Systematic Parasitology **54:** 13–23, 2003.

Genetic evidence for two sibling species within *Contracaecum ogmorhini* Johnston & Mawson, 1941 (Nematoda: Anisakidae) from otariid seals of boreal and austral regions

S. Mattiucci[1], R. Cianchi[2], G. Nascetti[3], L. Paggi[1], N. Sardella[4], J. Timi[4,5], S.C. Webb[6], R. Bastida[5,7], D. Rodríguez[5,7] & L. Bullini[2]

[1] *Department of Public Health Sciences, Section of Parasitology, University of Rome 'La Sapienza', P.le Aldo Moro, 5, 00185 Rome, Italy*

[2] *Department of Genetics and Molecular Biology, Section of Ecology, University of Rome 'La Sapienza', Via dei Sardi, 70, 00185 Rome, Italy*

[3] *Department of Environmental Sciences, Tuscia University, P. le dell'Università Viterbo, 01100 Italy*

[4] *Laboratorio de Parasitología, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. Funes 3350, 7600 Mar del Plata, Argentina*

[5] *Consejo Nacional de Investigaciones Cientificas y Tecnica (CONICET), Mar del Plata, Argentina*

[6] *Cawthron Institute, Nelson, New Zealand*

[7] *Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. Funes 3350, 7600 Mar del Plata, Argentina*

Accepted for publication 9th July, 2002

Abstract

Genetic variation of *Contracaecum ogmorhini* (*sensu lato*) populations from different otariid seals of the northern and southern hemisphere was studied on the basis of 18 enzyme loci as well as preliminary sequence analysis of the mitochondrial *cyt b* gene (260 bp). Samples were collected from *Zalophus californianus* in the boreal region and from *Arctocephalus pusillus pusillus*, *A. pusillus doriferus* and *A. australis* from the austral region. Marked genetic heterogeneity was found between *C. ogmorhini* (*sensu lato*) samples from the boreal and austral region, respectively. Two loci (*Mdh-2* and *NADHdh*) showed fixed differences and a further three loci (*Iddh, Mdh-1* and *6Pgdh*) were highly differentiated between boreal and austral samples. Their average genetic distance was $D_{Nei} = 0.36$ at isozyme level. At mitochondrial DNA level, an average proportion of nucleotide substitution of 3.7% was observed. These findings support the existence of two distinct sibling species, for which the names *C. ogmorhini* (*sensu stricto*) and *C. margolisi* n. sp., respectively, for the austral and boreal taxon, are proposed. A description for *C. margolisi* n. sp. is provided. No diagnostic morphological characters have so far been detected; on the other hand, two enzyme loci, *Mdh-2* and *NADHdh*, fully diagnostic between the two species, can be used for the routine identification of males, females and larval stages. *Mirounga leonina* was found to host *C. ogmorhini* (*s.s.*) in mixed infections with *C. osculatum* (*s.l.*) (of which *C. ogmorhini* (*s.l.*) was in the past considered to be a synonym) and *C. miroungae*; no hybrid genotypes were found, confirming the reproductive isolation of these three anisakid species. The hosts and geographical range so far recorded for *C. margolisi* n. sp. and *C. ogmorhini* (*s.s.*) are given.

Introduction

The pinniped parasite *Contracaecum ogmorhini* Johnston & Mawson, 1941, was first described from the leopard seal *Hydrurga leptonyx* (Blainville) in South Australian waters. *C. ogmorhini* was later synonymised with *C. osculatum* (Rudolphi, 1802) by Johnston & Mawson (1945) and Hartwich (1975). On the other hand, *C. ogmorhini* was considered as a valid species by Campana Rouget & Paulian (1960), who recorded it from the Antarctic fur seal, *Arctocephalus gazella* (Peters). These authors also indicated

C. corderoi Lent & Freitas, 1948, a parasite described from *Arctocephalus australis* (Zimmermann) off the coast of Uruguay, as a synonym of *C. ogmorhini*. Fagerholm & Gibson (1987) examined holotype and allotype specimens of *C. ogmorhini* from *Hydrurga leptonyx* and compared them with samples newly collected from different otariid pinnipeds: *Zalophus californianus* (Lesson) from Baja California, Mexico, *Eumetopias jubatus* (Schreber) from the Californian Pacific coast, and *Arctocephalus tropicalis* (Gray) from Gough Island and the South African coast (southern Atlantic). On the basis of this comparison, they confirmed the validity of *C. ogmorhini* and provided a new description of this anisakid nematode. The species was structurally differentiated from other related taxonomic units by Fagerholm (1988).

Genetic heterogeneity between boreal and austral populations of *C. ogmorhini* was first suggested based on enzyme electrophoresis (D'Amelio et al., 1994) and later supported by single-strand conformation polymorphism (SSCP) of ribosomal DNA analysis (Zhu et al., 2001).

In the present paper, the genetic variation of *C. ogmorhini* samples, collected from different otariid species in the boreal and austral regions, was analysed at nuclear level (18 enzyme loci). A preliminary investigation of mitochondrial DNA sequence divergence (a 260 bp sequence of *cytochrome b* gene) was also performed. Aims of the paper are: (*i*) to confirm the genetic heterogeneity of *C. ogmorhini* (*sensu lato*); (*ii*) to quantify the genetic divergence of *C. ogmorhini* from the boreal and austral regions; (*iii*) to evaluate possible morphological differences between specimens from these two geographical areas; (*iv*) to give a formal description for the boreal form; (*v*) to provide diagnostic genetic markers for a routine identification of the taxa included in *C. ogmorhini* (*s.l.*), at any life-history stage and for both sexes; and (*vi*) to present data on the geographical distribution and definitive hosts of *C. ogmorhini* (*sensu stricto*) and the boreal form, *C. margolisi* n. sp.

Materials and methods

A total of 131 adult specimens of *C. ogmorhini* (*s.l.*) were collected from stomachs of different otariid pinniped species from the boreal and austral regions. These definitive hosts are: the California sea lion *Zalophus californianus* (Lesson), the South American fur seal *Arctocephalus australis* (Zimmermann), the South African fur seal *A. pusillus pusillus* (Schreber) and the Australian fur seal *A. pusillus doriferus* (Lesson). Some specimens were also collected from the phocid seal *Mirounga leonina* L. caught on the southern Argentinean coast. Collection sites and hosts of *C. ogmorhini* (*s.l.*) are reported in Table 1. Frozen adult nematodes were stored at −70 °C and then transported in dry ice to Rome for their genetic analysis. Portions of tissue were used for allozyme and DNA analysis. In addition, their cephalic and caudal ends were mounted in lactophenol for morphological identification based on the description given for *C. ogmorhini* by Fagerholm & Gibson (1987) and Fagerholm (1988).

Multilocus allozyme electrophoresis

Genetic variation of *C. ogmorhini* (*s.l.*) samples was investigated by standard horizontal starch gel electrophoresis at 18 enzyme loci: they are reported in Table 2 with their code number, electrophoretic migration and references for the procedures used. As in our previous papers (e.g. Nascetti et al., 1993), 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: *Contracaecum osculatum* A (see Nascetti et al., 1993) from the bearded seal of the North East Atlantic Ocean.

The statistical significance of departures from the Hardy-Weinberg equilibrium was estimated using the chi-square test (χ^2). The genetic divergence of populations and species was estimated using the following indices: standard genetic distance, D_{Nei} by Nei (1972) and D_T, by Rogers (1972, modified by 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}) level; and expected mean heterozygosity per locus (He). Indirect estimation of gene flow among the populations studied was calculated using the parameter Nm, from the values of Fst (Wright, 1943, 1951). Population genetic analyses were performed using BIOSYS software (Swofford & Selander, 1989).

Mitochondrial DNA analysis

A 260 bp sequence of mitochondrial *cytochrome b* (*cyt b*) gene was analysed in specimens of *C. ogmorhini* (*s.l.*) from *Zalophus californianus* (4), *Arcto-*

Table 1. Collection data of *C. ogmorhini* (*s.l.*) and *Contracaecum* spp. samples studied.

Anisakid species	*Np*	Host	*Nh*	Collecting site	Collector	Date of collection
C. ogmorhini (s.l.)	38	*Zalophus californianus* (Otariidae: Otariinae)	1	Vancouver Island, Canada (North Eastern Pacific)	L. Margolis	1991
C. ogmorhini (s.l.)	30	*Arctocephalus pusillus doriferus* (Otariidae: Arctocephalinae)	1	New Zealand coast (South Pacific)	I. Beveridge	1988
C. ogmorhini (s.l.)	25	*Arctocephalus australis* (Otariidae: Arctocephalinae)	2	Mar del Plata, Argentina (South Western Atlantic)	D. Romero	2000
C. ogmorhini (s.l.)	30	*Arctocephalus pusillus pusillus* (Otariidae: Arctocephalinae)	2	South African coast (South Western Atlantic)	S.C. Webb	1997
Contracaecum spp.	53	*Mirounga leonina* (Phocidae: Monachinae)	1	Valdés Peninsule, Argentina (South Eastern Atlantic)	R. Bastida	2000

Nh, number of infected hosts.
Np, number of parasites studied by multilocus allozyme electrophoresis and sequenced at the *cyt b* mtDNA gene.

Table 2. Enzyme studies, listed with their code number, encoding loci, electrophoretic migration (+ anodal, −cathodal) and procedures in *C. ogmorhini (s.l.)* samples from otariids.

Enzyme	Code number	Encoding loci	Buffer* systems	Electrophoretic migration	Reference
Idditol dehydrogenase	1.1.1.14	*Iddh*	3	+	Nascetti et al., 1993
Malate dehydrogenase	1.1.1.37	*Mdh-1*	5	+	Nascetti et al., 1993
		Mdh-2	5	+	Nascetti et al., 1993
		Mdh-3	5	−	Nascetti et al., 1993
		Mdh-4	5	−	Nascetti et al., 1993
Isocitrate dehydrogenase	1.1.1.42	*Icdh*	3	+	*Idh* in Nascetti et al., 1993
6-Phosphogluconate dehydrogenase	1.1.1.43	*6Pgdh*	3	+	*Idh* in Nascetti et al., 1993
NADH dehydrogenase	1.2.1.12	*NADHdh*	4	+	Nascetti et al., 1993
Superoxide dismutase	1.15.1.1	*Sod-1*	4, 3	+	Nascetti et al., 1993
Nucleoside phosphorylase	2.4.2.1	*Np*	4	+	Mattiucci et al., 1998
Aspartate amino transferase	2.6.1.1	*Aat-2*	3	+	Nascetti et al., 1993
Adenylate kinase	2.7.4.3	*Adk-2*	3,5	−	Nascetti et al., 1993
colorimetric Esterase	3.1.1	*cEst-1*	4,1	+	Nascetti et al., 1993
fluorescent Esterase	3.1.1	*fEst-1*	4	+	Mattiucci et al., 1998
Mannose phosphate isomerase	5.3.1.8	*Mpi*	4	+	Nascetti et al., 1993
Glucose phosphate isomerase	5.3.1.9	*Gpi*	4	+	Nascetti et al., 1993
Phosphoglucomutase	5.4.2.2	*Pgm-1*	6	+	Nascetti et al., 1993
		Pgm-2	6	+	Nascetti et al., 1993

**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 & Sing, 1970; 5. Phosphate citrate, Harris, 1966; 6. tris-maleate, modified from Brewer & Sing, 1970; 7.0.1M tris-maleate pH=7.8, Richardson et al., 1986.

cephalus australis (4) and *A. pusillus pusillus* (4). Total DNA was extracted from a 1 mg portion of individual nematode tissue using a Promega Wizard Genomic DNA purification kit (1440) following the manufacturer's instructions. A 260 bp fragment of mtDNA *cyt b* was amplified using the following primers: 5502 (forward) 5′AATTTTGGTAGTATGTTGGG3′ and 5787 (reverse) 5′ACTAAAACATAACCCATAAAAGC3′, designed from the complete sequence of *Ascaris suum* and *Caenorhabditis elegans* (see Okimoto et al., 1992). PCR amplification was carried out in a final volume of 50 μl containing 3 μl of a 200 μl resuspension of total DNA extracted from 1 mg of tissue, 30 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.2 μM dNTP, 0.2 μM of each primer, 2.5 units of *Taq* polymerase (Promega) and double distilled water up to 50 μl. The mixture was denatured at 96°C for 2 min. and subjected to 35 cycles of 30 s at 94°C, 45 s at 50°C and 1 min at 72°C, followed by a final step at 72 °C for 10 min. A Perkin Elmer-Cetus Thermal Cycler Model 480 was used. Products were separated in 2% agarose gels containing 0.2 μg/ml ethidium bromide and observerd under UV. PCR products were purified with SIGMA GenElute PCR clean-Up kit (Product code NA 1020) and resuspended in 30 μl of ultrapure distilled water; 0.6 ng of PCR product per bp and 4.5 pmol of each primer were used with an automated ABI 373 sequencer. The sequences obtained were observed using the program ABI EditView. Alignments were performed using ClustalX software (Jeanmougin et al., 1998). Sequence analysis was performed using MEGA version 2.1 (Kumar et al., 2001).

Results

Allozyme data

Of the 18 enzyme loci analysed, 14 (*Iddh, Mdh-1, Mdh-2, Mdh-4, Icdh, 6Pgdh, NADHdh, Adk-2, cEst-1, fEst-1, Mpi, Gpi, Pgm-1, Pgm-2*) were found to be variable in *C. ogmorhini* (*s.l.*) from different collection sites and definitive hosts. The total sample of *C. ogmorhini* (*s.l.*) exhibited significant deviations from the Hardy-Weinberg expectations at most polymorphic loci (*Iddh, Mdh-1, Mdh-2, Mdh-4, Icdh, 6Pgdh, NADHdh, cEst-1, fEst-1* and *Mpi*), with heterozygote deficit. This was mainly due to significant heterogeneity between the boreal and austral samples of *C. ogmorhini* (*s.l.*). Indeed, at the *Iddh* locus, the most common alleles in the austral samples ($Iddh^{135}$ and $Iddh^{140}$) were not found in the samples from the boreal region; at *Mdh-1* and *6Pgdh* different alleles were the most common in the boreal ($Mdh\text{-}1^{92}$, $6Pgdh^{84}$) and austral ($Mdh\text{-}1^{106}$ and $6Pgdh^{90}$) populations, respectively. At *Mdh-2*, 2 alternative alleles were found in the boreal ($Mdh\text{-}2^{95}$) and in the austral ($Mdh\text{-}2^{105}$) samples; similarly, *NADHdh* showed 2 distinct alleles: $NADHdh^{80}$ in the boreal sample and $NADHdh^{85}$ in the austral ones. When the total *C. ogmorhini* (*s.l.*) sample was divided into subsam-

Table 3. Allele frequencies at polymorphic enzymatic loci of *C. ogmorhini* (*s.l.*) samples from Boreal and Austral Regions. *CAN* = Pacific Canada, in *Zalophus californianus*; *NZE* = New Zealand, in *Arctocephalus pusillus doriferus; SAF:* = South African coast, in *A. pusillus pusillus; ARG* = Argentinian coast, in *A. australis*.

Locus	Alleles	*CAN*	*NEZ*	*SAF*	*ARG*
Iddh	*115*	0.11	0.06	0.01	–
	120	0.61	–	–	–
	125	0.28	0.25	0.19	0.10
	135	–	0.38	0.41	0.40
	140	–	0.31	0.39	0.50
Mdh-1	*82*	0.04	–	–	–
	92	0.85	0.05	0.07	0.18
	106	0.11	0.92	0.93	0.82
	116	–	0.03	–	–
Mdh-2	*95*	1.00	–	–	–
	105	–	1.00	1.00	1.00
Mdh-4	*85*	–	0.37	–	0.21
	92	–	–	–	0.25
	98	1.00	0.63	1.00	0.54
Icdh	*105*	0.40	0.92	0.86	1.00
	110	0.57	0.08	0.14	–
	115	0.03	–	–	–
6Pgdh	*78*	0.03	–	–	–
	84	0.91	0.10	0.25	0.15
	88	–	0.03	–	–
	90	0.06	0.87	0.75	0.85
NADHdh	*80*	1.00	–	–	–
	85	–	1.00	1.00	1.00
Adk-2	*110*	0.58	1.00	1.00	1.00
	116	0.42	–	–	–
cEst-1	*100*	0.47	0.61	0.95	1.00
	105	0.53	0.39	0.05	–
f Est-1	*75*	0.05	–	0.25	0.14
	90	0.95	1.00	0.69	0.70
	102	–	–	0.06	0.10
	115	–	–	–	0.06

Table 3. (Continued)

Locus	Alleles	*CAN*	*NEZ*	*SAF*	*ARG*
Mpi	*95*	–	0.03	0.06	0.06
	100	0.10	–	–	–
	105	0.06	0.06	0.02	0.02
	108	0.20	0.10	0.04	0.05
	112	–	0.14	0.21	0.16
	115	–	0.05	0.04	0.03
	118	–	0.44	0.33	0.43
	120	0.60	0.11	0.20	0.06
	122	–	0.05	0.04	0.16
	125	–	0.02	0.06	0.03
	130	0.04	–	–	–
Gpi	*103*	1.00	1.00	0.98	1.00
	108	–	–	0.02	–
Pgm-1	*90*	0.89	1.00	1.00	0.97
	96	–	–	–	0.03
	100	0.11	–	–	–
Pgm-2	*83*	1.00	1.00	0.98	1.00
	93	–	–	0.02	–

Table 4. Genetic distance values according to Nei's (1972, below the diagonal) and Rogers (1972, mod. Weight, 1978) indices, observed among *C. ogmorhini* samples studied. For population codes, see Table 3.

Population	*CAN*	*NZE*	*SAF*	*ARG*
CAN	–	0.498	0.485	0.512
NZE	0.357	–	0.156	0.137
SAF	0.332	0.029	–	0.132
ARG	0.385	0.023	0.021	–

ples, according to their geographical locations, all the polymorphic loci were found to be in Hardy-Weinberg equilibrium. These findings show that two distinct gene pools exist within *C. ogmorhini* (*s.l.*), one from the northern and the other from the southern hemisphere. Allele frequencies at variable loci in population samples of these two taxa are given in Table 3.

The average genetic distance between boreal and austral *C. ogmorhini* (*s.l.*) taxa were $D_{Nei} = 0.36$ and $D_T = 0.50$ (Table 4). Low values of genetic distance were found between populations of the austral taxon, ranging from $D_{Nei} = 0.02$ (Argentina sample *vs* New Zealand) to $D_{Nei} = 0.03$ (South Africa *vs* New Zealand sample). The corresponding interpopulation genetic diversity values are $F_{ST} = 0.05$ (Argentina *vs* both New Zealand and South Africa) and $F_{ST} = 0.07$ (South Africa *vs* New Zealand); while, the average *Fst* between boreal and austral samples is 0.37. An indirect estimate of gene flow for *C. ogmorhini* from the Southern Ocean was $Nm = 4.1$. On the other hand, virtually no gene flow was estimated between boreal and austral samples $Nm = 0.42$.

Table 5. Parameters of genetic variability in samples of *C. ogmorhini (s.l.)* so far studied.

Population	*n*	*A*	P_{99}	P_{95}	*He*
CAN	38	*2.0*	*50.0*	*50.0*	*0.19*
		(0.4)			(0.06)
NZE	30	*1.8*	*33.3*	*33.3*	*0.15*
		(0.4)			(0.06)
SAF	30	*1.7*	*50.0*	*38.9*	*0.15*
		(0.2)			(0.05)
ARG	33	1.8	*38.9*	*33.3*	*0.17*
		(0.3)			(0.06)

N, number of specimens tested.
For population codes, see Tables 1 and 3.
A, mean number of alleles per locus; P_{99} and P_{95}, proportion of polymorphic loci at the 0.99 and 0.95 criteria; *He*, expected mean heterozygosity per locus. Standard error in parentheses.

The values observed for genetic variability: *A*, P_{99}, P_{95}, and *He*, for boreal and austral taxa are summarised in Table 5; a slightly higher variability was found in the sample from The Canadian Pacific.

mtDNA data

A 260 bp sequence of the mitochondrial *cytb* gene was analysed preliminarily on a small available sample. Austral and boreal samples consistently differed by at least 7 bp (Figure 1), with 2 transitions in the 3rd position, 1 transversion in the 3rd position and 1 transversion in the 1st position, leading to a non-synonymous aminoacidic substitution. No variation was detected between specimens from the same geographical location. The average proportion of nucleotide difference between austral and boreal samples was p=0.037; the Kimura-2 parameter distance was 0.038.

Diagnostic characters between austral and boreal C. ogmorhini *(s.l.)*

No significant differential morphological character was detected between the boreal and austral samples of *C. ogmorhini* (*s.l.*) At allozyme level, 2 loci proved to be fully diagnostic between these 2 taxa:

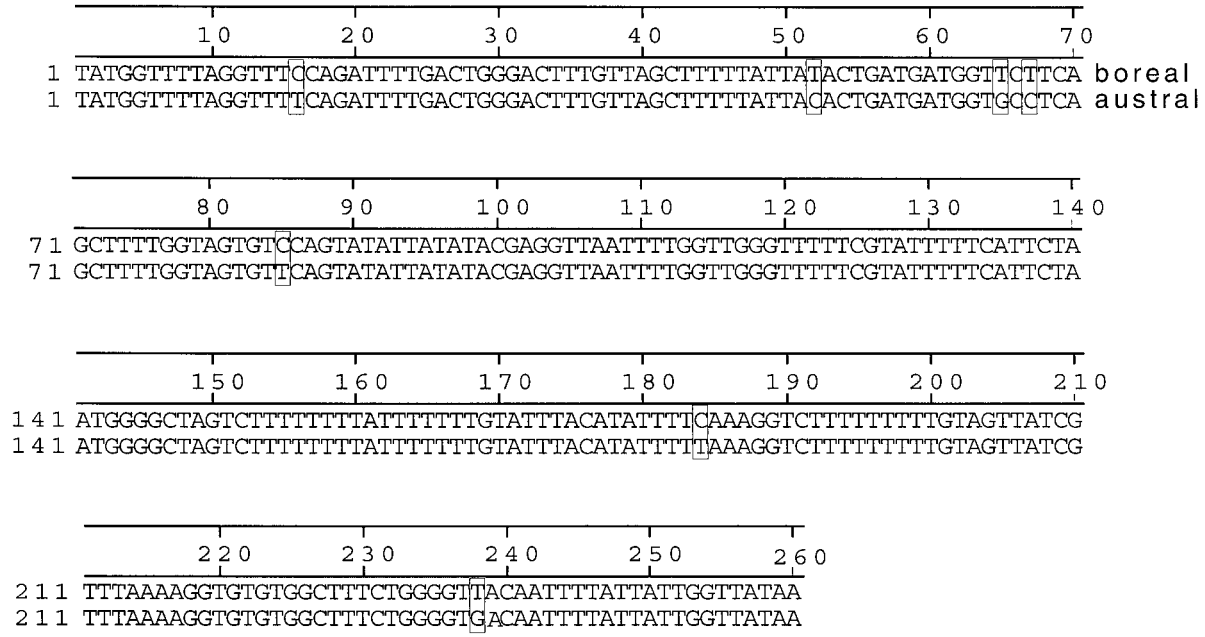

Figure 1. mtDNA *cyt b* sequences of *C. ogmorhini* (*s.l.*) from boreal and austral samples.

Mdh-1 and *NADHdh* (Table 3), allowing the routine identification of males, females and larval stages. At mtDNA level, they can be distinguished using restriction enzymes *Ban*I or *Dde*I, which cut into 2 fragments (62-198 bp and 67-193 bp, respectively) the sequence of the austral taxon, but not the boreal one.

Because *C. ogmorhini* was originally described from *Hydrurga leptonyx* in the austral region, we propose to retain the name of *C. ogmorhini* (*s.s.*) for the austral taxon. The taxon from the boreal region is here described as a new species under the name *Contracaecum margolisi* n. sp., with a morphological description, rather than indicating it with a letter (e.g. A, B) as has been done for some other anisakid species.

Contracaecum margolisi n. sp.

Type-material: Holotype: anterior and posterior ends of one male from stomach of *Zalophus californianus* (Lesson), stranded at Nanaimo, Vancouver Island, Canada; collector Dr. Leo Margolis; deposited in the collection of the NHM London, BMNH No. 2002.11.25.1 (Figure 2). *Paratypes*: posterior ends of 3 males collected from the same host and locality as the holotype, deposited in the collection of the Department of Public Health Sciences, Parasitology Section, University of Rome 'La Sapienza'. The description is based on the only suitable specimens for the morphological analysis, which were limited due to the scarcity of the material and the various genetic methodologies performed on the same specimens.

*Description (*Figure 2*)*

Based on anterior and posterior ends of holotype (measurements in mm). Body length 24. Lips with antero-median rounded notch and antero-lateral auricles with lateral rounded knob, devoid of dentigerous ridges. Dorsal lip with 2 sublateral double papillae. Subventral lips with 1 large double papilla and 1 small externo-lateral papilla plus an amphid. Interlabia almost as long as lips. Nerve-ring situated at 0.48 from anterior end; deirids 0.57 from anterior end. Oesophagus 3.85 × 0.45; ventriculus 0.15 × 0.18; intestinal caecum 2.95 × 0.25; ventricular appendage 1.49 × 0.12. Tail conical, with pointed tip. Spicules: right 6.10; left 6.30. Pattern of caudal papillae (*sensu* Fagerholm, 1989) as follows: 1 median papilla lens-shaped; 2 large paracloacal papillae, close to cloaca, situated side by side; 4 pairs of distal papillae (2 subventral and 2 sublateral); 1 pair of papilla-like phasmids situated laterally between 2 sublateral distal papillae (Figure 2b).

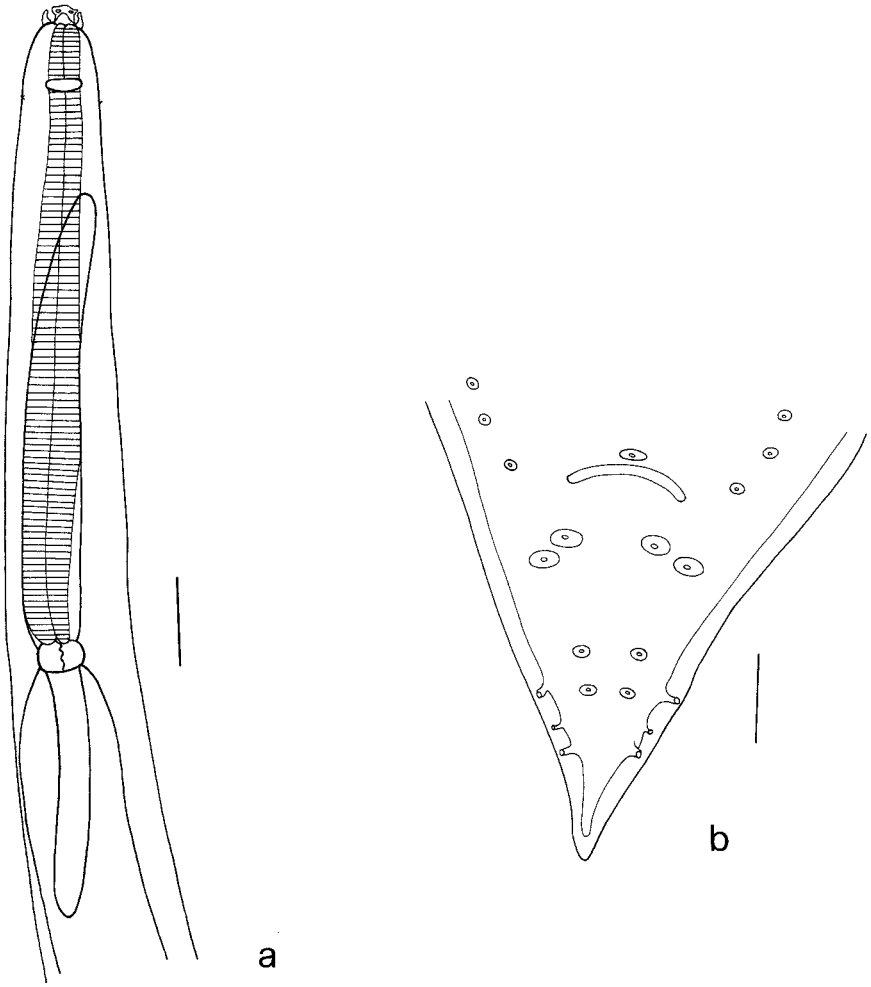

Figure 2. Contracaecum margolisi n. sp. (holotype, male): a. anterior body; b. tail, ventral view. *Scale-bars*: a, 0.5 mm; b, 0.05 mm.

Etymology: The species is named *Contracaecum margolisi* for Dr Leo Margolis, an outstanding scientist who made a valuable contribution to our research on anisakid nematodes.

Definitive hosts and geographical distribution

According to the available literature and the present data, definitive hosts so far identified for the *C. ogmorhini* complex are otariids from the northern and southern hemispheres. In particular, according to the present results, the sea lion *Zalophus californianus* is a definitive host for the sibling species *C. margolisi* n. sp., whereas the fur seals *Arctocephalus pusillus pusillus*, *A. pusillus doriferus* and *A. australis* are definitive hosts for *C. ogmorhini* (*s.s.*). The above-mentioned hosts were previously considered to be parasitised by *C. ogmorhini* (*s.l.*) (Fagerholm & Gibson, 1987; Dailey, 1978; Lent & Freitas, 1948). The elephant seal *Mirounga leonina* from the coast of Argentina was also found, in the present study, to host *C. ogmorhini* (*s.s.*) in mixed infections with *C. osculatum* (Rudolphi, 1802) (*s.l.*) and *C. miroungae* Nikolskii, 1974. Of the 53 anisakid nematodes identified from two mixed infections, eight specimens corresponded to *C. ogmorhini* (*s.s.*) and four to *C. miroungae*, while 41 belonged to a new taxon, yet to be described, genetically related to *C. osculatum* B (Nascetti et al., 1993; and unpublished data). Neither interspecific hybrids, nor recombinant genotypes were detected, which confirms the reproductive isolation of these three species, which are often considered synonymous. Moreover, *C. margolisi* n. sp. and *C. ogmorhini* (*s.s.*) were found to be highly differ-

Table 6. List of hosts found in the present study or reported in literature for *C. ogmorhini (s.l.)* from boreal and austral regions.

Host species	Collecting sites	References
Boreal region		
Zalophus californianus	Baja, Mexico (North Eastern Pacific)	Fagerholm & Gibson, 1987
Zalophus californianus	Southern California	Dailey, 1978
Eumetopias jubatus	Monterey Bay (California)	Fagerholm & Gibson, 1987
Zalophus californianus	Vancouver Island (North Eastern Pacific)	D'Amelio et al., 1994 Zhu et al., 2001; Present study;
Austral region		
Arctocephalus gazella	Amsterdam Island (Indian Ocean)	Campana-Rouget & Paulian, 1960
Arctocephalus tropicalis	South African coast (South Eastern Atlantic)	Fagerholm & Gibson, 1987
Arctocephalus tropicalis	Gough Island (South Atlantic)	Fagerholm & Gibson, 1987
Arctocephalus pusillus pusillus	New Zealand coast (South Pacific)	Zhu et al., 2001; Present study
Arctocephalus pusillus doriferus	South African coast (South Eastern Atlantic)	Zhu et al., 2001; Present study
Arctocephalus australis	Uruguay cost (South Western Atlantic)	Leint & Freitas, 1948; Present study
Mirounga leonina	Valdes Peninsula, Argentina (South Western Atlantic)	Present study

entiated genetically (on average, $D_{Nei} = 6.44$) from adult *C. septentrionale* Kreis, 1955 collected from a fish-eating bird, *Phalacrocorax olivaceus* (Gmelin), off Vancouver Island (our unpublished data).

Further definitive hosts reported in the literature for *C. ogmorhini* (*s.l.*) are the leopard seal *Hydrurga leptonyx* (see Jonston & Mawson, 1941), the Antarctic fur seal *Arctocephalus gazella* (see Campana-Rouget & Paulian, 1960) and the Amsterdam Island fur seal *A. tropicalis* (see Fagerholm & Gibson, 1987) from the southern hemisphere, and Steller's sea lion *Eumetopias jubatus* from the northern hemisphere (Fagerholm & Gibson, 1987; see also Table 6).

No intermediate hosts involved in the life-cycle of the *C. ogmorhini* complex have so far been identified. The collecting sites so far known for the two sibling species, *C. margolisi* n. sp. and *C. ogmorhini* (*s.s.*), as well as previous records of *C. ogmorhini* (*s.l.*), are shown in Figure 3.

Discussion

Two distinct gene pools were found within *C. ogmorhini* (*s.l.*) in the boreal and austral regions. No evidence of gene exchange has been so far detected between the two taxa, *C. margolisi* n. sp. and *C. ogmorhini* (*s.s.*), which parasitise different otariid seals in the two geographical areas. Only allopatric samples have been so far collected, which prevents a demonstration of reproductive isolation in the field (*biological species concept*). However, this is supported by the fixed differences observed between them. The genetic divergence observed between boreal and austral sibling species at nuclear level (average, $D_{Nei} = 0.36$) is similar to that often found between both allopatric and sympatric sibling species of anisakid nematodes, belonging to the genera *Anisakis* Dujardin, 1845, *Contracaecum* Railliet & Henry, 1912 and *Pseudoterranova* Mosgovoi, 1950 (see Nascetti et al., 1993; Bullini et al., 1997; Mattiucci et al., 1997, 1998). Also at mtDNA level, a divergence ($p = 3.7\%$ on 260 bp

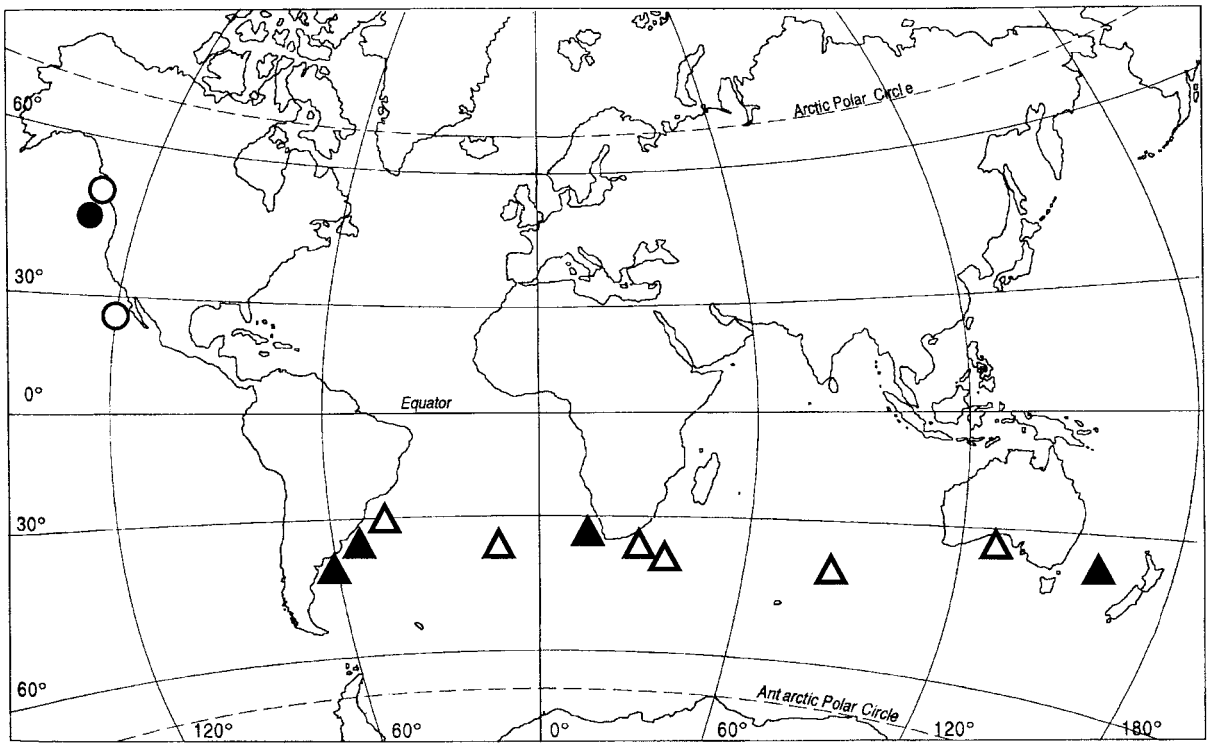

Figure 3. Map showing the collecting sites of populations of *C. ogmorhini* (*s.l.*) so far recorded from the literature (open symbols) and the present study (filled symbols). Circles, boreal region (corresponding to *C margolisi* n. sp.); triangles, austral region (corresponding to *C. ogmorhini* (*s.s.*)).

of *cyt b*) has been observed, for example, between the sibling species *Anisakis simplex* (Rudolphi, 1809) (*s.s.*) and *A. pegreffii* Campana-Rouget & Biocca, 1955 (our unpublished data). On the other hand, a high genetic homogeneity was detected among the *C. ogmorhini* (*s.s.*) populations from both South Atlantic and South Pacific waters (e.g. average $D_{Nei} = 0.02$), a value similar to those previously found among conspecific populations of marine anisakid nematodes (Nascetti et al., 1993; Mattiucci et al., 2001). An indirect estimate of gene flow within *C. ogmorhini* (*s.s.*) from the austral region ($Nm = 4.1$) was at the same level as that found within sibling species of other anisakid nematodes parasitising pinnipeds (Nascetti et al., 1993; Mattiucci et al., 1997).

Genetic heterogeneity within *C. ogmorhini* (*s.l.*) was also recently observed by Zhu et al. (2001) on the basis of nucleotide differences in the ITS-1 and ITS-2 sequences and SSCP profile. The authors showed that individuals from *Arctocephalus pusillus pusillus* from South African waters had the same SSCP profiles as those from *A. pusillus doriferus* from South Australia; whereas those from *Zalophus californianus* from the boreal region exhibited different profiles. Pairwise comparison of the nucleotide differences between the two groups of samples were low: 0.2% and 0.7% for ITS-1 and ITS-2 sequences, respectively (Zhu et al., 2001).

The allopatric distribution of these two species appears to be related to that of their distinct definitive hosts (Table 6, Figure 3), ranging from c. 20°-55° N in boreal Pacific waters, but from 20°-50° S in South Atlantic and South Pacific waters. Although no records of *C. ogmorhini* (*s.l.*) are available from regions between 20° N and 20° S, from either our surveys or literature data, the presence of *C. ogmorhini* off the western South America coast has been verified (Fagerholm, pers. comm.). Potential hosts in this area, which might permit contact between the boreal and austral taxa, are the South American Sea Lion *Otaria byronia* (=*O. flavescens*) (Shaw), *Zalophus californianus wollebaeki* (Siversten) and *Arctocephalus galopagoensis* (Heller). *C. ogmorhini* (*s.l.*) has not been reported from these potential definitive hosts, but *C. osculatum* (*s.l.*) has. Further studies of material from these intermediate regions will enable

a finer assessment of the distribution of *C. margolisi* n. sp. and *C. ogmorhini* (*s.s.*).

Acknowledgements

We are grateful to Dr David I. Gibson for his constructive comments on the draft manuscript and two anonymous referees whose remarks and suggestions were helpful in improving the manuscript. We thank Prof. I. Beveridge, for kindly providing some material, Dr J. David and Dr M. Meyer (Sea Fisheries Research Institute of Cape Town) for their help in collecting samples from South African waters, and to Lic. D. Romero (Museo Municipal de Ciencias Naturales 'Lorenzo Scaglia', Mar del Plata) for providing part of the material from Argentina. We are also grateful to Dr M. Iaconelli for his helpful technical collaboration. The research was co-supported by grants from the Ministero degli Affari Esteri (DG per la Promozione Culturale, Italia Sud-Africa), and from the Universidad Nacional de Mar del Plata (No. E117/97), ISF (No AA/12125) and Agencia Nacional de Promocion Científica y Técnica, Pict 99, 0107111.

References

Bullini, L., Arduino, P., Cianchi, R., Nascetti, G., D'Amelio, S., Mattiucci, S., Paggi, L., Orecchia, P., Plotz, J., Berland, B., Smith, J.W. & Brattey, J. (1997) Genetic and ecological research on anisakid endoparasites of fish and marine mammals in the Antarctic and Arctic-Boreal regions. *In*: Battaglia, Valencia & Walton (Eds) *Antarctic communities. Species, structure and survival*. Cambridge: Cambridge University Press, pp. 39–50.

Brewer, G.J. & Sing, C.F. (1970) *An introduction to isoenzyme techniques*. New York & London: Academic Press, 186 pp.

Campana-Rouget, Y. & Paulian, P. (1960) Mise en synonymie de deux espèces de *Contracaecum*, parasites de mammiferes marins. *Annales of Parasitologie Humaine et Comparée*, **35**, 191–192.

D'Amelio, S., Mattiucci, S., Paggi. L., Orecchia, P., Nascetti, G., Cianchi, R., Bullini. L. & Margolis, L. (1994) Genetic variation of the pinniped parasite *Contracaecum ogmorhini* (Ascaridida, Anisakidae). *Parassitologia*, **36** (suppl.), 40.

Dailey, M.D. (1978) Baseline data on parasites from marine mammals in Southern California. In: *Marine mammals and seabird survey of the Southern California Bight area*. Vol. III. *Principal investigator's reports*. Book 1. *Pinnipedia, Cetacea and Parasitology*. pp. 442–472.

Fagerholm, H.P. (1988) Patterns of caudal papillae in *Contracaecum osculatum* (Nematoda) and some related species from different regions of the world. *International Journal for Parasitology*, **18**, 1039–1051.

Fagerholm, H.P. (1989) Intra-specific variability of the morphology in a single population of the seal parasite *Contracaecum osculatum* (Rudolphi) (Nematoda: Ascaridida) with a redescription of the species. *Zoologica Scripta*, **18**, 33–41.

Fagerholm, H.P. & Gibson, D.I. (1987) A redescription of the pinniped *Contracaecum ogmorhini* (Nematoda, Ascaridoidea) with an assessment of its antiboreal circumpolar distribution. *Zoologica Scripta*, **16**, 19–24.

Harris, H. (1966) Enzyme polymorphism in man. *Proceedings of the Royal Society, Series B*, **164**, 298–310.

Hartwich, G. (1975) Rhabditida und Ascaridida. *Tierwelt Deutschland*, **62**, 1–256.

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G. & Gibson, T.J. (1998) Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences*, **23**, 403–405.

Johnston, T.H. & Mawson, P.M. (1941) Nematodes from Australian marine mammals. *Records of the South Australian Museum* **6**, 429–434.

Johnston, T.H., & Mawson, P.M. (1945) Parasitic nematodes. *Reports of the British, New Zealand and Australian Antarctic Research Expedition*, Series B, **5**, 74–159.

Kumar, S., Tamura, K., Jakobsen, I.B. & Nei, M. (2001) MEGA2: Molecular evolutionary genetics analysis software. *Bioinformatics* (in press).

Lent, H. & Freitas, J.F.T. (1948) Uma colecao de nematodoes, parasitos de vertebrados, do Museu de Historia Natural de Montevideo. *Memórias do Instituto Oswaldo Cruz* **46**, 1–71.

Mattiucci, S., Nascetti, G., Cianchi, R., Paggi, L., Arduino, P., Margolis, L., Brattey, J., Webb, S.C., D'Amelio, S., Orecchia, P. & Bullini, L. (1997) Genetic and ecological data on the *Anisakis simplex* complex with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *Journal of Parasitology*, **83**, 401–416

Mattiucci, S., Paggi, L., Nascetti, G., Ishikura, H., Kikuchi, K., Sato, N., Cianchi, R. & Bullini, L. (1998) Allozyme and morphological identification of *Anisakis, Contracaecum and Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). *Systematic Parasitology*, **40**, 81–92.

Mattiucci S., Paggi L., Nascetti G., Abollo E., Webb, S.C., Pascual., S., Cianchi, R. & Bullini, L. (2001) Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). *International Journal for Parasitology*, **31**, 9–14.

Nascetti, G., Cianchi, R., Mattiucci, S., D'Amelio, S., Orecchia, P., Paggi, L., Brattey, J., Berland, B, Smith, J.W. & Bullini, L. (1993) Three sibling species within *Contracaecum osculatum* (Nematoda, Ascaridida, Ascaridoidea) from the Atlantic Arctic Boreal region: reproductive isolation and host preferences. *International Journal for Parasitology*, **23**, 105–120.

Nei, M. (1972) Genetic distance between populations. *The American Naturalist*, **106**, 283–292.

Okimoto, R., Macfarlane, J.L., Clary, D.O. & Wolstenhome, D.R. (1992) The mitochondrial genomes of two nematodes, *Caenorhabditis elegans* and *Ascaris suum. Genetics*, **130**, 471–498.

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*. Sydney: Academic Press, 410 pp.

Ridgway, S.H. & Harrison, F.R.S. (1981) *Handbook of marine mammals*. Vol. 1. *The walrus, sea lions, fur seals and sea otter*. London: Academic Press, 235 pp.

Rogers, J.S. (1972) Measures of genetic similarities and genetic distance. *Studies in Genetics VII, University of Texas Publications*, **7213**, 145–153.

Selander, R.K, Smith, M.H., Yang, S.Y. & Gentry, J.B. (1971) Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation of the old-field mouse (*Peromyscus po-*

lionotus). Studies in Genetics VI, University of Texas Publications, **7103**, 49–90.

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. Champaign: Illinois Natural History Survey, 31 pp.

Wright, S. (1943) Isolation by distance. *Genetics*, **28**, 114–138.

Wright, S. (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

Wright, S. (1978) *Evolution and the genetics of populations*. Vol. 4. *Variability within and among natural populations*. Chicago: The University of Chicago Press, pp. 580.

Zhu, X., D'Amelio, S., Hu, M., Paggi, L. & Gasser, R. (2001) Electrophoretic detection of population variation within *Contracaecum ogmorhini* (Nematoda: Ascaridoidea: Anisakidae). *Electrophoresis*, **22**, 1930–1934.