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The parasitism of *Flamingolepis liguloides* (Gervais, 1847) (Cestoda, Hymenolepididae) in *Artemia salina* (Crustacea, Branchiopoda) in two saline lakes in Algeria

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Abstract

Studies revealed the role of *Artemia salina* as intermediate host in the life-cycle of a cestode species parasitizing flamingos, i.e. *Flamingolepis liguloides*. Cysticercoids of this parasite were found for the first time in the Algerian populations of *Artemia salina* in winter of 2000 and 2001 in Chott Marouane and spring of 2003 in Sebkha Ez-Zemoul. The prevalence ranged between 10 and 33% for the two examined *Artemia* populations. The intensity of infection was 1–3 cysticercoids per individual. The abdomen was the most targeted site of infection (95% of the population of Sebkha Ez-Zemoul) followed by the thorax and the ovisac. Infected females were less fertile than uninfected ones (24.83 *vs* 43.70 cysts/brood) in Sebkha Ez-Zemoul or castrated in Chott Marouane.

Keywords

Cestoda, *Flamingolepis liguloides*, cysticercoid, *Artemia*, Chott, Sebkha, Algeria

Introduction

The crustaceans of the genus *Artemia* Leach, 1819 (Branchiopoda, Anostraca) are intermediate host of 14 cestode species (Georgiev *et al*. 2005, Vasileva *et al*. 2009). Among them, the genus *Flamingolepis* (Cyclophyllidea, Hymenolepididae) is represented by five species (Maksimova 1973) and *F. liguloides* (Gervais, 1847) is the most widespread.

Flamingolepis liguloides was found in several populations of *Artemia*: from Tengiz Lake in Kazakhstan (Maksimova 1973), the French Camargue (Thiéry *et al*. 1990, Robert and Gabrion 1991), Bonmati Salterns in Spain (Amat *et al*. 1991a, b) and in salt works of Sardinia (Mura 1995) and Andalucia (Georgiev *et al*. 2005, 2007). *Artemia* individuals filter water and consume cestode eggs containing a larva called oncosphere (20 μm) (Robert and Gabrion 1991). It penetrates through the intestinal wall into the hemocoel where it develops into a cysticercoid (larva with scolex) (Sanchez *et al*. 2007). The latter continues its life-cycle by becoming mature worm in the digestive tract of the final host (flamingo), which has been infected by predating infected *Artemia*. The adult parasites produce eggs, which are then released with faeces to the environment.

There is little information on the cestode infections and their pathogenic effect on *Artemia*, with the exception of reports by Maksimova (1973), and those by Gabrion *et al*. (1982), Thiéry *et al*. (1990) and Robert and Gabrion (1991). The latter authors studied the distribution of the parasites in various populations of *Artemia* from the French salterns and highlighted the role of brine shrimp as intermediate host of cestodes parasitizing flamingos. Amat *et al*. (1991a, b), Varó *et al*. (2000), Georgiev *et al*. (2005, 2007) and Sánchez *et al*. (2006, 2007, 2009) studied cestode parasitism in Spanish *Artemia* populations and their effect on the behaviour of the intermediate host.

Our investigations on the biology and the ecology of *Artemia salina* (L., 1758) from Chott Marouane (Amarouayache 2002) and Sebkha Ez-Zemoul (Amarouayache *et al*. 2004) revealed the presence of *F. liguloides* cysticercoids. We describe the first data on the parasitism in these populations in the present paper. The species identification of *A. salina* (unpublished data) has been performed by using scanning electron microscopy, i.e. by the lack of a spine at the basis of the penes (Triantaphyllidis *et al*. 1997). The site of cysticercoids in *Artemia* specimens, their effect on fecundity and some parasite infection indices are also approached.

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Materials and methods

Studied areas

The studied populations of *A. salina* are from Chott Marouane (34°02´N, 05°58´E) and Sebkha Ez-Zemoul (35°53´N, 06°27´E, altitude, 900 m) (Fig. 1). With an area of 350 km^2 and a depth of 9 m, Chott Marouane fills with water gradually starting from November and is drying up in May and the next several months (depending on the specific annual conditions). The salinity of the water is high and varies between 230 and 360 $g.l^{-1}$. Its avifauna is represented by Anatidae and particularly by flamingos *Phoenicopterus ruber roseus* (Pallas, 1811), of which 14,000 individuals were documented in 1999 (Anonymous 2001). Sebkha Ez-Zemoul has an area of 61 km^2 and a mean depth of 0.6 m. It is generally full of water from September to June and dries up, depending on the year, in July forward (Anonymous 2004). The salinity of the water varies between 30 and 255 $g.l^{-1}$. *Artemia* lives with 4 other crustacean: *Cletocamptus retrogressus*, *Phallocryptus spinosa*, *Moina salina* and *Heterocypris* sp. (unpublished data). Among birds occurring at Sebkha, the flamingo *P. ruber roseus* is the most common (Samraoui *et al*. 2006), with 1,200 to 1,400 individuals recorded between 2003 and 2004 (Saheb *et al*. 2006).

Sampling and examination

The samples of *A. salina* were collected in February 2000 and during all the wet period of 2001 (February, March and April) in Chott Marouane. In Sebkha Ez-Zemoul, the presence of cysticercoids was verified monthly during all the wet period between January 2003 and February 2005 (18 months). Samples of 1–10 l, according to *Artemia* density, were filtered by using 125 µm mesh plankton net and fixed in 4% formaldehyde. Observations and measurements were made using an optical microscope provided with an ocular micrometer (× 100). The parasites were identified according to the criteria suggested by Georgiev *et al*. (2005) and their site in the body of *Artemia* was recorded. Data on the Chott Marouane *Artemia* population concerned the prevalence in females (n = 135). In the population of Sebkha Ez-Zemoul, the prevalence, mean intensity and mean abundance were calculated (see Bush *et al*. 1997 for definitions). The uninfected and infected individuals of the same sample were measured from the top of the head to the anus. The offspring contents in the ovisac were counted in uninfected and infected females of the same sample. For statistical analysis, non parametrical *Z*-test was performed to compare prevalence between males and females. Mann-Whitney test (*W*-values) was used to compare mean intensity and mean abundance between the two sexes and mean fecundity between infected and uninfected females. Student *t*-test was used to compare between mean sizes of infected and uninfected individuals. A significance threshold was set at $P<0.05$ (Dagnélie 1975). Minitab software has been used for calculations.

Fig. 1. Geographical situation of the studied sites: Sebkha Ez-Zemoul (■), Chott Marouane (●)

Results

In both sites, only preadult and adult individuals of *A. salina* were infected by *F. liguloides* (Fig. 2), that fits to overall length higher than 4 mm in the population of Chott Marouane and higher than 6 mm in the population of Sebkha Ez-Zemoul. In *A. salina* specimens from Chott Marouane, cysticercoids were found at the beginning of the wet period, in February 2000 and 2001 and in March 2001, when monthly average salinity was at its minimum (233–287 $g.l^{-1}$). They were located in the abdomen, thorax or ovisac. The prevalence was 10, 11 and 13% in February 2000, February and March 2001, respectively. The infected females from Chott Marouane were completely unfertile, regardless of the site of the infection (abdomen, thorax or ovisac).

In Sebkha Ez-Zemoul, *A. salina* was infected only in April and May 2003 (50–70 $g.l^{-1}$), when the density was at its maximum (17–29 $ind.l^{-1}$). Prevalence, mean intensity and mean abundance of males and females are presented in Table I. The values found between April and May were similar. The prevalence was ranging between 17.14 and 33.33% for females and males, respectively. Generally, only one cysticercoid was found per individual, with a maximum of 3 in both sexes. No significant differences were found between infection indices of the two sexes ($Z = 1.64$, $P = 0.101$ for prevalence; $W = 105.5$, $P = –0.000$ for mean intensity and $W = 1,563.5$, $P = 0.0000$ for mean abundance). Mean body size was significantly bigger in infected males (9.12 ± 0.94 mm) than in uninfected ones (8.35

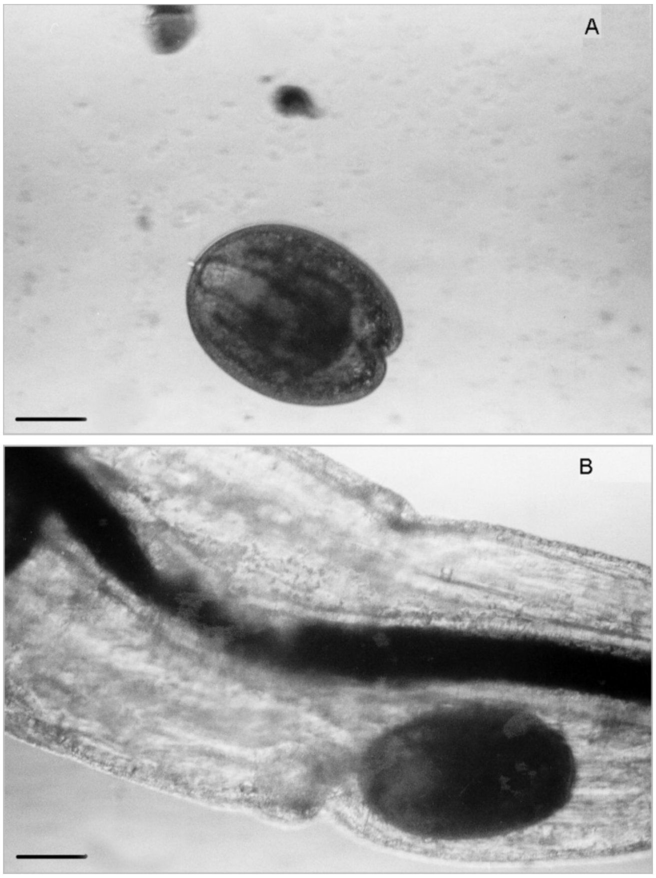

Fig. 2. Cysticercoids of *Flamingolepis liguloides* from *Artemia salina*: Isolated cysticercoid (**A**) and cysticercoid in the abdomen of the host (**B**). Scale bars = 150 µm (A) and 250 µm (B)

± 0.73 mm) (Student's *t*-test: 2.56, $P \leq 0.001$). This was not the case in females or when gathering the two sexes. The parasites were located in the abdomen (95%) and sometimes (males only) in the thorax (5%). The infected females were significantly less fertile than the uninfected ones, with mean numbers of offspring 24.83 ± 13.27 cysts/brood and 43.70 ± 18.61 cysts/brood ($W = 416$, $P = 17$), respectively.

Discussion

In the Mediterranean area, the cysticercoids of the hymenolepidids *F. liguloides* were identified for the first time in *Artemia* by Robert and Gabrion (1991). It was found in *A. salina* from Chott Marouane at the end of winter (February/March), whereas the population of Sebkha Ez-Zemoul was infected in spring (April/May) with relatively high prevalence (25%). This period coincides with the presence of flamingos in great numbers, attracted by the abundant crustaceans in Sebkha (personal observations). In the Camargue, three species of parasites of the genus *Flamingolepis* were identified together with *Gynandrotaenia stammeri* (family Progynotaeniidae), with a prevalence of 4.5% (Robert and Gabrion 1991). In Iberian Peninsula, several (5–10) cestode species were found in *Artemia* populations with very high prevalence (attaining 89%) and *F. liguloides* was the dominant species in most of the cases (Georgiev *et al*. 2007). Even if at first sight males seem to be more infected than females, probably because of the sex-ratio (2.79) being in favour of males in this period (Amarouayache *et al*. 2004), statistical data showed no significant differences between the two sexes. Georgiev *et al*. (2007) brought back the same conclusions for Iberian *Artemia* populations. Parasitic intensity is low in Algerian populations (less than 3 cysticercoids per individual). The intensity can reach 9 cysticercoids in Fos-Sur-Mer, 11 in Fangassier (France) (Thiéry *et al*. 1990), attaining 13 in the populations of the South-West of Spain (Georgiev *et al*. 2005). The accumulation of cysticercoids would be progressive with the age and thus the body size (Thiéry *et al*. 1990) and this increase in the parasite intensity should not kill the intermediate host in order to ensure the transmission to the final host (Parker *et al*. 2003). According to these authors, the strategy used generally by larvae of helminths is the reduction of their volume in such a way that the total volume of parasites is constant, regardless of the infection intensity, that seems to be a cooperative solution.

The cysticercoid location in the body of *Artemia* would be related to the volume of the hemocoel (Thiéry *et al*. 1990). In the present study, the cysticercoids were found in the abdomen (95%) and seldom in the thorax (5%) in the population of Sebkha Ez-Zemoul and also in the ovisac in the population of Chott Marouane. The same parasites were found inside the head in the population of Fos-Sur-Mer (Thiéry *et al*. 1990). In the saltworks of Sardinia, the cysticercoids occurred in the thorax of *Artemia* juveniles, whereas in adults, they were found in the abdomen (Mura 1995). According to Thiéry *et al*. (1990), cysticercoids provoke host size increase, and the modification of the cysticercoids distribution is related to allometric changes during growth, which is thoracic in 1st phase and becomes ab-

Table I. Infection indices and body size of infected and uninfected individuals (mean ± SD) from Sebkha Ez-Zemoul (values in parentheses represent number of individuals)

Parameters	Females	Males	Adults
Prevalence (%)	17.14	33.33	25.67
Intensity range	1–3	1–3	1–3
Mean intensity	1.50 ± 0.83 (6)	1.16 ± 0.57 (12)	1.28 ± 0.67 (18)
Mean abundance	0.26 ± 0.64 (35)	0.39 ± 0.64 (39)	0.33 ± 0.64 (74)
Mean body size of infected individuals (mm)	10.27 ± 1.26 (6)	9.12 ± 0.94 (13)	9.49 ± 1.15 (19)
Mean body size of uninfected individuals (mm)	10.16 ± 0.91 (29)	8.35 ± 0.73 (26)	9.32 ± 1.23 (55)

dominal in the old individuals (Amat 1980). Gigantism of infected individuals was also reported in the Spanish population from Odiel Marshes (Sanchez *et al*. 2009). In our case, this was only observed in infected males.

Among the effects of cestode parasitism in *Artemia*, the reduction in the reproductive capacity (Amat *et al*. 1991a) or castration are the most dramatic ones (Amat *et al*. 1991b). In the Iberian populations, infected females produced only 10–12 offspring/brood against 80 offspring in uninfected ones. This is a way to increase the profitability of the available energy by parasites (Amat *et al*. 1991a). In the population of Sebkha Ez-Zemoul, the infected females were almost twice less fertile than the uninfected ones, whereas those of Chott Marouane, living in harsh conditions of salinity, were completely infertile.

Many other effects and modifications are reported in infected *Artemia* individuals by cestodes, which make them more profitable to predators (Poulin 1995, de Jong-Brink and Koene 2005, Helluy and Holmes 2005, Sanchez *et al*. 2009). The red colour associated with modifications of feeding behaviour and carotenoids accumulation make hosts more visible by water birds (Thiéry *et al*. 1990, Amat *et al*. 1991a, Robert and Gabrion 1991, Varó *et al*. 2000, Sánchez *et al*. 2006). Amat *et al*. (1991b) found that the parasitized individuals have a higher lipid level, attain bigger size and live longer than the uninfected ones in order to increase the profitability of the prey, which would be a phenomenon of "favorisation" (Combes 1991). Parasites would have a positive effect on buoyancy and drive their host to swim on the surface of water (Thomas *et al*. 1997, Helluy and Holmes 2005) to facilitate the predation by the water birds (Curio 1988). According to Thomas *et al*. (1997), the role of the parasites is not only pathogenic since their presence among intermediate hosts is directly related to the accessibility of food for the predatory species and the density regulation of the infected communities. More efforts are required to understand the distribution and also the effect of the parasites and their role in Chotts and Sebkhas ecosystems in Algeria and other North African regions.

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