

Research note

Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae)s

S. Mattiucci^a, L. Paggi^a, G. Nascetti^b, E. Abollo^c, S.C. Webb^d, S. Pascual^c, R. Cianchi^e,
L. Bullini^{e,*}

^aInstitute of Parasitology, University of Rome 'La Sapienza', P. le Aldo Moro, 5, 00185, Rome, Italy

^bDepartment of Environmental Sciences, Tuscia University, 01100 Viterbo, Italy

^cLaboratorio de Parasitologia, Facultad de Ciencias del Mar, University of Vigo, Vigo, Spain

^dDepartment of Zoology, University of Cape Town, Rondebosch 7701, South Africa

^eDepartment of Genetics and Molecular Biology, University of Rome 'La Sapienza', Via dei Sardi, 70, 00185 Rome, Italy

Received 8 August 2000; received in revised form 11 September 2000; accepted 11 September 2000

Abstract

In order to assess the taxonomic status of *Anisakis brevispiculata* Dollfus, 1966 population samples of this taxon from central and south-eastern Atlantic ocean were compared at 22 enzymatic loci with samples belonging to *Anisakis physeteris* Baylis, 1923 from the Mediterranean sea and central-eastern Atlantic ocean. Very low interpopulational genetic divergence was observed both within *A. brevispiculata* (average $D_{Nei} = 0.008$) and within *A. physeteris* ($D_{Nei} = 0.009$) despite the geographic distance among the samples, indicating high levels of gene flow in both taxa. On the other hand, the average genetic distance between *A. brevispiculata* and *A. physeteris* was found to be $D_{Nei} = 0.80$, a value generally observed between well differentiated congeneric species. The reproductive isolation between *A. brevispiculata* and *A. physeteris* is indicated by the following observations: (1) no F_1 hybrids or recombinant genotypes were until now observed; and (2) the two *Anisakis* species do not seem to share their definitive hosts. The main definitive host of *A. brevispiculata* is the pygmy sperm whale (*Kogia breviceps*), while for *A. physeteris* it is the sperm whale (*Physeter catodon*). Only adult males differ slightly in spicule length, while females and larval stages are not differentiated morphologically. Both *A. brevispiculata* and *A. physeteris* show a type II larva. The correct recognition of *A. brevispiculata* from *A. physeteris* and from other *Anisakis* species studied, in either sexes and at any life stage, is made easy by allozyme markers (e.g. *Icdh*, *Gapdh*, *Sod-1*, *Np*, *Aat-2*, *Adk-2*, *fEst-2*, *PepB*, *PepC-2*, *Mpi*). Diagnostic keys, which can be used for routine identification in the field of these *Anisakis* worms, based on genetic markers, are given. © 2001 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Molecular systematics, *Anisakis brevispiculata*, Allozyme electrophoresis, Genetic divergence, Reproductive isolation, Host preference, Type II larvae

The anisakid nematode *Anisakis brevispiculata* was originally described by (Dollfus, 1966) in the Atlantic ocean from the pygmy sperm whale, *Kogia breviceps* (de Blainville, 1838). (Davey, 1971), in his re-examination of the genus *Anisakis*, considered *A. brevispiculata* as a synonym of *Anisakis physeteris* (Baylis, 1923).

In the present paper, the genetic variation of *A. brevispiculata* is studied at 22 enzyme loci and compared with that of *Anisakis physeteris*. The taxonomic status, reproductive isolation, and genetic relationships of these two taxa are discussed. In addition, data on geographic distribution, definitive and paratenic hosts of *A. brevispiculata* and *A. physe-*

teris are provided, as well as diagnostic allozyme keys for their correct identification in either sex and at any life-stage. In such keys also other *Anisakis* species so far studied genetically are included.

The anisakid specimens genetically studied, their hosts, and collection data, are reported in Table 1. Adult and pre-adult specimens were collected from the stomach of pygmy sperm whales, *K. breviceps* stranded on the South African coast (south eastern Atlantic waters) and on the Spanish coast of Galicia (central eastern Atlantic). Male worms were found to correspond morphologically to *A. brevispiculata*, according to spicule length (Dollfus, 1966). Adults of *A. physeteris* were collected from the sperm whale, *P. catodon* (Linnaeus, 1758) from the central Tyrrhenian sea. Larval stages (L4) (Table 1) were collected from two individuals of *Ziphius*

* Corresponding author. Tel.: +39/06/4917825; fax: + 39/06/4940839.
E-mail address: bullini@uniroma1.it (L. Bullini).

Table 1

Collection data of adults (A) and larvae (L₃) of *Anisakis* spp. studied^a

<i>Anisakis</i> parasites	Host	Collecting site	<i>N_h</i>	<i>N_p</i>	Stage	Date of collection
<i>A. physeteris</i>	<i>Physeter catodon</i>	Tyrrhenian sea (Latium coast)	1	151	Adult	June 1982
<i>A. brevispiculata</i>	<i>Kogia breviceps</i>	Rio de Ferrol (La Coruña, North Atlantic Galician coast)	1	20	Adult	1997
<i>A. brevispiculata</i>	<i>Kogia breviceps</i>	De Hoop (East of Cape Town, Republic of South Africa)	1	8	Adult	May 1996
<i>A. brevispiculata</i>	<i>Kogia breviceps</i>	Elands Bay (North of Cape Town, Republic of South Africa)	19	20	Adult	September 1996
Type II larvae	<i>Ziphius cavirostris</i>	Ionian sea	1	10	L ₄	1986
Type II larvae	<i>Todarodes sagittatus</i>	Ustica Island (south Tyrrhenian sea)	1	10	L ₃	July 1985
Type II larvae	<i>Xiphias gladius</i>	south Tyrrhenian sea	6	20	L ₃	July 1997
Type II larvae	<i>Merluccius merluccius</i>	Balearic Islands (West Mediterranean sea)	17	45	L ₃	June 1998
Type II larvae	<i>Merluccius merluccius</i>	Atlantic coast of Morocco	13	29	L ₃	June–July 1998

^a *N_h*, Number of infected hosts; *N_p*, number of parasites collected.

cavirostris (Cuvier, 1823) in mixed infections with adult forms of *Anisakis ziphidarum* (Paggi et al., 1998). L3 larvae type II (Berland, 1961; Shiraki, 1974) were recovered in the squid *Todarodes sagittatus* (Lamarck, 1798) from Mediter-

anean waters, in European hake, *Merluccius merluccius* (Linnaeus, 1758) collected from Mediterranean and Atlantic waters and in specimens of swordfish *Xiphias gladius* (Linnaeus, 1758) from the Tyrrhenian sea (Table 1). *Anisakis*

Table 2

The enzyme studied, listed with their code number, encoding loci, electrophoretic migration (+anodal, -cathodal), buffer systems and staining procedures, for the samples studied

Enzyme/code number	Encoding loci	Migration	Buffer systems ^a	Staining procedure/reference
Idditol dehydrogenase (1.1.1.14)	<i>Iddh</i>	+	3	(Nascetti et al., 1986) designated as <i>Sdh</i>
Malate dehydrogenase (1.1.1.37)	<i>Mdh-1</i>	–	5	(Nascetti et al., 1986)
	<i>Mdh-2</i>	–	5	
Isocitrate dehydrogenase (1.1.1.42)	<i>Icdh</i>	+	3	(Nascetti et al., 1986)
6-Phosphogluconate dehydrogenase (1.1.1.44)	<i>6Pgdh</i>	+	5	(Nascetti et al., 1986)
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.1)	<i>Gapdh</i>	+	3	(Nascetti et al., 1986)
NADH dehydrogenase (1.6.99)	<i>NADHdh</i>	+	4	(Nascetti et al., 1986)
Superoxide dismutase (1.15.1.1)	<i>Sod-1</i>	+	3,6	(Nascetti et al., 1986)
	<i>Sod-2</i>	–	3,6	(Nascetti et al., 1986)
Nucleoside phosphorylase (2.4.2.1)	<i>Np</i>	+	4,7	Mod. from (Nascetti et al., 1986): staining sol., 0.05 M phosphate buffer, pH 7.5
Aspartate amino transferase (2.6.1.1)	<i>Aat-2</i>	–	3	(Nascetti et al., 1986)
Adenylate kinase (2.7.4.3)	<i>Adk-2</i>	–	5,3,1	(Nascetti et al., 1986)
Colorimetric esterase (3.1.1.1)	<i>cEst-2</i>	+	4,2	(Nascetti et al., 1986)
Fluorescent esterase (3.1.1)	<i>fEst-1</i>	–	7	Designated as carbonic anhydrase (<i>Ca</i>) in (Nascetti et al., 1986)
Leucine aminopeptidase (3.4.11)	<i>Lap-2</i>	+	2	(Nascetti et al., 1986)
Peptidase (Leu Leu) (3.4.11)	<i>PepB</i>	+	3	10 mg L-leucyl leucine, 3 mg peroxidase, 0.05 mg L-amino acid oxidase, 10 mg orthodanisidine, agar 0.8% in 30 ml Tris–HCl 0.05 M pH = 8
Peptidase (Leu Ala) (3.4.11)	<i>PepC-1</i>	+	3	20 mg L-leucyl alanine, 3 mg peroxidase, 0.05 mg L-amino acid oxidase, 10 mg orthodanisidine, 10 mg MnCl ₂ , agar 0.8% in 30 ml Tris–HCl 0.05 M pH = 8
	<i>PepC-2</i>	+	3	
Mannose phosphate isomerase (5.3.1.8)	<i>Mpi</i>	+	4	(Nascetti et al., 1986)
Glucose phosphate isomerase (5.3.1.9)	<i>Gpi</i>	+	4	(Nascetti et al., 1986)
Phosphoglucomutase (5.4.2.2)	<i>Pgm-1</i>	+	7	(Nascetti et al., 1986)
	<i>Pgm-2</i>	–	7	(Nascetti et al., 1986)

^a Buffer systems: 1, discontinuous Tris/citrate (Na) (Poulik, 1957); 2, Discontinuous Tris/citrate (Li), modified from (Poulik, 1957); 3, continuous Tris/citrate (Selander et al., 1971); 4, Tris/versene/borate (Brewer and Sing, 1970); 5, phosphate citrate (Harris, 1966); 6, Tris-maleate, modified from (Brewer and Sing, 1970); 7, 0.1M Tris-maleate pH = 7.8 (Richardson et al., 1986).

Table 3

Loci and alleles found diagnostic above the 99% level between *Anisakis brevispiculata* and *Anisakis physeteris*

Locus	<i>A. brevispiculata</i>	<i>A. physeteris</i>
<i>Icdh</i>	110, 114, 118	95, 105
<i>Gapdh</i>	98	115
<i>6Pgdh</i>	93, 97	78, 86, 88, 90
<i>Sod-1</i>	90	97
<i>Np</i>	70	> 60
<i>Aat-2</i>	80, 85	97
<i>Adk-2</i>	103	97
<i>fEst-2</i>	90	115
<i>PepB</i>	60	75 >
<i>PepC-1</i>	95	105
<i>PepC-2</i>	102	108
<i>Mpi</i>	80, 88	85, 93, 98, 105

specimens were stored at 70°C. Anterior and posterior portions of adults were mounted and clarified in lactic acid-phenol (1:1) for morphological studies, while the remaining part of the body was used for enzyme electrophoresis.

Tissue from single worm specimens was ground in distilled water. The homogenate was absorbed onto 5 × 5 mm chromatography paper wicks and inserted into 10–12% starch gel for horizontal electrophoresis. Electrophoresis was performed at 5°C at 7–9 V/cm for 3–6 h, according to the gene-enzyme system studied. Eighteen enzymes, putatively encoded by 22 loci, were studied: they are listed in Table 2, together with electrophoretic techniques utilised.

As in previous papers (Nascetti et al., 1986; Mattiucci et al., 1986, 1997), isozymes were numbered in order of decreasing mobility from the most anodal one. Allozymes were named with numbers indicating their mobility (in mm, standardised conditions) relative to the most common allele, designated as 100, found in a reference population (i.e. *Anisakis pegreffii* from the Mediterranean sea). The statistical significance of departures from the Hardy–Weinberg equilibrium was estimated using chi-square test (χ^2) and *F*-statistics (Wright, 1943, 1951). The genetic divergence of populations and species was estimated using the following indices: standard genetic distance D_{Nei} by Nei (Nei, 1972), and D_T , by (Rogers, 1972; Wright, 1978). The genetic variability of population samples was calculated using the following parameters: mean number of alleles per locus (*A*); proportion of polymorphic loci at the 99% (P_{99}) and 95% (P_{95}) criteria; expected mean heterozygosity per locus (*He*). Genetic analyses were performed using BIOSYS software (Swofford and Selander, 1989).

All the anisakid specimens collected from pygmy sperm whales were found to be genetically homogeneous, with polymorphic loci in Hardy–Weinberg equilibrium. Accordingly, the two populations belong to a same gene pool, characterised by various alleles, not present in either *A. physeteris* (Mattiucci et al., 1986), or other *Anisakis* species studied genetically so far (Paggi et al., 1998; Nascetti et al., 1986; Mattiucci et al., 1997, 1998). As mentioned above,

adult males corresponded to *A. brevispiculata*, according to the description given by (Dollfus, 1966). The loci and alleles found diagnostic above the 99% level between *A. brevispiculata* and *A. physeteris* are given in Table 3. Using such markers, type II larvae were identified: e.g. in European hake (*M. merluccius*), fished along the Moroccan Atlantic coast: five *A. brevispiculata* larvae were found together with 24 of *A. physeteris*.

Three examples of the possible allozyme diagnostic keys for the identification of the *Anisakis* species studied so far are given in Table 4. These and similar keys allow the accurate identification of *A. brevispiculata*, *A. physeteris*, as well as the sibling species of *A. simplex* complex and *A. ziphidarum*, in either sex and at any life stage.

Allele frequencies in three population samples of *A. brevispiculata* are given in Table 5 and compared with those observed in two samples of *A. physeteris*. A low genetic differentiation was detected among populations of *A. brevispiculata* (Table 6), in spite of their geographic distance. Intraspecific genetic distance values ranged from $D_{Nei} = 0.004$ between populations from Galician and from Moroccan Atlantic coasts, to $D_{Nei} = 0.012$ between South African and Moroccan Atlantic coasts (average $D_{Nei} = 0.008$) (Table 6). A similar low value of genetic distance was observed within *A. physeteris*: $D_{Nei} = 0.009$ (Table 6).

Reproductive isolation of *A. brevispiculata* and *A. physe-*

Table 4

Three examples of allozyme diagnostic keys for the identification of the *Anisakis* species so far studied genetically. The alleles utilised are diagnostic at the 99% level. Data from (Paggi et al., 1998; Nascetti et al., 1986; Mattiucci et al., 1986, 1997) and present paper

	Locus	Alleles		Species
1.	<i>Icdh</i>	95, 105 110, 114, 118 100, 93	→ → →	<i>A. physeteris</i> <i>A. brevispiculata</i> 2
2.	<i>Aat-2</i>	120 93, 100	→ →	<i>A. ziphidarum</i> 3
3.	<i>Adk-2</i>	105 100	→ →	<i>A. simplex</i> s.s. 4
4.	<i>Pep B</i>	100 60, 70	→ →	<i>A. pegreffii</i> <i>A. simplex</i> C
1.	<i>Adk-2</i>	90 97 103 105 100	→ → → → →	<i>A. ziphidarum</i> <i>A. physeteris</i> <i>A. brevispiculata</i> <i>A. simplex</i> s.s. 2
2.	<i>Mdh-1</i>	100 80, 90	→ →	<i>A. pegreffii</i> <i>A. simplex</i> C
1.	<i>PepC-1</i>	90 92 100 108 95, 105	→ → → → →	<i>A. simplex</i> s.s. <i>A. simplex</i> C <i>A. pegreffii</i> <i>A. ziphidarum</i> 2
2.	<i>Aat-2</i>	97 85	→ →	<i>A. physeteris</i> <i>A. brevispiculata</i>

Table 5

Allelic frequencies observed at 22 enzymatic loci in adult specimens (A) and larvae (L₃) of *Anisakis brevispiculata* in comparison with *A. physeteris* from Mediterranean sea and Atlantic waters^a

Loci	<i>A. brevispiculata</i>			<i>A. physeteris</i>	
	A	A	L ₃	A + L ₃ ^b	L ₃
Alleles	SA	GA	CA	ME	CA
<i>Iddh</i>					
97	1.00	1.00	1.00	0.89	1.00
103	–	–	–	0.11	–
<i>Mdh-1</i>					
105	1.00	1.00	1.00	1.00	1.00
<i>Mdh-2</i>					
113	1.00	1.00	1.00	0.70	1.00
118	–	–	–	0.30	–
<i>Icdh</i>					
95	–	–	–	0.75	0.79
105	–	–	–	0.25	0.21
110	0.32	–	–	–	–
114	0.62	1.00	1.00	–	–
118	0.06	–	–	–	–
<i>6Pgdh</i>					
78	–	–	–	0.52	0.50
86	–	–	–	0.29	0.35
88	–	–	–	0.07	–
90	–	–	–	0.07	0.15
93	0.79	0.98	0.99	0.04	–
97	0.21	0.02	0.01	–	–
105	–	–	–	0.01	–
<i>Gapdh</i>					
98	1.00	1.00	1.00	–	–
105	–	–	–	0.03	–
115	–	–	–	0.97	1.00
<i>NADHdh</i>					
70	1.00	1.00	1.00	1.00	1.00
<i>Sod-1</i>					
90	1.00	1.00	1.00	–	–
97	–	–	–	1.00	1.00
<i>Sod-2</i>					
100	1.00	1.00	1.00	1.00	1.00
<i>Np</i>					
60	–	–	–	1.00	1.00
70	1.00	1.00	1.00	–	–
<i>Aat-2</i>					
80	0.04	0.06	–	–	–
85	0.96	0.94	1.00	–	–
90	–	–	–	0.02	–
97	–	–	–	0.95	1.00
107	–	–	–	0.03	–
<i>Adk-2</i>					
97	–	–	–	1.00	1.00
103	1.00	1.00	1.00	–	–

cEst-1

102 1.00 1.00 1.00 1.00 1.00

fEst-2

90 1.00 1.00 1.00 – –

115 – – – 1.00 1.00

Lap-1

92 1.00 1.00 1.00 0.78 1.00

96 – – – 0.22 –

Lap-2

80 1.00 1.00 1.00 1.00 1.00

Pep B

60 1.00 1.00 1.00 – –

75 – – – 1.00 1.00

PepC-1

95 1.00 1.00 1.00 0.05 –

105 – – – 0.95 1.00

PepC-2

102 1.00 1.00 1.00 – –

108 – – – 1.00 1.00

Mpi

80 0.60 0.59 0.90 – –

85 – – – 0.06 0.11

88 0.40 0.41 0.10 – –

93 – – – 0.82 0.69

98 – – – 0.10 0.11

105 – – – 0.02 0.09

Gpi

90 0.10 0.06 0.02 0.04 0.07

98 0.90 0.94 0.98 0.92 0.91

108 – – – 0.04 0.02

Pgm-1

93 0.08 – – 0.91 1.00

95 0.92 1.00 1.00 0.09 –

Pgm-2

94 – – – 0.01 –

106 1.00 1.00 1.00 0.97 1.00

110 – – – 0.02 –

^a SA, South African coast; GA, Galician coast; ME, Mediterranean Sea; CA, Atlantic coast of Morocco.

^b Data from [6] and present paper.

teris is shown by the lack of F₁ hybrids and/or recombinant genotypes over the 313 specimens tested.

Some parameters of genetic variability for *A. brevispiculata* and *A. physeteris* are given in Table 7. The highest variability was found in *A. physeteris* from the Mediterranean sea (e.g. $P_{99} = 0.52$; $He = 0.13$). Less variable appears to be *A. brevispiculata*; the genetic variability of this species is higher in South African waters (e.g. $P_{99} = 0.26$; $He = 0.07$).

The main definitive host of *A. brevispiculata* is the pygmy sperm whale, *K. breviceps* (both from the literature and our

Table 6

Intra- and inter-specific values of genetic distance with the formula by Nei (D_{Nei}) below the diagonal, and by Rogers [12], modified by Wright [13] (above the diagonal), among populations of *Anisakis brevispiculata* and *A. physeteris*

Population	<i>A. brevispiculata</i> (South African coast)	<i>A. brevispiculata</i> (Galician coast)	<i>A. brevispiculata</i> (Atlantic coast of Morocco)	<i>A. physeteris</i> (Mediterranean sea)	<i>A. physeteris</i> (Atlantic coast of Morocco)
<i>A. brevispiculata</i> (South African coast)	–	0.085	0.108	0.700	0.705
<i>A. brevispiculata</i> (Galician coast)	0.007	–	0.066	0.716	0.721
<i>A. brevispiculata</i> (Atlantic coast of Morocco)	0.012	0.004	–	0.723	0.728
<i>A. physeteris</i> (Mediterranean sea)	0.787	0.812	0.820	–	0.091
<i>A. physeteris</i> (Atlantic coast of Morocco)	0.764	0.791	0.799	0.009	–

present data). There have been no *A. brevispiculata* specimens recorded from any other cetacean species examined in previous surveys (Paggi et al., 1998; Nascetti et al., 1986; Mattiucci et al., 1986, 1997). According to the available data, *A. brevispiculata* appears to be distributed in the Central and South Atlantic ocean. The European hake *M. merluccius* found infected by *A. brevispiculata* larvae, could play a role as paratenic/intermediate hosts in the life cycle of this anisakid nematode in the central-eastern Atlantic ocean. Nevertheless, the low prevalence ($P = 0.07$) and intensity ($I = 0.29$) of the infection by *A. brevispiculata* in this fish suggest that other fish and/or squids species are important in the life-cycle of *A. brevispiculata*. This agrees with the diet of the pygmy sperm whale which mainly consists of squids belonging to the family Cranchiidae (*Histiotheuthis* sp., *Onychoteuthis banksi*, *Enoploteuthis* sp., *Mastigoteuthis* sp., *Neoteuthis thieli*) and, secondly, by crustaceans and pelagic fish (Ross, 1979).

As to *A. physeteris*, its main definitive host is the sperm whale, *P. catodon* (present data and in the literature). No adults genetically identified have been recorded in other cetacean hosts. We found pre-adults (L4) of *A. physeteris* together with adults of *A. ziphidarum* in the beaked whale, *Ziphius cavirostris* from the Mediterranean sea, suggesting a similar feeding behaviour shared by the sperm whale and the beaked whale in the Mediterranean sea. At larval stages, *A. physeteris* was genetically identified in the fish *M. merluccius* and *X. gladius*.

Genetic studies show that *A. brevispiculata* and *A. physeteris* are two distinct biological species. Their genetic

distance is $D_{Nei} = 0.80$, a value within the range found among congeneric species of many different animal and plant groups. The reproductive isolation between *A. brevispiculata* and *A. physeteris* is indicated by the following observations (1) no F_1 hybrids or recombinant genotypes have been observed and (2) the two *Anisakis* species do not share their definitive hosts (the pigmy sperm whale *Kogia breviceps* for *A. brevispiculata*, and the sperm whale *Physeter catodon* for *A. physeteris*).

The main morphological diagnostic character between *A. brevispiculata* and *A. physeteris* appears to be the spicule length of male specimens which is significantly shorter in *A. brevispiculata*, as reported by (Dollfus, 1966) in his description of the species.

At the larval stages, both *A. brevispiculata* and *A. physeteris* show a type 2 larva (Berland, 1961). The genetic identification of the larvae showed that both *Anisakis* species infect the fish *M. merluccius*; *A. physeteris* was also found in the fish *X. gladius* and in the squid *T. sagittatus*.

The high level of genetic differentiation of both *A. physeteris* and *A. brevispiculata* from other species of the genus *Anisakis*, genetically studied so far by allozyme markers (average $D_{Nei} = 3.0$) indicates that the genus *Anisakis* is polyphyletic and highly heterogeneous. The species *A. physeteris* and *A. brevispiculata* should be assigned to the subgenus *Skrjabinisakis* proposed by Mosgovoi (Mosgovoi, 1951) for *A. physeteris*. Accordingly, type 2 larvae belong to *Anisakis* (*Skrjabinisakis*), while larvae type 1 belong to *Anisakis* (*Anisakis*) subgenus.

The paper confirms that allozymes, as well as other mole-

Table 7

Parameters of genetic variability (A , mean number alleles per locus; P_{99} and P_{95} , proportion of polymorphic loci at the 0.99 and 0.95 criteria; H_e , expected mean heterozygosity per locus) of *Anisakis brevispiculata* populations on the basis of 22 enzymatic loci in comparison with those observed in populations of *A. physeteris*^a

Populations	N	A	P_{99}	P_{95}	H_e
<i>A. brevispiculata</i> (South African coast)	28	1.3 (0.1)	0.26	0.22	0.075 (0.033)
<i>A. brevispiculata</i> (Galician coast)	20	1.2 (0.1)	0.17	0.13	0.033 (0.022)
<i>A. brevispiculata</i> (Atlantic coast of Morocco)	5	1.1 (0.1)	0.13	0.04	0.010 (0.008)
<i>A. physeteris</i> (Mediterranean sea)	236	1.9 (0.3)	0.52	0.43	0.126 (0.037)
<i>A. physeteris</i> (Atlantic coast of Morocco)	24	1.3 (0.2)	0.17	0.17	0.064 (0.035)

cular markers, (Andrews and Chilton, 1999; Nadler, 1990; Anderson et al., 1998; D'Amelio et al., 2000; McManus and Bowles, 1996) are useful for the validation of specific status in doubtful taxa and for endoparasite identification of larvae and adult females. The multilocus allozyme approach also allows studies on host preference and specificity, life-cycle and geographic distribution of sibling or incertae parasite species and can be especially useful for routine species identification in the field.

Acknowledgements

The Authors wish to thank Dr. Peter Best (South African Museum) for his valuable collaboration in collecting samples from the field. The work was carried out by grants from Italian Ministero per le Politiche Agricole e Forestali and within the European Community Grant FAIR CT97-3494 (Genhake).

References

- Anderson, J.T.C., Blouin, M.S., Beecher, R.N., 1998. Population biology of parasitic nematodes: applications of genetic markers. *Adv. Parasitol.* 41, 219–83.
- Andrews, R.H., Chilton, N.B., 1999. Multilocus enzyme electrophoresis: a valuable technique for providing answers to problems in parasite systematics. *Int. J. Parasitol.* 29, 213–53.
- Berland, B., 1961. Nematodes from some Norwegian marine fishes. *Sarsia* 2, 1–50.
- Brewer, G.J., Sing, C.F., 1970. *An Introduction to Isoenzyme Techniques*. Academic Press, New York and London.
- D'Amelio, S., Mathiopoulos, K., Santos, C.P., et al., 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. *Int. J. Parasitol.* 30, 223–6.
- Davey, J.T., 1971. A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridida). *J. Helminth.* 45, 51–72.
- Dollfus, R., 1966. Helminthofauna de *Kogia breviceps* (Blainville 1838) cetace odontocete. *Ann. Soc. Sci. Nat. Charete-Maritime* 4, 3–6.
- Harris, H., 1966. Enzyme polymorphism in man. *Proc. R. Soc. Ser. B* 164, 298–310.
- 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, 383–7.
- Mattiucci, S., Nascetti, G., Cianchi, R., et al., 1997. Genetic and ecological data on the *Anisakis simplex* complex with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *J. Parasitol.* 83, 401–16.
- Mattiucci, S., Paggi, L., Nascetti, G., et al., 1998. Allozyme and morphological identification of *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). *Syst. Parasitol.* 40, 81–92.
- McManus, D.P., Bowles, J., 1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. *Int. J. Parasitol.* 26, 687–704.
- Mosgovoi, A.A., 1951. Ascarids of mammals of the U.S.S.R. (Anisakidae). *Trud. Gelminth. Lab.* 5, 12–22.
- Nadler, S.A., 1990. Molecular approaches to studying helminth population genetics and phylogeny. *Int. J. Parasitol.* 20, 11–29.
- 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. Parasitol.* 16, 633–40.
- Nei, M., 1972. Genetic distance between populations. *Am. Nat.* 106, 283–92.
- Paggi, L., Nascetti, G., Webb, S., Mattiucci, S., Cianchi, R., Bullini, L., 1998. A new species of *Anisakis* Dujardin, 1845 (Nematoda, Anisakidae) from beaked whales (Ziphiidae): allozyme and morphological evidence. *Syst. Parasitol.* 40, 161–74.
- Poulik, M.D., 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature* 180, 1477.
- Richardson, B.J., Baverstock, P.R., Adams, M., 1986. *Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies*. Academic Press, Sydney.
- Rogers, J.S., 1972. Measures of genetic similarities and genetic distance. *Stud Gen VII*, 7213. University of Texas Press, pp. 145–53.
- Ross, G.J.B., 1979. Records of pygmy and dwarf sperm whales, genus *Kogia* from Southern Africa, with biological notes and some comparisons. *Ann. Cape Prov. Museum Nat. Hist.* 11, 259–327.
- Selander, R.K., Smith, M.H., Yang, S.Y., et al., 1971. I. Variation of the old-field mouse (*Peromyscus polionotus*). *Stud Genet VI*, 7103. University of Texas Press, pp. 49–90.
- Shiraki, T., 1974. Larval nematodes of family Anisakidae (Nematoda) in the northern sea of Japan as a causative agent of eosinophilic phlegmon or granuloma in the human gastro-intestinal tract. *Acta. Med. Biol.* 22, 57–98.
- Swofford, D.L., Selander, R.B., 1989. BYOSYS-1, A Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics, Version 1.7, Natural History Survey, Champaign: IL.
- Wright, S., 1943. Isolation by distance. *Genetics* 28, 114–38.
- Wright, S., 1951. The genetical structure of populations. *Ann. Eugen.* 15, 323–54.
- Wright, S., 1978. *Evolution and the Genetics of Populations*. 4. Variability within and among Natural Populations. University of Chicago Press, Chicago, IL.