



## Characterisation of *Crassicauda fuelleborni* nematode infection in Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) using postmortem computed tomography

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### ABSTRACT

Nematodes of the genus *Crassicauda* are parasites that infect various body tissues of cetaceans, including the mammary glands which can influence the reproductive output and hence threaten the survival of endangered cetacean populations. In this study, postmortem computed tomography (PMCT) was used to characterise lesions related to *Crassicauda fuelleborni* infections in stranded Indo-Pacific finless porpoises (*Neophocaena phocaenoides*) from Hong Kong waters. Using PMCT and subsequently verified by conventional necropsy, *Crassicauda*-related lesions were found in 52% of finless porpoises examined (n = 13/25), including both males and females. These parasitic lesions were mostly located in the ventral abdominal muscles in both sexes and situated in proximity to the mammary glands in females. *C. fuelleborni* infections were also found in the male reproductive organs, which to our knowledge have not been reported in this cetacean species previously. PMCT characteristics of the lesions were also correlated with the gross appearance observed at necropsy and the chronicity of the parasitic infections. In conclusion, this study established the use of virtopsy, particularly PMCT, to characterise *C. fuelleborni* infections in stranded finless porpoises for the first time, which is non-invasive and can be used prior to conventional necropsy to aid disease diagnosis and targeted sampling. This technique can be extended to other species of cetaceans and parasites, as well as being used in the retrospective analysis of past PMCT scans to deepen our understanding of the prevalence, health impacts, and ecological implications of parasitic infection in cetaceans.

### 1. Introduction

Parasitic infections are common among wild cetaceans and have been documented for decades (Fraija-Fernández et al., 2016; Raga et al., 2018). While parasites are often found as incidental findings in stranded cetaceans, severe parasitic infections can be detrimental to host fitness and contribute to the natural mortality of wild cetacean populations (Aznar et al., 2001; Balbuena and Simpkin, 2014; Geraci and Aubin, 1987; Gibson et al., 1998; Lehnert et al., 2005; Oliveira et al., 2011; Perrin and Powers, 1980; Siebert et al., 2001). Aside from the

pathogenic effects, parasites also have wider ecological implications like serving as biomarkers to reveal habitat use and social structure (Aznar et al., 1995; Balbuena and Raga, 1994) and indicating changes in the health of cetacean populations and marine ecosystem (Aznar et al., 2005; Van Bressemer et al., 2009).

Nematodes of the genus *Crassicauda* (Spirurida: Tetrameridae) infect the vasculature, urogenital system, muscles, mammary glands, and cranial sinuses of cetaceans, including both mysticetes and odontocetes (Balbuena and Simpkin, 2014; Dailey, 1985; Fraija-Fernández et al., 2016; Geraci and Aubin, 1987; Keenan-Bateman et al., 2016;

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Lambertsen, 1985, 1986, 1992). While the occurrence of crassicaudid infection has been widely documented, there is limited knowledge on the life cycle of crassicaudid nematodes in relation to the cetacean host. For crassicaudids that infect the vasculature and urinary system, migration of larvae from the intestines via arteries to the kidneys where the nematodes mature into adults has been suggested based on pathological and parasitological evidence (Díaz-Delgado et al. 2016; Lambertsen, 1992; Marcer et al. 2019a, 2019b). Apart from this, different possible transmission pathways for crassicaudids such as transplacental, through milk or urine, or via intermediate hosts have been hypothesised (Geraci et al., 1978; Lambertsen, 1986; Suárez-Santana et al., 2018). As marine spirurids typically require intermediate hosts (Anderson, 2000), transmission via intermediate hosts involved in the diet of cetaceans (e.g., crustaceans, cephalopods, fishes) was speculated (Díaz-Delgado et al. 2016; Marcer et al. 2019b). For crassicaudids that infect the mammary glands of smaller odontocetes, with the presence of adult nematodes and nematode ova observed in the mammary glands, direct transmission from mother to offspring via contaminated milk has been proposed (Geraci et al., 1978; Mauroo, 2017).

Crassicaudids that infect the mammary glands have attracted much attention as they may influence the reproductive success of cetaceans. Geraci et al. (1978) examined 30 female Atlantic white-sided dolphins *Lagenorhynchus acutus* and found *C. grampicola* infection of the mammary glands in 47% of animals, which were associated with inflammation and the replacement of secretory tissues by fibrosis or necrosis, thus affecting milk production and thereby calf survival and herd productivity of the population. Crassicaudid infections of the mammary glands and adjacent muscles were also found in other species like the harbour porpoise *Phocoena phocoena*, Dall's porpoises *Phocoenoides dalli*, and Indo-Pacific bottlenose dolphins *Tursiops aduncus* (Brook et al., 2002; Conlogue et al., 1985; Mauroo et al., 2008; Lehnert et al., 2014). In Hong Kong (HK) (between 22°08' and 22°35'N, 113°49' and 114°31'E), parasitic mastitis caused by *Crassicauda* sp. has been reported in both residential cetacean species, the Indo-Pacific humpback dolphin *Sousa chinensis* and the Indo-Pacific finless porpoise *Neophocaena phocaenoides* Cuvier, 1829 (hereafter NP) (Kot et al., 2021; Mauroo 2017; Mauroo et al., 2020). Hence, crassicaudid infections may present as one of the many natural and anthropogenic threats that contribute to the decline of cetacean populations in HK waters.

With the advantage of non-invasiveness, virtopsy, the postmortem use of imaging modalities including computed tomography (PMCT) and magnetic resonance imaging (Thali et al., 2003a), has been increasingly applied in the postmortem examination of cetaceans in recent years (e.g., Danil et al., 2014; Hamel et al., 2020; Kot et al., 2018, 2019, 2020a, 2020b, 2021; Tsui et al., 2020; Yuen et al., 2017; Zucca et al., 2004). One of the applications of virtopsy, particularly PMCT, is to detect pathological lesions, including parasitic infections, especially in cumbersome regions that are difficult and not routinely examined thoroughly during conventional necropsy (e.g., cranial sinuses or muscles across the entire body), and therefore allowing precise examination and targeted sampling (Tsui et al., 2020). However, PMCT studies focusing on the detection of parasitic infection in cetaceans are currently limited. Zucca et al. (2004) applied PMCT to assess the cranial sinuses of a Risso's dolphin *Grampus griseus* and found calcification and inflammation related to crassicaudid infection, which was later confirmed at necropsy. PMCT has preliminarily been used to detect and characterise different parasites in stranded cetaceans in HK waters, including *Crassicauda*-related lesions in the mammary glands and abdominal muscles (Kot et al., 2016, 2020a, 2021), which assisted in locating the lesions during post-mortem examination for pathological assessment and parasitology studies.

To strengthen the use of virtopsy, particularly PMCT, in the surveillance of crassicaudid infections in stranded cetaceans, this study aimed to characterise the PMCT features of *Crassicauda*-related lesions in stranded NP from HK waters. Prospective PMCT examinations were conducted to detect *Crassicauda*-related lesions, which were

subsequently verified and correlated with findings from conventional necropsy. The normal anatomy of the mammary glands in NP, as well as the PMCT appearances of *Crassicauda*-related lesions in both males and females, were illustrated in this study.

## 2. Materials and methods

### 2.1. Study subjects

This study was conducted between August 2020 and April 2021. During this period, 33 stranded NP cases were recovered by the local cetacean stranding response programme in HK waters (Tsui et al., 2020). Eight cases with incomplete carcasses or undetermined sex were excluded, and the remaining 25 fully intact NP carcasses were included in this prospective study. These 25 cases consisted of 14 males and 11 females, with total body length (TBL) ranging from 73 to 180 cm. These 25 cases were classified into age groups based on body length and radiological assessment of osteological features in the pectoral flipper bones (Kwan et al., 2022), including 3 neonates (TBL <90 cm, with no to limited epiphyseal ossification), 7 juveniles (TBL of 90–150 cm, with progressive epiphyseal ossification), 9 subadults (TBL >150 cm, with epiphyseal closure and remnants of epiphyseal line), and 6 adults (TBL >150 cm, with complete epiphyseal closure). These 25 NP carcasses were found in code 3 (moderate decomposition; n = 3), code 4 (advanced decomposition; n = 21), and code 5 (mummified; n = 1) (Geraci and Lounsbury, 2005).

Using PMCT and conventional necropsy, 13 out of these 25 cases (52%) were diagnosed with confirmed or probable crassicaudid infection in the reproductive organs, mammary glands, or adjacent muscles, which formed the main subject group of this study (Table 1). These 13 cases consisted of 6 males and 7 females, including 2 juveniles, 6 subadults, and 5 adults, and were all code 4.

### 2.2. PMCT assessment

As part of the local cetacean stranding response programme, all recovered stranding cases undergo routine PMCT scanning prior to conventional necropsy (Tsui et al., 2020). For all 25 stranded NP cases in this study, PMCT scans were conducted using a Siemens 64-row dual-source SOMATOM go.Up CT scanner (Siemens Healthineers, Erlangen, Germany) or a Philips 16-slice Brilliance Big Bore CT scanner (Philips Healthcare, Amsterdam, Netherlands), with the following scan parameters: 100–140 kV, 69–303 mA, 0.6–0.8 mm slice thickness, and scan field of view of 401–621 mm. PMCT scans were reconstructed and interpreted in the TeraRecon Aquarius iNtuition workstation ver.4.4.12 (TeraRecon Inc., San Mateo, CA, USA) with a consistent window setting across all CT scans (WW: 2200 and WL: 200). The PMCT scan of each NP carcass was assessed for abnormalities that indicate *Crassicauda*-related lesions with reference to preliminary studies (Kot et al., 2020a, 2021). For lesions found with PMCT, the location and PMCT characteristics (shape, outline, CT attenuation, and homogeneity) were qualitatively recorded, while the sizes of the lesions were measured in three dimensions using the in-built linear measurement function of the TeraRecon workstation.

### 2.3. Conventional necropsy and parasite identification

PMCT findings of each stranded NP case were reported to certified veterinarians, who then carried out the necropsies following standard protocols (Geraci and Lounsbury, 2005) and with reference to the PMCT findings. *Crassicauda*-related lesions detected on PMCT were searched with reference to the location noted on PMCT. Gross characteristics of the lesions were described, recorded, and supplemented with photographs and measurements whenever appropriate. The lesions were then examined for nematodes, which were collected and stored in 70% ethanol whenever available. Owing to the advanced decomposition

**Table 1**

Demographics and the lesion locations of the 13 examined stranded Indo-Pacific finless porpoises *Neophocaena phocaenoides* with confirmed or probable *Crassicauda fuelleborni* infection.

Case No.	Sex	Body length (cm)	Age group	Morphological identification	Location(s) of lesions found			
					<i>M. rectus abdominis</i>	<i>Mm. abdominales</i>	<i>M. hypaxialis lumbordum</i> (caudal)	Other
1	F	156	Subadult	<i>Crassicauda</i> sp.	Y	Y	N	N
2	M	170	Adult	NA (probable <i>Crassicauda</i> infection)	Y	N	N	N
3	F	167	Adult	<i>Crassicauda</i> sp.	Y	Y	N	N
4	F	162	Subadult	<i>Crassicauda</i> sp.	Y	N	N	N
5	M	156	Subadult	<i>Crassicauda fuelleborni</i>	Y	N	N	N
6	M	147	Juvenile	<i>Crassicauda</i> sp.	N	N	N	Y (right epididymis; penis)
7	F	145	Juvenile	<i>Crassicauda</i> sp.	Y	Y	N	N
8	F	167	Adult	<i>Crassicauda fuelleborni</i>	Y	Y	Y	Y
9	M	175	Adult	NA (probable <i>Crassicauda</i> infection)	Y	N	Y	N
10	F	157	Subadult	NA (probable <i>Crassicauda</i> infection)	Y	N	N	N
11	M	155	Subadult	<i>Crassicauda fuelleborni</i>	Y	N	N	Y (prostate)
12	F	161	Subadult	NA (probable <i>Crassicauda</i> infection)	Y	N	N	N
13	F	180	Adult	NA (probable <i>Crassicauda</i> infection)	Y	N	N	Y (penis)

condition of these cases, only gross examination and sampling for parasitological analysis were conducted, and no histopathological findings were available. Viable nematodes found were identified based on established morphological keys for the genus *Crassicauda* (Hoepli et al., 1929; Raga and Balbuena, 1990; Shiozaki and Amano, 2017). In 3 cases, the viable nematode was morphologically identified as *Crassicauda fuelleborni* Hoepli & Hsü 1929 (Table 1). In 5 cases, the viable nematode was only identified down to genus level as *Crassicauda* sp. (Table 1). In the remaining 5 cases where no viable nematodes were available (Table 1), the lesions were assigned as probable *Crassicauda*-related based on the consistent characteristics with the confirmed lesions and previous descriptions (Geraci et al., 1978; Mauroo, 2017; Kot et al., 2021).

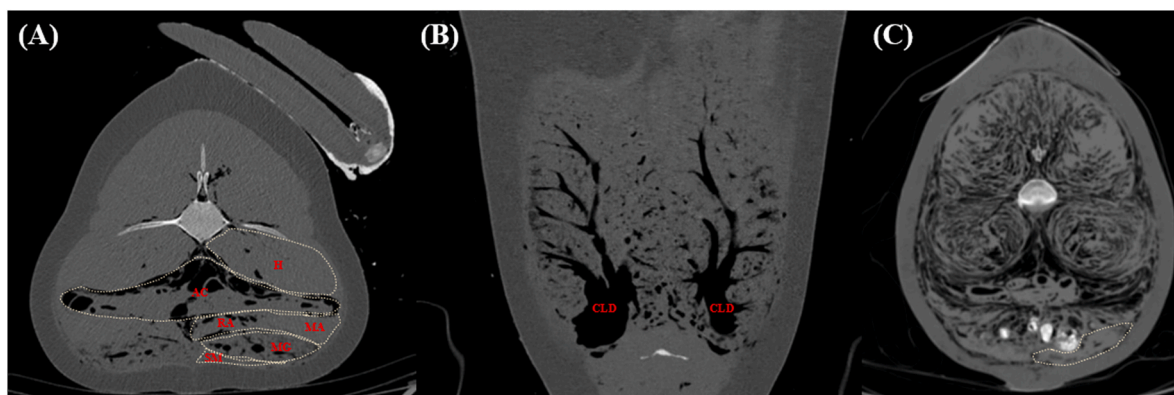
### 3. Results

#### 3.1. Normal anatomy and *Crassicauda*-related pathology of the mammary glands on PMCT

To assist with the detection of crassicaudid infection using PMCT, the

CT anatomy of the mammary glands and adjacent structures were first described with reference to literature (Huggenberger et al., 2019; Rommel et al., 2018; Plón and Bernard, 2007) (Fig. 1). On PMCT, the mammary glands of NP appeared as a pair of glandular tissues on the ventral abdominal surface, located between the ventral abdominal muscles (*m. rectus abdominis* and *mm. abdominales*) and a layer of superficial muscles overlying the blubber (Fig. 1A). The mammary glands opened into the nipples within the mammary slits that were situated lateral to the genital slit, while the glands extended cranially from the opening. The size of the glands was dependent on sexual maturity and reproductive status and most prominent in lactating individuals. The mammary glands of sexually immature females were harder to distinguish on PMCT owing to the smaller size and similar attenuation with surrounding muscles. In a lactating female (Fig. 1A–B), the mammary glands were enlarged (measured  $\sim 23 \times 8 \times 2$  cm in Fig. 1) and can be readily seen on PMCT. In addition, postmortem gas accumulation may allow the visualisation of the internal structure of the mammary glands, consisting of smaller branch-like ducts that converge into central lactiferous ducts towards the opening (Fig. 1B).

The typical presentation of *Crassicauda*-related mammary gland



**Fig. 1.** PMCT images showing the anatomy of the mammary glands and adjacent structures, as well as an example of *Crassicauda*-related mammary gland pathology in Indo-Pacific finless porpoises *Neophocaena phocaenoides*. (A) Recently pregnant adult female, axial cross-section of the mammary glands (MG) and adjacent structures (H: *m. hypaxialis lumborum*, AC: abdominal cavity, RA: *m. rectus abdominis*, MA: *mm. abdominales*, SM: superficial muscles between the mammary glands and blubber). (B) Recently pregnant adult female, longitudinal cross-section of the mammary glands, with branch-like ducts converging into the central lactiferous ducts (CLD). (C) Sexually mature adult female with severe parasitic infection of the mammary glands and adjacent muscles, notably with multiple hyperattenuated lesions in the muscles dorsal to the mammary glands with one invading and compressing the mammary gland tissues on the right side.

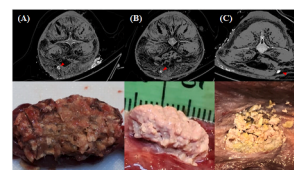
pathology in NP was also illustrated with an example (Fig. 1C). In NP, *Crassicauda*-related lesions were typically found in the caudoventral abdomen, particularly in the mammary glands and ventral abdominal muscles that situate immediately dorsal to the glands. On PMCT, these lesions presented as well-defined and ovoid-shaped nodules that were isoattenuated or hyperattenuated, and can be single to generalized multifocal depending on the severity of infection. The lesions were often seen in the ventral abdominal muscles in proximity to the mammary glands, and sometimes invading and compressing the gland tissues.

### 3.2. Occurrence of *Crassicauda*-related lesions in stranded NP

Using PMCT and subsequently verified by conventional necropsy, *Crassicauda*-related lesions were diagnosed in 52% of stranded NP cases ( $n = 13/25$ ) in HK waters between August 2020 and April 2021. *Crassicauda*-related lesions were found in both males ( $n = 6$ ) and females ( $n = 7$ ) (Table 1). Among these 13 cases, *C. fuelleborni* infections were confirmed using morphological identification in 3 cases (23%), and the nematodes were grossly identified to genus level as *Crassicauda* sp. in 5 cases (38.5%). No viable nematodes were found in the remaining 5 cases (38.5%) and these were assigned as probable *Crassicauda*-related lesions based on the consistent characteristics with the confirmed lesions and previous descriptions (Geraci et al., 1978; Mauroo, 2017; Kot et al., 2021) (Table 1).

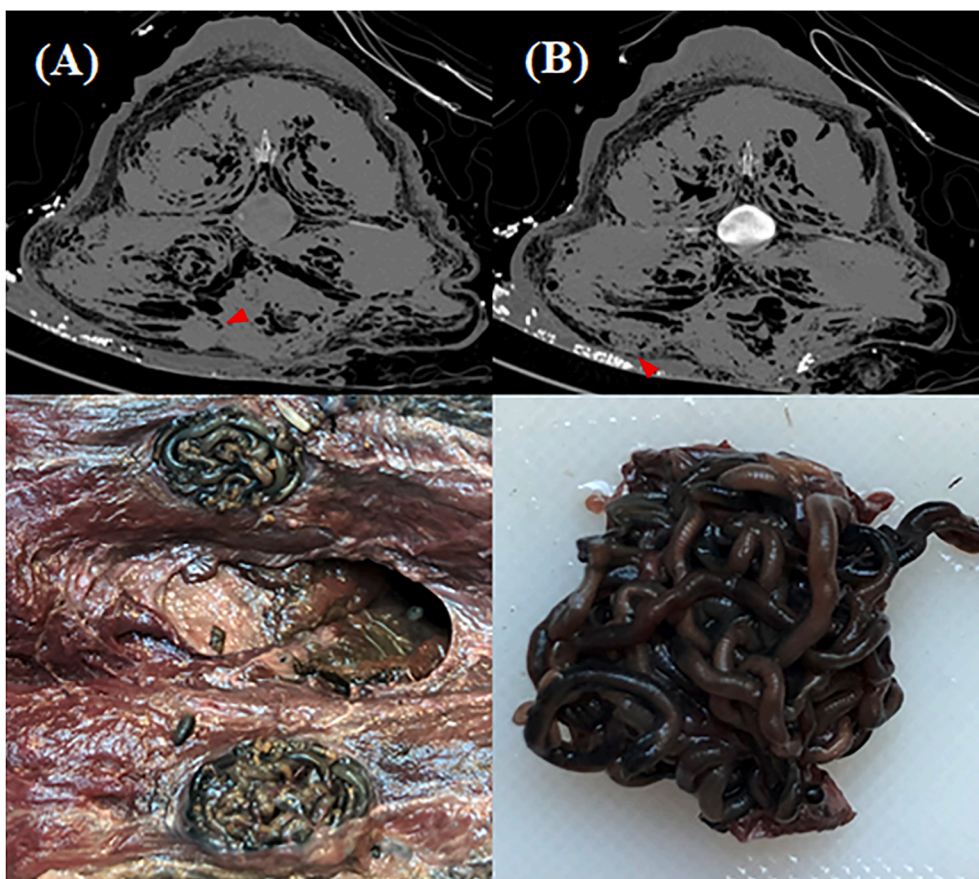
### 3.3. PMCT and gross characteristics of *Crassicauda*-related lesions

*Crassicauda*-related lesions in NP appeared on PMCT as ovoid-shaped nodules that were typically measured with diameters of 1.0–1.5 cm on the axial PMCT images and lengths of 1.5–3.0 cm on the coronal PMCT images. The lesions were classified into two types as ‘isoattenuated lesions’ (Fig. 2) and ‘hyperattenuated lesions’ (Fig. 3) based on the CT



**Fig. 3.** PMCT and necropsy images of probable *Crassicauda*-related lesions in Indo-Pacific finless porpoises *Neophocaena phocaenoides* stranded in Hong Kong waters. (A) Case 8, adult female, heterogeneous lesion (arrowhead) with a hyperattenuated rim and isoattenuated content in the ventral abdominal muscle (upper) and the corresponding nodule containing decomposed worms among caseous debris (lower). (B) Case 8, adult female, heterogeneous lesion (arrowhead) with both hyperattenuated and isoattenuated content in the ventral abdominal muscle (upper) and the corresponding nodule containing creamy pink caseous debris (lower). (C) Case 1, subadult female, homogeneous hyperattenuated lesion (arrowhead) in the ventral abdominal muscle (upper) and the corresponding nodule containing dried and pale yellow caseous debris (lower). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

attenuation measured in Hounsfield Unit (HU) in comparison to adjacent muscles and tissues. The isoattenuated lesions were less well-defined with similar attenuation as adjacent soft tissues and muscles (~40–70 HU) and mostly homogeneous in attenuation (Fig. 2A) but can also appear heterogeneous with a mix of isoattenuated content and some hypoattenuated foci (~-1000 HU), interpreted as small gas collections within the nodules (Fig. 2B). At necropsy, the isoattenuated lesions corresponded to thinner-walled intramuscular nodules that contained intact or fragmented nematodes that were tightly coiled and viable for parasitological analysis (Fig. 2). In contrast, the hyperattenuated lesions exhibited a wider range of homogeneity in attenuation on PMCT, ranging from nodules that were heterogeneous with a mix of



**Fig. 2.** PMCT and necropsy images of lesions caused by the nematode *Crassicauda* sp. in an Indo-Pacific finless porpoise *Neophocaena phocaenoides* stranded in Hong Kong waters. (A) Case 1, subadult female, homogeneous isoattenuated lesion (arrowhead) in the ventral abdominal muscle (upper) and the corresponding nodule containing coiled and fragmented nematodes later identified as *Crassicauda* sp. (lower). (B) Case 1, subadult female, heterogeneous lesion (arrowhead) with isoattenuated content and hypoattenuated foci interpreted as small gas collections in the ventral abdominal muscle (upper) and the intact and coiled nematodes collected from the corresponding nodule and later identified as *Crassicauda* sp. (lower).

isoattenuated and hyperattenuated content (wide range of 40–1300+ HU) (Fig. 3A–B), to homogeneously hyperattenuated (~1300+ HU) (Fig. 3C). At necropsy, the hyperattenuated lesions corresponded to thicker-walled intramuscular nodules with content that were not viable for morphological analysis, including fragments of nematodes among caseous materials (Fig. 3A), or entirely of caseous materials that were pink or pale yellow in colour (Fig. 3B–C).

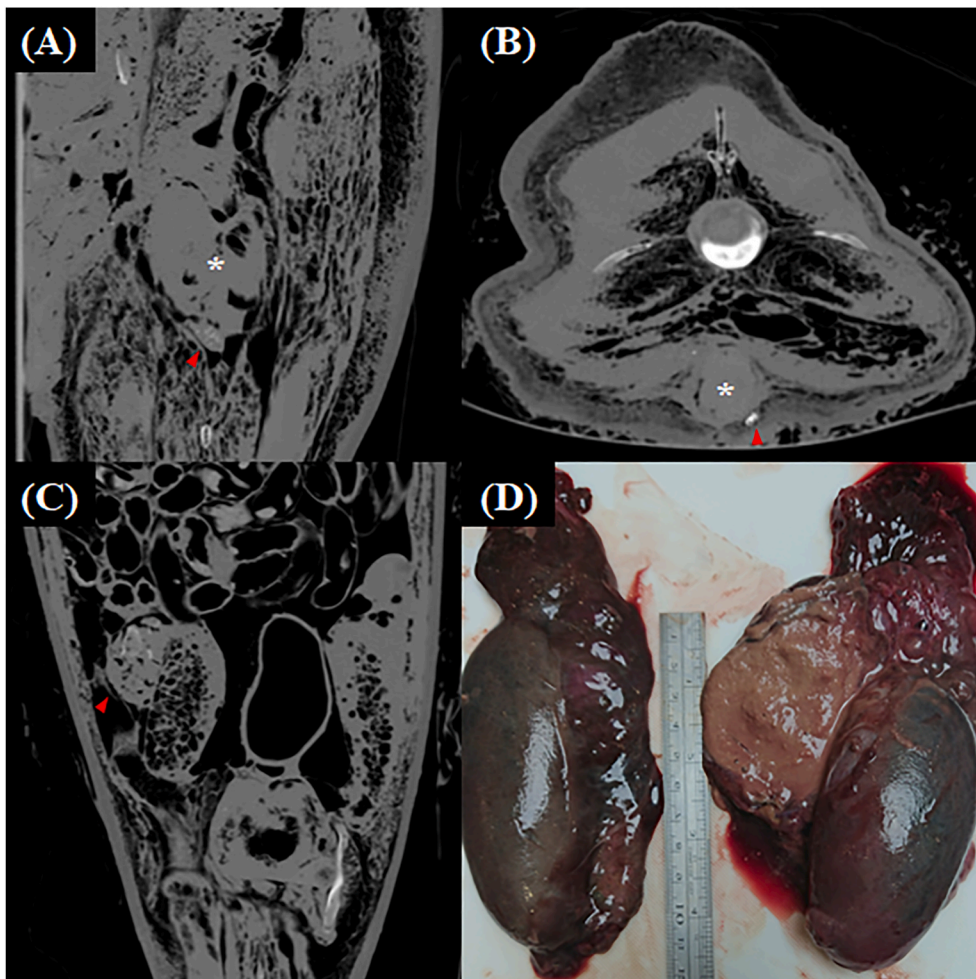
### 3.4. Locations of *Crassicauda*-related lesions

In the 7 female NP examined, confirmed or probable *Crassicauda*-related lesions were found in the ventral abdominal muscles overlying the mammary glands (n = 7/7), particularly in the more medial *m. rectus abdominis* region (n = 7/7; e.g., Fig. 3B) and the more lateral *mm. abdominales* region (n = 5/7). Most lesions in the ventral abdominal muscles were situated immediately dorsal and in proximity to the mammary glands as described earlier (e.g., Fig. 3A–B). In the 6 male NP examined, the confirmed or probable *Crassicauda*-related lesions were mostly found in the equivalent area of the *m. rectus abdominis* (n = 5/6). In one female and one male NP, *Crassicauda*-related lesions were found more caudal to the abdomen, situated between the caudal portions of *m. rectus abdominis* and *m. hypaxialis lumborum*. In addition, *C. fuelleborni* or *Crassicauda* sp. infections were found in the reproductive organs in 3 male NP cases, including the prostate (Fig. 4A; Case 11), tissues around the penis (Fig. 4B; Case 6 and 13), and an abscessed lesion in the epididymis (Fig. 4C–D; Case 6).

## 4. Discussion

In the current study, *Crassicauda*-related lesions in NP presented as parasitic nodules that were commonly found in the ventral abdominal muscles adjacent to the mammary glands in female NP (n = 7/7). The same region was also commonly infected in male NP (n = 5/6). The parasitic nodules exhibited a range of CT attenuation and were classified into isoattenuated and hyperattenuated lesions (Figs. 2 and 3). Upon necropsy, these parasitic nodules were found to encapsulate different contents, with isoattenuated nodules contained fresher nematodes viable for parasitological analysis, while the hyperattenuated nodules contained caseous materials and occasionally decomposed fragments of nematodes that were not viable for parasitological analysis. PMCT was able to non-invasively and effectively identify the locations of the parasitic nodules for the first time, and allowed targeted examination and sampling at necropsy, which greatly improved the efficiency of stranding postmortem investigations.

Lesions detected using PMCT in these 13 NP cases were subsequently verified using necropsy and parasitological analysis as either related to *C. fuelleborni* or *Crassicauda* sp. infections (for isoattenuated lesions with viable nematodes; Fig. 2), or as probable *Crassicauda*-related lesions based on consistent characteristics with the confirmed lesions and previous descriptions (for hyperattenuated lesions with caseous materials; Fig. 3). Although in this study, no alternative diagnosis was observed for these lesions, other potential differentials that may produce similar appearances on PMCT include abscessed or granulomatous lesions from fungal or bacterial infections, or neoplasm, which should also be considered when interpreting CT images. Some characteristics of



**Fig. 4.** PMCT and necropsy images of lesions caused by the nematode *Crassicauda fuelleborni* or *Crassicauda* sp. in the male reproductive organs of Indo-Pacific finless porpoises *Neophocaena phocaenoides* stranded in Hong Kong waters. (A) Case 11, subadult male, heterogeneous lesion (arrowhead) with both isoattenuated and hyperattenuated content associated with the prostate (asterisk), nematode found was later identified as *C. fuelleborni*. (B) Case 13, adult male, homogenous hyperattenuated probable *Crassicauda*-related lesion in the tissues surrounding the penis (asterisk). (C) Case 6, juvenile male, isoattenuated lesion with some hypoattenuated and hyperattenuated foci associated with the right testis (arrowhead). (D) Case 6, juvenile male, the corresponding abscessed lesion at the right epididymis with nematodes later identified as *Crassicauda* sp.

*Crassicauda*-related lesions as observed in this study that may assist this differentiation is that the lesions were often multifocal and concentrated at the ventral caudal abdominal region (although singular lesions have been observed), and typically well-defined from surrounding tissues. The PMCT presentation of these *Crassicauda*-related lesions may be specific to *C. fuelleborni* infections in NP, as crassicaudids infect different organ systems in various cetacean species including both odontocetes and mysticetes. Histopathological analysis should be performed whenever possible for definitive diagnosis, and the results should be correlated with the PMCT findings. Further PMCT investigation on more NP cases and mammary gland-infecting crassicaudids in other odontocetes is required to develop this technique for characterising parasites in cetaceans.

Our findings agreed with the previous descriptions by Mauroo (2017), which documented *Crassicauda*-related lesions in NP also as nodules that either contained fresher nematodes, or with thicker walls and contained degenerated and calcified nematodes with caseous materials. The variation in gross characteristics of *Crassicauda*-related lesions was suggested to be related to the age of the host and chronicity of the infection, with caseous nodules being more common in NP with longer body length and presumptively older in age, while chronic granulomatous inflammation and fibrosis were found in tissues around the nodules with caseous and calcified materials (Mauroo, 2017). Similar histological findings were also reported in intramuscular crassicaudid infections in harbour porpoises, which were characterised by thick fibrotic walls and focal chronic non-suppurative inflammation in the adjacent tissues (Lehnert et al., 2014). In this study, histopathological analysis was not performed owing to the high level of decomposition for all 13 cases (code 4; Geraci and Lounsbury, 2005). A high proportion of advanced decomposed cetacean carcasses has been established as one of the main difficulties that hindered stranding research in HK, as decomposition affects the interpretation of pathological findings (Jefferson et al., 2006; Tsui et al., 2020). However, PMCT was able to effectively discern the differences in gross characteristics between *Crassicauda*-related lesions (i.e., fresher lesions with viable nematodes versus more chronic lesions with degenerated and caseous materials) due to the differences in attenuation values produced by the calcified materials within the lesions. This revealed the potential use of PMCT to study the chronicity of *Crassicauda* infections which is currently unknown. In addition, this application of PMCT in advanced decomposed cetacean carcasses also highlighted the strength of virtopsy in the examination of decomposed carcasses, as the non-invasive imaging modalities allowed assessment of the carcass without tampering with the anatomy which can be significantly altered from decomposition (Bolliger and Thali, 2015; Thali et al., 2003b). While well-established in humans, systematic analysis of postmortem changes using virtopsy is still limited in animals, and such research would significantly improve the value of virtopsy in cetacean postmortem investigations (Kot and Martelli, 2017).

*C. fuelleborni* infections associated with the male reproductive organs including the epididymis, prostate, and penis were found in the 3 male NP cases using PMCT and conventional necropsy (Fig. 4), which to our knowledge have not been reported from NP in HK waters. In males of other odontocete species, *Crassicauda* sp. was found infecting the urogenital organs including the kidneys, urethra, prostate, and penis (Suárez-Santana et al., 2018; Raga and Balbuena, 1990). Compared to the mammary gland infections which influence milk production in female cetaceans (Geraci et al., 1978), little is known about crassicaudid infections of the male reproductive organs and the consequences on male reproductive output, as well as the role of males in the transmission of crassicaudid nematodes. Various transmission pathways for crassicaudids, including transplacental, through milk or urine, or via intermediate hosts, have been proposed (Geraci et al., 1978; Lambertsen, 1986; Suárez-Santana et al., 2018). Notably for crassicaudids that infect the mammary glands, given the observation of adult nematodes and nematode ova in the mammary gland tissues, vertical transmission

through contaminated milk has been hypothesised (Geraci et al., 1978; Mauroo, 2017). In odontocetes, Suárez-Santana et al. (2018) suggested that the tropism of *Crassicauda* infections towards the reproductive organs and adjacent muscles, which was also observed in this study, might be related to the transmission through milk, semen, or urine. Given the diverse nature of *Crassicauda* nematodes and the fact that *C. fuelleborni* infects both males and females in NP, whether hosts of both sexes play a role in the life cycle and transmission of this parasite requires further studies.

Using PMCT and conventional necropsy, lesions attributed to *Crassicauda* infections were detected in 52% of stranded NP cases ( $n = 13/25$ ) in HK waters during the study period. In previous studies that were solely based on necropsy, Lehnert et al. (2014) reported crassicaudid infections in the perimuscular fascia of 45% of the examined harbour porpoises ( $n = 9/20$ ), including both males and females. Prevalence of *C. grampicola* infection in the mammary glands and nearby muscles were reported in females of other species such as the Atlantic white-sided dolphin (47%;  $n = 14/30$ ; Geraci et al., 1978) and Dall's porpoise (69%;  $n = 20/29$ ; Conlogue et al., 1985), but the prevalence in males of these species were not documented. In HK waters, parasitic mastitis caused by *Crassicauda* sp. were found in both *Sousa chinensis* ( $n = 2$ ) and NP ( $n = 9$ ) out of 31 animals examined between 2007 and 2014 by Mauroo (2017). However, only females and fresher cases (code 2–3) were included in the study, and thus may not present the full prevalence of crassicaudid infection affecting the cetacean populations in HK waters. The current study demonstrated that *C. fuelleborni* infects both male and female NP, and *Crassicauda*-related lesions can be effectively diagnosed using PMCT even in decomposed carcasses for the first time. Another advantage of using virtopsy is that the images are stored digitally and can be retrospectively and repeatedly analysed (Chan et al., 2017). Utilising the collected virtopsy datasets in the cetacean stranding response programme in HK since March 2014 (Tsui et al., 2020), an updated study to analyse both retrospective and prospective data is necessary to understand the epidemiology and health impacts of crassicaudid infections on the residential cetaceans in HK waters.

PMCT has been demonstrated here to be an effective tool for the detection of *Crassicauda*-related lesions in stranded cetaceans alongside conventional necropsy. Similar studies utilising PMCT to diagnose and characterise parasitic infections of other body regions in cetaceans, particularly for parasites in the cranial sinuses and respiratory system, are currently under development (Kot et al., 2021). As parasitic infections are one of the most common findings in stranded marine mammals (Raga et al., 2018), virtopsy can also be extended and applied to other cetaceans or marine mammals, to assist in the parasitological and pathological assessment during stranding investigations, as well as to provide corrective aids for necessary intervention during the rehabilitation of live stranded animals.

#### Declarations of competing interest

None.

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