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Steven A. Nadler

University of California - Davis, sanadler@ucdavis.edu

S. D'Amelio

University of Rome "La Sapienza"

H. P. Fagerholm

Åbo Akademi University

B. Berland

Universitetet i Bergen

L. Paggi

University of Rome "La Sapienza"

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Phylogenetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Høst, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data

S. A. NADLER^{1*}, S. D'AMELIO², H.-P. FAGERHOLM³, B. BERLAND⁴ and L. PAGGI²

¹Department of Nematology, University of California, Davis, California 95616-8668, USA

²Institute of Parasitology, University of Rome 'La Sapienza', Piazzale Aldo Moro, 5, 00185 Rome, Italy

³Institute of Parasitology, Department of Biology, Åbo Akademi University, Artillerigatan 6, BioCity, FIN-20520 Åbo, Finland

⁴Zoologisk Institutt, Universitetet i Bergen, Allegt. 41, N-5007, Bergen, Norway

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SUMMARY

Nuclear-encoded large-subunit ribosomal DNA sequences were used to infer a phylogenetic hypothesis for 17 taxa (16 nominal species) of the genera *Contracaecum* and *Phocascaris*. Phylogenetic trees based on these data have been used to assess the validity of the taxonomic distinction between these genera, which was based on the presence or absence of certain structural features, rather than on explicit hypotheses of evolutionary history. Phylogenetic hypotheses based on parsimony, likelihood, and neighbor-joining analyses of these sequence data strongly support the hypothesis that species of *Phocascaris* are nested within the clade of *Contracaecum* species hosted by phocid seals, and are more closely related to species of the *Contracaecum osculatum* complex than to other *Contracaecum* species. Alternative tree topologies representing *Phocascaris* as not nested within the *C. osculatum* complex were significantly worse interpretations of these sequence data. Phylogenetic analysis also provides strong support for the monophyly of all taxa (*Contracaecum* and *Phocascaris*) from phocid seals, which is consistent with Berland's (1964) proposal that such species form a natural group; however, his proposal to recognize all species in phocid seals as *Phocascaris*, with all species from birds as *Contracaecum* would result in a paraphyletic *Contracaecum*, according to the molecular phylogenetic hypothesis.

Key words: phylogeny, *Contracaecum*, *Phocascaris*, Ascaridoidea, ribosomal DNA.

INTRODUCTION

The nematode superfamily Ascaridoidea contains approximately 52 genera, and many species of these parasites are of medical or economic significance. Within the family Anisakidae Skrjabin & Karokhin, 1945, the subfamily Contracaecinae contains 3 genera: *Contracaecum* Railliet & Henry, 1912, *Phocascaris* Høst, 1932 and *Galeiceps* Railliet, 1916. Species in these genera have aquatic life-cycles and homeotherm final hosts. Adult *Contracaecum* are commonly reported from birds and seals in all regions of the world, and more rarely from dolphins, whereas species of *Phocascaris* occur only in phocid seals. Species of *Galeiceps* are mainly found in sea otters, but because specimens were unavailable for our molecular research, this genus is not discussed further herein. *Contracaecum* and *Phocascaris* are distinguished by the presence of interlabia in species of the former, and their absence or strong reduction

in *Phocascaris*. The presence of labial denticulation in *Phocascaris* but not in *Contracaecum* is another diagnostic feature. Notwithstanding such structural differences, Berland (1964) suggested that the species of *Contracaecum* occurring in seals and the species formerly described as *Phocascaris* must all be referred to one genus, *Phocascaris*, with those species maturing in fishes and birds remaining in the genus *Contracaecum*. Species from fishes were subsequently reassigned to *Thynnascaris*, and are now, in part, *Hysterothylacium* Ward and Magath, 1917 (Deardorff & Overstreet, 1980). Berland's proposal was not accepted by Hartwich (1974) in producing a classification and key for the Ascaridoidea, and the systematics of these genera remains unresolved. This uncertainty has remained primarily because it is unknown if the presence and absence of interlabia and denticles demarcates distinct evolutionary lineages for *Contracaecum* and *Phocascaris*, or conversely, if some species of *Contracaecum* share a more recent common ancestor with *Phocascaris* than with other *Contracaecum* species.

Orecchia *et al.* (1986) and Nascetti *et al.* (1990), based on allozyme data, showed that *Contracaecum*

* Corresponding author: Department of Nematology, University of California, Davis, CA 95616-8668, USA. Tel: +530 752 2121. Fax: +530 752 5809. E-mail: sanadler@ucdavis.edu

species from seals appeared to be genetically most similar to each other, sharing no alleles with congeneric species from birds. In addition, genetic distances indicated that the genus *Phocascaris* was not only more similar to seal species of *Contracaecum*, but nested within the cluster representing *Contracaecum osculatum sensu lato*, which includes a complex of 6 sibling species (Nascetti *et al.* 1993; Orecchia *et al.* 1994; D'Amelio *et al.* 1995). The species *C. osculatum sensu stricto* (from the Baltic Sea), was observed to be more genetically dissimilar from the other sibling species of the complex than *Phocascaris*. These genetic results demonstrated that revision of these genera was required (Paggi & Bullini, 1994). With respect to additional morphological evidence obtained by scanning electron microscopy, Fagerholm's (1991) data on the distribution of papillae in the male tail was consistent with 2 groups; one that mainly included *Phocascaris* and *Contracaecum* species from phocid seals, and another that primarily included *Contracaecum* species from birds and *C. ogmorhini* from otariid seals. These groups conflict with those defined strictly by interlabia and labial denticulation, which reinforces the need for additional data to address this systematic problem.

In the present study representative species of *Contracaecum* and *Phocascaris* were used to develop a phylogenetic hypothesis based on nuclear ribosomal DNA sequences. This phylogenetic hypothesis is used to evaluate existing taxonomic proposals that were based on morphological similarity, and provides a framework for interpreting patterns of character evolution.

MATERIALS AND METHODS

PCR and sequencing of specimens

Taxa sequenced, collection localities, host, and GenBank accession numbers of sequences are provided in Table 1. The study was conducted using specimens previously identified to the species level by isoenzyme analysis, except for *Phocascaris* sp. (provided by J. Bratney) and *C. eudyptulae* (provided by R. Gasser). Tissue samples from these nematode specimens were preserved in 95% ethanol for subsequent nucleic acid extraction. Immediately prior to extraction, a piece of the ethanol-preserved worm was rehydrated in TE buffer (pH 8.0) at 4 °C. Nucleic acids were extracted from the rehydrated tissue using a DNA binding method ('glass milk') employing isothiocyanate and guanidinium (ID Pure Genomic DNA Kit, ID Labs Biotechnology). DNA for outgroup species was obtained from frozen tissues by phenol-chloroform extraction as reported in Nadler & Hudspeth (1998). Nucleic acids obtained from *Contracaecum* and *Phocascaris* specimens were not quantified prior to use in polymerase chain reaction (PCR) amplifications, instead, each extract

was concentrated by vacuum evaporation to 14 µl, and 2 µl of this solution was used for each PCR. For the outgroup species, 100 ng of DNA was used per PCR.

A region within the 5'-end of the nuclear large subunit ribosomal DNA (26/28S rDNA) containing the D2 and D3 domains was amplified by PCR. Design of the forward PCR primer (no. 391, 5'-AGCGGAGGAAAAGAACTAA, positions 3745-3764 in *Caenorhabditis elegans* GenBank X03680) was described in Nadler & Hudspeth (1998). Alternative forward PCR primers (primer no. 538, 5'-AGCATATCATTTAGCGGAGG, positions 3733-3752, primer no. 542, 5'-CCATCG-GCCACTGGTCGC, positions 3817-3834), were also used for 5 species (*C. osculatum sensu stricto*, *C. osculatum* B, *C. microcephalum*, *C. radiatum*, and *C. rudolphii* A) that yielded minor non-target products with primer no. 391). The reverse primer (no. 501, 5'-TCGGAAGGAACCAGCTACTA, positions 4681-4700) was designed by Thomas *et al.* (1997). An alternative reverse PCR primer (no. 535, 5'-TAGTCTTTTCGCCCTATAC, positions 4651-4669), was used for 2 species (*C. rudolphii* A and *C. septentrionale*), that yielded minor non-target products with primer no. 501.

PCR conditions were adjusted empirically to optimize reaction specificity for individual species. Typical conditions for 25 µl reactions included an initial DNA denaturation at 94 °C for 3 min, followed by 33 cycles of 94 °C for 30 sec, 55 °C for 20 sec, and 72 °C for 45 sec, and a post-amplification extension at 72 °C for 7 min. Proofreading polymerase (DyNAzyme EXT, Finnzymes Oy) was used for amplification, with a final MgCl₂ concentration of 2 mM. A 1 µl aliquot of the PCR product was subjected to agarose gel electrophoresis (1.3% agarose in 1 × TBE buffer) to confirm product size (approximately 1 kb) and yield. PCR products were prepared for direct sequencing by enzymatic treatment using exonuclease I and shrimp alkaline phosphatase (PCR product Presequencing Kit, Amersham Corporation). Sequencing reactions were performed using BigDye (Perkin-Elmer) terminator cycle sequencing chemistry and reaction products were separated and detected using an ABI 377 automated DNA Sequencer. Sequences for each species were completely double-stranded for accuracy using the PCR primers and 2 or more internal sequencing primers as required. Internal sequencing primers (no. 116, < 5'-AAGCTCTCAGCGCATA-CC, no. 117, > 5'-AGAGAGTTCAAGAGGGC-GT, and no. 503, < 5'-CCTTGGTCCGTGTTT-CAAGACG) were designed based on data obtained during this and previous (Nadler & Hudspeth, 1998) studies. Contig assembly and sequence ambiguity resolution was performed with the aid of the Sequencher program ver.3.0 (Gene Codes, Ann Arbor MI).

Table 1. Specimen information and GenBank accession numbers for ingroup and outgroup species studied

Species	Definitive host	Stage	Collection locality	GenBank Accession No.
(1) <i>Phocascaris</i> sp.	<i>Phoca groenlandica</i>	Adult	Gulf of St Lawrence, Newfoundland, Canada	AF226575
(2) <i>Phocascaris cystophorae</i>	<i>Cystophora cristata</i>	Adult	Front, Newfoundland, Canada	AF226578
(3) <i>Phocascaris phocae</i>	<i>Phoca groenlandica</i>	Adult	Sotra, Norway	AF226584
(4) <i>Contracaecum osculatum</i> A	<i>Erignathus barbatus</i>	Adult	St Anthony, Newfoundland, Canada	AF226583
(5) <i>Contracaecum osculatum</i> B	<i>Phoca groenlandica</i>	Adult	Front, Newfoundland, Canada	AF226580
(6) <i>Contracaecum o. baicalensis</i>	<i>Phoca sibirica</i>	Adult	Lake Baikal, Russia	AF226589
(7) <i>Contracaecum osculatum sensu stricto</i>		Larva	Geta, Åland, Finland	AF226576
(8) <i>Contracaecum radiatum</i>	<i>Leptonychotes weddelli</i>	Adult	Weddell Sea, Antarctica	AF226577
(9) <i>Contracaecum miroungae</i>	<i>Mirounga leonina</i>	Adult	King George Island, Antarctica	AF226581
(10) <i>Contracaecum ogmorhini</i>	<i>Arctocephalus pusillus pusillus</i>	Adult	South Africa	AF226582
(11) <i>Contracaecum microcephalum</i>	<i>Phalacrocorax pygmaeus</i>	Adult	Scutari lake, Yugoslavia	AF226573
(12) <i>Contracaecum multipapillatum</i>	<i>Pelecanus crispus</i>	Adult	Psatatopi, Greece	AF226574
(13) <i>Contracaecum micropapillatum</i>	<i>Pelecanus onocrotalus</i>	Adult	Assuan, Egypt	AF226587
(14) <i>Contracaecum rudolphii</i> A	<i>Phalacrocorax carbo</i>	Adult	Policoro, Italy	AF226585
(15) <i>Contracaecum rudolphii</i> B	<i>Phalacrocorax carbo</i>	Adult	Policoro, Italy	AF226579
(16) <i>Contracaecum eudyptulae</i>	<i>Eudyptula minor</i>	Adult	Philip Island, Victoria, Australia	AF226586
(17) <i>Contracaecum septentrionale</i>	<i>Phalacrocorax carbo</i>	Adult	Husavik, Iceland	AF226588
(18) <i>Hysterothylacium pelagicum</i>	<i>Coryphaena hippurus</i>	Adult	Gulf Coast of Mississippi, United States	AF226590
(19) <i>Hysterothylacium auctum</i>	<i>Zoarces viviparus</i>	Adult	Geta, Åland, Finland	AF226591
(20) <i>Heterocheilus tunicatus</i>	<i>Trichechus manatus</i>	Adult	Citrus County, Florida, United States	AF226592

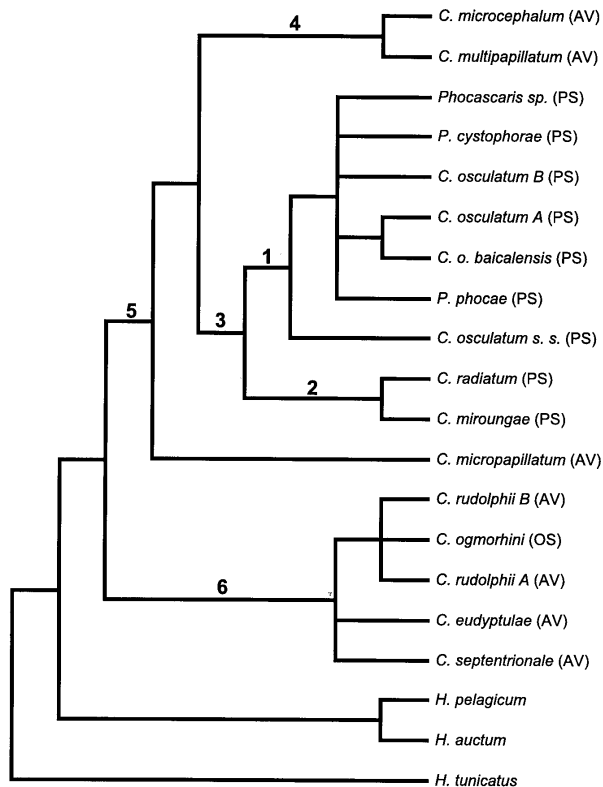


Fig. 1. Strict consensus of 2 most-parsimonious trees inferred from analysis of all 3 datasets (FA, MUNC, GR) by branch-and-bound searches. This tree is also one of the 2 most parsimonious trees recovered for each dataset (the other tree in each case depicts resolution of the *edyptulae/septentrionale* polytomy as (*Contracaecum septentrionale*, (*C. edyptulae*, (*C. rudolphii* B, *C. rudolphii* A, and *C. ogmorhini*))). Numbers refer to clades discussed in the text. Two-letter host designation follows ingroup names (AV, avian host; PS, phocid seal host; OS, otariid seal host).

Sequence analysis

Regions corresponding to the PCR amplification primers were removed from the sequences prior to multiple sequence alignment and phylogenetic analysis because primer incorporation during amplification masks potential mismatches (substitutions) that may occur in priming sites. Sequences were aligned initially using CLUSTAL X ver. 1.53b (Thompson *et al.* 1997), and the resulting output was adjusted manually to improve homology statements. This process yielded an alignment of 985 characters (the 'full alignment', abbreviated FA). A second data set ('gap recoded', abbreviated GR) was produced from the FA wherein inferred gaps that were potentially parsimony-informative were recoded conservatively such that each unambiguous contiguous gap was represented as one character and 'nucleotide present' was coded as the alternative character state (Swofford, 1993; Crandall & Fitzpatrick, 1996). This GR dataset was used for parsimony analysis; all unrecoded gaps (the original inferred indels) were treated as missing data in

parsimony analyses. The effect of alignment ambiguity on phylogenetic analysis was explored by producing a third data matrix ('minus uncertainty', abbreviated MUNC) from the FA that excluded 23 characters where inferences of positional homology were judged to be tenuous. Datasets and tree-files have been deposited in TreeBASE (Sanderson *et al.* 1994).

Data were analysed by 3 tree inference methods: unweighted maximum parsimony (MP) and neighbor-joining (NJ) trees were inferred using PAUP* 4.0 (beta version 4.0b2; Swofford, 1998). PUZZLE 4.0.2 (Strimmer & von Haeseler, 1996) was used to approximate a maximum likelihood tree; this program was also used to estimate the proportion of invariable sites used for calculating pairwise (log-determinant) distances for the neighbor-joining tree, and testing the nucleotide composition of individual species for departure from homogeneity (stationarity). Parsimony analyses were performed using the branch-and-bound search option. Bootstrap parsimony analyses were performed using heuristic searches (simple stepwise addition, TBR branch-swapping, MULPARS); all bootstrap trees were inferred using 2000 replicates. Reported consistency and homoplasy indices (C.I., H.I.) do not include uninformative characters. Selected alternative evolutionary hypotheses were compared to the most parsimonious trees using the winning sites test (Prager & Wilson, 1988) and Templeton's modified parsimony test (Templeton, 1983) using PAUP* 4.0. Three outgroup species were included in all analyses (*Heterocheilus tunicatus*, *Hysterothylacium pelagicum*, and *Hysterothylacium auctum*), and the trees rooted by *H. tunicatus*, a choice supported by more taxonomically comprehensive phylogenetic analyses of ascaridoid nematodes (Nadler & Hudspeth, 1998, 2000).

RESULTS

For the full alignment (985 characters), 719 sites were constant among all 20 taxa (850 sites among the *Contracaecum* and *Phocascaris* ingroup), and 144 of the variable sites were parsimony-informative (73 among the ingroup). The MUNC and GR alignments had 139 and 147 parsimony-informative characters, respectively. Average nucleotide frequencies were, A (0.221), C (0.236), G (0.320), T (0.223). Chi-square tests showed no statistically significant departures from homogeneity of base frequencies among these 20 taxa. Sequences of *P. cystophorae*, *P. phocae*, and *Phocascaris* sp. were identical; all other species had a minimum of 1 difference (maximum of 85 among ingroup taxa) in pairwise comparisons. Comparison of log likelihoods for trees inferred with (−3452.73) and without (−3422.94) the constraint of a molecular clock by likelihood ratio test revealed that the tree with

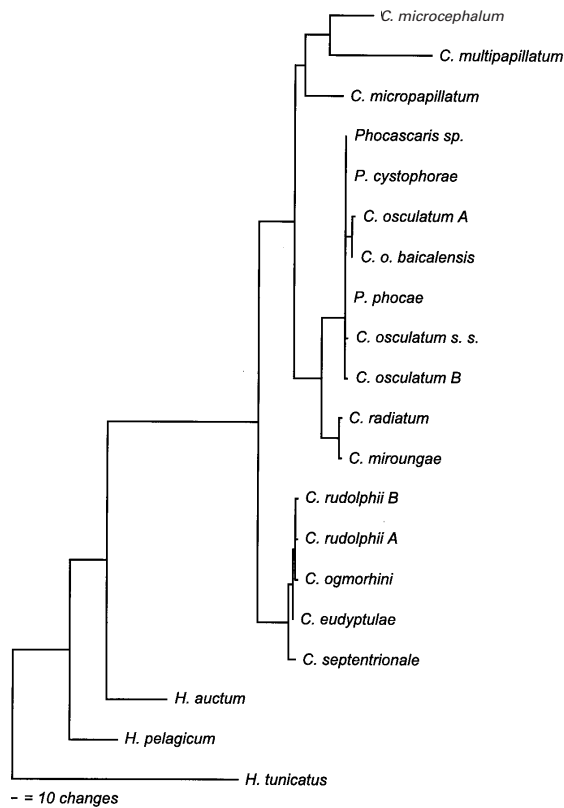


Fig. 2. Neighbor-joining tree inferred for the full alignment dataset using log-determinant distances. Branch lengths are scaled to the expected number of substitutions per site.

molecular clock assumption is significantly worse ($P < 0.05$).

Maximum parsimony analysis yielded the same 2 trees for all 3 datasets (FA, MUNC, and GR), with C.I./H.I. in each of 0.67/0.33 and respective lengths of 421, 406, and 435 steps. The 2 tree topologies differed only with respect to the resolution of *C. eudyptulae* and *C. septentrionale* within the clade containing these species and *C. rudolphii* B, *C. rudolphii* A, and *C. ogmorhini*. In 1 of the trees, *C. eudyptulae* and *C. septentrionale* were members of a basal polytomy within this larger clade (Fig. 1), whereas in the second tree their relationships were resolved as (*C. septentrionale*, (*C. eudyptulae*, (*C. rudolphii* B, *C. rudolphii* A, and *C. ogmorhini*))). The strict consensus of these 2 most parsimonious trees includes clades (Fig. 1, numbers refer to Fig. labels) with taxa representing: (1) sampled species from the *C. osculatum* complex and *Phocascaris* taxa (all from phocid hosts), (2) *C. radiatum* and *C. miroungae* (sampled species from antarctic phocids), (3) all sampled species from pinniped hosts except *C. ogmorhini*, (4) *C. microcephalum* and *C. multipapillatum* (2 of 7 sampled species that mature in birds), (5) clades 3 and 4 plus *C. micropapillatum*, (6) the remaining 4 species that mature in birds, plus *C. ogmorhini* (from the south african fur seal *Arctocephalus pusillus*). Trees inferred by NJ (Fig. 2) and

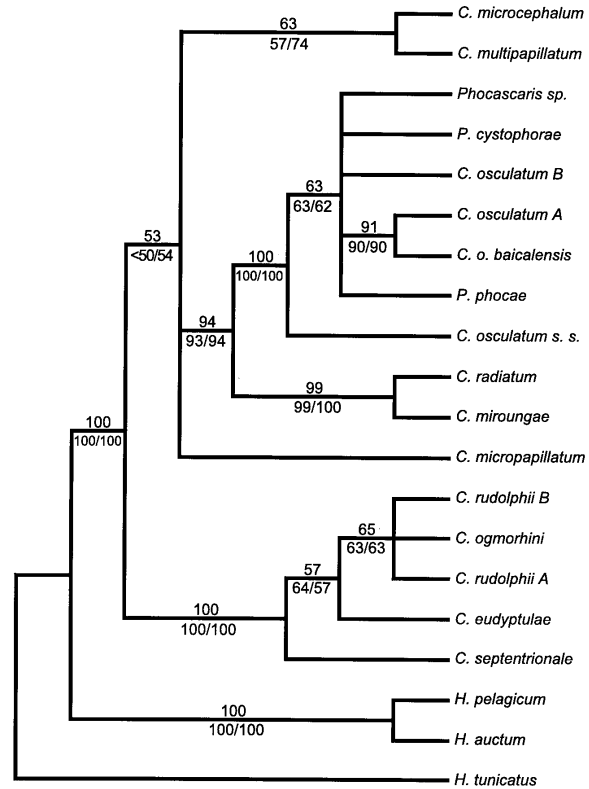


Fig. 3. Maximum parsimony 50% majority-rule bootstrap consensus tree obtained from analysis of 2 of the 3 datasets (FA and GR). The MP bootstrap tree inferred for the MUNC dataset differed only in the position of *Contraceacum micropapillatum*, which was collapsed in a polytomy to the next most basal node (representing *Contraceacum* and *Phocascaris* monophyly). Bootstrap percentages of clades (based on 2000 iterations) are shown above (FA) and below (MUNC/GR) internal nodes.

ML (not shown) for the FA dataset also recovered clades 1–4 and 6; the NJ tree inferred for the MUNC dataset included clades 1, 4, and 6. Bootstrap parsimony analysis of the three datasets (FA, MUNC, GR) revealed strong support (Fig. 3) for clades 1–3 and 6, whereas clade 4 received weak-moderate support, and clade 5 very weak support (in the MUNC dataset, this clade was not recovered in the 50% majority rule bootstrap consensus tree). Other groups receiving strong support in all bootstrap MP trees (FA, MUNC, GR datasets) included a clade of *C. osculatum* A. and *C. o. baicalensis*, monophyly of all *Contraceacum* plus *Phocascaris*, and a clade representing the 2 *Hysterothylacium* species (Fig. 3). In trees inferred by all methods, including bootstrap consensus trees, *Phocascaris* species are within the *osculatum* complex and nested in the clade containing *Contraceacum* from phocids. Likewise, *C. ogmorhini* (hosted by an otariid) is always part of a clade that otherwise includes only species with avian definitive hosts.

Alternative tree topologies were compared to the 2 most parsimonious trees (listed as Trees 1 and 2 in Table 2) using statistical methods. Alternative

Table 2. Alternative topology tests based on 28S sequence data and the full alignment dataset

(Rejection and acceptance of alternative topologies was the same for the MUNC and GR datasets. Topologies 1 and 2 are the 2 equally parsimonious trees; alternative topologies 3–9 are explained in the Results section and defined by standard parenthetical notation.)

Tree no.*	Tree length	Length difference from MP tree	Templeton's test <i>P</i>	Winning-sites <i>P</i>
1	431	—	—	—
2	431	—	—	—
3	434	3	0.4054	0.5811
4	467	36	< 0.0001	< 0.0001
5	464	33	< 0.0001	< 0.0001
6	443	12	0.0005	0.0005
7	449	18	0.0001	< 0.0001
8	475	44	< 0.0001	< 0.0001
9	465	34	< 0.0001	< 0.0001

* Numbers within parentheses refer to species in the sequence listed in Table 1 (with tree rooted by *H. tunicatus*, number 20).

1 = (20, ((18, 19), (((15, 10, 14), 16), 17), (13, ((11, 12), ((8, 9), (7, (1, 2, 5, (4, 6), 3))))))))))

2 = (20, ((18, 19), (((15, 10, 14), 16), 17), (13, ((11, 12), ((8, 9), (7, (1, 2, 5, (4, 6), 3))))))))))

3 = (20, ((18, 19), ((((((15, 10, 14), 16), 17), 13), (11, 12)), ((8, 9), (7, (1, 2, 5, (4, 6), 3))))))))))

4 = (20, ((18, 19), ((((((15, 14), 16), 17), 13), (11, 12)), (10, ((8, 9), (7, (1, 2, 5, (4, 6), 3))))))))))

5 = (20, ((18, 19), ((3, 2, 1), (((15, 10, 14), 16), 17), (13, ((11, 12), ((8, 9), (7, (5, (4, 6))))))))))

6 = (20, ((18, 19), (((15, 10, 14), 16), 17), (13, ((11, 12), ((3, 2, 1), ((8, 9), (7, (5, (4, 6))))))))))

7 = (20, ((18, 19), (((15, 10, 14), 16), 17), (13, (11, ((8, 9), (12, (7, (1, 2, 5, (4, 6), 3))))))))))

8 = (20, ((18, 19), (((15, 14), 16), 17), (13, ((11, 12), (((8, 9), 10), (7, (1, 2, 5, (4, 6), 3))))))))))

9 = (20, (((15, 10, 14), 16), 17), (18, 19)), (13, ((11, 12), ((8, 9), (7, (1, 2, 5, (4, 6), 3))))))

topologies compared included (numbers refer to trees in Table 2): (3) 2 clades, 1 including all parasites of birds plus *C. ogmorhini*, the other including all sampled phocid parasites, (4) *C. ogmorhini* as the sister taxon to the species from phocids, (5) monophyletic *Contracecum* as the sister clade to a monophyletic *Phocasaris*, (6) *Phocasaris* as the sister group to *Contracecum* from phocids, (7) *C. multipapillatum* as sister to members of the *osculatum* complex plus *Phocasaris*, (8) *C. ogmorhini* as sister to the species from antarctic phocids, and (9) *Hysterothylacium* nested within ingroup. When alternatives existed for defining these trees, the most parsimonious topology that represented the alternative was used. With the exception of alternative tree number 3, all other tested tree topologies were significantly worse (Table 2) by both statistical tests for all three datasets (FA, MUNC, and GR).

DISCUSSION

The 5'-end of the nuclear 28S rDNA contains 2 variable domains (D2 and D3) that appear to have an

appropriate rate of substitution for inferring evolutionary relationships among species of the superfamily Ascaridoidea (Nadler & Hudspeth, 1998). This region of rDNA from *Contracecum* and *Phocasaris* species showed moderate levels of conservation, such that multiple sequence alignment (and inference of positional homology) was relatively straightforward. Furthermore, alignment ambiguity had little effect on the resulting phylogenetic hypotheses. For example, the dataset without regions of potential alignment ambiguity (MUNC) excluded only 2.3% of sites in the full alignment, and the MP trees recovered from the MUNC dataset were the same as those inferred from the full alignment and the gap-recoded data. However, the relative conservation of 28S sequences among these species was also reflected in the fraction of parsimony-informative sites (7.4%) among *Contracecum* and *Phocasaris* species, and by the small number of sequence differences between certain species. Accordingly, polytomies for several closely related species resulted from the absence of shared-derived characters, rather than from conflict among different

informative characters. These 28S sequences were useful for developing phylogenetic hypotheses among relatively distantly related lineages within the *Contracaecinae*. An advantage of this 28S region among the studied taxa is that the sequences did not show significant departures from a model of nucleotide frequency homogeneity, which is a condition necessary for maximum likelihood tree inference and the use of certain DNA distances for inferring trees. However, comparison of likelihood scores with and without the constraint of a molecular clock revealed that a model of clock-like sequence evolution was significantly worse for these taxa. Increasing the resolution within certain clades (e.g. [*C. rudolphii* A, *C. rudolphii* B, *C. ogmorhini*]) will require data from sequences that evolve more rapidly, such as the rDNA internal transcribed spacers (Powers *et al.* 1997). Likewise, since no sequence differences were observed between *P. cystophorae* and *P. phocae*, more rapidly evolving genes should be sequenced to test if these taxa can be delimited as species using nucleotide sequences.

Bootstrap parsimony analysis showed that certain clades recovered in MP and NJ analyses were strongly supported, and statistical tests revealed that certain alternative hypotheses of relationships were significantly worse interpretations of these data. Bootstrap analysis strongly supports the hypothesis that species of *Phocascaris* are more closely related to members of the *C. osculatum* complex than to other *Contracaecum* species, a result that is consistent with isoenzyme evidence (Paggi & Bullini, 1994). In MP, NJ, and ML trees, *Phocascaris* species were nested within the clade of *Contracaecum* spp. from phocids. Alternative topologies depicting *Phocascaris* as the sister group to *Contracaecum* from phocids, or as the sister group to a clade of all sampled *Contracaecum* spp. were significantly worse in statistical comparisons, and these results are inconsistent with recognizing *Phocascaris* as comprising a distinct evolutionary lineage (such as a separate genus). Although these 28S data are uninformative regarding whether these *Phocascaris* taxa are each others closest relatives within the *osculatum* complex, it is possible that the shared characters proposed to diagnose *Phocascaris* (absence or reduction of interlabia, and presence of labial denticles) result from recurrent similarity (homoplasy) rather than homology. Such an explanation (high homoplasy) has been invoked to explain the presence and absence of interlabia and labial denticles in more taxonomically comprehensive phylogenetic analyses of ascaridoids (Nadler & Hudspeeth, 1998, 2000).

These data provide strong support for the monophyly of all *Contracaecum* and *Phocascaris* of phocid seals, which is consistent with Berland's proposal that all such species form a natural group. Fagerholm (1988, 1989, 1991) also reported data showing that the pattern of caudal papillae is distinct

in species from phocids. In addition, the 28S-based trees depicted *C. osculatum sensu stricto* as the most basal species of the *osculatum* complex, a finding that agrees with results from protein electrophoretic data (Orrechia *et al.* 1994). However, Berland's proposal to recognize all species in phocid seals as *Phocascaris*, with all species from birds as *Contracaecum*, is not congruent with the inferred 28S trees, because it would result in a paraphyletic *Contracaecum*. With respect to Berland's proposal, the phylogenetic trees do not depict avian *Contracaecum* as monophyletic (some are more closely related to phocid parasites), and there is strong bootstrap support indicating that the otariid parasite, *C. ogmorhini*, shares a more recent common ancestor with certain avian species (i.e. *C. rudolphii* taxa). From a statistical viewpoint, alternative tree topologies representing *C. ogmorhini* as more closely related to the parasites of phocids, or as the sister taxon to the *Contracaecum* spp. from antarctic phocids, were significantly worse. Thus, the finding that pinniped *Contracaecum* are not monophyletic is very strongly supported, and the tree topologies indicate that the otariid, *Arctocephalus pusillus*, has 'captured' a parasite of the avian lineage. Similarities between caudal papillae patterns of avian *Contracaecum* and *C. ogmorhini* are also consistent with this interpretation (Fagerholm & Gibson, 1987; Fagerholm, 1991).

It is also notable that the bootstrap MP tree (MUNC dataset) revealed that support for considering certain avian *Contracaecum* more closely related to phocid *Contracaecum* is influenced by some 'alignment ambiguous' characters, and in general, bootstrap support for this result is very weak in all datasets. In addition, although estimates of phylogeny (MP, NJ, ML) do not recover a clade including all avian *Contracaecum* (plus *C. ogmorhini*), an alternative hypothesis depicting a clade of phocid ascaridoids and a sister-clade of avian *Contracaecum* (plus *C. ogmorhini*) was not significantly worse than the most parsimonious trees. This result indicates that these data are insufficient to discriminate between these particular alternative hypotheses in a statistical framework. Thus, it remains possible that sequence data from other genes might yield character support for the monophyly of avian *Contracaecum* plus *C. ogmorhini*. Only evidence of reciprocal monophyly would provide a basis for nomenclatural changes to distinguish between ascaridoids of phocids, versus those in birds plus *C. ogmorhini*. Clearly, sampling additional loci and taxa (particularly parasites of Otariidae) is warranted in this context.

The *Contracaecum* taxa from 2 southern-hemisphere phocid hosts (Phocidae, Monachinae) were also strongly supported as a sister clade to the *osculatum* complex (plus *Phocascaris*), and this topology is consistent with the geographical distributions of these boreal and austral phocids. How-

ever, it is important to note that no antarctic species of the *osculatum* complex were included in this study. Like previous analyses of nuclear rDNA sequences (Nadler & Hudspeth, 1998), or combined analyses of rDNA, cytochrome oxidase subunit 2, and morphology (Nadler & Hudspeth, 2000), these 28S data are not consistent with representing *Hysterothylacium* spp. as sharing a more recent common ancestor with anisakids (Zhu, Gasser & Chilton, 1998). Among phocid parasites, the other strongly supported clade was *C. osculatum* A and *C. o. baicalensis*, the latter subspecies obtained from the endemic freshwater seal of Lake Baikal (*Phoca sibirica*). The phylogenetic tree indicates these nematodes shared an immediate common ancestor, presumably prior to the colonization of Lake Baikal by the ancestors of *P. sibirica*. The relationship between *C. osculatum* A and *C. o. baicalensis* is also consistent with genetic distances inferred from isoenzymes (D'Amelio *et al.* 1995); however, isoenzyme results indicate that an *osculatum* species not sampled for 28S rDNA (*C. osculatum* D) is also very similar genetically to *C. o. baicalensis*. Phylogenetic analysis of rDNA sequences and distance data from isoenzymes provide complementary and independent evidence for the monophyly of *Contracaecinae* from phocid seals, and indicates that species of *Phocascaris* do not form a sister-group to *Contracaecum*, but instead are more closely related to species of the *C. osculatum* complex.

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