The Biology of Echinostomes

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Editors

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From the Molecule to the Community

Springer

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Preface

Echinostomes are parasitic intestinal trematodes that infect a wide range of verte-brate host species, including humans, in their adult stage, and also parasitize numer-ous invertebrate and cold-blooded vertebrate hosts in their larval stages. Echinostomes have been studied for many years in relation to their role as basic research models in biodiversity and the systematics of helminths, particularly since the systematics of echinostomes are problematic because of interspecific homogeneity of character-istics to distinguish species. Apart from these aspects, echinostomes have been used extensively as experimental models in parasitology. In a pragmatic sense, echinos-tomes offer many advantages in experimental parasitology, i.e., easy maintenance of their life cycles in the laboratory, wide distribution of larval and adult stages, and a broad spectrum of intermediate and definitive hosts. For these reasons, echinostomes have contributed significantly to numerous developments in many areas studied by parasitologists and experimental biologists.

In this context, the application of novel techniques is moving the echinostomes into the frontline of parasitology in several areas. In recent years, a number of find-ings of importance for present and future developments in parasitology have been made using echinostomes as experimental models. Of particular interest are advances in fields such as immunobiology of snails and rodent hosts against parasitic infections, effects of echinostomes and echinostome-like trematodes on natural populations of amphibians, metabolic profiling of trematodes, assessment of anthelminthic drugs, and proteomic studies of intestinal helminth infections. Extensive coverage of the aforementioned topics is included in this book.

A book which presents echinostomes as experimental models should be well received by research workers and advanced students. This book reviews the recent literature on echinostomes, mainly from 1998 to 2007, supplementing the literature covered in the coedited book by B. Fried and T.K. Graczyk in 2000 on Echinostomes as Experimental Models for Biological Research (Kluwer, Dordrecht). In this new book several chapters cover the research and literature on such echinostome-like trematodes as Ribeiroia spp. Emphasis is placed on recent advances and gaps in knowledge that must be filled to determine the importance of this group of digene-ans as experimental models. This is critical to gain a full understanding of the potential role of echinostomes in the field of experimental parasitology.

The list of chapters includes some basic subject matter as well as some new topics. All chapters are covered from a modern point of view, considering matters such as the applications of novel techniques and analysis of data in the context of host-parasite interactions. In summary, the main goal of this book is to present the echinostomes in the context of modern parasitology and to show applications of new methodologies and concepts to a group of trematodes that may be useful to obtain information of great value in both parasitology and general biology.

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Chapter 1
Echinostomes: Systematics and Life Cycles

José Guillermo Esteban and Carla Muñoz-Antoli

Contents

Abstract This chapter provides a review of the most significant literature in the last decade on the systematics and biology of echinostomes and echinostome-like digeneans. This review is primarily concerned with members of the genus *Echinostoma*, although members of other genera (*Echinoparyphium, Echinochasmus, Himasthla, Hypoderaeum, Petasiger, Euparyphium, Stephanoprora, Isthmiophora,* and *Acanthoparyphium*) and echinostome-related genera (*Parorchis, Philophthalmus* and *Ribeiroia*) are also considered. The literature on molecular systematics and morphometrics of these trematodes is reviewed. Specific mention is made of the life cycle patterns of echinostome and echinostome-like digeneans along with an overview of recent advances on different topics in the biology of these trematodes; the review covers various aspects of the different stages of these organisms, i.e., free-living stages (miracidia and cercariae), and parasitic stages in the invertebrate hosts (sporocysts, rediae, and metacercariae) and vertebrate hosts (adults).

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1.1 Echinostomes: Systematics and Life Cycles

1.1.1 Introduction

Echinostomes are intestinal trematodes with an extensive literature dealing with them. In the last decade, three major reviews (Fried 2001; Fried and Graczyk 2004; Toledo et al. 2006), and the edited book by Fried and Graczyk (2000), provided ample evidence of the importance of these digeneans. Furthermore, the use of echinostomes as experimental models in host-parasite relationships between adult parasites and vertebrate hosts, and between larval trematodes and invertebrate and cold-blooded vertebrate hosts, has been highlighted by Toledo and Fried (2005) and Toledo et al. (2007), respectively.

In order to conduct studies in different areas of research, such as systematics, experimental, manipulative, ecological, physiological, biochemical, immunological, molecular, treatment, and control, two important features are needed: knowledge of which echinostome species are involved and the ability to maintain life cycles to do research on all echinostome stages, i.e., free-living, intramolluskan, and the adult stages.

Relative to these topics, this chapter provides an overview of the most relevant literature on the systematics and biology of echinostomes and echinostome-like digeneans. This review is mainly focused on the most significant literature of the last decade, although other previous papers are mentioned if needed; this review is mainly concerned with members of the genus *Echinostoma* because most research has focused on them; however, members of the other genera of the Echinostomatidae are also mentioned, including the echinostome-like digeneans.

1.1.2 Systematic Studies

Since the previous book edited by Fried and Graczyk (2000), some new papers and significant exhaustive revisions have appeared relative to the echinostomes. Of these, Cribb et al. (2001), and particularly Kostadinova and Jones (2005) and Kostadinova (2005a) in the recent volume 2 of the "Keys to the Trematoda," have defined the current classification of the Echinostomatoidea to the generic level. Among the eight families recognized by Kostadinova and Jones (2005), Echinostomatidae is characterized by the presence of a circumoral head-collar armed with one or two crowns of large spines interrupted ventrally; theses spines are larger than the tegumental spines (Fig. 1.1).

At the generic level, relatively few changes have been made, mainly in relation to the genus *Singhia*, reallocated to Echinostomatinae (see Table 1.1), whereas at the specific level, two new 37-collar spined species of *Echinostoma* belonging to the "*revolutum*" group have been described in the last decade. *E. friedi* was described from Spanish material on the basis of several morphological and biological

Fig. 1.1 Scanning electron micrograph of the anterior third region of an 11-day-old adult of *Euparyphium albuferensis* recovered from the intestine of an experimentally infected albino mouse. Scale bar: 100 μm

features of the life cycle stages and in its cercarial chaetotaxy (Toledo et al. 2000). *E. deserticum* was described from African materials (Kechemir et al. 2002). *E. luis-reyi* has been described from Brazilian materials on the basis of the oral corner spines that increased in size from the latero-oral to the ventro-oral regions, as well as the excretory pore radially wrinkled and dorsally subterminal (Maldonado et al. 2003).

The remaining published studies refer to descriptions of new species belonging to other genera of Echinostomatidae, and to refined diagnoses and redescriptions of both type specimens and/or newly collected materials, and critical evaluation of the published data belonging to different species of genera, i.e., *Stephanoprora* (Ostrowski de Nuñez and Quintana 2007) and *Uroproctepisthmium* (Kostadinova and Gibson 2001a) (Echinochasminae), *Drepanocephalus* (Kostadinova et al. 2002), *Echinoparyphium* (Kostadinova et al. 2003), *Echinostoma* (Kostadinova et al. 2000a, b, 2003; Maldonado et al. 2001a), *Euparyphium* (Kostadinova and Gibson 2002; Kostadinova et al. 2003), *Hypoderaeum* (Kostadinova et al. 2003), *Isthmiophora* (Kostadinova and Gibson 2002; Kostadinova et al. 2003), *Paryphostomum* (Kostadinova et al. 2002), *Petasiger* (King and Van As 2000;

Table 1.1 Type species, genera, and subfamilies included in the last Echinostomatidae classification according to Kostadinova (2005a)

Subfamily	Genus	Type species
Chaunocephalinae	*Chaunocephalus* Dietz, 1909	*C. ferox* (Rudolphi, 1795) Dietz, 1909
	Balfouria Leiper, 1908	*B. monogama* Leiper, 1908
Echinochasminae	*Dissurus* Verma, 1936	*D. farukhabadi* Verma, 1936
	Echinochasmus Dietz, 1909	*E. coaxatus* Dietz, 1909
	Mehrastomum Saksena, 1959	*M. minutum* Saksena, 1959
	Microparyphium Dietz, 1909	*M. facetum* Dietz, 1909
	Pulchrosomoides Freitas et Lent, 1937	*P. elegans* Freitas et Lent, 1937
	Stephanoprora Odhner, 1902	*S. ornata* Odhner, 1902/ *S. spinulosa* Dietz, 1909
	Saakotrema Skrjabin et Bashkirova, 1956	*S. metatestis* (Saakova, 1952)
	Uroproctepisthmium Fischtal et Kuntz, 1976	*U. taiwanense* Fischtal et Kuntz, 1976
Echinostomatinae	*Bashkirovitrema* Skrjabin, 1944	*B. incrassatum* (Diesing, 1850)
	Drepanocephalus Dietz, 1909	*D. spathans* Dietz, 1909
	Echinodollfusia Skrjabin et Bashkirova, 1956	*E. stenon* (Dollfus, 1950)
	Echinoparyphium Dietz, 1909	*E. elegans* (Looss, 1899)
	Echinostoma Rudolphi, 1809	*E. revolutum* (Frölich, 1802)
	Euparyphium Dietz, 1909	*E. capitaneum* Dietz, 1909
	Hypoderaeum Dietz, 1909	*H. conoideum* (Bloch, 1782)
	Isthmiophora Lühe, 1909	*I. melis* (Schrank, 1788)
	Longicollia Bykhovskaya-Pavlovskaya, 1954	*L. echinata* Bykhovskaya-Pavlovskaya, 1954
	Lyperorchis Travassos, 1921	*L. lyperorchis* Travassos, 1921
	Moliniella Hübner, 1939	*M. anceps* (Molin, 1859)
	Neocanthoparyphium Yamaguti, 1958	*N. petrowi* (Nevostrueva, 1953)
	Pameileenia Wright et Smithers, 1956	*P. gambiensis* Wright et Smithers, 1956
	Parallelotestis Belopol'skaya, 1954	*P. horridus* Belopol'skaya, 1954
	Paryphostomum Dietz, 1909	*P. radiatum* (Dujardin, 1845)
	Petasiger Dietz, 1909	*P. exaeretus* Dietz, 1909
	Prionosoma Dietz, 1909	*P. serratum* (Diesing, 1850)
	Prionosomoides Freitas et Dobbin, 1967	*P. scalaris* Freitas et Dobbin, 1967
	Singhia Yamaguti, 1958	*S. thapari* (Singh, 1953)
Himasthlinae	*Acanthoparyphium* Dietz, 1909	*A. phoenicopteri* (Lühe, 1898)
	Aporchis Stossich, 1905	*A. croaticus* (Stossich, 1905)
	Artyfechinostomum Lane, 1915	*A. sufrartyfex* Lane, 1915
	Caballerotrema Prudhoe, 1960	*C. brasiliense* Prudhoe, 1960
	Cloeophora Dietz, 1909	*C. micata* Dietz, 1909
	Curtuteria Reimer, 1963	*C. numenii* Reimer, 1963
	Hismasthla Dietz, 1909	*H. rhigedana* Dietz, 1909
Ignaviinae	*Ignavia* Freitas, 1948	*I. venusta* Freitas, 1948
Neophrostominae	*Nephrostomum* Dietz, 1909	*N. ramosum* (Sonsino, 1895)

(continued)

Table 1.1 (continued)

Subfamily	Genus	Type species
	Patagifer Dietz, 1909	*P. bilobus* (Rudolphi, 1819)
Pegosominae	*Pegosomum* Ratz, 1903	*P. saginatum* (Ratz, 1897)
Pelmatostominae	*Pelmatostomum* Dietz, 1909	*P. episemum* Dietz, 1909
Ruffetrematinae	*Ruffetrema* Saxena et Singh, 1982	*R. indirae* Saxena et Singh, 1982
Sodalinae	*Sodalis* Kowalewski, 1902	*S. spathulatus* (Rudolphi, 1819)

Zamparo et al. 2005; Kostadinova and Skirnisson 2007), and *Singhia* (Kostadinova and Gibson 2001b) (Echinostomatinae), and *Acanthoparyphium, Caballerotrema, Curtuteria* and *Himasthla* (Himasthlinae) (Kostadinova and Gibson 2001b; Díaz and Cremonte 2004; Desclaux et al. 2006; Martorelli et al. 2006).

The echinostome-like trematode group has had a confusing and controversial taxonomic history. Numerous authors have commented on the close relationship between this group, basically the genus *Ribeiroia*, and echinostomatids based on morphological ("wish-bone" type of intestine with solid esophagus and cecae; excretory bladder shape, collecting tubule pattern and concretion character and amount; and flame cell pattern) and ecological (infecting amphibians, fishes, birds, and mammals) similarities. However, the genus *Ribeiroia* appears to be more species specific at the level of the first intermediate host and is not known to form metacercariae within snails (Johnson et al. 2004). In addition, *Ribeiroia* lacks a typical echinostome collar and circumoral spines, and possesses characteristic esophageal cecae or diverticula. All these features have allowed Kostadinova (2005b) to consider the genus *Ribeiroia*, and 12 other genera within the family Psilostomidae. Other genera also considered echinostome-like digeneans, such as *Parorchis* and *Philophthalmus*, are included within the family Philophthalmidae (Kanev et al. 2005).

1.1.2.1 Recent Information on Molecular Systematics and Morphometrics

The echinostomes constitute a group of digeneans characterized by great confusion regarding their systematic classification. This is attributable to a number of factors, including misidentified species or species that have been insufficiently described, as well as the existence of substantial interspecific homogeneity of the morphological characteristics of the adult stage. Such confusion is evidenced not only in the 37-collar-spined *Echinostoma* belonging to the "*revolutum*" group, but also in members of 43- and 45- collar-spined *Echinoparyphium* (*E. elegans* complex and *E. recurvatum* complex, respectively). However, the studies published in the last decade, and basically those of Kostadinova and coauthors, have contributed to further our understanding of the systematics of the echinostomes.

Kostadinova and Gibson (2000), in their exhaustive and rigorous review, contributed an update to our knowledge on the systematics of echinostomes, particularly in relation to the 37-collar-spined "*revolutum*" group of *Echinostoma*. These authors studied morphologically some of the materials used by different workers in their descriptions and analyzed the causes of the errors made in many of the diagnoses. In addition, they established the characteristic features of the species-rich genera (*Echinochasmus*, *Echinoparyphium*, *Echinostoma*, *Himasthla*, and *Stephanoprora*) and the features of the species recognized to date within the "*revolutum*" group: *E. revolutum* Frölich 1802, *E. echinatum* (Zeder 1803), *E. jurini* (Skvortsov 1924), *E. caproni* Richard 1964, *E. trivolvis* (Cort 1914), *E. paraensei* Lie et Basch 1967, *E. parvocirrus* Nassi et Dupouy 1988, *E. miyagawai* Ishii 1932, and *E. friedi* Toledo, Muñoz-Antolí et Esteban 2000.

Of relevance is the exhaustive analysis made to date by Kostadinova and Gibson (2000) on the main features used for differentiation at the specific level in the "*revolutum*" species group, that is: number and disribution of the paraesophageal gland-cell outlets in cercariae; patterns of the distribution of sensilla in cercariae; adult morphology; final host-parasite compatibility; behavior related to distribution of adults along the intestine of definitive host; mating behavior; geographical distribution; and finally genetic characteristics, related to karyotyping or chromosome counting and comparison, isoelectric focusing, and enzyme electrophoresis.

These criteria have been the subject of a recent critical comment by Fried and Toledo (2004), who point out that these characters considered individually are of limited value overall when trying to identify the 37-collar-spined *Echinostoma* group, and suggest that for the diagnosis and description of new species integrated and comparative studies involving adequate morphological and biological features are necessary.

However, morphological and biological studies are complicated *a priori*, since in many cases they imply the development and maintenance of the full life cycle, as well as the description of each of the different parasite stages in the cycle.

As an alternative to the classical morphological studies, molecular tools, especially primary sequence comparisons, are now being used for life cycle elucidation, the examination of potential cryptic species, the examination of species complexes and their phylogeographical genetic structure, and phylogenetic studies (Nolan and Cribb 2005; Vilas et al. 2005). Logically, the molecular data have the capacity to allow comparisons that remove confounding factors of age, host or geographically based variation; and the nature of molecular techniques means that it is possible to bypass studies of the biology of parasites.

However, relatively few molecular-based studies on echinostomes have been carried out to date. Earlier studies involved molecular techniques such as karyotyping and enzyme electrophoresis. More recent studies are based on the analysis of different DNA sequences and their utility for the species differentiation, to analyze the echinostome phylogenetics, and for the diagnosis of the echinostomes.

The random amplification of polymorphic DNA (RAPD) using polymerase chain reactions (PCR) is useful for identifying closely related echinostome species, i.e., *E. trivolvis* and *E. caproni* (Fujino et al. 1995), *E. caproni* and *E. paraensei*

(Petrie et al. 1996), *and Echinochasmus fujianensis* (conspecific to *E. liliputanus*) *and E. japonicus* (Cheng et al. 1999).

Sequence data from both mitochondrial DNA (mtDNA), nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) and cytochrome c oxidase subunit 1 (CO1), and nuclear ribosomal DNA (rDNA), internal transcribed spacers 1 and 2 (ITS1 and ITS2), have also been used as markers for identifying species of echinostomes and several strains of echinostomes isolated from different geographical locations, particularly for the four most studied species in the 37-collar-spined "*revolutum*" group, i.e., *E. caproni*, *E. paraensei*, *E. revolutum*, and *E. trivolvis* (Sorensen et al. 1998; Morgan and Blair 1995, 1998a,b, 2000; Maldonado et al. 2001a; Kostadinova et al. 2003).

Molecular studies on genera other than *Echinostoma*, or those on echinostome-like genera, are scant. Grabda-Kazubska et al. (1998) sequenced ITS1 from rediae and cercariae to distinguish *Echinoparyphium elegans, E. recurvatum, E. pseudor-ecurvatum, Pseudechinoparyphium echinatum, Neocanthoparyphium echinatoides*, and *Hypoderaeum conoideum.* These authors suggested a close relationship between *H. conoideum* and the three species of *Echinoparyphium*.

Special mention should be made of the results obtained by Kostadinova et al. (2003). These authors sequenced the ND1 and ITS genes of six species in the genera *Echinostoma* (including *E. revolutum*), *Echinoparyphium, Hypoderaeum and Isthmiophora,* and concluded that ND1 sequence provided useful information for the construction of a phylogenetic tree, which agrees with morphological identifi-cations, and that the ITS gene provided insufficient resolution for distinguishing echinosome species. In addition, these authors reviewed the morphology of selected individuals and demonstrated congruence between morphological and molecular identification of the species examined.

In relation to the echinostome-like group, i.e., *Ribeiroia*, studies on ITS2 sequences have allowed for the recognition of three species within this genus: *R. ondatrae*, *R. congolensis*, and *R. marini* (Wilson et al. 2005). These results are in accord with the previous morphological studies and systematic classification of Yamaguti (1971, 1975). However, Wilson et al. (2005) noted that further molecular studies will be needed to elucidate the correct classification of *Ribeiroia* spp. into either Psilostomidae or Cathaemasiidae.

However, molecular techniques for the identification of echinostomes also have problems. Probably, the most relevant of which are questions related to materials used for these studies. The molecular studies require the availability of materials morphologically referred to a genus and species, in order to allow for adequate sequencing and an introduction of the data in the GenBank. It is therefore clear that the deposited type sequence of each echinostome species should be obtained from the "voucher" of type material, or alternatively from materials corresponding to the type locality ("terra typica"). This would allow comparative analysis of the results of the sequences of materials presumably based on morphological grounds to the same or other species, and from the same or different geographical origins. The rigorous retention of morphological vouchers for all sequenced samples, and ide-ally their placement in museums, would be the most correct approach.

However, some type specimens cannot be located, are inaccessible, or are in too poor a state of preservation to allow for detailed morphological examination. In this case, efforts must be made to obtain materials from the type locality, or alternatively, the material must be described morphologically before applying molecular studies since several problems may occur. It should possibly be considered that there exists more than one species in a sample and/or that two different samples represent the same species as a consequence of a misidentification error, mislabeling, or contamination. These aspects have been addressed by Kostadinova and coworkers, who observed that even the type material vouchers deposited in museums may in fact contain a pool of different species, rather than a single species only (Kostadinova and Gibson 2000; Kostadinova et al. 2000a,b, 2003).

Molecular studies are not usually accompanied by morphological studies to confirm the identity of the material. This represents an added problem, making it difficult to do comparative studies in situations where the application of molecular techniques may not be feasible.

Overall, molecular studies for species differentiation have not yielded results truly different from those already known through traditional morphological research – when the latter research has been exhaustive and rigorous. Moreover, in those cases where there is no agreement between schemes of classification based on morphology and those based on molecular techniques there are problems fundamentally related to voucher of type material and/or the incorrect identification of samples used.

Although the new technologies tend to displace the traditional systematic methods, the most reasonable approach in view of the aspects commented is that both systematic studies, i.e., based on morphological and molecular data, should be regarded as complementary and should coexist.

1.1.3 An Overview and Recent Advances in the Biology of Echinostomes and Echinostome-Like Digeneans

In the present section, information published from 2000 to date will be considered. Most of this information is concerned with four *Echinostoma* species of the "*revolutum*" group, i.e., *E. caproni*, *E. paraensei*, *E. trivolvis*, and *E. friedi*. For other *Echinostoma* species (*E. revolutum, E. miyagawai, and E. luisreyi*), other echinostomatid genera (*Echinoparyphium, Echinochasmus, Himasthla, Hypoderaeum, Petasiger, Euparyphium, Stephanoprora, Isthmiophora,* and *Acanthoparyphium*) or echinostome-related genera, such as *Parorchis, Philophthalmus*, and mainly *Ribeiroia*, the literature is not extensive.

The most relevant reviews on the biology of echinostomes and echinostome-like digeneans are as follow:

Huffman and Fried (1990) reviewed the significant earlier literature on *Echinostoma* and echinostomiasis to about 1988 and covered, among others, different aspects related with the biology of 37-collar-spined echinostomes, basically *on E. caproni*,

E. trivolvis, *E. paraensei*, *E. revolutum*, and *E. echinatum*, as well as the infectivity studies in first and second intermediate hosts, and the infectivity, growth, and development in vertebrate hosts. Later on, Fried and Huffman (1996) published an exhaustive monographic review of all the citations relating to *E. caproni* up to that date.

The review of Kanev et al. (2000), in the edited book by Fried and Graczyk (2000), compiled the echinostome citations from 1970 to 1998. Moreover, the most significant contributions to the biology of echinostomes from the eighteenth to the twentieth century are listed, and specific mention is made on the life cycle pattern of echinostomes and on the form and function for each of the different echinostome stages: egg, miracidium, sporocyst, redia, cercaria, metacercaria, and adult. The most recent reviews of Fried (2001) and Fried and Graczyk (2004) have served as a background for this overview of biology of echinostomes.

Among the echinostome-like digeneans, the genus *Ribeiroia* has the largest number of citations, because it can cause severe pathology and mortality in snails and amphibian intermediate hosts. The recent revision of Johnson et al. (2004) also serves as a compilation for this genus. For *Philophthalmus* and philophthalmiasis, the reviews of Nollen and Kanev (1995) and Nollen (1997) serve as an overview of this genus. Moreover, Graczyk and Fried (2007) discussed the transmission of human and animal philophthalmids by contaminated water.

1.1.3.1 Life Cycles

Members of echinostome and echinostome-related genera follow a three-host life cycle: a vertebrate definitive host, an invertebrate first intermediate host (usually an aquatic gastropod mollusk), and a second intermediate host carrying the encysted metacercarial stage. The life cycle consists of six phases (Fig. 1.2):

1. Passage of eggs from the definitive host to the outside environment and their subsequent development;
2. Hatching of miracidia, and search for and penetration of the first intermediate snail host;
3. Development and multiplication of the parasites inside the snail;
4. Emergence of the cercariae from the snails and search for the second intermediate host;
5. Penetration and encystment of the cercariae in the second intermediate host;
6. And, finally, ingestion of infective metacercariae by the final hosts and development to adult worms.

Some differences in relation to this general life cycle pattern may be observed. For example, fully developed eggs of *E. caproni* may hatch in the snail gut (Idris and Fried 1996), or even lymnaeid snails may become infected by ingestment of unhatched embryonated *E. revolutum* eggs (Davis 2005). The cercariae of *Himasthla quissetensis* infect directly domestic chicks through the cloaca, with worm development occurring in the ileum (Herman and Bacha 1978). Cercariae of *Echinochasmus* spp. infect humans and other mammals when they are ingested by
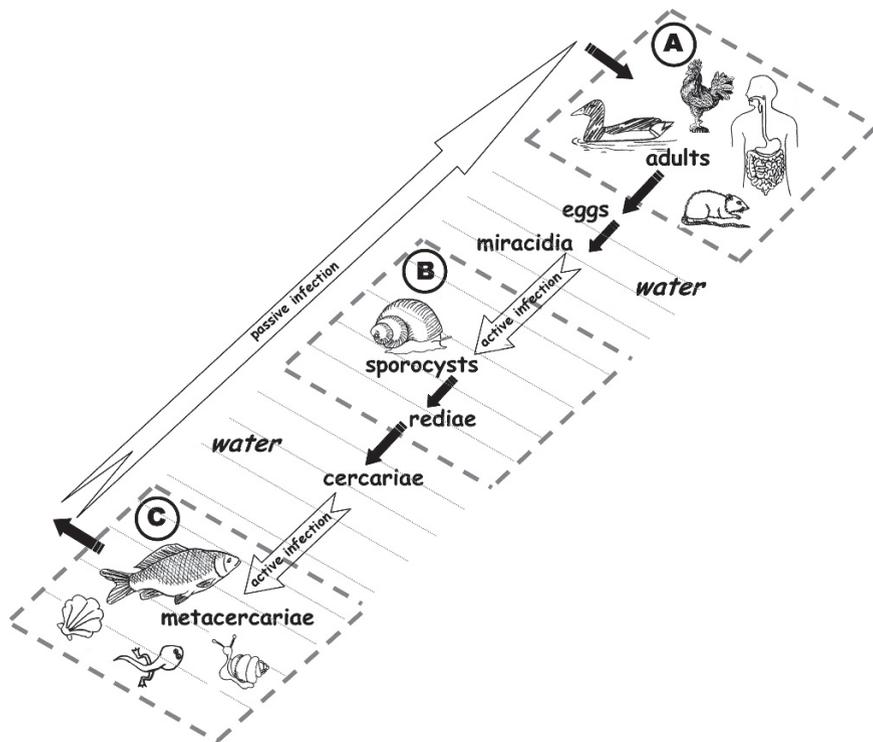
Fig. 1.2 Generalized life cycle of an echinostome. (**a**) definitive hosts; (**b**) first intermediate hosts; (**c**): second intermediate hosts

the host in contaminated water (Xiao et al. 1992, 1995). Cercariae of *Parorchis acanthus*, an echinostome-like species, emerge from infected dogwhelks (*Nucella lapillus*) and after a brief period of free swimming, they encyst on solid objects in the nearby locality (Rees 1966).

In the period reviewed, some studies have involved the description of life cycles: *E. friedi* in Spain, *E. miyagawai* in Bulgaria, *E. deserticum* in Niger, and *E. luisreyi* in Brazil (Toledo et al. 2000; Kostadinova et al. 2000b; Kechemir et al. 2002; Maldonado et al. 2003); *Echinoparyphium recurvatum* in Korea (Sohn 1998), and *E. megacirrus* and *Echinoparyphium* sp. in Argentina (Semenas et al. 1999; Prepelitchi and Ostrowski de Nuñez 2007); *Hypoderaeum conoideum* in Spain (Muñoz-Antolí et al. 2000); and *Petasiger variospinosus* in South Africa (King and Van As 2000), *P. combesi* in Costa Rica (Zamparo et al. 2005), and *P. islandicus* from Iceland (Kostadinova and Skirnisson 2007); *Stephanoprora uruguayense* and *S. aylacostoma* in Argentina (Ostrowski de Nuñez 2007; Ostrowski de Nuñez and Quintana 2007); *Acanthoparyphium tyosenense* in Korea (Chai et al. 2001; Kim et al. 2004) and *Himasthla escamosa* in Argentina (Diaz and Cremonte 2004); and *Philophthalmus lucipetus* and *P. distomatosa* in Israel (Radev et al. 1999, 2000).

Additional scant literature has covered reports of new first and second interme-diate hosts of some echinostome species, i.e., *E. revolutum* in Finland (Vayrynen et al. 2000); *E. cinetorchis* in Korea (Chung et al. 2001a, b; Park et al. 2006), and *Curtuteria australis* in New Zealand (McFarland et al. 2003)

1.1.3.2 Free-Living Stages

Miracidia and cercariae are the two free-living stages in the life cycle of the echinostomes.

Miracidium

The echinostome egg is released by a definitive host in an aquatic environment. The egg is undeveloped when laid and consists of a fertilized ovum surrounded by yolk granules. It is typically oval in shape, variable in size and yellow, dark brown, or silver-white in color. It has an operculum at one end and a distinct knob at the abopercular end (Fig. 1.3a, b).

Fujino et al. (2000) provided a comparative study of ultrastructure of eggs in *E. paraensei*, *E. caproni*, and *E. trivolvis* using light microscopy, scanning electron microscopy, and transmission electron microscopy. Differences among these spe-cies are observed in relation to size and the aboperculum region, but no difference in eggshell structure was detected in any species.

The egg takes about 10–21 days at 22–28°C, depending on the echinostome spe-cies, to reach the fully developed miracidial stage. Several physicochemical factors influence embryonation, and the eggs can be maintained for at least 5 months at 4°C and still retain their ability to develop and hatch.

The miracidium is about 100 μm in length, broad anteriorly, and tapering poste-riorly to a blunt end. The tegument is ciliated, and possesses a variable number of the ciliated epidermal plates and argentophilic papillae with a special arrangement. Retractile apical papillae followed by a pyriform gland have been described. Two dark-brown eye-spots, consisting of two pairs of pigmented bodies are located side by side, and the excretory system consists of two flame cells, two excretory ducts, and two excretory pores (Fig. 1.3c).

Pinheiro et al. (2004a) described the morphology and topography of *E. paraensei* miracidium by light and electron microscopy. The 19 papilla-like structures are arranged in three axes and four groups are observed at the terebratorium of this miracidium, which differs from the number of argentophilic papilla-like structures observed in *E. caproni*, *E. jurini* and *E. trivolvis*. The ultrastructural organization of the *E. paraensei* miracidium has been studied by Pinheiro et al. (2005) using transmission electron microscopy.

Among the large cells located in the posterior of *E. caproni* and *E. paraensei* miracidia are secretory cells, germinal cells, and undifferentiated cells. Secretory cells do not give rise to progeny, whereas germinal cells do. Undifferentiated cells
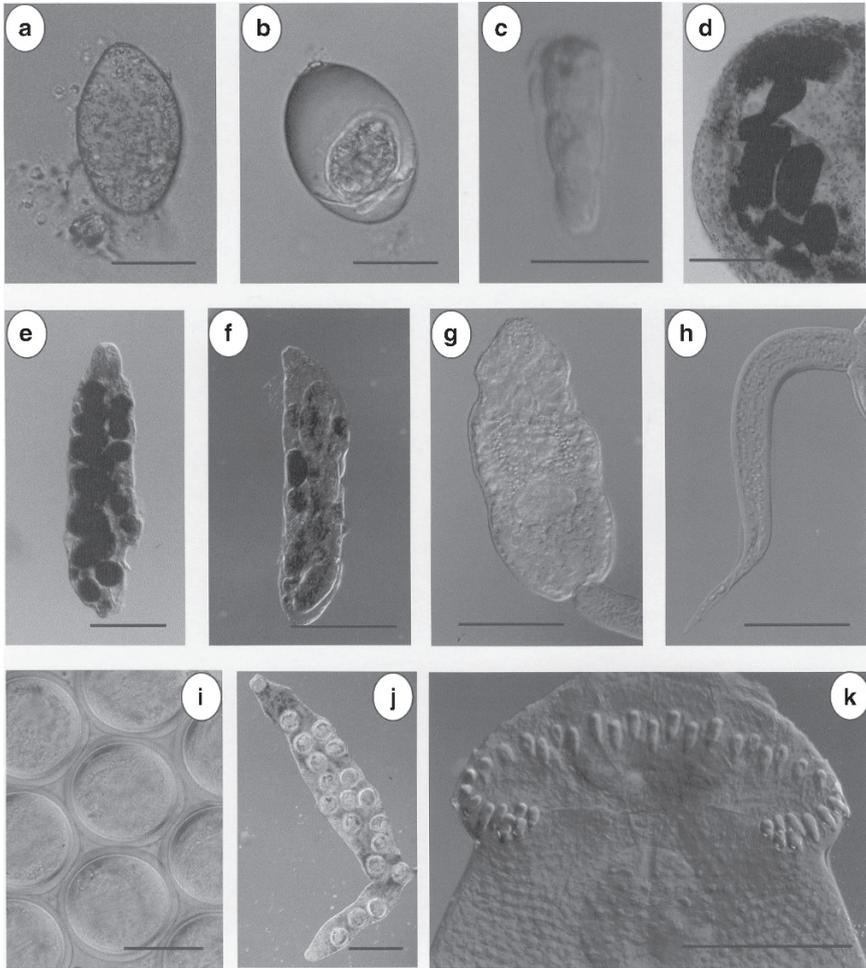
Fig. 1.3 Parasitic stages of the life cycle of *Echinostoma friedi*: (**a**) undeveloped egg; (**b**) embry-onated egg; (**c**) miracidium recently hatched from an egg; (**d**) sporocyst in ventricle of snail; (**e**) first generation redia; (**f**) second generation redia; (**g**) cercarial body; (**h**) cercarial tail; (**i**) metacercariae; (**j**) redia containing encysted metacercariae; (**k**) anterior third of an adult. A, B, C, G, H, I, and J: fresh-living eggs, miracidia, cercaria, metacercariae, and metacercariae encysted in redia observed between slide and cover-slip; D, E, and F: fixed in Bouin's fluid under coverslip pressure, stained with Grenacher's borax carmine, mounted in Canada balsam, and observed with interference contrast microscopy; K: head collar region of a fresh-living adult observed between a slide and cover-slip with lactophenol fluid by interference contrast microscopy. Scale bars: A, B, and C: 50 μm; D, G, H, I, and J: 100 μm; E: 200 μm; F: 300 μm; K: 150 μm

develop into germinal cells that can also divide to produce embryos. Differences into the germinal cells of miracidia help to explain differences detected in the next of the intramolluskan echinostome stages (Ataev et al. 2001).

The behavioral patterns of echinostome miracidia have been reviewed by Haas (2000). The hatching of miracidia from eggs is stimulated by different factors; light is the major stimulus, although the pattern is different according to species. Whereas *E. paraensei* and *E. caproni* miracidia hatch in a strict diurnal pattern between 11.00 and 16.00 h, *E. trivolvis* does not show a daily hatching pattern.

Development, hatching, and infectivity of *E. caproni* eggs were described by Idris and Fried (1996). No significant differences in development were seen in the eggs maintained under conditions of light or darkness. The eggs maintained in the dark for 10 days and exposed to incandescent light produced a large synchronous hatch of miracidia within 3 h of exposure to light.

Maldonado et al. (2001b) found that miracidia of *E. paraensei* hatched after 10 days of incubation in the dark, and began to hatch 1 h after exposure to incandescent light, although most of them hatched between 3 and 4 h of exposure.

The effect of snail-conditioned water from *B. glabrata* snails on hatching rates of *E. caproni* miracidia was studied by Fried and Reddy (1999). Significantly greater hatching was obtained when snails were maintained in intact or perforated dialysis sacs in multiwell chambers as compared with sacs without snails.

The effects of salinity, pH, and temperature on the half-life and longevity of *E. caproni* miracidia have been analyzed by Ford et al. (1998). Miracidia tolerated pH ranges from 3 to 11 showing half-lives of 2.4 h, or greater under these conditions. At lower than ambient temperatures, *E. caproni* miracidia lived longer, the greatest being a half-life of 5.0 and a maximum life span of 15 h at 5°C.

After hatching, the miracidia swim and respond to environmental stimuli, such as light and gravity. The miracidia of *E. caproni* and *E. trivolvis* showed a negative geotaxis which was dominated by a positive phototaxis; meanwhile, *E. paraensei* miracidia showed no distinct geo-orientation and only weak photo-orientation.

The miracidia also respond to stimuli emanating from the host. The main host-finding signals for *E. caproni* miracidia detected have been complex macromolecules from snail mucus (Haberl et al. 2000).

The presence of nonhost snails, in a given snail community, may interfere with the ability of miracidia to orientate, reach, and successfully penetrate their normal host snail. These effects on echinostome miracidial host-finding behavior are poorly understood. A radioisotope assay system was used for testing the host finding of *E. revolutum* miracidium (Christensen 1980); two studies examined the response of *E. caproni* (Behrens and Nollen 1991) and *E. trivolvis* (Nollen 1994) miracidia to chemicals; a study was done on the different suitabilities of two lymnaeid snails for *H. conoideum* miracidia (Toledo et al. 1999a); a complete study was done on the host-finding process in *E. caproni* miracidia and cercariae (Haberl et al. 2000); and a recent study was done on the interactions related to host, nonhost snails, and water-conditioned snail on the host-finding processes of the miracidia of *Euparyphium albuferensis* and *E. friedi*; both species share the same natural habitat (Esteban et al. 1997; Muñoz-Antoli et al. 2003).

Muñoz-Antoli et al. (2002) studied the survival and infectivity of *E. friedi* miracidia, which was determined to be age dependent. This finding was in accord

with the previous study on the survival of *H. conoideum* miracidia (Muñoz-Antolí et al. 2000). The maximum life span and the time to 50% mortality were determined to be 10.0 and 6.8 h, respectively. A gradual increase of infectivity in the few first hours after hatching was not observed, and the lack of a preinfective period in *E. friedi* miracidia was related to the low specificity of this echinostome species for its intermediate host (Muñoz-Antoli et al. 2002).

Toledo et al. (2004) found that the age of adult worms significantly influenced *E. friedi* miracidial infection in *Lymnaea peregra* snails. Infective miracidia only were obtained from adult worms in the age range from 4 to 9 weeks p.i., and the infectivity was maximal in those miracidia derived from adults collected 8 and 9 weeks p.i., which corresponds with maximum egg output. These authors suggested that adult worms producing viable eggs require additional maturation in order to yield eggs containing infective miracidia. However, these results differed from those obtained by Fried and Bandstra (2005) for *E. caproni* miracidia. These authors determined the percentage of fully developed miracidia in terms of eggs derived from adult worms obtained from laboratory mice at 2, 4, 6, and 8 wk postinfection, and obtained a percentage of fully developed miracidia >90% and 60–80% of the eggs hatched.

Although it was suggested that each species of echinostome infects only one or a few closely related snail species (Huffman and Fried 1990), more recent studies show marked differences in that the spectrum of first intermediate host species for each echinostome species may be broader than previously expected. Maldonado et al. (2001b) infected three sympatric snail species, belonging to the Planorbidae (*Biomphalaria glabrata*), Physidae (*Physa marmorata*), and Lymnaeidae (*Lymnaea columella*) families, with a Brazilian isolate of *E. paraensei*. Muñoz-Antolí et al. (2006) studied the infectivity of *E. friedi* miracidia in a range of sympatric and allopatric laboratory-reared snails, and the results showed that the miracidia were able to infect and develop in snail species belonging to three different families (Lymnaeidae, Planorbidae, and Bulinidae) and from different geographical origins, although the rates of infection were low for all these species.

In relation to echinostome-like digeneans, *Ribeiroia* is more species specific than echinostomes in its use of first intermediate hosts. The members of this genus use only 12 species of Planorbidae belonging to the genera *Biomphalaria, Helisoma*, and *Planorbella* (Johnson et al. 2004).

The effect of *E. friedi* miracidial infection on survival, growth, and fecundity of two susceptible first intermediate host snails, *Radix peregra* and *Biomphalaria glabrata*, has been studied by Muñoz-Antoli et al. (2007). Infected *B. glabrata* showed gigantism and had a shorter life span that *R. peregra*; infected *R. peregra* reduced normal development; *E. friedi* produced total parasitic castration of both infected snails species. These differences allowed the establishment of the level of host compatibility: *R. peregra* would be considered as the required snail host, and it is able to transmit the parasite and to maintain the parasite population without other snail hosts; *B. glabrata* would be considered only an adequate experimental snail host, being able to maintain the *E. friedi* life cycle in the absence of *R. peregra*.

Cercaria

The echinostome cercaria is typically distomate, gymnocephalus with an oral collar of spines, not always visible, and a simple tail (Fig. 1.3g, h). Differences in size among echinostome species are common. The main excretory ducts of the excretory system are simple and short, containing a large number of excretory granules. The number (from 10 to 100) and the arrangement (in pairs, by three or by four) of flame cells are of importance in the diagnosis echinostome cercariae. In addition to openings of penetration and cystogenous glands, some cercariae contain paraesophageal and esophageal glands. The number and distribution of tegumentary papillae, the so-called cercarial chaetotaxy patterns, has proved to be of taxonomic value for echinostomatid species (Toledo et al. 1998a, b, 2000; Kostadinova 1999; Nakano et al. 2003). The cercarial tail of most *Echinostoma* species has fin-fold structures, not described on the cercarial tail of other genera such as *Echinoparyphium, Euparyphium*, *Hypoderaeum*, and *Isthmiophora*. The general morphology of cercariae of different echinostome genera has been reviewed by Kanev et al. (2000).

The presence of two laterally projecting esophageal diverticula, located approximately halfway along the length of the esophagus, and the presence of a conspicuously rose-pink-colored structure located between the oral sucker and the pharynx, constitute two relevant morphological structures of the echinostome-like cercaria of *Ribeiroia* (Johnson et al. 2004).

Using indirect immunocytochemistry to demonstrate neuroactive substances and the phalloidin-fluorescence technique for staining myofibril F-actin, the muscle systems and aminergic and peptidergic innervation of *E. caproni* cercariae have been examined (Sebelová et al. 2004). Combined studies of high-performance thin-layer chromatography with lipid histochemistry of neutral lipids in cercariae of *E. caproni* showed the major lipid fraction to be free sterol, and this parasite stage had 6.5 times more free sterol than the encysted metacercaria (Marsit et al. 2000).

The free-swimming cercariae escape from their first intermediate host 4–6 weeks postinfection. The cercariae are short lived and rarely survive beyond 48 h at ambient temperatures.

Numerous papers have been published focusing on different aspects of echinostome cercariae. The movement patterns of cercariae (in *Echinoparyhphium, Himasthla, Pseudechinoparyphium, Echinostoma*, and *Hypoderaeum* species), the chemo-orientation of swimming cercariae (in *Echinostoma, Pseudechinoparyphium*, and *Hypoderaeum* species), and the cercarial attachment and host invasion (in *Echinostoma, Hypoderaeum, Pseudechinoparyphium*, and *Isthmiophora* species) have been extensively reviewed by Haas (2000).

McCarthy (1999a) studied phototactic responses of the cercariae of *E. recurvatum* from infected *L. peregra* snails and found that cercariae have an initially positively phototactic, low infectivity, dispersal phase followed by a negatively phototactic, maximally infective host location, and infection phase.

The effect of light and gravity on orientation of cercariae was studied in *Echinostoma, Hypoderaeum, Pseudechinoparyphium*, and *Isthmiophora* species (Loy et al. 2001). These authors demonstrated that each of the 4 species studied has

an individual behavior pattern of horizontal photo-orientation and geo-orientation, with distinct changes during the time after emergence. This diversity of behavioral responses corresponds with that of their chemo-orientation toward the intermediate hosts. *Hypoderaeum* approaches its host snails by direct chemotactic orientation along concentration gradients of snail-emitted peptides, whereas *Pseudechinoparyphium* and *Echinostoma* swim back when the concentration of snail-emitted amino acids, urea, and ammonia decreases, and *Isthmiophora* seems to show no chemo-orientation at all toward its amphibian and fish hosts. The geo-orientation was controlled differently in each species by the intensity and the direction of light radiation, and the different orientation patterns suggest functions such as leaving the habitats of the host snails emitting the cercariae, dispersal, and frequenting the microhabitats of potential hosts (Loy et al. 2001).

Temperature is a determinant factor in survival and longevity, and survival is shorter at higher temperatures (Evans 1985). Schmidt and Fried (1996) studied the emergence of *E. trivolvis* cercariae from naturally infected *H. trivolvis* snails. Of the numerous laboratory conditions tested, the only significant factors that had an impact on cercarial emergence were temperature related. Recently, Fried and Ponder (2003) observed that the effects of temperature on cercarial survival in artificial spring water and on infectivity of *E. caproni* are temperature dependent, and the infective life span of the cercariae is shorter than the total life span. Moreover, the temperature has also a major effect on in vitro encystment of the cercariae of *E. caproni*.

Schmidt et al. (1996) studied the effect of storage on the survival of the intramolluskan stages of *E. trivolvis* in *H. trivolvis* (Pennsylvania strain) snails. Twenty snails were stored at 4°C in artificial spring water for 10 months; three snails were alive at 10 months and released cercariae. The number of cercariae released and their infectivity to a Colorado strain of *H. trivolvis* snails was significantly lower than that of cercariae from freshly collected snails.

The survival and infectivity characteristics of echinostome cercariae have been the subject of several studies. McCarthy (1999b) studied the influence of temperature on the survival and infectivity of *E. recurvatum* cercariae; cercarial survival was temperature dependent with the maximum survival time being reduced from 68 h at 10°C to 12 h at 30°C. However, the survival and infectivity of *E. friedi* cercariae was found to be markedly age dependent (Muñoz-Antoli et al. 2002). The maximum life span at 20°C and time to 50% mortality was determined at 28.00 and 23.5 h, respectively. These results are consistent with those observed for other sympatric echinostome species, such as *H. conoideum* and *E. albuferensis* (Toledo et al. 1999b), but the times observed in the latest two species were significantly shorter than those reported for other echinostome cercariae such as *E. caproni, E. trivolvis*, and *E. recurvatum* for which the time to 50% mortality ranged from 29 to 31 h at similar temperatures. Moreover, the cercarial infectivity of *E. friedi* gradually increased during the first few hours, and reached a peak after a prior period of aging. The existence of this preinfective period may represent a dispersal phase that aids cercarial dissemination, thus reducing superinfection and parasite associated mortality of the first intermediate host (Muñoz-Antoli et al. 2002).

The emergence pattern under different conditions has been analyzed in the *E. trivolvis-H. trivolvis*, *E. recurvatum-L. peregra*, and *E. albuferensis-G.chinensis* systems. Schmidt and Fried (1996) described the number of *E. trivolvis* cercariae released during 1 h under different conditions, whereas McCarthy (1999c) studied the photoperiodic cercarial emergence of *E. recurvatum* from *L. peregra*. Cercariae emerged during the light phase of the experiment from 08.00 to 20.00 h. Toledo et al. (1999d) studied the production and chronobiology of the emergence of cercariae of *E. albuferensis* from *G. chinensis* experimentally infected with a single miracidium. These authors noted that although the daily cercarial shedding rates were very variable, a progressive increase in cercarial production was observed in the first several weeks of cercarial shedding. Under a 12-h light-dark cycle, the cercariae emerged in the light and the rhythm was circadian. A sudden change in the light-dark cycle resulted in corresponding changes in the emergence patterns, showing that cercarial emergence in this species is correlated to light-dark changes.

Nevertheless, in the echinostome-like *Ribeiroia*, the emergence is rhythmic under continuous darkness. The cercariae emerge at night, usually between 7 PM and 3 AM, and may swim actively for at least 12 h or more. Infected snails typically release around 300–400 cercariae per night for 3–7 months (Théron and Moné 1986), though some individuals produced more than 1,000 cercariae per night. However, significant diurnal emergence of cercariae was also observed (Fried pers. com. to Johnson).

Maldonado et al. (2001b) studied the kinetics of cercarial emergence for the sympatric snails infected with cercariae of a Brazilian isolate of *Echinostoma paraensei*. Differences in relation to the miracidial doses (one vs five miracidia) were detected, and the length of the prepatent period differed significantly between snail species. Fried, LaTerra and Kim (2002) examined various physicochemical factors related to optimal release of *E. caproni* from experimentally *B. glabrata* snails. Among the numerous conditions tested, e.g., the addition of lettuce, the use of snail-conditioned water from *B. glabrata*, and a high temperature (35°C) significantly increased the shedding of *E. caproni* cercariae.

Snail size is another relevant factor in determining the mortality associated with echinostome cercarial penetration and encystment in the second intermediate host (Ponder and Fried 2004b; Schneck and Fried 2004). In general, neonatal snails are more susceptible to infection but show a significant decrease in survival.

Studies on the effects of pollutants on echinostome cercariae are limited. Evans (1982) and more recently Morley et al. (2002) studied the toxic effects of copper and zinc, and cadmium and zinc, respectively, on the transmission of *E. recurvatum* cercariae toward the second intermediate host snails. These authors detected a differential response in infectivity of cercariae dependent on the snail species to be infected; the exposure of different snails to toxic pollutants caused a different susceptibility to *E. recurvatum* cercariae depending on the snail species exposed. Similar results were obtained in the study on effects of tributyltin and copper on cercariae of *Parorchis acanthus* (Bennett et al. 2003). Reddy et al. (2004) analyzed the effects of copper toxicity on cercariae of *E. caproni* and *E. trivolvis*, as well as on the survival of *B. glabrata* snails, and suggested that copper sulfate, used in

concentrations sufficient to kill juvenile snails, was also sufficient to eliminate the cercariae of both echinostomes. More recently, Koprivnikar et al. (2006) analyzed the effects of the herbicide Atrazine, commonly used in North America, on longevity, activity, and infectivity of *E. trivolvis* cercariae, and observed that the viability of these cercariae is compromised by exposure to this herbicide.

Free-swimming cercariae normally come in contact with a compatible second intermediate host. The specificity of echinostomes toward the second intermediate host is low, and usually numerous species of snails, clams, tadpoles, frogs, and even fishes, other invertebrates, and natural products such as snail mucus may serve as second intermediate hosts of echinostomes (Huffman and Fried 1990). Recently, an ectosymbiotic flatworm, *Temnocephala chilensis*, was found naturally parasitized with metacercariae of *Echinoparyphium megacirrus* (Viozzi et al. 2005).

Cercarial preference toward second intermediate hosts has been the subject of some studies (Anderson and Fried 1987; McCarthy 1990d; Fried 2001). Studies on *E. albuferensis* and *E. friedi* cercarial infectivity toward different snail communities composed of combinations of 4 sympatric snail species were performed to evaluate the level of parasite-snail host compatibility. The results obtained from single- to four-host exposures showed that high densities of low compatible hosts may reduce the level of parasite transmission; however, the presence of gastropods of high compatibility in the communities contributes to an increase in the susceptibility of those snail species showing low compatibility (Muñoz-Antolí et al. 2008).

Effects of snail diet on transformation from cercaria to metacercaria in the second intermediate host have been poorly studied. Recent studies have demonstrated that snail diet may affect larval development in the first intermediate host (Sandland and Minchella 2003). Glucose added to artificial spring water extended the survival time of *E. trivolvis* and *E. caproni* cercariae (Fried et al. 1998; Ponder and Fried 2004a), whereas in *E. caproni*, the glucose decreased the cercarial ability to infect snails or move in a linear direction. Fried et al. (1997a) showed that the lipophilic fraction of a *Helisoma trivolvis* dialysate significantly enhanced cercarial chemoattraction and penetration in this planorbid by *E. caproni* and *E. trivolvis*. However, Ponder and Fried (2004b) did not detect an apparent difference in *E. caproni* encystment in juvenile *H. trivolvis* fed either with hen's egg yolk or with Romaine leaf lettuce diet; in consequence, lipophilicity was not a factor in cercarial encystment. Moreover, the diet did not enhance larval development in the snail.

In the absence of a second intermediate host, in vitro encystment of echinostome cercariae can occur. *E. revolutum* and *E. liei* have been reported to encyst on snail mucus (Fried and Bennett 1979; Christensen et al. 1980), *E. revolutum* in Locke's solution with or without glucose (Fried and Bennett 1979; Fried et al. 1997c), *E. paraensei* and *E. caproni* in cultures with *B. glabrata* embryonic cells (Stein and Basch 1977; Loker et al. 1999), *E. caproni* in Locke's-ASW (1:1) medium with or without copper sulfate (Fried and LaTerra 2002; Fried and Schneck 2004), *Echinostoma cinetorchis* in RPMI 1640 plus 10% fetal bovine serum (Park et al. 2006), *Himasthla quissetensis* in casein hydrolysate supplemented with glucose (Laurie 1974), and *Echinochasmus liliputanus* in different solutions (Xiao et al. 2005). The results showed differences in relation to echinostome species and conditions of

culture, but the cysts formed in vitro became infective to the definitive host. However, the infectivity was lower than those obtained from experimentally infected snails (Fried and LaTerra 2002; Park et al. 2006).

Recently, Schotthoefer et al. (2007) tested the cercariophagic activity of several freshwater invertebrates on the echinostome-like species, *Ribeiroia ondatrae*. From the species tested, *Hydra* sp., damselfly (Odonata, Coenagrionidae) larvae, dragonfly (Odonata, Libellulidae) larvae, and copepods (Cyclopoida) consumed cercariae. In some cases, 80–90% of the cercariae exposed to damselfly and dragonfly larvae were consumed within 10 min.

1.1.3.3   Parasitic Stages in the Invertebrate Host

Sporocysts and rediae in the first intermediate host and metacercariae in the second intermediate host are the parasitic stages in the invertebrate host.

Sporocysts and Rediae

These intramolluskan stages are formed after the invasion of an aquatic snail by miracidia and their later transformation. Kanev et al. (2000) compiled the morphological characteristics of these parasitic stages in different echinostome genera, whereas Ataev and coauthors contributed greatly to the knowledge of echinostome stage development in the first intermediate host (Ataev et al. 1997, 1998, 2001, 2005, 2006). Miracidia usually enter the head foot region of the snail, shed their ciliated plates, and transform into sporocysts at the site of penetration, typically the mantle collar, the foot and head covering (including velum and tentacles), the mantle cavity, and the oral cavity. These sporocysts are sac-like structures, about 100 mm long, that after 5–8 days, develop and produce the next parasite stage, the rediae. The ventricle and the common aorta are the final sites of infection for the mother sporocysts after migration (Fig. 1.3d). The behavior patterns and host cues responsible for this site-finding remain unknown, though it was suggested that the ventricle and aorta may be recognized by sporocysts (Haas 2000).

In vitro studies by Ataev et al. (1998) showed that the presence of *B. glabrata* embryonic cells in the cultures is essential for the development of *E. caproni* mother sporocyst in the snail host. During *E. caproni* sporocyst development, every primary germinal cell gave rise to a redial embryo, whereas undifferentiated cells gave rise to both somatic and secondary generative cells. Each mother sporocyst produced about 15 rediae. Intramolluskan development of the *E. caproni* mother sporocysts consists of 5 development steps (resting, migration, growth, reproduction, and degeneration) that are not temperature dependent (Ataev et al. 1997). The mother sporocyst produces the first generation, which consists of maternal rediae forming only redoid embryos (Fig. 1.3e). These initially reside within the ventricle and aorta of the snail, although when these sites became filled, these rediae begin to colonize the ovotestis of the snail. The next generations are represented by