

Habitat use pattern of the giant parasitic nematode *Crassicauda magna* within the pygmy sperm whale *Kogia breviceps*

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ABSTRACT: The giant (>3 m) parasitic nematode *Crassicauda magna* infects kogiid whales, although only 3 studies to date have provided detailed descriptions of these worms, all based upon fragmented specimens. These fragments were found within the neck region of kogiids, an unusual anatomic site for this genus of parasites. *C. magna* is a species-specific parasite among kogiids, infecting only pygmy sperm whales *Kogia breviceps*, and with a primarily cervico-thoracic distribution. To date, however, the pattern of habitat use within the host and transmission path of this parasite remain unknown. We used detailed dissections (n = 12), histological examination of host tissues (n = 2), and scanning electron microscopy of excised nematodes (n = 7) to enhance our understanding of this host–parasite relationship. Results revealed that a critical habitat for the parasite is an exocrine gland in the whale’s ventral cervical region. *C. magna* male and female tails were found intertwined within the glandular lumen, and eggs were observed within its presumed secretion, illuminating the transmission path out of the host. The cephalic ends of these worms were often meters away (curvilinearly), embedded deeply within epaxial muscle. A single worm’s complete, tortuous 312 cm course, from the gland to its termination in the contralateral epaxial muscle, is described for the first time. This study also provides the first scanning electron micrographs of *C. magna*, which illustrate taxonomically important features of the heads and tails of both male and female worms.

KEY WORDS: Parasitic nematode · *Crassicauda magna* · *Kogia breviceps* · Cetacean · Stranding · Morbidity · Scanning electron microscopy

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INTRODUCTION

The genus *Crassicauda*, comprised of large parasitic nematodes, is endemic to both mysticete and odontocete cetaceans (Lambertsen 1985, 1986, Lambertsen et al. 1986, Geraci & St. Aubin 1987, reviewed by

Keenan-Bateman et al. 2016). *Crassicaudids* infect the urogenital system of mysticetes and odontocetes and the cranial pterygoid sinuses of odontocetes (Ridgway 1972, Dailey & Stroud 1978, Geraci et al. 1978, Perrin & Powers 1980, Gibson et al. 1998, Lopez et al. 1999). *Crassicauda* infection has been directly linked

to morbidity and mortality in both suborders of cetaceans (Lambertsen 1985, 1986, 1992, Geraci & St. Aubin 1987, Gibson et al. 1998, Oliveira et al. 2011).

Crassicaudids were first described within members of the family Kogiidae by Johnston & Mawson (1939) and next by Dollfus (1966) as infecting the 'connective tissues' and 'subcutaneous musculature' of the neck, an unusual site of infection for this genus of nematodes. Both studies identified the host species as *Kogia breviceps*, although both were published before Handley's (1966) delineation of kogiids into 2 separate species, *K. breviceps* and *K. sima*.

Subsequently, several surveys of metazoan parasites of cetaceans, from multiple geographic regions, have identified crassicaudids within *K. breviceps*, generally infecting cervical tissues (Abollo et al. 1998, Mignucci-Giannoni et al. 1998, Cardona-Maldonado & Mignucci-Giannoni 1999, Carvalho et al. 2010). These studies, in total, report on 6 infected *K. breviceps* specimens.

Keenan-Bateman et al. (2016) carried out a large-scale, retrospective analysis of over 100 records of kogiids that had stranded along the US mid-Atlantic coast. Those authors demonstrated crassicaudids to be a species-specific parasite among kogiid whales in this region, infecting only pygmy sperm whales *K. breviceps* and not dwarf sperm whales *K. sima*. This work also confirmed the parasite's general cervico-thoracic distribution reported by earlier authors, and identified a specific site of infection as a previously undescribed exocrine gland, located at the ventral terminus of the pigmented 'false gill slit' in the whale's cervical region. This gland and its associated pigmentation pattern, like crassicaudid infection, are features unique to *K. breviceps* (Keenan-Bateman et al. 2016).

To date, only 3 studies have provided detailed descriptions of the nematodes that infect *K. breviceps*, all of which were based upon fragmented specimens (Johnston & Mawson 1939, Dollfus 1966, Jabbar et al. 2015). For example, the determination of the species *C. magna* by Johnston & Mawson (1939) was based upon a partial worm, collected from the cervical tissues of an adult female *K. breviceps* stranded in South Australia. Based upon similarities in general head morphology (e.g. rounded head, strongly chitinized buccal cavity, and 2 simple lateral lips), the large size of the specimen relative to *C. boopis* described from the humpback whale *Megaptera novaeangliae* (Baylis 1920, 1922), and the specificity of the genus *Crassicauda* to cetaceans, these authors named and described the sixth crassicaudid species, *C. magna*, from this anterior fragment of a female worm. Johnston &

Mawson (1939) did not, however, observe the 2 lateral and 4 submedial papillae described by Baylis (1920) as characteristic of the genus *Crassicauda*.

Dollfus (1966) later named *C. duguyi* based solely upon the morphological features of a posterior fragment of a male specimen recovered from cervical tissues of a *K. breviceps* stranded in southwestern France. Unable to identify this specimen as that of *C. magna* as described by Johnston & Mawson (1939), Dollfus instead compared the posterior fragment to available descriptions of other known spiculated forms of the genus. Dollfus (1966) found all previously described forms to be incompatible with his specimen and therefore erected and described in detail the new species *C. duguyi*.

Recently Jabbar et al. (2015) suggested that these 2 species are synonymous. Those authors provided new morphological information on the heads of crassicaudids from museum collections, and on the tails of male crassicaudids from both museum collections and from a 2014 stranding of *K. breviceps* from Moreton Bay, Australia, the same location as the host specimen examined by Johnston & Mawson (1939). Jabbar et al. (2015) identified their specimen as *C. magna* based upon the similar (1) cephalic morphology of the parasite to that described by Johnston & Mawson (1939), (2) occurrence of the parasite in the subcutaneous tissues of the neck, (3) host species (*K. breviceps*), and (4) geographic locality of the host stranding. Those authors found their description of the male tail to be very similar to that of Dollfus (1966), except for minor differences in the placement and number of caudal papillae, and, thus, suggested that *C. duguyi* was a synonym of *C. magna*.

Jabbar et al. (2015) also provided an illustration of the apical portion of the anterior end of this nematode, which was not included in the original description by Johnston & Mawson (1939); and described an anterior cuticular plate (ptilina) unique to the genus *Crassicauda*, but, like Johnston & Mawson (1939), were unable to confirm the presence of the 2 lateral and 4 submedial papillae described by Baylis (1920) as characteristic of the genus. Jabbar et al. (2015) suggested that scanning electron microscopy would be a valuable tool to confirm the presence or absence of these characteristic features and could serve to increase our understanding of crassicaudid morphology.

While there exists new information on the identity of the *Crassicauda* nematode that infects *K. breviceps* and on its general sites of infection, to date, very little is known about how the parasite specifically uses its host tissues. Due to the tortuous course these nema-

todes take through its host, no study has yet included an intact *Crassicauda* specimen. Heads and tails of the worm have been described, but where they are specifically found in the host body is still unknown. Thus, to date, no study has identified the pattern of 'habitat' use of this very large worm within its kogiid host. Habitat is defined by Bush et al. (1997), as the host organs and tissues that make up the local environment of endoparasites. The goal of this study, which builds upon previous work (Keenan-Bateman et al. 2016), was to use multiple methods, including gross and micro-dissection, histological examination of specific host tissues, and scanning electron microscopy of cephalic and tail ends of both male and female nematodes, to describe the pattern of habitat use of *C. magna* within its *K. breviceps* host. The complete, tortuous 312 cm course of a single worm through the host body is also described for the first time.

MATERIALS AND METHODS

To identify the pattern of habitat use of *Crassicauda magna* within *Kogia breviceps*, we used systematic gross dissections carried out on 12 *K. breviceps* (Table 1), and histological analyses of selected host tissues (n = 2). In addition, morphological examination of isolated crassicaudid nematodes (n = 7), collected from multiple sites within the host, was completed using scanning electron microscopy (SEM).

Table 1. *Kogia breviceps* specimens used in this study (n = 12) specifically to investigate *Crassicauda magna* infection (see footnotes) and to describe the 'gill slit' gland (gross morphology: all specimens; histology: 2 specimens)

Field ID	Strand date	Length (cm)	Sex
KLC 210	16-Sep-14	155.0	Male
NCARI 024 ^{a,b}	19-Jan-14	232.0	Male
VAQS 20141093 ^a	18-Nov-14	240.0	Male
VAQS 20131067 ^{a,b,c}	21-Jun-13	268.7	Male
KLC 212 ^{a,c}	3-Oct-14	293.5	Male
CAHA 207 ^a	5-Nov-13	316.5	Male
GA 2013040	15-Oct-13	335.0	Male
VAQS 20141094 ^{a,c}	1-Dec-14	236.9	Female
VAQS 20071081 ^{a,d}	4-Oct-07	258.0	Female
VAQS 20131386 ^{a,b,e}	1-Oct-13	289.8	Female
CAHA 267 ^{a,b,e}	6-Jul-14	293.0	Female
KLC 211 ^{a,b,c}	16-Sep-14	301.0	Female

^aUsed for *C. magna* habitat utilization; ^bUsed for scanning electron microscopy of *C. magna*; ^cUsed for identification of eggs in 'gill slit' gland fluid; ^dArchived (frozen) specimen made available and investigated during this study; ^eUsed for 'gill slit' gland histology

All *Kogia breviceps* specimens used in this study were necropsied in fresh to moderate carcass condition (Smithsonian Institution codes 1–3; Geraci & Lounsbury 1993). Individual body condition ranged from emaciated to robust, and no individual displayed evidence of human interactions that may have contributed to mortality. Whales were considered positive for infection if crassicaudids were observed grossly and/or histologically. When crassicaudids were present, details regarding their anatomic position within the host were documented.

Gross descriptions of *C. magna* habitat use

In each *K. breviceps* specimen, detailed gross dissections were carried out to identify specifically the host tissues used by *C. magna*. Dissections began with an external examination of the cervical region of the whale to identify the position of the opening of the duct into the 'gill slit' gland. Once the duct was located, an Argyle 14.0 cm long, open-end catheter (1.17 mm diameter) (Covidien) was inserted into the lumen to mark the location of the gland. The left gland was routinely approached via tangential sectioning through the blubber (superficial to deep), and the right gland was approached via cross-sectioning (cranial to caudal). As each blubber section and underlying muscle layer was removed, it was investigated for nematodiasis caused by crassicaudid parasites. When present, the location of the crassicaudid(s) was documented, and selected host tissues were collected for histological examination. Once the 'gill slit' gland was exposed, its large central lumen was examined for the presence of crassicaudids. In 4 *K. breviceps* infected with *C. magna*, the contents of the gland — presumed to be the secretion — were collected and examined using standard wet mount techniques and the Fecasol flotation technique (specific gravity 1.200) (Vetoquinol). A subsample of the secretion was also archived (at –80°C) for future analyses.

Following the removal of the entire blubber layer, the cervical, shoulder, and epaxial musculature was examined grossly. Due to their large size (over 2.5 m in length in adults), the epaxial muscles were systematically serially cross-sectioned every 5–10 cm and excised to search for the presence of crassicaudids. When either a cephalic or tail end of a crassicaudid was recovered within host tissues, its position was documented. The crassicaudid specimen was then removed and preserved in either acidified formol alcohol or 70% ethanol for later examination using SEM (see below).

In 1 kogiid specimen (CAHA 267, adult female, 293 cm), the entire course of a single crassicaudid worm through host tissues was investigated using both gross and micro-dissection techniques. This dissection began in the 'gill slit' gland because it is a common site of infection and presents the tail end of the parasite (Keenan-Bateman et al. 2016), which can be followed retrograde. During a 2 d dissection, the course of this worm was followed until both its cephalic and tail ends were identified. All dissections were documented using digital photography and videography (either a Nikon DX camera or Nikon D600 with a Nikkor 28–30 mm lens).

Histological examination of the 'gill slit' gland

A preliminary histological examination of the 'gill slit' gland was undertaken using samples collected from 2 adult female *K. breviceps* (VAQS 20131386, CAHA 267) infected with *C. magna*. Tissues were collected from the deepest portions of the gland, to sample an area not in direct contact with the nematode. Serial paraffin sections (10–15 µm) were stained with hematoxylin and eosin, to examine the general anatomy of the tissue. Slides were examined using an Olympus BX60 microscope, and micrographs were collected utilizing a SPOT RT color camera (Olympus Scientific Solutions Americas; SPOT Imaging Solutions).

Description of *C. magna* using SEM

Preserved cephalic and tail ends of recovered crassicaudids (n = 7) were prepared for SEM by first rehydrating in deionized water. These specimens were then examined and photographed using a Zeiss dissecting scope and SPOT RT color camera to verify the identity of the recovered segments. Once identified, the heads and tails were cut to approximately 0.5–1 cm lengths and dehydrated in ascending concentrations of ethanol (EtOH) (50, 70, 90, 95, and 2 times at 100%) in 1 h increments. The specimens were then transferred to a 1:1 concentration of 100% EtOH and hexamethyldisilazane (HMDS) for 1 h, and then to 100% HMDS for an additional hour. Specimens were then placed on filter paper and allowed to dry for 1–2 h. Once dry, specimens were mounted on aluminum stubs using carbon tabs. Mounted specimens were placed in a Cresington 208 HR High Resolution Sputter Coater for coating with a 10 nm thick conducting layer of platinum/palladium, and ana-

lyzed using a Philips XL 305 FEG Scanning Electron Microscope (FEI) to provide detailed surface images of cephalic and tail structures.

RESULTS

Necropsy findings

The cause of stranding was not determined in all cases and could have been multifactorial. Suspect contributing factors included pregnancy-related metabolic derangements, lymphadenitis related to viral and/or infectious disease, and hepatic congestion. Seven individuals were screened for morbillivirus using PCR and all tested negative for infection. Of note, all but 1 female investigated in this study were pregnant, and the 1 non-pregnant female was with calf.

Gross description of *Crassicauda magna* habitat use and the course of a single worm

The habitat of *C. magna* includes the epaxial musculature and vasculature, the cervical 'gill slit' gland, and multiple tissues between these 2 anatomically distant sites within the host (Fig. 1). The most conspicuous site of *C. magna* infection was within the cervico-thoracic epaxial musculature, extending from the back of the skull to the end of the ribcage. Nematodes were discovered during dissections at multiple positions within the muscle. On the superficial surface of the muscle, hardened and flattened tortuous tubes of tissue were often observed. These worm 'sheaths' most often did not contain viable worms. Whether these sheaths were secreted by the worm, or produced as a host tissue response, is not yet known. Worms that appeared to be in good condition (i.e. circular cross-section, turgid, light cream to pink in color) were often observed deep within the epaxial muscle. A common site of infection within the epaxial muscle was near the atlanto-occipital joint, although crassicaudids were observed throughout the cervico-thoracic region (Fig. 2). These worms displayed the capacity to alter grossly the morphology of the skeletal muscle in which they were embedded. Coiled lengths of worms were routinely recovered from within cavernous spaces, surrounded by seemingly unaffected muscle. These smooth-walled, trabeculated chambers, containing multiple loops of worm, were a characteristic feature of *C. magna* infection within the epaxial musculature. Despite the vast length (>1 m) of worm that could be removed from within some of these

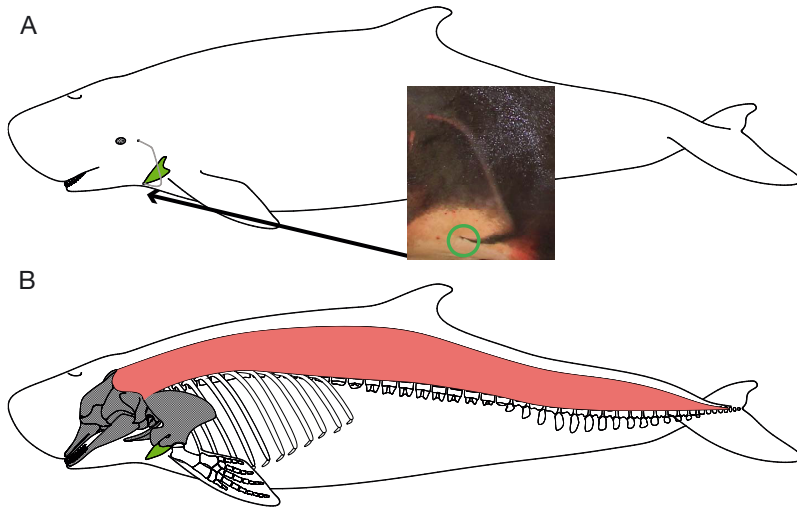


Fig. 1. Illustrations depicting the position of the cervical 'gill slit' gland (shown in green) and the epaxial locomotor muscles (shown in pink) within *Kogia breviceps*. (A) Externally, the duct of the 'gill slit' gland is located at the cranial tip of the ventral arm of the false 'gill slit' pigmentation pattern (see inset photo). (B) Internally, the gland is situated between 2 skeletal landmarks (shown in gray); the gland lies ventral to the mastoid process of the skull and extends dorsally and caudally to lie just deep to the scapula

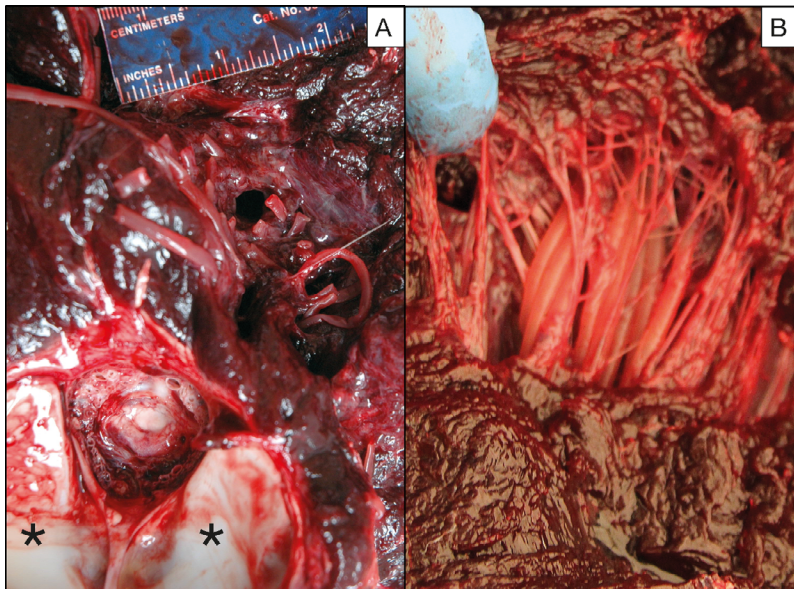


Fig. 2. *Crassicauda magna* within the epaxial musculature of its host, *Kogia breviceps*. In these images, cranial is to the top of the page. (A) Dorso-cranial view of the cervical epaxial muscle at the level of the occipital condyles (marked by asterisks) (specimen TKB 002). Note the tissue damage surrounding the nematode, which has been cut at multiple positions along its length. (B) Dorsal view of multiple loops of a single coiled nematode within a sac-like cavernous space within the cervical epaxial muscle (VAQS 20141094)

chambers in the epaxial muscles, only 1 end of the worm—the anterior end—was recovered there. Seven ends of crassicaudid worms were dissected from the epaxial muscles of 5 *K. breviceps* specimens, and each was identified as a cephalic end.

The tails of crassicauidids were discovered during dissections in an entirely different anatomic location within the host, the cervical 'gill slit' gland. Externally, the duct of the 'gill slit' gland opens at the cranial tip of the ventral arm of the false 'gill slit' pigmentation pattern (see Fig. 1A). Internally, the 'gill slit' gland is located between 2 skeletal landmarks—ventral to the mastoid process of the skull and extending dorsocaudal to lie just beneath the cranial margin of the scapula (see Fig. 1B). The duct of the gland extends from its external opening at the skin surface caudo-medially through the blubber layer at an approximate 45° angle to the central chamber of the gland, which lies between 2, vertically oriented, layers of cutaneous muscles. The muscle layer superficial to the gland is richly invested with fat and a loose connective tissue matrix, and is separated from the gland by a fascial layer. The superficial muscle layer continues dorsally to insert into a large fat pad that is situated between the mastoid process of the skull and leading edge of the scapula. The cutaneous muscle layer deep to the gland has a more typical skeletal muscle appearance, and overlays the sternomastoid and sternohumeralis muscles. Both cutaneous muscle layers surrounding the gland appear to be distinct from, but very similar in appearance to, the panniculus muscle described by Shulte et al. (1918).

Male and female tails have only been observed hanging freely and entwined within the lumen of the 'gill slit' exocrine gland of infected *Kogia breviceps* (Fig. 3A). To date, adult male tails have also been found in the gland without female tails being present, but no adult female tails have been found without being entwined by a male tail. The presumed secretion of

the gland, collected from 4 *K. breviceps* parasitized by *C. magna*, contained eggs that were extremely abundant, 40–44 $\mu\text{m} \times 20\text{--}26 \mu\text{m}$, thick-shelled and ovoid, and contained larvae (Fig. 3B). Additional histological and molecular analyses of the kogiid 'gill slit'

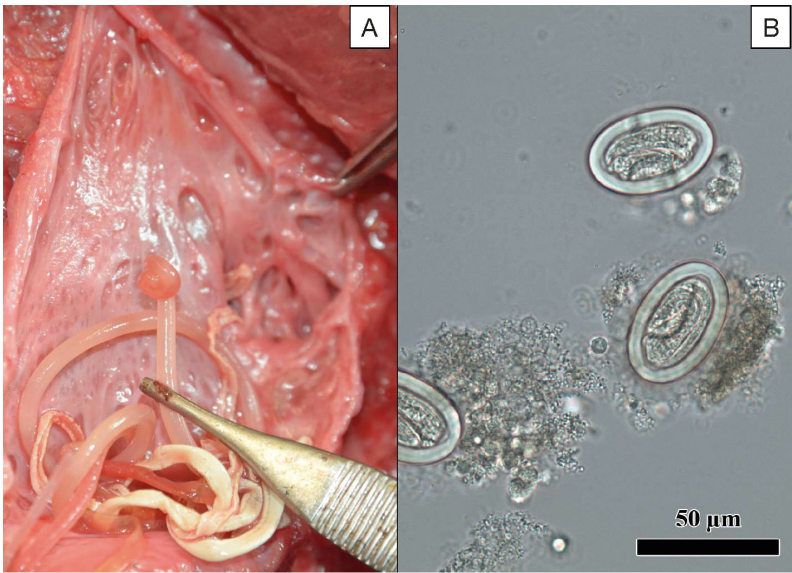


Fig. 3. *Crassicauda magna* within the cervical 'gill slit' gland of *Kogia breviceps*. (A) Tails of multiple males hanging freely within the lumen of the cervical 'gill slit' gland of *K. breviceps* (specimen CAHA 267). (B) Eggs with developing larvae isolated from the fluid within the 'gill slit' gland of an infected whale (VAQS 20131386)

gland and secretions are ongoing under a separate study.

The epaxial muscles and cervical 'gill slit' gland are distant anatomic sites, yet they are the only locations where *C. magna* cephalic and tail ends, respectively, were discovered. The entire course of a single worm was followed, using micro-dissection techniques, to elucidate its path through its *K. breviceps* host. Within the gland, the worm's tail was free of any covering/sheath (see Fig. 3A). In all other host tissues, however, the worm's body was covered in a sheath, or was found looped within smooth walled chambers. Within these chambers, some portions of the worm's body could also be ensheathed. These structures, and the surrounding host tissues, had to be carefully dissected away to follow the worm's course. To permit a directional description, the worm's path is described from the 'gill slit' gland to the epaxial muscle and terms such as 'moved' and 'traveled' are used for descriptive ease. An overview of the worm's path, and views of its morphology at multiple points along that path, are provided in Figs. 4 & 5, respectively.

The dissection began by locating the tail of a robust, male crassicaudid in the left 'gill slit' gland of an adult female *K. breviceps* (CAHA 267) (Fig. 5A). The worm's body exited the gland by traveling some centimeters out the duct of the gland, through the duct wall and into the surrounding blubber. After traveling a short distance in the blubber layer, in what appeared to be an irregular circular path near the duct, the worm traversed the blubber thickness to re-enter the body at the fascial layer between the deep slip of the cutaneous muscle and superficial surface of the sternohyoid muscle. It then continued circumferentially around the ventral neck and dorsally up the contralateral (right) side of the body, traveling within the fascial plane between the blubber layer and superficial muscles. Within this fascial

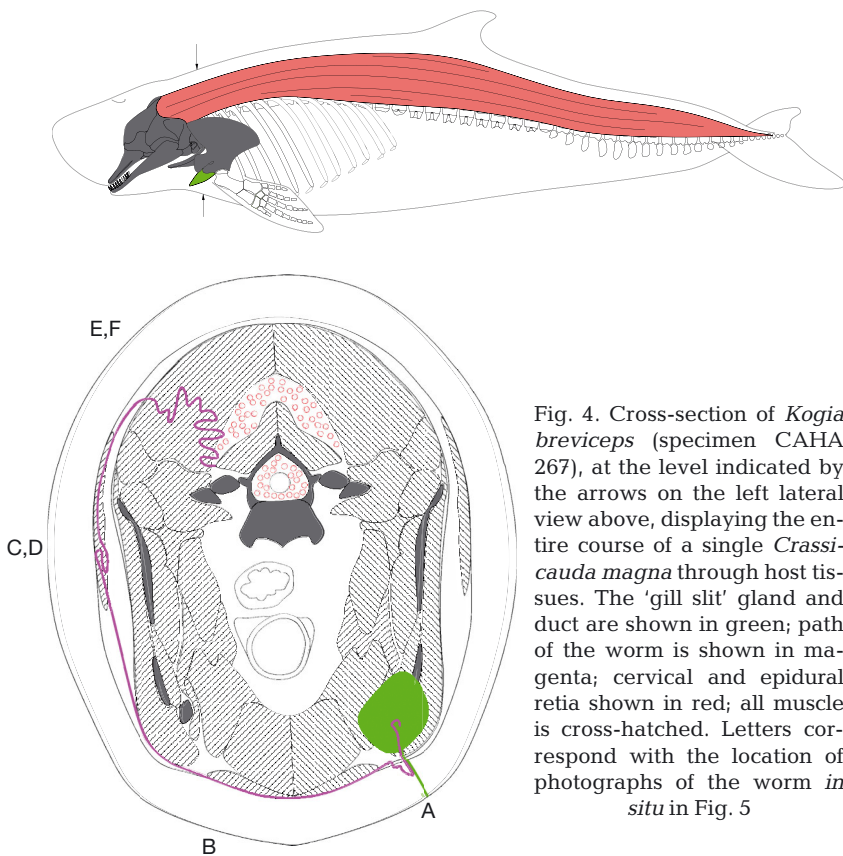


Fig. 4. Cross-section of *Kogia breviceps* (specimen CAHA 267), at the level indicated by the arrows on the left lateral view above, displaying the entire course of a single *Crassicauda magna* through host tissues. The 'gill slit' gland and duct are shown in green; path of the worm is shown in magenta; cervical and epidural retia shown in red; all muscle is cross-hatched. Letters correspond with the location of photographs of the worm *in situ* in Fig. 5

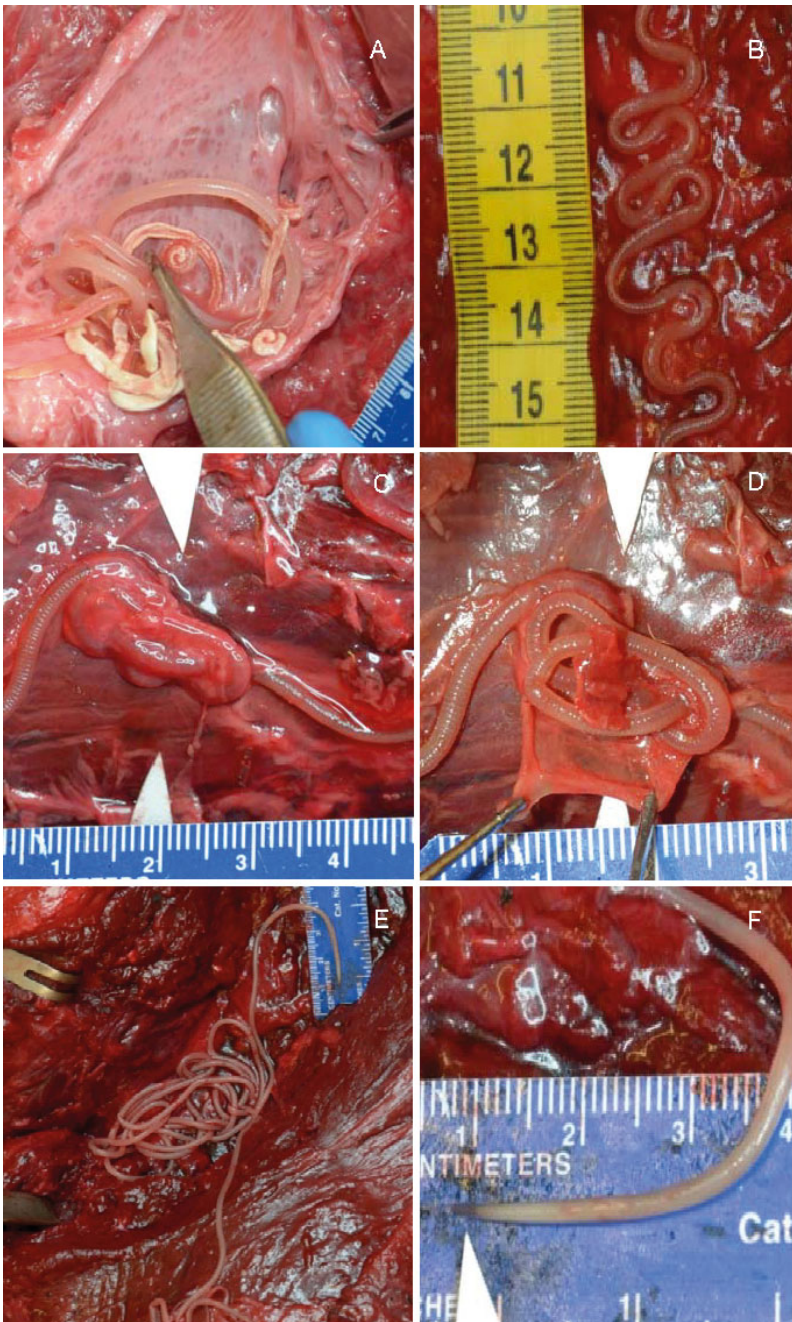


Fig. 5. *In situ* photographs displaying the pattern of habitat use of *Crassicauda magna* within *Kogia breviceps* (specimen CAHA 267). The letters correspond to specific positions along the path of the worm shown in Fig. 4. (A) *Crassicauda* tails hanging freely within the central lumen of the 'gill slit' gland. (B) Sinusoidal path of the worm in the subcutaneous fascial plane. (C) Knot-like loops along the worm's path covered by a sheath and (D) with the sheath covering removed. (E) Final chamber within the epaxial musculature where the worm terminated, and (F) its anterior end (tip of the white arrow)

plane, the worm's body maintained a very regular sinusoidal path with an average peak to peak amplitude of 1.5 cm and period of approximately 2 peaks per 5 cm (Fig. 5B).

At the level of the right sternomas-toid, the worm's body was arranged into several knot-like loops along its path, which were encased within a thin walled sac-like extension of the sheath surrounding the worm (Fig. 5C,D). Within the sac, the worm's body was looped upon itself in a convoluted fashion, giving the appearance of a knot. These pseudo-knots appeared to function to anchor the worm in place, as they were often observed at positions where the worm's trajectory changed, or where it entered a new tissue type or muscle. As the worm's body continued dorsally, it entered the superficial cutaneous muscle where its path became less sinusoidal and more straightened, a trajectory that was similar to the circumferentially oriented (i.e. dorso-ventrally vertical) muscle fibers through which it traveled. At the level of insertion of the rhomboideus capitis at the caudal skull, the worm's body turned deep, and entered the fascial plane between the medial surface of the rhomboideus capitis and an extensive occipital venous plexus. Within this fascial plane, the path of the worm again became sinusoidal, although with a more gently curved and wider path. These observations suggested that the trajectory of the worm was dependent, at least in part, upon the tissue through which it was traveling. Through fascial planes, the worm's path was sinusoidal, while within the muscle, its path appeared more constrained to spaces between fascicles.

At the deep surface of the rhomboideus capitis, the worm's body, which had been oriented generally within a transverse body plane as it traversed around the circumference of the whale, took a virtual 90° turn caudad. The worm then entered a rich vascular space dorsolateral to the semispinalis muscle and continued

caudally. At approximately 15 cm caudal to the back of the skull, the worm's body again appeared to become knotted within a thin-walled sac. Upon fine micro-dissection of the sac, these knots were simi-

larly determined to be multiple, tightly wound loops within a sac that appeared to be a dilation and thinning of the sheath. From this pseudo-knot, the worm's body burrowed deeply into the epaxial muscles (Fig. 4E,F) and traveled mediad, in a path perpendicular to the orientation of the epaxial muscle fibers. The worm's body then moved obliquely cranial and entered into a third, larger, encapsulated pseudo-knot deep within the caudal semispinalis muscle. This smooth-walled chamber was near the level of the vertebral articulation with the first rib (capitulum), just caudal to the transverse processes of the first 2 cervical vertebrae, and very near to a specialized vascular structure known as the cervical rete. Upon opening, this third sac differed in appearance from those observed earlier. It was a larger and more open trabeculated chamber. Within this chamber, vast lengths of the worm's body were observed, loosely coiled in multiple layers of loops rather than forming a knot-like structure (Fig. 5E). The worm's cephalic end was found in this chamber, facing cranially (Fig. 5F).

Histological anatomy of the 'gill slit' exocrine gland

Preliminary histological analyses indicate that the 'gill slit' gland is a compound tubuloalveolar exocrine gland with a large central chamber (see Figs. 3A & 5A) and many branching ducts, surrounded by a fibrous connective tissue capsule (Fig. 6). The epithelial lining of the ducts appears to vary between

stratified columnar and stratified cuboidal. The glandular portions, which take the form of both branched tubules and alveoli, maintain a double cuboidal epithelium and contain an eosinophilic luminal product (Fig. 6B).

Description of *C. magna* using SEM

The descriptions below follow the format of both Johnston & Mawson (1939) and Jabbar et al. (2015). The maximum diameter of preserved material was 3×4 mm. The head was rounded, and the stoma was surrounded by 2 small lateral lips (Fig. 7). Each lip displayed a prominent labial papilla (pseudolabium) that merged laterally with a presumed surrounding cuticular plate (ptilina). The apical surface of the pseudolabia did not possess papillae as suggested by Jabbar et al. (2015), but, rather, had a porous, pit-like appearance (Fig. 7B). The 2 lateral and 4 submedial papillae described by Baylis (1916, 1922) as characteristic of *Crassicauda* (Fig. 7A,B) were observed, although these features appeared somewhat reduced in size relative to Baylis' (1916, 1920, 1922) illustrations (Fig. 7C) and descriptions. Each of the 2 lateral papillae displayed an apical pit, which is presumably an amphid (Fig. 7B,D). Amphids are sensory pits found on the anterior end of nematodes (Chitwood & Chitwood 1952, Roberts & Janovy 2005).

The male tail possessed prominent caudal alae, and 2 spicules of unequal length that protruded from a cloaca (Fig. 8A,B). Submedial to the cloaca, lateral

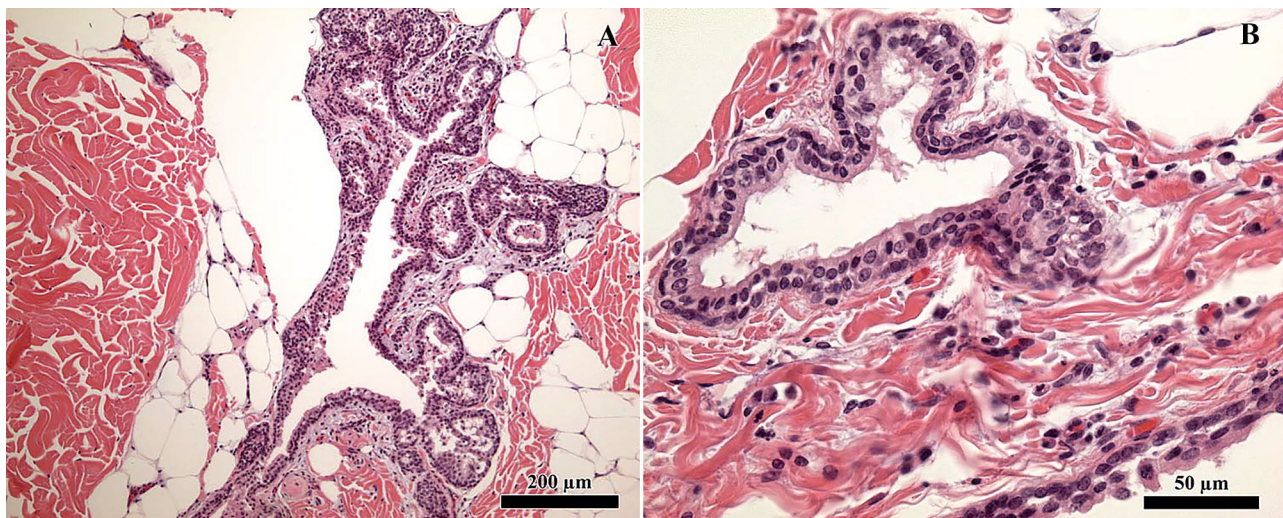


Fig. 6. Micrographs of the 'gill slit' exocrine gland of *Kogia breviceps* (specimen VAQS 20131386) stained with hematoxylin and eosin. (A) The 'gill slit' gland is a compound tubuloalveolar exocrine gland. (B) The glandular portions, which take the form of both branched tubules and alveoli, maintain a double cuboidal epithelium and contain an eosinophilic luminal product

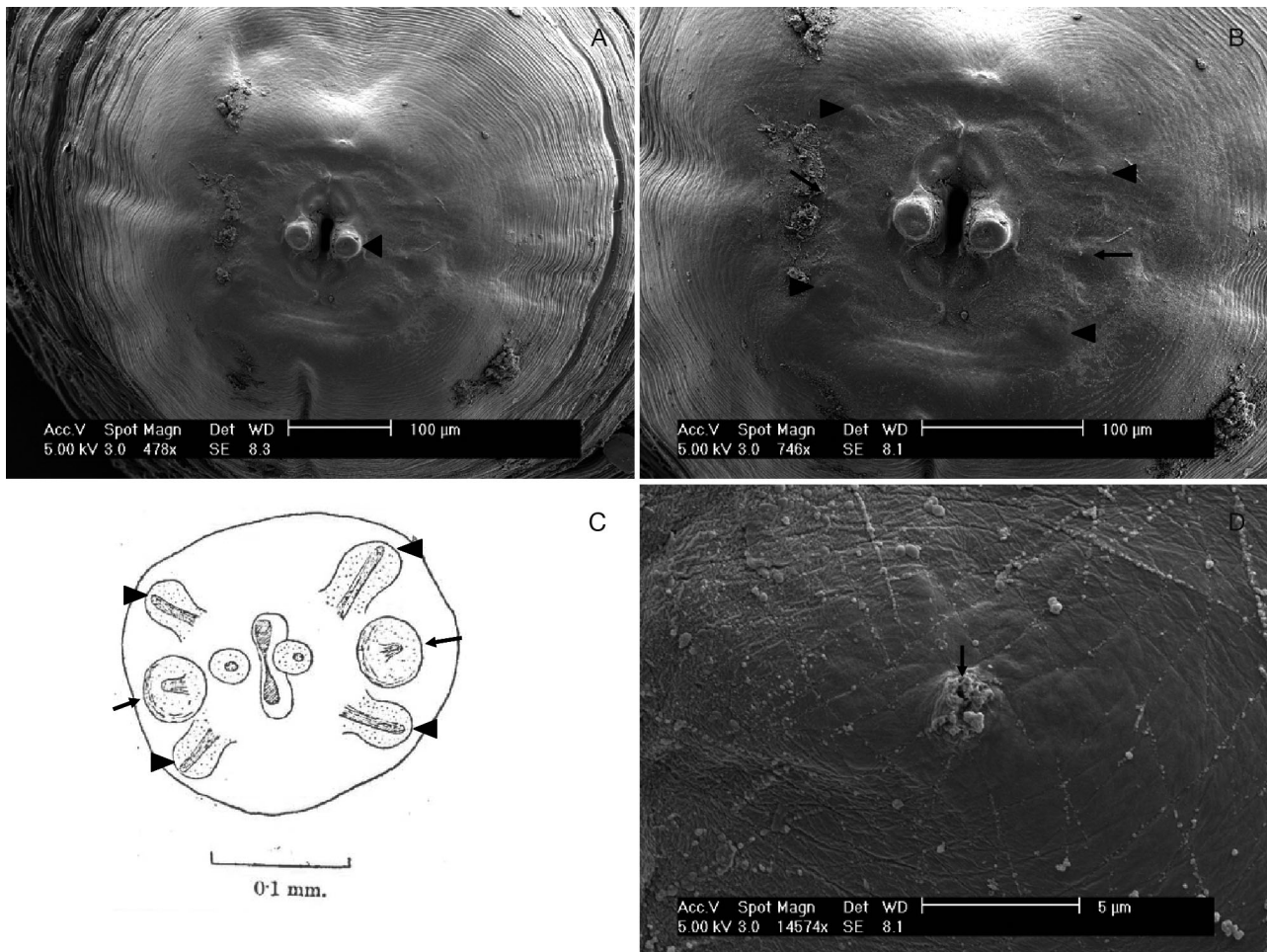


Fig. 7. Scanning electron micrographs displaying the cephalic morphology of *Crassicauda magna* collected from *Kogia breviceps*. (A) En face view showing 2 small lips in lateral positions, each displaying prominent labial papillae (arrowhead) (pseudolabia) that merge with a presumed cuticular plate. (B) En face view displaying 2 lateral (arrows) and 4 submedial (arrowheads) papillae situated along the margin of the cuticular plate. (C) Illustration from Baylis (1916; used by permission) describing aforementioned structures as characteristic of all crassicaudids. (D) The 2 lateral papillae each display an apical pit (arrow) and are therefore presumed amphids

caudal papillae were present, with 5 on the right side and 3 on the left. A single, median pre-cloacal papilla was also present (Fig. 8B). Directly posterior to the cloaca were 2 pores, presumably phasmids (Fig. 8C,D). Phasmids are sensory pits found on the tails of nematodes (Chitwood & Chitwood 1952, Roberts & Janovy 2005). In addition, there were 4 unidentified pores, a pair on each side of the cloaca's posterior lateral margin (Fig. 8B,C).

The female genital pore was found approximately 5–10 mm cranial to the tip of the tail within a circumferential constriction common to mature female crassicaudids (Skrjabin 1969) (Fig. 9A). An unidentified pore was present just cranial to the genital pore (Fig. 9B,C). The anal pore was located at the terminus of the tail (Fig. 9D).

DISCUSSION

Crassicauda magna habitat use within its *Kogia breviceps* host

This study provides the first detailed description of how the parasitic nematode *C. magna* uses *K. breviceps*, its definitive host. The 'gill slit' gland serves as an important habitat for *C. magna*. Adult male and female tails were found entwined and hanging freely within the lumen of this gland, in a manner similar to crassicaudids infecting the urinary system of mysticetes and the mammary glands of odontocetes (e.g. Baylis 1916, 1920, 1922, Lambertsen 1985, 1986, Geraci et al. 1978, Mead & Potter 1995). Larvated eggs, of a size consistent with those described for

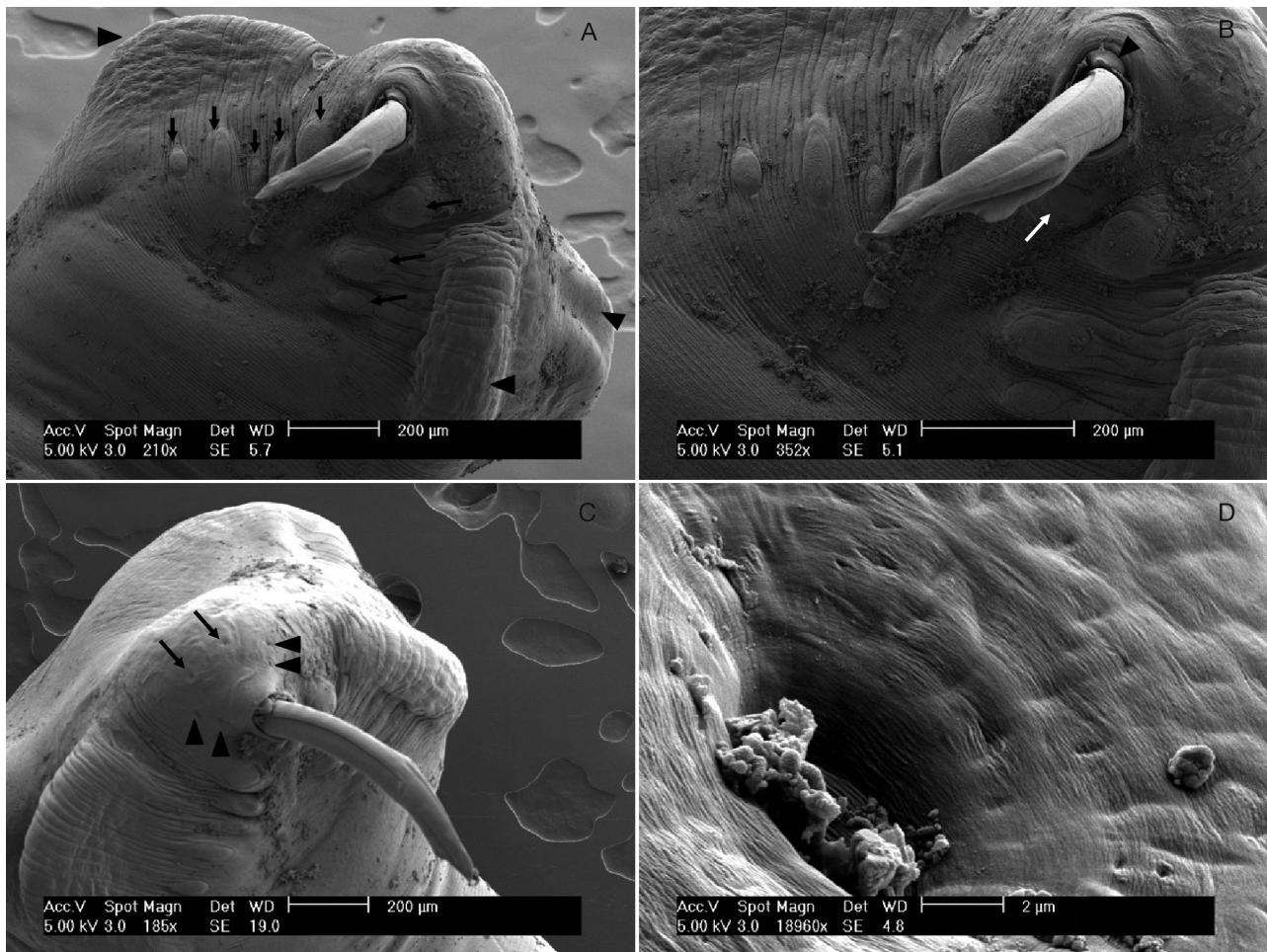


Fig. 8. Scanning electron micrographs displaying the tail morphology of a male *Crassicauda magna* collected from the 'gill slit' gland. (A) Prominent caudal alae (arrowheads) and 2 spicules of unequal length; submedial to the cloaca are lateral caudal papillae, with 5 on the right side and 3 on the left (arrows). (B) The smaller of the 2 spicules protrudes only slightly past the cloacal opening (arrowhead). Note also that there is a single median pre-cloacal papilla (arrow). (C) Directly posterior to the cloaca are 2 pores, presumably phasmids (arrows). Note that there are also 4 unidentified pores, a pair on each side of the cloaca's posterior-lateral margin (arrowheads). (D) Higher magnification view of 1 of the presumed phasmids directly caudal to the cloaca

other crassicaudids (Johnston & Mawson 1939, Margolis & Pike 1955, Lambertsen 1986, Geraci et al. 1978), have also been observed in the presumed secretion of the gland, illuminating the likely transmission path out of the host.

The cephalic ends of these parasitic nematodes are often found meters away (curvilinearly), embedded deeply within the host's epaxial musculature, often near large vascular beds that feed the central nervous system. Thus, *C. magna* is capable of penetrating through multiple host tissues, including epithelium, blubber, skeletal muscle, connective tissue, fascia, and vasculature. These worms also appear capable of significantly altering the morphology of the tissues in which they are embedded. Specifically, dissections revealed extensive tissue damage to the whale's

cervico-thoracic epaxial muscles, the primary locomotor muscles that power the upstroke (Pabst 1993). Multiple loops of worm were observed within cavernous spaces that appeared 'excavated' or 'digested' out of the surrounding muscle. The absence of muscle within these chambers suggests that the worms are consuming skeletal muscle, and potentially vascular tissue and blood.

Additionally, along its length within the *K. breviceps* host, the crassicaudid was surrounded by a sheath. Empty sheaths were also observed within host tissues, apparently devoid of living worm tissue. Whether the sheath is secreted by the parasite or is a host reaction to the parasite is not yet known. Similar structures, associated with *Crassicauda* infection in other cetaceans, have been described. For example, Geraci & St. Aubin

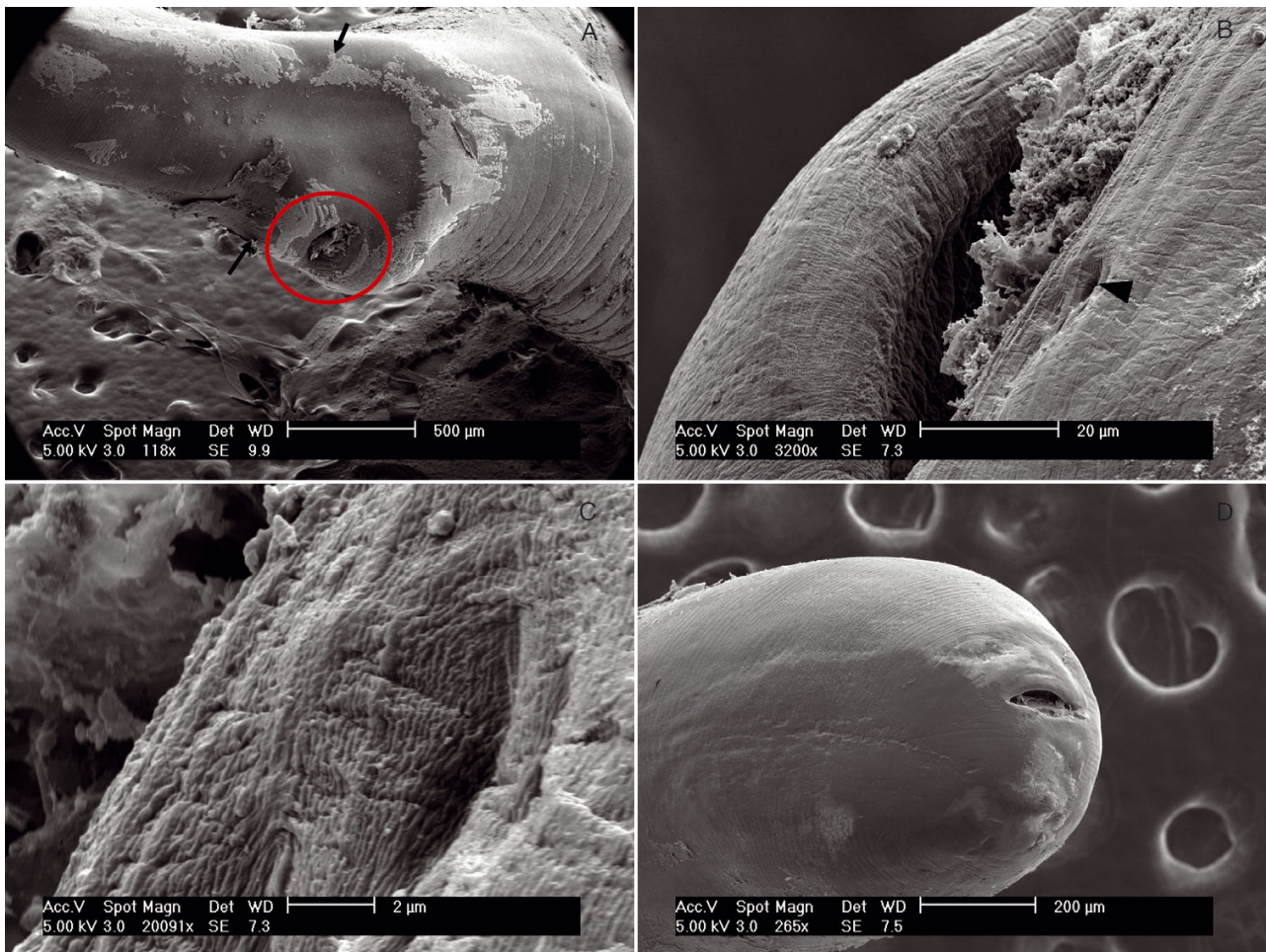


Fig. 9. Scanning electron micrographs displaying the tail morphology of a female *Crassicauda magna* collected from the 'gill slit' gland. The anal pore is located at the terminus of the tail, which is beyond the edge of the micrograph in (A). (A) Genital pore (red circle) is found on the presumed ventral surface, approximately 5–10 mm cranial to tail tip, within a circumferential constriction (arrows) of the tail common to mature female crassicaudids. (B) Higher magnification view of female genital pore. An unidentified pore (arrowhead) is present just cranial to the genital pore. (C) Higher magnification view of unidentified pore shown in (B). (D) The anal pore is located at the terminus of the tail

(1979) reported finding tortuous *Crassicauda* 'tracts' in the blubber of the lateral body wall of delphinids, with no worm *in situ*. Blaxter et al. (1992) provided a review of the role the nematode surface coat plays in evading host immunity in parasitic species. These researchers suggest that while the surface coat secreted by the excretory-secretory system of migrating parasitic nematodes can be used to directly evade the host's immune mechanisms, they can also be shed and left behind as deposits to divert the host's immune response away from the parasite as it progresses forward.

At multiple points along the worm's progression through host tissues, its movement appeared to change. At these positions, pseudo-knots were often observed and resembled anchoring or stabilizing points. The sac-like structure covering the pseudo-

knots appeared to be continuous with, and composed of, the same material as the sheath surrounding the worm. Similar structures have been described by other authors in cetaceans from both suborders. For example, Baylis (1916) described the disposition of crassicaudid heads embedded in 'masses of connective tissue' within the kidneys of fin whales. The author described the worm as having a very tortuous course within these masses, and later provided illustrations that were similar in appearance to what is described here as pseudo-knots, suggesting they may be homologous structures. Similar masses containing coiled worms have been described in mysticetes by several authors (Hamilton 1916, Rees 1953, Cockrill 1960, Skrjabin 1966, Lambertsen 1986, Geraci & St. Aubin 1987).

Thus, *C. magna* appears to use its *K. breviceps* host in much the same way as has been described for crassicaudids in other cetacean species. They consume host tissues (likely muscle and vascular tissue); occupy an excretory organ through which their eggs can exit the body (the 'gill slit' gland); are covered in a sheath of tissue that surrounds their body; and periodically, along their impressive length, arrange themselves into complicated, looped masses, surrounded by distinct, but as of yet unidentified tissue. How these crassicaudid nematodes initially infect *K. breviceps*, how they successfully traverse their path, what cues they use to navigate, and how they progress through host tissues are unanswered questions that warrant further attention.

***Crassicauda magna* SEM studies and taxonomic implications**

This study provided the first detailed scanning electron micrographs of taxonomically important features of the cephalic and tail ends of male and female *C. magna* infecting *K. breviceps*. It also provided the first description of any female tail of this nematode species.

The presence of cephalic papillae, which have been identified as a diagnostic characteristic of the genus *Crassicauda* by Baylis (1920), was confirmed in *C. magna* infecting *K. breviceps* from the mid-Atlantic. These structures were not reported in descriptions by earlier authors using light microscopy (Johnston & Mawson 1939, Jabbar et al. 2015).

SEM also illuminated several taxonomically important features of the male tail, including caudal papillae and caudal alae. The males investigated in this study possessed asymmetry in the number of lateral caudal papillae (Fig. 8A). This pattern differs from the most recent light microscopy study by Jabbar et al. (2015), who reported 5 pairs of lateral cloacal papillae. Caudal alae are used to differentiate between the 2 genera within the subfamily Crassicaudinae—*Crassicauda* and *Placentonema*. *Placentonema* are very large nematodes (greater than 8 m in length) that infect the placenta of the sperm whale *Physeter macrocephalus* and are identified by the presence of caudal alae, while *Crassicauda* lack this feature (Gubanov 1951). The male *Crassicauda* infecting *K. breviceps* in this study, and those illustrated by Dollfus (1966) and Jabbar et al. (2015), clearly possessed caudal alae, unlike any other *Crassicauda* species described to date in any cetacean species. This result suggests that either the

crassicaudid nematodes that infect *K. breviceps* may belong to the genus *Placentonema*, or that this feature is not diagnostic of the genus. This morphological observation, and molecular data from Jabbar et al. (2015) that put *Crassicauda* within a new family, the Acuariidae, suggest that the taxonomy of this group of nematodes requires additional clarification. Correct phylogenetic placement of these nematodes could also provide insight into the life cycle of this inadequately studied genus of parasites (Anderson 2000).

CONCLUSION

This study provides the first detailed description of the use of the *Kogia breviceps* host habitat by the parasitic nematode *Crassicauda magna*. This nematode appears to feed on skeletal muscle and vascular tissues that are meters away from the exocrine gland used as its site of reproduction and egg transmission to the environment. SEM revealed taxonomically important features on the head of *C. magna*, not previously observed in this *K. breviceps*-dependent species. The presence of caudal alae on the male tail, in contrast, bring into question the genus identity of this parasite. The gross and histological morphology of the 'gill slit' gland suggests that it produces a secretion that may be stored and actively expressed into the environment, and that further investigation into its function is warranted. Although the function of the 'gill slit' gland is not yet known in *K. breviceps*, this study has identified its crucial role for its crassicaudid parasite. We hope that the basic description of the parasite's habitat provided in this work proves useful for future studies of the pathogenesis of crassicaudosis in this kogiid whale.

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