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Some adult and larval nematodes from fishes off

New Caledonia

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ABSTRACT

A collection of nematodes from the Muséum National d'Histoire Naturelle (National Museum of Natural History), Paris, France was studied. The nematodes had previously been collected from a range of marine fish hosts. The aim of this study was to investigate the identity of these nematodes. Detailed body measurements of the nematodes were taken via light microscopy and where possible first and second internal transcribed spacers (ITS-1 and ITS-2) of ribosomal DNA were subjected to PCR, purified and sequenced. Six species and three larval types were found from a range of fish species and included 13 new host records and two new geographical records. Of the taxa found, *Hysterothylacium kajikiae* is the only species which may have zoonotic potential. The majority of host fish in this study are edible species, commercially and recreationally fished and frequently incorporated into the cuisine of New Caledonia as raw fish dishes. This study will allow better understanding of the diversity, life cycles, distribution and host-parasite relationships in the New Caledonia area.

Keywords: New Caledonia; Nematoda; Parasites; Ascaridoids; Seafood safety; Aquatic Animal Health; Environment

Introduction

A large number of studies have described the diversity of parasites in marine fishes from New Caledonia (Justine 2010) and cover a broad range of parasitic phyla. However, these studies have rarely focused primarily on nematodes which are considered to be of high zoonotic importance. The larval form of nematodes are difficult to accurately identify from morphology alone (Justine et al. 2010a; Justine et al. 2010b; Justine et al. 2012a) and molecular studies are thus required, particularly for zoonotic marine ascaridoids (Shamsi et al. 2015; Shamsi et al. 2017; Shamsi et al. 2018a). A more comprehensive understanding of host-parasite interactions is necessary to determine the overall and potential impact marine nematodes may have on wildlife and human health. This is particularly important due to high consumption of fish/seafood in this area and the potential risk associated with a culinary preference for raw fish dishes. Information about these parasites from New Caledonia remains incomplete and new species and parasites are still being described (Moravec and Justine 2018a; Moravec and Justine 2018b; Moravec and Justine 2019a; Moravec and Justine 2019b; 2020a,b). The aim of the present study was to identify adult and larval nematode parasites from marine fish in New Caledonia.

Materials and methods

Hosts

Nematodes were collected from a variety of marine fish hosts from New Caledonian waters during 2003-2011. by one of us (JLJ) with the help of his students and sometimes visiting colleagues in the years 2003-2011. The nematodes were collected from many species of marine fish hosts from New Caledonian waters and are deposited now in the collections of the Muséum National d'Histoire Naturelle in Paris, France (MNHN). Most fish specimens were from the vicinity of Nouméa, New Caledonia and were collected by various means (fishing and

purchasing from the fishmarket of Nouméa); however, *Kajikia audax*, a pelagic fish, was caught far off the coast. Host morphological identification was performed by available keys and sometimes confirmed by ichthyologists. In rare instances, precise identification of fish hosts was problematic as these fish did not perfectly match descriptions of existing species and therefore have only been identified to the genus level.

Parasite collection

Parasites (larvae and adults) were collected via a variation of the wash method (Justine et al. 2012b) from the abdominal cavities of the fish. Most were found in the stomach and intestinal lumen but some were found in encapsulations on surfaces of the abdominal organs. In this case, were removed with fine tissue forceps. Specimens were washed in saline and fixed in 70% ethanol.

Morphology

A small cross-section of the mid-body from each parasite was excised and frozen until subsequent molecular studies. The anterior and posterior ends were then mounted on microscope slides and cleared in lactophenol for morphological examination. For larger, thicker worms, the anterior and posterior ends were soaked in Berland's fluid for 48 hours prior to being mounted for additional clearing. Specimens were assigned into distinct groups based on the morphology of lips (labia), tail, reproductive, digestive and excretory systems. A number of representatives from each group were selected for detailed measurement of important morphological features. A drawing of each parasite was made using a microscope equipped with a drawing tube.

Molecular study

For the groups that were subject to molecular study, one representative from each parasite group per host species was selected for molecular examination. Genomic DNA (gDNA) was isolated

from each individual specimen via sodium dodecyl-sulphate/proteinase K treatment, column-purified (Wizard™ DNA Clean-Up, Promega, Wisconsin, USA) and eluted into 45 µl of water. PCR was used to amplify the ITS-1 and ITS-2 regions using previously used primer sets (Ghadam et al. 2018). Cycling conditions: initial 94 °C/5', then 94 °C/30", 55 °C/40", 72 °C/40" × 30 cycles, 72 °C/5' extension and 4 °C. A 4 µl aliquot of each amplicon was examined on a 1.5% w/v agarose gel. Amplicons were purified over mini-columns (Wizard™ PCR Prep, Promega, WI, USA), eluted in 35 µl H₂O and then subjected to automated sequencing using the same primers as for PCR. Sequences were aligned using the computer programme ClustalX and then adjusted manually. Polymorphic sites were designated using International Union of Pure and Applied Chemistry (IUPAC).

Results

Table 1 summarises the parasite taxa found in each host in the present study. Results are given only for the specimens measured and sequenced. For the following results sections, all measurements are in millimetres unless otherwise stated. Mean measurements are given followed by range and number of specimens measured in parentheses. Some features were not measurable due to poor quality or incompleteness, therefore number of features measured may not always match number of specimens for that taxon. As mentioned previously, 10 representatives from each group were selected for measurement. If in the case some features were not determinable, additional specimens may have been measured to compensate for this.

Adult Nematoda

Hysterothylacium kajikiae Shamsi, 2017

Origin of specimens: The nematodes were collected during a general survey of the diet of pelagic fish undertaken by the Pacific Community (CPS) in Nouméa. Numerous nematodes were collected from frozen fish stomach/s. Precise information on the catch was: CPS number:

CHS0307-57; date 18-08-2003; coordinates: 22°26.952S, 165°11.162E, i.e. ca. 120 km West off New Caledonia). Material communicated by Valérie Allain, CPS, Nouméa. The nematodes were collected by Ian Beveridge.

Males: n=6; from *Kajikia audax* (Philippi); Accession number: MNHN JNC1402; Specimen numbers: 243-2, 243-4, 243-6, 243-9, 244-1 and 244-5.

Description (Figure 1 a to d): Males body length 39.02 (24.98-52.75; n=6). Maximum body width 0.65 (0.5-0.83; n=6). Cuticle lightly annulated, with numerous parallel cuticular ornamentations on ventral surface near posterior end. Three protruding labia with deep postlabial grooves and prominent lateral flanges. Dorsal lip with two double papillae, ventrolateral lips both with a lateral amphid, a single papilla and a double papilla. Height of lips 0.1 (0.09-0.12; n=5), width 0.09 (0.06-0.13; n=5). Interlabia well developed. Nerve ring 0.54 (0.5-0.61; n=6) from anterior end. Excretory pore just posterior to nerve ring position. Lateral alae absent. Cervical papillae present, around location of nerve ring. Muscular oesophagus, 5.03 (3.3-6.7; n=6) long, slightly broader at posterior end, 12.9% (8.5-17.2%; n=6) of total body length, ending in glandular ventriculus. Ventriculus subglobular, 0.23 (0.13-0.34; n=6) long. Ventricular appendix length 3.34 (1.63-4.75; n=5). Intestinal caecum length 4.36 (2.28-6.1; n=6). Ratio of intestinal caecum to ventricular appendix 1:0.77. Spicules subequal and alate, 3.7 (2.47-6.55, n = 6) and 3.7 (2.5-6.0, n=6) long and 11% (6-23%) and 11% (6-20%) of body length. Tail conical, relatively short and aspinose. Caudal papillae small and 21 pairs in total, 16 pairs precloacal, one pair of double paraclacal and 4 post cloacal pairs. Cloaca 0.22 (0.19-0.28; n=6) from posterior end.

Females: n = 8; from *Kajikia audax* (Philippi); Accession number: MNHN JNC1402, MNHN JNC1403; Specimen numbers: 243-3, 243-5, 243-7, 243-8, 244-2, 244-3, 244-4 and 244-6.

Description (Figure 1c & d): Body length 63.73 (21.58-87.95; n=7). Maximum body width 1.11 (0.78-1.3; n=8). Cuticle finely annulated. Labia height 0.12 (0.06-0.23; n=8), width 0.12

(0.08-0.15; n=8). Lips/papillae/amphids as per male. Nerve ring 0.6 (0.33-0.76; n=6) from anterior end. Lateral alae absent. Excretory pore not observed. Muscular oesophagus, length 5.7 (2.65-7.9; n=8), slightly broader at posterior end, 8.94% (4.16-12.4%; n=8) of total body length, ending in glandular ventriculus. Ventriculus subglobular, length 0.25 (0.1-0.39; n=8), 0.39% (0.16-0.61%; n=8) of total body length. Ventricular appendix length 5.63 (4.3-7.23; n=3). Intestinal caecum length 8.21 (5.35-14.7; n=5). Ratio of intestinal caecum to ventricular appendix 1:0.69 (8.21:5.63). Vulva located 19.25 (7.93-29.45; n=3) from anterior end. Eggs oval-shaped, length 0.1 (0.07-0.16; n=5), width 0.07 (0.06-0.08; n=5). Tail conical, aspinose and relatively long. Anus located 0.87 (0.45-1.13; n=7) from posterior end. No caudal papillae.

Genetic characterisation: Sequences of the ITS-1 and ITS-2 were obtained separately for one male 243-1 and one female 243-3. ITS-1 was 429 bp and identical among specimens (GenBank accession numbers: MT428328-9). ITS-2 was 271 bp and identical among specimens (GenBank accession numbers: MT428344-5).

***Raphidascaris* sp.**

Immature females: n= 5; from *Lutjanus* sp. Bloch; Accession number: MNHN JNC3369; Specimen numbers: 245-5, 245-6, 245-7, 245-8, 245-9 and 245-10.

Description (Figure 1 e & f): All five specimens were immature females. Body length 3.82 (3.18-4.18; n=5). Maximum body width 0.27 (0.22-0.36; n=5). Cuticle lightly annulated transversely. 3 protruding labia, height 0.03 (0.03-0.04; n=5), width 0.05 (0.05-0.05; n=5). Anterior lateral alae present. Nerve ring 0.16 (0.11-0.2; n=5) from anterior end. Muscular oesophagus, more expanded at distal end, 0.5 (0.43-0.57; n=5) long, 13.09% (11.26-14.92%; n=5) of total body length, ending in glandular ventriculus. Ventriculus transversely oval, 0.05 (0.04-0.05; n=5) long, 1.31% (1.05-1.31%; n=5) of total body length. Ventricular appendix 0.36 (0.25-0.54; n=4) long. Vulva located 1.13 (1.1-1.15; n=2) from anterior end. Eggs absent. Tail conical with multispinous tip. Anus 0.19 (0.15-0.21; n=5) from posterior end.

Adult female: n=1; from *Nemipterus furcosus* (Valenciennes); Accession number: MNHN JNC214D; Specimen number 212-1.

Description (Figure 1g & h): Gravid female. Body length 8.68. Maximum body width 0.49. Cuticle finely annulated. 3 protruding labia, height 0.05, width 0.05. Anterior lateral alae present. Nerve ring 0.25 from anterior end. Muscular oesophagus, slightly more expanded at caudal end, ending in glandular ventriculus. Oesophagus length 1.05, 12.1% of total body length. Ventriculus transversely oval, 0.06 in length, 0.69% of total body length. Ventricular appendix 0.39 long. Vulva 1.83 from anterior end. Egg length 0.04 and egg width 0.03. Tail with multispinous tip. Anus 0.33 from posterior end.

Adult male: n=1; from *Nemipterus furcosus* (Valenciennes); Accession number: MNHN JNC214D; Specimen number: 212-2. This specimen was not in good morphological condition to be described.

Genetic characterisation: Eight specimens including 245-5, 245-6, 245-7, 245-8, 245-9, 245-10, 212-1, 212-2 were sequenced for ITS-1. The ITS-1 was 468 bp and 100% identical among specimens (GenBank accession numbers: MT428337-43). The ITS-2 sequences were obtained for seven specimens (245-5, 245-6, 245-7, 245-8, 245-9, 245-10 212-1 and 212-2) (GenBank accession numbers: MT428354-61). They were 100% identical among specimens and 287 bp long. An identification as *R. lophii* was made based on the identical sequence available in the GenBank (Table 1).

***Cucullanus* sp.**

Adult female: n=1; from *Epinephelus cyanopodus* (Richardson); Accession number: MNHN JNC1888; Specimen number: 282-3.

Description (Figure 1 i & j): Gravid, medium sized with whitish body. Total body length 6.9. Maximum body width 0.32. Cuticle covered in fine, closely situated annulations. Lateral alae absent. Nerve ring 0.43 from anterior end, surrounding oesophagus at narrowest point.

Excretory pore not observed. Head rounded, somewhat asymmetrical in lateral view. Oral aperture dorsoventrally elongate, surrounded by raised narrow membranous ala (collarete) supported by a row of minute basal teeth. Four submedian cephalic papillae (two pairs) and one pair of lateral amphids present. No marginal oral papillae. One pair of deirids small and hooked, situated just posterior to level of oesophago-intestinal junction, 1.32 from anterior end. Oesophagus muscular and double club-shaped, width 0.09 at narrowest point and width 0.22 at widest points. Oesophagus expansion at anterior end forming a large elongate pseudobuccal capsule (oesophastome). Expansion at posterior end of oesophagus roughly same width as pseudobuccal capsule. Oesophagus length 1.1, 15.94% of total body length. Oesophagus opens to intestine through a large valve. Oesophageal-intestinal valve 0.05 long, extending into lumen of anterior intestine. Vulva slightly elevated, located 2 mm from posterior end (could not be measured from anterior end due to cross section taken). Vagina directed anteriorly, weakly muscular. Uteri opposed. Eggs oval shaped, 0.06 long (0.04-0.7; n=7), 0.04 wide (0.04-0.05; n=7). Tail conical and aspinose but with pointed tip, one pair of caudal phasmids near mid length of tail. Anus 0.25 from posterior end.

Genetic characterisation: No molecular work was completed on this specimen due to preservation method.

Procamallanus (Spirocamallanus) monotaxis (Olsen 1952)

Males: n = 5; from *Lethrinus genivittatus* (Valenciennes); Accession number: MNHN JNC221B; Specimens numbers: 200-2, 200-7, 200-8, 200-9 and 200-12.

Description (Figure 1 k & l): Total body length 9.81 (9.6-10.2; n=4). Body width 0.25 (0.22-0.28; n=5). Cuticle covered in fine transverse annulations. Nerve ring 0.26 (0.25-0.28; n=4) from anterior end, wrapped around junction of muscular oesophagus and glandular oesophagus. Excretory pore 0.4 (0.39-0.43; n=4) from anterior end. Deirids small and simple, 0.18 (0.12-0.19, n=3) from anterior end. Mouth aperture oval to square provided with narrow circumoral

flange and surrounded by six flat, crescent-shaped elevations and 12 submedian cephalic papillae arranged in three circles, each formed by four papillae; papillae of external circle distinctly larger. One pair of small lateral amphids present. Large orange-brown buccal capsule with smooth spiral grooves, some of which incomplete. Length of buccal capsule including basal ring 0.09 (0.09-0.09; n=4). Maximum width of buccal capsule 0.07 (0.06-0.07; n=4). Muscular oesophagus length 0.18 (0.16-0.19; n=4). Glandular oesophagus length 0.22 (0.2-0.24; n=4). No caeca or diverticula. Caudal alae present. Three pairs of equally spaced preanal pedunculate papillae. Postanal papillae: 4 pairs of anterior subventral and 2 pairs of posterior lateral pedunculate papillae; additional 2 pairs of small transversely elongate sessile papillae surrounding cloacal opening. Spicules unequal, longer and more sclerotised right spicule length 0.3 (0.29-0.33; n=5), left spicule length 0.21 (0.17-0.28; n=5). Both spicules with pointed tips.

Females: n=5; from *Lethrinus genivittatus* (Valenciennes); Accession number: MNHN JNC221B; Specimens numbers: 200-1, 200-3, 200-4, 200-5 and 228-1

Description (Figure 1 m & n): Total body length 13.8 (12.7-15.95; n=3). Body width 0.47 (0.44-0.51; n=5). Cuticle covered in fine transverse annulations. Nerve ring 0.27 (0.25-0.29; n=4) from anterior end, wrapped around junction of muscular oesophagus and glandular oesophagus. Excretory pore 0.38 (0.32-0.5; n=3) from anterior end. Deirids slightly unequal, located 0.18 (0.17-0.19; n=4) to 0.2 (0.19-0.21; n=4) from anterior end. Buccal capsule and oral aperture same as male. Length of buccal capsule including basal ring 0.1 (0.1-0.1; n=4). Muscular oesophagus length 0.19 (0.19-0.21; n=4). Glandular oesophagus length 0.3 (0.28-0.33; n=4). No caeca or diverticula. Numerous larvae in uterus. Vulval lips not elevated. Muscular vagina directed posteriorly. Tail rounded with a digit-like projection with two (dorsal and ventral) terminal cuticular spikes on tip. Anus 0.12 (0.1-0.16; n=4) from posterior end.

Genetic characterisation: No molecular work was completed on this specimen due to preservation method.

***Camallanus carangis* Olsen, 1954**

Male: n=1; from *Parupeneus heptacanthus* (Lacepède); **Accession number:** MNHN JNC364E;

Specimen number: 231-19

Description (Figure 1 o & p): Total body length 6.3. Maximum body width 0.22. Body cuticle with fine transverse annulations. Nerve ring 0.24 from anterior end, located just posterior to tips of tridents. One pair of deirids both 0.4 from anterior end. Excretory pore 0.39 from anterior end. Buccal capsule large and orange-brown in colour with smooth longitudinal ridges, some incomplete. Length of buccal capsule including basal ring 0.15, maximum width of buccal capsule 0.15. Mouth aperture slit-shaped, surrounded by four submedian cephalic papillae and two lateral amphids. Length of tridents between 0.12 and 0.15. Muscular oesophagus 0.69 in length. Glandular oesophagus 0.63 in length. Ratio of muscular oesophagus to glandular oesophagus $0.69:0.63 = 1:0.91$. Buccal capsule and oesophagi representing 23.25% of total body length. No caeca or diverticula. Posterior part of body with caudal alae. Spicules unequal, right (longer) spicule length 0.33 (appears bent dorsally at point of anus as result of physical manipulation, not a taxonomic feature), left shorter and less sclerotized, spicule length 0.15. Length ratio of spicules $0.33:0.15 = 1:0.45$. Two pairs of sessile paracloacal papillae. Seven pairs preanal and six pairs of postanal pedunculate papillae. Anus 0.1 from posterior end.

Genetic characterisation: No molecular work was completed on this specimen due to preservation method.

Nematode larvae:***Hysterothylacium* larval type VI of Shamsi et al. 2013**

Materials examined: 14 specimens from *Saurida undosquamis* (Richardson) (Accession number: MNHN JNC570B; Specimen number: 196-6), *Cheilinus fasciatus* Bloch (Accession number: MNHN JNC129C; Specimen number: 204-1), *Nemipterus furcosus* (Valenciennes)

(Accession number: MNHN JNC250A&B, JNC260H and JNC259G; Specimen number: 216-17, 216-25, 221-11, 222-6), *Parupeneus heptacanthus* (Lacepède) (Accession number: MNHN JNC364E; Specimen number: 231-17, 231-23), *Chaetodon auriga* Forsskål (Accession number: MNHN JNC384A; Specimen number: 234-5), *Moolgarda seheli* (Forsskål) (Accession number: MNHN JNC3359; Specimen number: 246-1, 246-4), *Gymnocranius grandoculis* (Valenciennes) (Accession number: MNHN JNC1726; Specimen number: 280-1, 280-4 and 280-7).

Description (Figure 2 a & b): Third stage larvae (Figure 2 i&j). Total body length 5.63 (3.13-9.68; n=14), maximum body width 0.18 (0.08-0.28; n=14). Boring tooth absent. Labia poorly developed. Nerve ring 0.23 (0.12-0.38; n=13) from anterior end. Excretory pore located between nerve ring and tip of intestinal caecum, closer to intestinal caecum, 0.4 (0.34-0.46; n=2) from anterior end. Oesophagus muscular, length 0.52 (0.35-0.79; n=13), 9.24% (6.22-14.03%; n=13) of total body length, ending in glandular ventriculus. Ventriculus subglobular, length 0.08 (0.04-0.18; n=13), 1.42% (0.71-3.2%; n=13) of total body length. Ventricular appendix 0.48 (0.16-0.85; n=11) long. Intestinal caecum length 0.22 (0.1-0.45; n=12). Ratio of intestinal caecum to ventricular appendix 0.22:0.48 = 1:2.18. Tail conical, slightly swollen at tip, decorated with a single minute spine. Anus 0.19 (0.11-0.25; n=14) from posterior end.

Genetic characterisation:

ITS-2 was obtained for one specimen (280-1) from *Gymnocranius grandoculis* (Valenciennes). It was 262 bp (GenBank accession number: MT428346). Attempts to obtain sequences from the remaining specimens was not successful. There was no identical comparable sequence from adult specimens available in the GenBank to allow specific identification.

***Hysterothylacium* larval type XIV of Shamsi et al. 2015**

Materials examined: 14 specimens from *Saurida undosquamis* (Richardson) (Accession numbers: MNHN JNC570B, JNC571B, JNC502C; Specimen number: 196-1, 196-3, 196-10,

197-2, 197-4, 197-5, 219-19) and *Nemipterus furcosus* (Valenciennes) (Accession numbers: MNHN JNC254D, JNC379C; Specimen numbers: 233-3, 233-7, 233-9, 239-6, 239-7, 239-9 and 239-10).

Description (Figure 2 b & c): Third stage larvae (Figure k & i). Body length 4.85 (2.4-7.35; n=14). Maximum body width 0.17 (0.13-0.2; n=13). Labia poorly developed. Boring tooth absent. Nerve ring 0.25 (0.09-0.36; n=11) from anterior end. Excretory pore 0.3 (0.22-0.36; n=3) from anterior end, located between tip of intestinal caecum and nerve ring (closer to nerve ring). Oesophagus muscular, length 0.66 (0.5-1.07; n=10), 13.61% (10.31-22.06%; n=10) of total body length, ending in glandular ventriculus. Ventriculus subglobular, length 0.06 (0.06-0.07; n=8), 1.24% (1.24-1.44%; n=8) of total body length. Ventricular appendix length 0.47 (0.23-0.58; n=8). Intestinal caecum length 0.18 (0.11-0.34; n=11). Ratio of intestinal caecum to ventricular appendix 0.18: 0.47 = 1:2.61. Tail conical, with tip bent dorsally and decorated with 3-4 minute triangular spines. Anus 0.15 (0.12-0.2; n=14) from posterior end.

Genetic characterisation: Seven specimens 216-8, 197-2, 197-4, 197-5, 197-8, 256-6 and 197-9 were subjected to sequencing. ITS-1 was 436 bp and identical among all specimens (GenBank accession numbers: MT428330-6). ITS2 was 276 bp long (GenBank accession numbers: MT428347-53) and identical among all specimens as well. This larval type was identified as *Hysterothylacium persicum* based on the identical ITS sequence available in the GenBank (Table 1).

***Raphidascaris* larval type**

Materials examined: 5 specimens from *Saurida undosquamis* (Richardson) (Accession number: MNHN JNC570B, JNC571B; Specimen numbers: 196-5, 196-9, 197-3, 197-6) and *Parupeneus heptacanthus* (Lacepède) (Accession number: MNHN JNC345B; Specimen number: 229-2).

Description (Figure 2 e & f): Third stage larvae. Body length 3.4 (2.58-4.68; n=5). Maximum body width 0.18 (0.16-0.2; n=5). Cuticle lightly annulated. Labia poorly developed. Boring tooth present. Excretory pore not observed. Nerve ring 0.19 (0.15-0.25; n=5) from anterior end. Muscular oesophagus, significantly more expanded at distal end, length 0.51 (0.47-0.6; n=5), 15% (13.82-17.65%; n=5) of total body length. Oesophagus ending in transversely oval, glandular ventriculus. Ventriculus 0.04 (0.03-0.06; n=5) long, 1.18% (0.9-1.76%; n=5) of total body length. Ventricular appendix 0.27 (n=5) long. Tail conical and aspinose with finely pointed tip. Anus 0.13 (0.09-0.17; n=5) from posterior end (Figure 2f).

Genetic characterisation: ITS-1 and ITS-2 sequences were obtained for one specimen from this group (197-6). The length of the ITS-1 and ITS-2 was 468 and 287 bp (GenBank accession number: MT428360). They were identical with those of mature and immature female *Raphidascaris* found in the present study and similarly were identified as *R. lophii*.

Discussion

Despite the high number of studies on parasitic nematodes in New Caledonian fish, it is remarkable that new species (Moravec and Justine 2020a) and new occurrences (present study) still occur. The Nematoda found in the present study were in adult and larval stages and due to the preservation conditions, performing molecular work on all of them was not possible.

Among the adult nematodes found in the present study, the anisakid *H. kajikiae* is reported for the first time in New Caledonian waters. The parasite was previously reported as a new species from the same host in Australian waters (Shamsi 2017) and based on the description of one male specimen. The present study provided opportunity for examination of more male specimens. Shamsi (2017) considered spicule morphology and size as one of the most distinguishing characteristics for the new species. In the present study, the original description was confirmed, however, spicules were shorter in the smaller (presumably younger) specimens.

The specimens in the present study had identical ITS-1 and ITS-2 sequences with *H. kajikiae* reported in Australian waters (Table 1).

This study also provided opportunity to examine and describe a number of adult *Raphidascaris* specimens. There was no male specimen in good morphological condition in the collection to allow for specific identification. However, ITS sequences of the specimens in the present study collected from the three different host species were identical among specimens suggesting all belong to the same species. A Blast search in GenBank resulted in identical sequence assigned to *R. lophii* in an unpublished study from China (Table 1). In addition, there were a number of other sequences in GenBank (Table 2) which were highly similar to sequences of the *Raphidascaris* specimens in the present study. Of the sequences listed in Table 2, Zhao et al. (2016) and Li et al. (2016) provided both morphological description and sequence data for *R. lophii*. *Raphidascaris lophii* along with *R. trichiuri* was considered as junior synonyms of *R. chirocentri* by Sheenko (1992). However, this was later contradicted by Moravec and Justine (2020a) who considered *R. lophii* and *R. trichiuri* as valid species. Based on the high level of similarity in the ITS sequences of the specimens in the present study with those available in GenBank we consider the identity of these nematodes as *R. lophii*. This study is the first record of *R. lophii* in New Caledonian waters. It is noteworthy that from the three hosts that we report *R. lophii* (Table 1) from, *Raphidascaris (Ichthyascaris) nemipteri* has been reported in *Nemipterus furcosus* (Valenciennes) (Moravec and Justine 2005), and *Raphidascaris (Ichthyascaris) nudicauda* has been reported in *Saurida undosquamis* (Richardson) (Moravec and Justine 2020a). These reports are based on morphology only. We did not have a male specimen in our study to provide comparable morphological description with those reported by Moravec and Justine (2020a).

Another adult specimen found in the present study was a female *Cucullanus* sp. found in *Epinephelus cyanopodus* (Richardson). The only other *Cucullanus* species reported in groupers

(serranid fish) in the New Caledonian waters have been *Cucullanus epinepheli*, described from male specimens only, from *Epinephelus chlorostigma* (Valenciennes) (Moravec and Justine 2017) and *C. petterae* from *Epinephelus merra* described from one male and one nongravid female (Moravec and Justine 2020c). Therefore, comparable morphological information is not available. Although comparison of the morphometrics between the nongravid female of *C. petterae* and the single female specimen found in the present study does not suggest the difference is significant, we observed in our specimen cranial surface is more rounded, intestine is wider than the posterior end of the oesophagus and tail is broader.

Of larvae found in this study, *Hysterothylacium* larval type VI of Shamsi et al. (2013) was one of the most abundant parasites. The main distinguishing feature of larval type VI from other congeneric larval types is the slightly bulbous rounded tail tip decorated with a single minute spine (Shamsi et al. 2013). The hosts for this larval type in the present study were: *Chaetodon auriga* Forsskål (threadfin butterflyfish), *Cheilinus fasciatus* (Bloch) (red-breasted wrasse), *Gymnocranius grandoculis* (Valenciennes) (blue-lined large-eye bream), *Moolgarda seheli* (Forsskål) (bluespot mullet), *Nemipterus furcosus* (Valenciennes) (fork-tailed threadfin bream), *Parupeneus heptacanthus* (Lacepède) (cinnabar goatfish) and *Saurida undosquamis* (Richardson) (brushtooth lizardfish). *Hysterothylacium* larval type VI has been reported from New Caledonia previously (Shamsi et al. 2015) from hosts *Gymnocranius euanus* (Günther) (Japanese large-eye bream), *Gymnocranius superciliosus* Borsa, Béarez, Pajjo & Chen (eyebrowed large-eye bream), *Rastrelliger kanagurta* (Cuvier) (Indian mackerel) and *Sphyraena forsteri* (Cuvier) (bigeye barracuda). None of these host records match the present study. Therefore, the present study is a new host record for seven host species. *Hysterothylacium* larval type VI has also been found in Australian waters in *Chaetodon auriga* Forsskål (Shamsi et al. 2013) and also other fish species in Queensland waters (Shamsi et al. 2018b). However, the present study is the first report of *Hysterothylacium* larval type VI from

C. auriga Forsskål from New Caledonia. Apart from *Hysterothylacium* larval type reported in Lizard Island (Table 1) in which the ITS sequences were identical, *Hysterothylacium* larval type VI in the present study had considerably different ITS-1 and ITS-2 sequences from those previously reported from New Caledonia, Australia and elsewhere. The specific identity of this larval type remains unknown until a comparable sequence data from a well described male nematode becomes available.

Another *Hysterothylacium* larval type found in the present study was *Hysterothylacium* larval type XIV. This larval type was first reported in 2015, from New Caledonian waters from hosts *Herklotsichthys quadrimaculatus* (Rüppell) (bluestripe herring), *Rastrelliger kanagurta* (Cuvier) (Indian mackerel) and *Trichiurus lepturus* Linnaeus (largehead hairtail) (Shamsi et al. 2015). The key feature that distinguishes larval type XIV from other *Hysterothylacium* larval types is the tail ornamentation. Type XIV possesses a tail tip that is bent dorsally (Figure 2 d) and is decorated with three to four minute conical spines (Shamsi et al. 2015). The specimens of the present study all had this distinguishing feature and measurements that fall within given ranges (Shamsi et al. 2015). The hosts for this larval type in the present study are new host records. Molecular analysis of specimens in this larval type revealed ITS sequences identical to those previously reported from different developmental stages and other parts of the world suggesting their identity as *Hysterothylacium persicum* (Table 1).

Nematodes reported in the present study are not of zoonotic significance. However, some of the fish species studied in the present study are known to harbour zoonotic nematodes. For example, *Anisakis* larval type I has been reported from *Nemipterus furcosus* (Valenciennes) and *Saurida undosquamis* (Richardson) in New Caledonian waters (Shamsi et al. 2015; Shamsi et al. 2018a). Nematode species reported in the present study may represent only a small sample of the diversity expected in the highly biodiverse range of hosts and parasite taxa in New Caledonian waters (Justine et al. 2010a; Justine et al. 2010b; Justine et al. 2012a). Completion

of molecular works on remaining groups in the future will shed further light on their specific identity and taxonomic status. Scanning electron microscopy where enough specimens in good quality are available would also assist in detecting more minute taxonomic details that are difficult to discriminate under a light microscope.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1- Comparison of the sequences generated in the present study and the associated identification, with identical sequences in GenBank. This table includes information only for those specimens for which ITS sequences were successfully obtained.

Details of the genetically characterised taxa in the present study					Details of the closely related taxa with comparable sequences in the GenBank					
Parasite found	Developmental stage	Host	GenBank accession no (Specimen no), ITS-1	GenBank accession no (Specimen no), ITS-2	Identified as (developmental stage)	Similarity (region)	GenBank accession no	Host	Locality	Reference
<i>Hysterothylacium kajikiae</i>	Adult male and female	<i>Kajikia (Tetrapturus) audax</i>	MT42832	MT42834	<i>Hysterothylacium kajikiae</i>	429/429 (ITS-1)	HE862220	<i>Kajikia (Tetrapturus) audax</i>	Nelson Bay, NSW, Australia	Shamsi (2017)
			8 (243-1), MT42832	4 (243-1), MT42834		271/271 (ITS-2)	-1 HE862226			
<i>Raphidascaris</i> sp.	Immature female	<i>Lutjanus</i> sp.	MT42834	MT42835	<i>R. lophii</i>	468/468 (ITS-1)	MF803224 -6, MF803204 -5 and KU991875	-	-	unpublished
			0 (245-5)	7 (245-5)						
			MT42834	MT42835						
			2 (245-6)	9 (245-6)						
			MT42833	MT42835						
			7 (245-7)	5 (245-8)						
			MT42833	MT42835						
			8 (245-8)	6 (245-9)						

			MT42833 9 (245-9)	MT42835 8 (245-10)						
			MT42834 1 (245-10)							
<i>Raphidascaris</i> sp.	Adult male and female	<i>Nemipterus</i> <i>furcosus</i>	MT42834 3 (212-2) (male)	MT42836 1 (212-1) (female)	<i>R. lophii</i>	468/468 (ITS-1)	MF803224 -6, MF803204 -5 and KU991875	-	-	unpublishe d
			212-1 (female)	212-2 (male)						
<i>Raphidascaris</i> sp.	L3	<i>Saurida</i> <i>undosquamis</i>	197-6	MT42836 0 (197-6)	<i>R. lophii</i>	468/468 (ITS-1)	MF803224 -6, MF803204 -5 and KU991875	-	-	unpublishe d
<i>Hysterothylaciu</i> <i>m</i> larval type VI	L3	<i>Gymnocraniu</i> <i>s grandoculis</i>	-	MT42834 6 (280-1)	<i>Hysterothylaciu</i> <i>m</i> sp. type VA	262/262 (ITS-2)	JX848691	Unknown *	Lizard Is., Queenslan d, Australia	Jabbar <i>et</i> <i>al.</i> (2012)
<i>Hysterothylaciu</i> <i>m</i> larval type XIV	L3	<i>Saurida</i> <i>undosquamis</i>	MT42833 0 (216-8)	MT42834 7 (216-8)	<i>Hysterothylaciu</i> <i>m</i> larval type XIV	436/436 (ITS-1); 276/276 (ITS-2)	MK161424 MK161449	<i>Engraulis</i> <i>australis</i>	NSW, Australia	(Hossen & Shamsi, 2019)
		<i>Nemipterus</i> <i>furcosus</i>	MT42833 1 (197-2)	MT42834 8 (197-2)						
			MT42833 2 (197-4)	MT42834 9 (197-4)						

MT42833	MT42835
3 (197-5)	0 (197-5)
MT42833	MT42835
4 (197-8)	1 (197-8)
MT42833	MT42835
5 (256-6)	2 (256-6)
MT42833	MT42835
6 (197-9)	3 (197-9)

<i>Hysterothylacium persicum</i>	436/436 (ITS-1); 276/276 (ITS-2)	LT576366-8; LT576370-1	<i>Scomberomorus commerson</i>	Persian Gulf, Iran	Shamsi <i>et al.</i> (2016)
<i>Hysterothylacium</i> larval type X [#]	276/276 (ITS-2)	JX848679	Unknown ^	Lizard Is., Queensland, Australia	Jabbar <i>et al.</i> (2012)
<i>Hysterothylacium</i> larval type XIV	436/436 (ITS-1); 276/276 (ITS-2)	MG594310-12; MG594333	<i>Tripodichthys angustifrons</i>	Green Is., Moreton Bay, Queensland, Australia	Shamsi <i>et al.</i> (2018b)
<i>Hysterothylacium</i> larval type XIV	276/276, and 275/276 (ITS-2)	LN651105-7	<i>Herklotsichthys quadrimaculatus</i> , <i>Rastrelliger kanagurta</i> ,	New Caledonia	Shamsi <i>et al.</i> (2015)

Trichiurus
lepturus

* The authors listed *A. polyacanthus*, *C. citrinellus*, *S. commerson*, *S. apicalis* as host for all what they labelled as type V. However only one sequence is available in the GenBank and it is not known which one of them is the host for the parasite. For issues relate with their identification see the Discussion.

Erroneous identification.

^The authors listed *A. endrachtensis*, *C. cyanostigma*, *Hyporhamphus affinis* as host for all what they labelled as type x. However only one sequence is available in the GenBank and it is not known which one of them is the host for the parasite. For issues relate with their identification see the Discussion.

Table 2- Comparison of the ITS-1 and ITS-2 sequences of the *Raphidascaris* specimens in the present study with the highly similar sequences in the in the GenBank

Identified as (developmental stage)	Similarity (region)	GenBank accession no	Host	Locality	Reference
<i>R. lophii</i> (not mentioned)	466/468 (ITS-1)	MF422212 and KX110077	-	-	Unpublished
<i>R. lophii</i> (L3)	466/468 (ITS-1); 286/287 (ITS-2)	MH211575-84	<i>Lophius litulon</i>	East China Sea	Zhang <i>et al.</i> (2018)
<i>R. lophii</i> (Adult males, a female and L3)	466/468 (ITS-1); 286/287 (ITS-2)	KP262035–KP262037 KP262038, KP262039	Starry batfish, <i>Halieutaea stellate</i>	South China Sea	Li <i>et al.</i> (2016)
<i>Raphidascaris</i> (larval type)	287/287 (ITS-2)	LN651108-9	<i>Branchiostegus wardi</i> , <i>Carangoides chrysophrys</i> , <i>Chirocentrus dorab</i> and <i>Diodon hystrix</i>	New Caledonia	Shamsi <i>et al.</i> (2015)
<i>R. trichurii</i> * (not mentioned)	287/287 (ITS-2)	FJ009682	<i>Muraenesox cinereus</i>	Taiwan Strait, Fujian, China	Damin and Heqing (2001)
<i>R. lophii</i> (L3)	466/468 (ITS-1); 286/287 (ITS-2)	MF539818-20	<i>Conger myriaster</i>	South China Sea	Chen <i>et al.</i> (2018)
<i>R. lophii</i> (L3)	286/287 (ITS-2)	KX110077	<i>Pseudorhombus cinnamoneus</i>	Yellow Sea, China	Li <i>et al.</i> (2017)
<i>R. lophii</i> (female and L3)	286/287 (ITS-2)	KP419720 , KP326520-31 and KP326533-8	<i>Uroconger lepturus</i> <i>Scomberomorus niphonius</i>	South China Sea	Zhao <i>et al.</i> (2016),

			<i>Scomber japonicas</i>		
			<i>Mene maculate</i>		
			<i>Pterois russelii</i>		
			<i>Lepidotrigla japonica</i>		
			<i>Rhynchoconger ectenurus</i>		
<i>R. lophii</i> (male)	285/287 (ITS-2)	KP326532	<i>Uroconger lepturus</i>	South China Sea	Zhao <i>et al.</i> (2016)
<i>R. lophii</i> (larvae)	286/287 (ITS-2)	JX974559	<i>Sebastes inermis</i>	Jeju, South Korea	Kim <i>et al.</i> (2012)
<i>R. lophii</i> (-)	285/287 (ITS-2)	JF809816	-	-	Li <i>et al.</i> (2012)

* There is no mention of the GenBank accession number in the paper, hence it is not known whether it was obtained from a male, female or larva. As a result identification as *R. trichurii* is dubious.

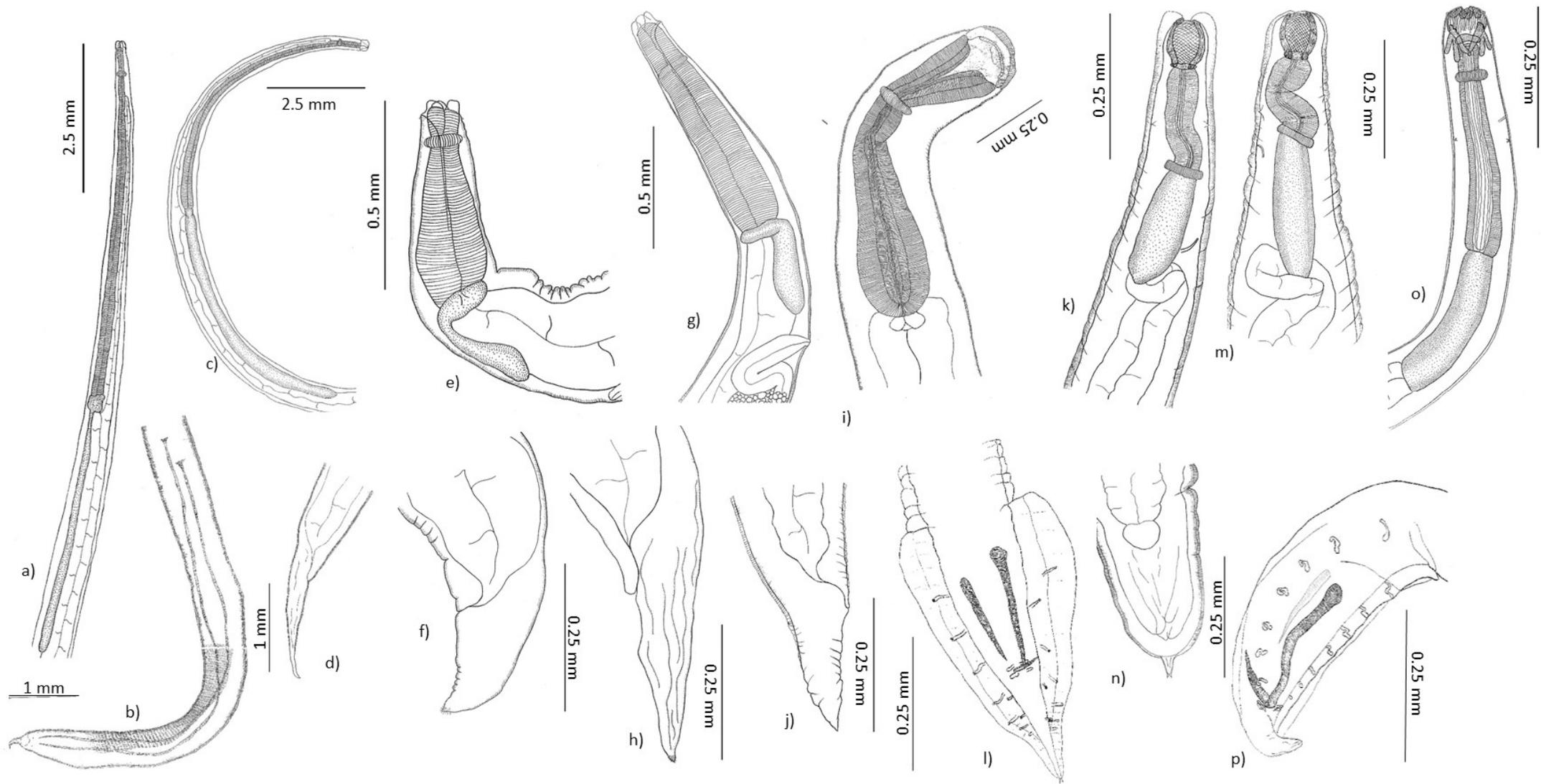


Figure 1) Adult nematodes found in the present study; a to d) *Hysterothylacium kajikiae* from *Kajikia (Tetrapturus) audax*, a & b) anterior and posterior ends

of male #243-6, c & d) anterior and posterior ends of female #244-3; e & f) anterior and posterior ends of an immature female *Raphidascaris* sp. from *Lutjanus* sp. specimen number #245-5; g & h) anterior and posterior ends of a mature female *Raphidascaris* sp. from *Nemipterus furcosus*, specimen number #212-1; i & j) *Cucullanus* sp. female from *Epinephelus cyanopodus*, specimen number #282-3, i) anterior end lateral view and j) posterior end; k & l) anterior and posterior ends of *Procamallanus (Spirocamallanus) monotaxis* male from *Lethrinus genivittatus*, specimen number #200-7; m & n) anterior and posterior ends of *Procamallanus (Spirocamallanus) monotaxis* female from *Lethrinus genivittatus*, specimen number #200-1; o & p) *Camallanus carangis* male from *Parupeneus heptacanthus*, specimen number 231-19 o) anterior end, p) posterior end dorso-lateral view; q, r, s) Rhabdochonidae gen. sp., q) anterior end of a female (lateral view), specimen numbers #234-9, r) posterior end of a male (lateral view), specimen numbers #237-5, s) posterior of a female (lateral view), specimen number #234-9.

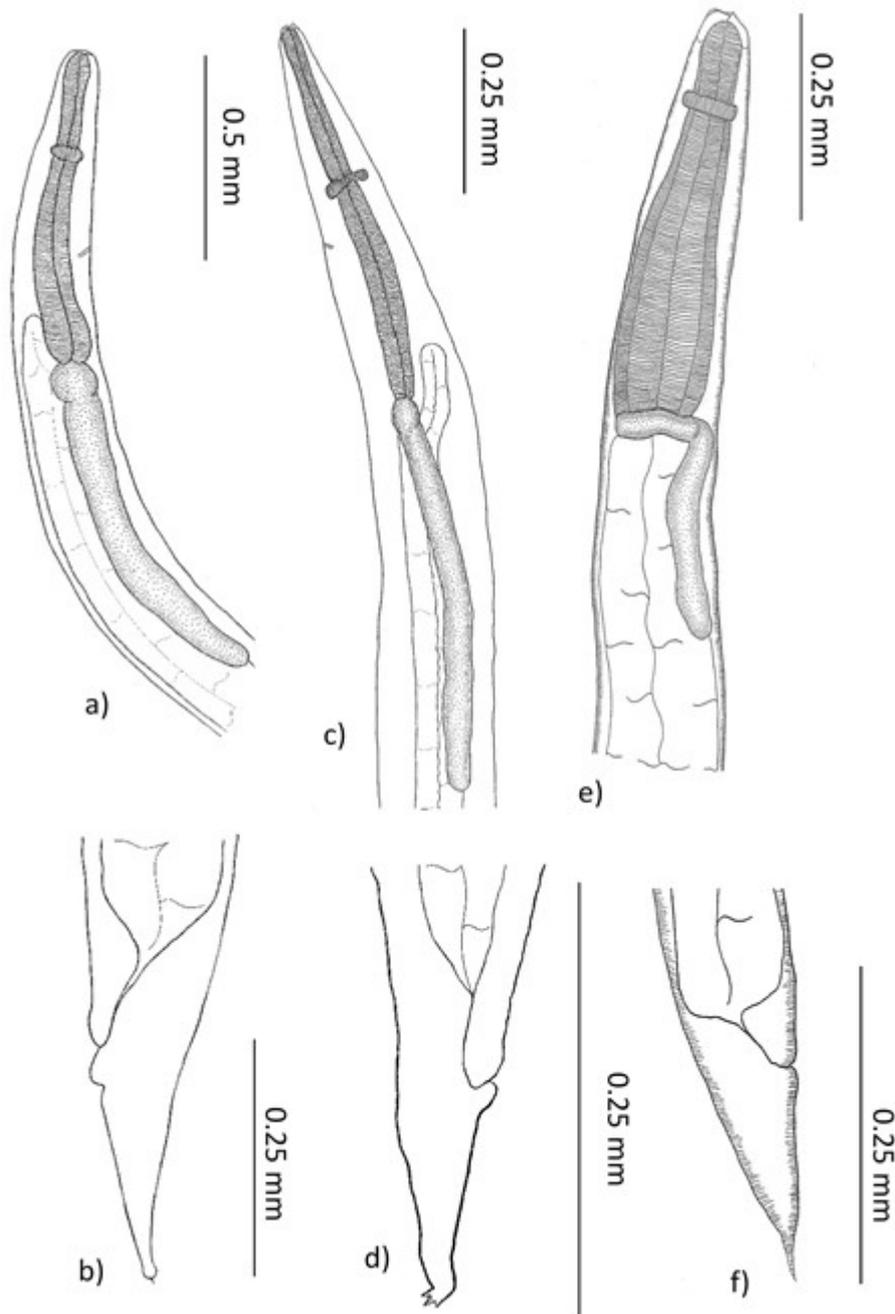


Figure 2 a & b) anterior and posterior ends of *Hysterothylacium* larval type VI of Shamsi et al. (2013) from *Nemipterus furcosus*, specimen number #222-6; c & d) anterior and posterior ends of *Hysterothylacium* larval type XIV of Shamsi et al. (2015), from *Saurida undosquamis*, specimen number #197-4; e & f) *Raphidascaaris* larval type from *Parupeneus heptacanthus*, specimen number #229-2.