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CONTENTS

(*Continued on Outside Back Cover*)

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Synlophe of *Cooperia neitzi* (Trichostrongylidae: Cooperiinae) with Comments on Vulval Inflations and Hypertrophy of Cuticular Ridges among the Trichostrongylids

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ABSTRACT: The synlophe of *Cooperia neitzi* is characterized by a closed pattern in the cervical region (most similar to *C. punctata* and *C. pectinata*), a minuscule lateralmost ridge, 20 ridges at the level of the excretory pore and cervical papillae in males and females, and sequential addition of ridges laterally starting near the midbody (20 and 24 ridges at the midbody of males and females, respectively, with a maximum of approximately 32 adjacent to the copulatory bursa and vulva). The characteristic bilateral vulval fan in females has a consistent structure, being supported by a pair of hypertrophied ridges in each subdorsal field adjacent to the lateralmost ridge. Three species typical of bovids in sub-Saharan Africa (*C. neitzi, C. verrucosa,* and *C. okapi*) share the characters of minuscule lateralmost ridges, a closed cervical synlophe, and cuticular inflations at the level of vulva. Comparisons to other species of Cooperiinae (*Parostertagia heterospiculum, Cooperia verrucosa,* and *Cooperia okapi*) indicate homology in the bilateral and symmetrical structure of the vulval fans recognized in species of the subfamily. In contrast, it appears that the irregular and asymmetrical cuticular inflations reported or observed at the level of the vulva among certain Ostertagiinae (*Longistrongylus* spp., *Mazamastrongylus* spp., and *Camelostrongylus mentulatus*) have a fundamentally different configuration. It is suggested that vulval inflations in the Cooperiinae and Ostertagiinae had independent origins and thus are convergent.

KEY WORDS: *Cooperia* spp., Cooperiinae, Ostertagiinae, Trichostrongylidae, synlophe, morphology.

Cooperia neitzi Mönnig, 1932, was described from kudu (*Tragelaphus strepsiceros* (Pallas)) in the Transvaal, South Africa (Mönnig, 1932, 1933). Travassos (1937) and Skrjabin et al. (1954) included this species in monographs on the Trichostrongylidae but did not augment the description. Gibbons (1981) provided a redescription of males and females based on material from the type host in Zimbabwe. However, synoptic accounts of the synlophe are lacking, although some aspects including the disposition of ridges ventrally and the form of prominent bilateral inflations at the level of the vulva in females were depicted in the original description by Mönnig (1933), and Gibbons (1981) documented the structure and numbers of ridges near the midbody.

The current study arose from the necessity to understand the structural basis for cuticular inflations in the vulval regions of some species of the Cooperiinae (*Parostertagia heterospiculum* Schwartz and Alicata, 1933; *Cooperia neitzi*; *C. okapi* Leiper, 1935; *C. verrucosa* Mönnig, 1932; and perhaps others) and the Ostertagiinae (species of *Mazamastrongylus* Cameron, 1935, and *Longistrongylus* Le Roux, 1931, and *Camelostrongylus mentulatus* (Railliet and Henry, 1909)) and their relationship to the synlophe (Mönnig, 1933; Gibbons, 1977, 1981; Hoberg and Lichtenfels, 1992; Lichtenfels et al., 1993). Specifically, a requisite for the phylogenetic analysis of the trichostrongylids resides in determining the homology for these and other characters among the 6 subfamilies currently recognized as valid (see Gibbons and Khalil, 1982b; Durette-Desset, 1983; Hoberg and Lichtenfels, 1992).

In the current study we describe the synlophe in males and females of *Cooperia neitzi*. The cervical synlophe is compared among *C. neitzi* and those species of *Cooperia* previously evaluated (Lichtenfels, 1977; Gibbons, 1981). Additionally, observations of the structure of vulval inflations (based on transverse sections near the level of the vulva) including position and relationship to the synlophe are presented for *C. neitzi, Mazamastrongylus* sp., *Longistrongylus sabie* (Mönnig, 1932), and *Camelostrongylus mentulatus*. These latter data provide a basis for preliminary comparisons of vulval fans or inflations reported among the Cooperiinae and Ostertagiinae.

Methods and Materials

Nematodes were examined as temporary whole mounts cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol). Observa-

tions concentrated on *C. neitzi*, and wholemounts were used to study the configuration of the longitudinal ridges laterally and dorsoventrally in the cervical zone, to determine the extent of the synlophe posteriad in males and females, and to examine the structure of vulval inflations in females. Transverse sections from single specimens of female *C. neitzi*, *C. okapi*, and *Longistrongylus sabie* and from two specimens each of *C. mentulatus* and *Mazamastrongylus* sp. were prepared freehand with a cataract knife and embedded in glycerine jelly. Sections were used to study the structure of the synlophe, with particular reference to the configuration of the vulval inflations characteristic of these species. Line figures and photomicrographs of sections are as viewed from the anterior and oriented with dorsal toward the top of the plates; all line figures were prepared with aid of a camera lucida. Terminology for the structure of the synlophe is consistent with that developed by Lichtenfels (1977) for *Cooperia* spp. The term *cuticular strut* follows Lee (1965).

Specimens examined

Cooperia neitzi: material included 5 female and 3 male specimens from the type host collected in Zimbabwe (listed as Rhodesia) by J. B. Condy and made available from the collection of the International Institute of Parasitology, St. Albans, U.K. (No. 904).

Specimens examined for comparative purposes are the following. *Cooperia okapi*: material included 6 female specimens from *Okapia johnstoni* (Sclater) in Zaire (listed as Epulu, Belgian Congo) and deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland (No. 61409). These specimens were originally included in the type series of *Cooperia okapiae* van den Berghe and Vuylsteke, 1937, a synonym of *C. okapi* (see van den Berghe and Vuylsteke, 1937). *Cooperia punctata* (von Linstow, 1906) and *C. pectinata* Ransom, 1907: material included 5 male specimens of each species from *Bos taurus* Linnaeus representing unaccessioned material from the U.S. National Parasite Collection. *Longistrongylus sabie*: material included 5 females from *Aepyceros melampus* (Lichtenstein) in Kruger National Park, South Africa, and deposited in the U.S. National Parasite Collection (No. 77484). *Camelostrongylus mentulatus*: material included 5 females each from *Camelus* sp. in the U.S. National Zoo and from *Lama glama* (Linnaeus) in Oregon, deposited in the U.S. National Parasite Collection (Nos. 32079 and 82440, respectively). *Mazamastrongylus* spp. (including both *M. odocoilei* (Dikmans, 1931) and *M. pursglovei* (Davidson and Prestwood, 1979)): material included 5 females examined by Lichtenfels et al. (1993) from *Odocoileus virginianus*.

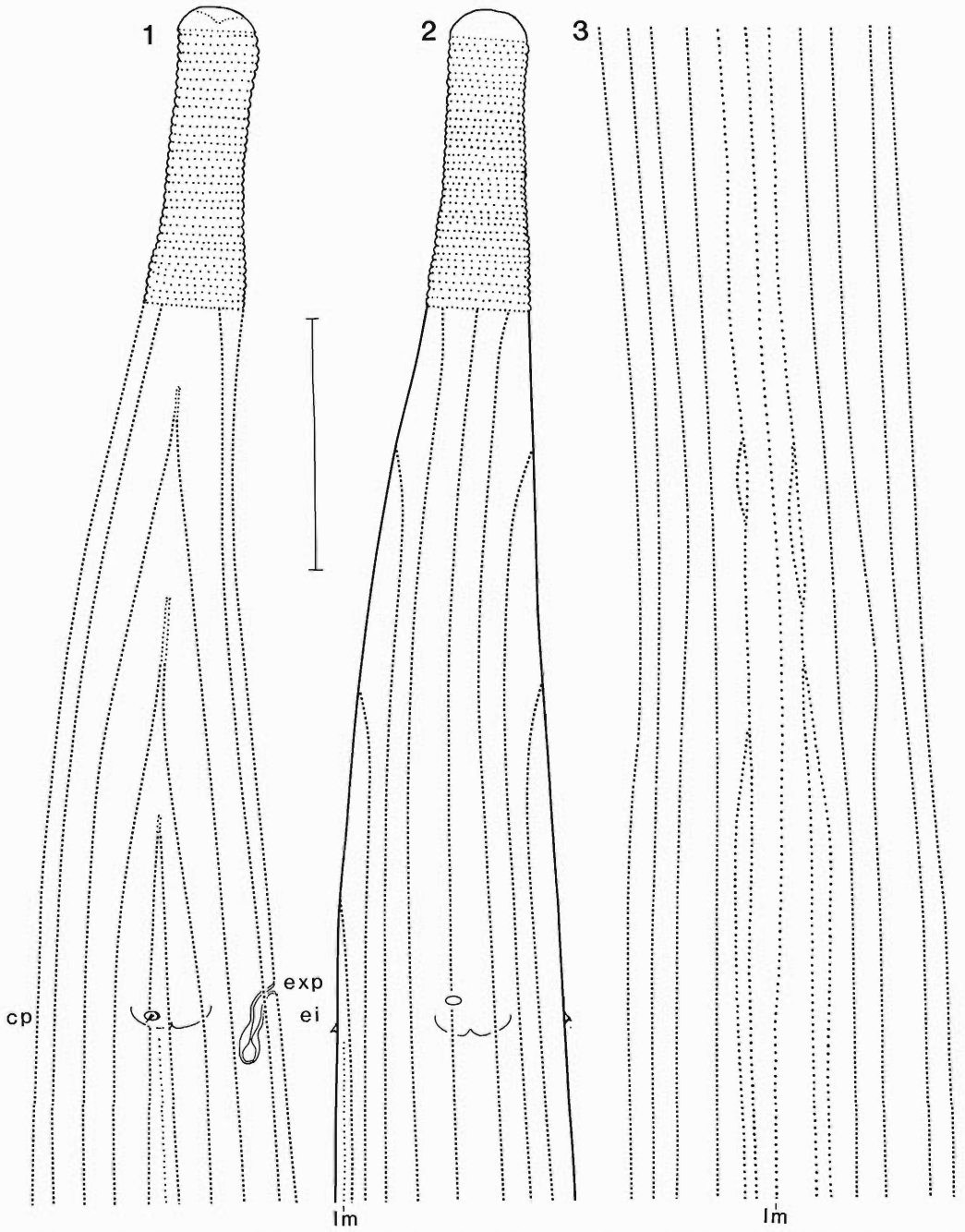
Results

Synlophe of *C. neitzi*

The structure of the synlophe in males and females of *Cooperia neitzi* shares basic similarities. A bilaterally symmetrical system of well-defined ridges extends from the base of the cephalic expansion to the anterior margin of the bursa in males and beyond the anus in females (Figs. 1–3). The striated or beaded appearance of the synlophe is attributable to the structure of underlying struts supporting individual ridges.

In the cervical zone of males and females (level of excretory pore and base of esophagus), there are 20 ridges (7 ventral and 7 dorsal with broad inter-ridge intervals, and 3 smaller, narrowly spaced ridges in each lateral field) that are continuous and extend into the posterior ¼ of the body to terminate adjacent to the caudal extremity (Figs. 1–3). Those in the lateral field are arranged bordering a minuscule lateralmost ridge (Figs. 1, 3). A closed pattern is typical for 3 pairs of ridges in each lateral field in the cervical zone (Fig. 1). As these pairs converge toward a caretlike point in the anterior, the narrowly spaced ridges become parallel and diminished in height and may continue for 20–30 μm before termination. Ventrally and dorsally, 3 ridges extend to the base of the cephalic expansion, and the ventral ridge is interrupted at the level of the excretory pore (Fig. 2).

Posteriad from the cervical zone, there is a sequential increase in the numbers of ridges beginning near the midbody in females and in the posterior ¼ of the body in males. Addition of new ridges consistently occurs adjacent to the 3-ridge lateral system, with initiation of ridges usually being convergent on the pair that directly borders the minuscule lateralmost ridge (Fig. 3). Anterior to the point of initiation of each new pair of ridges, irregularities develop, with short regions where individual ridges bifurcate and anastomose (Fig. 3). In males there are 20 ridges (7 large dorsal and ventral, 3 small in each lateral field) at the midbody; posteriad there are 6 pairs of ridges that originate laterally in the posterior ¼ of the nematode (originating in 3 consistent zones at approximately 77, 90, and 95% of body length from the anterior) for a maximum of 32 (7 dorsal and ventral, 9 in each lateral field). In females there are 20–25 ridges at the midbody (Fig. 5); posteriad 4–6 pairs of continuous ridges are added in the region from the midbody to anterior to the vulva for a maximum of 32 (attained at 75–80% of body length from the anterior). Only rarely do new lateral ridges originate beyond the 3-ridge lateral system; variation in females is also due to sporadic occurrence of short, discontinuous ridges in the lateral field. All ventral ridges are interrupted at the level of the vulva; the numbers of ridges then increase to approximately 30 posterior to the vulva with irregular loss of lateral ridges occurring in the



Figures 1–3. Drawings of the synlophe in female and male specimens of *Cooperia neitzi* (scale bar = 100 μm). 1. Cervical region of female specimen, lateral view showing typical closed pattern (see Lichtenfels, 1977), and minuscule lateralmost ridge (exp = excretory pore, cp = cervical papilla, ei = esophageal–intestinal junction). 2. Cervical region of female specimen, ventral view showing 3 continuous ventral ridges extending to the base of the cephalic expansion, and minuscule lateralmost ridges (lm). 3. Posterior quarter of a male specimen, lateral view showing typical pattern of addition of ridges in the lateral field. Note minuscule lateralmost ridge (lm) and irregularities in the synlophe anterior to the point of origin of new ridges.

region anterior to the anus; posteriad extension of the synlophe occurs beyond the anus.

Bilateral vulval fan in *C. neitzi*

A prominent bilateral inflation, 200–230 μm in length, occurs at the level of the vulva (approximately 80% of the body length from the anterior). The form and orientation of the inflation is consistent in all specimens (Figs. 4, 6, 7). Each fan is formed by the hypertrophy of struts supporting a pair of specific lateral ridges in each subdorsal field (Figs. 4, 6, 7). Origin of the inflation in the subdorsal field adjacent to the minuscule lateralmost ridge is accompanied by hypertrophy of the ridges (Figs. 4, 6), inflation of the cuticle, and substantial disruption of the synlophe with interruption of a number of ridges in the lateral fields, including the lateralmost. At the greatest width of the fans (Figs. 4, 7), near the level of the vulva, considerable curvature is observed as bilaterally the inflations and supporting struts attain a ventrally directed orientation. Posterior to the ovejectors, the structure of the synlophe regains the symmetry and orientation evident in the midbody of the nematode.

Vulval inflations among other species

Transverse sections at the level of the vulva among representatives of the Ostertagiinae revealed cuticular inflations (distinct from vulval flaps) to be variable in extent, generally asymmetrical, and disposed dorsally, laterally, or lateroventrally. Hypertrophied struts do not provide direct support for these irregular inflations.

Ornamentation adjacent to the level of the vulva in *Mazamastrongylus* spp. is relatively complex, and there is no specific orientation or symmetry in the disposition of cuticular inflations. Inflations are highly irregular, being composed of dorsally, ventrally, or laterally directed cuticular crests or broader hypertrophied regions (Figs. 8, 9). One to several ridges of the synlophe (occasional fusion of ridges is observed) may be associated with each inflation. Enlarged struts are absent or poorly defined and only indirectly constitute the foundation for inflated regions (Fig. 9).

Substantial inflations at the vulva typical of *Longistrongylus sabie* are to some extent bilateral to ventrolateral in disposition. Each major inflation is a multiridge system variable in development, and irregular hypertrophy of adjacent dorsal ridges is also evident (Fig. 10). The synlophe is superficial with respect to the bilateral inflations, and prominent struts providing direct support of hypertrophied regions and ridge systems are absent (Fig. 10).

Broad, rounded inflations (1–4 in number) disposed dorsally or laterally are typical of the vulval region in *Camelostrongylus mentulatus* (Fig. 11). Hypertrophied regions of the cuticle and synlophe represent multiridge systems lacking specific orientation or symmetry. Strutlike formations are evident but appear as irregular rodlike structures deep within the inflated cuticle (Fig. 11).

Discussion

The synlophe in males and females of *Cooperia neitzi* has never been completely characterized, nor has the structure of the cervical syn-

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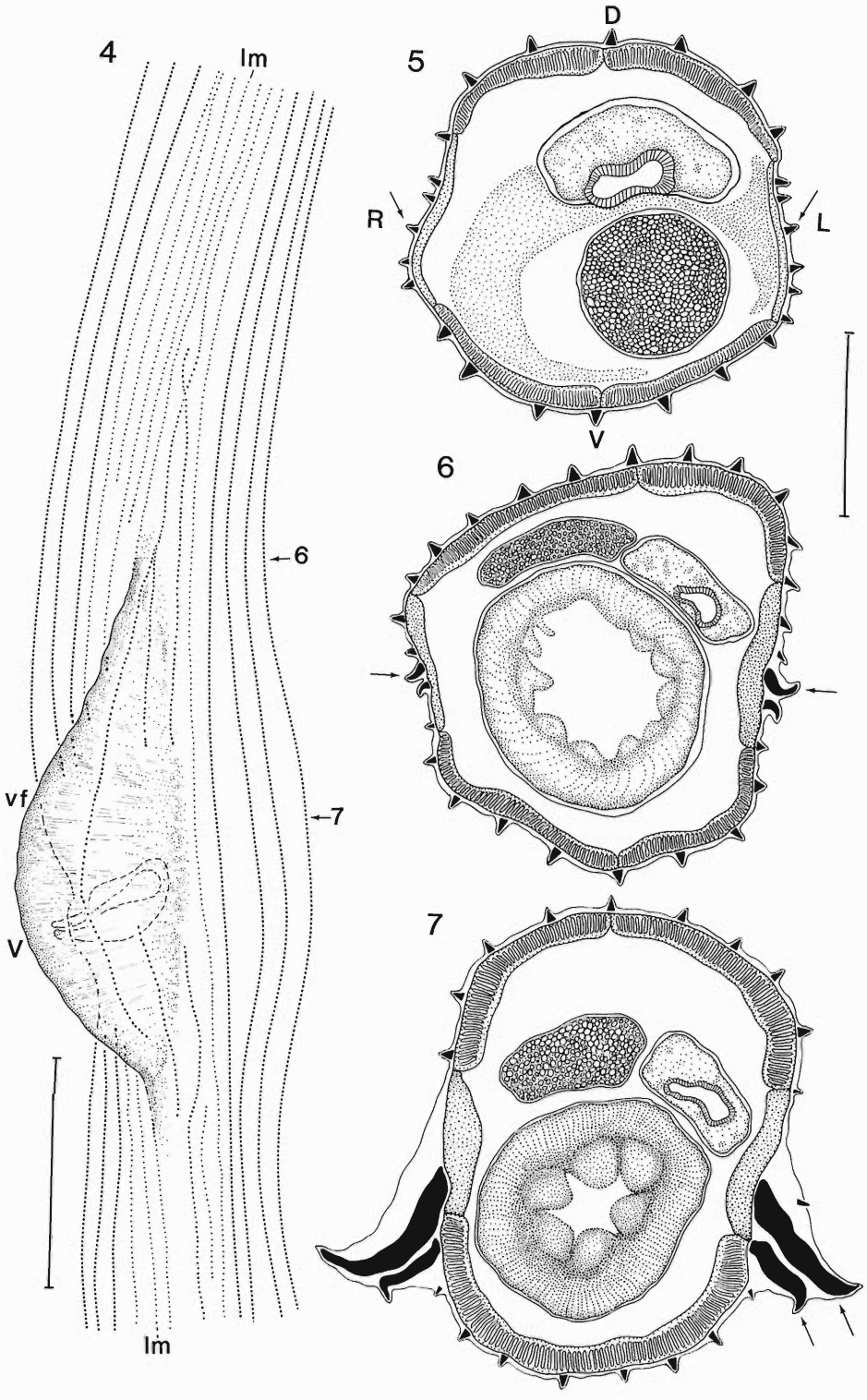
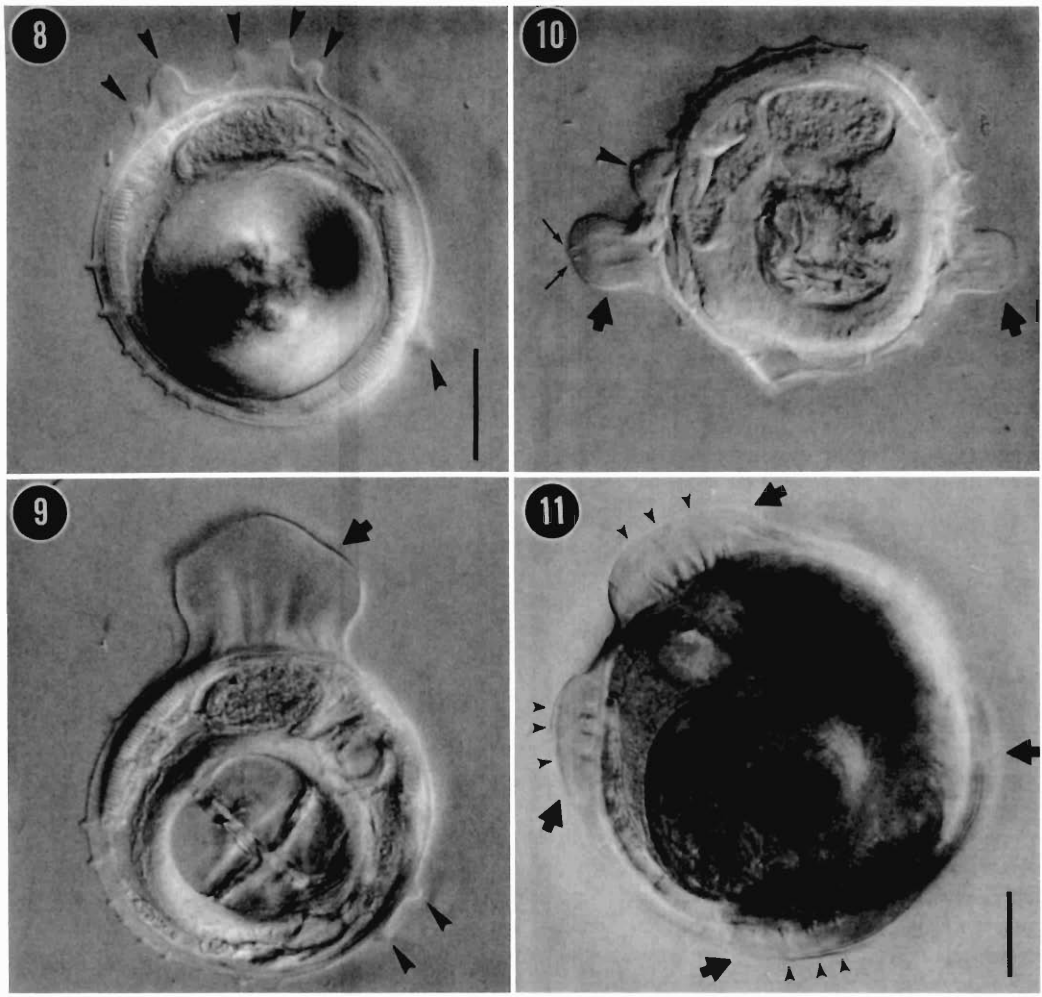
Figures 4–7. Synlophe and cuticular fans at the level of the vulva in females of *Cooperia neitzi* (scale bar = 100 μm for Fig. 4; 50 μm for Figs. 5–7). 4. Vulval region in left lateral view showing configuration of the synlophe and origin (in the subdorsal field adjacent to the lateralmost ridge) and structure of the vulval fans (only left lateral fan is depicted). Note that the ridges of the lateral fields are typically smaller than those in the ventral or dorsal fields (lm = lateralmost ridge, v = vulva, vf = vulval fan). The figure is orientated with anterior toward the top; positions of transverse sections depicted in Figures 6 and 7 are indicated by arrows. 5. Synlophe at the midbody (transverse section as viewed from the anterior, with dorsal [D] oriented toward the top, ventral [V] toward bottom, and left [L] and right [R] indicated) showing position of lateralmost ridges (arrows); 25 ridges are present with 7 large ventral and dorsal ridges and 6 and 5 smaller laterals. 6. Synlophe near the point of initiation of the fans showing subdorsal hypertrophied struts and lateral ridges (arrows). There are 29 ridges present with 7 large ventral and dorsal and 7 and 8 laterals. Interruption of the lateralmost has occurred slightly anterior to the position of this section. 7. Synlophe near level of vulva and widest extent of bilateral fans showing massive hypertrophied struts (arrows) supporting the cuticular inflation (directed slightly ventrad); 23 ridges are present with 7 dorsal, 6 ventral, and 6 and 4 laterals. In this section a ventral ridge adjacent to the vulva has been interrupted.





Figures 8–11. Cuticular inflations in females among some species of the Ostertagiinae shown in transverse section near the level of the vulva (scale bars = 25 μm; same scale for Figs. 8–10). 8. *Mazamastrongylus* sp., sectioned at the level of the anterior sphincter, showing initial development of irregular cuticular crests and hypertrophy of ridges dorsally and lateroventrally (pointers) that constitute the prominent vulval inflations. 9. *Mazamastrongylus* sp., sectioned at the level of the anterior vestibula, showing full extent of a dorsal vulval inflation (arrow), lacking highly distinct supporting struts, that has developed from the fusion of the cuticular crests shown in Figure 8; note hypertrophied ridges in lateroventral field (pointers). 10. *Longistrongylus sabie,* sectioned through the vestibule, showing laterally oriented inflations (large arrows) and hypertrophy of a single laterodorsal ridge (pointer). Note the minuscule struts of two ridges (small arrows) that indicate the synlophe is superficial with respect to the inflations. 11. *Camelostrongylus mentulatus,* sectioned anterior to the vulva, showing 4 rounded cuticular inflations (arrows), with one being beyond the plane of focus. Note the irregular rodlike struts deep within the inflations (pointers).

lophe in any of the species typical of African ruminants been compared in detail to those occurring in North America (Lichtenfels, 1977; Gibbons, 1981). Results of the present study are in general agreement with previous reports but provide additional details of the structure and distribution of longitudinal ridges. Mönnig (1933) described 20–30 continuous longitudinal striations in males and females and depicted the interruption of the ventral synlophe and the presence of a pair of lateral alae at the level of the vulva in females. Gibbons (1981) found 20 (7 dorsal and ventral, 3 in each lateral field) and 19 ridges, respectively, at the midbody in females

and males but did not evaluate the cervical zone. In the present study we found 20 ridges at the midbody of males and 20–25 in females and a maximum of 30–32 in the posterior ¼ of the body. With respect to Gibbons (1981), the discrepancies in the number of midbody ridges relate to variation in the point of origin (near the midbody) for lateral ridges in females and an apparent irregular discontinuity in a left lateral ridge in the male specimen examined in her study (see Fig. 49 of Gibbons, 1981).

The cervical synlophe has been evaluated in detail for 6 species of *Cooperia* from ruminants in North America (Lichtenfels, 1977). The "closed pattern" was designated for 4 species (*C. pectinata, C. punctata,* and *C. oncophora* (Railliet, 1898) and *C. surnabada* Antipin, 1931) where pairs of ridges converge symmetrically and terminate laterally in the cervical zone (Lichtenfels, 1977). The pattern apparent in *C. neitzi* is similar to that described for *C. pectinata* and *C. punctata,* except in the former species there are 20 cervical ridges (compared to 14, with 5 large dorsal and ventral ridges and 2 small ridges in each lateral field), 3 pairs converge and terminate laterally (compared to 2 pairs), and a minuscule lateralmost ridge in each lateral field is not evident in the other species (Lichtenfels, 1977). Absence of the lateralmost ridge in *C. pectinata* and *C. punctata* was confirmed by midbody sections depicted by Gibbons (1981).

Variation in the configuration of the closed pattern (structure of the "caretlike" anteriorly directed points where ridges converge and terminate) was evident in a comparison of *C. neitzi* and specimens of *C. punctata* and *C. pectinata* examined during the present study and is greater than that previously documented by Lichtenfels (1977). Specifically, in *C. neitzi* and these other species, ridges converge laterally to form a prominent caretlike point but then may extend parallel (with a very narrow interval separating the ridges) for 20–105 μm before terminating (Fig. 1). Thus, although the typical closed pattern is evident (in contrast to the "open pattern" typical of *C. curticei* (Railliet, 1893) and *C. spatulata* Baylis, 1938), the anteriorly directed "caret-like points" described by Lichtenfels (1977) are not always sharply delineated. Additionally, in both *C. pectinata* and *C. punctata,* the most anterior pair of ridges (labeled D-1 and V-1 in Fig. 1 by Lichtenfels, 1977) may converge and extend to the base of the cephalic capsule as a single ridge.

Among species of *Cooperia* endemic to Africa in which the cervical zone has been examined, the closed pattern (consistent with that observed in *C. neitzi*) has been depicted for *C. verrucosa* from *Tragelaphus oryx* (Pallas) (see Gibbons, 1981) and is apparent in *C. okapi* from *Okapia johnstoni* (Hoberg, unpubl. data). These species also possess a single minuscule lateralmost ridge in each lateral field, discernible in wholemounted specimens extending the length of the nematode and also evident in sections at the midbody, similar in form to that described in *C. neitzi* (see Gibbons, 1981). In contrast, *C. rotundispiculum* Gibbons and Khalil, 1980, from *Redunca redunca* (Pallas) is characterized by an open system (similar to that described for *C. curticei*) and lacks a diminutive lateralmost ridge (Lichtenfels, 1977; Gibbons, 1981). Although requiring confirmation, it appears that the diminutive lateralmost ridge may be correlated with the closed pattern of the cervical synlophe and may potentially indicate a more inclusive group of *Cooperia* spp. occurring in bovids endemic to sub-Saharan Africa. This contention is further supported by the presence of 20 midbody ridges (7 large dorsal and ventral, 3 small ridges in each lateral field) in *C. neitzi* and *C. verrucosa,* although the number in *C. okapi* (14–16 in males and females) is more typical of other *Cooperia* spp. (Gibbons, 1981).

Posterior to the cervical zone, Lichtenfels (1977) noted a sequential increase in the numbers of ridges in the lateral fields of the synlophe among the 6 species considered from North America. This pattern of ridge addition (laterally in the posterior) was also observed in the present study and appears to be a uniform character among *C. neitzi, C. okapi, C. verrucosa,* and other *Cooperia* spp. Addition of new ridges typically occurs adjacent to the pair of ridges that border the lateralmost (or the pair of lateralmost ridges when the minuscule ridge is absent). Ridges are either initiated in the space between ridges (e.g., *C. pectinata*) or appear to originate directly from the edges of the lateral ridges (e.g., *C. neitzi* [Fig. 3], *C. punctata*).

Cuticular inflations or fans at the level of the vulva have been recognized in a number of *Cooperia* spp. but are particularly well developed in *C. neitzi* and *C. verrucosa* and to a lesser extent in *C. okapi* (Mönnig, 1933; Travassos, 1937; Gibbons, 1981; Hoberg and Lichtenfels, 1992). The bilateral fans typical of *C. neitzi* are supported by 2 hypertrophied struts (see Lee, 1965) and represent 2 specific lateral ridges in each

subdorsal field (see Figs. 4, 6, 7). Although the origins of the inflations are in the subdorsal field, at the maximum extent of development there is a ventral orientation for these ridge systems (see Figs. 4, 7). Mönnig (1933) clearly depicted the subdorsal origin of the fans while describing a pair of prominent lateral alae at the level of the vulva (also shown by Travassos, 1937), whereas Gibbons (1981) showed the fans with an origin in the ventrolateral field. Neither of these earlier studies provided a lateral view of the cuticular ridges in the vulval region.

The structure of the fan in *C. verrucosa* is highly similar to that described in the present study for *C. neitzi*. The lateral view of the synlophe in the vulval region of the former species by Mönnig (1933) unequivocally shows the origin of the fan from a single ridge in the subventral field (ventral to the lateralmost ridge). Although it is not evident whether or not more than a single ridge is involved in the structure of each fan, Mönnig (1933) indicated that "... one of the longitudinal striations is raised into an alar expansion. ..." Gibbons (1981) did not evaluate the configuration of the synlophe in the vulval region of *C. verrucosa*.

In contrast to *C. neitzi* and *C. verrucosa*, fans evident in *C. okapi* differ in form (see Gibbons, 1981). In the latter species, all ventral and dorsal ridges become hypertrophied. However, it is specifically the ventrolateral ridges that attain the greatest height and thus form the impression of a small, elongate, bilateral fan at the level of the vulva (Gibbons, 1981; Hoberg, unpubl. obs.). At the level of the vulva, all ridges remain discrete and there are no irregular enlargements (inflations) of the cuticle associated with fusion of individual ridges comprising the synlophe. The ridges ventral in position to the lateral field are consistently the largest. Thus, in these 3 species of *Cooperia*, in which fans are recognized, minuscule lateralmost ridges and a closed pattern in the cervical synlophe are also consistently present (see Gibbons, 1981).

In the Cooperiinae, vulval fans are typically bilateral and symmetrical and appear to arise from specific ventral or dorsal ridges in the lateral fields (intergeneric and interspecific differences are apparent, but intraspecific variation is minimal) (Hoberg and Lichtenfels, 1992). There appears to be a general consistency in the configuration of the fans among the Cooperiinae where this character has been examined (e.g., *Parostertagia heterospiculum, C. neitzi, C. verrucosa,* and *C. okapi*) (Mönnig, 1933; Gibbons, 1981; Hoberg and Lichtenfels, 1992). Other characters largely restricted to the Cooperiinae include a small number of ridges in the synlophe and convergent addition and increase posteriad in the numbers of ridges along with specific attributes of the bursa (Durette-Desset, 1982, 1983; Gibbons and Khalil, 1982b; Hoberg and Lichtenfels, 1992).

Prominent inflations at the level of vulva are relatively rare among the trichostrongylids, being reported only among the Cooperiinae as indicated earlier and among some of the Ostertagiinae (specifically, *Mazamastrongylus* spp., *Longistrongylus* spp., *Camelostrongylus mentulatus,* and possibly *Cervicaprastrongylus malviyai* (Chaturvedi and Kansal, 1977)) (Gibbons, 1973, 1977; Gibbons and Khalil, 1982a; Lichtenfels et al. 1993; Hoberg, unpubl. data). Inflations and bilateral fans appear distinct from vulval flaps known among the Ostertagiinae, the Haemonchinae, and the Cooperiinae (e.g., structures present in some *Ostertagia* spp., *Haemonchus* spp., and *Paracooperia* spp.) as the latter typically represent a posteriad extension of the body wall that partially or completely envelops the region of the vulva in females (see Skrjabin et al., 1954). In contrast, inflations are hypertrophied regions of the cuticle often intimately associated with the synlophe, as described previously. This distinction is particularly evident among some of the Ostertagiinae, where inflations and vulval flaps may be present concurrently in females of *Mazamastrongylus* spp.

Vulval inflations among the Ostertagiinae appear to be fundamentally different from those characteristic of the Cooperiinae. Whereas bilateral and symmetrical fans are typical of the latter subfamily, irregular inflations associated with the synlophe have been evaluated in *Mazamastrongylus, Longistrongylus,* and *Camelostrongylus* (Gibbons, 1972, 1973, 1977; Lichtenfels et al., 1993) and recently recognized in *Hyostrongylus rubidus* (Hassall and Stiles, 1892) (Hoberg, unpubl. data; see Hassall and Stiles, 1892; Goodey, 1924). In these genera of the Ostertagiinae, inflations are asymmetrical, irregular systems of multiple or discontinuous ridges disposed dorsally, laterally, and lateroventrally (Figs. 8–11). Fusion of ridges is also often associated with the development of inflations among species of these genera. Additionally, the direct relationship of hypertrophied struts and inflation of the cuticle, established for the Cooperiinae, is

apparently not as well defined among the Ostertagiinae (Hoberg and Lichtenfels, 1992). Thus, it is suggested that vulval inflations among the Cooperiinae and Ostertagiinae are convergent. However, among genera and species within each subfamily, characteristic cuticular inflations may represent putative homologies (synapomorphies) indicative of more inclusive relationships. Results of the current study provide additional support for placement of *Parostertagia heterospiculum* in the subfamily Cooperiinae (see Hoberg and Lichtenfels, 1992).

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Syncoelium regaleci sp. n. (Digenea: Syncoeliidae) from the Branchial Cavity of the Oarfish (*Regalecus glesne*)

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ABSTRACT: *Syncoelium regaleci* sp. n. (Trematoda: Syncoeliidae) is described from the gill rakers of the oarfish, *Regalecus glesne,* from Baja California Sur, Mexico. The new species differs from all others in the genus in number of testes. The size range of testes in *S. spathulatum* overlaps *S. regaleci* sp. n. but differs in body size and ovary shape.

KEY WORDS: Trematoda, *Syncoelium regaleci,* Syncoeliidae, oarfish, *Regalecus glesne,* Baja, Mexico.

During July 1988, a moribund oarfish (*Regalecus glesne* (Ascanius) was found washed up on the beach in the Bay of La Paz, Baja California Sur, Mexico (24°N, 111°W). The fish was necropsied and examined for parasites. Forty specimens of digenean trematodes that are new to science were collected from the gill rakers of this fish. These worms are described in this paper.

Materials and Methods

The parasites were fixed in cold alcohol–formalin–acetic acid for 12 hr and stored in 70% ethanol. Whole mounts were stained with trichrome, Delafield's hematoxylin, or Semichon's acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. All measurements are in micrometers unless otherwise indicated. Illustrations were made with the aid of a microprojector and drawing tube.

Results

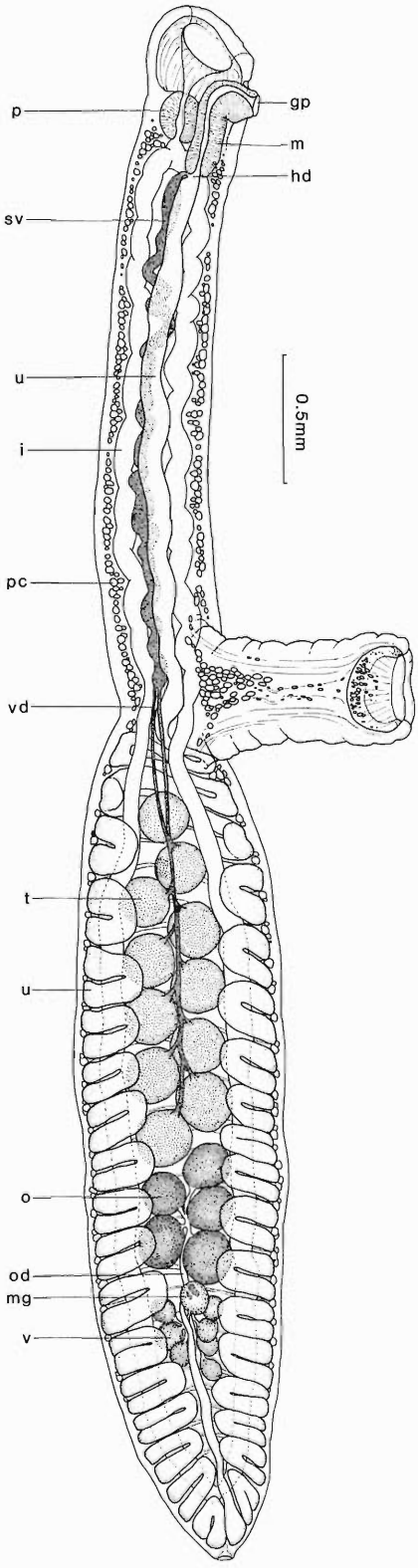
Syncoelium regaleci sp. n. (Fig. 1)

SPECIFIC DIAGNOSIS (based on measurements of 5 entire, mature specimens): Syncoeliidae (Looss, 1899) Odhner, 1927; Sycoeliinae Looss, 1899. Body elongate, 6.0–8.2 mm long, maximum width 0.90–1.0 mm wide. Cuticle thick, mesenchymal cells numerous from pharynx to acetabulum, few in hindbody. Oral sucker subterminal 250–350 long by 250–500 wide, prepharynx absent, pharynx muscular, 150–250 long by 150–200 wide. Acetabulum pedunculate, in midbody. Peduncle 0.75–1.75 mm long by 250–583 ($\bar{x}$ = 317) wide, acetabular sucker 250 long by 500 wide. Esophagus short, cecum bifurcates 0.50–1.0 mm from anterior end extending length of body where it is contiguous, forming loop near posterior end. Testes 11 in number, in anterior half of hindbody, round, intercecal, in 2 irregular rows, 150–350 in diameter. Cirrus pouch and cirrus absent. Vas deferens paired, seminal vesicle long (268–280), joining metraterm at level of posterior end of pharynx to form hermaphroditic duct. Genital pore ventral to oral sucker. Ovary median, divided into 5 rounded, dendritic acini, posttesticular, in posterior third of body. Oviduct extends posterior to ootype. Mehlis' gland present. Laurer's canal not observed. No seminal receptacle. Vitellaria consist of 7 glands united by a collecting duct, which extends anteriorly to ootype Mehlis' gland complex. Uterus runs posteriorly from ootype joining posteriormost loop of cecum. Uterus extremely sinuous in hindbody, then extending as a straight tube anteriorly to hermaphroditic duct. Excretory vesicle Y-shaped with terminal pore. Eggs oval, operculate, thick-shelled, 31–39 long by 25 wide (N = 10).

TAXONOMIC SUMMARY:

Host	Oarfish, *Regalecus glesne*
Locality	Punta Colorado, Bay of La Paz, Baja California Sur, Mexico
Habitat	Gill rakers
Holotype	USNM Helm. Coll. No. 82607
Paratype	USNM Helm. Coll. No. 82608
Etymology	Species named for genus of host fish

REMARKS: *Syncoelium regaleci* differs from all species in the genus except *S. spathulatum* by number of testes (*S. thyrisitae, S. cypseluri katuwo,* and *S. filiferum* all with 18; *S. cypseluri,*



16–18; *S. priacanthi,* 15). *Syncoelium spathulatum* is described as having a range of 10–17 testes. *Syncoelium regaleci* differs from *S. spathulatum* by exact number of testes (11, $N = 30$), smaller body size (*S. spathulatum* 320–530), shape and size of ovary (lobate 420–540 for *S. spathulatum*).

Discussion

The giant oarfish, or king of the herrings, is the origin of many sea serpent stories. This fish occurs worldwide and reaches a length of 10.7 m and a weight of 227 kg. It is seldom seen and is listed as rare (Miller and Lea, 1972). This is the first helminth parasite recorded from this host in the eastern Pacific Ocean. Other species of *Syncoelium* have been reported from other hosts in eastern Pacific waters. Yamaguti (1970) described *S. cypseluri* from the Hawaiian flying fish (*Cypselurus spilonotoplerus*), and *Syncoelium filiferum* was reported from the humpback salmon (*Onchorhynchus garbuscha*) by Lloyd and Guberlet (1936). However, Manter (1954) considered the parasite reported from *O. garbuscha* to be a synonym of *S. katuwo* Yamaguti, 1938.

The digenetic trematodes of the genus *Syncoelium* are unique in several ways. They are found outside the internal organs of the host and have a cyclocoel-type gut. Coil and Kuntz (1963) discussed their observations on the histochemistry of *S. spathulatum,* including the numerous glandlike bodies found throughout the parasite. Yamaguti (1970) also dicussed these structures in his description of *S. cypseluri,* referring to them as "obviously modified parenchymatous cells" equivalent to the "drüsenartige Zellennester" described by Looss (1899). *Syncoelium regaleci* contains the same dark-staining structures; however, they are primarily restricted to the forebody and acetabulum, with very few found in the hindbody.

The complex life history of members of this genus has not been published; however, metacercariae have been reported on the surface of marine copepods (Schell, 1985).

←

Figure 1. ***Syncoelium regaleci*** **sp. n. Entire worm. gp = genital pore, p = pharynx, m = metraterm, hd = hermaphroditic duct, sv = seminal vesicle, *u* = uterus, i = intestinal cecum, pc = parenchymatous cells, vd = vas deferens, t = testis, o = ovary, od = oviduct, mg = Mehlis' gland, v = vitellarium.**

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New Editor

Volume 60 marks the completion of my 5-year term as editor of the *Journal.* It is with mixed feelings of regret and relief that I step down. I have learned much. I have enjoyed working with the members of the Editorial Board, the authors, and the staff of Allen Press. I thank them all for maintaining the *Journal* as a first quality scientific journal.

The new editor is Dr. Sherman Hendrix. Beginning immediately all correspondence concerning the *Journal* should be sent to him at Department of Biology, Gettysburg College, Gettysburg, PA 17325.

Ralph P. Eckerlin

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Gastrointestinal Helminths of Night Lizards, Genus *Xantusia* (Xantusiidae)

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ABSTRACT: Examination of the gastrointestinal tracts of 278 *Xantusia vigilis,* 40 *Xantusia henshawi,* and 8 *Xantusia bolsonae* revealed the presence of 1 species of nematode, *Parapharyngodon californiensis* (prevalences 1, 28, and 50%, respectively). *Xantusia henshawi* and *X. vigilis* also harbored 1 species of cestode, *Oochoristica bezyi* (prevalences 35 and 16%, respectively). *Xantusia bolsonae* is a new host for *P. californiensis. Xantusia henshawi* and *X. vigilis* are new hosts for *O. bezyi.* Examination of the gastrointestinal tracts of 21 *Xantusia riversiana* revealed the presence of 6 species of nematodes: *Alaeuris clementensis, Alaeuris riversianae, Parapharyngodon pseudothaparius, Parapharyngodon xantusi, Thubunaea iguanae,* and an unidentified oxyurid (prevalences 71, 81, 100, 90, 14, and 5%, respectively). One species of cestode, *Oochoristica islandensis* (prevalence 52%), also was present. *Xantusia riversiana* is a new host for *T. iguanae.* Compared to the mainland species of *Xantusia,* the helminth fauna of the insular *X. riversiana* is both unique and diverse. The high prevalences of helminths in *X. riversiana* may be due to the increased opportunity for infection and reinfection presented by its unusually dense populations and overlapping home ranges.

KEY WORDS: Cestoda, *Oochoristica bezyi, Oochoristica islandensis,* Nematoda, *Alaeuris clementensis, Alaeuris riversianae, Parapharyngodon californiensis, Parapharyngodon pseudothaparius, Parapharyngodon xantusi, Thubunaea iguanae,* Xantusiidae, *Xantusia bolsonae, Xantusia henshawi, Xantusia riversiana, Xantusia vigilis,* prevalence, intensity.

The 20 living species of the New World lizard family Xantusiidae are arrayed into 3 genera. The northernmost, *Xantusia,* consisting of 4 habitat-specialized species, comprises the focus of the present study. The only widely distributed member of the genus is the desert night lizard, *Xantusia vigilis* Baird, 1858, which ranges discontinuously from central California and southern Utah, south to Baja California Sur and Zacatecas, Mexico, and inhabits yuccas, agaves, and other plants (Bezy, 1982). The cestode *Oochoristica scelopori* Voge and Fox, 1950, and the nematode *Parapharyngodon californiensis* (Read and Amrein, 1952) Adamson, 1981, have been reported from *X. vigilis* by Amrein (1951) and Telford (1970).

The granite night lizard, *Xantusia henshawi* Stejneger, 1893, lives exclusively beneath exfoliations of boulders in the peninsular ranges of southern California and northern Baja California (Lee, 1976). Read and Amrein (1952) and Telford (1970) reported *O. scelopori* and *P. californiensis* in *X. henshawi.* In addition, Telford (1970) recovered the nematode *Thubunaea iguanae* Telford, 1965.

A second rock-crevice specialist, the Bolsón night lizard, *Xantusia bolsonae* Webb, 1970, is known from only 2 localities in the southern Chihuahuan desert of Durango, Mexico (Flores-Villela et al., 1990). To our knowledge, there are no reports of helminths from this species.

The island night lizard, *Xantusia riversiana* Cope, 1883, is endemic to 3 of the California Channel Islands, where it occurs beneath rocks (Fellers and Drost, 1991). Amrein (1951) found *O. scelopori, Parapharyngodon bicaudatus* (Read and Amrein, 1952) Adamson and Nasher, 1984, and *Alaeuris waltoni* (Read and Amrein, 1952) in *X. riversiana.* Lucker (1951) reported *Parapharyngodon pseudothaparius* (Lucker, 1951) Adamson and Nasher, 1984, and *P. xantusi* (Lucker, 1951) Adamson and Nasher, 1984. Telford (1970) found the cestode *Mesocestoides* sp. in addition to *O. scelopori* and 4 nematodes: *Alaeuris clementensis* (Telford, 1965) Baker, 1987, *Alaeuris riversianae* (Telford, 1965) Baker, 1987, *Parapharyngodon pseudothaparius,* and *P. xantusi.* Goldberg (1985) described the histopathology of infection by *Mesocestoides* sp.

In this paper we report the results of examination of gastrointestinal tracts from a total of 347 individuals of the 4 species of *Xantusia,* in-

Table 1. Helminth prevalences in 4 species of *Xantusia*, night lizards.

	Mainland species			Insular species
	X. bolsonae	*X. henshawi*	*X. vigilis*	*X. riversiana*
Cestoda				
Oochoristica bezyi	—	35% (14/40)	16% (44/278)	—
Oochoristica islandensis	—	—	—	52% (11/21)
Nematoda				
Alaeuris clementensis	—	—	—	71% (15/21)
Alaeuris riversianae	—	—	—	81% (17/21)
Parapharyngodon californiensis	50% (4/8)	28% (11/40)	1% (2/278)	—
Parapharyngodon pseudothaparius	—	—	—	100% (21/21)
Parapharyngodon xantusi	—	—	—	90% (19/21)
Thubunaea iguanae	—	—	—	14% (3/21)
Unidentified oxyurid	—	—	—	5% (1/21)

cluding the previously unsurveyed *X. bolsonae*, summarize the taxonomy, occurrence, and prevalence of the helminth parasites, and discuss these from ecological and evolutionary perspectives.

Materials and Methods

Two-hundred seventy-eight *Xantusia vigilis* (40.2 mm snout–vent length [SVL] ± 6.8 SD) were collected in August 1972 by the third author (R.L.B.). Two-hundred eight were from 5.9 km (by Highway N6) south of Pearblossom, Los Angeles County, California (34°27′N, 117°54′W, elevation 1,219 m) (Los Angeles County Museum of Natural History [LACM] 138685–138687, 139140, 139143, 139145–139146, 139148–139149, 139152, 139154–139175, 139177–139352). Seventy were from Hesperia, San Bernadino County, California (34°26′N, 117°17′W, elevation 975 m) (LACM 139353–139385, 139387–139390, 139392–139395, 139397–139425). Forty *X. henshawi* (55.5 mm SVL ± 5.4 SD) were examined. They were collected in the late 1960's at Cabazon (33°55′N, 116°47′W, elevation 1,790 m) Riverside County, California (LACM 100713–100716, 100718–100719, 100727–100730, 100732, 100735–100737, 100739–100741, 100743, 100745–100758, 100760–100763, 100765, 100767–100769). Eight *X. bolsonae* (46.5 mm SVL ± 7.1 SD) were examined. They were collected in the early 1970's at 16 km NNW of Pedriceña (25°15′N, 103°42′W, elevation 1,340 m) Durango, Mexico (LACM 72324–72325, 76156–76157, 76159, 106805–106806, 116260). Twenty-one *X. riversiana* (69.6 mm SVL ± 15.1 SD) collected by the senior author (S.R.G.) in June 1970 on the northwest end of San Clemente Island (33°2′N, 118°36′W, elevation 30 m) were examined (LACM 139106–139126). All specimens had been preserved in 10% formalin. None of the preceding samples were from sympatric populations.

The esophagus, stomach, small intestine, and large intestine were examined separately under a dissecting microscope. Each cestode was identified utilizing a glycerol wet-mount procedure. Selected specimens were stained with Delafield's hematoxylin and mounted in Canada balsam for study as whole mounts. Nematodes were identified utilizing a glycerol wet-mount procedure.

Representative helminths were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Oochoristica bezyi* (81873–81874), *Oochoristica islandensis* (82224–82225), *Alaeuris clementensis* (82174), *A. riversianae* (82175), *Parapharyngodon californiensis* (82172–82173, 82179), *P. pseudothaparius* (82176), *P. xantusi* (82177), *Thubunaea iguanae* (82178), unidentified oxyurid (82211).

Results

Prevalence of infection for each species of night lizard is presented in Table 1. *Xantusia vigilis* harbored 1 species of cestode, *Oochoristica bezyi* Bursey and Goldberg, 1992, and 1 species of nematode, *Parapharyngodon californiensis*, A total of 134 *O. bezyi* were removed from the small intestines of 44 lizards (prevalence 16%). Seven *P. californiensis* were recovered from the large intestines of 2 lizards (prevalence 1%). By locality, 35 of the 208 lizards from Pearblossom (prevalence 17%) and 9 of the 70 lizards from Hesperia (prevalence 13%) were infected; prevalences were not significantly different ($\chi^2 = 0.45$, 1 df, $P > 0.05$). For sex, 13 of 84 males (prevalence 15%) and 31 of 194 females (prevalence 16%) were infected ($\chi^2 = 0.01$, 1 df, samples not significantly different, $P > 0.05$). The mean intensity of *O. bezyi* for the sample was 3.0 (range 1–11); by subsample, Pearblossom, 3.2 (range 1–11), Hesperia, 2.6 (range 1–6). When the intensities of infection by subsample were analyzed statistically, significant differences were found (ANOVA, $F = 5.86$, 1 and 42 df, $P < 0.05$). One female lizard from Pearblossom ($N = 208$) contained 1 *P. californiensis* (prevalence <1%) and 1 female lizard from Hesperia ($N = 70$) contained 6 *P. californiensis* (prevalence 1%).

Xantusia henshawi harbored 1 cestode, *Oochoristica bezyi,* in the small intestines and 1 nematode, *Parapharyngodon californiensis* in the large intestines. Prevalence of *O. bezyi* was 35% (14/40) and mean intensity was 2.0 (range 1–6). There was no significant difference for prevalence of infection ($\chi^2 = 0.43$, 1 df, $P > 0.05$) nor mean intensity of infection (Kruskal-Wallis statistic = 0.41, 1 df, $P > 0.05$) between male and female lizards. Prevalence of *P. californiensis* was 28% (11/40) and mean intensity was 1.9 (range 1–7). There was no significant difference for prevalence ($\chi^2 = 0.12$, 1 df, $P > 0.05$) for mean intensity (Kruskal-Wallis statistic = 0.07, 1 df, $P > 0.05$) between male and female lizards.

Xantusia bolsonae harbored only the nematode, *Parapharyngodon californiensis,* in the large intestines. Prevalence was 50% (4/8) and mean intensity was 2.5 (range 1–5). Due to the small sample size, no statistical analyses were attempted.

Xantusia riversiana harbored 1 species of cestode, *Oochoristica islandensis* Bursey and Goldberg, 1992, in the small intestines and 6 species of nematodes: *Alaeuris clementensis, A. riversianae, Parapharyngodon pseudothaparius, P. xantusi,* and an unidentified oxyurid nematode in the large intestines and *Thubunaea iguanae* in the stomachs. Prevalence, mean intensity, and range for each species of helminth are as follows: *Oochoristica islandensis,* 52%, 1.3, 1–44; *A. clementensis,* 71%, 199.7, 6–85; *A. riversianae,* 81%, 218.8, 2–1,027; *P. pseudothaparius,* 100%, 82.5, 5–198; *P. xantusi,* 90%, 38.5, 2–145; *Thubunaea iguanae,* 14%, 1.3, 1–2; and unidentified oxyurid, 5%, 3. The *X. riversiana* sample contained 11 males and 10 females and was tested statistically for differences in prevalence of infection by *Oochoristica islandensis, A. clementensis, A. riversianae,* and *P. xantusi* between male and female lizards. All 21 lizards were infected by *P. pseudothaparius. Thubunaea iguanae* was recovered from 1 male and 2 female lizards; the unidentified oxyurid was recovered from 1 female lizard. There was no significant difference (1 df, $P > 0.05$ each test) in prevalence between male and female lizards ($\chi^2 = 0.44$ for *O. islandensis,* 3.22 for *A. clementensis,* 0.01 for *A. riversianae,* and 0.0 for *P. xantusi*). Male and female lizard subsamples were also tested statistically for differences in mean intensity of infection by *O. islandensis, A. clementensis, A. riversianae, P. pseudothaparius,* and *P. xantusi.* There was no significant difference (1 df, $P > 0.05$ each test) in mean intensity between male and female lizards (Kruskal-Wallis statistic, 0.14 for *O. islandensis,* 1.38 for *A. clementensis,* 0.83 for *A. riversianae,* 0.83 for *P. pseudothaparius,* and 0.80 for *P. xantusi*). The 3 lizards infected by *T. iguanae* had intensities of 1, 1, and 2. The unidentified oxyurid (3 nematodes) was recovered from a single lizard.

Discussion

With the exception of *Thubunaea iguanae,* the helminths recovered in this study are apparently restricted to xantusiid lizards. *Thubunaea iguanae* has been recovered from New World gekkonid, phrynosomatid, crotaphytid, teiid, and xantusiid lizards. Telford (1970) speculated that the infection period for *T. iguanae* was concentrated in 2 parts of the year: December–January and May–June. The periods September–December and March–April were passed as eggs outside of the definitive hosts or as developing larvae in the arthropod intermediate hosts. Our collecting period for *X. riversiana* was June, thus within Telford's (1970) stated infective period. The unidentified oxyurid recovered from *X. riversiana,* we believe, is not a typical lizard parasite and most likely an incidental infection.

Both Amrein (1951) and Telford (1964) reported *Oochoristica scelopori* from *Xantusia henshawi, X. vigilis,* and *X. riversiana,* although the measurements of the cestodes were strikingly different. In Amrein's (1951) study, the average length of 25 mature worms from *X. vigilis* and *X. henshawi* was 16 mm, whereas those from *X. riversiana* were 33–37 mm. Telford (1964) reported that his cestode specimens from xantusiid lizards were less than 45 mm. Our measurements for these cestodes from the same host species are similar to those of the preceding authors and are much less than those given in the original description of *O. scelopori* by Voge and Fox (1950). The smaller *Oochoristica* found in *X. vigilis* and *X. henshawi* was described as *O. bezyi* by Bursey and Goldberg (1992b), whereas the larger one in *X. riversiana* was described by Bursey and Goldberg (1992c) as *O. islandensis.* Thus, we believe that all 3 species of *Xantusia* should be removed from the host list for *O. scelopori,* leaving only phrynosomatid and crotaphytid lizards as hosts for this cestode (see Bursey and Goldberg, 1992b).

All but 1 helminth species appear to be unique to lizards of the genus *Xantusia,* and none is shared with *Cricosaura typica,* the only other xantusiid that has been examined (Barus and Coy-

Otero, 1974; Baker, 1987). Two genera, *Parapharyngodon* and *Oochoristica,* are particularly interesting in terms of their occurrences within *Xantusia. Parapharyngodon pseudothaparius* and *P. xantusi* have been recovered only from the insular *X. riversiana,* whereas *P. californiensis* has been found exclusively in the 3 mainland species of *Xantusia* (*X. bolsonae, X. henshawi,* and *X. vigilis*). *Oochoristica islandensis* is known only from *X. riversiana,* whereas *O. bezyi* has been found exclusively in *X. henshawi* and *X. vigilis.* As a phylogeny is not currently available for these helminths, it is not possible at this point to evaluate whether the sharing of unique helminths among the mainland species of *Xantusia* results from co-speciation or from independent acquisition and co-accommodation (Brooks, 1979).

The helminth data are also interesting from a biogeographical and ecological viewpoint. The insular endemic *X. riversiana* is found to have a unique and relatively diverse oxyurid fauna that is not shared with any of the mainland species. Bezy et al. (1980) estimated that *Xantusia riversiana* may have been isolated on 1 or more of the Channel Islands for as much as 5 million years. The high level of helminth endemism in *X. riversiana* is an additional source of evidence for a long period of insular isolation. Insular endemism is also characteristic of these oxyurid genera. Of the 33 species of *Parapharyngodon* (see Baker, 1987), 10 (30%) are found only on islands. Similarly, 12 (36%) of the 33 species of *Alaeuris* are known exclusively from islands (see Baker, 1987).

Interestingly, such helminth endemism is not characteristic of *Uta stansburiana,* the only other species of lizard with which *Xantusia riversiana* co-exists on San Clemente Island. Telford (1970) examined 51 *U. stansburiana* from San Clemente Island and found only *Spauligodon giganticus.* This nematode occurs in 6 mainland lizard species (Bursey and Goldberg, 1992a) but is not currently known from mainland *U. stansburiana. Xantusia riversiana* and *U. stansburiana* do not have any helminth species in common, although these 2 lizards are sympatric on San Clemente Island and may even be found under the same rock. The founding of an island population of *U. stansburiana* may be relatively recent, whereas the helminths of *X. riversiana* may be of longer standing.

Brattstrom (1952) examined stomach contents of species of *Xantusia* and found that *X. riversiana* has the most varied diet, which includes insects, spiders, and substantial amounts of vegetation. Fellers and Drost (1991) reported *X. riversiana* to have a diverse diet and to consume an unusually large proportion of plant material. Our measurements of stomach contents at necropsy are also in agreement (14.5% plant and 85.5% animal matter by dry weight). *Xantusia henshawi* was found to eat primarily spiders and other arthropods, whereas *X. vigilis* is mainly insectivorous (Brattstrom, 1952). We found that *Xantusia bolsonae* is also insectivorous. That there is an association between the herbivory of *X. riversiana* and a more diverse helminth fauna is strongly suggested by the high percentage of primarily herbivorous reptiles that are hosts for species of *Alaeuris* (87%, 20/23) (see Baker, 1987).

We also believe that certain aspects of the ecology of *X. riversiana* are responsible for their greater helminth prevalences than found in mainland xantusiids, which, in comparison, have a depauperate helminth fauna. *Xantusia riversiana* have very small home ranges, about 17.2 m^2, they are slow growing, and some individuals live to be at least 12 yr old (Fellers and Drost, 1991). Due to their diet, low metabolism, and small overlapping home ranges, they reach densities (1,700–3,200/ha) greater than any other ground-dwelling lizard (Fellers and Drost, 1991). In contrast, *X. vigilis* has a much lower density of 49/ha (Zweifel and Lowe, 1966). Also, all 3 mainland species occupy spatially disjunct habitats (boulders and yuccas) in which home ranges are not continuously overlapping.

All of the nematodes so far recovered from night lizards are oxyurids with the exception of the spirurid *Thubunaea iguanae.* Oxyurid nematodes have, as far is known, direct life cycles and infection is probably due to fecal contamination (Telford, 1971). Spirurids presumably require an insect intermediate host and *Thubunaea iguanae,* in particular, is thought to have a short life cycle (Telford, 1964). The population density reached by *X. riversiana* as well as overlapping home ranges may allow fecal buildup, which could provide numerous opportunities for infection and reinfection of oxyurids. Also, the larger body size of *X. riversiana* may provide greater opportunity for helminth infection without undue damage to the host.

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Errata

Publication cost of the paper titled, A coprological survey of parasites of wild muriquis, *Brachyteles arachnoides,* and brown howling monkeys, *Alouatta fusca,* by M. D. Stuart et al., which appeared in the January issue of this journal, Volume 60(1):111–115, was supported by the Brayton H. Ransom Memorial Trust Fund. The Editor regrets the oversight.

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The Effect of Temperature, pH, Sodium Chloride, and Glucose on the Survival of Female *Thelastoma bulhoesi* (Nematoda: Oxyurata)

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ABSTRACT: Female *Thelastoma bulhoesi,* parasites of the hindgut of *Periplaneta americana,* were exposed to a variety of temperatures and solutions of variable pH and sodium chloride and glucose concentrations. *Thelastoma bulhoesi* survived more than 10 days at 27°C, in a neutral pH and 1.0% sodium chloride. Most worms could not survive 7°C for longer than 30 min. Other concentrations of hydrogen ion, sodium chloride, and glucose were less well tolerated but gave unusual bimodal results.

KEY WORDS: *Thelastoma bulhoesi, Periplaneta americana,* bionomics, Nematoda, Oxyuroidea.

Thelastoma bulhoesi is a pinworm (Oyxurata) inhabiting the large intestine of the American cockroach, *Periplaneta americana.* The nematode is common in many laboratory colonies of this host and has been used as an experimental model by many researchers (Lee, 1959, 1960; Guthrie and Tindall, 1968; McCallister and Schmidt, 1983, 1984). It is possible to maintain *Thelastoma bulhoesi* in sterile saline for many hours, making them useful as research and teaching tools. This study presents data that show the effect of temperature and concentrations of sodium chloride (NaCl), glucose, and hydrogen ion on the survival of *T. bulhoesi.* These bionomic data have not been reported previously for this species.

Materials and Methods

Cockroaches, *Periplaneta americana,* were killed in a killing jar using ethyl acetate fumes. The large intestine was removed and teased apart in a 0.75% NaCl solution at a pH of 7. This saline concentration was used when preliminary studies showed best survival of *T. bulhoesi* at this concentration. Female worms were transferred manually, using a bent number 1 insect pin attached to a wooden applicator stick, to autoclaved 65-mm watch glasses containing approximately 2 ml sterile test solutions. These watch glasses were, in turn, maintained in 100% humidity. All experiments were repeated 3 times with between 20 and 30 worms per experiment.

Temperature studies

To determine the effect of temperature on the survival of *T. bulhoesi,* female worms were exposed to temperatures of 0, 5, 15, 25, 35, and 45°C while in 0.75% NaCl at pH 7 and 100% humidity. Worms were removed and examined at ×100 with a compound microscope for motility after 1, 2, 4, 8, 16, 32, 64, 128, and 256 hr. Worms that were not moving spontaneously were agitated with a probe. If they did not respond to the probe, death was assumed. In a separate experiment, female worms were exposed to 5°C for 0.25, 0.5, 1, 2, 4, 8, 16, and 36 hr. At the end of these times, the worms were removed to 26°C incubation chambers, and the length of survival time following cold stress was determined by observing the parasite for motility, as already described, at 1, 2, 4, 8, 16, 32, 63, 128, and 256 hr.

Osmotic and pH studies

Worms were exposed to NaCl at strengths of 0, 0.03, 0.06, 0.12, 0.25, 0.5, 0.75, 1.0, 2.0, and 4.0% at 25°C. These solutions were tested at pH 7 and again at pH 5 to investigate pH effects within the range normally found within the cockroach hindgut (Guthrie and Tindall, 1968). The worms were also exposed to pH 1, 3, 5, 7, 9, and 11 in 0.75% NaCl. Hydrogen ion concentration was adjusted using 1 M HCl or 1 M NaOH. No buffer was added. Glucose was tested at the same weight per volume concentrations as the NaCl, but only at pH 7.

Results

The length of survival of female *T. bulhoesi* at various temperatures is shown in Table 1. Maximum survival occurred at 27°C, where an average of 13% of the organisms survived for more than 10 days. Temperatures of 47 and 7°C were about equally lethal with few worms surviving more than 16 hr. The length of time *Thelastoma bulhoesi* can survive after exposure to 7°C is shown in Table 2. Exposure to 7°C for 30 hr or longer was fatal, but exposure to this low temperature for as little as 30 min affected the survival of the worm, even after it was removed from the stress. Most worms stressed in this manner did not survive past 32 hr postexposure.

The survival of female worms in various concentrations of NaCl is depicted in Tables 3 and 4. Worms were first exposed to NaCl at pH 7 and another group was exposed at pH 5. Survival, as determined by numbers surviving and length of survival, was optimum in 1.0% NaCl at pH 7, where 15% of the worms survived 256 hr. At pH 5 maximum length of survival was in

Table 1. Mean % survival at different temperatures of female *Thelastoma bulhoesi* cultured in 0.75% NaCl, pH 7, 25°C.

Temperature (°C)	Hours								
	1	2	4	8	16	32	64	128	256
0	0	0	0	0	0	0	0	0	0
7	100	100	100	100	27	0	0	0	0
17	100	92	92	92	69	31	7	0	0
27	100	100	95	87	60	60	33	20	13
37	91	82	63	63	64	0	0	0	0
47	100	100	96	39	0	0	0	0	0

0.03% NaCl, where a mean of 10 worms survived for 256 hr. A greater number of worms (40) survived to 128 hr at this pH.

Table 5 shows the results when female worms were exposed to a wider range of pH at 0.75% NaCl. The nematodes survived a large range of pH concentrations. Optimum survival was at pH 7, but about half the worms could survive 64 hr in any pH between 3 and 9.

The mean percentage of survival of female *Thelastoma bulhoesi* in glucose concentrations (wt./vol.) is shown in Table 6. Maximum survival was in 2.0% glucose, and minimum survival was in 0.5% glucose. This creates an interesting bimodal distribution.

Discussion

It is not surprising that parasites of homeothermic animals are limited in the temperature range that they can tolerate. Parasites of poikilotherms might be expected to be more tolerant of temperature extremes because their host is susceptible to environmental temperatures. Much of the research on the effects of temperature on nematodes deals with free-living or plant parasitic forms. Several authors have reviewed the literature on this topic (Lee, 1965; Zuckerman et al., 1971; Nicholas, 1975; Croll, 1976).

Guthrie and Tindall (1968) reported the optimum temperature of *Periplaneta* species to be between 26 and 28°C. It is not surprising to see that the greatest parasite survival was at 27°C. The cockroach host survives temperatures down to 1°C, although with decreased activity. Because *T. bulhoesi* does not survive long at 7°C, holding the host at this temperature for 48 hr might prove to be a method of obtaining worm-free cockroaches for experimental purposes. Nematodes were also affected by temperatures of 47°C, whereas cockroaches can withstand these temperatures depending on the relative humidity. In summary, the temperature tolerance range of female *T. bulhoesi* is within that of their host, generally being more susceptible to extremes than

Table 3. Mean % survival of female *Thelastoma bulhoesi* in concentrations of Nacl incubated at pH 7, 25°C, for varying lengths of time.

NaCl (%)	Hours								
	1	2	4	8	16	32	64	128	256
0.00	100	62	0	0	0	0	0	0	0
0.03	100	88	88	88	88	75	13	0	0
0.06	100	88	77	77	77	77	33	0	0
0.12	100	100	100	89	89	89	38	0	0
0.25	100	100	83	83	83	66	50	0	0
0.50	100	100	100	100	85	66	0	0	0
0.75	100	100	95	87	60	60	35	20	13
1.00	100	100	94	94	94	88	41	29	15
2.00	1	0	0	0	0	0	0	0	0

Table 2. Mean % survival of female *Thelastoma bulhoesi* cultured in 0.75% NaCl, pH 7, 25°C, after exposure to 7°C for different lengths of time.

Exposure time (hr)	Hours								
	1	2	4	8	16	32	64	128	256
0.25	100	90	90	90	65	61	30	15	9
0.50	100	80	80	75	70	60	0	0	0
1.00	100	100	100	85	71	29	0	0	0
2.00	100	100	90	90	80	30	0	0	0
4.00	100	100	90	64	24	19	0	0	0
8.00	100	45	18	18	9	0	0	0	0
16.00	27	27	27	27	18	18	9	0	0
32.00	50	50	42	33	33	0	0	0	0
64.00	8	0	0	0	0	0	0	0	0
128.00	0	0	0	0	0	0	0	0	0

Table 4. Mean % survival of female *Thelastoma bulhoesi* in concentrations of NaCl incubated at pH 5, 25°C, for varying lengths of time.

NaCl (%)	Hours								
	1	2	4	8	16	32	64	128	256
0.00	80	60	20	18	10	0	0	0	0
0.03	100	90	90	90	80	70	40	20	10
0.06	100	80	80	70	70	70	60	40	0
0.12	100	100	83	83	66	66	0	0	0
0.25	100	100	100	100	88	75	50	0	0
0.50	100	100	100	100	100	90	64	18	0
0.75	100	100	100	92	85	62	62	0	0
1.00	100	80	80	70	50	50	30	0	0
2.00	100	73	45	45	27	18	0	0	0
4.00	0	0	0	0	0	0	0	0	0

Table 5. Mean % survival of female *Thelastoma bulhoesi* in variable pH incubated in 0.75% NaCl, 25°C, for varying lengths of time.

Exposure time (hr)	Hours								
	1	2	4	8	16	32	64	128	256
1	38	0	0	0	0	0	0	0	0
3	100	100	91	91	66	64	25	0	0
5	100	100	100	92	85	62	62	0	0
7	100	100	95	87	60	60	33	20	13
9	100	100	92	87	66	55	44	0	0
11	100	100	91	91	64	9	0	0	0

Table 6. Mean % survival of female *Thelastoma bulhoesi* in glucose concentrations maintained at pH 7, 25°C, for varying lengths of time.

Glucose	Hours								
	1	2	4	8	16	32	64	128	256
0.00	80	60	20	18	10	0	0	0	0
0.03	100	64	18	0	0	0	0	0	0
0.06	100	38	38	20	11	0	0	0	0
0.12	100	55	55	32	17	7	0	0	0
0.25	100	35	23	0	0	0	0	0	0
0.50	100	8	0	0	0	0	0	0	0
0.75	100	100	100	100	82	49	0	0	0
1.00	100	100	100	58	58	22	8	0	0
2.00	100	100	100	100	100	100	73	0	0
4.00	100	100	100	100	88	88	55	0	0
8.00	100	100	100	100	51	51	0	0	0
16.00	0	0	0	0	0	0	0	0	0

the cockroach. These ranges also correspond to data reported for other nematodes. The cockroach hindgut varies in osmotic pressure due to drying of the peritrophic membrane during molting. Thus, the nematode must be able to withstand some variation in osmotic pressure in order to parasitize the host continuously through its life cycle. Lee (1966) investigated this phenomenon for *Hammerschmidtiella diesingi,* an oxyurid nematode parasite of the hindgut of *Blatta orientalis.* He showed that the worm could survive the host molt and had some limited abilities of osmoregulation.

The host molting procedure itself takes only 10–20 min (Guthrie and Tindall, 1968), but physiological differences can be noted as early as 2 days before and after (Patton and Flint, 1959; Patton, 1962). While optimum survival of *Thelastoma bulhoesi* occurs at 1% NaCl, the worm can survive for at least 32 hr at any concentration of NaCl tested greater than 0%. It is possible that changes in osmotic pressure may inhibit transtadial transmission of cockroach pinworms. This may account for the results reported by McCallister (1988) that adults are more often, and more heavily, parasitized than nymphs.

The colon of *P. americana* has been determined to have a pH of 7.3 in females and 7.4 for males, using indicator dyes. Using glass electrodes, the pH for both sexes was 7.7 (Guthrie and Tindall, 1968). This probably fluctuates with diet, age, and molting. Because changes in pH can affect solubility of many compounds, it may also have an effect on osmotic pressure. When *T. bulhoesi* is exposed to the same concentrations of NaCl at both pH 5 and 7, the spectrum of survival shifts to lower concentrations of NaCl. Maximum survival concentration at pH 5 is 0.03% NaCl, whereas at pH 7 it is 1.0% NaCl. Optimum survival under normal osmotic pressure appears to occur in solutions of pH 7. This is in keeping with the normal environment in the cockroach hindgut.

Several authors have published data that suggest that the cuticle of nematodes is impervious to sugars (Lee, 1966; Croll and Viglierchi, 1969). This is presumably due to the large size of the molecule and its consequent inability to pass through the cuticle of the nematode. Best survival in glucose solutions in this study was at 2.0%. Death in other concentrations was most likely due to osmotic pressure as nematodes tended to eviscerate or collapse in other concentrations.

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Report on the Brayton H. Ransom Memorial Trust Fund

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Financial Report for 1992

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Receipts:		
Interest received in 1992	$	989.61
Donations		125.00
Total	$	1,114.61
Disbursements:		
Grant to the Helminthological Society of Washington for 1992	($	50.00)
Membership in the American Association for Zoological Nomenclature for 1992	($	50.00)
Page Charge Support	($	400.00)
Total	($	500.00)
On hand, December 31, 1992		$13,143.48

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Developmental Stages of a Smooth-Walled Filamentous Bacterium Associated with Equine Cyathostomes

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ABSTRACT: Communities of microorganisms colonize the anal and vulvar pores on the posterior extremities of female cyathostomid nematodes recovered from Burchell's zebras, *Equus burchelli antiquorum*. Cyathostomes with attached filamentous microorganisms were processed for scanning and transmission electron microscopy using standard methods. The adherence and in situ development of a filamentous bacterium, designated as a smooth-walled multicellular organism, or trichome-forming bacterium, is described. A vegetative cell complex that adheres to the cyathostome cuticle gives rise to unbranched aerial filaments. These filaments develop by means of multiple transverse and longitudinal septation to form a multicellular filament enclosed in a common cell-wall profile. New cellular units (microgonidia) may be released from mature filaments into the ingesta of the hindgut, where they attach to a cyathostome cuticle and develop new daughter filaments. This is the first known report on the development and adherence of such a trichome-forming filamentous bacterium. The significance of the structure, development, and association of this filamentous bacterium and nematode are discussed. Its exact classification is still unknown.

KEY WORDS: Burchell's zebra, equine cyathostomes, filamentous bacterium, trichome-forming bacteria, microorganisms, SEM, TEM, morphology, developmental cycle, microbial communities.

Free-ranging equids (i.e., zebras) are host to large numbers and an extensive diversity of nematodes (Krecek et al., 1987a, b). Microbial communities have been observed colonizing the anal and vulvar regions of cyathostomid nematode females without apparent pathological consequences (Krecek et al., 1987b; Mackie et al., 1989; Els and Krecek, 1990). Previous studies have described the ultrastructure and proposed developmental stages of some of these microorganisms as well as their relationship to their cyathostome hosts. Attempts have also been made to isolate and characterize some of these bacteria (Krecek et al., 1987b; Mackie et al., 1989; Els and Krecek, 1990; Els et al., 1991).

Studies on the structure and developmental cycle of a segmented filamentous bacterium (Els and Krecek, 1990), a helical bacterial filament (Els et al., 1991), and other components of these microbial communities (Mackie et al., 1989; Krecek et al., 1992) have contributed to the knowledge needed to understand these microorganism–nematode host relationships. Attempts to culture these organisms have as yet been unsuccessful. A smooth-walled multicellular filamentous bacterium constitutes the third known constituent of filamentous structures associated with cyathostomes (Els and Krecek, 1990). Although some features resemble those of other filamentous or trichomous bacteria observed in a number of animals (Chase and Erlandsen, 1976; Trentini, 1981; Savage, 1983; Hirsch, 1989; Strohl, 1989), the adherence process and developmental stages appear to be complex and unique. This report proposes some developmental phases and an adherence process of this filamentous bacterium to the cuticle of the cyathostome.

Materials and Methods

Electron microscopy

Cyathostomes were collected and processed for electron microscopy according to methods described by Els and Krecek (1990).

Microbiology

To cultivate the microorganisms and reduce contamination from hindgut flora, the nematodes were rinsed with phosphate-buffered solution and cultured in several enriched media. The media included blood tryptose agar (BTA) (tryptose agar with 10% bovine blood), BTA with 50 mg/liter nalidixic, and worm-agar, which were intended for anaerobic incubation (Krecek et al., 1992). Because the smooth-walled multicellular filament resembled some features characteristic of trichomes in the genus *Caryophanon*, attempts were also made to isolate this filamentous organism or any *Caryophanon* spp. from zebra feces. The procedure of enrichment of *Caryophanon* described by Trentini (1981) was followed.

Terminology

To avoid confusion, we used the terminology for filamentous bacteria referred to by Hirsch (1989), Sayre

and Starr (1989), Strohl (1989), and Trentini (1986). In addition, some mycological terminology was used to describe this filamentous microorganism. Such terms include macrogonidia and microgonidia to indicate the disclike and spherical propagation cells observed, respectively (Hirsch, 1989), as well as thallus (Krecek et al., 1987b).

Results

Electron microscopy

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) revealed a diverse group of microorganisms associated with the reproductive and digestive tract openings of female cyathostomes of zebras. Among these microorganisms, SEM consistently revealed a smooth-walled multicellular filamentous bacterium (Fig. 1) that showed internal septate structures apparently in various stages of development when viewed by TEM (Figs. 2–14).

Filament morphology

Filamentous bacteria varied from 1.6 to 2.3 μm (average 1.8 μm) in width and measured up to 500 μm in length. The wall, about 100 nm thick, was longitudinally continuous in appearance in thin sections. The cell-wall complex included several layers shown in transverse and longitudinal sections (Figs. 2, 3). Both light and electron microscopy revealed a type of Gram-positive structure. The outer part of the filament wall included both a moderately electron-dense fibrillar layer (F = 55 nm) and a denser inner layer (D = 20 nm). This was contiguous to a faint, moderately electron-dense layer (N = 30 nm), often resolved as a faint nonmembranous structure. An electron-lucent space (L = 15 nm) separated the outer layers from an inner layer (CM), which demarcated the discs or inner cellular compartments (Figs. 2, 3). The CM was resolved as a typical unit cell membrane or cytoplasmic membrane (CM = 8–10 nm) forming the septal walls of the enclosed disc material. When ruthenium red (RR) stain was added to the fixative, the outer layers appeared more electron-dense and their fibrillar nature was clearly observed, especially when observed in cross-section (Fig. 2). RR thus revealed a polysaccharide component present in the fibrils (Handley et al., 1988).

Developmental cycle of the filamentous bacterium

A developmental sequence of the filamentous bacterium is proposed in Figs. 4–14. Filaments appeared to develop from a vegetative growth complex that is either cauliflowerlike or random grapelike aggregates of cellular units. Each unit consisted of an outer electron-dense fibrillar structure that surrounded an electron-lucent space with a moderately dense core (Figs. 4, 5). The fibrillar layer of the units adhered superficially to the cuticle of the cyathostome without penetration. The complex stained more electron-dense with RR indicating a polysaccharide content. Unbranched filamentous structures were observed to originate from the growth complex. These filaments initially developed as single barrel-shaped cells (thalluslike) anchored to the cuticle by means of 1 or more rooting structures in the growth complex (Figs. 4, 5). From the thallus, elongation proceeded at the free end by the formation of internal undifferentiated cellular units separated by intracellular septa (Fig. 5).

At the junction of the thallus and the adjoining cell, a ring of closely arranged spherical or club-shaped structures were noted, each consisting of a clear halo around a moderately electron-dense center. The ring may be involved in the addition of cells to the developing filament (at least in the initial stage). The appearance and number of components in the ring varied according to the plane of section (Figs. 4, 5 and inset in Fig. 5). During initial cell formation, each completed cell unit within the actively growing filament also exhibited growth of secondary septa with the CM invaginating like the closure of an iris diaphragm (Fig. 6). This resulted in the formation of new discs (macrogonidia) and apparent elongation of the filament (Figs. 7, 8).

A set of septate walls that formed perpendicularly to the existing septa was the subsequent stage of development. These walls separated the macrogonidia (Fig. 9) and resulted in numerous spherical-shaped units, microgonidia (Figs. 10, 11) bound by a common cell wall (hence the designation multicellular filament). In the final stage of development, mature individual microgonidia (daughter cells) may be released from the mother filament into the hindgut environment (Figs. 12, 13) to be dispersed to new sites of attachment (Fig. 14).

Discs (Fig. 3, macrogonidia) showed homogeneous cytoplasm and nuclear areas with the appearance of prokaryotic cells. Mesosomelike structures occasionally observed were considered fixation artifacts (Hobot et al., 1985). No intrasegmental bodies (holdfast segments) simi-

lar in morphology to the initial thallus were observed in any segment or disc.

The actual membrane structures involved in the formation of the discs are shown in Figure 15. In septum formation, the external fibrillar layer remained unindented, involving only the cell unit membranes (CM) adjacent to its internal side. The CM replication at the site of septum growth gave rise to an internal budding profile of newly formed CM (S in Fig. 15). Invaginations of these membranes preceded the annular ingrowth of the future cell septa. Shortly before the process of septum formation was completed, the ingrowing CM skirting the septa joined to form 2 club-shaped structures (Fig. 15). Fusion of these shapes (with the final splitting of the replicating CM and subsequent filling with septum material) resulted in a complete transverse septum to form 2 new cells.

Microbiology

None of these culture techniques was successful with regard to the isolation of any recognizable filamentous bacteria or *Caryophanon* spp.

Discussion

Previous studies have shown that the cuticle of cyathostomes recovered from the zebra hindgut supports a large and diverse population of bacterial forms including 3 filamentous types (Krecek et al., 1987a, b; Mackie et al., 1989; Els and Krecek, 1990). Using TEM, morphological evidence of a filamentous smooth-walled multicellular bacterium or trichome-forming bacterium is presented with a detailed developmental sequence.

Ultrastructural evidence exists for developmental cycles of segmented, filamentous bacteria attached to intestinal epithelial cells in various hosts (Davis and Savage, 1974; Chase and Erlandsen, 1976; Breznak and Pankratz, 1977; Bracke et al., 1979; Savage, 1983). These bacteria differ from those in the present study by having an association with epithelial linings in various mammalian, avian, and insect intestinal tracts and the ability to form endospores or specialized holdfast cells. In contrast, in the present study the bacteria are associated with an invertebrate host inside a mammalian hindgut and a thallus (Krecek et al., 1987b) appears to be the initial generating reproductive cell.

The filamentous bacterium of the present study differs from other known members of the microbial community in the zebra hindgut in its bacterial wall structure, mode of septation, and means of adherence to the nematode cuticle (Mackie et al., 1989; Els and Krecek, 1990; Els et al., 1991). Although extracellular fibrous struc-

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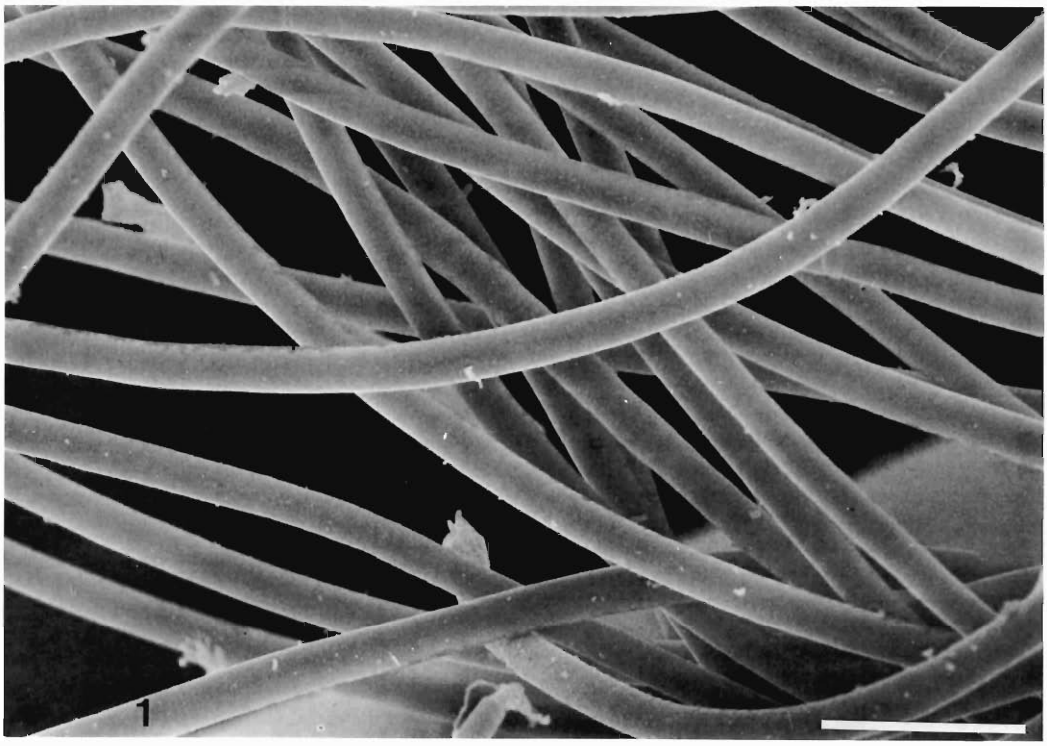
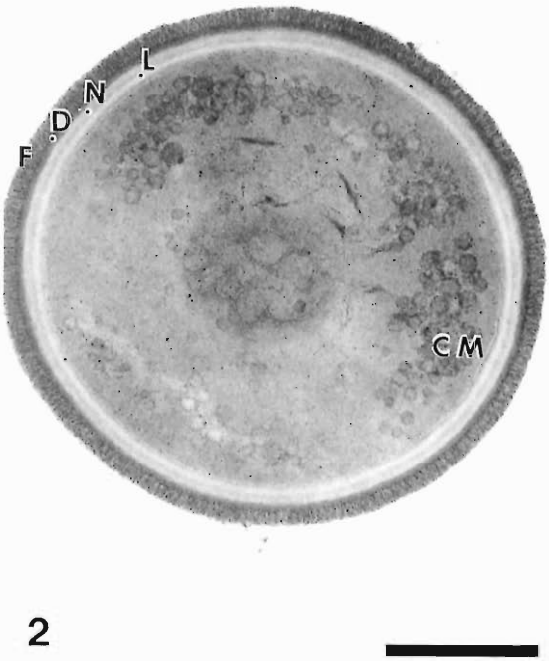
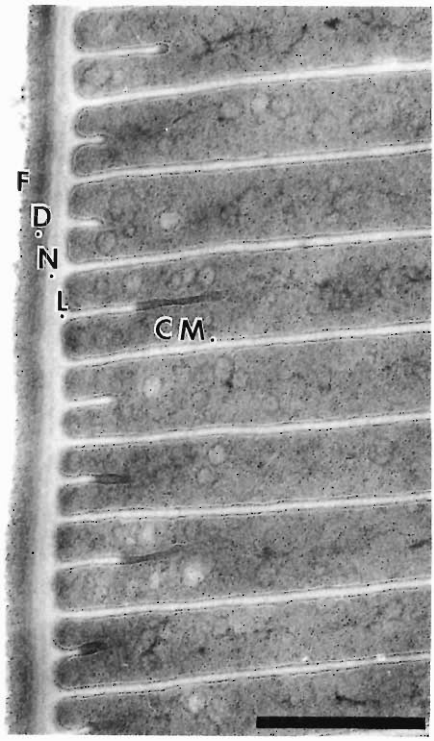
Figures 1–3. Electron microscope views of bacteria associated with cyathostomid nematodes. 1. SEM of the aerial trichome-forming bacteria close to the cyathostome cuticle. The continuous smooth-walled nature of the filaments is evident. Bar = 10 μm. 2. TEM showing the cell-wall structure of a filament in cross-section. The various layers are indicated as follows: F = outer fibrillar layer, D = dense layer, N = nonmembranous layer, L = electron-lucent space (see text), CM = cytoplasmic membrane. Bar = 0.5 μm. 3. TEM of longitudinal section of a filament showing the cell-wall layers as well as the nature of septation. The CM associated with the formation of discs is clearly visible. The outer layers (F–L) of the cell wall are free from indentations. Bar = 0.5 μm.

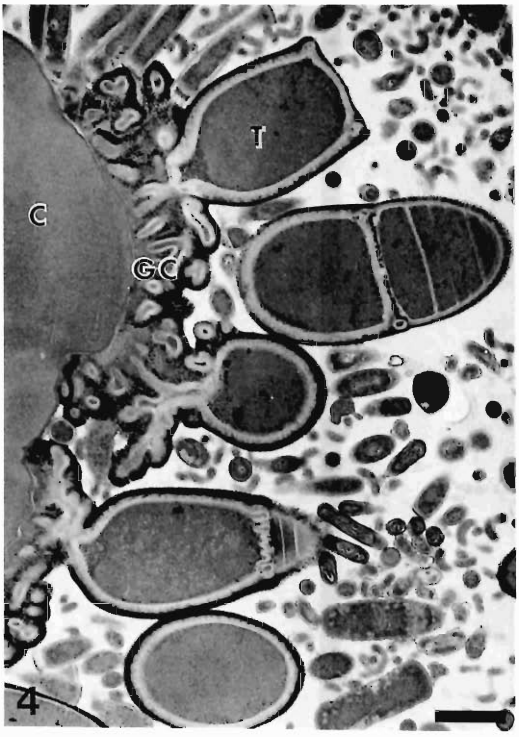
Figures 4–7. TEM of filaments of bacteria associated with cyathostomid nematodes. 4. Multicellular filaments portraying their initial growth phases. Note the growth complex (GC) and its fibrillar nature, the initial barrel-shaped thallus (T) anchored in the GC via a number of roots, or fused branches of the GC giving rise to the thallus. C = cuticle of cyathostome. Bar = 1 μm. 5. Aerial filament showing further internal growth of the thallus with the addition of further cell units (discs). Bar = 1 μm. Inset: Club-shaped structures observed between thallus and first cell. Bar = 1 μm. 6. Filament showing further development in growth with secondary septa at different stages of development growing simultaneously. Bar = 1 μm. 7. A long septate filament showing more stages of secondary annular ingrowth of the CM in various cell units. Bar = 1 μm.

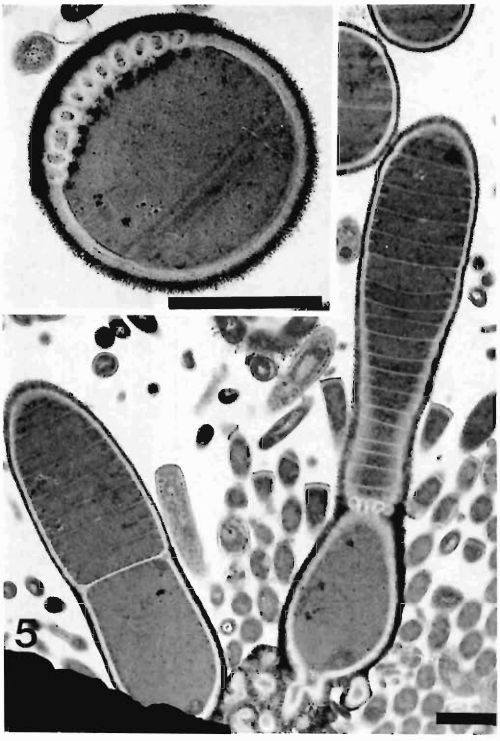
Figures 8–13. TEM of filaments of bacteria associated with cyathostomid nematodes. 8. Longitudinal section showing extensive internal CM ingrowth resulting in numerous thin discs (macrogonidia). Bar = 1 μm. 9. Longitudinal section with more advanced division: septation occurs perpendicular to previous transverse annular ingrowth. Bar = 1 μm. 10. Result of previous 2 directions of septation: spherical or ovoid-shaped elements (microgonidia) are formed giving rise to a multicellular filament. Bar = 1 μm. 11. Cross-section equivalent to the stage in Figure 10. Bar = 1 μm. 12. The last developmental phase: it suggests eventual release of the microgonidia. Bar = 1 μm. 13. Cross-section equivalent to the stage in Figure 12. Note the appearance of fibrillar components on some cells. Bar = 1 μm.

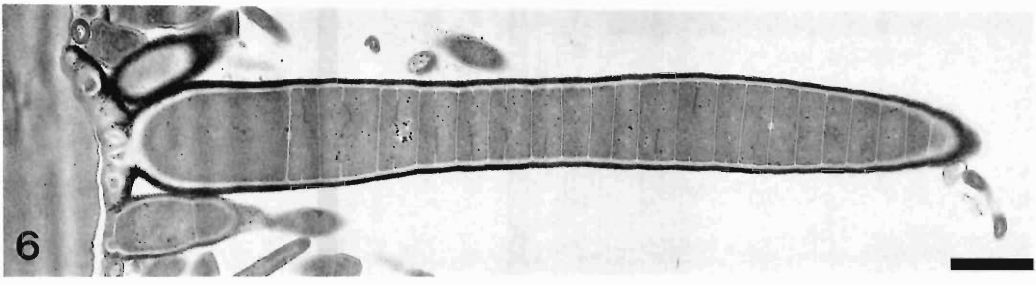


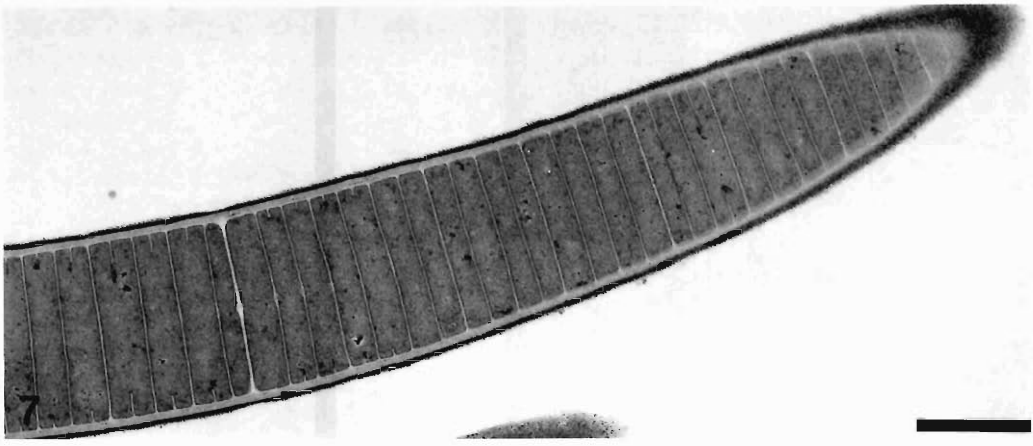


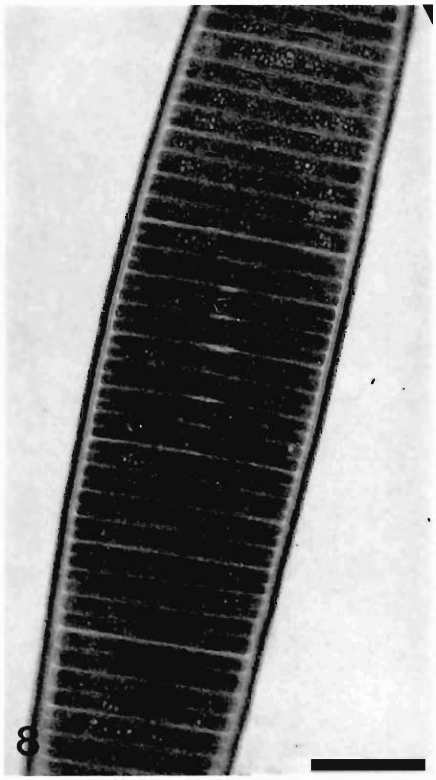


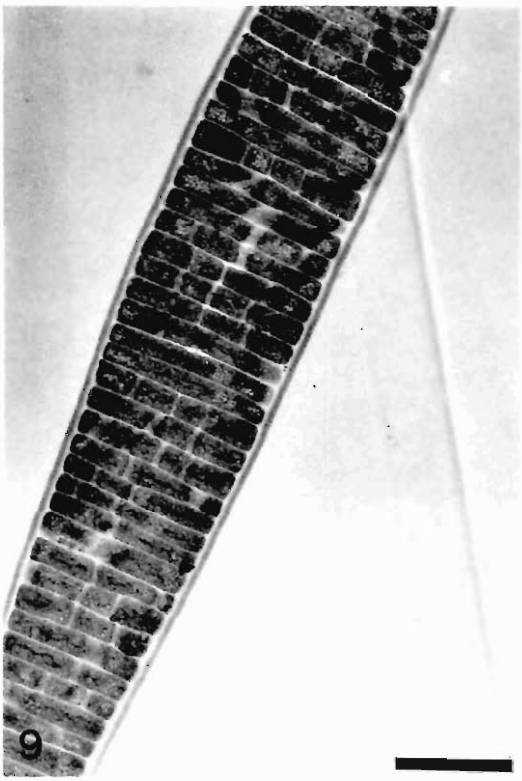


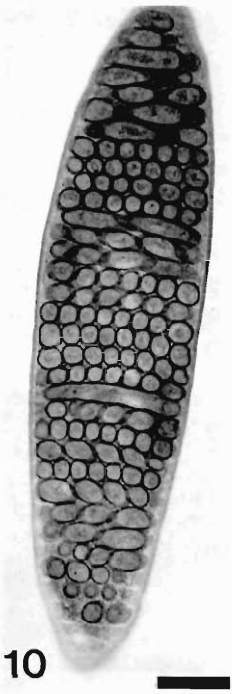


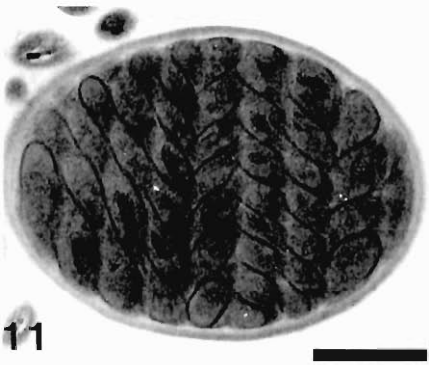


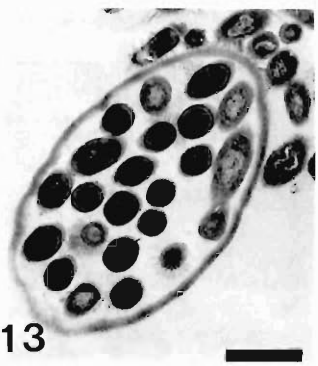


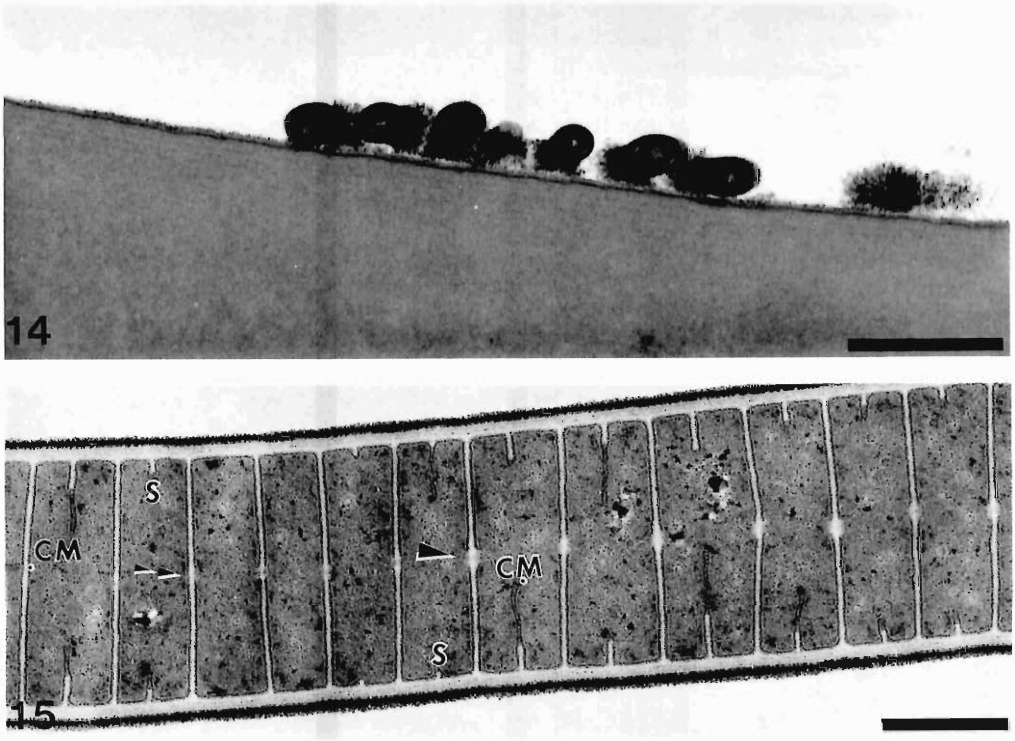












Figures 14, 15. TEM of bacteria of cyathostomes. 14. Microgonidia apparently attaching to the cuticle. Bar = 1 μm. 15. The process of septation in more detail. S = site of septa formation. Note the inward growing CM skirting the septa, the joining of opposing CM in club-shaped (bulbous) structures (arrow), and their fusion (double arrows). Bar = 1 μm.

tures similar to those in many of the other microorganisms are also used for adherence (Els and Krecek, 1990), a growth complex such as that noted here by aid in propagation in this habitat and colonization of the cuticular surface of the cyathostome.

Although RR staining indicates some polysaccharide content, the exact composition and function of the growth complex has not been established. Cauliflowerlike vegetative colonies that form endospores have been described for the genus *Pasteuria* by Sayre and Starr (1989). The vegetative complex of the present study differs morphologically from that of *Pasteuria* and does not appear to share any ultrastructural characteristics.

The sequence of events that follows the release of daughter cells (microgonidia) from the mature filaments, their transformation, and the structure and function of the growth complex is incomplete. The cell-wall structure of the mature filament (Figs. 2, 3) differs from that of the microgonidia (Figs. 10, 12, 13), suggesting that there may be a process of transformation of the wall of the latter during the stages of attachment and development (Figs. 4, 5, 14). It is possible, as with cyanobacteria, that synthesis of the fibrous wall layer is repressed during septation. Also, it is possible that at the time of their release microgonidia may possess walls containing only peptidoglycan and inner membrane layers (Rippka et al., 1979) or that synthesis of the fibrils in the outer wall may accompany release of the microgonidia (Fig. 13). Microgonidia do not appear to resemble spores (Chase and Erlandsen, 1976) nor do the filaments that they form resemble other sporulating filamentous organisms (Bracke et al., 1979; Savage, 1983).

Although morphological characteristics alone are not sufficient to classify the bacterium described here, the developmental stages resemble those demonstrated in other studies of trichomes. These stages correspond to the properties given by Trentini (1981, 1986) that a completed cell unit within the actively growing trichome shows the growth of 1 or more devel-

oping septa and results in new cells and an extension of the trichome length. These cells are usually closely appressed and wider than they are long. Previous reports (Krecek et al., 1987b; Mackie et al., 1989) suggested that filaments resembling those of the present study may belong to the family *Arthromitus* in the order Caryophanales (Peshkoff and Marek, 1973; Trentini, 1981, 1986). Caryophanales is a group of typical filamentous segmented sporeformers (e.g., *Arthromitus* Leidy). Members belonging to *Arthromitus* are trichomes that are observed attached to the intestinal walls of insects and tadpoles (Davis and Savage, 1974). Several trichome-forming bacteria occurring in the alimentary tract of animals have been reported to form endospores but have not been obtained in pure culture (Savage, 1983). The bacterium described here did not exhibit evidence of such endospores.

The microorganisms discussed in this paper may comprise a small part only of the community existing in the zebra's hindgut. In one population, almost 70% of the female cyathostomes exhibited filamentous bacteria attached to their posterior extremities (Krecek et al., 1994). The bacterium in the present study has developed an adaptation for its survival in the production of the numerous daughter cells instead of single endospores. The dispersal of these cells may be the means by which these organisms ensure survival by colonizing a highly specific niche restricted to a precise region on selected cyathostomes.

Further study of this microbial community–cyathostome relationship may aid in our understanding of these bacteria and their function in the zebra hindgut environment. Light microscopical studies suggest that colonization of these filamentous microorganisms is in the vulvar region of female cyathostomes and not in the anal, oral, and excretory orifices (Krecek et al., 1994). The role that the presence of the microorganisms at the vulva bears (i.e., hinders the reproductive activities of the female) has yet to be determined.

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Ultrastructure of the Infective-Stage Larva of *Toxocara canis* (Nematoda: Ascaridoidea)

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ABSTRACT: The ultrastructural morphology of the infective-stage larva of *Toxocara canis* is described. Seven weeks after eggs were placed in culture in 0.5% formalin, larvae were hatched mechanically and collected 2 days later. Larvae were fixed 3 days at 4°C in aldehyde fixative, postfixed in osmium tetroxide, embedded, sectioned, and stained. The cuticle has several layers of fibers, and lateral alae extend the length of the body. The lateral cord hypodermis has multiple nuclei, mitochondria, and lipid granules. Muscle cells are meromyarian and platymyarian. A neuronal bundle that innervates the cephalic sensillae runs anteriad from the nerve ring on each side of the worm. The ventral nerve cord has numerous nuclei, mitochondria, and neural fibers. The excretory cell has a single large nucleus, extensive rough endoplasmic reticulum (RER), Golgi bodies, mitochondria, and vesicles presumably containing protein; the 2 excretory columns also have vesicles surrounding a collecting duct. The dorsal sector of the esophagus is much larger than the 2 subventral sectors and contains RER, Golgi bodies, and vesicles with variable density suggesting a maturation of their content. The intestine has no lumen and is composed of a single row of cells containing lipid granules. The rectum is lined with cuticle.

KEY WORDS: *Toxocara canis,* larval morphology, ultrastructure, nematode.

Larval toxocariasis in humans is caused in most instances by the larvae of *Toxocara canis* (Beaver et al., 1984). In humans and other paratenic hosts, the larvae that persist in the tissues are morphologically the same as the larvae that hatch from infective eggs (Nichols, 1956a; Beaver et al., 1984). The morphology of these larvae has been described in detail at the level of the light microscope (Nichols, 1956a). Although other workers have examined various aspects of the ultrastructural morphology of these larvae (Rockey et al., 1983; Ghafoor et al., 1984; Vegni-Talluri et al., 1986; Vegni-Talluri and Dallai, 1990), there has been no overview of the fine structure of these larval nematodes presented. Thus, the purpose of this work was to provide a generalized description of the fine structure of these larval nematodes.

Materials and Methods

Infective-stage larvae from eggs that had been in culture for 2 mo were collected for in vitro cultures using the methods of Bowman et al. (1987). One day after the larvae were placed in culture, they were transferred to 1-ml centrifuge tubes. The tubes containing the larvae were centrifuged at 7,000 *g* for 1 min, and the larvae were resuspended in modified Karnovsky's fixative (1.25% glutaraldehyde and 20% paraformaldehyde in 0.1 M phosphate buffer, pH 7.0) and fixed at 4°C for 3 days. The aldehyde fixative was removed using an overnight wash of 0.1 M phosphate at a pH of 7.0; this and all the other solution changes made prior to the addition of agar as described below were done by centrifuging the larvae at 7,000 *g* for 1 min and suspension in new solution. The larvae were postfixed for 1 hr in 1% osmium tetroxide and then washed twice with 2 10-min changes of distilled water. After removing the water from the second wash, a small portion of 2% agar, about 150 μl, at 55–60°C was added to the pellet of larvae. After the agar hardened, it was removed from the tubes and cut into small blocks. Blocks of larvae were dehydrated using a graded ethanol series and then infiltrated with the plastic embedding mixture of Mollenhauer (1964). Infiltration was performed using mixtures of plastic resin in propylene oxide: the blocks were in the 25% resin mix for 1 hr, the 50% resin mix for 2 hr, the 75% resin mix for 3 hr, and the pure epoxy mix overnight. The infiltrated blocks of larvae were embedded in fresh plastic resin.

Sections were cut using a Reichert Ultracut E microtome and were either mounted on mesh grids or transferred using a formvar film suspended across a small wire hoop, to slot grids. Sections were stained with uranyl acetate, or uranyl acetate and lead citrate, and were examined using a Philips 410 electron microscope. Photographs were recorded on Kodak electron image plates.

Description (Figs. 1–25)

STOMA AND ESOPHAGUS: The stoma is composed of cuticle that is more electron translucent than that of the anterior end (Fig. 1). It is triradiate, and the external surface of the stoma is lined with cuticle that is of the same density as

that of the surface of the worm (Figs. 1, 2). At the level of the vestibule, the internal surface of the cuticle, as described by Vegni-Talluri et al. (1986), is lined with numerous interdigitations of the lamellae composed of the plasma membranes of the vestibular cells (Fig. 1). The cuticle lining the lumen of the esophagus extends the length of this structure (Figs. 1–18). Esophageal cells appear to attach to the esophageal luminal cuticle via zonular junctions arranged parallel to the long axis of the esophagus (Figs. 3–16).

The esophagus is divided by the cuticle-lined, triradiate lumen into 3 sectors, 1 dorsal and 2 subventral, and there is no significant torsion away from the dorsoventral axis throughout the length of the esophagus (Figs. 3–16). The esophagus can be divided into 4 region on the basis of morphology: a slender procorpus (Figs. 3, 4), a thickened metacorpus (Figs. 5–7), a slender elongated isthmus (Figs. 8–14), and a terminal ventriculus (Figs. 15–17). Posterior to the excretory pore, the esophagus is apparently pushed into the dorsal portion of the body by the large excretory cell (Figs. 10–16).

The esophagus contains 3 gland cells; there is 1 gland cell in each sector. The dorsal gland cell is the largest and most extensive; it extends from the beginning of the metacorpus to the esophageal valve (Figs. 5–17). At the level of the ventriculus, the enlarged dorsal gland cell causes the dorsal sector to be several times larger than the subventral esophageal sectors. The nucleus of the dorsal gland cell is quite large and is located at the very posterior portion of the ventriculus (Fig. 17). The subventral esophageal gland cells are located almost exclusively within the ventriculus extending only slightly anteriad into the isthmus (Fig. 13). The nuclei of the subventral gland cells are also located within the ventriculus at a level slightly anteriad to that of the dorsal gland cell (Fig. 17). The cytoplasm of the dorsal and 2 subventral gland cells are similar; the cytoplasm contains mitochondria, rough endoplasmic reticulum (RER), Golgi lamellae, and vesicles of varying densities. The collecting cisternae described by Vegni-Talluri et al. (1986) were not seen in these sections.

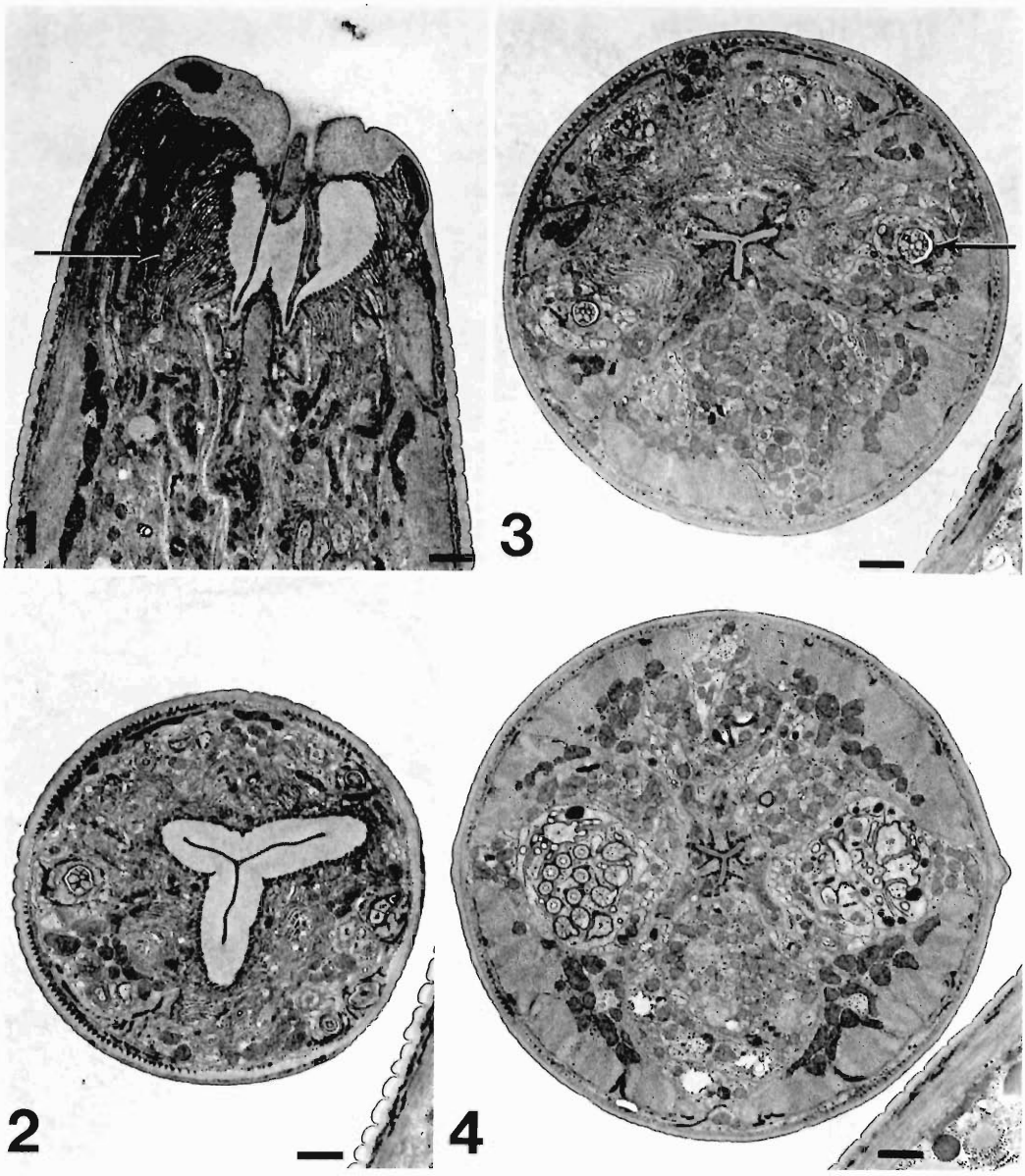
INTESTINE AND PROCTODEUM: The intestine consists of a single chain of cells, each with 1 large nucleus (Figs. 18–25). Also observed within the intestinal cells are numerous, smaller inclusions that have an appearance similar to small nuclei (Fig. 25). Near the esophagus, the intestinal cells are laterally compressed by the excretory columns (Fig. 18), but more posteriorly, the excretory columns are smaller and the intestinal cells become more circular in cross section (Figs. 19–21). The cytoplasm of the intestinal cells contains large lipid granules and scattered deposits of glycogen.

The proctodeum is lined with cuticle that is similar to that lining the lumen of the esophagus (Fig. 24). This cuticle appears continuous with the surface of the worm. This cuticle-lined channel extends between the anus and the last intestinal cell and is surrounded by numerous nuclei with very small amounts of associated cytoplasm (Figs. 22, 24).

CUTICLE: The cuticle is composed of 4 layers that are designated herein as the epicuticle, cortical, fibrillar (medial), and matrix (basal) layers (Fig. 26). The cuticle is about 0.4 μm thick at midbody. Striae are present from near the anterior end of the worm (Fig. 1) to the very tip of the tail (Fig. 24); the striae are about 0.8 μm apart. The outermost layer of the cuticle is the epicuticle; it is a thin, electron-dense layer that covers the entire external surface of the cortical layer. Progressing internally, the next layer is the cortical layer, and running throughout this layer is the slightly more electron-dense fibrillar layer. The fibrillar layer is present near the external layer of the cortical layer and forms thickenings at the base of each stria (Fig. 17). The matrix layer extends the length of the worm under the cortical layer as a flat, homogeneous layer that has the same thickness throughout the body.

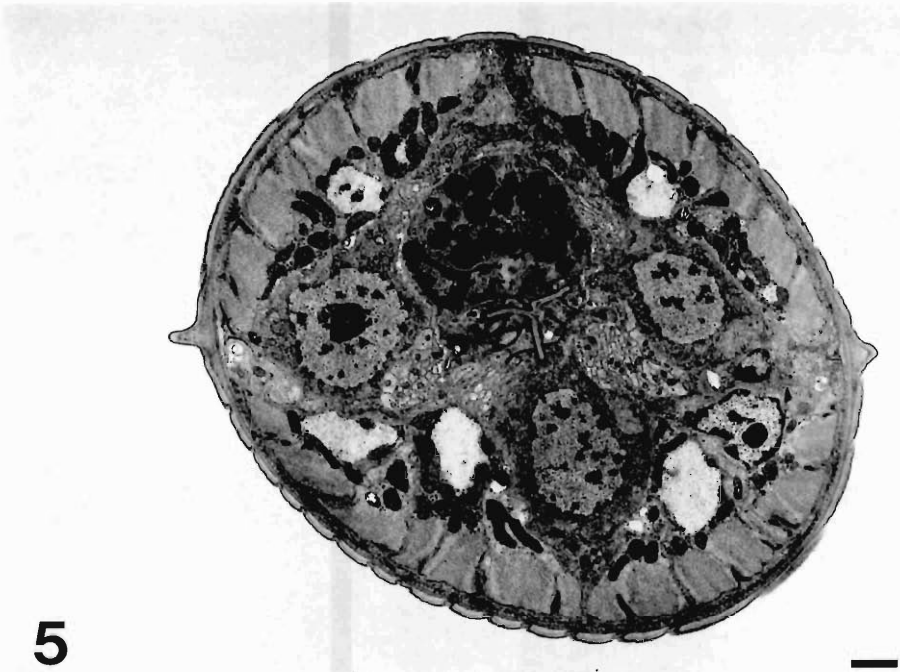
Lateral alae begin slightly posteriad to the buccal capsule (Fig. 4), become very prominent at the level of the nerve ring (Fig. 9), and extend posteriad past the rectum to near the tip of the tail (Figs. 22, 23). At the base of each ala, the dorsal and ventral cortex is modified by the addition of an electron-opaque, V-shaped layer that divides the less dense cortex (e.g., Fig. 12); this more dense layer is evident from the most anterior extent of the ala (Fig. 4) to its most posterior extent (Fig. 23). The V-shaped layer is external to the unchanged matrix and fibrillar layers of the cuticle that are also present in each ala (Fig. 27). Alae are about 3 μm tall at midbody (Fig. 18).

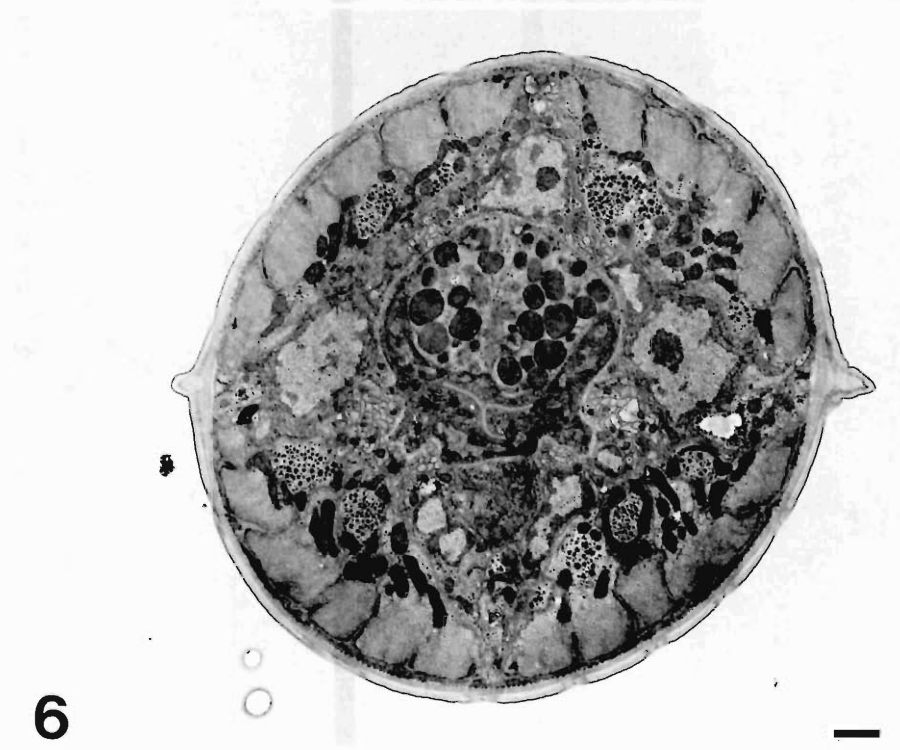
HYPODERMIS: At a level just posterior to the buccal capsule, the hypodermis of the lateral cords is composed of cytoplasm containing numerous mitochondria (Figs. 3, 4). This same cytoplasm is contiguous with that between the muscle cells and cuticle in the 4 body quadrants, and at this level it forms the areas of the dorsal and ventral cords (Figs. 3, 4). Slightly posterior to this level,



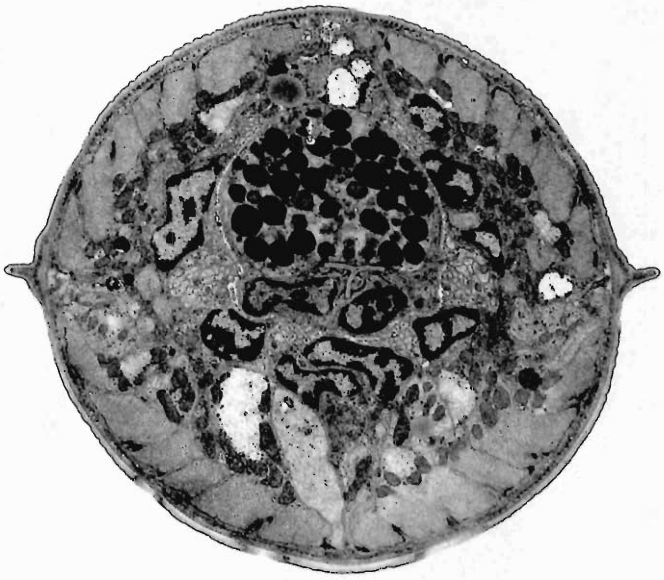
Figures 1–4. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 1. Anterior end, lateral view. Note the prolonged ventral surface and the translucent vestibule attached to the lamellae of the vestibular cells (arrow). 2. Transverse section at the level of the vestibule. Note the various prolongations of the anterior sensory neurons. 3. Transverse section just posterior to the vestibule; again note the anterior projections of the sensory neurons (arrow at 1 amphid) and the thick fibrillar areas attached to the cuticular lining of the esophagus. 4. Transverse section at the level of the esophageal procorpus. Note the large bundles of sensory neurons located laterally on the worm, the presence of lateral alae with inner cuticular bars, the large numbers of mitochondria in the lateral cords, and the small number (2) of muscle cells per quadrant.

the lateral cord hypodermis has a small central area next to the cuticle that is separated from its adjacent areas by desmosomes (Fig. 5). These desmosomes are present throughout the length of the worm, being present even posterior to the anus (Figs. 5–13, 15, 16, 18–23). This medial area of hypodermis extends into the pseudocoelom between the sublateral portions of the cord





Figures 5, 6. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 5. Transverse sections at the level of the metacorpus. Note the enlargement of the dorsal sector of the esophagus, the presence of a centrally demarcated area within the lateral cord, and the large cell nuclei within the pseudocoelom. 6. Transverse section slightly posteriad to Figure 5. Note the ventral and dorsal cords.



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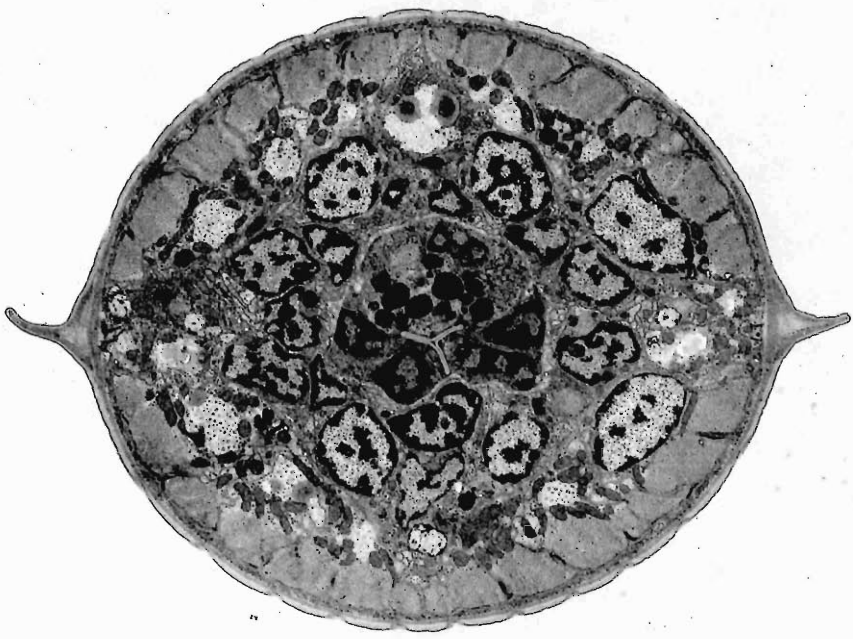

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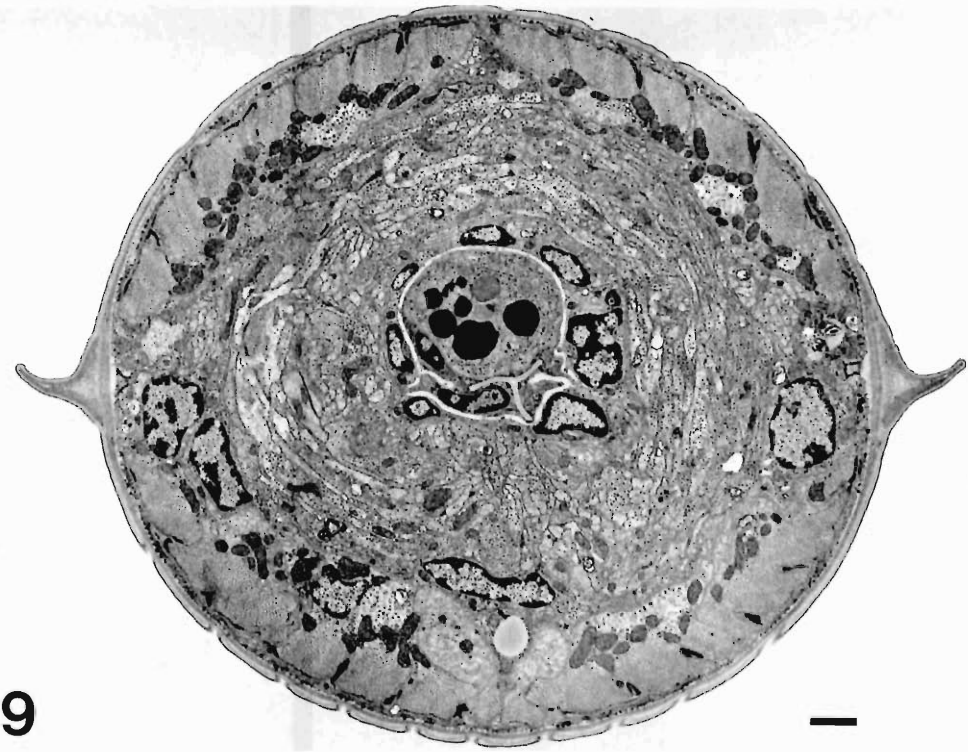
Figures 7, 8. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 7. Transverse section at the level of the metacorpus. Note the appearance of numerous nerve bundles and nuclei within the pseudocoelom. 8. Transverse section just anterior to the nerve ring and posterior to the esophageal *metacorpus*. Note the large number of nuclei within the pseudocoelom, the well-demarcated areas within the lateral cords, and the large number of nerve fibers that are present.



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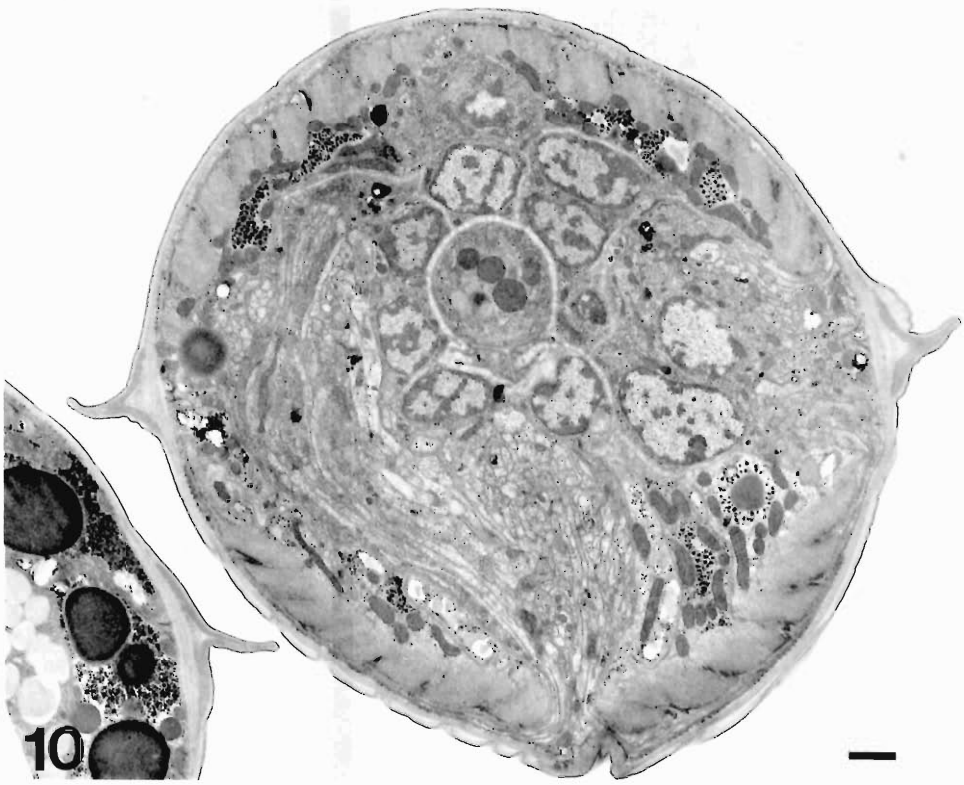

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(Figs. 12, 16, 20, 28). At the level of the deirid (Fig. 11), this same area of cytoplasm extends into the subcuticular papillary prominence. Mitochondria were sometimes observed in this portion of the hypodermis (Figs. 8, 11, 12, 15, 16, 20), but nuclei were observed only at the level of the rectum (Fig. 22). There were no similar regions demarcated by desmosomes in the dorsal and ventral cords, although the hypodermis extends into the pseudocoelom in these areas (Figs. 15, 16). The sublateral portions of the lateral cords are apparent at the level of the nerve ring as granular cytoplasm containing nuclei and numerous mitochondria (Figs. 8, 9). At the level of the excretory cell nucleus (Fig. 12), these portions of the cord are quite prominent and contain mitochondria, nuclei, and large lipid droplets; this morphology is consistent throughout the remainder of the body of the worm (Figs. 13–16, 18–24). Posterior to the anteriormost occurrence of desmosomes in the lateral cords, the sublateral portions of the lateral cord are the portions of hypodermis that appear contiguous with that lying between the cuticle and muscle cells. In the hypodermis under the muscle cells of the body quadrants, numerous tonofilamentlike densities extend between the cuticle and the underlying muscle cells; these densities are not present under the lateral alae (e.g., Fig. 12).

SOMATIC MUSCULATURE: The somatic musculature begins just posterior to the buccal capsule (Figs. 1, 3) and extends to near the tip of the tail (Fig. 25). The muscle cells are meromyarian and platymyarian in type. Anteriorly, there are 2 cells per quadrant (Fig. 3); at the nerve ring through the base of the esophagus, 3 cells per quadrant (Figs. 9–13, 15, 16); at the beginning of the intestine, 3 cells per quadrant (Fig. 18); in the region of the posterior intestine, 2 cells per quadrant (Fig. 19); and posterior to the anus, 1 cell per quadrant (Figs. 22, 23). There are usually several bundles of myofibrils per muscle cell (e.g., Fig. 16). The cytoplasmic portions of the muscle cells contain numerous mitochondria and glycogen (e.g., Figs. 11, 15) as well as the muscle cell nucleus (e.g., Fig. 16).

NERVOUS SYSTEM: The nerve ring is a circular bundle of fibers surrounding the esophagus (Fig. 9). Although a few nuclei are present in the nerve ring, most nuclei are confined to areas just anterior to and just posterior to the nerve ring (Figs. 8 and 10, respectively). Within the fibers of the nerve ring are numerous small vesicles and mitochondria (Fig. 9).

The ventral cord is the major nerve trunk running throughout the worm. In the area of the stoma, the cytoplasm of the cells in the cord contain Golgi apparati, numerous mitochondria, and multivesicular bodies (Figs. 2–4). Posterior to this level, but anterior to the nerve ring, the cord typically contains a single nucleus or a bundle of nuclei surrounded by an area of mitochondria and RER (Figs. 5–7). In the anterior nucleated portion and the nonnucleated portion of the nerve ring, it is similar to the other nervous tissue (Figs. 8, 9). At the level of the excretory pore, numerous fibers from the nerve ring extend into the ventral cord (Fig. 10); more posteriad, neuronal fibers and nuclei surround the serpentine excretory duct (Fig. 11). From the excretory cell commissure to the tail, the ventral cord contains neurons and cell bodies with nuclei, mitochondria, and neuronal fibers (Figs. 18, 23). Posterior to the rectum, the ventral cord fills most of the pseudocoelom (Fig. 23). The dorsal nerve cord is similar to the ventral cord throughout its length but is smaller in diameter.

The sensory structures of the anterior end are innervated by fascicles of fibers extending anteriad from the nerve ring (Figs. 2–8); the largest fascicles are laterally located (Figs. 4–8). At the anterior of the lateral alae, some of the nerves in the lateral fascicles contain microtubules in the pattern of a modified ciliary axoneme, a circle of 9 doublet microtubules and an inner group of 2–6 single microtubules (Fig. 4). There are at least 13 of these tubule-bearing cells at the base of each amphidial socket (Fig. 4), but not all extend to the anterior extremity of the worm (Figs. 2, 3). The nerves that innervate the outer labial pupillae also have a similar, microtubular

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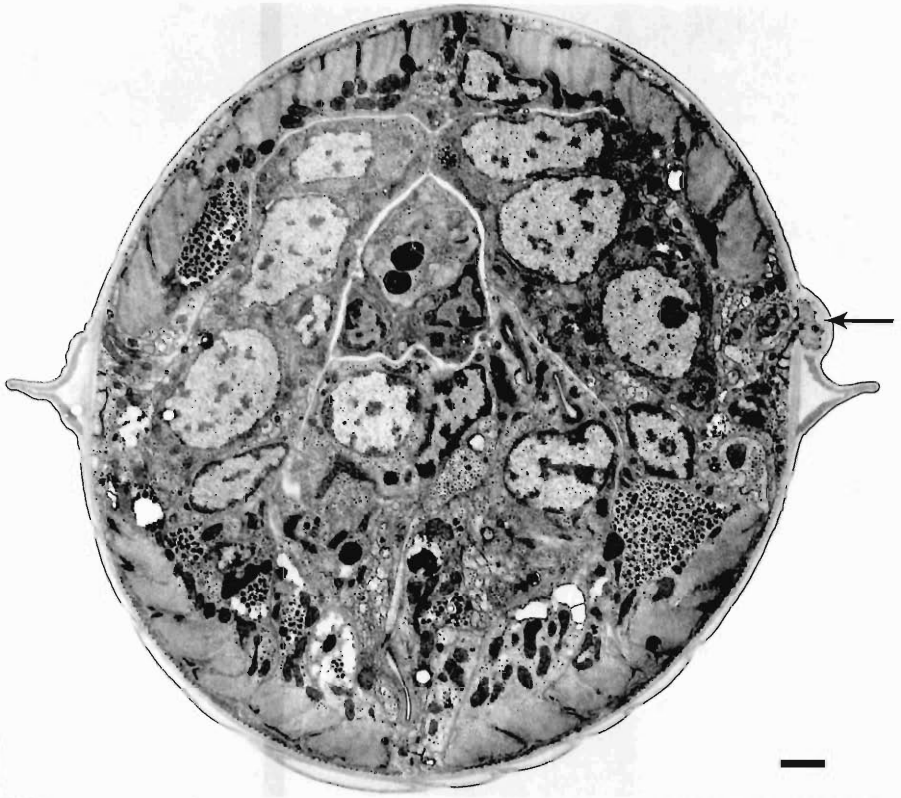
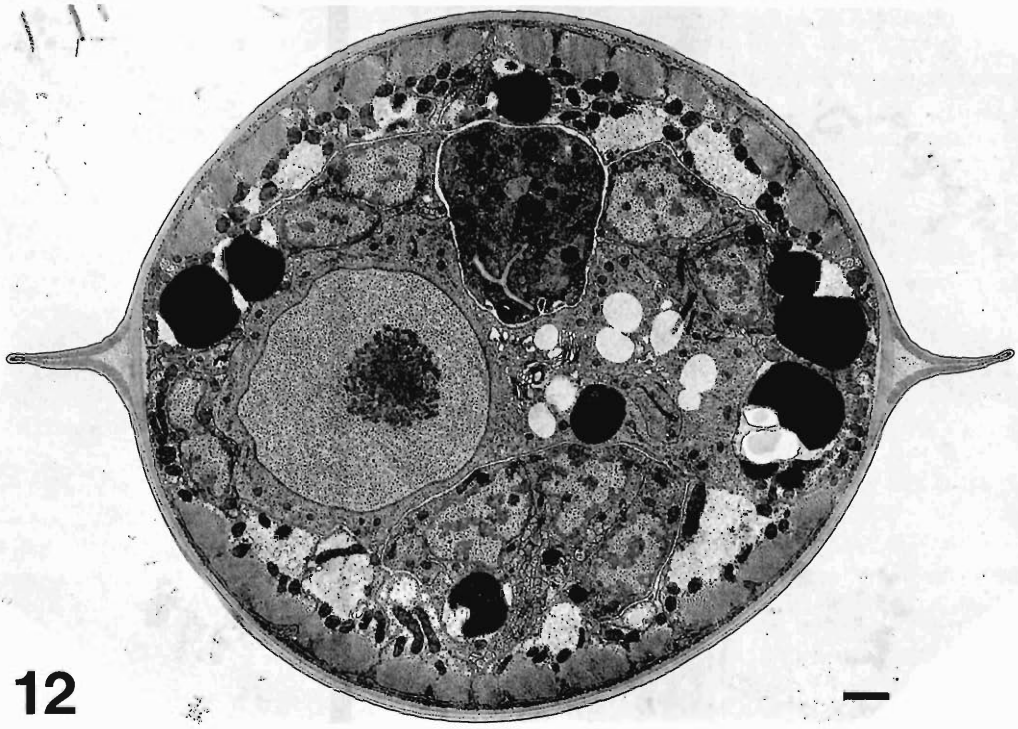
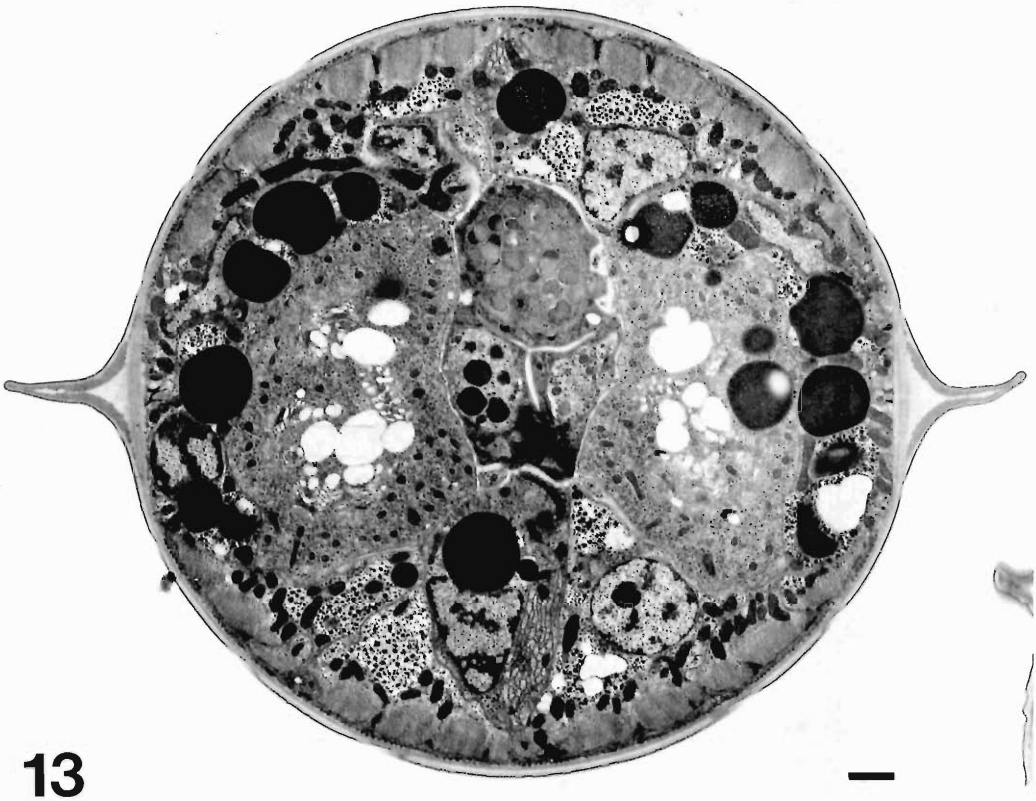
Figures 9, 10. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 9. Transverse section at the level of the nerve ring. Note the lack of nuclei within the nerve ring that fills the pseudocoelom. Also note the highly expanded lateral alae. 10. Transverse section at the level of the excretory pore. Note the large numbers of nerve fibers extending into the ventral cord at this level and the appearance of more neural nuclei within the pseudocoelom.





Figures 11, 12. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 11. Transverse section at the level of the left deirid (arrow). Note the extension of nervous tissue through the cuticle dorsal to the lateral ala. Sinuous tracts of the excretory duct can also be seen. 12. Transverse section at the level of the excretory cell commissure. Note the large excretory cell nucleus and the numerous Golgi bodies within the excretory cell cytoplasm. The lateral alae are probably most pronounced at this level.



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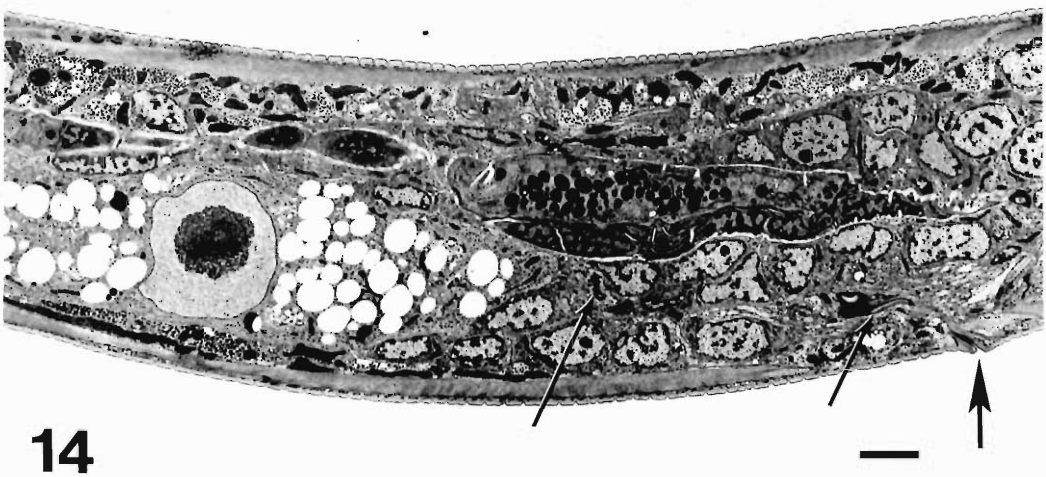

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Figures 13, 14. Electron micrographs of the infective-stage larva of *Toxocara canis*. **13.** Transverse section just posterior to the excretory cell commissure. Note the beginning of the posteriorly directed excretory columns, the large ventral ganglion, and the anterior extension of the subventral gland cells of the esophagus in their respective subventral sector. Bar = 1 μm. **14.** Longitudinal section through the anterior end of the worm from the level of the excretory cell nucleus to the excretory pore. From the excretory pore (large arrow) the excretory duct continues posteriad (small arrows) to the large portion of the excretory cell where the large excretory cell nucleus is located. Bar = 2 μm.

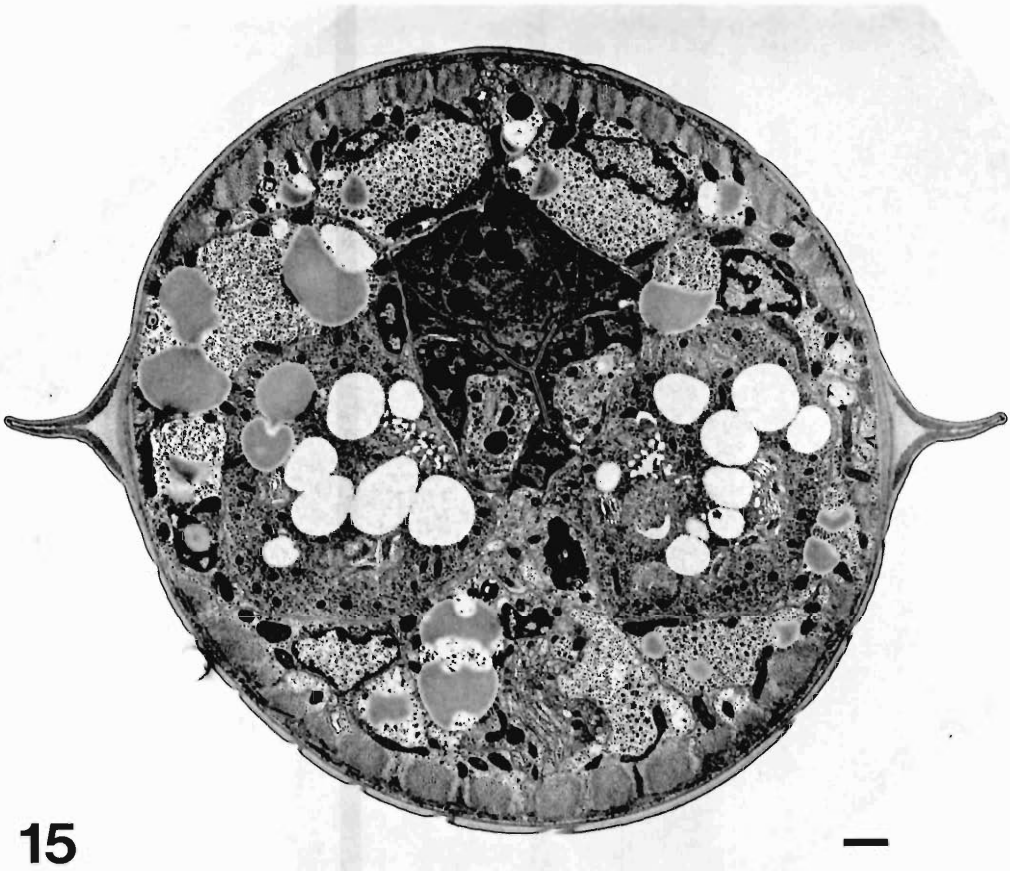

Figure 15. Transverse section just anterior to the ventriculus of the esophagus of the infective-stage larva of *Toxocara canis*. Note the large number of nuclei composing the esophagus and the presence of dorsal and subventral gland cell cytoplasm in the 3 esophageal sectors. Bar = 1 μm.

pattern (Fig. 2); the nerves of the inner labial papillae were not seen.

Deirids were located just posteriad to the excretory pore and just dorsal to the lateral alae. Each deirid was formed by a subcuticular protrusion of hypodermis and nervous tissue through the matrix and fibrillar layers of the cuticle (Fig. 11). Phasmids were not seen in sections.

EXCRETORY SYSTEM: The unicellular excretory system has the appearance of a shortened "H" as described by Nichols (1956a). The large nucleus (Figs. 12, 14) occurs at the level of the excretory cell commissure, i.e., where the anteriorly and posteriorly directed lateral columns join. From the excretory pore (Fig. 10), the sinuous excretory duct extends posteriad to the cell commissure (Figs. 11, 12). The excretory duct is lined with an electron-dense material that appears similar to the epicuticle of the body (Fig. 14).

The cytoplasm of the excretory cell contains numerous mitochondria, Golgi bodies, RER, and large numbers of vesicles that presumably contain protein. The vesicles are found throughout the excretory cell cytoplasm (Figs. 12–20), except in the lateral arms that extend anteriad from the

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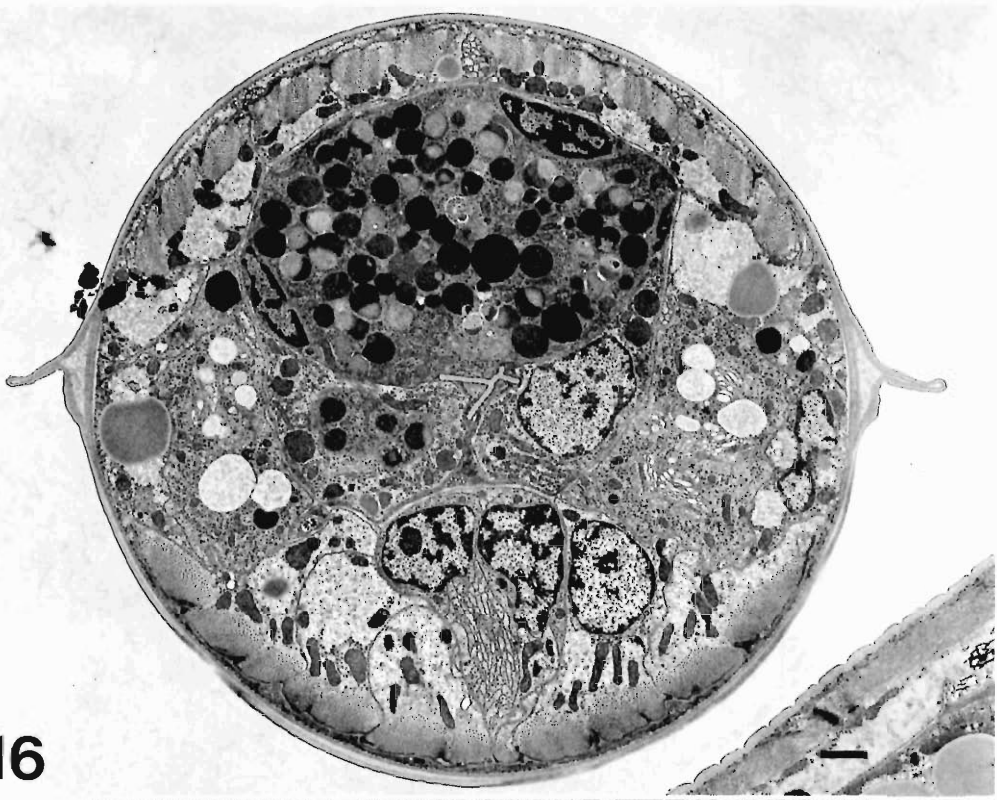
Figures 16, 17. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 16. Transverse section through the ventriculus at the level of the left subventral gland nucleus. Note the dorsal displacement of the esophagus and the large subventral gland cell nucleus. The dorsal sector of the esophagus is enlarged and



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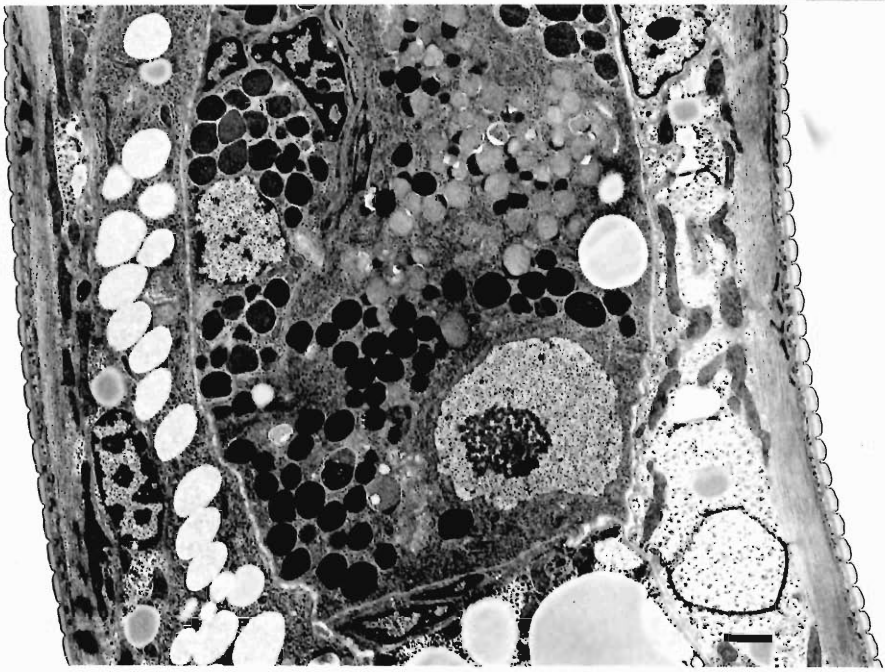

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filled with the dorsal esophageal gland. 17. Longitudinal section through the ventriculus of the esophagus. This section shows the relationship of the dorsal and subventral gland cell nuclei with respect to each other and with respect to the beginning of the intestine.

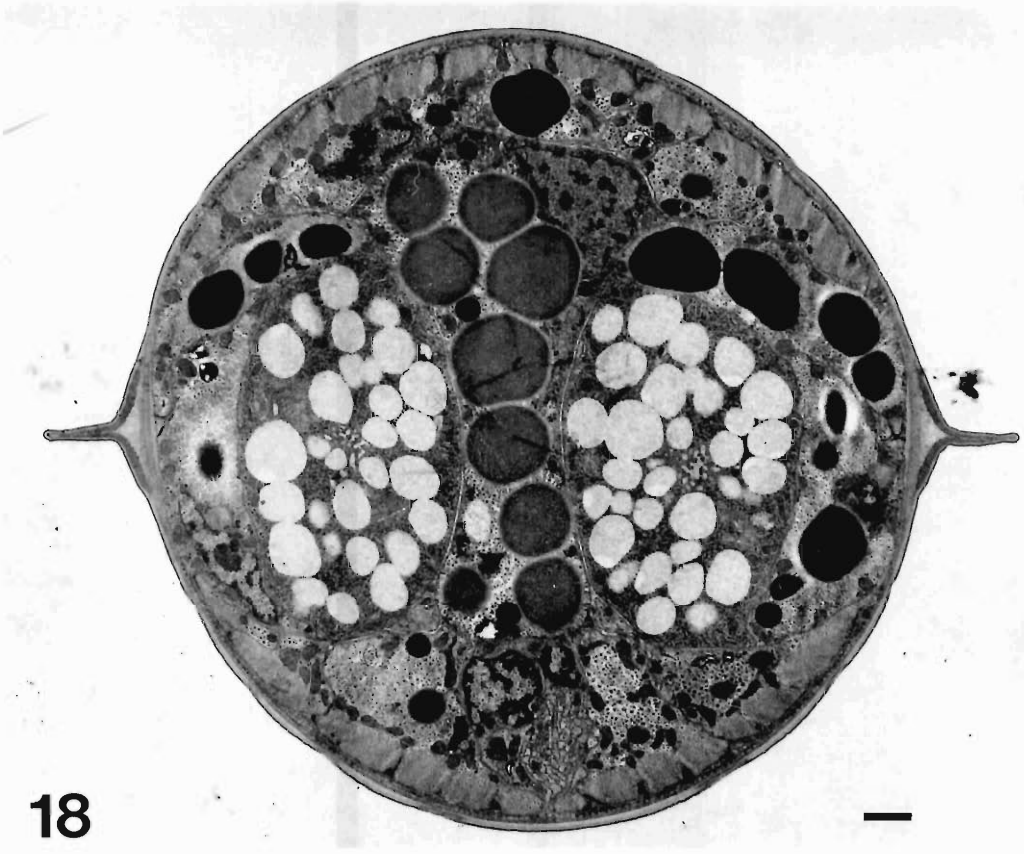


Figure 18. Transverse section at the level of midbody of the infective-stage larva of *Toxocara canis* showing the compression of the intestinal cell by the excretory cell processes. Also note the large ventral cord and the thickened cuticular bars within the lateral alae. Bar = 1 μm.

cell commissure. In the lateral columns posterior to the nucleus, the vesicles surround the collecting canaliculi. These canaliculi may join in the commissure to form the excretory duct, but this was not noted in any of the sections that were observed. The lateral columns extend further posteriad on the left side of the worm than on its right (Fig. 20).

GENITAL PRIMORDIUM AND PSEUDOCOELOMOCYTES: Although all sections in the region of the intestine were examined for these structures, cells were never identified that could be considered part of the genital primordium or that morphologically resembled pseudocoelomocytes.

Discussion

The present description extends the observations of Nichols (1956a) to the ultrastructural level. Overall, the description that was made with the light microscope was found to be very complete and is only supplemented by details.

The stoma of the infective stage larva of *T. canis* has been described previously (Vegni-Talluri et al., 1986). Those authors first reported on the lamellar system surrounding the vestibular cuticle and suggested that the resemblance of this lamellar system to the transporting epithelia of other animals indicates a possible function in ionic or osmotic regulation. They speculated that this osmotic regulation might be important as the larva encounters different environments within the infected host. All osmotic shifts would occur via diffusion through the overlying layer of thickened cuticle. The stomas of other infective-stage larvae of ascaridoid nematodes have not to our knowledge been examined for a similar type of structure. Such a structure is lacking in

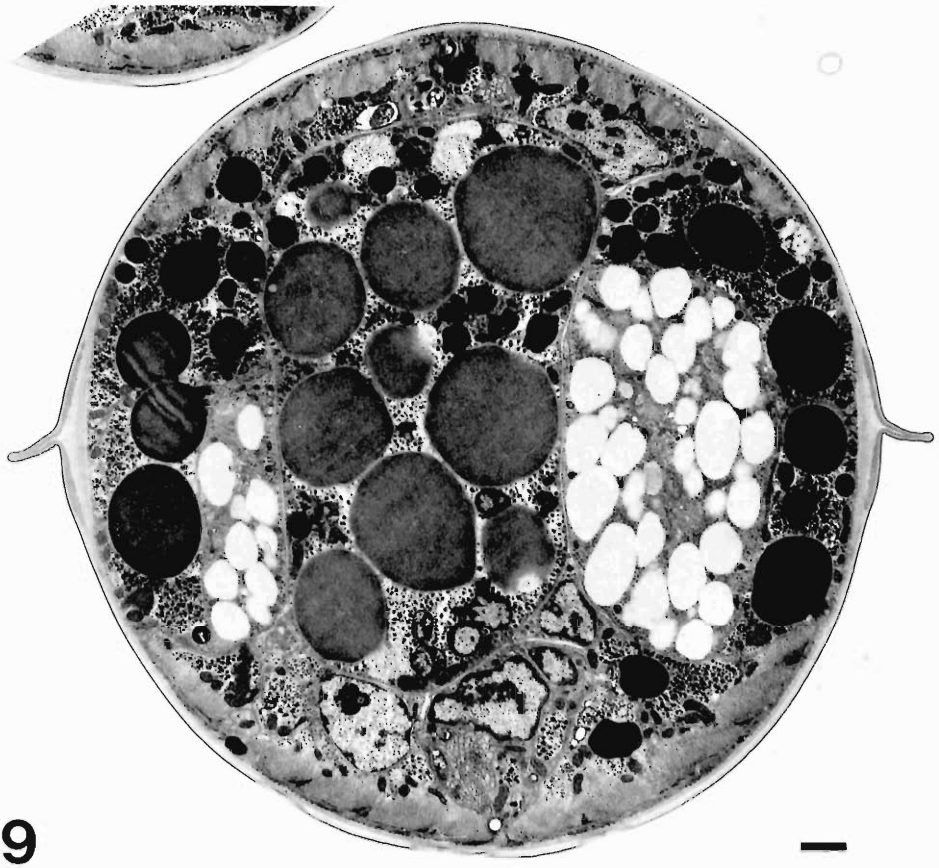
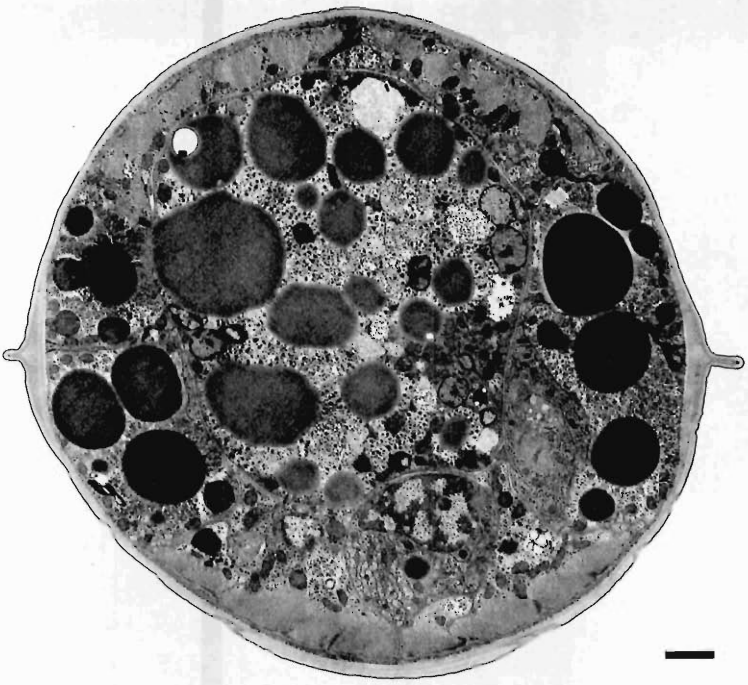


Figure 19. Transverse section of the infective-stage larva of *Toxocara canis* showing the diminution of the excretory cell toward the posterior of the body. Note that the process of the excretory cell on the worm's left side is larger than that on the right and that the sublateral portions of the lateral cords are prominent and contain large numbers of lipid droplets. At this level, the intestine is no longer compressed by the excretory cell columns. Bar = 1 μm.

the buccal capsules of fourth-stage larvae of *Caenorhabditis elegans* (Wright and Thomson, 1981) and second-stage larvae of *Meloidogyne incognita* (Endo and Wergin, 1988).

There was no indication of the presence of a cephalic septum at the front of the esophagus, as has been described for adult ascaridoids by Inglis (1964); this could be considered as further evidence that the lips that develop in the fourth-stage larvae and the adult nematodes are due to a process of elongation of the cells of the lips, clavate cells, and lobus impar, rather than to the inward growth of the hypodermis. Studies on the morphology of the cephalic structures in developing larvae would be required to resolve the details of the origin of the structures.

The ultrastructure of the esophagus of the infective-stage larva of *T. canis* has been examined by Vegni-Talluri et al. (1986), who described the duct emptying the dorsal esophageal gland cell as occurring near the anterior portion of the esophagus. The subventral gland cells are noted by Vegni-Talluri et al. (1986) to empty into the esophageal lumen near the base of the esophagus. The anatomy of the esophagus in the larval *T. canis* is very similar to that reported by Hsü (1933) for the adult worm. Hsü found that the ducts of the gland cells emptied in the same areas of the esophagus in the adult as they do in the larva, as described by Vegni-Talluri et al. (1986). Hsü reported that within the adult, the nucleus of the dorsal esophageal gland was in the ventral portion of the ventriculus and that the subventral gland cell nuclei were found in dorsal positions.



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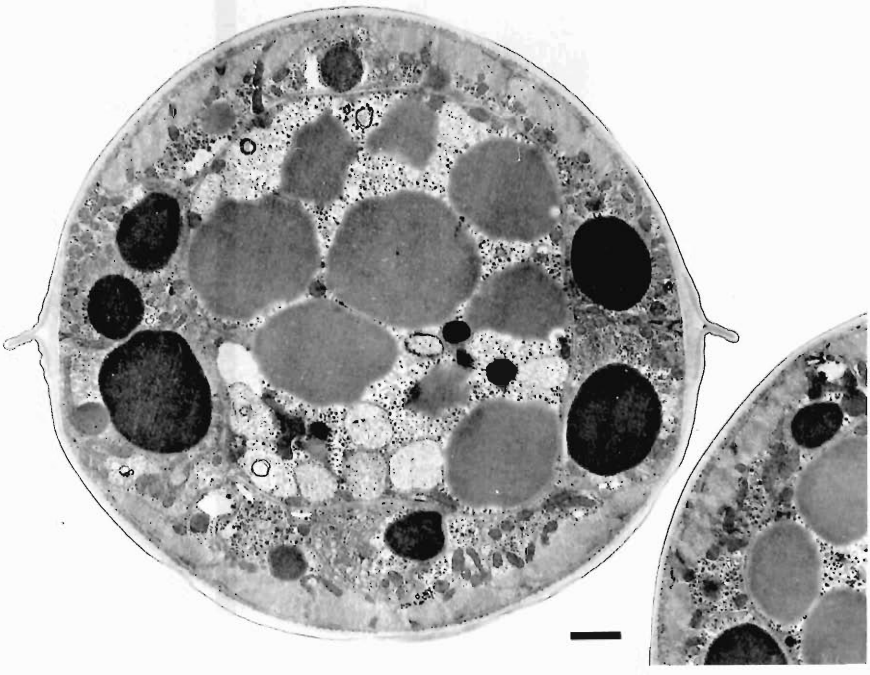

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Figures 20, 21. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 1 μm. 20. Transverse section near the end of the left posteriormost extension of the excretory column. Note that there is no excretory cell process on the worm's right side. The medial and sublateral portions of the lateral cord are quite prominent in this section. 21. Transverse section posterior to the excretory columns. The lateral cords at this level contain large numbers of mitochondria, and lipid droplets are present.

The movement of the dorsal esophageal gland nucleus to a ventral position was noted in developing larvae that Sprent (1958) recovered from experimentally infected dogs. Schacher (1957), alternatively, noted the dorsal esophageal gland within the dorsal sector of the esophagus in fourth-stage larvae recovered from dogs but found that it had migrated to the ventral sector within the adult worms. Overall, however, it would appear that sometime during the development of the worm that these nuclei migrate to different positions within the ventriculus.

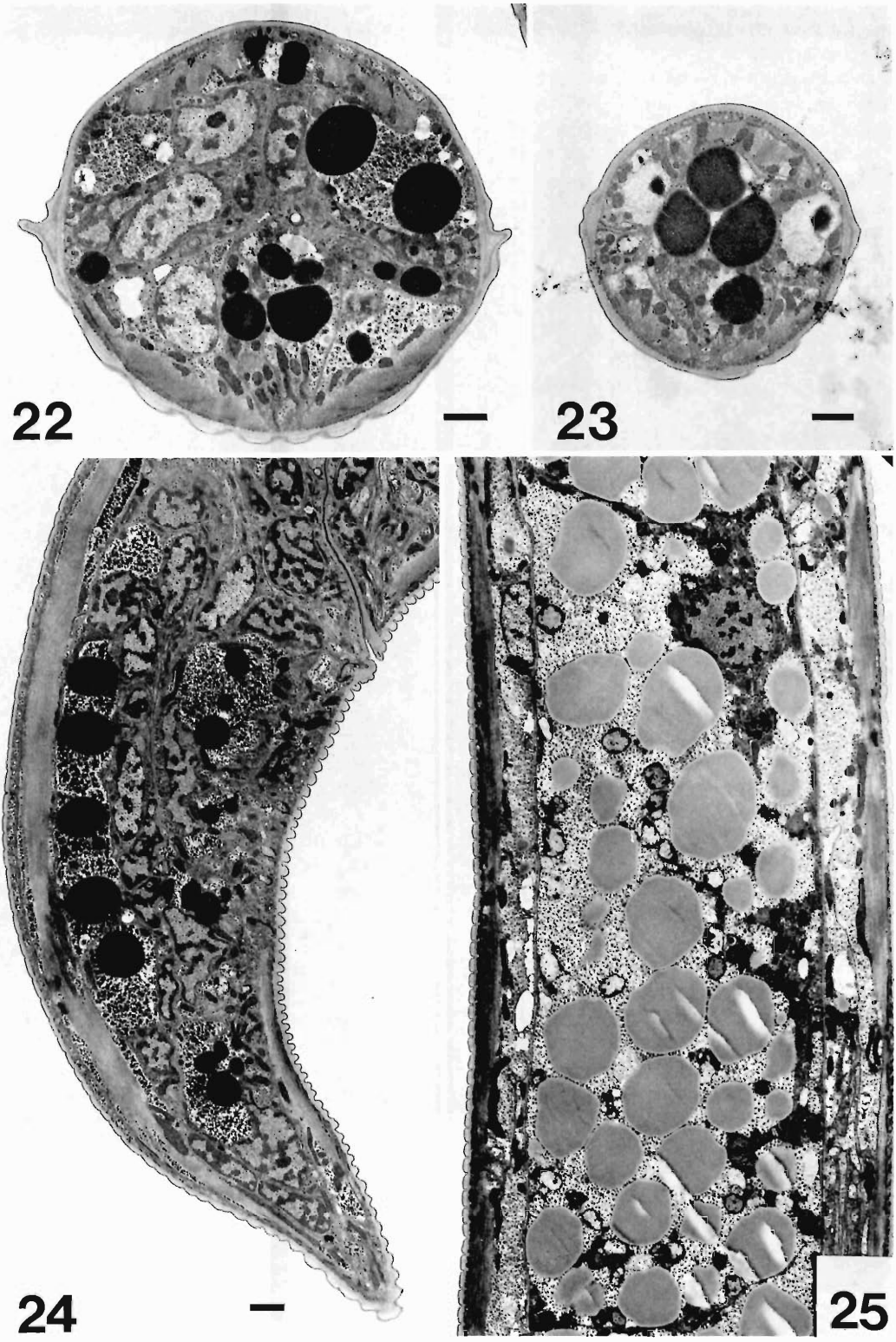
The granules within the esophageal glands of ascaridoids have received attention since they were first described by Looss (1896). Mueller (1931) examined the vesicles within the esophageal glands of the adult *Ascaris lumbricoides* and *Ascaris megacephala* and based on their fixation and staining reactions was convinced that they contained protein. The work of Drum (1966) on the secretory granules of the esophagus of adult *T. canis* and *A. lumbricoides* showed that they were membrane-bound vesicles containing endopeptidases with optimal activity similar to that of chymotrypsin. Work with larvae of the ascaridoid *Anisakis simplex* has also shown that the esophageal glands contain a trypsinlike proteolytic enzyme that was not present in the excretory cell (Matthews, 1984). Recent work with *Ostertagia circumcincta* (McGillivery et al., 1990) has shown that a stage-specific glycoprotein recovered from larval worms is located within the secretory vesicles of the esophagus. Similarly, work with the second-stage larva of *Meloidogyne incognita* has shown by immunogold labeling that a large molecular weight, secreted glycoprotein is located in the secretory granules of the subventral esophageal glands (Hussey and Mims, 1990; Hussey et al., 1990). The function of these glycoproteins has yet to be determined.

The intestine of the infective-stage larva of *T. canis,* as originally described by Nichols (1956a), is composed of a chain of single cells. The lack of a lumen has also been verified by Bai et al. (1978). In the canine final host, the cells of the intestine begin to multiply soon after infection. Schacher (1957) noted an intestinal lumen in larvae recovered from the stomach of dogs 3 days after infection with infective eggs. Similarly, Sprent (1958) noted larvae with intestinal lumina in worms recovered from naturally, prenatally, infected puppies during the first week of life. Griesemer et al. (1963) show a figure of larva of *T. canis* with a patent lumen in the lung of a puppy that was 2 days old. The intestines of the fourth-stage and adult worms are polycytous, as is typical of ascaridoid nematodes (Argeseanu, 1934; Chitwood and Chitwood, 1950; Fujino and Ishii, 1988).

The ultrastructure of the larval intestine of *T. canis* is very similar to that reported for the intestine of the infective-stage larva of *Ascaris suum* (Jenkins and Erasmus, 1971; Rubin and Trelease, 1975). The major feature of the intestine of both these worms is the large amount of stored glycogen and lipid. It has been postulated that the larvae use the glycogen for supplying energy during their migration in the host (Fairbairn, 1970); however, the ability of larvae to survive in cultures where they are metabolically active for periods of over a year (de Savigny, 1975) would indicate that these products might also be used for periods of low level activity within the eggshell when temperatures are warm enough to allow metabolism by the larvae. It has also been noted that the intestine of the lung-stage larva of *Ascaris suum* contains large quantities of phosphorylcholine within its intestinal tract (Gutman and Mitchell, 1977). Examinations as to the localization of phosphorylcholine within the larva of *T. canis* have not been performed.

The cuticle of the larval *T. canis* was found to correspond with the cuticle of the third-stage larvae of *Ascaris lumbricoides* as described by Thust (1966, 1968). It was also found to be very similar to the third-stage cuticle of *Ascaris suum* as described by Rockey et al. (1983) and Thompson et al. (1977). Unlike the larva of *T. canis,* the third-stage larva of *A. lumbricoides* does not have prominent lateral alae in the esophageal region (Nichols, 1956a; Thust, 1968).

The structure of the cuticle on the body was found to be different from that of the adults of both *Toxocara cati* (as described by Glaue, 1910a, b; and Erlich, 1937) and *Toxocara canis* (as described by Inglis, 1964). These authors reported a thick fiber layer as occurring under the matrix layer. Erlich and Inglis also described a series of "punctation canals" composed of fibers running from the fibrillar layer through the matrix layer to layers of dense fibers in the supporting fiber layers that are external to a basal lamellar layer. Neither thickened fiber layers nor punctation canals were observed in the cuticle of the infective larva. The morphology of the cuticle of the larvae is such that it would appear that the matrix layer



is the innermost layer of the cuticle on the larva and that the other more internal layers do not develop either until the third-stage larva begins to grow or metamorphose to the fourth stage or perhaps even the adult stage.

The lateral alae of the infective-stage larva of *Toxocara canis* seen here were similar in morphology to those in the electron micrographs of Bai et al. (1978) and Kondo et al. (1987). Also, there were no differences seen in the alar morphology of the infective-stage larva when it was compared to the micrographs of lateral alae of larvae recovered from mice either 10 days (Bai et al., 1978) or sometime between 6 and 56 days (Ghafoor et al., 1984) after infection. A photomicrograph of a midbody section of an advanced larval stage in the lungs of a 2-day-old puppy shows alae that are different from those of the infective-stage larva in that they appear more robust and equilateral in shape (Griesemer et al., 1963). The alae are also significantly different in the adult *Toxocara canis* based on the figures of Höppli (1925) and those of the adult *Toxocara cati* by Glaue (1910a). The major difference is that the V-shaped electron-dense area (the "Flügelleiste" of these authors) of the adult extends internally from the periphery of the ala only about halfway toward its base.

The hypodermis of the infective-stage larva was similar to that described by Nichols (1956a, b) for the larvae of *Toxocara canis* and *Ascaris lumbricoides*. It was also found to be quite similar to the hypodermis of the third-stage larvae of *Toxascaris leonina, Baylisascaris procyonis, Hexametra leidyi,* and *Lagochilascaris sprenti,* as described by Bowman (1987). Allgen (1943a, b) described the morphology of the hypodermal cords of larval *Toxascaris leonina* and found that it was composed of single cells and that the synctium did not form until the worms reached sexual maturity. This was not found to be the case with larval *T. canis,* nor was it found to be the case in larval *Toxascaris leonina* that had been recovered from mice (Bowman, 1987).

The morphology of the lateral cords of the larval *Toxocara canis* are quite similar to those found in the adult worm. Höppli (1925) described that of the adult *T. canis* and found a morphology similar to that of the larvae. Glaue (1910a, b) and Martini (1909) described the morphology of the hypodermis of *Toxocara cati.* They found that there were 2 types of nuclei present in the lateral cords, 1 type in the medial portion and the other in the sublateral portions. Although the basic morphology is the same, no nuclei were noted in the present study in the medial line of the lateral cords except in areas posterior to the anus. Martini found very few nuclei in the lateral cords of specimens that were less than 6 cm long with the occasional nucleus that was found in the sublateral portions of the cord. It would thus appear that the nuclei present in larger adults develop after the worm has begun its growth as an adult.

Hinz (1962) described the ultrastructure of the hypodermis of the adults of *Parascaris equorum* whereas Bogoyavlenskii (1973) described the structure of the hypodermis of this and several other adult ascaridoids, including *Ascaris suum* and *Toxascaris leonina.* Bogoyavlenskii divided the hypodermis of the adult into 3 layers based on the appearance of the fibrils contained therein. The layer closest to the cuticle, one-fifth the total thickness of the hypodermis, contained large numbers of annularly arranged fibrils. The widest zone, being more than one-half the entire width of the hypodermis, was the middle zone, which contained large numbers of vacuoles and a branched, plexus organization of fibrils. The area adjacent to the musculature was found to contain large numbers of annular and longitudinal fibrils. Hinz noted that the hypodermis was rich in endoplasmic reticulum and that the nuclei were concentrated around the nervous tissue of the ventral nerve cord. There was no attempt made to determine the various layers of the hypodermis in the study reported here.

The ultrastructure of the muscle cells of the

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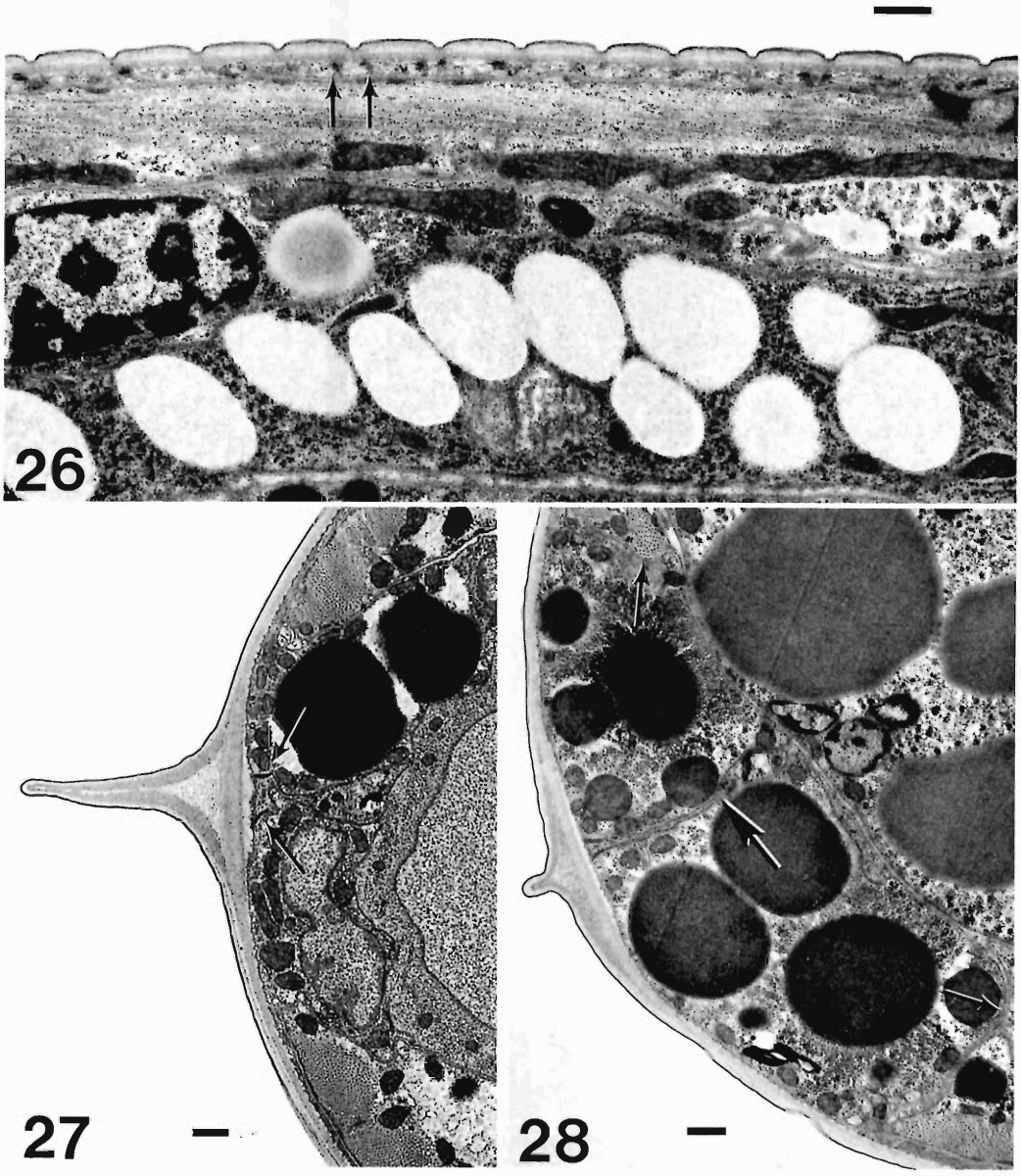
Figures 22–25. Electron micrographs of the infective-stage larva of *Toxocara canis.* Bars = 1 μm for Figures 22–24 and 2 μm for Figure 25. 22. Transverse section posterior to the rectum. Note the inward expansion of the lateral cords and the presence of nuclei within the pseudocoelom. 23. Transverse section near the tip of the tail. Note the large ventral cord containing lipid granules that fill most of the pseudocoelom. 24. Longitudinal section through the tail. Note the rectum, the large number of nuclei encircling the rectal canal, and the numerous nuclei posterior to the rectum. 25. Longitudinal section through an intestinal cell. The large nucleus of this cell can be noted at one end of the cell.



Figures 26–28. Electron micrographs of the infective-stage larva of *Toxocara canis*. Bars = 0.5 μm. 26. Enlargement of the cuticle on the dorsal surface (Fig. 17) showing the various cuticular layers; thickenings at the base of each stria are formed from the fibrillar layer (arrows). 27. Enlargement of the left lateral ala (Fig. 12) showing its cuticular composition and desmosomes marking the connection with the medial portion of the lateral cord (arrows). 28. Enlargement of the left lateral cord (Fig. 20) showing the medial (large arrow) and sublateral portions of the cord (dorsal and ventral extent marked with a small arrow).

infective-stage larva of *T. canis* revealed that there were few myocytes per quadrant and that these cells were of a meromyarian and platymyarian type. This differs from adult ascaridoids, wherein the merocyte organization is polymyarian and coelomyarian (Wright, 1966). Nichols (1956a) did not distinguish individual muscle cells in the infective-stage larvae of *T. canis*. Stretton (1976), however, noted that the infective-stage larva of *Ascaris suum* had a total of 83 stomatic muscle cells while the adult had a total of about 50,000 such cells. Thus, it would appear that an increase in myocyte number along with an accompanying change in myocyte morphology may be a common phenomenon within polymyarian ascaridoidea.

The muscle bridges of *Toxocara canis* adults were examined by Wright (1966), who showed that cytoplasmic portions of individual muscle cells often connected to both the ventral and lateral cords. They were found to be much like the muscle cells of the adults of *Ascaris lumbricoides* and *A. suum*, which have received considerable attention since Schneider (1866) noted that they differed from the muscles of other animals in that the cytoplasmic portions of the muscle fibers extend from the muscles to the nerves in the ventral or dorsal nerve cords (for review, see DeBell, 1965; also, Stretton, 1976).

Work by Bartnik et al. (1986) and Francis and Waterston (1991) has shown that the somatic musculature of nematodes is attached to the cuticle by desmosome-linked tonofilaments that are immunochemically similar to intermediate filaments of mammals. These filaments are believed to cross the hypodermal cell and act as a means of attachment between the muscle cell and the cuticle. The presence of tonofilaments in the hypodermis of the larva of *T. canis* suggests that the mode of muscle attachment to the cuticle is similar in this larval nematode.

The nervous system of the infective-stage larva of *T. canis* was much like that described by Nichols (1956a). Nichols, however, was not able to distinguish the dorsal, ventral, and lateral cords, although he did note that prominent nuclei could be observed in the ventral line. The nervous system of the adult of *A. suum* was extensively mapped by Goldschmidt (1903, 1908, 1909, 1910) and more recently by Stretton et al. (1978) and has been shown to consist of about 250 neurons, of which there are 162 in the nerve ring and anterior ganglia. As of this time, the number of nerve cells in the larva has not been counted, but it is believed that the number is very similar to that of the adult worm (Stretton, 1976).

The ultrastructure of the excretory cell described here for the larva of *T. canis* is similar to that described by Kondo et al. (1987) and by Vegni-Talluri and Dallai (1990). The ultrastructure of the excretory cell of the *T. canis* larva is also very similar to what has been described for other larval ascaridoids, i.e., the infective-stage larva of *Ascaris suum* (Jenkins, 1971), larval *Anisakis* (Lee et al., 1973), and larval *Phocanema* (*Pseudoterranova*) *decipiens* (Davey and Sommerville, 1974). The most striking feature of these cells is the large nucleus, the large numbers of membrane-bound vesicles, and the presence of numerous mitochondria, Golgi bodies, and RER, all features of a metabolically active secretory cell. Davey and Sommerville (1974) postulated that enzymes in the cell remain dormant until the cell is stimulated by neurosecretory cells at the time of molting. It would appear that this may be one function of the cell, although other functions have been suggested, including osmoregulation (Beherenz, 1956), exodigestion (Mueller, 1929; Lee, 1970), acetylcholine inhibition (Ogilvie and Jones, 1971), and substrate secretion to assist in motility (Bird, 1990). In culture, *T. canis* larvae produce large amounts of protein (Badley et al., 1987a), and antibodies to these proteins bind strongly to the excretory cells of larval *T. canis* in histological sections (James Parsons, unpubl. obs.). These data suggest that the excretory cell of the larval *T. canis* is actively producing proteins during this phase of the life cycle that is usually found within a paratenic host. Robertson et al. (1989) showed that proteases are a component of the excretory–secretory antigens produced by cultured larval *T. canis*. This enzymatic activity and the immunolocalization of these antigens to the excretory cell suggests that this cell is involved in the production and exportation of proteolytic enzymes to the external environment of the worm. For these reasons, it has been suggested that the cell should be termed a secretory cell (Maizels and Page, pers. comm.). However, it may be that the proteolytic portion of the excretory–secretory product is actually being produced by the esophageal gland cells, which, as already described, are known to produce proteases. In fact, some monoclonal antibodies to excretory–secretory antigens bind to the secretory cell, whereas others bind to

the esophageal region; thus, the origin of the proteases may be either, or both, sources (Maizels and Page, pers. comm.). Certain monoclonal antibodies that are reactive with the excretory–secretory products are also reactive with the larval surface (Bowman et al., 1987), and component(s) of polyclonal antibody that mediate cellular attachment to larvae in vitro can be removed by preabsorption of the antibody with excretory–secretory product (Badley et al., 1987b). This sharing of antigenic epitopes by the surface of the larvae and the excretory cell suggest the possibility of the export of excretory–secretory proteins to the larval surface; however, the mechanisms underlying transport of these antigens to the larval surface from the excretory cell has not been elucidated.

The general ultrastructural morphology of the excretory cell is similar to that reported for other nematodes. The work of Narang (1970, 1972) with *Enoplus brevis, Pangrellus redivivus, Dity-lenchus* spp., and *Heterodera rostochiensis,* Waddell (1968) with *Stephanurus dentatus,* Lee (1970) with *Nippostrongylus brasiliensis,* Nelson et al. (1983) with *Caenorhabditis elegans,* and Endo and Wergin (1988) with *Meloidogyne incognita* all show a similar pattern of morphological organization. This pattern consists of a single large cell that contains a large nucleus, large quantities of endoplasmic reticulum, membrane-bound vesicles, and canaliculi lined with a thickened cuticlelike material. Associated with this cell are several supporting cells that number only 3 in the case of *C. elegans* but are possibly more numerous in other nematode species.

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Bolbosoma capitatum and *Bolbosoma* sp. (Acanthocephala) from Sperm Whales (*Physeter macrocephalus*) Stranded on Prince Edward Island, Canada

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ABSTRACT: Specimens of *Bolbosoma capitatum* (von Linstow, 1880) and *Bolbosoma* sp. were recovered from 2 male sperm whales (*Physeter macrocephalus* L.) that died following a mass stranding on Prince Edward Island, Canada. Some aspects of previous descriptions of *B. capitatum* have been incomplete, particularly with characteristics of the hooks of the proboscis being poorly defined. Females of *B. capitatum* were found to have 16–18 longitudinal rows of hooks with either 7–8 or 8–9 hooks in each row. The largest hooks with strongly curved blades were apical to median (overall range 69–122 μm long), whereas the basal hooks were spinelike (68–91 μm long). The basal hooks had a unique transverse orientation of the roots, an attribute apparently shared only with *B. physeteris* Gubanov, 1952, among the 14 species of *Bolbosoma* from cetaceans and pinnipeds. Although *Bolbosoma capitatum* had apparently been reported from *Physeter macrocephalus* in the eastern Atlantic Ocean, none of these records could be substantiated. The current report constitutes a new geographic record (Gulf of St. Lawrence, Canada) and the first account of this parasite in sperm whales from North American waters.

KEY WORDS: *Bolbosoma capitatum, Physeter macrocephalus,* acanthocephalans, cetaceans, stranding.

Acanthocephalans have seldom been reported from large cetaceans in North American waters (Van Cleave, 1953; Margolis and Dailey, 1972; Margolis and Arai, 1989; Measures, 1992), although some species of *Corynosoma* Lühe, 1905, and *Bolbosoma* Porta, 1908, are considered typical of whales and dolphins (Van Cleave, 1953; Deliamure, 1955). Additionally, relatively little is known about the helminth faunas of sperm whales (*Physeter macrocephalus* L.; Physeteridae) in either the eastern North Pacific or the western North Atlantic oceans (Margolis and Dailey, 1972; Margolis and Arai, 1989), and recent studies have suggested that acanthocephalan parasites may be useful in the definition of cetacean populations (Dailey and Vogelbein, 1991). In the current study, we present new records for species of *Bolbosoma* from sperm whales in the southern region of the Gulf of St. Lawrence, Canada. Additionally, new details of the morphology of *Bolbosoma capitatum* (von Linstow, 1880) are described.

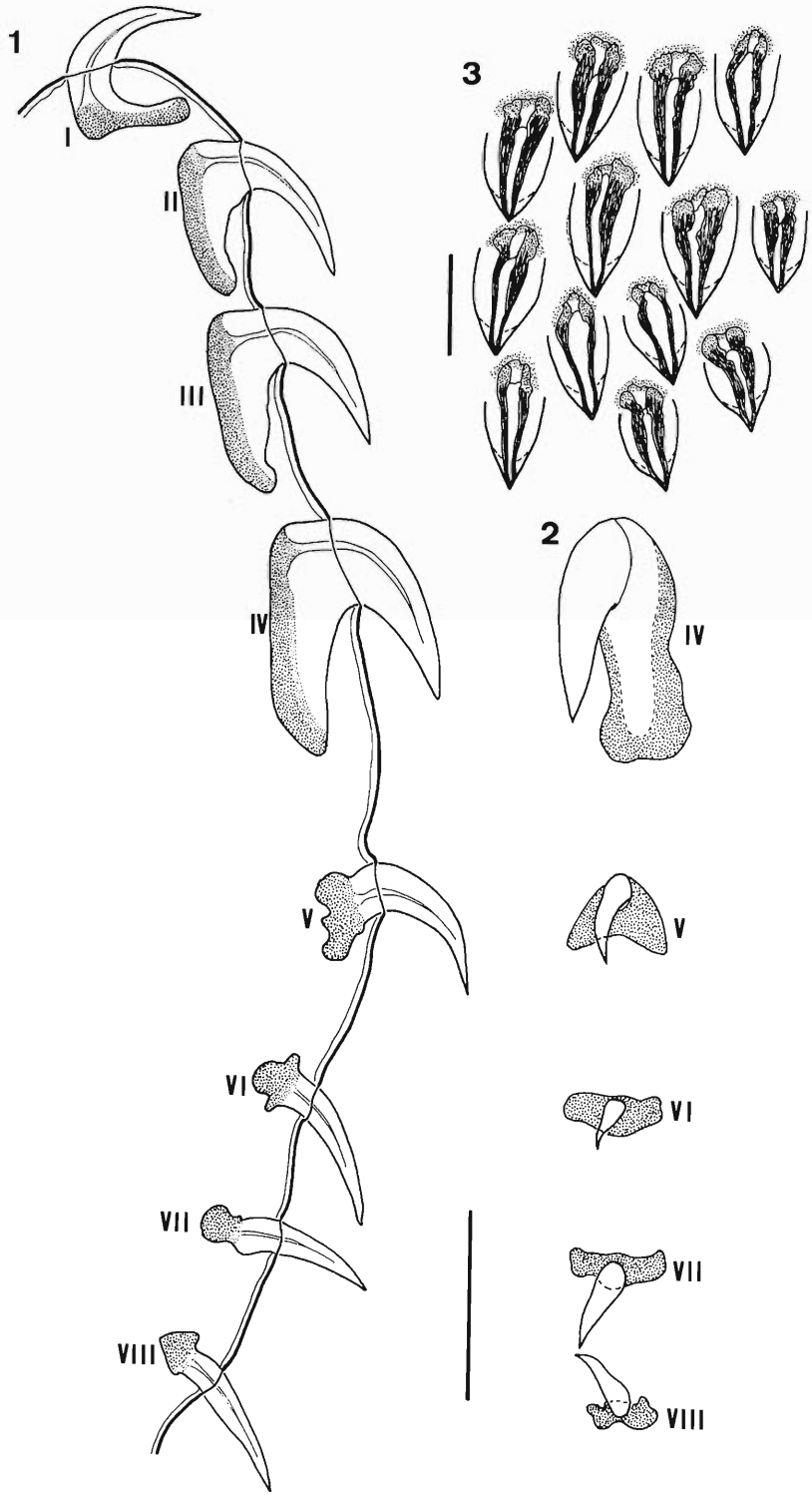
Materials and Methods

On the morning of 1 October 1989, 6 sperm whales stranded near Covehead Harbor on the northern shore of Prince Edward Island, Canada (46°24′N, 63°10′W), approximately 200 m from a bridge under repair. Two of these whales, both male, subsequently died and were examined for parasites. The first animal, an adult, 13.6 m long (males reach sexual maturity near 12.5–12.8 m [Best et al., 1984; Whitehead and Waters, 1990]), died on the afternoon of 1 October, and a necropsy was performed on the morning of 4 October. All internal organs, except those of the head, but including the entire gastrointestinal tract, were examined. The second animal, 11.4 m long, was towed offshore, with the 4 remaining whales, in the early evening of 1 October but died shortly afterward. It was found beached, approximately 50 km west of Covehead Harbor, 4 days later and examined at necropsy on the morning of 7 October. It was in an advanced state of postmortem decomposition, and only portions of the small intestine were examined for the presence of parasites. No disease process was identified in either whale, and mishap was considered a plausible cause of the stranding.

Acanthocephalans were fixed in 10% formalin, stained with Semichon's acetic carmine, dehydrated, and mounted entire. The proboscises from 2 specimens were dissected for examination of the structure of the hooks. Measurements are in micrometers unless specified otherwise; sample sizes (*N*) are followed by the range with the mean and standard deviation in brackets.

Results

Acanthocephalans of the genus *Bolbosoma* were the only helminthic parasites recovered from either whale. Only 9 female specimens of *Bolbosoma capitatum* were found in the small intestine of the first whale (deposited in the U.S.



Figures 1–3. ***Bolbosoma capitatum*** **(von Linstow, 1880). Scale bars = 100 μm; same scale in Figures 1 and 2. 1. Armature of proboscis in lateral view showing structure and distribution of hooks in a row of 8 (numbered from the anterior, I–VIII). Note typical apical to median hooks (I–IV) with strongly curved blades and elongate**

National Parasite Collection, U.S. Department of Agriculture, Beltsville, Maryland, No. 82700, and in the New Brunswick Museum, St. John, New Brunswick, Canada, No. 10211). Three specimens of a small *Bolbosoma* sp. were recovered from the second whale but could not be definitively identified because the proboscis was not extended in any of these immature females (U.S. National Parasite Collection, No. 82701). The short descriptions presented below augment current knowledge of species of the genus *Bolbosoma* in cetaceans.

Among 5 specimens of *B. capitatum* examined in detail, all were females, measuring 51.5–69.0 mm long by 2.0–2.3 mm in maximum width in the trunk and showing early development of ovarian balls. The forebody was distinctly separated from the trunk by a narrow constricted region up to 5.0 mm in length. The proboscis was cylindrical and rounded, measuring ($N = 3$) 575–760 (662 ± 92.99) × 380–475 (428 ± 47.52) and armed with 16–18 longitudinal rows of 7–8 (2 specimens) or 8–9 (1 specimen) hooks (usually 8). Hooks, apical to median in position, had prominent curved blades, were 4 in number, with the 4th hook being the largest in each row (numbered from the anterior hooks measured: I [$N = 6$] 68–88 [78 ± 8.16], II [$N = 13$] 78–101 [91 ± 7.28], III [$N = 13$) 91–114 [103 ± 6.98], IV [$N = 16$] 96–122 [112 ± 8.61]); these hooks had strongly developed elongate roots (I [$N = 5$] 52–55 [53 ± 1.64], II [$N = 12$] 70–96 [81 ± 7.56], III [$N = 10$] 88–104 [97 ± 8.49], IV [$N = 16$] 91–122 [110 ± 10.32]) (Fig. 1). Basal hooks numbered from the anterior, began with the 5th hook, were 3–4 or 4–5 in number and spinelike (V [$N = 1$] 78, VI [$N = 1$] 81, VII [$N = 4$] 70–81 [77 ± 5.32], VIII [$N = 9$] 78–91 [82 ± 3.88], IX [$N = 1$] 68]) and with prominent roots oriented transverse to the longitudinal axis of the proboscis (V [$N = 6$] 55–57 [57 ± 0.82], VI [$N = 6$] 44–55 [50 ± 4.22], VII [$N = 6$] 39–52 [46 ± 4.46], VIII [$N = 6$] 31–40 [37 ± 3.14], IX [$N = 1$] 34) (Figs. 1, 2). The enlarged cephalic bulb was 2.22–2.35 mm wide and armed with 2 prominent fields of spines that were not confluent ventrally. In the anterior field, there were about 10 transverse rows with a gradient in length from anterior to posterior; 35–85 in length. The posterior field of spines was situated across the broadest region of the bulb, also arranged in approximately 10 transverse rows and with an irregular gradient in length from the anterior to the posterior ([$N = 30$] 80–165 [115 ± 20.30]) (Fig. 3). The proboscis receptacle was 2,100–2,150 × 405–520 and extended beyond the posterior margin of the bulb. Lemnisci were broad, convoluted, and relatively short, not exceeding 3.4 mm, and not extending into the narrow constricted region. The uterine bell was located in the far posterior; combined length of the vagina, uterus, and uterine bell was 5.3–7.7 mm.

Three immature female specimens of *Bolbosoma* from the second whale measured 18.7–28.0 × 1.0–1.07 mm. The cephalic bulb was 1.0–1.25 mm in width; a prominent constriction separating the forebody from the trunk was lacking. The proboscis receptacle was 1,580–2,100 × 390 and the lemnisci were 750 in length; neither extended beyond the base of the bulb. The combined length of the vagina, uterus, and uterine bell was 3.05–4.45 mm. It could not be determined whether these specimens were immature *B. capitatum* or referable to another species.

Discussion

Bolbosoma capitatum was described briefly by von Linstow (1880) from false killer whales (*Pseudorca crassidens* (Owen)). It was subsequently redescribed by Porta (1906, 1908, 1909) with this information being perpetuated relatively unaltered by Meyer (1932), Deliamure (1955), Petrochenko (1958), and Yamaguti (1963). Edmonds (1957, 1987) and Machado Filho (1964) have augmented this redescription, but some pertinent information is lacking for the structure of the armature on the proboscis and bulb.

In contrast to some keys (Meyer, 1932; Deliamure, 1955; Petrochenko, 1958), *B. capitatum* is similar to other species of *Bolbosoma* in having a distinct armature on the proboscis (spinelike basal hooks with reduced or transversely expanded roots and strongly curved apical to median hooks with more typical elongate roots) and the cephalic bulb (simple spines) (in agreement

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roots and spinelike basal hooks (V–VIII) with reduced roots. 2. Armature of proboscis showing structure of roots on the largest median hook (IV) and spinelike basal hooks (V–VIII). Note that roots of the basal hooks are transversely elongate with respect to the longitudinal axis of the proboscis. 3. Armature of forebody showing pattern and structure of spines in the midregion of the posterior field of the inflated cephalic bulb.

with Porta, 1906, 1908, 1909; see Van Cleave, 1953) (Figs. 1–3). The range for numbers of hooks and hooks per row on the proboscis in the current specimens was in general agreement, particularly with more recent detailed reports (14–16 longitudinal of 8 hooks [Edmonds, 1957], 18 longitudinal of 8 [Machado Filho, 1964], and 15–17 longitudinal of 6–8 [Edmonds, 1987]).

Although the length and distribution of spines on the cephalic bulb agreed with those depicted by Machado Filho (1964), von Linstow (1880) and Porta (1906, 1909) have described 2 fields of spines that were confluent ventrally on the bulb of *B. capitatum.* Voucher specimens of *B. capitatum* examined during the current study (collected by C. Parona from *Globicephalus svineval* = *G. malaena* (Traill) in the Mediterranean Sea; confirmed by H. J. Van Cleave [USNM 6299]) were found to have 2 distinct fields of spines, similar to specimens from the Canadian sperm whale. Thus, it is possible that the distribution of spines on the bulb is variable or that *B. capitatum* represents a complex of at least 2 poorly defined species.

The dimensions and structure of the hooks and roots of *B. capitatum* have not previously been determined accurately. Machado Filho (1964) measured hooks on a proboscis that was not fully extended and apparently obscured (I = 65 μm, II = 70, III = 70, IV = 72, V = 60, VI = 56, VII = 40) and indicated that the largest hooks were median in position. Although the lengths of hooks found in the current study exceeded those reported by Machado Filho (1964), the general distribution was similar. The transverse orientation of the roots of basal hooks, depicted by Porta (1906, 1909), appears to be rare among species of the genus *Bolbosoma.*

Two species, *B. capitatum* and *B. physeteris* Gubanov, 1952, are the only members of the genus where a transverse orientation of the roots of basal hooks of the proboscis has been recognized (Porta, 1906; Deliamure, 1955). These species are similar in overall dimensions (females of *B. physeteris* are 47–87 mm in length) and most mensural characters overlap. They may differ in the number of longitudinal rows of hooks but there are conflicting data for this attribute with respect to *B. physeteris* (original description: 18–20 rows of 6–8 hooks [Deliamure, 1955; also in Yamaguti, 1963], 18–20 of 6–8 or 20–24 of 7–8 [Petrochenko, 1958], 20–24 of 7–8 [Skrjabin, 1970]). Relatively few characters exist to distinguish adequately between *B. capitatum* and *B. physeteris,* and apparent differences in the bulb armature require confirmation (numbers of transverse rows of spines and spine length) (Deliamure, 1955).

Among the 14 species of *Bolbosoma* listed by Amin (1985), 4 have been reported from sperm whales: *B. brevicolle* (Malm, 1867), *B. physeteris, B. tuberculata* Skrjabin, 1970, and *B. capitatum* (from the eastern Atlantic basin, Mediterranean Sea, southern Australia; primarily from delphinids and ziphiids). Although many of these species have apparently broad host and geographic ranges (Shipley, 1899; Porta, 1909; Baylis, 1929, 1932; Meyer, 1932; Deliamure, 1955; Edmonds, 1957, 1987; Yamaguti, 1963; Skrjabin, 1970; Dailey and Vogelbein, 1991), records of species of *Bolbosoma* from the northwestern Atlantic and the Gulf of St. Lawrence are limited (Van Cleave, 1953; Measures, 1992). Cowan (1967) provided the only report of *B. capitatum* in this region (in *Globicephalus melaena* on the coast of Newfoundland), but it has apparently not been found in the Gulf of St. Lawrence and appears to be rare in North American waters.

Records of *B. capitatum* from *P. macrocephalus* could not be substantiated. Baylis (1932), in a review of cetacean helminths, apparently was the first to specify sperm whales as a host. However, accounts cited by Baylis (1932) did not refer to *B. capitatum* from *P. macrocephalus* (see von Linstow, 1880; Shipley, 1899; Porta, 1906, 1908, 1909; Hamilton, 1916; Meyer, 1931) and there is no indication that new material was examined. Although commonly listed as a parasite from sperm whales (Deliamure, 1955; Petrochenko, 1958; Dailey and Brownell, 1972), none of these appear to constitute an original record. Notably, neither Meyer (1932) nor Yamaguti (1963) recognized *P. macrocephalus* as a host. Thus, our discovery of *B. capitatum* may represent a new host record; otherwise, it suggests that this helminth is a rare or an incidental parasite of sperm whales.

The occurrence of immature females, lacking copulatory caps, further indicates that *P. macrocephalus* may be an atypical host for *B. capitatum.* Typically, in early infections of other polymorphids (e.g., *Corynosoma* spp.), the sex ratio is close to 1:1 and males are rapidly passed from the host following copulation (Van Cleave, 1953; Valtonen and Helle, 1982; Adams, 1988).

There is only a single, wide-ranging stock of

sperm whales recognized in the North Atlantic (Mitchell, 1975), with males usually found at latitudes above 40°S and 45°N, either singly or in small bachelor pods (Reeves et al., 1986; Rice, 1989). Generally highly pelagic and found over deep water, they regularly occur in areas less than 200 m in depth on the Scotian Shelf (Whitehead et al., 1992), but in the shallow region of the Gulf of St. Lawrence (100 m or less, except for the Laurentian Channel) they appear relatively uncommon (other strandings of *P. macrocephalus* involved a whale on the northern shore of Prince Edward Island in December 1988 and 3 whales in December 1991 [Daoust, pers. obs.]). Thus, it is likely that the mass stranding at Covehead Harbor involved a bachelor group that included at least some adults.

Life cycles for species of *Bolbosoma* are considered to involve pelagic marine zooplankton (euphausiids and copepods) as intermediate hosts and a taxonomically broad array of fishes as paratenic hosts (Buron and Golvan, 1986; Edmonds, 1987; reviewed in Measures, 1992). Among male sperm whales, demersal fishes such as gadids and scorpaenids (known paratenic hosts for species of *Bolbosoma*) are a frequent but insubstantial component of the diet (Rice, 1989). Additionally, monkfish (*Lophius americanus* Valenciennes) (Lophiidae), a demersal predator in the western North Atlantic and the Gulf of St. Lawrence (Leim and Scott, 1966), has been found commonly in the stomach contents of sperm whales off Nova Scotia (Mullins et al., 1988). The wide range of piscine prey (including gadids, scorpaenids, cottids, osmerids, and others) and invertebrates exploited by monkfish indicate its potential as a paratenic host. Thus, the occasional exploitation of marine fishes could account for sporadic infections by species of *Bolbosoma* in sperm whales. Although mesopelagic squids of medium to large size are the primary prey of sperm whales throughout the world (Rice, 1989), they apparently have not been implicated in transmission of these acanthocephalans (Hochberg, 1990).

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Description and Surface Topography of a Larval Didymozoid (Trematoda) from *Apogon uninotatus* (Apogonidae) in Kuwait Bay

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ABSTRACT: A new larval didymozoid (Trematoda: Didymozoidae) was found in the stomach of the cardinalfish, *Apogon uninotatus,* in Kuwait Bay. The larva is characterized by the presence of a "stomach" and relatively short moniliform ceca comprising 6 chambers. The surface microtopography of the larva is basically similar to that of other digenean metacercariae. The larval surface is folded into a complex network of interconnecting lamellae. Only domed papillae were observed, presumably sensory organs. No spines were observed on the body tegument. The observed microtopographical features possibly facilitate migration in the definitive host tissue.

KEY WORDS: Trematoda, Digenea, Didymozoidea, larva, *Apogon uninotatus,* scanning electron microscopy, Kuwait Bay.

Didymozoids are tissue-dwelling parasites of marine predatory fishes (reviewed by Nikolaeva, 1985). The taxonomic position of this unique group of trematodes is debatable and the life cycles are obscure, although the larvae are known to occur in the alimentary tract and body muscles of a variety of invertebrates and small fishes. The small fishes presumably act as third intermediate host, acquiring the infection by ingesting infected crustacean second intermediate hosts or planktonic invertebrate paratenic hosts. Infection of the definitive host probably occurs by ingestion of infected small fishes. It is not known whether or not molluscs are involved in the life cycles of didymozoids, although this seems likely.

In this study, a new didymozoid larva from the stomach of *Apogon uninotatus* in Kuwait Bay was described, and its surface topography was examined by scanning electron microscopy (SEM).

Materials and Methods

Among fishes collected from intertidal pound-traps in Kuwait Bay, approximately 20 km west of Kuwait City, 10 specimens of *Apogon uninotatus* (Apogonidae), 6–10 cm long, harbored didymozoid larvae. Living larvae recovered from stomachs of the fish were washed in 0.7% saline and either prepared for light microscopy (LM) or SEM. For LM, larvae were fixed in alcohol-formalin-acetic acid, stained in Mayer's acid carmine or Ehrlich's hematoxylin, and mounted in Canada balsam. The larva was drawn with the aid of a camera lucida. Measurements were taken from stained specimens and are given in micrometers with averages in parentheses. For SEM, larvae were fixed for 1 hr in cold 2.0% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate. The larvae were then washed several times, postfixed for 10 min in cold 1% osmium tetroxide in the same buffer, and dehydrated in acetone. Larvae suspended in acetone were dried in a Technics critical-point drying apparatus using liquid CO_2 as a transitional medium. The larvae were sputter-coated with gold-palladium and viewed under a JEOL JSM-840 scanning electron microscope at an accelerating voltage of 15 kV. Approximately 50 larvae were examined in this study.

Results

Didymozoidae Poche, 1907

Immature larva

Description

Type host: *Apogon uninotatus* Smith and Radcliffe.

Site of infection: Stomach.

Type locality: Doha, Kuwait Bay.

Date of collection: March 1989.

Specimens: Deposited in the helminth collections of the Department of Zoology, University of Kuwait, and CAB International Institute of Parasitology, No. S-1087.

Diagnosis

Description, based on 7 specimens (Fig. 1): body slender 580.0–1180.0 (783.8) by 80.0–130.0 (108.8). Eyespots absent. Oral sucker 47.5–62.5 (56.7) by 25.0–52.5 (44.3), pyriforms entirely muscular. Pharynx 12.5–15.0 (13.6) by 10.0–15.0 (13.3). Ventral sucker 62.5–75.0 (68.2) by 57.5–70.0 (62.5); 15.00-34.0 (21.6) from anterior end of body. Esophagus sinuous, 130.0–177.5 (156.7) long. Stomach 37.5–85.0 (56.6) by 32.5–55.0 (42.9), thick-walled, surrounded by gland cells. Ceca each composed of 6 dilated, thin-walled chambers sequentially becoming larger, terminating at different levels. Excretory vesicle

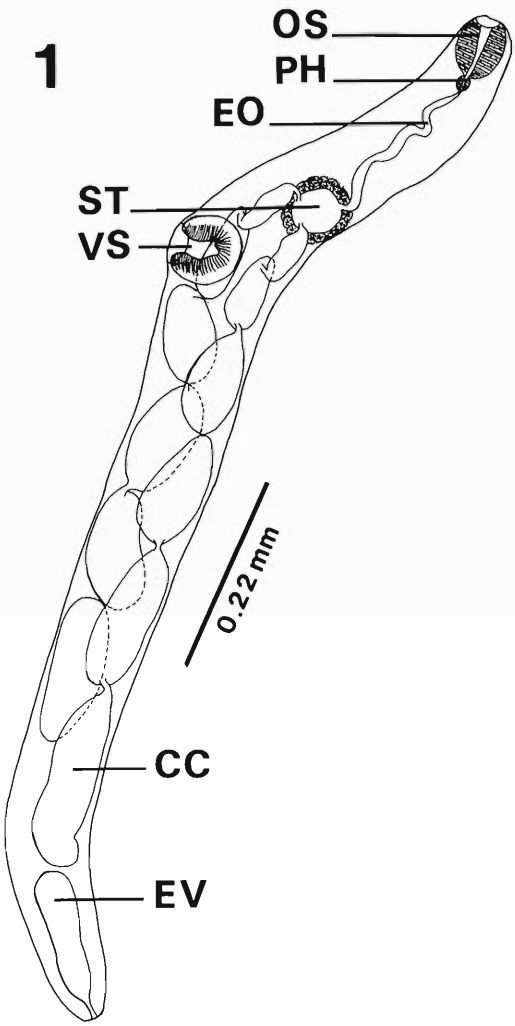


Figure 1. A didymozoid larva from *Apogon uninotatus*. CC = cecal chamber, EO = esophagus, EV = excretory vesicle, OS = oral sucker; PH = pharynx, ST = stomach; VS = ventral sucker.

postcecal 60.0–140.0 (108.0) by 25.0–67.5 (44.6), pore terminal.

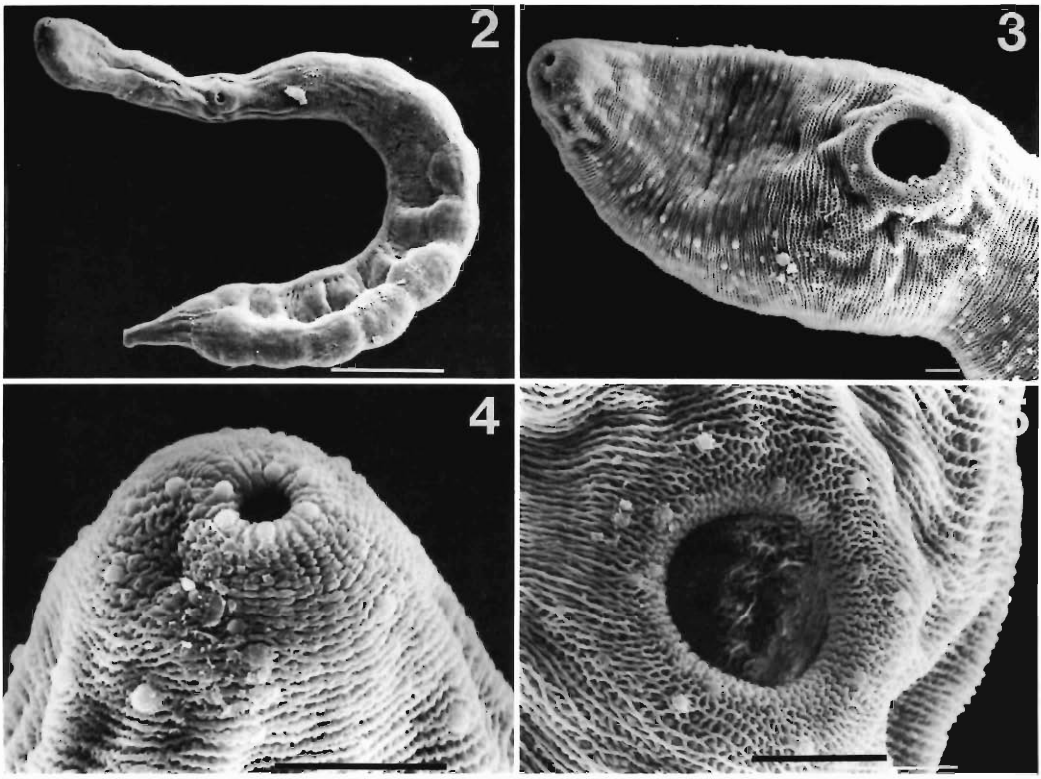
Surface microtopography

The body of the larva can be divided into 3 parts based on the body shape and microtopography (Fig. 2): (1) dorsoventrally flattened to subcylindrical forebody from the oral sucker to the ventral sucker region, (2) laterally expanded hindbody, and (3) sharply tapered short posterior part resembling the ecsoma of hemiurids. The prominence of this division depends on the state of muscular contraction. Domed papilla are concentrated on the ventral and lateral aspects of the body, particularly in the region between the suckers (Figs. 3, 4, 7). The oral sucker is subterminal, surrounded by 6 domed papillae (Fig. 4). The ventral sucker is surrounded by 2 circles of domed papillae, each with 6 papillae radially arranged on the outer margin of the sucker (Fig. 5). The forebody and the hindbody bear a series of circumferentially oriented ridges. Within the oral and ventral suckers, the ridges are arranged radially (Figs. 4, 6). The tegument of the posterior part of the larva is distinctly separated from the general pattern of the body by longitudinally oriented ridges (Fig. 8). At high magnification, tegumental ridges appear as a complex network of interconnecting lamellae changing in appearance according to state of body contraction (Figs. 9, 10). An invagination, the opening of the excretory pore, is observed at the posterior end of the larva (Fig. 8). No spines or ciliated papillae were observed on the tegument.

Discussion

Species of larval didymozoids with "stomach" and moniliform ceca have been reported from a variety of small fishes in tropical and subtropical waters (Yamaguti, 1942, 1970, 1975; Fischthal and Kuntz, 1964; Nikolaeva, 1965, 1970; Fischthal and Thomas, 1968; Madhavi, 1968; Kurochkin and Nikolaeva, 1978; Køie and Lester, 1985). The present species is characterized by the presence of a relatively short cecum with small number of chambers. It is most similar to immature didymozoid species 1 recovered from several species of small fishes in Moreton Bay, Queensland, Australia (Køie and Lester, 1985) and a didymozoid metacercaria from a copepod in Bay of Bengal (Madhavi, 1968). However, the former has 5 cecal chambers and the latter lacks a pharynx. This report is the second on larval didymozoids from fish in Kuwait Bay. Abdul-Salam et al. (1990) described a new species from *Nemipterus peronii,* which differs from the present species in anatomical and topographical features.

Several attempts have been made to develop a scheme for the classification of larval didymozoids (Nikolaeva, 1965; Yamaguti, 1970, 1975; Kurochkin and Nikolaeva, 1978). However, the proposed schemes were not successful because they were based on extremely variable criteria that change with age such as body size, ratio of body length to width, distance between

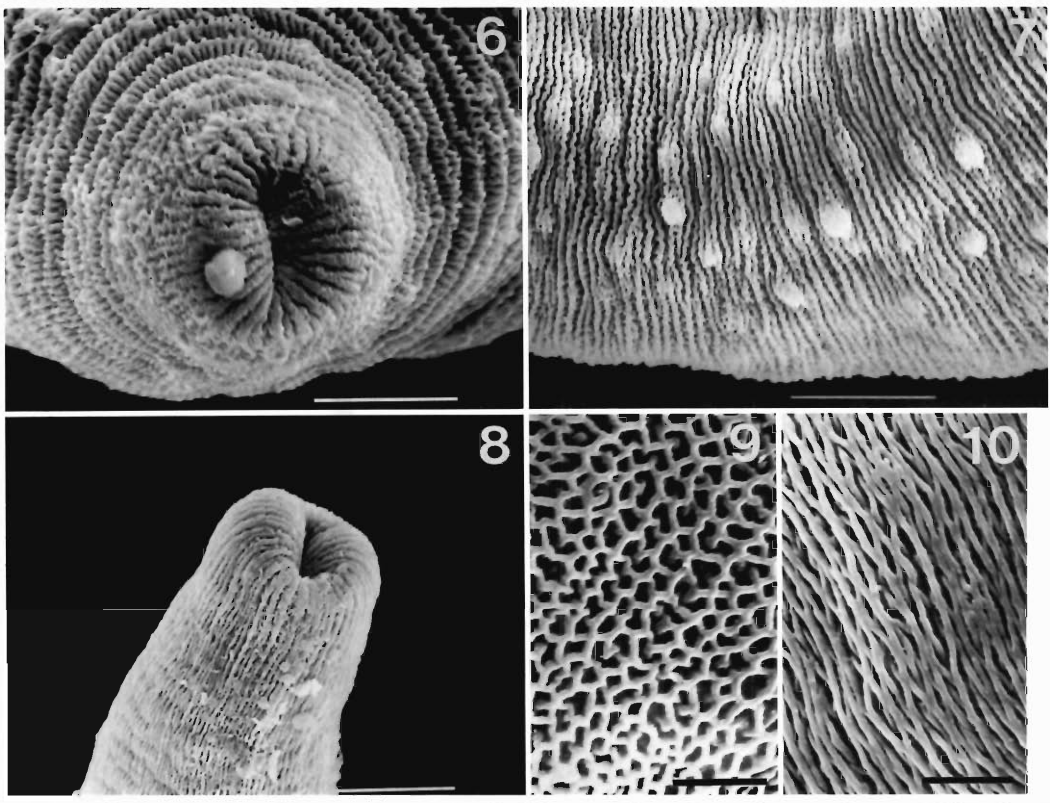


Figures 2–5. Scanning electron micrographs of a didymozoid larva from *Apogon uninotatus*. 2. Whole view, ventral surface showing dorsoventrally flattened forebody bearing oral and ventral suckers, laterally expanded hindbody showing cecal chamber impressions, and sharply pointed posterior part. Scale bar = 100 μm. 3. Forebody, ventral view showing oral sucker, ventral sucker and papillar distribution. Scale bar = 10 μm. 4. Anterior end, showing papillar distribution on and around oral sucker. Scale bar = 10 μm. 5. Ventral sucker surrounded by concentrically arranged domed papillae. Scale bar = 10 μm.

suckers, shape of the digestive tract, and species of host. Køie and Lester (1985) concluded that with the present knowledge it is impossible to classify the larval didymozoids to generic or higher taxonomic levels. SEM studies on surface topography of larval didymozoids, in particular, number and distribution of papillae on the sucker(s), may introduce reliable taxonomic features. LM investigations on other species of trematodes have demonstrated that papillar arrangement remains constant during development (Goodchild, 1943; Thomas, 1958). Fischthal (1951) made considerable use of papillar arrangement in the taxonomy of rhopalocercariae, and Bakke and Lien (1978) suggested the use of SEM images of papillar arrangement in the oral sucker as a basis for the taxonomy of *Phyllodistomum* species.

Although it is not possible to associate the larval didymozoid reported herein with any adult didymozoids, it is of interest to note that SEM images of the posterior part of the larva show a structure resembling the ecsoma of hemiurids described in *Neometadidymozoon helicis* (Lester, 1979). The presence of such a structure in a larval didymozoid lends support to the view that didymozoids are digenetic trematodes related to the hemiurids (Cable, 1974; Brooks et al., 1985).

The surface topography of the didymozoid larvae did not differ essentially from that of other digenean metacercariae examined by SEM (Køie, 1985). The most distinctive characteristics of the tegument are the extensive formation of ridges covering the entire body, concentration of domed papillae on the ventral surface, particularly around the suckers, and absence of spines or ciliated sensory structures. The highly ridged tegumental surface of the larva possibly allows greater strength and flexibility essential during



Figures 6–10. Scanning electron micrographs of a didymozoid larva from *Apogon uninotatus*. 6. Invaginated ventral sucker showing pattern of tegumental ridges. Scale bar = 10 μm. 7. Body tegument showing circumferentialy oriented ridges and papillae. Scale bar = 10 μm. 8. Posterior part showing terminal invagination of the excretory pore and longitudinally oriented tegumental ridges. Scale bar = 10 μm. 9. High magnification of tegumental ridges in stretched state. Scale bar = 4 μm. 10. High magnification of tegumental ridges in contracted state. Scale bar = 4 μm.

migration in cavities and tissues of the paratenic or definitive host. Comparable patterns of ridges have been observed on the surface of juvenile *Schistosoma mansoni,* and it has been suggested that they facilitate increase in size and volume of the worm during growth and provide flexibility during movement (Voge et al., 1978; Crabtree and Wilson, 1980; Basch and Basch, 1982). The domed papillae present on the tegument of the didymozoid larva are similar in structure to those described in other larval and adult digeneans (Smyth and Halton, 1983). From their structure and location, they could have a mechanoreceptive function involved in orientation during larval migration in the definitive host tissue.

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Obituary Notice

JUSTUS F. MUELLER

20 November 1902 — 1 April 1993
Honorary Member 1978

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A New Zoogonid Cercaria (Trematoda: Digenea) from the Florida Horse Conch, *Pleuroploca gigantea*, in the Northwestern Gulf of Mexico

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ABSTRACT: A seventh known species of larval zoogonid is reported, which was found parasitizing the gonad and digestive gland of the large carnivorous gastropod mollusc *Pleuroploca gigantea* collected in the Gulf of Mexico southeast of Galveston, Texas. Descriptions of the sporocyst and cercarial stages are given. The larva is assigned the temporary designation of "zoogonid *Cercaria* A" until further information concerning its life cycle and specific identity becomes available. The morphology of the cercaria most nearly resembles that of the cercaria of *Zoogonoides viviparus* (Olsson, 1868) Odhner, 1902, from which it differs by its lack of a prepharynx and lack of anterolateral indentations in the stylet. Other differences are in body size, host species, and host geographic range.

KEY WORDS: Zoogonidae, cercaria, marine cercaria, *Pleuroploca*.

Adult zoogonid trematodes are parasites of the digestive tracts of marine fishes. In those species for which life cycles are known, tailless xiphidiocercariae develop in sporocysts in marine snails and subsequently encyst to become metacercariae in a variety of benthonic invertebrates of limited mobility such as polychaete annelids and echinoderms (Stunkard, 1938, 1940, 1941, 1943; Prevot, 1966; Koie, 1976). In one species, however, cercariae apparently encyst within the sporocyst in the snail host (Palombi, 1930, 1934).

Of the known species of zoogonid cercariae, as tabulated by Madhavi and Shameem (1991), 2 have been reported from the western Atlantic Ocean (Stunkard, 1940, 1941). None has been reported from the Gulf of Mexico.

Materials and Methods

Trawl samples from the Gulf of Mexico 18–20 km southeast of Galveston, Texas, at a depth of 5–6 m yielded 5 specimens of the large carnivorous gastropod *Pleuroploca gigantea,* which were examined for parasites. The snails ranged from 83 to 263 mm in total shell length.

Figures of sporocysts and cercariae were prepared freehand from living material stained with neutral red in seawater under light coverslip pressure at magnifications of ×100–1,000. Measurements (in micrometers) were taken from 10 naturally shed heat-killed specimens under light coverslip pressure. Measurement ranges are followed by mean values in parentheses. Specimens were fixed in formalin–acetic acid–alcohol, stained with acetocarmine, dehydrated in alcohol, cleared in xylene, and mounted in Permount medium.

Results

The smallest of the 5 *Pleuroploca gigantea* examined (83 mm) was infected with a new zoogonid larva, which is described below.

Zoogonid *Cercaria* A (Figs. 1, 2)

DESCRIPTION: Body of tailless cercaria (Fig. 1) 220–315 (271.3) long, 55–77 (65.9) wide. Tegument aspinose anteriorly, becoming minutely spinose posteriorly. Posterior spines up to 1 in length. Mouth ventral and subterminal, oral sucker circular, 35–43 (39.2) in diameter. Stylet anterodorsal to oral sucker, lanceolate, 8–12 (10) long, 4–6 (5) wide. Prepharynx absent, pharynx doliiform, 15–27 (21) long, 9–16 (12.8) wide. Esophagus bifurcating anterior to ventral sucker, forming crura that extend posterolaterally terminating anterior to midlevel of ventral sucker. Contents of crura staining red in neutral red vital dye. Ventral sucker circular, 48–61 (53.3) in diameter, its anterior margin located at midlevel of body. Six pairs of granular penetration glands located in anterolateral portion of body, their ducts extending forward on each side in a bundle dorsolateral to oral sucker, terminating in anterior pores. Penetration glands and ducts staining light pink in neutral red stain as do 3 irregular and indistinct genital primordia posterior to ventral sucker. Excretory bladder oval, 45–57 (53.8) long, 30–45 (37.7) wide, thin-walled, loosely packed with evenly distributed spherical concre-

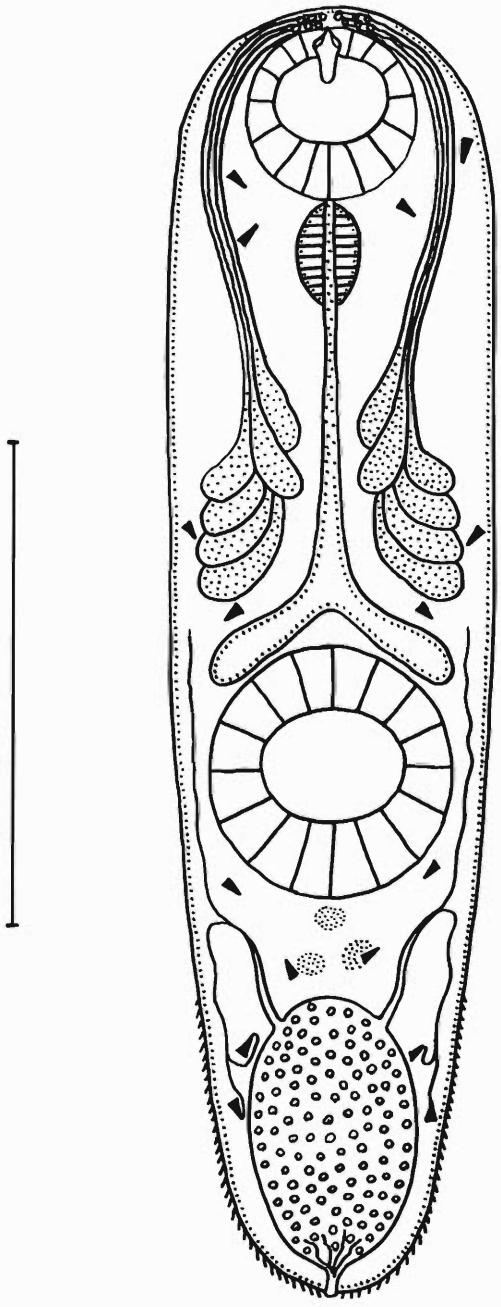

Figure 1. Zoogonid *Cercaria* A from *Pleuroploca gigantea*. Ventral view. Scale bar = 100 μm.

tions about 2 in diameter. Excretory pore posterior and terminal, communicating with bladder through excretory tube 8 in length. Flame cell formula 2[(2 + 2) + (2 + 2)] = 16. Right and left common excretory tubules enter bladder anterolaterally.

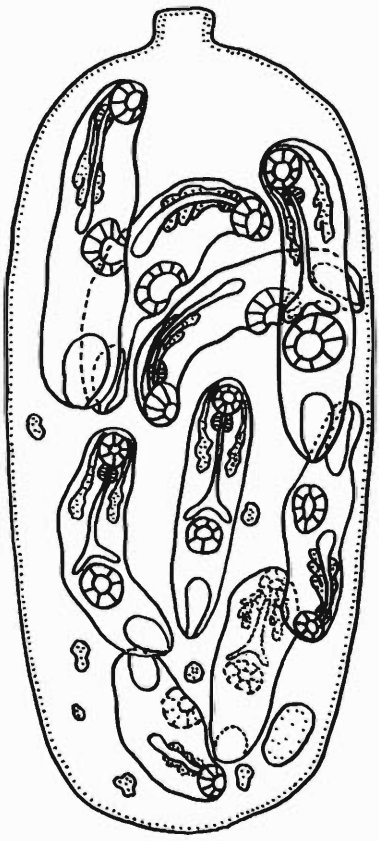

Figure 2. Sporocyst stage of Zoogonid *Cercaria* A from *Pleuroploca gigantea*. Lateral view. Scale bar = 100 μm.

Cercariae, which emerged from sporocysts, were observed crawling vigorously on substrate surface.

Sporocysts (Fig. 2) located in gonad and digestive gland of host snail, thin-walled, transparent and nonpigmented, 650–750 (685) long and 280–370 (315) wide, each containing up to 10 cercariae in various stages of development.

HOST: *Pleuroploca gigantea* (Kiener, 1840), Florida horse conch.

LOCALITY: Gulf of Mexico, 29°10′N, 94°42′W, southeast of Galveston, Texas.

HABITAT: Sand-mud substrate, depth 5–6 m.

PREVALENCE OF INFECTION: 1 of 5 snails (20%).

VOUCHER SPECIMEN: U.S. National Parasite collection, Beltsville, Maryland, 20705. USNM No. 82738.

Discussion

When compared morphologically to the previously reported zoogonid larvae, as tabulated

by Madhavi and Shameem (1991), *Cercaria* sp. A appears to be a separate species. *Cercaria* sp. A differs from the cercaria of *Diphterostomum brusinae* Stossich, 1904 (synonym: *Cercaria inconstans* Sinitzin, 1911), and from *Cercaria chilkaensis* Madhavi and Shameem, 1991, in having more than 3 pairs of penetration glands. It differs from *Cercaria brachycaeca* Shimura and Ito, 1980, and from *Cercaria crispata* Pelseneer, 1906, in having intestinal crura that extend to the level of the ventral sucker and in having an excretory bladder with a convex anterior margin. It differs from the cercaria of *Zoogonoides laevis* Linton, 1940, and from the cercaria of *Zoogonus lasius* (Leidy, 1891) Stunkard, 1940, in having intestinal crura that do not extend posterior to the ventral sucker.

Cercaria sp. A appears to be most similar to the cercaria of *Zoogonoides viviparus* (Olsson, 1868) Odhner, 1902, from which it differs in its lack of a prepharynx and anterolateral indentations of the stylet (as illustrated by Lebour, 1918). Other differences are in body size, host species, and host geographic range.

This constitutes the first report of a zoogonid larva from the Gulf of Mexico and the second report of a trematode species parasitizing the Florida horse conch, *Pleuroploca gigantea*. Wharton (1939) reported the occurrence of the nymph stage of the aspidogastrean *Lophotaspis vallei* (Stossich, 1899) Looss, 1902, from Florida, employing the older synonymous name for the horse conch, *Fasciolaria gigas* Linne.

It is interesting to note that, with the exception of the turbinid archaeogastropod host (*Batillus*) of *Cercaria brachycaeca,* all known gastropod hosts for the sporocyst and cercarial stages of zoogonids (Madhavi and Shameem, 1991) belong to the predaceous and carnivorous neogastropod superfamily Buccinoidea, which includes the families Buccinidae (*Buccinum*), Columbellidae (*Mitrella*), Nassariidae (*Nassarius, Ilyanassa*), Naticidae (*Natica*), and Fasciolariidae (*Pleuroploca*). It is quite likely that the movement of these predatory host gastropods between their invertebrate prey population centers may facilitate acquisition of second intermediate hosts by the tailless, nonswimming zoogonid cercariae, which are capable of crawling only short distances among the benthos during transmission. *Cercaria brachycaeca,* although tailless and bearing overall morphological similarities to zoogonid cercariae, might ultimately be proven to be a tailless opecoelid larva due to similarities in excretory system morphology, development in an elongated sporocyst with many cercariae, and utilization of an archaeogastropod first intermediate host, which is the host type used by the majority of typical opecoelid larvae.

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Affiliation of *Hyostrongylus rubidus* (Nematoda: Trichostrongylidae) with the Ostertagiinae, and Evaluation of the Synlophe and Other Structural Characters

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ABSTRACT: Classifications of the Trichostrongylidae have referred *Hyostrongylus* Hall, 1921, to the Ostertagiinae or the Graphidiinae. The genital cone and synlophe of *Hyostrongylus rubidus* (Hassall and Stiles, 1892) were studied to clarify the subfamilial position of the genus and to assess hypotheses for the origin of the Ostertagiinae. Paired "0" papillae, a putative synapomorphy for the Ostertagiinae, are located on the ventral aspect of the genital cone in *H. rubidus*. This character, along with the structure of the bursa, confirmed placement of *Hyostrongylus* in the Ostertagiinae rather than the Graphidiinae. The synlophe was composed of a largely symmetrical system of continuous ridges extending from the cervical zone to near the caudal extremity in males and females. At the midbody there were 40–58 ridges; in females the vulval region was modified by irregular cuticular inflations. It was concluded that current concepts for independent origins of genera of the Ostertagiinae from the Graphidiinae were not supportable, as such would result in polyphyly for the former and paraphyly for the latter subfamily. Additionally, the genus *Cervicaprastrongylus* Gibbons and Khalil, 1982, was considered to be distinct from *Hyostrongylus*.

KEY WORDS: Trichostrongylidae, *Hyostrongylus,* Ostertagiinae, Graphidiinae, synlophe, genital cone.

Hyostrongylus rubidus (Hassall and Stiles, 1892) Hall, 1921, the type for the genus (synonyms: *Strongylus rubidus* Hassall and Stiles, 1892; *Ostertagia rubida* Travassos, 1918; *Haemonchus rubidus* Sluiter and Swellengrebel, 1912; and *Trichostrongylus rubidus* Fiebeger, 1923) was originally recognized as a nematode parasite of the white-lipped peccary (*Tayassu pecari* (Link) = *Dicotyles albirostris* Illiger) in Brazil. Later described from domestic swine (*Sus scrofa* Linnaeus), it is now recognized as a characteristic parasite of the Suidae and Tayassuidae (Molin, 1860; Hassall and Stiles, 1892; Levine, 1980).

Hyostrongylus rubidus is considered as a cosmopolitan parasite of domestic swine and other suids and has been rarely reported from ruminants or other herbivores (Levine, 1980). The nematode is essentially absent in sylvatic ruminants from sub-Saharan Africa (Round, 1968) and has only recently been reported from bush pigs (*Potamochoerus porcus* (Linnaeus)) and red duikers (*Cephalophus natalensis* Smith) in South Africa (Boomker, 1990; Boomker et al., 1991). There are apparently only 3 records from cattle (South America, The Netherlands, Ukraine) and 2 from sheep (North America, Ukraine) (Becklund and Walker, 1967; Da Costa and Benevenga, 1971; Borgsteede, 1978; Levine, 1980; Trach, 1986). Roe deer (*Capreolus capreolus* (Linnaeus)) in Bulgaria have been the only cervids recognized as hosts (Ianchev, 1973). Records from lagomorphs are limited to European hares (*Lepus capensis* Linnaeus; reported as *L. europaeus*) in Austria (Kutzer and Frey, 1976).

Although the genus *Hyostrongylus* Hall, 1921, was established for *H. rubidus* from swine (Hall, 1921), Travassos (1921) referred this species to *Ostertagia* Ransom, 1907. Goodey (1924), Alicata (1935), and Travassos (1937) considered *Hyostrongylus* to be valid, as the latter author relegated the genus to the subfamily Trichostrongylinae. Possible affinities to *Ostertagia* and related genera were again indicated by the decision of Skrjabin and Shul'ts (1937; cited *in* Skrjabin et al., 1954) to place *Hyostrongylus* in the tribe Ostertagiea of the subfamily Trichostrongylinae. However, the tribe Hyostrongylea was later established for *H. rubidus* and several other genera within the Cooperiinae (Skrjabin et al., 1954). Subsequently, *Hyostrongylus* was transferred to the Graphidiinae by Durette-Desset and Chabaud (1977, 1981) and since has been retained in this subfamily (Durette-Desset, 1982, 1983, 1985, 1989). In contrast, Gibbons and Khalil (1982a) and Jansen (1989) supported recognition of *Hyostrongylus* within the Ostertagiinae, and Trach (1986) referred the tribe Hyostrongylini with *H. rubidus* to this subfamily.

Nematodes of this genus have been considered to hold an intermediate position with respect to

these latter subfamilies (Durette-Desset and Chabaud, 1977, 1981; Jansen, 1989) and, as a consequence, have been referred in recent literature to either the Graphidiinae (Durette-Desset, 1982, 1983, 1985, 1989) or the Ostertagiinae (Khalil and Gibbons, 1981; Gibbons and Khalil, 1982a, b). Thus, the systematics of the genus *Hyostrongylus* remains problematic but must be clarified to promote examination of hypotheses for the evolution of the subfamilies Graphidiinae and the Ostertagiinae of the family Trichostrongylidae (see Durette-Desset and Chabaud, 1977, 1981). Placement of the genus *Hyostrongylus* has a bearing on concepts for the validity and relationships of the Ostertagiinae and the Graphidiinae (Hoberg and Lichtenfels, 1992).

In the present study, we provide a detailed description of the synlophe and genital cone of *H. rubidus* that augments studies by Trach (1986). These data promote an assessment of the subfamilial placement of *Hyostrongylus*. Initial character analysis of the synlophe and genital cone, requisite for phylogenetic studies (Hennig, 1966; Wiley, 1981) among the trichostrongylid subfamilies, was conducted. Synapomorphic characters for definition of the Ostertagiinae were identified and constitute the basis for evaluating previous hypotheses for the relationship of the Ostertagiinae and the Graphidiinae. Comments on the validity of the genus *Cervicaprastrongylus* Gibbons and Khalil, 1982 (a putative synonym of *Hyostrongylus* according to Jansen [1989] and Durette-Desset et al. [1992]) are presented. Additionally, a lectotype, allolectotype, and paralectotypes are designated for *H. rubidus*, as Hassall and Stiles (1892) did not formally select and deposit a holotype and allotype in the original description.

Materials and Methods

Specimens were studied as temporary whole mounts cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol), or in glycerine, and examined with differential interference contrast light microscopy. Transverse sections were prepared freehand with a cataract knife and embedded in glycerine jelly. Sections were used to study the structure of the synlophe in the cervical zone (including the region of the esophageal–intestinal [EI] junction), anterior quarter, midbody, and posterior region of 6 males (sections prepared to the level proximal to the spicules) and 5 females. The configuration of irregular cuticular inflations at the level of the vulva in females was evaluated in whole mounts and sectioned specimens. Photographs of sections were oriented with dorsal surface toward the top of the plate and shown as if viewed from the anterior. Throughout the manuscript, measurements are presented in micrometers unless specified otherwise and presented as a range with $\bar{x} \pm 1$ SD in parentheses.

The current study focused on the configuration of the synlophe, esophageal valve, and genital cone (following Lichtenfels and Pilitt, 1991; Hoberg et al., 1993a). Other mensural and structural characters are included in the redescription (measurements of the ovejectors follow Lichtenfels and Pilitt, 1991). Genital papillae and bursal rays are numbered according to the methodology developed by Chabaud et al. (1970), and the orientation of the synlophe follows concepts presented by Durette-Desset (1985). The term "cuticular strut" follows Lee (1965).

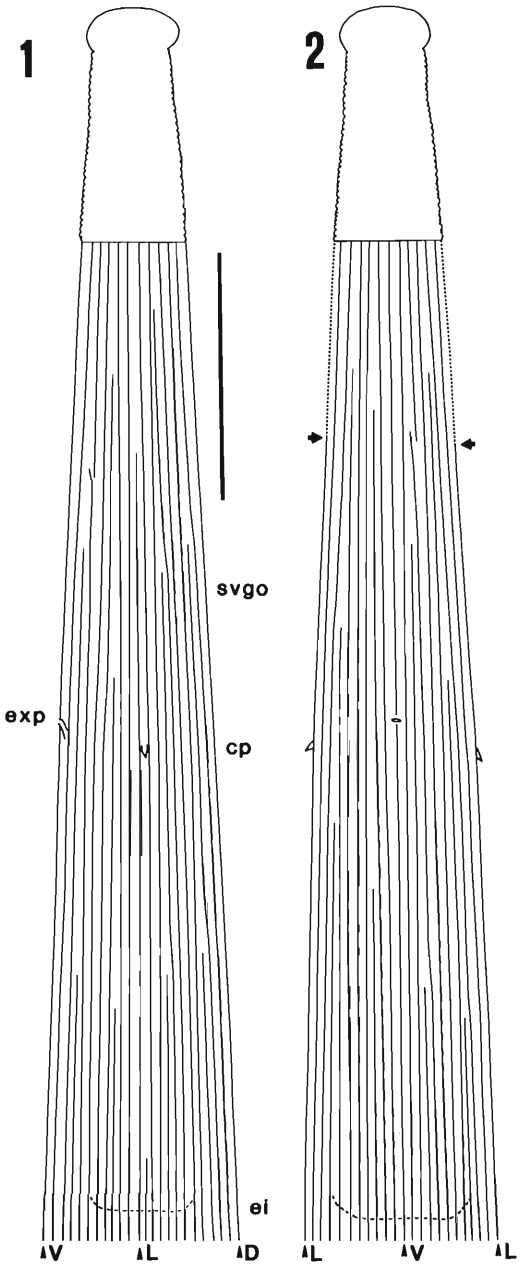
Specimens Examined

Specimens were obtained from the U.S. National Parasite Collection maintained at the Biosystematic Parasitology Laboratory, United States Department of Agriculture, Beltsville, Maryland, and included material from a variety of locations in North America and Central America collected between 1892 and 1981 (Table 1). Hassall and Stiles (1892) did not formally designate a holotype and allotype in the original description and such were not indicated among the specimens denoted as syntypes (USNM No. 14). Consequently a male specimen from this lot was selected as the lectotype and a female as the allolectotype for *Hyostrongylus rubidus*, with the remaining male and female syntypes becoming paralectotypes in accordance with the third edition of the International Code of Zoological Nomenclature (1985).

Results

General characters (synlophe and esophagus)

The synlophe in *Hyostrongylus rubidus* is composed of a largely symmetrical system of continuous parallel cuticular ridges that extends from the base of the cephalic expansion to near the caudal extremity in males and females (Figs. 1, 2). Ridges are perpendicular to the body wall, and a gradient or orientation is absent. In the cervical zone (anterior to the base of the esophagus), 18–22 ridges attain the base of the cephalic expansion. There are 34–42 and 32–44 ridges at the level of the prominent, thorn-like cervical papillae in males and females, respectively. Variation in numbers is attributable to differences in the levels of origin for individual ridges in the anterior. At the limit of the EI junction, there are 38–50 ridges in males and 42–55 in females. The synlophe is of uniform height, and there is minimal variation in the interval between ridges, as determined from sections, laterally, ventrally, or dorsally. A slight dorsoventral asymmetry is evident in the numbers of ridges posterior to the



Figures 1, 2. Cervical synlophe of *Hyostrongylus rubidus*. Scale bar = 100 μm. 1. Typical male specimen showing left lateral view and pattern of evenly spaced parallel ridges. Note the 2 pairs of continuous ridges that border the lateralmost ridge (L) and the anterior position of the subventral gland orifices (svgo). Other attributes depicted include the ventral (V) and dorsal (D) ridges, excretory pore (exp), cervical papilla (cp), and esophageal–intestinal junction (ei). 2. Male specimen showing ventral view and pattern of parallel ridges. Note that the lateral ridges (L) do not extend to the base of the cephalic expansion (indicated by dotted lines and arrows to denote region of termination). There are 3 continuous ventral ridges (similar to the Type A ventral system defined by Lichtenfels et al. [1988]) and the ventral ridge is interrupted at the level of the excretory pore.

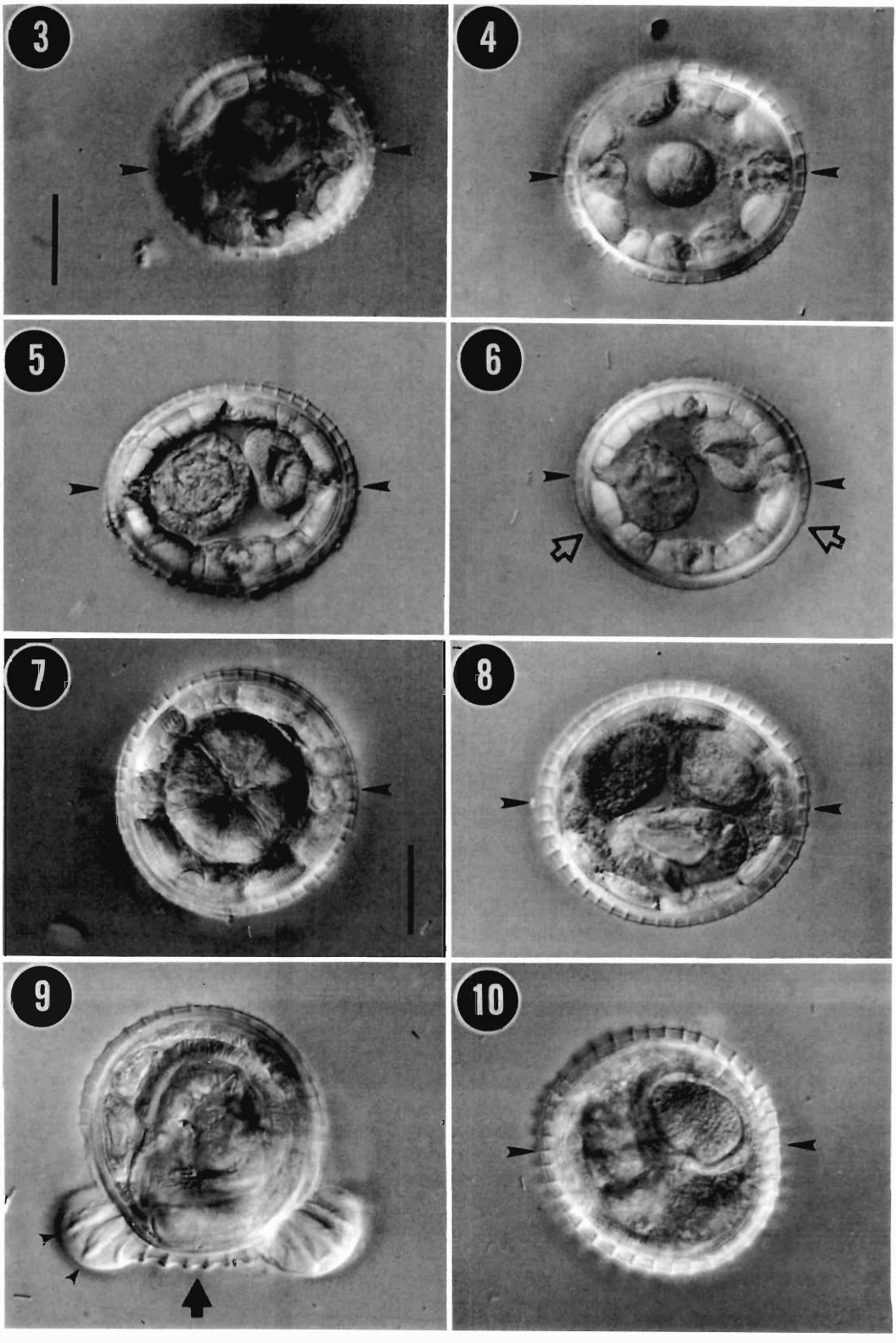
Table 1. List of specimens of *Hyostrongylus rubidus* with hosts and geographic localities.

USNM No.*	Locality	Host	♂†	♀†
82538‡	Maryland	*Sus scrofa*‖	1	1
14§	Maryland	*Sus scrofa*‖	6	5
5355	Maryland	*Sus scrofa*‖	2	1
18136	Virginia**	*Sus scrofa*‖	2	0
24540	Virginia	*Sus scrofa*‖	6	3
26226	Iowa	*Sus scrofa*‖	2	0
29398	Louisiana	*Sus scrofa*‖	5	5
31457	Puerto Rico	*Sus scrofa*‖	4	5
32649	Florida	*Sus scrofa*‖	6	4
56796	Illinois	*Ovis aries*‖	1	3
58403	Panama	*Sus scrofa*‖	6	6
61117	Maryland	*Sus scrofa*‖	11	12
68480	Maryland	*Sus scrofa*‖	11	12
69787	Alabama	*Sus scrofa*‖	5	5
76758	Florida	*Sus scrofa*††	3	3

* Collection number from U.S. National Parasite Collection.
† Numbers of specimens examined.
‡ Designated lectotype and allolectotype, selected from syntypes examined by Hassall and Stiles (1892).
§ Paralectotypes, representing remaining syntypes from Hassall and Stiles (1892).
‖ Domestic hosts.
** Animals originated in Kansas.
†† Sylvatic hosts.

cervical papillae, where often 1–2 unpaired ridges are present in either the dorsal or ventral fields.

Laterally, 2 pairs of continuous ridges bordering the lateralmost ridges (Fig. 1) extend from the cephalic expansion usually to near the caudal extremity, resulting in 5-ridge lateral fields (lacking a narrow interval between ridges, and not definable as the Type II pattern of Lichtenfels et al. [1988, 1990]). The left and right lateralmost ridges extend <75% of the distance anterior from the cervical papillae to the cephalic expansion and are slightly smaller than those in the adjacent lateral fields (Figs. 1, 2). Origins of ridges ventral and dorsal to these lateral fields usually occur adjacent to the 5-ridge system. However, single ridges may originate directly adjacent to the lateralmost near or posterior to the base of the esophagus and only rarely in the region near the cervical papilla. Ventrally in the cervical zone there are 3 continuous parallel ridges (Fig. 2) with



the ventralmost being interrupted at the excretory pore (similar to the Type A pattern of Lichtenfels et al. [1988]).

Posterior to the cervical zone there is considerable variation in the numbers of ridges and extent of the synlophe in males and females (Figs. 3–10). Ridges may originate in the lateral, dorsal, or ventral fields. Among males there are 41–57 ridges at the end of the first quarter, generally increasing to 40–58 at the midbody (Fig. 4). Posterior to the midbody there is sequential loss of ridges (Fig. 6), with the synlophe terminating dorsally at 67–96% of the body length from the anterior, ventrally at 65–92%, and laterally at 67–98% (prebursal papillae are situated at approximately 98% from the anterior); posteriad extent of the synlophe is not correlated with total length of the nematode. Consistently, the synlophe extends further posteriad laterally and dorsally than ventrally (Fig. 6). Although the synlophe may occasionally extend to near the prebursal papillae, usually prominent ventral and dorsal arcuate gaps are evident in the posterior third of the body. The interval between lateral ridges remains relatively constant; however, spacing of the dorsal and ventral ridges increases posteriad, coinciding with the termination of the synlophe. In contrast, among females there are 50–55 ridges at the end of the first quarter, 43–56 at the midbody (Fig. 8), 45–55 in the posterior third quarter, and 41–49 near the point of termination adjacent to the anus (Fig. 10); some lateral and dorsal ridges may extend onto the tail. Modification of the synlophe occurs at the level of the vulva where ridges may be interrupted ventrally and/or hypertrophied to form irregular cuticular inflations (Fig. 9), described in detail later. In females the interval among the lateral, ventral, and dorsal ridges remains relatively constant until termination of the synlophe near the anus.

The EI valve is relatively short in males and females (55–86 μm) (Tables 2, 3; Fig. 11). The orifices of the subventral esophageal glands are usually substantially anterior to both the cervical papillae and the excretory pore (Tables 2, 3; Fig. 1). A minuscule, triangular, dorsal esophageal tooth is present.

Female characters

Specimens with a synlophe and esophageal structures identical to those found in males were considered to represent *H. rubidus*. The tail varied considerably in length (Table 3), and consistently several prominent annulations were present (Fig. 12). Modification of the cuticle at the level of the vulva was variable in extent (Figs. 13–15), with inflations, as described later, being the most common ornamentation. However, flaplike structures that slightly overlapped the vulva were observed in 3 of 50 specimens (Fig. 16). The anterior infundibulum and vestibule + sphincter were consistently longer than those in the posterior (Fig. 17).

Among specimens examined (32 of 50), 64% exhibited irregular cuticular inflations at the level of the vulva (Figs. 9, 14, 15). The extent of the inflations was variable, with the most common form being a single broadened ventral zone immediately posterior to the vulva. However, additional prominent inflations occurred ventrally, anterior to the vulva, and as paired or single zones ventrolateral in position (Figs. 14, 15). Inflations are associated with irregular hypertrophy of the synlophe but lack specific orientation, form, or symmetry. Inflations are supported by single or multiple systems of enlarged ridges (fusion of ridges is occasionally observed), with erratically formed struts providing some internal foundation (Fig. 9). However, ridges are not evident superficially on the surface cuticle of most inflations. Posterior to the vulval region, the synlophe regains a typical configuration, as already described (Fig. 10).

Male characters

The bursal ray formula is 2–2–1 (see Durette-Desset, 1983) with the tips of rays 2 and 3 being

←

Figures 3–10. Synlophe of a male and female of *Hyostrongylus rubidus* in transverse section shown as viewed from the anterior with dorsal oriented toward the top of the figure and lateral ridges indicated by pointers. Scale bars = 25 μm. 3–6. Male specimen. 3. Level of esophageal–intestinal junction showing 39 ridges. 4. Midbody, showing 40 ridges. 5. Posterior region of third quarter showing 42 ridges. 6. Anterior region of fourth quarter showing approximately 33 ridges; note absence of ridges across ventral aspect (between arrows). 7–10. Female specimen. 7. Level of esophageal–intestinal junction showing 48 ridges. 8. Midbody showing 48 ridges. 9. Level of vulva (arrow) showing prominent inflations supported by irregularly hypertrophied cuticular struts (small pointers). 10. Posterior region of fourth quarter anterior to the anus showing 41 ridges.

Table 2. Morphometrics (in micrometers; range with $\bar{x}$ and SD in parentheses) of males of *Hyostrongylus rubidus*.*

Characters	1	2	3	4	5	6	7		8		
Number examined	—	—	—	—	—	—	20			50	
Body length	5,000	4,400–5,000	5,000–7,000	3,800–4,900	3,400–5,000	4,000–7,000	5,479–6,929	(6,130)	[49]†	3,047–7,025	(5,608 ± 863.9)
Cephalic vesicle length	—	—	—	—	—	—	—		[47]	60–88	(70 ± 6.76)
Esophagus length	—	—	—	524–539	570–590	—	590–768	(668)	[48]	484–750	(637 ± 50.19)
Esophagus as % of body length	—	—	—	11.0–13.8‡	12–17‡	—	10.7–11‡	(10.9)	[47]	8.9–16	(11.5 ± 1.47)
Esophageal–intestinal valve length	—	—	—	—	—	—	—	—	[49]	55–78	(68 ± 6.31)
Esophageal–intestinal valve width	—	—	—	—	—	—	—	—	[48]	36–57	(45 ± 5.29)
Subventral esophageal gland orifices§	—	—	—	—	—	—	—	—	[45]	186–305	(263 ± 24.5)
Excretory pore§	—	—	—	258–281	209–323	—	219–344	(277)	[46]	252–398	(321 ± 36.78)
Cervical papillae§	—	—	400	273–281	320–360	—	221–367	(290)	[48]	268–433	(339 ± 37.98)
Spicule length‖	130	127–134	—	114–121	127–139	127–134	123–140	(134)	[49]	103–144	(125 ± 9.53)

* 1 = Hassall and Stiles (1892), original description from natural infections in *Sus scrofa* from Washington, D.C. 2 = Travassos (1921), redescription of specimens in *Sus scrofa* from Brazil. 3 = Goodey (1924), redescription of specimens from naturally infected *Sus scrofa* from Great Britain. 4 = Alicata (1935), specimens from experimental infection in guinea pig (*Cavia porcellus*). 5 = Skrjabin et al. (1954). 6 = Sprehn (1957). 7 = Sarashina and Taniyama (1986), redescription from naturally infected *Sus scrofa* in Hokkaido, Japan. 8 = Present study, redescription from natural infections in *Sus scrofa* and *Ovis aries* from North America and Central America.

† *n* for individual measurements.

‡ Calculated from numerical values in previous description.

§ Measured from anterior.

‖ Typically the right and left spicules are equal in length.

convergent. The genital cone is typical of the Ostertagiinae (Fig. 18). The ventral of "0" papillae are paired (Fig. 19) and located on the ventral portion of the genital cone. The accessory bursal membrane is small and rectangular, positioned transversely on the dorsal aspect of the genital cone and supported by widely separated, minuscule "7" papillae (Figs. 18, 20). The dorsal ray (rays 9/10) is 39–47 μm in length, with a pair of large processes at 50–63% of the ray length from the anterior and a diminutive pair of lateral processes slightly anterior to the terminal bifurcation at about 80% from the anterior; additional small processes arise from the tips posterior to the bifurcation (Fig. 21). The dorsal ray is situated ventrally with reference to the externodorsal rays (ray 8) and contained in a small lobe (Figs. 21, 22).

The spicules are relatively short and not of great complexity (Fig. 23). Single dorsal and ventral processes of unequal length originate from the respective ala at 50–60% of the spicule length from the anterior. The dorsal process is long and slender, extending to near the tip of the main shaft (Figs. 18, 22, 23); the obscure ventral process is short and does not approach the spicule tip. The primary shaft of each spicule tip has an obscure hyaline foot and is surrounded by a membrane (Figs. 18, 21). The gubernaculum, highly elongate and narrow, is located dorsal to the lateral plates of telamon (Fig. 18).

Discussion

Morphology of *Hyostrongylus rubidus*

The synlophe in males and females was found to be largely identical, consisting of 38–55 continuous parallel ridges at the level of the EI junction. The numbers of ridges were found to increase posteriad and usually attained a maximum of 40–58 near the midbody. Although the synlophe had not been previously evaluated in detail, Hassall and Stiles (1892) noted 40–45 "longitudinal striae" in the original description of nematodes from North America but did not indicate at what level the ridges were counted. Goodey (1924), Skrjabin et al. (1954), and Thoonen and Vercruysse (1951) also reported the occurrence of ridges but did not specify the number present on specimens from Europe. Durette-Desset et al. (1992) reported approximately 50 indistinct ridges near the level of the midbody in specimens from North America. Sarashina and Taniyama (1986) indicated the presence of 40–45 ridges in specimens from Hokkaido, Japan.

There was a general agreement in morphometrics of most diagnostic characters among specimens representing populations from Asia, Europe, Central America, and North America (see Tables 2, 3). The results of the current study indicate a broader range in the length of the spicules in males and in the tail of females, but other mensural characters did not differ substantially. The spicules in males examined during the current study were found to be trifurcate (dorsal and ventral processes arising from the main shaft), agreeing with recent redescriptions presented in Trach (1986) and Govorka et al. (1988). However, males of *H. rubidus* were previously considered to have relatively unmodified spicules each with a single elongate dorsal process (see Hall, 1921; Skrjabin et al., 1954; Gibbons and Khalil, 1982a). Additionally, irregular vulval inflations in females appear to have been a variable character among most populations of *H. rubidus* examined in the current study. Inflations are rare among congeners (Durette-Desset et al., 1992), having been described only in *H. kigeziensis* Durette-Desset, Chabaud, Ashford, Butynski, and Reid, 1992.

Although specimens examined and redescribed by Travassos (1921) from Brazil appeared similar in all other major details to those from diverse regions, the length of the esophagus was markedly short (Table 3). The basis or significance of this difference in esophageal length of Travassos' (1921) specimens is unknown but could potentially indicate a lapse or, alternatively (but less likely), a regional isolation of this parasite in South America.

The broad morphological similarity of apparently disjunct populations of *H. rubidus* supports the concept of a widely distributed cosmopolitan species that has been disseminated extensively with the movement of domestic swine (e.g., to Australia [Pavlov, 1988] and North America). This may also be reflected in the relatively recent first reports of *H. rubidus* from Belgium in 1951, Japan in 1987, and South Africa in 1991 (Thoonen and Vercruysse, 1951; Sarashina and Taniyama, 1986; Boomker et al., 1991).

Validity of the genus *Cervicaprastrongylus*

Based on a comparison of the spicules, bursa, and genital cone of *H. rubidus* and details of

Table 3. Morphometrics (in micrometers; range with $\bar{x}$ and SD in parentheses) of females of *Hyostrongylus rubidus*.*

Characters	1	2	3	4	5	
Number examined	—	—	—	—	—	
Body length	8,000–8,500	5,300–8,000	8,000–9,000	4,800–8,000	7,340–9,360	(8,000)
Cephalic vesicle length	—	—	—	—	—	
Esophagus length	640	230–280	—	530–608	621–769	(700)
Esophagus as % of body length	7.5–8.0‡	3.5–5.3‡	—	7.6–11‡	8.2–8.5‡	
Esophageal–intestinal valve length	—	—	—	—	—	
Esophageal–intestinal valve width	—	—	—	—	—	
Subventral esophageal gland orifices§	—	—	—	—	—	
Excretory pore§	230–290	240	—	234–266	—	
Cervical papillae§	670	200	—	296–315	247–422	(352)
Vulva position§	—	4,350–6,500‡	6,300–7,100‡	3,880–6,628‡	6,080–6,628‡	(6,500)
% of body length to vulva	—	81–82‡	79‡	81–83	81–83‡	(81)
Anterior infundibulum length	—	—	—	—	—	
Posterior infundibulum length	—	—	—	—	—	
Anterior sphincter length‖	—	—	—	—	—	
Posterior sphincter length‖	—	—	—	—	—	
Ovejector length	—	—	—	—	—	
Tail length	680	100	200	129–152	—	

* 1 = Hassall and Stiles (1892), original description from natural infections in *Sus scrofa* from Washington, D.C. 2 = Travassos (1921), redescription of specimens in *Sus scrofa* from Brazil. 3 = Goodey (1924), redescription of specimens from naturally infected *Sus scrofa* from Great Britain. 4 = Alicata (1935), specimens from experimental infections in guinea pig (*Cavia porcellus*). 5 = Thoonen et al. (1951), redescription from natural infections in *Sus scrofa* in Belgium. 6 = Sprehn (1957). 7 = Sarashina and Taniyama (1986), redescription from natural infections in *Sus scrofa* in Hokkaido, Japan. 8 = Present study, redescription from natural infections in *Sus scrofa* and *Ovis aries* from North America and Central America.

† *n* for individual measurements.

‡ Calculated from numerical values in previous descriptions.

§ Measured from anterior.

‖ Measurements include the sphincter and vestibula as the muscular portion of the sphincter could not be clearly differentiated from the distal vestibula.

descriptions of those species currently referred to *Cervicaprastrongylus,* it is apparent that the latter genus cannot be reduced as a synonym of *Hyostrongylus.* The spicules characteristic of *C. gabonensis* (Durette-Desset and Chabaud, 1974), *C. moreli* (Durette-Desset and Denke, 1978), and *C. malviyai* (Chaturvedi and Kansal, 1977) (type for the genus) all have a dorsal and ventral process extending posteriad from the alae along the main shaft and a characteristic "eyelet" at the level of the trifurcation of the spicule tips (Durette-Desset and Chabaud, 1974; Durette-Desset and Denke, 1978; Gibbons and Khalil, 1982b). In contrast, spicules of *H. rubidus* are relatively simple (although trifurcate), being composed of a main shaft and slender ventral and dorsal processes extending posteriad from the alae; an eyelet is absent. In addition, most species of *Hyostrongylus* (exclusive of those referred to *Cervicaprastrongylus*) apparently have only a single prominent dorsal process on each spicule (but see Durette-Desset et al. [1992], who suggest it is necessary to confirm this by dissection). Although there is similarity in the 2–2–1 pattern of the bursa, the structure of the dorsal ray may differ in the location and number of lateral processes and in position of the bifurcation (see Trach, 1986; Govorka et al., 1988). Additionally, the simple, rectangular accessory bursal membrane, supported by 2 widely separated dorsal raylets (#7 papillae), differs from that in species of the genus *Cervicaprastrongylus* (Gibbons and Khalil, 1982b; Trach, 1986). However, placement of both genera in the Ostertagiinae is supported by the presence of paired "0" papillae on the ventral aspect of the genital cone (see Hoberg and Lichtenfels, 1992; Lichtenfels and Hoberg, 1992).

Recognition of *Cervicaprastrongylus* and *Hyostrongylus* requires comment on the species referred to these genera. In addition to *H. rubidus,* 5 other species of *Hyostrongylus* have been recognized including *H. okapiae* (Van den Berghe, 1937) from *Okapiae johnstoni* (Sclater) in central

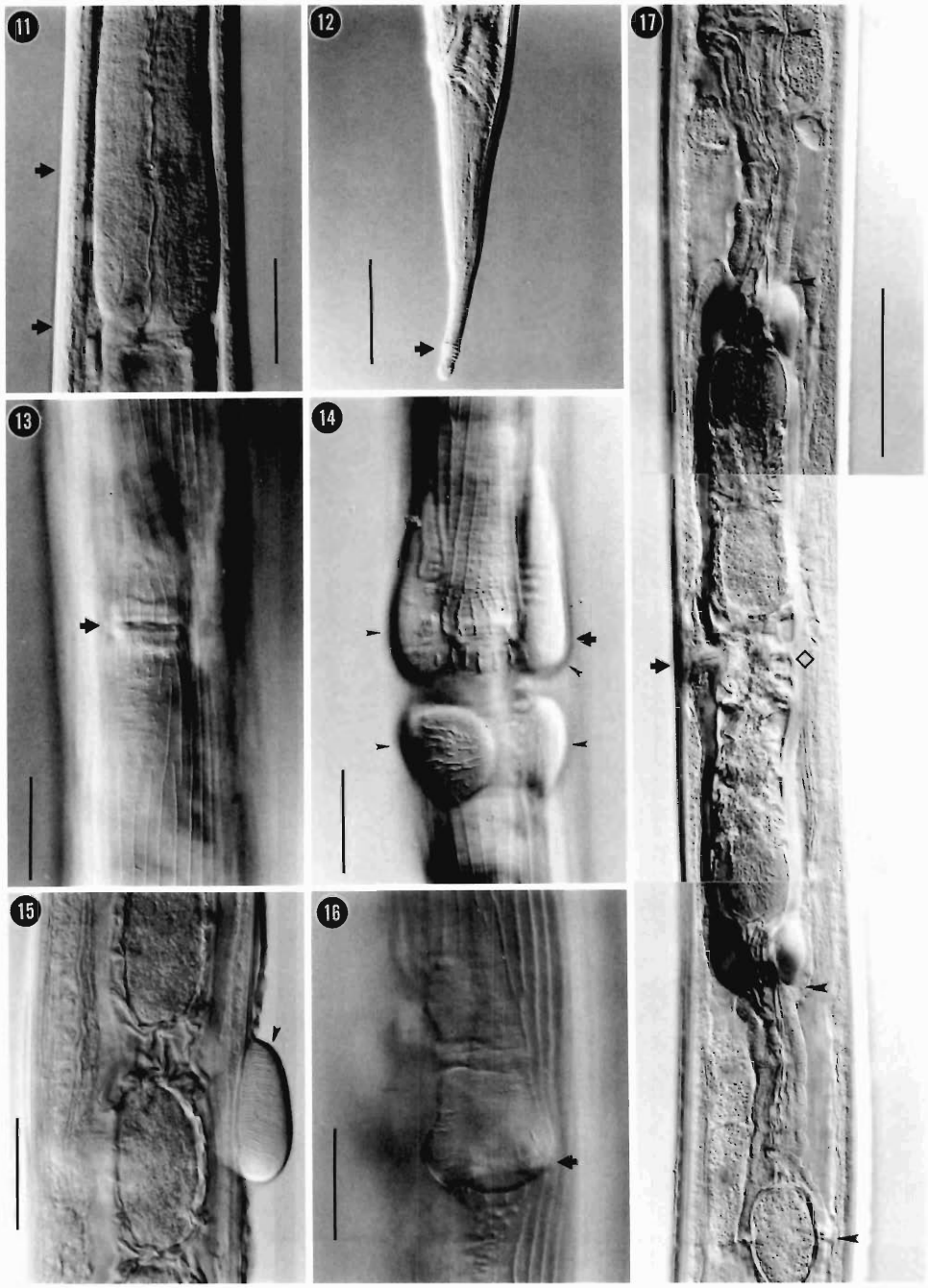
Table 3. Continued.

6	7		8		
—	20			50	
5,000–10,000	7,396–8,796	(8,070)	[50]†	4,554–10,275	(7,574 ± 1,274.2)
	—	—	[46]	57–88	(69 ± 6.76)
660–750	640–807	(713)	[50]	570–770	(677 ± 47.11)
7.5–13.2‡	8.6–9.2‡	(8.8)	[50]	7–13	(9.13 ± 1.3)
—	—	—	[49]	57–86	(71 ± 7.17)
—	—	—	[49]	36–57	(49 ± 6.23)
—	—		[47]	237–328	(278 ± 23.47)
240	226–357	(287)	[48]	244–403	(323 ± 44.23)
400–670	231–376	(306)	[48]	248–433	(344 ± 47.16)
—	6,284–7,241‡	(6,773)	[50]	3,883–8,600	(6,265 ± 1,085.43)
—	82–85‡	(84)	[50]	77–85	(83 ± 1.46)
—	—	—	[39]	94–161	(127 ± 15.2)
—	—	—	[40]	73–151	(115 ± 15.91)
—	—	—	[44]	99–255	(171 ± 41.89)
—	—	—	[44]	88–225	(149 ± 34.67)
—	—	—	[39]	413–735	(566 ± 86.89)
68–100	118–157	(140)	[45]	104–174	(142 ± 16.77)

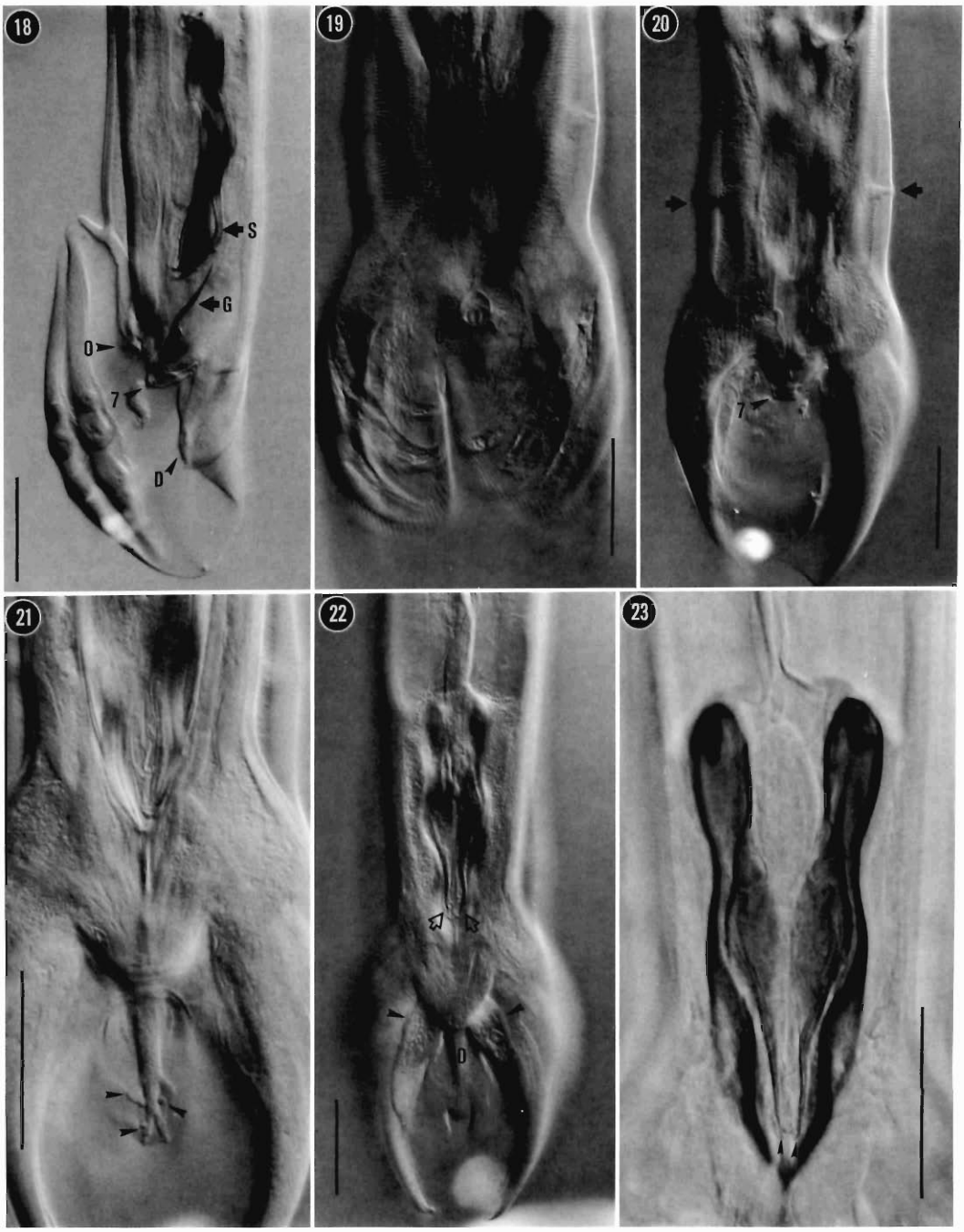
Africa, *H. vinnica* Trach, 1986, from sheep and cattle in the Ukraine, *H. gabonensis* from the tragulid *Hyemoschus aquaticus* Ogilby in Gabon, *H. moreli* from the leporid *Lepus capensis* Linnaeus in Mali, and *H. kigeziensis* from *Gorilla gorilla* (Savage and Wyman) in Uganda (Durette-Desset and Chabaud, 1974; Durette-Desset and Denke, 1978; Trach, 1986; Durette-Desset et al., 1992). *Hyostrongylus gabonensis* and *H. moreli* were subsequently transferred to the Ostertagiinae when *Cervicaprastrongylus* Gibbons and Khalil, 1982, was established for several trichostrongylids from lagomorphs and ruminants (Gibbons and Khalil, 1982b). The validity of this genus has been questioned by Jansen (1989) and Durette-Desset et al. (1992), who considered it to be a synonym of *Hyostrongylus*. The concepts of Gibbons and Khalil (1982a, b) would refer 4 species to *Hyostrongylus* and 3 to *Cervicaprastrongylus* and relegate both genera to the Ostertagiinae, whereas Durette-Desset et al. (1992) would refer 7 species (if *H. vinnica* is included) to the former genus and apparently (based on exclusion from the Ostertagiinae) place them in the Graphidiinae.

Referral of *Hyostrongylus* to the Ostertagiinae

Recent studies of the Trichostrongylidae have referred *Hyostrongylus* to the Ostertagiinae (Gibbons and Khalil, 1982a; Trach, 1986; Jansen, 1989), although Durette-Desset and Chabaud (1977, 1981) and Durette-Desset (1983, 1985, 1989) placed this genus among the Graphidiinae. The presence of paired "0" papillae (raylets) on the ventral aspect of the genital cone in *H. rubidus* supports placement of the genus in the Ostertagiinae. In this regard, Chabaud et al. (1970) considered that the minuscule paired "0" papillae of many rhabditea represented the ancestral condition for these nematodes. However, fusion of these papillae is typical of the Strongyloidea, Ancylostomatoidea, Metastrongyloidea, and all Trichostrongyloidea except the members of the Ostertagiinae. Hypertrophy of these papillae among the Ostertagiinae is substantial, such that they are definably different from the putative ancestral condition. Consequently, within the context of outgroup comparison with all other strongylates, the paired ventral raylets of the Ostertagiinae represent a putative synapomorphy for the subfamily (see Hoberg and Lichtenfels, 1992; Lichtenfels and Hoberg, 1993) and are uniformly present only among *Hyostrongylus* and those genera collectively referred to the Ostertagiinae by Durette-Desset (1983, 1989), Gibbons and Khalil (1982a, 1983), and Jansen (1989). Additional characters that may support placement in the Ostertagiinae include relatively short spicules, irregular cuticular inflations at the level



Figures 11–17. Esophageal, cuticular and internal characters of females of *Hyostrongylus rubidus*. Scale bars = 50 μm for Figures 11–16 and 100 μm for Figure 17. 11. Esophageal valve (between arrows) showing typical structure and relatively short length. 12. Tail showing usual cuticular rings at tip (arrow). 13. Vulva in ventral view (arrow) showing simple structure in the vulval region without prominent cuticular inflations. 14. Vulva in ventral view (arrow) showing 4 complex and prominent cuticular inflations (pointers), each formed by hypertrophy of several ridges, and irregular pattern of synlophe in the vulval region. 15. Single lateral inflation near the level of the vulva (pointer). 16. Flap-like structure covering vulva (arrow). 17. Ovejectors (anterior directed toward top of figure) showing position of vulva (arrow) anterior and posterior vestibula and sphincters (between diamond and pointers) and the anterior and posterior infundibula (between pointers); note absence of cuticular modification at level of vulva in this specimen.



Figures 18–23. Characters of male specimens of *Hyostrongylus rubidus*. Scale bars = 50 μm. 18. Lateral view of the genital cone showing position of "0" papillae (0), "7" papillae (7), dorsal lobe with dorsal ray (D), gubernaculum (G), and dorsal process of spicule tips (S). 19. Ventral view of bursa showing position and structure of the paired "0" papillae (0). 20. Ventral view of bursa showing position and structure of "7" papillae (7) and the position of the prebursal papillae (arrows). 21. Dorsal view of bursa showing configuration of the dorsal ray with 2 pairs of laterally directed processes anterior to the bifurcation from which additional processes arise (pointers). 22. Dorsal view of bursa showing dorsal position of externodorsal rays (pointers) with respect to the dorsal lobe and dorsal ray (D); note also the elongate dorsal processes of the spicules (arrows). 23. Spicules, dorsal view, showing prominent dorsal processes extending from the dorsal alae.

of the vulva (Hoberg and Lichtenfels, 1992; Hoberg et al., 1993b), and a 2–2–1 bursa.

Comparisons among other Ostertagiinae in which the synlophe and genital cone have been evaluated indicate the need for critical assessment of these and other characters (e.g., esophageal valve) in developing a concept for relationships within the subfamily. Considering species with a 2–1–2 bursa, 2 forms of the lateral synlophe are recognized. Some species of *Ostertagia* (*O. ostertagi* (Stiles, 1892) and *O. bisonis* Chapin, 1925 and associated minor morphotypes), *Longistrongylus* (*L. sabie* (Mönnig, 1932) and *L. curvispiculum* (Gibbons, 1973)), and *Camelostrongylus mentulatus* (Railliet and Henry 1909) have a tapering cervical synlophe (largely definable as the Type I pattern of Lichtenfels et al. [1988]). A parallel cervical synlophe (Type II pattern of Lichtenfels et al. [1988]) is present in *Ostertagia leptospicularis* Assadov, 1953, *O. gruehneri* Skrjabin, 1929, *O. mossi* Dikmans, 1931, and *Marshallagia marshalli* (Ransom, 1907) and the putative minor morphotypes associated with these species (Hoberg et al., 1993a). In contrast, among those species known to have a 2–2–1 bursa (species of *Teladorsagia* Andreeva and Satubaldin, 1954, *Spiculopteragia* (Orloff, 1933), and *Mazamastrongylus* Cameron, 1935), the synlophe has a tapering pattern laterally in the cervical zone (Type I of Lichtenfels et al. [1988] is definable only in *Teladorsagia*). However, the form of the bursa is 2–2–1 in *H. rubidus,* and although the lateral synlophe has 5 continuous parallel ridges the Type II lateral pattern (3–5 narrowly spaced parallel ridges in each lateral field) is absent. The cervical synlophe of *H. rubidus* differs in having a constant interval between ridges. The presence of a 2–2–1 bursa and a parallel synlophe constitute a combination of characters not compatible with the patterns defined for some of the genera and species already outlined. Elucidation of the relationships of these and other genera referred to the Ostertagiinae thus requires development of explicit hypotheses for homology for elements of the synlophe and genital cone along with the definition of character-state transformation series.

Relationship of the Ostertagiinae and Graphidiinae

Recognition of *Hyostrongylus* and the species currently referred to *Cervicaprastrongylus* within the Ostertagiinae (Gibbons and Khalil, 1982a) requires reconsideration of hypotheses for multiple origins of the former subfamily from the Graphidiinae (Durette-Desset and Chabaud, 1977, 1988; Durette-Desset, 1983, 1985). Whether or not *Hyostrongylus* is basal within the Ostertagiinae remains to be determined. However, there is a parallel synlophe that is unmodified, a relatively high number of ridges, and a 2–2–1 bursa. In contrast, the Graphidiinae (according to Gibbons and Khalil [1982a], and with the removal of *Hyostrongylus* sensu Durette-Desset et al. [1992] and *Parostertagia* Schwartz and Alicata, 1933) would be defined by a 2–1–2 bursa (rather than 2–2–1 and 2–1–2) and an unmodified parallel synlophe (Hoberg and Lichtenfels, 1992). Both of these characters are widespread within the Molineidae (see Durette-Desset 1983, 1985) and, thus (by outgroup comparison), may constitute the plesiomorphic condition with respect to the Graphidiinae and Ostertagiinae and as such are not phylogenetically informative. With an unmodified parallel synlophe as plesiomorphic, Type II and Type I patterns would be derived within the Ostertagiinae. Additionally, the 2–2–1 pattern of the bursa appears to be limited to the Ostertagiinae (within the Trichostrongylidae) and a few distantly related heligmosomes (Durette-Desset, 1983, 1985). These latter characters of the synlophe and bursa along with the paired "0" papillae are postulated as apomorphic within the Ostertagiinae, with the latter attribute representing a putative synapomorphy for the subfamily, as presented earlier. *Hyostrongylus* could thus be basal within the Ostertagiinae. However, the relationship for *Hyostrongylus* as the basal member of a more inclusive group within the Ostertagiinae continues to require confirmation, as indicated by previous workers (Dróżdż, 1965, 1967; Durette-Desset and Chabaud, 1977, 1981; Durette-Desset, 1982, 1983, 1985; Jansen, 1989).

Durette-Desset and Chabaud (1977, 1981) in their classic essays on the classification of the Trichostrongyloidea proposed multiple origins for the Ostertagiinae from 2 genera of the Graphidiinae. *Hyostrongylus* (within the Graphidiinae) was considered the basal member of a lineage that included 3 genera of the Ostertagiinae with a 2–2–1 bursa: *Spiculopteragia* (Orloff, 1933), *Teladorsagia* Andreeva and Satubaldin, 1934, and *Gazellostrongylus* Yeh, 1956 (the latter now referred by Durette-Desset [1983] to the Cooperiinae). Jansen (1989) also included *Mazamastrongylus* Cameron, 1935, with those genera postulated as being derived from a *Hyostron-*

gylus-like trichostrongylid. Concurrently, *Graphidium* Railliet and Henry, 1909, was postulated as the basal member of a lineage of 3 other genera of ostertagiines with a 2–1–2 bursa: *Marshallagia* (Orloff, 1933), *Longistrongylus* (Le Roux, 1931), and *Ostertagia* Ransom, 1907. If this view is correct, then it would become necessary to synonymize the Ostertagiinae and Graphidiinae. Otherwise, in the currently accepted classification of the Trichostrongyloidea (Durette-Desset, 1983, 1985), the Ostertagiinae would be polyphyletic (derived independently from 1 or more ancestors referred to another taxon), whereas the Graphidiinae would become paraphyletic (a taxon with a common ancestor, but with exclusion of 1 or more descendants) (Hennig, 1966; Wiley, 1981; Wiley et al., 1991). In either case, these taxa would be artificial with a resulting classification (cladistic or otherwise) being inconsistent with the phylogenetic history of these trichostrongylid subfamilies (see opinions on classification in Khalil and Gibbons, 1981; Jansen, 1989).

However, recognition of an unequivocal synapomorphy defining the Ostertagiinae (to the exclusion of the Graphidiinae) refutes the hypothesis for multiple origins. Alternative hypotheses suggest that (1) the Ostertagiinae and Graphidiinae may be sister-groups (sharing a common ancestor; also refuted by absence of a synapomorphy for both subfamilies) or (2) the Ostertagiinae and Graphidiinae are more closely related to other subfamilies of Trichostrongylidae. Although monophyly appears established for the Ostertagiinae, any putative relationship with the Graphidiinae or other subfamilies of the Trichostrongylidae must yet be clarified within the context of phylogenetic analyses of the family currently in progress.

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Meeting Schedule

HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1993–1994

(Wednesday) 6 October 1993	Anniversary Dinner Meeting hosted by the Uniformed Services University of the Health Sciences. Time and place to be announced.
(Wednesday) 10 November 1993	National Institutes of Health, Bethesda, MD
(Wednesday) 6 February 1994	Animal Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD
(Wednesday) 6 April 1994	Johns Hopkins University, Baltimore, MD
(Saturday) 7 May 1994	Joint Meeting with the New Jersey Society for Parasitology, at the New Bolton Center, University of Pennsylvania, Kennett Square, PA

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Gastrointestinal Helminths of Five Horned Lizard Species, *Phrynosoma* (Phrynosomatidae) from Arizona

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ABSTRACT: Five species of horned lizards of the genus *Phrynosoma* from Arizona were examined for gastrointestinal helminths. *Phrynosoma cornutum* ($N = 7$) and *Phrynosoma solare* ($N = 8$) harbored the cestode *Diochetos phrynosomatis* and the nematodes *Atractis penneri* and *Skrjabinoptera phrynosoma*. *Phrynosoma douglassii* ($N = 19$) and *Phrynosoma platyrhinos* ($N = 5$) contained both species of nematodes, whereas *Phrynosoma modestum* ($N = 5$) harbored only *S. phrynosoma*. *Phrynosoma cornutum* and *P. douglassii* are new hosts for *A. penneri*; *P. modestum* is a new host for *S. phrynosoma*. It appears that gastrointestinal helminths of Arizona horned lizards are restricted to 3 species. *Diochetos parvovaria* is placed in synonymy with *D. phrynosomatis*.

KEY WORDS: Phrynosomatidae, *Phrynosoma cornutum, Phrynosoma douglassii, Phrynosoma mcallii, Phrynosoma modestum, Phrynosoma platyrhinos, Phrynosoma solare,* Cestoda, *Diochetos parvovaria, Diochetos phrynosomatis,* Nematoda, *Atractis penneri, Skrjabinoptera phrynosoma,* prevalence, intensity.

Six species of horned lizards, *Phrynosoma,* occur in Arizona (see Stebbins, 1985). The Texas horned lizard, *Phrynosoma cornutum* (Harlan, 1825), ranges from Kansas through the Gulf Coast of Texas and extreme southeastern Arizona to Durango and Tamaulipas, Mexico, in dry areas of open country from sea level to 1,830 m elevation. The short-horned lizard, *Phrynosoma douglassii* (Bell, 1828), ranges from southern Canada through the western United States to Durango, Mexico, in open rocky or sandy plains and forests at elevations of 170–3,440 m. The flat-tail horned lizard, *Phrynosoma mcallii* (Hallowell, 1852), occurs in the Coachella Valley of southern California to northeast Baja California and southwestern Arizona in regions of windblown sand from below sea level to 180 m elevation. The roundtail horned lizard, *Phrynosoma modestum* Girard, 1852, ranges from west Texas, northern New Mexico and southeastern Arizona to San Luis Potosí, Mexico, in semiarid regions of scrub vegetation from 210 to 1,850 m elevation. The desert horned lizard, *Phrynosoma platyrhinos* Girard, 1852, ranges from southern Idaho and southeastern Oregon to northeastern Baja California and northwestern Sonora, Mexico, in areas of sandy, gravelly soil with scrub vegetation, from sea level to 1,980 m elevation. The regal horned lizard, *Phrynosoma solare* Gray, 1845, ranges from central Arizona to northern Sinaloa, Mexico, in scrub vegetation at elevations from sea level to 1,460 m.

Helminths have been reported previously from *Phrynosoma cornutum, P. douglassii, P. mcallii, P. platyrhinos,* and *P. solare* (Table 1), but to our knowledge there are no reports of helminths from *P. modestum*. The purpose of this report is to present data on helminth prevalences and intensities for the 6 species of *Phrynosoma* occurring in Arizona. Our specimens of *P. mcallii* were collected from Sonora, Mexico, just south of the Arizona border.

Materials and Methods

Specimens of *Phrynosoma cornutum, P. douglassii, P. mcallii,* and *P. solare* were borrowed from the Herpetology Collection, Natural History Museum of Los Angeles County (LACM); *P. modestum* and *P. platyrhinos* were borrowed from the Herpetology Collection, Department of Zoology, Arizona State University (ASU). The number of specimens of each species examined, body size as snout–vent length (SVL), and collection dates are given in Table 2. Museum accession numbers and collection site longitudes, latitudes, and elevations are given in Appendix 1.

The body cavity was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was excised by cutting across the esophagus and rectum. The esophagus, stomach, small intestine, and large intestine were slit longitudinally and examined under a dissecting microscope. Each helminth was placed on a microscope slide in a drop of undiluted glycerol. A coverslip was added, and the slide was set aside until the helminth became transparent. Each helminth was identified using this glycerol wet-mount method. Selected cestodes were stained with Delafield's hematoxylin and mounted in Canada balsam. Selected intact specimens were placed in vials of 70% ethanol and

Table 1. Previously reported helminths from the species of *Phrynosoma* occurring in Arizona.

Host Helminth	Locality	Prevalence	Reference
Phrynosoma cornutum			
Diochetos phrynosomatis	Kansas	17% (1/6)	Loewen, 1940
	Oklahoma	100% (1/1)	Steelman, 1939
	Texas	57% (4/7)	Harwood, 1932
	Texas	48% (13/27)	Vincent, 1948
Skrjabinoptera phrynosoma	Mexico, Arizona	Not given	Caballero, 1937
	New Mexico	100% (8/8)	Morgan, 1942
	Oklahoma	30% (12/40)	Morgan, 1942
	Texas	95% (19/20)	Morgan, 1942
	Texas	75% (18/24)	Lee, 1955
	Texas	43% (3/7)	Harwood, 1932
	Texas	63% (17/27)	Vincent, 1948
Phrynosoma douglassii			
Skrjabinoptera phrynosoma	Mexico, Arizona	Not given	Caballero, 1937
	Mexico	Not given	Morgan, 1942
Phrynosoma mcallii			
Skrjabinoptera phrynosoma	California	100% (2/2)	Telford, 1970
Phrynosoma platyrhinos			
Diochetos phrynosomatis	Idaho	40% (4/10)	Lyon, 1986
	Nevada	11% (11/104)	Babero and Kay, 1967
	Utah	Not given	Grundmann, 1959
Atractis penneri	California	100% (1/1)	Telford, 1970
	Idaho	33% (1/3)	Waitz, 1961
	Idaho	40% (4/10)	Lyon, 1986
	Nevada	43% (45/104)	Babero and Kay, 1967
	Utah	Not given	Grundmann, 1959 (see Telford, 1964)
Skrjabinoptera phrynosoma	California, Idaho, Utah	Not given	Morgan, 1942
	California	67% (2/3)	Telford, 1970
	Idaho	33% (1/3)	Waitz, 1961
	Idaho	60% (6/10)	Lyon, 1986
	Nevada	97% (101/104)	Babero and Kay, 1967
	Utah	100% (7/7)	Woodbury, 1934
	Utah	Not given	Grundmann, 1959
Phrynosoma solare			
Diochetos phrynosomatis	Arizona	29% (4/14)	Benes, 1985
Atractis penneri	Arizona	21% (3/14)	Benes, 1985
Skrjabinoptera phrynosoma	Arizona	75%	Hannum, 1941
	Arizona	79% (11/14)	Benes, 1985
	Mexico, Arizona	Not given	Caballero, 1937

deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (for accession numbers, see Appendix 1).

Results and Discussion

One species of cestode, *Diochetos phrynosomatis* Harwood, 1932, and 2 nematodes, *Atractis penneri* (Gambino, 1957) Baker, 1987, and *Skrjabinoptera phrynosoma* (Ortlepp, 1922) Schulz, 1927, were found. Prevalences, mean intensities, and site of infection are given in Table 3.

Diochetos phrynosomatis was originally described from *P. cornutum* from Houston and Anderson counties, Texas (Harwood, 1932), and is currently known only from the genus *Phrynosoma*. A second species, *D. parvovaria* Steelman, 1939, taken from a single *P. cornutum* collected in Stillwater, Oklahoma, has been described but has not been reported since. Steelman (1939) based his description on 52 specimens of *D. parvovaria* ranging in length from 5 to 22 mm and having 52–87 testes per segment. Harwood (1932) reported 55–70-mm lengths and 125–180 testes

Table 2. Collection location, year of collection, and mean size of the 6 species of *Phrynosoma* examined in this study.

Species	Arizona County	*N*	Collection year	Mean SVL (range in mm)
Phrynosoma cornutum	Cochise	7	1966–1967	76 (30–89)
Phrynosoma douglassii	Pima	17	1966–1967	65 (32–91)
	Graham	1	1966	83 (—)
	Santa Cruz	1	1967	82 (—)
Phrynosoma mcallii	—*	2	1963	64 (62–65)
Phrynosoma modestum	Graham	2	1966	59 (58–59)
	Cochise	1	1973	58 (—)
	Maricopa	2	1971	64 (58–69)
Phrynosoma platyrhinos	Yuma	3	1974	73 (72–74)
	Pinal	2	1956	70 (69–71)
Phrynosoma solare	Pima	8	1966	94 (77–113)

* Mexican side of the Mexico–Yuma County border.

per segment for *D. phrynosomatis*. Egg and oncosphere diameters were reported to be similar for both cestodes: 49–71 and 26–46 μm, respectively, for *D. parvovaria* as compared to 55 and 30 μm, respectively, for *D. phrynosomatis*. In our samples, gravid *D. phrynosomatis* from a single host ranged from 20 to 65 mm in length and had 70–150 testes per segment. The differences between *D. parvovaria* and *D. phrynosomatis* as enumerated by Steelman (1939) were related to size: *D. parvovaria* was about one-third the length of *D. phrynosomatis*, the scolex and suckers were smaller, the segments became mature relatively nearer the scolex, and the testes were half as numerous. Because there are no unique morphological characteristics, the differences can be explained by dwarfing, and because our measurements overlap both descriptions we have placed *D. parvovaria* in synonymy with *D. phrynosomatis* and included it in Table 1. The dwarfing of helminths by crowding has been well documented (Morgan, 1942; Babero and Kay, 1967; Brooks and Mayes, 1976; Bursey and Goldberg, 1992). In addition to the hosts listed in Table 3, *D. phrynosomatis* has been reported from the Mexican horned lizards, *Phrynosoma bracon-*

Table 3. Helminths recovered from 5 species of *Phrynosoma* collected in Arizona.

Host Helminth	Prevalence	Mean intensity (range)	Site
Phrynosoma cornutum			
Diochetos phrynosomatis	71% (5/7)	86 (22–181)	Small and large intestine
**Atractis penneri*	14% (1/7)	137	Large intestine
Skrjabinoptera phrynosoma	86% (6/7)	611 (9–1,579)	Stomach
Phrynosoma douglassii			
**Atractis penneri*	11% (2/19)	476 (323–636)	Small and large intestine
Skrjabinoptera phrynosoma	11% (2/19)	47 (34–60)	Stomach and small intestine
Phrynosoma modestum			
**Skrjabinoptera phrynosoma*	80% (4/5)	5 (1–13)	Stomach, small intestine, and lung
Phrynosoma platyrhinos			
Atractis penneri	40% (2/5)	511 (396–625)	Large intestine
Skrjabinoptera phrynosoma	40% (2/5)	8 (6–10)	Stomach
Phrynosoma solare			
Diochetos phrynosomatis	100% (8/8)	30 (21–70)	Small intestine
Atractis penneri	63% (5/8)	1,113 (2–2,364)	Large intestine
Skrjabinoptera phrynosoma	100% (8/8)	524 (16–1,804)	Stomach and small and large intestines

* New host record.

nieri (prevalence 9%, 1/11) and *Phrynosoma taurus* (prevalence 20%, 1/5) by Goldberg and Bursey (1991). The mean prevalence for the 5 species harboring *D. phrynosomatis* is 27% (53/200). The life cycle for *D. phrynosomatis* is not known; however, insects and mites serve as intermediate hosts for anoplocephalid cestodes (Schmidt, 1986).

Atractis penneri is 1 of 2 species of *Atractis* reported to occur in the United States. The other species, *Atractis scelopori,* has been reported from southern California (Kern, Los Angeles, and Riverside counties) and southern Nevada (Clark County) as well as Mexico from large herbivorous lizards (see Gambino and Heyneman, 1960). It is distinguished from *A. penneri* in that it has equal spicules and 6 prominent lip papillae. *Atractis penneri* is a parasite of carnivorous phrynosomatid and crotaphytid lizards infecting some 21 North American species from Idaho to Texas. These 2 species of *Atractis* overlap only in southern California. The life cycle of *A. penneri* is apparently direct, like that of other atractids (Cheng, 1986). Baer (1951) has suggested that these nematodes, which occur in large numbers and in all stages of development, are possibly living on partially digested vegetable matter and should be considered as commensals rather than true parasites. *Phrynosoma cornutum* and *P. douglassii* are new hosts for *A. penneri.*

Skrjabinoptera phrynosoma, the only member of the genus *Skrjabinoptera* reported from the United States, is the most commonly found nematode of horned lizards, although it has been reported from both crotaphytid, phrynosomatid, polychrid, and teiid lizards (see Goldberg and Bursey, 1991). Lee (1957) showed experimentally that the ant *Pogonomyrmex barbatus* served as an intermediate host for *S. phrynosoma.* Pearce and Tanner (1973) suggested that several species of ants may serve as intermediate hosts for this nematode. The number of *S. phrynosoma* present has been found to be roughly related to the size of the lizard. Lee (1957) reported never finding *S. phrynosoma* in the stomachs of *P. cornutum* under 50 mm SVL, at which size they change their diet from exclusively small ants to include the larger ant *P. barbatus.* Benes (1985) found *S. phrynosoma* in the stomachs of *P. solare* measuring 35 mm SVL and suggested they may begin to feed on *P. barbatus* at an earlier age than does *P. cornutum.* Although our specimens ranged from 30 to 113 mm SVL, the smallest infected horned lizard in our study was a *P. modestum* of 58 mm SVL. *Phrynosoma modestum* is a new host for *S. phrynosoma.* The 2 *P. mcallii* that we examined from the state of Sonora, Mexico, were also infected with 67 and 90 *S. phrynosoma.*

Based on our observations and previous reports from the literature (Table 1), we conclude that the gastrointestinal helminth community of Arizona horned lizards is restricted to 3 helminths: *D. phrynosomatis, A. penneri,* and *S. phrynosoma.* The similarity of helminth faunas among species of horned lizards may be related to diet. Pianka and Parker (1975) found the bulk of the diet of species of *Phrynosoma* to consist of ants, and for the 6 species that we examined the percentage of ants by number of prey items in their study ranged from 97% in *P. mcallii* to 69% in *P. cornutum.* Because ants serve as the intermediate host (Lee, 1957), a high prevalence of *S. phrynosoma* in horned lizards might be expected. As a corollary, because *D. phrynosomatis* is restricted to horned lizards, it is conceivable that an ant may also serve as the intermediate host for this species. Finally, because *A. penneri* has a direct life cycle (Cheng, 1986) and occurs in many North American lizard species (see Baker, 1987), it is possible that these infections may be passed, perhaps by fecal contamination of substrate or food, among sympatric lizard species.

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Appendix 1: Museum Accession Numbers, Locality Data, and USNM Helminthological Collection Numbers

P. cornutum: Cochise County, LACM 140076–140080, 32°14′N, 109°45′W; elevation 1,269 m; LACM 140075, 32°31′N, 109°58′W, elevation 1,345 m; LACM 140081, 31°41′N, 109°08′W, elevation 1,335 m. USNM Helminthological Collection numbers: *Diochetos phrynosomatis* 82641; *Atractis penneri* 82642; *Skrjabinoptera phrynosoma* 82640.

P. douglassii: Pima County, LACM 140056–140059, 140061–140066, 140068–140074, 32°26′N, 110°45′W, elevation 2,438 m; Santa Cruz County, LACM 140067, 31°42′N, 110°46′W, elevation ca. 1,524 m; Graham County, LACM 140060, 32°41′N, 109°52′W, elevation 3,108 m. USNM Helminthological Collection numbers: *A. penneri* 82644; *S. phrynosoma* 82643.

P. mcallii: Sonora, Mexico, LACM 140054–140055, 32°27′N, 115°18′W, elevation 50 m. USNM Helminthological Collection number: *S. phrynosoma* 82645.

P. modestum: Graham County, ASU 7267, 7866, 32°44′N, 109°42′W, elevation 1,036 m; Cochise County, ASU 14317, 32°12′N, 109°34′W; elevation ca. 1,950 m; Maricopa County, ASU 21471, 21472, 33°32′N, 111°39′W; elevation ca. 420 m. USNM Helminthological Collection number: *S. phrynosoma* 82646.

P. platyrhinos: Yuma County, ASU 15966, 15969–15970, 33°58′N, 114°28′W, elevation 203 m; Pinal County, ASU 5841, 5843, 32°33′N, 111°31′W, elevation 506 m. USNM Helminthological Collection numbers: *A. penneri* 82651; *S. phrynosoma* 82650.

P. solare: Pima County, LACM 140082–140083, 140086–140089, 32°20′N, 110°49′W, elevation ca. 907 m; LACM 140084, 32°01′N, 111°37′W, elevation 975 m, LACM 140085, 32°18′N, 111°09′W, elevation 796 m. USNM Helminthological Collection numbers: *D. phrynosomatis* 82648; *A. penneri* 82649; *S. phrynosoma* 82647.

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Helminths of Varied Thrushes, *Ixoreus naevius*, and Robins, *Turdus migratorius*, from British Columbia

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ABSTRACT: From 1986 to 1992, 48 varied thrushes, *Ixoreus naevius* (Gmelin), and 17 robins, *Turdus migratorius* L., were collected in British Columbia and examined for helminths. Twenty-one helminth species were found in 46 varied thrushes, 11 in 17 robins. Four acanthocephalan species were found in varied thrushes with *Prosorhynchus cylindraceus*, the most prevalent helminth. It was the only species of acanthocephalan in robins. Five cestode species were found in varied thrushes including 2 (*Aploparaksis dujardini neoarcticus* and *Dilepis undula*) that were common to robins. Eight digenetic trematodes were found in varied thrushes; *Lutztrema monenteron* had high prevalence but 3 (*Tamerlania zarudnyi, Urotocus rossitensis,* and *Mosesia chordeilesia*) consisted of single infections. Only 2 digenetic trematodes, *L. monenteron* and *Brachylecithum mosquense*, were found in robins. Of 5 nematodes found, *Capillaria obsignata* and *Capillaria quiscali* were most prevalent in varied thrushes and robins.

KEY WORDS: *Ixoreus naevius, Turdus migratorius,* British Columbia, Acanthocephala, Cestoidea, Nematoda, Digenea.

This paper is a report on the helminths found in varied thrushes, *Ixoreus naevius,* and robins, *Turdus migratorius,* in British Columbia. These passeriform birds are common on the Pacific coast of North America, but very little is known of their parasites.

Materials and Methods

From 1986 to 1992, 48 varied thrushes and 17 robins were examined from collections in southern British Columbia. The birds were collected at Pearson College on Vancouver Island, at Lynn Canyon Ecology Centre in North Vancouver, and in suburban areas of Vancouver. Seven birds with no localities indicated were presumed to have been collected in southern British Columbia. Most of the birds had died accidentally as a result of collisions against windows, and 83% of them were found during September through March. The birds were classified as males, females, and juveniles. Twice as many male varied thrushes were found than females with a total of 31 males, 14 females, and 3 juveniles. Four male, 5 female, and 8 juvenile robins were collected.

The birds were frozen for later examinations for parasites. The digestive, respiratory, and excretory organs were examined by standard methods. Large organs such as the intestine were divided into 4 sections and scraped, and the sediment was examined under a stereoscope. Acanthocephalans were placed in tap water until the proboscides were extended. All of the helminths except nematodes were fixed in alcohol–formalin–acetic acid and then stored in 70% ethanol. Nematodes were placed directly into 70% ethanol. Scolices of cestodes and whole digeneans were mounted in Berlese chloral gum. These specimens were measured and drawn immediately after mounting. Nematode lengths were taken from outlines drawn with aid of a camera lucida and extrapolated with a map measurer. Taxonomic references used were Amin (1985) for acanthocephalans, Schmidt (1986) for cestodes, Schell (1986) for digeneans, and McDonald (1974) for nematodes.

Accession numbers for specimens deposited in the U.S. National Museum (USNM) Parasite Collection, Beltsville, Maryland, are as follows: *Prosorhynchus cylindraceus* (USNM 82745), *Prosorhynchus paulus* (USNM 82746), *Aploparaksis dujardini neoarcticus* (USNM 82747), *Dilepis undula* (USNM 82748), *Monopylidium iola* (USNM 82749), *Passirilepis crenata* (USNM 82750), *Leucochloridium cardis* (USNM 82751), *Leucochloridium turdi* (USNM 82752), *Mosesia chordeilesia* (USNM 82753), *Tamerlania zarudnyi* (USNM 82754), *Urotocus rossitensis* (USNM 82755), *Ascaridia galli* (USNM 82756), *Capillaria obsignata* (USNM 82757), *Capillaria quiscali* (USNM 82758), *Porrocaecum ensicaudatum* (USNM 82759), and *Syngamus merulae* (USNM 82760).

Results

Twenty-two helminth species are listed in Table 1 with the numbers of infected varied thrushes and robins, mean intensities, and ranges. New host records are noted with asterisks: 15 for varied thrushes and 2 for robins. A total of 21 species was found in 46 or 96% of varied thrushes. Two of these hosts had a maximum of 7 parasite species and totals of 44 and 68 worms. The heaviest worm load in a varied thrush was 108 individuals. Only 11 helminth species were found in the 17 robins sampled. One robin had a maximum number of 7 parasite species with a total of 35 individuals. Two juvenile robins had heavy worm loads of 220 and 518. Ten of the 11 helminth species in robins were also found in varied thrushes, with *Brachylecithum mosquense* the

Table 1. Helminths of varied thrushes and robins from British Columbia.

Helminth taxon	Thrushes ($N = 46$) No. infected	Thrushes ($N = 46$) Mean intensity ± SD	Thrushes ($N = 46$) Range	Robins ($N = 17$) No. infected	Robins ($N = 17$) Mean intensity ± SD	Robins ($N = 17$) Range
Acanthocephala						
Prosorhynchus cylindraceus (Goeze, 1782) Schmidt and Kuntz, 1966	29*	4 ± 6	1–32	9	5 ± 5	1–17
Prosorhynchus paulus Van Cleave and Williams, 1951	1*	5				
Pseudolueheia boreotis (Van Cleave and Williams, 1951) Schmidt and Kuntz, 1967	8	2.5 ± 1.6	1–5			
Sphaerirostris lancea (Westrumb, 1821) Golvan, 1956	9	6 ± 9	1–31			
Cestoidea						
Aploparaksis dujardini neoarcticus Webster, 1955	22	5 ± 4	1–18	2*	4 ± 2	2–6
Aploparaksis turdi Williamson and Rausch, 1965	2	29.5 ± 10.5	19–40			
Dilepis undula (Schrank, 1788) Weinland, 1858	9*	3 ± 3.9	1–13	9	14 ± 32	1–105
Monopylidium iola (Lincicome, 1939) Schmidt, 1986	2*	19.5 ± 18.5	1–38			
Passirilepis crenata (Goeze, 1782) Sultanov and Spaskaja, 1959	3*	2 ± 0.8	1–3	5	20 ± 29	2–77
Unidentified cestodes	3	1 ± 0.5	1–2	3	1 ± 0.5	1–2
Digenea						
Brachylaima fuscatum (Rudolphi, 1819) Joyeux, Baer, and Timon-David, 1932	6	2.7 ± 1.5	1–5			
Brachylecithum mosquense (Skrjabin and Issaitschikoff, 1927) Shtrom, 1940				3	7.7 ± 5	1–14
Lutztrema monenteron (Price and McIntosh, 1935) Travassos, 1941	21	25.8 ± 24	2–76	5	2.2 ± 1.7	1–5
Leucochloridium cardis Yamaguti, 1939	2*	1				
Leucochloridium turdi Yamaguti, 1939	4*	20.8 ± 28.5	1–70			
Mosesia chordeilesia McMullen, 1936	1*	37				
Tamerlania zarudnyi Skrjabin, 1924	1*	3				
Urotocus rossitensis (Mühling, 1898) Looss, 1899	1*	2				
Nematoda						
Ascaridia galli (Schrank, 1788) Freeborn, 1923	1*	1		1	21	
Capillaria obsignata Madsen, 1945	12*	6.6 ± 4.8	1–14	7	14 ± 14	1–44
Capillaria quiscali Read, 1949	21*	4 ± 4.6	1–18	8*	9 ± 9.7	1–31
Porrocaecum ensicaudatum (Zeder, 1800) Lopez-Neyra, 1946	2*	1				
Syngamus merulae Baylis, 1927	2*	1		6	1.5 ± 0.8	1–3
Total 22 species	46/48	27 ± 27	1–108	17/17	30.5 ± 51.5	1–220

* New host record.

exception. Taxonomic notes and comparisons of morphological data are arranged according to the helminths listed in Table 1.

ACANTHOCEPHALANS: Van Cleave and Williams (1951) described and illustrated 4 species of acanthocephalans from Alaska, which are now reported in British Columbia. *Lueheia adlueheia,* a species described by Werby (1938) in Washington, was not found in this study. While only *Prosorhynchus cylindraceus* was found in robins, 4 species were found in varied thrushes. Mixed species infections consisted of 4 varied thrushes with *P. cylindraceus* and *Pseudolueheia boreotis,* 3 with *P. cylindraceus* and *Sphaerirostris lancea,* 2 with *P. cylindraceus, P. boreotis,* and *S. lancea,* 1 with *P. boreotis* and *S. lancea,* and 1 with *P. boreotis* and *Prosorhynchus paulus.* The most prevalent parasite was *P. cylindraceus,* and it occurred in 59% of the birds as the only acanthocephalan infection. All of the acanthocephalans were found at the junction of the second and third sections of the intestine. Hemor-

rhagic damage to the intestine was observed in 1 host.

CESTODES: *Aploparaksis dujardini neoarcticus* was described from a varied thrush by Webster (1955) from an unknown locality. In British Columbia, this tapeworm occurred in 48% of varied thrushes and 12% of robins. Nodules surrounding the scolices of *A. dujardini* were observed in the intestine of 1 varied thrush. *Aploparaksis turdi* was reported from robins and varied thrushes in Alaska by Williamson and Rausch (1965). It was not found in robins, and only 2 varied thrushes were infected in this study. *Dilepis undula, Monopylidium iola,* and *Passirilepis crenata* are parasites of *Turdus* spp. according to Schmidt (1986) and are now reported from varied thrushes. Infections of *D. undula* occurred in 6 juvenile robins with 105 of these massive tapeworms found in 1 host. Some cestodes were listed as unidentified because they lacked scolices and had missing hooks or immature proglottids.

DIGENEA: Although Canaris (1967) reported *Brachylaima pellucidum* Werby, 1938, from the intestine of a varied thrush in Oregon, I regard this species as *B. fuscatum. Brachylecithum mosquense* and *Lutztrema monenteron* were reported from the gall bladders and bile ducts of robins and varied thrushes in Idaho by Schell (1957). In this study, *B. mosquense* was found in 3 robins, with 2 having co-infections with *L. monenteron. Lutztrema monenteron* was the most abundant digenean in varied thrushes. *Leucochloridium cardis* and *L. turdi* were originally reported from robins from Japan and occurred in 4 varied thrushes. Because the worms found in the cloaca tended to be desiccated in frozen birds, intensity and prevalence may be greater than if fresh birds had been examined.

Three unusual digeneans were found in varied thrushes. *Mosesia chordeilesia* (Lecithodendriidae) was found in a single host with 37 specimens in the intestine. This species was originally reported in Michigan from insectivorous nighthawks (*Chordeiles minor*) and purple martins (*Progne subis*), with mayfly naiads as the intermediate hosts (Hall, 1959). Two specimens of *Tamerlania zarudnyi* (Eucotylidae) were found in the kidney tubule of 1 varied thrush. Because the eggs and gonads were larger than measurements from the single specimen reported by Penner (1939) as *Tamerlania melospizae,* I regard the specimens as belonging to the type species. Six specimens of *T. melospizae* were found in 1 robin in 1965 from Montana according to Canaris (pers. comm.). Two specimens of *Urotocus rossitensis* (Leucochloriidae) were recovered from the bursa Fabricius of 1 varied thrush. Although similar to *U. fusiformis* in body shape, the body lengths and egg size conform to the type species, whose anatomy was described by Williams (1960). Measurements of 3 newly reported digeneans are as follows:

1. *Leucochloridium turdi* ($N = 10$; range in micrometers followed by the mean in parentheses): body length, 1,285–1,938 (1,637); body width at midbody, 469–816 (639). Oral sucker length by width, 196–388 by 262–428 (335 by 367). Pharynx length by width, 59–155 by 82–155 (121 by 128). Acetabulum length by width, 306–469 by 278–491 (384 by 392). Anterior testis, 98–188 by 71–164 (136 by 105); posterior testis, 82–205 by 65–131 (154 by 88). Ovary, 98–180 by 82 by 155 (144 by 122). Eggs ($N = 65$), 20–28 by 10–18 (24 by 13.7).
2. Because the original description of *L. cardis* was based on 1 specimen, measurements are given for the 2 specimens found: body lengths, 1,540 and 2,162; body widths, 775 and 826. Oral sucker diameters, 425 and 449. Acetabulum widths, 458 and 490. Testes, 90–188 by 115–139. Ovary, 131–180 by 131–147. Eggs ($N = 20$), 23–31 by 14–18 (25 by 16.5).
3. *Mosesia chordeilesia* ($N = 10$; range in micrometers followed by the mean in parentheses): body length, 229–335 (288); body width at midbody, 123–196 (161). Oral sucker transverse width, 31–46 (38). Pharynx diameter, 13–26 (17). Acetabulum transverse width, 28–43 (35). Testes diameters ($N = 12$), 38–59 (49). Ovary diameter ($N = 6$), 28–38 (36). Cirrus pouch length ($N = 5$), 64–102. Eggs ($N = 30$), 18–26 by 10–14 (20.7 by 11.2).

NEMATODES: Four of the 5 species reported here have also been reported in robins (Read, 1949; Slater, 1967). *Capillaria obsignata* occurred as single infections in 14 varied thrushes and robins and as 8 co-infections with *C. quiscali* in varied thrushes and as 5 co-infections in robins. Varied thrushes and robins are new hosts for *C. quiscali,* originally described from bronzed grackles, *Quiscalus quiscula aeneus,* in Wisconsin by Read (1949). The 2 capillarids were differentiated from each other by the presence or absence of a vulvar appendage, the surfaces of the eggs in the females, and the shape and type

of sheath of the spicules in the males. The vulvar appendage of *C. quiscali* appeared more extended than originally described.

Measurements of *C. quiscali* are as follows: Females ($N = 10$; range followed by the mean in parentheses in micrometers unless stated otherwise): body length, 6.9–11.6 mm (9.2); body width, 49–65 (54.6) at level of vulva. Proportion of anterior length at vulva to posterior length, 1:2. Number of stichocytes ($N = 8$), 36–51. Vulvar appendage ranging in length from body width to 3 times the width ($N = 8$), 57–164 by 33–57. Eggs with papillate surfaces ($N = 20$), 50–64 by 24–31 (57 by 27). Males ($N = 4$): body length, 4.6–9.5 mm; width at midbody, 37–65; spicule with broad tip, enclosed in cuticular, non-spinous sheath, lengths, 836–1,000.

Discussion

It is difficult to sample "normal" hosts to determine their parasitic fauna, and in this study the findings were probably skewed because of the accidental and often violent nature of the deaths, the sex ratios, and ages of the birds. Twice as many male varied thrushes were examined than females, and it is interesting to note a similar sex skew in Slater's (1967) study. More juvenile robins than adults were examined in this small sample, and perhaps sampling more adults would have resulted in finding more helminth species.

In comparisons of parasites of turdid birds by James and Llewellyn (1967), *P. cylindraceus* was the dominant parasite in two-thirds of the song-thrushes, redwings, and blackbirds sampled. The generic composition of the parasite fauna was similar to that found in this study, reflecting the similarity of invertebrate intermediate hosts used as food items such as land snails, earthworms, and insects in the diets of robins and varied thrushes in the Northern Hemisphere. It is surprising that more studies on passeriform birds are not done because comparisons of their helminth fauna could indicate interesting differences in localities, food preferences, and interactions with intermediate hosts.

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Three New Species of Nematodes Associated with Endemic Grape (*Vitis*) in California

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ABSTRACT: Three new species of nematodes were encountered during a study of natural diversity of nematode associates of native species of *Vitis* L. in California. *Achromadora walkeri* sp. n. was found in rhizosoil of the native California grape *Vitis californica* Bentham and is characterized by the position of the amphid (within the vicinity of both dorsal and ventral teeth), a relatively long stoma, and the absence of a prerectum. The other 2 species were plant parasitic criconematids: *Criconemoides featherensis* sp. n., found in association with roots of *V. californica,* is characterized by possessing strongly retrose annuli posterior to the vulva, a long stylet, the shape of the first annulus of the head, and rare anastomosis of annuli of the body. Specimens of *Hemicycliophora armandae* sp. n. were recovered from the rhizosoil of the desert grape *Vitis girdiana* Munson and are characterized by having 3 cephalic annuli, a lateral field marked by interruption of the striae, a long stylet, and a digitate tail. The study of symbiotic associations of native species of crop plants is important in studies of faunal and floral biodiversity.

KEY WORDS: *Achromadora walkeri* sp. n., California, *Criconemoides featherensis* sp. n., *Hemicycliophora armandae* sp. n., *Vitis californica, Vitis girdiana,* taxonomy, biodiversity, native plant species.

During a study of the diversity of soil nematode communities associated with native grape (*Vitis*: Vitaceae) of California, several new species were encountered. Nematodes of the family Criconematidae were common in most localities that were sampled, and a new species of *Criconemoides* was found around the roots of *Vitis californica* Bentham in the central valley of California. A second new species of Criconematidae (genus *Hemicycliophora*) was recovered from the rhizosoil of *Vitis girdiana* Munson in desert habitat of southern California. In addition, a new species of *Achromadora* was found in rhizosoil of *Vitis californica* in the coast range west of the Sacramento Valley. Descriptions of these 3 new species are presented herein.

Materials and Methods

Soil samples from the rhizosphere of the roots of *Vitis* species were collected and transported to the laboratory in plastic bags. Extraction of nematodes from soil followed the sugar floatation–centrifugation method (Niblack and Hussey, 1985) for species of the genera *Criconemoides* and *Hemicycliophora.* Nematodes of the genus *Achromadora* were recovered using both sugar floatation–centrifugation and Baermann funnel methods (Christie and Perry, 1951). Nematodes recovered were killed and fixed in hot buffered formalin. Permanent slides were made using the rapid method of Seinhorst (1959). Nematodes were stained with Rose Bengal. Measurements were taken using both the ocular micrometer and the JAVA® image analysis program. Drawings were made from either permanent mounts or formalin-fixed nematodes using a drawing tube. All measurements are given in micrometers unless otherwise stated; ranges are in parentheses. For the descriptions, abbreviated measurements are reported as follows: a = ratio of total length to maximum width; b = ratio of total length to esophagus length; c = ratio of total length to length of tail; R = total number of annuli; R_{st} = number of annuli from anterior extremity to base of stylet knobs; R_{ex} = number of annuli from anterior extremity to the excretory pore; R_v = number of annuli from anterior extremity to vulva; R_{vp} = number of annuli from vulva to posterior extremity.

Results

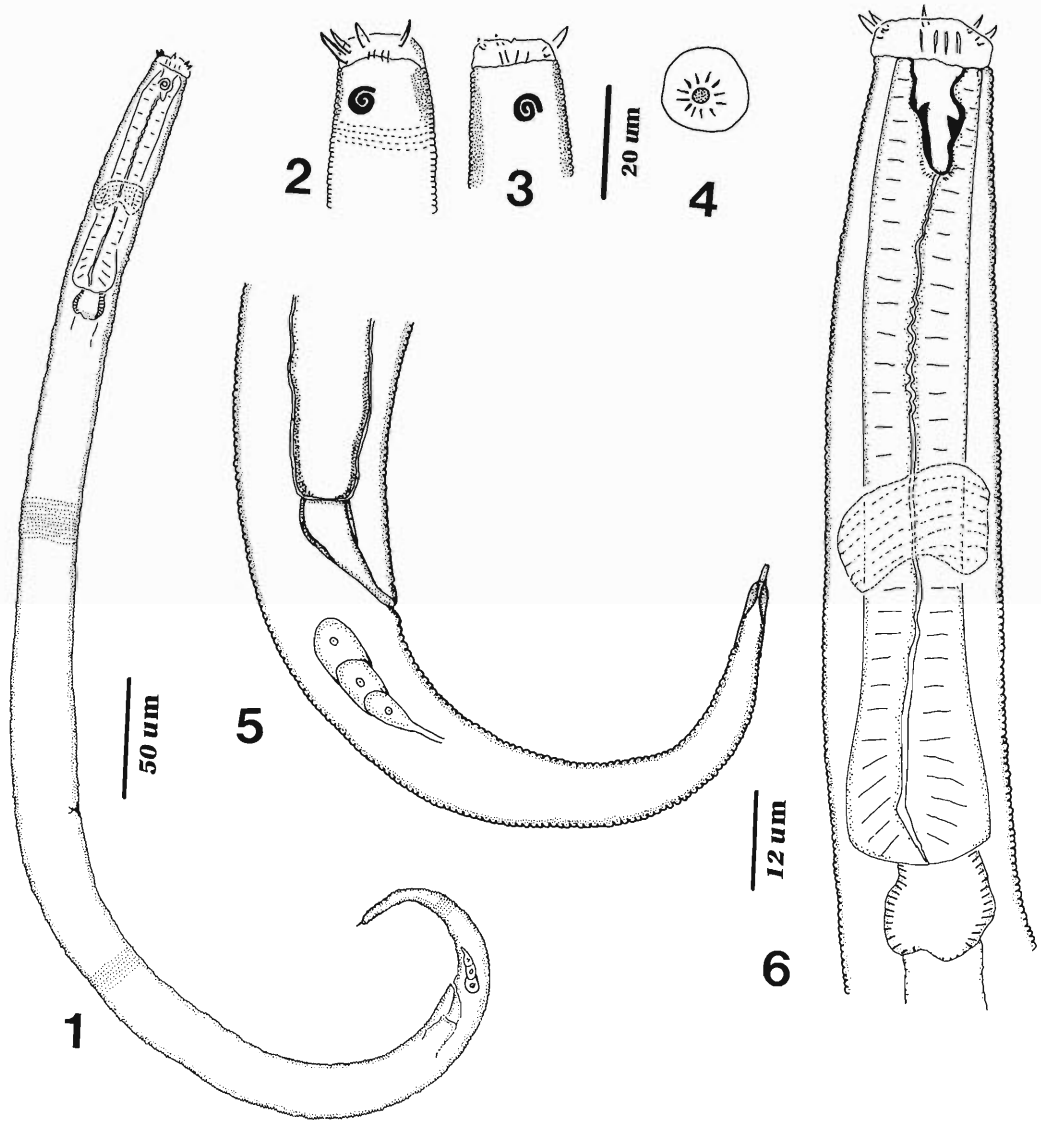
Achromadora walkeri sp. n. (Figs. 1–6)

Description

HOLOTYPE (female): Length 698; maximum width 27; esophagus length 105; tail length 77; width at anus 15; rectum length 17; V% = 48; buccal cavity 13 × 5; anterior extremity to amphid 8.6; amphid diameter 4.5; a = 26; b = 6.6; c = 8.9.

FEMALES (N = 6): Length 640 (590–698); maximum width 26 (24–28); esophagus length 101 (91–107); tail length 72 (63–80); width at anus 17 (15–20); rectum length 17 (15–20); V% 47 (43–49); buccal cavity 12 × 4.7 (10–13 × 4–5); anterior extremity to amphid 8.5 (7.5–9); amphid diameter 4.3 (3.4–4.8); a = 25 (23–26); b = 6.4 (5.8–7); c = 8.9 (8–10).

Anterior body straight, posterior half curved then coiled postanal (Fig. 1). Cuticle annuli very fine, transverse rows of punctation exist along the body cuticle (Fig. 2). Head with 12 cephalic setae visible in en face view (Fig. 4). Stoma infundibular, dorsal tooth located in upper third



Figures 1–6. ***Achromadora walkeri*** **sp. n. 1. Female, entire. 2, 3. Female, anterior region, amphid as seen on right-lateral side and left lateral side, respectively (view of body from right-lateral side). 4. Female, en face view of the head showing 12 well-developed setae. 5. Female, posterior region. 6. Female, anterior region showing development of esophagus and buccal capsule.**

of buccal cavity (38%). Subventral tooth located posteriorly in buccal cavity (47%). Amphid helical, 8.5 from anterior extremity, diameter approximately 4.3 (Figs. 2, 3). Esophagus with valved posterior bulb, 16% of total body length. Nerve ring approximately 64 from anterior extremity (Fig. 6). Esophago–intestinal valve present. Vulva not protruding, located 43–49% of total body length. Prerectum absent. Tail curved ventrally, tapered to a rounded terminus, with cuticular spinneret (Fig. 5).

MALES: Not found.

TYPE LOCALITY: Mix Canyon (38°24′N, 122°02′W), Solano County, California, U.S.A.

SYMBIOTYPE (see Frey et al., 1992): *Vitis californica* Bentham, University of California Davis, J. M. Tucker Herbarium No. 120481.

SITE: Soil around the roots of *Vitis californica.*

TYPE MATERIAL (holotype): Female on slide, University of California, Davis Nematode Collection (UCDNC) No. 2925.

PARATYPES (females): On slide, UCDNC No. 2926.

ETYMOLOGY: This nematode was named after Dr. Andrew Walker, who helped obtain samples used in this study.

Diagnosis

Achromadora walkeri sp. n. appears morphologically similar to *A. ruricola* (de Man, 1880), from which it can be differentiated by the position of the amphid. The amphid in *A. ruricola* is located at the base of the stoma (Mulvey, 1969), whereas in *A. walkeri* sp. n. it is more anteriad (level of sub-ventral tooth) and the buccal cavity is larger (12 × 4) than that in *A. ruricola* (7 × 4). *Achromadora walkeri* differs from both *A. micoletzkyi* Steiner, 1916, and *A. pseudomicoletzkyi* van der Linde, 1938 (see Mulvey, 1969), in lacking a prerectum, and it differs further from *A. micoletzkyi* in having a longer body (0.59–0.7 vs. 0.48–0.61 mm) and larger buccal cavity (12 × 4 vs. 8 × 4) (Mulvey, 1969). *Achromadora walkeri* also differs from *A. pseudomicoletzkyi* in tail length (72 vs. 100) and in vulva position (43–49 vs. 53%) and from *A. semiarmata* Altherr, 1952, in total body length (0.59–0.70 mm vs. 0.45–0.46 mm), length of the stoma (12 vs. 8), c-value (8–10 vs. 5.5–6), and the position of the vulva (43–49 vs. 44%).

Criconemoides featherensis sp. n. (Figs. 7–10)

Description

HOLOTYPE (female): Length 391; maximum width 35; esophagus length 141; stylet length 101; V% = 84; a = 11.2; b = 2.8; R = 83; R_{st} = 27; R_{vp} = 13.

FEMALES (N = 15): Length of body 325 (263–391); maximum width 33 (31–35); esophagus length 117 (106–141); stylet length 95 (87–103); V% = 85 (80–90); a = 9.4 (8.9–11.2); b = 2.8 (2.6–3.2); R = 80 (75–84); R_{st} = 27 (22–31); R_{vp} = 13 (11–14).

Body curved ventrally (open C-shape) (Fig. 10), tapering at posterior extremity. Annuli along body retrose, strongly retrose behind vulva to posterior extremity (Fig. 9). Anastomosis very rare, annuli mostly smooth without interruption. Lip region with 2 annuli (Fig. 7). Labial plate almost flat, slightly elevated. Stylet long, ending with anchor-shaped knobs. Esophagus length 36% of total body length, basal bulb not offset from isthmus. Vulval slit-position variable found between annulus numbers 11 and 14 from posterior extremity. Body tapers posterior to vulva to a terminus with protruding knob-like structure.

MALES: Not found.

JUVENILES: Annuli serrated (Fig. 8).

TYPE LOCALITY: Bobelaine Wildlife Preserve, 32 km by road south of Yuba City (38°55′N, 121°34′W), Placer County, California, U.S.A.

SYMBIOTYPE: *Vitis californica* Bentham.

SITE: Soil from the rhizosphere of the roots of *Vitis californica*.

TYPE MATERIAL (holotype): Female, UCDNC No. 2923.

PARATYPES (females): UCDNC No. 2924 from type locality and host.

ETYMOLOGY: This nematode was named after the Feather River, near the type locality.

Diagnosis

Criconemoides featherensis sp. n. differs from *C. grassator* Adams and Lapp, 1967, in having very strong retrose annuli on the posterior body. In *C. featherensis*, the first annulus is thick and relatively wide, whereas the first ring in *C. grassator* has edges that project anteriad. In addition, the tail of *C. featherensis* tapers gradually to a point, whereas that of *C. grassator* tapers to a sharp postanal cone (Adams and Lapp, 1967).

Criconemoides featherensis differs from *C. annulifer* (de Man, 1921) and *C. calvus* Raski and Golden, 1966, by having a shorter body length (325 vs. 386 and 390 [mean], respectively, a tail that tapers gradually to a conspicuous knob without attenuation, and a greater number of annuli (80 [mean]) (Raski and Golden, 1966).

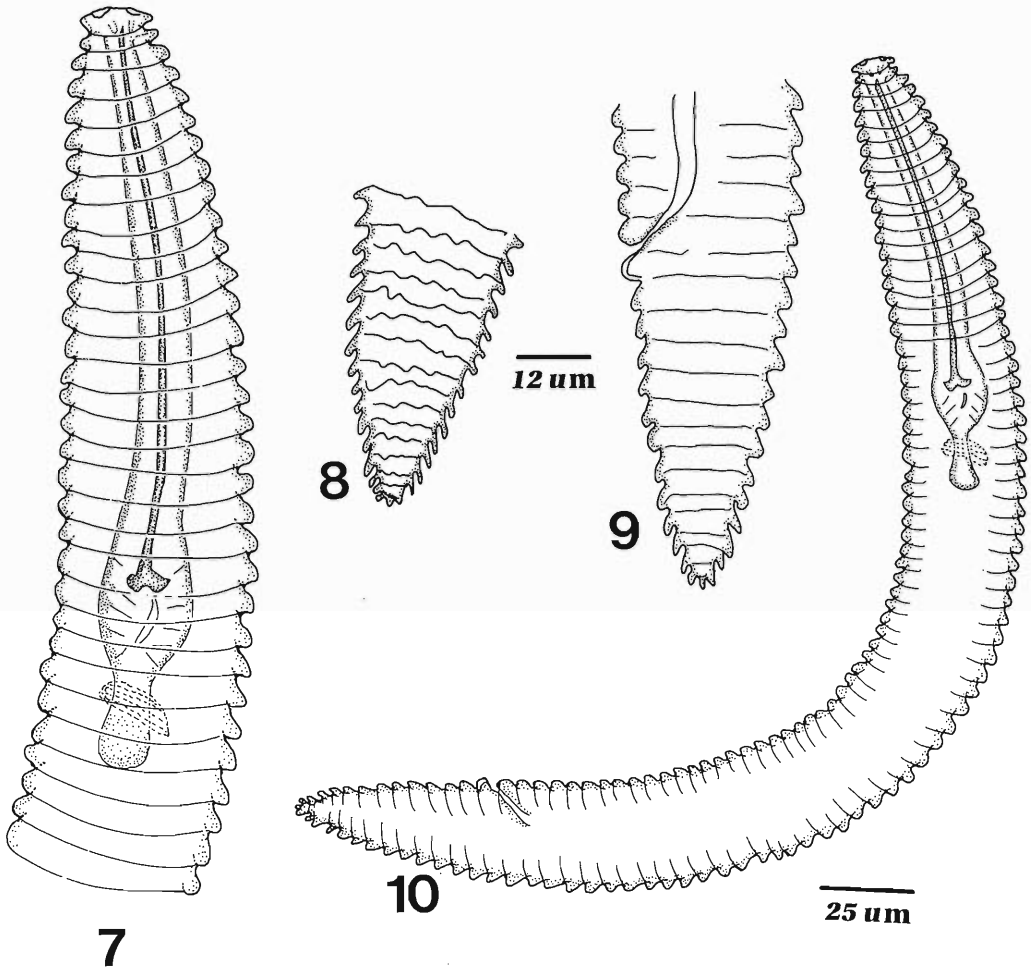
Hemicycliophora armandae sp. n. (Figs. 11–15)

Description

HOLOTYPE (female): Length 986; maximum width 53; esophagus length 187; V% = 85; a = 19; b = 5.3; stylet length 112; R = 250; R_{st} = 27; R_{ex} = 56; R_{v} = 233.

FEMALES (N = 8): Length 980 (790–1,090); maximum width 46 (38–54); esophagus length 190 (175–214); V% = 85 (82–86); a = 21 (19–25); b = 5.1 (4.5–5.7); stylet length 111 (95–119); R = 269 (250–280); R_{st} = 29 (25–30); R_{ex} = 54 (47–60); R_{v} = 220 (211–233).

Body curved slightly ventrally when killed in hot formalin (Fig. 11). Cuticular sheath close to



Figures 7–10. ***Criconemoides featherensis*** **sp. n. 7. Female, anterior region showing esophagus and stylet. 8. Juvenile, tail. 9. Female, tail. 10. Female, entire.**

inner body cuticle. Sheath and body annuli flattened especially on posterior region. Lateral fields marked by breaks in striae (Fig. 15). Cephalic region 16 wide × 1 high, with 3 annuli. Labial plate raised slightly. Stylet long and thin (95–119 × 1.2). Stylet knobs rounded, sloping slightly posteriad, located usually around annulus 29. Esophagus relatively long 190 (175–214). Esophago–intestinal valve present (Fig. 12). Excretory pore generally 2 annuli posterior to the end of esophagus, 196 (181–220) from the anterior extremity. Female gonad single, anteriorly directed and out-stretched without flexures. Spermatheca oblong, no spermatozoa visible (Fig. 13). Vulval lips modified, anterior lip extending over the posterior (Fig. 14). Body narrowing posterior to vulva but evenly conoid, ending with a digitate tail with a rounded terminus. Anus located about 16 annuli posterior to vulva.

MALES: Not found.

TYPE LOCALITY: Grapevine Mountain (33°07′N, 116°28′W) in Anza Borego Desert State Park, Riverside County, California, U.S.A.

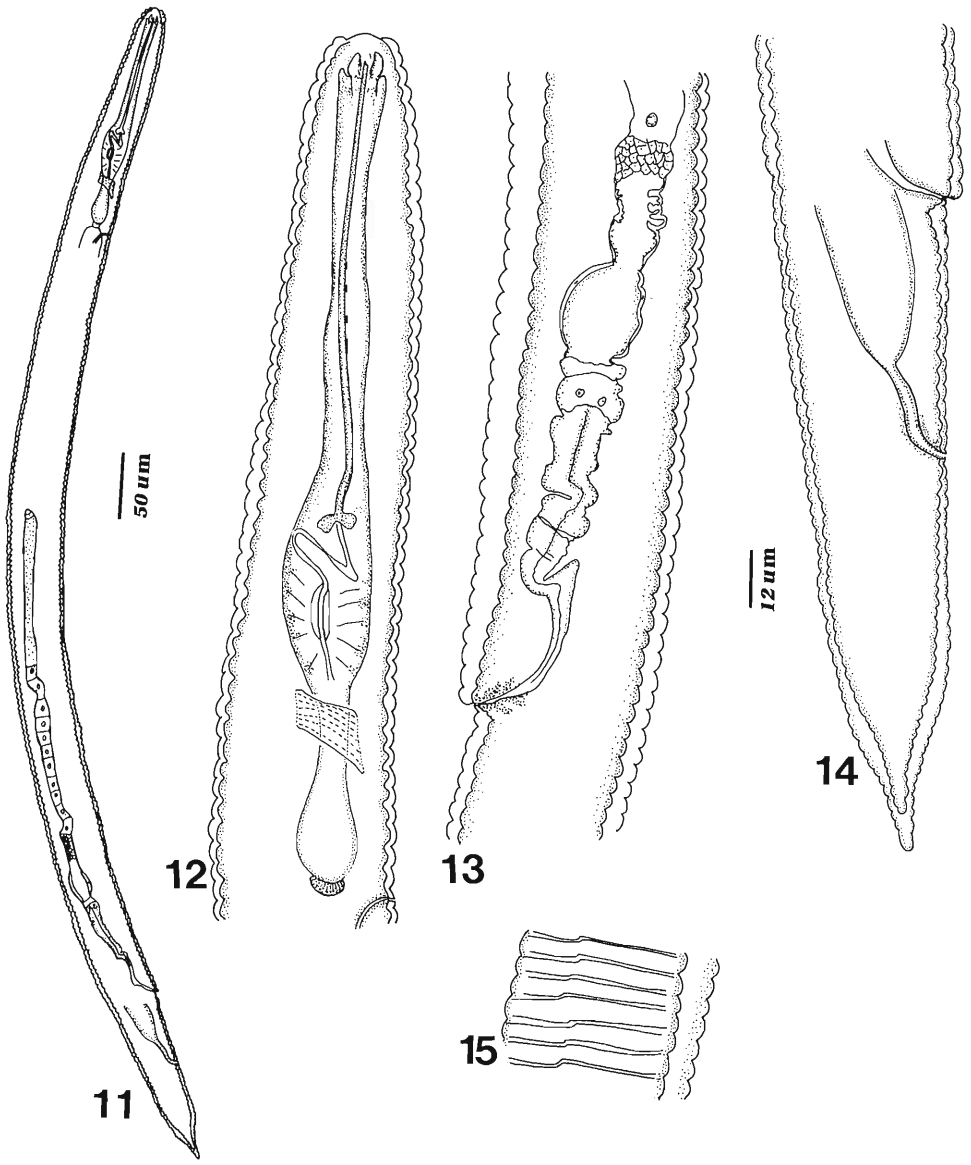
SYMBIOTYPE: *Vitis girdiana* Munson. University of California Davis, J. M. Tucker Herbarium No. 120480.

SITE: Soil around the roots of *Vitis girdiana.*

TYPE MATERIAL (holotype): Female on slide, UCDNC No. 2927.

PARATYPES (females): On slide, UCDNC No. 2928.

ETYMOLOGY: This nematode was named in honor of Dr. Armand Maggenti, a leader in the systematics of nematodes.



Figures 11–15. ***Hemicycliophora armandae*** **sp. n. 11. Female, entire. 12. Female, anterior region. 13. Female, region of vulva. 14. Female, tail. 15. Female, cuticular annulation.**

Diagnosis

Hemicycliophora armandae sp. n. can be recognized as distinct from *H. californica* Brzeski, 1974, *H. halophila* Yeates, 1967, *H. iwia* Brzeski, 1974, *H. minora* Wu, 1966, *H. shepherdi* Wu, 1966, *H. similis* Thorne, 1955, and *H. thornei* Goodey, 1963, in having 3 annuli in the cephalic region vs. 2 in all others (Thorne, 1955; Goodey, 1963; Wu, 1966; Yeates, 1967; Brzeski, 1974). The position of the excretory pore of *H. armandae* is located within the 47th to the 60th annulus while in the preceding species (except *H. shepherdi*), the excretory pore is located between the 38th and 49th annuli (inclusive). In addition, *H. armandae* differs from *H. minora, H. shepherdi,* and *H. similis* in having a prominent spermatheca, in the shape of the vulval lips, and in the tail terminus. *Hemicycliophora armandae* can be differentiated further from *H. thornei* in having no lateral lines, a longer stylet (111 vs. 63), and a vulva more posteriad (82–86 vs. 80–82%) (see

Goodey, 1963). *Hemicycliophora armandae* differs from *H. californica* in having larger values for R (250–280 vs. 210–236), R_v (211–233 vs. 172–195), cuticular sheath (close vs. very close to body cuticle), and stylet length (95–119 vs. 85–98) and a lower a-value (19–25 vs. 24–29) and lower V% (82–86 vs. 86–87%). The tail terminus is rounded in *H. armandae* but is sharply pointed in *H. californica* (see Brzeski, 1974) and the vulval lips are more protruding and the contraction behind the vulva is greater than in *H. californica*. *Hemicycliophora armandae* can be differentiated from *H. iwia* in shape of tail terminus (more finger-like in *H. armandae*), by greater total number of annuli (269 vs. 204), and in the absence of lateral lines. *Hemicycliophora armandae* differs from *H. halophila* in having a modified vulva, shorter body length (790–1,090 vs. 1,030–1,210), and greater total number of body annuli (269 vs. 230 [mean]) and in the absence of longitudinal markings; *Hemicycliophora halophila* possesses "delicate longitudinal markings along each edge" (Yeates, 1967).

Discussion

The diversity and systematic relationships of nematodes parasitic on the majority of plants grown as crops are fairly well known. In contrast, little is known of the symbiotic associates of wild or native relatives of presently cultivated crop plants (e.g., see the volume edited by Nickle, 1984). Despite the economic threat to cultivated crops posed by nematodes worldwide, there are few scientists with sufficient training capable of collecting, identifying, and describing new species of nematodes.

Up to the present time, very little work has been conducted on the nematode associates of native species of plants that are close relatives of presently cultivated crop plants. In the case of grapevine, of the more than 637 published reports of nematodes associated with grapes of the genus *Vitis* L., only 3 studies include data on nematodes of native species of *Vitis* (see González and Valenzuela, 1968; Siddiqui et al., 1973; Al Banna, 1992). The dagger nematode, *Xiphinema index* (Thorne and Allen, 1950), the vector of the grape fan-leaf virus, has a cosmopolitan distribution and has been studied intensively in California (Raski et al., 1983); however, there is no clear picture of the area of origin of this nematode and, therefore, no information is available concerning the community of nematode associates in which *X. index* may have evolved. This is just one of many examples that demonstrates how little is known of the biological characteristics of nematode associates of native plants in nonagricultural ecosystems.

Because we know so little of the nematode associates of wild native plants, it is difficult to make generalizations concerning the ecological and trophic relationships of nematode associates of cultivated crop plants. We feel that new emphasis should be placed on studies of the relationships among plants and their symbionts.

Acknowledgments

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Coccidiosis Conference

The annual Coccidiosis Conference will be held on 31 October 1993 from 2–5 pm in the afternoon of the first day of the joint meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists, in Atlanta, Georgia. The purpose of the conference is to bring together scientists from disparate research areas who are studying mechanisms of immunity against parasites. Topics include developmental stages that induce and are targeted by protective immunity, the role of lymphokines in the immune response, and evasion mechanisms that the parasite may use to evade host immunity. The title of the conference is, "Parasite-Host Interactions: Immunity and Evasion Mechanisms."

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Studies on Indian Marine Cercariae: Two New Echinostome Cercariae

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ABSTRACT: Two new echinostome cercariae parasitizing *Cerithidea cingulata* Gmelin in India are described. *Cercaria bengalensis* II sp. n. is characterized by 23 collar spines and primary excretory tubules with lateral branches between the ventral sucker and pharynx. *Cercaria bengalensis* III sp. n. has 35 collar spines arranged in an uninterrupted semicircle with a gap on the ventral side and primary excretory tubules without lateral branches.

KEY WORDS: *Cercaria bengalensis* II sp.n., *Cercaria bengalensis* III sp. n., *Cerithidea cingulata,* Bay of Bengal, Echinostomatidae, Trematoda, India.

In a survey of marine and brackish water cercariae from the Coromandel coast, Bay of Bengal, India, 2 new species of echinostome cercariae were obtained from the snail *Cerithidea cingulata* Gmelin. One has 23 collar spines and may develop into adults of an *Acanthoparyphium* species; the other has 35 collar spines. The cercariae are designated here as *Cercaria bengalensis* II and *Cercaria bengalensis* III, respectively, after the geographical region from which the snails were collected.

Materials and Methods

Naturally emerged cercariae and developmental stages were obtained from snails collected during 1975–1979. The methods of Cable (1956) were used to study cercariae. Azure I–Schiff stain was employed to determine the number of collar spines (Hanumantha Rao and Murthy, 1972). Measurements are in micrometers and were taken for each species from 10 heat-killed specimens. Figures were drawn with the aid of a camera lucida from heat-killed specimens to show general features; other details were added freehand.

Results

Cercaria bengalensis II sp. n. (Figs. 1–4)

HOST: *Cerithidea cingulata* Gmelin.

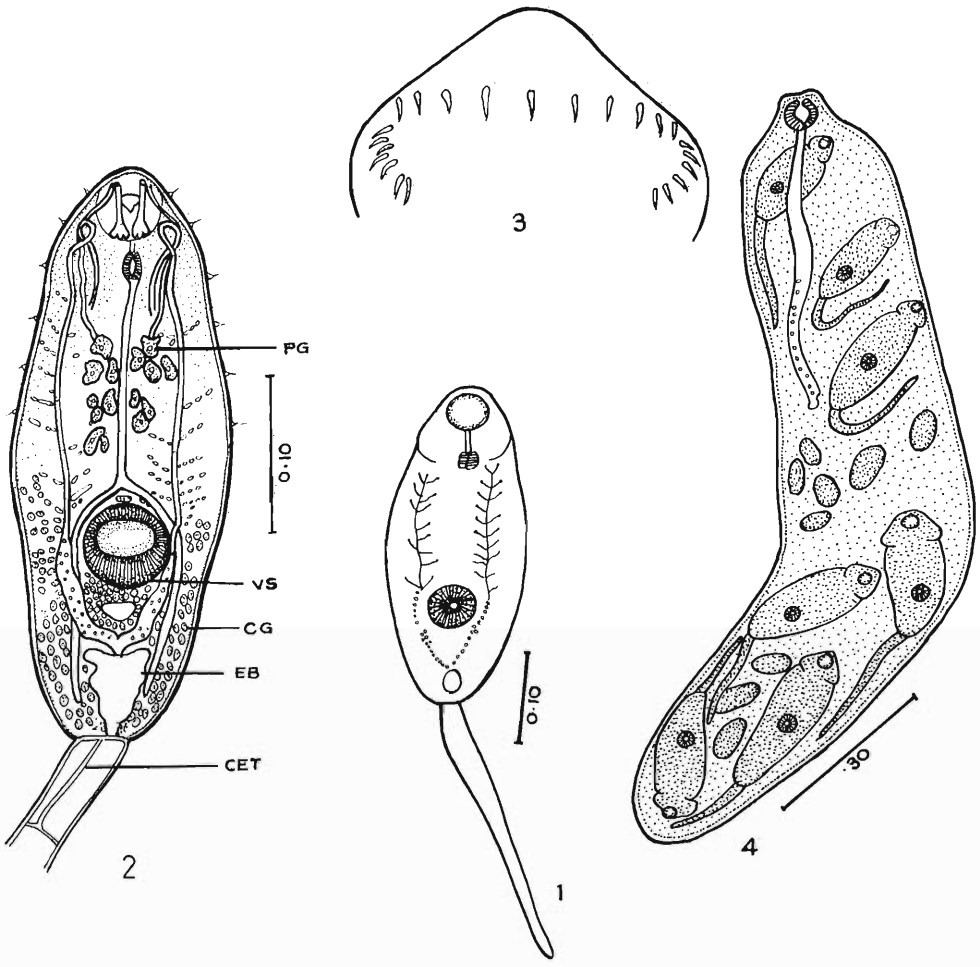
LOCALITY: Mangrove area near Visakhapatnam Harbour, brackish water of Bheemunipatnum (Bay of Bengal).

PREVALENCE OF INFECTION: 108 out of 9,717 snails.

SPECIMENS DEPOSITED: USNM Helminthological Collection Accession No. 79547.

Description

Body elongate 336–368 long, 140–160 wide, slightly tapering anteriorly; with distinct collar near pharyngeal level. Tegument spinulate, provided with few bristles set on papillae. Tail shorter than body, 288–304 long, 32 wide, attached subterminally. Oral sucker terminal, 42–44 in diameter, weakly muscular. Ventral sucker circular, 54–60 in diameter, about two-thirds body length from anterior end. Collar with 23 spines in single row forming a semicircle with wide ventral gap. Spines largest dorsally, decreasing in size toward ventral gap. Corner spines not distinct as such. Prepharynx short. Pharynx 20 in diameter followed by long, narrow esophagus bifurcating immediately anterior to ventral sucker. Ceca slender, extending to excretory bladder. Penetration glands of 2 types; 1 pair of lobed glands situated within and near posterior border of oral sucker, with ducts opening separately at pores near anterior end; the other type consisting of 12 large glands on either side of esophagus, with ducts passing anteriorly close to oral sucker to open at distinct pores near dorsal lip. Cystogenous glands with opaque, granular cytoplasm throughout body posterior to oral sucker. Excretory bladder expansive with thick muscular wall. Caudal excretory tubule extending into tail about one-fifth its length to bifurcate and open laterally. Primary excretory tubules voluminous, originating from midanterior portion of bladder but tapering gradually, tubules bearing lateral diverticula before turning posteriorly near oral sucker. Flame cells obscured by dense cystogenous glands. Genital rudiment represented by preacetabular and postacetabular cell masses.



Figures 1–4. ***Cercaria bengalensis*** **II sp. n. Abbreviations: CET = caudal excretory tubule, CG = cystogenous gland, EB = excretory bladder, PG = penetration gland, VS = ventral sucker. 1. Entire cercaria, ventral view. Scale bar = 100 μm. 2. Ventral view of cercarial body showing details (cystogenous glands omitted from preacetabular level). Scale bar = 100 μm. 3. Collar showing arrangement of spines (freehand drawing). 4. Redia. Scale bar = 300 μm.**

Behavior

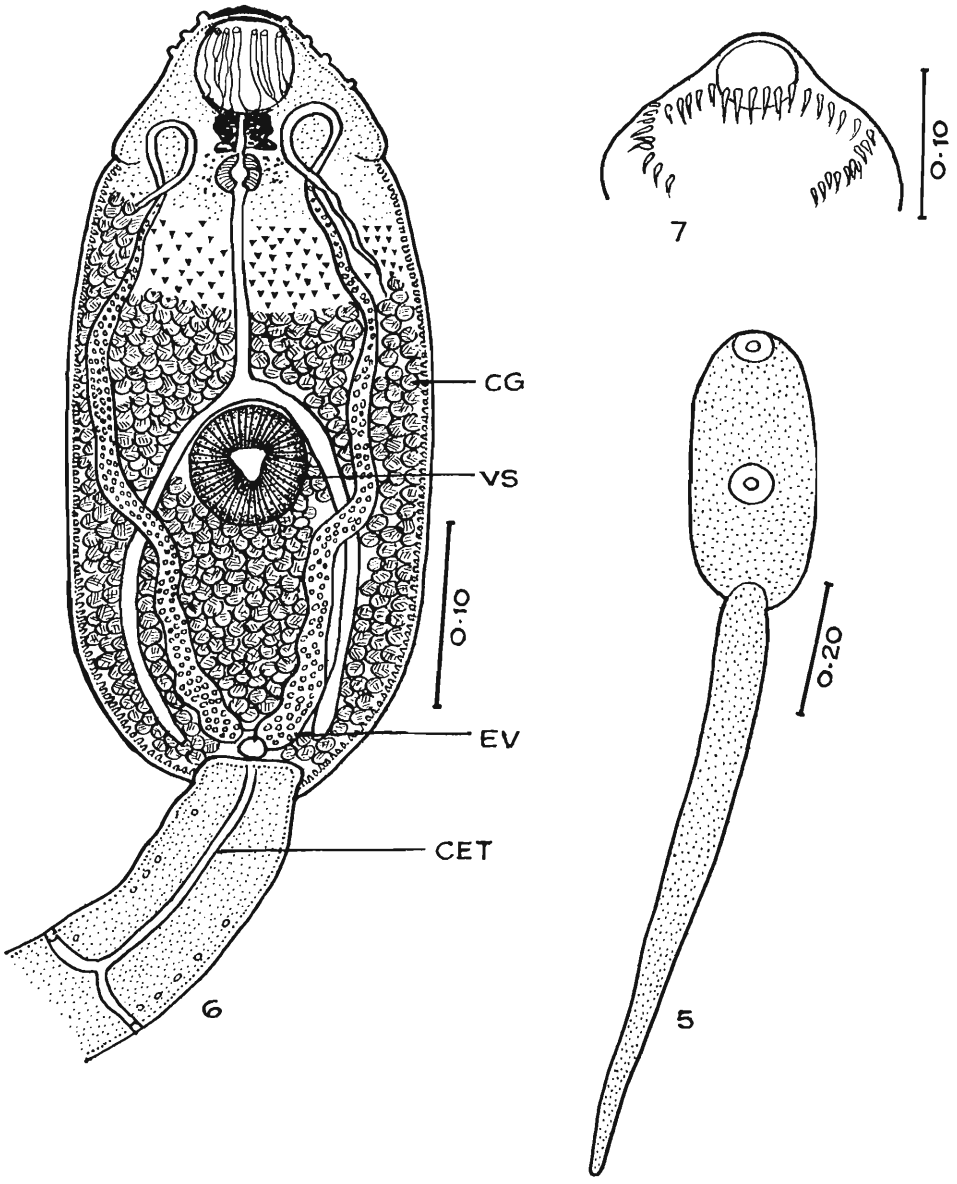
Cercariae emerging in large numbers throughout day and swimming actively with body strongly contracted and tail lashing vigorously. Rest periods very short with body stretching for a moment.

Redia

Redia sac-like, with distinct collar, 1,418–1,440 long by 293–336 wide; pharynx 52–54 long, gut narrow, one half body length long. Containing 10–15 cercariae and germ balls in various stages of development.

Discussion

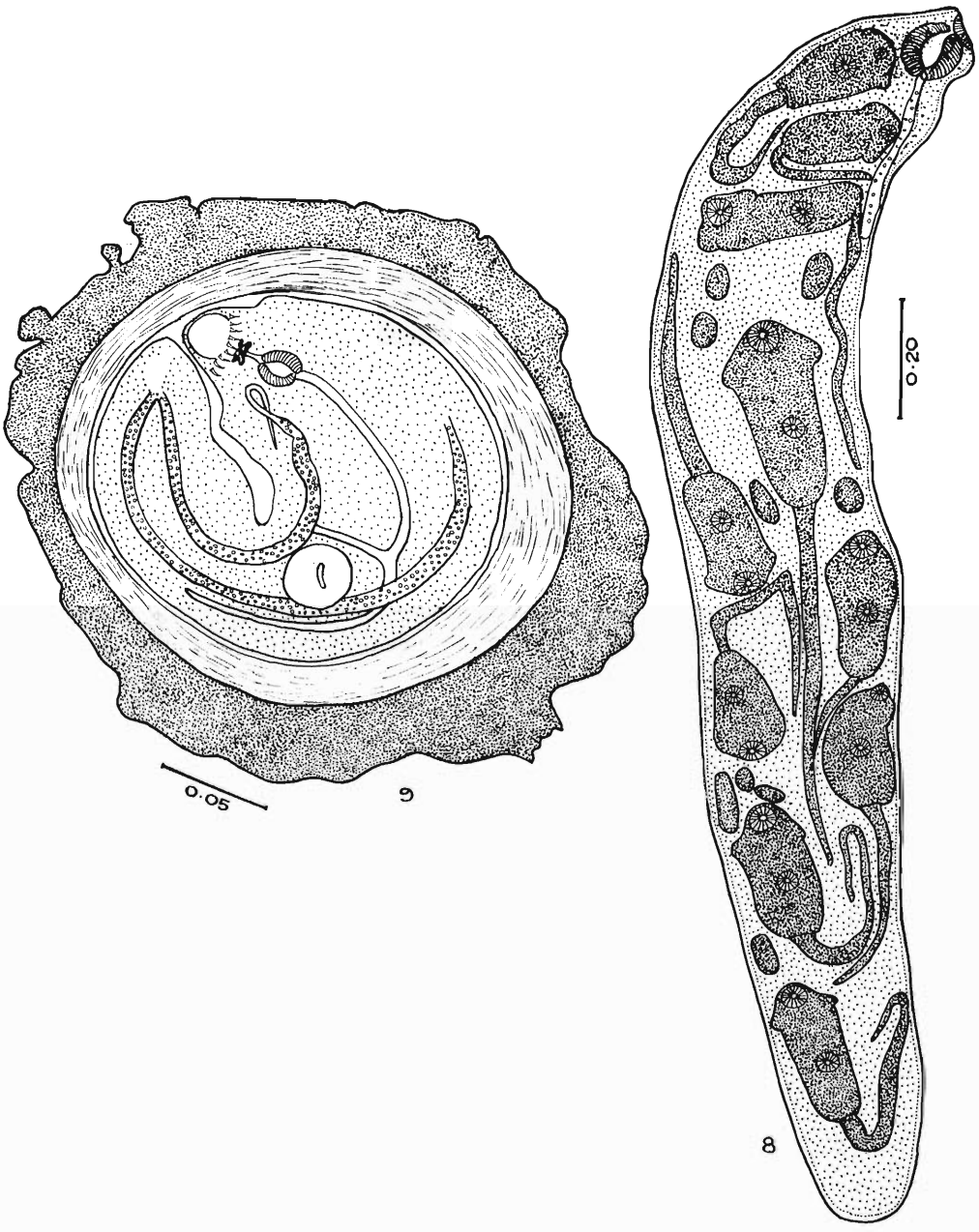
Marine echinostome cercariae possessing 23 collar spines are *Acanthoparyphium cercaria* Yamaguti, 1934, *Cercaria yamagutii* Ito, 1957, *Cercaria* III Maxon and Pequegnat, 1949, and *Cercaria caribbea* II Cable, 1956; cercaria of *Acanthoparyphium spinulosum* Johnston described by Bearup (1960) from Australia and Martin and Adams (1961) from United States; and cercaria of *Acanthoparyphium paracharadrii* described by Velasquez (1964). *Cercaria caribbea* II and the cercaria of *A. paracharadrii* differ from *C. bengalensis* II in body–tail proportions,



Figs. 5–7. ***Cercaria bengalensis*** **III sp. n. Abbreviations: CET = caudal excretory tubule, CG = cystogenous gland, EV = excretory vesicle, VS = ventral sucker. 5. Entire cercaria, ventral view. Scale bar = 200 μm. 6. Ventral view of body of cercaria. Scale bar = 100 μm. 7. Ventral view of cephalic end showing collar spines. Scale bar = 100 μm.**

by having a smooth tegument and in lacking cephalic glands. *Cercaria yamagutii* differs in the larger size of body and tail and in having 3 pairs of penetration glands (Ito, 1957). The cercaria of *A. spinulosum* has 4 penetration glands. *Cercaria* III differs in body–tail measurements, sucker ratio, and its encystment within and outside rediae (Maxon and Pequegnat, 1949).

Cercariae of species of *Acanthoparyphium* so far recorded have 23 collar spines in a single row and a characteristic excretory system with the primary excretory tubules bearing about 10 pairs



Figures 8, 9. ***Cercaria bengalensis*** **III sp. n. 8. Redia. Scale bar = 200 μm. 9. Metacercaria. Scale bar = 50 μm.**

of lateral diverticula filled with concretions. The available knowledge on the life cycles of *Acanthoparyphium* species indicates that larval stages develop in prosobranch snails and encyst as metacercariae either in the same snail or in lamellibranchs, and that the adults occur in birds. It is likely that *Cercaria bengalensis* II sp. n. may develop into a species of *Acanthoparyphium* in

local birds, but neither adults nor metacercariae belonging to this genus have so far been recorded from India.

Cercaria bengalensis III sp. n. (Figs. 5–9)

HOST: *Cerithidea cingulata* Gmelin.

LOCALITY: Mangroves near harbor area of Visakhapatnam, brackish water area of Bheemunipatnam, Balacheruvu and Kakinada (Bay of Bengal).

PREVALENCE OF INFECTION: 9 out of 9,717 snails.

SPECIMENS DEPOSITED: USNM Helminthological Collection Accession No. 79548.

Description

Body 400–422 long and 160–208 wide, region posterior to pharyngeal level with triangular spines in quincuncial arrangement, diminishing in size and density toward posterior end. Pigment granules brownish, scattered throughout body, concentrated near posterior border of oral sucker. Tail simple, 896–914 long and 64–80 wide, twice as long as body, attached subventrally. Oral sucker terminal, 45–48 in diameter. Ventral sucker circular, 60–69 in diameter, situated below midline. Mouth subterminal, leading into small prepharynx and globular pharynx, 18–20 in diameter, situated beneath pigment mass. Esophagus long, bifurcating just anterior to ventral sucker; ceca narrow extending to posterior end of body. Collar with 35 rod-shaped spines, nonuniform in size, in single continuous row ending with irregularly arranged corner spines with gap on ventral side. Cephalic glands limited to oral sucker, their number not determined but with ducts opening as 12 distinct pores at tip of oral sucker. Body with dense, rod-filled cystogenous cells. Excretory bladder small, oval, thin-walled. Primary excretory tubules broad at base, ascending segment filled with numerous refractile bodies. Descending segment ciliated, extending to posterior end of body and turning anteriorly before receiving secondary tubules. Caudal excretory duct extending one-sixth of tail length, bifurcating to open at lateral pores on tail.

Behavior

Cercariae negatively phototactic, emerging in moderate numbers throughout day and swimming actively with body contracted into spherical mass and tail lashing vigorously, describing characteristic 8-shaped movements.

Redia

Elongate, 1,952–2,128 long by 320–400 wide; with distinct collar and procruscula at level of posterior third of body. Pharynx well developed, 96–108 by 86–100. Cecum one-sixth body length containing yellowish-orange pigment granules. Birth pore below pharynx.

Metacercaria

Cercariae encysting freely on solid objects and on sides, as well as bottom, of container. Metacercarial cysts spherical, 240–272 by 240–256 in size, provided with thin hyaline layer and thick fibrous inner layer. Metacercaria lying folded and showing active movements inside cyst.

Discussion

Cercaria bengalensis III sp. n. closely resembles *Cercaria* I, described by Maxon and Pequegnat (1949) from *Cerithidea californica,* in possessing 35 collar spines and in other body features, but it clearly differs from the latter in lacking lytic gland cells between oral and ventral suckers, in the larger size of the tail, and in showing negative phototaxis. Among the other known echinostome cercariae bearing collar spines, the present cercaria shows resemblance to *Cercaria fuscata* described by Holliman (1961) and to cercaria of *Himasthla rhigedana* described by Adams and Martin (1963) and *Himasthla littorinae* described by Stunkard (1966). However, unlike *C. bengalensis* III sp. n., *C. fuscata* has 49 collar spines, with smooth tegument. The cercaria of *H. rhigedana* has 39 collar spines and a saccular excretory bladder, and that of *H. littorinae* has 29 collar spines.

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Presentation of the 1991 Anniversary Award to Dr. Frank G. Tromba

It is indeed an honor and an immense pleasure to have the privilege of presenting to Dr. Frank G. Tromba the 1991 Anniversary Award. Dr. Tromba was elected to membership in 1951 and has since served the Helminthological Society of Washington in a variety of capacities. He was elected Recording Secretary in 1957, served as Council Member-at-Large from 1960 through 1961, elected Vice-President in 1962, and President in 1963.

It is of interest to note, that his election to President of this Society occurred on November 16, 1962, almost 30 years to the date of this present meeting. The election took place at the Log Lodge on the campus of the Agricultural Research Station. Dr. Tromba continued to serve the Helminthological Society in a selfless manner and was Editor of the Proceedings from 1966 to 1970. He authored or co-authored about a dozen papers in the Proceedings, many of them on sundry aspects of the biology of *Ascaris,* and also contributed several presentations at our local meetings. He was elected a Life Member in 1983.

On behalf of the membership of the Helminthological Society of Washington and the Anniversary Award Committee, I would like to thank Dr. Tromba for his contributions to the Society and congratulate him on receiving this award.

Edward H. Michelson
Chairman,
Anniversary Award Committee, 1991

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Research Note

Coccidian Parasites of Heteromyid and Murid Rodents from Baja California del Sur, Mexico

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ABSTRACT: Thirty-one heteromyid and murid rodents were collected from 3 sites in Baja California del Sur, Mexico, and their feces examined for coccidia. Of the 31 rodents examined, 10 (32%) were found to be harboring 1 of 3 eimerians. Infected hosts included 2 of 7 *Peromyscus eva eva* (Muridae) with *Eimeria arizonensis* and 1 of 7 *P. e. eva* with *Eimeria langebarteli*; and 3 of 6 *Chaetodipus baileyi extimus* (Heteromyidae), 2 of 8 *Chaetodipus spinatus broccus,* and 2 of 3 *C. s. peninsulae* with *Eimeria reedi.* This note documents new host and distribution records for *Eimeria* species from murid and heteromyid rodents in Baja California del Sur, Mexico.

KEY WORDS: Rodentia, Heteromyidae, Muridae, *Eimeria arizonensis, Eimeria langebarteli, Eimeria reedi,* survey, Baja California del Sur, Mexico.

Much has been published on coccidian parasites of rodents (see Levine and Ivens, 1990), particularly those belonging to the family Heteromyidae (Doran and Jahn, 1949, 1952; Doran, 1953; Levine et al., 1957, 1958; Ivens et al., 1959; Ernst et al., 1967a, b, 1968, 1970; Short et al., 1980; Stout and Duszynski, 1983; Hill and Best, 1985; Ford et al., 1990). Although some information is available on coccidia of heteromyids from Baja California Norte, Mexico (Stout and Duszynski, 1983; Ford et al., 1990), nothing, to our knowledge, has been written on coccidians from rodents of Baja California del Sur, Mexico. Here, we report new host and distributional records for *Eimeria* species in heteromyid and murid rodents from that region.

During January 1992, 31 rodents, including 9 murids and 22 heteromyids, were collected by 2 of us (R.R.H. and K.M.H.) from 3 localities in Baja California del Sur, Mexico (Table 1), and their feces examined for coccidia. General habitat of the area is desert shrub and creosote bush (*Larrea* sp.) or the lower Sonoran life zone of Merriam (cited *in* Odum, 1945). Mice were collected with Sherman live traps and killed by cervical dislocation. Feces from the rectum were placed in individual vials of 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and stored on ice. On return to the United States, samples were mailed to the VA Medical Center–Dallas, where unsporulated oocysts were sporulated at room temperature (ca. 22°C) in Petri dishes in a thin layer of $K_2Cr_2O_7$. Sporulated oocysts were concentrated by centrifugation in Sheather's sugar solution (sp. gr. 1.30) and examined microscopically. Measurements were made on up to 30 oocysts of each species and compared to previously published descriptions. Voucher specimens of hosts are on deposit in the Museum, Texas Tech University, and the Texas Cooperative Wildlife Collection, Texas A&M University.

Of the 31 rodents examined, 10 (32%) were found to be passing oocysts of 1 of 3 eimerians (Table 1); all infected hosts harbored a single species of *Eimeria.* Although our sample size is modest, prevalence of infection compares favorably to data provided by Ford et al. (1990), who reported 84 of 223 (38%) heteromyids infected with 11 species of eimerians from the southwestern United States, Baja California Norte, and Sonora, Mexico.

Eimeria arizonensis Levine, Ivens, and Kruidenier, 1957, appears to be one of the most ubiquitous coccidians in North American murid rodents. It has been reported previously from Piñon mice, *Peromyscus truei* (Shufeldt, 1885), in Arizona (Levine et al., 1957) and New Mexico (Reduker et al., 1985, 1987; Wash et al., 1990; Upton et al., 1992); white-footed mice, *Peromyscus leucopus* (Rafinesque, 1818), in Illinois (Levine and Ivens, 1960) and Texas (Upton et al., 1992); deer mice, *Peromyscus maniculatus* (Wagner,

Table 1. Rodents surveyed for coccidia from Baja California del Sur, Mexico, and the eimerian species collected.

Rodents	Locality*	Prevalence	Coccidian
Muridae			
Peromyscus eva eva	1	2/7	*Eimeria arizonensis*
	1	1/7	*E. langebarteli*
Neotoma lepida pretiosa	1	0/2	—
Heteromyidae			
Chaetodipus baileyi extimus	1	3/5	*E. reedi*
	2	0/1	—
C. dalquesti	3	0/3	—
C. spinatus broccus	1	2/8	*E. reedi*
C. s. peninsulae	3	2/3	*E. reedi*
Dipodomys merriami melanurus	2	0/2	—

* Localities: 1 = El Juncalito; 2 = 9.7 km S, 17.7 km W La Paz; 3 = Migriño, 25.7 km NW Cabo San Lucas.

1845), in British Columbia (Levine and Ivens, 1963), Illinois (Levine and Ivens, 1960), and New Mexico (Reduker et al., 1987); cactus mice, *Peromyscus eremicus* (Baird, 1858), in New Mexico (Reduker et al., 1987); canyon mice, *Peromyscus crinitis* (Merriam, 1891), in Utah (McAllister et al., 1991); northern rock mice, *Peromyscus nasutus* (J. A. Allen, 1891), in Texas (McAllister et al., 1991); brush mice, *Peromyscus boylii* (Baird, 1855), in New Mexico (Wash et al., 1990); and fulvous harvest mice, *Reithrodontomys fulvescens* J. A. Allen, 1894, and plains harvest mice, *Reithrodontomys montanus* Baird, 1855, in Texas (Upton et al., 1992). As noted recently by Upton et al. (1992), the report by Ford et al. (1990) of *E. arizonensis* in California pocket mice, *Chaetodipus californicus* Merriam, 1899, from Baja California Norte, Mexico, and Texas kangaroo mice, *Dipodomys elator* Merriam, 1894, from Texas, may be a misidentification. Our finding of sporulated oocysts that we could not distinguish from published descriptions of *E. arizonensis* in Eva's desert mice, *Peromyscus eva eva* Thomas, 1898, from Baja California del Sur, Mexico, is not surprising; however, it represents a new host and distributional record for the coccidian.

Eimeria langebarteli Ivens, Kruidenier, and Levine, 1959, was originally described from *P. boylii* in Chihuahua, Mexico (Ivens et al., 1959). It has since been reported from *P. leucopus* and *P. truei* from California (Reduker et al., 1985) and hispid pocket mice, *Chaetodipus hispidis* Baird, 1858, from Texas (Ford et al., 1990). In addition, Upton et al. (1992) suggested that the report of *Eimeria taylori* McAllister and Upton, 1988, in *P. leucopus* from Texas (McAllister and Upton, 1992) was a misidentification and probably represented the morphologically similar *E. langebarteli.* In the present survey, sporulated oocysts that were structurally identical to those of *E. langebarteli* were found in a new host, *P. eva eva.* Baja California del Sur, Mexico, is also a new locality for the coccidian.

Eimeria reedi Ernst, Oaks, and Sampson, 1970, is a common eimerian of heteromyid rodents. The species was originally reported from long-tailed pocket mice, *Chaetodipus formosus* Merriam, 1889, from California (Ernst et al., 1970; Ford et al., 1990) as well as *C. californicus* and desert pocket mice, *Chaetodipus penicillatus* Woodhouse, 1852, from California and Baja California Norte, Mexico, San Diego pocket mice, *Chaetodipus fallax* Merriam, 1889, and spiny pocket mice, *Chaetodipus spinatus* Merriam, 1889, from Baja California Norte, Mexico, *C. hispidis* from Texas, and silky pocket mice, *Perognathus flavus* Baird, 1855, from New Mexico and Texas (Ford et al., 1990). The sporulated oocysts we observed from the new hosts, Bailey's pocket mice, *Chaetodipus baileyi extimus* Nelson and Goldman, 1930, and 2 subspecies of spiny pocket mice, *Chaetodipus spinatus broccus* Huey, 1960, and *C. s. peninsulae* Merriam, 1894, were indistinguishable from those in the preceding descriptions. In addition, this is the first time the coccidian has been reported from Baja California del Sur, Mexico.

In summary, new host and distributional records are reported for 3 rodent eimerians from murid and heteromyid rodents from Baja California del Sur, Mexico. Given the hostile environment and desert extreme in which the rodents and their coccidian oocysts occur (<25 cm pre-

cipitation/yr), we did not expect to find a third of the hosts infected. However, data are similar to those reported by Ford et al. (1990), who reported a moderate prevalence of infection for western rodents inhabiting arid environments.

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Research Note

Strongyle Control after Multiyear Use of Ivermectin in Horses on a Farm in Central Kentucky

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ABSTRACT: Counts of strongyle eggs per gram of feces (epg) were determined biweekly for an 8-wk period in 1991 and 1992 for 83 thoroughbred horse mares (N = 21/yr) and yearlings (N = 20–21/yr) on a farm in central Kentucky. Historically, horses on this farm have been on a regular deworming program for nearly 5 decades. Ivermectin has been used approximately every 8 wk since 1983 when it was first marketed. There was occasional usage of pyrantel pamoate between routine ivermectin treatments. For the 2 evaluation periods, strongyle epg counts for mares were all negative and for yearlings were all negative except pretreatment for 1 yearling (epg = 10) in 1991 and 3 yearlings (epg = 10, 10, and 30) in 1992.

KEY WORDS: horses, strongyles, control, ivermectin.

Chemotherapy is the primary method for control of internal parasites of equids. Opinions vary regarding frequency of treatment (e.g., every 8 wk or strategically) and as to whether classes of compounds should be alternated fast (e.g., every 8 wk) or slow (e.g., annually) (Drudge et al., 1989). There is general consensus, however, that 1 class of compound should not be used for indefinite periods. Recently, there has been opportunity to evaluate a parasite control program in thoroughbred horses on a farm in central Kentucky where the same compound has been used for several years.

This farm's management has made a program of parasite control a priority for several decades (E. T. Lyons, unpubl. data, 1992). Ivermectin has been given to the horses about every 8 wk since 1983. Occasionally, once or twice a year, pyrantel pamoate was given in between the routine ivermectin treatments for removal of tapeworms (i.e., at about 4 wk after and before an 8-wk ivermectin treatment).

For a pre- and posttreatment period in 1991 and 1992, fecal samples were collected from 83 horses (mares: N = 21/yr; yearlings: N = 21 in 1991 and 20 in 1992). Collections were on the day of treatment (2 April 1992; 30 April 1992) and every 2 wk for 8 wk posttreatment. Strongyle epg counts were determined on all fecal samples (Lyons et al., 1976). In addition, in 1991 strongyle larval counts per gram of feces (lpg) (Drudge et al., 1963) were completed on a composite culture of feces from a group of 10 mares and a similar one for a group of 10 yearlings.

The epg counts for all mares were negative for both sample periods. Also, the lpg counts for the composite fecal cultures for the 10 mares in 1991 were negative. For the yearlings, epg counts were negative except pretreatment (day 0) for 1 yearling (epg = 10) in 1991 and for 3 yearlings (epg = 10, 10, and 30) in 1992. The lpg counts for the group of 10 yearlings in 1991 were negative except for the pretreatment day (day 0), when 2 small strongyle larvae were found in the culture.

The strongyle epg counts were negligible overall. These findings for this particular farm are of interest because, after usage for 8 (1991) and 9 (1992) yr, ivermectin continues its highly effective control of strongyles. As already stated, parasite control on the farm was excellent even before use of ivermectin. This factor, no doubt, has contributed to the effectiveness of ivermectin. It should be reemphasized that, because of potential or actual drug resistance, most parasitologists, including the present authors, do not advocate exclusive use of a single antiparasitic compound or class of compounds.

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Research Note

Helminths from Some Minnesota and Wisconsin Raptors

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ABSTRACT: Seventy-seven hawks of 10 species (*Accipiter cooperii, Accipiter striatus, Accipiter gentilis, Circus cyaneus, Buteo lagopus, Buteo jamaicensis, Buteo platypterus, Pandion haliaetus, Falco peregrinus, Falco sparverius*) and 49 owls of 8 species (*Bubo virginianus, Strix nebulosa, Strix varia, Aegolius acadicus, Otus asio, Asio flammeus, Asio otus, Cryptoglaux funereus*) from Minnesota and Wisconsin were examined for helminths. *Echinoparyphium* sp., *Echinostoma trivolvis, Neodiplostomum* sp., *Ribeiroia thomasi, Strigea falconis* (Trematoda), *Capillaria* sp., *Cyrnae* sp., and *Porrocaecum* sp. (Nematoda) were common to both hawks and owls. *Paruterina* sp. (Cestoda) was found only in the great-horned owl. *Lyperosomum* sp., *Parastrigea* sp. (Trematoda), *Centrorhynchus spinosus* (Acanthocephala), *Contracaecum pandioni, Microtetrameres* sp., *Physaloptera* sp., *Serratospiculoides amaculata,* and *Tetrameres* sp. (Nematoda) were recovered from hawks. New host records include *Lyperosomum* sp. from the gall bladder of a kestrel and *Ribeiroia thomasi* from the proventriculi of great-horned owls and red-tailed and broad-winged hawks. The only instance of pathology was a tissue reaction to *S. amaculata* in the air sacs of a Cooper's hawk.

KEY WORDS: hawks, owls, Minnesota, Wisconsin, Acanthocephala, Cestoda, Nematoda, Trematoda, prevalence, pathology.

No recent studies of helminth parasites observed in Minnesota and Wisconsin raptors have been published. Chandler and Rausch (1947) and Dubois and Rausch (1948, 1950a, b) concentrated on strigeoids from the midwest. Morgan (1943, 1946, 1948) discussed nematode parasites. Rausch (1948) reported on cestode parasites from owls in North America. This paper presents information about helminths from 10 species of hawks and 8 species of owls obtained from Minnesota and Wisconsin.

Seventy-seven hawks of 10 species and 49 owls of 8 species were examined for helminth parasites. Five were fresh road kills collected by the authors in Wisconsin, and the remaining were obtained frozen from the Raptor Center of the University of Minnesota; the Northwoods Wildlife Rehabilitation Center, Minocqua, Wisconsin; Wisconsin Department of Natural Resources, Madison, Wisconsin; and Fran Hamerstrom, Plainfield, Wisconsin. Complete necropsies were performed on all remains.

All helminths, other than nematodes, were preserved in alcohol–formalin–acetic acid and stained in Semichon's carmine, dehydrated, and mounted in Canada balsam. Nematodes were cleared in glycerine alcohol and stored, or mounted on slides using the double coverslip method.

Selected specimens in good condition were deposited in the University of Nebraska State Museum, Harold W. Manter Laboratory Collection (HWML Coll.) as follows: HWML No. 35098, *Centrorhynchus spinosus* (Kaiser, 1893) Van Cleave, 1924, ex *Buteo platypterus*; HWML Nos. 35099, 35100, and 35101, *Ribeiroia thomasi* (McMullen, 1938) Yamaguti, 1958, ex *Pandion haliaetus, Buteo platypterus,* and *Bubo virginianus,* respectively; HWML No. 35102, *Parastrigea* sp. ex *Falco peregrinus*; HWML No. 35103, *Microtetrameres* sp. ex *Buteo lagopus*; HWML No. 35546, *Strigea falconis* Szidat, 1928, ex *Buteo platypterus*; HWML No. 35547, *Strigea falconis* ex *Buteo jamaicensis*; HWML No. 35548, *Neodiplostomum* sp. ex *Buteo jamaicensis*; HWML No. 35549, *Neodiplostomum* sp. ex *Accipiter striatus*; HWML No. 35550, *Neodiplostomum* sp. ex *Accipiter cooperi*; HWML No. 35551, *Strigea falconis* ex *Circus cyaneus*; HWML No. 35552, *Physaloptera* sp. ex *Accipiter striatus*; HWML No. 35553, *Contracaecum pandioni* Sobolev and Sudarikow, 1939, ex *Pandion haliaetus*; HWML No. 35554, *Serratospiculoides amaculata* Wehr, 1938, ex *Accipiter cooperi*; and HWML No. 35555, *Porrocaecum* sp. ex *Buteo jamaicensis.*

Prevalences of infections and ranges of numbers of worms found are given in Table 1 for hawks and Table 2 for owls.

Carcasses examined by us had usually been

Table 1. Prevalence of helminths in hawks from Minnesota and Wisconsin. Numbers in parentheses are ranges of worms per positive host. Goshawk (*Accipiter gentilis*, $N = 1$) was examined but found to be negative.*

	Cooper's hawk (*Accipiter cooperii*)	Sharp-shinned (*Accipiter striatus*)	Northern Harrier (*Circus cyaneus*)	Rough-legged (*Buteo lagopus*)	Red-tailed (*Buteo jamaicensis*)	Broad-winged (*Buteo platypterus*)	Osprey (*Pandion haliaetus*)	Peregrine (*Falco peregrinus*)	Kestrel (*Falco sparverius*)
Echinoparyphium sp.[1]						5/16 (1–169)			
Echinostoma trivolvis[1]					1/11 (1)	1/16 (37)			
Lyperosomum sp.[2]									1/9† (2)
Neodiplostomum sp.[1]	2/7 (20–39)	7/8 (1–20)	2/2 (3–12)		4/11 (4–41)	3/16 (1–5)			
Parastrigea sp.[1]						3/16 (1–6)		1/1 (4)	
Ribeiroia thomasi[3]					1/11† (1)	3/16† (1–27)	1/1 (21)		
Strigea falconis[1]	1/7 (6)	1/8 (3)	2/2 (1–9)	1/21 (5)	6/11 (20–87)	7/16 (2–5)			
Centrorhynchus spinosus[1]						2/16			
Contracaecum pandioni[4]							1/1 (4)		
Cyrnae sp.[3]	2/7 (1–2)	1/8 (1)	2/2 (3–31)			4/16 (1–16)			
Microtetrameres sp.[3]				1/21 (29)		1/16 (1)			1/9 (5)
Physaloptera sp.[4]		6/8 (3–5)		1/21 (1)		2/16 (4)			
Porrocaecum sp.[1]	3/7 (1–50)	1/8 (5)		2/21 (3)	5/11 (3–5)	6/16 (5–19)			
Serratospiculoides amaculata[5]	1/7 (27)								
Tetrameres sp.[3]									1/9 (5)

* Superscripts indicate location in host: 1 = intestine; 2 = gall bladder; 3 = proventriculus; 4 = stomach; 5 = air sacs.
† New host records.

frozen and in some cases refrozen. They exhibited slight to severe autolysis. Schoop et al. (1987) generally condemned the use of frozen hosts for parasitological surveys because trematodes and small cestodes are underrepresented and nematodes and acanthocephalans are overrepresented. The use of frozen hosts generally in poor condition made it difficult to identify parasites beyond the generic level. Pence et al. (1988) condoned the practice with certain caveats, especially when dealing with rare or endangered hosts. Raptors fit into this category. As a result, prevalence rates in Table 1 and 2 may be low, especially in regard to trematodes and cestodes. A thorough search of the raptor parasite literature was undertaken in order to compare numbers of helminths found by us to those reported by others. Comparisons proved difficult for the following reasons: only about one-half of the papers reported parasite numbers; when numbers were given they were often from different hosts; and some numbers reported were from helminth taxa differing from ours. Numbers and kinds of parasites found in our samples of great-horned owls are compared to those of Ramalingam and Samuel (1978), who also used frozen carcasses. They listed 13 genera, whereas species in 8 genera were found in our study. The studies had 7 genera and/or species in common (*Capillaria, Cyrnae, Echinoparyphium, Echinostoma revolutum, Pa-*

Table 2. Prevalence of helminths in owls from Minnesota and Wisconsin. Numbers in parentheses are ranges of worms per positive host. Great grey (*Strix nebulosa*, $N = 1$) and Screech (*Otus asio*, $N = 4$) owls were examined and found to be negative.*

	Great-horned (*Bubo virginianus*)	Barred (*Strix varia*)	Saw-whet (*Aegolius acadicus*)	Short-eared (*Asio flammeus*)	Long-eared (*Asio otus*)	Boreal (*Cryptoglaux funereus*)
Echinoparyphium sp.[1]	1/19 (67)					
Echinostoma trivolvis[1]	1/19 (8)					
Neodiplostomum sp.[1]	1/19 (7)	1/7 (22)				
Ribeiroia thomasi[2]	2/19† (1–8)					
Strigea falconis[1]	4/19 (3–23)	1/7 (1)				
Paruterina sp.		2/7 (7–19)				
Capillaria sp.[1]	2/19 (5–9)	2/7 (3–6)	1/8 (2)		1/3 (2)	
Cyrnae sp.[2]	4/19 (1–8)	2/7 (10–24)				
Porrocaecum sp.[1]	9/19 (1–6)	2/7 (1–10)		1/2 (1)	1/3 (5)	3/5 (2–16)

* Superscripts indicate location in host: 1 = intestine; 2 = proventriculus.
† New host records.

ruterina, Porrocaecum, and *Strigea*). Of the taxa in common, we report higher numbers only for the genus *Strigea.* The greater diversity and higher intensities of infection reported by them may simply reflect their sample size of 69 versus ours of 19.

Specimens of *Lyperosomum* sp. were collected from the gall bladder of a kestrel. Although a new host record, it should not be considered unusual because insects commonly make up a large part of the diet of these birds (Alcorn, 1934). Insects also serve as the second intermediate host for several members of the genus *Lyperosomum.*

Previously reported only in Cooper's hawk and ospreys, *R. thomasi* has now been recovered from great-horned owls, red-tailed and broad-winged hawks, and again from an osprey. The second intermediate hosts of this parasite are either fish or amphibians (Beaver, 1939), indicating a broad food base for these birds.

Newsom and Stout (1933) observed proventriculitis in chickens infected with *R. thomasi.* Proventriculitis was not observed in our study. The only instance of pathology in any of the birds was a tissue reaction in the air sacs of a Cooper's hawk due to the presence of 27 adult *Serratospiculoides amaculata.* Sterner and Espinosa (1988) reported *Serratospiculoides amaculata* from a Cooper's hawk and noted a similar tissue reaction surrounding the worms in the thoracic air sac. Ours is the second published report of a species of this genus from a Cooper's hawk.

The greatest diversity of parasites (Table 1) observed by us was found in the broad-winged hawks. According to Mosher and Palmer (1988), these hawks have some of the most diverse food habits among the raptors, feeding on invertebrates, fish, amphibians, reptiles, birds, and mammals.

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Research Note

Gastrointestinal Helminths of the Crevice Spiny Lizard, *Sceloporus poinsettii* (Phrynosomatidae)

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ABSTRACT: Twenty-one *Sceloporus poinsettii* from Texas and New Mexico were examined for helminths. Helminth faunas of the 2 lizard populations differed. The Texas population contained *Skrjabinoptera phrynosoma* (80% prevalence, mean intensity 27), *Thubunaea iguanae* (20% prevalence, mean intensity 1), and *Oochoristica scelopori* (30% prevalence, mean intensity 7). The New Mexico population contained *Physaloptera retusa* (55% prevalence, mean intensity 25) and *Spauligodon giganticus* (82% prevalence, mean intensity 30). All represent new host records. Xeric conditions of the Texas *S. poinsettii* habitat may partly account for the absence of *S. giganticus.*

KEY WORDS: *Sceloporus poinsettii,* Phrynosomatidae, Cestoda, *Oochoristica scelopori,* Nematoda, *Skrjabinoptera phrynosoma, Thubunaea iguanae, Physaloptera retusa, Spauligodon giganticus,* prevalence, intensity.

The crevice spiny lizard, *Sceloporus poinsettii* Baird and Girard, 1852, occurs from southern New Mexico and Texas to Zacatecas, Mexico, at elevations of 300–2,560 m (Stebbins, 1985). Gambino (1958) and Gambino and Heyneman (1960) previously reported the nematode *Atractis penneri* (Gambino, 1957) Baker, 1987, from *Sceloporus poinsettii.* The purpose of this note is to report 5 new host records: *Oochoristica scelopori* Voge and Fox, 1950, *Skrjabinoptera phrynosoma* (Ortlepp, 1922) Schulz, 1927, *Thubunaea iguanae* Telford, 1965, *Physaloptera retusa* Rudolphi, 1819, and *Spauligodon giganticus* (Read and Amrein, 1953) Skrjabin, Schikhobalova, and Lagodovskaja, 1960.

We examined 11 *Sceloporus poinsettii* (mean snout–vent length 87 ± 20 mm SD, range 38–105 mm) from New Mexico. Seven were borrowed from The Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico, MSB 13468, 17563–17565, and 40945–40947, and 4 were from the Herpetology Collection, Natural History Museum of Los Angeles County, LACM 139718–139721. The specimens were collected in 1958, 1965, or 1966 near Silver City (32°26′N, 108°16′W; elevation 809 m), Grant County, New Mexico. We also examined 10 *S. poinsettii* (mean snout–vent length 94 ± 13 mm SD, range 73–108 mm) from the Laboratory for Environmental Biology, The University of Texas at El Paso, UTEP 1648, 2586, 2587, 2590, 2621, 2622, 2650, 2796, 2854, and 2855. Specimens were collected from Hueco Tanks, Hueco Mountains, El Paso County, Texas (31°55′N, 106°09′W; elevation 1,493 m) during 1971–1975.

The abdomen was opened and the esophagus, stomach, and small and large intestines were removed, slit longitudinally, and examined individually under a dissecting microscope. Nematodes were identified using a glycerol wet mount. Selected cestodes were stained with Delafield's hematoxylin and mounted in Canada balsam.

Eight of the 10 Texas *S. poinsettii* (80% prevalence) were infected with helminths. Eight of the 10 had stomach and/or esophageal infections with *S. phrynosoma* (80% prevalence, mean intensity and range 27, 4–68); 2 of 10 (20% prevalence, mean intensity 1) contained *T. iguanae* in the stomach; and 3 of 10 (30% prevalence, mean intensity and range 7, 3–15) contained *O. scelopori* in the small intestine. Nine of the 11 New Mexico *S. poinsettii* (82% prevalence) were infected with helminths. Six of the 11 (55% prevalence, mean intensity and range 25, 1–116) had stomach infections of *P. retusa* and 9 of 11 (82% prevalence, mean intensity and range 30, 4–79) had large intestine infections of *S. giganticus*. These helminths represent new host records for *S. poinsettii*. Selected intact specimens were placed in vials of 70% ethanol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *Physaloptera retusa* (82480), *Spauligodon giganticus* (82481), *Skrjabinoptera phrynosoma* (82602), *Thubunaea iguanae* (82603), and *Oochoristica scelopori* (82604).

It is noteworthy that these 2 populations of *S. poinsettii* contain different parasites: *O. scelopori*, *S. phrynosoma*, and *T. iguanae* in the Texas population and *P. retusa* and *S. giganticus* in the New Mexico population. Neither population harbored the previously reported *A. penneri*.

Oochoristica scelopori occurs in crotaphytid and phrynosomatid lizards of the western United States (Telford, 1970). Its occurrence in Texas is a new locality record. *Skrjabinoptera phrynosoma* has been reported from Cuba, northern Mexico and the western United States from phrynosomatid, gekkonid, teiid, crotaphytid, polychrid, and tropidurid lizards (see Baker, 1987). Lee (1957) showed experimentally that the ant *Pogonomyrmex barbatus* served as an intermediate host for *S. phrynosoma*. Pearce and Tanner (1973) suggested that several species of ants may serve as intermediate hosts for this parasite. The degree of infection by *S. phrynosoma* may well be determined by the dietary preferences of lizards.

Thubunaea iguanae has previously been reported from gekkonid, xantusiid, crotaphytid, phrynosomatid, and teiid lizards from California and Utah (Telford, 1970; Pearce and Tanner, 1973). The life cycle of *T. iguanae* has not been determined, but Telford (1970) speculated that the infective period for adults is concentrated in 2 parts of the year: December–January and May–June. The occurrence of *T. iguanae* in Texas (a new locality record) suggests that distribution of this parasite may be more widespread than previously thought.

Physaloptera retusa is widely distributed in the Americas—Brazil, Venezuela, West Indies, and western North America (see Baker, 1987)—occurring in phrynosomatid, teiid, scincid, and anguid lizards. *Sceloporus poinsettii* is the thirteenth species of lizard in North America from which *P. retusa* has been reported (see Bursey and Goldberg, 1991). In *Sceloporus graciosus*, this parasite causes inflammatory lesions in the gastric mucosa (Goldberg and Bursey, 1989). The life cycle of *P. retusa* has not been determined, but the life cycles of several related species have been studied: *Physaloptera hispida* by Schell (1952), *Physaloptera rara* and *Physaloptera praeputialis* by Petri and Ameel (1950) and *Physaloptera maxillaris* by Hobmaier (1941) and Lincoln and Anderson (1975). In each case, an insect intermediate host is required to complete development.

Spauligodon giganticus has been reported only from the western United States (see Baker, 1987) and is apparently a parasite of only phrynoso-

matid lizards. Bursey and Goldberg (1992) listed 7 North American lizard hosts. Since then it has been found in *Sceloporus clarkii* by Goldberg and Bursey (1992) and in *Urosaurus ornatus* by Goldberg et al. (1993). Of the 10 known lizard hosts (including *S. poinsettii*), 8 are sceloporines, suggesting that lizards of the genus *Sceloporus* are prone to infection by this parasite. *Spauligodon giganticus* has a direct life cycle and infection may occur from fecal contamination of the substrate (Telford, 1971). A substrate licking behavior has been reported for many lizard species (DeFazio et al., 1977). In neonates of *S. jarrovii,* substrate licking behavior begins shortly after birth. It may be responsible for the almost immediate *S. giganticus* infection (Goldberg and Bursey, 1992) as well as for the maintenance of high monthly prevalences in adult *S. jarrovii* populations (Bursey and Goldberg, 1992).

The distribution of *S. giganticus* may be related to climatic conditions, especially soil moisture. The Hueco Mountains of Texas are much drier than the collection sites of the New Mexico population. We previously reported significantly lower prevalences of *S. giganticus* in New Mexico *Urosaurus ornatus* from the xeric Doña Ana Mountains as compared to a population from the more mesic Aguirre Spring area (Goldberg et al., 1993). These findings suggest that lack of soil moisture may conceivably be a limiting factor in the distribution of *S. giganticus.* Additional data on the geographic distribution of *S. giganticus* will be needed to answer this question.

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Research Note

Experimental Infections with the Tasmanian Isolate of *Trichinella pseudospiralis* Using a Non-enzymatic Recovery Technique

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ABSTRACT: Laboratory rats and mice, cats, brushtail possums (*Trichosurus vulpecula*), and 2 species of raptor (marsh harrier, *Circus aeruginosus,* and brown falcon, *Falco berigora*) were either infected orally with larvae of *Trichinella pseudospiralis* isolated by a non-enzymatic technique or by feeding infected muscle tissue. Muscle from a naturally infected Tasmanian devil (*Sarcophilus harrisii*) and an eastern quoll (*Dasyurus viverrinus*) resulted in infections in cats, rats, and marsh harriers. Similarly, larvae derived from feline muscle were infective for mice and a brown falcon. Infected muscle tissue from marsh harriers was also infective for the same species. The reproductive capacity index (RCI) for rats fed larvae from an eastern quoll was 34.5, whereas the RCI for mice infected with larvae derived from a cat was 31.6.

KEY WORDS: *Trichinella pseudospiralis,* experimental infections, non-enzymatic digestion.

Following the discovery of *Trichinella pseudospiralis* Garkavi, 1972, on the island of Tasmania in 1987, experimental infections were conducted at the Australian Animal Health Laboratory (AAHL) in Geelong, Victoria (Obendorf et al., 1990). Those studies demonstrated that laboratory rats and mice, pigs, and chickens were susceptible to infection; however, the reproductive capacity index (RCI; the ratio of larvae recovered from tissues of the experimentally infected individuals/number of larvae fed) in rodents and chickens was low. Additional rodent infections using muscle larvae liberated by rapid digestion of infected meats in a 1% pepsin/0.5% concentrated hydrochloric acid solution were unsuccessful, whereas brushtail possums (*Trichosurus vulpecula*) fed freshly minced muscle from the same source became infected (unpubl. data). Because the means for establishing these experimental infections were different, no worthwhile conclusions could be drawn about the relative susceptibility of placental and marsupial mammals.

Recent evidence indicates that when *T. pseudospiralis* larvae are exposed twice to low pH and pepsin, once during isolation by the conventional enzymatic digestion method and a second time when larvae are inoculated into the stomach of a host animal, their infectivity declines dramatically (Stewart and Deford, 1989; Stewart et al., 1990). In light of these reports, further experimental infections of placental mammals, brushtail possums, and birds of prey were attempted. The primary aims were to ascertain (1) whether or not the brushtail possum (a primarily herbivorous marsupial that will take other food, including meat) and laboratory rodents (rats and mice) could be infected with the Tasmanian isolate of *T. pseudospiralis* using larvae recovered by the non-enzymatic method of Stewart and Deford (1989), (2) whether or not the introduced feral cat *Felis catus* is susceptible to infection, and (3) whether or not bird-to-bird transmission is possible.

Trichinella pseudospiralis larvae were recovered from muscle tissues of two naturally infected dasyurid marsupials, a Tasmanian devil, *Sarcophilus harrisii,* and an eastern quoll, *Dasyurus viverrinus,* according to the method of Stewart and Deford (1989). Larvae were used to infect 4 wild-caught brushtail possums (380 larvae each) and 12 8-wk-old laboratory-reared rats (200 larvae each). The remaining muscle tissue was fed to 2 unweaned 6-wk-old kittens (*Felis catus*) and 2 wild-caught marsh harriers (*Circus aeruginosus*). Forty-five days postinfection (DPI), the marsh harriers were killed and muscle tissues fed to another marsh harrier. One kitten was killed at 8 DPI; the small intestine was examined for the presence of adult *T. pseudospiralis.* Larvae, recovered from the other cat (60 DPI), were fed orally to 6 8-wk-old mice (28 larvae/mouse). Two infected mice were killed at 60 DPI and fed, as whole carcasses, to a brown falcon (*Falco berigora*).

The brushtail possums were killed at between 52 and 82 DPI, and 10-g samples of selected muscles were digested for 12 hr in a solution of 1% pepsin and 0.5% concentrated hydrochloric acid. The mice and rats were killed 60 DPI, and

the entire body musculature was digested. Digest fluids were passed through a 53-μm sieve, and the material collected was examined by light microscopy at 40× magnification. Digests of pectoral and limb muscles were also performed on the marsh harrier 45 DPI infected with muscle from the 2 original marsh harriers and on the brown falcon 30 DPI infected with mice.

Irrespective of the source of *T. pseudospiralis* larvae, infections were established in all hosts. Although no RCI for the experimentally infected possums was obtained, the larval recovery per gram from selected muscles was very high (Table 1). Two cats became infected after eating meat from a Tasmanian devil. Eight DPI, adult *T. pseudospiralis* worms were recovered from the small intestine of 1 kitten. Of the worms recovered (n = 168), 53% were in the distal third of the small intestine. Infected rats, each dosed with 200 larvae from an eastern quoll, had an RCI of 34.5 (SD ± 6.8; n = 3). Two marsh harriers fed minced muscle tissue from the same eastern quoll had 0.8 and 2.2 larvae/g in their muscles. Subsequently, when muscle tissue from these harriers was fed to another marsh harrier, 5.6 muscle larvae/g were recovered. Laboratory mice originally infected with larvae derived from a cat had an RCI of 31.6 (SD ± 17.7; n = 4). Infection was also established when mice infected with cat-derived *T. pseudospiralis* larvae were fed to a brown falcon; 18.7 larvae/g were recovered.

These findings suggest that a wide range of hosts is potentially capable of becoming infected with the Tasmanian isolate of *T. pseudospiralis*, and this is in agreement with previous experimental studies using Northern Hemisphere isolates of *T. pseudospiralis* (Garkavi, 1974; Meerovitch and Chadee, 1982; Tomasovicova and Hovorka, 1982; Bober and Dick, 1983).

Rodents were readily infected with the Tasmanian isolate with RCI values exceeding 30. This value is considerably higher than 0.1–3.2 obtained in earlier studies at the AAHL using larvae derived by a rapid pepsin/HCl digestion technique (Obendorf et al., 1990). As demonstrated by Stewart et al. (1990), experimental studies that more closely reflect the natural mode of infection, namely, ingestion of muscle tissues or larvae recovered by a non-enzymatic extraction technique, enhance the infectivity of *T. pseudospiralis*.

No *T. pseudospiralis* infections were detected in a sample of 22 feral cats (Obendorf et al., 1990); however, the study presented here shows that cats are capable of becoming infected with this parasite.

Table 1. Recovery of *Trichinella pseudospiralis* larvae from selected muscles in brushtail possums (*Trichosurus vulpecula*) each dosed with 380 larvae.*

Possum number	DPI	Selected muscles (larvae/g)						
		dia.	int.	mass.	abdo.	quad.	neck	subcut.
#1	52	215	192	106	90	69	96	5
#2	82	102	ND	27	26	25	56	15
#3	56	23	3	16	5	4	14	6
#4	61	444	266	1,278	269	191	638	100

* Abbreviations: DPI = days postinfection; dia. = diaphragm, int. = intercostal, mass. = masseter, abdo. = abdominal, quad. = quadriceps, neck = cervical muscles, and subcut. = subcutaneous muscles; ND = not done.

At least 2 species of carnivorous or carrion-feeding bird (masked owl, *Tyto novaehollandiae*, and marsh harrier) are known to be naturally infected in Tasmania (Obendorf and Clarke, 1992). An obvious limitation with these experimental infections of the birds and possums is not knowing whether or not the 3 marsh harriers, the brown falcon, and the brushtail possums were uninfected prior to these studies.

In these experiments, brushtail possums were readily infected with *Trichinella pseudospiralis*, yet in the survey of Obendorf et al. (1990) infection was detected in only 1 of 145 free-living possums. These differences may reflect the infrequency with which brushtail possums actually feed on infected carcasses.

These experimental infections were conducted using dasyurid carcasses obtained as road kills. The assistance of N. Mooney, Department of Parks, Wildlife and Heritage, in making disabled and injured raptors available is also gratefully acknowledged. I wish to thank particularly Jason Wiersma for caring for these birds after they were fed with infected muscle tissues.

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Research Note

Acanthocephalans from the Orangethroat Darter, *Etheostoma spectabile,* from the Wabash Lowlands

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ABSTRACT: Two species of acanthocephalan parasites infected orangethroat darters collected from a stream in southwestern Indiana. *Acanthocephalus dirus* and *Pomphorhynchus bulbocolli* infected 85 and 15% of the darters examined, respectively. The difference in prevalence may be a function of food item selection patterns of the piscine host with regard to the parasites' intermediate host.

KEY WORDS: *Etheostoma spectabile,* orangethroat darter, *Acanthocephalus dirus, Pomphorhynchus bulbocolli,* Indiana.

The parasites of darters (Pisces: Percidae) have been casually mentioned by authors in the course of life history studies (see review by Page, 1983). However, except for a report by Buckner et al. (1985), little is known about the parasites of the darters inhabiting the Wabash Lowlands of southwestern Indiana. This note presents new information on the parasites infecting the orangethroat darter, *Etheostoma spectabile.*

Twenty orangethroat darters were collected from Road Brook, a first-order tributary of the Wabash River in Posey County, Indiana. Collections were made by seine between 20 December 1990 and 20 February 1991. Darters were preserved in 10% formalin and necropsied within 24 hr of collection. Darters were examined for endoparasites by dissecting through the gastrointestinal tract from the cardiac valve to the anus. Parasites were transferred to alcohol–formalin–acetic acid, stained with Semichon's acetocarmine, and mounted whole in Permount. Food items were quantified and identified to lowest practical taxon. Voucher specimens of *Acanthocephalus dirus* (USNM Helm. Coll. No. 82689) and *Pomphorhynchus bulbocolli* (USNM Helm. Coll. No. 82690) have been placed in the USNM Helminthological Collections, Beltsville, Maryland 20705. Specimens of the orangethroat darter hosts have been placed in the Southern Illinois University Ichthyology Collection (SIUC 20246 and 20247).

Food items of this orangethroat darter population consisted primarily of chironomid larvae (67.1% of total items, 80% freq.) and isopod crustacea (22.5% of total items, 65% freq.). Amphipod crustacea (4.6% of total items, 40% freq.), tricopteran larvae (3.9%, 25% freq), and oligochaete worms (1.7%, 5% freq.) were minor constituents of the diet. Seventeen of the 20 darters examined were parasitized by *Acanthocephalus dirus* Van Cleave, 1931 (85% prevalence), with

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a mean intensity of 5.6 worms per darter (range = 2–11). Heavy predation by this darter population upon the isopod intermediate host would seem to account for the high prevalence of this parasite (Seidenberg, 1973; Amin et al., 1980). Locally, *A. dirus* has been reported infecting the spottail darter, *Etheostoma squamiceps* (Strange, 1993) as well as 21 other species of fish (Amin, 1985; Buckner et al., 1985). Five specimens of *Pomphorhynchus bulbocolli* Linkins *in* Van Cleave, 1919, were collected from 3 individuals (prevalence = 15%) with a range of 1–2 worms per infected darter (mean intensity 1.7 worms per darter). The lower prevalence of this parasite within the orangethroat darter population may be related to less predation on the amphipod intermediate host. No flukes, tapeworms, or nematodes were found.

Although *A. dirus* and *P. bulbocolli* co-occur within the orangethroat darter, it is doubtful that significant interspecific competition occurs. The co-occurrence may be due to the overdispersal of either species within its definitive host populations (Dobson, 1985), because both have lower definitive host specificity than intermediate host specificity (Amin, 1978). In Kentucky, the rainbow darter, *Etheostoma caeruleum,* was also found to be host to both *A. dirus* and *P. bulbocolli* with little evidence of competitive exclusion (McDonough and Gleason, 1981). Darters are opportunistic in their feeding habits (Page, 1983), and the co-occurrence of these parasites may simply represent an overlap in resource utilization.

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Research Note

Some Acanthocephala and Digenea of Marine Fish from Grand Cayman, Cayman Islands, British West Indies

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ABSTRACT: A survey of 17 fishes belonging to 11 species from Grand Cayman, Cayman Islands, West Indies, led to the recovery of 2 species of acanthocephalans and 9 of digeneans. The acanthocephalans found were *Acanthogyrus (Acanthosentis) acanthuri* and *Dollfusentis ctenorhynchus*. The digeneans included *Monorchimacradena acanthuri* in *Acanthurus bahianus* (new host record), *Bucephalus varicus, Hurleytrematoides chaetodoni, Hurleytrematoides curacaensis, Multitestis chaetodoni, Lecithophyllum pyriforme, Stephanostomum sentum, Podocotyle oscitans,* and *Helicometra equilata* in *Holocentrus marianus* (new host record).

KEY WORDS: Acanthocephala, Digenea, marine fish, Grand Cayman, West Indies.

During a short research trip in summer (19 July–3 August) of 1991, 17 fishes belonging to 11 species were captured using traps and angling and examined for parasites. To the best of my knowledge, this is the first report of parasites of fish from Grand Cayman. Two species of acanthocephalans and 9 species of digeneans were recovered. After washing the parasites in 0.7% saline, they were processed as follows: the acanthocephalans were transferred to a dish containing tap water and placed overnight in a refrigerator to allow protrusion of the proboscis. The following day, the water was removed and quickly replaced with hot alcohol–formalin–acetic acid (AFA). The digenetic trematodes were studied alive under slight coverslip pressure and then fixed with cold AFA. Both groups of parasites were stained with acetocarmine, dehydrated in an ascending series of isopropanol, cleared in methyl salicylate, rinsed in xylol, and mounted in Kleermount (Carolina Biological Supply Co., Burlington, North Carolina).

One sergeant major, *Abudefduf saxatilis* (Linnaeus) family Pomacentridae, and 1 smooth trunkfish, *Lactophrys triqueter* (Linnaeus) family Ostraciidae, lacked parasites.

The species of fish, their parasites, and the number examined and found are listed in Table 1.

Representatives of some of the species are deposited in the United States National Museum (USNM) parasite collection, Beltsville, Maryland, and Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, under the listed accession numbers.

Even though the present study is limited in scope, it indicates the presence of a rich parasitic fauna of marine fishes of Grand Cayman. Fifteen (88%) of 17 fishes, representing 9 (82%) of 11 host species, were infected. Of those infected, 2 host species (22%) harbored acanthocephlans and 7 (78%) had digeneans. The intensity of infection with acanthocephlans was 5 for *Acanthogyrus (Acanthosentis) acanthuri* and 54 for *Dollfusentis ctenorhynchus*. For digenetic trematodes, the intensity ranged from 1 to 25. The exact number for each species is given in Table 1.

No new species were found in this study, but all the parasites represent new locality records. *Acanthogyrus (Acanthosentis) acanthuri* was originally described from Puerto Rico and redescribed by Schmidt (1975) from 8 specimens recovered from *Acanthurus coeruleus* (type host) and *A. chirugrus* from Tobago, West Indies. I agree with the revised description and measurements given by Schmidt (1975). Golvan (1959) had relegated *Acanthosentis* Verma and Datta, 1929, to subgeneric status, which, apparently, was not accepted by Schmidt (1975) but recognized by Amin (1985). The present finding is, therefore, the third for this species and extends its distribution to the northwestern part of the Caribbean. This is the second report of *Dollfusentis ctenorhynchus,* an acanthocephalan originally reported from Jamaica.

Two new hosts are reported in this paper: *Acanthurus bahianus* for *Monorchimacradena acanthuri* and *Holocentrus marianus* for *Helicometra equilata. Monorchimacradena acanthuri* is known from both Jamaica and Curaçao. *Helicometra equilata,* originally described from *Holocentrus ascensionis* in Tortugas, Florida, is probably widely distributed in the Caribbean, having been reported from Puerto Rico, Bimini,

Table 1. Parasites of marine fishes from Grand Cayman, Cayman Islands, British West Indies.

Host (number examined/number infected)	Parasite (number of parasites)	Deposited at:	Accession No.
Acanthurus bahianus Castelnau, 1855, ocean tang (1/1)	*Monorchimacradena acanthuri* Nahhas and Cable, 1964 (1), intestine		
Acanthurus coeruleus Block and Schneider, 1801, Blue tang 1/1	*Acanthogyrus (Acanthosentis) acanthuri* (Cable and Quick, 1954) Golvan, 1959 (5: 2 males, 3 females), intestine	HWML	35110
Caranx bartholomaei (Cuv. and Val., 1833) (1/1)	*Bucephalus varicus* Manter, 1940 (25), ceca	USNM HWML	82471 35109
Chaetodon ocellatus Bloch, 1787, common butterfly fish (4/1)	*Hurleytrematoides chaetodoni* (Manter, 1942) Yamaguti, 1950 (13), intestine	USNM HWML	82472 35207
	H. curacaensis Nahhas and Cable, 1964 (17), intestine	USNM HWML	82473 35208
	Multitestis chaetodoni Manter, 1947 (4), intestine	USNM	82474
Chaetodon striatus (Linn., 1758), banded butterfly fish (4/1)	*Multitestis chaetodoni* (4), intestine	HWML	35209
Haemulon flavolineatum (Desmarest, 1823), yellow grunt (1/1)	*Lecithophyllum pyriforme* (Linton, 1910) Yamaguti, 1958 (1), intestine		
Haemulon sciurus (Shaw, 1803) blue-striped grunt (1/1)	*Stephanostomum sentum* (Linton, 1910) Manter, 1947 (1), intestine *Podocotyle oscitans* (Linton, 1910) Yamaguti, 1971 (4), intestine		
Holocentrus marianus (Cuv. and Val., 1829), long-jaw squirrelfish (1/1)	*Helicometra equilata* (Manter, 1933) Siddiqi and Cable, 1960 (17), intestine	USNM HWML	82475 35210
Mulloidichthys martinicus (Cuv. and Val., 1829), yellow goatfish (1/1)	*Dollfusentis ctenorhynchus* (Cable and Linderoth, 1963) Golvan, 1969 (54: 28 females, 26 males), intestine	USNM HWML	82476 35111

and Jamaica. *Bucephalus varicus* has been recovered predominantly from carangid fishes of Grand Isle (Louisiana), Apalachee Bay, Tortugas, Tampa Bay, and Biscayne Bay; it is also known from Bimini, Curaçao, and Jamaica. It is of interest to note that this species has also been reported from Brazil, but neither Siddiqi and Cable (1960) nor Dyer et al. (1985, 1992) found it in Puerto Rican fishes. *Bucephalus varicus* has a worldwide distribution, having been reported from fishes in the Red Sea, the Philippines, and the Pacific and the Atlantic oceans. It is quite possible that these reports represent more than 1 species. Characteristic features of this species include 7 tentacles, which often are not protruded; instead, 7 "knob-like" structures may be counted on the anterior sucker. A slight pressure on live specimens may cause partial or complete protrusion of the tentacles. *Hurleytrematoides chaetodoni* is known from Tortugas, Puerto Rico, Curaçao, and Jamaica. *Hurleytrematoides curaçaensis* described from *Chaetodon capistratus* and *C. ocellatus* from Curaçao was not found in Jamaica. This species may be distinguished from *H. chaetodoni* chiefly by absence of eye-spot pigments and wider eggs with shorter filaments. Seventeen individuals were found in a mixed population with 13 *H. chaetodoni*. When several worms were being observed live, it was clear that 2 species were represented. *Multitestis chaetodoni* has been reported from Tortugas, Bermuda, the Atlantic coast of Panama, and Jamaica. *Lecithophyllum pyriforme* is widely distributed in the Gulf of Mexico, the Caribbean, adjacent waters, and as far south as Brazil. It has been reported from the Louisiana coast, Tortugas, Biscayne Bay, Puerto Rico, Jamaica, Curaçao, Bimini, and Brazil. *Stephanostomum sentum* is known from Apalachee Bay in the northern Gulf of Mexico, Tortugas, Biscayne Bay, Puerto Rico, Cuba, Jamaica, and Curaçao. It is also known from the Panamanian Pacific. *Podocotyle oscitans* is known from Tortugas, Biscayne Bay, Jamaica, Curaçao, and Puerto Rico. It is also known from the Galapagos Islands.

Although Grand Cayman lies well isolated in the Caribbean and separated from the nearest islands of Jamaica (southeast), Cuba (north and

northeast), Honduras, Guatemala, Belize, and the Yucatan Peninsula (west) by deep channels, many of its fishes are widely distributed along the shores and reefs of these lands and other Caribbean waters. This is undoubtedly true of their invertebrate fauna in general and the molluscs in particular. Extensive parasitological investigations of these areas will, in all likelihood, reveal an equally similar parasitic fauna.

When compared to the 2 closest islands of Jamaica and Cuba, Cayman's parasitic fauna is closely related to the former with 9 (81.8%) of 11 species common to both areas but only 1 species (9.1%) to Cuba. Pérez Vigueras' studies between 1940 and 1958 "described as new several (species) which were not adequately compared with known ones and probably are not distinct from them" (Nahhas and Cable, 1964, p. 217). Additional studies from Cuba are needed. Even though deep waters and great distances separate Grand Cayman from Curaçao, Puerto Rico, and Tortugas, a strong relationship of the parasite fauna of these fishes is evident: with Curaçao (7 or 63.6%) and Puerto Rico and Tortugas (6 each or 54.5%).

The author wishes to thank Dr. Thomas Byrnes, Director of the Grand Cayman Marine Laboratory, for the loan of a microscope.

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Research Note

Calyptospora funduli (Apicomplexa, Calyptosporidae) in the Liver of the Gulf Toadfish, *Opsanus beta*

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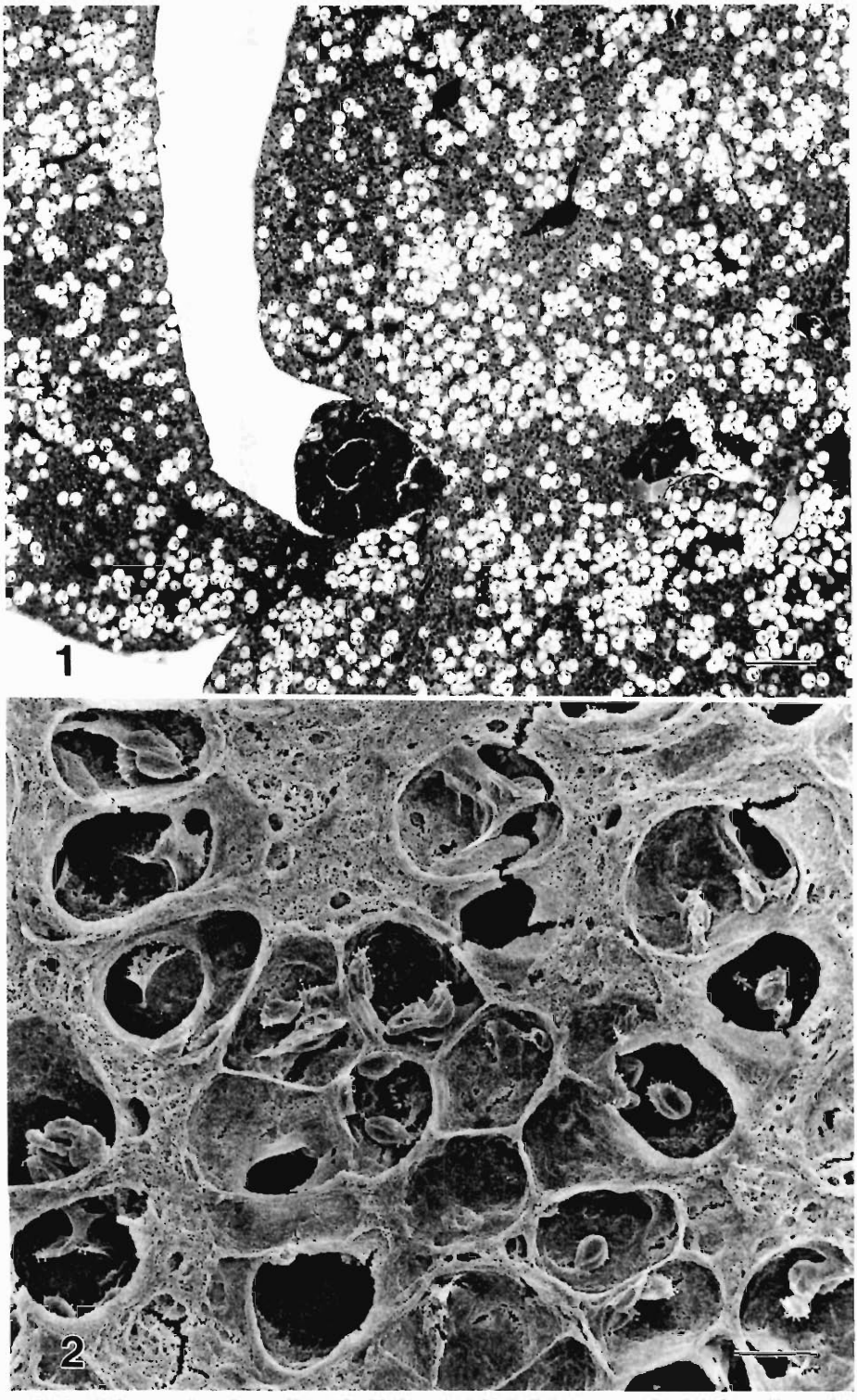
ABSTRACT: Oocysts of the apicomplexan protozoan *Calyptospora funduli* were found in the liver of a gulf toadfish (*Opsanus beta*). The infected specimen was 1 of 54 (1.9%) toadfish livers examined histologically. The paraffin-embedded specimen containing the infection as well as similar material from *Fundulus similis* were processed for scanning electron microscopical (SEM) examination to view diagnostic surface features of *C. funduli* sporocysts. SEM examination confirmed sporopodia and a thin veil surrounding each of the 4 sporocysts per oocyst. Although a single case, the toadfish infection expands the broad host specificity of *C. funduli* to include a host other than an atheriniform fish species.

KEY WORDS: Protozoa, Coccidia, *Calyptospora funduli*, fish, liver, host specificity, scanning electron microscopy.

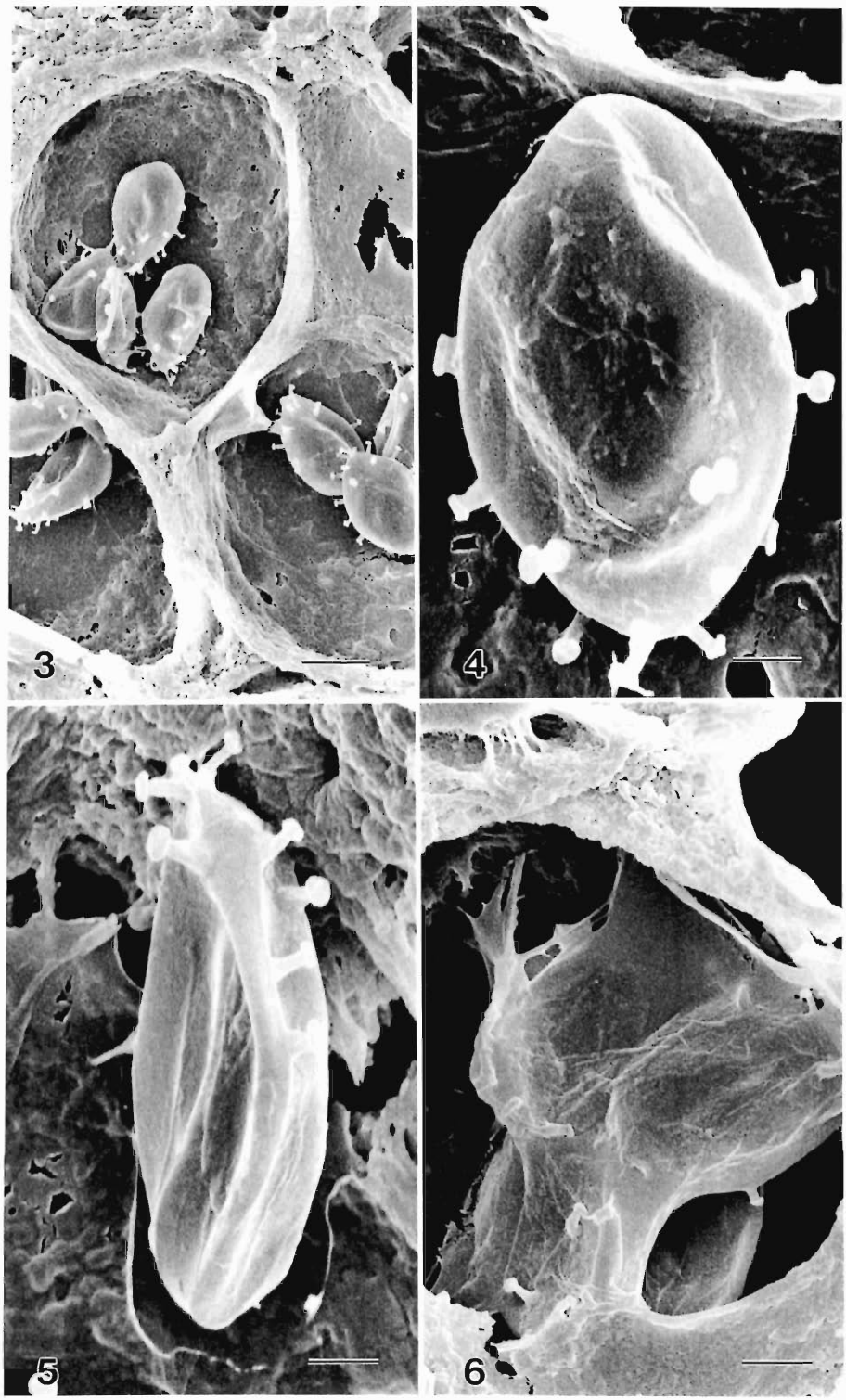
Sporocysts of coccidian species in the genus *Calyptospora* Overstreet, Hawkins, and Fournie, 1984, lack a Stieda body and are enclosed in 2 incompletely separated valves, and each is surrounded by a membranous veil that is supported by projections from the sporocyst wall. Members of the genus appear to require an invertebrate intermediate host (Fournie and Overstreet, 1983; Overstreet et al., 1984). The genus includes 4 described species: *C. funduli* (Duszynski, Solangi, and Overstreet, 1979), *C. empristica* Fournie, Hawkins, and Overstreet, 1985, *C. serrasalmi* Cheung, Nigrelli, and Ruggieri, 1985, and *C. tucunarensis* Békési and Molnár, 1991. All infect mainly liver parenchymal cells. Both *Calyptospora funduli* (Overstreet et al., 1984) and *C. empristica* (Fournie et al., 1985) commonly infect estuarine and freshwater killifishes of the genus *Fundulus* in North America, and in freshwater of Brazil, *C. serrasalmi* infects the black piranha (*Serrasalmus niger*) (Cheung et al., 1985) and *C. tucunarensis* infects the tucunare (*Cichla ocellaris*) (Békési and Molnár, 1991). Although piscine coccidians are generally expected to have a narrow host specificity, *Calyptospora funduli* naturally infects at least 6 estuarine species of atheriniform fishes (Fournie and Overstreet, 1982). Experimental infectivity studies on *C. funduli* confirm a rather broad host specificity within atheriniform fishes (Fournie and Overstreet, unpubl.). Here we report the occurrence of *C. funduli* infecting the liver of a gulf toadfish, *Opsanus beta* (Goode and Bean, 1879), from Mississippi.

Toadfish were captured by trawling from waters of the Mississippi Sound near Ocean Springs, Mississippi (30°24′N, 88°51′W). Specimens were brought alive to the laboratory, where they were anesthetized in 0.1% MS-222 (tricaine methanesulfonate) and examined for external lesions. Liver, kidney, spleen, and a gill arch from each specimen were removed, fixed in Lillie's fixative (formalin, picric acid, and formic acid), and embedded in paraffin. Paraffin sections were cut, placed on glass slides, and stained with hematoxylin and eosin. After the infection was detected by light microscopy, paraffin sections approximately 10 μm thick were cut and processed for examination by scanning electron microscopy (SEM) following modifications of techniques described by Oshel (1985) and Felgenhauer (1987). The paraffin sections were placed on round glass coverslips, deparaffinized in Shandon xylene substitute (Shandon Inc., Pittsburgh, Pennsylvania), rinsed in 100% ethanol, air-dried, and sputter-coated with gold-palladium. The coated coverslips were mounted on aluminum stubs with double-faced adhesive tape and examined with a JEOL T-330 scanning electron microscope. For comparison, a paraffin block containing sporulated oocysts of *C. funduli* from the liver of the longnose killifish (*Fundulus similis*) was processed similarly.

A single toadfish specimen from a total of 54



Figures 1, 2. Micrographs of paraffin-embedded material of *Calyptospora funduli* from liver of toadfish *Opsanus beta*. 1. Hematoxylin-and-eosin-stained section showing oocysts replacing much of the liver parenchyma. ×125. Bar = 80 μm. 2. SEM-prepared material showing scattered oocysts. ×600. Bar = 20.0 μm.



Figures 3–6. SEM micrographs of *Calyptospora funduli* from liver of toadfish *Opsanus beta*. 3. Three oocysts containing sporocysts of which 2 of them exhibiting 4 sporocysts are visible. ×2,000. Bar = 5.0 μm. 4. Sporocyst showing sporopodia arranged along the lateral margins and clustered at the posterior end. ×10,000. Bar = 1.0 μm. 5. Lateral view of a partially collapsed sporocyst. Posterior end (arrowhead). ×10,000. Bar = 1.0 μm. 6. Oocyst with sporocysts obscured by sporocyst veils. ×5,000. Bar = 2.0 μm.

toadfish specimens from which livers were examined histologically was infected with *Calyptospora funduli*. The infected specimen was collected from offshore waters of about 25‰ salinity near Horn Island, approximately 18 km from the mainland. The toadfish was a juvenile, 85 mm in total length and weighed 9 g.

Examination of paraffin sections revealed oocysts that were about 20 μm in diameter occurring singly or in clusters and replacing more than 75% of the liver parenchyma (Fig. 1). Exocrine pancreatic cells did not appear to be infected. There was no evidence of a host inflammatory response. All oocysts examined were sporulated. Oocysts contained 4 ovoid sporocysts (about 8–9 × 2–3 μm). A Stieda body could not be resolved in the sporocysts, although there was a dense structure in the sporocyst wall at the posterior end of the sporocyst. Projections (sporopodia) of the sporocyst wall supported a thin membranous veil that was attached at the anterior end of the sporocyst. Each sporocyst had 2 elongated sporozoites that were partly coiled together. Examination by SEM confirmed the heavy infection (Fig. 2). Oocysts and the enclosed sporocysts were well preserved but not the surrounding host tissues. The oocyst wall appeared thin in places where it could be seen between adjacent oocysts. Sporocysts were more pointed at the posterior than at the anterior end (Fig. 3). The sporocyst wall was smooth except where it gave rise to sporopodia. Sporopodia were about 1 μm long and knobbed at the distal end (Fig. 4). Because we could count 10 or 11 sporopodia on one side in an SEM specimen, we estimated that each sporocyst had about 20 sporopodia. The sporopodia appeared numerous along an anterior–posterior line and were especially numerous at the posterior (pointed) end. Figure 5 shows this line interpreted as the site of an underlying suture in a sporocyst that was partially collapsed. In Figure 6, 4 sporocysts are obscured by what appear to be remnants of the membranous veil. Examination by SEM revealed no morphological differences between *Calyptospora funduli* from the liver of a longnose killifish and the organism in the toadfish.

The method of SEM examination of paraffin-embedded material utilized in this study yielded considerable ultrastructural detail of this coccidian. The coccidian in the toadfish liver appeared to be *Calyptospora funduli*, and this was confirmed by comparing similarly prepared material of *C. funduli* in *Fundulus similis*. The infection in the toadfish, even though rare, appeared to be well tolerated because the parasite was fully developed and there was no evidence of degenerating stages of the parasite or a host inflammatory response.

Most eimerian coccidians have a strong host affinity and rarely do they naturally infect more than 1 genus (Long and Joyner, 1984). *Calyptospora funduli*, however, has a broad host specificity, and experimental infections can be produced in several species. With the exception of the batrachoidiform toadfish, all the hosts, whether natural or experimental, belong to the order Atheriniformes, primarily the Cyprinodontidae (Fournie and Overstreet, unpubl.). Possibly, the infected toadfish represents an abnormally susceptible individual rather than the result of feeding behavior. This would have to be investigated by experimental transmission studies using laboratory-reared toadfish. The coccidian requires a palaemonid shrimp intermediate host (Fournie and Overstreet, 1983), and the toadfish readily feeds on those grass shrimps (R. W. Heard and R.M.O., pers. obs.). A relatively high percentage of grass shrimp in enzootic areas have *C. funduli* infections (Solangi and Overstreet, 1980; Fournie and Overstreet, 1983). Consequently, if the toadfish were a susceptible species, the prevalence of infected individuals should have been higher.

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Research Note

Sarcocystis felis in Captive Cheetahs (*Acinonyx jubatus*)

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ABSTRACT: *Sarcocystis felis* was detected in the musculature of 7 of 10 cheetahs (*Acinonyx jubatus*) from a captive breeding colony in Winston, Oregon. This is the first report of *Sarcocystis felis* from cheetahs.

KEY WORDS: *Sarcocystis felis*, cheetah, *Acinonyx jubatus*.

Species of the genus *Sarcocystis* have a predator–prey cycle consisting of a definitive carnivore (predator) host and intermediate herbivore (prey) host. In the intermediate host, schizonts or muscle sarcocysts are the result of asexual reproduction, and in the definitive carnivore host, sexual reproduction occurs in intestinal cells, with oocysts or sporocysts passed in feces (Dubey et al., 1989). Carnivores infrequently develop sarcocysts in muscles or function as intermediate hosts. Definitive hosts have not been identified for *Sarcocystis* spp. with sarcocysts in carnivore muscles. In North America, sarcocysts identified as *Sarcocystis felis* Dubey, Hamir, Kirkpatrick, Todd, and Rupprecht, 1992, have been reported from bobcats (*Felis rufus*), domestic cats (*Felis domesticus*), Florida panther (*Felis concolor coryi*), and cougar (*Felis concolor*) (Kluge, 1967; Kirkpatrick et al., 1986; Everitt et al., 1987; Edwards et al., 1988; Fiori and Lowndes, 1988; Hill et al., 1988; Greiner et al., 1989; Anderson et al., 1992; Dubey et al., 1992). This report documents *S. felis* in the musculature of captive cheetahs (*Acinonyx jubatus*) from a wildlife facility in Winston, Oregon.

All cheetahs were part of a captive breeding program at Wildlife Safari, Winston, Oregon. All animals had been born in the United States, ranged in age from 5 to 14 yr, and had been in captivity all of their lives. Muscle biopsy specimens from 8 cheetahs, 1 male and 7 females, were obtained from the biceps femoris after administration of lidocaine. Necropsy specimens of biceps femoris were collected from 2 additional male cheetahs. Tissues were fixed in 10% buffered formalin, sectioned at 5 μm, and stained with hematoxylin and eosin. Tissues were examined microscopically ($\times$400), and sarcocysts were counted within a 1-cm^2 marked section of randomly chosen tissue.

Additional muscle specimens were processed for electron microscopy by methods described previously (Foreyt, 1989) and viewed with a transmission electron microscope (Hitachi H600, Hitachi, Santa Clara, California 95044).

Sarcocysts of *S. felis* were detected in 7 of 10 cheetahs (Fig. 1). Mean size of 48 sectioned sarcocysts was 251 $\times$ 121 μm (range, 64–997 $\times$ 49–

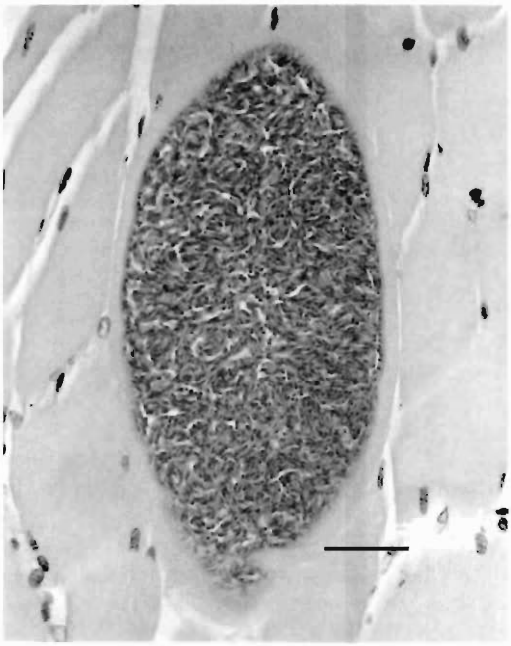

Figure 1. ***Sarcocystis felis*** **in the biceps femoris of a cheetah. Scale bar = 50 μm.**

220 μm). Mean intensity was 6.9 sarcocysts/cm². No inflammatory reaction was associated with the sarcocysts, and adjacent muscle fibers were histologically intact.

The septate sarcocysts (Fig. 2) were identified as *S. felis* based on published descriptions by Dubey et al. (1992). The primary vacuole membrane of the primary cyst wall was folded irregularly into short bumps and villar projections (Fig. 2).

Infections with *Sarcocystis* sp. in the musculature of carnivores are uncommon, because carnivores are the usual definitive hosts and herbivores are the usual intermediate hosts. In the present report, sarcocysts of *S. felis* were detected in 70% of the cheetahs sampled, but the importance of the infection could not be determined, Many of the cheetahs subsequently died from a variety of diseases, particularly renal and hepatic failure, and virtually all cheetahs exhibited signs of muscle wasting. Cheetahs lack genetic diversity (O'Brien et al., 1985) and are highly susceptible to infectious diseases such as feline infectious peritonitis virus and feline leukemia virus, which are capable of compromising the immune system of the host (Briggs and Ott, 1986; Briggs

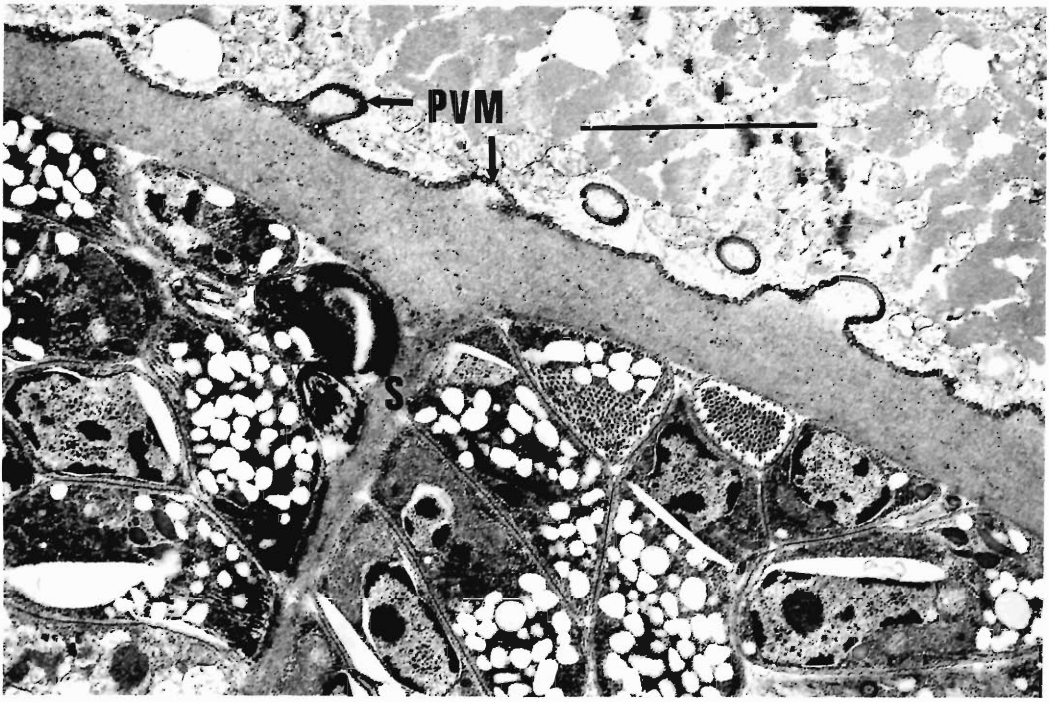

Figure 2. Transmission electron micrograph of the cyst wall of ***Sarcocystis felis*** **in the biceps femoris of a cheetah. Note the septum (S) and the folded parasitophorous vacuole membrane (PVM). Scale bar = 3 μm.**

et al., 1986). The effect of a compromised immune system on the development of sarcocysts in the carnivore host has not been investigated (Edwards et al., 1988) but may be important. The life cycle of *S. felis*, including the definitive host, has not been documented.

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Research Note

Larval *Ascarops* sp. (Nematoda: Spirurida) in Introduced Mediterranean Geckos, *Hemidactylus turcicus* (Sauria: Gekkonidae), from Texas

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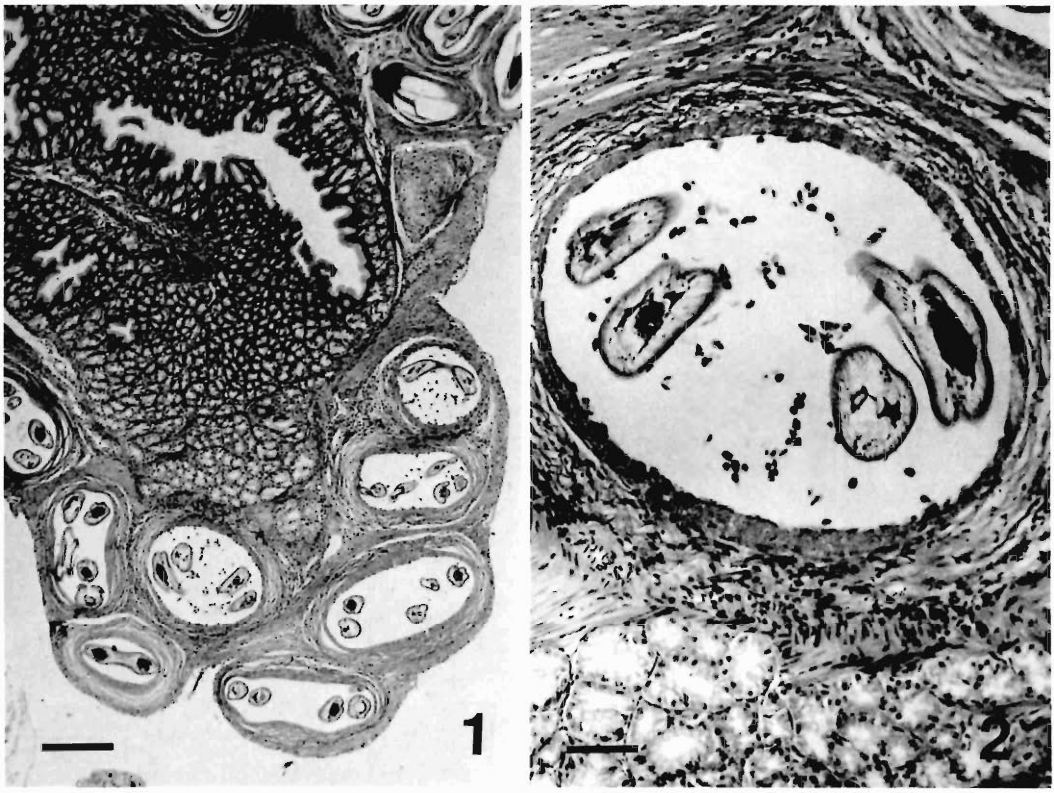
ABSTRACT: Third-stage larval spirurid nematodes, *Ascarops* sp., were found encysted in the stomach, pancreas, small intestine, and liver of 9 of 98 (9%) Mediterranean geckos, *Hemidactylus turcicus*, from Houston, Harris County, Texas. Histopathological effects of the parasite on tissues of *H. turcicus* were studied. The Mediterranean gecko represents a new host and the third saurian species reported to be infected by this nematode.

KEY WORDS: *Ascarops* sp., Gekkonidae, *Hemidactylus turcicus*, histopathology, Mediterranean gecko, Nematoda, prevalence, Spirurida, Texas.

The Mediterranean gecko, *Hemidactylus turcicus* (Linnaeus, 1758), is a small, mostly nocturnal, Old World lizard that ranges from western India and Somalia, west along the coastal regions of the Mediterranean basin to Spain, Morocco, and the Canary Islands (Conant and Collins, 1991). This gecko was inadvertently introduced into the New World around the turn of the century and is now well established at numerous localities around the Gulf Coast of the United States from Florida west to southern California and south into Mexico, Hispaniola, Cuba, and Panama (McCoy, 1970; Conant and Collins, 1991). A moderate amount of information is available on parasites of natural (McMillan, 1965; Tinar, 1982; Groschaft and Moravec, 1983; Paperna, 1989; Paperna and Landsberg, 1989a, b) and introduced populations of *H. turcicus* (Baruš and Coy Otero, 1974; Coy Otero and Baruš, 1979; McAllister et al., 1988; Pence and Selcer, 1988; Riley et al., 1988; Upton et al., 1988; McAllister et al., 1990). This note reports, for the first time, the occurrence of a larval spirurid in *H. turcicus* and provides prevalence data and a description of the cysts associated with the infection in this host.

Between December 1986 and March 1989, 98 (50 males, mean ± SE snout–vent length [SVL] = 46.3 ± 1.4, range 28–58 mm; 48 females, 47.0 ± 1.2, range 32–56 mm) hatchling, juvenile, and adult *H. turcicus* were collected by hand from within the reptile and amphibian facility of the Houston Zoological Gardens, Harris County, Texas ($N = 57$), Dallas Zoo, Dallas County, Texas ($N = 12$), on the walls of St. Anne's Catholic Church in Houston ($N = 22$), and at a private residence in Houma, Terrabonne Parish, Louisiana ($N = 7$). Geckos were killed within 48 hr with an overdose of sodium pentobarbital and examined for tissue-dwelling nematodes. Some encysted nematodes were fixed in situ in alcohol–formalin–acetic acid, sectioned at 7 μm, and stained with Harris' hematoxylin and eosin counterstain. Some nematodes were teased from infected tissues, transferred to 70% ethanol, and cleared in glycerol for examination as temporary mounts. Voucher specimens of *H. turcicus* are deposited in the Arkansas State University Museum of Zoology (ASUMZ 6329–6334, 6392–6402, 6442–6465, 8649–8665, 8667–8673, 8535–8541). Voucher specimens of *Ascarops* sp. are on deposit in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705, as USNM Helm. Coll. No. 82673.

Nine of 98 (9%) of the *H. turcicus* harbored third-stage larval *Ascarops* sp. within cysts in the stomach, pancreas, small intestine, and liver. One infected adult male gecko (54 mm SVL) came from the Houston Zoo while 2 adults and 1 ju-



Figures 1, 2. ***Ascarops*** **sp. larvae within gastric cysts in the Mediterranean gecko,** ***Hemidactylus turcicus.*** **Scale bar 1 = 250 μm; 2 = 70 μm.**

venile male (49.3 ± 3.3, range 43–54 mm) and 5 adult females (53.0 ± 1.4, range 48–56 mm) were collected at the St. Anne's locality in Houston. Only 1 of 27 (4%) of the immature versus 8 of 71 (11%) of the adult *H. turcicus* were infected.

In the stomach, thick-walled cysts were located in the submucosa and the layers of the muscularis externa. They were round to oblong in shape and approximated 420 μm in diameter. There was distortion and displacement of the muscle layers (Fig. 1), giving a nodular appearance to the serosa. A few mononuclear inflammatory cells were scattered in the centers of the cystic spaces. The inner walls were composed of a hyaline-like matrix, whereas the middle portion was composed of thick concentric layers of laminated collagenous connective tissue (Fig. 2). The outermost connective tissue layers contained only a small inflammatory response consisting of occasional clusters of mononuclear cells (macrophages).

In the liver, the parasitic cysts were larger and approximated 800 μm in diameter. Their structure was similar to those noted in the stomach and caused a mild compression of the surrounding liver tissue. No inflammatory response was elicited. *Ascarops* sp. cysts were also present in the pancreas, where they caused minimal compression of the pancreatic acini and in the small intestine where they occurred in the muscularis externa.

Each granuloma contained a third-stage *Ascarops* sp. larva. Larvae were approximately 2.2 mm long and 85 μm wide. The distinguishing differential features of a third-stage larva of *Ascarops* sp. are (1) the right and left anterolateral body walls are prolonged into dorsoventral liplike projections and (2) the tip of the tail possesses a smooth knoblike process (see Goldberg and Bursey, 1988). Alicata (1935) reported that the only difference between larval *Ascarops* and similar third-stage larvae of *Physocephalus sexalatus* (Molin, 1860) Diesing, 1861, is that the latter has a tail knob with several digitiform processes. Only smooth knobs were observed in our specimens, and fourth-stage larvae or adult nematodes were not found.

The gastric *Ascarops* sp. cysts in *H. turcicus*

were somewhat similar to those seen in the stomach of the sagebrush lizard, *Sceloporus graciosus,* by Goldberg and Bursey (1989). However, the cysts were more numerous and disseminated in *H. turcicus* than in *S. graciosus.* Minimal granulomatous host response was evident in both of these lizards. Goldberg and Bursey (1988) examined granulomas containing larval *Ascarops* sp. in the liver of the western fence lizard, *S. occidentalis.* These granulomas were smaller (approximately 330 μm in diameter) than those seen in *H. turcicus.* There was a much more mature granulomatous response to this parasite in *S. occidentalis,* including giant cells and epithelioid macrophages.

The life history of *Ascarops strongylina* (Rudolphi, 1819) Alicata and McIntosh, 1933, was first elucidated by Seurat (1915). Third-stage larvae have been recovered previously from 2 species of mammals, 4 species of birds, and 2 species of lizards (Goldberg and Bursey, 1989). Definitive hosts are mammals of the orders Artiodactyla, Lagomorpha, and Rodentia; first intermediate hosts include insects of the orders Coleoptera and Odonata (Alicata, 1935). About 20 species from 14 genera of beetles have been identified as intermediate hosts of nematodes in the genus *Ascarops* (Goldberg and Bursey, 1989). However, the specific insect host of *Ascarops* sp. ingested by *H. turcicus* has not yet been determined.

We thank E. A. Liner and D. M. Boyer for providing specimens of *H. turcicus* from Louisiana and the Dallas Zoo, respectively, and J. Furman, G. Migues, and K. Neitman for assistance in collecting in Houston.

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Research Note

Aplectana macintoshii (Nematoda: Cosmocercidae) in *Eumeces latiscutatus* (Sauria: Scincidae), from Japan

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ABSTRACT: Examination of 5 *Eumeces latiscutatus* revealed the presence of the nematode, *Aplectana macintoshii* (prevalence 20%, intensity 3) in the large intestine. This is a new host record and extends the range of *A. macintoshii* to the Palaearctic zoogeographic region of Japan.

KEY WORDS: Nematoda, *Aplectana macintoshii*, Scincidae, *Eumeces latiscutatus*, Palaearctic.

Eumeces latiscutatus (Hallowell, 1860) is a scincid lizard that is restricted to Japan (Welch et al., 1990) where it is found on Hokkaido, Honshu, Shikoku, Kyushu, and Osumi Gunto islands (Nakamura and Uéno, 1970). The purpose of this note is to report the presence of the nematode, *Aplectana macintoshii* (Stewart, 1914) Travassos, 1931, in *E. latiscutatus*. This finding represents a new host and locality record and what we believe to be the first nematode species recovered from *E. latiscutatus*. The report of *Entomelas markovi* (Szczerbak and Sharpilo, 1969) Baker, 1980, in *E. latiscutatus* from the Kuril Islands, Russia (Baker, 1987), most likely represents a finding in a different species of *Eumeces* (see Welch et al., 1990).

Five adult *E. latiscutatus* (2 males, 3 females), mean snout–vent length (SVL) 69 ± 8 mm SD (range 52–72 mm), were collected at Mount Rokko (34°46′N, 135°16′E, ca. 900 m elevation), Hyogo Prefecture, Honshu Island, 9 June 1992. Specimens were deposited in the herpetology collection of the Los Angeles County Museum of Natural History (LACM 140105–140109). The body cavity was opened ventrally and the esophagus, stomach, small intestine, and large intestine were slit longitudinally and examined under a dissecting microscope. The liver and body cavity were also examined for helminths. Nematodes were identified utilizing a glycerol wet mount.

One of 5 (20% prevalence) *E. latiscutatus* (LACM 140109, female, 71 mm SVL) harbored 1 male and 1 intact and 1 partial female *A. macintoshii* in the large intestine. The nematodes were identified using the key to species of *Aplectana* provided by Baker (1980) and are consistent with the description of *A. macintoshii*. The male measured 2.7 mm total length, with a spicule length of 255 μm compared to measurements of 2.0–2.6 mm total length, spicules 205–257 μm, for male *A. macintoshii* from Baker (1980). The 1 intact female measured 4.2 mm compared to measurements of 4.2–5.1 mm for *A. macintoshii* from Baker (1980). Published measurements (Baker, 1980) are for *A. macintoshii* from *Rana tigrina* collected in India. The 3 nematodes were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): USNM Helm. Coll. No. 82710.

There are approximately 41 species of *Aplectana*, the majority of which parasitize frogs and toads (see Baker, 1987). *Aplectana macintoshii* is the most cosmopolitan species of the genus and occurs in South America, Europe, Africa, India, Malaysia, and China, where it is known from 37 species of frogs and toads, 2 species of lizards, and 1 species of snake (see Baker, 1987). Because *A. macintoshii* occurs primarily in amphibians, it is possible that our recovery of this nematode from *E. latiscutatus* may represent pseudoparasitism. Unfortunately, our small sample size prevents consideration of this question.

Aplectana sp. (perhaps *A. macintoshii*) was recovered in 2 *Rana ishikawae* frogs from Amami Ō Shima Island, Japan (Hasegawa, 1990). Amami Ō Shima Island belongs to the Oriental zoogeographic region. Our finding of *A. macintoshii* in *E. latiscutatus* extends the range of this nematode into the Palaearctic zoogeographic region of Japan.

We thank Ronald I. Crombie (Division of Amphibians and Reptiles, Smithsonian Institution) for identifying *E. latiscutatus* and Fumiko K. Goldberg for field assistance.

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Research Note

Hemogregarines and *Sarcocystis* sp. (Apicomplexa) in a Western Green Rat Snake, *Senticolis triaspis intermedia* (Serpentes: Colubridae), from New Mexico

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ABSTRACT: A western green rat snake, *Senticolis triaspis intermedia* (Boettger, 1883), was collected from southwestern New Mexico and examined for endoparasites. Gamonts of 3 different hemogregarines were found in erythrocytes, and oocysts and free sporocysts of a *Sarcocystis* sp. were present in intestinal contents and feces. Measurements of small, medium, and large forms of intraerythrocytic gamonts were 12.8 × 3.4 (10.4–14.4 × 2.8–4.2) μm ($N = 20$), 17.0 × 4.0 (16.0–18.4 × 3.2–4.8) μm ($N = 20$), and 17.8 × 7.4 (16.0–20.0 × 6.2–8.8) μm ($N = 20$), respectively. Sporocysts of the *Sarcocystis* sp. were 12.7 × 10.6 (12.0–13.6 × 10.0–11.2) μm ($N = 20$) and had a shape index (length/width) of 1.20 (1.07–1.24). Although anecdotal information is available on parasites of *E. triaspis intermedia,* this is the first documentation of detailed information.

KEY WORDS: Apicomplexa, coccidia, gamonts, hemogregarines, Protozoa, *Sarcocystis* sp., *Senticolis triaspis intermedia,* western green rat snake, Colubridae, New Mexico.

The western green rat snake, *Senticolis triaspis intermedia* (Boettger, 1883), is a moderately large colubrid that ranges from southeastern Arizona, southwestern New Mexico, and southern Tamaulipas, Mexico, southward along the western Mexican highlands to Costa Rica (Stebbins, 1985; Garrett and Painter, 1992). It inhabits wooded and rocky canyon bottoms near streams in mountainous areas. Little is known about the biology of this snake (Wright and Wright, 1957; Dowling, 1960; Dowling and Fries, 1987; Cranston, 1989, 1990), and only anecdotal data are available on its parasites (Cranston, 1990). Herein, we report detailed information on 4 species of apicomplexan parasites found in a *S. triaspis intermedia.*

On 27 April 1992, an adult male *S. triaspis intermedia* (snout–vent length = 734 mm, University of New Mexico Museum of Southwestern Biology, MSB 54161) was collected by 1 of us (C.M.G.) in Guadalupe Canyon of the Peloncillo Mountains of extreme southwestern Hidalgo County, New Mexico (31°21′N, 109°03′W). This snake represented the first voucher specimen from the state (Garrett and Painter, 1992). The spec-

Table 1. Measurements of 20 gamonts of 3 types of hemogregarines found in erythrocytes of *Senticolis triaspis intermedia*.

Morphological type	Length (μm) $\bar{x}$ ± SD (range)*	Width (μm) $\bar{x}$ ± SD (range)†
Small form	12.8 ± 1.0 (10.4–14.4)	3.4 ± 0.4 (2.8–4.2)
Medium form	17.0 ± 1.0 (16.0–18.4)	4.0 ± 0.4 (3.2–4.8)
Large form	17.8 ± 1.3 (16.0–20.0)	7.4 ± 0.8 (6.2–8.8)

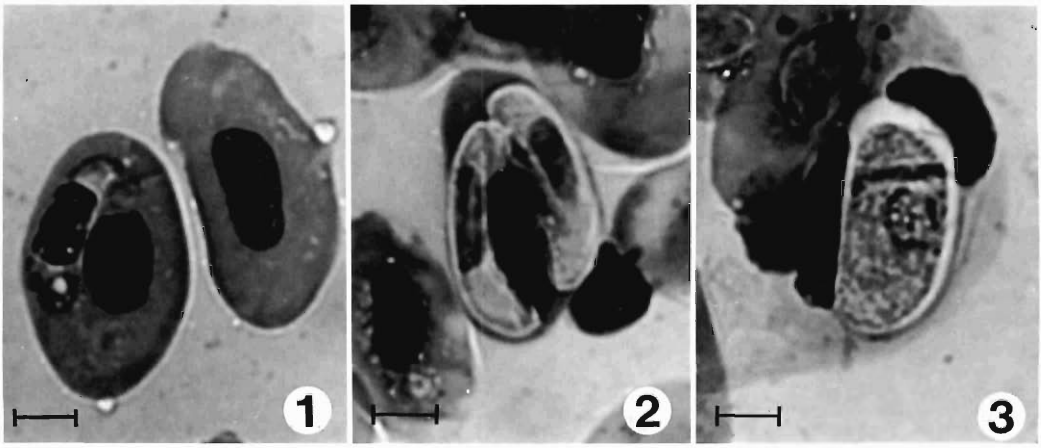
* For lengths, $P < 0.001$ from small form to medium form, $P < 0.001$ from small form to large form, and $P < 0.05$ from medium form to large form.

† For widths, $P < 0.005$ from small form to medium form, $P < 0.001$ from small to large form, and $P < 0.001$ from medium form to large form (paired Student's t-test, df = 19).

imen was returned to the laboratory and killed with an overdose of sodium pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, Illinois). Prior to killing, blood was obtained from the heart and films were air-dried, fixed in absolute methanol, stained with Wright's stain, and rinsed in phosphate buffer (pH = 7.2). Intestinal contents and feces were collected, placed in 2.5% (w/v) aqueous potassium dichromate, and processed further for coccidians using previously described methods (Upton and McAllister, 1990). Measurements were made on gamonts and sporocysts using a calibrated ocular micrometer. All measurements represent the mean of 20 ± 1 SD under a ×100 oil immersion lens and are in micrometers followed by the ranges in parentheses. A blood film has been deposited in the USNM Helminthological Collection, United States Department of Agriculture, Beltsville, Maryland 20705, as USNM 82744.

Gamonts of 3 distinct morphological and statistically significant different types of hemogregarines were observed in blood smears (Table 1). The most commonly encountered gamonts were short, elongate parasites with a central nucleus containing dark-staining cytoplasmic granules (Fig. 1). Another form was elongate, with curved ends, a central nucleus, and pale blue cytoplasm (Fig. 2). The third form differed by having large, robust gamonts, lightly staining cytoplasm, and an eccentric nucleus, usually at the posterior end but occasionally centrally located (Fig. 3). Since hemogregarines cannot be consistently distinguished solely by erythrocytic stages, generic designation is not possible (Telford, 1984). Although parasites described herein may represent either a species of *Hepatozoon* or *Haemogregarina,* we refrain from assigning generic designations without complete knowledge of the life cycles.

Cranston (1990) reported hemogregarines and trypanosomes from 2 *S. triaspis intermedia* from southeastern Arizona without giving specific morphological information. In addition, related



Figures 1–3. Gamonts of 3 forms of hemogregarines in erythrocytes of *Senticolis triaspis intermedia* from New Mexico. 1. Small form. 2. Medium or elongate form. 3. Large or robust form. Scale bars = 5.0 μm.

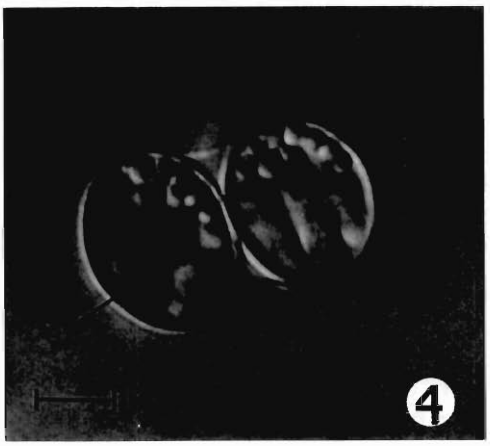


Figure 4. Oocyst of *Sarcocystis* sp. from *Senticolis triaspis intermedia* from New Mexico. Abbreviations: ow = oocyst wall, sp = sporocyst, sr = sporocyst residuum, sz = sporozoite. Scale bar = 5.0 μm.

rat snakes, *Elaphe obsoleta* (Say, 1823), from Arkansas, Louisiana, Illinois, and Ohio, and Great Plains rat snakes, *E. guttata emoryi* (Baird and Girard, 1853), from Texas have been reported to be hosts of hemogregarines (Hilman and Strandtmann, 1960; Hull and Camin, 1960; Marquardt, 1966; Daly et al., 1984; Lowichik and Yeager, 1987).

Oocysts and free sporocysts of a *Sarcocystis* sp. (Fig. 4) were recovered from intestinal contents and feces. Measurements of 20 sporocysts were 12.7 ± 0.43 × 10.6 ± 0.38 (12.0–13.6 × 10.0–11.2) μm and had a shape index (length/width) of 1.20 ± 0.06 (1.07–1.29). Numerous species of *Sarcocystis* have been reported from snakes (Upton et al., 1992), and it is impossible to distinguish species without tissue stages in the intermediate host. Although Cranston (1990) reported "coccidia" from *S. triaspis intermedia,* we are not sure whether or not he was referring to a species of *Sarcocystis.*

In conclusion, other than previously published anecdotal information, this is the first report of endoparasites from *E. triaspis intermedia.* Further study surveying a larger sample size of this snake for parasites throughout its range is warranted.

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J. Helminthol. Soc. Wash.
60(2), 1993, pp. 287–288

MINUTES

Six Hundred Twenty-Ninth Through Six Hundred Thirty-Sixth Meetings

629th Meeting: University of Maryland, Center for Adult Education, College Park, MD, 14 October 1992. The Anniversary Dinner Meeting was held with the Trustees of the Brayton H. Ransom Memorial Trust Fund. Morgan Golden, chairman of the Brayton H. Ransom Memorial Trust Fund, presided over the program honoring the long service of Gilbert F. Otto and Aurel O. Foster as trustees of the Ransom Fund. Nancy Pacheco gave a history of Dr. Ransom. Harley Sheffield recognized the achievements of Gilbert Otto and Ralph Lichtenfels cited those of Aurel Foster. Certificates of Achievement were presented to Gilbert Otto and Aurel Foster. The slate of officers for 1993 was presented: Ruth M. Kulstad, President; Mark Jenkins, Vice-President; Joan E. Jackson, Secretary-Treasurer; Eileen D. Franke, Recording Secretary.

630th Meeting: Animal Parasitology Unit, ARS, USDA, Beltsville, MD, 10 November 1992. David J. Chitwood presided over the meeting. The slate of officers was elected unanimously. The Chairman of the 1991 Award Committee, Ed Michelson, presented the 1991 Anniversary Award to Francis G. Tromba. The Chairman of the 1992 Award Committee, Jeff Bier, presented the 1992 Anniversary Award to Thomas K. Sawyer. Ralph Lichtenfels presided over the mini-symposium on the study of phylogenetic relationships. The following papers were presented: Why cladistics?, by Gregory Klassen; Molecular data and phylogenetic inference, by Mark C. Jenkins; and Phylogenetic reconstruction, evolution and historical biogeography—the basis for comparative biology, by Eric P. Hoberg.

631st Meeting: Nematology Laboratory, ARS, USDA, Beltsville, MD, 16 December 1992. David Chitwood presided over the business meeting and the scientific program. The following papers were presented: The life cycle of the cat liver fluke, *Platynosomum fastosum* (Digenea: Dicrocoeliidae), by Ralph P. Eckerlin; Some current research on entomopathogenic nematodes, by William R. Nickle; Alternative management strategies for soybean cyst nematode, by Susan L. F. Meyer; Briefing from the President of the American Society of Parasitologists, by K. Darwin Murrell. The new officers were installed.

632nd Meeting: Walter Reed Army Institute of Research, Washington, DC, 13 January 1993. Ruth Kulstad presided over the business meeting. Willis Reid presided over the scientific session. The following presentations were made: The new WRAIR—finally a reality?, by Henry Fein; Technology transfer—government innovation and the market place, by Willis Reid; Assessing region-specific health risks to U.S. soldiers deploying overseas, by Bruno Petruccelli.

633rd Meeting: Naval Medical Research Institute, Bethesda, MD, 10 February 1993. Ruth Kulstad presided over the business meeting and Trevor Jones presided over the scientific session. The following papers were presented: The safety and efficacy of Pentostam in the treatment of mucosal leishmaniasis in Perú, by Eileen Franke; The history of malaria in U.S. naval forces at war: World War I to the Vietnam War, by Christine Beadle; Malaria vaccine field site: transmission, diagnosis and clinical trial design, by Trevor Jones and Preston Church.

634th Meeting: National Institutes of Health, Bethesda, MD, 10 March 1993. Ruth Kulstad presided over the business meeting and Frank Neva presided over the scientific session. The following papers were presented: Molecular analysis of receptor–ligand interaction involved in the invasion of erythrocytes by *Plasmodium vivax,* by Dr. Chetan Chitinis; Attachment and release of leishmanial promastigotes in the sandfly gut, by Paulo Pimenta; In vivo cytokine mRNA expression during infection with *Schistosoma mansoni,* by Thomas Wynn.

635th Meeting: Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, The Johns Hopkins University, 7 April 1993. Ruth Kulstad presided over the business meeting and Noel Rose presided over the scientific session. Alan Scott gave a talk on Sper-

matogenesis and embryogenesis in filarial nematodes. The proposed amendments to the Bylaws of the Constitution were presented in writing to the members attending the meeting.

636th Meeting: New Bolton Center, University of Pennsylvania, Kennett Square, PA, 1 May 1993. Ruth Kulstad presided over the business meeting. The proposed amendments to the Bylaws of the Constitution were approved by unanimous vote of the Executive Committee. Gerhard Schad presided over the scientific session. The subject of the session was vertical transmission. The topics and speakers were: Vertical transmission of trematodes, by Wesley L. Shoop; *Sarcocystis, Toxoplasma* and malaria: effects of infection during pregnancy, by Ronald Fayer; Vertical transmission of nematodes in mammals, by Eugene T. Lyons; Mammary cestodiasis and vertical transmission?, by D. Bruce Conn. Support for the meeting was provided by SmithKline Beecham Animal Health.

The Helminthological Society of Washington welcomed 5 new members to the Society during the meetings indicated. *632nd:* Ruobing Wang and Jan Boyd. *633rd:* Stephen Hoffman, Peter Weina and Maki Ujiie.

Respectfully submitted,

Eileen D. Franke, Ph.D.
Recording Secretary

J. Helminthol. Soc. Wash.
60(2), 1993, p. 289

AUTHOR INDEX FOR VOLUME 60

J. Helminthol. Soc. Wash.
60(2), 1993, pp. 290–292

KEY WORD AND SUBJECT INDEX FOR VOLUME 60

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	* Leo A. Jachowski, Jr.	1976
Mildred A. Doss	1961	* Horace W. Stunkard	1977
* Allen McIntosh	1962	Kenneth C. Kates	1978
* Jesse R. Christie	1964	* Everett E. Wehr	1979
* Gilbert F. Otto	1965	* O. Wilford Olsen	1980
* George R. LaRue	1966	Frank D. Enzie	1981
* William W. Cort	1966	Lloyd E. Rozeboom	1982
* Gerard Dikmans	1967	Leon Jacobs	1983
* Benjamin Schwartz	1969	Harley G. Sheffield	1984
* Willard H. Wright	1969	A. Morgan Golden	1985
Aurel O. Foster	1970	Louis S. Diamond	1986
Carlton M. Herman	1971	Everett L. Schiller	1987
May Belle Chitwood	1972	Milford N. Lunde	1988
* Elvio H. Sadun	1973	J. Ralph Lichtenfels	1989
E. J. Lawson Soulsby	1974	A. James Haley	1990
David R. Lincicome	1975	Francis G. Tromba	1991
Margaret A. Stirewalt	1975	Thomas K. Sawyer	1992

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* Norman R. Stoll	1976	E. J. Lawson Soulsby	1990
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* Justus F. Mueller	1978	Louis Euzet	1992
John F. A. Sprent	1979		

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* Henry E. Ewing	1945	John T. Lucker, Jr.	1979
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* Allen McIntosh	1963	Paul C. Beaver	1986
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* Albert L. Taylor	1975	Judith H. Shaw	1990
David R. Lincicome	1976	Milford N. Lunde	1991
Margaret A. Stirewalt	1976	Everett L. Schiller	1991
* Willard H. Wright	1976	Harley G. Sheffield	1991

* Deceased.

CONTENTS

(*Continued from Front Cover*)

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