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ORIGINAL RESEARCH ARTICLE

Micropores of Acanthocephala, a Scanning Electron Microscopy study

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Abstract. Micropores, used for nutrient exchange, from the integument of 16 species of Acanthocephala were viewed with scanning electron microscopy (SEM) to determine number and pore size. SEM scans were completed for the anterior, mid, and posterior body regions for each specimen. For one species, *Corynosoma strumosum* (Rudolphi, 1802), both SEM and TEM (Transmission Electron Microscopy) were used to further understand the role of the micropore- canalicular system of the helminth. On an average, the highest number and largest pore size occurred in the mid body of the acanthocephalans. Micropores were present for all acanthocephalans examined for this study. Micropore counts and sizes for those helminths of the same family are similar. The TEM micrographs display a definite connection of the micropore with the inner fibrous canalicular system.

Keywords: Micropores; Pore-canal; Acanthocephala; Absorption.

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Introduction

Acanthocephalans (thorny-headed worms) lack a mouth or alimentary canal a feature they share with the cestodes. Thus they have to absorb host nutrients via the outer integument or body surface. Both microtrichs and micropores are present on the integument of the Acanthocephala (Crompton and Nikol, 1985; Amin et al., 2009). There are numerous papers published pertaining to the tegument (cuticle) of the acanthocephalans. Micropores are more common than microtrichs for the thorny-headed worms.

Micropores, which are part of the lacunar system of the Acanthocephala, are located in the tegument which covers the outer surface of the thorny-headed worm. The tegument is pierced with numerous canals or crypts (micropores, microcrypts).

The objective of this study was to determine the location, number, and size of micropores on the surface of 16 species (table 1) of Acanthocephala. Electron microscopy has been a common tool for research studies of the authors in which micropores and surface variations have been included (Amin et al., 2009; Amin et al., 2011; Amin et al., 2012; Amin and Heckmann, 2012b; Heckmann et al., 2012; Oguz et al., 2012; Amin et al., 2013).

Butterworth (1969) studied the body wall ultrastructure of developing cystacanths of *Polymorphus minutus.* Her data indicates that a system of pores and canals are present in these forms, which is similar to adult forms of Acanthocephala. The number of pores increases with growth of larva. Pore canals become evident towards the end of the late acanthella (Amin et al., 2010).

For *Moniliformes moniliformes* (Wright and Lumsden, 1968) canalicular crypts are continuous with pores of the free surface of the body wall and are present for a newly hatched acanthor. Infolding of the plasmalemma increases surface area and represents the beginning of the micropores.

Materials and methods

Specimens of 16 different species of Acanthocephala were processed for this study. A minimum of 5 body areas for each species was viewed with SEM. One species was duplicated *(Neoechinorhynchus zabensis)* since it was taken from 2 different host species. The other species were taken from a single host.

Table 1. Micropore Counts. 20,000x magnification Area: I = 2 cm^2, II = 3 cm^2

Species	I			II			Family
	A	M	P	A	M	P	
Acanthocephaloides irregularis	6	7	6	9	16	15	Arhythmacanthidae
Acanthocephalus ranae	9	9	13	15	23	21	Echinorhynchidae
Acanthocephalus rhinensis	13	11	9	23	23	19	Echinorhynchidae
**Acanthogyrus barmeshoori*	6	-	6	11	-	11	Quadrigyridae
Centrorhynchus globocaudatus	1	3	1	3	7	1	Centrorhynchidae
**Neoechinorhynchus dimorphospinus*	4	-	11	19	-	21	Neoechinorhynchidae
Neoechinorhynchus manubrianus	16	16	16	25	19	39	Neoechinorhynchidae
Neoechinorhynchus zabensis	1	2	1	3	5	4	Neoechinorhynchidae
Neoechinorhynchus zabensis	3	2	1	6	4	3	Neoechinorhynchidae
**Nephridiacanthus major*	7	-	5	14	-	8	Oligacanthorhynchidae
Plagiorhynchus nicobarensis	9	8	11	16	14	17	Plagiorhyneidae
Pomphorhynchus kashmirensis	1	4	3	1	9	5	Pomphorynchidae
Polymorphus spindlatus	15	18	11	23	37	19	Polymorphidae
Pomphorhynchus spindletruncatus	9	11	9	14	18	17	Pomphorynchidae
Radhinorhynchus laterospinosus	1	9	5	3	14	8	Rhadinorhynchidae
Sphaerirostris picae	5	5	4	6	9	6	Centrorhynchidae
Total	**106**	***105**	**115**	**191**	***198**	**213**	
Average	**7**	**8**	**7**	**12**	**15**	**13**	

*No mid body count, reflects on Average; A = Anterior Body; M = Middle Body; P = Posterior Body.

The selected specimens for each species previously fixed in 70% ethanol were placed in CPD baskets and dehydrated in 95% and 100% ethanol for at least 10 min. per soak followed by a critical point drying (Lee, 1992). Samples were then mounted on SEM sample mounts, gold coated and observed with a scanning electron microscope (FEI XL30 ESEM FEG). Some of the specimens were double coated (18 to 20 nm) with gold and palladium using a Quorum, Q750 TES. Digital images of the structures were obtained using digital imaging software attached to a computer and then transferred to an 8GB USB. Emphasis was placed on the outer integument.

The integument of each species was observed and recorded for the anterior, middle and posterior regions of 13 species and the anterior and posterior regions for all 16 species. Duplicate images were taken for each region at 20,000x magnification. The images were printed on an 8 x 10 inches photographic sheet and then studied. Five (n=5) counts for integument micropores were made for both a 3 cm^2 grid and a 2 cm^2 grid placed randomly over the picture for each species. An average micropore count was recorded. For one micrograph both 20,000 and 40,000x magnification were used *(Acanthocephalus ranae).* The size (diameter) of the micropores was measured for each specimen using the

micron bar attached to each SEM micrograph. A range was established for the micropores for each micrograph based on averaging 10 measurements for each. For TEM only one acanthocephalan was sectioned, *Corynosoma strumosum*.

Samples of *C. strumosum* for transmission electron microscopy (TEM) included sections from various trunk parts of 4 worms. Specimens previously fixed in both 5% buffered formalin and 70% ethanol were dehydrated in an ascending series of ethanol solutions to 70% and then stored until processed. Samples were then rehydrated for post-fixation in 1% buffered osmium tetroxide and dehydrated in an ascending series of ethanol, followed by 2 changes of 100% acetone. Specimens were then embedded in Spurr's resin and sectioned with a diamond knife, using an automated ultra-microtome, to a thickness of 80 to 100 nm. After post-staining with Reynolds lead citrate and 5% urinal acetate in 50% ethanol, sections were examined in an FEI Technai T-12 High Resolution TEM (FEI Company). Images at varying magnifications were recorded with a digital camera attached to a computer and then stored on a 8GB USB.

Results

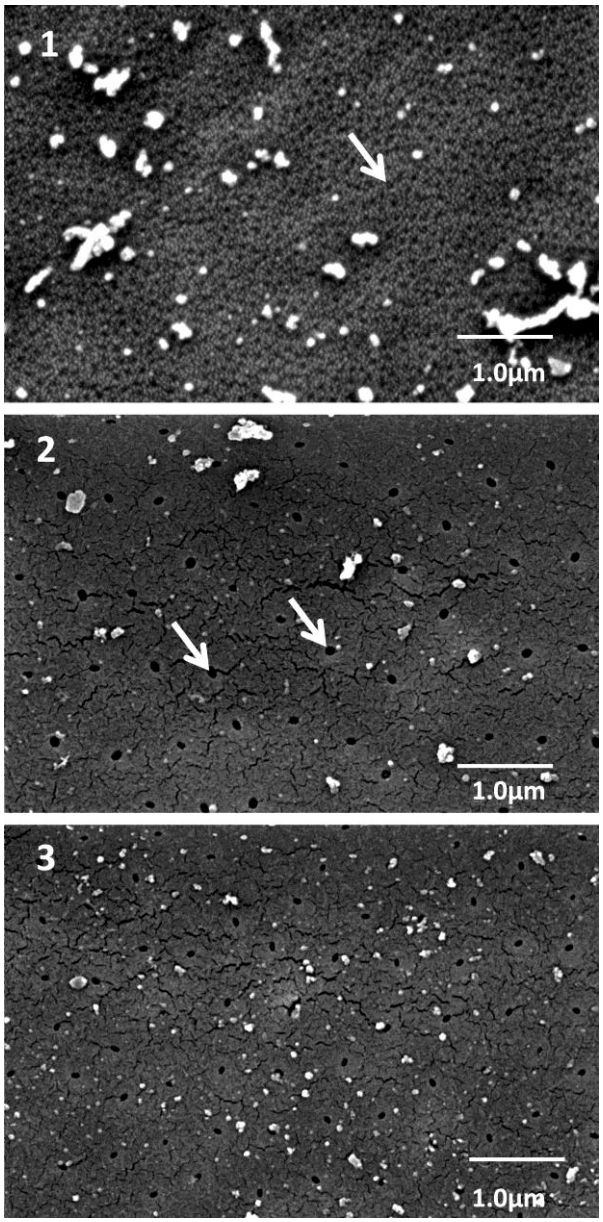
Tables 1 and 2 and figures 1 to 18 represent the results of this study. Micropores were present in all 16 species of Acanthocephala. Each region of the body (Anterior, Middle, Posterior) of the examined acanthocephalans had micropores. Only one species, *Corynosoma strumosum* was examined by both SEM and TEM.

Table 2. Micropore size, range for 10 counts (µm, micrometers)

Species	A (µm)	M (µm)	P (µm)
Acanthocephaloides irregularis	0.05-0.07	0.09-0.12	0.06-0.07
Acanthocephalus ranae	0.07-0.09	0.12-0.15	0.06-0.07
Acanthocephalus rhinensis	0.02-0.03	0.04-0.07	0.03-0.06
**Acanthogyrus barmeshoori*	0.05-0.06	----	0.06-0.07
Centrorhynchus globicaudatus	0.07-0.08	0.08-0.09	0.05-0.07
**Neoechinorhynchus dimorphospinus*	0.06-0.07	----	0.04-0.05
Neoechinorhynchus manubrianus	0.02-0.03	0.04-0.05	0.03-0.09
Neoechinorhynchus zabensis I	0.05-0.06	0.09-0.10	0.05-0.07
Neoechinorhynchus zabensis II	0.06-0.08	0.07-0.09	0.07-0.08
**Nephridiacanthus major*	0.03-0.05	----	0.05-0.06
Plagiorhynchus nicobarensis	0.04-0.06	0.06-0.08	0.07-0.09
Pomphorhynchus kashmirensis	0.02-0.03	0.06-0.08	0.05-0.08
Polymorphus spindlatus	0.03-0.04	0.02-0.03	0.03-0.08
Pomphorynchus spindletruncatus	0.07-0.08	0.06-0.08	0.04-0.06
Rhadinorynchus laterospinosus	0.02-0.03	0.07-0.09	0.07-0.08
Sphaerirostris picae	0.06-0.08	0.08-0.09	0.05-0.07
Total	**0.72-0.94**	**0.83-1.11**	**0.78-1.11**
Average	**0.04-0.06**	**0.07-0.09**	**0.05-0.07**

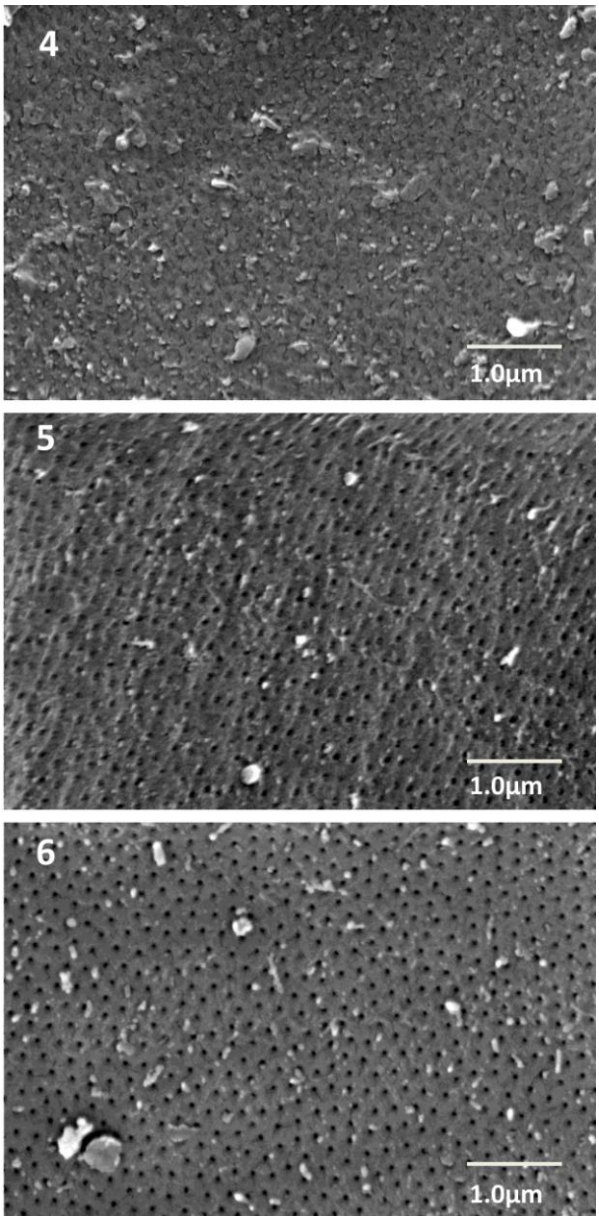
*No middle body examination; A = Anterior part of body; M = Middle part of body; P = Posterior part of body.

Every region of the body (anterior, middle, posterior) of the examined Acanthocephala had micropores. The number and size of the micropores varies from species to species. Table 1 contains the counts of micropores per given area and table 2 the average micropore size. In general the greatest number and size of micropores are in the mid body area of the worm (totals and averages from tables 1 and 2).



Figures 1-3. Variations in the size and pattern of acanthocephalan micropores. Figure 1: the micropores (arrows) are small and form a definite pattern *(Acanthocephalus ranae),* mid body), while Figure 2 (arrows) and 3 show fewer micropores but of a much larger size. Figure 2 is mid body for *Neoechinorhynchus zabensis* and Figure 3 the same species *(N. zabensis)* for the hind body with a smaller micropore opening.

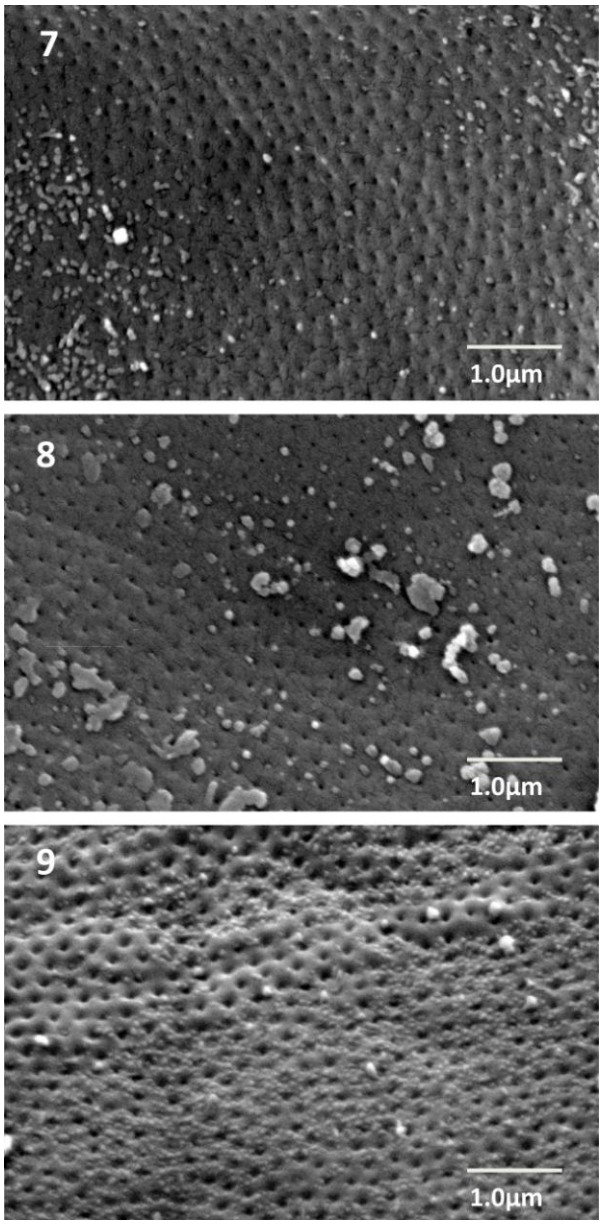
An example for pore counts is *Pomphorhynchus kashmirensis* and *Rhadinorynchus laterospinosus*. Exceptions for this comment about size and number would be *Acanthocephalus ranae* and *Plagiorhynchus nicobarensis.* For pore size *A. ranae*, *N. manubrianus*, and *Acanthocephaloides irregularis* had the largest size in the mid body. Figures 1, 2 and 3 represent variation in pore size (table 2) and number (table 1). Figure 1 *(A. ranae)* shows numerous, small pores while figures 2 *(Neoechinorhynchus zabensis* I) and 3 *(Neoechinorhynchus zabensis* II) show larger pores and more scattered across the integument.



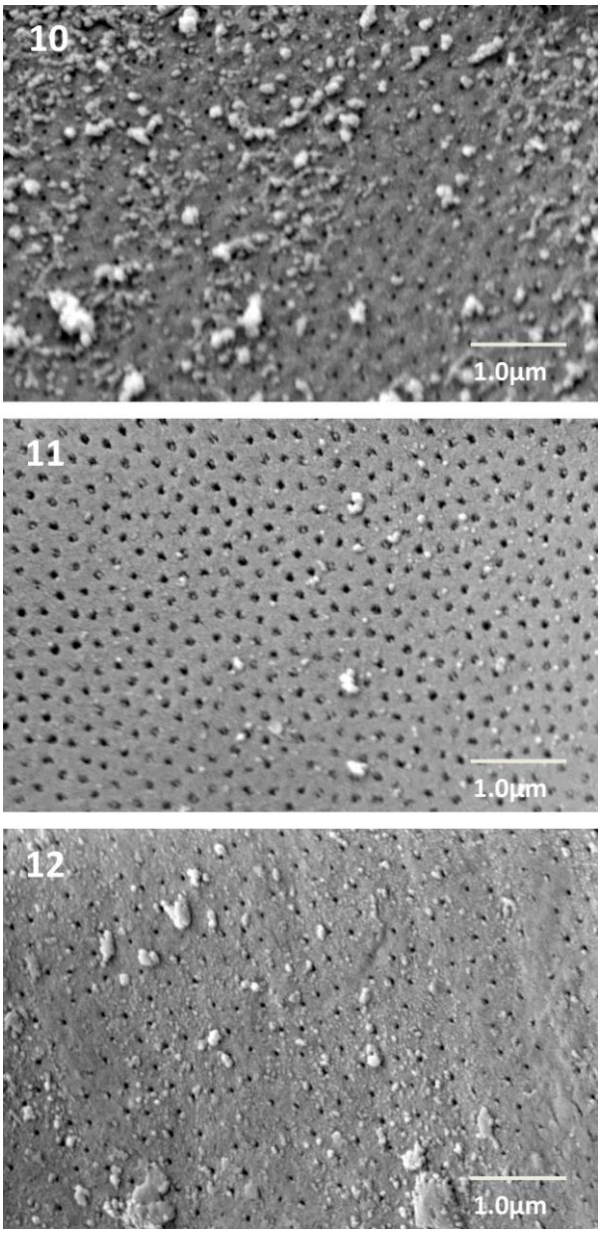
Figures 4-6. Anterior, middle and posterior body of *Acanthocephalus ranae* showing the micropores for the three body regions

Figures 4, 5 and 6 *(A. ranae)* represent the variation in number of micropores by region with the highest number in the posterior part of the body. The largest pore size was in the mid body. Figures 7, 8 and 9 *(Plagiorhynchus nicobarensis)* depict another variation from the

overall pattern. For *P. nicobarensis* the highest number of micropores was noted for the posterior body as well as the size of the micropore opening. Figures 10, 11, 12 are micrographs for the 3 body regions of *Acanthocephaloides irregularis* which follows the average for the specimens both for counts and size of micropores. Note the size and pattern of the micropores for figure 11.



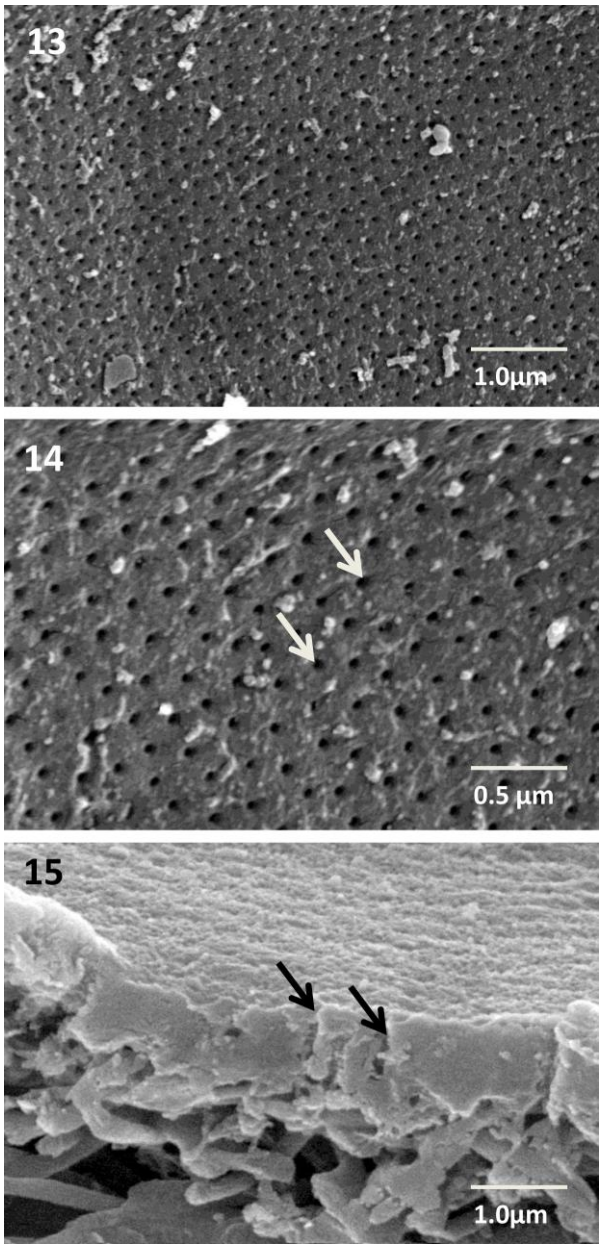
Figures 7-9. Micrographs for the 3 body regions (anterior, mid and posterior) of *Plagiorhynchus nicobarensis*



Figures 10-12. Micrographs from the 3 body regions of *Acanthocephalus irregularis*. Note the size and number of micropores for Figure 11 (mid body) in relation to the other two sections.

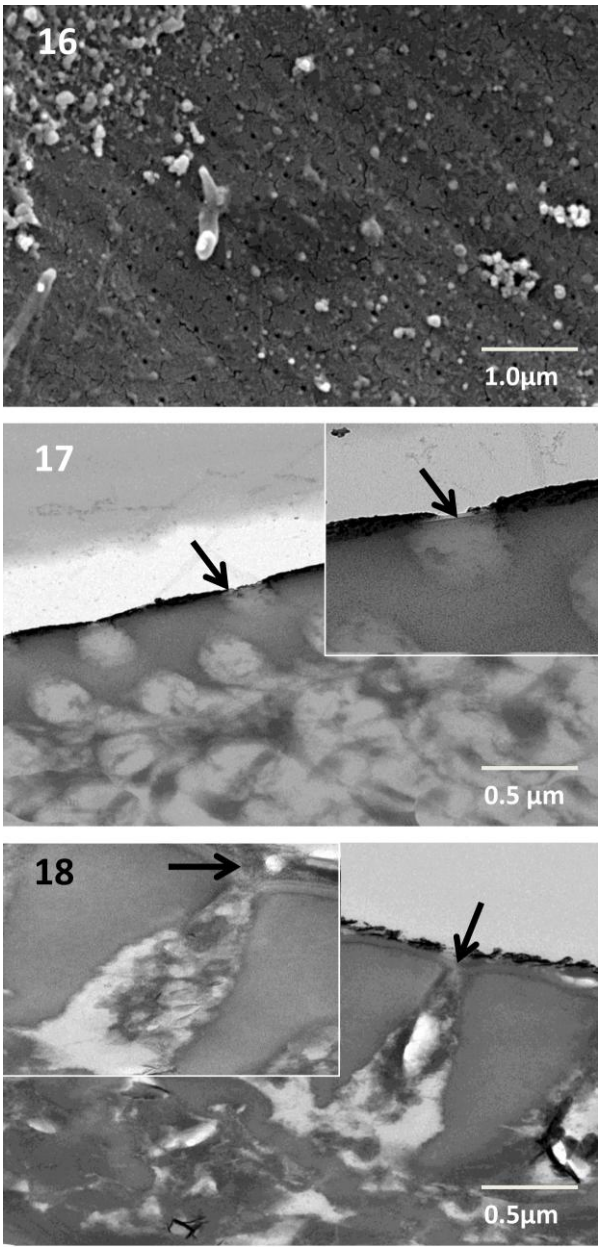
Figures 13 and 14 represent *Neoechinorhynchus zabensis* I (specimen from host I) at two magnifications. All of the specimens were evaluated at 20,000x magnification represented by figure 13. Figure 14 is magnified at 40,000x. Both figures show prominent micropores. Figure 15 is a cut section of the integument *N. zabensis* I whereby the route of the pore into the lower reaches and canaliculi of the integument is visible (see arrows). The results also show close similarity between micropore counts for the same

species *(N. zabensis* I and *N. zabensis* II) which varies more between species of the same genus *(Acanthocephalus rhinensis*, *Acanthocephalus ranae*, and *Neoechinorhynchus dimorphospinus*, *Neoechinorhynchus manubrianus)* and within the same Family *(Centrorhynchus globicaudatus*, *Sphaerirostris picae*, and *N. dimorphospinus*, *N. manubriensis*, *N. zabensis* I and *N. zabensis* II).



Figures 13-15. Figure 13 and 14 represent *Neoechinorynchus zabensis* at 20,000 (Figure 13) and 40,000 (Figure 14) at two magnifications. All figures except Figure 14 are magnified 20,000x. Figure 15 shows the micropores as they enter the lower levels of the integument (arrows).

Corynosomum strumosum was examined with both SEM and TEM. This sectioned acanthocephalan represented the extent of the internal canals and was the only one of the group examined with TEM. Figure 16 is an SEM micrograph of the mid body of *C. strumosum* displaying numerous micropores over the body integument.



Figures 16-18. Micrographs for *Corynosomum strumosum*. Figure 16 is a mid body scan with SEM for *C. strumosum* showing numerous micropores (20,000x) while Figures 17 and 18 represent the results of TEM examination of the same species showing micropores (arrows) as they extend into the lower reaches and canalicular system of the worm. Insets represent isolated micropores for *C. strumosum*.

Figures 17 and 18 represent TEM micrographs of the sectioned integument from two different samples of *C. strumosum*. The micropores extend into the lower fibrous layers of the outer integument which extend into canals and then to the muscle regions and interior layers to the pseudocoel.

Discussion

There has been a long-term interest concerning the ability of helminth parasites lacking a digestive system to absorb nutrients from their host (Lumsden, 1975a; 1975b; Cheng, 1986). This absorption usually occurs across a highly modified integument. Cestodes, lacking a body cavity, have microtriches which are modified microvilli while the intergument of a digenean trematode contains spines on the surface where the absorption surface is increased. The acanthocephalan, pseudocoelomate animals, have micropores and microtriches (Crompton and Lee, 1963; 1965; Amin et al., 2009). Micropores are part of the lacunar system of Acanthocephala (Hammond, 1968; Graeber and Storch, 1978). Microtriches are a major component for cestodes as they absorb nutrients from the host (Gobert et al., 2003; Venkatesh et al., 2006; Chervy, 2009). Microtriches in cestodes vary in number (Heckmann, 2008) similar to micropores in Acanthocephala.

The tegument is an outer covering of the acanthocephalans, cestodes and trematodes. Once considered to be a non-living component it is now know to be a dynamic cellular structure. It forms a protective layer and the host-parasite interface for the worms, serving both secretive and absorptive functions. The integument or tegument for Acanthocephala consists of a cuticle, syncytial hypodermis and underlying parietal layer of muscle fibers (Crompton and Lee, 1963; Yamaguti, 1963; Crompton and Nikol, 1985). This paper emphasizes the micropores and lacunar or canal system of the Acanthocephala. There are both microtriches and micropores for the spiny-headed worms (Amin et al., 2009). Microtriches are highly specialized microvilli covering the entire surface of the tegument of cestodes and some acanthocephalans. We stressed the importance of micropores and there absorptive qualities.

Micropores are part of the lacunar system of the Acanthocephala (Hammond, 1968). The tegument is pierced with numerous canals (micropores) or crypts in the surface area. The micropores or crypts have been calculated to give a 20 to 60 fold increase in the surface area (Lee, 1966). These pores may serve as pinocytic invaginations (Edmonds and Dixon, 1966). These openings represent an entrance into an underlying canalicular system (Nicholas and Mercer, 1965).

The fine structure of the acanthocephalan body wall has been described by previous authors (Nicholas and Mercer, 1965). Early history of this work includes Rothman and Rosario (1961) who published a brief note on the appearance of the body wall of *Macrocanthorhynchus hirudinaceus* under the transmission electron microscope (TEM). Nicholas and Mercer (1965) included an electron micrograph of a section through the body wall of *Moniliformes moniliformes*. Researchers made some observations of an Acanthor (Wright and Lumsden, 1970). Nicholas and Mercer (1965) studied the tegument of *Moniliformes moniliformes* showing presence of numerous organelles and fibers in the syncytial layer with muscle formation at the surface (micropores) suggesting a pinocytic assimilation of nutrients into the canalicular system. There is a fluid-filled canal system within the deeper parts of the hypodermis. Light microscopy techniques further expanded the concepts of the canalicular system. Crompton (1963) made a comprehensive histochemical study of *Polymorphus minutus* using light microscopy.

Since Acanthocephala lack an alimentary system, the body surface establishes the interface for chemical interchange with the host (Wright and Lumsden, 1968). Previous studies have shown the presence of mucopolysachrides in the epicuticle which serves as a shield against the host's digestive enzymes (Monné, 1959; Crompton, 1963).

Butterworth (1969) has studied the body wall ultrastructure of developing acanthella and

cystacanths of *Polymorphus minutus*. Her images and descriptions indicate a system of surface pores (micropores) and canals similar to those described for the adults of various acanthocephalan species (Crompton and Lee, 1965; Wright and Lumsden, 1969).

Butterworth (1969) also suggested that the number of pores increases with growth of the larva, with a pore to canal (canaliculi) system becoming evident toward the end of the late acanthella. The infolding of the outer plasmalemma defines the beginning of a pore-canal system. The folds of the plasmalemma defines the intrahypodermal crypts lined by the membrane and invaginate to form microvilli like structures (Wright and Lumsden, 1970). The above description is similar to the invagination of pinocytic vesicles. We observed surface pores (micropores) for all species studied. Lee (1966) suggested that these structures represent a system of canals and pores specialized for an absorptive function.

Electron microscopic studies by Koehler (1965) have demonstrated that the rotifer integument to be a membrane-limited symplasm with a superficial filamentous "cuticle" and bulblike invaginations of the surface plasmalemma similar to the Acanthocephala.

The plasmalemma beneath the epicuticle is continuous with the lining of the hypodermal canals representing the pore-canal network for the acanthocephalans. The pore-canal system has been considered a more permanent surface specialization for transport (Wright and Lumsden, 1969).

Variations in pore diameter and frequency distribution may reflect differential absorption of nutrients in the anterior, middle and posterior trunk regions of the same worm.

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