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The Phylogeny of the Cercomeria Brooks, 1982 (Platyhelminthes)

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ABSTRACT: A new classification of the parasitic platyhelminths is presented. It is derived by phylogenetic analysis of 39 morphological characters drawn from 19 putative homologous series. The construction and interpretation of phylogenetic trees using Hennigian phylogenetic systematics is discussed briefly to provide a reference point for the conclusions drawn. Parasitic platyhelminths having, at some time in ontogeny, a posterior adhesive organ formed by an expansion of the parenchyma into, minimally, an external pad, form the subphylum Cercomeria. Three superclasses are recognized within the Cercomeria: the Temnocephalidea, which is the most plesiomorphic of the three; the Udonellidea; and the Cercomeridea. Within the Cercomeridea, two classes are recognized: the Trematoda and the Cercomeromorphae. The Trematoda contains two subclasses, the Aspidocotylea and the Digenea, and the Cercomeromorphae contains the Monogenea, Gyrocotylidea, Amphilinidea, and Eucestoda. The Monogenea is the sister-group of the latter three, which together form the Cestodaria. The Gyrocotylidea is the sister group of the Amphilinidea and Eucestoda, which together form the Cestoidea. Several of the character complexes examined are discussed in detail, and all are listed. The number of hooks on the larval cercomer is concluded to be of little help in analyzing phylogenetic relationships among the cercomeromorphs. Other characters are more informative and provide additional support for the phylogeny proposed herein. These include the relative positions of the genital openings, and the structure of the anterior and posterior parts of the nervous system. New homologies are proposed for posterior adhesive organs and anterior body invaginations. The complex life cycles of digeneans and eucestodes are concluded to differ from each other in manner of origin. Eucestodes exhibit terminal addition of ontogenetic stages and nonterminal addition of an invertebrate host. Digeneans exhibit nonterminal addition of ontogenetic stages and terminal addition of a vertebrate host. Comparison of the classification presented with eight previous classifications shows that it provides the best fit to the data considered.

In the past 75 years there have been at least eight proposals for the phylogenetic relationships and classification of the parasitic platyhelminths. Two of the earliest, by Sinitsin (1911) and Fuhrmann (1928, 1931), postulated a dichotomy between those with a gut and those without a gut. The remaining six studies (Spengel, 1905; Janicki, 1920; Bychowsky, 1937, 1957; Llewellyn, 1965; Price, 1967; Malmberg, 1974) viewed those with a gut as either paraphyletic or polyphyletic. No general agreement has emerged. The present study is an application of Hennigian phylogenetic systematics (Hennig, 1950, 1966) to the problem.

Previous attempts at phylogenetic analysis of certain parasitic platyhelminths (Brooks, 1977, 1978a, b, 1981a; Brooks and Overstreet, 1978; Brooks et al., 1981a, b; Brooks and Caira, 1982) have been hampered by the lack of well-corroborated outgroups (see the next section). This has made character analysis difficult. Before attempting more analyses of particular groups, we considered it necessary to construct an hypothesis of the higher level relationships among the platyhelminths. Brooks (1982) provided a brief discussion of the results presented here, and proposed that parasitic platyhelminths possessing a posterior adhesive organ be included in a subphylum, the Cercomeria. This study extends and supports that hypothesis.

Materials and Methods

To provide a reference point for some of the conclusions we will draw, we present a précis of the theory and methodology of Hennigian phylogenetic systematics. See Wiley (1981a) and Brooks et al. (1984) for a more complete discussion. This précis is followed by a list of the characters we analyzed, as well as a brief discussion of diagnoses and keys.

Phylogenetic systematics

Organisms from different species may resemble each other by possessing similar traits that either have been inherited from a common ancestor or have evolved independently. The first types of traits are homologies, the second types are analogies. In a system produced by evolution, only homologies indicate phylogenetic (i.e., genealogical) relationships; thus, only homologies can be used for a phylogenetic classification. Given that homologues covary in greater numbers than do analogues, such a classification will be the most efficient information storage and retrieval system for systematics (see Farris, 1979; Brooks, 1981b).

Phylogenetic systematics avoids two types of ad hoc assumptions that can be introduced into classificatory studies. The first is the use of a unique trait to highlight

a particular taxon, with the concomitant grouping of the remaining taxa. Unique traits do set a taxon off by itself, but it does not follow that the remaining taxa not so highlighted form an evolutionary group. The second type of ad hoc assumption concerns explanations of ambiguities in the data. Ambiguities are caused by *homoplasy,* or parallel and convergent evolution, which is not recognized as such beforehand. This is caused by the independent acquisition or loss of a trait by two or more species. Homoplasious characters allow more than one classification to be inferred from the same data set. They will be inconsistent with the phylogenetic classification that explains their true origins, and consistent with at least one classification based on particular parallel or convergent traits. For example, in a group of organisms in which a trait is primitively absent, then evolves, and then is lost by some members, those lacking the trait could all be classified together, or they could be classified separately to recognize the primitive and derived conditions.

Phylogenetic systematics recognizes that both of the above problems require additional data for their resolution. It eliminates the first type of ad hoc reasoning by grouping taxa only by *shared* derived characters. Unique derived characters may be reported in a taxon's diagnosis, but they are not used to make groupings. The second type of ad hoc reasoning is minimized by considering the largest subset of the data that covaries in a single pattern to consist of homologous traits, and the exceptions to that pattern to indicate true homoplasy. Phylogenetic systematics thus neither denies the existence of homoplasy, nor assumes its rarity. Its only assumption is that these false indicators of phylogeny do not themselves covary in a pattern better supported than that of the homologues.

Three levels of homologous traits may be discerned. Some may be found in all members of a group being classified. These help establish that group's identity, but give no clues to the relationships of its members. Others may be present in all individuals of one member taxon, but absent in the rest. These establish the identity of single taxa, but provide no clues to relationships with other group members. Finally, there may be homologues shared by two or more of the taxa in the study group. These indicate particular relationships within the group. Traits that are general to the group being studied are *plesiomorphies.* Those found only within part of the study group and not in any taxa outside the group are *apomorphies.* Shared plesiomorphies are *symplesiomorphies.* Unique apomorphies are *autapomorphies.* Shared apomorphies are *synapomorphies.* Two aspects of phylogenetic systematics make this nomenclature necessary. First, Hennig rejected "idealized morphology" and its stress on the search for archetypal forms, and postulated that any species is a composite of ancestral and derived traits. Second, traits produced as novelties at one time can become generalized traits of a descendant group. Plesiomorphy and apomorphy therefore refer to *relative* primitiveness and derivation, depending on the level of generality of the investigation. For example, hair is a synapomorphy (shared derived trait) for mammals when discussing tetrapod evolution, but a symplesiomorphy (shared ancestral trait) when discussing the evolution of rodents.

Phylogenetic classifications are based on the discovery of appropriate levels of generality for homologous traits, and the recognition of groupings supported by synapomorphic traits. A number of protocols have been advanced for determining the plesiomorphy and apomorphy of traits in a study group (see, e.g., Stevens, 1980). To date all have been found either to be capable of giving incorrect estimates (such as the principle of "common equals primitive" of Estabrook, 1971, 1978, and Crisci and Stuessy, 1980), or to be special cases of the more general method of "outgroup comparisons." Outgroup comparisons are based on the concept that a trait found in at least one member of the study group and in a taxon outside the group (the outgroup—a close relative of the group being analyzed) is plesiomorphic. Such a trait is considered to have evolved prior to the existence of the ancestral species from which the study group evolved. By contrast apomorphic traits are those found only in some members of the study group. Because outgroups can themselves evolve, it is sometimes necessary to use more than one outgroup to confirm the plesiomorphy of a trait (Wiley, 1981a).

Homologous series of platyhelminth characters examined

Nineteen kinds of larval and adult platyhelminth traits are given below. Each is considered to be a potential series of homologues produced through evolutionary transformation. Each component of a series is a character. It is these characters (e.g., bifurcate gut), rather than the series (e.g., intestine), that individual taxa display. Homologous series may consist of two characters (e.g., the presence or absence of locomotor cilia) or more (e.g., the various types of posterior adhesive organs). The derived state of a two-state series will be a shared derived character for a single monophyletic group. A multistate character is produced when the derived character of a two-state series undergoes further evolution. This may produce either further structural modification or loss (vs. primitive absence) of the character. The result is an internesting of monophyletic groups (decreasing inclusiveness of taxa), each of which inherited a particular character of the series. The discovery of this internesting is made possible by determining the relative primitiveness and derivation of the characters. Outgroup comparisons help to establish the direction, or *polarization,* of this transformation.

a. Locomotor cilia: Among platyhelminths adult dalyelloid rhabdocoels exhibit restricted locomotor cilia, whereas adults of the Temnocephalidea, Udonellidea, and the trematode and cercomeromorphan groups (sensu nobis) lack locomotor cilia altogether (see Williams, 1981). The secondary loss of cilia in adult cercomerians is further corroborated by the presence of ciliated larvae in some members of all groups.

b. Vagina: In dalyelloids and temnocephalideans a single duct extends from the ovary. In some taxa this duct joins the uterus, and in others it opens directly into the genital atrium. Udonellideans, trematodes (sensu nobis) and cercomeromorphs (sensu nobis) possess two ducts extending from the ovary. One of these connects with the uterus, contains the ootype region, and receives the vitelline ducts. The other duct may open to the exterior by means of a separate pore or a

common atrium. Following traditional terminology, we call the first duct the oviduct (Hyman [1951] called it the ovovitelline duct), and the second the vagina. We do not believe that this vaginal duct has a counterpart among other turbellarians. Udonellideans, gyrocotylideans, amphilinideans, and eucestodes possess well-developed vaginae. Digeneans and aspidocotyleans have a small duct, called the Laurer's canal, extending from the oviduct region. This usually opens to the dorsal surface, but can end blindly in either taxon (almost always in aspidocotyleans), or connect with the excretory system (in some aspidocotyleans). Monogeneans have paired lateral vaginal openings, with apparent secondary loss of vaginae in many groups.

c. OVARY AND TESTES NUMBER: Dalyelloids have single ovaries and paired testes. Among various groups of parasitic platyhelminths, testes number has either decreased to one, or increased to many. The dalyelloid condition is nevertheless found in some temnocephalideans, digeneans, aspidocotyleans, and monogeneans. Because of the widespread occurrence of changes in testes number, we have not used any of the derived states in our analysis.

d. EXCRETORY SYSTEM: Paired lateral excretory ducts joining posteriorly into a vesicle comprise an additional trait linking dalyelloids with the rest of the parasitic platyhelminths.

e. PHARYNX: The doliiform pharynx (barrel-shaped, see Hyman, 1951) found in dalyelloid rhabdocoels occurs in temnocephalideans, udonellideans, digeneans, aspidocotyleans, and monogeneans. It has generally been considered that there is no pharynx in gyrocotylideans, amphilinideans and eucestodes (but see Discussion).

f. COPULATORY STYLET: Some form of sclerotized copulatory apparatus is part of the male genitalia of dalyelloids, temnocephalideans, udonellideans, and monogeneans. Digeneans, aspidocotyleans, gyrocotylideans, amphilinideans, and eucestodes lack such structures.

g. INTESTINE: Dalyelloids have a saccate gut, as do the majority of turbellarians. This trait is found in temnocephalideans, udonellideans, and most aspidocotyleans. Digeneans and monogeneans, for the most part, possess bifurcate guts. Gyrocotylideans, amphilinideans, and eucestodes lack a gut.

h. MEHLIS' GLAND: This gland is lacking in dalyelloids, at least as a centralized glandular structure. Although it is not unambiguously clear that temnocephalideans possess this gland (see Williams, 1981), all other groups in this study have been shown to have it.

i. POSTERIOR ADHESIVE ORGAN: Posterior attachment structures in the platyhelminths consist of adhesive glandular secretions, suckers, and hooks (see Hyman, 1951). The first primarily involve tegumental modifications, and the other two involve extensive parenchymal modifications as well. Adhesion can thus be achieved by chemical action, vacuum principle, and mechanical embedding, respectively. There appear to be four basic types of morphological modifications to the posterior holdfast organ: (1) glandulo-epidermal secretions, (2) expansion of the parenchyma into an external "pad" of some sort, (3) development of suckers by muscularization of the pad, and (4) the presence of hooks. Different combinations of these modifications exist in the taxa studied (see Table 1). Dalyelloids (e.g., *Dalyellia*; see Hyman, 1951) have only glandular secretions (see also the discussion for behavioral observations). Temnocephalideans and udonellideans have a parenchymal expansion as well (Williams, 1981). In digeneans this expansion has become muscularized to form a sucker that is midventral in many species, but posterior in a number of apparently primitive groups. Aspidocotyleans possess a posterior ventral sucker early in ontogeny that becomes a rugose or loculate disk in the adults. The adhesive organ reaches its maximum complexity in monogeneans, which possess hooks in addition to the three earlier modifications. The early larval stages of gyrocotylideans, amphilinideans, and eucestodes possess a posterior expansion bearing hooks, but apparently lacking adhesive secretions. The hooks may persist in a disarrayed configuration near the dorsal pore of the rosette funnel in gyrocotylidean adults (e.g., *Gyrocotyle urna*; see Lynch, 1945) and at the rounded posterior end of the body in amphilinidean adults (e.g., *Austramphilina elongata*; see Rohde and Georgi, 1983).

Table 1. The four basic modifications to the posterior adhesive organ in the platyhelminth taxa studied. Adhesive secretions are produced by glandulo-epidermal modifications, whereas the remaining three traits involve parenchymal modifications as well. The expansion of the parenchyma into an external "pad" at the posterior end of the body is considered to mark the first appearance of a cercomer. This organ is relatively unmodified in the Temnocephalidea and Udonellidea, and augmented with muscularization and/or hooks in the more derived taxa.

	Adhesive secretions	Parenchymal expansion	Muscles	Hooks
Dalyelloidea	*			
Temnocephalidea	*	*		
Udonellidea	*	*		
Aspidocotylea	*	*	*	
Digenea	*	*	*	
Monogenea	*	*	*	*
Gyrocotylidea		*	*	*
Amphilinidea		*	*	*
Eucestoda		*	*	*

j. TENTACLES: Temnocephalideans possess tentacles on their anterior ends, although in some cases these may be very small and possibly secondarily reduced (Williams, 1981). Some digenean (e.g., bucephalids) and eucestode (trypanorhynchs) groups possess "tentacles" as well, but these are neither common to all digeneans and eucestodes, nor of the same structure as those in temnocephalideans.

k. GENITAL PORES LOCATION: Turbellarians possess genital pores at the posterior end of the body. With the exception of amphilinideans the rest of the taxa in this study generally have all genital pores in or near the anterior half of the body, or proglottid (in eucestodes).

Amphilinideans have the male pore and the vagina in the posterior half, and the uterine pore in the anterior half (see Fig. 3).

l. GENITAL PORES ASSOCIATION: In dalyelloids and many other turbellarians the genital openings occur together, often in a common genital atrium. In the digeneans and monogeneans the vagina is separate from the male and uterine openings. The latter two may open to a common genital atrium and exit through a common genital pore. In the aspidocotyleans the vaginal opening is lacking altogether, but the relative positions of the male opening and uterine pore are as in digeneans. Gyrocotylideans have all three genital pores separated but in close proximity in the anterior half of the body. Amphilinideans have the uterine pore in the anterior half of the body, and the vagina and male pore separate in the posterior end of the body. Eucestodes possess closely associated male and vaginal openings (see Fig. 3).

m. ORAL SUCKER: Most digeneans, aspidocotyleans, and monogeneans have oral suckers. Gyrocotylideans, amphilinideans, and eucestodes possess, at some period in ontogeny, invaginations at the anterior end that we interpret as vestigial mouths and associated structures (see Discussion).

n. LIFE CYCLE PATTERNS: All of the platyhelminths considered here are associated with a host of some sort. Temnocephalideans, udonellideans, and aspidocotyleans are associated primarily with invertebrates; although some aspidocotyleans occur in vertebrates, these hosts are not necessary for completion of the life cycle (see Rohde, 1971). Monogeans, gyrocotylideans, and amphilinideans are associated primarily with vertebrates, although at least one species of amphilinidean has an intermediate arthropod host (see Rohde and Georgi, 1983). Both digeneans and eucestodes have, with few exceptions, life cycles involving both an invertebrate and a vertebrate host. For reasons elucidated in the discussion we consider the digenean life cycle to involve secondary addition of a vertebrate host, and the eucestode life cycle to involve secondary addition of an invertebrate host.

o. NERVOUS SYSTEM: Monogeneans, gyrocotylideans, amphilinideans, and eucestodes have doubled nervous commissures at the anterior and posterior ends of the body (see Tower, 1900; Watson, 1911; Lynch, 1945; Rohde, 1968, 1975; Allison, 1980; Fairweather and Threadgold, 1983). All other taxa in the study group have single anterior and posterior commissures, as do all rhabdocoels (Bullock and Horridge, 1965).

p. INVAGINATION OF THE POSTERIOR ADHESIVE ORGAN: Gyrocotylideans, amphilinideans, and eucestodes exhibit partial to complete invagination of the posterior adhesive organ when it occurs during ontogeny. This trait appears to be unique to them.

q. POLYZOIC BODY: Eucestodes are the only taxon in the study group that may possess a polyzoic body.

r. OSMOREGULATORY SYSTEM: Eucestodes are the only members of the study group whose larvae have protonephridia in the posterior half, rather than anterior half, of the body. In addition, whereas adult gyrocotylideans, amphilinideans, and eucestodes possess a reticulate osmoregulatory system, their early ontogenetic stages have paired lateral ducts joining in a posterior vesicle. This latter condition is characteristic of the rest of the study group throughout ontogeny.

s. LARVAL HOOKS ON THE POSTERIOR ADHESIVE ORGAN: Monogeneans have 12–16 hooks, gyrocotylideans 10 equal-sized hooks, amphilinideans six large and four small hooks, and eucestodes six equal-sized hooks. The other groups have no such hooks.

Method of character analysis

Dalyelloids were chosen as the putative outgroup because of the characters they shared with some members of the study group. This choice agrees with Karling's (1974) cladistic analysis of the Turbellaria. Our cladogram was initially constructed by Hennigian argumentation (Hennig, 1966; see Wiley, 1981a), and then checked with WAGNER analysis from the PHYSYS computer program of Drs. J. S. Farris and M. F. Mickevich. Those character series that were entirely within the study group, and thus not amenable to polarization by a *taxonomic* outgroup (the dalyelloids), were polarized by the *functional* outgroup method of Watrous and Wheeler (1981); Farris' (1982) expansion of this method was taken into consideration.

Diagnoses and keys

A phylogenetic analysis produces a cladogram, or a tree diagram, depicting the inferred genealogical relationships of taxa. From this tree, a verbal classification may be constructed. The main purpose of this form of classification is to describe the topology of the tree and the internested monophyletic groups it contains. This isomorphism produces an efficient information storage and retrieval system. Such a classification may be written as a Linnaean hierarchy, and when augmented with character information a diagnosis of sorts is produced. But it is not intended to be a traditional, inclusive diagnosis of the traits of each taxon. It is instead a description of the characters used in the analysis and the hierarchical level at which they are postulated to be synapomorphic. The diagnosis encompasses characters from homologous series that are two-state (the derived state remains a synapomorphy) or multistate (derived state I evolves into derived state II, and thus becomes synapomorphic at one level and symplesiomorphic at another). Hull (1979) and Wiley (1981a) have discussed these properties extensively. For example, a traditional "taxon" diagnosis of the Cercomeria would include "gut saccate, bifurcate, or absent." As we show in our results, a cladistic diagnosis separates the plesiomorphy and apomorphy in this statement and diagnoses the Cercomeria as "gut saccate" because that is concluded to be the primitive gut condition. The other two gut characters are introduced at the less inclusive taxonomic levels where they are postulated to have evolved.

Cladistic diagnoses are also often inappropriate for immediate use as a key. The important attributes of a key are clarity and convenience to allow rapid identification of specimens. It is in classifications, not keys, that phylogenetic relationships are proposed. Sometimes a key can be both succinct and natural (i.e., it follows the classificatory groupings). But this will be so only when the character series involved are all two-state (e.g., if a saccate gut were primitively present and always present in the Cercomeria). However, the existence of multistate character series (i.e., continuing evolution) usually makes an artificial key (i.e., no evolutionary connotations) preferable.

Results

Figure 1 gives the cladogram representing the best supported phylogenetic hypothesis for the cercomerians, based on 39 characters drawn from the 19 putative homologous series listed above. For reasons given in the discussion, some of the characters and series were not used. The same cladogram is obtained whether one uses dalyelloids as the outgroup or calculates the most parsimonious tree possible regardless of the outgroup (see Farris, 1979). We will show later that the use of acoel turbellarians as an outgroup for some of the members of our study group gives a poorer fit to the data than does our use of rhabdocoels. Numbers accompanying the slash marks on each branch of the cladogram refer to characters that are postulated to be synapomorphic at the level of the branch where they occur. The numbered characters are identified in the classification.

Classification and cladistic diagnosis for the subphylum Cercomeria Brooks, 1982

Subphylum Cercomeria Brooks, 1982

DIAGNOSIS: Rhabdocoelous platyhelminths with no vagina (1), single ovary and paired testes (2), paired lateral excretory vesicles (3), doliiform pharynx (4), saccate gut (5), copulatory stylet (6), no locomotor cilia in adults (7), Mehlis' gland (8), and posterior adhesive organ formed by an expansion of the parenchyma into an external pad, called a cercomer (9). All associated with at least one host type.

Superclass Temnocephalidea Benham, 1901

DIAGNOSIS: Cercomeria with anterior tentacles (10). Ectoparasites or commensals of invertebrates.

Superclass Udonellidea Ivanov, 1952

DIAGNOSIS: Cercomeria with genital pores in anterior half of body (11) and vagina (12). Ectoparasites of arthropods.

Superclass Cercomeridea taxon novum

DIAGNOSIS: Cercomeria with genital pores in anterior half of body (11), vagina (12), male genital pore and uterus proximate (13), and oral sucker (14). Ecto- and endoparasites of vertebrates, invertebrates, or both.

Class Trematoda Rudolphi, 1808

DIAGNOSIS: Cercomeridea with dorsal vagina a Laurer's canal (15), cercomer a sucker (16), and copulatory stylet lost (17).

Subclass Aspidocotylea Monticelli, 1892

DIAGNOSIS: Trematoda with vaginal opening lost (18) and ventral sucker modified in adults into ventral adhesive disk (19).

Subclass Digenea Van Beneden, 1858

DIAGNOSIS: Trematoda with bifurcate gut (20) and complex life cycle with vertebrate host secondarily acquired (21).

Class Cercomeromorphae Bychowsky, 1937

DIAGNOSIS: Cercomeridea with armed cercomer (22), doubled cerebral commissures (23), and doubled posterior commissures (24).

Subclass Monogenea Carus, 1863

DIAGNOSIS: Cercomeromorphae with paired lateral vaginae (25), bifurcate gut (26), and 12–16 hooks on larval cercomer.

Subclass Cestodaria Monticelli, 1891

DIAGNOSIS: Cercomeromorphae with osmoregulatory system becoming reticulate in later ontogeny (27), no intestine (28), posterior body invagination (29), no copulatory stylet (30), cercomer reduced in size and partially or totally invaginated (31), male genital pore not proximate to uterine opening (32), and vestigial oral structures (33).

Infrasubclass Gyrocotylidea Poche, 1926

DIAGNOSIS: Cestodaria with rosette at posterior end (34) and ten equal-sized hooks on larval cercomer.

Infrasubclass Cestoidea Rudolphi, 1808

DIAGNOSIS: Cestodaria with vagina and male genital pore proximate (35) and cercomer totally invaginated (36).

Superorder Amphilinidea Poche, 1922

DIAGNOSIS: Cestoidea with uterine pore in anterior half of body and genital pores in posterior half (37) and with six large and four small hooks on larval cercomer. At least one species known with complex life cycle with invertebrate host secondarily acquired.

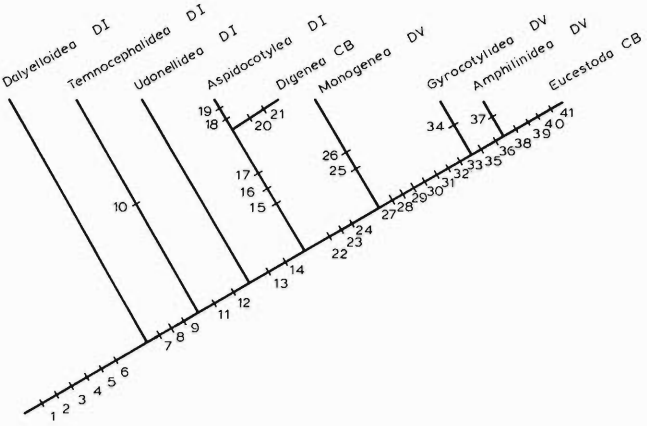


Figure 1. Cladogram representing the hypothesized phylogenetic relationships of the parasitic platyhelminth taxa examined. Characters are denoted by numbered slash marks and identified in the diagnoses in the text. Each slash mark postulates a synapomorphy, or evolutionary novelty, common to all the taxa above that branch. In some cases subsequent evolution has modified the character, creating an homologous, or transformation, series. There are 41 postulated changes for 39 characters indicating two cases of parallel evolution: loss of copulatory stylet (#17 and #30) and acquisition of bifurcate gut (#20 and #26). The taxa are identified as having life cycles that are direct in an invertebrate host (DI) or vertebrate host (DV) or complex with both an invertebrate and vertebrate host (CB) (see text for comments on the life cycles of amphilinideans).

Superorder Eucestoda Southwell, 1930

DIAGNOSIS: Cestoidea with polyzoic body (38), complex life cycles with invertebrate host secondarily acquired (39), protonephridia in posterior half of larva (40), cercomer lost during ontogeny (41), and six hooks on larval cercomer.

Artificial Key for the Subphylum Cercomeria Brooks, 1982

1a. Platyhelminths with double nervous commissures at the anterior and posterior ends of body; larvae with hooks on the posterior adhesive organ ..... Class Cercomeromorphae . . . 2

b. Platyhelminths with single nervous commissures at the anterior and posterior ends of body; larvae without hooks on the posterior adhesive organ 5

2a. Cercomeromorphae in which the posterior adhesive organ is an opisthaptor, bearing suckers and hooks; larval opisthaptor armed with 12–16 hooks; direct life cycles; usually ectoparasites of fish ..... Subclass Monogenea

b. Cercomeromorphae with an invagination at the posterior end of the body in the adult; gut absent; pharynx absent; copulatory stylet absent; reticulate osmoregulatory system developing later in ontogeny ..... Subclass Cestodaria . . . 3

3a. Cestodaria in which the posterior adhesive organ of the adult forms a rosette; larval cercomer with ten equal-sized hooks; parasitic in Holocephali ..... Infrasubclass Gyrocotylidea

b. Cestodaria in which the cercomer of the adult is totally invaginated; vaginal and male genital pores are proximate ..... Infrasubclass Cestoidea . . . 4

4a. Cestoidea in which the larval cercomer has six large and four small hooks; uterine pore is in the anterior half of the body, the genital pores are in the posterior half; parasitic in fish and turtles ..... Superorder Amphilinidea

b. Cestoidea in which the cercomer is lost in ontogeny; larval cercomer has six hooks; adults usually with polyzoic body; protonephridia in anterior half of the larvae; complex life cycles ..... Superorder Eucestoda

5a. Copulatory stylet absent; modified vagina forming a Laurer's canal; oral sucker usually present ..... Class Trematoda . . . 6

b. Copulatory stylet present; oral sucker absent 7

6a. Trematodes in which the posterior adhesive disk is modified to form a large ventral adhesive disc; saccate gut present; direct life cycles; host usually an invertebrate ..... Subclass Aspidocotylea

b. Trematodes in which the posterior adhesive organ is a sucker, which can be terminal, midventral, or secondarily lost; gut present, usually bifurcate; complex life cycles; definitive host usually a vertebrate ..... Subclass Digenea

7a. Vagina lacking; genital pores in the posterior half of body; anterior tentacles present (may be secondarily reduced) ..... Superclass Temnocephalidea

b. Vagina present; genital pores in the anterior half of body; tentacles lacking ..... Superclass Udonellidea

Discussion

Character evolution

The majority of the characters used in this analysis have been used previously by other workers studying the parasitic platyhelminths. This was done partly to facilitate comparisons with earlier classifications (discussed later), and partly to offer a phylogenetic hypothesis for further comparative work on newer characters. The study by Jamieson and Daddow (1982) on the ultrastructure of platyhelminth spermatozoa appears to be compatible with our analysis, although more work is required (see Rohde, 1971, 1980). Observations on platyhelminth excretory systems by Rohde (1980) and Rohde and Georgi (1983) offer another synapomorphy for the Cercomeridea (flame cell weir apparatus), the Trematoda (lamellated walls in the protonephridial ducts), and the Cestoidea (microvilli in the protonephridial ducts).

We must comment on several of the characters we have used, in light of their arrangement on the cladogram. First, we have broadened the use of the term *cercomer*. This is not simply an a priori decision, but an indication of the homology that we postulate to exist among the posterior adhesive organs of the taxa studied. There are thus two aspects to the matter: the hypotheses of homology drawn from structural studies, and the terms that are used to refer to those structures. The former must have precedence over the latter. Standard usage of the nomenclature would be maintained if the term *cercomer* was restricted to the armed posterior adhesive organ found in all larval and some adult cercomeromorphs, the term *ventral sucker* or *ventral adhesive disk* was restricted to the adhesive organs of trematodes, and the term *posterior adhesive disk* was restricted to the structure found in udonellideans and temnocephalideans. If the posterior adhesive organ of each taxon is given a different name, this can obscure the question of homology, whereas at the same time reinforcing arguments for convergent evolution of holdfast organs. But if all of the posterior holdfast organs of the platyhelminths are derived from a common ancestor, there then exists a multistate homologous series, each of whose characters are synapomorphic at certain levels of the cladogram. If this is the case, then all such organs should have the same name, with appropriate modifiers for further derived states.

Character interpretations build cladograms, and cladograms can be used to interpret other characters. We can justify our cladogram in Figure 1 by noting that even if all characters relating to posterior adhesive organs were removed (nos. 9, 16, 19, 22, 29, 31, 34, 36, and 41), the classification would still be fully supported. We can justify our interpretation of the homology of posterior adhesive organs, and our resulting broadening of the cercomer appellation, by referring to the four basic series of character development in that organ, discussed earlier and summarized in Table 1. Muscularization and hooks appear to be modifications of the phylogenetically earlier posterior parenchymal expansion. We therefore suggest that this expansion be called a cercomer, that modifiers be used to describe further derived states (loculate, sucker, armed, etc.), and that all of the taxa postulated to have inherited it or its modifications be united into a monophyletic group called the Cercomeria.

Some recent discussions of cercomeromorph relationships (Llewellyn, 1965; Malmberg, 1974) have placed emphasis on the number and structure of hooks on the larval cercomer. There are four major characters: 12–16 hooks in monogeneans, ten equal-sized hooks in gyrocotylideans, six large and four small hooks in amphilinideans, and six hooks in eucestodes. An initial interpretation from our cladogram would be that of a linear homologous series, from largest number to smallest, indicating an evolutionary trend towards reduction in number of hooks (Fig. 2a). Llewellyn (1965) proposed another se-

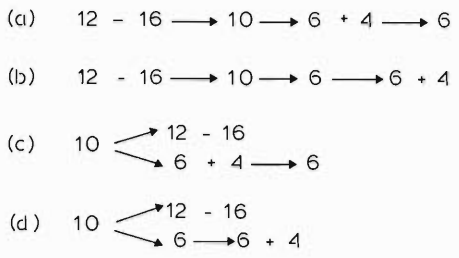


Figure 2. Four possible evolutionary transformations for the number of hooks on the larval cercomer in the Cercomeromorphae. All four series fit equally well on the phylogenetic tree in Figure 1 (see text for discussion).

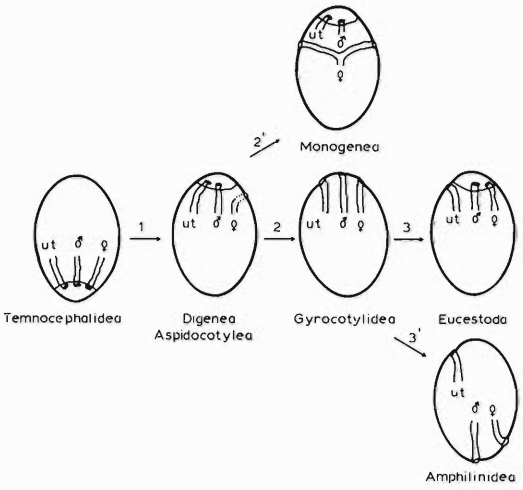


Figure 3. Diagrammatic representation of postulated evolutionary transformations of the relative positions of the genital pores in the Cercomeria. With the exception of the Temnocephalidea, the three pores are the male pore, the vagina, and the uterine pore (see character series k and l in Materials and Methods). Temnocephalideans possess a common genital atrium into which open a male canal, a uterine canal, and a canal from the ovary. This last canal functions as both an oviduct and a vagina—functions that become separated in the other taxa with the evolution of a vaginal canal. The anterior end of the diagrammatic bodies is at top. Transformations: (1) pores relocate in anterior, uterine and male pores meet in atrium, vagina ends in parenchyma, opens to dorsal surface, or joins excretory system; (2′) uterine and male pores remain together, vagina bifurcates; (2) pores open separately; (3′) male pore and vagina relocate in posterior; (3) male pore and vagina meet in atrium, uterine pore remains separate.

ries (Fig. 2b), suggesting that the four small hooks on the amphilinidean cercomer were secondarily evolved. Malmberg (1974) proposed a series opposite to that of Figure 2a, proceeding from smallest to largest number of hooks. We did not use the larval hooks as characters because we found that a number of different series fit the classification equally well (Fig. 2). That is, they all give equally parsimonious interpretations of the data without the need for postulating parallel or convergent evolution. Mickevich's (1982) Transformation Series Analysis would recognize cercomer hook number as a "trivial" character. This does *not* mean that it is of no phylogenetic significance, only that current analytic techniques allow too many evolutionary sequences to be considered equally likely. The data cannot be used to choose between hypotheses.

One might consider, however, the series in Figures 2b and 2d to be less likely because they require that the four small hooks of amphilinideans be derived from a different embryonic source than the other hooks when no empirical support for this exists. In fact, studies by Malmberg (1974) suggest the opposite, that all larval hooks are homologous. But this is still an open question, pending comparative developmental studies. Regardless of the resolution of this ambiguity, there would still be more than one equally likely series. This character appears at present to be of little help in studies of platyhelminth phylogenetic relationships beyond the recognition that those flatworms possessing an armed cercomer form a monophyletic group.

On the other hand, we found that the various characters pertaining to the genital openings in cercomerians provided useful evidence not fully utilized previously. To highlight this, we have illustrated the putative homologous series in Figure 3.

Previous studies of cercomerian relationships that attempted to link the cercomeromorphs as a group were restricted to one character: the armed cercomer. However, for at least some monogeneans (Rohde, 1968, 1975), gyrocotylideans (Watson, 1911; Allison, 1980), and eucestodes (Tower, 1900; Fairweather and Threadgold, 1983) enough is known of the anatomy of the nervous system to give two additional characters. Digeneans, aspidocotyleans, and all groups of rhabdocoels for which we could obtain data (see Bullock and Horridge, 1965) possess a central nervous system comprised of two bilateral longitudinal trunks with an anterior (cerebral) and posterior commissure. The cercomeromorphs differ in their possession of doubled cerebral and

posterior commissures. Some eucestodes may even have a third anterior commissure. It was the discovery of doubled posterior commissures in *Gyrocotyle* that led Watson (1911) to postulate that the eucestode scolex was derived from the posterior end of the body. This theory is falsified by the observation that in gyrocotylideans the rosette develops at the same body end bearing the larval cercomer, whereas in eucestodes (e.g., the cysticercus of *Hymenolepis*; see Alicata and Chang, 1939) the scolex and cercomer develop at opposite ends of the body. Some ambiguity in character analysis of the nervous system remains, particularly about the condition in amphilinideans, and the differences between ring and bridge commissures (see Lynch, 1945).

Our analysis also offers new interpretations of the evolution of the gut in platyhelminths. We have considered three characters: saccate gut, bifurcate gut, and the lack of a gut. Our cladogram in Figure 1 postulates that the bifurcate condition is convergent, having evolved separately in the Digenea and Monogenea. The same number of character steps (see the Comparisons with Other Classifications section) results if it is postulated that a bifurcate gut arose once as a synapomorphy for the Cercomeridea, then reverted to a saccate condition in the Aspidocotylea. This alternate interpretation must be considered, especially in view of the existence of aspidocotyleans (e.g., *Zonocotyle*) with bifurcate guts, and digeneans (e.g., Haplosplanchnidae) and monogeneans (e.g., Bothitrematidae) with saccate guts. These anomalies may be atavistic traits—characters from an earlier phylogenetic position in a homologue series. The likelihood of this is greater when the organisms are one of the most plesiomorphic taxa in their clade. The more derived a taxon is, the more likely it becomes that an apparent atavism is actually a further derived state with superficial similarity to the plesiomorphic state. A cladistic analysis of the Digenea to the family level by Brooks et al. (1985) suggests that haplosplanchnids are a relatively derived taxon and therefore nonatavistic in their gut condition. Similar studies are necessary for the Aspidocotylea and Monogenea before any further conclusions can be made.

The third gut character we have examined is that of the lack of a gut. We have suggested that this condition, which occurs in the Cestodaria (sensu nobis), is best interpreted as a derived state within the Cercomeria, rather than evidence that a gut never existed in the ancestors of gyrocotylideans, amphilinideans, and eucestodes (we discuss other authors' hypotheses of an acoel ancestry for the Cercomeromorphae in a later section). Our interpretation of secondary loss can be supported by two lines of evidence. First, those taxa lacking a gut are placed as highly derived groups by synapomorphic characters other than those involving the condition of the gut. All of the cercomerian taxa postulated to be plesiomorphic to the Cestodaria possess a gut. Second, it may not be the case that cestodarians lack any vestige whatsoever of a *digestive system* (as opposed to a gut). By this we do not refer to the question of the existence of entoderm or entoderm precursor cells in eucestode development (see Mackiewicz, 1981, and references therein). If these are present, there is simply a confirmation of the symplesiomorphic trait of the presence of a gut (with the derived character no. 28 in Fig. 1 becoming "gut components present in ontogeny" instead of "no gut"). But given our first line of evidence above, this a secondary point whose perceived importance to phylogenetic study arises from assumptions of the necessity for recapitulatory ontogeny (as well as assumptions about the formation of germ layers during cestodarian embryogenesis—see below). We refer instead to the question of structures associated with a gut in the more plesiomorphic flatworm taxa. Trematodes and monogeneans exhibit, as do rhabdocoels, a form of ectolecithal embryogenesis (see Hyman, 1951; Rees, 1940). There is no folding of germ layers to form body cavities. Instead, a part of the ectoderm grows inward, then hollows out and forms a mouth (which may become surrounded by an oral sucker) and pharynx invagination, and parts of the parenchyma hollow out and form the intestine. The embryogenesis of the Cestodaria is not so easily categorized, mainly because of changes in the relative sizes of the blastomeres. The basic pattern is nevertheless ectolecithal or hemiectolecithal (see Douglas, 1963, and references therein).

Cestodarian embryos clearly do not develop into adults possessing an intestine formed by an internal parenchymal cavitation (the larvae of the Cyclophyllidea possess another cavity, called the *primary lacuna* [see Freeman, 1973]). They do, however, display, at some stage in ontogeny, an anterior invagination of the ectoderm with varying degrees of muscularization and paren-

chymal modification. In gyrocotylideans and most amphilinideans this persists throughout life. We have observed living *Gyrocotyle* in the spiral valve of ratfish using this structure to take in host gut contents. If functional definitions are used, the absence of an intestine precludes calling the anterior invagination a mouth. In gyrocotylideans and amphilinideans it is termed an *anterior invagination,* and in eucestodes it is called an *apical sucker.* But, as with existing terminology for posterior holdfast organs, the nomenclature may be obscuring homologies. All of the Cercomeria possess an anterior invagination of the ectoderm at some time in ontogeny, and in all groups except the Cestodaria it becomes a functional pharynx and mouth when it connects with an intestine formed by a parenchymal cavity. Given these observations, we postulate that the anterior invagination of cestodarians is a vestigial mouth, and is therefore evidence of the primitive presence of a complete digestive system.

Adaptive significance of characters

Under current evolutionary theory, perceptions of the adaptive significance of characters will affect hypotheses of their origin and their relationship to similar characters in other taxa. In the cercomerian nervous system, the doubled commissures can be examined. Although it is relatively easy to envisage plausible functional explanations of what these structures do today, we suggest that there is no adaptive significance in their evolution, that is, the reasons for their appearance in the first place. Digeneans and aspidocotyleans have oral suckers and pharynges that operate with a single cerebral commissure, so the second commissure in monogeneans cannot be an adaptation for oral function. The two cerebral commissures are intimately involved in the development of the eucestode scolex, yet the doubled structure seems to have evolved before either the loss of the gut or the modifications of the mouth and anterior end into a holdfast organ. We interpret this to imply that the doubled commissures first evolved without affecting the functioning of the body ends of ancestral cercomeromorphs. That is, the single commissures in trematodes and the doubled commissures in cercomeromorphs are not only characters in the same homologous series, but functional equivalents (different structures performing the same function) as well.

Adaptationist explanations require either that functional equivalents be nonhomologous (e.g., birds' wings and insects' wings are adaptations for flying but are not homologues; monogenean cercomers and eucestode scolices are adaptations for holding onto the host but are not homologues), or that parts of an homologous series have different functions (e.g., shrew forelimbs are adaptations for burrowing, bat forelimbs are adaptations for flying, yet the two are homologues; monogenean cercomers are adaptations for holding onto the definitive host, eucestode cercomers are adaptations for penetrating the intermediate host, yet the two are homologues). It is undoubtedly the structure of the central nervous system in eucestodes that allows the functioning of the rostellum and four suckers, or of the four bothridia, but that does not mean that the structure is an adaptation for that function (i.e., that it was ever the focus of selection).

Gould and Vrba (1982) proposed the term *exaptation* to refer to structures that arose evolutionarily prior to the time they were coopted by natural selection for a particular function. This term was offered as a refinement of the more general concept of "pre-adaptation." We suggest that there is another way to interpret such character evolution. Some traits may evolve without any functional difference from the ancestral trait, i.e., the two would be functional equivalents. However, these new traits might allow other evolutionary changes to take place, which in turn could have major evolutionary consequences. For example, dalyelloid rhabdocoels kept alive in our laboratory adhere to a substrate by flattening the posterior end of their body and applying their adhesive secretions (an indication that posterior adhesive *behavior* was present in the outgroup before complex posterior adhesive *organs* evolved in the study group). With innervation capable of functioning in such a manner, many structural modifications of the posterior end could act as holdfasts. Similarly, the occurrence of doubled anterior nerve commissures would provide the innervation basis for the subsequent evolution of scolices.

Our alternate interpretation can also be applied to explanations of the evolution of tegumental structures in the Cercomeria. Finger-like projections of the tegumental surface are especially well developed in those parasitic platyhelminths lacking a gut. This has led to explanations that such structures evolved in order to

produce an alternative nutrient absorptive surface. But these projections have been found, to one degree or another, in temnocephalideans, trematodes, monogeneans, gyrocotylideans, amphilinideans, and eucestodes (e.g., Lee, 1966, 1972; Williams, 1981; Rohde and Georgi, 1983). They have, in fact, been reported to be present in all platyhelminths (Bedini and Papi, 1974). Lee called all such projections microvilli. Other workers (see Jarecka et al., 1981, and references therein) have adopted Rothman's (1963) distinction between those projections containing only cytoplasm (microvilli) and those with electron-dense caps as well (microtriches). The latter are especially prevalent around the scolex and neck in eucestode adults. Jarecka et al. (1981) demonstrated that although microtriches are present on the necks and scolices of procercoids, cercoscolices, cysticercoids and cysticerci, there are microvilli (sensu Rothman) on the "tails" of procercoids, cercoscolices and cysticercoids, the cyst wall of cysticercoids, and the bladder wall of cysticerci. An intermediate type of projection occurs in gyrocotylideans (Lyons, 1969) and amphilinideans (Rohde and Georgi, 1983). Developmental studies (e.g., MacKinnon and Burt, 1984) on eucestodes indicate that microtriches are derived from microvilli. Other studies suggest that rostellar hooks are derived from microtriches (Mount, 1970).

Malmberg (1974) (see Fig. 7) noted that tegumental similarities could be the result of (1) convergent evolution in digeneans and cercomeromorphs, with parallel evolution within the Cercomeromorphae (a postulate that is more parsimoniously interpreted to be a synapomorphy); or (2) the result of a common ancestor for digeneans and cercomeromorphs. Rohde and Georgi (1983) considered the projections in amphilinideans to be an adaptation to food absorption and of "no great phylogenetic significance" (but see Rohde, 1980). We suggest that not only are digitiform tegumental projections in the Cercomeria homologous, but that their functional utility in cestodarians is a consequence of their primitive occurrence in platyhelminths with a gut, which allowed the survival of descendants lacking a gut. No postulates of convergent or parallel evolution are necessary.

The evolution of life cycles

Our classification allows some hypotheses to be formed about the origin of life cycles in the Cercomeria (see also O'Grady, 1985). The life cycle criteria for the nine taxa can be mapped onto the cladogram in Figure 1 as direct life cycles involving invertebrate or vertebrate hosts, or complex life cycles involving both invertebrates and vertebrates. A direct cycle in an invertebrate is concluded to be plesiomorphic because it is the life cycle of the most plesiomorphic taxa. This conclusion is reached by both direct inspection of the taxa and Farris optimization (Farris, 1970) of the nodal values. The questions asked are (1) in what manner did a direct cycle in a vertebrate arise, and (2) are the complex cycles of digeneans and eucestodes (as well as some species of amphilinideans) an example of convergent evolution? Certainly the life cycles in the Digenea and Eucestoda appear functionally similar, with larval stages developing in invertebrates and the adults in vertebrates. In each case, a dissemination of life stages results. Differences arise, however, when the morphological traits and sister-group relationships are examined.

In eucestodes, it is the *larvae* that bear the closest resemblance to the adults of the sister-groups, the Amphilinidea, Gyrocotylidea, and Monogenea. The armed cercomer, for example, synapomorphic for the Cercomeromorphae, is present in only the early developmental stages of eucestodes. These include the hexacanth, procercoid, cysticercoid, and exceptionally the cysticercus (see Jarecka, 1975, Fig. 4). The characters representative of most eucestodes, such as a scolex and polyzoic body, are best interpreted as adult characters added to the end of the ancestral developmental sequence by *terminal addition.* Furthermore, whereas adults of other members of the Cercomeromorphae develop in invertebrate hosts, the comparable (i.e., of the same phylogenetic origin) developmental stage in eucestodes—the larva—develops in a more recently acquired invertebrate host. Because this relatively newer host harbors an intermediate developmental stage, its means of acquisition is one of intercalation, or *nonterminal addition* to the ancestral direct life cycle in vertebrates. Rohde and Georgi's (1983) discovery that *Austramphilina elongata* develops through an intermediate arthropod host indicates that the life cycle changes hypothesized above may actually be synapomorphic for the Cestoidea. The study by Janicki (1928) on *Amphilina foliacea* indicates this as well (see Rohde and Georgi, 1983).

The data in Figure 1 suggest that the evolution

of the life cycle in digeneans occurred in a reverse manner to that of eucestodes. Adult digeneans most closely resemble the *adults* of their sister-groups, the Aspidocotylea, Udonellidea, and Temnocephalidea. For example, these groups possess an unarmed cercomer (as defined earlier), synapomorphic for the Cercomeria. The only nonadult digenean stages to have this structure are the cercariae and metacercariae. The larval stages associated with the complex life cycle—miracidia, sporocysts and rediae—lack this character and do not appear to have comparable developmental stages in the most closely related taxa. This suggests an intercalation of developmental stages by *nonterminal addition.* With respect to the hosts, adults of dalyelloids, temnocephalideans, udonellideans, and aspidocotyleans develop in association with invertebrates (some aspidocotyleans occur with vertebrates). In digeneans, this plesiomorphic host group contains the relatively new larval stages, whereas the plesiomorphic developmental stage, the adult, is found in the more recently acquired vertebrate host. This acquisition is best interpreted as a *terminal addition* to the ancestral life cycle.

Our analysis therefore suggests that the complex life cycles of digeneans and eucestodes evolved by different means. Eucestodes exhibit terminal addition of ontogenetic stages and nonterminal addition of an invertebrate host. Digeneans exhibit nonterminal addition of ontogenetic stages and terminal addition of a vertebrate host. Vertebrates also appear to have been colonized by the ancestral cercomeromorphs.

A number of general conclusions are implied by this hypothesis. First, as with morphological characters, Hennigian analysis allows life cycle components to be treated as composites of plesiomorphic and apomorphic states, inherited and modified at different times. It is not necessary to search for the "archetypal" digenean or eucestode. Second, the term "complex life cycle" is too broad to be applied to both the Digenea and Eucestoda when evolutionary conclusions are to be drawn from the comparison. Third, the terms "intermediate host" and "definitive host" may have two meanings. One is functional, the other is historical. Our study has shown that the functional usage may obscure evolutionary differences. The historical usage, however, does not obscure the functionality of the hosts. Thus, we would call the plesiomorphic host for digeneans (the mollusc) the *functional intermediate host* and the plesiomorphic host for eucestodes (the vertebrate) the *functional definitive host.* This retains information about both the history and the function of the taxa involved.

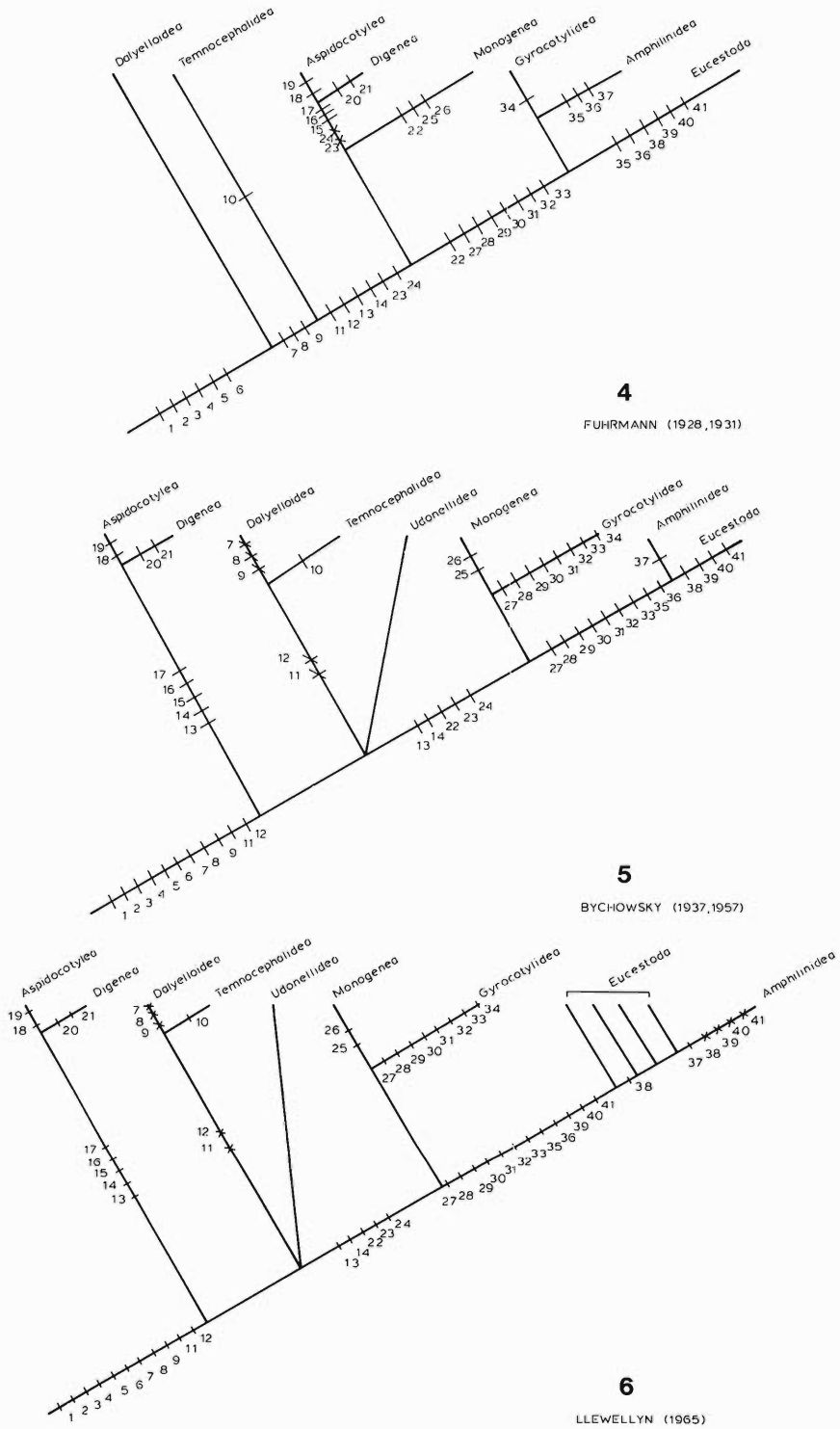
Comparisons with other classifications

Previous classifications for these platyhelminths include those by Spengel (1905), Janicki (1920), Fuhrmann (1928, 1931), Bychowsky (1937, 1957; see also Beklemishev, 1969; Dubinina, 1974), Llewellyn (1965), Price (1967), and Malmberg (1974). For reasons we will discuss later, it is difficult to make comparisons with the work of Spengel and Janicki. The classifications of the remaining authors are presented in Figures 4–9, rendered in a form comparable with our results. Numbers accompanying the slash marks refer to the characters from our cladogram in Figure 1, optimized with Farris' (1970) method in order to obtain the best fit possible. In some cases this resulted in hypotheses of evolution and inclusion of groups which the original author did not suggest, but with which a better fit to the stated hypotheses could be obtained. We have thus attempted to ensure that differences result from the classifications themselves and not from differential treatment of the data. At the same time we recognize that by adding data to an earlier classification we are not examining that proposal exactly as its originator formulated it. For systematics, however, these concerns are superseded by the necessity to treat every classification as an hypothesis subject to testing with new information.

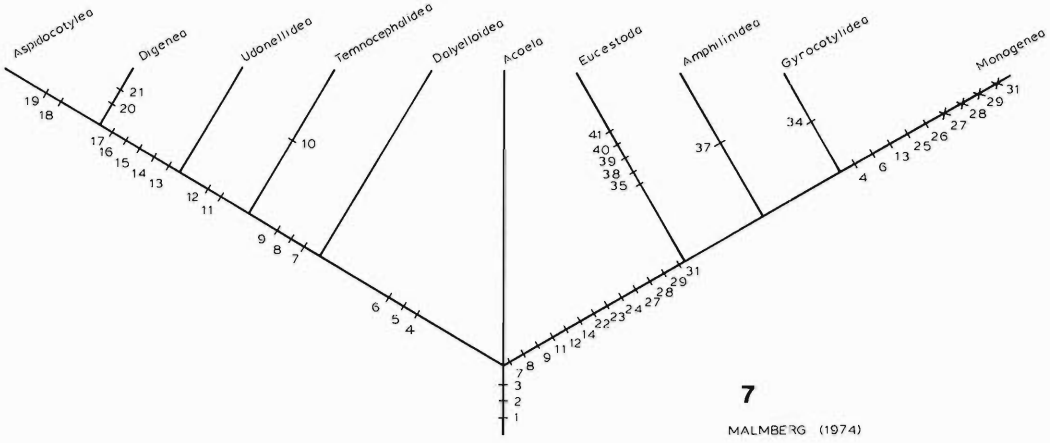
Classifications can be assessed in two ways. The first is the efficiency with which the data are described. This amounts to postulating the least amount of homoplasy or minimizing the type 2 ad hoc assumptions discussed earlier. Homopla-

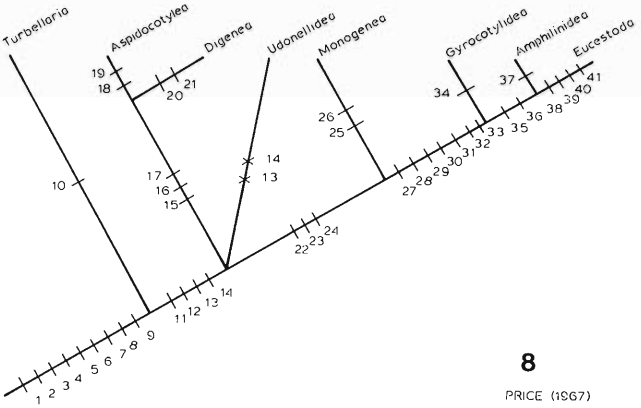
→

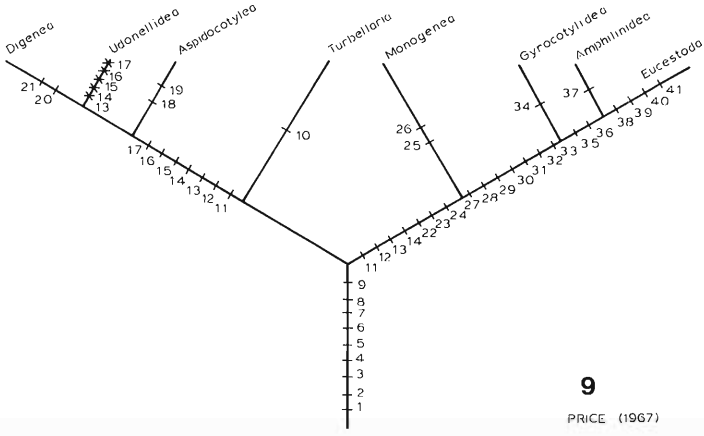
Figures 4–9. Cladograms depicting previous classifications of the parasitic platyhelminths, and the postulations of character evolution they require. Numbered slash marks refer to characters in Figure 1, "X" indicates an evolutionary reversal. Branches ending in a node (rather than a terminal taxon) that bear no slash marks propose a grouping that has no character support.



Figures 4-6. 4. Fuhrmann (1928, 1931). 5. Bychowsky (1937, 1957). 6. Llewellyn (1965).







Figures 7–9. 7. Malmberg (1974). 8. Price (1967) (phylogenetic tree). 9. Price (1967) (classification).

sy shows up in two ways in the classifications. These are: (1) postulations of parallel or convergent evolution, tabulated by counting the number of duplicated numbers on the trees; and (2) postulations of evolutionary reversals, or the secondary loss of a trait, tabulated by counting the number of characters denoted by an "X" on the trees. The fewer of either of these, the better the summary of the data. Efficiency can also be measured by the *consistency index* (CI) of Kluge and Farris (1969). This is calculated by dividing the minimum numbers of steps needed to represent the data (i.e., each character evolves once) by the actual number of steps required to support a particular classification. The closer the CI is to 1.0, the better the fit of the data to the tree. Our classification, for example, has a minimum number of 39 steps (39 characters), and an actual number of 41 steps, giving a CI of 39/41, or 0.95. The departure from maximum efficiency comes from the postulation of two cases of homoplasy: the loss of a copulatory stylet in the Trematoda and Cestodaria, and the development of a bifurcate gut in the Digenea and Monogenea.

The second way in which classifications can be assessed is by examining them for groupings of taxa for which there are no distinguishing characters; that is, the branch uniting those taxa on the tree has no character support (slash marks in Figs. 4–9). This can result from a type 1 ad hoc assumption, discussed earlier, in which taxa of secondary importance in a study group are grouped together by default because they do not possess a structure that has been used to highlight the taxa of primary interest (see Price, 1967, on the Acercomeromorphae). An unsupported grouping can also be included in a classification for heuristic, rather than empirical, reasons (see Inglis, 1983, on the Aschelmintha). Clearly, it is not helpful for taxonomic groupings to be devoid of diagnostic features; nevertheless, a number of higher taxa have been defined by what they are not. For example, "reptiles" are amniotes without bird-like or mammal-like features; the reptiles as a group have no distinguishing traits. Inspection of a phylogenetic tree for the Tetrapoda shows that reptiles are not an evolutionary group (Wiley, 1981b), that is, not all reptiles are each others' closest relatives. Birds and crocodiles (the Archosauria) are more closely related to each other than they are to any other group of organisms. Portions of classifications, therefore, that have empty branches when represented as a phylogenetic tree may be suspected of postulating artificial, nonevolutionary groups. Parenthetically, the extent to which such artificial groups have been defined by functional criteria is the extent to which departures from a strictly phylogenetic classification have been justified by reference to adaptive scenarios.

Table 2. Comparisons of previous classifications of parasitic platyhelminths with the present study. Three criteria are used to evaluate the fit of the classifications to the data set of 39 characters in Figure 1: the efficiency of character representation (number of steps), the degree to which the classification is supported by ambiguous characters [the consistency index, or CI, of Kluge and Farris (1969)—see text], and the number of unsupported groupings (number of empty branches). A good fit to the data is indicated by a high CI and a low number of steps and empty branches. Two of the four empty branches in Llewellyn's classification come from the postulation of paraphyly in the Eucestoda.

Author	No. of steps	CI	No. of empty branches
Fuhrmann, 1928, 1931	46	0.85	2
Bychowsky, 1937, 1957	55	0.71	2
Llewellyn, 1965	59	0.66	4
Price, 1967 (tree)	43	0.91	0
Price, 1967 (classification)	50	0.78	2
Malmberg, 1974	50	0.78	2
Present study	41	0.95	0

Table 2 summarizes the step length, CI, and empty branch statistics for the classifications we examined. Those by Fuhrmann (1928, 1931), Llewellyn (1965), Bychowsky (1937, 1957), and Malmberg (1974) appear to have made unnecessary postulates of convergent evolution. Such empirically unjustified departures from the data are difficult to detect when they can be considered to support perceptions of the adaptive plasticity (i.e., capacity for homoplasious evolution) of parasites (e.g., Price, 1980).

The classification by Malmberg (1974) is especially interesting because it is the only explicit, rather than anecdotal, attempt to support the hypothesis that cercomeromorphs are more closely related to acoel turbellarians than they are to trematodes. A review of this hypothesis is beyond the scope of this paper; it has gained recent support from Malmberg (1974), Logachev and Sokolova (1975), Freeman (1982), and Mackiewicz (1981, 1982). Its basic argument cites the absence of a digestive system in cestodarians (sensu nobis). Malmberg (1974) stated:

> "If the absence of mouth, pharynx and intestine in the ontogeny of cestodes, the amphilinideans and the gyrocotylideans implies that these body parts were never evolved here, then these groups cannot have originated from rhabdocoelan creatures."

Freeman (1982) gave the most succinct statement of the evidence:

> "Incidentally not even the suggestion of endoderm let alone a tube-like gut in the ontogeny of any present-day cestode (e.g. see Logachev and Sokolova, 1975), suggests that it never had such a gut . . . ."

We are dissatisfied with this type of reasoning. It is not possible to decide, *by reference to a character's absence alone,* whether that condition is due to primitive absence or subsequent loss (i.e., whether it is a primitive or derived character). Other characters must be examined. We suggest that the perceived necessity for all ancestral characters to remain in a descendant's ontogeny comes from perceptions of the ubiquity of recapitulatory development and evolution by nothing but terminal addition of developmental stages. The necessity of corroborative characters, however, may be a moot point in this case, in light of our earlier suggestion that the anterior invaginations of cestodarians may be vestigial mouths and pharynges.

Malmberg (1974) recognized the problems that secondary loss and expectations of recapitulatory development could create for phylogenetic studies, but his proposed solution—the primitive absence of a gut in the cercomeromorphs—creates problems of another sort. Let us assume for the sake of argument that anterior invaginations in cestodarians are convergent traits having nothing to do with a gut. There are still 38 other characters to be explained. Confirmation of the primitively gutless nature of cestodarians could come from those other characters analyzed independently. But, as we have shown, the most efficient and least ad hoc interpretation of those other characters supports relationships with the trematodes, udonellideans, temnocephalideans, and rhabdocoels—not the acoels. Malmberg's scheme (Fig. 7) requires 9 cases of convergent evolution to account for the structural similarities among the parasitic platyhelminths that we include in the Cercomeria.

Karling (1974), in a cladistic analysis of the Turbellaria, considered trematodes and cercomeromorphs to be derived from rhabdocoels. Also, platyhelminths may not be primitively gutless at all. The Cnidaria, Ctenophora, Gnathostomulida, and Nemertinea, as well as most platyhelminths, have intestines. Even some acoels, such as *Nemertoderma,* have intestines (see Karling, 1967). If the cercomeromorphs are grouped with acoels, *on the basis of their gutless condition alone,* there are still some gutless platyhelminths excluded from that grouping. Some dalyelloid rhabdocoel (Fecampiidae) parasites of crustaceans, such as *Kronborgia amphipodicola,* have no mouth, pharynx, or intestine (Christensen and Kanneworff, 1964). *Levenseniella* (*Monarrhenos*) *capitanea* is a microphallid digenean that lacks a pharynx and has only a few fibrous intestinal tissues (Overstreet and Perry, 1972). Clearly, one does not classify these taxa with the cercomeromorphs and acoels. Such a decision would require the postulation of a very large amount of homoplasious evolution of rhabdocoel-like and digenean-like traits. Our hypothesis of the phylogeny of the cercomeria is an attempt to maintain consistency in the method of inference of genealogical relationships, namely, to apply parsimony considerations to character analysis at every level of generality in the study group. We do not believe that previous classifications can offer adequate justification for the departures from parsimony that some of their groupings require.

The classifications by Spengel (1905) and Janicki (1920) are too incomplete to be fully compared with ours. We have attempted an analysis of Fuhrmann's work even though it is only slightly more complete. Spengel and Janicki considered four taxa: rhabdocoels, digeneans, monogeneans, and eucestodes. Spengel's placement of these four corresponds not only to ours, but also to almost every other classification we have considered. Our analysis of Bychowsky's classification, which is an expansion of Spengel's, shows that subsequent taxa and data were interpreted in a suboptimal manner.

Janicki's classification was connected with his cercomer theory. He proposed that monogeneans arose from rhabdocoels, then gave rise to digeneans, which gave rise to eucestodes. This interpretation was supported by the conclusion that (1) the cercomer of procercoids, the cyst and tail of cysticercoids, and the bladder of cysticerci are homologues; (2) these are homologous with

the opisthaptor of monogeneans; and (3) these are homologous with the tail of digenean cercariae. Janicki termed these cercomer-homologue-bearing taxa the "Cercomeromorphae." Bychowsky removed the Digenea from this grouping and retained the term to denote those platyhelminths with an armed cercomer. We have used the term in Bychowsky's sense but altered the inferred relationships of the taxa involved (cf. Figs. 1 and 5). Jarecka et al. (1981) drew from earlier work by Jarecka (e.g., 1975) and suggested that the presence of microvilli on the posterior body expansions of eucestode larvae provided support for Janicki's postulates of cercomer homology with the exception of the Digenea. Freeman (1973) noted the importance of distinguishing between the cercomer and the midbody of eucestode larvae when examining such homology. We have offered evidence for extending the series of cercomer homologues first to the trematodes (on the basis of the ventral adhesive disk, rather than the cercarial tail)—to form the Cercomeridea, and then to the Temnocephalidea—to form the Cercomeria.

A third problematic classification is that by Price (1967), who presented both a phylogenetic tree and a classification (see Table 2). The tree is not resolved at its base and is ambiguous as to the monophyly of the Cercomeria. Price's discussion, however, suggests that monophyly was being postulated. If we assume this, his tree can be represented by Figure 8. It differs from ours in two ways. First, it places *Udonella* in a trichotomy (three branches arising from one node) with trematodes and cercomeromorphs. Second, it does not distinguish between dalyelloid and temnocephalid turbellarians. This creates a problem for the mapping of characters 7, 8, 9, and 10 (see Fig. 1). We think it best, and fairest, to minimize the number of steps by maintaining Price's Turbellaria category, and simply note that not all of its members possess characters 7, 8, and 9, or 10. New problems arise when the phylogenetic inferences of Price's classification are made explicit by converting it into a tree (Fig. 9). The result gives a poorer fit to the data than does his original phylogenetic tree. The classification is therefore inconsistent with the original tree (see Wiley, 1981b). This means that the evolutionary relationships it implies are not the same as those given by the tree from which it was constructed. This is caused by the creation of a type 1 ad hoc grouping—the Acercomeromorphae. This grouping may have some "pragmatic" utility for the recognition of flatworms whose larvae do not possess an armed cercomer, but *given the phylogenetic tree whose relationships the classification is intended to communicate,* this trait cannot be considered to be an indicator of monophyly, as can an armed cercomer in the Cercomeromorphae.

Conclusions

Based primarily upon characters previously used to classify parasitic platyhelminths, we have derived a classification that best fits the data. It embodies elements common to most earlier proposals, and some aspects of each previous classification agree with ours. In total, however, neither our classification nor its justifications have been presented before. Some characters used previously are concluded to be too ambiguous for phylogenetic inference. Others, notably genital pores, posterior adhesive organs, and anterior invaginations are each united into homologous series. Revised terminology is proposed for the latter two character series. The sequence of origin for some traits previously taken to be of special taxonomic importance is considered to be inconsistent with explanations that they evolved as adaptations for a particular function. The classification indicates that the plesiomorphic life cycle of the Cercomeria is direct and in an invertebrate. Vertebrates appear to have been colonized twice: by the Digenea and by the ancestral cercomeromorphs. Invertebrates, especially arthropods, appear to have been recolonized by the Eucestoda or possibly the Cestoidea. The functional terms of "complex life cycle," "intermediate host," and "definitive host" obscure phylogenetic relationships. The postulate that cestodarians and monogeneans are descended from acoel turbellarians is rejected on the basis that it derives its support from unjustified assumptions of convergent evolution. The present study also offers new character analyses that conflict with this hypothesis. The evidence presented herein links monogeneans and cestodarians with trematodes and these three groups with dalyelloid rhabdocoels.

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Lecithodendriidae (Trematoda) from *Taphozous melanopogon* (Chiroptera) in Perlis, Malaysia

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ABSTRACT: Five species of Lecithodendriidae (Trematoda) were recovered from *Taphozous melanopogon* (Chiroptera) in Perlis, Malaysia: *Fontius molenkampi, F. klausrohdei, Papillatrium parvouterus, Paralecithodendrium longiforme,* and *P. ovimagnosum.* The genus *Fontius* is erected for lecithodendriids that possess a bulbous hermaphroditic organ and *Paralecithodendrium molenkampi* is designated as the type species. *Fontius klausrohdei* sp. n. can be distinguished from *F. molenkampi* because *F. klausrohdei* has the hermaphroditic organ drawn into a permanent nipple-like structure and has a smooth-margined ovary. *Paralecithodendrium parvouterus* is transferred to the genus *Papillatrium* because it has a genital atrium that contains a papilla. *Castroia kamariae* (type 2) is a junior synonym of *P. parvouterus. Castroia kamariae* (type 1) and *Paralecithodendrium cysticircum* are junior synonyms of *Paralecithodendrium ovimagnosum.*

Twenty-five black-bearded tomb bats *Taphozous melanopogon* Temmink, 1841, were collected from a cave 5 km south of the city of Kangar, state of Perlis, Malaysia. Five species of Lecithodendriidae were recovered from their small intestines. Herein we review the taxonomic status of these species and tabulate their host and locality records.

Trematodes were fixed in AFA (alcohol-formalin-acetic acid) without flattening, stained (Van Cleave's hematoxylin or Mayer's paracarmine), and examined as permament whole mounts or 10-μm-thick serial sections. All measurements are in micrometers with ranges followed by means in parentheses. USNM Helm. Coll. refers to the National Parasite Collection, United States Department of Agriculture, Beltsville, Maryland 20705, USA.

Fontius gen. n.

DIAGNOSTIC CHARACTER: Terminal genitalia consist of a common genital duct surrounded by a protrusible, bulbous, hermaphroditic organ (Figs. 2, 8).

DIAGNOSIS: Lecithodendriidae. Body small, pyriform to oval. Ceca short, widely divergent, end near anterior margin of testes. Acetabulum in middle third of body. Testes opposite in acetabular or preacetabular zone. Genital pore medial, preacetabular. Cirrus pouch membranous contains seminal vesicle, pars prostatica, and well-developed prostatic gland. Terminal genitalia consist of a common genital duct surrounded by a hermaphroditic organ. Ovary submedian in acetabular or testicular zone, lobed or entire. Laurer's canal arises from seminal receptacle. Vitellaria pretesticular. Uterus occupies most of hindbody. Eggs small, numerous. Excretory vesicle V- or Y-shaped.

ETYMOLOGY: The genus is named for Professor William F. Font, parasitologist, University of Wisconsin–Eau Claire, USA.

TYPE SPECIES: *Fontius molenkampi* (Lie, 1951) comb. n.

Fontius molenkampi (Lie, 1951) comb. n. (Figs. 1, 2)

=*Paralecithodendrium molenkampi* Lie, 1951.
=*Prosthodendrium molenkampi:* Dubois, 1962.

SPECIMENS EXAMINED: Two individuals loaned by Dr. Kian Joe Lie, Hooper Foundation, University of California, San Francisco, California 94143, USA.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 78377.

REMARKS: Our specimens agree with the original description by Lie (1951) and the redescription by Manning et al. (1971). From serial sections we determined that Laurer's canal arises from the seminal receptacle and opens on the dorsal surface.

We disagree with the use of "genital sucker" by Lie (1951) and Manning et al. (1971) for the organ surrounding the terminal genital duct (Fig. 2). The structure, as seen in serial sections, is not

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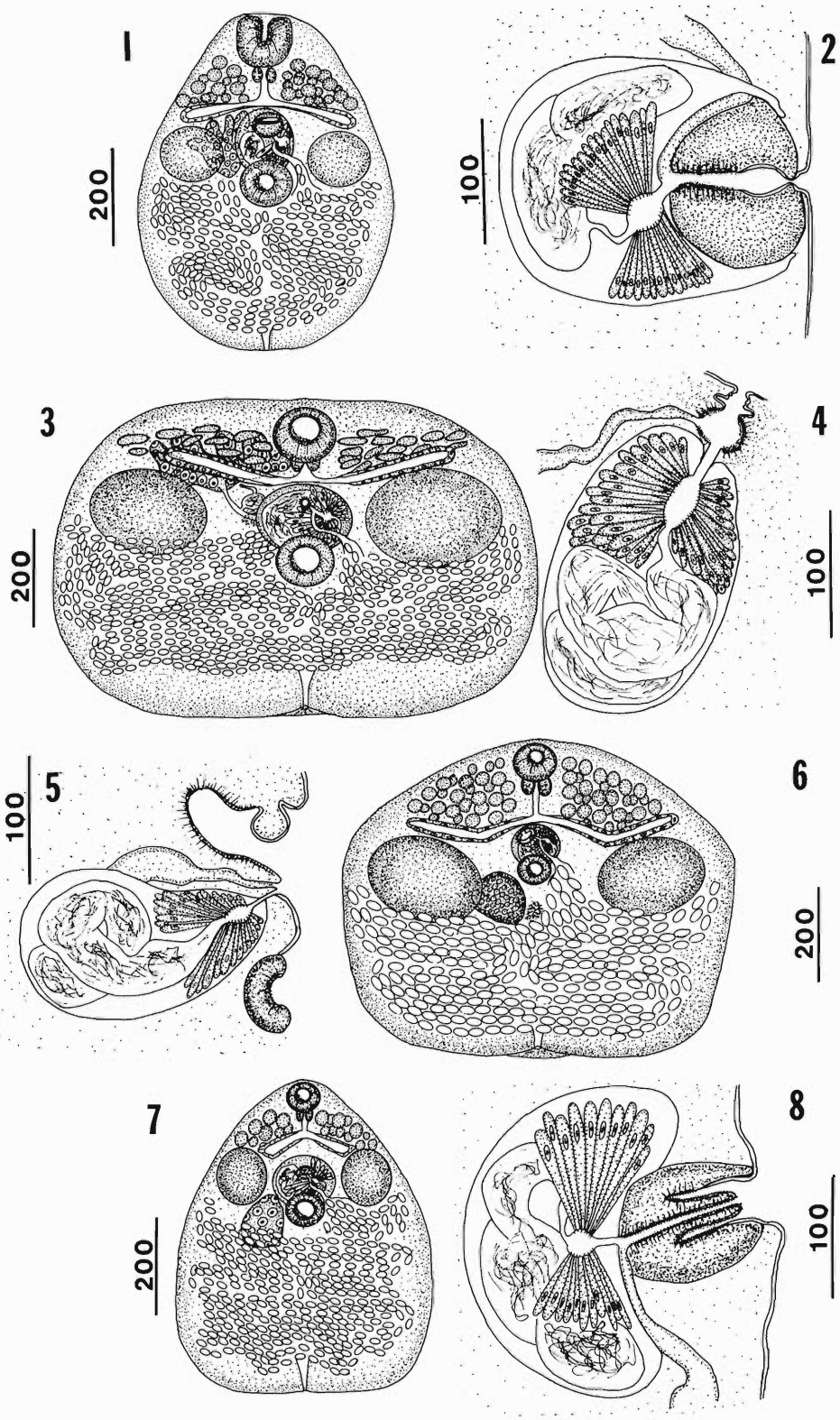

muscular and because it contains the common genital duct we prefer "hermaphroditic organ."

Dubois (1962) suggested that the presence of the hermaphroditic organ might preclude *F. molenkampi*'s assignment to the family Lecithodendriidae. Nonetheless, we consider *F. molenkampi* to be a lecithodendriid. A hermaphroditic duct is present in many species of Lecithodendriidae (Richard, 1966, pers. obs.) and the hermaphroditic organ appears to be merely a modification of the tissue surrounding the hermaphroditic duct.

Fontius klausrohdei sp. n.
(Figs. 7, 8)

=*Prosthodendrium swansoni:* Rohde, 1963.
Not *Prosthodendrium swansoni* Macy, 1936.
Not *Prosthodendrium swansoni:* Odening, 1968.
Not *Prosthodendrium swansoni:* Matskasi, 1973.

SPECIMENS EXAMINED: Six specimens from Dr. Klaus Rohde, Department of Zoology, University of New England, Armidale, New South Wales 2351, Australia.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 78372 (holotype and one paratype from *Taphozous melanopogon*). USNM Helm. Coll. No. 78373 (paratypes collected from *Hipposideros bicolor* (Temmink, 1834) by Dr. Klaus Rohde in Selangor, Malaysia).

ETYMOLOGY: The species is named for Dr. Klaus Rohde.

DESCRIPTION (based on three specimens from *Taphozous melanopogon* and six specimens from *Hipposideros bicolor,* the measurements of the two lots of specimens were not different from each other): Tegument finely spined. Body 531–944 (751) long by 448–826 (604) wide. Mouth subterminal. Oral sucker 58–78 (68) by 78–116 (97). Ventral sucker in anterior half of body 78–130 (99) by 84–122 (98). Oral sucker : ventral sucker ratio 0.77–0.95 (0.85). Pharynx 29–32 (31) by 29–43 (35). Esophagus 35–58 (50). Ceca short 130–203 (158) extend to or slightly overlap anterior margin of testes. Testes opposite, in acetabular zone, equal sized, 113–174 (141) by 110–174 (132). Cirrus sac membranous 101–160 (136) by 116–240 (161) medial mostly anterior to acetabulum, contains sinuous seminal vesicle, pars prostatica, and well-developed prostatic gland. Short ejaculatory duct extends from pars prostatica unites with metraterm to form hermaphroditic duct. Ovary 87–215 (130) by 58–107 (82), entire, dextral. Oviduct exits ovary posteriorly, unites with seminal receptacle and common vitelline duct. Laurer's canal arises from seminal receptacle, courses toward dorsal surface near midbody (opening not seen). Ootype surrounded by Mehlis' gland. Uterus occupies most of hindbody. Vitellaria, in two groups of follicles on either side of midline, extend from level of pharynx to overlap anterior margin of testes. Metraterm courses mediad to enter hermaphroditic duct. Hermaphroditic organ bulbous, not muscular, contains hermaphroditic duct. Hermaphroditic duct drawn into permanent nipple-like form that extends into genital atrium. Genital pore large, medial, preacetabular. Excretory pore terminal. Excretory bladder V- to Y-shaped. Egg operculate 22–23 (23) by 10–11 (11).

REMARKS: We have examined nine individuals of *Fontius klausrohdei.* Three specimens were recovered from *Taphozous melanopogon* in Perlis, Malaysia. In addition, we examined six specimens from *Hipposideros bicolor* collected in Selangor, Malaysia. These six specimens had been assigned provisionally to *Paralecithodendrium swansoni* (Macy, 1936) by Rohde (1963). A study of the holotype (USNM Helm. Coll. No. 8957) and additional material of *P. swansoni* convinced us that *P. swansoni* is distinct from *F. klausrohdei. P. swansoni* lacks the hermaphroditic organ and possesses tiny papillae surrounding the genital pore. *F. klausrohdei* possesses the hermaphroditic organ and lacks the tiny periporal papillae.

Groschaft and Tenora (1971) thought that Rohde's *P. swansoni* (=*F. klausrohdei*) was conspecific with *Paralecithodendrium brachyurna* (Groschaft and Tenora, 1971). We do not agree. *Fontius klausrohdei* possesses the hermaphroditic organ and an oral sucker: ventral sucker

←

Figures 1–8. Adult specimens and terminal genitalia of four species of Lecithodendriidae. Figures 1, 2. *Fontius molenkampi.* 1. Whole mount, ventral view. 2. Terminal genitalia, cross section. Figures 3, 4. *Paralecithodendrium ovimagnosum.* 3. Whole mount, ventral view. 4. Terminal genitalia, cross section. Figures 5, 6. *Papillatrium parvouterus.* 5. Terminal genitalia, sagittal section. 6. Whole mount, ventral view. Figures 7, 8. *Fontius klausrohdei.* 7. Whole mount, ventral view. 8. Terminal genitalia, sagittal section.

ratio less than 1.00. *P. brachyurna* lacks the hermaphroditic organ and has an oral sucker: ventral sucker ratio greater than 1.00.

Odening (1968) and Matskasi (1973a) reported *P. swansoni* from bats in Vietnam. Their figures and descriptions indicate a species that is much smaller than either *P. swansoni* or *F. klausrohdei*. In addition their specimens possess a cirrus sac that is smaller than or equal to the size of the ventral sucker. The cirrus sac is much larger than the ventral sucker in both *P. swansoni* and *F. klausrohdei*.

Papillatrium Richard, 1966

TYPE SPECIES: *Papillatrium inversus* Richard, 1966.

Papillatrium parvouterus (Bhalerao, 1926) comb. n. (Figs. 5, 6)

=*Lecithodendrium cordiforme parvouterus* Bhalerao, 1926.
=*L. cordiforme:* Mödlinger, 1930 (fide Dubois, 1955).
=*L. pyramidum:* Azim, 1936 (fide Dubois, 1960).
=*Prosthodendrium cordiforme:* Bhalerao, 1936 (fide Dubois, 1955).
=*P. pushpai* Bhalerao, 1936 (fide Dubois, 1955).
=*Lecithodendrium pyramidum:* Lukasiak, 1939 (fide Dubois, 1955).
=*Prosthodendrium pyramidum orientale* Yamaguti and Asada, 1942 (fide Dubois, 1955).
=*P. pyramidum* form *maroccana* Dollfus, 1954 (fide Dubois, 1955).
=*P. cordiforme:* Chen, 1954 (fide Kifune and Sawada, 1980).
=*P. parvouterus:* Dubois, 1955.
=*P. cordiforme:* Yeh, 1957 (fide Dubois, 1955).
=*Castroia kamariae* (type 2) Palmieri, Krishnasamy, and Sullivan, 1980, new synonym.

SPECIMENS EXAMINED: Two specimens from Dr. Klaus Rohde. Type material of *Castroia kamariae* (type 2) (USNM Helm. Coll. Nos. 73119 and 73120).

SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 78375 and 78374 (one of Dr. Rohde's specimens).

REMARKS: Richard (1966) erected the genus *Papillatrium* for lecithodendriids that possess a papilla in the genital atrium. She placed *Acanthatrium atriopapillatum* Capron, Deblock, and Brygod, 1961, and a new species *Papillatrium inversus* in the genus. Khotenovski (1974) rejected *Papillatrium,* and placed *Acanthatrium atriopapillatum,* which possesses pretesticular vitellaria, in the genus *Prosthodendrium* (junior synonym of *Paralecithodendrium* see Lotz and Font, 1983). He placed *Papillatrium inversus,* which possesses posttesticular vitellaria, in the genus *Lecithodendrium.* We prefer Richard's taxonomy. The terminal genitalia of *Paralecithodendrium* and *Lecithodendrium* consist of a simple hermaphroditic duct (Richard, 1966, pers. obs.). Species in the genus *Papillatrium* possess an expanded atrium that contains a papilla (Richard, 1966; Fig. 5, this paper). Species in the genus *Acanthatrium* possess an expanded atrium that contains spines but lacks the inta-atrial papilla (Cheng, 1959; Richard, 1966, pers. obs.). We therefore retain the genus *Papillatrium* and suggest that it is more closely related to the genus *Acanthatrium* than to either *Paralecithodendrium* or *Lecithodendrium.*

We transfer *Paralecithodendrium parvouterus* (Bhalerao, 1926) to the genus *Papillatrium* because of the structure of the terminal genitalia (Fig. 5). Specimens of *P. parvouterus* sent to us by Dr. Rohde from Malaysian bats also possess a papilla in the genital atrium; however, the papilla is difficult to see because his specimens appear to have been flattened before fixation.

We consider that the type specimens of *Castroia kamariae* (type 2) belong to *Papillatrium parvouterus.* From serial sections of our specimens we have determined that Laurer's canal arises from the seminal receptacle and opens on the dorsal surface of the worm.

Paralecithodendrium (Odhner, 1911) Travassos, 1921

TYPE SPECIES: *Lecithodendrium anticum* Stafford, 1905.

Paralecithodendrium longiforme (Bhalerao, 1926)

=*Lecithodendrium longiforme* Bhalerao, 1926.
=*L. orospinosa* Bhalerao, 1926 (fide Dubois, 1960).
=*L. luzonicum* Tubangui, 1928 (fide Dubois, 1960).
=*L. bhalerao* Pande, 1935 (fide Dubois, 1960).
=*L. kitazawai* Ogata, 1939 (fide Kifune and Sawada, 1980).
=*Prosthodendrium magnum* Rysavy, 1956 (fide Dubois, 1960).

Table 1. Host and locality records of five species of Lecithodendriidae recovered from *Taphozous melanopogon* in Perlis, Malaysia.

Species	Host	Locality	Reference
Fontius klausrohdei	Emballonuridae		
	*Taphozous melanopogon**	Malaysia: Perlis*	Present study
	Rhinolophidae		
	Hipposideros bicolor	Malaysia: Selangor	Rohde, 1963
Fontius molenkampi	Hominidae		
	*Homo sapiens**	Indonesia: Java*	Lie, 1951
		Thailand: Udon Thani	Manning et al., 1971
		Nong Khai	Manning et al., 1971
		Laos	Manning et al., 1971
	Muridae		
	Rattus rattus	Thailand: Udon Thani	Manning et al., 1971
		Nong Khai	Manning et al., 1971
		Laos	Manning et al., 1971
	Vespertilionidae		
	Scotophilus kuhli	Thailand: Udon Thani	Manning et al., 1971
		Nong Khai	Manning et al., 1971
		Laos	Manning et al., 1971
	Emballonuridae		
	Taphozous melanopogon	Thailand: Udon Thani	Manning and Lertprasert, 1973
		Malaysia: Perlis	Present study
Papillatrium parvouterus	Rhinopomatidae		
	Rhinopoma hardwickei	Egypt: "Jebe Al-Ahmar"	Saoud and Ramadan, 1977
	Emballonuridae		
	Taphozous melanopogon	Malaysia: Perlis	Present study
	T. nudiventris	Afghanistan: "Chak-Naur"	Groschaft and Tenora, 1971
	Megadermatidae		
	Megaderma lyra	Afghanistan: Kabul	Groschaft and Tenora, 1971
	Rhinolophidae		
	Asellia tridens	Egypt: Cairo	Saoud and Ramadan, 1977
	Hipposideros armiger	Vietnam: "Luc Yen"	Matskasi, 1973a
	Rhinolophus euryale	Egypt: S. Desert	Azim, 1936
		China: Manchuria	Yamaguti and Asada, 1942 (*in* Dubois, 1955)
	R. ferrumequinum	Japan: Kyushu	Kifune and Sawada, 1980
		Honshu	Kifune and Sawada, 1980
	Vespertilionidae		
	Eptesicus nilssoni	Poland	Lukasiak, 1939 (*in* Dubois, 1955)
	E. serotinus	Hungary	Matskasi, 1975
	Miniopterus schreibersi	Hungary	Mödlinger, 1930 (*in* Dubois, 1955)
		Morocco	Dollfus, 1954
		Zambia	Dollfus, 1954
		Japan: Kyushu	Kifune and Sawada, 1980
		Honshu	Kifune and Sawada, 1980
	Myotis blythi	Hungary	Matskasi, 1968
	M. hosonoi	Japan: Honshu	Kifune and Sawada, 1980
	Nyctalus noctula	Hungary	Matskasi, 1975
	Otonycteris hemprichi	Egypt: Giza	Saoud and Ramadan, 1977
	Pipistrellus kuhlii	Egypt: S. Desert	Azim, 1936

Table 1. Continued.

Species	Host	Locality	Reference
	Scotophilus heathi	Afghanistan: Nangarhar	Groschaft and Tenora, 1971
	Molossidae		
	Cheiromeles torquatus	Malaysia: Pahang	Rohde, 1963
	Tadarida mops	Malaysia: Ampang	Palmieri et al., 1980
	*T. plicata**	Burma: Rangoon*	Bhalerao, 1926
Paralecithoden-drium longiforme	Rhinopomatidae		
	Rhinopoma hardwickei	Egypt: Cairo	Saoud and Ramadan, 1977
	Emballonuridae		
	Taphozous melanopogon	Malaysia: Perlis	Present study
	T. nudiventris	Afghanistan: "Chuk-Naur"	Groschaft and Tenora, 1971
		Egypt: Cairo	Saoud and Ramadan, 1977
	Megadermatidae		
	Megaderma lyra	Afghanistan: Kabul	Groschaft and Tenora, 1971
	Rhinolophidae		
	Rhinolophus affinis	Vietnam: "Mong Son"	Matskasi, 1973a
	R. ferrumequinum	Czechoslovakia	Rysavy, 1956 (*in* Dubois, 1960)
	Vespertilionidae		
	Eptesicus nilssoni	Poland	Zdzitowiecki, 1969
	E. serotinus	Afghanistan: Nangarhar	Groschaft and Tenora, 1971
		France: Bouches-du-Rhone	Dubois, 1956
	Miniopterus schreibersi	Japan: Honshu	Kifune and Sawada, 1980
		Kyushu	Kifune and Sawada, 1980
	Myotis adversus	Vietnam: Lao Cai	Matskasi, 1975
	M. blythi	Hungary	Matskasi, 1975
	M. dasycneme	Hungary	Matskasi, 1975
	M. daubentoni	Hungary	Matskasi, 1975
		Poland	Zdzitowiecki, 1969
		USSR	Andreiko and Skvortsov, 1968 (*in* Saoud and Ramadan, 1977)
	M. myotis	Hungary	Matskasi, 1975
	M. mystacinus	Poland	Zdzitowiecki, 1969
	M. nattereri	Poland	Zdzitowiecki, 1969
	"*Nycticeius kuhli*"	India: Uttar Pradesh	Pande, 1935
	Pipistrellus abramus	Vietnam: Hadong	Odening, 1968
	Plecotus auritus	USSR	Andreiko and Skvortsov, 1968 (*in* Saoud and Ramadan, 1977)
	P. austriacus	Hungary	Matskasi, 1975
	Scotophilus heathi	Vietnam: Hadong	Matskasi, 1973a
	S. kuhli	Philippines	Tubangui, 1928 (*in* Dubois, 1956)
	Molossidae		
	Cheiromeles torquatus	Malaysia: Pahang	Rohde, 1963
	*Tadarida plicata**	Burma: Rangoon*	Bhalerao, 1926
Paralecithodendri-um ovimagnosum	Pteropodidae		
	Rousettus leschenaulti	India	Gupta and Mehta, 1970
	Rhinopomatidae		
	Rhinopoma hardwickei	India: Madhya Pradesh	Matskasi, 1973b
		Maharashtra	Matskasi, 1973b
	Emballonuridae		
	Taphozous longimanus	India: Orissa	Matskasi, 1973b
		West Bengal	Matskasi, 1973b

Table 1. Continued.

Species	Host	Locality	Reference
	T. melanopogon	India: Orissa	Matskasi, 1973b
		"Ricchai"	Matskasi, 1973b
		Madhya Pradesh	Matskasi, 1973b
		Mayaysia: Perlis	Present study
	Megadermatidae		
	Megaderma lyra	India: Andhra Pradesh	Salem, 1971
	Rhinolophidae		
	Hipposideros armiger	Vietnam: Luc Yen	Matskasi, 1973a
	H. fulvus	India: Orissa	Matskasi, 1973b
	Vespertilionidae		
	Miniopterus schreibersi	India: Maharashtra	Matskasi, 1973b
	"*Nycticeius kuhli*"	India: Uttar Pradesh	Agrawal, 1969
	Pipistrellus dormeri	India	Gupta and Mehta, 1970
	P. pipistrellus	USSR: Kazakhstan	Matskasi, 1973a
	P. sp.	Vietnam: Hadong	Matskasi, 1973a
	"*Scotophilus castaneus*"	Vietnam: Hadong	Odening, 1968
	Scotophilus heathi	Vietnam: Hadong	Matskasi, 1973a
		India: West Bengal	Matskasi, 1973b
		Mahableshwar	Matskasi, 1973b
	Scotophilus kuhli	China: Kwangtung	Chen, 1954 (*in* Dubois, 1962)
		Philippines: Luzon	Tubangui, 1928 (*in* Dubois, 1962)
		Vietnam: Hadong	Odening, 1968
		India: Orissa	Matskasi, 1973b
		West Bengal	Matskasi, 1973b
	S. leucogaster or *S. nigrita*	Ethiopia	Joyeux et al., 1937 (*in* Dubois, 1962)
	Vespertilio murinus	China: Manchuria	Fukui and Ogata, 1941 (*in* Dubois, 1962)
	Molossidae		
	Tadarida mops	Malaysia: Ampang	Palmieri et al., 1980
	*T. plicata**	Burma: Rangoon*	Bhalerao, 1926

* Type host or type locality.

Table 2. Prevalences and intensities of five species of Lecithodendriidae from *Taphozous melanopogon* in Perlis, Malaysia.

Helminth	Prevalence* Observed (95% CI)	Intensity† Range (mean)
Fontius molenkampi	0.76 (0.55–0.91)	1–283 (37.6)
F. klausrohdei	0.08 (0.01–0.26)	1–2 (1.5)
Papillatrium parvouterus	0.64 (0.42–0.82)	1–126 (25.9)
Paralecithodendrium ovimagnosum	0.68 (0.46–0.85)	1–8 (2.8)
P. longiforme	0.24 (0.09–0.45)	1–6 (2.2)

* Prevalence = number of infected hosts/number of hosts examined. CI = confidence interval.

† Intensity = number of helminths per infected host.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 78378.

Paralecithodendrium ovimagnosum
(Bhalerao, 1926)
(Figs. 3, 4)

=*Lecithodendrium ovimagnosum* Bhalerao, 1926.

=*Paralecithodendrium ovimagnosum:* Joyeux, Baer, and Martin, 1937.

=*Lecithodendrium asadai* Fukui and Ogata, 1941 (fide Dubois, 1962).

=*Prosthodendrium hepaticum* Chen, 1954 (fide Matskasi, 1973a).

=*Paralecithodendrium magnioris* Gupta and Bhardwaj, 1958 (fide Dubois, 1962).

=*Prosthodendrium kasakhstanin* Tschun-Sjun and Genis, 1962 (fide Matskasi, 1973a).
=*Paralecithodendrium thapari* Agrawal, 1966 (fide Salem, 1971).
=*P. cysticircum* Salem, 1971, new synonym.
=*Castroia kamariae* (type 1) Palmieri, Krishnasamy, and Sullivan, 1980, new synonym.

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 73117 and 73118 (*Castroia kamariae* [type 1]).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 78376.

REMARKS: *Paralecithodendrium ovimagnosum* was originally described by Bhalerao (1926) from *Tadarida plicata* (Buchanan, 1800) in Burma. Chen (1954) described *Paralecithodendrium hepaticum* from *Scotophilus kuhli* Leach, 1822, in China. Dubois (1962) considered *P. ovimagnosum* to be distinct from *P. hepaticum*. Matskasi (1973a) recovered *P. ovimagnosum* from bats in Vietnam and because of the observed variation in the ovary and body shape considered *P. hepaticum* to be a junior synonym of *P. ovimagnosum*. Our specimens exhibit the same degree of variation that Matskasi reported, and we agree with his conclusion.

Salem (1971) described *Paralecithodendrium cysticircum* from *Megaderma lyra* Geoffroy, 1810, in India. He noted that the ovary in his specimens extended transversely from the right testis only to the midline, whereas in *P. ovimagnosum* it continued across the midline to overlap partially the left testis. We think this represents intraspecific variation because our specimens of *P. ovimagnosum* exhibit the same variability. The reported measurements of *P. cysticircum* correspond to the measurements of *P. ovimagnosum*. Therefore we consider *P. cysticircum* to be a junior synonym of *P. ovimagnosum*.

Palmieri et al. (1980) described *Castroia kamariae* (type 1) from *Tadarida mops* (de Blainville, 1840) in Malaysia. We have examined the type material of this form and consider the specimens to belong to *P. ovimagnosum*.

Examination of serial sections of several of our specimens has revealed that Laurer's canal arises from the seminal recaptacle and opens on the dorsal surface of the worm.

Ecological Considerations

Table 1 lists the known hosts and localities of the five lecithodendriids recovered in this study. *Fontius klausrohdei* has only been reported from Malaysia. However, the apparent endemicity of a so recently described species must remain doubtful. *Fontius molenkampi* has been reported from Indonesia, Laos, Thailand, and Malaysia. Because *F. molenkampi* has been known since 1951 and detailed in the literature (Dubois, 1962; Manning et al., 1971) correct identifications can readily be made. We therefore think that the reported localities reflect the real distribution of *F. molenkampi* and consider the species to be endemic to Southeast Asia. *Papillatrium parvouterus* and *Paralecithodendrium longiforme* appear to be found throughout the Old World. *Paralecithodendrium ovimagnosum* is less extensively distributed. *Paralecithodendrium ovimagnosum* has not been reported from Europe or Japan although several studies of lecithodendriids have been undertaken in these areas.

Prevalences and intensities of the five lecithodendriids are given in Table 2.

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Black-spot Caused by *Uvulifer ambloplitis* (Trematoda) Among Juvenile Centrarchids in the Piedmont Area of North Carolina

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ABSTRACT: Prevalence and range of intensity of metacercariae of *Uvulifer ambloplitis* were determined from juvenile centrarchids (all <70 mm t.l.) in 43 lakes, streams, and ponds in the Piedmont area of North Carolina during the summer months of 1980–1982. The parasite occurred within 63% of the habitats checked. Metacercariae were most prevalent in bluegill, *Lepomis macrochirus*. The parasite was present in five of eight species of centrarchids examined. Because juvenile centrarchids are restricted to the shallow parts of the littoral zone by predatory largemouth bass, similar levels of infection should be expected among the various host species. There was, however, a great amount of variability in prevalence and range of infection. It is proposed that such variability is due to differences in inherent susceptibility among the different species of host.

Uvulifer ambloplitis (Hughes, 1927) is a widespread diplostomatid trematode that causes a disease in fish intermediate hosts, commonly known as black-spot. Studies on the population biology of *U. ambloplitis* in bluegill and largemouth bass in a North Carolina farm pond indicated exceedingly high prevalence and large mean intensities when compared to other areas of North America (Lemly and Esch, 1984a). This observation prompted an effort to survey the prevalence and range of intensities among different host species in a variety of streams, lakes, and ponds throughout the Piedmont area of North Carolina. The present report presents data from this survey and suggests an explanation for the large variability in infection among the various hosts examined.

Materials and Methods

Juvenile centrarchids and one percid (all <70 mm t.l.) were sampled from a variety of streams, ponds, and lakes in the Piedmont area of North Carolina; see Lemly (1983) for a description of the size of the various sites. A total of nine species of Centrarchidae and one species of Percidae were collected between May and October from 1980 to 1982. Most sites were sampled only once, but 10 were sampled twice. The species collected included bluegill, *Lepomis macrochirus* (Rafinesque); green sunfish, *Lepomis cyanellus* (Rafinesque); pumpkinseed, *Lepomis gibbosus* (Linnaeus); redbreast sunfish, *Lepomis auritus* (Linnaeus); warmouth, *Lepomis gulosus* (Cuvier); largemouth bass, *Micropterus salmoides* (Lacepede); red ear, *Lepomis microlophus* (Gunther); white crappie, *Pomoxis annularis* (Rafinesque); black crappie, *Pomoxis nigromaculatus* (Lesueur); and yellow perch, *Perca flavescens* (Mitchill).

All fish were collected using an 8.0 × 1.5-m seine having a mesh size of 0.5 cm². All fish were preserved, transported to the laboratory, and black-spot cysts enumerated. According to Hoffman (1967), black-spot may be caused by either *Crassiphilia bulboglossa* Van Haitsma, 1925 or *Uvulifer ambloplitis*. Based on the extensive experience of Lemly and Esch (1984b) in Reed's Pond, Davidson County, North Carolina, it is believed that the black-spot observed in other areas within the Piedmont of North Carolina is *U. ambloplitis*. Using the procedures of Hoffman and Putz (1965), metacercariae from freshly killed hosts were dissected from tissues and digested in pepsin. Live metacercariae were transferred to physiological saline and observed on slides with an ordinary light microscope. Full details of the techniques are described by Lemly and Esch (1984b).

Results

The prevalence and range of intensity of black-spot cysts of *U. ambloplitis* in eight species of Centrarchidae (all <70 mm t.l.) were determined from 43 ponds, lakes, and streams in the Piedmont area of North Carolina (Table 1). The sizes of the ponds and lakes ranged from 0.5 ha to 6,000 ha and the stream lengths from 1.7 km to 210 km. The parasite was widespread, occurring in 63% of the habitats sampled. By far the highest prevalence, range of intensity, and mean intensity occurred in Reed's Pond, Davidson County (Lemly and Esch, 1984a). Prevalence and range of intensity were relatively high in only four other habitats, one an unnamed farm pond in Davidson County, another an unnamed farm pond in Rowan County, and two others in Tyler County.

In species other than the bluegill, prevalence of *U. ambloplitis* was highest (53%) among green

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sunfish in Hedrick's Pond, Davidson County (Table 1). In this regard, it is interesting to note that Hedrick's pond was the only habitat sampled in which metacercariae were not found in bluegill, but were present in other centrarchids. Prevalence of *U. ambloplitis* did not exceed 12% among pumpkinseed, 7% among redbreast sunfish, and 4% among largemouth bass (except in Reed's Pond where prevalence was 21%; see Lemly and Esch, 1984a). The parasite was not seen in white or black crappie, nor in warmouth, all centrarchid species. Yellow perch were also sampled in seven different habitats, but they too were uninfected by *U. ambloplitis.*

Discussion

Metacercariae of *U. ambloplitis* have been reported in fish from the northeastern U.S. to California and from Florida into southern Ontario in Canada (Bangham and Hunter, 1939; Bangham, 1940; Bangham and Venard, 1946; Miller et al., 1973). For the most part, both the prevalence and range of intensity of black-spot among bluegill in Reed's Pond were exceptionally high when compared to other locations on the North American continent as well as to other sites in the Piedmont area of North Carolina. Lemly and Esch (1984a) attributed the high prevalence and mean intensity of metacercariae in Reed's Pond bluegill to two factors. First, they hypothesized that kingfishers congregated at the site during their nesting period in the spring and, presumably, deposited large numbers of parasite eggs. Second, they observed the highest numbers of *Helisoma trivolvis,* the highest prevalence of infected *H. trivolvis,* and the highest numbers of juvenile centrarchids during July, all of which served to maximize transmission rates during that time.

Based on the results of foraging studies by Werner et al. (1983a, b), it is expected that juvenile centrarchids of all species should be confined to shallow, littoral zone habitats if predatory largemouth bass are also present. It can also be assumed that confinement to the littoral zone would increase the probability of recruitment of *U. ambloplitis* equally among the juveniles of any centrarchid that might be present in the vicinity of a snail shedding cercariae of *U. ambloplitis.* However, based on the study by Lemly and Esch (1984a) and on the much more extensive observations presented here, there appears to be considerable variability in prevalence of black-spot among the various species of centrarchids. With a single exception, bluegill were the most heavily and consistently parasitized intermediate host species, followed by green sunfish, pumpkinseed, largemouth bass, and redbreast sunfish, in that order. In the single exception to this pattern (Hedrick's Pond), bluegill, green sunfish, redear sunfish, and largemouth bass were all present, but only green sunfish were infected. As suggested by an anonymous reviewer, it is certainly possible that the black-spot in Hedrick's Pond may represent metacercariae of *C. bulboglossa,* or even perhaps another, as yet undescribed, species. It is also conceivable that the bluegill in Hedrick's Pond are genetically distinct from others in the Piedmont and that they have developed some form of natural resistance to the parasite. Evidence to support or reject either or both of these hypotheses is at present unavailable.

There were 23 sites in which largemouth bass were present along with bluegill and at least one other species of centrarchid. In these locations, black-spot was present in bluegill and a centrarchid other than largemouth bass on 13 occasions. In the 17 sites where largemouth bass were absent, black-spot was present in a centrarchid other than bluegill only once. This observation indirectly supports the contention of Werner et al. (1983a) that the presence of predatory largemouth bass tends to confine juveniles of all centrarchid species to the shallow littoral zones of ponds and lakes and should thus enhance the probability of recruiting *U. ambloplitis.* In the absence of predation pressure, centrarchids would be released from confinement to shallow areas and thus reduce the risk of parasite recruitment.

If one assumes that juveniles of various centrarchids share a similar habitat, then they should also have similar potentials for recruitment of the parasite. This, however, is clearly not the case, at least in most of the sites that were sampled in the Piedmont area of North Carolina. Lemly and Esch (1984a) suggested that there is a differential capacity of *U. ambloplitis* cercariae to penetrate and encyst within bluegill and largemouth bass. This observation can now be extended to six other species of centrarchid, several of which are congeneric. It seems reasonable to suggest that species differences in inherent susceptibility among the eight species of centrarchids are responsible for the pattern of differential infections observed in the Piedmont area of North Carolina.

Lemly and Esch (1984b) generated substantive

Table 1. Prevalence (P) and range of intensity (I) of black-spot in juvenile centrarchids (<70 mm t.l.) collected in various habitats in the Piedmont area of North Carolina.

Location	Bluegill (*Lepomis macrochirus*)			Green sunfish (*Lepomis cyanellus*)			Pumpkinseed (*Lepomis gibbosus*)			Redbreast (*Lepomis auritus*)			Warmouth (*Lepomis gulosus*)			Redear (*Lepomis microlophus*)			Largemouth bass (*Micropterus salmoides*)			White crappie (*Pomoxis annularis*)			Black crappie (*Pomixis nigromaculatus*)		
	N*	P†	I‡	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I
Davidson County																											
Power Line Pond	93	9.7	0–3	70	1.5	0–3	32	0	0	—	—	—	—	—	—	49	2.1	0–2	—	—	—	—	—	—	—	—	—
Warfford's Pond	120	6.2	0–5	30	0	0	10	0	0	—	—	—	—	—	—	—	—	—	37	0	0	—	—	—	—	—	—
Hedricks Pond	100	0	0	81	53.0	0–9	—	—	—	—	—	—	—	—	—	7	0	0	50	0	0	—	—	—	—	—	—
Farm Pond 6	110	13.4	0–11	70	4.1	0–5	—	—	—	—	—	—	—	—	—	31	0	0	80	3.1	0.6	—	—	—	—	—	—
Farm Pond 7	40	0	0	40	0	0	—	—	—	37	0	0	—	—	—	6	0	0	25	0	0	—	—	—	—	—	—
Farm Pond 8	57	5.1	0–1	27	0	0	—	—	—	—	—	—	—	—	—	12	0	0	30	0	0	—	—	—	—	—	—
Farm Pond 9	70	16.0	0–7	39	13.1	0–2	—	—	—	—	—	—	—	—	—	3	0	0	65	1.1	0–1	—	—	—	—	—	—
Farm Pond 10	65	39.0	0–12	49	14.0	0–5	—	—	—	—	—	—	—	—	—	19	1.1	0–2	60	4.9	0–2	—	—	—	—	—	—
High Rock Lake	80	11.0	0–4	80	3.5	0–1	80	1.0	0–1	45	0	0	—	—	—	—	—	—	80	1.2	0–1	—	—	—	—	—	—
Tuckertown Lake	40	8.8	0–4	39	1.0	0–2	19	1.0	0–1	20	0	0	16	0	0	—	—	—	30	0	0	25	0	0	11	0	0
Cat's Creek	31	0	0	12	0	0	—	—	—	15	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lemly Creek	24	0	0	12	0	0	7	0	0	11	0	0	—	—	—	—	—	—	7	0	0	—	—	—	—	—	—
Briggstown Creek	15	0	0	67	0	0	3	0	0	40	0	0	—	—	—	—	—	—	11	0	0	—	—	—	—	—	—
Yadkin River	37	4.0	0–3	44	2.1	0–3	19	1.0	0–3	9	0	0	12	0	0	—	—	—	12	0	0	14	0	0	—	—	—
Abbott's Creek	35	1.0	0–2	19	0	0	21	0	0	30	0	0	19	0	0	—	—	—	6	0	0	12	0	0	—	—	—
Forsyth County																											
Minorca's Creek	20	0	0	25	0	0	15	0	0	19	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Table 1. Continued.

Location	Bluegill (*Lepomis macrochirus*)			Green sunfish (*Lepomis cyanellus*)			Pumpkinseed (*Lepomis gibbosus*)			Redbreast (*Lepomis auritus*)			Warmouth (*Lepomis gulosus*)			Redear (*Lepomis microlophus*)			Largemouth bass (*Micropterus salmoides*)			White crappie (*Pomoxis annularis*)			Black crappie (*Pomixis nigromaculatus*)		
	N*	P†	I‡	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I
Grandview Pond	39	22.0	0–5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	25	4.0	0–1	–	–	–	–	–	–
Oak Summit Pond	234	15.4	0–4	50	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Salem Lake	50	12.9	0–3	50	2.2	0–2	40	0	0	15	0	0	–	–	–	–	–	–	30	3.3	0–1	–	–	–	–	–	–
Belews Lake	94	13.7	0–2	35	2.8	0–1	32	0	0	20	0	0	–	–	–	15	0	0	21	0	0	–	–	–	–	–	–
Winston Lake	50	20.4	0–6	31	9.7	0–3	30	0	0	30	6.7	0–2	25	0	0	–	–	–	31	0	0	–	–	–	–	–	–
Salem Creek	15	0	0	19	0	0	5	0	0	13	0	0	–	–	–	–	–	–	3	0	0	–	–	–	–	–	–
Silas Creek	4	0	0	34	0	0	–	–	–	6	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Winston Creek	12	0	0	39	0	0	2	0	0	12	0	0	–	–	–	–	–	–	3	0	0	–	–	–	–	–	–
East Belews Creek	–	–	–	16	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Farm Pond 3	50	12.0	0–1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	70	0	0	–	–	–	–	–	–
Stokes County																											
Charlie's Pond	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cabarrus County																											
Foil's Pond 1	38	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Foil's Pond 2	106	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Foil's Pond 3	281	5.7	0–3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dutch Buffalo Creek	20	0	0	11	0	0	–	–	–	16	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Little Buffalo Creek	19	0	0	7	0	0	–	–	–	21	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 1. Continued.

Location	Bluegill (*Lepomis macrochirus*)			Green sunfish (*Lepomis cyanellus*)			Pumpkinseed (*Lepomis gibbosus*)			Redbreast (*Lepomis auritus*)			Warmouth (*Lepomis gulosus*)			Redear (*Lepomis microlophus*)			Largemouth bass (*Micropterus salmoides*)			White crappie (*Pomoxis annularis*)			Black crappie (*Pomixis nigromaculatus*)		
	N*	P†	I‡	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I	N	P	I
Scotland County																											
Farm Pond 1	100	8.0	0–2	50	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Farm Pond 2	100	28.0	0–7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rowan County																											
Farm Pond 4	80	18.9	0–4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	69	1.0	0–2	—	—	—	—	—	—
Farm Pond 5	120	63.0	0–34	59	37.0	0–10	66	12.0	0–5	—	—	—	—	—	—	16	0	0	69	0	0	—	—	—	—	—	—
Crane Creek	57	6.8	0–9	65	3.0	0–4	60	3.1	0–4	20	0	0	13	0	0	—	—	—	35	3.2	0–2	24	0	0	—	—	—
Tyler County																											
Farm Pond 11	37	62.0	0–37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Farm Pond 12	71	69.0	0–24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Montgomery County																											
Badin Lake	43	5.2	0–6	84	3.0	0–1	87	0	0	43	0	0	42	0	0	—	—	—	20	0	0	16	0	0	7	0	0
Mecklenburg County																											
Lake Norman	64	2.5	0–3	40	1.0	0–1	35	0	0	20	0	0	11	0	0	—	—	—	57	0	0	32	0	0	50	0	0
Martin County																											
Dan River	—	—	—	31	0	0	—	—	—	19	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Catawba County																											
Catawba River	19	0	0	29	0	0	16	0	0	12	0	0	4	0	0	—	—	—	11	1.1	0–1	15	0	0	—	—	—

* N = Number of fish examined for black-spot.
† P = Prevalence of black-spot.
‡ I = Range of black-spot cysts.

laboratory and field evidence to indicate that *U. ambloplitis* was capable of causing mortality among juvenile *L. macrochirus* in Reed's Pond, located in the Piedmont area of North Carolina. Prevalence of the parasite was found to be 100% in many months of a study lasting four years. Intensities of the parasite reached as high as 269 cysts/host. Their data indicate that juvenile bluegill with >50 cysts cannot survive cold water temperatures and that a combination of lowered body condition, depletion of total body lipid, and decreased feeding during the winter months all contribute to host mortality. If a lethal level of 50 cysts can be extrapolated to the other habitats in the Piedmont area, then it is clear from the present study that *U. ambloplitis* is having a regulatory impact in only one location, Reed's Pond, and on only a single species of centrarchid, namely the bluegill, *L. macrochirus*.

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Comparative Seasonal Dynamics of *Alloglossidium macrobdellensis* (Digenea: Macroderoididae) in Wisconsin and Louisiana

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ABSTRACT: Seasonal population dynamics of *Alloglossidium macrobdellensis* were studied in Wisconsin and compared with previous reports for this species in Louisiana. Differences observed in the two states are due to the apparently unequal influences of climate and life histories of the leech definitive hosts. In the nearly subtropical climate of south Louisiana the period of recruitment is prolonged and worms rapidly attain sexual maturity and produce eggs. The seasonal dynamics of *A. macrobdellensis* are influenced primarily by the one-year life span of the southern leech *Macrobdella ditetra*. In Wisconsin, climate more directly affects the dynamics of *A. macrobdellensis* than does the multi-year life span of its northern host *M. decora*. The cold temperate climate provides only a short growing season for *A. macrobdellensis*. Recruitment of parasites in *M. decora* is confined to midsummer, and growth ceases when leeches become dormant in winter. Egg production by *A. macrobdellensis* occurs one year after recruitment during the midsummer period of warmest water temperatures.

Seasonal temperature changes may influence seasonal population dynamics of parasites, especially those parasites that use poikilothermic hosts. The same species of parasite may exhibit different seasonal dynamics in different areas of its geographic range where climatic differences occur. The trematode *Alloglossidium macrobdellensis* Beckerdite and Corkum, 1974 provides an opportunity for a comparative study of such local adaptations. Corkum and Beckerdite (1975) described the life cycle and seasonal dynamics of *A. macrobdellensis* in the near subtropical climate of south Louisiana where the parasite uses the leech *Macrobdella ditetra* Moore, 1953 as its definitive host. We studied the dynamics of *A. macrobdellensis* in the American medicinal leech, *M. decora* (Say, 1824), in northern Wisconsin where cold temperate climatic conditions prevail.

Materials and Methods

Leeches were collected in Boot Bog, Chippewa Co., about 15 mi NW of Eau Claire, Wisconsin monthly from April through October 1977. Nocturnal collections of leeches actively swimming or clinging to sphagnum moss were made with dipnets. In October, only five leeches, all buried in bottom sediment, could be collected despite extensive effort. Ice covered Boot Bog from November until late March. Leeches were maintained in the laboratory at 5°C until they were measured and dissected. Leeches were examined for parasites, and permanent whole mounts of parasites were made as described by Corkum and Beckerdite (1975). Specimens No. 78026–78032 have been deposited in the USNM Helminthological Collection. Ecological terms used in this paper conform to definitions of Margolis et al. (1982).

Results

Morphology

Specimens of *Alloglossidium macrobdellensis* were recovered from coelomic tissues, from cysts embedded in crop mucosa, and from the intestinal lumen of *Macrobdella decora*. Measurements are given in Table 1. Size and degree of maturation of coelomic worms, metacercariae (=crop cysts of Corkum and Beckerdite, 1975), and intestinal worms displayed a large amount of overlap (Figs. 1–6). The smallest specimens from all three habitats within the leech were only slightly larger and more developed than *A. macrobdellensis* cercariae. In the largest coelomic worms, testes, cirrus sac, ovary, and uterus were evident. Vitellaria were not developed, cirrus sac contained no spermatozoa, and uterus was devoid of eggs. Largest metacercariae displayed greater development of genitalia than the largest coelomic worms, and Mehlis' gland was conspicuous. Vitellaria attained only partial development, and spermatozoa and eggs were not present. The intestinal lumen was inhabited by specimens ranging from immature worms similar to the smallest coelomic worms in size and organogenesis, to large, fully gravid adults.

Seasonal dynamics

Alloglossidium macrobdellensis exhibited a pronounced seasonal pattern of abundance (=relative density) and maturation in Wisconsin (Table 2; Figs. 7, 8). Coelomic worms occurred only in June, July, and August, infecting both

Table 1. Morphometrics of *Alloglossidium macrobdellensis* from *Macrobdella decora* in Wisconsin. (Measurements in micrometers; range followed by mean in parentheses.)

Character	Coelomic worm $N = 36$	Metacercaria $N = 106$	Intestinal worm $N = 306$
Body length	288–647 (431)	373–1,389 (705)	390–4,288 (1,046)
Body width	53–122 (88)	32–134 (88)	38–224 (85)
Oral sucker length	25–46 (34)	23–60 (42)	28–92 (46)
Oral sucker width	28–53 (37)	23–64 (44)	25–83 (47)
Pharynx length	14–30 (20)	14–39 (29)	14–58 (33)
Pharynx width	15–32 (24)	18–40 (30)	18–64 (33)
Acetabulum length	25–44 (33)	28–62 (41)	25–90 (45)
Acetabulum width	25–46 (32)	25–55 (39)	25–90 (43)
Anterior testis length	12–37 (23)	14–88 (36)	16–232 (61)
Anterior testis width	10–28 (16)	12–45 (24)	10–134 (40)
Posterior testis length	14–46 (28)	18–95 (40)	16–272 (72)
Posterior testis width	10–32 (18)	12–55 (25)	12–160 (40)
Ovary length	12–23 (15)	12–38 (22)	12–168 (37)
Ovary width	10–16 (12)	9–28 (16)	9–140 (25)
Egg length ($N = 20$)	–	–	23–32 (28)
Egg width	–	–	16–20 (18)

newly hatched leeches and leeches beginning their second year of life. In August and September, most coelomic worms completed migration to the crop and encysted as metacercariae. More metacercariae were recovered than were coelomic worms because crop cysts were much more apparent in the alimentary mucosa than were tiny coelomic worms buried in botryoidal and vasofibrous tissues. Some metacercariae excysted and migrated to the intestine in late summer and fall. Remaining metacercariae excysted either during the period of winter dormancy or as leeches emerged from dormancy in spring. The relative density of intestinal worms began to increase in late summer and fall.

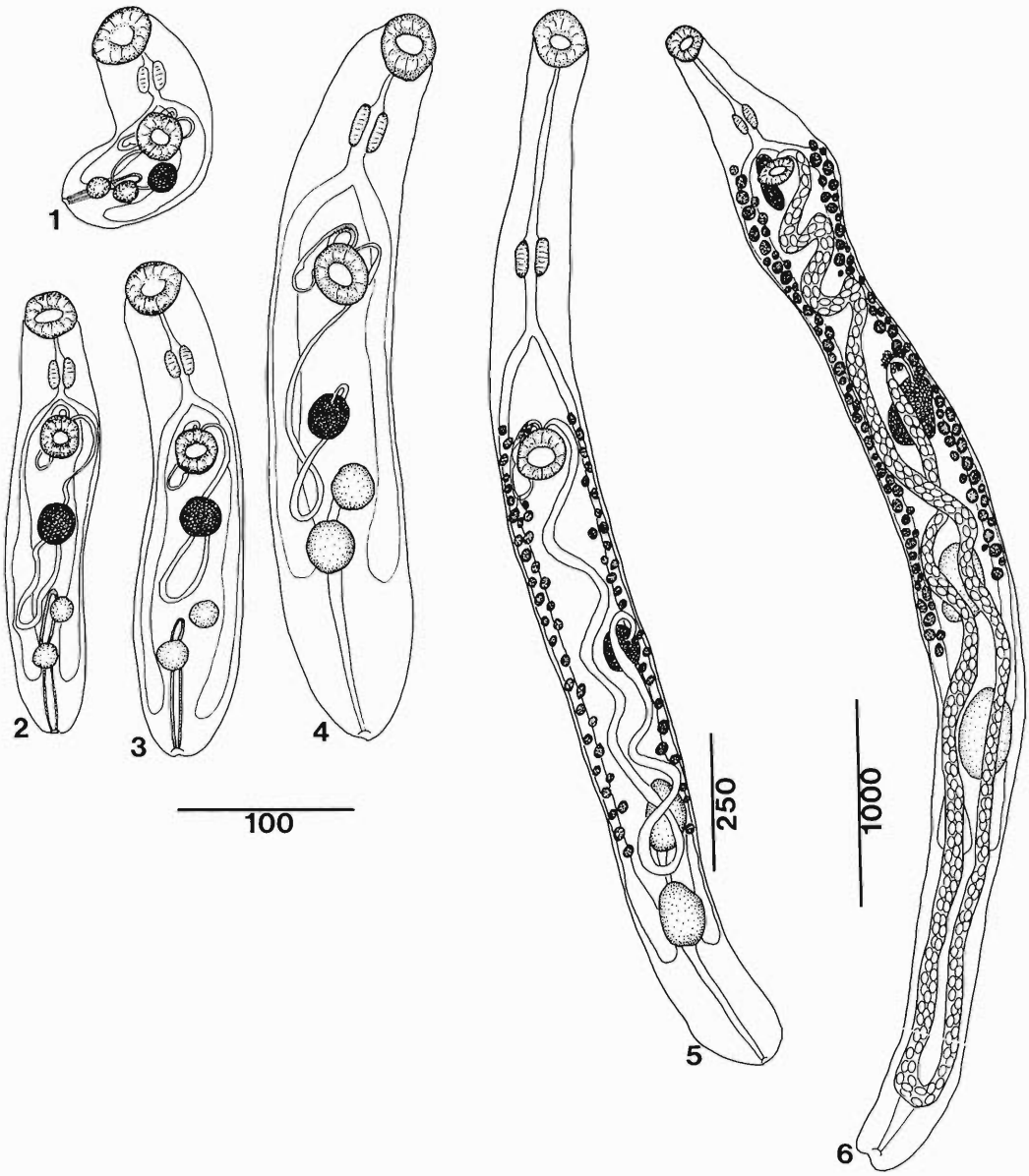
In order to determine their seasonal pattern of maturation, intestinal worms were divided into three discrete nonarbitrary phases as follows: Phase A—nongravid worms (body length 390–1,850 μm); Phase B—gravid worms with 1–10 eggs in uterus (body length 1,148–1,766 μm); and Phase C—gravid worms with more than 10 eggs in uterus (body length 1,434–4,288 μm).

Small immature worms first appeared in the intestine in late summer, simultaneously with the excystment of metacercariae. Remnants of empty metacercarial cysts were seen in crop mucosa at this time. In late September and October, as leeches entered dormancy, approximately 90% of intestinal worms were identified as Phase A (Fig. 8). Leeches that emerged from dormancy shortly after the melting of ice in April also harbored over 90% Phase A worms in the intestine. These specimens matured and began egg production in late spring and early summer as seen from the rise in Phase B (newly gravid) worms. Most intestinal worms were fully gravid (Phase C) in August, the period of warmest water temperature in Boot Bog. By September, very few gravid worms remained in the intestine. The loss of gravid worms coincided with the arrival of new (Phase A) intestinal worms.

Discussion

Alloglossidium macrobdellensis displayed the same life cycle pattern in *Macrobdella decora* in Wisconsin as that reported by Corkum and Beckerdite (1975) from *M. ditetra* in Louisiana, i.e., after cercarial penetration, worms migrated from coelomic tissues and encysted as metacercariae in crop mucosa, then excysted and commenced egg production in intestinal lumen. Differences were observed in the amount of morphogenesis that occurred in each habitat of the leech and with respect to temporal patterns of development.

In Louisiana, very little overlap apparently occurred in size and development attained by coelomic worms, metacercariae, and intestinal worms. Metacercariae exceeded coelomic worms in size and degree of organogenesis and became fully developed, short of egg production, while still encysted. Complete genital development presumably was required for excystment because Beckerdite and Corkum (1974) reported no immature or nongravid worms from the intestinal



Figures 1–6. Developmental stages of *Alloglossidium macrobdellensis* from *Macrobdella ditetra* in Wisconsin. 1. Smallest coelomic worm. 2. Smallest metacercaria. 3. Smallest intestinal worm. 4. Largest coelomic worm. 5. Largest metacercaria. 6. Largest intestinal worm. Figures 1–4 drawn to same scale. Body spination omitted from all figures.

lumen of *M. ditetra*. In Louisiana, therefore, all intestinal-dwelling *A. macrobdellensis* could be properly called adults.

In contrast, *A. macrobdellensis* in Wisconsin displayed more overlap in body size and organogenesis within each of the three habitats in the leech. Specimens were able to migrate to the next habitat prior to reaching a developmental state equal to that seen in Louisiana. Metacercariae never displayed complete development of the vitellaria. Because intestinal worms ranged in development from the equivalent of the smallest coelomic worm to fully gravid specimens, only a portion of intestinal worms truly can be considered adults in contrast with Louisiana where all intestinal worms were adults.

Table 2. Seasonal abundance of *Alloglossidium macrobdellensis* coelomic worms, metacercariae, and intestinal worms (Phase A, B, C) in *Macrobdella decora* in Wisconsin. Range of intensity followed by relative density in parentheses.

Month	Leeches examined	Coelomic worms	Metacercariae	Intestinal worms		
				Phase A	Phase B	Phase C
April	28	0	0–4 (0.7)	0–34 (12.4)	0	0–3 (0.8)
May	—	—	—	—	—	—
June	14	0–1 (0.3)	0–2 (0.4)	0–14 (7.8)	0–3 (0.5)	0–5 (0.2)
July	12	0–6 (2.2)	0–1 (0.1)	0.5 (1.6)	0–6 (0.8)	0–1 (0.3)
August	15	0–11 (3.2)	0–20 (4.0)	0–2 (0.4)	0–1 (0.1)	0–7 (1.0)
September	10	0	6–60 (18.8)	0–11 (8.1)	0	0–1 (0.7)
October	5	0	0–41 (12.0)	0–7 (4.4)	0	0–4 (0.8)

Important differences also occurred with regard to the seasonal dynamics of *A. macrobdellensis* in Louisiana and Wisconsin. Differences are attributable to the life history patterns of the two species of leeches and to different climatic conditions in the two states. Beckerdite and Corkum (1973) described the life history of *M. ditetra* and regarded the one-year life span of the leech as the greatest influence on the seasonality of the parasite (Corkum and Beckerdite, 1975).

Briefly, they reported that leeches are in reproductive condition in late spring. Significantly, after cocoons are deposited, all adult leeches die and no leeches are present in the water until July when young leeches emerge from cocoons. Shortly thereafter, leeches become infected with coelomic worms, and the first cysts and adults appear within a month. Because the number of coelomic worms increases throughout the year, presumably there is an extended period of cercarial emergence from the first intermediate host *Helisoma trivolvis*. Intensity of adult specimens peaks in late spring, shortly before the death of the entire generation of leeches. Because there are no leeches available for infection in June, the population of *A. macrobdellensis* at that time consists entirely of dispersed eggs and sporocysts in *H. trivolvis*.

In contrast, seasonal dynamics in Wisconsin are affected only in part by the multi-year life span of *M. decora*. Population dynamics are influenced directly to an even greater extent by a climate that is characterized by long, cold winters when ice covers bodies of standing water from

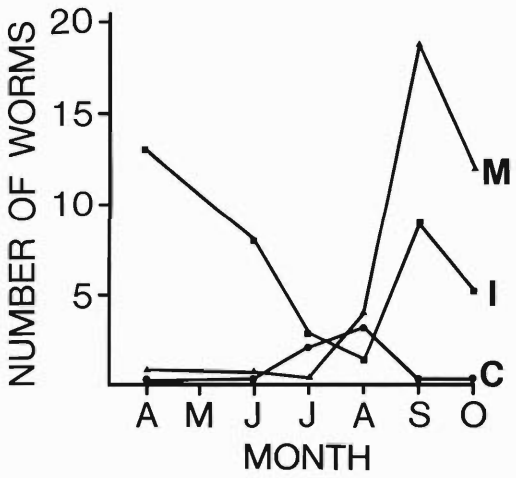


Figure 7. Seasonal relative density (mean number of parasites per leech) of *Alloglossidium macrobdellensis* coelomic worms (C), metacercariae (M), and intestinal worms (I) in *Macrobdella decora* in Wisconsin.

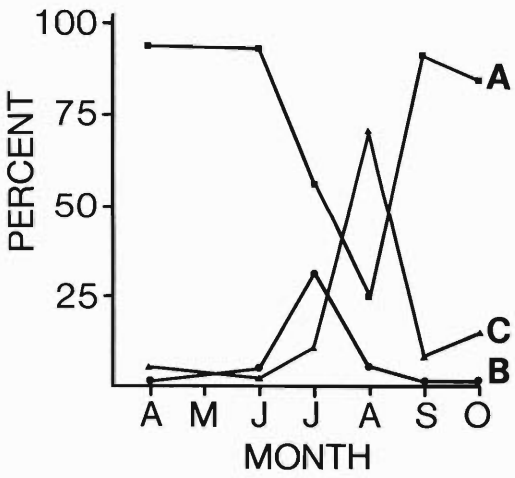


Figure 8. Seasonal relative abundance (expressed in percent) of *Alloglossidium macrobdellensis* intestinal worms, Phases A, B, and C in *Macrobdella decora* in Wisconsin.

November until April and short, cool summers that provide only a brief growing season. Leeches become infected only during June, July, and August, indicating that cercarial emergence is influenced primarily by this brief period of warm water temperatures. Unlike the situation in Louisiana, cercariae simultaneously infect two generations of leeches and there is never a period when specimens of hatched *M. decora* are not present in the water.

In spite of declining water temperatures in late summer and fall, some trematodes rapidly complete their migration to the intestinal lumen even though their morphogenesis remains incomplete when compared with Louisiana specimens. It is not known why the trematodes in Wisconsin enter the intestine so quickly and in a less developed condition. Leeches harbored over 12 times as many immature (Phase A) worms in the intestine compared with the number that successfully attained maximum egg production (Phase C). Perhaps intraspecific competition for intestinal space or nutrients occurs and those specimens that reach the intestine first are those that ultimately reproduce successfully.

Most specimens found in late fall, as leeches entered dormancy, were immature intestinal worms. Similarly, most worms were in the same state of development in early spring, indicating that little growth occurs while leeches remain dormant under the ice. Commencement of egg production is coincidental with increasing water temperature and maximum egg production occurs in the warmest part of the year. Shortly after egg production, adult specimens are lost from leeches' intestines and are immediately replaced by the next generation of immature *A. macrobdellensis* that arrive to occupy the intestine.

In summary, in Louisiana where the climate is nearly subtropical, the period of both cercarial emergence and egg production of *A. macrobdellensis* is prolonged and seasonal dynamics are affected directly by the one-year life span of *M. ditetra.* In contrast, the cold temperate climate and short growing season in Wisconsin has a more direct effect on the seasonal dynamics of *A. macrobdellensis* than does the life history of *M. decora,* restricting cercarial emergence and egg production to a brief period during midsummer.

The differences observed in the seasonal dynamics of *A. macrobdellensis* in Louisiana and Wisconsin are a testament to the wisdom of Holmes' (1983) words regarding host–parasite coevolution. "Helminths are capable of rapid evolutionary change and track local conditions. As a result, individual species may have very different characteristics in separated localities, and coevolution should be looked for at the local populational level."

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Proterogynotaenia texanum sp. n. (Cestoidea: Progynotaeniidae) from the Black-bellied Plover, *Pluvialis squatarola*

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ABSTRACT: *Proterogynotaenia texanum* sp. n. is described from the black-bellied plover, *Pluvialis squatarola* (Linnaeus) from the Texas Gulf coast. It is similar in proglottid number to *P. branchiuterina* Belopol'skaya, 1973, *P. daugi* Sandeman, 1959, *P. flaccida* (Meggitt, 1928) Baer, 1940, *P. polytestis* Belopol'skaya, 1973, and *P. variabilis* Belopol'skaya, 1953. *Proterogynotaenia texanum* differs from these primarily in posterior hook size. The posterior hooks are 18 μm long in *P. texanum* compared to 13–14 μm long in *P. branchiuterina, P. daugi,* and *P. polytestis,* and 8 μm long in *P. flaccida* and *P. variabilis.* There are also differences in testes number and cirrus pouch size.

The black-bellied plover, *Pluvialis squatarola* (Linnaeus), a migratory wading bird, is found in Texas primarily from August to December. From November 1978 to November 1980, five plovers were collected on the Intercoastal Waterway in Galveston County, Texas. Specimens of Cestoidea representing a species of *Proterogynotaenia* Fuhrmann, 1911 were found in three of the plovers. Species of *Proterogynotaenia* have been recovered from black-bellied plovers by Belopol'skaya (1953, 1973).

Live cestodes were taken from the intestine and heat fixed with AFA under slight coverslip pressure. Whole mounts were stained with Semichon's carmine and mounted in Kleermount. Unless otherwise indicated, measurements are in micrometers, the mean followed by the range in parentheses. All figures were drawn with the aid of a drawing tube. Types deposited in National Parasite Collection, USDA, Beltsville, Maryland.

Proterogynotaenia texanum sp. n. (Figs. 1-6)

DESCRIPTION (based on 10 mature specimens): Strobila 1.4–3.3 mm long, craspedote, apolytic, composed of 6–8 proglottids. Scolex 180 (159–210) long by 271 (210–405) wide. Suckers 108–230 in greatest diameter. Everted rostellum with two rows of six hooks each; anterior hooks 38 (33–42) long; posterior hooks 18 (no range) long. Rostellar sac 152 (132–210) long by 86 (78–99) wide. Mature proglottids 576 (330–830) long by 620 (520–670) wide; gravid proglottids longer than wide. Testes arranged in two groups, 16 (13–18) poral and 19 (15–29) aporal; ovoid 53 (27–60) long by 68 (37–84) wide. Cirrus pouch in anterior half of proglottid passing between longitudinal excretory canals, 400 (312–501) long by 122 (102–150) wide with proximally spined cirrus and large ovoid internal seminal vesicle 165 (126–204) long by 105 (69–141) wide. External seminal vesicle elongate. Genital pores irregularly alternating. Vagina absent. Seminal receptacle aporal, slightly anterior to vitelline gland, 152 (114–204) long by 89 (57–117) wide. Ovary lobate, aporal, 172 (129–240) long by 96 (66–150) at widest point. Vitelline gland postovarian, ovoid, 63 (45–90) long by 53 (30–78) wide. Uterus first appearing in posterior of proglottid, ovoid; becoming lobed, filling gravid proglottid. Eggs ovoid, 55 (51–60) long by 30 (27–39) wide, larvated. Larval hooks six, 12 in length.

HOST: *Pluvialis squatarola* (L.).

LOCATION: Intestine.

LOCALITY: Galveston, Texas.

PREVALENCE: Found in three of five plovers examined.

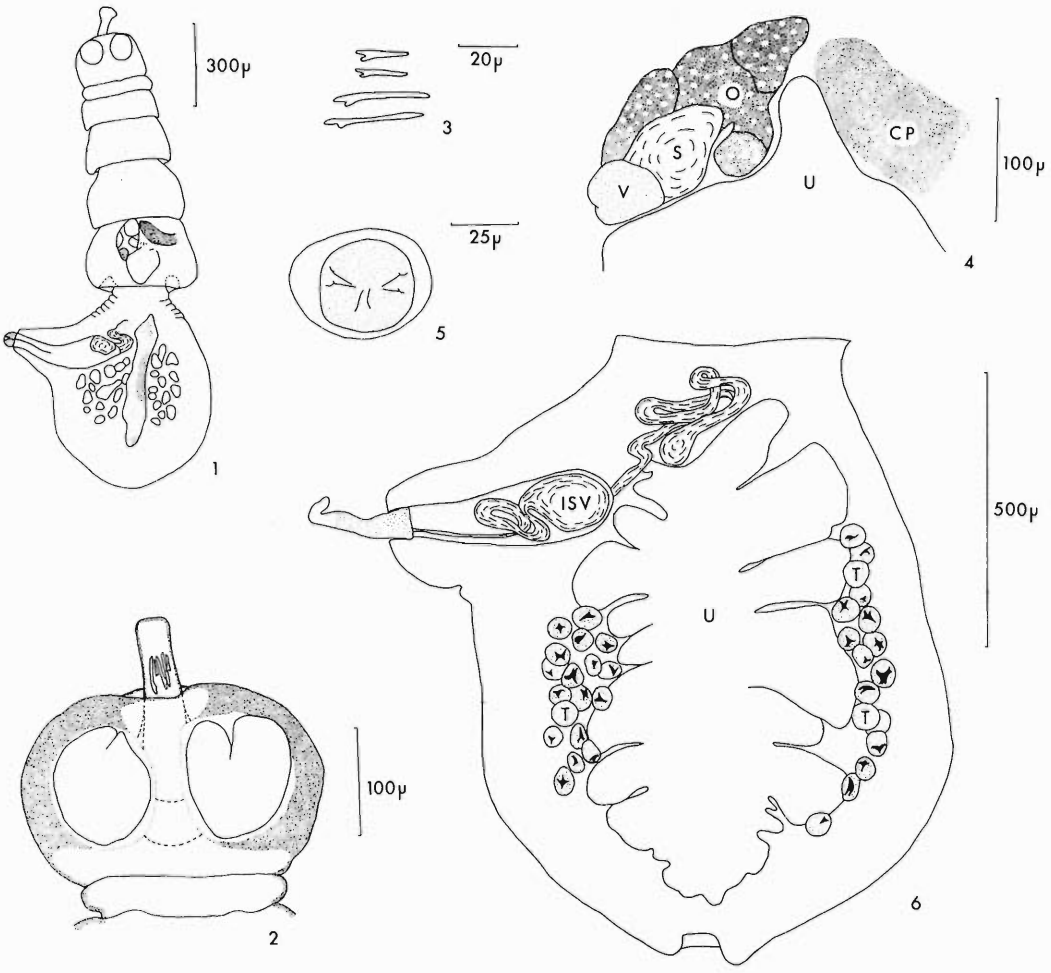
WORM BURDEN: Two to four per host.

HOLOTYPE: USNM Helm. Coll. No. 77131.

PARATYPES: USNM Helm. Coll. No. 77132; Texas A&M University Regional Invertebrate Collection No. 80-13.

ETYMOLOGY: Species name refers to the type locality.

REMARKS: The genus *Proterogynotaenia* is represented by seven previously described species: *P. rouxi* Fuhrmann, 1911, *P. flaccida* (Meggitt, 1928) Baer, 1940, *P. neoarctica* Webster, 1951, *P. variabilis* Belopol'skaya, 1953, *P. daugi* Sandemann, 1959, *P. branchiuterina* Belopol'skaya, 1973, and *P. polytestis* Belopol'skaya, 1973. On the basis of proglottid number, *P. texanum* is closest to *P. flaccida* from the avocet; *P. varia-*



Figures 1–6. ***Proterogynotaenia texanum*** **sp. n. from the black-bellied plover. 1. Mature worm. 2. Scolex. 3. Posterior and anterior hooks. 4. Reproductive structures in mature proglottid. Abbreviations: CP, developing cirrus pouch; O, ovary; S, seminal receptacle; U, uterus; V, vitelline gland. 5. Egg. 6. Gravid proglottid, excretory canals deleted for clarity. Abbreviations: ISV, internal seminal vesicle; T, testes; U, uterus.**

bilis, P. branchiuterina, and *P. polytestis* from black-bellied plover; and *P. daugi* from greater golden plover.

The primary difference appears in the hook size. *Proterogynotaenia texanum* and the five similar species have large hooks of approximately the same size, 30–40 μm long. The small hooks, however, of *P. texanum* are 18 μm long, whereas those of the other species are reported to be 13–14 μm in *P. branchiuterina, P. daugi,* and *P. polytestis,* or 8 μm in *P. flaccida* and *P. variabilis.*

The validity of using hook sizes as the primary differentiating characteristic could be questioned, particularly in view of *P. variabilis.* Belopol'skaya (1953) in the description of *P. variabilis* gives a broad range of both hook numbers and sizes. It is questionable though, as to its validity. The description is based upon only two complete specimens, with the hooks being either 34–35 μm or 8 μm. The variability of the hook number and size is based on 50 scolices from another bird from a different geographic region. The only reason given for assigning these 50 scolices to the species is that 56% of them had the same number of hooks as the original two complete specimens. Because no complete strobilae were found with the 50 scolices, validity of this assignment to *P. variabilis* can be questioned.

Testes number and cirrus pouch size also vary

with these species. *Proterogynotaenia texanum* has 16 poral and 19 aporal testes. *Proterogynotaenia daugi* and *P. flaccida* are very similar, both with 14 poral and 16 aporal testes. *Proterogynotaenia variabilis* has 7 poral and 11–12 aporal testes. The cirrus pouch of *P. texanum* is 400 μm by 122 μm. That of *P. daugi* is reported as 230–280 μm by 90–120 μm. The description of *P. flaccida* states only that the cirrus pouch is large and extends past the midline of the proglottid. The other three similar species have differing testes numbers and cirrus pouch measurements.

The genus *Proterogynotaenia* is very similar to the genus *Paraprogynotaenia* Rysavy, 1966. The only difference is the number of rows of rostellar hooks, with *Paraprogynotaenia* possessing a single row of hooks. *Proterogynotaenia texanum* possesses two rows of hooks, but the second row of small hooks is easily lost in the process of collecting, fixing, and mounting. In the *P. texanum* specimens collected, only one retained both rows of hooks.

Acknowledgments

The author thanks the Texas Parks and Wildlife Department without whose cooperation this study would not have been possible. Thanks are also given to Dr. Harold Underwood and Dr. Norman Dronen for technical assistance.

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Description of *Otobothrium insigne* Plerocercus (Cestoda: Trypanorhyncha) and Its Incidence in Catfish from the Gulf Coast of Louisiana

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ABSTRACT: One hundred fifty-eight (90%) of 175 *Arius felis* catfish collected from the Gulf of Mexico off the coast of Louisiana were infected with trypanorhynch plerocerci identified as *Otobothrium insigne.* This finding represents the first report of this cestode since its original description in 1905, and the only report of its plerocercus. The oncotaxy of this species is redescribed, based on the plerocercus, through the use of scanning electron microscopy.

In 1905, Edwin Linton published a report listing more than 100 different parasites of marine fishes collected near Beaufort, North Carolina during 1901 and 1902. A new cestode, *Otobothrium insigne,* from *Carcharhinus obscurus* (Lesueur) was described in Linton's study based on criteria developed from two immature specimens. In this species description, he omitted any mention of oncotaxy except in the form of two rather vague illustrations. Yet, the form and arrangement of the tentacular hooks is a major character presently used to differentiate taxa among this group of cestodes. We have found the metacestode stage of *O. insigne* in *Arius felis* (Linnaeus) catfish collected from the Gulf of Mexico south of Louisiana. This particular metacestode has proved to be a convenient model for studying a variety of ultrastructural and cytophysiological features of plerocercus-type metacestodes. Accordingly, we felt it necessary to ascertain its taxonomic status, and clarify its oncotaxy.

Materials and Methods

Plerocerci used in this study were obtained from the skeletal musculature of medium- to large-sized (greater than 300 g) *Arius felis* catfish collected by baited hook and line from the Gulf of Mexico in the vicinity of Grand Isle, Louisiana. The catfish were killed by pithing, and the body cavity cut open along its midventral longitudinal axis from anal pore through branchial arches. Upon removal of the visceral organs and gill arches, plerocerci were easily located and removed from their sites within the branchiomeric musculature. Numerous closely spaced incisions were also made within the thicker body wall and tail musculature to locate plerocerci within these regions.

Plerocerci for whole mounts were flattened, fixed with 4% neutral buffered formalin, dehydrated in an ethanol gradient, stained with Mayer's paracarmine, counterstained with fast green, and mounted in Permount. Whole mounts of manually excysted juvenile scolices were prepared as described for the entire plerocercus except that they were intially fixed, unflattened, in 70% ethanol and then postfixed in formalin. Initial fixation of specimens in ethanol resulted in a greater frequency of everted tentacles. Several formalin-fixed juvenile scolices were prepared for scanning electron microscopy by dehydrating the specimens in an ethanol gradient and liquid freon, critical-point drying them in liquid CO_2, and coating them with gold in an Edwards S-150 sputter coater. These scolices were then viewed with a Cambridge Stereoscan 600 scanning electron microscope. Two whole-mount voucher specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (No. 78166) and in the Division of Parasitology, University of Nebraska State Museum, Manter Laboratory, Lincoln, Nebraska 68588 (No. 22312).

Results

Otobothrium insigne Linton, 1905 (Figs. 1–7 and Table 1)

DESCRIPTION OF PLEROCERCUS (measurements based on 38 specimens; nomenclature after Dollfus, 1942; hook dimensions given as the mean in mm ± one standard deviation): Unflattened, fully developed plerocerci are pyriform, 8–10 mm long and 4–5 mm in maximum diameter. Juvenile scolex craspedote, 3.3–4.4 mm long. Two patelliform to subcircular bothridia, 0.88–1.22 mm long; posterolateral margin of each bothridium contains two relatively large auxiliary pits, 0.15–0.20 mm long. Tentacle diameter 0.07–0.09 mm excluding hooks, 0.11–0.16 mm including hooks; tentacular sheaths highly coiled. Tentacular retractor muscles insert near the middle of

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Table 1. Selected species characteristics.*

	Louisiana specimens†	*Otobothrium insigne*	*Otobothrium dipsacum*	*Otobothrium penetrans*
Source of measurements		Linton, 1905	Cruz-Reyes, 1973; Dollfus, 1942	Linton, 1905, 1907, 1924
Length of the juvenile scolex	3.8 ± 0.28 (3.3–4.4)	4.2‡	3.0–4.9	4–5
Appendix length	1.7 ± 0.16 (1.3–2.0)	N.R.§	N.R.	N.R.
Velum length	0.62 ± 0.09 (0.41–0.78)	0.30	N.R.	N.R.
Bothridium length	1.04 ± 0.06 (0.88–1.22)	0.70	1.0–1.4	1.4
Length of the auxiliary pit	0.179 ± 0.012 (0.15–0.20)	0.15	0.062–0.117	N.R.
Length of the tentacular bulb	0.772 ± 0.055 (0.68–0.87)	0.72	1.37–1.69	1.35–1.40
Width of the tentacular bulb	0.232 ± 0.016 (0.19–0.28)	0.28	0.161–0.322	0.40–0.55
Tentacle diameter (without hooks)	0.080 ± 0.004 (0.07–0.09)	0.09	0.092–0.103	0.15–0.18
Tentacle diameter (with hooks)	0.134 ± 0.013 (0.11–0.16)	0.15	0.21	0.24–0.28
Length of the longest hook	0.045 ± 0.002 (0.040–0.048)	0.045	0.050	0.09–0.14

* Measurements given in mm.

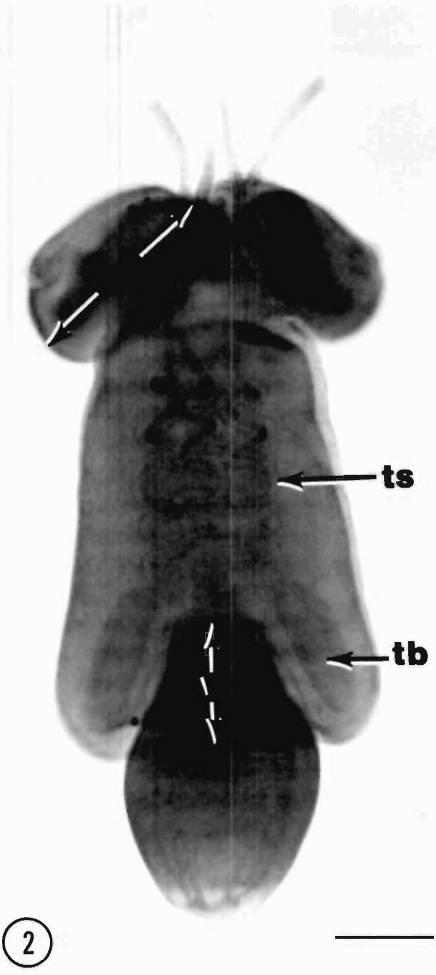
† Measurements based on 38 specimens: first value in each category represents the mean ± 1 SD; the second value (in parentheses) represents the range.

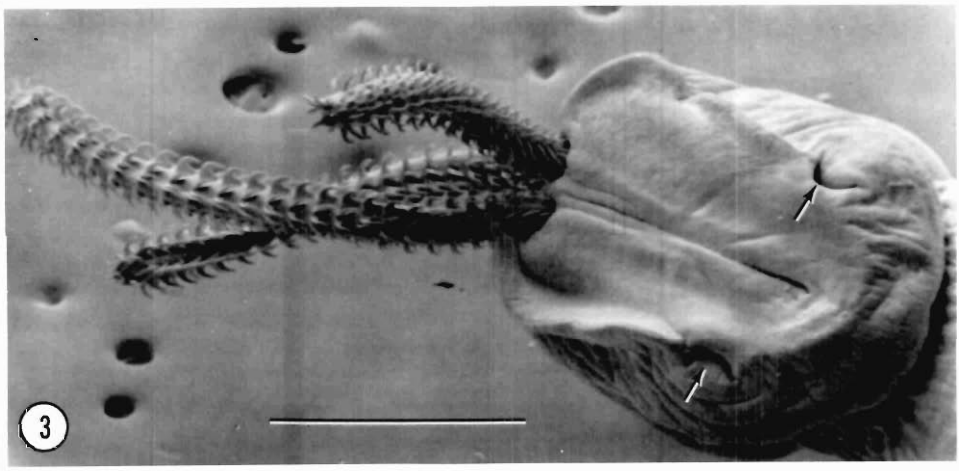
‡ Value represents the length of the adult scolex, and thus does not include the appendix.

§ N.R. = value was not reported.

the tentacular bulbs; latter ovoid, about three times longer than wide (0.772 × 0.232 mm), and mildly divergent posteriorly. Velum 0.41–0.78 mm long; appendix 1.3–2.0 mm long. Distal and metabasal tentacular armature consisting of 18 longitudinal rows of hooks. Hooks within each longitudinal row identical. Principal hooks arranged in numerous, obliquely horizontal rows occupying three-fourths of total tentacular circumference; each horizontal row divisible into two half spiral rows; each half row begins on central internal face as large rosethorn-shaped hook (hook 1,1′: total length 0.0425 ± 0.0027; base length 0.0363 ± 0.0025), and continues externally over either the bothridial or abothridial face as four large spiniform hooks with transverse bases. Each set of four spiniform hooks decreases sequentially in relative size; lengths of spiniform hooks include: 2(2′) 0.0451 ± 0.0019, 3(3′) 0.0424 ± 0.0022, 4(4′) 0.0351 ± 0.0038, and 5(5′) 0.0292 ± 0.0058. Principal hooks not continuing to the external face; latter region occupied by longitudinal band of small hooks roughly arranged in horizontal rows containing four hooks per row (hooks A, B, C, D or A′, B′, C′, D′). Two small spiniform hooks occupy terminal positions within each horizontal row (hook length: A,A′ 0.0130 ± 0.0016; D,D′ 0.0229 ± 0.0028); two central positions occupied by small spiniform hook (B,B′; length of 0.0093 ± 0.0015) and by small rosethorn-shaped hook (C,C′; length of 0.0156 ± 0.0029). Latter two hooks alternate positions relative to one another within each two neighboring rows. One horizontal row of small hooks present for each half spiral row of principal hooks. Rows of principal hooks extend to base of tentacle; rows of small hooks on external face end 0.15–0.25 mm from base. External face of basal region occupied by randomly arranged, small spiniform hooks with slightly curved tips (0.008–0.024 mm long).

HOST RECORD INFORMATION: A total of 3,656





fully developed *Otobothrium insigne* plerocerci were recovered from 175 medium- to large-sized *Arius felis* during six different collecting trips conducted from 1979 through 1981. Out of the 175 catfish posted, only 17 fish were free of fully developed plerocerci. The average yield of *O. insigne* plerocerci was 21 per fish with a high of 351. In addition to large fully developed plerocerci, smaller ones (presumably of the same species since auxiliary pits were present), were occasionally observed within these catfish. They were, however, in vast minority to the larger plerocerci; intermediate-sized plerocerci were encountered even less frequently.

Among 61 catfish collected during 1981, fully developed plerocerci were located in the following sites: branchiomeric musculature (22%), pectoral musculature along the dorsal surface of the cleithrum and coracoid (19%), body wall musculature just posterior to the coracoid (27%), posterior body wall musculature adjacent to the coelomic cavity (18%), tail musculature (6%), tissues bordering the pericardium (7%), and other tissues of the viscera (1%). In heavily infected fish, much of the musculature associated with the pectoral girdle was often replaced by plerocerci and host inflammatory tissue.

Discussion

Bothridial auxiliary pits occur exclusively in the family Otobothriidae, which contains three genera. The relatively short appendix, the highly coiled tentacular sheaths, and a blastocyst without a long, posterior extension all indicate that the present specimens are not members of the genus *Poecilancistrum* Dollfus, 1929. The genus *Diplootobothrium* Chandler, 1942 is characterized by the presence of a double set of reproductive organs in each mature proglottid, however, because only the plerocercus stage was available for this study, secondary characteristics of the two species (*D. springeri* Chandler, 1942; *D. tamilnadensis* Reimer, 1980) were compared to those of the present specimens. Major differences exist in the oncotaxy of the two *Diplootobothrium* species versus that of the species reported in this paper. For example, in *D. springeri* all of the principal hooks are rosethorn-shaped. In *D. tamilnadensis* there is a paucity of small hooks, and its largest principal hooks are roughly half the length of those of the present specimens.

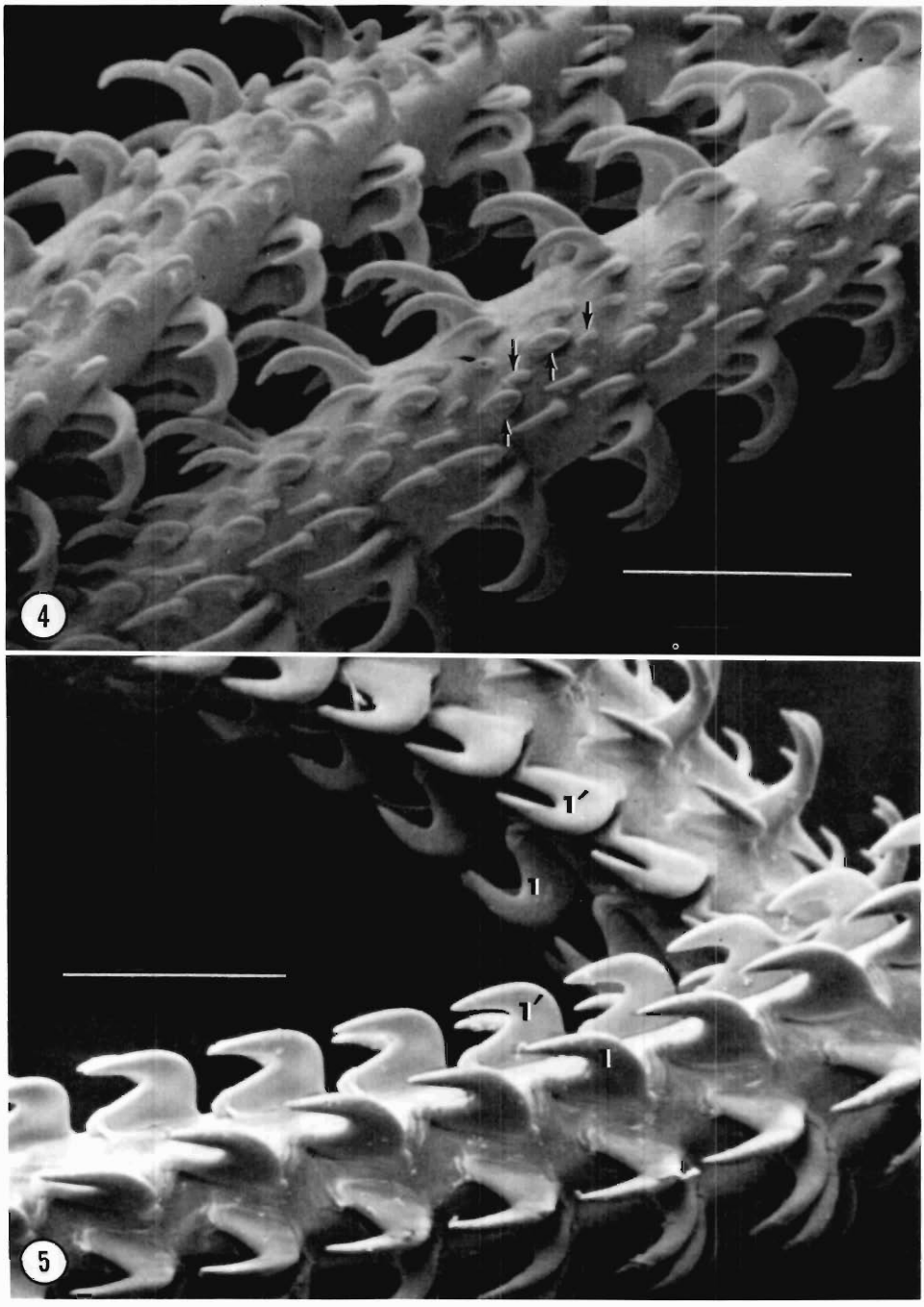
Sixteen species from the genus *Otobothrium* Linton, 1890 have been described throughout the world. The plerocercus described in this paper displays a very strong resemblance to *O. insigne* Linton, 1905. As shown in Table 1, there are very strong similarities between the morphometry of the juvenile scolex from the Louisiana plerocerci and that of the adult scolex of *O. insigne.* The morphometry of the tentacles, tentacular bulb muscles, and tentacular hooks are generally regarded as being particularly reliable species characters for both the adult and plerocercus stages of trypanorhynchs. In the present case, these characters are particularly similar, especially considering that Linton's measurements were based on only two individuals. Minor differences in the morphometry of the velum and bothridium from Linton's (1905) description versus that of the Louisiana specimens may represent normal variations in the population, may be due to adult versus plerocercus differences or simply due to specimen preparation differences.

The tentacular hook arrangement of *O. insigne,* as figured by Linton (1905; plate XIX, figs. 144, 145), is consistent with the hook arrangement of the specimens from Louisiana. Linton's illustrations (figs. 144 and 145) indicate that the armature consists of principal hooks arranged in horizontal rows that are divisible into two half spiral rows. It also appears that each half row begins with a large rosethorn-shaped hook, and

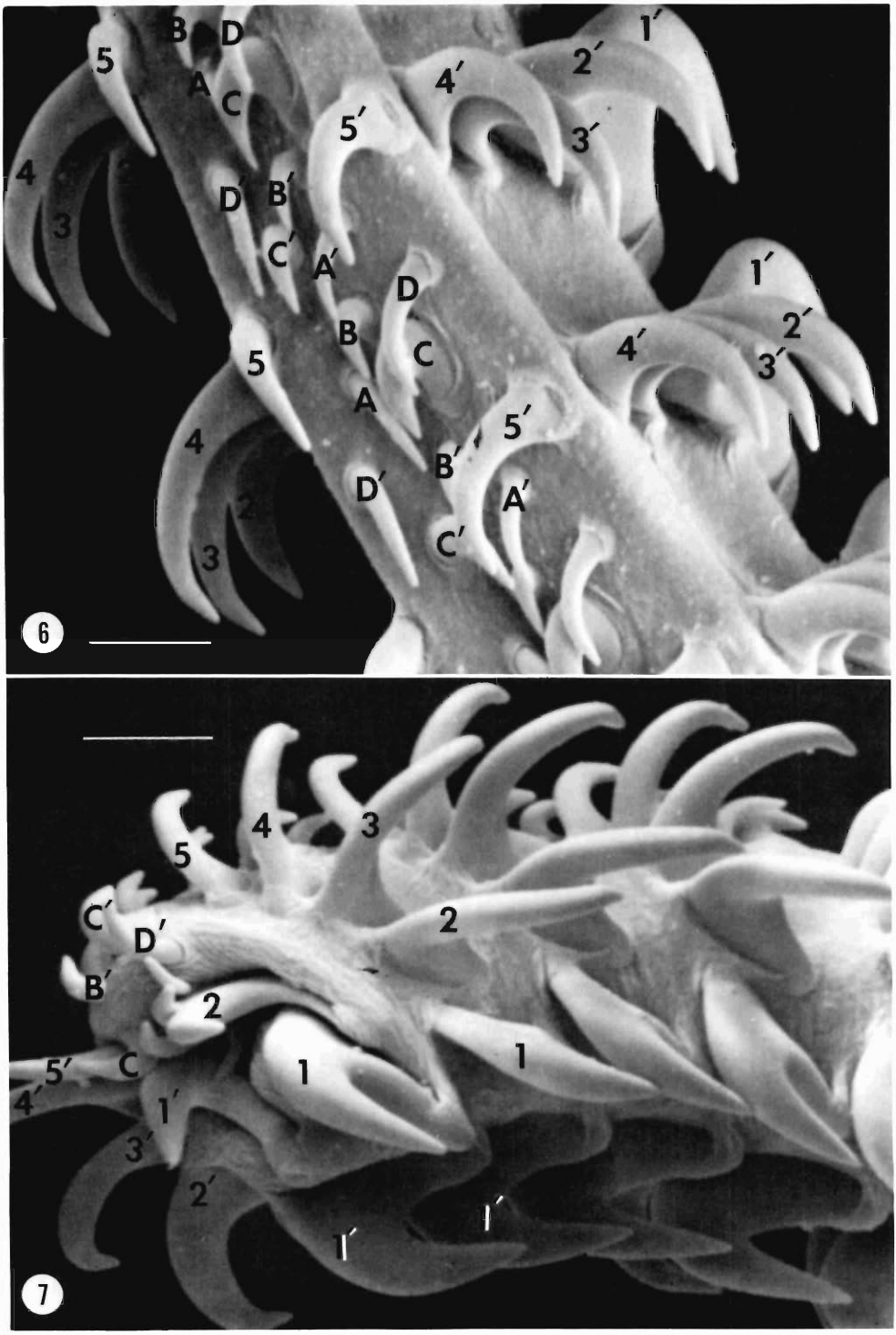
←

Figures 1, 2. Manually excysted juvenile scolices of *Otobothrium insigne.* Bar = 0.5 mm. 1. Scanning electron micrograph demonstrating the external morphology of the three basic regions: *pars bothridialis* (b), *pedunculus scolecis* (ps) and appendix or *pars proliferens* (pp). Only three of the four tentacles (t) are everted. 2. Whole-mount photomicrograph showing the highly coiled tentacular sheaths (ts) and the oval tentacular bulb muscles (tb); also indicated are the distances recorded in Table 1 as "bothridium length" (pb) and "velum length" (v).

Figure 3. Scanning electron micrograph of the *pars bothridialis* region showing the shape of the bothridium and the location of the auxiliary pits (indicated by arrows). The arrangement of the tentacular hooks into longitudinal rows is apparent. Bar = 0.5 mm.



Figures 4, 5. External and internal view of two tentacles. Bar = 0.1 mm. 4. External view showing the longitudinal band of small hooks from the metabasal region. Upward directed arrows indicate two small rose-thorn-shaped hooks from two neighboring rows of small hooks. Note that, within neighboring rows, the small thorn-shaped hooks alternate relative positions with that of the small central spiniform hooks (two of the small spiniform hooks are indicated by downward directed arrows). 5. Internal view showing the large rosethorn-shaped hooks. The thorn-shaped hook labeled "1" exists on the bothridial side of the internal midline, and the hook labeled "1′" exists on the abothridial side.



Figures 6, 7. Metabasal tentacular hook arrangement. Bar = 0.02 mm. 6. Somewhat collapsed tentacle demonstrating 17 of the 18 longitudinal rows of hooks. The labeled principal hooks include: the rosethorn-shaped hook from the abothridial side of the internal midline (1′), and the spiniform hooks from both the bothridial (2, 3, 4, 5) and abothridial (2′, 3′, 4′, 5′) faces. Small hooks in the external longitudinal band are labeled such that the horizontal row with the small rosethorn-shaped hook on the abothridial side of the external midline (C) is represented by letters A–D, whereas the row with the rosethorn-shaped hook on the bothridial side of the midline (C′) is represented by letters A′–D′. 7. Distal end of a tentacle viewed from the internal face and labeled as described in Figure 6. Several hooks remain partially inverted inside the distal end of the tentacle.

that each row continues as several large spiniform hooks. Based on these two illustrations, it is impossible to determine the number of spiniform hooks within each half row, though it appears to be either three, four, or five. The external face of the tentacle is occupied by a longitudinal band of small hooks. Unfortunately, the arrangement of these small hooks is also impossible to determine from Linton's illustration (plate XIX, fig. 144). Identification of the plerocerci from *Arius felis* would be greatly facilitated by a clarification of two parameters omitted from Linton's (1905) description: the number of large spiniform hooks within each half spiral row of principal hooks, and the arrangement of hooks in the external band of small hooks. Attempts to locate the type specimens for *O. insigne* Linton, 1905 were unsuccessful. However, due to the very strong similarities between all of the various other parameters from the Louisiana specimens and those from Linton's description of *O. insigne* (Table 1), the Louisiana specimens are identified as *O. insigne.*

Some controversy exists concerning the status of the species *O. insigne* Linton, 1905. Yamaguti (1959) and Cruz-Reyes (1973) list *O. insigne* as being a synonym of *O. dipsacum* Linton, 1897. Southwell (1929) and Dollfus (1942) state that the species of *Otobothrium* from the pearl banks of Ceylon, which Southwell (1912) misidentified as *O. insigne,* is synonymous with *O. dipsacum.* However, both authors maintain *O. insigne* Linton, 1905 from Beaufort, North Carolina as a valid species. As indicated in Table 1, the bulb muscles from *O. dipsacum* are roughly twice the length of those from *O. insigne,* whereas the auxiliary pits are roughly half the length. The tentacular armature of *O. dipsacum* is also very different from *O. insigne;* in *O. dipsacum,* the metabasal hooks are arranged in half spiral rows that begin in the middle of the internal face and extend to the middle of the external face. The various differences clearly demonstrate that *O. insigne* and *O. dipsacum* are separate species. *Otobothrium penetrans* Linton, 1907 also slightly resembles *O. insigne,* especially with respect to its hook arrangement; however, the dimensions of the various hooks, the dimensions of the tentacular bulbs, and the diameter of the tentacles (with and without hooks) from *O. penetrans* are all roughly twice that of *O. insigne.*

The present survey indicates that *O. insigne* is a common parasite of *Arius felis* from the Gulf of Mexico south of Louisiana. This finding represents the first report of this species since its original description, and the only report of its plerocercus. Plerocerci are located within the catfish skeletal musculature, particularly muscles associated with the gills, mandibles, and pectoral girdle. Two other *Otobothrium* species have been reported from the Gulf of Mexico: the adult stage of *O. penetrans* in *Carcharhinus lamia* (Risso), *C. limbatus* (Valenciennes), and *Rhizoprionodon terraenovae* (Richardson) from Dry Tortugas, Florida (Linton, 1924; Shuler, 1938) and the plerocercus stage of *O. dipsacum* in *Mycteroperca falcata* (Poey) from Pensacola, Florida (Linton, 1924).

Acknowledgment

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A Consideration of Alternative Intermediate Hosts for *Moniezia expansa* (Cestoda: Anoplocephalidae)

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ABSTRACT: Arthropods, other than oribatid mites, were examined as potential intermediate hosts of *Moniezia expansa* (Rudolphi, 1810). Collembolans (1,125) and mites in the families Glyciphagidae (917), Uropodidae (887), and Sejidae (298) were collected from north-central Florida from three pens known to harbor goats infected with these cestodes. None of the arthropods examined contained metacestodes.

Fresh proglottids of *M. expansa* were fed to a colony of the fire ant, *Solenopsis invicta* Buren. Worker ants readily accepted the proglottids and fed them only to 4th instar larvae. The latter were also fed eggs of *M. expansa* by hand. Within the ant larval gut, oncospheres remained in the pyriform apparatus even when eggs were ruptured. Prior to pupation, ant larvae voided the tapeworm eggs in the meconium. Meconium pellets contained some live tapeworm eggs.

In the past 43 years no one has examined arthropods other than oribatid mites as possible intermediate hosts of *Moniezia* spp. This reluctance to consider other invertebrates reflects a general trust in the negative results obtained by previous investigators. A review of the literature, however, revealed that early studies on various possible hosts were inadequate in both scope and methodology (Fritz, 1982).

Prior to Stunkard's (1937) discovery that oribatid mites are intermediate hosts of *M. expansa* (Rudolphi, 1810), Joyeux (1920), Flattely (1922), Daubney (1923), Joyeux and Kobozieff (1929), and Mönnig (1929) examined other arthropods as potential intermediate hosts of *Moniezia* spp. Their investigations, for the most part, were inadequate for two reasons: (1) too few individuals of too few species were examined from pastures and from laboratory experiments, and (2) attempts to infect arthropods experimentally were too short in time to allow for the development of cysticercoids. Although it is relatively easy to detect mature cysticercoids within oribatid mites, the early stages of development are more indistinct in shape and can be difficult to distinguish from host tissues. These stages can also be overlooked because of their small size and tendency to burst during dissection of the host. In addition, since rates of parasitism of intermediate hosts with *Moniezia* spp. can be very low (Graber and Gruvel, 1969), it is important to examine large numbers of individuals of a species being considered.

Prior to 1937, only Mönnig (1929) had examined mites as possible intermediate hosts of *M. expansa*. He collected two species of mites from a pasture and kept these in a jar with proglottids for 14 days. Krull (1940) further examined mites other than oribatids, but merely fed four individuals collected from a pasture to a lamb. Similarly, investigations of other arthropods often considered few individuals of a given species. Mönnig (1929) included only 77 ants, 11 dung beetles, and 22 fly larvae in his dissections of arthropods from a pasture. In his attempts to infect insects experimentally, he used only 7 collembolans, 14 psocopterans, and 23 ants.

Nonspecific intermediate hosts in the life cycles of other anoplocephaloid cestodes suggest that *M. expansa* might also form cysticercoids in arthropods other than oribatid mites. For example, both psocopterans and oribatid mites have been reported as intermediate hosts for tapeworms in the family Thysanomatidae (Sengbusch, 1977). Furthermore, collembolans and oribatic mites are intermediate hosts for *Paranoplocephala* spp. (Cestoda: Anoplocephalidae) (Sengbusch, 1977; Smirnova and Kontrimavichus, 1977). This study investigates further the possibility that arthropods other than oribatid mites are intermediate hosts for *M. expansa*.

Materials and Methods

Soil and surface debris were collected by hand from inside and from the immediate perimeter of three pens known to harbor goats parasitized by *M. expansa* (see Fritz, 1982). Two pens were located in Alachua County near Newberry town, and the third pen was located in Levy County near Williston, Florida. Arthropods were extracted from the samples in Tulgren funnels and were kept in 70% ethanol.

Collembola and mites in the families Glyciphagidae,

Table 1. The number of mites and collembolans collected from three goat pens in north-central Florida and dissected for the presence of *Moniezia expansa* metacestodes.

	Pens				Metacestodes
	1	2	3	Total	
Glyciphagidae	414	418	85	917	0
Uropodidae	328	93	466	887	0
Sejidae	0	0	298	298	0
Collembola	230	184	711	1,125	0

Uropodidae, and Sejidae were dissected for the presence of metacestodes. Collembolans share some of the same food preferences (fungi, detritus, feces) as many of the oribatid mites that are intermediate hosts of *M. expansa* (see Wallwork, 1970). Many fungivorous uropodids are ubiquitous inhabitants of dung. Glyciphagids commonly inhabit processed feeds and animal nests. They have been shown to be intermediate hosts for tapeworms in the family Catenotaeniidae (Cestoda: Anoplocephaloidea). Sejids occur in humus and litter. Little is known about their feeding habits, but Krantz (1978) stated that they do not appear to be adapted for predation. Dissections were accomplished by crushing individuals in saline or Hoyer's mounting medium under a coverslip on a microscope slide.

Attempts were made to infect the fire ant, *Solenopsis invicta* Buren, with *M. expansa*. *S. invicta* was chosen for this study because it is one of the more common ants found on pastures in the southeast (Hung et al., 1977). These ants are omnivorous and workers live long enough for the complete development of cysticercoids; minum workers live 50.7 ± 13.1 days and major workers live 124.3 ± 52.3 days under laboratory conditions (Mirenda and Vinson, 1981).

A colony of ca. 1,000 ants was housed in an enamel pan in which the inner surfaces were coated with Fluon (Northeast Chemical Co., Woosocket, Rhode Island) to prevent ants from escaping. Clear plastic petri dishes with plaster-castone bottoms were used for nest chambers. These were kept moist, and four holes around the sides of the dishes allowed ants to enter the enamel pan feeding area. The colony was kept at room temperature (23–27°C) and was fed a diet of mole crickets, honey, dog food, and water. Twenty-one proglottids of *M. expansa* were fed to the colony over a period of 2 weeks. They were introduced on the same day that they were removed from the feces of a goat. Proglottids were given to the ants both mixed with their food and separately. After 40 and 60 days, 200 and 500 ants, respectively, were dissected for cysticercoids.

In addition, 54 4th instar larvae were removed from a separate colony of *S. invicta*, were hand-fed eggs of *M. expansa*, and were placed in covered Stender dishes. These ants were dissected at various stages throughout their development into adults.

Results

The majority of the glyciphagid mites examined came from the two goat pens near Newberry, whereas all the sejid mites and most of the uropodid mites were collected from the pen near Williston. None of the mites or the collembolans contained metacestodes of *M. expansa* (Table 1).

Foraging workers of *S. invicta* readily accepted whole proglottids, which they fragmented and carried into the brood chamber. Here they were met by other workers who further apportioned the tapeworm eggs. Only 4th instar larvae received eggs; this feeding behavior agrees with observations by Petralia and Vinson (1978) that only the last larval instar is fed solid foods.

Three days after the initial feeding of proglottids to the ant colony, 6 of 10 4th instar larvae examined had eggs in their gut. Most of the tapeworm eggs were intact and oncospheres were not seen within the hemocoel or loose in the gut. Oncospheres in ruptured eggs were never seen outside the pyriform apparatus. None of the 700 adult ants dissected contained cysticercoids.

No oncospheres were found in the gut or hemocoel of the hand-fed 4th instar ant larvae or in the subsequent pupae. Eggs of *M. expansa* were voided in the meconium just prior to pupation, and some of the intact eggs still contained live oncospheres.

Discussion

In all three pastures the highest concentration of goat feces was evident in and around the pens. Tapeworm eggs should then have been relatively more abundant in these areas, and intermediate hosts of *M. expansa* could reasonably be expected to have their highest rates of infection here. In one of these pens oribatid mites of four species were found infected with cysticercoids at rates ranging from 0.2 to 3.4% (Fritz, 1982). If the collembolans and mites examined in this study are capable of serving as intermediate hosts of *M. expansa*, their rates of infection were too low to be detected by the numbers of individuals examined.

Solenopsis invicta is incapable of serving as an intermediate host for *M. expansa*. Although 4th instar larvae will ingest the cestode eggs, the majority of these remain intact within the ant gut. Presumably, therefore, the few ruptured eggs observed were damaged prior to or during feeding; the ant larval gut itself appears incapable of rupturing eggs. Furthermore, conditions within the gut are such that the oncosphere in a ruptured egg does not emerge from the pyriform apparatus.

Krull (1940) observed ants dragging proglottids into their nest in a pasture. In the present study, pupating 4th instar ant larvae released the meconium containing the ingested tapeworm eggs. It is not known whether the meconia with ingested eggs in ant refuse piles play any further role in the life cycle of *M. expansa.*

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Redescription of *Trypanosoma cervi* (Protozoa) in Moose, *Alces alces,* from Alaska and Wyoming[1]

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ABSTRACT: *Trypanosoma cervi* Kingston and Morton, 1975 is redescribed from 38 bloodstream trypomastigotes concentrated from the blood of a single Alaskan moose, *Alces alces gigas,* in 1981. These trypanosomes were compared with trypanosomes from moose in Wyoming, *A. a. shiras,* and with trypanosomes from elk, mule deer, white-tailed deer, and reindeer. Statistical analysis confirmed the conspecificity of cervid trypanosomes.

Unidentified trypanosomes were first recovered from cervids by Kistner and Hanson (1969). These were found in white-tailed deer (*Odocoileus virginianus*) from the southeastern United States. Trypanosomes subsequently have been reported from this host (Stuht, 1975; Kingston and Crum, 1977; Davidson et al., 1983) as well as from elk (*Cervus canadensis*) in Wyoming, Michigan, and New Mexico (Kingston and Morton, 1973; Davies and Clark, 1974; Stuht, 1975), mule deer (*O. hemionus*) (Clark, 1972; Kingston et al., 1975; Matthews et al., 1977), and reindeer (*Rangifer tarandus*) (Kingston et al., 1982). Morphometric analyses of bloodstream trypomastigotes from these hosts have demonstrated (Kingston and Morton, 1975; Kingston and Crum, 1977; Matthews et al., 1977; Kingston et al., 1982) conspecificity of these parasites and that they are *Trypanosoma cervi* Kingston and Morton, 1975. Although we have collected blood samples from moose (*Alces alces shirasi*), whenever possible (Kingston et al., 1981), and a few bloodstream trypomastigotes have been observed, an adequate comparison with trypanosomes from other hosts has not been possible. Blood collected from an Alaskan moose, *Alces alces gigas,* provided sufficient numbers of bloodstream trypomastigotes to allow comparison with those from moose in Wyoming and from other cervids in North America.

Materials and Methods

About 10 ml of jugular blood from an anesthetized moose was drawn into a heparinzed vacutainer tube. This was chilled until examination. Part of the sample was centrifuged in microhematocrit tubes and the plasma buffy-coat interface was examined by brightfield microscopy (DE, single concentration, using a 3.5–16× objective and a 12.5× ocular) for active trypanosomes. When trypanosomes were observed, the tube was scored just above the buffy-coat and some plasma, the trypanosomes, the buffy-coat, and some red cells were expressed onto a microscope slide by pushing on the hematocrit tube sealant with a stylet. The expressed material was mixed on the slide and conventional thin blood films were prepared. Thirty-eight slides were made, air-dried, fixed with absolute methanol, stained with Giemsa's stain, and decolorized in cold running tap water. Slides were coated with a thin film of immersion oil and the blood film completely scanned (16× objective, 12.5× ocular) for trypanosomes. When observed, trypanosomes were photographed with color transparency film under the oil immersion objective with a 10× ocular substituting for the lens of a Zeiss SLR camera mounted on the microscope. Thirty-eight trypanosomes were adequate for further study. The transparencies were projected and images drawn at a standard distance. A transparency of a stage micrometer at the same magnification was projected on the drawing and the scale was indicated. The drawings were measured using a calibrated map wheel reader (Alvin, 1112, Switzerland). The mensural parameters of the trypanosomes derived from these measurements were analyzed using a computerized single analysis of variance (Statistical Package for the Social Sciences [SPSS], version 8). These were compared with mensural values of trypanosomes from other cervid species similarly analyzed.

Results

Fourteen captive, and/or free-ranging moose from Wyoming have been examined for trypanosomes since 1977. Of these, two calves and eight adults, including both sexes, were positive for trypanosomes, either by culture (veal infusion medium, VIM) or by direct examination (DE) of concentrated blood (Kingston et al., 1981). Four trypomastigotes were recovered on slides from such infected blood examined DE. Trypano-

[1] Published with the approval of the Director, Agriculture Experiment Station Paper No. JA 1275, College of Agriculture, University of Wyoming, Laramie.

Table 1. Comparison of means of mensural values* between Wyoming moose and Alaska moose trypanosomes and trypanosomes from other deer.†

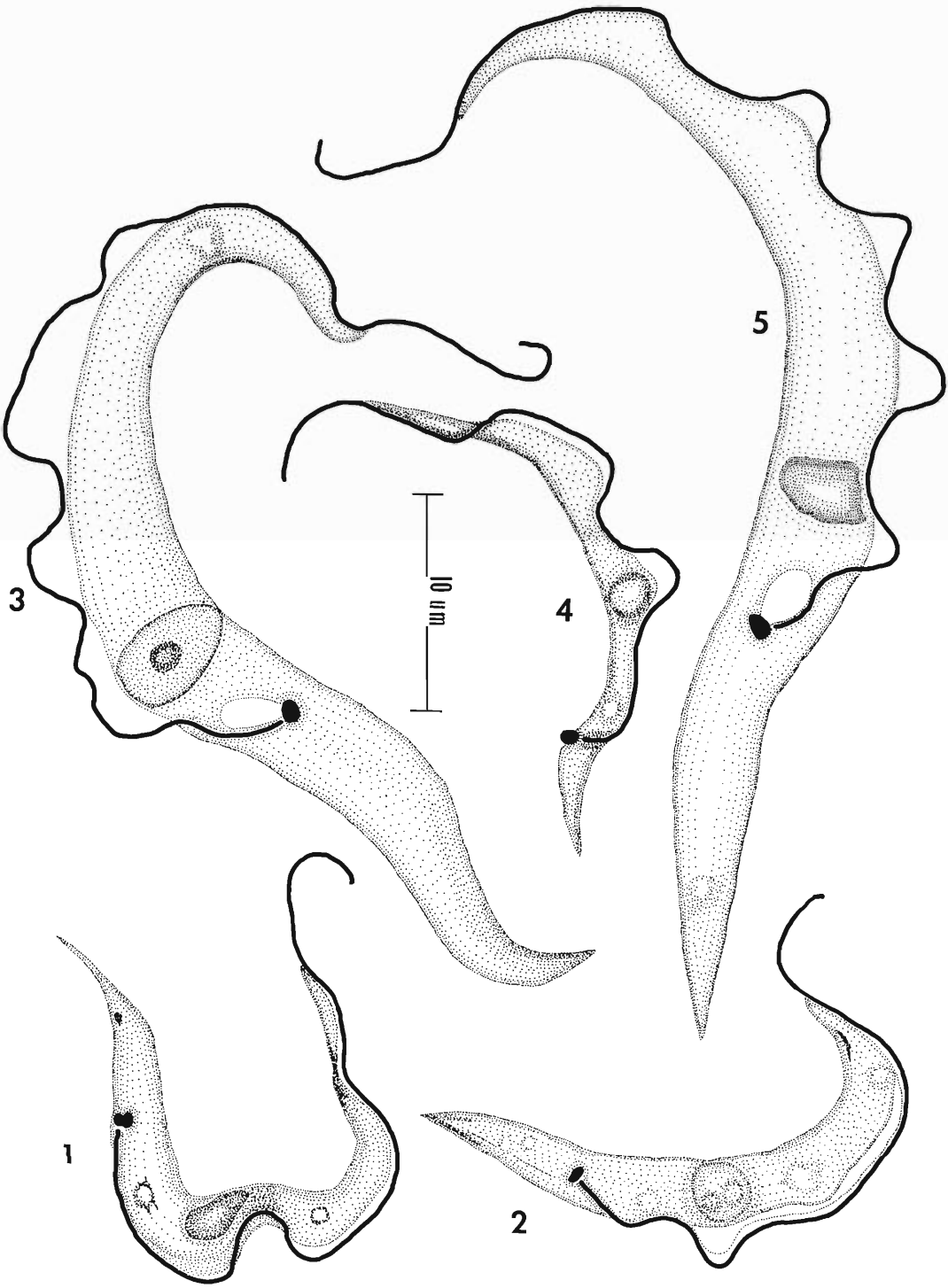
	$\bar{x}$										
	PK‡	KN	PN	NA	BL	FF	L	W	FF:BL	NI	KI
Wyoming moose ($N = 4$)	7.5 ± 0.96§	7.0 ± 0	14.5 ± 0.96	15.2 ± 1.03	30.3 ± 1.7	8.3 ± 0.75	38.5 ± 1.85	2.8 ± 0.25	1:3.8 ± 0.36	0.9 ± 0.06	1:2.1 ± 0.14
Alaska moose ($N = 38$)	16.7 ± 0.85	7.5 ± 0.28	24.0 ± 0.93	30.4 ± 0.99	54.5 ± 1.54	9.8 ± 0.59	64.0 ± 1.56	7.0 ± 0.50	1:6.5 ± 0.50	0.8 ± 0.37	1:3.3 ± 0.14
Composite: Wyoming moose + Alaska moose ($N = 42$)	15.8 ± 0.86	7.5 ± 0.26	23.1 ± 0.95	29.0 ± 1.13	52.2 ± 1.78	9.6 ± 0.54	61.6 ± 1.84	6.6 ± 0.49	1:6.2 ± 0.47	0.8 ± 0.35	1:3.2 ± 0.14
Other deer†: Composite ($N = 132$)	10.2 ± 0.42	6.9 ± 0.19	17.1 ± 0.50	21.5 ± 0.55	38.8 ± 0.95	7.7 ± 0.29	46.4 ± 1.05	5.1 ± 0.18	1:6.1 ± 0.35	1:0.8 ± 0.02	1:2.6 ± 0.08
Composite grand mean ($N = 174$)	11.5 ± 0.43	7.0 ± 0.16	18.5 ± 0.48	23.3 ± 0.55	42.0 ± 0.94	8.2 ± 0.26	50.1 ± 1.03	5.5 ± 0.19	1:6.1 ± 0.29	0.8 ± 0.2	1:2.7 ± 0.07

* Expressed in μm.

† Other deer include values from elk, mule deer, white-tailed deer, and reindeer.

‡ Abbreviations: PK = posterior end to kinetoplast, KN = kinetoplast to nucleus, PN = posterior end to nucleus, NA = nucleus to anterior end, BL = body length, FF = free flagellum, L = length, W = width, FF:BL = free flagellum to body length ratio, NI = PN/NA, KI = PN/KN.

§ Standard error.



Figures 1, 2. Small trypanosomes recovered from the blood of moose from Wyoming ($\bar{x}$ BL = 30 μm). Figures 3, 5. Large bloodstream trypomastigotes from moose from Alaska ($\bar{x}$ BL = 54 μm). Figure 4. Small trypomastigote from blood of moose from Alaska.

somes were not observed in two adult moose from Utah. One captive yearling male moose from the Kenai Moose Research Center, on the Kenai National Moose Refuge, Soldatna, Alaska was positive for trypanosomes on DE. Forty-two trypanosomes were found on 38 slides. The mensural values of the four trypanosomes from Wyoming moose and of the 38 usable trypanosomes from the Alaska moose are presented in Table 1. Both the small forms from Wyoming moose (Figs. 1, 2) in which the body length (BL) exclusive of the flagellum averaged 30 μm, or larger forms (Figs. 3–5) from Alaskan moose (BL $\bar{x}$ = 54 μm) are typical *Megatrypanum* trypanosomes. All specimens have (1) a centrally to slightly posteriorly located nucleus, (2) a long pointed "tail," and (3) the short free flagellum characteristic of other cervid trypanosomes. Duncan's Multiple Range Test for similarities of the means ($P \leq 0.05$) of mensural values of trypanosomes from different hosts, viz., Alaska moose, moose, elk, mule deer, white-tailed deer, and reindeer indicated that for: (1) posterior end to kinetoplast (PK) values, trypanosomes from Alaskan moose differ only from trypanosomes recovered from mule deer; (2) kinetoplast to nucleus (KN) distances, trypanosomes from Alaskan moose differ significantly only from trypanosomes found in mule deer; (3) distances from posterior end to nucleus (PN), trypanosomes from Alaskan moose differ significantly from trypanosomes from all species except white-tailed deer; (4) distances from nucleus to anterior end (NA), trypanosomes from Alaskan moose significantly differ from those found in all other host species; (5) body length (BL), length (L), and width (W) values, trypanosomes from Alaskan moose differ significantly from trypanosomes found in all other species; (6) lengths of free flagella (FF), trypanosomes from Alaskan moose are similar to trypanosomes from all other host species; (7) free flagellum to body length index (FF:BL) values and for nuclear index (NI = PN/NA) values, trypanosomes from Alaskan moose are similar to trypanosomes from all other host species; (8) kinetoplast index (KI = PN/KN) values, trypanosomes from Alaskan moose are similar to trypanosomes from elk and white-tailed deer but differ significantly from trypanosomes from other host species; and (9) width to body length index (W:BL) values, trypanosomes from Alaskan moose are similar to trypanosomes from all other host species.

Differences in absolute measurements observed for trypanosomes from Alaskan moose compared with absolute measurements of other *T. cervi* trypanosomes (PN, AN, BL, and L) are all functions of body size. It therefore seems unreasonable to separate the moose trypanosomes from the other cervid trypanosomes based on, essentially, that single criterion. We conclude therefore that the trypanosomes from moose are conspecific with *Trypanosoma cervi* Kingston and Morton, 1975 described from elk in Wyoming (Kingston and Morton, 1975). This species is also known from mule deer (Matthews et al., 1977), white-tailed deer (Kingston and Crum, 1977), and reindeer (Kingston et al., 1982) in North America.

Discussion

Examination and comparison of the mensural data derived from measurements of trypanosomes from moose in Wyoming and Alaska (Table 1) may suggest to some that these are widely divergent forms and, possibly, different species owing to the apparent great disparity in body size and position of various organelles. We argue that these differences in mensural values are more apparent than real. The small numbers of specimens (four) from Wyoming moose constitute an inadequate sample for comparison. Moreover, samples of trypanosomes from Wyoming moose were taken at seasons of the year when small forms predominate, causing them to resemble chronic (winter) forms in many respects (see mule deer, Fig. 6; Matthews et al., 1977). Such winter forms tend to be smaller than spring/summer forms as observed in trypanosomes from mule deer (Matthews et al., 1977), white-tailed deer (Kingston and Crum, 1977), and elk (Kingston and Morton, 1975) collected between April and June. Values for reindeer trypanosomes are composites of summer and fall specimens (Kingston et al., 1982).

The 38 specimens of trypanosomes from a single Alaskan moose (Table 1) were recovered in July and thus these large forms represent, we think, predivision stages that are generally much longer (BL), wider (W), with the kinetoplast closer to the nucleus (KI = >3), and often with polychromatic inclusions that are food reserves. Such stages have been seen in spring/summer-collected mule deer (Matthews et al., 1977), white-tailed deer (Kingston and Crum, 1977), and reindeer

Table 2. Examination of the "fit" of trypanosomes from moose with the standard population of deer trypanosomes.

	Standard population*			No. cases excluded/ % cases included† (%)	No. moose excluded/ % cases included†/% excluded moose‡ (%)
	−2 SD	$\bar{x}$	+2 SD		
PK§	0.45	10.17	19.90	15/91	11/94/26
KN	2.49	6.92	11.36	11/94	1/99/2
PN	5.71	17.08	28.44	18/90	11/94/26
NA	8.88	21.52	34.15	17/90	12/93/29
BL	16.97	38.75	60.53	15/91	12/93/29
FF	1.05	7.73	14.41	8/95	5/97/2
L	22.31	46.42	70.54	14/92	10/94/24
W	0.91	5.08	9.26	12/93	9/95/21
FF:BL	−1.86	6.09	14.04	9/94	2/99/5
NI	0.38	0.81	1.25	10/94	2/99/5
KI	1.05	2.59	4.89	11/94	3/98/7

* Standard population of 132 cervid trypanosomes measured from elk, mule deer, white-tailed deer, and reindeer (moose not included) showing distribution of 95% of the population of trypanosomes for the value measured.

† $N = 174$, moose included.

‡ $N = 42$.

§ Abbreviations: PK = posterior end to kinetoplast, KN = kinetoplast to nucleus, PN = posterior end to nucleus, NA = nucleus to anterior end, BL = body length, FF = free flagellum, L = length, W = width, FF:BL = free flagellum to body length ratio, NI = PN/NA, KI = PN/KN.

in part (Kingston et al., 1982). In these latter three host species (Kingston and Crum, 1977; Matthews et al., 1977; Kingston et al., 1982; Davidson et al., 1983) dividing epimastigotes also have been recovered, which indicate that multiplication of these parasites is enhanced during the warmer months of the year. When FF and indices of certain values of trypanosomes (FF:BL, NI = PN/NA, and KI = PN/KN) from the five host species are compared there is a general concurrence in these indices for trypanosomes from Wyoming moose, Alaska moose, and the other deer. The FF:BL is lower (3.75) in trypanosomes from Wyoming moose than expected for cervid trypanosomes (where it is usually greater than 4). This finding may be a further reflection of the small sample size, and this value is grouped (statistically) with FF:BL indices of trypanosomes from Alaska moose, reindeer, white-tailed deer, and mule deer though not with that index for trypanosomes from elk.

Moreover, conspecificity of trypanosomes from Wyoming moose and those from Alaska moose may be inferred from the occurrence of these trypanosomes in the same host species though from different subspecies: *Alces alces shiras* and *A. a. gigas* from widely separated geographical regions; also, *Trypanosoma cervi* exists in both geographical regions, in reindeer in Alaska, and in mule deer and elk in Wyoming.

Mayer et al. (1953) in discussing the problem of subspecific determination, suggest the application of the "75% rule" where "A population A is subspecifically distinct if 75% of its individuals differ from a "standard population" . . . ." Although the 75% rule cannot be strictly applied in an analysis of moose trypanosomes ($N = 42$) and trypanosomes from other North American deer ($N = 132$), some interesting relationships are found when these two subgroups of trypanosomes are examined.

In Table 2, the "standard population" of *Trypanosoma cervi* consists of the 132 specimens derived from the blood of elk, mule deer, white-tailed deer, and reindeer. The mean value is given along with the value for 2 SD on either side of the mean. Then the number of cases excluded from the total population ($N = 174$) is presented and the number of these cases that comprise moose is presented. Certain of these values may exceed 25% of moose (x/42) parameters but all of these are functions of body length merely indicating that the sample is made up of mostly large individuals. What is significant in this analysis is that for the indices compared (FF:BL, NI, KI) only 5–7% of moose are excluded. Put another way this says that 93–95% of moose trypanosomes examined by these index values are included in the standard deer population of trypanosomes.

Acknowledgments

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Isospora masoni sp. n. (Apicomplexa: Eimeriidae) from the Cotton Rat, *Sigmodon hispidus*

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ABSTRACT: *Isospora masoni* sp. n. is described from the cotton rat, *Sigmodon hispidus*, from Alabama. Infections of *I. masoni* were transmitted to coccidia-free cotton rats by oral inoculation of sporocysts that were sporulated at the time they were passed in the feces or by oral inoculation of mucosal scrapings from infected rats. Unsporulated and sporulated oocysts were found in enterocytes in the jejunum and ileum of experimentally infected cotton rats. The prepatent period varied from 4 to 7 days; the patent period was extended and was at least 40 days. The oocyst wall was thin and usually ruptured releasing free sporocysts in the feces. Sporocysts from the feces were ovoid, 8.1 × 5.4 μm, with a thin, colorless wall. Stieda and substieda bodies were present. Sporozoites were 7.0 × 1.8 μm and contained anterior and posterior refractile bodies.

In June 1982, cotton rats *Sigmodon hispidus* were collected alive (in Sherman traps) from Lee County, Alabama as part of a study on the prevalence of coccidia in cotton rats. Fecal flotations from one of the cotton rats revealed *Isospora*-like sporocysts. In the present study we report experimental transmission of the coccidium to cotton rats and describe the structure of the oocysts and sporocysts of *Isospora masoni* sp. n. from the cotton rat.

Materials and Methods

Fully sporulated sporocysts passed in the feces of wild-trapped or experimentally infected cotton rats were concentrated by flotation using Sheather's sugar solution (sp. gr. 1.18), washed free of sugar by centrifugation and stored in a 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) solution at 4°C until used. Prior to oral inoculation by stomach tube into coccidia-free cotton rats, sporocysts were washed three times in water to remove the potassium dichromate. Approximately 100,000 sporocysts, as calculated by a hemacytometer, were used to inoculate each rat.

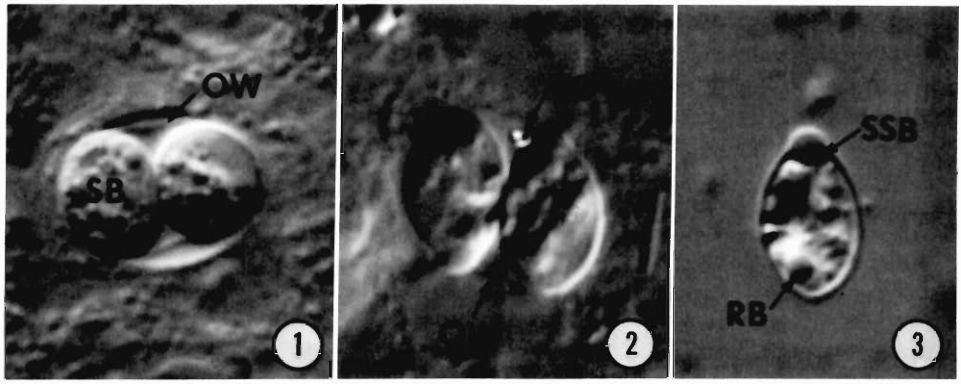
Four groups of 4–6-wk-old, coccidia-free cotton rats were used for transmission studies. The sex and weight of the rats were not determined. All cotton rats were housed individually in wire-bottom cages and were fed commercial rodent chow and water ad libitum. Feces were collected in pans containing paper towels moistened with 2.5% $K_2Cr_2O_7$ solution. Feces were examined by coverslip flotation using Sheather's sugar solution and Nomarski interference contrast (NIC) microscopy.

Group I consisted of 10 cotton rats; eight were inoculated with sporocysts obtained from a single, wild-trapped cotton rat and two served as uninoculated controls. Feces obtained daily on days 1–40 postinoculation (PI) and on day 70 PI were examined by NIC microscopy for the presence of oocysts or sporocysts.

Group II consisted of six cotton rats; four were inoculated with sporocysts collected from feces passed on day 10 PI from experimentally infected animals from group I, and two served as uninoculated controls. Feces on days 1–20 PI were obtained and examined daily for the presence of oocysts or sporocysts.

Group III consisted of 10 cotton rats; eight were inoculated with sporocysts collected from the feces on day 10 PI from group II and two served as uninoculated controls. Individual cotton rats were killed on days 2 and 8 PI and mucosal scrapings of the duodenum, jejunum, ileum, and squash preparations of mesenteric lymph nodes were examined by NIC microscopy for parasites. Additional portions of the above tissues were placed in 10% neutral buffered formalin, processed for routine histologic sectioning in paraffin, and stained with H&E. All remaining cotton rats in group III were killed on day 10 PI and mucosal smears were made from the duodenum, jejunum, ileum, cecum, and colon and examined with NIC microscopy. Additional mucosal scrapings obtained from the jejunum and ileum of these rats were placed individually in phosphate buffered saline (PBS) and used for the following portion of the experiment.

Group IV consisted of six cotton rats; four were inoculated with mucosal scrapings obtained from the ileum of the four infected animals killed on day 10 PI in group III, and two were inoculated with mucosal scrapings from the two uninoculated control cotton rats in group III. Before inoculation, portions of the jejunum and ileum were opened lengthwise and the mucosal surface was rinsed vigorously with PBS to remove



Figures 1–3. Nomarski interference contrast photomicrographs of *Isospora masoni* sp. n., 10 days PI, ×2,200. 1. Unsporulated oocyst in the sporoblast stage from mucosal scraping. 2. Sporulated oocyst from mucosal scraping. 3. Sporocyst from fecal flotation. Abbreviations: OW, oocyst wall; PG, polar granule; RB, refractile body; SB, sporoblast; SSB, substieda body.

extracellular parasites. Mucosal scrapings were incubated in 2.5% $K_2Cr_2O_7$ at 4°C for 2 hr to kill all stages except sporozoites within sporocysts and then washed by centrifugation in deionized water prior to oral inoculation. Feces obtained from cotton rats in group IV on days 1–10 PI were examined daily for the presence of oocysts or sporocysts.

Thirty sporulated sporocysts in fecal flotations were examined and measured with a NIC microscope equipped with a calibrated ocular micrometer. Measurements are in micrometers (μm) with the mean followed by the range in parentheses.

Results

All eight cotton rats in group I, inoculated with *I. masoni* sporocysts obtained from the live-trapped animal, passed sporulated sporocysts in their feces beginning on days 5–7 PI. Seldom were intact oocysts observed in fecal flotations. Sporocysts were still present 40 days PI, but not on day 70 PI. Sporocysts or oocysts were not found in the feces of control cotton rats.

The four cotton rats in group II, inoculated with *I. masoni* sporocysts obtained from animals in group I, began passing sporulated sporocysts on days 5–6 PI. Sporocysts were still present 20 days PI, the last day of fecal examination. Oocysts or sporocysts were not found in the feces of control rats.

Cotton rats in group III, inoculated with *I. masoni* sporocysts obtained from infected animals in group II, began passing sporulated sporocysts 4–6 days PI. Mucosal smears of the jejunum and ileum obtained 10 days PI had unsporulated and sporulated oocysts within villus enterocytes (Figs. 1, 2). Infections were heaviest in the ileum; no parasite stages were observed in the duodenum, cecum or colon. The oocyst wall was thin and membrane-like, and a highly refractile polar granule was present within the oocysts (Fig. 2). An oocyst residuum was not present. Parasite stages were not observed in intestinal tissues obtained from control animals.

Endogenous stages were not seen in the cotton rat killed on day 2 PI. However, examination of histologic sections of the cotton rat killed on day 8 PI revealed meronts, gamonts, and oocysts in the distal one-half of the villus epithelium of the jejunum and ileum. Parasite stages were not observed in the lamina propria or in other sites examined.

The four cotton rats in group IV, inoculated with mucosal scrapings from infected cotton rats in group III, passed sporocysts in their feces beginning 4–6 days PI. The 2 control cotton rats, inoculated with mucosal scraping from uninfected animals in group III, did not pass sporocysts or oocysts.

Description of Sporocysts

Isospora masoni sp. n. (Figs. 1–4)

Oocysts usually found in enterocytes of the jejunum or ileum. Sporocysts from the feces are ovoid, 8.1 × 5.4 (7.2–9.0 × 4.8–5.6), with a thin, colorless wall, ~0.3 thick. Shape index 1.5 (1.3–1.8). Stieda and substieda bodies present. Stieda body 1.7 wide (1.2–2.0), flattened distal to substieda body, consisting of a bottle-neck extension of the sporocyst wall. Substieda body homogeneous, 1.0 × 1.8 (0.9–1.0 × 1.4–2.0). Sporocyst

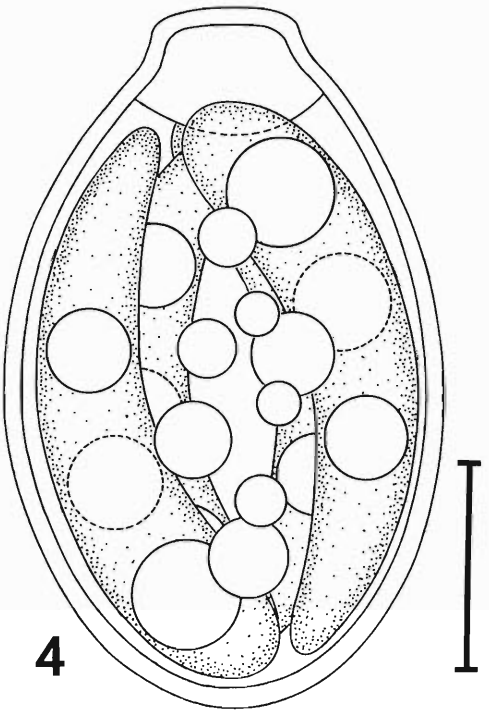


Figure 4. Composite line drawing of sporocyst of *Isospora masoni* sp. n. Scale bar = 2 μm.

residuum present, consisting of 3–12 scattered granules 0.5–1.8 in diameter. Sporozoites elongate, 7.0 × 1.8 (6.3–8.1 × 1.5–2.0) in situ, lying lengthwise and parallel within the sporocyst. Two sporozoites lie in one direction, whereas the other two are oriented in the opposite direction. Anterior and posterior refractile bodies present. Anterior refractile body spherical, 1.0 (0.8–1.2), located slightly anterior to the midpoint of the sporozoite. Posterior refractile body spherical or ovoid, 1.4 × 1.5 (0.9–1.8 × 1.0–2.0). The nucleus is located between the refractile bodies.

TYPE HOST: *Sigmodon hispidus* (cotton rat), Lee County, Alabama.

SPORULATION: Endogenous.

LOCATION IN HOST: Endogenous stages in villus epithelium of jejunum and ileum.

PREPATENT PERIOD: Four to seven days.

ETYMOLOGY: This species is named in honor of Dr. William H. Mason, Coordinator, General Biology, Auburn University, Alabama.

TYPE SPECIMENS: USNM Helm. Coll. No. 78130. Sporocysts deposited at the National Parasite Collection, USDA, Beltsville, Maryland.

REMARKS: Only one other *Isospora* sp. has been described from *Sigmodon hispidus*. Barnard et al. (1974) found oocysts of an isosporan in the feces of 47 of 193 (24.4%) cotton rats in Barbour and Macon counties, Alabama. Sporocysts of this *Isospora* sp., however, are larger and always enclosed within a thick, brownish oocyst wall. Additionally, the sporocyst residuum is larger than in *I. masoni*. Barnard et al. (1974) were unable to infect cotton rats with the *Isospora* sp. they described.

Discussion

Isospora masoni infections can be transmitted to cotton rats both by sporocysts obtained from fresh feces of infected rats and by sporulated oocysts within villus enterocytes. Therefore, the life cycle is direct and sporogony outside the host is not required. The unusually long patent period of *I. masoni* (>40 days) is not unique among rodent coccidia. Long patent periods have been reported for *Eimeria ontarioensis* Lee and Dorney, 1971 (>30 days), *E. scholtysecki* Ernst, Frydendall, and Hammond, 1967 (>30 days), *E. utahensis* Ernst, Hammond, and Chobotar, 1968 (>70 days), *E. vermiformis* Ernst, Chobotar, and Hammond, 1971 (17–25 days), and *E. zapi* Gerard, Chobotar, and Ernst, 1977 (~22 days). No satisfactory explanations of these unusually long patent periods have been provided; however, they may be due to ingestion of feces containing infective sporocysts, reinfection by sporozoites that excyst from newly formed sporocysts immediately after sporocysts are discharged into the intestinal lumen, recycling of one or more asexual generations, or because some oocysts are somehow retained in tissues longer than for most known species of intestinal coccidia.

Isospora masoni resembles *Cryptosporidium, Frenkelia,* and *Sarcocystis* because it sporulates endogenously. A thin oocyst wall, which ruptures easily releasing free sporocysts in the feces, is also a feature common to the Sarcocystinae. Although endogenous sporulation is uncommon for mammalian Eimeriidae, *E. neitzi* has been shown to sporulate in the uterus of the impala (McCully et al., 1970). However, because sporocysts of *I. masoni* have stieda and substieda bodies, and because this species has a direct life cycle, it is properly placed in the genus *Isospora.*

Acknowledgment

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Factors Contributing to Clinical Illness in Calves Experimentally Infected with a Bovine Isolate of *Cryptosporidium*

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ABSTRACT: In seven experiments, 23 neonatal calves were used to determine some of the conditions under which infection with a bovine isolate of *Cryptosporidium* might produce clinical illness. *Cryptosporidium* oocysts were stored in potassium dichromate, or water, or were antibiotic-treated; dosage levels from 3.2×10^6 to 30×10^6 oocysts were compared in colostrum-fed (CF) versus colostrum-deprived (CD) calves; interaction with virus, pathogenic bacteria, and bacterial toxin were ascertained. In Exps. 1–3 great individual variation and inconsistency were observed among calves experimentally infected with oocysts stored in 2.5% potassium dichromate solution: some calves infected with 3.2×10^6 oocysts died, whereas none infected with 30×10^6 oocysts died, responses to infection in CF and CD calves ranged from none to severe diarrhea, none to numerous oocysts shed, none to moderate fever, and complete recovery to death. Differences in the response of CD versus CF calves were not discernable. In Exp. 4 a CD calf infected with fresh untreated oocysts developed diarrhea, shed large numbers of oocysts, and died; although viruses were not found, *Clostridium perfringens,* which may have contributed to the clinical illness, was isolated from intestinal contents. The same contents, containing numerous oocysts of *Cryptosporidium,* were used as a source of inoculum for calves in Exps. 5–7. In Exp. 5 all four CD calves died after two were fed the centrifuged pellet containing oocysts and two were fed the supernatant in which no oocysts were found. Again, *C. perfringens* was isolated from intestinal contents of three calves; rotavirus antigen was detected in one of these three. In Exp. 6, a repeat of Exp. 5 with CF calves, one calf fed oocysts developed severe diarrhea, shed oocysts, and died; an excessive number of yeasts, but no other pathogens, were found in its intestinal contents at postmortem. The three surviving calves were not seriously affected. In Exp. 7, in which oocysts were cleaned of most fecal debris and were antibiotic-treated before infecting two CF and two CD calves, none of the calves became seriously ill although rotavirus and small numbers of *C. perfringens* were found in all of them.

The lack of consistent production of clinical illness related to oocyst dosage and the finding of rotavirus and/or *C. perfringens* in calves with clinical cryptosporidiosis as well as the marked reduction in severity of illness when oocysts were cleaned and exposed to antibiotic suggests that the isolate of *Cryptosporidium* used in this study interacted with other enteropathogens to produce clinical illness. Whether other isolates of *Cryptosporidium* might be more virulent remains to be determined.

The protozoan parasite *Cryptosporidium* has been associated with diarrhea in neonatal calves in Australia, Canada, Czechoslovakia, The Federal Republic of Germany, Israel, the Republic of South Africa, the United Kingdom, and the United States (Barker and Carbonell, 1974; Morin et al., 1976; Pavlasek et al., 1983; Heine and Boch, 1981; Nobel et al., 1982; Howerth, 1981; Snodgrass et al., 1980; Panciera et al., 1971).In experimental studies in which calves were artificially infected with *Cryptosporidium* it was concluded that cryptosporidia are pathogenic as evidenced by finding oocysts in diarrhetic calves but not in nondiarrhetic calves, by the absence of other known enteric pathogens in some calves, and by morphologic lesions associated with the organism (reviewed by Anderson, 1982).

However, upon careful examination the cause-effect relationship of the parasite to the clinical illness does not appear to be firmly established. Surveys of neonatal calves in Germany and the United States reported concurrent diarrhea in less than half the calves that were shedding oocysts of *Cryptosporidium* (Jungmann and Hiepe, 1983; Leek and Fayer, 1984). In surveys as well as experimental studies assessments of the viral and bacterial populations of affected calves are incomplete. Furthermore, because the minute size of oocysts renders them difficult to identify microscopically there either has been no attempt to quantify the number of oocysts used in inocula for studies on experimentally induced cryptosporidiosis (Tzipori et al., 1981a, b, 1982) or quantities have been extrapolated indirectly from counts of stained smears (Tzipori et al., 1983). Because information is lacking on both the minimum number of oocysts required to consistently produce diarrhea and the actual number admin-

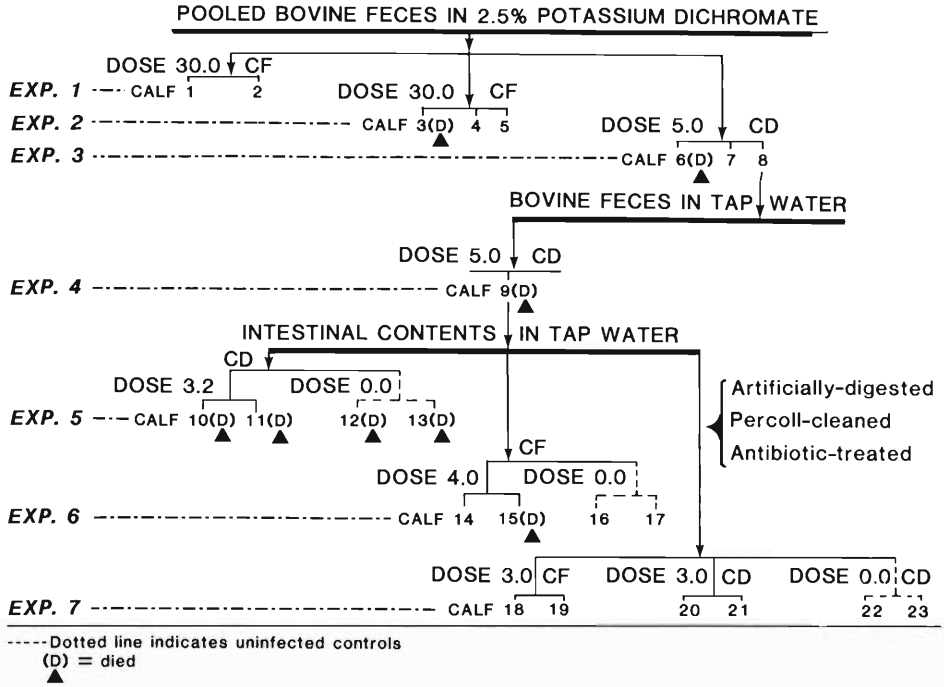

Figure 1. Experimental design for Exps. 1–7: the sources of oocysts, their treatment, the infective dose ($\times 10^6$), and identification of colostrum-fed (CF) and colostrum-deprived (CD) calves.

istered per animal the interpretation of results from these experiments must be questioned. The present study was designed to determine the conditions under which a bovine isolate of *Cryptosporidium* might exhibit pathogenicity and produce diarrhea in neonatal calves.

Materials and Methods

Experimental animals

Twenty-three Holstein-Friesian male calves were obtained within 24 hr of birth. Eleven calves were allowed to suckle their dams to obtain colostrum, 12 were colostrum deprived. All were housed in individual pens with cement walls and floors in isolated barns. Wood shavings, used for bedding, were removed daily and replaced with clean shavings. Milk replacer was fed in nippled bottles twice daily. Water was continuously available from individual automatic waterers. Calves were 1–3 days of age when experimentally infected.

Parasites

Based on recent cross transmission studies of *Cryptosporidium* among numerous host species there appears to be a single species of *Cryptosporidium* that lacks host specificity. Because the first named species in this genus was *Cryptosporidium muris* Tyzzer, 1907 we assume that *C. muris* is the species of the bovine isolate used in the present study. The source, treatment, and number of oocysts used to infect each calf are presented in Figure 1. Oocysts of *Cryptosporidium* in pooled bovine feces from naturally infected calves were mixed with tap water containing 2.5% potassium dichromate and stored at 5°C for less than 3 mo when used in Exps. 1–3. This method of storage has historically been used for intestinal coccidia of mammals and birds (Hammond and Davis, 1944). Oocysts in feces from calf 8 were mixed with tap water and stored at 5°C for less than 2 wk when used in Exp. 4. Oocysts in intestinal contents from calf 9 were mixed with tap water and stored at 5°C for less than 6 mo when used in Exps. 5–7. For Exp. 7, oocysts were cleaned from intestinal debris by artificial digestion in a mixture of pepsin-HCl (500 ml H_2O, 7 ml conc. HCl, 2.6 g pepsin 1–10,000) at 37°C with constant stirring for 30 min. The mixture was centrifuged at 1,100 *g* for 10 min, the supernatant decanted, and the pellet resuspended in tap water. Aqueous oocyst suspensions were mixed with Percoll to obtain a 45% (v/v) Percoll suspension that was centrifuged at 1,100 *g* for 10 min. Supernatant contained relatively clean oocysts that were incubated at 37°C for 1 hr in sterile distilled water containing 100 μg/ml mitomycin C. These were centrifuged at 1,100 *g* for 10 min, the pellet resuspended in sterile distilled water and stored at 5°C until used. Before infecting calves in Exp. 7, these oocysts were subjected to in vitro excystation as described by Fayer and Leek (1984) to confirm their viability. After 45 min in excysting fluid at 37°C, 70% excystation was obtained and sporozoites appeared normal.

To determine the number of oocysts in aqueous sus-

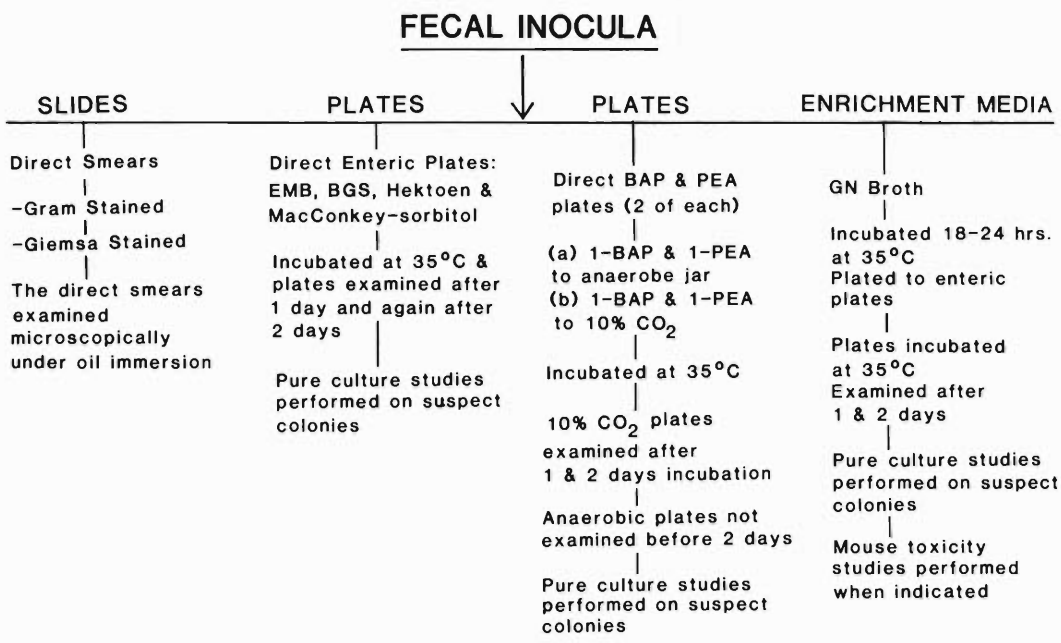

Figure 2. Procedures used to detect pathogenic bacteria and toxin in Exps. 4–7.

pensions used to infect each calf duplicate or triplicate counts were made with the aid of a thin hemacytometer and phase-contrast microscopy. Calves were infected with the aqueous suspension of oocysts via a nippled bottle.

Experimental design

The experimental design for Exps. 1–7 is outlined in Figure 1. In all experiments, feces were collected daily and examined by phase-contrast microscopy after sugar flotation-concentration. In Exps. 2, 3, 4, 5, and 7 body temperature was recorded once or twice daily. Selected calves were necropsied and portions of small and large intestine were fixed in 10% neutral buffered formalin for histologic examination. Three experiments were conducted to determine the susceptibility of colostrum-fed (CF) versus colostrum-deprived (CD) calves to infection with oocysts of *Cryptosporidium*. In Exps. 1, 2, and 3, oocysts rinsed of the 2.5% potassium dichromate storage solution were used to infect 2 CF calves, 3 CF calves, and 3 CD calves at the rates of 30×10^6, 30×10^6, and 5.0×10^6 oocysts per calf, respectively.

In Exp. 4, CD calf 9 was infected with 5×10^6 oocysts recently obtained from the feces of calf 8. These had not been exposed to antibacterial or antifungal agents. Calf 9 was necropsied, tissues taken for histology, and intestinal contents examined for pathogenic viruses, bacteria, and toxins.

Three experiments were conducted to determine the susceptibility of CF and CD calves to infection with oocysts recently obtained from the intestinal contents of calf 9. These also has not been exposed to antibacterial or antifungal agents. In Exps. 5 and 6 intestinal contents mixed with tap water were centrifuged at 1,100 *g* for 10 min to obtain a pellet of oocysts and a supernate free of oocysts. In Exp. 5 the pellet of oocysts was used to infect CD calves 10 and 11 with 3.2×10^6 oocysts each, and the supernate that contained no apparent oocysts was fed to CD control calves 12 and 13 (Fig. 1). All calves were necropsied and tissues from calves 11 and 13 were taken for histology. Feces obtained from calves 10, 11, and 13 on the day they died were examined for pathogenic viruses, bacteria, and toxins.

In Exp. 6, the pellet containing oocysts was used to infect CD calves 14 and 15 with 4.0×10^6 oocysts each and the supernate that contained no apparent oocysts was fed to CF control calves 16 and 17 (Fig. 1). Calf 15, which died, was necropsied, tissues were taken for histology, and intestinal contents were collected for virology and bacteriology. In Exp. 7, oocysts that remained after the artificial digestion, cleaning, and antibiotic exposure of intestinal contents were used to infect CF calves 18 and 19 and CD calves 20 and 21 at the rate of 3.0×10^6 oocysts each; CD calves 22 and 23 remained as uninfected controls (Fig. 1). At 6 and 14 days after inoculation, feces were collected from each calf and examined for pathogenic viruses, bacteria, and toxins. The cleaned, antibiotic-treated inoculum was also subjected to complete examination for pathogenic bacteria as outlined in Figure 2.

Tests for pathogenic viruses were conducted by the Maryland State Health Laboratory, College Park, Maryland, for Exps. 4, 5, and 6 and by the Athens Diagnostic Laboratory, Athens, Georgia, for Exp. 7. These tests included attempted isolation and identification in cell cultures (Exps. 4, 5, and 6) and identification of rotavirus antigen by the Rotazyme test (Exps. 4, 5, 6, and 7). Tests for pathogenic bacteria as outlined

Table 1. Clinical signs of experimental cryptosporidiosis in calves.

Exp. no.	Calf no.	Colostrum fed	No. of oocysts inoc. per calf ($\times 10^6$)	Days of diarrhea/days of exp.	Avg. no. of oocysts ($\times 10^6$)*/no. days shed	No. of days fever	Day calf died
1	1	+	30	6/12	0.92/5	ND†	
	2	+	30	9/12	0.11/3	ND	
2	3	+	5	6/10	0/0	0	10
	4	+	5	0/14	0.11/2	1	
	5	+	5	2/14	0/0	3	
3	6	−	5	5/8	0.16/6	2	8
	7	−	5	4/13	0.22/5	0	
	8	−	5	9/13	1.28/4	0	
4	9	−	5	4/5	1.15/3	0	5
5	10	−	3.2	3/6	0.65/3	2	6
	11	−	3.2	3/8	0.59/3	6	8
	12	−	0	NS‡	ND	0	1
	13	−	0	NS	ND	1	2
6	14	+	4	3/14	0/0	ND	
	15	+	4	5/8	0.94/5	ND	8
	16	+	0	2/14	0.20/2	ND	
	17	+	0	5/14	0.39/4	ND	
7	18	+	5	0/21	0.02/2	2	
	19	+	5	3/21	0.08/5	11	
	20	−	5	2/21	0.16/6	6	
	21	−	5	3/21	0.17/6	5	
	22	−	0	0/21	0.13/5	4	
	23	−	0	0/21	0.13/5	5	

* Number of oocysts per ml of feces determined each day and then divided by the number of days on which they were shed.
† ND = No data.
‡ NS = No samples obtained.

by flow chart in Figure 2 included Giemsa and Gram-stained direct smears (which were examined microscopically to differentiate bacteria by cellular morphology and spore formation), direct plating on two BAP (blood agar plates, 5% sheep blood in Columbia Agar base; Grand Island Biological Company), two PEA plates (5% sheep blood in phenol ethyl alcohol agar base, DIFCO Laboratories), one plate each of the following enteric plates: EMB (Levine's eosin-methylene blue agar; GIBCO), Hektoen enteric agar (GIBCO), BGS (brilliant green sulfa agar; Baltimore Biological Laboratories), and MacConkey agar base with 1% D-sorbitol (DIFCO Laboratories); and inoculated into GN broth (Gram-negative broth, Hajna formula; DIFCO Laboratories). All media were incubated at 35°C. One BAP and PEA set was incubated anaerobically for not less than 2 days. Colonial morphology and hemolysis on BAP were used to differentiate *C. perfringens* from other clostridia (Koneman et al., 1983). Another BAP and PEA set was incubated under 10% CO_2 and examined at 1 and 2 days. The enteric plates were incubated under aerobic conditions and examined at 1 and 2 days. The GN broth was plated after 1 day on enteric plates that were incubated and examined as described above. *Escherichia coli* was tested for its ability to utilize sorbital as an indicator of pathogenicity. To indicate the presence of *C. perfringens* toxin, two to four mice were injected intraperitoneally with supernate fluid from tripsinized and nontrypsinized fecal matter or intestinal contents. Other mice were inoculated as appropriate with supernate from suspect isolated colonies grown in anaerobic cooked meat broth.

Results

Experiments 1–3

With the exception of calf 2, the duration and severity of diarrhea, the duration and number of oocysts shed, the duration and severity of fever, and the number of deaths were similar between colostrum-fed (CF) and colostrum-deprived (CD) calves after infection with oocysts of *Cryptosporidium* (Table 1). Calf 2 had mild diarrhea for 3 days and moderate to severe diarrhea for 6 days. All other calves, except those that died (calves 3 and 6), had relatively mild transient clinical signs, appeared healthy, and were not shedding oocysts at the end of each experiment.

Both calves that died had developed a very liquid diarrhea, and feces from the CD calf also contained blood for 2 days.

Experiment 4

The CD-inoculated calf 9 had a very liquid diarrhea for 3 days (1 day with blood) and died 5 days after inoculation (DAI). Postmortem examination revealed that the small and large intestine were filled with liquid feces and the mucosal surface of the lower jejunum and upper ileum were reddened. No other gross lesions were observed. Microscopic examination of histologic sections of duodenum, jejunum, ileum, and cecum revealed the loss of epithelial cells in all sections. Virologic examination was negative. Moderate numbers of *C. perfringens* were isolated and the isolate produced a toxin that was lethal for mice.

Experiment 5

All calves died (Table 1). Calves 10 and 11 given pelleted oocysts had only mild or moderate diarrhea for a short duration. Calf 10 became moribund with a temperature of 40.4°C and was euthanatized 6 DAI. Calf 11, after 6 consecutive days with body temperatures of 40.0 and 40.9°C, died 8 DAI. Except for dehydration no other lesions were seen at necropsy. Histologic sections of the small intestine, cecum, and colon of calf 11 revealed the loss of all villous epithelium. Virologic examination was negative for both calves. However, moderate numbers of murine toxigenic *C. perfringens* were isolated from the intestinal contents of calf 11.

Control calves 12 and 13 were found dead in the morning 1 and 2 days, respectively, after ingesting the aqueous supernate in which *Cryptosporidium* oocysts were not observed. No lesions were seen at necropsy. Microscopic examination of histologic sections from calf 13 revealed unidentified fungi in lung and kidney and Gram-negative rods in kidney. Rotavirus antigen was detected and many murine toxigenic *C. perfringens* were isolated from the intestinal contents of calf 13.

Experiment 6

One of the two CF calves (15) given an aqueous suspension of oocysts died 8 DAI. The other calf (14) and the two control calves (16 and 17) given supernate without visible oocysts developed only a mild diarrhea (Table 1).

Calf 15 had a moderate to severe diarrhea that lasted for 3 days and contained blood for 1 day. No lesions were seen at necropsy. Although oocysts were shed for 5 consecutive days before death, histologic specimens of intestine revealed intact epithelium without parasites. Rotavirus antigen was detected. Although neither pathogenic bacteria nor toxins were found in intestinal contents, the intestinal flora population appeared abnormal. Most notable was the complete absence of Gram-negative rods and the presence of apparently excessive numbers of yeasts.

Oocysts were not seen in feces of the other inoculated calf (14). In contrast, control calves 16 and 17 began shedding oocysts 4 and 5 days after ingesting the aqueous supernate.

Experiment 7

None of the four calves (18–21) given clean antibiotic-treated oocysts became severely ill and the two uninfected control calves (22, 23) remained clinically normal.

Diarrhea lasting 2 or 3 days was observed in three of the four inoculated calves although all four calves shed oocysts. Feces from CF calf 19 and CD calf 20 were liquid and both had elevated temperatures of 39.6–40.5°C for 11 and 6 days duration, respectively. Feces from CD calf 21 were less markedly diarrhetic and this calf had an elevated temperature of 39.6–40.1°C for 5 days. Of the eight fecal specimens subjected to the Rotazyme test at 6 and 14 DAI all were positive at levels of 1+ or 2+. Examination of the same specimens for pathogenic bacteria revealed that five specimens contained moderate numbers of *C. perfringens*. These were isolated only from calves 19, 20, and 21 (the only calves with diarrhea) and each of these isolates produced a toxin that was lethal for mice.

Uninfected control calves 22 and 23 had no diarrhea although both calves shed oocysts. Of the four fecal specimens subjected to the Rotazyme test when the calves reached 6 and 14 days of age, those for calf 22 reached levels of 1+ and 3+, whereas those for calf 23 reached levels of 1+ and ±. Pathogenic bacteria were not found on any direct smears from fecal specimens but a small number of *C. perfringens* was isolated on direct plates from calf 23 at 6 days. Because so few colonies were found, toxicity studies on mice were not conducted. The inoculum itself, after cleaning and antibiotic treatment still contained a small number of *C. perfringens*.

Discussion

Experiments 1, 2, and 3 had great within-group variation rendering any differences between doses of 5×10^6 vs. 30×10^6 or of CF vs. CD indiscernable. Responses within treatments ranged from none to severe diarrhea, none to numerous oocysts shed, none to moderate fever, and complete recovery to death. Because the inoculum had been stored in 2.5% aqueous potassium dichromate at 5°C and the effects of this storage medium on the viability of oocysts of *Cryptosporidium* were unknown, the subsequent four experiments were designed using oocysts stored in water.

Experiment 4 utilized CD calf 9 inoculated with an aqueous suspension of oocysts from the feces of CD calf 8 in Exp. 3. Calf 9 developed a severe diarrhea, shed large numbers of oocysts, and died. This was the first calf in this study examined for pathogenic viruses and bacteria. Moderate numbers of *C. perfringens* were found that produced a toxin lethal for mice. The finding of murine toxigenic *C. perfringens* in this and other calves in this study must be interpreted carefully. This organism, which is more widespread than any other pathogenic bacterium, is part of the flora commonly found in the intestine of neonatal calves and most strains are toxigenic for mice (Smith and Holdeman, 1968). Although this species is divided into five types, A to E, based on production of major lethal toxins, we did not attempt to differentiate types in this study. The type(s) and possible interaction with *Cryptosporidium* as an enteropathogen in calves should be thoroughly evaluated.

The liquid intestinal contents from calf 9, containing *Cryptosporidium* oocysts as well as a variety of fungi and bacteria including *C. perfringens,* were used as inoculum for Exps. 5, 6, and 7. Experiments 5 and 6 were designed to test the effects of this inoculum on CD (Exp. 5) and CF (Exp. 6) calves. In each of these experiments the inoculum was centrifuged to concentrate the oocysts in the pellet while removing them from the supernate, thereby providing for control purposes any pathogens that might be present in the inoculum minus *Cryptosporidium.* Because all four CD calves in Exp. 5 died, and those receiving the supernate died as early as 24 and 48 hr after infection, and because *C. perfringens* as well as rotavirus antigen were detected in feces from these calves, the contribution of these agents to the clinical findings in these calves cannot be overlooked. Although a similar pellet and supernate inoculum from the same stock source was given to CF calves in Exp. 6, these calves were apparently less susceptible to the clinical manifestations of infection. Only one of these calves died (calf 15). This calf received the pellet inoculum containing the oocysts. However, a few oocysts might have remained in the supernate because calves 16 and 17 that received only the supernate began to shed oocysts after a normal prepatent period had elapsed. The possible presence of such oocysts in the supernate would not explain the deaths of calves 12 and 13 in Exp. 5 because they died too early in the prepatent period for *Cryptosporidium* to be considered as a contributing factor.

The composite findings of Exps. 1–6 suggested that in addition to our isolate of *Cryptosporidium,* or perhaps exclusive of it, other pathogenic organisms or toxins contributed to the severity of the clinical illness in these experimental neonatal calves. Experiment 7 was designed to determine if the oocysts of *Cryptosporidium,* freed from as much fecal debris and other contaminants as possible, could produce clinical illness. Despite artificial digestion, Percoll cleaning, and antibiotic treatment, a few *C. perfringens* were detected in the inoculum used in this experiment. However, none of the calves became severely ill even though patent infections with *Cryptosporidium* and rotavirus antigen were detected in all calves. Diarrhea and fever were found in three of the inoculum-fed calves and *C. perfringens* was detected in their feces but not in the feces of one CF calf that remained completely free of clinical infection. Neither uninfected CD control calf had signs of illness although both must have become infected with *Cryptosporidium* because both shed oocysts. One of these calves had very low numbers of *C. perfringens* isolated from its feces. These findings add support to those of Exps. 1–6 that suggested that other pathogens or toxins might contribute to clinical illness in the presence of *Cryptosporidium.*

The question of whether *Cryptosporidium* is a pathogen in the absence of other infectious agents or toxins remains unanswered. The problem of testing this question remains unsolved. Even if gnotobiotic animals are utilized, the inoculum containing oocysts of *Cryptosporidium* must be rendered bacteria and virus free. Although Tzipori et al. (1982, 1983) attempted to conduct

experiments utilizing pigs and calves infected with an isolate of *Cryptosporidium* freed of other microorganisms, the validity of these studies must be placed in doubt. Their inoculum ". . . following bacterial sterility checks was believed to be free of other microorganisms" after exposure to 60% ethyl alcohol (Tzipori et al., 1982). The specific bacterial sterility tests were not described. In contrast, bovine feces containing *Cryptosporidium* oocysts stored in 70% ethyl alcohol at 5°C for 1 wk still contained many *C. perfringens* as determined by direct anaerobic blood plate cultures (Fayer and Miller, unpubl.).

In natural outbreaks of neonatal calf diarrhea, neither Morin et al. (1976) nor Pohlenz et al. (1978) were able to demonstrate pathogens accompanying *Cryptosporidium* in five calves and in one calf, respectively. However, no reports mention examination of specimens specifically for anaerobic bacteria.

A field survey of neonatal calves from dairy farms in Maryland indicates that *Cryptosporidium* is often present in healthy calves with no diarrhea as well as in diarrhetic calves (Leek and Fayer, 1984). Thus, *Cryptosporidium* exists commonly in nature in the absence of clinical signs.

As suggested by Pohlenz et al. (1978) there remains a need to conduct transmission studies (in gnotobiotic animals) with a *Cryptosporidium* inoculum from feces containing no other infectious agents (or toxins). Such studies must be thoroughly controlled to eliminate the possibility of any extraneous infectious agents. Specific agents such as *C. perfringens* isolates could be added to such an inoculum to determine possible interaction with *Cryptosporidium* in elucidating the cause of neonatal diarrhea. Attention must also be focused on the possibility that different isolates of *Cryptosporidium* could vary greatly in virulence.

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Host-Parasite Relationships of *Tanaorhamphus longirostris* (Acanthocephala: Neoechinorhynchidae) in the Intermediate Host, *Diaptomus pallidus* (Crustacea: Copepoda)

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ABSTRACT: *Tanaorhamphus longirostris* has been studied in relation to its intermediate host, the limnetic calanoid copepod, *Diaptomus pallidus*. The study area was Caesar Creek Lake located in southwestern Ohio. Approximately 12,800 adult copepods were examined from a June 27, 1981 collection. A total of 620 (4.8%) adult copepods was infected with *T. longirostris*. Prevalence decreased to 1.5% on July 18, 1981. No statistical difference in susceptibility to infection between sexes of adult copepods could be demonstrated. Based on 604 single infections, 400 (66.2%) of the cystacanths were directed anteriorly and 204 (33.8%) were found facing posteriorly within the copepod. A significant difference in cystacanth orientation from the expected 50:50 distribution was demonstrated ($\chi^2 = 63.6$, $P < 0.001$). No statistical difference was found between the mean length of infected and uninfected adult male or adult female copepods. The mean length of cystacanths in adult male and adult female copepods was 608 μm ± 51.9 and 693 μm ± 63.3, respectively. There was a significant difference in the mean length between cystacanths found in adult male and adult female copepods (*t*-test, $P < 0.001$).

Acanthocephalans parasitizing freshwater fishes are known to utilize crustaceans as intermediate hosts. Most life cycles known involve either amphipods, isopods, or ostracods, although a few species may develop in cyclopoid copepods. Hubschman (1983) demonstrated that the calanoid copepod *Diaptomus pallidus* Herrick, 1879 serves as an intermediate host for *Tanaorhamphus longirostris* (Van Cleave, 1913) Ward, 1918, a parasite of the gizzard shad *Dorosoma cepedianum* LeSueur, 1818. It appears from a literature survey that *Diaptomus pallidus* is a new intermediate host record for an acanthocephalan.

The present study was undertaken to investigate some aspects of the host–parasite relationships of larval *T. longirostris* in the copepod intermediate host, *D. pallidus*. The primary objectives were to determine (1) the prevalence and intensity of the parasite in the *D. pallidus* population; (2) differences in infection between male and female copepods; and (3) size of the parasite relative to its intermediate host.

Materials and Methods

The population of copepods studied was that of Caesar Creek Lake, Ohio (39°30′N, 84°05′W). The 1,145-hectare reservoir, located in Warren, Clinton, and Greene counties, was impounded in 1978 by damming Caesar Creek, a tributary of the Little Miami River. Lake depths range from <1 m to 33.5 m with an average depth of 11 m.

Qualitative samples of zooplankton were collected at 24 stations (depths ranged from 2.5 to 31 m) by taking two vertical tows through the water column using a Wisconsin-style plankton net with a 12-cm mouth opening and 80-μm netting. Sampling dates were June 27, July 18, July 25, August 8, August 18, August 28, September 19, September 26, and October 17, 1981. Zooplankters were washed with distilled water into sample bottles, then anesthetized with 12 ml carbonated water to reduce body distortions during fixation, and then were fixed in 4% formalin at the time of collection. Later in the laboratory, samples were serially dehydrated and preserved in 70% alcohol with 2% glycerin.

Specimens of zooplankton were placed into a glass culture dish and examined under a stereoscopic microscope. Acanthocephalan larvae could be seen through the relatively transparent copepod cuticle. Infected copepods were transferred to an embryological watch glass. Within 24 hr the alcohol preservative evaporated, leaving the copepods in glycerin concentrate. Glycerin jelly slide mounts of infected copepods were then made.

Body measurements (μm) of copepods (excluding the urosome) and cystacanths in situ were made using a compound microscope equipped with a calibrated ocular micrometer. Distinguishing the sex of cystacanths within the copepods was difficult with glycerin jelly mounts; therefore, a number of infected copepods was stained with Semichon's carmine. It was hoped that staining would enhance cystacanth internal morphology to examine the sex ratio of *T. longirostris* in an intermediate host; however, upon examination of the stained specimens it was evident that cystacanth sex could not be determined in all specimens.

Prevalence is the percentage of infected copepods in a given sample and intensity is the number of parasites per host. All measurements of copepods and cystacanths are given as μm ± SD unless indicated otherwise.

Two specimens of *Diaptomus pallidus* with cyst-

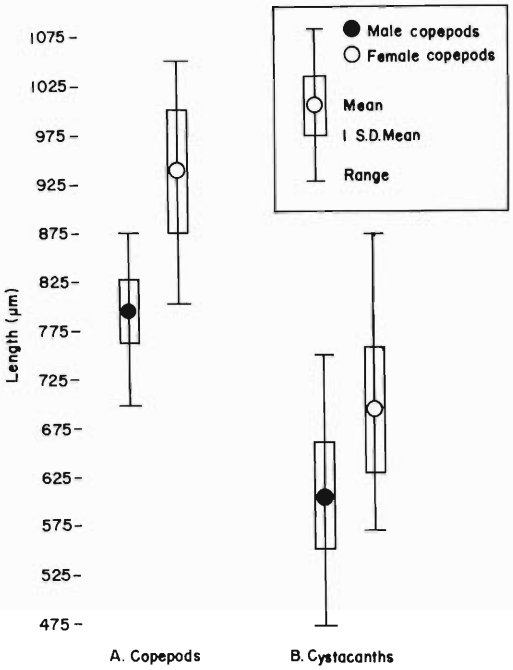


Figure 1. (A) Comparative data of copepod metasome lengths (µm) between parasitized *Diaptomus pallidus* adult males and females; (B) comparative data of body lengths (µm) of *Tanaorhamphus longirostris* within the hemocoels of adult male and female copepods.

acanths were deposited (by J.H.H.) in the USNM Helm. Coll. as No. 77544 (two slides).

Results

The distribution of copepods infected with *Tanaorhamphus longirostris* was irregular in both time and space. Based on the data, the calculated range of occurrence at the 24 stations sampled on June 27, 1981, was 0–435 infected copepods per cubic meter (mean = 125/m³). We could detect no pattern in the occurrence of infected copepods related either to depth or station location.

Of approximately 12,800 adult *Diaptomus pallidus* examined from the June 27, 1981 collection, 620 (4.8%) contained cystacanths of *T. longirostris*. Of those with cystacanths, 604 (97.4%) had single infections, 14 (2.3%) contained two cystacanths, and 2 (0.3%) had three cystacanths. The prevalence of *T. longirostris* in adult copepods decreased from 4.8% on June 27, 1981, to 1.5% (8,000 copepods examined) on July 18, 1981. Parasitized copepods were not observed in zooplankton samples following the July 18, 1981 collection with the exception of one infected copepod found in an October 17, 1981 sample.

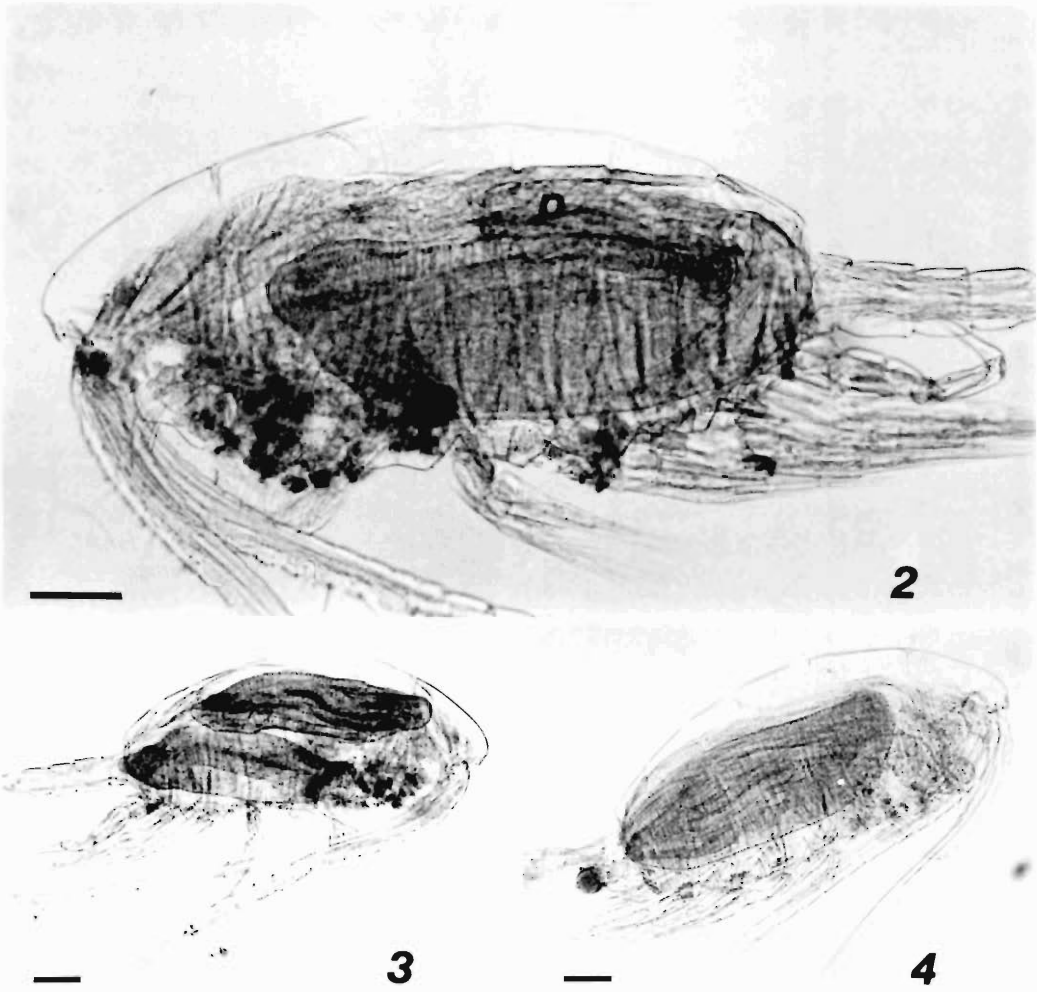
The sex distribution of infected *D. pallidus* was 318 (51.3%) females and 302 (48.7%) males. Mean lengths of parasitized female and male copepods were 937 ± 59.5 and 798.6 ± 33.5, respectively. A random selection of infected and uninfected adult male and adult female copepods was measured. There were no statistical differences in the mean lengths of infected and uninfected adult male or adult female copepods (*t*-test).

Cystacanths were oriented in the copepod's hemocoel with the presoma either facing anteriorly or posteriorly. Based on 604 single infections, there was a significant difference between the number directed anteriorly (400) and posteriorly (204) within the copepod ($\chi^2 = 63.6$, $P < 0.001$). There was no statistical difference in cystacanth orientation between the sexes of copepods.

Cystacanth lengths within adult copepods ranged from 475 to 875 with a mean length of 650.5 ± 72.4. In order to investigate the possibility that body length of the host may be a limiting factor in the growth (length) of the parasite, cystacanth lengths among infected male and female copepods were examined. The mean length of cystacanths within infected male copepods ($N = 253$) was 608 ± 51.9. The mean length of cystacanths within infected female copepods ($N = 256$) was 693 ± 63.3 (Fig. 1). There was a significant difference in the mean lengths of cystacanths ($t = 16.6$, $P < 0.001$). Correlation analysis indicated a statistically significant association (5% level for H_0: $r = 0$) between host length and parasite length. Correlation coefficients for male and female copepods were $r = 0.41$ and $r = 0.45$, respectively. The results suggest a general trend in a positive direction.

In addition to adult copepods, immature stages (copepodids) were found to be parasitized. Based on observations of approximately 7,000 copepodids, 90 (1.3%) from the June 27, 1981 collection were infected. The mean length of infected copepodids was 689 ± 66.7. Cystacanth lengths within copepodid hemocoels ranged from 400 to 700 with a mean of 551.6 ± 66.0. These could not be properly included in the length analysis above because the copepodids had not yet achieved terminal growth.

Based on single infections, eighty-three (13.7%) of the cystacanths found in adult *D. pallidus* were



Figures 2–4. Scale bars = 100 μm. 2. *Tanaorhamphus longirostris* with everted proboscis (p) within *Diaptomus pallidus*. 3. Double infection of *T. longirostris* within copepod hemocoel. 4. Parasitized *D. pallidus* (adult female) with one egg attached.

observed to have everted proboscides (Fig. 2). Of the cystacanths found in copepodids, 10 (11.4%) were seen with proboscides everted.

Discussion

Tanaorhamphus longirostris that developed within the hemocoel of *Diaptomus pallidus* occupied most of the host's body cavity (Figs. 2–4). The copepod's internal organs were often displaced by the cystacanth. Buckner et al. (1978) reported comparable findings when investigating acanthocephalan larval development in amphipod intermediate hosts.

It is not known if mating behavior of copepods is affected by parasitism. However, during this study some infected copepods were observed with attached spermatophores. This suggests that parasitized copepods do participate in sexual activity. None of the infected mature female copepod specimens was seen carrying a clustered egg-sac, which is typical of the species. Approximately 5% of the parasitized females did appear to have one egg attached to the genital segment (Fig. 4). Although experience has shown that, in preserved zooplankton samples, calanoid copepod egg-sacs are often found detached from females, the sacs are generally seen as a complete unit or "cluster of eggs." This observation suggests that

the one-egg condition is not the consequence of sequential detachment of individual eggs as a result of fixation. Anderson (1978) reported helminth infections in vertebrate or invertebrate intermediate hosts can effect changes in host reproduction. He stated that although total loss of the host's ability to reproduce is relatively uncommon, parasitic infections tend to diminish host reproduction. Muzzall and Rabalais (1975) reported isopods infected with *Acanthocephalus jacksoni* were never observed carrying eggs. Reinhard (1956) discussed the suppression of oogenesis when the amphipod, *Gammarus pulex,* is parasitized with *Polymorphus minutus.*

The first samples of zooplankton were obtained on June 27, 1981, and by then parasitized copepods already contained late acanthellae and cystacanth stages. Therefore, no analysis of infection by acanthors or early acanthellae can be reported here. Obviously prior to June 27, 1981, copepods ingested eggs (shelled acanthors) of *T. longirostris.* One has to address the question of the availability of infective shelled acanthors (hereafter: eggs) to the *Diaptomus* population. Assuming eggs from the definitive host settle to the lake bottom, it is not difficult to conceive of benthic detritus-feeders ingesting eggs. This would be true for amphipods, isopods, and ostracods. *Diaptomus pallidus,* a limnetic filter-feeder, is not likely to feed on infective eggs on the lake bottom. It is possible that the eggs may remain dormant in the lake sediment until the fall or spring overturn thus making the eggs accessible as other materials are known to be circulated through the water column during this period. Several investigators have reported that acanthocephalan eggs stored in water at 4°C remain viable for 6–9 mo (DeGiusti, 1949; Merritt and Pratt, 1964; Harms, 1965). It may be in a resistant egg stage that *T. longirostris* overwinters at Caesar Creek Lake retaining viability and becoming available to copepods the following spring.

In addition to availability, egg size in relation to food particle size for *D. pallidus* must be considered. Shelled embryos of *T. longirostris* have been reported to be 27 × 8–10 μm (Van Cleave, 1913). Friedman (1980) has shown that *D. pallidus* can ingest particles whole up to 30 μm in diameter. He also suggests that the presence of 40–80 contact chemoreceptors, within the setae located over the mouth region of these copepods, argues for a more direct selective pattern than the passive "fixed-sieve" feeding mechanism described in the literature.

Muzzall and Rabalais (1975) and Nickol and Heard (1973) investigated the position and orientation of cystacanths within isopod intermediate hosts. In both studies, cystacanths were most often found with presomas facing posteriad. Contrary to their findings, cystacanths of *T. longirostris* within *D. pallidus* were more often found facing anteriorly; the biological significance of this orientation remains unclear.

During this study, developmental anomalies of the cystacanth proboscis were observed in a few specimens with everted proboscides. The most noticeable abnormality was misalignment or random positioning of hooks. These hooks were often pointed forward in contrast to the normally recurved hooks. Uznanski and Nickol (1980) observed similar anomalies in larval acanthocephalans developing in amphipods. They suggested that abnormalities may have been induced by high temperatures (>30°C) in the laboratory during their experimental work. Cystacanths observed in our copepods were from a natural population; therefore, direct comparison may not be appropriate. The U.S. Corps of Engineers reported maximum surface temperatures of 22.0°C and 26.5°C on June 2, 1981, and July 7, 1981, respectively. Factors other than high temperatures may contribute to larval anomalies.

It can be stated with reasonable assurance that most cystacanths observed in this study had entered a quiescent state as evidenced by the invaginated proboscides; therefore, no further growth in size would be expected. The mean lengths of cystacanths within adult copepod intermediate hosts (Fig. 1) coincided with increased sizes of female copepods when compared to male copepods. Wilson (1959) reported that female copepods are generally larger than males. This is true for *D. pallidus.* Because copepods do not continue to grow after molting to the adult stage, the data presented here represent lengths of adults that have terminated growth. There is no evidence to suggest that the presence of *T. longirostris* affects the growth (length) of the intermediate host, *D. pallidus.*

Harms (1965) and Amin et al. (1980) reported that sizes of acanthocephalan larvae are affected by intermediate host size. It is evident from our work that copepod hemocoel space is a limiting factor in the growth of *T. longirostris* larvae.

Another variable that can affect parasite development is intensity or parasites/host as reported by Uglem and Larson (1969) and Keppner (1974). Cystacanths of multiple infections (Fig. 3) observed in this study were smaller in overall size than those occurring singly.

It was the intent of this investigation to examine the sex ratio of developing *T. longirostris* within the intermediate host; however, it became evident that most specimens could not be sexed. In specimens that could be sexed it was observed that: (1) males had small, rounded contiguous testes but the remaining reproductive organs were not well differentiated, and (2) females had a single ovary usually seen in the anterior region of the pseudocoel. No fragmentation of the ovary was observed.

It appears from the data that infected copepods may not be available to the definitive host throughout the summer months at Caesar Creek Lake, but may exhibit seasonal occurrence with a peak prevalence in June. Uninfected copepods were seen in zooplankton samples throughout the 1981 sampling period. In order to elucidate the periodicity of infection of the intermediate host of *T. longirostris,* additional copepod specimens must be obtained fromCaesar Creek Lake during winter and early spring months.

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Raillietnema longicaudata (Walton, 1929) n. comb. (Nematoda: Cosmocercidae) from North American Frogs

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ABSTRACT: *Raillietnema longicaudata* (Walton, 1929) n. comb. (=*Aplectana longicaudata* Walton) from frogs in Illinois, U.S.A. is redescribed from the type specimens. The species was transferred to *Raillietnema* Travassos, 1927, because the uteri in female worms contain few markedly large eggs. *R. longicaudata* is the first *Raillietnema* species reported from North America (other species in South America, Africa, Madagascar, Malaysia). It most closely resembles *R. rhacophori* Yuen, 1965, from frogs of Malaysia, but may be distinguished from it by its shorter spicules. The host for *R. longicaudata* was identified as *Rana pipiens*. This must be doubted because recent information indicates that in Illinois the "*Rana pipiens*" complex of leopard frogs is represented by three distinct species: *R. pipiens, R. blairi,* and *R. utriculari.*

Aplectana longicaudata Walton, 1929, was described originally from frogs identified as *Rana pipiens* of Illinois, U.S.A. The original description lacks the details required for classification to genus in the subfamily Cosmocercinae. Fortunately the type specimens are still available for study and they are sufficiently well preserved to permit a redescription and evaluation of the systematic position of the species.

Materials and Methods

Type specimens were borrowed from the National Parasite Collection of the United States. They were cleared for study in lactophenol. Measurements are given in micrometers unless otherwise specified.

Results

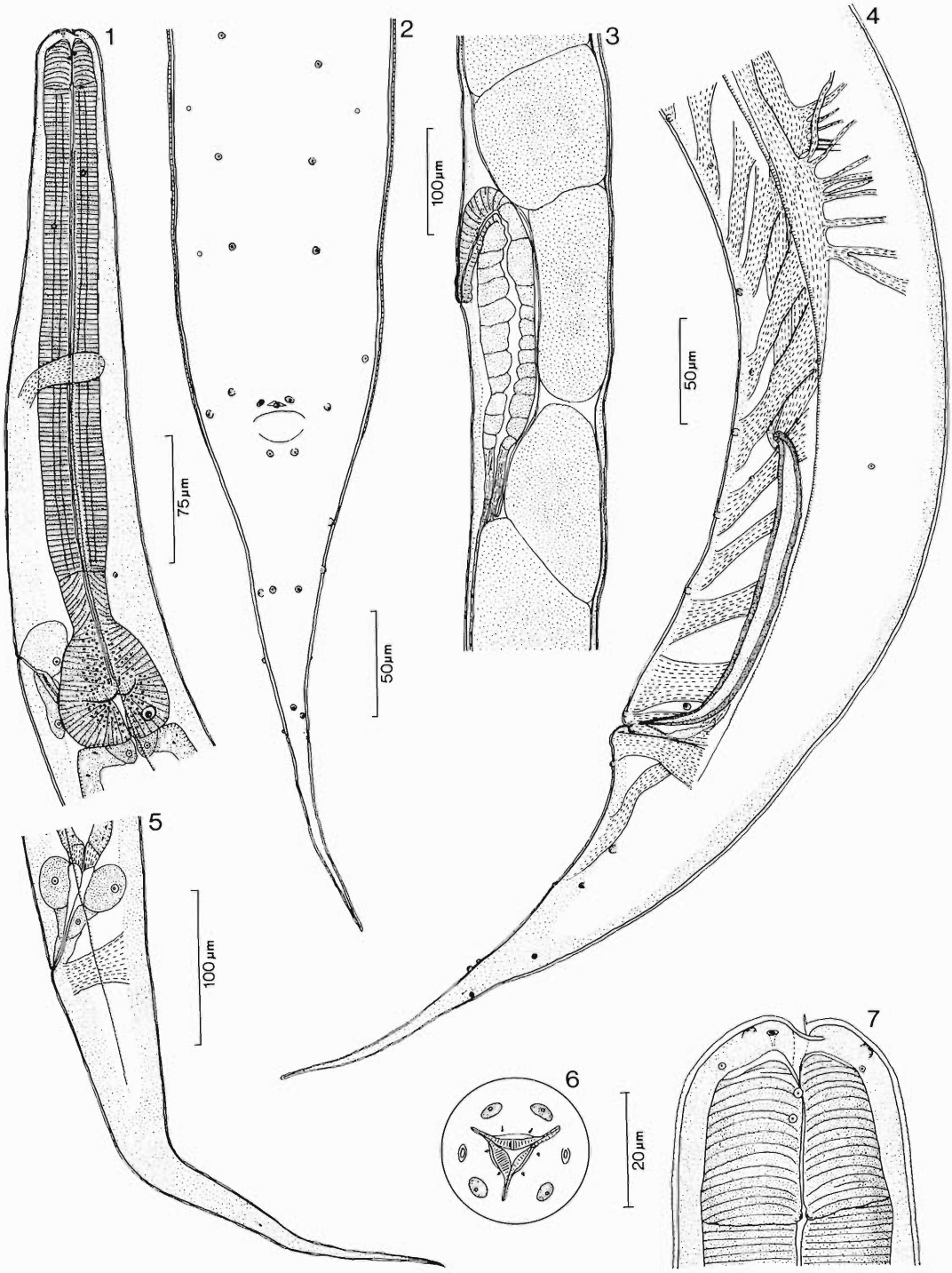
Raillietnema longicaudata (Walton, 1929) n. comb.

Syn. *Aplectana longicaudata* Walton, 1929.

GENERAL: Cosmocercidae, Cosmocercinae. Cuticle of body with fine transverse striations. Lateral alae present, maximum width 2, extending from level just anterior to nerve ring to near anus in males and to anterior third of tail in females. Somatic papillae present over body surface, most numerous near cephalic extremity. Cephalic end with three small lips each bearing slender cuticular flange overlapping mouth. Six minute labial papillae and four large outer cephalic papillae present. Anterior extremity of esophagus in form of three blunt projections. Esophagus divided into short anterior pharyngeal portion, elongate corpus, short and narrow isthmus, and large bulb bearing three valves.

MALES (measurements of lectotype followed by two paralectotypes in parentheses): Total length 2.42 (2.42–2.5) mm. Esophagus total length 409 (387–409); pharyngeal portion 32 (27–31), corpus 277 (276–280), isthmus 33 (24–34), bulb 67 (56–68) long. Nerve ring 210 (190–226) and excretory pore 341 (331–340) from anterior extremity. Tail conical, 253 (235–246) long, with long, slender terminal point. Phasmids in posterior half of tail. Caudal papillae distributed as follows: posterior half of tail with one pair of subventral, one pair of lateral and one pair of subdorsal papillae; anterior half of tail with two pairs of adjacent subventral papillae located near mid-tail, one pair of subventral papillae located between mid-tail and the anus (one of this pair lacking in lectotype specimen, but present in other specimens), one pair of subventral papillae located near posterior edge of anus; preanal region with one unpaired papilla on lip of anus, two pairs of papillae adjacent to unpaired papilla, and six pairs of subventral papillae in two rows extending anteriorly from the level of the anus. Thirteen pairs of subventral muscle cells located anterior to anal region. Spicules 179 (168–180) long, strongly arcuate in lateral view, with sharply pointed distal extremity and ventrally curved capitulum, spicular muscles prominent. Gubernaculum 30 (25–28) long, weakly sclerotized.

FEMALES (measurements of six paralectotypes): Total length 3.04–3.58 mm. Esophagus total length 430–455; pharyngeal portion 22–36, corpus 307–316, isthmus 29–34, bulb 63–73 long. Nerve ring 202–210, excretory pore 334–428, and vulva 1.60–1.90 mm from anterior extremity. Tail 340–420 long, conical, slender throughout length. Phasmids in posterior half of tail. Vagina 400 long, muscular, directed anteriorly



Figures 1–7. ***Raillietnema longicaudata*** **(Walton, 1929) n. comb. 1. Anterior end of male, lateral view. 2. Caudal end of male, ventral view. 3. Vagina, lateral view. 4. Caudal end of male, lateral view. 5. Tail of female, lateral view. 6–7. Cephalic extremity of male, apical and lateral view, respectively.**

in first quarter and flexed to posterior in terminal three-quarters. Amphidelphic, ovary of anterior uterus terminating anterior to uterus, ovary of posterior uterus terminating just anterior to vulva. Uteri containing few eggs (maximum number of 14 observed in one female). Eggs variable in shape, 125–200 long and 65–125 wide. In two worms hatched larva observed in utero.

HOST OF TYPE: *Rana pipiens* Latreille. The identification of the host species must be doubted. In 1929 it was not known that throughout North America "*Rana pipiens*" includes a complex of several near sibling species, each with a characteristic geographical distribution. Three species of this complex (*Rana pipiens, R. blairi, R. utricularia*) occur in Illinois, with distributions that overlap slightly (Conant, 1975). Since Walton (1929) did not specify a locality in Illinois where frogs were collected, the host species cannot be determined.

LOCATION: Intestine.

LOCALITY: Illinois, U.S.A. (not specified more precisely).

SPECIMENS: U.S. Helm. Coll. No. 50768 (syntypes). The type series consists of four male (one broken) and eight female worms. The male worms all correspond to *R. longicaudata* based on the original description. Unfortunately the female worms include two species: six worms representing females of *R. longicaudata* and two females of the subfamily Cosmocercinae that cannot be identified to species (see Discussion below). One male specimen is designated a lectotype, and the three remaining males and six conspecific females are therefore paralectotypes. The two unidentified females are removed from the type series and accessioned as Cosmocercinae gen. sp. under U.S. Helm. Coll. No. 78229.

Discussion

Aplectana longicaudata Walton has been transferred to the genus *Raillietnema* Travassos, 1927, because the possession of few markedly large eggs (egg length >120 μm) in the uteri of female worms is characteristic of this genus. In contrast, in the genus *Aplectana,* eggs are relatively small (<120 μm long) and numerous in the uteri (Chabaud, 1978).

Raillietnema longicaudata represents the first *Raillietnema* species reported from North America. The 19 other species in the genus occur in various amphibians and reptiles from South America, Africa, Madagascar, and Malaysia. The four New World species are: (1) *R. simples* (Travassos, 1925) Travassos, 1927, from *Hyla faber* of Brazil; (2) *R. baylisi* (Walton, 1933) Baker, 1980, from *Ceratophrys dorsata* of Brazil; (3) *R. gubernaculatum* Freitas and Ibanez, 1965, from *Bufo spinulosus limensis* of Peru and *Bufo itericus* of Brazil (Gomes, 1964b); (4) *R. spectans* Gomes, 1964, from *Bufo crucifer* of Brazil and *Bufo paracnemis* of Brazil (Baker, 1982). *R. longicaudata* may be differentiated from all four species in possessing spicules less than the length of the male tail. *R. longicaudata* is similarly differentiated by marked differences in spicule length and shape and disposition of male caudal papillae from all Old World species except *R. rhacophori* Yuen, 1965, from *Rhacophorus leucomystax* and *Rhacophorus* sp. of Malaysia. *R. longicaudata* and *R. rhacophori* are similar in male and female tail shape, number and disposition of male caudal papillae, and spicule shape. In fact these species may be differentiated only by a small difference in spicule length. Thus *R. rhacophori* males were recorded by Yuen (1965) as being 2.0–2.7 mm long and with spicules 200–230 μm long, whereas males of *R. longicaudata* are 2.42–2.58 mm long and with spicules 168–180 μm long.

The presence in the type series of *R. longicaudata* of two distinct kinds of female cosmocercids (one with few relatively large eggs in utero and the other with numerous relatively small eggs in utero) raises the question of which females correspond to the male worms. Differences in esophageal shape and dimensions, and cephalic morphology made this identification possible. The female worms identified herein as *R. longicaudata* have an esophagus which in shape of its various parts and in relative length (compared to body length) is close to the male worms. In contrast the esophagus of the other females was relatively shorter and with a relatively smaller anterior pharyngeal portion and thicker corpus. Finally the cephalic lips of the female worms identified as *A. longicaudata* are indistinguishable from the male worms, whereas the lips are more prominent in the other female worms.

The two female worms removed from the type series of *R. longicaudata* cannot be identified to species. In morphology of the reproductive organs, esophagus, and tail they do not correspond to any of the following Cosmocercinae species presently reported from eastern North America: *Aplectana hamatospicula* Walton, 1940, from

Microhyla carolinensis of Florida; *Cosmocercoides dukae* (Holl, 1928) from numerous amphibians and reptiles throughout North America (see Anderson, 1960); and *Cosmocercella haberi* Steiner, 1924, from hylid frogs of Canada and the eastern United States (see Baker and Adamson, 1977).

Since the original description, *R. longicaudata* has been reported from *Rana catesbeiana* of North Carolina (Brandt, 1936) and Indiana (Lank, 1971), and from *Plethodon glutinosus* and *Gyrinophilus porphyriticus* of New York State (Fischthal, 1955).

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Stimulation of Ingestion in *Trichostrongylus colubriformis* (Nematoda)[1]

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ABSTRACT: Feeding in vitro by the adult sexes of *Trichostrongylus colubriformis* was stimulated significantly by doses of histamine and dopamine, based on the uptake of the fluorescent dye Rhodamine B. Octopamine significantly decreased the uptake of dye by male, but not female, nematodes. Oral administration of histamine to the host also significantly increased in vivo feeding of the helminths. Aspirin and antihistamine had no effect on the in vivo feeding of *T. colubriformis*. Serotonin increased the nematode's ingestion during in vitro and in vivo tests but no dosage-dependency was found. In vitro feeding was not altered by various concentrations of host bile or chyme, or host dietary components such as sugars, amino acids, and enzymes.

Little information is available about feeding by zooparasitic nematodes. Croll (1976) proposed that *Nippostrongylus brasiliensis* exhibited an orthokinetic response to food in the intestine of the rat and monitored its surroundings by mechano- and chemoreceptors for chyme. Glassburg et al. (1983) reported that females of *N. brasiliensis* moved more rapidly than males in food-deprived hosts. Neither report concerned the compounds that may serve as sensory information for regulation of ingestive behavior of the helminths.

The chemical components that modulate feeding by nematodes are unknown. Serum may contain an unknown stimulant for feeding by *Ancylostoma canimum* (Warren et al., 1962; Fernando and Wong, 1964; Roberts and Fairbairn, 1965). This feeding stimulus was less effective in vitro for *N. brasiliensis* (Roberts and Fairbairn, 1965).

Knowledge of feeding stimuli is limited for other helminths. Mettrick and Cho (1981) reported that exogenous serotonin regulated absorptive feeding by the tapeworm *Hymenolepis diminuta*. In contrast, considerable information is available about feeding attractants and deterrents in various aquatic invertebrates (Bardach, 1975) and insects (Harborne, 1982).

This study examined the effect of selected biological compounds on feeding by *Trichostrongylus colubriformis*, a nematode parasite of the small intestine in ruminants, based on in vitro and in vivo uptake of dye (Bottjer and Bone, 1984a, b). Knowledge of compounds that stimulate or inhibit feeding by nematodes may facilitate in vitro cultivation, contribute to an understanding of the basis of pathogenicity, or enable novel methods of helminth control.

Materials and Methods

Trichostrongylus colubriformis was maintained in cross-bred male goats that were obtained from commercial sources. Animals were treated with anthelmintic (Omnizole, Merck) on arrival and isolated from uninfected goats. Fecal examinations were performed periodically to determine any occurrence of helminth infection. Uninfected animals were housed in a pen with a concrete floor that was cleaned daily with bleach. Animals were fed a commercial grain mixture and hay pellets. Experimentally infected goats averaged 20 kg at infection and were free of helminths, based on the absence of ova in the feces and helminths, other than *T. colubriformis* when the hosts were killed.

Trichostrongylus colubriformis was obtained originally from Animal Parasitology Institute, USDA, Beltsville, Maryland, and has been maintained in goats at the Regional Parasite Research Laboratory, Auburn, Alabama, for 4 years. Adults of *T. colubriformis* were recovered from hosts at 21 days postinfection for experimentation.

Feeding in vitro was determined by uptake of the fluorescent dye Rhodamine B (Bottjer and Bone, 1984b). Triplicate samples of 250 worms of each sex were collected by mechanical pipettor, rinsed several times, and placed in 2 ml of glucose-free Tyrode's solution (GFTS) at 37°C. Dye was added at a final concentration of 100 μg/ml. After 30 min for feeding, worms were rinsed repeatedly to remove the remaining dye. These procedures were completed within 2 hr after killing the host. The helminths were macerated in a mechanical tissue grinder prior to flurometric determination of ingested dye versus standard solutions of Rhodamine B in macerated, undyed worms. Background fluorescence was corrected by analysis of undyed worms. Feeding was determined as ng of ingested dye/μg dry body weight according to previous procedures (Bottjer and Bone, 1984a). Ingestion of dye in untreated worms that were held in glucose-free Tyrode's solution (GFTS) was determined in all in vitro tests as controls.

[1] Mention of a trademark of proprietary product does not constitute a guarantee of warranty of the product of the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

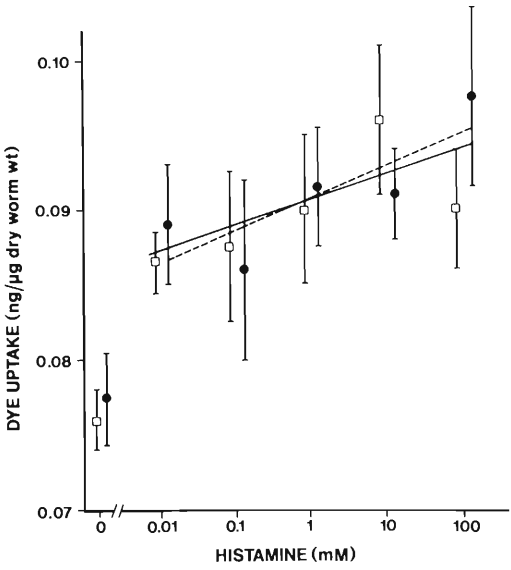

Figure 1. In vitro uptake of dye (ng/µg dry body weight ± SEM) by male (●) and female (□) *T. colubriformis* ($r = 0.73, 0.88$, respectively) in the indicated concentrations of histamine.

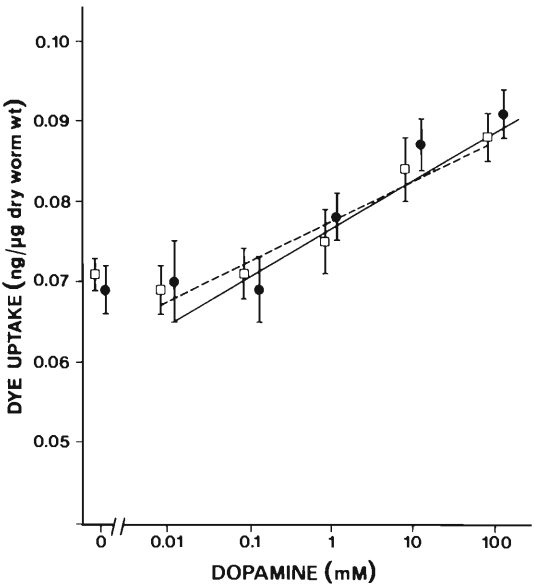

Figure 2. In vitro uptake of dye (ng/µg dry body weight ± SEM) by male (●) and female (□) *T. colubriformis* ($r = 0.78, 0.83$, respectively) in the indicated concentrations of dopamine.

Various compounds were examined for their effect on in vitro feeding by adults of *T. colubriformis* according to the above procedures. Sugars tested included L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-mannitose, L-sorbose, sucrose, and D-xylose (Merck). Sugars were added to glucose-free Tyrode's solution at concentrations of 0.1 and 10 mg/ml. Amino acids (L- or DL-isomers) were tested similarly. These included alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, leucine, methionine, phenylalanine, proline, serine, tyrosine, and valine (Merck). Twenty-two animals were used in these studies to provide helminths for testing in vitro.

Host chyme and bile were collected from uninfected hosts for determination of any effect on the helminth's feeding in vitro. Intestinal contents were obtained by gently scraping the anterior intestine. Bile was taken by syringe from the gall bladder. Worms were incubated in each fluid to determine their feeding after addition of Rhodamine B. Additionally, feeding by the nematodes was tested in 25, 50, and 75% concentrations of bile and chyme after dilution with Tyrode's solution. Several digestive enzymes were tested also at various dosages. These included the following: chymotrypsin (5.5–22 units/ml), trypsin (10–10,000 units/ml), pepsin (0.001–10 mg/ml), and lipase (0.1–5 mg/ml) (Sigma). Twelve animals were used in these studies.

Various biologically active compounds were tested at dosages that ranged from 10^{-5} to 10^{-1} M. These included adenosine 3′,5′ cyclic monophosphoric acid (c-AMP), guanosine 3′,5′ cyclic monophosphoric acid (c-GMP), acetylcholine chloride, dopamine, 5-hydroxytryptamine (creatinine sulfate complex), histamine (diphosphate), gamma-amino-n-butyric acid (GABA), DL-norepinephrine, DL-octopamine, and reduced glutathione. These compounds were obtained from Sigma. Ten goats were killed for recovery of helminths.

Based on the above results, selected compounds were tested in vivo. Various dosages of these substances were given orally to individual goats in a 67-ml volume (20-kg animal) that contained 7.5 mg of Rhodamine B per ml of Tyrode's solution. Animals were killed after 4 hr to determine feeding in vivo by the helminths, based on uptake of dye (Bottjer and Bone, 1984a). Three groups of 250 adult worms of each sex were recovered and rinsed repeatedly prior to maceration and determination of their ingestion of dye.

Histamine was given orally in Tyrode's solution at concentrations of 23, 46, 93, 138, and 183 mg/kg body weight. Aspirin was administered orally (36 mg/kg body weight, twice-a-day) as an anti-inflammatory agent. The antihistamine, tripelennamine HCl (Re-Covr, Squibb) was given by intramuscular injection, twice daily, at 0.8 mg/kg body weight. Both compounds were given for 5 days prior to sacrificing the host at 21 days post-infection. Additionally, selected dosages of other neurotransmitters were tested in vivo after oral administration according to the above procedures. Doses were selected based on the comparative effects of histamine in vivo and in vitro. These compounds included octopamine (93 mg/kg body weight), serotonin (138 mg/kg body weight), and dopamine (93 mg/kg body weight). Ten animals were used for study of the helminth's feeding in vivo. An additional ten animals were given only dye solution as controls for in vivo feeding by the helminths and as drug placebo for the hosts.

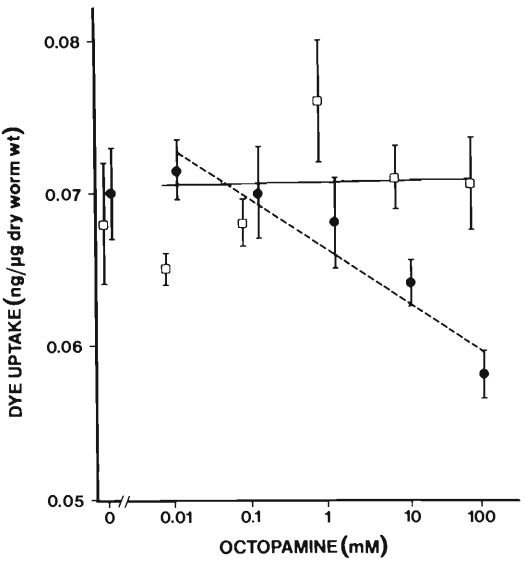

Figure 3. In vitro uptake of dye (ng/µg dry body weight ± SEM) by male (●) and female (□) *T. colubriformis* ($r = -0.85$, 0.09, respectively) in the indicated concentrations of octopamine.

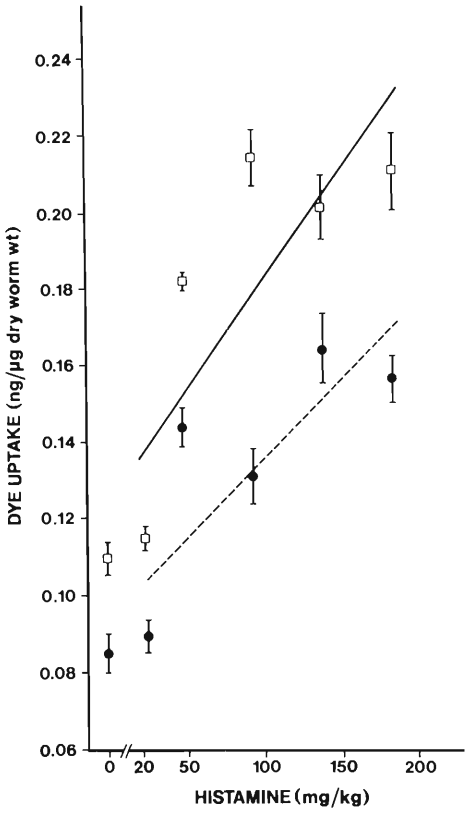

Figure 4. In vivo uptake of dye (ng/µg dry body weight ± SEM) by male (●) and female (□) *T. colubriformis* ($r = 0.84$, 0.83, respectively) in the indicated concentrations of histamine.

Data were evaluated by linear regression or analysis of variance. The 0.05 probability level was considered significant. Data are presented as the mean (±SEM) of the three replicates of each treatment or control.

Results

Feeding by adults of *T. colubriformis* was not significantly elevated in host chyme or bile. Males and females ingested 0.074 ± 0.006 and 0.073 ± 0.004 ng of dye/µg body weight, respectively, in the GFTS control. Incubation of male and female helminths in 100% chyme yielded ingestion of 0.071 ± 0.005 and 0.081 ± 0.003 ng of dye/µg body weight, respectively. Similar results were obtained in various dilutions of host bile. Thus, these host materials may not modulate feeding by helminths, based on in vitro results.

Likewise, the tested concentrations of sugars and amino acids showed no inhibition or stimulation of in vitro feeding by *T. colubriformis.* Uptake of dye in these substances was not significantly different from the uptake of dye in the GFTS controls. Feeding by the helminths in chymotrypsin, trypsin, pepsin, and lipase was tested also. These enzymes had no effect on feeding by either sex at the examined dosages.

Many of the other compounds that were tested were inactive also, based on similar uptake of dye in these compounds and their controls, even though they have biological effects in various vertebrates and invertebrates. However, three of the tested biogenic amines altered feeding by *T. colubriformis* in vitro. Feeding was elevated significantly in male and female helminths ($F_{29}^{5} = 7.63$ and 9.38, respectively) during incubation in 0.01 mM histamine when compared to the control (Fig. 1). Higher concentrations caused significant increases in feeding by male and female nematodes ($r = 0.73$, 0.88, respectively).

Dopamine also increased feeding of the helminths (Fig. 2). Linear increases of dye uptake were seen in both males ($F_{29}^{5} = 27.83$, r = 0.78) and female ($F_{29}^{5} = 19.62$, $r = 0.83$) as the dopamine concentration was raised. Serotonin increased feeding in both sexes of *T. colubriformis,* but the results were not dosage-dependent, and, thus, were insignificant.

Octopamine had no effect on feeding by female *T. colubriformis* ($F_{29}^{5} = 1.69$, $r = 0.09$) at the

tested doses (Fig. 3). However, feeding by male helminths was reduced significantly ($F_{2,9}^{5} = 8.38$, $r = 0.85$).

Several of the neurotransmitters had effects in vivo that were similar to their in vitro influence. Histamine increased feeding significantly in male and female helminths ($F_{2,9}^{5} = 26.14$ and 13.72, respectively) (Fig. 4). However, feeding by helminths in animals that were treated with aspirin or antihistamine was similar to the untreated controls. Serotonin at 138 mg/kg of host weight increased uptake of dye in females (0.21 ± 0.16 ng/μg dry body weight) and males (0.128 ± 0.01 ng/μg dry body weight). No significant effect in vivo was found for dopamine or octopamine at the tested in vivo levels.

Discussion

Any influence of dietary components of the host on the feeding of *T. colubriformis* seems remote, because bile, chyme, enzymes, sugars, and amino acids failed to alter feeding in this study. However, these compounds may have an effect in vivo, may act synergistically as a complex of the host's food, secretions, and enzymes, or may function independently at untested dosages.

Alternatively, feeding by *T. colubriformis* may be regulated by a simpler mechanism, such as an active substance from the host. Mettrick and Cho (1981) showed that movement of the tapeworm *H. diminuta* to the anterior of the rat's intestine was caused by increases in serotonin and host feeding. Exogenous serotonin may modulate also the tapeworm's carbohydrate metabolism (Mettrick et al., 1981).

However, feeding by *T. colubriformis* in vitro was not stimulated significantly by serotonin, which suggests that other informational compounds may govern feeding. The 28% increase of feeding in vitro that was induced in females by histamine at 100 mM indicates that this compound, rather than other amines, may be involved. Mettrick and Podesta (1982) found that histamine induced a distal movement by *H. diminuta* in the intestine. Histamine also increased the average weight of individual tapeworms, but not the total biomass of worms, whereas fecundity was decreased (Cho and Mettrick, 1982).

Free-living nematodes are affected also by various biogenic amines. Pharyngeal pumping by the free-living *Caenorhabditis elegans* is stimulated and depressed, respectively, by serotonin and octopamine (Horvitz et al., 1982). Croll (1975) found that histamine increased pharyngeal activity in several free-living nematodes.

Histamine was elevated in the intestine during infections of *T. colubriformis,* and may be involved in immune expulsion of the helminth (Jones et al., 1978). Release of histamine in the intestine may signal also the presence of a feeding site to the helminth. Thus, increased feeding by the nematode may anticipate the impending immune response of the host and enhance the competitive status of the helminth.

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Survey or Taxonomic Papers

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository and include the accession numbers in the manuscript. The following are acceptable, and others are described in the "Guide to Parasite Collections of the World," prepared by the ASP and available from Allen Press ($3.00).

Helminths and Protozoans

U.S. National Parasite Collection
Dr. J. Ralph Lichtenfels, Curator
USDA, ARS, BARC-East No. 1180
Beltsville, Maryland 20705
(Phone: 301-334-2444)

Protozoans

International Protozoan Type Slide Collection
Dr. Klaus Ruetzler, Curator
Department of Invertebrate Zoology
National Museum of Natural History, W-325
Smithsonian Institution
Washington, D.C. 20560
(Phone: 202-357-2486)

Mites

USDA and USNM Acari Collections
Dr. E. W. Baker, Curator
USDA, ARS, BARC-West No. 004
Beltsville, Maryland 20705
(Phone: 301-344-3890)

Ticks

Dr. James E. Keirans, Curator
Museum Support Center
Smithsonian Institution
Washington, D.C. 20560
(Phone: 301-423-1085)

Leeches

Hirudinea Collection
Department of Invertebrate Zoology
National Museum of Natural History
Smithsonian Institution
Washington, D.C. 20560
(Phone: 202-357-2472)

Helminths and Protozoans
(voucher specimens or paratypes)

University of Nebraska State Museum
Prof. Mary Hanson Pritchard, Curator
16th and W Streets
Lincoln, NE 68588
(Phone: 402-472-3334)

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Digenetic Trematodes of Marine Fishes of the Western and Southwestern Coasts of Puerto Rico

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ABSTRACT: A comprehensive study was made in coastal waters of western and southwestern Puerto Rico from 1974 to 1984 to inventory the digenetic flukes of marine fishes. A total of 1,019 fishes representing 76 families, 155 genera, and 252 species were examined. Nineteen families of digenetic flukes representing 52 genera and 66 species were recorded, including 11 digenea not previously known from Puerto Rico. Four new host records were established. Most infections were of a single species and although prevalence and intensity were low, host specificity was high.

Major contributions to knowledge of the digenetic flukes of marine fishes of Puerto Rico stem from the early studies of Cable (1954a, b, 1956a, b), LeZotte (1954), and the more recent comprehensive report by Siddiqi and Cable (1960).

Between April 1974 and January 1984 additional data were obtained from examining 1,019 marine fishes representing 69 families of bony fishes and 7 families of elasmobranchs from coastal waters of western and southwestern Puerto Rico and Mona Island for digenetic flukes. Scientific and common names used in this report are the names of fishes recognized by the American Fisheries Society (Robins et al., 1960).

Materials and Methods

A variable mesh gill net, trawl net, seine, traps, spearfishing and hook and line supplemented by quinaldine, and rotenone to obtain species inhabiting reefs and shallow water were all used to collect fishes. Fishes were placed in plastic bags containing seawater and held in styrofoam containers for transport to the laboratory where they were refrigerated and usually necropsied immediately.

Flukes were washed in 0.7% saline, flattened with minimal coverslip pressure, fixed with warm AFA, and stored in 70% ethanol. Specimens were stained with either Harris' hematoxylin or Grenacher's alcoholic borax carmine and prepared as whole mounts in Canada balsam. Voucher specimens of most species have been deposited in the National Parasite Collection, USDA, Beltsville, Maryland, under the accession numbers listed in Table 1. Other specimens are in the authors' collections.

Results

One or more species of digenetic flukes were recovered from 128 (12.6%) of 1,019 fishes representing 41 (53.9%) of 76 families, 59 (38.1%) of 155 genera, and 70 (27.8%) of 252 species. The 66 species of flukes detected represented 19 families and 52 genera (Table 1). Of the 70 species of fishes that were infected, 56 (80%) harbored 1 species of digenetic fluke, 8 species (11.4%) 2, and 2 species (2.9%) with 3 to a maximum of 5 species of flukes. Fish negative for digeneans are listed in Appendix 1.

The intensity of a given species ranged from 1 to 100 flukes per host. Each of 88 fishes yielded 1–5 specimens; 18, 6–10; 4, 11–15; 3, 20; 5, 21–25; 3, 30–35; 3, 50; and 4 yielded 58, 76, 90, and 100 specimens, respectively.

For most of the fish species examined, the prevalence of infection with digenetic flukes was low. However, 20.8% of 24 French grunts, *Haemulon flavolineatum* (Desmarest) were infected with *Infundibulostomum spinatum* Siddiqi and Cable, 1960; 33.3% of 6 manytooth conger, *Conger triporiceps* Kanazawa with *Dinurus barbatus* (Cohn, 1903); and 37.5% of 8 *Bothus* sp. were infected with *Lecithochirium monticellii* (Linton, 1898).

Fifty-two (78.8%) of the 66 species of digenetic flukes recovered occurred in 1 host species, 10 (15.2%) in 2, and the remaining 4 (6%) in 3, 5, 6, and 8 host species, respectively.

Eleven of the 66 fluke species are reported from Puerto Rico for the first time: *Rhagorchis odhneri* Manter, 1931; *Lecithostaphylus nitens* (Linton, 1898); *Opechona chloroscombri* Nahhas and Cable, 1964; *Bathycotyle coryphaenae* Yamaguti, 1938; *Opisthadena dimidia* Linton, 1910; *Rhynchopharynx paradoxa* Odhner, 1928; *Genitocotyle atlantica* Manter, 1947; *Barisomum erubescens* Linton, 1910; *Deretrema fusillus* Linton, 1910; *Tubulovesicula pinguis* (Linton, 1940); and *Plagioporus crassigula* (Linton, 1910). To our knowledge, the following are new host records:

Table 1. Digenetic flukes of marine fishes from coastal water of western and southwestern Puerto Rico.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Bony fishes				
Acanthuridae (surgeonfishes)				
Acanthurus chirurgus (Bloch) 9/4	*Hapladena acanthuri* Siddiqi and Cable, 1960	Desecheo Island, October 11, 1975	1/1/2	77797
	Pseudodichadena lobata Yamaguti, 1971 nec. *Dichadena acuta* Linton, 1910	Cayo Enrique, LaParguera, September 8, 1975	1/1/3	
		Turrumote reef, LaParguera, March 8, 1975	1/1/1	
	Tetrochetus aluterae (Hanson, 1955)	Turrumote reef, LaParguera, August 23, 1975	1/1/7	77728
Anomalopidae (flashlight fishes)				
Kryptophanaron alfredi Silvester and Fowler 3/2	*Lecithochirium monticellii* (Linton, 1898)	Margarita reef, LaParguera, May 3, 1978	2/1/5	
	Lecithochirium parvum Manter, 1947	Margarita reef, LaParguera, May 3, 1978	2/1/2	
Antennariidae (frogfishes)				
Antennarius scaber (Cuvier) 2/2	*Lecithochirium parvum*	Mouth of Añasco River, Mayagüez Bay, March 1, 1975	2/2/4.5	77737
Apogonidae (cardinalfishes)				
Phaeoptyx xenus (Böhlke et Randall) 1/1	*Pseudopecoelus tortugae* von Wicklen, 1946	Salinas, December 25, 1976	1/1/1	
Balistidae (leatherjackets)				
Aluterus schoepfi (Walbaum) 3/1	*Rhagorchis odhneri* Manter, 1931	Channel between Isla Magueyes and LaParguera, July 20, 1977	1/1/4	77772
Monacanthus ciliatus (Mitchill) 5/2	*Megapera gyrina* (Linton, 1907)	LaParguera, May 2, 1981	2/2/2	77774
	Multitestis (*Multitestoides*) *brasiliensis* Amato, 1983	LaParguera, May 2, 1981	2/2/1.5	77764
Belonidae (needlefishes)				
Platybelone argalus (Lesueur) 3/1	*Lecithostaphylus nitens* (Linton, 1898)	Isla LaCueva, March 5, 1975	1/1/1	77799
Bothidae (lefteye flounders)				
Bothus lunatus (Linnaeus) 4/2	*Lecithochirium monticellii*	Cayo Enrique, LaParguera, February 7, 1979	1/1/11	
	Hamacreadium mutabile Linton, 1910	Playa Sardinero, Mona Island, April 12, 1975	1/1/4	78004
Bothus sp. 8/3	*Lecithochirium monticellii*	Mouth of Añasco River, Mayagüez Bay, March 26, 1981	8/3/1.7	77745

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Carangidae (jacks)				
Caranx lugubris Poey 1/1	*Lecithochirium microcercus* (Manter, 1947)	Carbinero Point, Mona Island, October 13, 1974	1/1/2	
Chloroscombrus chrysurus (Linnaeus) 5/1	*Opechona chloroscombri* Nahhas and Cable, 1964	Mayagüez Bay, February 16, 1979	1/1/4	77771
Decapterus macarellus (Cuvier) 2/1	*Tergestia laticollis* (Rudolphi, 1819)	Crashboat Pier, Aguadilla, September 27, 1983	2/1/8	77887
Oligoplites saurus (Schneider) 2/1	*Manteria brachyderus* (Manter, 1940)	LaParguera, October 7, 1977	1/1/1	77723
Selene setapinnis (Mitchell) 39/1	*Tergestia pectinata* (Linton, 1905)	Bahiá de Guánica, September 24, 1974	15/1/2	
Trachinotus goodei Jordan and Evermann 1/1	*Neolepidapedon* (*Neolepidapedoides*) *trachinoti* Siddiqi and Cable, 1960	Mona Island, May 25, 1977	1/1/1	77770
Centropomidae (snooks)				
Centropomus undecimalis (Bloch) 7/1	*Theletrum fustiforme* Linton, 1910	Mouth of Añasco River, Mayagüez Bay, March 12, 1975	1/1/1	77756
Chaetodontidae (butterflyfishes)				
Chaetodon ocellatus Bloch 7/1	*Lecithochirium parvum*	Mario reef, LaParguera, March 16, 1976	1/1/7	77874
Clupeidae (herrings)				
Harengula humeralis (Cuvier) 1/1	*Parahemiurus merus* (Linton, 1910)	Crashboat Pier, Aguadilla, January 22, 1984	1/1/5	
Congridae (conger eels)				
Conger triporiceps Kanazawa 6/4	*Dinurus barbatus* (Cohn, 1903)	Mouth of Añasco River, Mayagüez Bay, October 9, 1975	6/2/80	77739
	Elytrophallus chloroscombri (Siddiqi and Cable, 1960)	Mouth of Añasco River, Mayagüez Bay, October 9, 1975	6/1/1	77740
	Lecithochirium monticellii	Mouth of Añasco River, Mayagüez Bay, October 9, 1975	6/1/20	77747
Coryphaenidae (dolphins)				
Coryphaena hippurus (Linnaeus) 2/2	*Bathycotyle coryphaenae* Yamaguti, 1938	LaParguera, March 20, 1976	1/1/1	77729
		LaParguera, March 23, 1976	1/1/4	77729
Diodontidae (porcupinefishes)				
Diodon hystrix Linnaeus 6/1	*Opistholebes diodontis* Cable, 1956	Ahogados reef, LaParguera, April 8, 1981	4/1/20	77789
Ephippidae (spadefishes)				
Chaetodipterus faber (Broussonet) 9/6	*Aponurus elongatus* Siddiqi and Cable, 1960	Media Luna reef, LaParguera, February 4, 1981	4/1/22	77734
	Lecithaster sp.	Mario reef, LaParguera, February 24, 1976	1/1/6	77958
	Multitestis (*Multitestis*) *inconstans* (Linton, 1905)	Mario reef, LaParguera, September 2, 1977	1/1/5	77955

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
		Media Luna reef, LaParguera, February 4, 1981	4/1/6	77763
	Multitestis (*Multitestoides*) *brasiliensis*	LaParguera, November 25, 1974	1/1/1	77765
		LaParguera, November 17, 1974	1/1/100	77765
	Vitellibaculum spinosum (Siddiqi and Cable, 1960)	Bahiá de Guánica, September 24, 1974	1/1/1	77798
Haemulidae (grunts)				
Anisotremus virginicus (Linnaeus) 6/1	*Stephanostomum coryphaenae* Manter, 1947	Isla Magueyes, LaParguera, August 15, 1975	1/1/3	77726
Haemulon flavolineatum (Desmarest) 24/10	*Infundibulostomum spinatum* Siddiqi and Cable, 1960	19 km south of Isla Magueyes, August 23, 1974	8/5/30	77731
	Leurodera decora Linton, 1910	19 km south of Isla Magueyes, August 22, 1974	6/2/3	77748
		Western end of Cayo Enrique, LaParguera, April 4, 1975	1/1/24	77749
	Postmonorchis orthopristis Hopkins, 1941	Cayo Enrique, LaParguera, October 26, 1974	1/1/76	77775
	Pseudoplagioporus brevivitellus Siddiqi and Cable, 1960	Medusa dock, LaParguera, September 16, 1977	1/1/25	77953
Haemulon parrai (Desmarest) 1/1	*Leucodera decora*	Playa Sardinero, Mona Island, April 14, 1974	1/1/3	77750
Holocentridae (squirrelfishes)				
Flammeo marianus (Cuvier and Valenciennes) 7/1	*Lecithochirium microcercus*	19 km south of Isla Magueyes, August 22, 1974	1/1/1	77743
Holocentrus rufus (Walbaum) 6/1	*Helicometra equilata* (Manter, 1933)	Mario Island, July 23, 1981	1/1/2	77783
Kyphosidae (sea chubs)				
Kyphosus sectatrix (Linnaeus) 2/1	*Opisthadena dimidia* Linton, 1910	Carbinero Point, Mona Island, October 13, 1974	1/1/6	77751
Labridae (wrasses)				
Clepticus parrai (Bloch and Schneider) 8/1	*Tergestia laticollis*	Turrumote reef, LaParguera, August 31, 1975	1/1/3	77732
Lachnolaimus maximus (Walbaum) 11/5	*Myzoxenus lachnolaimi* Manter, 1947	Margarita reef, LaParguera, September 4, 1977	1/1/10	77956
		LaParguera, February 5, 1981	2/1/2	77766
		Turrumote reef, LaParguera, August 15, 1981	1/1/1	77767
		Turrumote reef, LaParguera, December 22, 1974	1/1/12	77768
		Isla LaCueva, LaParguera, March 5, 1975	1/1/7	77769
Lutjanidae (snappers)				
Lutjanus apodus (Walbaum) 3/1	*Hamacreadium mutabile* Linton, 1910	Media Luna reef, LaParguera, April 17, 1981	1/1/1	77780
Lutjanus buccanella (Cuvier) 4/1	*Lecithochirium parvum*	Crashboat pier, Aguadilla, October 22, 1975	1/1/4	77735
Lutjanus cyanopterus (Cuvier) 1/1	*Stephanostomum casum* (Linton, 1910)	LaParguera, January 23, 1978	1/1/2	77725

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Lutjanus griseus (Linnaeus) 8/7	*Hamacreadium mutabile*	Ahogado reef, LaParguera, March 18, 1981	2/2/2	77781
		9.7 km south of Isla Magueyes, December 14, 1974	1/1/5	77782
	Hamacreadium sp.	Medusa dock, LaParguera, August 22, 1977	1/1/50	77954
	Metadena adglobosa Manter, 1947	Isla Magueyes, LaParguera, January 3, 1975	1/1/2	
		Isla Magueyes, LaParguera, August 22, 1977	1/1/6	77875
	Stephanostomum casum (Linton, 1910)	LaParguera, January 3, 1975	1/1/50	77724
Ocyurus chrysurus (Bloch) 6/2	*Lepocreadium trulla* (Linton, 1907)	Cayo Enrique, LaParguera, April 14, 1981	2/2/20	77761
Molidae (molas)				
Mola lanceolata Lienard 1/1	*Rhynchopharynx paradoxa* Odhner, 1928	LaParguera, August 26, 1983	1/1/35	77888
Mugilidae (mullets)				
Mugil curema Valenciennes 1/1	*Hysterolecitha rosea* Linton, 1910	Guayanilla Bay, September 16, 1976	1/1/1	77741
Mullidae (goatfishes)				
Mulloidichthys martinicus (Cuvier) 1/1	*Opecoeloides elongatus* Manter, 1947	Cayo Enrique, LaParguera, April 1, 1981	1/1/1	77787
Muraenidae (morays)				
Gymnothorax funebris Ranzani 1/1	*Sterrhurus fusiformis* (Lühe, 1901)	Desecheo Island, February 28, 1975	1/1/58	77753
Gymnothorax moringa (Cuvier) 1/1	*Sterrhurus fusiformis*	Turrumote reef, LaParguera, November 23, 1974	1/1/2	77752
Ophichthidae (snake eels)				
Myrichthys oculatus (Kaup) 1/1	*Lecithochirium monticellii*	South shore of Desecheo Island, February 28, 1975	1/1/6	77746
Opistognathidae (jawfishes)				
Opistognathus aurifrons (Jordan and Thompson) 3/1	*Genitocotyle atlantica* Manter, 1947	Media Luna reef, LaParguera, March 10, 1979	1/1/1	
Ostraciidae (boxfishes)				
Lactophrys bicaudalis (Linnaeus) 5/1	*Megapera gyrina* (Linton, 1907)	Mario Island, January 23, 1981	1/1/3	77773
Pempheridae (sweepers)				
Pempheris schomburgki Müller and Troschel 10/1	*Lecithochirium monticellii*	Laural reef, LaParguera, January 21, 1981	1/1/4	
Pomacanthidae (angelfishes)				
Holacanthus ciliaris (Linnaeus) 4/1	*Barisomum erubescens* Linton, 1910	Laural reef, LaParguera, September 7, 1974	1/1/1	77796
Pomacanthus arcuatus (Linnaeus) 17/7	*Antorchis urna* (Linton, 1910)	Turrumote reef, LaParguera, September 14, 1974	3/2/1	77730
	Barisomum candidulum (Linton, 1910)	Turrumote reef, LaParguera, September 14, 1974	3/1/1	77793
	Barisomum erubescens	Bird Island, March 16, 1981	5/2/3	77795

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
	Cleptodiscus reticulatus Linton, 1910	Bird Island, March 16, 1981	5/2/25	77791
		8.1 km southwest of Isla Magueyes, August 23, 1973	1/1/24	77792
	Theletrum fustiforme	Laural reef, LaParguera, September 7, 1974	1/1/31	77755
		Laural reef, LaParguera, September 21, 1974	2/1/90	77755
Pomacanthus paru (Bloch) 3/2	*Barisomum candidulum*	Carbinero Point, Mona Island, October 13, 1974	2/2/50	77794
Pomacentridae (damselfishes)				
Abudefduf saxatilis (Linnaeus) 8/1	*Deretrema fusillus* Linton, 1910	Media Luna reef, LaParguera, September 25, 1976	1/1/1	
Chromis multilineatus (Guichenot) 11/1	*Lepocreadium trulla*	Mouth of Añasco River, Mayagüez Bay, March 1, 1975	2/1/2	77762
Priacanthidae (bigeyes)				
Priacanthus cruentatus (Lacapède) 6/2	*Lecithochirium parvum*	Laural reef, LaParguera, August 11, 1977	1/1/4	77736
	Lecithochirium microcercus	Laural reef, LaParguera, February 1, 1975	1/1/6	77744
Scaridae (parrotfishes)				
Sparisoma aurofrenatum (Valenciennes) 1/1	*Schikhobalotrema* sp.	19 km south of Isla Magueyes, August 22, 1974	1/1/2	77733
Sciaenidae (drums)				
Cynoscion jamaicensis (Vaillant) 29/1	*Tubulovesicula pinguis* (Linton, 1940)	Mouth of Añasco River, Mayagüez Bay, October 9, 1975	6/1/4	77757
Equetus acuminatus (Schneider) 9/1	*Manteriella crassa* (Manter, 1947)	Crashboat Pier, Aguadilla, February 7, 1981	2/1/2	77785
Equetus punctatus (Schneider) 3/1	*Pinguitrema lobatum* Siddiqi and Cable, 1960	Mario Island, January 23, 1981	1/1/6	77790
Larimus breviceps (Cuvier) 10/1	*Sterrhurus* sp.	Mouth of Añasco River, Mayagüez Bay, October 9, 1975	1/1/1	77754
Micropogonias furnieri (Desmarest) 2/1	*Lecithochirium microcercus*	Bahiá de Guánica, September 24, 1974	2/1/4	77877
Odontoscion dentex (Cuvier) 12/2	*Manteriella crassa*	Cayo Enrique, LaParguera, February 7, 1975	1/1/1	77786
	Opecoeloides sp.	Cayo Enrique, LaParguera, April 14, 1981	1/1/1	77788
Scombridae (mackerels)				
Acanthocybium solanderi (Cuvier) 5/5	*Hirudinella* sp.	LaParguera, April 1, 1976	1/1/2	77758
	Hirudinella ventricosa Pallas, 1774	Media Luna reef, LaParguera, April 25, 1981	1/1/3	
		Desecheo Island, October 15, 1978	1/1/1	77759
		Desecheo Island, October 22, 1977	1/1/1	77760
		Mona Island, March 23, 1975	1/1/4	

Table 1. Continued.

Host (no. examined/ no. infected)	Parasite	Locality, date	No. hosts examined/no. infected/avg. no. worms per infected host	USNM Helm. Coll. No.
Scorpaenidae (scorpionfishes)				
Scorpaena plumieri (Bloch, 1789) 3/2	*Lecithochirium parvum*	LaParguera, January 15, 1978	1/1/9	77738
	Lecithochirium microcercus	Aguadilla, March 15, 1981	1/1/2	
Serranidae (sea basses)				
Epinephelus adscensions (Osbeck) 4/2	*Helicometra torta* Linton, 1910	Playa Sardinero, Mona Island, April 13, 1975	1/1/10	77957
		Media Luna reef, LaParguera, February 23, 1981	1/1/1	77784
Epinephelus guttatus (Linnaeus) 9/3	*Cainocreadium longisaccum* (Siddiqi and Cable, 1960)	Media Luna reef, LaParguera, February 9, 1981	1/1/2	77778
		Laural reef, LaParguera, February 22, 1981	4/2/3.5	77779
Epinephelus striatus (Bloch) 6/2	*Neolepidapedon (Neolepidapedoides) epinepheli*	Mona Island, April 15, 1975	1/1/6	77873
	Cainocreadium lintoni (Siddiqi and Cable, 1960)	Carbinero Point, Mona Island, October 13, 1974	1/1/12	77776
Mycteroperca interstitialis (Poey) 1/1	*Stephanostomum dentatum* (Linton, 1901)	Cayo Enrique, LaParguera, February 16, 1979	1/1/2	77727
Sparidae (porgies)				
Archosargus rhomboidalis (Linnaeus) 4/2	*Plagioporus crassigula* (Linton, 1910)	Isla Magueyes, LaParguera, February 20, 1981	1/1/1	77876
		Phosphorescent Bay, December 9, 1974	1/1/5	
Sphyraenidae (barracudas)				
Sphyraena barracuda (Walbaum) 10/1	*Bucephalopsis attenuata* (Siddiqi and Cable, 1960)	Isla LaCueva, LaParguera, March 5, 1975	5/1/1	
Synodontidae (lizardfishes)				
Saurida normani Longley 1/1	*Parahemiurus merus*	LaParguera, September 4, 1976	1/1/2	
Synodus foetens (Linnaeus) 3/1	*Lecithochirium parvum*	Mayagüez Bay, February 16, 1979	1/1/7	
Synodus intermedius (Agassiz) 1/1	*Sterrhurus musculus* Looss, 1907	0.4 km southwest of Isla Magueyes, September 21, 1974	1/1/9	77742
Tetradontidae (puffers)				
Sphoeroides spengleri (Bloch) 1/1	*Pseudomegalophallus* sp.	LaParguera, January 10, 1975	1/1/15	
Triglidae (searobins)				
Prionotus punctatus (Bloch) 3/2	*Lecithochirium parvum*	Mayagüez Bay, October 9, 1975	3/2/4	

Lecithostaphylus nitens in *Platybelone argalus* (Lesueur), *Rhynchopharynx paradoxa* in *Mola lanceolata* (Lienard), *Genitocotyle atlantica* in *Opisthognathus aurifrons* (Jordan and Thompson), and *Tubulovesicula pinguis* in *Cynoscion jamaicensis* (Vaillant).

Discussion

The only extensive study of digenetic flukes of marine fishes from the western and southwestern coasts of Puerto Rico as well as Mona Island, approximately 72.4 km distant, is that of Siddiqi and Cable (1960). Seventy-six species of fishes

(63.3%) examined by them were infected with a total of 123 digenean species. Because of major differences in the various groups of fishes examined in their study and ours and because the prevalence and intensity for each species of digenetic fluke could not be ascertained from the data presented by them, comparison could not be initiated. They described 45 new, and reported 78 previously known species, whereas the present study reports only 66 species, none of which is new. In addition to differences mentioned above, some of this variance might be attributed to differences in specific localities, as it is well known that parasites abundant in one area may be absent from the same host species only a short distance away (Van Cleave and Mueller, 1934).

Siddiqi and Cable (1960) found that of 76 species harboring digeneans, 42 were infected with only 1 species (55.2%), 12 with 2 (15.8%), 9 with 3 (11.8%), 5 with 4 (6.6%), 3 with 5 (3.9%), 3 with 6 (3.9%), 1 with 7 (1.3%), and 1 with 9 (1.3%). In both studies, the majority of infections encountered constituted single infections. The number of hosts with mixed infections decreased as the variety of parasites increased. Calculated from their data, 90 (73.2%) of the 123 species of digenetic flukes found occurred in but 1 host species, 28 (22.8%) in 2 host species, 3 (3.4%) in 3, and 2 (1.6%) in 4. None was found to have five or more host species.

Comparison of these findings with those reported in the present study show that whereas differences exist for the various numerical categories of hosts, both studies reveal that digenetic flukes have a high degree of host specificity in marine fishes. These results substantiate the findings of Manter (1957) who summarized the extent to which digenetic trematodes have been reported from one or more species of marine fishes in Japan, Tortugas, the Mediterranean, and the British Isles as well as information given by Nahhas and Cable (1964) on host specificity of the digenetic flukes of marine fishes of Curącao and Jamaica.

Eleven digenetic flukes reported in the present study and at least 45 reported by Siddiqi and Cable (1960) represent new geographic localities. Environmental factors such as water temperature, depth, velocity of water currents, the presence or absence of intermediate hosts, and others that are of a more complex nature and as yet not well understood, contribute to the geographic distribution of digenetic trematodes. The phenomenon of geographic distribution of digenetic flukes of marine fishes from widely separated geographic localities has been summarized by Manter (1947, 1955). Siddiqi and Cable (1960) compared the trematode fauna of marine fishes from the western and southwestern coasts of Puerto Rico with that of marine fishes off Mona Island. Of the 105 species of fish from Puerto Rico and the 24 species from Mona Island, only 6 were common to both areas. Although differences in sample sizes as well as differences in host species were contributing factors in the diversity of flukes, speciation resulting from isolation of land masses by deep water channels also attributed to this variance. Our sample size from Mona Island is at present inadequate to provide data by which any valid conclusions concerning differences in the diversity of flukes from these two areas can be formulated.

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Appendix 1
Species of Fish Negative for Digeneans Listed Alphabetically by Family with the Number of Individuals Examined in Parentheses

Bony fishes

Acanthuridae—*Acanthurus bahianus* Castelnau (1), *A. coeruleus* Schneider (2); Albulidae—*Albula vulpes* (Linnaeus) (2); Antennariidae—*Antennarius multiocellatus* (Valenciennes) (1); Apogonidae—*Apogon lachneri* Böhlke (10), *A. maculatus* (Poey) (1), *A. planifrons* Longley and Hildebrand (1), *A. townsendi* (Breder) (2), *Astrapogon stellatus* (Cope) (5), *Phaeoptyx pigmentaria* (Poey) (1); Atherinidae—*Allanetta harringtonensis* (Goode) (1); Aulostomidae—*Aulostomus maculatus* Valenciennes (8); Balistidae—*Aluterus scriptus* (Osbeck) (4), *Balistes vetula* Linnaeus (3), *Cantherhines macrocerus* (Holland) (1), *C. pullus* (Ranzani) (9), *Canthidermis sufflamen* (Mitchill) (1), *Melichthys niger* (Bloch) (1), *Monacanthus tuckeri* Bean (1), *Xanthichthys ringens* (Linnaeus) (4); Belonidae—*Tylosurus crocodilus crocodilus* (Peron and Lesueur) (4); Blenniidae—*Entomacrodus nigricans* Gill (1), *Ophioblennius atlanticus* (Valenciennes) (2); Bothidae—*Etropus crossotus* Jordan and Gilbert (3), *Syacium gunteri* Ginsburg (13), *S. micrurum* Ranzani (1); Callionymidae—*Callionymus pauciradiatus* Gill (1); Carangidae—*Caranx crysos* (Mitchill) (1), *C. latus* Agassiz (9), *C. ruber* (Bloch) (7), *Selar crumenophthalmus* (Bloch) (1), *Selene vomer* (Linnaeus) (1); Chaetodontidae—*Chaetodon aculeatus* (Poey) (3), *C. capistratus* Linnaeus (4), *C. striatus* (Linnaeus) (4); Cirrhitidae—*Amblycirrhitus pinos* (Mowbray) (1); Clinidae—*Acanthemblemaria betinensis* Smith-Vaniz and Palacio (1), *A. maria* Böhlke (1), *Chaenopsis limbaughi* Robins and Randall (1), *Emblemaria bahamensis* (Stephens) (1), *Emblemariopsis signifera* (Ginsburg) (1), *Labrisomus bucciferus* (Poey) (1), *L. filamentosus* Springer (1), *Lucayablennius zingaro* (Böhlke) (1), *Malacoctenus macropus* (Poey) (2), *Stathmonotus gymnodermis* Springer (2), *S. stahli* (Evermann and Marsh) (1); Clupeidae—*Chirocentrodon bleekerianus* (Poey) (6), *Harengula clupeola* (Cuvier) (6), *Opisthonema oglinum* (Lesueur) (3); Cynoglossidae—*Symphurus arawak* Robins and Randall (9), *S. plagiusa* (Linnaeus) (9); Dactylopteridae—*Dactylopterus volitans* (Linnaeus) (1); Dactyloscopidae—*Platygillellus rubrocinctus* (Longley) (1), Echeneidae—*Echeneis naucratus* (Linnaeus) (3); Eleotridae—*Eleotris pisonis* (Gmelin) (5); Elopidae—*Megalops atlanticus* (Valenciennes) (2); Engraulidae—*Anchoa filifera* (Fowler) (2), *Anchovia clupeoides* (Swainson) (21), *A.* sp. (5); Exocoetidae—*Hemiramphus balao* Lesueur (5), *H. brasiliensis* (Linnaeus) (9), *Hyporhamphus unifasciatus* (Ranzani) (1); Fistulariidae—*Fistularia tabacaria* Linnaeus (1); Gerreidae—*Diapterus olisthostomus* (Goode and Bean) (1), *D. rhombeus* (Cuvier) (35), *Eucinostomus argenteus* Baird and Girard (3), *Gerres cinereus* (Walbaum) (4), *Mojarra* sp. (1); Gobiesocidae—*Arcos macrophthalmus* (Günther) (1); Gobiidae—*Coryphopterus glaucofraenum* Gill (8), *C. personatus* (Jordan and Thompson) (1), *Elacatinus chancei* (Beebe and Hollister) (1), *Gnatholepis thompsoni* Jordan (11), *Gobiosoma* sp. (7); Grammidae—*Gramma loreto* Poey (3); Grammistidae—*Rypticus saponaceus* (Schneider) (5); Haemulidae—*Anisotremus surinamensis* (Bloch) (3), *Conodon nobilis* (Linnaeus) (1), *Haemulon album* Cuvier (5), *H. aurolineatum* Cuvier (3), *H. bonariense* Cuvier (2), *H. carbonarium* Poey (2), *H croicensis* Bloch (1), *H. macrostomum* (Günther) (4), *H. plumieri* (Lacepède) (4), *H. sciurus* (Shaw) (3), *Pomadasys corvinaeformis* (Steindachner) (8); Holocentridae—*Holocentrus ascensionis* (Osbeck) (1), *Myripristis jacobus* Cuvier (6); Inermiidae—*Inermia vittata* Poey (2); Labridae—*Bodianus rufus* (Linnaeus) (1), *Decodon puellaris* (Poey) (1), *Halichoeres bivittatus* (Bloch) (4), *H. garnoti* (Valenciennes) (4), *H. maculipinna* (Müller and Troschel) (1), *H. pictus* (Poey) (1), *H. poeyi* (Steindachner) (1), *H. radiatus* (Linnaeus) (2), *Hemipteronotus splendens* (Castelnau) (3), *Thalassoma bifasciatum* (Bloch) (15); Lutjanidae—*Apsilus dentatus* Guichenot (1), *Lutjanus analis* (Cuvier) (1), *L. mahogoni* (Cuvier) (1), *L. synagris* (Linnaeus) (13), *L. vivanus* (Cuvier) (1); Malacanthidae—*Malacanthus plumieri* (Bloch) (1); Mullidae—*Pseudupeneus maculatus* (Bloch) (3); Muraenidae—*Enchelycore* sp. (1), *Muraena miliaris* (Kaup) (1); Ogcocephalidae—*Ogocephalus nasutus* (Valenciennes) (1); Ophidiidae—*Ophidion* sp. (9); Ostraciidae—*Lactophrys polygonia* (Poey) (3), *L. quadricornis* (Linnaeus) (3), *L. trigonus* (Linnaeus) (4), *L. triqueter* (Linnaeus) (3); Polynemidae—*Polydactylus oligodon* (Günther) (7); Pomacanthidae—*Holacanthus tricolor* (Bloch) (4); Pomacentridae—*Abudefduf taurus* (Müller and Troschel) (3), *Chromis cyaneus* (Poey) (2), *C. insolatus* (Cuvier) (5); *Pomacentrus fuscus* Cuvier (5), *P. leucostictus* Müller and Troschel (1), *P. mellis* (Emery and Burgess) (1), *P. partitus* Poey (6), *P. planifrons* Cuvier (8), *P. variabilis* (Castelnau) (1); Priacanthidae—*Priacanthus arenatus* Cuvier (2), *Pristigenys alta* (Gill) (1); Scaridae—*Scarus coeruleus* (Bloch) (1), *S. croicensis* Bloch (2), *S. vetula* Schneider (1), *Sparisoma radians* (Valenciennes) (2), *S. rubripinne* (Valenciennes) (5), *S. viride* (Bonnaterre) (8); Sciaenidae—

Ophioscion adustus (Agassiz) (1), *Stellifer colonensis* (Meek and Hildebrand) (22); Scombridae—*Katsuwonus pelamis* (Linnaeus) (1), *Scomberomorus regalis* (Bloch) (7); Scorpaenidae—*Pontinus castor* Poey (1); Serranidae—*Diplectrum formosum* (Linnaeus) (1), *D. radiale* (Quoy and Gaimard) (1), *D.* sp. (2), *Epinephelus afer* (Bloch) (1), *E. cruentatus* (Lacepède) (3), *E. fulvus* (Linnaeus) (5), *E. itajara* (Lichtenstein) (1), *Hypoplectrus aberrans* Poey (6), *H. chlorurus* (Valenciennes) (6), *H. guttavarius* (Poey) (2), *H. indigo* (Poey) (1), *H. unicolor* Walbaum (4), *Liopropoma mowbrayi* Woods and Kamazawa (3), *L. rubre* Poey (1), *Mycteroperca bonaci* (Poey) (1), *M. rubra* (Bloch) (1), *M. tigris* (Valenciennes) (1), *Paranthias furcifer* (Valenciennes) (2), *Serranus baldwini* (Evermann and Marsh) (1), *S. phoebe* Poey (1), *S. tabacarius* (Cuvier) (2), *S. tigrinus* (Bloch) (2); Soelidae—*Achirus lineatus* (Linnaeus) (4); Sparidae—*Calamus calamus* (Valenciennes) (5); Sphyraenidae—*Sphyraena picudilla* (Poey) (1); Tetradontidae—*Canthigaster rostrata* (Bloch) (4), *Lagocephalus laevigatus* (Linnaeus) (2), *Sphoeroides greeleyi* Gilbert (4), *S. testudineus* (Linnaeus) (6); Xiphiidae—*Xiphias gladius* Linnaeus (2).

Batoid fishes

Dasyatidae—*Dasyatis americana* Hildebrand and Schroeder (1), *Urolophus jamaicensis* (Cuvier) (2); Mobulidae—*Mobula hypostoma* (Bancroft) (1); Myliobatidae—*Aetobatus narinari* (Euphrasen) (1).

Sharks

Carcharhinidae—*Carcharhinus falciformis* (Bibron) (2), *C. obscurus* (Lesueur) (1), *Galeocerdo cuvieri* (Peron and Lesueur) (6), *Negaprion brevirostris* (Poey) (2), *Rhizoprionodon porosus* (Poey) (1); Hexanchidae—*Hexanchus vitulus* Springer and Waller (1); Orectolobidae—*Ginglymostoma cirratum* (Bonnaterre) (2); Squalidae—*Squalus cubensis* (Howell and Rivero) (1).

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Parasites of the Florida Panther (*Felis concolor coryi*)

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ABSTRACT: Between 1978 and 1983 12 Florida panthers (*Felis concolor coryi* Bangs) were examined for parasites. Seven were examined at necropsy and the other five were live animals examined during capture operations. Findings included 1 species of protozoan, 2 trematodes, 3 cestodes, 7 nematodes, 6 ticks, and 1 flea. All panthers were infected with at least six species of parasites. Intensities varied from 263 to 10,094 parasites per animal. The two most prevalent and abundant parasites were the diplostomatid trematode *Alaria marcianae* (LaRue, 1917) and the hookworm *Ancylostoma pluridentatum* (Alessandrini, 1905).

Today the only documented population of the Florida panther exists in remote areas in southern Florida where there are an estimated 20–50 animals. In 1967 it was declared an endangered species by the U.S. Department of Interior (FR 48). Currently there is no published information on the parasites and diseases of this rare cat. In 1978 a study was undertaken to determine the parasite fauna of the Florida panther and a summary is presented herein.

Materials and Methods

From March 1978 through December 1983 parasites were obtained from 12 different Florida panthers in southern Florida (Dade, Collier, and Glades counties). Seven of these animals were examined at necropsy (5 road-killed animals, 1 illegally killed animal, and 1 animal killed accidentally during capture operations) and five were animals from which ectoparasites and blood and fecal samples were obtained during a radiotelemetry project. Techniques for the recovery of ectoparasites, helminths, intestinal protozoans, and blood parasites were as described by Forrester et al. (1974) including the use of fine-mesh screens for obtaining small helminths. Samples of diaphragm (and in some cases other muscles) were examined for the presence of larvae of *Trichinella* sp. by tissue squash (three cats) or by both tissue squash and pepsin-HCl digestion (four cats). Serum samples were tested for antibodies to *Toxoplasma gondii* by the indirect hemagglutination test (Jacobs and Lunde, 1957) with titers $\geq$1:256 considered positive.

Representative specimens of the helminths have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705 (USNM Helm. Coll. Nos. 78380–78393) and specimens of the ticks have been deposited in the U.S. National Museum, Smithsonian Institution, Washington, D.C. 20560 (Accession Nos. RML 112157, 112391, 112392, 115550, 115551, 115581, 115582, 115897–115900, and 116845–116849).

Results and Discussion

Twenty species of parasites were detected including 1 protozoan, 2 trematodes, 3 cestodes, 7 nematodes, 6 ticks, and 1 flea. Table 1 shows the locations within the host and prevalences and intensities of infection for each of these parasites. All panthers were infected with at least six species of parasites (mean, 9; median, 8; range, 6–12). The total number of parasites per cat varied between 263 and 10,094 (mean 3,388; median, 2,373).

The two most prevalent and abundant parasites were the diplostomatid trematode *Alaria marcianae* and the hookworm *Ancylostoma pluridentatum*. *Alaria marcianae* has been reported previously from a mountain lion (*Felis concolor acrocodia*) in Paraguay and is a common parasite of a number of other felids such as the bobcat, *Felis rufus* (Schreber), and also several canids and mustelids (Johnson, 1968). In Florida it has been reported by Conti (1984) from gray foxes, *Urocyon cinereoargenteus* (Schreber), and coyotes, *Canis latrans* Say.

The hookworm (*Ancylostoma pluridentatum*), however, never has been reported from free-ranging *Felis concolor,* but is known from a captive cougar in the New York Zoological Park (McClure, 1933) and from a number of wild feline hosts in Panama and Colombia (Thatcher, 1971). It has also been found in several captive felids originating from Central and South America that died in the National Zoological Park in Washington, D.C. (Schwartz, 1927). Although the parasites of bobcats in southern Florida have not been studied, we were able to examine the intestines of three bobcats trapped in Florida

Table 1. Prevalence and intensity of parasites from Florida panthers (*Felis concolor coryi*), 1978–1983.

Parasite	No. panthers		Intensity[a]		
	Examined	Positive	Mean	Median	Range
Protozoa					
Toxoplasma gondii (Nicolle and Manceaux, 1908) (1)[b]	5[c]	1[d]	—	—	—
Trematoda					
Alaria marcianae (LaRue, 1917) (2)	7	7	2,972	1,090	238–9,689
Heterobilharzia americana Price, 1929 (3)	7	5	4	3	1–7
Cestoda					
Mesocestoides sp. (2)	7	4	212	6	1–833
Spirometra mansonoides (Mueller, 1935) (2)	7	4	13	8	4–33
Taenia omissa Lühe, 1910 (2)	7	4	7	6	1–15
Nematoda					
Ancylostoma pluridentatum (Alessandrini, 1905) (2)	7	6	254	160	36–744
Trichinella sp. larvae (4)	7	4[e]	—	—	—
Dirofilaria striata (Molin, 1858) (4)[f]	7	4	3	2	1–7
Capillaria aerophila (Creplin, 1839) (5)	7	2	2	2	1–3
Strongyloides sp. (2)	7	2	9	9	1–16
Toxocara cati (Schrank, 1788) (2)	7	1	2	2	2
Toxocara sp. larvae (2)	7	1	25	25	25
Molineus barbatus Chandler, 1942 (2)	7	1	2	2	2
Arthropoda					
Dermacentor variabilis (Say, 1821) (6)	12	9	8	4	1–26
Ixodes scapularis Say, 1821 (6)	12	7	49	39	3–222
Ixodes affinis Neumann, 1899 (6)	12	4	5	5	1–10
Amblyomma americanum (Linnaeus, 1758) (6)	12	1	8	8	8
Amblyomma maculatum (Koch, 1844) (6)	12	1	2	2	2
Dermacentor nitens Neumann, 1897 (6)	12	1	1	1	1
Ctenocephalides felis (Bouché, 1835) (6)	12	1	1	1	1

[a] Number of parasites per infected or infested host.

[b] Numbers in parentheses indicate site in host: (1) blood, (2) small intestine, (3) blood vessels of liver and lungs, (4) connective tissue between muscles, (5) lungs, and (6) skin and pelage.

[c] Information on *Toxoplasma* is based on serologic studies. Data on one of these negative serum samples were given in an earlier paper (Burridge et al., 1979).

[d] The titer on this animal was 1:256.

[e] Intensity of infection was determined for one panther that had <1 larva per gram of diaphragm and psoas muscle.

[f] Values for *Dirofilaria striata* are based on adults found at necropsy. The prevalence of this filariid is probably greater than indicated because the adult forms are difficult to locate.

panther habitat (Collier County). Two of the three bobcats had infections of *A. pluridentatum.*

The other species of helminths except *Strongyloides* sp. have been reported previously from other subspecies of *Felis concolor* (Lee, 1962; Stone and Pence, 1978; Anderson, 1983; Rausch et al., 1983). *Trichinella* has been reported several times from the puma in western North America (Anderson, 1983). In Florida it is known from foxes (species not given), opossums (*Didelphis virginiana* Kerr), raccoons (*Procyon lotor* (L.)), and skunks (species not given) (Scholtens and Norman, 1971). In 1982 and 1983 we digested samples of diaphragm and/or tongue from 26 wild hogs (*Sus scrofa* L.) from Collier County where Florida panthers occur and found larvae of *Trichinella* sp. in one hog. Wild hogs are one of the main food items of the Florida panther (Belden, 1984) and it is probable that infections of panthers are acquired from these animals.

Although one of five panthers examined serologically was found to have antibodies to *Toxoplasma gondii,* no oocysts were detected in fecal samples taken from eight different panthers including the seropositive animal. *Toxoplasma* infections have been found to be widespread among wildlife in Florida, especially among armadillos (*Dasypus novemcinctus* L.), raccoons, and roof rats (*Rattus rattus* (L.)) (Burridge et al., 1979). No blood protozoans were found in blood films

examined from five panthers although microfilariae were found in all five. Three of the five cats with microfilariae were examined at necropsy and adults of *Dirofilaria striata* were found in each. No other filarial worms were detected and we presume, therefore, that these microfilariae are *D. striata.*

Of the six species of ticks found on panthers only two (*Dermacentor variabilis* and *Amblyomma americanum*) have been reported previously from *Felis concolor* (Bishopp and Trembley, 1945; Anderson, 1983). With the exception of *Dermacentor nitens,* which is a parasite of horses in southern Florida (Strickland et al., 1976), the ticks found on the Florida panther also occur in Florida on wild hogs (Greiner et al., 1984) and/or on white-tailed deer (*Odocoileus virginianus* (Zimmermann)) (Smith, 1977).

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Morphogenesis of Developmental Stages of *Dirofilaria immitis* (Nematoda) in the Dog

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ABSTRACT: Morphogenesis of the dog heartworm, *Dirofilaria immitis,* through the third and fourth molts to the fifth stage is described. Specimens were collected from mosquitoes 14 or 15 days after infection (DAI) or from dogs 3–79 DAI. Infective larvae from mosquitoes were 0.7–1.0 mm long with a tapered blunt anterior end, bilayered cuticle, refractile granules in the anterior ⅔ of the glandular esophagus, and a pair of small, broadly based, subventral bumps near the larger conical tail tip. Sexes were distinguished by the position of the oval-shaped genital primordium (GP) (near the anterior part of the glandular esophagus in females and just anterior to midbody in males), and by the presence of spicular primordia in males. When larvae were first collected from dogs 3 DAI most had completed the third molt. Fourth-stage larvae (L-4) at 3–6 DAI were 1.0–1.5 mm long, had untapered anterior ends, and had sharply defined, angular submedian papillae near a button-like tail tip. Posterior growth of the male GP was seen first in L-4's 9 DAI and it reached the rectum 30 DAI. Genital papillae and developing spicules of the fifth stage were evident 41 DAI. Formation of the vagina was initiated 9 DAI by hypodermal invasion of the GP at its attachment point. By 12 DAI the female GP was enlarged and swollen posteriorly with two large nuclei near the posterior tip. By 21 DAI two branches of the GP developed, each with a large nucleus at its posterior tip; by 30 DAI each branch extended beyond the junction of the esophagus and intestine (E–I). The vulva was located near but anterior to the E–I 41–58 DAI. The fourth molt occurred 50–58 DAI. Some specimens of both sexes completed ecdysis by 58 DAI. Early fifth-stage specimens at 58 DAI were 12–14.8 mm long with a thick, finely striated cuticle. Spicules were almost completely sclerotized and as large as in mature males by 79 DAI. Spermatozoa were present in the testis but not in the vas deferens 79 DAI.

The development of the dog heartworm, *Dirofilaria immitis,* in the dog has been described by Kume and Itagaki (1955), Orihel (1961), and Kotani and Powers (1982). The only description of the morphogenesis of *D. immitis* in the dog was by Orihel (1961). Because of the high cost of dogs for research and humane considerations much current research on *D. immitis* is carried out in model animal systems or in vitro rather than in dogs. Studies by Taylor (1960), Yoeli et al. (1964), Sawyer (1965), Sawyer and Weinstein (1965), Wong et al. (1982), and Lok et al. (1984) in vitro; and by P. Supakorndej, J. J. Jun, and J. W. McCall (pers. comm.) in a model animal system all have reported developmental differences from that described by Orihel (1961) in the dog, especially in the time of the third molt. The objective of this study was to redescribe the morphogenesis of *D. immitis* in its normal definitive host, the dog. The results should be useful for evaluating the development of the nematode in vitro and in model animal systems.

Materials and Methods

Specimens

Specimens of *Dirofilaria immitis* for study were collected in a previous project on development in dogs reported by Kotani and Powers (1982). Nematodes were dissected from two beagle dogs 3, 6, 9, 12, 15, 21, 30, 41, 50, 70, and 79 days after infection (DAI) as described previously (Kotani and Powers, 1982). Some specimens collected by Kotani and Powers (1982) were used in other studies and were unavailable for this study. All available specimens 3–15 DAI were studied. Fewer than the total number available from 21 to 79 DAI were studied. The numbers of specimens studied (*N*) are given with the Results. Kotani and Powers (1982) also collected specimens from 84–196 DAI, but those specimens were not part of this study.

Infective larvae (third stage) were allowed to emerge from the mouthparts of cold-inactivated mosquitoes, *Aedes aegypti* (Liverpool strain), into Hanks' balanced salt solution 14 or 15 DAI (Kotani and Powers, 1982). Additional specimens were collected for study from *A. aegypti* (Liverpool selected strain) at 12 DAI (Lok et al., 1984) and from in vitro cultures (Wong et al., 1982;

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Table 1. Measurements* of *Dirofilaria immitis*: infective larvae from mosquitoes; third molt from in vitro cultures; and third, fourth, and early fifth stages from dogs by stage of development and days after infection.

Stage of development (age of infection)	Total length (mm)	Diameter at level of			Distance from anterior extremity to			Length of			
		Anterior extremity	Base of esophagus	Anus	Nerve ring	Genital primordium			Reproductive system		
						Male	Female	Esophagus	Male	Female	Tail
Infective third (14 or 15 DAI)	0.74–1.04	8–13	22–49	17–22	74–100	336–528	112–216	256–400	14–22	11–23	29–42
Late third (6 DAI)†	0.83	9	—	15	69	—	—	—	—	—	28
Third molt (2 or 3 days)	0.72–1.17	10–20	19–38	14–28	71–95	365–496	144–200	256–400	16–20	15–20	32–46
Early fourth (3 DAI)	0.98–1.30	14–20	21–30	18–23	79–100	352–592	144–288	272–448	20–28	15–42	34–46
(6 DAI)	1.14–1.56	15–22	15–36	16–28	82–121	464–640	200–224	256–560	19–23	20–38	29–52
Middle fourth (9 DAI)	1.33–1.66	17–27	25–35	20–29	72–110	448–688	200–240	256–432	22–105	27–43	35–47
(12 DAI)	1.33–1.84	19–25	24–38	19–28	83–101	416–784	192–236	288–448	36–275	25–52	35–52
(15 DAI)	1.69–2.21	19–31	29–38	21–31	89–113	672–864	196–256	304–448	273–496	41–55	37–54
(21 DAI)	1.32–3.24	28–40	37–50	24–37	88–120	704–1,490	224–288	272–495	1.09–1.64 mm	74–129	37–57
(30 DAI)	3.12–5.63	34–58	41–74	29–52	99–131	1.37–2.69 mm	265–368	320–496	1.38–2.30 mm	239–425	41–68
Late fourth (41 DAI)	5.63–9.20	39–84	60–98	36–79	110–137	2.24–3.27 mm	300–432	368–560	2.90–6.96 mm	1.01–2.46 mm	41–74
(50 DAI)	8.65–12.8	50–84	90–138	48–92	125–147	1.75–2.24 mm	424–520	448–624	7.20–9.20 mm	4.08–7.19 mm	60–82
(58 DAI)	8.71–12.8	60–68	103–126	42–84	128–152	0.73–1.04 mm	453–528	435–608	8.48–8.77 mm§	6.24–7.36 mm	58–92
Fourth molt (50 DAI)‡	8.29–10.4	58–74	85–119	63–84	126–157	1.27–2.35 mm	—	480–560	6.77–8.35 mm	—	63–79
(58 DAI)	8.89–13.2	46–89	90–145	52–87	120–140	0.85–1.94 mm	454–665	540–665	7.93–9.26 mm	8.13–11.1 mm	63–84
Early fifth (58 DAI)	11.4–14.8	59–89	115–144	58–84	126–152	2.84–4.42 mm	448–588	540–797	8.47–10.8 mm	8.93–10.2 mm‖	71–89
(70 DAI)	19.2–29.4	59–96	147–229	76–102	128–168	7.92–11.8 mm	496–693	666–896	12.0–14.6 mm	12.7–20.6 mm	66–92
(79 DAI)	34.5–47.1	82–114	178–256	85–108	166–220	18.6–22.5 mm	726–1,400	787–975	20.8–24.6 mm	20.3–28.8 mm	80–145

* Measurements in micrometers unless noted otherwise.
† One specimen.
‡ Males only.
§ Two males measured.
‖ Four females measured.

Lok et al., 1984). Terminology for the phases of development follows that used by Douvres et al. (1969) for *Ascaris suum*. At least 10 specimens of each sex were measured for larvae collected 0–41 DAI. Five specimens of each sex were measured for specimens collected 50–79 DAI. Additional morphometrics of specimens from this study were reported by Kotani and Powers (1982). Voucher specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (Nos. 78343–78365).

Fixing and clearing

Infective larvae were studied alive after heat inactivation at 37°C for 1 hr on a slidewarming table; the remaining specimens were fixed in hot (60°C) 5% neutral buffered formalin; hot 70% ethanol-glycerine (20:1), or cold (4°C) 3% glutaraldehyde buffered with 0.2 M potassium phosphate at pH 6.8. Specimens for light microscopy were cleared for study in tinted glycerine (<0.01% cottonblue). Specimens studied by electron microscopy were postfixed 2 hr in 2% buffered osmium tetroxide, dehydrated in ethanol, and critical-point-dried in liquid CO_2 (Humphreys, 1975).

Microscopy

Most specimens were studied and photographed as whole mounts in glycerine with an oil immersion lens and interference-contrast microscopy. SEM specimens were attached with adhesive to a stub, coated with gold/palladium, and viewed at 5–20 kV (Madden and Tromba, 1976). TEM specimens were stained with uranyl acetate and lead citrate and viewed at 60 kV with a 20-μm aperture (Endo and Wergen, 1973; Wergin and Endo, 1976).

Results

All available *Dirofilaria immitis* specimens (80) recovered from the two dogs necropsied 3 DAI had completed the third molt. Among the 84 available specimens collected at 6 DAI was a single specimen in the third stage; all others were in the fourth stage through 41 DAI. Some specimens collected at 50 and most at 58 DAI were in the fourth molt; and some specimens of both sexes ecdysed to fifth stage by 58 DAI. By 70 DAI all specimens were fifth stage. Sexes could be distinguished in infective larvae by the position of the genital primordia (GP) and by the presence of spicular primordia in males. The morphogenesis of *D. immitis* will be described in seven phases: infective third stage, third molt, early fourth stage, mid-fourth stage, late fourth stage, fourth molt, and early fifth stage.

Infective third stage (Figs. 1–9) ($N = 73$)

Anterior fifth of infective *D. immitis* larvae (emerged from mouthparts of mosquitoes) tapers gradually (Fig. 1) so anterior extremity 50–70% of width at level of nerve ring. Fixed infective larvae 0.74–1.04 mm long. (Additional morphometrics listed in Table 1.) Tail tapers slightly to bluntly rounded posterior end that bears three prominences: conical tail tip and two smaller subventral bumps (Fig. 9). Buccal capsule consists of thin-walled narrow tube. Glandular esophagus filled with fine refractile granules except for posterior fifth; esophageal–intestinal (E–I) valve absent (Figs. 2, 3). Intestine narrower than glandular esophagus (Fig. 3), sometimes with prominent lumen and thin walls. Male GP oval-shaped, located ventrally just anterior to mid-body (Fig. 4); female GP attached ventrally by anterior end just posterior to junction of muscular and glandular esophagus (Fig. 2). In males, spicular primordia dorsolateral to rectum (Figs. 6, 7), absent in females (Fig. 8). Cuticle with transverse striations, thick and bilayered in some specimens (Fig. 3). Lateral chord nuclei large, oval-shaped, in two rows (one row in each of two hypodermal columns of each chord) (Fig. 5).

Third molt (Figs. 10–15) ($N = 34$)

Only a single third-stage larva (collected 6 DAI) was among the *D. immitis* specimens collected from dogs; all others had completed the third molt to fourth stage by 3 DAI when the earliest collections were made. The following description is based on in vitro grown specimens (Wong et al., 1982) from cultures that were 2 and 3 days old. After 2 days in culture the cuticle of molting specimens was separated from the underlying cuticle on one or both ends (Figs. 10–12). After 3 days in culture some specimens had completed ecdysis.

Anterior end tapers only slightly (Fig. 10). Larvae (not including sheaths) 0.73–1.17 mm long. Tail of fourth stage beneath cuticle of third stage

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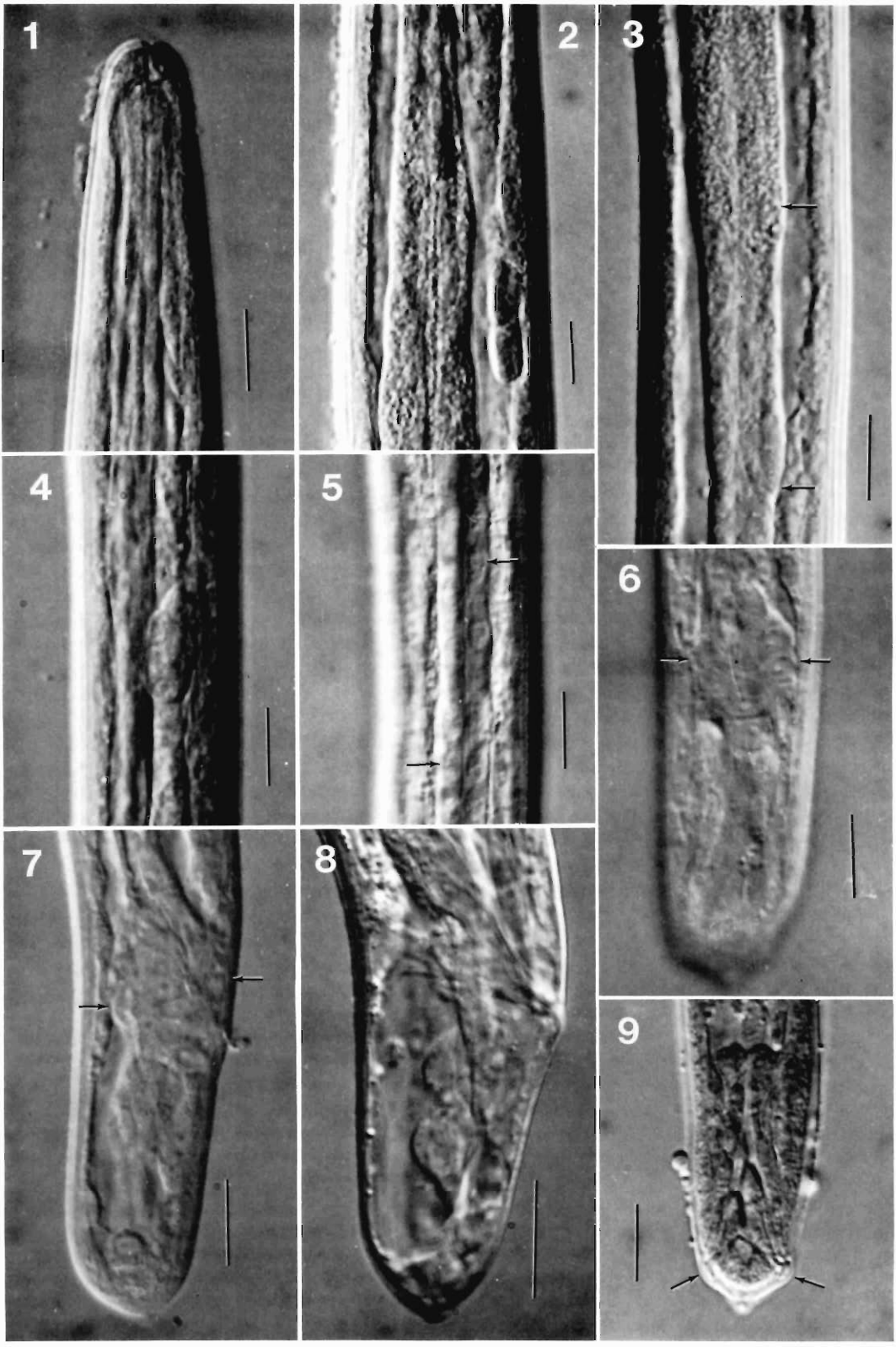
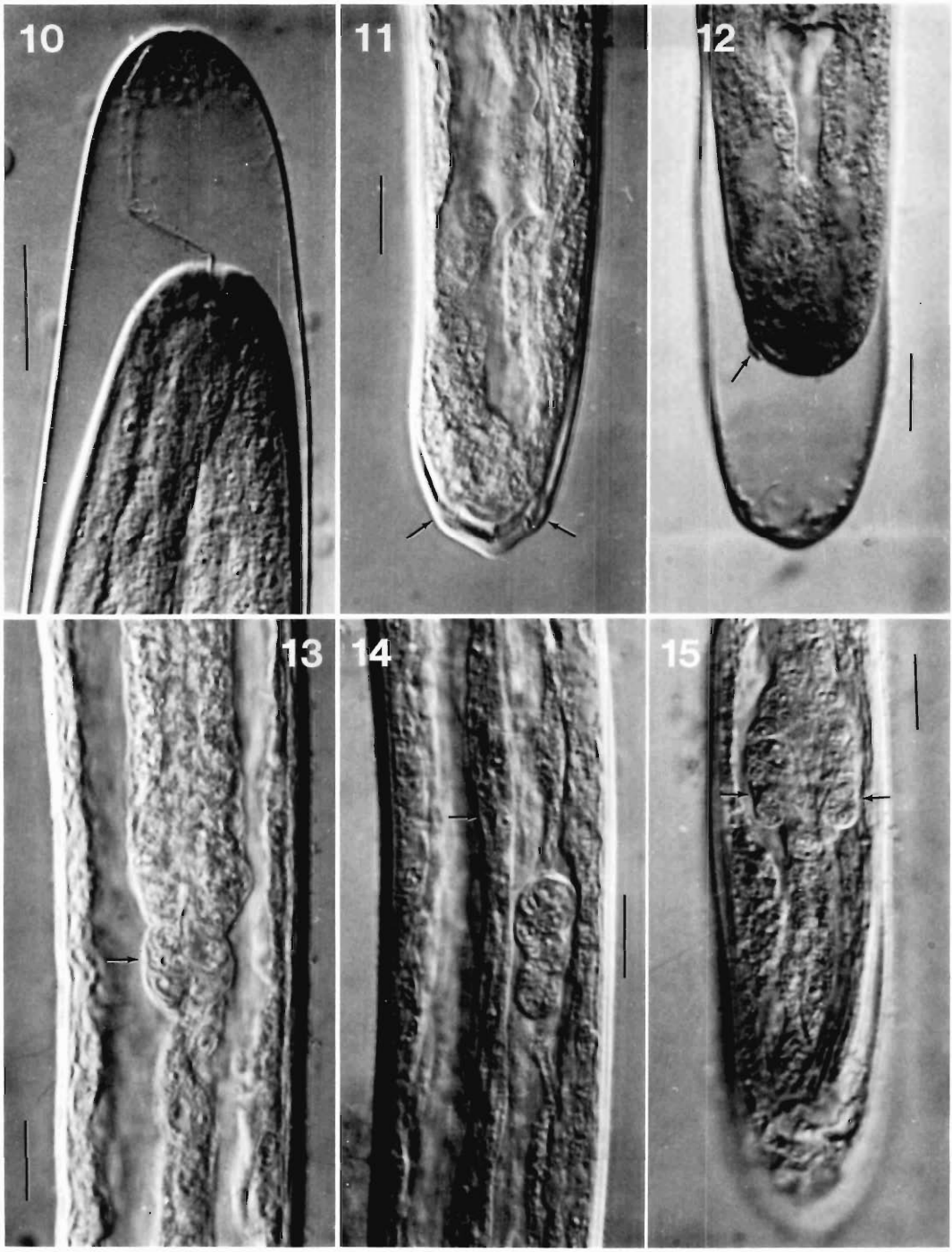
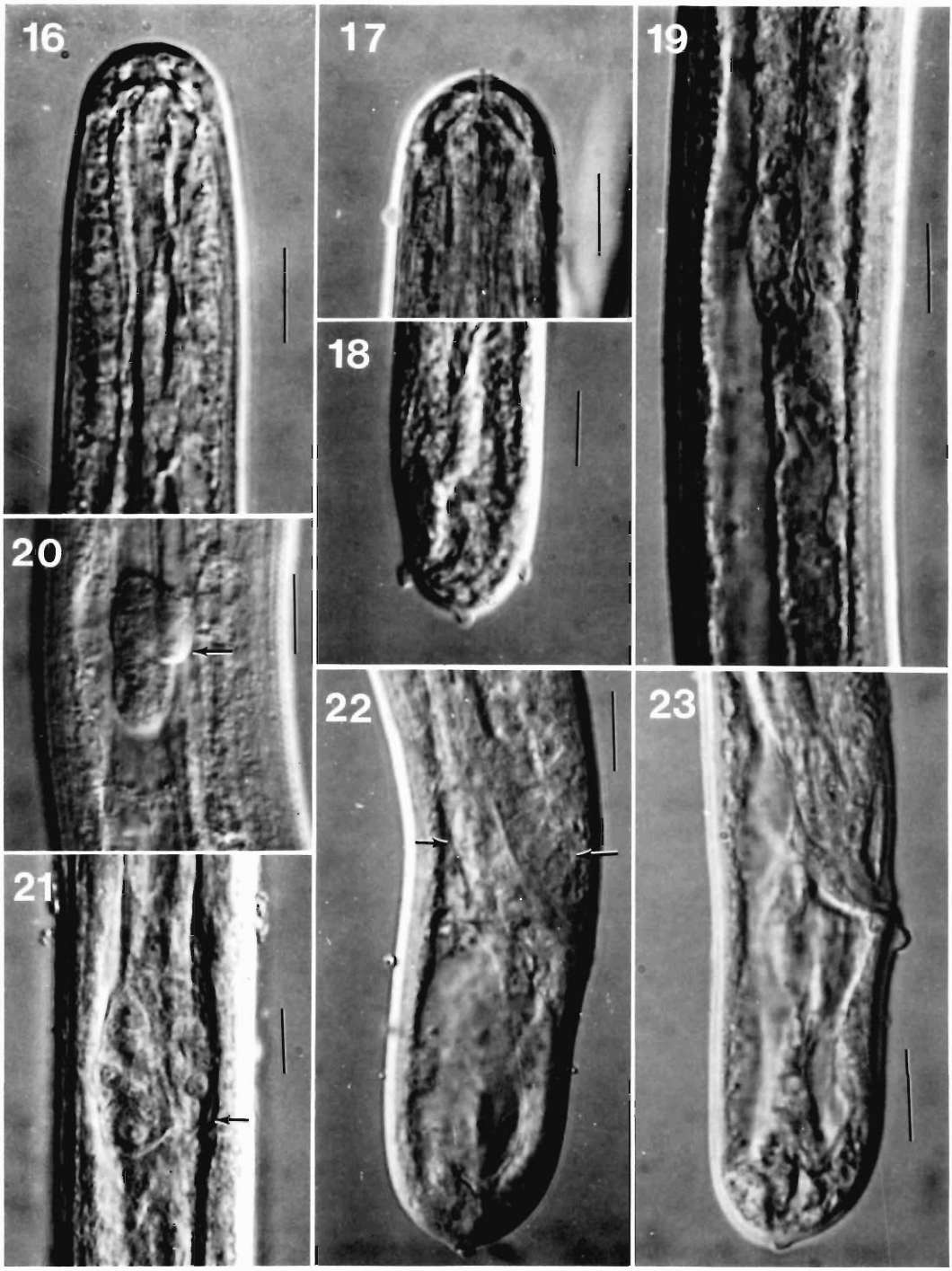
Figures 1–9. ***Dirofilaria immitis,*** **infective, third-stage larvae, from** ***Aedes aegypti*** **14 or 15 DAI. Scale bars 10 μm. 1. Anterior fifth of female. 2. Genital primordium of female. 3. Glandular esophagus with granules (arrow) and E-I junction (arrow). 4. Genital primordium of male. 5. Lateral chord showing oval-shaped nuclei (arrows). 6. Spicular primordia, dorsal view (arrows). 7. Spicular primordia, lateral view (arrows). 8. Female tail, lateral view. 9. Tail of male, ventral view (arrows at subventral bumps).**





Figures 10–15. ***Dirofilaria immitis,*** **third molt from in vitro cultures 2 or 3 days after inoculation. Scale bars 10 μm. 10. Anterior end with separated third-stage cuticle. 11, 12. Posterior ends showing submedian papillae (arrows) of fourth-stage larvae within cuticle of third stage. 13. Esophageal–intestinal junction showing valve (arrow). 14. Genital primordium of male with anterior end reflexed slightly, and intestine (arrow) without a lumen. 15. Spicular primordia (arrows), dorsal view.**



Figures 16–23. ***Dirofilaria immitis,*** **early fourth stage, from dogs. Scale bars 10 μm. 16. Anterior ⅙ of female, 3 DAI. 17. Anterior extremity of male, 6 DAI, showing buccal capsule. 18. Tail of female, ventral view, 3 DAI. 19. Esophageal-intestinal junction of female, 6 DAI. 20, 21. Male genital primordia, anterior end partially reflexed (arrow), and with anterior end completely reflexed (arrow), 6 DAI. 22. Tail of male, showing spicular primordia (arrows), 6 DAI. 23. Tail of female, 6 DAI.**

more bluntly rounded than in infective stage, with smaller tail button and larger well-defined submedian papillae (Figs. 11, 12). Buccal capsule still thin but wider posteriorly than anteriorly. Glandular esophagus still contains refractile granules; E–I valve present (Fig. 13). Intestine narrower than glandular esophagus, thick walled, lumen closed (Fig. 13). Male GP with anterior end reflexed (Fig. 14). Each spicular primordium consists of about eight cells (Fig. 15). Key morphological features for separating third- and fourth-stage larvae of *D. immitis* are given in Table 3.

Early fourth stage (Figs. 16–23) (N = 164: 80, 3 DAI; 84, 6 DAI)

At 3 and 6 DAI all (except one third-stage) specimens were in an early phase of fourth-stage development identical to *D. immitis* specimens having ecdysed a third-stage cuticle at 2 or 3 days in vitro. The following description is based on specimens collected from dogs 3 and 6 DAI.

Anterior body with almost parallel sides, untapered, with bluntly rounded anterior end (Fig. 16). Fixed larvae 0.98–1.30 mm long 3 DAI and 1.14–1.56 mm long 6 DAI. Tail bluntly rounded with three prominent projections—the button-like tail tip and the slightly larger angular submedian papillae (Fig. 18). Buccal capsule narrow, tubular, wider posteriorly; walls thicker (Fig. 17) than in third stage. Glandular esophagus without refractile granules; E–I valve present (Fig. 19). Intestine almost as wide as glandular esophagus, thin-walled with lumen open (Fig. 19). Male GP almost round (Figs. 20, 21) from anterior end reflexing to level of its posterior end. Spicular primordia evident as mass of cells around rectum of male (Fig. 22), absent in female (Fig. 23). Nuclei of lateral chords still oval-shaped with single row in each hypodermal column.

Mid-fourth stage (Figs. 24–35) (N = 815: 115, 9 DAI; 358, 12 DAI; 235, 15 DAI; 59, 21DAI; 48, 30 DAI)

Development from 9 through 30 DAI in *D. immitis* specimens collected from dogs is described as mid-fourth stage.

Shape of body 9 DAI uniformly cylindrical with bluntly rounded ends, but in some specimens anterior and posterior extremities slightly swollen or broader than rest of body. Swollen ends more common 12, 15, and 21 DAI and most prominent 30 DAI (Figs. 24–29). Fixed larvae 1.3–1.7 mm long 9 DAI to 3.1–5.6 mm long 30 DAI (Table 1). Tail papillae unchanged from early fourth stage but appear smaller in relation to larger body. Buccal capsule unchanged from earlier phase (Fig. 25). Glandular esophagus without refractile granules, about as wide as intestine. Male GP in shape of shepherd's crook 9 DAI due to posterior growth of its anterior end (Fig. 30). Original anterior end (now posterior end) reaches rectum 30 DAI, not joined to rectum. Vagina formation by hypodermal invagination began 9 DAI at attachment point of GP (Fig. 31). Female GP enlarged and swollen posteriorly with two large nuclei near posterior tip that each extend posteriorly in branch at 21 DAI (Fig. 32) and extend beyond the E–I junction 30 DAI (Fig. 35). Lateral chord nuclei large, oval-shaped, and in a single row through 21 DAI (Fig. 33); smaller, round, and numerous 30 DAI (Fig. 34).

Late fourth stage (Figs. 36–42) (N = 129: 54, 41 DAI; 68, 50 DAI; 7, 58 DAI)

According to Kotani and Powers (1982) all *D. immitis* specimens collected from dogs 41 DAI, 98% of 317 collected 50 DAI, and 35% of 152 collected at 58 DAI were in the late phase of fourth stage.

Head and tail slightly narrower than rest of body (Figs. 36, 37). Tail tip and subventral papillae clustered at posterior extremity, proportionally smaller because of increase in size of nematode (Fig. 38). Fixed larvae 41 DAI 5.6–9.2 mm long and 50 DAI 8.6–12.8 mm long. Posterior extension of male GP joined ventrally to rectum, anterior end of GP (original posterior end) triangular- or knob-shaped, full of round cells (Fig. 41). Spicules and genital papillae partially formed and visible in cleared specimens (Fig. 38). Vagina lumen lined with cuticle 41 DAI (Fig. 39); large cells line posterior ⅔ expanded

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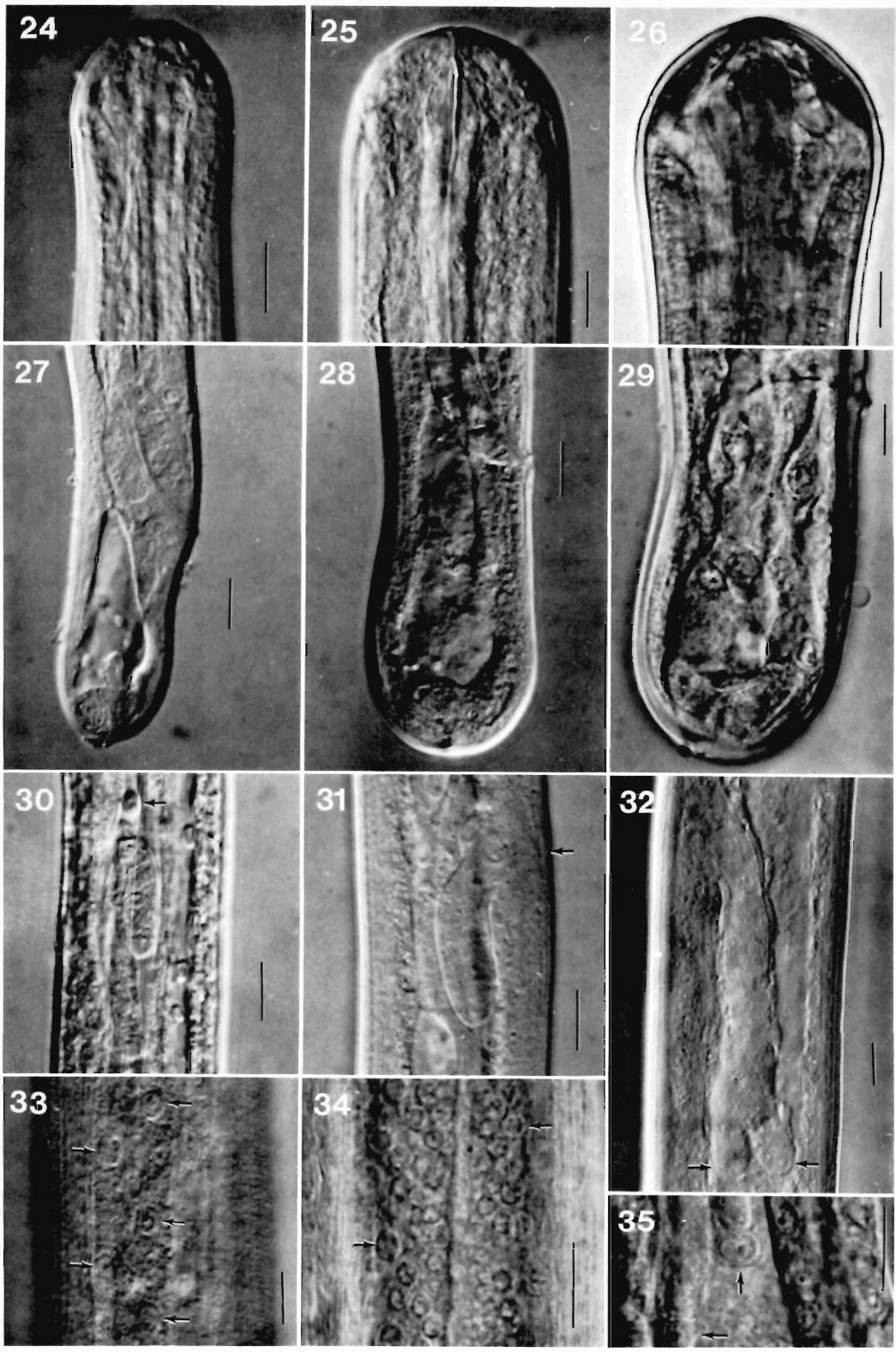
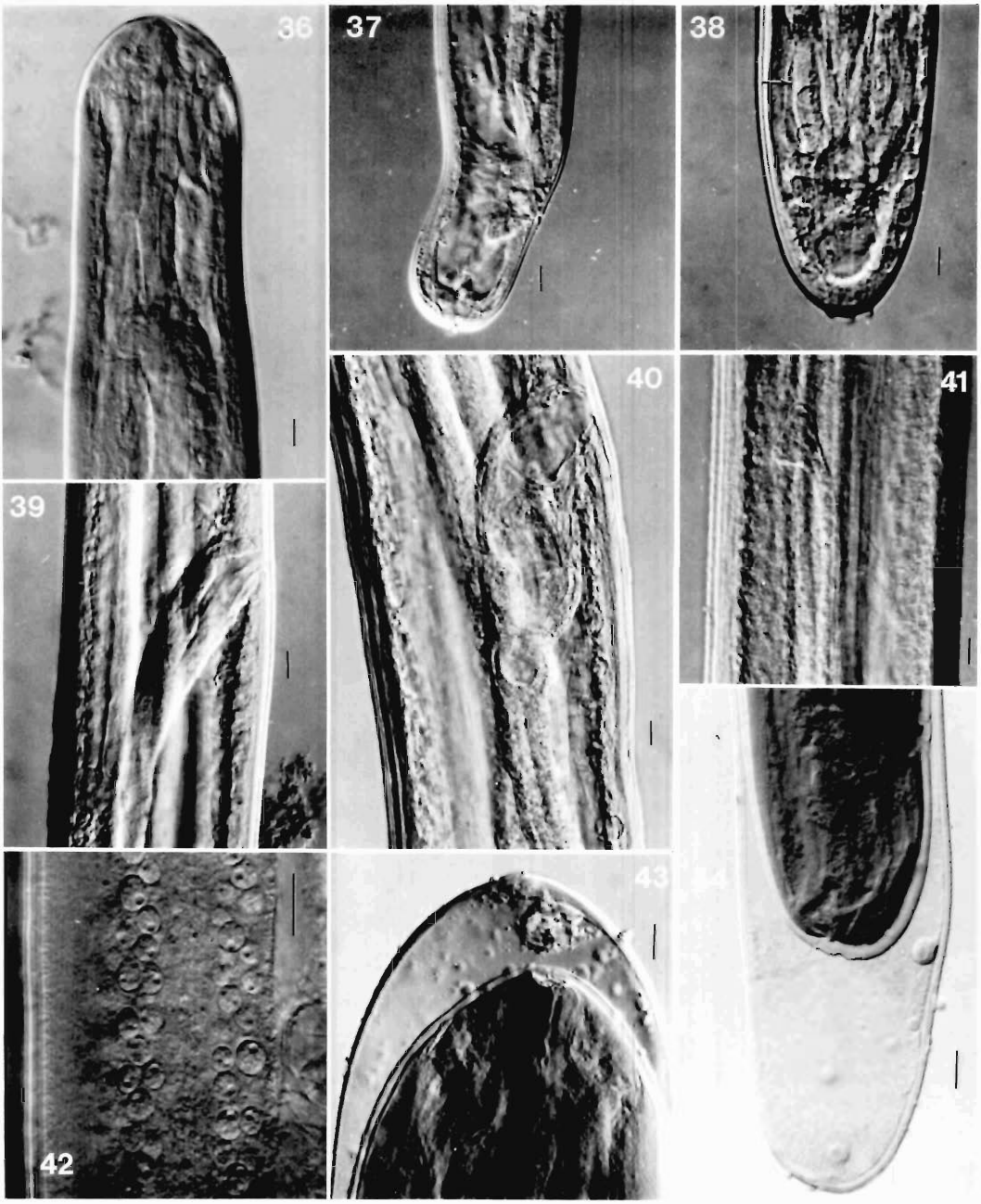
Figures 24–35. ***Dirofilaria immitis*****, mid-fourth stage, from dogs. Scale bars 10 μm. 24–26. Anterior extremities of male, female, and female, 9, 21, and 30 DAI, showing extremity broader than adjacent body. 27–29. Posterior extremities of male, female, and female 9, 21, and 30 DAI showing extremity broader than adjacent body. 30. Male genital primordium and pseudocoelomocyte (arrow), 9 DAI. 31. Female genital primordium (arrow at attachment), 9 DAI. 32. Female genital primordium (arrows at branches), 21 DAI. 33, 34. Nuclei (arrows) in lateral chord, 21 DAI and 30 DAI. 35. Posterior extremities (arrows) of branches of female genital primordium, 30 DAI.**





Figures 36–42. ***Dirofilaria immitis,*** **late fourth stage, from dogs. Scale bars 10 μm. 36, 37. Anterior extremity of male, and posterior extremity of female, 41 DAI. 38. Male tail, ventral view showing the genital papillae and spicule (arrow) of fifth stage beneath the cuticle of the fourth stage, 41 DAI. 39. Vulva and vagina vera, 41 DAI. 40. Vulva, expanded vagina vera, and tubular vagina uterina, 50 DAI. 41. Proximal end of male reproductive system, 50 DAI. 42. Nuclei in lateral chord of male, 50 DAI.**

Figures 43, 44. ***Dirofilaria immitis,*** **fourth molt, 58 DAI. Scale bars 25 μm. 43. Anterior extremity of female. 44. Posterior extremity of female.**

Table 2. Measurements* of spicules and the female reproductive system of *Dirofilaria immitis* from dogs in the late fourth, fourth molt, and early fifth stages.

Characteristic lengths of	Late fourth		Fourth molt	Early fifth stage		
	50 DAI	58 DAI	58 DAI	58 DAI	70 DAI	79 DAI
Left spicule (μm)	nd†	nd	nd	209–399	304–332	340–388
Right spicule (μm)	nd	nd	nd	164–220	188–192	200–222
Vagina vera (μm)	105–147	128–144	115–144	126–132‡	100–120	111–170
Vagina uterina (μm)	176–640	400–688	384–688	560–624	704–1,090	940–1,110
Uterus (mm)	2.14–3.82	4.36–5.44	4.36–5.44	4.59–4.86	5.84–6.84	4.64–5.84
Oviduct and ovary (mm)	1.23–2.96	3.21–4.36	3.21–4.36	3.60–4.84	7.30–8.21	14.4–16.6

* Other measurements of these stages in Table 1.
† nd = Not done; spicules not formed sufficiently.
‡ Four females measured.

muscular vagina vera 50 DAI (Fig. 40). Branches of female GP ½–¾ body length. Additional morphometrics are given in Table 2. Lateral chord nuclei small and numerous (Fig. 42). Lateral excretory tubule evident between columns of lateral chords. Cuticle appears to be bilayered in some specimens (Fig. 41).

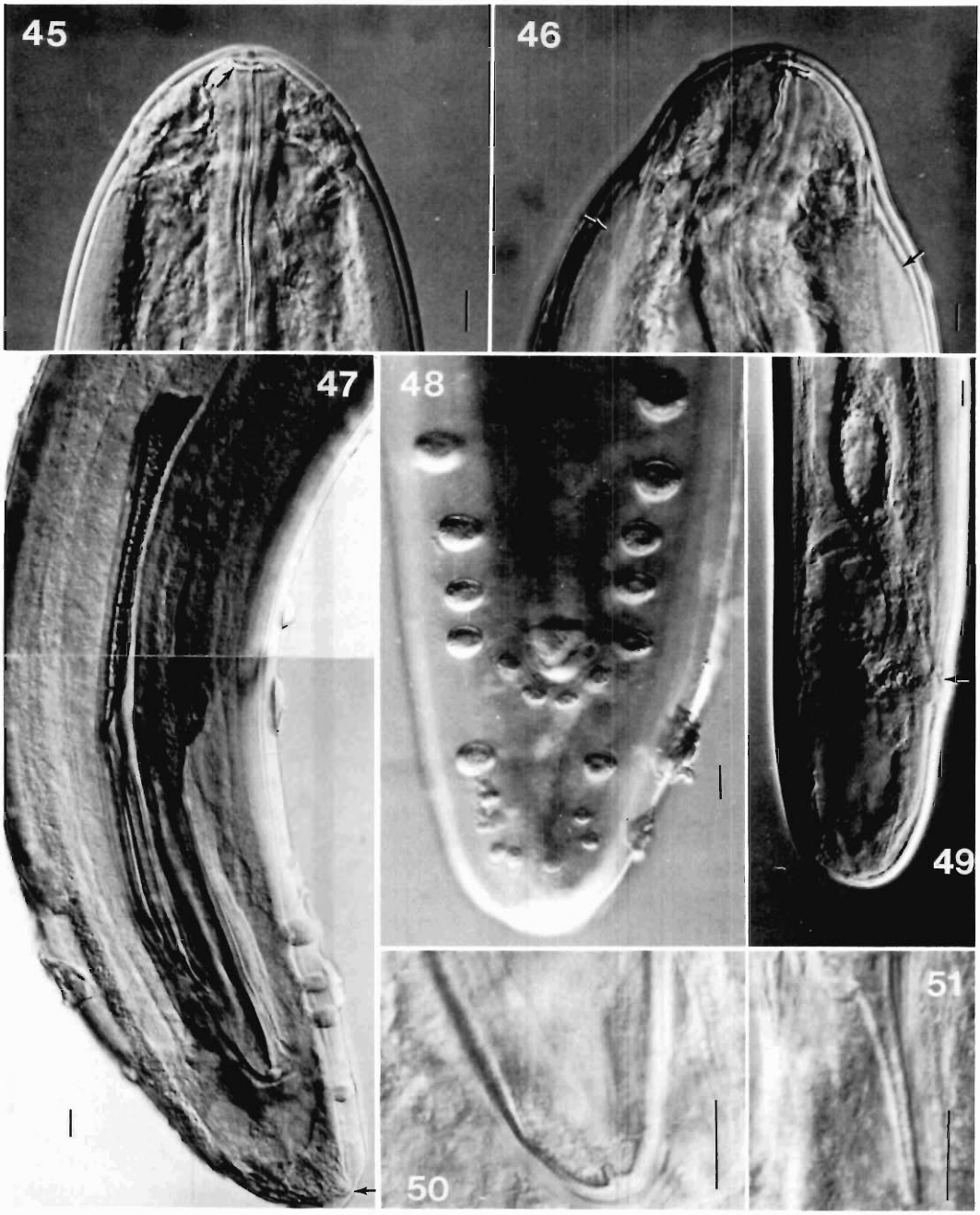
Fourth molt (Figs. 43–44) ($N = 17$: 6, 50 DAI; 11, 58 DAI)

Specimens of *D. immitis* with the fourth-stage cuticle separated from the underlying fifth-stage cuticle at either or both extremities were designated as fourth molt (Figs. 43, 44). This phase of development was in 2% of 317 specimens at 50 DAI and in 20% of 152 at 58 DAI (Kotani and Powers, 1982). Molting specimens were 8.3–12.8 mm long exclusive of fourth-stage cuticle. Female reproductive tract extended to within 1 mm of posterior extremity. Spermatocytes present in anterior portion of testis.

Early fifth stage (Figs. 45–59) ($N = 58$: 23, 58 DAI; 17, 70 DAI; 18, 79 DAI)

This phase extended from 58 DAI through 79 DAI. At 58 days 45% of 152 *D. immitis* specimens were in fifth stage; at 70 and 79 DAI 100% were fifth stage (Kotani and Powers, 1982).

Anterior extremity bluntly rounded at ecdysis (Fig. 45), but narrower than adjacent body by 70 DAI (Fig. 46) because of thick dorsal and ventral somatic musculature; head shape persists in mature specimens. Buccal capsule absent, but peribuccal ring present at anterior end of esophagus (Figs. 45, 46). Female tail short, tapers from anus, bluntly rounded, phasmids near tip (Fig. 49). Male tail spirally coiled 79 DAI with diagonal cuticular ridges on ventral surface of posterior ⅛ of body, gradually become almost transverse before ending anterior to genital papillae. Genital papillae of male usually asymmetrically arranged, left side usually anterior to right side; arranged in three to five pairs, pedunculate, beginning preanally and ending adanally; single pedunculate pair in middle of tail; single large ventral papilla on anterior edge of semilunar-shaped vent; two pairs of smaller unstalked papillae in semicircle around posterior edge of vent; three pairs of small stalked papillae in semicircle in posterior ½ of tail (Fig. 48); phasmids at tip of tail (Fig. 47). Spicules, especially proximal ½ of left spicule, not completely sclerotized 70 DAI; sclerotization nearly complete 79 DAI. Right spicule 164–222 μm long (Table 2), thick, boat- or scoop-shaped with narrow ventral groove in tapered, rounded distal tip (Figs. 47, 50). Left spicule longer (304–399 μm), thinner, divided at about 60% of length by bend in shaft, distal tip spike-shaped (Figs. 47, 51). Testis ends in knob-shape near middle of body (Fig. 52), contains round cells (spermatocytes); male reproductive tract long, usually straight tube except for some reflexing posteriorly, of uniform diameter with epithelial covering that is clear in cleared specimens (Fig. 53); contains spermatozoa in proximal portion 79 DAI. Vulva usually near, but anterior to E–I junction; vagina vera lined with cuticle, reflexed within thick muscular capsule (Fig. 55); vagina uterina elongate (Table 2), undivided with thinner muscle coat (Figs. 55, 56); uterus divided into two long posteriorly directed branches, with muscle coat (Fig. 56); oviduct and ovaries abruptly narrower than uteri, lack muscle coat (Fig. 57). Nuclei of lateral chord numerous, round with prominent nucleoli (Fig. 54). Posterior deirids laterally 0.1–3.0 mm at differing levels



Figures 45–51. ***Dirofilaria immitis,*** **early fifth stage. Scale bars 10 μm. 45. Anterior extremity of male showing peribuccal ring (arrow), 58 DAI. 46. Anterior extremity of male with narrow head anterior to somatic muscles (arrows), 70 DAI. 47. Montage showing spicules and male tail, lateroventral view, 79 DAI (arrow indicates phasmid). 48. Male tail, ventral view, 70 DAI. 49. Female tail, lateral, (arrow at anus), 79 DAI. 50. Distal tip of right spicule, ventral view, 58 DAI. 51. Distal tip of left spicule, lateral view, 58 DAI.**

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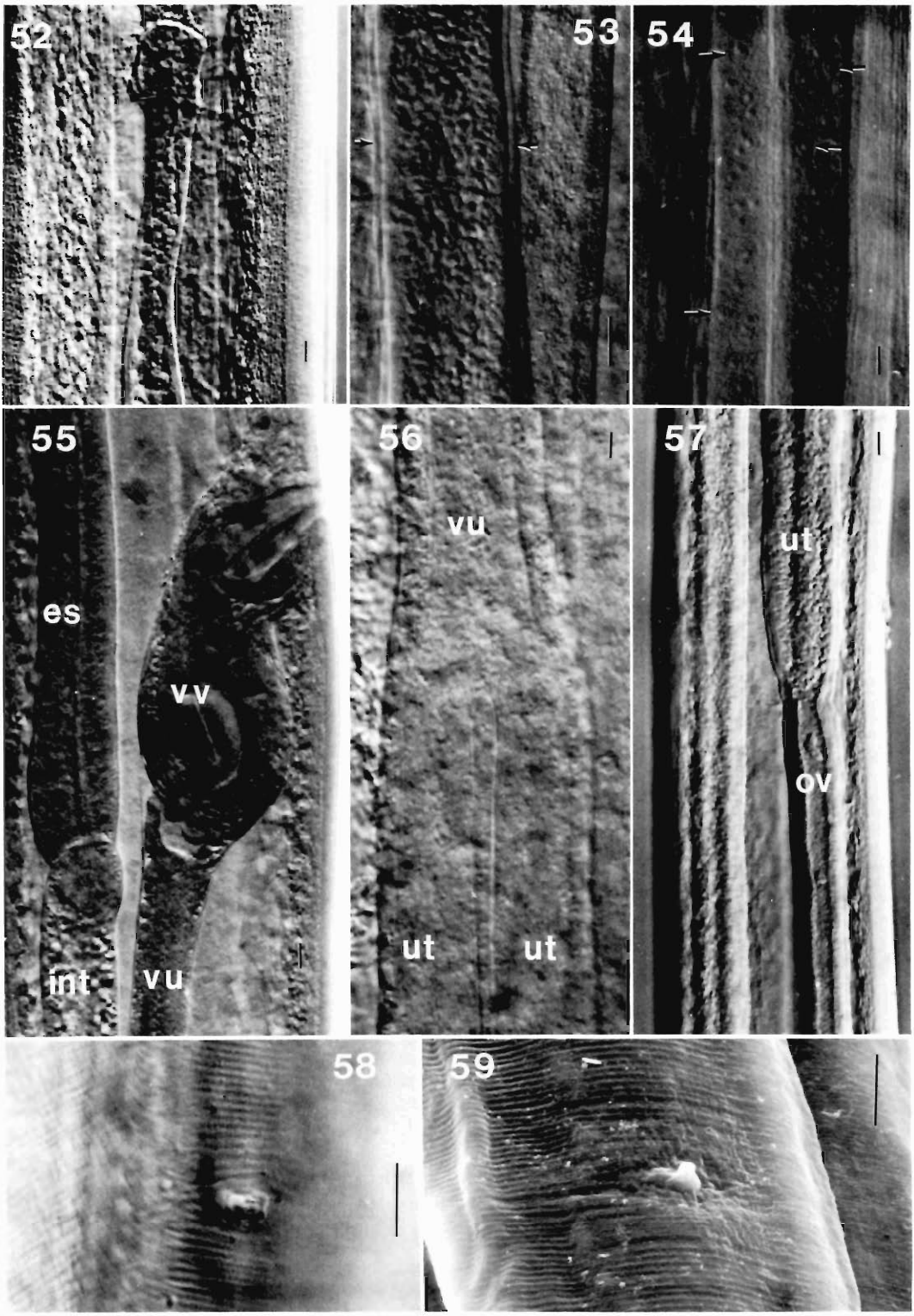
Figures 52–59. ***Dirofilaria immitis,*** **early fifth stage. Scale bars 10 μm. 52. Proximal end of testis, 70 DAI. 53. Male reproductive tract (between arrows), in proximal half, containing spermatozoa, 79 DAI. 54. Nuclei (arrows) of lateral chord, 79 DAI. 55. Vagina vera (vv), anterior portion of vagina uterina (vu), esophagus (es) and intestine (int), lateral view, 79 DAI. 56. Junction of vagina uterina (vu) and uteri (ut), 79 DAI. 57. Junction**



of uterus (ut) and oviduct (ov), 70 DAI. 58. Right postdeirid of female, 70 DAI. 59. Scanning electron micrograph of left postdeirid of male, 70 DAI.

Table 3. Key morphological features* of early developmental stages of *Dirofilaria immitis*.

Morphological feature	Third stage	Early fourth stage
Anterior extremity	Tapered	Untapered
Esophageal–intestinal valve	Undeveloped	Developed
Submedian caudal papillae	Broadly based bumps formed by papillae beneath cuticle	Sharply defined, angular papillae project from surface of tail
Tail tip	Conical, larger than submedian papillae	Button-like, smaller than submedian papillae

* See text for additional details.

from posterior extremity on both sides of males and females (Figs. 58, 59). Usually the left postdeirid is more anterior than the right. Cuticle with fine transverse striation (Figs. 58, 59).

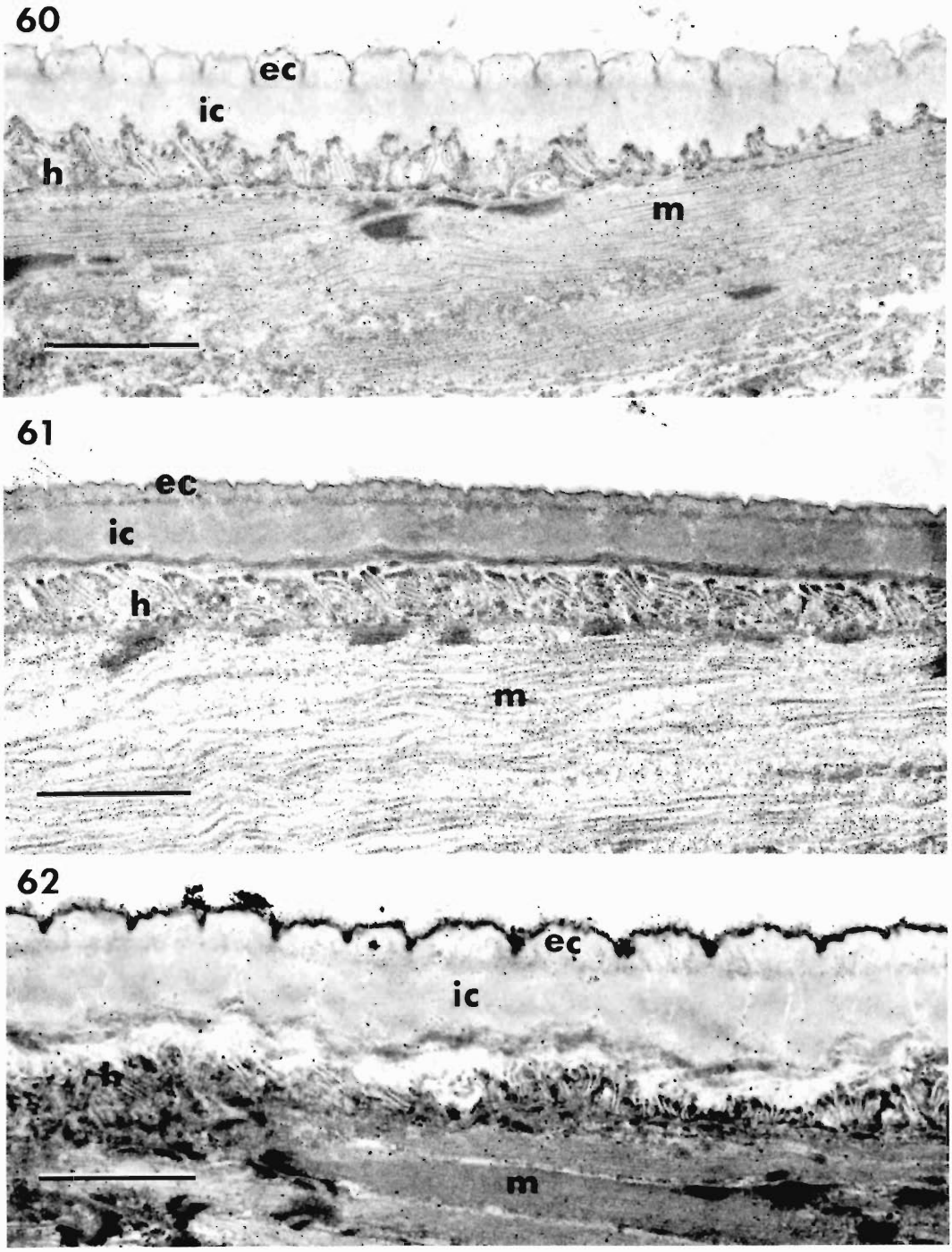
Discussion

Apparently, investigators of the development of *Dirofilaria immitis* in dogs have not observed the third molt because they have not looked early enough. Kume and Itagaki (1955) examined dogs no earlier than 23 DAI. Orihel (1961) examined dogs no earlier than 5 DAI; and the earliest examinations by Kotani and Powers (1982) were 3 DAI. In vitro studies, however, demonstrated that a molt occurs from 2 to 5 days after third-stage larvae emerge from mosquitoes (Yoeli et al., 1964; Sawyer, 1965; Sawyer and Weinstein, 1965; Wong et al., 1982; Lok et al., 1984). In addition, P. Supakorndej, J. J. Jun, and J. W. McCall (pers. comm.) reported the third molt in ferrets occurred 3 DAI. In in vitro and model animal systems all observers of the third molt reported it to occur much earlier than the 9–12 DAI third molt in the dog reported by Orihel (1961).

We conclude that the third molt was completed in dogs earlier than 3 DAI. Although the third molt was not observed in dogs in this study, all available specimens collected 3 DAI from dogs were early fourth-stage larvae identical with those that emerged from the third molt 2 and 3 days in vitro. We did not find molting larvae at 3, 6, 9, 12, 15, 21, 30, or 41 DAI. A single third-stage larva in poor condition was found 6 DAI, and we observed a few fourth-stage larvae at 6, 9, and 12 DAI that were in poor condition with cellular debris adhered to slightly swollen cuticles. We believe these were dead or moribund specimens, but such specimens resemble a premolting condition as seen in infective larvae. They were identified as early fourth-stage larvae by the presence of sharply defined submedian tail papillae and untapered anterior ends (Table 3). However because some specimens collected at 3–15 DAI were unavailable for this study, it is possible that additional specimens in third stage or third molt were present at those times and were not seen by us. In any case, all of the 872 available specimens collected from dogs at 3–15 DAI (except one third-stage larva) were in the fourth stage, indicating that those specimens had already completed the molt observed at 2 or 3 days in vitro.

Because of the differences between our observations and those of Orihel (1961) regarding the third molt of *D. immitis* in dogs, we conducted two additional studies. If the molt reported by Orihel (1961) at 9–12 DAI in dogs was a different molt from the one we observed at 2–3 days in vitro, then one or both of the following must also be true: (a) because more than four molts would have been accounted for (two in the mosquito and three in the dog), the molt observed in vitro and in ferrets would have to be an ecdysis of an earlier molt in the mosquito; and (b) molting lethargic specimens at 9–12 DAI may have been retained in the tissues of the dog and missed in the present study. Therefore, to determine whether (a) and/or (b) might be true: (1) we reexamined the development of *D. immitis* in *Aedes aegypti,* as described by Taylor (1960), by dissecting developing larvae from mosquitoes fixed in alcohol 12 DAI; and (2) we examined with transmission electron microscopy the cuticle of specimens collected at the time Orihel (1961) reported the third molt to occur to determine whether any indication of a molt, as described by Howells and Blainey (1983) for *Brugia pahangi* (Buckley and Edeson, 1956), could be seen.

The results of our reexamination of the development of *D. immitis* in *A. aegypti* found it to be as described by Taylor (1960) and by Bartlett (1984a) for *Dirofilaria scapiceps* (Leidy, 1886). We observed a first-stage larva with a pointed cap over the stoma, a first molt and ecdysis



Figures 60–62. Electron micrographs of early and middle phases of fourth-stage larval *Dirofilaria immitis* from dogs. Scale bars 5 μm. 60. Early fourth phase, 3 DAI, showing an annulated, convoluted external cuticle (ec) with deep striae separated from the thicker internal layer of cuticle (ic) by a thin electron-dense line; a thin, convoluted hypodermis (h) with electron-dense beads adjacent to the cuticle, and somatic musculature (m). 61. Mid-fourth phase, 9 DAI, showing cuticle with broader annules and more shallow striae, and a less convoluted hypodermis than in the earlier phase of development. 62. Mid-fourth phase, 12 DAI, with the cuticle somewhat thicker than earlier and with an electron-lucent layer between the cuticle and the hypodermis.

(sometimes simultaneously with the second ecdysis), a second-stage larva with elongate buccal capsule and anal plug, and clear evidence that both the buccal capsule and anal plug are shed with the ecdysis of the second molt in the mosquito as described by Taylor (1960) for *D. immitis* and by Bartlett (1984a) for *D. scapiceps*. We concluded, therefore, that the molt observed by several workers at 2–5 DAI in vitro and in ferrets 3 DAI by P. Supakorndej, J. J. Jun, and J. W. McCall (pers. comm.) is the third molt and not an ecdysis of the second molt.

The results of our transmission electron microscope study of the cuticle of developmental stages (3, 9, and 12 DAI) of *D. immitis* in the dog showed no indication of either a recent molt or an impending molt at 9–12 DAI (Figs. 60–62); rather the cuticle was typical of an intermolt period (Lee, 1970; Howells and Blainey, 1983). Furthermore, with the molt at 2 DAI, the previous two molts in the mosquito (Taylor, 1960) and the fourth molt at 50–58 DAI, all molts in the life cycle of *D. immitis* are accounted for.

The third molt in *D. scapiceps* in rabbits was recently reported by Bartlett (1984b) to occur 6 DAI. Bartlett (1984b) described a buccal capsule more strongly developed in the fourth stage than in the third stage. We also observed thicker walls in the buccal capsule of the fourth stage of *D. immitis* than in the third stage. Spicular primordia were visible in the third stage of both species. The anterior development of a narrow germinative portion of the male reproductive tract as described for *D. scapiceps* by Bartlett (1984b) was not seen in *D. immitis* which retained the knob-shaped anterior end of the original genital primordium as described by Orihel (1961).

From a survey of the literature it appears that there are considerable differences among the larvae of the Onchocercidae in the presence of submedian papillae and tail tip. Unlike *D. immitis,* in which the submedian caudal papillae of the fourth stage are more prominent than in the third stage, the submedian caudal papillae of *D. scapiceps* are more prominent in the third stage (Bartlett, 1984a, b, and a study of specimens by us). However, the submedian caudal papillae of third-stage *D. scapiceps* are smaller than the tail tip (Bartlett, 1984a) as in *D. immitis.* The cuticles of both third- and fourth-stage *D. scapiceps* are much thicker than in *D. immitis,* which may account for the difference in prominence of the papillae. In *Brugia pahangi* and *Wuchereria bancrofti* (Cobbold, 1877) the caudal papillae of the third stage are three in number, but in the fourth stage the terminal papilla or tail tip is absent or greatly reduced in size leaving only the two submedian papillae (Aoki et al., 1980; Franz and Zielke, 1980). This difference was used by Aoki et al. (1980) to identify the larvae of *B. pahangi* to stage.

In the present study refractile granules were present in the glandular esophagus of infective larvae and larvae in the third molt, but were not seen at any other times. Refractile granules were observed in the glandular esophagus of late fourth and early fifth stages of *D. scapiceps* in rabbits by Bartlett (1984b). She suggested that the granules may be secretory and of use to the migrating early fifth stage of the nematode.

Our description of sexual dimorphism in third-stage *D. immitis* from mosquitoes agrees with the earlier description by Orihel (1961). Sexual dimorphism has been described also in third-stage *D. scapiceps* from mosquitoes by Bartlett (1984a).

A single pair of bilateral postdeirids were present in all stages of *D. immitis* in the present study; they were described in adults previously by Uni (1978). Bartlett (1984b) also reported postdeirids in *D. scapiceps.* A similar lateral papilla is present on the tail of all stages of *B. pahangi* according to Aoki et al. (1980). The function of the postdeirids is unknown.

The principal contribution of the present study is the redescription of the morphogenesis of *Dirofilaria immitis* in dogs. With this basic information on the morphogenesis in the normal definitive host, the success of in vitro and model animal systems and treatment programs can be evaluated, and a series of reference specimens is available for the identification of developmental stages of the dog heartworm.

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Resistance to Monensin Medication in *Eimeria tenella*

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ABSTRACT: A strain of *Eimeria tenella* from broilers from North Carolina was markedly less sensitive to three ionophores (monensin, narasin, and lasalocid) than was a laboratory isolate that had never been exposed to anticoccidials. Parameters used to measure drug efficacy were weight gain, cecal lesion score, packed cell volume, plasma pigmentation, and oocyst production. The infection with the field strain was better controlled by medication with arprinocid, amprolium, nicarbazin, clopidol, or halofuginone. Best control was with robenidine and buquinolate. When the field strain was passed in monensin-medicated chicks, the sensitivity to ionophores was further reduced. In addition, compounds that were previously effective, such as buquinolate, were no longer effective. Passing mixed strains of *E. tenella* through monensin-medicated birds six times gave similar results, although the loss of sensitivity to monensin was not as dramatic as with the field strain. Conversely, passing a laboratory strain through monensin-medicated chicks for 50 successive passages did not reduce monensin efficacy.

One method of predicting the likelihood of avian coccidia developing drug resistance involves the serial propagation in birds fed a ration containing the anticoccidial in question. With monensin, such attempts have been uniformly unsuccessful with chicken coccidia (Shumard et al., 1970; Chapman, 1976a). Some strains isolated from the field have been less sensitive to monensin medication than others (Jeffers, 1974; Chapman, 1976b; Weppelman et al., 1977b) and were sometimes termed "resistant." However, attempts to enhance resistance by serial passage under drug pressure in chickens have been unsuccessful. Recent reports seem to indicate that field strains with reduced sensitivity to monensin are increasing (Jeffers, 1981; McDougald, 1981; Chapman, 1982). In addition, monensin-resistant strains of the turkey coccidia, *Eimeria meleagrimitis,* have been isolated from the field (Jeffers and Bentley, 1980a) and developed in the laboratory from a monensin-sensitive parent stock (Jeffers and Bentley, 1980b). The development of partial resistance in the Houghton strain of *Eimeria tenella* has been reported since this paper was submitted for publication (Chapman, 1984).

The first purpose of this study was to characterize a strain of *E. tenella* recovered from chickens in a commercial flock medicated with monensin and to see if monensin resistance in that strain could be enhanced by serial passage. The second purpose was to see if monensin resistance could be developed (1) in a laboratory strain of *E. tenella* that readily developed resistance to a variety of other anticoccidials (McLoughlin, 1970) or (2) in a mixture of *E. tenella* from several sources, not all of which were derived from a single oocyst isolation.

Materials and Methods

Coccidia

Eimeria tenella oocysts were recovered from commercial broilers from North Carolina that had been on continuous medication with 121 ppm monensin. At the time of collection (4 wk of age), 3 of 18 broilers examined had severe cecal lesions. Oocysts from the ceca were pooled, passed once in unmedicated chicks, and designated the field strain (FS, API #47). No other species of *Eimeria* were found in this culture. The second strain used in these studies was a laboratory strain (LS) of *E. tenella* originally isolated from a single oocyst and maintained by cecal collection. This laboratory strain (API #49) has never been exposed to anticoccidials, although it has been used in numerous studies to demonstrate development of drug resistance (McLoughlin, 1970). A mixture of *E. tenella* oocysts from five sources was used in one study. These strains were all known susceptible to monensin. Three of the strains were field collections that had not been single oocyst isolated. Therefore, this mixture (MS) was considered to have a greater genetic diversity than the laboratory strain.

Tests for drug efficacy

Drug efficacy was tested in 3- or 4-wk-old White Leghorn cockerels. These were weighed and divided into groups of 10 birds each based on the weight distribution method of Gardiner and Wehr (1950). Birds were placed on medicated ration at this time. One day later all inoculated chickens received 10^5 sporulated oocysts each. Six days postinoculation (DPI), birds were reweighed. Five or six birds from each group were bled via cardiac puncture, killed, and cecal lesions scored. Packed cell volume (PCV), and plasma pigment (Wilson, 1956) were measured.

Table 1. Control of a field strain of *E. tenella* by various medications (Exp. 1).*

Medication	Level (ppm)	Weight gain (g/bird)	Lesion score	PCV (%)	Plasma pigment (β-carotene equiv.)
None (uninoculated control)	0	151 ± 6^{ab}†	0^{a}	30.8 ± 0.6^{a}	18.6 ± 1.6^{a}
None (inoculated control)	0	67 ± 9^{e}	3.9 ± 0.1^{e}	17.7 ± 0.7^{d}	7.0 ± 0.8^{c}
Monensin	100	109 ± 14^{d}	2.4 ± 0.2^{cd}	17.5 ± 2.3^{d}	9.4 ± 2.5^{c}
Monensin	121	86 ± 9^{e}	2.8 ± 0.2^{d}	17.2 ± 1.4^{d}	8.9 ± 1.7^{c}
Lasalocid	75	68 ± 6^{e}	3.7 ± 0.2^{e}	18.8 ± 1.2^{d}	8.4 ± 1.8^{c}
Amprolium	125	134 ± 7^{bc}	2.1 ± 0.1^{c}	24.3 ± 1.1^{c}	11.8 ± 1.1^{bc}
Nicarbazin	125	115 ± 7^{cd}	0.4 ± 0.2^{b}	26.4 ± 0.7^{bc}	16.2 ± 1.4^{ab}
Buquinolate	82.5	142 ± 7^{ab}	0^{a}	29.4 ± 1.2^{ab}	17.4 ± 3.2^{a}
Robenidine	33	159 ± 5^{a}	0^{a}	27.2 ± 0.5^{bc}	20.2 ± 1.7^{a}
Clopidol	125	156 ± 5^{ab}	0^{a}	26.5 ± 0.8^{bc}	15.6 ± 1.2^{ab}

* Medication begun on D −1; inoculation D 0; weight gain D 0 to D 6; cecal lesion score, PCV, and plasma pigment at D 6.
† Values within a column followed by a common letter are not significantly different ($P \leq 0.05$). Each value is $\bar{x}$ ± SEM.

Drug sensitivity of field strain

One hundred chicks were divided into 10 groups (Exp. 1). Each group was given a ration containing levels of medication as shown in Table 1. Chicks were inoculated with the FS. In a second experiment (Exp. 2), 13 groups were started on medicated rations as shown in Table 2. Chicks were inoculated 1 day later with either the FS or the LS. The LS was included to confirm drug presence and efficacy.

Passage of field strain under monensin pressure

Ten White Leghorn cockerels, 3 wk of age, were placed on feed containing 363 ppm monensin. One day later they were each given 100,000 sporulated oocysts of the FS. Oocysts were collected from the feces on days 6–8 PI. This procedure was repeated for five consecutive passages. At the time of the fifth passage, harvests were also made of the parental FS and the LS from unmedicated chicks.

One hundred sixty chicks were divided into 16 groups to test for enhanced resistance in the monensin-passed field strain (MPFS) (Exp. 3). Three groups were each given feed containing one of four different medications; 121 ppm monensin, 66 ppm salinomycin, 75 ppm lasalocid, or 82.5 ppm buquinolate. Four groups were given unmedicated feed. The following day, chicks from one unmedicated group and one group on each medication were inoculated with the MPFS. Chicks from a corresponding set of groups were inoculated with the

Table 2. Control of a field or laboratory strain of *E. tenella* by various medications (Exp. 2).*

Strain	Medication	Conc. (ppm)	Weight gain (g/bird)	Lesion score	PCV (%)	Plasma pigment (β-carotene equiv.)
None	None (uninoculated control)	0	105 ± 3^{a}†	0^{a}	26.1 ± 0.8^{abc}	12.9 ± 2.2^{a}
Field	None (inoculated control)	0	33 ± 5^{e}	3.0 ± 0.2^{d}	12.3 ± 0.6^{f}	4.4 ± 1.4^{c}
	Monensin	121	71 ± 5^{cd}	3.4 ± 0.2^{d}	19.2 ± 1.2^{de}	8.2 ± 2.2^{bc}
	Narasin	60	41 ± 3^{e}	3.5 ± 0.2^{d}	17.0 ± 2.7^{e}	4.7 ± 0.8^{c}
	Lasalocid	75	59 ± 7^{d}	1.1 ± 0.6^{bc}	16.7 ± 0.8^{e}	6.2 ± 1.0^{c}
	Arprinocid	60	84 ± 4^{bc}	0.4 ± 0.3^{ab}	26.6 ± 0.5^{abc}	12.1 ± 1.6^{a}
	Halofuginone	3	75 ± 8^{c}	0^{a}	25.1 ± 1.3^{abc}	12.3 ± 1.0^{a}
Laboratory	None (inoculated control)	0	58 ± 8^{d}	2.7 ± 0.2^{d}	17.6 ± 2.3^{e}	5.6 ± 1.1^{c}
	Monensin	121	93 ± 3^{ab}	0.1 ± 0.1^{a}	24.5 ± 0.9^{abc}	12.6 ± 2.1^{a}
	Narasin	60	94 ± 4^{ab}	1.2 ± 0.3^{bc}	22.2 ± 2.5^{cd}	12.9 ± 2.0^{a}
	Lasalocid	75	92 ± 4^{ab}	1.6 ± 0.4^{c}	23.7 ± 1.6^{bc}	11.8 ± 1.5^{ab}
	Arprinocid	60	98 ± 2^{ab}	0.1 ± 0.1^{a}	28.0 ± 0.8^{ab}	13.4 ± 1.1^{a}
	Halofuginone	3	95 ± 3^{ab}	0^{a}	28.9 ± 0.4^{a}	12.3 ± 0.6^{a}

* Medication begun on D −1; inoculation D 0; weight gain D 0 to D 6; cecal lesion score, PCV, and plasma pigment at D 6.
† Values within a column followed by a common letter are not significantly different ($P \leq 0.05$). Each value is $\bar{x}$ ± SEM.

Table 3. Efficacy of medication against the original field strain of *E. tenella* (parent) and the same strain passed 5 successive times in chicks medicated with monensin (Exp. 3).*

Parameter	Uninoculated unmedicated control	Strain	Medicated (ppm) None	Monensin (121)	Lasalocid (75)	Salinomycin (66)	Buquinolate (82.5)
Weight gain (g)	130 ± 5[a]†	Parent	78 ± 10[c]	106 ± 5[b]	102 ± 5[b]	80 ± 6[c]	125 ± 3[b]
		Passed	4 ± 11[e]	32 ± 9[d]	16 ± 8[de]	20 ± 5[de]	33 ± 9[d]
Lesion score	0[a]	Parent	2.8 ± 0.2[cd]	2.2 ± 0.1[bc]	2.2 ± 0.1[bc]	2.5 ± 0.2[bc]	0[a]
		Passed	3.0 ± 0.3[d]	3.0 ± 0.2[d]	3.2 ± 0.2[d]	2.9 ± 0.3[cd]	2.0 ± 0[b]
PCV (%)	26.3 ± 0.8[a]	Parent	21.7 ± 2.6[bc]	22.9 ± 1.8[bc]	22.3 ± 0.8[bc]	19.0 ± 1.9[bc]	27.7 ± 0.5[a]
		Passed	14.1 ± 1.6[d]	14.7 ± 1.5[d]	14.0 ± 1.5[d]	17.5 ± 2.1[cd]	21.0 ± 1.4[bc]
Plasma pigment	12.8 ± 1.7[a]	Parent	4.0 ± 1.1[de]	4.9 ± 0.6[cd]	7.8 ± 1.1[bc]	4.2 ± 1.1[de]	8.9 ± 1.4[b]
		Passed	1.7 ± 0.2[e]	4.2 ± 0.8[de]	3.0 ± 0.8[de]	1.3 ± 0.2[e]	5.4 ± 0.2[cd]

* Against the laboratory strain, average protection by medication was 90% for weight gain; 75% for lesion score; 94% for PCV; 72% for plasma pigment.

† Values within a parameter not followed by a common letter are significantly different ($P \leq 0.05$). Each value is $\bar{x} \pm$ SEM.

FS or the LS. The remaining unmedicated group was not inoculated.

Passage of laboratory strain under monensin pressure

The LS was passed for 50 successive generations in unmedicated chicks and in chicks fed 363 ppm monensin (Exp. 4). The procedure was essentially that described by McLoughlin and Chute (1978) using oocysts from the cecal cores at 8 days PI. After each passage, the recovered oocysts were tested for resistance to monensin by inoculating chicks with oocysts of the monensin passage and the corresponding unmedicated passage.

Passage of mixed strains under monensin pressure

A mixture of five strains of *E. tenella* was passed for six successive generations in chicks medicated with 121 ppm monensin. Oocysts were harvested at 8 DPI from cecal contents. After four passages, the oocysts were tested for monensin resistance in comparison to the original FS strain, the LS, and the MPLS (Exp. 5). Two trials were run to test susceptibility to monensin after six passages (Exp. 6).

Statistical analysis

Data was analyzed using analysis of variance and Duncan's Multiple Range Test.

Results

The FS of *E. tenella* used in these trials produced lesions and markedly reduced weight gain, PCV, and plasma pigment in unmedicated chicks (Tables 1, 2). In Exp. 1, monensin or lasalocid produced no or only slight improvement in these parameters of infection (Table 1). Amprolium and nicarbazin gave some improvement, whereas buquinolate, robenidine, and clopidol gave good control of the infection. In Exp. 2, the efficacy of monensin, narasin, lasalocid, arprinocid, and halofuginone was readily demonstrated against the LS (Table 2). Conversely, these same compounds, especially the ionophores, failed to control infection with the FS. Weight gain with arprinocid and halofuginone remained significantly less than in uninoculated control chicks, even though they improved PCV and plasma pigment, and reduced lesion score.

The total feed consumption of chicks fed 363 ppm monensin in Exp. 3 was 60% that consumed by chicks given unmedicated feed and never less

Table 4. Effect of medication with 121 ppm monensin on infections of a laboratory strain of *Eimeria tenella* successively passed in medicated (Mon) or unmedicated (None) chicks (Exp. 4).

Passage	Weight gain* Mon	Weight gain* None	Lesion score Mon	Lesion score None	Oocysts/bird (×10⁶) Mon	Oocysts/bird (×10⁶) None
1–20	79 ± 6	94 ± 5	1.1 ± 0.2	0.8 ± 0.1	5.5 ± 0.6	8.4 ± 1.5
21–40	88 ± 3	95 ± 4	1.3 ± 0.2	1.1 ± 0.1	6.3 ± 1.1	5.9 ± 0.8
41–50	90 ± 5	92 ± 4	1.4 ± 0.2	1.0 ± 0.3	4.9 ± 1.0	6.5 ± 1.6

* Expressed as a % of unmedicated, uninoculated controls. Weight gain of unmedicated, inoculated birds averaged 29.5 ± 2.9% and 22.1 ± 1.9% of the unmedicated, uninoculated controls for the 50 passages of the monensin-medicated and unmedicated passed oocyst, respectively.

Table 5. Efficacy of 121 ppm monensin against several strains of *E. tenella* (Exp. 5).

Strain	Medication	Weight gain (g)	Lesion score	PCV (%)	Plasma pigment (β-carotene equiv.)
Uninoculated	Unmedicated control	123 ± 6[a]†	0[a]	26.4 ± 0.5[a]	11.9 ± 0.6[ab]
Laboratory	None	−18 ± 8[d]	3.4 ± 0.3[c]	19.2 ± 1.4[cd]	4.8 ± 0.7[b]
	Monensin	122 ± 5[a]	0	24.8 ± 0.6[a]	13.1 ± 1.9[a]
Laboratory-passed*	None	−23 ± 11[d]	3.2 ± 0.2[c]	20.1 ± 2.6[bcd]	5.4 ± 1.1[b]
	Monensin	114 ± 10[a]	0.4 ± 0.3[a]	25.2 ± 0.8[a]	11.2 ± 1.2[a]
Mixed-passed*	None	13 ± 12[c]	3.0 ± 0.2[c]	23.0 ± 1.0[abc]	6.2 ± 0.6[b]
	Monensin	104 ± 9[ab]	2.0 ± 0[b]	23.9 ± 1.7[ab]	11.7 ± 2.1[a]

* 50 passages under monensin pressure for the laboratory strain and four passages for the mixed.

† Values within a column followed by a common letter are not significantly different ($P \leq 0.05$). Each value is $\bar{x}$ ± SEM.

than 31% on any day. Thus, the exposure of the coccidia to monensin at 363 ppm was equivalent to, or exceeded, the exposure level that would have existed at 121 ppm. Monensin, lasalocid, and salinomycin were less effective in controlling the MPFS than in controlling the FS (Table 3, Exp. 3). Even buquinolate, which was highly effective against the FS was not as efficacious against the MPFS. The MPFS was more pathogenic than the FS in unmedicated birds, especially based on weight gain and PCV.

Attempts to develop monensin resistance in the LS by 50 passages through monensin-medicated chicks were unsuccessful (Table 4, Exp. 4). Control of the infection based on weight gain, lesion score, and oocyst production was equally as good in the 41–50 passages as in the 1–20 passages. An additional test (Exp. 5) confirmed these results (Table 5). In the same trial, monensin was effective in controlling weight and plasma pigment loss with the mixed strain passed four times, but did not eliminate lesions or protect against the decrease in PCV (Table 5). After six passages of the mixed strain, two separate trials showed that monensin no longer protected against the depressed weight gain caused by infection with the passed mixed strain, although monensin was effective against the parent mixed strain (Table 6).

Discussion

In the present study, the recovery of a field strain of *E. tenella* that was not well controlled by monensin is not unique. What was novel, however, was that a wide range of compounds, including non-ionophores, gave less than expected control with this strain (Tables 1, 2). In addition, the lack of control was increased by passage under monensin pressure. Furthermore, a similar decrease in control was produced by passing mixed strains of *E. tenella* six times in monensin-medicated chickens. This is the first

Table 6. Efficacy of 121 ppm monensin against mixed strains of *E. tenella* (parent) or the same mixture passed six times in chicks medicated with monensin (Exp. 6).

			Parameter			
Trial	Strain	Medication	Weight gain (g)	Lesion score	PCV (%)	Plasma pigment (β-carotene equiv.)
1	Uninoculated	None	94 ± 3[a]*	0[a]	26.0 ± 0.4[a]	6.5 ± 0.6[a]
	Parent	Monensin	84 ± 6[a]	0.2 ± 0.1[a]	25.4 ± 1.3[a]	7.3 ± 0.6[a]
	Passed	Monensin	67 ± 7[b]	0.7 ± 0.1[a]	26.5 ± 0.8[a]	6.2 ± 0.6[a]
2	Uninoculated	None	109 ± 6[a]	0[a]	30.3 ± 1.0[a]	—
	Parent	Monensin	115 ± 4[a]	0[a]	28.6 ± 0.8[ab]	—
	Passed	Monensin	68 ± 12[b]	2.6 ± 0.2[b]	25.3 ± 1.4[b]	—

* Values within a column and trial followed by a common letter are not significantly different ($P \leq 0.05$). Each value is $\bar{x}$ ± SEM.

report of the enhancement of resistance to monensin in a field strain of chicken coccidia. Chapman (1984) developed partial resistance in the Houghton laboratory strain of *E. tenella.*

The speed with which resistance developed was dependent on the particular strain used initially. Although the LS readily developed drug resistance to a variety of other anticoccidials (McLoughlin, 1970; McLoughlin and Chute, 1978), including amprolium, quinolones, robenidine, and nicarbazin, even 50 passages under monensin pressure failed to give any indication of resistance (Table 4). Conversely, passage of the FS under monensin pressure readily lessened drug control (Table 3), whereas the development of increased pathogenicity in the mixed strain was somewhat slower (Tables 5, 6). Chapman (1984) required 16 passages to develop partial resistance in the Houghton strain of *E. tenella.* This is in agreement with Weppelman et al.'s (1977a), Jeffers' (1981), and Chapman's (1982) conclusions that chicken coccidia do not readily develop resistance to monensin.

Acknowledgments

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Development of the Parasitic Stages of *Nematodirus abnormalis* in Experimentally Infected Sheep and Associated Pathology

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ABSTRACT: The development of *Nematodirus abnormalis* May, 1920 was examined in 10 worm-free lambs each infected with 60,000 larvae and killed 2–20 days after infection (DAI). Most worms developed in the anterior 4 m of the small intestine, with the third and fourth molts occurring 4–6 and 12–14 DAI respectively. A large proportion of larvae remained inhibited at the early fourth stage. Morphological development of the larvae including the genitalia and body ridges is described in detail. Pathological changes were associated with worms coiling around villi and distorting but not breaking the continuity of the epithelium. *N. abnormalis* is thought to be only mildly pathogenic for sheep.

Nematodirus abnormalis May, 1920 is a cosmopolitan parasite of sheep, goats, and other ruminants, but in spite of its possible economic importance, it has been little studied. Frequently it occurs in mixed infections with other species and is usually present as only a small proportion of the total number of *Nematodirus* (Brunsdon, 1961; Becklund and Walker, 1967). However, in sheep in South Australia, *N. abnormalis* is frequently the dominant species in mixed infections or may occur in monospecific infections (Beveridge and Ford, 1982). A similar situation occurs in other areas of the world with hot, dry Mediterranean type climates (Guralp and Oguz, 1967).

Aspects of development of the free-living stages of *N. abnormalis* have been studied by Onar (1975) and Neiman (1977), and adult morphology has been described in detail by Becklund and Walker (1967), Stringfellow (1968), Lichtenfels and Pilitt (1983), and Rossi (1983). In this paper we describe for the first time the development of the parasitic stages together with the pathological changes associated with infection by a single dose of *N. abnormalis* larvae.

Materials and Methods

Nematodirus abnormalis was obtained from naturally infected sheep from Myrtle Springs Station, Leigh Creek, South Australia (30°27′S, 138°13′E). Two naturally infected sheep were killed and nematodes from the upper small intestine were collected in tissue culture medium (Medium 199, C.S.L. Melbourne, Australia) and held at 37°C for no more than 2–3 hr. Males were identified individually by the characteristic morphology of the spicule tip (Becklund and Walker, 1967), and males of *N. abnormalis* and all females present were surgically transplanted into the duodenum of a lamb that had been raised worm-free. Feces from the recipient sheep were collected and cultured beginning 21 days after surgery. Feces were soaked in water, homogenized, and sieved to remove gross particulate material. The washings were allowed to sediment, the supernatant removed, and the residue mixed with a saturated sugar solution. The top layer of the flotation containing the eggs was removed and washed in a 44-μm sieve to remove the sugar. The eggs were then cultured in water in large petri dishes at 25°C for up to 5 wk, until all the eggs had hatched. Larvae were concentrated using a Baermann technique and stored in water at 4°C until used. The isolate was passaged through several generations in worm-free sheep prior to use in this experiment. Larvae used for experimental infections were fresh larvae, and were used within 4 wk of culturing.

Ten 12-wk-old Merino lambs, raised worm-free, were each infected with 60,000 *N. abnormalis* larvae by stomach tube. A single worm-free lamb served as a non-infected control, and was killed at the commencement of the experiment. The infected sheep were killed 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after infection (DAI). Sheep were killed using an intravenous barbiturate overdose, and the anterior small intestine was removed rapidly, freed of mesenteric attachments, and divided into six, 1-m-long sections using large artery forceps. Two small sections (4 cm) of tissue at the anterior end of each of the first four segments were separated with forceps and gently distended by injection of 10% buffered formol-saline or 2.5% glutaraldehyde in cacodylate buffer. As soon as the segments had been filled with fixative (within 3–10 min of the death of the sheep), the segments were opened, attached to small pieces of card, and placed in the appropriate fixative. Specimens fixed in glutaraldehyde were washed gently to remove debris, and after 4–6 hr were stored in cacodylate buffer with added sucrose.

The content of the six 1-m lengths and the remainder of the small intestine were washed out with normal saline, sedimented at room temperature, and the sediment from two of the sectors fixed with hot 70% ethanol for morphological study of the nematodes. The remainder were fixed with cold formol-saline. The number of nematodes in each segment of the intestine

Table 1. Distribution of *Nematodirus abnormalis* in 1-m segments of the small intestine of lambs experimentally infected with 60,000 larvae.

Time of killing (DAI)	Percentage of worm burden in each small intestine segment						
	Segment						
	1	2	3	4	5	6	Remainder
2	61.6*	28.0	5.6	0.9	0	0.9	2.8
4	21.5	55.4*	10.4	3.6	2.0	2.0	5.2
6	7.4	16.5	20.5	24.2*	21.5	7.1	2.7
8	3.3	9.5	15.2	5.4	12.8	27.2*	26.7
10	0	0	1.7	3.3	0	1.7	93.3*
12	0	0.6	29.0	39.3*	16.2	7.6	7.3
14	1.0	14.0	23.7	41.6*	17.2	2.5	0
16	0.4	13.1	41.2*	36.5	7.1	1.8	0
18	0	3.7	49.4*	19.2	6.57	13.4	7.7
20	8.8	35.1*	28.5	21.6	2.7	1.1	2.2
Total	10.4	17.6	22.5*	19.6	8.6	6.5	14.8
SE of mean	6.1	5.0	4.8	5.0	2.6	2.6	9.0

* Mode.

was estimated by duplicate counts on 10% samples. Nematodes fixed in 70% ethanol were mounted in glycerine for morphological examination and a series of standard measurements made on 10 males and 10 females from each sheep using a micrometer eyepiece, or by drawing the worm with the aid of a drawing tube attached to the microscope, and obtaining the measurements from the drawing using a map measurer.

For quantitative studies on the growth of worms, both larvae and adults were selected for measurement. Prior to 6 DAI, the sex of larvae could not be determined, and measurements were made on 20 randomly selected larvae. At 6 and 8 DAI, 20 male and 20 female larvae were measured. From 12 DAI onwards, 20 male and 20 female adults were selected together with a number of early fourth-stage larvae and fourth-stage larvae close to the final molt. The measurements of each group of larvae were treated separately.

Table 2. Worm counts of Merino lambs experimentally infected with 60,000 larvae of *Nematodirus abnormalis* and killed 2–20 days after infection (DAI).

Time of killing (DAI)	Total no. worms	Percent established	Proportions of larval stage (%)			
			L_3	Early L_4	Late L_4	L_5 adult
2	10,700	18	100	0	0	0
4	30,700	51	100	0	0	0
6	29,700	50	0	100	0	0
8	24,300	41	0	100	0	0
10	6,000	10	0	100	0	0
12	32,800	55	0	90	10	0
14	40,100	67	0	74	25	1
16	45,200	75	0	91	2	7
18	35,000	58	0	83	4	13
20	36,500	61	0	91	3	6

Drawings of genital organs were made with the aid of a drawing attachment to the microscope. Hand-cut sections of the nematodes were made in the esophageal, mid-body, and caudal regions and were examined in glycerine using Nomarski interference contrast to determine the pattern of the body ridges. Numbering of the body ridges follows the system of Lichtenfels and Pilitt (1983).

Formalin-fixed tissues were trimmed longitudinally, embedded in wax, sectioned at a thickness of 6 μm, and the sections stained with hematoxylin and eosin. Tissues fixed in glutaraldehyde were sectioned similarly and stained to demonstrate alkaline phosphatase (Bancroft and Stevens, 1977), and larger pieces of tissue were dehydrated in graded ethanols, critical-point dried using CO_2, mounted, and coated with a 200-nm layer of gold. Specimens were examined in a JEOL JSM-P15 scanning electron microscope (SEM).

Results

Distribution within the intestine

The majority of nematodes was recovered from the first 6 m of the small intestine (Table 1). There was considerable variation in parasite distribution between individual animals, with an apparent posterior migration prior to the final molt followed by an anterior migration. Because only one lamb was killed at each examination, the significance of this observation is uncertain and the results were combined. Most of the nematodes were recovered from meters 2–4 of the small intestine (Table 1). The higher mean percentage in the remainder compared with meter 6 was due to a single animal (10 DAI) in which the majority of its nematode burden was recovered from the lower small intestine.

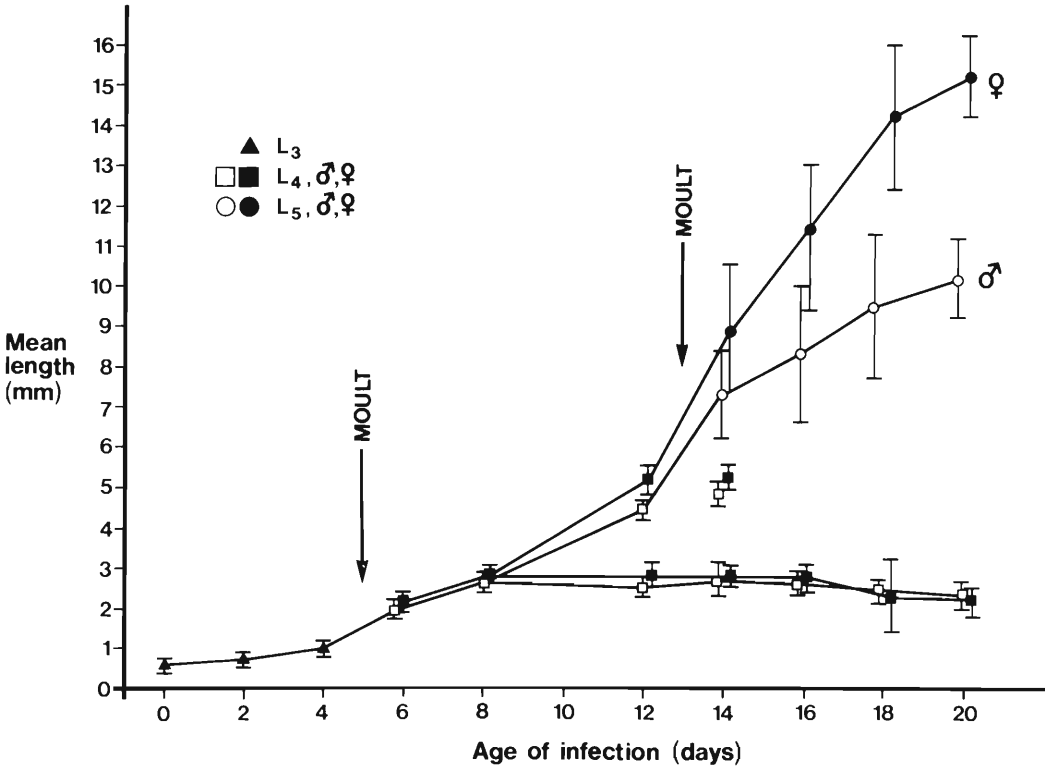


Figure 1. Growth of *Nematodirus abnormalis* in lambs experimentally infected with 60,000 larvae. Each point in main curve of development represents mean length of 20 larvae ± SD.

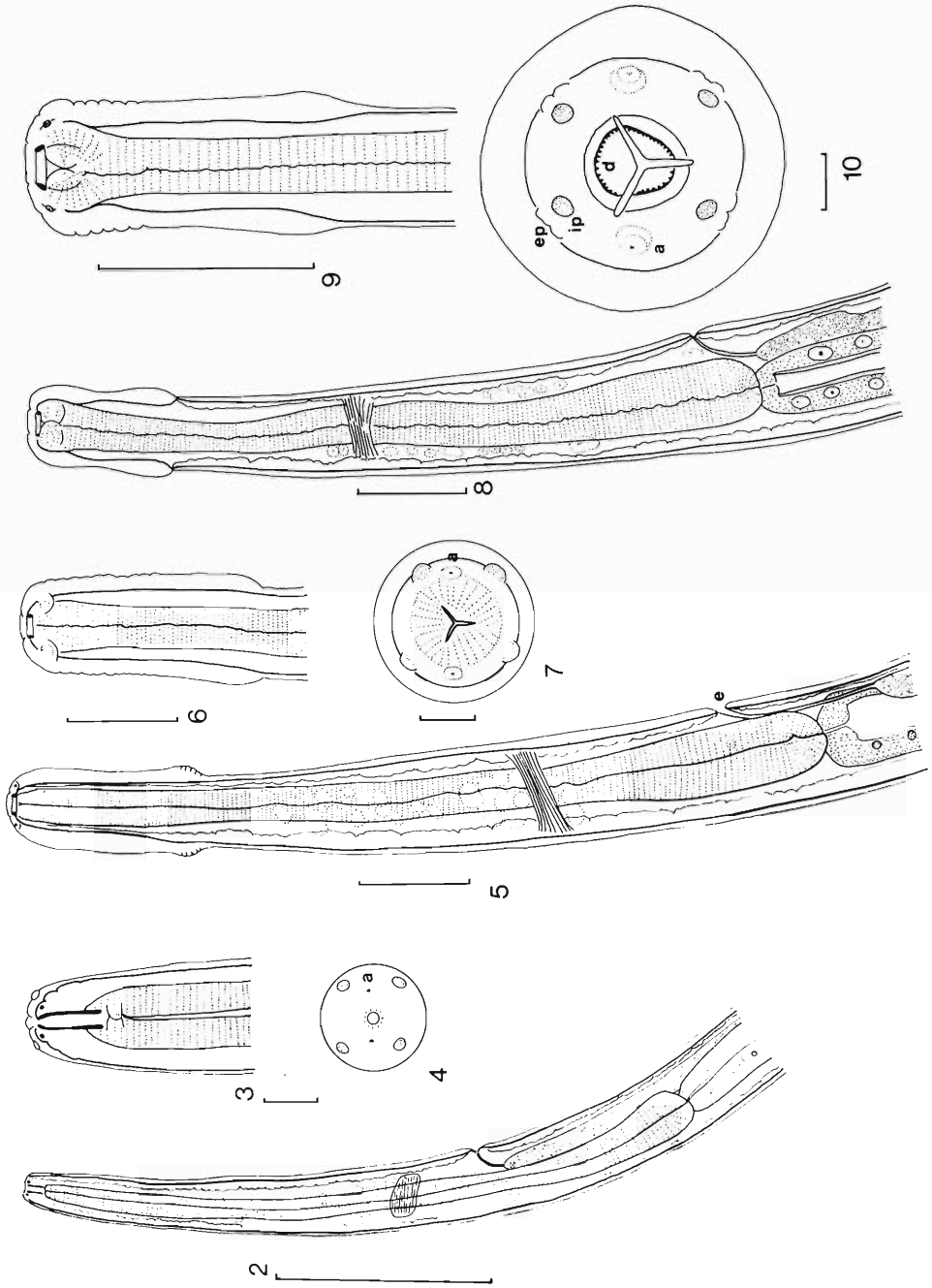
Establishment and growth within the intestine

Between 10 and 75% (mean 49%) of larvae established and developed in the small intestine (Table 2). The single lamb with the lowest nematode burden (10 DAI) had the majority of its nematodes in the posterior region of the small intestine. Development within the small intestine was synchronous until after the third molt, with considerable variation (74–91% at 14–20 DAI) in the numbers of inhibited larvae occurring between individual sheep. At 12 and 14 DAI a large number of nematodes was found approaching the fourth molt (Table 2). Their percentage in the total count declined dramatically from 16 to 20 DAI as most developing nematodes became adults, however a small percentage was always present. It was not clear whether these were larvae that were developing more slowly, had been inhibited, and were resuming their development or, indeed, were inhibited prior to the final molt.

Following infection, worms grew slowly until 4 DAI. The third molt occurred between 4 and 6 DAI without any obvious prior lethargus and the final molt occurred between 12 and 14 DAI, apparently not affecting the rapid rate of growth during this period. Larvae could be differentiated sexually from 6 DAI onwards. Lack of alcohol-fixed larvae 10 DAI precluded making measurements comparable with those made on the other nematodes. The inhibited larvae failed to increase in length, and were slightly shorter in the 18 and 20 DAI samples, when compared with earlier measurements. From 12 DAI onwards, female nematodes grew at a significantly greater rate than males (Fig. 2, Table 2). Patency was achieved 18 DAI.

Morphogenesis

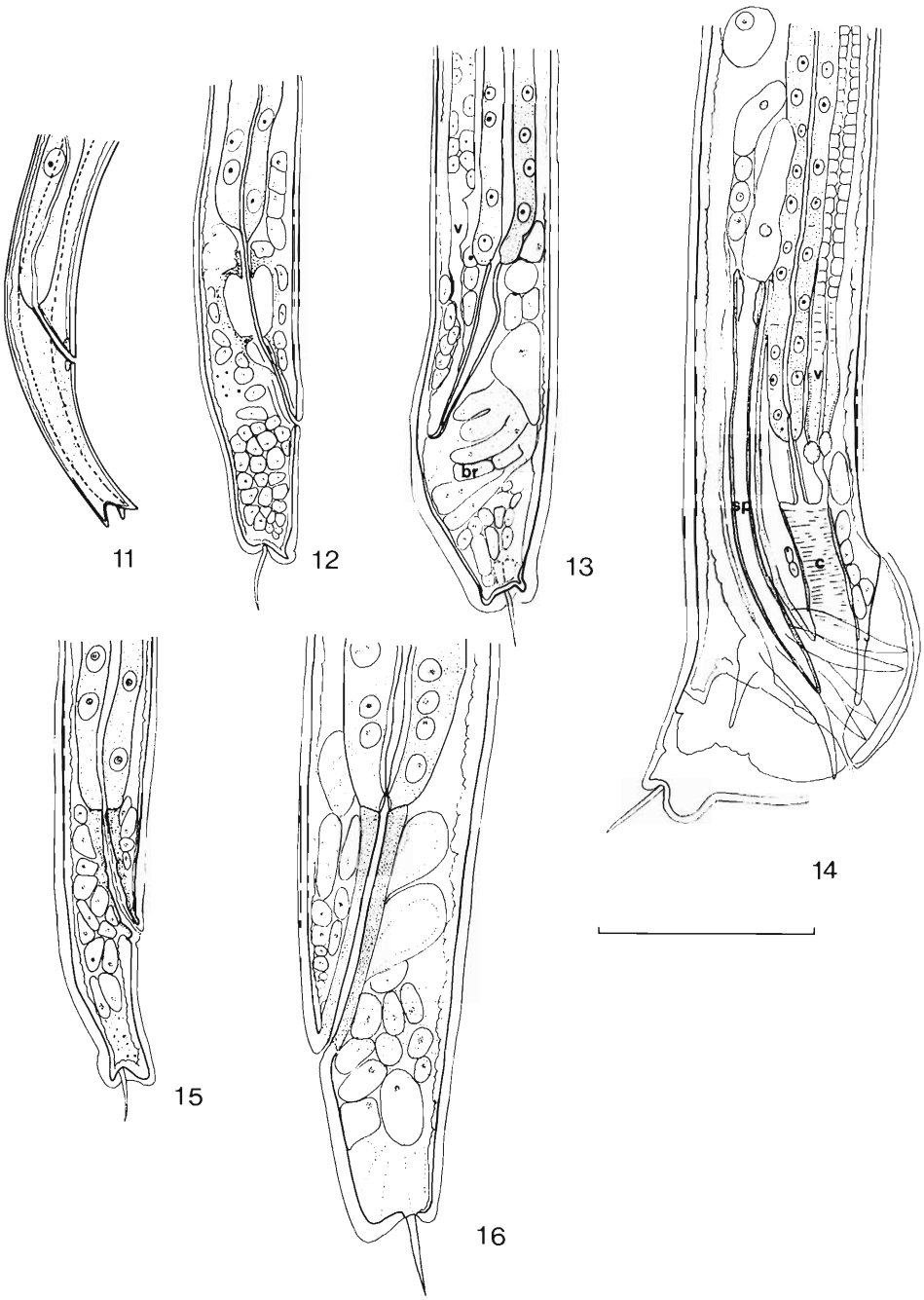
Features of the morphogenesis are summarized below, under age of infection (DAI) and larvae stage (L_3, L_4, or L_5). Measurements (mm) of organs are presented as the range of 10 measurements followed by the mean in parentheses.



Figures 2-10. Anterior extremities of larval and adult stages of *Nematodirus abnormalis*. 2-4. Third larval stage. 2, 3. Lateral views. 4. En face view. 5-7. Fourth larval stage. 5, 6. Lateral views. 7. En face view. 8-10. Adult. 8, 9. Lateral views. 10. En face view. Legend: a, amphid; d, denticles; e, excretory pore; ep, external papilla; ip, internal papilla. Scale lines: Figures 2, 5, 8, 9, 0.1 mm; Figures 3, 4, 6, 7, 10, 0.01 mm.

0 DAI: L_3: Genital primordium oval, 0.016–0.030 (0.020) × 0.006–0.012 (0.010), 6–8 cells visible (Fig. 28).

2 DAI: L_4: Cephalic vesicle absent (Fig. 2); mouth opening circular; 2 amphids and 4 submedian papillae visible in en face view (Fig. 4); buccal capsule cylindrical, longer than wide (Fig.3); esophagus filiform; excretory pore prom-



Figures 11–16. Caudal extremities of larval and adult stages of *Nematodirus abnormalis*. 11. L_3, 4 DAI. 12. L_4, 6 DAI, ♂, showing vacuolation around rectum indicating formation of bursa. 13. L_4, 8 DAI, ♂, showing opening of vas deferens into future cloaca and vestigial bursal rays. 14. L_4, 12 DAI, ♂, about to undergo final molt, showing spicule primordium with associated cells, and cloaca. 15. L_4, 6 DAI, ♀. Note lack of vacuolation around rectum. 16. L_5, 15 DAI, ♀. Legend: br, bursal rays; c, cloaca; sp, spicule; v, vas deferens. Scale: 0.1 mm.

inent, anterior to esophago–intestinal junction. Two body ridges visible in lateral view (Fig. 19); extend to within 0.08 from anterior end, replaced by single lateral ala (Fig. 17). Body ridges extend onto dorsal and ventral tail projections (Fig. 11). Slight enlargement of genital primordium evident, 0.022–0.032 (0.024) × 0.008–0.014 (0.012) with up to 14 cells visible (Fig. 29). Tail composed of three subequal triangular projections, two ventral, one dorsal (Fig. 11).

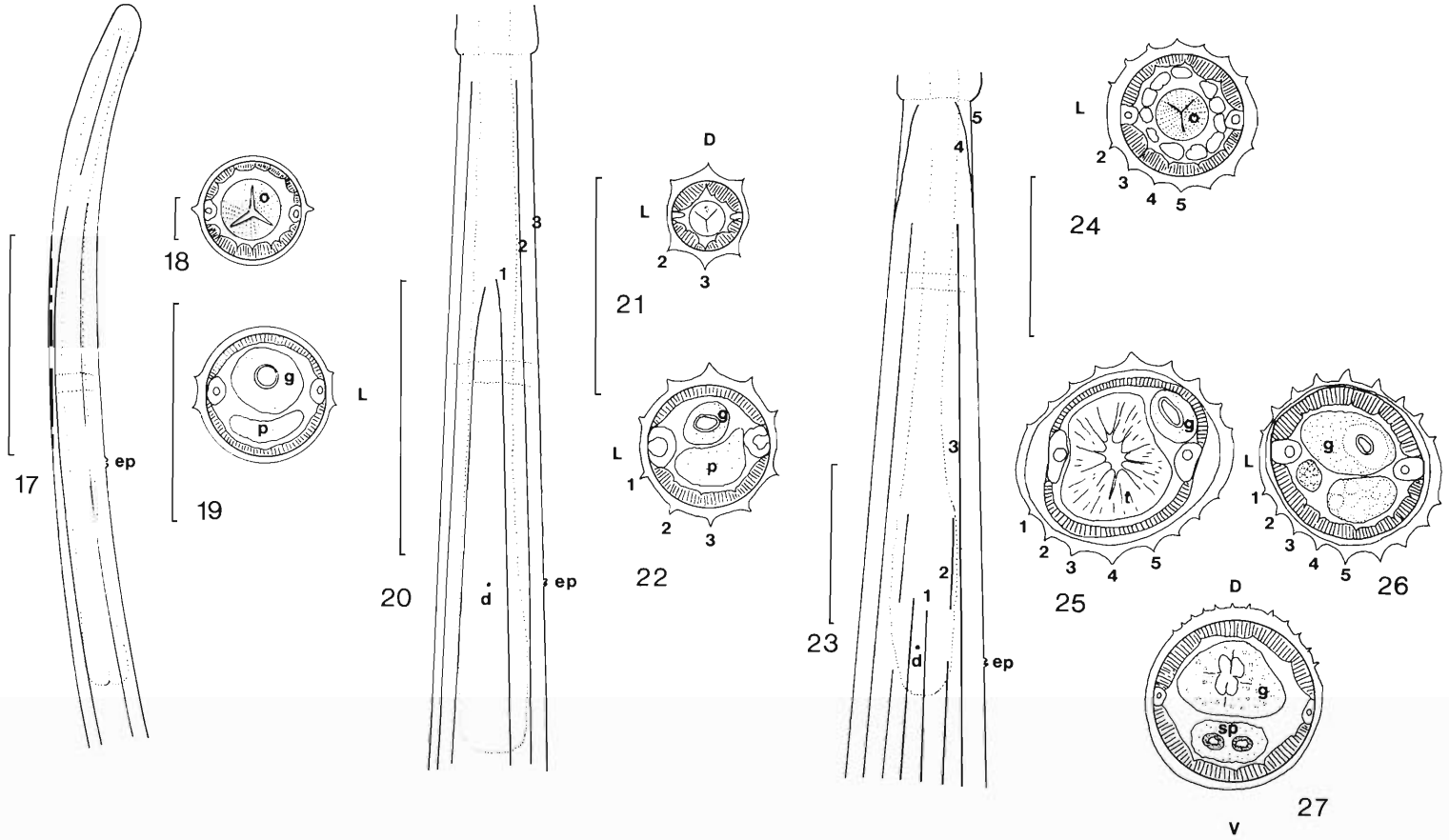
4 DAI: L_3: Just prior to molt, separation of cuticle evident at anterior and posterior ends; structures such as buccal ring and tail spike of L_4 faintly visible within. Two types of anlagen present: majority oval or elongated, slight increase in cell number; cells larger, nuclei prominent, anlagen 0.060–0.100 (0.071) × 0.012–0.016 (0.014); some anlagen with prominent central body 0.070–0.090 (0.080) × 0.014–0.018 (0.016) and with tapering arms 0.050–0.140 (0.071) at either end (Figs. 30, 31).

6 DAI: L_4: Cephalic vesicle present (Fig. 6); two amphids and four submedian papillae visible in en face view (Fig. 7); buccal capsule shallow, ring-like, peri-oral denticles absent (Fig. 6); esophagus claviform; excretory pore on slight eminence anterior to esophago–intestinal junction (Fig. 5). Five body ridges present on each side of body in mid-body region and posterior to it (Figs. 20, 22); ridges 1 and 5 terminate anterior to nerve ring; transverse sections anterior to termination show three ridges on each side (Fig. 21). Male anlage differentiated into elongate testis, 0.13–0.36 (0.25) × 0.014–0.018 (0.016), and narrow vas deferens 0.090–0.160 (0.120) × 0.008–0.018 (0.014); vas deferens terminates distally in two elongate cells, not reaching developing cloaca (Fig. 33). Female anlage differentiated into body of ovejector 0.070–0.110 (0.090) × 0.016–0.028 (0.021), formed of two parallel rows of cells; two vulval cells prominent on ventral aspects of ovejector; vaginae uterinae not differentiated (Fig. 32). Remainder of gonoduct undifferentiated; anterior branch, 0.060–0.132 (0.102) long, posterior branch reflexed, 0.064–0.110 (0.086) long. Tail of male and female with subtriangular dorsal and ventral lobes, and terminal spike (Fig. 12); area surrounding rectum and posterior to it occupied by large, prominent cells. Males distinguishable by slight swelling of posterior end and formation of cavity around rectum.

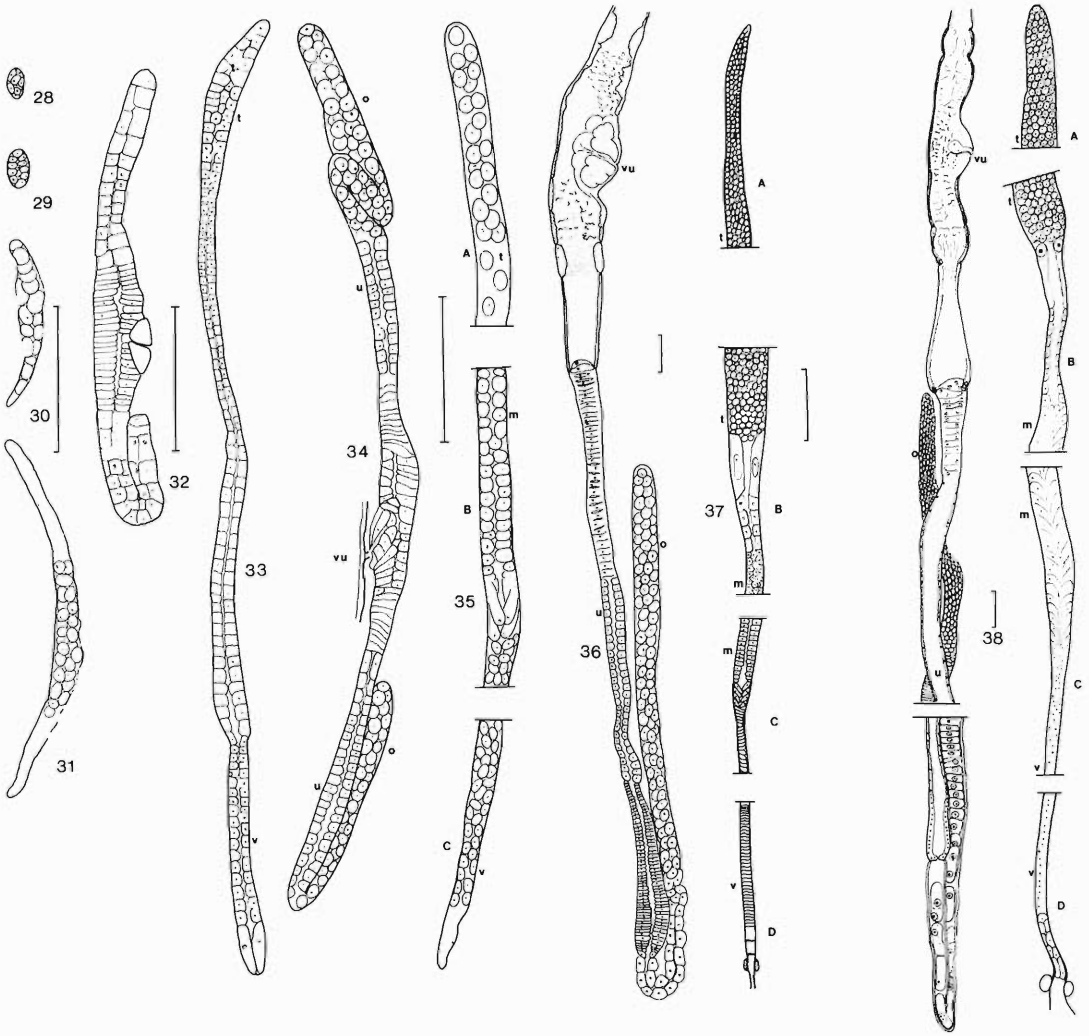
8 DAI: L_4: Testis greatly elongated, 0.52–0.62 (0.57) × 0.012–0.020 (0.016); vas deferens 0.32–0.52 (0.39) × 0.008–0.016 (0.011) separated from testis by series of elongate, radially arranged cells; vas deferens enters developing cloaca (Fig. 35). Female genitalia: Ovejector 0.072–0.110 (0.090) × 0.016–0.028 (0.021); vulva composed of two rows of four cells; vaginae uterinae differentiated from ovejector as series of elongate, transverse cells; uteri short, lined with columnar cells; ovary distinct from uterus; anterior ovary and uterus flexed; posterior ovary and uterus reflexed, 0.25–0.32 (0.30) (Fig. 34). Male tail swollen, primordial bursa present with vestigial bursal rays present in some specimens (Fig. 13).

12 DAI: L_4: Larvae just about to enter final molt. Major components of testis fully developed; germinative zone 0.68–1.28 (0.94) long, filled with germinative cells, separated from maturation zone by two elongate cells and two short rows of large cuboidal cells; maturation zone 0.32–0.56 (0.42) long; vas deferens 0.68–1.16 (0.96) long (Fig. 37). Elements of ovejector differentiated; infundibulum 0.24–0.38 (0.31) long; vulva a dense mass of cells around genital opening; sphincters of infundibulum partly formed; vaginae uterinae 0.10–0.18 (0.12) long; posterior uterus 0.53–0.70 (0.68) long; oviduct differentiated; ovary 0.38–0.72 (0.50) long, reflexed, anterior branch reaches vagina uterina (Fig. 36). Tail of male greatly swollen (Fig. 14); lobes and rays of bursa fully developed; cloaca present with openings to intestine and vas deferens; spicule primordium present, short, poorly sclerotized; large cells anterior to tip of spicule move anteriorly as spicule elongates.

14 DAI: L_5: Cephalic vesicle present (Fig. 9); two amphids, four internal, four external cephalic papillae visible in en face view (Fig. 10); peri-oral denticles present; esophagus claviform; excretory pore just anterior to esophago–intestinal junction (Fig. 8). Nine body ridges present on each side of body in mid-body region (Figs. 25, 26); ridges 1 and 9 terminate anterior to deirid (Fig. 24); ridges 2 and 8 interrupted before termination in mid-esophageal region (Fig. 23); ridges 3 and 7 terminate posterior to cephalic vesicle; posterior end of male with 12 dorsal ridges, no ventral ridges (Fig. 27). Testis fully formed; germinative zone 1.67–2.86 (2.02) long; maturation zone 0.60–0.82 (0.73) long; vas deferens patent, 1.28–2.16 (1.68) long (Fig. 39). Infundibulum, vulva, sphincters fully developed; infundibulum 0.35–0.59 (0.50) long; posterior uterus almost entirely patent 0.85–2.40 (1.74) long; ovary reflexed, 0.76–2.40 (1.71) long, ex-



Figures 17-27. Body ridges of larval and adult stages of *Nematodirus abnormalis*. 17-19. L_3. 17. Lateral view of anterior end. 18. Transverse section in anterior esophageal region. 19. Transverse section in mid-body region. 20-22. L_4. 20. Lateral view of anterior end. 21. Transverse section in anterior esophageal region. 22. Transverse section in mid-body region. 23-27. Adult. 23. Lateral view of anterior end. 24. Transverse section in mid-esophageal region. 25. Mid-body section of male. 26. Mid-body section of female. 27. Transverse section of posterior extremity of male. Numbering of ridges following Lichtenfels and Pilitt (1983). Legend: d, deirid; ep, excretory pore; g, gut; o, esophagus; p, excretory gland; sp, spicule; t, testis; D, dorsal; L, lateral; V, ventral. Scale lines: Figure 18, 0.01 mm; Figures 17, 19-27, 0.1 mm.



Figures 28–39. Development of the genital organs of the larval and adult stages of *Nematodirus abnormalis*. 28. Genital primordium, infective larvae. 29. Genital primordium, L_3, 2 DAI. 30. Genital primordium, L_3, ♂, 4 DAI. 31. Genital primordium, L_3, ♀, 4 DAI. 32. Genital system, L_4, ♀, 6 DAI. 33. Genital system, L_4, ♂, 6 DAI. 34. Genital system, L_4, ♀, 8 DAI. 35. Genital system, L_4, ♂, 8 DAI; (a) anterior extremity of testis, (b) junction of maturation zone with vas deferens, (c) vas deferens. 36. Posterior ramus of genital system, L_4, ♀, 12 DAI. 37. Genital system, L_4, ♂, 12 DAI; (a) anterior extremity of testis, (b) junction of testis with maturation zone, (c) junction of maturation zone with vas deferens, (d) posterior region of vas deferens. 38. Posterior ramus of genital system, adult, ♀, 14 DAI. 39. Genital system, adult, ♂, 14 DAI; (a) anterior extremity of testis, (b) junction of testis with maturation zone, (c) junction of maturation zone with vas deferens, (d) posterior region of vas deferens. Legend: m, maturation zone; o, ovary; t, testis; u, uterus; v, vas deferens; vu, vulva. Scale line: 0.1 mm.

tending to vagina uterina (Fig. 38). Spicule clear to pale yellow in color, incompletely sclerotized; bursa with inconspicuous internal bosses.

16 DAI: L_5: Testis: Germinative zone of increased length, 2.56–5.36 (3.98) long; maturation zone 0.75–1.36 (1.01) long; amoeboid sperm present in vas deferens, vas deferens 1.92–3.72 (2.69) long. Sperm present in uteri of females; some females with partially developed eggs in uteri; infundibulum 0.50–0.58 (0.55) long; posterior uterus longer, 2.00–3.36 (2.56) long; ovary also of greater length, 3.84–5.92 (4.68) long, extending anterior to infundibulum. Spicules fully sclerotized, dark brown in color; bosses on bursa distinct.

18 DAI: L_5: Main changes involve increases

Table 3. Measurements (mm) of *Nematodirus abnormalis* from experimentally infected lambs (means of 10 measurements ± SD).

Time after infection (days)	Stage of development	Sex of nematodes	Total length	Length of esophagus	Length from excretory pore to anterior end	Tail	Length from vulva to posterior end
2	L_3	—	0.66 ± 0.14	0.18 ± 0.03	0.12 ± 0.01	0.05 ± 0.02	—
4	L_3	—	0.98 ± 0.10	0.23 ± 0.02	0.15 ± 0.02	0.05 ± 0.03	—
6	L_4	♂	2.04 ± 0.17	0.27 ± 0.02	0.21 ± 0.03	0.05 ± 0.01	—
		♀	2.13 ± 0.10	0.24 ± 0.09	0.22 ± 0.03	0.05 ± 0.01	0.4 ± 0.03
8	L_4	♂	2.75 ± 0.23	0.29 ± 0.02	0.23 ± 0.02	0.06 ± 0.02	—
		♀	2.81 ± 0.16	0.28 ± 0.03	0.28 ± 0.03	0.05 ± 0.01	0.52 ± 0.06
12	L_4	♂	4.38 ± 0.18	0.30 ± 0.02	0.30 ± 0.01	—	—
		♀	5.25 ± 0.44	0.32 ± 0.02	0.31 ± 0.01	0.60 ± 0.02	1.13 ± 0.08
14	L_5	♂	6.94 ± 0.98	0.38 ± 0.02	0.33 ± 0.04	—	—
		♀	8.55 ± 1.40	0.40 ± 0.03	0.34 ± 0.02	0.06 ± 0.01	2.35 ± 0.55
16	L_5	♂	8.9 ± 0.85	0.36 ± 0.02	0.36 ± 0.05	—	—
		♀	11.5 ± 1.67	0.39 ± 0.02	0.41 ± 0.04	0.07 ± 0.01	3.51 ± 0.82
18	L_5	♂	9.5 ± 1.79	0.39 ± 0.02	0.38 ± 0.03	—	—
		♀	13.6 ± 2.04	0.42 ± 0.04	0.37 ± 0.03	0.06 ± 0.01	4.78 ± 0.85
20	L_5	♂	9.4 ± 0.83	0.38 ± 0.01	0.39 ± 0.03	—	—
		♀	15.5 ± 0.52	0.42 ± 0.02	0.38 ± 0.03	0.07 ± 0.01	5.64 ± 0.69

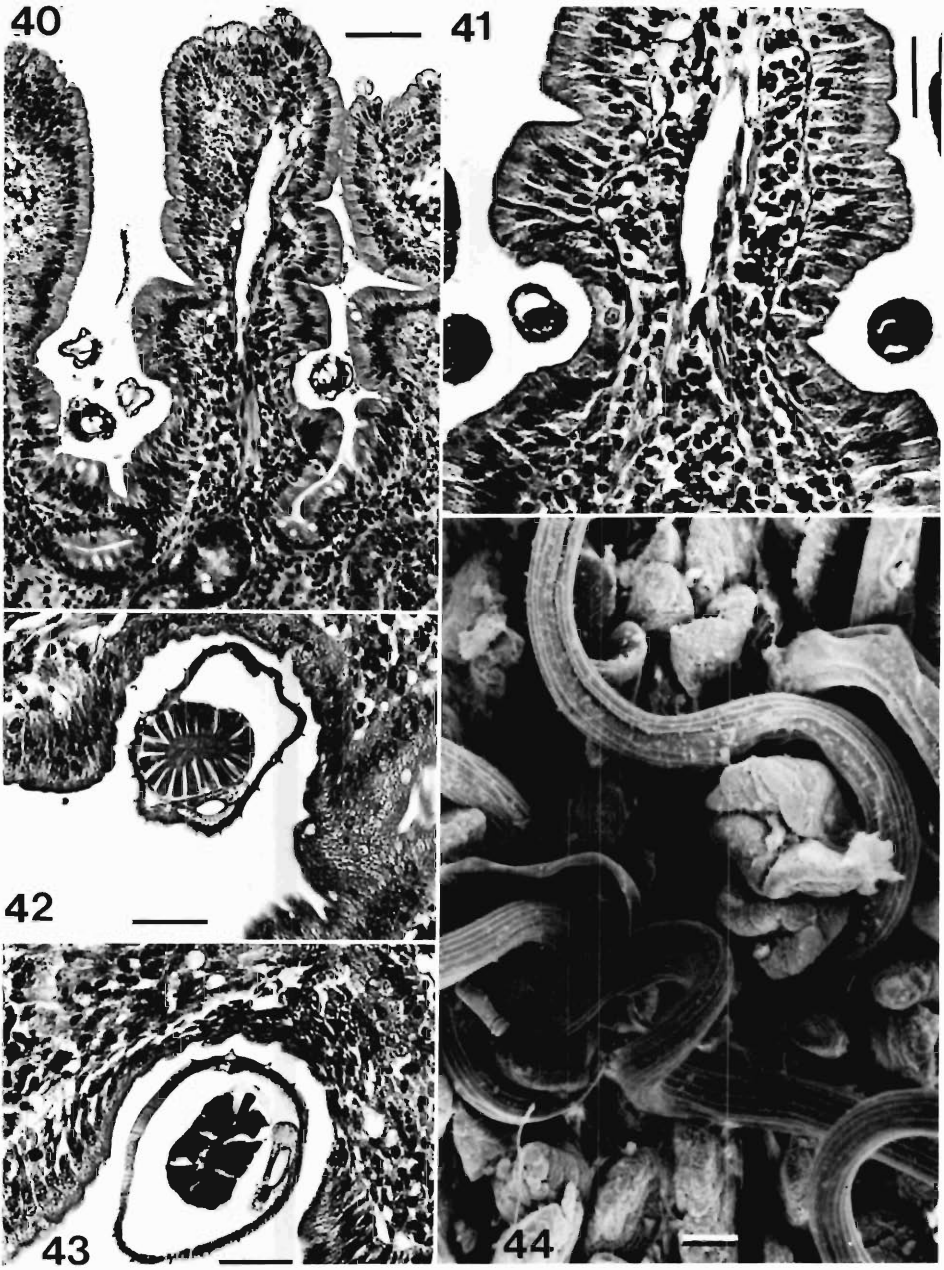
in size of most components of genital system. Germinative zone of testis 4.16–5.60 (4.89); maturation zone 0.96–1.68 (1.28) long; vas deferens 1.92–2.56 (2.26) long; infundibulum 0.60–0.72 (0.68) long; posterior uterus 2.72–4.00 (3.18) long; ovary 8.16–12.4 (10.3) long; females fully gravid.

20 DAI: L_5: Morphological changes limited to changes in size of genital organs; germinative zone of testis 3.44–6.00 (4.96) long; maturation zone 1.20–2.24 (1.45) long; vas deferens 1.92–2.40 (2.12) long. Infundibulum 0.53–0.73 (0.59) long; posterior uterus 2.56–3.52 (3.12) long; ovary 10.4–13.4 (11.8) long.

Pathology

SCANNING ELECTRON MICROSCOPY: Worms were found lying coiled or uncoiled on the surface of the intestine, with some worms lying between villi towards their bases. Some worms were coiled spirally around villi causing obvious compression. A small number of nematodes had their anterior ends deeply buried in crypts. There was no evidence of villous atrophy.

HISTOLOGY: Histological changes were related to the position of nematodes. Nematodes were frequently seen coiled around a single villus (Fig. 40), resulting in compression of the epithelium at the site of attachment and a noticeable indentation in the side of the villus. In the case of third-stage larvae, 4 DAI, the compression of the villus was out of all proportion with the diameter of the larvae (Fig. 41). The epithelial cells were reduced to a cuboidal form but there was little loss of the brush border. In infections with adult worms (Figs. 42, 43) there was similar compression of villi. The epithelium was reduced to a cuboidal form (Fig. 42) or even to a squamous epithelium (Fig. 43) with, occasionally, a break in the continuity of the epithelium. The body ridges of the nematodes made marked indentations into the epithelium (Fig. 43) and there was a complete loss of brush borders. A mild infiltration of mononuclear cells was evident in the lamina propria, and eosinophils were more prominent than in the control. In situations where the nematode body lay adjacent to the epithelium but was not constricting it (in crypts), there were no obvious changes in the epithelium. In a few instances where nematode heads were found deeply buried in a crypt, there was a marked accumulation of mononuclear cells in the lamina propria, but no obvious penetration of the epithelium by the parasite. There was some variation in villus height between individual sheep, but no marked villus atrophy was detected in infected sheep. The villi of sheep killed 16–20 DAI were marginally shorter than those in sheep killed 2–6 DAI, but were no shorter than the control. No differences were noted in the alkaline



Figures 40–44. Histological changes associated with infection of *Nematodirus abnormalis* in lambs. 40. Villus with fourth-stage larva (18 DAI) coiled around base, causing indentation of epithelium. 41. Third-stage larvae (4 DAI) coiled around villus; note extent of indentation. 42. Adult male coiled around villus causing reduction of columnar epithelium to cuboidal. 43. Adult male coiled around villus reducing epithelium to squamous layer; note indentations in epithelium produced by body ridges. 44. Scanning electron micrograph of adult *N. abnormalis* (16 DAI) coiling around tips of villi. Scale bars: 0.1 mm.

phosphatase activity in the mucosa of parasitized sheep.

Discussion

The distribution of larval and adult *N. abnormalis* in the anterior portion of the small intestine of experimentally infected sheep compares favorably with earlier studies on *N. filicollis* and *N. battus* by Thomas (1959b) in which most nematodes were recovered from the anterior 20 ft of the small intestine, the mode being in the 10–15-ft sector. Detailed quantitative investigations of distribution during development have been made only on *N. battus* in rabbits (Gallie, 1972), and in this study nematodes were distributed throughout the small intestine and were thought to select sites for attachment and undergo development at that site. Our data suggest that *N. abnormalis* larvae attach in the first few meters of the small intestine and that during development there is a general posterior migration of nematodes, followed after the final molt by an anterior migration. Because only a single lamb was killed at each time interval, these results must be treated with some caution and the phenomenon requires further study. The combined results indicate that the majority of nematodes occur in the first few meters of the small intestine, and this result agrees with data published for other trichostrongyloid nematodes in sheep (Barker, 1974; Taylor and Kilpatrick, 1980; Beveridge and Barker, 1983).

The general pattern of development of *N. abnormalis* in sheep was similar to that described for *N. battus* in rabbits, except that the development of *N. abnormalis* was more synchronous. The first molt occurred between 4 and 6 DAI in *N. abnormalis,* whereas Gallie (1972) noted the presence of some fourth-stage larvae of *N. battus* as early as 4 DAI. Likewise, *N. abnormalis* underwent the final molt between 12 and 14 DAI, whereas *N. battus* molted over a period of 10–14 DAI. With *N. abnormalis,* the percentage of late fourth-stage larvae reached a maximum of 25% 14 DAI. In spite of minor differences, the growth patterns of the two species are quite similar, with a percentage of early fourth-stage larvae remaining inhibited. In lambs killed 16, 18, and 20 DAI, there were two obvious populations of nematodes, one of developing adults, and a second of small inhibited larvae, with only a few larvae in the late fourth and early fifth stages.

A striking difference between this and earlier studies is the high proportion of nematodes that remained inhibited. In the lambs killed 16–20 DAI, only 6–13% of the nematodes were adult, with 74–91% remaining as inhibited at the fourth stage. By contrast, in Gallie's (1972) studies on *N. battus* less than 10% of the nematodes became inhibited. However, Thomas (1959a) reported that a lamb infected with *N. filicollis* and slaughtered 15 DAI contained mainly immature nematodes, with only 25% of adults present, a level approaching that seen in our study.

The mechanisms responsible for larval inhibition in trichostrongyloid nematodes are not clear, but work with certain species has shown that exposure of the infective larvae to cold may increase the percentage that became inhibited (Armour et al., 1969; Hutchinson et al., 1972; Michel et al., 1975). The larvae used in this experiment were stored at 4°C prior to use, and this treatment may have affected their subsequent development.

The morphogenesis of *Nematodirus* spp. has not previously been described in detail as early studies utilized few hosts and were little concerned with the detail of morphological development. A number of features of the development of *N. abnormalis* are of interest. In the male, the essential features of the genital system are formed prior to the final molt. Sperm are first present in the vas deferens 16 DAI, yet the germinative zone continues to increase from a mean length of 3.98–4.96 20 DAI, whereas the vas deferens and maturation zones remain at approximately the same size. Similarly, the posterior ovary elongates in the same period from a mean length of 4.68 at 16 DAI when partially formed eggs are visible in the uteri to a mean of 11.8 long 20 DAI when fully gravid. In the case of the female genitalia, only the posterior uterus and ovary were clearly visible throughout their development and consequently they are the only parts of the genitalia described. The spicule of the male is first visible 12 DAI, though poorly sclerotized, and continues to increase in length until 18 DAI. The spicule apparently grows from its anterior, knobbed extremity, and several large, prominent cells associated with the anterior end of the spicule move anteriorly as the spicule elongates, suggesting a direct connection with spicule formation.

The body ridges of adult *Nematodirus* spp. have been studied in detail by Durette-Desset

(1979), Rossi (1983), and particularly by Lichtenfels and Pilitt (1983). Limited observations on the morphology of ridges in the fourth-stage larva have been made by Durette-Desset (1979) and Rossi (1983), but the morphogenesis of the ridges has not previously been followed through all parasitic larval stages. The fourth larval stage of *N. abnormalis* has only five ridges on each side of the body compared with nine in the adult, and in this respect resembles *N. spathiger* (Durette-Desset, 1979). However, just as ridges 1 and 2 terminate anterior to the excretory pore in the adult, leaving five ridges that continue to the cephalic vesicle, so in the fourth stage, ridge 1 terminates anterior to the nerve ring, with only ridges 2 and 3 continuing to the cephalic vesicle. In the third larval stage only two lateral ridges are present, and anterior to the nerve ring these terminate and are replaced by a single lateral ridge. Thus, through the parasitic larval stages, there is a regular increase in the number of body ridges (2–5–9), but always with a major discontinuity in the esophageal region. The recent use of body ridges in a cladistic analysis of certain *Nematodirus* spp. by Lichtenfels and Pilitt (1983) is therefore supported by the present examination of the ontogenesis of ridges in *N. abnormalis,* since both *N. abnormalis* and *N. spathiger,* which form a monophyletic pair in their cladistic analysis, have similar body ridge patterns in the fourth larval stage.

Only limited conclusions can be made about the pathogenicity of *N. abnormalis* since a single lamb was killed at each time interval and the number of nematodes established in these lambs was not high. However, the basic pathogenetic mechanisms appeared to be coiling around villi, deformation of the epithelium, but lack of penetration into the lamina propria.

By contrast, *N. battus,* the most pathogenic species known from sheep, penetrates the lamina propria as far as the muscularis mucosae in both sheep and rabbits (Thomas, 1959a; Gallie, 1972, 1973) during the fourth larval stage and is responsible for diarrhea and weight loss prior to patency. *N. spathiger,* which causes severe diarrhea in lambs (Kates and Turner, 1953; Seghetti and Senger, 1958), also undergoes an extensive migration into the lamina propria during the fourth larval stage (Kates and Turner, 1953). Data on *N. filicollis* are too scanty for comparison (Thomas, 1959a). *N. helvetianus,* a parasite of cattle, is reported to burrow deeply into the intestinal mucosa causing severe pathological changes (Herlich, 1954; Samizadeh-Yazd and Todd, 1978).

The experimental lambs used in this experiment exhibited no clinical symptoms, no diarrhea, and no obvious weight losses. Effects on epithelium ultrastructure were not investigated, but no alteration in mucosal alkaline phosphatase activity was detected. *N. battus* by contrast causes a marked reduction in the activity of this enzyme in infected lambs (Coop et al., 1973). These observations coupled with the failure of the larval stages to penetrate the epithelium suggest that *N. abnormalis* will prove to be only mildly pathogenic in sheep.

Acknowledgments

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Research Note

Observations on the Morphology of the Eyeworm, *Ceratospira inglisi* (Nematoda: Thelaziidae) from a Moluccan Cockatoo

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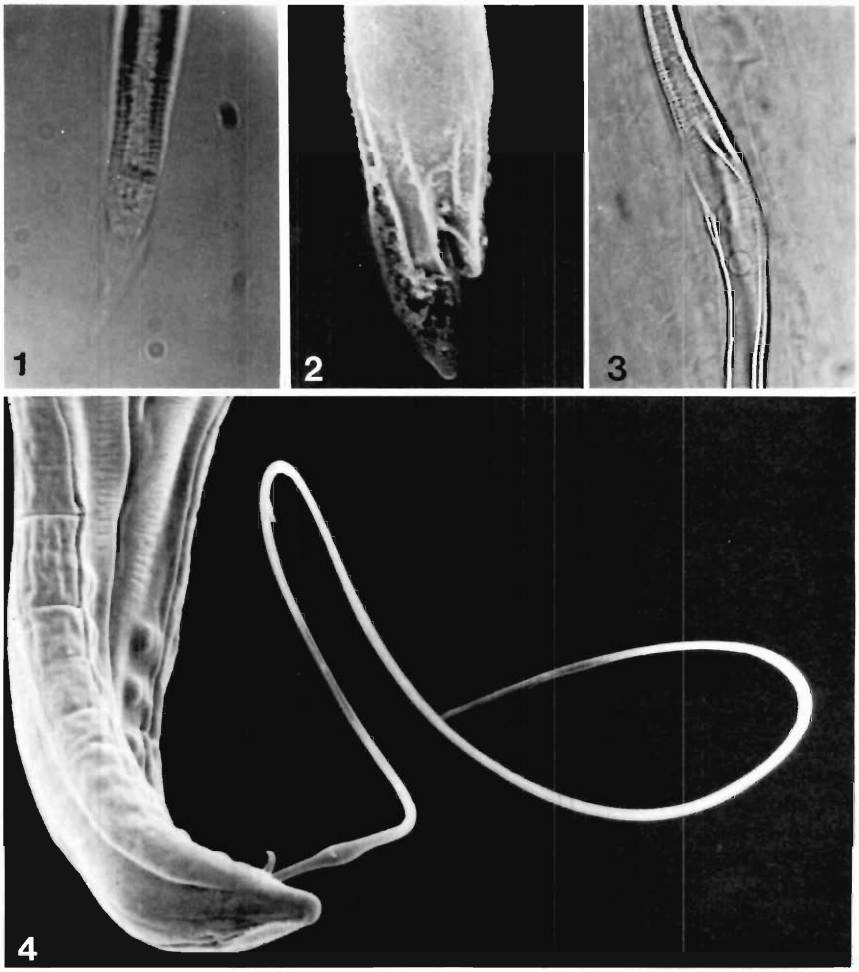
Ceratospira inglisi Schmidt et Kuntz, 1971 were recovered from the eyes of a captive Moluccan cockatoo, *Kakatoe moluccensis,* in Florida. The nematodes were weakly attached beneath the nictitating membranes, and their tails extended from beneath the membrane to the middle of the corneas. The only lesion noted was a tiny nodule at the point of attachment of each



Figures 1–4. ***Ceratospira inglisi*** **spicule. 1. A delicate membranous tip extends beyond the tip of the left spicule. The spicule tip is bluntly rounded and closed (×1,500). 2. The spicule tip in this example is open, with a bilobed membranous tip (SEM ×3,200). 3. Midway along the length of the left spicule, the sclerotization of the median wall bends toward the lateral wall, leaving the median wall as a membrane that gradually becomes more sclerotized distad (×1,000). 4. The left spicule protrudes, showing the midshaft swelling (SEM ×130).**

nematode. The parasites did not seem to bother the cockatoo, although numerous parasites were present. Eleven male and 20 female nematodes (not the entire population in the eyes) were placed in 70% ethanol and subsequently examined.

Our specimens of *C. inglisi* had some differences from the original description (Schmidt and Kuntz, 1971, Parasitology 63:91–99) regarding spicular and vulvar structure. All other morphologic characteristics agreed with those published for *C. inglisi.*

The left spicule of our specimens varied from the original description in total length, structure of the tip, and structure of the midshaft. Measurements of the left spicules in 10 males were 2.07–2.72 mm, considerably longer than the originally described 1.60–1.64 mm. The right spicules measured 0.193–0.274 mm, which is comparable to the 0.188–0.206 mm described. The spicular ratio of our specimens ranged from 1:8.28 to 1:12.5, compared to the range of 1:7.96 to 1:8.88 in the original description.

The tip of the left spicule was originally described as being square. In our specimens, the sclerotized portion of the spicule was always very bluntly rounded terminally. Usually the tip was closed by sclerotization across the end, but sometimes the tip remained unsclerotized and open. A delicate membrane extended for about 10 μm beyond this end, coming to a sharp point. In several specimens, this membranous tip was bifid, having a major and a minor lobe (Figs. 1, 2). The membrane appeared to be an extension of a delicate sheath that invested the spicule. Examination of the type material from the U.S. National Museum showed that this membranous tip was also visible in those instances where the spicule was protruding.

Another finding was the presence of a break in the continuity of the sclerotization of the median wall of the left spicule at the midpoint along its length (Fig. 2). At this same point, the spicule widened slightly (Figs. 2, 3). This same undescribed feature was also found during re-examination of the type material.

A fourth variation our specimens showed from the original description concerned the vulva, which was originally described as inconspicuous and illustrated as a simple round pore. In our specimens, the vulva is a short transverse slit with sclerotized, nonsalient lips.

The original collections of *C. inglisi* were made from a Philippine cockatoo, *Kakatoe haematopygia,* and a mountain imperial pigeon, *Ducula b. badia.* Since that time, it has not been reported. Our recovery from *Kakatoe moluccensis* represents a new host record for *C. inglisi.* Some of the morphologic variations in our specimens may have been caused by host influences. Specimens from this case have been placed in the USNM Helm. Coll. (No. 77832).

The authors thank Dr. G. J. Harrison, Lake Worth, Florida, for submitting the specimens and supplying the clinical data, and Dr. G. D. Schmidt, University of Northern Colorado, Greeley, for confirming the identification of the nematodes.

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Research Note

Monogenea (Platyhelminthes) of Various Freshwater Fishes in Louisiana

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Between August 1981 and October 1982, more than 500 specimens representing 20 species of freshwater fishes were collected from several freshwater localities in southern Louisiana by electroshocking, seining, gill netting, or hook and line. Each host species was isolated at the time of collection, transported to the lab in separate containers, and examined for monogeneans. The gills were removed from the hosts and placed in a 1:4,000 formalin solution for approximately 1.5 hr to facilitate the removal of parasites. The worms were then pipetted into vials of 10% for-

malin and allowed to remain in the fixative for at least 24 hr. Subsequently, the parasites were mounted in glycerin jelly and identified.

Nineteen described species of monogeneans have been reported from freshwater fishes in Louisiana. Summers and Bennett (1938, Proc. La. Acad. Sci. 4:247–248) reported 12 described and five undescribed species of monogeneans. Seamster (1948, Am. Midl. Nat. 39:165–168) reported six additional described and two undescribed species. Chien (1974, J. Parasitol. 60: 585–594) later described a single species from the state.

In the present study we are following the classification proposed by Yamaguti (1963, Systema Helminthum. IV. Monogenea and Aspidocotylea. Intersci. Publ., New York. 699 pp.) and establish 20 new state and three new host records. A compilation of all described species of monogeneans reported from freshwater fishes in Louisiana is provided in Table 1. All host identifications were based upon keys by Eddy and Underhill (1978, How to Know the Freshwater Fishes. Wm. C. Brown Co., Dubuque, Iowa. 215 pp.). Representative specimens were deposited in the National Parasite Collection, USDA, Beltsville, Maryland 20705 (USNM Helm. Coll. Nos. 78288–78333).

Table 1. Compilation of all monogeneans reported from freshwater fishes in Louisiana with pertinent references.

Monogenean	Hosts	No. infected/ no. examined	References	USNM no.
*Actinocleidus articularis** (Mizelle, 1936)	*Lepomis megalotis*	12/12	Present study	78288
*A. bifidus** Mizelle and Cronin, 1943	*Lepomis microlophus*	8/8	Present study	78289
*A. bifurcatus** Mizelle, 1941	*Lepomis gulosus*†	3/22	Present study	78298
	Lepomis megalotis	4/12	Present study	78299
	Lepomis microlophus	1/8	Present study	78300
*A. brevicirrus** Mizelle and Jaskoski, 1942	*Lepomis punctatus*	5/5	Present study	78290
*A. fergusoni** Mizelle, 1938	*Lepomis humilis*	26/26	Present study	78291
	Lepomis macrochirus	273/276	Present study	78292
*A. flagellatus** Mizelle and Seamster, 1939	*Lepomis gulosus*	22/22	Present study	78293
A. fusiformis (Mueller, 1934)	*Micropterus salmoides*	—	Summers and Bennett, 1938	—
	Micropterus salmoides	20/20	Present study	78294
A. longus Mizelle, 1938	*Lepomis cyanellus*	10/16	Present study	78295
	Lepomis macrochirus	—	Summers and Bennett, 1938	—
A. oculatus (Mueller, 1934)	*Lepomis macrochirus*	—	Summers and Bennett, 1938	—
Anchoradiscus triangularis (Summers, 1937)	*Lepomis macrochirus*	99/276	Present study	78296
	Lepomis microlophus	1/8	Present study	78297
	Lepomis symmetricus	—	Summers and Bennett, 1938	—
*Cleidodiscus bedardi** Mizelle, 1936	*Lepomis megalotis*	6/12	Present study	78301
C. capax Mizelle, 1936	*Pomoxis annularis*	18/19	Present study	78302
	Pomoxis nigromaculatus	—	Seamster, 1948	—
	Pomoxis nigromaculatus	11/11	Present study	78303
*C. floridanus** Mueller, 1936	*Ictalurus punctatus*	11/12	Present study	78304
*C. longus** Mizelle, 1936	*Pomoxis nigromaculatus*	4/11	Present study	78305
*C. miniatus** (Mizelle and Jaskoski, 1942)	*Lepomis punctatus*	5/5	Present study	78330
C. nematocirrus Mueller, 1937	*Lepomis macrochirus*	—	Summers and Bennett, 1938	—
	Lepomis macrochirus	43/276	Present study	78306
	Lepomis megalotis	—	Seamster, 1948	—
	Lepomis megalotis	3/12	Present study	78307
C. pricei Mueller, 1936	*Ictalurus melas*	—	Summers and Bennett, 1938	—
	Ictalurus melas	—	Seamster, 1948	—
	Ictalurus melas	8/8	Present study	78304; 78308
	Ictalurus punctatus	12/12	Present study	78309

Table 1. Continued.

Monogenean	Hosts	No. infected/ no. examined	References	USNM no.
C. robustus Mueller, 1934	*Lepomis macrochirus*	—	Summers and Bennett, 1938	—
	Lepomis macrochirus	48/276	Present study	78310
	Lepomis punctatus†	1/5	Present study	78311
C. vancleavei Mizelle, 1936	*Pomoxis annularis*	—	Summers and Bennett, 1938	—
	Pomoxis annularis	19/19	Present study	78312
	Pomoxis nigromaculatus	11/11	Present study	78313
Dactylogyrus aureus Seamster, 1948	*Aphredoderus sayanus*	—	Seamster, 1948	—
D. mississippiensis Chien, 1974	*Nocomis leptocephalus*	—	Chien, 1974	—
Haplocleidus acer (Mueller, 1936)	*Lepomis macrochirus*	—	Seamster, 1948	—
	Lepomis macrochirus	235/276	Present study	78317
*H. attenuatus** (Mizelle, 1941)	*Lepomis microlophus*	8/8	Present study	78319
	Lepomis punctatus	4/5	Present study	78320
*H. dispar** (Mueller, 1936)	*Lepomis macrochirus*	161/276	Present study	78324
H. furcatus Mueller, 1937	*Micropterus salmoides*	—	Summers and Bennett, 1938	—
	Micropterus salmoides	17/20	Present study	78327
*Microcotyle spinicirrus** MacCallum, 1918	*Aplodinotus grunniens*	1/4	Present study	78315
*Neodactylogyrus minutus** (Kulwiec, 1927)	*Cyprinus carpio*	4/4	Present study	78314
Onchocleidus acuminatus Mizelle, 1936	*Lepomis megalotis*	—	Seamster, 1948	—
	Lepomis megalotis	10/12	Present study	78318
*O. cyanellus** Mizelle, 1938	*Lepomis cyanellus*	16/16	Present study	78323
*O. distinctus** Mizelle, 1936	*Lepomis megalotis*	10/12	Present study	78325
	Lepomis microlophus	7/8	Present study	78333
O. ferox (Mueller, 1934)	*Lepomis macrochirus*	—	Summers and Bennett, 1938	—
	Lepomis macrochirus	—	Seamster, 1948	—
	Lepomis macrochirus	263/276	Present study	78326
O. principalis Mizelle, 1936	*Micropterus salmoides*	—	Summers and Bennett, 1938	—
	Micropterus salmoides	20/20	Present study	78331
*Pseudanthocotyle banghami** (Price, 1958)	*Dorosoma cepedianum*	3/30	Present study	78316
*Urocleidus chaenobryttus** Mizelle and Seamster, 1939	*Lepomis gulosus*	22/22	Present study	78321
	Lepomis humilis†	20/26	Present study	78322
*U. interruptus** (Mizelle, 1936)	*Morone mississippiensis*	6/6	Present study	78328
*U. mimus** (Mueller, 1936)	*Morone chrysops*	3/3	Present study	78329
U. seculus Mizelle and Arcadi, 1945	*Gambusia affinis*	—	Seamster, 1948	—
	Gambusia affinis	9/10	Present study	78332
U. umbraensis Mizelle, 1938	*Fundulus notti*	—	Summers and Bennett, 1938	—
U. wadei Seamster, 1948	*Centrarchus macropterus*	—	Seamster, 1948	—
Negative	*Amia calva*	0/10	Present study	—
Negative	*Esox niger*	—	Seamster, 1948	—
Negative	*Lepisosteus oculatus*	0/7	Present study	—
Negative	*Notemigonus crysoleucas*	—	Seamster, 1948	—

* Indicates new state record.
† Indicates new host record.

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Research Note

Prevalence of *Nanophyetus salmincola,* the Vector of Salmon Poisoning Disease in Steelhead Trout (*Salmo gairdneri*) in Idaho

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The digenetic trematode, *Nanophyetus salmincola* Chapin, 1927 is the vector of *Neorickettsia helminthoeca,* the etiologic agent of salmon poisoning disease (SPD), a highly fatal rickettsial disease of domestic and wild Canidae. Indigenous infections of SPD occur only in the Pacific Northwest, principally on the western slopes of the Cascade Mountains from northwestern California to southwestern Washington (see Knapp and Millemann, 1981, pages 376–387 *in* J. W. Davis, L. H. Karstad, and D. O. Trainer, eds. Infectious Diseases of Wild Mammals. Iowa State University Press, for review). The geographic range of the disease is primarily dependent on the distribution of the first intermediate host, the snail *Oxytrema silicula* (Fig. 1). However, migratory salmon and trout may become infected with metacercariae if they spend part of their life cycle in aquatic systems that support *O. silicula.* Therefore, it is postulated that anadromous salmonid species migrating into eastern Washington, Idaho, and eastern Oregon (Fig. 1) from the Pacific Ocean could become infected as they pass through waters where the disease is enzootic.

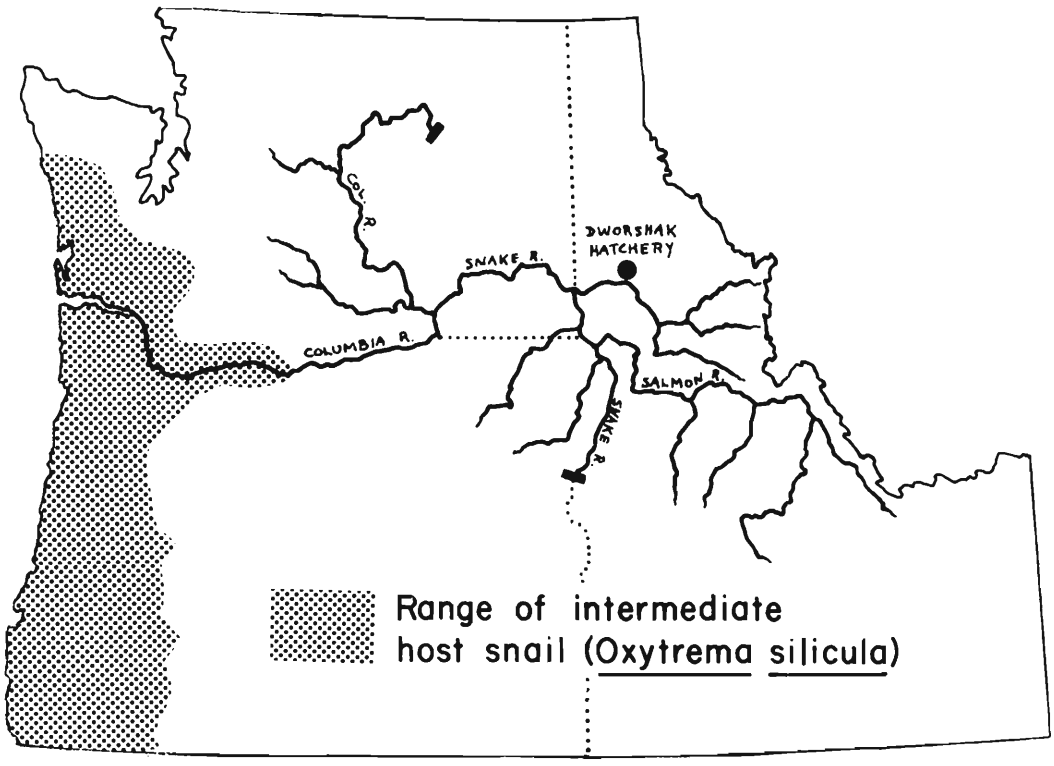

Figure 1. River systems of Idaho, Washington, and Oregon where anadromous salmonids migrate. The range of the snail intermediate host is superimposed (from Knapp and Millemann, 1981, op. cit.).

Figure 2. Metacercariae (approximately 125 μm in diameter) of *Nanophyetus salmincola* in the kidney of a steelhead trout (*Salmo gairdneri*).

In April 1983, we conducted a survey at the Dworshak National Fish Hatchery at Ahsahka, Idaho, to determine the prevalence of metacercariae of *N. salmincola* in migrating steelhead trout (*Salmo gairdneri*). These adult trout, weighing approximately 5–7 kg, were returning from the Pacific Ocean, a distance of approximately 800 km (Fig. 1). The 3½-yr-old fish usually return to the place of hatching; therefore, they presumably were hatched in 1980 and were released from the hatchery for their ocean migration in 1981.

Kidneys were removed from 251 individual fish. The posterior one-third of each kidney was squashed between two glass plates, 15 cm × 15 cm and examined under a dissecting microscope (15×) for the presence of metacercariae (Fig. 2). In addition, we examined 45 kidneys from fingerling steelhead (15 cm) that had been raised in the hatchery for almost 1 yr to determine if the fluke infection was indigenous to the area and the hatchery. The water used in the hatchery is natural river water.

Metacercariae were observed in 128 of 251 (51%) of the kidneys of returning steelhead (Fig. 2). Numbers of metacercariae were not counted, but five or more per kidney was a common finding. Metacercariae were not detected in the fingerlings.

Kidneys from the 128 infected fish were fed to five 8-wk-old captive coyotes (*Canis latrans*) to determine whether the fish were carriers of SPD. Six days after the kidneys were eaten, eggs of *N. salmincola* were detected in feces (sugar flotation, sp. gr. = 1.27) and adult flukes were recovered from the small intestine of one coyote that was euthanized 14 days after inoculation (USNM Helm. Coll., USDA, Beltsville, Maryland 20705, No. 78226). Although coyotes are highly susceptible to SPD (Foreyt et al., 1982, J. Wildl. Dis. 18:159–162), none died even though elevated temperatures of 40.5°C (105°F) in two of five coyotes were detected approximately 10 days after ingestion of the fish. Enlarged popliteal and prescapular lymph nodes were palpable at this time. One coyote was euthanized 14 days after inoculation and histologic examinations (Giemsa stain) revealed a histiocytosis characteristic of SPD (Cordy and Gorham, 1950, Am. J. Pathol. 26:617–637), but rickettsiae were not observed. It is possible that either the typical rickettsia (*N. helminthoeca*) was not present, that the inoculum was not of sufficient quantity, or that these coyotes were resistant to the fatal effects of the disease. It is also possible that another rickettsia such as Elokomin fluke fever (EFF) was involved. EFF is less pathogenic than SPD in dogs and the mortality is lower (Farrell et al., 1973, Am. J. Vet. Res. 34:919–922).

Although typical SPD was not demonstrated in our study, the high percentage of fish returning to Idaho with *N. salmincola* (51%) indicates that anadromous salmonids in the Pacific Northwest have the potential of carrying SPD to all major rivers that support anadromous salmonids (Fig. 1). Occasional reports of SPD in dogs in eastern Washington, eastern Oregon, and Idaho emphasize the importance for awareness of SPD in these regions.

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Research Note

Attempts to Stimulate Resistance to *Fasciola hepatica* in Calves with *Schistosoma mansoni* Cercariae

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Sirag et al., demonstrated resistance to *Fasciola hepatica* in calves that harbored patent *Schistosoma bovis* infections (1981, J. Helminthol. 55:63–70). Cross-reactive antigens have been reported in *F. hepatica* and *Schistosoma mansoni* (Hillyer, 1981, Bol. Asoc. Med. Puerto Rico 73:150–161), and mice have been protected from challenge infections with *S. mansoni* by prior inoculation with various *F. hepatica* antigens (Hillyer and Serrano, 1982, J. Inf. Dis. 145: 728–732). Christensen et al. (1980, Exp. Parasitol. 49:116–212) reported patent *S. mansoni* infections in mice resulted in resistance to *F. hepatica* infections. Natural infections of cattle with *S. mansoni* have been reported in Brazil (Barbosa et al., 1962, Science 138:831), and because cattle become resistant to reinfection with *F. hepatica* (Nansen, 1974, Nord. Vet. Med. 26:18–20; Kendall et al., 1978, J. Comp. Pathol. 88: 115–122), conceivably a protective resistance to infection by *F. hepatica* through prior inoculation with *S. mansoni* could be developed. Reported herein are results of tests attempted to produce such a protective resistance in calves against *F. hepatica* by inoculations with *S. mansoni* cercariae.

Two experiments were conducted using *F. hepatica*-naive Holstein bull calves ranging in age from 6 mo to 1 yr. They were fed a maintenance ration of alfalfa hay and water ad lib.

The *S. mansoni* cercariae were freshly shed when obtained from the Biomedical Research Institute in Rockville, Maryland. Cercariae were counted and calves were inoculated subcutaneously by syringe with cercariae suspended in distilled water. After the experimental regimen for schistosome exposure, all calves within an experiment were challenged with oral inoculation of *F. hepatica* cysts on filter paper in gelatin capsules. Necropsies of challenged calves were made 15 wk postinoculation of cysts. Fecal samples were collected for egg counts (Knight, 1985, Proc. Helminthol. Soc. Wash. 51:349–351) 13 weeks postinoculation with *F. hepatica*. Calves inoculated with *S. mansoni* cercariae were examined at necropsy for schistosomes. Results were subjected to analysis of variance for significance.

EXPERIMENTAL DESIGN: Experiment 1. Five calves were assigned to groups of three and two calves each. The three calves of Group 1 were inoculated with *S. mansoni* cercariae as follows: one received 17,000 cercariae 6 wk before *F. hepatica* challenge; one received 36,000 cercariae 3 wk before challenge; one received 17,000 and 36,000 cercariae 6 and 3 wk, respectively, before challenge. Calves of Group 2 were not inoculated with *S. mansoni* cercariae. Three weeks after the

Table 1. Numbers of *Fasciola hepatica* recovered at necropsy from calves inoculated with *Schistosoma mansoni* cercariae compared with control calves.

Calves	No. *F. hepatica*	% Inoculum
Experiment 1		
S. mansoni calves	78	16
	73	15
	86	17
Average	79	16
Control calves	100	22
	155	31
Average	133	27
Experiment 2		
S. mansoni calves	103	29
	111	32
	62	18
	48	14
	53	15
Average	75	21
Control calves	44	13
	54	15
	54	15
	43	12
	42	12
Average	42	12

last administration of cercariae all calves were challenged with 500 *F. hepatica* cysts.

Experiment 2. Ten calves were assigned to two groups of five calves each. The calves of Group 1 were each inoculated weekly with 12,500 *S. mansoni* cercariae for 6 wk, totaling 75,000 cercariae per calf; calves of Group 2 were not inoculated with *S. mansoni* cercariae. Three weeks after the last administration of cercariae all calves were challenged with 350 *F. hepatica* cysts.

The numbers of flukes at necropsy are presented in Table 1. In Experiment 1, the average number of *F. hepatica* recovered from control calves was higher than the average number recovered from calves inoculated with *S. mansoni,* and though the difference was not statistically significant it seemed to warrant further tests. In Experiment 2, calves were inoculated with a greater number of *S. mansoni* cercariae over a longer period of time to provide greater antigenic stimulation. At necropsy, however, no significant difference was found between experimental and control groups.

None of the calves in either experiment developed a patent *S. mansoni* infection; only eggs of *F. hepatica* were found, and no schistosomes were recovered at necropsy. Christensen et al. (1980, op. cit.) found that patent infections of both sexes of *S. mansoni* with resultant egg production were necessary to produce resistance in mice to *F. hepatica.* Likewise, resistance reported by Sirag et al. (1981, op. cit.) resulted from a patent infection of *S. bovis.* In the tests reported here, no resistance to *F. hepatica* was produced by exposure to *S. mansoni* cercariae, supporting the concept that patent infections with *S. mansoni* are necessary to stimulate resistance to *F. hepatica.*

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Research Note

Two Rare Helminths in an Osprey, *Pandion haliaetus,* in Mexico

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In October 1981, an adult male osprey, *Pandion haliaetus,* was found impaled on a television antenna in the town of Puerto Peñasco, Sonora, Mexico. This small village is located near the northern end of the Gulf of California, 98 mi south of Lukeville, Arizona. The still-living bird was brought by a resident to the Center for the Study of Deserts and Oceans, where it died the same night. Examination of the digestive tract revealed two species of helminths, both so rare that their occurrence should be recorded.

Eighteen adult *Sexansocara skrjabini* Sobolev et Sudarikov, 1939 (Nematoda: Acuriidae) were found in the proventriculus. This species was first discovered in Gorkii, Russia (Sobolev and Sudarikov, 1939, Tr Gor'k. Gos. Sel'skokhoz. Inst. 39:97–103) in the same host. It was also reported from the osprey from Georgian SSR (Kurashvili, [1954], Rabot. Gel'mintol. 75-Let. Skrjabin, pp. 340–346; 1956, Trudy Inst. Zool. Akad. Nauk Gruzinsk. SSR, 14:105–145). This, then, is its first record in North America. Voucher specimens are deposited in USNM Helm. Coll. (USDA, Beltsville, Maryland) No. 77183.

Thirty-five *Scaphanocephalus expansus* (Creplin, 1842) Jägerskiold, 1903 (Digenea: Heterophyidae) were recovered from the small intestine. This parasite has been reported from ospreys in Europe, Asia, and Africa. There are three prior records of it in North America. Hoffman (1953, J. Parasitol. 39:568) found adult worms in an osprey in Iowa (USNM Helm. Coll. No. 46437). This is the only prior record of adults in North America. Metacercariae were found encysted in the skin, fins, gills, and eyes of marine fishes in Florida by Hutton (1964, Trans. Am. Microsc. Soc. 83:439–447), and by Skinner (1979, Bull. Mar. Sci. [for 1978] 28:590–595). This is the second record of adult *S. expansus* in the Western Hemisphere and the first of the species

in Mexico. Voucher specimens are deposited in USNM Helm. Coll. No. 77184.

Thanks are expressed to Dr. Peggy Turk, Education Director, CEDO, for providing the osprey carcass.

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Research Note

Parasites of Limpkins, *Aramus guarauna*, in Florida

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The limpkin, *Aramus guarauna* (L.), is a medium-sized long-legged wading bird of the order Gruiformes (cranes, rails, gallinules, coots, etc.) and is the sole member of the family Aramidae. It is limited to freshwater habitats primarily in Florida and southeastern Georgia in the U.S.A. (American Ornithologists' Union, 1957, Checklist of North American Birds. 5th ed., Baltimore). Limpkins feed primarily on apple snails (*Pomacea paludosa* (Say)), a behavior shared by the snail kite, *Rostrhamus sociabilis* Vieillot, although unlike snail kites they will take also other foods such as lizards, frogs, insects, crustaceans, mussels, and other snails (Snyder and Snyder, 1969, Living Bird 8:177–223). Whereas the food habits of limpkins have been well studied, very little is known about their parasites. The present report concerns the parasites of limpkins from central Florida.

Fifteen limpkins were examined. Most (13 adults and one hatchling) were collected from October 1975 to February 1976 at Rodman Pool in the Oklawaha River, Marion County, Florida. One additional adult was collected in February 1980 approximately 32 km south of this locality at Alexander Springs (Lake County).

Nine adult birds and one hatchling were necropsied after having been frozen for up to 3 mo. Blood samples were obtained from only four other adult birds during banding and release oper-

Table 1. Location, prevalence, and intensity of helminths of nine limpkins, *Aramus guarauna*, from the Oklawaha River, Florida.

Parasite	Prevalence (%)	Intensity*		
		Mean	Median	Range
Trematoda				
Cyclocoelidae (1)†‡§	100	429	34	2–3,554
Lyperorchis lyperorchis Travassos, 1921 (4)‖	56	4	1	1–12
Echinostomatidae (3)‖§	33	2	1	1–3
Prionosoma serratum (Diesing, 1850) Dietz, 1909 (3)‖	11	2	2	2
Nematoda				
Amidostomum acutum (Lundahl, 1848) Seurat, 1918 (2)‡	56	6	5	2–10
Strongyloides sp. (3)‡	33	59	75	25–78

* Intensity = no. parasites/infected host; values ≥0.5 rounded to next highest number.
† Numbers in parentheses indicate site in host: (1) lungs, air sacs; (2) gizzard; (3) small intestine; (4) cloaca.
‡ New host record.
§ Immature forms.
‖ New locality record.

ations. Biting lice were collected from three of the ten birds examined at necropsy and from one additional limpkin that was captured, banded, and released.

Procedures for collecting and studying parasites followed those given by Forrester et al. (1974, Proc. Helminthol. Soc. Wash. 41:55–59). Voucher specimens of each parasite have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland, Nos. 78046–78053).

Four trematodes, two nematodes, and two biting lice were recovered. No blood parasites were detected on blood films.

The biting lice, *Laemobothrion cubense* Kellogg and Ferris, 1915 and *Rallicola funebris* (Nitzsch, 1866), were found on two and three of 11 birds, respectively. Only one of the limpkins harbored specimens of both species. Both of these lice have been reported previously from limpkins (Emerson, 1972, Checklist of the Mallophaga of North America (North of Mexico), Part IV. Bird Host List, Dugway, Utah).

Table 1 lists the sites, prevalences, and intensities of infection for the helminths collected from nine limpkins originating from the Oklawaha River. A tenth bird from Alexander Springs was free of helminths and was not included in Table 1 since it was collected from a different locality.

All limpkins were infected with specimens of at least one species of helminth (range 1–4, $\bar{x}$ = 3, med. = 2). The total number of helminths per infected limpkin ranged from 10 to 3,586 ($\bar{x}$ = 454, med. = 58); however, these values are skewed upward because of a large number (3,554) of immature cyclocoelids in the hatchling. Intensity values did not exceed 83 specimens of this trematode in any of the adult limpkins, of which all were infected. In fact, none of the other limpkins had a total parasite count >165. Because only immature specimens were recovered, this suggests that the limpkin may be an abnormal host for this helminth.

Only two specimens of *Prionosoma serratum* were found in the intestine of one limpkin. Three other birds possessed immature forms of an echinostome that may have been *P. serratum*. This species was recovered from limpkins in Venezuela, Brazil, and Cuba (Nasir and Diaz, 1972, Riv. Parassitol. 33:245–276). A closely related species of trematode, *P. pricei* Perez Vigueras, 1944, was shown experimentally to infect snail kites in Cuba via apple snail intermediate hosts (Nasir and Diaz, 1972, op. cit.). Apple snails may serve as intermediate hosts for both *P. pricei* and *P. serratum* in their respective definitive hosts, but this remains to be determined.

Amidostomum acutum was more prevalent than *Strongyloides* sp., but intensities of infection were much lower. Both are pathogenic in various avian hosts (Levine, 1980, Nematode Parasites of Domestic Animals and of Man, 2nd ed., Minneapolis), but the effects of these infections on limpkins are unknown. Because the life cycles of *A. acutum* and *Strongyloides* sp. are direct, limpkins probably acquire infections through ingestion of infective stages or also by skin penetration of infective stages in the case of *Strongyloides* sp.

Helminths have been reported from other gruiform relatives of the limpkin in Florida, i.e., American coots (*Fulica americana* (Gmelin), common moorhens (*Gallinula chloropus* (L.)), purple gallinules (*Porphyrula martinica* (L.)), and wintering and resident sandhill cranes (*Grus canadensis* (L.)) (Kinsella, 1973, Proc. Helminthol. Soc. Wash. 40:240–242; Kinsella et al., 1973, Am. Midl. Nat. 89:467–473; Forrester et al., 1974, op. cit.; Forrester et al., 1975, J. Parasitol. 61:547–548). Limpkins appear to have few helminths in common with their relatives, sharing only the nematode genera *Amidostomum* and *Strongyloides*. The immature cyclocoelid trematodes may represent the species that occur in coots and gallinules (*Cyclocoelum mutabile* (Zeder, 1800) or *C. oculeum* Kossack, 1911) or perhaps the species in snail kites, *Bothrigaster variolaris* (Fuhrmann, 1904) (Kinsella, 1973, op. cit.; Kinsella et al., 1973, op. cit.; Travassos et al., 1969, op. cit.; Sykes and Forrester, 1983, Fla. Field Nat. 11:111–116). Apple snails may be involved as intermediate hosts of the cyclocoelids of limpkins and snail kites. Although our sample size was small, it appears that limpkins have fewer species of helminths (seven or eight) than other gruiforms in Florida such as the American coot (17 species), common moorhen (17), purple gallinule (18), Florida sandhill crane (13), and Greater sandhill crane (14) (Kinsella, 1973, op. cit.; Kinsella et al., 1973, op. cit.; Forrester et al., 1974, op. cit.; Forrester et al., 1975, op. cit.). This may result from the limpkin's more restricted diet.

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Research Note

Toxascaris leonina (Nematoda: Ascarididae) from the Pronghorn Antelope, *Antilocapra americana*, in Wyoming

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[2] Wyoming Game and Fish Commission, Warden, Kaycee, Wyoming

Although the genera *Toxocara* Stiles, 1905 and *Toxascaris* Leiper, 1907 are common in canines and felines, only occasionally are they found in ruminants or other artiodactylids. John R. Talbott, Game Warden, Wyoming Game and Fish Commission, Lusk, Wyoming, killed a doe pronghorn antelope, *Antilocapra americana* (Ord), in Niobrara County, Wyoming, November 3, 1981 because the animal was weak and probably would have died within a short time. While completing a postmortem examination of the doe, the warden noted a poor body condition, deterioration of bone marrow, and emaciation. Nematode worms were present in the posterior portion of the small intestine. He collected nearly a dozen live nematodes, put them in ethanol, and submitted them to Dr. E. Tom Thorne, Wildlife Research Veterinarian with the Wyoming Game and Fish Dept., Research Laboratory, University of Wyoming, Laramie. Dr. Thorne relayed the worms to two of us (R.C.B. and N.K.). The nematodes appeared to be of normal color and morphology. Female worms ranged from 4.5–6.0 cm in length and were 0.8–1.2 mm in width at midbody. Lengths of male worms ranged from 3.4 to 3.7 cm and measured 0.7–0.9 mm at greatest width. Cervical alae were typical of nemas of the *Toxascaris–Toxocara* genera in the family Ascarididae Baird, 1853. Nematodes of this group can be separated by the fact that *Toxascaris* spp. (Ascaridinae) have a simple esophagus without a ventriculus, but *Toxocara* spp. (Toxocarinae) have a ventriculus. Lengths of the female and male worms were near the middle of the range of *Toxascaris* as given by Levine, 1980 (Nematode Parasites of Domestic Animals and of Man. Burgess Publishing Co., Minneapolis, Minnesota). However, the widths of both the female and male worms were less than those given by Levine and other authors. Most female worms had no eggs in the uteri so there may be some question whether the females ever would have produced viable ova. Dr. J. Ralph Lichtenfels, Animal Parasitology Institute, Agricultural Research Service, USDA, Beltsville, Maryland, confirmed our identification of the ascarids as *Toxascaris leonina* (Linstow, 1902) Leiper, 1907. Lichtenfels noted that no previous record exists of *Toxascaris* sp. in pronghorn antelope or in any other ruminant in the United States.

Recent literature indicates that *Ascaris suum* Goeze, 1782 and *Toxocara canis* (Werner, 1782) Stiles, 1905 will infect domestic cattle (calves) causing extensive pulmonary lesions (Greenway and McGraw, 1970, Can. J. Comp. Med. 34(3): 227–237) and that *Toxocara cati* (Schrank, 1788) Brumpt, 1927 = *T. mystax* (Zeder, 1800) has been found in an equine host in northern Iran (Mirzayans, 1973, Vet. Rec. 92:262, letter). Grinberg (1961, Med. Parazitol. Moskva 30.626: English trans. #568, U.S. Namru 3) found an abscess containing about 100 adult *Toxascaris leonina* in a male human in the U.S.S.R. Since *Toxocara vitulorum* (Goeze, 1782) Warren, 1971 (syn. *Neoascaris vitulorum*) occurs in the small intes-

tine of bovids in many parts of the world (Soulsby, 1982, Helminths, Arthropods and Protozoa of Domesticated Animals, 7th ed. Lea and Febiger, Philadelphia, Pennsylvania, p. 155), perhaps the genera *Toxocara–Toxascaris* may infect ruminants more commonly than previously surmised.

One male and two female specimens have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705 as USDA Helm. Coll. No. 69448.

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52(1), 1985, p. 143

Research Note

A Trypanosome in Roe Deer, *Capreolus capreolus*, in Southern Poland[1]

NEWTON KINGSTON[2,3] AND BOLESŁAW BOBEK[4]

[2] W. Stefański Institute of Parasitology, Polish Academy of Sciences, 00-973 Warszawa, ul. L. Pasteura 3 Skr. p. 153, Poland and

[4] Jagiellonian University, Institute of Biology, 30-060 Kraków, ul. M. Karasia 6, Poland

Eight roe bucks (*Capreolus capreolus*, 1–8 yr of age, weights 14–20 kg), culled as part of a management program during rut in August 1984 in Puszcza Niepołomicka near Kraków, Poland, were examined for bloodstream hemoflagellates by direct examination of heparinized blood in microhematocrit tubes (DE, ca. 0.05–0.07 ml of blood) and by blood culture in veal infusion medium (VIM, ca. 10 ml of blood). Trypanosomes seen by DE were transferred to microscope slides and conventional thin blood films prepared, air dried, fixed in methanol, and stained with Giemsa's stain (Matthews et al., 1977, J. Wildl. Dis. 13:33–39). Cultures were examined 1–2 wk after inoculation.

Seven of the eight roe deer (88%) were positive by DE and examination of some of the parasites on slides indicates that these parasites were *Trypanosoma* (*Megatrypanum*) sp. characterized by their large size, typical long posterior end with the kinetoplast distant from the posterior end (Hoare, 1972, The Trypanosomes of Mammals, Blackwell), and bearing a short free flagellum at the anterior end. It was not possible to photograph and analyze these trypanosomes at the time. Species identification must await further discovery, examination, and analysis of more bloodstream stages on microscope slides. For unknown reasons no trypanosomes were recovered from any cultures of roe deer blood samples.

Trypanosomatids have been reported from roe deer by Knuth (1909, Zeits. Infekt. Haust. 6:357–362), who found a species of *Herpetomonas* in this host in Germany, and by Friedhoff et al. (1984, Zbl. Bakt. Hyg. A 256:286–287) who found 10 of 27 roe deer near Hannover, Federal Republic of Germany, infected by using culture (NNN and NCTC media) techniques. These authors speculate that the species in these deer is *Trypanosoma cervi* Kingston and Morton, 1975 (J. Parasitol. 61:17–23), but they have no evidence for this assertion inasmuch as they have seen only culture forms. Wróblewski (1908, Cbl. Bakteriol. 1 Abt. Orig. BD. XLVIII. Heft. 2:162–163) reported on the presence of *Trypanosoma theileri* in the wisent (European bison, *Bison bonasus*) in Puszcza Białowieska, in eastern Poland, but his material was also derived from culture. It is unlikely that the parasite in the roe deer is *T. theileri* as that species appears highly host specific (Hoare, 1972, op. cit.).

This is a first report of a species of *Trypanosoma* from roe deer in Poland.

[1] Research sponsored by the U.S. National Academy of Sciences–Polish Academy of Sciences Scientific Exchange Program, 1984.

[3] Permanent address: Division of Microbiology and Veterinary Medicine, Box 3354, University Station, University of Wyoming, Laramie, Wyoming 82071, USA.

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52(1), 1985, pp. 144–145

Research Note

Trypanosoma sp. in Red Deer (*Cervus elaphus*) and Elk (*Alces alces*) in Poland[1]

NEWTON KINGSTON,[2] JAN DRÓŻDŻ, and MARIA RUTKOWSKA
W. Stefański Institute of Parasitology, Polish Academy of Sciences, 00-973 Warszawa, ul. L. Pasteura 3 Skr. p. 153, Poland

In late May 1984, at three experimental deer farms, Popielno, Wesjuny, and Baranowa, in northeastern Poland, blood samples from 27 anesthetized (Rompun or acetyl choline) red deer (*Cervus elaphus*), three elk (*Alces alces*), one fallow deer (*Dama dama*), and one Père David's deer (*Elaphurus davidianus*) were collected in Vacutainor blood tubes (one heparinized and one serum tube from each animal). Direct examination of blood (DE) centrifuged in microhematocrit tubes within hours of collection did not reveal blood parasites nor were trypanosomes recovered following double concentration procedures (Kingston and Morton, 1975, J. Parasitol. 61:17–23); subsequent culture of these blood samples in veal infusion medium (VIM) (Kingston and Morton, 1973, J. Parasitol. 59:1132–1133) revealed 10 of 14 red deer from Popielno (70%) (3 bulls, 5 cows, and 2 castrates), and 5 of 13 red deer (39%) from Wesjuny and Baranowa to be infected with trypanosomes. The elk, fallow deer, and Père David's deer did not show infection. Ages of animals examined ranged from less than 1 yr (two animals) to 18 yr; infected animals ranged in age from less than 1 yr to 16 yr.

In early July, on a second visit to these research stations, seven of the positive deer at Popielno, two of the positive deer at Wesjuny plus two other deer not previously examined, as well as two of the three bull elk previously examined were reexamined by DE and VIM cultures. Trypanosomes were seen moving in the plasma by DE of microhematocrit tubes centrifuged from two red deer and in one elk. The microhematocrit tubes were scored just above the buffy coat, and the trypanosomes, some plasma, the white cells, and some red blood cells were expressed onto microscope slides and conventional thin blood films were prepared, air dried, fixed in absolute methanol, and stained with Giemsa's stain. All cultures from these animals when examined 11–12 days later were positive for trypanosomes making a total of 17+/29 red deer (59%) and 2+/3 elk (67%). Typical *Trypanosoma* (*Megatrypanum*) sp. have been seen on slides prepared from concentrated red deer blood but it was not possible to photograph and analyze the trypanosomes at the time; the specific identity of these trypanosomes and those from elk awaits the further examination of blood films and analysis of parasites.

The occurrence of *Trypanosoma* sp. in the blood of these hosts is noteworthy as no previous reports of trypanosomes are known from red deer or elk in Poland, though trypanosomes are known from all species of cervids in North America (Kingston, Morton, and Dieterich, 1982, J. Protozool. 29:588–591). Friedhoff et al. (1984, Zbl. Bakt. Hyg. A 256:286–287) reported on the recovery of trypanosomes from two fallow deer, 10 of 27 roe deer (*Capreolus capreolus*) and one of three red deer from the vicinity of Hannover, Federal Republic of Germany, by culture methods; they were unable to recover trypanosomes on slides; no trypanosomes were found in four elk examined. Though these authors refer to the parasite as *Trypanosoma cervi* Kingston and Morton, 1975, the parasite must remain as species inquirenda until bloodstream stages are found and subjected to morphometric analysis. The only other previous report of trypanosomes from a wild ruminant in Europe was that of Wróblewski (1908, Cbl. Bakteriol. 1 Abt. Orig. BD XLVIII Heft. 2:162–163) who found trypanosomes by

[1] Research sponsored by the U.S. National Academy of Sciences–Polish Academy of Sciences Scientific Exchange Program, 1984.

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culture from the blood of a wisent (European bison, *Bison bonasus*) from Puszcza Białowieska, in eastern Poland.

This is the first report of trypanosomes from *Cervus elaphus* in Poland and the first record of trypanosomes in *Alces alces* on the European continent.

We acknowledge the kind cooperation, in collecting blood samples, of Prof. Dr. Zbiegniew Jaczewski, Popielno, and Dr. Andrezej Krzywiński, Wesjuny, Institute of Genetics and Animal Breeding PAN. We also acknowledge Prof. Dr. Andrzej Malczewski, W. Stefański Institute of Parasitology PAN, who generously gave of his time to make this study possible. Bayer AG, Leverkusen, FRG, generously supplied Rompun, and Peter Ott AG, Basel, Switzerland, contributed cartridges and syringes.

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PRESENTATION OF THE ANNIVERSARY AWARD TO HARLEY G. SHEFFIELD

By Richard L. Beaudoin, 10 October 1984 Helminthological Society of Washington

Dr. Beaudoin (right) presenting Anniversary Award to Dr. Sheffield.

It is the responsibility of the Chairperson of the Awards Committee to present the Society's Anniversary Award. This year that charge is also a distinct pleasure because the recipient is not only deserving because of his notable contributions to parasitology and because of his long and outstanding service to the Society but also because of the personal warmth and friendship he has extended to our membership throughout the years. His humor and smile are infectious and, I think everyone would agree, have exerted a very positive influence on the tone of our meetings, an influence certainly in keeping with the philosophy of fellowship fostered by the founders of our Society. For these reasons it is an honor to be in a position to present to Dr. Harley G. Sheffield the 1984 Anniversary Award of the Helminthological Society of Washington.

Dr. Sheffield was born in Detroit, Michigan, where he received his early education in the public school system. He attended Wayne State University where he earned a Bachelor of Science degree in 1953. From 1953 to 1955, he served on active duty in the Army Signal Corps following which he to returned to Wayne State where he completed a Master of Science degree in 1958. He then interrupted his formal education by taking a position as a research biologist at Parke Davis & Co. where he remained until 1959. He enrolled at Louisiana State University where, in 1962, he was awarded a Ph.D. degree. That same year, he accepted a position in the Laboratory of Parasitic Diseases, NIAID, where he stayed until 1978. During the last 10 years of this period, he was Head of the Section on Cell Biology and Immunology. In 1978, Dr. Sheffield left the laboratory behind him and took on the position of Health Scientist Administrator in the extramural

program of NIAID, a position he has held with compassion until the present.

Dr. Sheffield has been a very active member of the Society for many years and has held both appointed and elected offices including member of the Executive Committee, Vice President, and President as well as Editor of the Proceedings from 1970–1975. When he held the office of President, he initiated the September Dinner Meeting for officers and members of the Executive Committee, a meeting which has since become a tradition. Although it may be common knowledge, I believe it should be noted that few if any have been as faithful or as ready to serve as Dr. Sheffield. His attendance at meetings is near perfect and if he has not served on every committee of this Society, I would be hard pressed to point out the omission.

In the arena of research, Dr. Sheffield is probably best remembered as a student of the parasitic Protozoa about which he made many significant contributions. It may interest you to know, however, that he can lay honest claim to being a helminthologist, having worked on a variety of nematodes. I noted in going over his publications that he has even studied from close up the fine points of the seminal bursa as well as the dorsal vaginal valve of *Aedes aegypti.*

In closing, I want to repeat myself in saying that seldom have I had a responsibility which I was as pleased to discharge as that of awarding you, Dr. Harley G. Sheffield, the 1984 Anniversary Award of the Helminthological Society of Washington.

Report on the Brayton H. Ransom Memorial Trust Fund

Balance on hand, 1 January 1984	$7,196.75
Receipts: Net interest received in 1984	1,116.90
Contribution	2.00
	$8,315.65
Disbursements:	
Grant to the Helminthological Society of Washington for 1984	50.00
Membership, Am. Soc. Zool. Nomenclature	50.00
Publication Support	400.00
	$ 500.00
On hand, 31 December 1984	$7,815.65

HARLEY G. SHEFFIELD
Secretary-Treasurer

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Field of interest: _____

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Signature of sponsor: _____
(a member)

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