Proc. Zool. Inst. Russ. Acad. Sci, 310. 2006: 51-58

Phenomenon of parthenogenetic metacercariae in gymnophallids and aspects of trematode evolution

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Gymnophallids are parasites of coastal birds and use marine bivalve molluscs as first intermediate hosts and molluscs (bivalve or gastropod) or polychaetes as second intermediate hosts. The so-called germinal sacs, which produce cercariae and metacercariae, have been described for six gymnophallid species but confusion has existed as to their nature and if the host in which they were found is the first or second one (Szidat, 1962; James, 1964; Chubrik, 1966; Tsimbaljuk *et al.*, 1978; Ching, 1982). Our experimental work on *Cercaria margaritensis* Ching, 1982 confirmed that the germinal sacs are found in the second intermediate host and that the sporocysts develop in the bivalve *Turtonia minuta* (Galaktionov, 1996). Further investigation has shown that *Cercaria margaritensis* Ching, 1982 represented a new species *Parvatrema margaritense* Galaktionov, Irwin et Saville, 2006. Daughter sporocysts of *P. margaritense* produce typical gymnophallid furcocercariae that are shed from *T. minuta* and penetrate the prosobranch *Margarites* spp., the second intermediate host (Fig. 1). Each cercaria migrates to the extrapallial cavity, drops its tail, and changes into a germinal sac considered as a parthenogenetic metacercaria (PM). This primary parthenogenetic metacercaria (PM1) produces the second generation of metacercariae (PM2) in its brood sac. The PM2 metacercariae leave the PM1 and parasitize the extrapallial cavity of the *M. helicinus.* The PM2 are also parthenogenetic and produce the third generation of metacercariae (M3) which are infective to the definitive host. The birds are infected when they eat molluscs containing PM2 with mature M3 inside them. Life cycle transmission in other gymnophallids with parthenogenetic metacercariae appears to follow a similar scenario.

At present the life cycle described above seems to be one of the most complex among trematodes and includes two periods of parthenogenetic reproduction. The first takes place in the first intermediate (molluscan) host on the stages of mother and daughter sporocysts and results in production of a huge number of intective stages (cercariae). This is typical for trematodes.

However the life cycle under discussion acquires additional stages (PM) for clonal multiplication of the next infective stages, the metacercariae. Undoubtedly this increases the transmission success of these parasites.

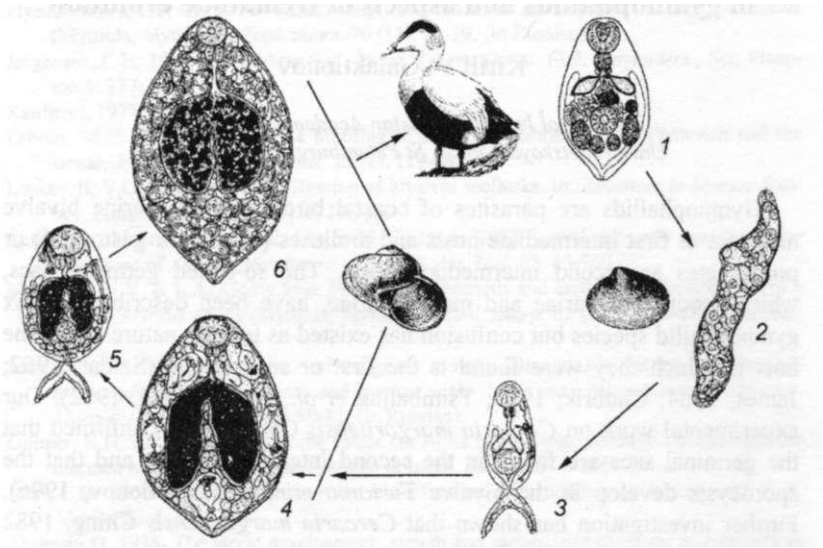

Fig. 1, The life-cycle of *Parvatrema margeritense*. 1 - adult in the common eider *(Somateria mollissima); 2* - daughter sporocyst in the first intermediate host, bivalve *Turtonia minuta;* 3 - cercaria; 4-6 - stages in the second intermediate host, gastropod *Margarites helicinus* (4 - PM1; 5 - young PM2; 6 - mature PM2 filled with M3) (see text for details)

Phenomenon of PM is of special interest from the evolutionary point of view because it may be considered as a model demonstrating the early steps of trematode life cycle evolution when proto-trematodes started to colonize the first intermediate (molluscan) host. A great similarity in bauplane of PM and rediae is most pronounced (Fig. 2). Both of them can be characterized as organisms possess vast brood sac and well developed digestive system. Unlike rediae the PM have a bifurcated digestive caeca, excretory bladder, oral sucker and undeveloped ventral sucker. Development of gonads and reproductive system ducts has been completely suppressed. Instead, germinal cells appear to arise from undifferentiated cells in the genital primordium of young PM1 and PM2. The germinal cells, in turn, cleave to give rise to embryo cells. The same can be observed in developing rediae and daughter sporocysts (up to the point of brood sac development), when some

cells in the undifferentiated central cell mass become specialized as germinal cells (the so called primary germinal cells) and begin to cleave (Galaktionov & Dobrovolskij, 2003).

This would suggest that the parthenogenetic mode of reproduction may have originated in association with retardation and eventual cessation of reproductive organ formation and provokes formation of the morpho-functional organization which is characteristic for redia. This supports the opinion of Ginetzinskaya (1968) and Pearson (1972) that it was rediae that recapitulated most fully the ancestral organization features of ancient forms. Thus it seems likely that the first parthenitae in proto-trematode life cycles were rediae-like organisms. Later they evolved in sporocysts owing to morphological simplification which is the main trend of redial morphological evolution. As was shown recently by Galaktionov & Dobrovolskij (2003) rediae evolved into sporocysts several times independently in different phylogenetic branches of trematodes.

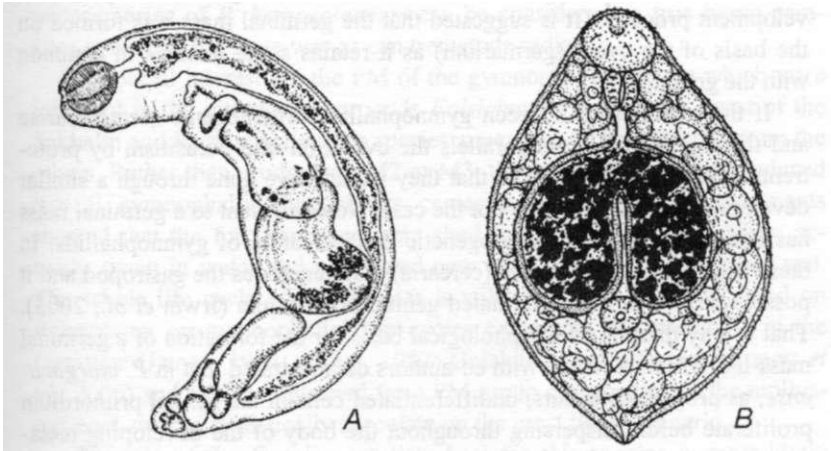

Fig. 2. Redia of *Cercaria fennica I* (Notocotyiidae) (from Odening, 1963) (A) and developing parthenogenetic cercaria (PM2) of *Parvatrema margeritense* (B)

The question arises why only gymnophallid metacercariae possess parthenogenesis though metacercariae of some other trematodes, such as echinostomatids, renicolids, etc., are also parasitic in molluscs? Gymnophallid metacercariae do not encyst and, unlike most digenean metacercariae, are able to make use of their oral suckers and gut caeca to actively ingest and digest their molluscan host tissues. This contrasts with the majority of digenean metacercariae that depend on provision of their energy resources from their

molluscan hosts through their teguments (Smyth & Halton, 1983; Galaktionov & Dobrovolskij, 2003). The period during which gymnophallid metacercaria may feed on second intermediate host tissue is not limited and that could be a reason why they have adopted parthenogenetic reproduction.

Analysis of the morpho-functional organization of PM can also elucidate what stage of the pro-trematodes life cycle adopted parasitism in gastropods. Some authors (Heyneman, 1960; Cable, 1965; Ginetsinskaya, 1968; Pearson, 1972) have suggested that larvae of proto-trematodes, rather than reproductively mature forms, were first to parasitize gastropods. James and Bowers (1967) and Galaktionov and Dobrovolskij (2003) contend, on the other hand, that it was reproductively mature proto-digeneans that took the first step to parasitism. Significant to this argument is the fact that the organization of digenean rediae and sporocysts is characterized by the presence of a distinct structure, the germinal mass, in which the reproduction and "maturation" of reproductive cells and initial stages of embryonic development proceed. (It is suggested that the germinal mass was formed on the basis of the ovary (germarium) as it retains some features in common with the gonad).

If the relationship between gymnophallid parthenogenic metacercariae and their gastropod hosts parallels the evolution into parasitism by proto-trematodes, one might expect that they would have gone through a similar developmental stage. This is not the case. No equivalent to a germinal mass has been observed in parthenogenetic metacercariae of gymnophallids. In these organisms it is the larva (cercaria) that penetrates the gastropod and it possesses only an undifferentiated genital primordium (Irwin *et al.,* 2003). That is why there is no morphological basis for the formation of a germinal mass-like structure. Irwin with co-authors demonstrated that in *P. margaritense,* as progenesis occurs, undifferentiated cells in the genital primordium proliferate before dispersing throughout the body of the developing metacercaria and giving rise to embryos of the next generation.

One can assume that the state of the reproductive system of gymnophallid parthenogenic metacercariae (devoid of germinal mass or any gonad-like structure) and rediae/sporocysts (possessing a germinal mass) illustrates that distinctly different developmental stages acquired the ability to reproduce parthenogenetically. In gymnophallids the cercaria made use of its undifferentiated genital primordium whereas proto-digeneans, probably having already developed gonads, transformed the ovary into the germinal mass in the course of evolution. Thus the absence of a germinal mass in gymnophallid parthenogenetic metacercariae supports the contention of

James and Bowers (1967) and Galaktionov and Dobrovolskij (2003) that adult proto-digeneans, rather than larval forms, were first to parasitize gastropods.

When all the species of gymnophallid posses PM are considered, it is apparent that they include species that demonstrate many of the successive stages of transition to tissue parasitism that have been postulated for prototrematodes. The PM of *P. margaritense* and the gymnophallid species from *Falsicingula* molluscs (see below) are commensals in the extrapallial fluid of their gastropod hosts. PM of *Cercaria quadriramis* Chubrik, 1966 are parasitic in seminal vesicles of *Littorina* spp. These cercariae probably penetrate the mollusc's male reproductive ducts from the extrapallial cavity via the genital pore. Finally *Parvatrema homoeotecnum* James, 1964 PM1 localize in female *Littorina saxatilis* gonoducts and the PM2 penetrate the haemocoelic space of the digestive gland and gonad, destroying these organs and feeding on tissue debris (James, 1964). Thus, the parthenogenetic metacercariae of *P. homoeotecnum* can be considered as true tissue parasites in exactly the same way as can trematode rediae.

Of special interest are the PM of the gymnophallid species which were recorded in the intertidal gastropods *Falsicingula* spp. on the coast of the Sakhalin and Kuril islands. This species appears to differ distinctly from the others. Rather than producing PM2 or M3, the PM of this species produced typical gymnophallid furcocerous cercariae (Fig. 3). Our experiments showed that the furcocercariae were shed from their gastropod hosts, actively swam in water and penetrated new individuals of *Falsicingula* spp. The whole life cycle of this species is still unknown. However based on information on gymnophallid life cycles containing PM available in the literature (James, 1964; Ching, 1982; Galaktionov, 1996; Galaktionov *et al.,* 2006) and evidence gleaned from PM group composition in the molluscan host, we can realistically speculate on the most likely scenario.

The role of the first intermediate host for this species is most likely played by an as yet unidentified intertidal or upper sub-tidal bivalve. Sporocysts developing in that bivalve produce cercariae that are released into the environment and infect the second intermediate host, the prosobranchs *Falsicingula* spp. After penetrating a second intermediate host the cercariae drop their tails, grow and transform into individuals of the first generation of PM (i.e. PM1) that produce cercariae (see Fig. 3). The later are released from the PM1 and are shed from infected *Falsicingula*. However, some of these cercariae remain in the extrapallial cavity of the molluscan host and develop into the PM of the next generation (i.e. PM2).

Therefore, at least some of the PM observed in groups in *Falsicingula* spp. may be individuals of the first generation of parthenogenetic metacercariae, i.e. PM1, whereas others are represented by the PM of the next parthenogenetic generations, i.e. PM2 and progeny (see below).

The results of our experiments show that at least a significant percentage of the cercariae produced by the PM, successfully leave the molluscan host and can infect other falsicinguls. Since not a single larva in the infected molluscs showed any signs, however small, of hermaphroditic reproductive system development, we can conclude that, once in the *Falsicingula* extrapallial cavity, cercariae launch down the path of transformation into PM. Such individuals are to become, at least, metacercariae of the second parthenogenetic generation (corresponding to PM2 of *Parvatrema margaritense* - see above). In their turn, they will start to produce cercariae. Very possibly, some will develop into PM of the next generation in the same mollusc. Others will be shed and will infect other *Falsicingula* individuals (see Fig. 3).

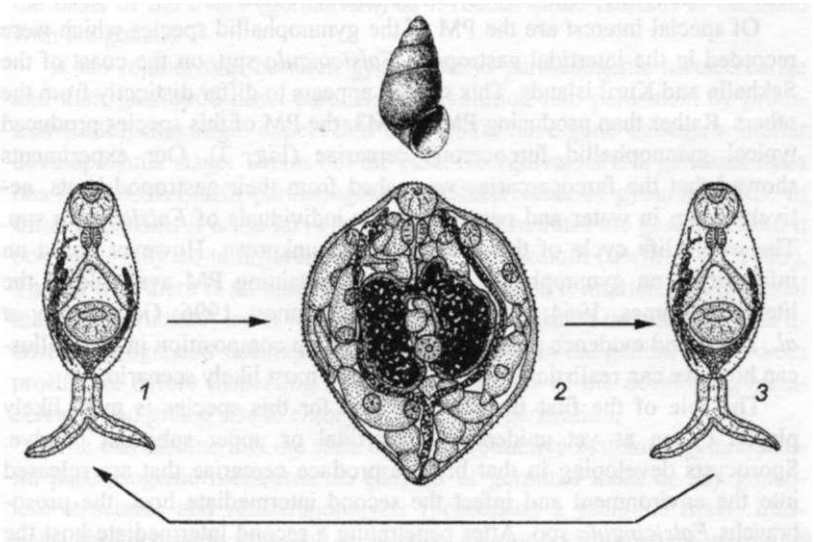

Fig. 3. The "cercaria - parthenogenetic metacercaria - cercaria" part in the life cycle of gymnophallid species from *Falsicingula* spp. 1 - cercaria; 2 - PM in *Falsicingula;* 3 - cercaria

It is impossible to realistically estimate the number of times that the "cercaria - parthenogenetic metacercaria - cercaria" component of the life cycle might be repeated. Obviously, at some stage in the life cycle, a meta-

cercaria that is infective for the definitive host must emerge. Most probably that production of metacercariae infective for birds is seasonal. For instance, during the warm summer period (during which, incidentally, the material of the present investigation was collected) the "cercaria - parthenogenetic metacercaria - cercaria" cycle is constantly repeated. Following this, in response to lower temperatures, the PM start to produce and accumulate infective metacercariae in their broad sacs. This type of seasonal switch has already been observed in gymnophallid larval development. Loos-Frank (1969) discovered that the daughter sporocysts of *Gymnophallus choledochus* in the cockle *Cardium edule* from the North Sea in summer produce cercariae that actively emerge into the environment, while in winter the cercariae do not leave the sporocysts, developing in the latter into metacercariae infective for birds.

Although the variations in the life cycle of the gymnophallid species from *Falsicingula* postulated above are only based on our observations and comparisons with the life cycles of closely related species, one thing appears quite certain: The "cercaria - parthenogenetic metacercaria - cercaria" part in the life cycle has the potential for autonomy. In fact, this part could well constitute an autonomous sub-cycle or loop. Once established, it no longer depends on other life cycle components associated with the first intermediate or the definitive host. Hypothetically, this sub-cycle could be maintained in *Falsicingula* populations indefinitely. As a further development of this idea, one can imagine that under certain circumstances (e.g., if a PM-infected falsicingul were transferred to a location where a *Falsicingula* population is present but one of the hosts required for completion of the full life cycle is absent) a new species, with a simple life cycle, could be formed on the basis of the above sub-cycle. In the simple life cycle of this new species, *Falsicingula* would play the role of the only host, in which the sexual (parthenogenetic) individuals would develop. Transmission would be achieved by the cercariae shed into the environment. It is possible that this has already occurred in case of gymnophallid species under consideration.

The latter proposition makes one think about the limits of complexity of the life cycles. According to May (1972) large systems undergo the very sharp transition from stable to unstable behaviour as the complexity exceeds a critical value. Applied to life cycles this assumption may be interpreted in that way that extraordinary complexity of life cycles leads to their instability. This may be the case of gymnophallid species from falsicinguls. The life cycles of some parasitic protists, such as Microsporidia (e.g., *Nosema), Toxoplasma,* Myxozoa, may be also mentioned in this connection.

The work was supported by the grants of INTAS and RFBR (project no. 04-04-49439).

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