MORPHOLOGY AND LIFE CYCLE OF *DIPLOZOON*

Systematic Position

Phylum:	Platyhelminths
Class:	Trematoda
Order:	Monogenea
Sub-order:	Polyopisthocotylea
Family:	Discocotylidae
Subfamily:	Discocotylinae

Yamaguti (1963) however, gives slightly modified classification of *Diplozoon*. He gives the same classification upto sub-order, but gives superfamily Diplozoidea; family Diplozoidae and sub-family Diplozoidinae. Under this family he recognised two genera namely *Diplozoon* with four pairs of clamps and a pair of inconspicuous posterior anchors on a rectangular opisthaptor and *Neodiplozoon* with numerous clamps and without posterior anchors on bilobed opisthaptor.

Definition: *Diplozoon* is defined as a genus of the order Monogenea, super-family Diplozoidea Yamaguti (1963) family Diplozoidae Yamaguti (1963), in which two adult specimens are in every case united in X-form. With the new description of the species *Neodiplozoon barbi* Tripathi (1959), another genus with this characterization was found. This genus *Neodiplozoon* differs from *Diplozoon* by the higher number of clamps, mostly 16. The definition of the genus *Diplozoon* therefore must include the sign: 4 pairs of clamps and a pair of inconspicuous posterior anchors (hamuli) on a rectangular opisthaptor.

Historical: The genus *Diplozoon*, the so called "***double animalcule***", was created by Nordmann (1832) who reported *D. paradoxum* from freshwater teleosts in Europe. At present the number of species described is more than 20. The first report of the genus from Indian region was by Dayal (1941) who described *D. indicum* from the gills of *Barbus sarana*.

Next Indian record of the genus was that of Kaw (1950) who reported *D. kashmirensis* from the gills of *Schizothorax* sp. in Kashmir and proposed a key to the four species of *Diplozoon* known till then.

Tripathi (1957) described two more species *D. soni* and *D. cauvery* from *Oxygester baciala* and *Cirrhina cirrhosa* respectively from India and gave key to seven species. The author also proposed a new genus *Diplotrema* from a new Diplozoid species *D. barbi* Tripathi, 1957 from *Barbus chagunio* in river Son (Bihar). This species is peculiar in having a large number of hold-fast clamps (18-28 pairs) instead of typical four pairs in genus *Diplozoon*. The same author proposed a separate family Diplozoidae including under it the genus *Diplozoon* and his new genus *Diplotrema* but the latter name has been found pre-occupied and has since been replaced by *Neodiplozoon*.

Location and Host: The adults of *Diplozoon* are reported from the gills of freshwater fishes, without extensive pathogenicity combined with fish losses, from different parts of the world. They remain attached to the gill filaments by opisthaptor and cause chocking.

Morphology: *Diplozoon* is a unique monogenetic trematode in which adult individuals remain united in pairs in a permanent copulation in the form of a cross (x-shape). Each individual has a long foliate fore-body comprising of numerous minute vitelline follicles and major part of intestinal caecae, bearing lateral diverticulae. Anterior end of each individual bears a prohaptor possessing two suckers followed by a mouth and a pharynx leading into a short oesophagus; while as the hind body has three regions – anterior region bearing gonads, middle region bearing termination of intestinal caecae and the hinder region bearing the opisthaptor (consisting of four pairs of clamps and a median pair of small hooks). Thus each individual retains separate digestive tract, reproductive organs and also the opisthaptor.

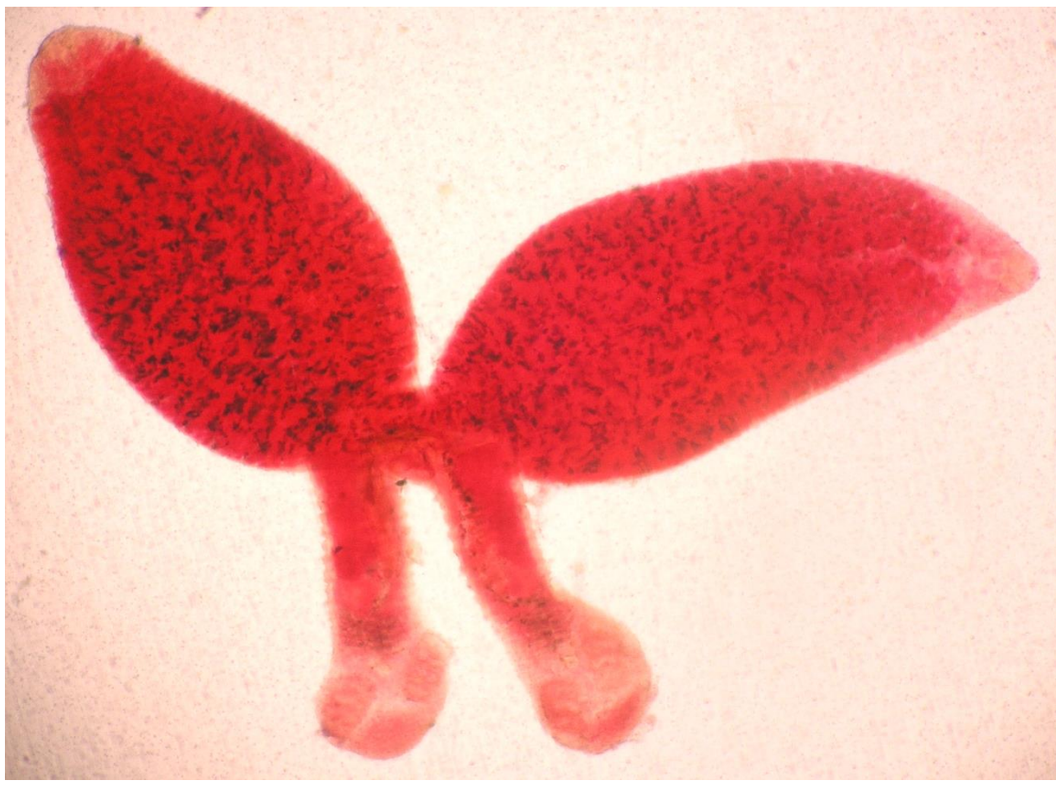
Alimentary Canal: It consists of mouth, pharynx and intestine. Mouth is small situated at the anterior end, bearing a pair of anterior suckers also referred to as oral suckers, which are located one on each side of the mouth. The mouth leads into a short but well developed and muscular pharynx. Oesophagus is very well developed. The intestine is long and single upto a long distance, but bifurcates near the posterior end into 2 branches as in *D. indicum*. The 2 branches unite again forming a single branch in the opisthaptor. The 2 branches are united posterior to the testis. Mouth in *D. indicum* is triangular on ventral side. Prepharynx and pharynx well represented. According to Tripathi (1959) the portion of intestine anterior to the place of union of the 2 individuals bears dichotomous branches. The intestine ends blindly at the posterior end. According to Yamaguti characteristic feature of Diplozoidae is that intestine is not bifurcate. Lateral diverticalue according to him may be reticulate.

Reproductive System: The two individuals in a cross although hermaphrodite live in permanent copulation. Each individual has a single testis situated between the two intestinal caeca at the anterior region of the opisthaptor. Testis is rounded or oval and from each testis arises a vas deferens or sperm duct which opens just opposite to the vaginal opening of the other.

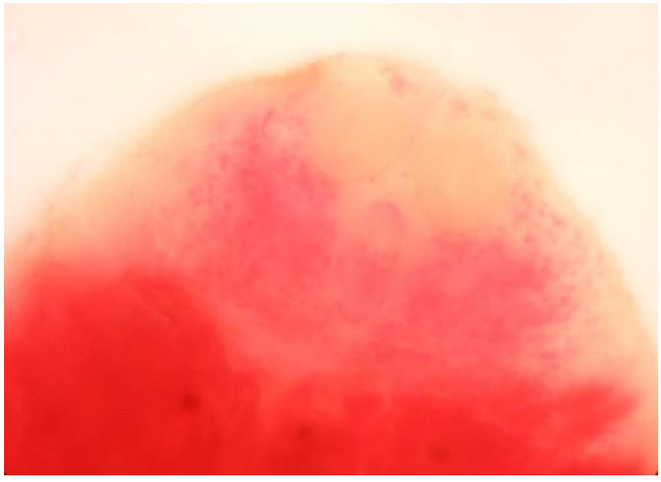
The female reproductive organs consist of a single ovary which is in the form of a long band which bends twice on itself, thus forming an inverted, overlapping double U as in *D. indicum*. From the ovary arises a short oviduct. The vitellaria are scattered in the pre-ovarian region of the body. They are co-extensive with intestinal branches. The small vitelline ductules arising from them unite to form a long common vitelline duct which unites with the oviduct forming ovo-vitelline duct. Just near the oviduct arises a copulation canal as shown by Hyman (1951). But according to Yamagut (1954) vagina is absent. According to Schang (1954) also vagina is present, as shown by him in his diagramme.

The common ovo-vitelline duct continues and forms a short uterus. It has a basal muscular part and it continues forward as a tubular structure to open by the genital pore. According to Yamaguti (1971) a genito-intestinal canal is present. It connects the genital organs with the intestine and is present in most of the Polyopisthocotylea. It probably diverts excess yolk into the intestine or it may be a legacy from the past *i.e*., it might have been inherited from some turbellarian ancestors where it was present.

Opisthaptor: It is rectangular, concave ventrally. It is provided with four pairs of clamp like structures or lateral clamps which are set close together along the postero-lateral margin symmetrically in thick capsules. There is present one pair of posterior anchors. The anterior pair of suckers is the largest and those which follow are smaller, the last pair being smallest. In *D. kashmirensis* the 2nd pair of clamps is the largest.



Pmg. 1: Two Adult worms in Copula



Pmg. 2: Fore body showing suckers and pharynx

Pmg. 3: Hind body showing clamps

Pmgs. 1 – 3: ***Dilozoon kashmirensis*** **Kaw, 1950**

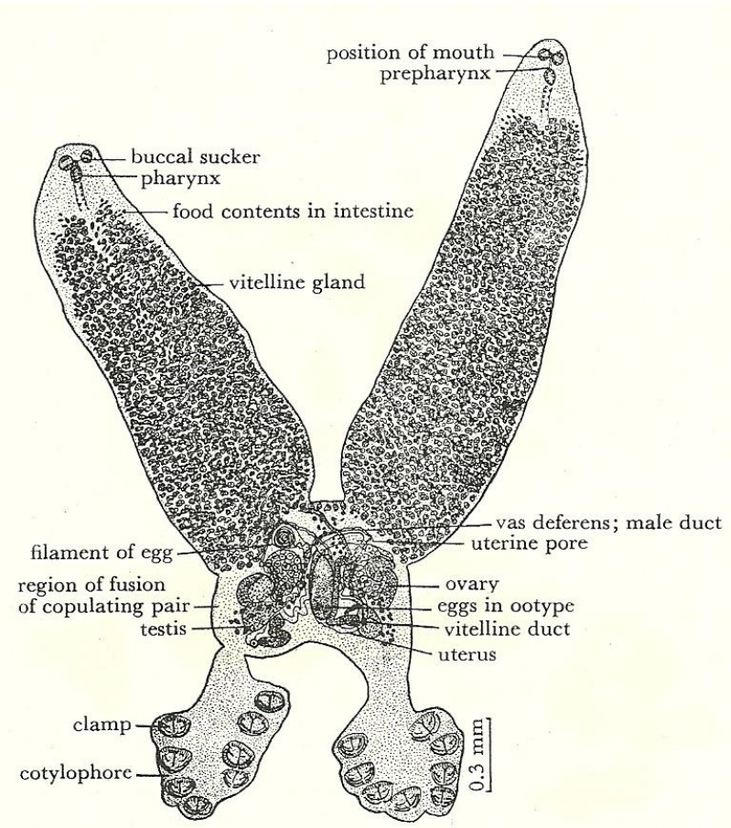


Fig. 6-7. Two *Diplozoon ghanense* in permanent copulation. (Thomas, courtesy J. West. African Sci. Assoc.)

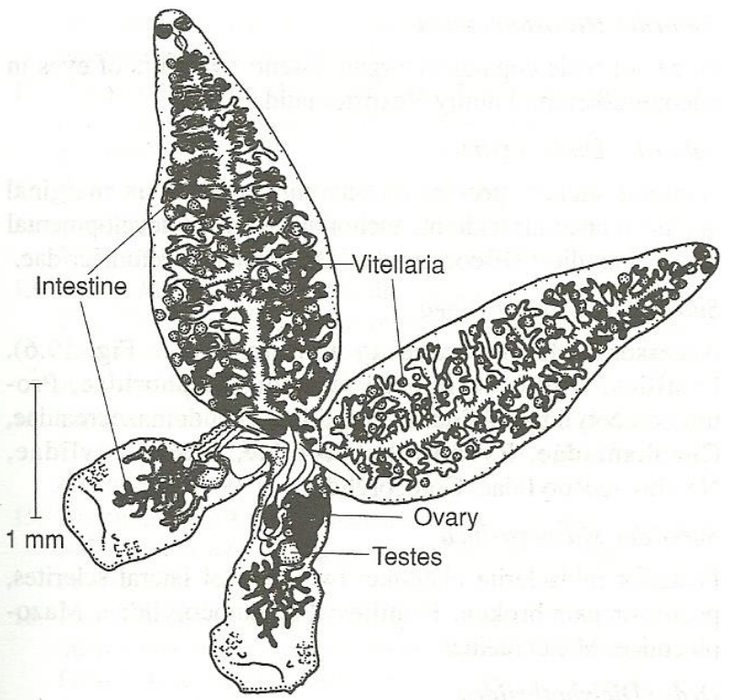


Figure 19.15
Diplozoon paradoxum, a parasite of freshwater fishes in Europe and Asia.

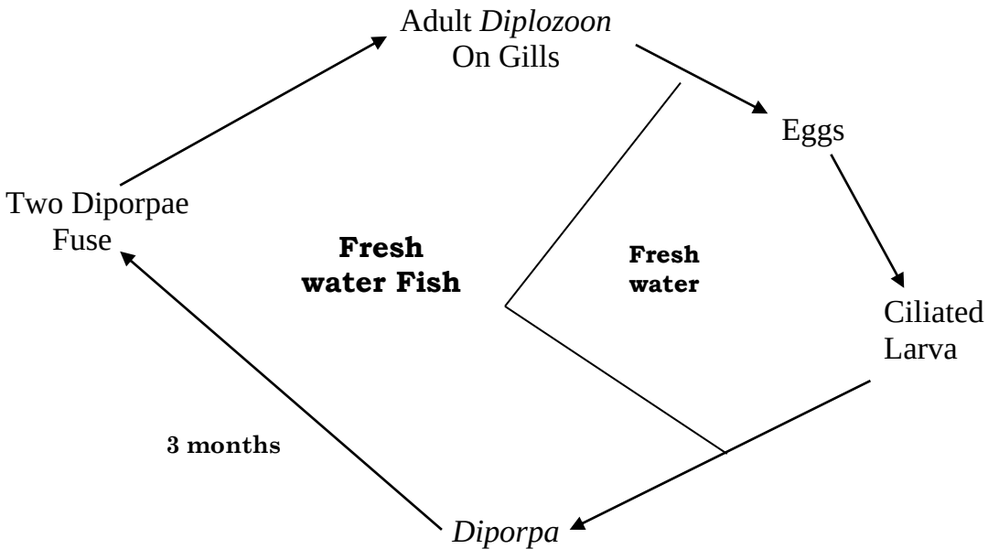
Life Cycle

The life-history of *Diplozoon* is unique. For whereas the larvae of most animals grow up, each into a single adult, in *Diplozoon*, of the few larvae that survive the dangers of their free-swimming existence, only those become mature which conjugate permanently with another individual. But although there are thus only half as many adult *Diplozoon* as there were conjugating larvae (or *Diporpa*, as they were called when they were considered distinct forms), yet the total number of eggs produced is probably as great as if each larva became individually mature.

The life cycle is of typical Monogenetic pattern. The egg develops into a ciliated larva that quickly fastens to a host of the same sort, where it grows and alters to the adult form; hence a simple life history which does not involve a change of host.

The uterus usually contains a single mature egg. Eggs are large, oval and operculate. They range in size from 0.27 to 0.29 mm by 0.07 – 0.09 mm. The eggs are provided with thick shells and a large coiled filament. Inside the capsule there are present a number of yolk cells surrounding the zygote which in turn are covered by a perivitelline membrane and the egg shell.

The eggs are deposited in water but according to Hyman (1951) the capsule remains attached to the host gills by a long tangled filament. The ovum divides rapidly at the expense of the yolk cells, and in a fortnight a ciliated larva (0.2 mm. long) hatches out, which, however, succumbs if it does not meet with a Minnow (host of *D. paradoxum*) in five or six hours. It has 2 pigmented eye spots, a well developed simple alimentary canal and ciliated epidermis. The alimentary canal is represented by a mouth, pharynx, and a blind sac like intestinal caecum. At the anterior end there are present 2 small suckers. The opisthaptor is well developed and bears a pair of adhesive clamps. The ciliated larva swims freely in water and if it comes in contact with the host gills it attaches to them. It loses the eye spots and cilia and develops branched intestinal caeca. The posterior end of the body becomes elongated and wider. The larva develops a small sucker in the midventral region and a small conical projection or papilla at the corresponding point in the dorsal surface. Now this stage larva is known as *Diporpa*. According to Schang (1964) the *Diporpa* is developed after attachment to the host gills. But according to Hyman (1951) the larva after becoming *Diporpa* attaches to the host gills. These *Diporpa* may acquire a third and even a fourth pair of suckers, and continue to live three months, but they only develop and mature their reproductive organs, if each conjugates with another *Diporpa* and this only occurs in a small percentage of instances. The two Diporpae become associated intimately in such a way that ventral sucker of one individual fits into the conical dorsal papilla of the other. In order to fit the two structures mentioned, the two Diporpae twist and at the point of contact the two worms fuse organically *i.e.*, complete fusion occurs, the united Diporpae (or *Diplozoon* as the product is now called) decussate, each forming one limb of the X-shaped *Diplozoon*, within which the two sets of complex genitalia develop. The two animals grow permanently together in this position and apparently can survive only as such fused pairs. When the reproductive system develops, the sperm duct of each opens directly into the yolk duct of the other by a connection probably representing a copulation canal, so that the two flukes are in a permanent state of copulation (Zeller, 1872a). Schang (1954) reports that “It is usually after 2 worms thus united that 2 additional pair of clamps take form, first pair being formed prior to union. 3 pairs of clamps are characteristic of sexually mature adult but actually there are 4 pairs of adhesive clamps in adult stage. Larvae which fail to find a partner subsequently die and never attain sexual maturity. A physiological explanation for the failure of single individuals to become mature has not yet been produced.



Graphic Life Cycle of *Diplozoon*

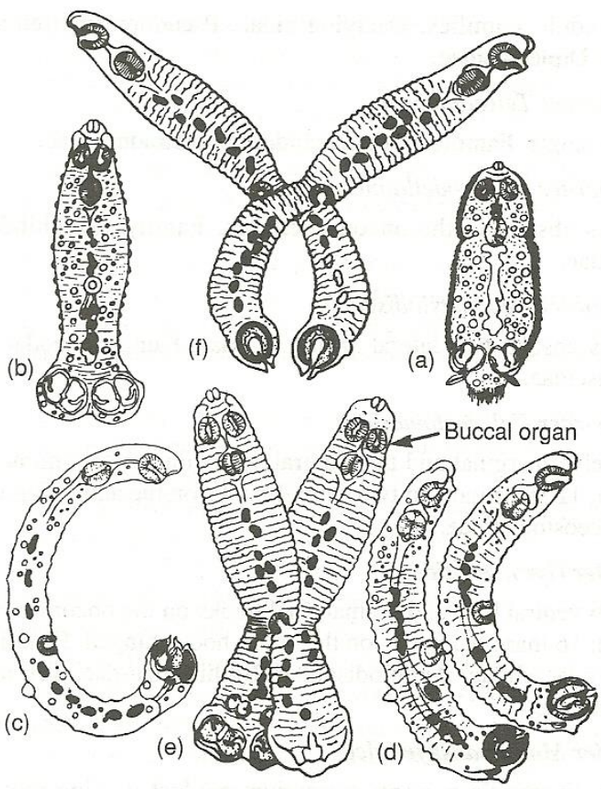


Figure 19.16
Development of *Diplozoon paradoxum*. (*a*) Freshly hatched, free-swimming juvenile. (*b*) Diporpa juvenile. (*c–f*) Diporpa juveniles attaching themselves to one another.

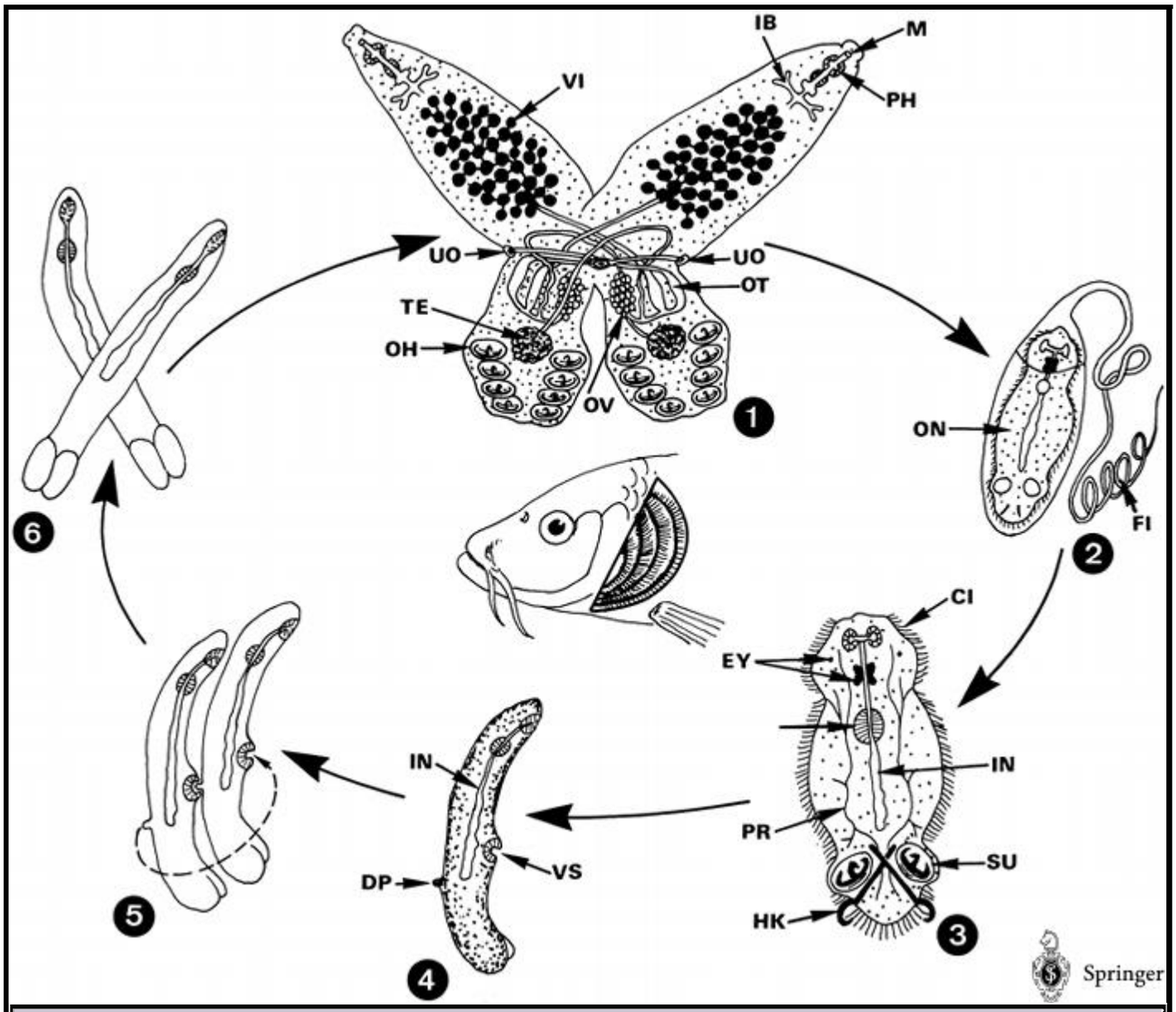


Fig.: **Life cycle of *Diplozoon paradoxum* on the gills of cyprinid fish.**
1: Adults on the gills of fish. ***2:*** Egg with a larva. ***3:*** Free Oncomiracidium. ***4:*** After attachment to the gills of a host the Oncomiracidium is transformed into the Diporpa larva. ***5 & 6:*** Fusion of two diporpas on the host; each 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: Opisthohaptor* with suckers; *ON*: Oncomiracidium; *OT:* Ootyp; *OV:* Ovary; *PH:* Pharynx; *PR: Pharynx*; *SU:* Sucker (Clamps); *TE: Testis; UO:* Uterus Opening; *VI:* Vitelline Gland; *VS:* Ventral Sucker

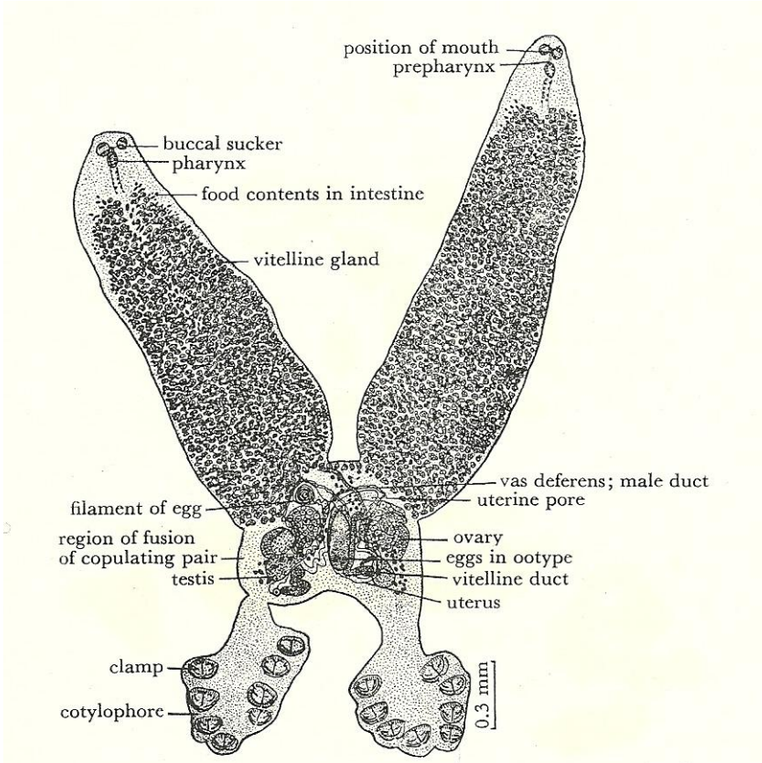


Fig. 6-7. Two *Diplozoon ghanense* in permanent copulation. (Thomas, courtesy J. West. African Sci. Assoc.)

Diplozoon ghanense

This species lives on the gills of the fish *Alestes macrolepidotus.* Its greatest distinction is that, although it is hermaphroditic, a permanent union of the worms occurs. During the larval stage of this fluke a small fleshy knob appears on the dorsal surface. Eventually this knob becomes fitted into a ventra1 sucker of another larval worm. The two worms become securely fused together and cannot be separated. The gonads then begin to develop, and finally the vagina of one individual opens in the region of the uterus and vas deferens of the other. This arrangement is reciprocal and cross fertilization is made easy.

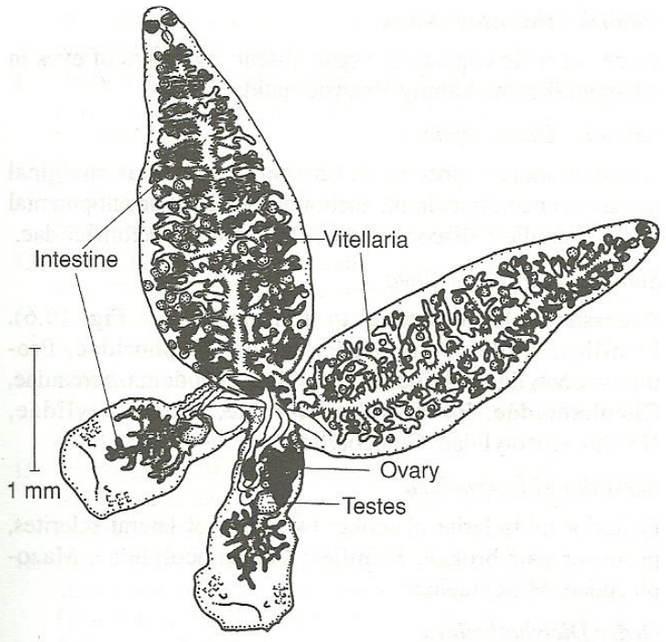


Figure 19.15
Diplozoon paradoxum, a parasite of freshwater fishes in Europe and Asia.

Diplozoon paradoxum

Diplozoon paradoxum is a common parasite of the gills of European cyprinid fishes. *Diplozoon paradoxum* exhibits a strong seasonal variation in its reproductive activity. Virtually no gametes are produced during winter, but gonads begin to function during the spring, reaching a peak during May to June and continuing through the summer. Eggs, which have a long, coiled filament at their ends, can hatch about 10 days after deposition; light intensity and water turbulence, as might be caused by host feeding or spawning activity, stimulate hatching. Oncomiracidia bear two clamps on their opisthaptor with which they attach to a gill filament; they lose their cilia almost immediately. Worms feed and begin to grow, adding another pair of clamps to the opisthaptor. A small sucker also appears on the ventral surface and a tiny papilla appears on the dorsal surface, slightly more posterior than the sucker. When this stage was first discovered, it was thought to represent a new genus and was named *Diporpa.* When *Diporpa* was recognized as a juvenile stage of *Diplozoon*, the term **diporpa** was applied to the stage. Curiously, *Diplozoon paradoxum*, even its diporpae, rarely infect young-of-the-year fish.

A diporpa juvenile can live for several months, but it cannot develop further until encountering another diporpa; unless this happens, the diporpa usually perishes by winter. When one diporpa finds another, each attaches its sucker to the dorsal papilla of the other. Thus begins one of the most intimate associations of two individuals in the animal kingdom. The two worms fuse completely, with no trace of partitions separating them. The fusion stimulates maturation. Gonads appear; the male genital duct of one terminates near the female genital duct of the other, permitting cross-fertilization. Two more pairs of clamps develop in the opisthaptor of each. Adults apparently can live in this state for several years.

Control

For parasite control in fish ponds it is proposed that:

- A restricted contact with natural waters,
- The creation of a healthy ecosystem and fish hygiene,
- A periodic parasitical examination,
- Chemical-based treatments must be restricted,
- Using plant extracts could be an alternative control measure of fish parasites.