

## STUDIES ON MALAYAN FILARIASIS IN CHE-JU IS., KOREA

3 Microfilarial surface architecture of *Brugia malayi* (Che-ju strain)  
in comparison with that of *Brugia pahangi*

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**Abstract:** Scanning electron microscopic observations have been made on the microfilariae of *Brugia malayi* and *B. pahangi* which already cast off their sheaths *in vitro*. The microfilariae of both species have striated appearances. *B. malayi* microfilaria varies in number of striations between 402 and 442 with an average of 423, and *B. pahangi* microfilaria between 443 and 485 with an average of 458. Except for the difference in number of striations, *B. malayi* presents close morphological resemblances to *B. pahangi*. The anterior end of the microfilaria forms the bluntly rounded cap provided with a wedge-shaped hook and two small pores. The hook measures 0.7-1.1 microns in length and projects backward. Of the two pores, one, rectangular in shape, exists at the center of the cephalic cap and the other, round in shape, lies between the base of the hook and the rectangular pore. Three fang-like spines are situated on the first annulus opposite to the hook side. The spines are equal in length, 0.6-0.7 micron, to one another. The spines usually point backwards, though they are occasionally directed forwards by the withdrawal of the cephalic cap into the cephalic space. The excretory pore lies on around the 150th annulus from the anterior end, but the opening of the anal pore has not been found in the present study. The tail tapers gradually showing striations, though near the end a few annuluses become expanded. The terminal appendage, connected to the last expanded annulus, is 6-7 microns long, club-shaped and striated.

It was already described that some morphological differences were discernible between *Brugia malayi* and *Brugia pahangi* at the microfilarial stage (Laing *et al.*, 1960; Schacher, 1962; Sivanandam *et al.*, 1966, 1972), the larval stages in mosquitoes (Beckett *et al.*, 1971, 1972), and the adult stage (Buckley *et al.*, 1956).

These papers dealt primarily with the characteristics of internal organs of the worms and the over-all lengths of the bodies. Only a few knowledge on the surface organs were derived from them. Recently, scanning electron microscope has been informing of some important structures on the surfaces of the helminthes, and Aoki and Katamine (1975) have pointed out some interesting surface organs on the microfilaria of *Dilofilaria immitis*.

In this paper, the fine surface structures of the microfilariae of *B. malayi* and *B. pahangi* are described, and a comparison is made on the microfilarial surface architecture between the two species.

## MATERIALS AND METHODS

Specimens of *B. malayi* microfilariae were obtained from the microfilaria carriers dwelling in Che-ju Island, Korea and those of *B. pahangi* microfilariae were recovered from the dogs infected experimentally. Because the microfilariae of both species were enclosed within delicate membranous sheaths, it was necessary to let them exsheathe in order to make the clear demonstration of the cuticular surfaces. Numbers of sheathless microfilariae were obtained as follows:

Hemolysis was attained by mixing venous blood with saline containing one per cent saponin, equal in volume. After repeated centrifugations and washing with saline several times, the microfilariae suspension was obtained. It contained numerous active microfilariae and a few blood components. Following the procedure that was described by Aoki (1971a, 1971b), the microfilariae suspension was spread over the agar pads in which saline was used as diluent. During the two hours incubation at room temperature, more than 50% of microfilariae cast off their sheaths. Sheathless microfilariae were recovered by washing out the surface of the agar pad.

Specimens were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, dehydrated in ascending series of acetone, and finally dropped over the 1 cm<sup>2</sup> glass slides for air drying. All specimens were rotary-coated in the vacuum evaporator with gold and examined with a JSM-U3 scanning electron microscope.

## RESULTS

On the cuticles of the microfilariae of the two species, there were numerous transverse striations running across regularly all over the body (Figs. 1, 2). As shown in Table 1, *B. malayi* microfilaria varied in number of striations between 402 and 442 with an average of 423, and *B. pahangi* microfilaria between 443 and 485 with an average of 458.

The anterior end of *B. pahangi* microfilaria had the appearance of a bluntly rounded cap, where three special external structures were recognized. One was

TABLE 1 Number of striations of microfilariae of *B. malayi* and *B. pahangi*

<i>B. malayi</i>	<i>B. pahangi</i>
440	443
434	455
412	468
434	450
406	445
442	482
402	445
432	476
407	

a single wedge-shaped cephalic hook. It extended backwards from one edge of the cephalic cap, probably on the ventral surface, and was 0.7 to 1.1 microns long. Others were two small pores. One of them was situated at the center of the cephalic cap. It was rectangular in shape and about 0.4 micron by 0.2. The other pore was round in shape. It measured 0.2 to 0.3 micron in diameter and lay just in the middle between the base of the hook and the rectangular pore. The rest was a bow-shaped groove on the side of the cephalic cap opposite to the hook (Fig. 3).

Each annulus presented a flat columnar or a disk-like appearance. Surface of the annulus was completely smooth and any special surface structure was not observed on all but the first annulus and the one where the excretory pore opened.

The fang-like spines lay at the posterior side of the first annulus opposite to the hook side, probably on the dorsal surface. Most of the *B. pahangi* microfilariae bore three spines lying a row transversely with regular intervals of 1 micron. The spines were almost equal in length to one another, measuring 0.6 to 0.7 micron. Some microfilariae, however, had only two spines, and others as many as four. The spines usually pointed backwards, though they were occasionally everted and directed forwards by the withdrawal of the cephalic cap into the cephalic space (Figs. 6-10).

*B. malayi* microfilariae were essentially identical in cuticular appearance of the cephalic region with *B. pahangi* microfilariae (Fig. 4). There were little differences in size and shape of hook, two pores and spines between the two species (Figs. 3-6). Interestingly enough the spines of *B. malayi* microfilariae were constant in number, fixed at three.

The excretory pore lay on the definite portion of around 150th annulus from the anterior end in the microfilariae of both species. It had a round opening and a diameter of 0.3 micron (Fig. 11). The microfilariae of two species did not differ in size and morphology of the excretory pore.

On the caudal surface of the microfilariae of *B. malayi* and *B. pahangi*, such an anal pore was not detected as evident in *D. immitis* microfilaria.

The tail tapered gradually showing regular striations, though the last three to four annuluses expanded abruptly into spheres, where grooves became shallow and obscure. The terminal appendage, 6-7 microns long, was connected to the last expanded annulus. It was club-shaped and had the characteristic striated appearance (Figs. 12, 13).

#### DISCUSSION

It has already been demonstrated that numerous transverse grooves exist on the cuticle of microfilaria at the level of either the light or the electron microscopy. Scanning electron microscopic observations have, for the first time, clarified the number of the striations engirdling the cuticular surfaces of the microfilariae of *B. malayi*, *B. pahangi* (present paper) and *D. immitis* (Aoki and Katamine, 1975). Both *B. malayi* and *B. pahangi* microfilariae have striations one and a half times as many as *D. immitis* microfilariae have. The number of the striations in *B. pahangi* microfilaria is somewhat greater than that in *B. malayi* microfilaria, and there is

no overlap in the range of numbers. This finding would be considered to be due to the fact that the former is somewhat longer than the latter (Laing *et al.*, 1960; Schacher, 1962).

Except for small differences in number of the striations, the general surface architecture of *B. malayi* microfilaria conforms exactly to that of *B. pahangi* microfilaria.

Laurence and Simpson (1968) demonstrated the hook and three spines at the anterior tips of both *B. malayi* and *B. pahangi* microfilariae by means of the special staining. The findings of the present study are not only in agreement with the results obtained by them, but also elucidate, for the first time, the three dimensional features and arrangement of these structures: The hook extends backwards from the cephalic cap and the spines extend backwards from the side of the first annulus opposite to the hook side. According to Laurence and Simpson, though the hook is observed in varieties of microfilariae, the spines seem to exist in the microfilariae of limited species only, varying in number from species to species. As reported previously by authors (1975), *D. immitis* microfilariae lack the spine. It is generally accepted that the species with sheathed microfilariae do not differ from those with sheathless microfilariae in the structure of microfilarial body itself. These results, however, suggest that the spines may be the characteristics of the sheathed microfilariae. For further studies into this problem, it is necessary to investigate the cephalic structures of many species of sheathed and sheathless microfilariae.

Another point of interest is that the number of spines is constant in the microfilariae of *B. malayi*, whereas it varies from two to four in those of *B. pahangi*. The significance of these is not yet fully understood.

From the observation on living microfilariae, Taylor (1960) reported the movements of the hook, and later Eslinger (1962) described in detail that the cephalic hook of *B. pahangi* was alternatively erected and relaxed at a varying rate. Evidence has been also provided in fixed microfilariae by Laurence and Simpson (1968). In the present study, all the three spines of some microfilariae which exsheathed *in vitro* have been found to be everted forwards by the withdrawal of the cephalic cap into the cephalic space. It is reasonable to presume that the further withdrawal of the cephalic cap may cause also the forward direction of the hook which has been observed by the previous authors. Anterior muscle cells which are probably associated with the hook have been demonstrated by Kozek (1971), McLaren (1972) and Tongu (1974) in the microfilariae of some species at the ultrastructure level. On the basis of available evidence it seems likely that the hook and spines are mainly instrumental in their exsheathing and penetrating into the midgut wall of mosquitoes.

The rectangular pore most probably corresponds to the opening of one of the paired cephalic channels which were reported in the microfilariae of several species by Kozek (1968, 1971) and McLaren (1969, 1972) as well as in those of *B. malayi* by Tongu (1974), judging from its location on the cephalic cap. The opening of the other cephalic channel seems to be hidden beneath the hook. The location of the round pore in the middle between the base of the hook and the rectangular pore suggests that it is in agreement with the opening of the buccal cavity reported by the previous authors. The openings of the anal pore and the caudal channels

which were described by Tongu (1974) have not been observed under the scanning electron microscope, probably due to the closure of the openings by dense materials like plugs. Another possible explanation of our failure to detect them is that the small pores might have been closed by the shrinkage of the worms during the dehydrating process.

Significant morphological difference in the tail has not been detected between the microfilariae of the two species belonging to the genus *Brugia*. *Brugia* microfilariae, however, differ markedly from the microfilaria of *D. immitis* in having the swollen last three to four annuluses and the striation of the terminal appendage. Thus, these results support the view that the caudal structures of the microfilariae afford an important clue to identify "genera", to say the least.

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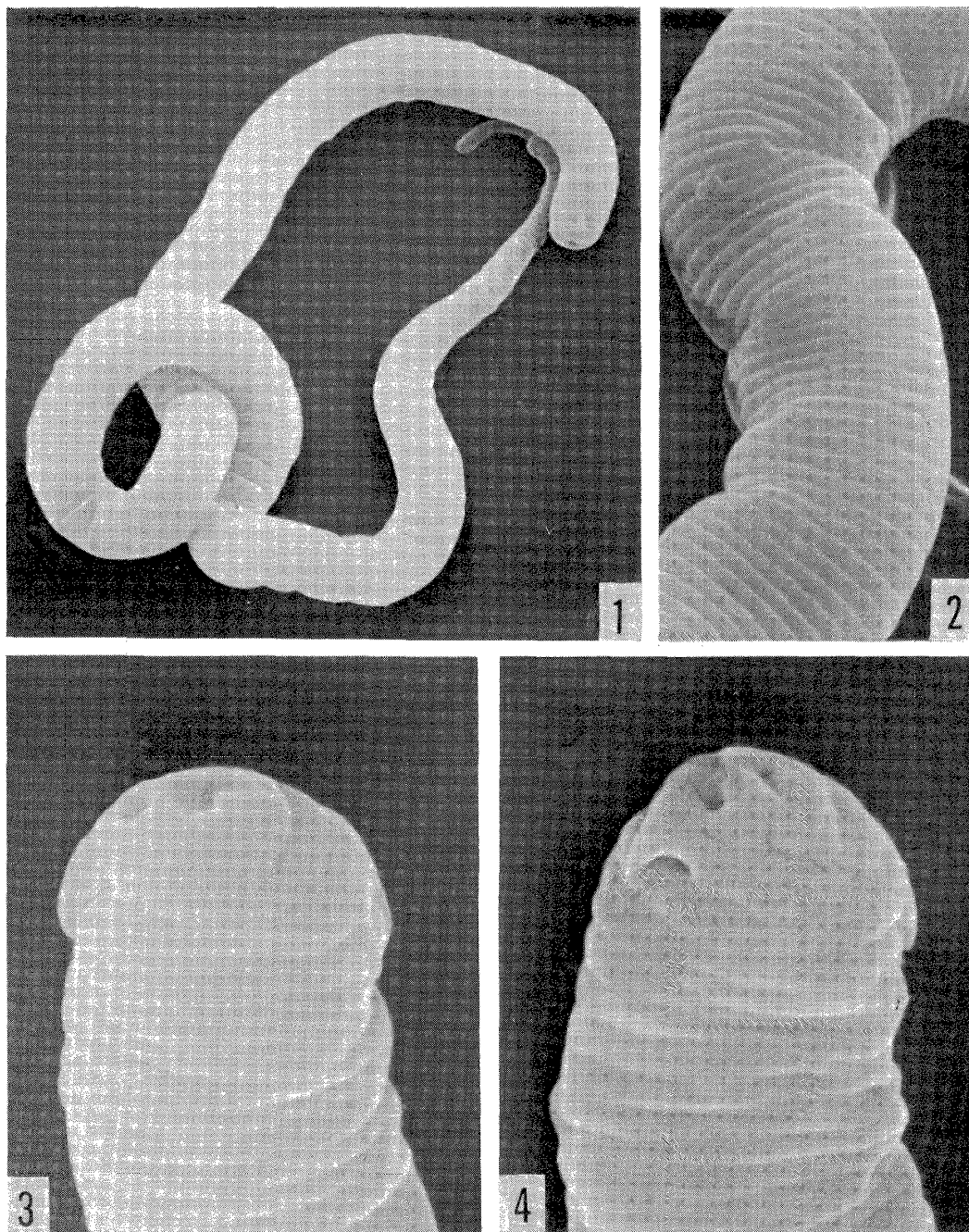
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## 韓国濟州島のマレー糸状虫に関する研究

### 3 *Brugia malayi* (Che-ju strain) 仔虫と *B. pahangi* 仔虫の体表微細構造

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生体外で脱鞘をおこした *Brugia malayi*, *B. pahangi* 仔虫を走査電顕にて観察し, その体表微細構造を観察した。両種とも角皮表面には多くの輪状溝が存在し, その数は *B. malayi* で 402-442 (平均 423), *B. pahangi* で 443-485 (平均 458) であった。*B. pahangi* 仔虫で輪状溝数がやや多いことを除いては, 両種仔虫の間には基本的体表構造の違いは認められなかった。仔虫頭端は半球状を呈し, 1本の鉤と2つの小孔が存在する。鉤は“くさび”状, 0.7-1.1  $\mu$  の長さで, 後方にのびる。2つの小孔の内, 1つは矩形で頭端部の中央に存在し, 他の1つは円形で鉤基部と矩形小孔の間に存在する。半球状頭端に続く第1体環の鉤と対側面には3本の刺突起がみられる。その長さは互に等しく, 0.6-0.7  $\mu$  である。刺突起は通常は後方に向かっているが, 時折, 頭端部が虫体内方に陥凹して, 刺突起が前方に反転している虫体が観察された。排泄孔は頭端より約150番目の体環に開くが, 肛門孔は本観察ではみられなかった。尾部は輪状溝構造を保ちながら徐々に細くなるが, 最後の数個の体環は膨瘤する。この膨瘤した最後の体環に長さ6-7  $\mu$  の棍棒状尾端部が続く。ここにも著明な輪状溝がみられる。



- Fig. 1 General profil of *B. pahangi* microfilaria.  $\times 2,000$   
 Fig. 2 Transverse striation at midportion of *B. malayi* microfilaria.  $\times 6,000$   
 Fig. 3 Anterior end of *B. pahangi* microfilaria showing the hook, the spines and the two pores.  $\times 12,000$   
 Fig. 4 Anterior end of *B. malayi* microfilaria.  $\times 12,000$

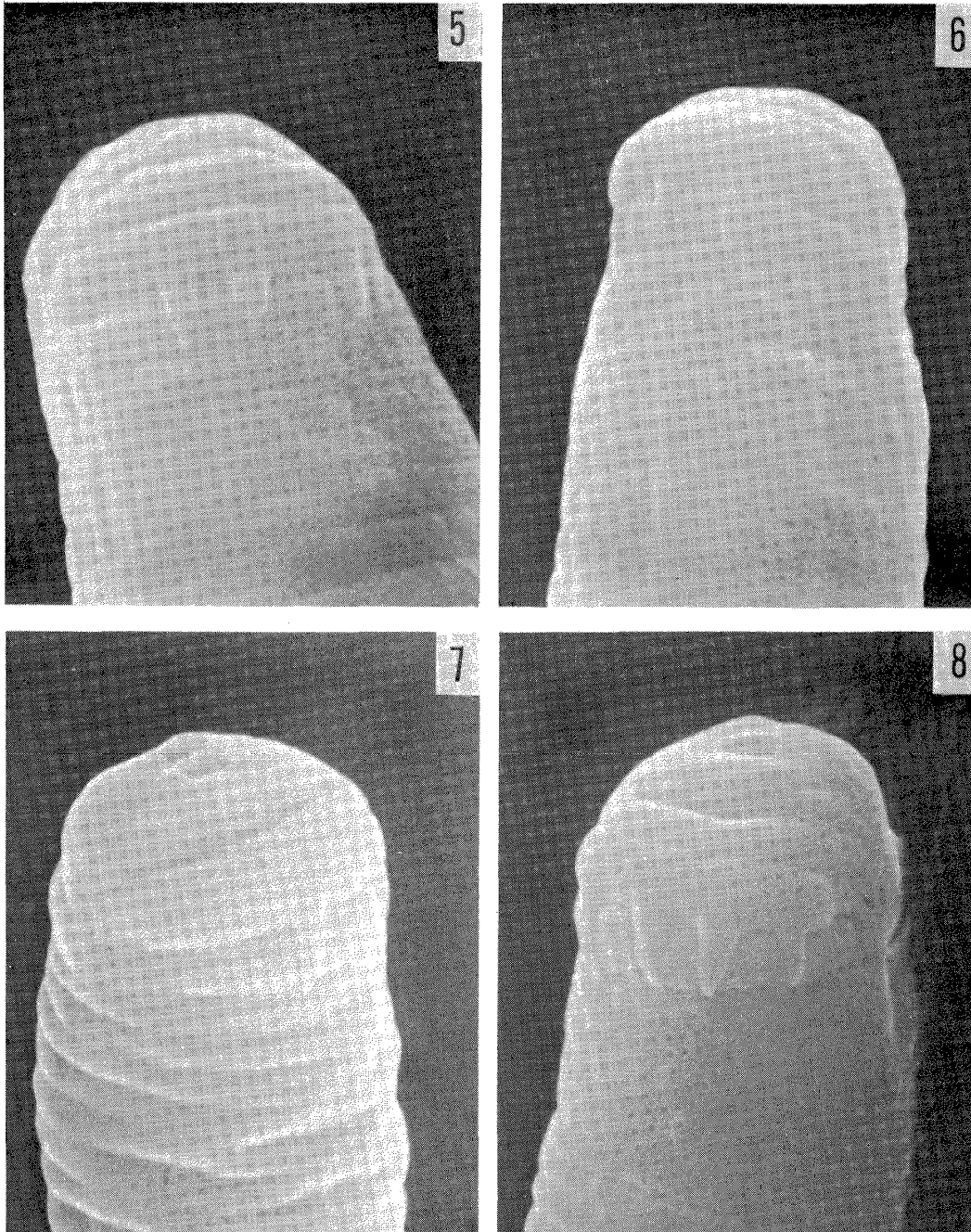


Fig. 5 Three spines on the first annulus of *B. malayi* microfilaria.  $\times 18,000$

Fig. 6 Three spines on the first annulus of *B. pahangi* microfilaria.  $\times 14,000$

Fig. 7 *B. pahangi* microfilaria with two spines.  $\times 14,000$

Fig. 8 *B. pahangi* microfilaria with four spines.  $\times 14,000$



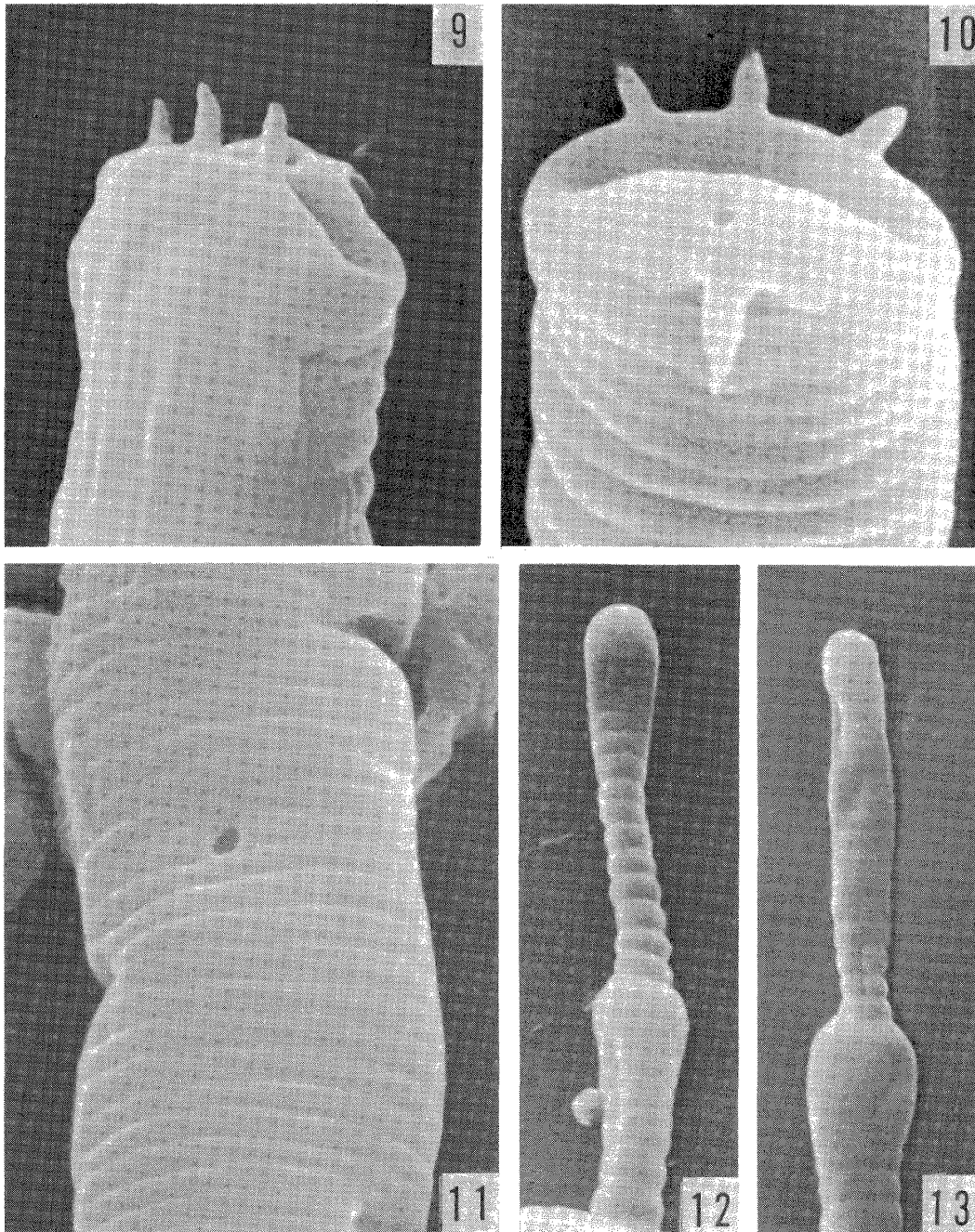


Fig. 9 and 10 Erected spines of *B. pahangi* microfilaria.  $\times 14,000$ ;  $\times 16,000$

Fig. 11 Excretory pore of *B. malayi* microfilaria.  $\times 10,000$

Fig. 12 Tail of *B. malayi* microfilaria.  $\times 8,000$

Fig. 13 Tail of *B. pahangi* microfilaria.  $\times 8,000$