Trematode Parasites: What Is Their Genesis?

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Abstract

An overview of trematode[1] parasitology from the evolution and creation perspectives is presented, including a discussion of the "design-like" features of these parasites. No credible evolutionary explanations are found in the evolutionary literature to account for these "design-like" aspects. Histological microtechnique for electron microscopy is also reviewed and some cyst ultrastructural data are reported. A caring God may have designed trematodes, now recognized as parasites, to serve other functions before the Fall of man (Genesis 3).

Introduction

The purposes of this study are to review some of the "design-like" behaviors and structures of this group of parasites, to search for a reasonable explanation for their existence in the evolutionary literature, and to provide a creation-based explanation for their origins.

As shown previously (Lumsden and Armitage, 1999), digenetic, heterophyid trematode parasitic worms of the genus *Ascocotyle* infect certain amnicolid snails as first intermediate hosts (such as *Littoradinops*). They also infect certain cyprinodont and poeciliid estuarine fishes (*Cyprinodon*, *Poecilia*, *Fundulus*, *Gambusia*), as second intermediate hosts in a three-step life cycle. The hermaphroditic, adult trematode worms mature in the intestines of definitive hosts, most often piscivorous birds, but also certain mammals such as the raccoon. The *Ascocotyle* group comprises some 30 different species, which vary by mostly minute morphological differences, such as spine count and shape, organ position and size, and organ shape within adults. They also vary in metacercarial cyst shape and thickness, location of infection within the second intermediate host, and the specific host type.

The cyst wall configuration, as observed by TEM (this paper) also can serve as a diagnostic species characteristic. It is on the basis of these morphological features and not reproductive isolation alone that species are identified within this group. These parasites cannot be classified on the single basis of reproductive isolation, as many animal species are, because trematodes are hermaphroditic and several species often inhabit the same estuary.

All of these observed differences are considered by creationists to be at the microevolutionary level of variation, or normal variation within a created kind. The creation model of origins predicts small changes within a kind based on the genome designed by the Creator at the beginning. In contrast, very large scale genomic changes, as espoused by the evolution model of origins, would be necessary to change the *Ascocotyle* worm into a cestode or an annelid, if it could be done at all.

Ascocotyle parasites have been described from Argentina (Ostrowski de Nunez, 1976; 1992; 1993), Brazil (Travassos, 1930), the Caribbean (Price, 1935), Egypt (Looss, 1899), Eastern U.S. (Stunkard and Uzman, 1955; Burton, 1956; 1958; Leigh, 1954; 1956; 1959; 1974; Hutton and Sogandares-Bernal, 1959; 1960; Harkema and Miller, 1962, Miller and Harkema, 1962, Stein, 1968), Gulf Coastal U.S. (Font, Overstreet and Heard, 1984; Font, Heard and Overstreet, 1984; Sogandares-Bernal and Bridgman, 1960; Sogandares-Bernal and Lumsden, 1963; 1964; Lumsden, 1963a, b; 1968), and California (Armitage, 1997b; 1999) but there is a significant paucity of reports of incidence from the Western U.S.

Some workers have reported on the apparent pathogenicity of the *Ascocotyle* genus (Martin and Steele, 1970; Snyder et al., 1989; Font, Overstreet and Heard, 1984; Font, Heard and Overstreet, 1984), but many more have shown large numbers of parasites within infections of no pathogenicity (Stunkard and Uzman, 1955; Burton, 1956; Lenhoff et al., 1960; Lumsden, 1963a; Schroeder and Leigh, 1965; Skinner, 1975; Coleman, 1993; Ostrowski de Nunez, 1993) other than occasional blood flow impediment caused by mechanical occlusion within the heart or

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[1]This term and other technical terms are defined in a list of definitions at the end of this paper.

the circulatory system. Some significant ultrastructural observations (Lumsden, 1968; Stein and Lumsden, 1971a, b; this paper) using TEM, show that there is absolutely no pathogenicity or host immune response resulting from the presence of the parasite within host tissues. Many workers have even reported that heavily infected snails and fish live for up to a year or more in laboratory aquaria (Leigh, 1956; Stein, 1968; Font, Heard and Overstreet, 1984, and my own observations over many months).

There has been very little discussion by previous workers regarding the complexity of the *Ascocotyle* life cycle (which requires parasitization of three distinct hosts for completion of its life cycle) and what mechanisms may have brought this multifarious system into place. Most, if not all of the evolutionary literature on digeneans in general and ascocotylids in particular fails to develop a credible, empirically-based phylogeny for these organisms.

Some aspects of the *Ascocotyle* life cycle exhibit "design-like" features in behavior, morphology, and structure. These features take the form of behaviors which guide the microscopic parasite to the appropriate host (even in the presence of other fishes), behaviors and sensory papillae which guide the parasite to the appropriate organ for encystment, and specialized structures which allow tissue penetration. There are also other complex structures which control safe passage of the parasite through the digestive or circulatory systems of intermediate and definitive hosts and minimize host immune response.

Workers at the University of California Santa Barbara, have collected some of the same hosts used in this study and have described the actual alteration of host behavior by trematode parasites to ensure predation by the definitive host (Lafferty and Morris, 1996). Their failure to provide an evolutionary explanation for such a highly designed system, however, is evident. Other limited discussions of evolution within the *Ascocotyle* group are offered (Sogandares-Bernal and Lumsden, 1964; Skinner, 1975; Font, Heard and Overstreet, 1984), but no serious explanation has been proposed for how these complex life cycles were initiated and how they arrived at their present state.

The real question is, can parasites be designed? If they can be designed, what would constitute a design feature and how would it be recognizable as such? If certain features are attributed to design, can evolutionary explanations likewise be made for them? If parasites such as *Ascocotyle* were designed, can that design be attributed to a Master-Designer within a Biblical creationist model of origins?

Implications for Creationism

The heteroecious life cycle of the *Ascocotyle* parasite is complex and it clearly shows that it is obligated to a cycle requiring the timely intervention of at least three different hosts for it to achieve fecundity. Many questions are raised by such life cycles with respect to possible natural selection or environmental pressures which may have driven the *Ascocotyle* parasite (and most trematodes) to seek refuge within these very different hosts in order to reach maturity.

There is no reference in the ascocotylid literature to any significant mechanism which may account for the presence of such complex life cycles, except the usual vague homage paid to an evolutionary "parasite strategy," and/or "selective advantage." Some discussion is made in defense of possible evolution-based parasite-host relationships (Sogandares-Bernal and Lumsden, 1964; Yamaguti, 1971; Skinner, 1975; Font, Heard and Overstreet 1984; Lafferty and Morris, 1996), but answers are often speculative. Statements such as Yamaguti's (1971) prevail:

> The present information indicates that the adoption of the parasitic habit occurred at an extremely remote period (of earth history) and that the evolution of parasitic life cycles with accompanying adaptions of the parasites, has proceeded hand in hand with the evolution of their hosts. (brackets mine).

There is no question that worm parasitism has been a part of the biosphere since early times (Poinar, 1984; Ferreira, et al., 1993), and in the creationist model, it certainly pre-dates the Flood. To relegate its origins to a misty past, however, is to avoid the obvious question: Where, when and how did it arise?

The evolutionary-based, trial and error method of adaptation proposed by these authors, fails to serve as an acceptable explanation for the presence of heteroecious life cycles and likewise falls flat in explaining the complex biochemical and sanctuary interactions between these parasites and their hosts. Evolutionary progression would require countless failed random experiments on the part of a parasite to make the transition from a free-living state to life within three completely different host environments, something that an ascocotylid now freely enjoys. Evolution appears to fail in this regard because it cannot be in an organism's "best interest" to "fetter itself" to another organism, upon which it must depend for its very survival, let alone three different organisms in multiple habitats. Behe (1996) has shown that complex biochemical interactions (which themselves are based upon irreducibly complex biochemical structures) — cannot be formed on a trial and error basis. If these relationships were designed by a Master-Planner from inception, however, then a possible symbiotic exchange may be taking place (or occurred once in the past) and the relationship could be explained on that basis.

Except for one brief note in Smith (1984) describing some alteration of host behavior by parasites, and my recent papers (Armitage, 1997a; 1998; Lumsden and Armi-

tage, 1999), no worker has seriously studied trematodes from a strictly creationist viewpoint. Furthermore, there is no reference in ascocotylid literature which states that a possible positive relationship might exist between this group of worms and their hosts. On the contrary, these infections are often characterized by researchers as merely being benign or minimally harmful (Stein and Lumsden., 1971a, b; Coleman, 1993). This study represents the first attempt to show that these heteroecious life cycles and specialized structures are too complex to have developed by chance, and to present a creationist design argument for the presence of such parasites.

On the other hand, it is most difficult to account for these apparently created structures on an evolutionary basis. The very fact that these organisms can invade a host and go undetected by the immune system implies that certain biocompatibilites were in place before the life cycle was initiated.

Evolutionary Explanations for Apparently Designed Features

A search of the ascocotylid literature failed to produce a satisfactory evolutionary explanation which can account for the complex life cycle and the "design-like" structures employed within this group. One is hard-pressed to synthesize any meaningful evolutionary rationale from this literature, particularly in the field of host behavior modification. Comments like: "a parasite can parlay a small (host) behavioral modification into a large increase in predation" abound (Lafferty and Morris, 1996, p. 1394) (brackets mine).

Stunkard (1946) reviews Odhner's contention that similarities in the reproductive and excretory systems of digenetic trematodes indicate a common origin of all digeneans but he does not elaborate further. Sogandares-Bernal and Lumsden (1964) do not offer a mechanistic solution for the origin or complexity of the ascocotylids, but they do state that a significant and complex behavior of the worm "to remain in the definitive host long enough to produce, but not release a potentially dangerous (to the host) burden of eggs" may be an evolutionary adaptation by the parasite (brackets mine). Cable (1974) does attempt a phylogenetic survey of the trematodes, but does not focus on the digeneans or design features *per se*. Skinner (1975, p. 342) contends (with little argument) that parasites evolve slower than their hosts. He focuses on the intricate features of ascocotylids, saying, "their narrowly defined habitat and high specialization...(makes them) good material for the exploration of evolutionary development...", and "...similarities in the parasite fauna point to close host relationships..." (brackets mine). Overstreet (1978) suggests that the evolutionary relationships of some fishes can be explained on the basis of the similar types of parasites which infect them. With respect to the complexity of the life cycle, he then states, however, "The more complicated the life cycle and the greater the variation in the stages, the more a cycle can be influenced by the environment" (Overstreet, 1993, p. 127), indicating that possibly the environment molded the life cycle.

In a section of their book describing the staggering complexity of digenetic trematode eyespots, chemosensory papillae and other sensory structures, Schmidt and Roberts (1989, p. 234) exclaim that the "sensory endings (in one larval stage) are strikingly similar to the olfactory receptors of the vertebrate nasal epithelium!" but offer no mechanism of how they came to be that way (brackets mine). This would support a strange phylogeny indeed!

As to why trematode cercariae typically manifest abundantly more sense organs than the supposedly more highly developed adults, they surmise that "(this is) undoubtedly related to the adaptive value of finding a host quickly" (p. 233) (brackets mine). The assumption here is that once the worm adapted to finding a host by using these sense organs, the energy required to maintain the sense organs in the adult became less adaptive than just losing them. This is just one example in a long string of the typically imaginative explanations offered by evolutionists, but then Schmidt and Roberts (1989, p. 240) admit that the complexity of the life cycle has fueled the imagination for a long time:

> This alternation of sexual and asexual generations in different hosts is one of the most striking biological phenomena. The variability and complexity of life cycles and ontogeny have stimulated the imaginations of zoologists for more than 100 years, creating a huge amount of literature on the subject. Even so, many mysteries remain, and research on questions of trematode life cycles remains active.

With respect to the wildly different environments this parasite must deal with as it passes from host to host, Schmidt and Roberts (1989, p. 248) state, that they go through a "sequence of totally different habitats in which the various stages must survive, with physiological adjustments that must often be made extremely rapidly." There are wide swings in osmotic pressures from host to host. The chemical nature of the host skin must be detected. They need to penetrate host skin using leukotrienes and prostaglandins (which are very sophisticated proteins) and they must possess a myriad of ways to evade host immunological detection once they are on board. Although the synthesis of these highly specific proteins and enzymes by microscopic parasites is currently unexplainable, evolutionary authors are reluctant to relinquish the supposed materialistic origin:

> When one considers that *chance* governs the successful completion of much of the life cycle of any

> given parasite, it becomes apparent that the odds against success are nearly overwhelming (Schmidt and Roberts, 1989, p. 12) (*emphasis* mine).

The use of sophisticated macromolecules by these parasites to alter host behavior is also discussed by Lafferty and Morris (1996, p. 1395) who admit that, "We know little about the mechanisms parasites use to alter host behavior, but some evidence exists for sophisticated manipulation of (host) hormones and neurochemicals..." (brackets mine). How this remarkable manipulative ability came about is not discussed.

Schmidt and Roberts (1989, p. 254) emphasize the fact that natural selection provides little help in understanding trematode origins:

> Whatever the ancestral digenean, any system of their phylogeny must rationalize the evolution of their complex life cycles in terms of natural selection, a most perplexing task.

The subsequent evolutionary reconstruction is padded with phrases like, 'most authorities today believe', 'this may imply', 'it is not difficult to imagine', 'it may be assumed', 'was probably', 'it is likely that', and 'less difficult to visualize'. It can be seen that the origin of these parasites from an evolutionary point of view is indeed perplexing.

With respect to the supposed evolutionary development of symbiosis and parasitism, McLaughlin and Cain (1983, pp. 189–190) also tender some less than convincing arguments, and frankly state that the "data are scarce". They reference just one laboratory study in which a bacterium and an amoeba established a mutualistic relationship after 100 generations in a controlled laboratory environment. These authors offer four general principles for the origin of symbiosis and admit that:

> Naturally, the...model is speculative...
>
> 1) Symbioses originate rapidly and frequently in nature. Partners evolve rapidly under the pressures of adapting to the symbiotic relationship. Once a complete, free-living life cycle is impossible for one of the partners, it is committed to the evolutionary progression described.
>
> 2) One partner, the host...eventually gains control of the relationship after it becomes obligatory to the other partner, the symbiont.
>
> 3)The evolutionary progress is unidirectional; the symbiont often becomes less pathogenic, then nonparasitic, then actually beneficial to the host (if this is possible; if the symbiont has nothing to offer, then it simply becomes extinct).
>
> 4)Eventually, the desirable features of the symbiont which can be incorporated by the host are so incorporated. The symbiont becomes either extinct or a diminishing part of the host.

To their credit, the authors are candid about the lack of experimental support for their logic. The problem with this kind of evolutionary scenario is that generous anthropomorphisms are ascribed to microscopic creatures which can in no way be self-aware. They cannot be aware of the concepts of symbiosis, parasitism, "interesting ploys", "desirable features", "nothing to offer", "selective advantage", etc.—all of which are teleological value judgments which are constantly and readily made. How, indeed, can a parasite "know" what is in its "best interest" from an evolutionary (or any) point of view? Perhaps these authors only mean that these seemingly directed behaviors and structures really occur as a result of natural selection in gene pools, but if that is the case, they do not say so.

Further, the whole idea that a microscopic trematode or other parasite can "guide" its intermediate host to the actual definitive host by "scheming" to alter its behavior via an evolutionary "strategy" is absurd. Carney (1969) discusses the alteration of formicine ant behavior by the lancet fluke trematode (*Dicrocoelium dendriticum*). He writes about this and the liver fluke (*Brachylecithum mosquensis*) which "ensure" their own predation by the herbivorous sheep which serves as the definitive host. These trematodes evidently cause the ant to climb to the tips of grass during the period when sheep graze. With no supporting material, Carney states:

> Both flukes have parallelly evolved the ability to alter their intermediate host's behavior such that their own chance of survival is enhanced... Bizzare adaptations to parasitism such as these are one of the most interesting aspects of biology, although often the least known, and indicate a long association between these flukes and their respective hymenopteran hosts (p. 610).

Curio (1988) comments on this as well, stating:

> To manipulate hosts behavior patterns seems to ask a lot in evolutionary novelties. However, the brainworm when inducing an ant to cling to the top of plants capitalizes on an apparently ancient behavior... the parasite needed "merely" to reactivate a hidden potential of the ant (p. 151).

Moore (1984) surveys several parasites, particularly in the acanthocephalans (spiny headed worms), which are known to alter intermediate host behavior, and he laments:

> ...the parasites do not induce novel behavior patterns but merely elicit existing patterns at disastrously inappropriate times. Nevertheless, this is quite a feat, and a general physiological explanation of how an acanthocephalan accomplishes it while floating in the body cavity of the host has yet to be found. The realization that parasites can change host behavior has intriguing implications. Biologists observing certain animals in the field must now take into account the possibility that the observed behavior may have been 'rigged' (p. 115).

Moore, however, does not elaborate on who or what may have done the 'rigging'. In a discussion of host castration by parasites, Hurd (1990, p. 274) writes:

> Baudoin (1974), considered parasitic castration in the wider sense, outlined above as an evolutionary strategy, and concluded that a parasite-induced manipulation of host resources away from reproduction may produce increased host survival, thus leading to increased parasite fitness as a result of an improved environment.

In this case it is assumed either that the parasite has "understood" what host resources are and has "devised" a "strategy" to ensure its own fitness, or that chance, natural selection, and mutation caused it.

Aeby (1991) discusses trematodes which encyst in coral polyps as an intermediate stage, changing the polyp appearance and behavior. These trematodes later mature in the definitive host, a coral-eating fish attracted by these very changes.

In the discussion, she writes:

> One might question why fish would evolve to feed on infected polyps... I can only speculate about this, but there are several hypothetical explanations... The parasite residing in the fish may have adopted the 'prudent parasite' strategy (Holmes 1983) in which the parasite produces minimal damage to the host (p. 267).

The fact that a parasite induces minimal damage on its fish host hardly seems a compelling reason for a fish to begin feeding on infected polyps in the first place!

Lafferty and Morris (1996, p. 1390) state,"Three main lines of evidence currently support the hypothesis that behavior modification is a parasite strategy evolved to increase transmission..." The authors then go on to point out that the very fact that increased predation by the definitive host is occurring in conjunction with parasitism is evidence enough that such a "strategy" has evolved. One clue to an evolutionary origin for these parasites would be the discovery of a free-living variety or finding a parasitic trematode that completed all stages of the life cycle in one host. There is, however, only one example in the literature of all three stages of this life cycle occurring in one host (Barger and Esch, 2000), but there are no free-living forms. A snail is required as a first intermediate host, followed by a fish or frog, and finally a piscivorous bird or mammal. All digenetic heterophyid trematodes are endoparasitic and obligated to these hosts. Why would a parasite initially become completely dependent upon a host for its very survival? How could such a relationship develop over time from a free-living state to a parasitic state? Why would an *Ascocotyle* tie itself to such a risky developmental route, where not one, but three hosts are required?

Overstreet (1978) classifies all organisms which live together as symbionts. He states that a symbiont becomes a parasite, "when (it) depends entirely upon a host, occasionally harming it..." (p. 2). Commensals live together and "eat from the same table", and mutualism occurs when "...both parties benefit and both metabolically depend on each other" (Overstreet 1978, pp. 2–4; 1993). There is no doubt that an *Ascocotyle* is a parasite and a commensal. Quite possibly it even has a metabolic dependence upon one or more of its hosts to dissolve its cysts. Some question remains as to whether the *Ascocotyle* worm is involved in a mutualistic relationship in which it actually benefits its host. All members of this genus have an oral coronet of large spines at the adult stage, some having two rows, some having one row, etc. (Armitage 1997a). Of what purpose are these spines? The adult worm appears to lodge in the intestinal mucosal crypts of the definitive host, but does not penetrate the mucosal layer (Font, Overstreet and Heard, 1984; Font, Heard and Overstreet, 1984). Worms hold on in a cup-shaped fashion, over the host villi, by using both the acetabulum and the oral sucker. Yet no pathology to the villi is observed so the oral spines are possibly not used as holdfasts. But of what value are two rows of spines rather than one, or of one additional incomplete row in preference to a complete one? Why is a single incomplete row never observed? The tegument of these worms is entirely covered with a carpet of fine body spines which certainly must aid in anchoring the parasite and resisting host peristalsis.

In experiments conducted with cercaria of *A. mcintoshi* Price, Leigh (1974) discovered that cercarial penetration glands are HCl-sensitive and fully evert in weak solutions of HCl. Of what purpose is a set of penetration glands that are activated only in the presence of HCl, glands that are required for entry into host tissues, unless the parasite in question "anticipates" the gastric juices of the fish which swallows it as the second intermediate host? Could a trial and error method account for this elegant penetration gland? This biochemical functional system seems to fall within Behe's (1996) category of "irreducible complexity." If the sensitivity to HCl were removed, would the parasite fail to penetrate the host tissue? To bequeath this biocompatibility to the ancient processes of time and chance strains one's scientific credibility to the breaking point. One of the most compelling arguments for design within this *Ascocotyle* group comes from the structure of the metacercarial cyst, which is HCl resistant, and yet, temperature, pH and trypsin sensitive (Stein, 1968). Without a temperature of 37° C, a solution adjusted to pH 7.5, and the presence of trypsin, the cyst will not dissolve. Only with this combination can the parasite, encased within its miniature ark, successfully pass the definitive host stomach and dissolve only within the somewhat protective confines of the intestine, which is perfectly matched to its required conditions. Schmidt and Roberts (1989, p. 248) observe, "This combination of conditions is not likely to be present

anywhere but in the intestine of a homoiothermic vertebrate...".

The evolutionary literature has failed to supply us with a proper explanation for how these "design-like" features may have come about by the chance, random processes of evolutionary descent.

Intelligent Design Explanations

The questions which confront us have to do with the relationships, behaviors and specialized structures observed in this parasite — all of which appear to have been designed (Armitage, 1998). Such designs exhibit irreducible complexity as observed by Behe (1996) for the chemical basis of human vision and blood clotting. Behe's conclusion is that a gradualistic, Darwinian mechanism could never have produced these features:

> The impotence of Darwinian theory in accounting for the molecular basis of life is evident not only from the analysis in this book, but also from the complete absence in the professional scientific literature of any detailed models by which complex biochemical systems could have been produced...the scientific community is paralyzed. No one at Harvard University, no one at the National Institutes of Health, no member of the National Academy of Sciences, no Nobel prize winner—no one at all can give a detailed account of how the cilium, or vision, or blood clotting, or any complex biochemical process might have developed in a Darwinian fashion (p. 187).

And:

> There is an elephant in the roomful of scientists who are trying to explain the development of life. The elephant is labeled 'intelligent design.' To a person who does not feel obligated to restrict his search to unintelligent causes, the straightforward conclusion is that many biochemical systems were designed. They were designed not by the laws of nature, not by chance and necessity; rather they were *planned*. (p.193) (emphasis in the original).

Ascocotylids are able to rapidly manufacture macromolecules necessary to render osmotic potentials harmless. They can synthesize host penetration macromolecules used for swiftly and painlessly entering their intermediate hosts. They also produce the macromolecules which will envelop them with a immune-transparent cyst that will not dissolve in HCl but which will come apart readily in a warm, pH adjusted, environment, bathed in trypsin and bile salts. All these characters demand planning just as much as does Behe's blood clotting mechanism. But the question remains of whether or not a loving God would plan invaders such as these. Were these organisms designed from the start to perform functions they no longer perform? Answers to these questions may vary but we can be sure that a loving God did not intend the rampant parasitism we observe today.

The only acceptable alternative to the evolution explanation is that these complex life cycles and "design-like" structures were planned by the Creator, at the point in history when He designed all of the other living organisms. Why and how some symbionts have today become pathogenic is open for speculation, but in a creation scenario, pathobiology must certainly be related somehow to the Fall of Adam and the subsequent Curse (Genesis 1:31; 3:18).

Delving Deeper into Trematode Biology: Microtechnique

In an effort to explain the basis of this and other studies, in which TEM has been applied to the cyst walls of trematode worms, a primer on the preparation of biological material is presented in the appendix. The appendix and the illustrations will also serve to orient the reader to the field of microtechnique and to show contrasting features in *Ascocotyle* cysts.

The Ultrastructure of Metacercarial Cyst Walls

At one time researchers believed that the thick-walled metacercarial cyst of trematodes encysted in various hosts was a direct response by the host to the presence of the parasite within tissues (Sogandares-Bernal and Lumsden, 1964). It now is known however, that cyst walls are produced by secretions from the tegument of the metacercaria and may even be used to delineate differing species based upon its unique structure (Stein and Lumsden, 1971b; Stein and Basch, 1977). But certain other authors have not agreed that this is a species characteristic (Huffman, 1968; Wittrock et al., 1991; Walker and Wittrock, 1992). The extent of host response to the presence of cysts varies considerably. The typical host response is the production of a fibrous collagen capsule surrounding the cyst, along with associated host fibroblasts, but there is little or no host immune response (Lumsden, 1968; Stein and Lumsden, 1971a, b; Mitchell, 1974; Higgins et al., 1977; Stein and Basch, 1977; Gulka and Fried, 1979; So and Wittrock, 1982; Galaktionov et al., 1997).

The variation in layers of cyst walls within the ascocotylids is seen in the following examples. *Ascocotyle pachycystis* (Figure 1), produces a four-layered cyst up to 35 micrometers in thickness (Stein and Lumsden, 1971b) with two major, bilayered lamellae. *A. chandleri* (not shown) exhibits a 15 micrometer-thick cyst with two layers (Lumsden, 1968). *A. leighi* (Figure 2), was shown to have a 10 to 11 micrometer thick cyst which also has two layers

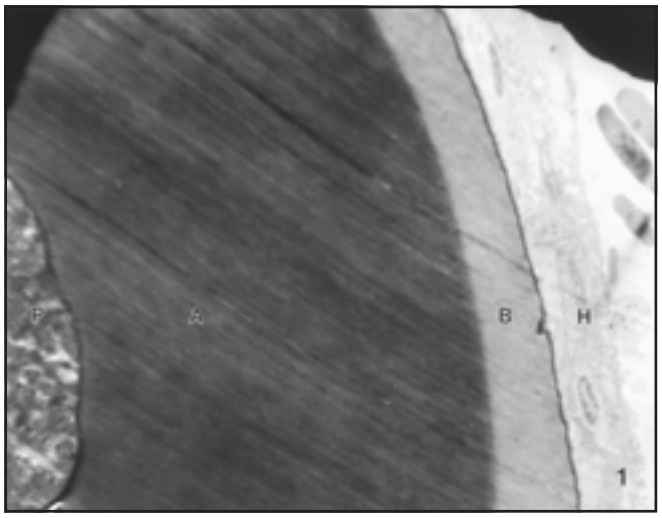

Figure 1. Ascocotyle pachycystis *in* Cyprinodon *heart, (2,000X). This figure shows a cross section of a fluke parasite (*Ascocotyle*) cyst, growing in the heart of a sheepshead minnow (*Cyprinodon*). Note the very thick layers of the cyst which protect the parasite. Legend for figures 1–4: P = parasite tissue, A = cyst wall 1, B = cyst wall 2, H = host tissue).*

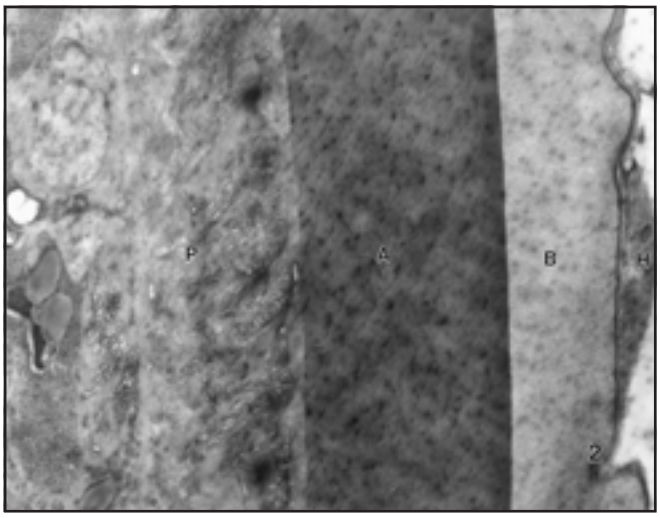

Figure 2. Ascocotyle leighi *in* Poecilia *heart, (7,000X). This is a cross section of another species of* Ascocotyle *fluke (*A. leighi*) in the heart of the sailfin molly (*Poecilia*). The cyst in this case is much thinner; yet this parasite, when mature is almost identical in shape to* A. pachycystis.

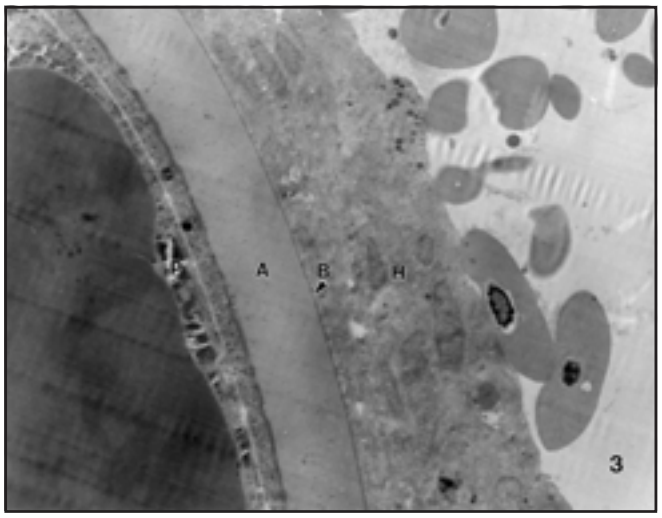

Figure 3. Ascocotyle tenuicollis *in* Gambusia *heart, (4,000X). Within the heart of* Gambusia, *the mosquitofish, is the cyst of another fluke,* A. tenuicollis, *also shown in cross section. This cyst closely resembles that of* A. leighi; *yet it is a different species found in a different fish host.*

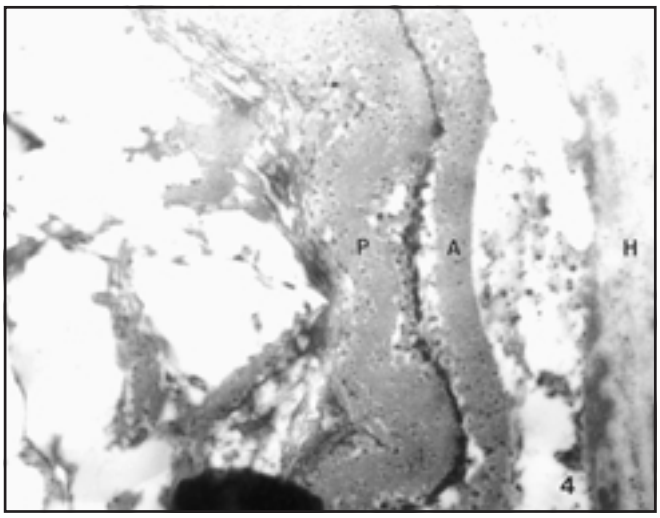

Figure 4. Ascocotyle (Phagicola) diminuta *in* Fundulus *gill, (8,000X). This fluke,* A. diminuta *is found in the gill tissue of the Killifish (*Fundulus*) and one or two others. Note that the cyst wall and the parasite outer skin are almost identical in appearance.*

(Stein and Lumsden, 1971a). The cysts of *A. tenuicollis* (Figure 3), collected in Mississippi come closer in structure to *A. chandleri* than any other member of this genus because of a three-layered, nine micrometer-thick cyst. *A. sexidigita* has a 16 micrometer-thick, three layered cyst (not shown). *A. (P.) diminuta* has a single cyst wall of 1.5 to 3 micrometers in width (Figure 4), which in every respect resembles the very tegument of the metacercaria it is harboring. The wall is comprised of a spongy matrix of tissue interspersed with dense nuclei and a very thin granular outer border.

Definitions

Acetabulum: The ventral sucker on a trematode worm.

Amnicolid and Hydrobiid: fresh or brackish water snails belonging to the family Hydrobiidae which are charac-

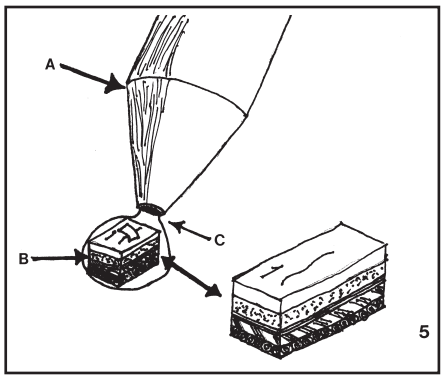

Figure 5. Dissected fish tissue block is chemically processed. Tissues for electron microscopy must first be stabilized in a fixative, such a formalin. This preserves, as close to the living specimen as possible, the ultrastructure of the tissue for the microscope. Legend for figures 5–9: A = dropper, B = tissue block, C = solution, D = capsule, E = polymer, F = thin sections, G = knife, H = grid, I = specimen chamber).

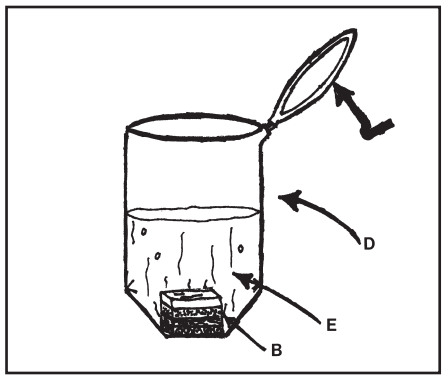

Figure 6. Processed tissue is placed in a liquid polymer. A liquid polymer (plastic) is used to infiltrate the tissues after they have been preserved properly. Once the tissue is thoroughly impregnated with the polymer, it is placed into an oven overnight for hardening. The tissues can then withstand the harshness of the electron microscope.

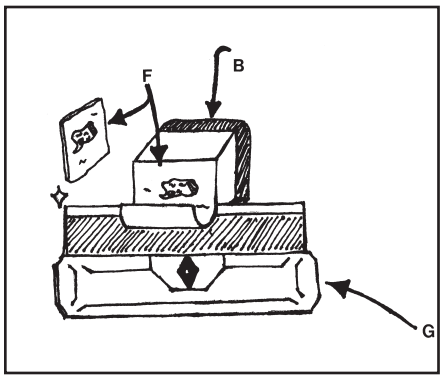

Figure 7. Hardened polymer block with tissue is sectioned into ultra thin sections. Very thin sections of the tissue block are made with a glass or diamond knife of surgical sharpness. These thin sections will go into the microscope.

terized by true gills and opercula, versus pulmonate, or air-breathing, structures.

Cercarial: The last larval stage of trematodes, free swimming, from the first intermediate host, to the second intermediate host, where penetration of that host occurs.

Creationism: The belief that God created all things as described in Genesis.

Cyprinodont: the killifish family of fresh and saltwater minnows.

Digenetic: Subclass Digenea, parasitic worms, a subclass of the Trematoda, having two or more asexual generations, an alternation of hosts, the first almost always a mollusc, and which are endoparasitic in vertebrates such as birds.

Evolutionism: the belief that all life forms arose over millions of years from a common ancestor due to mutations in the genetic code and a stochastic "system" of selection called Natural Selection.

Fecundity: The capacity of an organism to produce offspring.

Hermaphroditic: (often) self-fertilizing organisms which contain both male and female sexual organs .

Heteroecious: "many houses" or complex cycles which require several different hosts to complete.

Heterophyid: having a bifurcated intestinal organ or ceca usually long and truncated.

Metacercaria: the third, or preadult stage of the trematode life cycle, often involving an encystment within the second intermediate host.

Micromorphology: body structures which can only be seen by means of magnification.

Pathogenicity: The causing of disease or toxic response in another organism.

Poeciliid: the topminnow or live-bearer family of fresh and brackish water fishes which hatch young internally and bear live young.

Piscivorous: fish eating vertebrates such as herons, egrets, and raccoons.

Redia: An intermediate larval stage of trematodes that develops asexually within a sporocyst inside the first intermediate host, usually a snail.

SEM: Scanning electron microscope, which images metal coated whole mounts, in magnifications of 100:20,000 diameters using secondary electron capture.

Sensory Papillae: Sophisticated sensory projections from cells or tissues, often containing cilia.

Symbiosis: The intimate and protracted association of individuals of different species.

Tegument: The outer layer or skin of a trematode parasite.

TEM: Transmission electron microscope, which images metal coated thin sections, to magnifications up to 200,000 diameters using primary or direct electron capture.

Trematode: Class Trematoda of helminths which are parasitic flatworms (flukes) mainly in the digestive tract of all classes of vertebrates. These trematodes possess a digestive tract, specialized sensory organs, and muscular sucking disks which serve to attach the fluke to the host.

Appendix

Biological tissues such as *Ascocotyle* cysts must be processed properly in order to be viewed in an electron microscope. First, the tissue of interest must be dissected and chemically processed in fixative, buffers, and alcohols (Lumsden, 1970). Then it must be embedded in a liquid polymer that will harden upon heating (Figures 5–6). Once hardened, the tissues can be thin sectioned on an ultramicrotome (Figure 7: blade and tissue block shown). Finally, tissue sections are transferred to thin metal grids on which they are stained with metal salts and placed into a special chamber in the TEM (Figures 8–9).

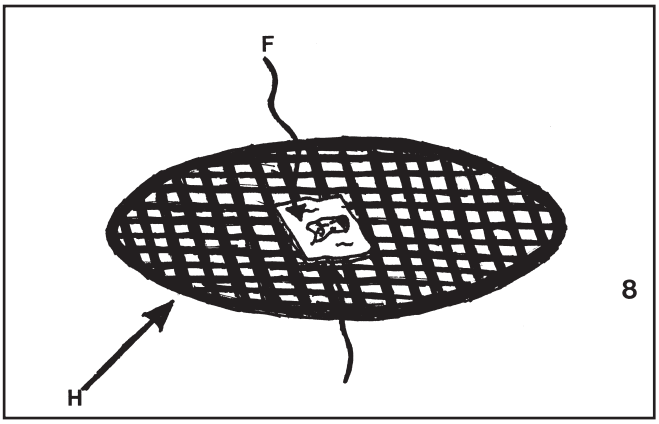

Figure 8. Individual sections are placed onto TEM grids and stained. The grids are metal substrates used to support the thin sections and for handling into and out of the heavy metal staining solutions (lead and uranium salts) as well as the microscope.

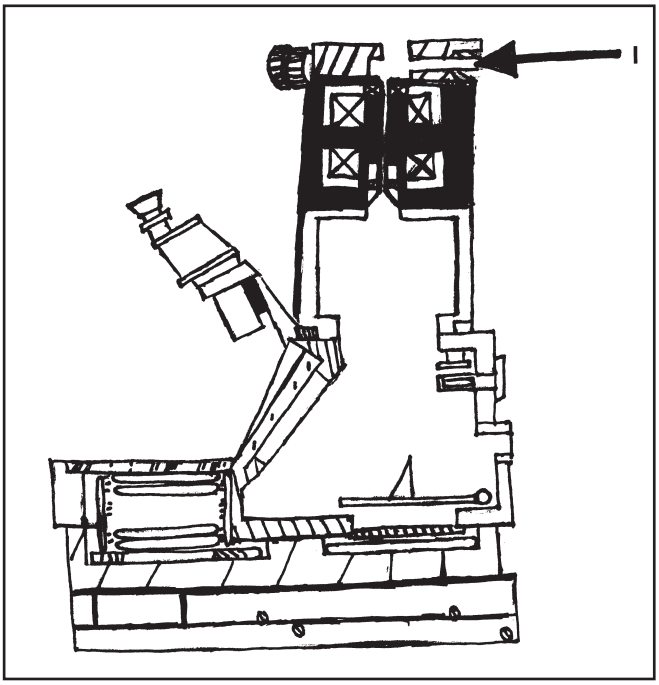

Figure 9. Grids are placed into the TEM for imaging. Once stained, the grids, holding the stained sections are placed into the electron beam. Tissues which took up the metal stain block the passage (transmission) of electrons and appear dark under the microscope.

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JP: Journal of Parasitology

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