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A NEW SPECIES OF *PARAFILAROIDES* (NEMATODA: FILAROIDIDAE) IN THREE SPECIES OF FUR SEALS (CARNIVORA: OTARIIDAE) FROM THE SOUTHERN HEMISPHERE

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ABSTRACT: Blocks of frozen lungs of 2 Australian fur seals (*Arctocephalus pusillus doriferus* Wood Jones, 1925), 2 New Zealand fur seals (*A. forsteri* [Lesson, 1828]), and 1 sub-Antarctic fur seal (*A. tropicalis* [Gray, 1872]) from 3 different locations (Australia, New Zealand, and South Africa, respectively) were examined and found to contain lung parasites. This represents the first thorough description and identification of a new species, *Parafilaroides normani*, from an eared seal (Otariidae) in the Southern Hemisphere. The new species is described, illustrated, and differentiated from the 6 recognized species in the genus by body size, spicule shape and size, vulva to anus length, and vaginal sphincter musculature.

Parafilaroides Dougherty, 1946 (Metastongyloidea: Filaroididae) comprises small, delicate lungworms in pinniped marine mammals. Dailey (2006) recognized 6 valid species of *Parafilaroides*: *P. gymnurus* Railliet 1899; *P. decorus* Dougherty and Herman 1947; *P. hydrurgae* Mawson 1953; *P. hispidus* Kennedy 1986; *P. measuresae* Dailey 2006; and *P. gullandae* Dailey 2006. All species of *Parafilaroides* are from pinnipeds in the Northern Hemisphere except for *P. hydrurgae*, which was reported from the leopard seal (*Hydrurga leptonyx* Blainville 1820) taken near Antarctica at Heard Island. Also, with the exception of *P. decorus*, which infects the California (*Zalophus californianus* Lesson 1828) and Steller (*Eumatopias jubatus* (Schreber, 1776) (Otariidae) sea lions, all previously reported *Parafilaroides* spp. are from earless or true seals (Phocidae). The present study describes and illustrates a new species of *Parafilaroides* from specimens of Southern Hemisphere lungworms, infecting 3 species of fur seals (Otariidae): Australian fur seal, *Arctocephalus pusillus doriferus* Wood Jones, 1925; New Zealand fur seal, *A. forsteri* (Lesson, 1828); and sub-Antarctic fur seal, *A. tropicalis* (Gray, 1872).

MATERIALS AND METHODS

The lungworms in this study were collected by Dr. Richard Norman, Massey University, Palmerston North, New Zealand, between August 1993 and August 2000. The specimens were frozen with the host animal and subsequently made available for examination under the following circumstances. Two dead yearling males, *Arctocephalus pusillus doriferus*, were recovered from Dog Beach, Ocean Grove, Victoria, Australia, on 22 August 1993 and 2 September 1993. The animals were collected and worms made available under Permit No. RP-92-141 from the Department of Conservation and Environment (DCE), Victoria, Australia. Two yearling males, *Arctocephalus forsteri*, were found stranded on Foxton Beach, Palmerston North, New Zealand 12 August 2000. The animals were found alive, but in poor condition. They were shot by a member of the DCE. Worms were made available for study from the DCE on 14 August 2000. No permit number was provided. One *Arctocephalus tropicalis* (age and sex not provided) was found in poor condition and shot on 26 August 1996 in Cape Town, South Africa. Access to parasites was provided by Sea Fisheries Research Institute, Cape Town, South Africa. No permit number was provided.

Following necropsy, frozen blocks of infected lung were immersed either in formalin or 0.9% saline and individual worms were teased out of the tissue using a dissecting microscope. Saline collected worms were fixed in 70% ethanol. Specimens were cleared in glycerin and studied as temporary mounts.

Samples of the *A. forsteri* material were sequenced for rDNA comparison to *Parafilaroides decorus* from the California sea lion (*Zalophus californianus* Lesson, 1828) by the following method. Nucleic acids were extracted from 1–3 nematodes using 1 of 2 commercial kits (DNAzol, Molecular Research Center, Inc., Cincinnati, Ohio; ID Pure Genomic DNA Kit, ID Labs Biotechnology, London, Ontario, Canada). Extracts were concentrated by vacuum evaporation to 20 μl, and 1.5–3 μl of this preparation was used in each polymerase chain reaction (PCR) reaction.

Two regions of 1 nuclear gene (large-subunit rDNA or LSU) and 1 mitochondrial gene (cytochrome *c* oxidase subunit 1) were amplified by PCR. A region of the LSU containing the D2 and D3 divergent domains was amplified using forward primer 5′-AGCGGAGGAAAAGAAACTAA and reverse primer 5′-TCGGAAGGAACCAGCTACTA. A second region of LSU was amplified using forward primer 5′-GATCCGTAACTTCGGGAAAAGGAT and reverse primer 5′- CTTCGCAATGATAGGAAGAGCC. Approximately 45% of the mitochondrial *cox* 1 gene (positions 7,893–8,596 in *Caenorhabditis elegans*), was amplified using forward primer 5′-AGTTCTAATCATAA(A/G)GATAT(C/T)GG and reverse primer 5′-TAAACTTCAGGGTGACCAAAAAATCA.

PCR reactions (25 μ) consisted of 0.5 μM of each primer, 200 μM deoxynucleoside triphosphates, 0.5 unit Finnzymes DNAzyme EXT proofreading polymerase, and 3 mM $MgCl_2$. For the LSU regions, PCR parameters included denaturation at 94 for 3 min, followed by 36 cycles of 94 C for 30 sec, 54 C for 30 sec, and 72 C for 1 min, followed by a post-amplification extension at 72 C for 7 min. For the *cox* 1 region, PCR parameters included denaturation at 94 C for 3 min, followed by 36 cycles of 94 C for 1 min, 40 C for 1 min, and 72 C for 1 min. followed by post-amplification extension at 72 C for 7 min.

PCR products were prepared for direct nucleotide sequencing using enzymatic treatment with exonuclease I and shrimp alkaline phosphatase (PCR product pre-sequencing kit, USB Corporation, Cleveland, Ohio) and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer (PE Applied Biosystems, Foster City, California). Sequences were double-stranded for verification using reactions primed from the PCR or internal sequencing primers. Contigs were assembled using CodonCode Aligner software (CodonCode Corporation, Dedham, Massachusetts).

Type material of *P. hydrurgae* described by Mawson (1953) was received on loan from the South Australian Museum, New Terrace, Adelaide, South Australia (AHC #3272, #42905) for examination during this study.

Specimens used for the present study are deposited in the U.S. National Parasite Collection in Beltsville, Maryland, and the South Australian Museum, New Terrace, Adelaide, S. A.

Drawings were made with a drawing tube. Measurements are presented in μm, unless otherwise indicated, as range values, followed by mean values in parentheses.

DESCRIPTION

Parafilaroides normani n. sp.

(Figs. 1–4)

Diagnosis: Based on 9 entire and 12 incomplete specimens. Body small, delicate, thin nematode with narrow cuticle. Buccal capsule 2.1–

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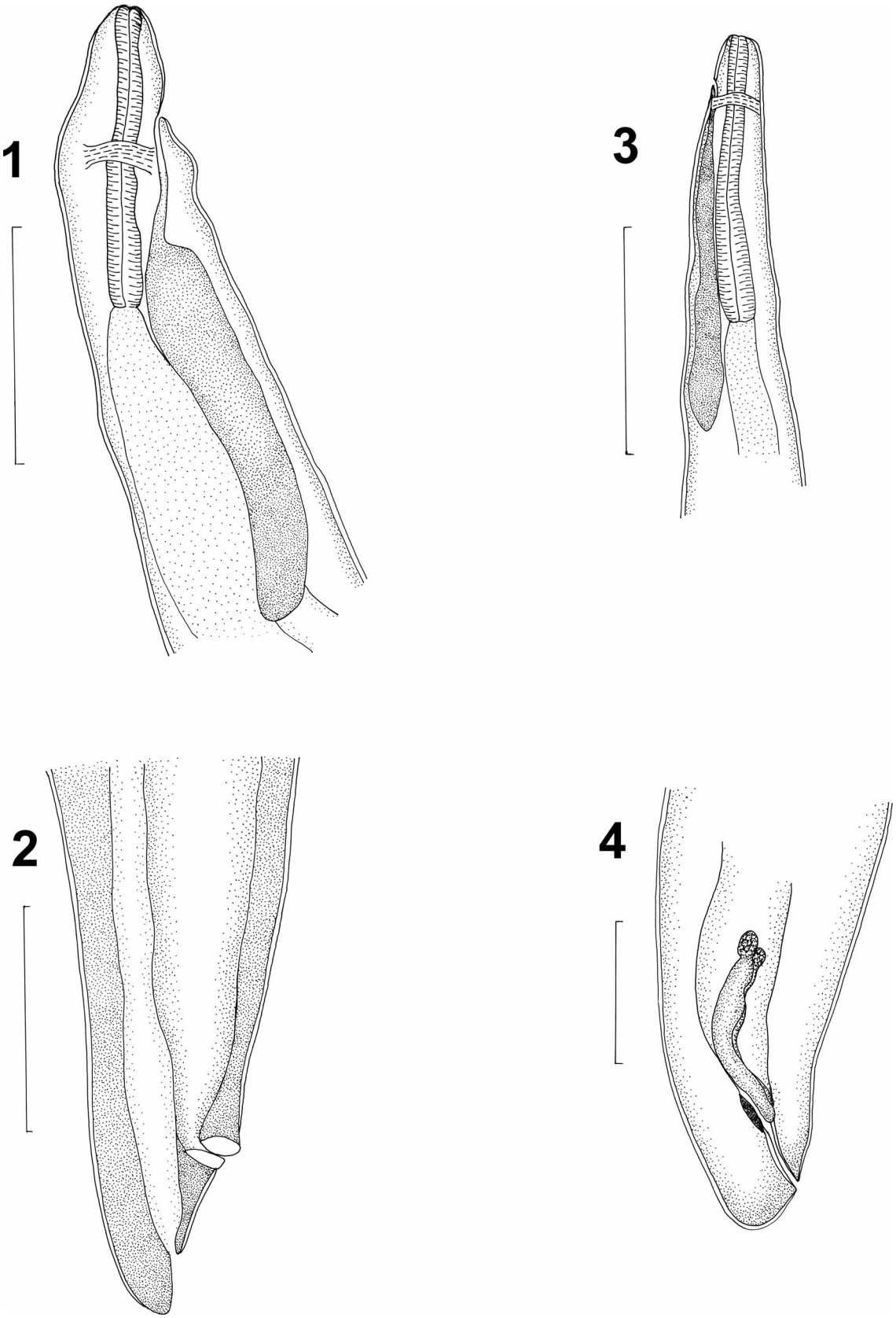

FIGURES 1–4. *Parafilaroides normani* n. sp. (**1**) Anterior end of female. (**2**) Posterior end of female. (**3**) Anterior end of male. (**4**) Posterior end of male. Bar = 110 μm (Figs. 1, 2, 3); Bar = 30 μm (Fig. 4)

TABLE I. A comparison of species in Parafilaroides.*

	P. measuresae		*P. gullandae*		*P. gymnurus*		*P. hispidus*		*P. decorus*		*P. hydrurgae*		*P. normani*	
	Northern elephant seal		Pacific harbor seal		Atlantic harbor seal		Ringed seal		California sea lion		Leopard seal		Fur Seal	
	Dailey, 2006		Dailey, 2006		Gosselin/Measures, 1997		Kennedy, 1986		Dailey, 2006		Mawson, 1953		This study	
	California, U.S.		California, U.S.		Canada		Beaufort Sea		California, U.S.		Antarctica		New Zealand	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Body length (mm)	22–25	42–44	12–15	23–37	6.5–10.9	6.4–16.6	4.5–6.3	11.9–13.9	4.5–6.3	9.3–16.5	25–36.7	(up to) 90	9–14	31–41
Max. body width	52–67	69–92	100–136	132–154	93–165	95–221	109–140	251–397	83–100	130–191			80–100	120–175
Esophagus length	145–157	125–163	132–157	154–176	128–144	132–147	200–252	207–303	129–151	154–174	180	250	137–180	140–190
Max. esophagus width	14–18	14–20	11–15	14–15	10–14	14–16	31–42	45–56	21–24	26–31			17–30	25–30
Nerve ring	60–66	57–65	44–57	55–65	39–53	37–59	29–37	60–80	66–72	65–85			60–85	87–105
Left spicule	44-56		33–46		46–63		67–88		36–37		55–60		35–50	
Right spicule	60–63		33–46		42–52		67–88		36–37		55–60		35–50	
Capitulum length	10–12		6–11		7–16								8–13	
Gubernaculum	18–20		8–10		12–19		20–28		10		30		7–11	
Vulva to anus length		65–67		28–29		18–34		9		41–66		60		87–110
Tail length	30–52		26–30		26–29				26–49		30		40–63	

* All measurements in μm unless otherwise indicated.

3.2 long. Esophagus muscular, nearly straight, with slight flare at intestinal junction. Tail moderately attenuated terminally in females, bluntly rounded in males, anus subterminal with transverse opening. Phasmids terminal, posterior to anus.

Male (holotype; n = 4): Body thin, cylindrical, 9–14 (13.8) mm long by 80–100 (93) wide at maximum width. Anterior extremity bluntly rounded, not narrowing abruptly at level of excretory pore. Oral opening circular, labia lacking. Cephalic papillae not observed. Esophagus 137–180 (162) long by 17–30 (24) wide at maximum width. Nerve ring 60–85 (69) from anterior end. Excretory pore 29 from anterior extremity. Spicules equal 35–50 (42), thin, and minute. On lateral view, capitulum rounded, knob like, 8–13 (11) followed by 2 swellings and contractions before long arcuated lamina. Gubernaculum 7–11 (9.2), slightly concave, attenuated on proximal and distal ends. Caudal papillae not observed.

Female (allotype; n = 5): Body thin, cylindrical, 31–41 (38.2) mm long by 120–175 (138) at maximum width. Anterior and posterior body extremity gradually tapered, narrowing abruptly at level of excretory pore. Oral opening circular, labia lacking, cephalic papillae not observed. Esophagus 140–190 (167) long by 25–30 (27) wide at maximum width. Nerve ring 87–105 (95) from anterior end. Vulva anterior to subterminal anus, both with transverse openings. Vaginal sphincter consists of single undivided muscle. Distance from vulva to posterior end 127–158 (148), vulva to anus 87–110 (98), tail length 40–63 (48). Larvae measured 200–202 (201) long by 8–11 (10) wide.

Taxonomic summary

Type host: New Zealand fur seal, *Arctocephalus forsteri* (Lesson, 1828).

Other hosts: Australian fur seal, *Arctocephalus pusillus* (Schreber, 1775); sub-Antarctic fur seal, *Arctocephalus tropicalis* (Gray, 1872).

Type locality: Waikanae Beach, New Zealand, 41°17′S, 174°47′E.

Site of infection: Lung parenchyma, alveoli.

Type specimens: Holotype, USNPC No. 100360; Allotype, USNPC No. 100361; Paratypes: USNPC No. 100362 and South Australian Museum, Adelaide, South Australia, No. AHC 34812.

Etymology: Species named for Dr. Richard Norman in appreciation of his veterinary work with marine mammals and collection of lungworm samples.

Remarks

Parafilaroides normani n. sp. differs from the other previously described species in the genus in a number of features (Table I). *Parafilaroides gymnurus* females are shorter than the new species (ranging from 31 to 41 mm compared to 6.4–16.6 mm) and more attenuated at the posterior end (vulva to anus length 87–110 compared to 18–34). Females of *P. gymnurus* also have a well-developed bipartite and vaginal sphincter, while those of *P. normani* consist of a single individual muscle.

Parafilaroides normani is a longer, thinner worm (9–14 mm long by 80–100 wide in males [m], 31–41 mm by 120–175 wide in females [f]) compared to *P. hispidus* (4.5–6.3 mm by 109–140 wide [m], 11.9–13.9 mm long by 251–397 wide [f]). In addition, the females differ in position of the vulva and anus (subterminal in *P. normani*, terminal in *P. hispidus*).

The new species is longer in both sexes (9–14 mm [m], 31–41 mm [f]) than *P. decorus* (4.5–6.3 [m], 9.3–16.5 [f]). Spicules are generally larger in *P. normani* (35–50) than in *P. decorus* (36–37), and the vulva to anus length is greater in *P. normani* (87–110), than in *P. decorus* (41–66).

Parafilaroides measuresae is longer and thinner (22–25 mm long, 52–67 wide [m], 42–44 mm long, 69–92 wide [f]) than *P. normani* (9–14 mm long, 80–100 wide [m], 31–41 long [m], 120–175 wide [f]). They also differ in size and shape of spicules (right spicule 60–63 with a square capitulum and a posterior spur in *P. measuresae*, 35–50, with a rounded capitulum and no posterior spur in *P. normani*). The length of the gubernaculum is 18–20 in *P. measuresae*, and 7–11 in *P. normani*. In addition, female *P. measuresae* have a bipartite vaginal sphincter, while *P. normani* has a single undivided muscle.

Parafilaroides gullandae males are wider (100–136) than *P. normani* (80–100); the esophagus width is thinner in *P. gullandae* in both sexes (11–15 [m], 14–15 [f]) than in *P. normani* (17–30 [m], 25–30 [f]). The

nerve ring is more anterior in *P. gullandae* (44–57 [m], 55–65 [f]) than in *P. normani* (60–85 [m], 87–105 [f]). The posterior extremity is more truncate in *P. gullandae* (vulva to anus length 28–29, tail length 26–30 [f]) than in *P. normani* (vulva to anus length 87–110, tail length 40–63).

Sequences from 3 loci comparing *P. decorus* from a California sea lion and *P. normani* revealed differences at all loci (rDNA 28S5′ end, rDNA 28S3′ end, and mitochondrial cytochrome oxidase 1). The rDNA sequences and cytochrome sequences showed 5 and 50 differences, respectively.

DISCUSSION

A single species of *Parafilaroides* (*P. hydrurgae*), has been described from a marine mammal in the Southern Hemisphere (Mawson, 1953). Other reports of pulmonary nematodes from the area have been published, but none has been identified to species. Nicholson and Fanning (1981) mentioned an ''undescribed, apparently new species,'' of *Parafilaroides* from the Australian sea lion (*Neophoca cinerea* Péron, 1816). Baker and McCann (1989) reported pulmonary nematodes from the Antarctic fur seal (*Archtocephalus gazella* Peters, 1875).

Life histories of lungworms in pinnipeds are not well known. *Parafilaroides decorus* in the California sea lion has been experimentally demonstrated to use a tide pool fish (*Girella nigricans*) as an intermediate host (Dailey, 1970). Bergeron et al. (1997) experimentally infected American plaice (*Hippoglossoides platessoides*) with *Otostrongyluss circumlitus* (Railliet, 1899) de Bruyn, 1933 from the Atlantic gray seal, *Halichoerus grypus* (Fabricius, 1791).

Each of 3 sympatric hosts from the Northern Hemisphere (California sea lion, northern elephant seal, and Pacific harbor seal) is infected with a different *Parafilaroides* (*P. decorus*, *P. measuresae*, *P. gullandae*, respectively); this distribution is related to the unique dispersal of each species' prey (Dailey, 2006). The fact that the 3 *Arctocephalus* spp. examined in the present study were infected with the same species of *Parafilaroides* is understandable, considering that all fur seals feed on fish, crustaceans, and cephalopods from similar habitats (Rand, 1959).

The zoogeography of the 3 species demonstrates an overlap in foraging activity. *Arctocephalus pusillus doriforus* ranges from southeastern Australia (Victoria) to southern Tasmania, then north to New South Wales. *Arctocephalus tropicalis* ranges from Capetown, South Africa, west to the southern coast of Australia and the South Island of New Zealand. *Arctocephalus forsteri* has 2 distinct populations, 1 along the southern coast of Australia, the other around New Zealand (Gentry and Kooyman, 1986). Further studies are needed on the biodiversity of *Parafilaroides* spp. infecting additional species of pinnipeds from the Southern Hemisphere.

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