Parassitologia 31: 45-49, 1989

ELECTROPHORETIC IDENTIFICATION OF *ANISAKIS* SP. LARVAE (ASCARIDIDA: ANISAKIDAE) FROM *CLUPEA HARENGUS* L. IN BALTIC SEA

S. MATTIUCCI[1], S. D'AMELIO[1], J. ROKICKI[2]

[1] *Istituto di Parassitologia, Università di Roma "La Sapienza", Italia;* [2] *Department of Invertebrate Zoology, University of Gdansk, Poland.*

Abstract. On the basis of electrophoretic studies carried out on 15 gene-enzyme systems, 80 *Anisakis* sp. larvae from the herring *Clupea harengus*, fished in South Baltic Sea and Gdanisk Bay, were identified as *Anisakis simplex* B. This is the first record of *Anisakis simplex* B in the Baltic Sea. The spawning migration of the herrings from the North Sea to the Baltic Sea and the distribution of *A. simplex* B are briefly discussed.

Key words: multilocus electrophoresis, *Anisakis* sp. larvae, *Clupea harengus*, Baltic Sea.

INTRODUCTION

Several records have been reported for larval forms of *Anisakis* Dujardin, 1845, in fishes from the Baltic Sea. *Anisakis* sp. in *Clupea harengus* was investigated for the first time in this area by Rokicki in 1972; later Grabda (1974, 1976) studied the life-cycle and morphogenesis of *Anisakis simplex* and its occurrence in *Gadus morhua callarias*. Recently, *Anisakis* larvae have been reported in *Stizostedion lucioperca* by Feiler and Winkler (1981) and in *Belone belone* by Grabda (1981) for the Baltic Sea. The species identification of larvae belonging to the genus *Anisakis* is difficult because of the lack of morphological characters at the specific level. During the last years, the analysis of the genetic structure of *Anisakis* populations from the Mediterranean Sea and North-East Atlantic, by means of multilocus electrophoresis, has provided interesting results for the taxonomy of the genus. The main observations involved: i) the detection of two sibling species within the *Anisakis simplex* complex (Nascetti *et al.*, 1986); ii) the evaluation of the genetic divergence of these sibling species from *Anisakis physeteris* (Mattiucci *et al.*, 1986); iii) the biochemical identification of *Anisakis* both at larval and adult stages (Orecchia *et al.*, 1986). The aim of this note is to provide results on the electrophoretic identification of *Anisakis* sp. recovered from *Clupea harengus* of the Baltic Sea.

Materials and methods

Anisakis sp. larvae collected from *Clupea harengus*, fished in April and May 1988, in the South Baltic Sea and Gdanisk Bay, were preserved in distilled water at —70°C for the electrophoretic tests. Horizontal starch gel electrophoresis was carried out on homogenates obtained from 80 specimens, according to the techniques described, in detail, by Nascetti *et al.* (1986). The following 15 enzymatic loci were studied: *Sorbitol dehydrogenase* (*Sdh*), *Lactate dehydrogenase* (*Ldh*), *Malate dehydrogenase* (*Mdh*), *Isocitrate dehydrogenase* (*Idh*), *6-Phosphogluconate dehydrogenase* (*6-Pgdh*), *Superoxide dismutase* (*Sod*), *Nucleoside phosphorilase* (*Np*), *Glutamate-oxaloacetate transaminase* (*Got*), *Adenilate kinase-2* (*Adk-2*), *Phosphoglucomutase-2* (*Pgm-2*), *Esterase-2* (*Est-2*), *Leucine aminopeptidase-1* (*Lap-1*), *Carbonic anhydrase* (*Ca*), *Mannose phosphate isomerase* (*Mpi*), *Glucose phosphate isomerase* (*Gpi*). The electrophoretic patterns of the *Anisakis* larval population studied were compared with those of *Anisakis simplex* B from the North-East Atlantic Ocean and those of *Anisakis simplex* A from the Mediterranean Sea. Isozymes were numbered in order of decreasing mobility from the most anodal; allozymes were named numerically according to their mobility relative to the most common one in *A. simplex* A, designated as *100* (Nascetti *et al.*, 1986).

The statistical analysis of genotype frequencies was carried out on the polymorphic loci. For the enzymatic locus *Idh*, the χ^2 values were calculated without any correction. For the enzymatic loci *6-Pgdh* and *Got*, we pooled the classes whose expected values were lower than 1 (see Ayala and Kiger, 1980). In *Est-2*, *Lap-1* and *Gpi*, rare alleles were pooled for the application of Wright's fixation index (Brown, 1970). This index was also applied to *Mpi*.

Results and discussion

The sample of *Anisakis* sp. larvae collected from *Clupea harengus* in South Baltic Sea and Gdanisk Bay was found to be genetically homogeneous at all the 15 loci studied. Out of the 15 loci analysed, eight were monomorphic (*Sdh*, *Ldh*, *Mdh*, *Sod*, *Np*, *Adk-2*, *Pgm-2*, *Ca*). The remaining polymorphic loci (*Idh*, *6-Pgdh*, *Got*, *Est-2*, *Lap-1*, *Mpi*, *Gpi*) proved to be in Hardy-Weinberg equilibrium (Table 1), thus indicating that all the individuals belong to a single species. The genetic structure of the population studied corresponds to that of *A. simplex* B collected in North-East Atlantic previously studied (Nascetti *et al.*, 1986).

As far as it is known, the geographic distribution of *Anisakis simplex* B involved mainly the North-Eastern part of the Atlantic (Nascetti *et al.*, 1986). The results presented in this note would extend the distribution of this species to the South Baltic Sea. Nevertheless, Grabda (1974) hypothesized that the spring-spawning herrings found heavily infected with *Anisakis* larvae in the south-western Baltic Sea, were those which spawned in this

TABLE 1 - Genotype distributions, allelic frequencies and probabilities for expected Hardy-Weinberg equilibrium distributions for the polymorphic loci of *A. simplex* B from Baltic Sea.

Genotype	Observed	Expected	χ^2 contributions
Idh			
[*93/ 93*]	12	12.02	0.000
[*100/100*]	4	4.02	0.000
[*93/100*]	14	13.99	0.001
		$\chi^2 = 0.001$	$0.95 \leq p \leq 0.98$
93: 0.633 *100*: 0.367			
6-Pg			
[*93/ 93*]	12	10.84	0.124
[*97/ 97*]	1	0.49	0.531
[*100/100*]	12	9.92	0.436
[*93/ 97*]	5	4.61	0.033
[*93/100*]	18	20.73	0.359
[*97/100*]	3	4.41	0.451
		χ^2 corrected 2DF = 1.117	$0.50 \leq p \leq 0.70$
93: 0.441 *97*: 0.098 *100*: 0.461			
Got			
[*88/ 88*]	0	0.14	0.143
[*93/ 93*]	20	19.75	0.004
[*97/ 97*]	2	0.81	1.748
[*100/100*]	2	0.81	1.748
[*88/ 93*]	4	3.36	0.122
[*88/ 97*]	0	0.68	0.682
[*88/100*]	1	0.68	0.150
[*93/ 97*]	8	8.02	0.000
[*93/100*]	7	8.02	0.130
[*97/100*]	0	1.63	1.628
		χ^2 corrected 2DF = 3.017	$0.20 \leq p \leq 0.30$
88: 0.057 *93*: 0.670 *97*: 0.136 *100*: 0.136			
Est-2			
[*96/ 96*]	0	0.03	0.026
[*100/100*]	30	30.54	0.009
[*105/105*]	0	0.31	0.309
[*96/100*]	2	1.79	0.025
[*96/105*]	0	0.18	0.180
[*100/105*]	7	6.14	0.120
	F = —0.13	$1.96/\sqrt{N} = 0.31$	
96: 0.026 *100*: 0.885 *105*: 0.089			

TABLE 1 - Genotype distributions, allelic frequencies and probabilities for expected Hardy-Weinberg equilibrium distributions for the polymorphic loci of *A. simplex* B from Baltic Sea.

Genotype	Observed	Expected	χ^2 contributions
Idh			
[*93/ 93*]	12	12.02	0.000
[*100/100*]	4	4.02	0.000
[*93/100*]	14	13.99	0.001
		$\chi^2 = 0.001$	$0.95 \leq p \leq 0.98$
93: 0.633 *100*: 0.367			
6-Pg			
[*93/ 93*]	12	10.84	0.124
[*97/ 97*]	1	0.49	0.531
[*100/100*]	12	9.92	0.436
[*93/ 97*]	5	4.61	0.033
[*93/100*]	18	20.73	0.359
[*97/100*]	3	4.41	0.451
		χ^2 corrected 2DF = 1.117	$0.50 \leq p \leq 0.70$
93: 0.441 *97*: 0.098 *100*: 0.461			
Got			
[*88/ 88*]	0	0.14	0.143
[*93/ 93*]	20	19.75	0.004
[*97/ 97*]	2	0.81	1.748
[*100/100*]	2	0.81	1.748
[*88/ 93*]	4	3.36	0.122
[*88/ 97*]	0	0.68	0.682
[*88/100*]	1	0.68	0.150
[*93/ 97*]	8	8.02	0.000
[*93/100*]	7	8.02	0.130
[*97/100*]	0	1.63	1.628
		χ^2 corrected 2DF = 3.017	$0.20 \leq p \leq 0.30$
88: 0.057 *93*: 0.670 *97*: 0.136 *100*: 0.136			
Est-2			
[*96/ 96*]	0	0.03	0.026
[*100/100*]	30	30.54	0.009
[*105/105*]	0	0.31	0.309
[*96/100*]	2	1.79	0.025
[*96/105*]	0	0.18	0.180
[*100/105*]	7	6.14	0.120
	F = —0.13	$1.96/\sqrt{N} = 0.31$	
96: 0.026 *100*: 0.885 *105*: 0.089			

TABLE 1 - Genotype distributions, allelic frequencies and probabilities for expected Hardy-Weinberg equilibrium distributions for the polymorphic loci of *A. simplex* B from Baltic Sea.

Genotype	Observed	Expected	χ^2 contributions
Lap-1			
[90/90]	15	15.14	0.001
[94/94]	0	0.05	0.054
[97/97]	0	0.01	0.014
[90/94]	2	1.81	0.020
[90/97]	1	0.92	0.007
[94/97]	0	0.05	0.055
	F = —0.09	$1.96/\sqrt{N} = 0.46$	
90: 0.917	94: 0.055 97: 0.028		
Mpi			
[95/ 95]	0	0.01	0.013
[100/100]	78	77.93	0.002
[95/100]	2	2.05	0.005
	F = —0.01	$1.96/\sqrt{N} = 0.22$	
95: 0.013	100: 0.987		
Gpi			
[88/ 88]	0	0.004	0.004
[93/ 93]	1	0.25	2.250
[100/100]	56	55.35	0.008
[88/ 93]	0	0.06	0.063
[88/100]	1	0.95	0.003
[93/100]	6	7.38	0.258
	F = + 0.16	$1.96/\sqrt{N} = 0.24$	
88: 0.008	93: 0.062 100: 0.930		

area, from the North Sea, from November to May. These fish acquired their infection during the summer months. Herrings that had spent all their lives within the Baltic were only occasionally infected. The agreement between the allelic frequencies found in the *Anisakis simplex* B populations from North Sea and those from Baltic Sea could confirm Grabda's hypothesis.

According to these results the use of *Anisakis* larvae as a biological tag (Smith and Wootten, 1978) may prove to be a powerful approach for investigations on the dynamics of fish stock populations.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Marina Ortis for the technical support in the course of the work.

ɔected Hardy-Weinberg
Baltic Sea.

χ^2 contributions
0.001
0.054
0.014
0.020
0.007
0.055
0.013
0.002
0.005
0.004
2.250
0.008
0.063
0.003
0.258

fish acquired their
pent all their lives
greement between
lations from North
hypothesis.
as a biological tag
ɔproach for investi-

the course of the work.

REFERENCES

AYALA F. J., KIGER J. A. Jr. (1980). *Modern genetics*. The Benjamin/Cummings Publishing Company.

BROWN A. H. D. (1970). The extimation of Wright's fixation index from genotypic frequencies. *Genetica 41*: 399-406.

FEILER K., WINKLER H. M. (1981). First occurrence of *Anisakis* larvae in *Stizostedion lucioperca* from the coastal waters of the Baltic Sea. *Angew. Parasit. 22*: 124-130.

GRABDA J. (1974). The dynamics of the nematode larvae, *Anisakis simplex* (Rud.) invasion in the south-western Baltic herring (*Clupea harengus* L.). *Acta Ichthyol. Pisc. 4*: 3-21.

GRABDA J. (1976). Studies on the life-cycle and morphogenesis of *Anisakis simplex* (Rudolphi, 1809) (Nematoda: Anisakidae) cultured *in vitro*. *Acta Ichthyol. Pisc. 6*: 119-141.

GRABDA J. (1981). Parasitic fauna of garfish *Belone belone* (L.) from the Pomeranian Bay (southern Baltic) and its origin. *Acta Ichthyol. Pisc. 11*: 75-85.

MATTIUCCI S., NASCETTI G., BULLINI L., ORECCHIA P., PAGGI L. (1986). Genetic structure of *Anisakis physeteris*, and its differentiation from the *Anisakis simplex* complex (Ascaridida: Anisakidae). *Parasitology 93*: 383-387.

NASCETTI G., PAGGI L., ORECCHIA P., SMITH J. W., MATTIUCCI S., BULLINI L. (1986). Electrophoretic studies on the *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North-East Atlantic. *Int. J. Parasit. 16*: 633-640.

ORECCHIA P., PAGGI L., MATTIUCCI S., SMITH J. W., NASCETTI G., BULLINI L. (1986). Electrophoretic identification of larvae and adults of *Anisakis* (Ascaridida: Anisakidae). *J. Helminth. 60*: 331-339.

ROKICKI J. (1972). Larwy *Anisakis* sp. u sledzi *Clupea harengus* L. w Baltykn. *Wiad. parazyt. 18*: 89-96.

SMITH J. W., WOOTTEN R. (1978). *Anisakis* and Anisakiasis. *Adv. Parasit. 16*: 93-163.