Community structure of metazoan parasites of silverside, *Odontesthes bonariensis* (Pisces, Atherinopsidae) from Argentina

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ABSTRACT. The helminth communities of silverside, *Odontesthes bonariensis* (Valenciennes, 1835), from two Argentinean lagoons were studied and compared at component community and infracommunity levels. Nine helminth species were found: five digeneans (*Austrodiplostomum* **cf**. *mordax*, *Ascocotyle (Phagicola)* **cf**. *diminuta*, *Ascocotyle* sp., *Thometrema bonariensis* and *Saccocoelioides* sp.); two nematodes (*Contracaecum* sp. and *Hysterothylacium* sp.); one acanthocephalan (*Wolffhugelia matercula*) and one cestode (*Cangatiella macdonaghi*). *Odontesthes bonariensis* is a new host record for five parasite species. Richness, diversity and number of helminths in silversides from Salada Grande lagoon were higher than in those from Lacombe lagoon. This could be related with lagoon size, abundance of mollusks and fish-eating birds, and size and diet of silversides captured in each lagoon. In Salada Grande lagoon the helminth community of silversides was dominated by the allogenic and generalist species *A.* **cf**. *mordax*; while the autogenic and intermediate specialist species *C. macdonaghi* was dominant in Lacombe lagoon. Host sex did not affect richness, diversity or total abundance, whereas host size was positively correlated with these attributes, except diversity in Salada Grande lagoon.

KEYWORDS. Helminth community, infracommunity, Platyhelminthes, Nematoda, Acanthocephala.

RESUMEN. Estructura de la comunidad de metazoarios parazitos del pejerrey, *Odontesthes bonariensis* (Pisces, Atherinopsidae) from Argentina. Las comunidades de helmintos del pejerrey, *Odontesthes bonariensis* (Valenciennes, 1835), procedentes de dos lagunas argentinas fueron estudiadas y comparadas a nivel de comunidad componente e infracomunidad. Se hallaron nueve especies de helmintos, cinco digeneos (*Austrodiplostomum* **cf**. *mordax*, *Ascocotyle (Phagicola)* **cf**. *diminuta*, *Ascocotyle* sp., *Thometrema bonariensis* y *Saccocoelioides* sp.); dos nematodes (*Hysterothylacium* sp. y *Contracaecum* sp.); un acantocéfalo (*Wolffhugelia matercula*) y un cestode (*Cangatiella macdonaghi*). *Odontesthes bonariensis* es reportado como un nuevo hospedador para cinco de estas especies. La riqueza específica, la diversidad y la carga parasitaria fueron mas elevadas en la laguna Salada Grande que en la Laguna Lacombe. Esto podría estar relacionado con atributos del cuerpo de agua (tamaño, abundancia de moluscos y aves ictiófagas) y del hospedador (talla y dieta de los pejerreyes capturados en cada laguna). En la laguna Salada Grande, la comunidad de helmintos fue dominada por una especie alogénica y generalista, *A.* **cf**. *mordax*; mientras que en la laguna Lacombe fue dominada por un a especie autogénica y especialista intermedia, *C. macdonaghi*. El sexo del hospedador no estuvo correlacionado con la riqueza específica, diversidad y carga parasitaria, mientras que la talla del hospedador presentó correlación positiva con estos atributos, excepto la diversidad en la laguna Salda Grande.

PALABRAS-CLAVE. Comunidad de helmintos, infracomunidad, Platyhelminthes, Nematoda, Acantocephala.

The silverside, *Odontesthes bonariensis* (Valenciennes, 1835) is one of the most important freshwater commercial and sport fishing resources in the Pampean region of Argentina. It is the species most used for restocking due to its high adaptability and economic importance, and it has been introduced in numerous freshwater environments in Argentina and other countries (Japan, Italia, Peru, Bolivia and Chile) (Grosman, 2001).

Silversides are zooplanktivores, although capable of exploiting other habitat resources such as insects, mollusks, other invertebrates and fishes, and even cannibalism, when plankton is scarce (Escalante, 2001).

Previous surveys of parasites of silversides from Argentina have focused mainly on taxonomy. At present, the records of helminths in this host species consist of metacercariae of *Austrodiplostomum mordax* Szidat & Nani, 1951 and *Tylodelphys destructor* Szidat & Nani, 1951 (Digenea-Diplostomidae) found in the brain; metacercariae of Heterophyidae (Digenea) in gills; third-stage larvae of *Contracaecum* sp. (Nematoda-Anisakidae) in the body cavity, adults of *Cangatiella macdonaghi* (Szidat & Nani, 1951) (Cestoda-Proteocephalidae) and the monogeneans *Dactylogyrus* sp. (Dactylogyridae) and *Gyrodactylus* sp. (Gyrodactyldae) in the intestine (Mac Donagh, 1932; Szidat & Nani, 1951; Szidat, 1969; Gil de Pertierra & Viozzi, 1995; Mancini *et al*., 2006). A few studies were focused on helminth populations of silverside (Mancini *et al*., 2008), however, no previous studies have focused on the parasite communities of this fish species.

The goal of this work is to analyze the helminth community of *O. bonariensis*, at the component and infracommunity levels, in two lagoons from Buenos Aires Province, Argentina.

MATERIALS AND METHODS

The fish were sampled in two sites in Buenos Aires Province, Argentina: Salada Grande lagoon (SGL), General Lavalle (36º55'S, 56º58'W) and Lacombe lagoon (LL), Lezama (35º49'S, 57º49'W). The SGL has a surface area of 6,078 ha and LL has a surface area of 140 ha. A total of 653 silversides were captured between April 1996 and May 1998; 262 in SGL [fall 1996 (n=22), winter 1996 (n=31), spring 1996 (n=29), summer 1997 (n=30), fall 1997 (n=30), winter 1997 (n=30), spring 1997 (n=30), summer 1998 (n=60)] and 391 in LL [winter 1996 (n=26), spring 1996 (n=31), summer 1997 (n=30), fall 1997 (n=32), winter 1997 (n=29), spring 1997 (n=65), summer 1998 (n=94), fall 1998 (n=84)]. The fish were captured using coastal net and serial gill-net. Each individual was sexed by direct

examination of gonads (102 males, 128 females in SGL; 135 males, 117 females in LL; juveniles could not be reliably sexed due to insufficient gonad development) and measured, 3.8-46.8 cm (22 ± 8.8) standard length in SGL and 1.7-40.8 cm (16.4 ± 7.4) in LL. The Student "t" test was used to verify the existence of significant differences among standard length of silversides from both lagoons. The 10% of the hosts was examined in the field for helminths, which were removed for taxonomic study. The rest of hosts were fixed in formalin 10%, except the specimens larger than 15 cm of standard length, which were dissected in the field, their heads and viscera were fixed in 10% formalin and examined in the laboratory. Digeneans, acantocephalans and cestodes were stained with a 1:6 dilution in 96% ethanol of hydrochloric carmine, dehydrated and mounted in Canada balsam. Nematodes were cleared in lactophenol or glycerine. The helminths studied were deposited in the Helminthological Collection of Museo de La Plata (MLP), La Plata, Argentina (MLP 3977-3985, 4069-4268, 4374-4549, 4557-4561, 4591-4597, 5286-5287, 5293-5295). The food items were identified under a stereoscopic microscope. The results were expressed as relative frequency of occurrence (FO=number of samples with occurrence of a given food item / number of samples analyzed).

Ecological terminology follows Bush *et al.* (1997) and the specificity was classified according to Desdevises *et al*. (2002). The description of infracommunity structure was based on helminth species richness, mean number of parasites, diversity, evenness, and dominance. Shannon's Diversity Index (H) was calculated using base 10 logarithms, and the Berger-Parker index of dominance was calculated following Magurran (1988). All helminth species, irrespective of their rareness in helminth faunas, were considered for the calculation of these attributes. Possible effect of host sex on helminth species richness, diversity and total abundance was assessed using Mann-Whitney test (U), and possible influence of host standard length was tested by Spearman's rank correlation coefficient (r_s). A Kruskal-Wallis test (KW) was used to compare mean values of richness, diversity and number of helminth per infracommunity among seasons. The correlation between standard length of silversides and number of metacercariae of *A*. **cf**. *mordax* was tested by Spearman's rank correlation coefficient (r_s).

RESULTS

Composition of the helminth community. Nine species of helminths were found parasitizing *O. bonariensis*, five of them as larval stages. Five species were found in silversides from both lagoons: *A*. **cf**. *mordax*, *C. macdonaghi, Wolffhugelia matercula* Mañé Garzón & Dei-Cas, 1974 (Acanthocephala-Neoechinorhynchidae), *Contracaecum* sp. and *Hysterothylacium* sp. (Nematoda-Anisakidae). Four species were found exclusively in silversides from SGL: *Thometrema bonariensis* Lunaschi, 1988 (Digenea-Derogenidae), *Saccocoelioides* sp. (Digenea-Haploporidae), *Ascocotyle (Phagicola)* **cf**. *diminuta* (Stunkard & Haviland, 1924) and *Ascocotyle* sp. (Digenea-Heterophyidae). Three digenean species, one nematode species and one acantocephalan species are reported for the first time in *O. bonariensis* (Tab. I). The highest prevalence values were observed for *Ascocotyle* sp. and *C. macdonaghi* in silversides from SGL and LL, respectively; while the most abundant species were *A*. **cf**. *mordax* and *C. macdonaghi* in SGL and LL, respectively (Tab. II).

Colonization strategy. All helminth species found in this study have complex life cycles with at least

Tab. I. Helminth parasites found in *Odontesthes bonariensis* from Salada Grande and Lacombe lagoons.

	Infection site	Development stages	Colonization strategy
Digenea			
Austrodiplostomum **cf**. *mordax*	brain (meninges, ventricles), optic nerve and spinal chord.	metacercariae	allogenic
Ascocotyle (P.) **cf**. *diminuta**	gills	metacercariae	allogenic
Ascocotyle sp.	heart (bulbus arteriosus)	metacercariae	allogenic
*Thometrema bonariensis**	intestine	adults	autogenic
Saccocoeliodes sp.*	intestine	immature	autogenic
Cestoda			
Cangatiella macdonaghi	intestine	adults	autogenic
Nematoda			
Hysterothylacium sp.*	mesentery	L_3	autogenic
Contracaecum sp.	mesentery	L_3	allogenic
Acanthocephala			
*Wolffhugelia matercula**	intestine	immature	autogenic

* New host records

Tab. II. Prevalence (P), mean intensity (IM) and mean abundance (AM) of helminths in *Odontesthes bonariensis* from Salada Grande and Lacombe lagoons.

	SGL (n = 262)			LL (n = 391)		
	P (%)	IM (range)	AM (X ± sd)	P (%)	IM (range)	AM (X ± sd)
Digenea						
Austrodiplostomum **cf**. *mordax*	62	184 (1-1871)	114 ± 276	24	60 (1- 481)	14.3 ± 49
Ascocotyle (P.) **cf**. *diminuta*	89	17.4 (1-66)	15 ± 13	-	-	-
Ascocotyle sp.	98	7.6 (1-25)	7.5 ± 5.7	-	-	-
Thometrema bonariensis	2.3	4.7 (1-8)	0.11 ± 0.8	-	-	-
Saccocoeliodes sp.	0.8	47 (2-92)	0.36 ± 5.7	-	-	-
Cestoda						
Cangatiella macdonaghi	61	152 (1-3387)	94 ± 304	50	78 (1-1606)	39 ± 127
Nematoda						
Hysterothylacium sp.	2.3	1 (1-1)	0.02 ± 0.1	2	1.1	0.02 ± 0.17
Contracaecum sp.	41	3.3 (1-30)	1.38 ± 3.4	19	2.9	0.6 ± 2.2
Acanthocephala						
Wolffhugelia matercula	1.9	2 (1-3)	0.04 ± 0.3	2	1.3	0.02 ± 0.17

one intermediate host; four species are allogenic and five autogenic (Tab. I). The autogenic species represents 41% of the helminths found in SGL and 73% of helminths found in LL.

Diet. The diet of *O. bonariensis* in both lagoons consisted primarily of microcrustaceans (Copepoda and Cladocera), while others items were rare (Tab. III). However, higher variety of food items was found in the silversides from SGL.

Component community structure. Table IV shows summarized data on helminth component community structure. Silversides from SGL showed higher helminth species richness and diversity compared to those from LL. Evenness was nearly equal for both lagoons, with very low values. The Berger Parker index was higher in LL, due to the high abundance of *C. macdonaghi* that represented 73% of the total number of helminths.

Infracommunity structure. Table V shows the results of the analysis of composition of the infracommunities. At site LL, 34% of the silversides were uninfected, whereas all silversides caught at SGL harbored at least one helminth species. In SGL, individual fish harbored between 1 and 6 (most frequently 4) helminth species, the maximum species richness (9) was not reached, and *A*. **cf**. *mordax* was the most abundant species in the infracommunities. In LL, individual fish harbored between 1 and 3 (most frequently 1) helminth species, the maximum richness (5) was not reached, and *C. macdonaghi* was the most abundant species in the infracommunities.

Host size (standard length) was positively and significantly correlated with helminth species richness, diversity and total abundance, according to Spearman's rank correlation coefficient, with the exception of diversity in SGL (Tab. VI). Helminth species richness, diversity and total abundance were not significantly correlated with sex of the silversides examined (Tab. VI).

Infracommunity diversity (H) was significantly higher in SGL than in LL ($U=6496$, $p < 0.001$), even when uninfected individuals were taken into account ($U=7094$, $p < 0.001$).

The seasonal differences in mean values of richness, diversity and number of helminths per

Tab. III. Relative frequency of occurrence of food items (FO) in the diet of *Odontesthes bonariensis* from Salada Grande and Lacombe lagoons.

Food items	SGL	LL
Microcrustaceans (Cladocera and Copepoda)	95.9%	98.4%
Macrocrustaceans (*Palaemonetes argentinus*)	1.8%	1.1%
Fishes (*Bryconamericus* sp. and *Astyanax* sp.)	0.7%	0.5%
Mollusks (*Heleobia parchappei*)	0.8%	-
Ostracods	0.5%	-
Chironomids larvae	0.2%	-
Plant parts	0.1%	-

Tab. IV. Comparison of helminth community in *Odontesthes bonariensis* from Salada Grande and Lacombe lagoons.

	SGL	LL
Number silversides	262	391
Number helminths	60978	20947
Richness	9	5
H	0.46	0.28
Evenness	0.15	0.12
Dominant species	*A*. **cf**. *mordax*	*C. macdonagh*
Berger Parker index	49%	73%
Autogenic species	5	2
Allogenic species	4	3

infracommunity (Figs 1-3), were not significant (KW=1.3, p=0.7; KW=1, p=0.8; KW=7, p=0.43 in SGL, and KW=7, p=0.43; KW=7.5, p=0.48; KW=6.9, p=0.3 in LL).

DISCUSSION

Some of the helminth species found are not habitual members of the helminth communities of *O. bonariensis*, but occur regularly in other freshwater fishes from other bodies of water in Buenos Aires Province. These include *T. bonariensis*, a common parasite of *Cichlasoma facetum* (Jenyns, 1842) (Lunaschi, 1988); *W. matercula*, commonly found as parasite of *Cnesterodon decemmaculatus* (Jenyns, 1842) and *Jenynsia lineata* (Jenyns, 1842) (Lunaschi & Drago, 1995), and *Saccocoelioides* sp., a genus with species reported from numerous freshwater fishes (Szidat, 1973; Lunaschi, 1984, 2002; Martorelli, 1986). At the infracommunity level, the presence of these species does not contribute significantly to the evaluation of species richness and mean number of individuals due to their low prevalence and abundance (Tab. II). In addition, *W. matercula* and *Saccocoelioides* sp. do not reach sexual maturity when parasitizing *O. bonariensis*. Similar results were found in the population of *Hyphessobrycon meridionalis* Ringuelet, Miquelarena & Menni, 1978 from Saladita lagoon, Buenos Aires Province, where *W. matercula* do not reach sexual maturity, in despite being present throughout the year (Drago, 1997).

Helminth assemblages in fish are generally depauperate and isolationist compared with those in endothermic vertebrates; factors such as ectothermy, low vagility and structural simplicity of the gut, among others, have been cited as possible reasons for these differences (Kennedy *et al*., 1986; Kennedy, 1990). In addition, the freshwater habitats are essentially isolated systems, in which the fish may move more or less freely within them, but natural movements of fish and their parasites between systems necessitates overcoming barriers are thus more limited and infrequent (Kennedy *et al*., 1991).

Richness and diversity of helminths of *O. bonariensis* were higher in SGL, despite the smaller sample size in this lagoon. This could be related with several factors: (1) the larger size of SGL and of the silversides collected in this lagoon (significant differences were found between the standard length of silversides from both lagoons; t=8.4; p <0.001). The influence of the size of aquatic environments has been discussed by several authors. According to the island-size hypothesis (island size being defined as the area of an aquatic environment, number of hosts within a population, or the size of an individual host) large islands should contain more parasitic species (Holmes & Price, 1986). Nevertheless, conflicting results have been obtained from this approach (Marcogliese & Cone, 1991). Some authors have argued that larger areas may sustain larger host populations and thus may favour the existence of higher parasite diversity (Kennedy, 1978). Moreover, localities with high diversity of host species may be more favourable for parasites with complex life cycles, because of the availability of more definitive host species in which the parasites can achieve full development (Guégan *et al*., 2005); (2) greater abundance of mollusks and fish-eating birds in SGL, which increases the chances of allogenic species such as, *A. (P.)* **cf**. *diminuta* and *Ascocotyle* sp.; (3) the greater variety of food items found in the gut of silversides captured in SGL; which increases the chances of autogenic species such as *T. bonariensis* and *Saccocoelioides* sp., that were found only in hosts that had gastropods [*Heleobia parchappei* (d'Orbigny, 1835)] in the gut. The life cycles described for other derogenids and haploporids indicate that fishes become parasitized when they eat infected snails (Martorelli, 1986, 1989).

Tab. V. Comparison of helminth infracommunities in *Odontesthes bonariensis* from Salada Grande and Lacombe lagoons.

	Salada Grande lagoon	Lacombe lagoon
Number of silversides	262	391
Richness	3.59 ± 1.10	0.95 ± 0.88
Mean number of parasites	233 ± 409	53.57 ± 139.17
H	0.31 ± 0.18	0.04 ± 0.09
Evenness	0.01± 0.005	0.002 ± 0.01
Berger–Parker Index	0.71 ± 0.19	0.94 ± 0.12
Dominant species	*A.* **cf**. *mordax*	*C. macdonaghi*
Uninfected fishes	-	34.0%
Percent of coexistent helminth species		
1 species	3.4%	44.0%
2 species	14.1%	14.6%
3 species	25.2%	7.4%
4 species	36.3%	-
5 species	19.5%	-
6 species	1.5%	-
7, 8, 9 species	-	-

Tab. VI. Association of richness, H and number of helminths, with standard length and sex of hosts in Salada Grande and Lacombe lagoons. (r_s = values of Spearman's rank correlation coefficient and U= values of Mann-Whitney test).

		Richness	H	Number of helminths
SGL	r_s	0,59**	-0,02 ns	0,64**
	U	5181 ns	4669 ns	4900 ns
LL	r_s	0,56**	0,54**	0,53**
	U	7529 ns	7785 ns	7385 ns

** p < 0.01; ns: not significant

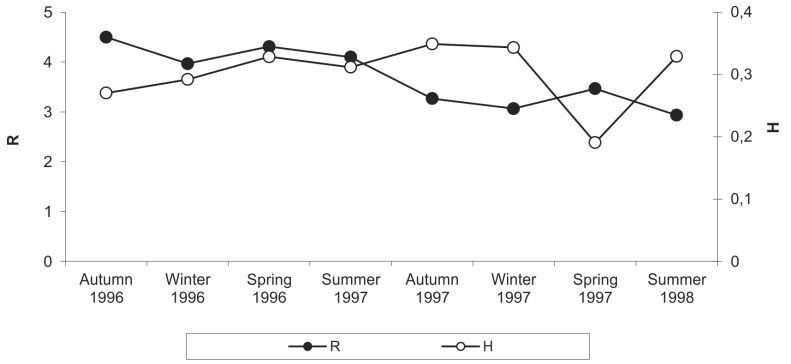

Fig. 1. Seasonal variations of mean richness (R) and mean diversity expressed as Shannon Index (H) per infracommunity in *Odontesthes bonariensis* (Valenciennes, 1835) from Salada Grande lagoon.

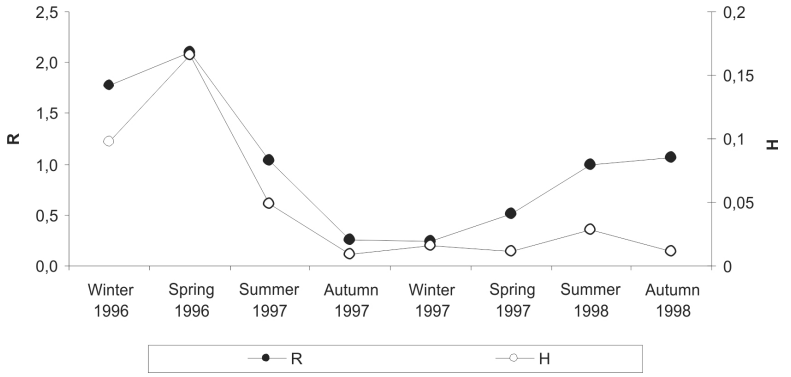

Fig. 2. Seasonal variations of mean richness (R) and mean diversity expressed as Shannon Index (H) per infracommunity in *Odontesthes bonariensis* (Valenciennes, 1835) from Lacombe lagoon.

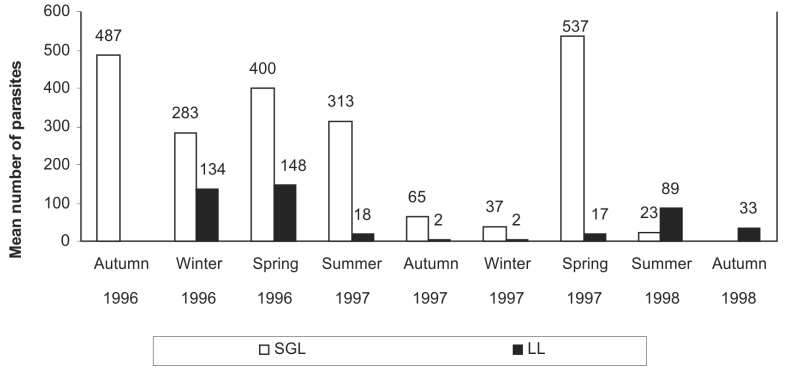

Fig. 3. Seasonal variations of mean number of helminths per infracommunity in *Odontesthes bonariensis* (Valenciennes, 1835) from Salada Grande and Lacombe lagoons.

The helminth community of silversides is dominated by the autogenic intermediate specialist species *C. macdonaghi* at the component and infracommunity levels in LL; while it is dominated by the allogenic generalist species *A.* **cf**. *mordax* in SGL. Is interesting to note that in LL the dominant species was too the most prevalent, but in SGL the dominant species was not the most prevalent. The specificity of dominant species has been studied in several communities of freshwater fishes; still, no clear pattern has been established because the dominant species may be specialist, generalist, or change across geographic regions for the same host species (KENNEDY, 1990, 1995, 1997; SURES *et al*., 1999; KENNEDY & HARTVIGSEN, 2000). The allogenic-autogenic condition of dominant species has been analyzed in several opportunities, and in spite of the supposed limitations of autogenic life-style, these species can dominate helminth communities (ESCH *et al*., 1988, LYNDON & KENNEDY, 2001). The differences regarding dominant species in both lagoons suggest that specificity and colonization strategy of silverside parasites do not represent important factors for community dominance. The dominance of the allogenic species *A.* **cf**. *mordax* in SGL could be attributed to the greater body size of silversides caught in this lagoon. Moreover, the standard length of silversides from both lagoons has positive correlation with the number of metacercariae of this species (r_s=0.8, $p < 0.001$ in SGL; r_s=0.57, $p < 0.001$ in LL).

In some host-parasite systems, community structure may be influenced by host size through changes in diet or volume of ingested food, ontogenetic changes in immunocompetency and changes in the probability of contact with intermediate hosts (Esch *et al*., 1990). The higher richness and number of helminths in the infracommunities from larger silversides could be related with the higher variety of food items consumed by these fishes, particularly gastropods and their distribution in the lagoon (larger fish inhabit deep waters while smaller individuals inhabit the coastal region). Moreover, the increased abundance of larval endoparasites in larger fishes can be attributed to the occurrence of cumulative infection processes (Isaac *et al*., 2000; Guidelli *et al*., 2003). These processes can explain the increasing number of helminths throughout the life cycle of silversides, especially for larval stages (*A*. **cf**. *mordax*, *A. (P.)* **cf**. *diminuta*, *Ascocotyle* sp., *Hysterothylacium* sp. and *Contracaecum* sp.).

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