DOI: 10.2478/s11686-014-0226-2

Acta Parasitologica, 2014, 59(1), 165–172; ISSN 1230-2821

VERSITA

Anisakids of seals found on the southern coast of Baltic Sea

Michał Skrzypczak[1], Jerzy Rokicki[1]*, Iwona Pawliczka[2], Katarzyna Najda[1] and Joanna Dzido[1]

[1]University of Gdansk, Faculty of Biology, Department of Invertebrate Zoology and Parasitology, Laboratory of Parasitology and General Zoology, Wita Stwosza 59, 80-308 Gdańsk, Poland;
[2]University of Gdansk, Institute of Oceanography, Hel Marine Station, Morska 2, 84-150 Hel, Poland

Abstract

In the present study 5 grey seals (*Halichoerus grypus*), 3 common seals (*Phoca vitulina*) and 1 ringed seal (*Pusa hispida*) by-caught or stranded on the Polish Baltic Sea coast in years 2000–2006 were investigated for the infestation of parasitic anisakid nematodes. 749 of anisakids were found. The most common were: *Contracaecum osculatum* (59.3%) and *Pseudoterranova decipiens* (31.0%). There were also small numbers of *Anisakis simplex* (0.8%). After performing RFLP three sibling species were found. *C. osculatum* was identified as *C. osculatum* C, *P decipiens* was identified as *P. decipiens* sensu stricto and *A. simplex* – *A. simplex* sensu stricto. Nematodes found in seals were mostly in L4 and adult life stage – both of them were equal with some minor variations among the specimens. Sex ratio was also equal, but there was slight excess of males in some cases. There was a minority of L3 larvae belonging to *A. simplex* species (0.8%).

Keywords

Anisakid nematodes, seal, Baltic Sea

Introduction

Parasitic nematodes belonging to family Anisakidae are important group of organisms that are capable of infecting marine animals. Over several decades, many studies about them were performed. There were also many studies covering the problems of their hosts including Pinnipedia and seals (Phocidae).

In Baltic Sea there are three seal species: grey seal (*Halichoerus grypus* (Fabricius, 1791)), ringed seal (*Pusa hispida* (Schreber, 1775)) and common seal (*Phoca vitulina* (Linnaeus, 1758)). The grey seals are found mainly in the central and northern part of the Baltic region (Harding *et al*. 2007). Ringed seals live mainly in the eastern parts of Baltic Sea – the Gulf of Riga and Gulf of Finland. Common seals can be found in the very western parts of the Baltic Sea. In the hosts mentioned above there were found three Anisakidae parasites: *Pseudoterranova decipiens* (Krabbe, 1878), *Contracaecum osculatum* (Rudolphi, 1802) and *Anisakis simplex* (Rudolphi, 1802).

The life cycle of those nematodes still remains not fully discovered. There are some important differences between the parasites that should be pointed. It is known that *P. decipiens* hatches in benthic and epibenthic habitats and is already in L3 larval stage (Køie *et al.* 1995, McClelland 2002). A larva adheres to the substrate of seabed by its caudal end (Klimpel and Palm 2011, McClelland 2002) and then it is ingested by tiny benthic crustaceans and penetrates into the host's body cavity. Secondarily, after infesting the crustacean, larva gains an ability to infect polychaets, molluscs (Martell and McClelland 1995, McClelland 2002) or fishes belonging to families: Gadidae, Pleuronectidae, Zoarcide, Clupeidae, Salmonidae and Cottidae (Grabda-Kazubska and Okulewicz 2005) that act as the paratenic hosts. The main vectors of this parasite in the southern Baltic Sea area may be cod (*Gadus morhua*) (Myjak *et al.* 1994, Sobecka *et al.* 2011) and – less likely – herring (*Clupea harengus*) (Szostakowska *et al.* 2005). Subsequently a specimen gains an ability of infesting *Phocidae* host where L3 larva moults into L4 stage and then – adult stage (McClelland, 2002).

An early stage development of *C. osculatum* is associated with pelagic environment. L3 free-swimming larva hatches from the egg and becomes ingested by an intermediate host like an amphipod or copepod. In contrast to two other nematode species mentioned in this article, a specimen after hatching may infect small fishes (Køie and Fagerholm 1995,

*Corresponding author: rokicki@univ.gda.pl

Rokicki 2006). Although the larvae of *C. osculatum* were rarely collected from herrings and cods in southern Baltic Sea, these fish species may be a major source of seals' infestations with this parasitic helminth (Myjak *et al.* 1994; Szostakowska *et al.* 2005). The seals are known to be definitive hosts where L3 larva moults into L4 stage and then becomes mature (Grabda-Kazubska and Okulewicz 2005).

A. simplex emerges from an egg as a vagile L3 larva (Køie *et al.* 1995). After being ingested by pelagic crustaceans – mainly krill (Euphasiacea) – in their haemocoel they develop L3 stage that is infective to paratenic hosts – fishes (herrings, mackerels) and cephalopods (Guz *et al.* 2005, Grabda-Kazubska and Okulewicz, 2005). On the contrary, Anderson (2000) suggests that L3 larva grows in the paratenic host. In the southern Baltic Sea it was proven that herring migrating from Danish Straits for spawning may be heavily infected with *A. simplex* larvae (Rokicki *et al.* 2009, Sobecka *et al.* 2011). This fish species seems to be main carrier of *A. simplex* that infects marine mammals. Final hosts of *A. simplex* are cetaceans (Klimpel *et al.* 2004, Rokicki 2006). The seals are only accidental hosts for *A. simplex* and maturation of worms does not take place.

Due to advances in molecular diagnostics it became possible to determine what sibling species are the anisakids of Baltic Sea and the neighbouring regions. In eastern Atlantic Ocean a presence of *P. decipiens* s.s. and *P. krabbei* among grey seals and common seals was confirmed (Aspholm *et al*. 1995, Mattiucci *et al.* 2007, Mattiucci and Nascetti 2007). It has also been discovered that *C. osculatum* C is a parasite of grey seal living in Baltic Sea (Mattiucci *et al.* 2007, Mattiucci and Nascetti 2007). *A. simplex* s.l. found in seals belongs in fact to sibling species *A. simplex* s.s. (Mattiucci and Nascetti 2007).

This research was performed in order to complete the data about the anisakid parasites infecting seals of Baltic Sea. There had been made an approach to determine species of nematodes mentioned above (complexes and sibling species), their life stages and genders among the seals found on the southern coast of Baltic Sea.

Materials and Methods

The nematodes' specimens that were identified had been collected from 9 seals bycaught (Hg39, Hg66, Pv80) or stranded (Hg71, HgI, HgS, Pv73, Pv88, Ph55) in the Polish Baltic coastal zone between years 2000 and 2006. There were: 5 grey seals (4 adults and 1 juvenile HgS), 3 common seals (all adults) and 1 ringed seal (adult). They were trans-

Table I. Numbers (and percentage) of anisakids found in the seals from southern Baltic Sea. The numbers are counts of complex species. The counts of sibling species are shown next to the number of the whole complex (if available). Hg – *Halichoerus grypus*, Pv – *Phoca vitulina*, Ph – *Phoca hispida*, C – *C. osculatum* C, s.s. – *P. decipiens* s.s or *A. simplex* s.s. (according to head of table)

Host Hg	*C. osculatum*		*P. decipiens*		*A. simplex*		Unknown	Total
71	**364** (61.7%)		**176** (29.8%)		**2** (0.3%)		**48** (8.1%)	**590**
66	**14** (87.5%)	C **10** (62.5%)	–		**2** (12.5%)	s.s. **2** (12.5%)	–	**16**
39	**10** (47.6%)		**9** (42.9%)		–		**2** (9.5%)	**21**
I	**14** (82.4%)		**1** (5.9%)		–		**2** (11.8%)	**17**
S	**17** (77.3%)		**3** (13.6%)		–		**2** (9.1%)	**22**
Total	**419** (62.9%)	C 10 (1.5%)	**189** (28.4%)		**4** (0.6%)	s.s. **2** (0.3%)	**54** (8.1%)	**666**
Pv								
88	**1** (50%)		**1** (50%)		–		–	**2**
80	**19** (27.5%)		**39** (56.5%)	s.s. **14** (20.3%)	**2** (2.9%)	s.s. **2** (2.9%)	**9** (13.0%)	**69**
73	–		–		–		**3** (100%)	**3**
Total	**20** (27.0%)		**40** (54.1%)	s.s. **14** (18.9%)	**2** (2.7%)	s.s. **2** (2.7%)	**12** (16.2%)	**74**
Ph								
55	**5** (62.5%)		**3** (37.5%)		–		–	**8**
Total Hosts	**444** (59.3%)	C **10** (1.3%)	**232** (31.0%)	s.s. **14** (1.9%)	**6** (0.8%)	s.s. **4** (0.5%)	**67** (8.9%)	**749**

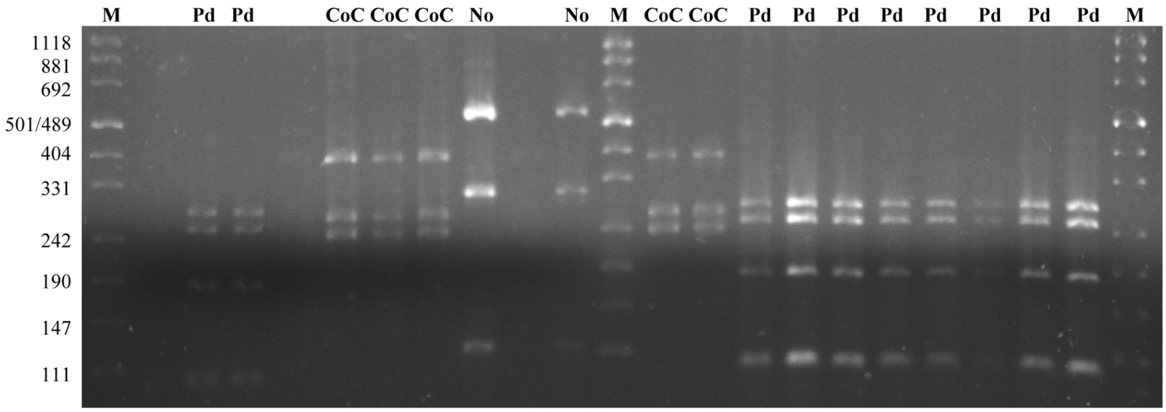

Fig. 1. The RFLP results with RsaI in use. CoC – *Contracaecum osculatum* C, Pd – *Pseudoterranova decipiens* s.s., No – unknown, M – A&A Biotechnology pUC Mix molecular marker

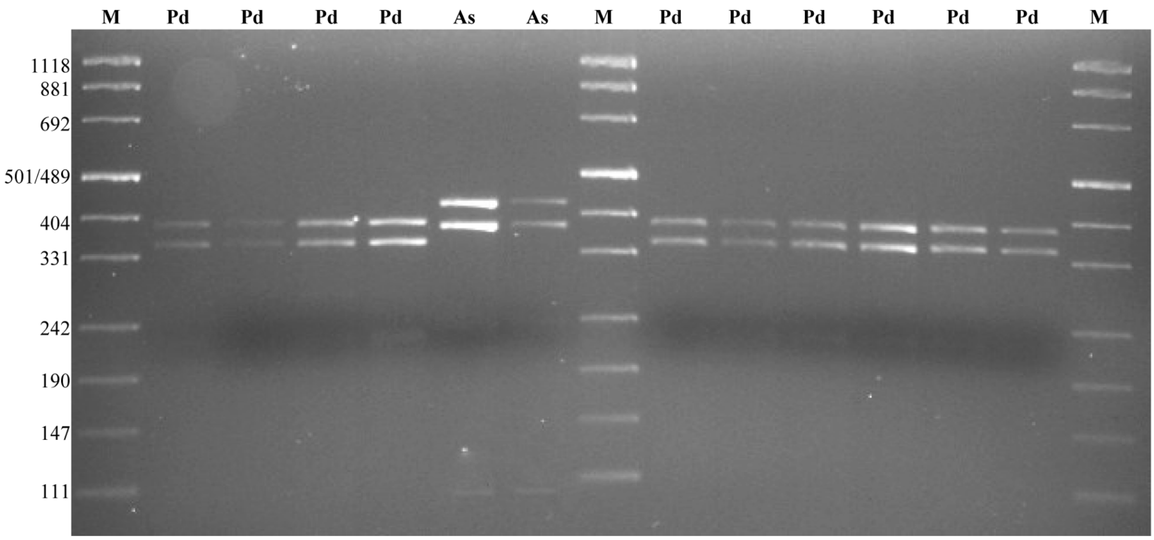

Fig. 2. The RFLP results with HhaI in use. Pd – *Pseudoterranova decipiens* s.s., As – *Anisakis simplex* s.s., M – A&A Biotechnology pUC Mix molecular marker

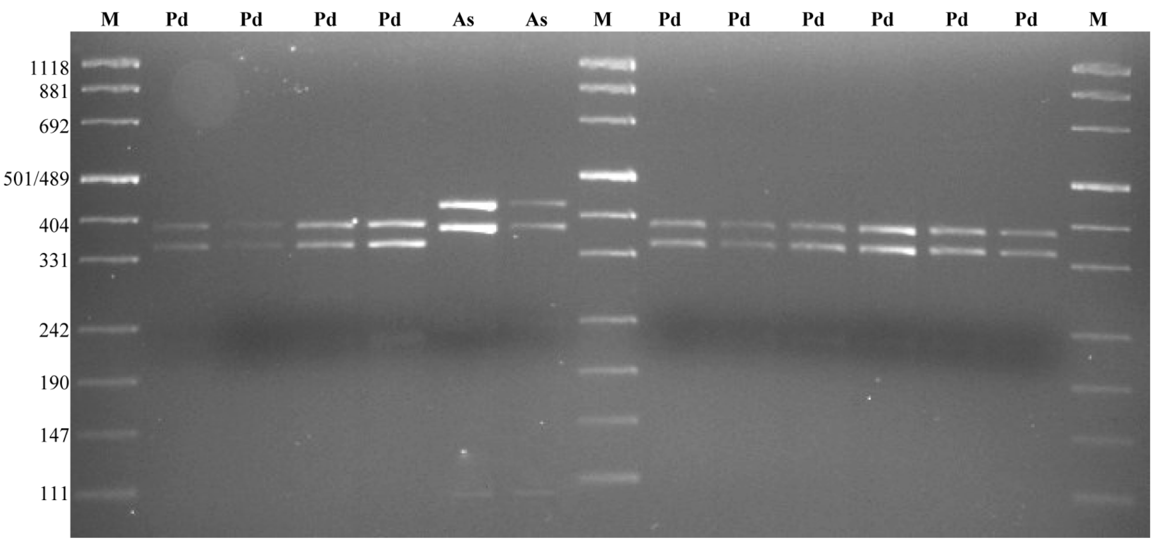

Fig. 3. The RFLP results with TaqI in use. Pd – *Pseudoterranova decipiens* s.s., Astr – *Anisakis simplex* s.s., M – A&A Biotechnology pUC Mix molecular marker

ported to the Hel Marine Station where after freezing to –21°C carcasses were necropsied in order to perform several analyses. The contents of digestive tract were examined in case of any parasites' occurrence. There were anisakid nematodes found. They were rapidly conserved in 70% ethanol and transported to Invertebrate Zoology Department belonging to University of Gdansk in order to undertake further analysis.

Preliminary processing of the specimens was made in order to separate anterior and posterior parts – that were about to undergo morphological identifications – and central parts – that were left in 70% ethanol for additional molecular diagnostics. Anterior and posterior fragments had been left in separate Eppendorf containers with 5% alcohol-glycerine solution until the evaporation of alcohol and clarification process has ended. In some cases lac-

Table II. Parasites' life stages for the *Halichoerus grypus* specimens (Hg). The numbers are counts of parasite species in selected life stage. Percentage means a part of total parasite species count in selected host specimen and selected life stage. × – unknown adult

Host Hg	**Species**	**L3**	**L4**	**Adults**				**Unknown**
				♀	♂	×	Total	
71	*C. osculatum*	–	218 (59.9%)	50 (13.7%)	94 (25.8%)	–	144 (39.6%)	2 (0.5%)
	P. decipiens	–	58 (33.0%)	34 (19.3%)	82 (46.6%)	–	118 (67.0%)	–
	A. simplex	–	2 (100.0%)	–	–	–	–	–
	Unknown	–	12 (25.0%)	6 (12.5%)	14 (29.2%)	2 (4.2%)	22 (45.8%)	14 (29.2%)
	Total	**–**	**290 (49.2%)**	**90 (15.3%)**	**190 (32.2%)**	**4 (0.7%)**	**284 (48.1%)**	**16 (2.7%)**
66	*C. osculatum*	–	10 (71.4%)	4 (28.6%)	–	–	4 (28.6%)	–
	A. simplex	2 (100.0%)	–	–	–	–	–	–
	Total	**2 (12.5%)**	**10 (62.5%)**	**4 (25.0%)**	**–**	**–**	**4 (25.0%)**	**–**
39	*C. osculatum*	–	7 (70.0%)	3 (30.0%)	–	–	3 (30.0%)	–
	P. decipiens	–	5 (55.6%)	3 (33.3%)	1 (11.1%)	–	4 (44.4%)	–
	Unknown	–	1 (50.0%)	–	1 (50.0%)	–	1 (50.0%)	–
	Total	**–**	**13 (61.9%)**	**6 (28.6%)**	**2 (9.5%)**	**–**	**8 (38.1%)**	**–**
I	*C. osculatum*	–	10 (71.4%)	3 (21.4%)	1 (7.1%)	–	4 (28.6%)	–
	P. decipiens	–	–	–	1 (100.0%)	–	1 (100.0%)	–
	Unknown	–	1 (50.0%)	–	–	–	–	1 (50.0%)
	Total	**–**	**11 (64.7%)**	**3 (17.6%)**	**2 (11.8%)**	**–**	**5 (29.4%)**	**1 (5.9%)**
S	*C. osculatum*	–	6 (35.3%)	7 (41.2%)	4 (23.5%)	–	11 (64.7%)	–
	P. decipiens	–	–	1 (33.3%)	2 (66.7%)	–	3 (100.0%)	–
	Unknown	–	1 (50.0%)	–	–	1 (50.0%)	1 (50.0%)	1 (50.0%)
	Total	**–**	**7 (31.8%)**	**8 (36.4%)**	**6 (27.3%)**	**1 (4.5%)**	**14 (63.6%)**	**1 (4.5%)**
Total		**2 (0.3%)**	**331 (49.7%)**	**108 (16.2%)**	**200 (30.0%)**	**5 (0.8%)**	**315 (47.3%)**	**18 (2.7%)**

tophenol and Berland's Solution have been used (Berland 2005).

First phase of research was performed in order to identify complex species. It was done observing (via microscope) some major morphological distinctions like: ventricular and intestinal appendices and/or posterior parts' shape (Grabda-Kazubska and Okulewicz 2005). Life stage was determined due to observation of mucron, larval tooth, spicule or uterus filled with ovas. A development of lips surrounding the mouth cavity was also an essential diagnostic feature (Grabda-Kazubska and Okulewicz 2005, Weerasooriya *et al.* 1986).

The second phase of research was connected with usage of Restriction Fragments Length Polymorphism technique (RFLP). Central parts of the specimens were processed due to DNA isolation. Subsequently some specific rDNA fragments (ITS-1, ITS-2) were amplified using a PCR technique. NC2 and NC5 primers were used (Zhu *et al.* 2002). The next step was a digestion of PCR products using restriction enzymes: RsaI, HhaI, HinfI and TaqI. Results of RFLP were shown during a high resolution electrophoresis on 4% agarose gel with ethidium bromide added. An image was then compared with templates (D'Amelio *et al.* 2000, Kijewska *et al*. 2002; La Rosa *et al*. 2006).

Results

A general outcome of the research was that seals were infected with *Contracaecum osculatum* C, *Pseudoterranova decipiens* s.s. and *Anisakis simplex* s.s.

749 of nematodes were collected (see Table I). All of them were examined excepting those collected from Hg71 grey seal. This specimen was infested with 590 anisakids. Only 295 of them were examined and the final result was obtained by extrapolation (multiplying species' counts by 2).

In grey and common seals there were found: *C. osculatum*, *P. decipiens* and *A. simplex*. In a ringed seal there were found: *C. osculatum* and *P. decipiens*. All grey seals were massively infected with *C. osculatum*. The heaviest infection of *C. osculatum* reached 364 with prevalence 61.7% in Hg71. Total number of nematodes found in digestive tracts of the other grey

Table III. Parasites' life stages for the *Phoca vitulina* specimens (Pv) and *Pusa hispida* (Ph). The numbers are counts of parasite species in selected life stage. Percentage means a part of total parasite species count in selected host specimen and selected life stage

Host Pv	Species	L3	L4	Adults			Unknown
				♀	♂	Total	
88	*C. osculatum*	–	–	–	1 (100.0%)	1 (100.0%)	–
	P. decipiens	–	1 (100.0%)	–	–	–	–
	Total	**–**	**1 (50.0%)**	**–**	**1 (50.0%)**	**1 (50.0%)**	**–**
80	*C. osculatum*	–	9 (47.4%)	1 (5.3%)	9 (47.4%)	10 (52.6%)	–
	P. decipiens	1 (2.6%)	16 (41.0%)	9 (23.1%)	12 (30.8%)	21 (53.8%)	1 (2.6%)
	A. simplex	1 (50.0%)	1 (50.0%)	–	–	–	–
	Unknown	2 (22.2%)	4 (44.4%)	–	–	–	3 (33.3%)
	Total	**4 (5.8%)**	**30 (43.5%)**	**10 (14.5%)**	**21 (30.4%)**	**31 (44.9%)**	**4 (5.8%)**
73	Unknown	–	–	–	–	–	3 (100.0%)
Total		**4 (5.4%)**	**31 (41.9%)**	**10 (13.5%)**	**22 (29.7%)**	**32 (43.2%)**	**7 (9.5%)**
Ph							
55	*C. osculatum*	–	4 (80.0%)	–	1 (20.0%)	1 (20.0%)	–
	P. decipiens	–	–	1 (33.3%)	2 (66.7%)	3 (100.0%)	–
	Total	**–**	**4 (50.0%)**	**1 (12.5%)**	**3 (37.5%)**	**4 (50.0%)**	**–**

seals was approximately 20 times less. There was a large minority of specimens that remained unidentified – for Hg71 this count equalled approximately 8.1% (48 out of 590 specimens). *C. osculatum s*pecimens collected from Hg66 were identified as sibling species C (10 counts). There were also *P. decipiens* s.s. (2 counts) and *A. simplex* s.s. (2 counts) detected (see Table I).

The most infected common seal was specimen Pv80. This host was infected mainly with *P. decipiens*. Proportion of infestation (*P. decipiens* : *C. osculatum*) is like 2:1. Sibling species *P. decipiens* s.s. (14 identified specimens) and *A. simplex* s.s. (2 specimens) were identified using RFLP. In the other seals only single nematodes were found. A problem with identification has occurred during examination of Pv73 anisakids. RFLP results were not specific. The restriction enzymes digestion products were as follows: RsaI – ~600, ~530 bp, TaqI – ~450, ~380, ~170 bp, HhaI – the matrix remained undigested.

The only examined ringed seal was infected with 8 nematodes: 5 of them were members of complex *C. osculatum* and 3 of them were *P. decipiens*. There was no case of positive DNA isolation so there was no possibility to determine sibling species.

Development stages and gender proportions

The L3 larvae were very rare in the whole material. There was a majority of L4 and adult specimens, generally in equal proportions. Variations could be observed mainly among low infected hosts. The most common observation is an unequal distribution of genders – particularly significant among Hg71 specimens – with females to males proportion being about 1 : 2. Detailed informations can be found in Table II and Table III.

L4 and adult stages proportions are like 1:1 for *C. osculatum* found in digestive tract of both common seals and ringed seal. There is only a slight variation among the grey seals – the proportion is 3 : 2. It has been found that there is a minor excess of males belonging to *C. osculatum* collected from grey seals. Examination of common seals showed that there is a domination of *C. osculatum* males. Similar tendency has been proven to ringed seal – there was a majority of males.

Two thirds of *P. decipiens* specimens collected from grey seals were adult. It has also been noticed that there were very few specimens in L3 stage found in common seals, but there is still domination of adult nematodes. Gender proportions are not like 1:1 in any studied case. Males are more numerous than females. This tendency is significant especially for grey seals – only 30% of *P. decipiens* are females.

A. simplex found in the grey seals remained in L3 stage – so did the specimens collected from common seals. There were two L4 larvae found in grey seal. Only single L4 specimen was found in common seal. There was no occurrence of adult *A. simplex* worms.

Discussion

Recent studies showed a high infection rate of *C. osculatum* among the grey seals of Baltic Sea (Liskins 2002, Mattiucci and Nascetti 2007, Valtonen *et al*. 1988). The same observation has been made during this research. Occurrence of *P. decipiens* among the grey seals of North Sea (Aspholm *et al.* 1995, Lehnert *et al.* 2007) makes a possibility of finding the parasite in Baltic Sea that could migrate with the paratenic hosts. The seals living in Baltic Sea feed mainly on herrings (Lundström *et al*. 2010) which are vectors for *A. simplex* (Guz *et al.* 2005, Sinisalo 2007). It might be a main reason of seals' minor infestation with this parasitic species.

Results of infection and gender proportions examinations were similar to Sinisalo (2007) and Liskins (2002) which noticed that the adult specimens of *C. osculatum* and *P. decipiens* are present in the material collected from grey seals living in Baltic Sea. L4 and adult stages proportions of grey seals from Iceland were like 2:1 for both parasites mentioned above (Ólafsdóttir and Hauksson 1997). It has also been noticed that the males were more numerous than the females.

Infestation proportions for common seal differ from the data gathered during examination of grey seals. In common seals there was a majority of *P. decipiens*. This anisakid species had been found several times in common seals of eastern Atlantic (McClelland 2002) including Oslofjord (Aspholm *et al.* 1995) and Wadden Sea (Lehnert *et al.* 2007), L4 and adult *C. osculatum* and *P. decipiens* numbers were equal. During the research made in Oslofjord (Aspholm *et al.* 1995) it has been proven that L3, L4 and adult worms can be found in common seals. The adults were the most numerous. It has also been noticed that in north-eastern Atlantic Ocean there is only an occurrence of adult *P. decipiens* specimens (Mattiucci and Nascetti 2007).

There were only 8 Anisakidae specimens collected from the single ringed seal. Both *C. osculatum* and *P. decipiens* have been found. There was no occurrence of *A. simplex*. It is difficult to compare such modest data to any results already published. It is known that *C. osculatum* may be found among ringed seals from Bothnian Bay (Sinisalo 2007, Valtonen *et al.* 1988).

No *A. simplex* specimens were found in adult stage. This observation is expected due to host specificity of this parasitic species – it does maturate only in cetacean hosts (Klimpel *et al.* 2004, Rokicki 2006).

Molecular diagnostics have proven that *P. decipiens* from seals found in southern Baltic Sea belong to *P. decipiens* s.s. sibling species. The same sibling species may be found in common seals from eastern Atlantic Ocean (Mattiucci *et al.* 2007, Paggi *et al.* 2000). There were also made some positive identifications of *C. osculatum*. It has been proven that the specimens belong to *C. osculatum* C sibling species. It is an expected result. Data collected from fish during previous studies in Baltic Sea confirm this observation (Mattiucci *et al.*

2007, Mattiucci and Nascettii 2007, Zhu *et al.* 2002). There were also some positive identifications of *A. simplex* – it has been defined as *A. simplex* s.s. It is also an expected result (D'Amelio 2000, Mattiucci and Nascettii 2007). There were no any differences between results already published and those gathered during this research. However, it can't be left unnoticed, that few specimens were identified in a molecular way, so their significance is quite low.

In the past time there were not many studies on anisakids in seals occurring in southern Baltic. Despite the low numbers of examined hosts, during this preliminary study significant and new data have been collected. It is important to further investigate seals' infestations levels in southern Baltic Sea. Data collected during such researches may be crucial for understanding anisakids' life cycles and hosts relationships in this area.

Acknowledgements. This work was supported by National Science Centre Dec nr: 2011/01/B/NZ8/04194.

References

Anderson R.C. 2000. Nematode parasites of vertebrates, their development and transmission. CABI, Wallingford, 650 pp.

Aspholm E.P., Ugland K.I., Jødestøl K.A., Berland B. 1995. Sealworm (*Pseudoterranova decipiens*) infection in common seals (*Phoca vitulina*) and potential intermediate fish hosts from outer Oslofjord. *International Journal for Parasitology*, 25, 367–373. DOI: 10.1016/0020-7519(94)00133-9.

Berland B. 2005. Whole Mounts. KUSTEM, Terengganu, 53 pp.

D'Amelio S., Mathiopoulos K.D., Santos C.P., Pugachev O.N., Webb S.C., Picanço M., Paggi L. 2000. Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. *International Journal for Parasitology*, 30, 223–226.

Grabda-Kazubska B., Okulewicz A. 2005. Pasożyty ryb Polski (klucze do oznaczania) – Nicienie (Nematoda). Polskie Towarzystwo Parazytologiczne, Warszawa, 169 pp.

Guz L., Studzińska M.B., Sadzikowski A.B., Gundłach J.L. 2005. Anisakioza. *Annales Universitatis Mariae Curie-Skłodowska*, LX(10), 74–83.

Harding K.C., Härkönen T., Helander B., Karlsson O. 2007. Status of Baltic grey seals: Population assessment and extinction risk. *NAMMCO Scientific Publications*, 6, 33–56.

Kijewska A., Rokicki J., Sitko J., Węgrzyn G. 2002. Ascaridoidea: a simple DNA assay for identification of 11 species infecting marine and freshwater fish, mammals, and fish-eating birds. *Experimental Parasitology*, 101, 35–39. DOI: 10.1016/S0014-4894(02)00031-0.

Klimpel S., Palm H.W., Rückert S., Piątkowski U. 2004. The life cycle of *Anisakis simplex* in the Norwegian Deep (northern North Sea). *Parasitology Research*, 94, 1–9.

Klimpel S., Palm H.W. 2011. Anisakid nematode (Ascaridoidea) life cycles and distribution: increasing zoonotic potential in the time of climate change? In: (Ed. H. Mehlhorn) Parasitology Research Monographs, Vol. 2. Heidelberg, 201–222. DOI: 10.1007/s00436-004-1154-0.

Køie M., Berland B., Burt M.D.B. 1995. Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Fisheries and Aquatic Sciences*, 52, 134–139. DOI: 10.1186/1756-3305-1-23.

Køie M., Fagerholm H.P. 1995. The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. *Parasitology Research*, 81, 481–489. DOI: 10.1007/BF00931790.

La Rosa G., D'Amelio S., Pozio E. 2005. Molecular identification of nematode worms from seafood (*Anisakis* spp. and *Pseudoterranova* spp.) and meat (*Trichinella* spp.). *Methods in Biotechnology*, 21, 217–232. DOI: 10.1385/1-59259-990-7:217.

Lehnert K., Raga J.A., Siebert U. 2007. Parasites in harbour seals (*Phoca vitulina*) from the German Wadden Sea between two phocine distemper virus epidemics. *Helgoland Marine Research*, 61, 239–245. DOI: 10.1007/s10152-007-0072-9.

Liskins N. 2002. Dissection results of dead seals perished in fishing gear in the Gulf of Riga. *Acta Zoologica Lituanica*, 12, 369–371. DOI: 10.1080/13921657.2002.10512526.

Lundström K., Hjerne O., Lunneryd S-G., Karlsson, O. 2010. Understanding the diet composition of marine mammals: grey seals (Halichoerus grypus) in the Baltic Sea. *ICES Journal of Marine Science*, 67, 1230–1239. DOI: 10.1093/icesjms/fsq022

Martell D.J., McClelland G. 1995. Transmission of *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) via benthic macrofauna to sympatric flatfishes (*Hippoglossoides platessoides*, *Pleuronectes ferrugineus*, *P. americanus*) on Sable Island Bank, Canada. *Marine Biology*, 122, 129–135. DOI: 10.1007/BF00349286.

Mattiucci S., Nascetti G. 2007. Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. *Veterinary Parasitology*, 148, 43–57. DOI: 10.1016/j.vetpar.2007.05.009.

Mattiucci S., Paoletti M., Damiano S., Nascetti G. 2007. Molecular detection of sibling species in anisakid nematodes. *Parassitologia*, 49, 147–153.

Myjak P., Szostakowska B., Wojciechowski J., Pietkiewicz H., Rokicki J. 1994. Anisakid larvae in cod from the southern Baltic Sea. *Archive of Fishery and Marine Research*, 42, 149–161.

McClelland G. 2002. The trouble with sealworms (*Pseudoterranova decipiens* species complex, Nematoda): a review. *Parasitology*, 124, 183–203. DOI: 10.1017/S0031182002001658.

Ólafsdóttir D., Hauksson E. 1997. Anisakid (Nematoda) infestations in Icelandic grey seals (*Halichoerus grypus* Fabr.). *Journal of Northwest Atlantic Fishery Science*, 22, 259–269. DOI: 10.1155/2011/235832.

Paggi L., Mattiucci S., Gibson D.I., Berland B., Nascetti G., Cianchi R., Bullini L. 2000. *Pseudoterranova decipiens* species A and B (Nematoda, Ascaridoidea): nomenclatural designation, morphological diagnostic characters and genetic markers. *Systematic Parasitology*, 45, 185–197. DOI: 10.1023/A:1006296316222.

Rokicki J. 2006. Anisakidoza (anizakidoza, *anisakiasis*). In: (Ed. A. Kotłowski), Medycyna i higiena tropikalna. Akademia Medyczna w Gdańsku, Gdynia, 488–507.

Rokicki J., Rolbiecki L., Skóra A. 2009. Helminth parasites of twaite shad, *Alosa fallax* (Actinopterygii: Clupeiformes: Clupeidae) from the southern Baltic Sea. *Acta Ichthyologica et Piscatoria*, 39, 7–10.

Sinisalo T. 2007. Diet and foraging of ringed seals in relation to helminth parasite asemblages. Jyväskylä University Printing House, Jyväskylä, 41 pp.

Sobecka E., Łuczak E., Więcaszek B., Antoszek A. 2011. Parasite community structure of cod from Bear Island (Barents Sea) and Pomeranian Bay (Baltic Sea). *Polish Polar Research*, 32, 253–262. DOI: 10.2478/v10183-011-0016-6.

Szostakowska B., Myjak B., Wyszyński M., Piętkiewicz H., Rokicki J. 2005. Prevalence of anisakin nematodes in fish from southern Baltic Sea. *Polish Journal of Microbiology*, 54, 41–45.

Valtonen E. T., Fagerholm H. P., Helle E. 1988. *Contracaecum osculatum* (Nematoda: Anisakidae) in fish and seals in Bothnian Bay (northeastern Baltic Sea). *International Journal for Parasitology*, 18, 365–370. DOI: 10.1186/1756-3305-1-23

Weerasooriya M. V., Fujipo T., Ishii Y., Kagei N. 1986. The value of external morphology in the identification of larval anisakid nematodes: a scanning electron microscope study. *Parasitenkunde*, 72, 765–778. DOI: 10.1007/BF00925097

Zhu X. Q., D'Amelio S., Palm H. W., Paggi L., George-Nascimento M., Gasser R. B. 2002. SSCP-based identifications of mem bers within the *Pseudoterranova decipiens* complex (Nematoda: Ascaridoidea: Anisakidae) using genetic markers in the internal transcribed spacers of ribosomal DNA. *Parasitology*, 124, 615–623. DOI: 10.1017/S0031182002001579

Received: May 28, 2013
Revised: December 4, 2013
Accepted for publication: January 20, 2014