60

Platyhelminthes

Monogenea (Class)

Griselda Pulido-Flores

Phylum Platyhelminthes

    Class Monogenea

Chapter 60

Monogenea (Class)

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Introduction

The phylum Platyhelminthes, known as flatworms, includes the class Monogenea, mainly ectoparasites of the skin, fins, gills, and urinary bladder of fishes, amphibians, and some reptiles (Kearn, 2014). However, there is one species that is a parasite of mammals, *Oculotrema hippopotami* Stunkard, 1924, from the eye of the African hippopotamus *Hippotamus amphibius* (see Stunkard, 1924; Yamaguti, 1963). There also are a few species of monogeneans that infect cephalopods (Rohde, 2011). *Isancistrum loliginis* has been reported from squids, (*Loligo* spp.) and *Polystoma loliginum* has been reported and collected from other cephalopods (Overstreet and Hoschberg, 1975). Sometimes, instead of living as ectoparasites as is usual, a few monogeneans may be found living within the stomodeum, proctodeum, bladder, or diverticula of a host (Roberts and Janovy, 2008).

Classification: Historical Review

Entobdella hippoglossi was the first species of Monogenea described. Müller described it as *Epibdella hippoglossi*, a parasite from the skin of the Atlantic halibut *Hippoglossus hippoglossus*. In the original descriptions of this monogenean, it was mistaken for a leech and the author named it *Hirudo hippoglossi* (see Kearn, 2014).

There is controversy about whether the name that refers to this group of Platyhelminthes should be "Monogenea" or "Monogenoidea." The Latin term Monogenea derives from van Beneden's (1858) use of the French term "monogénèses" in French (cited in Carus, 1863) and is now the generally-used term for this group (Carus, 1863; Wheeler and Chisholm, 1995). Monogenoidea sensu Bychowsky (1937) is not the correct name because its use predates use of the term Monogenea. In addition, the ending of -oidea in animal taxonomy always refers to superfamily designations. Some have argued

for the use of Monogenoidea as the valid name of the class; however, this is based on erroneous assumptions of authorship, priority, and rank as defined in the International Code of Zoological Nomenclature (ICZN, 2012). The resolutions adopted at the Fourth International Congress of Parasitology (ICOPA IV) in Warsaw, Poland in 1978 during the Round Table "Monogenea: Problems of Systematics, Biology, and Ecology" resulted in an agreement supported by all participants to adopt Monogenea as the name of the class rather than Monogenoidea. For more information on this process, see Wheeler and Chisholm (1995).

The Monogenea have been divided into 2 major subgroups: **Polyopisthocotylea** (which means, in adults, possession of a more complex opisthaptor) and **Monopisthocotylea** (which means possession of a single opisthaptor). The morphology of the adult's attachment organs is what distinguishes these subgroups. The morphology of the attachment organ in the larval forms is what distinguishes the **Oligonchoinea** (**oligo** = few; Greek) and **Polyonchoinea** (**poly** = many; Greek) (Justine, 1998). The groups do not overlap because of the position of the polystomatids and sphyranurids.

A phylogenetic analysis using morphological data, the ultrastructure of spermiogenesis, and spermatozoa of the taxon Rhabdocoela (Platyhelminthes) produces a hypothesis that Monogenea is a monophyletic group that is more closely related to tapeworms than other platyhelminths (Justine, 1991; Zamparo et al., 2001); however, analyses of molecular data (18S or 28S rDNA sequences) do not support the monophyly of the Monogenea (Mollaret et al., 1997). The analyses conducted by Mollaret and colleagues (1997) suggest that Monogenea is a paraphyletic group, although the monophyly of Monopisthocotylea and Polyopisthocotylea were suggested (Mollaret et al., 2000). The molecular data agree with studies of the ultrastructure of spermiogenesis of Polyopisthocotylea, all of which share the synapomorphy of having lateral microtubules present in the principal region of the spermatozoon. In the monopisthocotyleans, dorsal and ventral microtubules are absent from the principal region of the spermatozoon (Justine, 1991). However, an analysis with both the morphological and molecular data of 18S rDNA analyses supports the monophyly of the group as Monogenea (Mollaret et al., 2000).

Current Classification

The current classification of Monogenea divides the class into 3 subclasses: **Polyonchoinea**, **Oligonchoinea**, and **Polystomatoinea** (Boeger and Kritsky, 1993). The monophyly of Monogenea as a class is supported by the following morphological synapomorphic (shared derived) characteristics: Adult and oncomiracidium possessing 2 pairs of eyespots, 16 marginal hooks in the haptor, a haptor with a single ventral

pair of hamuli (= anchors; Boeger and Kritsky, 1993), and an oncomiracidium with 3 rows of ciliary epidermal bands present (Brooks, 1989; Boeger and Kritsky, 1993).

The monophyly of Polyonchoinea is supported by the mouth being on the ventral surface, the reduced numbers of subsurface sperm microtubules, the oncomiracidium, and adults having 14 marginal hooks and 2 central hooks in the haptor (Boeger and Kritsky, 1993). The monophyly of Oligonchoinea is supported by having a crochet en fléau present that is hook-like (the crochet en fléau is the form of the termination of the central part of the clamp of the haptoral sclerite), and the presence of a single pair of lateral sclerites, 4 pairs of haptoral suckers, and diverticula in the walls of the intestine (Boeger and Kritsky, 1993).

The monophyly of subfamily Polystomatoinea is supported by the absence of egg filaments (Boeger and Kritsky, 1993). Polystomatoinea is the sister group of Oligonchoinea. The relationship is supported by 6 shared synapomorphies, namely: Having more than 2 testes; the presence of a gastrointestinal canal; the presence of haptoral suckers in the adults; the presence of hooks in the adults' haptoral sucker; that there are 3 parts of the haptoral suckers; and the presence of 2 lateral vaginal ducts (Boeger and Kritsky, 1993). The clade formed by Oligonchoinea + Polystomatoinea is the sister group of Polyonchoinea (see Figure 1) (Boeger and Kritsky, 1993).

Brabec et al. (2023) show 2 different arrangements of the phylogenetic relationships of the flatworms. They elevated the Monopisthocotylea and Polyopisthocotylea to the level of class. For additional clarification see the modified trees given in the introduction to the Platyhelminthes in this book as well the paper by Brabec and colleagues (2023).

Body Wall

The monogeneans, like the digeneans (trematodes/flukes) and cestodes (tapeworms), possess an external layer called a tegument. The surface of this is a syncytial stratum laden with vesicles and mitochondria. This layer is enclosed externally by a plasma membrane and glycocalyx and internally by a membrane and basal lamina. This stratum is the distal cytoplasm and it is connected by trabeculae (internuncial processes) to the cell bodies, or cytons (perikarya), located inside a layer of superficial muscle. Often, the outer surface of the tegument has scattered short microvilli. In some species the microvilli are absent and in their place shallow pits occur (Roberts and Janovy, 2008).

The tegument is the site of the exchange by diffusion of gases and nitrogenous waste between the body and the environment. Some nutrients in the form of amino acids are taken in by pinocytosis or the cellular mechanism of taking liquids
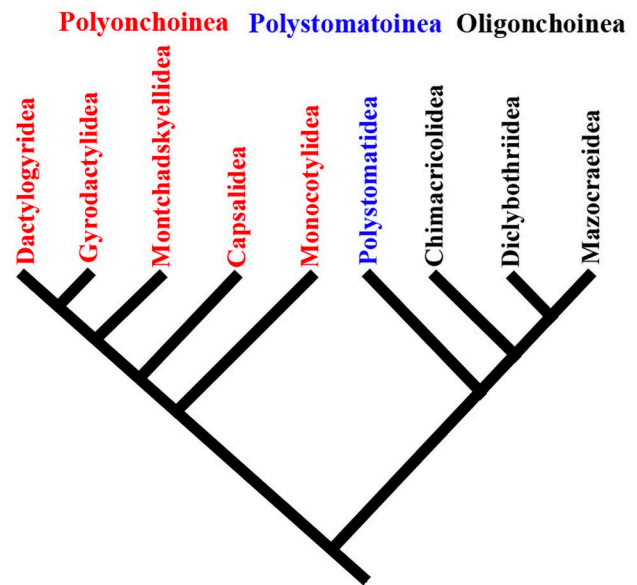

Figure 1. Relationships of the orders of Monogenea; synapomorphies of each other. Source: Adapted from morphologies in Boeger and Kristky, 1993. License: CC BY-NC-SA 4.0.

through the cellular membrane and forming a vesicle (Brusca and Brusca, 2003).

Life Cycles of Monogeneans

All monogeneans have a direct life cycle, which means that they do not have an intermediate host. They have tiny, free-swimming ciliated larvae called oncomiracidia (singular: miracidium) that hatch directly from an egg. Some life cycles have been studied, particularly those of *Dactylogyrus*, *Polystoma, Diplozoon*, *Benedenia*, and *Microcotyle* (see Bychowsky, 1957). For example, *Polystoma nearcticum*, a parasite of North American hylid frogs, lives in the urinary bladder of adult frogs and tadpoles of *Hyla versicolor* (= urinary bladder generation) and on the gills of their tadpoles (= branchial generation) (Bentz et al., 2006). In the urinary bladder of toads, the adults of the bladder generation release embryonated eggs into the urinary bladder and are voided with urine. The development of the eggs begins in the water and fully developed larvae enter the gill chambers of the tadpoles, thereby ending the urinary bladder generation and initiating the branchial (gill) generation. These larvae attach to gills of tadpoles and mature in about 22 days (see Figure 2) (Olsen, 1962).

The life cycle of monogeneans has been shown to be influenced by water temperature. For example, in *Neobenedenia girellae* infections, parasite growth, egg production, and emerging second generations stay on the same host. Infection levels and growth change on the skin corresponding with
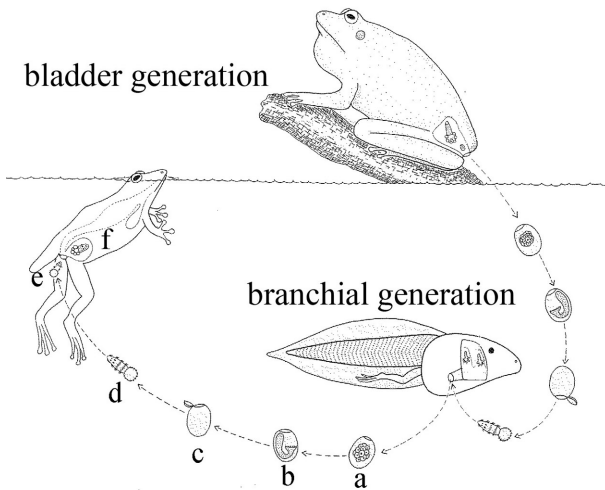
Figure 2. Life cycle of *Polystoma nearcticum* showing 2 generations. Note: a) unembryonated eggs laid on gills of tadpoles are washed into the water; b) fully developed larva, identical to those from the bladder generation; c) empty egg shell; d) the larva free in the water; e) the larva enters the cloaca of the metamorphosing toad eventually ending up in the urinary bladder; f) developing monogeneans enter the bladder and initiate the urinary bladder generation, reaching sexual maturity simultaneously with the toad. Source: Adapted from Olsen, 1962. License: CC BY-NC-SA 4.0.

differences in water temperatures. At 30 °C, the body length of worms is significantly greater than worms from fish reared at 20 °C or 25 °C. In the same manner, the number of eggs produced by adults is greater at 30° C than 20 °C or 25 °C (Hirazawa et al., 2010).

In most species of monogeneans, new hosts are infected directly by the oncomiracidia, the tiny, free-swimming ciliated larva (the adults are oviparous). The exceptions to this involve members of the Gyrodactylidae, most of which are viviparous; that is, small, unciliated larval individuals, similar to the parent, are produced within the body of the parent. After they have developed sufficiently, these young worms spread to new hosts by contagion. They use the substrate of the water body as a staging post where feeding fish may pick up the parasites. In some species the young worms float in the water until they come in contact with fish. When an infected fish dies, its parasites will infect a new host that comes close to the dead fish. Adult members of *Gyrodactylus* have several generations of embryos (young worms) within them, and each embryo has another embryo inside, even before it is released from the adult. In this manner, each adult worm produces fully developed offspring that may attach to either the same or a different host. This produces exponential population growth, which proves to be particularly problematic in freshwater fish farms (see Figure 3) (Cable and Harris, 2002).

Body Form

Monogeneans are flatworms, more or less dorsoventrally flattened, with bilateral symmetry and small sizes. The majority of them are tiny, but some species have larger bodies. In general, size range of the body is from 0.2 mm to 10.0 mm, but sometimes can be even larger. Usually, they are lanceolate, elliptical, or discoid in outline shape. The body may be clear to whitish or gray, depending upon the species, and the **eggs** generally are yellowish. The body is subdivided into
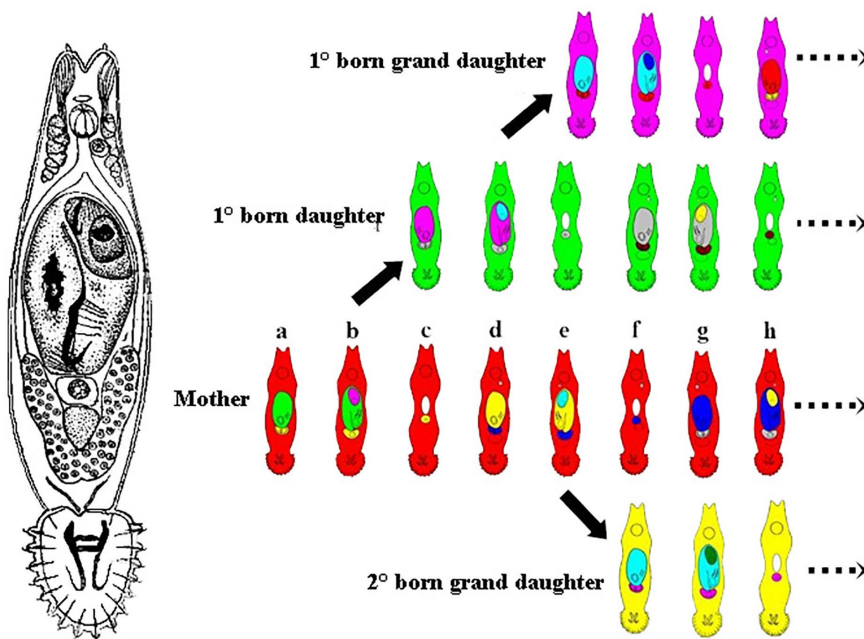

Figure 3. Life cycle of *Gyrodactylus* sp. Source: Adapted from Cable and Harris, 2002. License: CC BY-NC-SA 4.0.
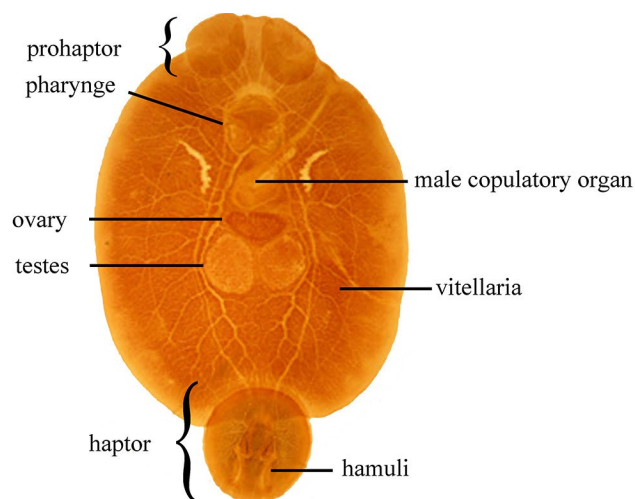
Figure 4. Subclass Polyopisthocotylea *Protomicrocotyle manteri* Bravo-Hollis, 1966, parasite of the Crevalle jack *Caranx hippos* from Campeche, Mexico. Source: G. Pulido-Flores. License: CC BY-NC-SA 4.0.

3 regions: The **cephalic region** (anterior to the **pharynx**), the **trunk** (body proper), and the **haptor** (sometimes called the **opisthaptor**; the organ used to attach to the host).

Cephalic Region

The anterior end of the body, usually called the **prohaptor**, includes the feeding and adhesive organs. Sometimes the prohaptor structures are called **head lappets**, **cephalic glands**, **head organs**, and/or **pre-oral suckers**. For example, in *Protomicrocotyle manteri* and *Benedeniella posterocolpa*, the prohaptor is formed by 2 large suckers (Figures 4 and 5), and in *Polystomoidella oblongum*, the prohaptor has an oral sucker (Figure 6).

Haptor

The haptor of monogeneans is the posterior attachment organ. In the past, the majority of the papers referred to the attachment organ as an opisthaptor (meaning posterior haptor). Malmberg (1990) called the attachment organ of the oncomiracidium a haptor and he referred to the organ in adults as an opisthaptor. In most of the recent literature, the authors refer to the attachment organ as a haptor without regard to the developmental state.

The haptor of adults may be a single unit forming a simple muscular disc or a muscular sucker with 1 or 2 pairs of **hamuli** (Figure 5) and may have 1 or 2 transverse bars. Or they may have a complex attachment organ consisting of 2 or more muscular **suckers** or **clamps**. In some taxa, the haptor also has a **haptoral appendix** and the suckers are armed with **sclerites** (Figures 4 and 6) (Yamaguti, 1963;
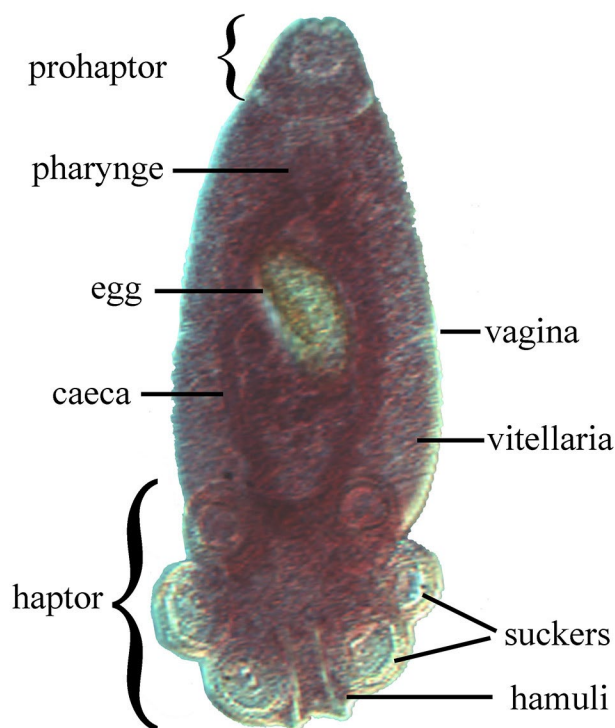

Figure 5. *Benedeniella posterocolpa* (Hargis, 1955) Yamaguti, 1963 (subclass Polyonchoinea), parasite of *Rhinoptera bonasus* from Ciudad del Carmen, Campeche, Mexico. Source: G. Pulido-Flores. License: CC BY-NC-SA 4.0.
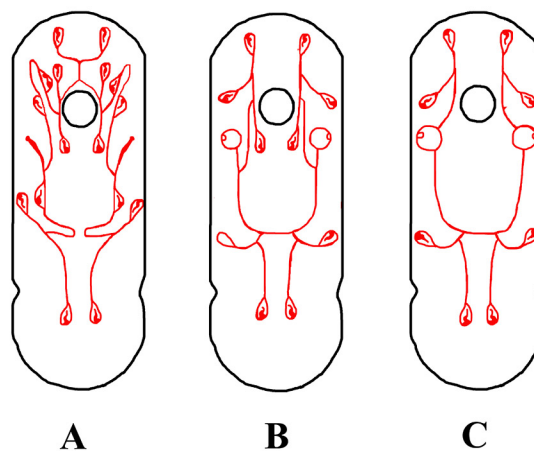
Figure 6. *Polystomoidella oblongum* (Wright, 1879) (subclass Polystomatoinea), parasite of *Kinosternon hirtipes* from Tezontepec de Aldama, Hidalgo, Mexico. Source: G. Pulido-Flores. License: CC BY-NC-SA 4.0.

Schell, 1970; Malmberg, 1990). For example, *Denarycotyle gardneri* has a haptor with a central loculus, an additional loculus on either side of the central loculus, and 10 peripheral loculi. There are 2 accessory structures (for which the func-

tion is unknown) on the dorsal surface of the haptor and on each hamulus is a sclerotized accessory piece. The margin of the haptor has 14 hooklets (for a visual depiction, see Figure 1A from Pulido-Flores et al., 2015). *Neonchocotyle violantei* has an asymmetrical haptor with 3 paired sucker-sclerite complexes with the longitudinal axis of the haptor forming an angle of approximately 45° from the midline of the body and a dorsal haptoral appendix with pairs of microhooks (for a visual depiction, see Figures 1 and 4 from Quiterio-Rendon et al., 2018).

For all monogeneans, the haptor is the principal attachment organ. Even a larva has a tiny haptor when it hatches from an egg. It might be armed with sclerotized unhinged or hinged marginal hooks or spines that give it a strong capacity for attachment. This structure is retained in adults in the majority of the species and, as it grows, it expands into the characteristic haptor of the adult.

The total number of marginal hooks on the haptor differs among species. Some species have unhinged marginal hooks that number 10, 14, 16, or 18. They present in a symmetrical manner, such as, in species with 10 hooks, they are arranged with 5 hooks on each side of the hamuli; that is, 5 lateral + 5 lateral = 10 total hooks. In species with hinged marginal hooks, the number is either 10 or 16. The details of how these patterns of hooks were defined can be seen in Malmberg (1990). Generally, the unhinged and hinged marginal hooks retain their shape during ontogeny, but certain marginal hooks can move from their original position or sometimes even disappear (for more information, see Malmberg, 1990). The various patterns of marginal hooks are consistent among each different group of monogeneans.

Osmoregulatory System

The osmoregulatory system in monogeneans is similar to that of other Platyhelminthes and composed of **flame cells** interconnected by tubular **ducts**. Malmberg (1990) described 3 types in monogeneans and related them to 3 groups characterized by the different patterns of marginal **hooks**. Members of group A have 10 marginal hooks and a type of **spermatozoa** that is in taxa more basal in the cladogram of Monogenea. Members of group B (called the intermediate type) also have 10 marginal hooks but the spermatozoa is more derived than those of group A. Members of group C, called the Dactylogyrid type, are those with other patterns of marginal hooks (not 5 + 5 = 10) (see detailed characterizations in Malmberg, 1990).

Group A has the most simple type of osmoregulatory system, consisting of an anterior and a posterior protonephridial arrangement in the body of the oncomiracidium that has few flame bulbs, both arrangements opened laterally, either separately or by a common bladder. Members of group B,

the intermediate type (also with 5 + 5 = 10 haptoral hooks), has an osmoregulatory system consisting of an anterior protonephridial arrangement (which extends through one half of the body) that opens into the posterior arrangement. The members of group C, the Dactylogyrid type, have an osmoregulatory system consisting of propulsive flame cells in the anterior and the posterior main canals (see the figure in Malmberg, 1990).

It is interesting to reflect on how the patterns of haptoral hooks and the patterns of the osmoregulatory systems are consistent with each other. Of course, that is the type of evolutionary pattern that one should expect—groups of characteristics/features that show patterns of evolution that are the same. This subject cannot be dealt with here, but it is sufficient to note that this type of similar pattern of characters (character evolution) is the basis of modern hypotheses of the phylogenetic relationships of the taxa (natural groups) of organisms. For those interested in the evolution of species and the methodology used to discover patterns of character evolution, see Brooks and McLennan (1991; 1993; 2002), as well as the studies cited within those, and those who have cited these works.

Digestive System

In general, in most species of monogeneans the digestive system is incomplete (they do not have an anus). Often, the **mouth** is surrounded by an **oral sucker** that opens in a short **prepharynx**, which connects to the muscular, glandular **pharynx**. In turn, the pharynx connects to the **esophagus**, which leads to the **intestine**. The intestine is divided into 2 **cecae** in most species; however, some species have an intestine composed of only a single cecum. Species of the genera *Tetraonchus* and *Udonella* are examples of those with only a single cecum (Schell, 1970). The cecae may be branched or unbranched, and they may end blindly or they may anastomose (connect) posteriorly.

Nervous System

The nervous system in monogeneans is ganglionic; that is, it is formed by 2 **cerebral ganglia** located in the anterior region of the body that are united by a transverse commissure. From each node arise 2 **nerves**: 1 dorsolateral and 1 ventrolateral, that run toward the posterior end of the body. From these, numerous secondary branches lead from the lateral nerves then anastomose with each other, forming a complex, ladder-like network. Also, some anterior nerves run out from the cerebral ganglia, in particular, those associated with the **sense organs**, such as ocelli, which are located in the anterior region. Many larval or juvenile forms have **ocelli** (**eyespots**) that provide orientation using light. The adults of

some taxonomic groups retain the larval ocelli and others lose them, sometimes leaving fragments of retinal pigment where they were.

Male Reproductive System

Monogeneans are hermaphroditic, but cross-fertilize. In general, the male and female **gonopores** are located some distance from each other, making self-fertilization difficult to impossible, although in some taxa they are located close together. The male reproductive system consists of 1 to several **testes**, which are located anterior or posterior to the single **ovary**. A **vas efferent duct** (**vas efferens**) runs out from each testis, if there is more than one; the vasa efferentia join together to form a single duct, the **vas deferens** that connects to the **seminal vesicle**. That in turn is connected to the male copulatory organ. Sometimes the **genital atrium** (that is, the area where the male and female gonopores can be found) may be present or absent. The **male copulatory organ** (called a **cirrus**) can be armed or unarmed, is sometimes sclerotized, and extends out of the common genital pore, which usually opens ventrally. Sometimes **prostatic glands** are present. For example, *Denarycotyle gardneri* has 1 testis with the vas deferens arising from the left side of the testis. The vas deferens is enlarged to form a spherical reservoir to hold sperm, and it leads to a smaller reservoir that is curved toward the left side of the body. The vas deferens is a loosely coiled, narrow duct that ascends dorsally, posterior to the genital pore, to connect to a seminal vesicle, then to the ejaculatory bulb and the male copulatory organ. In this species, the male copulatory organ is a short, sclerotized tube (for a visual depiction, see Figure 1B from Pulido-Flores et al., 2015). *Neonchocotyle violantei* has 8 testes. Its seminal vesicle is elongate, extending anteriorly to the proximal male copulatory organ, which is located within a pouch that is longer than the male copulatory organ (for a visual depiction, see Figure 1A from Quiterio-Rendon et al., 2018).

Female Reproductive System

The female reproductive system consists of 1 **ovary** of variable shape and position among the different species. The **oviduct** connects the ovary with the **ootype** and the **vitelline duct**, and the **vagino-** and **genitointestinal ducts** also open out. Associated with these structures is the **Mehlis' gland**, a duct that runs from the ootype and ends in the **genital pore**. Monogeneans usually have 1 **vagina**, but some groups have 2 vaginas that usually are connected to the **seminal receptacle**.

Denarycotyle gardneri has an ovary that is elongate, V-shaped, with the lateral arm of the "V" encircling the right intestinal cecum dorsoventrally, and then it narrows to form the oviduct. The oviduct, the seminal receptacle, and the

common vitelline duct all join at the ootype. In this species, the vagina is muscular, unsclerotized, and sac-like. The seminal receptacle is present and the vitellaria (yolk-producing glands) extend from the level of the posterior portion of the pharynx to the posterior of the body proper (for a visual depiction, see Figure 1B from Pulido-Flores et al., 2015).

Neonchocotyle violantei has 2 vaginae that run parallel in the proximal portion and non-parallel in the distal portion. The proximal region, connected to the vitelline reservoir, is glandular and the muscular distal region connects to the vaginal pore (female gonopore). The vaginal pores open ventrally. The ovary of this species is tubular, with deep lobes and ascending and descending branches that reach to the region of the oviduct. The descending branch is coiled and connects posteriorly to the ootype. The ootype is dorsal to the ovary, but ventral to the vas deferens, and it leads to the uterus and the seminal receptacle (for a visual depiction, see Figures 1A and 2, and the detailed description in Quiterio-Rendon et al., 2018).

In *Neonchocotyle violantei*, the vitellaria are abundant, follicular, and they are arranged laterally along the entire body, and sometimes into the haptor. An efferent duct extends from the vitellaria and fuses to form the vitelline duct in close proximity to the oviduct. Near this point, they form a vitelline reservoir. In this species, the transverse vitelline ducts are dorsal, forming a Y-shaped reservoir; the proximal region of the vaginae are connected to the anterior branches of the reservoir and the posterior region of the reservoir is joined to the oviduct (for a visual depiction, see Figures 1A and 2 from Quiterio-Rendon et al., 2018).

Fertilization of the ova occurs in the ootype. Fully developed eggs are operculated and they have 2 polar filaments (some species have a single filament, others have none). The number of eggs is variable among the species; these are released to the outside through the genital pore.

The structural details of the various species of Monogenea are complex and sometimes difficult to envision. Studying the descriptions of several different species will provide a better understanding of this complexity.

Taxonomic Classification

The taxonomic classification of the Class Monogenea follows the phylogenetic analysis of Boeger and Kritsky (1993).

Class Monogenea van Beneden, 1858

    Subclass Polyonchoinea Bychowsky, 1937

      Order Monocotylidea Lebedev, 1988

          Family Monocotylidae Taschenberg, 1879

          Family Loimoidae Price, 1936

Order Capsalidea Lebedev, 1988
    Family Acanthocotylidae Price, 1936
    Family Capsalidae Baird, 1853
    Family Dionchidae Johsnton & Tiegs, 1922
Order Montchadskyellidea Lebedev, 1988
    Family Montchadskyellidae Bychowsky, Korotajeva & Gusev, 1970
Order Gyrodactylidea Bychowsky, 1937
    Family Gyrodactylidae Van Beneden & Hesse, 1863
    Family Anoplodiscidae Tagliani, 1912
    Family Bothitrematidae Price, 1936
    Family Tetraonchoididae Bychowsky, 1951
Order Dactylogyridea Bychowsky, 1937
  Suborder Calceostomatinea Gusev, 1977
    Family Calceostomatidae Parona & Perugia, 1890
  Suborder Neodactylodiscidae Kamegai, 1972
    Family Neodactylodiscidae Kamegai, 1972
  Suborder Amphibdellatinea Boeger & Kritsky, 1993
    Family Amphibdellatidae Carus, 1885
  Suborder Tetraonchinea Bychowsky, 1937
    Family Tetraonchidae Monticelli, 1903
    Family Neotetraonchidae Bravo-Hollis, 1968
  Suborder Dactylogyrinea Bychowsky, 1937
    Family Dactylogyridae Bychowsky, 1933
    Family Pseudomurraytrematidae Krtisky, Mizelle, & Bilqees, 1978
    Family Diplectanidae Monticelli, 1903
Subclass Polystomatoinea Lebedev, 1986
  Order Polystomatidea Lebedev, 1988
    Family Polystomatidae Gamble, 1896
    Family Sphyranuridae Poche, 1926
Subclass Oligonchoinea Bychowsky, 1937
  Order Chimaericolidea Bychowsky, 1957
    Family Chimaeridolidae Brinkmann, 1942
  Order Diclybothriidea Bychowsky, 1957
    Family Diclybothriidae Price, 1936
    Family Hexabothriidae Price, 1942
  Order Mazocraeidea Bychowsky, 1957
   Suborder Mazocraeinea Bychowsky, 1957
    Family Plectanocotylidae Monticelli, 1903
    Family Mazoplectidae Mamaev & Splipchenki, 1975
    Family Mazocraeidae Price, 1936
   Suborder Gastrocotylinea Lebedev, 1972 sedis mutabilis
   Infraorder Anthocotylina Boeger & Kritsky, 1993

    FamilyAnthocotylidae Price, 1936
   Infraorder Gastrocotylina Lebedev, 1972
    Family Pseudodiclidophoridae Yamaguti, 1965 incertae sedis
   Superfamily Protocomicrocotyloidea Johnston & Tiegs, 1922 sedis mutabilis
    Family Protomicrocotylidae Johnston & Tiegs, 1922
    Family Allodiscocotylidae Tripathi, 1959
    Family Pseudomazocraeidae Lebedev, 1972
    Family Chauhaneidae Euzet & Trilles, 1960
   Superfamily Gastrocotyloidea Price, 1943 sedis mutabilis
    Family Bychowskycotylidae Lebedev, 1969
    Family Gastrocotylidae Price, 1943
    Family Neothoracocotylidae Lebedev, 1969
    Family Gotocotylidae Yamaguti, 1963
  Suborder Discocotylinea Bychowsky, 1957 sedis mutabilis
    Family Discocotylidae Price, 1936
    Family Diplozoidae Tripathi, 1959
    Family Octomacridae Yamaguti, 1963
  Suborder Hexostomatinea Boeger & Kritsky, 1993
    Family Hexostomatidae Price, 1936
  Suborder Microcotylinea Lebedev, 1972
   Superfamily Microcotyloidea Taschenber, 1879
    Family Axinidae Monticelli, 1903
    Family Diplasiocotylidae Hargis & Dillon, 1965, sedis mutabilis
    Family Heteraxinidae Unnithan, 1957, sedis mutabilis
    Family Microcotylidae Taschenberg, 1879, sedis mutabilis
   Superfamily Diclidophoroidea Cerfontaine, 1895, sedis mutabilis
    Family Diclidophoridae Cerfontaine, 1895
    Family Pyragraphoroidea Yamaguti, 1963, sedis mutabilis
    Family Pterinotrematidae Caballero y Caballero & Bravo-Hollis, 1955
    Family Rhinecotylidae Lebedev, 1979, sedis mutabilis
    Family Pyragraphoridae Yamaguit, 1963, sedis mutalibis
    Family Heteromicrocotylidae Unnithan, 1961, sedis mutabilis
Taxa incertae sedis: Sudanonchidae Malmberg, 1990 [Polyonchoinea]; Iagotrematidae Mañé-Garzón & Gil, 1962 [Polyonchoinea]; Microbothriidae Price, 1936 [Monogenea].

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