47

Digenea

Summary of the Digenea (Subclass): Insights and Lessons from a Prominent Parasitologist

Robin M. Overstreet

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

doi:10.32873/unl.dc.ciap047
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 47

Summary of the Digenea (Subclass): Insights and Lessons from a Prominent Parasitologist

Robin M. Overstreet

Gulf Coast Research Laboratory, University of Southern Mississippi, Ocean Springs, Mississippi, United States

Reviewer: Michael Barger, Department of Biology, Health Science, and Integrative Human Biology, School of Health Sciences, Stephens College, Columbia, Missouri, United States

Introduction

Digeneans serve as marvelous parasites to study because they are so very diverse. Part of this diversity exists because they infect both intermediate and final hosts. Intermediate hosts include the first intermediate host, a mollusc, except for a few that use polychaetes. Second intermediate hosts include many groups of invertebrates, as well as many vertebrates. Final hosts include fishes, amphibians, reptiles, birds, and mammals.

Considerable knowledge about digeneans has resulted from United States federal funding directed toward schistosomiasis. This has helped understanding of the group, but species of the genus *Schistosoma* are somewhat unusual for digeneans in that they have separate male and female individuals, rather than each individual being hermaphrodites, and that they live in blood vessels where direct competition among other helminths is limited or nonexistent for the most part.

Several higher level taxa will be discussed in this chapter, primarily following the classification of Littlewood and colleagues (2015), and will be discussed with some addenda toward the end. When reading through the chapter, note that there will often be exceptions for any statement.

Morphology

Very little is actually known about most morphological structures of digeneans. In some cases, the structures are very similar to those found in other taxa of Platyhelminthes but offer opportunities for intriguing studies for students to compare features of specific digeneans with those of the other digeneans or other Platyhelminthes. And in other cases, the function of the various structures offers wonderful opportunities for investigation.

To learn more than what is covered here, names and features of morphological structures of adult digeneans occur in many other textbooks, albeit those for the same or similar structures may differ and for different structures may be referenced as the same (for example, Ginetsinskaya, 1988; Noble et al., 1989; Roberts and Janovy, 2013). Names and morphological features may also be found in reference books (for example, the CAB International 3-volume series Keys to the Trematoda, edited by Gibson et al., 2002; Jones et al., 2005, and Bray et al., 2008, as well as important treatments by Yamaguti, 1971; 1975) and articles (for example, Manter, 1970; Gibson and Bray, 1979; and Bullard and Overstreet, 2008).

Following are descriptions of some tegumental features, including spines (and other attachment structures), the alimentary tract, and the reproductive system, including eggs.

Tegumental Features

The tegument of a trematode is the outer body structure that interfaces with the host, providing both protection and some structural integrity to the trematode. The tegument (integument) is a complex structure. Over 100 years ago, Pratt (1909) described the cuticle and subcuticule of trematodes and cestodes. He, however, showed that rather than being cuticles, the outer layer of tissue in both served as teguments formed from parenchymal cells or their secretions, with the subtegument (as subcuticle) actually consisting of parenchymal cells.

Long after Bils and Martin (1966) set a groundwork for study of the ultrastructure, Świderski and colleagues (2013) described the tegument of the microphallid *Maritrema feliui* as consisting of a 2-layered syncytial epithelium. The outer layer consists of an external anucleate cytoplasmic region connected to an inner layer of nucleate perikarya (cytons) deeply embedded in the cortical parenchyma. The plasma membrane of the surface contains deep invaginations in which pinocytosis occurs and also 2 types of tegumental spines. The inner layer produces disc-shaped granules that are passed on to the surface layer. Those authors remind us of the publication by Schulte and colleagues (2013), who point out that the most efficacious schistosome vaccines thus far developed are directed against tegumental structures. Published research on the tegument of spineless paramphistomes suggests there exist 4 layers, with the innermost layer resting on and coupling with a thick basal lamina (Anuracpreeda et al., 2014). It may be that the abundance of negative

Box 1. Notes on the Study of Digenean Biology

Nollen conducted a variety of relatively simple elegant studies providing valuable results on the biology of digeneans. Nollen (1983) also reviewed patterns of sexual reproduction among the digeneans, monogeneans, and cestodes as well as isotopic labeling techniques useful to evaluate spermatogenesis, oogenesis, and mating in a variety of different species. Fried also conducted simple elegant studies, usually collaborating with students (for example, Fried and Rosa-Brunet, 1991). For example, they describe a simple way of cultivating echinostome metacercariae into relatively large, ovigerous adults, albeit with eggs not necessarily producing developed embryos. The method involved excysting metacercariae on a chick chorioallantoic membrane maintained at 38.5 °C and a relative humidity of 60 to 65%. They noted worm length increased from 0.5 mm at 2 days to about 3.0 mm at 6 days post-inoculation. When they transferred the immature adult to a second membrane, it reached 6 mm and was producing over 100 eggs in 17 days. These worms can be used for a variety of studies.

Nollen extracted adult philophthalmids, also relatively large worms related to the echinostomes, from under the nictitating membrane of chickens, conducted initial phase experiments on them, and then replaced the individuals adjacent to the eye.

Overstreet found that he could obtain adult microphallids of some species to study by placing the encysted progenetic metacercariae in saline in a glass stender dish into a 40 °C waterbath for a few hours to days. If the metacercariae did not hatch on their own, he added a small amount of trypsin or trypsin with ox bile salts, which rapidly digest the outer host portion of the cyst wall; once hatched, the metacercariae have to be maintained in fresh, warm saline or a cell culture solution like medium 199 and allowed to produce eggs over a 1–5-day period. There are lots of tricks enabling one to obtain healthy, living worms for study. Since it is difficult to rear most trematodes, Overstreet found that placing 1 or more individuals into dialysis tubing; tying off both ends; surgically implanting the tube into the body cavity of an appropriate host such as a chicken, rat, or large fish; and then later, surgically removing the tube with the developed worms protected from the host cellular response provides a useful method.

charges on the surface could protect paramphistomes from immune attacks by the host. This research as well as that of a related species also show the variety of papillae and tegumental folds (Panyarachun et al., 2010). These structures can be compared with those of the spined tegument of *Deropristis inflata* (Deropristidae) from the eel *Anguilla anguilla* (see Filippi et al., 2013).

Spines (and other attachment structures)

As mentioned above, spines occur in the tegument of many digeneans. Different digeneans have different shaped body spines that presumably have different functions, including moving, attaching, and feeding. Usually in the adult there exists an embedded basal portion of the spines, but some have completely embedded spines. Radev and colleagues (1998) described 4 different types of spines in the eye fluke *Philophthalmus hegeneri*: 1) Circular and scale-like; 2) scale-like with distal points; 3) oval with a distal spine; and 4) spine-like with 1–6 segments. Some spines are covered and do not protrude above the tegumental surface while others have the basal portion only embedded. Overstreet and Heard (1995) show scanning electron microscope images (SEMs) of differences of scale-like spines at 3 levels of an individual of *Megalophallus reamesi* (Microphallidae). *Maritrema madrynensis* also has a variety of scales and spines (Diaz and Cremonte, 2010). The spines of *Cryptocotyle lingua* cercariae are shed shortly after penetration and encystment in the fish host (Køie, 1977). Some body spines in adults do not occur near the posterior end, near the ventral sucker, or on the dorsal surface; these placements are often important taxonomic features in closely related species, and in some species they occasionally occur inconsistently. See Table 1 for an example of a comparison of body spines in members of one genus.

Not all trematodes have external spines, especially those like hemiuroids, most of which inhabit the high-acid stomachs of their fish hosts. Species of other families have an enlarged ring of oral tegumental spines such as some echinostomes, heterophyids, cryptogonimids, and others. Monorchiids have various tegumental spines as well as various shaped spines in the terminal genitalia, including beautiful rose-thorn-shaped spines used in copulation (for example, Overstreet and Brown, 1970). The shape, size, and

Table 1. Examples of body spines in members of the genus *Homalometron* (see Overstreet, 1969; Curran et al., 2013a; Fayton et al., 2016).

Species name	Description of tegumental spines
Homalometron foliatum	Cover the entire immature specimen but just near the testicular level
H. cryptum	Entirely absent in the thick tegument
H. robisoni	Limited to anterior 13%
H. palmeri (phenotypically similar to *H. pallidum*)	Measure 12–17 µm long, broad, and scale-like over the entire body
H. pallidum (phenotypically similar to *H. palmeri*)	Measure 6–9 µm long, delicate, and mostly devoid in the posterior half

distribution of spines on the cirrus of *Maritrema madrynensis* are the primary means of distinguishing it from a closely related species (Diaz and Cremonte, 2010). Students of parasitology interested in structure and importance of terminal genitalia (including spines on the cirrus) should also read the literature dealing with these features in other groups (for example, Doe and Smith, 2016).

Some trematodes attach to various places in the host by means of adhesive organs. For example, Erasmus and Öhman (1965) conducted ultrastructural studies of the gland cells and host-parasite interface of the adhesive organ of the diplostomoid *Cyathocotyle bushiensis* in its bird host. The pear-shaped gland cells and their ducts within the parenchyma of the adhesive organ produce a complex secretion comprising densely granular bodies, finely granular material, and mitochondria. When the retracted organ everted to attach to its host, the microvillus external surface attached to the host mucosal tissue and served to discharge the secretions into the host and perhaps also allow absorption of nutrient materials from the host.

In some individuals of the apocreadiid *Crassicutis archosargi* (Apocreadiidae) that attach to the intestine of its only-known definitive host, *Archosargus probatocephalus* (Sparidae, the sheepshead fish), the permanently attached region of the trematode consists of a modification of the tegument on either the dorsal or ventral side and extends among the host's intestinal villi. This modified tegument can be studied easily as it stains distinctively by the PAS (periodic acid-Schiff) method digested with diastase, as well as by other methods (Overstreet, 1976b).

Papillae

Different types of externally protruding papillae occur in different locations in the tegument on most digeneans and, in many cases when relatively consistent, can be used as a diagnostic characteristic (if the specimens are heat-killed and clean), meaning that identification of the species can be determined based on observation of this character.

Several researchers have studied the ultrastructure of sensory receptors in both adult and cercariae of different species. For example, Torimi and colleagues (1989) described 4 types of sensory structures in adults of *Echinostoma hortense* using SEM and transmission electron microscopy (TEM). Two contained ciliated papillae and 2 contained papillae without cilia; each occurred near either the oral sucker or ventral sucker. Although electrophysiological studies on this or other worms have not been conducted by cited researchers, most of them speculated that the morphological features and the distribution of these papillae indicate that they function as contact or stretch receptors during attachment and feeding of the worms. Many studies are waiting to be conducted!

Neurophysiological aspects

Because digeneans do not have a true circulatory system nor true endocrine organs, an understanding of the numerous neuropeptides becomes important for determining current anthelmintic and other drug-target selection. Adequate nerve and muscle function for many key behavioral determinates involves sensory perception/host location, invasion, locomotion/orientation, attachment, feeding, and reproduction (McVeigh et al., 2012). Some trematodes possess a type of lymphatic or osmoregulatory system that is metabolically active, suggesting a circulatory or excretory role (Sharma, 1978; Fried and Haseeb, 1991); in some cases, the structure and overall shape of this system serve as a taxonomic character. McVeigh and colleagues (2012) reviewed neuropeptide signaling in the Nemata and Platyhelminthes, including digeneans, highlighting a suite of 19 protein families that affect phenotypes in helminth reverse genetic screens. The types and organization of neurosecretory cells, nerve fibers, and perikarya have been reported from several digeneans, including light microscopy, ultrastructure, histochemistry, and immunochemistry (for example, Sharma and Sharma, 1981; Riddell et al., 1991; Mohammed and Al-Attar, 2000).

Even though dealing with a gill monogenean, Maule and colleagues (1990) tackled a well-reviewed

Box 2. Digeneans, Morphology: Alimentary Tract — Study It

There are a variety of ways for a student to investigate the alimentary tract of digeneans. Nollen (1968b) approached this with a rather simple system. He used species of *Philophthalmus* that as adults resided in the ocular sac of birds. That made it possible for him to remove the worms for experimental treatment and then, when necessary, return them to the host without surgery. In the cited paper, he exposed *Philophthalmus megalurus* to tritiated compounds later processed for autoradiography, using freeze-dried specimens embedded and sectioned in epoxy resin, with standard histological techniques for paraffin sections as controls, to detect the incorporated compounds (glucose, tyrosine, leucine, and thymidine). Absorbed glucose through the tegument became distributed widely within 1 min; then within 15 min, glucose converted to glycogen. The limitation of alkaline phosphatase within the excretory system showed the unimportance of that enzyme to glucose absorption. Tyrosine and leucine entered the worm, mostly through the gut within 5 min, and became distributed throughout the body within 15 min. Those 2 amino acids became incorporated in the vitellarium within 10 min. In contrast, it took 8 hr for tyrosine injected into infected birds to become incorporated by the vitelline cells, which in turn formed egg-shell material. It took 10 days for the eggs to be laid by the worm. However, within 30 min, the thymidine became incorporated within the reproductive system. Rapid entrance and incorporation of thymidine, glucose, tyrosine, and leucine into developing miracidia within the eggs demonstrated that the uterus was much more than a passive conduit for eggs.

immunocytochemical study using confocal scanning laser microscopy that would provide a splendid background for a digenean study. Moczoń and Świderski (1983) took an opposite approach. They infected specimens of a male South African pouched mouse (*Saccostomus campestris*) with 200 cercariae of the Tanzanian strain of *Schistosoma haematobium*. After 3 months, they treated the mice with a series of doses of niridazole, and then examined the region of the schistosomes posterior to the ovary for the presence of any ultrastructural and histochemical pathological changes from untreated specimens.

Alimentary Tract

Unlike cestodes, which only acquire nutrients through their tegument, most trematodes acquire nutrients through both the tegument and an alimentary tract.

The cellular tissue that lines the cecum is called the cecal epithelium, or gastrodermis, and it differs among different digenean species and also among different groups of Platyhelminthes. For example, the monogenean *Calicotyle kroyeri*, from rays, ingests epidermal tissues and associated mucus from the skin of its fish host. Halton and Stranock (1976) studied the common columnar cell in *C. kroyeri* that is filled with heterogeneous vacuoles from the viewpoint of both histochemistry and ultrastructure. This cell in the monogenean has an apical endocytotic complex comprising cell surface lamellae, apical vesicles, and numerous tubular invaginations of the plasmalemma. The luminal surface bears a highly organized array of peg-like structures that take up particulate food material from the gut lumen for transfer by means of other vesicles to the vacuoles in the columnar cells for digestion. The digestive elements of the cell are histologically reactive for protein, mucus, and carboxylic esterases. Indigestible residues and lipid droplets accumulate in the large apical vacuole and are periodically released into the lumen by exocytosis. The resulting distinction of the lumen of this worm involves an outflow of digestive secretions from the gastrodermis cells. These enzymes are secreted into an environment that is slightly acidic and important in several ways for regulating digestive processes, such as those involving cysteine and aspartic proteases.

Molecular and cellular studies of proteases (which are best characterized as a series of cathepsins) were instrumental in the discovery that they are vital in nutrient uptake from the host by degrading blood tissue proteins and other tissues into free amino acids (Dalton et al., 2005). Proteases involved in tegumental turnover, parasite excystment, egg hatching, and host penetration have played a pivotal role in the development of parasitism.

Since trematodes may produce peptidases that are specific for certain species, these enzymes may be targeted by researchers to develop practical applications in diagnostics, chemotherapy, and perhaps vaccination. For example, cysteine proteases such as cathepsin B1 serve as a primary target for small-molecule cysteine protease inhibitors. For example,

Box 3: Studying Digeneans: Anomalies — Study It

Numerous anomalous conditions occur in trematodes, but relatively few are reported. When a researcher finds an anomaly, they usually just toss the affected worms away or do not use them for descriptive purposes. In some researchers' experience, most anomalies appear to be found in unfertilized specimens, resulting in a poorly formed vitellarium, Mehlis' gland, or other reproductive structure. For example, Stunkard and Nigrelli (1930) noted that 1 of many specimens of *Lintonium vibex* contained 1 rather than 2 testes, and the worm appeared otherwise normal. Sometimes, these abnormalities may create an informative genetic situation like the number of testes in the common opecoelid *Helicometrina execta* from wrasses in Florida (Overstreet, 1969). Collections of 55 mature specimens contained individuals with from none to 5 testes, and some additional specimens from a total of 8 species of wrasses and non-wrasses contained 4, 7, 8, and the predominate 9 testes.

Out of about 1,500 specimens of *Philophthalmus megalurus*, a single 6-day old experimentally produced specimen had a portion of the anterior testis containing typically appearing ovarian tissue (Nollen, 1970). In a different case, an individual of a digenean presently known as the apocreadiid *Homalometron cryptum* in Florida without evidence of injury or degeneration exhibited 1 anomalous cecum interrupted to form a short branch joining the normal cecum and another portion with 2 blind ends extending the length of the vitelline follicles and lacking the well-developed epithelium of the normal cecum (Overstreet, 1969).

administering this protease to human test subjects infected with *Schistosomes* has been shown to decrease worm burdens of *Schistosoma mansoni*. Other cathepsins may serve as targets for other trematodes. Nevertheless, several trematodes other than blood flukes obtain their nutrition from blood and parasitologists have long questioned the source of hemoglobin in adults and metacercariae of digeneans (Cain, 1969a; 1969b; Vandergon et al., 1988).

Reproductive System

With the exception of schistosomes from mammals and a few didymozoids from fishes, digeneans are hermaphroditic. Species having a longevity of months to years continuously produce ova and sperm. In fact, some hemiuroids and didymozoids possess a Juel's organ, a modified appendage to the Laurer's canal and an organ that serves as a disposal unit, which recycles excess or unused reproductive material (Gibson and Bray, 1979). The Laurer's canal, which in most other digeneans links the oviduct dorsally with the exterior or to a seminal receptacle, seems to serve as a drainage conduit for excess or spent seminal and vitelline material.

In trematodes with a short lifespan, such as some microphallids (Digenea: Microphallidae) that occur in migratory birds, some species known to use a crustacean intermediate host are progenetic (meaning that they mature very quickly) and develop quickly and are able to rapidly develop and produce eggs in the feces of the bird before the bird acquires a good meal and leaves the area, continuing it migration. The area is often inhabited by endemic snails or crustaceans hosts of the microphallids and perpetuation of the worm's life cycle requires the use of a similar flyway (geographic area of migration) each season for the bird to maintain the microphallid species.

As mentioned elsewhere in this chapter, some microphallids as well as hemiurids and other digeneans in their intermediate host are progenetic and exhibit precocious development. Jackson and colleagues (1997) considered the condition in *Hemiurus levinseni* in mysidaceans from cold bays in Nova Scotia to result from accelerated gamete production or a shortened life cycle where the usual obligate vertebrate host is no longer required.

The relative position and size of gonads provide important taxonomic features. Sperm ultrastructure of various digeneans also provides informative taxonomic and phylogenetic features. There are several characters that provide useful features, and these should be compared among close species and other taxa (for example, Ternengo et al., 2009; Quilichini et al., 2016).

Some researchers have found the Mehlis' gland, a cluster of gland-cells, to be an important taxonomic structure as well as one producing materials necessary for lubricating the uterus, forming and protecting the egg and eggshell, and probably activating the spermatozoa. Depending on the species, up to 5 different types of cells may be observed in the 'gland' that appear morphologically different and can be differentiated when stained with neutral red.

Box 4. Digeneans, Morphology: Reproductive System — Study It

The ultrastructure of the oviduct of the lung fluke *Paragonimus ohirai* was studied in detail by Orido (1990), showing there are 5 principal regions with cilia confined to 2 separate areas. His investigation (1991) also included the ultrastructure of the Mehlis' gland from which 2 types of secretory products were produced.

Smyth and Halton (1983) assumed the secretion played an important role in eggshell formation, and a variety of studies involving different trematodes have suggested the presence of a complex polysaccharide.

Moczoń and Świderski (2000) determined using ultrastructure that the secretions produced by the gland as well as by the wall of the distal ootype in *Schistosoma mansoni* included neutral glycoproteins.

Several researchers have used histochemistry and light microscopy to study the Mehlis' gland and its secretions in a variety of trematodes (for example, Del Conte, 1970; Sharma et al., 1981).

Those interested in this field of study should also investigate the similar structures, including the vitellarium, in cestodes (for example, Smyth and Halton, 1983; Świderski et al., 2011b), monogeneans (for example, El-Naggar et al., 1990), turbellarians (for example, Chandler et al., 1992), and other trematodes (for example, Świderski et al., 2011a).

Table 2. Selected general keys to genera, some accompanied by lists of digenean species.

First-listed author(s)	Year	Description of resource
Yamaguti	1971	Vertebrates: List of species, descriptions of genera
Yamaguti	1975	Life cycles
Schell	1985	North America north of Mexico
Gibson et al.	2002	Generic and higher level keys – Keys to the Trematoda, Volume 1
Jones et al.	2005	Generic and higher level keys – Keys to the Trematoda, Volume 2
Bray et al.	2008	Generic and higher level keys – Keys to the Trematoda, Volume 3

Eggs

Little is known about the ultrastructure of digenean eggs, especially when compared with those of cestodes, which are much more diverse. Conn and colleagues (2018) described some features from small operculated eggs from microphalloids. Those consist of an embryo surrounded by eggshell, with the shell material derived from vitellocyte secretions similar to that process found in the eggs of bothriocephalidean and caryophyllidean cestodes. Fried and Haseeb (1991) described eggs and miracidia of several trematodes. Nollen (1971) reported an early study on the quinone tanning system in eggshells. Future studies assessing eggs and miracidia of those trematode groups with large eggs without an operculum should provide biologically and taxonomically useful results.

As a general rule, trematodes that have large operculated eggs have a diagnostic miracidium that hatches from the egg and uses chemoreception to locate the molluscan first intermediate host. Stimulation for hatching usually differs among species and depends on various environmental conditions. See the text box (Box 6) for examples of the variations that have been observed.

Trematodes with tiny eggs such as hemiurids often contain many thousands of them and deposit them in the feces of their host, which in the case of hemiurids are fishes. The eggs, which usually spread out over a wide geographic area, have the statistical chance for the first intermediate host to feed on them. In the case of what was reported as *Hirudinella ventricosa* from a mackerel in India, the eggs were released from the worm in strings, with each string containing active spermatozoa and numerous oval, thick-shelled, translucent eggs containing fully-developed miracidia (Murugesh and Madhavi, 1990).

Note for Tables 2, 3a, 3b, and 3c: Be aware that in many cases the taxa names are not acceptable to recent authorities, so be sure to examine online sources like WoRMS for marine species or recent literature for accepted names.

Box 5. Digeneans, Morphology: Reproductive System, Eggs — Study It

Eggs of *Echinostoma caproni* under either light or dark conditions at 27 °C from an experimental mouse infection developed fully in 9 days, compared with 10 days when from a hamster (Behrens and Nollen, 1993). When exposed to light as a trigger for hatching, with incandescent light providing more consistent stimulation than florescent light, miracidia from eggs from mice took 13 days to hatch, whereas those from hamsters took 11 days. Both lots hatched between 11:00 and 16:00 hr, indicating a diurnal circadian rhythm, and, when stored in the dark for over 56 days, the miracidia from those eggs displayed abnormal swimming behavior; those eggs stored for 70 days did not hatch when exposed to light.

When developed eggs of the same echinostome species were experimentally exposed by Fried and Reddy (1999) to snail-conditioned (*Biomphalaria glabrata*) water, a greater number of eggs hatched than when maintained in artificial springwater.

Ford and colleagues (1998) studied the effects of salinity, pH, and temperature on the half-life and longevity of the same species of miracidia and found they were unable to tolerate much salt and lived longer at lower temperatures but never longer than 15 hr. Unlike eggs of echinostomes that require time to embryonate in an aquatic environment, those from schistosomes released from the minimally salty gut into a freshwater environment are already developed and hatched immediately.

When the chemosensitivity of miracidia of 2 species of *Philophthalmus*, which are positively phototactic and geotactic, were compared by Nollen (1990a), they showed an opposite response to some of the tested chemicals. The miracidium of the blood fluke *Schistosoma mansoni* also exhibited a positive phototactic response but was negatively geotactic in contrast to that of *Schistosoma haematobium*, which responded oppositely for both sensitivities (Shiff, 1974).

Biology

General

Numerous species of digeneans exist, and, with proper attention, students can find infections in most vertebrate hosts although infection depends on habitats and general prevalence. There are about 150 families, and identification at the superfamily, family, subfamily, and genus levels of members can be best accomplished by using the 3 volume keys entitled Keys to the Trematoda (Gibson et al., 2002; Jones et al., 2005; Bray et al., 2008) or updated articles on specific genera or groups. Details of many of these families are included in other chapters in this online textbook. Nevertheless, mention of a few examples will occur elsewhere in this chapter.

Digeneans are especially powerful for students to study because they come in all sizes and shapes; they exhibit a variety in feeding, reproducing, moving, and surviving; some can be harmful to their hosts, and, consequently, many have economical or medical importance. The taxon provides laboratory features that can utilize a variety of tools to investigate them. What a wonderful experience to have the opportunity to spend hours extracting an adult specimen of *Nematobibothrioides histoidii* over 6–12 m-long and in tangled masses extending from 1 side to the other just under the skin of a moribund *Mola mola* (ocean sunfish) (Noble and Noble, 1964; Bullard and Overstreet, 2008, personal experience).

A clear understanding of the biology of most digeneans remains poorly understood because of their lack of medical importance; little actual work has been done on the wildlife infecting species that are of little medical or veterinary importance. For example, most emphasis for intense study of trematodes has been focused on blood flukes, however, blood flukes and are not the normal run of the mill trematodes that comprise 95% of the species that exist. The blood flukes are truly exceptional because some groups even have separate sexes. However, understanding of trematodes without great medical importance can be had as shown with the following example: Many years ago, Nollen (1968a; 1968b; 1978) used radioactive ^{3}H-thymidine, as mentioned elsewhere, to determine exciting aspects such as development of stages within eggs still in the uterus, mating of different sized individuals, and other examples. Most of his studies were conducted on worms that matured under the nictitating membrane in the eyes of baby chickens (chicks); he could remove

Table 3a. Selected literature sources regarding digeneans in marine fishes.

First-listed author(s)	Year(s)	Location(s)
Linton	1910	Dry Tortugas
Manter	1947	Dry Tortugas
Yamaguti	1934, 1954, 1958, 1971, 1975	Worldwide
Sogandares-Bernal	1959	Gulf of Panama and Bimini
Siddiqi and Cable	1960	Puerto Rico
Nahhas and Cable	1964	Curaçao and Jamaica
Overstreet	1969	Biscayne Bay, Florida
Williams and Bunkley-Williams	1996	Puerto Rico lists
Yamaguti	1970	Hawaii
Palm and Bray	2014	Hawaii
Madhavi and Bray	2018	India

Table 3b. Selected literature sources regarding digeneans in freshwater fishes.

First-listed author(s)	Year	Location
Bunkley-Williams and Williams	1994	Puerto Rico
Hoffman	1999	North American (keys)
Thatcher	2006	Amazon fishes
Kohn et al.	2007	South American

Table 3c. Selected literature sources regarding digeneans of other animals.

First-listed author(s)	Year	Location and/or type of source and/or subtopic(s)
Travassos et al.	1969	Brazil, vertebrates
McDonald	1969a, 1969b	Bibliography and catalog, anatid birds
McDonald	1981	Keys, waterfowl
Prudhoe and Bray	1982	Amphibians
Forrester and Spalding	2003	Florida, wild birds
Jacobson	2007	Reptiles
Atkinson et al.	2008	Wild birds
Samuel et al.	2008	Wild mammals
Overstreet et al.	2009	Gulf of Mexico, lists of adult digeneans from all marine vertebrates
Fernandes et al.	2015	South America, birds and mammals
De Baets et al.	2015	Fossil evidence
Overstreet and Hawkins	2017	Gulf of Mexico, diseases of fishes and other animals prior to 2010

the individuals, treat them, replace them to their original sites, remove them again, and analyze them. With modern tools, students can now investigate many more aspects of the biology of digeneans.

After a 6-hour exposure to ^{3}H-thymidine, isolated adult specimens of *Philophthalmus gralli* were transplanted to chicks and labeled oogonia became primary oocytes within 4 days and then enclosed in newly formed eggs by day 12. In adults labeled in vitro and transplanted singly to chicks, only 2 of 28 self-inseminated. Labeled adults transplanted with unlabeled ones never self-inseminated but cross-inseminated with approximately 40% of the available individuals. Transplanted adults localized in 3 micro-habitats within the chicks' orbit. In only 1 of 21 attempts, did a labeled worm inseminate an un-labeled one outside of the micro-habitat where it was found (Nollen, 1978). Nollen (1984) also conducted mating studies with

Box 6. *Hirudinella ventricosa* — Learn More

These relatively large marine worms when constricted are about the size of a human thumb and are commonly known as walnut worms.

There are probably several species presently known as *Hirudinella ventricosa*, but they occur primarily in offshore predatory fishes.Ribosomal DNA from specimens from 3 different pelagic fishes in the Gulf of Mexico shows that there are at least 4 species in the Gulf of Mexico (Calhoun et al., 2013), including what tentatively is supported by morphological differences (specimens from type localities were not sequenced) to be *Hirudinella ventricosa* from the wahoo (*Acanthocybium solandri*), *Hirudinella ahi* from the yellowfin tuna (*Thunnus albacares*), and 2 different unidentified species of *Hirudinella* sp. from the blue marlin (*Makaira nigricans*) with 1 of those also infecting the benthic yellow goatfish (*Mulloidichthys martinicus*).

One would probably win a bet with a fisherman who just caught a wahoo shorter than 160 cm by wagering that exactly 2 individuals of *Hirudinella ventricosa* will infect the stomach of their catch (Overstreet, 1978).

P. gralli and *P. megalurus* and determined when single, transplanted, labeled *P. megalurus* were transplanted into chicks with unlabeled *P. gralli*, interspecies mating occurred, but there was no evidence of hybrids. Opposite studies with labeled *P. gralli* differed because interspecies mating did not occur. When Nollen (1999) recovered young adults of *Echinostoma trivolvis* and *E. paraensei*, he labeled the sperm and transplanted those individuals singly to uninfected hamsters that contained several unlabeled worms of the same or opposite species or both species. After 5 days, when no recipient worm of the same species was present, only 1 interspecies mating occurred out of 113 possible recipients. When single donor worms had a choice of either species of recipient worm, no interspecies mating took place, but self-insemination occurred.

Each species has its own biological eccentricities, and it is up to the readers to see how to untangle those of the species presently under the objective lenses of their microscopes. For example, *Cyclocoelum oculeum* occurs in the nasal sinuses of coots. McLaughlin and Marcogliese (1983) studied the migration, growth, and development of the species in *Fulica americana.* They orally intubated 40 encysted metacercariae to each of 1 group and artificially excysted another batch and injected 40 intraperitoneally to that group. Those injected into the body cavity migrated through the air sacs and air passages to the sinuses and migrated faster and grew larger than the others in an asynchronous manner. In fact, no infection resulted from orally fed worms after 6 weeks! Infection with the related *C. mutabile* involved an invasion of the liver after penetrating the intestine, and it remained there about 2 weeks before migrating to the air sacs where specimens matured synchronously, with that species exhibiting a more complex cycle.

A Note on Preparation Methods

Morphological characters used to identify worms to the level of genus or species can be modified by methods of fixation and preservation. For example, workers fixed digeneans decades ago in alcohol, formalin, and acetic acid (AFA) and killed them under coverslip pressure. The acetic acid in AFA, along with ethanol and formalin, eroded spines, especially in specimens left in the fixative for a long period. Also, some workers relax trematodes in distilled or tap water and killed them with a cold fixative. That may be acceptable for acanthocephalans that will not be sequenced or used for ultrastructure. However, structures in digeneans may degenerate or otherwise result in shifted features or otherwise altered structures.

For example, a study by Curran and colleagues (2001) involved digeneans from colubrid snakes in Vietnam. When the specimens of *Singhiatrema vietnamensis* were bathed in fresh water and then cold-killed, they were wider, the pharynx and esophagus were distorted, the ceca were shorter, and the cirrus sac was oriented differently. These alterations could have resulted in a misidentification to species. However, when treated similarly, the vitellarium of *Szidatia taiwanensis* exhibited distorted, confluent follicles rather than separate ones, a feature of *Gogatea* rather than of *Szidatia*. Before you go to collect parasites be sure to use the most current and up to date methods for collecting and preserving parasites and their hosts. Some sources include Gardner and Jiménez-Ruiz (2009), Gardner and colleagues (2012), and Galbreath and colleagues. (2019).

Phylogeny and Classification

Olson et al. (2003) combined their sequences from various digeneans to develop species-level phylograms based

Box 7: Personal Note from the Author, Robin M. Overstreet (from 2018)

Readers should be aware that the late Ray Cable was probably the most "forceful international inspiration for contemporary cercarial studies," with a long list of cercarial studies (for example, Overstreet, 1997b) and my copy of several (for example, Cable, 1956; 1965) stand well worn. He was also an interesting parasitologist!

Another contemporary authority on cercariae and life cycles is Marianne Køie (for example, 1985).

And I am embarrassed to confess that Richard Heard and I have amassed one of the largest collections of unpublished digenean experimental life cycles, which we hope to publish before either of us expire.

on Bayesian inference of combined data, ssrDNA + lsrDNA, and a revised classification based on the phylograms showing relationships among the different higher level taxa. Since that time, information gaps have been filled and much more is known about the relationships among families, genera, and species. A few general updates on methods and relationships followed (Nolan and Cribb, 2005; Olson and Tkach, 2005) and many have added to and sorted out the relationships and are cited where the corresponding taxa are treated below.

Before the availability of marvelous molecular tools, various researchers used morphological and developmental means to show those relationships. Some were highly inaccurate, though a relationship tree developed by Cable (1974) was unexpectedly close. Cable's accomplishment is truly amazing when one finds that worms that appear very similar morphologically are not phylogenetically related. Cable also showed convergent evolution of distantly related Microphallidae, Heterophyidae, and Fellodistomatiidae. On the other hand, worms that appear distinctly different may be shown to be closely related, as established by molecular means. Clearly, improvements in molecular tools, including entire genomes, will open new doors. They will also allow researchers to much better understand the biology and history of the digeneans.

Life cycles also have been used to assess the evolution of digeneans. A discussion of the importance of morphological features of adults and cercariae in understanding the phylogeny of digeneans occurs elsewhere in this chapter. Sinitsin (1931) and others, who considered that digeneans originated as gastropod parasites, were challenged by Heyneman (1960) who considered flatworms evolved from dalyelloid rhabdocoels. Cribb and colleagues (2001b, 2003) critically examined the nature and evolution of digeneans, looked at Diplostomida and Plagiorchiida separately, and still could not be definitive about how the complex cycle arose and how variation within the group evolved. Is a gastropod or vertebrate the primary original host? There is still lots of good reading such as that by Pearson (1972; 1988; 1992), who presumed that digeneans evolved from free-living rhabdocoels with a mollusc first origin, Cable (1965; 1974; 1982) and Gibson (1987) to compare with the molecular data that places the major helminth taxa (Trematoda, Monogenea, and Cestoda) and minor ones (Gyrocotylidea and Amphilinidea) together as the monophyletic Neodermata (Littlewood et al., 1999a; 1999b). That monophyly allowed the use of parsimony but has not definitively settled the origin of digeneans.

LaRue (1957) established a grouping based on embryological aspects of the excretory system of cercariae, which, with modifications, is similar to the accepted scheme used today. His suborders Anepitheliocystidia and Epitheliocystidia are no longer accepted because the cellular structure of the excretory vesicle as assumed by light microscopy for some members of Epitheliocystidia was shown to be a syncytium with transmission electron microscopical evaluation, the development of the excretory system in the tail was not clear cut, and most important, these features do not fit an acceptable phylogeny.

Classification and phylogeny evolved into using morphological adult characters and characteristics, and characteristics of all life stages combined to produce cladograms from cladistic analysis (for example, Brooks et al., 1985; 1989; Brooks and McLennan, 1993). Classification developed further by utilizing phylogenetic relationships determined from genetic sequences of specific genetic fragments (Tkach et al., 2000; Olson et al., 2003). The 3 publications by Brooks and colleagues pointed out the ambiguous data for the unresolved Plagiorchiata and tried to better establish members of the clade. Tkach and colleagues (2000) considered those works valuable and an important basis for other investigations. In fact, those molecular works supported some of the conclusions and straightened out other relationships.

Molecular analyses during the next 2 decades have clarified the higher level digenean taxa. Presumably, this classification will be perfected even more by using entire genomic sequences in the near future. Nevertheless, many articles on specific groups have added to or corrected the earlier phylogram of Olson and colleagues (2003). For example, Overstreet and Curran (2005a; 2005b) classified the haploporoids, all known to infect fishes only, based on morphological features. But once they collected and analyzed molecular sequences, they straightened out several aspects of the early classification (see the classification of Haploporoidea elsewhere in this chapter).

Classification

Littlewood and colleagues (2015) updated Olson's work from the point of view of diversity, showing that at that time there were 24 major groups (superfamilies) of Digenea, with 150 families, 1,777 described genera, and 12,012 described species. Not all families include any sequenced individual, and, in most groups, fewer than 5% or 10% have been sequenced. Looking at the numbers from a different point of view, Bullard and Overstreet (2008) estimate that they amass the largest group of monozoic plathyhelminths, perhaps about 18,000 nominal species, with fishes hosting an astonishing number of digeneans. Considering there exist about 27,977 extant fish species, accounting for just over half of all living vertebrates, and considering the number of new digeneans named yearly, with approximately half the species being named and examined for digeneans, the number of digeneans infecting fishes will soon probably exceed the number of fish species. Moreover, most sequenced species from fishes represent fewer than 5% of the known members in their representative families (Littlewood et al., 2015).

Subclasses Diplostomida and Plagiorchiida

As indicated above, the Digenea contains the subclasses Diplostomida and Plagiorchiida, with the former containing 19 families in 3 superfamilies, with most attention directed toward the Schistosomatoidea. Orélis-Riberio and colleagues (2014) provided a helpful tree illustrating phylogenetic relationships among all 3 blood fluke families using 83 blood fluke partial D1–D2 domains of 28S sequences.

Family Schistosomatidae

Members of the Schistosomatidae infect birds and mammals, those of the Spirorchiidae infect turtles, and those of the Aporocotylidae infect fish. The family for those in fishes has been considered both Sanguinicolidae and Aporocotylidae, but Bullard and colleagues (2009) determined it should be Aporocotylidae. Bullard and colleagues (2008) and Orélis-Ribeiro and colleagues (2014) showed that plesiomorphic members of the Aporocotylidae and maybe other blood flukes are some of the only digeneans that can show an association with some primitive hosts.

Family Diplostomatidae

Barcodes using cytochrome *c* oxidase 1 were analyzed by Locke and colleagues (2015) on 52 species of Diplostomatidae based on larval forms from fishes with more success than using the barcode on other digenean groups. That study was useful for detecting 23 of 40 unidentified species supported by at least 1 additional line of evidence.

Superfamily Brachylaimoidea

The superfamily Brachylaimoidea, according to Littlewood et al. (2015) is the sister group of Schistosomatoidea and Diplostomoidea, even though Heneberg and colleagues (2016; 2018) considered it in the Plagiorchiida. Those latter authors, however, provided trees with several brachylaimoids and showed that they really belonged in the Diplostomida. Locke and colleagues (2012) provided molecular and morphological information on the Holarctic distribution of *Urogonimus macrostomus*, confirming that several 'prior' species and individuals showing a wide degree of biological and geographical variation did indeed belong to this leucochloridiid brachylaimoid. The brachylaimoids occur in 7 families, 29 genera, and 227 species. Some leucochloridiids have furcocercariae, and some have colorful branched sporocysts that are visible within their land snail host, attracting their definitive hosts. The cercaria is often a cercarium (without a developed tail), and the metacercaria, usually encysted in the intermediate host, has a well-developed reproductive system. The definitive hosts for members of this superfamily are amphibians, reptiles, birds, and mammals.

Superfamilies Bivesiculoidea and Transversotrematoidea

The subclass Plagiorchiida contains 21 superfamilies of which the Bivesiculoidea and Transversotrematoidea each contain a single-family, each with 5 or fewer genera, and the 2 superfamilies not being significantly related to each other. These constitute the most primitive plagiorhiids. The bivesiculids are atypical in they have a single testis and completely lack ventral and oral suckers, assuming one accepts the anteriorly located muscular structure as a pharynx; those with a known life cycle have a furcocystocercous cercaria that swims and is eaten directly by the definitive marine or freshwater fish host, resulting in a 2-host cycle. The presence of *Bivesicula claviformis* in large groupers presented a challenge. Cribb and colleagues (1998) found immature specimens in a wrasse that compared with adults from the grouper, but not with specimens of 2 other species, using both molecular sequencing and morphological structures. They suggest that the immature specimens constituted a true metacercaria and an obligate stage in a 3- rather than 2-host cycle. The family was originally proposed by Yamaguti (1934) as a subfamily of the Monorchiidae, which, of course, it is not.

The transversotrematids are transversely elongate or pyriform digeneans with a cyclocoel gut (posterior portions join, making a cyclocoel), and those with a known life cycle have a furcocercous cercaria with distinctive arm processes at its bases that allow them to attach directly on the skin of their marine or freshwater fish host, allowing them in turn to mature into an adult under the host scales without passing through a metacercarial form. The family is unique.

Superfamily Azygioidea

One of the next 5 related plagiorchiid superfamilies also has members with a forked-tailed cercaria, even though the tails of some of the 5 differ considerably. Azygioidea is confined to 1 family, Azygioidae, with 4 genera and 40 species that mature in the stomach or body cavity of elasmobranchs and in the stomach of freshwater teleost and holosteans. The cercaria of some is an active, large, colorful, usually yellowish or orangish, and appears as an insect to a hungry fish. The superfamily is a sister superfamily with that of the related Hemiuroidea.

Superfamily Hemiuroidea

The Hemiuroidea contains 13 families, 212 genera, and 1,334 species. They infect the gut, especially the stomach, of marine teleosts, but they are also common in freshwater teleosts and less common in elasmobranchs, amphibians, and reptiles as well as progenetic in invertebrates. For example, Overstreet and Hochberg (1975) reported adults of the fish hemiuroid *Derogenes varicus* in the cuttlefish, *Sepia officinalis,* and included a reference for egg-bearing specimens in an arrowworm (Chaetognatha); when Køie (1979) described the life cycle of *D. varicus*, she found natural infections of immature metacercariae in the arrowworm *Sagitta elegans*. Along the same vein, Overstreet (1969) found a 3.2% prevalence of progenetic metacercariae of a different hemiuroid in the coelom of 250 specimens of *Sagitta hispida*. When 2 of 3 of the arrowworms were maintained in separate beakers for 29 days, the egg-bearing digenean migrated into the host's uterus. A hemiuroid's body surface is usually smooth (without spines) but can be rugate or plicate. As indicated elsewhere, some hirudinellids are quite large and occur in the stomach of large, carnivorous, marine teleosts (Overstreet, 1978; Bullard and Overstreet, 2008). Other hemiuroids occur in the esophagus of frogs and are well known because they have 3 intermediate hosts (for example, Yamaguti, 1975).

Family Didymozoidae

Members of another related family, Didymozoidae, are atypical because some are not hermaphroditic and most occur in tissues, embedded on gills, or in body cavities of oceanic pelagic fishes (for example, Yamaguti, 1971; Bullard and Overstreet, 2008). A relatively early comparison of phylogenies of genomes versus morphology of hemiuroids was conducted by Blair and colleagues (1998).

Family Heronimoidea

Heronimoidea is a single family with 1 accepted species, *Heronimus mollis*. Rather than a relic of an ancestral form, the species appears to be an aberrant form with a secondarily reduced life cycle and not related with the paramphistomoids. It is found in the lungs and trachea of freshwater turtles, and its eggs are retained in the adult and hatch when the adult migrates to the mouth of the turtle host and escapes into the water; the hatched miracidia may already contain cercarial embryos in the mother sporocyst. The cercaria does not encyst (Jones, 2005).

Superfamily Bucephaloidea

The next recognized superfamily, Bucephaloidea, has 2 reported families, 29 genera, and 416 species. Curran and Overstreet (personal communications) find that the 28S sequences of several genera do not match the morphological findings (Overstreet and Curran, 2002); perhaps extensive genomic sequences will clarify the phylogeny of this group. Known cercariae possess oxbow-shaped tails unlike an atypical forked-tail cercaria. Overstreet and Curran (2002) provide descriptions of the genera; tentatively do not accept but describe and discuss the second family, Nuitrematidae; a reader should note that bivalves serve as the first intermediate host, fishes as the second intermediate hosts, and teleosts as definitive hosts. One species is known from a salamander, and I suspect adult worms that we encountered in elasmobranchs off Mississippi (Overstreet et al., 2009) as being acquired from sharks eating teleosts containing adults of those species. This family deserves extensive study, although life cycle investigations and other studies would make good student projects.

Superfamily Gymnophalloidea

The superfamily Gymnophalloidea with a forked tail is quite complicated and deserves extensive study. Bray (2002) listed 5 families rather than the 4 by Littlewood and colleagues (2015), but all should be investigated molecularly. The family Gymnophallidae contains relatively small worms in birds and mammals; molecular studies are needed to distinguish several of those species. The rest of the families occur in fishes, and most of the species in those have been placed in the Fellodistomidae, a family that has been considered a 'catch-basket group.' Many of its members require molecular attention and investigation of their life cycles.

Superfamilies Paramphistomoidea and Pronocephaloidea

The next 2 superfamilies have gymnocephalus cercariae that attach on vegetation or some other substratum. The first, Paramphistomoidea, contains 11 families, 135 genera, and 431 species, and most have their ventral sucker located at or near the posterior end. In some species there is a modified attachment organ. Many are quite large and occur in the rectum of their vertebrate hosts. The second, Pronocephaloidea, fits as a sister-group of above superfamily and members are commonly referred to as monostomes because they lack a ventral sucker or a typical pharynx; many have a head-collar or longitudinal rows of papillae on the ventral surface. Many occur in the digestive tract, but many others also occur in the respiratory tract, oviduct, urinary and gallbladders, pancreas, liver, and tissue sites of their teleost, reptiles (turtles and iguanid lizards), birds, and mammals. There are 6 families, 49 genera, and 293 species.

Superfamilies Haplosplanchnoidea and Echinostomatoidea

The above 2 superfamilies are sister groups to the Haplosplanchnoidea and Echinostomatoidea. The Haplosplanchnoidea has but a single family, and it was once thought closely related to Haploporoidea, members of which are also found in the gut of fishes only. It has 9 genera and 50 species that have a smooth tegument, and nearly all have a single cecum.

The Echinostomatoidea is a much better known superfamily because it is larger, and members infect primarily birds, even though some occur in reptiles and mammals in addition to fishes. Tkach and colleagues (2016) updated the list of Littlewood and colleagues (2015) with its 1,098 described species included in 9 families and 105 genera. It is the last of the related superfamilies with gymnocephalus cercariae; however, the cercariae also occur in haploporoids as discussed below. Tkach and colleagues (2016), using partial genetic sequences for 80 species, representing 8 families and 40 genera, elevated 2 subfamilies to families, created a new family, and abolished 2 families and 3 subfamilies as well as refined the generic boundaries within 3 abundant families. In addition to illustrating the phylogenetic relationships among the taxa, they also provide a schematic representation of that tree including intermediate and final hosts, making it one of the best known superfamilies.

Superfamily Opisthorchioidea

The superfamily Opisthorchioidea contains numerous species (839), and, which by itself, has most of its members with pleurolophocercus cercariae that infect fish as second intermediate hosts. The superfamily Opisthorchioidea in this study comprises the Heterophyidae, Opisthorchiidae, and Cryptogonimidae. Several of the species of the first 2 families infect humans and other mammals as well as birds and reptiles, and many have been sequenced (for example, Dao et al., 2017). Recently, several cryptogonimids, a taxon with members infecting freshwater and marine fishes as well as crocodilians (for example, Brooks and Holcman, 1993), the odd snake (Tkach and Bush, 2010), and amphibians (Miller and Cribb, 2008) have been sequenced and studied. Fishes provide a variety of different model systems such as lutjanids (for example, Miller and Cribb, 2007b), haemulids (Miller and Cribb, 2007a), and Mexican cichlids (for example, Razo-Mendivil et al., 2008). Martínez-Aquino and colleagues (2017) provided a nice phylogenetic tree from Bayesian inference analysis of the concatenated data involving larval forms of crocodilian species from Mexico as well as an acanthostomine from a Southeast Asian snake along with numerous fish cryptogonimids and species reported as members of Heterophyidae/Opisthorchiidae. There are many species and genera just being seen for the first time and many more to be seen in the future. Some are cryptic species, some have a wide distribution, and some make exceptional indicators—more areas and hosts should be studied (Miller and Cribb, 2007b; Overstreet, personal observations). Several species in both families infect humans.

Superfamily Apocreadioidea

The Apocreadioidea represents a superfamily infecting primarily fishes with a few reptiles. Pulis and colleagues (2014) were the first to investigate the phylogenetic position of the Megaperidae. In doing so, they changed the rank of the latter family to a subfamily within the Apocreadiidae. Blend and colleagues (2017) then reorganized the

Schistorchiinae and considered Megaperidae as a synonym of Apocreadiidae, a decision not accepted by Gibson (2017 in the WoRMS database because it was non-compliant with Article 35.5 of the International Code of Zoological Nomenclature (ICZN, 2012)). In any event, Apocreadiidae is the sole family in the superfamily and contains many cryptic species that had been misidentified for many years. For example, the genus *Homolometron* contains 34 accepted species, with 6 similar species described since 2010 (Parker et al., 2010; Curran et al., 2013a; 2013b; Barger and Wellenstein, 2015; Fayton et al., 2016). One of those, *Homolometron palmeri*, described in 2013 (Curran et al., 2013b) had been reported by a few workers as *Homolometron pallidum* since 1958, and it infects at least 7 fish hosts. Species of other apocreadiid genera also have been sequenced (for example, Scholz et al., 2004; Curran et al., 2013a; Tkach et al., 2013), and the genera are now better understood than when reported earlier based on morphological characteristics (for example, Caira, 1989).

Superfamily Lepocreadioidea

The Lepocreadioidea is sister of the above and others; members infect marine fishes and coastal birds. Bray and colleagues (2009) assessed partial *lsr*DNA and *nad1* sequences of 55 species and found the group, with the exception of 2 species of the putative Enderidae genus *Cadenatella*, formed a monophyletic polytomy of 5 clades. There occurred some odd findings: a significant proportion of *nad1* did not necessarily evolve under positive selection, all deep sea species were not related, different life cycles existed and perhaps a representative lepocreadioid cycle included a bivalve, encystment of vegetation, and a herbivorous fish host. Morphological features did not indicate strong value when relating higher level relationships, but many similarly appearing species infected related hosts.

Superfamily Monorchioidea

The Monorchioidea consists of 2 families, both of which infect bony fishes. The Lissorchidae, the most primitive, infects freshwater fishes, and the Monorchiidae infects both marine and freshwater fishes. The superfamily is sister to several others, all of which have cercariae with a stylet (xiphidiocercaria) except for the Haploporoidea. There are several paraphyletic species/taxa published and unpublished (for example, Wee et al., 2018; Cribb et al., 2018), and students under Thomas H. Cribb (Nicholas Q.-X. Wee) off the coast of Australia and Robin M. Overstreet (Apryle Panyi) in and off the coast of the Southeast United States have recently studied various species, and the combination of the findings should provide a much better understanding of the Monorchiidae. Bray and colleagues (2005) determined that *Cableia pudica* was a basal monorchiid rather than an acanthocolpid. A variety of life cycles are known.

Superfamily Haploporoidea

As indicated above, the superfamily Haploporoidea has a gymnocephalus type cercaria that differs from the remaining 5 superfamilies in the subclass Plagiorchiida. All members infect fishes, both marine and freshwater, and most of those members contain a single testis like most monorchiids and lissorchids. Overstreet and Curran (2005a; 2005b) presented keys for the Haploporidae and Atractotrematidae before any of the tentatively allotted species had been sequenced. We erected a new subfamily to make 4 in the Haploporidae. Since then, our group has collected many species throughout the world, sequenced many of them, described new species and genera, and published on most but not all of them. We were correct in accepting Atractotrematidae and including the apparently dissimilar genera in it (Andres et al., 2016a; Andres et al., 2018). Seven subfamilies are now accepted in the Haploporidae. Additional species of *Cadenatella* to those removed from the Lepocreadioidea by Bray and colleagues (2009) were sequenced, and the monophyletic group was a clear haploporid. *Hapladena*, which was originally in the Magasoleninae belongs in a separate subfamily. Blasco-Costa and colleagues (2009), who sequenced many haploporids from the Mediterranean Sea, erected Forticiculcitinae without the type species, but we accept the taxon, and Andres and colleagues (2015) included new species from Argentina and freshwater in Florida as well as a newly erected genus (*Xiha*) for *Dicrogaster fastigatus* from the northern Gulf of Mexico; that species had previously been assumed to be a haploporine associated with members from the Mediterranean Sea. Several haploporid species when sequenced (Pulis and Overstreet, 2013; Andres et al., 2014a; Andres et al., 2018), including *D. fastigatus*, turned out to be grouped into subfamilies different from those in which they were originally placed by Overstreet and Curran (2005b). As more haploporids are sequenced, additional changes in classification may be made in the freshwater chalcinotrematines (Pulis et al., 2013; Curran et al., 2018), 'megasolines,' and other subfamilies, but present data support a common marine ancestor with 2 testes, shifting from a primarily marine life history with eupercarian hosts to a more euryhaline one with diadromous, mostly mullet, hosts as originally suggested by Manter (1957).

Superfamily Gorgoderoidea

The Gorgoderoidea contains 12 families, but members of most from the variety of hosts (Elasmobranchii, Chondrostei, Teleostei, Amphibia, Reptilia, Aves, and Mammalia) have not been sequenced. Cutmore and colleagues (2013) examined members of 3 subfamilies of Gorgoderidae from teleosts, elasmobranchs, and deep sea teleosts and combined specimens from frogs and detected a variety of clades, including 4 in Gorgoderinae. These will provide a good baseline for additional specimens from different host groups for a future dissertation. The true allocreadiids, according to Curran and colleagues (2006) belong in this superfamily as sister to the Gorgoderidae. The 'Allocredioidea' indicated by Olson and colleagues (2003) was split into Brachycladioidea and Opecoeloidea, which will be discussed below (Littlewood et al., 2015).

Superfamily Opecoeloidea

The Opecoeloidea includes 1 family split into several subfamilies, including the Opistholebetinae, and infects freshwater, marine, and deep sea fishes (Cribb, 2005; Bray et al., 2016). The family, Opecoelidae, is the largest digenean family and contains over 90 genera with nearly 900 described species. It had been suggested by Curran and colleagues (2006) to be considered a member in the Brachycladioidea, but, to avoid confusion, I am accepting Opecoeloidea. Recent articles based on genetic sequences demonstrate the complex nature of the various clades in the family (Andres and Overstreet, 2013; Andres et al., 2014b; 2014c; Bray et al., 2014; Bray et al., 2016). The cercariae of many opecoelids contain a short, suckered tail that allows them to move in a leech-like manner to infect their typically crustacean hosts. Køie (1981) described the ultrastructure of 2 related species exhibiting differences in their tegument and a few other features.

Superfamily Brachycladioidea

Bray and colleagues (2005) sequenced several species of Brachycladioidea in *Stephanostomum* and related genera (Acanthocolpidae) from fishes and 1 species in Brachycladiidae from marine mammals and showed the relationship among them; also, they used those plus other data on sister taxa from Olson and colleagues (2003) to suggest that the brachycladiids from fish-eating marine mammals were derived from piscivorous marine fish parasites. As an example of recent uncertainty, Fernandez and colleagues (1998) pointed out that the marine mammal campulids (so-called) had historically been associated with Fasciolidae or Acanthocolpidae (the most speciose genus being *Stephanostomum*) on the basis of morphology. Orecchia and colleagues (2006, now unavailable online) considered them as Diplostomoidea. As indicated above and in WoRMS, the several genera are now separated into the subfamilies Brachycladiinae and Nasitrematinae of the Brachycladiidae (Superfamily Allocreadioidea) with 7 prior subfamilies considered as synonyms. Additional data should further clarify the relationships.

Superfamily Plagiorchioidea

The Plagiorchioidea includes 26 families infecting freshwater fishes and amphibians as well as terrestrial reptiles, birds, and mammals. However, a few members of Macroderoididae occur in brackish and marine environments in addition to fresh water. Tkach and Kinsella (2011) reported 4 species of *Macroderoides* plus 1 of the closely related *Paramacroderoides* in the same individual of the Florida gar. Three of those also infected bowfin from the same locality. A closely related species in 1 of the 2 North American clades is specific to pickerel, and it is the only 1 in a North American teleost rather than a holostean host. Moreover, the single member of *Paramacroderoides* mentioned above showed fewer differences in the number of variable sites with 1 particular species of *Macroderoides* than between it and the other species (Tkach et al., 2010). Analysis of additional species should show whether *Macroderoides* is a junior synonym or an additional genus is warranted. Many studies await attention of parasitologists. The Plagiorchioidea are sister to the Microphalloidea, and because the nearly 1,000 species in freshwater and terrestrial habitats and their available hosts that could serve as a large source of parasitology projects. Tkach and colleagues (2003) provided a good phylogenetic analysis of the superfamily.

Superfamily Microphalloidea

The Microphalloidea is considered sister of the Plagiorchioidea, and both have many families, genera, and species. Of the over 1,335 species, many have had their life cycles determined. Since many microphallid metacercariae from crustacean hosts are progenetic and can be cultured, the adults from a wide range of hosts as similar for the plagiorchioids can readily be compared with their metacercariae morphologically and molecularly. Speciation is complex; that of *Micophallus pygmaeus* complex with a derived 2-host cycle in the Holarctic speciated by host switching rather than co-speciation (Galaktionov et al., 2012). Since intermediate hosts of zoogonids and faustulids include echinoderms, cnidarians, and other invertebrates, infections with these serve as interesting indicators of feeding behavior. Good articles on analyses of sequences include those by Tkach and colleagues (2003), Bray and colleagues (2005), and Kudlai and colleagues. (2015).

Life Cycles

Sporocyst Stage

Miracidia hatch from the egg, either after the molluscan first intermediate host eats the egg or directly in the water. Several means can trigger this hatching, usually through an operculum. Most known infections result from chemosensitivity allowing attraction of the miracidium to the mollusc. Once in the mollusc, the miracidium enters tissue where germ cells undergo asexual reproduction as a mother sporocyst or redia. In fact, in a few cases, redia develop in some miracidia even when still in the definitive host. Several cited general textbooks and cited articles treat the asexual reproduction process that results from a variety of taxonomically specific ways in the production of cercariae (see other sections in this book for citations to these).

Redial Stage/Generations

Redial generations are intriguing because, unlike the more abundant sporocyst stage, this stage has an intestinal cecum, pharynx, and birth pore through which a cercaria can exit. Køie and colleagues (1977) provided an ultrastructural study on the microvillus-like and cilia-like projections on the redia of *Fasciola hepatica* as well as that of the cercaria, external cysts, metacercaria, and migratory stages. A study by Dönges (1971) using chain-transplantations of daughter rediae of an echinostomatid (*Isthmiophora melis*) from infected to uninfected snails can pass through a minimum of 42 successive generations. Similar results have been obtained using redia of at least 2 other echinostomes. Dönges thought the limiting factors for redial multiplication in the intermediate host must be the size and lifespan of the intermediate host.

Preformed redia are known to occur in miracidia of the chicken eye flukes *Philophthalmus megalurus* and *P. gralli* based on ultrastructural studies by West (1961) and Nollen (1990a). This redial stage escapes and actively moves about when the miracidium stops swimming in pond water. Nollen (1990b) found that rediae escaped much earlier when in certain culture media than when in certain salt solutions or pond water. Because some rediae can feed on sporocysts of other trematodes, creating competition among trematodes in snail hosts, the technique described by Nollen can be used to obtain rediae for such studies. Sporocysts differ from rediae by

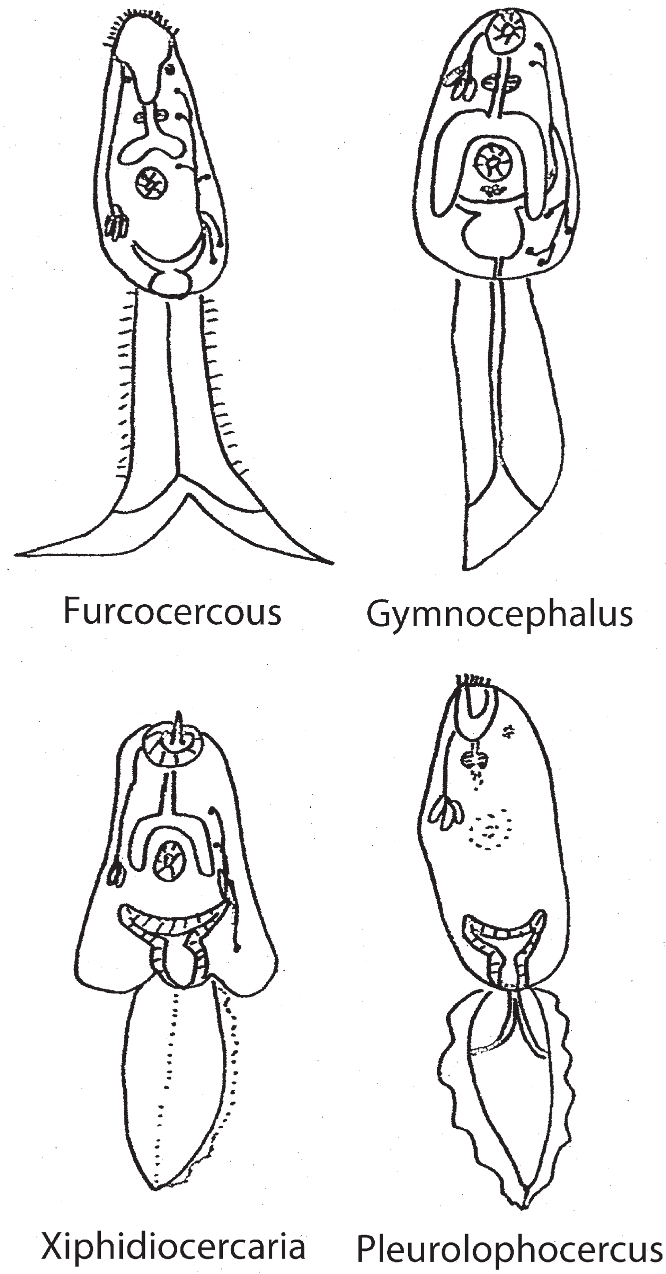

Figure 1. Furcocercous and gymnocephalus cercariae, mostly having thin-walled excretory vesicles, and xiphidiocercaria and pleurolophocercus, mostly having thick, putative epithelial excretory vesicles. The name of the furcocercous type refers to its forked tail. Source: R. M. Overstreet. License: CC BY.

lacking the intestinal cecum, and consequently the nutrients for the developing cercariae must be absorbed through the sporocyst's syncytial tegument (Køie, 1985).

Box 8. Host-Digenean Relationships — Study It

Asexual reproduction of the blood fluke *Trichobilharzia ocellata* affects reproduction of *Lymnaea stagnalis*, its snail host. Under laboratory conditions, an uninfected snail laid from 35 to 85 eggs per week and died before reaching 28 weeks, and an infected snail produced 24,500 cercariae/week, laid only 1 egg/week, grew more rapidly, had 90% survival at 28 weeks, and lost 2.9 mg of carbohydrate and 5.1 µg of protein (Bourns, 1974).

Cercarial Stage

Life histories differ among digenean families, and the cercarial type for a family plays an important role in dictating that history. Various textbooks describe or illustrate numerous types of cercariae (for example, Schell, 1985; Olson, 1974; Roberts and Janovy, 2013; Bullard and Overstreet, 2008). To avoid complexity, for purposes of this chapter, only 4 general types are covered, namely, furcocercous and gymnocephalus, mostly having thin-walled excretory vesicles, and xiphidiocercaria and pleurolophocercus, mostly having thick, putative epithelial excretory vesicles (Figure 1). Most of the 4 general types have a well-developed excretory system with flame cells that occur in specific patterns, a poorly-developed alimentary tract, oral and ventral suckers, and a tail. Remember that all sorts of exceptions exist.

The name of the furcocercous type refers to its forked tail (Figure 1). The anterior end has small spines and penetration glands with ducts that empty near the spines. The gymnocephalus type, relatively large so that it usually can be detected without a dissecting microscope, has cystogenous glands producing cysts that typically attach to or entwine on vegetation, shells of intermediate hosts, or other external substratum. A genital primordium is usually apparent. These types with relatively thin-walled excretory vesicles (bladders) differ from the next 2 with thicker walls and representing what was previously termed Epitheliocystida of LaRue (1957) rather than Anepithetheliocystida (**an** = without; Latin). Thus, a xiphidiocercaria with its 'epithelial' excretory vesicle, a feature that relates to its ability to encyst within the intermediate host, and a movable stylet anteriorly that assists in penetration into the intermediate host. There are typically 2 types of glands with ducts that exit anteriorly, 1 for penetration and 1 for encystment. The fourth—but not necessarily final—type is the pleurolophocercus; its name refers to a fin fold located on the tail which assists in both swimming and allowing the cercaria to maintain its position near the surface of the water. This type has rasping spines on the anterior end that associate with penetration glands; cells lining the excretory vesicle sometimes assist in forming material to create the inner wall of the cyst.

Most of what is known about the excretory, or paranephridial system, is known from the cercarial stage. Pearson (1986) published a good chapter on the subject that also mentions a distinct lymphatic system. Some digeneans have corpuscles and others have concretions. Both constitute important taxonomic features and both beg to be studied. Only a few trematodes, but some in different families, contain excretory concretions, and these may have different functions. Martin and Bils (1964) initiated studies by using ultrastructure of these structures in the metacercaria of *Acanthoparyphium spinulosum*. They determined that these concretions were composed chiefly of calcium carbonate and a trace of phosphate. Initially, calcium salts were delivered to the main collecting excretory vesicles in a flocculent state and ultimately deposited in concentric layers. They thought this material may be useful to fix carbon dioxide and buffer acids. Whether this is true, whether they involve lipid metabolism (Erasmus, 1967), whether they have an osmoregulatory function (Gibson, 1973), or whether these structures in different species have different functions create wonderful questions for students to ask and answer. For sure, the concretions have important biological functions and, at least for some investigators, are also important taxonomic features in adults and larval stages of some species of monorchiids, haploporids, opecoelids, hemiurids, and other digeneans. The corpuscles fill the main excretory vessels of some diplostomatids, echinostomatoids, hemiurids, and other digeneans.

Biological aspects of cercariae differ for each species and for each species under different environmental conditions. This aspect makes cercariae useful as indicators or fun to assess their ecology. Cercarial emergence of different species from their molluscan host, worms both related and not, occurs at different times related to light: dark cycling and other factors, usually related to the necessary presence of the intermediate hosts (Bell et al., 1999; Ginetsinskaya, 1988; Yamaguti, 1975).

Box 9. Metacercaria, Cysts — Study It

Weinstein and Fried (1991) used 2 species of *Echinostoma*, *Echinostoma trivolvis* and *E. caproni,* which infect the kidney of the snail second intermediate host *Biomphalaria glabrata* to experimentally infect 6–8-week-old ICR mice. The intestine of mice infected with *E. caproni* exhibited dilation, atrophied villi, a large loss of goblet cells, and retained worms compared with the expulsion of *E. trivolvis* from the mice intestine, which had an increase of goblet cells and of collagen. In another study with *E. caproni* with ICR mice, Hosier and Fried (1991) fed 25 cysts to each of 40 mice and recovered about 15 worms per mouse weekly for 20 weeks. The number of worms decreased after that with only 2 mice infected, one with 8 worms at 24 weeks and one with 1 worm at 29 weeks.

In a different researcher's study using NMRI mice, initial rejection occurred at week 12 PI, with body area per worm increasing from week 4 to week 12; the body area of worms in ICR mice was much less. In NMRI mice, the worms located in the posterior 80% of the intestine through the first 8 weeks and then occurred in the first 60 and 80% of the intestine and thereafter compared with the first 60 to 80% until week 12 and then occurred in the first 40%, demonstrating that differences in growth, body area, and distribution of the species differed between the 2 mouse strains. Once again, there are a lot of different digenean species for students to study in different hosts.

Differentiation of cercarial gland cells and the function of each will make exciting student projects!

Metacercarial Stage

The metacercariae can be free in the tissue or encysted in their second intermediate host, and the cyst wall differs among species and occasionally among families. Also, occasionally when most in a group are encysted, others are free. For example, the diplostomatid *Hysteromorpha triloba* can be situated free or encapsulated by host fibrotic tissue in tissues, usually deep within the musculature and often associated with the vertebral column, whereas *Bolbophorus damnificus* and other species in the genus and most in the family Diplostomidae are encysted.

Some metacercariae are relatively small such as that of *Paragonimus kellicotti* in the pericardial sac or soft tissues of crayfish; and these, like *Paragonimus westermani* (more common in humans in Asia), develop from a 0.4–0.7 mm metacercaria in a 0.4 mm diameter cyst into a large, 7–15 mm adult encapsulated in the lungs of their mink, feral cat, or other mammal (including human) host in the Midwest to Southeast United States. There are many species.

Some metacercariae of other families develop precociously into a stage that matures within hours. For example, some microphallids can deposit eggs in the feces of their migratory bird hosts shortly after the bird feeds on its crustacean intermediate host prey. This allows that gastropod first intermediate host to get infected. In some cases, both the snail and crustacean hosts have restricted geographical ranges, but they permit infection of the bird, which will migrate thousands of kilometers within a few days or weeks.

Cysts

Cyst walls are often similar in related species, but that is not always true. For example, in *Parorchis acanthus,* the wall is bilayered with 5 sublayers detectable with light microscopy and histochemistry. However, ultrastructural examination shows 3 layers without detectable sublayers. Perhaps some of the sublayers may constitute interfaces at surfaces and between layers. The innermost layer is laminated and formed by secretions from bâtonnet glands (Cable and Schutte, 1973) which have spiraled layers that unroll, and these layers also occur in cysts of *Fasciola hepatica.* The cyst wall of the related *Philophthalmus megalurus* has 2 layers, missing the 1 formed by bâtonnet glands. Secretions from glands in the encysting cercaria are excreted to form the outer layers. These cysts, like most from gymnocephalus-type cercariae, encyst on vegetation or external to host tissue.

Cable and Schutte (1973) considered 1 set of glands that originated in the parenchyma to be involved with encystment of the cercaria, but Haas and Fried (1974) considered those to function during post-metacercarial development. Some echinostomatids (*Echinostoma* spp.) that form cysts within the kidneys of ranid frogs, second intermediate hosts, some restricted to Bowman's capsule if in ranid frogs (Bowman, 2014), but all had a fibrous capsule of host origin. Some of those cysts with thicker encapsulations turned brownish, and the metacercariae in these often died (Martin and Conn, 1990).

Cysts formed by xiphidiocercariae in crustacean intermediate hosts also show a variety of types of cyst walls. Strong and Cable (1972) described the ultrastructure of a 4-layered wall in *Microphallus opacus* carried from the crayfish intermediate host gill where the cercaria penetrated into the digestive gland where the cyst embedded. Heard and Overstreet (1983) studied the cercariae of *M. basodactylophallus* infecting the blue crab and *M. turgidus* from several species of *Palaemonetes.* Cercariae of these 2 species both deposit what we called penetration cysts on a gill lamella to allow leverage in penetrating through the gills after shedding their tails. Both have 2 pairs of cystogenous glands. The larger anterior ones stain dark red with neutral red until the penetration cysts (pseudocysts by Prévot [1974]) form. The smaller glands located near the midbody also have ducts emptying near the stylet similarly to where the anterior gland ducts exit, take on a lighter stain with neutral red, and do not change color until the beginning of encystment.

Cyst walls of most trematodes exhibit resistance to host inflammatory cells. Howell (1973) demonstrated the resistance of cysts of *Stictodora lari* (Heterophyidae) to encapsulation by cells of the fish host. When he implanted glass beads into the abdominal cavity of the western mosquitofish, he noted encapsulation in 3 days as opposed to 21–23 days for encapsulation of the trematode in the same site, presumably because the outer layer of the cyst made up of host material acts as 'self.' The rigid cyst wall of the related *Cryptocotyle concavum* has 4 layers of parasitic origin surrounded by a host-derived capsule (El-Mayas and Kearn, 1995). Some species in a variety of digenean families in some specific hosts produce chromatophores in the outer host encapsulations (for example, Overstreet and Heard, 1995). Whether in fishes or crustaceans, these black spots can be involved in attraction of predators or in degeneration of the metacercariae.

Entering the Definitive Host

The digenean usually enters the final, or definitive, host as a free or encysted metacercaria when that final host feeds on the second intermediate host. Numerous exceptions exist such as some haploporoid and bivesiculid cercaria whose bodies withdraw into the base of their tails and are eaten directly unencysted. Also, the blood fluke cercaria penetrates the final host. In the case of some blood flukes, a stage known as a schistosomulum undergoes development in some site different than from where it resides as an adult. This name is sometimes reserved for the stage of mammalian members of Schistosomatidae; however, when parasitologists carefully examine infected fishes, they find immature specimens developing in a site different from where the adult resides. They should be considered a schistosomulum, a counterpart of metacercariae.

In the final host, the typical adult digenean usually occurs in the alimentary tract, but they can occur in almost any tissue, depending on the species. Examples are muscle tissues, lungs, bile duct, body cavity, gills, and others (for example, Yamaguti, 1971; Bullard and Overstreet, 2008).

Types of Digenean Life Cycles

There are many different digenean life cycles, such as a 4-host cycle (for the hemiurids *Halipagus* spp.: *Physa*-like snail, cyclops-like copepod, dragonfly, and frog). Hundreds of such cycles exist (Yamaguti, 1975) and more than that have yet to be discovered and offer wonderful opportunities for students such as those reading this chapter. Molecular sequencing has certainly been able to piece different stages of a cycle. For example, it clearly demonstrated that the cercaria, known as *Cercaria sevillana*, was the metacercaria of the microphallid *Gynaecotyla longiintestinata* as reported by Pina and colleagues (2007).

Host-Digenean Relationships

Host-digenean relationships typically result in little pathological alteration, but when a cycle necessitates the intermediate host to be attractive to the definitive host, pathological alterations can be involved. In numerous cases, a definitive host may respond heavily to a parasite, but it usually relates to an abnormal/accidental host for the parasite or a condition that helps complete the cycle, like in aquaculture discussed later.

When a large number of individuals harms the host, this relationship is usually referred to as a disease rather than an infection. For example, Lumsden (1979) reports a fibrotic response to an egg of *Schistosoma mansoni* in a mouse liver, a condition that can allow the ultimate passage to the intestinal lumen to be passed externally and does not cause disease unless heavily infected. He also shows an electron-micrograph of *Paragonimus kellicotti* in a cat lung. Responses occur around eggs and the worm; apparently if just 1 specimen obtained from a crayfish infection gets in a lung, it will migrate searching for a mate and create extensive host cellular response and, if the mate is found, the pair becomes protected from further response by fibrotic encapsulation (Lumsden and Sogandares-Bernal, 1970). Many cases of pathological responses are shown to occur in humans (for example, Beaver et al., 1984; Ash and Orihel, 2007) and wildlife (for example, Takashima and Hibiya, 1995; Randall and Reece, 1996; Jacobson, 2007).

In some cases, large numbers of cercariae penetrating into a host or of miracidia initially penetrating and then migrating within a host can cause pathological alterations or even kill the host (for example, Bullard and Overstreet, 2002; 2008). In the case of cercariae of *Diplostomum* sp., its odors did not have an effect on juvenile rainbow trout; however, when odors—alarm substances—from infected juvenile fish encircled free cercariae, the number of penetrations and length of time spent motionless by the cercariae increased (Poulin et al., 1999).

A different diplostome, *Ornithodiplostomum ptychocheilus*, acts differently in the brain of its fathead minnow host (Matisz et al., 2010). The cercaria can reach the brain within 3 hours by using different nerves, it then utilizes specific nerve tracts to reach the outermost tissue layer of the optic lobes where it grows for 4 weeks, and finally shifts its location to the adjacent meninges where it encysts. Associated with the shift in location occurred a massive inflammation, which lasted about 9 weeks and affected the health of the fish, with the amount apparently depending on the intensity of infection.

Effects on Host Behavior

When a host-parasite relationship is critically examined, one often finds the behavior of the host is altered. Whereas harmful effects often characterize the heavily infected final host, one can more often detect a harmful effect in the second intermediate host. This happens because the alteration influences the ability for the final host to prey on the infected intermediate host and for the parasite to successfully conclude its life cycle than would occur by chance alone. A wonderfully simple example that can be observed along the Gulf of Mexico and Atlantic coastline is infection of the talitrid marsh amphipods *Uhlorchestia spartinophilia, Orchestia grillus*, and *Chelorchestia forceps* by *Levinseniella byrdi.* (Curiously, however, note that this microphallid could not experimentally infect other tested talitrids (Bousfield and Heard, 1986; Overstreet and Lotz, 2016). The outstanding thing about this infection is that about a month after being infected, the amphipod turns from a greenish, grayish, or brownish color to a translucent or bright orangish and becomes negatively phototactic, not always hiding under thatch layers or wracks of dead, dissociated leaves and stems of marsh grass or other of their dietary debris-shelters. The carotenoids in the amphipod with infective metacercariae seem to become unbound from their protein, resulting in free and unmasked pigments and a color that makes the amphipod attractive to predatory birds and an ability to tolerate more direct light than an uninfected amphipod. Moreover, disorientation apparently caused by metacercarial physical effect on the ventral nerve ganglia can make the infected amphipod especially available to the seaside and marsh sparrows, clapper rail, willet, and semipalmated sandpiper definitive hosts. Most species of *Levinseniella* do not cause the orangish coloration, but a similar color occurred in *Austochiltonia australis* in Tasmania when infected with *L. tasmaniae* according to Smith (1981), and in other infections.

The example becomes more complex and ecologically important when researchers examine infections in a large-scale, 11-year, marsh study in Massachusetts, United States where nutrients were added to 3 large marsh areas containing tidal creeks flooded twice a day, but not added to 3 otherwise similar reference locations (Johnson and Heard, 2017). *Orchestia grillus* was the numerically dominant arthropod in the ecosystem, and along with *Uhlorchestia spartinophilia*, which was limited to the low marsh, were the only amphipods infected with *Levinseniella byrdi*. Looking at only *O. grillus,* the authors found similar amphipod densities between the enriched and reference locations during the first 4 years, but the densities were significantly higher in enriched areas in years 5–11. The densities of infected amphipods ranged from 0–3/m^2 with an average prevalence of 2.4% in reference marshes compared with 0–24/m^2 with an average prevalence of 15% across all years in the enriched marshes. The mean intensity of 1–5 metacercarial cysts did not differ among locations, but the prevalence increased each enrichment year, and 1 metacercaria would produce the orange coloration in the amphipod. After a decade, the mean prevalence of infection was up to 30% in the nutrient-enriched marshes compared with 2.4% in reference marshes. The biomass of infected amphipods was 11 times higher in the enriched compared with reference marshes. Infected and uninfected amphipods occurred in the high marshes, but only infected ones inhabited open areas such as vertical creek walls when exposed at low tides in enriched areas where the sandpipers could be observed feeding on them.

A myriad of cases, some critically described, may be found in books, chapters, and articles, some of them cited here (for example, Barnard and Behnke, 1990; Combes, 2001; Moore, 2002), but most cited elsewhere and others waiting to be investigated. Such studies attract readers, and they incorporate many fields of biology and general science for the investigator to adequately assess.

Hyperparasitism

A hyperparasite is a parasite that occurs either in or on another parasite. Dollfus (1946) reviewed literature at that time on hyperparasites of helminths as well as added further information. Examples include an ectoparasite copepod attached to the hemiuroid *Derogenes varicus* in the buccal cavity of what is known today as the American plaice (*Hippoglossoides platessoides*) located in Northumberland, England, United Kingdom, and studied by Marie Lebour.

Box 10. Personal Recollection from the Author Robin Overstreet (from 2018)

I had considerable data on infections in the Atlantic croaker and Gulf killifish collected since 1969 from a variety of locations, a few the same stations on a continual basis. Presentations on some aspects of analyzed data were given, but I had continued collecting samples for intended long-term studies until 2005 and maintained them in various freezers at University of Southern Mississippi and at commercial freezers in Biloxi. As it turns out, in 2005 Hurricane Katrina destroyed all properties containing those freezers, and lack of power for weeks allowed those frozen fish in salvaged freezers to spoil. Nevertheless, my student Andrew Claxton and I hope to report on additional materials and on materials that had been analyzed periodically over the years, and those are substantial. Sometimes even carefully protected host and parasite specimens as well as data can still be destroyed by floods, tornadoes, or fire.

In the pharyngeal cavity of a puffer near Woods Hole, Massachusetts, United States, a trichodinid ciliate was noted by Edwin Linton infesting *Lintonium vibex*. Probably more likely not accidental are a multitude of internal 'protozoans.' He covered a variety of microsporidians from all stages of trematodes, including members of a few different families; a few haplosporideans; a flagellate; opalinids from amphistomes in frogs; and even a nematode. Canning (1975) provided more information on the same and additional microsporidians. She also described others, and Overstreet described yet more with Yuliya Sokolova (Sokolova and Overstreet, 2018; 2020).

Overstreet (1976b) found a flagellate species of *Hexamita* in the cecum of *Crassicutis archosargi* different than one from an acanthocolpid by Hunninen and Wichterman (1938) and others mentioned (Overstreet, 1976b). Overstreet has searched for ciliates and flagellates in digeneans from herbivorous fish like mullets and rabbitfishes without success, but opportunities exist for future researchers.

The myxosporidian *Fabespora vermicola* infects *C. archosargi* (see Overstreet, 1976a) and probably more digeneans will be infected by myxosporidians. A microsporidian has even been described from a myxosporidian in a rabbitfish (Diamant and Paperna, 1985). The haplosporidean *Urosporidium crescens* infects cercariae and metacercariae of microphallids in grass shrimp and the blue crab causing a condition called blackspot in the crab and shrimp when the metacercariae become greatly hypertrophied (for example, Overstreet, 1978; 1983).

Whether an accidental infection or not, Graham (1969) reported an alarid mesocercarium in *Styphlodora magna*. Overstreet has often witnessed these mesocercariae rapidly invade helminths in a stender dish containing saline, but has never seen an infected helminth when immediately transferred into saline.

Bacterial infections can occasionally be seen in digeneans. Overstreet has often seen the Brownian movement of a bacterium in the excretory vesicle of some haploporids from mullets. He tried unsuccessfully to obtain and culture specimens with a drawn out capillary tube and regular tryptic soy broth. Others are encouraged to use a similar technique with a micro-manipulator and a combination of different culture media and sequencing procedures.

As discussed elsewhere in this chapter (Curran and Overstreet, 2004; Bullard and Overstreet, 2008), the diplostomatid *Bolbophorus damnificus* has caused millions of dollars of loss of cultured catfish annually. Infections can be associated with nephrotic pathological alterations in the catfish host. However, when as few as 4 metacercariae of *B. damnificus* are experimentally hyperparasitised by the bacterium *Edwardsiella ictaluri,* the commercial channel catfish (*Ictalurus punctatus*) died (Labrie et al., 2004). About 10% died by day 8 and cumulative mortality of 85% by day 21 compared with 45% mortality when exposed with the bacterium only (without the digenean) and 0% with controls and just the digenean group at day 21. Other studies reveal that different bacterial strains and different fluke genotypes influence host mortality, and interactions affect virulence and host health in surprising ways (Louhi et al., 2015).

A Few Notes on Ecological Methods in Parasitology

Although ecological studies take a long time to complete, they attract a lot of students and their mentors. With careful planning, a parasitologist can accompany an entomologist, ichthyologist, mammologist, ornithologist, or herpetologist, and gather material—hopefully fresh—so it can be examined under a microscope and fixed properly. Of course, the parasitologist will probably spend the days collecting hosts and the nights collecting parasites. Studies can involve those parasites inhabiting specific hosts, those comparing infections in different hosts or the same host or hosts in different localities or under different conditions.

For a chapter on patterns and processes in parasite communities, Esch and colleagues (1990a) introduced the historical aspects by saying that perhaps most ecological parasitologists agree that the earliest body of ecological studies was conducted by the Russian academician V. A. Dogiel and colleagues (for example, 1966), that H. D. Crofton (1971a; 1971b) introduced quantitative approaches to population dynamics, and that J. C. Holmes (for example, 1979) initiated a quantitative approach to helminth community dynamics. That chapter (Esch et al., 1990b) and other books (for example, Combes, 2001; Bush et al., 1997; 2001) can be used separately or in conjunction to understand terms and approaches. There exist a variety of books and publications that treat different aspects of ecology. For example, Poulin and Morand (2004) wrote a good general book on parasite diversity and models. Chapters should encourage readers to ask themselves many questions regarding their research and course topics. Diversity of trematodes in freshwater fishes is poorly understood and requires more research (Choudhury et al., 2016).

General Digenean Ecology

Marcogliese (2004) presented an opening address to a group of fish researchers entitled "Parasites: Small players with crucial roles in the ecological theater." He told how parasites could have pronounced or subtle effects on the behavior, growth, fecundity, and mortality of the host as well as regulate host population dynamics and influence community structure.

Digeneans seldom kill their definitive host. They occasionally harm their intermediate hosts but seldom kill them unless the hosts are being reared, such as in aquaculture. The majority of the commercial channel catfish (*Ictalurus punctatus*) grown in the United States comes from ponds in Mississippi. Eggs from *Bolbophorus damnificus* are deposited with the feces of its host, the American white pelican, into the ponds where the pelican feeds on the catfish along its flyway. Snail intermediate hosts in the shallow water of the ponds along their borders obtain heavy infections and produce very large numbers of cercariae. Consequently, since fingerling catfish occupy the shallow water, they become heavily infected, and losses of over US $10 million in catfish have occurred annually. As discussed elsewhere in this chapter, hyperinfection with the bacterium *Edwardsiella ictaluri* can kill the catfish when only 4 metacercariae occur per fish. Normally, the hyperparasitized metacercariae kill the fish. The surviving fingerlings usually have about 40 to 50 metacercariae per fish, suggesting that more—and there can be hundreds—kill the fish intermediate host (Overstreet and Curran, 2004; Bullard and Overstreet, 2008). Infected fish often have necrotic kidneys.

In addition to the adult of *Bolbophorus damnificus* in the American white pelican occurs a cryptic species, *Bolbophorus* sp., often just a few centimeters away in the same individual bird's intestine. That digenean uses sunfishes and *Gambusia* spp. as a second intermediate host, and it readily kills them in the same ponds (Overstreet et al., 2002).

As shown elsewhere, infected hosts serve as indicators of many biological activities as well as historical biogeography and phylogenetics (Brooks and Hoberg, 2000). Parasites can indicate trophic interactions over weeks or months as opposed to 24 hours or less when analyzing gut contents. When mullet fry enter the estuary from offshore plankton, the parasites reflect a copepod diet, but when the same sized fry is sampled from the nearby bottom, it adds haploporid trematodes acquired by feeding on the bottom (Paperna and Overstreet, 1981).

On the basis of 1 short collecting trip, Bush and colleagues (1993) collected metacercariae from 2 crab species from a small key in the Florida Keys, found that 1 crab species had 5 different microphallid species, and a few individuals of the other crab harbored 1 microphallid clumped in masses of a few thousand. They suggested that a single definitive host bird briefly feeding on the first crab species may be colonized by 6 different species and that the infrapopulation can increase rapidly by feeding on the other crab species. Consequently, understanding colonization processes in definitive hosts may be a critical underpinning to many community level studies. Consequently, community-level studies on invertebrate hosts (intermediate hosts as source communities) may be easier and more informative than conducting such studies on definitive hosts.

Long term studies on 1 or more parasites are important in understanding many aspects of ecological relationships. Esch and colleagues (1986) and Marcogliese and colleagues (1990) investigated *Crepidostomum cooperi* in the burrowing mayfly for 16–20 years and determined the dynamics were driven by eutrophication.

Digeneans as Indicators

Several studies have involved parasites as indicators, or tags, and most involve marine fishes because of the difficulties answering many fisheries questions. Some studies deal with specific fishes (Gibson, 1972). A few of the many recent studies include those by MacKenzie and colleagues (1995), MacKenzie (1999; 2002), and Marcogliese and Jacobson (2015). Others are cited elsewhere in the chapter.

Feeding Behavior of Hosts

The same approach can provide information about feeding habits and other biological parameters of the hosts. For example, studies on parasites of 21 species of grebes worldwide by Storer (2000), and those by Overstreet and Curran (2005c) investigating the American white pelican and brown pelican, relate digeneans and other parasites to specific feeding habits. Both studies also show how the digeneans, digenean hosts, and other parasites show evolution of the hosts, evolution of the parasites, health of bird hosts, health of intermediate hosts, public health risks, migratory patterns, and other aspects.

Variations in results from sampling hosts for digeneans obviously differ when the presence of necessary hosts differ. However, when compared ecosystems have variation in temperature and other environmental factors, the prevalence of infection (percentage of hosts infected divided by those examined in a sample) and mean intensity of infection (the number of a specific parasite divided by the number of hosts infected by the specific parasite) may also exhibit variation. Note that high prevalence and mean intensity of the digeneans indicate a healthy host and environment.

Collections made during different seasons from the same locations will usually reflect differences in infections of some of the parasites, depending on the longevity of the infection and other factors. There are also unusual conditions such as collections from near a nuclear power plant discharging hot water. Cercariae shed a month earlier in that water than those not in the heated location (Höglund and Thulin,1988).

Overstreet (1993) discusses a variety of natural and anthropogenic cases involving temperature as well as other environmental factors on host-parasite relationships. Another example reveals dynamics of infections of *Metadena* cf. *spectanda* in the Atlantic croaker (*Micropogonias undulatus*) during subsequent similar seasons. This worm may be the same as *Metadena spectanda* in Brazil (Overstreet, 1971a). However, sequencing a few Brazilian specimens and comparing them with the larger specimens from Mississippi will probably show that the specimens from the northern Gulf of Mexico represent a new species. Both the prevalence and mean intensity reached high values in the early 1970s in Mississippi. The fish fed on a wide variety of prey, but crustaceans, annelids, molluscans, and small fishes serve as the principal diet, at least in inshore water (Overstreet and Heard, 1978). Prevalence of infection with *M.* cf. *spectanda* became increasingly higher in fish over 60 mm-long (standard length) demonstrating when the croaker fed more on fishes. These trematode infections probably differ seasonally and annually because when the temperature and salinity is high, anchovies are abundant, and they are a favorite prey for the croaker but not a host of the trematode. In contrast, when the salinity and temperature are low, anchovies are rare or absent, and the croaker is more energy efficient when searching out gobies as their fish prey. A few different gobies serve as the second intermediate host for *M.* cf. *spectanda*, and during these periods, the croaker served as a super host for that parasite (Overstreet, 1973; 1982; personal observations).

Feeding studies provide a good background for studies dealing with indicators, zoogeography, diversity, and other fields. In a presentation at a symposium, Marcogliese (2003) asked whether parasites were the missing link to food webs and biodiversity. He also pointed out the need for integrating several disciplines (as was done in classical parasitology) and how these fields are no longer highly regarded. This is a shame considering the importance of using digeneans as indicators as discussed elsewhere in this chapter.

Digeneans, especially when in combination with nematodes represent an ecological link between mesozooplankton and relatively large pelagic animals (Noble, 1973; Campbell, 1983; 1990; Marcogliese, 1995; Klimpel et al., 2010; Andres et al., 2016a).

Health of Ecosystem (Including Toxicology)

Using digeneans as monitors of environmental health requires selecting the appropriate animal host. Considerable work has been conducted with fish model systems (for example, Overstreet, 1997). Criteria for a good fish model include having a restricted home range, serving as host for a relatively large number of digenean species, and being common and easily sampled. Depending on how good a model fish is will determine whether it will answer questions and solve problems. Additional features are usually needed to support and refine a study such as parasites other than digeneans, histological findings, or genetic markers.

Overstreet (1997a) used the western mosquitofish, *Gambusia affinis,* in Mississippi as an indicator of parasitism because it was host for many different metacercarial and other larval species that showed that the environment contained many specific teleosts, birds, mammals, turtles, snakes, and the alligator as well as many specific gastropods and bivalves. It shows this because specific harsh conditions can eliminate

Table 4. List of parasitology textbooks that cover digeneans. See the References for the full citations.

First-listed author(s)	Year	Pertinent topic(s) covered, with respect to digeneans
Smyth	1962	Biology
Dogiel et al.	1966	Revised classic tome on biology and ecology
Olsen	1974	Life cycles and ecology
Nickol	1979	Host-parasite interfaces
Smyth and Halton	1983	Physiology
Ginetsinskaya	1988	Life cycles, biology, evolution
Noble et al.	1989	Biology
Esch et al.	1990	Communities
Barnard and Behnke	1990	Parasitism and host behavior
Toft et al.	1991	Coexistence or conflict?
Williams and Jones	1994	General helminthology of fishes
Halton et al.	2001	Practical exercises
Bush et al.	2001	Diversity and ecology
Combes	2001	Diversity, genetics, ecology
Littlewood and Bray	2001	Interrelationships among flatworms)
Moore	2002	Behavior of hosts and ecology
Combes	2005	Ecology
Thomas et al.	2005	Parasitism and ecosystems
Maule and Marks	2006	Molecular biology, biochemistry, immunology, and physiology
Woo	2006	Fish diseases
Poulin	2007	Evolutionary ecology
Schmid-Hempel	2011	Integrated study of infections, immunology, ecology, and genetics
Roberts and Janovy	2013	Foundations
Goater et al.	2014	Diversity and ecology
Loker and Hofkin	2015	Concepts and principles

1 of those specific hosts (break a link in the parasite life cycle) and consequently the associated digenean. He determined that metacercaria of many different species remain in the fish for periods of over a year. Consequently, the relative number of animals in the environment can be determined by sampling the model fish just once or maybe twice a year, whereas sampling the biota requires numerous collections and a variety of biologists to identify the different animals.

If all the parasites in the model in addition to the digeneans are sampled, the number of non-parasitic invertebrate and vertebrate hosts in the ecosystem can be detected, making assessing parasitic data much more economically valuable than sampling animals monthly or bimonthly because many of those animals may remain in the environment for just a short time. Of course, reference stations are necessary for comparisons. When trying to evaluate specific areas, a variety of similar locations containing the model fish with and without the suspected conditions have to be sampled as those reference locations.

Anthropogenic contaminants

Anthropogenic contaminants can act in a distinct manner relative to host, parasites, and each other as well as being influenced by natural environmental conditions. When a sample of a specific fish host from a specific area exhibits a lower number or mean intensity of 1 or more digenean species than in samples from nearby localities, that finding suggests contamination. Further assessment of the samples for bacterial contamination, histopathological alterations, and other parasites can often pinpoint the source of contamination. Multiple samples of the western mosquitofish from one Back Bay, Mississippi, United States location designated as a superfund toxic clean-up site revealed a low prevalence and mean intensity of digeneans compared with samples from reference sites. In another nearby site contaminated with specific chemicals used to treat timbers, a low number of only one of the local digeneans occurred, and a myxosporidian with associated histopathological alterations was also unique to that location. When a live sample from that location was transferred

Table 5. List of books that deal with digeneans in the context of public health or veterinary science. See the References for the full citations.

First-listed author(s)	Year	Pertinent veterinary or health topic(s) covered, with respect to digeneans
Beaver et al.	1984	Clinical parasitology
Deardorff and Overstreet	1991	Seafood transmission
Coles	2006	Chemotherapy
Garcia	2007	Diagnostic medical parasitology
Bullard and Overstreet	2008	Human marine trematode diseases
Noga	2010	Fish disease, diagnosis, treatment
Overstreet	2012	Human marine diseases
Bowman	2014	Veterinary medicine

to a laboratory and reared, about 50% died from the myxosporidian infection. No fish from 2 of the reference sites died or exhibited the infections when reared concurrently (Overstreet, 1997).

In another example from a Texas river using the same fish model, the same group of researchers determined that contamination occurred upstream from an integrated pulp and paper mill effluent canal, primarily on the basis of the mean intensity of a digenean metacercaria, which was most prevalent in the effluent canal, and invasion of a usually free-living ciliate and macrophage aggregates in the spleen, both of which occurred at the upstream location. The effluent canal, which had been incorrectly accused of being a toxic site because of the coffee-like appearance, gave the impression of being the healthiest of the 5 sampled locations (Overstreet et al., 1996).

In another study, Sun and colleagues (1998; 2009) were charged with assessing a large number of sampling locations along 2 contaminated rivers in southern Taiwan. As it turned out, because of the pollution, only fish species and hybrids of tilapia could tolerate the rivers and no intermediate hosts of expected parasites could tolerate the conditions. Results had to be obtained from the amount of morphological and histopathological abnormalities in the fishes.

Bioaccumulation

In addition to parasites indicating the presence of toxicants in the ecosystem, parasites can also concentrate toxins from host tissues. Sures (2001) reviewed this problem in fishes where helminths, primarily acanthocephalans and secondarily cestodes and nematodes, can concentrate numerous heavy metals to concentrations several orders of magnitude higher than those in host tissues or the environment. Most digeneans do not concentrate as much as other helminths, but *Fasciola hepatica* inhabiting the bile ducts of cattle has been shown to accumulate lead concentrations 172 and 115 times higher than values in muscle and liver, respectively (Sures et al., 1998). Perhaps this occurs because lead binds to the erythrocytes, is transported to the liver where the majority of lead is stripped from the blood, and is excreted into the intestine by means of bile. Apparently the site of *F. hepatica* with high concentrations of lead allows the worm good access to it. As a point of interest, this ability of many helminths protects hosts from acquiring too high of concentrations of many heavy metals shows that parasites/digeneans can be good guys!

Catastrophes

By using similar methods for determining biological richness, Overstreet (2007) sampled a variety of locations and known hosts continually for digeneans after a hurricane to assess habitat recovery. Hurricane Katrina in August 2005 reached gusts of 433 km/hour and surges penetrating 20 km inland along bays, rivers, and bayous of coastal Mississippi, Louisiana, and Alabama in the United States. Resulting devastation covered a landmass of about the same size as that of the island of Great Britain, United Kingdom. They investigated a variety of situations involving hurricanes, but regarding digeneans, they noted how long it took various digeneans to become reestablished following Hurricane Katrina. Loss of biota resulted from perturbations of sediments and surge of high salinity water into estuarine and freshwater habitats. Clay and sandy sediments were lost from some areas and added to others, with the storm's energy being most influential offshore and at a depth of 25–30 m, where 1 m of sediment was scoured from the bottom and re-suspended, with the corresponding loss of the infauna. The surge of over 9 m in some locations with water of 32 ppt replacing water of 15–0 ppt saline, flushing out and killing nearly all of the biota.

The reader must keep in mind that it may take 1 or more years for the invertebrates serving as intermediate hosts to become reestablished and additional years for those invertebrates to become infected by their digenean parasites. Of course, migrating fishes that acquire infections in Texas or Florida in the United States do not show a loss of infections nor do local fishes that migrated to avoid the effects of the storm. In the latter case, the authors considered reestablishment as infections in juvenile fish that had not been born until after the storm.

By the time of the first scientific presentation on reestablishment (Overstreet, 2007), only a few fish species became infected, and with a low mean intensity of digeneans. Sampling continued, and updated results on specific digeneans and other parasites were presented at various scientific meetings, and finally the compiled data were published (Overstreet and Hawkins, 2017), showing that reestablishment can take a short period for some species and many years for others.

Climate Change

Parasites, and digeneans in particular, allow researchers to investigate large scale events. Since change takes place over evolutionary and ecological time scales resulting from natural and anthropogenic causes, Marcogliese (2001) considered temperature and parasites of boreal regions of North America as a good focal point for investigations of climate change. Because different hosts in a cycle follow range constrictions, the presence of a parasite will also become modified in unpredictable ways since the host-parasite systems are intricately interwoven with the environment, and changes in physical processes at different temporal and space scales will affect parasite populations differently.

Introduced Species

Occasionally when a megafaunal organism becomes introduced outside its typical location, other organisms are included or the range of the organism spreads. Also, a parasite can be included in the transfer or spread. As an example, tropical fishes are reared in outside facilities and are shared with other growers. This has happened probably on numerous occasions and has involved vegetation and the invasive snail *Melanoides tuberculatus* (common name, red rim melenia). The snail became introduced at least in the 1970s into southern Florida, United States (Roessler et al., 1977). Unfortunately, the heterophyid *Centrocestus* cf. *formosanus* infects the snail and follows it around the southeast United States, and probably elsewhere. This parasite has an unusual characteristic of promoting proliferation of cartilage surrounding the metacercarial cyst, usually in the gills of the host. This abnormal proliferation occurs extensively in a few of the many fishes the cercariae can infect. Some of the fishes are rare, such as the federally listed endangered fountain darter (*Etheostoma fonticola*), which is highly susceptible to and easily killed by the infection. Mitchell et al. (2005) reported on the history of the introduction and the life cycle of the worm.

Digenean species that had once been considered to be introduced are occasionally determined by molecular comparisons to be sister species. For example, what had thought to be *Bolbophorus confusus* introduced from Europe appears to be *B. damnificus* or *Bolbophorus* sp. of Overstreet and colleagues (2002), who discuss the introductions.

Migration of Model Host

Using parasites of pelicans and grebes as a variety of indicators, including migration, was mentioned elsewhere in the chapter. Most species of these are useful to examine because they host many digeneans. However, the use of digeneans and of other parasites has also been very useful for determining migration of fish hosts and stock separation. For example, Blaylock and colleagues (1998) examined Pacific halibut from 15 localities from northern California to the northern Bering Sea for all parasites, including many digeneans. The fish clustered into 3 groups on the basis of parasites, and these depended on temperature and geography, features that have a large effect on digeneans. These and associated data suggest 3 separate stocks of this commercially important fish.

Host Stocks

Not all fishes make good models for using parasites to separate or distinguish fish stocks, and often digeneans do not provide the best parasite indicator. The sablefish, *Anoplopoma fimbria*, off Canada's west coast is an example of a good model (Kabata et al., 1988). These fish contained 7 digeneans, and their prevalence, mean intensity, relationship with host age, and locations (13) differed enough to show the seamount and slope host populations constituted separate stocks. That development of the localized fisheries provided a significant yield to Canadian fishermen.

There are other cases where salmonids infected with a single freshwater digenean species that has a lengthy longevity in both freshwater and marine phases, such as the metacercaria of *Nanophyetes salmincola* and adult of *Plagioporus shawi* in juvenile trout from the United States Pacific Northwest, then tag the fish. They allow researchers to know from which specific or group of freshwater sources the infected individuals arose.

Dalton (1991) reported tagged steelhead trout 5,000–5,500 km from their area of origin in the central North Pacific Ocean. Monitoring of chinook salmon smolts from the Trinity River, California, United States detected annual differences, possibly because of differences in temperature and the resulting shed of cercariae (Foott et al., 1997). In this study, fish and snails were placed in a shallow trough, and 10 fish were examined and sectioned. In a wet mount of the most infected tissue, the mid-kidney, the most infected individual contained 10,220 cysts/gram, and the mean number of metacercariae in sections of the posterior kidney was 28.0 ± 14.7. The Puget Sound Steelhead Marine Survival Workgroup (Berejikian et al., 2018) reported abstracts on various projects on *Nanophyetes salmincola,* including cumulative mortality of fish at 46 days in seawater (mortality leveled at 7% after day 12 for infected individuals versus 0% for uninfected ones), susceptibility of waterborne cercariae to chemotherapeutics (100 ppm hydrogen peroxide, Perox-Aid®, and various doses of formalin), plus others.

Detective Work/Forensics

Many of the findings resulting from using parasites, primarily digeneans and other helminths with complicated life cycles, as indicators can be considered detective work. However, some cases clearly can be defined as detective work in the literal sense. An example concerns a truckload of red drum (*Sciaenops ocellatus*) that had been stopped and examined by different authorities, including United States Customs officials. The fishermen operating the truck said the fish, which they planned to sell, came from the Carolinas, from where the catch would have been legal. Professionals had Overstreet examine a sample of the fish, and he found a bucephalid endemic to the northern Gulf of Mexico where limits and seasons were stricter than along the Atlantic coast. Neither Overstreet nor other researchers who had examined the red drum from the Carolinas found any infection with that worm. That evidence was used to find the fishermen guilty of illegally catching and trying to sell Gulf fish (Overstreet et al., 2009).

Ichthyologists considered the Pascagoula River in the late 1960s to be free of striped bass. Consequently, a few hatchery-reared individuals fed commercial feed were released in the area, and 1 year later Overstreet (1971b) discovered several specimens of a new digenean species, *Neochasmus sogandaresi*, in a specimen of the fish. Then and later, a great deal of effort was unsuccessfully spent trying to see if the parasite also occurred in another host. None was discovered, suggesting that a small wild stock of striped bass had occurred in the area and represented at least enough striped bass to maintain a population of the digenean.

Digeneans as a Human Food Source

Numerous books and articles treat public health. Overstreet (2003) took the other point of view. He wrote about people eating parasites on purpose, with the assumption that there was no public health risk. For example, different people eat, or have eaten in earlier times, the giant liver fluke of various species of deer, *Fascioloides magna*: Hunters eating what they call little livers, Cajuns eating double-fried puffed flukes, Native Americans of the southeast United States eating what they call little flapjacks, and some members of the Sioux Nation in North America eating them and other liver flukes as a portion of their game or domesticated mammal with the intention to transfer the life force. Some indigenous people in Africa eat the paramphistomes from the stomach lining of hippopotamus calling them the juicy part of the hippo, and members of the tribes of Meghalaya, India relish paramphistomes from the rumen of cattle and buffaloes. Lots of parasites other than flukes are eaten fresh or cooked with smiles on the face of the consumers.

A Note on the Literature on Digeneans

Some early literature is intentionally being presented because it, mixed with recent studies, allows a good starting point for a variety of studies that can be readily tackled. These older approaches include those by Paul Nollen, Bernie Fried, Robin Overstreet, and others. Several general parasitology textbooks treat various aspects of digeneans, some in more detail or from a different point of view than presented in this chapter. Examples of some of those include are listed in Table 4.

Some students will address this chapter with public health or veterinary medicine viewpoints. A few of the many references treating such information are listed in Table 5.

Acknowledgements and Disclosure

The author thanks Jean Jovonovich and Janet Wright for their tireless help with the references and reading over portions of the text. Some of the investigations described in this section were supported in part by a grant from BP Exploration and Production, Inc.

Literature Cited

Andres, M. J., and R. M. Overstreet. 2013. A new species of *Podocotyloides* (Digenea: Opecoelidae) from the grey conger eel, *Conger esculentus*, in the Caribbean Sea. Journal of Parasitology 99: 619–623. doi: 10.1645/12-155.1

Andres, M. J., S. S. Curran, T. J. Fayton, and R. M. Overstreet. 2015. An additional genus and two additional species of Forticulcitinae (Digenea: Haploporidae). Folia Parasitologica 62: 025. doi: 10.14411/fp.2015.025

Andres, M. J., M. S. Peterson, and R. M. Overstreet. 2016a. Endohelminth parasites of some midwater and benthopelagic stomiiform fishes from the northern Gulf of Mexico. Gulf and Caribbean Research 27: 11–19. doi: 10.18785/gcr.2701.02

Andres, M. J., E. E. Pulis, T. H. Cribb, and R. M. Overstreet. 2014a. Erection of the haploporid genus *Litosaccus* n. g. and its phylogenetic relationship within the Haploporidae Nicoll, 1914, Systematic Parasitology 89: 185–194. doi: 10.1007/s11230-014-9521-4

Andres, M. J., E. E. Pulis, S. S. Curran, and R. M. Overstreet. 2018. On the systematics of some marine haploporids (Trematoda) with the description of a new species of *Megasolena* Linton, 1910. Parasitology International 67: 805–815. doi: 10.1016/j.parint.2018.08.002

Andres, M. J., E. E. Pulis, and R. M. Overstreet. 2016b. Description of three species of *Isorchis* (Digenea: Atractotrematidae) from Australia. Acta Parasitologica 61: 590–601. doi: 10.1515/ap-2016-0079

Andres, M. J., E. E. Pulis and R. M. Overstreet. 2014b. New genus of opecoelid trematode from *Pristipomoides aquilonaris* (Perciformes: Lutjanidae) and its phylogenetic affinity within the family Opecoelidae. Folia Parasitologica 61: 223–230. doi: 10.14411/fp.2014.033

Andres, M. J., C. L. Ray, E. E. Pulis, S. C. Curran, et al. 2014c. Molecular characterization of two opecoelid trematodes from fishes in the Gulf of Mexico, with a description of a new species of *Helicometra*. Acta Parasitologica 59: 405–412. doi: 10.2478/s11686-014-0258-7

Anuracpreeda, P. S. Phutong, A. Ngamniyom, B. Panyarachun, et al. 2014. Surface topography and ultrastructural architecture of the tegument of adult *Carmyerius spatiosus* Brandes, 1898. Acta Tropica 143: 18–28. doi: 10.1016/j.actatropica.2014.12.003

Ash, L. R., and T. C. Orihel. 2007. Ash and Orihel's Atlas of Human Parasitology, 5th edition. American Society for Clinical Pathology Press, Chicago, Illinois, United States, 540 p.

Barger, M., and D. Wellenstein. 2015. Morphological confirmation of *Homalometron* (Trematoda: Apocreadiidae) species in freshwater fishes in southeastern Texas, USA, with description of two species. Comparative Parasitology 82: 248–253. doi: 10.1654/4754.1

Barnard, C. J., and J. M. Behnke, eds. 1990. Parasitism and Host Behavior. Taylor and Francis, London, United Kingdom, 332 p.

Beaver, P. C., R. C. Jung, and E. W. Cupp. 1984. Clinical Parasitology, 9th edition. Lea and Febiger, Philadelphia, Pennsylvania, United States, 825 p.

Bell, A. S., C. Sommerville, and D. I. Gibson. 1999. Cercarial emergence of *Ichthyocotylurus erraticus* (Rudolphi, 1809), *I. variegatus* (Creplin, 1825) and *Apatemon gracilis* (Rudolphi, 1819) (Digenea: Strigeidae): Contrasting responses to light, dark cycling. Parasitology Research 85: 387–392. doi: 10.1007/s004360050564

Berejikian, B., C. Elings, E. Connor, E. Neatherlin, et al. 2018. Puget Sound Steelhead Marine Survival, 2013–2017: Research Findings Summary. Salish Sea Marine Survival Project, Seattle, Washington, United States, 83 p. https://marinesurvivalproject.com/wp-content/uploads/PS-Steelhead-Marine-Survival-Research-Summary-Report-2013-2017-13April20....pdf

Bils, R. F., and W. E. Martin. 1966. Fine structure and development of the trematode integument. Transactions of the American Microscopical Society 85: 78–88. doi: 10.2307/3224777

Blair, D., R. A. Bray, and S. C. Barker. 1998. Molecules and morphology in phylogenetic studies of the Hemiuroidea (Digenea: Trematoda: Platyhelminthes). Molecular Phylogenetics and Evolution 9: 15–25.

Blasco-Costa, I., J. A. Balbuena, A. Kostadinova, and P. D. Olson. 2009. Interrelationships of the Haploporinae (Digenea: Haploporidae): A molecular test of the taxonomic framework based on morphology. Parasitology International 58: 263–269. doi: 10.1016/j.parint.2009.03.006

Blaylock, R. B., L. Margolis, and J. C. Holmes. 1998. Zoogeography of the parasites of Pacific halibut (*Hippoglossus stenolepis*) in the northeast Pacific. Canadian Journal of Zoology 76: 2,262–2,273. doi: 10.1139/z98-172

Blend, C. K., Y. F. M. Karar, and N. O. Dronen. 2017. Revision of the Megaperidae Manter, 1934 n. comb. (Syn. Apocreadiidae Skrjabin, 1942) including a reorganization of the Schistorchiinae Yamaguti, 1942. Zootaxa 4358: 1–44. doi: 10.11646/zootaxa.4358.1.1

Bousfield, E. L., and R. W. Heard. 1986. Systematics, distributional ecology, and some host-parasite relationships of *Uhlorchestia uhleri* (Shoemaker) and *U. spartinophila*, new species (Crustacea: Amphipoda), endemic to salt marshes of the Atlantic coast of North America. Journal of Crustacean Biology 6: 264–274. doi: 10.1163/193724086X00082

Bowman, D. D. 2014. Georgis' Parasitology for Veterinarians, 10th edition. Elsevier Health Sciences, St. Louis, Missouri, United States, 496 p.

Bray, R. A. 2002. Superfamily Gymnophalloidea Odhner, 1905. *In* D. I. Gibson, A. Jones and R. A. Bray, eds. Keys to the Trematoda, Volume 1. CAB International, Wallingford, United Kingdom, p. 243–244.

Bray, R. A., T. H. Cribb, D. T. J. Littlewood, and A. Waeschenbach. 2016. The molecular phylogeny of the digenean family Opecoelidae Ozaki, 1925 and the value of morphological characters, with the erection of a new subfamily. Folia Parasitologica 63: 013. doi: fb.2016.013

Bray, R. A., D. I. Gibson, and A. Jones. 2008. Keys to the Trematoda, Volume 3. CAB International, Wallingford, United Kingdom, 824 p.

Bray, R. A., A. Waeschenbach, T. H. Cribb, G. D. Weedall, et al. 2009. The phylogeny of the Lepocreadiidae (Platyhelminthes: Digenea) inferred from nuclear and mitochondrial genes: Implications for their systematics and evolution, Acta Parasitologica 54: 310–329. doi: 10.2478/s11686-009-0045-z

Bray, R. A., A. Waeschenbach, P. Dyal, D. T. J. Littlewood, et al. 2014. New digeneans (Opecoelidae) from hydrothermal vent fishes in the southeastern Pacific Ocean, including one new genus and five new species. Zootaxa 3768: 73–87. doi: 10.11646/zootaxa.3768.1.5

Bray, R. A., B. L. Webster, P. Bartoli, and D. T. J. Littlewood. 2005. Relationships within the Acanthocolpidae Lühe, 1906 and their place among the Digenea. Acta Parasitologica 50: 281–291. http://www.actaparasitologica.pan.pl/archive/PDF/Bray.pdf

Brooks, D. R., and E. P. Hoberg. 2000. Triage for the biosphere: The need and rationale for taxonomic inventories and phylogenetic studies of parasites. Comparative Parasitology 67: 1–25.

Brooks, D. R., and B. Holcman. 1993. Revised classification and phylogenetic hypothesis for the Acanthostominae Looss, 1899 (Digenea: Opisthorchiformes: Cryptogonimidae). Proceedings of the Biological Society of Washington 106: 207–220. http://biostor.org/reference/65590

Brooks, D. R., and D. A. McLennan. 1993. Parascript. Parasites and the Language of Evolution. Smithsonian Institution Press, Washington, DC, United States, 429 p.

Brooks, D. R., S. M. Bandoni, C. A. MacDonald, and R. T. O'Grady. 1989. Aspects of the phylogeny of the Trematoda Rudolphi, 1808 (Platyhelminthes: Cercomeria). Canadian Journal of Zoology 67: 2,609–2,624. doi: 10.1139/z89-370

Brooks, D. R., E. P. Hoberg, W. A. Boeger, S. L. Gardner, et al. 2014. Finding them before they find us: Informatics, parasites, and environments in accelerating climate change. Comparative Parasitology 81: 155–164.

Brooks, D. R., R. T. O'Grady, and D. R. Glen. 1985. Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. Canadian Journal of Zoology 63: 411–443. doi: 10.1139/z85-062

Bullard, S. A., and R. M. Overstreet. 2008. Digeneans as enemies of fishes. *In* J. C. Eiras, H. Segner, T. Wahli, and B. G. Kapoor, eds. Fish Diseases, Volume 2. Science Publishers, Enfield, New Hampshire, United States, p. 817–976.

Bullard, S. A., and R. M. Overstreet. 2002. Potential pathological effects of blood flukes (Digenea: Sanguinicolidae) on pen-reared marine fishes. Proceedings of the Gulf and Caribbean Fisheries Institute 53: 10–25.

Bullard, S. A., K. Jensen, and R. M. Overstreet. 2009. Historical account of the two family-group names in use for the single accepted family comprising the "fish blood flukes." Acta Parasitologica 54: 78–84. doi: 10.2478/s11686-009-0012-8

Bullard, S. A., S. D. Snyder, K. Jensen, and R. M. Overstreet. 2008. New genus and species of Aporocotylidae (Digenea) from a basal actinopterygian, the American paddlefish, *Polyodon spathula*, (Acipenseriformes: Polyodontidae) from the Mississippi Delta. Journal of Parasitology 94: 487–495. doi: 10.1645/GE-1323.1

Bush, A. O., J. C. Fernández, G. W. Esch, and J. R. Seed. 2001. Parasitism: The Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, United Kingdom, 566 p.

Bush, A. O., R. W. Heard, Jr., and R. M. Overstreet. 1993. Intermediate hosts as source communities. Canadian Journal of Zoology 71: 1,358–1,363. doi: 10.1139/z93-186

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83: 575–583. doi: 10.2307/3284227

Cable, R. M. 1956. Marine cercariae of Puerto Rico. New York Academy of Sciences Scientific Survey of Porto Rico and the Virgin Islands, Volume XVI, Part 4, p. 491–577.

Cable, R. M. 1982. Phylogeny and taxonomy of the malacobothrean flukes. *In* D. F. Mettrick and S. S. J. Desser, eds. Parasites: Their World and Ours. Elsevier Biomedical Press, Amsterdam, Netherlands, p. 194–197.

Cable, R. M. 1974. Phylogeny and taxonomy of trematodes with reference to marine species. *In* W. B. Vernberg, ed. Symbiosis in the Sea. University of South Carolina, Columbia, South Carolina, United States, p. 173–193.

Cable, R. M. 1965. "Thereby hangs a tail." Journal of Parasitology 51: 3–12. doi: 10.2307/3275635

Cable, R. M., and M. H. Schutte. 1973. Comparative fine structure and origin of the metacercarial cyst in two philophthalmid trematodes, *Parorchis acanthus* (Nicoll, 1906) and *Philophthalmus megalurus* (Cort, 1914). Journal of Parasitology 59: 1,031–1,040. doi: 10.2307/3278639

Cain, G. D. 1969b. The source of hemoglobin in *Philophthalmus megalurus* and *Fasciolopsis buski* (Trematoda: Digenea). Journal of Parasitology 55: 307–310. doi: 10.2307/3277395

Cain, G. D. 1969a. Studies on hemoglobins in some digenetic trematodes. Journal of Parasitology 55: 301–306. doi: 10.2307/3277394

Caira, J. N. 1989. Revision of the North American papillose Allocreadiidae (Digenea) with independent cladistics analyses of larval and adult forms. Bulletin of the University of Nebraska State Museum 11: 1–58 + 34 p. https://digitalcommons.unl.edu/museumbulletin/114/

Calhoun, D. M., S. S. Curran, E. E. Pulis, J. M. Provaznik, et al. 2013. *Hirudinella ventricosa* (Pallas, 1774) Baird, 1853 represents a species complex based on ribosomal DNA. Systematic Parasitology 86: 197–208. doi: 10.1007/s11230-013-9439-2.8

Campbell, R. A. 1983. Parasitism in the deep sea. *In* G. T. Rowe, ed. Deep-Sea Biology, the Sea, Volume 8. Wiley, New York, New York, United States, p. 473–552.

Canning, E. U. 1975. The microsporidian parasites of Platyhelminthes: Their morphology, development, transmission and pathogenicity. Commonwealth Institute of Helminthology Miscellaneous Publication Number 2. Commonwealth Agricultural Bureaux, Bucks, United Kingdom, 32 p.

Choudhury, A., M. L. Aguirre-Macedo, S. S. Curran, M. Ostrowski de Núñez, et al. 2016. Trematode diversity in freshwater fishes of the globe, II: New World. Systematic Parasitology 93: 271–282. doi: 10.1007/s11230-016-9632-1

Combes, C. 2001. Parasitism: The Ecology and Evolution of Intimate Interactions, I. De Buron and V. A. Connors, transl. University of Chicago Press, Chicago, Illinois, United States, 728 p.

Conn, D. B., Z. Świderski, and J. Miquel. 2018. Ultrastructure of digenean trematode eggs (Platyhelminthes: Neoophora): A review emphasizing new comparative data on four European Microphalloidea. Acta Parasitologica 63: 1–14. doi: 10.1515/ap-2018-0001

Cribb, T. H. 2005. Family Opecoelidae Ozacki, 1925. *In* A. Jones, R. A. Bray, and D. I. Gibson, eds. Keys to the Trematoda, Volume 2. CAB International, Wallingford, United Kingdom, p. 443–531.

Cribb, T. H., G. R. Anderson, R. D. Adlard, and R. A. Bray. 1998. A DNA-based demonstration of a three-host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). International Journal for Parasitology 28: 1,791–1,795. doi: 10.1016/S0020-7519(98)00127-1

Cribb, T. H., N. Q.-X. Wee, R. A. Bray, and S. C. Cutmore. 2018. *Monorchis lewisi* n. sp. (Trematoda: Monorchiidae) from the surf bream, *Acanthopagrus australis* (Sparidae), in Moreton Bay, Australia. Journal of Helminthology 92: 100–108. doi: 10.1017/S0022149X1700102X

Crofton, H. D. 1971a. A model of host-parasite relationships. Parasitology 63: 343–364. doi: 10.1017/S0031182000079890

Crofton, H. D. 1971b. A quantitative approach to parasitism. Parasitology 62: 179–193. doi: 10.1017/S0031182000071420

Curran, S. S., R. M. Overstreet, D. T. The, and N. T. Le. 2001. *Singhiatrema vietnamensis* sp. n. (Digenea: Ommatobrephidae) and *Szidatia taiwanensis* (Fischthal and Kuntz, 1975) comb. n. (Digenea: Cyathocotylidae) from colubrid snakes in Vietnam. Comparative Parasitology 68: 219–227.

Curran, S. S., E. E. Pulis, M. J. Andres, and R. M. Overstreet. 2018. Two new species of *Saccocoelioides* (Digenea: Haploporidae) with phylogenetic analysis of the family, including species of *Saccocoelioides* from North, Middle, and South America. Journal of Parasitology 104: 221–239. doi: 10.1645/17-189

Curran, S. S., V. V. Tkach, and R. M. Overstreet. 2013a. Molecular evidence for two cryptic species of *Homalometron* (Digenea: Apocreadiidae) in freshwater fishes of the southeastern United States. Comparative Parasitology 80: 186–195. doi: 10.1654/4626.1

Curran, S. S., V, V. Tkach, and R. M. Overstreet. 2013b. A new species of *Homalometron* (Digenea: Apocreadiidae) from fishes in the northern Gulf of Mexico. Journal of Parasitology 99: 93–101. doi: 10.1645/GE-3169.1

Curran, S. S., V. V. Tkach, and R. M. Overstreet. 2006. A review of *Polylekithum* Arnold, 1934 and its familial affinities using morphological and molecular data, with description of *Polylekithum catahoulensis* sp. nov. Acta Parasitologica 51: 238–248. doi: 10.2478/s11686-006-0037-1

Cutmore, S. C., T. L. Miller, S. S. Curran, M. B. Bennett, et al. 2013. Phylogenetic relationships of the Gorgoderidae (Platyhelminthes: Trematoda), including the proposal of a new subfamily (Degeneriinae n. subfam.). Parasitology Research 112: 3,063–3,074. doi: s00436-013-3481-5

Dalton, T. J. 1991. Variation in prevalence of *Nanophyetus salmincola*, a parasite tag indicating U. S. Northwest origin, in steelhead trout (*Oncorhynchus mykiss*) caught in the central North Pacific Ocean. Canadian Journal of Fisheries and Aquatic Sciences 48: 1,104–1,108. doi: 10.1139/f91-131

Dalton, J. P., C. R. Caffrey, M. Sajid, C. Stack, et al. 2005. Proteases in trematode biology. *In* A. G. Maule and N. J. Marks, eds. Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology. CAB International, Wallingford, United Kingdom, p. 348–368.

Dao, T. T. H., T. T. G. Nguyen, S. Gabriël, K. L. Bui, et al. 2017. Updated molecular phylogenetic data for *Opisthorchis* spp. (Trematoda: Opisthorchioidea) from ducks in Vietnam. Parasites and Vectors 10: 575. doi: 10.1186/s13071-017-2514-9

Diamant, A., and I. Paperna. 1985. The development and ultrastructure of *Nosema ceratomyxae* sp. nov., a microsporidian hyperparasite of the myxosporean *Ceratomyxa* sp. from Red Sea rabbitfish (Siganidae). Protistologica 21: 249–258.

Diaz, J. I., and F. Cremonte. 2010. Development from metacercaria to adult of a new species of *Maritrema* (Digenea: Microphallidae) parasitic in the kelp gull, *Larus dominicanus*, from the Patagonian coast, Argentina. Journal of Parasitology 96: 740–745. doi: 10.1645/GE-2343.1

Doe, D. A., and J. P. S. Smith III. 2016. Structure of the male copulatory apparatus in *Prognathorhynchus busheki* (Platyhelminthes, Kalyptorhychia). Invertebrate Biology 135: 150–162. doi: 10.1111/ivb.12125

Dogiel, V. A., Y. I. Polyanski, E. M. Kheisin, and Z. Kabata. 1966. General Parasitology. Academic Press, New York, New York, United States, 516 p.

Dollfus, R. P. 1946. Parasites (animaux et végétaux) des helminthes. *In* P. Lechevalier, ed. Encyclopédie biologique, XXVII: Hyperparasites, ennemis et prédateurs des helminthes parasites et des helminthes libres: Essai de comliation méthodique. Jouve and Cie, Imprimeurs, Paris, France, 482 p.

Dönges, J. 1971. The potential number of redial generations in echinostomatids (Trematoda). International Journal for Parasitology 1: 51–59. doi: 10.1016/0020-7519(71)90046-4

El-Mayas, H., and G. C. Kearn. 1995. In vitro excystment of the metacercaria of *Cryptocotyle concavum* from the common goby *Pomatoschistus microps*. Journal of Helminthology 69: 285–297. doi: 10.1017/S0022149X00014851

Erasmus, D. A. 1967. Ultrastructural observations on the reserve bladder system of *Cyathocotyle bushiensis* Khan, 1962 (Trematoda: Strigeoidea) with special reference to lipid excretion. Journal of Parasitology 53: 525–536. doi: 10.2307/3276710

Erasmus, D. A., and C. Öhman. 1965. Electron microscope studies of the gland cells and host-parasite interface of the adhesive organ of *Cyathocotyle bushiensis* Khan, 1962. Journal of Parasitology 51: 761–769. doi: 10.2307/3276153

Esch, G. W., A. Bush, and J. M. Aho. 1990a. Parasite Communities: Patterns and Processes. Chapman and Hall, London, United Kingdom, 335 p.

Esch, G. W., T. C. Hazen, D. J. Marcogliese, T. M. Goater, et al. 1986. A long-term study on the population biology of *Crepidostomum cooperi* (Trematoda: Allocreadidae) in the burrowing mayfly, *Hexagenia limbata* (Ephemeroptera). American Midland Naturalist 116: 304–317. doi: 10.2307/2425738

Esch, G. W., A. W. Shostak, D. J. Marcogliese, and T. M. Goater. 1990b. Patterns and processes in helminth parasite communities: an overview. *In* G. W. Esch, A. O. Bush and J. M. Aho, eds. Parasite Communities: Patterns and Processes. Chapman and Hall, London, United Kingdom, p. 1–19.

Fayton, T. J., S. S. Curran, M. J. Andres, R. M. Overstreet, et al. 2016. Two new species of *Homalometron* (Digenea: Apocreadiidae) from Nearctic freshwater fundulids, elucidation of the life cycle of *H. cupuloris*, and molecular phylogenetic analysis of some congeners. Journal of Parasitology 102: 94–104. doi: 10.1645/15-862

Fernandez, M., D. T. J. Littlewood, A. Latorre, J. A. Raga, et al. 1998. Phylogenetic relationships of the family Campulidae (Trematoda) based on 18S rRNA sequences. Parasitology 117: 383–391. doi: 10.1017/S0031182098003126

Filippi, J.-J., Y. Quilichini, and B. Marchand. 2013. Topography and ultrastructure of the tegument of *Deropristis inflata* Molin, 1859 (Digenea: Deropristidae), a parasite of the European eel *Anguilla anguilla* (Osteichthyes: Anguillidae). Parasitology Research 112: 517–528. doi: 10.1007/s00436-012-3162-9

Foott, J. S., D. Free, W. Talo, and J. D. Williamson. 1997. Physiological Effects of *Nanophyetus* Metacercaria Infection in Chinook Salmon Smolts (Trinity River): FY96 Investigational Report. United States Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, California, United States, 19 p.

Fried, B., and M. A. Haseeb. 1991. Platyhelminthes: Aspidogastrea, Monogenea, and Digenea. *In* F. W. Harrison and B. J. Bogitsh, eds. Microscopic Anatomy of Invertebrates, Volume 3: Platyhelminthes and Nemertinea. Wiley-Liss, New York, New York, United States, p. 141–209.

Galaktionov, K. V., I. Blasco-Costa and P. D. Olson. 2012. Life cycles, molecular phylogeny and historical biogeography of the '*pygmaeus*' microphallids (Digenea: Microphallidae): widespread parasites of marine and coastal birds in the Holarctic. Parasitology 139: 1,346–1,360. doi: 10.1017/S0031182012000583

Galbreath, K. E., E. P. Hoberg, J. A. Cook, B. Armién, et al. 2019. Building an integrated infrastructure for exploring biodiversity: Field collections and archives of mammals and parasites. Journal of Mammalogy 100: 382–393. https://doi.org/10.1093/jmammal/gyz048

Gardner, S. L., and M. L. Campbell. 1992. Parasites as probes for biodiversity. Journal of Parasitology 78: 596–600. doi: 10.2307/3283534

Gardner, S. L., and F. A. Jiménez-Ruiz. 2009. Methods of endoparasite analysis. *In* T. Kunz and S. Parsons, eds. Ecological and Behavioral Methods for the Study of Bats. Johns Hopkins University Press, Baltimore, Maryland, United States, p. 795–805.

Gardner, S. L., R. N. Fisher, and S. J. Barry. 2012. Field parasitology techniques for use during reptile surveys. *In* R. McDiarmid, M. Foster, C. Guyer, and J. W. Gibbons, eds. Reptile Biodiversity: Standard Methods for Inventory and Monitoring. Smithsonian Publications, University of California Press, Oakland, California, United States, p. 114–121.

Gibson, D. I. 1972. Flounder parasites as biological tags. Journal of Fish Biology 4: 1–9.

Gibson, D. I. 1987. Questions in digenean systematics and evolution. Parasitology 95: 429–460. doi: 10.1017/S0031182000057851

Gibson, D. I. 1973. Some ultrastructural studies on the excretory bladder of *Podocotyle staffordi* Miller, 1941 (Digenea). Bulletin of the British Museum of Natural History, Zoology Series 24: 461–465.

Gibson, D. I., and R. A. Bray. 1979. The Hemiuroidea: Terminology, systematics and evolution. Bulletin of the British Museum of Natural History, Zoology Series 36: 35–146.

Gibson, D. I., A. Jones, and R. A. Bray, eds. 2002. Keys to the Trematoda, Volume 1. CAB International, Wallingford, United Kingdom, 521 p.

Ginetsinskaya, T. A. 1988. Trematodes, Their Life Cycle, Biology and Evolution. Translated for the United States Department of the Interior and the National Science Foundation, Washington, DC, United States. Amerind Publishing, New Delhi, India, 559 p.

Graham, L. C. 1969. Hyperparasitism by alariid mesocercariae: Fact or artifact? Journal of Parasitology 55: 1,094–1,095. doi: 10.2307/3277189

Haas, M. R., and B. Fried. 1974. Observations on cephalic glands in *Philophthalmus hegeneri.* Journal of Parasitology 60: 1,041–1,043. doi: 10.2307/3278548

Halton, D. W., and S. D. Stranock. 1976. The fine structure and histochemistry of the caecal epithelium of *Calicotyle kröyeri* (Monogenea: Monopisthocotylea). International Journal for Parasitology 6: 253–263. doi: 10.1016/0020-7519(76)90043-6

Heard, R. W., and R. M. Overstreet. 1983. Taxonomy and life histories of two North American species of "*Carneophallus*" (= *Microphallus*) (Digenea: Microphallidae). Proceedings of the Helminthological Society of Washington 50: 170–174.

Heneberg, P., J. Sitko, and J. Bizos. 2016. Molecular and comparative morphological analysis of central European parasitic flatworms of the superfamily Brachylaminoidea Allison, 1943 (Trematoda: Plagiochiida). Parasitology 143: 455–474. doi: 10.1017/S003118201500181X

Heneberg, P., J. Sitko, and J. Bizos. 2018. Molecular and comparative morphological analysis of central European parasitic flatworms of the superfamily Brachylaimoidea Allison, 1943 (Trematoda: Plagiorchiida): Corrigendum. Parasitology: 1–5. doi: 10.1017/S0031182018001610

Heyneman, D. 1960. On the origin of complex life cycles in the digenetic flukes. *In* Libro Homenaje al Dr. Eduardo Caballero y Caballero, Jubileo 1930–1960. Editorial Politécnica, Secretaría de Educación Pública, México, p. 133–152.

Höglund, J., and J. Thulin. 1988. Parasitangrepp i ögon hos fisk, som leveri kylvatten från kärnkraftsreaktorer. Naturvårdsverket Rapport 3539: 11, 47 p.

Holmes, J. C. 1979. Parasite populations and host community structure. *In* B. B. Nickol, ed. Host-Parasite Interfaces. Academic Press, New York, New York, United States, p. 27–46.

Howell, M. J. 1973. The resistance of cysts of *Stictodora lari* (Trematoda: Heterophyidae) to encapsulation by cells of the fish host. International Journal for Parasitology 3: 653–659. doi: 10.1016/0020-7519(73)90090-8

Hunninen, A. V., and R. Wichterman. 1938. Hyperparasitism: A species of *Hexamita* (Protozoa, Mastigophora) found in the reproductive systems of *Deropristis inflata* (Trematoda) from marine eels. Journal of Parasitology 24: 95–101. doi: 10.2307/3272490

ICZN (International Commission on Zoological Nomenclature). 2012. International Code of Zoological Nomenclature. https://www.iczn.org/the-code/the-code-online/

Jacobson, E. R. 2007. Infectious Diseases and Pathology of Reptiles. CRC Press, Boca Raton, Florida, United States, 716 p.

Jackson, C. J., D. J. Marcogliese, and M. D. B. Burt. 1997. Precociously developed *Ascarophis* sp. (Nematoda: Spirurata) and *Hemiurus levinseni* (Digenea: Hemiuridae) in their crustacean intermediate hosts. Acta Parasitologica 42: 31–35.

Johnson, D. S., and R. Heard. 2017. Bottom-up control of parasites. Ecosphere 8: e01885. doi: 10.1002/ecs2.1885

Jones, A. 2005. Superfamily Heronimoidea Ward, 1917. *In* A. Jones, R. A. Bray, and D. I. Gibson, eds. Keys to the Trematoda, Volume 2. CAB International, Wallingford, United Kingdom, p. 185–187.

Jones, A., R. A. Bray, and D. I. Gibson, eds. 2005. Keys to the Trematoda, Volume 2. CAB International, Wallingford, United Kingdom, 745 p.

Kabata, Z., G. A. McFarlane, and D. J. Whitaker. 1988 Trematoda of sablefish, *Anoplopoma fimbria* (Pallas, 1811), as possible biological tags for stock identification. Canadian Journal of Zoology 66: 195–200. doi: 10.1139/z88-027

Klimpel, S., M. W. Busch, T. Sutton, and H. W. Palm. 2010. Meso- and bathy-pelagic fish parasites at the Mid-Atlantic Ridge (MAR): Low host specificity and restricted parasite diversity. Deep Sea Research Part I: Oceanographic Research Papers 57: 596–603. doi: 10.1016/j.dsr.2010.01.002

Kudlai, O., S. C. Cutmore, and T. H. Cribb. 2015. Morphological and molecular data for three species of the Microphallidae (Trematoda: Digenea) in Australia, including the first descriptions of the cercariae of *Maritrema brevisacciferum* Shimazu et Pearson, 1991 and *Microphallus minutus* Johnston, 1948. Folia Parasitologica 62: 053. doi: 10.14411/fp.2015.053

Køie, M. 1979. On the morphology and life-history of *Derogenes varicus* (Müller, 1784) Looss, 1901 (Trematoda, Hemiuridae). Zeitschrift für Parasitenkunde Parasitology Research 59: 67–78.

Køie, M. 1981. On the morphology and life-history of *Podocotyle reflexa* (Creplin, 1825) Odhner, 1905, and a comparison of its developmental stages with those of *P. atomon* (Rudolphi, 1802) Odhner, 1905 (Trematoda: Opecoelidae). Ophelia 20: 17–43.

Køie, M., 1977. Stereoscan studies of cercariae, metacercariae, and adults of *Cryptocotyle lingua* (Creplin 1825) Fischoeder 1903 (Trematoda: Heterophyidae). Journal of Parasitology 63: 835–839. doi: 10.2307/3279888

Køie, M. 1985. The surface topography and life-cycles of digenetic trematodes in *Limanda limanda* (L.) and *Gadus morhua* L. (Summary). PhD dissertation—Marine Biological Laboratory, University of Copenhagen, Copenhagen, Denmark, 20 p.

Køie, M., P. Nansen, and N. Ø. Christensen. 1977. Stereoscan studies of rediae, cercariae, cysts, excysted metacercariae, and migratory stages of *Fasciola hepatica*. Zeitschrift für Parasitenkunde/Parasitology Research 54: 289–297. doi: 10.1007/BF00390120

Labrie, L., C. Komar, J. Terhune, A. Camus, et al. 2004. Effect of sublethal exposure to the trematode *Bolbophorus* spp. on the severity of enteric septicemia of catfish in channel catfish fingerlings. Journal of Aquatic Animal Health 16: 231–237. doi: 10.1577/H04-011.1

LaRue, G. R. 1957. The classification of digenetic Trematoda: A review and a new system. Experimental Parasitology 6: 306–349. doi: 10.1016/0014-4894(57)90025-5

Littlewood, D. T. J., R. A. Bray, and A. Waeschenbach. 2015. Phylogenetic patterns of diversity in cestodes and trematodes. *In* S. Morand, B. R. Krasnov, and D. T. J. Littlewood, eds. Parasite Diversity and Diversification: Evolutionary Ecology Meets Phylogenetics. Cambridge University Press, Cambridge, United Kingdom, p. 304–319. doi: 10.1017/CBO9781139794749.018.7

Littlewood, D. T. J., K. Rohde, R. A. Bray, and E. A. Herniou. 1999a. Phylogeny of the Platyhelminthes and the evolution of parasitism. Biological Journal of the Linnean Society 68: 257–287. doi: 10.1111/j.1095-8312.1999.tb01169.x

Littlewood, D. T. J., K. Rohde, and K. A. Clough. 1999b. The interrelationships of all major groups of Platyhelminthes: Phylogenetic evidence from morphology and molecules. Biological Journal of the Linnean Society 66: 75–114. doi: 10.1111/j.1095-8312.1999.tb01918.x

Locke, S. A., F. S. Al-Nasiri, M. Caffara, F. Drago, et al. 2015. Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. International Journal for Parasitology 45: 841–855. doi: 10.1016/ijjpara.2015.07.001

Locke, S. A., A. R. Lapierre, K. Byers, H. Proctor, et al. 2012. Molecular and morphological evidence for the Holarctic distribution of *Urogonimus macrostomus* (Rudolphi, 1803) Monticelli, 1888 (Digenea: Leucochloridiidae). Journal of Parasitology 98: 880–882. doi: 10.1645/GE-3043.1

Louhi, K.-R., L.-R. Sundberg, J. Jokela, and A. Karvonen. 2015. Interactions among bacterial strains and fluke genotypes shape virulence of co-infection. Proceedings of the Royal Society B: Biological Sciences 282: 20152097. doi: 10.1098/rspb.2015.2097

Lumsden, R. D. 1979. Morphological aspects of host-parasite interaction: some observations on the mammalian inflammatory response to helminth parasitism. *In* B. B. Nickol, ed. Host-Parasite Interfaces. Academic Press, New York, New York, United States, p. 49–70.

Lumsden, R. D., and F. Sogandares-Bernal. 1970. Ultrastructural manifestations of pulmonary paragonimiasis. Journal of Parasitology 56: 1,095–1,109. doi: 10.2307/3277553

MacKenzie, K. 1999. Parasites as biological tags in population studies of marine organisms. Qatar University Science Journal 19: 117–127.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: An update. Parasitology 124: 153–163. doi: 10.1017/S0031182002001518

MacKenzie, K., H. H. Williams, B. Williams, A. H. McVicar, et al. 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *In* Advances in Parasitology, Volume 35. Academic Press, New York, New York, United States, p. 85–144. doi: 10.1016/S0065-308X(08)60070-6

Manter, H. W. 1957. Host specificity and other host relationships among the digenetic trematodes of marine fishes. *In* First Symposium on Host Specificity among Parasites of Vertebrates. Institut de Zoologie, Université de Neuchâtel, Neuchâtel, Switzerland, p. 185–198.

Manter, H. W. 1970. The terminology and occurrence of certain structures of digenetic trematodes, with special reference to the Hemiuroidea. *In* K. S. Singh and B. K. Tandon, eds. H. D. Srivastava Commemoration Volume. Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India, p. 27–33.

Marcogliese, D. J. 2003. Food webs and biodiversity: Are parasites the missing link? Journal of Parasitology 89 (Supplement): S106–S113.

Marcogliese, D. J. 2001. Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology 79: 1,331–1,352. doi: 10.1139/z01-067

Marcogliese, D. J. 2004. Parasites: Small players with crucial roles in the ecological theater. EcoHealth 1: 151–164. doi: 10.1007/s10393-004-0028-3

Marcogliese, D. J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. Reviews in Fish Biology and Fisheries 5: 336–371. doi: 10.1007/BF00043006

Marcogliese, D. J., and K. C. Jacobson. 2015. Parasites as biological tags of marine, freshwater and anadromous fishes in North America from the tropics to the Arctic. Parasitology 142: 68–89. doi: 10.1017/S0031182014000110

Marcogliese, D. J., T. M. Goater, and G. W. Esch. 1990. *Crepidostomum cooperi* (Allocreadiidae) in the burrowing mayfly, *Hexagenia limbata* (Ephemeroptera) related to trophic status of a lake. American Midland Naturalist 124: 309–317. doi: 10.2307/2426180

Martin, T. R., and D. B. Conn. 1990. The pathogenicity, localization, and cyst structure of echinostomatid metacercariae (Trematoda) infecting the kidneys of the frogs *Rana clamitans* and *Rana pipiens*. Journal of Parasitology 76: 414–149. doi: 10.2307/3282677

Martin, W. E., and R. F. Bils. 1964. Trematode excretory concretions: Formation and fine structure. Journal of Parasitology 50: 337–344. doi: 10.2307/3275837

Martínez-Aquino, A., V. M. Vidal-Martínez, and M. L. Aguirre-Macedo. 2017. A molecular phylogenetic appraisal of the acanthostomines *Acanthostomum* and *Timoniella* and their position within Cryptogonimidae (Trematoda: Opisthorchioidea). PeerJ 5: e4158. doi: 10.7717/peerj.4158

Matisz, C. E., C. P. Goater, and D. Bray. 2010. Migration and site selection of *Ornithodiplostomum ptychocheilus* (Trematoda: Digenea) metacercariae in the brain of fathead minnows (*Pimephales promelas*). Parasitology 137: 719–731. doi: 10.1017/S0031182009991545

Maule, A. G., D. W. Halton, C. F. Johnston, C. Shaw, et al. 1990. The serotoninergic, cholinergic, and peptidergic components of the nervous system in the monogenean parasite, *Diclidophora merlangi*: A cytochemical study. Parasitology 100, Part 2: 255–273. doi: 10.1017/S0031182000061266

McLaughlin, J. D., and D. Marcogliese. 1983. The migration, growth and development of *Cyclocoelum oculeum* (Kossack, 1911) (Trematoda: Cyclocoelidae) in *Fulica americana* (Gm.). Parasitology 87: 239–247. doi: 10.1017/S0031182000052604

McVeigh, P., L. Atkinson, N. J. Marks, A. Mousley, et al. 2012. Parasite neuropeptide biology: Seeding rational drug target selection? International Journal for Parasitology Drugs and Drug Resistance 2: 76–91. doi: 10.1016/j.ijpddr.2011.10.004

Miller, T. L., and T. H. Cribb. 2008. Family Cryptogonimidae Ward, 1917. *In* R. A. Bray, D. I. Gibson, and A. Jones, eds. Keys to the Trematoda, Volume 3. CAB International, Wallingford, United Kingdom, p. 51–112.

Miller, T. L., and T. H. Cribb. 2007a. Two new cryptogonimid genera *Beluesca* n. gen. and *Chelediadema* n. gen. (Digenea: Cryptogonimidae) from tropical Indo-West Pacific Haemulidae (Perciformes). Zootaxa 1543: 45–60. doi: 10.11646/ZOOTAXA.1543.1.2

Miller, T. L., and T. H. Cribb. 2007b. Two new cryptogonimid genera (Digenea: Cryptogonimideae) from *Lutjanus bohar* (Perciformes: Lutjanidae): Analyses of ribosomal DNA reveals wide geographic distribution and presence of cryptic species. Acta Parasitologica 52: 104–113. doi: 10.2478/s11686-007-0019-y

Mitchell, A. J., R. M. Overstreet, A. E. Goodwin, and T. M. Brandt. 2005. Spread of an exotic fish-gill trematode: A far-reaching and complex problem. Fisheries 30: 11–16. doi: 10.1577/1548-8446(2005)30[11:SOAEFT]2.0.CO;2

Moczoń, T., and Z. Świderski. 1983. *Schistosoma haematobium*: Oxidoreductase histochemistry and ultrastructure of niridazole-treated females. International Journal for Parasitology 13: 225–232. doi: 10.1016/0020-7519(83)90017-6

Mohammed, M. S. A., and H. Y. Al-Attar. 2000. Neurocytological and histochemical studies on the neurosecretory materials of *Sonsinotrema tecapence* (Trematoda: Digenea). Rivista di Parassitologia 17: 113–117.

Moore, J. 2002. Parasites and the Behavior of Animals. Oxford University Press, New York, New York, United States, 315 p.

Murugesh, M., and R. Madhavi. 1990. Egg and miracidium of *Hirudinella ventricosa* (Trematoda: Hirudinellidae). Journal of Parasitology 76: 748–749. doi: 10.2307/3282998

Noble, E. R. 1973. Parasites and fishes in a deep-sea environment. *In* F. S. Russell and M. Yonge, eds. Advances in Marine Biology, Volume 11. Academic Press, London, United Kingdom, p. 121–195. doi: 10.1016/S0065-2881(08)60269-2

Noble, E. R., and G. A. Noble. 1964. Parasitology: The Biology of Animal Parasites, 2nd edition. Lea and Febiger, Philadelphia, Pennsylvania, United States, 724 p.

Noble, E. R., G. A. Noble, G. A. Schad, and A. J. MacInnes, eds. 1989. Parasitology: The Biology of Animal Parasites, 6th edition. Lea and Febiger, Philadelphia, Pennsylvania, United States, 574 p.

Nolan, M. J., and T. H. Cribb. 2005. The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. Advances in Parasitology 60: 101–163. doi: 10.1016/S0065-308X(05)60002-4

Nollen, P. M. 1968a. Autoradiographic studies on reproduction in *Philophthalmus megalurus* (Cort, 1914) (Trematoda). Journal of Parasitology 54: 43–48. doi: 10.2307/3276870

Nollen, P. M. 1990a. Chemosensitivity of *Philophthalmus megalurus* (Trematoda) miracidia. Journal of Parasitology 76: 439–440. doi: 10.2307/3282685

Nollen, P. M. 1971. Digenetic trematodes: quinone tanning system in eggshells. Experimental Parasitology 30: 64–72. doi: 10.1016/0014-4894(71)90071-3

Nollen P. M. 1990b. Escape of rediae from miracidia of *Philophthalmus megalurus* and *Philophthalmus gralli* during *in vitro* culture. Journal of Parasitology 76: 725–729. doi: 10.2307/3282989

Nollen, P. M. 1984. Mating behavior of *Philophthalmus megalurus* and *P. gralli* in concurrent infections of chicks. International Journal for Parasitology 14: 71–74. doi: 10.1016/0020-7519(84)90014-6

Nollen, P. M. 1999. Mating behaviour of *Echinostoma trivolvis* and *E. paraensei* in concurrent infections in hamsters. Journal of Helminthology 73: 329–332. doi: 10.1017/S0022149X99000542

Nollen, P. M. 1978. Studies on the reproductive system of *Philophthalmus gralli* using techniques of transplantation and autoradiography. Journal of Parasitology 64: 613–616. doi: 10.2307/3279944

Nollen, P. M. 1968b. Uptake and incorporation of glucose, tyrosine, leucine, and thymidine by adult *Philophthalmus megalurus* (Cort, 1914) (Trematoda), as determined by autoradiography. Journal of Parasitology 54: 295–304. doi: 10.2307/3276939

Olsen, O. W. 1974. Animal Parasites: Their Life Cycles and Ecology, 3rd edition. University Park Press, Baltimore, Maryland, United States, 562 p.

Olson, P. D., and V. V. Tkach. 2005. Advances and trends in the molecular systematics of the parasitic Platyhelminthes. Advances in Parasitology 60: 165–243. doi: 10.1016/S0065-308X(05)60003-6

Olson, P. D., T. H. Cribb, V. V. Tkach, R. A. Bray, et al. 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). International Journal for Parasitology 33: 733–755. doi: 10.1016/S0020-7519(03)00049-3

Orecchia, P., M. Ortis, and L. Paggi. 2006. Digenei, Revisione della Checklist della fauna marina italiana. http://www.faunaitalia.it/checklist/

Orélis-Riberio, R., C. R. Arias, K. M. Halanych, T. H. Cribb, et al. 2014. Diversity and ancestry of flatworms infecting blood of nontetrapod craniates "fishes." Advances in Parasitology 85: 1–64. doi: 10.1016/B978-0-12-800182-0.00001-5

Overstreet, R. M. 1982. Abiotic factors affecting marine parasitism. *In* D. R. Mettrick and S. S. Desser, eds. Parasites: Their World and Ours. Fifth International Congress of Parasitology (August 7–14, 1982, Toronto, Canada): Proceedings and Abstracts, Volume 2. Elsevier Biomedical Press, Amsterdam, Netherlands, p. 36–39.

Overstreet, R. M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. Tulane Studies in Zoology 15: 119–176.

Overstreet, R. M. 2007. Effects of a hurricane on fish parasites. Parassitologia 49: 161–168.

Overstreet, R. M. 1976a. *Fabespora vermicola* sp. n., the first myxosporidan from a platyhelminth. Journal of Parasitology 62: 680–684. doi: 10.2307/3278937

Overstreet, R. M. 2003. Flavor buds and other delights [American Society of Parasitologists Annual Meeting, Presidential address]. Journal of Parasitology 89: 1,093–1,107. doi: 10.1645/GE-236.7

Overstreet, R. M. 1978. Marine maladies? *In* Worms, Germs, and Other Symbionts from the Northern Gulf of Mexico. Mississippi-Alabama Sea Grant Consortium, MASGP-78-021, 140 p.

Overstreet, R. M. 1971a. *Metadena spectanda* Travassos, Freitas, and Bührnheim, 1967 (Digenea: Cryptogonimidae) in estuarine fishes from the Gulf of Mexico. Proceedings of the Helminthological Society of Washington 38: 156–158.

Overstreet, R. M. 1983. Metazoan symbionts of crustaceans. *In* A. J. Provenzano, ed. The Biology of Crustacea: Pathobiology, Volume 6. Academic Press, New York, New York, United States, p. 155–250.

Overstreet, R. M. 1971b. *Neochasmus sogandaresi* n. sp. (Trematoda: Cryptogonimidae) from the Striped Bass in Mississippi. Transactions of the American Microscopical Society 90: 87–89. doi: 10.2307/3224902

Overstreet, R. M. 1973. Parasites of the Atlantic croaker as biological indicators. *In* Thirty-seventh Annual Meeting (Mississippi Academy of Sciences, Biloxi, Mississippi, March 15–17, 1973). Mississippi Academy of Sciences, Jackson, Mississippi, United States.

Overstreet, R. M. 1993. Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. *In* J. A. Couch and J. W. Fournie, eds. Pathobiology of Marine and Estuarine Organisms. CRC Press, Boca Raton, Florida, United States p. 111–156.

Overstreet, R. M. 1997. Parasitological data as monitors of environmental health. Parassitologia 39: 169–175.

Overstreet, R. M. 1976b. A redescription of *Crassicutis archosargi*, a digenean exhibiting an unusual tegumental attachment. Journal of Parasitology 62: 702–708. doi: 10.2307/3278945

Overstreet, R. M., and C. E. Brown. 1970. *Lasiotocus trachinoti* sp. n. (Digenea: Monorchiidae) from the pompano, *Trachinotus carolinus* (Linnaeus), along the east coast of Florida. Journal of Parasitology 56: 941–943.doi: 10.2307/3277510

Overstreet, R. M., and S. S. Curran. 2004. Defeating diplostomoid dangers in USA catfish aquaculture. Folia Parasitologica 51: 153–165. doi: 10.14411/fp.2004.019

Overstreet, R. M., and S. S. Curran. 2005a. Family Atractotrematidae Yamaguti, 1939. *In* A. Jones, R. A. Bray, and D. I. Gibson, eds. Keys to the Trematoda, Volume 2. CAB International, Wallingford, United Kingdom, p. 167–174.

Overstreet, R. M., and S. S. Curran. 2005b. Family Haploporidae Nicoll, 1914. *In* A. Jones, R. A. Bray, and D. I. Gibson, eds. Keys to the Trematoda, Volume 2. CAB International, Wallingford, United Kingdom, p. 129–165.

Overstreet, R. M., and S. S. Curran. 2005c. Parasites of the American white pelican. Gulf and Caribbean Research 17: 31–48. doi: 10.18785/gcr.1701.04

Overstreet, R. M., and S. S. Curran. 2002. Superfamily Bucephaloidea La Rue, 1926. *In* D. I. Gibson, A. Jones, and R. A. Bray, eds. Keys to the Trematoda, Volume 1. CAB International and Natural History Museum, Wallingford, United Kingdom, p. 67–110.

Overstreet, R. M., and W. E. Hawkins. 2017. Diseases and mortalities of fishes and other animals in the Gulf of Mexico. *In* C. Ward, ed. Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill. Springer, New York, New York, United States, p. 1,589–1,738. doi: 10.1007/978-1-4939-3456-0_6

Overstreet, R. M., and R. W. Heard. 1978. Food of the Atlantic croaker, *Micropogonias undulatus,* from Mississippi Sound and the Gulf of Mexico. Gulf Research Reports 6: 145–152. doi: 10.18785/grr.0602.05

Overstreet, R. M., and R. W. Heard. 1995. A new species of *Megalophallus* (Digenea: Microphallidae) from the clapper rail, other birds, and littoral isopod *Ligia baudiniana*. Canadian Journal of Fisheries and Aquatic Sciences 52 (Supplement 1): 98–104. doi: 10.1139/f95-515

Overstreet, R. M., and F. G. Hochberg, Jr. 1975. Digenetic trematodes in cephalopods. Journal of the Marine Biological Association of the United Kingdom 55: 893–910. doi: 10.1017/S0025315400017781

Overstreet, R. M., and J. M. Lotz. 2016. Host-symbiont relationships: Understanding the change from guest to pest. *In* C. J. Hurst., ed. The Rasputin Effect: When Commensals and Symbionts Become Parasitic. Advances in Environmental Microbiology, Volume 3. Springer International, Cham, Switzerland, p. 27–64. doi: 10.1007/978-3-319-28170-4_2

Overstreet, R. M., J. O. Cook, and R. W. Heard. 2009. Trematoda (Platyhelminthes) of the Gulf of Mexico. *In* D. L. Felder and D. K. Camp, eds. Gulf of Mexico: Origin, Waters, and Biota, Volume 1: Biodiversity. Texas A & M University Press, College Station, Texas, United States, p. 419–486.

Overstreet, R. M., S. S. Curran, L. M. Pote, D. T. King, et al. 2002. *Bolbophorus damnificus* n. sp. (Digenea: Bolbophoridae) from the channel catfish *Ictalurus punctatus* and American white pelican *Pelecanus erythrorhynchos* in the USA based on life-cycle and molecular data. Systematic Parasitology 52: 81–96. doi: 10.1023/A:1015696622961

Overstreet, R. M., W. E. Hawkins, and T. L. Deardorff. 1996. The western mosquitofish as an environmental sentinel: Parasites and histological lesions. *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. Environmental Fate and Effects of Pulp and Paper Mill Effluents. St. Lucie Press, Delray Beach, Florida, United States, p. 495–509.

Panyarachun, B., P. Sobhon, Y. Tinikul, C. Chotwiwatthanakun, et al. 2010. *Paramphistomum cervi:* Surface topography of the tegument of adult fluke. Experimental Parasitology 125: 95–99. doi: 10.1016/j.exppara.2009.12.020

Paperna, I., and R. M. Overstreet. 1981. Parasites and diseases of mullets (Mugilidae). *In* O. H. Oren, ed. Aquaculture of Grey Mullets, International Biological Programme 26. Cambridge University Press, Cambridge, United Kingdom, p. 411–493.

Parker, J. H., S. S. Curran, R. M. Overstreet, and V. V. Tkach. 2010. Examination of *Homalometron elongatum* Manter, 1947 and description of a new congener from *Eucinostomus currani* Zahuranec, 1980 in the Pacific Ocean off Costa Rica. Comparative Parasitology 77: 154–163. doi: 10.1654/4451.1

Pearson, J. C. 1988. Nature and origin of the fluke life-cycle [Inaugural lecture, September 21]. University of Queensland, St. Lucia, Queensland, Australia.

Pearson, J. C. 1992. On the position of the digenean family Heronimidae: An inquiry into a cladistic classification of the Digenea. Systematic Parasitology 21: 81–166. doi: 10.1007/BF00010255.7

Pearson, J. C. 1986. The paranephridial system in the Digenea: Occurrence and possible phylogenetic significance. *In* M. Cremin, C. Dobson, and D. E. Moorhouse, eds. Parasite Lives. University of Queensland Press, Queensland, Australia, p. 56–68.

Pearson, J. C. 1972. A phylogeny of life-cycle patterns of the Digenea. *In* B. Dawes, ed. Advances in Parasitology, Volume 10. Academic Press, London, United Kingdom, p. 153–189. doi: 10.1016/S0065-308X(08)60174-8

Pina, S. M. R., F. Russell-Pinto, and P. Rodrigues. 2007. Clarification of *Cercaria sevillana* (Digenea: Microphallidae) life cycle using morphological and molecular data. Journal of Parasitology 93: 318–322. doi: 10.1645/GE-836R1.1

Poulin, R., and S. Morand. 2004. Parasite Biodiversity. Smithsonian Books, Washington, DC, United States, 216 p.

Poulin, R., D. J. Marcogliese, and J. D. McLaughlin. 1999. Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. Journal of Fish Biology 55: 47–53. doi: 10.1111/j.1095-8649.1999.tb00655.x

Pratt, H. S. 1909. The cuticula and subcuticula of trematodes and cestodes. American Naturalist 43: 705–728. doi: 10.1086/279105

Prévot, G. 1974. Recherches sur le cycle biologique et l'ecologie de quelques trematodes nouveaux parasites de *Larus argentatus michaellis* Naumann dans le midi de la France. PhD dissertation—Université de droit, d'Economie et des sciences. D'Aix-Marseille, France, 319 p.

Pulis, E. E., and R. M. Overstreet, 2013. Review of haploporid (Trematoda) genera with ornate muscularisation in the region of the oral sucker, including four new species and a new genus. Systematic Parasitology 84: 167–191. doi: 10.1007/s11230-012-9401-8

Pulis, E. E., S. S. Curran, M. J. Andres, and R. M. Overstreet. 2014. Change in rank of Megaperidae (Trematoda) to Megaperinae within the Apocreadiidae and description of *Haintestinum amplum* n. g., n. sp. Parasitology International 63: 269–274. doi: 10.1016/j.parint.2013.11.007

Pulis, E. E., T. J. Fayton, S. S. Curran, and R. M. Overstreet. 2013. A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*, Journal of Parasitology 99: 501–508. doi: 10.1645/12-106.1

Quilichini, Y., A. J. S. Bakhoum, J.-L. Justine, R. A. Bray, et al. 2016. Spermatozoon ultrastructure in two monorchiid digeneans. PeerJ 4: e2488. doi: 10.7717/peerj.2488

Radev, V., I. Kanev, and P. Nollen. 1998. Body spines of the eye flukes *Philophthalmus hegeneri* Penner et Fried, 1963 (Trematoda: Philophthalmidae). Helminthologia 35: 83–85.

Randall, C., and R. L. Reece. 1996. Color Atlas of Avian Histopathology. Mosby-Wolfe, London, United Kingdom, 232 p.

Razo-Mendivil, U., R. Rosas-Valdez, and G. Pérez-Ponce de León. 2008. A new Cryptogonomid (Digenea) from the

Mayan cichlid, *Cichlasoma urophthalmus* (Osteichthyes: Cichlidae), in several localities of the Yucatán Peninsula, Mexico. Journal of Parasitology 94: 1,371–1,378. doi: 10.1645/GE-1546.1

Riddell, J. H., P. J. Whitfield, M. A. Balogun, and M. C. Thorndyke. 1991. FRMFamide-like peptides in the nervous and endocrine systems of the digenean helminth *Echinostoma liei*. Acta Zoologica 72: 1–5. doi: 10.1111/j.1463-6395.1991.tb00311.x

Roberts, L. S., and J. J. Janovy, Jr. 2013. Gerald D. Schmidt and Larry S. Roberts' Foundations of Parasitology, 9th edition. McGraw-Hill, New York, New York, United States, 670 p.

Roessler, M. A., C. L. Beardley, and D. C. Tabb. 1977. New records of the introduced snail, *Melanoides tuberculata* (Mollusca: Thiaridae) in South Florida. Florida Scientist 40: 87–94.

Schell, S. C. 1985. Handbook of Trematodes of North America North of Mexico. University Press of Idaho, Moscow, Idaho, United States, 263 p.

Scholz, T., M. L. Aguirre-Macedo, and A. Choudhury. 2004. *Auriculostoma astyanace* n. gen., n. sp. (Digenea: Allocreadiidae), from the banded astyanax, *Astyanax fasciatus* (Characiformes: Characidae), from Nicaragua, with a reevaluation of Neotropical *Crepidostomum* spp. Journal of Parasitology 90: 1,128–1,132. doi: 10.1645/GE-3275

Schulte, L., E. Lovas, K. Green, J. Mulvenna, et al. 2013. Tetraspanin-2 localisation in high pressure frozen and freeze-substituted *Schistosoma mansoni* adult males reveals its distribution in membranes of tegumentary vesicles. International Journal for Parasitology 43: 785–793. doi: 10.1016/j.ijpara.2013.04.003

Sharma, P. N. 1978. Histochemical distribution of succinic dehydrogenase in the lymphatic system of a trematode *Ceylonocotyle scoliocoelium*. Journal of Helminthology 52: 159–162. doi: 10.1017/S0022149X00005290

Sharma, P. N., and A. N. Sharma. 1981. Cytochemical characteristics of the neurosecretory cells of *Ceylonocotyle scoliocoelium* (Trematoda: Digenea). Journal of Helminthology 55: 223–229. doi: 10.1017/S0022149X00026882

Sinitsin, D. 1931. Studien über die Phylogenie der Trematoden, IV: The life histories of *Plagioporus siliculus* and *Plagioporus virens*, with special reference to the origin of Digenea. Zeitschrift für Wissenschaftliche Zoologie 138: 409–456.

Smith, S. J. 1981. The trematode fauna of a brackish coastal lagoon in Tasmania. PhD dissertation—University of Tasmania, Hobart, Tasmania, Australia, 450 p. https://eprints.utas.edu.au/402/

Sokolova, Yu. Ya., and R. M. Overstreet. 2020. Hyperparasitic spore-forming eukaryotes (Microsporidia, Haplosporidia, and Myxozoa) parasitizing trematodes (Platyhelminthes). Invertebrate Zoology 17: 93–117. doi: 10.15298/invertzool.17.2.01

Sokolova, Yu. Ya., and R. M. Overstreet, 2018. A new microsporidium, *Apotaspora heleios* n. g., n. sp. from the riverine grass shrimp *Palaemonetes paludosus* (Decapoda: Caridea: Palaemonidae). Journal of Invertebrate Pathology 157: 125–135. doi: 10.1016/j.jip.2018.05.007

Storer, R. W. 2000. The Metazoan parasite fauna of grebes (Aves: Podicipediformes) and its relationship to the birds' biology. Miscellaneous Publications, Museum of Zoology, University of Michigan 188: 1–90.

Strong, P. L., and R. M. Cable. 1972. Fine structure and development of the metacercarial cyst in *Microphallus opacus* (Ward, 1894). Journal of Parasitology 58: 92–98. doi: 10.2307/3278248

Stunkard, H. W., and R. F. Nigrelli. 1930. On *Distomum vibex* Linton, with special reference to its systematic position. Biological Bulletin 58: 336–343.

Sun, P. L., N. J. Brown-Peterson, W. E. Hawkins, R. M. Overstreet, et al. 1998. Morphological and histological abnormalities in tilapia (*Oreochromis spp.)* from two contaminated rivers in southern Taiwan. Environmental Sciences 6: 129–152.

Sun, P. L., W. E. Hawkins, R. M. Overstreet, and N. J. Brown-Peterson. 2009. Morphological deformities as biomarkers in fish from contaminated rivers in Taiwan. International Journal of Environmental Research and Public Health 6: 2,307–2,331. doi: 10.3390/ijerph6082307

Sures, B. 2001. The use of fish parasites as bioindicators of heavy metals in aquatic ecosystems: A review. Aquatic Ecology 35: 245–255. doi: 10.1023/A:1011422310314

Sures, B., G. Jürges, and H. Taraschewski. 1998. Relative concentrations of heavy metals in the parasites *Ascaris suum* (Nematoda) and *Fasciola hepatica* (Digenea) and their respective porcine and bovine definitive hosts. International Journal for Parasitology 28: 1,173–1,178. doi: 10.1016/S0020-7519(98)00105-2

Świderski, Z., I. Montoliu, C. Feliu, D. I. Gibson, et al. 2013. A transmission electron microscopical study of the tegument of *Maritrema feliui* (Digenea: Microphallidae). Acta Parasitologica 58: 478–485. doi: 10.2478/s11686-013-0161-7

Takashima, F., and T. Hibiya, eds. 1995. An Atlas of Fish Histology: Normal and Pathological Features. Kodansha, Tokyo, Japan, 195 p.

Ternengo, S., Y. Quilichini, P. Katharios, and B. Marchand. 2009. Sperm ultrastructure of the gallbladder fluke *Anisocoelium capitellatum* (Digenea: Cryptogonimideae), a parasite of *Uranoscopus scaber* (Pisces: Uranoscopidae). Parasitology Research 104: 801–807. doi: 10.1007/s00436-008-1259-y

Tkach, V. V., and S. E. Bush. 2010. *Serpentoanisocladium sinense* n. g., n. sp. (Digenea: Cryptogonimideae) from the eastern water snake *Sinonatrix percarinata* (Boulenger) (Serpentes: Colubridae) in Guizhou Province, China. Systematic Parasitology 76: 205–210. doi: 10.1007/s11230-010-9246-y

Tkach, V. V., and J. M. Kinsella. 2011. New Macroderoides (Digenea: Macroderoididae) from Florida gar, with molecular phylogeny of the genus. Journal of Parasitology 97: 210–923. doi: 10.1645/GE-2704.1

Tkach, V. V., S. S. Curran, J. A. Bell, and R. M. Overstreet. 2013. A new species of *Crepidostomum* (Digenea: Allocreadiidae) from *Hiodon tergisus* in Mississippi and molecular comparison with three congeners. Journal of Parasitology 99: 1,114–1,121. doi: 10.1645/13-279.1

Tkach, V. V., O. Kudlai, and A. Kostadinova. 2016. Molecular phylogeny and systematics of the Echinostomatoidea Looss, 1899 (Platyhelminthes: Digenea). International Journal for Parasitology 46: 171–185. doi: 10.1016/j.ijpara.2015.11.001

Tkach, V. V., D. T. J. Littlewood, P. D. Olson, J. M. Kinsella, et al. 2003. Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Systematic Parasitology 56: 1–15. doi: 10.1023/A:1025546001611

Tkach, V. V., J. Pawlowski, and J. Mariaux. 2000. Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial lsrDNA sequences. International Journal for Parasitology 30: 83–93. doi: 10.1016/S0020-7519(99)00163-0

Tkach, V. V., E. E. Pulis, and R. M. Overstreet. 2010. A new *Paramacroderoides* species (Digenea: Macroderoididae) from two species of gar in the Southeastern United States. Journal of Parasitology 96: 1,002–1,006. doi: 10.1645/GE-2385.1

Torimi, I., T. Tsuboi, W. Hirai, and H. Nishida. 1989. Ultrastructure of sensory receptors of adult *Echinostoma hortense* (Trematoda: Echinostomatidae). Japanese Journal of Parasitology 38: 353–360.

Vandergon, T. L., G. Pittman Noblet, and J. M. Colacino. 1988. Identification and origin of hemoglobin in a gymnophallid metacercaria (Trematoda: Digenea), a symbiote in the marine polychaete *Amphitrite ornata* (Annelida: Terebellidae). Biological Bulletin 174: 172–180. doi: 10.2307/1541784

Wee, N. Q.-X., S. C. Cutmore, and T. H. Cribb. 2018. Two monorchiid species from the freckled goatfish, *Upeneus tragula* Richardson (Perciformes: Mullidae), in Moreton Bay, Australia, including a proposal of a new genus. Systematic Parasitology 95: 353–365. doi: 10.1007/s11230-018-9789-x

West, A. F. 1961. Studies on the biology of *Philophthalmus gralli* Mathis and Leger, 1910 (Trematoda: Digenea). American Midland Naturalist 66: 363–383. doi: 10.2307/2423036

Yamaguti, S. 1934. Studies on the Helminth Fauna of Japan, Part 2: Trematodes of Fishes 1. Japanese Journal of Zoology 5: 249–541.

Yamaguti, S., 1971. Synopsis of Digenetic Trematodes of Vertebrates, Volumes 1 and 2. Keigaku Publishing, Tokyo, Japan, 1,074 p.

Yamaguti, S. 1975. A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates. Keigaku Publishing, Tokyo, Japan. 590 p.

Supplemental Reading

Atkinson, C. T., N. Thomas and D. B. Hunter, eds. 2008. Parasitic Diseases of Wild Birds. Wiley-Blackwell, Ames, Iowa, United States, 595 p. doi: 10.1645/12-155.1

Behrens, A. C., and P. M. Nollen. 1993. Hatching of *Echinostoma caproni* miracidia from eggs derived from adults grown in hamsters and mice. Parasitology Research 79: 28–32. doi: 10.1007/BF00931214

Bourns, T. K. 1974. Carbohydrate and protein in *Lymnaea stagnalis* eggs and *Trichobilharzia ocellata* cercariae. Journal of Parasitology 60: 1,046–1,047. doi: 10.2307/3278551

Bunkley-Williams, L., and E. H. Williams. 1994. Parasites of Puerto Rican freshwater sport fishes. Puerto Rico Department of Natural and Environmental Resources, San Juan, Puerto Rico and Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico, United States, 168 p.

Campbell, R. A. 1990. Deep water parasites. Annales de parasitologie humaine et comparée 65: 65–68. doi: 10.1051/parasite/1990651065

Chandler, R. M., M. B. Thomas, and J. P. S. Smith III. 1992. The role of shell granules and accessory cells in eggshell formation in *Convoluta pulchra* (Turbellaria, Acoela). Biological Bulletin 182: 54–65. doi: 10.2307/1542180

Coles, G. C. 2006. Developments in the chemotherapy of parasitic flatworms. *In* A. G. Maule and N. J. Marks, eds. Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology. CAB International, Wallingford, United Kingdom, p. 243–255.

Combes, C. 2005. The Art of Being a Parasite. D. Simberloff, transl. University of Chicago Press, Chicago, Illinois, United States, 291 p.

Cribb, T. H., R. A. Bray, and D. T. J. Littlewood. 2001. The nature and evolution of the association among digeneans, molluscs and fishes. International Journal for Parasitology 31: 997–1,011. doi: 10.1016/S0020-7519(01)00204-1

Cribb, T. H., R. A. Bray, P. D. Olson, and D. T. J. Littlewood. 2003. Life cycle evolution in the Digenea: A new perspective from phylogeny. *In* D. T. J. Littlewood, J. R, Baker, R. Muller, and D. Rollinson, eds. The Evolution of Parasitism: A Phylogenetic Perspective. Advances in Parasitology 54. Elsevier, Oxford, United Kingdom, p. 197–254. doi: 10.1016/s0065-308x(03)54004-0

Deardorff, T. L., and R. M. Overstreet. 1991. Seafood-transmitted zoonoses in the United States: The fishes, the dishes, and the worms. *In* D. R. Ward and C. R. Hackney, eds. Microbiology of Marine Food Products. Van Nostrand Reinhold, New York, New York, United States, p. 211–265.

De Baets, K., P. Dentzien-Dias, I. Upeniece, O. Verneau, et al. 2015. Constraining the deep origin of parasitic flatworms and host-interactions with fossil evidence. Advances in Parasitology 90: 93–135. doi: 10.1016/bs.apar.2015.06.002

Del Conte, E. 1970. Études cytologiques et histochimiques sur la glande de Mehlis' chez *Corpopyrum* sp. (Trematoda, Digenea). Archives d'anatomie microscopique et de morphologie expérimentale 59: 9–20.

El-Naggar, M. M., A. A. Khidr, and G. C. Kearn. 1990. Ultrastructural observations on the oviduct, Mehlis' glands and ootype of the monogenean *Cichlidogyrus halli* typicus (Price & Kirk, 1967) Paperna, 1979. International Journal for Parasitology 20: 203–209. doi: 10.1016/0020-7519(90)90102-S

Fernandes, B. M., M. C. N. Justo, M. Q. Cárdenas, and S. C. Cohen. 2015. South American Trematodes Parasites of Birds and Mammals. Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, 516 p.

Font, W. F., R. W. Heard, and R. M. Overstreet. 1984. Life cycle of *Ascocotyle gemina* n. sp., a sibling of *A. sexidigita* (Digenea: Heterophyidae). Transactions of the American Microscopical Society 103: 392–407. doi: 10.2307/3226476

Ford, D. M., P. M. Nollen, and M. A. Romano. 1998. The effects of salinity, pH, and temperature on the half-life and longevity of *Echinostoma caproni* miracidia. Journal of Helminthology 72: 325–330. doi: 10.1017/S0022149X00016680

Forrester, D. J., and M. G. Spalding. 2003. Parasites and diseases of wild birds in Florida. University Press of Florida, Gainesville, Florida, United States, 1,132 p.

Fried, B., and A. Reddy. 1999. Effects of snail-conditioned water from *Biomphalaria glabrata* on hatching of *Echinostoma caproni* miracidia. Parasitology Research 85: 155–157. doi: 10.1007/s004360050526

Fried, B., and L. C. Rosa-Brunet. 1991. Cultivation of excysted metacercariae of *Echinostoma caproni* (Trematoda) to ovigerous adults on the chick chorioallantois. Journal of Parasitology 77: 568–571. doi: 10.2307/3283161

García, L. S. 2007. Diagnostic Medical Parasitology, 5th edition. ASM Press, Washington, DC, United States, 1,202 p.

Gibson, D. I. 2002. Family Bathycotylidae Dollfus, 1932. *In* D. I. Gibson, A. Jones, and R. A. Bray eds. Keys to the Trematoda, Volume 1. CAB International, Wallingford, United Kingdom, p. 349–350.

Goater, T. M., C. P. Goater, and G. W. Esch. 2014. Parasitism: The Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, United Kingdom, 497 p.

Halton, D. W. 1968. Light and electron microscope studies of carboxylic esterase activity in the trematode *Haplometra cylindracea*. Journal of Parasitology 54: 1,124–1,130. doi: 10.2307/3276975

Halton, D. W., J. M. Behnke, and I. Marshall, eds. 2001. Practical Exercises in Parasitology. Cambridge University Press, Cambridge, United Kingdom, 461 p.

Hoffman, G. L. 1999. Parasites of North American Freshwater Fishes, 2nd edition. Cornell University Press, Ithaca, New York, United States, 539 p.

Hosier, D. W., and B. Fried. 1991. Infectivity, growth, and distribution of *Echinostoma caproni* (Trematoda) in the ICR mouse. Journal of Parasitology 77: 640–642. doi: 10.2307/3283176

Howell, M. J. 1971. Some aspects of nutrition in *Philophthalmus burrili* (Trematoda: Digenea). Parasitology 62: 133–144. doi: 10.1017/S0031182000071341

Kohn, A., B. M. M. Fernandes, and S. C. Cohen. 2007. South American Trematodes Parasites of Fishes. Fundação Oswaldo Cruz, Oficina de Livros, Rio de Janeiro, Brazil, 318 p.

Larson, O. R., G. L. Uglem, and K. J. Lee. 1988. Fine structure and permeability of the metacercarial cyst wall of *Clinostomum marginatum* (Digenea). Parasitology Research 74: 352–355. doi: 10.1007/bf00539457

Linton, E. 1910. Helminth fauna of the Dry Tortugas, II: Trematodes. Papers from the Tortugas Laboratory of the Carnegie Institute of Washington 4: 11–98.

Littlewood, D. T. J., and R. A. Bray, eds. 2001. Interrelationships of the Platyhelminthes. Taylor and Francis, London, United Kingdom, 356 p.

Loker, E., and B. Hofkin. 2015. Parasitology: A Conceptual Approach. Garland Science, Taylor and Francis, New York, New York, United States, 576 p.

Madhavi, R., and R. A. Bray. 2018. Digenetic Trematodes of Indian Marine Fishes. Springer Nature, Dordrecht, Netherlands, 693 p. doi: 10.1007/978-94-024-1525-3

Manter, H. W. 1947. The digenetic trematodes of marine fishes of Tortugas, Florida. American Midland Naturalist 38: 257–416. doi: 10.2307/2421571

Maule, A. G., and N. J. Marks, eds. 2006. Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology. CAB International, Wallingford, United Kingdom, 448 p.

McDonald, M. E. 1969a. Annotated bibliography of helminths of waterfowl (Anatidae). Special Scientific Report Wildlife, Number 125. United States Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, DC, United States, 333 p.

McDonald, M. E., 1969b. Catalogue of helminths of waterfowl (Anatidae). Special Scientific Report, Wildlife, Number 126. United States Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, DC, United States, 692 p.

McDonald, M. E. 1981. Key to trematodes reported in waterfowl. Resource Publication, Number 142. United States Fish and Wildlife Service, Washington, DC, United States, 156 p.

Moczoń, T., and Z. Świderski. 2000. *Schistosoma japonicum*: cytochemistry of the Mehlis' gland and of the ootype wall. Acta Parasitologica 45: 22–28.

Nahhas, F. M., and R. M. Cable. 1964. Digenetic and aspidogastrid trematodes from marine fishes of Curaçao and Jamaica. Tulane Studies in Zoology 11: 169–228. doi: 10.5962/bhl.part.7052

Nickol, B. B., ed. 1979. Host-Parasite Interfaces. Academic Press, New York, New York, United States, 144 p.

Noga, E. J. 2010. Fish Disease: Diagnosis and Treatment, 2nd edition. Wiley-Blackwell, Hoboken, New Jersey, United States, 536 p.

Nollen, P. M. 1970. An ovotestis in *Philophthalmus megalurus*. Journal of Parasitology 56: 1,033. doi: 10.2307/3277533

Nollen, P. M. 1983. Patterns of sexual reproduction among parasitic platyhelminths. Parasitology 86: 99–120. doi:10.1017/S0031182000050861

Nollen, P. M., and M. J. Nadakavukaren. 1974. Observations on ligated adults of *Philophthalmus megalurus, Gorgoderina attenuata*, and *Megalodiscus temperatus* by scanning electron microscopy and autoradiography. Journal of Parasitology 60: 921–924. doi: 10.2307/3278512

Orido, Y. 1991. Ultrastructure of Mehlis' gland in the lung fluke, *Paragonimus ohirai* (Trematoda: Troglotrematidae). Journal of Morphology 207: 9–16. doi: 10.1002/jmor.1052070103

Orido, Y. 1990. Ultrastructure of the oviduct of the lung fluke, *Paragonimus ohirai* (Trematoda: Troglotrematidae). Journal of Morphology 204: 247–255. doi: 10.1002/jmor.1052040303

Overstreet, R. M. 1997. In Memoriam: Raymond Millard Cable, 1909–1995. Journal of Parasitology 83: 337–343.

Overstreet, R. M. 2012. Waterborne parasitic diseases in the ocean. *In* R. A. Meyers, ed. Encyclopedia of Sustainability Science and Technology, W–Z, Volume 17. Springer, New York, New York, United States, p. 12,018–12,062. doi: 10.1007/978-1-4419-0851-3

Palm, H. W., and R. A. Bray. 2014. Marine Fish Parasitology in Hawaii. Westarp and Partner Digitaldruck, Hohenwarsleben, Germany, 320 p.

Poulin, R. 2007. Evolutionary Ecology of Parasites, 2nd edition. Princeton University Press, Princeton, New Jersey, United States, 360 p.

Prudhoe, S., and R. A. Bray. 1982. Platyhelminth parasites of the Amphibia. British Museum of Natural History/Oxford University Press, Oxford, United Kingdom, 217 p.

Ruszkowski, J.-S. 1925. Sur quelques anomalies des trématodes. Annales de parasitologie humaine et comparée 3: 388–191.

Samuel, W. M., M. J. Pybus, and A. A. Kocan. 2008. Parasitic Diseases of Wild Mammals, 2nd edition. Iowa State University Press, Ames, Iowa, United States, 559 p. doi: 10.1002/9780470377000

Schmid-Hempel, P. 2011. Evolutionary Parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics. Oxford University Press, Oxford, United Kingdom, 516 p.

Schrandt, M. N., M. J. Andres, S. P. Powers, and R. M. Overstreet. 2016. Novel infection site and ecology of cryptic *Didymocystis* sp. (Trematoda) in the fish *Scomberomorus maculatus*. Journal of Parasitology 102: 297–305. doi: 10.1645/15-772

Sharma, P. N., and S. Mandawat. 1982. A comparison of morphology, acid phosphatase and ATPase activity in *Ganeo tigrinum* from hibernating and non-hibernating *Rana cyanophlyctis* and *R. tigrina*. Journal of Helminthology 56: 5–10. doi: 10.1017/S0022149X00034921

Sharma, P. N., S. Mandawat, and A. N. Sharma. 1981. Cytochemistry of Mehlis' gland in *Ceylonocotyle scoliocoelium*. Journal of Helminthology 55: 141–148. doi: 10.1017/S0022149X0002561X

Shiff, C. J. 1974. Seasonal factors influencing the location of *Bulinus (Physopsis) globosus* by miracidia of *Schistosoma haematobium* in nature. Journal of Parasitology 60: 578–583. doi: 10.2307/3278710

Siddiqi, A. H., and R. M. Cable. 1960. Digenetic trematodes of marine fishes of Puerto Rico, Scientific Survey of Porto Rico and the Virgin Islands 17: 257–369.

Smyth, J. D. 1962. Introduction to Animal Parasitology. C. C. Thomas, Springfield, Illinois, United States, 470 p.

Smyth, J. D., and D. W. Halton. 1983. The Physiology of Trematodes, 2nd edition. Cambridge University Press, Cambridge, United Kingdom, 445 p.

Sogandares-Bernal, F. 1959. Digenetic trematodes of marine fishes from the Gulf of Panama and Bimini, British West Indies. Tulane Studies in Zoology 7: 69–117.

Świderski, Z., A. J. S. Bakhoum, I. Montoliu, C. Feliu, et al. 2011a. Ultrastructural study of vitellogenesis in *Maritrema feliui* (Digenea, Microphallidae). Parasitology Research 109: 1,707–1,714. doi: 10.1007/s00436-011-2444-y

Świderski, Z., D. I. Gibson, A. M. Marigo, E. Delgado, et al. 2011b. Ultrastructure and cytochemistry of vitellogenesis and the vitellocytes of the bothriocephalidean cestode *Clestobothrium crassiceps* (Rudolphi, 1819), a parasite of the teleost fish *Merluccius merluccius* (L., 1758) (Gadiformes, Merlucciidae). Acta Parasitologica 56: 392–405. doi: 10.2478/s11686-011-0071-5

Thatcher, V. E. 2006. Amazon Fish Parasites, Volume 1, 2nd edition. Aquatic Biodiversity in Latin America. Pensoft Publishers, Sofia, Bulgaria, 508 p.

Thomas, F., F. Renaud, and J.-F. Guegan, eds. 2005. Parasitism and ecosystems. Oxford University Press, New York, New York, United States, 221 p.

Toft, C. A., A. Aeschlimann, and L. Bolis. 1991. Parasite-Host Associations: Coexistence or Conflict? Oxford University Press, New York, New York, United States, 384 p.

Travassos, L., J. F. Texeira de Freitas, and A. Kohn. 1969. Trematódeos do Brasil. Memorias do Instituto Oswaldo Cruz 67: 1–886.

Uglem, G. L., and O. R. Larson. 1987. Facilitated diffusion and active transport systems for glucose in metacercariae of *Clinostomum marginatum* (Digenea). International Journal for Parasitology 17: 847–850. doi: 10.1016/0020-7519(87)90068-3

Weinstein, M. S., and B. Fried. 1991. The expulsion of *Echinostoma trivolvis* and retention of *Echinostoma caproni* in the ICR mouse: pathological effects. International Journal for Parasitology 21: 255–257. doi: 10.1016/0020-7519(91)90018-3

Williams, E. H., and L. Bunkley-Williams. 1996. Parasites of offshore big game fishes of Puerto Rico and the western Atlantic. Puerto Rico Department of Natural Environmental Resources and the University of Puerto Rico, Mayaguez, Puerto Rico, United States, 382 p.

Williams, H., and A. Jones. 1994. Parasitic Worms of Fish. Taylor and Francis, London, United Kingdom, 593 p.

Woo, P. T. K. 2006. Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections, 2nd edition. CAB International, Cambridge, Massachusetts, United States, 791 p.

WoRMS Editorial Board. 2019. World Register of Marine Species. http://www.marinespecies.org. doi: 10.14284/170

Yamaguti, S. 1970. Digenetic Trematodes of Hawaiian Fishes. Keigaku Publishing, Tokyo, Japan, 436 p.

Yamaguti, S. 1954. Systema Helminthum, Part 1: Digenetic Trematodes of Fishes. Satyû Yamaguti, Tokyo, Japan, 403 p.

Yamaguti, S. 1958. Systema Helminthum, Volume 1: Digenetic Trematodes of Vertebrates, Parts 1 and 2. Interscience, New York, New York, United States, 1,575 p.