 (13)	41-52 (12)	20-29 (11)	9-13 (12)
	Java	7-10 (15)	12–18 (15)	9-18 (12)	15-30 (14)	19–28 (14)	11-24 (13)	6-13 (13)	34-36 (13)	19-29 (13)	0-8 (10)
	Peninsular Malaysia*	6-9 (20)	11-18 (23)	10-15 (20)	16-32 (55)	16-28 (55)	10-24 (55)	6-9 (20)	29–52 (55)	17-25 (20)	0-10 (23)
campestris	Peninsular Malaysia*	6-9 (10)	8-17 (20)	9-16 (20)	27-45 (55)	24-45 (55)	21-39 (55)	6-13 (20)	48-81 (55)	17-27 (20)	9-23 (20)

the numbers examined are from the unpublished Tables (see p. 5). From Reid (1962);

*

All of the 38 pupae from Sulawesi had this seta spinose in the normal way, and there appeared to be no correlation of the setose state with any of the other characters examined in any life stage.

Pupal seta 2, III. While verifying a series of adult barbirostris (with pupal skins) from peninsular Malaysia collected by E.P. Hodgkin, 2 specimens were found which had a higher sum of branches on the setal pair 2, III than recorded by Reid (1968). The range he gives of 11-19 (key, p.122) should be revised to read 11-22.

Hindtarsal pale bands. Reid (1962, p.14; 1968, p. 129) was unable to recognize Venhuis' Sulawesi vector form with the 3rd hindtarsal pale band confined to the apex of tarsomere 3. Out of 12 specimens seen by Reid, 10 had this band extending onto the base of the 4th tarsomere in the usual manner, and the pupal and larval characters of these specimens were those of ordinary barbirostris. He therefore suggested that there might be 2 distinct forms on Sulawesi confused under the name barbirostris, one being Venhuis' vector form.

Consequently, when the material of the vector forms from Sulawesi and Flores, listed in this paper, became available, Reid at once looked at the 3rd hindtarsal pale bands. He found that in the specimens from Bobo, an endemic area of Brugian filariasis in Sulawesi, many of the specimens did indeed lack an extension of the 3rd hindtarsal pale band onto the base of tarsomere 4, and clearly were the form described by Venhuis. Harrison found 4/5 of his specimens from Sulawesi lacked a pale band on the base of hindtarsomere 4. But only a small proportion of the Flores specimens lacked a basal pale band on hindtarsomere 4 and, as already explained, all specimens agreed in pupal and larval characters with typical *barbirostris*. Thus the extension or non-extension of the third hindtarsal pale band across the joint onto the base of hindtarsomere 4 appears to be a normal variation, and not correlated with vector status, at least outside Sulawesi.

However, the measurements made of the 3rd hindtarsal pale band of females have produced some interesting information (Table III). Venhuis refers to small variations in *barbirostris* from different parts of Sulawesi, and it appears that in the southernmost parts, such as Makassar and Kaboena Island, the proportion of specimens with the pale band crossing the joint onto the base of tarsomere 4 may be higher than further north in Sulawesi. Specimens from Kaboena (Reid, 1962, p. 15) and the 2 specimens recently examined from Makassar (= Ujung Pandang) (LSHTM) had the band extending onto tarsomere 4, while in the 34 specimens from Bobo that were measured only 12 (35 percent) had the band extending onto tarsomere 4.

The most obvious point shown by Table III is that the specimens from peninsular Malaysia and Cambodia had the longest 3rd hindtarsal pale bands, which always crossed the joint onto the base of tarsomere 4 (Fig. 2). When this is examined in more detail (Table IV) it is seen that on the mainland of Asia the length of the pale band appears to be correlated with latitude, being longest near the equator and shorter further north where not all specimens have the band extending across the joint onto tarsomere 4. Presumably this variation is a north-south cline. South of the equator, on the islands, there is no obvious correlation with latitude. Table III

Measurements of 3rd hindtarsal pale bands of females of Anophelas barbinostris from various

parts of the range. Figures in () are numbers of specimens examined.

	1				فكالبار المتحد بالم				
ale band ction ength on t4	Mean	0.21(14)	0.25	0.37	0.38(20)	0.38	0.42	0.29	0.31
Those with F on t4; fra of total 1 of band	Range	0.10 - 0.41	0.18 - 0.30	0.25 - 0.47	0.20 - 0.47	0.32 - 0.42	0.36 - 0.45	0.27 - 0.30	0.25 - 0.43
ending 2 of t4	%	37	77	87	100	100	57	67	80
Band exte onto base	Number	15/(41)	10/13	7/8	30/(30)	9/9	4/7	2/3	8/10
Ratio Length/Width*	Mean	2.35	2.61	2.81	3.73	3.53	2.32	2.49	2.54
	Range	1.53 - 3.67	1.75 - 3.75	2.00 - 3.64	2.21 - 5.55	2.67 - 4.92	1.17 - 3.85	2.33 - 2.67	1.76 - 3.33
of	Mean	0.12	0.12	0.13	0.16	0.17	0.12	0.13	0.13
Total length band, mm	Range	0.09 - 0.17	0.10 - 0.15	0.08 - 0.14	0.11 - 0.22	0.13 - 0.20	0.07 - 0.17	0.12 - 0.14	0.10 - 0.17
, 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 2000 - 200	,	(36)	(13)	(8)	(20)	(9)	(2)	(3)	(10)
Place		Sulawesi	Flores	Java	Peninsular Malaysia	Cambodia	N. Vietnam (Tonkin)	N. Thailand (Chiangmai)	Burma (Rangoon)

Measured at the proximal end of the band on tarsomere 3.

*

Mosquito Systematics

Vol. 11(3) 1979

The above suggests that the presence or absence of a pale band on the base of hindtarsomere 4 is a function of the total length of the band, but the figures from Sulawesi and Flores (Table III) suggest a less simple relation for these 2. From these islands, though the average total length of the third hindtarsal pale band is the same, 0.12 mm., the proportion extending onto the base of tarsomere 4 is very different; 77 percent, in the Flores sample compared to 37 percent in the sample from Sulawesi. This suggests that the whole pale band in the specimens from Flores is displaced distally compared to those from Sulawesi (Fig. 2). There is slight confirmation for this suggestion from those specimens with the band extending onto tarsomere 4. In these specimens the proportion of the band which lies on the base of tarsomere 4 averages one quarter (0.25) in the Flores specimens compared to one fifth (0.21) in those from Sulawesi.

Table IV

Anopheles barbirostris from mainland Asia. Relation between latitude and the length of the 3rd hindtarsal pale band on females. Figures in () are the numbers of specimens examined.

		3rd hindta	arsal pale band	
Place	°N latitude	Mean length	Band extending or	nto base
		in mm	of t4	
			Number	%
Peninsular Malaysia	3 - 6	0.16 (20)	30/30	100
Cambodia	11 - 12	0.17 (6)	6/6	100
Burma(Rangoon)	17	0.13 (10)	8/10	80
N. Thailand (Chiangmai)	19	0.13 (3)	2/3	67
N. Vietnam (Hanoi)	21	0.12 (7)	4/7	57

On the other hand, in the specimens from Flores, the mean total length of the band is greater 0.12 mm (0.122) when it crosses the joint onto tarsomere 4, than when it does not 0.10 mm (0.103). The length is nearer the same in both classes of specimens from Sulawesi; 0.11 mm (0.106) in those with the band confined to the apex of tarsomere 3 and 0.12 mm (0.115) in those with the band extending onto tarsomere 4. As the mean fraction of the total length of the band that lies on the base of tarsomere 4 in the relevant specimens from Sulawesi is one fifth, this would be one fifth of 0.12 mm, i.e., 0.024 mm. But

the difference in length between the two classes is less than 0.01 mm (0.115 – 0.106 = 0.009). So it appears, as with the specimens from Flores, that there is slight element of distal displacement of the whole band when it extends onto the base of tarsomere 4.

A search for characters correlated with vector status. Although the morphological evidence suggests that the vector forms of Flores and Sulawesi (and presumably also Timor) are *barbirostris* sensu stricto, one would like to know whether these vector forms can be recognized and differentiated from the non-vector forms of Java, Malaysia, etc. As already explained, the absence of a pale band on the base of hindtarsomere 4 does not appear to be correlated with vector status. This is shown by the large differences in this character as between Sulawesi and Flores, and the small difference between specimens from Flores and Java. Characters of the wings have not yet been examined.

We have already shown that so far as the key identifying characters of the pupal and larval setae are concerned, these show, at most, only slight overlapping differences from the non-vector forms. Other pupal and larval setae have not been examined in any systematic fashion, except for pupal setae 9,II and 1,VII on the abdomen. Some interesting differences were detected when the number of branches on seta 1,VII were tabulated (Table V).

Table V

An. barbirostris pupae. Variation in the number of branches on seta 1,VII in specimens from different places. Figures in () are the numbers of setae examined*

Place		No. branch	es	With less t branch	hat 20 es
	-	Range	Mean	No.	%
Burma (Rangoon) (20)	23 - 55	33.7	0	0.0
Peninsular					
Malaysia (26)	18 - 41	28.2	1	3.8
Java (25)	15 - 40	28.7	3	12.0
Sulawesi ((42)	10 - 37	22.8	13	30.9
Flores ((30)	9 - 25	16.0	25	83.3

*All were counted by Reid during the present study using the same microscope and standards. Table V shows that the range and mean number of branches on 1,VII are less in the specimens from the 2 vector areas, Sulawesi and Flores, than in the 3 non-vector areas, Burma, peninsular Malaysia and Java. In particular the proportion of specimens having fewer than 20 branches on this seta is much greater in specimens from Sulawesi and Flores than in those from Burma, Malaysia and Java. Harrison noted that 7/20 of his specimens from Sulawesi had fewer than 20 branches on 1,VII, a result in close agreement with that in Table V.

These results fit with a curious general trend in the An. barbirostris species group, whereby the pupae of species from the Philippines have few branches on seta 1 compared to those from further west where this seta is usually bushy (p.246). It may be significant that the members of the related An. bancroftii group, which overlap with the barbirostris group in the Philippines and Sulawesi, also have very few branches on seta 1. Compare also the bancroftii-like character of a setose 9,II on one third of the Flores barbirostris specimens (p.238), see discussion.

It would seem from Table V that on present knowledge any population of *barbirostris* having more than 20 percent of specimens with less than 20 branches on pupal seta 1,VII might be regarded as a possible vector population. This does not imply any causal connection between the number of branches on this seta and vector status. It is simply an observed correlation that requires further testing.

Discussion

Taxonomic status. Although the foregoing morphological evidence points strongly to the vector forms of Sulawesi and Flores as being conspecific with typical non-vector barbirostris, we realize that tests by crossing experiments are desirable. It had been hoped to perform such experiments, but though live eggs of barbirostris from Java were successfully sent to G. Davidson at the Ross Institute, LSHTM, logistic difficulties prevented the sending of live eggs from Sulawesi or Flores. Davidson was unable to maintain colonies of the barbirostris from Java.

Assuming that we are correct in believing that the vector forms of Sulawesi, Timor and Flores are only local variants of *barbirostris*, then the morphological differences known so far between them and typical *barbirostris* from Java are much too small and overlapping to warrant subspecific status. Thus the name *innominata* Stoker and Koesoemawinangoen 1949 (Knight and Stone 1977), and *innominata* Stoker and Waktoedi of Stone, Knight and Starcke (1959), is best considered a synonym of *barbirostris*. Stoker and Koesoemawinangoen attached the name *innominata* to *An. vanus* Walker which Bonne-Wepster and Swellengrebel (1953) had incorrectly assumed to be the proper name for the vector form of *barbirostris* described by Venhuis. *Anopheles vanus* is an entirely different species closely related to *An. barbumbrosus* Strickland and Chowdhury as explained by Reid (1962, 1968).

Behaviour and genetics. The large differences of behaviour between the vector and non-vector forms of barbirostris, particularly in their degree of association with man, need consideration. There seems to be a close parallel with An. maculatus Theobald which is the principal vector of malaria in peninsular Malaysia, but harmless in Borneo and over much of the rest of its wide range. Only minimal morphological differences have been found between maculatus from peninsular Malaysia and from Borneo (Reid, 1968). Evolutionary selection pressure is so universal, potent and yet sensitive that we can be sure that the habits of the vector forms of barbirostris have survival value in their environments. But we do not know why this should be so. It may be that the climate and other environmental factors favour a genotype which is also best suited to biting indoors at night where humidities will generally be lower than outside - the aridity factor of Coluzzi et al. (in press). 0r it might be that the theory of Gabaldon (1952) applies. He suggested that when an anopheline species, which near the center of its range antedates man and is zoophilous, spreads with man into new territory it may become a manbiter. This could have happened to barbirostris if it spread eastwards from Java in the wake of man, but this is only speculation. However, it would be interesting if series of specimens with skins could be examined from all the main islands of Indonesia from Sumatra in the west to the Moluccas in the east. Recent remarks by Gillett (1979) seem relevant here. He believes that "as far as medical entomology is concerned we must now think in terms of vector populations and not vector species for the vector concept must be accepted as a statistical one concerning gene frequencies in populations."

It seems possible that if the giant polytene chromosomes of *barbirostris* were examined for polymorphisms and any were found, their incidence, like that of blood groups in genetically different human populations, might differ markedly between vector and non-vector populations. One or more identifiable polymorphisms associated with the manbiting house-frequenting habits might be present as was suggested by Reid (1970), and as has been found in An. arabiensis Patton (= gambiae sp. B) by White (1974) and in arabiensis and gambiae Giles (= gambiae sp. A) by Coluzzi et al. (in press).

Preparations of the giant salivary chromosomes of *barbirostris* are said to be easy to make (Chowdaiah et al. 1967), and a map has been published by Chowdaiah et al. (1970). However, it should be pointed out that there is a slight element of uncertainty as to the identity of the *'barbirostris'* studied by them, as there are certainly 2 species (Reid, personal communication with N.L. Kalra, August 1975), and possibly 3 (Wattal et al. 1962) confused under the name *barbirostris* in India.

The tendency by populations of *barbirostris* from Flores and Sulawesi, and by all members of the *barbirostris* group in the Philippines, to display some *bancroftii*-like characters in the pupae (p. 238), deserves comment. It calls to mind the views of Belkin (1962) that hybridization has played an important part in speciation among South Pacific mosquitoes. The *barbirostris* and *bancroftii* groups are sympatric in the above areas, except for Flores where the *bancroftii* group has not been recorded. Without going as far as Belkin in his views on hybridization as a cause of speciation, one may suppose that during the many changes of land form in the Malaysian/Indonesian archipelago in recent geological times, closely related species of mosquitoes Mosquito Systematics

may have been brought together and interbred before effective isolating mechanisms evolved to prevent further interbreeding. We know that even today some interbreeding occurs between An. gambiae and An. arabiensis in the An. gambiae species group (White, 1971; Coluzzi et al. in press). The males of such crosses are sterile, but the females are fertile and can maintain a small amount of gene-flow between the 2 species.

Vector status. The populations of barbirostris in Sulawesi, Timor, Flores and associated islands are clearly important, as they are efficient vectors of human malarial and filarial parasites. Van Hell (1952) regarded barbirostris as 3rd in importance after sundaicus Rodenwaldt and ludlowae var. torakala Stoker and Koesoemawinangoen as a vector of malaria in south Celebes where he found 9 species infected. In Portuguese Timor, Ferriera and Breda (1962) found barbirostris an important vector along with the main vectors sundaicus and subpictus Grassi. More recently Lien et al. (1977) stated their belief that barbirostris is an important vector of malaria and periodic Brugian filariasis in the Margolembo area of S. Sulawesi.

With regard to filaria transmitted by barbirostris in Sulawesi, the early records of Jurgens in 1932 in S. Sulawesi and of Burg in 1937 in Central Sulawesi (Kalawara) are briefly reviewed by Atmosoedjono et al. (1976), who record finding barbirostris still the vector of periodic Brugia malayi Brug in an area near Kalawara. In S. Sulawesi, Partono et al. (1972) found barbirostris to be the vector of B. malayi in the Margolembo area of Luwu Regency, from where the disease had not been reported before. In Timor, David and Edeson (1965) found the Timor filaria (Brugia timori Partono et al. 1977) widespread and since barbirostris is a malaria vector in Timor it is presumably also the vector of this filaria. This supposition is supported by the work of Kanda et al. (1976) on Timor, where they found barbirostris to be one of the commonest mosquitoes attracted to two men indoors, in a village where both malaria and Timor filariasis were present. They found what looked like Brugia sp. larvae in 1/42 barbirostris dissected. In Flores, investigations in 2 villages in different parts of the island showed that in both, barbirostris was transmitting B. timori for which it was an efficient host (Atmosoedjono et al. 1977; Atmosoedjono and Dennis 1977).

Identifying immigrant strains. The efficiency of barbirostris as a vector of malaria and filariasis in these eastern islands of Indonesia, despite its harmlessness over the rest of its wide range (from India to S.E. China, and south and east to the Moluccas), makes it a matter of interest and importance to try to identify the probable source of specimens of this species which may be found from time to time outside its normal range. For example, Ward et al. (1976) record the capture of 3 females of barbirostris in light traps between May and October 1975 on Guam Island in the Pacific. The species had not been found there before and clearly had been introduced. Larvae were searched for but not found. If a series of specimens from Guam with associated skins had been available, the geographic variation reported in the present paper might have enabled one to say with a fair degree of confidence whether the specimens belonged to a probable vector or non-vector strain.

Acknowledgments

It is obvious from the list of specimens examined in this study that we owe much to past and present members of the World Health Organization who have collected or helped to collect a considerable number of the specimens examined. In alphabetic order they are: P.F. Beales, W. Büttiker, E.I. Coher, R.T. Collins, P.F. Mattingly and C.T. O'Connor.

The senior author is grateful to the authorities of the British Museum (Nat. Hist.) for working space and the use of the Museum's great resources, and to a member of the staff, Mr. J. Chainey, who mounted the larval and pupal skins from Sulawesi.

We are very grateful to Dr. G. Davidson of the Ross Institute, London School of Hygiene and Tropical Medicine, who reared the eggs of *barbirostris* sent from Java and preserved material for study; also to Mr. J. Lane and Professor W.W. Macdonald of the Department of Entomology of the same school for the loan of specimens. We thank Dr. G.B. White for help and encouragement.

References

- Atmosoedjono, S., and D.T. Dennis, 1977. Anopheles aconitus and An. subpictus naturally infected with Wuchereria bancrofti in Flores, Indonesia. Mosq. News 37: 529.
- Atmosoedjono, S., F. Partono, D.T. Dennis and Purnomo. 1977. Anopheles barbirostris as a vector of the Timor filaria on Flores island: preliminary observations. J. Med. Ent. 13: 611-613.
- Atmosoedjono, S., P.F.D. Van Peenen and J. Putrali. 1976. Anopheles barbirostris (Van der Wulp) still a vector of Brugia malayi in Central Sulawesi (Celebes), Indonesia. Trans. R. Soc. Trop. Med. Hyg. 70: 259.
- Baisas, F.E. 1935. Notes on Philippine mosquitoes, V. The pupal characters of anophelines under the *Myzorhynchus* series and group *Neocellia*, with further comments on the larvae and adult of *sinensis*. Mon. Bull. Bur. Hlth. Philipp. 15: 291-339.
- Belkin, J.N. 1962. The Mosquitoes of the South Pacific. 2 vols., Berkeley and Los Angeles, Univ. Calif. Press, 1020 pp.
- Bonne-Wepster, J., and N.H. Swellengrebel. 1953. The Anopheline Mosquitoes of the Indo-Australian Region. Amsterdam, de Bussy, 504 pp.
- Chowdaiah, B.N., T.T. Avirachan and P.L. Seetharam. 1967. Salivary gland chromosomes in four species of tropical anophelines: A. subpictus, A. vagus, A. hyrcanus nigerrimus and A. barbirostris. Proc. Indian Acad. Sci. 65: 181-184, 2 pl.
- Chowdaiah, B.N., T.T. Avirachan and P.L. Seetharam. 1970. Chromosome studies of oriental anophelines. I. The salivary gland chromosomes of *Anopheles barbirostris*. Experientia, 26: 315-317.

Mosquito Systematics

- Coluzzi, M., A. Sabatini, V. Petrarca, and M.A. Di Deco. In press. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. Trans R. Soc. Trop. Med. Hyg.
- David, H.L., and J.F.B. Edeson. 1965. Filariasis in Portuguese Timor, with observations on a new microfilaria found in man. Ann. Trop. Med. Parasit. 59: 193-204.
- Ferreira, A.P., and A.V.M.A. Breda. 1962. Endemic malaria in Timor. 4. Investigations made in 1962. Annais Inst. Med. Trop. Lisbon, 20: 73-98.
- Gabaldon, A. 1952. The effect of DDT on the population of anopheline vectors in Venezuela. Riv. Parassit. 13: 29-41.
- Gillett, J.D. 1979. The vector concept. Antenna 3: 17-22.
- Harrison, B.A., and J.E. Scanlon. 1975. The subgenus Anopheles in Thailand (Diptera: Culicidae). Contr. Amer. Ent. Inst. (Ann Arbor) 12(1): 1-307.
- van Hell, J.C. 1952. The anopheline fauna and malaria vectors in South Celebes. Documenta Med. geogr. trop. 4: 45-56.
- Kanda, T., A. Joesoef, Y. Oguma, T. Tadano and J.S. Saroso. 1976. A record of biting cycles of the possible vectors of "Timor Filaria' on Timor Island, Indonesia. Mosq. News 36: 364-365.
- Knight, K.L. and A. Stone. 1977. A catalog of the mosquitoes of the World (Diptera: Culicidae). 2nd Edition. Thomas Say Found., Entomol. Soc. Amer. 6: 1-611.
- Lien, J.C., B.A. Kawengian, F. Partono, Borahima Lami and J.H. Cross. 1977 A brief survey of the mosquitoes of South Sulawesi, Indonesia, with special reference to the identity of *Anopheles barbirostris* from the Margolembo area. J. Med. Ent. 13: 719-727.
- Partono, F., Hudojo, Sri Oemijati, N. Noor, Borahina, J.H. Cross, M.D. Clarke, G.S. Irving and C.F. Duncan. 1972. Malayan filariasis in Margolembo, South Sulawesi, Indonesia. Southeast Asian J. trop. Med. pub. Hlth. 3: 537-547.
- Partono, F., Purnomo, D.T. Dennis, S. Atmosoedjono, Sri Oemijati and J.H. Cross. 1977. Brugia timori sp. n. (Nematoda: Filarioidea) from Flores Island, Indonesia. J. Parasit. 63: 540-546.
- Peters, W. 1962. Mosquitoes of New Guinea. III. Some undescribed pupae of Anophelini and a revised pupal key. Proc. R. ent. Soc. Lond. (B). 32: 163-169.
- Reid, J.A. 1962. The Anopheles barbirostris group. Bull. ent. Res. 53: 1-57.

_____. 1968. Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. med. Res. Malaysia, No. 31, 520 pp.

- Reid, J. A. 1970. Systematics of malaria vectors. Anopheline systematics and malaria control, with special reference to Southeast Asia. Misc. Publ. ent. Soc. Amer. 7(1): 56-62.
- Stone, A., K.L. Knight and H. Starcke. 1959. A synoptic catalog of the mosquitoes of the world (Diptera: Culicidae). Thomas Say Found., Entomol. Soc. Amer., 6: 1-358.
- Venhuis, W.B. 1939. Voorloopige entomologische mededeelingen omtrent A. barbirostris van Celebes. Geneesk. Tijdschr. Ned.-Indie 79: 2515-2519.
- Ward, R.A., B. Jordan, A.R. Gillogly and F.J. Harrison. 1976. Anopheles litoralis King and A. barbirostris group on the island of Guam. Mosq. News 36: 99-100.
- Wattal, B.L., N.L. Kalra and R.R. Gopal. 1962. A study of Anopheles barbirostris in the collection of the Malaria Institute of India with first record of A. barbumbrosus and A. vanus in India. Indian J. Malar. 16: 63-74.
- White, G.B. 1971. Chromosomal evidence for natural interspecific hybridization by mosquitoes of the *Anopheles gambiae* complex. Nature, Lond. 231: 184-185.
 - . 1974. Polytene karotypes and biting times of *Anopheles gambiae* species B and C in the highlands of Ethiopia. Proc. 3rd internat. Congr. Parasit. 2: 857.

Legends for Figures

- Fig. 1 Setae 9, II on the pupa of specimen 07 from Flores; note left one setose, right one spinose.
- Fig. 2 Diagrams of typical 3rd hindtarsal pale bands to scale based on mean lengths and widths. A, peninsular Malaysia, all specimens; B, Flores, those with the band extending onto T_4 ; C, Sulawesi, those with the band confined to T_3 . (T_3 , T_4 , tarsomeres 3 and 4.)



