

Man, Micro-Parasites, and Electron Microscopy of Trematodes

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ABSTRACT

The very thought of parasites and parasitology is usually enough to conjure up images of exotic tropical diseases at the very least, and to make one's skin 'crawl' with 'cold clammies' at most, but parasites are in many instances a necessity for normal life. Parasites in general have been given 'bad press' in recent years, but the fact is that every human being is constantly predated upon by many (mostly benign) organisms which are only detected with sophisticated scientific equipment, and yet we are no worse for the wear. Some parasites, such as the trematode Ascocotyle group, exhibit such complex and well-designed life cycles that it is difficult to visualise how these inter-relationships arose by chance, or even the 'pressures' of natural selection to survive.

Heterophyid trematode parasitic worms of the genus Ascocotyle which infect certain Cyprinodont and Poeciliid fishes have been described at length by many authors using the standard light microscope technique. Some authors have studied the metacercarial cyst embedded in the heart and other host body tissues under transmission electron microscopy (TEM) at very high magnification, but to-date scanning electron microscopy (SEM) has not been applied to these interesting parasites. In this study, SEM as well as the standard light microscope microtechnique are applied to some members of the Ascocotyle complex. SEM is shown to be a valuable tool for the species level identification of Ascocotyle sibling species. Additionally, parasitism can add to a creationist understanding of design in the biota.

INTRODUCTION

The term 'biodiversity' has, of late, become a 'buzz-word' among the ecologically sensitive to denote the staggering array of types of organisms which now exist or have existed at one time on Earth. As may be expected, there are as many different types of habitats as there are organisms which live in them, and the same can be said for the ways in which those many organisms seek nourishment and a platform from which to multiply. Many organisms are designated as either strictly land or strictly water dwelling (although there are some very rare atmospheric hitch-hikers), but there is another very rich habitat which is not often considered. As Dr Richard Lumsden was fond of saying, 'My boy, there are three habitats in the biosphere —

the aquatic, the terrestrial, and then there is that of other living things!'

In general, a **predator** will attack another organism and consume all or only part of its body, which may or may not kill that prey.¹ In contrast, **scavengers** will derive sustenance from an already dead organism — whether the leftovers of a predator, or one which has expired of natural causes. A unique group of 'critters', comprised of both predators and scavengers, is that group which can draw nutrition (or even housing) only in direct association with members of another species. This association, known as symbiosis, may confer an advantage to one or even both of the partners. In some symbiotic relationships, however, the host organism harbours another, usually smaller species, and provides some beneficial metabolic resources to that

species. This is known as parasitism (although some authors prefer to group all invaders under 'symbionts'). Parasites can be pathogenic (that is, causing injury), or non-pathogenic. If the host is injured in some way by the visitor during close and prolonged association with that host, then the relationship is called an infection. A disease or a train of morbid symptoms often ensues within the host. All parasites, whether pathogenic or not, in very large numbers are capable of injuring a host, but some, like *Escherichia coli*, are necessary for a healthy human life.

Organisms which can only survive by parasitising another organism, whether for food or to complete their life cycle, are known as **obligate** parasites. Some obligate parasites are so 'adapted' that they can only reside within a particular organ or tissue type of their host.² If a specific host population dies or becomes unavailable for whatever reason to the parasite, the parasite (potentially the entire population) may perish or at the least become extinct in a specific area. **Facultative** parasites, on the other hand, may at some point be free-living individuals which can revert to parasitism when the opportunity presents itself.

Not all parasites are bad, however, as some are beneficial to the hosts they live with, and for some hosts even a requirement which they cannot live without.³ As a clinical microbiology manual states:

*'Microorganisms, i.e., bacteria, yeasts, fungi, protozoa, and viruses, are not only ubiquitous in man's environment; they also abound in enormous numbers on and in his body while he is in the best of health.'*⁴

We would not be able to enjoy milk, for example, if it were not for the bacteria living within the bovine gut which can easily digest plant cellulose, something which a cow cannot accomplish on its own. Termites likewise would perish without similar bacteria which live within them to break down the massive volume of wood cellulose they consume. Young termites are hatched without these flagellated partners, but remedy this by eating the faeces of their adult brothers soon after hatching.⁵⁶ Some parasites, however, can become fatal to the host if allowed to multiply in large numbers.

Parasites may or may not have special environmental requirements within which they can live. Those which can complete their entire life cycle within one host are called **autoecious** (one house). Others, known as **heteroecious**, need several different hosts to fulfil all the steps in a complex life cycle or they will not live to produce offspring. Additionally, these visitors can be selective about where on a host they might choose to live. **Ectoparasites**, such as many arthropods, may temporarily visit or even permanently live on the outer layer of their host, while **endoparasites** find refuge somewhere within the host body tissues.

Most major phyla of plants and animals include parasite representatives, and they come in a bewildering array of shapes and sizes. Some well-known examples include bacteria such as *Escherichia coli*, which are found normally

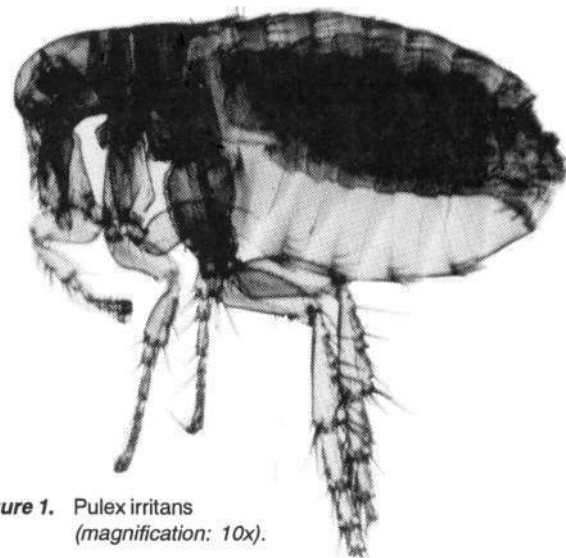


Figure 1. *Pulex irritans*
(magnification: 10x).

(and abundantly) in the human digestive system. It has been estimated by some that the total number of bacteria found living at any one time on the skin of one human is more than all the humans that have ever lived.⁷ All viruses, as far as we know, are strictly parasitic. Among the fungi are the rusts which wreak havoc on many commercial plant crops globally, and also (and perhaps, more well-known) the fungus *Tinea pedis*, which you may find growing contentedly on the moist, warm skin cells between your toes. Many worms are parasitic, such as the tapeworms or *Cestodes* which inhabit the small intestine. What is most amazing about these particular worms is their complete apparent lack of a digestive system, including a mouth. On first examination, this appeared to be a serious problem until electron microscope studies showed that they are literally an intestine turned 'inside out', with all of the full absorptive capabilities of the normal human intestine.⁸⁹

Among the arthropods, we find the well-distributed itch mite or *Sarcoptes scabiei*,¹⁰ which causes scabies, a highly contagious skin disease. Many vertebrates are parasitic, including fish such as the lamprey, which has nearly devastated commercial fish stocks in the Great Lakes region of the United States. Even the tradition of 'kissing under the mistletoe' plant is not untouched by parasitism. The mistletoe itself is a well-known parasite with over 900 species,¹¹ which attach to the bark of a host and then bore deep into the cortex, robbing the host of water and nutrients.

COMMONLY KNOWN MICRO-PARASITES

Man is well acquainted with the flea, *Pulex irritans* (see Figure 1), particularly if one has flea-ridden pets at home, although some fleas will lay in wait until a warm-blooded mammal passes by, and with a grand leap will hitch a ride directly from the sand or ground. They are quite capable jumpers, able to jump hundreds of times their body length, hundreds of times per hour for days, courtesy of a special protein called resilin which has properties closer to

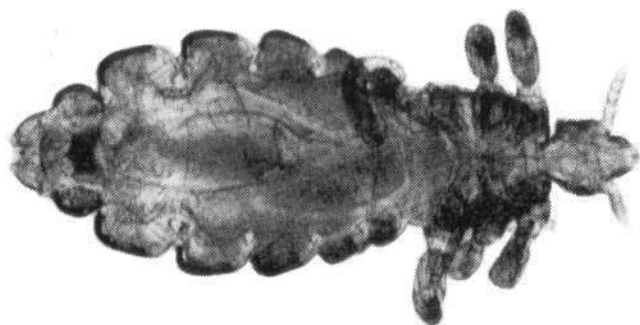


Figure 2. *Pediculus humanus* (magnification: 10x).

an ideal rubber than any other known to man.¹²¹³ Fleas, which live exclusively on a diet of vertebrate blood, feed by burrowing their heads (and sharp mouth-parts) into flesh and sucking away blood and body fluids for a meal. The problem here is that the fleas themselves are parasitised by tapeworms, and also can carry plague and typhus which are then passed to the host while feeding. They can infect cats, dogs and even humans.

Pediculus humanus (see Figures 2 and 3) may also be a familiar pest if your children have ever come home from school with head or body lice, although they may be transferred from person to person by direct contact in any public place.¹⁴ They have very strong claws for gripping, and mouth-parts for chewing, and because they also feed on blood, can transmit typhus and trench fever. The female will lay tiny, white and very hardy eggs on hair shafts, and the body louse can survive for weeks away from a host on discarded clothing.

The hard tick, *Dermacentor* (see Figure 4), is another arthropod ectoparasite, which is again parasitised by certain microorganisms which are harmful to man. In addition to



Figure 4. *Dermacentor* (magnification: 8x).

possibly inflicting paralysis on humans, they can transmit one of the several varieties of treponeme bacteria such as *Borrelia* (see Figure 5), which cause debilitating relapsing fever,¹⁵ as well as up to 25 other pathogens. Because they require what is called a 'blood meal' to grow to the next life cycle stage, they can pick up and transmit any microbes which may be circulating within the blood of any temporary host they encounter. Exhibiting what is called 'questing behaviour', ticks can wait for up to decades for an appropriate blood meal if one is not immediately available. The hypostome, or mouth-parts (see Figure 6), seem rather large to be buried into your skin for feeding, but the tick

has the ability to anaesthetise its new host so that it won't feel a thing.

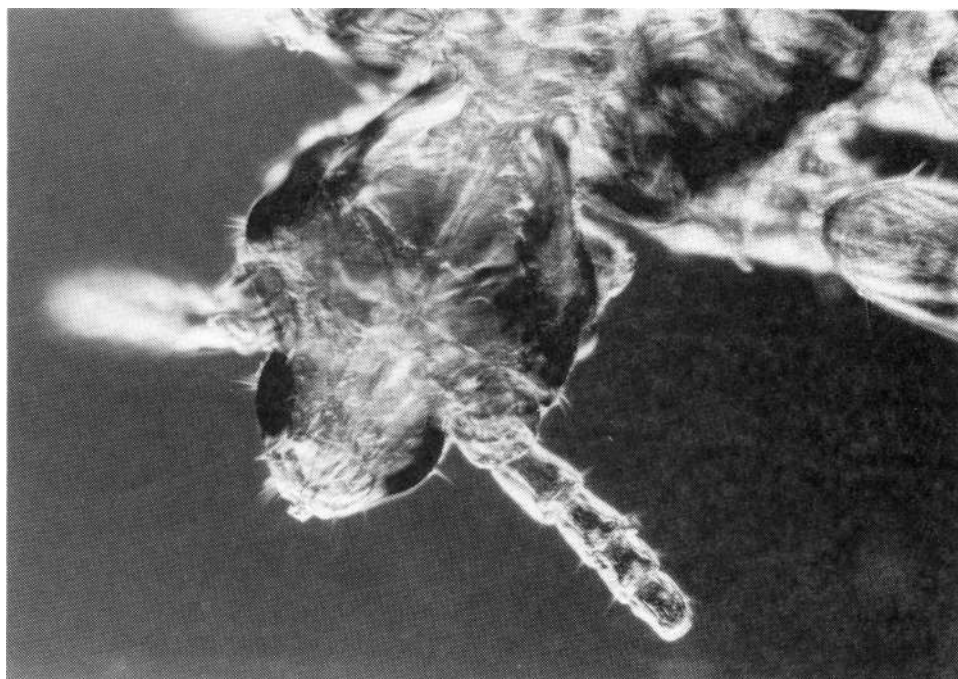


Figure 3. Enlargement of head, *Pediculus* (magnification: 115x).

OTHER SINGLE-CELLED INVADERS

Some species of the lowly amoeba (see Figure 7) are completely benign and may even prevent tooth decay in one instance.¹⁶ Others, however, have been linked to serious problems. *Acanthamoeba* can cause corneal ulcers if allowed to grow on the eye, a particular problem for wearers of contact lenses. *Entamoeba histolytica*, transmitted in faecally contaminated water, will multiply in the host colon and eventually find its way to the liver, lungs, brain and heart.

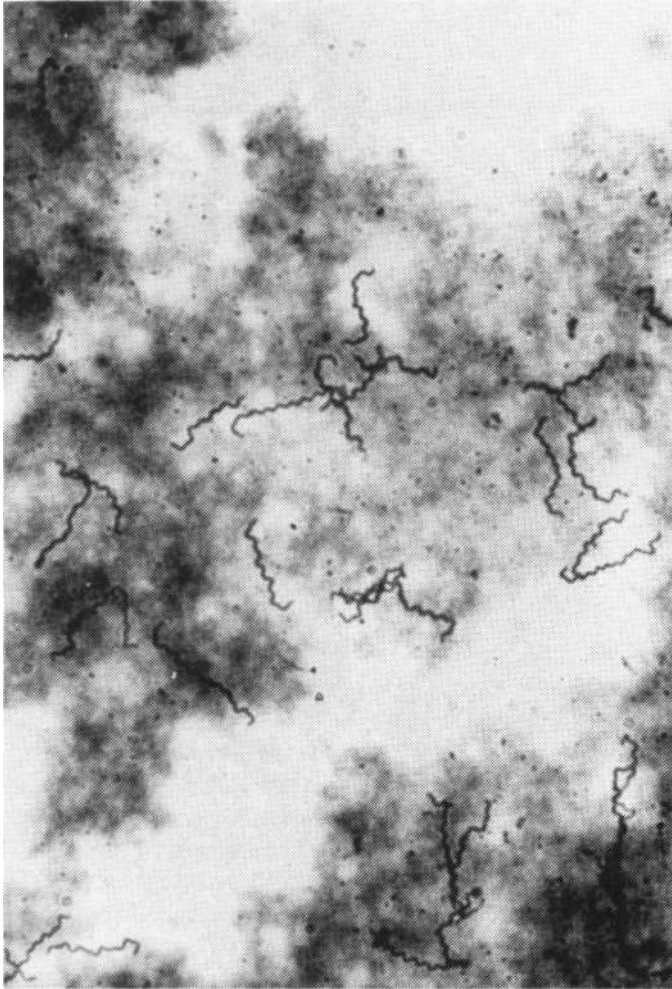


Figure 5. *Borrelia* (magnification: 625x).

AIDS researchers have found that some amoebas may even participate in the spread of the HIV virus, by rupturing immune cells which have attached themselves to the virus in an attempt to destroy it, while *Endolimax nana* may have been recently implicated as the causal agent for rheumatoid arthritis.^{17,18}

Cryptosporidium parvum, a tiny (4 micron) oval protozoan, is turning out to be one of the most dangerous little parasites of the 1990s. Although many cases have been reported as a result of risky sexual behaviour, the main transmission route appears to be faecally contaminated water (human and animal) which contains the cysts. Many have become seriously ill and almost one hundred people have died recently from contaminated city water, where chlorination has no effect on these cysts or on those of *Giardia lamblia* (see Figure 8); they can only be screened out by filtering with 1-5 micron pore filters, an expensive measure which many municipal water suppliers are reluctant to implement.

Many other microscopic parasites affect the human condition, including *Leishmania* (now a famous U.S. visitor from the sands of Operation Desert Storm against Saddam Hussein), *Plasmodium* (malaria), which requires at least

two and sometimes even three separate hosts, many single-celled flagellates, and another well-known treponeme known as *Treponema pallidum* (syphilis) which is experiencing an all-around major comeback with the proliferation of promiscuity. And not to be forgotten are the nematodes, cestodes, and trematodes, all of which also have highly specialised and very complex life cycles.

DESIGNED DIVERSITY

Very few of these parasites share the same transmission and pathogenic characteristics. Just as we see tremendous biodiversity in the macroscopic world, we see a staggering number of body and infection plans in the microscopic world. Parasites look like they were designed. Some parasites require such a specific set of hosts that one wonders how the life cycle ever got started. Once associated with these hosts, the intermediate stage of the parasite is so finely tuned, that to lose the host would mean extinction.

Some parasites can alter the behaviour and even the appearance of the host so as to make the host more susceptible to predation by the exact next host in the life cycle.¹⁹ Researchers at the University of California Santa Barbara have found that a flatworm, *Euhaplorchis*, which requires three separate hosts, infects the brain of small marshwater baitfish and alters their behaviour in such a way as to make them more likely to be eaten by the actual host required for the next stage in the life cycle of the parasite.

Parasites have even been found to exhibit mimicry in behaviour or appearance, which could never have developed by chance. The *Volucella* fly, for example, has the same body-banding pattern as the very wasps which it parasitises.²⁰ The fly will enter the brood comb and lay its eggs on the waste pile where its young can develop, undisturbed by the sentries which guard the nest.

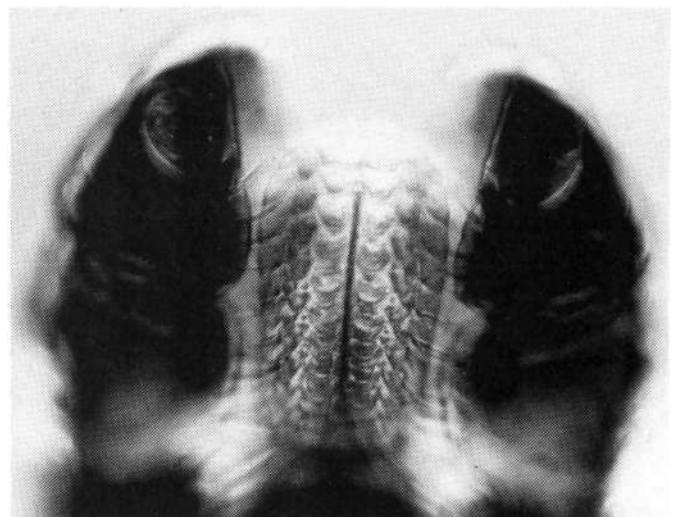


Figure 6. Enlargement of mouth-parts, *Dermacentor* (magnification: 100x).

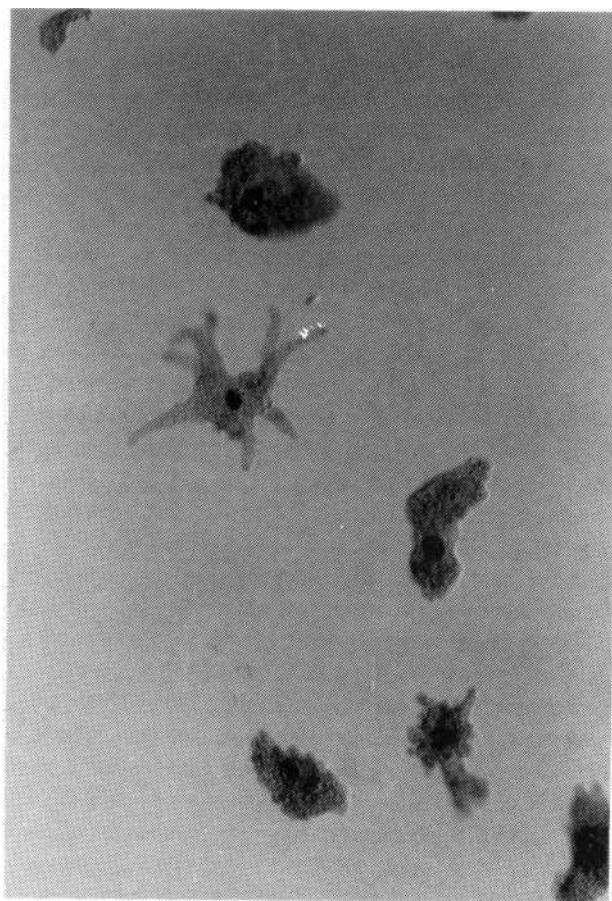


Figure 7. *Amoeba* (magnification: 100x).

A STUDY OF A NON-PATHOGENIC TREMATODE

It has been well established for many years that brackish water fishes are heavily parasitised by several types of opportunistic and obligate endoparasites, including but certainly not limited to the trematodes (flukes).²¹⁻²³ In fact, some authors have referred to fish such as *Gambusia* and *Fundulus* as 'floating zoos', since they can often harbour simultaneous trematode infections of the heart, gill, liver, body cavity, intestine and even the brain, with seemingly little effect on the host.^{24,28} Much understanding has been advanced by several workers using light microscopy on the differing species of trematode parasites and their hosts inhabiting South Eastern coastal, Gulf coastal, Pacific coastal and even South American coastal brackish waters.²⁹⁻³³

One non-pathogenic parasite called the *Ascocotyle* complex of trematodes has an amazing life cycle. This microscopic worm begins its development within certain aquatic snails inhabiting saltwater mudflats, which take up the eggs from the mud they travel over. These eggs and the larvae within them will fail to develop if the right type of snail does not happen to come along and ingest them. After a period of time within the snail in which the redia or brood

sac develops, a host of free-swimming cercaria burst free from the snail and swim towards small bait fish such as *Gambusia* (mosquitofish), where they lose their tails and either enter the mouth and are swallowed or penetrate the gill systems to quickly form cysts in the fish gill, heart, stomach or liver. After some growth, the worms' further development is arrested until the fish is consumed by an endothermic vertebrate, such as fish-eating (piscivorous) birds or mammals (for example, raccoons). It is there that the adult worms mature and, since they are hermaphroditic, or self-fertilising, they produce eggs which leave the intestine of the definitive host to fall into the mudflats where the cycle begins again. There are some 25 varieties of this worm, with only minute differences, such as body length and shape, number and rows of spines, cyst wall shapes, etc. Amazingly, some varieties are host specific for a singular species of fish, even in the presence of other similar fish which they will, for some reason, not infect. My preliminary research indicates that infected (parasitised) fish tend to out-live their uninfected counterparts, suggesting that the parasite may confer some advantage to the fish.

Studies of the particular life cycles for certain species of these trematodes, which include the particular fish and the particular snail required as intermediate hosts and the definitive host (such as the racoon, the egret as well as experimental animals), have been carefully observed and described.^{34,38} These life cycles, and the complete

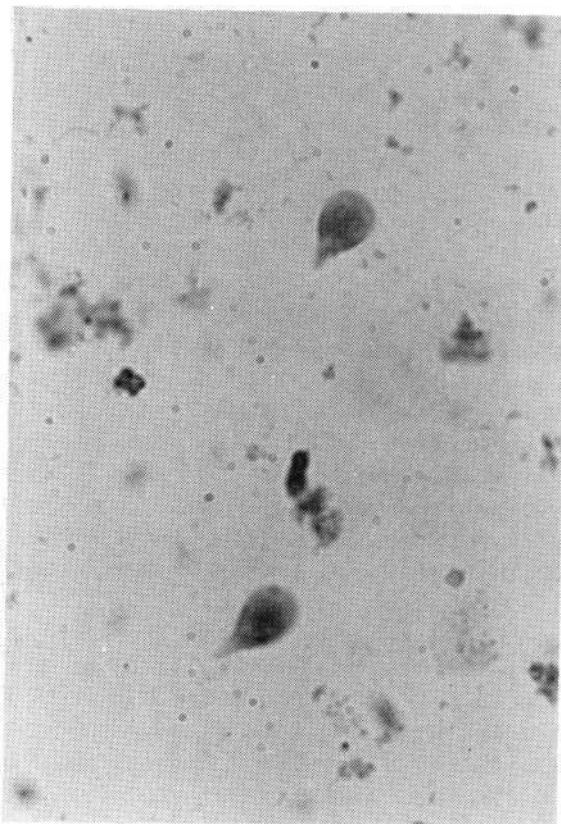


Figure 8. *Giardia lamblia* (magnification: 625x).

description of the adult trematodes found in the vertebrate guts, often include light micrographs, particularly of cross-sections of the infected host tissues, the oral coronet of spines, and hand drawings of the adult whole mount by means of a microscope drawing tube or camera lucida attachment. To date, however, only transmission electron microscopy (TEM), and not scanning electron microscopy (SEM), has been applied to these organisms, and that only to the ultrastructure of the parasite metacercarial cyst wall and surrounding host tissue.³⁹⁻⁴¹ TEM requires that the trematode and the host tissues be destructively sectioned and stained, and only allows minute inner parasite/host tissue relationships and morphology to be studied at very high magnifications, and only in two dimensions. In contrast, SEM affords the opportunity for outer surface micromorphology and details to be clearly imaged in a three-dimensional aspect, with no sectioning required and destructive processing kept to a minimum.

In this paper the application of SEM as well as standard light microscopy and microtechnique will be made to several species of the *Ascocotyle* complex of parasitic trematodes, including whole mounted parasites.

MATERIALS AND METHODS

Sheepshead minnow (*Cyprinodon variegatus*), Sailfin Molly (*Poecilia latipinna*), Mosquitofish (*Gambusia affinis*) and Killifish (*Fundulus parvipinnis*) specimens were collected by seine, dip net and trap method from Ocean Springs, Mississippi on the Gulf coast and from Back Bay, Newport Beach and Point Mugu, California on the West coast. The hearts, gills and livers of these fish were harvested and examined under a Carl Zeiss GSZ dissecting microscope for the presence of metacercarial cysts, indicating possible trematode infections. Some of the hearts and gills were fixed in glutaraldehyde fixative for embedment in both epon and glycol methacrylate for later thin sectioning. Some cysts were gently removed from the heart and gill tissue for mechanical as well as enzymatic excystment and further study under high magnification.

Approximately 25 cysts each of *Ascocotyle leighi* and *Ascocotyle pachycystis* were placed into petri dishes with a 7.5 pH adjusted mixture of saline, RPMI media and trypsin, and introduced into an incubator at 37°C for 3-5 hours. Upon removal, the cysts had dissolved away and free trematode worms moved about actively on the petri dishes. Using fine needle forceps, some of the other cysts were forcibly popped open, releasing the live trematodes for observation under high magnification light microscopy. Some of these were fixed in hot AFA fixative and stained with Van Cleave's hematoxylin stain. They were then mounted with permount on glass slides for further study. Approximately 50 of each of the *A. pachycystis* and the *A. leighi* which had been excysted were then fixed in acrolein/glutaraldehyde and stained for further processing and study under the SEM, as were some 25 *A. angrense* and other, as

yet unidentified, worms from the *Fundulus* gills collected at Newport Beach and Point Mugu, California.

Heart and gill material which had been fixed and dehydrated for embedment was prepared by dicing into 1 mm cubes and placing into BEEM capsules, which were then filled with glycol methacrylate and allowed to heat cure. After curing for a period of 24 hours, the hardened blocks were removed, faced for sectioning and labelled.

Glass knives for sectioning were made from glass knife strips on a LKB knifemaker, and a diamond knife was also secured. Two to five micron (µm) sections of Molly and Sheepshead minnow hearts were cut with diamond and glass knives on a Sorvall, Porter-Blum MT-2 microtome and a Reichert OM U-2 ultramicrotome, affixed to heated glass slides, stained with Methylene Blue and Azure II, and cover-slipped.

Light photomicrographs were taken of whole live material, of sections, and of fixed, stained worms on a Carl Zeiss Jenaival Contrast microscope in bright-field, dark-field, phase contrast and Nomarski modes.

Trematodes which had been fixed for SEM study were processed through Osmium, Thiocarbohydrazide and a graded series of alcohols up to absolute alcohol.⁴² Individual specimens were at first dehydrated by the critical point drying method, but many specimens were lost when the chamber was vented. Finally, specimens were dehydrated in acetone, and then transferred by hand under the dissecting microscope to SEM stubs, where they were sputter-coated for four minutes at 30 mA deposition. Stubs were then transferred to a Jeol JSM 35 Scanning Electron Microscope and were observed and photographed at 100-2000X magnification at 15 and 25 kV.

RESULTS

Figure 9 shows a typical 50 mm (2 inches) long example of *Fundulus parvipinnis* collected at Point Mugu, California. These fish were observed in a laboratory aquarium for a week before examining for parasites, and although it was not uncommon to find every gill filament in these fish harbouring cysts, the observed fish behaviour demonstrated no obvious impairment as a result of the infection. In contrast to *Fundulus* collected elsewhere, those collected at Point Mugu, California showed no parasitic infection in the heart or liver. The gut was not examined. Figure 10 shows a cyst bearing a metacercaria of *A. angrense* laying along the cartilage shaft found normally inside each gill filament. Worms were found to have penetrated this shaft and even to have encysted inside of it, as shown in Figure 11. Whole live cysts of *A. pachycystis* in heart tissue of *Cyprinodon variegatus* collected from Mississippi are shown in Figure 12. Some of these hearts contained over 1,000 cysts and yet the fish was living. No evidence of pressure necrosis, due to the pressure of the cysts on any of the tissues, was found in any of the infected fish. Material that was thin sectioned is shown in Figures 13-16. The

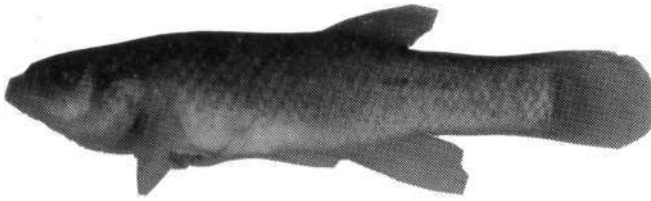


Figure 9. *Fundulus parvipinnis* (magnification: 1x).

marked differences in cyst structure between *A. leighi* and *A. pachycystis* are readily apparent, in that *A. pachycystis* seems to not only have a thicker cyst wall, but one composed of multiple layers as reported by Stein and Lumsden.^{43,44}

Figure 17 shows a whole, excysted *Ascocotyle gemina*. Mechanical excystment was a time-consuming process, as the cyst first had to be removed from the host tissue and then opened. Cysts removed from host tissues had very active worms inside, which rolled and turned actively inside their cramped quarters. Very often the mechanical excystment process using fine needle forceps resulted in the complete tearing of the worm, because the cysts were so elastic and hard to handle. This often rendered the worm unusable for both light and scanning electron microscopy.

Figures 18 and 19 show SEM micrographs of a whole, excysted *A. pachycystis*. Note that the body is completely

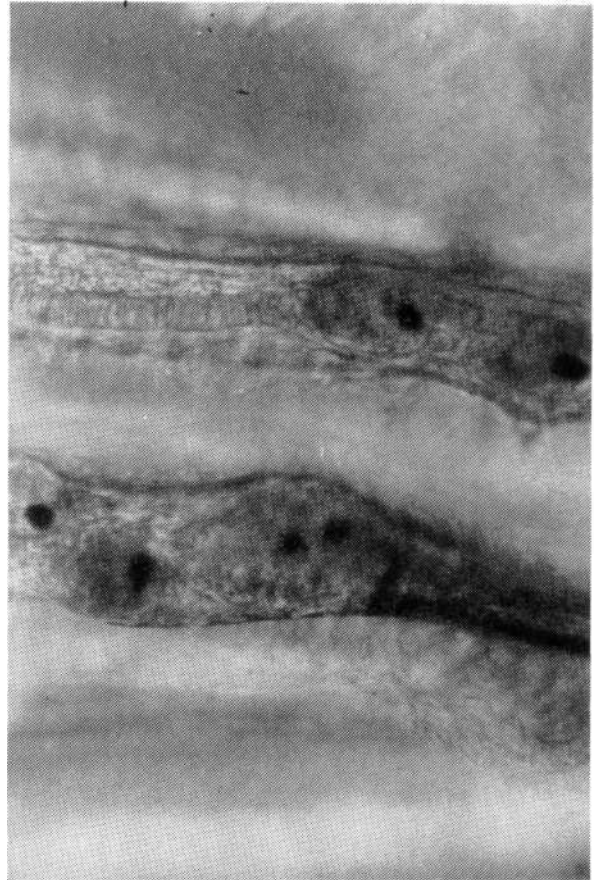


Figure 11. *A. angrense* cysts, inside gill filament shaft (magnification: 150x).



Figure 10. *Ascocotyle angrense* cyst, on gill filament (magnification: 312x).

spinose and that two complete rows of large spines encircle the oral sucker. In contrast, *A. leighi* is shown in Figures 20 and 21. Note the well-defined acetabulum or ventral body sucker, as well as the two rows of spines around the oral sucker. One of the few morphological differences between these two worms, which may represent sibling species, shows up clearly in these micrographs. *A. leighi* demonstrates a pronounced anterior lip-like projection,⁴⁵ which is much less pronounced and more rounded in *A. pachycystis*. This feature shows up very well here, in contrast to the light micrography used by other authors. *A. angrense* was expected to be found in *Fundulus* collected on the West coast, based on the work of Sogandares-Bernal and Lumsden,⁴⁶ and indeed, they showed up in great numbers. Note that these worms (see Figures 22 and 23) exhibit a single row of spines around the oral sucker. What was unexpected was the find shown in Figures 24, 25 and 26, which represents an as-yet-unidentified *Ascocotyle* trematode with two complete rows of spines, found within the gills of the *Gambusia affinis* collected at Back Bay, Newport Beach. This worm is much smaller than *A. angrense*, and in fact, may even represent a new, undescribed species.



Figure 12. *A. pachycystis* cysts, heart tissue (magnification: 80x).

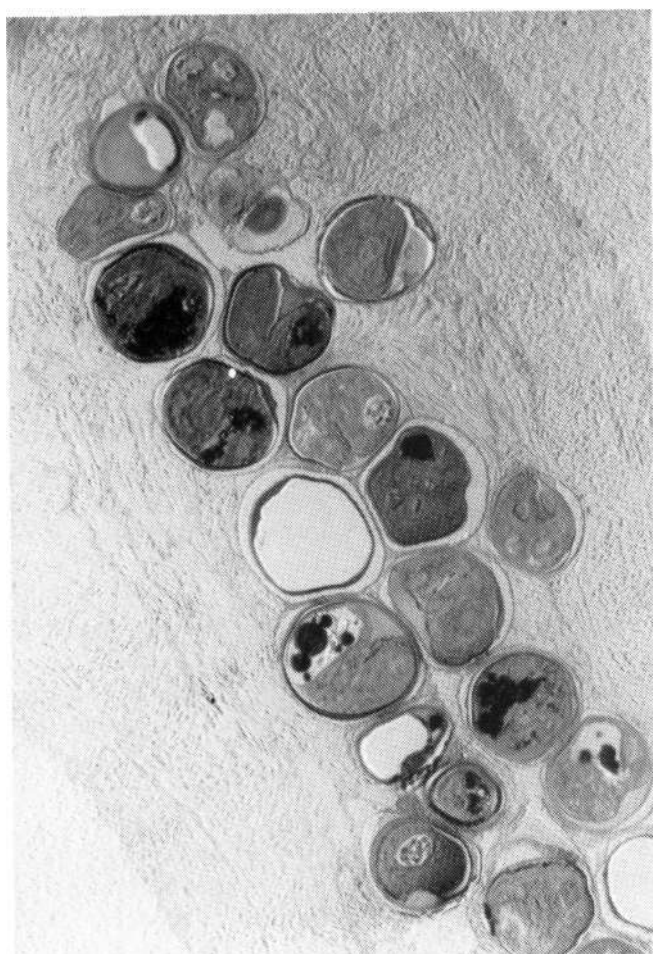


Figure 13. Cross section, heart tissue with *A. leighi* cysts (magnification: 80x).

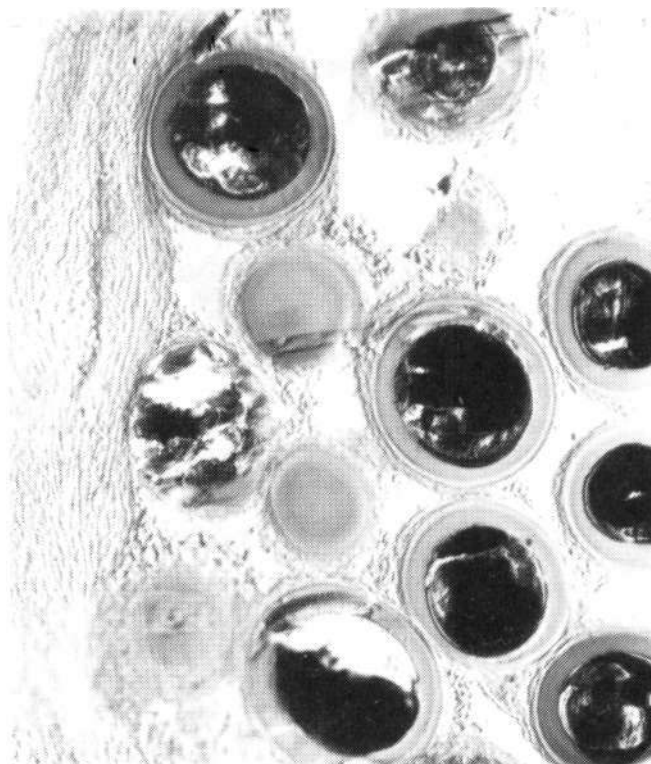


Figure 14. Cross section, heart tissue with *A. pachycystis* cysts (magnification: 80x).

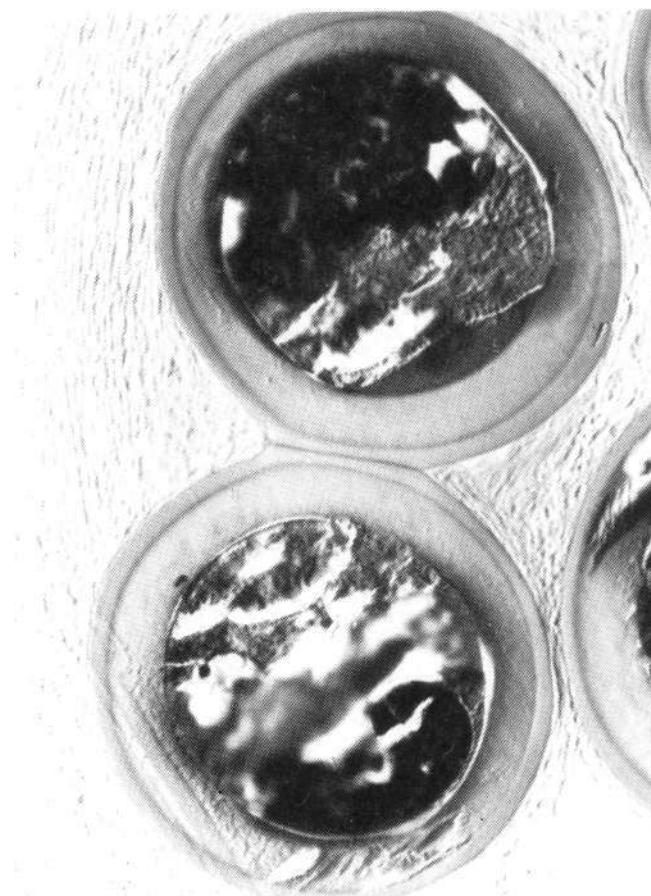


Figure 15. Enlargement, *A. pachycystis* cysts (magnification: 200x).

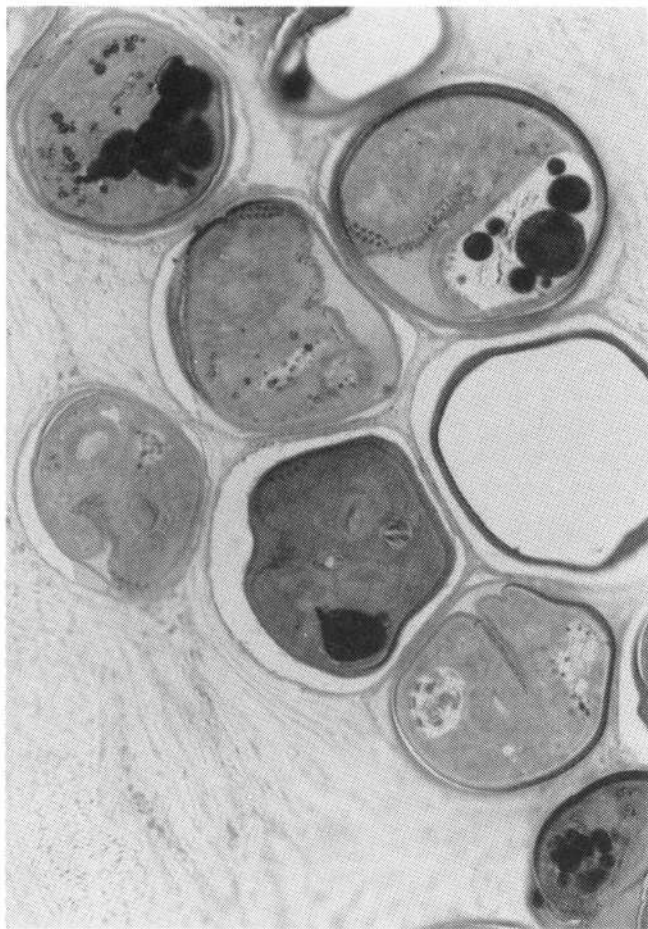


Figure 16. Enlargement, *A. leighi* cysts (magnification: 200x).

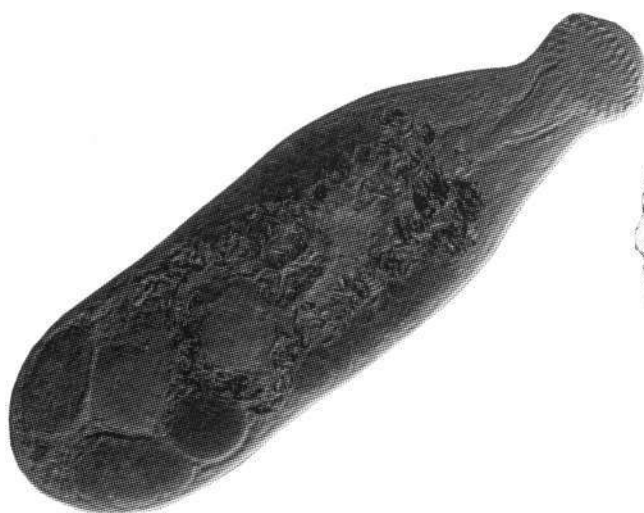


Figure 17. Whole mount, *A. gemina* (magnification: 150x).

DISCUSSION

It was readily apparent that the cysts removed from the *Cyprinodon* hearts were *Ascocotyle pachycystis*, due to the unusually thick cyst wall as described by Lumsden,⁴⁷ while those removed from the *Poecilia* hearts were *A. leighi*, showing a much thinner cyst wall. A heavy parasitic

CEN Tech. J., vol. 11, no. 1, 1997

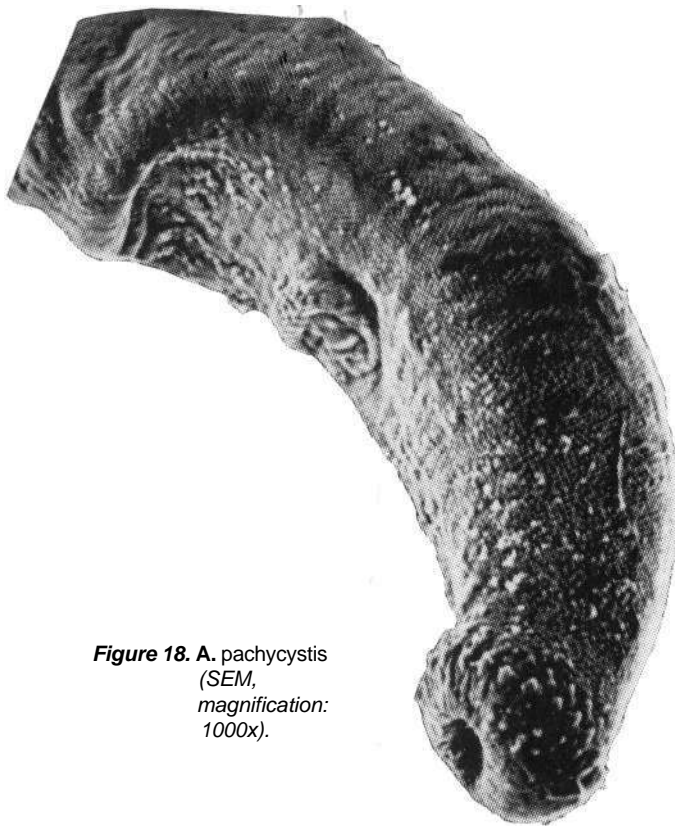


Figure 18. *A. pachycystis* (SEM, magnification: 1000x).

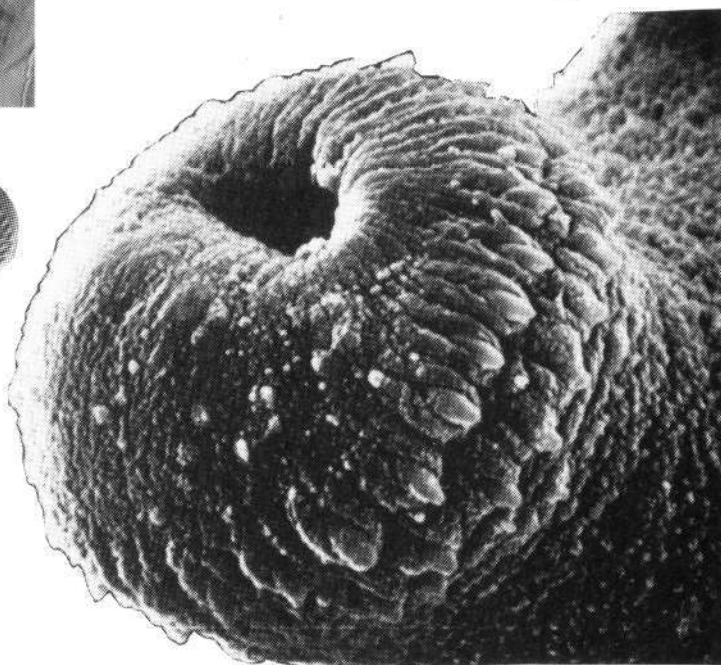


Figure 19. *A. pachycystis* (SEM, magnification: 1800X).

infestation of the heart of these fish may enlarge the heart by some 20 times with over 1,000 and up to 8,000 cysts, as it can come to resemble a bag full of marbles with little apparent deleterious effect on the fish.^{48,49}

The *A. pachycystis* cysts were very hard to mechanically break and took up to six hours to dissolve in trypsin at 37°C, while the *A. leighi* cysts dissolved completely within

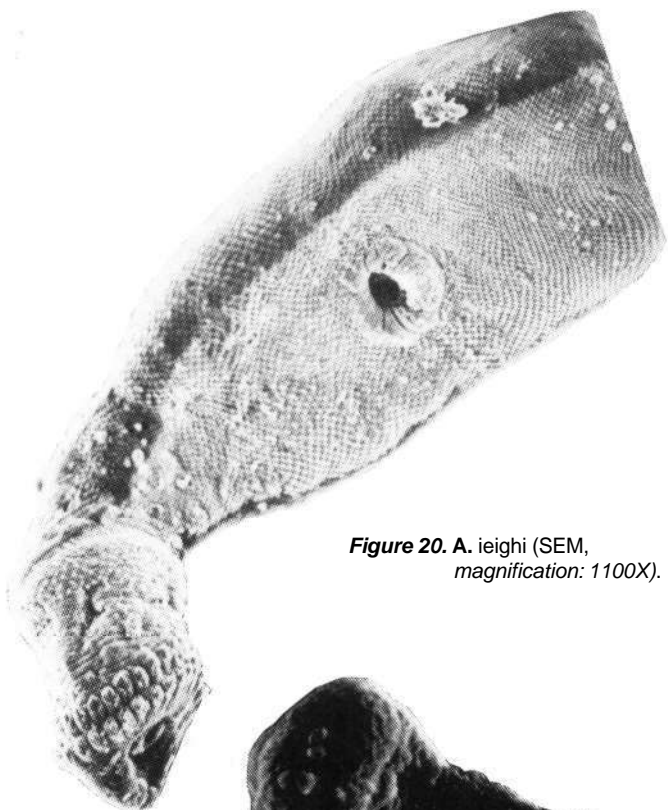


Figure 20. *A. ieighi* (SEM, magnification: 1100X).



Figure 23. *A. angrense* (SEM, magnification: 2000X).



Figure 21. *A. leighi* (SEM, magnification: 1800X).

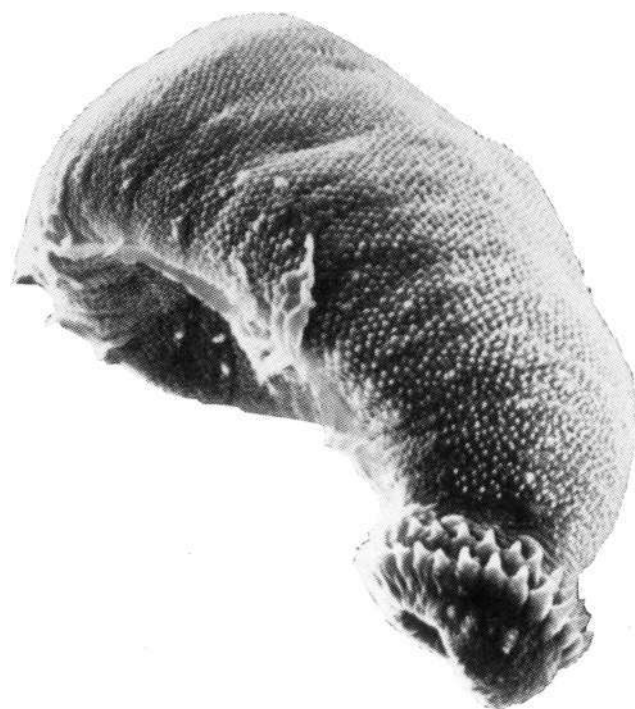


Figure 24. *Ascocotylesp.* (SEM, magnification: 780X).

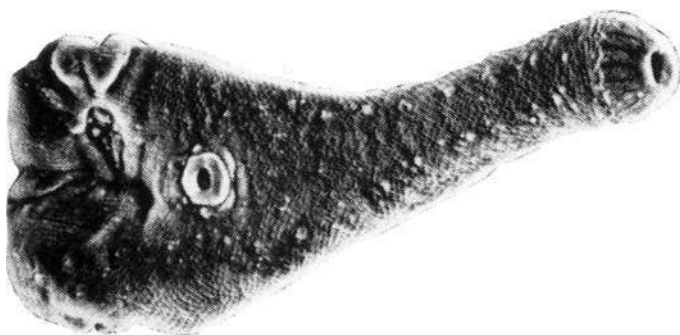


Figure 22. *A. angrense* (SEM, magnification: 440X).

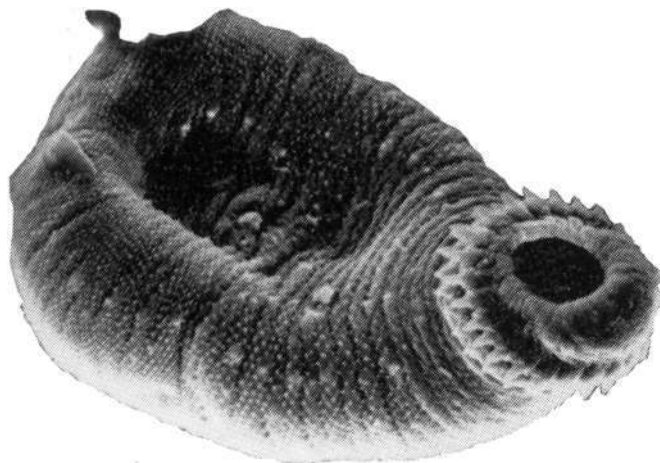


Figure 25. *Ascocotyle* sp. (SEM, magnification: 720X).

two hours. This suggests that the thickness alone may not be the only factor in the dissolution of the cysts, but that a different chemical matrix may inhibit ready digestion by trypsin. In addition, the *A. pachycystis* cysts seemed to 'balloon up' much more than the *A. leighi* did during this process, with the worms moving about very actively inside the cysts, as if searching for an opening or pushing on the inner cyst walls as if to break them. In fact, some workers have reported that cyst dissolution begins on the inside and is aided mostly by the active parasite.⁵⁰

There are few morphological differences between *A. leighi* and *A. pachycystis*, but the host specificity and cyst differences are dramatic, as seen here and as reported by Burton,⁵¹ Schroeder and Leigh⁵² and Stein.⁵³ Some species of *Ascocotyle* are also organ specific within the same host, such as reported by Stein and others.^{54,56}

Periodic Acid Setoff's (PAS) staining, which should accentuate the glycoprotein layers in the cyst wall, was attempted with limited and varied results. In some instances whole cyst layers were differentiated, in others the stain was taken up by different and unrelated portions of the cyst and trematode tissue.

Great care must also be taken in processing tissues for SEM, as contaminants, however small, show up readily under high magnification. Failure to maintain ultra-clean glassware and to adequately buffer-wash away excess thiocarbonylhydrazide (TCH) during the OTO method of post-fixative staining, can occlude very small surface structures including spines, rendering them invisible to the SEM.⁵⁷ The orientation of the specimen on the stub was very time-consuming and often ruined many hours of prior work when not done properly and under an adequate dissecting microscope of sufficient magnification and illumination.

Species of the *Ascocotyle* complex are identified by organ placement (such as ovaries, testes and seminal receptacle) within the body of the worm. In addition, host type and host organ specificity and spine shape also play a part in identification.⁵⁸ SEM micrographs show that the

spine shapes and other external structures are clear and well-defined and thus can serve to assist in the species identification, but the processing of the worms through osmium and sputter-coating prevents any look inside the body for organ examination.

PARASITES, SCRIPTURE AND CREATIONISM

Parasites are dealt with extensively in Scripture. God gave the Jews very strict and very effective dietary and personal hygiene laws, and this was several thousand years before we became 'smart' enough to treat our water and sewerage systems. In fact, most human parasites can be avoided if one eats safe foods and washes his face and hands twice a day! There are over 170 references in the Pentateuch to safe foods for eating and cleanliness. The concept or command to wash is used 44 times, while there are rules governing sexual relations 27 times, and 26 references to mildew or moulds. An apparently strange command given by God to Moses in Deuteronomy 23:12-13 is that of digging holes for excrement. Other than possibly eliminating bad odours from the camp and reducing the numbers of flies, there was no other logical reason to take a shovel and cover up the excrement. Only with sophisticated microscopes, which began their development in the late 1700s, did man fully come to comprehend the microbiological epidemiology behind this simple but highly effective command.⁵⁹

Parasites are found in both the plant and animal kingdoms and across most animal phyla. Many parasites

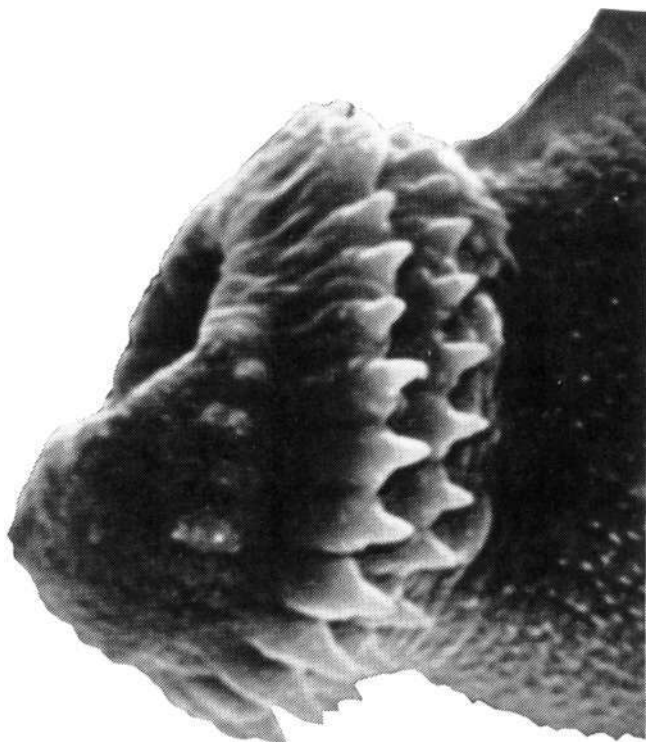


Figure 26. *Ascocotyle* sp. (SEM, magnification: 1800X).

confer a distinct advantage to the host within which they reside. That fact, and the fact that multiple and often completely disparate hosts are necessary for these organisms to complete their complex life cycles, is at once bewildering from an evolutionary perspective and indicative of the design principle held by creationists. One might ask, 'By what evolutionary mechanisms or selection pressures were *Ascocotyle* trematodes urged to cycle through snails, fishes and birds?' Or, 'What cosmic chemistry professor of chance produced a multi-layered cyst, capable of HCl (and therefore stomach acid) resistance, and yet susceptible to hot digestive enzyme dissolution in a pH of 7.4 (as found within the lower intestine of herons and egrets)?'

Furthermore, it does not make strategic sense from an evolutionary point of view for a highly specialised organism such as *Plasmodium* (malaria) to base its complete existence upon a mosquito which must feed on warm-blooded animals not once, but twice, in order to complete the malarial life cycle. Without the Anophelene mosquito, the sporozites cannot be transferred into a warm-blooded animal where the asexual developmental stages take place inside of its red blood cells. Could this feeding specificity represent a post-Fall change in mosquito behaviour? Again, without the mosquito, the gametes produced in the asexual stages cannot be transferred back to the mosquito stomach where the malarial zygote is formed. It is most difficult to accept that such elegant design parameters arose from trial and error. Such inter-relationships speak not of quick and dirty 'survival of the fittest', but of elegantly designed biocompatibilities, which only God is capable of producing.

On the other hand, many a student of theology would shudder at the idea that parasites were expressly designed by God to invade (for whatever reason) and reside within unsuspecting biological hosts. This simply does not conform to their picture of God. And yet, there they are — at the end of an electron microscope. Parasites must have been a part of the original creation, since '*... the heavens and the earth were completed in all their vast array*' at the end of Day Six of the Creation Week (Genesis 2:1). No new organisms were created after this point in Earth history. Since parasites are often beneficial to their host, this is consistent with a 'very good' creation (Genesis 1:31).

What role then did malaria play in the pre-Fall world? Does the idea of a mosquito extracting blood from a garden-animal violate our concept of the shedding of blood taking place only after Adam and Eve sinned? (The mosquito only extracts a minute quantity of blood, whereas in Scripture the term 'shedding blood' means killing, that is, taking of life.) Did warm-blooded organisms happily offer an arm or a leg to a hungry mosquito, or did these parasites feed on plants as well?

Post-Fall differences could have resulted in a change in parasite behaviour, resulting in harmful forms. Or could it be that their original design parameters were 'robust' enough to function in a 'very good' as well as a sinful world? It is not in a parasite's selective interest to harm its host, so it is

quite possible that these parasitic organisms once provided a benefit to them. Parasitism contributes nothing to evolutionism save the many questions left unanswered by stunning life cycles and highly specialised structures.⁶⁰ Parasitism, however unsettling its theological implications, serves creationism by enlarging our understanding of the biota without compromising our foundational principle of design by the Creator. To be consistent with Scripture in understanding parasites, however, it must be acknowledged that pre-Fall life cycles could not have included transferrals of parasites through carnivorous activities, such as we see with piscivorous birds and the *Ascocotyle* parasite. Somehow, this complex life cycle must have been completed within a system which did not involve the death of an intermediate host.

The strangest twist of all is that the only way of gaining eternal life for ourselves is to cling (parasitise if you will) to Him Who offers us that life.

CONCLUSIONS

Parasites, including micro-parasites, are a part of everyday life on and in the human body, and in the world around us. An SEM study of the parasitic trematodes of the *Ascocotyle* complex of species has yielded significant results, showing the bodies to be completely spinose and providing a clear image of the size and shape of oral spines and other unique features, such as the pronounced anterior lip. Much further work must be done, however, including the acquisition of better micrographs of the oral coronet and acetabulum, micrographs of the inner and outer cyst surfaces, and of the host tissues which are in close proximity to the cyst. In addition, SEM study of the other unique stages in the life cycle of this parasite, such as the cercarial and redial stages, as well as the tissues of its other hosts, would further develop our understanding of these amazing organisms.

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DEDICATION

This paper is dedicated to the memory of Richard Dick

Lumsden, Ph.D., my mentor, dear friend, brother in Christ and fellow soldier on the Creation battlefield, who went to be with his Lord on January 12, 1997.

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