Occurrence of *Terranova* larval types (Nematoda: Anisakidae) in Australian marine fish with comments on their specific identities

Shokoofeh Shamsi and Jaydipbhai Suthar
School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

**ABSTRACT**

Pseudoterranovosis is a well-known human disease caused by anisakid larvae belonging to the genus *Pseudoterranova*. Human infection occurs after consuming infected fish. Hence the presence of *Pseudoterranova* larvae in the flesh of the fish can cause serious losses and problems for the seafood, fishing and fisheries industries. The accurate identification of *Pseudoterranova* larvae in fish is important, but challenging because the larval stages of a number of different genera, including *Pseudoterranova*, *Terranova* and *Pulchrascaris*, look similar and cannot be differentiated from each other using morphological criteria, hence they are all referred to as *Terranova* larval type. Given that *Terranova* larval types in seafood are not necessarily *Pseudoterranova* and may not be dangerous, the aim of the present study was to investigate the occurrence of *Terranova* larval types in Australian marine fish and to determine their specific identity. A total of 137 fish belonging to 45 species were examined. *Terranova* larval types were found in 13 species, some of which were popular edible fish in Australia. The sequences of the first and second internal transcribed spacers (ITS-1 and ITS-2 respectively) of the *Terranova* larvae in the present study showed a high degree of similarity suggesting that they all belong to the same species. Due to the lack of a comparable sequence data of a well identified adult in the GenBank database the specific identity of *Terranova* larval type in the present study remains unknown. The sequence of the ITS regions of the *Terranova* larval type in the present study and those of *Pseudoterranova* spp. available in GenBank are significantly different, suggesting that larvae found in the present study do not belong to the genus *Pseudoterranova*, which is zoonotic. This study does not rule out the presence of *Pseudoterranova* larvae in Australian fish as *Pseudoterranova decipiens E* has been reported in adult form from seals in Antarctica and it is known that they have seasonal presence in Australian southern coasts. The genetic distinction of *Terranova* larval type in the present study from *Pseudoterranova* spp. along with the presence of more species of elasmobranchs in Australian waters (definitive hosts of *Terranova* spp. and *Pulchrascaris* spp.) than seals (definitive hosts of *Pseudoterranova* spp.) suggest that *Terranova* larval type in the present study belong to either genus *Terranova* or *Pulchrascaris*, which are not known to cause disease in humans. The present study provides essential information that could be helpful to identify Australian *Terranova* larval types in future studies. Examination and characterisation of further specimens, especially adults of *Terranova* and *Pulchrascaris*, is necessary to fully elucidate the identity of these larvae.
INTRODUCTION

Psudoterranovosis (Hochberg & Hamer, 2010), the seafood borne parasitic disease, caused by larvae of *Pseudoterranova*, is another form of anisakidosis, that has caused concern to human beings. The disease is most common in the United States followed by Japan and Europe (Hochberg & Hamer, 2010). With the increased popularity of eating raw or slightly cooked seafood dishes, the number of cases have increased globally (Chai, Darwin Murrell & Lymbery, 2005). The symptoms of the disease vary and may include nausea, severe epigastric pain and other abdominal discomforts, “tingling throat syndrome” from a worm crawling in the upper esophagus or oropharynx, cough and vomiting up live or dead worms (Margolis, 1977). The life cycle of the *Pseudoterranova* spp. includes crustaceans and fish as their intermediate hosts and marine mammals as their definitive hosts (Anderson, 2000). Human infection occurs after eating infected seafood, therefore the presence of *Pseudoterranova* larvae in the flesh of fish can cause serious losses and problems for fish and fisheries industry across the world. For example, up to 36 worms per fish have been reported in cod populations from Norwegian waters (Jensen, Andersen & Desclers, 1994) or Icelandic cod fillets provided by the industry have been reported to be infected with 2.5–17.6 worms per kg fillet (Hafsteinsson & Rizvi, 1987). It has been estimated that detection and removal of the larvae thought to be *Pseudoterranova* from the flesh of Atlantic cod (*Gadus morhua*) and other demersal species, and the resultant downgrading and discard of product, cause an annual loss of $50 million in Atlantic Canada (McClelland, 2002). This implies the need for detection and accurate identification of these larvae in fish. One of the challenges in diagnosing of parasitic diseases is the specific identification of larval stages of parasites. Larval stages of nematodes cannot be identified reliably using morphological characters alone. This is a consequence of the small size of larval stages and the lack of a sufficient number of characteristic features (Shamsi, Gasser & Beveridge, 2011). Molecular approaches have gained prominence for accurate identification of anisakids, irrespective of developmental stage and sex of the parasite, and for establishing systematic relationships (e.g., Orecchia et al., 1986). Several studies showed that ITS-1 and ITS-2 are useful genetic markers for specific identifications of nematodes irrespective of their developmental stage or sex and to study their life cycle (e.g., Shamsi, Gasser & Beveridge, 2011). However, this approach relies on presence of ITS sequences for well identified adults.

In several countries other than Australia, the ability to recognise and diagnose anisakidosis/pseudoterranovosis caused by these larvae has been improved, resulting in progress towards understanding its epidemiology and clinical manifestations of the disease. In Australia, however, little is known about the disease, the causative agent and its epidemiology. Australia is an increasingly multicultural country where seafood prepared in all its forms is very popular. A confirmed case of human anisakidosis was published recently by Shamsi & Butcher (2011) and several unpublished cases are on record
Therefore, there has been an increasing awareness of anisakidosis in humans and the presence of anisakid larval types in marine fish in Australia (Shamsi, 2014).

A review of the literature shows that Terranova larval types have been reported quite often in Australian marine fish (e.g., Cannon, 1977; Doupe et al., 2003; Lester, Barnes & Habib, 1985; Moore et al., 2011) but there is no information on the specific identity of Terranova larval types reported in Australia. The dilemma with Terranova larval types is that it could belong to any of three genera of anisakid nematodes, including Terranova, Pulchrascaris or Pseudoterranova, whose adult stages have been reported from Australian waters. Members of Terranova and Pulchrascaris become adult in elasmobranchs and are not known to cause harm to humans whereas Pseudoterranova spp. become adult in marine mammals and there are numerous publications about their pathogenicity and human health impacts. The larval stages of all these genera, i.e., Terranova, Pseudoterranova and Pulchrascaris are morphologically very similar. The typical characteristic of these larvae is the location of the excretory pore at the anterior end of the nematode, presence of a ventriculus without an appendix and having an intestinal caecum (Deardorff, 1987; Gibson & Colin, 1982). Therefore, distinction between larval stages of these genera based solely on morphology can be challenging. With recent increasing awareness about the presence of anisakid larvae in Australian fish as well as the presence of human cases in the country, knowing the specific identity of Terranova larval types becomes very important. In the last decade, molecular tools have provided the opportunity for specific identification of larval stages of parasites and there have been several works in the Americas, European countries and Antarctica on specific identification of Terranova larval types (Arizono et al., 2011; Paggi et al., 1991). Therefore, the aim of the present study is to employ a combined molecular and morphological approach to investigate the occurrence of Terranova larval types in Australian marine fish and to determine their specific identity.

MATERIALS AND METHODS

Parasite collection

A total of 137 fish belonging to 45 species, Abudesduf whitleyi (n = 2), Aldrichetta forsteri (n = 1), Atherinomorus vaigiensis (n = 1), Caesio cuning (n = 8), Carangoides fulvoguttatus (n = 1), Caranx ignobilis (n = 2), C. melampygus (n = 1), Carcharias taurus (n = 1), Chaetodon aureofasciatus (n = 1), C. auriga (n = 1), C. flavirostris (n = 2), C. lineolatus (n = 1), C. melannotus (n = 1), Chaetodon sp (n = 1), Coryphaena hippurus (n = 1), Engraulis australis (n = 2), Epinephelus cyanopodus (n = 1), Grammatorcynus bizarinatus (n = 3), Haplophryne sp. (n = 1), Heniochus monoceros (n = 2), H. singularius (n = 1), Istiopex indica (n = 3), Kajikia audax (n = 3), Lutjanus argentimaculatus (n = 2), L. bohar (n = 1), L. carponotatus (n = 4), L. fulviflamma (n = 1), L. sebae (n = 4), Makaira mazara (n = 3), Mugil cephalus (n = 5), Pastinachus sephen (n = 1), Platyccephalus laevigatus (n = 8), Platyccephalus sp. (n = 2), Pristipomoides multidens (n = 3), Rhombosolea tapirina (n = 3), Sardinops sagax neopilchardus (n = 8), Scomber australasicus (n = 11), Seriola hippos (n = 2), S. lalandi (n = 17), Siganus fuscescens (n = 1), S. punctatus (n = 1), Sillago flindersi (n = 13), Sphyraena novaehollandiae (n = 4), Taeniomembras microstomus (n = 1),
and *Thunnus albacares* (*n* = 1) were examined for infection with anisakid larval types. Fish were collected off Australian coasts, including Queensland, New South Wales, Victoria, South Australia and Western Australia. No fish were caught or killed for the purpose of this study. All fish were either already euthanized as part of other research projects or were bought from fishermen in various fish markets.

Dead fish were cut open and first examined for presence of larval nematodes in the surface of the internal organs and also for gross pathology. Then the gastro-intestinal tract from mouth to anus was examined for the presence of nematodes. All nematodes found were washed in physiological saline and then preserved in 70% ethanol. A small piece of the mid-body of each nematode was excised for molecular study, and the rest of the nematode were used for microscopy.

**Morphological examination**

The anterior and posterior parts of each nematode were cleared in lactophenol and examined under a light microscope. *Terranova* larvae were identified according to the identification key proposed by *Cannon (1977)* and were selected for description and further molecular analyses. Illustrations were made using a microscope equipped with camera lucida.

**Molecular study**

Genomic DNA (gDNA) was isolated from all individual larvae identified morphologically as *Terranova* larval type, by sodium dodecyl-sulphate/proteinase K treatment, column-purified (Wizard™ DNA Clean-Up; Promega, Madison, WI, USA) and eluted into 45 µl of water. PCR was used to amplify the ITS-1 and ITS-2 regions using primer sets SS1: 5′-GTTCGTCATGCTACCTGCG-3′ (forward) and NC13R: 5′-GCTGCGTTCTCTTCATG-3′ (reverse) for the former and SS2: 5′-TTGCAGACACATTGAGCAGC-3′ (forward) and NC2: 5′-TTAGTTTCTTTTTCCTCCGT-3′ (reverse) for the latter region, and 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 (*Shamsi & Butcher, 2011*). An aliquot (4 µl) of each amplicon was examined on a 1.5% w/v agarose gel, stained with GelRed™ and photographed using a gel documentation system.

Representative samples based on host species and geographical locations were selected for sequencing. Sequences were aligned using the computer program ClustalX (*Thompson et al., 1997*) and then adjusted manually. Polymorphic sites were designated using International Union of Pure and Applied Chemistry (IUPAC) codes. Pair-wise comparisons of sequence differences (*D*) were determined using the formula *D* = 1 − (*M/L*), where *M* is the number of alignment positions at which the two sequences have a base in common, and *L* is the total number of alignment positions over which the two sequences are compared (*Chilton, Gasser & Beveridge, 1995*).

Phylogenetic analysis of the nucleotide sequence data for combined ITS-1 and ITS-2 regions were conducted in PAUP 4.0. Table 1 shows details of the taxa used to build phylogenetic trees. Two tree-building methods, neighbour-joining and maximum parsimony were employed for phylogenetic analysis. The outgroup employed was *Heterakis*
| Abbreviation       | Scientific name                  | Specimen/Accession no. | Reference                          |
|--------------------|----------------------------------|------------------------|-----------------------------------|
|                    |                                  | ITS-1 | ITS-2                              |                                   |
| A.brevispiculata   | Anisakis brevispiculata          | AY826719 | AY826719                           | Nadler et al. (2005)              |
| A.brevispiculata1  | Anisakis brevispiculata          | PSW4-1 | PSW4-2                             | Shamsi, Gasser & Beveridge (2012) |
| A.pegreffi         | Anisakis p egregii               | FN391850 | FN556997                           | Shamsi, Gasser & Beveridge (2012) |
| A.pegreffi1        | Anisakis p egregii               | FN391851 | FN556998                           | Shamsi, Gasser & Beveridge (2012) |
| A.physeteris       | Anisakis physeteris              | AY826721 | AY826721                           | Nadler et al. (2005)              |
| A.physeteris1      | Anisakis physeteris              | AY603530 | AY603530                           | Kijewska et al. (2008)            |
| A.simplexC         | Anisakis simplex C               | FN391883 | FN391884                           | Shamsi, Gasser & Beveridge (2012) |
| A.simplexs.s.      | Anisakis simplex sensu stricto   | AJ225065 | AB196672                           | Abe, Ohy & Yanagiguchi (2005)     |
| A.TMTP             | Larva of Anisakis sp. (TMTP)     | AY260555 | AY260555                           | Pontes et al. (2005)              |
| A.typica           | Anisakis typica                  | AY826724 | AY826724                           | Nadler et al. (2005)              |
| A.typica1          | Anisakis typica                  | FN391887 | FN391889                           | Shamsi, Poupa & Justine (2015)    |
| A.ziphidarum       | Anisakis ziphidarum              | AY826725 | AY826725                           | Nadler et al. (2005)              |
| C.bancrofti        | Contracaecum bancrofti           | EU839572 | FM177883                           | Shamsi et al. (2009b)             |
| C.bancrofti1       | Contracaecum bancrofti           | EU839573 | FM177887                           | Shamsi et al. (2009b)             |
| C.eudyptulae       | Contracaecum eudyptulae          | FM177531 | FM177562                           | Shamsi et al. (2009b)             |
| C.margolisi        | Contracaecum margolisi           | AY821750 | AY821750                           | Nadler et al. (2005)              |
| C.microcephalum    | Contracaecum microcephalum       | FM177524 | FM177528                           | Shamsi et al. (2009b)             |
| C.multipapillatum  | Contracaecum multipapillatum     | AM940056 | AM940060                           | Shamsi et al. (2008)              |
| C.ogmorhini        | Contracaecum ogmorhini sensu stricto | FM177542 | FM177547                           | Shamsi et al. (2009b)             |
| C.osculatumaA      | Contracaecum osculatum A         | AJ250410 | AJ250419                           | Zhu et al. (2000)                 |
| C.osculatumB       | Contracaecum osculatum B         | AJ250411 | AJ250420                           | Zhu et al. (2000)                 |
| C.osculatumbaicalensis | Contracaecum osculatum baicalensis | AJ250415 | AJ250416                           | Zhu et al. (2000)                 |
| C.osculatumC       | Contracaecum osculatum C         | AJ250412 | AJ250421                           | Zhu et al. (2000)                 |
| C.osculatumD       | Contracaecum osculatum D         | AJ250413 | AJ250418                           | Zhu et al. (2000)                 |
| C.osculatumE       | Contracaecum osculatum E         | AJ250414 | AJ250417                           | Zhu et al. (2000)                 |
| C.radiatum         | Contracaecum radiatum            | AY603529 | AY603529                           | Kijewska et al. (2002)            |
| C.rudolfiiA        | Contracaecum rudolfii A          | AJ634782 | AJ634911                           | Li et al. (2005)                  |
| C.rudolfiiB        | Contracaecum rudolfii B          | AJ634783 | AJ634911                           | Li et al. (2005)                  |
| C.rudolfiiD        | Contracaecum rudolfii D          | FM210253 | FM210267                           | Shamsi et al. (2009a)             |
| C.rudolfiiD1       | Contracaecum rudolfii D          | FM210254 | FM210265                           | Shamsi et al. (2009a)             |
| C.rudolfiiE        | Contracaecum rudolfii E          | FM210257 | FM210269                           | Shamsi et al. (2009a)             |
| C.rudolfiiE1       | Contracaecum rudolfii E          | FM210258 | FM210273                           | Shamsi et al. (2009a)             |
| C.septentrionale   | Contracaecum septentrionale      | AJ634784 | AJ634787                           | Li et al. (2005)                  |
| C.variegatum       | Contracaecum variegatum          | FM177531 | FM177537                           | Shamsi et al. (2009b)             |
| Contracaecumn.sp.  | Contracaecum pyripapillatum      | AM940062 | AM940066                           | Shamsi et al. (2009b)             |
| H.aduncum1         | Hysterothyacum aduncum           | AJ225068 | AJ225069                           | Zhu et al. (1998)                 |
| H.aduncum2         | Hysterothyacum aduncum           | AB277826 | AB277826                           | Umehara et al. (2008)             |
| H.auctum           | Hysterothyacum auctum            | AF115571 | AF115571                           | Szostakowska et al. (2001)        |
| H.III              | Hysterothyacum larval type III   | FN811721 | FN811678                           | Shamsi, Gasser & Beveridge (2013) |

(continued on next page)
Table 1 (continued)

| Abbreviation | Scientific name          | Specimen/Accession no. | Reference                        |
|---------------|--------------------------|------------------------|---------------------------------|
| H.III-1       | *Hysterothylacium* larval type III | FN811723 FN811681 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVA         | *Hysterothylacium* larval type IV Genotype A | FN811724 FN811690 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVB         | *Hysterothylacium* larval type IV Genotype B | FN811730 FN811682 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVGA        | *Hysterothylacium* larval type IV Genotype A | FN811729 FN811690 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVGA1       | *Hysterothylacium* larval type IV Genotype A | FN811729 FN811691 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVGA2       | *Hysterothylacium* larval type IV Genotype A | FN811729 FN811692 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVGB        | *Hysterothylacium* larval type IV Genotype B | FN811730 FN811683 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVGB1       | *Hysterothylacium* larval type IV Genotype B | FN811731 FN811684 | *Shamsi, Gasser & Beveridge* (2013) |
| H.IVGB2       | *Hysterothylacium* larval type IV Genotype B | FN811733 FN811685 | *Shamsi, Gasser & Beveridge* (2013) |
| H.V           | *Hysterothylacium* larval type V | FN811738 FN811699 | *Shamsi, Gasser & Beveridge* (2013) |
| H.VI          | *Hysterothylacium* larval type VI | FN811740 FN811701 | *Shamsi, Gasser & Beveridge* (2013) |
| H.VII         | *Hysterothylacium* larval type VII | FN811749 FN811709 | *Shamsi, Gasser & Beveridge* (2013) |
| H.VIII        | *Hysterothylacium* larval type VIII | FN811750 FN811710 | *Shamsi, Gasser & Beveridge* (2013) |
| Heterakis gallinarum | *Heterakis gallinarum* | JQ995320 JQ995320 | *Jimenez et al.* (2012) |
| P.azarasi     | *Pseudoterranova* azarasi | AJ413973 AJ413974 | *Zhu et al.* (2002) |
| P.bulbosa     | *Pseudoterranova* bulbosa | AJ413970 AJ413971 | *Zhu et al.* (2002) |
| P.cattani     | *Pseudoterranova* cattani | AJ413982 AJ413984 | *Zhu et al.* (2002) |
| P.decipiens   | *Pseudoterranova* decipiens | AJ413979 AJ413980 | *Zhu et al.* (2002) |
| P.decipiens1  | *Pseudoterranova* decipiens | AJ413979 AJ413978 | *Zhu et al.* (2002) |
| R.acus        | *Raphidascaris* acus | AY603537 AY603537 | *Kijewska et al.* (2008) |
| Terranova sp. | *Terranova* sp. | LN795828 LN795872 | The present study |
| Terranova sp.1| *Terranova* sp. | LN795851 LN795871 | The present study |

gallinarum* (Nematoda: Heteakoidea; GenBank accession numbers *JQ995320* and *JQ995320* for ITS-1 and ITS-2, respectively).

**RESULTS**

Of 45 species of fish examined in the present study, third stage *Terranova* type larvae (*n* = 93) were identified as type II based on the presence of intestinal caecum and ventriculus, absence of developed labia and ventricular appendix, and location of the excretory pore being at the anterior end (Fig. 1). Morphological description of these larvae was summarized in Table 2. *Terranova* type larvae were found in 13 species of fish collected from North-Eastern, Eastern and south eastern coasts of Australia. Material morphologically examined were 10 larvae in good condition from *Caesio cuning* (*n* = 3), *Caranx ignobilis* (*n* = 2), *Grammatorcynus bicarinatus* (*n* = 1), *Lutjanus argentimaculatus* (*n* = 3) and *L. carponotatus* (*n* = 1) from Heron Island, Queensland.
Table 2  Morphological description of Terranova larval type found in the present study. All measurements are given in millimetres. Mean measurements are given, followed by the range in parentheses.

| Taxonomically important morphological character | Measurement/description |
|------------------------------------------------|-------------------------|
| Body length                                     | 6.6 (3.0–9.0)           |
| Body width                                      | 0.24 (0.18–0.28)        |
| Tooth                                           | Present                 |
| Lips morphology                                | Inconspicuous           |
| Distance of nerve ring from anterior end        | 0.37 (0.22–0.72)        |
| Location of excretory pore                     | At anterior end         |
| Oesophagus length                              | 0.88 (0.4–1.14)         |
| Ratio of oesophagus length to body length       | 14.3 (9.5–26.5%)        |
| Ventriculus length                             | 0.38 (0.24–0.54)        |
| Intestinal caecum length                       | 0.71 (0.50–0.90)        |
| Tail morphology                                | Strongly annulated, conical, tapering smoothly |
| Tail length                                     | 0.13 (0.12–0.14)        |
| Ratio of tail length to body length             | 2.2% (1.3–4.0%)         |

A total of 93 specimens from various fishes, including Abudesdulf whitleyi, Caesio cuning, Carangoides fulvoguttatus, Caranx ignobilis, Caranx melampygus, Epinephelus cyanopodus, Grammatorcynus bicaudatus, Lutjanus argentimaculatus, L. bohar, L. carponotatus and L. fulviflamma and Scomber australasicus were subjected to PCR amplification. Based on the species of hosts and their geographical locations, 25 and 21 specimens were selected and sequenced for ITS-1 and ITS-2 respectively.

The length of the ITS-1 was 437 bp except for two specimens which were 436 bp long. The difference in length was due to a gap at alignment position 20 in the latter specimens (Fig. 2). Also, sequence polymorphism was detected at alignment position 426 in one specimen (Fig. 2). Sequence variation in the ITS-1 among specimens was 0–0.4% and the G + C content was 47.6–47.9%. The length of the ITS-2 was 252 bp. Sequence polymorphism was detected at alignment position 22 in two specimens. Sequence variation among individuals was 0–0.4% and the G + C content was 46.4–46.8%. ITS-1 and ITS-2 sequences of Terranova larval type found in the present study were almost identical among all larvae.

DISCUSSION

Previously, Cannon (1977) described two distinct Terranova larval types, I and II, in Queensland waters which were later reported by other authors from other parts of Australia (e.g., Doupe et al., 2003; Moore et al., 2011). According to Cannon (1977), the main difference between larval types I and II is the ratio of intestinal caecum to ventriculus being 1:1 in the former and 2:1 in the latter morphotype. Based on the similarity in the ratio of intestinal caecum to ventriculus and considering the geographical location of larvae and matching it with presence of adult nematodes, he suggested Terranova larval type I in his study could be Terranova chiloscyiti and Terranova larval type II could be T. galeocerdonis.
Table 3  Taxa listed under genera Terranova, Pulchrascaris and Pseudoterranova.

| Taxa | Host common name | Host scientific name | Location | Reference |
|------|------------------|-----------------------|----------|-----------|
| Terranova Leiper & Atkinson, 1914 | | | | |
| T. amoyensis Fang & Luo 2006 | Red string ray | Dasyatis akajei | Taiwan Strait | Fang & Luo (2006) |
| T. antarctica (Leiper & Atkinson, 1914) | Gummy shark | Mustelus antarcticus | Bay of Islands, New Zealand | Leiper & Atkinson (1914) |
| T. brevicapitata (Linton, 1901) | Tiger sharkk | Galeocerdo cavier | Woods Hole, Massachusetts, USA | Deardorff (1987) |
| T. caballeroi Diaz-Ungria, 1967 | Porcupine river stingray | Potamotrygon hystrix | Delta of the Orinoco River, Venezuela | Diaz-Ungria (1967) |
| T. cephaloscyllii (Yamaguti, 1941) | Blotchy swell shark | Cephaloscyllium umbratilis | Nagasaki, Japan | Yamaguti (1941) |
| T. circularis (Linstow, 1907) | Common sawfish | Pristis pristis | Cameroon | Bruce, Adlard & Cannon (1994) |
| T. crocodili (Taylor, 1924) | West African crocodile | Crocodylus sp | Ghana | Sprent, (1979) |
| | Malayan crocodile | Crocodylus johnstoni | Northern Australia; Queensland; Malaya | |
| T. draschei (Stossich, 1896) | Arapaima | Arapairna gigas | Rivers of northern South America | Bruce, Adlard & Cannon (1994) |
| T. galeocerdonis (Thwaite, 1927) | Tiger shark | Galeocerdo cavier | Twynams Paar, Ceylon; South Australia and Queensland, Australia; Natal, northern Brazil. | Bruce, Adlard & Cannon (1994) |
| | Scalloped hammerhead | Sphyra lewini | | |
| | Smooth hammerhead | S. zygaena | | |
| | Blacktai reef shark | Carcharinus amblyrhynchos | | |
| T. ginglymostomae Olsen, 1952 | Nurse shark | Ginglymostoma cirratum | Tortugas, Florida, USA; off Queensland, Australia | Bruce, Adlard & Cannon (1994) |
| | Spotted wobblegong | Orectolobus maculatus | | |
| | Zebra shark | Stegostoma fasciatum. | | |
| T. lanceolata (Molin 1860) | Black caiman | Melanorhynchus niger | Brazil | Sprent (1979) |
| | American alligator | Alligator mississippiensis | | |
| T. nidifex (Linton, 1900) | Tiger shark | Galeocerdo tigrinus | Woods Hole, Massachusetts, USA | Deardorff (1987) |
| T. pristis (Baylis & Daubney, 1922) | Large tooth sawfish | Pristis microdon (P. perotteti) | Ulubaria, India; Balgal, Queensland, eastern Australia | Bruce, Adlard & Cannon (1994) |
| | Snaggettooth shark | Hemipristis elongatus Walle la attu | | |
| | Wallago | | | |
| T. petrovi Mozgovoi, 1950 | Shark | Raja longirostris | Kamchatka, USSR | Bruce, Adlard & Cannon (1994) |
| T. quadrata (Linstow, 1904) | The saltwater crocodile | Crocodylus porosus | Belgrade | Mozgovoi (1950) |
| T. rochalimai (Pereira, 1935) | Shark | Scientific name was not mentioned in the original description | Brazil | Mozgovoi (1950) |
| T. scoliodontis (Baylis, 1931) | Shark | Scoliodon sp. | Cleveland Bay, Townsville, Australia | Bruce, Adlard & Cannon (1994) |
| T. secundum (Chandler, 1935) | Largehead hairtail | Trichiurus lepturus. | Galveston Bay, Texas, USA; La Paloma, Uruguay | Chandler (1935) |

(continued on next page)
Table 3 (continued)

| Taxa                                      | Host common name | Host scientific name | Location                                      | Reference                          |
|-------------------------------------------|------------------|-----------------------|-----------------------------------------------|------------------------------------|
| T. serrata (Drasche, 1896)\(^b\)          | Arapaima         | Arapaima gigas        | Rivers of northern South America              | Bruce, Adlard & Cannon (1994)      |
| Terranova trichiuri (Chandler, 1935)\(^a\) | Indian threadfin | Polycyclus indicus    | Galveston Bay, Texas, USA; Khulna, Pakistan   | Bruce, Adlard & Cannon (1994)      |
| Pulchrascaris Vicente and dos Santos, 1972|                  |                       |                                               |                                    |
| P. caballeroi Vicente and dos Santos, 1972| Angelshark       | Squatina squatina\(^a\) | Rio de Janeiro, Brazil                        | Bruce, Adlard & Cannon (1994)      |
| P. chiloscyllii (Johnston and Mawson, 1951)| Brownbanded bamboo shark | Chiloscyllium punctatum | Halfway Island, Australia; Hawaii, Alabama, USA; South Africa | Bruce, Adlard & Cannon (1994)      |
| P. secunda (Chandler, 1935)                | Largehead hairtail| Trichiurus lepturus   | Galveston Bay, Texas, USA; La Paloma, Uruguay | Bruce, Adlard & Cannon (1994)      |
| Pseudoterranova Mozgovoi, 1951             |                  |                       |                                               |                                    |
| Pseudoterranova azarasi (Yamaguti & Arima, 1942) | Steller’s sea lion | Eumetopias jubatus    | Japanese and Sakhalinese waters of the North Pacific Ocean | Mattiucci & Nascetti (2008)       |
| P. bulbosa (Cobb, 1888)                    | Bearded seal     | Erignathus barbatatus | Barents and Norwegian Seas, the Canadian Atlantic and the Sea of Japan, | Mattiucci & Nascetti (2008)       |
| P. cattani George-Nascimento and Urrutia, 2000 | South American sea lion | Otaria byronia      | South-East Pacific, Chilean coast             | Mattiucci & Nascetti (2008)       |
| P. decipiens (Krabb, 1868) (sensu stricto) | California sea lion | Zalophus californianus | North-East and North-West Atlantic            | Mattiucci & Nascetti (2008)       |
| P. krabbei Paggi, Mattiucci et al., 2000   | Northern elephant seal | Phoca vitulina richardsii |                                               | Mattiucci & Nascetti (2008)       |
| P. decipiens E of Bullini et al., 1997     | Antarctic Weddell seal | Leptonychotes weddellii | Antarctica                                      | Mattiucci & Nascetti (2008)       |

Notes.

\(^a\)The species has been described based on a single female and should be redescribed.
\(^b\)Mozgovoi (1953) lists this species as Terranova serrata (Drasche 1884) while Bruce, Adlard & Cannon (1994) listed it as Porrocaecurn draschei (Stossich, 1896) and noted that there is some doubt as to which name has priority for this species.
\(^c\)This taxon was considered as junior synonym of T. galeocerdonis by Tanzola & Sardella (2006).
\(^d\)According to Johnston & Mawson (1945) T. nidifex may be identical to T. galeocerdonis.
\(^e\)This taxon was regarded as species inquirenda by Gibson & Colin (1982).
\(^f\)Now is known as Pulchrascaris secunda (Deardorff, 1987).
\(^g\)This species was considered as a synonym of T. secundum (Chandler, 1935) by Olsen (1952).
\(^h\)According to Deardorff (1987) this is a misidentification of host.
\(^i\)In the original description Cação panan was stated as type host which could not be assigned to any specific elsamobranch.
or *T. scoliodontis*. Although some species within *Pseudoterranova* (e.g., *P. cattani*) have the same ratio of intestinal caecum to ventriculus and although *Pulchrascaris* has been reported from the same general location (Table 3), the possibility of these larvae being *Pulchrascaris* spp. or *Pseudoterranova* spp. was not discussed in Cannon’s work. In addition, assigning larval type to adults based on the ratio of intestinal caecum to ventriculus has been considered to be unreliable. *Huizinga* (1967) showed that the length of the intestinal caecum is shorter in smaller/younger larvae and increases as the larvae grow in length. This can affect the ratio of intestinal caecum to other organs, such as ventricular appendix or ventriculus. As a result the specific identity of *Terranova* larval types remains unknown. For the same reasons, despite of morphological resemblance between *Terranova* larval type in the present study and those described by *Cannon* (1977) there is no certainty that they
Figure 2  Alignment of the sequences of the ITS-1 and ITS-2 regions of Terranova larval type II of Cannon, 1977c found in the present study. The left column indicates the GenBank accession number of specimens. Numbers to the right of alignment indicate the alignment position. Polymorphic sites were designated using IUPAC codes.
are genetically similar or belong to the same species due to lack of comparable molecular data for Cannon’s specimens.

In an attempt to specifically identify Terranova larval type in the present study, we genetically characterised all Terranova larval type found in the present study from broad geographical region as well as a broad variety of fish species, based on their ITS-1 and ITS-2 sequences followed by phylogenetic analyses.

The nucleotide variation within Terranova larval type in the present study was very low (0–0.4% for both ITS-1 and ITS-2), and was within the range for nucleotide variation (0–0.2% and 0–0.4% for ITS-1 and ITS-2 respectively) calculated for members of the same species in the family Anisakidae (Shamsi et al., 2009b). This suggests they all should be the same genotype/species.

To reveal the specific identity of the Terranova larval type found in the present study comparable ITS sequences from well identified adults must be available. To date, there is no such sequence in the GenBank database. Among reliably identified species whose ITS-1 and ITS-2 sequences were available in the GenBank database, there was no identical or highly similar sequence to ITS-1 and ITS-2 sequences found in the present study. Alignment of ITS-1 and ITS-2 sequences of Terranova larval type in the present study with those available in GenBank database did not result in finding identical or highly similar sequences. Although the closest ITS sequences in the GenBank database belonged to Pseudoterranova azarasi, P. bulbosa, P. cattani and P. decipiens sensu strict the nucleotide difference between ITS sequences of the larvae in the present study and those of Pseudoterranova spp. in the GenBank was too great (38.9–39.8% and 46.7–48.4% for ITS-1 and ITS-2, respectively) to be considered within the genus Pseudoterranova (Table 4). The distinction between Terranova larval type in the present study and Pseudoterranova spp. was also supported by phylogenetic analyses (Fig. 3).

ITS-1 and ITS-2 sequences of well identified closely related taxa were selected to build the phylogenetic tree to investigate the association of larvae in the present study with other taxa within family Anisakidae. Both neighbour joining and maximum parsimony (the latter is not shown) trees had similar profile and grouping of taxa were the same among both trees. In the neighbour joining phylogenetic tree (Fig. 3), Terranova larval type found in the present study were resolved as a distinct clade with strong bootstrap support of 100%. None of the anisakid species (Pseudoterranova spp., Anisakis spp. and Contracaecum spp. becoming adult in marine mammals) with similar morphology to Terranova spp. (i.e., having excretory pore opened at the base of the labia) were grouped in the same clade as Terranova larval type found in the present study. Closely related species becoming adult in teleost fishes (Hysterothylacium spp. and Raphidascaris acus) were also included in the phylogenetic tree, although the excretory system in this group has a different feature to Terranova spp. These anisakids also resolved as a distinct clade to Terranova spp.

In both phylogenetic trees produced in the present study based on the combined ITS-1 and ITS-2 sequences, the Terranova larval type was resolved separately from Pseudoterranova spp. suggesting they do not belong to the genus Pseudoterranova.

As reviewed in the Introduction, Australian Terranova larval types could potentially be larval stages of Pseudoterranova spp., Terranova spp., or Pulchrascaris spp. Species
Figure 3  Phylogenetic analysis of the combined ITS-1 and ITS-2 sequence data for members of the Anisakidae with Heterakis gallinarum as outgroup, using the neighbour-joining method. Bootstrap support values are indicated. See Table 1 for detailed abbreviations. Note that Terranovasp and Terranova1 both belong to the same taxon and only different in polymorphic sites as shown in Fig. 2. They are representative of 93 Terranova larval type examined in the present study.
Table 4  Pairwise comparisons of the nucleotide differences (%) in the consensus sequences of ITS-1 and ITS-2 between Terranova larval type found in the present study and Pseudoterranova spp. (the only taxa with closest ITS sequence similarity available in GenBank database).

| Terranova larval type in the present study | ITS-1 | ITS-2 |
|-------------------------------------------|-------|-------|
| *P. azarsi*                               | 39.8  | 46.7  |
| *P. bulbosa*                              | 38.9  | 48.4  |
| *P. cattani*                              | 39.3  | 47.2  |
| *P. decipiens sensu tricto*               | 39.0  | 48.3  |

Figure 4  Map shows reported cases of Terranova larval types (circles), Adult Terranova spp (asterisk), adult Pseudoterranova spp (square), adult Pulchrascaris (triangle), distribution of Australian sea lion (solid line), Australian fur seal (square dots) and New Zealand fur seal (round dot).

Thus, comparison of ITS sequence of Terranova larval type found in the present study with those of Pseudoterranova spp. available in GenBank (Table 4) shows a considerable nucleotide difference of 38.9–39.8% in both ITS-1 and ITS-2 regions. This is greater than nucleotide difference found for distinct species within a genus of family Anisakidae (Shamsi et al., 2009b) suggesting Terranova larval type in the present study does not belong to the genus Pseudoterranova.

To date, four species of Terranova have been reported from Australian sharks, *T. galeocerdonis*, *T. ginglymostomae*, *T. pristis* and *T. scoliodontis* (Bruce & Cannon, 1990). In addition, *T. crocodyli* was found in Australian crocodiles (Sprent, 1979). They all have
a similar relationship between length of the intestinal caecum and ventriculus to the 
Terranova larval type in the present study. Pulchrascaris is a small genus in terms of number 
of species under family Anisakidae. Like members of the genus Terranova, Pulchrascaris 
spp. become adult in elasmobranches. There is intra/inter specific variation in the ratio of 
the intestinal caecum to ventriculus of Pulchrascaris spp. (Bruce & Cannon, 1990). Since 
there is no ITS sequences available for Terranova spp. or Pulchrascaris spp. in the GenBank database, the specific identity of the Terranova larval type found in the present study remains unknown and we are not able to associate these larvae to any Terranova spp. or 
Pulchrascaris spp. however, the present study, particularly the ITS sequence data, provides 
the essential information for future studies when the adult form is found and characterised. 

This is the first report of a Terranova larval type from Abudefduf whitleyi, Carangoides fulvoguttatus, Caranx ignobilis, C. melampygus, Chaetodon flavirostris, Lutjanus argenti-
maculatus, L. bohar, Pristipomoides multidens, Scomber australasicus. Some of these fish, 
such as Australian mackerel (Scomber australasicus) are popular edible fish. Infection of 
those fish species that are not edible is also very important due to their role in the survival 
and transmission of Terranova larval type in the ecosystem.

Although the present study could not specifically identify the Terranova larval type 
in Australian waters, it could rule out the possibility of them being Pseudoterranova 
larvae which would have different implications for seafood and consumers’ safety and 
policy development in the country. It should be emphasized that it is very likely that 
Pseudoterranova larvae exist in Australian waters, infect some fish and await discovery. 
Their definitive hosts, Australian sea lion, Australian fur seal and New Zealand fur seal 
are found in southern coast of Australia (Fig. 4) and have been found to be infected 
with Pseudoterranova decipiens E (Bullini et al., 1997). However, given that in Australian 
waters the diversity of elasmobranch species is considerably higher (approximately 200 
species, www.fishbase.net) than that of marine mammals (3 species of seals) our suggestion 
is that Terranova larval type in Australian waters is more likely to be a Terranova or a 
Pulchrascaris. To date there is no evidence that larval stage of Terranova or Pulchrascaris 
can cause infection in humans.

ACKNOWLEDGEMENTS

Authors are grateful to Prof. Tom Cribb (University of Queensland, Australia) and his 
research group for their generous cooperation in collecting specimens from Heron Island.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work had partial financial support by School of Animal and Veterinary Sciences, 
Charles Sturt University and University of Melbourne. Shokoofeh Shamsi received support 
from the Graham Centre for Agricultural Innovations. The funders had no role in study 
design, data collection and analysis, decision to publish, or preparation of the manuscript.
**Grant Disclosures**
The following grant information was disclosed by the authors:
School of Animal and Veterinary Sciences.
Charles Sturt University.
University of Melbourne.
Graham Centre for Agricultural Innovations.

**Competing Interests**
The authors declare there are no competing interests. Shokoofeh Shamsi is a member of the Graham Centre for Agricultural Innovations.

**Author Contributions**
- Shokoofeh Shamsi conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper, drawings of the parasite.
- Jaydipbhai Suthar performed the experiments, analyzed the data, prepared figures and/or tables, reviewed drafts of the paper.

**DNA Deposition**
The following information was supplied regarding the deposition of DNA sequences:
Materials examined morphologically were deposited in South Australian Natural History Museum. The isolate numbers are as below: 301-1, 304-18, 304-2, 316-2, 324-4, 333-10, 336-4, 336-6, 340-4 and 341-3.
Nucleotide sequence data reported in this paper are available in the GenBank database under the accession numbers LN795828–LN795873.

**Data Availability**
The following information was supplied regarding data availability:
The research in this article did not generate any raw data.

**REFERENCES**

Abe N, Ohya N, Yanagiguchi R. 2005. Molecular characterization of *Anisakis pegreffii* larvae in Pacific cod in Japan. *Journal of Helminthology* **79**:303–306 DOI 10.1079/JOH2005290.

Anderson RC. 2000. *Nematode parasites of vertebrates: their development and transmission*. Wallingford: CABI Publishing, 284.

Arizono N, Miura T, Yamada M, Tegoshi T, Onishi K. 2011. Human infection with *Pseudoterranova azarasi* roundworm. *Emerging Infectious Diseases* **17**:555–556 DOI 10.3201/eid1703.101350.

Bruce NL, Adlard RD, Cannon LRG. 1994. Synoptic checklist of ascaridoid parasites (Nematoda) from fish hosts. *Invertebrate Taxonomy* **8**:583–674 DOI 10.1071/IT9940583.
Bruce NL, Cannon LRG. 1990. Ascaridoid nematodes from sharks from Australia and the Solomon Islands, Southwestern Pacific Ocean. *Invertebr. Taxon.* 4:763–783 DOI 10.1071/IT9900763.

Bullini L, Arduino P, Cianchi R, Nascetti G, D’Amelio S, Mattiucci S, Pagli L, Orecchia P, Plotz J, Berland B, Smith JW, Brattee J. 1997. Genetic and ecological research on anisakid endoparasites of fish and marine mammals in the Antarctic and Arctic -Boreal regions. In: *Antarctic communities.* Cambridge: Cambridge University Press, 39–44.

Cannon LRG. 1977. Some larval ascaridoids from south-eastern Queensland marine fishes. *International Journal for Parasitology* 7:233–243 DOI 10.1016/0020-7519(77)90053-4.

Chai J-Y, Darwin Murrell K, Lymbery AJ. 2005. Fish-borne parasitic zoonoses: status and issues. *International Journal for Parasitology* 35:1233–1254 DOI 10.1016/j.ijpara.2005.07.013.

Chandler AC. 1935. Parasites of fishes in Galveston Bay. *Proceedings of the United States National Museum* 83:123–157 DOI 10.5479/si.00963801.83-2977.123.

Chilton NB, Gasser RB, Beveridge I. 1995. Differences in a ribosomal DNA sequence of morphologically indistinguishable species within the *Hypodontus maccopi complex* (Nematoda: Strongyloidea). *International Journal for Parasitology* 25:647–651 DOI 10.1016/0020-7519(94)00171-J.

Deardorff TL. 1987. Redescription of *Pulchrascaris chiloscyllii* (Johnston and Mawson, 1951) (Nematoda: Anisakidae), with comments on species in *Pulchrascaris* and *Terranova.* *Proceedings of the Helminthological Society of Washington* 54:28–39.

Diaz-Ungria C. 1967. *Tres especies de nematodes de peces venezolanos, con descripcio* *n de Terranova caballeroi, n. sp. (Nematoda),* vol. 22. Maracay: Revista de Medicina Veterinaria y Parasitologia, 1–8.

Doupe RG, Lymbery AJ, Wong S, Hobbs RP. 2003. Larval anisakid infections of some tropical fish species from North-West Australia. *Journal of Helminthology* 77:363–365 DOI 10.1079/JOH2003193.

Fang WZ, Luo DM. 2006. Description of a new ascarid species in elasmobranchs from Taiwan Strait. *Journal of Parasitology* 92:822–825 DOI 10.1645/GE-694R1.1.

Gibson DI, Colin JA. 1982. The *Terranova* enigma. *Parasitology* 85:R36–R37.

Hafsteinsson H, Rizvi SSH. 1987. A review of the sealworm problem—biology, implications and solutions. *Journal of Food Protection* 50:70–84.

Hochberg NS, Hamer DH. 2010. Anisakidosis: perils of the deep. *Clinical Infectious Diseases* 51:806–812 DOI 10.1086/656238.

Huizenga HW. 1967. The life cycle of *Contracaecum multipapillatum* (Von Drasche, 1882) Lucker, 1941 (Nematoda: heterocheilidae). *The Journal of Parasitology* 53:368–375 DOI 10.2307/3276593.

Jensen T, Andersen K, Desclers S. 1994. Sealworm (*Pseudoterranova decipiens*) in dem- ersal fish from 2 areas in Norway. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 72:598–608 DOI 10.1139/z94-082.
Jimenez FA, Gardner SL, Navone G, Orti G. 2012. Four events of host switching in Aspidoderidae Nematoda involve convergent lineages of mammals. *Journal of Parasitology* **98**:1166–1175 DOI 10.1645/GE-3045.1.

Johnston TH, Mawson PM. 1945. Parasitic nematodes. British, Australian and New Zealand Antarctic research expedition. *Reports, Series B* **5**:73–159.

Kijewska A, Czarna A, Fernandez M, Zdzitowiecki K, Rokicki J, Wrobel B. 2008. Analysis of 5.8S rDNA and internal transcribed spacer 1 (ITS1) sequences of ascaroid nematodes: phylogenetic signal and hypothesis testing. *Genes & Genomics* **30**:291–306.

Kijewska A, Rokicki J, Sitko J, Wegrzyn G. 2002. *Ascaridoidea*: a simple DNA assay for identification of 11 species infecting marine and freshwater fish, mammals, and fish-eating birds. *Experimental Parasitology* **101**:35–39 DOI 10.1016/S0014-4894(02)00031-0.

Leiper RT, Atkinson EL. 1914. Helminthes of the British Antarctic Expedition 1910–1913. *Proceedings of the Zoological Society of London* **1914**:222–226.

Lester RJG, Barnes A, Habib G. 1985. Parasites of skipjack tuna, *Katsuwonus pelamis*: fishery implications. *Fishery Bulletin* **83**:343–356.

Li A, D’Amelio S, Paggi L, He F, Gasser RB, Lun Z, Abollo E, Turchetto M, Zhu X. 2005. Genetic evidence for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) and the validity of *Contracaecum septentrionale* (Kreis, 1955) (Nematoda: Anisakidae). *Parasitology Research* **96**:361–366 DOI 10.1007/s00436-005-1366-y.

Margolis S. 1977. Public health aspects of codworm infection: a review. *Journal of the Fisheries Research Board of Canada* **34**:887–898 DOI 10.1139/f77-140.

Mattiucci S, Nascetti G. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host—parasite co-evolutionary processes. *Advances in Parasitology* **66**:47–148 DOI 10.1016/S0065-308X(08)00202-9.

McClelland G. 2002. The trouble with sealworms (*Pseudoterranova decipiens* species complex, Nematoda): a review. *Parasitology* **124**:s183–s203.

Moore BR, Stapley J, Allsup Q, Newman SJ, Ballagh A, Welch DJ, Lester RJG. 2011. Stock structure of blue threadfin *Eleutheronema tetratactylum* across northern Australia, as indicated by parasites. *Journal of Fish Biology* **78**:923–936 DOI 10.1111/j.1095-8649.2011.02917.x.

Mozgovoi AA. 1950. Key to parasitic nematodes. In: Skrjabin KI, ed. *Oxyurata and Ascaridata*, vol 2. New Delhi: Academiya NAUK SSSR. Washington, D.C.: United States Department of Agriculture and the National Science Foundation, 1982.

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. *Journal of Parasitology* **91**:1413–1429 DOI 10.1645/GE-522R.1.

Olsen LS. 1952. *Some nematodes parasitic in marine fishes*. Vol. 2. Port Aransas: Institute of Marine Science of the University of Texas, 173–215.
Orecchia P, Paggi L, Mattiucci S, Smith JW, Nascetti G, Bullini L. 1986. Electrophoretic identification of larvae and adults of *Anisakis* (Ascaridida: Anisakidae). *Journal of Helminthology* **60**:331–339 DOI 10.1017/S0022149X00008580.

Paggi L, Nascetti G, Cianchi R, Orecchia P, Mattiucci S, D’Amelio S, Berland B, Brattey J, Smith JW, Bullini L. 1991. Genetic evidence for three species within *Pseudoterranova decipiens* (nematoda, ascaridida, ascaridoidea) in the north atlantic and norwegian and barents seas. *International Journal for Parasitology* **21**:195–212 DOI 10.1016/0020-7519(91)90010-5.

Pontes T, D’Amelio S, Costa G, Paggi L. 2005. Molecular characterisation of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. *Journal of Parasitology* **91**:1430–1434 DOI 10.1645/GE-565R1.1.

Shamsi S. 2014. Recent advances in our knowledge of Australian anisakid nematodes. *International Journal for Parasitology: Parasites and Wildlife* **3**:178–187.

Shamsi S, Butcher AR. 2011. First report of human anisakidosis in Australia. *Medical Journal of Australia* **194**:199–200.

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. *Molecular and Cellular Probes* **25**:13–18 DOI 10.1016/j.mcp.2010.09.003.

Shamsi S, Gasser R, Beveridge I. 2012. Genetic characterisation and taxonomy of species of *Anisakis* (Nematoda:Anisakidae) parasitic in Australian marine mammals. *Invertebrate Systematics* **26**:204–212 DOI 10.1071/IS11019.

Shamsi S, Gasser R, Beveridge I. 2013. Description and genetic characterisation of *Hysterothylacium* (Nematoda: Raphidascarididae) larvae parasitic in Australian marine fishes. *Parasitology International* **62**:320–328 DOI 10.1016/j.parint.2012.10.001.

Shamsi S, Gasser R, Beveridge I, Shabani AA. 2008. *Contracaecum pyripapillatum* n. sp. and a description of *C. multipapillatum* (von Drasche, 1882) from the Australian pelican, *Pelecanus conspicillatus*. *Parasitology Research* **103**:1031–1039 DOI 10.1007/s00436-008-1088-z.

Shamsi S, Norman R, Gasser R, Beveridge I. 2009a. Genetic and morphological evidences for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) (Nematoda: Anisakidae) in Australia. *Parasitology Research* **105**:529–538 DOI 10.1007/s00436-009-1424-y.

Shamsi S, Norman R, Gasser R, Beveridge I. 2009b. Redescription and genetic characterization of selected *Contracaecum* spp. (Nematoda: Anisakidae) from various hosts in Australia. *Parasitology Research* **104**:1507–1525 DOI 10.1007/s00436-009-1357-5.

Shamsi S, Poupa A, Justine J-L. 2015. Characterisation of Ascaridoid larvae from marine fish off New Caledonia, with description of new Hysteroythyaculum larval types XIII and XIV. *Parasitology International* **64**:397–404 DOI 10.1016/j.parint.2015.05.014.

Sprent JFA. 1979. Ascaridoid nematodes of amphibians and reptiles: *Terranova*. *Journal of Helminthology* **53**:265–282 DOI 10.1017/S0022149X00006088.
Szostakowska B, Myjak P, Kur J, Sywula T. 2001. Molecular evaluation of Hysterothylacium auctum (Nematoda, Ascaridida, Raphidascarididae) taxonomy from fish of the southern Baltic. *Acta Parasitologica* **46**:194–201.

Tanzola RD, Sardella NH. 2006. *Terranova galeocerdonis* (Thwaite, 1927) (Nematoda: Anisakidae) from *Carcharias taurus* (Chondrichthyes: Odontaspididae) off Argentina, with comments on some related species. *Systematic Parasitology* **64**:27–36 DOI 10.1007/s11230-005-9015-5.

Thompson JD, Gibson TJ, Plewniac F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface:flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**:4876–4882.

Umehara A, Kawakami Y, Araki J, Uchida A. 2008. Multiplex PCR for the identification of Anisakis simplex sensu stricto, *Anisakis pegreffii* and the other anisakid nematodes. *Parasitology International* **57**:49–53 DOI 10.1016/j.parint.2007.08.003.

Yamaguti S. 1941. Studies on the helminth fauna of Japan. Part 33. Nematodes of fishes, II. *Japanese Journal of Zoology* **9**:343–396.

Zhu X, D’Amelio S, Paggi L, Gasser RB. 2000. Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of the *Contracaecum osculatum* complex (Nematoda: Ascaridoidea: Anisakidae). *Parasitology Research* **86**:677–683 DOI 10.1007/PL00008551.

Zhu X, Gasser RB, Podolska M, Chilton NB. 1998. Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal dna sequences. *International Journal for Parasitology* **28**:1911–1921 DOI 10.1016/S0020-7519(98)00150-7.

Zhu XQ, D’amelio S, Palm HW, Paggi L, George-Nascimento M. 2002. SSCP-based identification of members within the Pseudoterranova decipiens complex (Nematoda: Ascaridoidea: Anisakidae) using genetic markers in the internal transcribed spacers of ribosomal DNA. *Parasitology* **124**:615–623.