

ELECTROPHORETIC STUDIES ON THE *ANISAKIS SIMPLEX* COMPLEX (ASCARIDIDA: ANISAKIDAE) FROM THE MEDITERRANEAN AND NORTH-EAST ATLANTIC

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Abstract—NASCETTI G., PAGGI L., ORECCHIA P., SMITH J. W., MATTIUCCI S. and BULLINI L. 1986. Electrophoretic studies on the *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North-East Atlantic. *International Journal for Parasitology* 16: 633–640. The genetic variation of the sibling species *Anisakis simplex* A and *A. simplex* B was investigated by electrophoretic analysis of 22 gene–enzyme systems. The two species are reproductively isolated and no gene flow takes place between them. Three loci, *Sod*, *Adk-2* and *Lap-1*, show distinct alleles in *A. simplex* A and *A. simplex* B, allowing their reliable identification both at the larval and adult stages. A fourth locus, *Got*, appears to be diagnostic at the 95% level. The value of Nei's genetic distance found between *A. simplex* A and *A. simplex* B is 0.28. Parameters of genetic variability (*He*, *P*, *A*) are given for both species. The geographic distribution of *A. simplex* A and *A. simplex* B appears to be mainly Mediterranean for the former, and mainly North Atlantic for the latter. Several paratenic hosts (fish and squid) and one cetacean definitive host are identified for each of the two species. The names *A. pegreffii* and *A. simplex* are tentatively proposed for *A. simplex* A and *A. simplex* B respectively.

INDEX KEY WORDS: Electrophoresis; gene–enzyme systems; genetic variation; biochemical taxonomy; sibling species; reproductive isolation; speciation; *Anisakis simplex* complex.

INTRODUCTION

ONE OF THE most important contributions of multi-locus electrophoresis to taxonomy is the detection of sibling species, that is biological species, reproductively isolated (no gene flow between them), which are virtually identical at the morphological level. This method affords a direct and reliable test of reproductive isolation between natural populations of bisexual organisms, allowing us to assess if they belong or not to distinct species; a quantitative estimate of their genetic divergence is also made possible by this approach (Coluzzi & Bullini, 1971; Nei, 1972; Avise, 1975; Ayala, 1975; Bullini & Sbordoni, 1980; Ferguson, 1980; Bullini, 1982, 1983 a, b). This can be relevant for taxonomic purposes in those groups to which, due to their mode of reproduction (parthenogenesis, selfing, fission, or other types of asexual reproduction), the biological concept of species based on their reproductive isolation cannot be applied.

On the basis of electrophoretic studies, we demonstrated that two sibling species are included within the bisexual ascaridoid nematode *Anisakis simplex*, the adult of which occurs in marine mammals, and the larvae in crustaceans, molluscs and fish (Nascetti, Paggi, Orecchia, Mattiucci & Bullini, 1981, 1983). These two species were provisionally indicated as

A. simplex A and *A. simplex* B; their larvae both correspond to Berland's (1961) type I (Paggi, Orecchia, Bullini, Nascetti, Mattiucci, 1983; Paggi, Nascetti, Orecchia, Mattiucci, Bullini, 1985; Orecchia, Paggi, Mattiucci, Smith, Nascetti, Bullini, submitted for publication). Detailed morphological analysis of adults of *A. simplex* A and *A. simplex* B, both from the field and from *in vitro* cultures of larvae, failed to reveal any significant difference between these two species; *in vitro* cultures also allowed exclusion of the possibility that the larvae studied were of *A. typica*, which also shows a larva type I (in preparation).

The aims of this paper are: (1) to analyze the genetic variation of *A. simplex* A and *A. simplex* B, and to test the possible existence of gene flow between them, in order to confirm their reproductive isolation; (2) to give diagnostic characters for their identification both at the larval and adult stages; (3) to provide data on their geographic distribution, and on their paratenic and definitive hosts; and (4) to discuss the names to be used to designate these species.

MATERIALS AND METHODS

The specimens of the *Anisakis simplex* complex studied electrophoretically were collected from various hosts from either the Mediterranean Sea or the North-East Atlantic

Ocean; numbers, life-history stage, hosts and geographic origin are listed in Table 1. The last information is available in detail only for part of the host specimens studied, most having been obtained from fishing-boats. For the electrophoretic tests, homogenates were obtained from single individuals crushed in distilled water. These were absorbed in 5 by 5 mm chromatography paper (Whatman 3MM) and inserted in 10% starch gel trays. Standard horizontal electrophoresis was carried out at $7-9 \text{ V cm}^{-1}$ for 3–6 h at 5°C . Gels were then sliced in two, and each part stained for a specific enzyme. The following enzymes were studied: sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGDH), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), superoxide dismutase (SOD), nucleoside phosphorylase (NP), glutamate-oxaloacetate transaminase (GOT), adenylate kinase (ADK), phosphoglucomutase (PGM), esterase (EST), acid phosphatase (ACPH), leucine aminopeptidase (LAP), carbonic anhydrase (CA), mannose phosphate isomerase (MPI), and glucose phosphate isomerase (GPI). Details of the enzymes studied and the electrophoretic techniques used are given in Tables 2–4. About 100 specimens per species were tested for each of the 22 loci analyzed. Loci and alleles designation was as follows: isozymes were numbered in order of decreasing mobility from the most anodal; allozymes were named numerically according to their mobility relative to the commonest one ($=100$) in *A. simplex* A (>100 = faster mobility; <100 = slower). The genetic variability of populations was

evaluated using the following parameters: expected mean heterozygosity per locus (H_e), proportion of polymorphic loci according both to the 1% and 5% criteria (P), and mean number of alleles per locus (A). Alleles with frequencies less than 0.01 were not considered for computations. The amount of genetic divergence between the two species was estimated with the indices of standard genetic identity (I) and distance (D) proposed by Nei (1972).

RESULTS AND DISCUSSION

Genetic variation

Genotype frequencies found in *Anisakis simplex* at some polymorphic loci gave the first evidence that two distinct gene pools exist within this taxon (Nascetti *et al.*, 1981). For example, two alleles were found at the *Sod* locus: 92 and 100, but no heterozygotes 92/100 were observed; similarly, at the *Got* locus, which showed the alleles 93 and 100 as the most common, with a frequency of 0.20 and 0.71 respectively, the heterozygotes 93/100 were only 29 out of the 92 expected. Two other polymorphic loci, 6-*Pgdh* and *Mpi*, deviated significantly from Hardy-Weinberg equilibrium. When the total sample was subdivided into two groups according to the *Sod* genotypes (92/92 and 100/100), all the polymorphic loci turned out to be in Hardy-Weinberg equilibrium in the two subsamples, in agreement with the hypothesis of two separate species.

TABLE 1—LIFE-HISTORY STAGE, HOSTS, AND GEOGRAPHIC ORIGIN OF THE SPECIMENS OF *Anisakis simplex* A AND *A. simplex* B ELECTROPHORETICALLY TESTED.
 n = NUMBER OF INDIVIDUALS TESTED

Species	Life-history stage	n	Hosts	Geographic origin
<i>A. simplex</i> A	L3	12	<i>Todarodes sagittatus</i>	Thyrrhenian Sea
	L3	87	<i>Micromesistius poutassou</i>	Thyrrhenian Sea
	L3	35	<i>Merluccius merluccius</i>	Thyrrhenian Sea
	L3	39	<i>Boops boops</i>	Thyrrhenian Sea
	L3	253	<i>Trachurus trachurus</i>	Thyrrhenian Sea (30 of which from Sardinian Sea)
	L3	50	<i>Scomber scombrus</i>	Thyrrhenian Sea
	L3	7	<i>Scomber scombrus</i>	NE Atlantic
	L3	17	<i>Scomber japonicus colias</i>	Thyrrhenian Sea
	L3	67	<i>Lepidopus caudatus</i>	Thyrrhenian Sea
	Adult	5	<i>Physeter macrocephalus</i>	stranded near Formia (Latium)
Total tested		572		
<i>A. simplex</i> B	L3	11	<i>Gadus morhua</i>	Norwegian Sea
	L3	36	<i>Pollachius virens</i>	North Sea: $58^\circ40'N$ $01^\circ11'E$
	L3	67	<i>Merlangius merlangus</i>	North Sea: $61^\circ26'N$ $01^\circ24'E$
	L3	65	<i>Micromesistius poutassou</i>	North Sea: $61^\circ26'N$ $01^\circ24'E$
	L3	130	<i>Clupea harengus</i>	North Sea: (22 of which from $58^\circ47'N$ $00^\circ21'E$)
	L3	50	<i>Salmo salar</i>	North Sea
	L3	271	<i>Scomber scombrus</i>	North Sea
	L3	4	<i>Scomber scombrus</i>	Thyrrhenian Sea
	Adult	25	<i>Phocaena phocaena</i>	North Sea, Bell Rock: $56^\circ25'N$ $02^\circ10'E$
Total tested		659		

TABLE 2—THE ENZYMES SCORED LISTED WITH THEIR CODE NUMBER, QUATERNARY STRUCTURE, ENCODING LOCI, AND ELECTROPHORETIC MIGRATION

Enzyme	Code number	Quaternary structure	Encoding loci	Migrtion
Sorbitol dehydrogenase	1.1.1.14	tetrameric	<i>Sdh</i>	anodal
Lactate dehydrogenase	1.1.1.27	tetrameric	<i>Ldh</i>	anodal
Malate dehydrogenase	1.1.1.37	dimeric	<i>Mdh</i>	anodal
Isocitrate dehydrogenase	1.1.1.42	dimeric	<i>Idh</i>	anodal
6-Phosphogluconate dehydrogenase	1.1.1.43	dimeric	<i>6-Pgdh</i>	anodal
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	tetrameric	<i>G3pdh</i>	anodal
Superoxide dismutase	1.15.1.1	dimeric	<i>Sod</i>	anodal
Nucleoside phosphorylase	2.4.2.1	trimeric	<i>Np</i>	anodal
Glutamate-oxaloacetate transaminase	2.6.1.1	dimeric	<i>Got</i>	anodal
Adenylate kinase	2.7.4.3	monomeric	<i>Adk-1</i>	anodal
		monomeric	<i>Adk-2</i>	cathodal
Phosphoglucomutase	2.7.5.1	monomeric	<i>Pgm-1</i>	anodal
		monomeric	<i>Pgm-2</i>	anodal
Esterase	3.1.1.1	monomeric	<i>Est-1</i>	anodal
		monomeric	<i>Est-2</i>	anodal
Acid phosphatase	3.1.3.2	monomeric	<i>Acph-1</i>	anodal
		monomeric	<i>Acph-2</i>	cathodal
Leucine aminopeptidase	3.4.11.1	monomeric	<i>Lap-1</i>	anodal
		monomeric	<i>Lap-2</i>	anodal
Carbonic anhydrase	4.2.1.1	dimeric	<i>Ca</i>	anodal
Mannose phosphate isomerase	5.3.1.8	monomeric	<i>Mpi</i>	anodal
Glucose phosphate isomerase	5.3.1.9	dimeric	<i>Gpi</i>	anodal

TABLE 3—BUFFER SYSTEMS (ANALYTICAL GRADE REAGENTS PER LITRE ; pH AT ROOM TEMPERATURE)

Buffer system	Electrodes	Gel	Reference
1. Discontinuous Tris/citrate (Na)	0.3 M sodium borate, pH 8.2 (18.55 g boric acid, 2.40 g NaOH)	0.076 M Tris/0.005 M citric acid, pH 8.7 (9.21 g Tris, 1.05 g monohydrate citric acid)	Poulík, 1957
2. Discontinuous Tris/citrate (Li)	0.05 M lithium hydroxide/ 0.19 M boric acid, pH 8.1 (1.2 g lithium hydroxide 11.8 g boric acid)	0.008 M citric acid/ 0.05 M Tris, pH 8.3 (1.60 g citric acid, 6.2 g Tris)	modified from Poulík, 1957
3. Continuous Tris/citrate	0.687 M Tris/0.157 M citric acid pH 8 (83.2 g Tris, 30 g monohydrate citric acid)	0.023 M Tris/0.005 M citric acid, pH 8 (2.77 g Tris, 1.10 g mono- hydrate citric acid)	Selander, Smith, Yang, Johnson & Gentry, 1971
4. Tris/versene/ borate	0.21 M Tris/0.15 M boric acid/ 0.006 M EDTA, pH 8 (25.4 g Tris, 9.24 g boric acid, 2.20 g EDTA)	0.021 M Tris/0.02 M boric acid/ 0.0007 M EDTA, pH 8 (2.5 g Tris, 1.24 g boric acid, 0.25 g EDTA)	Brewer & Sing, 1970
5. Phosphate/ citrate	0.15 M tri-sodium citrate/ 0.24 M sodium hydrogen phosphate, pH 6.3 (44.11 g sodium citrate, 33.12 g NaH ₂ PO ₄)	electrode buffer diluted 1:40, adjusted to pH 6.3 with 0.2 M citric acid	Harris, 1966
6. Tris/maleate	0.01 M Tris/0.01 M maleic acid/0.01 M EDTA/0.015 MgCl ₂ /0.125 M NaOH, pH 7.2 (12.11 g Tris, 11.61 g maleic acid, 3.72 g EDTA, 2.03 g MgCl ₂ , 5 g NaOH)	electrode buffer diluted 1:10, pH 7.4	modified from Brewer & Sing, 1970

TABLE 4—ELECTROPHORETIC PROCEDURES

Enzyme	Buffer system	V cm ⁻¹	Time	Staining buffer	Coenzymes	Linking enzymes	Substrates	Activators, inhibitors	Visualization methods
SDH	3	8	5 h	0.05 M Tris/HCl pH 8.30 ml	NAD 15 mg		sorbitol 500 mg		MTT 10 mg PMS 2 mg agar 0.8%
LDH	1 or 6	8	5 h	0.05 M Tris/HCl pH 8.30 ml	NAD 15 mg		L(+) lactic acid 50 mg		MTT 10 mg PMS 2 mg agar 0.8%
MDH	3	8	5 h	0.05 M Tris/HCl pH 8.30 ml	NAD 15 mg		L-malic acid 1 M pH 7.5 ml		MTT 10 mg PMS 2 mg agar 0.8%
IDH	3	8	5 h	0.05 M Tris/HCl pH 8.30 ml	NADP 5 mg		DL-isocitrate 30 mg	MgCl ₂ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
6PGDH	5	9	6 h	0.05 M Tris/HCl pH 8.30 ml	NADP 5 mg		gluconate-6-phosphate 20 mg	MgCl ₂ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
G3PDH	3	9	5 h	0.05 M Tris/HCl pH 8.50 ml	NAD 30 mg	EC 4.1.2.13 ALD 1 mg	fructose-1, 6-diphosphate 125 mg incubate with ALD for ½ h	sodium arsenate 150 mg	MTT 10 mg PMS 2 mg agar 0.8%
SOD	3 or 6	9	5½ h	0.05 M Tris/HCl pH 8.30 ml	NAD 15 mg				MTT 10 mg PMS 2 mg agar 0.8%
NP	4	8	5 h	0.05 M Tris/HCl pH 8.30 ml		EC 1.2.3.2 XO 0.1 mg	Inosine 10 mg		MTT 15 mg PMS 3 mg agar 0.8%
GOT	3	9	6 h	0.2 M Tris/HCl pH 8.50 ml			Aspartic acid 200 mg α -ketoglutaric acid 100 mg; adjust to pH 7.5 with 1 M Tris. Pour on gel, incubate for ½ h then add Fast Blue BB	Pyrydossal- 5-phosphate 10 mg	Fast Blue BB 150 mg

ADK	3	9	6 h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 2.7.1.1 HK 0.2 mg	ADP 10 mg Glucose 45 mg	MgCl ₂ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
PGM	6	8	6 h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	Glucose-1- phosphate 80 mg	MgCl ₂ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EST	1	7	3½ h	0.1 M Na ₂ HPO ₄ / 0.1 M KH ₂ PO ₄ pH 6.5, 50 ml			0.5% α and β naphthylacetate in 3.5 ml acetone + 1.5 H ₂ O		Fast Garnett GBC 100 mg
ACPH	2	9	6 h	0.125 M sodium acetate pH 5, 100 ml			α-Naphthylphosphate 100 mg		Fast Garnett GBC 100 mg
LAP	1	8	5 h	0.2 M tris-maleate adjust to pH 5.5 with 0.2 M NaOH			1-Leucil-β- naphthylamide 70 mg		Fast Garnett GBC 100 mg
CA	4	8	5 h	0.1 M Na ₂ HPO ₄ 0.1 M KH ₂ PO ₄ pH 6.5, 20 ml			4-Methyl-umbelliferyl acetate 10 mg in 1 ml acetone		UV, filter paper overlay
MPI	4	8	5 h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg EC 5.3.1.9 GPI 0.04 mg	Mannose-6- phosphate 25 mg	MgCl ₂ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
GPI	4	8	5 h	0.05 M Tris/HCl pH 8, 30 ml	NADP 5 mg	EC 1.1.1.49 G6PDH 0.02 mg	Fructose-6- phosphate 10 mg	MgCl ₂ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%

The present study, carried out on further gene-enzyme systems and on additional samples both of larvae and adults, fully confirms this finding. Beside *Sod*, two more loci were found, *Adk-2* and *Lap-1* with distinct alleles in the two species (diagnostic loci). On the basis of the genotypes of these loci, which showed the same electrophoretic pattern in larvae and adults, all the specimens could be assigned to either *A. simplex* A or *A. simplex* B. No hybrid specimens were found, as well as no evidence of gene flow between them.

The allele frequencies calculated for each of the two species at the 22 loci studied are given in Table 5. Since the samples from different hosts and locations proved to be genetically homogeneous within both *A. simplex* A and *A. simplex* B, they were pooled together for computations.

Three loci (*Ldh*, *Est-1*, *Acph-2*) were found to be monomorphic, with the same allele in the two species. A group of 10 loci (*Mdh*, *6-Pgdh*, *G3pdh*, *Np*, *Adk-1*, *Pgm-2*, *Est-2*, *Lap-2*, *Ca*, and *Gpi*) show allele frequencies either similar or poorly differentiated. A third group (*Sdh*, *Idh*, *Pgm-1*, *Acph-1* and *Mpi*) is significantly differentiated between *A. simplex* A and *A. simplex* B. Another locus, *Got*, appears to be diagnostic at the 95% level. Finally, *Sod*, *Adk-2* and *Lap-1* show, as mentioned above, distinct alleles in the two species, allowing their reliable identification both at the larval and adult stage.

Nei's genetic distance (*D*) found between *A. simplex* A and *A. simplex* B, calculated on the basis of the 22 loci tested, is 0.28. Similar values have been found between a number of sibling species in different animal groups (Bullini, 1985).

The parameters of genetic variability of *A. simplex* A and *A. simplex* B are reported in Table 6. *A. simplex* B appears to be more variable than *A. simplex* A. Both species show a considerably high genetic variability, as do other multiple-host ascaridoid species. The correlation between genetic variability and life cycle in ascaridoid worms is discussed by Bullini, Nascetti, Paggi, Orecchia, Mattiucci, Berland (in press).

Geographic distribution and hosts of A. simplex A and A. simplex B.

As shown in Table 1, most of the specimens of *A. simplex* A (565 out of 572) were from the Mediterranean Sea, and only seven from the North-East Atlantic Ocean. In contrast, 655 out of the 659 specimens of *A. simplex* B were from the North-East Atlantic, with only four from the Mediterranean. In spite of the fact that *A. simplex* A is mainly a Mediterranean species and *A. simplex* B is mainly North Atlantic, specimens of the two forms can coexist in the same area and even in the same host. As mentioned above, this does not lead to hybrid formation, nor to introgression between them, their gene pools remaining fully distinct. This confirms that they are good species, reproductively isolated by intrinsic anti-hybridization mechanisms.

The range of *A. simplex* B presumably extends throughout the North Atlantic. This is suggested by the correspondence of allele frequencies at an *Acph* locus (= *Acph-1* in the present paper) analyzed by Beverley-Burton (1978) in samples from both the western and eastern North Atlantic. Of course, a multilocus analysis of the genetic structure of *A. simplex* (s.l.) from this and other parts of its range would be necessary.

The speciation of *A. simplex* A and *A. simplex* B seems to have occurred by geographic isolation (allopatric speciation, sensu Mayr, 1970), possibly related to one of the regressions of the Mediterranean Sea.

The paratenic and definitive hosts so far detected for *A. simplex* A and *A. simplex* B are given in Table 1. According to Smith (1983), the little evidence so far available on the life cycle of *Anisakis* species suggests that the 2nd moult occurs in crustaceans, considered therefore as intermediate hosts, while fish and squids would be paratenic hosts: the 3rd and 4th moult apparently occur in marine mammals (definitive hosts). The fish *Scomber scombrus*, a paratenic host for both species, was responsible for the exceptional finding of larvae of *A. simplex* A in the North-East Atlantic and of *A. simplex* B in the Mediterranean. Larvae of both species were found together in two *S. scombrus* individuals, that had apparently shifted from one sea to the other. Obviously data on the hosts of *A. simplex* A and *A. simplex* B are far from complete. Euphausiids appear to be important intermediate hosts for *A. simplex* B in the North-East Atlantic (Smith, 1983), but information on the intermediate hosts of *A. simplex* A in the Mediterranean is lacking. Only a more complete picture of the life cycle of *A. simplex* A and *A. simplex* B will make it possible to assess if adaptation to different host species was involved in the speciation of these parasites.

Names to be applied to A. simplex A and A. simplex B

The discovery of sibling species often raises the problem of whether it is better to create new names, rather than use names already available in the literature. The latter solution has been often preferred, e.g. in the case of several *Anopheles* sibling species. This option can be used in the case of *A. simplex* A and *A. simplex* B, taking into account their differential geographic distribution. The name *A. simplex* is tentatively retained for *A. simplex* B, as it was originally assigned to specimens from the North Atlantic (Rudolphi, 1809; Krabbe, 1878). The name *A. pegrefii* is tentatively suggested for *A. simplex* A; this name was given by Campana-Rouget & Biocca (1955) to a Mediterranean *Anisakis* form, collected from the seal *Monachus albiventer*, and later considered by Davey (1971) to be a synonym of *A. simplex*. The electrophoretic study of *Anisakis* specimens from *M. albi-*

TABLE 5—ALLELE FREQUENCIES AT 22 ENZYME LOCI IN *Anisakis simplex* A, AND *A. simplex* B.
h = EXPECTED HETEROZYGOSITY

Loci	Alleles	<i>A. simplex</i> A	<i>A. simplex</i> B	Loci	Alleles	<i>A. simplex</i> A	<i>A. simplex</i> B
<i>Sdh</i>	90	—	0.06	<i>Pgm-1</i>	95	—	0.81
	95	—	0.17		100	1.00	0.19
	100	0.92	0.66		<i>h</i>	0.00	0.31
	105	0.08	0.11	<i>Pgm-2</i>	97	0.01	—
	<i>h</i>	0.15	0.52		100	0.99	1.00
<i>Ldh</i>	100	1.00	1.00		<i>h</i>	0.02	0.00
	<i>h</i>	0.00	0.00	<i>Est-1</i>	100	1.00	1.00
					<i>h</i>	0.00	0.00
<i>Mdh</i>	93	0.01	0.02	<i>Est-2</i>	90	0.03	0.01
	100	0.97	0.96		96	0.16	0.11
	105	0.01	0.01		100	0.62	0.69
	110	0.01	0.01		105	0.18	0.19
	<i>h</i>	0.06	0.08		108	0.01	—
<i>Idh</i>	93	0.31	0.61		<i>h</i>	0.56	0.48
	100	0.69	0.39	<i>Acph-1</i>	80	—	0.02
	<i>h</i>	0.43	0.48		90	0.11	0.41
<i>6-Pgdh</i>	86	—	0.02		100	0.87	0.51
	93	0.06	0.26		108	0.02	0.06
	97	0.07	0.15		<i>h</i>	0.23	0.57
	100	0.84	0.56	<i>Acph-2</i>	100	1.00	1.00
	103	0.02	0.01		<i>h</i>	0.00	0.00
	108	0.01	—	<i>Lap-1</i>	85	—	0.01
	<i>h</i>	0.28	0.60		90	—	0.85
<i>G3pdh</i>	100	1.00	0.98		94	—	0.13
	108	—	0.02		97	—	0.01
	<i>h</i>	0.00	0.04		100	0.96	—
<i>Sod</i>	92	—	1.00		102	0.04	—
	100	1.00	—		<i>h</i>	0.08	0.26
	<i>h</i>	0.00	0.00	<i>Lap-2</i>	98	0.02	—
					100	0.98	1.00
<i>Np</i>	92	—	0.08		<i>h</i>	0.04	0.00
	100	0.98	0.89	<i>Ca</i>	100	0.95	0.94
	104	0.02	—		108	—	0.01
	110	—	0.03		115	0.05	0.05
	<i>h</i>	0.04	0.20		<i>h</i>	0.09	0.11
<i>Got</i>	88	—	0.08	<i>Mpi</i>	97	0.04	—
	93	0.01	0.67		100	0.63	1.00
	97	0.01	0.02		103	0.32	—
	100	0.97	0.18		105	0.01	—
	105	0.01	0.04		<i>h</i>	0.50	0.00
	110	—	0.01	<i>Gpi</i>	88	0.01	0.02
	<i>h</i>	0.06	0.51		93	0.02	0.03
<i>Adk-1</i>	90	—	0.19		100	0.96	0.94
	100	1.00	0.81		106	0.01	0.01
	<i>h</i>	0.00	0.31		<i>h</i>	0.08	0.11
<i>Adk-2</i>	100	1.00	—				
	105	—	1.00				
	<i>h</i>	0.00	0.00				

TABLE 6—PARAMETERS OF GENETIC VARIABILITY IN *Anisakis simplex* A AND *A. simplex* B ON THE BASIS OF 22 ENZYME LOCI. *He* = EXPECTED MEAN HETEROZYGOSITY PER LOCUS; *P* = PROPORTION OF POLYMORPHIC LOCI, ACCORDING TO THE 1% AND 5% CRITERIA; *A* = MEAN NUMBER OF ALLELES PER LOCUS. ALLELES WITH FREQUENCIES LESS THAN 0.01 WERE NOT CONSIDERED FOR COMPUTATION

	<i>He</i>	<i>P</i> 1%	<i>P</i> 5%	<i>A</i>
<i>A. simplex</i> A	0.12	0.64	0.32	2.32
<i>A. simplex</i> B	0.21	0.64	0.50	2.60

venter would elucidate this point; however, this is made difficult by the near extinction of this seal.

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