Summary:
The genetic relationships among 11 taxa, belonging to the genus Contracaecum (C. osculatum A, C. osculatum B, C. osculatum (s.s.), C. osculatum D, C. osculatum E, C. osculatum baikalensis, C. mirounga, C. radiatum, C. agnornorini (s.s.), C. margolisi) and Phocascaris (Phocascaris cystophorae), parasites as adults of seals, were inferred from sequence analysis (519 bp) of the mitochondrial cytochrome c oxidase subunit II (mtDNA cox2) gene. Phylogenetic analyses obtained from Parsimony (MP) and Neighbour-Joining (NJ) K2P distance values generated similar topologies, each well supported at major nodes. All analyses delineated two main clades: the first encompassing the parasites of the phocid seals, i.e. the C. osculatum species complex, C. osculatum baikalensis, C. mirounga and C. radiatum, with the latter two species forming a separate subclade; the second including the parasites of otarids, i.e. C. agnornorini (s.s.) and C. margolisi. An overall high congruence between mtDNA inferred tree topologies and those produced from nuclear data sets (20 allozyme loci) was observed. Comparison of the phylogenetic hypothesis here produced for Contracaecum spp. plus Phocascaris with those currently available for their definitive hosts (pinnipeds) suggests parallelism between hosts and parasite phylogenetic tree topologies.

**KEY WORDS:** Contracaecum, Phocascaris, seals, mtDNA cox2, allozymes, phylogeny, host-parasite cophylogenetic aspects.

Specific morphological features in the cephalic part of the interlabia, present in the species of genus Contracaecum from birds and seals are considered as diagnostic with respect to species of genus Phocascaris in which interlabia are reduced or absent (Höst, 1932). Despite this morphological character, Berland (1964) suggested that all the species of Contracaecum from seals and those of Phocascaris should belong to the same genus, because of their similar life cycles and definitive hosts (seals). First allozyme studies, carried out on this group of anisakids, have demonstrated that the species of Phocascaris are genetically close to members of Contracaecum that are parasitic as adults in seals (Orecchia et al., 1986; Nascetti et al., 1990). Conversely, Contracaecum spp. maturing in pinnipeds are genetically very distant, sharing no alleles with those Contracaecum spp. maturing in fish-eating birds, even if they exhibit similar morphological features (presence of “interlabia”) (Nascetti et al., 1990; Orecchia et al., 1986). Indeed, allozyme studies have suggested that the genus Contracaecum is highly genetically heterogeneous and polyphyletic. Recent studies (Nadler et al., 2000) based on the LSU rDNA sequence data carried out on several taxa of Contracaecum (including species of Contracaecum from seals and from waterbirds previously identified by allozyme markers) supported the hypothesis that species of genus Phocascaris are nested within the clade of the species of Contracaecum hosted in phocids, thus supporting the monophyly of Phocascaris spp. with those Contracaecum species from phocids. In addition, previous studies using allozyme markers have demonstrated the reproductive isolation and absence of gene flow among sympatric and allopatric populations of Contracaecum spp. hosted by pinnipeds from Arctic and Antarctic regions. They have proved the existence, within some nominal species of the genus Contracaecum maturing in seals, of several biological species, often morphological very similar, and, at times, identical (sibling species). This is the case in C. osculatum sensu lato, considered previously as a cosmopolitan species and parasitic in various definitive seal hosts, which actually comprises at least six sibling species. They are: the arctic members C. osculatum A, C. osculatum B, C. osculatum (s.s.) (Nascetti et al., 1993;
Mattiucci et al., 1998) plus *C. osculatum baicalensis* (D’Amelio et al., 1995), and the two Antarctic members (*C. osculatum D* and *C. osculatum E*) (Orecchia et al., 1994). Single-strand conformation polymorphism (SSCP) based identification of members of the *Contraacaecum osculatum* complex using genetic markers in the internal transcribed spacers of ribosomal DNA (Zhu et al., 2000) and in the three mitochondrial DNA regions cytochrome-c oxidase subunit I (COD), small and large subunits of rRNA (ssrRNA and lsrRNA, respectively) (Hu et al., 2001) allowed the unequivocal differentiation of all the taxa previously disclosed by allozyme markers, except of the two Antarctic taxa, *C. osculatum D* and *C. osculatum E* (Zhu et al., 2000; Hu et al., 2001).

Allozymes and partial sequence analysis of the mitochondrial cytochrome oxidase b (mtDNA cytb) have also demonstrated that the morphospecies *C. ogmorhini* Johnston & Mawson, 1941, which is sometimes also considered a synonym of *C. osculatum* (s.l.) (Johnston & Mawson, 1945; Hartwick, 1964) includes two sibling species (*C. ogmorhini* (s.s.) and *C. margolisi*) (Mattiucci et al., 2003; Timi et al., 2003). The existence of two species within *C. ogmorhini* (s.l.) was also suggested by using molecular markers in the internal transcribed spacers of ribosomal DNA (Zhu et al., 2001). Moreover, allozyme markers have also demonstrated that these anisakid species are ecologically differentiated by their host preference, ecological niche, and life cycle pathway (Nascetti, 1992; Bullini et al., 1994, 1997; Mattiucci & Nascetti, 2007, 2008).

The present paper aims to: *i)* review the genetic structure of eleven taxa from pinnipeds belonging to the genus *Contraacaecum* and *Phocascaris*, based on allozyme markers; *ii)* infer a phylogenetic hypothesis for these species, based on the mitochondrial cytochrome-c oxidase 2 (mtDNA cox2) sequence analysis; *iii)* compare their genetic relationship based on mtDNA cox2 with that provided by allozyme data; and *iv)* gather preliminary data on host-parasite co-phylogenetic aspects.

### MATERIAL AND METHODS

**Parasite Material.**

Anisakid specimens belonging to 11 *Contraacaecum* taxa so far recognized genetically by allozyme markers (i.e. *C. osculatum A, C. osculatum B, C. osculatum (sensu stricto), C. osculatum D, C. osculatum E, C. osculatum baicalensis, C. radiatum, C. mirounga, C. ogmorhini (sensu stricto), C. margolisi*, plus *Phocascaris cystophorae*) were considered in the present paper. The anisakid specimens were first identified to species level by allozymes; they were then sequenced separately from individual nematodes at the mitochondrial DNA region of the cox2. All collection data and specimens analyzed are summarized in Table I. The pinniped hosts, reported in Table I, were stranded animals. They include: seven species of “true seals” (Family Phocidae) belonging to the subfamily Phocinae

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>N allozyme</th>
<th>N mtDNA and specimen code</th>
<th>Collection locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. osculatum A</em></td>
<td>Erignathus barbatus</td>
<td>20</td>
<td>5 (COA1-COA2-COA3)</td>
<td>Newfoundland (Canada)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. osculatum B</em></td>
<td>Pagophilus groenlandicus</td>
<td>10</td>
<td>10 (COB1-COB2-COB3-COB4-COB5-COB6-COB7-COB8-COB9-COB10)</td>
<td>Newfoundland (Canada)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. osculatum (s.s.)</em></td>
<td>Halichoerus grypus</td>
<td>10</td>
<td>2 (COC1-COC2)</td>
<td>Bothnian Bay (Baltic Sea)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. osculatum D</em></td>
<td>Leptonychotes weidellii</td>
<td>10</td>
<td>2 (COD1-COD2)</td>
<td>Ross Sea (Antarctica)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. osculatum E</em></td>
<td>Leptonychotes weidellii</td>
<td>10</td>
<td>5 (COE1-COE2-COE3-COE4-COE5)</td>
<td>Ross Sea (Antarctica)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. osculatum baicalensis</em></td>
<td>Phoca sibirica</td>
<td>5</td>
<td>4 (CBA1-CBA2-CBA3-CBA4)</td>
<td>Lake Baikal</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. cystophorae</em></td>
<td>Cystophora cristata</td>
<td>3</td>
<td>2 (PCYS1-PCYS2)</td>
<td>Newfoundland (Canada)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. radiatum</em></td>
<td>Leptonychotes weidellii</td>
<td>10</td>
<td>3 (CRA1-CRA2-CRA3)</td>
<td>Ross Sea (Antarctica)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. mirounga</em></td>
<td>Mirounga leonina</td>
<td>10</td>
<td>2 (CMI8-CMI9)</td>
<td>Peninsula Valdes (Argentina coast)</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. mirounga</em></td>
<td>Mirounga leonina</td>
<td>20</td>
<td>6 (CMI1-CMI2-CMI4-CMI5-CMI6-CMI7)</td>
<td>Antarctic</td>
</tr>
<tr>
<td></td>
<td>(Phocidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. ogmorhini (s.s.)</em></td>
<td>Arctocephalus australis</td>
<td>10</td>
<td>2 (COGM1-COGM2)</td>
<td>Argentine coast</td>
</tr>
<tr>
<td></td>
<td>(Otariidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. margolisi</em></td>
<td>Zalophus californianus</td>
<td>4</td>
<td>4 (CMAR1-CM22-CMAR3-CMAR4)</td>
<td>California coast</td>
</tr>
<tr>
<td></td>
<td>(Otariidae)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table I. – Taxa of *Contraacaecum* and *Phocascaris* from pinnipeds analyzed genetically. *N* allozyme = number of specimens studied at 20 enzyme loci; *N* mtDNA = number of specimens sequenced at the mtDNA cox2 gene.

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and Monachinae, and two species of fur seals (Family Otariidae) (i.e. Zalophus californianus and Arctocephalus australis) (Table I).

**MULTILOCUS ALLOZYME ELECTROPHORESIS**

All of the anisakids were adults. They were tested by multilocus allozyme electrophoresis. 20 enzyme loci shared by all the *Contracaecum* species here considered, were analysed in the present study. The following enzymes were studied: idditol dehydrogenase (IDDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICDH), 6-phosphoglurocinate dehydrogenase (6PGDH), glycaldehyde-3-phosphate dehydrogenase (G3PDH), superoxide dismutase (SOD), nucleoside phosphorylase (NP), aspartate aminotransferase (AAT), adenylyl kinase (AK), colorimetric esterase (cEST), peptidase (LEU-ALA) (PEP C), mannose phosphate isomerase (MPI), glucose phosphate isomerase (GPI), and phosphoglucomutase (PGM). Details on the enzyme-loci genetically analyzed and electrophoretic procedures used are given in previous papers (Nascetti *et al*., 1993; Mattiucci *et al*., 2003). The nematode specimens were identified to species level by diagnostic markers according to those reported elsewhere (Nascetti *et al*., 1993; Orecchia *et al*., 1994; Mattiucci *et al*., 2003).

**DNA AMPLIFICATION AND SEQUENCING**

45 *Contracaecum* specimens previously genetically characterized at allozyme level, were sequenced using mtDNA cox2. A 519 bp fragment of the cytochrome oxidase 2 (cox2) gene was analysed for all the specimens of *Contracaecum* spp. listed in Table I. Their GenBank accession numbers are: EU477205 (*Contracaecum osculatum A*), EU477204 (*Contracaecum osculatum B*), EU477206 (*Contracaecum osculatum* (s.s.)), EU477205 (*Contracaecum osculatum D*), EU477207 (*Contracaecum osculatum E*), EU477208 (*Contracaecum osculatum hircine*), EU477209 (*Phocascaris cystophorae*), EU477210 (*Contracaecum radiatum*), EU477213 (*Contracaecum miroungae*), EU477211 (*Contracaecum ogmorhini* (s.s.)), EU477212 (*Contracaecum margolis*). Total DNA was extracted from 2 mg of tissue from a single nematode using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin) or cetyltrimethylammonium bromide (Valentini *et al*., 2006). The cox2 gene from each species of *Contracaecum* was amplified using the primers 211F 5’-TTT TCT TCT AGT TAT ATA GAT TGR TTY AT-3’ and 210R 5’-CAC CAA CTC TTA AAA TTA TC-3’ from Nadler & Hudspeth (2000) spanning mtDNA nucleotide position 10,639-11,248 as defined in *Ascaris suum* (Genbank X54253). PCR (polymerase chain reaction) amplification was carried out in a volume of 50 µl containing 30 pmol of each primer, MgCl₂ 2.5 mM (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), PCR buffer 1 x (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), DMSO 0.08 mM, dNTPs 0.4 mM (Sigma-Aldrich, St. Louis, MO), 5 U of *Taq* Polymerase (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) and 10 ng of total DNA. The mixture was denatured at 94°C for 3 min, followed by 34 cycles at 94°C for 30 sec, 46°C for 1 min and 72°C for 1.5 min, followed by post-amplification at 72°C for 10 min. The PCR product was purified using PEG precipitation and automated DNA sequencing was performed by Macrogen Inc. (Seoul, Korea) using primers 210R and 211F. Reference specimens and isolated DNA samples were stored at the Section of Parasitology of the DSSP, “Sapienza” – University of Rome.

**GENETIC DATA ANALYSIS**

Allozyme data obtained here was combined with that gathered in previous allozyme studies performed by the authors. Genetic divergence at interspecific level was estimated using Nei’s indices of standard genetic distance (DNei (Nei, 1972) and chord distance (Dc, Cavalli-Sforza & Edwards, 1967)). Population genetic analyses were performed using BIOSYS-2 software (Swofford & Selander, 1989). Genetic relationships between the considered species of *Contracaecum* and *Phocascaris* were evaluated by UPGMA and Neighbour-Joining (NJ) cluster analysis using PHYLIP version 3.57 (Felsenstein, 1995). Bootstrap consensus analysis (500 replicates) was carried out to verify the robustness of the topologies obtained by PHYLIP (Felsenstein, 1995). The cox2 sequences were aligned using Clustal W (Thompson *et al*., 1994), and a square matrix based on p-distance and K2P was performed using MEGA 3.1 (Kumar *et al*., 2001). Phylogenetic analyses at the interspecific level were performed using “maximum parsimony” (MP) by PAUP* version 4.0 (Swofford, 2005). UPGMA and Neighbour-Joining (NJ) analyses, based on K2P values, were performed using the MEGA 3.1 program (Kumar *et al*., 2001). The reliabilities of the phylogenetic relationships were evaluated using nonparametric bootstrap analysis (Felsenstein, 1985) for the MP and NJ trees. Bootstrap values ≥ 70 were considered well supported (Hills & Bull, 1993). Sequences at the mtDNA cox2 of *Pseudoterranova ceticola* Deardoff & Overstreet, 1982 from *Kogia breviceps* (Genbank DQ116435) was included as outgroup to root the *Contracaecum* phylogenetic trees, based on the relationships of *Contracaecum* (s.s.), and *Pseudoterranova* spp., previously demonstrated in ribosomal and mitochondrial DNA analyses (Nadler & Hudspeth, 2000).

**RESULTS**

**ALLOZYME DIFFERENTIATION**

At allozyme level, the genetic divergence observed between the considered taxa of *Contracaecum* and *Phocascaris* are reported in Table II. The
B. Higher genetic distance values were those found from pinnipeds, so far characterized genetically. P-dissection of Contracaecum species complex (on average, K2P ≈ 0.56, respectively). Higher values were observed in the comparison between the members of the C. osculatum complex and C. mirounga (on average, K2P = 0.13), or with respect to C. radiatum (on average, K2P = 0.14). The highest values were those observed in the comparison of the sibling species of C. osculatum complex with respect to C. ogmorhini species complex (on average K2P = 0.15) (Table III).

**GENETIC RELATIONSHIPS AMONG Contracaecum spp.**

 Parsimony analysis (MP) based on mtDNA cox2 sequence data, using all the codon positions, generated a tree (Fig. 2) showing two main clusters. The first consists of the sibling species of C. osculatum complex, C. mirounga and C. radiatum, while the second cluster includes the species of the C. ogmorhini complex, always well supported. Interestingly, in the first clade, the species P. cystophorae is clustering within the same clade formed by the C. osculatum species complex. In addition, a subclade is produced by C. radiatum and C. mirounga, highly supported (Fig. 2). A congruent tree topology to MP was generated by NJ inferred from the K2P distance analysis (Fig. 3). The same two main clusters were produced: as with the previous analysis, the species of the C. osculatum complex plus P. cystophorae and C. oscul-
Fig. 1 – Alignment of mtDNA cox2 sequences (519 bp) for all currently recognized species of *Coronula asteroides* and *Phocascaris*. One representative of each unique sequence for each taxon was included in the comparison. Dots indicate identity.
Fig. 2 – Cox2 derived Maximum Parsimony (MP) tree using PAUP, for the *Contracaecum* and *Phocascaris* specimens sequenced. Specimen codes are those reported in Table I. Bootstrap values were calculated over 1,000 replicates, percentage ≥ 70% are shown at the internal nodes. *Pseudoterranova ceticola* is the outgroup.
Fig. 3. – Neighbour-Joining (NJ) tree inferred from K2P distance values obtained from mtDNA cox2 by MEGA, showing the genetic relationships among the *Contracaecum* and *Pho-
cascaris* from pinnipeds analyzed. Speci-
cimen codes are those reported in Table I. The scale-bar indicates the distance in substitutions per nucleotide. Bootstrap values were calculated over 1,000 replicates, percentage ≥ 70 % are shown at the internal nodes. *Pseu-
doterranova ceticola* is the outgroup.
latum baicalensis form a clade receiving a well supported bootstrap value. In all the elaborations, the species C. ogmorhini (s.s.) and C. margolisi form always a basal lineage in the tree topology, always well supported. Cluster analysis carried out by different methods (UPGMA) (data not shown) and NJ (Fig. 4) on the considered taxa of Contracaecum and Phocascaris from seals, based on the $D_{ot}$ and $D_{c}$ values, generated similar topologies. Two main clusters were found consistently supported by high bootstrap values, including respectively: cluster 1) a subclade formed by the five members of the C. osculatum complex (i.e. C. osculatum A, C. osculatum B, C. osculatum (s.s.), C. osculatum D and C. osculatum E), plus C. osculatum baicalensis and Phocascaris cystophorae; in this main cluster, C. mirounga is clustering with C. radiatum, forming a separate subclade; cluster 2) formed by the two members C. ogmorhini (s.s.) and C. margolisi (Fig. 4).

**DISCUSSION**

**COX2 BASED PHYLOGENETIC RELATIONSHIPS AMONG CONTRACAECUM SPP. AND COMPARISON WITH ALLOZYME DATA**

Allozymes have demonstrated reproductive isolation between Contracaecum taxa from pinnipeds and provided specific genetic markers for their recognition at any life-history stage (Nascetti et al., 1993; Orecchia et al., 1994; Mattiucci et al., 2003). In the present study, sequence data generated from the mitochondrial cox2 region support previous allozyme studies in recognizing the existence of distinct Contracaecum species based upon evidence of independent evolutionary lineages. The use of different genetic character states inferred from independent data sets provides strong evidence of high heterogeneity within the genus Contracaecum, and implies the presence of genetically distinct taxa of Contracaecum from seals. A phylogenetic hypothesis for all the taxa so far recognized genetically, was here provided by allozyme data and supported by mtDNA cox2 gene data. All the tree topologies derived from the phylogenetic analyses were in substantial agreement where each depicted C. ogmorhini (s.s.) and C. margolisi as a sister group to the remaining species of Contracaecum from the pinnipeds analyzed, forming a monophyletic grouping highly supported when analyzed by MP, and NJ based on K2P distance values, and also by NJ allozymes. Phocascaris cystophorae showed close genetic relationship with C. osculatum (s.s.) and nested within the subclade formed by the C. osculatum species complex. Moreover, sequences of the mtDNA cox2 provided unambiguous phylogenetic evidence for the two Antarctic members of the C. osculatum complex, C. osculatum D and C. osculatum E, as distinct species, whose
reproductive isolation was previously demonstrated in sympathy in the same definitive hosts by allosyme markers (Orecchia et al., 1994).

An overall high congruence was found between the tree topologies obtained from the mitochondrial data sets studied here and the phenetic clustering gathered from nuclear data sets (allozymes) generated previously (Arduino et al., 1995; Bullini et al., 1997) and here reviewed also including the taxa C. mirounga and C. osculatum bicalensis (Fig. 4). Indeed, allosyme clustering, obtained from 20 allosyme loci shared by all the Contracaecum taxa here considered, depicted the existence of two main clusters as well: one formed by the species of C. osculatum complex, plus P. cystophora and the species from the monachine seals, i.e. C. radiatum and C. mirounga. These latter two species form a separate subclade. Moreover, high congruence was found in showing P. cystophora as nesting in the subclade of the first cluster. The discordant placement of C. osculatum A as closely related to the Antarctic species C. osculatum E in the tree generated from allosymes is in contrast to that obtained from cox2 sequences (Fig. 4) where C. osculatum bicalensis shows a closer relationship.

Finally, both cox2 phylogeny derived and clustering based on allosymes clearly demonstrated that the species of the C. osculatum complex plus P. cystophora formed a monophyletic group well supported in all the elaborations, and that C. ogmorhini (s.s.) and C. margolis are basal sister taxa in all the elaborations from the two different data sets. High congruence was also found in showing the close genetic relationship between P. cystophora and C. osculatum (s.s.) as inferred from both the data analyses (Figs 2, 3 and 4).

These findings are in agreement with the phylogenetic hypothesis based on LSU rDNA sequence data sets by Nadler et al. (2000) inferred for seven among the eleven Contracaecum taxa from pinnipeds here considerred, in depicting the species of the C. osculatum complex (except C. osculatum D and C. osculatum E, which were not included in the elaboration by the Authors) plus Phocascaris spp., as forming a monophyletic group. In addition, high consistence between the phylogenetic hypothesis provided by LSU rDNA sequences (Nadler et al., 2000) and that furnished by mtDNA cox2, was also found in indicating C. osculatum bicalensis as the most related species to C. osculatum A, and showing C. radiatum and C. mirounga genetically closely related each other, forming a monophyletic group.

Host-parasite association

AND CO-PHYLGENETIC ASPECTS

The presence of the two major clusters evidenced in the phylogenetic hypothesis of Contracaecum from pinnipeds here proposed is supported also by ecolological evidences regarding specific host-parasite relationships and host preferences evidenced so far in the Contracaecum species from seals from Arctic and Antarctic regions (Nascetti, 1992; Nascetti et al., 1993; Bullini et al., 1997; Mattiucci et al., 2003; Mattiucci & Nascetti, 2007).

The true seals belonging to the Family Phocidae are the main definitive hosts of the members of the C. osculatum complex from the Arctic region; indeed, the bearded seal Erignathus barbatus is the main definitive host for the species C. osculatum A in both the North Atlantic and Pacific Ocean waters, while the harp seal Pagophilus groenlandicus, and the harbour seal Phoca vitulina are suitable hosts for C. osculatum B in both Atlantic and Pacific waters; finally, C. osculatum (s.s.) is the only species present in the grey seal Halichoerus grypus from Baltic Sea (Nascetti et al., 1993; Brattey & Stenson, 1993; Mattiucci et al., 1998; Paggi et al., 1998). In addition, the species C. osculatum bicalensis represents an endemic nematode anisakid species of the relict Baikal seal, Phoca sibirica (D’Amelio et al., 1995). The taxon C. radiatum was so far recognized genetically in the Antarctic region only in the Weddell seal Leptonychotes weddelli (Arduino et al., 1995), although the species was morphologically reported also in the leopard seal Hydrurga leptonyx (Baylis, 1937). The taxon C. mirounga has been so far characterized genetically as hosted by the southern elephant seal Mirounga leonina and, occasionally, also occurring in sympatry with C. ogmorhini (s.s.) in the southern fur seal Arctocephalus australis from the southern hemisphere (Mattiucci et al., 2003).

The otariids in the southern fur seals belonging to the genus Arctocephalus are the definitive hosts of the species C. ogmorhini (s.s.), while the northern fur seal (Zalophus californianus) is the main definitive host so far detected for C. margolis.

Phylogenetic relationships proposed here for the species of Contracaecum from pinnipeds seem to align that of their definitive hosts as suggested by Arnason et al. (1995) using the complete sequences of the mitochondrial cytochrome b gene (mtDNA cytb) of the Phocidae, Odobenidae and Otariidae, and recently by Démère et al. (2003) using a composite tree inferred from the basic topology of generic level, morphological and molecular data, and fossil taxa to propose an integrated hypothesis for pinniped evolutionary biogeography. According to these data elaborations, the Pinnipedia includes three major monophyletic clades: 1) the Otariidae (fur seals and sea lions), 2) the Odobenidae (walrus), and 3) the Phocidae (true seals), plus the extinct desmatophocids (Démère et al., 2003). In this combined tree, the fur seals and sea lions, including the Otariinae (Zalophus californianus) and the Arctocephalinae (Arctocephalus spp.), with the Arctocephalus spp. from the Southern hemisphere are repre-
sented, in the host phylogenetic tree inferred from different data sets (Arnason, et al., 1995; Démère et al., 2005), as the basal groups. In accordance with that analysis, the branching order here proposed for the *Contracaecum* taxa depicts that nematodes from the Otariidae (i.e. *C. ogmorhini* s.s.) from *Arctocephalus* spp. and *C. margolisi* from *Zalophus californianus* always occupy a basal lineage of the parasite phylogenetic tree, with the species of the *C. osculatum* complex from the Phocinae (true seals) as the most derived.

**CONCLUDING REMARKS**

The present study demonstrated clearly the usefulness of the mitochondrial *cox2* gene for unequivocal recognition of seal-parasitizing *Contracaecum* taxa so far genetically characterized by allozymes. On the other hand, analyses of the substitution patterns for mtDNA genes of nematodes have suggested that they are very useful markers for identifying and differentiating cryptic species and for determining relationships of closely related species (Blouin et al., 1998; Blouin, 2002; Hu et al., 2004 and references therein, Hu & Gasser, 2006). The same mtDNA region was demonstrated to be useful in distinguishing closely related anisakid taxa previously characterized by allozyme markers, belonging to the genus *Anisakis* (Valentini et al., 2006), and to the genus *Contracaecum* maturing in fish-eating birds (Mattiucci et al., 2008). The genetic divergence of mtDNA here estimated among *Contracaecum* from seals is of the same level as that previously found between other anisakid species (Valentini et al., 2006; Mattiucci et al., 2008). Bootstrap analysis has revealed an overall high congruence between the genetic relationships proposed for this group of anisakid species inferred from two different data sets (nuclear and mitochondrial). Bootstrap analysis is based on both genetic markers also strongly supported the hypothesis that species of *Phocascaris* are more closely related to members of the *C. osculatum* complex; a result consistent with nuclear rDNA evidence (Nadler et al., 2000). This finding supports Berland’s proposal that the *Contracaecum* species that have seals as definitive hosts should be included in a same genus with *Phocascaris* species. Moreover, as *Phocascaris* was erected for those species devoid of “interlabia”, the morphological character of “interlabia” has proved to be of no taxonomic value in the seal ascarids. Finally, the parallelism between host and parasite phylogenies so far discovered between *Contracaecum* spp. (plus *Phocascaris* spp.) and seals seems to suggest that some level of co-evolution, including co-divergence and host-switching events, could have accompanied the speciation of these group of nematodes and their definitive hosts. A broader set of data is needed to confirm these findings. Evidence for such co-phylogenetic events has recently been reported between *Anisakis* taxa and their cetacean definitive hosts by Mattiucci & Nascetti (2006, 2008).

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