

# Diplozoon

**Class : Monogenoidea** Bychowsky, 1937

**Sub Class : Oligonchoinea** Bychowsky, 1937

**Order : Mazocraeidea** Bychowsky, 1957

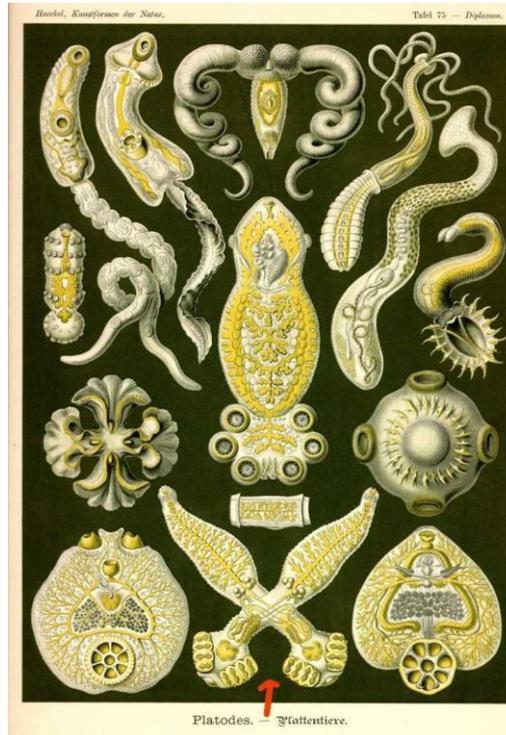
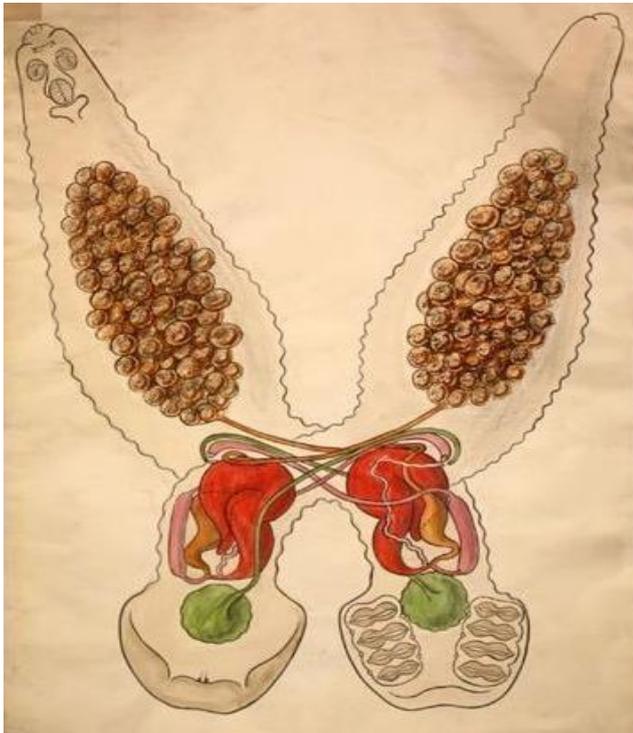
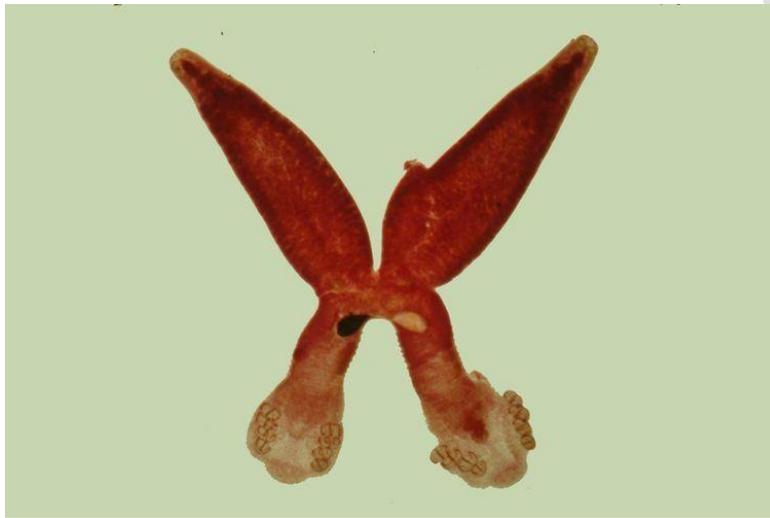
**Sub order : Discocotylinea** Bychowsky, 1957

**Super family : Gastrocotyloidea** Price, 1943

**Family : Diplozoidae** Tripathi, 1959

Ex. *Diplozoon* Nordmann, 1832

*Neodiplozoon* Tripathi, 1960



*Diplozoon*

## Geographic Range

*Diplozoon* is the parasite of various cyprinid fishes in Europe (and possibly Asia) (Schmidt & Roberts, 1989). (Schmidt and Roberts, 1989)

## Biogeographic Regions

- [Palearctic](#) (living in the northern part of the Old World. In other words, Europe and Asia and northern Africa.)



- [Oriental](#) (found in the oriental region of the world. In other words, India and south east Asia.)



## Habitat

*Diplozoon* parasitize various cyprinid fishes, including, but not limited to: *Abramis*, *Rutilus*, *Gobio*, and *Phoxinus*. A study in Northern England found that *Diplozoon* were absent entirely from fish of the genus *Leuciscus* (Wiles, 1968). (Wiles, 1968)



**Diplozoon paradoxum. Figure 2** Coloured stage showing both partners being united for ever.

### Physical Description

An adult specimen of *Diplozoon* is two permanently fused individuals, conjoined at the flat fusion bridge, so that their bodies form an H shape together (Wong & Gorb, 2013). Adult *Diplozoon* were measured at 8 to 10 mm in length (Schmahl & Taraschewski, 1987), and the fusion bridge is 0.37 mm in length (Wong & Gorb, 2013). The larva, or diporpa, reported by Bychowsky (1957) are roughly 0.23 mm in length. (Bychowsky, 1957; Schmahl and Tarachewski, 1987; Wong and Gorb, 2013)

The integument of *Diplozoon* is comparatively irregular in comparison to closely related species, likely attributable to many vacuoles and vesicles. It is a syncytium, measuring roughly 2 to 6  $\mu\text{m}$  deep, with mitochondria often lying near the base (Schmahl and Tarachewski, 1987). (Schmahl and Tarachewski, 1987)

The individuals of the mated pair each maintain their own anterior attachment apparatus, consisting of two buccal suckers lateral to the mouth. These suckers are aided by groupings of gland cells that secrete an adhesive substance. The mouth is located on the anterior ventral surface of the parasite, it is followed by a buccal cavity, where the oval shaped pharynx is slightly protruded (Halton & Jennings, 1965). The species is also in possession of a comparatively long trapeze spur (Matejusova et al., 2004). (Halton and Jennings, 1965; Matejusová, et al., 2004)

The opisthaptor of adults of this species, uses four pairs of clamps that are arranged in parallel lateral rows on the ventral posterior terminal end of the body. There is also a small pair of hooks on the ventral

side of the haptor, though the clamps are thought to play the most crucial role in attachment to the substrate. The clamps are supported by a framework of sclerites, and receive peripheral support by marginal sclerites. The anterior end of the clamps is fixed, and the posterior end is hinged. The sclerites of the clamps are covered in a fibrous tissue layer, that is roughly 0.01 mm thick (Owen, 1963). Recent studies have shown the clamps remain closed by nature of the elastic material of the clamps, not active muscular force. It is believed that the clamps are only opened by muscle force, which is a likely explanation of the long term fixed position of adult specimens. There is a positive relationship between the force required for removal from host tissue and the body mass of the parasite. The force to mass ratio is roughly 246, which is considered high among other studied invertebrate species (Wong & Gorb, 2013). Each clamp is able to grasp one or two gill lamellae, though the first, most posterior clamp is sometimes smaller than the other three (Wong & Gorb, 2013). All clamps of the opisthaptor of *D. paradoxum* were of the same size (Matejusova et al., 2004). In another study concerning abnormalities of the attachment clamps, the precedent was to consider any clamps of a smaller size, abnormal (Sebelova et al., 2002). The clamps are innervated by two nerves each, that both bifurcate, and travel one branch into the posterior, and one branch into the anterior portion of the clamp, where on both side they branch excessively, and end at the distal tip of the tissue lining (Owen, 1963). It is also noteworthy, that all eight clamps were observed by Wong and Gorb (2013) to be able to open and close independent of each other. (Matejusová, et al., 2004; Owen, 1963; Šebelová, et al., 2002; Wong and Gorb, 2013)

The excretory system of *Diplozoon* is comprised of two different types of cells, flame cells and canal cells. The flame cells are flagellated and the canal cells line the anterolaterally-placed excretory ducts. The collecting ducts are interwoven throughout the body, and are greatest in number in the anterior and posterior terminal regions. The portions of the canal cells lining the excretory ducts are ciliated to assist in the transportation of liquid through the canal (Konstanzova et al., 2016). (Konstanzová, et al., 2016)

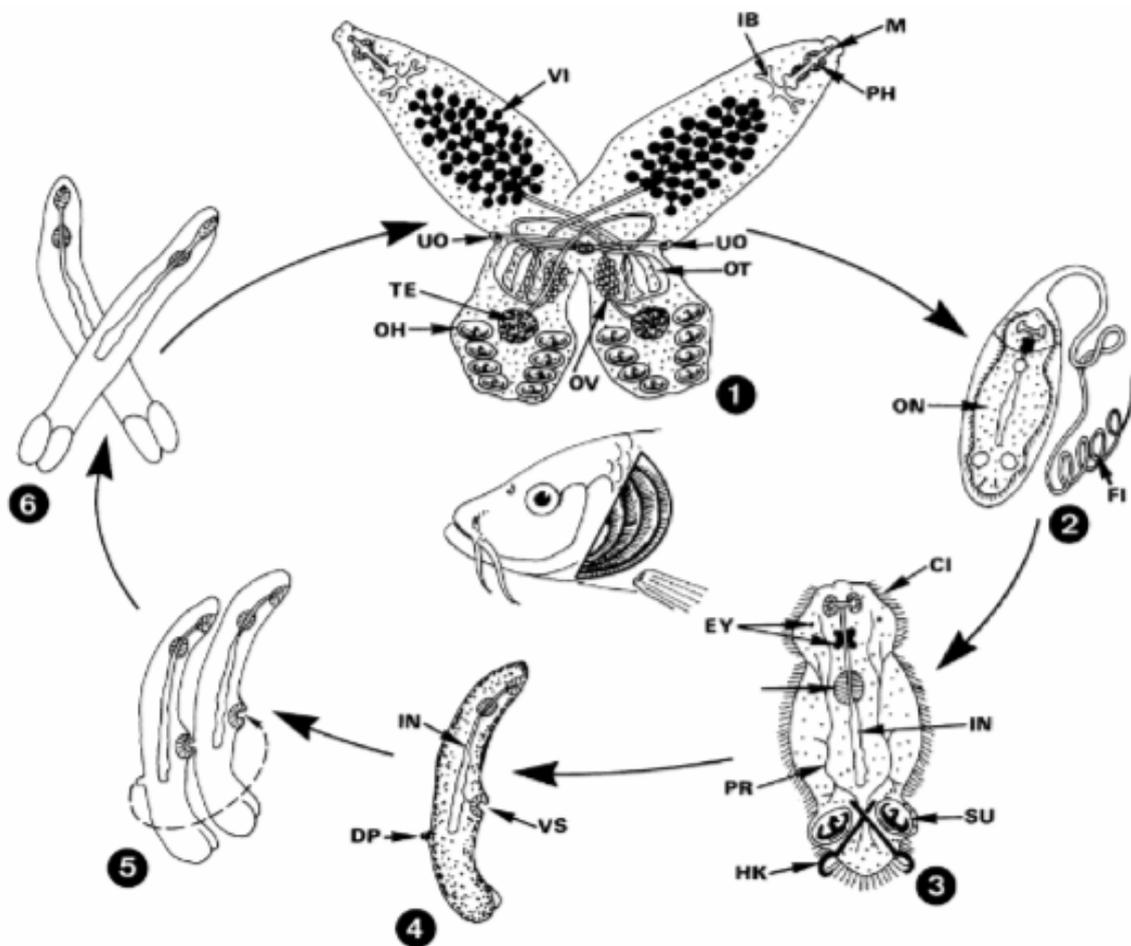
### **Development**

The eggs of the oviparous parasites have long coiled filaments at their ends (Schmidt & Roberts 1989), and can hatch nearly ten days after deposition (Bychowsky, 1957). Light intensity and turbulence of water, which might be caused by host spawning, or feeding activity, assist in stimulating hatching (Bychowsky, 1957). A newly-emerged oncomiracidium is ciliated, possesses two pair of clamps on the opisthaptor, and bears two medial hooks that are quite large, and assist in both the movement and the settlement of the larva (Bychowsky, 1957). The cilia are localized in three places on the body by five different groups of cells. The first group of cilia are on the lateral and dorsal surfaces of the body, anterior to the small band, but not on the anterior end of the organism. The second grouping of cilia, comprised of two sets of cells, begins posterior to the small band, and continues on the lateral and dorsal surfaces, until reaching the anterior end of the opisthaptor. The third grouping of cilia is behind the

posterior attachment apparatus on the lateral and dorsal surfaces of the animal, and continues posteriorly to the terminal end of the animal. (Bychowsky, 1957; Llewellyn, 1963). Bychowsky (1957) identified two fundamental phases of larval activity, a free swimming phase, making use of the cilia, and a somewhat attached phase where the larva creeps through the substrate using its musculature. Attachment to the host occurs early in diporpa stages of development (larval stages specific to this clade of parasites; Schmidt & Roberts, 1989), often utilizing only the buccal suckers (Kagel & Tarachewski, 1993). After attaching to a gill filament, the larva loses its cilia almost immediately. The worm will feed and grow, acquiring another set of clamps, a dorsal papilla, and a ventral sucker, posterior to the buccal sucker. The growth and development of the clamps occurs in order, with the first pair at the most posterior end of the opisthaptor to the fourth pair at the most anterior end (Sebelova et al., 2002). The growth of the third set of posterior clamps in one individual can be asymmetrical, with one side of the opisthaptor having two clamps, and the other side having three (Bychowsky, 1957). Structural abnormalities such as the opisthaptoral clamps can be traced to deviations in normal development, often from the embryonic state (Sebelova et al., 2002). Upon mating the pigmented eyes disappear, and as the larva grow into each other, and merge, they acquire their final sets of clamps and grow to their full size. Growth of the individual occurs rather slowly, and accelerates greatly after mating. If independent larvae do not mate they perish by the following winter. Mated pairs undergo rapid growth, and reach maturity by spring of the following year (Bychowsky, 1957). (Bychowsky, 1957; Kagel and Tarachewski, 1993; Llewellyn, 1963; Schmidt and Roberts, 1989; Šebelová, et al., 2002)

### **Reproduction**

*Diplozoonis* oviparous, and production of eggs is only achieved by paired, fully matured organisms (Koskova et al., 2011; Bychowsky, 1957). Two hermaphroditic individuals must meet and permanently fuse their bodies in order to achieve maturity (Avenant-Oldewage & Milne, 2014). Egg production occurs at its highest rates in the warm months, May through June, but does continue through the summer (Bychowsky, 1957). Virtually no gametes are produced during the winter (Schmidt & Roberts, 1989). According to Zeller (1872, as cited in Bychowsky 1957), the rapid development of the egg cells and vitelline cells takes place in the spring, along with the concurrent rise in temperature. An artificial introduction of the parasite and its host, to warmer temperatures saw egg formation begin about the fifth or sixth day. The vitelline glands are in close contact with the intestine for a majority its length (Halton & Jennings, 1965). Diporpa larva each develop a dorsal papilla and a ventral sucker, posterior to the buccal cavity. When the worms mate, one individual attaches its ventral sucker to the papilla on the



**Diplozoon paradoxum.** Figure 1 Life cycle of *Diplozoon paradoxum* on the gills of cyprinid fish. 1 Adults on the gills of fish. 2 Egg with an →oncomiracidium larva. 3 Free oncomiracidium. 4 After attachment to the gills of a host the oncomiracidium is transformed into the diporpa larva. 5, 6 Fusion of 2 diporpas on the host; each →diporpa attaches its sucker (VS) to the dorsal papilla (DP) of the other. This process stimulates their maturation and cross-fertilization. The blood-sucking adults can live for years in this form of complete copulation. CI, →cilia; DP, dorsal papilla; EY, eyes; FI, filament; HK, hook; IB, intestinal branch; IN, intestine; M, mouth; OH, →opisthaptor with suckers; ON, oncomiracidium; OT, ootype; OV, ovary; PH, pharynx; PR, →protonephridium; SU, sucker (clamps); TE, →testis; UO, uterus opening; VI, →vitellarium (vitelline gland); VS, ventral sucker.

dorsal surface of the other. This encounter stimulates fusion, maturation, and the gonads finally complete development (Schmidt & Roberts, 1989). According to Bovet (1967, as cited by Anderson 1974), once the hermaphroditic larvae mate, the vagina of one individual is opened in close proximity of the vas deferens of the other, resulting in cross fertilization between the adult, fused, mated pair. (Avenant-Oldewage and Milne, 2014; Bychowsky, 1957; Halton and Jennings, 1965; Košková, et al., 2011; Schmidt and Roberts, 1989; Zeller, 1872)

### Lifespan/Longevity

The infective stages of *Diplozoon* have a lifespan of approximately 10 hours (Bovet, 1967, as cited in Anderson 1974). The diporpa stage of the species is capable of living for several months, once attached to a host, but without a mate, will die in the winter months. Mated pairs are able to live off of the host for several years (Schmidt & Roberts, 1989). (Anderson, 1974; Bovet, 1967; Schmidt and Roberts, 1989)

## Behavior

A study of the common bream (*Abramis brama*) found that the number of parasites per host increases with the age of the fish, and that parasite burden is determined by three factors. First, Bovet (1967) suggests that adult specimens anchor themselves permanently to their respective hosts. Because of this permanent settlement, a single organism claims a defined space on the gill lamellae. The parasites compete for space on the gill filaments, and by association, the blood supply from which they obtain nutrients. Overcrowding of this environment will often result in failure of an organism to find a mate, hindering maturation and survival capability, or failure of the organism to attach to the substrate. Second, older fish are bound to accumulate more parasites over time, resulting in a larger burden. Third, a larger fish passes a larger volume of water over their gills. If this water is infected, then larger fish have higher rates of exposure to infection (Anderson, 1974). Above average densities of *D. paradoxum* have detrimental effects on the mass of the host in the winter and spring months, potentially resulting in the death of the host. This occurs especially where specific age groups of fish compete for limited resources. Different developmental stages of the parasite have different microhabitat preferences. Diporpa are most frequently found on the distal portion of the primary gill lamellae, and often only attached to the host by their anterior end. Usually unpaired diporpa only enclose one secondary gill lamellae with the underdeveloped opisthaptor, and are much less restricted in their mobility. Adult individuals have a preference for the basal portion of the primary gill lamellae, however, and each clamp is capable of compressing between one and four secondary gill lamellae (Kagel & Tarachewski, 1993). A more recent study found the monogeneans localized at the gill filaments "tightly adhering to the gills by the opisthaptor while also concentrating in small groups, with no preferences in localization between hemibranches or gill arches" (Yildirim & Üvner, 2012:246). Wiles (1968) found that infection rates of *Diplozoon* were highest in moving bodies of water, and lowest in stagnant water such as ponds and reservoirs. *Diplozoon* is known to infect more than 30 species of cyprinid fish and several species of Cobitidae. Bychowsky (1957) asserts that they do not parasitize any fish species except representatives from these two families. (Anderson, 1974; Bovet, 1967; Bychowsky, 1957; Kagel and Tarachewski, 1993; Wiles, 1968; Yıldırım and Üvner, 2012)

## Communication and Perception

The diporpa phase of the species is in possession of four eyes, each having a single rhabdomere. Two of these eyes are laterally arranged on the anterior midline, near the dorsal surface, and covered by a pigment shield. They are cup shaped and are oriented laterally. These eyes have no solid lenses and no oil droplets that might otherwise serve as temporary lenses. A single reticular cell is associated with each of these pigmented eyes. Muscle fibers are abundant in the vicinity of the eyes, but direct

articulation between those structures has not been confirmed. The eyes lacking pigment shields lie lateral to the pharynx, and closer to the surface of the body. They too are rhabdomeric and closely resemble the other pair of eyes, apart from lacking the pigment shield. No apparent movement has been observed in these eyes, but it is hypothesized that they are useful in allowing the animal to orient itself. It is likely that the lateral eyes are well equipped to detect sudden changes in light intensity, and this can be related to the perception of the shadow casting of a fish (Kearn, 1978). (Kearn, 1978)

### **Food Habits**

These sanguivorous parasites feed using negative pressure to suck a plug of gill tissue into the buccal cavity back to the pharynx, where prolonged strain on the tissue eventually causes it to rupture. This rupture is the result of negative pressure alone, and is not aided by any glandular secretions. The ruptured tissue permits the flow of blood, and some tissue fragments. This sanguivorous diet is relatively characteristic of the polyopisthocotylea, however *Diplozoon* was found to supplement the blood diet with variable amounts of gill tissue and mucus. Hemolysis occurs almost immediately within the buccal cavity, and is followed by "partial intraluminal digestion" within the posterior, laterally-branched intestine. This intestine extends, in each individual, to the fusion bridge, where the intestines of the two organisms merge. In the intestine, "soluble substances are absorbed by the gastrodermis," (Halton & Jennings, 1965:266) and subsequently the remainder of digestion occurs in the intracellular space. Once the erythrocytes have been stripped of the useful byproducts, heme is left in the intracellular space, which requires that the organism disintegrate older cells. This process likely results in the branched nature of the intestine, and indicates an incomplete evolution of the parasites toward the blood diet. A positive side effect is that this destruction of gastrodermal cells releases intracellular enzymes that aid in extracellular digestion (Halton & Jennings, 1965). (Halton and Jennings, 1965)

### **Primary Diet**

- carnivore
- sanguivore
- eats body fluids
- Animal Foods
- blood
- body fluids

### **Species Used as Host**

- carps and true minnows (Cyprinidae)

### **Economic Importance for Humans: Negative**

Gill parasitizing monogeneans often become a serious problem in commercial fish farms, and have been a serious issue in Eastern and southeastern Europe. First noticed in the early 1950s, the pathological

effects of these parasites can result in lethal damage to the host. Many treatments for monogeniasis, can be highly toxic for the fish as well. Praziquantel is the most effective means of killing monogenean parasites, without adverse effects to the host (Schmahl & Taraschewski, 1987). (Schmahl and Taraschewski, 1987)

### Other Comments

Although generally visible in the natural coloring of a specimen, Halton and Jennings (1964) found a strong reaction of the nervous system to non-specific esterase, and this reaction was specific to *Diplozoon*; it was observed minimally in other species of monogeneans. Koskova et al. (2011) provided a phylogenetic tree based on RNA ITS, which is corroborated by karyological analysis (Matejusova et al., 2004). (Halton and Jennings, 1964; Košková, et al., 2011; Matejusová, et al., 2004)

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