Rokkam Madhavi · Rodney A. Bray

Digenetic Trematodes of Indian Marine Fishes

Springer

Digenetic Trematodes of Indian Marine Fishes

Rokkam Madhavi · Rodney A. Bray

Digenetic Trematodes of Indian Marine Fishes

Springer

Rokkam Madhavi
Department of Zoology
Andhra University
Visakhapatnam, Andhra Pradesh
India

Rodney A. Bray
Life Sciences
Natural History Museum
London
UK

ISBN 978-94-024-1533-9 ISBN 978-94-024-1535-3 (eBook)
https://doi.org/10.1007/978-94-024-1535-3

Library of Congress Control Number: 2018941991

Printed on acid-free paper

This Springer imprint is published by the registered company Springer Nature B.V.
The registered company address is: Van Godewijckstraat 30, 3311 GX Dordrecht, The Netherlands

Preface

The present century is witnessing a sharp decline in taxonomy research and an acute dearth of taxonomic expertise all over the world, and this has been the cause of much concern to scientists. The reasons for this crisis are many, a major one being the difficulties in getting access to the literature which is making taxonomic research strenuous and unattractive to the younger workers. In particular, the taxonomy of digeneans of marine fishes has received lot of attention and India has produced many eminent taxonomists, not to speak of the large number of publications. A glance at the literature reveals, however, that it is widely scattered and some of it published in remote journals is not readily accessible to the taxonomists. The introduction of molecular methods in taxonomy has given a slight boost to taxonomic research but molecular taxonomy cannot be taken as a replacement to traditional taxonomy, it can only be an adjunct to it. Application of both basic and modern tools together in taxonomy makes it a strong and attractive field.

To revive the research interest in taxonomy, emphasis should be placed on the publication of monographs, checklists and reviews and databases. Concerted efforts of scientists from public and private institutions are needed to ignite the spirit of taxonomy in the young scientists. Keeping these facts in view, we have brought out this monograph on digenetic trematodes of Indian marine fishes. Almost all the species of digeneans so far recorded, numbering over 600, are covered in this monograph. For each species information on hosts, localities, geographic distribution, references and a brief description accompanied by an illustration are furnished. A host-parasite list is also presented. The pertinent areas for future research are indicated. We are hoping that taxonomists working in this field will find this manual to be a useful guide.

We have taken help directly or indirectly from a number of scientists in the context of preparing this monograph. The publications of Dr. Gibson, Dr. Cribb, Dr. Overstreet, Dr. Justine have proved to be extremely useful. We are thankful to them for providing the literature whenever it is needed. Madhavi extends her grateful thanks to a number of Indian scientists without whose help this monograph would not have been completed. She is particularly grateful to Prof. K. C. Pandey and Nirupama Agrawal who supplied most of the Indian literature and to the director of Zoological Survey of India for providing the holotype slides and the literature. She is thankful to her former research students Dr. Shameem, Dr. Umadevi, Dr. Janaki Ram, Dr. Jayasree and Dr. Sailaja for their strong support and continuous help in bringing the manual to the final stage. She remains for ever grateful to her teacher and guide late Professor K. Hanumantha Rao who introdued her to this field and offered valuble guidane and support. Finally, the financial assistance provided by funding agencies like UGC, CSIR, MOEF and DST is gratefully acknowledged.

The textbook includes quite a good number of figures taken from different journals. In this context, the authors express their grateful thanks to the publishers: Allen Press, Cambridge University Press, Springer and De Gruyter and the directors of Zoological Survey of India, *Pakistan Journal of Zoology* and Indian Society of Parasitology for permitting reproduction of figures published in their journals.

Visakhapatnam, India — Rokkam Madhavi
London, UK — Rodney A. Bray

Contents

Chapter 1
Introduction

Marine fishes by virtue of their inherent characteristics such as large body size, varied diet, high vagility and wide geographic distribution serve as hosts for a wide range of parasites belonging to diverse groups and occupying different niches as ecto- and endoparasites. The role of different species of marine fish as hosts also varies depending on a multitude of ecological and host factors. Based on the overwhelming number of parasite species recorded from marine fishes, it is assumed that the number of parasites far exceeds the number of species of marine fish. The ubiquitous nature of these parasites also indicates that they will have some commercial importance in fisheries and aquaculture. On the positive side, they can be used as biological tags for stock discrimination (Mackenzie, 2002) and they are also receiving importance as indicators in environmental monitoring. On the negative side, many parasites are severe pathogens of commercially important fishes and cause mass mortalities especially under culture conditions.

A vast literature now exists on marine fish parasites dealing with a variety of aspects including taxonomy, life cycles, ecology and host–parasite relationships. The main focus in research has been on taxonomic aspects devoted to morphological descriptions, identification up to species level and analysis of phylogenetic relationships of different taxa of marine parasites. In recent years, taxonomic research has made rapid advances because of the application of molecular methods.

So far as the Indian scenario is concerned, marine fish parasites have not received due recognition. Studies are yet to be undertaken on a large scale. India has a vast coastline of about 7520 km spread along the margins of the Bay of Bengal on the east coast and the Arabian Sea on the west coast. These two coasts join at the Gulf of Mannar, situated between India and Sri Lanka. Additionally, there are a few island territories such as the Andaman and Nicobar archipelagoes in the Bay of Bengal and Lakshadweep in the Arabian Sea (Fig. 1.1). There are 9 coastal states, 5 along the west coast namely Mumbai, Gujarat, Karnataka, Kerala and Goa and 4 along the east coast namely West Bengal, Orissa, Andhra Pradesh and Chennai (Madras coast). The coastal waters support a rich and diverse fish fauna. It is estimated that there are about 2564 species of marine fish inhabiting Indian coastal waters. Many

R. Madhavi and R. Bray, *Digenetic Trematodes of Indian Marine Fishes*,
https://doi.org/10.1007/978-94-024-1535-3_1

Fig. 1.1 Map of India showing the various coastal areas

institutions and universities situated in coastal districts are undertaking investigations on various aspects of marine biology on a large scale. One shortcoming in these studies is the lack of contributions to Marine Parasitology, although it is well recognized that knowledge on this aspect is very important and should form an integral part of Marine Biological studies. It is well known that a wide range of parasites belonging to Protozoa, Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala, Myxozoa and Crustacea infect marine fishes. Studies undertaken so far are confined to morphological aspects. Knowledge of the life cycles, pathogenicity and ecology is meagre. The present knowledge of marine parasites is at a basic level.

Among the various groups of parasites infecting marine fishes, the digeneans have received more attention, because of their common occurrence in marine fishes and the great diversity exhibited by them in their morphology and the life cycle patterns. They occur in a variety of sites, exhibiting rather variable, but often strict host specificity for each species or higher group of fishes. Approximately 18,000 nominal species are reported worldwide including well over 5,000 species from marine fishes alone (Cribb, 2005b), constituting perhaps the largest group of metazoan parasites (Cribb et al. 2001). A huge literature exists now on digenean parasites of marine fishes. The volumes on "Keys to the Trematoda" edited by Gibson, Bray and Jones served as a boon to scientists working on Digenean taxonomy. The contributions made by scientists of yesteryears, e.g. Yamaguti, Manter, Dawes, and the present century, e.g. Gibson, Bray, Cribb, Overstreet, are substantial.

Historical account: Research on Digenea of marine fishes in India has a long history. Investigations were initiated in 1906 but the major work commenced in the 1930s. Early workers like Lühe (1906) and Southwell (1913) identified a few digeneans collected from marine fish of Gulf of Mannar. The first detailed study was that of Srivastava (1936–1941) who published a series of papers on digeneans of marine fishes from the east and west coasts of India and reported several new species. Another major contribution was by Chauhan (1943, 1945) who reported digeneans belonging to Bucephalidae and Hemiuridae and published monographs on these groups (Chauhan, 1954a, b). After a gap of nearly 20 years, a comprehensive study of digenean parasites of marine fishes was undertaken by Hafeezullah (Hafeezullah and Siddiqi, 1970; Hafeezullah, 1971–1990; Hafeezullah and Dutta, 1998) who not only reported many species of digeneans but also discussed the taxonomic relations of the species and the validity of various species. Many surveys were undertaken especially during the 1960s, 1970s and early 1980s. Scientists from Lucknow University such as Ahmad, V. Gupta, P. C. Gupta, R. C. Gupta, S. P. Gupta, from Punjab University, e.g. N. K. Gupta and Andhra University, e.g. Hussain et al., reported several species. The senior author of this monograph (Rokkam Madhavi) carried out investigations on Digenea of marine fishes of the Visakhapatnam coast of the Bay of Bengal from 1970 to 1982 and published a series of papers. A comprehensive study on didymozoid trematodes of tunas of Visakhapatnam was undertaken and several new species were reported (Murugesh and Madhavi 1990–1993). Investigations were undertaken on metazoan parasites of *Rastrelliger kanagurta,* and helminth parasites of mullets (Madhavi and Trivenilakshmi, 2011; Rekharani and Madhavi, 1985).

Recently, there has been a general decline in studies on this aspect and just a few publications dealing with description of one or a few parasites have appeared (Gupta, 2007; Govind, 1985; Job, 1961–1965; Karyakarte, 1968–1969; Karyakarte and Yadav, 1976a, 1976b, 1977a, 1977b; Khan and Karyakarte, 1984, 1987a, 1987b; Kumar and Agrawal, 1984; Bijukumar, 1997; Dutta and Manna, 1993, 1998). At present, it has come to a standstill. Madhavi (2011) published a checklist on Digenea of Indian marine fishes by compiling all the published information. All the digeneans reported till then together with their hosts, localities and references were covered in this checklist.

A perusal of the literature reveals that many more species are yet to be described. The studies undertaken so far were confined to fish from just a few regions such as the Puri coast, Visakhapatnam and the Mumbai coast. Vast areas along the coast still remain to be explored. The validity of many of the species reported has been questioned and many species whose identifications are based on a single specimen have been placed in the species/taxon *inquirendae* category. In spite of these controversies, it is surprising that molecular studies have not been undertaken and not even a single species of digeneans of marine fish from this region has been subjected to molecular study, although this is receiving global attention. Further, practically no information is available on aspects such as host specificity, biodiversity, pathogenicity and geographic distribution.

The present monograph is an attempt to consolidate all the species of Digenea so far recorded from marine fishes of the Indian coasts of the Bay of Bengal and the Arabian Sea. The primary aim in preparing the monograph is to bring together all the information scattered in different journals, most of which is not readily accessible. It covers over 600 species of digeneans. Most species are illustrated and described briefly, information on the hosts, locality, geographic distribution, and the validity status is presented. Keys and definitions are given for families, subfamilies, genera and species. A host–parasite list is also presented. Attention is drawn to some poorly known or controversial species, but they are not necessarily described or illustrated.

In the preparation of the account, testing the validity of each and every species reported by referring the diverse body of literature has proved a herculean task. In this context, the data presented in World Register of Marine species (WoRMS) has proved to be extremely useful. These data are a tremendous resource for trematodes studies, created largely through the efforts of Dr. D. I. Gibson. The reviews published by Bray and Cribb on different families of digeneans were of great help.

We have attempted to cover all the valid species and provide means for their identification including diagnostic features for families, subfamilies and genera and keys for their separation. The "Keys to the Trematoda" volumes 1, 2 and 3 served as useful guides for preparing this document. The diagnostic characters of families, subfamilies and genera were derived from them. The classification suggested in these accounts is adopted except where superseded by recent molecular studies.

One problem encountered relates to the date of publication of some papers. In some cases, the journal was actually published later than the nominal date on the front page of the journal part. The actual date of publication may be listed, often in small print, on the last page or inside the cover. We have not been able to check this in every case, so there may be discrepancies between the dates given here and those quoted in other publications and online sources.

The purpose of the monograph is to serve as a guide to Indian scientists for the identification of digeneans. We hope it will stimulate studies on the taxonomy of fish parasites adopting modern taxonomic tools, as well as inspire workers to undertake studies on life cycles, ecology and allied aspects.

Chapter 2
Materials and Methods

Digeneans infect almost every species of marine fish. According to the estimates of the Zoological Survey of India, Fisheries Division there are approximately 1,400 species of marine fish in the Indian Ocean. It is reckoned that on average each species of marine fish harbours one species of digenean (Poulin & Morand, 2000). From the records, it is evident that only 50% of digeneans have so far been recorded and there is vast scope for future studies. For proper identification of digeneans it is necessary to see that uniform methods are followed for collection, fixation, staining and mounting of the parasite and that the slide prepared is of a high standard permitting detailed study of its organization on which depends the identification of the parasite.

Morphological analysis requires that the material is in good condition. For this purpose, it is essential that only live and actively moving individuals are used for the study. This can be achieved by following certain precautionary measures. The ideal method for collecting trematodes is to examine the host while still alive or freshly caught. Flukes that are dead and degenerating should be avoided. Proper fixation of parasites is also of crucial importance to the study. The most commonly used method is to fix the flukes under slight cover glass pressure. In this method, the fluke is placed on a slide, and flattened under a cover glass pressure, and fixed in AFA or 70% alcohol, or 10% formaldehyde; the pressure exerted depends on the size and thickness of the parasite. Usually, the pressure that is just sufficient to keep the fluke flat is applied. This method, although commonly followed, is criticized as disrupting the internal organs and causing damage to the parasite.

Cribb and Bray (2010) suggested fixation by pipetting live parasites into nearly boiling saline and immediately transferring them to the fixative. Cribb and Bray (2010) described the procedure in sufficient detail with illustrations. Most of the present-day scientists are adopting this method. Later, Justine et al. (2012) presented a slightly modified version of this method.

Another method suggested is fixation in Berland's fluid (95% glacial acetic acid + 5% formalin). The parasite is pipetted into Berland's fluid and almost immediately preserved in 80% ethanol. This method may be useful for the parasites having a thick muscular tegument, but the material will not be suitable for molecular study.

R. Madhavi and R. Bray, *Digenetic Trematodes of Indian Marine Fishes*,
https://doi.org/10.1007/978-94-024-1535-3_2

Fixation is followed by staining in carmalum or haematoxylin, dehydrating in alcohol series, clearing in creosote or methyl salicylate and mounting in Canada balsam.

In India the number of parasitologists trained in systematics is declining and this is particularly the case with digeneans. Only traditional taxonomic methods have so far been employed for identifying the digeneans. The present-day trend in parasite taxonomy is to use both traditional and molecular methods; the latter techniques are gaining special emphasis. In this method, DNA sequences are used to address a variety of questions on the relationships and species boundaries of organisms. This is found to be a very useful approach that complements traditional taxonomy based on morphology. The protocol for molecular taxonomy consists of DNA extraction from the parasite, amplification of the DNA extracted, sequencing and construction of phylogenetic tree using proper software. The database containing sequences of digenetic trematodes is quickly growing and being used for taxonomic identification of many species. In India, however, molecular methods are yet to find their place and not even a single species of digenean has been subjected to analysis of sequences. It is essential to initiate these studies on a priority basis. Technical details for molecular methods have been well established and a number of publications have appeared (e.g. Nolan & Cribb, 2005; Blasco-Costa et al. 2016).

Preparation of the document: The vast literature published in various journals on digenetic trematodes from marine fishes from different coastal regions of India is the main source for the preparation of this document. In this context, the data presented in world register of marine species (WoRMS) compiled mainly by David Gibson (2004–2016) has proved to be extremely useful in getting details of species of digeneans recorded under each digenean genus, their validity, taxonomic status and the relevant literature.

The major part of the document deals with systematic account on digenetic trematodes collected from marine fishes of the Indian region. Only adult digeneans are treated in this manual. The metacercarial stages and the cercariae are not covered. Almost all the species recorded are covered. Keys are provided for the differentiation of subfamilies, genera and species. Keys are designed to include only species occurring in Indian region. Diagnoses are provided for all the taxa down to generic level. Brief descriptions for each species including the key characters have been furnished. Illustrations have been copied from published literature after obtaining necessary permission from the publishers. Information on the hosts and geographical distribution is also provided for each species. The host scientific names given in the original description have been revised following ‘Fish Base’. All the measurements are given in microns. Information on the number of specimens collected by the author for each species is also provided. This information is felt desirable because for some species the descriptions are based on only one specimen and these are to be accepted with reservation.

Abbreviations are used for indicating the localities along east and west coast of India. These are: AS, Arabian Sea; BOB, Bay of Bengal; PR, Puri Coast; VSK Visakhapatnam coast; BOM, Bombay coast; GOM, Gulf of Mannar; MS, Madras coast.

Chapter 3
The Digenetic Trematodes

General Organization and Life Cycles (Fig. 3.1a–e)

The vast majority of digeneans are endoparasitic, except for a few such as *Transversotrema* spp. which are ectoparasitic. The preferred site of infection is the gut; there are also reports from the urinary bladder (gorgoderids), air bladder (*Elongoparorchis*) and blood vessels (aporocotylids). The didymozoid trematodes are exceptional in that they occur encysted in pairs in almost all the organs of the fish body where they appear as prominent yellowish cysts. Wide variations also exist in the life cycle patterns. As an adaptation to the site of infection, the digenean body organization varies in different species, while at the same time reflecting similarities to the basic structure of the group.

The body is leaf-like, oval or elongate, filamentous, transversely oval in some (*Transversotrema* spp.). Didymozoids are peculiar in that the body is often divisible into a filiform forebody and a cylindrical hindbody. In some hemiurids, the posterior part of the body is modified into retractable tail-like structure known as ecsoma. The body size also varies from less than a millimetre (bivesiculids) to more than 5 cm (*Hirudinella*).

The body typically bears two suckers, the oral and ventral suckers. The oral sucker surrounds the mouth at the anterior end, except in bucephalids where the mouth is located at the ventral midbody (gasterostomate condition) and the sucker at the anterior end is then known as the anterior sucker which may be simple or provided with a solid, muscular rhynchus with or without tentacles. The oral sucker may be simple or provided with a ring of spines (*Stephanostomum*) or a collar-like structure (*Orientodiploproctodaeum*). In the genus *Karyakartia*, the rim of the oral sucker is provided with anteriorly directed tentacles. The ventral sucker is usually located ventrally in the body proper (distome), or at the posterior end of the body (amphistome), or may be totally absent (monostomate). It is usually sessile or may be borne on a peduncle as is the case in the genus *Pseudopecoeloides* Yamaguti, 1940. In the genus *Opegaster* Ozaki, 1942, the anterior and posterior lips of the

R. Madhavi and R. Bray, *Digenetic Trematodes of Indian Marine Fishes*,
https://doi.org/10.1007/978-94-024-1535-3_3

ventral sucker are provided with digital papillae. Usually, the size relationships of the two suckers are indicated by the sucker ratio, which is the ratio of the transverse diameters of the two suckers, with the diameter of oral sucker taken as one. Similarly, the region of the body between anterior end and anterior margin of ventral sucker is known as the forebody and that between posterior margin of ventral sucker and posterior end is known as the hindbody.

The outer layer of the body wall is known as the tegument, a dynamic syncytial cytoplasmic layer connected to underlying subtegumental cells through cytoplasmic extensions. The tegument may be thin or thick, smooth, spined or annulated.

Almost all the digeneans possess a rudimentary digestive system. Mouth is usually at the anterior end, opening terminally or subterminally. In bucephalids, the mouth is located at about the ventral midbody (gasterostomatous condition). The mouth leads into prepharynx, pharynx and oesophagus, the relative sizes of which vary from species to species. The pharynx is a muscular, bulbous structure. The oesophagus usually bifurcates into two caeca, usually in the forebody, rarely in the hindbody. The caeca terminate blindly close to the posterior end of the body or open through two ani (e.g. *Bianium*) or open into the excretory bladder forming an uroproct (*Stephanostomum*). The caeca may also unite forming a cyclocoel (e.g. *Coitocaecum*) which may in turn open through a single anus (*Opegaster*). In the haplosplanchnids, the caecum is single and lined by prominent cells. In some hemiurids, the oesophagus is provided with Drüsenmargen, a glandular structure.

Most digeneans are hermaphroditic and protandrous. Only a few digeneans such as didymozoids and schistosomes are gonochoristic with separate male and female individuals either totally free or partially or fully fused. The genital pore is single and is usually located in the forebody, rarely in the hindbody, and may be median, submedian or lateral. The genital pore opens into a genital atrium into which open the male and female terminal ducts. Typically, there are two testes, oval, lobed or follicular, located in the hindbody or forebody, disposed symmetrically, obliquely or in tandem. The testis is single in some monorchiids, and multiple in *Pleorchis* spp. The vasa differentia lead from the testes into a seminal vesicle which is saccular, or bipartite or sinuous and either lies free in the parenchyma or is enclosed in a cirrus-sac. Sometimes, a part of the seminal vesicle lies outside the cirrus-sac, dividing it into two parts, the internal and external seminal vesicles (lepocreadiids). In some opecoelids, the cirrus-sac is absent and the seminal vesicle lies free in parenchyma. The seminal vesicle continues as the pars prostatica and then as an ejaculatory duct, which may extrude to form cirrus, which leads into genital atrium. The ejaculatory duct may be lined with spines (monorchiids). The pars prostatica is surrounded by prostatic cells that often fill the cirrus-sac. In some forms such as hemiurids, the cirrus-sac is absent and the metraterm joins the ejaculatory duct and forms a hermaphroditic duct which may be enclosed in a sac, the sinus-sac also called a hermaphroditic sac. The genital atrium maybe thin-walled and inconspicuous, long and tubular as in some acanthocolpids, and in some monorchiids its lumen as well as the cirrus bears prominent spines. In bucephalids, where the genital pore is at the posterior end of body, the genital atrium is spacious, and the cirrus-sac is extended into it and bears blunt papillae. In tandanicolids and *Monodhelmis* spp., the genital atrium is provided with an accessory copulatory organ consisting of a solid anteriorly directed digitiform process and associated glandular tissue.

The female reproductive system is complex, consisting of several organs and a system of ducts connecting the various organs. Typically, there is a single spherical or lobed ovary, from which arises the oviduct. Near the origin of the oviduct, there is a valve-like structure known as the ovicapt. The oviduct feeds into a seminal receptacle and also Laurer's canal. The latter usually opens through a dorsal pore; but in some worms, such as *Elongoparorchis pneumatis*, it opens into a blind sac known as Juel's organ which contains a thick fibrous wall and is filled with degenerating cellular components like sperm, ova and vitelline cells (Madhavi & Rao, 1974). In some digeneans, the seminal receptacle is absent and the sperm is stored in the proximal part of the uterus and is known as the uterine seminal receptacle. The oviduct joins the vitelline duct and forms the ootype surrounded by Mehlis' gland, consisting of a cluster of gland cells. The functions of Mehlis' gland are still not clearly established, but it is believed that the secretion helps in the release of shell globules from the vitelline cells, that ultimately fuse and form the eggshell in the ootype. The ootype continues as the uterus where the eggs constructed in the ootype accumulate. The uterus may be short, straight or long and coiled, may be confined to a small region of the body or occupy practically the entire body. The distal part of the uterus is known as the metraterm, a muscular tube which opens into the genital atrium or joins the ejaculatory duct to form the hermaphroditic duct. In monorchiids, there is a terminal organ associated with the genital atrium, which may be simple or bipartite and spined or not, and the metraterm opens into the terminal organ at its base or its middle part. The uterus contains few or numerous eggs. The eggs are oval, operculate or non-operculate, and the capsule may rarely extend to form a filament. Another prominent structure in the female reproductive system is the vitellarium, which may be follicular occupying a varying extent of the lateral fields of the body or may be reduced to one or two compact masses situated near the ovary.

The excretory system is of the protonephridial type and performs two functions: osmoregulation and removal of waste. Its main components are the excretory bladder, numerous flame cells and the connecting tubules. The excretory bladder may be I-shaped, V-shaped or Y-shaped and opens through a posterior pore situated terminally or subterminally. The shape of the excretory bladder and its anterior extent is of taxonomic importance. In *Prosogonotrema,* there occurs a long and wide accessory vesicle in the hindbody dorsal to the normal excretory vesicle, which is known as Manter's organ. Some digeneans also possess a lymphatic system (Apocreadiidae).

The nervous system is of a primitive type consisting of a pair of ganglia (brain) located in the pharyngeal region, three pairs of anterior and three pairs of posterior longitudinal trunks and several commissures. Some digeneans also retain eyespots from the cercarial stage.

Life Cycles of Digenetic Trematodes

The life cycles of digeneans involve an alternation of generations with the sexual generation represented by the adult fluke occurring in the definitive host and the asexual generation represented by the 'larval' stages (sporocyst and redia—known as parthenitae) occurring in the first intermediate host, usually a mollusc and occasionally an annelid. The adult stage with high fecundity produces large numbers of eggs, and the asexual multiplication in the first intermediate host results in the generation of numerous infective stages. Both of these processes ensure successful invasion of the definitive host and a built up of heavy infections.

A wide range of patterns exist in the life cycles of digeneans depending on the behaviour, the food and feeding habits of the definitive host, the nature of the intermediate host and the surrounding environment. These patterns are discussed in detail by Cribb et al. (2003) and Niewiadomska & Pojmanska (2011). These are discussed briefly here, with examples from digeneans infecting marine fishes. Usually, the

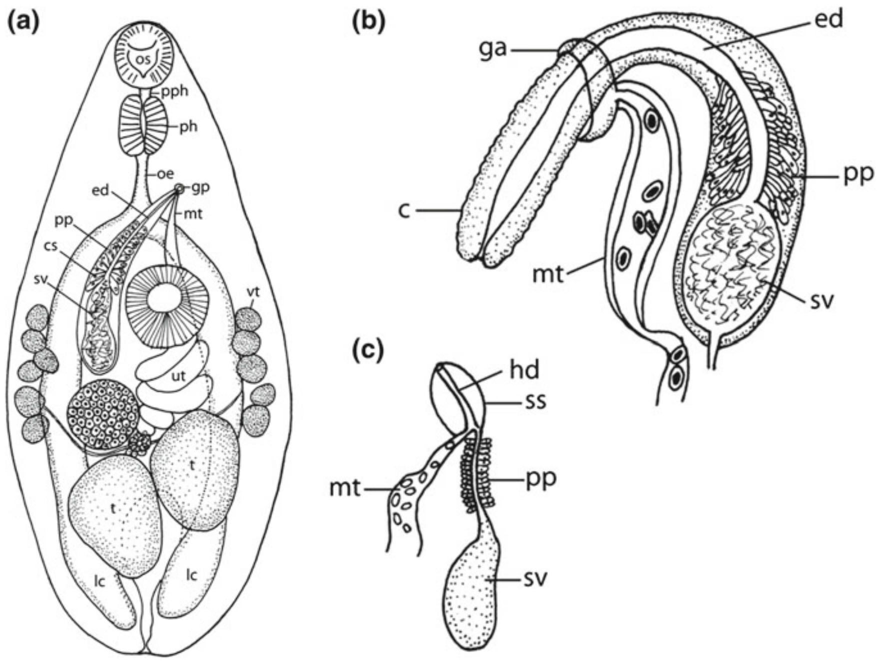

Fig. 3.1 **a**–**e** Organisation of digenetic trematodes. **a** Adult stage **b**, **c**, **e** Male terminal genitalia: **b** typical digenean **c** hemiurid **e** haploporid. **d** Female reproductive system. *Abbreviations c*, cirrus; *cs*, cirrus-sac; *ed*, ejaculatory duct; *ga*, genital atrium, *gp*, genital pore; *hd*, hermaphroditic duct; *hs*, hermaphroditic sac; *ic*, intestinal caecum; *lc*, Laurer's canal; *mg*, Mehlis' gland; *mt*, metraterm; *oc*, ovicapt; *od*, oviduct; *oe*, oesophagus; *oot*, ootype; *os*, oral sucker; *ph*, pharynx; *pp*, pars prostatica; *pph*, prepharynx; *rs*, seminal receptacle; *ss*, sinus sac; *sv*, seminal vesicle; *t*, testis; *ut*, uterus; *vd*, vitelline duct, *vr*, vitelline reservoir; *vt*, vitellarium

eggs of the parasite are released into the surrounding water medium, and they either hatch into a tiny motile stage known as the miracidium which actively penetrates the molluscan intermediate host or the eggs with the miracidium are swallowed by the molluscan host in the tissues of which they are stimulated to hatch. The miracidium develops into a mother sporocyst in the tissues, which in turn produces daughter sporocysts or rediae. The germ cells inside these stages multiply rapidly by asexual means and produce large numbers of cercariae which are released into the external environment. They infect the definitive host either through the active skin penetration (aporocotylids) or follow a passive mode of penetration by encysting either in the open environment or algae (haplosplanchnids, gyliauchenids) or in the tissues of a second intermediate host to develop into metacercariae which are consumed with the second intermediate host by the definitive host. The metacercariae excyst in the gut of the definitive host and the immature flukes migrate into the preferred habitat to develop into egg-producing stages. Details are given in Fig. 3.1.

The typical life cycle involves three hosts, two intermediate hosts and a definitive host, which pattern is followed in most digeneans, such as bucephalids, opecoelids, lepocreadiids, acanthocolpids, cryptogonimids and monorchids to mention a few. In some digenean families, abbreviation of the life cycle occurs usually through the elimination of the second intermediate host. The simplest type of life cycle is reported in bivesiculids such as *Paucivitellosus fragilis* and *P. hanumanthai*, which do not have a second intermediate host, but infect the fish host directly when the cercaria is ingested by the fish. In some digeneans such as hemiurids, the life cycle

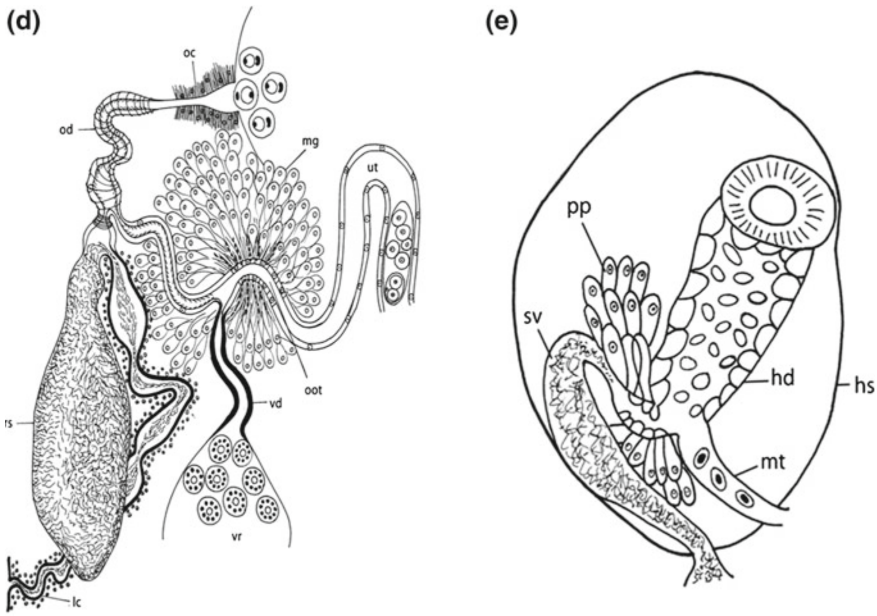

Fig. 3.1 (continued)

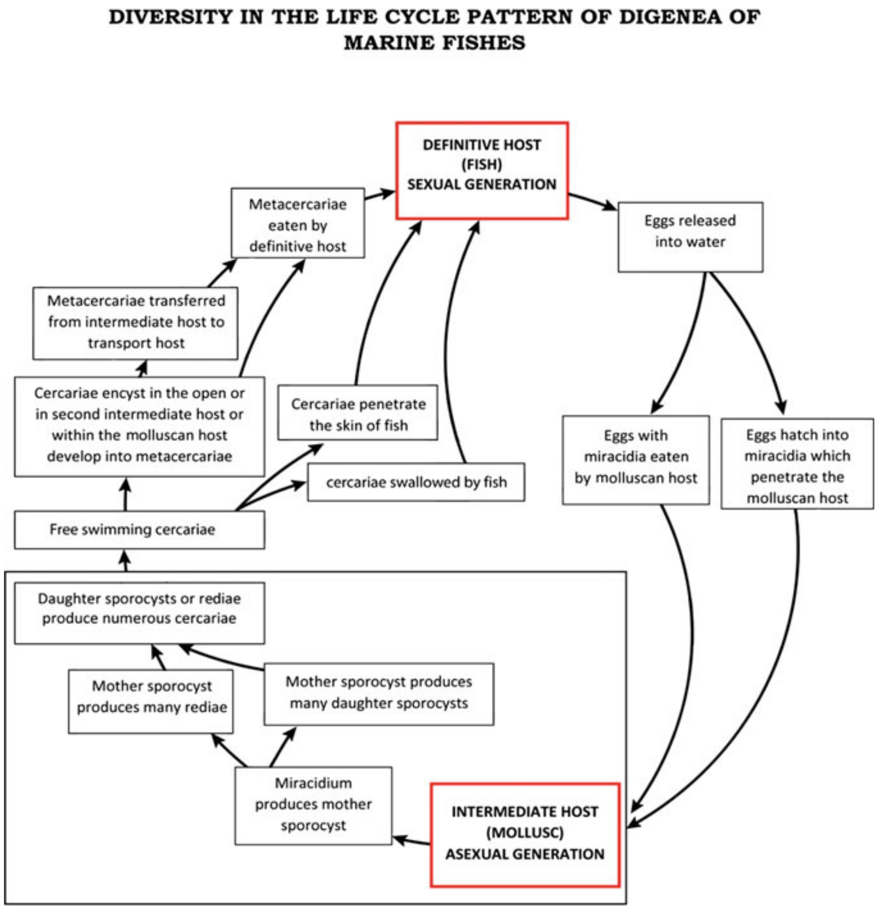

Fig. 3.2 Diversity in the life cycle pattern of Digenea of marine fishes

is extended by the addition of a paratenic (transport) host which helps in the transfer of the parasite from the second intermediate host to the definitive host.

Information available from India on the life cycles of marine digeneans is scanty. The life cycles have so far been described for three species; *Paucivitellosus hanumnathai* by Gnanamani (1989), *Stephanostomum cloacum* by Madhavi & Shameem (1993) and *Helicometra gibsoni* by Murugesh et al. (1993). Investigations on life cycles of these digeneans have to be undertaken on a large scale employing both experimental studies and molecular methods (Fig. 3.2).

Classification of Digenea

The Digenea is recognized as a subclass in the Class Trematoda along with the subclass Aspidogastrea. Aspidogastrea is a small group comprising about 80 species occurring as adults in molluscs, fishes and reptiles. On the other hand, the Digenea, comprising about 18,000 nominal species, represent the largest group of internal metazoan parasites (Cribb et al, 2001), capable of infecting members of all groups of vertebrates. They are especially common in marine fishes. In view of the great diversity exhibited by them in their morphology, life cycle pattern and the extraordinary variation in the host selection, classifying them in a form reflecting their phylogeny has proved to be a challenging issue. Cribb et al (2001) provided a historical review of provisional classifications suggested for Digenea. Early classifications relied mostly on adult morphological features such as the number and arrangement of suckers which are now considered as of limited taxonomic value. As knowledge on cercarial characters and the life cycles of digeneans has accumulated, this information along with a stress on the excretory system was used in later classifications. The system proposed by LaRue (1957) covers these various aspects and it was the most widely accepted classification for many years.

With the progress of knowledge on ultrastructure and DNA sequences of different species of digeneans, many changes have been suggested in the phylogenetic relationships of different taxa of digeneans and the overall classification. The best classification covering all families of digeneans and utilizing both morphological and molecular data is that followed in 'Keys to the Trematoda' volumes 1 to 3, proposed by Gibson, Bray and Jones (2002–2008). Although higher taxa were not covered, this classification dealing up to superfamilies is found to be most useful and comprehensive and served as the basis for other classifications proposed later. The important conclusion from this study is that both the morphological and molecular matrices are complimentary and both kinds of data are of value in inferring relationships among Digenea. Prior to this work, Cribb et al. (2001) attempted a combined approach using morphological characters for all stages of the digenean life cycle and complete18sRDNA sequences for 75 species of digeneans. The conclusions drawn from these two studies are similar. Another comprehensive study in this direction is that of Olson et al (2003) who, employing all the available molecular data on different taxa of digeneans, created a classification that is most acceptable and accurate. The notable feature in this classification is that only two orders were recognized instead of three as in the earlier classifications: order Diplostomida consisting of 3 suborders and Plagiorchiida with 13 suborders. Recently a number of families or species of digeneans have been subjected to molecular phylogenetic analysis employed mainly to determine the monophyly of the groups and the validity of different taxa and their interrelationships. This type of information is now available on digeneans of marine fishes belonging to the Lepocreadioidea by Bray et al. (2009), Opecoelidae by Bray et al. (2016), Bunocotylinae, by Perez-Ponce-de-Leon& Brooks (2016), Hemiuroidea by Blair et al (1998), Gyliauchenidae by Blair& Barker (1993), and many significant contributions were made on their phylogenetic relationships. This information is presented here in the accounts dealing with individual families of digeneans. Kostadinova & Perez-del-Olmo (2014) in the article entitled 'The Systematics of the Trematoda' provided a comprehensive review on the various classifications and other contributions on the molecular analysis of digeneans and compared the

Table 3.1 Classification of Digenea Adopted for Digenea of Indian marine fish

Superfamily	Family
Order Diplostomida Olson, Cribb, Tkach & Littlewood, 2003	
Schistosomatoidea Stiles & Hassall, 1898	Aporocotylidae Odhner, 1912
Order Plagiorchiida La Rue, 1957	
Apocreadioidea Skrjabin, 1942	Apocreadiidae Skrjabin, 1942
BivesiculoideaYamaguti, 1934	BivesiculidaeYamaguti, 1934
Bucephaloidea Poche, 1907	Bucephalidae Poche, 1907
Gymnophalloidea Odhner, 1905	Fellodistomidae Nicoll, 1909
	Tandanicolidae Johnston, 1927
Haplosplanchnoidea Poche, 1926	Haplosplanchnidae Poche, 1926
Hemiuroidea Looss, 1899	Hemiuridae Looss, 1899; Accacoelidae Odhner, 1911; Derogenidae Nicoll, 1910,DictysarcidaeSkrjabin&Guschanskaja, 1955; Didymozoidae Monticelli, 1888; Hirudinellidae Dollfus, 1932; Lecithasteridae Odhner, 1905; Ptychogonimidae Dollfus, 1937; Sclerodistomidae Odhner, 1927; Sclerodistmoididae Gibson &Bray, 1979; Syncoeliidae Looss, 1899.
Transversotrematoidea Witenberg, 1944	Transversotrematidae Witenberg, 1944
Haploporoidea Nicoll, 1914	Haploporidae Nicoll, 1914, AtractotrematidaeYamaguti, 1939
Lepocreadioidea Odhner, 1905	Lepocreadiidae Odhner, 1905; AephnidiogenidaeYamaguti, 1934; Enenteridae Yamaguti, 1958; Gyliauchenidae Fukui, 1929; LepidapedidaeYamaguti, 1958.
Brachycladioidea Odhner, 1905	Acanthocolpidae Luhe, 1906
Opecoeloidea Ozaki, 1925	Opecoelidae Ozaki, 1925; Opistholebetidae Fukui, 1929
Monorchioidea Odhner, 1911	Monorchiidae Odhner, 1911
Gorgoderoidea Looss, 1899	Gorgoderidae Looss, 1899
Microphalloidea Ward, 1910	Zoogonidae Odhner, 1902; Faustulidae Poche, 1926
Opisthorchioidea La Rue, 1957	Cryptogonimidae Ward, 1917; Cladorchiidae Fischoeder, 1901; MicroscaphidiidaeLooss, 1900; Cephalogonimidae Looss, 1899; OpisthorchiidaeLooss, 1899

two classifications namely the one followed in the Keys to the Trematoda and the other proposed by Olson et al (2001).

The relationships at the superfamily level appear to be well resolved, but several issues remain to be solved at the higher digenean taxa. In the present account, the classification of Digenea proposed in 'Keys to the Trematoda' is modified to include only the families relevant to the present work. Some other changes have also been made. All the superfamilies are distributed under two orders: the Diplostomida Olson, Cribb, Tkach & Littlewood, 2003, and Plagiorchiida La Rue, 1957. No attempt was made to include suborders (Table 3.1).

Part I
Systematic Account—Order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003

Chapter 4
Superfamily Schistosomatoidea Hassall, 1898

Family Aporocotylidae Odhner, 1912

[Syn. Sanguinicolidae von Graff, 1907]

Two different family names have been in use for the blood flukes of fishes: the Aporocotylidae Odhner, 1912, and the Sanguinicolidae von Graff, 1907. The Sanguinicolidae was widely accepted and is followed by Yamaguti (1958, 71) and by Smith (2002) in the Keys to the Trematoda. Overstreet & Køie (1989) considered Aporocotylidae to be a junior synonym of Sanguinicolidae. However, Bullard et al. (2009) from a deep and critical analysis of the literature recommended the family name Aporocotylidae for these flukes, and subsequently, this family name is used in all the publications.

The family comprises trematodes that principally parasitise the vascular system of fishes (Smith, 2002). The body organization shows several unique features such as a flat leaf-like body and the usual absence of suckers. The eggs are thin, transparent and the eggshell is made of elastin (Madhavi & Rao, 1971a, b).

Diagnosis: Body flat, leaf-like. Tegument with transverse rows of spines. Oral sucker absent or rudimentary. Ventral sucker absent. Pharynx absent. Oesophagus surrounded by gland cells. Intestinal caeca H- or X- or inverted U-shaped. Testis single, oval or follicular or transversely coiled, in middle third of body. Cirrus-sac present or absent. Ovary slightly lobed, post-testicular. Seminal receptacle present or absent. Uterus postovarian. Eggs thin-shelled. Genital pore dorsal, postovarian, near posterior end of body. Vitellarium follicular, mostly anterior to ovary. Type genus: *Aporocotyle* Odhner, 1900.

Only two species of aporocotylids have been reported from India: *Orchispirium heterovitellatum* Madhavi & Rao, 1970 from an elasmobranch fish and *Paradeontacylix megalaspium* Trivenilakshmi & Madhavi, 2007 from a teleost fish.

R. Madhavi and R. A. Bray, *Digenetic Trematodes of Indian Marine Fishes*,
https://doi.org/10.1007/978-94-024-1535-3_4

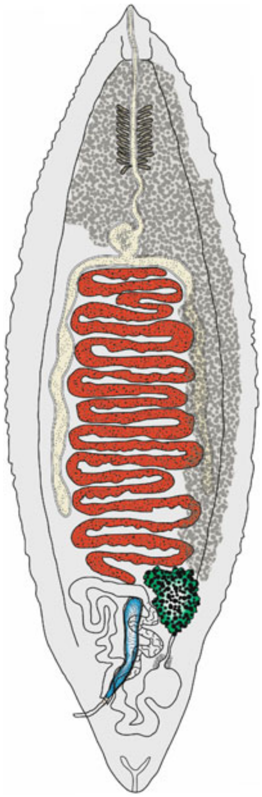

Fig. 4.1 *Orchispirium heterovitellatum* (after Madhavi & Rao, 1970, J. Parasitol, 56, p 42 Courtesy Allen Press)

Genus *Orchispirium* Madhavi & Rao, 1970

Diagnosis: Body lanceolate. Oesophagus long, surrounded by gland cells, enlarged posteriorly to form vesicle. Intestine with posterior caeca only, appearing as inverted U-shaped. Testis single, transversely coiled tube, between and posterior to caeca. Cirrus-sac poorly developed, encloses seminal vesicle and short, eversible cirrus. Ovary lobed, post-testicular. Uterus postovarian. Metraterm short. Eggs thin-shelled. Vitellarium follicular, with follicles much more extensive on right side than on left side. Type and only species: *O. heterovitellatum* Madhavi & Rao, 1970.

Orchispirium heterovitellatum Madhavi & Rao, 1970 (Fig. 4.1)
Host: Dasyatidae: *Brevitrygon imbricata* (Bloch & Schneider)
Location: Mesenteric veins
Locality: VSK, BOB
Reference: Madhavi & Rao (1970a).

Description: Body spatulate, 3230–4480 long 990–1280 wide, tapering gradually towards ends. Tegument tuberculated, spines absent. Mouth small, surrounded by small sucker, visible only under high magnification. Oesophagus long, surrounded by gland cells, with vesicle at posterior end. Caeca narrow, terminate preovarian. Testis large, transversely coiled, with 9–11 loops, anterior surface of loop smooth, posterior surface crenulated, situated in intercaecal space in front of ovary. Genital pore single, submedian, postovarian. Cirrus-sac thin-walled, slender, extends from posterior margin of ovary to genital pore, encloses long seminal vesicle and short cirrus. Ovary multi-lobed, immediately post-testicular, to right of midline. Uterus post-testicular. Metraterm short. Eggs small, thin-shelled, transparent, 23 by 16 in size. Vitelline follicles small, asymmetrical in distribution, extending from nerve commissure to level of ovary on right side, to level of intestinal bifurcation on left side.

Remarks: The important characteristics of *O. heterovitellatum* are considered to be: the flat leaf-like body, the lack of obvious suckers, and the presence of a vesicle at the posterior region of the oesophagus, the U-shaped intestinal caeca, the testis with transverse loops, the lobed ovary and asymmetrically distributed vitelline follicles. The original description of the species was revised by Bullard & Jensen (2008) based on a re-examination of holotype and paratype specimens. In the revised description, a rudimentary oral sucker is stated to be present. However, it is visible in only a few specimens, it is very small and almost indistinguishable. So far, this is the only species in the genus.

Genus *Paradeontacylix* McIntosh 1934

Diagnosis: Body small, flat. Tegument with transverse rows of spines. Suckers and pharynx absent. Intestinal caeca X or H-shaped. Testes 19–71, arranged in two or three rows between caeca. Cirrus-sac encloses seminal vesicle and pars prostatica. Male genital pore opens dorsally near left body margin. Ovary shield or heart shaped lying posterior to testis. Seminal receptacle absent. Uterus largely preovarian. Metraterm present. Female genital pore opens dorsally in midline, anterior to male pore. Eggs thin-shelled, ovoid. Vitellarium follicular, extensive, from near anterior end of body to near ovary.

Type species: *O. sanguinicoloides* McIntosh, 1934
Paradeontacylix megalaspium Trivenilakshmi & Madhavi, 2007 (Figs. 4.2 and 4.3)

Host: Carangidae: *Megalaspis cordyla* (Linnaeus)
Location: Gill blood vessels
Locality: VSK, BOB
Reference: Trivenilakshmi & Madhavi (2007).

Description: Body long, slender, worm-like, 1920–3520 long and 96–192 wide. Tegument thin, transparent, with spines arranged in 400–450 regular rows, each row with 8–10 spines. Suckers absent. Oesophagus long, sinuous. Caeca H-shaped, anterior branches short, posterior ones long. Testis follicular, approximately 70–86 follicles, arranged in two irregular rows in intercaecal region. Cirrus-sac encloses seminal vesicle and short cirrus. Male genital pore submedian on dorsal side, at some distance from posterior end. Ovary post-testicular appears as large irregular

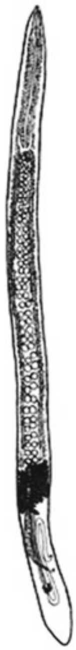

Fig. 4.2 *Paradeontacylix megalaspium* (after Trivenilakshmi & Madhavi, 2007, Zootaxa, 1512, p 67)

Fig. 4.3 *P. megalaspium* Enlarged view of posterior part of body (after Trivenilakshmi & Madhavi, 2007)

mass. Seminal receptacle absent. Uterus fills post-testicular area. Female genital pore submedian, just anterior to male pore. Eggs small, oval, 20–24 long 12 wide. Vitelline follicles small, occupy most of area between nerve commissure and genital pore.

Remarks: By possessing characters such as H-shaped caeca, a follicular testis, separate male and female genital pores and a postovarian distribution of uterine coils, the present form fits into the genus *Paradeontacylix*. This species differs from all the other species of the genus by possessing the following combination of characters: the long, slender worm-like body, with 400–450 rows of marginal tegumental spines, the absence of enlarged posterior tegumental spines and the presence of 70–86 testis follicles occupying the major part of the body.