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Digenean parasites of the marine gastropods *Littorina littorea* and *Gibbula umbilicalis* in the Northern Portuguese Atlantic coast, with a review of digeneans infecting the two gastropod genera

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ABSTRACT

Objective: To study the digenean parasites of the prosobranch gastropods, *Littorina littorea* (*L. littorea*) and *Gibbula umbilicalis* (*G. umbilicalis*) from rocky shores of the Northern Atlantic coast of Portugal.
Methods: A total of 413 *L. littorea* and 2603 *G. umbilicalis* were collected from rocky shores, at Aveiro estuary, Aguda Beach, and Foz Beach at Porto (Northern Atlantic coast of Portugal) from January to July 2014 and February 2014 to January 2015, respectively. Gonads and digestive glands of the gastropods were examined under light microscopy for the presence of digeneans. Infected gonads of *L. littorea* were prepared for histological observation, whereas digeneans found in *G. umbilicalis* were studied and identified with the aid of light and scanning electron microscopy.
Results: Two species of digeneans were found infecting the digestive gland and gonads of *G. umbilicalis*, namely, *Cainocreadium labracis* and *Lecithochirium furcolabiatum* with a combined prevalence of 3%. Histological observations of gonads of *L. littorea* revealed the infection with digeneans, with considerable replacement of gonadal tissues. These digeneans were not identified to species level. The prevalence of digeneans in this snail host was 0.7%.
Conclusions: The present study adds *G. umbilicalis* as another first intermediate host for *Cainocreadium labracis* and extends its geographical range to the Portuguese Atlantic coast. Cercariae of *Lecithochirium furcolabiatum* were previously reported from *G. umbilicalis*. A literature review of digeneans occurring in the gastropod genera *Gibbula* and *Littorina* is given.

1. Introduction

Molluscs are invertebrates that are part of the benthos in coastal and estuarine waters. Some species of molluscs, both gastropods and bivalves, due to their high economic value, have been exploited and cultivated all over the world for many decades[1]. Several species of prosobranch gastropods act as intermediate hosts for digenean parasites, being the final hosts either fish or fish-eating birds[2,3]. Although marine molluscs, in particular the littoral forms, are well adapted to the physical conditions controlling their habitats, some biological factors, such as infestation by diseases and parasites, may represent an impact to the survival of the affected molluscs[4-7]. Infections with digeneans, which parasitize the gonads of gastropods, can lead to castration, which obviously compromises the reproductive potential of the species[8-10]. Apart from the reduction in reproductive potential, infections with digeneans parasites can also lead to behavioural changes and mortalities[4,11-17]. The periwinkle *Littorina littorea* (Linnaeus, 1758) (Family Littorinidae) (*L. littorea*) and the purple topshell *Gibbula umbilicalis* (da Costa, 1778) (Family Trochidae) (*G. umbilicalis*) are marine gastropods which are common in the intertidal rocky shores of the Northeastern Atlantic Ocean and the North Sea[18,19]. *L. littorea* is native to European coastal waters and presently distributed from the White Sea to Southern Atlantic coast of Portugal, in the eastern side of the Atlantic, the western side from Labrador to Virginia[20] and the Pacific coasts of North America[21]. In the coasts of England and the North Sea coasts of Germany, it is distributed on rocky intertidal shores, whereas in the Baltic Sea, where salinity is reduced, it is located in the sub-tidal zones[4]. According to Barroso *et al.*[22] in

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Ria de Aveiro (Northern Atlantic coast of Portugal), it is found in the intertidal muddy flats with vegetal cover by *Zostera* sp., *Gracilaria* sp., or *Ulva* sp. *G. umbilicalis* is distributed in the Northeast Atlantic Ocean, North Sea and the Mediterranean Sea[23]. Both molluscs are feeding on small invertebrates and grazing on algae[24,25]. In this study, we presented the results of the occurrence of digeneans in the sea snails, *L. littorea* and *G. umbilicalis*, collected from rocky shores in the Northern Atlantic coast of Portugal, and reviewed the literature on the worldwide occurrence of digeneans in the two snail genera, *Littorina* and *Gibbula*.

2. Material and methods

Specimens of *G. umbilicalis* (n = 2603) were collected at the rocky shores of sandy beaches at Foz (Porto, Portugal) from January to July 2014, and *L. littorea* (n = 413) were collected from Aveiro estuary and sandy beach at Aguda (south of Porto, Portugal) (40°64'05"–41°15'17" N), from February 2014 to January 2015 (Figure 1). In the laboratory, gonads and digestive gland were excised and examined with a binocular microscope for infections with digeneans. Infected tissues were fixed in Bouin's fluid for 24 h, dehydrated in ascending alcohol series and xylene and embedded in paraffin wax. Sections of 4–5 µm were made with the aid of a rotary microtome, hydrated in descending alcohol series, stained with Mayer's haematoxylin and eosin, dehydrated in ascending alcohol series and xylene, and mounted in DPX (Merck). Live cercariae recovered from infected digestive gland and gonads of *G. umbilicalis* were also examined and measured alive in temporary fresh mounts stained with neutral red. For scanning electron microscopy, isolated cercariae collected from *G. umbilicalis* were fixed in 2.5% glutaraldehyde in 0.2 mol/L sodium cacodylate (pH: 7.2) at 4 °C for 3–4 h, washed in buffer overnight and post-fixed in 2% osmium tetroxide for 2 h, transferred to 70% ethanol and dehydrated to 100% ethanol. Specimens were placed in cylindrical stubs, allowed to dry, coated with gold and palladium and examined with a FEI QUANTA SEM. The prevalence of digeneans was calculated according to Bush *et al.*[26].

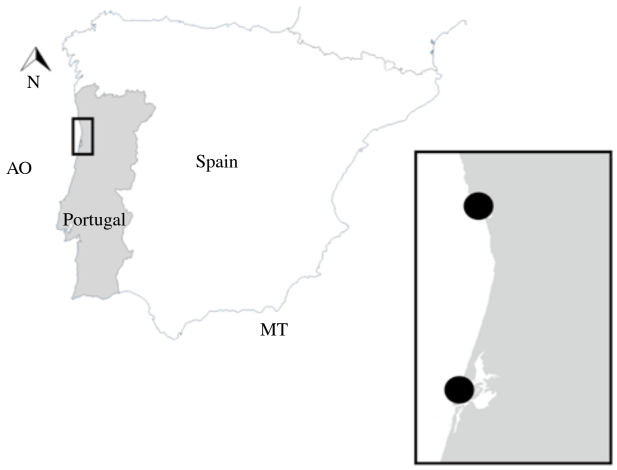

Figure 1. Location of the survey regions (●) in the Northern Portuguese Atlantic coast.

3. Results

Several sporocysts, white and orange, measuring 1700 µm in length and 190 µm in width, were observed replacing digestive gland tissues and gonadal tissues of *G. umbilicalis* (Figure 2A). They contained cercariae in number of at least 20 individuals. Two different types of cercariae were found infecting the gonads and the digestive gland (a cotylocercous cercariae and a cystocercous cercariae). The cotylocercous cercariae had a mean length of 267 µm and width of 67 µm with two rounded muscular suckers, oral and ventral suckers (40–45 µm to 53 µm) (Figure 2B). The oral sucker bearing a stylet of 13.5 µm, glandular cells, 12 in number, ending at the upper border of the ventral sucker, excretory bladder of 53 µm in length, with a caudal appendix of 25 µm in length and 30 µm in width. SEM observations revealed the structure of the oral and ventral suckers. Oral suckers with filiform and spiniform microtriches, and 6 large papillae on its anterior border, ventral sucker subglobular, with inner covered by several rows of spines and the caudal appendix (tail stump) with posterior end protrusible with glandular structures (Figure 2C, D). This posterior end measured 5 µm in length and 20 µm in width (Figure 2E).

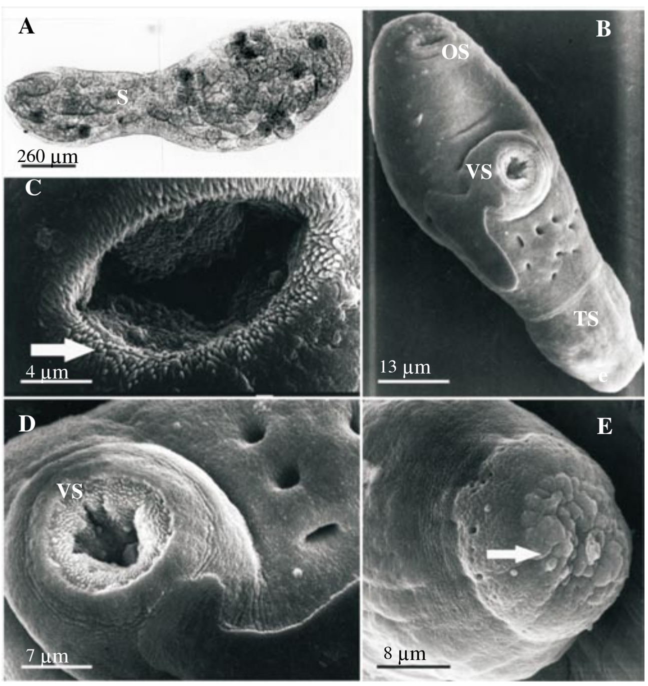

Figure 2. Light and scanning electron microscope observations of *C. labracis* infecting *G. umbilicalis*.
A: Light micrograph of a sporocyst (S) filled with cercariae; B: SEM of a cercaria of *C. labracis* from *G. umbilicalis* showing oral sucker (OS), ventral sucker (VS) and tail stump (TS); C: Detail of the oral sucker, with filiform microtriches (arrowed); D: Detail of the ventral sucker (VS), with several rows of spines; E: Detail of the tail stump with vesicular structures (arrowed).

Based on the microscopical observations, the cercariae were identified as *Cainocreadium labracis* Dujardin, 1845 (Opecoelidae) (*C. labracis*). The cystocercous type showed a cercarial body with two developed suckers, oral (30 µm) and ventral (40 µm) suckers, had a caudal cyst, which was anteriorly rounded and narrow at the end, with the length of 128–138 µm and width of 75 µm, and long caudal filaments (Figure 3). The cercarial body with the two suckers was 100 µm in length and 23 µm in width. Based on the microscopical observations, and measurements of the total body length, position, measurements and structure of suckers, we concluded that this cystocercous type corresponds to *Lecithochirium furcolabiatum* (Jones, 1933) Dawes, 1947 (Hemiuridae) (*L. furcolabiatum*). Digenean infections in *L. littorea* were only studied in paraffin sections. For this reason, it was not possible to identify the digenean species involved. Histological sections of infected gonads of periwinkles showed numerous sporocysts filled with a number of cercariae replacing the gonadal tissue without evidence of host tissue reaction (Figure 4A). In some sections, more than 8 cercariae per sporocyst could be counted. Some cercariae showed well-developed suckers (Figure 4B), others possessed smaller ventral suckers (Figure 4C), and a tail (Figure 4D), which suggested that more than one species of digeneans infected the gonads. The combined prevalence of digeneans in *G. umbilicalis* was 3% (n = 2 603), with some seasonal fluctuations (Figure 5). In *L. littorea, the* prevalence of digeneans was lower (0.7%, n = 413). Tables 1 and 2 are compilations of the digenean parasites infecting members of the genera *Littorina* and *Gibbula*.

Table 1

Digenean parasites reported from periwinkles of the genus *Littorina*.

Parasite species	First intermediate host	Prevalence	Geographic distribution	Final host	Source
C. lingua	*L. littorea* *L. saxatilis* *L. obtusata*	Up to 65% < 2.5% < 1%	German North Sea and Baltic Sea, Maine, North America, Atlantic Ocean, Wales	Gulls	[4,27-31]
H. elongata	*L. littorea* *L. saxatilis*	Up to 7.2%	German North Sea, German and Danish Baltic Sea	Gulls	[4,28,30,32]
Himasthla littorinae Stunkard, 1966	*L. littorea*	10.0%–18.0%	Atlantic Ocean, Wales	Gulls	[27]
Maritrema arenaria Hadley & Castle, 1940 (Microphallidae)	*L. saxatilis*	Up to 0.27%	Barents Sea	Waders, gulls	[30,33]
Microphallus pygmaeus Levinsen, 1881	*L. littorea* *L. saxatilis* *L. obtusata*	Up to 2.7% Up to 23.6% Up to 11.0%	German North and Baltic Sea, Danish Baltic Sea	Eiders, ducks, gulls	[4,30,34,35]
Microphallus piriformes Galaktionov, 1983	*L. saxatilis* *L. obtusata*	0.16% for both	Barents Sea	Ducks	[30,33,35]
Microphallus similis (Jägerskiöld, 1900) Nichol, 1906	*L. littorea* *L. saxatilis* *L. obtusata*	Data not found Up to 6% Up to 13.7%	Barents Sea	Gulls	[30,34]
Microphallus triangulatus Galaktionov, 1984	*L. saxatilis* *L. obtusata*		Barents Sea	Sea ducks	[30,33,35]
Paramonostomum chabaudi Van Strydonck, 1965 (Notocotylidae)	*L. littorea* *L. saxatilis*	1.9% Up to 0.8%	North Sea, Celtic Sea, Swedish west coast	Birds	[4,27,28,34,36]
Parapronocephalum symmetricum (Pronocephalidae)	*L. saxatilis* *L. obtusata*			Shore birds, oyster catchers	[30]
Parvatrema homoeotecnum James, 1964 (Gymnophallidae)	*L. saxatilis* *L. obtusata*	0.09% for both		Shore birds	[27,33]
Podocotyle atomon (Rudolphi, 1802) Odhner, 1905 (Opecoelidae)	*L. littorea* *L. saxatilis* *L. obtusata*	Up to 0.3% for three	German North Sea, German and Danish Baltic Sea	Marine fishes	[4,30]
R. roscovita	*L. littorea* *L. saxatilis* *L. obtusata*	Up to 3.7% for three	German North and Baltic Seas, North America	Gulls	[4,28,32,34]
Cercaria parvicaudata Stunkard & Shaw, 1931	*L. littorea* *L. saxatilis* *L. obtusata*	Up to 2.23% Up to 1.82% Up to 0.42%	Europe, North America		[27,33]
Cercaria linearis Lespés, 1857	*L. littorea*		North Atlantic Ocean		[27]
Cercaria emasculans Pelseneer, 1906 (Renicolidae)	*L. littorea* *L. saxatilis*	0.28%	Celtic Sea	Birds	[27,28,33]
Cercaria buccini Lebour, 1911	*L. littorea*		North Atlantic Ocean		[27]

C. lingua: *Cryptocotyle lingua* Creplin, 1825 (Heterophiidae); *H. elongata*: *Himasthla elongata* Mehlis, 1831 (Echinostomatidae); *R. roscovita*: *Renicola roscovita* Stunkard, 1932 (Renicolidae) *L. saxatilis*: *Littorina saxatilis*; *L. obtusata*: *Littorina obtusata.*

Table 2

Digenean parasites reported from top shells of the genus *Gibbula*.

Parasite species	First intermediate host	Prevalence	Geographic distribution	Final host	Source
C. labracis	*G. adansonii*, *G. umbilicalis*	22.04%–30.80%	Mediterranean Sea, Atlantic Ocean	*D. labrax*	[37-40]
Helicometra fasciata (Rudolphi, 1819) Odhner, 1902 (Opecoelidae)	*G. adansonii*	0.35%	North Atlantic, Mediterranean Sea	*Gobius niger*, *Zosterisessor ophiocephalus*, *A. anguilla*, *L. pholis*	[41-44]
Macvicaria obovata Molin 1859, (Bartoli, Bray & Gibson, 1989) (Opecoelidae)	*G. adansonii*	Up to 23.10%		*Sparus aurata*, *Blennius* spp., *Oblada melanura*	[38,40,45]
L. furcolabiatum	*G. umbilicalis*	1.13%	North Atlantic	*C. conger*, *A. anguilla*, *C. mustela*	[41,46-48]
Lecithochirium rufoviride (Rudolphi 1819) Lühe 1901	*G. umbilicalis*, *Gibbula cinerea*		North Atlantic	*C. conger*, *A. anguilla*	[46,49]
Lecithochirium fusiforme Lühe, 1901	*Gibbula varia*		North Atlantic	*C. conger*	[46,50]

G. adansonii: *Gibbula adansonii*; *M. obovata*: *Macvicaria obovata* Molin 1859, (Bartoli, Bray & Gibson, 1989) (Opecoelidae); *D. labrax*: *Dicentrachus labrax*; *A. anguilla*: *Anguilla anguilla*; *L. pholis*: *Lipophrys pholis*; *C. conger*: *Conger conger*; *C. mustela*: *Ciliata mustela.*

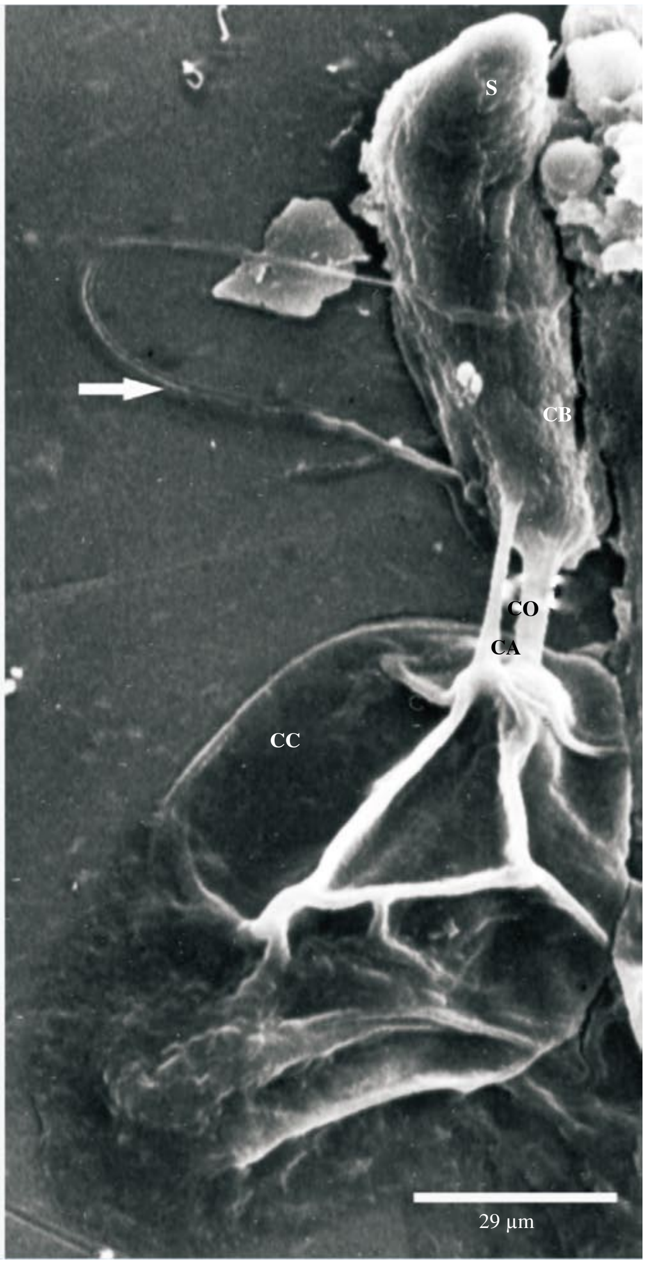

Figure 3. SEM of a cercaria of *L. furcolabiatum* from *G. umbilicalis*, showing cyst aberture (CA), cercarial body (CB), suckers (S), caudal cyst (CC), excretory appendage (arrowed), and the connection of cercarial body to caudal cyst (CO).

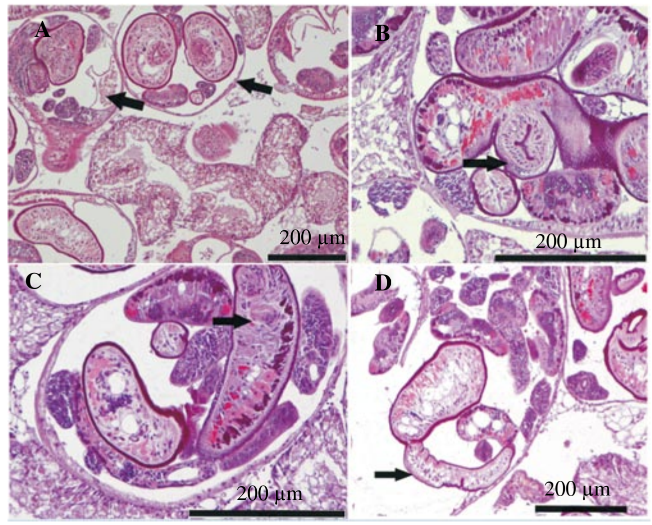

Figure 4. Histological sections of gonads of *L. littorea* infected with cercariae of digeneans.

A: Section of infected gonads of *L. littorea* showing several cercariae of digeneans (arrowed); B: Detail of cercariae inside sporocysts showing well-developed suckers (arrowed); C: Another cercarial type with small ventral sucker inside a sporocyst (arrowed); D: Cercarial type with a tail inside a sporocyst in the gonads of *L. littorea*.

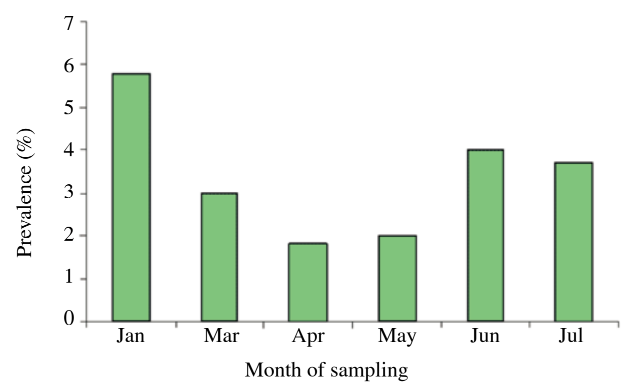

Figure 5. Prevalence of digeneans in *G. umbilicalis* from January to July 2014.

4. Discussion

The present study identified two different types of cercariae in the intermediate host, the purple top shell, *G. umbilicalis*, namely *C. labracis* and *L. furcolabiatum*. Measurements of the cercariae of *C. labracis* and SEM observations of its oral and ventral suckers, and tail stump corresponded to those recorded for this digenean from the snail host *G. adansonii*[37,38]. Metacercariae of *C. labracis* have been found encysted in the fins and skin of several different fish hosts in the North Atlantic and Mediterranean coastal waters, belonging to diverse fish families, such as Gobiidae, Syngnathidae and the adults are parasites of the intestine of sea bass *D. labrax* and other fish species belonging to the family Sparidae[37,51,52]. However, molecular studies conducted in *C. labracis* collected from *D. labrax*, the type host, and from *Dentex dentex* (Sparidae) demonstrated that two different digenean species were involved, leading to the description of the new species, *Cainocreadium dentecis* in *Dentex dentex*[52]. This emphasises the need for revision of previous records and the importance of coordinated morphological and molecular approaches.

The snail intermediate host for *C. labracis* can be *G. umbilicalis* and *G. adansonii*[37-39]. The life cycle of *C. labracis* thus involves a prosobranch snail as first intermediate host, where cercariae are produced, small rock-pool fish hosts harbouring the metacercariae and the demersal fish host, *D. labrax* as definitive host.

L. furcolabiatum is a parasite of the intestine and stomach of conger eel *C. conger*, rockling *C. mustela* and eel *A. anguilla*[46]. Cercariae of this digenean parasite were first known as *Cercaria vaullegeardii* Pelseneer, 1906[47,48,53]. Matthews[47,48] has published an extensive work on the development and ultrastructure of this cercarial type, collected from the first intermediate host, *G. umbilicalis*. Later, Gibson *et al.*[53] and Køie[49] have elucidated that this type corresponds to *L. furcolabiatum*. Our observations based on light and SEM are in accordance with those of Matthews[47,48]. The life cycle of *L. furcolabiatum* involves a snail host as the first intermediate host, *G. umbilicalis*, the second intermediate hosts, are benthic harpacticoid copepods, which harbour the metacercariae[48]. The third hosts are rock-pool fishes, and the final hosts are conger eel *C. conger* and rockling *C. mustela*[46,54]. Metacercariae of *L. furcolabiatum* were found infecting the mesenteries of blennies, *L. pholis* in the same location as we found the infected purple top shells[41], which indicated that in that particular location, *L. pholis* act as the third intermediate host.

Prevalence of *L. furcolabiatum* in the snail host is usually very low according to Matthews[47] (1.13%). This is in accordance with our findings, although as stated in the results section, we did not separate the prevalence of the infection with *C. labracis* from the prevalence with *L. furcolabiatum*. Digeneans infecting *L. littorea* were not identified to species level, because gonads were fixed and prepared for histology immediately after capture of specimens. Observations of the histological sections of infected gonads, showing replacement of gonadal tissue with masses of sporocysts with cercariae, suggested a decrease of the reproduction potential of the infected hosts (Figure 4A). Apparently, more than one species of digeneans infected the gonads of periwinkles in the present study (Figure 4B, C). Some of the cercariae possessing a tail structure could belong to the Microphallidae, which are common digenean parasites of this snail host, with crabs as second intermediate hosts and sea birds as definitive hosts[27,28,55].

Crabs and sea gulls were abundant in our sampling locations. The fact that we only found 3 infected periwinkles in 413 individuals (prevalence of infection = 0.7%) indicates that sampling effort should be increased in this sampling place. James[27] in his study of the occurrence of digeneans in periwinkles examined 6 165 snails to reach a 4.8% prevalence of infection, and Thieltges *et al.*[28] showed that increasing sample size effectively enhances the probability of finding infected snails. Low values of prevalence with digeneans are not unusual in the snail intermediate hosts[42,56]. Periwinkles, *L. littorea*, act as intermediate hosts for several trematode families, such as Echinostomatidae, Gymnophallidae, Heterophyidae, Microphallidae, Notocotylidae, Opecoelidae and Renicolidae[4,7,28]. Its digenean parasite fauna is very well documented and studied by several authors[4,7,27,57] (Table 1). The effects of digenean infections on *L. littorea* can be quite severe. For example, the infection with the digenean *C. lingua* can lead to a reduction in gonadal output and retardation of growth[5], and *R. roscovita* infections cause death of host snails[8]. For the digenean parasites, *C. lingua*, *H. elongata* and *R. roscovita*, for example, the periwinkle is the first intermediate host, where sporocysts and redia stages infect the digestive gland and gonads, whereas fish (for *C. lingua*) and mussels (for *H. elongata* and *R. roscovita*) are the second intermediate hosts harbouring metacercariae, and fish-eating birds are the end hosts[58-60]. Nevertheless, metacercariae of *R. roscovita* were found encysted in the visceral cavity of herring, *Clupea harengus*[61].

The parasite infestations can additionally reduce the overall resistance of molluscs to changing environmental conditions, such as temperature and salinity variations which occur in their habitat, the intertidal zone[5,13,62]. Experiments conducted by Lauckner on the temperature tolerance of *L. littorea* have shown that infected gastropods were less resistant to high temperature stress than uninfected ones[4]. Other authors observed that variations in prevalence of digeneans in the snail hosts were correlated with the density of the intermediate hosts as well as with the abundance of final hosts[63,64]. Furthermore, snails from higher shore levels were usually more heavily parasitized than those from lower levels[29,65]. This can be interpreted two fold: 1) snails from higher shore level are more susceptible to be parasitized because they are under environmental stress; or 2) as they inhabit higher shore level, they are easier prey for birds, which act as definitive hosts of a number of digenean species. Seasonal fluctuations in prevalence of digeneans also occur, either due to differential mortality or behavioural changes[14]. Experiments conducted on the Yorkshire coast (England) showed that periwinkles infected with *C. lingua* and *R. roscovita* moved significantly shorter distances than healthy individuals[66]. This reduced fitness was due to the damage inflicted by the parasites to the digestive gland and gonads, causing impairment of the host's vital functions, not due to the damage of the head-foot[57,58].

Purple top shells are also common intermediate hosts of a number of digeneans (Table 2). More than one species of *Lecithochirium* (*L. furcolabiatum*, *Lecithochirium rufoviride*) use this gastropod as the first intermediate host[47-49], and cotylocercous cercariae of the family Opecoelidae, namely *C. labracis* and *M. obovata* are found in this gastropod[37,39,40,45,52,67]. The two opecoelid cercariae, which do not possess a tail, have a sit-and-wait transmission strategy to find the next host, which in the case of *C. labracis* are gobiid fish, and in the case of *M. obovata* is another gastropod[37-39]. On the other hand, *L. furcolabiatum* cercaria has a complex tail structure, a cystophorous tail, which protects the cercariae from being destroyed by the mouth parts of the second intermediate host, a copepod, and helps to inoculate the infective portion into the crustacean haemocoel[48]. This mechanism of infection has been experimentally observed in other hemiurids, such as *Derogenes varicus*[68], and *Lecithocladium excisum*[69]. Although the pathological effect of the hemiurids in their snail hosts was not referred, the huge number of sporocysts filled with cercariae replacing the digestive gland and gonads of the host suggest an impairment of the host reproduction potential (see Køie[49] and present results). Some gastropod species appear to be more susceptible to infections with digeneans than others[70]. Pechenik *et al.*[70] studying three distinct snail species, *L. littorea*, *Ilyanassa obsoleta* and *Crepidula fornicata* for infections with digeneans, in Massachusetts and Rhode Island found that only the first two snail species were infected and the third one was uninfected. A literature and online search allowed him to conclude that digenean infections have been reported from only 10% of marine gastropod families. These results can be interpreted three fold: 1) not all gastropod species are susceptible to infections with digeneans; 2) there are still many species of gastropods that were not examined for digeneans; 3) many gastropods were already examined for digeneans and negative results were not published. Without question, digenean parasites are abundant in the intertidal zone, where they play an important role in the local ecology. They undergo different transmission pathways and have complex life cycles strategies, aiming at increasing their probabilities of success[71-75].

Molluscs can act either as the first or second intermediate hosts of digeneans and in some cases as the definitive hosts: 1) The periwinkle *L. littorea* acts as the first intermediate hosts for the digeneans, *C. lingua*[12,76]; 2) periwinkles acting as the second intermediate hosts for cercariae of *H. elongata*, *Himasthla littorinae*, *R. roscovita* and *Cercaria parvicaudata*[77]; 3) rarely gastropods can also have a role in the life cycle of digeneans as their final hosts. One example is the top shell *G. umbilicalis* acting as the final host for *Proctoeces progeneticus* from Atlantic coast of Morocco[78]. Nevertheless, common definitive hosts of digeneans infecting marine gastropods are fishes or sea birds and only rarely the snails are the definitive hosts[30,31,79].

Conflict of interest statement

We declare that we have no conflict of interest.

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