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ORIGINAL PAPER

Occurrence of anisakid parasites in marine fishes and whales off New Caledonia

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Abstract

Anisakid nematodes are the most infamous parasites occurring in seafood with ability to infect humans. In the present study, the infective stages of five anisakid larval types, including *Anisakis* types I and III, *Terranova* types I and II and *Contracaecum* larval type, as well as adult *Anisakis paggiae* are reported from 16 host species from New Caledonian waters. The specific identity of the larval types was investigated using ITS sequence data. *Anisakis* larval types I and III were identified as *Anisakis typica* and *Anisakis brevispiculata*, respectively, based on identical ITS sequences. However, the specific identity of the *Terranova* larval types and *Contracaecum* larval type remains unknown until a matching ITS sequence from a well-identified adult is available. Several fish host species are reported for the first time for anisakid larval types found in this study. Considering that third-stage larvae of anisakids are known to be the infective stage of the parasite for humans and the popularity of seafood in New Caledonia, presence of these parasites in New Caledonian fish is of high importance in terms of public health and raising awareness among various stakeholders. Although adult nematodes in the present study were identified as *Anisakis paggiae*, the spicule length is shorter in our specimens and falls within the range reported for *Anisakis oceanicus* previously reported in Pacific waters from black fish (genus *Globicephala*) and later synonymised with *Anisakis physeteris*. However, our specimens are different from *A. physeteris* in morphology of ventriculus. *Anisakis paggiae* has been reported from whales in southern hemisphere and this is the first report from the Pacific regions.

Keywords Anisakidae · Nematoda · Seafood · New Caledonia

Introduction

Seafood products account for more than 40% of the protein intake of people in New Caledonia (Gontard and de Coudenhove 2013). Although there have been studies on marine parasites from this area, most of these studies cover a range of parasitic phyla (Justine 2007, 2010; Justine et al. 2010a, b, 2012a; Myers et al. 2000) and the focus is rarely just on nematodes of zoonotic importance. Identification of nematodes, particularly for zoonotic marine ascaridoids, is often not completed down to the species level in these studies and molecular analyses are often not used to specifically identify larvae. Identification of potentially zoonotic parasites is especially important due to the high consumption of fish and seafood in this area, including raw fish. A series of lists of fish parasites from New Caledonia have been published, mainly for the fish families Epinephelidae, Lethrinidae and Lutjanidae (Justine et al. 2010a, b, 2012a) and other families (Justine 2010), including mainly coral reef fish and also some deep-sea fish from the external slope of the coral reef. However, the authors pointed out that the results were only about a fraction of the true biodiversity of an ecosystem as many species were yet unidentified. It was outlined that many records simply labelled as 'unidentified anisakid larvae' were certainly hiding a higher diversity. Our previous works have aimed at elucidating this hidden biodiversity of anisakid larvae (Shamsi et al. 2015, 2017).

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The aim of the present study was to identify nematode parasites found in marine fish and selected aquatic animals in New Caledonian waters using combined molecular and morphological approaches. This is an essential step if any future work is to be done on areas such as risk assessment, control, prevention and seafood safety. The focus of this study is on anisakid nematode parasites only, due to their zoonotic significance.

Materials and methods

Hosts

Fish were mostly purchased from the fish market and were dead at the time of purchase. Fish from other places were collected under permit from Province Sud, New Caledonia, given to Institut de Recherche pour le Développement (IRD), Nouméa, and were killed according to local and French laws and humane rules. Stranded mammals were dead at the time of the parasitological inspection. Host morphological identification was performed by Jean-Lou Justine using current literature (Froese and Pauly 2018) and then confirmed by ichthyologists (see acknowledgements). In some instances, the precise identification of fish hosts was problematic; 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 generally 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. Specimens were washed in saline and fixed in 70% ethanol. Specimens from marine mammals were collected by other scientists and given to Jean-Lou Justine years after their collection. All parasites were deposited in Museum National d'Histoire Naturelle, Paris.

Morphology

A small cross-section of the mid body from each parasite was excised and kept frozen for 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 h 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 (Cannon 1977; Jabbar et al. 2012; Murata et al. 2011; Shamsi et al. 2011, 2012; Shamsi and Suthar 2016). A number of representatives from each group were selected for detailed measurement of important bodily features. A drawing of each parasite was made using a microscope equipped with a drawing tube. Images were captured via a light microscope equipped with a digital camera.

PCR and sequencing

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) and eluted into 45 μl of water. PCR was used to amplify the ITS-1 and ITS-2 regions using primer sets SS1: 50-GTTTCCGTAGGTGAACCTGCG-30 (forward) and NC13R: 50-GCTGCGTTCTTCATCGAT-30 (reverse) for the former and SS2: 50-TTGCAGACACATTGAGCACT-30 (forward) and NC2: 50-TTAGTTTCTTTTCCTCCGCT-30 (reverse) for the latter region (Shamsi and Suthar 2016). 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 H_2O and then subjected to automated sequencing using the same primers as for PCR (Table 1).

Phylogenetic analyses

ITS-1 and ITS-2 sequences were either generated in our current study, or were obtained from GenBank (Table 2). If obtained from GenBank, only sequences from well-identified adults for which museum vouchers are available were considered. ITS-1 and ITS-2 sequence data were concatenated by using Geneious version 11.0.5 (Kearse et al. 2012). Combined sequences were aligned by Geneious alignment algorithm, and then were double checked with all variable sites in the original trace files for confirmation. Alignments were then truncated to 768, 794 and 711 characters, based on the shortest sequence of the alignment, for *Contracaecum* spp., *Anisakis* spp. and *Terranova* spp., respectively. Same gene region from *Heterakis gallinarum* was used as an outgroup. Phylogenetic relationship among species was calculated by MEGA7.0.26 (Kumar et al. 2016) using neighbour-joining method with p-distances model.

Results

Twenty-three host individuals from 16 species were examined. Five larval types belonging to three genera, *Anisakis*, *Contracaecum* and *Terranova* and one adult, *Anisakis paggiae* were found. Table 1 shows occurrence of anisakids in various hosts. Below detailed characteristics for each

Table 1 Details of hosts examined in the present study

Fish	MNHN JNC[a]	Locality[b]	Coordinates	Collection date	Fork length (mm)	Weight (g)	Anisakid
Balistidae							
Sufflamen fraenatum	JNC297	Not recorded	Not recorded	21/03/2003	265	500	*Terranova* type I
Berycidae							
Beryx splendens	JNC1576	Seamount "Mont J"	23° 54′ S, 169° 145′ E, 800 m depth	June 2002	Not recorded	Not recorded	*Anisakis* larvae type III
Carangidae							
Atule mate	JNC3365	Fish market	Not recorded	25/03/2011	290	417	*Terranova* types I and II
Carangoides cf. *orthogrammus*	JNC569	Off Pointe Bovis	22° 14,206 S, 166° 20,645 E	27/06/2003	281	388	*Terranova* type II
C. fulvoguttatus	JNC3298	Fish market	Not recorded	28/01/2011	265	409	*Anisakis* type I
Decapterus macarellus	JNC2018	Near Baie des Citrons	22°17′ 30″ S, 166°25′ 30″ E	10/10/2006	190	95	*Anisakis* type I
D. macarellus	JNC2019	As JNC2018	As JNC2018	As JNC2018	185	97	*Terranova* type I
Echeneidae							
Echeneis naucrates	JNC209	As JNC204	As JNC204	As JNC204	760	1900	*Anisakis* type I *Terranova* type II
Epinephelidae							
Epinephelus areolatus	JNC204	Near Ilôt Canard	22° 19,610 S, 166° 25,892 E	26/02/2003	390	750	*Anisakis* type I
E. areolatus	JNC205	As JNC204	As JNC204	As JNC204	330	550	*Terranova* type II
E. ongus	JNC3275	Fish market	Not recorded	13/10/2010	282	384	*Terranova* type I
Gobiidae							
Periophthalmus argentilineatus	JNC3320	Ouemo	Not recorded	16/02/2011	105	11.2	*Contracaecum* larvae
Lutjanidae							
Lutjanus rivulatus	JNC1864	Fausse passe de Uitoé	Not recorded	11/06/2006	580	4200	*Terranova* type I
Mammalia							
Kogia breviceps	JNC1531	Stranded in Nouméa	Not recorded	02/10/1997	Not recorded	Not recorded	*Anisakis paggiae*, adults
K. sima	JNC1735	Stranded in Plum	Not recorded	28/12/2005	Not recorded	Not recorded	*Anisakis paggiae*, adults
Mullidae							
Upeneus vittatus	JNC3347	Fish market	Not recorded	24/02/2011	260	237	*Anisakis* type I
U. vittatus	JNC3348	Fish market	Not recorded	24/02/2011	254	226	*Anisakis* type I
Nemipteridae							
Nemipterus furcosus	JNC252	Off Nouméa	22° 16,49S, 166° 20,83E	10/03/2003	200	127	*Anisakis* type I
N. furcosus	JNC279	Grande Rade	22° 15,10S, 166° 22,80E	13/03/2003	200	138	*Terranova* type II
Scombridae							
Scomberoides sp.	JNC3378	Fish Market	Not recorded	29/04/2011	610	>1000	*Anisakis* type I *Terranova* types I and II
Scomberomorus commerson	JNC898	Near Récif To	Not recorded	30/09/2003	900	6600	*Terranova* type II
Sphyraenidae							
Sphyraena qenie	JNC394	Not recorded	Not recorded	14/04/2003	630	1500	*Terranova* type II
Synodontidae							
Saurida undosquamis	JNC571	As JNC569	As JNC569	As JNC569	210	79	*Terranova* type I

[a] MNHN JNC stands for Museum National d'Histoire Naturelle

[b] All localities are near Nouméa, except JNC 1576 and JNC 1735

Table 2 Details of sequences obtained from GenBank to build the phylogenetic tree (Fig. 2)

Taxa name	GenBank accession number ITS1 and ITS2	Reference
Anisakis brevispiculata	JQ912694	Mattiucci et al. (2014)
Anisakis brevispiculata	AB592793	Murata et al. (2011)
Anisakis brevispiculata	KC342887	Quiazon et al. (2013)
Anisakis brevispiculata	FN391881	Shamsi et al. (2012)
Anisakis nascettii	JQ912692	Mattiucci et al. (2014)
Anisakis paggiae	EU624345	Quiazon et al. (2009)
Anisakis paggiae	AB592796	Murata et al. (2011)
Anisakis pegreffii	FN391853 and FN556991	Shamsi et al. (2012)
Anisakis physeteris	AY826721	Nadler et al. (2005)
Anisakis physeteris	AY603530	Kijewska et al. (2002)
Anisakis simplex	EU624342	Quiazon et al. (2009)
Anisakis typica	MF642334 and MF642335	Shamsi et al. (2017)
Anisakis typica	KY352230	dos Reis Sardella and Luque (2016)
Anisakis type I	MH190354-62 and MH190312-20	Present study
Anisakis type III	MH190363-6 and MH190321-4	Present study
Contracaecum bioccai	JF424598	D'Amelio et al. (2012)
Contracaecum eudyptulae	FM177550 and M177565	Shamsi et al. (2009)
Contracaecum fagerholmi	JF424599	D'Amelio et al. (2012)
Contracaecum bancrofti	EU839566 and FM177883	Shamsi et al. (2009)
Contracaecum microcephalum	FM177524 and FM177528	Shamsi et al. (2009)
Contracaecum variegatum	FM177531 and FM177541	Shamsi et al. (2009)
Contracaecum ogmorhini	FM177542 and FM177549	Shamsi et al. (2009)
Contracaecum rudolphii	JF424597	D'Amelio et al. (2012)
Contracaecum larva	MH190385-95 and MH190343-53	Present study
Terranova sp. type I	JX848667 and JX848681	Jabbar et al. (2012)
Terranova sp. type I	MH190367-75 and MH190325-33	Present study
Terranova sp. type II	JX848671 and JX848681	Jabbar et al. (2012)
Terranova sp. type II	MH190377-84 and MH190334-42	Present study
Terranova sp.	MG594307 and G594330	Shamsi et al. (2018)
Heterakis gallinarum	KT310099	Gu et al. (2016)

anisakid nematode found in this study is provided followed by a phylogenetic tree showing their genetic relationships. 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.

Morphological and genetic characterisation

Anisakis larval type I of Cannon 1977

Materials examined: Two from *Echeneis naucrates* (Museum accession number: JNC209A), one from *Epinephelus areolatus* (Museum accession number: JNC204H), one from *Nemipterus furcosus* (Museum accession number: JNC252E), two from *Upeneus vittatus* (Museum accession numbers: JNC3348A and 3347), one from *Carangoides fulvoguttatus* (Museum accession number: JNC3298), one from *Scomberoides* sp. (Museum accession number: JNC3378A) and one from *Decapterus macarellus* (Museum accession number: JNC2018).

Morphometrics: Third-stage larvae (Fig. 1a, b). Body length 19.38 (15.1–23.925, $n = 9$), maximum body width 0.46 (0.34–0.6, $n = 9$). Cuticle with fine transverse annulations, most apparent on tail. Poorly developed labia. Boring tooth present. Excretory pore immediately below tooth. Nerve ring 0.29 (0.24–0.4, $n = 9$) from anterior end. Muscular oesophagus, length 1.79 (1.425–2.05, $n = 9$), 9.27% (7.35–10.32%, $n = 9$) of total body length, ending in glandular ventriculus. Ventriculus 0.76 (0.475–1.05, $n = 9$) in length, 3.92% (2.45–5.42%, $n = 9$) of total body length and joined obliquely to intestine (Fig. 1a). No caeca or diverticula present. Rectum short and oblique to anus. Tail conical, short, with rounded tip, decorated with a single mucron. Anus 0.13 (0.09–0.23, $n = 9$) from posterior end.

Genetic characterisation: Nine specimens, one from each fish species, were selected to obtain their ITS-1and ITS-2 sequences. ITS-1 (accession numbers MH190354-62) to and ITS-2 (accession numbers MH190312-20) were 348 and 355 bp in length, respectively. No nucleotide differences were observed within ITS-1 or ITS-2 sequences among specimens in the present study. Sequences were identical to those of *Anisakis typica* (GenBank accession number: MF642334 and MF642335) and were grouped phylogenetically with previously characterised *Anisakis typica* (Fig. 2).

Anisakis larval type III (Murata et al. 2011)

Materials examined: Ten specimens from *Beryx splendens* (Museum accession number: JNC1576).

Morphometrics: Third-stage larvae (Fig. 1c, d). Body length 23.32 (15.95–27.9, $n = 10$). Maximum body width 0.83 (0.63–1.05, $n = 10$). Thick, transversely annulated

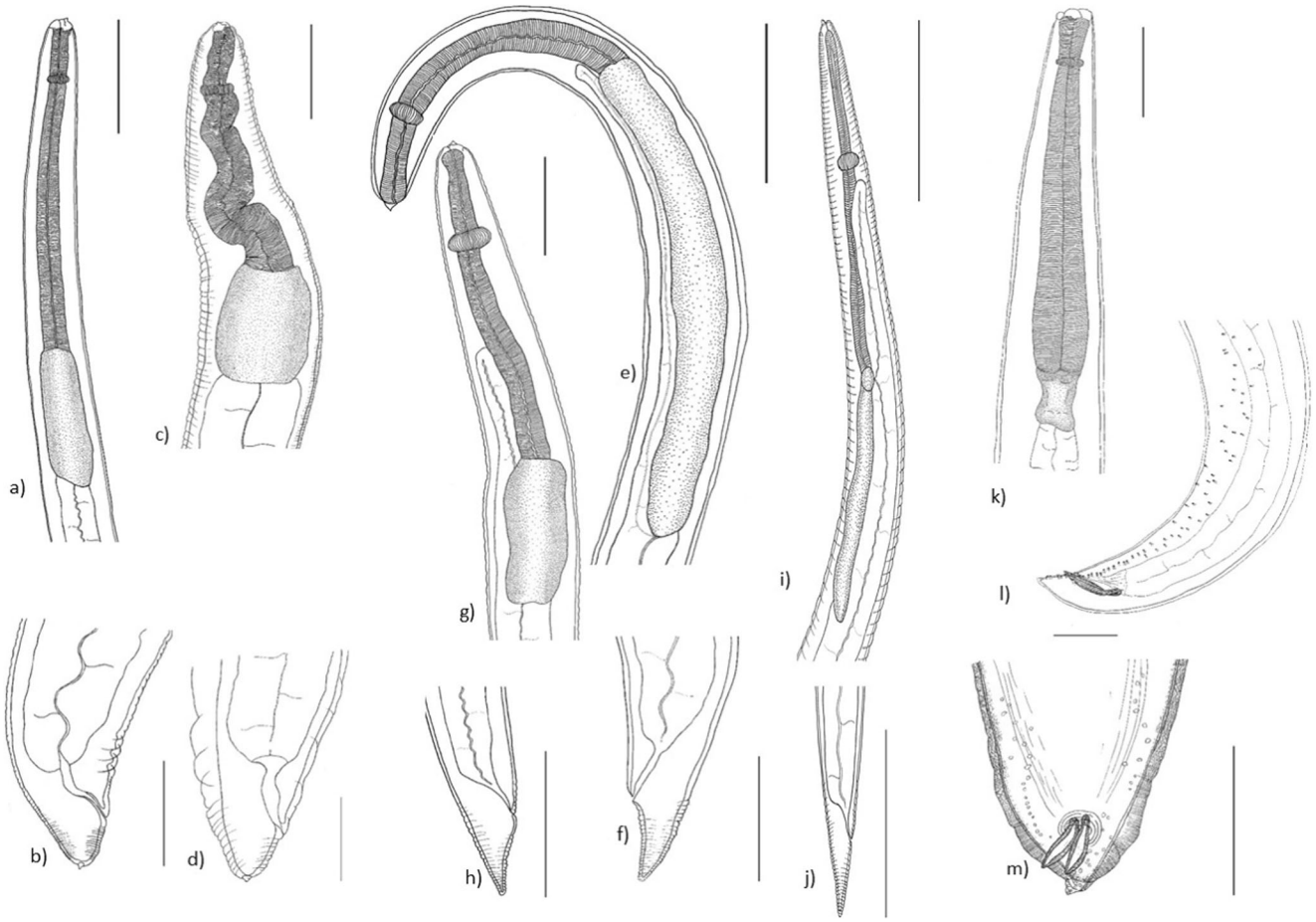

Fig. 1 Nematodes found in the present study. **a**, **b** *Anisakis* larval type I from *Echeneis naucrates* (specimen number: 206–10); **a** anterior end, scale bar 0.5 mm, **b** posterior end, scale bar 0.25 mm. **c**, **d** *Anisakis* larval type III from *Beryx splendens* (specimen number: 274–9); **c** anterior end, scale bar 0.5 mm; **d** posterior end, scale bar 0.25 mm. **e**, **f** *Terranova* larval type I from *Epinephelus ongus*, specimen number 266-1; **e** anterior end, scale bar 0.5 mm; **f** posterior end, scale bar 0.25 mm. **g**, **h** *Terranova* larval type II from *Echeneis naucrates*, specimen number 206-13; **g** anterior end, scale bar 0.25 mm; **h** posterior end, scale bar 0.25 mm. **i**, **j** *Contracaecum* larval type from *Periopthalmus argentilineatus*, specimen number 261-5; **i** anterior end, scale bar 0.25 mm; **j** posterior end, scale bar 0.25 mm. **k**–**m** Adult *Anisakis paggiae* males from *Kogia breviceps*; **k** anterior end, scale bar 1 mm; **l** posterior end lateral view, scale bar 0.25 mm; **m** posterior end ventral view, scale bar 0.25 mm

cuticle. Poorly developed labia. Boring tooth present. Excretory pore immediately below tooth. Nerve ring 0.33 (0.25–0.38, $n = 10$) from anterior end. Muscular oesophagus, 1.46 (0.425–2.18, $n = 10$) in length, 6.26% (1.82–9.35%, $n = 10$) of total body length, ending in glandular ventriculus. Ventriculus 0.65 (0.3–1.53, $n = 10$) long, 2.79% (1.29–6.54%, $n = 10$) of total body length. No caeca or diverticula present. Rectum short and oblique to anus. Tail conical, short with rounded tip, decorated with a single mucron. Anus 0.11 (0.05–0.18, $n = 10$) from posterior end.

Genetic characterisation: Four specimens were subjected to PCR for amplification and obtaining sequences of the ITS-1 and ITS-2. ITS-1 and ITS-2 were 375 and 272 bp long respectively (accession numbers MH190363-6 and MH190321-4). Apart from one specimen (GenBank accession number MH190365) which had different nucleotide at alignment position 117 (in the ITS-1 region), ITS sequences were identical among our specimens. A search in GenBank for matching sequences belonging to adult *Anisakis* spp. showed that adult *A. brevispiculata* (KC342887 and FN891881) from the Philippines (Quiazon et al. 2013) and Australian waters (Shamsi et al. 2012), respectively, are almost identical with our specimens.

***Terranova* larval type I of Cannon (1977)**

Materials examined: Eight specimens from *Saurida undosquamis* (numbers: JNC571B), *Sufflamen fraenatum* (JNC297B), *Scomberoides* sp. (JNC3378B), *Atule mate* (JNC3365), *Epinephelus ongus* (JNC3275), *Decapterus macarellus* (JNC2019A) and *Lutjanus rivulatus* (JNC1864).

Morphometrics: Third-stage larvae (Fig. 1e, f). Body length 9.92 (7.5–12.55, $n = 8$). Maximum body width 0.25 (0.19–0.32, $n = 8$). Delicately annulated cuticle. Poorly developed labia. Boring tooth present. Excretory pore immediately below tooth. Nerve ring 0.27 (0.2–0.33, $n = 8$) from anterior

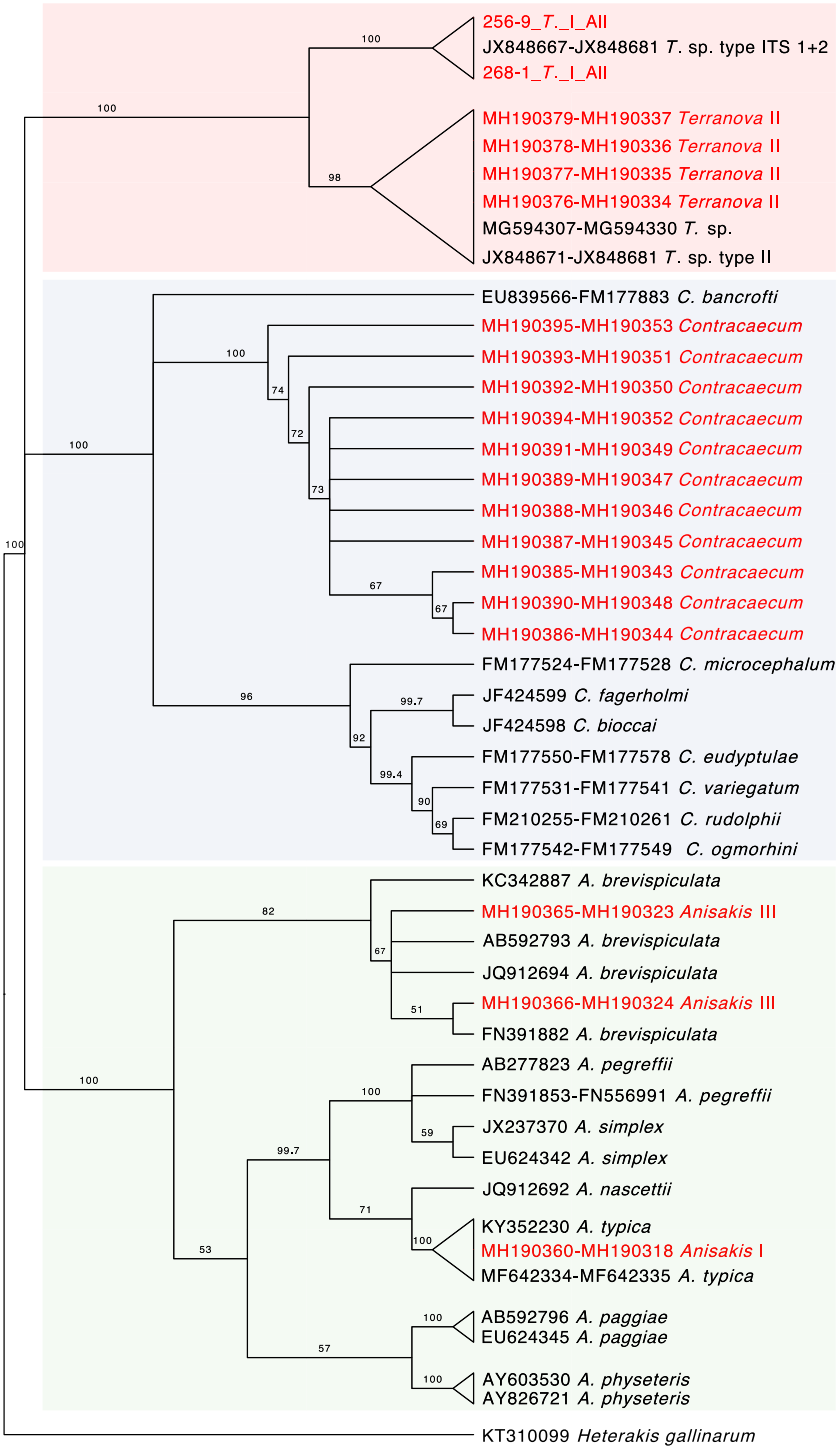

Fig. 2 Phylogenetic analysis of the concatenated ITS-1 and ITS-2 sequence data for *Contracaecum* spp., *Anisakis* spp. and *Terranova* spp., with *Heterakis gallinarum* as an outgroup, using the using neighbour-joining method with p-distances model. Bootstrap (1000 replicates) support values are indicated above the branches. Numbers after taxa refer to ITS-1 and ITS-2, respectively. If only one number is presented, it indicates that ITS sequence data were extracted from a bigger region of rDNA

end. Muscular oesophagus, 1 (0.7–1.28, $n = 8$) long, 10.08% (7.06–12.9%, $n = 8$) of total body length. Oesophagus ending in elongated glandular ventriculus, 1.09 (0.35–1.45, $n = 8$) long, 10.99% (3.53–14.62%, $n = 8$) of total body length. Intestinal caecum present, 1.23 (0.6–1.7, $n = 8$) long. Ratio of intestinal caecum to ventriculus 1.13:1 (1.23, 1.09, $n = 8$). Tail strongly annulated, conical, smoothly tapered. Anus 0.13 (0.11–0.17, $n = 8$) from posterior end.

Genetic characterisation: Eight specimens including one from each above mentioned host (accession numbers MH190367-74) plus one from *Variola albimarginata* (accession number MH190375) were sequenced in both ITS-1 and ITS-2. The length of ITS-1 was 437 bp. ITS-1 was identical among all sequences as well as with GenBank accession number KC437344, belonging to *Terranova* sp. type I from Western Australian waters (Jabbar et al. 2013). The length of

ITS-2 was 266 bp, identical among sequences (MH190325-33) including two nucleotide ambiguities at alignment positions 43 (Y) and 147 (R), as well as identical to JX848681 belonging to *Terranova* larval type I from Lizard Island in Australia (Jabbar et al. 2012). *Terranova* type I in the present study was grouped with previously known *Terranova* type I from southern hemisphere.

***Terranova* larval type II of Cannon (1977)**

Materials examined: Ten specimens from *Carangoides* cf. *orthogrammus* (JNC569C), *Scomberomorus commerson* (JNC898D), *Epinephelus areolatus* (JNC205E), *Echeneis naucrates* (JNC209A), *Nemipterus furcosus* (JNC279B), *Sphyraena qunie* (JNC394A), *Scomberoides* sp. (JNC3378B) and *Atule mate* (JNC3365).

Morphometrics: Third-stage larvae (Fig. 1g, h). Body length 6.63 (5.42–8.3, $n = 10$). Maximum body width 0.23 (0.18–0.28, $n = 10$). Delicately annulated cuticle. Poorly developed labia. Boring tooth present. Excretory pore immediately below tooth. Nerve ring 0.25 (0.22–0.32, $n = 10$) from anterior end. Muscular oesophagus, 0.85 (0.73–1.03, $n = 10$) long, 12.82% (11.01–15.54%, $n = 10$) of total body length, ending in oval-shaped glandular ventriculus. Ventriculus 0.34 (0.29–0.38, n = 10) long, 5.13% (4.37–5.73%, $n = 10$) of total body length. Intestinal caecum present, 0.68 (0.61–0.85, $n = 10$) long. Ratio of intestinal caecum to ventriculus 2:1 (0.68, 0.34, $n = 10$). Tail strongly annulated, conical, smoothly tapered. Anus 0.13 (0.09–0.15, $n = 10$) from posterior end.

Genetic characterisation: One specimen from each host mentioned above, except for *Nemipterus furcosus*, plus one from *Sufflamen frenatum* each were subjected to amplification of the ITS-1 and ITS-2 regions and subsequent sequencing.

ITS-1 was 437 bp, except for specimen from *Saurida undosquamis* (accession number MH190376); all had identical sequence (accession numbers MH190377-84). The difference was for alignment position 99 for which it was CC in MH190376 instead of CT for others. Blast in GenBank resulted showed our specimens have identical ITS-1 sequence as LN795828 previously reported in Australian waters. ITS-2 was 252 bp and identical among all sequences in the present study (MH190334-42). Blast in GenBank resulted showed our specimens have identical ITS-2 sequence as LN651110 previously reported in New Caledonian waters. Phylogenetic analyses showed that all *Terranova* type II are grouped together (Fig. 2).

***Contracaecum* larval type**

Materials examined: Eight specimens from *Periophthalmus argentilineatus* (JNC 3320).

Morphometrics: Third-stage larvae (Fig. 1i, j). Body length 3.43 (2.67–4.25, $n = 8$). Maximum body width 0.14 (0.11–0.2, $n = 8$). Cuticle strongly annulated. Poorly developed labia. Boring tooth present. Excretory pore located ventrally, immediately below boring tooth. Nerve ring 0.19 (0.17–0.24, $n = 8$) from anterior end. Muscular oesophagus 0.45 (0.38–0.53, $n = 8$) in length, 13.12% (11.08–15.45%, $n = 8$) of total body length. Oesophagus ending in glandular, subglobular ventriculus. Ventriculus length 0.04 (0.03–0.05, $n = 8$), 1.17% (0.87–1.46%, $n = 8$) of total body length. Intestinal caecum length 0.27 (0.2–0.33, $n = 8$). Ventricular appendix 0.35 (0.25–0.42, $n = 8$) long. Ratio of intestinal caecum to ventricular appendix 0.77:1 (0.27, 0.35, $n = 8$). Tail conical and without mucron. Anus 0.1 (0.09–1.1, $n = 6$) from posterior end.

Genetic characterisation: All specimens above plus four additional specimens from the same fish species were subjected to amplification of the ITS-1 and ITS-2 region followed by the sequences. ITS-1 was at least 443 bp (accession numbers MH190385-95) with two polymorphic sites including alignment positions 53 and 415 which were A and G, respectively in MH190392, MH190393, MH190395, and G and A in the remaining sequences. ITS-2 was 260 bp, identical among all specimens (accession numbers MH190343-53) except for alignment position 63 which was T in four specimens (MH190353, MH190343, MH190344 and MH190348) and C in the remaining specimens. No highly similar sequence was found in GenBank.

***Anisakis paggiae* adults**

Materials examined: Ten specimens; five male, five female (museum accession numbers: JNC 1735 and JNC 1531 from hosts *Kogia sima* and *Kogia breviceps*.

Morphometrics:

Males (Fig. 1k–m) with fine transverse annulated cuticle. Body length 35.12 (15.98–49.25, $n = 5$). Three protruding labia, 0.1 (0.07–0.15, $n = 5$) in height, 0.13 (0.12–0.16, $n = 5$) in width. Dorsal and ventro-lateral labia with low anterior projection with dentigerous ridges on inner surface. Dorsal lip with two double papillae. Ventro-lateral lips with one double papilla, one single papilla and one amphid. Deirids small and papillate, located around level of first third of oesophagus, posterior to nerve ring, 0.74 (0.52–0.9, $n = 8$) from anterior end. Nerve ring 0.55 (0.29–1.15, $n = 5$) from anterior end. Muscular oesophagus 2.63 (2.08–3.48, $n = 5$) in length, 7.49% (5.92–9.91%, $n = 5$) of total body length, ending in glandular ventriculus. Ventriculus violin-shaped with a distinct constriction in the middle, 0.53 (0.43–0.62, $n = 5$) in length, 1.51% (1.22–1.77%, $n = 5$) of total body length. No caeca or diverticula present. Two short, stout and slightly subequal spicules present. Right spicule 0.2 (0.14–0.31, $n = 5$), left spicule 0.22 (0.15–0.32, $n = 5$). Ratio between right and left spicule = 1:1.1. Tail 0.2 (0.16–0.25, $n = 5$) in length. Three narrow denticulate caudal plates (plectanes) present, posterior to cloaca. Pre-cloacal papillae pairs numerous and arranged in single rows. One median papilla and one pair of proximal papillae lateral to cloaca. One pair of double paracloacal papillae. Four pairs of distal papillae.

Females with fine transverse annulated cuticle. Body length 39.22 (25.43–46.83, $n = 5$). Maximum body width

0.82 (0.37–1.15, $n = 5$). Three protruding labia, 0.1 (0.08–0.12, $n = 5$) in height, 0.12 (0.1–0.15, $n = 5$) in width. Dorsal and ventro-lateral labia with low anterior projection with dentigerous ridges on inner surface. Dorsal lip with two double papillae. Ventro-lateral lips with 1 double papilla, one single papilla and one amphid. Deirids small and papillate, located around level of first third of oesophagus, posterior to nerve ring, 0.89 (0.58–1.13, $n = 8$) from anterior end. Nerve ring 0.54 (0.36–0.83, $n = 5$) from anterior end. Muscular oesophagus 3.03 (2.23–3.83, $n = 5$) in length, 7.73% (5.69–9.77%, $n = 5$) of total body length, ending in glandular ventriculus. Ventriculus violin-shaped with a distinct constriction in the middle, 0.57 (0.42–0.65, $n = 5$) long, 1.45% (1.07–1.66%, $n = 5$) of total body length. No caeca or diverticula present. Vulva small, 11.13 (8.75–13.38, $n = 5$) from anterior end. Tail conical with rounded tip. No mucron present. Anus 0.28 (0.18–0.35, $n = 5$) from posterior end.

Genetic characterisation: These specimens were fixed in formalin and therefore molecular work could not be carried out.

Phylogenetic analyses of taxa found in this study and comparison with congeners

Phylogenetic analysis of the ITS-1 and ITS-2 sequence data was performed using neighbour-joining method. Phylogenetic analyses showed that the five larval types found in the present study were genetically distinct (Fig. 2), divided into three distinct clades. Clade I (green highlighted) included members of *Terranova* larval types found in the resent study, revealing clear distinction between types I and II supported by a strong bootstrap value. Clade II (pink highlighted) included members of *Contracaecum* larva, all resolved in one group. The group was distinct from other *Contracaecum* spp. found in Australia and/or for which comparable data were available in GenBank. Clade III (blue highlighted) represented *Anisakis* spp. Two *Anisakis* larval types found in the present study were resolved separately. *Anisakis* type I was grouped with members of *Anisakis typica* and *Anisakis* type III was clustered with members of *A. brevispiculata*.

Discussion

Five morphotypes belonging to three genera of anisakid nematodes, including *Anisakis*, *Terranova* and *Contracaecum*, were found in New Caledonian waters during this study. The morphological distinction between larvae was supported by ITS sequence data. Phylogenetic analysis revealed presence of three distinct clades in which sequences from these three genera clustered. Clade I included the most common anisakid larvae in the present study, *Terranova* larval types, including larval types I and II which could be morphologically differentiated based on the ratio of intestinal caecum length to ventriculus length. They were considerably different in their ITS sequence data and resolved separately in the phylogenetic tree (Fig. 2). However, not much is known about the specific identity of *Terranova* larval types yet. Larvae referred to as '*Terranova* sp.' have the potential to belong to a number of different genera including *Pseudoterranova*, *Terranova* and *Pulchrascaris* (Shamsi and Suthar, 2016). Although adults of *Terranova scoliodontis* have been reported from the tiger shark, *Galeocerdo cuvier*, off New Caledonia (Moravec and Justine 2006), there is no comparable molecular data available to investigate its relationship with larvae found in the present study.

Terranova larval type I has not been previously reported in New Caledonian fish, therefore the present study is a new geographical record; however, previous publications have listed specimens found simply as '*Terranova* sp.' larvae with no further clarification as to type. *Terranova* larval type II was reported from New Caledonia previously (Shamsi et al. 2015) in 11 host species. Except for *Atule mate* and *Carangoides* cf. *orthogrammus*, the remaining six host species; *Echeneis naucrates* (live sharksucker), *Epinephelus areolatus* (areolate grouper), *Nemipterus furcosus* (fork-tailed threadfin bream), *Scomberoides* sp., *Scomberomorus commerson* (narrow-barred Spanish mackerel) and *Sphyraena qunie* (great barracuda) are new host records. Hosts *A. mate* and *Scomberoides* sp. were infected with both larval types I and II. ITS region sequences were a match for those of specimens obtained from larvae from 13 species of Australian fishes (Shamsi and Suthar 2016). ITS-1 and ITS-2 regions in specimens examined by Shamsi and Suthar (2016) showed a high degree of similarity, therefore suggesting they all belong to one species. However, there is a lack of comparable sequence data from well-described adults in GenBank, so the specific identity of specimens from the present study and from Shamsi and Suthar (2016) remain unknown.

Finding of *Contracaecum* larval type is another interesting finding of the present study. Human infection due to *Contracaecum* larval type can occur (Shamsi and Butcher 2011); however, *Periophthalmus argentilineatus* is not an edible fish. In the phylogenetic tree, all *Contracaecum* larvae found in the present study were grouped separately from all other known *Contracaecum* spp. reported in Australia and also from those with the highest similar ITS sequence (Fig. 2), suggesting it may belong to a new species or to a known species for which ITS sequence data is not available. There is no report of adult *Contracaecum* from New Caledonia but presence of the infective stage of the parasite in fish suggests that adults must be present in birds or marine mammals nearby. *Periophthalmus argentilineatus* is a mangrove fish and the parasite cycle may involve other hosts. Our specimens of *P. argentilineatus* were also infected with acanthocephalan larvae (Bray and Justine 2013) another parasite group that have marine mammals or birds as their definitive hosts.

Clade III included two different *Anisakis* larval types in New Caledonian fish, including types I and III. Although *Anisakis* larval types I and III could easily be differentiated morphologically based on the morphology of the ventriculus and tail, specific identification is not possible due to lack of characteristic features with taxonomic values, such as spicules. Based on morphology, *Anisakis* type I larva could be belong to *A. simplex*, *A. pegreffii*, *A. berlandi* or *A. typica*. Similarly, *Anisakis* type III could be *A. brevispiculata*, *A. paggiae* or *A. physeteris*. The grouping of taxa clade III of our phylogenetic tree suggests *Anisakis* larval type I to be *A. typica* and *Anisakis* larval type III to be *A. brevispiculata*. Previously, *Anisakis* type I larvae were reported from 16 species of fish in New Caledonia (Shamsi et al. 2015). There is also a report of *Anisakis* sp. larva in this geographical region from *E. areolatus* (Justine et al., 2010a); however, the larval type was not stated and specific identification was unknown as molecular work was not performed. Except for *C. fulvoguttatus*, the remaining five fish species are new host records.

In contrast, *Anisakis* larval type III was found only in one fish, *Beryx splendens* (splendid alfonsino), in the present study. In opposition to all other fish mentioned in the present study, which are coral reef-associated fish, this species is a deep-sea fish, collected from a Seamount off New Caledonia. This fish host was not included in previous bibliographic works of parasites from fish from New Caledonia. The present study is a new parasite-host record and first report of *Anisakis* type III larvae in New Caledonia. *Beryx splendens* has a circumglobal distribution and *Anisakis* parasites of this host species have been recorded in other geographical locations. ITS molecular work on *Anisakis* larval types II, III and IV from *Beryx splendens* from Japan revealed type II larvae were a 100% match to *A. physeteris*, type III larvae had a high level of similarity to *A. brevispiculata* and type IV larvae were 100% identical to *A. paggiae* (Murata et al., 2011). Third-stage larvae of *A. physeteris* have been found in *B. splendens* from the Mediterranean Sea (Psomadakis et al. 2012). In our study, *Anisakis* type III larvae had identical sequences with *A. brevispiculata* reported from whales in Australia and the Philippine waters. Alignment of ITS sequences of *A. brevispiculata* from southern hemisphere and those in the Philippine waters shows presence of 1 bp difference in both ITS-1 and ITS-2. For some *Anisakis* spp. (members of *Anisakis simplex* sensu *lato*), one base pair difference in either ITS-1 or ITS-2 region means a distinct species (Shamsi et al. 2012). It cannot be concluded at this stage that the base pair difference observed between *A. brevispiculata* from different parts of the world is indicative of the presence of distinct species. More studies using other genes are necessary to investigate this.

Although adult nematodes collected from whales in the present study were placed under *Anisakis paggiae*, a closely related species to *A. brevispiculata* (Mattiucci et al., 2018), the spicule length in our specimens is shorter than both *A. paggiae* and *A. brevispiculata*, fitting within the range reported for *Anisakis oceanicus*, previously reported in pacific waters from black fish (genus *Globicephala*) (Johnston and Mawson, 1951) and currently considered as *A. physeteris* (Davey, 1971). For the time being due to the lack of molecular data, the specimens in the present study are placed under *A. paggiae* and if this is correct then this is the first report of the species from the Pacific region.

In conclusion, this study shows that new and known anisakid nematodes are commonly found in New Caledonian waters. All of the specimens from fish were in third larval stage and adults were only found in whales. Third-stage larvae of anisakids are known to be the infective stage of the parasite for humans, therefore presence of these parasites in New Caledonian fish, where seafood consumption is high (Charlton et al. 2016), is very important. Similar to some of the neighbouring countries, e.g. in Australia (Shamsi and Shorey 2018), the absence of report from humans in this region could be due to the lack of awareness among health practitioners. Considering the high level of seafood consumption in New Caledonia, it is crucial to raise awareness among various stake holders about these parasites and risk they may pose to public health.

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References

Bray RA, Justine J-L (2013) A digenean parasite in a mudskipper: *Opegaster ouemoensis* sp n. (Digenea: Opecoelidae) in *Periophthalmus argentilineatus* Valenciennes (Perciformes: Gobiidae) in the mangroves of New Caledonia. Folia Parasitol 60: 7–16

Cannon LRG (1977) Some larval ascaridoids from south-eastern Queensland marine fishes. Int J Parasitol 7:233–243

Charlton KE, Russell J, Gorman E, Hanich Q, Delisle A, Campbell B, Bell J (2016) Fish, food security and health in Pacific Island countries and territories: a systematic literature review. BMC Public Health 16:285

D'Amelio S, Cavallero S, Dronen NO, Barros NB, Paggi L (2012) Two new species of *Contracaecum* Railliet & Henry, 1912 (Nematoda: Anisakidae), *C. fagerholmi* n. sp and *C. rudolphii* F from the brown pelican Pelecanus occidentalis in the northern Gulf of Mexico. Syst Parasitol 81:1–16

Davey JT (1971) A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridata). J Helminthol 45:51–72

dos Reis Sardella CJ, Luque JL (2016) Morphological and molecular diagnostic of *Anisakis typica* and *Anisakis brevispiculata* of southeastern coast of Brazil. Braz J Vet Med 38(Supl. 3):124–130

Froese, R., Pauly, D., (Eds) (2018) FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2018)

Gontard T, de Coudenhove G (2013) Economic monitoring study: the fisheries sector in New Caledonia. SPC Fish Newsletter 141:29–36

Jabbar A, Asnoussi A, Norbury LJ, Eisenbarth A, Shamsi S, Gasser RB, Lopata AL, Beveridge I (2012) Larval anisakid nematodes in teleost fishes from Lizard Island, northern Great Barrier Reef, Australia. Mar Freshw Res 63:1283–1299

Gu X, Zhu J-Y, Jian K-L, Wang B-J, Peng X-R, Yang G-Y, Wang T, Zhong Z-J, Peng K-Y (2016) Absence of population genetic structure in *Heterakis gallinarum* of chicken from Sichuan, inferred from mitochondrial cytochrome c oxidase subunit I gene. Mitochondrial DNA Part A 27:3612–3617

Jabbar A, Fong RWJ, Kok KX, Lopata AL, Gasser RB, Beveridge I (2013) Molecular characterization of anisakid nematode larvae from 13 species of fish from Western Australia. Int J Food Microbiol 161: 247–253

Johnston TH, Mawson PM (1951) Report on some parasitic nematodes from the Australian Museum. Rec Aust Mus 22:289–297

Justine J-L (2007) Parasite biodiversity in a coral reef fish: twelve species of monogeneans on the gills of the grouper *Epinephelus maculatus* (Perciformes: Serranidae) off New Caledonia, with a description of eight new species of *Pseudorhabdosynochus* (Monogenea: Diplectanidae). Syst Parasitol 66:81–129

Justine JL (2010) Parasites of coral reef fish: how much do we know? With a bibliography of fish parasites in New Caledonia. Belg J Zool 140(Suppl):155–190

Justine J-L, Beveridge I, Boxshall GA, Bray RA, Miller TL, Moravec F, Trilles J-P, Whittington ID (2010a) An annotated list of parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected in groupers (Serranidae, Epinephelinae) in New Caledonia emphasizes parasite biodiversity in coral reef fish. Folia Parasitol 57:237–262

Justine J-L, Beveridge I, Boxshall GA, Bray RA, Moravec F, Whittington ID (2010b) An annotated list of fish parasites (Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected from emperors and emperor bream (Lethrinidae) in New Caledonia further highlights parasite biodiversity estimates on coral reef fish. Zootaxa 2691:1–40

Justine J-L, Beveridge I, Boxshall GA, Bray RA, Miller TL, Moravec F, Trilles J-P, Whittington ID (2012a) An annotated list of fish parasites (isopoda, Copepoda, Monogenea, Digenea, Cestoda, Nematoda) collected from snappers and bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. Aquatic Biosystems 8:22

Justine J-L, Briand MJ, Bray RA (2012b) A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. Parasitol Res 111:341–351

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649

Kijewska A, Rokicki J, Sitko J, Węgrzyn G (2002) Ascaridoidea: a simple DNA assay for identification of 11 species infecting marine and freshwater fish, mammals, and fish-eating birds. Exp Parasitol 101(1):35–39

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874

Mattiucci S, Cipriani P, Webb SC, Paoletti M, Marcer F, Bellisario B, Gibson DI, Nascetti G (2014) Genetic and morphological approaches distinguish the three sibling species of the Anisakis simplex species complex with a species designation as *Anisakis berlandi* n. sp. for A. simplex sp C (Nematoda: Anisakidae). J Parasitol 100:199–214. https://doi.org/10.1645/12-120.1

Mattiucci S, Cipriani P, Levsen A, Paoletti M, Nascetti G (2018) Molecular epidemiology of *Anisakis* and Anisakiasis: an ecological and evolutionary road map. Adv Parasitol 99:93–263

Moravec F, Justine J-L (2006) Three nematode species from elasmobranchs off New Caledonia. Syst Parasitol 64:131–145

Murata R, Suzuki J, Sadamasu K, Kai A (2011) Morphological and molecular characterization of *Anisakis* larvae (Nematoda: Anisakidae) in *Beryx splendens* from Japanese waters. Parasitol Int 60:193–198

Myers N, Mittermeier RA, Mittermeier CG, da Fonesca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA (2005) Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern Pacific marine mammals. J Parasitol 91(6):1413–1429

Psomadakis PN, Stefanni S, Merella P, Ferrando S, Amato A, Vacchi M (2012) Additional records of *Beryx splendens* (Osteichthyes: Berycidae) from the Mediterranean Sea, with notes on molecular phylogeny and parasites. Ital J Zool 79:111–119

Quiazon KMA, Yoshinaga T, Santos MD, Ogawa K (2009) Identification of Larval *Anisakis* spp. (Nematoda: Anisakidae) in Alaska Pollock (Theragra chalcogramma) in Northern Japan Using Morphological and Molecular Markers. J Parasitol 95(5):1227–1232

Quiazon KMA, Santos MD, Yoshinaga T (2013) Anisakis species (Nematoda: Anisakidae) of dwarf sperm whale Kogia sima (Owen, 1866) stranded off the Pacific coast of southern Philippine archipelago. Vet Prasitol 197:221–230

Shamsi S, Butcher AR (2011) First report of human anisakidosis in Australia. Med J Aust 194:199–200

Shamsi S, Shorey H (2018) Seafood-borne parasitic diseases in Australia: are they rare or underdiagnosed? Int J Med 48:591–596

Shamsi S, Suthar J (2016) Occurrence of *Terranova* larval types (Nematoda: Anisakidae) in Australian marine fish with comments on their specific identities. PeerJ 4:e1722–e1722

Shamsi S, Norman R, Gasser R, Beveridge I (2009) Redescription and genetic characterization of selected *Contracaecum* spp. (Nematoda: Anisakidae) from various hosts in Australia. Parasitol Res 104(6): 1507–1525

Shamsi S, Gasser RB, Beveridge I (2011) Mutation scanning-coupled sequencing of nuclear ribosomal DNA spacers (as a taxonomic tool) for the specific identification of different *Contracaecum* (Nematoda: Anisakidae) larval types. Mol Cell Probes 25, 13–18

Shamsi S, Gasser R, Beveridge I (2012) Genetic characterisation and taxonomy of species of *Anisakis* (Nematoda:Anisakidae) parasitic in Australian marine mammals. Invertebr Syst 26:204–212

Shamsi S, Poupa AM, Justine J-L (2015) Characterisation of Ascaridoid larvae from marine fish off New Caledonia, with description of new *Hysterothylacium* larval types XIII and XIV. Parasitol Int 64:397–404

Shamsi S, Briand MJ, Justine J-L (2017) Occurrence of *Anisakis* (Nematoda: Anisakidae) larvae in unusual hosts in southern hemisphere. Parasitol Int 66:837–840

Shamsi S, Steller E, Chen Y (2018) New and known zoonotic nematode larvae within selected fish species from Queensland waters in Australia. Int J Food Microbiol 272:73–82