

SCANNING ELECTRON MICROSCOPIC STUDY OF MICROFILARIA AND THE THIRD STAGE LARVA OF *WUCHERERIA BANCROFTI*

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Abstract. The surface structures of microfilaria and of the third stage larva of *Wuchereria bancrofti* were studied by scanning electron microscopy. Distinct features were observed that could be used for differentiating species of this parasite. Specifically, the sheath of microfilariae of *W. bancrofti* projected beyond the head. The head region of the microfilaria was composed of a cephalic cap with hook, mouth and amphidial opening, and its cuticle showed annulation. Spines were absent at the first transverse annulation, and the tail end showed a slight constriction. In the infective stage larva, characters which are used for differentiating species, such as the two bubble-like ventro-lateral papillae and one dorso-terminal papilla were rather similar to each other in size, but the grooves seen around the base were absent. A previously unreported feature of the third stage larva of *W. bancrofti* that was discovered in this study is a papilliform process on the left side of the posterior region, between the anus and the tail end.

INTRODUCTION

Accurate identification of the infective larvae (the third stage larvae) of filariae of *Wuchereria bancrofti* from mosquitos is important for epidemiological studies (Nelson, 1959). At present, the differentiation of species is based on light microscopy alone and pictorial keys have been prepared for this purpose (Sasa, 1976). Species identification at the level of light microscopy may be subject to error. Electron microscopy has the potential of bringing out some features which are not visible in the light microscope and, therefore, of adding to the accuracy of identification.

Scanning electron microscopy (SEM) is ideal for the study of the surface structures of parasites and for revealing morphological features that are useful not only for distinguishing between species but also may help in the understanding of the functions of various structures. Scanning electron microscopy of *Brugia malayi* adults has been reported (Franz and Lenze, 1982; Chew *et al*, 1983; Lim *et al*, 1983). In Thailand, the surface morphology of *B. malayi* adults and microfilariae has been

studied by SEM (Choochote *et al*, 1987; Saitongdee, 1989). In addition, Maleewong *et al* (1987) and Saitongdee (1989) studied the structure of infective larvae of nocturnally sub-periodic *W. bancrofti* by using SEM. However, the parasites used in this study were obtained from Kanchanaburi Province in Western Thailand.

Zaman and Narayanan (1986) described the differentiation of the infective stage larvae of *W. bancrofti* from those of *B. malayi* using SEM. They concluded that the caudal papillae were the most useful diagnostic feature for differentiation between these species. There were three caudal papillae, two lateral and one terminal. Saitongdee (1989) reported that all three caudal papillae of *W. bancrofti* were distinctly protruding, and that the lateral papillae showed the absent gutter-like indentation around their base. In the present study, the surface structures of microfilariae and third stage larvae of nocturnally sub-periodic *W. bancrofti* strain from Tha Song Yang District, Tak Province, Thailand, were studied by SEM. The purpose of the study was to determine whether unique structural features listed could be used to distinguish species variation from different areas in Thailand.

MATERIALS AND METHODS

Microfilariae of *W. bancrofti* were collected from the peripheral blood of human volunteers after direct feeding. The microfilariae were collected by filtration through a five-micron-nucleopore filter (Dennis and Kean, 1971). The third stage larvae of *W. bancrofti* were collected from direct feeding of *Aedes (Finlaya) niveus* group.

The microfilariae and the infective larvae were re-suspended and fixed (12 hours, 4°C) immediately after collection with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer containing 0.1 M calcium acetate pH 7.4. The fixed organisms were washed three times in the same buffer, were post-fixed (1 hour, 4°C) with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer pH 7.4, and were washed three times in distilled water. The samples were then dehydrated with increasing concentrations of ethanol and dried in a Hitachi HCP-2 critical-point drying apparatus. Specimens were mounted on aluminium planchets and coated with gold for three minutes in an Poraron E-5000 ion-sputtering apparatus at 1.4 KV and 12 mA. The specimen were examined with a Hitachi S-430 scanning electron microscope operating at 15 KV.

RESULTS

Microfilariae

Each microfilaria of *W. bancrofti* strain (Tak Province) is enclosed within a sheath (Figs 1A, 1B), which appears as a thin flexible, translucent membrane. The sheath extends beyond the head and tail of the microfilaria. The surface structure of the body can be distinguished through the sheath (Fig 1B). Microfilariae generally form a graceful curve in appearance, bluntly rounded anteriorly and more finely pointed caudally (Figs 1C, 2C, 2D). The cuticle is striated (Figs 2A, 2B). The posterior extremity appears as a short tail demarked by a distinct constriction (Figs 2C, 2D).

The mean body length and width of microfilariae are about 208 and 4.32 μm , respectively (Table 1). In exsheathed microfilaria, the anterior end consists of a round cephalic cap, approximately

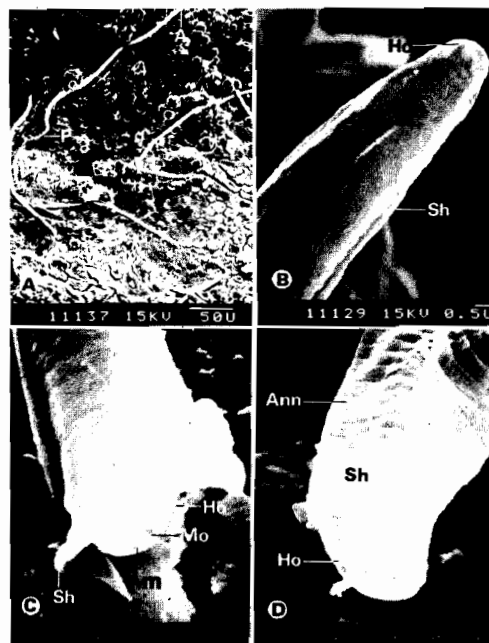


Fig 1—Scanning electronmicrographs of microfilaria of *W. bancrofti*. A) A low-power micrograph of several whole worms, showing blunted anterior end (A) and tapered posterior end (P). B) A high-magnification micrograph of the ensheathed anterior end showing a hook (Ho) and the sheath (Sh). C) A high-magnification micrograph of the cephalic cap of the exsheathed anterior end showing the mouth opening (Mo), right amphidial opening (Am), hook (Ho), annulations (Ann) and sheath (Sh). D) A high-magnification micrograph of the exsheathed anterior region, showing hook (Ho), annulations (Ann) with numerous folds and sheath (Sh).

two μm in diameter (Fig 1C). Fig 1D shows the hook of the cephalic cap, which is about 0.71 μm in length. It is partially hidden in the left amphidial opening. On the right border of the cephalic cap, the right amphidial opening is formed, approximately 0.17 x 0.08 μm in size, and situated at the tip of the cephalic cap of the microfilaria (Fig 1C). The mouth is located in the middle of the cephalic cap and measures about 0.23 x 0.1 μm in size (Fig 1C).

The cuticle of a microfilaria is marked by transverse annulations running from the anterior to the posterior end (Figs 1D, 2A-2D). Annulations are approximately 0.44, 0.61, and 0.58 μm in width at the anterior, middle and posterior regions, respectively (Figs 1D, 2A-2D). The short tail appears at the distal end of the body and starts after a distinct

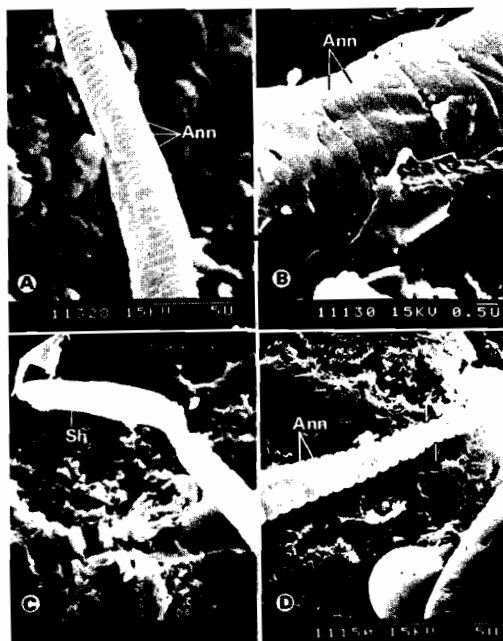


Fig 2—Scanning electronmicrographs of the middle region and posterior end of a microfilaria. A) A medium-magnification micrograph of middle region showing annulations (Ann) with numerous folds. B) A high-magnification micrograph of the middle region showing annulations (Ann). C) A view of ensheathed posterior end showing sheath (Sh). D) A medium-magnification micrograph of the posterior end showing annulations (Ann) and a slight constriction of the distal part of the body (arrows).

constriction. The tail measures approximately 5.13 μm in length, and is also annulated. Each annulation of the tail measures about 0.37 μm in width (Fig 2D).

Infective stage larva

Micrographs of the infective third stage larva of nocturnal sub-periodic *W. bancrofti* are shown in Fig 3. Larvae average 927.99 μm in length and 12.99 μm in width (Table 2, Fig 3A). The mouth appears formed as an extended fissure (mean = 1.42 μm in length) at the anterior end (Table 2, Fig 3B). There are four pairs of oral papillae arranged in two concentric rings, each ring consisting of two sub-dorsal and two sub-ventral papillae. The four smaller inner papillae are cone shaped (0.36 μm high) on flat bases (0.68 μm wide) (Fig 3B). The four outer

Table 1

Sizes of the surface structures of microfilaria of *W. bancrofti* (Tak Province).

Microfilaria	Dimensions (μm) mean \pm SD
Body length	208 \pm 5.25
Body width	4.32 \pm 0.23
Mouth diameter	0.23 \pm 0.07
Hook length	0.71 \pm 0.09
Mid-region	0.61 \pm 0.09
Annulation width	
Tail length	5.13 \pm 1.36

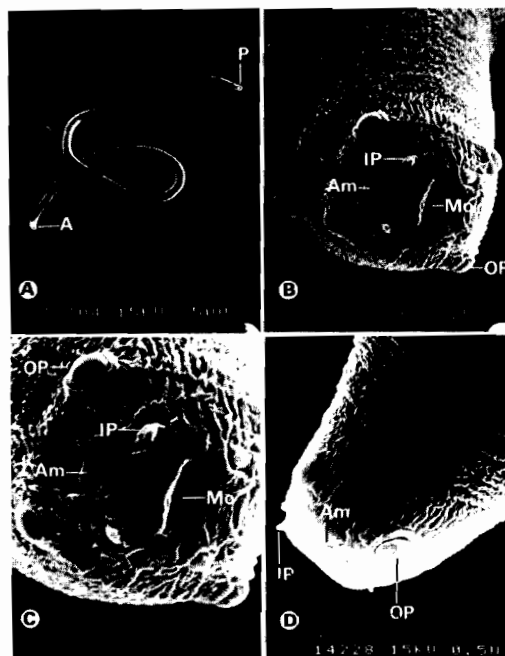


Fig 3—Scanning electronmicrographs of the third stage larva of *W. bancrofti*. A) A low-magnification micrograph of the whole worm, showing anterior (A) and posterior (P) ends. B-D) High-magnification micrographs of the anterior end showing the mouth (Mo), an amphid (Am), inner papilla (IP) and outer papilla (OP).

papillae are distinct and round, with an average diameter of 0.85 μm (Table 2, Figs 3B-3D). Apart from the oral papillae, the two amphidial openings are formed as a narrow fissure, measuring 0.8 μm in length (Table 2, Figs 3B-3D). Each papilla and

Table 2

Sizes of the surface structures of the infective stage larva of *W. bancrofti* (Tak Province).

Infective stage	Dimensions (μm) mean \pm SD
Body length	927.99 \pm 167.29
Body width	12.99 \pm 1.71
Mouth diameter	1.42 \pm 0.05
Inner cephalic papilla :	
cone length	0.36 \pm 0.04
base width	0.68 \pm 0.05
Outer cephalic papilla diameter	0.85 \pm 0.12
Amphidial opening length	0.80 \pm 0.05
Mid-region cuticular annulation width	0.56 \pm 0.09
Anus length	3.78 \pm 0.76
The left ventro-lateral papilla :	
diameter	2.10 \pm 0.35
height	2.19 \pm 0.02
The right ventro-lateral papilla:	
diameter	2.28 \pm 0.26
height	2.10 \pm 0.14
Dorso-terminal papilla :	
diameter	2.56 \pm 0.51
height	2.16 \pm 0.25
Small papilliform process diameter :	
base width	1.42 \pm 0.31
cone length	0.69 \pm 0.19

amphid is surrounded by a small groove with elevated rim (Figs 3B-3D).

The cuticle of the third stage larva shows transversed annulations throughout the body, except at the anterior and posterior ends (Figs 3D, 4). Annulations measure 0.56 μm in width (Table 2, Fig 4B). Lateral lines are observed as interruptions of the cuticular annulations and longitudinal folds are clearly observed on the surface over the body, except at the anterior and posterior ends. The lateral lines measure approximately 2.2 μm in width (Fig 4B).

The anus is located on the right side, approximately 22.5 μm in front of the posterior end, and measures 3.78 μm in length. It is prominent and crescent shaped, with a raised anterior border (Table 2, Figs 5, 6A). The folds of the anal field are directed towards the anal pore and are about 3.89

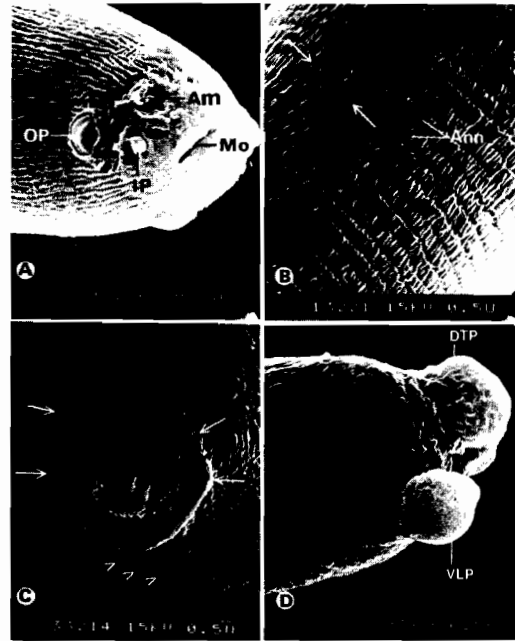


Fig 4—Scanning electronmicrographs of the third stage larva. A) Head region showing fissured mouth (Mo), inner papilla (IP), outer papilla (OP) and amphid (Am). B) High-magnification micrograph of the middle part showing striated annulations (Ann) and lateral line (arrows). C) Enlarged view of the anus (An), anal field (arrows) and longitudinal folds (arrowheads). D) High-magnification micrograph of the tail, indicating the two ventro-lateral papillae (VLP) and the dorso-terminal papilla (DTP), with a bubble-like structure distinctly protruding from the main body of the larva.

μm long (Fig 5A). The caudal extremity is round and carries three papillae of approximately the same size (Figs 5A-5D). There are two bubble-like ventro-lateral papillae with diameters of approximately 2.10 μm and 2.28 μm on the left and right sides (Figs 5A, 5B, 5D) and one dorso-terminal papilla with a diameter of approximately 2.5 μm (Table 2, Figs 5 A, 5B). A small papilliform process is found on the left side of the posterior region, and measures approximately 1.42 μm in diameter at the base width and 0.69 μm in cone length (Table 2, Figs 6B-6D).

DISCUSSION

Scanning electron microscopy reveals morpho-

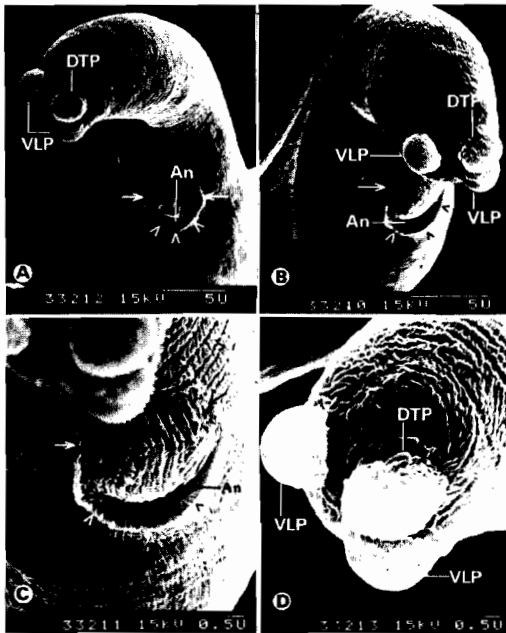


Fig 5—Scanning electron micrographs of the posterior region of the third stage larva. A-B) The posterior region of the third stage larva showing the right side (A) left side (B), anus (An), cowl-shaped ridges (arrowhead), anal field (arrows), two ventrolateral papillae (VLP) and dorso-terminal papilla (DTP). C) High-magnification micrograph of anus (An) showing the opening through cowl-shaped ridges (arrowheads) and anal field (arrows). D) An enlarged view of A, showing two ventrolateral papillae (VLP) and the dorso-terminal papilla (DTP).

logical features that are not visible by light microscope and, therefore, can add to the accuracy of identification of detailed structural differences in species. Aoiki *et al* (1980) reported a SEM study of the microfilariae of *Dirofilaria immitis*, *Brugia pahangi* and *B. malayi*, the infective larvae of *B. pahangi* and adult worms of *Setaria* spp. and *Dirofilaria* spp. These reports delineated some cuticular surface structures which were difficult to envision by either light microscopy or by transmission electron microscopy.

A similar approach has been used to study the detail surface morphology of the microfilariae. In the present study, the microfilariae of *W. bancrofti* were enclosed within a sheath which was originally a thin flexible, transparent ovoidal membrane. Microfilariae were bluntly rounded anteriorly and pointed caudally. The cuticle was striated (Suzuki,

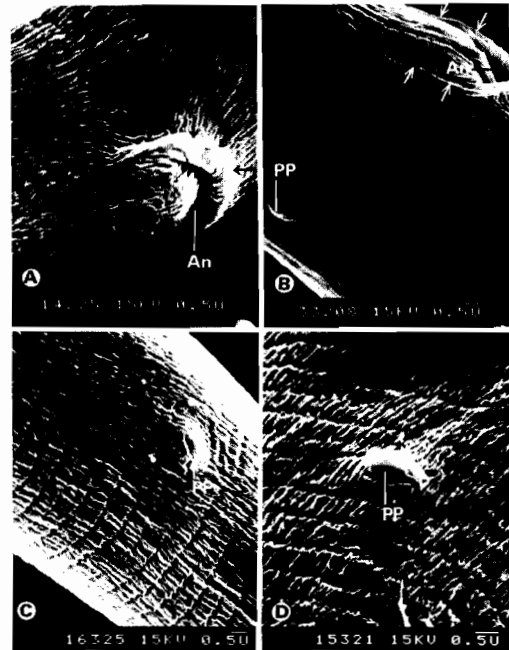


Fig 6—Scanning electron micrographs of lateral view of the third stage larva. A) Right side of the body showing anus (An), cowl-shaped ridges (arrow). B) The left side of the body showing anus (An), anal field (arrows) and small papilliform process (PP) located at the posterior to the anal opening. C-D) Medium and high-magnification micrographs of the left ventro-lateral region showing a small papilliform process (PP).

1981). The sheaths of *B. pahangi* and *W. bancrofti* microfilariae are secreted by the ovarian wall (McLaren, 1973). Saitongdee (1989) concluded that the microfilarial stages of *B. malayi* and *B. pahangi* were enveloped by sheaths. The unshathed microfilaria of both species has a similar pattern of the cuticular surface structure. The anterior end carried the cephalic cap with a hook, mouth, amphidial opening and spines. The cuticle showed annulations. The sheath of *B. pahangi* was granular, whereas that of *B. malayi* was translucent and rather smooth. These characters of *B. pahangi* and *B. malayi* were similar to those observed for *W. bancrofti* in this study. The important characters of *W. bancrofti* which can be used to differentiate species of worms are spines and tail end cuticle. The microfilariae of *W. bancrofti* (Tak Province) had no spines at the first transverse cuticular annulation, but these spines were observed on *B. pahangi* and *B. malayi* (Chew *et al*, 1983; Choochote

et al, 1987). In addition, the tail cuticle of *W. bancrofti* microfilariae appeared less constricted than that of *B. pahangi* and *B. malayi*.

The observations on the surface topography of the third stage larvae of the nocturnally sub-periodic *W. bancrofti* strain from Tha Song Yang District, Tak Province, Northwest Thailand used in the present study revealed that the morphological features of this species are generally the same as observed by previous investigators (Franz and Zielke, 1980; Maleewong *et al*, 1987; Saitongdee, 1989). The structure of the head (the mouth diameter and the cephalic papillae), body cuticle, anus and terminal caudal papillae are indistinguishable. The only different morphological characteristic that was found in this study was the presence of a small papilliform process on the left side, between the anus and the tail end. This process was absent in *W. bancrofti* strain from Kanchanaburi Province, Western Thailand, that was studied by Saitongdee (1989).

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