OCCURENCE OF THE PATHOGEN *PSOROSPERMIUM HAECKELI* (HILGENDORF) IN ASTACID POPULATIONS IN CROATIA

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ABSTRACT

Psorospermium haeckeli is a thick-walled unicellular organism widely reported in European astacid crayfish. It is typically referred to as a "parasite" although its pathogenic impact remains unclear. Until now there were no data about its presence in Croatian astacid crayfish. We examined 144 specimens of the three astacid species from 12 different locations and found sporocysts of *Psorospermium* in 13 preparations of epidermal connective tissue of *Astacus astacus* and *Astacus leptodactylus*. Preparations were made by scraping the epidermal-connective tissue from under the carapace, treating it with 10% KOH and viewing it under a microscope at 125×, 250× and 400× magnification. A relative scale was used to describe the degree of infection of the crayfish by *Psorospermium*. We found two types of mature sporocysts and also some developing stages of *Psorospermium*. In the *A. leptodactylus* we found many sporocysts that were fully melanised. Further investigations are needed to understand the distribution of infected crayfish in Croatia and patterns of their infection with *Psorospermium*.

Key words: *Psorospermium haeckeli*, *Astacus astacus*, *Astacus leptodactylus*, *Austropotamobius torrentium*, Croatia

APPARITION DU PATHOGÈNE *PSOROSPERMIUM HAECKELI* (HILGENDORF) DANS LES POPULATIONS D'ÉCREVISSES EN CROATIE

RÉSUMÉ

Psorospermium haeckeli est un organisme unicellulaire à parois épaisse très répandu chez les écrevisses européennes. *P. haeckeli* est considéré comme un « parasite » même si sa pathogénicité reste encore non clairement définie. Jusqu'à présent il n'y avait aucune donnée concernant sa présence chez les écrevisses en Croatie. Nous avons examiné 144 spécimens de trois espèces d'écrevisses provenant de 12 stations et trouvé des sporocystes de *P. Haeckeli* dans 13 préparations de tissu conjonctif épidermique prélevé sous la carapace, préparé avec du KOH 10 %, et observé sous microscope aux grossissements 125×, 250× et 400×. Une échelle relative a été utilisée pour décrire le degré d'infection des écrevisses par *P. haeckeli*. Nous avons trouvé deux types de sporocystes de *P. haeckeli* matures et quelques-uns en développement. Chez *A. leptodactylus* nous avons trouvé plusieurs sporocystes complètement mélanisés. De plus amples recherches sont nécessaires pour comprendre la distribution des écrevisses infectées en Croatie et le mode d'infection de *Psorospermium*.

Mots-clés : *Psorospermium haeckeli*, *Astacus astacus*, *Astacus leptodactylus*, *Austropotamobius torrentium*, Croatie.

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INTRODUCTION

Psorospermium haeckeli (Hilgendorf) is a thick-walled unicellular organism widely reported in European astacid crayfish (VOGT, 1999). Its taxonomic status has recently being elucidated; it is closely related to the similar organisms of aquatic pathobiology. They constitute the most basic branch of the Animalia provisionally named the DRIP clade according to the first letters of its member genera (*Dermocystidium*, *Ichthyophonus, Psorospermium* and rosette agent) (EVANS and EDGERTON, 2002). There are some speculations that DRIPs belong to the class Mesomycetozoea with *P. haeckeli* belonging to its lowest order of Ichthyophonida (MENDOZA, TAYLOR and AJELLO, 2002). At present, two morphotypes of *Psorospermium* sporocysts can be distinguished in European astacid on the basis of size, morphology and histology: an ovoid morphotype which includes numerous globules of heterogeneous size and an elongated morphotype which contains smaller and homogeneous globules (RUG and VOGT, 1995). *Psorospermium* is particularly abundant in the collagenous wall of the dorsal thoracic blood vessels and in the surrounding subepidermal connective tissue (VOGT and RUG, 1995). Compared to the other DRIP's *Psorospermium* seems to have very low pathogenicity (HENTTONEN, 1996) although it can interfere with the immune system of crayfish (THÖRNQVIST and SÖDERHAÄLL, 1993). Most publications dealing with *Psorospermium* describe these organisms as being relatively large (often over 100 µm long), oval or elongated, filled with globules of different sizes within an outer thick wall or capsule with a verticular pattern (BOSHKO, 1981; NYLUND, WESTMAN and LOUNATMAA, 1983). Many isolated observations of *P. haeckeli* have been reported in crayfish from different areas in Europe (HENTTONEN, HUNER and LINDQVIST, 1995; CERENIUS and SÖDERHÄLL, 1993; NOVOSELTCEV, PEPELYAEV and HENTTONEN, 1995; TAUGBØL and SKURDAL, 1992; BUCINSKIENE, 1996; VOGT, KELLER and BRANDIS, 1996; DIÉGUEZ-URIBEONDO *et al*., 1993). However, studies of the organism's distribution in the southeast of the continent are lacking. There were no data about its presence in astacid populations of Croatia. The objectives of this study were to investigate the possible presence of *Psorospermium* in some astacid populations in Croatia, and to determinate and describe the morphotype and life form, if possible. Twelve populations of the three native astacid species: noble crayfish *Astacus astacus* (Linne,1758), narrow–clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823) and stone crayfish *Austropotamobius torrentium* (Schrank, 1803) were screened for the presence/absence of *Psorospermium.*

MATERIAL AND METHODS

The presence/absence of *Psorospermium* in Croatian fresh waters was studied from 2001 to 2003. The majority of the collected crayfish came from the waters where other studies have been conducted at the same time and were fresh, living animals trapped during all seasons. Some examined specimens, mostly from streams and brooks, were collected during 1999 and were preserved in 70 % ethanol. Twelve collection sites are presented on the map (Figure 1) and listed in Table I. Each collection site was inhabited by only one crayfish species. Three astacid species were examined for presence/absence of *Psorospermium*: *Astacus astacus*, *Astacus leptodacylus* and *Austropotamobius torrentium*. Two species of the *Astacus* genera were trapped overnight by baited LiNi traps. Total lengths of *A. astacus* were from 5.35 to 14.2 cm while total lengths of *A. leptodactylus* were from 4.5 to 10.9 cm. Specimens of *A. torrentium* were collected by hand or trapped overnight by hand made small traps. Total lengths were from 5.5 to 11.1 cm.

Three pieces of epidermal and connective tissue under the carapace, per individual, were examined for *Psorospermium*. They were prepared by scraping the tissue from under the carapace, treating it with 10% KOH and pressing it slightly between a glass slide and a coverslip. Preparations were viewed under the microscope at 125×, 250× and 400× magnification. At 125× magnification, relative scale (0 = not present; + = 1-14 organisms per field of view; ++ = 15-60 organisms per field of view) was used to describe

Table I

Occurrence of *Psorospermium* in different waterbodies in Croatia during 1999-2003. The number of the waterbody corresponds to the number on the map (Figure 1). Proportion of *Psorospermium* is presented as the number of infected crayfish/the number of crayfish investigated. Collection dates are presented as month/year.

Tableau I

Présence de *Psorospermium* dans différentes eaux de Croatie entre 1999-2003. Le numéro des stations correspond au numéro sur la carte (Figure 1). La proportion de *Psorospermium* est présentée comme le nombre d'écrevisses infectées/le nombre d'écrevisses analysées. Les dates sont indiquées en format mois/année.

No.	Name of the waterbody	Collection month and/ or year	Species	Proportion infected by *Psorospermium*	Relative abundance
1.	River Drava - in Prelog	12/2001	*A. astacus*	2/2	++
2.	Lake Vukovina	04/2001 10/2001 10/2002 07/2003 08/2003	*A. astacus*	0/11 3/9 0/35 2/5 0/6	0 + 0 + 0
3.	Lake Crno	05/2001	*A. astacus*	1/4	+
4.	River Sava – Lonjsko Polje	05/2003 06/2003 07/2003	*A. leptodactylus*	0/3 3/5 2/10	0 + +
5.	Lake Bundek	03/2003	*A. astacus*	0/4	0
6.	Stream Mrzlak	1999	*A. torrentium*	0/9*	0
7.	Stream Sarni	05/1999	*A. torrentium*	0/2*	0
8.	Stream Veliki	05/2001	*A. torrentium*	0/10	0
9.	Stream Kraljevec	11/1999 05/2003 06/2003 07/2003	*A. torrentium*	0/1* 0/6 0/5 0/6	0 0 0 0
10.	Stream in Nature Park Plitvice	1999	*A. torrentium*	0/6*	0
11.	River Krapina	07/2001	*A. astacus*	0/4	0
12.	River Sava – Rajevo selo	07/2003	*A. leptodactylus*	0/1	0

Abbreviations:

* Specimens preserved in 70% ethanol.

Relative scale was used to describe abundance of the *P. haeckeli* in the subepidermal connective tissue at 125× magnification (0 = not present; + = 1–14 organisms per field of view; ++ = 15–60 organisms per field of view).

Abréviations :

* échantillon préservé dans 70 % d'alcool.

L'échelle relative a été utilisée pour décrire l'abondance de *P. haeckeli* dans le tissu sub-épidermal à l'agrandissement 125× (0 = non présent ; + = 1-14 organismes dans le champ de vue ; ++ = 15-60 d'organismes dans le champ de vue).

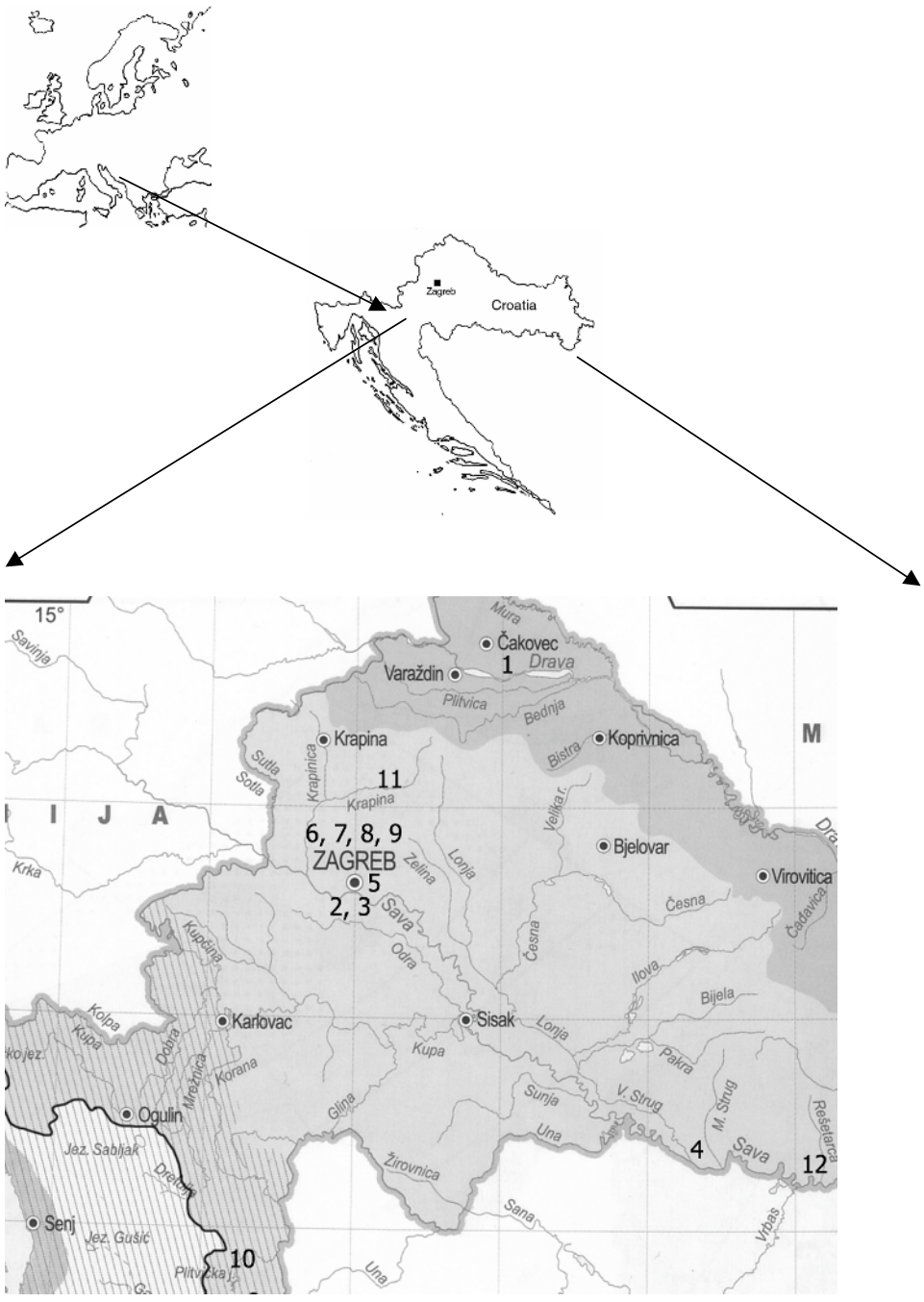


Figure 1

Location of the waterbodies where *Astacus astacus*, *Astacus leptodactylus* and *Austropotamobius torrentium* were collected from 1999 to 2003. In the waterbodies numbered 1-4 *Psorospermium* was present, in the waters number 5-12 *Psorospermium* was not found.

Figure 1

Localisation des stations où ont été prélevés les spécimens d'*Astacus astacus*, *Astacus leptodactylus* et *Austropotamobius torrentium* entre 1999–2003. Dans les stations 1 à 4 *Psorospermium* a été observé, dans les eaux 5 à 12 il n'y a eu aucune observation de *Psorospermium*.

parasite abundance per individual. Relative parasite abundance within the population was based upon the average abundance of parasite within all infected animals. Specimens of epidermal-connective tissue of *A. torrentium* preserved in 70% ethanol were viewed under the microscope without 10% KOH pretreament. The total number of crayfish examined was 144, of which there were 80 specimens of *A.astacus*, 19 specimens of *A. leptodactylus* and 18 ethanol-preserved/27 fresh specimens of *A. torrentium*.

RESULTS

Psorospermium was observed in 4 out of 12 investigated wild populations. Three of them were noble crayfish *A. astacus* populations from northwest Croatia and one was narrow-clawed crayfish *A. leptodactylus* population from the Sava River in the middle part of Croatia (Figure 1).

Of two examined specimens of noble crayfish from the Drava River, both were infected with the mature form of *Psorospermium* sporocysts with fully developed structures (Figure 2). They were "shuttle–shaped", quite long and narrow (approx. 140 µm long and 60 µm wide) and slightly angular. Three visible layers surrounded the sporocysts: an innermost double-membraned layer, the cell wall, which was thick and amorphous, and a thick outer layer of shell plates. The sporocyst contained some large and a few very small globules scattered inside the cell. In both specimens there were between 15 and 20 organisms per field of view.

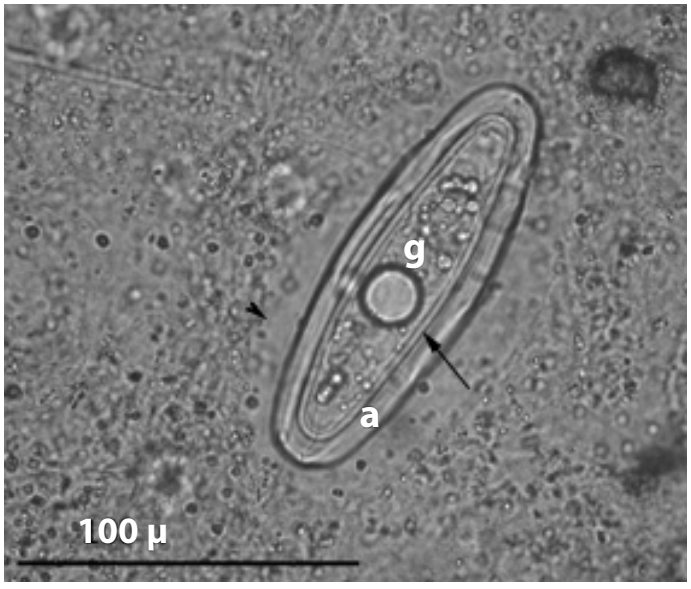


Figure 2

Elongated *Psorospermium* morphotype 2 found in the subepidermal connective tissue of *Astacus astacus* from the Drava River. Mature sporocyst with double-membraned layer (arrow), thick and amorphous layer (a), and thick layer of shell plates (arrowhead). Globules (g) of different sizes are scattered inside the sporocyst. Bar scale 100 µm.

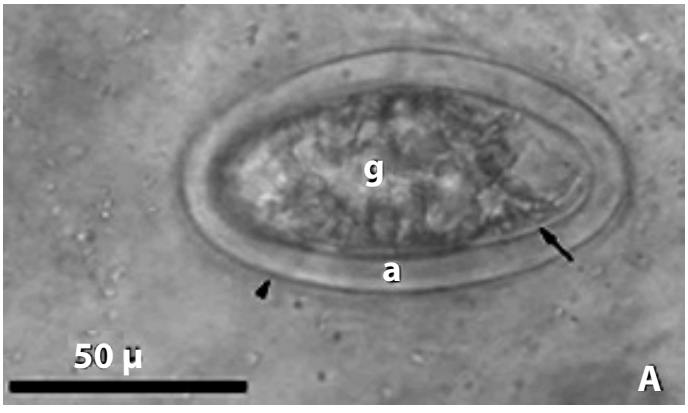
Figure 2

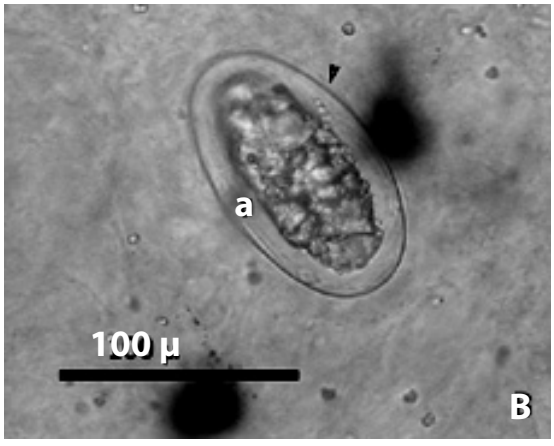
***P. haeckeli* de morphotype 2 de forme allongé trouvé dans le tissu conjonctif sub-épidermal d'*Astacus astacus* du fleuve Drava. Sporocyste mature avec la double couche membranaire (flèche), la couche épaise et amorphe (a) et la couche en feuillet épais (trait). Globules (g) de tailles différentes situés dans la cellule. Echelle 100 µm.**

Of 66 noble crayfish from the Vukovina Lake, only 5 specimens were infected with *Psorospermium*, and of 4 examined crayfish from the Crno Lake, one specimen was infected. Sporocysts from those samples were different from that previously described. We found mature sporocysts (Figure 3A) of ovoid shape (approx. 100 µm long and 50 µm wide) with the three characteristic layers. However, the outermost shell plate layer was much thinner than previously described. Globules of different sizes were mixed together inside the cell and they were not so prominent. Also, in crayfish trapped during July 2003 we noticed differentiating stages of *Psorospermium* sporocysts (Figure 3B), which were ovoid with two layers partly developed: thick medium amorphous layer and outermost thin layer of shell plates. Never were more then 5 organisms observed per field of view.

Of 19 examined narrow-clawed crayfish from the Sava River, 6 were infected with *Psorospermium*. Mature sporocysts (Figure 4A) were elongated and quite narrow (approx. 180 µm long and 70 µm wide), surrounded with the fully developed and visible three characteristic layers. The outermost layer was very thick and consisted of many shell plates. The cell was completely filled with numerous and uniform small globules or, in some cases, contained a few big and many small globules. When there were more than 10 sporocysts per field of view, many of them were melanised (Figure 4B). In one sample, taken in July 2003, we found differentiating stages of *Psorospermium* (Figure 4C), which were very similar to those, previously described for noble crayfish from the Vukovina Lake, but there were no mature sporocysts in that sample. In 5 cases there were between 5 and 10 organisms per field of view. Only one crayfish was more heavily infected and it had approximately 15 organisms per field of view.

Psorospermium sporocysts were not detected in crayfish from the other 8 locations. Moreover, no stone crayfish was found to be infected.





DISCUSSION

Our investigation represents the first survey for *Psorospermium* in Croatia. It shows that this parasite is present in two out of four native Croatian astacid crayfish (MAGUIRE, 2002). *P. haeckeli* was described in Central Europe in 1857 by HAECKEL (1857) and according to WIERZEJSKI (1888) it was found in many localities in Galizia (Poland) at the end of the 19th century. There were almost no data published about its presence in crayfish of southeast Europe or in countries neighbouring Croatia. HENTTONEN, HUNER and LINDQVIST (1997) examined one specimen of *A. leptodactylus* and one *A. astacus* from Hungary and failed to detect *P. haeckeli.* However, as we found *Psorospermium* in noble crayfish from the Drava River, which is bordering Hungary, we could assume that infection exists there but data are lacking. It is interesting that in those two specimens of noble crayfish we found the elongate *Psorospermium* morphotype that is frequently found in narrow-clawed crayfish (VEY, 1979) and only occasionally in noble crayfish (GRABDA, 1934). RUG and VOGT (1995) described two different morphotypes of *P. haeckeli* which may represent species: the ovoid *Psorospermium sp.* 1 which seems to be common in *Astacus astacus* and elongate *Psorospermium sp.* 2 which was usually found in *Astacus leptodactylus*. They, as well as other authors (NYLUND and WESTMAN, 1995), found that elongate *Psorospermium sp.* 2 morphotype could be mixed together with the ovoid *Psorospermium sp.* 1 morphotype in tissues of noble crayfish. However, in the two observed noble crayfish from the Drava River we found only elongate *Psorospermium sp. 2* morphotype. These sporocysts were slightly different from the typical elongated one described by RUG and VOGT (1995). Their globules were of different sizes, scattered around the cell and not as numerous (Figure 2). Due to the relatively high abundance of *Psorospermium* in noble crayfish from the Drava River (Table I), and the fact that both specimens were infected we could assume that this population has high infection rate. However, more detailed investigations are needed for confirmation.

The typical elongated *Psorospermium sp.* 2 morphotype was found in narrow-clawed crayfish from the Sava River in the middle part of Croatia. There are many reports of infection of *A. leptodactylus* by *P. haeckeli* (FÜRST and SÖDERHAÄLL, 1987; NOVOSELTCEV, PEPELYAEV and HENTTONEN, 1995; BUCINSKIENE, 1996). We found that 5 out of 19 observed crayfish were infected with elongated *Psorospermium sp.* 2 morphotype, typical for narrow-clawed crayfish (Figure 4A). This type of *Psorospermium*

◀ **Figure 3**

Mature and developing ovoid sporocysts of *Psorospermium* morphotype 1 found in the subepidermal connective tissue of *Astacus astacus* from the Vukovina Lake and Crno Lake. (A) Mature ovoid sporocyst of *Psorospermium sp.* 1 morphotype with double membraned layer (arrow), amorphous layer (a), and outermost thin shell plate layer (arrowhead). Less prominent globules (g) inside the sporocyst. Bar scale 50 µm. (B) Differentiating ovoid sporocyst with two layers: medium amorphous layer (a) and a thin layer of shell plates (arrowhead). Bar scale 100 µm.

Figure 3

Sporocystes ovoïdes matures et en développement de *Psorospermium* de morphotype 1, trouvés dans le tissu conjonctif sub-épidermal d'*Astacus astacus* dans les lacs Vukovina et Crno. (A) Sporocyste ovoïde mature du *Psorospermium sp.* Morphotype 1 avec une couche membranaire double (flèche), une couche amorphe (a) et une mince couche en feuillet (pointe de flèche). Globules (g) non-accentués dans la cellule. Echelle 50 µm. (B). Sporocyste ovoïde au stade avec deux couches : une couche médiane amorphe (a) et une couche en feuillet (pointe de flèche). Echelle 100 µm.

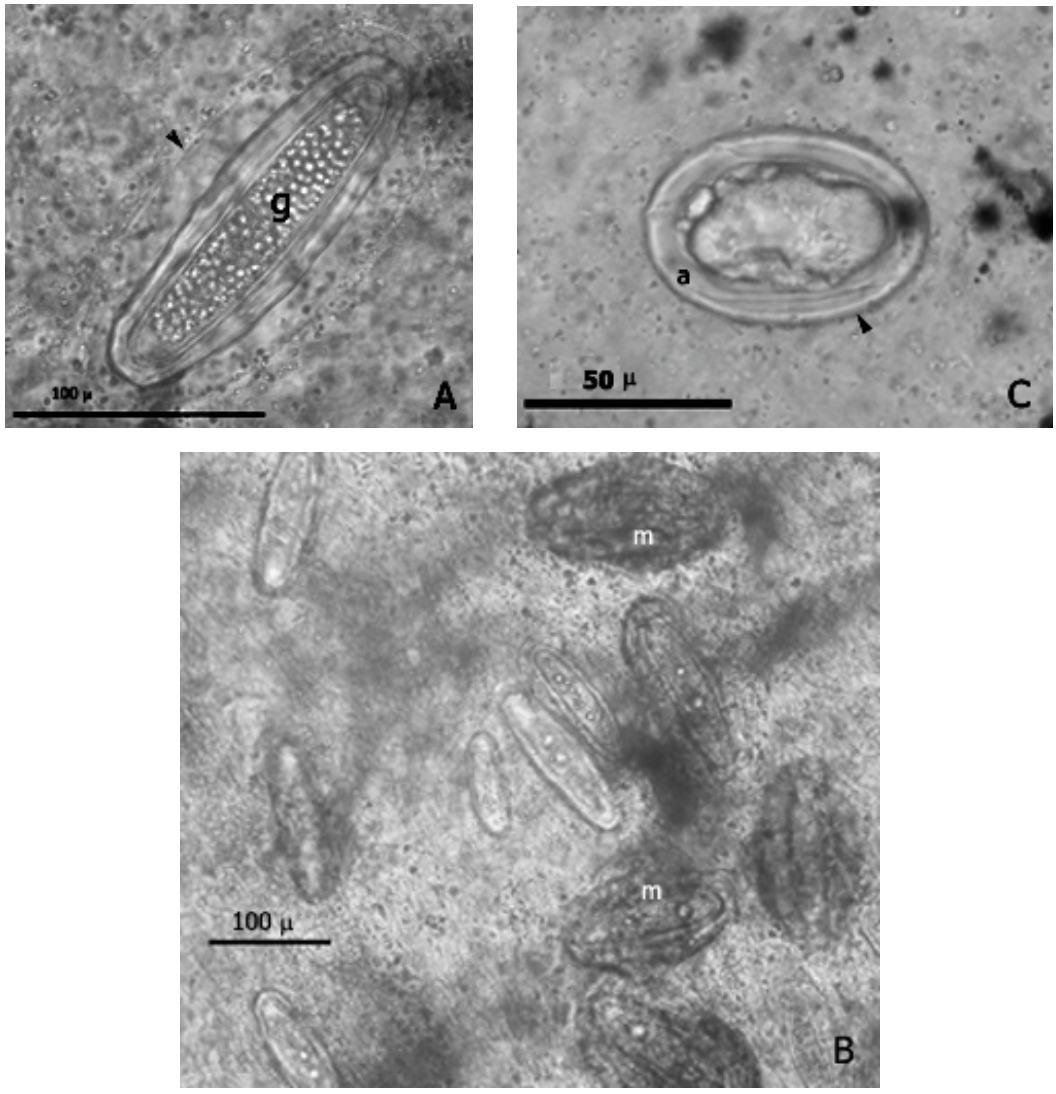


Figure 4

Mature and developing sporocyst of *Psorospermium* found in the subepidermal connective tissue of *Astacus leptodactylus* from the Sava River. (A) Elongated mature sporocyst of *Psorospermium sp.* 2 morphotype with a very thick layer of shell plates (arrowhead) and filled with many uniform small globules (g) Bar scale 100 µm. (B) Melanised *Psorospermium sp.* 2 morphotype sporocysts (m) Bar scale 100 µm. (C) Differentiating sporocyst of *Psorospermium sp.* 1 morphotype with an amorphous layer (a) and a shell plate layer (arrowhead). Bar scale 50 µm.

Figure 4

Sporocyste de *Psorospermium* mature et en phase de développement trouvé dans le tissu conjonctif sub-épidermal d'*Astacus leptodactylus* dans le fleuve Sava. (A) sporocyste mature allongé de *Psorospermium sp.* Morphotype 2, avec une très épaisse couche en feuillet (flèche), et rempli de petits globules (g). Echelle 100 µm. (B). Sporocystes mélanisés (m) de *Psorospermium sp*. 2 Echelle 100 µm. (C). Sporocystes de *Psorospermium sp.* de morphotype 1 en développement avec une couche amorphe (a) et une couche en feuillet (pointe de la flèche). Echelle 50 µm.

had a prominent envelope of connective tissue produced by the host consistent with the description given by RUG and VOGT (1995). In one heavily infected crayfish *Psorospermium* induced immune reactions including weak melanisation (Figure 4B). This is contrary to the findings of RUG and VOGT (1995) where only *Psorospermium sp*. 1 induced melanisation. Differentiating stages found in one crayfish sample were more similar to those of *Psorospermium sp*. 1 ovoid type described by VOGT and RUG (1999). However, as no mature sporocyst were observed in the sample it was not possible to determine if the crayfish was infected with the *Psorospermium sp*. 1 morphotype typical for noble crayfish.

Obviously, different morphotypes of *Psorospermium* are not exclusively present in particular astacid species which is consistent with findings of other authors (GRABDA, 1934; NYLUND and WESTMAN, 1995).

Noble crayfish from the two small and closely located gravel pits in the north-west of Croatia were also infected by the *Psorospermium*. Proportion of *Psorospermium* in the Vukovina Lake, where 66 crayfish were sampled, was not very high. Only 5 specimens were infected. Due to the very low abundance of the parasite in the subepidermal connective tissue of these crayfish (1–5 organisms per field of view) it is possible that others in the sample were infected at the level which did not allow detection by this method. In crayfish from both gravel pits, ovoid *Psorospermium sp*. 1 morphotype, described by RUG and VOGT (1995), was found. Typical sporocysts of ovoid shape, with a thin outermost shell plates layer and globules of different sizes, were found in many *A. astacus* populations in Scandinavian countries (HENTTONEN, HUNER and LINDQVIST, 1994; TAUGBØL and SKURDAL, 1995; CERENIUS and SÖDERHAÄLL, 1993). Our findings confirm that the ovoid *Psorospermium* is a typical morphotype for *A. astacus.* Differentiating stages of *Psorospermium* found in tissue samples of crayfish from the Vukovina Lake (Figure 3B) were also of *Psorospermium sp.* 1 type and similar to those described by VOGT and RUG (1999).

Although *P. haeckeli* was detected in the stone crayfish *A. torrentium by other authors* (VEY, 1979), especially when it coexisted with the noble crayfish *A. astacus* (VOGT, KELLER and BRANDIS, 1996) we have never found infected specimens in this species. One reason could be that many tissue specimens were not fresh but were preserved in ethanol. However, such specimens were used by Spanish authors (DIÉGUEZ-URIBEONDO *et al*., 1993) and resulted in positive findings of *Psorospermium*. On the other hand, as we found no evidence of infection in fresh specimens, we could assume that the stone crayfish populations studied were not infected with the *Psorospermium*.

CONCLUSION

This investigation confirmed that *Psorospermium* does infect astacid populations in Croatia. *Psorospermium* was detected in *A. astacus* and *A. leptodactylus*, and not in *A. torrentium*. Two characteristic and earlier described morphotypes of *Psorospermium* sporocysts were found: ovoid and elongated morphotypes. Both morphotypes were present in noble crayfish and narrow-clawed crayfish but were never present in the same crayfish sample.

It is difficult to make any kind of conclusions on the causes of the distribution of *Psorospermium* in Croatia observed in this study. Firstly, the data collected were the first of their kind and more data are required. Secondly, it is still unknown how the parasite is spreading between crayfish and research is required to determine how distribution is correlated with other population parameters and with hydrodynamics. Finally, there is no data on the occurrence of *Psorospermium* in neighbouring countries.

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