Digenean fauna of *Cerastoderma glaucum* (Veneroidae, Cardidae) from Tunisian coasts

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Abstract
The study conducted on the cockle *Cerastoderma glaucum* from Tunisian coasts (Bizerte lagoon and Gulf of Gabes) showed the presence of two digenean species (sporocyst and cercaria of *Bucephalus minimus* and metacercaria of *Meiogymnophallus strigatus*). A description of these larvae and the behaviour of the naturally emerging cercaria are reported. The examination of the frequency of *B. minimus* and *M. strigatus* reveals some seasonal variation. This variation becomes more significant by comparing the sites. *B. minimus* has the highest frequency in the Bizerte lagoon, by contrast to that of *M. strigatus* which is the highest in the Gulf of Gabes. This variation is statistically significant. These results indicate that *B. minimus* and *M. strigatus* can be considered as biological indicators making it possible to predict the capture area of the cockle.

Introduction
The cockle, *Cerastoderma glaucum*, which has a significant economic value, is commonly exploited in several European countries. In Tunisia, in spite of its abundance in several sites, in lagoons as well as in the sea, this species still remains unexploited. This bivalve, slightly hidden in the sandy-muddy sediment of the intertidal zone, is among the benthic organisms most exposed to organic and chemical pollutants debouched in the receiving environment. Studies carried out in the Bay of Saint Brieuc by Le Mao et al. (2002), on some species of bivalves, show a marked decrease of the cockle between 1988 and 2000. Various hypotheses were proposed to explain this reduction, among which, we can cite intensive fishing, as well as pollution and parasitism. Parasitism, as a process of regulating of the natural populations of marine organisms, is a theme which was the subject of little attention because of the durability of the relation parasite-host (Combes, 1995). Recently, De Montaudouin et al. (2000), Mouritsen (2002), Desclaux (2003) and Desclaux et al. (2002) studied the impact of Digenea on the cockle populations. These authors conclude that digenean parasitism does not necessarily explain the marked reduction in the molluscs, even if the presence of digeneans can indirectly increase the mortality rate of the cockle (*Cerastoderma edule, Austrovenus stutchburyi*) by reducing their capacity for burrowing in the sediment. A multifactorial effect is therefore suspected in cockle mortalities.

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Conscious of the ecological importance of the parasites, their action on the dynamics of populations and the behaviour of the host, we investigated the digenean fauna of the cockle, *Cerastoderma glaucum* and the spatiotemporal distribution of the parasites.

**Materials and methods**
The studied cockles (3200 specimens) were sampled from the Bizerte lagoon (north-eastern Tunisia) and the Gulf of Gabes (port of Sfax) (south-eastern Tunisia). The Bizerte lagoon, characterized by sandy-clay to clay-silt-sandy sediments, is rather deep (12 m) (Soussi et al., 1983). In Bizerte Lagoon, the mean annual water temperature is 18.28 °C and the mean annual salinity is 34 ‰. In the Gulf of Gabes, characterized by sandy-muddy bottom sediments (Amari, 1984), the annual mean temperature is 20.58 °C and the mean annual salinity is 38.68‰.

The collected lamellibranchs were placed in small aquaria, which were examined daily with a binocular microscope to detect spontaneous cercarial emergence. Each infected mollusc was then kept in its own aquarium. The number of cercariae produced daily was counted. The experiment was subjected to natural lighting conditions only, except during the period when cercariae were counted under artificial light. If these examinations appeared negative, after 15 days the molluscs were dissected to detect the possible presence of young sporocysts or metacercariae. The site of infection and the number of each parasite were noted. The parasites collected were either studied directly *in vivo* under the stereomicroscope, or treated for a later observation. The parasites were fixed in Bouin’s fluid between slide and coverglass, washed by distilled water and stained with boric carmine. After differentiation, the specimens were dehydrated in series of alcohol (70, 95, 100%), cleared in Gaulteria oil and mounted between slide and coverglass in Canada balsam.

Silversides collected from Kalaat El Andalous, a site known to be negative for the bucephalid parasite, were exposed to cercaria released from *C. glaucum* to try the experimental infection.

For histological preparations, cockles were fixed in Bouin’s fluid. After washing, samples were dehydrated through an ethanol series, treated with butyl alcohol, embedded in paraffin wax and sectioned. The 5-µm sections were stained with Masson Trichrome (Martoja and Martoja-Pierson, 1967).

Illustrations and measurements of parasites were made with the aid of Wild microscope drawing tube.

The nomenclature used in the analysis of the frequencies of the larval-stage parasites according to the seasons and sampling sites follows Margolis et al. (1982) and Bush et al. (1997).

To test the significance of the variation of the values of the frequency according to the localities of capture, $X^2$ test was applied.

**Results and discussions**
Examination of the cockle *Cerastoderma glaucum* collected from the Bizerte lagoon and the Gulf of Gabes (port of Sfax) reveal that this mollusc is parasitized by the same digenean fauna in both localities. Two species of digenean belonging to two distinct families were recorded: the sporocyst and cercaria of *Bucephalus minimus*.
(Stossich, 1887) Maillard, 1976 (Bucephalidae) and the metacercaria of *Meiogymnophallus stri-gatus* (Lebour, 1908) Ching, 1965 (Gymnophal-lidae).

**Bucephalus minimus**

**Sporocyst**

Branched sporocyst, white with constructions and containing, according to the degree of maturation, either the germinal balls or the cercariae (Figures 1 a, b). Sporocyst occurring in gonads and digestive gland of the cockle. With histological analysis, it was possible to confirm the presence of sporocyst in analyzed tissue (Figure 2 a, b). As reported by Khamdan (1998) and Desclaux et al. (2002), these larvae caused severe disruption such as the castration of the gonadal tissue, function alteration of the digestive gland or death of the mollusc. The development of the larval stages was, therefore, achieved at the expense of the parasitized host. The parasite diverts the metabolic activities of the mollusc for its own gain. The death of parasitized molluscs occurred before the total emergence of the cercariae.

**Cercaria**

**Morphology**

The gasterostome cercaria (Figure 3), measuring, on average, 360 µm in length and 90 µm in width. Anterior extremity armed with an organ of penetration, the rhynchus containing numerous glandular cells. The posterior margin, ending with the tail, composed of a caudal bulb, prolonged by two long retractile furca with numerous small transverse folds on surface. Glandular cells, present on caudal bulb and furca, secreting adhesive substance allowing the attachment of the cercaria to the second intermediate host. Digestive tract of the cercaria including an equatorial mouth followed by a muscular pharynx (30 µm diameter), a short oesophagus and a saccular sac. The main excretory tubules, resulting from the union of anterior and posterior collecting tubules, joining the excretory vesicle at the level of the mouth. Excretory vesicle, saccular, thin-walled, and measuring on average 60 µm in length. 72 flame cells (36 on the right, 36 on the left) present in the following arrangement:

\[2[(6+6+6) + (6+6+6)] = 72\]

Figure 1. Sporocyst containing cercariae of *Bucephalus minimus* from *Cerastoderma glaucum*, a: Drawing, b: photo
The Bucephalid cercaria, named *Bucephalus haimeanus* by Lacaze-Duthiers in 1954, was collected in the oyster *Ostrea edulis* L. 1758 in the Balearic Islands and in *Cardium glaucum* Bruguiere, 1789 in France coasts (Sete). Matthews (1973) found this parasite in *Cardium edule* and succeeded in obtaining experimentally the metacercaria by infecting the goby, *Pomatoschistus microps*. Maillard (1975, 1976) never found a bucephalid cercaria in *Ostrea edulis* at Sete whereas *Cardium glaucum* was parasitized. He concludes that the bucephalid parasite of the oyster and that of the cockle constitute two distinct species. However, this author, only on the basis of morphological criteria, attributed the bucephalid collected from *Cardium edule* and that taken from *Cardium glaucum* to the species which he named *Labratrema minimus*. However, all previous designations were considered as a synonym of *Bucephalus minimus* (Overstreet and Curran, 2002; Bartoli et al., 2006).

**Behaviour**
The cercaria left the mollusc and moved initially towards the water surface. The cercarial body

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**Figure 2.** a: Histological section showing the presence of *Bucephalus minimus* sporocyst in gonads. b: Histological section showing the presence of *Bucephalus minimus* sporocyst in digestive gland. Sp: sporocyst.

**Figure 3.** Cercaria of *Bucephalus minimus*. 100 µm
was directed downwards and floats due to its two long filamentous furcae. In the absence of an intermediate host, the larva remained at the bottom of the small aquaria. It carried out slow movements by contracting the body and the filamentous furcae. Finally, the cercaria encysted within a thin transparent envelope. The cercariae were released diurnally from the mollusc. The emergence of the cercariae is related to the increase of the temperature. Indeed, the mean number of emitted cercariae from parasitized mollusc, at temperatures of 15°C, 20°C and 25°C, is 45, 100, 250 individuals per day, respectively. However, temperature above 25°C shortens the longevity of the larvae (5 to 6 hours). The temperature of 15°C, on the other hand, maintains the life of the cercaria over 2 to 3 days. Graczyk and Fried (2001) suggest that the lifespan of the cercariae in water is determined by its glycogen reserves and water temperature. Higher temperatures involve an increased activity of the cercaria and thus a faster loss of its energy reserves, thus reducing its duration of survival (Evans and Gordon, 1983; Evans, 1985).

**Experimental infections**

Previous research (Gargouri Ben Abdallah, 2001) showed the presence of the metacercaria (Figure 4) in small teleost fishes (*Atherina boyeri, Aphanius fasciatus, Gobius niger, Gobius paganellus* and *Pomatoschistus microps*) and the adult parasite (Figure 5) in the sea bass, *Dicentrarchus labrax*. The highest frequency of this metacercaria was recorded in the silverside (*A. boyeri*) which seems to be the preferred host of this parasite. Accordingly, an experimental infection using 8 specimens of silversides was carried out. The fish were exposed, for one day, to naturally infested *Cerastoderma glaucum* which were emitting cercariae of *B. minimus*. The cercariae attached to the fish by the filamentous furcae and bulb and penetrated by their apical part. These fish were dissected, at a rate of 2 specimens, at 2, 4, 10, 20 days post exposure. Two days post exposure, the liver of the fish was entirely invaded by the metacercariae of *B. minimus*. These larvae have enormous digestive caeca, filled with hepatic and sanguineous cells,
occupying the major part of the parasite body. Four days after exposure, the anterior edge of the rhynchus forms protuberances, the volume of the digestive caeca decreased and the excretory vesicle increased in size. After ten days, the rhynchus carried, at its anterior margin, the well developed protuberances, while the digestive caeca continued to regress, and the excretory vesicle to dilate to be filled with fine granules. The silverside dissected after twenty days showed a metacercariae with immature gonads.

The number of larvae collected in autopsied fish varied from 20 to 35 specimens. These larvae give the liver a white colour and a granular aspect. Faliex (1991) and Faliex and Morand (1994), by studying the dynamics of the populations and the pathology of these larvae on the second host, concluded that the cercariae consume the hepatic cells before encystment. When the infestation is intense, the liver is presented by a compact mass of metacercariae.

The finding of the cercaria and the sporocyst of B. minimus, on the Tunisian coasts, allowed us to partially achieve the life cycle of this parasite as already described by Maillard (1975). The life cycle of this parasite was also achieved on the Portugal coasts (Pinna et al., 2009). However the first and the second intermediate hosts are respectively presented by Cerastoderma edule and Mugil cephalus. The larval and adult stage harvested on Tunisian and Portugal coast present the same morphology. Only molecular analysis will allow the confirmation of the attribution of the two sympatric parasites, harvested in different intermediate hosts, to the same species.

**Meiogymnophallus strigatus**
Metacercaria body (Figure 6 a, b), with rounded margin, measuring 406 µm in length and 279 µm in width and presenting a spinose tegument. Oral sucker (107 µm), subterminal, twice bread

![Figure 6. Metacercaria of Meiogymnophallus strigatus from Cerastoderma glaucum, a: Drawing, b: Photo.](image-url)
than ventral sucker (61 µm). Ventral sucker located, depending on the contraction, at the posterior limit of the mid third or at the anterior limit of the posterior third of the body. Mouth, opening in the middle of the oral sucker, followed by a small pharynx (37 µm). Oesophagus bifurcating into digestive caeca which reaching the level of the ventral sucker.

Immature gonads composed of two testes (80 µm), located in the area of the ventral sucker, near the lateral wall of the body and an ovary (65 µm) situated just anterior to the left testicle. Excretory system made of 16 flame cells presented as the following formula: 2 [(2+2) + (2+2)] = 16. Bladder, with two branches, extending to the level of the oral sucker and ending in two lobes.

The metacercaria of *M. strigatus* is gradually enveloped by the pallial proliferations. It is located between the mantle and the shell of the host, on the surface of the pallium or placed inside the furrow periostracal bordering the pallial lobes and generally, under the hinge. The number of parasite per infested mollusc does not exceed 35. This parasite does not seem to cause a disturbance at the host. This is undoubtedly due to the lower number of the larvae so their pathogenic effect can remain hidden.

The metacercaria of *M. strigatus*, never encysted, was originally described as being a cercaria. It was reported for the first time by Giard (1897) at Boulogne in *Donax trunculus* L., *Tellina fabula* Gronov, *Tellina tenuis* Da costa and *Tellina solidula* L. Lebour (1908) attributed the name of *Cercaria strigita* to this helminth. Dollfus (1912) described this parasite again and specified that it represents a metacercaria and not a cercaria.

This larva was found by Rees (1939) in *Cardium edule*. Bartoli (1983) reported the presence of this metacercaria in several species of lamellibranchs (*Donax trunculus* L., *Donax semistriatus* Poli., *Lentidium mediterraneum* (Costa), *Solen margintus* Pennant, *Ensis ensis* (L.), *Pharus legumen* (L.), *Spisula subtruncata* (Da costa), *Loripes lacteus* (L.)) from the coasts of the Camargue. The cercaria of *M. strigatus* was found in *Tellina tenuis* Da costa and the adult in *Melanitta nigra*, and *M. fusca* (Pérès and Picard, 1964; Bartoli, 1983).

The metacercaria collected from the cockle from the Tunisian coast, has an identical morphology to that described by Bartoli (1983) in the Camargue. However, measurements of the Tunisian specimens are slightly greater than those of the southern coasts of France. We also note that the anterior end of the testes, which does not pass the middle of the ventral sucker in the specimens from the Camargue, reached or exceeds the anterior margin of the ventral sucker in the larvae of our coasts. This parasite is extremely close to *Gymnophallus fossarum* collected on the Tunisian coast from *Tapes decussata* (Trigui el Menif et al., 2004). Our observations show that these two species are characterized by the form of the body; *G. fossarum* is generally oval at and *M. strigatus* is round. The saccular digestive caeca are more voluminous in *M. strigatus*. The size of the oral sucker, the form and the width of the bladder constitute the discriminating criteria of these two species. The subterminal oral sucker, twice the size of the ventral sucker at *M. strigatus*, is slightly broader than the ventral sucker in *G. fossarum*. The anterior end of the bladder is slightly forked and does not pass the pharynx in *G. fossarum*, but it reaches the anterior limit of the oral sucker and is divided into two lobes in *M. strigatus*. The
Seasonal frequencies of digenean larvae

The seasonal prevalences of the sporocysts of *B. minimus* show a perennial infestation in the cockle from Bizerte lagoon with maximum values in autumn which decrease gradually in winter then in spring and becoming minimal in summer. These values are zero in summer in the Gulf of Gabes (port of Sfax) and do not exceed 3% in spring (Figure 7). The absence of this bucephalid in summer at Sfax can be explained by the increase of the temperature which can be lethal for infested mollusc, exhausted by the energy spoliation caused by the parasite, or for the infecting miracidial stage. The highest frequencies noted in Bizerte lagoon may be due to the nature of the harvested area; indeed, the lagoon of Bizerte, qualified as confined environment, with high densities of organisms, represents a privileged endemiotope for the digeneans, thus allowing the successful completion of the life cycle of *B. minimus*. The hydrodynamism, more important at Sfax, plays a fundamental role in the dispersion of the infective stages thus restricting their chances of the meeting with their hosts. This variation can also be related with salinity and frequency, in the prospected area, of the intermediate and the final hosts allowing the completion of the heteroxenic life cycle of this parasite. However, Maillard (1976) noted that the emergence of the cercaria of *B. minimus*, in the ponds of Prevot, Vic and Arnel, is observed during the summer then decrease in autumn and disappears in winter. The development of the cercaria begins again in spring. The lag, of one season, of parasitic frequency peak between the Tunisian and French coasts and the absence of this larva in winter in the latter locality are, undoubtedly, because the water temperature in France is lower.

Metacercaria of *M. strigatus* presents generally low values of frequencies. These values which are more important in Sfax, are cancelled in autumn in the two prospected localities. The development of the metacercaria starts in winter reaching a maximum in spring to be reduced again in summer (Figure 8). The diminution of this frequency during the summer is, undoubtedly, due to the mortality of the cockle following the increase of the temperature of water. The lack of infestation during the autumn can be explained by the non emission of the cercaria during the summer season or by the fast death of these larvae, following the rise in the temperature, before reaching the second host. The space variability of the infestation is probably related to the environmental conditions of the sites and the frequency of the other hosts intervening in the life cycle.

Examination of the frequencies of *B. minimus* and *M. strigatus* reveals a low seasonal variability. The variation becomes more noticeable when we compare the sites. Indeed, *B. minimus* is more frequent in the Lagoon of Bizerte and *M. strigatus* is more common in the Gulf of Gabes. The variations of the distribution according to sampling localities are statistically significant ($X^2=3.84, df=1, p<0.05$) for the two species. These results make it possible to consider *B. minimus* and *M. strigatus* as biological indicators of cockles stocks. We think that parasitic fauna could be used to predict the harvesting area for unknown samples. This natural method of marking was used in the countries of the northern Mediterranean coast for distinction of fish stocks (Arthur and Albert, 1993; Margolis, 1993; Mackenzie, 2002; Power et al., 2005; Timi, 2007). In Tunisia, a similar approach was used to discriminate stocks of triglids (*Chelidonichthys*...
Figure 7. The seasonal variation of prevalence of *Bucephalus minimus* according to sampling localities

Figure 8. The seasonal variation of prevalence of *Meiogymnophallus strigatus* according to sampling localities
lastoviza, C. lucerna and C. obscurus) from the Gulfs of Gabes and Hammamet by using a differential space frequency of Monogenea (Plectanocotyle sp, P. major, Trochopus pini and Triglicola obscurum) (Neifar, 2008).

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