Advances in Experimental Medicine and Biology 1154

Rafael Toledo
Bernard Fried *Editors*

Digenetic Trematodes

Second Edition

Springer

Advances in Experimental Medicine and Biology

Volume 1154

More information about this series at http://www.springer.com/series/5584

Rafael Toledo • Bernard Fried
Editors

Digenetic Trematodes

Second Edition

Springer

Editors
Rafael Toledo
Área de Parasitología
Departamento de Farmacia y
Tecnología Farmacéutica y Parasitología
Facultad de Farmacia
Universidad de Valencia
Valencia, Spain

Bernard Fried
Department of Biology
Lafayette College
Easton, PA, USA

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-18615-9 ISBN 978-3-030-18616-6 (eBook)
https://doi.org/10.1007/978-3-030-18616-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Digenetic trematodes are a large group of parasites of vertebrates that have significant medical, veterinary, and economic importance. Over 100 species of digenetic trematodes have been recorded to be infecting humans, and the list is very extensive considering the trematode species that parasitize animals. The morbidity and mortality caused by several species in humans can be considered mild or even benignant, though other species have important implications for human health. For example, schistosomiasis affects more than 200 million people, and it has been estimated that schistosomes cause approximately 280,000–500,000 deaths every year and the DALY ("disability-adjusted life years") index of schistosomiasis is estimated as 3.3 million per year. Moreover, it is well established that several species of trematodes act as promoters of malignancy. Despite these facts, human trematode infections have been neglected for years, probably in relation to their limited distribution to low- and middle-income countries. However, this picture has been changing in recent years. Factors such as the migration flows, increased international tourism, changes in alimentary habits, and the globalization of food markets are expanding their geographical limits and the population at risk worldwide. Apart from their importance in human and animal health, digenetic trematodes have a great interest in experimental biology due to their other characteristics such as the following: (1) the complex systematics of this group of parasites in relation to the morphological similarity between members of different taxa and the inadequate or poor specific diagnosis (or both) of several newly established taxa and (2) the complexity of the life cycles that have led to the development of an important number of adaptive strategies to enhance parasite survival and transmission.

In 2014, the first edition of the present book was published to provide coverage to all these aspects. The main goal of the second edition is to present the scientific updates in the field during the latest years and to complete some aspects that could be overlooked in the first edition. To this purpose, it has been divided into four parts. The first part is devoted to analyze the general concepts on the biology and systematics of trematode. The second part focuses on the groups of trematodes that have important implications for human health. Each of the six major groups of human trematodes and the corresponding diseases (schistosomiasis, fascioliasis, paragonimiasis, opisthorchiasis, and clonorchiasis and the intestinal trematodes) are dealt with under separate chapters.

In these chapters, emphasis is placed on recent advances and gaps in our understanding that must be filled to complete the knowledge of these trematodes. In the third part of the book, the main groups of trematodes of veterinary and wildlife interest are analyzed. As mentioned above, the list of potential trematodes that might be discussed in this section is vast. For convenience, we have chosen to focus the chapters on those groups of trematodes that are also recognized to have implications for human health. In the fourth part, clinical aspects such as epidemiology and diagnosis of trematode infections are specifically addressed. Moreover, a new chapter on the genomics and proteomics of the trematode infections has been included in this fourth part to complete a modern view of these important parasitic diseases.

In summary, the main goal of the book is to provide an update of the current status of knowledge on these important parasites in the context of modern parasitology.

Valencia, Spain — Rafael Toledo
Easton, PA, USA — Bernard Fried

Contents

Part I General Aspects of the Trematodes

1 Form and Function in the Digenea 3
Alba Cortés Carbonell and Bernard Fried

2 The Systematics of the Trematoda 21
Aneta Kostadinova and Ana Pérez-del-Olmo

Part II Trematodes of Interest in Human Health

3 Schistosomiasis 45
Philip T. LoVerde

4 Fascioliasis 71
Santiago Mas-Coma, Mª Adela Valero,
and Mª Dolores Bargues

5 Paragonimiasis 105
David Blair

6 Liver Flukes: *Clonorchis* and *Opisthorchis* 139
Weerachai Saijuntha, Paiboon Sithithaworn, Nadda Kiatsopit,
Ross H. Andrews, and Trevor N. Petney

7 Intestinal Trematode Infections 181
Rafael Toledo, Maria Alvárez-Izquierdo, Carla Muñoz-Antoli,
and J. Guillermo Esteban

Part III Trematodes of Interest in Veterinary and Wildlife Disease

8 Schistosomatoidea and Diplostomoidea 217
Petr Horák, Jana Bulantová, and Libor Mikeš

9 Amphistomes 255
Veena Tandon, Bishnupada Roy, Jollin Andrea Shylla,
and Sudeep Ghatani

10 Dicrocoeliidae Family: Major Species Causing Veterinary Diseases 279
M. Yolanda Manga-González and M. Carmen Ferreras

11 Selected Wildlife Trematodes 321
Matthew G. Bolek, Jillian T. Detwiler, and Heather A. Stigge

Part IV Clinical Aspects of Trematode Infections

12 Epidemiology of Trematode Infections: An Update 359
Jong-Yil Chai and Bong-Kwang Jung

13 Trematode Genomics and Proteomics 411
Javier Sotillo, Mark S. Pearson, and Alex Loukas

14 Diagnosis of Human Trematode Infections 437
J. Guillermo Esteban, Carla Muñoz-Antoli, Rafael Toledo, and Lawrence R. Ash

Index 473

Contributors

Maria Alvárez-Izquierdo Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Ross H. Andrews CASCAP, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Faculty of Medicine, St Mary's Campus, Imperial College London, London, UK

Lawrence R. Ash Infectious and tropical Diseases, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA, USA

Mª Dolores Bargues Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

David Blair College of Science and Engineering, James Cook University, Townsville, QLD, Australia

Matthew G. Bolek, MS, PhD Department of Integrative Biology, Oklahoma State University, Stillwater, OK, USA

J. Bulantová Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia

Alba Cortés Carbonell Departament de Farmàcia, Tecnologia Farmacèutica i Parasitologia, Facultat de Farmàcia, Universitat de València, Valencia, Spain

Jong-Yil Chai Institute of Parasitic Diseases, Korea Association of Health Promotion, Seoul, Republic of Korea

Seoul National University College of Medicine, Seoul, Republic of Korea

Jillian T. Detwiler, MS, PhD Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

J. Guillermo Esteban Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

M. Carmen Ferreras Departamento de Sanidad Animal, Facultad de Veterinaria, Instituto de Ganadería de Montaña (CSIC-ULE), Universidad de León, León, Spain

Bernard Fried Department of Biology, Lafayette College, Easton, PA, USA

Sudeep Ghatani Department of Zoology, Sikkim University, Gangtok, Sikkim, India

Petr Horák Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia

Bong-Kwang Jung Institute of Parasitic Diseases, Korea Association of Health Promotion, Seoul, Republic of Korea

Nadda Kiatsopit Department of Parasitology, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Aneta Kostadinova Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Alex Loukas Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia

Philip T. LoVerde Department of Biochemistry and Structural Biology, University of Texas Health, San Antonio, TX, USA

Department of Pathology and Laboratory Medicine, University of Texas Health, San Antonio, TX, USA

M. Yolanda Manga-González Departamento de Sanidad Animal, Instituto de Ganadería de Montaña, Consejo Superior de Investigaciones Científicas (CSIC-ULE), León, Spain

Santiago Mas-Coma Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Libor Mikeš Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia

Carla Muñoz-Antoli Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Mark S. Pearson Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia

Ana Pérez-del-Olmo Unitat de Zoologia Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Parc Científic, Universitat de València, Valencia, Spain

Trevor N. Petney CASCAP, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Department of Paleontology and Evolution, State Museum of Natural History, Karlsruhe, Germany

Bishnupada Roy Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya, India

Weerachai Saijuntha Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham, Thailand

Jollin Andrea Shylla St. Anthony's College, Shillong, Meghalaya, India

Paiboon Sithithaworn Department of Parasitology, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Javier Sotillo Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia

Heather A. Stigge Department of Biology, College of Saint Mary, Omaha, NE, USA

Veena Tandon Biotech Park, Lucknow Uttar Pradesh, India

Rafael Toledo Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Mª Adela Valero Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Part I

General Aspects of the Trematodes

Form and Function in the Digenea

1

Alba Cortés Carbonell and Bernard Fried

1.1 Introduction

For our coverage on form and function in the Digenea, we have relied heavily on the former chapter on this topic in the first edition of this book (Peoples and Fried 2014). Herein, we have considered the major organ systems covered in that previous work, and followed the same strategy for literature search. Briefly, the words Digenea and Trematoda were each paired with the following search terms: tegument, tegumentary system, sense organs, sensory structures, parenchyma, lymph system, lymphatic system, nervous system, neuromuscular system, holdfast organs, alimentary tract, excretory system, osmoregulation, male reproductive system, and female reproductive system.

Emphasis in this chapter is based on the novel literature since the coverage by Peoples and Fried (2014) and places accent on salient findings between 2012 and the first half of 2018. The layout of this chapter will proceed as follows: for every body system, an overview is given followed by a discussion of the recent literature as it applies to (1) its morphology and (2) its functionality in adult digeneans and, if applicable, in some larval stages. For background information on the subject matter covered in this chapter, two earlier works by Fried and Haseeb (1991) and Fried (1997) should be consulted.

1.2 Tegumentary System

The tegument of digeneans is a living tissue consisting of a distal syncytial cytoplasm and the nucleated cell bodies (or cytons) that lie under a layer of peripheral muscles. A network of cytoplasmic bridges connects the cytons with the distal cytoplasm (Roberts and Janovy 2009). Tegument-associated cells are the primary differentiation progeny of somatic stem cells (or neoblasts) in the schistosomatid *Schistosoma mansoni* (Collins et al. 2016). In an elegant study, Collins and co-authors showed that depletion of stem cells resulted in a delayed downregulation of a set of genes encoding tegument-associated proteins, thus indicating that neoblasts are required to sustain the expression of these genes. Moreover, pulse-chase experiments labeling proliferating cells showed that neoblasts differentiate mainly into a population of short-lived tegument-associated cells, which is rapidly and continuously renewed. Whether this cell population represents

A. Cortés Carbonell (✉)
Departament de Farmàcia, Tecnologia Farmacèutica i Parasitologia, Facultat de Farmàcia, Universitat de València, Valencia, Spain
e-mail: alba.cortes@uv.es

B. Fried (✉)
Department of Biology, Lafayette College, Easton, PA, USA
e-mail: friedb@lafayette.edu

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_1

terminally differentiated tegumental cell bodies or short-lived progenitors to terminally differentiated cells is difficult to demonstrate; irrespective of this, the authors suggested that neoblasts contribute to parasite longevity by way of sustaining tegument turnover and regeneration of host-inflicted damage (Collins et al. 2016).

Key changes in tegument structure along the development of larval stages are described in the first edition of this book (Peoples and Fried 2014). Furthermore, topographic and ultrastructure descriptions of the tegument of digenetic trematodes can be found elsewhere. For instance, recent studies have provided insights on the tegumental organization of the adult stages of the microphallid *Maritrema feliui* (Świderski et al. 2013), the cryptogonimid *Aphallus tubarium* (Antonelli et al. 2014) or the paramphistomes *Carmyerius spatiosus* (Anuracpreeda et al. 2015), and *Orthocoelium parvipapillatum* (Anuracpreeda et al. 2016), among others. The tegumental surface is often corrugated with ridges and furrows, and penetrated by deep pits (Świderski et al. 2013; Anuracpreeda et al. 2015, 2016); some species bear short microvilli (Anuracpreeda et al. 2015, 2016). Ornamentation such as papillae and spines is commonly present in some areas of the body (Świderski et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016) although some species have been reported to be spineless (Anuracpreeda et al. 2016). The syncytial tegument normally contains organelles, such as mitochondria and lysosomes, as well as a number of morphologically diverse vesicular inclusions (commonly referred as tegumental granules or secretory bodies) that arise from the underlying cytons (e.g., Świderski et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016). The function of these vesicles still remain unclear although it has been suggested that they may contribute to the maintenance, restoration, and modification of tegumental membranes (Skelly and Wilson 2006).

Certain proteins with key roles in membrane biology are essential for tegument development and the maintenance of its structural integrity. Suppression of members of the tetraspanin family resulted in malformation of the tegument of *S. mansoni* (Tran et al. 2010) and the opisthorchiid *Opisthorchis viverrini* (Piratae et al. 2012), and impaired parasite survival (Tran et al. 2010). Similarly, suppression of vesicle-associated membrane protein 2 (VAMP2), which is presumably involved in the normal cargo trafficking across the tegument, affected tegument structure and transtegumental glucose uptake in the schistosomatid *S. japonicum*, thus reducing parasite viability (Han et al. 2017). Myoferlin, in contrast, has been regarded to participate in vesicle trafficking and fusion during the repair of the external membrane of *S. japonicum* (Xiong et al. 2013).

From a functional perspective, the tegument of digenetic trematodes is involved in multiple vital processes, including osmoregulation, nutrient acquisition, synthesis and secretion of substances, and protection from external damage (Roberts and Janovy 2009). Furthermore, the apical membrane also contains a number of proteins, lipids, and glycoconjugates that are important for host–parasite interactions across developmental stages (e.g., Skelly and Wilson 2006). Recently, a role for the tegument in the secretion of extracellular vesicles has been documented. Multivesicular bodies have been observed in the syncytial tegument of the echinostomatid *Echinostoma caproni*, the fasciolid *Fasciola hepatica*, and the dicroceliid *Dicrocoelium dendriticum* (Marcilla et al. 2012; Bernal et al. 2014). These structures, which have an endosomal origin and are likely to be constitutively produced by flukes (Cwiklinski et al. 2015a, b), fuse with the apical membrane to discharge exosome-like intraluminal vesicles into the environment (Marcilla et al. 2012; Bernal et al. 2014). Parasite-derived extracellular vesicles can transfer their cargo (mainly proteins and small RNAs) into mammalian host's cells (Marcilla et al. 2012; Chaiyadet et al. 2015) and alter their protein profiling (Chaiyadet et al. 2015); therefore, these tegument-derived elements are crucial to interspecific communication between parasites and their hosts.

Being the most external organ, the tegument plays also a pivotal role in immune evasion. Several tegumental proteins of *S. japonicum* are able to bind host immunoglobulins in a non-specific manner (i.e., via the Fc domain), which

may serve to mask specific antigen-recognition sites and avoid immune attachment (Wu et al. 2015). In contrast, host antibodies bind specifically to the surface of *E. caproni* and become trapped by excretory/secretory products; that facilitates antibody degradation by parasite-derived proteases and may hamper the antibody-mediated attack (Cortés et al. 2017). On the other hand, tegumental ATP diphosphohydrolase expressed by intravascular stages of *S. mansoni* works as an apyrase, degrading external pro-inflammatory and pro-thrombotic signals (ATP and ADP, respectively) and minimizing host immune responses (Da'dara et al. 2014).

1.3 Sensory System

Ultrastructure studies have revealed a vast array of presumed sensory receptors in the tegument of both larval and adult stages of digeneans. These organs harbor fine nervous terminations branched from the peripheral nervous system that end beneath the surface layer of the tegument. Although experimental evidence is lacking, a variety of functions have been ascribed to these receptors based on accurate structural descriptions. In particular, sensory endings have been regarded to be sensitive to touch (tangoreceptors), light (photoreceptors), fluid currents (rheoreceptors), and specific molecules and ions (chemoreceptors). Form, function, and distribution of these receptors throughout the body of the parasite largely depend on the species and life stage and may reflect the sources of stimuli to which the parasite have to react to Halton (2004).

Two types of sensory structures displaying different morphology and localization have been described on the tegument of the deropristid *Deropristis inflata* (Filippi et al. 2013) and the cryptogonimid *A. tubarium* (Antonelli et al. 2014). In both species, type 1 receptors are button-like uniciliated structures consisting of a nerve bulb from which a cilium extends from a centriole. The nerve bulb contains numerous mitochondria and nerve fibers, and is connected to the cytoplasm by a hemidesmosome located at the top of the bulb. A long striated rootlet lies underneath the centriole in type 1 receptors of *D. inflata* (Filippi et al. 2013), but is lacking in those of *A. tubarium* (Antonelli et al. 2014). Type 2 receptors are dome-like non-ciliated structures; however, their internal ultrastructure varies between the two species. While type 2 receptors of *D. inflata* enclose an ovoid electron-dense structure (Filippi et al. 2013), in the case of *A. tubarium*, nerve bulbs are filled with electron-lucent vesicles (Antonelli et al. 2014). Hemispherical electron-dense collars are observed on the top of the nerve bulbs in type 2 receptors of *A. tubarium*, and most of them display a gland-duct opening to the outside (Antonelli et al. 2014). Similar uniciliated (type 2) and non-ciliated (type 1) structures have been described on the surface of paramphistomes *C. spatiosus* (Anuracpreeda et al. 2015) and *O. parvipapillatum* (Anuracpreeda et al. 2016).

Sensory receptors show a specific distribution throughout the surface of the parasite and may appear both singly or in groups. For instance, either type 1 or type 2 receptors of *C. spatiosus* and *O. parvipapillatum* are grouped on the top of dome-shaped papillae displaying a nipple-like tip, which, in turn, can also appear clustered or isolated (Anuracpreeda et al. 2015, 2016). Type 1 receptors of *A. tubarium* are grouped (Antonelli et al. 2014), while in *D. inflata* this type of receptors can be observed either individually, or in groups of two to three in a single papilla (Filippi et al. 2013). Regarding their distribution throughout body of the parasite, the density of sensory receptors is generally higher in the ventral side of the flukes and, in particular, in the surroundings of the suckers, suggesting that they may have roles in feeding and attachment (Filippi et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016). Intrategumental receptors (i.e., non-ciliated) are suggested to act as tangoreceptors sensing pressure, while ciliated receptors could possibly be chemoreceptors, as well as rheo- or mechanoreceptors (Filippi et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016).

Sensory organs are particularly important for the free-living stages of digeneans (mainly miracidia and cercariae), which heavily rely on external stimuli, such as light and gravity, to rapidly

find an appropriate host and keep the life cycle going (Roberts and Janovy 2009). Three sensory structures have been described in the miracidia of the strigeid *Cardiocephaloides longicollis*: two types of peripheral sensory endings, namely uniciliated and multiciliated deep-pit-like sensory papillae, and a pair of eyespots (each one of a different size). These structures are located in the anterior part of the miracidium and are presumed to be adaptations for chemoreception, tangoreception, and photoreception in the active location of the snail host (Born-Torrijos et al. 2017).

1.4 Neuromuscular System

According to classical descriptions, the somatic musculature of digenetic trematodes generally consists of the body-wall musculature, which comprises a layer of circular muscles laying just under the basal lamina of the tegument plus longitudinal and diagonal muscular bundles beneath the circular muscles, and the internal or parenchymal musculature, mostly formed by dorsoventral muscle fibers (Roberts and Janovy 2009). However, a study conducted by Krupenko and Dobrovolskij (2015) on the organization of the muscular system in digeneans, suggests that further groups of muscle fibers do exist in both the body-wall and the inner musculature, which appear consistently across distant taxa (Krupenko and Dobrovolskij 2015). In particular, anteroradial, posteroradial, and anterolateral muscle fibers and U-shaped muscle sets are abundant in the body-wall musculature at the ventral surface, and are associated with the acetabulum. Furthermore, eight main types of additional internal musculature have been defined, with presumable functions in the movement of the suckers and pharynx (Krupenko and Dobrovolskij 2015). Indeed, both body-wall and parenchymal musculature are generally more prominent in the preacetabular part of the flukes, from cercaria to adult. Preacetabular musculature is regarded to be involved in the movements of the anterior part of the body, including ventral and oral suckers and the anterior organ in the cercarial stage (Krupenko and Dobrovolskij 2015).

Species lacking of a ventral sucker, such as notocotylids, display adaptations of the musculature that may aid in parasite attachment via the ventral concavity (Krupenko and Gonchar 2017a, b). Analogs for acetabulum-associated groups of muscles are found in the ventral concavity of these flukes, including muscle groups that are characteristic for the Notocotylidae family. For instance, a set of ventrolateral longitudinal musculature with a U-shaped pattern works like the circular musculature of a sphincter, while the oblique internal musculature may perform the function of the radial muscle fibers of a sucker. Moreover, additional body-wall and internal musculature at the anterior region are also specific for this family in Digenea and may provide the oral sucker with diverse movements (Krupenko and Gonchar 2017a, b).

The structure of the muscular system usually shows a general pattern through the hermaphroditic generations of digeneans (Krupenko and Dobrovolskij 2015; Krupenko and Gonchar 2017b). However, the musculature of diplostomids, and in particular that of *Diplostomum pseudospathaceum*, undergoes a substantial rearrangement during metacercarial development, which is particularly extensive in the body-wall and the anterior region of the parasite; this transformation may reflect the different roles the musculature plays in cercarial and adult worms (Petrov and Podvyaznaya 2016). Developmental changes in the body-wall musculature of *D. pseudospathaceum* occur in three consecutive phases, including (1) the expansion of the longitudinal musculature; (2) the replacement of cercarial musculature and the increase in the number of longitudinal and circular body-wall muscles; and (3) the separation of the diagonal muscles into two distinct sets on the dorsal and ventral sides of the body, each of them working in an antagonistic manner. Circular and longitudinal muscles seem also likely to form two separate sets although this configuration has not been confirmed. Furthermore, dorsoventral muscles become particularly abundant in the infective metacercariae (Petrov and Podvyaznaya 2016).

The digenean nervous system typically has an orthogonal, or ladder-like, conformation, with

pairs of longitudinal nerve cords extending anteriorly and posteriorly from the cerebral ganglia and cross-linked at intervals by transverse ring commissures. Nervous terminations branch from the longitudinal trunks and provide motor and sensory innervation to the muscles and tegument (Roberts and Janovy 2009). The nervous system of trematodes have been traditionally reported to consist of a single pair of cerebral ganglia; however, presence of two of these structures has been reported in adult *S. mansoni* by targeting specific neuronal markers, including the biogenic amine octopamine (El-Sakkary et al. 2018). Each pair of ganglia is associated to one of the two suckers (oral or ventral) in the adult worm, suggesting a particular adaptation for an independent control of each sucker (El-Sakkary et al. 2018).

Neuronal cell types in Digenea are mainly multi- and bipolar, and highly secretory in nature; the composition of these secretions have been reported to include cholinergic, aminergic, and peptidergic messenger molecules (Halton and Maule 2004). Although early experimental evidence supported an inhibitory role for acetylcholine (ACh) in trematodes (Barker et al. 1966; Holmes and Fairweather 1984), functional ACh-activated receptors were not identified until many decades later. A study conducted in *S. mansoni* characterized for the first time a family of ACh-gated chloride channels (SmACCs), which play an inhibitory role in the neuromuscular activity of this parasite (MacDonald et al. 2014). These channels are formed by chloride-selective nicotinic ACh receptor subunits and are located mainly in minor nerve fibers that innervate the body-wall musculature, rather than directly on the muscles. This pattern of distribution suggests that SmACCs mediate their inhibitory effects in an indirect manner, presumably modulating the release of other neurotransmitters (MacDonald et al. 2014). Genomic evidence exists for the presence of closely related putative nicotinic ACh receptor chloride channels in the opisthorchiid *C. sinensis* (Huang et al. 2013), which lends credit to the hypothesis of MacDonald and co-workers (2014) about the existence of a unique clade of platyhelminth-specific nicotinic chloride channels.

A second ACh receptor has been described in *S. mansoni* (SmGAR), a constitutively active G protein-coupled receptor, which suppression led to the unexpected inhibition of motility in the schistosomula (MacDonald et al. 2015). The way in which this receptor modulates the movement of the parasite remains unknown. Nonetheless, it seems plausible that, as in other organisms (Dittman and Kaplan 2008), SmGAR works to repress the activity of ACh via a mechanism of negative feedback; therefore, suppression of the negative feedback provided by the receptor would result in increased ACh signaling with the consequent reduction in motility (MacDonald et al. 2015).

Serotonin (5-hydroxytriptamine: 5-HT) is an important myoexcitatory neurotransmitter in trematodes, which is present both in the central nervous system, as well as in peripheral nerve fibers and the plexuses that supply the body-wall musculature. Furthermore, 5-HT stimulates glucose uptake and carbohydrate metabolism, thus increasing energy availability for muscle activity (reviewed by Ribeiro et al. 2005). Two studies have started to shed light on the mechanisms of signaling of 5-HT and its mode of action in *S. mansoni* (Patocka and Ribeiro 2013; Patocka et al. 2014). A functional receptor specific for 5-HT (Sm5THR) has been identified in this schistosomatid, which is involved in the control of motor activity (Patocka et al. 2014). Sm5THR is a rhodopsin-like G protein-coupled receptor, signaling through which involves coupling of a stimulatory G protein and activation of adenylate cyclase, with the subsequent increase in cytosolic cyclic AMP. This receptor is broadly distributed through the body of the worm although it is particularly abundant throughout the nervous system and, particularly, closely to 5-HT-containing neurons. Suppression of the gene encoding for Sm5HTR resulted in a reduced motility of both larval and adult schistosomes, which is consistent with the excitatory role of 5-HT. Overall, localization and functional features of Sm5HTR suggest that it controls parasite's movement by modulating the release of other myoexcitatory neurotransmitters, which in turn initiate muscle contraction. Moreover, its distribution in the

central nervous system indicates that additional 5-HT-regulated mechanisms may be operating at this level and contribute to motor control (Patocka et al. 2014). A second putative 5-HT receptor has been identified in the genome of *S. mansoni*, which has yet to be confirmed by functional analysis (Patocka et al. 2014).

Another component of the serotonergic system has been characterized in *S. mansoni*: a specific plasma membrane transporter (SmSERT), with a potential role in the inactivation of 5-HT. This transported is distributed parallel to 5-HT throughout the nervous system, in particular at neuronal varicosities, where neurotransmitters are released to exert their function and then sequestered back into the neurons. Furthermore, disruption of SmSERT activity caused schistosome larvae to become hyperactive, which is consistent with it mediating the re-uptake of 5-HT to terminate signaling. These features suggest that SmSERT is a key player in the serotonergic control of parasite motility, which main function should be removing 5-HT from the extracellular space to control the intensity and duration of signaling (Patocka and Ribeiro 2013).

Besides ACh and 5-HT, further neuroactive molecules such as dopamine, histamine, octopamine, and neuropeptides are also involved in nervous and neuromuscular activities in digeneans (e.g., Eriksson et al. 1996; El-Shehabi and Ribeiro 2010; Tolstenkov et al. 2010; El-Shehabi et al. 2012; El-Sakkary et al. 2018). In regard to the later, two groups of native neuropeptides have been identified in trematodes, including pancreatic polypeptide-like neuropeptide F and FMRFamide-related peptides, whose localization and potential functions were covered in the first edition of this book (Peoples and Fried 2014). Nevertheless, it is worth mentioning that genes with potential functions in neuropeptide signaling are highly transcribed in the gonads of adult *S. mansoni*, particularly in the testes, suggesting that neural processes may play roles in growth and differentiation of the gonads, and intervene the reproductive biology via endocrine and paracrine mechanisms (Lu et al. 2016).

1.5 Alimentary System

Trematode feeding varies depending on the habitat within the host, generally consisting in blood, mucus, or tissues. The alimentary system consists of two differentiated parts, the foregut and the gut, or ceca. The former typically includes the mouth (surrounded by the oral sucker), the pharynx, and the esophagus, which connects with the ceca. Some species display a short pre-pharynx, while others, however, have no pharynx. The lining of the foregut displays a tegument-like structure, but without spines, and is responsible for food suction and early break down. Right after the esophagus, the alimentary tract usually bifurcates in two ceca, where digestion and nutrient uptake occur. Digestion is predominantly extracellular, occurring at the lumen of the ceca, although intracellular events may occur in some species. The ceca are lined by a simple epithelium, called gastrodermis, which can be cellular or syncytial, and whose cytoplasm is highly folded with the purpose to increase the absorptive surface (Bogitsh et al. 2013; Roberts and Janovy 2009). Some genera in the Digenea, however, may not have a digestive system. In particular, while early descriptions of the dicroceliid genus *Metadelphis* reported these flukes to have a poorly visible, although still existing, digestive system (Travassos 1944, 1955), recent reexaminations have suggested that this statement was mistaken, and members of the genera *Metadelphis* and *Parametadelphis* actually lack of a digestive system (Tkach et al. 2018).

To our knowledge, the latest fine ultrastructural descriptions of the whole alimentary tract in Digenea were carried out on the cercarial stages of the diplostomid *D. pseudospathaceum* (Podvyaznaya 2006) and the bucephalid *Prosorhynchoides borealis* (Podvyaznaya 2011), and both were well covered in the first edition of this book (Peoples and Fried 2014).

In the past few years, studies on the alimentary system of digeneans have focused on the esophagus of human schistosomatids *S. mansoni* and *S. japonicum*. The esophagus of these flukes appears as a glandular tissue, organized in two

well-demarcated compartments. The lining of the anterior esophagus is typical of the syncytial tegument, with cytoplasmic folds projecting towards the lumen (Li et al. 2013). In *S. japonicum*, the surface area of the anterior esophagus is greatly increased by abundant corrugations displaying thread-like extensions of cytoplasm on their tips. These filiform projections have been proposed to work to entangle blood cells during the feeding process, thus increasing their retention time for interacting with esophageal secretions (Li et al. 2014). The posterior esophageal syncytium is characterized by the presence of copious plate-like extensions containing unique crystalloid vesicles that are emptied between the plates, where they form extracellular aggregates. These cytoplasmic extensions are closely disposed in such a way that only plasma proteins are allowed to enter the interplate spaces. This singular organization, typical of transporting epithelia, led the authors to suggest that it may serve for the generation massive ion-fluxes. Alternatively, it is also plausible that this structure functions to sequester the aforementioned crystalloid secretions, thus enabling a more efficient interaction between them and the ingested blood (Li et al. 2013).

Food ingestion in these schistosomes occurs in two steps (Li et al. 2013). First, blood accumulates in the lumen of the anterior esophagus, before being pushed as a bolus to the posterior compartment; a network of muscular fibers propels this transition. Red blood cells appear to be lysed rapidly after they enter the posterior esophagus. However, a plug of leucocytes at different degrees of degradation is tethered in the lumen of this compartment, while blood streams around. Presumably, this cellular mass works to minimize the entrance of leucocytes into the gut and avoid the detrimental consequences that this event might have for the parasite (Li et al. 2013).

Ultrastructural studies suggest that the esophagus of these blood flukes possesses a high secretory activity since different types of vesicular inclusions are abundant in the cytoplasmic projections of both the anterior and posterior compartments (Li et al. 2013, 2014). The importance of the secretory pathway in this organ is supported by transcriptomic data, showing the enrichment of transcripts from genes involved in the intracellular vesicle transport (Wilson et al. 2015). Other transcripts overrepresented in this region come from microexon genes and genes encoding proteases and lysosomal hydrolases, which are secreted into the esophagus lumen (Li et al. 2013, 2018; Wilson et al. 2015). Predicted structure for the protein products of microexon genes suggests that they may perform several functions, including leucocyte trapping in the esophageal lumen, protection against immune attack, hemolysis, and antimicrobial roles (Wilson et al. 2015; Li et al. 2018). Some families of microexon genes are also represented in the bird schistosomatid *Trichobilharzia regenti*, suggesting that these genes have an ancient origin related to blood feeding (Li et al. 2018). Lysosomal hydrolases and proteases are regarded to work in blood processing, and in preventing blood clotting (Wilson et al. 2015).

1.6 Respiratory System

The life cycle of digeneans consist in a sequence of several developmental stages inhabiting a range of aerobic/anaerobic environments; therefore, these organisms need to undergo constant transitions in energy metabolism to adapt themselves to the changing environment (Takamiya et al. 2010). The expression profile of key metabolic enzymes revealed that fasciolid *F. hepatica* shifts from an aerobic to an anaerobic metabolism during the development of newly excysted juveniles into adult flukes, which has been related to a reduction in the diffusion of oxygen into the parasite tissues as it grows (Cwiklinski et al. 2015a, b). Transcriptomic analyses of the opisthorchids *Clonorchis sinensis* (Huang et al. 2013) and *O. viverrini* (Young et al. 2014) showed that the adult stages of these liver flukes express genes associated with both aerobic and anaerobic respiration.

1.7 Excretory System

The excretory system in Digenea is of protonephridial type, i.e., a tubular system composed of flame cells that opens only at the distal end by way of a pore. Flame cells (or bulbs) are flask-shaped units, each containing a tuft of fused flagella and opening in a terminal tubule. Beating of the flagella creates a pressure gradient that draws the excretory fluid through a filtering weir out of the cell into the collecting tubule. Terminal tubules of several flame cells converge to form larger collecting tubules, eventually emptying into a common excretory bladder. In adult digeneans, the excretory bladder opens to the exterior through the excretory pore, usually located near the posterior end of the body. This system is presumed to serve both excretory and osmoregulatory functions. Notwithstanding, in some trematodes the walls of the collecting ducts display structural adaptations such as microvilli, thus suggesting that additional absorptive/secretory functions may also occur (Roberts and Janovy 2009).

Excretion comprises a series of processes aiming at (1) removing waste products of metabolism and other unnecessary substances; (2) regulating the internal osmotic pressure and ionic composition; and (3) inactivating and/or eliminating injurious compounds (Beklemishev 1969). The latter purpose raises a particular interest in the context of parasitic helminths due to its importance in the development of drug resistance (e.g., Sato et al. 2004; Kumkate et al. 2008). A study conducted on the opisthorchiid *O. felineus* identified four putative genes encoding for P-glycoproteins, which are active in the excretory system of the adult stage and are presumably involved in the elimination of drugs via this system (Mordvinov et al. 2017). P-glycoproteins belong to the ABC transporter superfamily of proteins and are responsible for the efflux of a wide range of endo- and xenobiotics across cellular membranes (reviewed by Greenberg 2013). Members of this family had been previously identified in other digeneans, including the fasciolids *F. gigantica* (Kumkate et al. 2008) and *F. hepatica* (Wilkinson et al. 2012), and the schistosomatid *S. mansoni* (Messerli et al. 2009). In these species, P-glycoproteins have been implicated in drug excretion and the generation of anthelmintic resistance (Kumkate et al. 2008; Messerli et al. 2009; Wilkinson et al. 2012; Kasinathan et al. 2014; Pinto-Almeida et al. 2015).

The metabolic system cytochrome P450 is also associated with the protonephridia in *O. felineus*, where it participates in the biotransformation of xenobiotics. Moreover, suppression of the gene encoding for this system caused a significant enlargement of the excretory channels and bladder, and increased worm mortality. These results suggest that cytochrome P450 is potentially involved in worm metabolism and detoxification, and evidence the importance of this system for the parasite lifestyle (Pakharukova et al. 2015).

Although the term excretory system is usually employed as a synonym for protonephridia, excretion in digeneans, as in other flatworms, involves additional body systems, such as the alimentary tract and the tegument (Hertel 1993). For instance, unlike in *O. felineus* (Mordvinov et al. 2017), the aforementioned P-glycoproteins have been localized in the ceca and tegumental cells of *F. gigantica* (Kumkate et al. 2008), and the gut of *S. mansoni* (Messerli et al. 2009).

The tegument play a particularly important role in osmoregulation throughout the various developmental stages of trematodes, and tegumental aquaporins are crucial for this function, facilitating the selective passage of water and small solutes through the external membrane (e.g., Faghiri and Skelly 2009; Thanasuwan et al. 2014). Furthermore, aside of maintaining the osmotic pressure, a role for some tegumental aquaporins in the excretion of nitrogenous waste products, such as urea and ammonia, have been reported in the opisthorchiid *C. sinensis* (Geadkaew et al. 2015) and the schistosomatid *S. japonicum* (Huang et al. 2016). In addition to transporter-assisted excretion, wastes can be also excreted via simple diffusion across the tegument and the gut epithelium, or by the means of exocytic vesicles that flush residues produced within the organism outwards (Hertel 1993).

1.8 Reproductive Systems

Digenetic trematodes display obligate alternation between sexual and clonal reproduction over their life cycles. With the exception of members of the family Schistosomatidae, which have evolved separate sexes, trematodes are hermaphroditic. However, although self-fertilization may occur in some species, cross-fertilization is the most extended form of reproduction during the adult stage. Within the definitive host, flukes find each other by means of chemoattractants, presumably cholesterol, or a closely related steroid, except in the case of schistosomes (Roberts and Janovy 2009). Exceptional cases of asexual reproduction by parthenogenesis have also been reported in adult trematodes (Whitfield and Evans 1983; Nollen 1997; Van Herwerden et al. 1999).

1.8.1 Male Reproductive System

The male reproductive system typically consists of two testes, although some species have only one testis, while others are multitesticular. The position, relative orientation, and shape of the testes vary among species and are generally useful for the identification of trematode species. Each testis has a vas efferens that connects with others to form a unique vas deferens, eventually ending in a genital pore, that is usually located within the common genital atrium on the midventral surface of the fluke. Before entering the genital pore, the deferent duct normally goes into a muscular cirrus sac, where it expands to form an internal seminal vesicle. Distally, this vas constricts again to form an ejaculatory duct, normally bounded by prostate gland cells; it extends until the end of the cirrus sac, forming the male copulatory organ, or cirrus, which can evaginate from the surrounding pouch to discharge the sperm into the female reproductive system. Specialized modifications of the distal part of the system, such as absence of cirrus sac and/or prostate glands, or external location of the seminal vesicle (i.e., out of the cirrus sac) are observed in some trematodes (Roberts and Janovy 2009).

The spermatological characteristics in Digenea have been extensively reviewed by Bakhoum and co-authors (Bakhoum et al. 2017), who proposed five types of spermatological models according to the main features of the mature spermatozoa. Commonly, male gametocytes are elongated cells without discernible external organization, containing two axonemes, four attachment zones, at least one mitochondrion, a posterior nucleus, and two longitudinal bundles of parallel cortical microtubules; membrane ornamentations and spine-like bodies can be observed in some species (Bakhoum et al. 2017). The axonemes typically exhibit a 9 + '1' trepaxonematan pattern, consisting in 9 peripheral doublets of microtubules disposed around a central core. Nonetheless, particular variations of this structure are found in schistosomes and some didyzomids (Bakhoum et al. 2017). Additional ultrastructural features such as presence or absence of a lateral expansion, the arrangement and localization of cortical microtubules, or the existence of external ornamentations on the plasma membrane, as well as their location and association with cortical microtubules, are also variable across the spermatozoa of digeneans and may be useful for phylogenetic purposes (Bakhoum et al. 2017). For instance, the ultrastructure of the posterior spermatozoon extremity supports molecular data suggesting that the family Acanthocolpidae is not closely related to the clade Opecoelidae + Opistholebetidae (Littlewood et al. 2015). Notably, while the posterior ending of spermatozoon lacks of cortical microtubules and only contains the nucleus in the studied acanthocolpids *Stephanostomum murielae* and *Stephanostomoides tenuis* (Bakhoum et al. 2015), the presence of cortical microtubules in the posterior extremity has been reported in opecoelids and opistholebetids (Miquel et al. 2000; Levron et al. 2004; Quilichini et al. 2010). In a like manner, the first ultrastructural characterization of the mature spermatozoa of a member of the genus *Sclerodistomum* (*S. italucum*) showed specific features that are observed only in other representatives of the superfamily Hemiuroidea. These features include the simultaneous presence of two types of extramembranous

ornamentations, the presence of short cortical microtubules in the anterior part of the spermatozoon, and a single bundle of longer cortical microtubules in the medium part of the sperm cell (Ndiaye et al. 2017).

Spermiogenesis is generally homogeneous across digenean species. It starts with the formation of the differentiation zone, which is bordered by cortical microtubules and contains numerous mitochondria, as well as the nucleus and a pair of centrioles that give rise to two initially free flagella. Then, a cytoplasmic process is formed between the two flagella, along which the nucleus, mitochondria, and cortical microtubules migrate. Finally, the flagella arrange parallel to and fuse with the median cytoplasmic process, and the spermiogenesis terminates with the constriction at the differentiation zone, and the release of the young spermatozoon (Bakhoum et al. 2017). High-throughput transcriptomics carried out in the opistorchiid *C. sinensis* (Huang et al. 2013) and the schistosomatid *S. mansoni* (Lu et al. 2016) has revealed that the vast majority of transcripts are concentrated in the gonads in these digeneans. According to Lu and co-authors, this observation could be explained by the storage of mRNA in spermatozoa and its transport into the oocyte via sperm (Lu et al. 2016).

1.8.2 Female Reproductive System

The female reproductive system consists of two parts: a structural complex called oogenotop (or egg-forming apparatus) and the uterus, a long, often convoluted tube, through which the eggs are transported to the exterior. Most trematodes have a single ovary, connected to a short oviduct via a proximal sphincter, called ovicapt, that regulates the passage of oocytes down into the oviduct. A seminal receptacle emerges as an out pocket of the oviduct wall and provides with sperm for fertilization. Vitelline glands communicate also with the oviduct and contribute with mature vitelline cells, which are essential for eggshell formation and nourishment of the forming embryos. Following sperm penetration and association with several vitelline cells, oocytes go into the ootype, an expansion of the oviduct surrounded by numerous unicellular Mehlis' glands that discharge their secretions into it via miniscule ducts. The function of the material provided by Mehlis' glands is not fully understood; presumed functions include contribution to eggshell formation and hardening, activation of sperm, and activation of vitelline cells to release shell material. Beyond the ootype, the female duct expands to form the uterus, which extends to the female genital pore. In some species, the distal end of the uterus becomes muscular and serves as an ovijector and a vagina; this structure is termed metraterm. During the copula, the cirrus is inserted into the distal end of uterus and the sperm swims towards the seminal receptacle, where it is stored. A narrow tube, called Laurer's canal, often arises at the base of the seminal receptacle; it ends either blindly in the parenchyma or opens outward through the tegument, and is thought to be a non-functional vestigial vagina (Roberts and Janovy 2009).

Oogenesis and vitellogenesis follow general patterns in all digeneans although some variations have been reported. For instance, Greani and co-authors (2016), studied for the first time the evolution of these two processes in a member of the family Allocreadiidae, namely *Crepidostomum metoecus.* Compared to other digeneans, fully mature vitellocytes of *C. metoecus* contain large amounts of nutritive reserves and material for shell development; this, together with the low protein composition of mature oocytes, led the authors to suggest that in this species oocytes do not participate in the nourishing of the developing embryo (Greani et al. 2016).

A comparative study of oogenesis between diploid and triploid isolates of the fasciolid *F. hepatica* evidenced that synaptonemal complexes are formed early in primary oocytes of all individuals, irrespective of their chromosomal complement (Hanna et al. 2016). The formation of these complexes in triploid specimens indicates that meiosis is also initiated in this type of flukes, which, a priori, are unable to undergo conventional meiosis (Fletcher et al. 2004). Furthermore, in all the isolates studied by these authors, the meiosis of oocytes completes within eggs in the

uterus, in which distal end, oocytes typically enclose two equal-size pronuclei; this represents the most advanced stage of development of eggs within the fluke, before being shed into the environment. In diploid individuals, each pronucleus originates from male and female gametocytes, respectively. In contrast, parthenogenesis is believed to occur in triploid organisms, which are aspermic (Hanna et al. 2016). In these specimens, besides the segregation of synapsed homologous chromosomes, meiosis 1 is thought to involve the allocation of the unsynapsed chromosomes (i.e., the third copy) in the two daughter nuclei, resulting into an unequal distribution of the genetic material between them. Hanna and co-authors (2016) postulated that in these flukes the zygote might be restored to the triploid condition prior to proceed into meiosis 2, by way of re-fusing the two pronuclei resultant from the first mitotic division. This mechanism might restore the unbalancing effects of meiosis 1 and ensure success in the cleavage division at meiosis 2, thus enabling the survival and clonal expansion of polyploids (Hanna et al. 2016). According to these authors, the same mechanism of parthenogenesis may operate in a facultative manner in diploid *F. hepatica* if sperm is not available (Hanna et al. 2016).

In regard to the functioning of the female reproductive system, particular attention has been paid in the last 5 years to specific molecules and pathways that play key roles in regulating the reproductive biology of dioecious trematodes of the genus *Schistosoma*, in which egg production has special relevance due to the role of eggs in chronic pathology. Several signal transduction processes are now known to play essential roles in controlling mitosis, cell differentiation, and egg production via the regulation of key transcriptional targets. In particular, cooperation between Src-kinase- and TGFβ receptor I-containing pathways has been reported to influence mitotic activity and egg production in female *S. mansoni* since the inhibition of these pathways suppressed both processes in paired females (Buro et al. 2013). Furthermore, the mitogen-activated protein kinase (MAPK) cascade also plays an important role in regulating the reproduction of this species of schistosome. Suppression of the gene coding for the extracellular signal-regulated kinase (SmERK) caused a significant reduction in egg production, in addition to morphological alterations in the female reproductive system, such as smaller ovaries that accumulated immature oocytes and accumulation of mature oocytes in the uterus (Andrade et al. 2014). Additional protein kinases, such as SmTK3, SmTK4, SjTK4, and SmTK6, have also been reported to play roles in gonad-associated processes in female schistosomes (Beckmann et al. 2010a, 2011; Buro et al. 2017; Hahnel et al. 2017; Ding et al. 2017).

Venus kinase receptors (VKRs) are unusual receptor tyrosine kinases of invertebrates, displaying a particular protein structure (Vicogne et al. 2003). Two VKRs are expressed in *S. mansoni* (SmVKR1 and SmVKR2), which play different roles in oogenesis and egg formation (Vanderstraete et al. 2014). Each of these receptors is activated by a different ligand; specifically, L-arginine activates SmVKR1, while Ca^{+2} activates SmVKR2 (Vanderstraete et al. 2014). However, a novel way of activation has been discovered for SmVKR1, which is independent of ligand binding and works by interaction of the receptor with a signaling complex, including a beta integrin receptor Smβ-Int1 (Gelmedin et al. 2017), for which a role in oogenesis had been previously reported (Beckmann et al. 2012). Ligand-independent activation of SmVKR1 results in downstream activation of several signaling pathways, and the blockade of this type of activation affects ovary structure, oocyte integrity, and egg formation (Gelmedin et al. 2017). In particular, formation of this signaling complex is crucial to control the differentiation status of oocytes by regulating apoptosis (Gelmedin et al. 2017).

Schistosome micro(mi)RNAs also play important regulatory roles in male–female pairing, gametogenesis, and egg production (Zhu et al. 2016). A number of miRNAs are differentially expressed between male and female *S. japonicum*, and across different stages of maturation; among them, miRNAs with regulatory roles on mRNA targets involved in signal transduction for sexual maturation and egg production have been identified. In particular, functional evidence has

been proved for miR-31 and bantam, whose suppression, as well as the suppression of their mRNA targets, resulted in severe defects in ovary development (Zhu et al. 2016). A role in regulating the sexual development of *S. mansoni* females have also been suggested for members of the miR-277 family of miRNA since their target genes display different expression profiles between paired (mature) and unpaired (immature) females (Protasio et al. 2017).

Beyond sexual maturation of worms, a proper formation of the eggshell is crucial for egg viability and successful transmission of the infection. In *S. mansoni*, the production of the major eggshell protein (Smp14) is controlled by epigenetic modifications at the promoter region of the corresponding gene. In particular, two histone acetyltransferases (SmGCN5 and SmCBP1) are recruited at the *Smp14* promoter by nuclear receptors and acetylate the H3 to activate the transcription of this gene, an essential step for egg development. Indeed, inhibition of these transferases resulted in disrupted eggshell integrity and defective egg structure (Carneiro et al. 2014). Moreover, this inhibition impacted negatively on the development of the ovary and vitellaria, and the maturation of oocytes (Carneiro et al. 2014). Other processes involving chromatin remodeling have been proved relevant for the function in the sexual development of *S. mansoni* females. Concretely, suppression of the members of the family of high mobility group box proteins (HMGBs: SmHMGB2 and SmHMGB3) impaired ovary development and egg laying (de Abreu da Silva et al. 2016).

1.9 Trematode Body Systems as Targets for Drug Discovery

One of the biggest issues faced by current human and veterinary parasitology is the limited range of drugs available for combating trematode infections in the face of emerging resistance against conventional chemotherapeutics (Kelley et al. 2016; Vale et al. 2017). This concern evidences the necessity of developing next-generation antitrematodal drugs, a challenging task that comprises not only the discovery and/or characterization of anthelmintic compounds, but also the identification of parasite molecules that can be exploited as drug targets (Keiser and Utzinger 2007; Ferreira et al. 2015). Each of the body systems covered in this chapter performs vital functions for the parasite biology, thus having the potential for novel treatment opportunities. Nonetheless, besides a critical functional relevance, drug-target candidates are required to meet additional criteria, such as known or predicted druggability and parasite specificity, i.e., lack of host orthologues or, at least, low structure similarity (McVeigh et al. 2011). Reviewing the recent advances in this field is out of the scope of this chapter. Notwithstanding, brief comments on some few highlights concerning the targeting of trematode body systems for the discovery of novel therapeutic and control opportunities are provided.

Aiming at identifying candidate molecules for their validation as new drug targets, particular emphasis is being placed on neural, excretory, and gonad-associated processes in the schistosomatid *S. mansoni*. Neurotransmitter signaling is subject of an extensive research (e.g., Patocka and Ribeiro 2013; Patocka et al. 2014; MacDonald et al. 2014, 2015; Chan et al. 2016a, b; Hahnel et al. 2018; McVeigh et al. 2018a). Neuropeptides and other small neuronal transmitters are key for the control of the neuromuscular activity, beside performing additional functions in metabolism and nutrient transport (reviewed by Ribeiro et al. 2005 and Marks and Maule 2010); therefore, by disrupting neural signaling, disabling effects on vital functions such as locomotion, attachment, feeding, or sensory perception can be achieved. Neurotransmitter transporters, transmitter-activated ion channels receptors, and G protein-coupled receptors (GPCRs) are known targets for a number of neuroactive compounds and have a well-contrasted druggability potential, thus becoming promising drug targets for anthelmintic development [see McVeigh et al. (2011, 2018b), Ribeiro and Patocka (2013) and Greenberg (2014) for reviews].

Targeting the reproductive function represents also an interesting treatment option for control purposes since it impacts directly on life cycle progression and parasite transmission. Moreover, this approach is particularly interesting to combat the egg-associated pathology of schistosomiasis (Beckmann et al. 2010b), which is reflected in the great number of publications on this subject that have arisen in the past few years (see Sect. 1.8.2). Several proteins have been proposed as potential therapeutic targets based on transcriptional profiling and/or functional evidence (e.g., Beckmann et al. 2010b; Buro et al. 2013; Andrade et al. 2014; Lu et al. 2016; Hahnel et al. 2018; McVeigh et al. 2018a). Among these options, GPCRs displaying essential functions in trematode reproductive and developmental biology (Hahnel et al. 2018; McVeigh et al. 2018a) are excellent candidates for applied studies due to their great structural diversity and pharmacological properties that enable the design of highly selective ligands (reviewed by McVeigh et al. 2018b).

On the other hand, although the inactivation and elimination of injurious compounds are key functions of the excretory system of all animals, xenobiotic-metabolizing enzymes and, specially, drug transporters are known to contribute to low drug efficacy and the development of anthelminthic resistance (Kotze et al. 2014; Matoušková et al. 2016). Biotransformation enzymes, such as cytochrome P450 or glutathione S-transferase, are potential drug targets due to their essential roles in parasite metabolism (reviewed by Matoušková et al. 2016). In turn, targeting drug transporters may represent an opportunity to increase the efficacy of other anthelmintics (reviewed by Kotze et al. 2014). Aside performing fundamental functions for parasite biology, these groups of proteins display additional desirable properties for being considered as potential therapeutic targets (Kotze et al. 2014; Matoušková et al. 2016).

In addition to the abovementioned biological functions, the maintenance of the structural integrity of the tegument is vital for trematodes; therefore, this organ also represents a prominent target for the rational development of flukicide drugs and, more important, of anti-helminth vaccines (see Leow et al. 2015 and Sotillo et al. 2017 for reviews).

References

Andrade LF, Mourao Mde M, Geraldo JA, Coelho FS, Silva LL, Neves RH, Volpini A, Machado-Silva JR, Araujo N, Nacif-Pimenta R, Caffrey CR, Oliveira G (2014) Regulation of *Schistosoma mansoni* development and reproduction by the mitogen-activated protein kinase signaling pathway. PLoS Negl Trop Dis 8:e2949

Antonelli L, Quilichini Y, Foata J, Marchand B (2014) Topography and ultrastructure of the tegument of *Aphallus tubarium* (Rodolphi, 1819) Poche, 1926 (Digenea: Cryptogonimidae), intestinal parasite of the common *Dentex dentex* (Linnaeus 1758) from Valinco Gulf. Acta Parasitol 59:615–624

Anuracpreeda P, Phutong S, Ngamniyom A, Panyarachun B, Sobhon P (2015) Surface topography and ultrastructural architecture of the tegument of adult *Carmyerius spatiosus* Brandes, 1898. Acta Trop 143:18–28

Anuracpreeda P, Chawengkirttikul R, Sobhon P (2016) Surface histology, topography, and ultrastructure of the tegument of adult *Orthocoelium parvipapillatum* (Stiles & Goldberger, 1910). Parasitol Res 115:2757–2769

Bakhoum AJ, Quilichini Y, Justine JL, Bray RA, Ba CT, Marchand B (2015) Ultrastructural study of sperm cells in Acanthocolpidae: the case of *Stephanostomum murielae* and *Stephanostomoides tenuis* (Digenea). Peer J 3:e744

Bakhoum AJS, Miquel J, Ndiaye PI, Justine JL, Falchi A, Ba CT, Marchand B, Quilichini Y (2017) Advances in spermatological characters in the Digenea: review and proposal of spermatozoa models and their phylogenetic importance. Adv Parasitol 98:111–165

Barker LR, Bueding E, Timms AR (1966) The possible role of acetylcholine in *Schistosoma mansoni*. Br J Pharmacol Chemother 26:656–665

Beckmann S, Buro C, Dissous C, Hirzmann J, Grevelding CG (2010a) The Syk kinase SmTK4 of *Schistosoma mansoni* is involved in the regulation of spermatogenesis and oogenesis. PLoS Pathog 6:e1000769

Beckmann S, Quack T, Burmeister C, Buro C, Long T, Dissous C, Grevelding CG (2010b) *Schistosoma mansoni*: signal transduction processes during the development of the reproductive organs. Parasitology 137:497–520

Beckmann S, Hahnel S, Cailliau K, Vanderstraete M, Browaeys E, Dissous C, Grevelding CG (2011) Characterization of the Src/Abl hybrid kinase SmTK6 of *Schistosoma mansoni*. J Biol Chem 286:42325–42336

Beckmann S, Quack T, Dissous C, Cailliau K, Lang G, Grevelding CG (2012) Discovery of platyhelminth-

specific alpha/beta-integrin families and evidence for their role in reproduction in *Schistosoma mansoni*. PLoS One 7:e52519

Beklemishev WN (1969) Origin and development of the excretory apparatus. In: Kabata Z (ed) Principles of comparative anatomy of invertebrates, vol 2. Oliver & Boyd, Edinburgh, pp 327–352

Bernal D, Trelis M, Montaner S, Cantalapiedra F, Galiano A, Hackenberg M, Marcilla A (2014) Surface analysis of *Dicrocoelium dendriticum*. The molecular characterization of exosomes reveals the presence of miRNAs. J Proteomics 105:232–241

Bogitsh BJ, Carter CE, Oeltmann TN (2013) General characteristics of the Trematoda. In: Bogitsh BJ, Carter CE, Oeltmann TN (eds) Human parasitology, 4th edn. Academic Press, Waltham, pp 153–178

Born-Torrijos A, Holzer AS, Raga JA, van Beest GS, Yoneva A (2017) Description of embryonic development and ultrastructure in miracidia of *Cardiocephaloides longicollis* (Digenea, Strigeidae) in relation to active host finding strategy in a marine environment. J Morphol 278:1137–1148

Buro C, Oliveira KC, Lu Z, Leutner S, Beckmann S, Dissous C, Cailliau K, Verjovski-Almeida S, Grevelding CG (2013) Transcriptome analyses of inhibitor-treated schistosome females provide evidence for cooperating Src-kinase and TGFbeta receptor pathways controlling mitosis and eggshell formation. PLoS Pathog 9:e1003448

Buro C, Burmeister C, Quack T, Grevelding CG (2017) Identification and first characterization of SmEps8, a potential interaction partner of SmTK3 and SER transcribed in the gonads of *Schistosoma mansoni*. Exp Parasitol 180:55–63

Carneiro VC, de Abreu da Silva IC, Torres EJ, Caby S, Lancelot J, Vanderstraete M, Furdas SD, Jung M, Pierce RJ, Fantappie MR (2014) Epigenetic changes modulate schistosome egg formation and are a novel target for reducing transmission of schistosomiasis. PLoS Pathog 10:e1004116

Chaiyadet S, Sotillo J, Smout M, Cantacessi C, Jones MK, Johnson MS, Turnbull L, Whitchurch CB, Potriquet J, Laohaviroj M, Mulvenna J, Brindley PJ, Bethony JM, Laha T, Sripa B, Loukas A (2015) Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. J Infect Dis 212:1636–1645

Chan JD, Acharya S, Day TA, Marchant JS (2016a) Pharmacological profiling an abundantly expressed schistosome serotonergic GPCR identifies nuciferine as a potent antagonist. Int J Parasitol Drugs Drug Resist 6:364–370

Chan JD, McCorvy JD, Acharya S, Johns ME, Day TA, Roth BL, Marchant JS (2016b) A miniaturized screen of a *Schistosoma mansoni* serotonergic G protein-coupled receptor identifies novel classes of parasite-selective inhibitors. PLoS Pathog 12:e1005651

Collins JJ III, Wendt GR, Iyer H, Newmark PA (2016) Stem cell progeny contribute to the schistosome host-parasite interface. Elife 5:e12473

Cortés A, Sotillo J, Muñoz-Antolí C, Molina-Durán J, Esteban JG, Toledo R (2017) Antibody trapping: a novel mechanism of parasite immune evasion by the trematode *Echinostoma caproni*. PLoS Negl Trop Dis 11:e0005773

Cwiklinski K, Dalton JP, Dufresne PJ, La Course J, Williams DJ, Hodgkinson J, Paterson S (2015a) The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biol 16:71

Cwiklinski K, de la Torre-Escudero E, Trelis M, Bernal D, Dufresne PJ, Brennan GP, O'Neill S, Tort J, Paterson S, Marcilla A, Dalton JP, Robinson MW (2015b) The extracellular vesicles of the helminth pathogen, *Fasciola hepatica*: biogenesis pathways and cargo molecules involved in parasite pathogenesis. Mol Cell Proteomics 14:3258–3273

Da'dara AA, Bhardwaj R, Ali YB, Skelly PJ (2014) Schistosome tegumental ecto-apyrase (SmATPDase1) degrades exogenous pro-inflammatory and pro-thrombotic nucleotides. Peer J 2:e316

de Abreu da Silva IC, Carneiro VC, Vicentino AR, Aguilera EA, Mohana-Borges R, Thiengo S, Fernandez MA, Fantappie MR (2016) The distinct C-terminal acidic domains of HMGB proteins are functionally relevant in *Schistosoma mansoni*. Int J Parasitol 46:253–262

Ding H, Liu F, Zhu L, Wu F, Liu Q, He S, Shao W, Du Y, Ren C, Shen J, Liu M (2017) Tyrosine kinase 4 is involved in the reproduction of the platyhelminth parasite *Schistosoma japonicum*. Parasit Vectors 10:498

Dittman JS, Kaplan JM (2008) Behavioral impact of neurotransmitter-activated G-protein-coupled receptors: muscarinic and GABAB receptors regulate *Caenorhabditis elegans* locomotion. J Neurosci 28:7104–7112

El-Sakkary N, Chen S, Arkin MR, Caffrey CR, Ribeiro P (2018) Octopamine signaling in the metazoan pathogen *Schistosoma mansoni*: localization, small-molecule screening and opportunities for drug development. Dis Model Mech 11:dmm033563. https://doi.org/10.1242/dmm.033563

El-Shehabi F, Ribeiro P (2010) Histamine signalling in *Schistosoma mansoni*: immunolocalisation and characterisation of a new histamine-responsive receptor (SmGPR-2). Int J Parasitol 40:1395–1406

El-Shehabi F, Taman A, Moali LS, El-Sakkary N, Ribeiro P (2012) A novel G protein-coupled receptor of *Schistosoma mansoni* (SmGPR-3) is activated by dopamine and is widely expressed in the nervous system. PLoS Negl Trop Dis 6:e1523

Eriksson KS, Johnston RN, Shaw C, Halton DW, Panula PA (1996) Widespread distribution of histamine in the nervous system of a trematode flatworm. J Comp Neurol 373:220–227

Faghiri Z, Skelly PJ (2009) The role of tegumental aquaporin from the human parasitic worm, *Schistosoma mansoni*, in osmoregulation and drug uptake. FASEB J 23:2780–2789

Ferreira LG, Oliva G, Andricopulo AD (2015) Target-based molecular modeling strategies for schistosomiasis drug discovery. Future Med Chem 7:753–764

Filippi JJ, Quilichini Y, Marchand B (2013) Topography and ultrastructure of the tegument of *Deropristis inflata* Molin, 1859 (Digenea: Deropristidae), a parasite of the European eel *Anguilla anguilla* (Osteichthyes: Anguillidae). Parasitol Res 112:517–528

Fletcher HL, Hoey EM, Orr N, Trudgett A, Fairweather I, Robinson MW (2004) The occurrence and significance of triploidy in the liver fluke, *Fasciola hepatica*. Parasitology 128:69–72

Fried B (1997) An overview of the biology of trematodes. In: Fried B, Graczyk TK (eds) Advances in trematode biology. CRC, Boca Raton, pp 1–30

Fried B, Haseeb MA (1991) Platyhelminthes, Aspidogastrea, Monogenea, and Digenea. In: Harrison FW, Bogitsh BJ (eds) Microscopic anatomy of invertebrates, vol 3. Wiley and Liss, New York, pp 141–209

Geadkaew A, von Bulow J, Beitz E, Tesana S, Vichasri Grams S, Grams R (2015) Bi-functionality of *Opisthorchis viverrini* aquaporins. Biochimie 108:149–159

Gelmedin V, Morel M, Hahnel S, Cailliau K, Dissou SC, Grevelding CG (2017) Evidence for integrin - venus kinase receptor 1 alliance in the ovary of *Schistosoma mansoni* females controlling cell survival. PLoS Pathog 13:e1006147

Greani S, Quilichini Y, Marchand B (2016) Ultrastructural study of vitellogenesis and oogenesis of *Crepidostomum metoecus* (Digenea, Allocreadiidae), intestinal parasite of *Salmo trutta* (Pisces, Teleostei). Parasite 23:47

Greenberg RM (2013) ABC multidrug transporters in schistosomes and other parasitic flatworms. Parasitol Int 62:647–653

Greenberg RM (2014) Ion channels and drug transporters as targets for anthelmintics. Curr Clin Microbiol Rep 1:51–60

Hahnel S, Parker-Manuel R, Dissous C, Cailliau K, Grevelding CG (2017) First characterization of SmOPG1, a novel protein involved in gonad-associated processes in *Schistosoma mansoni*. Mol Biochem Parasitol 213:22–25

Hahnel S, Wheeler N, Lu Z, Wangwiwatsin A, McVeigh P, Maule A, Berriman M, Day T, Ribeiro P, Grevelding CG (2018) Tissue-specific transcriptome analyses provide new insights into GPCR signalling in adult *Schistosoma mansoni*. PLoS Pathog 14:e1006718

Halton DW (2004) Microscopy and the helminth parasite. Micron 35:361–390

Halton DW, Maule AG (2004) Flatworm nerve-muscle: structural and functional analysis. Can J Zool 82:316–333

Han Q, Jia B, Hong Y, Cao X, Zhai Q, Lu K, Li H, Zhu C, Fu Z, Shi Y, Lin J (2017) Suppression of VAMP2 alters morphology of the tegument and affects glucose uptake, development and reproduction of *Schistosoma japonicum*. Sci Rep 7:5212

Hanna RE, Moffett D, Forster FI, Trudgett AG, Brennan GP, Fairweather I (2016) *Fasciola hepatica*: a light and electron microscope study of the ovary and of the development of oocytes within eggs in the uterus provides an insight into reproductive strategy. Vet Parasitol 221:93–103

Hertel LA (1993) Excretion and osmoregulation in the flatworms. Trans Am Microsc Soc 112:10–17

Holmes SD, Fairweather I (1984) *Fasciola hepatica*: the effects of neuropharmacological agents upon *in vitro* motility. Exp Parasitol 58:194–208

Huang Y, Chen W, Wang X, Liu H, Chen Y, Guo L, Luo F, Sun J, Mao Q, Liang P, Xie Z, Zhou C, Tian Y, Lv X, Huang L, Zhou J, Hu Y, Li R, Zhang F, Lei H, Li W, Hu X, Liang C, Xu J, Li X, Yu X (2013) The carcinogenic liver fluke, *Clonorchis sinensis*: new assembly, reannotation and analysis of the genome and characterization of tissue transcriptomes. PLoS One 8:e54732

Huang Y, Li W, Lu W, Xiong C, Yang Y, Yan H, Liu KC, Cao P (2016) Cloning and in vitro characterization of a *Schistosoma japonicum* aquaglyceroporin that functions in osmoregulation. Sci Rep 6:35030

Kasinathan RS, Sharma LK, Cunningham C, Webb TR, Greenberg RM (2014) Inhibition or knockdown of ABC transporters enhances susceptibility of adult and juvenile schistosomes to praziquantel. PLoS Negl Trop Dis 8:e3265

Keiser J, Utzinger J (2007) Advances in the discovery and development of trematocidal drugs. Expert Opin Drug Discov 2(sup1):S9–S23

Kotze AC, Hunt PW, Skuce P, von Samson-Himmelstjerna G, Martin RJ, Sager H, Krücken J, Hodgkinson J, Lespine A, Jex AR, Gilleard JS, Beech RN, Wolstenholme AJ, Demeler J, Robertson AP, Charvet CL, Neveu C, Kaminsky R, Rufener L, Alberich M, Menez C, Prichard RK (2014) Int J Parasitol Drugs Drug Resist 4:164–184

Kelley JM, Elliott TP, Beddoe T, Anderson G, Skuce P, Spithill TW (2016) Current threat of triclabendazole resistance in *Fasciola hepatica*. Trends Parasitol 32:458–469

Krupenko DY, Dobrovolskij AA (2015) Somatic musculature in trematode hermaphroditic generation. BMC Evol Biol 15:189

Krupenko D, Gonchar A (2017a) Musculature arrangement and locomotion in notocotylid cercariae (Digenea: Notocotylidae) from mud snail *Ecrobia ventrosa* (Montagu, 1803). Parasitol Int 66:262–271

Krupenko D, Gonchar A (2017b) Ventral concavity and musculature arrangement in notocotylid maritae (Digenea: Notocotylidae). Parasitol Int 66:660–665

Kumkate S, Chunchob S, Janvilisri T (2008) Expression of ATP-binding cassette multidrug transporters in the giant liver fluke *Fasciola gigantica* and their possible involvement in the transport of bile salts and anthelmintics. Mol Cell Biochem 317:77–84

Leow CY, Willis C, Hofmann A, Jones MK (2015) Structure-function analysis of apical membrane-associated molecules of the tegument of schistosome parasites of humans: prospects for identification of

novel targets for parasite control. Br J Pharmacol 172:1653–1663

Levron C, Ternengo S, Marchand B (2004) Spermiogenesis and sperm ultrastructure of *Poracanthium furcatum* (Digenea, Opecoelidae), a parasite of *Mullus surmuletus* (Pisces, Teleostei). Acta Parasitol 49:190–200

Li XH, de Castro-Borges W, Parker-Manuel S, Vance GM, Demarco R, Neves LX, Evans GJ, Wilson RA (2013) The schistosome oesophageal gland: initiator of blood processing. PLoS Negl Trop Dis 7:e2337

Li XH, Stark M, Vance GM, Cao JP, Wilson RA (2014) The anterior esophageal region of *Schistosoma japonicum* is a secretory organ. Parasit Vectors 7:565

Li XH, DeMarco R, Neves LX, James SR, Newling K, Ashton PD, Cao JP, Wilson RA, Castro-Borges W (2018) Microexon gene transcriptional profiles and evolution provide insights into blood processing by the *Schistosoma japonicum* esophagus. PLoS Negl Trop Dis 12:e0006235

Littlewood DTJ, Bray RA, Waeschenbach A (2015) Phylogenetic patterns of diversity in cestodes and trematodes. In: Morand S, Krasnov B, Littlewood DTJ (eds) Parasite diversity and diversification: evolutionary ecology meets phylogenetics. Cambridge University Press, Cambridge, pp 304–319

Lu Z, Sessler F, Holroyd N, Hahnel S, Quack T, Berriman M, Grevelding CG (2016) Schistosome sex matters: a deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. Sci Rep 6:31150

MacDonald K, Buxton S, Kimber MJ, Day TA, Robertson AP, Ribeiro P (2014) Functional characterization of a novel family of acetylcholine-gated chloride channels in *Schistosoma mansoni*. PLoS Pathog 10:e1004181

MacDonald K, Kimber MJ, Day TA, Ribeiro P (2015) A constitutively active G protein-coupled acetylcholine receptor regulates motility of larval *Schistosoma mansoni*. Mol Biochem Parasitol 202:29–37

Marcilla A, Trelis M, Cortés A, Sotillo J, Cantalapiedra F, Mínguez MT, Valero ML, Sánchez del Pino MM, Muñoz-Antolí C, Toledo R, Bernal D (2012) Extracellular vesicles from parasitic helminths contain specific excretory/secretory proteins and are internalized in intestinal host cells. PLoS One 7:e45974

Marks NJ, Maule AG (2010) Neuropeptides in helminths: occurrence and distribution. In: Geary TG, Maule AG (eds) Neuropeptide systems as targets for parasite and pest control. Springer Nature, Gewerbestrasse, pp 49–77

Matoušková P, Vokřál I, Lamka J, Skálová L (2016) The role of xenobiotic-metabolizing enzymes in anthelmintic deactivation and resistance in helminths. Trends Parasitol 32:481–491

McVeigh P, Atkinson L, Marks NJ, Mousley A, Dalzell JJ, Sluder A, Hammerland L, Maule AG (2011) Parasite neuropeptide biology: seeding rational drug target selection? Int J Parasitol Drugs Drug Resist 2:76–91

McVeigh P, McCammick E, McCusker P, Wells D, Hodgkinson J, Paterson S, Mousley A, Marks NJ, Maule AG (2018a) Profiling G protein-coupled receptors of *Fasciola hepatica* identifies orphan rhodopsins unique to phylum Platyhelminthes. Int J Parasitol Drugs Drug Resist 8:87–103

McVeigh P, McCusker P, Robb E, Wells D, Gardiner E, Mousley A, Marks NJ, Maule AG (2018b) Reasons to be nervous about flukicide discovery. Trends Parasitol 34:184–196

Messerli SM, Kasinathan RS, Morgan W, Spranger S, Greenberg RM (2009) *Schistosoma mansoni* P-glycoprotein levels increase in response to praziquantel exposure and correlate with reduced praziquantel susceptibility. Mol Biochem Parasitol 167:54–59

Miquel J, Nourrisson C, Marchand B (2000) Ultrastructure of spermiogenesis and the spermatozoon of *Opecoeloides furcatus* (Trematoda, Digenea, Opecoelidae), a parasite of *Mullus barbatus* (Pisces, Teleostei). Parasitol Res 86:301–310

Mordvinov VA, Ershov NI, Pirozhkova DS, Pakharukov YV, Pakharukova MY (2017) ABC transporters in the liver fluke *Opisthorchis felineus*. Mol Biochem Parasitol 216:60–68

Ndiaye PI, Quilichini Y, Marigo AM, Ba CT, Tkach VV, Marchand B (2017) Ultrastructural characteristics of the mature spermatozoon of the digenean *Sclerodistomum italicum* (Stossich, 1893) (Hemiuroidea, Sclerodistomidae) intestinal parasite of *Hypocanthus amia* (Teleostei, Carangidae). Tissue Cell 49:15–21

Nollen PM (1997) Reproductive physiology and behavior of digenetic trematodes. In: Fried B, Graczyk TK (eds) Advances in trematode biology. CRC, Boca Raton, pp 117–147

Pakharukova MY, Vavilin VA, Sripa B, Laha T, Brindley PJ, Mordvinov VA (2015) Functional analysis of the unique cytochrome P450 of the liver fluke *Opisthorchis felineus*. PLoS Negl Trop Dis 9:e0004258

Patocka N, Ribeiro P (2013) The functional role of a serotonin transporter in *Schistosoma mansoni* elucidated through immunolocalization and RNA interference (RNAi). Mol Biochem Parasitol 187:32–42

Patocka N, Sharma N, Rashid M, Ribeiro P (2014) Serotonin signaling in *Schistosoma mansoni*: a serotonin-activated G protein-coupled receptor controls parasite movement. PLoS Pathog 10:e1003878

Peoples RC, Fried B (2014) Form and function in the Digenea. In: Toledo R, Fried B (eds) Digenetic trematodes, 1st edn. Springer, New York, pp 3–20

Petrov A, Podvyaznaya I (2016) Muscle architecture during the course of development of *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Trematoda, Diplostomidae) from cercariae to metacercariae. J Helminthol 90:321–336

Pinto-Almeida A, Mendes T, Armada A, Belo S, Carrilho E, Viveiros M, Afonso A (2015) The role of efflux pumps in *Schistosoma mansoni* praziquantel resistant phenotype. PLoS One 10:e0140147

Piratae S, Tesana S, Jones MK, Brindley PJ, Loukas A, Lovas E, Eursitthichai V, Sripa B, Thanasuwan S, Laha T (2012) Molecular characterization of a tetraspanin

from the human liver fluke, *Opisthorchis viverrini*. PLoS Negl Trop Dis 6:e1939

Podvyaznaya IM (2006) An ultrastructural study of alimentary tract development in the cercariae of *Diplostomum pseudospathaceum* (Digenea: Diplostomidae). Parasitol Res 99:362–367

Podvyaznaya I (2011) An ultrastructural study of alimentary tract development in the cercariae of *Prosorhynchoides borealis* (Digenea, Bucephalidae). *Acta Zool* 92:170–178

Protasio AV, van Dongen S, Collins J, Quintais L, Ribeiro DM, Sessler F, Hunt M, Rinaldi G, Collins JJ, Enright AJ, Berriman M (2017) MiR-277/4989 regulate transcriptional landscape during juvenile to adult transition in the parasitic helminth *Schistosoma mansoni*. PLoS Negl Trop Dis 11:e0005559

Quilichini Y, Foata J, Justine JL, Bray RA, Marchand B (2010) Ultrastructural study of the spermatozoon of *Heterolebes maculosus* (Digenea, Opistholebetidae), a parasite of the porcupinefish *Diodon hystrix* (Pisces, Teleostei). Parasitol Int 59:427–434

Ribeiro P, El-Shehabi F, Patocka N (2005) Classical transmitters and their receptors in flatworms. Parasitology 131:S19–S40

Ribeiro P, Patocka N (2013) Neurotransmitter transporters in schistosomes: structure, function and prospects for drug discovery. Parasitol Int 62:629–638

Roberts LS, Janovy J Jr (2009) Trematoda: form, function, and classification of Digeneans. In: Roberts LS, Janovy J Jr (eds) Foundations of parasitology, 8th edn. McGraw-Hill, Dubuque, pp 219–245

Sato H, Kusel JR, Thornhill J (2004) Excretion of fluorescent substrates of mammalian multidrug resistance-associated protein (MRP) in the *Schistosoma mansoni* excretory system. Parasitology 128:43–52

Skelly PJ, Wilson AR (2006) Making sense of the schistosome surface. Adv Parasitol 63:185–284

Sotillo J, Doolan D, Loukas A (2017) Recent advances in proteomic applications for schistosomiasis research: potential clinical impact. Exp Rev Proteomics 14: 171–183

Świderski Z, Montoliu I, Feliu C, Gibson DI, Miquel J (2013) A transmission electron microscopical study of the tegument of *Maritrema feliui* (Digenea: Microphallidae). Acta Parasitol 58:478–485

Takamiya S, Fukuda K, Nakamura T, Aoki T, Sugiyama H (2010) *Paragonimus westermani* possesses aerobic and anaerobic mitochondria in different tissues, adapting to fluctuating oxygen tension in microaerobic habitats. Int J Parasitol 40:1651–1658

Thanasuwan S, Piratae S, Brindley PJ, Loukas A, Kaewkes S, Laha T (2014) Suppression of aquaporin, a mediator of water channel control in the carcinogenic liver fluke, *Opisthorchis viverrini*. Parasit Vectors 7:224

Tkach VV, Achatz TJ, Hildebrand J, Greiman SE (2018) Convoluted history and confusing morphology: molecular phylogenetic analysis of dicrocoeliids reveals true systematic position of the Anenterotrematidae Yamaguti, 1958 (Platyhelminthes, Digenea). Parasitol Int 67:501–508

Tolstenkov OO, Terenina NB, Serbina EA, Kreshchenko ND, Maklakova L, Jashina A (2010) The spatial relationship between the musculature and the 5-HT and FMRFamide immunoreactivities in cercaria, metacercaria and adult *Opisthorchis felineus* (Digenea). Acta Parasitol 55:123–132

Tran MH, Freitas TC, Cooper L, Gaze S, Gatton ML, Jones MK, Lovas E, Pearce EJ, Loukas A (2010) Suppression of mRNAs encoding tegument tetraspanins from *Schistosoma mansoni* results in impaired tegument turnover. PLoS Pathog 6:e1000840

Travassos L (1944) [Revisão da familia Dicrocoeliidae Odhner, 1910] *Imprensa Nacional* (in Portuguese)

Travassos L (1955) Sôbre dois novos Dicrocoeliidae de Chiroptera. An Acad Bras Cienc 27:561–565. (in Portuguese)

Vale N, Gouveia MJ, Rinaldi G, Brindley PJ, Gärtner F, Correia da Costa JM (2017) Praziquantel for schistosomiasis: single-drug metabolism revisited, mode of action, and resistance. Antimicrob Agents Chemother 61:e02582–16

van Herwerden L, Blair D, Agatsuma T (1999) Genetic diversity in parthenogenetic triploid *Paragonimus westermani*. Int J Parasitol 29:1477–1482

Vanderstraete M, Gouignard N, Cailliau K, Morel M, Hahnel S, Leutner S, Beckmann S, Grevelding CG, Dissous C (2014) Venus kinase receptors control reproduction in the platyhelminth parasite *Schistosoma mansoni*. PLoS Pathog 10:e1004138

Vicogne J, Pin JP, Lardans V, Capron M, Noel C, Dissous C (2003) An unusual receptor tyrosine kinase of *Schistosoma mansoni* contains a Venus Flytrap module. Mol Biochem Parasitol 126:51–62

Whitfield PJ, Evans NA (1983) Parthenogenesis and asexual multiplication among parasitic platyhelminths. Parasitology 86:121–160

Wilkinson R, Law CJ, Hoey EM, Fairweather I, Brennan GP, Trudgett A (2012) An amino acid substitution in *Fasciola hepatica* P-glycoprotein from triclabendazole-resistant and triclabendazole-susceptible populations. Mol Biochem Parasitol 186:69–72

Wilson RA, Li XH, MacDonald S, Neves LX, Vitoriano-Souza J, Leite LC, Farias LP, James S, Ashton PD, DeMarco R, Castro-Borges W (2015) The schistosome esophagus is a 'hotspot' for microexon and lysosomal hydrolase gene expression: implications for blood processing. PLoS Negl Trop Dis 9:e0004272

Wu C, Hou N, Piao X, Liu S, Cai P, Xiao Y, Chen Q (2015) Non-immune immunoglobulins shield *Schistosoma japonicum* from host immunorecognition. Sci Rep 5:13434

Xiong Y, Zhang M, Hong Y, Wei M, Ai D, Meng P, Han Y, Fu Z, Shi Y, Yang J, Lin J (2013) Characterization analysis of *Schistosoma japonicum* plasma membrane repair relative gene myoferlin. PLoS One 8:e66396

Young ND, Nagarajan N, Lin SJ, Korhonen PK, Jex AR, Hall RS, Safavi-Hemami H, Kaewkong W, Bertrand D, Gao S, Seet Q, Wongkham S, The BT, Wongkham C,

Intapan PM, Maleewong W, Yang X, Hu M, Wang Z, Hofmann A, Sternberg PW, Tan P, Wang J, Gasser RB (2014) The *Opisthorchis viverrini* genome provides insights into life in the bile duct. Nat Commun 5:4378

Zhu L, Zhao J, Wang J, Hu C, Peng J, Luo R, Zhou C, Liu J, Lin J, Jin Y, Davis RE, Cheng G (2016) MicroRNAs are involved in the regulation of ovary development in the pathogenic blood fluke *Schistosoma japonicum*. PLoS Pathog 12:e1005423