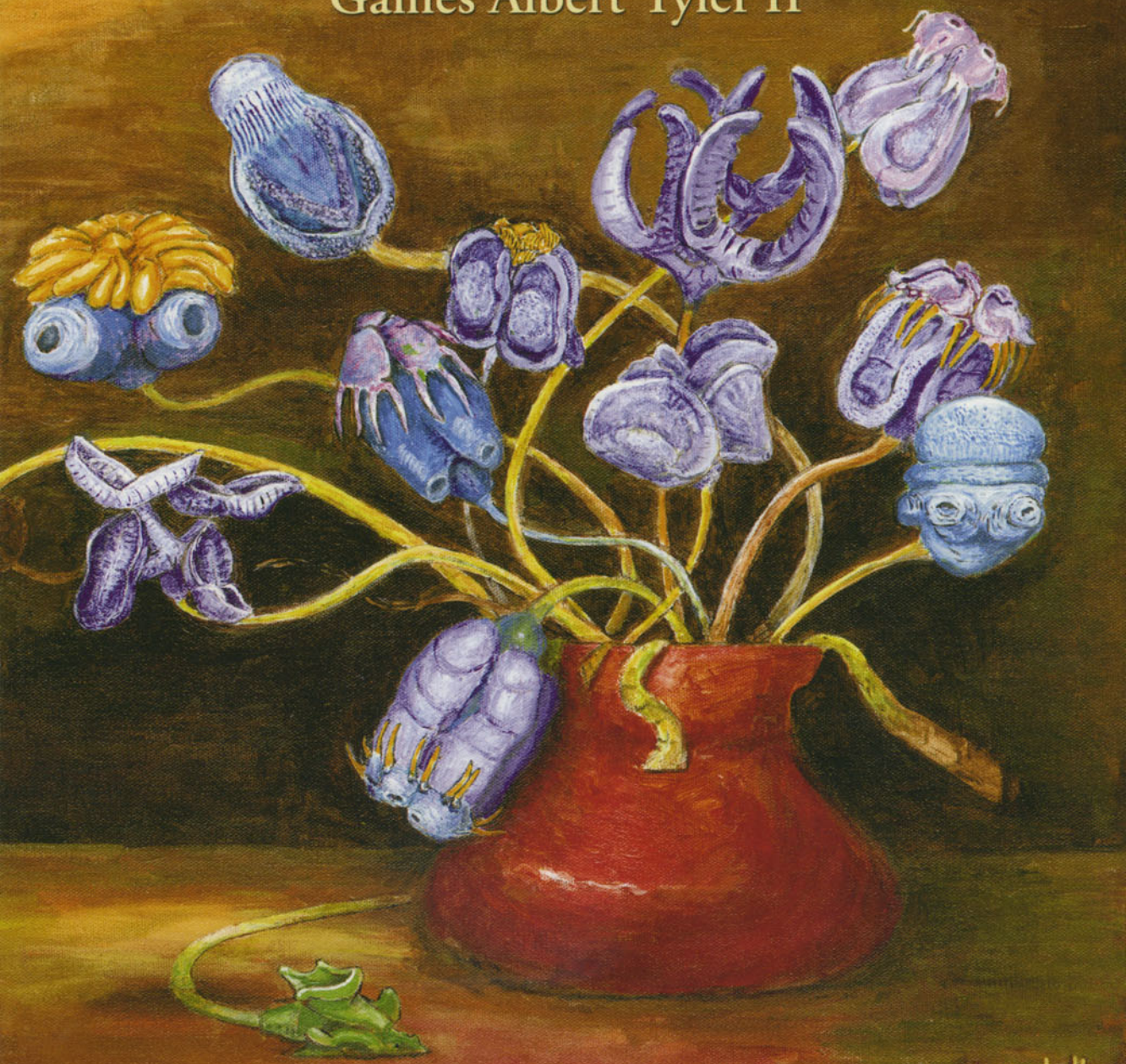
Tapeworms of Elasmobranchs (Part II)

A Monograph on the Diphyllidea

(Platyhelminthes, Cestoda)

Gaines Albert Tyler II



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Cover: Tapeworms and red vase. A fanciful representation of some tapeworms of sharks and rays. Painting by William C. Campbell (acrylic on canvas, 2004).

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TAPEWORMS OF ELASMOBRANCHS (Part II)
A Monograph on the Diphyllidea
(Platyhelminthes, Cestoda)

by

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Abstract. The main objective of this monograph was a synthesis of the available knowledge on the diversity, systematics, host associations, and biogeography of the Diphyllidea. A thorough review of the literature resulted in the transfer of *Diagonobothrium* into Diphyllidea as a *genus inquirendum*. The genus *Yogeshwaria* was also transferred to Diphyllidea as a synonym of *Echinobothrium*. Its only species, *E. nagabhushani* n. comb., is considered to be a *species inquirenda*. New collections resulted in the description of a new species of *Echinobothrium*. Type and/or voucher specimens for 32 of 36 valid diphyllidean species (including the new species) were examined using light microscopy and scanning electron microscopy. All 32 species examined were redescribed and figured. This work resulted in the elucidation of 55 morphological characters which were employed in cladistic analyses involving 34 diphyllidean and seven outgroup species. Outgroups included species belonging to the orders Tetraphyllidea, Pseudophyllidea, and Trypanorhyncha. Several phylogenetic analyses were performed using various data partitions. A 20% exclusion rule was applied to both taxa and characters. All characters were treated as unweighted and unordered. Maximum parsimony was the optimality criterion used in all analyses. The most parsimonious trees resulting from these analyses support *Ditrachybothridium* as a monophyletic taxon. All three species formerly assigned to *Macrobothridium* appeared among species of *Echinobothrium*. Thus, *Echinobothrium* is paraphyletic if *Macrobothridium* is excluded. As a consequence, *Macrobothridium* is synonymized with *Echinobothrium*, and its constituent species transferred to the latter genus. The trees obtained from these analyses failed to recover any of the topology of the only previously published phylogeny of the order. Constraining the tree resulting from this study to the topology of the

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previously published tree resulted in a substantially longer tree. A comparison of the tree resulting from the phylogenetic analyses among the diphyllideans to a composite tree of the relationships among batoid genera known to host *Echinobothrium* suggests that strict coevolution between the elasmobranchs and their diphyllidean tapeworms is unlikely to have occurred. However, because the sampling of potential elasmobranch hosts has not been comprehensive, this conclusion is considered preliminary.

INTRODUCTION

Overview

Cestodes, like all internal parasites, live in an environment unlike anything experienced by free-living organisms. By taking up residence in the intestine of a vertebrate host, cestodes escape the vagaries of living in either an aquatic or terrestrial habitat, such as daily light/dark cycles, rapid changes in temperature and pH, or ecological disturbances. Thus, even though the gut of a vertebrate is generally considered a harsh environment, it is relatively stable and protected, at least from a cestode's point of view. Perhaps as a result of living in this protected environment, cestodes have become very specific in their ecological needs, often being found in only a single host species, and usually in a very specific location within that host (Caira 1990; Caira and Jensen 2001). This high degree of host specificity may exist not only between the tapeworm and its vertebrate definitive host, but between the tapeworm and its (one or more) intermediate host(s) as well, but this has not yet been demonstrated. The result is an organism that can continue to exist only if a precise set of ecological conditions is met. An organism with such precise ecological needs is a good candidate for a biological sentinel, or indicator organism. In the case of these cestodes, their presence or absence may be used to make inferences about certain aspects of the biology of their elasmobranch hosts, such as feeding habits or migratory patterns (Caira 1990). As parasites, cestodes have the potential to harm their hosts. In addition, cestodes have been shown to affect host behavior (see *e.g.*, Taylor *et al.* 1998; Loot *et al.* 2001), growth (see *e.g.*, Pulkkinen and Valtonen 1999; Arnott *et al.* 2000) and perhaps, even evolution (Hamilton and Zuk 1982; Jackson and Tinsley 2001). The ability of cestodes to affect their hosts illustrates not only the importance of cestodes in an ecosystem, but also that tapeworms can be interesting, even fascinating organisms to study.

In order for any organism or group of organisms to serve as an indicator, we must first have a good understanding of their diversity and basic biology. Unfortunately, with the exception of medically important species in the orders Cyclophyllidea and Pseudophyllidea, little is known about most cestode groups. The order Diphyllidea is no exception. Even among cestodologists, there has not been a specialist in the Diphyllidea for over 100 years.

Anatomy

The anatomy of cestodes is unique among the invertebrates. In the simplest terms, a tapeworm consists of a scolex (primarily an attachment organ), neck (germinative region), and a strobila (primarily for nutrient absorption and reproduction). Basic diphyllidean morphology is illustrated in Figure 1. The most detailed descriptions of the anatomy and functional morphology of the Diphyllidea were published by Rees (1959, 1961a) for *Ditrachybothridium* and *Echinobothrium*, respectively. The reader is referred to those works for a more detailed description of the anatomy. The purpose of this section is to introduce only the anatomical terminology of the Diphyllidea relevant to the taxonomic treatment of the diphyllidean taxa.

The scolex of a diphyllidean consists of a scolex proper (rostellum and one dorsal and one ventral bothrium) and a cephalic peduncle. The bothria aid in attachment of the worm to the intestinal surface of the host. These structures have been reported to function in several ways, including wedging into the glandular crypts of the intestinal surface (see Rees 1961a), capping the ends of intestinal villi (see Neifar *et al.* 2001), or intertwining between adjacent villi (see Neifar *et al.* 2001). The scolex of diphyllideans has been regarded by many to consist of two bothridia, rather than bothria (see, *e.g.*, Schmidt 1986; Khalil 1994; Hoberg *et al.* 1997; Ivanov and Hoberg 1999; Hoberg *et al.* 2001). However, examination of cross sections through the scolices of several species of *Ditrachybothridium*, *Echinobothrium*, and *Macrobothridium*

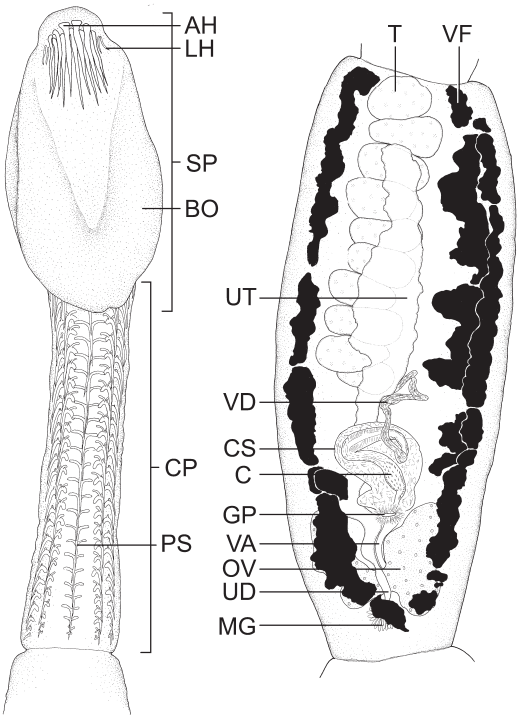


Fig. 1. General anatomy of a diphyllidean cestode. Abbreviations: AH, apical hook; BO, bothrium; C, cirrus; CP, cephalic peduncle; CS, cirrus sac; GP, genital pore; LH, lateral hooklet; MG, Mehlis' gland; OV, ovary; PS, cephalic peduncle spine; SP, scolex proper; T, testis; UD, uterine duct; UT, uterus; VA, vagina; VD, vas deferens; VF, vitelline follicle.

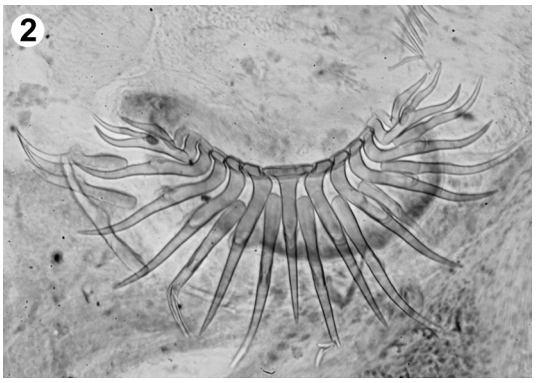


Fig. 2. Diphyllidean anatomy I: Apical hook symmetry. Type A hook symmetry.

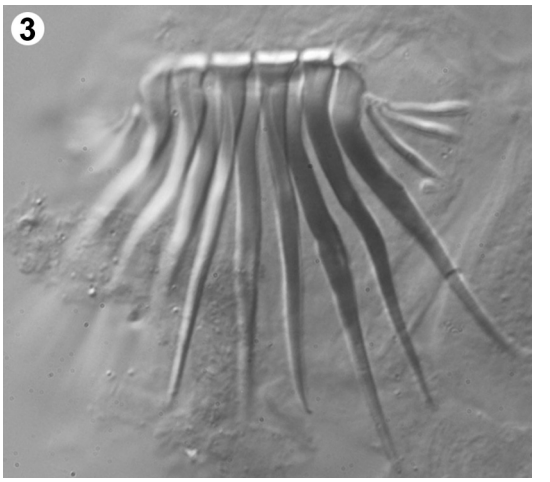


Fig. 3. Diphyllidean anatomy II: Apical hook symmetry. Type B hook symmetry.

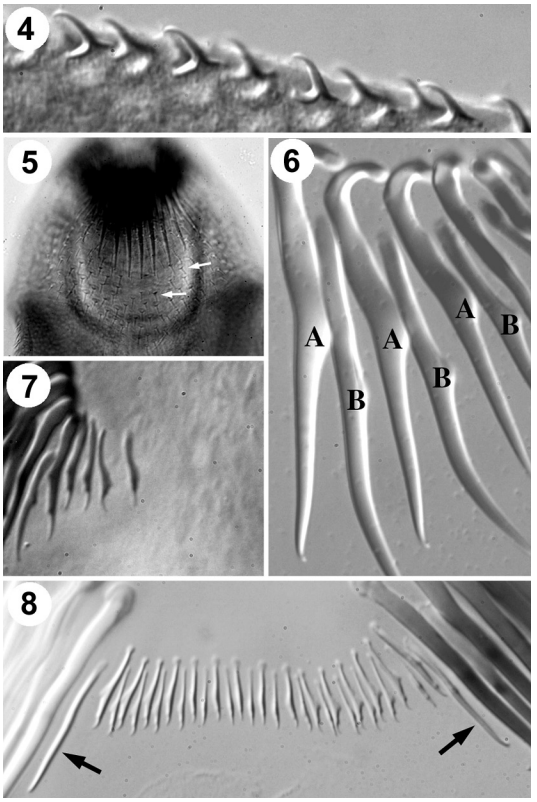
reveals that the structures that form much of the scolex are indeed bothria, as they lack the characteristic high degree of organization and muscularization of true bothridia (see Caira *et al.* 1999; Faliex *et al.* 2000; Neifar *et al.* 2001). In addition to bothria, most diphyllideans also possess an apical rostellum armed with two groups of large hooks and one or more groups of smaller lateral hooklets which are used to firmly anchor the worm in place.

Not all cestodologists agree with the terminology used here to describe this particular organ (see, *e.g.*, Ivanov 1997). The term rostellum has long been used to describe the apical structure found in cyclophyllidean tapeworms. In the Cyclophyllidea, this organ is usually armed with dual continuous coronas of hooks, attached at their bases to muscles which elevate or lower the hooks; the entire rostellum is often retractable. The rostellum of a diphyllidean is remarkably similar to that of a cyclophyllidean not only in function, but in form. Although the diphyllidean rostellum is bilaterally symmetrical rather than radially symmetrical (as in the Cyclophyllidea), the hooks are arranged in two rows, as in the Cyclophyllidea, and both use a combination of muscle contraction and a muscular fulcrum to elevate the hooks (see Rees 1961a). The rostellum (as an apical organ) of diphyllideans was considered as homologous to the apical organs found in other cestode groups, including those of the Cyclophyllidea (Caira

et al. 1999, 2001).

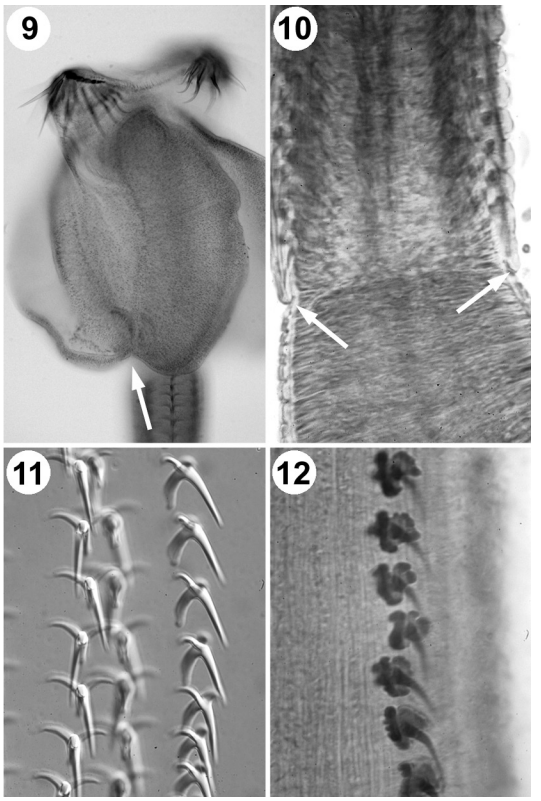
The armature of the rostellum has a very distinctive arrangement. It consists of two groups of apical hooks (one dorsal, one ventral), with or without smaller lateral hooklets on either side of each group of apical hooks (Figs. 2, 3). The lateral hooklets form either a single continuous row between the two groups of apical hooks (Fig. 8), or extend only partway between them (Fig. 7). Each group of lateral hooklets may be arranged either in a straight row (Fig. 7) or staggered (Fig. 8). Each group of apical hooks is arranged in two rows, one anterior and one posterior, the hooks of the anterior row alternating with those of the posterior row. The hooks of the anterior row have bases that are strongly recurved or geniculate, while the bases of the hooks in the posterior row are only slightly arched, or straight (Neifar *et al.* 2001). These different forms of hooks are referred to here as type A (recurved) and type B (arched or straight) (Fig. 6), following Neifar *et al.* (2001). The hook formula used here also follows that of Neifar *et al.* (2001), and is as follows: {LH AH(A)/AH(B) LH} where (LH) refers to the number (or range) of lateral hooklets in each group, AH(A) refers to the number (or range) of type A (anterior row) apical hooks, and AH(B) refers to the number (or range) of type B (posterior row) apical hooks. For example, Figure 3 shows a scolex with nine apical hooks, flanked on either side by three lateral hooklets. Because the nine apical hooks are comprised of six type A hooks in the anterior row, and three type B hooks in the posterior row, the hook formula for this species is {3 6/3 3}. Apical hooks centered about a type A hook (i.e., an odd number of type A hooks) are described as having type A symmetry (Fig. 2). When centered about a type B hook, they are described as having type B symmetry (Fig. 3). When the lateral hooklets form a single continuous row, the number expressed in the hook formula is half that in the entire row.

In most diphyliideans the cephalic peduncle, measured from the point of attachment of the bothria to the scolex to the densely staining neck region, is also armed, possessing eight columns of spines, each of which usually bears a triradiate base (Fig. 11). The bases of these hooks exhibit a lateral process on either side of the anterior end of a slender spine, with a third process perpendicular to the lateral processes and extending into the cephalic peduncle. The spine elevator muscles are attached to this process. The reader is referred to Rees (1961a) for an excellent description of the rostellar and cephalic peduncle armature and their associated musculature. The cephalic peduncle armature is strictly an adult feature, having never been observed in larval stages.



Figs. 4-8. Diphyllidean anatomy III: Other scolex features. 4. Spines on proximal bothrial surface. 5. Small spines between rostellum and bothria. 6. Type A and type B apical hooks. 7. Lateral hooklets, uniformly arranged, in two groups. 8. Lateral hooklets, staggered arrangement, in single continuous row; first and last hooklets longer than others (arrows).

The diphyllidean strobila consists of a neck, or germinative region, and a series of proglottids, each containing a complete set of both male and female reproductive organs. Although the strobila of some tapeworms



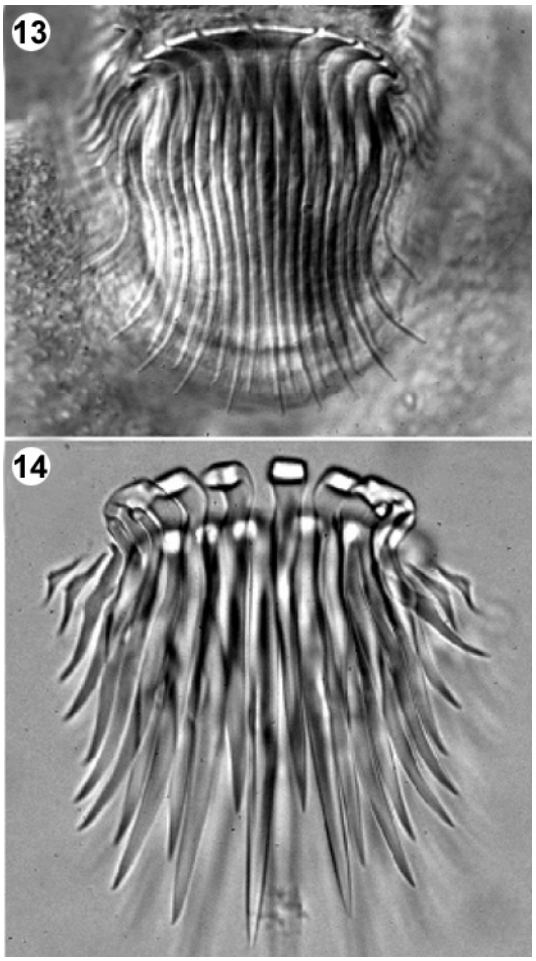
Figs. 9-12. Diphyllidean anatomy IV: Other scolex features. 9. Dorsal view of bothrium, showing cleft (arrow). 10. Cephalic peduncle, showing velum (arrows). 11. Cephalic peduncle spines with triradiate bases. 12. Cephalic peduncle spines with leaflike bases.

may reach extraordinary lengths with tens of thousands of proglottids (*e.g.*, *Hexagonoporus* Gubanov *in* Delyamure [1955] may reach 30 m; see Bray *et al.* [1994]), diphyllideans are generally small worms, less than a centimeter in length that possess fewer than 30 proglottids. The strobilae of diphyllideans are either apolytic (gravid proglottids remain on the strobila, but drop off before shedding eggs), or euapolytic (proglottids drop off the strobila when mature before becoming gravid), although one species (see Neifar *et al.* 2001) has been described as anapolytic. However, under the terminology followed here, that of Caira *et al.* (1999), that species would be considered apolytic.

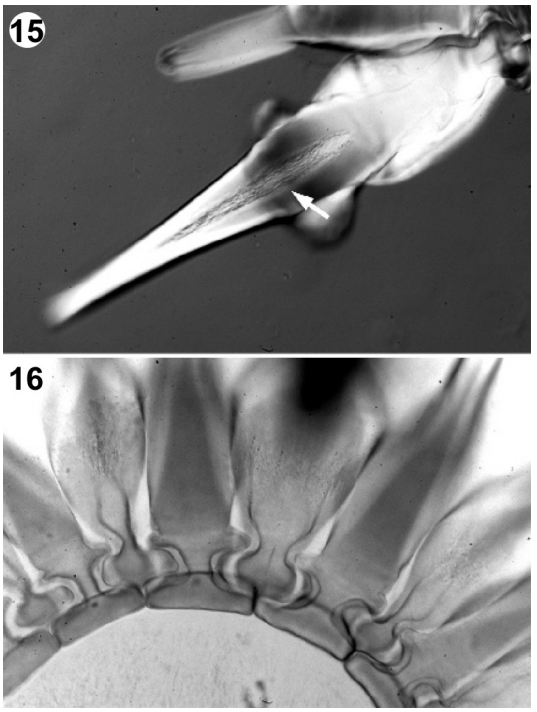
Tapeworms in general lack a mouth or gut, and thus, nutrition is acquired by absorption of nutrients through the tegument (Wardle and McLeod 1952). The tegument, while appearing to be a very simple structure, is actually a complex organ, consisting of a distal syncytial cytoplasm, tegumentary cytons (nuclei), and a basement lamella. Projecting from the outer surface of the tegument are structures called microtriches, which are generally considered to aid in absorption of nutrients by increasing the surface area of the tegument (see Lumsden and Hildreth 1983). After observing the enormous microtriches seen on some diphyllideans (*e.g.*, *Echinobothrium hoffmanorum* Tyler, 2001; Fig. 86), one cannot help but assume that the microtriches in such taxa also aid in attachment to the host, as has been reported by McVicar (1976) for some tetraphyllideans.

The fine structure of the tegument of diphyllideans has never been the focus of intensive research; it nonetheless exhibits some interesting and taxonomically useful features. As mentioned above, the tegument of diphyllideans, like that of all other cestodes, is covered with microtriches. Kuperman (1988) was the first to publish a description of the surface fine structure of a diphyllidean, noting the presence of pectinate microtriches on *Echinobothrium typus* Van Beneden. Caira *et al.* (1999) described various forms of microtriches on cestodes of elasmobranchs, recognizing two categories, filiform and spiniform. Those authors described two types of filiform microtriches, long and short, and several types of spiniform microtriches. Faliex *et al.* (2000) proposed to standardize the terminology used to describe the various forms and types of microtriches. Their terminology is followed here.

As is typical of most tapeworms, diphyllidean proglottids each contain a complete set of both male and female reproductive organs (Fig. 1). In diphyllideans, the male organs and genitalia usually begin their development first. However, there does not appear to be any temporal lag between maturation of the male and female organs. This arrangement would make self fertilization possible. Although never actually observed in the Diphyllidea, it may in fact occur, as single gravid specimens have been reported in the absence



Figs. 13-14. Diphyllidean anatomy V: Central apical hook sizes. 13. Hooks gradually increasing in length toward center of group. 14. Hooks conspicuously shorter in center of group.



Figs. 15-16. Diphyllidean anatomy VI: Apical hook morphology. 15. Hollow hook; arrow indicates channel. 16. Apical hooks with articulating bases.

of conspecifics (see, *e.g.*, Rees 1961b).

The form and function of the diphyllidean reproductive system was painstakingly described by Rees (1961a), and the reader is again referred to that work as the standard reference source for that information. What follows is a description of the diphyllidean reproductive system as observed during this study, supplemented by additional information from Rees (1961a).

The male reproductive organs consist of testes, which are always in the anterior part of the proglottid, and an invaginable cirrus contained within a cirrus sac. A short vas efferens connects each testis to the vas deferens. The vas deferens winds its way toward the cirrus sac, and may enter an external seminal vesicle just external to the cirrus sac. The seminal vesicle was defined by Caira *et al.* (1999) as a sac-like expansion of the vas deferens. An internal seminal vesicle may also be present inside the cirrus sac. There are often several coils of the vas deferens inside the cirrus sac, in addition to the coiled cirrus, which is usually armed with microtriches.

The female reproductive anatomy is as follows: a bilobed ovary resides in the posterior part of the proglottid; its two lobes are joined by an isthmus. The vagina opens distally into the genital atrium just posterior to the cirrus on the midventral surface and extends posteriorly to the region of the ovarian isthmus. The vagina may or may not have a seminal receptacle. The seminal receptacle, when present, comprises a sac-like expansion of the vagina with a constriction at either end. The vitelline (or yolk) glands are follicular. They are usually arranged in

two columns which extend laterally along the longitudinal axis of the proglottid, each connected to a median vitelline duct. Each ovum passes out of the ovary through a muscular oocapt, and into the oviduct where the vagina delivers sperm to fertilize the ovum. The fertilized ovum passes into the ootype, which is surrounded by the Mehlis' gland, where it receives two vitelline cells (containing yolk and some shell precursor material) from the median vitelline duct. The Mehlis' gland appears to function in the production of the egg shell, but the details are not well understood (see Smyth 1969). The egg leaves the ootype via the uterine duct, which extends anteriorly, entering the saccate uterus, usually near the level of the genital pore. The uterus expands to fill the proglottid when gravid.

Life Cycle

As adults, diphyllidean cestodes are obligate internal parasites of elasmobranch fishes (sharks, skates, and rays). These parasites live their adult lives attached to the mucosal lining of the spiral intestine of their hosts. No complete life-cycle is known for any diphyllidean cestode, although larval stages of several species have been found in invertebrate hosts. One of these was found in the body cavity of a shrimp within the gut of a skate (Ruszkowski 1927). If diphyllideans are like other cestodes of elasmobranchs for which some life cycle data have been collected (*e.g.*, Mattis 1986), then the life cycle is likely to involve three hosts, although a two host life cycle cannot be ruled out. To date, diphyllidean plerocercus larvae have been found in crustaceans including *Crangon* sp. and *Pagurus* sp. (Leuckart and Pagenstecher 1858; Bray and Olson 2004), *Gammarus locusta* (Van Beneden 1871), *Perioculoides longimanus* (Monticelli 1890), *Hippolyte varians* (Ruszkowski 1927), *Matuta victor* (Anantaraman, 1963), *Carcinus maenas* (Dollfus 1964), *Ethusa mascarone* (Vivares 1971, 1972-73, 1973), *Leptochela aculeocaudata* (Ramadevi and Rao 1974), *Leptochela* sp. (Shimazu 1975, 1982), *Penaeus longistylus* (Jones and Beveridge 2001); molluscs including *Nassa reticulata* (Lespés 1857), *Solen vagina* (Kunstler 1888), *Bullia malanoides* and *Murex tropa* (Anantaraman 1963), *Cantharus cancellarius* and *Nassarius vibex* (Cake 1976, 1977), and a teleost fish *Labrus merula* (Campos and Carbonell 1994). A hypothesized life cycle for the diphyllidean involving known intermediate hosts is as follows: Eggs are shed with the feces of the definitive host, and are eaten by a free-living filter-feeding invertebrate such as an amphipod or copepod. The egg hatches, releasing a hexacanth larva which then burrows through the gut wall, encysts, and develops into a procercoid larva in an organ such as the liver. The infected amphipod is then eaten by a second host such as a crab or a shrimp, burrows through the gut, encysts in the liver, and develops into a plerocercus larva. The second intermediate host may then be eaten by a third host, possibly a paratenic host such as a teleost, or may be directly eaten by the final elasmobranch host. Maturation occurs in the elasmobranch host, where sexual reproduction occurs between adults either via cross or self-fertilization in the spiral intestine of the elasmobranch, and the cycle begins again.

Although no studies have been undertaken to determine the lifespan and complete life history of diphyllideans, there is some evidence suggesting that the lifespan of adults is less than one year. Tyler (2001) observed seasonal fluctuation in the abundance of *Echinobothrium* in some host species in the Gulf of California, which he suggested may have been the result of seasonal mortality of the adults. Although McVicar (1976) did not observe any seasonal variation in parasite intensity, he did observe a negative correlation between host length in *Raja naevus* Müller and Henle, 1841 and prevalence of infection with *Echinobothrium harfordi* McVicar, 1976. McVicar attributed this correlation to a change in preferred prey associated with definitive host size. Assuming that the larger hosts had exhibited a higher prevalence of infection with *E. harfordi* when they were younger and smaller, the worms must have been shed by the host, indicating that either the hosts develop immunity or that the worms do not live long relative to their hosts.

Historical Summary

Given that the order Diphyllidea consisted only of the genus *Echinobothrium* Van Beneden, 1849 from its establishment in 1863 until 1959, all systematic treatments of the order during that period were tantamount to treatments of the genus. In order to avoid repetition, only the information pertinent to the Diphyllidea in general will be discussed here; the remainder will be addressed below. At the time of his description of the first diphyllidean cestode, *Echinobothrium typus* Van Beneden, 1849, Van Beneden (1849) had divided the "Cestoïdes" into two sections, the Acanthocephales and the Anacanthocephales, placing this new genus in the latter section. The origin of the taxon name Diphyllidea appears to derive from the classification of the cestodes proposed by Van Beneden (1850) in which he divided the "Cestoïdes" into four sections, Tetraphyllés, Diphyllés, Pseudophyllés, and Téniens, abandoning Acanthocephales and Anacanthocephales. Later, Van Beneden (1858) revised this classification, retaining the four sections he had proposed in 1850, but dividing the Cestoïdes into two orders, the Bothriadés, parasites of cold blooded vertebrates containing the families Tetraphyllés, Diphyllés, and Pseudophyllés; and Téniadés, parasites of warm blooded vertebrates, which included only the genus *Taenia*. Diphyllidea was established as a family by Van Beneden (*in* Carus 1863) for the single genus *Echinobothrium.* Although this name has existed for 138 years, it took some time for it to become widely accepted.

Perrier (1878) either ignored or did not recognize the classification proposed by Carus (1863) and published a classification based on Van Beneden's (1858) earlier work. He retained the two orders Bothriadés and Téniadés, but modified their diagnoses so that Téniadés housed all tetrafossate forms, and Bothriadés all difossate forms, including *Echinobothrium*, eliminating Diphyllés from Van Beneden's (1858) classification. Perrier (1897) later proposed a classification which differed from his 1878 work, but in which the name Diphyllidea was also not recognized. It is unclear how the classification used by Stossich (1898), was derived. Stossich (1898) treated the cestodes as an order, like Carus (1863), but did not use Carus' (1863) classification of families, and did not recognize the taxon name Diphyllidea. Braun (1894-1900) elevated the family Diphyllidea (and the other families in Carus [1863]) to ordinal status, creating the foundation for most modern classifications of the Cestoda. Nonetheless, not all workers accepted this classification. Lühe (1910) unwittingly contributed to the lack of acceptance of Diphyllidea (see below) when he published a guide to the freshwater fauna of Germany. In that work, he listed four of the five orders of Braun (1894-1900), but made little mention of Diphyllidea, stating only that the order would not be treated in his book because it was exclusively marine. The classification of Mola (1921) was loosely based on that of Braun (1894-1900), except that he divided the Diphyllidea into two families and added several other genera of pseudophyllideans, lecanicephalideans, and spathebothrideans to the order. Meggitt (1924) apparently did not recognize the Diphyllidea, as he failed to mention it (or *Echinobothrium*) in his "complete" list of cestode genera. Southwell (1925, p. 8) mistakenly claimed that Lühe (1910) "classified the true (Polyzootic) cestodes in four Orders viz., Pseudophyllidea, Tetraphyllidea, Cyclophyllidea, and Trypanorhyncha" and erected the new order Heterophyllidea for *Echinobothrium* and a number of other enigmatic tetraphyllidean and proteocephalidean genera. However, this taxonomic dumping ground failed to gain acceptance from other systematists, and soon disappeared into obscurity, but not before being harshly criticized by Poche (1926). Poche (1926) expressed dismay at Southwell for erecting a new order (Heterophyllidea) to replace an existing one, and including in that order a set of genera with no common characteristics and no apparent similarities to *Echinobothrium*. Ironically, rather than resurrecting Diphyllidea as the correct order for *Echinobothrium*, Poche (1926) erected the new order Echinobothriidea for that genus, and distributed the remaining genera among other orders. Given that most of the higher taxa in Poche (1926) were new, it would ap-

pear that this, and many of Poche's other taxonomic decisions, were inspired by something other than a desire to build a stable classification. With respect to the Diphyllidea, Mola's (1929) classification was identical to that in his 1921 work. In 1930, Southwell proposed yet another classification scheme, this time failing to recognize any of Braun's orders or, for that matter, his own order Heterophyllidea; *Echinobothrium* was omitted from this classification. Joyeux and Baer (1936) recognized the Diphyllidea as an order as circumscribed by Braun (1894-1900).

Wardle and McLeod (1952) devoted a great deal of ink to criticizing and even insulting the work of others, but were themselves guilty of confusing the taxonomic literature, at least with respect to the Diphyllidea. Following the path blazed by Southwell (1925), Wardle and McLeod (1952) stated that Lühe (1910) failed to recognize the Diphyllidea and subsequently rejected it themselves. They also rejected Southwell's (1925) Heterophyllidea, placing *Echinobothrium* in their new order Lecanicephala. In his classification scheme, Riser (1955) made no mention of the name Diphyllidea, and placed *Echinobothrium* in the Tetraphyllidea. Both Euzet (1959) and Yamaguti (1959) followed Braun's (1894-1900) scheme, retaining Diphyllidea as a valid order within which both authors placed *Echinobothrium*. Schmidt (1970) generally followed Braun's classification scheme, but also erected the new family Ditrachybothridiidae Schmidt, 1970 for the genus *Ditrachybothridium* Rees, 1959, which Rees (1959) only tentatively placed in Diphyllidea. In perhaps the single most confusing taxonomic decision ever to affect the Diphyllidea, Wardle *et al.* (1974) again misinterpreted the work of Lühe (1910), explicitly stating that he had rejected the Diphyllidea of Van Beneden (*in* Carus 1863). These authors also rejected the Diphyllidea of Mola (1921; 1929), and went on to claim that Diphyllidea was therefore a *nomen oblitum*, and used this fact to validate their resurrection of the name for their new order Diphyllidea to house the pseudophyllidean family Diphyllobothriidae Lühe. This classification has been followed by only a few authors (*e.g.*, Ferguson and Appleton 1988). Stunkard (1983) followed Braun's (1894-1900) classification, but, perhaps more importantly, he was the first to recognize that Lühe (1910) had not rejected the Diphyllidea. Khalil and Abdul-Salam (1989) also followed the classification scheme of Braun (1894-1900), recognizing the order Diphyllidea and adding to it the new family Macrobothridiidae Khalil and Abdul-Salam, 1989 for their new genus *Macrobothridium* Khalil and Abdul-Salam, 1989. In his widely used cestode keys, Schmidt (1986) also utilized Braun's (1894-1900) classification scheme.

Although the intent of Brooks and McLennan (1993) was admirable (*i.e.*, a classification based on a complete phylogenetic analysis of the parasitic platyhelminths), the result was at best confusing, and at worst, destabilizing to tapeworm systematics. These authors dismantled Braun's (1894-1900) classification scheme entirely, and replaced it with a complicated system employing the rarely used taxonomic ranks of cohort, subcohort and infracohort; in the process they did away with the name Diphyllidea. Brooks and McLennan (1993) treated only one of the three families of diphyllideans, Echinobothriidae, placing it in the order Pseudophylliformes along with several families of pseudophyllideans, ignoring the other two diphyllidean families. In the most recent comprehensive keys of the cestodes (Khalil *et al.* 1994), the order Diphyllidea Van Beneden in Carus, 1863 was recognized as comprising three families: Echinobothriidae Perrier, 1897; Ditrachybothridiidae Schmidt, 1970, and Macrobothridiidae Khalil and Abdul-Salam, 1989. The justification for maintaining Diphyllidea was strengthened by Caira *et al.* (1999, 2001) and Ivanov and Hoberg (1999), who demonstrated the monophyly of the order. The taxonomic status of the order is now fairly stable, and is accepted by most, if not all, cestode systematists worldwide (*e.g.*, Hoberg *et al.* 1997; Mariaux 1998; Caira *et al.* 1999; Olson and Caira 1999; Caira *et al.* 2001; Hoberg *et al.* 2001; Olson *et al.* 2001).

MATERIALS AND METHODS

Cataloging of Type and Voucher Material

At the inception of this project, the location of type material for only about 65% of described diphyllidean species was known. Therefore, it was necessary to contact or visit museums worldwide in order to locate missing types and all other cataloged or non-cataloged material. The search for diphyllideans included the institutions listed below. Each institution was contacted either through correspondence (*) or a formal visit (#). Not all institutions replied to written inquiries. These are denoted with a (?). The name of each museum is followed by the acronym used for that museum throughout the remainder of the text. Museums contacted:

U.S. National Parasite Collection (#), Beltsville, Maryland, U.S.A. (USNPC)
Harold W. Manter Laboratory (#), University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (HWML)
Canadian Museum of Nature (*), Aylmer, Quebec, Canada (CMNPA)
Institute of Parasitology (*), Macdonald College, Montreal, Quebec, Canada (IP)
Colleción Nacional de Helmintos (*), Universidad Nacional Autónoma de México, México City, Mexico (CNHE)
Instituto Oswaldo Cruz (*), Rio de Janeiro, Brazil (IOC)
Museo de Ciencias Naturales (*), La Plata, Argentina (MLP)
Colleción Parasitologica, Faculdad de Humanidades y Ciencias (?), Montevideo, Uruguay (CPU)
The Natural History Museum (#), London, England (BMNH)
Muséum d'Histoire Naturelle de Genève (#), Geneva, Switzerland (MHNG)
Muséum National d'Histoire Naturelle (#), Paris (MNHN)
Ecole Nationale Vétérnaire de Lyon (?), Lyon, France (ENVL)
Commonwealth Institute of Parasitology (*), St. Albans, Wales (CIP)
Naturhistorisches Museum Wien (#), Vienna, Austria (NMW)
Museum für Naturkunde der Humboldt-Universität (*), Berlin, Germany (MNB)
Polish Academy of Sciences (*), Warsaw, Poland (PAS)
Zoological Museum (*), Copenhagen, Denmark (ZMC)
Swedish Museum of Natural History (*), Stockholm, Sweden (SMNH)
Naturhistoriska Museet (*), Göteborg, Sweden (NMG)
Zoological Museum (*), Lund, Sweden (ZML)
Zoologisk Museum (*), University of Oslo, Norway (ZMO)
Zoological Museum (?), University of Bergen, Norway (ZMB)
Museum of Natural History (*), Reykjavíc, Iceland (MNHR)
Cátedra de Parasitología y Enfermadas Parasitarias (?), Córdoba Universidad, Córdoba, Spain (CPEP)
Departamento de Parasitología, Universidad de Barcelona (?), Barcelona, Spain (UB)
Institute of Biology of the Southern Seas (*), National Academy of Sciences of Ukraine, Crimea, Ukraine (IBSS)
Bulgarian Academy of Sciences (#), Sofia, Bulgaria (BAS)
Zoological Survey of India (?), Calcutta, India (ZSI)
Cestodology Laboratory, Department of Zoology, Marathwada University (?), Aurangabad, Maharashtra, India (MU)
Department of Parasitology, Guiyang Medical College (?), Guiyang, China (GMC)
Zhongshan Medical College Parasite Collection (?), Guangzhou, China (ZMCPC)
Meguro Parasitological Museum (#), Tokyo, Japan (MPM)
University of Philippines Natural Science Research Center (?), Quezon City, Phillippines (UPNSRC)
Ain Shams University (?), Cairo, Egypt (ASU)
Veterinary Research Institute (?), Onderstepoort, South Africa (VRI)
Queensland Museum (*), Brisbane, Austra-

lia (QM)
South Australian Museum (*), Adelaide, Australia (SAMA)
Museum of New Zealand (*), Wellington, New Zealand (ZW)

Collections

In order to adequately sample the global diversity and to obtain fresh specimens of diphyllideans, collections of elasmobranchs were made in the following localities (see Fig. 145): Chesapeake Bay, Maryland, U.S.A.; Core Sound, North Carolina, U.S.A.; northern Gulf of Mexico, U.S.A.; Gulf of Alaska; Gulf of California, México; Gulf of Carpenteria, Australia; New Zealand; Sea of Japan; Thailand; Sète, France; Tunisia; Madagascar.

Elasmobranchs were collected by commercial or recreational fishermen by trawling, seining, gill netting, spearing, or angling, and necropsied shortly thereafter. Each elasmobranch was dissected open with a longitudinal incision on the ventral surface, and the spiral intestine removed. Some spiral intestines were preserved in the field prior to inspection for parasites either by making an incision along the primary mesenteric artery and immersing the intestine in 10% formalin (3.7% formaldehyde), or by tying off both ends of the intestine, injecting it with 10% formalin, then immersing it in 10% formalin. Other spiral intestines were dissected open with a longitudinal incision along the primary mesenteric artery, and worms were removed in the field using forceps or curette. Worms were fixed in 10% formalin for at least 48 hours, then transferred into 70% ethanol for storage.

Specimen Preparation

Light microscopy: Selected worms were hydrated in a graded ethanol series, and stained in either Gill's or Delafield's hematoxylin for at least one hour. Worms were then dehydrated in a graded ethanol series, destained in 70% acid alcohol, cleared in methyl salicylate or xylene, and mounted in Canada balsam on glass slides. In some cases, scolices were counterstained with fast green in 95% ethanol. Hook preparations were made by hydrating scolices and mounting them in Berlese's medium on glass slides.

Serial sectioning: Selected worms were stained with fast green in 95% ethanol, dehydrated in a graded ethanol series, cleared in xylene, and embedded in Paraplast or TissuePrep (Fisher Scientific, Pittsburgh, Pennsylvania). Serial sections were cut at 6-10 µm intervals using an American Optics or Olympus CUT 4060 rotary microtome. Sections were attached to glass slides with sodium silicate, stained with Gill's or Delafield's hematoxylin and eosin, and mounted in Canada balsam.

Scanning electron microscopy: Selected worms were post-fixed overnight in 1% osmium tetroxide in distilled water, rinsed three times in distilled water, and dehydrated in a graded ethanol series. Worms were dried in hexamethyldisilazane (Ted Pella Inc., Redding California), mounted on carbon tape on aluminum stubs, sputter coated with approximately 100 Å of gold, and examined under a LEO/Zeiss DSM 982 Gemini field emission scanning electron microscope. Images were electronically captured and either photographed on Polaroid type 55, or printed on a laser printer.

Descriptions

Whole mounts and serial sections were examined using a Zeiss Axioskop, Axioskop II with DIC, or AusJena Jenaval with DIC. Light micrographs were taken with a Kodak DCS 410 digital SLR attached to one of the microscopes described above. Drawings were prepared with a drawing tube. Except where noted, all measurements are given in µm and expressed as ranges. If all type material for a species was examined, the range is followed in parentheses by the mean, standard deviation, number of worms examined (n), and number of observations (*n*) when more than one structure was measured per worm. Otherwise, only the range is presented, and is adjusted to reflect the new observations. Distribution maps were obtained using On-

line Map Creation (version 4.1) (http://www.aquarius.ifm-geomar.de/omc_intro.html) generating maps using GMT (The Generic Mapping Tools) (Wessel and Smith 1998). Elasmobranch common names follow FishBase (Froese and Pauly 2005).

SYSTEMATIC TREATMENT OF THE DIPHYLLIDEA

Diphyllidea Van Beneden *in* Carus, 1863

Diagnosis

Scolex consisting of two sessile bothria and cephalic peduncle; armed rostellum present or absent; cephalic peduncle armed or unarmed. Proglottids acraspedote. Genital pore mid-ventral, in posterior part of proglottid. Testes pre-ovarian. Cirrus sac piriform, opening midventrally into common genital pore. Vagina opening into common genital pore, posterior to cirrus. Vitellaria follicular, lateral, or circumcortical. Uterus saccate, medial, ventral. Uterine pore absent. Parasites of elasmobranchs. Cosmopolitan.

Problematic Genera

Diagonobothrium Shipley and Hornell, 1906

Diagnosis

This genus was described by Shipley and Hornell (1906, p. 58) as follows: "Head 2.3 millims. in length, about 1 millim. in breadth. There is a large terminal muscular sucker and two ear-like bothridia which run down right and left of the head. One edge of each of these bothridia runs forward obliquely, and loses itself in the crinkled membrane which surrounds the terminal sucker. There is only one edge on each side thus prolonged, and the two prolongations cross one another at about a right angle. The head is thus asymetrical. The neck is long and shows hardly any structure."

Type and only species: *Diagonobothrium assymetrum* Shipley and Hornell, 1906; in *Myliobatis maculatus* Gray; Dutch Bay, Sri Lanka.

Remarks

This genus was erected for a single incomplete specimen (scolex only) collected from *Myliobatis maculatus* in Sri Lanka, and even the authors questioned whether it was an abnormality. Shipley and Hornell (1906) did not place this genus within any order at that time, and in fact its placement was not addressed until Southwell (1925) transferred the genus into his now defunct Heterophyllidea. Having abandoned his Heterophyllidea (see Southwell 1925), Southwell (1930) left *Diagonobothrium* as a genus of uncertain systematic position. Wardle and McLeod (1952) dismissed the Heterophyllidea, and attempted to place *Diagonobothrium* within an accepted order. Failing to do so, as *Diagonobothrium* demonstrated affinities to both Lecanicephala and Tetraphyllidea, they considered this a *genus inquirendum*. Yamaguti (1959) did not discuss the genus, except to list it as a genus *incerta sedis* within the Lecanicephalidea. Joyeux and Baer (1961) ignored the genus. Neither Schmidt (1970, 1986) nor Euzet (1994a, b) addressed this genus directly. Euzet did mention, however, that *Diagonobothrium* probably represented a diphyllidean species which had lost all of its armature. This opinion was shared by Jensen (2005) who rejected inclusion of *Diagonbothrium* in the Lecanicephalidea.

The marked resemblance of *Diagonobothrium* to an *Echinobothrium* which has lost its armature, combined with the known suitability of *Myliobatis* species as hosts for *Echinobothrium*, suggests that the two genera are synonyms. However, without any type or other material available for examination, this decision cannot be made with any degree of certainty. Therefore, *Diagonobothrium* is considered here a *nomen dubium* within the Diphyllidea.

Yogeshwaria Chincholikar and Shinde, 1976

Diagnosis

Described by Chincholikar and Shinde (1976, p. 275) as follows: "Small worms, scolex with two simple, oval, sessile, bothria situated on hood-like structure, having all proglottids broader than long. Posteriorly

enlarged squarish region of the tape, having proglottization externally and not internally. Posterior proglottids increase in length and width. Parasitic in elasmobranchs."

Type and only species: *Yogeshwaria nagabhushani* Chincholikar and Shinde, 1976; in *Trygon* sp.; Ratnagiri, Maharashtra, India.

Remarks

This genus was erected by Chincholikar and Shinde (1976) and placed in the Lecanicephala as a genus *incerta sedis*. Schmidt (1986) treated it as a tetraphyllidean of doubtful status, pointing out that it was a junior synonym [sic] of *Yogeshwaria* Shinde, 1968. Euzet (1994b) treated the genus within the Lecanicephalidea, and, based on its inadequate description and uncertain identity of the host, suggested the name be suppressed. Jensen (2005) rejected inclusion of *Yogeshwaria* in the Lecanicephalidea.

Although Schmidt (1986) stated that this genus was a synonym, it is in fact a junior homonym of *Yogeshwaria* Shinde, 1968, a dilepidid cyclophyllidean. Because the genus is a junior homonym, the name must be rejected. Based on the presence of two bothria on the scolex and a dasyatid elasmobranch host, this worm, in all likelihood, represents a diphyllidean, specifically a species of *Echinobothrium* that has lost its rostellar armature. *Yogeshwaria* Chincholikar and Shinde, 1976 is therefore considered here to be a junior synonym of *Echinobothrium*.

Key to the Families of Diphyllidea Van Beneden

1(a) Scolex with apical armature.....**Echinobothriidae Perrier, 1897**
1(b) Scolex unarmed.....**Ditrachybothridiidae Schmidt, 1970**

Ditrachybothridiidae Schmidt, 1970

Diagnosis

Scolex with one dorsal and one ventral bothrium. Bothria covered with pectinate microtriches on proximal and/or distal surfaces. Weakly developed, unarmed apical organ present. Cephalic peduncle unarmed, terminating posteriorly with a velum. Strobila apolytic, cylindrical to laterally compressed. Genital pore ventral. Uterine pore absent. In North Atlantic and South Pacific Oceans. In rajid and scyliorhinid elasmobranchs.

Type and only genus: *Ditrachybothridium* Rees, 1959.

Ditrachybothridium Rees, 1959

Historical summary

Rees (1959) erected this genus for worms collected from two species of *Raja* and one species of *Scyliorhinus*. At that time, Rees (1959) was reluctant to place the genus into the Diphyllidea, citing the questionable status of that order (see Southwell, 1925; Wardle and McLeod, 1952). However, she did state that, regardless of the higher classification, *Ditrachybothridium* was closely related to *Echinobothrium*. Schmidt (1970) was not so deferential to Southwell or to Wardle and McLeod, and erected the family Ditrachybothridiidae Schmidt, 1970 for *Ditrachybothridium*, within Diphyllidea. Wardle *et al.* (1974) rejected Diphyllidea Van Beneden (see above) and suggested that both *Ditrachybothridium* and *Echinobothrium* be placed in the Lecanicephalidea. This classification has been largely ignored, however. Both Schmidt (1986) and Khalil (1994) retained *Ditrachybothridium*

in Ditrachybothridiidae in the Diphyllidea. A second species of *Ditrachybothridium* was described by Faliex *et al.* (2000), necessitating a slight revision of the diagnoses of both family and genus.

Diagnosis

With the characteristics of the family.

Type species: *Ditrachybothridium macrocephalum* Rees, 1959; in *Raja fullonica* L.; St. Kilda, Scotland.

Additional species: *Ditrachybothridium piliformis* Faliex, Tyler, and Euzet, 2000; in *Galeus* sp.; Vanuatu.

Key to the Species of *Ditrachybothridium*

1(a) Bothria with spines (Fig. 4) and microtriches on proximal surfaces ..... ***D. macrocephalum***

1(b) Bothria with microtriches only ..... ***D. piliformis***

Ditrachybothridium macrocephalum Rees, 1959

(Figs. 17-21)

Type host: *Raja fullonica* L., Shagreen ray (Rajidae, Rajiformes).

Additional hosts: *Raja circularis* Couch, Sandy ray (Rajidae, Rajiformes), *Scyliorhinus caniculus* (L.), Small-spotted catshark (Syliorhinidae, Carcharhiniformes), *Galeus melastomus* Rafinesque, Blackmouth catchark (Syliorhinidae, Carcharhiniformes), *Apristurus laurussonii* (Saemundsson), Iceland catchark (Syliorhinidae, Carcharhiniformes) (plerocercus), Rajidae - possibly *Rajella bigelowi* (Stehman), Bigelow's ray (plerocercus).

Site of infection: Spiral intestine.

Type locality: Near St. Kilda, Scotland 57˚50'N, 9˚15'W and 57˚50'N, 9˚00'W.

Additional localities: northern North Sea; Goban Spur (49˚47'N, 11˚58'W), northeastern Atlantic; Porcupine Seabight, 51˚09'N, 11˚55'W), northeastern Atlantic.

Type material: BMNH No. 1959.8.4.193-196 (holotype and paratypes).

Voucher specimens: BMNH Nos. 1973.6.11.11-13, 1976.4.13.39-40, 1976.4.13.41, 2004.1.-6.1-5, and 2004.1.6.6-11.

Specimens examined: Two paratypes (BMNH Nos. 1959.8.4.193-196); two vouchers (BMNH Nos. 1973.6.11.11-13 and 1976.4.13.39-40).

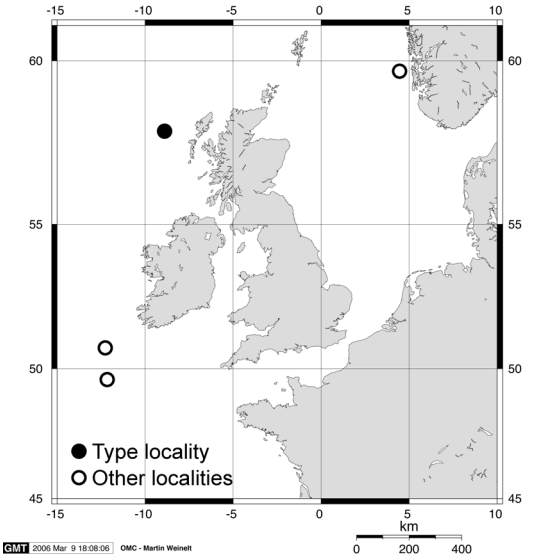


Fig. 17. Distribution of *Ditrachybothridium macrocephalum*.

Etymology: Not given.

Description (Modified from Rees [1959].)

Worms 31.5-56.06 mm long, up to 1.68 mm wide at terminal proglottid. Strobila apolytic, acraspedote, 49-59 proglottids, covered with long filitriches. Mature proglottids

2.28-4.35 mm long, 750-1,240 wide; gravid proglottids 4.33-5.44 mm long, 1.31-1.68 mm wide. Scolex bipartite, 1.29-1.43 mm long, consisting of scolex proper and short cephalic peduncle. Scolex proper 1.25-1.34 mm long, 414-480 wide, consisting of one dorsal and one ventral bothrium. Bothria 820-1,090 long, 414-480 wide, proximal surfaces armed with large spines and covered with long filitriches, with cleft at posterior margin. Distal bothrial surfaces with bifid and pectinate spinitriches and long filitriches. Cephalic peduncle short, unarmed, with small velum at posterior terminus, covered with long filitriches.

Testes 52-62 in number, anterior to cirrus sac, in two irregular columns, 1-2 layers deep. Vas deferens extensive, extending posterior to cirrus sac. Cirrus sac piriform, 260-320 long, 183-213 wide. Ovary bilobed, 345-530 long, up to 530 wide, H-shaped in dorsoventral view. Vagina thick-walled, muscular, posterior to genital pore, relatively uniform in width, undulating slightly and making a single coil at posterior terminus. Genital pore midventral, 22-26% of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, filling entire proglottid when fully gravid. Vitellaria follicular, lateral, extending to anterior margin of ovary. Egg shape not determined (collapsed in whole mounts). Excretory ducts lateral.

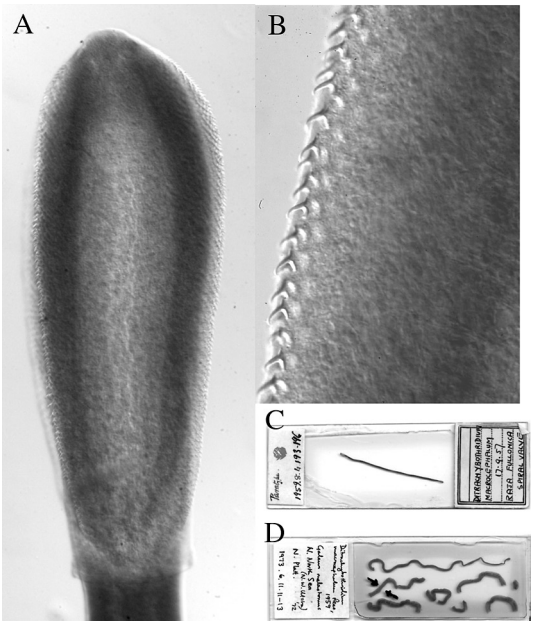


Fig. 18. Light micrographs of *Ditrachybothridium macrocephalum*. A. Scolex. B. Detail of spines on proximal bothrial surface. C. Paratype slide BMNH No. 1959.8.4.196. D. Voucher slide BMNH No. 1973.6.11.11-13.

Remarks

The presence of spines on the proximal bothrial surfaces in this species is sufficient to distinguish it from the only other species in the genus.

This species, described by Rees (1959), is the type species of the genus, which, at that time, was placed only tentatively into the order Diphyllidea. Rees' difficulty in classifying this species was due to the presence of a highly unusual unarmed scolex, unarmed cephalic peduncle, and several aspects of the genitalia. In particular, Rees (1959) noted that the vitelline follicles were distributed circumcortically, rather than in two lateral fields. However, observations on more mature specimens by Faliex *et al.* (2000) revealed that the vitelline follicles are in fact arranged in two lateral fields. Nevertheless, this species is unique within the genus (and in the order) in that the proximal bothrial surfaces are armed with spines. *Ditrachybothridium macrocephalum* was originally described from only 12 specimens (2 large, 10 small), none of which had fully mature proglottids. This led Faliex *et al.* (2000) to suggest that *Raja fullonica*, *Raja circularis,* and *Scyliorhinus caniculus* are all unsuitable hosts for this species, and that perhaps a deeper-water catshark is the normal definitive host, based on their discovery of a new species of *Ditrachybothridium* in a deep-water catshark (see below). Further evidence in support of this hypothesis comes from examination of a fully mature and gravid specimen of this species taken from *Galeus melastomus* in the North Sea. Because the scoleces on the worms from both the type and voucher specimens were identical, it is believed that these two specimens represent the same species. This hypothesis was further supported by the discovery of plerocercus larvae of *D. macroceph-*

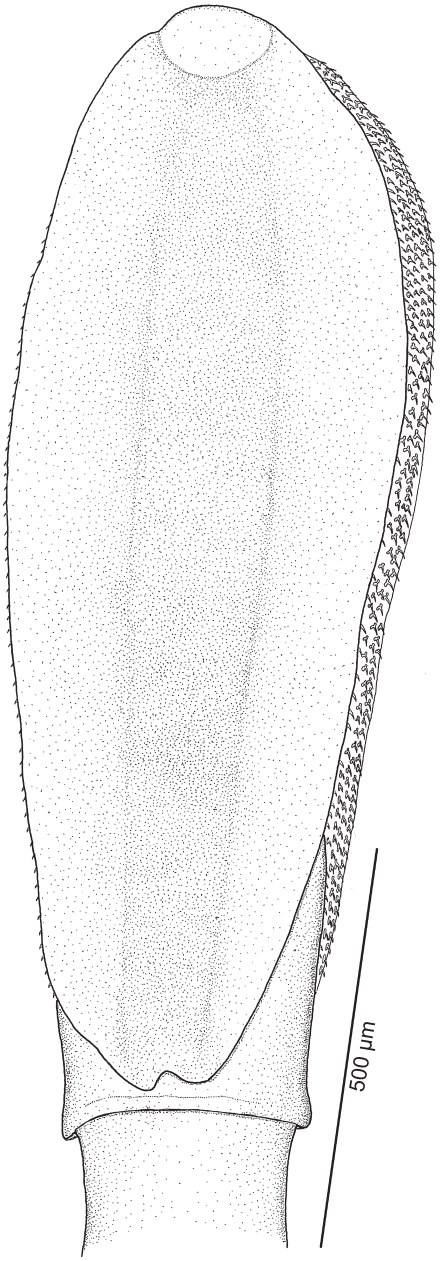


Fig. 19. Line drawing scolex of *Ditrachybothridium macrocephalum*.

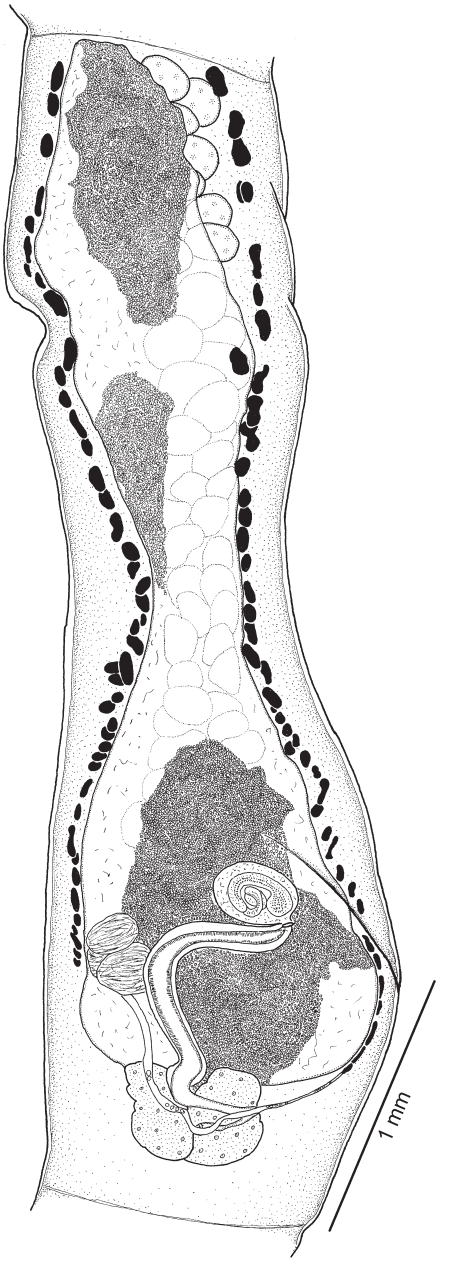


Fig. 20. Line drawing of proglottid of *Ditrachybothridium macrocephalum*.

alum from the deepwater shark *Apristurus laurussonii* and a deepwater skate by Bray and Olson (2004). Therefore, the description of the reproductive anatomy above is based primarily on this material (BMNH No. 1973.6.11.11-13).

Ditrachybothridium macrocephalum was included in a phylogenetic analysis of the Diphyllidea published by Ivanov and Hoberg (1999), and as an outgroup in the phylogenetic analyses of Caira *et al.* (1999; 2001), who examined these same specimens. In both

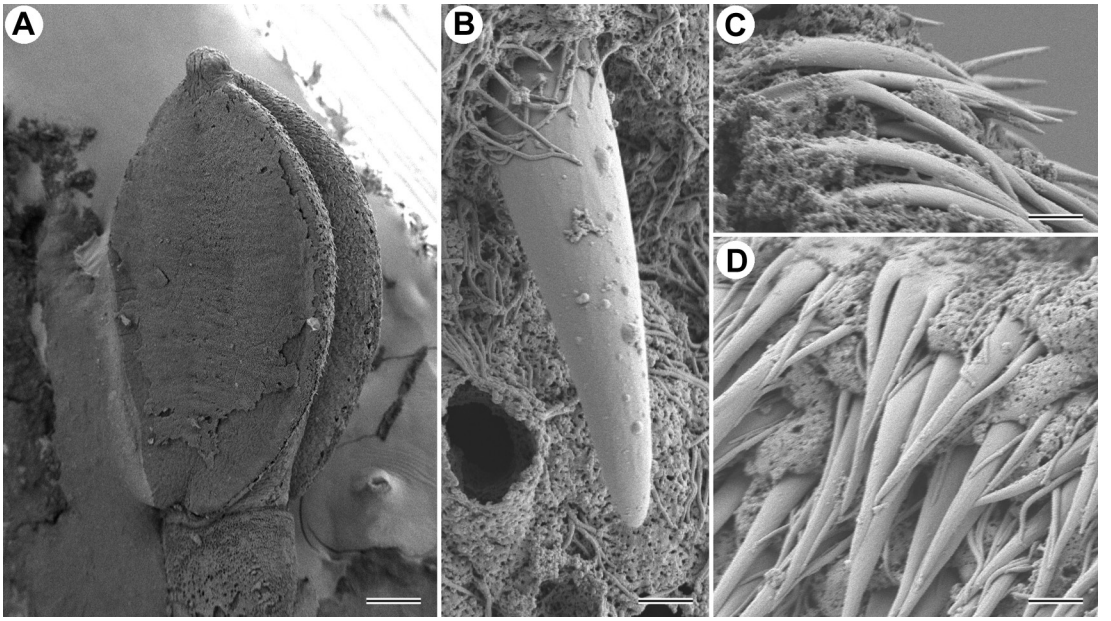


Fig. 21. Scanning electron micrographs of *Ditrachybothridium macrocephalum*. A. Scolex. B. Spine on proximal bothrial surface. C. Distal bothrial surface showing bifid spinitriches. D. Distal bothrial surface showing pectinate spinitriches. Scale bars: A, 100 µm; B-D, 1 µm.

cases, *D. macrocephalum* was basal in position among the Diphyllidea.

Ditrachybothridium piliformis Faliex, Tyler, and Euzet, 2000

(Figs. 22-24)

Type host: *Galeus* sp. (Scyliorhinidae, Carcharhiniformes).

Additional hosts: *Apristurus* sp. (Scyliorhinidae, Carcharhiniformes).

Site of infection: Spiral intestine.

Type locality: South Pacific Ocean near Vanuatu Island (15˚57'S, 166˚38'E).

Additional localities: Sandy Cape, Tasmania, Australia (41˚24'S, 144˚48'E).

Type material: MNHN No. 820 HF 69 C IX (holotype); MNHN Nos. 820 HF 70 C IX and 820 HF 71 C IX, BMNH No. 1999.10.6.1-2, USNPC No. 89166, and HWML No. 15150 (paratypes).

Voucher specimens: Eleven specimens on nine slides (SAMA No. S17638); one egg mount (SAMA No. AHC28402).

Specimens examined: Holotype (MNHN No. 820 HF 69 C IX); paratypes (MNHN Nos. 820 HF 70 C IX and 820 HF 71 C IX, BMNH No. 1999.10.6.1-2, USNPC No. 89166, and HWML No. 15150); 11 voucher specimens on nine slides from Tasmania (SAMA No. S17638, 17644); one egg mount, SAMA No. AHC28402.

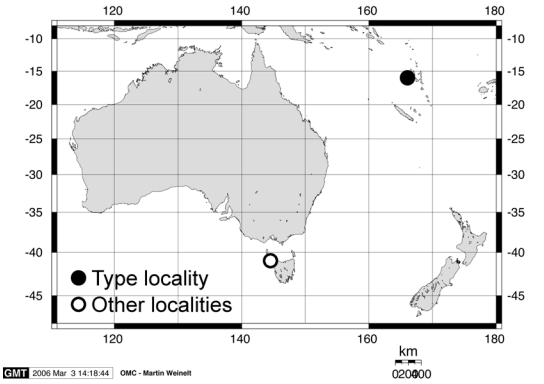


Fig. 22. Distribution of *Ditrachybothridium piliformis*.

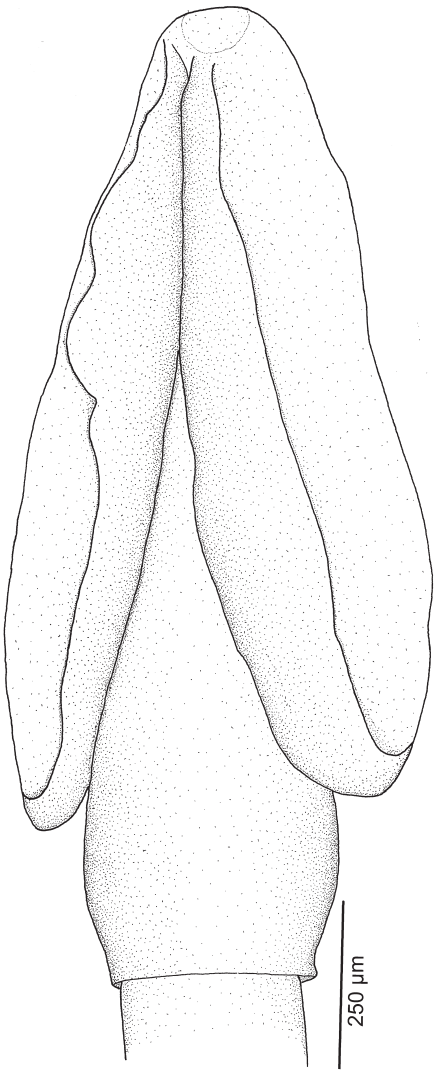


Fig. 23 Line drawing of scolex of *Ditrachybothridium piliformis*.

Description

Worms 11.3-95.6 mm long, 500-750 wide at terminal proglottid. Strobila anapolytic, acraspedote, with 18-97 proglottids, covered with long filitriches. Scolex 1.250-2.580 mm long, 550-1,610 (720 ± 200) wide, consisting of scolex proper and short cephalic peduncle. Scolex proper consisting of one dorsal and one ventral bothrium, weakly defined apical organ. Proximal bothrial surfaces with spatulate spinitriches and long filitriches, distal surfaces with pectinate spinitriches and long filitriches. Cephalic peduncle unarmed, 100-1,420 long, 260-500 wide, with small velum at posterior terminus, covered with pectinate spinitriches and long filitriches.

Testes 43-81 (54 ± 8) in number, overlapping cirrus sac, arranged in two irregular columns, one layer deep. Vas deferens extensive, extending posterior to cirrus sac. Cirrus sac piriform, 205-300 long, 150-200 wide. Ovary bilobed, 250-535 long, 245-300 wide, H-shaped in dorso-ventral view. Vagina thick-walled, muscular, posterior to genital pore, expanded distally, undulating slightly and coiled at posterior terminus. Genital pore midventral, 19-34% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, filling entire proglottid when fully gravid. Vitellaria follicular, lateral, extending from level of ovary to anterior margin of proglottid. Eggs oval, 60-88 long, 23-40 wide, with mucron at one end, not packaged. Excretory ducts lateral.

Remarks

This species, described by Faliex *et al.* (2000), is the second species assigned to this genus since its establishment in 1959 by Rees. It differs conspicuously from the type species in lacking spines on the proximal bothrial surfaces. Like *D. macrocephalum*, this species is found in at least two species of hosts, a phenomenon not typical of diphyllideans in general. Both host species for this parasite are catsharks (Scyliorhinidae), as is the host in which the most mature specimens of *D. macrocephalum* were found.

Echinobothriidae Perrier, 1897

Diagnosis

Scolex with one dorsal and one ventral bothrium, and armed rostellum. Bothria covered with pectinate microtriches on proximal and/or distal surfaces. Rostellum with one dorsal and one ventral group of hooks. Lateral hooklets present or absent between each dorso-ventral group of hooks. Cephalic peduncle unarmed or armed with eight columns of posteriorly directed spines, generally with triradiate bases. Strobila apolytic or euapo-

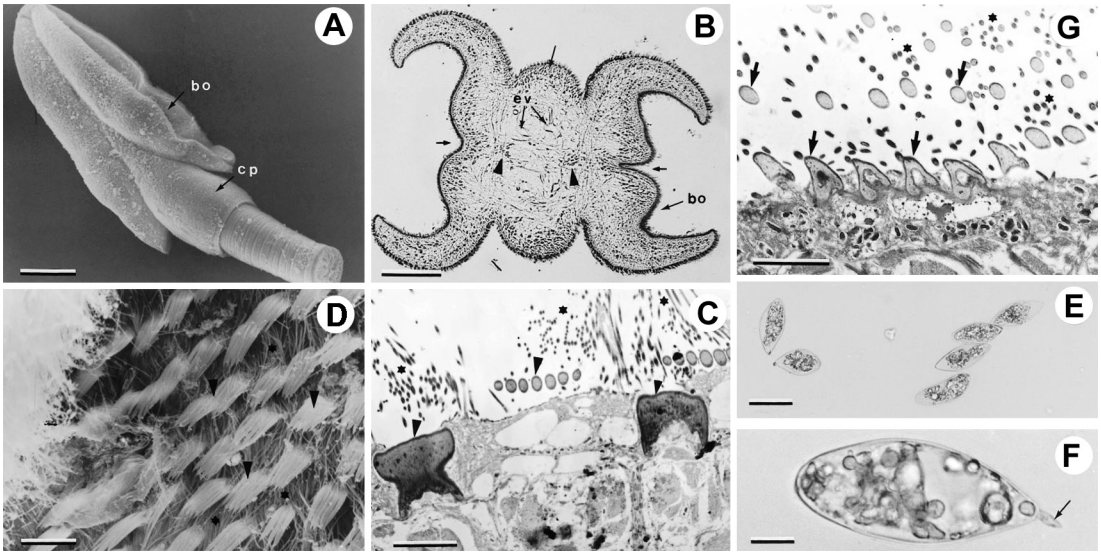


Fig. 24. Micrographs of *Ditrachybothridium piliformis*. A. Scolex. B. Cross section through scolex. C. TEM micrograph of section through scolex showing pectinate spinitriches (arrow) and filitriches (*). D. Distal bothrial surface. E. Eggs. F. Egg showing mucron (arrow). G. TEM micrograph of section through scolex showing spatulate spinitriches (arrow) and filitriches (*). Abbreviations: BO, bothrium; CP, cephalic peduncle; EV, excretory vessel. Scale bars: A, 215 µm; B, 100 µm; C, 0.25 µm; D, 6 µm; E, 5 µm; F, 1.25 µm; G, 15 µm. Modified from Faliex *et al.* (2000).

lytic. Genital pore ventral. Uterine pore absent. Eggs unembryonated. Cosmopolitan.

Type and only genus: *Echinobothrium* Van Beneden, 1849.

Echinobothrium Van Beneden, 1849

Synonyms: *Yogeshwaria* Chincholikar and Shinde, 1976 **n. syn.** (also a junior homonym of *Yogeshwaria* Shinde, 1968); *Macrobothridium* Khalil and Abdul-Salam 1989 **n. syn.**

Historical summary

The genus *Echinobothrium* was erected by Van Beneden (1849) for a peculiar form of worm that he found in a skate ("raie bouclée"). At that time, Van Beneden placed this new genus in the family Botrioïdes in the section Acanthocephales (bothridiate armed worms), along with several other genera now considered to belong in the Tetraphyllidea and Trypanorhyncha. When Van Beneden (1850) reclassified the cestodes, he placed *Echinobothrium* into the section Diphyllés. Diesing (1854) derived his own classification scheme, placing *Echinobothrium* into the subtribe Anaegocheila, of the tribe Gamoarhynchobothria, in the section Paramecocotylea. In 1858, Van Beneden reclassified the Cestoïdes once again, but retained *Echinobothrium* in the Diphyllés. Diesing (1863) subsequently placed it in the family Dibothria (subtribe Atrypanorhyncha, tribe Paramecocotlylea Aprocta, section Paramecocotylea, order Cephalocotylea), apparently ignoring both of Van Beneden's earlier classifications. In the same year, Van Beneden (*in* Carus 1863) erected the family Diphyllidea for *Echinobothrium*, which by that time included two species. Perrier (1878) was apparently unaware of the classification of Carus (1863), and placed *Echinobothrium* in the Bothriadés of Van Beneden (1858) along with several genera now considered to be pseudophyllideans.

In 1889, Pintner revised the genus, adding two species and providing a key to all four species of *Echinobothrium* known from adult

specimens. Later, Perrier (1897) erected the family Echinobothriidae Perrier, 1897, for *Echinobothrium* and the trypanorhynch genus *Hepatoxylon*, and again ignored Carus' (1863) classification, placing Echinobothriidae and *Echinobothrium* into the order Trypanorhyncha. However, the diagnosis Perrier (1897, p. 1848) provided for *Echinobothrium* was in error, defining *Echinobothrium* as possessing a "scolex avec deux bothridies et deux tentacules spinifères; cou armé de piquants." It seems that Perrier (1897) may not have examined any specimens of *Echinobothrium* or even the illustrations of Van Beneden (1849, 1850, 1858, 1871), Wagener (1854), or Pintner (1889). Stossich (1898) placed *Echinobothrium* in the family Echinobothridae [sic], but, because he did not give an authority citation for the family, it is unclear whether he erected this family or was referring to Echinobothriidae Perrier, 1897. Regardless, Stossich's (1898) concept of the family was different from that of Perrier (1897); Stossich described the family as possessing a large scolex with two opposed, unarmed dorso-ventral bothridia with a small armed terminal rostellum. Braun (1894-1900) proposed the classification that most closely resembles the classification widely accepted today. He retained *Echinobothrium* in Diphyllidea, revised the family Echinobothriidae, removing *Hepatoxylon*, and provided an accurate diagnosis of the family. Mola (1921) proposed yet another classification in which he did not recognize Echinobothriidae Perrier, 1897 and placed *Echinobothrium* in the newly erected family Dibothriacantidae Mola, 1921 (subfamily Echinobothrinae Mola, 1921). Meggitt (1924) claimed to have compiled the only complete list of tapeworm genera since Braun (1894-1900), but made no mention of *Echinobothrium* or Echinobothriidae. Southwell (1925) did not recognize the order Diphyllidea (see above) and placed *Echinobothrium* into his new order Heterophyllidea Southwell, 1925 along with several other genera now considered to belong in Proteocephalidea and Tetraphyllidea. Poche (1926) proposed a number of new orders including the order Echinobothriidea Poche, 1926 for *Echinobothrium*. Pintner (1928) did not mention the work of Poche (1926) and retained *Echinobothrium* in Echinobothriidae. In 1929, Mola revised his 1921 classification of the tapeworms, but did not change the position of *Echinobothrium*. Southwell (1930) abandoned his order Heterophyllidea and considered *Echinobothrium* a genus of uncertain position, and left it out of his classification.

Since 1930, the Braun classification scheme has been followed by most authors (Joyeux and Baer 1936; Euzet 1951; Euzet 1959; Yamaguti 1959; Schmidt 1970; Joyeux and Baer 1961; Schmidt 1986; Khalil *et al.* 1994). Wardle and McLeod (1952) however, did not recognize the order Diphyllidea and placed *Echinobothrium* in their new order Lecanicephala as a *genus inquirendum*. Riser (1955) retained *Echinobothrium* in Echinobothriidae, but placed the family in the order Tetraphyllidea in the superfamily Lecanicephaloidea (see above). Wardle *et al.* (1974) placed *Echinobothrium* tentatively in the Lecanicephalidea, following Wardle and McLeod (1952). Several keys to the species of *Echinobothrium* have been published, starting with Pintner (1889). Euzet (1951) provided a key which was later revised and updated by Rees (1961b). Probert and Stobart (1989) provided a key to the species in the genus, but, as noted by Campbell and Andrade (1997), omitted several species. The most recent and comprehensive key to date was provided by Ivanov and Campbell (1998a).

Based on the data of the phylogenetic analyses presented in this study, *Macrobothrium* is considered to be a synonym of *Echinobothrium*. Consequently, the species of *Macrobothrium* are transferred to *Echinobothrium* creating the following new combinations: *Echinobothrium euterpes* (Neifar, Tyler, and Euzet, 2001) **n. comb.,** *Echinobothrium rhynchobati* (Khalil and Abdul-Salam, 1989) **n. comb.**, and *Echinobothrium syrtensis* (Neifar, Tyler, and Euzet, 2001) **n. comb.**

Diagnosis

With the characteristics of the family.

List of Species of *Echinobothrium*

Problematic species:

Echinobothrium boisii Southwell, 1911; in *Aetobatis* [sic] *narinari* (Euphrasen); Portugal Bay, Sri Lanka.

Echinobothrium lateroporum Subhapradha, 1948; in *Mustelus manazo* Bleeker; Waltair coast, India.

Echinobothrium levicolle Lespés, 1857; in *Nassa reticulata* L.; Atlantic coast, France.

Echinobothrium nagabhushani (Chincholikar and Shinde, 1976) **n. comb.**; in *Trygon* sp.; Ratnagiri, Maharashtra, India.

Echinobothrium rhinoptera Shipley and Hornell, 1906; in *Rhinoptera javanica* Müller and Henle; Dutch Bay, Sri Lanka.

Echinobothrium scoliodoni Sanaka, Lakshmi, and Rao, 1986; in *Chiloscyllium indicum* (Gmelin); Waltair coast, India.

Type species:

Echinobothrium typus Van Beneden, 1849; in *Raja clavata* L.; Belgian coast.

Additional valid species:

Echinobothrium acanthinophyllum Rees, 1961; in *Raja montagui* Fowler; Plymouth, England.

Echinobothrium acanthocolle Wojciechowska, 1991; in *Raja georgiana* Norman; South Georgia Island.

Echinobothrium affine Diesing, 1863; in *Raja radula* Delaroche; Nice, France.

Echinobothrium benedeni Ruszkowski, 1927; in *Raja asterias* Delaroche; Roscoff, France.

Echinobothrium bonasum Williams and Campbell, 1980; in *Rhinoptera bonasus* (Mitchill); Sakonnet Point, Rhode Island, U.S.A.

Echinobothrium brachysoma Pintner, 1889; in "Rochenarten" (skates); Trieste, Italy.

Echinobothrium californiense Ivanov and Campbell, 1998; in *Platyrhinoidis triseriata* (Jordan and Gilbert); Newport Beach, California, U.S.A.

Echinobothrium chisholmae Jones and Beveridge, 2001; in *Rhinobatos typus* Bennett; Heron Island, Queensland, Australia.

Echinobothrium clavatum Probert and Stobart, 1989; in *Raja clavata* L.; Irish Sea.

Echinobothrium coenoformum Alexander, 1963; in *Dipturus nasuta* (Müller and Henle); Cook Strait, New Zealand.

Echinobothrium coronatum Robinson, 1959; in *Mustelus lenticulatus* Phillipps; Wellington Harbor, New Zealand.

Echinobothrium deeghai Gupta and Parmar, 1988; in *Pastinachus sephen* (Forsskål); Deegha, West Bengal, India.

Echinobothrium elegans Tyler, **n. sp.**; in *Taeniura lymma* (Forsskål); Gulf of Carpenteria, Northern Territory, Australia.

Echinobothrium euterpes (Neifar, Tyler, and Euzet, 2001); in *Rhinobatos rhinobatos* (L.); Zarzis, Tunisia.

Echinobothrium euzeti Campbell and Carvajal, 1980; in *Sympterygia lima* (Poeppig); Constitución, Chile.

Echinobothrium fautleyae Tyler and Caira, 1999; in *Rhinoptera steindachneri* Evermann and Jenkins; Puertecitos, México.

Echinobothrium harfordi McVicar, 1976; in *Raja naevus* Müller and Henle; Aberdeen, Scotland.

Echinobothrium helmymohamedi Saoud, Ramadan, and Hassan, 1982; in *Taeniura lymma* (Forsskål); Al Ghardaga, Red Sea, Egypt.

Echinobothrium heroniense Williams, 1964; in *Taeniura lymma* (Forsskål); Heron Island, Queensland, Australia.

Echinobothrium hoffmanorum Tyler, 2001; in *Urobatis maculatus* Garman; Isla San Esteban, México.

Echinobothrium longicolle Southwell, 1925; in *Dasyatis kuhlii* (Müller and Henle); Portugal Bay, Sri Lanka.

Echinobothrium mathiasi Euzet, 1951; in *Myliobatis aquila* (L.); Sète, France.

Echinobothrium megacanthum Ivanov and Campbell, 1998; in *Myliobatis goodei* Garman; San Matías Gulf, Argentina.

Echinobothrium mexicanum Tyler and Caira, 1999; in *Myliobatis longirostris* Applegate and Fitch; Bahía de Los Angeles, México.

Echinobothrium musteli Pintner, 1889; in *Mustelus mustelus* (L.); Trieste, Italy.

Echinobothrium notoguidoi Ivanov, 1997; in *Mustelus schmitti* Springer; Mar del

Plata, Argentina.

Echinobothrium pigmentatum Ostrowski de Núñez, 1971; in *Zapteryx brevirostris* (Müller and Henle); Mar del Plata, Argentina.

Echinobothrium raji Heller, 1949; in *Raja scabrata* Garman; Quebec, Canada.

Echinobothrium raschii Campbell and Andrade, 1997; in *Rhinoraja longi* Raschi and McEachran; Bering Sea.

Echinobothrium rayallemangi Tyler, 2001; in *Rhinobatos leucorhynchus* Günter; Bahía de Los Angeles, México.

Echinobothrium reesae Ramadevi, 1969; in *Himantura walga* (Müller and Henle); Waltair coast, India.

Echinobothrium rhynchobati (Khalil and Abdul-Salam, 1989); in *Rhinobatos granulatus* Cuvier; Kuwait City, Kuwait.

Echinobothrium syrtensis (Neifar, Tyler, and Euzet, 2001); in *Rhinobatos cemiculus* Geoffroy Saint-Hilaire; Djerba, Tunisia.

Problematic Species

Included here are several species which for various reasons were not considered valid. For the most part, these were species whose descriptions were not adequate to allow unambiguous identification of the species.

Echinobothrium boisii Southwell, 1911

Type host: *Aetobatis* [sic] *narinari* (Euphrasen), Spotted eagle ray (Myliobatidae, Myliobatiformes).

Status: *Species inquirenda.*

Site of infection: Spiral intestine.

Type locality: Portugal Bay, Sri Lanka.

Type material: None designated, but possibly in the National Museum of Natural History, Colombo, Sri Lanka.

Specimens examined: None.

Etymology: Named in honor of Sir Stanley Bois.

Description (Modified from Southwell [1911].)

Worm 10 mm long, 775 wide at terminal proglottid. Strobila acraspedote. No mature or gravid proglottids observed. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 2 mm long, 1.3 mm wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Hook formula unknown, figure shows 9-12 apical hooks in each group, with one large lateral hooklet on each side of each central apical group. Cephalic peduncle armed with eight longitudinal columns of 24 spines possessing triradiate bases.

Remarks

Southwell (1911) described this species from a single specimen he collected from *Aetobatis* [sic] *narinari*. Later, Southwell (1925) synonymized this species with *E. typus*. Neither Euzet (1951) nor Rees (1961b) included this species in their keys. Yamaguti (1959) and Schmidt (1986) both included this species in their lists of *Echinobothrium* species. Probert and Stobart (1989) neither mentioned nor included this species in their key. Campbell and Andrade (1997) considered it a *species inquirenda*; Ivanov and Campbell (1998a) concurred, omitting it from their key. This species was among the valid species listed by Tyler and Caira (1999).

The original description of this species, based on a single immature specimen, lacked any information on the reproductive anatomy. Data on other diagnostic characteristics, such as number of apical hooks or number of cephalic peduncle spines in each column, were also either missing or confused. Although Southwell (1925) considered this species to be a junior synonym of *E. typus*, there appear to be morphological differences between *E. boisii* and *E. typus*. In fact, the description of *E. boisii* does not match any of the descriptions provided for *E. typus* by Van Beneden (1849, 1858) or Pintner (1889). Most notably, Southwell (1911) figured *E. boisii* as possessing at least 9-12 apical hooks in each dorsoventral group, whereas Van Beneden (1849) described *E. typus* as possessing nine apical hooks in each group. Van Beneden (1858) later figured *E. typus* with either 9, 11, or 15

hooks in each group, but the revision of *E. typus* by Pintner (1889) established the hook number at seven per group. Therefore, the synonymy of *E. boisii* with *E. typus* should not stand, and this species should remain a *species inquirenda* until such time as either the type specimen, or other material, can be examined. This is but one of many species described from India and Sri Lanka which require redescription.

Echinobothrium lateroporum Subhapradha, 1948

Type host: *Mustelus manazo* Bleeker, Starspotted smoothhound (Triakidae, Carcharhiniformes).
Status: *Nomen nudum.*
Site of infection: Spiral intestine.
Type locality: Waltair coast, India.
Type material: None designated.
Specimens examined: None.
Etymology: The name for this species was derived from the alleged lateral position of the genital pore.

Remarks

The description of this species appeared in an unpublished thesis (Subhapradha 1948; University of Madras), but did not appear in Subhapradha's (1955) published work. All the above information on this species comes from Anantaraman (1963). Although references to this species appear in Anantaraman (1963) and Dollfus (1964), the validity of this species was not addressed until Campbell and Andrade (1997) considered it a *species inquirenda*. Tyler and Caira (1999) pointed out that this species name was not validly published.

The identity of the type host is questionable, given the type locality. Compagno (1984) notes that specimens from the waters around India reported to be *M. manazo* have mostly been correctly identified as *Mustelus mosis* Hemprich and Ehrenberg. A few specimens of *Echinobothrium* were collected from *M. manazo* in Tokyo Bay during this study, but all appear to represent *Echinobothrium musteli*. Because the name *Echinobothrium lateroporum* has not yet been validly published, it is considered here to be a *nomen nudum.*

Echinobothrium levicolle Lespés, 1857

Type host: *Nassa reticulata* L., Netted dog whelk (Gastropoda, Mollusca).
Status: *Species inquirenda.*
Site of infection: Hepatopancreas.
Type locality: Atlantic coast of France.
Type material: None designated.
Specimens examined: None.
Etymology: None given.

Description (Modified from Lespés [1857].)

Plerocercoid 1-3 long. Scolex with central apical armature present. Armature consists of more than 20 apical hooks in each group. Cephalic peduncle armature not yet developed.

Remarks

This species was described in 1857 by Lespés from larval forms taken from the mollusc *Nassa reticulata*. Pintner (1889) considered the possibility that this species could be the larval stage of *Echinobothrium musteli*, especially after finding several specimens of *N. reticulata* in the gut of some of his infected elasmobranchs. However, he did not feel he had sufficient evidence to make that decision. Southwell (1925, p. 349) considered the species "doubtful," stating "it is clear that this larval form cannot rank as a species" (p. 349). Joyeux and Baer (1936) considered *E. levicolle* to be the larva of *E. musteli*, implicitly synonymizing the two species. If this is the case however, *E. musteli* would be the junior synonym. Schmidt (1986) listed this species as a valid species, but Probert and Stobart (1989) did not include this species in their list or key. Campbell and Andrade (1997) considered this species a *species inquirenda*. Tyler and Caira (1999) considered this a *species inquirenda*, an opinion maintained in the present work.

Echinobothrium nagabhushani (Chincholikar and Shinde, 1976) n. comb.

Synonym: *Yogeshwaria nagabhushani* Chincholikar and Shinde, 1976 **n. syn.**
Type host: *Trygon* sp. (Dasyatidae, Myliobatiformes)
Status: *Species inquirenda.*
Site of infection: Spiral intestine.
Type locality: Ratnagiri, Maharashtra, India.
Type material: None designated.
Specimens examined: None.
Etymology: Named in honor of Dr. R. Nagabhushanam, Marathwada University.

Description (Modified from Chincholikar and Shinde [1976].)

This species was described by Chincholikar and Shinde (1976) as follows (p. 274): "The scolex consists of two well distinct, oval bothria. It measures 0.268 in length and 0.242 in width whereas bothria measure 0.268 in length and 0.20 in width. Following the scolex is present a hood-like structure, consisting of numerous broader proglottids. The proglottids measure 0.024 in length and 0.22 in breadth. Below this is present an enlarged squarish part in which the lateral proglottization is well marked whereas the inner proglottization is inconspicuous. This part consists of about 24-26 proglottids. The proglottids, posterior to this region, are very numerous but no mature and gravid proglottids are seen. The posterior proglottids gradually increase in length and breadth upto [sic] some extent measuring 0.057 in length and 0.332 in breadth whereas the posterior most proglottids increase in length rapidly and in breadth very slow. These proglottids measure 0.15 to 0.18 in length and 0.33 to 0.37 in width respectively."

Remarks

This species was originally placed into the new genus *Yogeshwaria* Chincholikar and Shinde, 1976. That name however, was a junior homonym of *Yogeshwaria* Shinde, 1968, and is therefore rejected (see above), and synonymized with *Echinobothrium*. Chincholikar and Shinde (1976) did not provide adequate information to allow *E. nagabhushani* n. comb. to be distinguished from other species in the genus, and there were no type specimens designated. Therefore, *Echinobothrium nagabhushani* n. comb. is considered to be a *species inquirenda*.

Echinobothrium rhinoptera Shipley and Hornell, 1906

Type host: *Rhinoptera javanica* Müller and Henle, Javanese cownose ray (Rhinopteridae, Myliobatiformes).
Status: *Species inquirenda.*
Site of infection: Spiral intestine.
Type locality: Dutch Bay, Sri Lanka.
Type material: None designated.
Specimens examined: None.
Etymology: The specific epithet was presumably derived from the genus name of the host.

Description (Modified from Shipley and Hornell [1906].)

Worms about 3 mm long, 2 mm wide at terminal proglottid. Strobila acraspedote, eight proglottids. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper approximately 2 mm long, unarmed, with one dorsal and one ventral bothrium. Cephalic peduncle approximately 5 mm long, with eight longitudinal columns of 12-13 spines. Spines with triradiate bases. Cirrus armed with recurved "spines."

Remarks

This species was poorly described and illustrated, and has not been reported since its original description. Although Southwell (1925) believed the lack of scolex armature to have been the result of the hooks having fallen off, he considered this species to be valid. Several authors used the partially armed cephalic peduncle as an identifying character in their keys (Euzet, 1951; Rees, 1961b; Probert and Stobart, 1989). Schmidt (1986) listed this species among the valid species of *Echinobothrium*, an opinion not shared by Campbell and Andrade (1997) or Ivanov and Campbell (1998a), who also excluded the spe-

cies from their key. Khalil (1994) did not specifically address the species. Tyler and Caira (1999) considered this a *species inquirenda*.

It is unclear how many specimens Shipley and Hornell (1906) examined in preparing the description of this species. The authors alternately referred to "the specimens" and "our specimen." They described this species as having an unarmed scolex and a "neck" between the scolex proper and the cephalic peduncle. Based on the original illustrations, it appears more likely that the armature in these two regions was lost during removal from the intestine of the host. Because no type or other specimens were available for study, and because the morphology of this species, as described, departs greatly from that generally accepted for *Echinobothrium*, it remains a *species inquirenda.*

Echinobothrium scoliodoni Sanaka, Lakshmi, and Rao, 1986

Type host: *Chiloscyllium indicum* (Gmelin), Slender bamboo shark (Hemiscylliidae, Orectolobiformes).
Status: *Species inquirenda.*
Site of infection: Spiral intestine.
Type locality: Waltair coast, India.
Type material: None designated.
Specimens examined: None.
Etymology: Not given.

Description (Modified from Sanaka *et al.* [1986].)

Worms 20-25 mm or 35-40 mm long. Strobila consists of 40-50 proglottids. Scolex described as follows (Sanaka *et al.* 1986, pp. 53, 56): "The scolex is having a well developed rostellum. In the terminal region of the rostellum a heart shaped pad-like structure is present with a narrow groove in the anterior region. Three types of hooks can be seen on the rostellum. 10-13 pairs of large hooks are arranged on either margin of the pad in 2 groups. 10-12 pairs of medium sized hooks are seen on either side of the groove arranged in two groups. The rest of the pad and the rostellar sac are embedded by small hooks."

Cephalic peduncle armed, described as follows (Sanaka *et al.* 1986, p. 56): "The cephalic peduncle where it attaches to the scolex bears 2 or 3 tiers of large spines. Each row bears 10-14 spines. Following this, several spines are arranged in 8 longitudinal rows which are smaller than the above spines." Testes 10-14 in number, oval to round in shape. Vas deferens entering cirrus sac anteriorly. Cirrus sac piriform, midventral. Ovary bilobed, U-shaped in dorsal view. Vitellaria numerous, lateral. Uterus saccate, extending to anterior margin of proglottid.

Remarks

This unusual species, described from a hemiscyllid shark by Sanaka *et al.* (1986), was not included in the key published by Probert and Stobart (1989). Campbell and Andrade (1997) considered it a *species inquirenda*. Ivanov (1997) implicitly considered the species valid. Later Ivanov and Campbell (1998a) agreed with Campbell and Andrade (1997), and they omitted the species from their key. This species was, however, included in the phylogenetic analysis of Ivanov and Hoberg (1999). In the tree resulting from this analysis, it grouped with *E. musteli*, also described from a shark, and placed in a position basal to other species in the order. This species was among those listed as valid by Tyler and Caira (1999).

Sanaka *et al.* (1986) stated that the specimens they collected could be sorted into two different sizes, large and small; the larger is described as being 35-40 mm long with 40-50 proglottids, and the smaller 20-25 mm long with 20-25 proglottids. The two size groups were also described as having different types of armature, the smaller of the two groups lacking lateral hooklets. This raises the question of whether the type series consisted of one or two species. Like other species of *Echinobothrium* found in sharks, this species has several rows of small spines or microtriches on the scolex just posterior to the apical armature, and anterior to the bothria. Much of the description is confusing and difficult to follow, even with the figures provided. For example, the cephalic peduncle armature of this species is somewhat peculiar as described. The authors describe "2 or 3 tiers of large spines"

with 10-14 spines per row, followed by the typical eight longitudinal columns of triradiate spines. In addition, the genital pores are described as alternating irregularly, but midventral. Because type or voucher specimens are unavailable, the original description is confusing, and the figures do not match the written description, this species is considered here to be a *species inquirenda*.

Key to the Valid Species of *Echinobothrium*

1(a) Cephalic peduncle unarmed 2
1(b) Cephalic peduncle armed with eight columns of spines 6

2(a) Bases of apical hooks articulating with one another via a system of interlocking knobs and sockets (Fig. 16)..... .....***E. rhynchobati*** n. comb.
2(b) Bases of apical hooks not articulating with one another..... 3

3(a) Worms large, over 5 mm in length..... 4
3(b) Worms small, under 5 mm in length 5

4(a) Rostellar armature with two groups of 14 hooks.....***E. deeghai***
4(b) Rostellar armature with two groups of 17 hooks.....***E. reesae***

5(a) Bothria large, foliaceous; ovary H-shaped; genital pore anterior to ovary .....***E. euterpes*** n. comb.
5(b) Bothria slender; ovary U-shaped; genital pore posterior to ovary .....***E. syrtensis*** n. comb.

6(a) Several rows of small spines between rostellum and bothria (Fig. 5) 7
6(b) No spines between rostellum and bothria 8

7(a) Ovary U-shaped; genital pore at same level as ovary.....***E. musteli***
7(b) Ovary H-shaped; genital pore anterior to ovary. .....***E. notoguidoi***

8(a) Apical hooks with type "A" symmetry (Fig. 2)..... 9
8(b) Apical hooks with type "B" symmetry (Fig. 3) 12

9(a) Cephalic peduncle with eight columns of 100 or more spines each 10
9(b) Cephalic peduncle with eight columns of fewer than 100 spines each 11

10(a) Lateral hooklets arranged in two groups (Fig. 7); cleft present on posterior margin of bothria (Fig. 9); ovary H-shaped, cephalic peduncle spines with triradiate bases (Fig. 11) .....***E. euzeti***
10(b) Lateral hooklets absent; cleft absent from posterior margin of bothria; ovary U-shaped; cephalic peduncle spines with leaflike bases (Fig. 12) .....***E. longicolle***

11(a) Hook formula {1 9/8 1}; cephalic peduncle with eight columns of 11-15 spines each; 9-11 testes .....***E. coenoformum***
11(b) Hook formula {(3-4) 13/14 (3-4)}; cephalic peduncle with eight columns of 57-60 spines each; 20-30 testes.....***E. mathiasi***

12(a) Genital pore anterior to ovary....13
12(b) Genital pore at same level as ovary....21

13(a) Lateral hooklets arranged in two distinct groups (Fig. 7)....14
13(b) Lateral hooklets arranged in single continuous row (Fig. 8) 16

14(a) Each group of lateral hooklets staggered in position (Fig. 8)..***E. coronatum***
14(b) Each group of lateral hooklets uniform in position (Fig. 7)....15

15(a) Hook formula {(3-4) 10/9 (3-4)}....***E. elegans*** Tyler, n. sp.
15(b) Hook formula {(2-3) 6/5 (2-3)}....***E. affine***

16(a) Testes arranged in single column ....***E. pigmentatum***
16(b) Testes in two or more columns....17

17(a) Vitelline follicles entirely pre-ovarian 18
17(b) Vitelline follicles extending full length of proglottid 19

18(a) First and last lateral hooklets in each group at least twice as long as other lateral hooklets (Fig. 8)....***E. fautleyae***
18(b) Lateral hooklets all relatively equal in length....***E. bonasum***

19(a) Lateral hooklets staggered in position (Fig. 8); cleft present on posterior margin of bothria (Fig. 9) ....***E. raschii***
19(b) Lateral hooklets uniform in position (Fig. 7); cleft absent from posterior margin of bothria 20

20(a) Hook formula {(5-7) 12/11 (5-7)}....***E. mexicanum***
20(b) Hook formula {6 14/13 6} .... ***E. megacanthum***

21(a) Lateral hooklets arranged in two distinct groups (Fig. 7) or lacking entirely 22
21(b) Lateral hooklets arranged in single continuous row (Fig. 8) 32

22(a) Ovary U-shaped 23
22(b) Ovary H-shaped **25**

23(a) Testes arranged in single column ....***E. chisholmae***
23(b) Testes arranged in two columns **24**

24(a) Hook formula {(2-4) 4/3 (2-4)}....***E. typus***
24(b) Hook formula {(3-4) 6/3 (3-4)}....***E. brachysoma***

25(a) Cephalic peduncle with velum at posterior terminus (Fig. 10).... **26**
25(b) Cephalic peduncle without velum at posterior terminus **27**

26(a) Lateral hooklets absent; cephalic peduncle with eight columns of 5-9 spines each....***E. raji***
26(b) Lateral hooklets present in two groups; cephalic peduncle with eight columns of 11-16 spines each .... ***E. clavatum***

27(a) Cleft present on posterior margin of bothria (Fig. 9)..... **28**
27(b) Cleft absent from posterior margin of bothria **30**

28(a) Testes arranged in single column .....***E. heroniense***
28(b) Testes arranged in two columns **29**

29a) Cephalic peduncle with eight columns of 11-14 spines each; 6-7 testes .....***E. harfordi***
29(b) Cephalic peduncle with eight columns of 16-17 spines each; 12-17 testes .....***E. helmymohamedi***

30(a) Testes arranged in 4-5 columns .....***E. acanthocolle***
30(b) Testes arranged in two columns **31**

31(a) Hook formula {(2-4) 12/11 (2-4)}; 11-14 testes .....***E. acanthinophyllum***
31(b) Hook formula {4 14/12 4}; 10 testes .....***E. benedeni***

32(a) Cleft present on posterior margin of bothria (Fig. 9).....***E. californiense***
32(b) Cleft absent from posterior margin of bothria **33**

33(a) Cephalic peduncle with eight columns of 2-5 spines each..***E. rayallemangi***
33(b) Cephalic peduncle with eight columns of 10-17 spines each .....***E. hoffmanorum***

Echinobothrium typus Van Beneden, 1849

TYPE SPECIES
(Figs. 25-27)

Type host: *Raja clavata* L., Thornback ray (Rajidae, Rajiformes).
Additional hosts: *Raja punctata*, Starry ray (Rajidae, Rajiformes) (see de Beauchamp 1905); *Raja batis* L., Blue skate (Rajidae, Rajiformes); *Myliobatis aquila* (L.), Common eagle ray (Myliobatidae, Myliobatiformes) (see Stossich 1898); *Dasyatis pastinaca*, Common stingray (Dasyatidae, Myliobatiformes) (see Van Beneden 1871); *Gammarus locusta* (L.) (Amphipoda, Decapoda) (larva; see Van Beneden 1870); *Perioculoides longimanus* (Amphipoda, Decapoda) (larva; see Monticelli 1890).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: Belgian coast, Atlantic Ocean.
Additional localities: Trieste, Italy; Sète, France; Banyuls-sur-mer, France; Wimereaux, France; Black Sea.
Type material: None designated.
Voucher specimens: Nine specimens col-

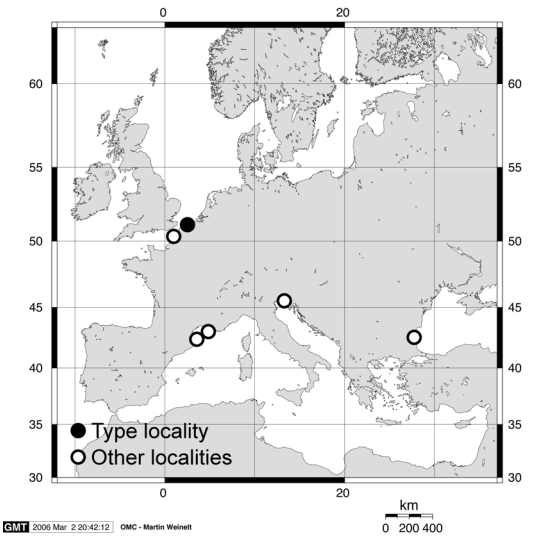


Fig. 25. Distribution of *Echinobothrium typus*.

lected from *R. clavata* in Sète, France on two slides in personal collection of L. Euzet.

Specimens examined: Nine specimens from L. Euzet.

Etymology: This is the type species of the genus.

Description (Modified from Pintner [1889].)

Worms 2-5.64 mm long, 330-380 wide at terminal proglottid. Strobila euapolytic, acraspedote, 14-16 proglottids. Mature proglottids 2-3 in number, 730-1,000 long, 290-380 wide. Scolex bipartite, 585-765 long, consisting of scolex proper and cephalic peduncle. Scolex proper 290-295 long, 233-240 wide, consisting of armed apical rostellum and two bothria. Seven apical hooks in each dorso-ventral group. Hook formula {(2-4) 4/3 (2-4)}, apical hooks solid, hooks increasing in length toward center of group. Bothria 198-245 long, 233-240 wide. Cephalic peduncle 338-500 long, 90-108 wide, armed with eight longitudinal columns of 16-18 spines. Spines with triradiate bases, 13-73 long.

Testes 7-12 in number, anterior to cirrus sac, in two columns, one layer deep. Vas deferens extensive, following a zig-zag course anterior to cirrus sac. Cirrus sac piriform, 143-160 long, 83-110 wide. Cirrus armed proximally with microtriches. Ovary 300-440 long, U-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, looping anterior to genital pore, relatively uniform in diameter along length, undulating slightly. Genital pore midventral, 14-24% of proglottid length from posterior end of proglottid, overlapping ovary. Excretory ducts lateral.

Remarks

Echinobothrium typus was the first described diphyllidean, and remained the only member of the genus until *E. levicolle* was described in 1857. Van Beneden described *E. typus* in 1849, then provided more descriptive information in 1850 and 1858. All of these descriptions were very cursory. In his original description, Van Beneden (1849) stated that there are nine apical hooks in each dorso-ventral group, and illustrated them in his figure 9, showing what appears to be a hook

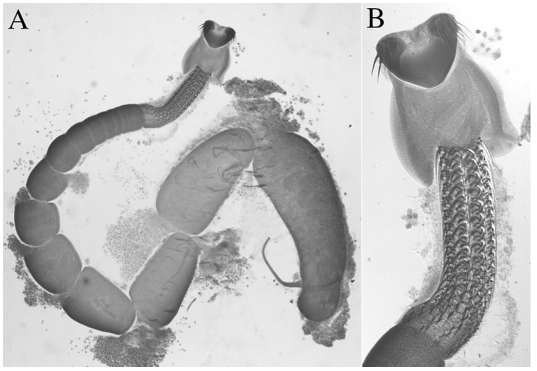


Fig. 26. Light micrographs of *Echinobothrium typus*. A. Whole worm. B. Scolex.

formula of {6/3}. Later, Van Beneden (1858) described 9-16 hooks in each group, and illustrated nine hooks per group (hook formula apparently {2 5/4 2}) in figure 1, 15 per group (hook formula {2 8/7 2}) in figure 2 and 11 per group (hook formula {2 6/5 3}) in figure 3. It now appears that Van Beneden (1849, 1858) may have been working with specimens of several species, possibly including *E. typus*, E. affine, and E. clavata. It also appears that there may have been two different forms each bearing nine-hooks per group. In his revision of *Echinobothrium*, Pintner (1889) redescribed the species, providing the description

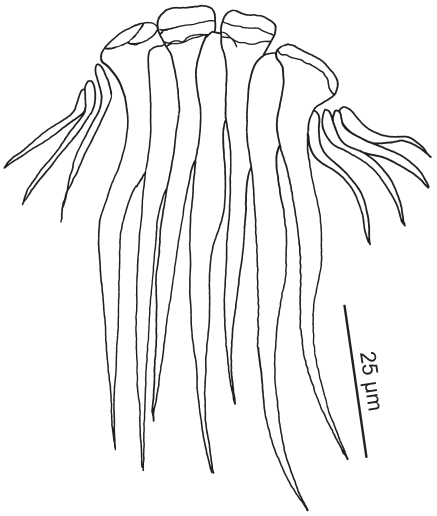


Fig. 27. Line drawing of apical hooks of *Echinobothrium typus*.

that has been widely accepted since. Pintner (1889) stated that Van Beneden's (1849) figure showing the hook formula {6/3} was probably an error and described seven hooks in each dorso-ventral group, then illustrated a hook formula of {4 4/3 4}. Whether or not Van Beneden (1849) was in error is unclear. However, because the various descriptions of this species published by Van Beneden are accompanied by illustrations of several different species, the accuracy of these descriptions is called into question. On the other hand, Pintner's (1889) redescription has been the standard for this species for well over 100 years. For example, in all published keys for *Echinobothrium* (Pintner 1889; Euzet 1951; Rees 1961b; Probert and Stobart 1989; Ivanov and Campbell 1998a), *E. typus* is identified in part by its possession of seven apical hooks per group. Therefore, it is Pintner's (1889) more detailed redescription of *E. typus* that is followed here.

The type host for this species was given by Van Beneden (1849) as "raie bouclée." In 1871, Van Beneden listed the common name of *R. clavata* as "raie bouclée." Thus, this species is given as the type host here.

Several other workers have reported *E. typus* from the Atlantic Ocean, Mediterranean Sea, and Black Sea (see Wagener 1854; Wedl 1855; Leuckart and Pagenstecher 1858; Olsson 1866-67; Lönnberg 1889; Stossich 1898; Dimitrov 1989). However, it appears that none of these workers deposited their specimens. Extensive searches for specimens of diphyllideans at museums worldwide have failed to produce any type material of this species. The only specimens available for study in the present work came from the personal collection of L. Euzet. None of those specimens, however, was of sufficient quality to be considered a neotype. Thus, it is imperative that this species be collected again, and a neotype designated and deposited in a museum.

Echinobothrium acanthinophyllum Rees, 1961

(Figs. 28-30)

Type host: *Raja montagui* Fowler, Spotted ray (Rajidae, Rajiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: English Channel, Plymouth England.

Additional localities: Roscoff, France.

Type material: BMNH No. 1962.28.14 (holotype).

Voucher specimens: Four specimens from *R. montagui* from Plymouth BMNH No. 1965.2.24.101-105; 13 specimens from *R. montagui* from Roscoff, France on one slide in the personal collection of L. Euzet.

Specimens examined: Holotype; two vouchers (BMNH No. 1965.2.24.101-105); 13 specimens from L. Euzet's collection.

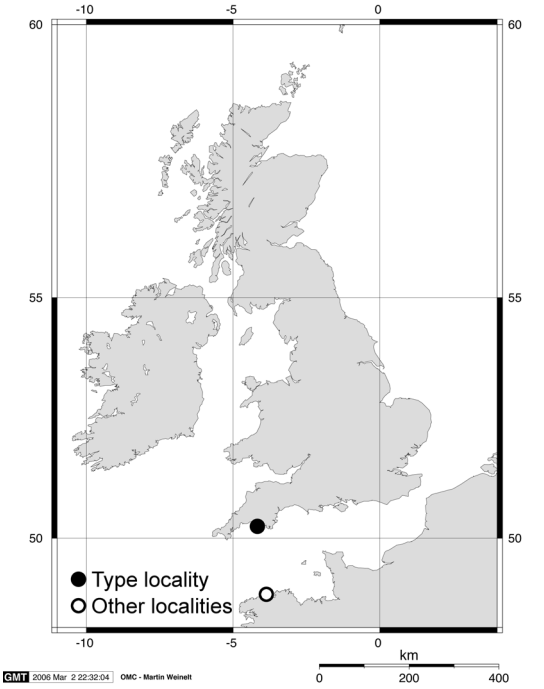


Fig. 28. Distribution of *Echinobothrium acanthinophyllum*.

Description (Modified from Rees [1961b].)

Worms 2.15-4.77 mm long, 370-780 wide at terminal proglottid. Strobila euapolytic, acraspedote, 7-9 proglottids. Mature proglottids 0-1 in number, 620 long, 240-300 wide. Gravid proglottids 0-3 in number, 875-1,380 long, 380-625 wide. Strobila occasionally with immature and gravid proglottids only. Scolex bipartite, 510-630 long, consisting of scolex proper and cephalic peduncle. Scolex proper 420-525 long, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Twenty-three apical hooks in each dorso-ventral group. Hook formula {(2-4) 12/11 (2-4)}, apical hooks solid, gradually increasing in length towards center of group. Lateral hooklets arranged in two groups. Bothria 400 long, proximal surfaces with large spinitriches. Cephalic peduncle 260-330 long, 105-130 wide, terminating in small velum, armed with eight longitudinal columns of 10-13 spines. Cephalic peduncle spines with triradiate bases, 15-96 long.

Testes 11-14 in number, 52-95 long, 95-113 wide, anterior to cirrus sac, in two irregular columns, one layer deep. Vas deferens extensive, extending laterally to cirrus sac. Cirrus sac oval, 125-160 long, 80-95 wide. Cirrus armed with small microtriches along its length. Ovary bilobed, 252-332 long, 196-208 wide, H-shaped in dorso-ventral view. Vagina thin-walled, posterior to genital pore, relatively uniform in width, coiling slightly. Seminal receptacle present. Genital pore midventral, 20-29% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development, expanding to fill gravid proglottid. Vitellaria follicular, forming two lateral columns extending entire length of proglottid, uninterrupted by ovary. Eggs round, 13-15 long, 11-13 wide, lacking appendages, packaged in cocoons of 5-10 eggs each. Excretory ducts lateral.

Remarks

The hook formula of this species distinguishes it from all others in the genus except *E. mexicanum*, *E. raschii*, and *E. rayallemangi*. *Echinobothrium acanthinophyllum* differs from all these species in having the lateral

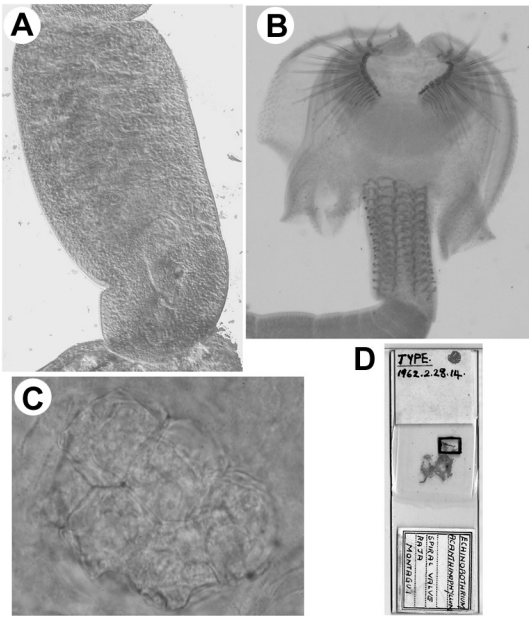


Fig. 29. Light micrographs of *Echinobothrium acanthinophyllum*. A. Mature proglottid. B. Scolex. C. Egg packet. D. Holotype slide BMNH No. 1962.2.28.14.

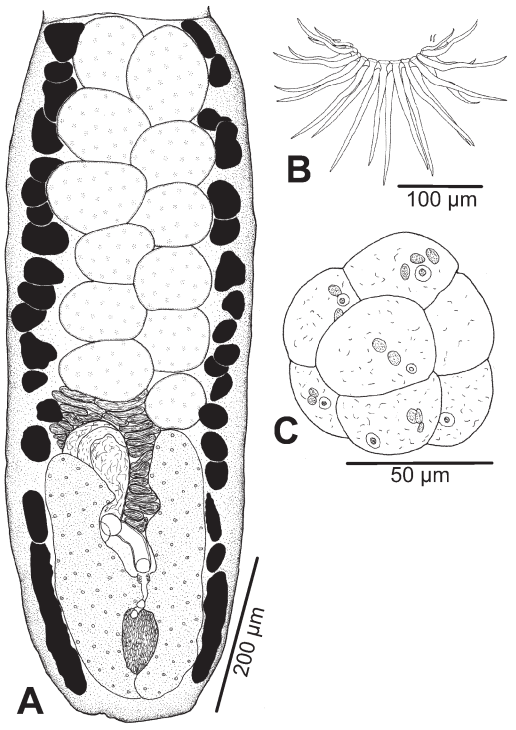


Fig. 30. Line drawings of *Echinobothrium acanthinophyllum*. A. Mature proglottid. B. Apical hooks. C. Egg packet.

hooklets arranged in two groups, whereas all these species have lateral hooklets arranged in a continuous row.

This species was described by Rees (1961b) from only a single specimen and has not been reported since. *Echinobothrium acanthinophyllum* is redescribed here from the holotype and several voucher specimens collected from the type host in Plymouth, England and Roscoff, France. The voucher specimens were originally identified as either *E. affine* or *Echinobothrium* sp. This species appeared in the keys published by Probert and Stobart (1989) and Ivanov and Campbell (1998a), and was included in the phylogenetic analysis published by Ivanov and Hoberg (1999). In their tree it appeared as part of a trichotomy with *E. brachysoma* and a clade comprising *E. acanthocolle*, *E. reesae*, *E. rhynchobati*, and *E. raji*. This species was considered valid by Tyler and Caira (1999).

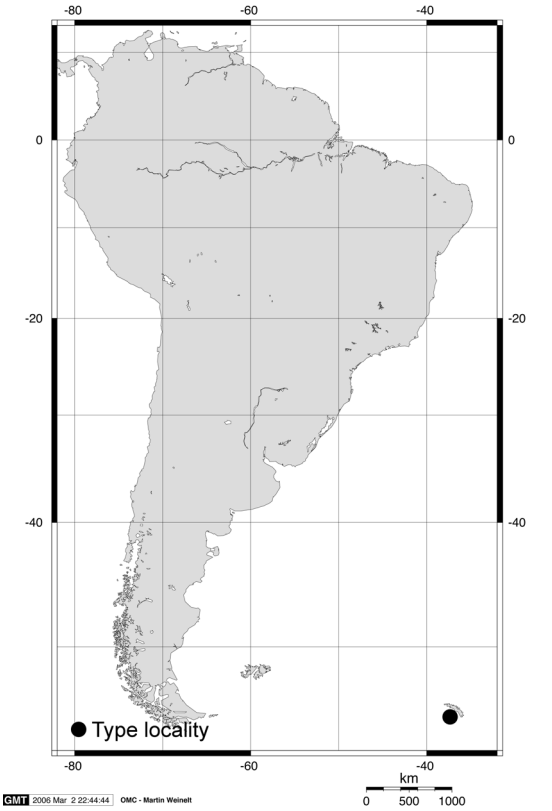


Fig. 31. Distribution of *Echinobothrium acanthocolle*.

Echinobothrium acanthocolle Wojciechowska, 1991

(Figs. 31-33)

Type host: *Raja georgiana* Norman, Antarctic starry skate (Rajidae, Rajiformes).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: South Georgia Island.
Type material: Holotype and paratype No. 1237 mounted on one slide in the personal collection of A. Wojciechowska at the Polish Academy of Sciences, Warsaw, Poland.
Specimens examined: Holotype; one paratype.
Etymology: Not given.

Description (Modified from Wojciechowska, [1991].)

Whole worm 4.96 mm long, 770 wide at terminal proglottid. Strobila acraspedote, euapolytic, nine proglottids. Two mature proglottids, 800-1,150 long, 670-770 wide. No gravid proglottids on these specimens. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Total length 800. Scolex proper 800 long, 590 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Hook formula apparently {3 16/15 3}, hooks solid. Hook increasing in length toward center of group. Lateral hooklets arranged in two groups. Bothria 773 long, 590 wide. Proximal bothrial surfaces and lateral surface of scolex proper with large spinitriches. Cephalic peduncle 118 long, 260 wide, armed with eight longitudinal columns of 2-5 spines. Spines with triradiate bases, 28 to 35 long.

Testes 19-27 in number, anterior to ovary, spherical to sub-spherical, 100-120 in diameter, in 4-5 irregular columns, one layer deep. Vas deferens minimal in size, entirely anterior to cirrus sac. Cirrus sac piriform, 198 long, 150 wide, slightly overlapping ovary. Ovary 210-350 long, 330-350 wide, H-shaped on dorso-ventral view. Vagina thin-walled, looping anterior to genital pore, surrounded by gland-like cells distally, lined with long cilia

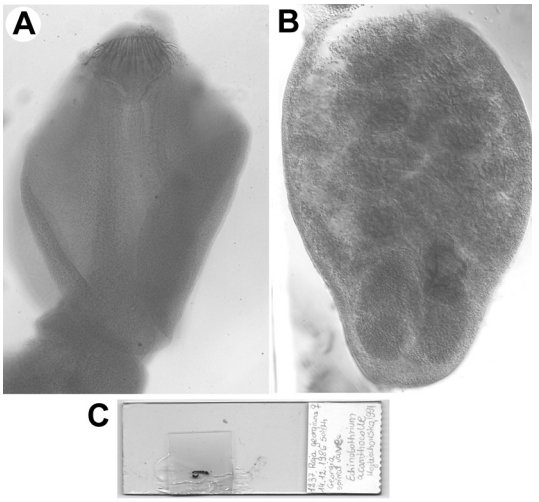


Fig. 32. Light micrographs of *Echinobothrium acanthocolle*. A. Scolex. B. Mature proglottid. C. Type slide.

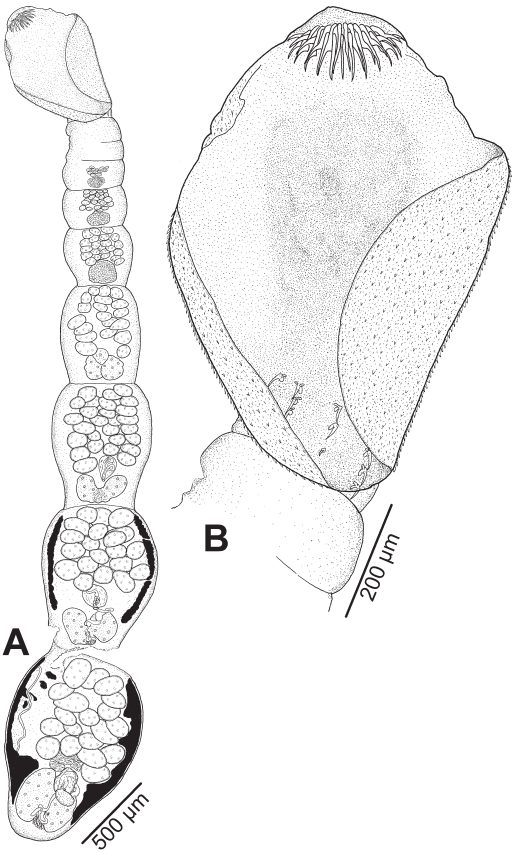


Fig. 33. Line drawings of *Echinobothrium acanthocolle*. A. Whole worm. B. Scolex.

or filitriches, with expanded lumen halfway between ootype and genital pore. Seminal receptacle absent. Genital pore midventral, 28% of proglottid length from posterior end of proglottid. Uterus not observed. Vitellaria lateral, follicular, densely packed into two lateral columns extending entire length of proglottid, uninterrupted by ovary. Eggs not observed. Excretory ducts lateral.

Remarks

The unique hook formula of this species is sufficient to distinguish it from all other species in the genus.

This species was described by Wojciechowska (1991) from the south Pacific Ocean off South Georgia Island. It has not been reported since. The single specimen identified only as "*Macrobothridium* sp." by Wojciechowska *et al.* (1995, p. 207) from *Bathyraja eatoni* from the subantarctic region resembles this species in its overall dimensions. However, as this specimen consisted of only a scolex with much of the apical armature damaged, it was not possible to identify it to species.

This species has been considered valid by all subsequent workers. Ivanov and Campbell (1998a) included this species in their key, and Ivanov and Hoberg (1999) included it in their phylogenetic analysis. In their tree it formed a polytomy with *E. reesae* and *E. rhynchobati*. This species was among those considered valid by Tyler and Caira (1999).

This species was described from two specimens, only one of which possesses a scolex. Both type specimens are on a single slide bearing the number 1237. The complete specimen was illustrated by Wojciechowska (1991), and is assumed here to be the holotype. However, the holotype was never explicitly differentiated from the paratype by the author.

Echinobothrium affine Diesing, 1863

(Figs. 34-36)

Type host: *Raja radula* Delaroche, Rough ray (Rajidae, Rajiformes).

Additional hosts: *Raja clavata* L., Thornback ray (Rajidae, Rajiformes) (as *R. batis* L.), *R. miraletus* L., Brown ray (Rajidae, Rajiformes), *R. alba* Lacepède, bottlenosed skate (Rajidae, Rajiformes) (as *R. marginata)*, *Dasybatis clavata,* and *Laeviraja oxyrhynchus* (Rajidae, Rajiformes) (see Stossich 1898); *Carcinus maenus* L., European green crab (Portunidae, Decapoda) (see Dollfus 1964); *Ethusa mascarone*, Stalkeye sumo crab (Dorippidae, Decapoda) (larva; see Vivares 1971).

Status: Valid.

Site of infection: Spiral intestine (as adult).

Type locality: Nice, France.

Additional localities: Trieste (see Wedl 1855; Pintner 1889); Butt of Lewis, Scotland (see Rees 1961a); Plymouth, England (see Rees 1961a); Sète, France (unpubl.).

Type material: BMNH No. 1976.4.13.32 (neotype).

Voucher material: BMNH Nos. 1976.4.13.33-36 and 1976.4.13.37-38.

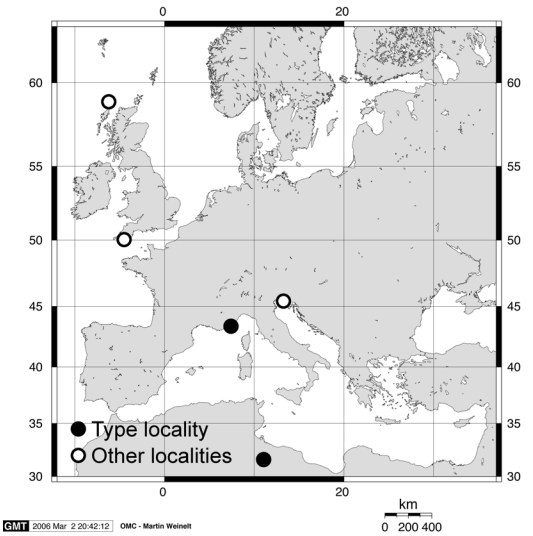


Fig. 34. Distribution of *Echinbobothrium affine*.

Specimens examined: Neotype; eight vouchers (BMNH No. 1976.4.13.32-36); six vouchers (BMNH No. 1976.4.13.37-38).

Etymology: Not given.

Description (Modified from Pintner [1889] and Rees [1961a].)

Worms 3-8.90 mm long, 460-800 wide at terminal proglottid. Strobila acraspedote, apolytic, 7-8 proglottids. Mature proglottids 1-2 in number, 705-1,540 long, 290-450 wide. Gravid proglottids 1-2 in number, 1.73-3.76 mm long, 370-800 wide. Scolex bipartite, 400-860 long, consisting of scolex proper and cephalic peduncle. Scolex proper 308-414 long, 198-238 wide. Eleven apical hooks in each dorso-ventral group. Hook formula {(2-3) 6/5 (2-3)}, hooks solid. Hooks increasing in length toward center of group. Lateral hooklets arranged in two groups. Bothria 623-350 long, 198-238 wide. Cephalic peduncle 438-558 long, 50-146 wide, armed with eight longitudinal columns of 20-30 spines each.

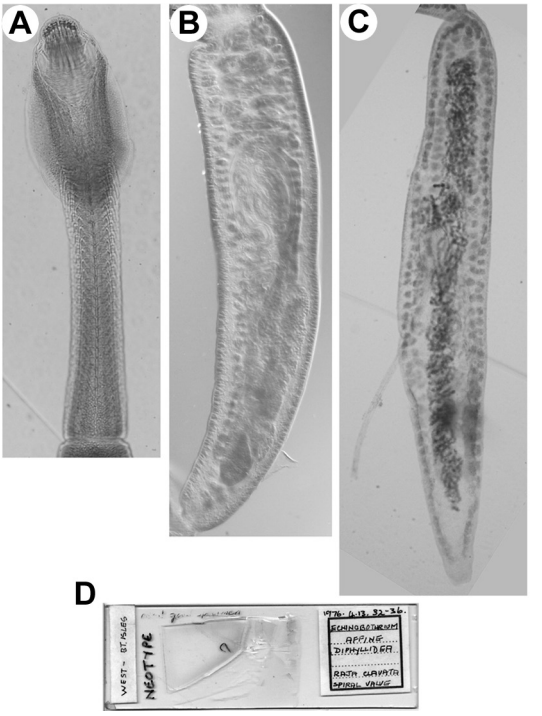


Fig. 35. Light micrographs of *Echinobothrium affine*. A. Scolex. B. Mature proglottid. C. Gravid proglottid. D. Neotype slide BMNH No. 1976.4.13.32.

Spines with triradiate bases, 11-13 to 68-73 long.

Testes 9-13 in number, anterior to ovary, 43-68 long, 110-138 wide, in two irregular columns, one layer deep. Vas deferens extensive, extending posterior to cirrus sac. Cirrus sac piriform, 240-335 long, 160-225 wide. Cirrus 1.12-2.89 mm long, 53-55 wide at base, covered with small microtriches. Ovary 465-750 long, H-shaped in dorso-ventral view. Mehlis' gland prominent, posterior to ovarian isthmus, 100-195 long, 83 wide. Vagina thin-walled, posterior to genital pore, uniform in width along its length, highly coiled. Seminal receptacle present. Genital pore midventral, 44-62% of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, extending to anterior end of gravid proglottid. Vitellaria lateral, follicular, approximately 50-65 long, 35-50 wide, forming two columns extending entire length of proglottid, uninterrupted by ovary. Eggs round to oval, 13-20 in diameter, with single terminal filament, packaged in groups of three, within chains. Excretory ducts lateral.

Remarks

The hook formula of this species distinguishes it from all others in the genus except *E. bonasum*, *E. fautleyae*, *E. harfordi*, and *E. syrtensis*. *Echinobothrium affine* can be distinguished from the former two species by its lateral hooklets, which are arranged in two groups as opposed to a single continuous row in the former two species. It is distinguished from *E. harfordi* by its distinctive eggs, which are packaged in chains; those of *E. harfordi* are not packaged. *Echinobothrium affine* is distinguished from *E. syrtensis* by its possession of cephalic peduncle armature, a feature lacking in *E. syrtensis*.

Echinobothrium affine was erected by Diesing (1863) for *Dibothrium typus* Van Beneden of Wagener (1854), *E. typus* of Wedl (1855), and possibly *E. typus* of Leuckart and Pagenstecher (1858) and Leuckart (1859). Diesing (1863) provided no information on the internal anatomy, apical hook or cephalic peduncle spine counts. The anatomy of this species remained largely unknown until Pintner (1889) revised the genus and more thoroughly described the species. Southwell (1927) reported *E. affine* from *Carcharias* sp. and *Rhinobatos halavi* (Forsskål, 1775) (as *Rhynchobatis* [sic] *helavi* [sic]) in Nagatmapur, India. Rees (1961a) provided a detailed description of the scolex and reproductive anatomy of specimens matching the description of Pintner (1889). Spermatogenesis and the ultrastructural anatomy of the spermatozoids of this species have been well studied, (Euzet *et al.* 1981; Azzouz-Draoui and Mokhtar-Maamouri 1986-1988). These data have been used as characters in several analyses of higher level cestode relationships (see Hoberg *et al.* 1997; Justine 1998).

The figures of Wagener (1854) and Leuckart and Pagenstecher (1858), upon which Diesing based his description, appear to represent more than one species, and both may actually represent species other than what is now considered to be *E. affine*, based on Pintner's (1889) redescription. For example, the scolex figured in Wagener's (1854) figure 87 shows eight apical hooks in each group, whereas the scolex in Leuckart and Pagenstecher's (1858) figure 5 has either nine or 12 apical hooks per group. In addition, the genital pore position shown in Wagener's figure 80 is anterior to the ovary, whereas in Leuckart and Pagenstecher's figure 5 it is posterior to the ovary (cf. *E. brachysoma*). Diesing (1863) provided little information about the anatomy of these forms, other than the overall dimensions, proglottid length to width ratio, and a description of the eggs. Apparently, it was this anatomical feature (filamented eggs in chains) which Pintner (1889) used to identify *E. affine*, and subsequently redescribe the species. Judging from Pintner's (1889) description, it appears that at least some of what Van Beneden (1858, 1861) considered to be *E. typus* was probably *E. affine*, based on central apical hook number and arrangement (in these papers, Van Beneden shows four different figures of "*E. typus*," two with nine hooks per group [1849, 1858], one with 11 hooks per group [1858], and one with 15 hooks per group [1858]).

Many specimens identified as *E. affine* and currently housed in museum collections were found to be misidentified. It appears

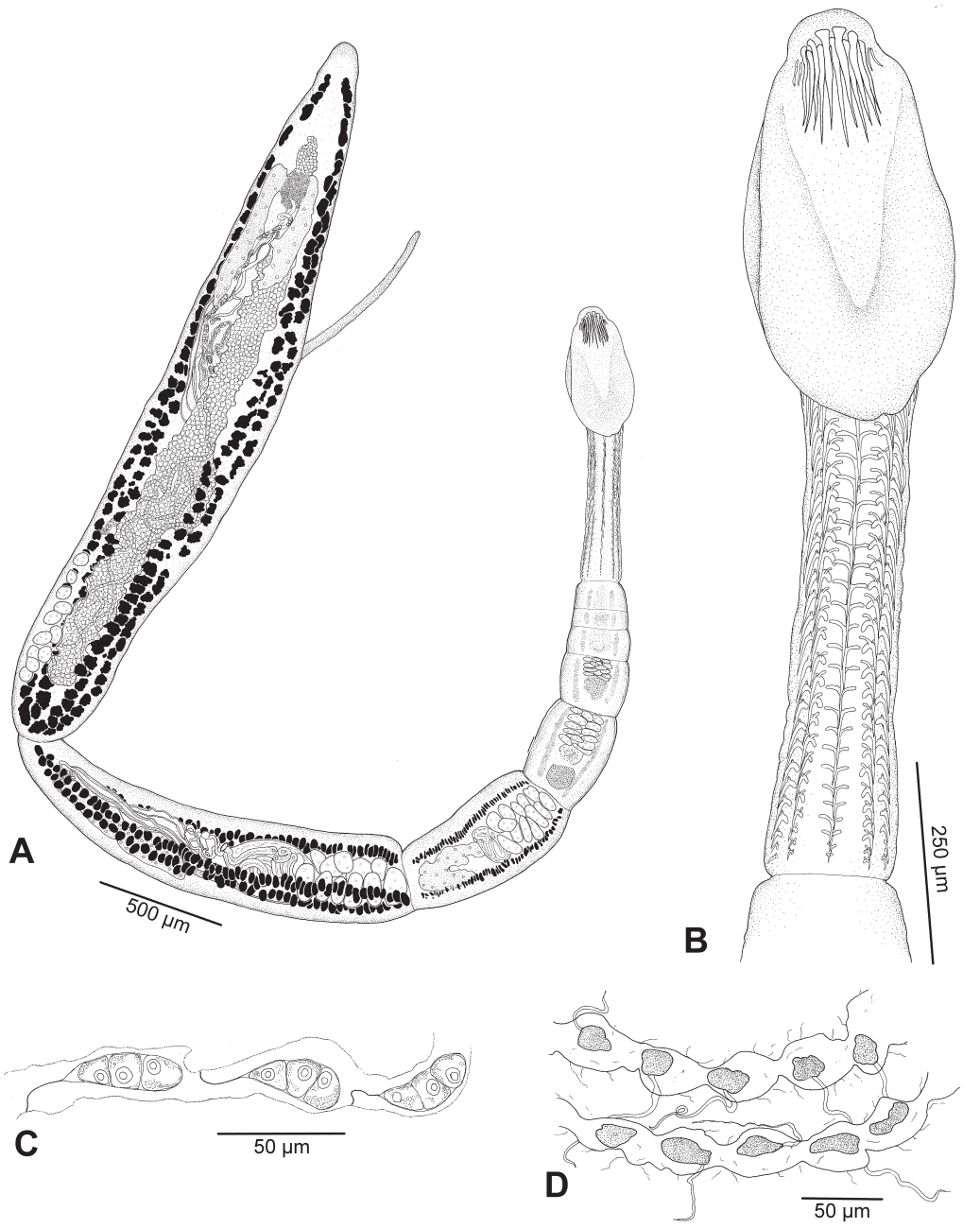


Fig. 36. Line drawings of *Echinobothrium affine*. A. Whole worm. B. Scolex. C. Eggs in uterine duct. D. Eggs in uterus.

that some workers identified their diphyllideans in haste or, owing to the relative scarcity of diphyllideans, had no experience with them. In other cases, it may be that the authors simply preferred to place their specimens into currently existing taxa. For example, the specimens Southwell (1927) identified as *E. affine* from *Carcharias* sp.

and *Rhinobatos halavi* (as *Rhynchobatis* [sic] *helavi* [sic]) *Rhynchobatis helavi* (BMNH No. 1977.11.9.54-55) were, in at least one of those cases, a different species. Examination of the specimens Southwell (1927) reported from *R. helavi*, revealed that they are not *E. affine*, and instead constitute a new species. Southwell's specimens from *Carcharias* sp. were not in good enough condition to identify to species. Dollfus (1964) reported a plerocercoid of *E. affine* from the green crab, *Carcinus maenas*. The actual identity of this larva cannot be verified, however, as larvae do not possess any of the cephalic peduncle or proglottid characters found in adult specimens. The larvae examined by Dollfus (1964) had the hook formula {3 6/5 3}. This hook formula is shared by two other diphyllidean species, *E. harfordi* and *E. syrtensis*, either of which (especially *E. harfordi*) could possibly be found in Roscoff, where Dollfus' material was collected.

Rees (1961a) provided a detailed description of the reproductive anatomy and the structure and function of the scolex of *E. affine*. Because the specimens used in that work were readily available (BMNH), these specimens were used as the basis for the redescription provided here. Given the lack of type material, one of Rees' (1961a) specimens (BMNH No. 1976.4.13.32) has been designated as the neotype in order to stabilize the nomenclature.

Echinobothrium benedeni Ruszkowski, 1927

(Figs. 37-38)

Type host: *Hippolyte varians* Leach, Chameleon prawn (Aristeidae, Decapoda) (intermediate host).

Additional hosts: *Raja asterias* Delaroche, Starry ray (Rajidae, Rajiformes).

Status: Valid.

Site of infection: Spiral intestine (as adult).

Type locality: Roscoff, France (Atlantic).

Type material: None designated.

Specimens examined: None.

Etymology: Not given, but presumably named in honor of P. J. Van Beneden.

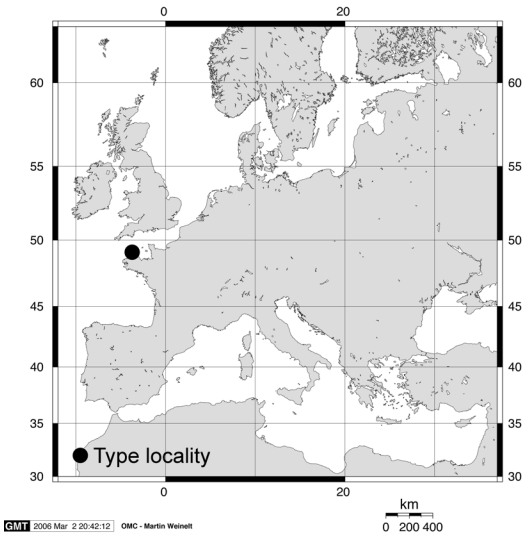


Fig. 37. Distribution of *Echinobothrium benedeni*.

Description (Modified from Ruszkowski [1927, 1928].)

Plerocercoid larva, 1.50-1.70 mm long, 500-700 wide. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper consisting of armed apical rostellum and one dorsal and one ventral bothrium. Hook formula as illustrated (Ruszkowski 1927; 1928) {4 14/12 4}, apical hooks solid. Lateral hooklets arranged in two groups. Cephalic peduncle 150 long, 200 wide, with eight longitudinal columns of pigmentation, believed to be precursors of spines.

Testes 10 in number, arranged in two columns. Ovary in posterior part of proglottids.

Remarks

The unique hook formula of this species distinguishes it from all others in the genus. This species has not been reported since its description by Ruszkowski (1927). As he did not designate type specimens in his published accounts, the whereabouts (or existence) of type material for this species is unknown at this time. All attempts to locate the specimens of Ruszkowski through correspondence have been unsuccessful. Probert and Stobart (1989) did not include this species in their key to the genus. Campbell and Andrade (1997) considered this species to be a *species*

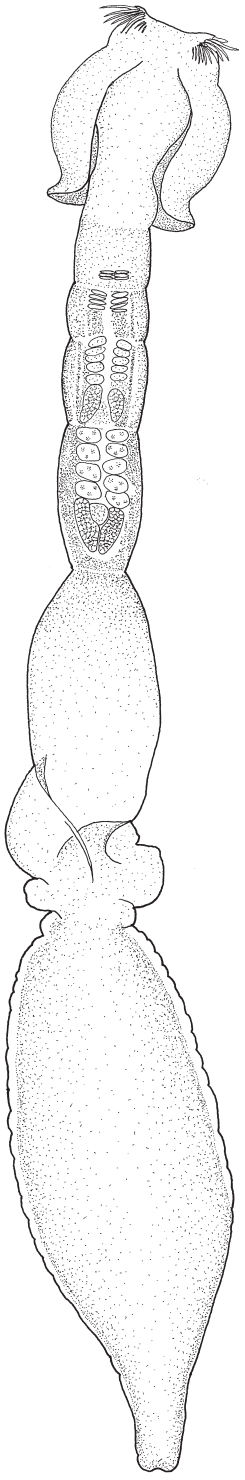


Fig. 38. Line drawing of *Echinobothrium benedeni*. Redrawn from Ruszkowski (1928) (no scale bar provided).

inquirenda because it was described primarily from larvae. Ivanov and Campbell (1998a) did not include this species in their key to the genus, nor was it included in the phylogenetic analysis of Ivanov and Hoberg (1999). However, Tyler and Caira (1999) recognized this species as valid.

Ruszkowski (1927) described this species from larvae found encysted in the shrimp *Hippolyte varians* within the gut of the vertebrate host *Raja asterias*. Among the specimens studied by Ruszkowski were several post-larvae which had been digested free from the intermediate host and had begun development of the cephalic peduncle armature and of the genitalia. It is not clear whether or not these specimens were actually embedded in the intestinal wall. However, the level of development of the larvae of *E. benedeni* within the gut of *R. asterias* provides sufficient evidence for this elasmobranch to be considered the definitive host for this species. Ruszkowski (1927) described the scolex armature as consisting of 26 apical hooks with a group of four lateral hooklets on either side. Since the central apical groups of hooks generally tend to be symmetrical, the actual hook count is most likely 27, rather than 26, with a hook formula of {4 14/13 4}. Although the anatomy of the mature proglottid has not yet been described in detail, the original description provides sufficient morphological detail, particularly of the apical armature, to distinguish it from all other species in the genus. Following Tyler and Caira (1999), this species is considered here to be valid.

Ruszkowski (1927; 1928) provided information for this species that is lacking for every other species of diphyllidean known, that is, the identity of both the definitive and final intermediate host. Whereas Ramadevi and Rao (1974) described larvae of *E. reesae* from the pasiphaeid shrimp *Leptochela aculeocaudata* Paulson, and Jones and Beveridge (2001) described the terminal larval stage of *Echinobothrium chisholmae* from another pasiphaeid, *Penaeus longistylus* Kubo, neither of these have been found within the gut of a definitive host.

Echinobothrium bonasum Williams and Campbell, 1980

(Figs. 39-41)

Type host: *Rhinoptera bonasus* (Mitchill), Cownose ray (Rhinopteridae, Myliobatiformes).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: Sakonnet Point, Rhode Island, U.S.A. (41°27'N, 71°12'W).
Additional localities: Chesapeake Bay, Virginia, U.S.A.
Type material: USNPC No. 75770 (holotype); USNPC Nos. 75771 and 75772 (paratypes).
Specimens examined: Holotype; two paratypes.
Etymology: Named after its host.

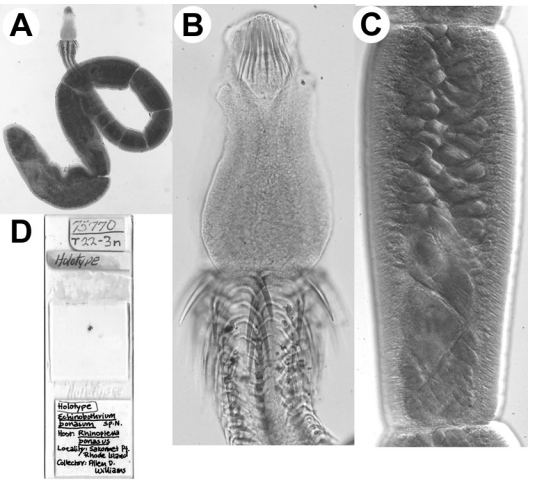


Fig. 40. Light micrographs of *Echinobothrium bonasum*. A. Whole worm. B. Scolex. C. Mature proglottid. D. Holotype slide USNPC No.75770.

Description (Modified from Williams and Campbell [1980].)

Worms 2.1-8.3 mm long, 110-365 wide at terminal proglottid. Strobila euapolytic, acraspedote, 10-18 proglottids. Mature proglottids two in number, 565-1,470 long, 224-365 wide. Gravid proglottids not observed. Scolex bipartite, 535-598 long, consisting of scolex proper and cephalic peduncle. Scolex proper 228-320 long, 126-152 wide, consisting of armed apical rostellum and two slender bothria. Eleven apical hooks per dorso-ventral group. Hook formula {(12-14) 6/5 (12-14)}, apical hooks solid, type A hooks gradually increasing in length toward center of group, central type B hooks shorter than adjacent type B hooks. Lateral hooklets arranged in continuous row, staggered in position relative to one another. Bothria 218-320 long, 126-152 wide, with spinitriches on proximal surfaces. Cephalic peduncle 237-416 long, 80-123 wide, armed with eight longitudinal columns of 22-26 spines. Spines with triradiate bases, 7-78 long.

Testes 24-31 in number, anterior to ovary, 11-46 long, 65-152 wide, in 2-3 irregular columns, one layer deep. Cirrus sac piriform, 95-280 long, 78-163 wide. Cirrus armed proximally with robust thorn-like microtriches. Ovary 188-330 long, 150-163 wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thick-walled, muscular, lined with cilia, looping anterior to genital pore, undulating slightly, with expanded lumen distally. Genital pore midventral, 27-38% of proglottid length from posterior end of proglottid, anterior to ovary. Uterus not observed beyond early developmental stages, thick-walled in early stages of development. Vitellaria follicular; follicles 17-46 long, 23-61 wide, lateral, but partially overlapping testes, extending to level of ovary. Eggs not observed. Excretory ducts lateral.

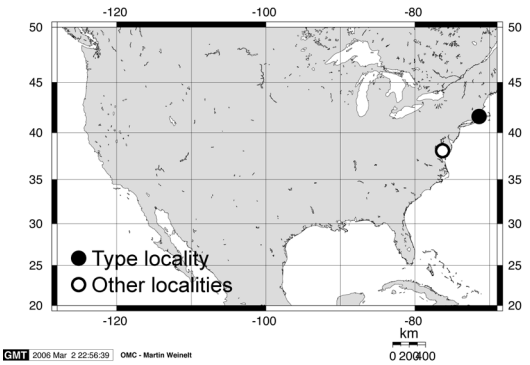


Fig. 39. Distribution of *Echinobothrium bonasum*.

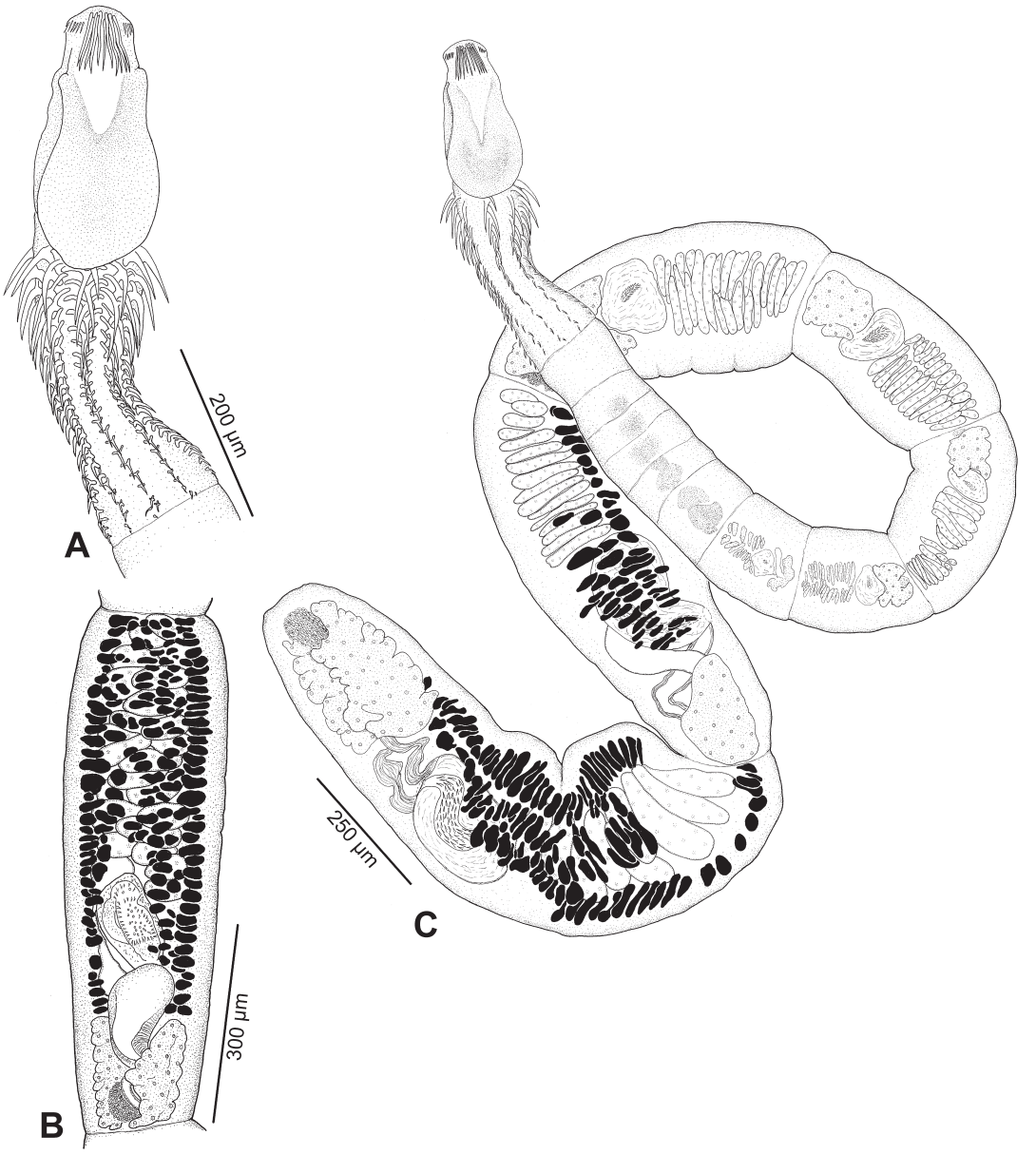


Fig. 41. Line drawings of *Echinobothrium bonasum*. A. Scolex. B. Whole worm. C. Mature proglottid, dorsal view.

Remarks

The hook formula of this species allows it to be distinguished from all other species in the genus except *E. affine*, *E. harfordi*, and *E. fautleyae*. *Echinobothrium bonasum* differs from the first two species in that the lateral hooklets are arranged in a single continuous row, as opposed to two groups in those species. *Echinobothrium bonasum* is distinguished from *E. fautleyae* by its lateral hooklet arrangement, having all of the hooklets relatively equal in length, whereas the first and last hooklet of each row on *E. fautleyae* are considerably longer than the others in the row.

This species was described by Williams and Campbell (1980) from the U.S. Atlantic coast. It is the first and only diphyllidean reported from eastern U.S. waters. This species was included in the keys of Probert and Stobart (1989) and Ivanov and Campbell (1998a). Schmidt (1986), Campbell and Andrade (1997), and Tyler and Caira (1999) all considered this species to be valid. Ivanov and Hoberg (1999) included this species in their phylogenetic analysis. It appeared in their tree as the sister species to the clade containing *E. affine*, *E. raschii*, *E. pigmentatum*, and *E. californiense*.

This species was originally described from 11 specimens, but only three type specimens were designated. In an attempt to obtain additional specimens of this species for the present study, cownose rays were collected from several locations including Chesapeake Bay, Maryland, Core Sound in North Carolina, and Ocean Springs, Mississippi (Gulf of Mexico). Several specimens of *Echinobothrium* were collected from rays in each location. However, the morphology of these specimens was not entirely consistent with either the type specimens or the published description of *E. bonasum*. These specimens have not been included in this description.

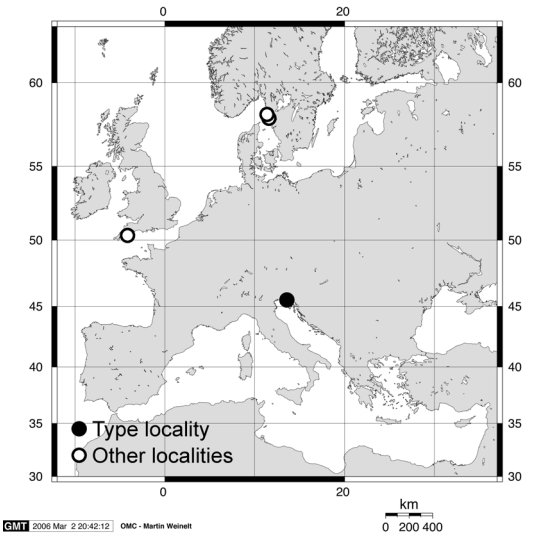


Fig. 42. Distribution of *Echinobothrium brachysoma*.

Echinobothrium brachysoma Pintner, 1889

(Figs. 42-44)

Type host: "Rochenarten" (skate species).

Additional hosts: *Raja clavata* L., Thornback ray (Rajidae, Rajiformes) (see Rees 1961b); *R. batis* L., Blues skate (Rajidae, Rajiformes) (see Stossich 1898).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Trieste, Italy.

Additional localities: Plymouth, England (Rees, 1961a); Bohuslän, Skagerrak, Gåsön, Sweden (Lönnberg, 1889); Bohuslän, Gullmarsfjorden, Sweden (Lönnberg, 1889).

Type material: None designated.

Voucher specimens: BMNH Nos. 1976.4.13.37-38 and 1976.4.13.25-31; SMNH Nos. 32640-32643, 32651-32658, and 32661.

Specimens examined: One voucher (BMNH No. 1976.4.13.37-38) from Plymouth; one voucher (BMNH No. 1976.4.13.25-31) from Plymouth; four vouchers (SMNH Nos. 32640-32643) from Skagerrak; eight vouchers (SMNH Nos. 32651-32658) from Gullmarsfjorden; two vouchers mounted for SEM (SMNH Nos. 32650 and 32661).

Etymology: This species derives its name from its small size.

Description (Modified from Rees [1961a].)

Worms 1.66-3.37 mm long, 168-258 wide at terminal proglottid. Strobila apolytic, acraspedote, 12-16 proglottids. Mature proglottids 0-1 in number, 615 long, 158 wide, gravid proglottids 0-1 in number, 428-1,000 long, 168-258 wide. Scolex bipartite, 483-931 long, consisting of scolex proper and cephalic peduncle. Scolex proper 188-313 long, 178-188 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper with short filitriches. Nine apical hooks in each dorsoventral group. Hook formula {(3-4) 6/3 (3-4)}, apical hooks solid, central type A hooks shorter than adjacent hooks, type B hooks increasing in length toward center of group.

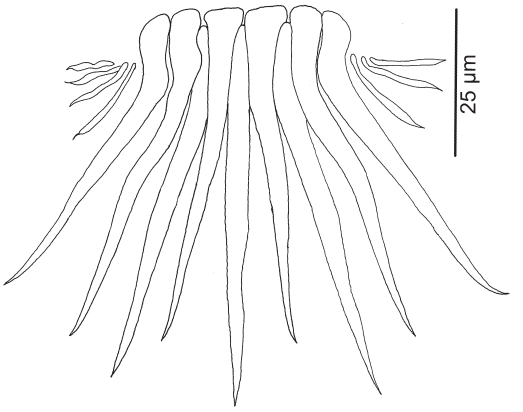


Fig. 43. Line drawing of apical hooks of *Echinobothrium brachysoma*.

Proximal bothrial surfaces with pectinate spinitriches each bearing 4-5 relatively equal length digits. Distal bothrial surfaces with pectinate spinitriches each bearing three digits; central digit conspicuously longer than others. Lateral surfaces of scolex proper with pectinate microtriches each bearing 3-4 relatively equal length digits. Cephalic peduncle 336-685 long, 68-138 wide, covered with short filitriches, armed with eight longitudinal columns of 14-18 spines. Spines with triradiate bases, 8-73 long.

Testes 6-9 in number, anterior to cirrus sac, in two irregular columns, one layer deep. Vas deferens extensive, anterior to cirrus sac. Cirrus sac piriform, 117-148 long, 67-93 wide. Cirrus 470 long, tapering from 44-8 wide, armed with fine microtriches. Ovary 170-219 long, U-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, looping anterior to genital pore, relatively uniform in diameter along length, undulating slightly. Genital pore midventral, 12-24% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, filling entire proglottid when gravid. Vitellaria follicular, 30-50 in diameter, forming lateral columns

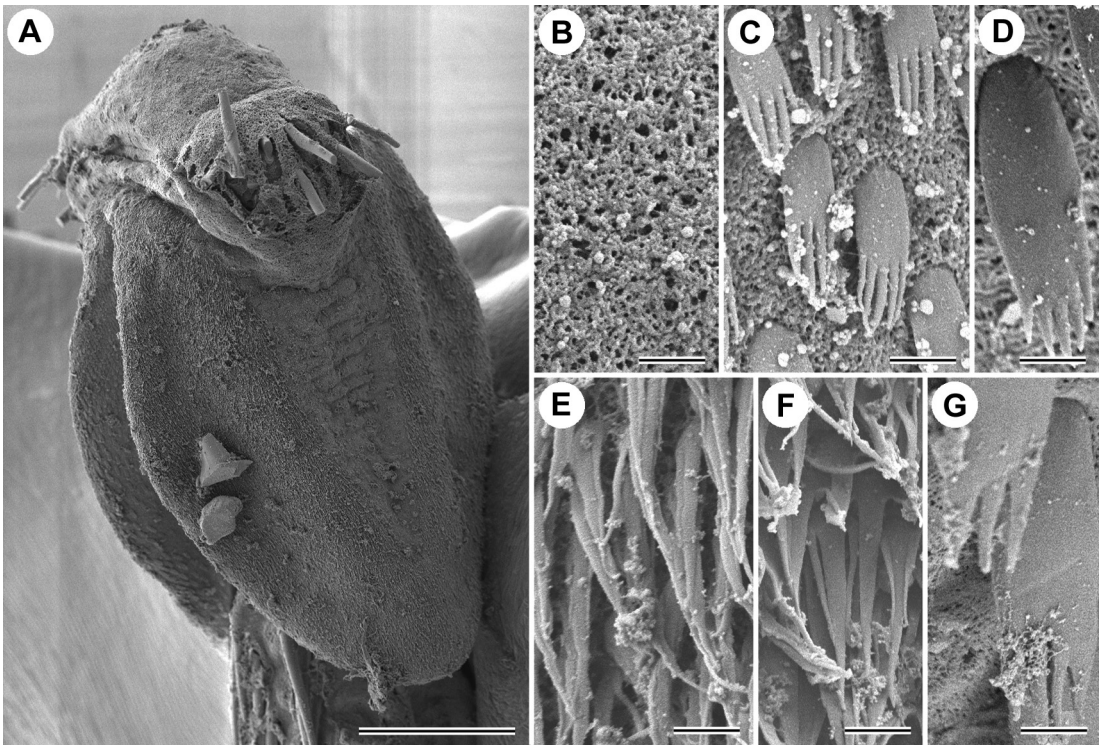


Fig. 44. Scanning electron micrographs of *Echinobothrium brachysoma*. A. Scolex. B. Apex of scolex. C. Proximal bothrial surface (anterior). D. Proximal bothrial surface (posterior). E. Distal (lateral) bothrial surface. F. Distal (medial) bothrial surface. G. Lateral surface of scolex proper. Scale bars: A, 50 µm; B-G, 1 µm.

extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 22 long, 15 wide, lacking appendages, packaged in cocoons of 2-5 eggs. Excretory ducts lateral.

Remarks

This species was described by Pintner (1889) for several specimens he collected from various skate species in Trieste. It was also reported by Stossich (1898) from Trieste, and by Rees (1961a) from the North Sea. Despite its interesting history (see below) and minimal description, the validity of this species has never been questioned It has been accepted as valid by Joyeux and Baer (1936), Euzet (1951), Rees (1961a, b), Schmidt (1970; 1986), Probert and Stobart (1989), Khalil (1994), Campbell and Andrade (1998), Ivanov and Campbell (1998a), Tyler and Caira (1999), and Ivanov and Hoberg (1999), who also included the species in their phylogenetic analysis. It appeared in their tree as part of a polytomy with *E. acanthinophyllum* and a clade comprising *E. acanthocolle*, *E. reesae*, *E. rhynchobati*, and *E. raji*.

Rees (1961a) stated that the type host for *E. brachysoma* was *Raja batis*. However, nowhere in Pintner's (1889) description of *E. brachysoma* is the type host given. The only indication of host provided by Pintner (1889, p. 397) is in the key, where the hosts for *E. brachysoma*, *E. affine* and *E. typus* are collectively referred to as "Rochenarten." *Echinobothrium brachysoma* was one of four species collected by Pintner in Trieste in 1888 and 1889. Based on the overall size and number of segments of these worms and a peculiar aspect of the excretory ducts in the scolex, Pintner (1889) considered this to be a new species. Rees (1961a) provided a detailed description of the function and anatomy of the scolex and genitalia

Because the description is much more thorough and supported by specimens deposited in the BMNH, the redescription here is based on that of Rees (1961a) and the specimens examined by Rees, together with material deposited by Lönnberg and Odhner at the Swedish Museum of Natural History.

Echinobothrium californiense Ivanov and Campbell, 1998

(Figs. 45-47)

Type host: *Platyrhinoidis triseriata* (Jordan and Gilbert), Thornback guitarfish (Rhinobatidae, Rhinobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Newport Beach, California, U.S.A.

Type material: HWML No. 31318 (holotype); HWML No. 31318 (paratypes, six specimens).

Specimens examined: Holotype; four paratypes on three slides.

Etymology: Not given, but presumably named for the type locality.

Description (Modified from Ivanov and Campbell [1998a].)

Worms 1.49-4.48 mm long, 260-422 wide, at terminal proglottid. Strobila apolytic, acraspedote, 4-9 proglottids. Mature proglottids 1-2 in number, 680-1,096 long, 284-352 wide. Gravid proglottids 1-2 in number, 1.056-1.104 mm long, 368-432 wide. Scolex bipartite, 365-550 long, consisting of scolex proper and cephalic peduncle. Scolex proper 288-316 long, 160-176 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Twenty-one apical hooks in each dorso-ventral group. Hook formula {(9-12) 10/11 (9-12)}, apical hooks solid, hooks gradually increasing in length toward center of group. Lateral hooklets arranged in

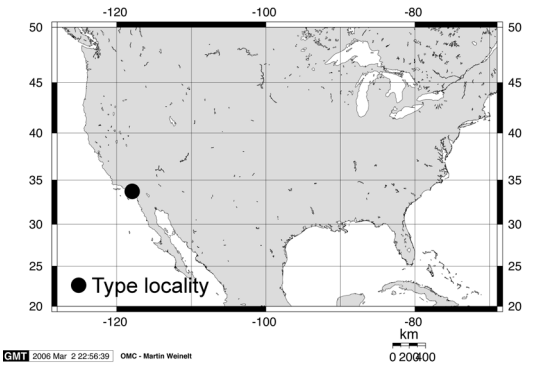


Fig. 45. Distribution of *Echinobothrium californiense*.

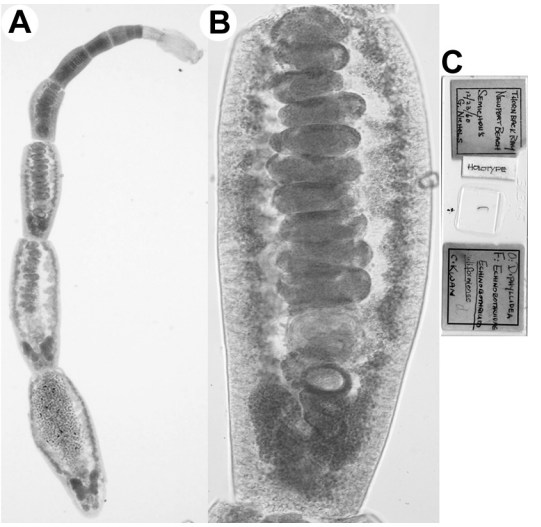


Fig. 46. Light micrographs of *Echinobothrium californiense*. A. Whole worm. B. Mature proglottid. C. Holotype slide HWML No. 31318.

single continuous row, uniformly arranged. Bothria 198-272 long, 106-176 wide, with cleft at posterior margin, proximal surfaces covered with spinitriches. Cephalic peduncle 116-147 long, 65-96 wide, armed with eight longitudinal columns of 5-7 spines. Cephalic peduncle spines with triradiate bases, 6-54 long.

Testes 8-11 in number, 34-72 long, 88-124 wide, anterior to cirrus sac, in single column, one layer deep. Vas deferens minimal, entirely anterior to cirrus sac. Cirrus sac oval, 57-144 long,72-128 wide. Cirrus 81-115 long. Ovary bilobed, 96-208 long, 160-222 wide, H-shaped in dorso-ventral view. Vagina thick-walled, muscular, posterior to genital pore, relatively uniform in width, undulating slightly. Mehlis' gland posterior to ovarian isthmus, 68 long, 72 wide. Genital pore midventral, 19-33% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development, expanding to fill gravid proglottid. Vitellaria follicular, 20-28 in diameter, forming two lateral columns extending entire length of proglottid, uninterrupted by ovary. Eggs unembryonated, with only one or two vitelline cells per egg. Excretory ducts lateral.

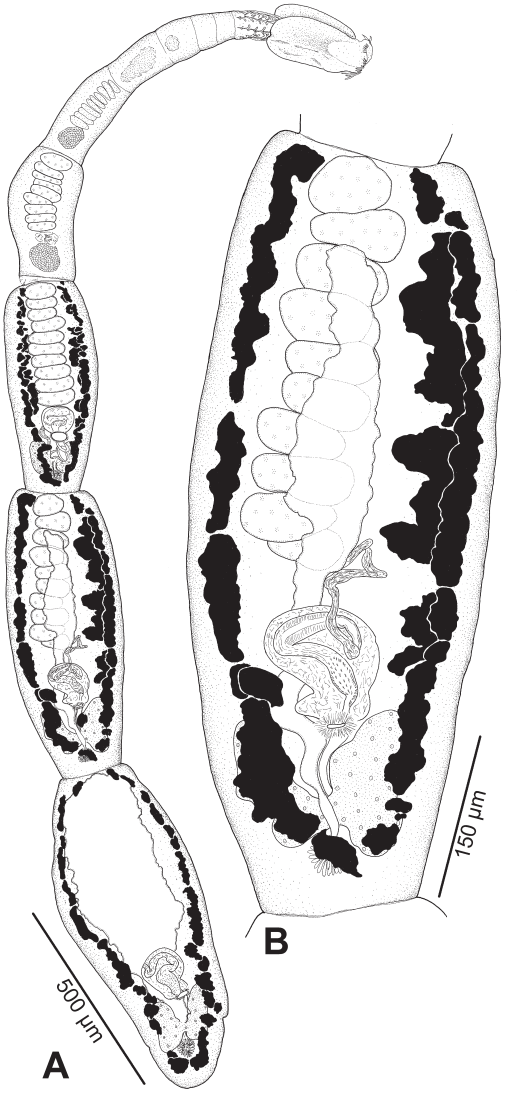


Fig. 47. Line drawings of *Echinobothrium californiense*. A. Whole worm. B. Mature proglottid.

Remarks

The hook formula of this species is sufficient to distinguish it from all other species in the genus except *E. hoffmanorum*. It can be distinguished from that species in possessing fewer spines per column on the cephalic peduncle (5-7 vs. 10-17).

Material of this species was collected in 1960 and deposited in the HWML where it remained undescribed until Ivanov and Campbell (1998a) described it as a new species. This species was also included in the

phylogenetic analysis presented by Ivanov and Hoberg (1999). In their tree, *E. californiense* was placed as the sister species to the clade containing *E. affine*, *E. raschii*, and *E. pigmentatum*.

Echinobothrium chisholmae Jones and Beveridge, 2001

(Figs. 48-49)

Type host: *Rhinobatos typus* Bennett, Giant shovelnose ray (Rhinobatidae, Rhinobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Type material: QM No. G218096 (holotype); QM Nos. G218097-106 (10 specimens), SAMA No. AHC 28330 (10 specimens), BMNH No. 2000.8.3.4-7 (five specimens), USNPC No. 90608 (five specimens), and MNHN Nos. 851 HF 141-143 CIX (five specimens) (paratypes).

Specimens examined: None.

Genbank accession numbers: AF286986 (18s rDNA); AF286922 (28s rDNA).

Etymology: This species was named in honor of Dr. L. Chisholm.

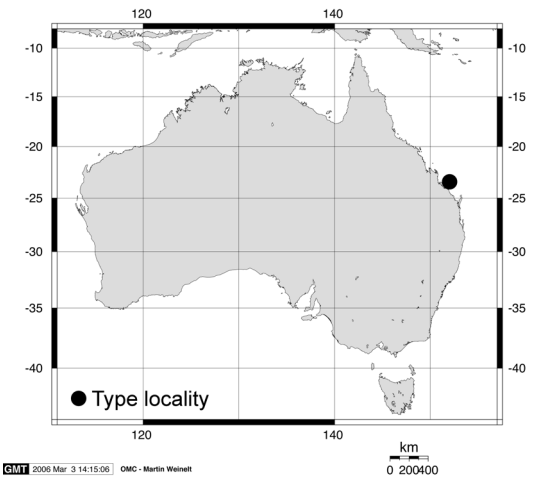


Fig. 48. Distribution of *Echinobothrium chisholmae*.

Description (Modified from Jones and Beveridge [2001].)

Worms 1.5-2.6 mm long, 50-220 wide at terminal proglottid. Strobila euapolytic, acraspedote, 4-6 proglottids. Mature proglottids 130-1,040 long, 50-220 wide. Scolex bipartite, 280-350 long, consisting of scolex proper and cephalic peduncle. Scolex proper consisting of armed apical rostellum and one dorsal and one ventral bothrium. Eleven apical hooks in each dorso-ventral group. Hook formula {(3-6) 6/5 (3-6)}, apical hooks solid, hook lengths all gradually increase toward center of group. Lateral hooklets arranged in two groups. Bothria 220-350 in diameter, proximal surfaces covered with pectinate spinitriches each bearing 3-5 relatively equal length digits. Cephalic peduncle 290-550 long, 50-100 wide, armed with eight longitudinal columns of 21-29 spines. Cephalic peduncle spines with triradiate bases, 10-12 to 60-96 long.

Testes 8-13 in number, 30-40 long, 50-110 wide, anterior to cirrus sac, in single column, one layer deep. Vas deferens minimal, entering cirrus sac anteriorly. Cirrus sac oval, 57-144 long, 72-128 wide. Cirrus 81-115 long. Ovary bilobed, 160-240 long, U-shaped in dorso-ventral view. Vagina thin-walled, looping anterior to genital pore, relatively uniform in width, coiling slightly. Mehlis' gland posterior to ovarian isthmus, approximately 50 long, 40 wide. Genital pore midventral, in posterior third of proglottid, overlapping or posterior to ovary. Vitellaria follicular, forming two lateral columns extending nearly entire length of proglottid, uninterrupted by ovary. Excretory ducts lateral.

Remarks

The hook formula of this species is sufficient to distinguish it from all others in the genus except *E. affine*, *E. harfordi*, *E. bonasum*, *E. fautleyae* and *E. syrtensis*. *Echinobothrium chisholmae* can be distinguished from *E. syrtensis* by its possession of cephalic peduncle armature, a feature lacking in that species. This species can be distinguished from both *E. bonasum* and *E. fautleyae* by its possession of testes arranged in a single column, and lateral hooklets arranged in two

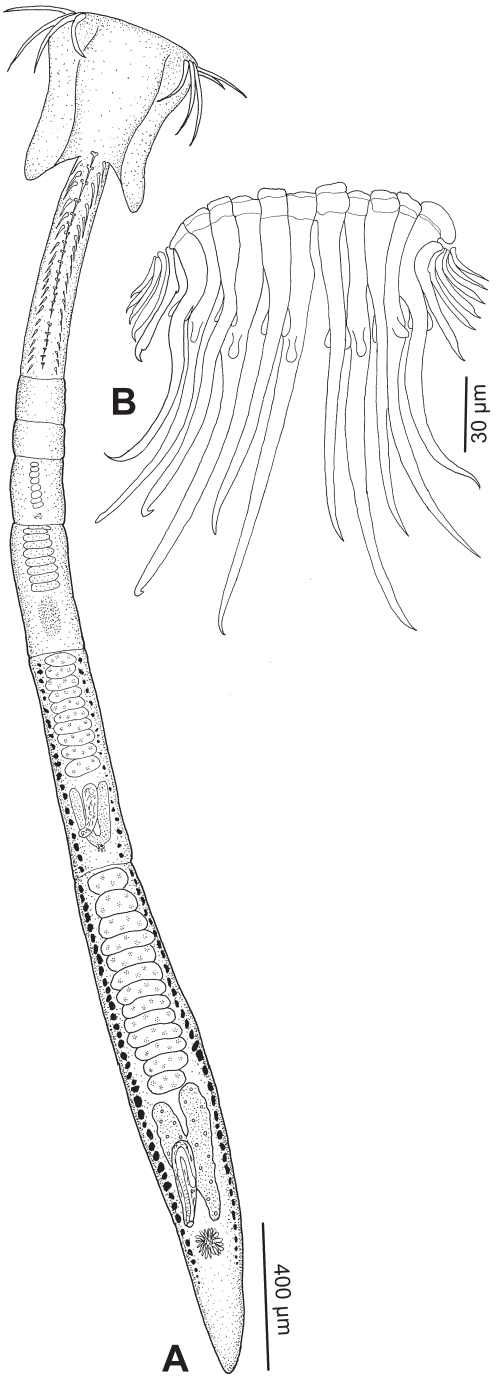


Fig. 49. Line drawings of *Echinobothrium chisholmae*. A. Whole worm. B. Apical hooks. Redrawn from Jones and Beveridge (2001).

groups; the latter two species have testes in two or more columns and lateral hooklets arranged in a single continuous row. This species most closely resembles *E. affine* and *E. harfordi*. It can be distinguished from *E. harfordi* in its possession of testes arranged in a single column, as opposed to two columns for both latter species, and in its possession of 21-29 cephalic peduncle spines per column versus 11-14. *Echinobothrium chisholmae* differs from *E. affine* in its possession of testes arranged in a single column, as opposed to two and in its possession of an ovary that is U-shaped rather than H-shaped.

This species is the second species of *Echinobothrium* to be recorded from Heron Island. DNA sequence data for this species was included in the phylogenetic analysis of Olson *et al.* (2001) who showed a closer relationship between this species and *E. rhynchobati* (as *Macrobothridium rhynchobati*) than between this species and *E. harfordi*. Specimens of *E. chisholmae* were not examined for this study, nor was it included in the phylogenetic analysis, as the description had just recently been published.

Echinobothrium clavatum Probert and Stobart 1989

(Figs. 50-52)

Type host: *Raja clavata* L., Thornback ray (Rajidae, Rajiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Irish Sea, north Wales.

Additional localities: North Sea, 17 Mi. N. of Troop Head (approx. 57.7˚N, 4.1˚W); English Channel at Plymouth (approx. 50.3˚N, 2.2˚W); Sète, France.

Type material: BMNH No. 1988.6.1.1 (lectotype); BMNH No. 1988.6.1.2-3 (syntypes).

Voucher material: BMNH No. 1987.6.23.29-54 (Troop Head); BMNH No. 1989.1.31.7-9 (Plymouth); two specimens on one slide in the personal collection of L. Euzet (Sète).

Specimens examined: Lectotype; five syntypes on one slide (BMNH No. 1988.6.1.1-

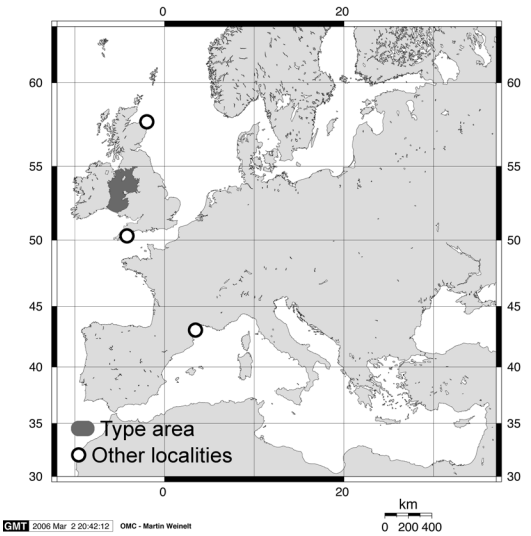


Fig. 50. Distribution of *Echinobothrium clavatum*.

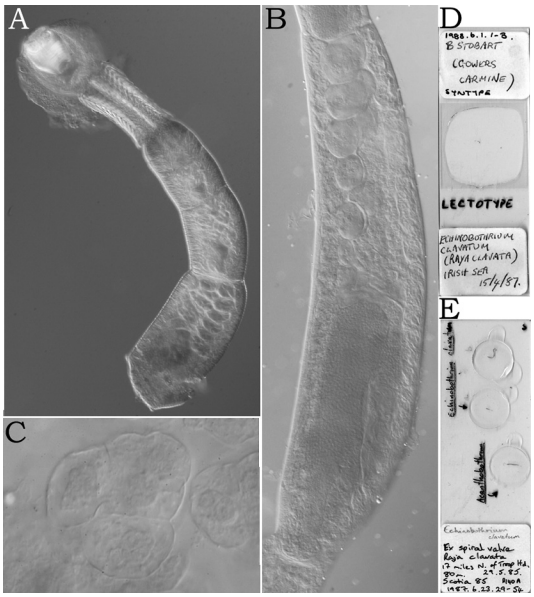


Fig. 51. Light micrographs of *Echinobothrium clavatum*. A. Lectotype. B. Mature proglottid. C. Egg packets. D. Lectotype slide BMNH No. 1988.6.1.1. E. Voucher slide BMNH No. 1987.6.23.29-54.

3); two vouchers on one slide (BMNH No. 1987.6.23-54); three specimens on one slide (BMNH No. 1989.1.31.7-9); both specimens from L. Euzet's collection.

Etymology: Not given, but presumably named after its host.

Description (Modified from Probert and Stobart [1989].)

Worms 2.97-5.44 mm long, 420-500 wide at terminal proglottid. Strobila apolytic, acraspedote, 7-8 proglottids. Mature proglottids 1-2 in number, 650-670 long, 233-300 wide. Gravid proglottids 0-1 in number, 690-1,870 long, 285-500 wide. Scolex bipartite, 340-790 long, consisting of scolex proper and cephalic peduncle. Scolex proper 225-323 long, 175-198 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Fifteen apical hooks in each dorsoventral group. Hook formula {(3-4) 8/7 (3-4)}, apical hooks solid, hooks gradually increasing in length toward center of group. Lateral hooklets arranged in two groups. Bothria 200-275 long, 175-198 wide, proximal surfaces covered with pectinate spinitriches each bearing 3-5 relatively equal length digits, distal surfaces with long filitriches. Cephalic peduncle 173-515 long, 58-98 wide, armed with eight longitudinal columns of 11-16 spines, terminating in a small velum. Cephalic peduncle spines with triradiate bases, 16-61 long.

Testes 11-14 in number, 50-98 long, 95-110 wide, anterior to cirrus sac, in single column, one layer deep. Vas deferens extensive, entirely anterior to cirrus sac. Cirrus sac oval, 125-195 long, 75-108 wide. Ovary bilobed, 288-615 long, 178 wide, H-shaped in dorso-ventral view. Vagina thick-walled, muscular, posterior to genital pore, relatively uniform in width, undulating slightly. Seminal receptacle present. Genital pore midventral, 16-33% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development, expanding to fill gravid proglottid. Vitellaria follicular, forming two lateral columns extending entire length of proglottid, uninterrupted by ovary. Egg shape not determined (eggs collapsed in whole mounts), in cocoons of approximately 4-7. Excretory ducts lateral.

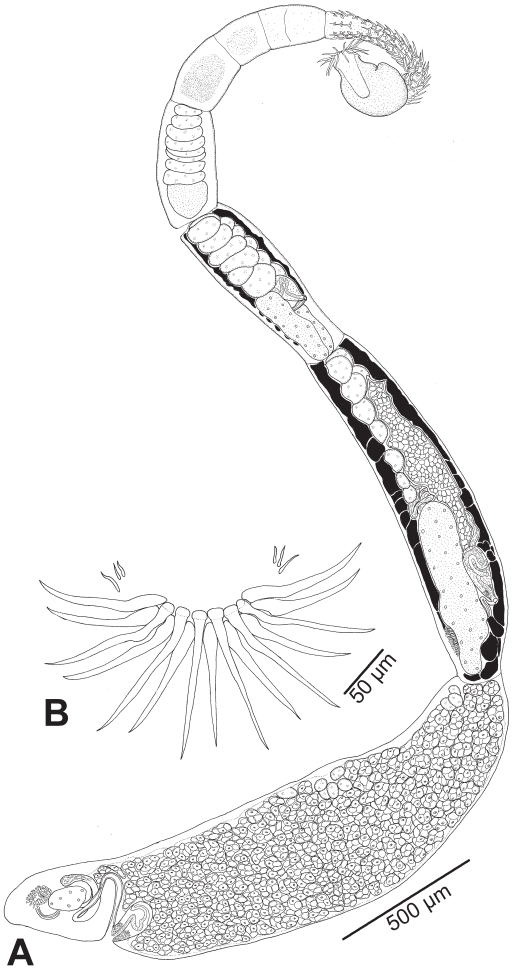


Fig. 52. Line drawings of *Echinobothrium clavatum*. A. Whole worm. B. Apical hooks.

Remarks

The unique hook formula of this species is sufficient to distinguish it from all other species in the genus.

This species was described by Probert and Stobart (1989), from several immature specimens. The description was the first to include scanning electron micrographs, and revealed the presence of pectinate spinitriches on the bothria. This species has been considered valid by all subsequent authors, including Ivanov and Hoberg (1999), but was excluded from their cladistic analysis due to lack of morphological data.

Along with *E. typus*, *E. affine*, and *E. brachysoma*, this is among four species of *Echinobothrium* reported from the host *Raja clavata*, and may be among the species illustrated as *E. typus* by Van Beneden (1858; plate XIX, fig. 2) (see remarks on *E. typus*). The authors of this species did not designate a holotype; rather, they deposited a syntype series. Examination of this type material revealed that one of the syntypes (BMNH No. 1988.6.1.1) was slightly more mature, and this specimen has been designated the lectotype here. However, none of the syntypes was fully mature, and therefore nearly all of the reproductive anatomy described here was observed in voucher specimens in the BMNH that had been collected from the same host in the North Sea and the English Channel.

Echinobothrium coenoformum Alexander, 1963

(Figs. 53-54)

Type host: *Dipturus nasuta* (Müller and Henle), New Zealand rough skate (Rajidae, Rajiformes) (as *Raja nasuta*).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Cook Strait, New Zealand.

Additional localities: Oamaru Harbor, New Zealand.

Type material: Holotype ZW No. 226.

Specimens examined: Holotype.

Etymology: The specific epithet of this species is derived from the Greek "koinos" (shared in common), referring to the commonalities shared by this species and others found in *Raja* species.

Description (Modified from Alexander [1963].)

Worms up to 3.0 mm long, 260-455 wide at terminal proglottid. Strobila euapolytic, acraspedote, 4-7 proglottids. Mature proglottids one in number, 802-1,683 long, 260-455 wide. Scolex bipartite, 580 long, consisting of scolex proper and cephalic peduncle. Scolex proper 376 long, 182-416 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Seventeen apical hooks in each dorso-ventral group. Hook formula {1 9/8 1}, apical hooks solid, hooks gradually

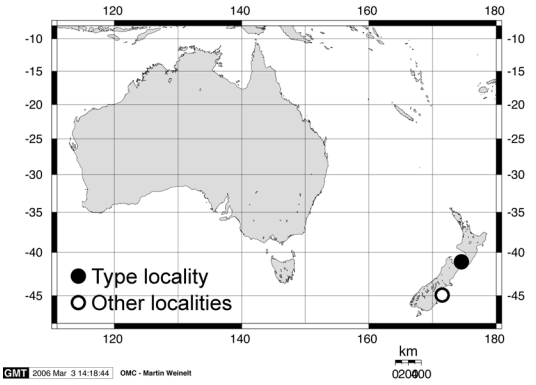


Fig. 53. Distribution of *Echinobothrium coenoformum*.

increasing in length toward center of group. Bothria 234-468 long, 182-416 wide, proximal surfaces covered with spinitriches. Cephalic peduncle 260-390 long, 100 wide, armed with eight longitudinal columns of 11-15 spines. Cephalic peduncle spines with triradiate bases, 10-88 long.

Testes 10-18 in number, 87-90 in diameter, anterior to cirrus sac, in three irregular columns, one layer deep. Vas deferens extensive, extending lateral to cirrus sac. Cirrus sac oval, 195-300 long, 120-180 wide. Cirrus armed along its length with stout recurved microtriches. Ovary bilobed, 298 long, H-shaped in dorso-ventral view. Vagina thick-walled, muscular, looping anterior to genital pore, expanded distally, coiling slightly. Genital pore midventral, 41% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development, expanding to fill gravid proglottid. Vitellaria follicular, forming two lateral columns extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 27-31 long, 22-25 wide. Excretory ducts lateral.

Remarks

The unique hook formula of this species distinguishes it from all other species in the genus except *E. reesae*. This species differs from *E. reesae* in its possession of cephalic peduncle armature, a feature lacking in the latter species.

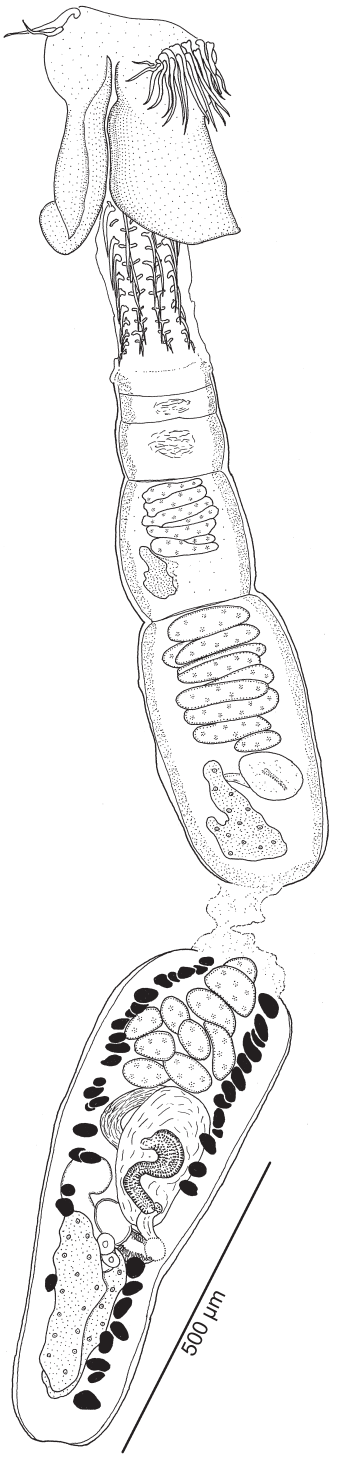


Fig. 54. Line drawing of *Echinobothrium coenoformum*.

This species, described by Alexander (1963), is one of two species of *Echinobothrium* reported from New Zealand. It has not been reported since the original description. It has been considered a valid species by all subsequent workers and was included in the keys of Probert and Stobart (1989) and Ivanov and Campbell (1998a). Ivanov and Hoberg (1999) included this species in their phylogenetic analysis of the order Diphyllidea. In their tree, *E. coenoformum* appeared as the sister species to a clade comprising *E. bonasum*, *E. californiense*, *E. pigmentatum*, *E. raschii*, and *E. affine*. Although the original description was based on several specimens, only the holotype specimen was deposited; the location of the remaining specimens is unknown (C. Alexander, pers. comm.). Collections of the type host made in New Zealand in 2000 as part of this study failed to yield additional specimens of this parasite species.

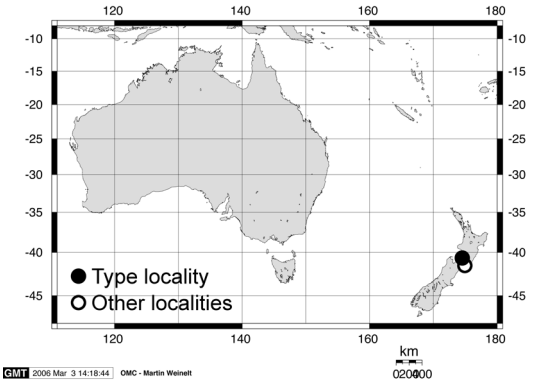


Fig. 55. Distribution of *Echinobothrium coronatum*.

Echinobothrium coronatum Robinson, 1959

(Figs. 55-56)

Type host: *Mustelus lenticulatus* Phillipps, Spotted estuary smooth-hound (Triakidae, Carcharhiniformes).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: Wellington Harbor, New Zealand.
Additional localities: Cook Strait, New Zealand.
Type material: ZW Nos. 202a, 202b (egg mount) (holotype).
Specimens examined: Holotype.
Etymology: Not given.

Description (Modified from Robinson [1959].)

Worms 6.5 mm long. Strobila acraspedote, proglottid apolysis unknown, 21 proglottids. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper consisting of armed apical rostellum and one dorsal and one ventral bothrium. Hook formula 14 (20) 14, (hook arrangement unknown), lateral hooklets in two groups, in a staggered arrangement. Bothria 380 long. Cephalic peduncle 1.30 mm long, armed with eight longitudinal columns of 32 spines. Spines with triradiate bases, up to 95 long.

Testes 9-11 in number, anterior to cirrus sac, up to 128 in diameter. Cirrus armed with microtriches. Genital pore midventral, anterior to ovary. Eggs irregular in shape, up to 27 in diameter.

Remarks

This species is distinguished from all others in the genus except *E. californiense*, *E. hoffmanorum*, and *E. pigmentatum* by the number of apical hooks (20) in each dorsoventral group. *Echinobothrium coronatum* is distinguished from *E. californiense*, *E. hoffmanorum*, and *E. pigmentatum* in its possession of lateral hooklets arranged in two groups as opposed to a single continuous row.

This species was described by Robinson (1959) from a single specimen. The next report of this species came from Alexander (1963), who also collected a single specimen from *M. lenticulatus* in New Zealand. The whereabouts of the specimen collected by Alexander (1963) is unknown (C. Alexander, pers. comm.). The type material of this species, while available for inspection, was in poor condition; useful information on its anatomy could not be obtained for this species. Fortunately, the original description does allow differentiation between this and other species based on scolex features. Attempts at re-collecting this species for this study have been unsuccessful.

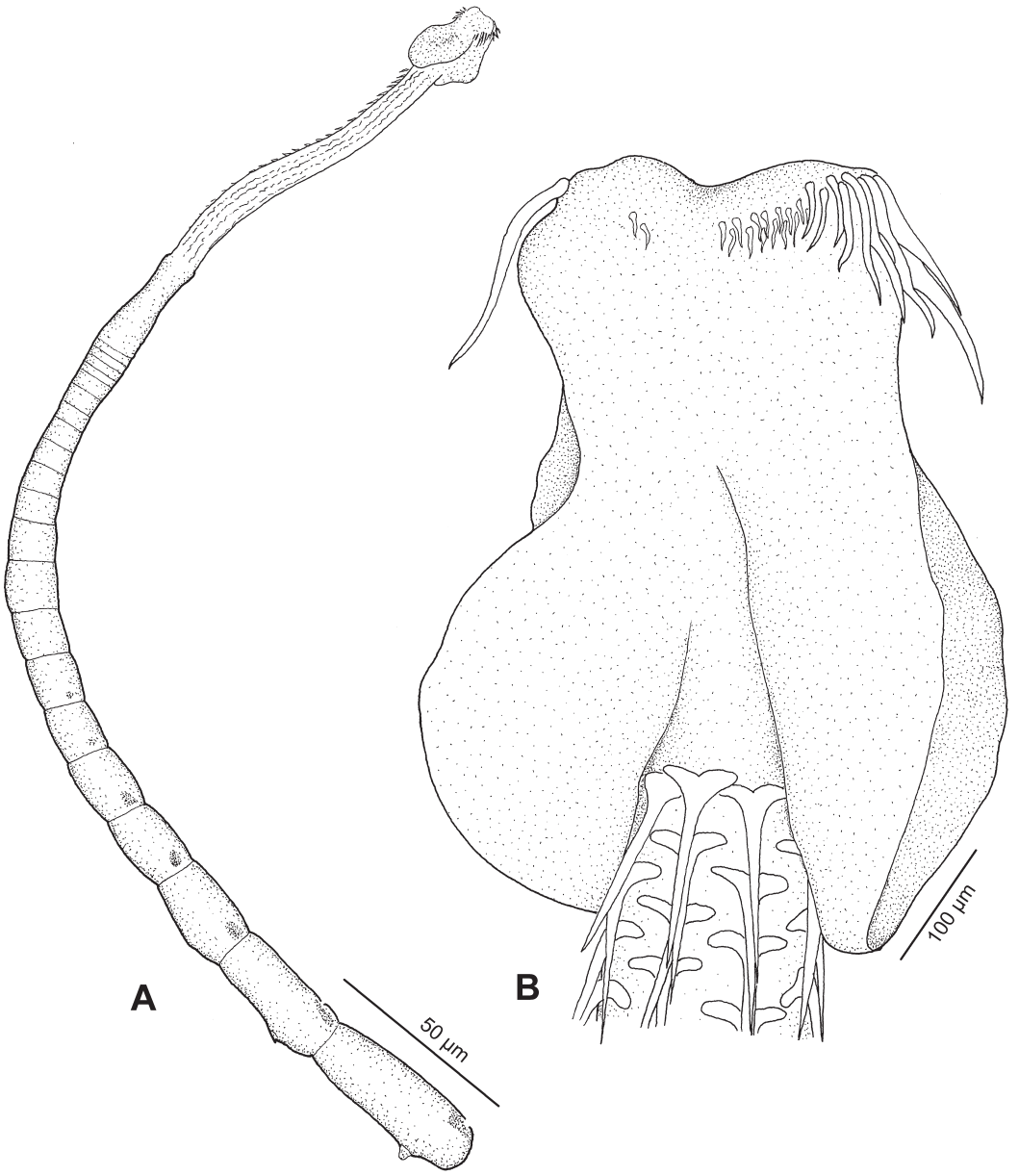


Fig. 56. Line drawings of *Echinobothrium coronatum*. A. Whole worm. B. Scolex. Redrawn from Robinson (1959).

Two lines of evidence suggest that this species is an accidental parasite in *M. lenticulatus*. The most compelling piece of evidence is that only two individual worms of this species have ever been reported, despite the necropsy of numerous specimens of the type host species from New Zealand. Specifically, examination of 77 specimens of *M. lenticulatus* collected from or near the type locality during the same season as specimens collected by Robinson (1959) and Alexander (1963) failed to recover even a single specimen of this worm. If *E. coronatum* is in fact present in this host, it now exhibits a prevalence of less than 1.3%, although Alexander reported it in one of just seven (14%) hosts he

examined. In addition, of the 33 valid species of *Echinobothrium*, only four are parasites of sharks (as opposed to batoids). Three of these species parasitizing sharks (*i.e.*, *E. musteli, E. scoliodoni, E. notoguidoi*) possess a distinctive feature not found in the species of *Echinobothrium* parasitizing batoids. They all possess several rows of small spines or microtriches on the scolex just posterior to the apical armature. Robinson (1959) neither mentioned nor illustrated this feature in his description, making this the only species of *Echinobothrium* reported from a shark that lacks this feature.

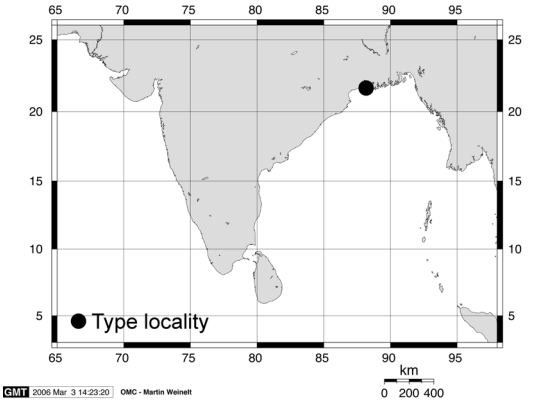


Fig. 57. Distribution of *Echinobothrium deeghai*.

Echinobothrium deeghai Gupta and Parmar, 1988

(Figs. 57-58)

Type host: *Pastinachus sephen* (Forsskål), Cowtail stingray (Dasyatidae, Myliobatiformes) (as *Trygon sephen* Günther).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: Deegha, Midnapur, West Bengal, India.
Type material: None designated.
Specimens examined: None.
Etymology: Named after the type locality.

Description (Modified from Gupta and Parmar [1988].)

Worms 20.22-23.60 mm long, 600-880 wide. Strobila apolytic, acraspedote, 40-50 proglottids. Mature proglottids 1.075-1.090 mm long, 600-720 wide, gravid proglottids 1.50-1.55 mm long, 840-880 wide. Scolex bipartite, 3.22-3.28 mm long, consisting of scolex proper and cephalic peduncle. Scolex proper consisting of armed apical rostellum and one dorsal and one ventral bothrium. Hook formula unknown. Apical rostellum with two groups of 14 hooks. Bothria 2.20-2.28 mm long, 1.72-1.98 mm wide. Cephalic peduncle 1.22-1.28 mm long, 400-430 wide, unarmed.

Testes 20-28 in number, anterior to cirrus sac, 50-80 long, 80-120 wide, in three to four irregular columns. Cirrus sac piriform, 220-260 long, 170-200 wide. Cirrus armed along its length with small microtriches. Ovary 380-420 long, U-shaped in dorso-ventral view, bilobed in cross section. Vagina coiling slightly. Genital pore midventral, in posterior third of proglottid, overlapping ovary. Uterus saccate. Vitellaria follicular, extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 30-35 long, 32-40 wide.

Remarks

The lack of cephalic peduncle armature separates this species from all but *E. euterpes*, *E. reesae*, *E. rhynchobati*, and *E. syrtensis*. This species differs from *E. euterpes* and *E. syrtensis* in its extraordinary size, being a much larger, longer worm. The number of hooks in each apical group (14) distinguishes this species from *E. reesae* which has 17 hooks per group, and *E. rhynchobati* with an entirely unique hook formula.

Although described by Gupta and Parmar in (1988), this species was not treated by Probert and Stobart (1989). Based on its lack of cephalic peduncle armature, Khalil (1994) implicitly considered this a *species inquirenda*; Campbell and Andrade (1997) were explicit in that judgment. Although it did not appear in the key published by Ivanov and Campbell (1998a), it was included in the phylogenetic analysis of the Diphyllidea by Ivanov and Hoberg (1999). Their analysis resulted in the placement of *E. deeghai* in a position basal to all other species of *Echinobothrium* on their tree. Neifar *et al.*

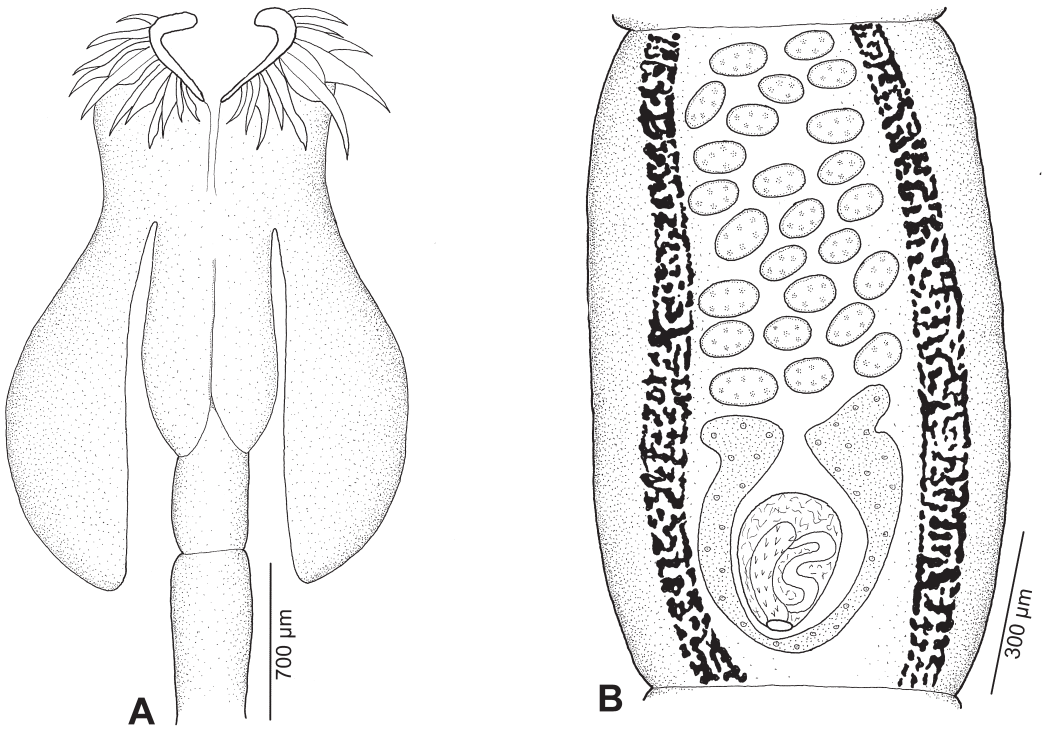


Fig. 58. Line drawings of *Echinobothrium deeghai*. A. Scolex (lateral view). B. Proglottid. Redrawn from Gupta and Parmar (1988).

(2001) suggested the transfer of this species into *Macrobothridium* based on its lack of cephalic peduncle armature, but refrained from doing so pending a more robust phylogenetic hypothesis than that provided by Ivanov and Hoberg (1999).

This species, like *Echinobothrium reesae*, was described as possessing an unarmed cephalic peduncle. Several aspects of the morphology, including its extraordinary size (for a diphyllidean), apparently robust hooks, and U-shaped ovary, bear a strong resemblance to *E. rhynchobati* described from the Persian Gulf. As with several other species described from that region of the world, this species requires further study. However, the description given by Gupta and Parmar (1988) is sufficient to distinguish this species from all others. Therefore, it is considered here to be valid.

Echinobothrium elegans **Tyler, n. sp.**

(Figs. 56-59)

Type host: *Taeniura lymma* (Forsskål), Bluespotted ribbontail ray (Dasyatidae, Myliobatiformes).

Status: Valid

Site of infection: Spiral intestine.

Type locality: Nhulunbuy-Gove, Northern Territory, Gulf of Carpenteria, Australia.

Type material: QM No. G 218426 (holotype); QM Nos. G 218427-218437 (11 specimens), SAMA No. AHC28401 (six specimens), USNPC No. 091661 (one specimen), BMNH No. 2001.7.31.1 (one specimen), and LRP No. 2199 (one specimen) (paratypes).

Etymology: The specific epithet "*elegans*" refers to the delicate morphology of this worm.

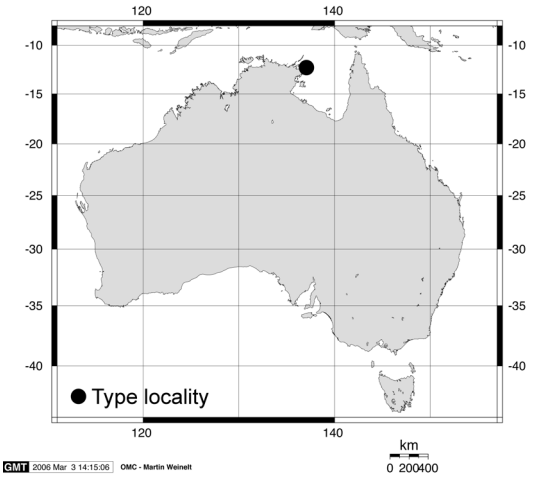


Fig. 59. Distribution of *Echinobothrium elegans* Tyler, n. sp.

Table 1. *Echinobothrium elegans* Tyler, n. sp. hook lengths.

		Apical hooks
Hook number	Hook type	
1	A	40-50 (44.6 ± 2.94; n=11)
2	B	45-55 (52.2 ± 3.52; n=11)
3	A	58-75 (68.4 ± 5.37; n=11)
4	B	70-80 (75.1 ± 3.24; n=11)
5	A	78-89 (86.0 ± 3.41; n=11)
6	B	85-98 (91.4 ± 3.35; n=11)
7	A	85-99 (93.1 ± 3.88; n=11)
8	B	94-108 (100.3 ± 3.44; n=11)
9	A	73-88 (82.1 ± 5.05; n=11)
10	B	95-110 (104.0 ± 3.77; n=11)
11	A	73-88 (81.7 ± 4.73; n=11)
12	B	94-105 (100.5 ± 2.88; n=11)
13	A	85-98 (93.5 ± 3.91; n=11)
14	B	88-95 (91.9 ± 2.59; n=11)
15	A	78-90 (86.0 ± 3.35; n=11)
16	B	70-78 (75.2 ± 2.49; n=10)
17	A	65-75 (71.9 ± 2.98; n=11)
18	B	48-56 (53.2 ± 2.52; n=11)
19	A	40-50 (45.1 ± 2.77; n=11)
		Lateral hooklets
Hooklet number		
1		30-18 (33.5 ± 1.98; n=15, *n*=24)
2		18-27 (23.0 ± 2.27; n=15, *n*=24)
3		15-23 (19.4 ± 1.70; n=15, *n*=23)
4		13-18 (16.0 ± 2.45; n=4)

Description

(Based on 21 mature worms, 6 mature proglottids in cross or longitudinal section, and 8 scolices prepared for electron microscopy)

Worms 985-1,580 (1,270 ± 167; n=21) long, 130-195 (159 ± 17; n=21) wide at terminal proglottid. Strobila euapolytic, acraspedote, 4-5 (5 ± 3; n=20) proglottids, with long filitriches. Mature proglottids 3-4 (4 ± 3; n=20) in number, 430-720 (554 ± 84; n=21) long, 125-195 (158 ± 19; n=21) wide, gravid proglottids not observed. Scolex bipartite, 288-378 (338 ± 28; n=20) long, consisting of scolex proper and cephalic peduncle. Scolex proper 200-268 (232 ± 19; n=19) long, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper covered with short filitriches. Nineteen apical hooks in each dorso-ventral group. Hook formula {(3-4) 10/9 (3-4)}, apical hooks solid, central type A hooks (odd numbers in Table 1) shorter than adjacent hooks, type B hooks (even numbers in Table 1) gradually increasing in length toward center of group. Hook lengths given in Table 1. Lateral hooklets arranged in two groups, lying in a single plane. Bothria 138-200 (172 ± 17; n=19) long, 143-202 (160 ± 17; n=14) wide, proximal surfaces covered with pectinate spinitriches and short filitriches. Pectinate spinitriches with three relatively equal length digits anteriorly, 5-6

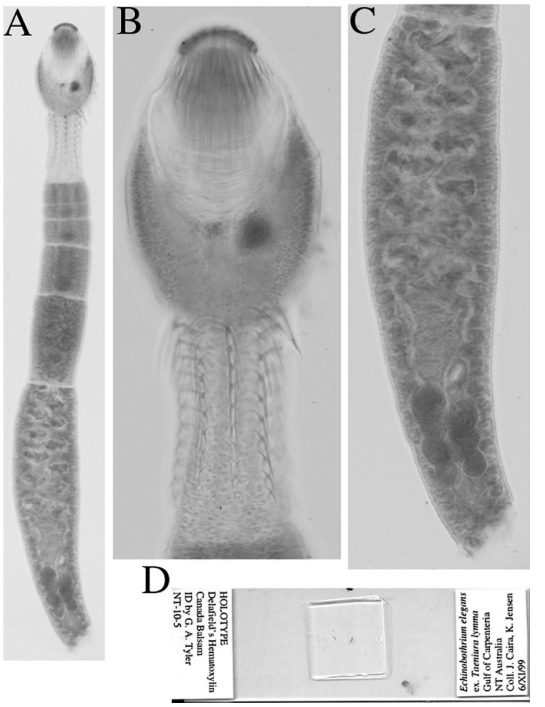


Fig. 60. Light micrographs of *Echinobothrium elegans* Tyler, n. sp. A. Whole worm. B. Scolex. C. Mature proglottid. D. Holotype slide QM No. G 218426.

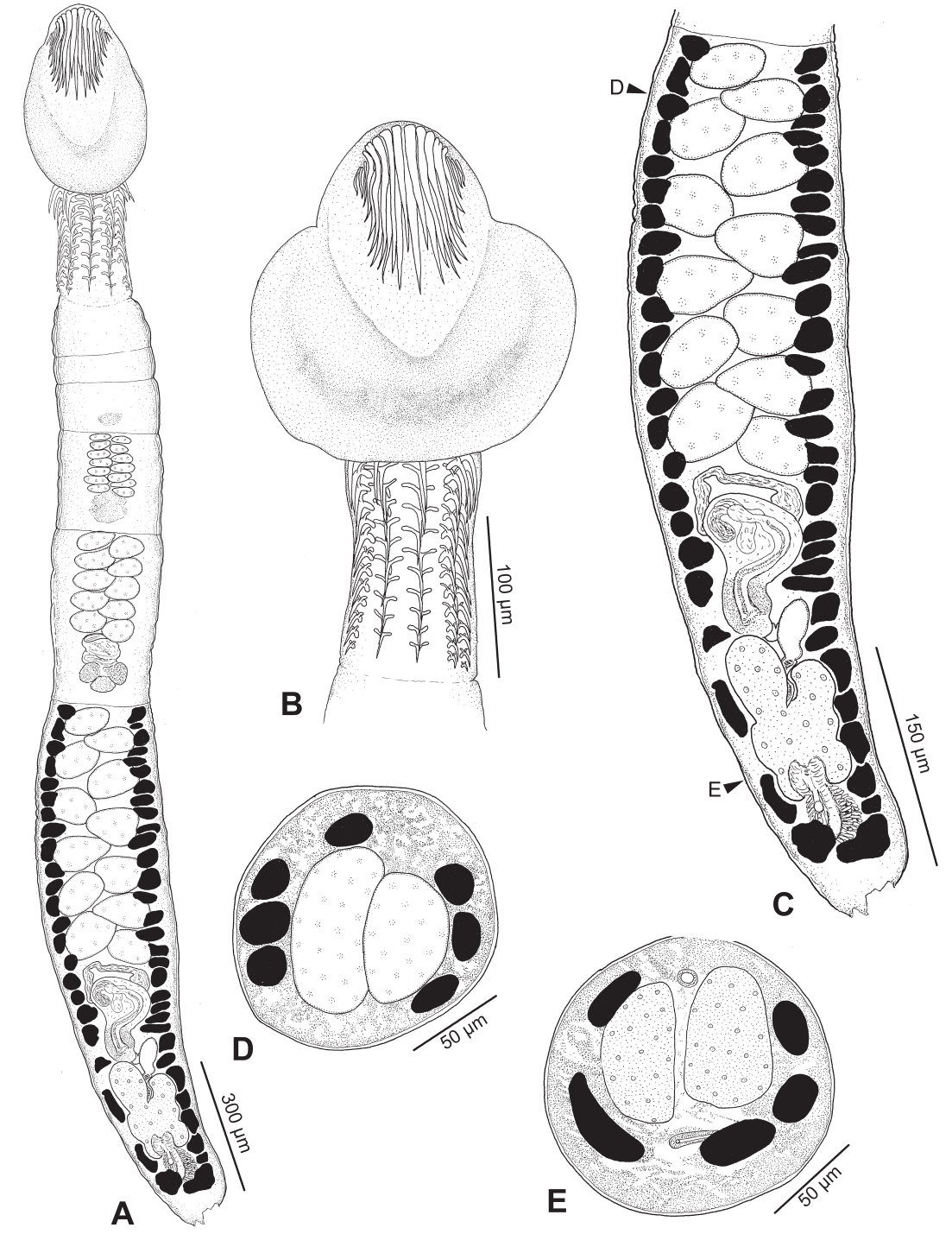


Fig. 61. Line drawings of *Echinobothrium elegans* Tyler, n. sp. A. Whole worm. B. Scolex. C. Mature proglottid. D. Cross section through proglottid at level indicated by “D” in C. E. Cross section through proglottid at level indicated by “E” in C.

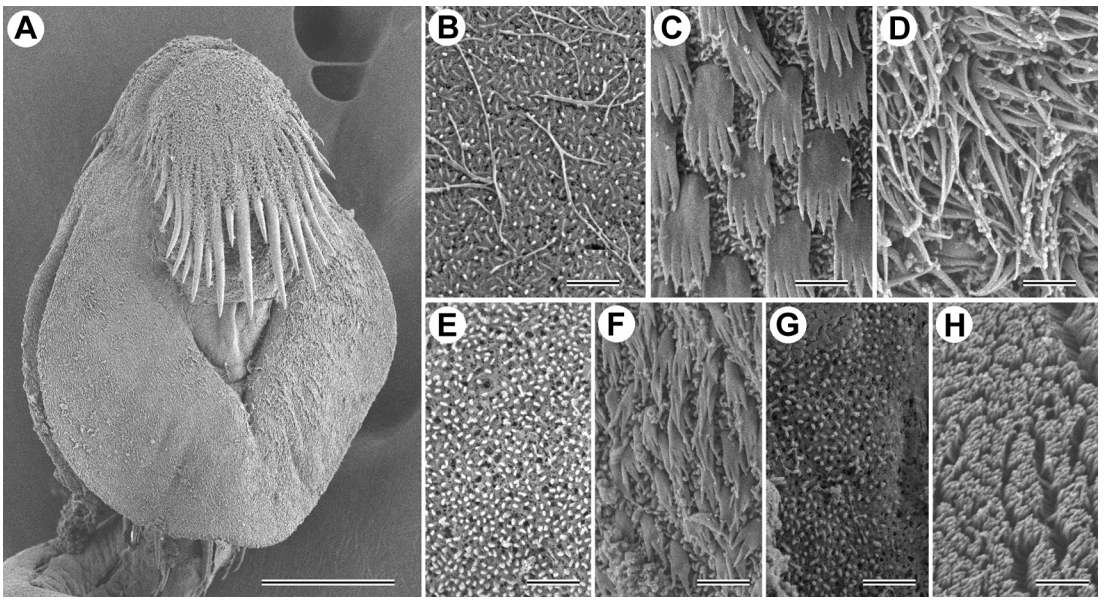


Fig. 62. Scanning electron micrographs of *Echinobothrium elegans* Tyler, n. sp. A. Scolex. B. Apex. C. Proximal bothrial surface. D. Distal (lateral) bothrial surface. E. Distal (medial) bothrial surface. F. Lateral surface of scolex proper. G. Cephalic peduncle. H. Strobila. Scale bars: A, 50 µm; B-H, 1 µm.

medially, and 3-5 posteriorly. Distal bothrial surfaces (except medial distal surface) with pectinate spinitriches each with three digits. Central digit conspicuously longer than others. Medial distal bothrial surface with short filitriches only. Cephalic peduncle 108-173 (140 ± 17; n=19) long, 58-95 (78 ± 9; n=20) wide, armed with eight longitudinal columns of 10-14 (12 ± 1; n=19, *n*=75) spines, covered with short filitriches. Spines with triradiate bases, 5-13 (8 ± 2; n=21) to 33-48 (39 ± 4; n=21) long.

Testes 12-18 (14 ± 1; n=17, *n*=22) in number, anterior to cirrus sac, 24-54 (37 ± 7; n=17, *n*=84) long, 44-75 (60 ± 7; n=15, *n*=74) wide, in two columns, one layer deep in cross section (Fig. 61D). Vas deferens minimal, anterior and lateral to cirrus sac. Cirrus sac piriform, 62-116 (87 ± 14; n=18) long, 40-75 (59 ± 10; n=17) wide. Cirrus armed along its length with fine microtriches. Ovary 68-135 (97 ± 19; n=20) long, 54-104 (75 ± 13; n=15) wide, H-shaped in dorso-ventral view, bilobed in cross section (Fig. 61E). Vagina thin-walled, posterior to genital pore, relatively uniform in diameter along length, coiling slightly. Mehlis' gland prominent, relatively large, 33-69 (47 ± 9; n=18) long, 26-50 (39 ± 6; n=14) wide. Genital pore midventral, 33-46% (40 ± 3; n=19) of proglottid length from posterior end of proglottid, anterior to ovary. Uterus thick-walled in early stages of development. Mature uterus not observed. Vitellaria follicular, forming two lateral bands with 2-4 follicles each in cross section, extending entire length of proglottid, uninterrupted by ovary, confluent posterior to ovary; follicles 10-30 (19 ± 5; n=20, *n*=100) long, 11-36 (23 ± 6; n=17, *n*=85) wide. Eggs not observed. Excretory ducts lateral.

Remarks

Echinobothrium elegans can be distinguished from all other species in the genus except *E. californiense*, *E. coronatum*, and *E. hoffmanorum* in its possession of rostellar armature of the formula {(3-4) 10/9 (3-4)}. *Echinobothrium elegans* differs from *E. californiense* in having fewer lateral hooklets in each group (3-4 vs. 9-12), more cephalic peduncle spines per column (10-14 vs. 5-8), and more testes per proglottid (12-18 vs. 8-11). This

new species differs from *E. coronatum* in possessing fewer lateral hooklets per group (3-4 vs. 14), fewer cephalic peduncle spines per column (10-14 vs. 32), fewer proglottids (4-5 vs. 21), and more testes (12-18 vs. 9-11). Finally, *E. elegans* differs from *E. hoffmanorum* in possessing fewer lateral hooklets per group (3-4 vs. 6-11) and more testes per proglottid (12-18 vs. 4-8).

Along with *E. heroniense and E. helmymohamedi*, this is the third species of *Echinobothrium* described from the blue-spotted stingray, *Taeniura lymma*. Williams' (1964) description of *Echinobothrium heroniense* was the first diphyllidean species described from this host. At that time, Williams (1964) discussed the possibility of misidentification of the host *T. lymma* because specimens caught in Australian waters hosted a different parasite fauna from those caught in the Red Sea. *Echinobothrium elegans* was found to co-occur with *E. heroniense* in the same host individual. This is the first published report of this phenomenon in diphyllideans.

Echinobothrium euterpes (Neifar, Tyler, and Euzet, 2001) n. comb.

(Figs. 63-65)

Synonym: *Macrobothridium euterpes* Neifar, Tyler, and Euzet, 2001 **n. syn.**

Type host: *Rhinobatos rhinobatos* (L.), Common guitarfish (Rhinobatidae, Rhinobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Zarzis, Tunisia (33˚15'N, 11˚10'E).

Additional localities: Sfax, Tunisia (34˚45'N, 10˚50'E).

Type material: MNHN No. 852 HF 144 CIX (holotype); MNHN No. 852 HF 145-147 CIX, BMNH No. 2000.7.28.1-4, and USNPC No. 90593 (paratypes).

Specimens examined: Holotype, all 24 paratypes.

Etymology: The name for this species, collected from a guitarfish, is derived from "Euterpe," a mythical music muse.

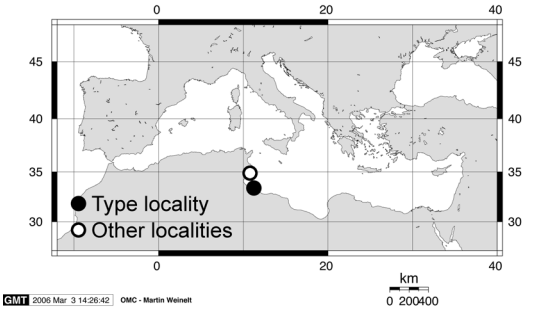


Fig. 63. Distribution of *Echinobothrium euterpes* n. comb.

Description (Modified from Neifar *et al.* [2001].)

Worms 2-4.5 mm (2.7 ± 0.29; n=25) in length, greatest width of strobila 200-350 (250 ± 19; n=25) generally at terminal proglottid. Strobila apolytic, acraspedote, 5-9 (6; n=25) proglottids. Immature proglottids 3-4 (n=22) in number. Mature proglottids 2-5 (3; n=22) in number, 500-2,100 (908 ± 46; n=25; *n*=34) long, 200-270 (256 ± 10; n=25; *n*=34) wide. Detached gravid proglottids 1.4-3.5 mm (2.4 ± 0.20; n=24) long, 250-500 (374 ± 26; n=24) wide. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 650-950 (792 ± 37; n=25) long, 400-800 (583 ± 57; n=25) wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex covered with long filitriches. Hook formula {(3-5) (13-15)/(14-16) (3-5)} , apical hooks solid, hooks gradually increasing in length toward center of group of group. Lateral hooklets arranged in two groups. Proximal bothrial surfaces with short filitriches and pectinate spinitriches each bearing 5-7 relatively equal length digits. Distal bothrial surfaces (except medial distal surface) with long filitriches. Medial distal bothrial surface with trifid pectinate spinitriches and long filitriches. Cephalic peduncle 100-150 (120 ± 12; n=25) long, 140-220 (175 ± 28; n=25) wide, unarmed, covered with small slender spinitriches.

Testes 27-46 (34; n=25; *n*=38) in number, anterior to cirrus sac, in 2-3 irregular columns, one layer deep. Vas deferens minimal, entirely anterior to cirrus sac. Cirrus sac piriform, 150-270 (217 ± 16; n=25) long, 100-

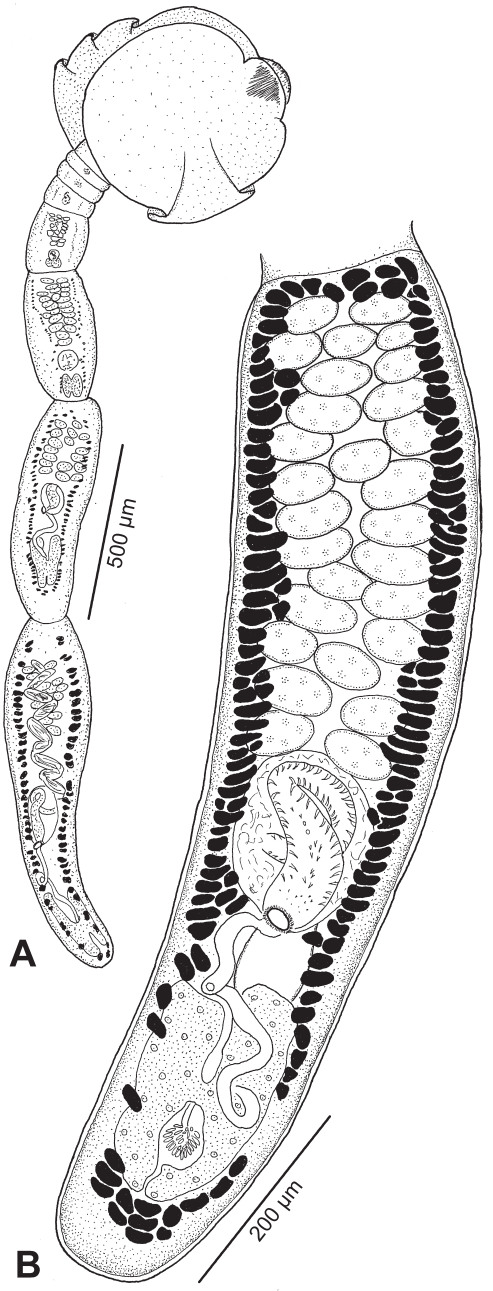


Fig. 64. Line drawings of *Echinobothrium euterpes* n. comb. A. Whole worm. B. Mature proglottid. Redrawn from Neifar *et al.* (2001).

180 (140 ± 10; n=25) wide. Cirrus 650-900 (820 ± 37; n=22) long, 80-100 (91 ± 3; n=22) in diameter, basal part armed with large "rose thorn" spinitriches, distal part lacking armature, narrow terminus with two spinitriches. Ovary 150-450 (258 ± 49; n=22) long, 100-180 (127 ± 10; n=22) wide, H-shaped in dorsoventral view, bilobed in cross section. Mehlis' gland dorsal, immediately posterior to ovarian isthmus, 45-65 (52 ± 3; n=12) long, 35-45 (41 ± 2; n=12) wide. Vagina thin-walled, entirely posterior to genital pore, relatively uniform in diameter along length, sinuous. Genital pore midventral, 25-40% (33.2 ± 2; n=22) of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, extending entire length of proglottid, in two lateral columns, uninterrupted by ovary, confluent posterior to ovary. Eggs round 24-30 (26 ± 2; n=24; *n*=27) in diameter, with single mucron, not packaged. Excretory ducts lateral.

Remarks

The unique hook formula of this species is sufficient to distinguish it from all others in the genus.

This species was described by Neifar *et al.* (2001) from *Rhinobatos rhinobatos* in Tunisian waters and assigned to the genus *Macrobothridium*. At that time, there was some question as to the validity of *Macrobothridium*, based on the results of a preliminary phylogenetic analysis by Ivanov and Hoberg (1999). Because of the preliminary nature of the results presented by Ivanov and Hoberg (1999), Neifar *et al.* (2001) considered *Macrobothridium* to be valid. In light of the phylogenetic analyses presented in this work, *Macrobothridium* is considered a junior synonym of *Echinobothrium*, with all the constituent species of the former transferred into the latter genus.

Echinobothrium euzeti Campbell and Carvajal, 1980

(Figs. 66-68)

Type host: *Sympterygia lima* (Poeppig), Filetail fanskate (Rajidae, Rajiformes) (as *Psammobatis lima*).

Status: Valid.

Site of infection: Spiral intestine.

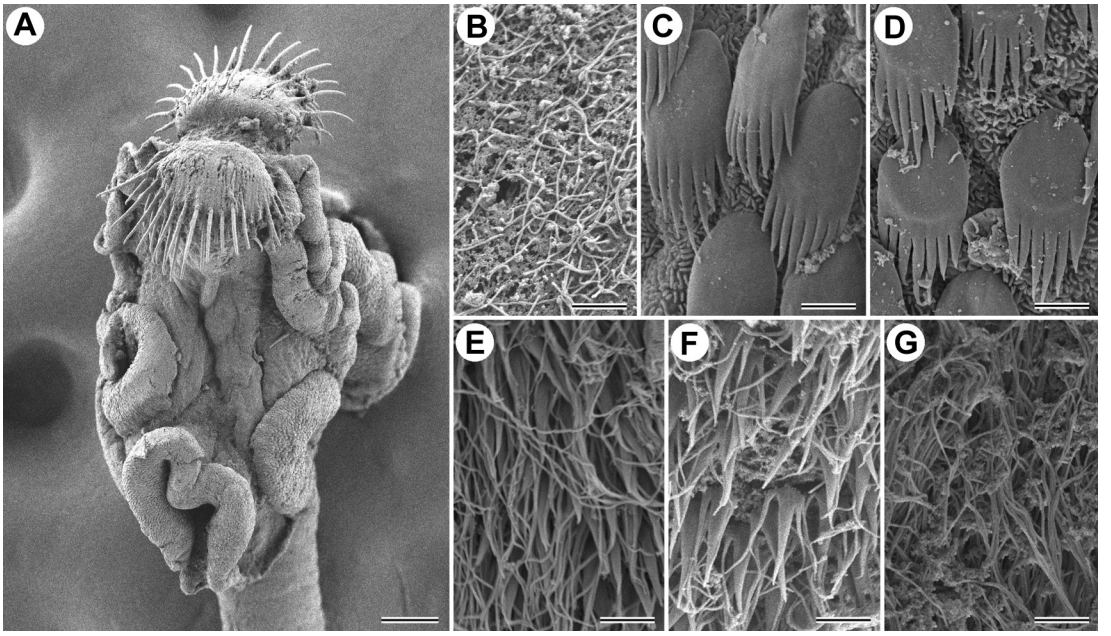


Fig. 65. Scanning electron micrographs of *Echinobothrium euterpes* n. comb. A. Scolex. B. Apex of scolex. C. Proximal bothrial surface (anterior). D. Proximal bothrial surface (posterior). E. Distal (medial) bothrial surface (anterior). F. Distal (medial) bothrial surface (posterior). G. Distal (lateral) bothrial surface. Scale bars: A, 50 µm; B-G, 1 µm.

Type locality: Constitución, Chile (35˚10'S, 72˚30'W).

Type material: USNPC No. 75773 (holotype); USNPC No. 75774 (one paratype).

Specimens examined: Holotype; one paratype.

Etymology: This species was named in honor of Professor L. Euzet.

Description (Modified from Campbell and Carvajal [1980].)

Worms up to 5.5 cm long, up to 900 wide at terminal proglottid. Strobila apolytic, acraspedote, 26-34 proglottids. Mature proglottids 1.12-2.96 mm long, 440-880 wide, gravid proglottids 1.25-2.00 mm long, 420-580 wide. Scolex bipartite, 2.100 mm long, consisting of scolex proper and cephalic peduncle. Scolex proper 1.00-1.04 mm long, 640-860 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Twenty-five apical hooks in each dorso-ventral group. Hook formula {(6-7) 13/12 (6-7)}, apical hooks solid, hooks gradually increasing in length toward center of each

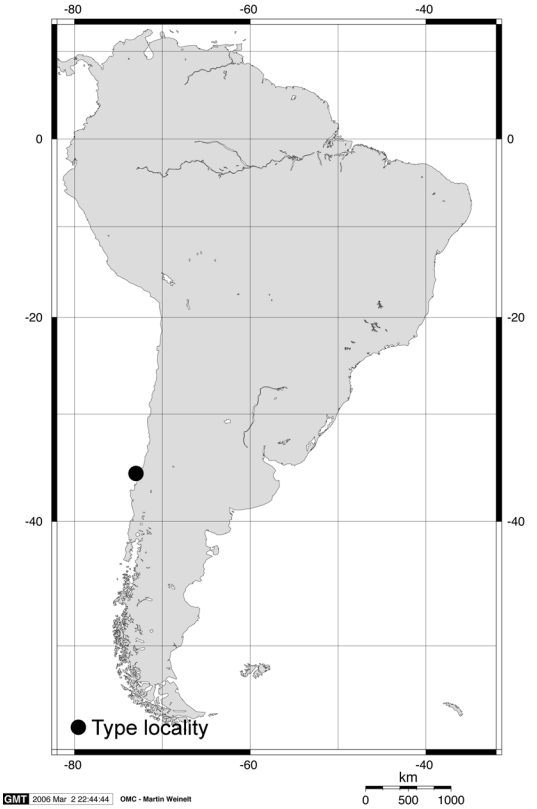


Fig. 66. Distribution of *Echinobothrium euzeti*.

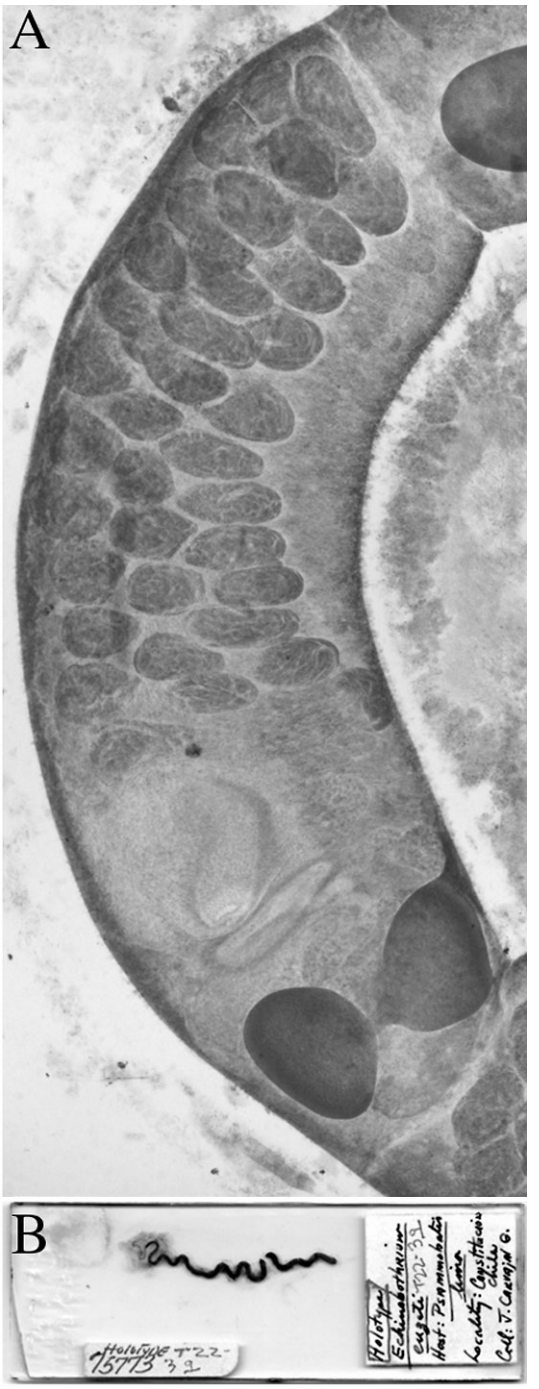


Fig. 67. Light micrographs of *Echinobothrium euzeti*. A. Mature proglottid. B. Holotype slide USNPC No. 75773.

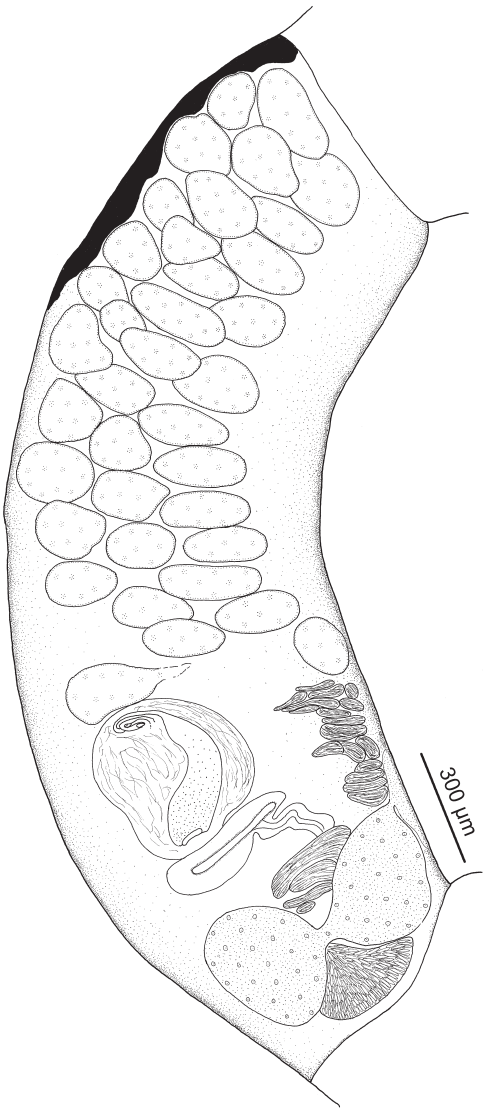


Fig. 68. Line drawing of proglottid of *Echinobothrium euzeti*.

group. Lateral hooklets uniformly arranged in continuous row. Bothria 835 long, with cleft in posterior margin, proximal and distal bothrial surfaces with pectinate spinitriches each bearing 5-6 relatively equal length digits. Cephalic peduncle up to 6.5 mm long, 289 wide, armed with eight longitudinal columns of 100-107 spines. Spines with triradiate bases, up to 56 long.

Testes 28-42 in number, anterior to ovary, 80-140 long, 160-260 wide, in 3-4 irregular columns, one layer deep. Vas deferens extensive, extending lateral to cirrus sac. Cirrus sac

piriform, 288-440 long, 168-296 wide. Cirrus armed with small, fine microtriches. Ovary 200-408 long, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thick-walled, posterior to genital pore, undulating slightly, relatively uniform in width. Genital pore midventral, anterior to ovary, 20-26% of proglottid length from posterior end of proglottid. Vitellaria follicular, extending entire length of proglottid, uninterrupted by ovary. Oncospheres approximately 20 in diameter.

Remarks

The unique hook formula and type A hook symmetry (see Fig. 2) of this species is sufficient to distinguish it from all other species in the genus.

This species was described by Campbell and Carvajal (1980), and has not been reported since. It has been considered to be valid by all subsequent workers, and was included in the keys of both Probert and Stobart (1989) and Ivanov and Campbell (1998a). This species was also included in the phylogenetic analysis of Ivanov and Hoberg (1999). In their tree, *E. euzeti* was the basal species in a clade also comprising *E. affine*, *E. raschii*, *E. pigmentatum*, *E. californiense*, *E. bonasum*, and *E. coenoformum*.

Although described from four specimens, there are only two specimens in the deposited type series. Both specimens were forcefully flattened between two glass slides, and neither was in particularly good condition. This process altered the dimensions of the worms, but perhaps more importantly, made observation under high magnification difficult because of the excessive thickness of the preparation.

Echinobothrium fautleyae Tyler and Caira, 1999

(Figs. 69-72)

Type host: *Rhinoptera steindachneri* Evermann and Jenkins, Pacific cownose ray (Rhinopteridae, Myliobatiformes).

Additional hosts: *Myliobatis californicus* Gill, Bat eagle ray (Myliobatidae, Myliobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Puertecitos, Gulf of California, México (30˚21'N, 114˚39'W).

Additional localities: Bahía de Los Angeles (28˚55´N, 110˚25´W), Santa Rosalia (27˚19'N, 112˚17'W), Loreto (26˚01' N, 111˚21'W), Punta Arenas (24˚08'N, 110˚25'W), Gulf of California, México; Puerto Viejo, Baja California Sur, México (24˚25'N, 111˚33'W).

Type material: CNHE No. 3340 (holotype); CNHE Nos. 3341-3342, USNPC Nos. 88217-88219, and HWML Nos. 33910-33911 (paratypes).

Voucher specimens: 47 specimens on seven slides from *R. steindachneri* from Puerto Viejo in the personal collection of L. Euzet.

Specimens examined: Holotype; all 22 paratypes; all 47 specimens from L. Euzet's collection.

Genbank accession numbers: AF124464 (18s rDNA).

Etymology: This species was named in honor of Dr. R. Fautley of Santa Rosa Junior College.

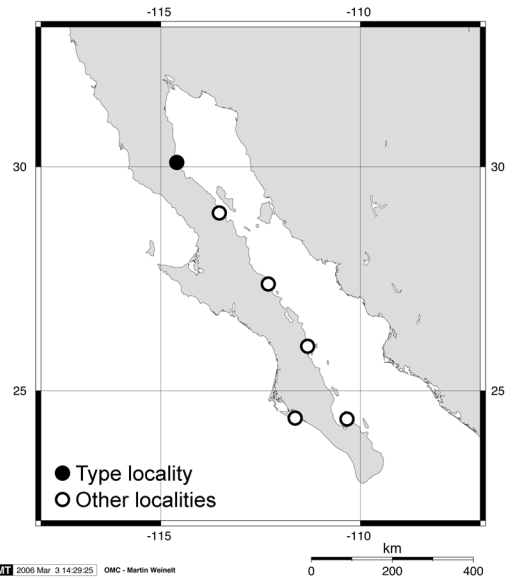


Fig. 69. Distribution of *Echinobothrium fautleyae*.

Description (Modified from Tyler and Caira [1999].)

Worms 920-2,795 (1,875 ± 488; n=16) long, 95-170 (116 ± 22; n=16) at terminal proglottid. Strobila acraspedote, apolytic, 4-6 (5.4 ± 7; n=16) proglottids, covered with long filitriches. Mature proglottids 1-2 (1.1 ± 0.3; n=16) in number, 348-1,000 (630 ± 176 n=16; *n*=17) long, 95-170 (120 ± 23; n=13; *n*=54) wide. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 163-294 (230 ± 37; n=23) long by 130-180 (156 ± 18; n=14) wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper covered with long filitriches. Eleven apical hooks in each dorso-ventral group. Hook formula {(11-12) 6/5 (11-12)}, apical hooks solid, hook lengths increasing toward center. First and last hooklet in each lateral group distinctly longer than others. Lateral hooklets arranged in continuous row, staggered in their arrangement. Bothria 125-238 (183 ± 35; n=20) long, 130-180 (156 ± 18; n=14) wide, proximal surfaces covered with pectinate spinitriches each bearing three digits, central digit conspicuously longer than others. Distal bothrial surfaces (except medial distal surface) with small pectinate spinitriches each bearing three relatively equal-length digits. Medial distal bothrial surface with pectinate spinitriches each bearing three digits, central digit conspicuously longer than others. Lateral surfaces of scolex proper with pectinate spinitriches each bearing three digits, central digit conspicuously longer than others. Cephalic peduncle 154-466 (311 ± 90; n = 22) long, 40-80 (60 ± 12; n=22) wide, armed with eight longitudinal columns of 19-36 (29.4 ± 4.6; n=23; *n*=34) spines, covered with short filitriches. Spines with triradiate bases, 5-13 (9 ± 2; n=23) to 45-83 (65 ± 11; n=23) long.

Testes 12-23 (17.7 ± 2.4; n=16; *n*=27) in number, anterior to cirrus sac, 24-69 (39 ± 9; n=13; *n*=64) long, 25-70 (47 ± 12; n=13; *n*=54) wide, in 2-3 columns, one layer deep. Vas deferens minimal, anterior to cirrus sac. Cirrus sac piriform, 49-138 (74 ± 25; n=12; *n*=13) long, 40-75 (53 ± 11; n=12; *n*=13) wide. Cirrus armed along its length with thorn-like microtriches. Ovary 75-200 (127 ± 35; n=13; *n*=14) long, 43-133 (71 ± 28; n=7; *n*=8) wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thick-walled, posterior to genital pore, uniform in diameter along length, coiling slightly. Mehlis' gland posterior to ovarian isthmus, 36-63 (46 ± 6; n=6; *n*=7) long, 29-48 (36 ± 6; n=6; *n*=7) wide. Genital pore midventral, 24-42% (32.9 ± 4.6; n=14; *n*=15) from posterior end of proglottid, anterior to ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, 10-28 (15 ± 6; n=1; *n*=10) long, 16-25 (20 ± 3; n=1; *n*=10) wide, forming two lateral bands consisting of 4-5 follicles each; bands nearly contiguous along midventral line in fully mature proglottids, extending from level of genital atrium to anterior margin of proglottid; vitelline follicles with non-distinct margins. Excretory ducts lateral.

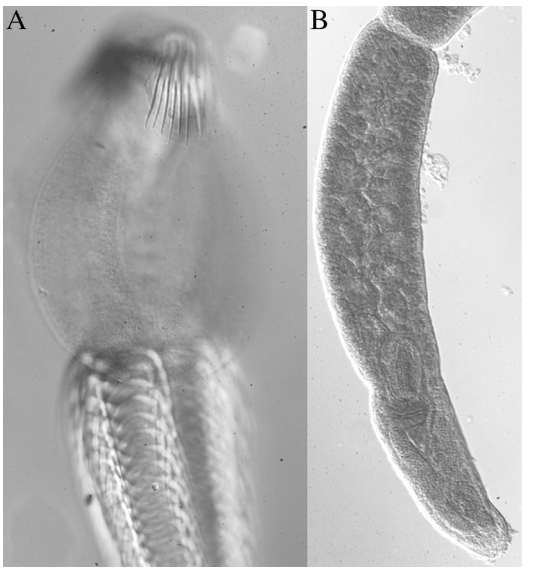


Fig. 70. Light micrographs of *Echinobothrium fautleyae*. A. Scolex. B. Mature proglottid.

Remarks

The hook formula of this species is sufficient to distinguish it from all other species in the genus except *E. affine*, *E. harfordi*, and *E. bonasum*. *Echinobothrium fautleyae* is distinguished from the former two species in its possession of lateral hooklets arranged in a single continuous row as opposed to two groups. *Echinobothrium fautleyae* is distin-

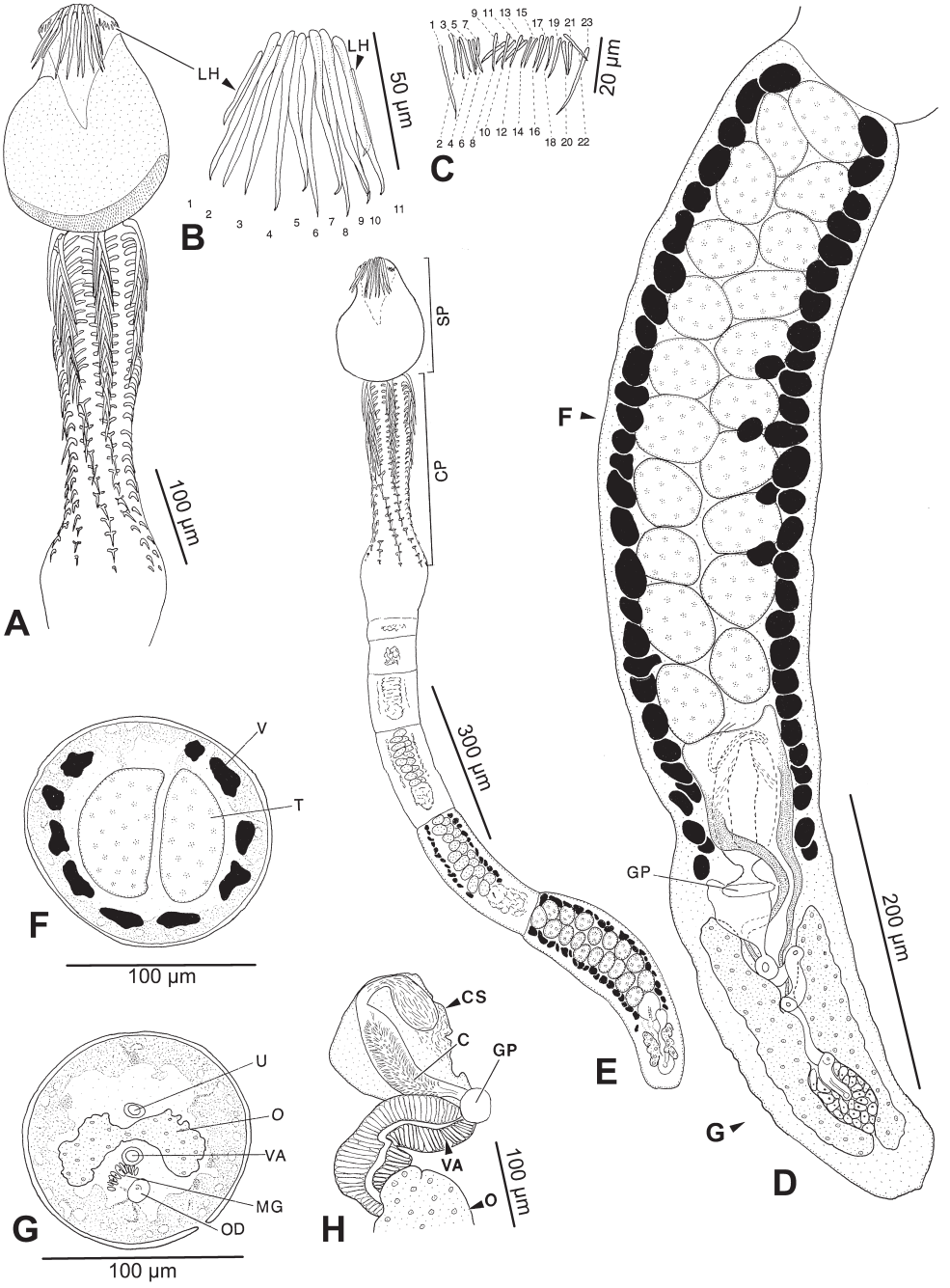


Fig. 71. Line drawings of *Echinobothrium fautleyae*. A. Scolex. B. Apical hooks. C. Lateral hooklets. D. Mature proglottid. E. Whole worm. F. Cross section through proglottid at level indicated by "F" in D. G. Cross section through proglottid at level indicated by "G" in D. H. Detail of terminal genitalia, lateral view. Abbreviations: C, cirrus; CP, cephalic peduncle; CS, cirrus sac; GP, genital pore; LH, lateral hooklets; MG, Mehlis' gland; O, ovary; OD, oviduct; SP, scolex proper; T, testis; U, uterus; V, vitelline follicle; VA, vagina. Modified from Tyler and Caira (1999).

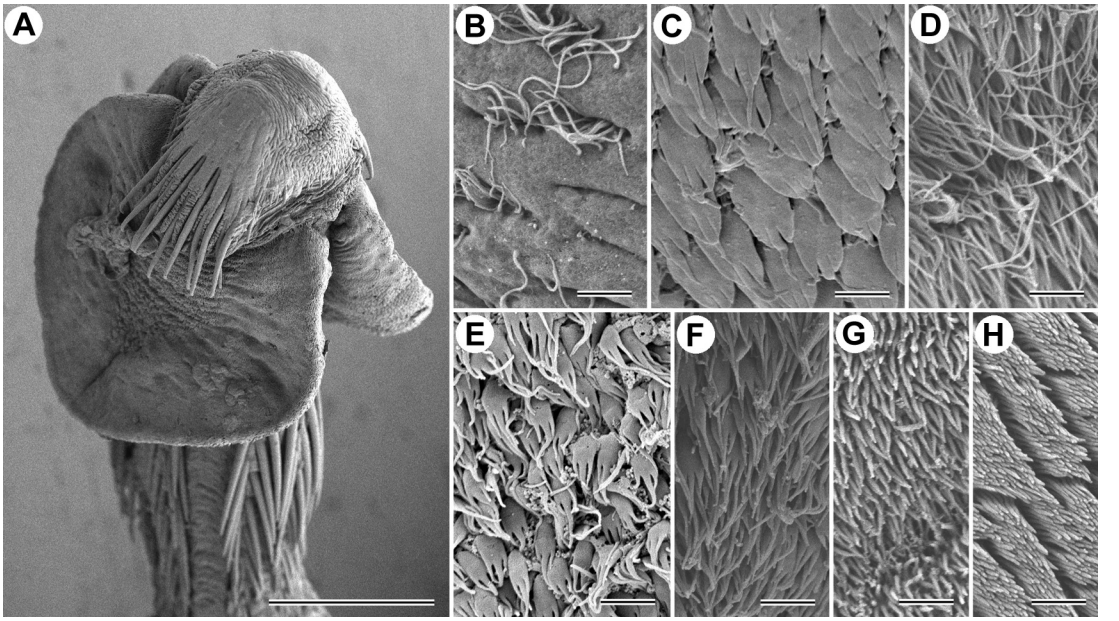


Fig. 72. Scanning electron micrographs of *Echinobothrium fautleyae*. A. Scolex. B. Apex of scolex. C. Proximal bothrial surface. D. Distal (lateral) bothrial surface. E. Distal (medial) bothrial surface. F. Lateral surface of scolex proper. G. Cephalic peduncle. H. Strobila. Scale bars: A, 50 µm; B-H, 1 µm.

guished from *E. bonasum* in that the first and last hooklet in each row of lateral hooklets is at least twice as long as the other hooklets in the row. In *E. bonasum*, all hooklets in a row are relatively equal in length.

This species was described by Tyler and Caira (1999) and was also included in a paper summarizing the diphyllideans in the Gulf of California (Tyler 2001). It was not included in the phylogenetic analysis of Ivanov and Hoberg (1999), as the description was not yet available. Olson and Caira (1999) included this species in a molecular phylogenetic analysis of the major lineages of tapeworms. Olson *et al.* (1999) also used this species as an outgroup in their analysis of host-parasite associations of tetraphyllideans based on ribosomal DNA sequence data.

Examination of numerous specimens of *Rhinoptera bonasus* in the Gulf of Mexico and Atlantic Ocean resulted in the collection of several specimens of *Echinobothrium* that appear to be intermediate between *E. fautleyae* and *E. bonasum*, suggesting that the two species may be conspecific or that the intermediates represent a new species. This question will likely remain unresolved until DNA sequences from the three morphotypes are compared.

Echinobothrium harfordi McVicar, 1976

(Figs. 73-75)

Type host: *Raja naevus* Müller and Henle, Cuckoo ray (Rajidae, Rajiformes.

Other hosts: *Raja clavata* L., Thornback ray (Rajidae, Rajiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Aberdeen, Scotland.

Additional localities: Plymouth, England; Sète, France.

Type material: BMNH No. 1975.9.16.1 (holotype); BMNH No. 1975.9.16.2-5 (paratypes).

Voucher specimens: four DNA vouchers verified by G. Tyler (BMNH No. 2001.1.23.4-7); one specimen from *R. clavata* in the personal collection of L. Euzet.

Specimens examined: Holotype; all 18 paratypes; one specimen from L. Euzet's collection.

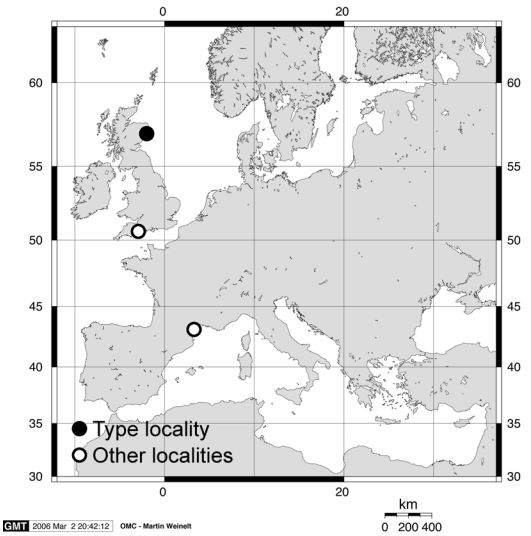


Fig. 73. Distribution of *Echinobothrium harfordi*.

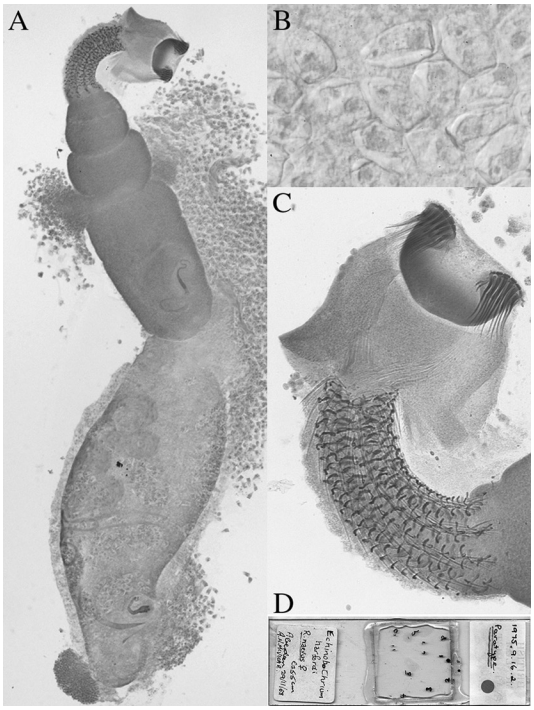


Fig. 74. Light micrographs of *Echinobothrium harfordi*. A. Whole worm. B. Eggs in utero. C. Scolex. D. Paratype slide BMNH No. 1975.9.16.2.

Genbank accession numbers: AF286985 (18s rDNA); AF286921 (28s rDNA).

Etymology: This species was named in honor of Dr. H. Harford Williams.

Description (Modified from McVicar [1976].)

Worms 600-3,630 long, greatest width at terminal proglottid. Strobila apolytic, acraspedote, up to 18 proglottids. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 150-220 long, consisting of armed apical rostellum and two bothria. Hook formula {(2-4) 6/5 (2-4)}, apical hooks solid, central type A hooks shorter than adjacent hooks, type B hooks gradually increasing in length toward center of group. Bothria 170-260 long, 110-220 wide. Cephalic peduncle 100-250 long, 80-100 wide, armed with eight longitudinal columns of 11-14 spines. Spines with triradiate bases, from 11-22 to 55-76 long.

Testes 6-7 in number, anterior to cirrus sac, in two irregular columns, one layer deep. Vas deferens extensive, anterior to cirrus sac. Cirrus sac piriform, approximately 110 long, 60 wide. Cirrus armed with thorn-like microtriches. Ovary H-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, posterior to genital pore, uniform in diameter along length, coiling slightly. Genital pore midventral, overlapping ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, lateral. Eggs oval, 28-33 long, 15-21 wide, with mucron at one pole, not packaged. Excretory ducts lateral.

Remarks

The hook formula of this species distinguishes it from all others in the genus except *E. affine*, *E. bonasum*, and *E. fautleyae*. This species differs from *E. affine* in the morphology and packaging of the eggs, having unpackaged eggs with a single mucron, whereas *E. affine* has filamented eggs packaged in chains. *Echinobothrium harfordi* differs from the latter two species in its possession of lateral hooklets arranged in two groups as opposed to in a single continuous row.

This species was described by McVicar (1976), but had not been reported again until it was collected by Olson *et al.* (2001), who used its DNA sequence in his phylogentic

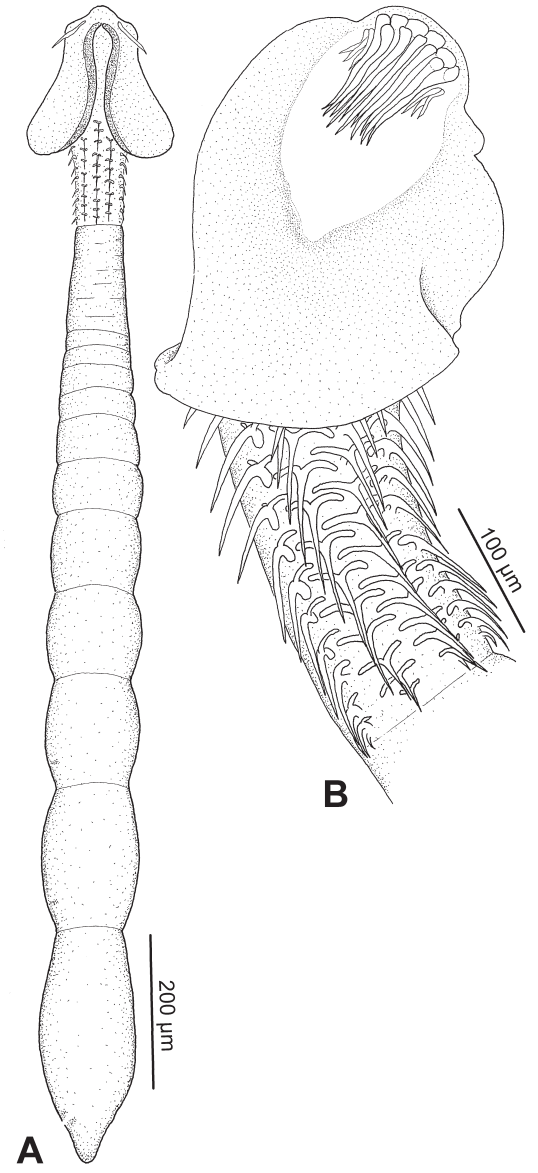


Fig. 75. Line drawings of *Echinobothrium harfordi*. A. Whole worm. B. Scolex. Redrawn from McVicar (1972).

analysis of cestode orders. *Echinobothrium harfordi* has been considered valid by all subsequent authors, and was included in the keys published by Probert and Stobart (1989) and Ivanov and Campbell (1998a). It was treated, but excluded from the phylogenetic analysis of Ivanov and Hoberg (1999), due to the lack of sufficient morphological data.

The type specimens of this species have all destained, making examination difficult. In addition, the mounting medium used has shrunken and pulled away from the specimens, leaving them nearly useless. Thus, some of the information above comes from the study of the single specimen from the collection of L. Euzet.

McVicar (1976) found this species in a number of specimens of *Raja naevus*. He noted that the prevalence of infection was greatest in smaller skates, and nearly non-existent in skates over 45 cm. McVicar (1976) attributed this change in prevalence to a change in feeding habits occurring over the life time of this particular host species.

Echinobothrium helmymohamedi Saoud, Ramadan, and Hassan, 1982

(Figs. 76-78)

Type host: *Taeniura lymma* (Forsskål), Bluespotted ribbontail ray (Dasyatidae, Myliobatiformes).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: Al-Ghardaga, Red Sea, Egypt.
Type material: BMNH No. 1998.10.19.113 (one paratype).
Specimens examined: Paratype (one specimen).
Etymology: This species was named in honor of Professor A. H. Helmy Mohamed.

Description (Modified from Saoud *et al.* [1982].)

Worms 3.88-5.13 mm long, greatest width 200-280. Strobila apolytic, acraspedote, 17 proglottids. Mature proglottids 1-2 in number, 760 long, 220 wide, gravid proglottids 0-1 in number. Scolex length 530-650. Scolex proper 350-410 long, 210-260 wide. Apical hooks solid. Hook formula unavailable; at least 29 apical hooks in each dorso-ventral group. Lateral hooklets absent. Type A hooks gradually increasing in length toward center of group, B type hooks shorter toward center of group. Bothria 330 long, 210-260 wide, cleft at posterior margin, proximal surfaces covered with spinitriches. Cephalic peduncle

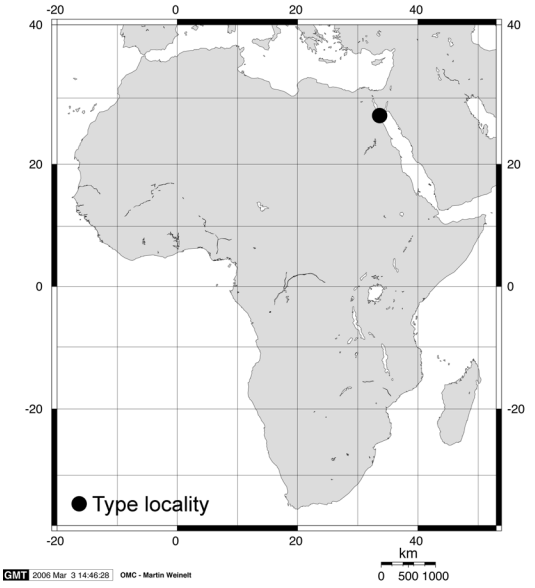


Fig. 76. Distribution of *Echinobothrium helmymohamedi*.

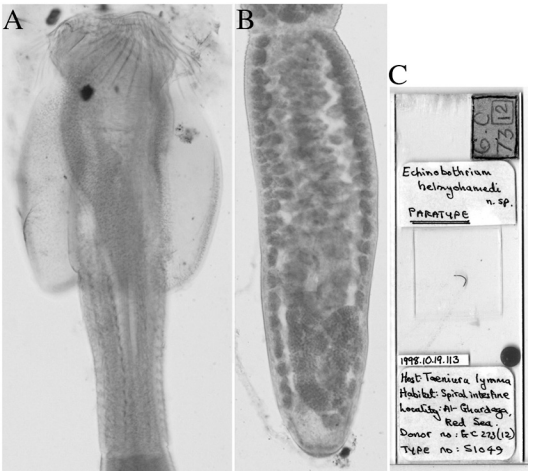


Fig. 77. Light micrographs of *Echinobothrium helmymohamedi*. A. Scolex. B. Mature proglottid. C. Paratype slide BMNH No. 1998.10.19.113.

21-58 long, 125 wide, armed with eight columns of 16-17 spines, each 20-44 long.

Testes 12-17 in number, 40-70 long, 50-90 wide, arranged in two columns, anterior to ovary. Vas deferens extensive, highly coiled, looping posterior to cirrus sac, entering sac at anterior end. Cirrus sac 95-110 long, 80-110 wide. Cirrus covered with fine spinitriches. Ovary bilobed, 130-220 long, each lobe 100-110 wide, H-shaped in dorso-ventral view. Mehlis' gland dorsal and anterior to ovarian isthmus, 70 long. Vagina thin-walled, coiling anterior to ovary, extending posteriorly to ovarian isthmus. Genital pore midventral, opening 38% from posterior end of proglottid, overlapping ovary, anterior to isthmus. Uterus saccate, filling anterior portion of proglottid when fully gravid. Vitellaria follicular, lateral, extending entire length of proglottid, uninterrupted by ovary. Follicles 20-37 in diameter. Eggs not observed in paratype, measuring (calculated from original description drawing) 14 long, 8 wide, oval, not packaged, no appendages.

Remarks

The hook arrangement for this species was never fully described. Unfortunately, examination of the only known type material provided little additional information on the hook arrangement. However, based on the unique combination of the following features, this species can be distinguished from all others in the genus: greater than 29 apical hooks in each dorso-ventral group, lateral hooklets lacking, 16-17 cephalic peduncle spines per column, and 12-17 testes.

This species was first alluded to by Williams (1964) in his description of *E. heroniense* from *Taeniura lymma* in Australia. It was described in 1982 by Saoud *et al.*, and has not been reported since. The validity of this species has never been questioned, although it was omitted from the key published by Probert and Stobart (1989). It did, however, appear in the key of Ivanov and Campbell (1998a), and was included in the cladistic analysis of Ivanov and Hoberg (1999). Interestingly, in the tree resulting from that analysis, *E. helmymohamedi* appeared as the sister species to *E. heroniense*, also a parasite of *T. lymma*. Saoud and Hassan (1983) collected specimens of *Echinobothrium* from this type host species in the Red Sea and the Mediterranean Sea. However, they did not identify the worms to species, so whether their specimens represented *E. helmymohamedi* is uncertain.

The original description of this species

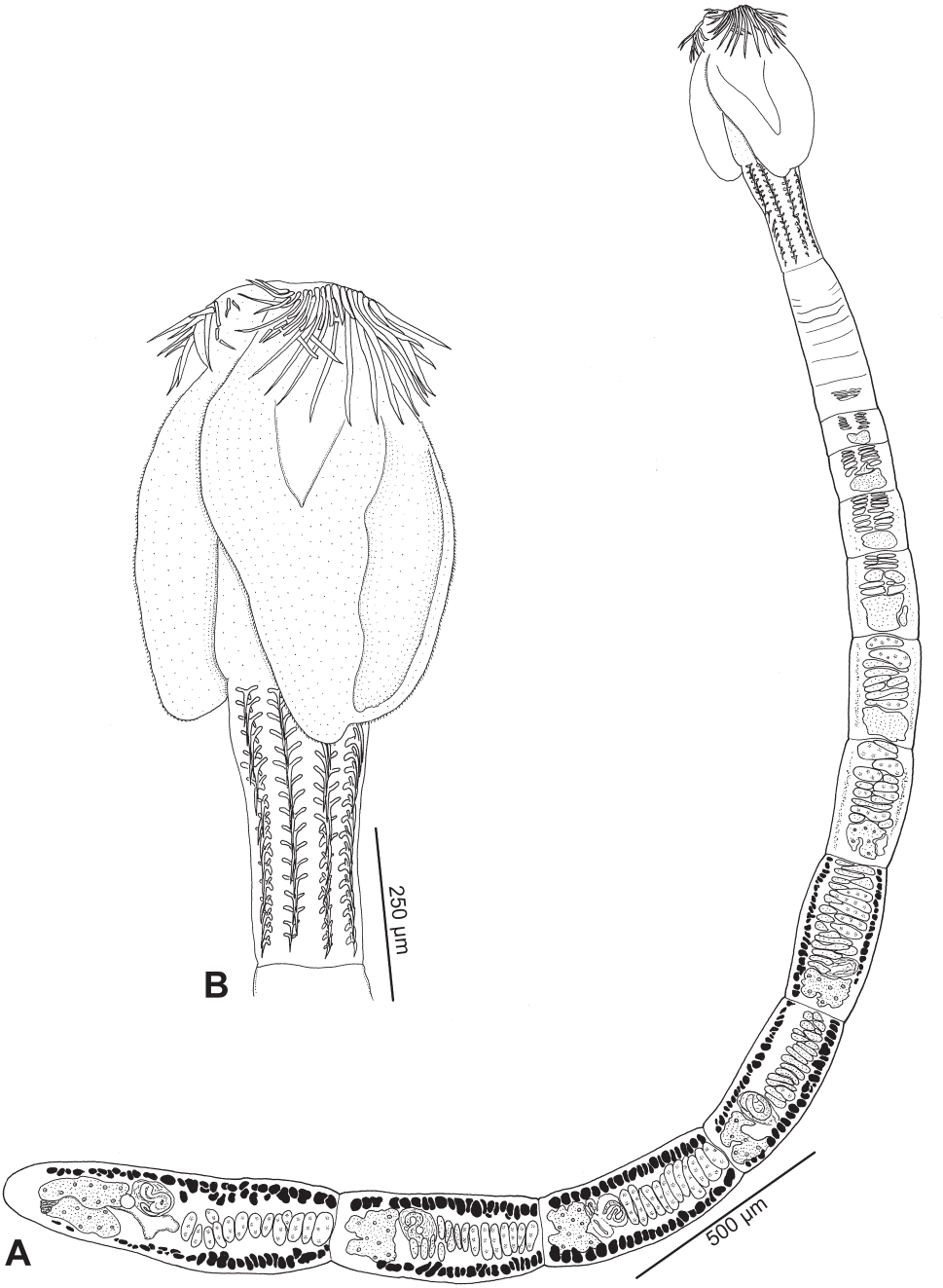


Fig. 78. Line drawings of *Echinobothrium helmymohamedi*. A. Scolex. B. Whole worm.

included several errors which require correction. The cephalic peduncle in this species was originally described as bearing only six columns of spines although there are actually eight. Each column was described as having 10-12 spines, but the paratype possesses 16-17 spines per column. The apical armature was described as consisting of 21 hooks in each dorso-ventral group, but there are at least 29 in each group on the paratype specimen

examined. Several of the type B hooks are broken in this preparation, however, giving the appearance of a smaller number of hooks. The eggs were originally described as being 140-150 long and 90-100 wide. There are no gravid proglottids on the specimen examined, but given the sizes of diphyllidean eggs reported elsewhere in the literature, it seems unlikely that the eggs are this large. When compared to the scale bar, the eggs shown in figure 6 of Saoud *et al.* (1982) appear to be approximately 14 long by 8 wide, suggesting that their error was merely typographical.

This species is the second of three species in the genus *Echinobothrium* described from the host *Taeniura lymma*. Williams (1964) described *E. heroniense* from this host in Australia, and suggested that the Australian *T. lymma* may in fact be a distinct subspecies, and that parasitological information from this host collected from both areas (western Pacific and Indian Ocean) could shed some light on this question. This question remains worthy of investigation. Specimens of *T. lymma* examined from the Gulf of Carpentaria, Australia and from Madagascar did not host *E. helmymohamedi*, although those from the Gulf of Carpenteria did host another species of diphyllidean, *E. elegans*.

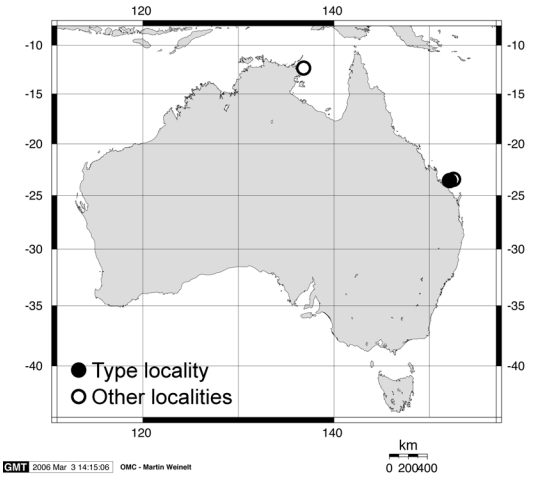


Fig. 79. Distribution of *Echinobothrium heroniense*.

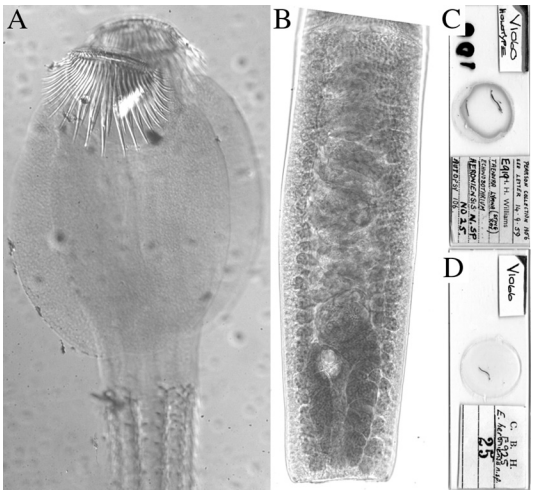


Fig. 80. Light micrographs of *Echinobothrium heroniense*. A. Scolex. B. Mature proglottid. C. Holotype slide SAMA No. V1060. D. Paratype slide SAMA No. V1066.

Echinobothrium heroniense **Williams, 1964**

(Figs. 79-82)

Type host: *Taeniura lymma* (Forsskål), Bluespotted ribbontail ray (Dasyatidae, Myliobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Additional localities: Nhulunbuy-Gove, Northern Territory, Gulf of Carpenteria, Australia; Winter Reef, Queensland, Australia.

Type material: SAMA No. 41060 (holotype); SAMA No. 41066 (paratypes).

Voucher specimens: Ten specimens from the Gulf of Carpenteria (LRP Nos. 2202-2211); five from Winter Reef (LRP Nos. 2212-2216).

Specimens examined: Holotype; two paratypes; 15 vouchers LRP 2202-2211 and 2212-2216.

Etymology: This species was named after its type locality.

Description (Modified from Williams [1964].)

Worms 5.25-7.70 mm long, 350-440 wide at first few or terminal proglottid. Strobila euapolytic, acraspedote, 13-25 proglottids per

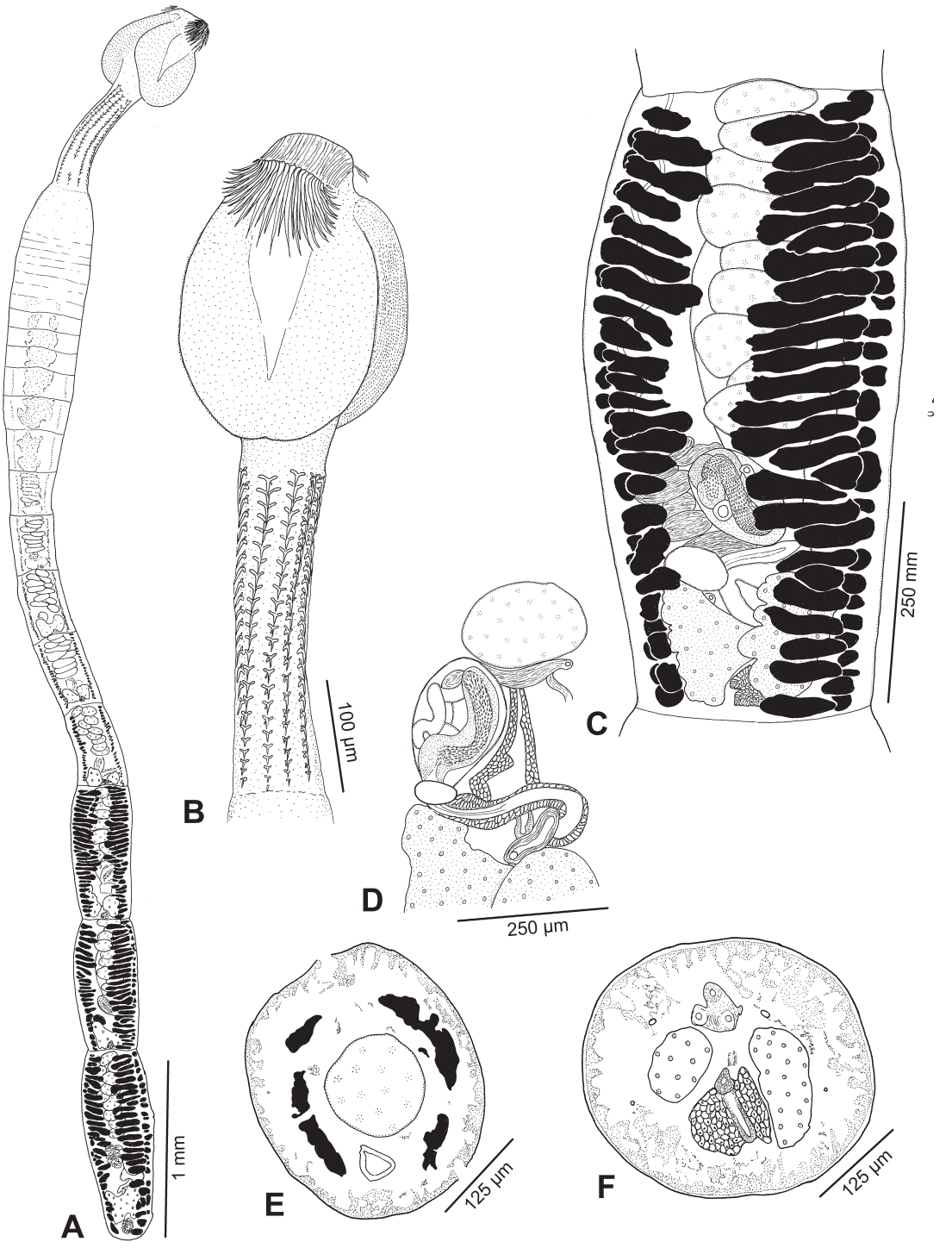


Fig. 81. Line drawings of *Echinobothrium heroniense*. A. Whole worm. B. Scolex. C. Mature proglottid. D. Detail of terminal genitalia, lateral view. E. Cross section through proglottid at level indicated by “E” in C. F. Cross section through proglottid at level indicated by “F” in C.

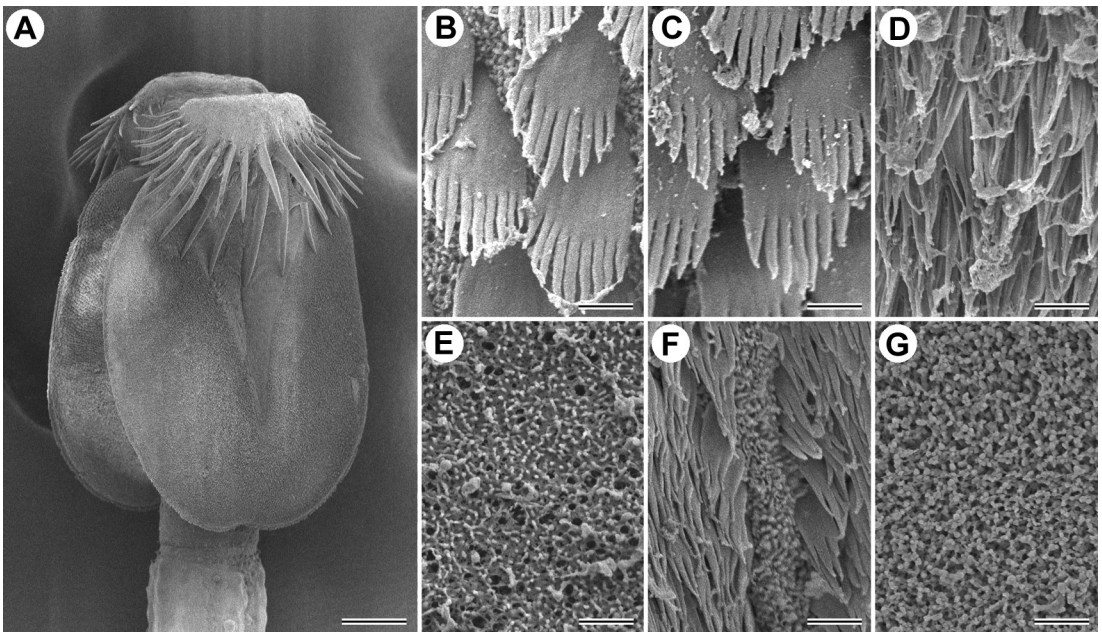


Fig. 82. Scanning electron micrographs of *Echinobothrium heroniense*. A. Scolex. B. Proximal bothrial surface (anterior). C. Proximal bothrial surface (posterior). D. Distal (lateral) bothrial surface. E. Distal (medial) bothrial surface. F. Lateral surface of scolex proper (right) and proximal bothrial surface (left). G. Strobila. Scale bars: A, 50 µm; in B-E, G, 1 µm; in F, 2 µm.

worm. Strobila covered with short filitriches. Mature proglottids 1-2 in number, 780-1,160 long, 400-410 wide, detached gravid proglottids 1,200 long, 250 wide. Scolex bipartite, 920-1,500 long, consisting of scolex proper and cephalic peduncle. Scolex proper 570-600 long, 360-500 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Forty-one apical hooks in each dorso-ventral group. Hook formula {(2-3) 22/19 (2-3)}, apical hooks solid to semihollow, hooks gradually increasing in length toward center of group. Lateral hooklets arranged in two groups. Bothria 390-550 long, 350-450 wide, with cleft in posterior margin, proximal surfaces covered with pectinate spinitriches each bearing seven relatively equal length digits. Distal bothrial surfaces (except medial distal surface) covered with pectinate spinitriches each with three digits; central digit conspicuously longer than others. Medial distal bothrial surface with short filitriches. Lateral surfaces of scolex proper with pectinate microtriches each bearing three relatively equal length digits. Cephalic peduncle 590-775 long, 136-200 wide, armed with eight longitudinal columns of 24-32 spines. Spines with triradiate bases, 20-28 to 39-50 long.

Testes 10-12 in number, anterior to cirrus sac, 75-80 long, in single irregular column, one layer deep. Vas deferens minimal, anterior to cirrus sac. Cirrus sac piriform, 101-150 long, 78-105 wide. Cirrus 200 long, armed proximally with thorn-like microtriches. Ovary 178-316 long, 108-200 wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, looping anterior to genital pore, uniform in diameter along length, undulating slightly. Genital pore midventral, 24-32% from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, large, measuring 28-35 long, 125-168 wide, circumcortical, occasionally interrupted medially, extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 35 long, 30 wide, lacking appendages, not packaged. Excretory ducts lateral.

Remarks

The unique hook formula of this species is sufficient to distinguish it from all other species in the genus.

This species was described by Williams (1964) from Heron Island on the Great Barrier Reef. It has been considered valid by all subsequent workers, appearing in the keys of Probert and Stobart (1989) and Ivanov and Campbell (1998a). Lester and Sewell (1989) listed this species in their checklist of parasites from Heron Island, and it was included in the cladistic analysis of Ivanov and Hoberg (1999), where it appeared in their tree as the sister species to another parasite of *T. lymma*, *E. helmymohamedi*.

This was the first species of *Echinobothrium* reported from the blue-spotted stingray *T. lymma*. Williams (1964) was aware of another species (*E. helmymohamedi*) from this host in the Red Sea, which had not yet been described, and based on his assumption of host specificity, considered the possibility of multiple cryptic host species all identified as *T. lymma*.

This species was found to co-occur with *E. elegans* in *T. lymma* collected in the Gulf of Carpenteria, Northern Territory, Australia.

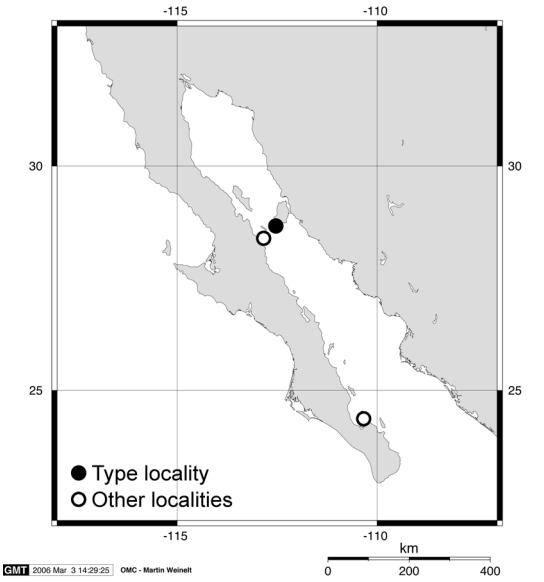


Fig. 83. Distribution of *Echinobothrium hoffmanorum*.

Echinobothrium hoffmanorum Tyler, 2001

(Figs. 83-86)

Type host: *Urobatis maculatus* Garman, Spotted round ray (Urolophidae, Myliob atiformes).

Additional hosts: *Urobatis halleri* (Cooper), Haller's round ray (Urolophidae, Myliobatiformes); *U. concentricus* Osburn and Nichols, Spot-on-spot round ray (Urolophidae, Myliobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Isla San Esteban, Gulf of California, México (28˚42'N, 112˚36'W).

Additional localities: San Francisquito, Baja California (28˚25'N, 112˚52'W); Punta Arenas, Baja California Sur (24˚04'N, 109˚50'W), México.

Type material: CNHE No. 3916 (holotype); CNHE No. 3917, USNPC No. 090148, and LRP Nos. 2022-2037 (paratypes from *U. halleri*); CNHE 3918-3919, USNPC Nos. 090146-090147, HWML No. 15490, and LRP Nos. 2038-2050 (paratypes from *U. maculatus*).

Specimens examined: Holotype; all 40 paratypes.

Etymology: This species was named in honor of the late Marion and Maximillian Hoffman in recognition of their support for undergraduate research at the University of Connecticut, through the Hoffman Foundation.

Description (Modified from Tyler [2001].)

Worms 1.205-3.475 mm (1.877 ± 0.601; n=39) long, 120-420 (236 ± 85; n=36) wide at terminal proglottid. Strobila apolytic, acraspedote, 4-10 (6.7 ± 1.3; n=41) proglottids, covered with long filitriches. Mature proglottids 0-2 (7 ± 6; n=41) in number, 273-835 (426 ± 120; n=26) long, 110-292 (174 ± 47; n=26; *n*=27) wide. Gravid proglottids 0-3 (0.9 ± 0.7; n=41) in number, 395-1,275 (716 ± 192; n=29; *n*=36) long, 145-420 (276 ± 78; n=24; *n*=29) wide. Strobila frequently with several immature proglottids, lacking mature

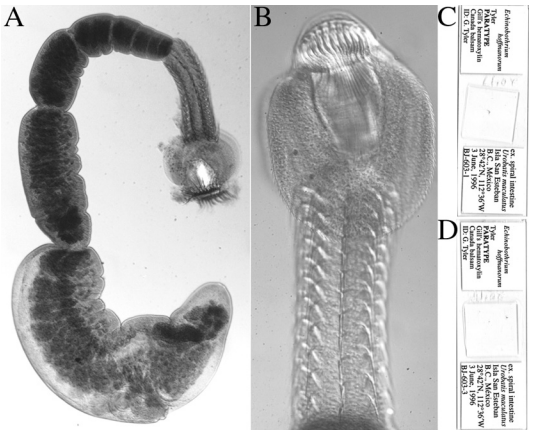


Fig. 84. Light micrographs of *Echinobothrium hoffmanorum*. A. Whole worm. B. Scolex. C-D. Paratype slides LRP Nos. 2048-2049.

proglottids and terminating with 1-2 gravid proglottids. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 185-278 (220± 24; n=29) long, 120-213 (174 ± 29; n=15) wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper covered with short and long filitriches, lateral regions anterior to lateral hooklets with short filitriches and small spinitriches. Nineteen or twenty-one apical hooks in each dorso-ventral group. Hook formula {(6-11) 10/(9-11) (6-11)}, apical hooks solid, hook lengths all increasing toward center of group. Lateral hooklets uniformly arranged in continuous row. Bothria 143-213 (170 ± 17; n=28) long, 120-212 (174 ± 29; n=15) wide, proximal surfaces covered with pectinate spinitriches, each bearing 9-11 relatively equal length digits, interspersed with short filitriches. Distal bothrial surfaces with the exception of medial region of distal surface and narrow longitudinal band on submarginal lateral surface with pectinate spinitriches each bearing 14-16 relatively equal length digits, interspersed with short filitriches and cilia; medial distal surface and narrow longitudinal bands on submarginal lateral surface with small filitriches only. Lateral region of scolex between bothria covered with short filitriches and small pectinate spinitriches each bearing 2-4 relatively equal length digits and cilia. Cephalic peduncle 145-375 (224 ± 59; n=40) long, 50-105 (70 ± 15; n=41) wide at midpoint, armed with eight longitudinal columns of 10-17 (13.0 ± 1.6; n=37; *n*=138) spines, covered with short filitriches. Spines with triradiate bases, from 10-28 (17 ± 5; n=38) to 45-62 (53 ± 5; n=38) long.

Testes 4-8 (5.5 ± 1.1; n=29; *n*=66) in number, anterior to cirrus sac, 26-83 (48 ± 11; n=20; *n*=108) long, 63-133 (94 ± 18; n=8; *n*=42) wide, in single column, one layer deep. Vas deferens extensive, extending lateral to cirrus sac. Cirrus sac piriform 48-188 (104 ± 30; n=24; *n*=26) long, 55-121 (81 ± 18; n=17) wide. Proximal portion of cirrus stout, approximately 90 long, 45 wide, armed with microtriches approximately 3 long; distal portion slender, unarmed, approximately 100 long, 18 wide. Ovary 70-168 (111 ± 29; n=26; *n*=32) long, 73-143 (109± 28; n=8; *n*=10) wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thick-walled, posterior to genital pore, relatively uniform in diameter along length, undulating slightly. Genital pore midventral, 21-45% (32.7 ± 6.3; n=25; *n*=30) of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development, expanding to fill gravid proglottid. Vitellaria follicular; follicles 20-45 (31 ± 6; n=5; *n*=23) long, 14-40 (20 ± 6; n=5; *n*=23) wide, forming two lateral bands; each band consisting of one dorsal and one ventral column of follicles; columns extending entire length of proglottid, occasionally joining ventrally, uninterrupted by ovary, confluent posterior to ovary. Eggs oval, 25-30 (27 ± 2; n=1; *n*=6) long, 23-25 (23 ± 1; n=1; *n*=6) wide, with single short terminal filament. Excretory ducts lateral.

Remarks

The hook formula of this species is sufficient to differentiate it from all other species in the genus except *E. californiense*, *E. coronatum*, *E. elegans*, *E. longicolle*, and *E. pigmentatum*. *Echinobothrium hoffmanorum* differs from all of the above species except *E. pigmentatum* in its possession of lateral hooklets arranged in a single continuous row rather than in two groups. *Echinobothrium*

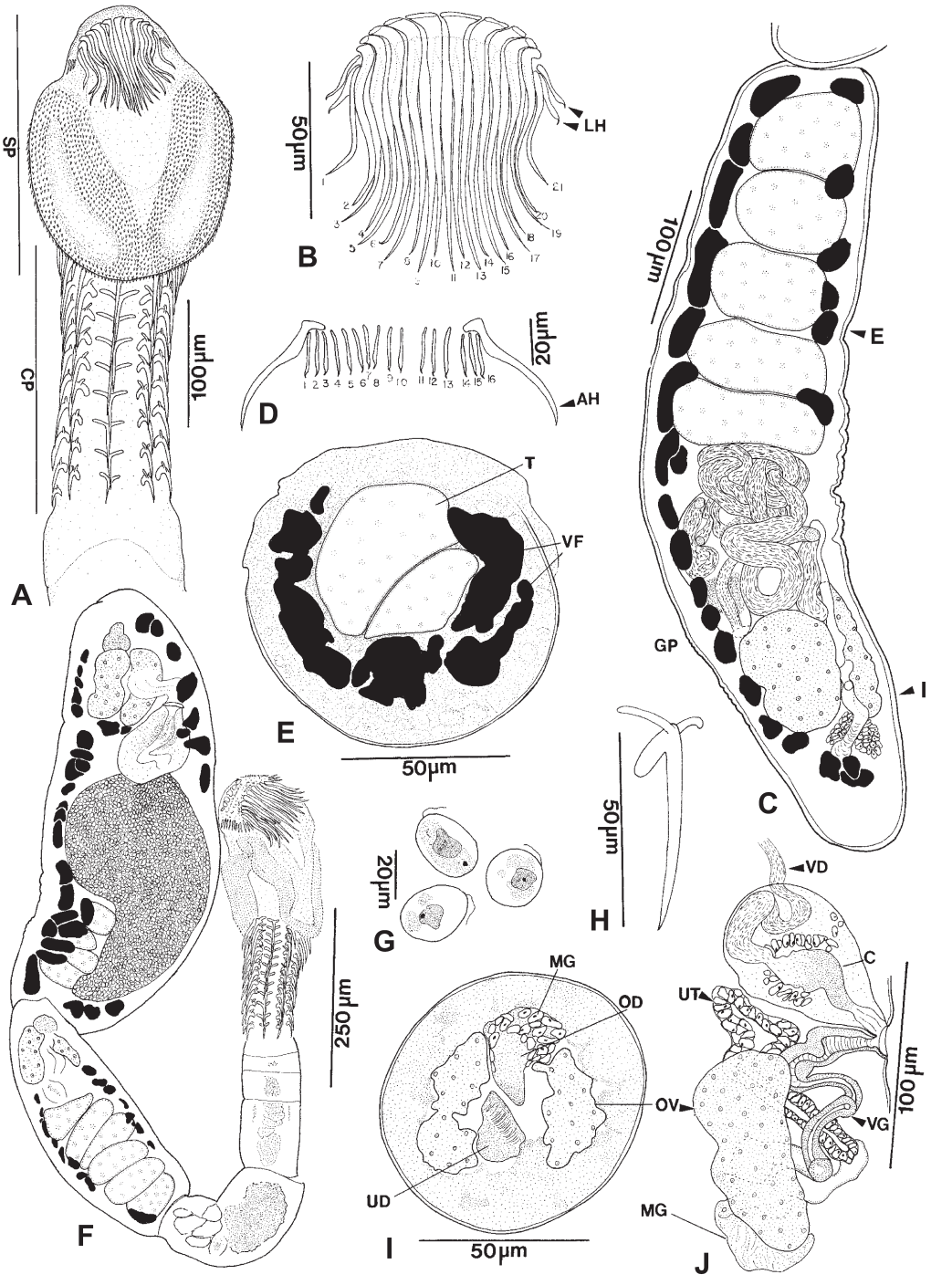


Fig. 85. Line drawings of *Echinobothrium hoffmanorum*. A. Scolex. B. Apical hooks. C. Mature proglottid. D. Lateral hooklets. E. Cross section through proglottid at level indicated by "E" in C. F. Whole worm. G. Eggs. H. Cephalic peduncle spine. I. Cross section through proglottid at level indicated by "I" in C. J. Detail of terminal genitalia, lateral view. Abbreviations: C, cirrus; CP, cephalic peduncle; CS, cirrus sac; GP, genital pore; LH, lateral hooklets; MG, Mehlis' gland; O, ovary; OD, oviduct; SP, scolex proper; T, testis; UD, uterine duct; UT, uterus; VD, vas deferens; VF, vitelline follicle; VG, vagina. Modified from Tyler (2001).

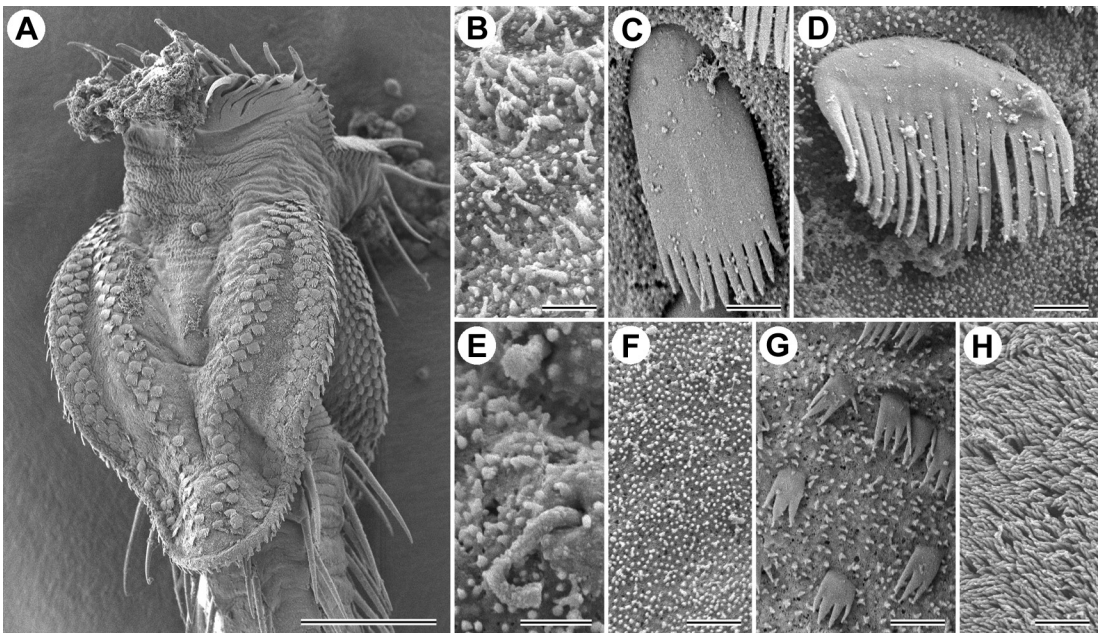


Fig. 86. Scanning electron micrographs of *Echinobothrium hoffmanorum*. A. Scolex. B . Apex C. Proximal bothrial surface. D. Distal (lateral) bothrial surface. E. Cilia on distal (lateral) surface. F. Distal (medial) bothrial surface. G. Lateral surface of scolex proper. H. Strobila. Scale bars: A, 50 µm; E, 500 nm; B-D, F-H, 1 µm.

hoffmanorum differs from *E. pigmentatum* in possessing a greater number of proglottids (4-10 vs. 5-7), eggs with single versus two filaments, and a genital pore overlapping the ovary versus anterior to the ovary.

This species was described by Tyler (2001) from collections made in 1996 in the Gulf of California, Mexico, and was the third diphyllidean species reported from that body of water. The discovery of this species in *Urobatis* was the first record of a diphyllidean from a ray in the family Urolophidae. This species was not reported by Tyler and Caira (1999) from earlier collections in the Gulf of California, possibly due to seasonal variation in parasite abundance or to environmental disturbance (Tyler, 2001). Interestingly, unlike most other diphyllideans, this species was found in not just one, but three host species. However, the taxonomy of the Urolophidae is not well understood at this time, and the validity of these three species has been questioned. For example, Thompson *et al.* (1987) suggested that all three species were conspecific.

Echinobothrium longicolle Southwell, 1925

(Figs. 87-89)

Type host: *Dasyatis kuhlii* (Müller and Henle), Bluespotted stingray (Dasyatidae, Myliobatiformes) (as *Trygon kuhlii*).
Status: Valid.
Site of infection: Spiral intestine.
Type locality: Portugal Bay, Sri Lanka.
Type material: Four syntype specimens currently in personal collection of L. Euzet.
Specimens examined: All four syntype specimens.
Etymology: Not given, but presumably descriptive of the extremely long cephalic peduncle, often mistakenly referred to as a neck.

Description (Modified from Southwell [1925].)

Worms 20-30 mm long. Strobila acraspedote, with approximately 50 proglottids. Scolex bipartite, 1.20 mm long, consisting of scolex proper and cephalic peduncle. Scolex proper 1.25 mm long, 1.05 mm wide,

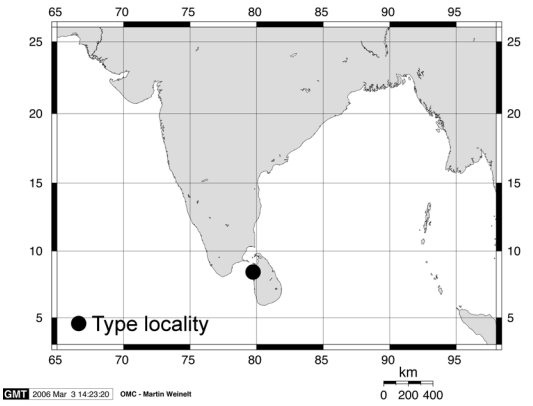


Fig. 87. Distribution of *Echinobothrium longicolle*.

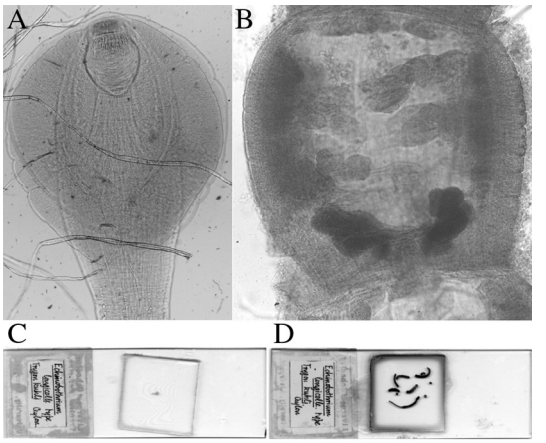


Fig. 88. Light micrographs of *Echinobothrium longicolle*. A. Scolex. B. Mature proglottid. C. Type slide. D. Type slide.

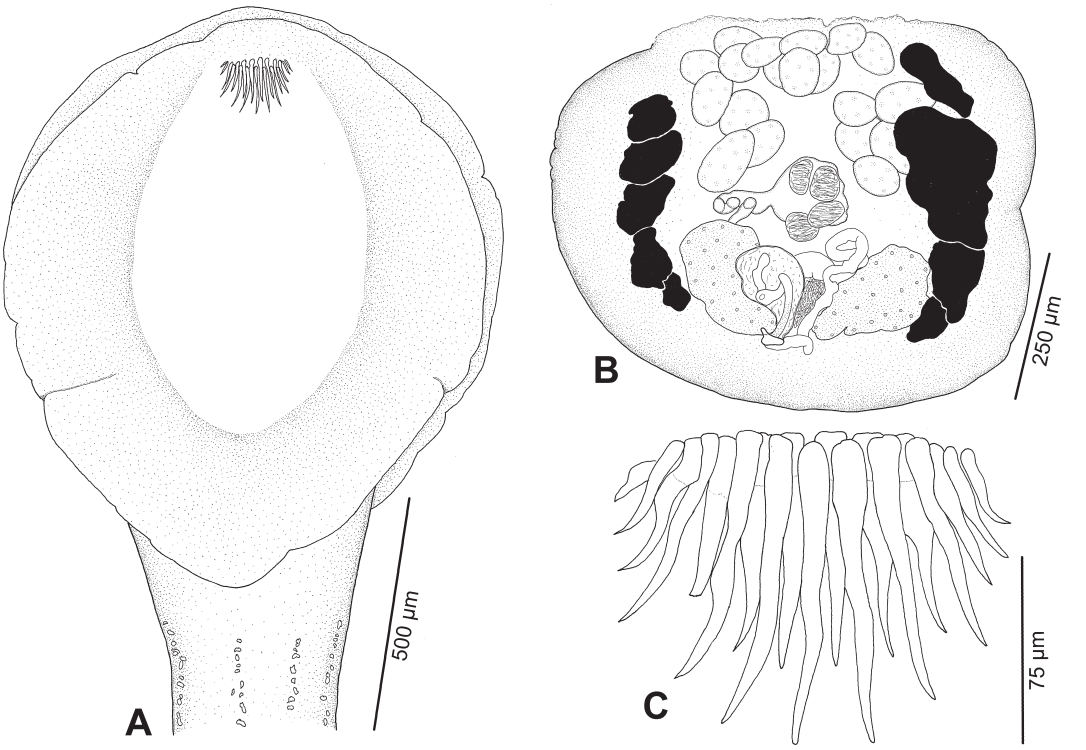


Fig. 89. Line drawings of *Echinobothrium longicolle*. A. Scolex. B. Mature proglottid. C. Apical hooks.

consisting of armed apical rostellum and two bothria. Hook formula unclear, 19 apical hooks in each group, lateral hooklets lacking. Apical hooks solid, gradually increasing in length toward center of group. Bothria 1.20 mm long, 1.05 mm wide. Cephalic peduncle

2.95-4.9 mm long, 65-96 wide, armed with eight longitudinal columns of 159-181 spines. Cephalic peduncle spines with leaflike bases (Fig. 12), 10-54 long.

Testes 19-27 in number, anterior to cirrus sac, in 4-5 irregular columns, one layer deep. Cirrus sac oval, 144 long, 68 wide. Ovary bilobed, 120 long, 420 wide, U-shaped in dorsoventral view. Vagina thin-walled, looping anterior to genital pore, relatively uniform in width, undulating slightly. Genital pore midventral, posterior to ovary. Vitellaria follicular, forming two lateral columns extending entire length of proglottid, uninterrupted by ovary.

Remarks

This species differs from all others in the genus in two distinctive characters. First, the cephalic peduncle of this species is exceedingly long, reaching about 5 mm, bearing up to 180 spines in each column. Second, the spines on the cephalic peduncle do not possess the typical triradiate bases (see Fig. 11) seen in other species of *Echinobothrium*, but have leaflike bases.

This species was described by Southwell (1925) from the host *Dasyatis kuhlii* in Sri Lanka. It has not been reported since. Collections made of the type host species in Madagascar and Australia as a part of this study have resulted in the collection of species of *Echinobothrium*, but did not include anything resembling this species. It is possible however, that the type host species collected by Southwell (1925) was different from that collected in the other two localities, if *D. kuhlii*, as currently circumscribed, actually comprises several cryptic species as suspected by Williams (1964) for *Taeniura lymma*, and by Jensen *et al.* (1999) for *Aetobatus narinari*.

The peculiar morphology of the cephalic peduncle spines in this species is deserving of further study. In addition to their unusual leaflike bases, many of the cephalic peduncle spines are directed forward, rather than backward, as in all other species in the genus. As the spines themselves are not yet protruding through the tegument, it appears that they are not yet fully developed. Both the unusual shape and orientation of these spines raise the possibility that they represent a developmental abnormality.

Echinobothrium mathiasi Euzet, 1951

(Figs. 90-93)

Type host: *Myliobatis aquila* (L.), Common eagle ray (Myliobatidae, Myliobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Mediterranean Sea, Sète, France.

Additional localities: Mediterranean Sea, Bizerte, Tunisia.

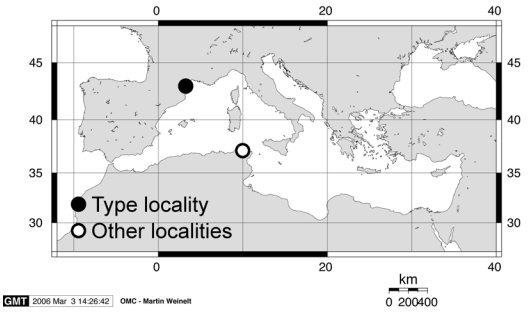


Fig. 90. Distribution of *Echinobothrium mathiasi*.

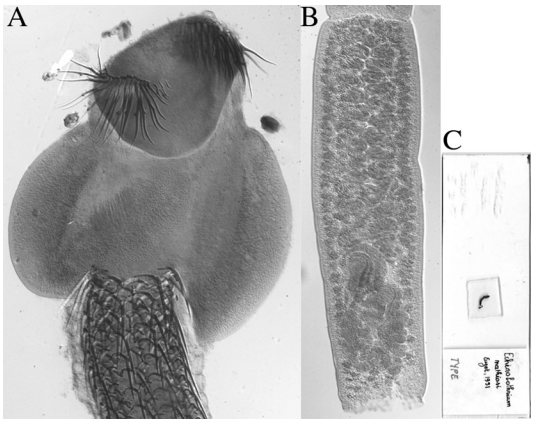


Fig. 91. Light micrographs of *Echinobothrium mathiasi*. A. Scolex. B. Mature proglottid. C. Holotype slide.

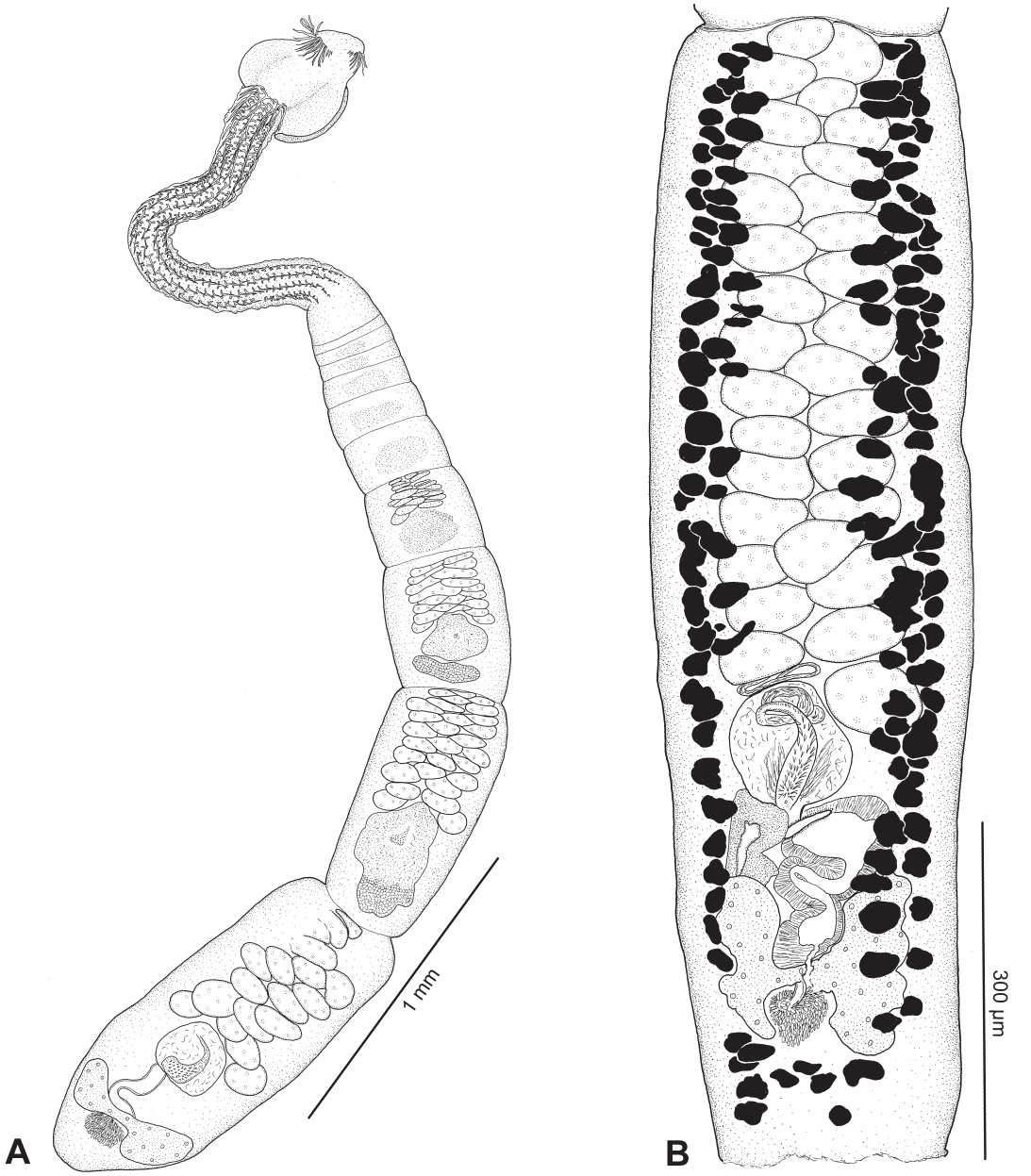


Fig. 92. Line drawings of *Echinobothrium mathiasi*. A. Whole worm. B. Mature proglottid.

Type material: Holotype and paratypes not accessioned in personal collection of L. Euzet.

Voucher specimens: Five specimens on one slide, several specimens in Berlese's medium, and three specimens mounted on two stubs for SEM, from Bizerte, Tunisia in the personal collection of L. Euzet.

Specimens examined: Holotype, two paratypes, all five vouchers, and all three SEM mounted specimens from L. Euzet's collection.

Etymology: This species was named in honor of Professor P. Mathias from the Université de Montpellier.

Description (Modified from Euzet [1951].)

Worms 5-6.26 mm long, up to 655 wide at terminal proglottid. Strobila euapolytic, acraspedote, 8-10 proglottids, covered with long filitriches. Mature proglottids one in number, 1.51-1.47 mm long, 640-650 wide. Scolex bipartite, up to 2.060 mm long, consisting of scolex proper and cephalic peduncle. Scolex proper up to 515 long, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper covered with long and short filitriches. Twenty-seven apical hooks in each dorso-ventral group. Hook formula {(3-4) 13/14 (3-4)}, apical hooks solid, hooks gradually increasing in length toward center of group. Lateral hooklets arranged in two groups. Bothria 355 long, proximal surfaces covered with short filitriches and pectinate spinitriches each bearing 8-9 relatively equal length digits. Distal surfaces except medial distal surface with pectinate spinitriches each bearing three relatively equal length digits. Medial distal surface with long and short filitriches. Cephalic peduncle 1-1.63 mm long, 150-175 wide, armed with eight longitudinal columns of 57-60 spines, covered with long filitriches. Spines with triradiate bases, 5-11 to 93-100 long.

Testes 20-31 in number, anterior to ovary, 93-123 long, 163-233 wide, in 2-3 irregular columns, one layer deep. Cirrus sac piriform, 260-400 long, 250-305 wide. Cirrus armed proximally with large spinitriches 20-35 long, small microtriches distally. Ovary 178-316 long, 108-200 wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, posterior to genital pore, undulating slightly, expanded distally. Genital pore midventral, 32-35% from posterior

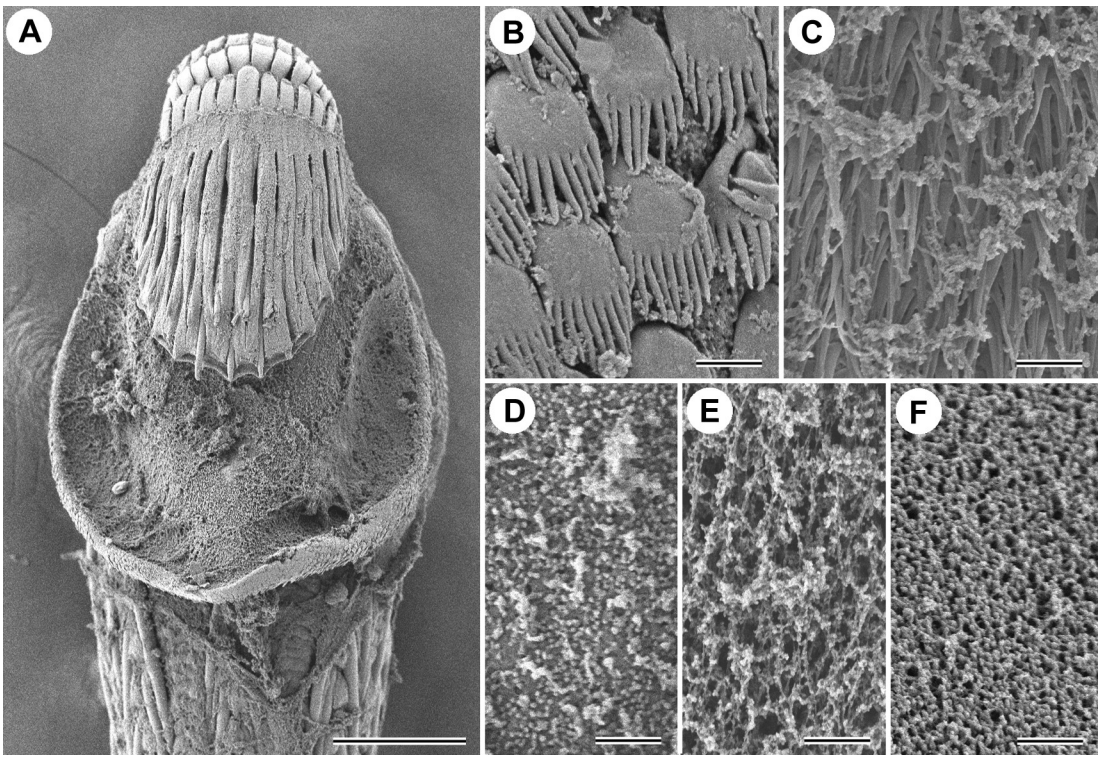


Fig. 93. Scanning electron micrographs of *Echinobothrium mathiasi*. A. Scolex. B. Proximal bothrial surface. C. Distal (lateral) bothrial surface. D. Distal (medial) bothrial surface. E. Cephalic peduncle. F. Strobila. Scale bars: A, 50 µm; B-F, 1 µm.

end of proglottid, anterior to ovary. Vitellaria follicular, in two lateral columns extending entire length of proglottid, uninterrupted by ovary. Eggs not packaged, oval, 40 long, 15 wide, with single polar appendage ending in a tuft. Excretory ducts lateral.

Remarks

The type A hook symmetry exhibited by this species distinguishes it from all other species in the genus except *E. euzeti*, *E. longicolle*, and *E. coenoformum*. This species is distinguished from *E. euzeti* in its possession of lateral hooklets in two groups as opposed to a single continuous row, and its possession of only 57-60 cephalic peduncle spines per column as opposed to over 100. *Echinobothrium mathiasi* differs from *E. longicolle* in its possession of lateral hooklets, which are lacking in *E. longicolle*, and in possessing only 57-60 cephalic peduncle spines per column as opposed to over 150. This species differs from *E. coenoformum* in the number of lateral hooklets, having 3-4 per group as opposed to only 1, and in possessing 57-60 cephalic peduncle spines per column as opposed to 11-15.

This species was described by Euzet (1951) and was among the species he collected during his doctoral research. The type specimens remain curated in his personal collection. Euzet (1951) also published a key to the species in the genus. The validity of this species has been accepted by all subsequent workers. This species has appeared in the keys of Rees (1961b), Probert and Stobart (1989), and Ivanov and Campbell (1998a). Ivanov and Hoberg (1999) included this species in their cladistic analysis where it appeared in their tree as the sister species to *E. megacanthum*, also a parasite of *Myliobatis*.

Examination of the type series of this species revealed that the smallest, posterior-most peduncle spines in each column on one of the specimens have distinctly leaflike bases (see Fig. 12), very similar to those seen on cephalic peduncle spines of *E. longicolle*. This species may be one to consider when investigating the hypothesis that the leaflike bases are a developmental abnormality (see *E. longicolle* above).

Echinobothrium megacanthum Ivanov and Campbell, 1998

(Figs. 94-96)

Type host: *Myliobatis goodei* Garman, Southern eagle ray (Myliobatidae, Myliobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: San Antonio Oeste, San Matías Gulf, Argentina (40˚44'S, 64˚56'W).

Type material: MLP No. 3958 (holotype); IPCAS No. C-288 and USNPC No. 87474 (paratypes).

Specimens examined: Holotype; one paratype (USNPC No. 87474).

Etymology: The specific epithet refers to the large armature associated with the cirrus.

Fig. 94. Distribution of *Echinobothrium megacanthum*.

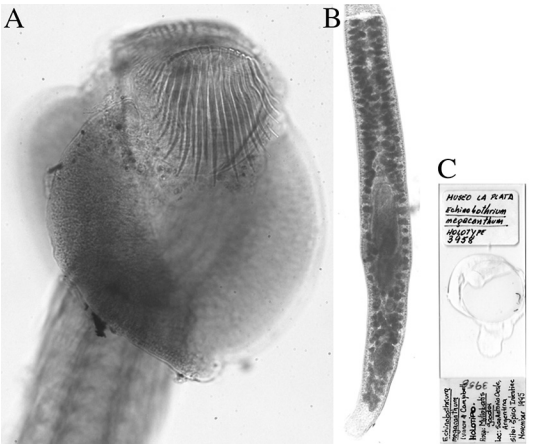


Fig. 95. Light micrographs of *Echinobothrium megacanthum*. A. Scolex. B. Mature proglottid. C. Holotype slide MLP No. 3958.

Description (Modified from Ivanov and Campbell [1998b].)

Worms 4.45-6.64 mm long, 205-288 wide at terminal proglottid. Strobila apolytic, acraspedote, 9-12 proglottids. Mature proglottids 1-2 in number, 1.21-1.65 mm long, gravid proglottids 0-1 in number. Scolex bipartite, 1.14-1.31 mm long, consisting of scolex proper and cephalic peduncle. Scolex proper 355-370 long, 227-282 wide, consisting of armed apical rostellum and two bothria. Twenty-seven apical hooks in each dorsoventral group. Hook formula {6 14/13 6}, apical hooks solid, hook lengths all increasing toward center of group. Lateral hooklets arranged in single continuous row. Bothria 288-330 long, 227-282 wide, proximal surfaces covered with long filitriches and pectinate spinitriches each bearing 5-7 relatively equal length digits. Distal bothrial surfaces (except medial distal surface) with long filitriches. Medial distal bothrial surface with long and short filitriches. Cephalic peduncle 780-1,027 long, 115-144 wide, armed with eight longitudinal columns of 38-43 spines. Spines with triradiate bases, 15-17 to 93-99 long.

Testes 13-18 in number, anterior to cirrus sac, 42-51 long, 51-54 wide, in two irregular columns, one layer deep. Vas deferens extensive, anterior to cirrus sac. External and internal seminal vesicles present. Cirrus sac piriform, 135-292 long, 74-144 wide. Cirrus armed proximally with thorn-like microtriches. Ovary 136-227 long, 90-180 wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thick-walled, anterior to genital pore, with muscular expansion distally, coiling slightly. Genital pore midventral, 33-40% of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, 16-29 long, 16-36 wide, forming lateral bands extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 17-19 long, 8-11 wide, without appendages, not packaged. Excretory ducts lateral.

Remarks

The hook formula of this species is sufficient to distinguish it from all other species in the genus except *E. euzeti*, *E. mathiasi*, and *E. raschii*. This species differs from the latter species in lacking a cleft in the posterior margin of the bothria, and from the former two species in exhibiting type B hook symmetry versus type A hook symmetry.

Ivanov and Campbell (1998b) described this species from *Myliobatis goodei*, making it the second *Echinobothrium* species described from that genus of host. At the time, the authors noted similarities between this species and the other described from *Myliobatis*, *E. mathiasi*, particularly noting the similarity in several genital characters. The phylogenetic analysis of Ivanov and Hoberg (1999) supported a close relationship between these two species.

Echinobothrium mexicanum Tyler and Caira, 1999

(Figs. 97-100)

Type host: *Myliobatis longirostris* Applegate and Fitch, Snouted eagle ray (Myliobatidae, Myliobatifdormes).

Additional host: *Myliobatis californicus* Gill, Bat eagle ray (Myliobatidae, Myliobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Bahía de Los Angeles, Gulf of California, México (28˚55'N, 110˚25'W).

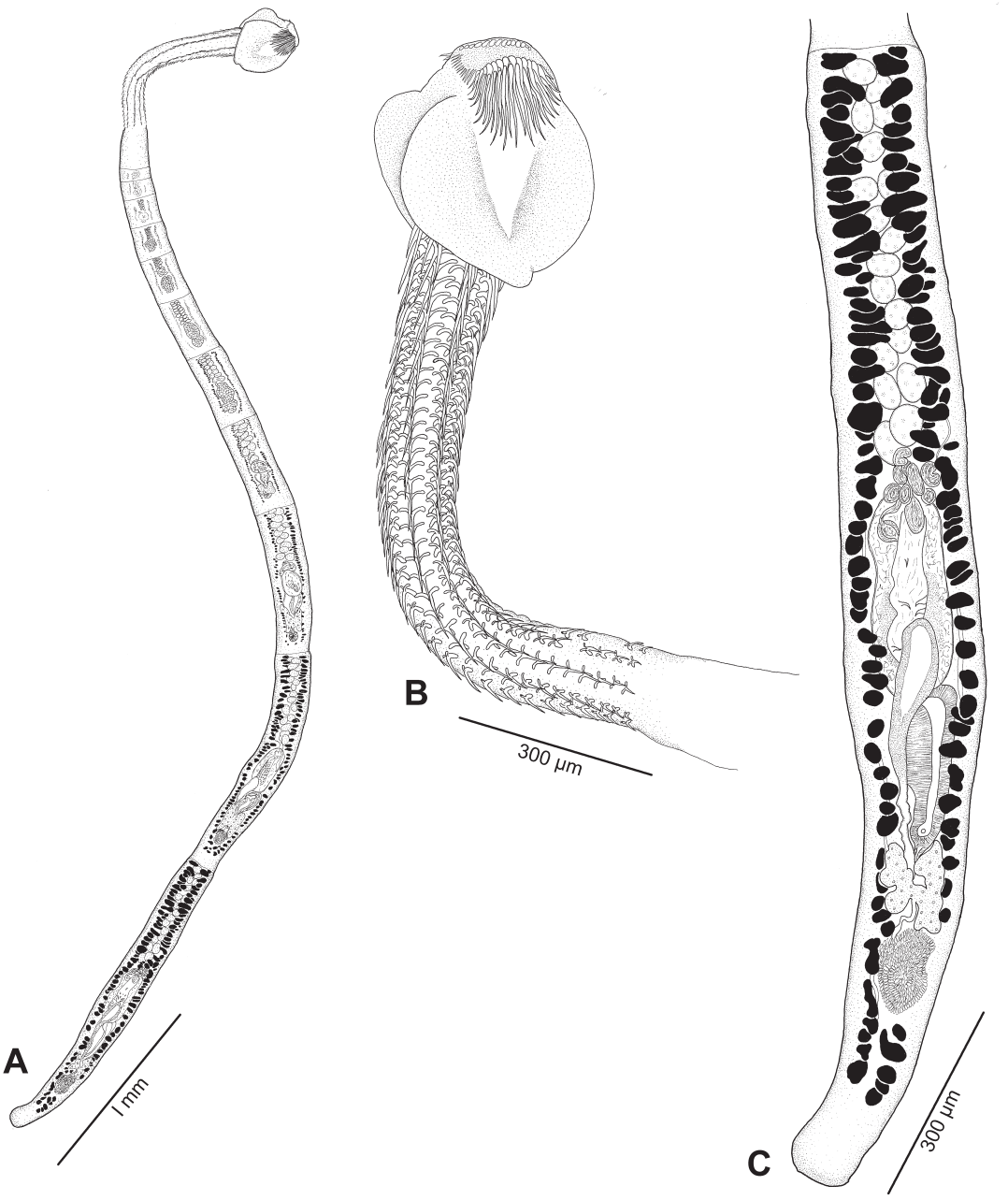


Fig. 96. Line drawings of *Echinobothrium megacanthum*. A. Whole worm. B. Scolex. C. Mature proglottid.

Additional localities: Puertecitos (30°21'N, 114°39'W), Santa Rosalia (27°19'N, 112° 17'W), and Loreto (26°01'N, 111°21'W), Gulf of California, México.

Type material: CNHE No. 3343 (holotype); CNHE Nos. 3344-3345, USNPC Nos. 88220-88221, and HWML Nos. 39914-39914 (paratypes).

Specimens examined: Holotype; all 37 paratypes.

Etymology: This species is named for the country where it was discovered.

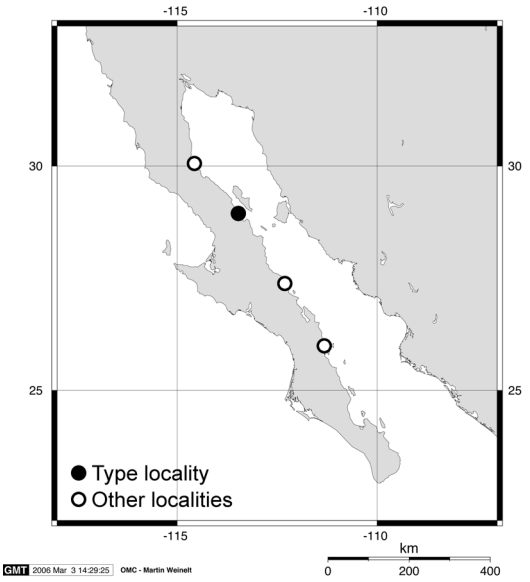


Fig. 97. Distribution of *Echinobothrium mexicanum*.

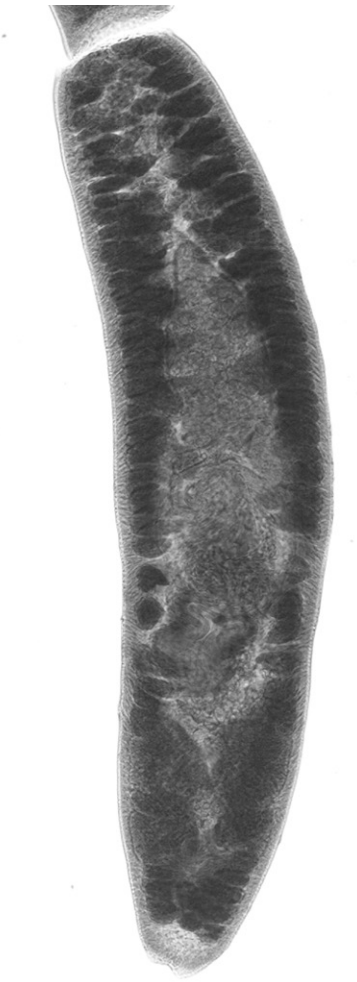


Fig. 98. Light micrograph of mature proglottid of *Echinobothrium mexicanum*.

Description (Modified from Tyler and Caira [1999].)

Worms 1.160-5.270 mm (2.892 ± 1.166; n=28) long, 110-440 (208 ± 086; n=28) wide at terminal proglottid. Strobila acraspedote, apolytic, 4-10 (8 ± 1; n=28) proglottids, covered with long filitriches. Mature proglottids 1-3 (2 ± 1; n=28) in number, 315-1,620 (747 ± 290; n=28; *n*=42) long, 103-250 (166 ± 39; n=28; *n*=42) wide. Gravid proglottids 0-1 (n=28) in number, 1.220-2.240 mm (1.573 ± 0.336; n=7) long, 240-360 (306 ± 45; n=7) wide. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 140-290 (236 ± 33; n=36) long, 138-230 (178 ± 26; n=10) wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper covered with long and short filitriches. Twenty-three apical hooks in each dorso-ventral group. Hook formula {(5-7) 12/11 (5-7)}, apical hooks solid, hook lengths increasing toward center. Lateral hooklets uniformly arranged in single continuous row. Bothria 95-230 (178 ± 38; n=35) long, 138-230 (178 ± 26; n=10) wide, proximal bothrial surfaces with short filitriches and pectinate spinitriches each bearing 4-6 relatively equal length digits. Distal bothrial surfaces (except medial distal surface) with cilia and pectinate spinitriches each bearing three relatively equal length digits. Medial distal bothrial surface with short filitriches. Lateral surfaces with short filitriches and pectinate spinitriches each bearing three digits; central digit longer than lateral digits. Cephalic peduncle 178-480 (324 ± 79; n=38) long, 35-100 (66 ± 15; n=38), armed with eight longitudinal columns of 23-40 (30.1 ± 3.6; n=36) spines. Spines with triradiate bases, 8-23 (13 ± 3; n=37) to 54-85 (66 ± 8; n=37) long.

Testes 10-20 (15.3 ± 1.9; n=27; *n*=29) in numer, anterior to cirrus sac, 26-63 (42 ± 9; n=15; *n*=67) long, 45-98 (67 ± 13; n=15; *n*=67) wide, in 2-3 irregular columns, one layer deep.

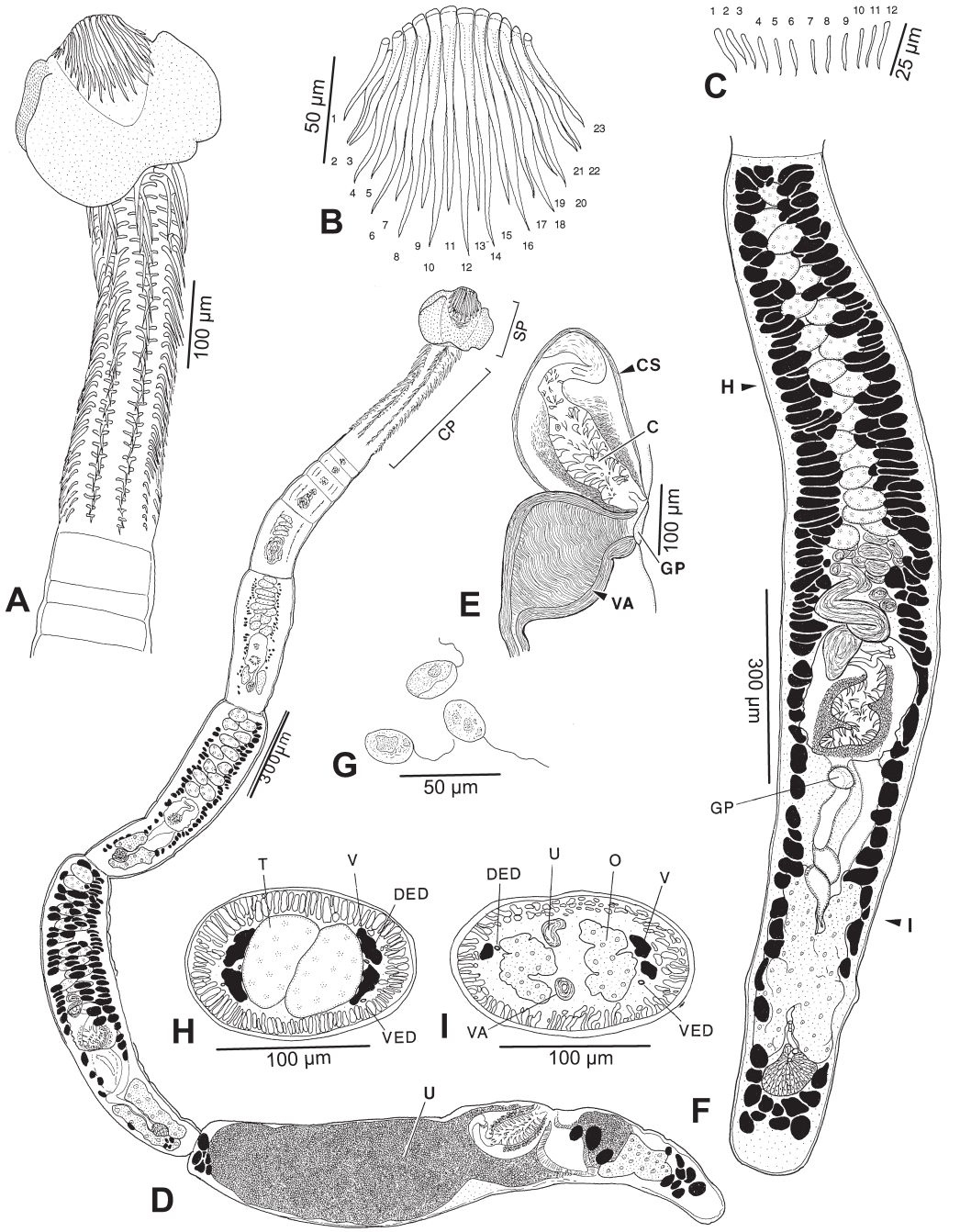


Fig. 99. Line drawings of *Echinobothrium mexicanum*. A. Scolex. B. Apical hooks. C. Lateral hooklets. D. Whole worm. E. Detail of terminal genitalia, lateral view. F. Mature proglottid. G. Eggs. H. Cross section through proglottid at level indicated by "H" in F. I. Cross section through proglottid at level indicated by "I" in F. Abbreviations: C, cirrus; CP, cephalic peduncle; CS, cirrus sac; DED, dorsal excretory duct; GP, genital pore; LH, lateral hooklets; MG, Mehlis' gland; O, ovary; OD, oviduct; SP, scolex proper; T, testis; U, uterus; V, vitelline follicle; VA, vagina; VED, ventral excretory duct. Modified from Tyler and Caira (1999).

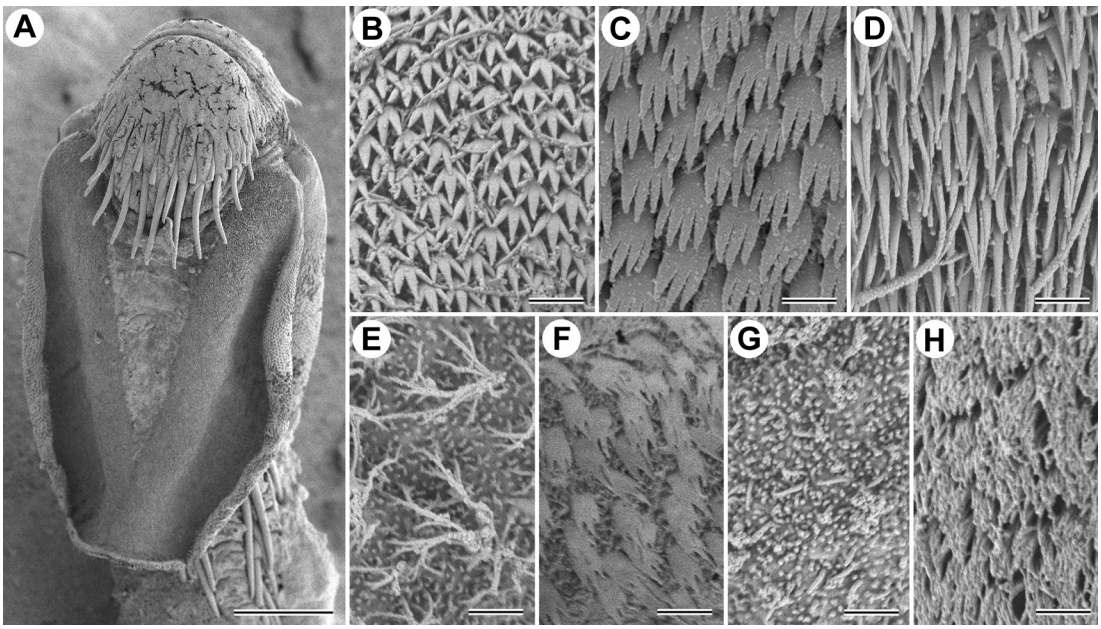


Fig. 100. Scanning electron micrographs of *Echinobothrium mexicanum*. A. Scolex. B. Proximal bothrial surface (anterior). C. Proximal bothrial surface (posterior). D. Distal (lateral) bothrial surface. E. Distal (medial) bothrial surface. F. Lateral surface of scolex proper. G. Cephalic peduncle. H. Strobila. Scale bars: A, 50 µm; B-H, 1 µm.

Vas deferens extensive, anterior to cirrus sac. External seminal vesicle present. Cirrus sac piriform, 80-263 (139 ± 56; n=24; *n*=25) long, 48-195 (101 ± 40; n=24; *n*=25) wide. Cirrus armed with thorn-like microtriches. Ovary 50-280 (153 ± 64; n=19; *n*=20) long, 33-200 (99 ± 35; n=19; *n*=20) wide, H-shaped in dorso-ventral view, bilobed in cross section. Mehlis' gland prominent, posterior to ovarian isthmus, 28-102 (49 ± 23; n=9; *n*=10) long, 20-80 (53 ± 17; n=9; *n*=10) wide. Vagina thick-walled, posterior to genital pore, expanded distally, coiling slightly. Genital pore mid-ventral, 25-48% (36.3 ± 5.2; n=27)of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, 10-38 (20 ± 6; n=10; *n*=49) long, 13-53 (33 ± 12; n=10; *n*=49) wide, forming two wide lateral bands; each band consisting of one dorsal and one ventral column of follicles, extending entire length of proglottid, uninterrupted by ovary, confluent posterior to ovary. Eggs with single short filament, not packaged. Excretory ducts lateral.

Remarks

The unique hook formula is sufficient to distinguish this species from all others in the genus except *E. acanthinophyllum*, *E. raschii*, and *E. rayallemangi*. This species differs from *E. acanthinophyllum* in its possession of lateral hooklets arranged in a single continuous row, as opposed to two groups. *Echinobothrium mexicanum* differs from *E. raschii* in lacking a cleft in the posterior bothrial margin as exhibited by *E. raschii*. This species differs from *E. rayallemangi* in its possession of 23-40 cephalic peduncle spines per column as opposed to 2-5 in *E. rayallemangi.*

This species, described by Tyler and Caira (1999), was the third species in the genus to be described from *Myliobatis* (see *E. mathiasi* and *E. megacanthum*). This species bears a marked resemblance to *E. megacanthum*. These species are similar in their overall slender shape, their possession of a robust, heavily armed cirrus and muscular vagina, and densely arranged vitelline follicles.

Echinobothrium musteli Pintner, 1889

(Figs. 101-102)

Type host: *Mustelus mustelus* (L.), Smooth-hound (Triakidae, Carcharhiniformes) (as "Hundshai").

Additional hosts: *Mustelus plebejus, Starry smooth-hound (Triakidae, Carch*arhiniformes); *M. laevus* (= *M. asterias*) (see Stossich 1898; Ruszkowski 1927).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Trieste, Italy.

Additional localities: Cape Blanc, Mauritania.

Type material: NMW Inv. No. 2047 (holotype).

Specimens examined: None.

Etymology: Not given, but presumably named for its host.

Description (Modified from Pintner [1889].)

Worms 4-5 mm long. Strobila euapolytic, acraspedote, at least 20 proglottids. Scolex bipartite, 850 long, consisting of scolex proper and cephalic peduncle. Scolex proper consisting of armed apical rostellum and one dorsal and one ventral bothrium. Thirty-one apical hooks in each dorso-ventral group. Hook symmetry undetermined, hook formula {6 31 6}. Apical hooks hollow. Lateral hooklets arranged in single continuous row, staggered in their arrangement. Bothria approximately 300 long. Several rows of small spines or large microtriches in region posterior to rostellar armature and anterior to bothria. Cephalic peduncle 480 long, armed with eight longitudinal columns of 20-22 spines. Spines with triradiate bases, 24-64 long.

Testes 22 in number, anterior to cirrus sac. Vas deferens extensive. Cirrus sac piriform. Ovary U-shaped in dorso-ventral view, bilobed in cross section. Vagina posterior to genital pore, uniform in diameter along length, undulating slightly. Genital pore midventral, overlapping ovary. Excretory ducts lateral.

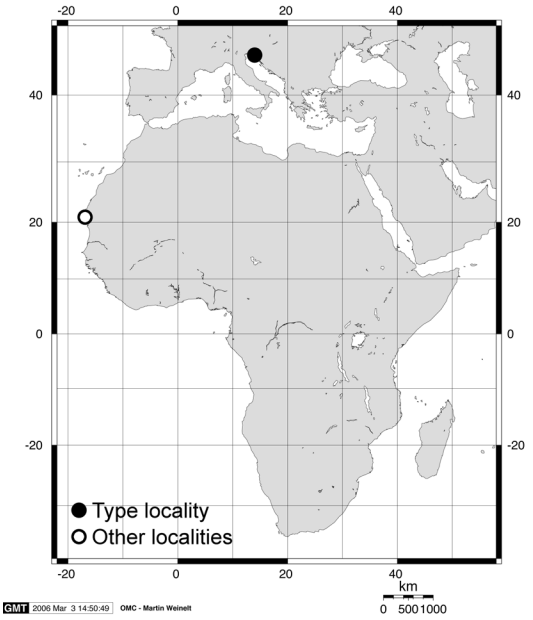


Fig. 101. Distribution of *Echinobothrium musteli*.

Remarks

This species can be distinguished from all other valid species in the genus except *E. notoguidoi* by its possession of 8-10 rows of small spines or microtriches between the rostellum and the bothria. This species differs from *E. notoguidoi* in possessing 22 versus 11-15 testes, and a U-shaped versus H-shaped ovary.

This was the first *Echinobothrium* species described from a shark. This species has been considered valid by all workers since its description, and its distinctive scolex armature was used to distinguish it from all other species in the genus in the keys of Euzet (1951), Rees (1961b), and Probert and Stobart (1989). Stossich (1898) and Ruszkowski (1927; 1928) both reported this species from *M. asterias*. Radulescu *et al.* (1972) reported 237 specimens of this species from a single specimen of *M. mustelus* caught off Mauritania. This species was included in the key to the species presented by Ivanov and Campbell (1998a) and also in the phylogeny published by Ivanov and Hoberg (1999). In their analysis, *E. musteli* surprisingly did not appear as sister to *E. notoguidoi* in their tree.

In the description of this species, Pintner (1889) noted that the worms are highly visible within the gut of the host due to the "blu-

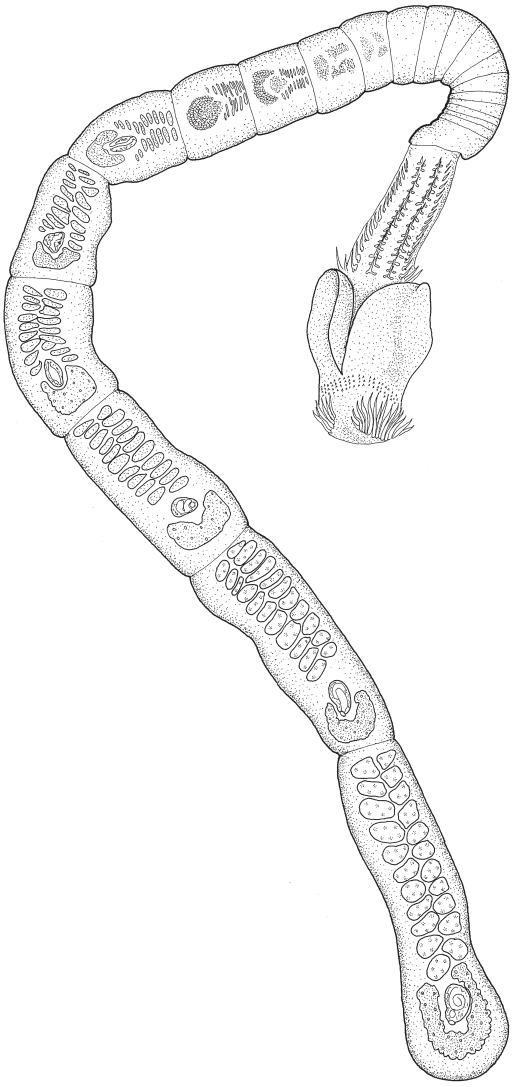


Fig. 102. Line drawing of *Echinobothrium musteli*. Redrawn from Pintner (1889).

trothe Färbung" (blood-red pigment) of the body. This pigment appears to be present in several other species in the genus (*e.g.*, *E. chisholmae*, *E. pigmentatum*). The detail with which Pintner (1889) described this species is astonishing. His description of the armature of the scolex was exceptionally detailed (except, ironically, for the hook formula). In that description he stated that, in his opinion, the spines present posterior to the apical armature were probably no different from the "peli setolosi" (= microtriches?) of Monticelli (not cited). Scanning electron microscope examination of similar armature in an undescribed species of *Echinobothrium* from a different triakid shark revealed these structures to be very large pectinate spinitriches with the lateral digits greatly reduced and fused to the central digit. It seems likely that this is the case for *E. musteli* (and *E. notoguidoi*), but these species were not examined using SEM.

The type host reported for this species was simply "Hundshai," or dogfish. However, in a footnote, Ruszkowski (1928) stated that while Braun (1894-1900) suggested that the "Hundshai" of Pintner (1889) was *Scyllium* [*Scyliorhinus*?] *canicula*, he (Ruszkowski) had never found *E. musteli* in that host, but did in fact find it in *Mustelus laevis* (= *M. mustelus*). In the footnote, Ruszkowski stated that he wrote directly to Pintner, who replied that *M. laevis* was indeed the type host, and that *E. musteli* was not found in any of the 100 specimens of *Scyllium* Pintner had dissected in Trieste.

Some workers (Ruszkowski, 1928; Dollfus, 1964; Jones and Beveridge, 2001) have stated that Pintner (1889) considered *E. levicolle* to be the larval stage of *E. musteli*. However, Pintner (1889) was not so certain, stating that it was not entirely impossible that the two were the same species, based on the fact that fragments of the gastropod *Nassa reticulata*, the host for *E. levicolle*, were among the gut contents of the sharks he had examined. Pintner (1889) also mentioned that the number of apical hooks described by Lespés (1857) for *E. levicolle* was not sufficient to unequivocally equate the two.

Echinobothrium notoguidoi Ivanov, 1997

(Figs. 103-105)

Type host: *Mustelus schmitti* Springer, Narrownose smooth-hound (Triakidae, Carcharhiniformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Mar del Plata, Argentina (38°00'S, 57°33'W).

Type material: MLP No. 3893C (holotype);

MLP Nos. 3894C and USNPC No. 87169 (paratypes).

Specimens examined: Paratypes (MLP No. 3894C, two specimens; USNPC No. 87169, two specimens).

Etymology: The specific epithet of this species is derived from the Greek "noto" meaning "austral," referring to its distribution, and "guidoi" in honor of Dr. Guido Pastorino, Universidad Nacional de La Plata.

Description (Modified from Ivanov [1997].)

Worms 4.16-9.73 mm long, 195-364 wide at terminal proglottid. Strobila euapolytic, acraspedote, 11-18 proglottids. Mature proglottids 845-1,480 long, 195-364 wide. Scolex bipartite, 1.22-1.49 mm long, consisting of scolex proper and cephalic peduncle. Scolex proper 480 long, consisting of armed apical rostellum and two bothria. Thirty-one apical hooks in each dorso-ventral group. Hook formula {13 16/15 13}, apical hooks solid, hook lengths all increasing toward center of group. Lateral hooklets arranged in two groups, staggered in position relative to one another. Region of scolex posterior to rostellum and anterior to bothria surrounded by 8-11 rows of small spines or microtriches. Bothria 210-285 long, 114-181 wide. Cephalic peduncle 481-585 long, 123-135 wide, armed with eight longitudinal columns of 24-26 spines. Spines with triradiate bases, 15-17 to 78-94 long.

Testes 11-15 in number, anterior to cir-

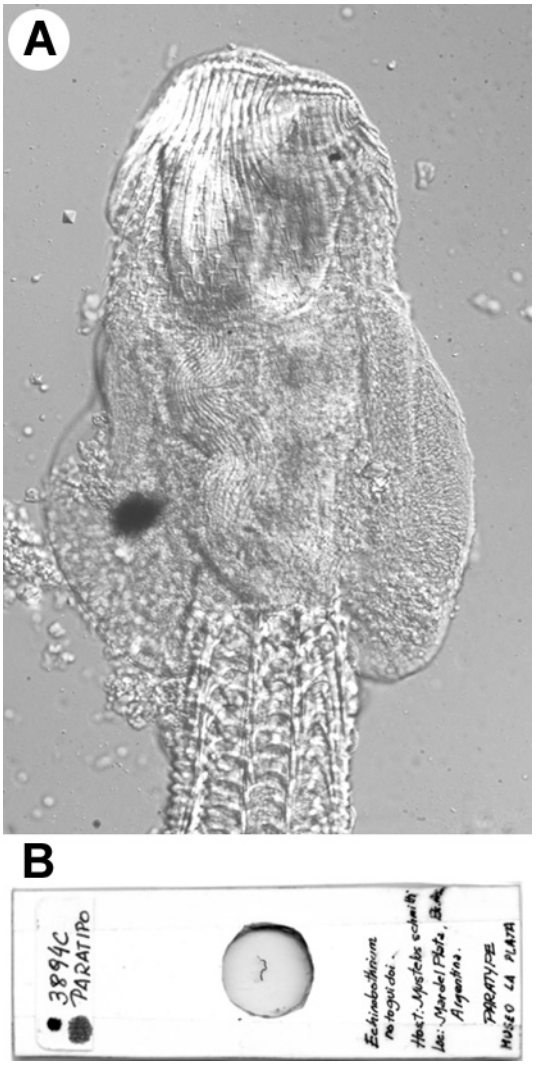


Fig. 104. Light micrographs of *Echinobothrium notoguidoi*. A. Scolex. B. Paratype slide MLP No. 3849C.

Fig. 103. Distribution of *Echinobothrium notoguidoi*.

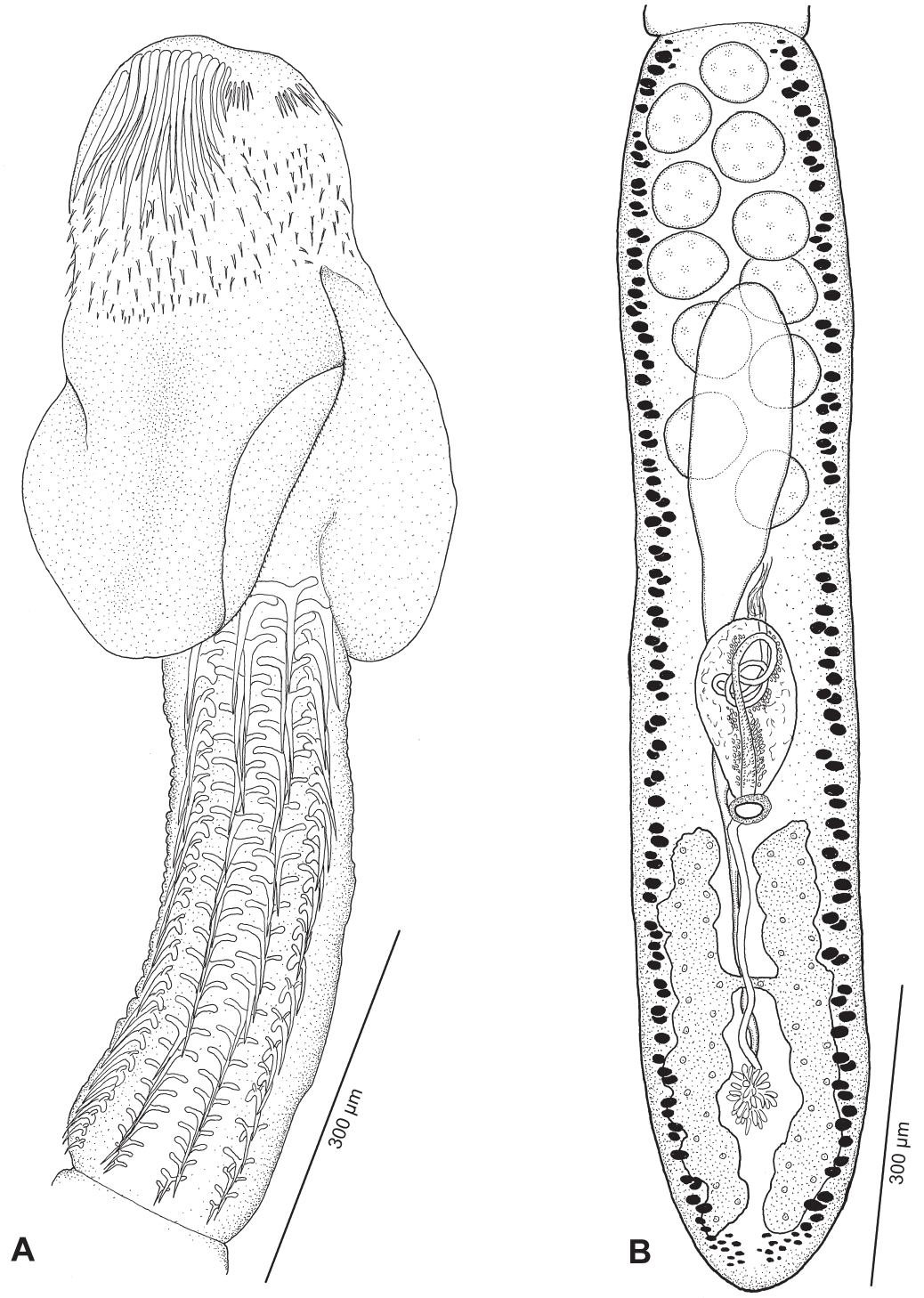


Fig. 105. Line drawings of *Echinobothrium notoguidoi*. A. Scolex. B. Mature proglottid. Redrawn from Ivanov (1997).

rus sac, 51-84 long, 72-84 wide, in two irregular columns, one layer deep. Vas deferens minimal, anterior to cirrus sac. Cirrus sac piriform, 135-195 long, 78-105 wide. Ovary 195-360 long, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, posterior to genital pore, relatively uniform in diameter along length, undulating slightly. Genital pore midventral, anterior to ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, forming two lateral bands extending entire length of proglottid, uninterrupted by ovary. Excretory ducts lateral.

Remarks

The unique armature between the rostellum and bothria differentiates this species from all other valid species in the genus except *E. musteli*. This species is distinguished from *E. musteli* in its possession of 11-15 rather than 22 testes, and an H-shaped rather than U-shaped ovary.

This species was the second to be described from the shark genus *Mustelus*. It has been considered valid by all subsequent workers, and was included in the key of Ivanov and Campbell (1998a) and in the cladistic analysis of Ivanov and Hoberg (1999). In the tree resulting from their analysis, this species did not group with *E. musteli* (also from *Mustelus*), but with two species from *Myliobatis*, *E. mathiasi* and *E. megacanthum*.

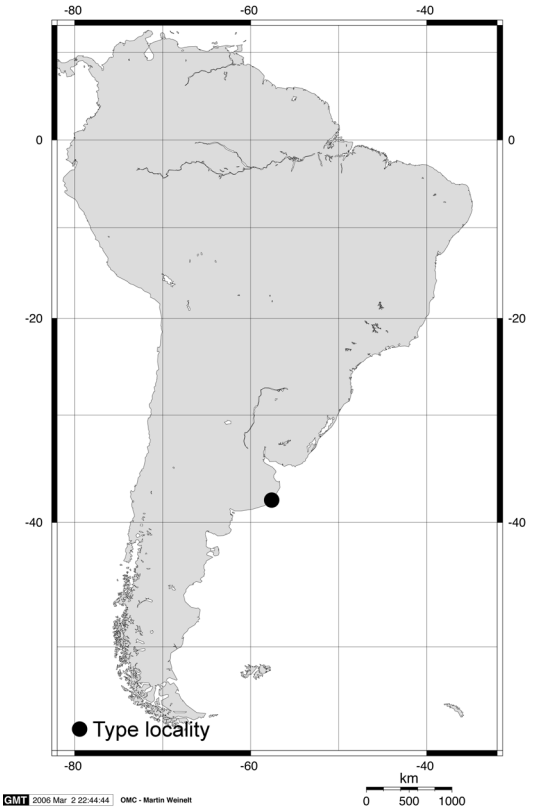


Fig. 106. Distribution of *Echinobothrium pigmentatum*.

Echinobothrium pigmentatum Ostrowski de Núñez, 1971

(Figs. 106-107)

Type host: *Zapteryx brevirostris* (Müller and Henle), Lesser guitarfish (Rhinobatidae, Rhinobatiformes).

Additional hosts: unidentified amphipod (intermediate host).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Mar del Plata, Argentina.

Type material: Holotype and 57 paratypes in the collection of M. Ostrowski de Núñez.

Specimens examined: Thirteen paratypes, Nos. 215/4 (7 immature, 5 mature, one free proglottid).

Etymology: Not given, but presumably refers to the red pigment observed in the cephalic peduncle/neck region.

Description (Modified from Ostrowski de Núñez [1971].)

Worms 900-1,790 long, 91-273 wide at terminal proglottid. Strobila euapolytic, acraspedote, 4-5 proglottids. Mature proglottids one in number, 593-1,080 long, 183-285 wide. Scolex bipartite, 268-340 long, consisting of scolex proper and cephalic peduncle. Scolex proper 168-175 long, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Nineteen apical hooks in each dorso-ventral group. Hook formula {(10-11) 10/9 (10-11)}, apical hooks solid, hooks gradually increasing in length toward center of group. Lateral hooklets uniformly arranged in continuous row. Bothria 91-155

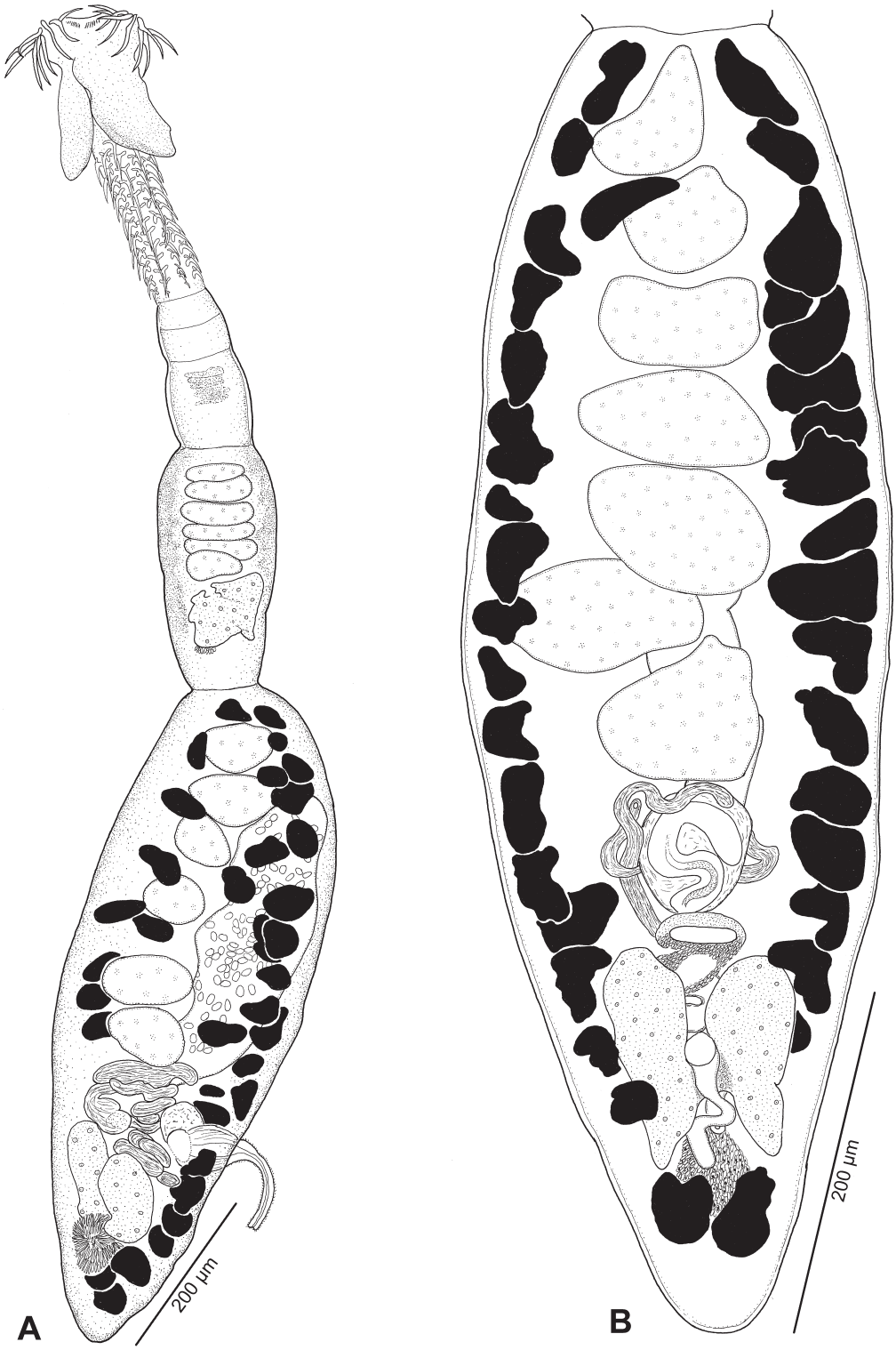


Fig. 107. Line drawings of *Echinobothrium pigmentatum*. A. Whole worm. B. Mature proglottid.

long, 130 wide, proximal surfaces covered with spinitriches. Cephalic peduncle 143-195 long, 39-55 wide, armed with eight longitudinal columns of 8-13 spines. Spines with triradiate bases, 12-23 to 33-49 long.

Testes 5-7 in number, anterior to cirrus sac, 19-93 long, 44-120 wide, in single irregular column, one layer deep. Vas deferens extensive, extending posterior to cirrus sac. Cirrus sac piriform, 36-117 long, 42-98 wide. Ovary 95-138 long, 108-110 wide, H-shaped in dorso-ventral view, bilobed in cross section. Vagina thick-walled, posterior to genital pore, expanded proximally, undulating slightly. Genital pore midventral, 25-30% of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, thick-walled in early stages of development. Mehlis' gland 45-63 long, 43-60 wide. Vitellaria follicular, large, measuring 30-55 long, 43-70 wide, in two lateral bands on either side of proglottid, extending entire length of proglottid, uninterrupted by ovary, confluent posterior to ovary. Eggs oval, 35 long, 30 wide, with filament on each end, not packaged. Excretory ducts lateral.

Remarks

The unique hook formula of this species distinguishes it from all others in the genus except *E. californiense*, *E. coronatum*, and *E. hoffmanorum*. This species differs from *E. californiense* in its lack of a cleft in the posterior bothrial margin, from *E. coronatum* in its possession of lateral hooklets arranged in a single continuous row rather than in two groups, and from *E. hoffmanorum* in possessing eggs with two, rather than one filament, and a genital pore that is anterior to, rather than overlapping, the ovary.

This species, although collected in fairly high numbers by Ostrowski de Núñez (1971) (56 plus one larva in an amphipod in one of just three hosts examined), has not been reported since. The validity of this species has never been questioned, and it has been included in the keys of both Probert and Stobart (1989) and Ivanov and Campbell (1998a). Ivanov and Hoberg (1999) included this species in their cladistic analysis of the order, where it appeared in a relatively derived position among *Echinobothrium* in their tree, as the sister species to *E. affine* and *E. raschii*.

Echinobothrium raji Heller, 1949

(Figs. 108-111)

Type host: *Amblyraja radiata* (Donovan), Thorny skate (Rajidae, Rajiformes) (as *Raja scabrata* Garman and *Raja radiata* Donovan).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Quebec, Canada: 3-4 miles off Grande Rivière (~48.5°N, 64.5°W); Miscou Bank, about 30 miles NE of Grande Rivière (~49°N, 63°W).

Additional localities: Kolbeinseyjargrunn Iceland; Labrador coast, Newfoundland, Canada.

Type material: CMNPA No. 1995-0010 (holotype); CMNPA Nos. 1995-0011, 1995-0012 (paratypes).

Voucher specimens: Two specimens, collected by A. F. Heller (LRP Nos. 2200-2201).

Specimens examined: Holotype; eight paratypes; both LRP vouchers.

Etymology: Not given, but presumably named for its host.

Description (Modified from Heller [1949].)

Worms up to 4.2 mm long, 775 wide at terminal proglottid. Strobila acraspedote, apolytic, 7-8 proglottids. One mature proglottid, 855-1,780 long, 295-710 wide. Single gravid proglottid 1.570-2.030 mm long, 775-

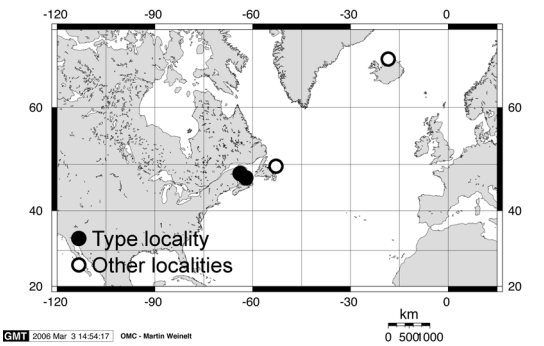


Fig. 108. Distribution of *Echinobothrium raji*.

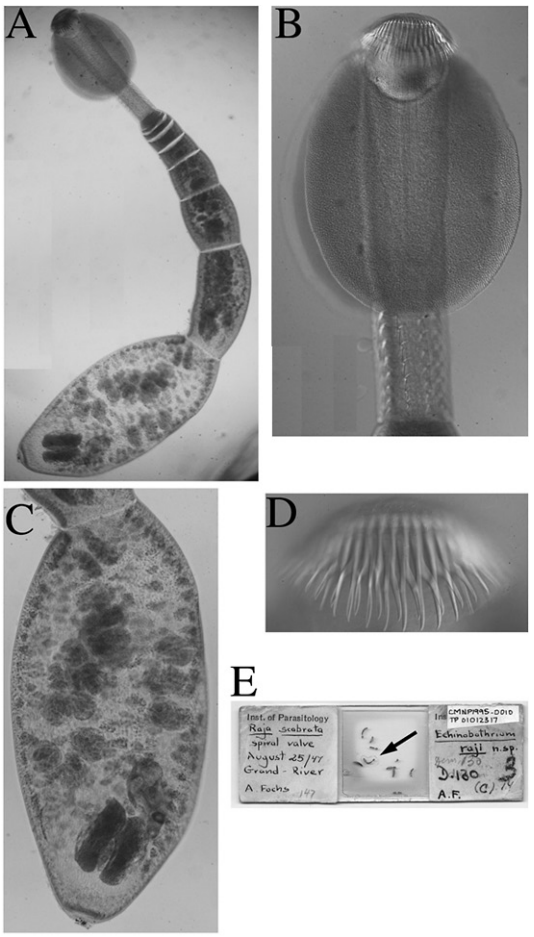


Fig. 109. Light micrographs of *Echinobothrium raji*. A. Whole worm. B. Scolex. C. Mature proglottid. D. Apical hooks. E. Holotype slide (arrow indicates holotype) CMNPA No. 1995-0010.

780 wide. Scolex bipartite, 850-980 long, consisting of scolex proper and cephalic peduncle. Scolex proper 680-735 long, 490-540 wide, consisting of armed apical rostellum and two bothria. Hook formula undetermined, at least 40 apical hooks per group. Apical hooks solid. Lateral hooklets absent. Hooks increasing in length toward center of group. Bothria 600-635 long, 490-540 wide, proximal bothrial surfaces with long filitriches and pectinate spinitriches each bearing 8-13 relatively equal length digits. Distal bothrial surfaces except medial distal surfaces with spatulate spinitriches only. Medial distal bothrial surface with pectinate spinitriches each bearing three digits; central digit conspicuously longer than others. Lateral surface of scolex proper with short filitriches and pectinate spinitriches each bearing 7-15 relatively equal length digits. Cephalic peduncle 250-300 long, 135-180 wide, armed with eight longitudinal columns of 5-9 spines, with velum at posterior terminus. Spines with triradiate bases, 18-38 to 55-60 long.

Testes 17-23 in number, anterior to ovary, spherical to sub-spherical, 100-150 long, 100-133 wide, in 2-3 irregular columns, one layer deep. Vas deferens extensive, looping posterior to cirrus sac. Cirrus sac piriform, 113-168 long, 100-133 wide, slightly overlapping ovary. Internal seminal vesicle present. Ovary 195-470 long, 255 wide, H-shaped in dorso-ventral view. Vagina thin-walled, looping anterior to genital pore, uniform in width along its length. Seminal receptacle present. Genital pore midventral, 28-29% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, extending to anterior end of gravid proglottid. Vitellaria follicular, 23-67 long, 42-65 wide, in two lateral bands extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 11 by 18, lacking appendages, not packaged. Excretory ducts lateral.

Remarks

This species has a greater number of apical hooks per group (at least 40) than all other species in the genus except *E. heroniense*. *Echinobothrium raji* differs from *E. heroniense* in its possession of a short peduncle with only 5-9 peduncle spines per column, versus 24-32 spines per column in *E. heroniense*.

Echinobothrium raji was described by Heller (1949), and was the first diphyllidean described from the western Atlantic. Although Euzet (1951) did not include this species in his key to the species of *Echinobothrium*, it has been regarded as a valid species by subsequent authors, having also been included in the keys of Rees (1961b), Probert and Stobart (1989), and Ivanov and Campbell (1998a), and included in the cladistic analysis of the order by Ivanov and Hoberg (1999). It was supported in their tree as the sister species to a polytomy comprising *E. acanthocolle*, *E. reesae*, and *E. rhynchobati*. *Echinoboth-*

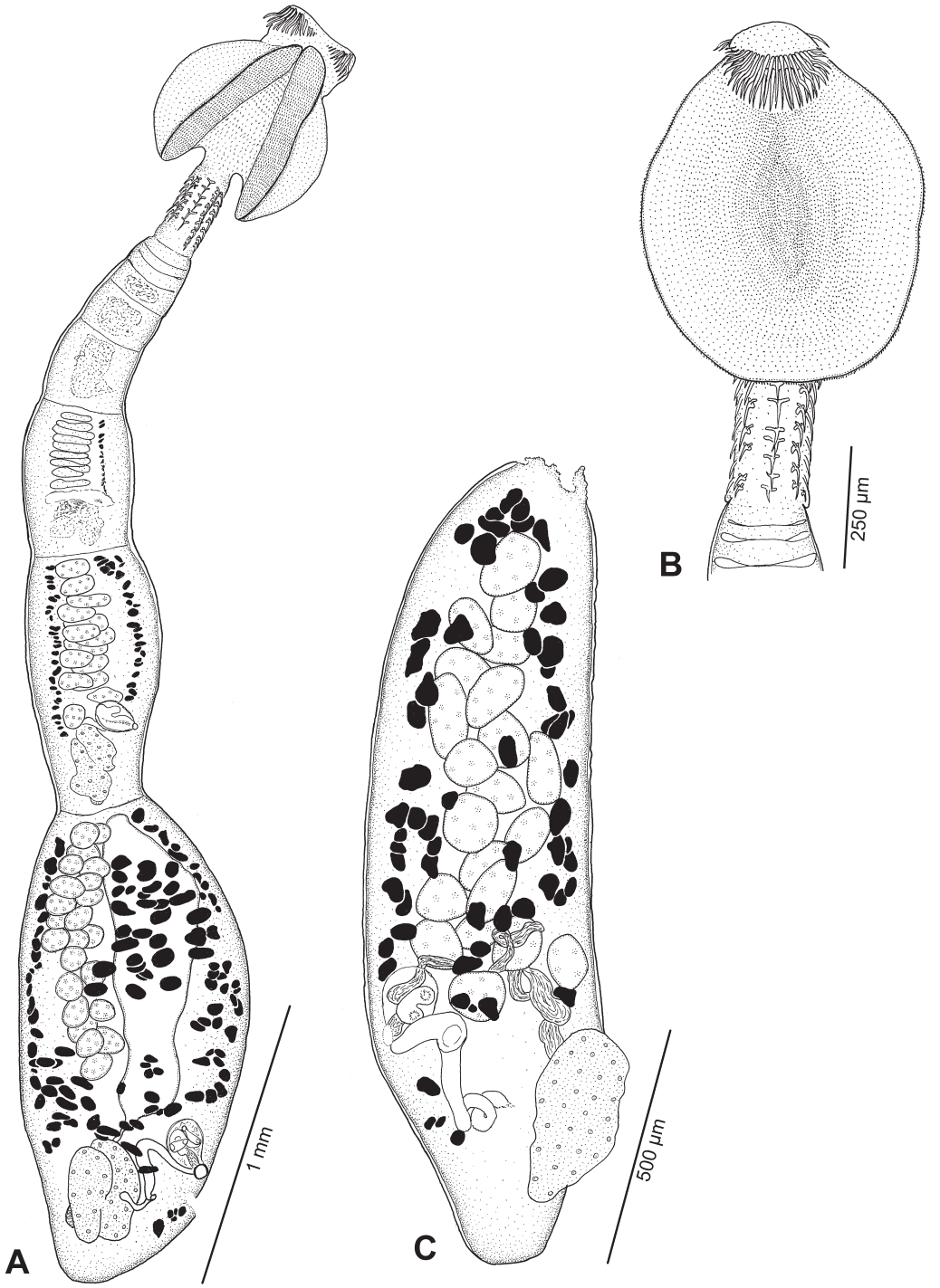


Fig. 110. Line drawings of *Echinobothrium raji*. A. Whole worm, terminal proglottid damaged. B. Scolex. C. Mature proglottid, ruptured.

rium raji has been reported on at least two other occasions since its description. Both reports are from the type host. Baer (1962) reported *E. raji* from Iceland, and Keeling

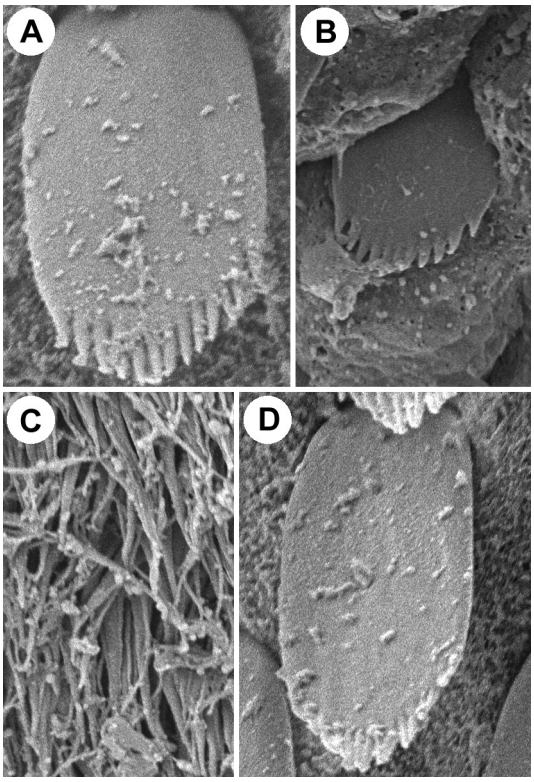


Fig. 111. Scanning electron micrographs of *Echinobothrium raji*. A.Proximal bothrial surface (anterior). B. Proximal bothrial surface (posterior). C. Distal bothrial surface. D. Lateral surface of scolex proper. Scale bars: A-D, 1 µm.

(1994, unpubl. thesis) from off the Labrador coast, Canada (bost as *Raja radiata*).

This species bears a remarkable resemblance to *E. acanthocolle*, also from cold waters at high latitudes. Both have a large, robust scolex with a large number of apical hooks, relatively few cephalic peduncle spines and a fairly short, robust strobila. The phylogenetic analysis presented in this volume does not, however, support a close relationship between the two.

Echinobothrium raschii Campbell and Andrade, 1997

(Figs. 112-114)

Type host: *Rhinoraja longi* Raschi and McEachran, Aleutian dotted skate (Rajidae, Rajiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Bering Sea (56˚08'N, 168˚21'W).

Type material: USNPC No. 86767 (holotype); USNPC Nos. 86768-86770 and BMNH No. 1996.7.26.3-6 (paratypes).

Specimens examined: Two paratypes (USNPC Nos. 86768, 86770).

Etymology: This species was named in honor of Dr. W. Raschi of Bucknell University.

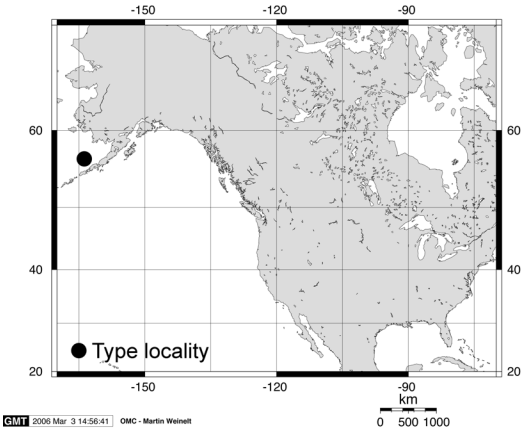


Fig. 112. Distribution of *Echinobothrium raschii*.

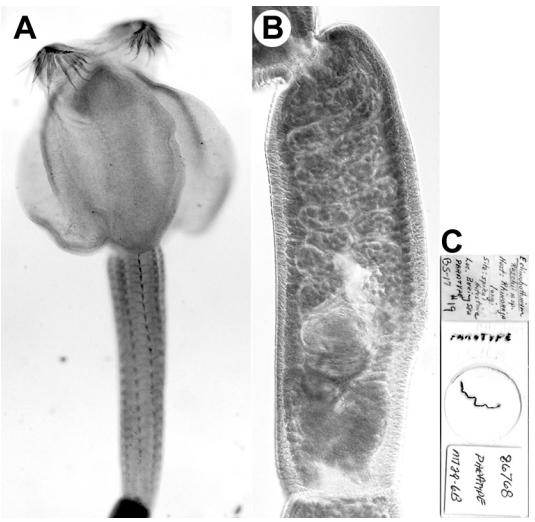


Fig. 113. Light micrographs of *Echinobothrium raschii*. A. Scolex. B. Mature proglottid. C. Paratype slide USNPC No. 86768.

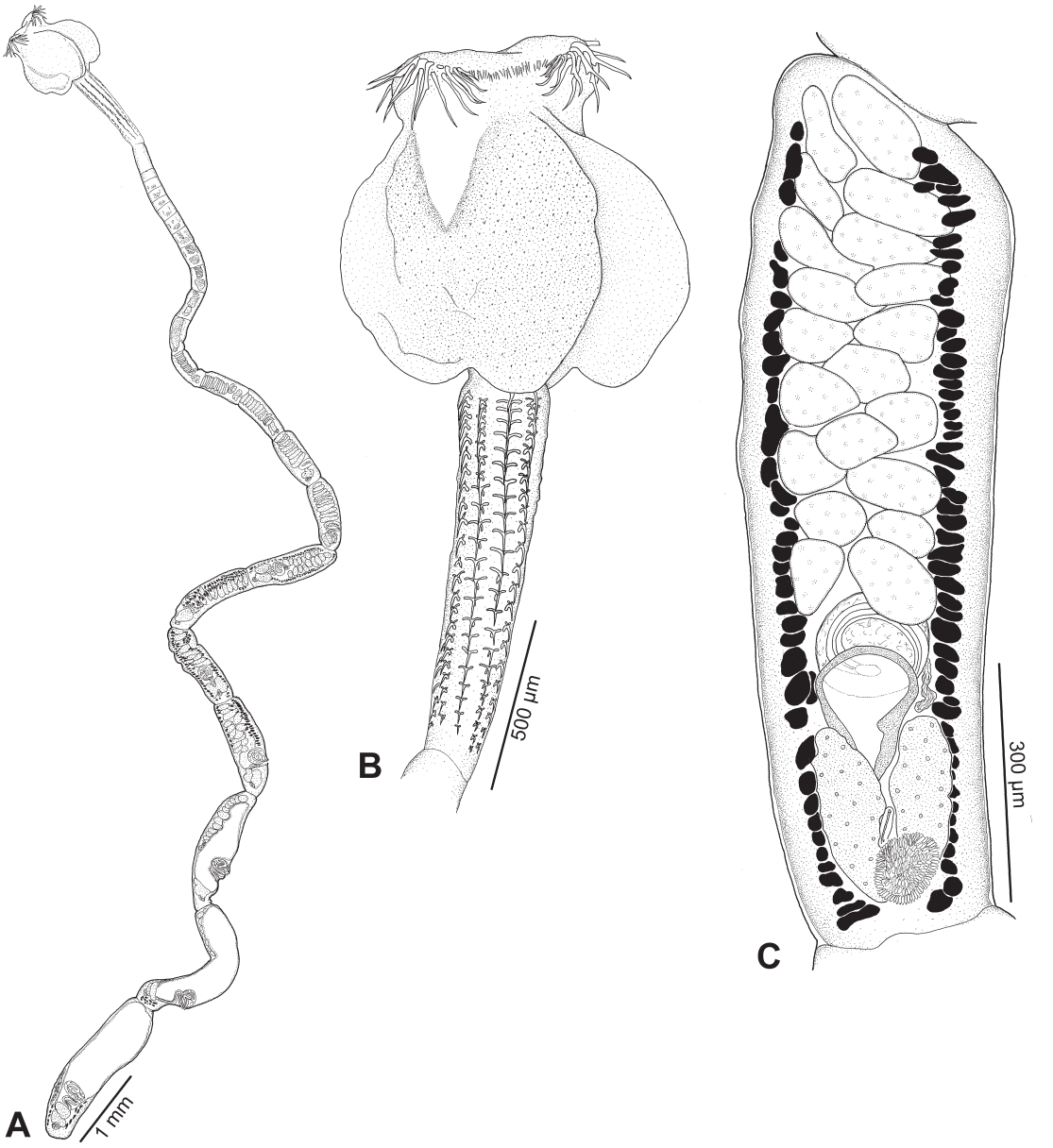


Fig. 114. Line drawings of *Echinobothrium raschii*. A. Whole worm. B. Scolex. C. Mature proglottid.

Description (Modified from Campbell and Andrade [1997].)

Worms 8.6-21.5 mm long, 500-580 wide at terminal proglottid. Strobila acraspedote, apolytic, 22-29 proglottids. Mature proglottids 2-7 in number, 1.0-1.150 mm long, 300-355 wide. Gravid proglottids 1-7 in number, 1.9-2.1 long, 420-580 wide. Scolex bipartite, 1.6-2.1 long, consisting of scolex proper and cephalic peduncle. Scolex proper 1.025-1.260 mm long, 688-736 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Hook formula {(12-18) (12-13)/(11-12) (12-18)}, hooks solid. Central apical hook lengths all increasing toward center of group. Lateral hooklets forming single continuous row, staggered in position relative to one another. Bothria 780-1,020 long, 688-

736 wide, with cleft in posterior margin, proximal surfaces covered with short filitriches. Distal bothrial surfaces except medial distal surfaces with short filitriches and pectinate spinitriches each bearing 15 relatively equal length digits. Medial distal bothrial surface devoid of microtriches. Cephalic peduncle 540-1,075 long, 200-275 wide, armed with eight longitudinal columns of 21-26 spines. Spines with triradiate bases, 14-22 to 69-79 long.

Testes 17-23 in number, anterior to ovary, 23-76 long, 68-122 wide, in two irregular columns, one layer deep. Vas deferens extensive, looping posterior to cirrus sac. Cirrus sac piriform, 148-251 long, 110-209 wide, anterior to ovary. Ovary 200-240 long, H-shaped in dorso-ventral view. Vagina thick-walled, entirely posterior to genital pore, uniform in width along its length. Seminal receptacle present. Genital pore midventral, 17-29% of proglottid length from posterior end of proglottid, anterior to ovary. Uterus saccate, extending to anterior end of gravid proglottid. Vitellaria lateral, follicular, 19-42 long, 15-30 wide, extending entire length of proglottid, uninterrupted by ovary. Eggs oval, 21-26 long, 13-17 wide, with single short filament, within intrauterine tube. Excretory ducts lateral.

Remarks

The unique hook formula of this species is sufficient to distinguish it from all other species in the genus except *E. euzeti*, *E. mathiasi*, *E. megacanthum*, *E. mexicanum*, and *E. rayallemangi*. *Echinobothrium raschii* differs from *E. euzeti* and *E. mathiasi* in exhibiting type B hook symmetry, as opposed to type A hook symmetry exhibited by the latter two species. *Echinobothrium raschii* can be distinguished from both *E. megacanthum* and *E. mexicanum* in its possession of a cleft in the posterior bothrial margin, a feature lacking in both *E. megacanthum* and *E. mexicanum*.

Campbell and Andrade (1997) described this species from the Bering Sea; this represented the first report of a diphyllidean from the eastern Pacific. This species has been considered valid by all subsequent workers. *Echinobothrium raschii* appeared in the key to the species of *Echinobothrium* published by Ivanov and Campbell (1998a), and in the phylogenetic analysis of the order published by Ivanov and Hoberg (1999). It appeared in their tree nested within *Echinobothrium*, as the sister species to *E. affine*.

Echinobothrium rayallemangi Tyler, 2001

(Figs. 115-118)

Type host: *Rhinobatos leucorhynchus* Günther, Whitesnout guitarfish (Rhinobatidae, Rhinobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Bahía de Los Angeles, Baja California, México (28˚55'N, 113˚32'W).

Additional localities: Santa Rosalia, Baja California Sur, México (27˚19'N, 112˚17'W).

Type material: CNHE No. 3920 (holotype); CNHE Nos. 3921-3922, USNPC Nos. 090149, 090150, HWML No. 15491, and LRP Nos. 2000-2021 (paratypes).

Specimens examined: Holotype; all 31 paratypes.

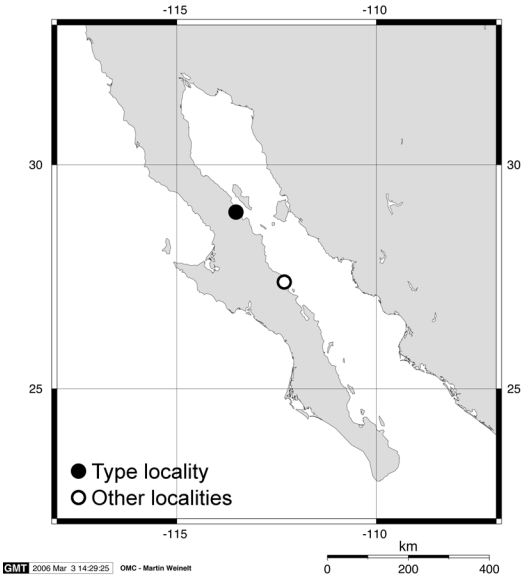


Fig. 115. Distribution of *Echinobothrium rayallemangi*.

Etymology: This species was named in memory of William Ray Allemang, Sr., the author's grandfather.

Description (Modified from Tyler [2001].)

Worms 940-1,630 (1,169 ± 169; n=30) long, 115-274 (180 ± 43; n=29) wide at terminal proglottid. Strobila acraspedote, euapolytic, 4-6 (4.6 ± 6; n=32) proglottids, covered with long filitriches. Mature proglottids 0-1 (0.9 ± 0.3; n=32) in number, 308-661 (504 ± 86; n=29) long, 115-275 (167 ± 36; n=26) wide, gravid proglottids 0-1 (0.2 ± 0.4; n=32) in number, 455-740 (596 ± 113; n = 6) long, 213-273 (246 ± 26; n=4) wide. Scolex bipartite, consisting of scolex proper and cephalic peduncle. Scolex proper 150-259 (203 ± 33; n=25) long, 100-158 (125 ± 14; n=18) wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Apex of scolex proper covered with long and short filitriches. Twenty-three apical hooks in each dorso-ventral group. Hook formula {(10-12) 12/11 (10-12)}, apical hooks solid, hooks increasing in length toward center of group. Lateral hooklets arranged in continuous row.

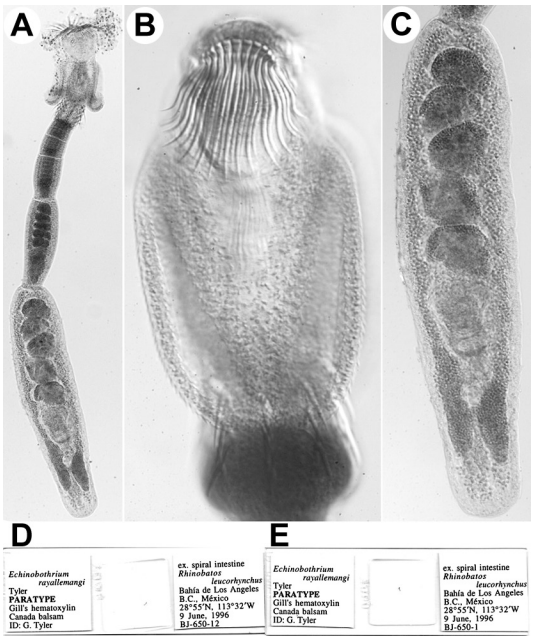


Fig. 116. Light micrographs of *Echinobothrium rayallemangi*. A. Whole worm. B. Scolex. C. Mature proglottid. D-E. Paratype slides LRP Nos. 2000, 2008.

Bothria 98-194 (153 ± 28; n=23) long, 100-158 (125 ± 14; n=18) wide, proximal bothrial surfaces with short filitriches, cilia and pectinate spinitriches each bearing 8-12 relatively equal length digits. Distal bothrial surfaces (except medial distal surface) with short filitriches and large pectinate spinitriches each bearing 10-15 relatively equal length digits. Medial distal bothrial surface with short filitriches only. Lateral surfaces of scolex proper with pectinate microtriches each bearing 3-5 relatively equal length digits. Cephalic peduncle 98-194 (153 ± 28; n=30) long, 100-158 (125 ± 14; n=31) wide, armed with eight longitudinal columns of 2-5 (4; n = 32, *n* = 122) spines, covered with short filitriches. Spines with triradiate bases, 13-28 (18 ± 4; n=31) to 28-48 (35 ± 4; n=32) long.

Testes 4-6 (5.1 ± 7; n=32; *n*=81) in number, anterior to cirrus sac, 33-93 (57 ± 12; n=25; *n*=111) long, 53-130 (88 ± 18; n=23; *n*=92) wide, in single column, one layer deep. Vas deferens extensive, looping posterior to cirrus sac. Cirrus sac piriform, 38-90 (68 ± 15; n=16; *n*=17) long, 48-83 (61.± 10; n=16; *n*=17) wide. Cirrus armed along length with fine microtriches. Ovary 75-170 (120 ± 22; n=30; *n*=31) long, 74-138 (103 ± 21; n=20; *n*=21) wide, H-shaped in dorso-ventral view, bilobed in cross section. Mehlis' gland posterior and dorsal to ovarian isthmus, 40-60 (50 ± 5; n=14) long, 35-54 (45 ± 6; n=13) wide. Vagina thick-walled, posterior to genital pore, uniform in diameter along length, coiling slightly. Genital pore midventral, 22-38% (31.4 ± 4.0; n=26) from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, 14-45 (28 ± 8; n=6; *n*=27) long, 15-36 (23 ± 6; n=6; *n*=27) wide, forming two latero-ventral bands, each consisting of two columns of follicles; columns extending from level of ovarian isthmus to anterior margin of proglottid. Eggs 10-13 (11 ± 1; n=2; *n*=7) long, 10-13 (11 ± 1; n=2; *n*=7) wide, lacking appendages, not packaged. Excretory ducts lateral.

Remarks

The unique hook formula of this species is sufficient to distinguish it from all other

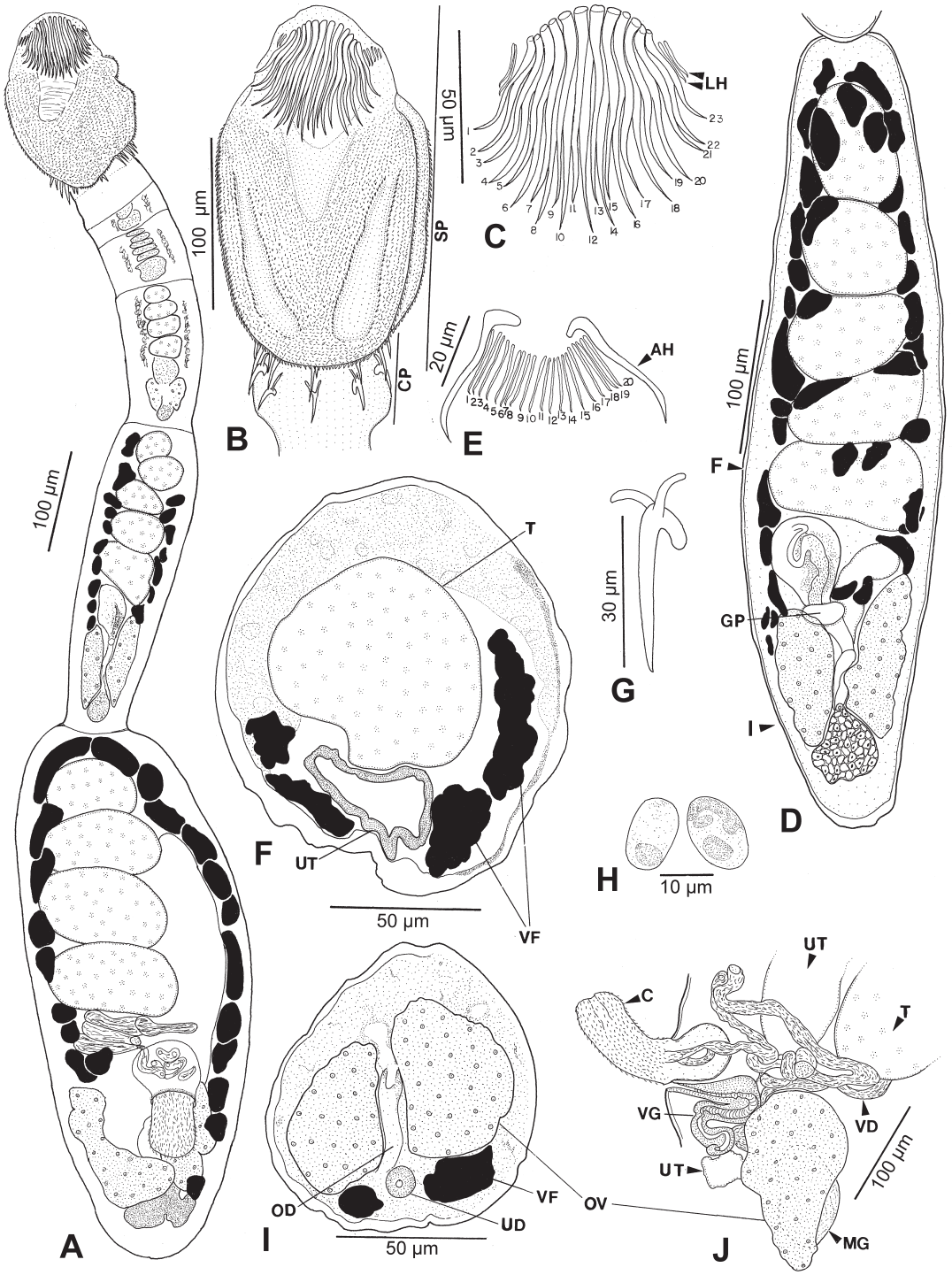


Fig. 117. Line drawings of *Echinobothrium rayallemangi*. A. Whole worm. B. Scolex. C. Apical hooks. D. Mature proglottid. E. Lateral hooklets. F. Cross section through proglottid at level indicated by "F" in D. G. Cephalic peduncle spine. H. Eggs. I. Cross section through proglottid at level indicated by "I" in D. J. Detail of terminal genitalia, lateral view. Abbreviations: C, cirrus; CP, cephalic peduncle; CS, cirrus sac; GP, genital pore; LH, lateral hooklets; MG, Mehlis' gland; O, ovary; OD, oviduct; SP, scolex proper; T, testis; UD, uterine duct; UT, uterus; VD, vas deferens; VF, vitelline follicle; VG, vagina.

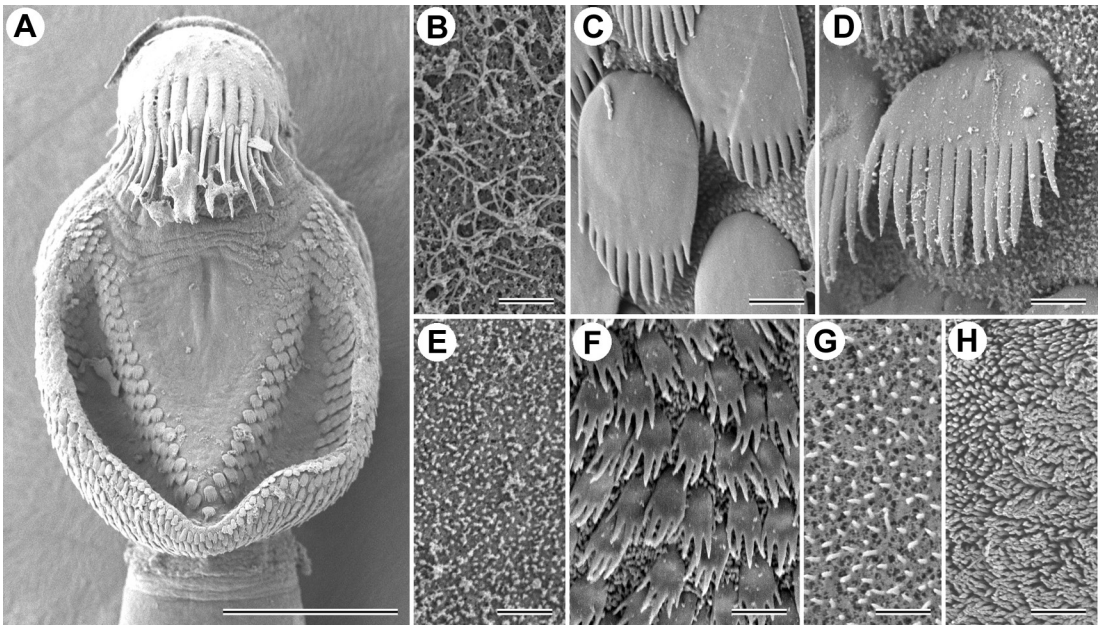


Fig. 118. *Echinobothrium rayallemangi* scanning electron micrographs. A . Scolex. B. Apex of scolex. C. Proximal bothrial surface. D. Distal (lateral) bothrial suface. E. Distal (medial) bothrial surface. F. Lateral surface of scolex proper. G. Neck. H. Strobila. Scale bars: A, 50 µm; B-H, 1 µm.

species in the genus except *E. acanthinophyllum*, *E. mexicanum*, and *E. raschii. Echinobothrium rayallemangi* differs from all three species in possessing testes arranged in one, rather than two columns.

This species was described by Tyler (2001) from the Gulf of California, making it the fourth diphyllidean species to be described from that body of water. It was collected during only one of two collecting trips, perhaps due to seasonal variation in parasite population levels or environmental disturbance (Tyler 2001).

Echinobothrium reesae Ramadevi, 1969

(Figs. 119-120)

Type host: *Himantura walga* (Müller and Henle), Dwarf whipray (Dasyatidae, Myliobatiformes); *Himantura uarnak* (Forsskål), Honycomb stingray (Dasyatidae, Myliobatiformes) (as *Trygon walga* and *T. uarnak*).

Additional hosts: *Leptochela aculeocaudata* Paulson (Pasiphaeidae, Decapoda) (intermediate host).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Waltair coast, India.

Type material: Not designated.

Specimens examined: None.

Etymology: This species was named in honor of Dr. Gwendolyn Rees, in recognition for

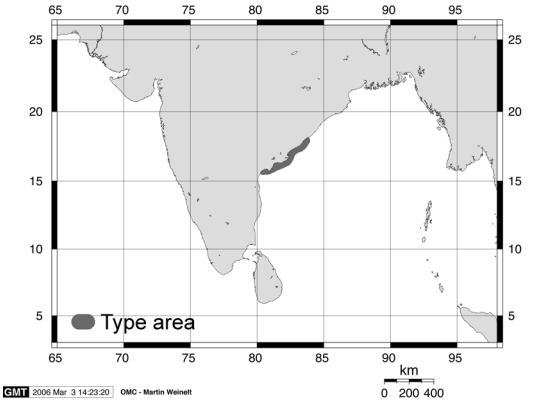


Fig. 119. Distribution of *Echinobothrium reesae*.

her contributions to the field of cestodology.

Description (Modified from Ramadevi [1969])

Worms approximately 10-15 mm long. Strobila acraspedote, euapolytic, 15-20 proglottids. Scolex bipartite, 351 long, consisting of scolex proper and cephalic peduncle. Scolex proper 223 long, 175 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Thirteen apical hooks per dorso-ventral group. Hook formula {2 7/6 2} , hook lengths increasing toward center of group. Cephalic peduncle 109 long, 97 wide, unarmed.

Testes 12 in number, anterior to cirrus sac, up to 183 in diameter, in two columns. Vas deferens extensive, looping posterior to cirrus sac. Cirrus sac oval, 312 in diameter. Cirrus armed along its length. Ovary 650 long, each lobe 170 wide, H-shaped in dorsoventral view, bilobed in cross section. Vagina thin-walled, looping anterior to genital pore, uniform in diameter along length, undulating slightly. Genital pore midventral, overlapping ovary. Uterus saccate. Vitellaria follicular, extending entire length of proglottid, uninterrupted by ovary. Eggs 31 in diameter.

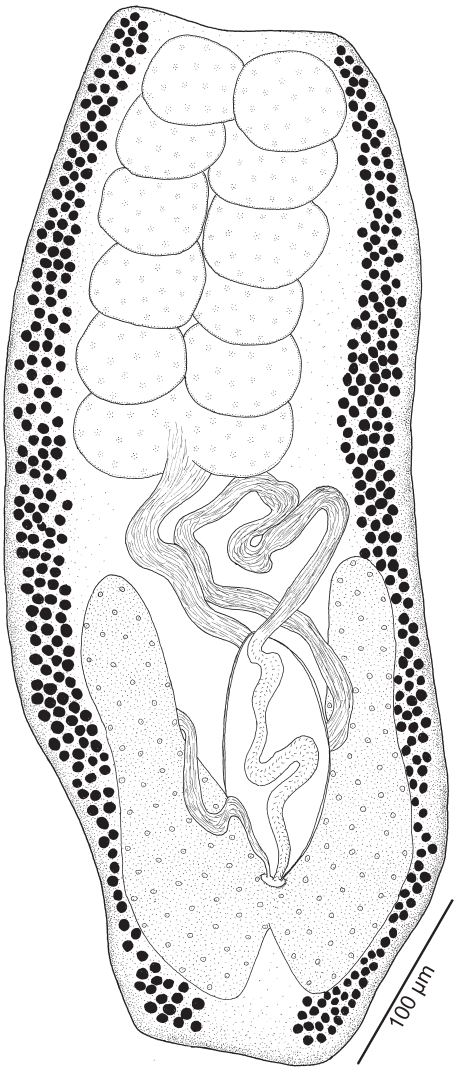


Fig. 120. Line drawing of proglottid of *Echinobothrium reesae*. Redrawn from Ramadevi (1969).

Remarks

This species is distinguished from all other species in the genus except *E. deeghai*, *E. euterpes*, *E. rhynchobati*, and *E. syrtensis* by its lack of cephalic peduncle armature. *Echinobothrium reesae* is distinguished from these four species by its unique hook formula.

Echinobothrium reesae was described by Ramadevi (1969), and the larval form was described by Ramadevi and Rao (1974). Probert and Stobart (1989) used the lack of cephalic peduncle armature as an identifying feature for this species in their key to the species of *Echinobothrium*. Khalil and Abdul-Salam (1989), in their description of *Macrobothridium*, stated that the validity and placement of *E. reesae* in *Echinobothrium* (versus *Macrobothridium*) required further investigation. Campbell and Andrade (1997) considered this a *species inquirenda*, and, because it was lacking cephalic peduncle armature, suggested it may actually belong in *Macrobothridium*. Ivanov and Campbell (1998a) agreed and excluded this species from their key. Although they acknowledged that Campbell and Andrade (1997) considered it a *species inquirenda*, Ivanov and Hoberg (1999) included this species in their cladistic analysis of the Diphyllidea, where it formed a polytomy with *E. acanthocolle* and *E. rhynchobati* in their tree. Neifar *et al.* (2001) considered transferring this species into *Macrobothridium*, but refrained from doing so, because there were

no type specimens available for examination to determine if cephalic peduncle spines were indeed lacking (which would necessitate its placement in *Macrobothridium*), or if their development was delayed (validating its current placement in *Echinobothrium*).

The larvae collected by Shimazu (1982) from *Leptochela* sp. in Malaysia strongly resemble this species, especially since the larvae were fully strobilated and possessed mature proglottids (see Ramadevi and Rao 1974). However, these larvae were not examined during this study.

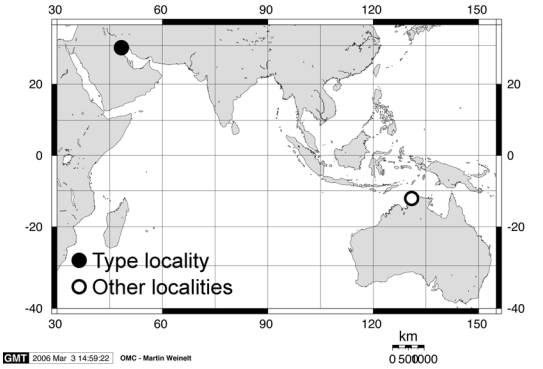


Fig. 121. Distribution of *Echinobothrium rhynchobati* n. comb.

Echinobothrium rhynchobati (Khalil and Abdul-Salam, 1989) n. comb.

(Figs. 121-124)

Synonym: *Macrobothridium rhynchobati* Khalil and Abdul-Salam, 1989 **n. syn.**

Type host: *Rhinobatos granulatus* Cuvier, Sharpnose guitarfish (Rhinobatidae, Rhinobatiformes).

Additional hosts: *Rhinobatos typus* Bennett, Giant shovelnose ray (Rhinobatidae, Rhinobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: A few miles east of Kuwait City, Persian Gulf.

Additional localities: Darwin, Northern Territory, Australia.

Type material: BMNH No. 1998.11.20.318 (holotype); BMNH No. 1998.11.20.319-323 (paratypes).

Voucher specimens: Thirteen whole mounts, 11 slides of serial sections, one scolex mounted in Berlese's medium and one egg preparation from *R. typus* from Darwin, Northern Territory, Australia (LRP Nos. 2217-2231); one DNA sequence voucher from *R. typus* from Darwin, Northern Territory, Australia (LRP No. D2149).

Specimens examined: Four paratypes (BMNH Nos. 1998.11.20.319-323); all 27 LRP vouchers.

Etymology: This species was named after its host (originally cited as *Rhynchobatus granulatus*).

Description (Modified from Khalil and Abdul-Salam [1989].)

Worms 30-43 mm long, 415-940 wide at terminal proglottid. Strobila acraspedote, apolytic, 78-115 proglottids, covered with long filitriches. Mature proglottids 3-9 in number, 750-3,910 long, 245-940 wide. Scolex bipartite, 2.22-3.35 mm long, consisting of scolex proper and cephalic peduncle. Apex of scolex proper covered with short and long filitriches. Scolex proper 2.43-3.11 mm long, 990-1,480 wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Bothria with extensive network of excretory vessels. Hook formula {(1-2) 6/(15-17) (1-2)},

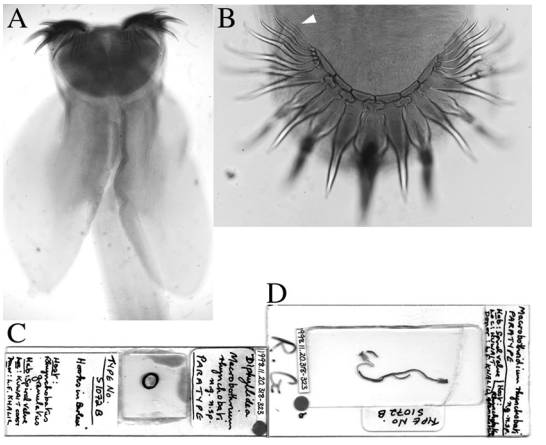


Fig. 122. Light micrographs of *Echinobothrium rhynchobati* n. comb. A. Scolex. B. Hooks (arrow indicates lateral hooklet). C-D. Paratype slides BMNH No. 1998.11.20.318-323.

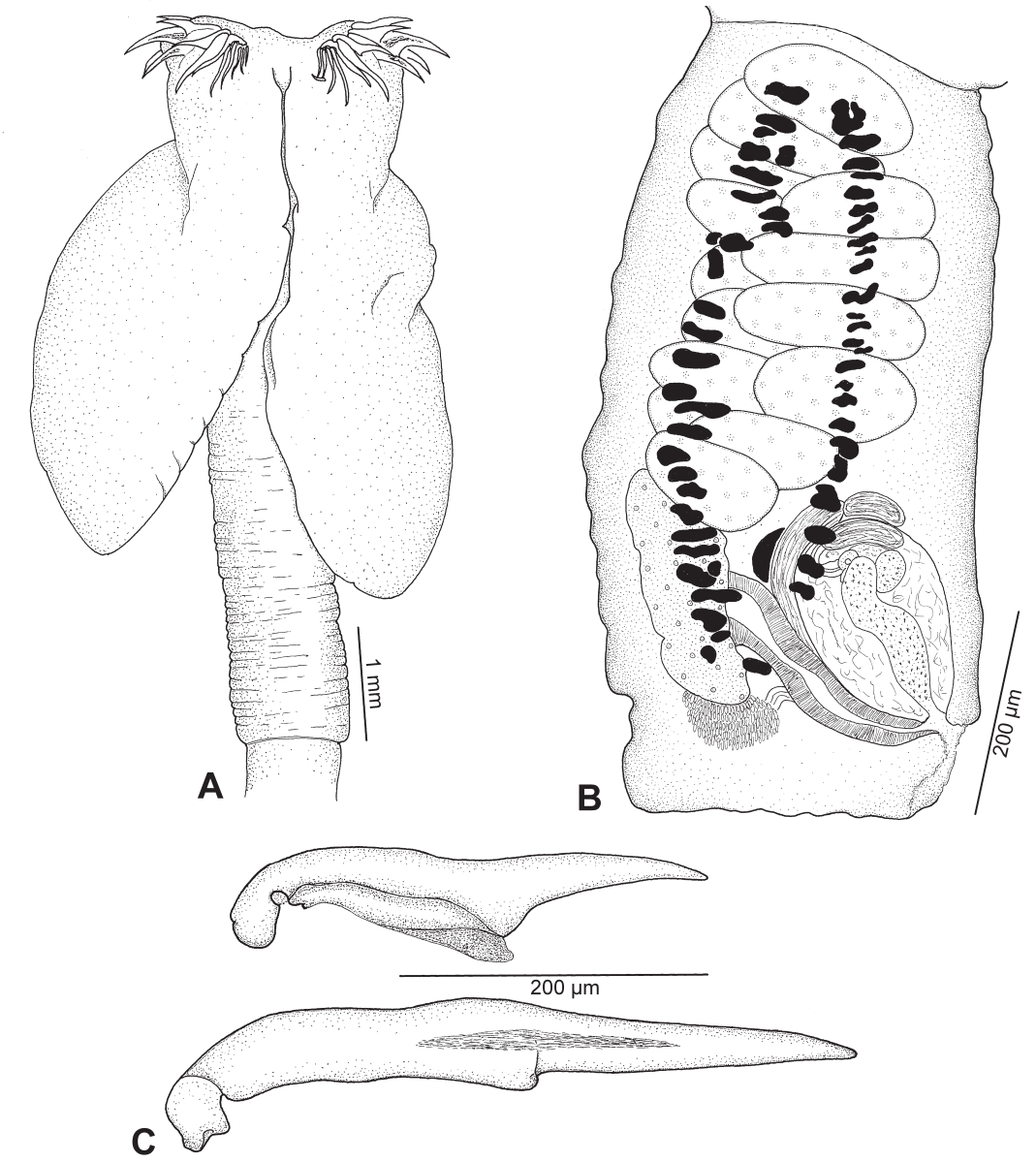


Fig. 123. Line drawings of *Echinobothrium rhynchobati* n. comb. A. Scolex, lateral view. B. Mature proglottid, lateral view. C. Detail of apical hooks.

apical hooks hollow, all hook lengths increasing toward center of group. Type "A" and "B" hooks articulating with one another at their bases with an intricate system of knobs and sockets (see Fig. 16). Bothria 1.52-2.48 mm long, 990-1,480 wide, proximal bothrial surfaces with short and long filitriches and pectinate spinitriches bearing 5-6 relatively equal length digits anteriorly, grading to 2-3 relatively equal length digits posteriorly. Distal bothrial surfaces with short filitriches and pectinate spinitriches each bearing three digits; central digit conspicuously longer than others. Lateral surfaces of scolex proper with short filitriches and pectinate microtriches each bearing 2-3 relatively equal length dig-

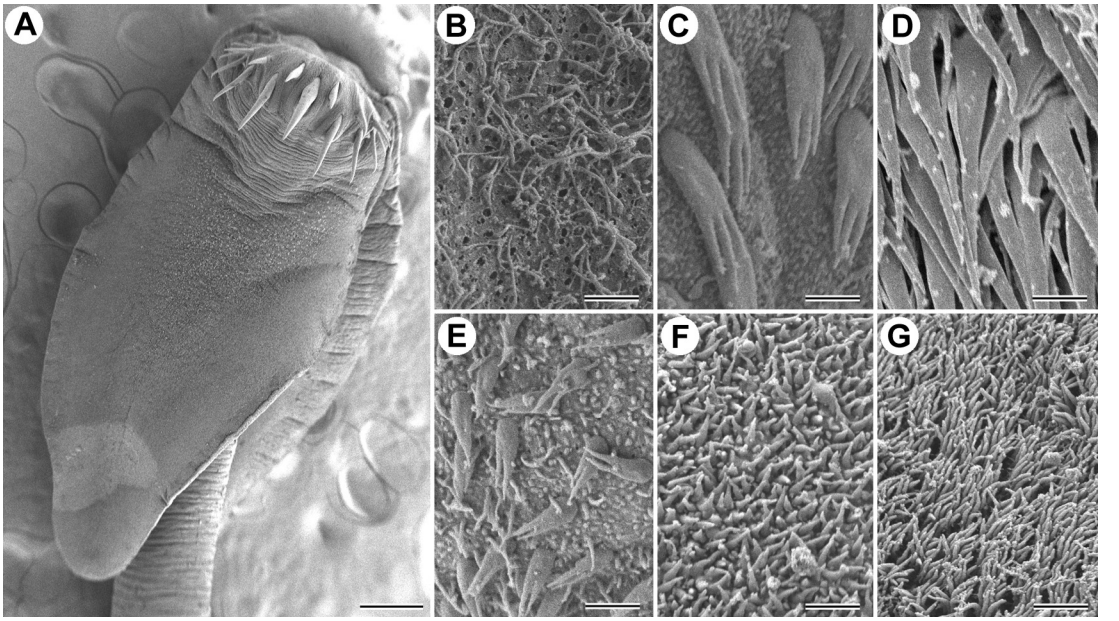


Fig. 124. Scanning electron micrographs of *Echinobothrium rhynchobati* n. comb. A. Scolex. B. Apex. B. Proximal bothrial surface. D. Distal bothrial surface. E. Lateral surface of scolex proper. F. Cephalic peduncle. G. Strobila. Scale bars: A, 250 µm; B-G, 1 µm.

its. Cephalic peduncle 460-1,660 long, 255-505 wide, unarmed, covered with long filitriches.

Testes 29-37 in number, anterior to cirrus sac, in 4-5 irregular columns, one layer deep. Vas deferens extensive, anterior to cirrus sac. Cirrus sac piriform, 206-243 long, 100-220 wide. Cirrus armed with small microtriches. Ovary 200-340 long, U-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, looping anterior to genital pore, relatively uniform in diameter along length, undulating slightly. Genital pore midventral, 10-20% of proglottid length from posterior end of proglottid, overlapping ovary. Uterus saccate, thick-walled in early stages of development, entirely filling gravid proglottid. Vitellaria follicular; follicles 13-20 long, 33-40 wide, in two lateral bands each comprising a dorsal and ventral column of follicles, extending entire length of proglottid, uninterrupted by ovary. Eggs unembryonated, spherical, 32-36 in diameter, lacking appendages, not packaged. Excretory ducts lateral.

Remarks

The unique hook formula of *Echinobothrium rhynchobati* distinguishes it from all other species in the genus.

This species was described by Khalil and Abdul-Salam (1989) as the type species of their new genus *Macrobothridium* in their new family Macrobothridiidae. Khalil (1994) maintained the validity of the genus and family. The phylogenetic analysis of the Diphyllidea published by Ivanov and Hoberg (1999) resulted in the placement of this species among species of *Echinobothrium*, rendering *Echinobothrium* paraphyletic if this species was excluded. Citing the preliminary nature of their results, those authors opted not to synonymize the two genera, pending a more rigorous analysis. Neifar *et al.* (2001) considered the genus valid, as there was no well corroborated phylogeny to suggest otherwise. In the trees resulting from the cladistic analysis presented in this volume, all three species of *Macrobothridium* appeared among species of *Echinobothrium*. The two genera are therefore synonymized.

DNA sequence data from Australian

specimens from *Rhinobatos typus* of this species was used by Olson and Caira (1999) in a phylogenetic analysis of the orders of eucestodes, which supported the monophyly of the Diphyllidea. Olson *et al.* (2001) also used the DNA sequence from this species in a similar analysis, but showed a closer relationship between *E. rhynchobati* (as *Macrobothridium rhynchobati*) and *E. chisholmae* than between that species and *E. harfordi*.

Khalil and Abdul-Salam (1989) reported the type host for *E. rhynchobati* as *Rhynchobatus granulatus* Cuvier. However, that name does not appear in the list of species names (as valid or otherwise) consulted for this work (Eschmeyer 1998). Neifar *et al.* (2001) suggested that the actual host for this species may be *Rhinobatos granulatus* Cuvier. Saoud and Hassan (1983) reported *Echinobothrium* sp. from *R. granulatus* in the Mediterranean Sea and Red Sea, and Al Kawari *et al.* (1996) reported *Echinobothrium* from this host species in the Arabian Gulf. However, these specimens were not identified to species, and their true identity remains unknown. Thus, the identity of the type host for *E. rhynchobati* remains to be verified.

Several of the observations made by Khalil and Abdul-Salam (1989) on this species require clarification. The hook formula for this species, when interpreted from the original description, is {6 6/5 6}. However, examination of several paratypes and voucher specimens indicates that what had previously been interpreted as six lateral hooklets are actually a series of type B hooks, which do not interdigitate with type A hooks, resulting in the peculiar hook formula given in the description above. The actual lateral hooklets observed in the type and voucher specimens are very small (see Fig. 122B), and appear to have been overlooked by Khalil and Abdul-Salam (1989) as they were not mentioned or illustrated in that paper.

Another character described for this species by Khalil and Abdul-Salam (1989) appears to have been in error. Examination of the type specimens suggests that the posterior sucker-like organ described by these authors on the posterior margin of the terminal proglottid is nothing more than a minor constriction in the terminus of the proglottid. There appears to be no musculature associated with this "organ" other than the longitudinal and circular musculature of the proglottid.

The bothria of this species have an extensive reticulating network of excretory vessels, which raises some questions about the function of the large structures. This type of excretory network was also described by Rees (1959) in *Ditrachybothridium macrocephalum*, another species with large bothria. As the functions of the excretory system are to maintain osmotic balance and remove metabolic waste (Smyth, 1969), one might assume that there is considerable metabolic activity occurring in the bothria of these two species. This remains to be investigated, however.

Echinobothrium syrtensis (Neifar, Tyler, and Euzet, 2001) n. comb.

(Figs. 125-128)

Synonym: *Macrobothridium syrtensis* Neifar, Tyler and Euzet, 2001 **n. syn.**

Type host: *Rhinobatos cemiculus* Geoffroy Saint-Hilaire, Blackchin guitarfish (Rhinobatidae, Rhinobatiformes).

Status: Valid.

Site of infection: Spiral intestine.

Type locality: Gulf of Gabès, Djerba, Tunisia (33˚20'N, 11˚15'E).

Additional localities: Zarzis, Tunisia (33˚15'N, 11˚10'E), Sfax, Tunisia (34˚45'N, 10˚50'E).

Type material: MNHN No. 853 HF 148 CIX (holotype); MNHN No. 853 HF 149 CIX, BMNH Nos. 2000.7.28.5-6, and USNPC Nos. 90594-90595 (paratypes).

Specimens examined: Holotype; all 24 paratypes.

Etymology: This species derives its name from "little Syrte," an alternate name for the Gulf of Gabès.

Description (Modified from Neifar *et al.* [2001].)

Worms 1.000-1.500 mm (1.278 ± 0.084; n=25) in length, greatest strobila width 200-350 (274 ± 20; n=25), generally at terminal proglottid. Strobila anapolytic, acraspedote,

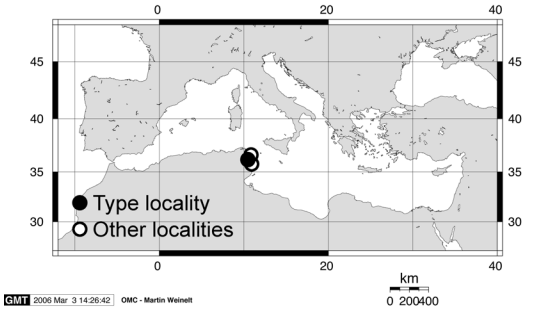


Fig. 125. Distribution of *Echinobothrium syrtensis* n. comb.

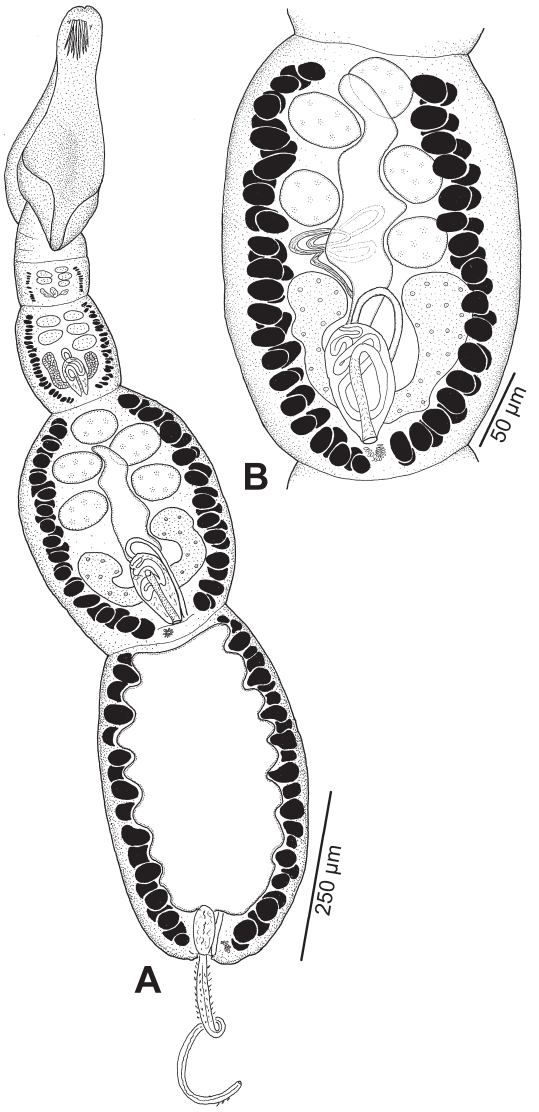


Fig. 126. Line drawings of *Echinobothrium syrtensis* n. comb. A. Whole worm. B. Mature proglottid. Redrawn from Neifar *et al.* (2001).

5-7 (6; n=25) proglottids per worm, covered with short filitriches. Immature proglottids 2-4 (3; n=25) in number. Mature proglottids 2-3 (2; n=25) in number, 120-230 (155 ± 11; n=25) long, 100-220 (134 ± 12; n=25) wide. Gravid proglottids 1-2 (1; n=25) in number, 180-450 (325 ± 25; n=25) long, 160-320 (234 ± 19; n=25) wide Scolex bipartite, consisting of scolex proper and cephalic peduncle. Apex of scolex proper covered with short and long filitriches. Scolex proper 240-380 (318 ± 20; n=25) long, 140-300 (219 ± 24; n=25) wide, consisting of armed apical rostellum and one dorsal and one ventral bothrium. Eleven apical hooks in each dorso-ventral group. Hook formula {(4-5) 6/5 (4-5)}, apical hooks solid, hook lengths gradually increasing toward center of group. Lateral hooklets arranged in two groups. Proximal bothrial surfaces covered with bifid and trifid pectinate spinitriches anteriorly, grading to 4-5 relatively equal length digits posteriorly. Posterior proximal surfaces also with small spinitriches and short filitriches. Distal bothrial surfaces with trifid pectinate spinitriches and long filitriches. Lateral surfaces of scolex proper with pectinate spinitriches each bearing 4-5 relatively equal length digits. Cephalic peduncle 40-70 (51 ± 8; n=25) long, 40-70 (63 ± 7; n=25) wide, unarmed, covered with short filitriches.

Testes 5-6 (5; n=25) in number, anterior to cirrus sac, 38-44 (40 ± 3; n=25) long, 23-25 (24 ± 2; n=25) wide, in two irregular columns, one layer deep. Vas deferens extensive, entirely anterior to cirrus sac. Cirrus sac piriform, 90-120 (107 ± 8; n=22) long, 45-60 (54 ± 5; n=22) wide. Cirrus 320-330 (325 ± 6; n=7) long, 10-15 (12 ± 3; n=7) in basal diameter, basal part armed with spinitriches, distal part unarmed or with 3-4 small spinitriches near tip. Ovary 120-180 (143 ± 8; n=22) long, 30-60 (42 ± 5; n=22) wide, U-shaped in dorso-ventral view, bilobed in cross section. Vagina thin-walled, looping anterior to genital pore, relatively uniform in diameter along length, undulating slightly. Genital pore midventral, 5-25% (16

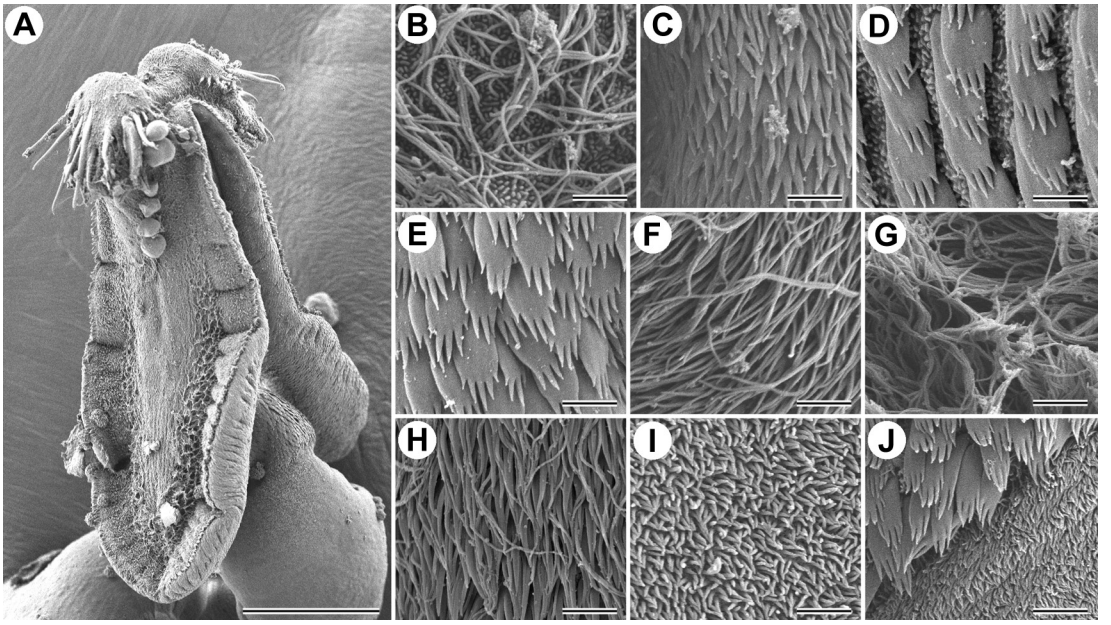


Fig. 127. Scanning electron micrographs of *Echinobothrium syrtensis* n. comb. A. Scolex. B. Apex of scolex. C. Proximal bothrial surface (anterior). D. Proximal bothrial surface (midway along length). E. Proximal bothrial surface (posterior). F. Distal surface, anterior to rostellum. G. Distal (lateral) bothrial surface. H. Distal (medial) bothrial surface. I. Strobila. J. Lateral surface of scolex, showing scolex proper and cephalic peduncle. Scale bars: A, 50 µm; B-I, 1 µm; J, 2 µm.

± 2; n=22) of proglottid length from posterior end of mature proglottid, posterior to ovary. Uterus saccate, thick-walled in early stages of development. Vitellaria follicular, in two lateral columns extending entire length of proglottid, uninterrupted by ovary, confluent posterior to ovary. Eggs oval, 35-40 (37 ± 2; n=18) long, 21-29 (25 ± 3; n=18) in diameter, with single polar mucron, not packaged. Excretory ducts lateral.

Remarks

The hook formula of this species is sufficient to distinguish it from all others in the genus except *E. affine*, *E. bonasum*, *E. chisholmae*, *E. fautleyae*, and *E. harfordi*. *Echinobothrium syrtensis* differs from all five in its lack of cephalic peduncle armature, a feature all five former species possess.

This was one of two new species of *Macrobothridium* described by Neifar *et al.* (2001). At the time, the authors considered the validity of the genus and, citing a lack of evidence to the contrary (but see Ivanov and Hoberg 1999), considered it valid. This species was transferred into *Echinobothrium* based on the results of the phylogenetic analysis presented in this work.

PHYLOGENETIC RELATIONSHIPS

Overview

Although a fair amount of attention has been given to the phylogenetic position of the Diphyllidea with respect to the other orders of cestodes, the relationships among the Diphyllidea have been largely ignored. Following the 2nd International Workshop on Tapeworm Systematics in Lincoln, Nebraska in 1996, Ivanov and Hoberg (1999) published the first phylogenetic hypothesis of the interrelationships among the Diphyllidea. However, as noted by Ivanov and Hoberg (1999), their analysis was supported by only a few morphological characters, and thus, their results required confirmation. Of particular interest with respect to these relationships is the monophyly of each of the three genera of diphyllideans, especially *Echinobothrium*. Ivanov and Hoberg (1999) noted that the tree resulting from their analysis showed *Macrobothridium* among the otherwise monophyletic *Echinobothrium*, and that as a consequence, the two genera should perhaps be synonymized. The authors refrained from taking this action however, because of the preliminary nature of their results. Neifar *et al.* (2001) also wrestled with the generic boundaries of *Macrobothridium* and *Echinobothrium*, questioning whether *E. reesae* and *E. deeghai*, both lacking cephalic peduncle armature, should be transferred to *Macrobothridium*. Those authors also chose to make no formal changes until a robust phylogeny was available. The examination of type or voucher specimens of 31 of the 40 described diphyllidean species conducted in this study has made a more comprehensive analysis of the interrelationships among diphyllideans possible. The results of these analyses are presented here.

Study Taxa

Phylogenetic analyses were performed to determine the relationships among 34 diphyllidean species. A total of 31 of the 40 described diphyllidean species was examined from type or voucher material. Three other species, *E. benedeni*, *E. musteli*, and *E. reesae*, were included in the analyses, but, as no specimens were available for study, these species were coded from the original descriptions and figures. In addition to examination of whole mounts, whenever possible, specimens were prepared for examination with SEM, and serial sections of proglottids were made and examined. Seven outgroup species (*Cathetocephalus* sp., *Diphyllobothrium cordatum* [Leuckart, 1863] Faust, 1929; *Grillotia similis*, [Linton, 1908] Caira and Gavarrinoo, 1990; *Mixodigma leptaleum* Dailey and Vogelbein, 1982; *Rhinebothrium urobatidium* [Young, 1955] Appy and Dailey, 1977; *Tentacularia* sp. and *Zyxibothrium kamienae* Hayden and Campbell, 1981) were chosen based on their hypothesized close relationships to the Diphyllidea (see Baer 1950; Euzet 1959; Brooks and McLennan 1993; Hoberg *et al.* 1997; Olson and Caira 1999).

Character Analysis and Coding

Ivanov and Hoberg (1999) based their phylogenetic analysis of the Diphyllidea on 21 morphological characters, 12 of which were used in the present study, either as originally interpreted by Ivanov and Hoberg (1999) or slightly modified. Caira *et al.* (1999) formulated a list of 120 morphological characters, including many fine structure (SEM) characters for their analysis of the tetraphyllidean, lecanicephalidean, and diphyllidean genera. Twenty-four of those characters were incorporated into this study. An additional 19 morphological characters, including six SEM characters, were added as the result of examination of the aforementioned material (see Table 2). The initial taxon/character data matrix consisted of 41 taxa and 55 characters and is shown in Table 3.

All characters were treated as unordered and were polarized using the outgroup method. Species exhibiting multiple states for a character were coded with both states, and interpreted as a polymorphism. Missing data

Table 2. Character list.

NO.	CHARACTER (Note: * Characters new to this analysis; not used in Ivanov and Hoberg [1999] or Caira *et al.* [1999].)
1	Type of holdfast: 0=neither bothria nor bothridia; 1=bothria; 2=bothridia. Modified from Caira *et al.* (1999) character 19.
2	Cephalic peduncle: 0=absent; 1=present. Caira *et al.* (1999) character 41.
3	Proglottid apolysis: 0=anapolytic; 1=apolytic; 2=euapolytic. Caira *et al.* (1999) character 84.
4	Proglottid margins: 0=acraspedote, 1=craspedote. Caira *et al.* (1999) character 83.
5	Apical organ on scolex: 0=absent; 1=present. Caira *et al.* (1999) character 11. An apical organ was defined as a modification of the apex of the scolex proper, with a discrete boundary consisting of a membrane. The excretory vessels of the scolex do not cross this membrane.
6	Apical hooks on scolex: 0=absent; 1=present; 9=inapplicable. Ivanov and Hoberg (1999) character 1.
7*	Number of apical hooks in each dorso-ventral group: 0=1-9; 1=10-19; 2=20-29; 3=30-39; 4=>39; 9=inapplicable. The inapplicable state for this character applies to all species lacking apical hooks. The divisions employed here were determined arbitrarily.
8*	Symmetry of apical hooks: 0="A" symmetry; 1="B" symmetry; 9=inapplicable. The dorso-ventral groups of apical hooks of *Echinobothrium* consist of two rows of hooks. The anterior row consisting of hooks each with a strongly recurved or geniculate base (type A in Fig. 6), and the posterior row consisting of hooks each with straight or slightly curved bases (type B in Fig. 6) (Neifar *et al.* 2001). The hooks in these two rows are interdigitated. Armed diphyllideans nearly always have an odd number of hooks in each dorso-ventral group. In such cases, the center hook is either of type A or type B. Species in which the center hook is a type A hook, are considered to exhibit type A symmetry (Fig. 2); those in which the center hook is of type B are considered to exhibit type B symmetry (Fig. 3). In species in which apical hook number varies intraspecifically, hook symmetry generally does not. For example, *Echinobothrium hoffmanorum* possesses either 19 or 21 hooks in each dorso-ventral group, but the symmetry is always about a type B hook. Thus, *E. hoffmanorum* is said to have type B hook symmetry. The inapplicable state applies to all taxa that lack apical hooks.
9*	Morphology of apical hooks: 0=solid; 1=hollow; 9=inapplicable. The inapplicable state applies to all taxa that lack apical hooks.
10	Articulation of apical hook bases: 0=bases articulated; 1= bases not articulated; 9=inapplicable. Ivanov and Hoberg (1999) character 4. The bases of the two types of apical hooks (see character 5 above) in some species articulate with one another by an intricate interlocking system as in *Echinobothrium rhynchobati* (see Fig. 122B). This is not the case in all hooked diphyllideans however. The inapplicable state applies to all taxa that lack apical hooks.
11*	Relative lengths of type A hooks: 0= increasing in length toward center of row; 1=decreasing in length towards center of row; 9=inapplicable. Generally, the hooks in each row of each dorso-ventral group of apical hooks are longest in the center of the group. That is, the length of the hooks gradually increases when moving from the more lateral hooklets towards the center of the group. In some species however, the length of the hooks gradually increases towards the center of the group, but the central one or two hooks are markedly shorter than those on either side. The inapplicable state applies to all taxa that lack apical hooks.
12*	Relative lengths of type B hooks: 0=increasing in length towards center of row, 1=decreasing in length towards center of row, 9=N/A. See explanation for character 11 above. The inapplicable state applies to all taxa that lack apical hooks.
13	Lateral hooklets on scolex proper: 0=absent; 1=present in two groups; 2=present in single continuous row. Ivanov and Hoberg (1999) character 2. The lateral hooklets in many species of *Echinobothrium* differ morphologically from the apical hooks. In some cases (*e.g.*, E. raji), the lateral-most hooks of the dorsal and ventral groups of apical hooks are very small and may be mistaken for true lateral hooklets. However, the lateral hooklets can be distinguished from the apical hooks by their more posterior point of attachment to the scolex and by the shape of their bases. In addition, these hooklets are straighter than the apical hooks, and lack a center muscle attachment point. Refer to Figs. 7, 8.
14*	Number of lateral hooklets in each group: 0=1-5; 1=6-10; 2=>10; 9= inapplicable. The number of lateral hooklets in each group was determined for taxa with a single continuous row by dividing the number of hooks in that single row by two. The inapplicable state applies to those taxa lacking lateral hooklets. The divisions used here were determined arbitrarily.
15*	Arrangement of lateral hooklets: 0=uniform; 1=staggered; 9= inapplicable. Lateral hooklets either lie in a uniform row (Fig. 7) or are staggered (Fig. 8). The inapplicable state applies to those taxa lacking lateral hooklets.
16*	Relative sizes of lateral hooklets: 0= relatively equal in size; 1=first and last hooklets much longer than others; 9=inapplicable. In some species (*e.g.*, *Echinobothrium fautleyae*) the first and last hooklets in each row are distinctly larger than the others; in these cases the first and last hooklets are at least twice as long as the other hooklets in the row (Fig. 8).
17	Several (8-12) rows of secondary armature forming a corona just posterior to the apex of the scolex: 0=absent; 1=present. Ivanov and Hoberg (1999) character 3. In several species of *Echinobothrium*, a corona of "spines" encircles the apex of the scolex (Fig. 5).
18	Cleft at posterior margin of bothria: 0=absent; 1=present; 9=inapplicable. Ivanov and Hoberg (1999) character 7. See Fig. 9.
19	Eight columns of spines on cephalic peduncle: 0=absent, 1=present; 9=inapplicable. Ivanov and Hoberg (1999) character 5.
20*	Shape of peduncle spine bases: 0=triradiate; 1=leaflike; 9=inapplicable. In most species of *Echinobothrium*, the bases of peduncle spines are triradiate (Fig. 11). However, in one species, *E. longicolle*, the bases of the peduncle spines are distinctly leaflike (Fig. 12). The inapplicable state applies to taxa lacking cephalic peduncle armature.
21	Velum at posterior terminus of cephalic peduncle: 0=absent; 1=present; 9=inapplicable. Ivanov and Hoberg (1999) character 8. See Fig. 10.

22 Number of columns of testes: 0=1 column; 1=2 columns; 2=greater than 2 columns. Modified from Caira *et al.* (1999) character 90.

23 Vas deferens size: 0=minimal; 1=extensive. Modified from Caira *et al.* (1999) character 93.
Taxa in which the vas deferens was inconspicuous were coded as minimal. Taxa in which the vas deferens was conspicuously expanded and full of sperm were coded as extensive

24 Position of vas deferens: 0=entirely anterior to cirrus sac; 1=extending lateral or posterior to cirrus sac. Modified from Caira *et al.* (1999) character 92.

25 External seminal vesicle: 0=absent; 1=present. Ivanov and Hoberg (1999) character 19.

26 Internal seminal vesicle: 0=absent; 1=present. Ivanov and Hoberg (1999) character 20.

27 Shape of ovary in dorso-ventral view: 0=H-shaped; 1=U-shaped. Modified from Ivanov and Hoberg (1999) character 11.

28* Morphology of vaginal wall: 0=thin-walled; 1=thick-walled.
If the walls of the vagina appear robust, muscular, or cellular, the vagina is considered thick-walled. If the vaginal wall appears simply membranous, it is considered thin-walled.

29 Position of vagina relative to genital pore: 0=entirely posterior to genital pore; 1=looping anterior to genital pore. Modified from Caira *et al.* (1999) character 104.

30* Shape of vagina: 0=uniform width along its length; 1=expanded lumen at distal terminus.
In several species of *Echinobothrium* the vagina is expanded at its distal end (see for example *E. mexicanum*, Fig. 99E). This usually occurs in taxa that also possess a stout, heavily armed cirrus.

31 Vaginal coils: 0=absent; 1=present.
If the vagina descends a meandering course from the genital pore to the ootype, it is considered coiled. The course of the vagina is considered straight only if it descends directly to the ootype without lateral deviation. Caira *et al.* (1999) character 106.

32 Seminal receptacle: 0=absent; 1=present. Ivanov and Hoberg (1999) character 21.

33 Position of genital pore relative to ovary: 0=anterior to ovary; 1=overlapping with ovary; 2=posterior to ovary. Modified from Caira *et al.* (1999) character 99.
Position relative to ovary was chosen rather than relative to ovarian isthmus (see Caira *et al.*, 1999 character 99) to accommodate the fact that the ovary may be either H- or U-shaped.

34 Arrangement of vitellaria: 0=exclusively lateral columns; 1=lateral columns converging on midline; 2=circumcortical. Modified from Caira *et al.* (1999) character 114.
Taxa exhibiting vitelline follicles arranged in straight, well defined lateral columns were coded as “0.” Taxa in which the lateral columns of vitellaria were very broad, and nearly touching one another near the midline of the proglottid were coded as “1.” Taxa in which the vitellaria completely encircled the proglottid were coded as “2.”

35 Position of vitellaria: 0=exclusively pre-ovarian; 1=full length of proglottid, uninterrupted by ovary. Modified from Caira *et al.* (1999) character 117.

36* Egg shape: 0=spherical; 1=oval; 2=piriform.

37 Appendages on eggs: 0=absent; 1=single filament; 2=2 filaments, 3=mucron at one pole. Modified from Ivanov and Hoberg (1999) character 16.

38* Egg packaging: 0=not packaged; 1=cocoons (sacs); 2=long chains.

39 Filitriches on apex of scolex proper: 0=absent; 1=short; 2=long. Modified from Caira *et al.* (1999) character 63.
Filitriches were considered to be short if their length/width ratio was less than 2.

40 Spinitriches on apex of scolex proper: 0=absent; 1=spatulate; 2=pectinate. Modified from Caira *et al.* (1999) character 62.

41* Cilia on apex of scolex proper: 0=absent; 1=present.

42 Spines on proximal bothrial surfaces: 0=absent; 1=present; 9=inapplicable.
Some taxa (e.g., *Ditrachybothridium macrocephalum*) have large (approximately 10 µm long) conical spines on the proximal bothrial surfaces. These spines are clearly visible with light microscopy or SEM. See Fig. 4. Caira *et al.* (1999) character 72.

43 Filitriches on proximal bothrial surfaces: 0=absent; 1=short; 2=long; 9=inapplicable. Modified from Caira *et al.* (1999) character 71.
See character 39 above.

44 Spinitriches on proximal bothrial surfaces: 0=absent; 1=spatulate; 2=pectinate; 9=inapplicable. Modified from Caira *et al.* (1999) character 70.

45* Cilia on proximal bothrial surface: 0=absent; 1=present; 9=inapplicable.

46 Filitriches on submarginal distal bothrial surfaces: 0=absent; 1=short; 2=long; 9=inapplicable. Modified from Caira *et al.* (1999) character 69.
See character 39 above.

47 Spinitriches on submarginal distal bothrial surfaces: 0=absent; 1=spatulate; 2=pectinate; 9=inapplicable. Modified from Caira *et al.* (1999) character 68.

48* Cilia on submarginal distal bothrial surface: 0=absent; 1=present; 9=inapplicable.

49 Filitriches on medial distal bothrial surface: 0=absent; 1=short; 2=long. Modified from Caira *et al.* (1999) character 69.
See character 39 above.

50 Spinitriches on medial distal bothrial surface: 0=absent; 1=spatulate; 2=pectinate; 9=inapplicable. Modified from Caira *et al.* (1999) character 68

51* Cilia on medial distal bothrial surface: 0=absent; 1=present; 9=inapplicable.

52* Filitriches on lateral surface of scolex proper: 0=absent; 1=short; 2=long; 9=inapplicable.
See character 39 above.

53* Spinitriches on lateral surface of scolex proper: 0=absent; 1=spatulate; 2=pectinate; 9=inapplicable.

54 Microtriches on cephalic peduncle: 0=absent; 1=short filitriches; 2=long filitriches; 3=spinitriches; 9=inapplicable. Modified from Caira *et al.* (1999) character 74.
See character 39 above. The inapplicable state applies to all taxa lacking a cephalic peduncle.

55 Filitriches on strobila: 0=absent; 1=short; 2=long. Modified from Caira *et al.* (1999) character 80.
See character 39 above.

Table 3. Complete species/character matrix.

	CHARACTER NUMBER
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
Ditrachybothridium macrocephalum	1 1 1 0 1 0 9 9 9 9 9 9 0 9 9 9 0 1 0 9 1 1 1 1 0 0 0 1 0 0 1 0 0 0 0 ? ? ? ? ? ? 1 2 0 0 2 ? 0 0 2 2 0 2 0 2 2
Ditrachybothridium piliformis	1 1 1 0 1 0 9 9 9 9 9 0 9 9 9 0 0 0 9 1 1 1 1 0 0 0 1 0 1 1 0 1 1 0 1 3 0 ? ? ? 0 2 1 0 2 2 0 2 0 2 0 2 2 2,3 2
Echinobothrium acanthinophyllum	1 1 1 0 1 1 2 1 0 1 0 0 1 0 0 0 0 0 1 0 0 1 1 1 0 0 0 0 0 0 1 1 1 0 1 0 0 1 ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium acanthocolle	1 1 2 0 1 1 3 1 0 1 0 0 1 0 0 0 0 0 1 0 0 2 0 0 0 0 0 0 1 0 1 0 1 0 1 ? ? ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? 1 ? ?
Echinobothrium affine	1 1 2 0 1 1 1 1 0 1 0 1 1 0 0 0 0 0 1 0 0 1 1 1 0 0 0 0 1 0 1 1 0 0 1 0 1 2 ? ? ? 0 ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium benedeni	1 1 ? 0 1 1 2 1 0 1 1 0 1 0 0 0 0 0 ? ? ? 1 ?
Echinobothrium bonasum	1 1 2 0 1 1 1 1 0 1 1 0 2 2 0 0 0 0 1 0 0 2 0 0 0 0 0 1 1 1 1 0 0 0 0 ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium brachysoma	1 1 1 0 1 1 0 1 0 1 0 0 1 0 0 0 0 0 1 0 1 1 0 0 0 1 0 1 0 1 0 1 0 1 1 0 1 ? 0 9 0 1 2 0 1 0,2 1 1 0 0 1,2 0 1 ?
Echinobothrium californiense	1 1 1 0 1 1 2 1 0 1 0 0 2 1,2 0 0 0 1 1 0 0 0 0 0 0 1 0 1 0 0 1 0 1 0 1 ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium clavatum	1 1 1 0 1 1 1 1 0 1 0 0 1 0 0 0 0 0 1 1 1 0 0 0 0 0 0 1 1 1 0 1 0 0 1 ? ? ? 0 0 2 0 2 0 ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium coenoformum	1 1 2 0 1 1 1 1 0 1 0 1 0 0 0 0 0 1 0 1 2 1 0 0 0 0 1 1 1 1 1 1 0 1 1 ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium coronatum	1 1 ? 0 1 1 2 ? 0 1 ? ? 1 2 1 0 0 ? 1 ? 0 ?
Echinobothrium euzeti	1 1 1 0 1 1 2 0 0 1 0 0 2 1 0 0 0 1 1 0 0 2 1 0 0 1 0 1 0 0 1 0 0 0 1 ? ? ? 0 ? 2 ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium fautleyae	1 1 2 0 1 1 1 1 0 1 1 0 2 2 1 1 0 0 1 0 0 0 0 0 1 0 0 1 0 0 0 0 1 0 1 0 2 ? ? ? 2 0 0 0 9 2 0 2 2 0 0 2 0 0 2 1 2
Echinobothrium harfordi	1 1 1 0 1 1 4 1 0 1 1 0 1 0 0 0 0 1 1 0 0 0 0 0 0 0 1 0 1 0 1 2 3 0 ? ? ? 0 0 1 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium helmymohamedi	1 1 1 0 1 1 3 ? 0 1 ? ? ? ? ? ? 0 1 1 0 1 1 1 0 0 0 0 0 0 1 0 1 0 1 1 0 0 ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium heroniense	1 1 2 0 1 1 4 1 0 1 0 0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 1 0 1 0 1 2 1 1 0 0 ? ? 0 0 2 0 0 1 0 0 0 2 0 1
Echinobothrium hoffmanorum	1 1 1 0 1 1 1,2 0 0 1 0 0 2 1,2 0 0 0 0 1 0 0 0 1 1 0 0 0 1 0 0 1 0 1 1 1 1 0 1,2 1 0 0 1 2 0 1 2 1 0 0 1 2 1 2
Echinobothrium longicolle	1 1 ? 0 1 1 2 0 0 1 0 0 0 9 9 9 0 0 1 1 0 2 0 ? ? ? 1 1 ? ? 1 ? 0 0 1 ? ? ? 1 ? ? 1 ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium mathiasi	1 1 2 0 1 1 2 0 0 1 0 0 1 0 0 0 0 1 0,1 0 2 0 0 0 0 0 0 0 1 1 0 0 0 1 1 1 0 1,2 0 0 0 1 2 0 0 1 2 0 0 1,2 0 0 ? ? 1 2
Echinobothrium megacanthum	1 1 1 0 1 1 2 1 0 1 0 0 2 1 0 0 0 1 1 0 1 1 0 1 0 1 0 0 1 1 1 0 ? ? ? 0 2 2 0 2 0 1,2 0 0 ? ? ?
Echinobothrium mexicanum	1 1 1 0 1 1 2 1 0 1 0 0 2 0,1 0 0 0 1 1 0 1 1 0 1 0 1 0 1 1 0 0 1 1 1 1 0 1,2 0 0 0 1 2 0 2 2 1 1 0 0 1 2 1 2
Echinobothrium musteli	1 1 2 0 1 1 3 ? 1 1 ? ? 2 1 1 0 1 0 1 0 0 1 1 0 ? ? 1 ? ? ? 1 ? 0 1 ? ? ? 1 ? ? ? 0 ? 1 ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium notoguidoi	1 1 2 0 1 1 3 1 0 1 0 0 1 2 1 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 0 ? 1 ? ? 1 ? ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium pigmentatum	1 1 1 0 1 1 2 1 0 1 0 0 2 2 0 0 0 0 1 0 0 0 1 1 0 0 0 1 0 1 1 0 0 0 1 1 2 0 ? ? ? 0 ? 1 ? ? 1 ? ? ? ? ? ? ?
Echinobothrium raji	1 1 1 0 1 1 4 ? 0 1 0 0 0 9 9 9 0 0 1 0 1 1 1 0 1 0 0 1 0 1 1 1 0 1 1 0 0 ? ? ? 0 2 2 0 0 1 0 0 2 0 1 2 ? ?
Echinobothrium raschii	1 1 1 0 1 1 2 1 0 1 0 0 2 1 0 0 0 1 1 0 0 2 1 1 0 0 0 1 0 1 1 1 0 0 1 1 ? 0 ? ? ? 0 1 0 ? 1 2 ? 0 0 0 0 ? ? ?
Echinobothrium rayallemangi	1 1 1 0 1 1 2 1 0 1 0 0 2 1,2 0 0 0 0 1 0 0 0 1 1 0 0 0 1 0 0 1 0 1 0 0 1 0 0 1,2 0 0 0 1 2 1 1 2 0 1 0 0 2 1 2
Echinobothrium reesae	1 1 1 0 1 1 1 1 0 1 ? ? 1 0 0 0 0 0 9 0 1 1 1 ? ? 0 0 1 0 0 0 1 0 1 0 0 ? ? ? 0 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium typus	1 1 2 0 1 1 0 1 0 1 0 0 1 0 0 0 0 1 0 1 1 1 ? ? ? 1 0 1 0 0 0 1 0 1 ? ? ? 0 ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Echinobothrium elegans	1 1 2 0 1 1 1 1 0 1 0 1 1 0 0 0 0 1 0 1 0 1 0 0 0 0 0 0 1 0 0 0 1 ? ? ? 1 0 0 0 1 2 0 0 1 0 0 1 2 1 2
Echinobothrium euterpes	1 1 1 0 1 1 2,3 0 0 1 1 0 0 1 0 0 0 0 0 9 0 1 1 0 0 0 0 0 0 0 1 0 0 0 1 0 3 0 2 0 0 0 1 2 0 2 ? ? 3 0
Echinobothrium rhynchobati	1 1 1 0 1 1 2 1 1 0 0 0 1 0 0 0 0 0 9 0 2 1 0 0 0 1 0 1 0 1 0 1 0 1 0 0 0 1,2 0 0 0 1 2 0 1 2 0 1 2 2 2
Echinobothrirum syrtensis	1 1 1 0 1 1 1 1 0 1 0 1 0 0 0 0 0 9 0 1 1 0 0 0 1 0 1 0 1 0 2 0 1 1 3 0 1,2 0 0 0 1 2 0 2 2 0 0 2 1 1
Grillotia similis	1 1 1 0 0 9 9 9 9 9 9 0 9 9 0 1 0 9 0 2 1 1 1 1 0 0 0 0 0 0 2 1 1 0 0 1 0 ? 0 1 2 ? 1 2 ? ? 9 9 9 ? ? 1 ?
Diphyllobothrium cordatum	1 0 0 1 0 9 9 9 9 9 9 0 9 9 0 0 9 9 2 0 1 1 0 0 0 0 0 0 0 0 0 1 0 0 0 ? ? ? 0 ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Rhinebothrium urobatidium	2 1 2 1 0 9 9 9 9 9 9 0 9 9 0 9 0 2 1 0 0 0 0 1 0 0 0 0 0 0 0 0 ? ? ? ? ? 9 9 9 9 9 9 9 9 ? ?
Mixodigma leptaleum	1 1 2 0 0 9 9 9 9 9 9 0 9 9 0 1 0 9 0 2 0 1 0 0 0 0 0 0 0 2 1 ? ? ? 0 ? ? ? ? 1 ? 9 9 9 ? ? ? ?
Tentacularia sp.	1 1 1 0 0 9 9 9 9 9 9 0 9 9 0 0 0 9 1 2 1 1 0 0 0 0 0 0 ? ? 0 2 1 ? ? ? 0 ? ? 0 2 ? ? 2 ? ? 9 9 9 ? ? ? ?
Cathetocephalus sp.	0 1 1 0 0 9 9 9 9 9 9 0 9 9 0 9 0 2 1 0 0 0 0 0 1 0 1 0 0 2 1 ? ? ? 0 0 ? 9 9 9 9 9 9 9 9 0 0
Zyxibothrium kamienae	2 0 1 0 0 9 9 9 9 9 9 0 9 9 0 9 9 9 2 0 1 0 0 1 1 0 0 1 0 0 0 1 0 1 2 ? ? ? 9 9 9 9 9 9 9 9 9 2

were coded with a "?," and inapplicable states were coded either with a "?" or with a "9" (see Caira *et al.* [1999] for a discussion of this technique). Characters not taken from Ivanov and Hoberg (1999) or Caira *et al.* (1999) are noted with an asterisk. Interpretation of the characters used, when different from that of the original authors, is noted. The characters and their states are found in Table 2.

Phylogenetic Analyses

Several phylogenetic analyses were performed using various partitions of the data set. In all cases, unknown character states were coded as "?." There was some question as to how to address the fact that some characters were inapplicable. For example, *Ditrachybothridium* species have no apical hooks. Therefore, characters describing the hooks are inapplicable in these species. Caira *et al.* (1999, 2001), citing the utility of different codings for inapplicable versus unknown character states, coded inapplicable characters as a separate character state, "9." In those papers, the authors stated the possible dangers of this technique, mainly the possibility of inappropriate groupings of taxa based on shared inapplicable characters. In 2001, Caira *et al.* examined the differences

in the results of cladistic analyses run using either "?" or "9" to denote inapplicable characters. Caira *et al.* (2001) showed that there was a slight, although noticeable difference in the topology of the consensus trees resulting from these two analyses. This would suggest that the use of a separate character state for inapplicable characters can adversely affect the outcome of a cladistic analysis, particularly when character support for a given topology is weak. This is important to note, given the paucity of characters (and therefore likelihood of weakly supported trees) used in this particular analysis.

A total of five analyses was run, the details of which follow.

Analysis 1. This analysis used the entire data matrix, with inapplicable character states coded as "9."

Analysis 2. This analysis also used the entire data matrix, but inapplicable characters were coded as "?" rather than "9."

Analysis 3. Because the presence of a large amount of missing ("?" coding) data can alter the outcome of a cladistic analysis (Platnick *et al.*, 1991), this analysis attempted to reduce the number of characters coded as unknown in the matrix by excluding all characters for which the states were unknown (*i.e.*, "?," but not "9") in more than 20% of the included taxa. This excluded characters 36-41, 43, and 45-55, which amounted to virtually all the SEM characters, leaving a total of 38 of the original characters in the analysis. In addition, after the poorly represented characters were excluded from the analysis, all taxa for which more than 20% of the characters were coded as unknown were excluded, resulting in the exclusion of *Echinobothrium benedeni*, *E. coronatum*, and *E. musteli* from the analysis. The outgroups used were the trypanorhynchs *Grillotia similis* and *Mixodigma leptaleum*. Although the seven outgroups originally included in the data matrix were chosen because of their putative close relationships with the Diphyllidea, *Tentacularia* sp., *Diphyllobothrium cordatum*, *Cathetocephalus* sp., *Zyxibothrium kamienae*, and *Rhinebothrium urobatidium* were excluded. These particular taxa were excluded after preliminary analyses demonstrated that the inclusion of these taxa greatly reduced the resolution in the resulting trees.

Analysis 4. This analysis used the same data matrix as employed in Analysis 2 above (*i.e.*, inapplicable characters coded as "?"), coding inapplicable characters as unknown. The 20% exclusion rule was utilized for both characters and taxa, resulting in the exclusion of characters 20, 36-43, and 45-55, leaving 36 characters in the analysis. The taxa *E. benedeni*, *E. coronatum*, *E. longicolle*, and *E. musteli* were deleted from the matrix.

Analysis 5. This analysis employed the same taxa and characters as Analysis 4 above, but constrained *Echinobothrium* to be monophyletic.

In each analysis, PAUP* 4.0b8 (Swofford, 2001) was employed to perform a heuristic search using maximum parsimony as the optimality criterion. Starting trees were generated by random taxon addition, with 100 replicates for each analysis, holding one tree at each step. Branch swapping was by TBR, with the MulTrees and Steepest descent options in effect. Resulting most parsimonious trees (MPT's) were combined into a strict consensus tree for each analysis. Nodal support was calculated using the decay tree commands option in MacClade 4.0 (Maddison and Maddison 2000).

Results

The tree statistics resulting from the five phylogenetic analyses are summarized in Table 4. The figures presented are strict consensus trees.

Table 4. Results of phylogenetic analyses.

	# MPTs	LENGTH	CI	RI	RC	FIGURE
Analysis 1	7	282	0.443	0.646	0.286	N/A
Analysis 2	660	245	0.396	0.521	0.206	N/A
Analysis 3	50	135	0.370	0.562	0.208	128
Analysis 4	10	128	0.367	0.567	0.208	129, 130
Analysis 5	50	130	0.362	0.556	0.201	131

The trees resulting from the first two analyses utilizing the entire data matrix of 41 taxa and 55 characters were near totally

unresolved, at least with respect to *Echinobothrium*. Application of the 20% exclusion rule for both characters and taxa greatly improved the resolution of the other three analyses. Although the consistency index of the trees resulting from Analysis 3 (Fig. 128) was slightly higher, the retention index was slightly lower, and the resolution of the consensus tree was not as great as in the trees resulting from Analysis 4 (Figs. 129, 130). Therefore, the consensus tree resulting from Analysis 4 is the one accepted here as the "best" tree. Interestingly, the coding of inapplicable states as "?" decreased resolution using the full data matrix, but increased it when the 20% exclusion rule was applied.

Several groupings of *Echinobothrium* were at least weakly supported in every tree of every analysis run. These groupings included *E. brachysoma* and *E. typus*, *E. californiense* and *E. euzeti*; *E. megacanthum* and *E. mexicanum* both parasites of bat rays of the genus *Myliobatis*.

Discussion of Relationships

The cladogram resulting from Analysis 4 (Figs. 129, 130) represents a conservative estimate of the phylogenetic relationships of the Diphyllidea. Although the tree is not fully resolved, several important patterns emerge. In the consensus tree resulting from each analysis performed, the two species of *Ditrachybothridium* are monophyletic. This is not surprising given the unique morphology of this genus within the Diphyllidea. Monophyly was not observed in the other genera of diphyllideans however. The monophyly of *Macrobothridium* was not supported by any of the above analyses. Both Analysis 3 (Fig. 128) and Analysis 4 (Figs. 129, 130) grouped all three species of *Macrobothridium* among species of *Echinobothrium*, and indicated that the genus was polyphyletic. Perhaps of greater concern though, was the fact that the placement of *Macrobothridium* species within *Echinobothrium* left that genus paraphyletic. Constraining the cladistic analysis to maintain a monophyletic *Echinobothrium* (Analysis 5) resulted in longer tree length and lower resolution (Fig. 131).

The status of *Macrobothridium* as a genus was first questioned by Ivanov and Hoberg (1999). In their analysis, *Macrobothridium rhynchobati* was grouped with several species of *Echinobothrium*, leaving the latter genus paraphyletic. The present analyses, which included two species of *Macrobothridium* not treated by Ivanov and Hoberg (1999), obtained similar results. These results are consistent with the limited analysis based on molecular data by Olson *et al.* (2001), who showed *Macrobothridium* sp. (= *M. rhynchobati*) to be the sister taxon to *Echinobothrium chisholmae*, with *E. harfordi* as a basal relative of the two.

The morphological differences between *Echinobothrium* and *Macrobothridium* have never been great, and have recently become even less clear. In the original description of the type species *Macrobothridium rhynchobati*, Khalil and Abdul-Salam (1989) distinguished the genus and species from *Echinobothrium* by its lack of cephalic peduncle armature, the presence of a sucker-like organ at the posterior terminus of the terminal proglottid, and its unusually large (for a diphyllidean) size. However, observations on the type specimens of this species revealed that the sucker-like organ described by Khalil and Abdul-Salam (1989) was only the constriction at the end of the terminal proglottid, similar to that observed in other diphyllideans. Those authors also discussed *E. reesae*, stating that because it lacked peduncle armature it too may belong in *Macrobothridium*, but that further investigation was necessary. Neifar *et al.* (2001) recently described two new species and assigned them to *Macrobothridium*. Neither of these species is particularly large. Thus, none of the three diagnostic characteristics proposed by Khalil and Abdul-Salam for the genus is observed in all members previously assigned to the genus, nor is exclusive to those species. Because the generic boundaries between *Macrobothridium* and *Echinobothrium* are completely muddled, and *Echinobothrium* is not monophyletic if *Macrobothridium* is excluded, *Macrobothridium* is synonymized with *Echinobothrium* herein.

Those species formerly assigned to *Mac-*

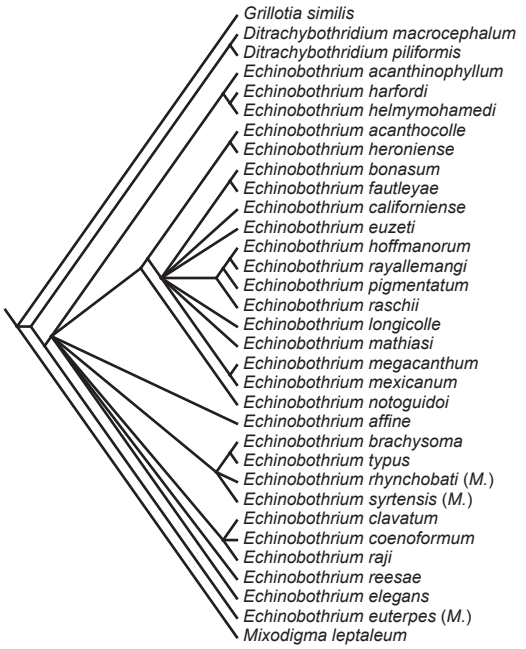


Fig. 128. Strict consensus tree resulting from Analysis 3. (*M.*) denotes species transferred from *Macrobothridium*.

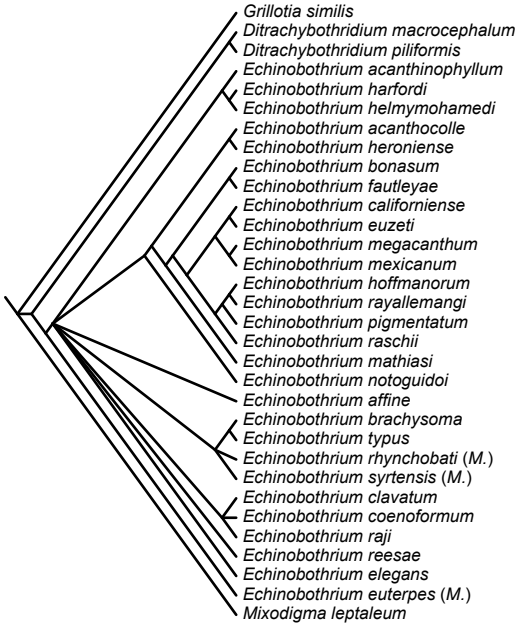


Fig. 129. Strict consensus tree resulting from Analysis 4. (*M.*) designates species transferred from *Macrobothridium*.

robothridium are deserving of special note. As mentioned above, cephalic peduncle armature in *Echinobothrium* is a feature strictly limited to adults, generally developing shortly after entering the definitive host (Alexander, 1963; McVicar, 1976; Jones and Beveridge, 2001). Some species originally assigned to *Echinobothrium* (before the establishment of *Macrobothridium*) have been described as lacking cephalic peduncle armature in the adult form (*e.g.*, *E. reesae*, *E. deeghai*). *Echinobothrium reesae* has also been described in larval form, encysted in a shrimp (Ramadevi and Rao, 1974). This encysted larval form appears to be progenetic, being fully strobilated, with the reproductive organs fully formed. Alternately, several adult specimens of *E. heroniense* collected from the blue-spotted stingray in Australia for this study appear paedomorphic, demonstrating a fully formed strobila with visible reproductive organs before the cephalic peduncle armature was fully developed. Clearly then, there is some plasticity in the developmental program of *Echinobothrium*, at least with respect to the cephalic peduncle armature and reproductive organ development. This developmental reaction norm may even be broad enough to encompass the total lack of cephalic peduncle armature in some members of *Echinobothrium*. Examination of these particular species and their hosts may provide some clues as to what signals trigger the development of the cephalic peduncle armature.

The phylogenetic analysis published by Ivanov and Hoberg (1999) did accomplish at least one of the intended goals of these authors, to stimulate further research on the Diphyllidea. One of the concerns of those authors was the relative paucity of morphological characters available for cladistic analysis of the order. Through the use of scanning electron microscopy, many more morphological characters have been elucidated. Sadly, nearly all of these characters were excluded after applying the 20% exclusion rule. However, even after the exclusion of these characters, these analyses retained a greater number of characters than those used by Ivanov and Hoberg (1999). In addition, eight species described since the publication of that paper

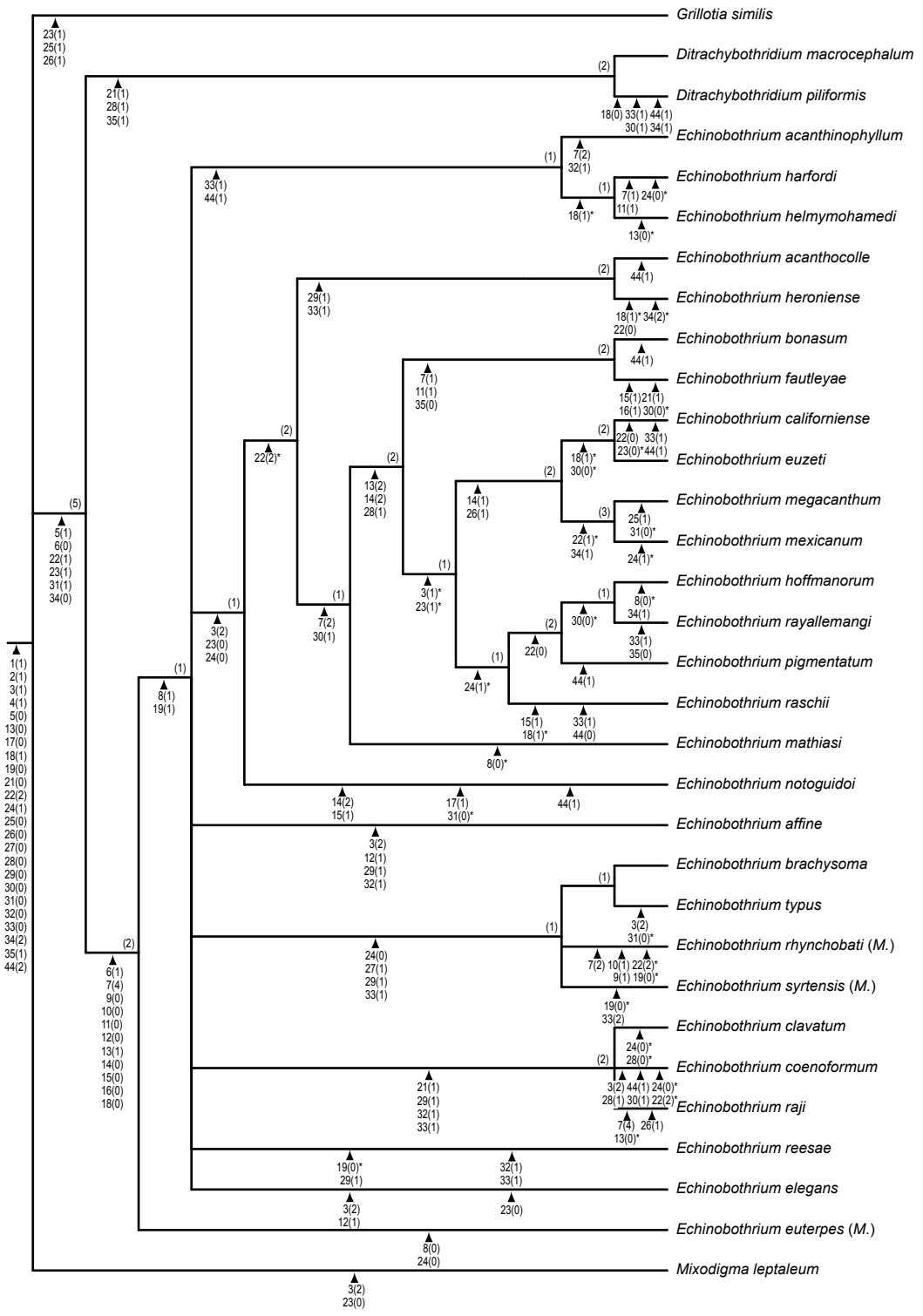


Fig. 130. Characters mapped on strict consensus tree resulting from Analysis 4. (*M.*) denotes species transfered from *Macrobothridium*. Characters are indicated below each branch, with states in parentheses. Numbers above branches indicate Bremer support values.

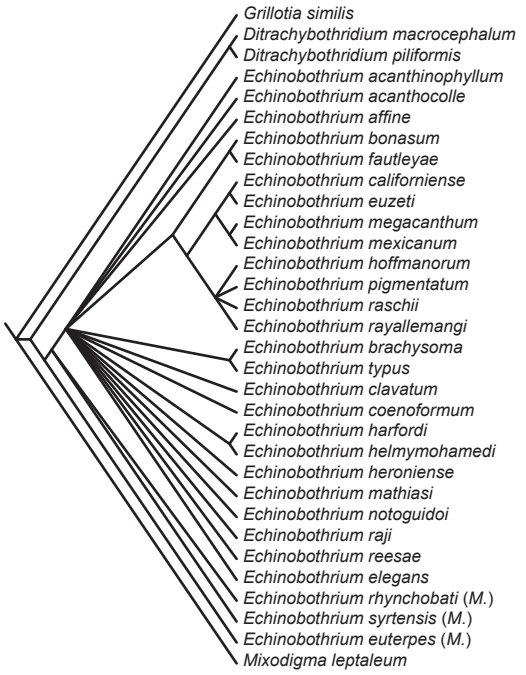


Fig. 131. Strict consensus tree resulting from Analysis 5. (*M.*) denotes species transferred from *Macrobothridium*.

were included in these analyses.

Perhaps as a result of using a different set of characters, and some different taxa, virtually none of the topology of the tree published by Ivanov and Hoberg (1999) was recovered by the analyses performed during this study. The only similarity between the two trees is the placement of *Ditrachybothridium* in a position basal to *Echinobothrium*.

Differences between the analyses presented here and in Ivanov and Hoberg (1999) go beyond the number of taxa and characters employed, and include several methodological differences. The outgroup used in the analysis of Ivanov and Hoberg (1999) was a hypothetical composite of a pseudophyllidean and haplobothriidean taxa, whereas the outgroups chosen for this study were actual species of pseudophyllideans, trypanorhynchs, and tetraphyllideans. Also, although Ivanov and Hoberg (1999) did employ a rule for the exclusion of taxa, excluding those with more than five (24%) unscored characters, they did not exclude characters which were unscored for a large number of taxa, which may have affected their results. Finally, the character coding of Ivanov and Hoberg (1999) was based primarily on a review of the literature, while the present work was based primarily on observation of actual specimens. This technique may be what led to the improper coding of a number of characters for some taxa, which became apparent during the examination of type specimens used in the present study.

In recent a molecular analysis of six species of diphyllideans, including *Ditrachybothridium macrocephalum*, Bray and Olson (2004) placed *Ditrachybothridium* in a relative derived postion within *Echinobothrium*. However, the limited number of taxa used in this study does not provide enough support for that position to warrant any changes in classification for that genus at this time.

The present phylogenetic analyses resulted in the most comprehensive phylogenetic estimate of the interrelationships of the Diphyllidea currently available. However, it is generally accepted, and demonstrated here, that the addition of more characters and/or more taxa can affect the outcome of an analysis. Therefore, because the diversity of the Diphyllidea has not yet been fully revealed (see below), the relationships among the species of diphyllideans presented in this study should be approached with caution, as these results are still somewhat preliminary.

EVOLUTION AND DIVERSIFICATION

Although the higher level phylogeny of the cestodes is beyond the scope of this monograph, the issue warrants some discussion here, at least with respect to the placement of the Diphyllidea. Throughout the past 200 years, many workers have attempted to resolve the higher level phylogeny of the cestodes. This discussion will be limited to those of most concern to modern cestode systematists: those that included explicit phylogenetic trees. While the relationships of some of the more derived cestode groups, particularly those of terrestrial vertebrates, are fairly well understood, the phylogenetic position of the Diphyllidea remains unclear even after over 100 years of study. The Diphyllidea share a number of morphological features with the Trypanorhyncha, Pseudophyllidea, and perhaps even the Haplobothriidea. For example, all three groups possess a difossate bothriate scolex (Hoberg *et al.* 1997). However, the Diphyllidea, Haplobothriidea, and some Pseudophyllidea possess a ventral (rather than a lateral) genital pore, a feature not present in the Trypanorhyncha (Hoberg *et al.* 1999). Their association with elasmobranch definitive hosts suggests a close relationship with the Trypanorhyncha, Tetraphyllidea, and Lecanicephalidea (Baer 1950), whereas similarities in egg structure ally them more closely with the Tetraphyllidea and Lecanicephalidea than to the Trypanorhyncha (see Euzet 1994a, b; Khalil 1994).

Baer (1950) relied on several lines of evidence including arrangement of the vitellaria (peripheral), the association with elasmobranch hosts, and the presence (at least presumed) of two intermediate hosts in the life-cycle, to ally the Diphyllidea with the Trypanorhyncha (see Fig. 132). Based primarily on early life-cycle data (larval forms), Euzet (1959) grouped the Diphyllidea in a basal polytomy with the Trypanorhyncha, Pseudophyllidea, and Haplobothriidea and the tetrafossate groups (see Fig. 133), but was reluctant to speculate further as so little was known about the young larval stages of diphyllideans. The advent of cladistics made the analysis of the higher level relationships among the cestodes more objective, but because of differences in taxon sampling and choice of characters, the results varied substantially from one study to another. For example, Brooks and McLennan (1993) placed the Diphyllidea with the Pseudophyl-

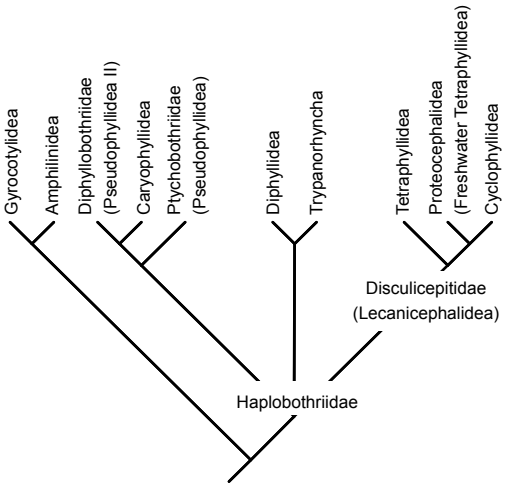


Fig. 132. Evolutionary tree of cestode orders from Baer (1950).

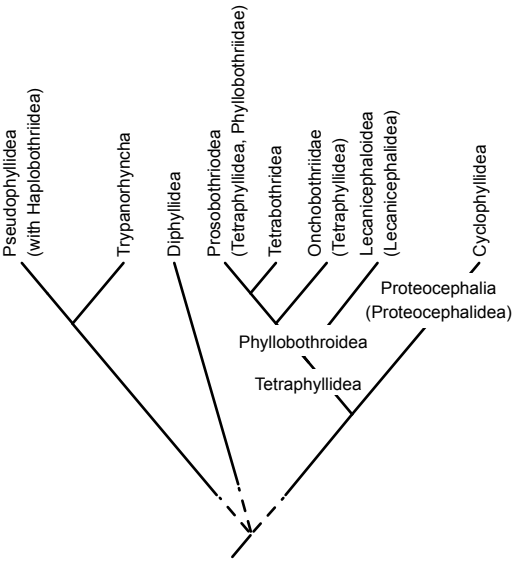


Fig. 133. Evolutionary tree of cestode orders from Euzet (1959).

lidea, Haplobothriidea, Caryophyllidea, and Spathebothriidea (see Fig. 134), and Hoberg *et al.* (1997, 1999, 2001) placed them as sister to a clade containing the trypanorhynchs and higher tetrafossate groups, in a derived position relative to Pseudophyllidea or Haplobothriidea (see Figs. 135-137).

The rapidly growing field of molecular systematics is expected to help elucidate the higher level cestode relationships, but as of yet, sampling of the Diphyllidea has been extremely limited; DNA sequences are available for only five species (Mariaux 1998; Olson and Caira 1999; Olson *et al.* 2001). To date, phylogenetic analyses based on DNA sequence data have offered wildly differing hypotheses. The Diphyllidea were allied with the Proteocephalidea by Mariaux (1998; see Fig. 139) and Hoberg *et al.* (2001; see Fig. 138) and found in a polytomy in the trees of Olson and Caira (1999) and Olson *et al.* (2001) (see Figs. 140, 141). It is likely that the true placement of the Diphyllidea within the Cestoda will become known only after morphological, developmental, and/or molecular data are considered for a larger subset of diphyllidean species.

The relatively low diversity of the Diphyllidea, when compared to other cestode groups known to parasitize elasmobranchs raises some puzzling questions. For example, the Diphyllidea currently comprise only 41 described species, compared to the Trypanorhyncha with well over 1,000 described species and the Tetraphyllidea with some 600+ described species. The Tetraphyllidea are considered by some to by polyphyletic (Olson and Caira 1999; Hoberg *et al.* 2001; Olson *et al.* 2001), which would result in an overestimate of their diversity, but each of the subgroups of tetraphyllideans is still substantially more speciose than the Diphyllidea. One possible explanation for this observed discrepancy is that it results from a lack of sampling. According to Caira and Jensen (2001), only 19.5% of the known elasmobranch species have been sampled for onchobothriid (tetraphyllidean) tapeworms. Assuming that these numbers are also fairly indicative of the sampling effort for the Diphyllidea, only 8.5% of the known diversity of Rajidae, the family

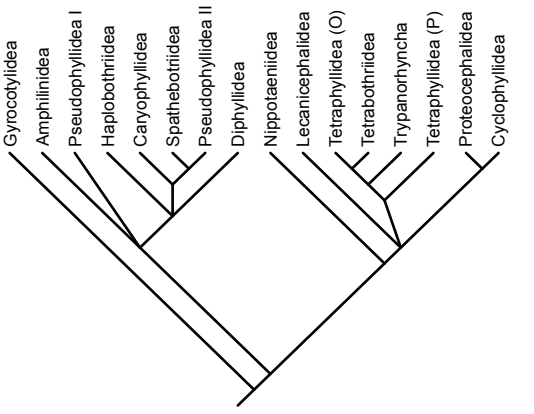


Fig. 134. Phylogenetic tree of cestode orders from Brooks and McLennan (1993).

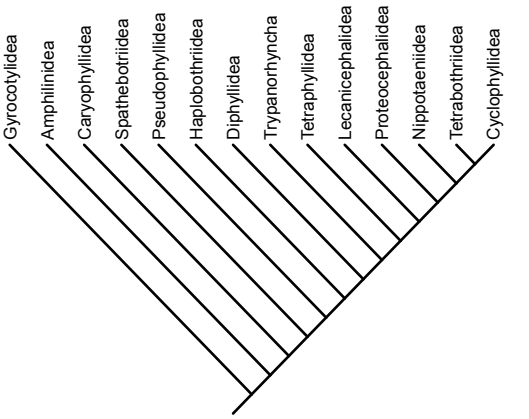


Fig. 135. Phylogenetic tree of cestode orders from Hoberg *et al.* (1997).

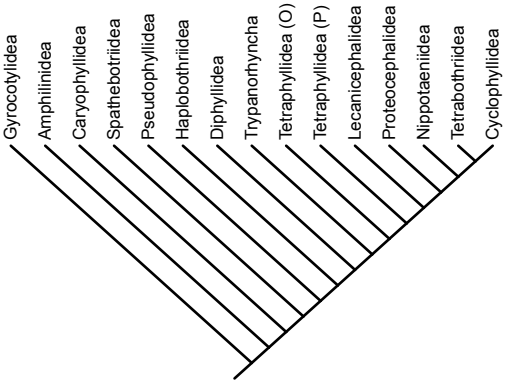


Fig. 136. Phylogenetic tree of cestode orders from Hoberg *et al.* (1999).

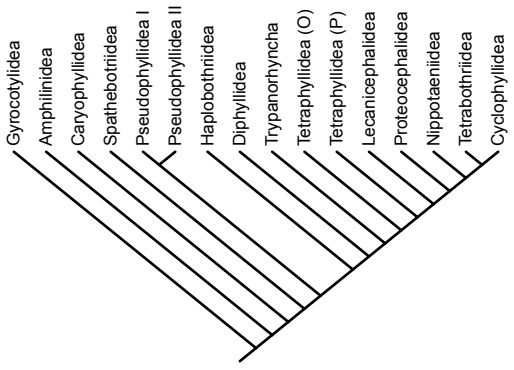


Fig. 137. Phylogenetic tree of cestode orders based on morphology from Hoberg *et al.* (2001).

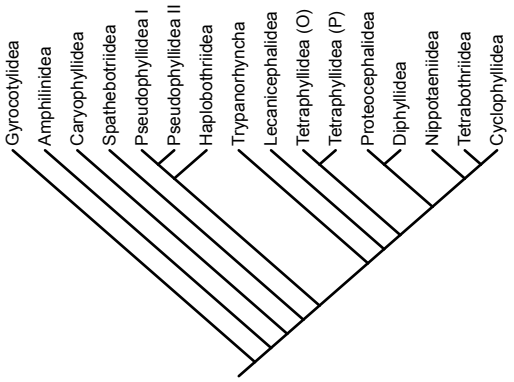


Fig. 138. Phylogenetic tree of cestode orders based on total evidence from Hoberg *et al.* (2001).

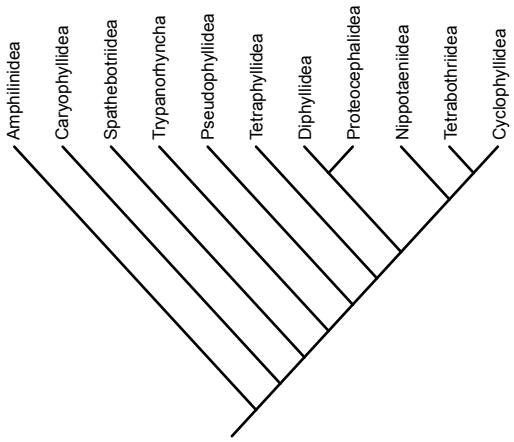


Fig. 139. Phylogenetic tree of cestode orders from Mariaux (1998).

best represented by diphyllideans, has been sampled for onchobothriids. While it is important to note here that negative data with respect to the Onchobothriidae may not appear in the literature, the fact remains that only a small subset of suitable host species for the Diphyllidea has been sampled for parasites. It seems likely that the current estimate of diphyllidean diversity is but the tip of an iceberg, and that additional sampling effort is necessary to gain a better understanding of the true diversity of this group.

One other issue arises with respect to sampling. The diphyllideans, with few exceptions, are very small worms, commonly attaining mature lengths of less than five millimeters; in this respect, they are much like the Lecanicephalidea, another group with relatively low known diversity. It is entirely possible that many researchers looking for trypanorhynchs or tetraphyllideans are overlooking the much smaller diphyllideans and lecanicephalideans.

The lingering question, then, is: What if the relatively low diversity of the Diphyllidea is not an artifact of poor sampling? One point to consider is the diversity of the hosts of diphyllideans, which is the equivalent of diversity of habitat types in free-living organisms. The ability to colonize a wide variety of habitat types has been shown to be a factor in the diversification of a taxon (Benton 1990). To date, diphyllideans are known from only eight families of elasmobranchs, whereas the trypanorhynchs are known from 25 (Bates 1990) and the Tetraphyllidea from 24 (Caira and Jensen 2001). If these data represent the true host associations of these orders, we are left to ask why diphyllideans are found in fewer families of elasmobranchs. If the Diphyllidea have more recently acquired their association with elasmobranch hosts than those other orders of cestodes, then we might expect that they would parasitize a narrower range of host families. However, most phylogenetic analyses place the Diphyllidea in a polytomy with, or in a basal position relative to, the Trypanorhyncha and the Tetraphyllidea. Clearly, this question needs further investigation. One difference between the Diphyllidea and these other orders is that

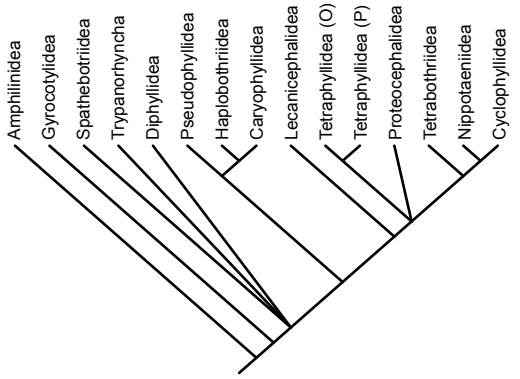


Fig. 140. Phylogenetic tree of cestode orders from Olson and Caira (1999).

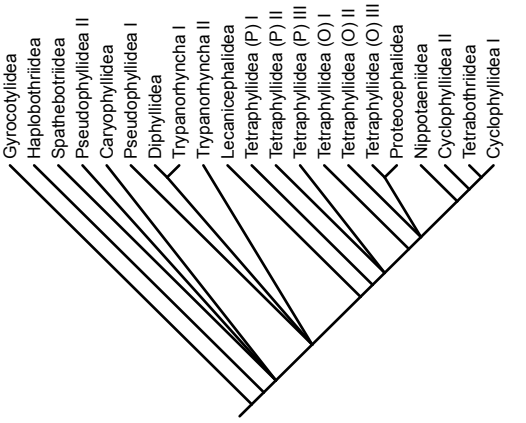


Fig. 141. Phylogenetic tree of cestode orders from Olson *et al.* (2001)

unlike those other orders, only one species of diphyllidean generally parasitizes each host species. In the Tetraphyllidea and Trypanorhyncha, multiple congeners are commonly found in a single host individual. This could lead to the great disparity observed in the species richness of these orders relative to one another, but there may well be other contributing factors that will be identified once the overall diversity of the Diphyllidea is more completely understood.

Although poor taxon sampling and relatively low host diversity are two possible explanations for the low diversity of the Diphyllidea, there is also the possibility that the Diphyllidea have exhibited either a low rate of speciation, or a propensity for extinction (see Tokeshi 1999). To address this question, however, the use of fossil data, entirely lacking for these soft bodied organisms, would be required.

HOST-PARASITE ASSOCIATIONS AND COEVOLUTION

One of the more fascinating aspects of parasitology is the evolutionary association between host and parasite. The ability of tapeworms to thrive in the guts of their hosts leads us to believe that they have long been associated with this environment. Observations on heavily infected elasmobranch hosts seem to show few observable effects from the infection (except occasionally at the point of attachment of the scolex see Borucinska and Caira [1993]), nor an immune response to the worms (but see McVicar and Fletcher 1970). The intimate association between the host and its parasite leads one to ponder the extent of the coevolutionary relationships between these two organisms. However, with the exception of some passing comments and observations made by Ivanov (1997), Ivanov and Campbell (1998a, b), and Ivanov and Hoberg (1999), the coevolutionary relationships between diphyllideans and their hosts have never been examined.

The study of host-parasite coevolution has a long history, and out of this history have come four general "rules." The first of these generalities or rules, coined by Eichler (1941) as Fahrenholz's Rule, states that the relationships of the hosts can often be inferred from the systematics of the parasites. The second generality, also coined by Eichler (1941) as Szidat's Rule, is that the relative phylogenetic age of the hosts can often be inferred by the level of organization of the parasites. The third rule, or set of rules, often referred to as Manter's Rules, were summarized by Brooks (1979, p. 299) as follows: "(1) parasites evolve more slowly than their hosts; (2) the longer the association with the host-group, the more pronounced the specificity exhibited by the parasite group; (3) a host species harbors the largest number of parasite species in the area where it has resided longest, so if the same or two closely-related species of host exhibit a disjunct distribution and possess similar parasite faunas, the areas in which the hosts occur must have been contiguous at a past time."

The fourth and final rule, named Eichler's rule by Stammer (1957), states that more diverse host groups have more diverse parasite faunas.

Caira and Jensen (2001) developed a set of criteria that should be considered before embarking on any study of host-parasite coevolution. These are first, that both host and parasite groups are monophyletic; second, that hosts and parasites are correctly identified; third, that there exist reasonably accurate phylogenetic hypotheses for both host and parasite groups; fourth, that all members of the host group have been examined for members of the parasite group; and last, that the parasites of interest exhibit high host specificity. Applying these five criteria to the Diphyllidea, there is little hope for a rigorous investigation of the coevolutionary relationships between them and their elasmobranch hosts. The first and last criteria are apparently met, but there are problems with the remaining three. The inability to study this system rigorously is due not to shortcomings in the parasite data per se, but to shortcomings in the host data. Many of the hosts from which diphyllideans have been collected are difficult to identify. Among the elasmobranch fishes, the batoids are the least well understood. Because most diphyllideans are hosted by batoids, misidentification of the hosts, leading to falsely deflated estimates of host specificity, may be rampant in this system. Most diphyllidean species are present in only one host species; that is, they exhibit oioxenic specificity (Euzet and Combes 1980). However, some species of *Echinobothrium* from the Mediterranean Sea and Great Britain (*e.g.*, *E. typus* and *E. affine*) have been reported from multiple host species, including *Raja clavata*, *R. radula*, and *R. alba*. In addition, some hosts (*e.g.*, *Raja clavata*) have been reported to harbor multiple species of *Echinobothrium* (*e.g.*, *E. typus*, *E. affine*, *E. brachysoma*, *E. clavatum*). The phylogenetic relationships among the host species in which diphyllideans are found are equally poorly understood. Although advances are

rapidly being made in the field of higher level elasmobranch systematics (Naylor 1992; de Carvalho 1996; McEachran *et al.* 1996; Shirai 1996; Naylor *et al.* 1997; McEachran and Dunn 1998), the species level phylogenies of these hosts, particularly the batoids, leaves much to be desired. Until the species level phylogenies of these host species are resolved, we cannot hope to study the host-parasite coevolutionary relationships accurately. Even with an accurate host phylogeny, there remains the problem (mentioned above) of gaps in sampling effort. Because less than 20% of the elasmobranch diversity has been sampled and examined (Caira and Jensen 2001), this host-parasite system does not make an especially good candidate for coevolutionary study, at least at the species level. However, as there are enough data in the literature to assemble a tree of the evolutionary relationships among the elasmobranch genera known to host *Echinobothrium*, and a phylogenetic hypothesis of the Diphyllidea is presented here (Figs. 129, 130), it is possible to examine the extent to which the two phylogenies are congruent. A composite tree of the batoid genera known to host diphyllideans was constructed by placing generic level trees of the rajoids (McEachran and Dunn 1998) and myliobatoids (Nishida 1990) onto the branches of the family level analysis of McEachran *et al.* (1996), and removing all taxa not reported as hosts of diphyllideans. Figure 142 shows the phylogeny of the Diphyllidea resulting from phylogenetic Analysis 4, and the composite batoid tree (see above). Host associations are indicated by lines connecting each diphyllidean species to its host genus. The degree to which these lines cross indicates the degree to which a strict cospeciation model is violated (*i.e.*, Fahrenholz's Rule is violated). However, as this comparison is based only on an incomplete composite tree of the batoids, the phenomenon deserves further investigation. Although strict coevolution between diphyllideans and their hosts can be ruled out at this point, there may yet be some coevolution occurring within genera. For example, the batoid family Rajidae hosts more diphyllidean species than any other (*i.e.*, 13). Once relationships are resolved among the species in this group, coevolutionary relationships may become apparent.

There are some host-parasite associations that deserve further investigation. For example, four species of *Echinobothrium* (*E. coronatum*, *E. musteli*, *E. notoguidoi*, and *E. scoliodoni*) have been described from sharks.

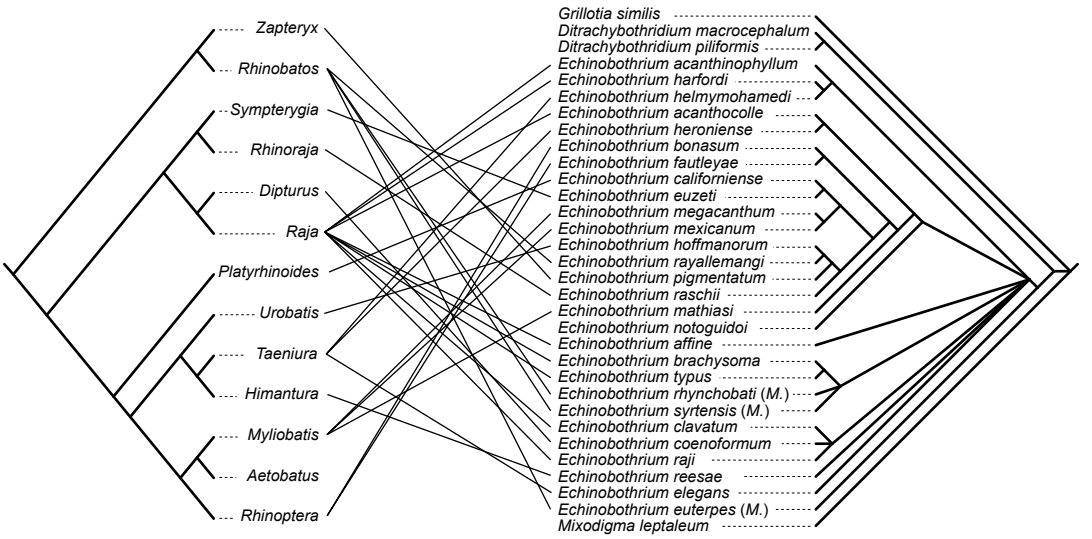


Fig. 142. Strict consensus cestode tree from Analysis 4 mapped on composite phylogram of batoid orders (from McEarchran and Dunn [1998]; Nishida [1990]; McEarchran *et al.* [1996]) known to host diphyllideans. Lines indicate host-parasite associations.

All except *E. coronatum* possess a peculiar form of scolex armature in which there are several rows of small spines or microtriches between the rostellum and bothria. This character has not been observed in any *Echinobothrium* species collected from a batoid. Although the lack of data prevented the inclusion of three of these species in the phylogenetic analysis, this unique character found among these parasites of sharks may be the result of a host switch from a batoid to a shark, followed by cospeciation with the shark hosts, as suggested by Ivanov (1997).

One interesting observation arising from this analysis concerns the genus *Ditrachybothridium* and its association with sharks, rather than rays. Admittedly, the type host of *Ditrachybothridium macrocephalum* (the generotype), was reported as a batoid (*Raja fullonica*), but evidence presented here, and by Faliex *et al.* (2000), strongly suggest that *D. macrocephalum*, like its congener *D. piliformis*, is a parasite of *Galeus* (catsharks). In the phylogenetic analysis presented above, *Ditrachybothridium* forms the sister clade to *Echinobothrium*. When the phylogeny of the Diphyllidea is compared to the higher level phylogeny of the elasmobranchs presented by Shirai (1996) (Fig. 143), a correlation is observed between the deep split in the diphyllidean genera *Echinobothrium* and *Ditrachybothridium* and the deep split in the elasmobranch superorders Galea and Squalea. This could represent an ancient cospeciation event between the common ancestor of the modern elasmobranchs and the common ancestor for the two diphyllidean genera. If this is the case, then given the evolutionary history of the elasmobranchs, the relationship between the elasmobranchs and the Diphyllidea dates back well into the Jurassic (Shirai 1996).

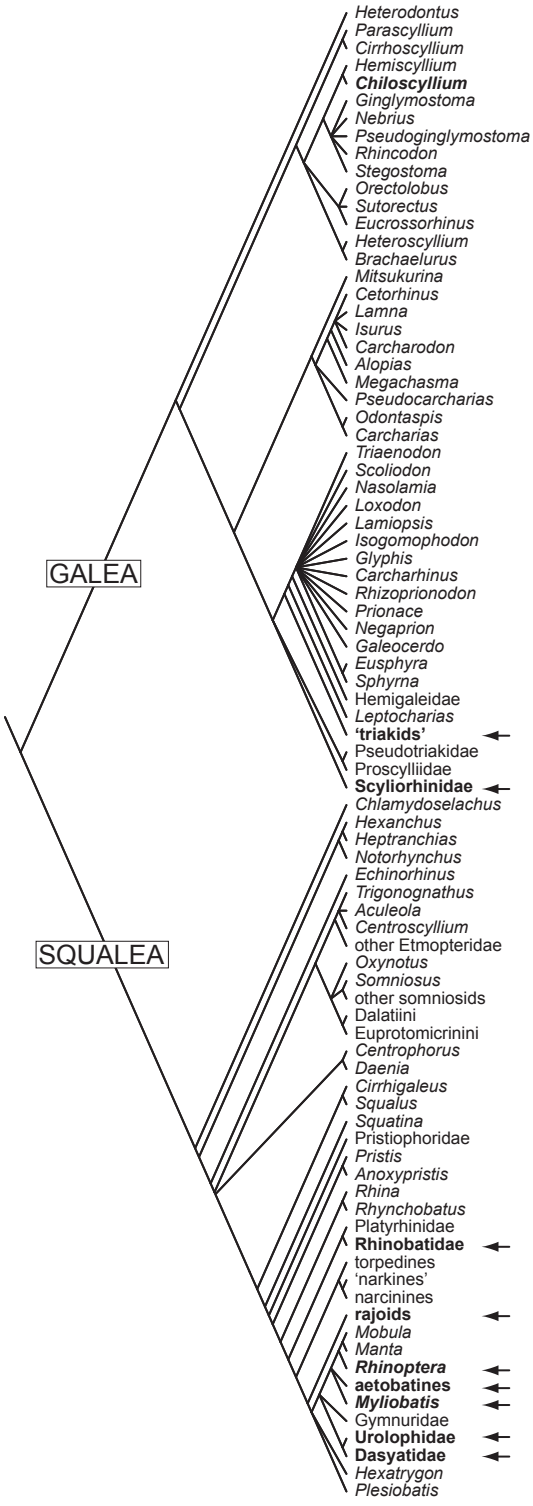


Fig. 143. Higher level phylogeny of elasmobranchs (after Shirai 1996). Note, arrows indicate groups hosting diphyllideans.

BIOGEOGRAPHY

No discussion of the evolution of a taxonomic group would be complete without an analysis of biogeography. The distribution of the Diphyllidea has been examined here in the context of the host relationships to detect any discernible patterns.

Diphyllideans are cosmopolitan (Figs. 141, 145), species having been described from all oceans and all continents except Antarctica (but see *E. acanthocolle*). Distributions are generally explained by dispersal, vicariance, or some combination of the two (Cox and Moore 2000). Cestode parasites of elasmobranchs present an interesting case in that the only free-living stages of the life-cycle (the egg, or in some orders a coracidium larva) are generally short lived (see Euzet 1959; Mattis 1986). Thus, chances for self-dispersal of these stages are extremely limited. However, dispersal is effected by living in one or more vagile host as an adult. On the other hand, most diphyllideans are parasites of skates and other batoids, which generally have highly restricted ranges (McEachran and Miyake 1990), a factor limiting their dispersal.

Given the cosmopolitan distribution and lack of recognized hotspots of diversity of diphyllideans, it is difficult to locate any regions of origin or dispersal. Moreover, when the area of endemism of each species in the phylogeny is mapped on the tree (Fig. 144), no distributional patterns are observed. However, it is expected that as the actual diversity of the Diphyllidea becomes better known, that some biogeographical patterns will begin to emerge.

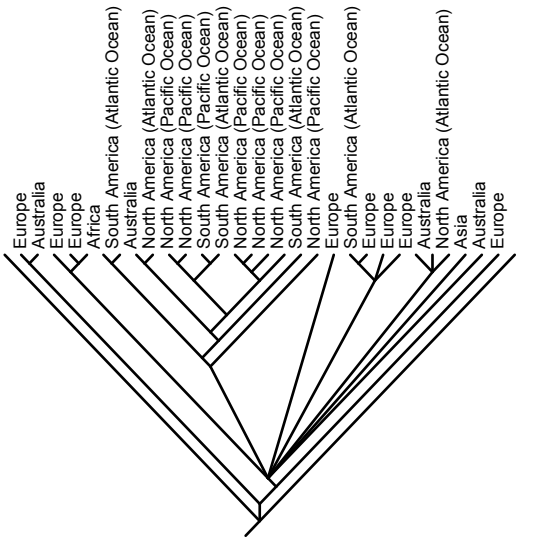


Fig. 144. Strict consensus tree of cestodes from Analysis 4 showing areas of geographic endemism.

COLLECTION OF PARASITES AND LACK OF TAXONOMIC REPRESENTATION

One of the most problematic issues arising during this study was the difficulty of obtaining fresh material for study. Many of the characters used in the descriptions and phylogenetic analysis were obtained with the use of scanning electron microscopy. Without fresh material, it was not possible to examine these characters, which in turn limited the number of characters used in the phylogenetic analysis. The reasons for the difficulties in recollecting many diphyllidean species were many and varied, both intrinsic and extrinsic to the parasites and/or their hosts. One problem, described above, is that of proper host identification, an issue discussed long ago by Williams (1961). Because these parasites appear to be highly host specific (but see below), our ability to locate and recollect them depends on our ability to identify the host.

Some hosts are inherently difficult to collect. For example, *Sympterygia lima* (host of *E. euzeti*) is only rarely encountered (M. Oliva, pers. comm.) in the western Pacific. Other hosts are collected easily enough, but frequently do not harbor diphyllideans. For example, *Mustelus lenticulatus* is the type host for *E. coronatum*, but, in spite of intensive collecting efforts (see above), this para-

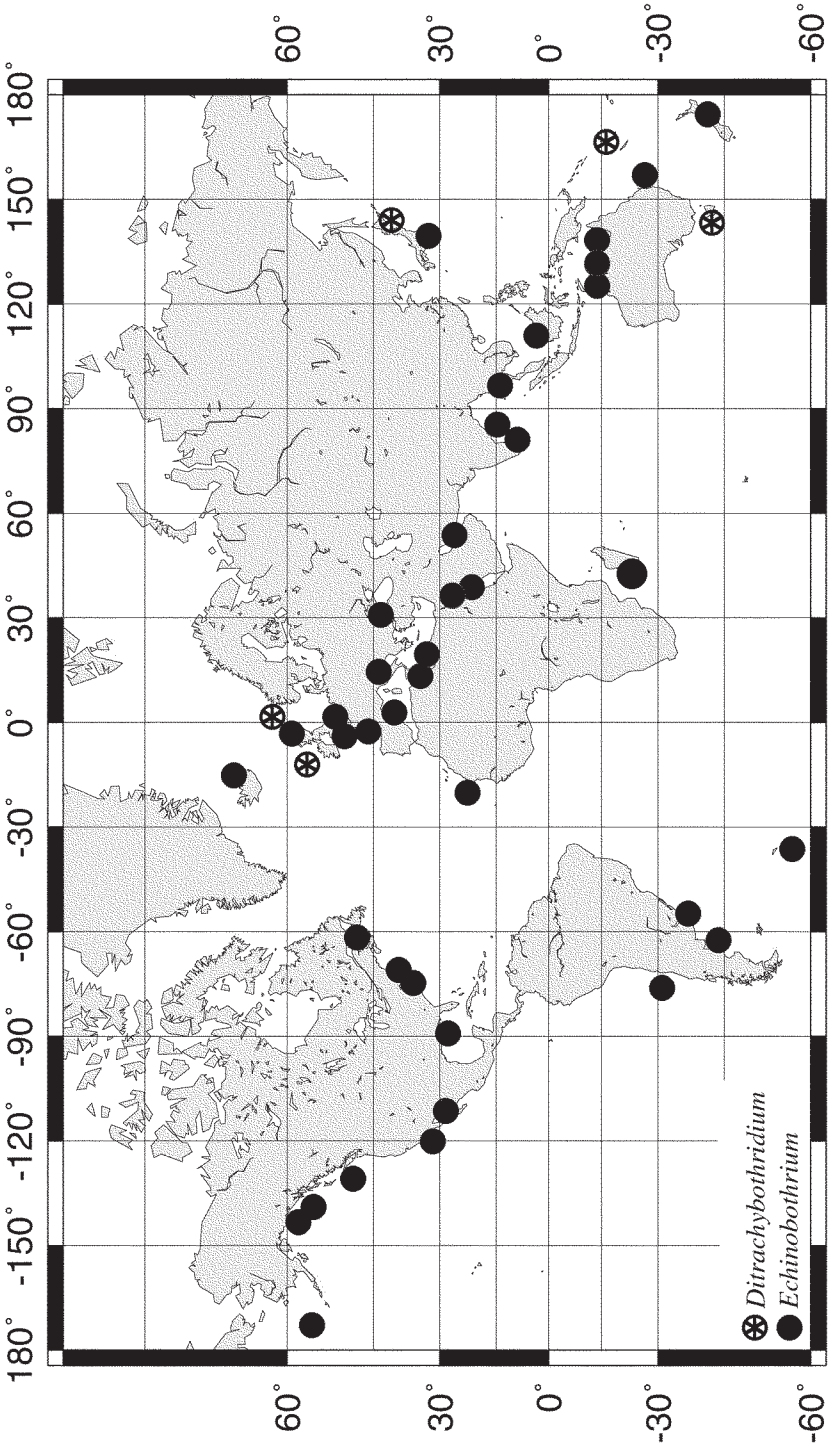


Fig. 145. Global distribution of diphyllidean species.

site was not recollected. Also, subsequent collections have failed to yield *E. coenoformum* from *Dipturus nasuta*.

In some cases, the type host species has been recollected, but not from the type locality, and has failed to yield the parasite of interest. This could be due to incorrect identification of the host (perhaps due to cryptic species) or, perhaps more likely, a restriction in the range of the parasite due to abiotic factors or lack of suitable intermediate hosts. Williams (1964) was the first to discover different species of diphyllidean tapeworms (*Echinobothrium*) in conspecific hosts (*Taeniura lymma*) from disjunct localities. He suggested that the hosts from the two localities were actually distinct subspecies, and that a careful study of the parasites might clarify this question. During the present study, seven specimens of *T. lymma* were collected from two localities in Australia. These rays were found to host *E. heroniense* as expected, as well as a new species, *E. elegans*. However, no examples of *E. helmymohamedi* were collected there, even though *T. lymma* is the host species for that particular parasite. Additionally, one specimen of *T. lymma* collected from Madagascar was found to host a species of *Echinobothrium*, but not any of the former species.

One region which has had several reports of diphyllideans is south central and western Asia (*i.e.*, Red Sea, Persian Gulf, Bay of Bengal). Although no collections were made from any of these areas during the present study, many species of elasmobranch reported to host diphyllideans in those regions were collected elsewhere (*e.g.*, Australia, Sea of Japan, Thailand, Madagascar). None of these, however, were found to harbor the same species of parasite as found in the type locality. For example, *Aetobatus narinari* was reported to host *E. boisii* in Sri Lanka (Southwell, 1911). However, of all the specimens collected during the present study (10 from the Gulf of California, Mexico; five from Florida; four from Northern Territory, Australia; and one from Thailand), none harbored this parasite, although those from Thailand and Australia did host, in very low numbers, other species of *Echinobothrium*. Evidence is mounting in support of the hypothesis that *A. narinari* is a species complex, rather than a single species (see Jensen *et al.* 1999), which could explain this phenomenon in *A. narinari*. This phenomenon is, however, observed in other species as well. *Dasyatis kuhlii* was reported to host *E. longicolle* in Sri Lanka (Southwell 1925), but five specimens of this host collected from Australia hosted a different species. A single specimen of *D. kuhlii* collected from Madagascar hosted no diphyllideans at all. Finally, *Pastinachus sephen* was reported as the type host of *E. deeghai* in West Bengal (Gupta and Parmar, 1988), but hosted a different diphyllidean species in Australia and none in Madagascar. Only one diphyllidean species from Asia, *Echinobothrium rhynchobati*, was re-collected during the course of this study. Interestingly enough, that record was from elasmobranch species other than the type host (*Rhinobatos typus* vs. *R. granulatus*)! It is also possible that the difficulty in recollecting previously described species was not due to problems with the definitive hosts, but with some other aspect of the parasite's life history or ecology. Given that most published reports of diphyllideans list prevalences of 15-50%, it would appear that the primary cause is not low prevalence of parasites, but some other external cause. Aside from the fact that collections have not been made from some of the type localities, several explanations for this observation seem plausible. First, it should be noted that any organism which relies on multiple hosts from different phyla to complete its life-cycle is much more sensitive to environmental disturbance, as it inhabits multiple environments over its lifetime. It is therefore possible that the absence of these species in the definitive hosts may be due to a disruption of the life-cycle resulting from some sort environmental disturbance, either natural or anthropogenic. This idea was raised by Tyler (2001) as a possible explanation for the difference in diphyllidean faunas in the Gulf of California between 1993 and 1996. Tyler (2001) speculated that changes in marine invertebrate communities associated with the 1992-93 El Niño event could have led to a temporary disruption of the life-cycle for two of the four species of diphyllideans reported there. If diphyllideans

are like some other cestode groups (*i.e.*, tetraphyllideans and trypanorhynchs), they may be able to withstand these temporary disruptions by remaining dormant in the intermediate hosts (T. Mattis, pers. comm.). Although the scenario hypothesized by Tyler (2001) was the result of a purely natural occurrence, anthropogenic disturbances are much more common and likely to be irreversible. Considering that cestode life-cycles are food chain driven, and that humans routinely decimate marine fish and invertebrate populations through overfishing, pollution or introducing exotic species, perhaps it is no surprise that a tapeworm species collected in 1959 and 1963 (*Echinobothrium coronatum* from *Mustelus lenticulatus*) can no longer be found. Invasive species, such as the European green crab *Carcinus maenus*, with their ability to displace indigenous crustacean and mollusc species, may play a role in the determination of local cestode communities, and thus may be responsible for the observed difficulty in re-collecting some of the species examined in this study. Admittedly, most of the above ideas are speculation, because the life-cycles of diphyllideans are unknown. Once the life-cycles of diphyllideans are understood, especially the identity and specificity for the intermediate hosts, it will be possible to address these issues more directly.

Given the known diversity and host associations of the Diphyllidea, it appears that there remain many undescribed species in this group. As noted by Caira and Jensen (2001), sampling of elasmobranchs for tapeworms, although especially intensive in recent years (see Caira and Burge 2001; Ghoshroy and Caira 2001; Jensen 2001; Neifar *et al.* 2001; Tyler 2001), covered less than 20% of described elasmobranch species. This limits the conclusions that can be made about the group, especially concerning global diversity and host-parasite coevolution. For example, only two species of *Ditrachybothridium* are known at this time; both have been reported from catsharks (Scyliorhinidae). However, only three of 106 species (2.8%) in the family Scyliorhinidae have been sampled, and one of these was a previously undescribed species (see Faliex *et al.* 2000)! Given that two species of *Ditrachybothridium* are known from only 2.8% of scyliorhinid species, there may be as many as 100 species of *Ditrachybothridium* yet to be discovered. Extrapolating from the host sampling figures of Caira and Jensen (2001) and considering the known diphyllidean diversity, less than 18% of diphyllidean diversity is believed to be described; there may be over 200 species of diphyllideans worldwide.

CONCLUSIONS

One overwhelming conclusion of this study of the Diphyllidea is that it is difficult to re-collect previously described species. The lack of new collections of known species from southern Asia has made the study of the Diphyllidea much more difficult than it might otherwise have been. Nine of 38 (*i.e.*, 23.7%) of the described species of diphyllideans were described from localities in southeast Asia (see Fig. 145). Type material is unknown for six of these (*i.e.*, 66.7%), and for most of them, the original descriptions are very poor. During this study, one neotype was designated, and missing type specimens were located for only one species. Sadly, this still leaves a significant gap in the understanding of the Diphyllidea, especially considering that there are probably many other hosts in the region which have never been examined for parasites. Fortunately, a project is currently underway to catalog and describe the cestode fauna of the elasmobranchs of Borneo, which should result in a better understanding of the diversity of diphyllideans in SE Asia.

This monograph has succeeded in compiling a substantial amount of information on the Diphyllidea; nevertheless, our understanding of the order is still limited by our lack of sampling of host taxa. In order to present the most accurate estimates of diversity, phylogeny, host-parasite associations, and biogeography, it is imperative that the hosts are better understood, beginning with phylogenies for the host groups and sampling of every species possible for the presence of diphyllideans.

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TAXONOMIC INDEX

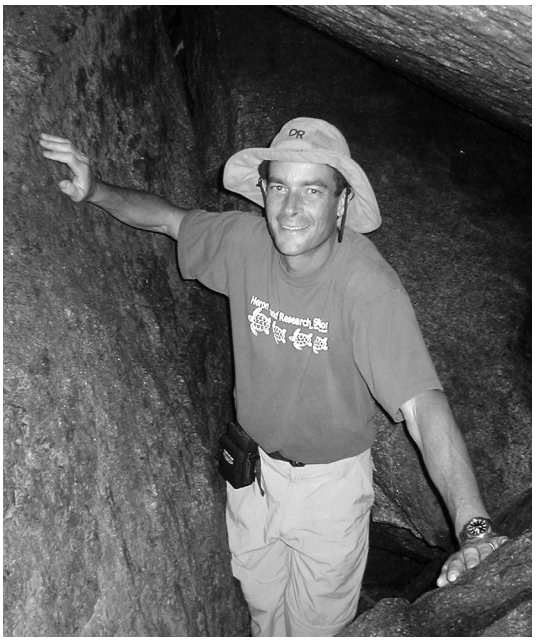
ABOUT THE AUTHOR

Gaines Tyler took a long and interesting road en route to receiving his Ph.D. in parasitology in 2001 from the Department of Ecology and Evolutionary Biology at the University of Connecticut. Prior to entering college in 1991, Gaines had been a nationally certified master automobile technician, a bartender, carpenter, and laborer. Desiring more from life than greasy hands and backaches, he entered college intent on a career in the biological sciences, perhaps as an environmental consultant to big businesses. By the end of his first semester, it became clear that organismal biology in general, and biodiversity in particular, were his main interests. By the end of his second semester, Gaines learned that his true love was teaching.

Gaines was first exposed to the world of parasitology by taking Janine Caira's parasitology course in 1994. Shortly thereafter, he entered the Honor's Program at UConn, and began doing research on *Echinobothrium* from the Gulf of California. Upon graduation *Summa Cum Laude* in 1996, Gaines joined a Caira led expedition of parasitologists from several institutions on a return trip to the Gulf of California. It was there that his skills as an auto mechanic were resharpened, as the rough terrain of Baja California proved too much for the team's vehicles.

After receiving his Ph.D. in 2001, Gaines was awarded an International Research Fellowship grant from NSF to study tapeworm lifecycles with Tom Cribb at the University of Queensland using a combined molecular and morphological approach. His notoriety followed him there as he learned some surprising facts about the bite force exerted by the porcupine fish *Diodon hystrix*.

Upon his return to the states, Gaines completed another postdoctoral appointment with Janine Caira before finally landing a teaching position at The Williams School in New London Connecticut, where he teaches biology and environmental science. Gaines' research interests have expanded to include ecosystem ecology, especially the role of parasites and insects in conservation biology. He currently resides in Mystic, Connecticut with his wife Melinda and two of his three children.



Gaines Tyler at Lost River Gorge, New Hampshire, 2004. Photo by Melinda Tyler.

Scanning electron micrograph of *Echinobothrium* sp.

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