

Research Note

Morphological Study on the Spicules of Hybrid Adult Males Between *Brugia malayi* and *B. pahangi*

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Larval stages of *Brugia malayi* and *B. pahangi* closely resemble each other in morphology, therefore the definitive identification of both species is based on the structural differences in the spicules of the male. In West Malaysia and Kalimantan (Borneo) these species are sympatric (Denham & McGreevy, 1977; Palmieri *et al.*, 1983). A natural human infection by *B. pahangi* in South Kalimantan was reported after determining human contact through examination of acid phosphatase (ACP) activity in microfilariae (Palmieri, *et al.*, 1983). In the sympatric areas of both species there is a possibility of natural hybridization occurring. Suswillo *et al.* (1978) investigated hybridization potential among *B. pahangi*, *B. patei* and *B. malayi* and demonstrated that a cross between male *B. pahangi* and female *B. malayi* was infertile, in spite of a capability among all other crosses of producing microfilariae. However, differentiation by ACP activity of each hybrid microfilariae was not attempted even though microfilariae of *B. malayi* and *B. pahangi* can be differentiated (Redington, *et al.*, 1975; Yen & Mak, 1978). The authors reported that the ACP activity patterns of the hybrid microfilariae between female *B. pahangi* and male *B. malayi* are similar to the patterns of paternal species (Kobayashi *et al.*, 1986). Hence it seems necessary to observe other morphological characteristics of the hybrid worms to identify species origin. Suswillo *et al.*,

(1978) reported that the left spicules of hybrid adult males were morphologically identical to those seen in males of the maternal species. However, no attempt was made to compare the male genitalia of hybrid adults with that of the other two species. The present paper was carried out to establish the morphological characteristics of the hybrid male genitalia, so as to distinguishable the hybrid from the parents.

Methods of hybridization have been described in a previous paper (Kobayashi, *et al.*, 1987). Hybrid microfilariae were collected from an infected jird by a peritoneal lavage with warmed Hank's balanced salt solution. Collected microfilariae were diluted with human serum to 20 microfilariae per μ l of the mixture. Mosquitoes, *Armigeres subalbatus* were membrane-fed the mixture and they were dissected at 10 days post-infection. From the dissection, an 250 infective hybrid larvae was obtained and injected into the peritoneal cavity of a naive male jird. No microfilariae however were detected in either peripheral blood or exudate of the peritoneal cavity during an 8 months post-infection period. The infected jird was then sacrificed to obtain the hybrid worms. In the second phase of the experiment adult males of *B. malayi* and *B. pahangi* were collected from the peritoneal cavity of respectively infected jirds. They were fixed in 10% formalin and then transferred into a hot (ca 55°C) 70% alcohol solution containing 5% glycerin. Specimens were made transparent by evaporation in pure glycerin, and then mounted in glycerin jelly (glycerin 100 ml, gelatin 20 g, distilled water 120 ml and carbolic

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acid 2 ml). Drawing of the spicules was performed with the aid of a camera lucida, and the drawn spicules were measured by a curvimeter. Body lengths were also measured by a curvimeter,

with the aid of a profile projector.

The left spicules of the hybrid males were morphologically identical to those described by Suswillo *et al.*, (1978). Body length and spicules

Table 1 Comparison of the measurements in spicules and body length of hybrid with those of *Brugia malayi* and *B. pahangi* male worm

Species of filariae	No. of males observed	Average length of spicules (\pm SD)		Spicule ratio l/r	Average body length (\pm SD)
		left (l)	right (r)		
Hybrid	8	241.8 μ m (38.8)	91.5 μ m (15.6)	2.64	16.6 mm (0.9)
<i>Brugia pahangi</i>	6	201.8 μ m (8.1)	83.2 μ m (3.1)	2.43	19.4 mm (1.3)
<i>Brugia malayi</i>	7	382.3 μ m (16.0)	117.8 μ m (9.2)	3.25	21.0 mm* (1.7)

* This average body length was obtained from 6 specimens.

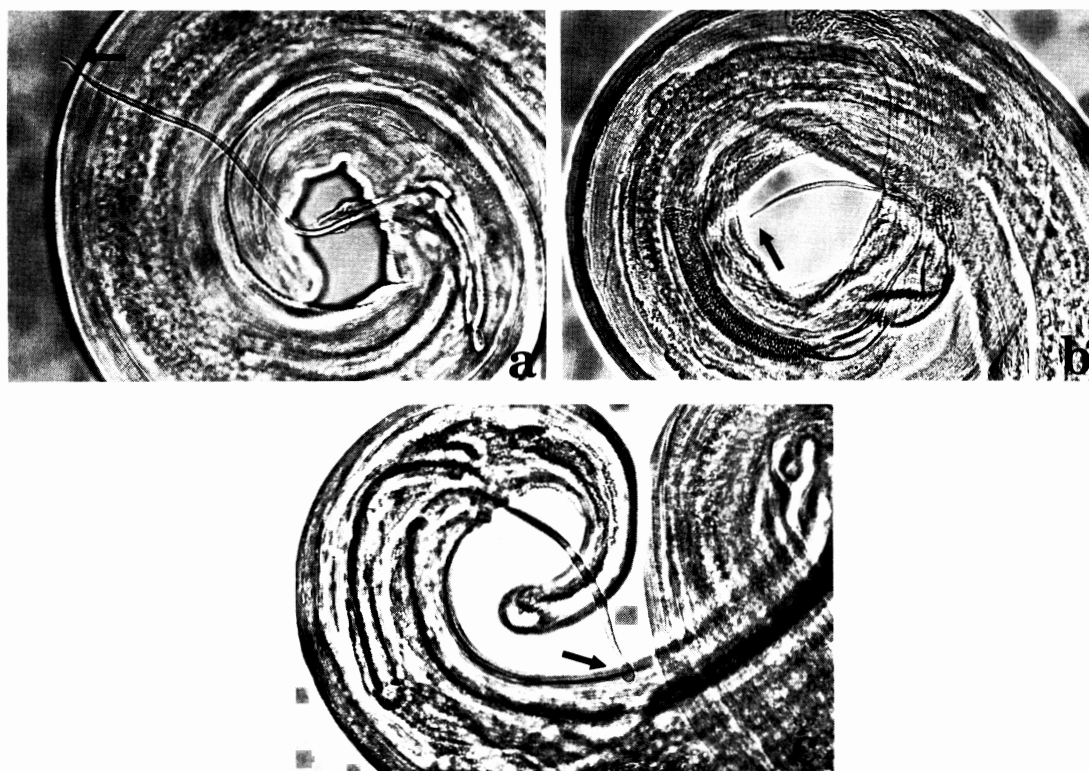


Fig. 1. Structural differences in left spicules of hybrid, *Brugia pahangi* and *B. malayi*
a: Hybrid, b: *B. pahangi*, c: *B. malayi*
All arrows show the termination of left spicules

measurements are shown in Table 1. They terminated in a pin point like shape, similar to those of *B. pahangi* whereas those of *B. malayi* terminates in a spatula like shape (Fig. 1-a,b,c). Body length and left and right spicule measurements of *B. pahangi* and *B. malayi* compared well with results appearing in other published papers (Rao & Maplestone, 1940; Buckley & Edeson, 1956). The average length of the left spicules of the hybrid was 241.8 μm , while those of *B. malayi* and *B. pahangi* 382.3 μm and 201.8 μm , respectively.

A statistically significant difference in the average length of the left spicules between the hybrid and *B. pahangi* was observed using Welch's method ($p = 0.05$). The ratio between the average length of the right spicules and the left spicules was 1:2.64 in the hybrid, 1:2.48 in *B. pahangi* and 1:3.25 in *B. malayi* respectively. There were significant differences in the average spicule ratios between *B. malayi* and the hybrid, and *B. malayi* and *B. pahangi*, however, no differences in the ratios between the hybrid and *B. pahangi* were observed. The average body length of the hybrid males was 16.6 mm which was clearly shorter than those of *B. malayi* and *B. pahangi* (significant at $p = 0.01$ by Welch's method). The tip of left spicules of the hybrid were morphologically identical to those of maternal *B. pahangi*, but they were longer in length than those of *B. pahangi*.

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