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AUTHORS: Elson-Riggins, J G; Gibbons, L M; Van Liere, D W; Zinkstok, E W; Blake, D P; Alegre, F; Spittle, H; Brakefield, P M; Udo de Haes, H A; and Osinga, N

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Surprisingly long body length of the lungworm *Parafilaroides gymnurus* from common seals of the Dutch North Sea

Elson-Riggins, J.G[1,2,3*], Gibbons, L.M.[4], Van Liere, D.W.[5], Zinkstok, E.W.[2,6], Blake, D.P.[1], Alegre, F.[2,7], Spittle, H.[1], Brakefield, P.M.[8,9], Udo de Haes, H.A.[8], Osinga, N.[8]

[1]The Royal Veterinary College, The University of London, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK; [2]Seal Rehabilitation and Research Centre (currently: Seal Centre Pieterburen), Hoofdstraat 94a, 9968 AG Pieterburen, The Netherlands; [3]Dengrove Scientific Services, Donhead St. Andrew, Shaftesbury, Dorset, SP7 9EW, UK; [4]Institute of Parasitology, Justus Lieberg University Giessen, 35392 Giessen, Germany; [5]CABWIM, Wildlife Research and Consultancy, Heuvelweg 7, 7128 BD Almen, The Netherlands; [6]Significant Science Communication, Rembrandtlaan 3, 1272 GM, Huizen, The Netherlands; [7]Jaguar Rescue Center Foundation, Playa Chiquita 70403, Costa Rica; [8]Institute of Environmental Sciences (CML) and Institute of Biology Leiden (IBL), Leiden University, Sylviusweg 72, 2333 BE, Leiden, The Netherlands; [9]Department of Zoology, Cambridge University, Downing Street, Cambridge, CB2 3EJ, UK

*Corresponding author – Jocelyn Elson-Riggins (jriggins@rvc.ac.uk) (+441747829225) (0000-0001-9909-1966)

ORCID Numbers: J.G. Elson-Riggins (0000-0001-9909-1966); L.M. Gibbons (0000-0003-1605-051); D.W. Van Liere (0000-0002-9314-522X); D.P Blake (0000-0003-1077-2306); P.M. Brakefield (0000-0001-6564-8914)

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Abstract

Lungworms of the genera *Parafilaroides* and *Otostrongylus* are responsible for parasitic bronchopneumonia, the foremost disease of eastern Atlantic common seals (EACS, *Phoca vitulina vitulina*) in the Dutch North Sea. Recently, there have been increased reports of lungworm cases and observations of unusually long *Parafilaroides* sp. adults in this location. The initial aim of this study was to confirm the identity of the *Parafilaroides* species infecting this population. *Parafilaroides* are usually small and delicate, making them difficult to extract from host tissue and there is often difficulty accessing fresh specimens for morphological study. The large size of the Dutch worms and the accessibility of specimens from numerous animals enabled the description and measurement of many intact specimens (N=64) from multiple host animals (N=20). Species identity was confirmed by targeted sequencing of ribosomal and mitochondrial DNA amplicons from a subset of worms. Worm morphology was consistent with descriptions for *P. gymnurus*, but the mature females were 1.9-fold and 3.4-fold longer than those recovered from French EACS ($P \leq 0.001$) and Canadian western Atlantic common seals (*Phoca vitulina concolor*; $P \leq 0.0001$). They were also significantly longer than mature female *P. gymnurus* described from other seal species, with the exception of those from harp seals of Les Escoumins, Quebec. We speculate that intraspecific genetic differences in *P. gymnurus* and the environment within the host could contribute to the variation reported here. This study is the first to describe *P. gymnurus* using morphological and molecular methods and should serve as a reference for identification of the species.

Keywords

Parafilaroides gymnurus, common seal, *Phoca vitulina*, lungworm, North Sea, morphology

Introduction

Parasitic bronchopneumonia is currently the primary cause of disease in eastern Atlantic common seals (harbour seals) (EACS, *Phoca vitulina vitulina*) of the Dutch North Sea (Osinga and ’t Hart 2010). Lungworms occur mainly in seals under 1 year old and they are most likely transmitted horizontally via the food chain, after weaning (Measures 2001). The Metastrongyloid genera *Otostrongylus* (Railliet 1899) and *Parafilaroides* (Railliet 1899) are the causative nematodes in this population (Borgsteede et al. 1991). Since the late 1990s, there has been a sharp increase in the number of young stranded EACS admitted to Seal Centre Pieterburen (Previously: Seal Rehabilitation and Research Centre), The Netherlands, with severe verminous pneumonia (Fig. 1) (Osinga and ‘t Hart 2010). The proportion of admitted animals with this condition rose from 22% during stranding period 1971-1997 to 53% during 1997-2009 and to 70% during 2009-2013. Also, this was a common cause of death in EACS that stranded dead along the Dutch Wadden Sea coast after seal year 1997-1998 (Osinga and ‘t Hart 2010). Such high morbidity and mortality would be expected to impact recruitment of the EACS population, since about a third of the roughly 1,500 pups born annually in Dutch waters strand (TSEG 2013). However, partly because of rehabilitation efforts, the total Dutch EACS population rose from 680 in 1971-1972 to 7,029 in 2012-2013, and there were 8,351 animals in 2015-2016 (Jensen et al. 2017; CBS, PBL, RIVM, WUR 2017; Reijnders et al. 1996).

North Sea EACS can be infected with either 1 or both lungworm genera (Claussen et al. 1991). *Parafilaroides* spp. are described as small nematodes embedded in the respiratory parenchyma (Measures 2001). Railliet (1899) first described *P. gymnurus* in an EACS from Baie de Somme, France, naming it *Pseudalius gymnurus*. Dougherty (1946) established the genus *Parafilaroides*, but Anderson (1978) made *Parafilaroides* a subgenus of *Filaroides*. He distinguished the 2 subgenera of *Filaroides* based on the smaller spicules and lack of caudal papillae in *Parafilaroides*. Dailey (2006) restored *Parafilaroides* to full generic status due to the identification of caudal papillae and the 28S/18S ribosomal DNA (rDNA) data of Carreno and Nadler (2003). Based on these findings, we follow Dailey (2006) in treating *Parafilaroides* as a genus. The *Parafilaroides* is composed of 7 species (Dailey 2009): 2 parasitize the eared seals (Otariidae), *P. decorus* and *P. normani*, and 5 parasitize the true seals (Phocidae), *P. measuresae*, *P. gullandae*, *P. hispidus*, *P. hydrurgae* and *P. gymnurus*. Only *P. gymnurus* and *P. gullandae* occur in common seals: *P. gymnurus* infects both western (WACS, *Phoca vitulina concolor*) and eastern Atlantic common seals (Claussen et al. 1991; Gosselin and Measures 1997), whilst *P. gullandae* has been identified only from Pacific common seals (PCS, *Phoca vitulina richardsi*) (Dailey 2006).

Gosselin and Measures (1997) redescribed *P. gymnurus* from Canadian WACS, ringed (*Pusa hipsida*), harp (*Pagophilus groenlandicus*), and grey (*Halichoerus grypus*) seals. It is the only *Parafilaroides* species to have been reported from EACS (Railliet 1899; Borgsteede et al. 1991; Claussen et al. 1991; Lehnert et al. 2010). Thus, we hypothesized that the species in Dutch EACS would be *P. gymnurus*. However, Gosselin and Measures (1997) observed that the *P. gymnurus* described from EACS in France (Railliet 1899) were longer than those from WACS in Canada. This was also observed by staff at Seal Centre Pieterburen, but the morphology of the parasite from EACS had not been described since Railliet's 1899 work.

The sharp increase in lungworm-infected EACS admitted to Seal Centre Pieterburen in recent years, the observations of long *Parafilaroides* sp. and the lack of recent morphological work on *Parafilaroides* from Europe were the impetuses for this study. We examined a large number of specimens to investigate whether they were a variant of *P. gymnurus* or a new species. We provide a morphometric and molecular description of *Parafilaroides* sp. from EACS of the Dutch North Sea. We also compare it morphologically to *P. gymnurus* descriptions and molecularly to sequences of *Parafilaroides* sp. obtained from PCS and California sea lion (CSL, *Zalophus californianus*) and to the *Parafilaroides* species available on the GenBank database. Finally, we explore the possible reasons for the unusually long *Parafilaroides* sp. in EACS of the Dutch North Sea.

Materials and methods

Samples

Parafilaroides sp. were retrieved from stranded EACS under 1 year of age during 2009-2012 at Seal Centre Pieterburen. Thirty-four entire and 4 partial mature males, 27 entire and 12 partial mature females, 3 complete and 1 incomplete immature adult females (no embryonated eggs visible) and 1 complete and 1 partial female L5 were retrieved from 20 seals for morphology. Nematodes were retrieved *post-mortem* or from the floor if they were expectorated (Supplementary Table S1). Dead nematodes and those used for DNA extraction were stored in 70% ethanol. Live nematodes used for microscopy were killed in 0.15 M saline at 60 °C before fixation. Nematodes were fixed in glycerin-alcohol (9 parts 70% ethanol: 1 part glycerin), cleared by alcohol evaporation, and mounted in glycerine jelly (Cable 1977). Faeces from PCS were collected at The Marine Mammal Centre (TMMC; Sausalito, California, USA) in 1997 and used in Baermanns to obtain nematode larvae. *Parafilaroides* sp. adults were collected *post-mortem* from CSL at TMMC in 1999 and they were separated from released larvae. All TMMC samples were stored in 0.15 M saline at -80 °C. Samples for molecular work were shipped overnight to The Royal Veterinary College (RVC), UK, by FedEx: on dry ice from the USA in 2006, and on ice from The Netherlands in 2011. They were stored at -80 °C, thawed and washed in either 0.15 M saline or phosphate buffered saline prior to larval screening and/or DNA extraction. *Parafilaroides* sp. and *O. circumlitus* larvae were separated based on size using a stereomicroscope (Zoomaster 65, Prior, Cambridge, UK). They were placed in 100 µl fresh Millipore Direct-Q® 3 water (Millipore (UK) Limited, Watford, UK) and stored at -80 °C.

Microscopy and statistical analysis

Nematodes were examined and measured using bright field microscopy with a Leitz Laborlux 11 compound microscope (Leica Microsystems Ltd, Milton Keynes, Buckinghamshire, UK) equipped with an eyepiece graticule. If a character was unclear within a specimen that measurement was excluded. They were photographed with an Olympus CX41 compound microscope (Olympus, Southend on Sea, Essex, UK) equipped with an Olympus DP20-5 camera. Spicule measurements were made for samples in all orientations but the gubernaculum was measured only in specimens where it was orientated laterally.

We first applied ANOVA to test for an individual host animal effect on the nematodes in our dataset. Several variables showed a significant host effect (see Results section). As we required independent samples and as some of the variables were not normally distributed, we applied the median of the measurements of the different worms gathered within a host as the sample estimate. T-tests were used to compare our estimates with previous descriptions of *P. gymnurus*. Railliet (1899) provided only means or ranges. For ranges, we assumed a non-skewed distribution and calculated the average of the minimum and maximum value as the central estimate. To determine if the spicules were equal, a matched pair t-test compared the left and right spicule within each male. The sample size was 1 for male *P. gymnurus* from Les Escoumins grey seal and Salluit ringed seal (Gosselin and Measures, 1997). We therefore calculated the chance for these sample values to occur under the distribution as estimated by the mean and standard deviation of our own sample estimates.

DNA Extraction, PCR and sequencing

DNA was extracted from 9 adult North Sea EACS *Parafilaroides* sp. preparations; 5 using several worms per preparation (total tissue mass 6 to 11.9 mg) and 4 using 1 worm per preparation. Four host animals were represented, which stranded during 2010-2011, and all single worm preparations came from the same seal. One Baermann extract from 1 juvenile PCS was used to prepare 3 tubes containing 20 *Parafilaroides* sp. larvae each. From 1 CSL we made 1 adult (approximately 20 mg tissue) and 2 larval (89 and 100 released larvae) *Parafilaroides* sp. preparations. DNA was extracted from the Dutch nematodes using a DNeasy blood and tissue kit (QIAGEN, Crawley, UK), following a slightly modified protocol: the sample was homogenized using a stainless steel bead in a MM300 mixer mill (Retsch GmbH, Haan, Germany) at 30 oscillations per second for 2 min before overnight incubation with proteinase K at 37 °C. DNA was extracted from CSL adult nematodes using a Wizard®genomic DNA purification kit (Promega UK, Southampton, UK), following the manufacturer's instructions. The quantity and quality of extracted DNA were assessed using a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). Larvae were thawed, then disrupted using a Soniprep 150 ultrasonic disintegrator (MSE, London, UK). Three 20 second pulses at 28 microns were used with 1 minute between pulses, when the sample was cooled on ice. This was used for PCR without a DNA extraction step.

The rhabditid primers NC1 and NC2 amplified the entire second internal transcribed spacer (ITS-2) region of ribosomal DNA (rDNA) (Gasser et al. 1993) using a 55 °C annealing temperature. The D3 expansion region of 28S rDNA was amplified using D3A and D3B (Al-Banna et al. 1997) at 60 °C. The cytochrome c oxidase subunit 1 (COI) gene of mitochondrial DNA (mtDNA) was amplified using CCOIF and CCOIR (Dailey 2009) at 40 °C. All PCR reactions were performed in a G-Storm GS1 thermal cycler (GRI, Braintree, UK) in a 25 µl reaction volume prepared using either a KAPA2G Robust kit (Kapa Biosystems, Woburn, MA, USA) or a MyTaq HS DNA polymerase kit (Bioline, London, UK), according to the enzyme manufacturer's instructions. In all experiments, positive (*Parafilaroides sp.* DNA from EACS) and negative (no DNA) controls were included. Products were visualized on 1.5% agarose gels stained with either SYBR® safe (Life Technologies, Paisley, UK) or GelRed™ (Biotium, Hayward, CA, USA). PCR products were purified using a QIAquick PCR purification kit (QIAGEN) and sequenced at either GATC-Biotech (London, UK) or Source BioScience (Cambridge, UK). Sequence analysis was performed using CLC Main Workbench 6 version 6.6.5, 7, and 8 (CLC bio, Swansea, UK). Sequences were compared to the NCBI database using BLASTn (Basic Local Alignment Tool for nucleotides).

Results

The EACS worm variables that showed a significant difference between individual host animals (host effect) were body length ($P<0.05$), maximum oesophagus width ($P<0.01$), distance from NR to SEP ($P<0.01$) and width at vulva level ($P<0.01$) for females and nucleus length in the short SE gland ($P<0.05$) for males. The worms corresponded qualitatively to *P. gymnurus* and morphometric comparisons to previous *P. gymnurus* descriptions are in Tables 1 and 2. The bipartite vaginal sphincter (Figs. 2a-c) was composed of a wide distal and narrow proximal muscle in lateral view. The vulva and anus were subterminal (Figs. 2a-d) and the female reproductive system was didelphic and prodelphic. The spicules were equal (total length, $P = 0.206$; capitulum length, $P = 0.1$; total width, $P = 0.815$) with the proximal ends wide apart and the distal ends close together in ventral view, forming a "V" shape (Fig. 2e). The capitula were bent ventrally and were followed by a narrow calomus before leading to the long arcuated lamina (Figs. 2f-g). The calomus was shorter on the ventral side than on the dorsal side (Fig. 2g). A terminal papilla and gubernaculum were visible in some males (Fig. 2f) and the gubernaculum decreased in thickness from the distal to the proximal end (Fig. 2f).

One SE gland was shorter than the other (Tables 3 and S2), with the nucleus of the shorter gland located anterior to the nucleus of the other gland. In mature females containing larvae, the distal vaginal sphincter muscle was often patent (Fig. 2b). There appears to be a supplementary valve at the proximal end of the vaginal sphincter, which was visible in many specimens (Figs. 2a and c). The uteri sometimes contained hatched larvae, which were usually interspersed with unhatched ova. Fig. 2d shows the vulva and anus in ventral view in a mature specimen. Vulva and vaginal sphincter measurements for this specimen and a ventrally orientated immature adult and a complete early stage L5 are in Supplementary Table S3. In the L5, the vaginal sphincter was starting to develop (21 µm length), and the body length was 11.2 mm (Tables S3 and S4). The shape of the posterior end in the mature females ranged from bluntly rounded (Fig. 2b) to attenuated (Fig. 2h; Table 3).

Although our nematodes were clearly morphologically *P. gymnurus*, the size of several characters differed significantly from previous descriptions of *P. gymnurus* from common seals (Tables 1 and 2). The mature female body length (Fig. 3; Table 1) was significantly greater than that described from WACS of Canada (3.4-fold; $P \leq 0.0001$) (Gosselin and Measures, 1997) and EACS of France (1.9-fold; $P \leq 0.001$) (Railliet, 1899). Our mature males were significantly shorter than our mature females ($P < 0.0001$). Our males were significantly longer than the males from WACS of Canada ($P \leq 0.001$), but they were comparable in length to those from EACS of France (Fig. 3; Table 2). The oesophagus length ($P \leq 0.0001$) and width ($P \leq 0.001$) of our mature females were significantly larger than those of the WACS females (Gosselin and Measures, 1997) (Table 1). Railliet's (1899) EACS females were significantly wider ($P \leq 0.001$) and the vulva to anus distance ($P \leq 0.0001$) and the larvae ($P \leq 0.01$) were significantly longer than ours (Table 1). The oesophagus length ($P \leq 0.01$) and width ($P \leq 0.0001$) of our males were significantly greater than those of the WACS *P. gymnurus* (Table 2). However, our males had significantly smaller total spicule ($P \leq 0.05$) and capitulum lengths (left, $P \leq 0.0001$; right, $P \leq 0.001$). Both EACS male characters measured in addition to body length by Railliet (1899) were significantly larger than ours (maximum width, $P \leq 0.01$; spicule length, $P \leq 0.001$)) (Table 2).

Our females were also significantly longer than female *P. gymnurus* described from other Canadian seal host species (Gosselin and Measures 1997), except those from harp seals collected in Les Escoumins (Table 1). This included our females being significantly longer than those from harp seals collected in St. Brides ($P \leq 0.05$). Our other female worm measurements were comparable to those of both harp seal populations, with the exception of the SEP and the vulva to anus distance, which were significantly longer in the females from harp seals. The maximum width and the oesophagus length and width were significantly greater in our females than those from grey and Holman ringed seals. However, the female measured from a Salluit ringed seal was significantly wider and the vulva to anus distance significantly longer than ours.

Our males were significantly longer than the male *P. gymnurus* from Canadian harp, grey, and ringed seals (Table 2) (Gosselin and Measures, 1997). With the exception of oesophagus length and width, all other measurements of the male *P. gymnurus* from Les Escoumins harp seals were however greater than ours. The spicules of the St. Brides harp seal *P. gymnurus* were larger than ours and the other significant differences were SEP distance (longer in harp seal) and oesophagus width (greater in ours). The spicule lengths of the *P. gymnurus* from grey and ringed seals were comparable to ours, although the capitula were mostly longer than ours. The other male measurements for these 2 host species varied, some smaller than ours, some larger.

Our immature adult female body lengths did not overlap with those of mature females previously described from common seals (Railliet 1899; Gosselin and Measures 1997) (Table S2). They were on average 2.6 times as long as the mature females from Canada and 1.4 times as long as the mature females from France.

We added to GenBank: ITS-2, D3, and COI sequences for Dutch EACS and PCS *Parafilaroides* sp., and ITS-2 and COI sequences for CSL *Parafilaroides* sp. (Table 4). The ITS-2 region of our EACS nematodes was 520 bp (Table 4) and 3 genotypes were represented, all of which differed from the *P. gymnurus* ITS-2 sequence already on GenBank (FJ87304) (Tables 4 and 5). The single nucleotide polymorphisms for the 3 genotypes were at positions 210, 211, 330, and 385 of the ITS-2 region (Table 5). The ITS-2 sequence of 1 of our 5 pooled samples had heterozygous peaks of equal height at some of these polymorphic sites that were not possible to base call, so our findings are based on the other 8 samples. PGHOLITS2GEN1 (genotype 1) (LT984653) was seen in 5 of our samples and was represented in all 4 host animals. PGHOLITS2GEN2 (genotype 2) (LT984651) was seen in 2 samples and PGHOLITS2GEN3 (genotype 3) (LT984652) was seen in 1 sample. All 3 genotypes were represented in the animal from which the single nematode preparations were prepared and that was the only seal hosting genotypes 2 and 3. All the pooled samples were genotype 1. Using BLASTn, genotypes 1 and 3 compared to the ITS-2 region of *P. gymnurus* from German Wadden Sea EACS (FJ787304) revealed 99.6% identity (Table 4), differing by 2 nucleotides (Table 5). Genotype 2 compared to FJ787304 with 99.4% identity (Table 4), differing by 3 nucleotides (Table 5). A sequence of 453 bp was produced within the ITS-2 region of the PCS *Parafilaroides sp.* (Table 4). This had 99.6% identity to FJ787304 (Table 4), differing by 2 nucleotides (Table 5). It had a unique base (T) at position 373 of the Dutch *Parafilaroides* ITS-2 sequence (Table 5). The Dutch and German worms had an A at this position. The PCS *Parafilaroides* sp. had 0.4% to 1.1% differences from the Dutch worms. A sequence of 421 bp was obtained within the ITS-2 region of the CSL *Parafilaroides* sp. (Table 4). Although this compared to FJ787304 with only 64% coverage and 75% identity (208/276 bases) (Table 4), it compared to an unknown species of *Parafilaroides* (KP402084) with 93% coverage and 93% identity (368/396 bases). The D3 sequences for the *Parafilaroides sp.* from PCS and Dutch EACS were identical. They were 310 bp and compared to the 28S rDNA of *P. decorus* (AM039757) with 97.1% identity (Table 4). A D3 sequence of 315 bp was produced for the CSL *Parafilaroides sp.*, which compared to *P. decorus* (AM309757) with 100% identity (Table 4). A 645 bp sequence was produced with the COI primers for *Parafilaroides* sp. from both subspecies of common seal (Table 4). There were 2 allelic types for Dutch EACS *Parafilaroides* sp., but only 1 for PCS *Parafilaroides* sp.. One of the Dutch allelic types (LT591890) had a T at nucleotide 85, in common with the PCS *Parafilaroide*s *sp*. (LT591893), and these sequences differed from each other by a total of 8 nucleotides (1.24%). The second allelic type for the Dutch worms (LT591891) had a C at nucleotide 85 and differed from the PCS *Parafilaroides* sp. by 9 nucleotides (1.4%). The Dutch allelic types compared to *P. normani* mtDNA (KJ801815) with identities of 89.8% (LT591890) and 89.6% (LT591891) and PCS *Parafilaroides* sp. compared with 89.5% identity. The CSL *Parafilaroides* sp. produced a 595 bp sequence, which compared to KJ801815 with 91.4% identity and differed from Dutch EACS *Parafilaroides* sp. by 12.6% (LT591890) and 12.8% (LT591891) and PCS *Parafilaroides* sp. (LT591893) by 13.1%.

Discussion

The results of this study support the hypothesis that the *Parafilaroides* sp. found in EACS of the Dutch North Sea were *P. gymnurus*. There was however a significant difference in mature female *P. gymnurus* body length between individual host animals and over time (current compared to 1899) in EACS, between common seals from different geographic locations (western versus eastern Atlantic), and between different seal host species. The *Parafilaroides* have historically been described morphologically and thus few nucleotide sequences are available. This study is the first to describe *P. gymnurus* using both morphological and molecular methods. Morphological study of the *Parafilaroides* is difficult, the males are abursate and few morphological characters are available for species differentiation (Dougherty 1946; Gosselin and Measures 1997). They are small and delicate, difficult to extract, and since they parasitize wild animals, it can be tricky to access fresh specimens. Here, the long *P. gymnurus* and availability of specimens from numerous individual animals at Seal Centre Pieterburen have facilitated the description and measurement of many specimens. Also, we describe worms expectorated by living animals and obtained from fresh and frozen carcasses. Our description did not therefore suffer from a particular preservation method and should serve well as a reference for this species.

Despite the length of our specimens, their morphology was consistent with *P. gymnurus* (Railliet 1899; Gosselin and Measures 1997). We confirm the presence of the disputed caudal papillae in the males of this genus. We also describe additional features not previously recorded for *P. gymnurus*: the supplementary valve at the proximal end of the vaginal sphincter and the shorter calomus length on the ventral side of the spicules. However, although the latter was not mentioned in previous descriptions, the spicule illustration in Gosselin and Measures (1997) appears to show this feature. The SE glands have not previously been described in detail. As for *O. circumlitus* (Elson-Riggins 2002), they were different in size and offset with respect to one another. We do not consider the attenuation of the female posterior end to be a valid character for species differentiation within the *Parafilaroides* since our specimens ranged from bluntly rounded to attenuated. The attenuation ratio facilitated comparison of specimens. Sample preparation methods and/or a smaller number of host animals could have resulted in the degree of attenuation appearing to be a useful character in previous studies.

The only *Parafilaroides* sequences previously available on the GenBank database were *P. gymnurus* for ITS-2, *P. decorus* for D3, and *P. normani* for COI. Thus, all the *Parafilaroides* sp. we sequenced from different hosts most closely matched the *Parafilaroides* sequences available for each region sequenced, but with different percentage identities. Unfortunately, no sequences were available on Genbank for WACS *P. gymnurus*. The ITS-2 results appear to agree with the morphology that the Dutch EACS *Parafilaroides* sp. were *P. gymnurus*. However, although these sequences exhibited high BLAST identity to *P. gymnurus* from Germany (Lehnert et al. 2010), these authors did not undertake a gold standard morphological study to prove the identity of their specimens. Interestingly, the ITS-2 data suggest that the PCS *Parafilaroides* sp. were also *P. gymnurus*. Despite efforts to obtain adult worms, we only had access to larvae from PCS and thus were not able to morphologically identify them. This is important because it is not clear in the literature whether PCS are infected by *P. gullandae* only or both *P. gullandae* and *P. gymnurus*. Thus, we suggest that morphological and molecular methods should be used in future studies to confirm which *Parafilaroides* species infect PCS. Our D3 results suggest that, as expected, the CSL *Parafilaroides* sp. were *P. decorus*. Although there was no D3 sequence available for *P. gymnurus* on GenBank, our nematodes from EACS and PCS presented with lower identity to the D3 expansion region of *P. decorus* than did the CSL nematodes. Since there were no COI sequence data available for *P. gymnurus* or *P. decorus* on GenBank, our results will be useful as references. The COI sequence differences (1.24 to 1.4%) between the *Parafilaroides* sp. from the 2 common seal subspecies supports the ITS-2 and D3 results in that they were within the range considered likely for conspecifics (up to 2%) (Blouin 2002). As expected, the COI sequence difference between *Parafilaroides* sp. from common seals and CSL confirmed that these were different species, and distinct from *P. normani*. Blaxter (2004) recommended that a nematode barcoding system should obtain data for at least 1 nuclear and 1 organellar gene. Here, we have data for 2 nuclear regions and 1 organellar gene. In our hands we recommend D3 and COI to provide the most robust data if sample quality or resources are limiting.

Generally, with the exception of body length, the morphological characters of the nematodes described by Railliet (1899) were larger than ours, but his sample size was limited and he only described 4 characters in addition to body length for females and 2 for males. Spicule length appears to be a variable measurement across host species. However, due to the curve of the structure, this can be difficult to measure. In the current study, each spicule was always measured more than once and our standard deviation was less for this character than for WACS *P. gymnurus* (Gosselin and Measures 1997). Within the spicules, the longer capitulum lengths of *P. gymnurus* from most other host species (including WACS) might be explained by the measurement method. We always measured our capitula on the dorsal side, where the calomus was longer and the capitulum was therefore shorter than on the ventral side.

While it is difficult in a mixed infection to separate the effects of *P. gymnurus* from *O. circumlitus*, the differences between individual hosts could be indicative of differences in body condition and/or immune response to the parasite and they should be the subject of future studies. Such studies should involve measuring and genotyping the same individual worms from each host, something that was not possible in the current study due to the requirements for full morphological examination.

It is not clear whether there is a relationship between *P. gymnurus* body length and pathogenicity. However, nematode fecundity can be positively associated with mature female length (Morand 1996) and the pathogenic effects of nematodes can depend on both their number and length (Mair et al. 2015). It is tricky to separate the effects of long worms from those of large numbers of worms and we suggest that future studies relating to *P. gymnurus* burden should account for both worm number and length.

The reasons for the unusually long mature female *P. gymnurus* in EACS of the Dutch North Sea are unknown. Here, we present 4 hypotheses.

There were limitations in earlier morphological studies: Sample sizes were limited in previous studies. Railliet (1899) described *P. gymnurus* using an unknown number of worms that were taken from 1 seal. Gosselin and Measures (1997) studied 5 males and 4 females from an undisclosed number of common seals. Also, these authors suggested that differences in body length between studies could be attributed to specimen maturity not being clearly indicated. However, Railliet (1899) and Gosselin and Measures (1997) clearly described mature worms, their female body lengths did not overlap with ours, and it is to their work that we made our comparisons. Therefore, we feel this is an unlikely explanation.

There are intraspecific genetic differences within P. gymnurus: The *P. gymnurus* in our dataset may be genetically different on a population level from *P. gymnurus* in WACS. Despite a concerted effort, we were unable to obtain specimens from WACS to sequence them ourselves. Also, it is not clear whether our females were longer than previously described from the same host subspecies (Railliet 1899) because of a recent evolution to longer body lengths. We therefore suggest that future studies compare our results to *P. gymnurus* from WACS and to museum specimens collected from EACS of the Dutch North Sea prior to 2009.

The host species affects nematode growth: Host-parasite compatibility is an important factor determining infection rates of parasites (Lagrue et al. 2011). While parasites infect a wide variety of host species, they often reach maturity in only a subset of hosts. However, all host species recorded here and in Gosselin and Measures (1997) and Railliet (1899) were infected with mature females. Interspecific host differences in infection levels can be related to morphological and/or physiological compatibility, affecting parasite growth and fecundity (Lagrue et al. 2011). Gosselin and Measures (1997) suggested that their differences in *P. gymnurus* body length between seal species could have been due to a host species effect. However, this hypothesis cannot explain the difference in *P. gymnurus* body length between WACS and EACS, since they are common seal subspecies, and it also cannot explain the difference between EACS *P. gymnurus* from The Netherlands and France. Also, although our females were not significantly longer than the females from the harp seals of Les Escoumins, they were significantly longer than those from the harp seals of St. Brides. We do not think therefore that this hypothesis is a likely explanation.

The environment within the host affects nematode growth: Although the size of an organism is partially determined genetically, the environment can also affect body size (Tuck 2014). In nematodes, substantial growth in organismal volume can occur via cell size during the adult stage, after cytokinesis has ended (Nyström et al. 2002). Dietary restriction in the eutelic free-living nematode, *C. elegans*, is associated with reduced DBL-1 signalling, so that it will not grow to its expected size (Tuck 2014). Growth is also modulated by signals from chemosensory neurons and from the gonad that are DBL-1 independent. Thus, it is clear that in free-living nematodes, within a species, environmental cues can affect body length.

In parasitic nematodes, the environment within the host can affect adult body length, particularly of the females. This has been well studied in *Teladorsagia circumcincta* and *Haemonchus contortus* from sheep. Immunity to both these species includes modulating adult worm length and hence fecundity by the interaction of eosinophils and parasite-specific IgA (Henderson and Stear 2006; Hernández et al. 2016). Generally, these worms have more severe effects on growing lambs than mature sheep, and nematode mass rather than number determines the severity of the infection (Stear et al. 1999; Mair et al. 2015). It has been proposed that immunity to *T. circumcincta* develops in 2 stages; first by the control of nematode growth and thus fecundity in lambs and subsequently by control of nematode number in sheep (Stear et al. 1999). Genetic variation in individual lambs has been shown to account for most of the variation in *T. circumcincta* adult length, including genetic variation in the nematodes themselves. Thus, the heritability of worm length is strong and within an individual lamb most of the adult female worms are of similar length. Lambs with long females also have long males, but the males are generally shorter. Jacobs and Rose (1990) found that the occurrence of "giant" adult *Teladorsagia* spp. in Greenlandic compared to British sheep was due to environmental rather than nematode genetic factors. Hong and Timms (1986) found that overall body length of adult *T. circumcincta* in sheep varied inversely to the degree of host resistance to the infection.

Since nematode growth generally stops or slows after maturity, a long prepatent period is usually correlated with large body size (Morand 1996). Maturity occurs at the age that maximizes reproductive success and thus when mortality rate is low, such as in an immunosuppressed host, a long maturation time is favoured. This has implications for the effects of drugs that select for changes in parasite life histories (Skorping 2007). Leignel and Cabaret (2001) showed that both susceptible and resistant *T. circumcincta* increased in size when exposed to selective pressure by anthelmintics. The rehabilitation treatment at Seal Centre Pieterburen involved a regime including anthelmintics. A worm response to these drugs could explain some, but not all, of the current results because 3 of our study animals coughed mature female worms within 1-2 days of admittance. A modelling study by Jensen et al. (2017) suggested that rehabilitation and release of common seals could negatively affect the genetic diversity of the recipient seal population. Rehabilitation treatment might select for the survival of seals that lack immunity to *P. gymnurus*, thus allowing the worms to reach long body lengths over generations of seals. This may only partially explain our results though because the number of lungworm cases admitted to Seal Centre Pieterburen increased sharply only in recent years (Fig. 1), which would not have allowed enough time to impact the entire Dutch EACS population, and none of our animals had mature female *P. gymnurus* of the expected size.

Hoffman et al. (2014) showed that genome-wide heterozygosity was reduced in almost 50% of the lungworm infected young EACS (under 1 year of age) compared to uninfected young EACS they tested from the Dutch Wadden Sea. This may have implications regarding the immune response of the infected animals. Indeed, the genetic diversity of Wadden Sea common seals is amongst the lowest for the species (Kappe et al. 1997). Also, severe disease, such as *Parafilaroides* spp. induced pneumonia may occur in hosts immunocompromised by co-infection with other agents (Measures, 2001). Thus, simultaneous infections may favour parasite establishment. Furthermore, exposure to toxic chemicals can increase the risk of deleterious effects, such as immunosuppression, in aquatic organisms (Measures 2001; Grieg et al. 2011; Lehnert et al. 2016). Persistent exposure to heavy metals and organic pollutants is associated with modulation of both innate and adaptive immunity in marine mammals and the prevalence and severity of their infectious diseases has increased in recent decades (Desforges et al. 2016). The immunotoxic threat to organisms in the Dutch North Sea is well documented (Rijks 2008; Laane et al. 2013; Mattig 2017). Lehnert et al. (2016) reported a correlation between pollutant exposure and transcription patterns of immune-relevant biomarkers in EACS and thus immunosuppression could play a role in the length of adult female *P. gymnurus* in this seal population. As top predators, seals bioaccumulate contaminants up the food chain and nursing pups are at a high trophic level (Frouin et al. 2011). The highest concentrations of persistent organic pollutants (POPs) in PCS pups from central California were those that had nursed in the wild and then lost mass post-weaning, when POPs were mobilized from blubber into blood (Greig et al. 2011). Thus, they have the potential to cause deleterious effects precisely when the pups are learning to forage and are exposed to some of their first parasitic infections, such as lungworms. And, although no recent studies have examined contaminant concentrations in Dutch EACS blubber, little is known about the concentration and effects of emerging contaminants and the combined effects of contaminant mixtures on marine organisms (Laane et al. 2013).

Measures (2001) stated that during times of stress, *Parafilaroides* spp. infections may predispose healthy animals to respiratory disease. Indeed, Siebert et al. (1999) found an association between high mercury levels and the prevalence of parasitic infections and pneumonia in harbour porpoises from the North and Baltic Seas. Stress could be multifactorial and may also include climate change, hunting pressure, changes in prey abundance, habitat disturbance and noise. In this regard, it is interesting to compare the long female *P. gymnurus* of harp seals from Les Escoumins (Gosselin and Measures 1997), a region known to be polluted (Frouin et al. 2011). However, at least at the time of sampling by Gosselin and Measures (1997), the common and grey seals from Les Escoumins were not infected by unusually long female *P. gymnurus* and they had a lower *P. gymnurus* prevalence than the harp seals from this location (Gosselin et al. 1998). The authors attributed this to the Arctic part of the harp seal life cycle. The harp seal was a new host record for *P. gymnurus*. But infected harp seals were in better body condition than uninfected harp seals, which the authors suggested could be due to more intensive or earlier feeding post-weaning. Canadian Northwest Atlantic harp seals are subject to hunting pressure, averaging approximately 52,000 animals per year taken between 1982 and 1995 (Stenson 2014). Also, Gosselin and Measures (1997) sampled *P. gymnurus* between 1990 and 1994, which coincided with the collapse of groundfish species in the Gulf of St Lawrence and thus the diets of harp and grey seals changed (Morissette et al. 2009). We cannot pin down one exact reason for the long female *P. gymnurus* in Les Escoumins harp seals, but we can conclude that they were sampled at a time of flux for the St. Lawrence marine ecosystem, when the seals were under multiple stresses, which could have affected their ability to suppress lungworm growth. The EACS of the Dutch North Sea have also been exposed to multiple stresses and ecosystem change in recent years. The water temperature of the western Wadden Sea, which is an important nursery area for many fish species, rose by 1.5 °C over 25 years (van Aken 2008). Corresponding changes in fish phenology have occurred, including a general trend for fish to delay their annual immigration to and advance their emigration from the Wadden Sea (van Walraven et al. 2017; Tulp et al. 2017). There have been changes in fish habitat, coastal sand nourishments and nutrient dynamics, and fisheries have partially been responsible for declines in both large and small fish (Tulp et al. 2017). Also, rehabilitation has occurred at high levels in recent years (Jensen et al. 2017) and it has been suggested that this EACS population may be approaching or have reached the current capacity of the trilateral Wadden Sea (Brasseur et al. 2018). Population estimates for the Dutch Wadden Sea were however 16,000 animals in 1900, after centuries of hunting (Dankers et al. 1990). We suggest that multiple anthropogenic stresses in Dutch EACS may provide an optimal environment for *P. gymnurus* and enable them to reach unusually long body lengths.

The hypotheses proposed here should be tested with further studies. These should include a comparison of the current *P. gymnurus* measurements with museum specimens collected from Dutch EACS. It should be determined whether mature female *O. circumlitus* from Dutch EACS also differ in length from those in the literature. Studies examining potential associations between lungworm length and number and host stress markers, tissue contaminant concentration, body condition, heterozygosity and markers of immune function should be performed. Finally, clues to the dynamics of *P. gymnurus* infection in Dutch EACS may be revealed by comparing the diet and other important parameters, such as immunity in grey seals of the Dutch North Sea, since despite the presence of *P. gymnurus* in Canadian grey seals (Gosselin and Measures 1997), grey seals of the Dutch coast have parasitic pneumonia that is caused solely by *O. circumlitus* (Seal Centre Pieterburen, unpublished data). Parasites link different ecosystem trophic levels and in addition to affecting host fitness, they can be responsible for indirect effects on species interactions and ecosystem functioning (Philippart et al. 2017).

Our knowledge regarding how anthropogenic changes affect the impact of parasites on coastal ecosystems is however limited. The presence of unusually long lungworms in a top predator that is under multiple anthropogenic stressors could therefore be a useful indicator of ecosystem change for future studies.

Conclusions

We found no apparent morphological differences, except body length, between Dutch North Sea EACS *Parafilaroides sp*. and earlier descriptions of *P. gymnurus*, leading us to conclude that they were *P. gymnurus*. On a molecular level, the *P. gymnurus* from Dutch EACS were the same species as those recorded from German EACS, but since Lehnert et al. (2010) did not morphologically confirm the identity of their *Parafilaroides*, this does not verify the identity of our worms. The *P. gymnurus* in Dutch EACS were conspecific with those we sequenced from PCS. There was a significant difference in body length of mature female *P. gymnurus* between seal host species, geographic location (western versus eastern Atlantic) and over time in EACS. There was also an individual host effect on mature female *P. gymnurus* length in Dutch EACS and, with the exception of the harp seals of Les Escoumins (Gosselin and Measures 1997), this host had the longest female body lengths that have been described to date. Intraspecific genetic differences in *P. gymnurus* and environmental conditions within the host may provide an optimal environment for *P. gymnurus* and thus enable them to reach unexpectedly long body lengths.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and samples were collected during the standard care and handling of rehabilitating seals.

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Tables

Table 1 Morphometric characteristics of mature (uteri contained embryonated ova) female *Parafilaroides gymnurus* in eastern Atlantic common seals (EACS) from the Dutch North Sea compared to female *P. gymnurus* from western Atlantic common seals of Canada, EACS of France, and harp, grey and ringed seals of Canada

Character	Host and Geographic Location								
	Common Seals			Harp Seals[b]		Grey Seals[b]		Ringed Seals[b]	
	Dutch North Sea[a]	Les Escoumins[b]	Baie de Sommes[c]	Les Escoumins	St. Bride's	Les Escoumins	Port Hood	Holman	Salluit[d]
Body Length (mm)	43.72 ± 10.77 (13), 25.43 - 69.73 (27)	12.55 ± 4.30 (4) ****	22.5 ***	35.18 ± 17.27 (5)	29.58 ± 8.32 (5) *	20.12 ± 1.85 (3) **	20.99 ± 4.47 (4) **	13.32 ± 3.24 (5) ****	17.46 (1) ***
Maximum Width[e]	149 ± 13 (13), 100 - 202 (28)	128 ± 53 (5)	170 ***	173 ± 55 (5)	152 ± 44 (5)	106 ± 20 (3) ***	109 ± 16 (4) ****	96 ± 37 (5) ***	171 (1) ***
Oesophagus Length	165 ± 8 (12), 140 - 200 (26)	139 ± 7 (5) ****	N/M	170 ± 20 (5)	164 ± 4 (5)	144 ± 5 (3) ***	151 ± 15 (4) *	141 ± 6 (5) ****	N/M
Oesophagus Width	19 ± 1 (11), 17 - 22 (22)	15 ± 1 (5) ***	N/M	18 ± 3 (5)	17 ± 1 (5)	16 ± 1 (3) *	14 ± 2 (4) ***	12 ± 2 (5) ****	N/M
Nerve Ring[f]	59 ± 12 (12), 30 - 91 (25)	48 ± 8 (5)	N/M	70 ± 5 (5)	70 ± 9 (5)	59 ± 9 (2)	61 ± 7 (4)	53 ± 2 (5)	N/M
Secretory-Excretory Pore[f]	38 ± 7 (12), 22 - 56 (23)	39 ± 11 (5)	N/M	54 ± 5 (4) **	57 ± 8 (5) ***	49 (1) ***	46 ± 22 (4)	42 ± 7 (5)	N/M
Tail Length	31 ± 6 (12), 17 - 54 (26)	27 ± 2 (5)	30	31 ± 4 (5)	29 ± 3 (5)	28 ± 1 (3)	32 ± 7 (3)	26 ± 6 (5)	24 (1) **
Vulva to Anus	30 ± 7 (12), 17 - 90 (25)	25 ± 7 (5)	48 ***	51 ± 17 (5) **	42 ± 6 (5) **	32 ± 13 (2)	34 ± 10 (3)	26 ± 14 (5)	42 (1) ***
Length of Larvae[g] (L1)	223 ± 14 (3), 207 - 234	N/A	290 **	254					

Measurements in µm unless otherwise stated. [a]Unless otherwise stated, measurements given as per host mean ± SD followed by host sample size (parentheses), range for all worms measured followed by total sample size (parentheses). [b]Gosselin and Measures (1997): mean ± SD, followed by sample size (parentheses) for all individuals measured. [c]Railliet (1899): mean for all individuals measured. [d]N=1, data was compared by calculating chance for this data given the estimates of the distribution given by the mean and SD of our own data. [e]Includes cuticle. [f]Measured from anterior end. [g]North Sea larval measurements given as mean ± SD followed by sample size (parentheses) and range for all individuals measured, Gosselin and Measures (1997) reported an average value for all host species. N/M = not measured. *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

Table 2 Morphometric characteristics of male *Parafilaroides gymnurus* in eastern Atlantic common seals (EACS) from the Dutch North Sea compared to *P. gymnurus* from western Atlantic common seals of Canada, EACS of France, and harp, grey and ringed seals of Canada

Character	Host and Geographic Location								
	Common Seals			Harp Seals[b]		Grey Seals[b]		Ringed Seals[b]	
	Dutch North Sea[a]	Les Escoumins[b]	Baie de Sommes[c]	Les Escoumins	St. Bride's	Les Escoumins[d]	Port Hood	Holman	Salluit
Body Length (mm)	15.87 ± 3.00 (11), 10.32 - 22.22 (34)	9.37 ± 1.77 (5) ***	16.5	11.95 ± 2.55 (5) *	9.41 ± 3.97 (5) **	8.84 (1) ***	9.94 ± 1.54 (4) **	8.87 ± 1.28 (7) ****	10.57 ± 1.9 (5) **
Maximum Width[e]	108 ± 10 (12), 80 - 135 (35)	112 ± 30 (5)	120 **	133 ± 24 (5) **	110 ± 21 (5)	100 (1) *	100 ± 12 (9)	96 ± 27 (7)	103 ± 11 (5)
Oesophagus Length	151 ± 7 (10), 129 - 189 (30)	136 ± 7 (5) **	N/M	152 ± 15 (5)	144 ± 13 (5)	152 (1)	137 ± 7 (6) **	138 ± 19 (7)	137 ± 6 (5) **
Oesophagus Width	17 ± 1 (11), 14 - 20 (27)	13 ± 2 (5) ****	N/M	18 ± 2 (5)	15 ± 0 (5) ****	18 (1) *	14 ± 3 (6) **	15 ± 3 (7) *	16 ± 2 (5)
Nerve Ring[f]	56 ± 14 (11), 30 - 86 (33)	46 ± 8 (5)	N/M	71 ± 9 (5) *	66 ± 7 (5)	63 (1)	55 ± 8 (5)	58 ± 7 (7)	62 ± 6 (5)
Secretory-Excretory Pore[f]	33 ± 9 (8), 21 - 58 (26)	32 ± 15 (5)	N/M	47 ± 5 (3) *	54 ± 6 (5) ***	48 (1) **	41 ± 5 (5)	44 ± 5 (6) *	44 ± 6 (5) *
Tail Length	12 ± 3 (11), 5 - 17 (21)	13 ± 3 (5)	N/M	17 ± 3 (5) **	15 ± 3 (4)	14 (1)	15 ± 2 (7) *	15 ± 6 (8)	13 ± 2 (5)
Left Spicule Length[g]	42 ± 3 (8), 37 - 52 (17)	51 ± 8 (4) *		46 ± 2 (5) *	46 ± 2 (4) *	40 (1)	41 ± 5 (6)	42 ± 5 (8)	45 ± 4 (5)
Right Spicule Length[g]	41 ± 3 (11), 37 - 45 (23)	46 ± 4 (5) *	44.5 ***	46 ± 3 (5) **	47 ± 4 (4) **	43 (1)	40 ± 5 (6)	43 ± 5 (8)	44 ± 4 (5)
Left Capitulum Length[h]	6 ± 1 (9), 5 - 7 (19)	12 ± 2 (5) ****	N/M	10 ± 1 (5) ****	9 ± 1 (5) ****	10 (1) ***	9 ± 1 (7) ****	9 ± 4 (8)	9 ± 2 (5) **
Right Capitulum Length[h]	6 ± 1 (11), 5 - 8 (22)	9 ± 1 (4) ***	N/M	9 ± 1 (5) ****	9 ± 2 (5) **	9 (1) ***	8 ± 1 (6) **	9 ± 3 (7) *	9 ± 1 (5) ****
Gubernaculum Length	15 ± 2 (11), 11 - 18 (15)	16 ± 2 (5)	N/M	19 ± 2 (5) **	14 ± 1 (4)	13 (1) *	13 ± 3 (6)	14 ± 2 (7)	13 ± 1 (5)

Measurements in µm unless otherwise stated. [a]Unless otherwise stated, measurements given as per host mean ± SD followed by host sample size (parentheses), range for all worms measured followed by total sample size (parentheses). [b]Gosselin and Measures (1997): mean ± SD, followed by sample size (parentheses) for all individuals measured. [c]Railliet (1899): mean for all individuals measured. [d]N=1. Data was compared by calculating chance for this data given the estimates of the distribution given by the mean and SD of our own data. [e]Includes cuticle. [f]Measured from anterior end. [g]Following curve of the structure. [h]Measured on the dorsal side. N/M = not measured. *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

Table 3 Morphometric characteristics of mature female (uteri contained embryonated ova) and male *Parafilaroides gymnurus* obtained from eastern Atlantic common seals of the Dutch North Sea

Character	Female	Male
Width[a] at Intestine	74 ± 11 (12), 49 - 111 (26)	52 ± 9 (10), 36 - 83 (30)
Secretory-Excretory (SE) Pore to Nerve Ring	23 ± 10 (11), 7 - 41 (20)	15 ± 9 (8), 2 - 41 (26)
Long SE Gland Length	691 ± 163 (9), 457 - 978 (14)	541 ± 60 (12), 436 - 715 (23)
Short SE Gland Length	608 ± 163 (10), 357 - 911 (14)	464 ± 55 (10), 322 - 642 (21)
Long SE Gland Nucleus Length	24 ± 3 (4), 17 - 31 (7)	21 ± 7 (8), 12 - 35 (15)
Short SE Gland Nucleus Length	24 ± 3 (5), 17 - 30 (9)	18 ± 8 (7), 7 - 30 (17)
Long SE Gland Nucleus Width	18 ± 1 (4), 16 - 20 (7)	13 ± 3 (8), 6 - 17 (15)
Short SE Gland Nucleus Width	20 ± 5 (5), 12 - 27 (9)	13 ± 3 (7), 9 - 20 (17)
Vulva Position[b] (mm)	45.46 ± 10.01 (12), 29.29 -69.66 (24)	N/A
Vulva, % Body Length	99.85 ± 0.04 (12), 99.66 - 99.90 (24)	N/A
Vulva to Posterior	61 ± 10 (12), 37 - 123 (26)	N/A
Vaginal Sphincter Length[c]	49 ± 7 (12), 35 - 62 (23)	N/A
Width[a] at vulva	79 ± 12 (12), 52 - 104 (24)	N/A
Width[a] at anus	54 ± 14 (12), 30 - 89 (23)	N/A
Attenuation Ratio[d]	0.59 ± 0.13 (12), 0.39 - 0.88 (23)	N/A
Left Spicule Maximum Width	N/A	8 ± 1 (7), 5 - 11 (19)
Right Spicule Maximum Width	N/A	8 ± 1 (9), 5 - 11 (22)

Measurements in μm unless otherwise stated and given as per host mean ± SD followed by host sample size (parentheses), range for all worms measured followed by total sample size (parentheses). [a]Includes cuticle. [b]Measured from anterior end. [c]Orientated in lateral view. [d]Tail length/width (at anus). N/A = not applicable.

Table 4 GenBank BLASTn results for the ITS-2 region of rDNA, D3 expansion loop (28S rDNA) and COI region of *Parafilaroides* sp. from eastern Atlantic common seal (EACS) of the Dutch North Sea and Pacific common seal (PCS) and California sea lion (CSL) from the California coast

Region of DNA	Host	Accession	Sequence Length	Identity to *P. gymnurus* (FJ787304)		
				% Cover	% ID	E value
ITS-2	EACS	LT984653	520	100	99.6	0.00E+00
		LT984651	520	100	99.4	0.00E+00
		LT984652	520	100	99.6	0.00E+00
	PCS	LT984654	453	100	99.6	0.00E+00
	CSL	LT984655	421	64	75.4	7.00E-45
D3 Expansion Loop (28S)	Host	Accession	Sequence Length	Identity to *P. decorus* (AM039757)		
				% Cover	% ID	E value
	EACS	LT98456	310	100	97.1	6.00E-146
	PCS	LT984657	310	100	97.1	6.00E-146
	CSL	N/A	315	100	100	2.00E-158
COI	Host	Accession	Sequence Length	Identity to *P. normani* (KJ801815)		
				% Cover	% ID	E value
	EACS	LT591890	645	100	89.8	0.00E+00
		LT591891	645	100	89.6	0.00E+00
	PCS	LT591893	645	100	89.5	0.00E+00
	CSL	LT591892	595	99	91.4	0.00E+00

Table 5 Polymorphic sites in the ITS-2 region of rDNA in *Parafilaroides* sp. from eastern Atlantic common seal (*Phoca vitulina vitulina)* of the Dutch North Sea (PGHOLITS2GEN1-3) (LT984653, LT984651, LT984652) compared to the German *P. gymnurus* reference sequence (FJ787304) and *Parafilaroides sp.* from Pacific common seal (*Phoca vitulina richardsi*) of California, USA (PSPPVUSAITS2) (LT984654).

Genotype	SNP Position[a]				
	210	211	330	373	385
FJ787304	T	T	A	A	G
LT984653	T	A	A	A	A
LT984651	C	T	G	A	A
LT984652	C	T	A	A	A
LT984654	T	A	A	T	G

[a] Sequence begins from base 1 of the Dutch *Parafilaroides* sp. sequences

Figure Legends

Fig. 1 Number of live-stranded eastern Atlantic common seals admitted to Seal Centre Pieterburen (1971-2013). Each year starts with the stranding of the first orphaned pup, which is usually in May

Fig. 2 Morphology of female (a-d, h) and male (e-g) *Parafilaroides gymnurus* from eastern Atlantic common seals of the Dutch North Sea. Bar is 50 µm unless otherwise stated. A = anus; Ca = capitulum; Co = calomus; DM = distal vaginal sphincter muscle; G = gubernaculum; L = lamina; P = papilla; PM = proximal vaginal sphincter muscle; S = spicule; SV = supplementary valve, V= vulva; VS = vaginal sphincter, labelled at indentation between distal and proximal sphincters. a Bipartite sphincter in an immature female (no embryonated ova visible), lateral view, attenuation ratio 0.57; b Bipartite vaginal sphincter of a mature female (containing larvae), lateral view, with patent distal muscle and bluntly rounded tail (ratio 0.39); c Mature female showing supplementary valve at proximal end of vaginal sphincter, lateral view, attenuation ratio 0.63; d Ventral view of mature female, showing vulva and anus; e Ventral view of mature male showing spicules: proximal ends are wide apart and distal ends are close together, forming a ‘V’ shape; f Lateral view of mature male showing both spicules, gubernaculum, and terminal caudal papilla; g Lateral view of right spicule showing capitulum, calomus, and lamina. h Attenuated tail (ratio 0.88) of mature female, lateral view

Fig. 3 Histogram showing the total body length of mature adult *Parafilaroides gymnurus* from eastern Atlantic common seals (EACS, *Phoca vitulina vitulina*) of the Dutch North Sea compared to *P. gymnurus* from western Atlantic common seals (*Phoca vitulina concolor*) of Canada (Gosselin and Measures 1997) and EACS of France (Railliet 1899). **** $P<0.0001$, *** $P<0.001$

All figures were created using Adobe Illustrator.